The Chemistry of Fragrances

From Perfumer to Consumer

2nd Edition

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From Perfumer to Consumer

2nd Edition

Edited by
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Preface

Chemistry is defined as the study of the properties of substances and their interactions with each other. It is easy to see how the word has also developed a colloquial meaning related to the effect of animate, inanimate and abstract entities on humans. So in a phrase such as 'the chemistry is right', the word takes on almost the meaning of 'magic'. Chemistry, in both senses of the word, is central to perfumery. A modern perfumery company will house specialists in all branches of chemistry from physical chemists through analysts and synthetic organic chemists to biochemists. Indeed, the spectrum of skills will range right across into biological fields such as toxicology and sensory and behavioural sciences. All of these specialists work together with perfumers, accountants, marketers and salespeople. No one skill is sufficient to meet the needs of the business and all must pull together as a team. The sensory scientists and marketers are particularly aware of the other sense of 'chemistry' in fragrance since they study and use the hedonic effects of perfume in designing the right fragrance to support each individual consumer product.

Our primary aim in this book is to show the use of chemistry in an exciting and rewarding business environment. However, we also felt that we should attempt to convey the interdisciplinary teamwork that is essential for success. In order to do this, we have invented a story that runs through the chapters and, we hope, links them together in a way that will show how different specialists work together in a modern perfumery company. Chemistry will remain a core discipline of the fragrance industry for the foreseeable future. The sustainability of the industry and its ability to provide for people's needs and wishes relies on chemistry to provide solutions for such issues as dwindling mineral oil reserves, conservation of oil-bearing plants, increasing need for performance (especially on safety and environmental grounds) in fragrances and an ever stricter regulatory environment.

I would like to thank all of my fellow authors for their enthusiasm and for the effort that they have put in. In turn, all of us thank the many other colleagues whose hard work and commitment were necessary in order to make this book possible.

Charles Sell

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Acknowledgement

We would like to acknowledge the special role of David Pybus, now of Pandora Ltd., in the inspiration and design of this book. Although he has been less involved in the editorial work of the second edition, the concept stems directly from that of the first and so is still the fruit of our collaboration.

On behalf of Quest and the team of authors,
Charles Sell

Glossary

ABSOLUTE The alcoholic extraction of the concrete.

ACCORD A blend of perfume ingredients balanced in odour intensity and having a pleasing effect. Generally used as a perfume building block.

ALCOHOLIC A perfumed product designed for application to the skin in which the carrier used is aqueous ethanol. *e.g.* Chanel 5. Concentration of perfume can vary from *ca.* 2% for an eau de cologne to *ca.* 30% for an extrait.

ALDEHYDIC An odour descriptor used to define the effect of using relatively large amounts of aliphatic aldehydes *e.g.* C10 aldehyde.

ANOSMIA Inability to perceive an odour generally or specifically for certain molecules. Can be genetically inherited, be induced by drugs or be the result of damage to the nose or brain.

BALANCE A combination of perfume notes such that no particular note dominates the others.

BASE Can be a confusing term as it is used in two ways in the perfume industry (1) to define a perfume sub-unit or building block (unlike an accord, a perfume base is balanced in terms of top, middle and end notes) or (2) to define the unperfumed medium *e.g.* soap noodles.

BOTTLE-NOTE The perception of the perfumed product on opening the bottle closure. Consists of perfume plus chemicals used in base (*e.g.* shampoo) manufacture.

BRIEF Document provided by the customer defining their perfume requirements.

CLOG P Calculated value for log P (q.v.).

CONCRETE The hydrocarbon extraction of the plant material.

END-NOTES The substantive part of a perfume comprising the less volatile components of the fragrance composition. Often crystalline, resinous or high relative molecular mass (low volatile) liquid.

ESSENTIAL OIL The steam distilled oil obtained from the plant material.

EXPRESSED A cold process in which the oil contained in the outer skin of a citrus fruit is released by rasping or compression of the citrus fruit *e.g.* lemon, orange, bergamot. Sometimes known as ‘cold pressed’ oil. Citrus oils would degrade if a normal steam distillation process were to be used.

FIXATION A fixative is used to prolong the effect of the more volatile ingredients in a perfume formula in an attempt to equalise the rate of evaporation of the component ingredients. Molecules with low vapour pressure are used; these are often resinous, crystalline or high relative molecular mass liquids.

FORMULA The list of ingredients with their proportions required to produce the desired odour effect.

HEADSPACE Headspace is the air above or surrounding a fragrant substance, which contains volatile compounds. Any form of analytical procedure that samples and analyses this vapour is termed ‘Headspace Analysis’.

HEDONIC Concerning the pleasurable sensation associated with fragrance.

LFER Linear free energy relationship.

LOG P The logarithm (base 10) of the octanol/water partition coefficient of a molecule.

MIDDLE-NOTES The heart of a perfume; the main theme. Last for a few hours on the skin.

MUGUET Lily-of-the-valley.

NATURAL Perfume materials of natural origin. Derived directly from nature.

NATURE IDENTICAL Perfume ingredients identical to those, which occur in nature but derived synthetically.

NATURAL PRODUCTS Plants, which can provide odiferous materials, *e.g.* rose, sandalwood, grapefruit, *etc.*

OSMOPHORE For a series of active molecules detected by the same mechanism it is assumed that there is a common conformation in which key atoms or functional groups are placed at certain relative distances from one another. The spatial arrangement is known as a biophore and specifically an osmophore in odorant perception.

QSAR Quantitative structure/activity relationship.

QSPR Quantitative structure/property relationship.

RMM Relative molecular mass i.e. molecular mass relative to H = 1.

SCHIFF'S BASE Organic compound formed by reaction between an aldehyde or ketone with a primary amine *e.g.* hydroxycitronellal will form a Schiff's base with methyl anthranilate to give Aurantiol®.

SMELLING BLOTTER Thin strip of highly absorbent paper used to assess the effect of a fragrance as it evaporates from top-note through to the end-notes. Also known as 'smelling strip' or 'mouillette'. One end of the blotter is dipped into the straight perfume oil or the oil diluted in alcohol.

SVP Saturated vapour pressure i.e. the vapour pressure above a pure liquid, measured under standard conditions.

SYNTHETIC INGREDIENTS Those materials obtained synthetically which are not identical to those found in nature *i.e.* neither Natural *q.v.* nor Nature Identical *q.v.*

TOP-NOTES The most volatile components of a perfume. Generally last a matter of minutes on the skin.

TRICKLE-DOWN The fragrance oil used in the alcoholic variant in a range is adapted for use in other variants in the range such as soap, antiperspirant, *etc.* This usually involves cheapening and substitution of perfume ingredients for stability and performance.

TROPICAL Used as an adjective to describe the type of fruit (*e.g.* mango) or flower (*e.g.* ylang ylang) found in the tropics. Sometimes also described as exotic.

WHITE FLOWERS Flowers with white petals such as jasmine, tuberose and gardenia. Often contain indole, which causes the characteristic browning of the petals with time.

CHAPTER 1

The Human Relationship with Fragrance

LINDA HARMAN
Quest International

> *'Smell is a potent wizard that transports you across thousands of miles and all the years you have lived'.*
>
> Helen Keller

Fragrance – the very word represents indulgence; pleasure; luxury. For centuries we have had a very special relationship with scent, with smell the most elusive of the senses. Helen Keller had an acute sensitivity to smell and touch, deprived as she was of sight and sound. The psychologist Sigmund Freud said that when mankind got together to cultivate the land, build ziggurats and temples, one of the casualties of civilisation was a diminution of the sense of smell. Certainly most of the greatest recent inventions have been linked to the more dominant informative senses of vision or hearing: television, telephone, computers, iPod … but when we want to retreat it is to the more emotional senses of smell, touch or taste that we turn.

Why is fragrance so evocative? Simply because of the way we are structured. Smell is the one sense whose exact mechanism remains a mystery – but which we do know is plugged directly into the part of the brain that is responsible for memory and emotion.

Barillé wrote: 'The magic of fragrance comes from the relationship between man and nature'.

Despite civilisation, the magic remains today. Fragrance is a sensory pleasure and a vital part of brand communication. Odour surrounds us from the first waft of coffee or burst of citrus in the shower to those indulgent mood-setting candles or soothing bath oils in the evening, whispering messages through associations stored deep in the human subconscious. Whether it be seeking relaxation through the fabled qualities of lavender or camomile, being revived by the bracingly medicinal scent of rosemary, soothed by the luxury of rose, Ylang Ylang, or seduced by the sensuality of sandalwood, scent is an emotional catalyst. In our increasingly pressured society smell is the emotional sense, fragrance the key to managing the, often turbulent, human psyche.

Fragrance is both an art and a science. Bringing the fragrant messages to products today is complex and relies on the craft of both perfumer and chemist. The industry behind fragrance is global and worth 152 Billion USD every year. It is dominated by six international companies who account for 57% of the total market: Givaudan (Swiss), IFF (American), Firmenich (Swiss), Symrise (German), Quest International (Anglo-Dutch), and Takasago (Japanese). The fragrances and flavours created by these companies are incorporated by manufacturers into every aspect of our daily lives, providing a plethora of choice: creamy shampoos and conditioners with soft sensual smell or astringent ones with herbal extract to reassure functional benefits, laundry products with built-in freshness designed to cling to clothes, cleaning products that sparkle olfactively as well as functionally and a whole array of personal fragrance products to convey invitations or stay-away messages to others.

Luxury, prestige, indulgence, warmth, reassurance, freshness, clean; fragrance stands for them all. Its role in modern life has however come under question by some elements of society who feel scent is not a necessity. The reader can draw his own conclusion as the psychology and science of scent is explored, the detail of the industry described, the role of natural *versus* synthetic ingredients investigated, all in the context of the creation of an imaginary scent – Eve. The creation of Eve mimics the process followed by the fragrance industry today, from receipt of the initial brief to the creation, evaluation, toxicology testing and application into product that takes place. Sound science and striking work by chemists is fundamental to the creation of all modern products. Fragrance is no exception.

This book sets out to explore the roots of this fascinating industry from alchemy to modern artisan. The chemistry behind fragrance as well as the work of the many professionals involved in bringing the sensory art of fragrance into our modern lives is described. From plant through lab. to production, the detailed process is followed and explained by industry experts working for leading fragrance supplier Quest International, a member of the ICI Group of companies.

CHAPTER 2

The History of Aroma Chemistry and Perfume

DAVID H. PYBUS
Pandora Ltd.

> *In chemistry also, we are now conscious of the continuity of man's intellectual effort; no longer does the current generation view the work of its forerunners with a disdainful lack of appreciation; and far from claiming infallibility, each successive age recognises the duty of developing its heritage from the past.*
>
> August Kekulé von Stradonitz (1821–1896)

The discovery, exploitation and use of fragrant materials began with an elite few, the priesthood, and had religious connotations. The very word perfume is derived from the Latin '*per fumum*', meaning 'by' or 'through' smoke, as it was with the use of burning incense that the prayers of the ancients were transported to the heavens for the contemplation of Gods. From thence came the priest-kings, and a wider audience, though still restricted, of pharaohs, emperors, conquerors and monarchs with their attendant courtesans and alchemists, and use of perfume took on a hedonistic mantle as well as a spiritual one. In the twentieth century, a combination of Chemistry and the Industrial Revolution brought the revelation of fragrances to the rest of mankind and acted as catalysts for the furtherance of their usage.

The great world religions of Islam, Christianity, Buddhism, Hinduism, Shintoism and Zoroastrianism employ fragrance in pursuance of their faiths. Finally, there is some evidence that human pheromones exist and that they are detected, perhaps subliminally, using the sense of smell. Thus it is that religious and pleasurable pursuits have been the main drives in the phenomenal growth of perfume usage throughout the centuries. After man's prehistoric past, with the dawning of civilisation, the use of fragrances developed within the four great centres of culture in China, India, Egypt and Mesopotamia, extending in time to sophisticated societies in Greece, Palestine, Rome, Persia and Arabia.

The seven ages of 'aromatic' man in Western Culture began when Crusaders brought back three magical gifts from the East to the Dark Ages of Europe, which had 'not bathed for a thousand years'. Delicate aromatics, distilled alcohol and refined glass were the physical manifestation of thousands of years of alchemical research. The three together, a beautiful smell, a solvent to extend it and a bottle to conserve this 'gift from the Gods' were gladly accepted in the medieval West, and their use blossomed through the seven ages of Chivalry, Alchemy, Discovery, Revolution, Empire, Fashion and into the New Millennium.

2.1 EARLY USE OF FRAGRANCE

In prehistoric times, the hunter-gatherer tribes of man, in their explorations of nature, found many wonderful substances of great use in everyday living. Our ancestors from earliest times lived in makeshift tree and ground nests, much as chimpanzees do today, and it may be that our abiding appreciation of greenery stems from this long homestead association with it. Animal products in great variety, by-products of the hunt, were employed for clothing, shelter and tools as well as for food. Some pleasant aromas were also perceived, not least the smell of cooked meat! Similarly, the collection of herbs, spices and grasses unearthed familiar and fragrant compounds that were put to good use by the clans. An elite few were given especial reverence to hold in trust the lore of the tribe. These sorcerers, or medicine men, knew the power of use and misuse of nature's pharmacopoeia, and over the centuries, by word of mouth, their store of wisdom increased. Craftsmen and artisans developed new and varied uses for materials as man's drive to extend and expand his knowledge knew no bounds.

Eventually, a drift and concentration of tribes founded the great civilisations of the Nile in Egypt, Mesopotamia between the Tigris and Euphrates in modern-day Iraq, the Hwang-Ho valley in China and the Indus civilisation of Mohenjo Daro and Harappa – all of which came into their own between 4000 and 2000 BC.

With them, over the centuries, came knowledge of glass, alcohol and aroma chemicals. Glass is made by fusing silica together that forms some

60% of the earth's surface, with sodium carbonate and calcium carbonate, or limestone, as evident from the fossilised remains of marine life. Mesopotamians and Egyptians had discovered that when sand and ashes were heated together, a hard, brittle, transparent substance was produced. The addition of limestone hardened the glass and gave it more durability. Thus, from SiO_2, Na_2CO_3 and $CaCO_3$, the vessel was formed to hold a yet greater chemistry.

The first alcoholic wines were most likely an accidental discovery from fermented grapes. Most fruits are contaminated with microbes that form the surface bloom, resulting in a natural alcoholic fermentation, given the right climatic environment, which was certainly present in Mesopotamia. Earliest references to the production of distilled spirits appear to have originated in China around 1000 BC, and it is believed that the production of beer developed in Egypt some 7000 years ago – involving the hydrolytic breakdown of starch in cereal extracts. The acid beer so produced, boozah, having modern-day connotations of Alehouses. Thus, with the earliest production of C_2H_5OH, ethanol, we have another key compound in our fragrant mix.

Meanwhile, a myriad of fragmented clues hint of ancient olfactory indulgence. Incense statuettes thousands of years old have been unearthed in the ruins of the Indus civilisation, which was known to trade with both Egypt and Mesopotamia, while in China, around 500 years before Christ, Confucius was proclaiming that both incense and perfume mitigate bad smells. Herbs, spices and flowers were used to ward off evil spirits, and flower-strewn graves over 5000 years old have been discovered in Iraq. Burning of incense in Japan, according to legend, attracted the Jiki-Ko-Ki – 'spirits which eat smoke'. In Mesopotamia, the fabled Garden of Eden, was where the sons of Noah were said to have settled in the land of Sumer after the flood. Fragrant wood was used to build temples and the fine smelling essences of cedarwood, myrtle and calamus reeds (sweet flag) were offered up for the pleasure of the Gods. It was here in Mesopotamia that the classic techniques of pressing, maceration and enfleurage, discussed in detail in Chapter 3, were developed. In supplication to the God Marduk, Nebuchadnezzar II, King of Chaldea announced, 'I anoint myself everyday with oil, burn perfumes and use cosmetics that make me worthier of worshipping thee'.

Early records detail King Sahure's trip to the fabled land of Punt, believed to be modern day Somalia or Ethiopia, around 2400 BC, bringing back, amongst other riches, 80,000 measures of myrrh. Temple pictographs dated around 1500 BC detail Queen Hatshephut's journey to

Punt with the objective of bringing back frankincense trees for replanting in Egypt.

Pictographs show courtesans wearing bitcones on their heads, consisting of animal fat impregnated with aromatic materials. In the Egyptian heat, this fat melted down the neck covering the body in an oily, pleasant layer which, while workable as an early form of deodorant, could have caused a great deal of inconvenience to the wearer – and was a far cry from modern day shampoo. Other hieroglyphs depict the great Ramses of Egypt offering incense in thanks to the Gods, while Nefertiti joined Semiramis of Babylon as one of the earliest women to demonstrate the liking of particular products, such as honey and orchid-leaf, in her fragrant formulations. Perfume concoctions appear on the wall of the temple of Horus, at Edfu, among which is Kyphi, or Kephri, the 'twice-good' fragrance, burnt in the early morning and at eventide. A listing of the key ingredients demonstrates sophistication in the formulae at this time, and the wealth of products used. The formula of Kyphi contained spikenard, another prized material, a calcite vase of which was found by Howard Carter in 1922 at King Tutankhamun's tomb. Two of the most powerful and earliest reputed alchemists, Hermes Trismegitus and Zosimus, hailed from the land of the Nile, and did much to set down the records of their arts for future generations to develop.

The Christian *Bible* is chock-full of descriptions of fragrances from the early days of the tribes of Israel, when Joseph's brothers sold him for a slave:

> *'A company of Ishmaelites came from Gilead with their camels bearing spicery and balm and myrrh, going to carry it down to Egypt'*.

In *Exodus*, God gave Moses instructions for a holy perfume for himself and a different one for his priests; while the visit of the Queen of Sheba to Solomon was motivated by her wish to keep open the trade routes of the Arabian peninsula, her source of frankincense and myrrh, through Palestine to Egypt and Mesopotamia.

The story of Jesus of Nazareth is populated by fragrant materials, from the frankincense and myrrh, his gifts at birth, through the use of spikenard to wash his feet during life and finally, the use of myrrh in the binding sheets of his body after crucifixion. Through trade and cultivation Palestine became a great source of aromatic wealth, and a key trade route for the caravanserai.

The Greeks further developed the use of fragrances, not only in praise of their Gods, but also for purely hedonistic purposes and for use in exercises and games – the first beginnings of early forms of aromatherapy.

Their myths were full of references to aromas. Tear-shaped drops of the resin Myrrh were the tears of a girl transmuted into a tree by the gods. The Hyacinth flower grew from the blood of dying Hyacinthus, struck by a discus during a feud between two other Gods. The Iris grew at the end of a rainbow, while the Narcissus flower grew at the spot near a mountain pool where its erstwhile namesake drowned. The especial fragrance formulation for the goddess Aphrodite created such sensual desire that the term 'aphrodisiac' was used in its praise.

The sciences of medicine and herbalism developed with Hippocrates and Theophrastus, while Alexander the Great, tutored by Aristotle, conquered half the known world, acquiring a love of fragrance from his defeated Persian kings. But it was Aristotle who, in the third century BC, arguably advanced the cause of alchemy and related it to the psychology of aroma substances. It was he who observed the production of pure water from the evaporation of seawater. He translated the *Book of Hermes* written by an Arab, Al-Makim. It expounded the theory, first suggested by Empedocles around 450 BC, that all substances are made of the four elements: fire, earth, air and water (Figure 2.1). By varying

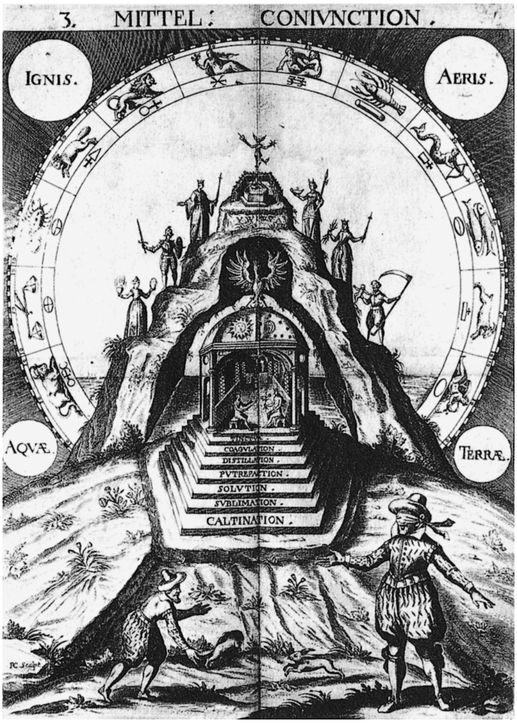


Figure 2.1 *The four elements, fire, air, water and earth*

Hot	Summer	Dry	Autumn
Fire	**Choleric**	Earth	**Melancholic**
Wet	Spring	Cold	Winter
Air	**Sanguine**	Water	**Phlegmatic**

Figure 2.2 *Elements and moods*

the amounts of the different elements in each compound, all other elements could be made.

The theory developed further to discuss moods related to the elements and the seasons as illustrated in Figure 2.2. The four key moods described were phlegmatic (solid, calm, unexcitable), choleric (irascible, hot-tempered), sanguine (optimistic, confident) and melancholic (sad, pensive). Combination of the four moods at their boundaries gives eight mood poles, and it is around these that some modern day theories of aromatherapy have evolved.

The fragrances most used by the Greeks were rose, saffron, frankincense, myrrh, violets, spikenard, cinnamon and cedarwood, and to obtain these aromatics they traded far and wide throughout the Mediterranean and Middle East.

Meanwhile in Rome, Pliny the Elder outlined a primitive method of condensation that collected oil from rosin on a bed of wool, and also made the first tentative experiment in chromatography. Meanwhile, the Romans had developed techniques of enamelling glass, and one of the most fundamental discoveries was made – that glass could be blown. Roman contribution to perfumes consisted mainly in making an industry for the supply of raw materials and production of a large variety of fragrances in different forms. Military conquests secured new sources and supply routes to fit the steady demands of a far-flung empire, and the key products in demand were:

- Hedysmata: solid unguents, normally in the form of gums and resins.
- Stymata: liquid toilet waters infused with flower petals.
- Diapasmata: powdered perfumes using aromatics disposed in talc or gypsum.

The Roman elite kept Acerra, small incense caskets, in their homes and carried ampullae, perfume containers and strigils, wooden blades for scraping oils off the skin at hot baths. Petronius wrote,

Wines are out of fashion, Mistresses are in
Rose leaves are dated
Now Cinnamon's the thing.

The first professional perfumers – unguentarii – plied their trade in Capua, which became a trading centre of the industry. Perfume was used in abundance at the Games, both as a present for the crowds and a mask for the malodours of a bloodstained and offal-dappled arena. It is estimated that in the first century AD, Romans were consuming nearly 3000 tonnes of frankincense and over 500 tonnes of the more expensive myrrh. Roman Emperors of course used perfume to excess, as instanced by Nero and his wife Poppaea who had a kind of 'perfumed plumbing' in their palaces, with false ceilings designed to drop flower petals onto dinner guests and scented doves to fragrance the air with their perfumed wings. It was said that when Poppaea died, Nero burned a whole year's supply of incense on her funeral pyre; a fragrant fortune that would have amounted to hundreds of tonnes. Towards the end of the Empire, Heliogabalus showed the true excesses of wealth and power. According to contemporary accounts, he sported gilded lips, henna-dyed hands and feet and eyes decorated in concentric rings of blue and gold. This Emperor of Rome hailed from Syria, Land of Roses, which, as with Nero, were his favourite blooms.

When Rome succumbed to the barbarian hordes, the lights went out in all the incense burners throughout Europe, and the rose petals went out with the bath water.

2.2 THE AGE OF CHIVALRY

While Crusaders became the implacable foes of Islam in the Holy Land throughout the eleventh and twelfth centuries, they admired many of the material possessions of Muslims, and brought back wall hangings, carpets, spices, eating forks, glass vessels and fragrances to their dank, dark and gloomy castles in Europe. Empress Zoe in the Christian stronghold of Constantinople, employed court perfumers, certain that incense and perfumes drove out demons. From there the practice spread, with Normans strewing flowers and rushes onto the floors of castles and churches to keep the air fragrant and acceptable.

It was common to employ a washerwoman or lavenderess (from which laundress is derived) to place sprigs and sachets of lavender around the rooms, and sweet-smelling packets of herbs amongst the bed linen. Not so pleasant odours were important too. Knights jousting for a lady's favour were not after a pretty handkerchief, but a 'pretty' smell (that of the lady's armpit odour) since there was a practice of holding a 'kerchief there to retain some of the smell and remembrance of the wearer.

2.3 THE AGE OF ALCHEMY

In a perverse way, the Black Death of 1347–1351 and subsequent pandemics were huge catalysts to the growth in usage of aromatic products, which had already shown signs of flourishing from its eastern alchemical practice. It was believed that plague would be caught by breathing foul air (*mal aria* – malaria also got its name from the seeming connection between death from this disease and the stink of stagnant water where mosquitoes might breed). Dead bodies lying in the street gave off the odour of decay, and to counteract this people carried nosegays and small floral bouquets (posies) from when the 'ring a ring of roses', children's nursery rhyme was derived (red rings being a primary visual symptom of plague). To visit important people during times of epidemic, one went through the trial of fire and water at their doors. Washing with water and then enveloping the body in smoke or incense was felt to be an effective defence against Black Death. In addition, torchbearers with plague torches (brands of burning fragrant herbs) walked ahead of important and rich personages.

Gentile of Foligno, a medicinal practitioner of Padua, made the connection between smell and death, thus:

> *Poisonous material is generated about the heart and lungs. Its impression is not for excess in degree of primary quality, but through the properties of poisonous vapours having been communicated by means of air breathed in and out, great extention and transition of the Plague takes place, not only from man to man, but from country to country.*

Plague doctors wore full leather coats, hats and crystal goggles to ensure that they did not catch the disease from patients, and to be doubly sure touched them only with examining wands. They wore large cones, or nosegays, resembling a bird's beak or bill, and thus it is believed the derogative 'quack' applied to fake doctors.

In an effort to keep disease at bay the first efforts to adopt more hygienic customs were made, at least with aristocracy. The English King Henry III (1207–1272) sent the following message ahead during one trip to London:

> *Since the privy chamber in London is situated in an undue and improper place, wherefore it smells badly, we command you on the faith and love by which you are bounden to us that you in no way omit to cause another privy chamber, to be made in such more fitting and proper place that you may select there, even though it should costs a hundred pounds…*

Guilds for the supply of aroma products began to be formed between the twelfth and thirteenth centuries. Related crafts included the London guild of Pepperers and Spicers, and in 1268 the Glovers' Guild was recognised. King Henry I of France and England granted a heraldic shield, which was essentially silver with three red gloves and a gold spice box on a blue background, to the Guild of Perfumers. The connection with gloves came from the need for leather tanners to hide the vile smell of their profession and from their expertise in fine aromas the development of perfume grew. A charter to glover perfumes had been granted by Philip Augustus of France (1165–1223) as early as 1190.

Venice was an important centre for trade and commerce between Europe, the Middle East and the Orient, was the funnel through which many spices and aromatic raw materials reached Europe, and its domination in trade for these products lasted for a few hundred years.

Glass saw major strides in its development. In medieval times a green tint, caused by iron in the silicate, was common. Antimony, used as a decolouriser, was replaced by manganese and the Renaissance produced rapid development in the art of glassmaking in Venice. By 1400, a rock-crystal-like glass was produced and perfected (cristillo).

Distillation as an art was well known in the eleventh century, but the first European treatise on distilling was written by the Catalonian Arnald of Villanova around 1310. Different types of distilled spirit were identified such as aqua vitae (life), aqua vini (wine) and aqua ardens (burning water); and the book on the practice – 'The Vertuose Boke of Distyllacyon' was translated in English from the German 1500 original version of Jerome Brunschweig by Lawrence Andrews. This book dealt in detail with the essential oils of lavender, juniperwood, pine and rosemary.

Early processes of distillation used alembics usually made of copper, iron or tin, since lead and silver had the characteristic of tainting the distillation vapour.

Arnald of Villanova showed interest also in the sulfur baths of Montpellier, and it was around this time that the great fragrance raw material and production centre of Grasse, in the south of France, began to develop strongly. Along with Arnald of Villanova came such famous alchemical names as Roger Bacon (attributed to the invention in Europe of gunpowder), Ramon Lull of Spain, Nicolas Flamel of France and George Ripley of England, who kept the flames of knowledge alive, moving technique and thought apace. Table 2.1 gives a more detailed list of key figures in the history of alchemy, which furthered the development of an art that eventually became true chemistry. Alchemists, as we shall see, figured in the furtherance of aromatic knowledge for the next two centuries.

2.4 THE AGE OF DISCOVERY

The sixteenth and seventeenth centuries saw an explosion in world exploration. It had been preceded by the Italian Columbus's voyages to the Americas with John Cabot, the Englishman, close on his heels. Verrazzano searched for a Northern Strait (1524–1528), while Cartier too plied the coast of North America (1534–1536) in search of riches.

Queen Elizabeth's master mariners, Gilbert, Frobisher, Drake and Raleigh trawled the seas in search of treasure for her coffers, while Magellan and da Gama probed the southern seas.

All this marine activity brought back many new aroma products to the tables and laboratories of Europe or increased the supplies of existing ones. And at the other end of the voyage the alchemists were waiting to add to their store of knowledge.

Court alchemists included Giordano Bruno (Henry III of France 1551–1589), Arthur Dee (Elizabeth I, Charles I) and Joseph Duchesne (Henry IV of France 1553–1610).

Bruno was an early atomist, writing on '*The Principles, elements and causes of things*' (1590), while Andreas Libavius (1597) developed the study of alchemy in two directions: encheiria, the manipulation of materials, and chymia, the preparation and classification of chemicals. Biringuccio (1540, *Pirotechnia*) wrote of fireworks, Agricola (1556, *De re metallica*) of metals, Neri (1612, *L'Arte Vetraria*) of glass

Table 2.1 *Alchemists of history*

Date	*Provenance*	*Alchemist or related science*	*Major work*
~1550 BC	Egypt	Maria Prophetessa	Development of Chemical Apparatus
~1550 BC	Egypt	Hermes Trismegitus	The Emerald Tablet
460–370 BC	Greece	Democritus	Formulated early Atomic Theory
~384–322 BC	Greece	Aristotle	Secretum Secretorum$^+$
~300–250 BC	Egypt	Bolos of Mendes	Phusika Kai Mustika
~130 AD	China	Wei Po Yang	Ts' An T′ung Ch'i
721–815	Persia	Jabir Ibn Hayyan	Summa Perfectionis
866–921	Persia	Rhazes	Book of the Secret of Secrets
980–1037	Persia	Avicenna (Ibn Sina)	Book of The Remedy
1193–1280	Germany	Albertus Magnus	Libellus de Alchimia
1214–1294	England	Roger Bacon	Opus Maius
1235–1311	Spain	Arnald of Villanova	A Treatise on the Preservation of Youth
1235–1294	Spain	Ramon Lull	Dignitates Dei
~1330	Italy	Petrus Bonus	Pretiosa Margarita Novella
1330–1417	France	Nicolas Flamel	Work on Transmutary Alchemy
~1450s	England	George Ripley	Medulla Alchimiae
~1470s	England	Thomas Norton	The Ordinall of Alchimy
1462–1516	Germany	Abbot Trithemius of Sponheim	Steganographia (Angel Magic)
1486–1535	Germany	Henry Agrippa	Occulta Philosophia
1493–1541	Switzerland	Paracelsus	Der Grosseren Wundartzney
1494–1555	Germany	Georgius Agricola	De Metallica
1527–1608	England	John Dee	Monas Hieroglyphica
1544–1609	France	Joseph Duchesne	On the Material of the Medicine of Ancient Philosophers
1548–1600	Italy	Giordano Bruno	De Umbris Idearum
1550–1627	England	Anthony Francis	Panacea Aurea
1568–1622	Germany	Michael Maier	Atalanta
1575–1624	Germany	Jacob Boehme	On the Three Principles of Divine Being
1586–1654	Germany	Johann Valentin Andrea	Chemical Wedding of Christian Rosencreutz
1560–1603	Germany	Heinrich Khunrath	Amphitheatre of Eternal Wisdom
1560–1616	Germany	Andreas Libavius	Alchemia
1574–1637	England	Robert Fludd	Physics and Technics
1580–1609	Germany	Oswald Croll	Basilica Chemica
1603–1665	England	Sir Kenelm Digby	A Choice Collection of Chymical Secrets

Continued

Table 2.1 *Continued*

Date	*Provenance*	*Alchemist or related science*	*Major work*
1616–1654	England	Nicholas Culpeper	A Physical Directory
1617–1669	England	Elias Ashmole	Theatrum Chemicum Britannicum
~1620s	Hungary	Daniel Stolz	Chemical Garden
1627–1666	USA	George Starkey	The Marrow of Alchemy
1627–1691	England	Robert Boyle	Sceptikal Chymist
1642–1727	England	Sir Isaac Newton	Principia
1593–1662	England	William Backhouse	The Magister

manufacture and Robert Fludd (1574–1637) covered nearly everything in his manifest works.

Meanwhile, Paracelsus (1493–1541) worked on distillation to separate the ‘essential’ from the ‘non-essential’ parts of a compound, and further developed the ‘*quinta essentia*’ (quintessence) theory of a fifth element, involved in imbuing life. His radical ideas greatly influenced medicine during the Renaissance. According to Paracelsus, God makes medicine but not in a prepared form. In nature, medicine is found compounded with ‘dross’ that must be taken away by distillation, setting the medicine free. The process became refined.

It was at the end of the seventeenth century that a German Johann Kunckel, discovered how to use gold chloride to manufacture ruby glass and also how to ‘strike’ it red (a re-heating technique to develop the colour).

In 1573, Edward de Vere, Earl of Oxford, brought Elizabeth I not only scented sachets but also perfumed gloves and jerkins. This sold Elizabeth, the Queen who had ‘a bath every three months whether she needed it or no’ on the use of fragrances, which gradually became *de rigeur* in court.

Around this time the first books and manuscripts describing perfumery techniques surfaced, and court perfumers took the stage. A contemporary of Elizabeth, Catherine de Medici (1519–1589) travelled to France to marry Henry II, and in her entourage were two skilled artisans, Tombarelli and Renato Bianco, skilled in the crafts of perfumes and poisons since court intrigue mixed affairs of the heart with affairs of the sword. Diane de Poitiers, a rival of Medici, was said to dabble herself in philtres, potions, perfumes and poisons. Nostradamus, the personal astrologer of Catherine, was known to inhale smoke and incense as part of his preparations for prophecying. As a plague doctor, Nostradamus used rose petal pills as palliative and part herbal remedy for removing bad breath and cleaning teeth. The recipe for these pills included red roses picked before dawn, sawdust from fresh green cypress, iris, cloves, calamus, tiger lily and lignaloes. His second wife, Anne Ponsard Gemelle, was famed as a maker of herbalised perfumes.

Throughout the ages, perfume has provided a pathway to happiness; like history itself, the odours are intangible and often fleeting. History is littered with examples of the famous and their perfumed preferences: Henry III was said to have fallen head over heels in love with Mary of Cleeves after breathing the odour of her just-removed clothing. Henry IV of France was reputed to smell so ripe that his intended, Marie de Medici (1573–1642), keeled over when she first met him, while Henry himself, revelling in his own natural odour and those of others, once reputedly wrote to his mistress Gabrielle d'Estree, 'Don't wash my love, I'll be home in eight days'. The French kings and their courts greatly indulged the use of fragrance: Louis XIII favouring neroli, based on orange blossom, while his chief adviser Cardinal Richelieu had the fragrant scent of flowers 'bellowed' through his apartments. Louis XIV, the Sun King, with his mistress Madame de Montespan compounded his own fragrances, while Louis XV lavished wealth on '*La Cour Parfumee*' – the Perfumed Court, with his mistresses Madame de Pompadour and Madame Barry, where even the fountains did not escape a fragrant dousing. Eventually the Madames of France, ending with Marie Antoinette, wife of Louis XVI (1774–1793) paid for their indulgence in meeting another Madame – the guillotine.

Meanwhile in England, Charles I (1600–1649) had Anne of Denmark as fragrant advisor, while Charles II (1630–1685) was torn between the aristocracy of Catherine of Braganza and the earthiness of Nell Gwynne. Perfume rings, filigree pomanders and vinaigrettes found new ways to perfume the air, much needed since the strong smell of valerian musk and civet was more desirable to the lack of personal hygiene which existed at the time. The animalic link to humans had also been made, as instanced by this sage advice of a Mr Wecker in his '*Secrets of Art and Nature*':

> *If any man would provoke a woman, let him sprinkle his Glans with Oyl, Musk or Civet, Castoreum or Cubeba, or any of these, for these so quickly provoke.*

2.5 THE AGE OF REVOLUTION

The rejection of monarchy, fuelled by an English Civil War earlier (1642–1651) grew apace in the revolutions of North America (1775–1783) and France (1789–1795), leading to fundamentally new politics and world order.

Meantime, a quieter revolution was occurring, as apothecaries, spicerers and chymists began to develop professionally a new trade – that of perfumery. In 1708 Charles Lilly, a London perfumer, introduced scented snuffs and a revolutionary fragrance consisting of Orange Flower, Musk, Civet, Violet and Amber, while in 1711 William Bayley opened a perfumery in Long Acre, moving later to Cockspur Street under the sign of 'Ye Olde Civet Cat'. Juan Floris (1730) and William Yardley (1770) added to the groundswell, matched in France by Houbigant (1774) and Lubin (1798).

These half dozen perfumers catalysed the marketplace, expanding their clientele to common folk in such a way that George III (1738–1820) became concerned enough to issue the following edict:

> *All women whether of rank or professional degree, whether virgins, maids or widows, that shall from after this Act impose upon, seduce and betray into matrimony any of his majesty's subjects by the use of scents, potions, cosmetics, washes, artificial teeth, false hair, Spanish wool, iron stays, hoops, high heels, shoes or bolstered hips, shall incur the penalty of the law now in force against witchcraft and like misdemeanors, and that the marriage, upon conviction, shall be null and void.*

Two famous compounded fragrances, *La Poudre de Marechale* (1670) and *Eau de Cologne* (1710) grew in popularity, marking a slight move in preference from the heavy animalic scents of the times; but with pox and pestilence to counter, aromas of all descriptions were slapped on, sprinkled over and carried in nosegays or tussie mussies. House atmospheres were refreshed using pomanders, pot pourris and cassolettes. The churches frowned and Oliver Cromwell did his best to put a stamp on the use of fragrances, but the eighteenth century saw a fragrance backlash of mighty proportion.

Fragrances were needed in profusion to combat the olfactory disaster zones of prisons, hospitals, ships, churches, theatres, workshops and indeed anywhere where there was a gathering of humming humanity.

Major cities sported ooze-smelling rivers, malignant vapours and rank-smelling fogs from industry. Dyers, tanners, butchers and various artisans added to the smoke, coal, sulfur and fetid aromas in the air, and the environment was further fouled by the lax sanitary conditions of the time.

Balsams and light florals were in with a vengeance, with rose, violet and lavender leading the way. The Elizabethan 'effluvia' maskers were on their way out, and, after 300 years, not before their time. Like Casanova, European gentry had palled to the smells, which evoked this comment from their distant ancestors

Lady I would descend to kiss thy hand
But 'tis gloved, and civet makes me sick.

Meanwhile, the glass-cutter borrowed techniques from the gem cutter, and the Venetian style of soda-lime glass was copied through Christendom, but without the same brilliance of metal. In 1673, the Glass Seller's Company of England commissioned George Ravenscroft to produce an acceptable substitute, and using up to 30% lead oxide in the mix, a brilliant glass with a high refractive index was manufactured, establishing England as a leader in the production of clear, decorative glass.

For colour, lithyalin glass, with slight metallic inclusions, gave colours ranging from reddish brown through leek green and olive green to bluish mauve, the better to hide impurities in a perfume mix. Hyalinth gave black glass, while selenium produced pink, oxides of cobalt and copper blue, cadmium sulphate, antimony and gold chloride yellow, while oxides of chromium and copper produced ruby glass. Apart from hiding impurities, dark glass had a useful purpose of matching costumery and fashion at the time, and also in protecting a fragrant mix against ultraviolet light. At first glass was seen as somewhat of a luxury, and thus its use focused around perfumes, cosmetics and toiletries. Four main types of container evolved: the cylindrical or cigar-shaped alabaster, the pear-shaped armphoriskos, the short-necked globe-like aryballos and the simple jug with a handle and flat base.

By the seventeenth century, perfumes had begun to be stored in lightly blown glass bottles, and the eighteenth century saw the appearance of pear-shaped bottles in opaque white glass, decorated similarly to porcelain ware. Weight was reduced, and decorative appeal achieved by colour, cutting and applique decoration, which made perfume bottles truly treasured possessions and worth much for today's collectors.

2.6 THE AGE OF EMPIRE (NINETEENTH CENTURY)

Napoleon Bonaparte loved aromas, even dispelling a revolution with his 'whiff of grapeshot'. Hailing from Corsica, Bonaparte liked the fresh citrus and herbal smells, and favoured Eau de Cologne, using by all accounts several bottles a day and more than 60 a month! In an echo back to the days of the French King Henry, he too extolled Josephine by messenger, from his campaigns, not to wash as he was returning home (the anecdotal accepted origin of the name for '*Je Reviens*').

Josephine, a Creole from Martinique, employed a different pot pourri of smells. She favoured animalics, and was particularly fond of musk oil, to the point that when Napoleon left her for another woman, she smeared the inside walls and curtainings of their house, Malmaison, with the all-pervading tenacious smell as a constant reminder of her. Needless to say, Napoleon was not too enamoured of that Parthian shot, but he still nevertheless kept a place in his heart for Josephine.

The old warrior carried a necklace with her cameo portrait and a pressed violet, one of her favourite flowers, around with him, and on her death visited her grave to strew it with violets. One of his own dying wishes was to be buried in a certain spot at St. Helena where his soul would be soothed by the sweet smell of tuberose.

Queen Victoria can be credited with two 'smell' revolutions which hit Britain. The first involved her dress style, which often in casual mode featured a knitted shawl. The shawls she wore were steeped in patchouli, imbuing a rich woody fragrance to the garment. Since half the populace wished to emulate the monarch, patchouli shawls were *de rigueur*, and we see here one of the first instances of smell being used as a product and in the marketing of a fashion item.

Victoria's second claim to fame was marrying Prince Albert from the German house of Saxe-Coburg. In doing so, the marvellous custom of the Christmas tree, long favoured in Central Europe and Scandinavia, was brought into British homes. The Christian festival of Christmas, founded on a much older Roman festival of Saturnalia, and before that the ancient pagan festivals welcoming the return of the sun, brought much of greenery into the home, as this signalled the return of springtime and the renewed growth of plant life on which we all depend. The Christmas tree brings an evocative smell of nature to life in our households, and along with clove, cinnamon, candle wax, mistletoe, holly and a cornucopia of scents, ensures that the memory of a merry Christmas is retained.

The wearing of perfume itself, however, was strictly controlled: just a little dab, or carried on a handkerchief, never on oneself, was the rule of the day. Victorian ladies had to be 'proper' and so scent was too evocative to be worn by a lady. As the Victorian era drew to a close, new names cropped up to cater to the mass-market demand for quality and reliability of scents. Scientists and artisans developed into perfumers of both integrity and repute. To famous names such as Lillie Yardley, Lentheric and Floris were added Savoury and Moores, Atkinsons, Chardin, Crown, Coty, Hougibant, Guerlain, Roger and Gallet, Penhaligon and Piver. Names which are familiar in households today.

Perfumery developed in three fundamental ways: the technique used, the structure and synthetics employed and the industrialisation (massification) of the process.

2.6.1 Technique

In his book '*Odours, Fragrances and Cosmetics*' (1865), S. Piesse developed theories relating specific odours to notes on a musical scale in an attempt to categorise the spectrum of smells, while in 1890 Atkinsons produced one of the first books on perfume technology, essentially concerned with the production of absolutes by the cryoscopic removal of fats. Perfumery was beginning to be investigated in depth.

2.6.2 Structured Perfumes, and Use of Synthetics

In 1861, Guerlain created Eau Imperiale for Empress Eugenie, the influential wife of Napoleon III, whose gowns were designed by the House of Worth. By the end of the century this redeveloped fragrance was shown to be created around Neroli, Rose, Geranium, Sandalwood, Musk and the synthetic chemical coumarin. Fragrances began to be described in a structural form with the adoption of top, middle and bottom note terminology.

Two other fragrances, Fougere Royale (1882, Houbigant) and Jicky (1889, Coty) were in vogue at the end of the century. Fougere Royale was arguably the first fragrance to use a synthetic – coumarin, while Jicky is held to be the first truly vertically structured fragrance, with a fresh citrus top based on lemon, bergamot and mandarin; middle based on floral notes of rose and jasmine and woody notes in vetiver, orris and patchouli; and base notes of coumarin, benzoin, civet, amber and vanillin (a second synthetic). The use of synthetics stemmed from a drive to lower costs and improve quality, while at the same time chemists were bringing to the fore, as described earlier in this chapter, a vast array of novel aromas to increase the olfactory palette.

2.6.3 Industrialisation and 'Massification'

By 1879 it was listed that Yardley exported over a score of different varieties of scented soaps to the United States, while the British company Crown Fragrances was exporting 49 different fragrances to 47 different countries. Perfumers focused on mass production techniques for aroma chemicals, glass bottles and alcohol to service an ever-growing market demand. Products were branded to encourage consumer loyalty, and the first tentative steps for marketing their olfactory wonders were being made by the giants of their time. Perfumers of France, England and Spain widened their horizons to a global marketplace. At the end of the nineteenth century, science, industrialisation, market demand and men of the moment had conspired to catalyse growth in the use of scent and the pleasure gained from it. Perfume was finally reaching the masses. This set the scene for the twentieth century, the age of fashion, which spurred truly explosive growth in the use of fragrance in many forms. In the background of this flurry of activity on the perfume front, major strides had been taken in the synthesis of aroma chemicals, which greatly influenced and aided the formulation chemists and perfumers in their choice and cost of materials. Table 2.2 gives, in summary form, some of the key compounds of interest to the industry, with their discovery dates and the chemists involved.

2.7 THE AGE OF FASHION (TWENTIETH CENTURY)

> *I am no longer interested in dressing a few hundred women, private clients; I shall dress thousands of women. But … a widely repeated fashion, seen everywhere, cheaply produced, must start from luxury.*
>
> Gabrielle (Coco) Chanel

Table 2.3 underscores a prodigious growth in the use of fragrances, where for each decade of the twentieth century, against dramatically different social backdrop, novel chemistry was developed, which gave new strength, depth and vision to the world of perfume. However, it

Table 2.2 *Important dates in the history of aroma chemicals*

Year	*Event*	*Attributed to*
1701	Observations that some flowers provided no essential oils on steam distillation	Nicholas Lemery
1759	Reaction of oil of amber with fuming nitric acid gave a musky odour	Berlin Academy
1800	Investigations into ambra component chemistry	
1833	Empirical formulae reported for anethole, borneol and camphor	Dumas
1834	Isolation of cinnamic aldehyde	Dumas, Peligot
	Preparation of nitrobenzene	Mitscherlich
1837	Isolation of benzaldehyde	Liebig, Wohler
1843	Methyl salicylate determined as main component of wintergreen oil	Cahours
1853	Preparation of benzyl alcohol	Cannizzaro
	Synthesis of aliphatic aldehydes	Piria
1856	Synthesis of cinnamic aldehyde	Chiozza
1859	Preparation of aldehydes from pyrolysis of calcium formate mixtures	Bertagnini
1859–1860	Large-scale preparation of salicylic acid	Kolbe
1863	Preparation of benzaldehyde	Cahours
1865	Determination of structure of benzene	Kekule
1866	Structure of cinnamic acid determined	Erlenmeyer
1868	Synthesis of coumarin	Perkin
1869	Discovery of heliotropin	Filtig and Mielk
1871	Structure of heliotropin determined	Barth
1874	Synthesis of vanillin from guaiacol	Reimer and Tiemann
1876	Discovery of phenylactetic aldehyde	Radziszewski
1875–1877	Synthesis of cinnamic acid	Perkin
1877	Production of anisaldehyde from *p*-hydroxybenzaldehyde	Tiemann, Herzfeld
1878	Structure of terpin hydrate determined	Tilden
1880	Quinolines discovered	Skraup
1884	Identification of d-limonene and dipentene	Wallach*
1888	Discovery of nitro musks	Baur
1889	Discovery of citronellal	Dodge
1890	Synthesis of heliotropin from saffrole	Eykmann
1893	Synthesis of ionone	Tiemann, Kruger
1894	Structure of alpha-pinene determined	Wagner
1885	Structure of terpineol determined	Wallach, Tiemann, Semler
1891	Discovery of Rhodinol	Eckhart
1898	Discovery of Musk Ketone	Baur, Thurgau
1903	Discovery of methyl heptin carbonate and homologues	Moureau, Delange
	Fundamental work on aromaticity	Von Baeyer*
	Methyl nonyl acetic aldehyde	Darzens
1904	Isolation of muscone	Walbaum
	Glycidic method of synthesising aldehydes	Darzens

Continued

Table 2.2 *Continued*

Year	*Event*	*Attributed to*
1905	Synthesis of cinnamic alcohol	Leser, Barbier
1905–1908	Hydroxycitronellal prepared and marketed	
1908	Discovery of gamma-undecalactone	Jukov, Schestakow
1913	Discovery of farnesol	Kerschbaum
1919	Discovery of cyclamen aldehyde	Blanc
	Synthesis of linalool	Ruzicka* Fomasir
1923	Discovery of alpha amyl cinnamaldehyde	Lesech, Descollonges
	Discovery of nerolidol	Ruzicka*
1926	Identification of muscone structure	Ruzicka* and Kirschbaum
	Structural determination work on ambra, civet	
	Exaltone®	Ruzicka*
1927	Ambrettolide®	Kerschbaum
1928	Civetone	Ruzicka*
	Exaltolide®	
1933	Structural determination of jasmone	Treff, Werner
	Jasmone	
1934	Synthesis of muscone	Weber, Ziegler
1946	Perfection of Wallach's isoprene rule for terpenoids	Robinson*
1947	Structural determination of irone	Ruzicka*, Naves
1949	Ambrox®	
1953	New Synthesis of linalol	Caroll, Kimel
1959	*cis*-3-Hexenol	Bedoukian
1962	Methyl dihydro-jasmonate (MDJ)	
1970	Damascones alpha and beta	Demole
	Damascenones alpha and beta	Demole

*Nobel prize winners – four in the first half of this century involved with aspects of aroma chemistry.

took another phenomenon to catalyse the industry of fine fragrances to a level that we see today; and that was of the fashion designer and the rise of the consumer.

While the perfume companies brought the baton of perfume into the twentieth century, and still run in the games, it was the designers who took the baton and ran a different kind of race – a race to bring a name to the masses.

In the first 20 years of the twentieth century, about a score of fine fragrances were developed, including Violette Purpre (1907, Houbigant), L'Origan (1905, Coty), English Lavender (1910, Atkinsons), L'Heure Blue (1912, Coty) and Old English Lavender (1913, Yardley). In the last decade of that century, over 100 fine fragrances a year were being launched. Towards the end of the twentieth century fine fragrances had

Table 2.3 *Twentieth century – the age of fashion*

Decade	*Background*	*Development and exploitation*	*Fragrance used*	*Emergent fashion designers*
1900s	Fin de Siecle, emancipation	Coumarin, heliotropin, ambreine	L'Origan	Worth
1910s	Peace and war	Undecalactone	Mitsouko	Poiret, Caron
1920s	Prohibition, exhibition	Aldehyde C10,C11,C12	Chanel 5	Chanel, Patou
1930s	Recession, depression	Phenyl ethyl acetate, civettone	Tabu	Schiaparelli, Dana
1940s	War and peace	Hydroxycitronellal, musk ketone	L'Air du Temps	Dior, Balenciaga, Balmain
1950s	Rock and roll	Amyl salicylate, cedryl acetate,	Youth Dew	Rochas, Nina Ricci, Nitromusks
1960s	Flower power	PTBCHA, *cis*-3-hexyl salicylate	Fidji	Laroche, Quant
1970s	Global village	Methyl dihydro jasminate	Chanel 19	Cacharel, Paco Rabanne
1980s	King consumer	Ethylene brassylate, Helional®	Obsession	Montana, Jean Paul Gaultier
1990s	Fin de Siecle, millennium	Dihydromyrcenol, synthetic musks	Cool Water	Thierry Mugler, Hugo Boss, Joop
2000s	Naughties	Macrocyclic musks	Beyond Paradise J'Adore	Hilfiger, McQueen Prada, Klein
		Headspace	Retroscent	Varvatos

five main platforms for display and sale to the general public, these being the small artesanal brands and shops, centred mainly in large cities, major retailer brands and fashion designer fragrances, fine fragrance houses and international corporations. Perfume had finally come to the people; chemistry and creativity had brought it there.

In 1905, Francois Coty said, 'Give a woman the best product you can compound, present it in a container of simple, but impeccable taste, charge a reasonable price for it, and a great business will arise such as the world has never seen'.

Thus, the man was not only a genius, but a visionary as well.

CHAPTER 3

Perfumery Materials of Natural Origin

CHARLES SELL
Quest International

3.1 PERFUMES AND ODOURS IN NATURE

3.1.1 Introduction

Like the pharmaceutical industry, the fragrance industry uses nature as its guide and source of inspiration. All the perfumes and perfume ingredients, which we produce in our factories, are modelled to a greater or lesser extent on those found in nature. We observe nature, analyse it to find out how it does the job and then modify and adapt its methods to suit our needs.

Smell and taste are the oldest of our senses. They probably developed in very primitive organisms as a means of obtaining information about chemical changes in their environment. Diurnal birds and aquatic animals rely heavily on sound; man and a few primates rely on vision; but all other species use smell and taste, the chemical senses, as the dominant medium through which they obtain information about the world in which they live. Since smell is such an important source of information for us, it is not surprising that nature has developed a very sensitive and sophisticated system for analysis of the chemicals, which make up our environment. It is intriguing that we can detect not only the natural odours, whether they have been put there deliberately or are simply artefacts of degradation processes, but also chemicals to which we have never been exposed before. Our understanding of how this wonderful sense works has advanced significantly in recent years and is described in more detail in Chapter 13.

Animals use smell and taste to find food and to assess its quality. The smell of food has a powerful effect on animals, whether it is a lion smelling out a herd of wildebeest or a shopper being drawn to the in-store bakery at the back of the supermarket. Watch your cat when you give him a bowl of food. He will carefully sniff it before eating to check that it is fresh and good. Our aversion to the smell of amines and mercaptans is, doubtless, related to their presence in food that has been spoiled by bacterial decomposition, a strong warning signal against sources of food poisoning. Some degradation reactions are responsible for the development of flavour in food. For example, autoxidation of fatty acids can lead to the formation of characteristic aldehydic flavour materials. It is important to note that the tongue (*i.e.* the sense of taste), only detects sweet, salt, sour, bitter and umami (savoury, or monosodium glutamate taste). The rest of taste is, in fact, smell. The volatile flavour ingredients are vaporised in the mouth and reach the nose through the airways behind the roof of the mouth.

Living organisms also use the chemical senses as a means of communication. If the communication is between different parts of the same organism, the messenger is referred to as a hormone. Chemicals used to carry signals from one organism to another are known as semiochemicals. These can be grouped into two main classes. If the signal is between two members of the same species, the messenger is called a pheromone. Pheromones carry a variety of information. Not all species use pheromones. In those that do, some may use only one or two pheromones while others, in particular the social insects such as bees, ants and termites, use an array of chemical signals to organise most aspects of their lives. Sex pheromones are among the most widespread. Male moths can detect females by smell at a range of many miles. Androstenone is the compound that produces ‘boar taint’ in pork. It is produced by boars and is released in a fine aerosol when the boar salivates and champs his jaws. When the sow detects the pheromone in the air, she immediately adopts what is known as ‘the mating stance’ in readiness for the boar. Ants and termites use trail pheromones to identify a path between the nest and a food source. This explains why ants are often seen walking in single file over quite long distances. The social insects also use alarm, aggregation, dispersal and social pheromones to warn of danger and control group behaviour. Chemicals that carry messages between members of different species are known as allelochemicals. Within this group, kairomones benefit the receiver of the signal, allomones its sender and with synomones both the sender and receiver benefit. Thus, the scent of a flower is a synomone since the attracted insect finds nectar and the plant gets a pollinator. Some plants produce compounds known as antifeedants, the taste which insects find repulsive. These are allomones since the signal generator, the plant, receives the benefit of not being eaten.

Unlike pheromones, many odorous chemicals in nature are produced for properties other than their odour. Many plants, when damaged, exude resinous materials as a defence mechanism. The shrub *Commiphora abyssinica*, for example, produces a resin, which contains a number of antibacterial and antifungal compounds. The role of the resin is to seal the wound and prevent bacteria and fungi from entering and damaging the plant. The resin has a pleasant odour and so was put to use by man as a perfumery ingredient. It is known as myrrh. Because of its antimicrobial properties, myrrh was also used as an antiseptic and preservative material, for instance, in the embalming of corpses. Frankincense has been used in religious rites for thousands of years, and so two of the three gifts brought to the Christ Child by the magi were perfume ingredients. Knowledge of perfumery thus helps us understand the symbolism involved; gold, frankincense and myrrh represent, king, priest and sacrifice, respectively.

3.1.2 Biosynthesis

Plants and animals produce odorous materials for a wide variety of reasons. How do they generate them? All living organisms produce a variety of chemicals through a process known as biosynthesis. The materials thus produced can be classified into two major groups, *viz.* primary and secondary metabolites. Primary metabolites are those that are common to all species and these can be subdivided into proteins, carbohydrates, lipids and nucleic acids. The materials used as perfume ingredients are mostly secondary metabolites though a few are derived from primary metabolites by degradative processes. The four categories of secondary metabolites, in decreasing order of importance as sources of perfume ingredients, are: terpenoids, shikimic acid derivatives, polyketides and alkaloids. There are very few odorous materials derived from the alkaloid family and so they will not be discussed further here. Among others, the terpenes are, by far, the most important. The terpenoids, shikimates and polyketides are all originally derived from glucose as can be seen in Figure 3.1. (Sometimes the letter P, written inside a circle, is used to represent a single phosphate unit.) It is worthwhile spending some time considering how the natural perfume ingredients are put together since, through this, the patterns of nature can be understood and used to assist

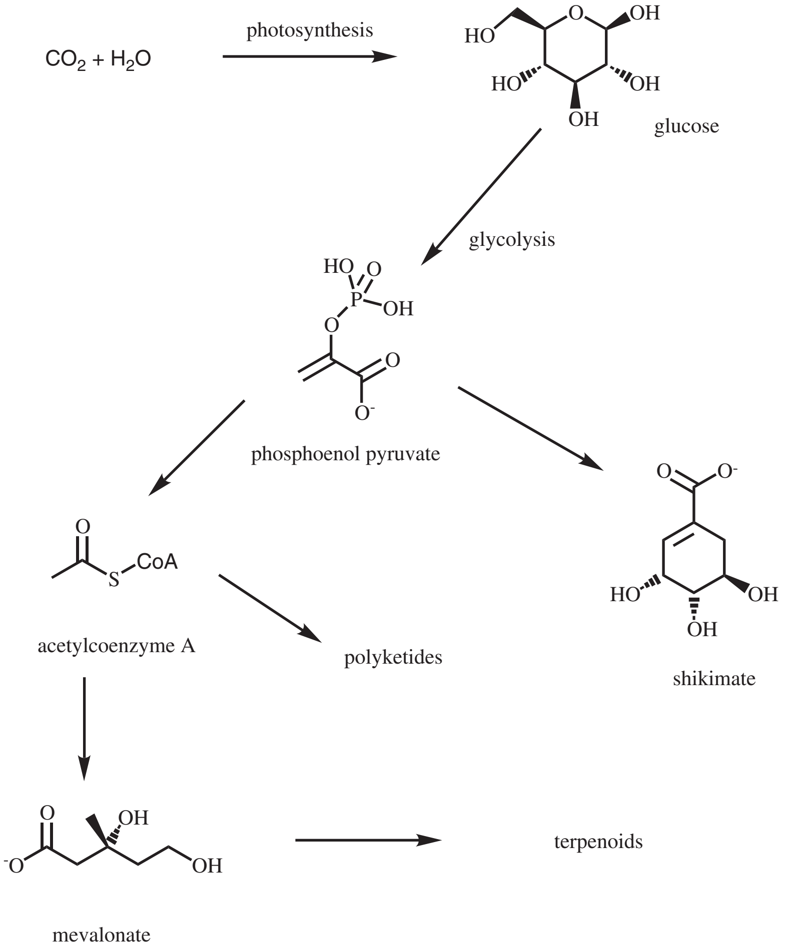


Figure 3.1 *Basic biosynthesis routes*

in identifying the structures of newly isolated materials and in producing new compounds with similar odour properties. More detail on biogenesis can be found in the book by Mann *et al.* (1994).

Green plants and photosynthetic algae synthesise glucose from carbon dioxide and water using sunlight as the energy source to drive this energetically unfavourable process, which is known as photosynthesis. Glucose can be broken down, either by the plant, which made it or by another species, which obtains it by eating the plant, to give the enol form of pyruvic acid, in which the enolic hydroxyl group is protected by the formation of a phosphate ester. One metabolic pathway builds shikimic acid from the phosphoenol pyruvate and another converts it to

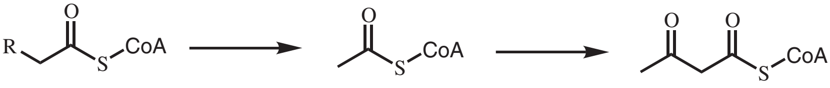
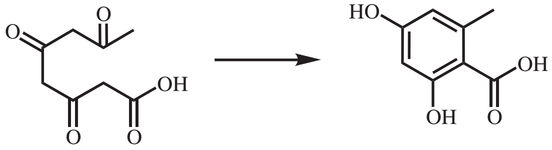


Figure 3.2 *Building of carbon chains from acetate units*



orsellinic acid

Figure 3.3 *Typical polyketide cyclisation*

acetyl coenzyme-A. The thiol function of coenzyme-A serves both as an activating group and an efficient leaving group, thereby facilitating aldol-type chemistry and leading to long chain compounds in which every second carbon existed, at some point, as a ketone. Self condensation of these chains then leads to the polyketides. Acetyl coenzyme-A can also be used to synthesise mevalonic acid, the precursor to the terpenoids.

Lipids and polyketides are biosynthesised by aldol-type reactions of esters with coenzyme-A as shown in Figure 3.2. The coenzyme-A ester of a fatty acid undergoes reaction with acetyl coenzyme-A to give a β-ketoester. Reduction of the ketone group followed by elimination of the resultant alcohol and addition of hydrogen gives an acid with two more carbon atoms in the chain. This is why natural fatty acids contain even numbers of carbon atoms in their chains. If the polyketoacids undergo condensation reactions rather than reduction, the result is a phenolic material of the polyketide family. Thus, in Figure 3.3, we see the formation of orsellinic acid, which is the precursor for some odorous components of plants.

One lipid of interest is arachidonic acid. This polyunsaturated fatty acid undergoes a radical cyclisation reaction involving oxygen as shown in Figure 3.4. This cyclisation leads to an important group of compounds known as prostaglandins. The prostaglandins are hormones in the animal kingdom. Degradative reactions lead to shortening of the chains to give jasmonic acid, a plant hormone and precursor for two important odorous materials, jasmone and methyl jasmonate.

Addition of phosphoenol pyruvate to erythrose-4-phosphate leads, through a number of reaction steps, to shikimic acid. The 3,4,5-trihydroxybenzoic skeleton of shikimic acid can be seen in many perfume

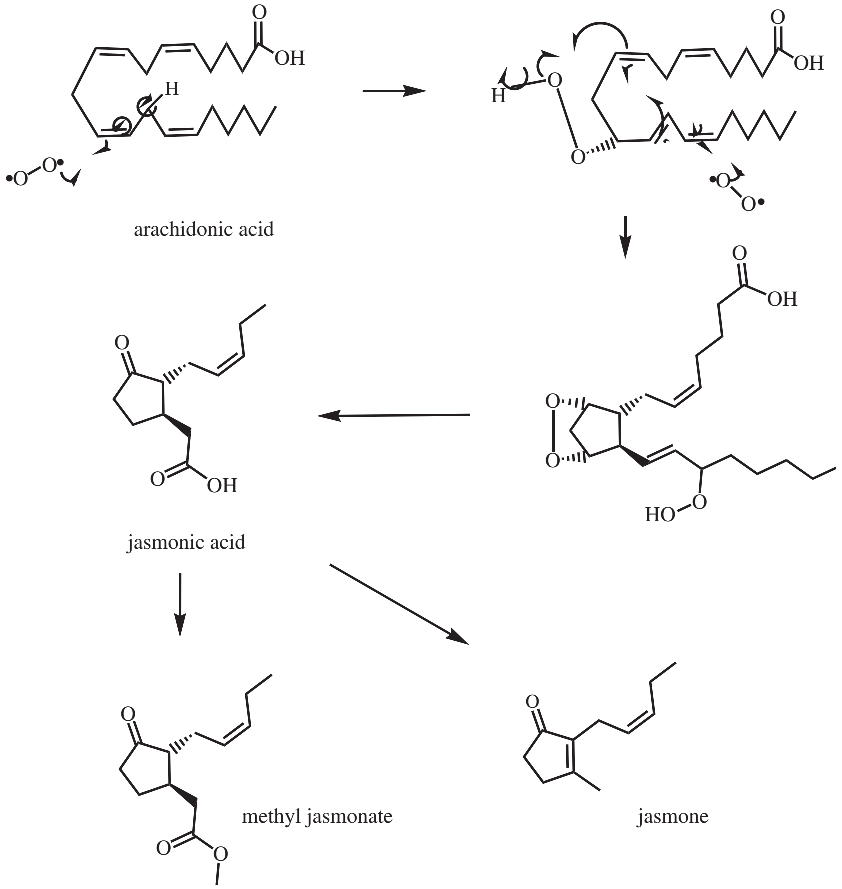


Figure 3.4 *Biosynthesis of jasmones*

components, although the oxygen atoms in the product are not usually those of the original shikimic acid. The original oxygen atoms are lost during biosynthesis and others reintroduced into the same sites at a later stage by oxidation. Addition of a further unit of phosphoenol pyruvate adds a three-carbon chain to the carbon carrying the carboxyl group. The latter is then lost by decarboxylation. An abridged scheme for the biosynthesis of eugenol, the characteristic odorant of cloves, from shikimic acid is shown in Figure 3.5.

Terpenoids are defined as materials which are made up of isoprene (2-methylbuta-1,3-diene) units. More detail on terpenoid chemistry can be found in the book by Sell (2003). Strictly speaking, the word 'terpene' is used to describe terpenoid hydrocarbons. In the perfumery industry, it

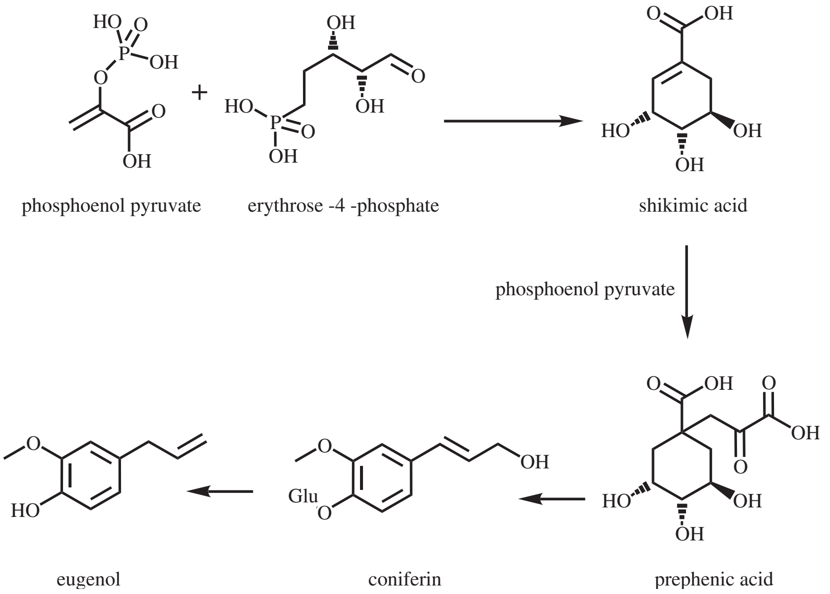


Figure 3.5 *Biosynthesis of eugenol*

Table 3.1 *Classification of terpenoids*

Name	*Number of isoprene units*	*Number of carbon atoms*
Hemiterpenoids	1	5
Monoterpenoids	2	10
Sesquiterpenoids	3	15
Diterpenoids	4	20
Sesterterpenoids	5	25
Triterpenoids	6	30
Carotenoids	8	40
Steroids	Terpenoids which produce Diels's hydrocarbon when distilled from zinc dust	

is often used when referring to mixtures containing monoterpenoid hydrocarbons only. Sometimes it is used to refer to any terpenoid. One must judge by context as to which meaning is intended. Terpenoids are classified into families according to the way in which their skeletal were formed. The broad classes of terpenoids are shown in Table 3.1.

Figure 3.6 illustrates how, through phosphorylation, elimination and decarboxylation, mevalonic acid is converted to isopentenyl pyrophosphate, which can be isomerised enzymically to pentenyl pyrophosphate.

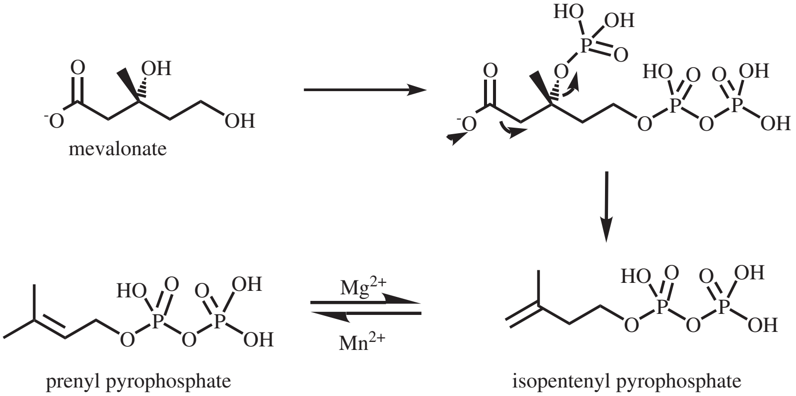


Figure 3.6 *Biosynthesis of hemiterpenoid building blocks*

Coupling of these two isomeric materials gives geranyl pyrophosphate as shown in Figure 3.7, whereas hydrolysis leads to the hemiterpenoid series. Addition of a further molecule of isopentenyl pyrophosphate to geranyl pyrophosphate gives farnesyl pyrophosphate. These coupled units then lead to the monoterpenoids and sesquiterpenoids respectively. Addition of further units of isopentenyl pyrophosphate leads, in the same manner, to the higher terpenes. The reactions shown in Figure 3.7 give rise to what is referred to as the head-to-tail coupling in which the ' "head" ' of one isoprene unit is connected to the ' "tail" ' of another. This is, by far, the most common way of joining isoprene units together though tail-to-tail couplings also occur, the best example being the tail-to-tail fusion of two geranyl pyrophosphate units to produce squalene and the carotenoids. The terpenoid pyrophosphates undergo cyclisation reactions under the influence of appropriate enzymes. Other enzymes then carry out further chemical conversions, such as oxidation, on the terpenoids. This leads to a vast array of complex structures depending on the exact nature of the enzymic reactions involved. Since the enzymes are often unique to one species, the terpenoids (and, of course, other metabolites also) produced by a plant can be used by botanists to classify it. Such classification of plants is referred to as chemotaxonomy.

Figure 3.8 shows how the isoprene units and the original backbone can be traced out in a number of terpenoids, which are important in perfumery. Sometimes skeletal rearrangements occur, which make this process more difficult, and fragmentation or degradation reactions can

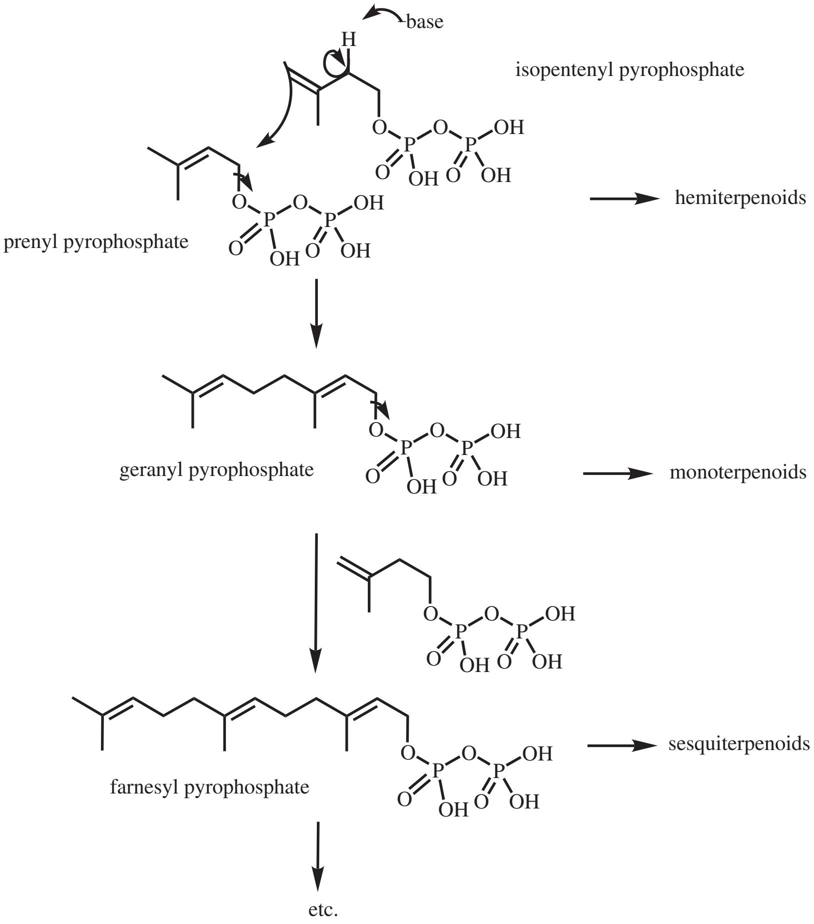


Figure 3.7 *Biosynthesis of terpenoids*

reduce the number of carbon atoms so that the empirical formula does not contain a simple multiple of five carbons. Nonetheless, the natural product chemist will still quickly recognise the characteristic terpenoid framework of the structure.

The book by Mann *et al.* (1994) on natural products provides a good introduction to the biogenesis of natural perfume ingredients and the book by Sell (2003) and the review by Croteau (1987) give further detail on the biosynthesis of monoterpenes.

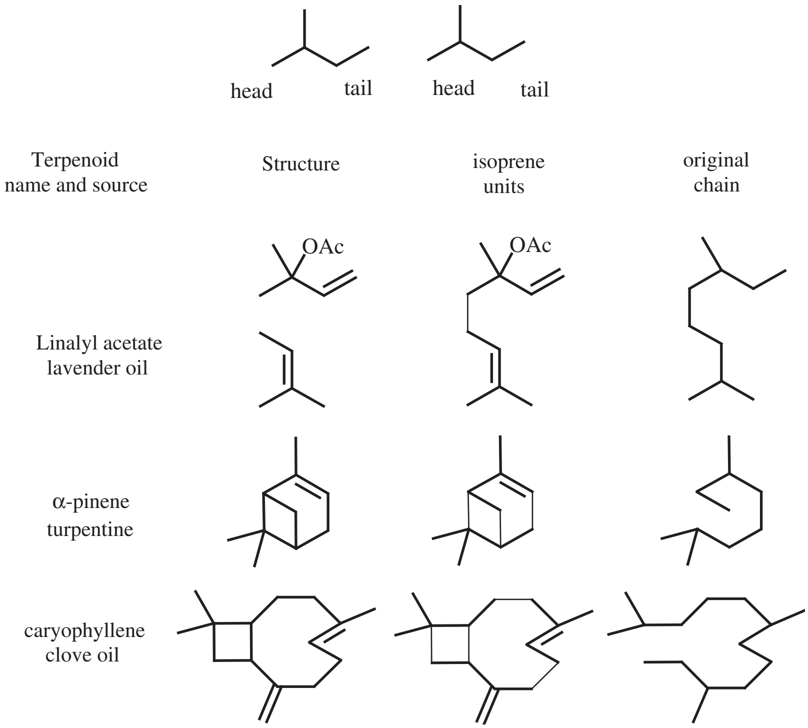


Figure 3.8 *Isoprene units in terpenoids*

3.2 EXTRACTION OF NATURAL PERFUME INGREDIENTS

The methods used to extract perfume ingredients from their natural sources have changed over time as technology in general has advanced. However, both old and new methods fall into three basic classes: expression, distillation and solvent extraction.

3.2.1 Expression

Expression is the simplest of the three techniques. When odorants are forced out of the natural source by physical pressure, the process is referred to as expression and the product is called an expressed oil. If you squeeze a piece of orange peel, you will see the oil-bearing glands burst and eject a fine spray of orange oil. Many commercially available citrus oils are prepared in this way.

3.2.2 Distillation

Distillation of perfume ingredients from their natural sources can be done in three ways: dry (or empyrumatic) distillation, steam distillation or hydrodiffusion. Dry distillation involves high temperatures since heat, in most cases this will be direct flame, is applied to the surface of the vessel containing the plant material. Usually this technique is reserved for oils of highest boiling point, typically those derived from wood, because the high temperatures are necessary to vaporise their chemical components. Cade and birch tar are the major oils obtained by dry distillation. Cade and birch tar oils contain distinctive burnt, smoky notes as a result of pyrolysis of plant material. In steam distillation, water or steam is added to the still pot and the oils are codistilled with the steam. The oil is separated from the water by means of a Florentine flask, which separates them on the basis of their differing densities. Figure 3.9 shows simple schematic representation of a still and a Florentine and Figure 3.10 shows a still charged with jasmine flowers ready for the top to be fitted prior to distillation.

The waters which codistil with the oil are called the waters of cohobation. In most cases, these are a waste product and are either discarded or recycled to the still pot. The waters of cohobation obtained from rose distillation are different. Rose oil is somewhat water-soluble and so the

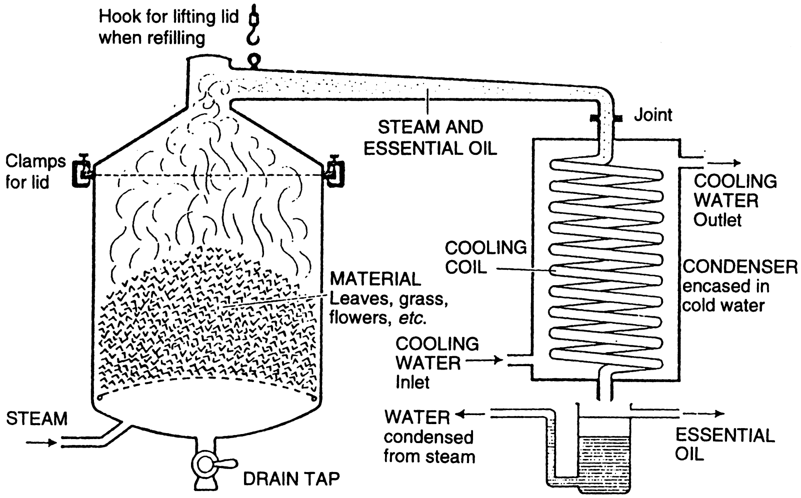


Figure 3.9 *Still and Florentine flask*

'rose water' is kept as a perfume and flavour ingredient. The presence of water in the pot during steam distillation limits the temperature of the process to 100°C. This means that much less degradation occurs in this process than in dry distillation. However, some degradation does occur. For example, high-boiling tertiary alcohols present in the plant often dehydrate in the pot and distil as the corresponding hydrocarbons.

Hydrodiffusion is a relatively new technique. It is somewhat like a steam distillation carried out upside down since the steam is introduced at the top of the pot and the water and oil taken off as liquids at the bottom. The function of the steam in hydrodiffusion is largely to break

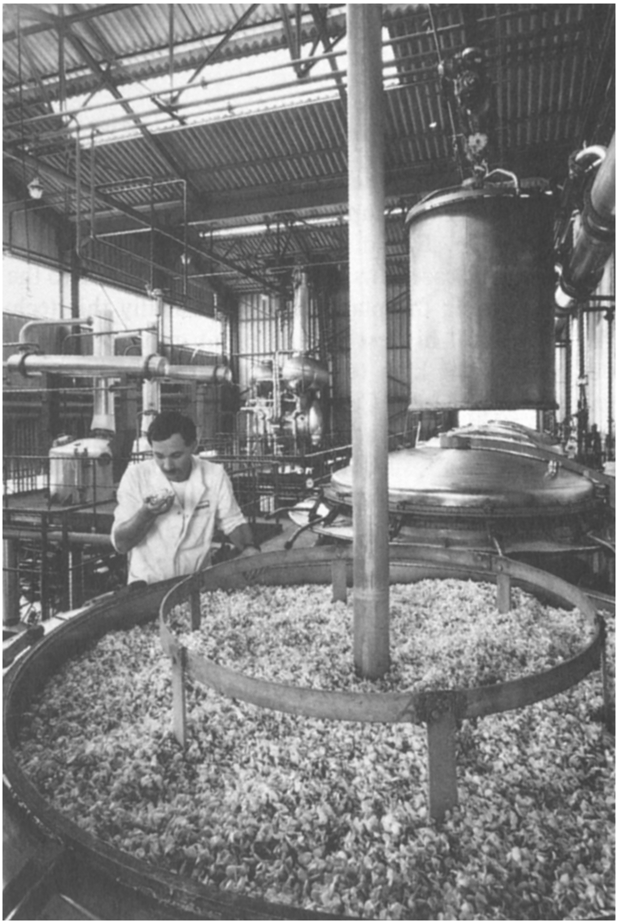


Figure 3.10 *Still charged with jasmine flowers*

open the plant cells containing the oil. Unlike steam distillation, the oil is not distilled and so it is not necessary to use the heat of the steam to vaporise the oil. Microwave radiation is another recent development for breaking the oil-bearing cells of the plant. In this case, heating with microwaves causes the cell contents to expand and burst from the cell, thereby releasing the oil.

Perfume materials obtained by distillation are referred to as essential oils. Thus, for example, the oil obtained by steam distillation of lavender is known as the essential oil of lavender or lavender oil. Sometimes, the monoterpenoid hydrocarbons are removed from the oils by distillation or solvent extraction to give a finer odour in the product. The process is known as deterpenation and the product is referred to as a terpeneless oil or folded oil.

3.2.3 Solvent Extraction

Figure 3.11 summarises the various possible processes using distillation and/or solvent extraction to obtain perfume ingredients. The processes are written in cursives and the technical names for the various products in capitals.

Ethanolic extraction is not used very much for plant materials because of the high proportion of water compared to oil in the plant. Vanilla

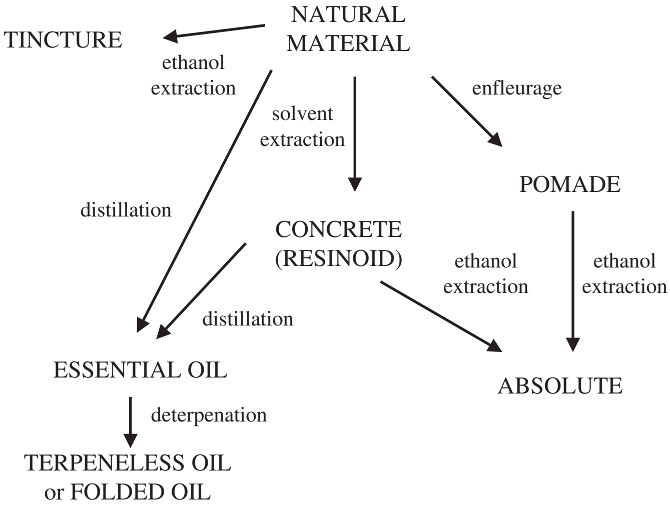


Figure 3.11 *Types of natural extracts*

beans are an important exception. It is more important with materials such as ambergris. The sperm whale produces a triterpene known as ambreine in its intestinal tract. This is excreted into the sea and, on exposure to salt water, air and sunlight, undergoes a complex series of degradative reactions, which produce the material known as ambergris. (More detail of this chemistry will be given in Chapter 4.) This waxy substance can be found floating in the sea or washed up on beaches. Extraction of it with ethanol produces tincture of ambergris.

Enfleurage was used by the ancient Egyptians to extract perfume ingredients from plant material and exudates. Its use continued up to this century but is now of no commercial significance. In enfleurage, the natural material is brought into intimate contact with purified fat. For flowers, for example, the petals were pressed into a thin bed of fat. The perfume oils diffuse into the fat over time and then the fat can be melted and the whole mixture filtered to remove solid matter. On cooling, the fat forms a pomade. Although the pomade contains the odorous principles of the plant, this is not a very convenient form in which to have them. The concentration is relatively low and the fat is not the easiest or most pleasant material to handle, besides which, it will eventually turn rancid. The ancient Egyptians used to apply the pomade directly to their heads but in more recent times, it was usual to extract the fat with ethanol. The odorous oils are soluble in alcohol because of their degree of oxygenation. The fat used in the extraction and any fats and waxes extracted from the plant along with the oil, are insoluble in ethanol and so are separated from the oil. Removal of the ethanol by distillation produces what is known as an absolute.

The most important extraction technique nowadays is simple solvent extraction. The traditional solvent for extraction was benzene, but this has now been superseded by other solvents because of concern over possible toxic effects of benzene on those working with it. Petroleum ether, acetone, hexane and ethyl acetate, together with various combinations of these, are typical solvents for extraction. Recently, there has been a great deal of interest in the use of carbon dioxide as an extraction solvent. Both supercritical carbon dioxide and subcritical liquid carbon dioxide are used, depending on circumstances. The pressure required to liquify carbon dioxide at ambient temperature is considerable and thus the necessary equipment is expensive. This is reflected in the cost of the oils produced but carbon dioxide has the advantage that it is easily removed and there are no concerns about residual solvent levels. The major applications of liquid carbon dioxide extraction are in decaffeination of coffee and extraction of hops.

The product of such solvent extractions is called a concrete or resinoid. It can be extracted with ethanol to yield an absolute or distilled to give an essential oil. The oil can then be deterpenated. With some particularly viscous concretes, such as those from treemoss or oakmoss, it is more usual to dissolve the concrete in a high boiling solvent and then codistil the product with this solvent.

Essential oils and other extracts vary considerably in price and in the volume used each year. Lavender, for example, is a relatively inexpensive oil, costing £15–30 per kg and 250–300 tonnes are used annually. Rose and jasmine are much more expensive and are used in much smaller quantities. The total annual production of rose oil is 15–20 tonnes and it costs between £1000 and £3000/kg depending on quality. About 12 tonnes of jasmine extracts are produced annually at prices up to £2000/kg. Eucalyptus oil (from *Eucalyptus globulus*) has one of the biggest production volumes, almost 2000 tonnes/annum and is one of the cheapest oils at around £5 per kg. The exact balance between volume and price depends on various factors such as ease of cultivation, ease of extraction and usefulness. For example, eucalyptus trees grow well, the leaves are easy to harvest, trimmed trees grow back vigorously, the oil is easily distilled and it is useful as a disinfectant as well as a camphoraceous fragrance ingredient. All of these factors combine to make it a high tonnage oil.

Before this century, perfumes commanded such a price that only the wealthiest people could afford them. This is because perfumers had to rely on natural sources for their ingredients. Most of these ingredients are in limited supply and are expensive to produce. For instance, it takes about 7,000,000 jasmine flowers to produce 1 kg of oil. The flowers have to be picked by hand (no-one has yet devised a mechanical method of harvesting jasmine) in the first few hours of the day when their oil content is at its highest (Figure 3.12). In view of the costs of cultivation and extraction, it is not surprising to find that jasmine oils cost in the region of £2000 per kg.

Nowadays, some natural oils are much less expensive because of automated farming methods. For instance, rows of lavender in a field can be cut almost to ground level and fed directly into a still pot carried on the tractor (Figure 3.13). The pot is then fitted under a field still and the oil extracted while the harvesting continues. The cost of lavender oil is thus tens, rather than thousands, of pounds per kg. Despite this, the modern perfumery industry would be unable to function as it does if it were to rely solely on natural ingredients. Cost alone would be prohibitive,

Figure 3.12 *Hand picking of jasmine*

Figure 3.13 *Cultivation/harvesting of lavender*

regardless of problems of stability in products or availability in view of limits on land use, *etc*.

Since essential oils are usually present in the botanical source at the level only a percent or two, at most, of the dry weight of the harvested plant, it is more economic to extract the oil at the location where the plant grows and ship oil, rather than plant material, to the customer. The degree of sophistication of the harvesting and extraction technology varies widely depending on the country of origin. The mint production of the United States and the lavender production of England and Tasmania are highly automated; indeed they must be to remain economically feasible in countries with such high labour costs. In some other countries, simple bush stills constructed from waste oil drums and drainpipes are the most cost-effective means of production. Table 3.2 lists some of the

Table 3.2 *Some of the more important natural fragrance materials*

Oil	*Types of process used*[*]	*Plant part extracted*	*Approximate annual production* (tonnes)	*Typical country of origin*
Ambrette	S	Seed	0.5	China, Colombia
Angelica	S	Root	1	Bulgaria
Anise	S	Seed	1200	China, Vietnam
Artemisia	S	Aerial parts	16	Morocco, Tunisia, India
Basil	S	Flowering tops	15	Reunion
Bay	S	Leaf	20	Dominica, Puerto Rico
Bergamot	E	Fruit	120	Italy
Benzoin	C	Exudate	3	Thailand, Indonesia
Birch tar	D	Wood	50	Austria, Germany, Russia
Cabrueva	S	Wood	10	Brazil, Paraguay
Cade	D	Wood[†]	12	Portugal, Yugoslavia
Cajeput	S	Leaves and twigs	50	Indonesia
Calamus	S	Rhizome	10	N. Korea, India
Camphor	S	Wood	250	China
Cananga	S	Flowers	45	Indonesia, Comores, Reunion
Caraway	S	Seeds	10	Bulgaria, Egypt, Australia
Cassia	S	Leaves	160	China
Cedarwood[‡]	S	Wood	2200	China, USA
Cedar leaf	L	Leaf	25	USA Canada
Celery	S	Seed	25	Bulgaria, India
Chamomile	S	Flowers	10	Morocco, France
Cinnamon bark	S	Bark	5	Sri Lanka
Cinnamon leaf	S	Leaf	100	Sri Lanka, India, Seychelles

Continued

Table 3.2 *Continued*

Oil	*Types of process used*[*]	*Plant part extracted*	*Approximate annual production* (tonnes)	*Typical country of origin*
Citronella	S	Leaves	2300	Sri Lanka, Indonesia
Clary sage	A, S	Flowers/leaves	5	Russia, USA, Bulgaria, France
Clove bud	S	Flower bud	70	Indonesia, Madagascar
Clove leaf	S	Leaf	2000	Indonesia, Madagascar
Copaiba balsam	U	Exudate	40	Brazil
Coriander	S	Seeds	100	Russia
Cornmint	S	Aerial parts	3000	China, Brazil
Cumin	A, S	Seeds	10	India
Dill	S	Aerial parts	140	USA, Hungary, Bulgaria
Elemi	C, S	Exudate	10	Philippines
Eucalyptus[§]	S	Leaves	See individual species	
E. citriodora			800	Brazil, S. Africa, India
E. dives			50	Australia
E. globulus			1600	Spain, Portugal
E. staigeriana			50	Australia, Brazil, S. Africa
Fennel	S	Seeds	80	Spain
Fir needle	S	Leaves	55	Canada, USA, Russia
Galbanum	C, S	Exudate	10	Iran, Lebanon, Turkey
Geranium	A, S	Leaves/stems	150	Reunion, Egypt
Ginger	S	Root	55	China, Jamaica
Grapefruit	E	Fruit	250	Israel, Brazil, USA
Guaiacwood	S	Wood	60	Paraguay
Ho	S	Leaf and wood	30	China
Jasmine	A, C	Flower	12	Egypt, Morocco
Juniper	S	Fruit	12	Yugoslavia, Italy
Labdanum[¶]	A, U, S	Exudate	20	Spain
Lavender[**]	S	Aerial parts	1000	France, Spain, Tasmania
Lemon	E	Fruit	2500	USA, Italy, Argentina, Brazil
Lime	E	Fruit	1200	Mexico, Haiti
Litsea cubeba	S	Fruit	900	China
Mandarin	E	Fruit	120	Italy, China
Marjoram	S	Leaves and flowers	30	Morocco
Neroli[*†]	S	Flowers	3	Tunisia
Nutmeg	S	Fruit	200	Indonesia, Sri Lanka
Oakmoss	A, C	Aerial	100	Yugoslavia, Italy, France
Olibanum	C	Exudate	10	Ethiopia, Yemen
Orange[*†]	E	Fruit	15,000	USA, Brazil, Israel, Italy
Origanum	S	Aerial parts	10	Spain, France
Orris	C, S	Rhizome	5	Italy, France, Morocco
Palmarosa	S	Leaves	55	India, Brazil

Continued

Table 3.2 *Continued*

Oil	*Types of process used*[*]	*Plant part extracted*	*Approximate annual production* (tonnes)	*Typical country of origin*
Patchouli	S	Leaf	800	Indonesia
Pennyroyal	S	Aerial parts	10	Morocco, Spain
Peppermint	S	Aerial parts	2200	USA
Petitgrain[*†]	S	Leaves	280	Paraguay
Peru balsam	C, S	Exudate	45	San Salvador, Brazil
Pimento	S	Fruit	50	Jamaica
Pine oil	S	Wood	1000	USA, Mexico, Finland, Russia
Rosemary	S	Aerial parts	250	Spain, Morocco, Tunisia
Rose	C, S	Flower	20	Bulgaria, Turkey, Morocco
Rosewood	S	Wood	250	Brazil, Peru, Mexico
Sage	S	Aerial parts	45	Yugoslavia, Spain, Greece
Sandalwood	S	Wood	250	Indonesia, India
Sassafras	S	Roots	750	Brazil
Spearmint	S	Aerial parts	1400	USA, China, Brazil
Styrax	C, S	Exudate	25	Turkey, Honduras
Tangerine	E	Fruit	300	Brazil
Tarragon	S	Aerial parts	10	Italy, Morocco
Thyme	S	Aerial parts	25	Spain
Ti tree	S	Leaves	10	Australia
Vanilla	C, T	Fruit	2500 (dried beans)	Reunion, Madagascar
Vetiver	S	Root	260	Reunion, Haiti, Indonesia
Ylang ylang	A, C, S	Flower	90	Comores, Madagascar

[*] A = absolutes; C = concretes and resinoids; D = dry distilled oil; E = expressed oil; S = steam distilled oil; T = tincture; U = untreated (however, these products are often boiled to free them from plant material).

[†] The wood is juniper.

[‡] There are two main types of cedarwood sources for perfumery. One comprises plants of the *juniperus* family and the other of the *cedrus* family. The former are known as English, Texan or Chinese cedarwood and are produced principally in China and U.S.A. The leaf oils are extracted from this family. Chemically, the major components of these oils are based on the cedrane skeleton. The products obtained from members of the *cedrus* family are known as Atlas or Himalayan cedarwoods. They are produced in North Africa and the Himalayas and the chemical structure of their major components are based on the bisabolane skeleton.

[§] The different Eucalyptus species of importance to the perfumery industry contain different terpenes as their major components. The terpenes of each are related to the following major components: *E. citriodora*, citronellal; *E. dives*, piperitone; *E. globulus*, cineole; *E. staigeriana*, citral.

[¶] The oil and absolute are known as cistus.

[**] There are three species of lavender which give oils of differing quality. They are known as lavender, lavandin and spike.

[*†] It is interesting to note that, in the case of bitter orange, three different oils are produced from the same species.

more important of the essential oils used in perfumery today. The table includes information on the plant parts and extraction techniques used to produce the fragrance products and also some of the more important countries of origin for each.

3.3 ADULTERATION OF NATURAL PERFUME INGREDIENTS

The high prices which essential oils command leads, inevitably, to the temptation for less scrupulous producers and dealers to adulterate the product. Adulteration is sometimes referred to, euphemistically, as sophistication. By adding lower cost materials but still asking the same high price for the mixture, the person perpetrating the fraud can stand to make considerable sums of money from an unsuspecting buyer. However, the major fragrance companies are sufficiently astute and technically competent to uncover almost all attempts at such fraud. The techniques used in adulteration vary from the crude to the very sophisticated as is evidenced by the following examples. The examples also illustrate some of the quality control (QC) techniques, which are used routinely by the industry.

A supplier of ylang ylang oil once tried to sell drums, which contained only a small amount of the oil, the remainder of the drum contents being river water. This attempt at deceit was easily discovered since QC samples are normally drawn from top, middle and bottom of drums, so one sample would be oil and the other two, water.

Lavender oils are relatively inexpensive essential oils but their major components are available as even less expensive chemicals and so the possibility to cheat does exist. Like all natural products, the composition varies. The percentage of the individual components present in the lavender oils will depend on, for example, the area where the plant was grown, the rainfall during that season, the harvesting method and so on. The analytical chemist responsible for QC of lavender oil will therefore not expect to see major components present at fixed levels, but rather within an acceptable range. Linalyl acetate, for example, is normally present in lavender oil at between 30 and 60%. Synthetic linalyl acetate is available for a fraction of the price of lavender oil and so, a supplier might be tempted to add some synthetic material to the oil and charge the full price for the mixture. This fraud is also relatively easily detected. Synthetic linalyl acetate is made, as will be seen later, from dehydrolinalool. The dehydrolinalool is hydrogenated over a Lindlar catalyst to linalool. This hydrogenation in theory, stops at linalool. However, a small amount of the substrate is fully saturated to dihydrolinalool. This compound does not occur in nature. Thus, the analyst will examine the gas chromatography (GC) trace of lavender oil to look for dihydrolinalyl acetate. If it is present, then adulteration would be suspected. The telltale component can be detected at extremely low concentrations, using GC–mass spectrometry (GC–MS) if necessary, since that technique is even more sensitive than GC alone.

The most important component of the vanilla bean, as far as flavour is concerned, is vanillin. Synthetic vanillin costs only a few pounds per kilogramme but vanillin extracted from vanilla beans can cost £5000 or more per kg. Food labelling laws are very strict and the penalties for declaring a flavour to be natural when it is not, are very high. Not only can companies be fined for false declaration but also their directors are liable to imprisonment in certain countries, in particular, in the United States. It is therefore very important that a company buying vanilla should be able to verify for itself whether or not the goods for sale are of natural origin.

One simple test is to measure the level of radioactivity from the sample. Synthetic vanillin is not radioactive. However, natural vanilla, like all natural products, is. This is, of course, because atmospheric carbon dioxide contains some radioactive ^{14}C formed by exposure to cosmic radiation in the upper atmosphere. Plants then incorporate this into their photosynthetic pathway and produce metabolites, which exhibit a low level of radioactivity. Synthetic vanillin is prepared from coal tar, which is not radioactive since the ^{14}C has long since decayed. However, unscrupulous dealers know this and can synthesise radiolabelled or ‘hot’ vanillin and dose it into synthetic material so that the level of radioactivity matches that of a natural sample. Another method of checking for naturalness must therefore be found. When plant enzymes synthesise molecules, they, like all catalysts, are susceptible to isotope effects. The vanilla plant is no exception and examination of the distribution of hydrogen and carbon isotopes in the vanillin molecule reveals that the heavier deuterium and ^{13}C isotopes accumulate at certain specific sites. A suitable NMR spectrometer can determine the isotopic distribution in a sample and the cost of using ^{2}H, ^{13}C and ^{14}C labelled synthetic materials to replicate the NMR spectra and radioactivity of natural vanillin in a synthetic sample would not be financially attractive. Furthermore, the ^{2}H and ^{13}C labelling patterns in the vanilla bean are different from those of other natural shikimate sources and so the NMR technique can also distinguish between vanillin from vanilla and vanillin produced by degradation of lignin. Lignin is the structural component of wood and is therefore very inexpensive. The use of isotopes for verifying the origin of chemicals is known as isotope dilution analysis. This technique is now so advanced, that the analytical chemist can even tell from the isotopes present in the vanillin it contains, whether a vanilla bean was grown on the island of Reunion, in Mexico or in Indonesia.

As each opportunity for adulteration is blocked by analysts, the crooks will seek new methods and so the QC analyst must proactively think to keep ahead.

3.4 FROM NATURAL TO SYNTHETIC

Until the middle of the nineteenth century, perfumes were largely for personal application and, furthermore, that use was restricted mostly to the wealthiest strata of society. This was because of the cost of producing the natural materials required as ingredients. The development of organic chemistry in the nineteenth century began to make synthetic chemicals available and their use in fragrances began to grow. The incorporation of synthetics into perfumery received a huge fillip in 1921 when Coco Chanel launched her famous perfume, No. 5. Chanel 5 owes its unique character to the inclusion of synthetic aliphatic aldehydes alongside the natural oils such as rose and jasmine. The success of this fragrance inspired other fragrance houses to experiment with synthetic materials and the modern age of perfumery was born. The synthetic materials were cheaper to produce than natural materials and their supply was much more secured, thus making perfume accessible to all. Furthermore, more robust molecules could be produced, which would survive in acidic, basic and even oxidising media. Thus, it became possible to put perfume into household products in which natural oils could not be used because of degradation of their components and resultant changes in odour and colour.

The use of essential oils is restricted by their price, availability and chemical stability. Many of the components of natural oils do not survive in products such as bleaches, laundry powders and even soaps. For example, the major component in jasmine oil is benzyl acetate, which is hydrolysed in all of these owing to their high pH (13–14, 10–11 and 9–10, respectively) and it is also susceptible to the oxidants present in the first two. The indole present in jasmine will cause soap to discolour. The discovery and application of synthetic fragrance materials towards the end of the nineteenth century and throughout the twentieth was therefore a momentous event in the history of the industry. Nowadays, fragrances can be used in all the consumer goods produced for personal and household care and they are affordable to everyone. More detail of the use and performance of fragrances in products will be given in other chapters, the reason for mentioning this at this point is to highlight the importance of economic consideration.

Initially, the synthetic perfumery materials were introduced through serendipitous use of the products discovered through advances in chemical technology. For example, the nitromusks were discovered by A. Baur while he was working on explosives related to TNT. As techniques for isolation, characterisation and synthesis of organic chemicals improved, the search for new fragrance materials became more structured. In this, the fragrance industry follows the same path as the pharmaceutical industry. The first step is to identify the materials which nature uses. Thus the chemical components of natural oils were separated by distillation and/or chromatography and their structures determined by chemical analysis and/or spectroscopy. (Details of the application of these techniques will be given in a separate chapter.) Having identified the molecular structure of an odourant, the next task is to synthesise a sample, which is identical to it. Synthesis serves as the final proof of the correct determination of the structure, but it also makes it possible to produce the material without relying on the natural source. Synthetic compounds, whose structures are the same as those of the natural material, are referred to as ‘nature identical’. This classification of materials is important in legislative terms and it is easier to obtain clearance for a nature-identical material than for one that has no natural counterpart. However, the natural materials may contain structural features which make them difficult to synthesise or susceptible to degradation in the products to which perfumes are added. The next step is therefore to synthesise materials which are close in structure but not identical to the natural one. The effect of changes of structure on the odour, and other properties, of the materials can then be studied and further analogues synthesised to produce an optimum balance of odour, performance and cost. A more detailed account of this process will be dealt with in Chapter 14. For the moment, the example of the chemistry of jasmine compounds will serve to illustrate the overall path from natural to synthetic material.

The components of an essential oil may be classified into three groups. Some components add little or even nothing to the odour of the oil but may serve another purpose. For instance, they could be fixatives. The components in the next group, add odour and are important in forming the total impression of the oil but, smelt in isolation, would not be associated immediately with the oil. The third group of compounds are the character-impact compounds. These are the materials which give the characteristic notes to the oil and which, when smelt in isolation, would be instantly associated with the oil. Figure 3.14 shows a simplified GLC trace of jasmine oil, and materials of each type can be seen in it. Isophytol and benzyl benzoate have very little intrinsic odour and serve mostly as fixatives. Benzyl acetate is the major component of jasmine oil and plays a significant part in the total odour. However, it possesses a fruity note, which could be, and indeed is, found in many other oils. The character impact compounds of jasmine are jasmone and methyl jasmonate. These two are instantly recognisable as jasmine in character and are essential to the odour of the oil. Their structures are shown in Figure 3.15 which shows their synthesis through a common intermediate. Jasmone was first synthesised by Crombie and Harper (1952), the synthesis in Figure 3.15 is that of Büchi and Egger (1971). Büchi's synthesis illustrates the main problem in the synthesis of nature-identical jasmone and methyl jasmonate, that is, the inclusion of the *cis*-double bond in the side chain. The most convenient method of introducing this feature is through the Lindlar hydrogenation of an acetylenic compound. In terms of total synthesis of natural products, this is a relatively trivial step and is easy to carry out on a laboratory scale. However, several synthetic steps will be required to prepare the five-carbon unit for the side chain and two more are needed to introduce and hydrogenate it. On manufacturing scale, this leads to high process costs, especially since two of the stages involve the handling of hazardous reagents, *viz.* acetylene and hydrogen. If the side chain of jasmone is replaced by a saturated one, the synthesis is made much easier and so dihydrojasmone is much less expensive than jasmone. Stetter and Kuhlmann's (1975) two-step synthesis of dihydrojasmone from readily available starting materials is shown in Figure 3.16. If the endocyclic double bond and the methyl substituent on the ring are also ignored, the synthesis becomes even more amenable to operation on a commercial scale as shown in Figure 3.17. This figure shows the preparation of pentylcyclopentanone but, in addition, use of different aldehydes in the initial aldol condensation gives rise to a series of homologous compounds each with a unique blend of jasmine and fruity notes. Figure 3.17 also shows the route used to prepare methyl dihydrojasmonate commercially. This chemistry is described in more detail in Chapter 4. Cyclopentanone is available in bulk at low cost by the pyrolysis of the calcium or barium salts of adipic acid, the precursor of nylon 6. This is an example of how

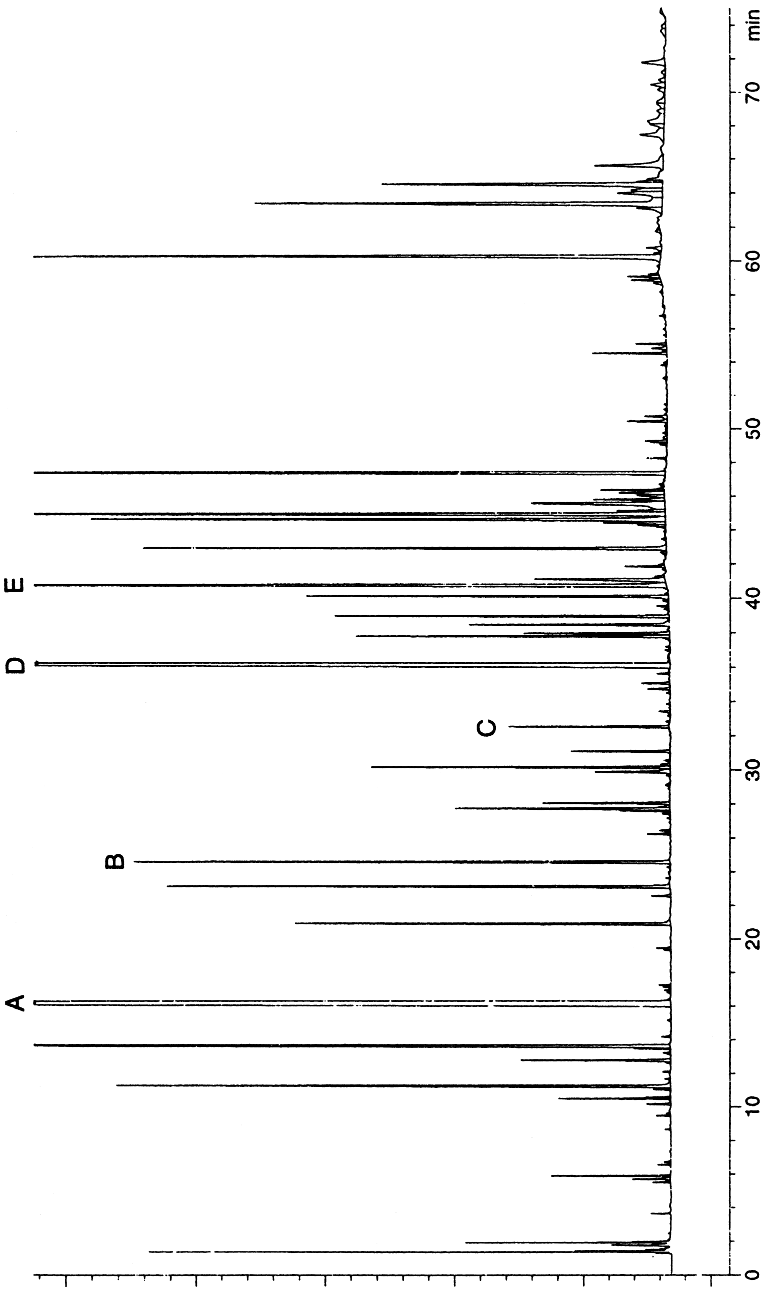


Figure 3.14 *GLC trace of jasmine absolute*

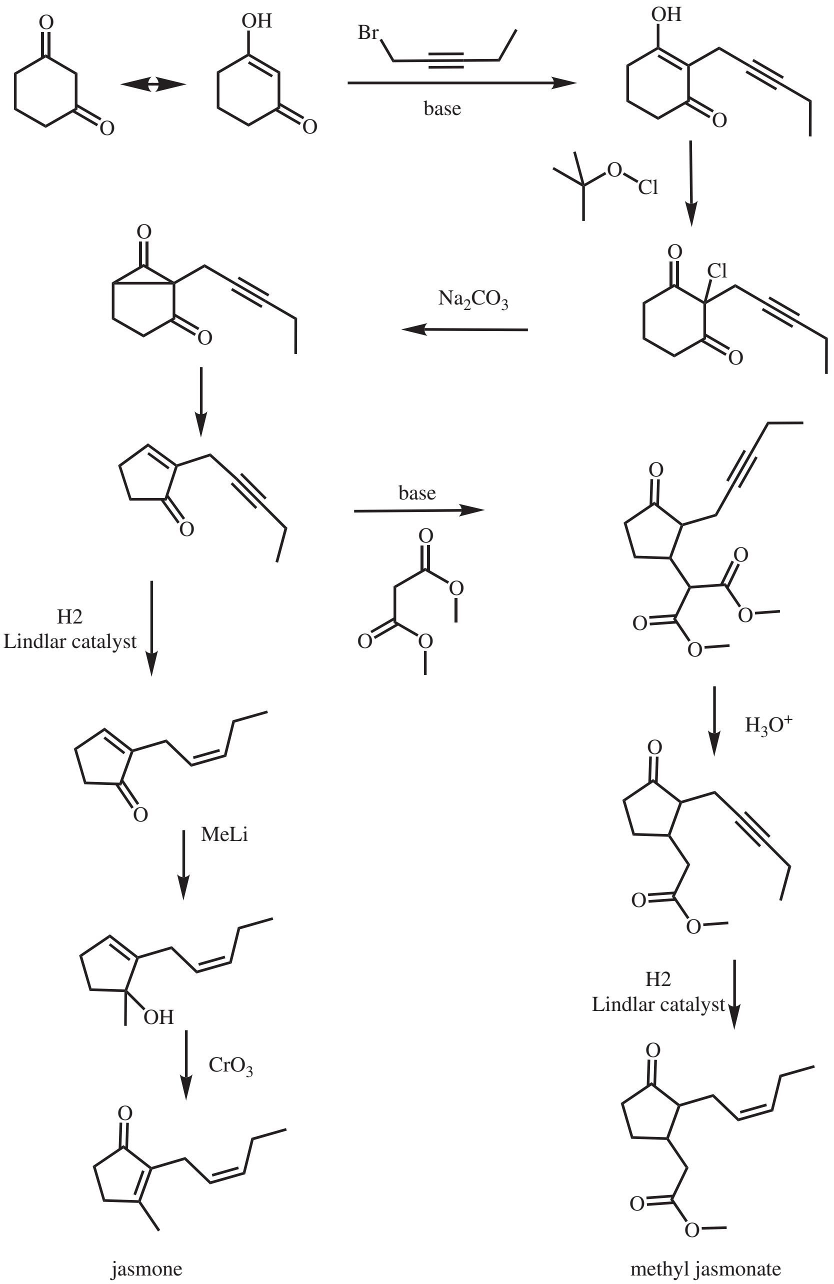


Figure 3.15 *Büchi's synthesis of jasmone and methyl jasmonate*

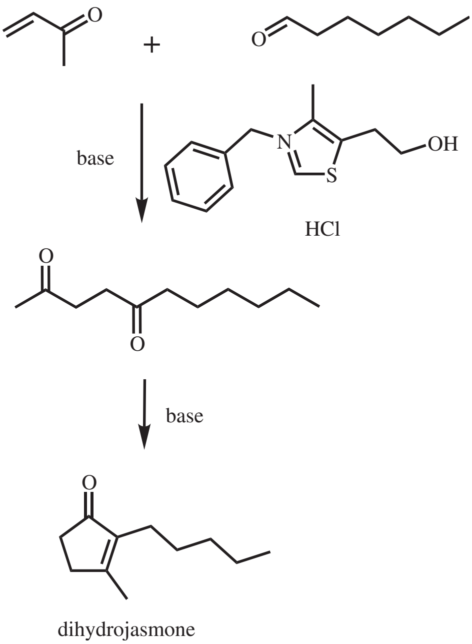


Figure 3.16 *Stetter's synthesis of dihydrojasmone*

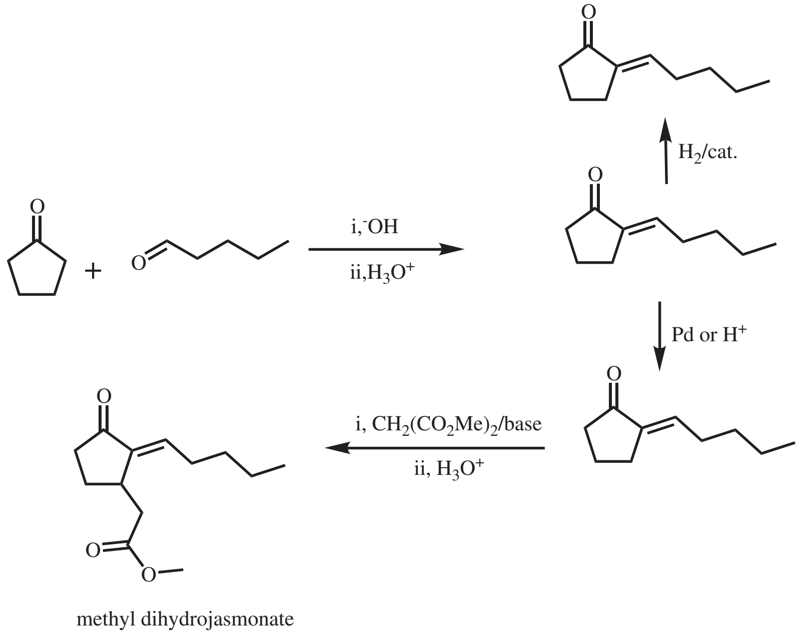


Figure 3.17 *Commercial synthesis of methyl dihydrojasmonate*

the fragrance industry capitalises on the availability of inexpensive feedstocks from much larger scale industries, in this case the textile industry.

Natural jasmine oils cost £3000–£5000 per kg, the nature-identical materials will be about one-tenth of that price and the price of the simpler analogues will be a further order of magnitude, or even more, lower in price. In addition, because they lack the double bonds, the synthetic materials are more stable in products, such as laundry powder, which contain bleaching agents. All of these materials are used in fragrances but there is a correlation between price and tonnage. Obviously, the less expensive a material is, the more it will be used. Jasmine absolute can only be used economically, in more than trace amounts, in the most expensive fine fragrances whereas, 2-heptylcyclopentanone is cheap enough to allow its use in reasonably higher proportions in most fragrances, including low-cost ones for use in laundry powders and household cleaners. The example of jasmine is typical of the interplay of inspiration from nature, technical possibility and economic pressure, which has given rise to the variety of fragrance materials which are in use today and which are described in Chapter 4.

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CHAPTER 4

Ingredients for the Modern Perfumery Industry

CHARLES SELL
Quest International

4.1 ECONOMIC FACTORS AFFECTING PERFUME INGREDIENT PRODUCTION

The four main factors which affect the volume of use of a fragrance ingredient are its odour contribution to a fragrance, its stability and performance in the products to be perfumed, its safety in use and its cost. The first three factors are discussed in the chapters on perfumery, applications, safety and ingredient design (Chapters 7, 9, 10 and 14, respectively). The fourth factor, cost, depends on raw material availability and chemical process technology. These topics will be discussed in this chapter.

The fragrance industry lies between the petrochemical and pharmaceutical industries in terms of scale of production and cost per kg of product. The production scale is closer to that of the pharmaceutical industry, but the prices are closer to those of the bulk chemicals industry.

The largest-volume fragrance ingredients are produced in quantities of 5000–6000 tonnes worldwide per annum and some ingredients, mostly those with extremely powerful odours, which limit their use in a fragrance, are required in only kilogramme amounts. These figures are dwarfed by products such as nylon 66 and nylon 6 (polycaprolactam), which are each produced at around 4 million tonnes annually. About 15,000 tonnes of aspirin, acetylsalicylic acid, are produced annually while the total annual production of salicylate esters used in perfumery amounts to about 5000 tonnes. The terpenoid vitamins – A, E and K – are produced in comparable quantities to the ionone family of fragrance ingredients with which they share a common precursor, citral.

The cost restraints on the industry are ultimately imposed by the consumer. In many products, such as soap and laundry powder, the fragrance may be a significant contributor to the overall cost of the finished goods. If the price of one product is not acceptable, the consumer will select a competitive brand. The manufacturer of these products therefore puts considerable pressure on the fragrance supplier to develop the most effective fragrance at the lowest possible cost. The highest-volume fragrance ingredients cost only a few pounds per kg. This is not far above the cost of many basic petrochemical building blocks, which are, typically, in the £0.5–1 per kg price range.

This means that the fragrance chemist has to work hard and think creatively and opportunistically in order to provide materials at an acceptable cost and without the advantages of scale that the bulk chemicals industry enjoys. One method by which the desired result is achieved is by seeking out materials which the bulk industries use as intermediates or produce as by-products and using them as feedstocks for the preparation of perfume ingredients. The application of this approach will be obvious in many of the following sections of this chapter. These sections are mostly based on family trees of materials produced from a common feedstock or group of feedstocks.

The experienced fragrance chemist will build economic considerations into his thinking, even at the stage of designing new molecules, since he knows that a material will not be successful if it cannot be produced at a competitive cost.

Each major fragrance company runs essentially two businesses: one producing ingredients and the other selling fragrances. Indeed, the major companies sell ingredients to each other. The ingredients business of each fragrance company therefore has to compete for internal business with its rival companies. To complicate matters still further, perfume ingredients are also produced by non-fragrance companies and these sources also compete for the ingredients business. The three main reasons why chemical companies from outside the fragrance industry produce perfume ingredients are feedstock availability, common intermediates and technology.

Companies whose origins were in the wood pulp and paper industry may well have diversified into terpenoid fragrance ingredients because of their generation of turpentine. A petrochemical company running a process on huge scale might find that a by-product, which is small on their scale, is large enough to serve as a major source for the fragrance industry, as is the case with phenylethanol, for example. These are examples of feedstock-driven entry into the perfume ingredients business.

Pharmaceutical companies, which are interested in the terpenoid vitamins – A, E and K – will use citral as an intermediate and may therefore branch out into fragrances. For this reason, Hoffmann-La Roche once owned the fragrance company Givaudan. Similarly, salicylic acid was a common intermediate for some of the fragrance ingredients produced by Haarmann and Reimer and the aspirin produced by their parent company Bayer. The Reimer in question is the Reimer of the Reimer–Tiemann reaction and it was his chemistry upon which Bayer's business was built. Similarly, the Japanese company, Kuraray, manufactures synthetic rubber from butadiene and isoprene and so has diversified into terpenoid aroma chemical manufacture from these basic feedstocks.

Fine chemical companies will often have strengths in one or more aspects of chemical technology and, if their technology offers a very economically attractive route to a fragrance ingredient, then they are likely to produce it.

One major feature of the industry in the early part of the twenty-first century is the emergence of the chemical industry of the developing world, and India and China in particular. Companies in these countries operate from a much lower cost base than those in Europe and North America and competition from them is making life very difficult for those in the West.

Biotechnology has had a significant effect on the flavour industry but two factors have limited its application to fragrance. The first is cost, as biotechnological processes are usually quite expensive. The second is selectivity. Individual enzymic reactions are very selective, but biochemical redox reactions require expensive co-factors and so the usual technique is to run whole cell fermentations so as to allow the cell's chemical factory to recycle the co-factors. However, the cell does much chemistry in addition to the reaction we wish it to do and the result is a horrendous effluent problem. In flavours, the problem is often simpler as the whole cell, *e.g.* a yeast cell, can be used as the product.

4.2 PERFUME INGREDIENTS DERIVED FROM TERPENOIDS

4.2.1 Introduction

The terpenoids form the largest group of natural odorants, so it is only to be expected that they also form the largest group of modern fragrance ingredients.

Thousands of different terpenoid structures occur in perfume ingredients, both natural and synthetic. The chemistry of terpenoids is rich and varied, and attempts to understand it have, on many occasions, contributed fundamentally to our total understanding of chemistry. One example is the work of Wagner and Meerwein, whose studies in terpenoid chemistry led, among many discoveries, to the elucidation of the rearrangement that bears their names. This work made a very significant contribution to our fundamental understanding of the properties and reactions of carbocations. For those who wish to know more than what is covered in this volume, the book by Sell (2003) will serve as an introduction to the exciting field of terpenoid chemistry. It also contains chapters on commercial production of terpenoids and the design of novel structures.

As far as perfume materials are concerned, the most important members of the terpenoid family are the oxygenated monoterpenoids. The terpenoid hydrocarbons generally have weaker odours and are used mainly as feedstocks. The higher molecular weight of the sesquiterpenoids results in their having lower vapour pressures than their monoterpenoid counterparts. Thus, sesquiterpenoids are present at a lower concentration in the air above a perfume than are monoterpenoids with the result that they must have a greater effect on the receptors in the nose in order to be detected. Hence, a lower percentage of sesquiterpenoids have useful odours than monoterpenoids. For the same reason, very few di- or higher terpenoids have odours. However, those sesqui- and higher terpenoids, which do have odours, are very tenacious because their lower volatility means that they are lost more slowly from perfumes. Such materials form the base of perfumes and serve also to fix the more volatile components.

Table 4.1 shows the odour type of some of the more important terpenoid fragrance ingredients. Volumes of these materials range from about 5000

Table 4.1 *Some of the more important terpenoid fragrance materials*

Material	*Odour*
Amberlyn®/Ambrox®/Ambroxan®	Ambergris
Carvone	Spearmint
Citronellol and esters	Rose
Dihydromyrcenol	Citrus, floral
Geraniol/nerol and esters	Rose
Hydroxycitronellal	Muguet
Borneol/*iso*borneol and acetate	Pine
Linalool	Floral, wood
Linalyl acetate	Fruit, floral
Menthol	Mint, coolant
(Methyl)ionones	Violet
α-Terpineol and acetate	Pine
Acetylated cedarwood	Cedar

tonnes per annum for materials such as linalool and citronellol down to the expensive specialities such as Amberlyn®, Ambrox® and Ambroxan®, which together are produced at around 10–20 tonnes per annum.

The remainder of this section is divided into 20 sub-sections. The first 9 describe the main approaches to the production of the large-volume terpenoids and the remaining 11 describe individual groups of terpenoids, sometimes classified biogenetically and otherwise, when more appropriate, by odour type. There are so many terpenoid-derived fragrance ingredients that it would be impossible to mention them all in this book. This chapter will, therefore, include only a selection of the more important and interesting ones.

4.2.2 Five Key Terpenoids

Geraniol/Nerol, linalool, citronellol, citronellal and citral are five of the most important terpenoids as far as the perfume industry is concerned. Apart from citral, they are all used as such in perfumes. The alcohols and their esters are particularly important. All of them are key starting materials for other terpenoids, and will be discussed later. Figure 4.1

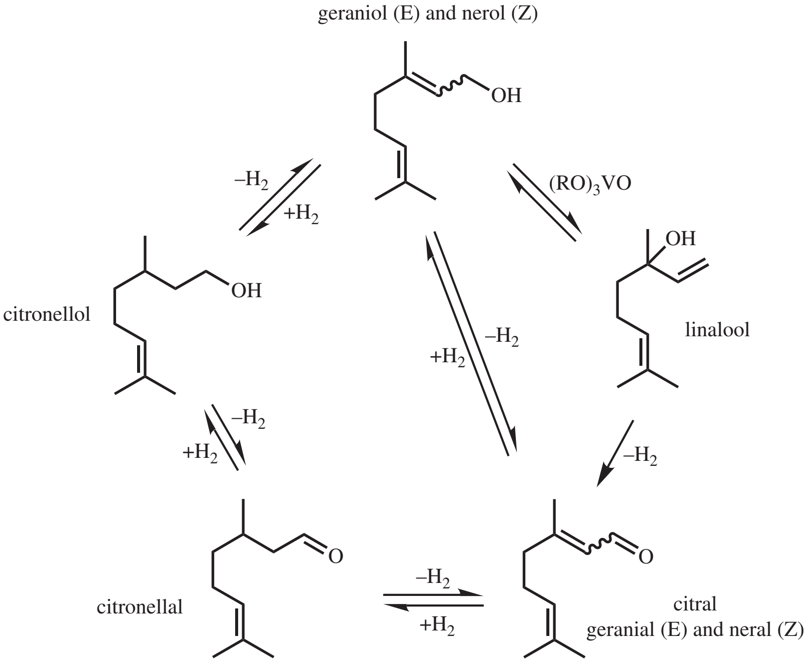


Figure 4.1 *Five key terpenoids*

shows the structures of these materials and how they can be interconverted simply by isomerisation, hydrogenation and oxidation. The ability to manufacture any one of these, therefore, opens up the potential to produce all of them and, hence, a wide range of other terpenoids. Obviously, if one company produces geraniol/nerol initially and another linalool and both do so at the same cost per kg, then they will not be able to compete with each other on both products. The first will be restricted to geraniol/nerol and the second to linalool. Thus, the range of products which any terpene producer can market effectively will depend on a fine balance of its feedstock and process costs *vis-à-vis* those of its competitors.

4.2.3 Feedstocks

Routes to the major synthetic terpenoids start from either turpentine or petrochemical sources.

When softwood (pine, fir, spruce) is converted into pulp in the Kraft paper process, the water-insoluble liquids which were present in it are freed and can be removed by physical separation from the process water. This material is known as crude sulfate turpentine (CST). Fractional distillation of CST gives a number of products as shown in Table 4.2. The residue is known as tall oil and contains diterpenoids such as abietic acid.

Turpentine obtained by tapping living trees is known as gum turpentine. The major components of both gum turpentine and CST are α- and β-pinenes. These are usually present in a ratio of about 7:3, α:β, the exact ratio depending on the species of tree involved. Pure α- and β-pinenes can be obtained by fractional distillation of turpentine. The two can be interconverted by catalytic isomerisation but this leads to an equilibrium mixture. The equilibrium can be driven in one direction by continuous removal of the lower boiling component through distillation. However, α-pinene is the lower boiling of the two and is already the more abundant. To increase the yield of β-pinene, it is necessary to fractionate, isomerise α into β fractionate again, isomerise α into β and

Table 4.2 *Products in distillate from CST*

Product	*Percentage in CST*
Lights	1–2
α-pinene	60–70
β-pinene	20–25
Dipentene	3–10
Pine oil	3–7
Estragole, anethole, caryophyllenes	1–2

so on. This is obviously a costly process in terms of time and energy. As a result of these factors, β-pinene is about twice the price of α-pinene and this affects the economics of the processes described below.

The petrochemical building blocks used for terpenoid synthesis are all readily available bulk feedstocks and are therefore free from concerns regarding availability and security of supply, until the supply of mineral oil is exhausted.

4.2.4 Pinene Pyrolysis

One of the earliest commercial routes into this key group of terpenoids involved pyrolysis of β-pinene as shown in Figure 4.2. When β-pinene is heated to 500°C, the cyclobutane ring breaks *via* a *retro* −2 +2 cycloaddition reaction. This ring opening is regioselective and produces the triene, myrcene. Addition of hydrogen chloride to myrcene gives a mixture of geranyl, neryl and linalyl chlorides. These can be hydrolysed but the reaction with acetate anion is more efficient than that with hydroxide and so the acetate esters are usually the initial products in commercial syntheses. Fractional distillation is used to separate the various products but is complicated by the presence of traces of chlorinated impurities and isomers formed from the opening of the cyclobutane ring in the 'wrong' direction.

The two main disadvantages of this route are the cost of β-pinene and the presence of trace amounts of chlorinated materials which must be removed from the product.

4.2.5 Pinane Pyrolysis

Hydrogenation of the less expensive α-pinene gives pinanol, which can be oxidised by air under radical conditions to give the hydroperoxide, which is then reduced to 2-pinanol. Pyrolysis of this alcohol gives

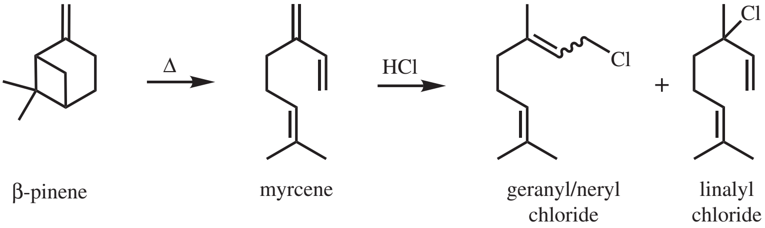


Figure 4.2 *Production of geranyl, neryl and linalyl chlorides*

linalool as shown in Figure 4.3. This process is operated by Millennium at their plant in Georgia in the USA.

The disadvantage of this process lies in a side reaction. Linalool is not stable under pyrolysis conditions and some of it undergoes an *ene* reaction to give a mixture of isomeric alcohols, known as plinols. These have boiling points close to that of linalool, making separation by distillation difficult. The pyrolysis is therefore run at below total conversion in order to minimise plinol formation. The mechanism of the *ene* reaction and the structure of the plinols are shown in Figure 4.4.

4.2.6 The Carroll Reaction

2-Methylhept-2-en-6-one, usually simply referred to as methylheptenone, is a useful synthon for total synthesis of terpenoids. One early

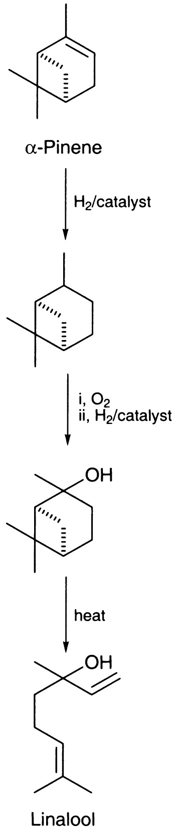


Figure 4.3 *Linalool from α-pinene*

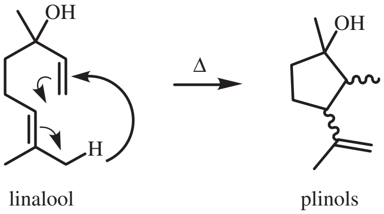


Figure 4.4 *Formation of plinols*

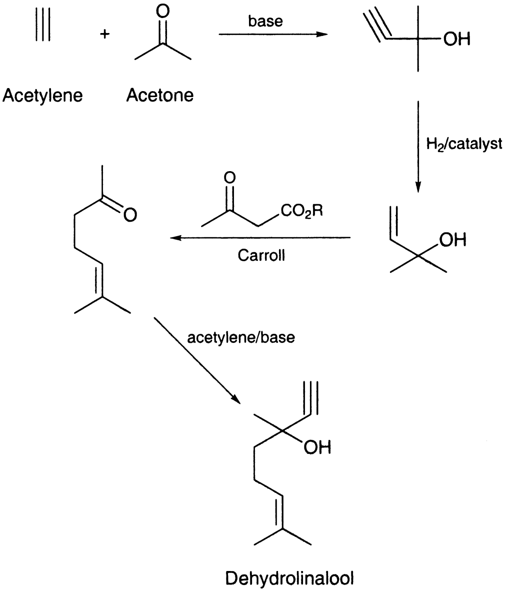


Figure 4.5 *Linalool from acetylene and acetone*

synthesis of this intermediate employed the Carroll reaction. The substrate for this reaction is prepared by addition of acetylene to acetone and subsequent partial hydrogenation to 2-methylbut-3-en-2-ol as shown in Figure 4.5. Addition of acetylene to methylheptenone gives dehydrolinalool, which can be hydrogenated to linalool using a Lindlar catalyst.

The Carroll reaction occurs when a β-ketoester is treated with an allylic alcohol in the presence of base or when an allyl ester of a β-ketoacid is heated. Figure 4.6 shows the mechanism of the latter.

The disadvantage of this process is that it is not very atom efficient. The elimination of carbon dioxide means that bulk is carried through the process only to be lost towards the end. This is undesirable from both the cost and environmental impact standpoints.

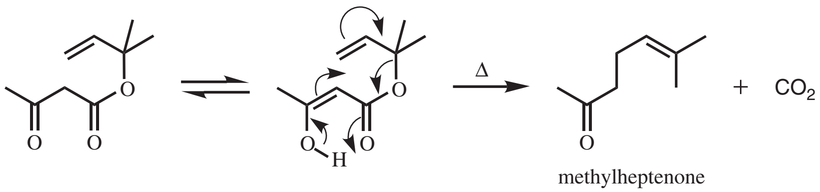


Figure 4.6 *Methylheptenone via the carroll rearrangement*

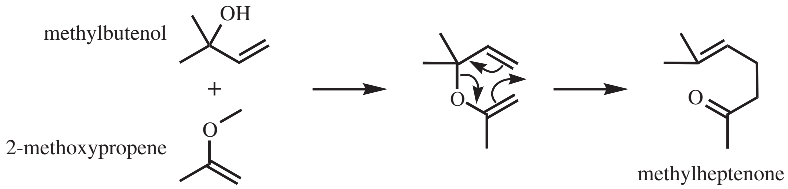


Figure 4.7 *Methylheptenone from methylbutenol and methoxypropene*

4.2.7 The Claisen Rearrangement

Use of the Claisen rearrangement enables the same conversion of methylbutenol to methylheptenone as does the Carroll reaction but without the loss of carbon dioxide. The methanol, which is produced in its place, can be recovered and recycled. In this process, as shown in Figure 4.7, methylbutenol is treated with the readily available 2-methoxypropene to give the allyl vinyl ether, which then undergoes a Claisen rearrangement to give methylheptenone.

4.2.8 Prenyl Chloride

Addition of hydrogen chloride to isoprene gives prenyl chloride, together with some of the allylic isomer 2-chloro-2-methylbut-3-ene. The presence of this tertiary chloride is not deleterious since, while prenyl chloride reacts *via* an S_N2 reaction, it reacts by an S_N2' mechanism to give the same product, methylheptenone. This is shown in Figure 4.8. Further elaboration to linalool, *etc.* is the same as in the processes described above.

4.2.9 The Ene Reaction

Aldol condensation of acetone with formaldehyde gives methyl vinyl ketone. This can undergo the *ene* reaction with *iso*butylene to give an

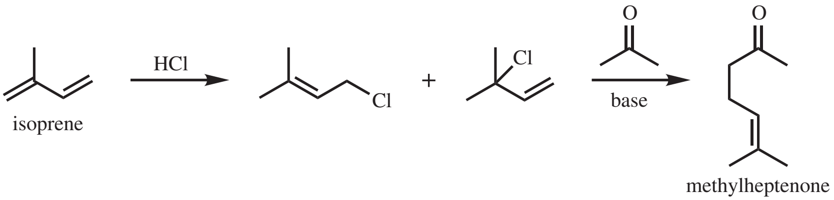


Figure 4.8 *Methylheptenone via hydrochlorination of isoprene*

isomer of methylheptenone, 2-methylhept-1-en-6-one. Isomerisation to methylheptenone is easy using an acidic catalyst and acetylene can be added to either isomer. From methylheptenone, the process leading to linalool is the same as in the examples above. If the acetylene is added to 2-methylhept-1-en-6-one, then iso-dehydrolinalool results. This is actually advantageous when the monoterpene unit is to be used as a precursor for ionones and vitamins, since the 1,1-disubstituted double bond is more reactive in the cyclisation reaction than is the normal 1,1,2-trisubstituted bond. (The cyclisation reaction is described in detail below.) The preparation of these two acetylenic ketones is shown in Figure 4.9 and is the basis of a process patented by BASF.

4.2.10 Elegance, a Four-Step Process

Another process patented by BASF is shown in Figure 4.10. This process uses only *iso*butylene, formaldehyde and air as reagents; the only by-product is two molar equivalents of water per mole of product and it gives citral in just four steps, two of which run in parallel. Such elegance not only has intellectual appeal but also is an excellent example of how industrial chemical synthesis should be carried out, producing valuable products efficiently, at low cost and with minimal environmental impact.

The reaction between *iso*butylene and formaldehyde produces 2-methylbut-1-en-4-ol, *iso*prenol. This can be isomerised to prenol or oxidised to *iso*prenal. Two moles of prenol and one of *iso*prenal are combined to give the ketal, which then eliminates one prenol (recovered and recycled) to give the enol ether. This ether undergoes a Claisen rearrangement to give an aldehyde containing two double bonds, which are perfectly placed to undergo the Cope rearrangement to give citral.

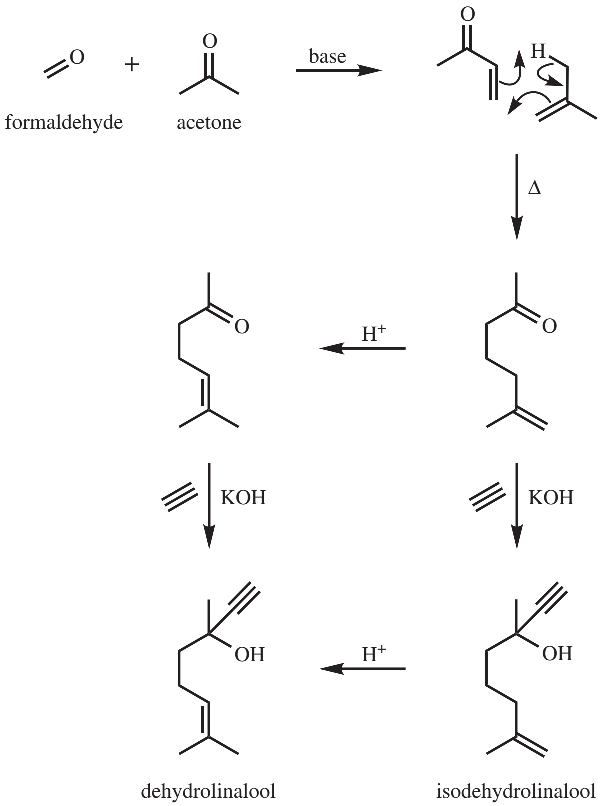


Figure 4.9 *Linalool from formaldehyde and acetone*

4.2.11 Hemiterpenoids

A small number of hemiterpenoids are used in perfumery, the most important of these are prenyl acetate and benzoate. Thioesters, such as those shown in Figure 4.11, have extremely intense green odours reminiscent of galbanum, in which they occur naturally. The esters are usually prepared from prenyl chloride and the thioesters from the corresponding thiol and acid chloride as shown in Figure 4.11.

4.2.12 Acyclic Monoterpenoids

The alcohols geraniol/nerol, linalool, citronellol and their esters are the largest tonnage materials of this class. (People in the fragrance industry tend to use nomenclature rather loosely. For example, to the chemist,

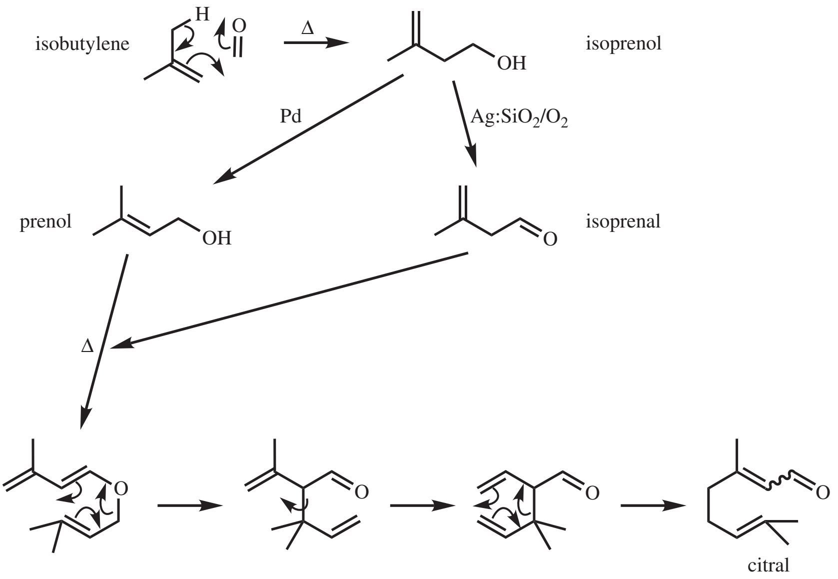


Figure 4.10 *Citral from formaldehyde and isobutylene*

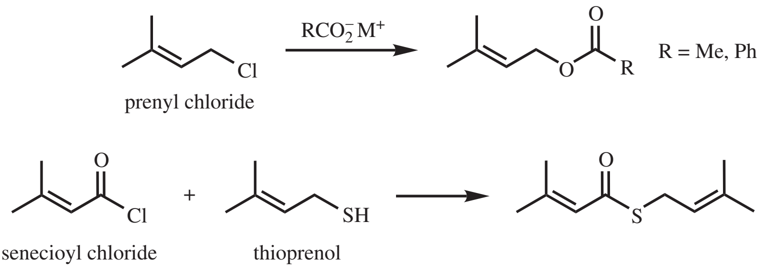


Figure 4.11 *Production of hemiterpenoid esters and thioesters*

geraniol is *E*-3,7-dimethylocta-2,6-dien-1-ol and nerol is *Z*-3,7-dimethylocta-2,6-dien-1-ol; whereas, in the fragrance industry, the word geraniol is often given to mixtures of the two isomers.) The syntheses of these key materials are described above and no further detail is necessary here. The chemical stability of these materials is limited by the unsaturation in them. In order to improve stability, particularly in oxidative media such as bleaches and laundry powders, various hydrogenated analogues have been developed. In some, one of the double bonds is reduced, in others both are.

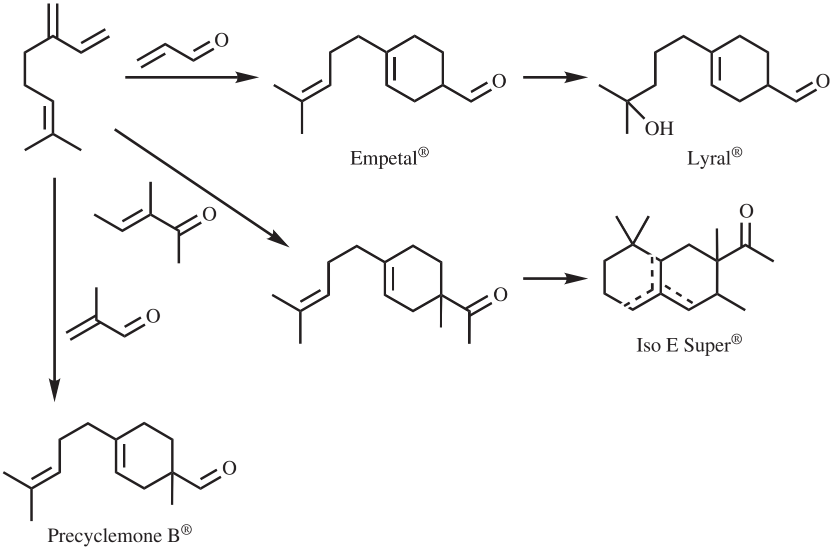


Figure 4.12 *Fragrance ingredients from myrcene*

A number of hydrocarbons of this family add oily, green or herbaceous notes to essential oils. Two hydrocarbons, myrcene and dihydromyrcene (also known as citronellene), deserve mention as feedstocks for other fragrance ingredients.

Pyrolysis of β-pinene gives myrcene as described above. Since it is a 1,3-diene, myrcene readily undergoes the Diels–Alder reaction with a variety of dienophiles. Addition of acrolein gives a mixture of regioisomers, the major one shown in Figure 4.12. This mixture is known as Myrac Aldehyde® or Empetal®. Hydration of the double bond in the tail gives Lyral®, a widely used muguet ingredient. The reaction with 3-methylpent-2-ene-3-one (from the aldol reaction of methyl ethyl ketone with acetaldehyde) is more complex in that a greater number of isomeric products are produced. Acid-catalysed cyclisation then gives an even more complex mixture, known as Iso E super®. This mixture has a pleasant, woody, amber odour, which is believed to arise predominantly from only a few of its components. The adduct of myrcene with methacrolein is known by the slightly misleading name of Precyclemone B®.

Pyrolysis of pinane gives dihydromyrcene, also known as citronellene. Hydration of the more reactive, trisubstituted double bond gives dihydromyrcenol as shown in Figure 4.13. Direct hydration is difficult, so usually a two-stage process is used. The first involves acid-catalysed

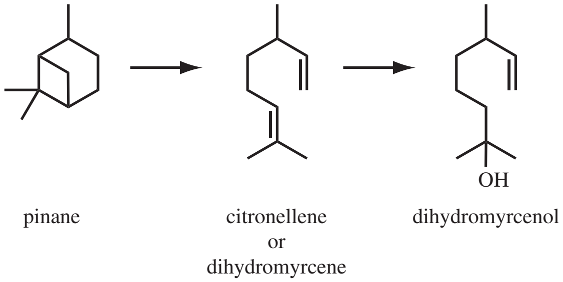


Figure 4.13 *Dihydromyrcenol from pinane*

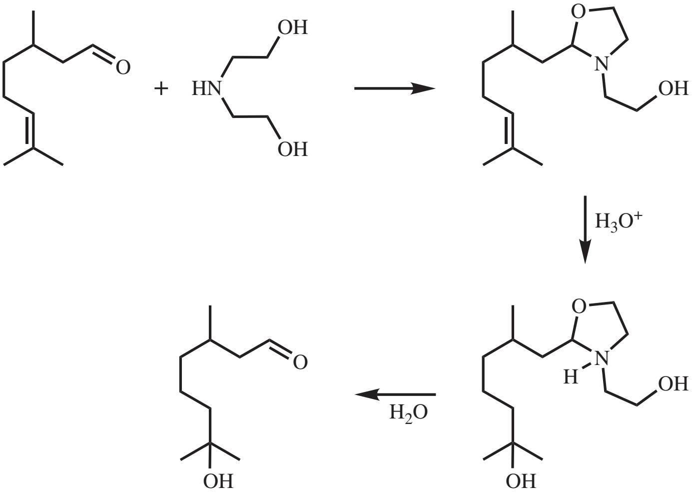


Figure 4.14 *Production of hydroxycitronellal*

addition of chloride, sulfate or acetate (from the corresponding acids), followed by hydrolysis to give the alcohol. This alcohol was first introduced as a stable, fresh floral/muguet note for functional products, but its success in the after-shave Drakkar Noir caused trickle-up to the fine fragrance market, the reverse of the usual trend with new ingredients.

Hydration of the double bond of citronellal gives the compound known as hydroxycitronellal in a reaction analogous to that used to prepare Lyral®. Like Lyral®, hydroxycitronellal has a muguet odour. In acidic conditions, citronellal cyclises to isopulegol and so the aldehydic group must be protected during the hydration stage. A typical sequence is shown in Figure 4.14. The hydration is carried out in concentrated acid, under which conditions the oxazolidine ring is stable. Dilution of

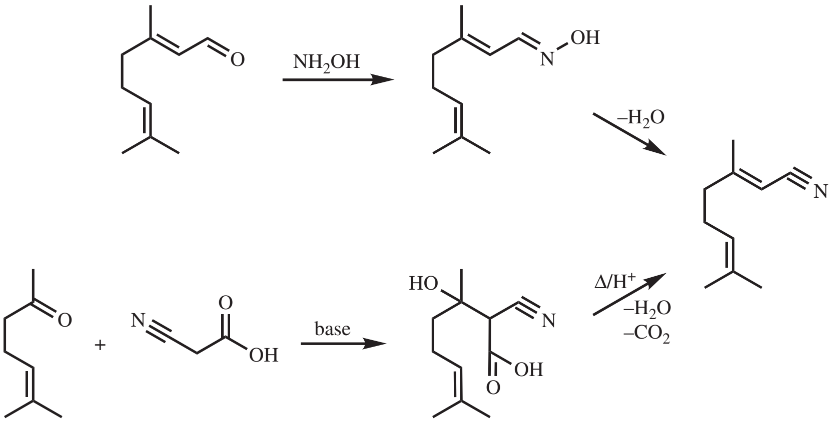


Figure 4.15 *Production of geranyl nitrile*

the medium allows hydrolysis of the protecting group to occur. Similar protection is required when hydrating Empetal® in order to prevent *trans*-annular reactions.

Citral is the key odoriferous principle of lemon oil and is therefore potentially very useful in perfumery. However, it is not stable to oxidation and therefore cannot be used in functional products containing bleach. Since lemon is associated with cleanliness and freshness, this represents a serious challenge for household products. One of the solutions which has been found is to convert citral into the more stable nitrile, known as geranyl nitrile. Often, nitriles have odours which closely resemble the corresponding aldehyde, this being a case in point. Geranyl nitrile can be prepared from either citral or methylheptenone as shown in Figure 4.15.

There are several cyclic ethers derived from acyclic monoterpenoids which are of importance at lower levels in fragrances.

Allylic oxidation of citronellol can be used to introduce a leaving group, which allows cyclisation to form the pyran, rose oxide. Chlorination was one of the first oxidation techniques employed; various others, including electrochemical methods, have since been developed. An outline of the synthesis is given in Figure 4.16. Rose oxide occurs in rose and geranium oils, to which it imparts a characteristic dry, green, rosy top-note.

Structurally related to rose oxide is the hydroxypyran shown in Figure 4.17. This material is known under the trade names Florosa® and Florol®. It is prepared by the Prins reaction between *iso*prenol and *iso*valeraldehyde.

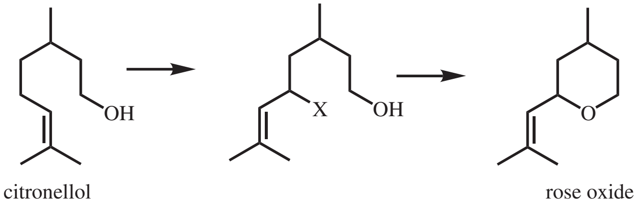


Figure 4.16 *Production of rose oxide*

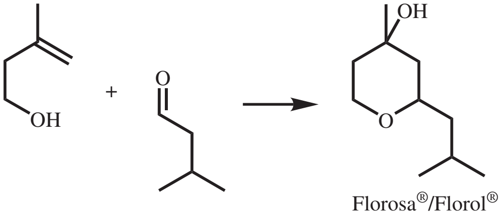


Figure 4.17 *Production of Florosa®/Florol®*

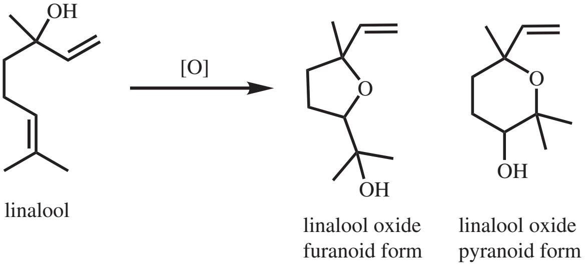


Figure 4.18 *Production of linalool oxide*

Oxidation of the trisubstituted double bond of linalool followed by cyclisation leads to the isomeric linalool oxides (Figure 4.18). The furanoid form is commercially available as a powerful, sweet, woody/floral odorant.

4.2.13 Cyclic Monoterpenes

The three most important cyclic monoterpenoids are l-menthol, l-carvone and α-terpineol (including its esters). l-Menthol occurs in a number of mint oils and is used not only for its minty odour, but also, and more importantly, for its physiological cooling effect. Its chemistry is of such interest and significance that it warrants a section of its own, which will be found at the end of this one.

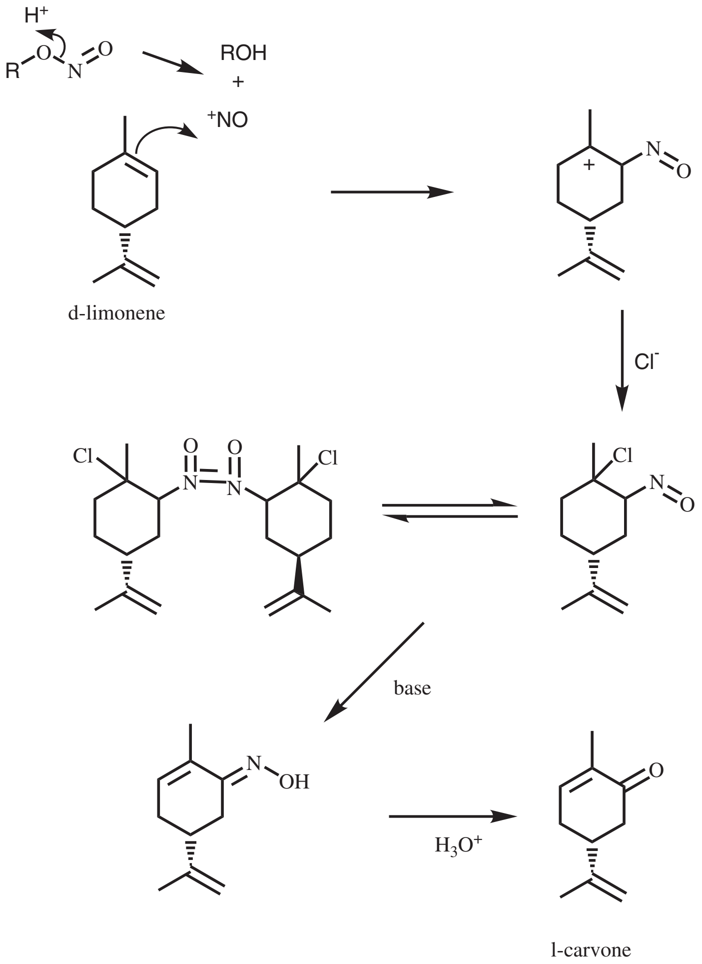


Figure 4.19 *l-Carvone from d-limonene*

l-Carvone is the principal odour component of spearmint oil. Both the oil and synthetic l-carvone are used as ingredients in mint flavours. The synthetic material is made from d-limonene, which is the major component of orange oil and therefore is available as a by-product of orange juice production. Quest International is the world's major producer of l-carvone. The classical chemistry used to produce l-carvone is shown in Figure 4.19. The chirality of the carvone is crucial to the odour, since the enantiomeric d-carvone has an odour reminiscent of dill or caraway rather than spearmint. It is therefore important that any synthesis of carvone leads to an enantiomerically pure product. It can be seen from Figure 4.19 that both l-carvone and d-limonene owe their chirality to the carbon atom at which the *iso*propenyl group is attached to the cyclohexene ring. By maintaining the chiral integrity of this centre through controlling the regiochemistry of the reactions, which take place at the opposite end of the ring, it is possible to obtain pure l-carvone from d-limonene. The first step involves addition of the nitrosyl cation to d-limonene. This cation can be obtained by heterolysis of nitrosyl chloride, but in practice it is more convenient to generate it by cleavage of *iso*propyl nitrite using hydrochloric acid. *Iso*propyl nitrite is easily prepared from sodium nitrite and *iso*propanol. The nitrosyl cation reacts preferentially with the more electron rich *endo*cyclic double bond of d-limonene. It also adds regioselectively to the less substituted end, thus generating the more stable tertiary carbocation. This carbocation is trapped by the chloride ions present from the hydrochloric acid. The initial adduct is a blue liquid, which is in equilibrium with the dimeric white solid, as shown in Figure 4.19. Treatment of this product mixture with a base leads to elimination of hydrogen chloride and rearrangement of the nitrosyl group to give an oxime. Thus, the reaction product is l-carvone oxime, which can be hydrolysed to give l-carvone.

Treatment of turpentine with aqueous acid leads to the formation of α-terpineol. The mechanism of this reaction is shown in Figure 4.20, in which α-pinene is used as an example. Some hydration of α-terpineol to give the diol, terpin hydrate, can also occur, the balance between the products depending on the severity of the reaction conditions. The crude mixture is known as pine oil and is the main ingredient of pine disinfectants. Terpin hydrate can be easily converted to α-terpineol since the ring hydroxyl group is more readily eliminated than that in the side chain.

Monocyclic monoterpenoid hydrocarbons occur in many essential oils and their by-products. They have relatively weak odours, although some add dryness and green notes to the oils containing them. This is particularly so for lime and petitgrain. The structures of four typical hydrocarbons are shown in Figure 4.21. d-Limonene occurs in citrus oils, whereas the l-isomer is found in pine. If limonene or other terpenes break down during processing to produce isoprene, then racemic limonene, dipentene, will be found in the product as a result of the Diels–Alder reaction. Terpinolene is the dehydration product of α-terpineol and so it is often present as an artefact. α-Phellandrene occurs in eucalyptus oil. Since it is a 1,3-diene, it is an obvious precursor for Diels–Alder reactions and a number of speciality ingredients are prepared from it in this way.

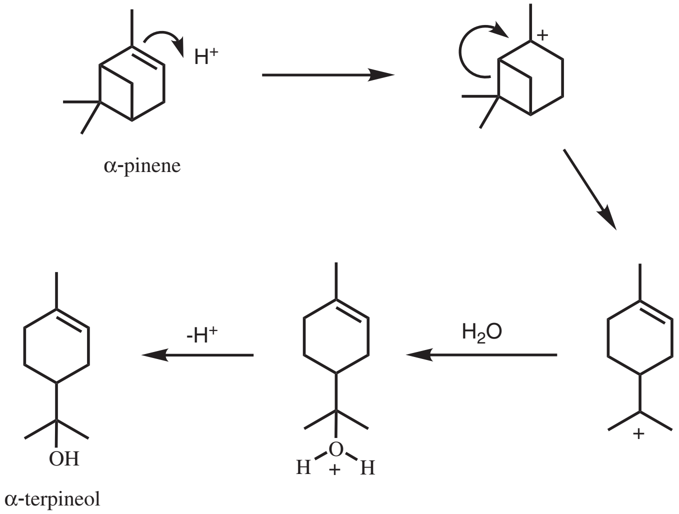


Figure 4.20 *α-Terpineol from α-pinene*

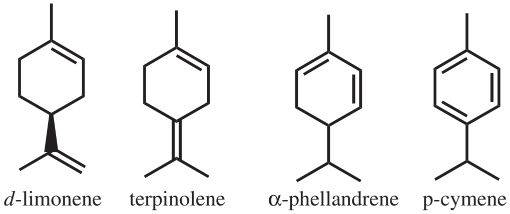
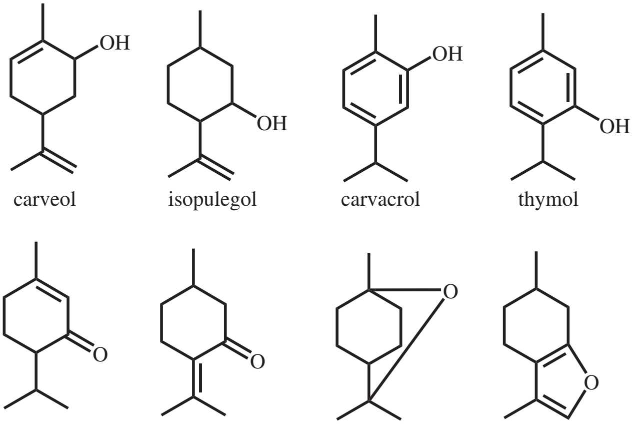


Figure 4.21 *Typical monoterpenes*

During processing of turpentine and other terpene sources, often a variety of acid-catalysed reactions and aerial oxidations occur. *p*-Cymene is often produced as a result of these processes since it is one of the most thermodynamically stable of terpenoid structures. It does occur in essential oils and fragrances, but its main uses are as a thermally stable heat transfer fluid and as a precursor for musks (see Section 4.3).

Many oxygenated monocarbocyclic monoterpenoids are of use to the flavour and fragrance industry. Some are extracted from natural sources, and others are prepared from the major members of the family, usually by straightforward functional group interconversions. Some examples are shown in Figure 4.22.



carveol isopulegol carvacrol thymol

pulegone piperitone 1,8-cineole menthofuran

Figure 4.22 *Typical oxygenated cyclic monoterpenoids*

Carveol is one of the minor components responsible for the odour of spearmint. It is easily prepared by reduction of carvone. *Iso*pulegol is prepared from citronellal, as discussed in the section on menthol, and is a precursor to other materials in the group. The phenols carvacrol and thymol are important in some herbal odour types, but the major use for thymol is as a precursor for menthol *q.v.* Piperitone and pulegone are strong minty odorants. The latter is the major component of pennyroyal oil. 1,8-Cineole is the major component of such eucalyptus oils as *Eucalyptus globulus*. These oils are inexpensive and so there is no need to prepare cineole synthetically. Menthofuran is a minor component of mint oils and can be prepared from pulegone, though normally, its presence in mint is undesirable because of its odour properties.

4.2.14 Menthol

The synthesis of menthol makes an interesting study since it nicely illustrates the balance of economic and technological factors governing the range of production methods, which can be employed commercially.

Menthol (1-methyl-4-isopropylcyclohexan-3-ol) is a monocyclic monoterpenoid, which possesses three asymmetric carbon atoms and therefore exists in eight stereoisomeric forms as shown in Figure 4.23. l-Menthol is the most highly desired of these since it produces a physiological cooling effect. That is, when applied to skin or mucus membranes, l-menthol creates the sensation of cooling independent of the

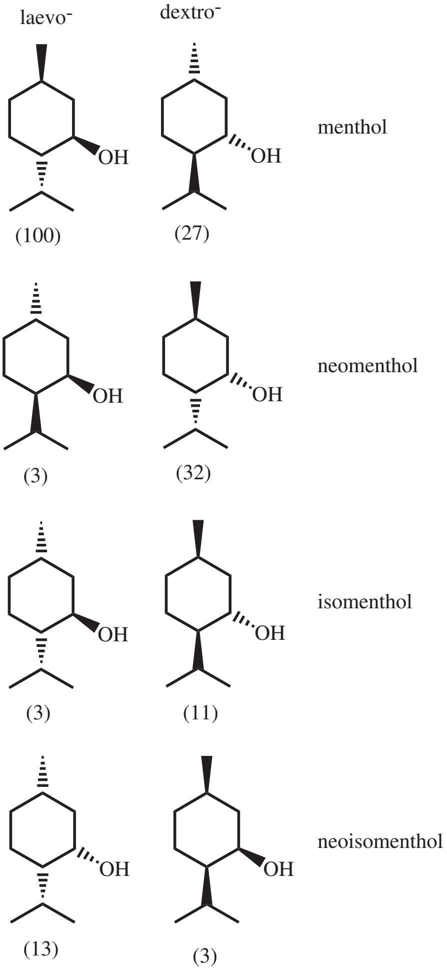


Figure 4.23 *Menthol isomers*

actual temperature of the tissue concerned. It is used in toothpaste and other oral-care products, in confectionery, tobacco and some cosmetic products, largely because of this effect. The mint taste/odour can be achieved with other materials, but the cooling effect of l-menthol is much greater than that of any of its isomers and is matched only by a few synthetic compounds, which have been found as the result of extensive research. The relative cooling power of each of the eight stereoisomers is shown in Figure 4.23. Isomeric mixtures of menthols are less useful than pure l-menthol because the cooling effect per unit weight is lower.

Therefore, any synthesis of menthol must be capable of delivering enantiomerically pure l-menthol to be commercially attractive.

Menthol can be extracted from various species of mint. Cornmint (*Mentha arvensis*) contains the highest levels of l-menthol and therefore is the major variety cultivated for menthol production. Mint is grown in China, India, Brazil and the United States. Because of the vagaries of climate and competition for land from other agricultural products, the supply of natural menthol is not stable. Price and availability fluctuate and these movements have a major impact on the economics of the various synthetic processes for l-menthol. When natural menthol is scarce, the synthetic materials command a high price and marginal processes become economically attractive. When the natural material is in abundant supply, only the more efficient of the synthetic processes will compete. The most competitive synthetic processes are those of Symrise and Takasago; hence their market domination.

The fact that menthol is produced from both renewable and fossil feedstocks allows for an interesting study in sustainability. In order to produce the same crop year after year, it is necessary to use fertilisers to replenish the nitrogen and minerals which the plant takes from the soil. Secondary metabolites such as menthol and essential oils occur at a level of, at most, only a few per cent of the dry weight of the herb. Therefore, in order to produce an economic return, it is necessary to use efficient, mechanical methods of cultivation and harvesting. A full life cycle analysis of menthol production reveals that production from cultivation of mint plants consumes more fossil fuel, produces more carbon dioxide effluent and has more environmental impact than either of the leading synthetic routes.

The process by which the German company Symrise produces l-menthol is shown in Figure 4.24. Addition of propylene to *m*-cresol produces thymol. Hydrogenation of thymol gives a mixture of menthol isomers. Treatment of any one of the eight isomers with the same copper chromite catalyst which is used for thymol hydrogenation will cause racemisation to the same equilibrium mixture of isomers. This fact is used to good effect in the process. The hydrogenation product is optically inactive, being composed of equal amounts of d- and l-isomers of each of the four conformational isomers. The balance between these is 62–64% menthol, 18–20% *neo*menthol, 10–12% *iso*menthol and 1–2% *neoiso*-menthol. Since these are pairs of diastereomers, their physical properties differ. Thus, at atmospheric pressure, d,l-menthol boils at 216.5 °C, d,l-*neo*menthol at 212 °C, d,l-*iso*menthol at 218 °C and d,l-*neoiso*menthol

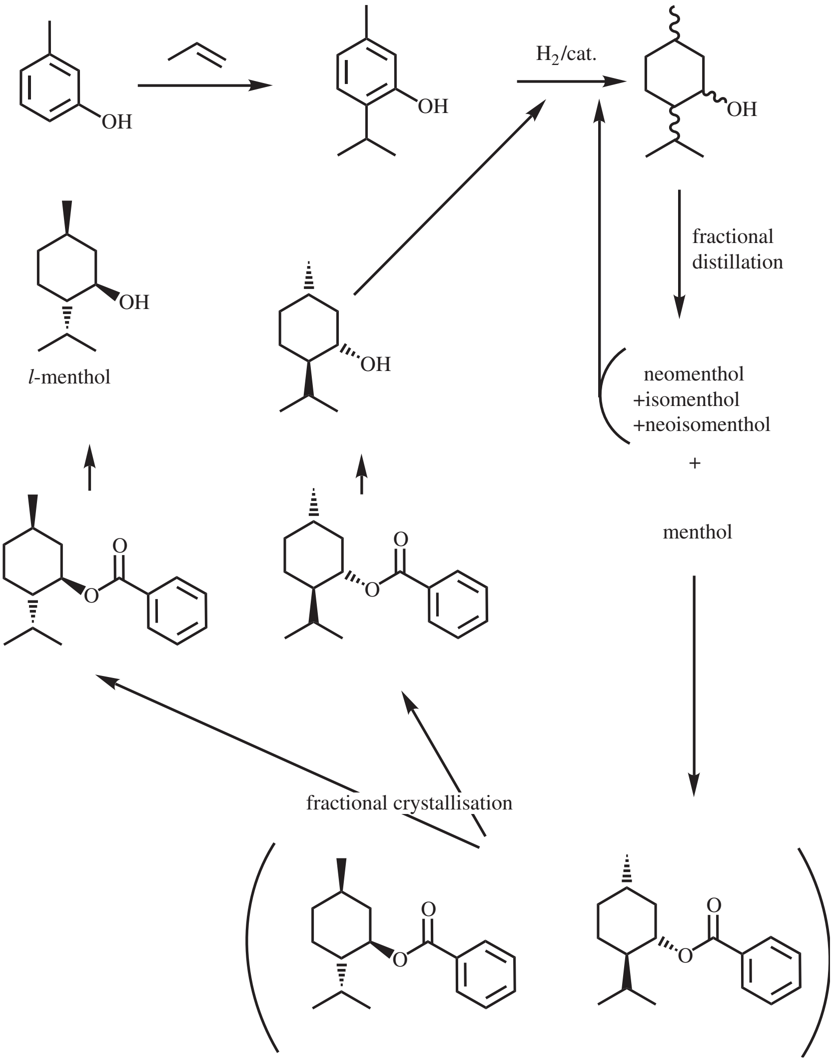


Figure 4.24 *The Symrise route to l-menthol*

at 214.6 °C. This means that the diastereomeric pairs can be separated by distillation through a high efficiency column and d,l-menthol obtained from the mixture. This mixture is resolved by fractional recrystallisation of the benzoate ester followed by saponification. Recrystallisation of the desired isomer gives pure l-menthol and all of the other seven isomers can be fed back into the hydrogenation stage with fresh thymol where they will be equilibrated as the thymol is hydrogenated. Since *m*-cresol and propylene are inexpensive feedstocks, the menthol produced by this process has a low raw material cost. However, recycling to the hydrogenation, esterification and hydrolysis and crystallisation stages all consume time, labour and reactor capacity, so the low raw material cost is offset by relatively high process costs. It is estimated that about 2500 tonnes of l-menthol are produced by this process annually.

The other major producer of synthetic l-menthol is the Japanese company Takasago. They produce over 2000 tonnes per annum using elegant chemistry developed by Professor Noyori, who won the 2001 Nobel Prize for chemistry in recognition of this work. The synthetic scheme is shown in Figure 4.25. Pyrolysis of β-pinene gives myrcene, to which diethylamine can be added in the presence of a catalytic amount of strong base. This produces *N*,*N*-diethylgeranylamine. Isomerisation of this with the rhodium BINAP complex produces the enamine of citronellal. The elegance of this route stems from the fact that the rhodium complex is chiral and so the proton is added to only one face of the intermediate, thus ensuring that only the enamine of d-citronellal is produced. Hydrolysis of the enamine gives d-citronellal which can be cyclised into isopulegol by a Lewis acid-catalysed ‘ene’ reaction. The chirality of the citronellal imposes itself on the transition state of the ene reaction and thus pure l-isopulegol is produced. This can then be hydrogenated to l-menthol.

The route shown in Figure 4.26 is used by Camphor and Allied, an Indian company, which has access to plentiful supplies of carene from Indian turpentine. High customs tariffs make imported menthol very expensive in India and so this process benefits from local economics. It uses the natural chirality of the turpentine-derived carene to produce d-*iso*terpinolene through the isomerisation–pyrolysis–isomerisation sequence. Hydrogenation over a poisoned catalyst gives d-3-*p*-menthane. This can then be epoxidised and the epoxide rearranged to give a mixture of l-menthone and d-*iso*menthone. Epimerisation with base increases the percentage of the former in the mixture since it is the di-equatorial, and hence more thermodynamically stable, isomer. Hydrogenation then gives a mixture of isomers, the major one being the desired l-menthol, which can be separated from the d-isomers by distillation or crystallisation. About 200 tonnes per annum has been produced in this way.

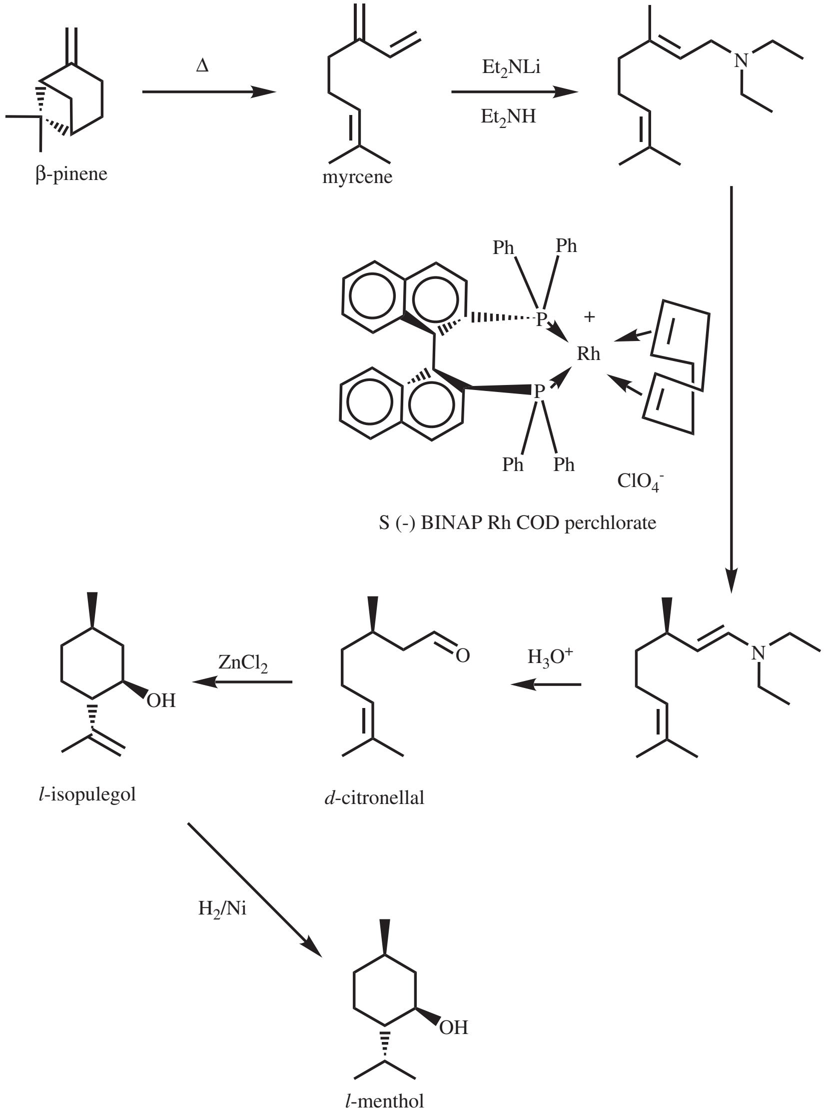


Figure 4.25 *The Takasago route to l-menthol*

Pennyroyal oil can be grown commercially in Southern Europe and North Africa. Its major constituent is d-pulegone and so another minor source of menthol relies on the chirality of this natural product in order to produce isomerically pure material. As shown in Figure 4.27,

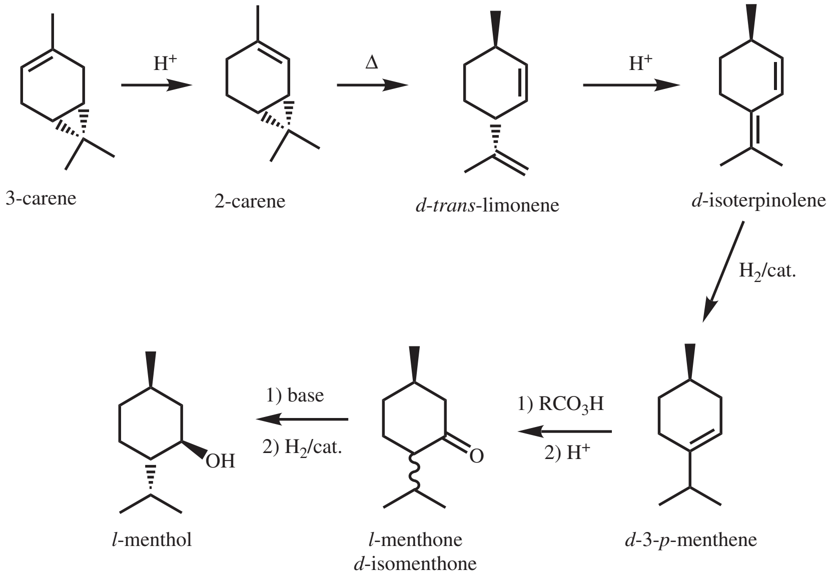


Figure 4.26 *l-Menthol from 3-carene*

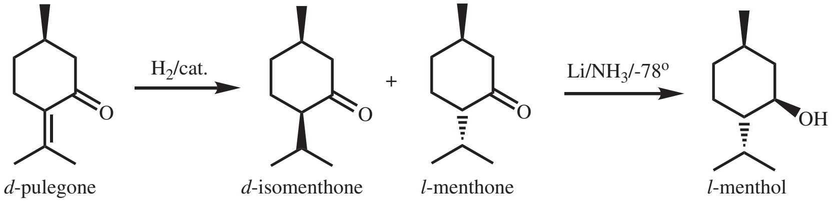


Figure 4.27 *l-Menthol from d-pulegone*

hydrogenation of d-pulegone gives a mixture of d-isomenthone and l-menthone. This mixture can be separated by distillation and the l-menthone reduced to l-menthol. The Spanish company Bordas, has produced menthol by this method.

The essential oil of *Eucalyptus dives* contains l-piperitone and this provides a starting material for l-menthol using the process shown in Figure 4.28. The l-piperitone is reduced to a mixture of piperitols which are separated and the major isomer, d-*trans*-piperitol, hydrogenated to give d-*iso*menthol containing a small amount of d-menthol. After purification, the former can be isomerised into l-menthol using aluminium

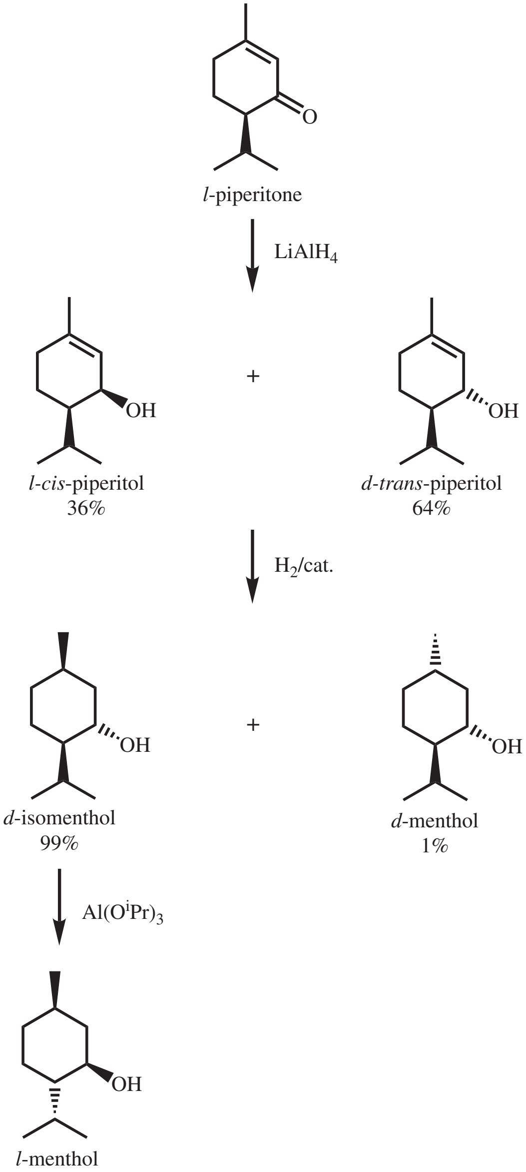


Figure 4.28 *l-Menthol from l-piperitone*

isopropoxide as catalyst. About 30 tonnes of l-menthol per annum were once produced by Keith Harris & Co. in Australia using this route.

This selection of menthol processes shows how the major producers are those with the most cost-effective processes, but that local economic

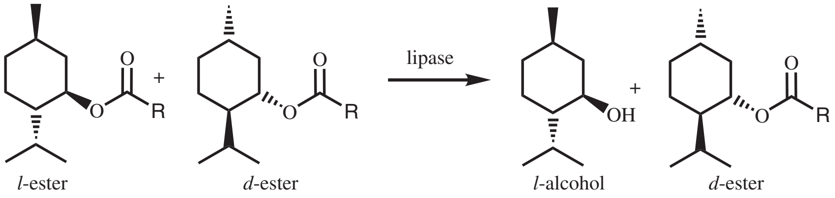


Figure 4.29 *Enzymic resolution of menthol*

conditions or feedstock availability can provide niche opportunities for less efficient processes.

The development of biotechnology is nowadays opening up new approaches for synthesis, particularly of chiral target molecules. An example is a recent patent (EP 1223223, **2002**) in which Symrise claim a process for the resolution of menthol using a lipase. When presented with a mixture of enantiomeric esters, the enzyme hydrolyses only the l-enantiomer and then the l-menthol is easily separated from the unreacted d-ester. The reaction scheme is shown in Figure 4.29.

4.2.15 Bicyclic Monoterpenoids

Camphene can be obtained from α-pinene through acid-catalysed rearrangement. The details of the mechanism are shown in Figure 4.30. It was the study of this reaction and related conversions that led to the elucidation of the Wagner–Meerwein rearrangement. Acid-catalysed addition of acetic acid to camphene gives *iso*bornyl acetate; the first carbocation formed in this conversion is identical to that from which camphene was formed. In the conversion of α-pinene to camphene, no good nucleophile is present and so the cation eliminates a proton. In the second reaction, the presence of acetic acid allows the cation to be trapped as the acetate. In such carbocationic rearrangements, the outcome of the reaction is usually determined by a delicate balance of steric and thermodynamic factors and by the nature of other species present in the medium. Hydrolysis of *iso*bornyl acetate gives *iso*borneol, which can be oxidised to camphor. Camphor can also be isolated from camphorwood, of which it is the major volatile constituent. The synthetic material is usually the less expensive. Camphor, *iso*borneol and *iso*bornyl acetate are all used for their woody odours.

Another bicyclic monoterpenoid derivative of use in perfumery is nopyl acetate, which has a sweet woody/fruity odour. Prins reaction of

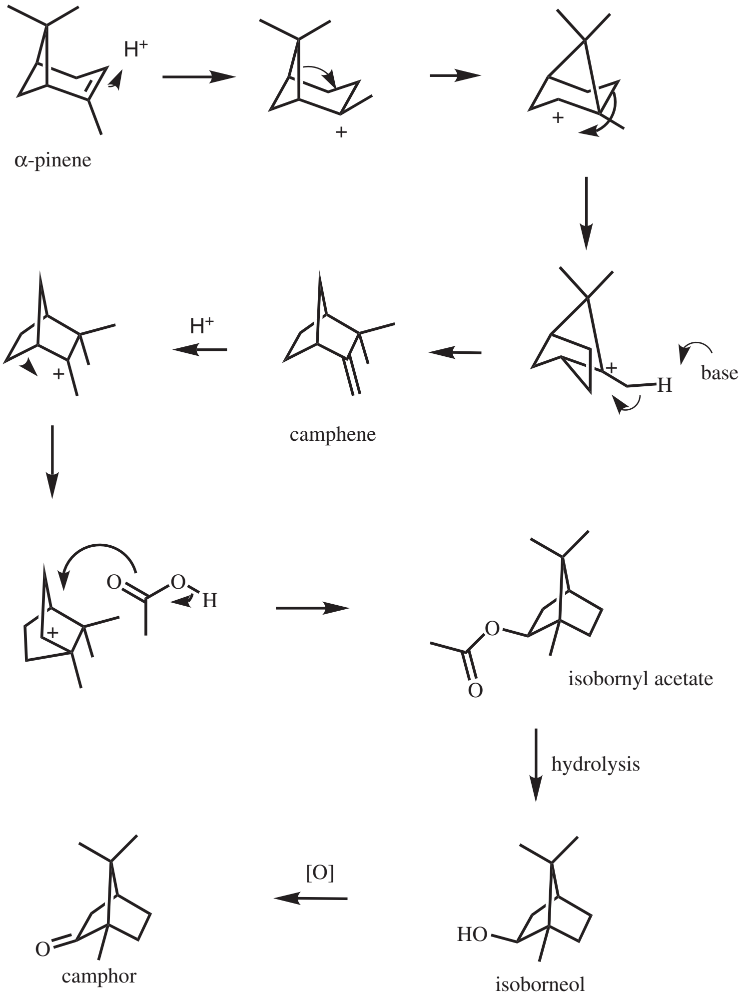


Figure 4.30 *Camphor from α-pinene*

β-pinene with formaldehyde gives nopol which is easily esterified to the acetate as shown in Figure 4.31.

4.2.16 Sesquiterpenoids

The Carroll, or an equivalent, reaction between linalool and an acetoacetic ester gives geranylacetone. Further elaboration as shown in Figure 4.32 produces sesquiterpenoids such as nerolidol, farnesol and bisabolol, which have some perfumery use although their odours are

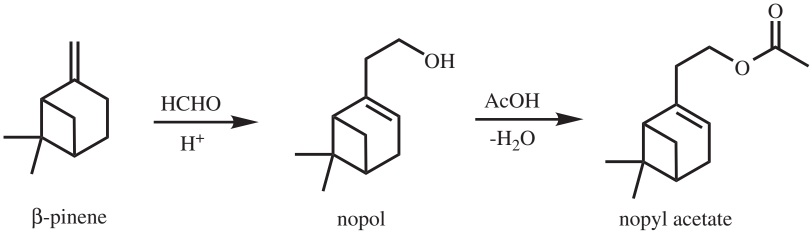


Figure 4.31 *Nopyl acetate from β-pinene*

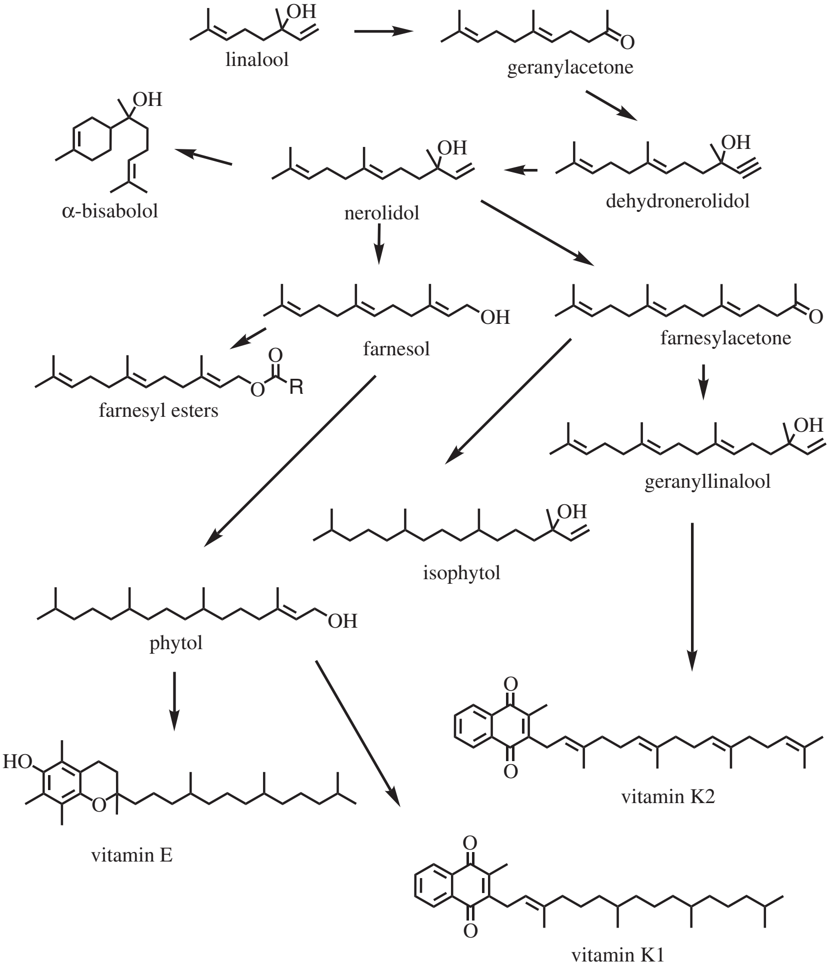
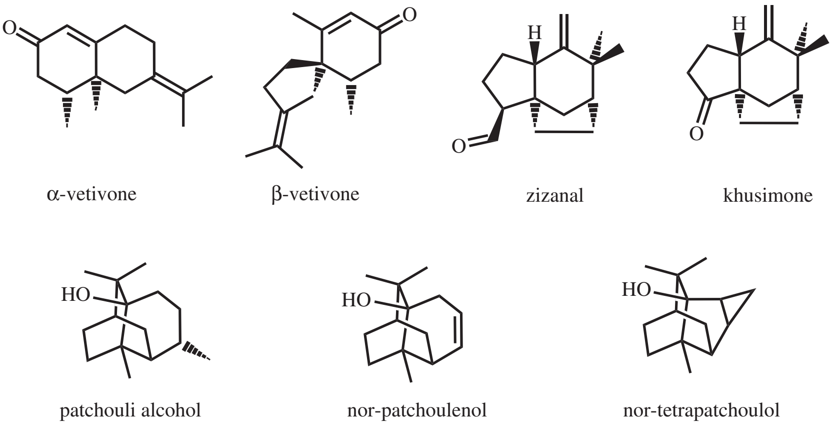


Figure 4.32 *Production of vitamins E and K*

relatively weak. Bisabolol is used mostly because of its anti-inflammatory and anti-bacterial properties. The main use for these materials is as intermediates for vitamins E and K.

The sesquiterpenoids that are important in odour terms mostly have complex cyclic structures. The problem of elimination of alcohols to produce olefins on attempted isolation is even more acute with sesquiterpenoids than with monoterpenoids because of their higher boiling points, which require more vigorous distillation conditions. The sesquiterpenoids responsible for the odours of vetiver and patchouli oils have complex structures which can only be reached by lengthy and hence uneconomic syntheses. Some of these components are shown in Figure 4.33. The vetivones are the major components of vetiver oil but contribute little to the odour. Minor components such as zizanal and khusimone are much more important. Similarly in patchouli oil, minor components such as nor-patchoulenol and nor-tetrapatchoulol are more important than the major constituent, patchouli alcohol.

Caryophyllene is the main hydrocarbon component of clove oil from which it is produced as a by-product of eugenol extraction. The endocyclic double bond of caryophyllene is highly strained and reacts readily with a variety of reagents. Usually both double bonds become involved in a *trans*-annular reaction, followed by rearrangements to give mixtures of polycyclic products. Some of these mixtures find use as woody ingredients in perfumes. Caryophyllene and two typical reaction products from it are shown in Figure 4.34.



α-vetivone β-vetivone zizanal khusimone

patchouli alcohol nor-patchoulenol nor-tetrapatchoulol

Figure 4.33 *Some odorous sesquiterpenes from vetiver and patchouli*

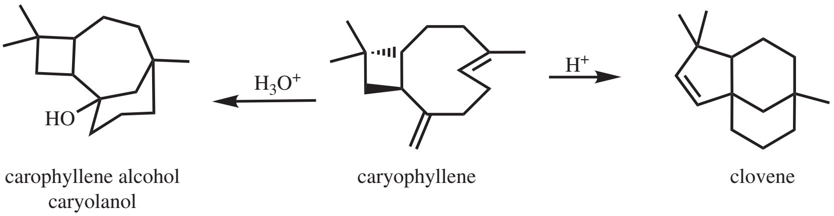


Figure 4.34 *Products from caryophyllene*

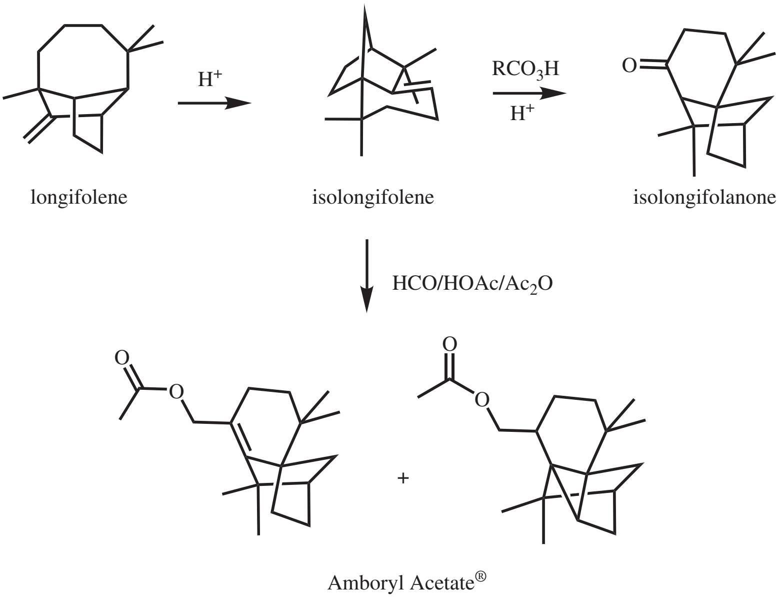


Figure 4.35 *Products from longifolene*

Longifolene is present in Indian turpentine, which is obtained from the species *Pinus longifolia.* This hydrocarbon also has a strained skeleton and treatment with acid causes rearrangement to *iso*longifolene. Treatment of this with a peracid under acidic catalysis gives isolongifolanone, which has a rich woody/amber odour. *Iso*longifolene can also be hydroformylated to give materials such as Amboryl Acetate®, which also has a woody/amber character. These reactions are shown in Figure 4.35.

4.2.17 Cedarwood

Two main families of cedarwood oils are used in perfumery. The first is extracted from trees of the family *Juniperus*. These oils are known as

English, Texan or Chinese Cedarwood and their components are derived from the cedrane and thujopsane groups of sesquiterpenoids. Atlas and Himalayan cedarwoods are obtained from *Cedrus* species and their terpenoids are mostly from the bisabolane family. The cedrane/thujopsane derivatives are much more widely used than the bisabolanes.

The major components of the *Cedrus* wood oils are cedrol, cedrene and thujopsene, and a number of perfume ingredients are made from these. The most important is acetylated cedarwood. This product is used because it possesses a much stronger cedarwood odour than the natural oil and is available under various trade names such as Lixetone® and Vertofix®. The acetylation can be carried out using acetyl chloride in the presence of a Lewis acid or by using a Bronsted acid system such as polyphosphoric acid/acetic anhydride or sulfuric acid/acetic anhydride. The cedrol dehydrates into cedrene under the reaction conditions and so the major component in the product is acetylcedrene. However, the main contributor to the odour of the complex reaction product mixture is the ketone derived from acetylation of thujopsene (Däniker *et al.*, 1972). These reactions are shown in Figure 4.36.

Sesquiterpenoid chemistry is always full of surprises. For example, an attempt to use titanic chloride in place of aluminium chloride to produce acetylcedrene resulted in a hitherto unknown compound (Mc Andrew *et al.*, 1983). In this case, as shown in Figure 4.37, the carbocation produced by addition of the acetyl cation to cedrene, instead of eliminating a proton as normal, underwent a Wagner–Meerwein rearrangement to a second cation, which was then trapped by *trans*-annular addition of the carbonyl group.

The methyl ether of cedrol is also used as a woody/amber note. The Atlas and Himalayan cedarwoods have a sweeter and less ambery woody smell than the materials described above. This odour is due to materials such as atlantone and deodarone which are shown in Figure 4.38.

4.2.18 Sandalwood

Sandalwood oil is obtained by dry distillation of the parasitic tree *Santalum album*. The major components of the oil are the santalols (see Figure 4.39). Many syntheses of these and related chemicals have been reported but, elegant as they are, none would compete economically with the oil itself. The synthetic sandalwood materials fall into two main classes: the so-called terpenophenols and the materials derived from campholenic aldehyde.

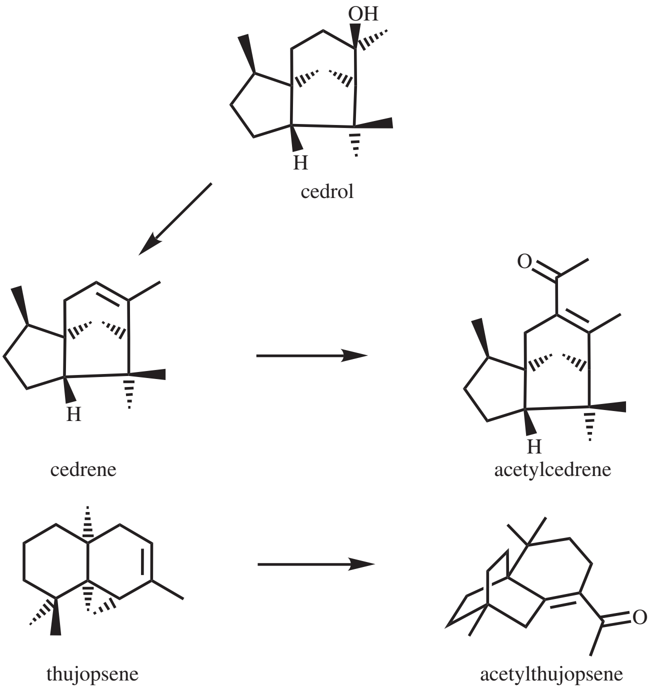


Figure 4.36 *Acetylation of cedarwood*

Addition of camphene or borneol to guaiacol gives a mixture of products. These vary in the nature of the terpenoid unit, since many rearrangements are possible, and in its position of attachment to the aromatic ring. Hydrogenation under high pressure produces an even more complex mixture. Hydrogenation leads to hydrogenolysis of one of the oxygen atoms and a variety of positional and conformational isomers of the resultant substituted cyclohexanol. In all, over 100 isomers are formed and only two are thought to contribute to the odour. These are those with an *iso*camphyl group attached to the 3-position of the cyclohexanol in a *trans* configuration relative to the hydroxy group as shown in Figure 4.40. The odour quality of the mixture depends on the exact balance of all of the components and there are many products on the market with trade names such as Sandela®, Sandel N®, Santalix®, Santalidol® and Indisan®.

Rearrangement of α-pinene oxide, catalysed by zinc chloride or bromide, gives a product known as α-campholenic aldehyde. Aldol condensation of this with a second aldehyde or a ketone gives an α, β-unsaturated carbonyl compound that can then be reduced to an

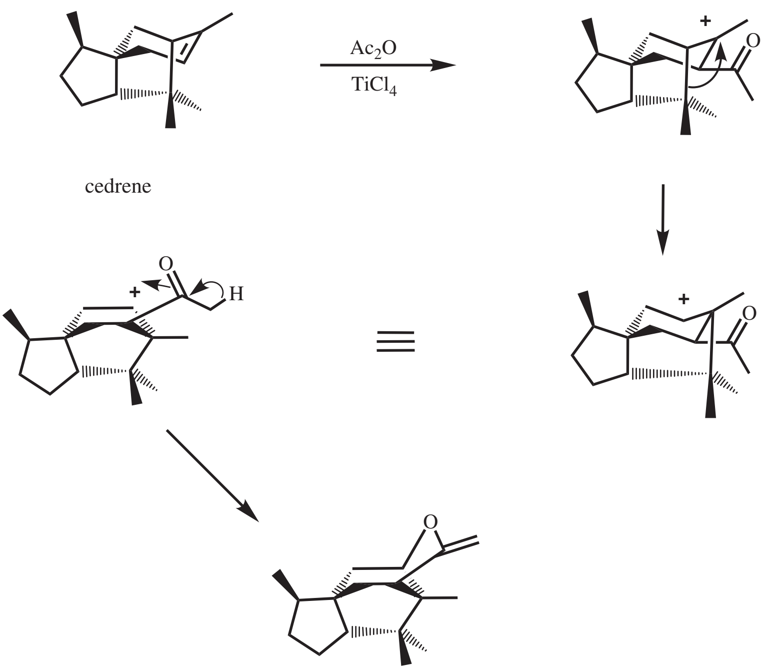


Figure 4.37 *Reaction of cedrene with titanic chloride*

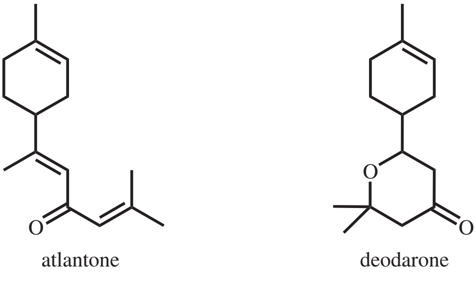


Figure 4.38 *Sesquiterpenoid ketones from cedarwood*

allylic alcohol. Many materials of this type are on the market, some of which have also undergone further modification. Some examples, with their trade names, are given with the reaction scheme in Figure 4.41.

One unusual structure for a synthetic sandalwood is that of Osyrol®, which is produced from dihydromyrcene as shown in Figure 4.42.

Figure 4.39 *Santalols*

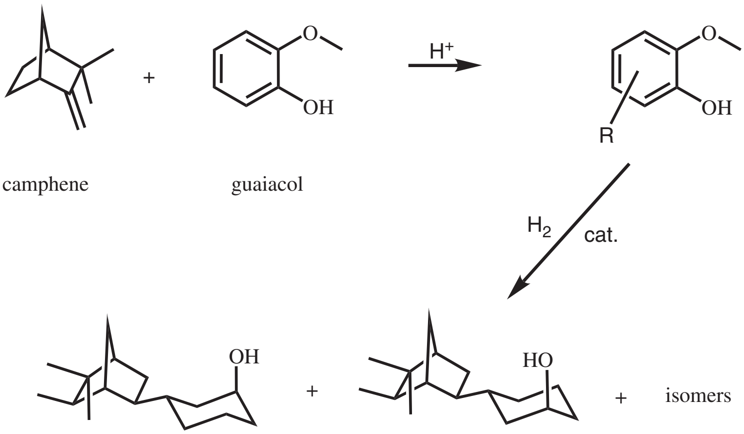


Figure 4.40 *Sandalwood terpenophenols*

4.2.19 Diterpenoids

Diterpenoids have, by definition, 20 carbon atoms in their structure. This means that very few are sufficiently volatile to possess an odour. One diterpenoid is used in perfumery because it and the derivatives concerned are odourless. That is, they are used as solvents. In view of their hydrophobicity and low volatility, these solvents also have fixative properties. Abietic acid is a major component of tall oil, the residue from distillation of turpentine. Esterification and hydrogenation produces two solvents as shown in Figure 4.43.

4.2.20 Ambergris

These last two sections deal with two very important groups of terpenoid fragrance ingredients which arise, in nature, from degradation of larger units.

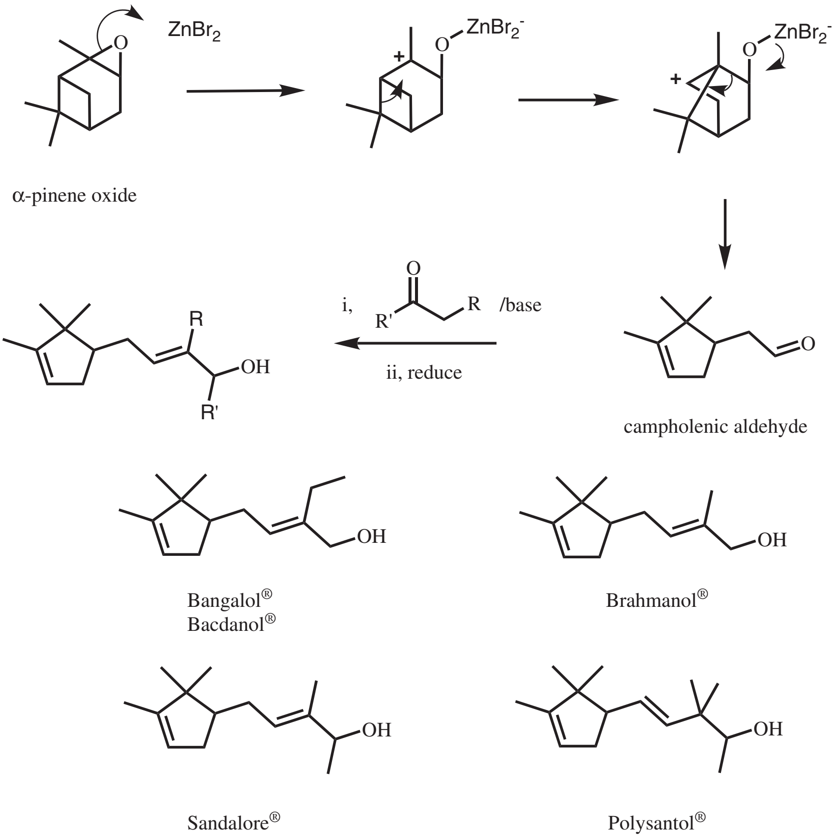


Figure 4.41 *Preparation and use of campholenic aldehyde*

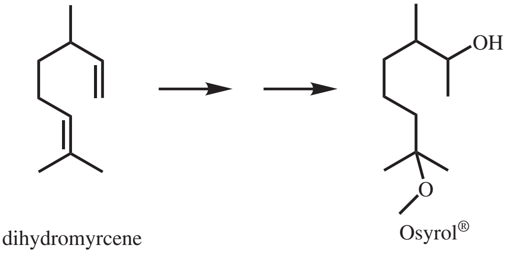


Figure 4.42 *Production of Osyrol®*

The sperm whale produces, in its intestinal tract, a triterpenoid called ambreine. The structure of this material is shown in Figure 4.44. It is not known exactly why the whale produces ambreine; it is possibly in response to some irritation. Lumps of ambreine, which can weigh up to

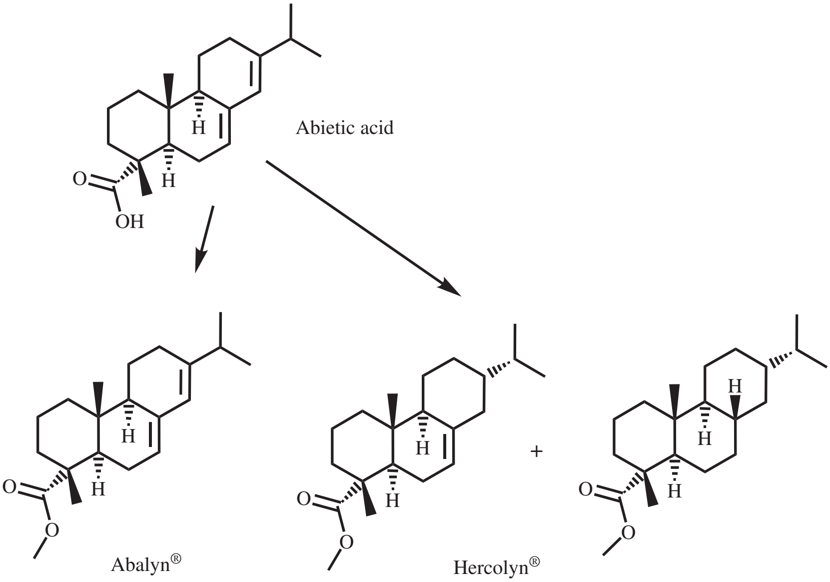


Figure 4.43 *Solvents from abietic acid*

100 kg, are excreted into the sea. There, in the presence of salt water, air and sunlight, the ambreine undergoes a variety of degradation reactions to produce a complex mixture of breakdown products. The mixture is known as ambergris, from the French *ambre gris* – grey amber. This name arises because ambergris is found washed up on beaches, as is the case with amber (*ambre brun* – brown amber); the fossilised resin which ambergris resembles to some extent. Some of the more organoleptically important of the degradation products are shown in Figure 4.44. The most important of all is the perhydronaphthofuran, which possesses the characteristic animalic note of ambergris.

Ambergris has always been very expensive and the decline in the whale population has exacerbated the situation. The price and availability of the natural material essentially preclude its use in fragrance and a lot of work has been done on synthetic substitutes. The naphthofuran is prepared from sclareol, a diterpenoid found in clary sage (*Salvia sclarea*). Clary sage oil is used in perfumery and sclareol is extracted from the distillation residues. Oxidative degradation of sclareol gives the lactone sclareolide as shown, by two different routes, in Figure 4.45. Reduction of the lactone with lithium aluminium hydride or borane or some similar reagent gives a diol, which can be cyclised to the furan. This is known

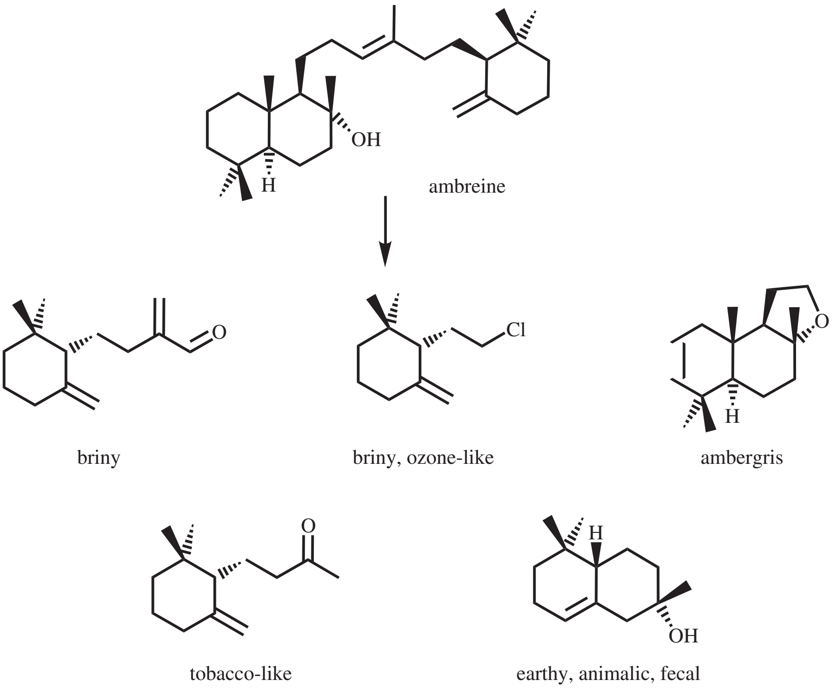


Figure 4.44 *Ambergris components*

commercially under trade names such as Amberlyn®, Ambrox® and Ambroxan®. In addition to the nature identical materials, there are a number of synthetic ambergris substitutes on the market such as Karanal®, which is described in Section 4.8.

4.2.21 Ionones and Related Compounds

This group of terpenoid materials are derived in nature from degradation of carotenoids. The major products are known as ionones and are synthesised from citral by aldol condensation with a ketone to form what are known as ψ-ionones, which are then cyclised using an acid catalyst, as shown in Figure 4.46. Some specific syntheses are included also in Figure 4.49, along with the syntheses of vitamin A and carotene. The ionones possess odours which are reminiscent of violet, sometimes also with woody notes.

The nomenclature system used for ionones is also shown in Figure 4.46. The Greek letters α-, β- and γ- refer to the position of the double

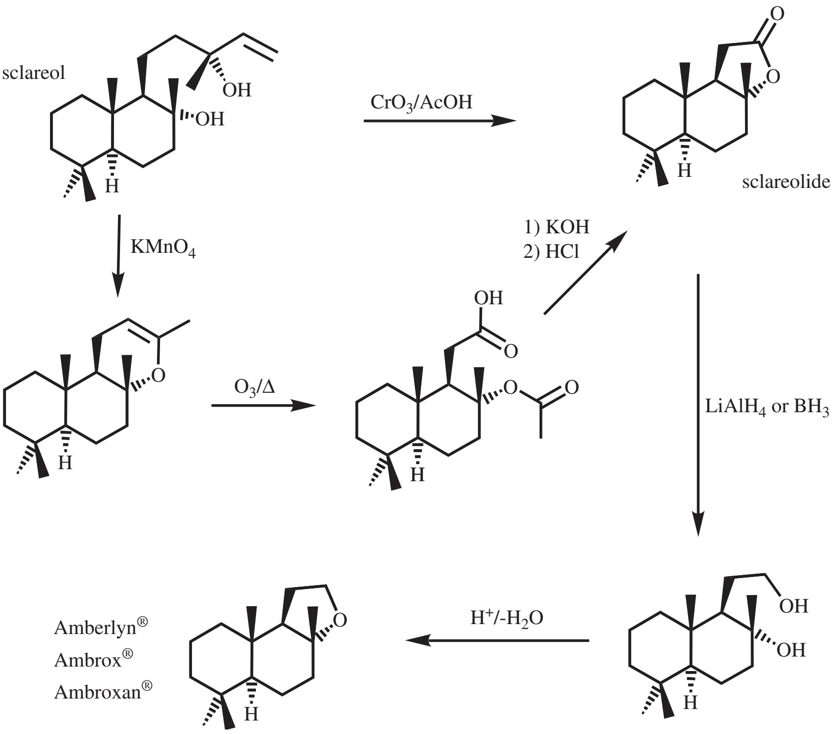


Figure 4.45 *Ambergris naphthofuran from sclareol*

bond after ring closure. The prefixes *n*- and *iso*- refer to the position of the alkyl group if a higher ketone than acetone is used in the aldol condensation. For example, the ionone, which is produced in greatest quantity, and which is also shown in Figure 4.46, is known as α-*iso*-methylionone. (It should be noted that some older texts erroneously refer to this material as γ-ionone.) With any given feedstock, the reaction conditions can be manipulated to produce a desired isomer predominantly. It is important to maintain strict control of process and distillation conditions in order to ensure reproducible odour quality. Quite a range of ionone derivatives are used in perfumery, each having its own particular odour character.

The damascones are a group of materials related to the ionones, but in which the enone unit is transposed. They are components of rose oils and have very intense fruity/floral odours. The damascenones, which contain a second double bond in the ring, are also very much sought after. One synthesis of α-damascone from methyl α-cyclogeranate is shown in Figure 4.47. A double Grignard addition gives the tertiary alcohol.

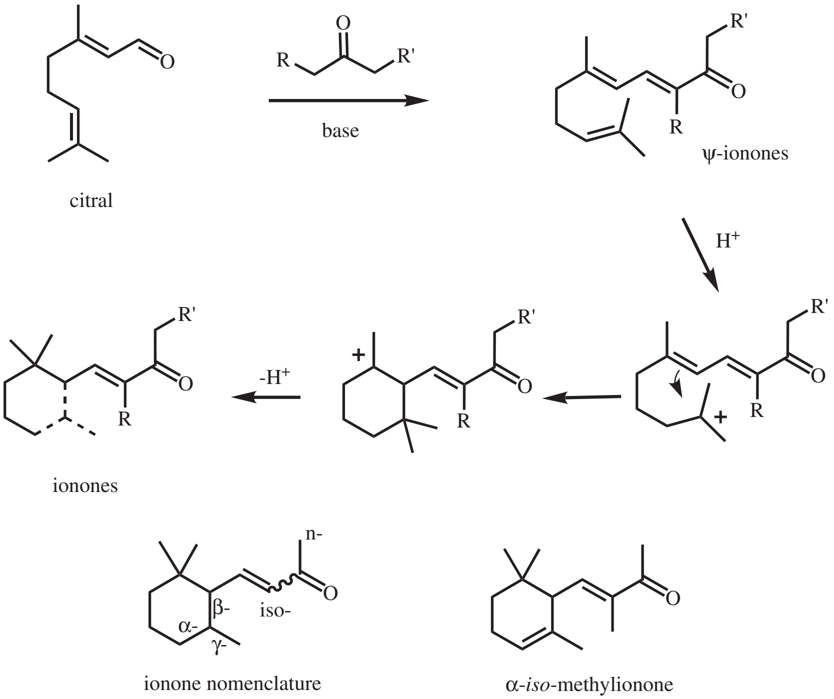


Figure 4.46 *Ionones, synthesis and nomenclature*

Heating of this in the presence of base, to prevent elimination of the alcohol, gives the ketone *via* an *ene* reaction. The prefixes α-, β- and γ- have the same meaning in the damascone series as in the ionones.

The irones are higher analogues of ionones, in that they contain an additional methyl group in the cyclohexane ring. They are components of orris and have odours reminiscent of that source. One example of a synthesis of an irone, starting from pinene, is given in Figure 4.48. Decarbonylation of the ozonolysis product of α-pinene gives a methyl trimethylcyclobutyl ketone. Addition of acetylene to this, followed by the Carroll reaction, gives (trimethylcyclobutyl)heptadienone. Pyrolysis of this opens the cyclobutane ring to give a methylated ψ-ionone, which, on cyclisation, produces α-irone. Once again, the Greek prefixes have the same significance as in the ionone series.

It is obvious that the syntheses of the damascones and irones are more difficult than those of the ionones, with the inevitable result that these are much more expensive products.

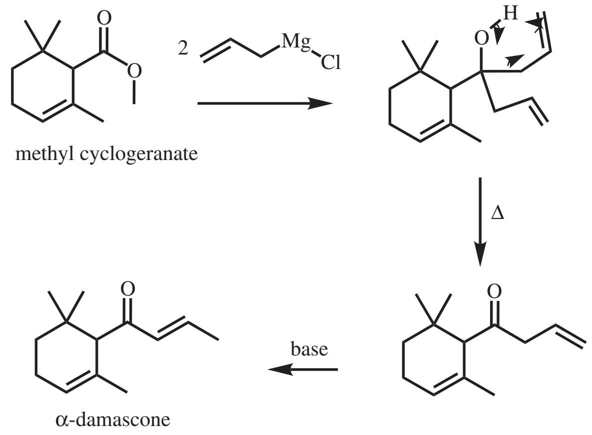


Figure 4.47 *Production of α-damascone*

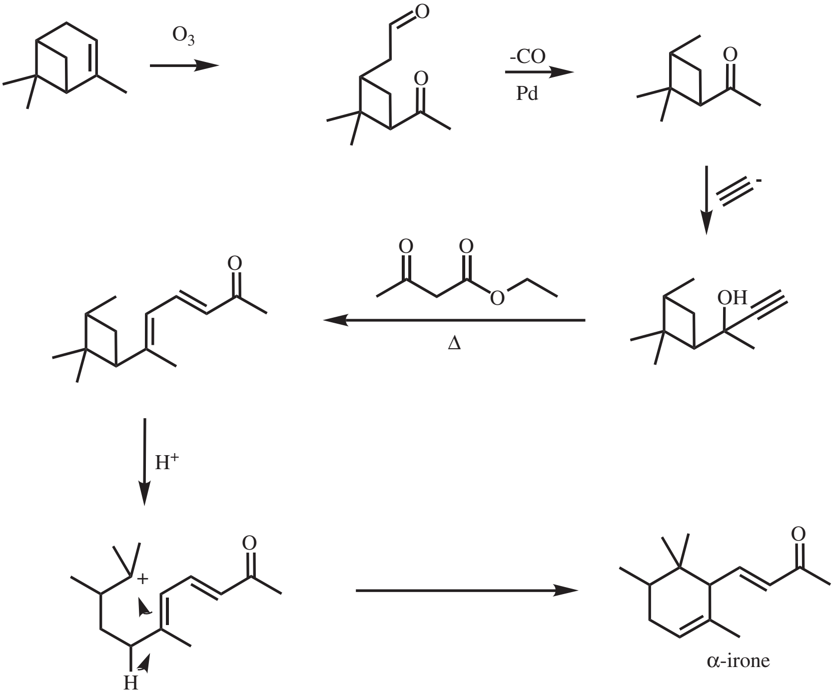


Figure 4.48 *Synthesis of α-irone*

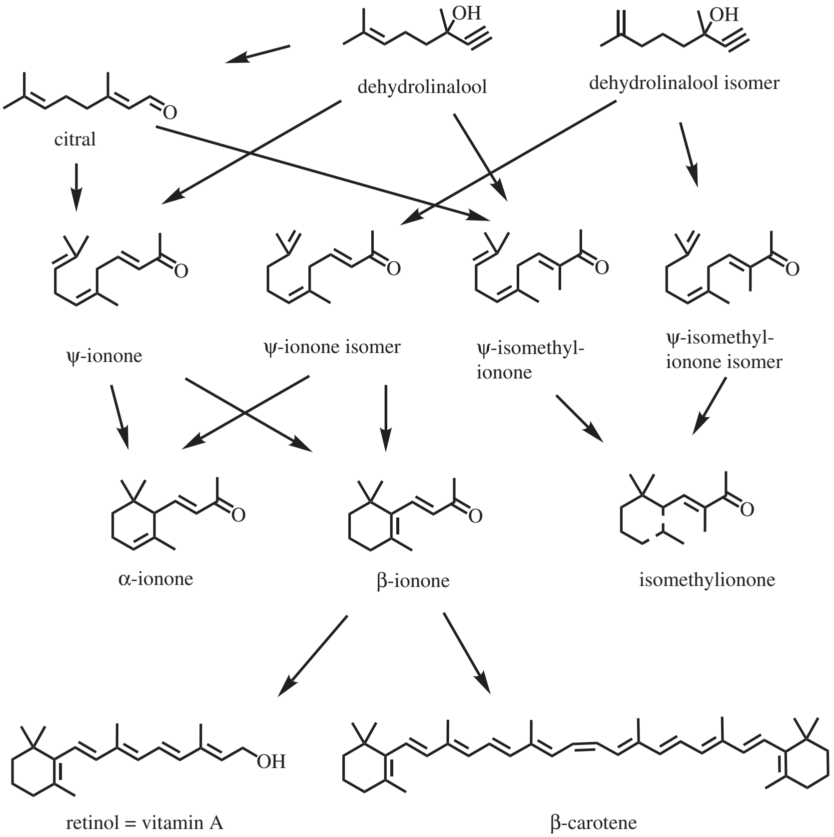
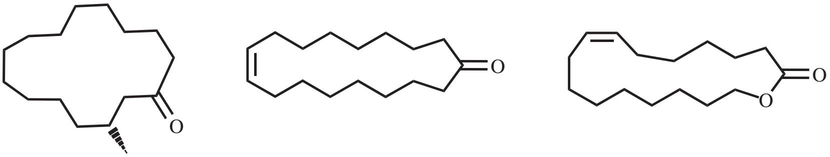


Figure 4.49 *Production routes for ionones and vitamin A*

The economics of ionones production is affected by the fact that ionone is an intermediate for vitamin A and carotene production. However, the volumes of production of ionones are not far from those of the vitamin A and so, while some companies produce both, others can compete effectively although they are only active in one. Figure 4.49 shows typical routes used to produce vitamin A and β-carotene from the various monoterpene precursors.

4.3 MUSKS

The original musk components of perfumes were extracted from animal sources. Nowadays, such sources are obsolete on both ethical and economical grounds. The two major ones were musk and civet, which were



muscone civetone ambrettolide

Figure 4.50 *Natural musk components*

extracted from the anal glands of the musk deer and the civet cat, respectively. In both cases, the extracts have a strong animalic character arising from compounds such as indole and skatole (2-methylindole). The musk deer (*Moschus moschiferus*) is found in central Asia and natural musk was often called musk Tonkin, to denote the region of its origin. The civet cat (*Viverra civetta*) is found in Africa and Asia. Somehow, early perfumers recognised that under this animalic, and not entirely pleasant, note, lay a rich, sweet character, which serves not only as a perfume component in its own right, but also to 'fix' other notes. Fixation is a property of some perfume components, usually the higher boiling ones, which enables them to fix or hold back the more volatile notes so that they do not evaporate so quickly. The fixatives are therefore important in blending all of the perfume components so that the character does not change rapidly as each ingredient evaporates in turn. The structures of the three key natural musks are shown in Figure 4.50. Muscone and civetone are the most important odour components of musk and civet, respectively. Ambrettolide is a plant product, occurring in the seeds of the ambrette plant (*Abelmoschus moschatus* Moench, syn. *Hibiscus abelmoschus*), which is cultivated in Madagascar, the Seychelles, Colombia and Equador.

The natural musks were always very expensive and their macrocyclic structures presented synthetic challenges, which were not conquered, even on laboratory scale, until the pioneering work of Ruzika (1926). It was therefore of major importance to the fragrance industry when, in 1888, Baur discovered the nitromusks. He had actually been working on explosives and noticed that the product of *t*-butylation of trinitrotoluene (TNT) had a pleasant, sweet, musky odour. The compound was named Musk Baur, although the alternative name, Musk Toluene, eventually became more common. For a while it was also known as Tonkinol, because of the similarity of its odour to that of musk Tonkin. Baur then searched for analogues of this material and discovered Musk Xylene,

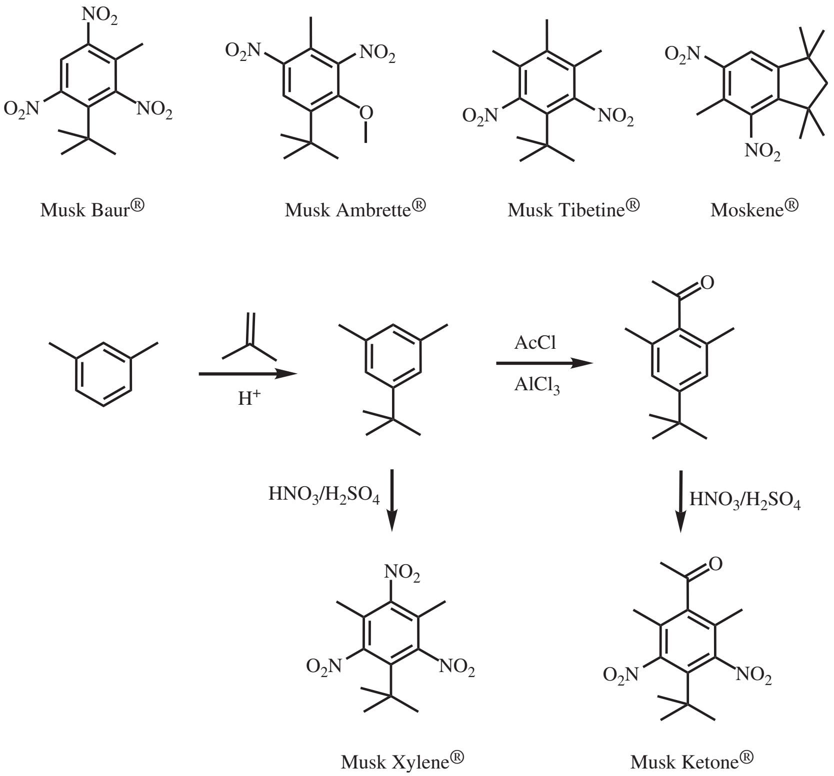
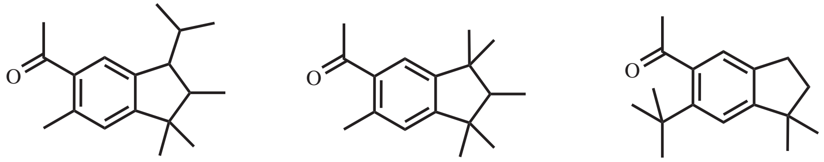


Figure 4.51 *Nitromusks*

Musk Ketone and Musk Ambrette. Musk Ketone is considered to have the closest odour to that of natural musk, and Musk Ambrette, as its name implies, has an odour reminiscent of ambrette seeds. Other nitromusks, which were discovered later, include Musk Tibetine and Moskene. The structures of these, together with the synthetic routes to Musk Xylene and Musk Ketone, are shown in Figure 4.51. Both of these are prepared from *m*-xylene through initial *t*-butylation. Nitration of the *t*-butyldimethylbenzene thus produced gives Musk Xylene, and acetylation followed by nitration gives Musk Ketone. The other nitromusks are prepared by similar combinations of classical aromatic reactions.

The nitromusks became the main contributors of musk notes in perfumery and maintained that position until the middle of the twentieth century. However, nitromusks suffer from a number of disadvantages and the discovery of the polycyclic musks in the middle of the twentieth



Traseolide®

Phantolide®

Celestolide®
or
Crysolide®

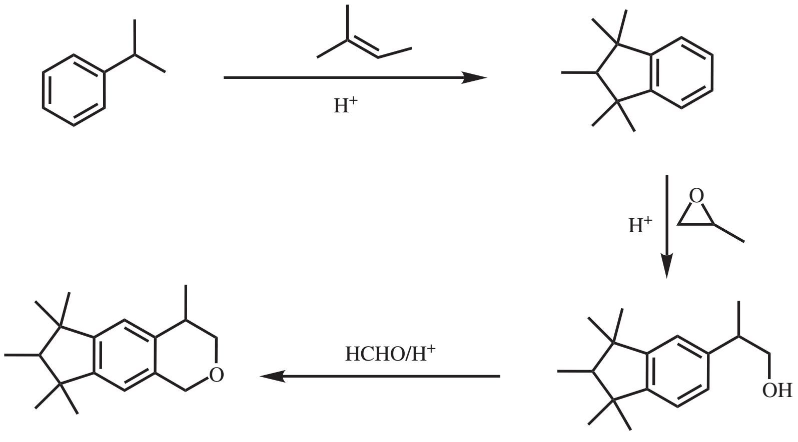
Figure 4.52 *Polycyclic musks*

century led to their demise. The preparation of nitromusks can be hazardous, after all they are related to explosives such as TNT and, although the final products are not explosive, some of the intermediates and reagents are not free of hazard. Moreover, some nitromusks have been found to be phototoxic; that is, when exposed to sunlight, they produce species, which can cause allergic reactions on skin. The combined effect of all of these problems, coupled with the ready availability of good alternatives, has made the nitromusk family obsolete.

In the 1950s, a new class of musks was discovered, the polycyclic musks. These materials mostly have indane, tetralin or isochroman structures, heavily substituted by methyl or other small alkyl groups. Typical members of this family are shown in Figure 4.52.

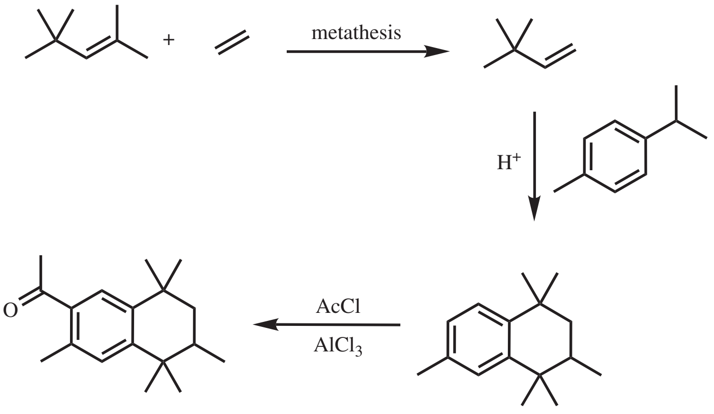
The most important polycyclic musks, in commercial terms, are 6-acetyl-1,1,2,4,4,7-hexamethyltetralin and 4,6,6,7,8-hexamethyl-1,3,4, 6,7,8-hexahydrocyclopenta[*g*]isochromene. These two compounds are each known by a variety of trade names, depending on the manufacturer. The commonest trade names for the tetralin are Fixolide®, Tetralide ® and Tonalide® and for the isochroman, Abbalide® and Galaxolide®. Both fragrance materials were of major importance to the perfume industry throughout the second half of the twentieth century though their use is falling nowadays as new routes to macrocyclic materials are making that class of material more readily accessible. It is worthwhile looking in more detail at the chemistry of the syntheses of Galaxolide® and Tonalide®. The synthesis of Galaxolide® is shown in Figure 4.53.

Cumene is prepared on a large scale from benzene and propylene as an intermediate in the synthesis of acetone and phenol. This makes it an inexpensive and readily available starting material for the production of Galaxolide. Three further electrophilic addition reactions complete the synthesis. First, *iso*amylene is added to form pentamethylindane to



Galaxolide®

Figure 4.53 *Production of Galaxolide®*



Tonalide®

Figure 4.54 *Production of Tonalide®*

which propylene oxide is added. Finally, treatment with formaldehyde leads, *via* the hemiacetal, to the isochroman.

The synthesis of Tonalide® is shown in Figure 4.54. The starting materials, as for Galaxolide®, are all inexpensive and readily available chemicals. *p*-Cymene is a terpene and is found as a by-product in many processes which involve heating of terpenes since it lies at the bottom of a potential energy well. Further details are given in the section on

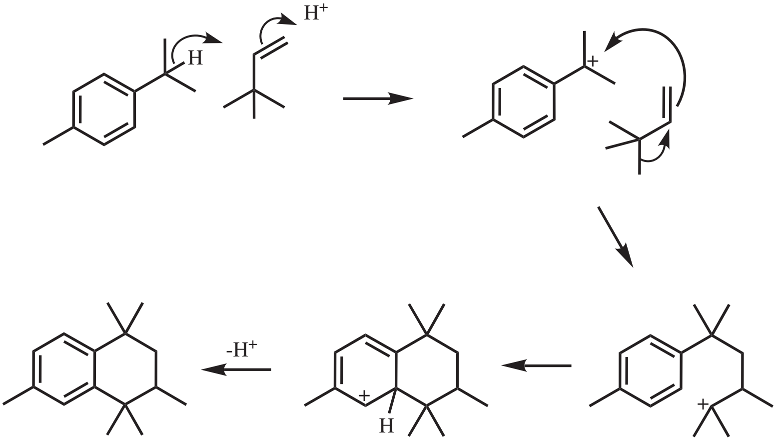


Figure 4.55 *Formation of hexamethyltetralin*

terpenes. Dimerisation of butylene gives di-*iso*-butylene, which undergoes olefin metathesis with ethylene to give *neo*-hexene. The Friedel–Crafts reaction between *p*-cymene and *neo*-hexene gives hexamethyltetralin, which can then be acetylated to produce Tonalide®. The cyclialkylation stage in this sequence is interesting and more detail of the mechanism is shown in Figure 4.55. As shown in Figure 4.55, the reaction requires two moles of *neo*-hexene per mole of *p*-cymene. Half of the olefin serves as a hydride abstraction agent (*i.e.* an oxidant) and the other half as the alkylating species. Protonation of the olefin generates a carbocation. This abstracts a hydride ion from the *p*-cymene to give the more thermodynamically stable *p*-cymyl cation. The latter then adds to a second molecule of *neo*-hexene. This addition occurs with a concomitant methyl shift, so that the next cation to be formed is tertiary rather than secondary and hence somewhat more stable. This product cation can then add to the aromatic ring to give the hexamethyltetralin. *Neo*-hexene is by far the more expensive of the two starting materials, and so the scheme shown in Figure 4.55 is unsatisfactory since it consumes twice as much of this reagent as is desirable. One way of overcoming this problem is to add a sacrificial oxidant, which is less expensive than *neo*-hexene. For example, *tert*-butyl chloride will, under the influence of aluminium chloride, lose its chlorine to produce the *tert*-butyl cation, which can abstract the hydride ion from *p*-cymene and thus save one molar equivalent of *neo*-hexene. An alternative solution is shown in Figure 4.56.

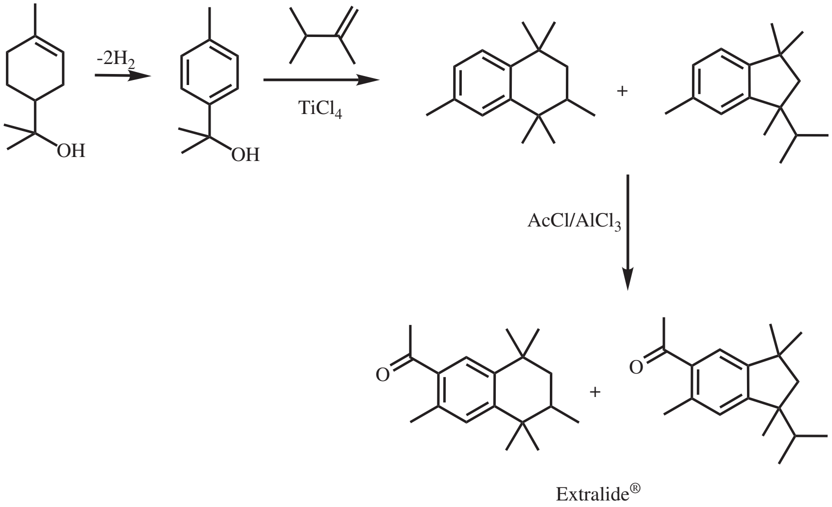


Figure 4.56 *Production of Extralide®*

Figure 4.56 shows the Quest synthesis of a musk mixture known under the trade name Extralide®. In this synthesis, no oxidation is necessary in the cyclialkylation stage because the appropriate carbon atom of the *p*-cymenyl structure is already at the correct oxidation level since it carries a hydroxyl group. This is achieved by dehydrogenation of α-terpineol to give *p*-cymenol. Reaction of this with 2,3-dimethylbut-1-ene, as an alternative to *neo*-hexene, gives a mixture of hexamethyltetralin and *iso*propyltetramethylindane. Acylation of this mixture gives a mixture of two musks. This mixture performs almost identically with pure Tonalid® in perfumes and has the advantage of a lower melting point, which makes dissolution in perfumes easier.

The problem in the synthesis of macrocyclic musks lies with entropy. The simplest approach to building a large ring is to make a long chain with functionality at each end such that the two ends of the chain can react to close the ring through the formation of a new carbon – carbon bond. However, entropy dictates that the likelihood of the two ends of chain meeting is lower than that of one end reacting with the end of another chain. Repetition of this process leads to polymerisation. Thus, for example, if 15-hydroxypentadecanoic acid is subjected to esterification conditions, the product will be a polyester rather than the musk cyclopentadecanolide. In the 1920s, Ruzicka solved this problem through the use of extremely high dilution, which reduces the chance of intermolecular reaction and therefore gives the functional groups at each end of the reagent molecule time to find each other and undergo intramolecular reaction. The high dilution principle works well on laboratory scale, but it is not satisfactory as a manufacturing process because of the poor reactor utilisation and the need to recover and recycle very large volumes of solvent. Both of these features lead to unacceptably high process costs.

Many methods have been developed in an attempt to overcome the problem posed by entropy in the formation of large rings. Inclusion of unsaturation in the substrate reduces the number of degrees of freedom in it and consequently increases the possibility of the two ends of the chain meeting. In the acyloin reaction, electrostatic attraction is used to restrict movement of the alkyl chain. A dicarboxylic acid is added to a suspension of sodium (or another alkali metal) particles in an inert solvent. The acid functions react with the metal to form carboxylate anions, which are then held to the positive surface of the metal by electrostatic attraction. This means that the chain becomes a loop, 'fastened' to the metal particle at each end until the two carboxylate groups approach close enough to allow the acyloin reaction to take place between them.

Another method was developed by Caruthers in the 1930s. He began by preparing polyesters from hydroxy acids or from mixtures of dicarboxylic acids and diols. He then depolymerised these to give monocyclic lactones. The lactones are much more volatile than the oligomers and polymers in the mixture and so can be separated from it by distillation. Obviously, this steady removal of the lactones also helps to force the equilibrium in the desired direction. The dicarboxylic acids are available by oxidation of unsaturated fatty acids or cyclic olefins or ketones. For example, ozonolysis of erucic acid, the major fatty acid of oilseed rape, gives brassylic acid (tridecane dicarboxylic acid). His first method of depolymerisation was to heat the polymer to a high temperature and allow the chains to bite back on themselves and thereby the volatile lactones to be removed by distillation from the mixture. He later developed the useful technique of using high-boiling alcoholic solvents to achieve the depolymerisation. The main alcohol used was glycerol. Glycerol serves two purposes in the depolymerisation. First, it provides hydroxy groups to help keep the interesterification equilibrium reactions in progress. Second, the boiling point of glycerol is in the same range as those of lactones in the 15–18 carbon range. By maintaining the system under reflux of glycerol, removal of the lactones by distillation is made more efficient. Furthermore, the lactones are only poorly soluble in liquid glycerol and the distillate readily separates

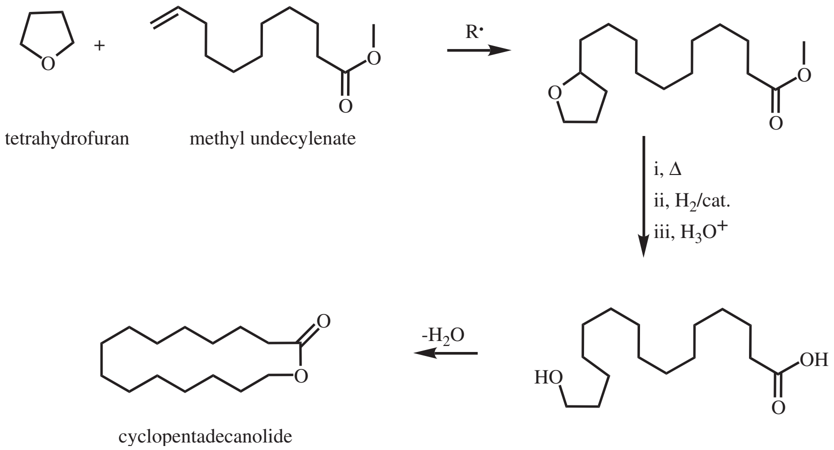


Figure 4.57 *Production of cyclopentadecanolide*

into two phases, making removal of the lactones easy through use of a Dean – Stark trap. Calcium oxide or hydroxide is usually employed as the catalyst in these reactions. Unfortunately, glycerol is not very stable under the conditions and much of it is lost, adding to costs in terms of both materials used and waste disposal. Ethylene brassylate is prepared by this type of process from brassylic acid and ethylene glycol, as is shown later. The low cost of the starting materials and the simplicity of the process make this the least expensive of the macrocylic musks at present and therefore the one which is used in the highest tonnage.

The second largest macrocyclic musk, in tonnage terms, is cyclopentadecanolide. Its synthesis is shown in Figure 4.57. The key starting material is methyl undecylenate, which is obtained from castor oil by pyrolysis and esterification. Exposure of tetrahydrofuran to a radical initiator generates the radical at a carbon next to the oxygen atom. This radical then adds to the terminal double bond of methyl undecylenate and the radical produced then abstracts a hydrogen atom from another tetrahydrofuran and so propagates the radical chain reaction. The methyl 11-(2′-tetrahydrofuryl)undecanoate thus produced is then subjected to elimination, hydrogenation and hydrolysis to give 15-hydroxypentadecanoic acid. Polymerisation/depolymerisation then gives cyclopentadecanolide.

In 1959, Wilke discovered that butadiene could be trimerised round a metal template to give cyclododecatriene. This could be converted into the mono-olefin, the ketone, the alcohol, *etc.* by obvious means.

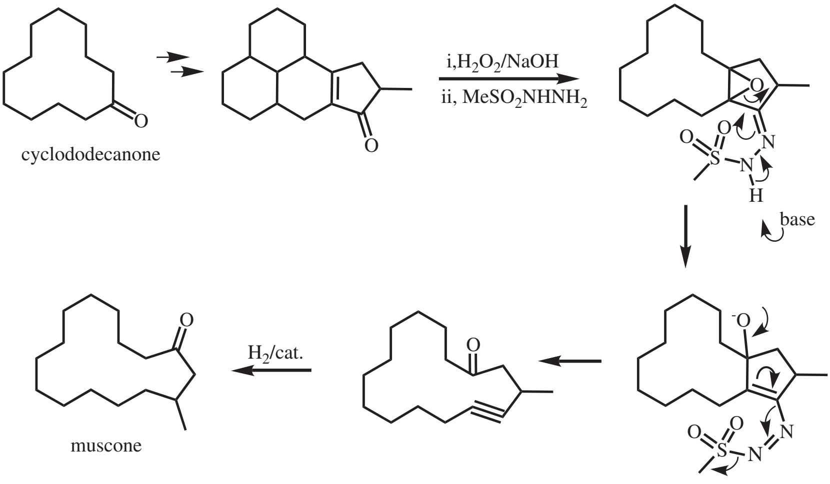


Figure 4.58 *Synthesis of muscone*

The ready availability of an inexpensive supply of cyclododecane derivatives set in train a new direction in musk research. The 12-carbon ring could be broken open, with or without addition of a side chain, to provide new linear precursors for macrocyclisation. Furthermore, the ring could be enlarged by fusing a second ring to it, and then breaking the bridgehead bond to produce a larger ring. This latter option offers an elegant means of overcoming the entropic problem of macrocyclisation. An example of this type of approach is shown in Figure 4.58.

Eschenmoser's synthesis of muscone shown in Figure 4.58 uses cyclododecanone as the starting material and employs the Eschenmoser fragmentation reaction in the key bridgehead-breaking step. First, a methacrylate unit is fused to the 12-membered ring using conventional anionic chemistry. This leads to the bicyclic ketone, which can be converted to the epoxide with alkaline hydrogen peroxide. Addition of methane sulfonylhydrazine gives the hydrazone. When treated with base, the hydrogen attached to the nitrogen atom is lost and the resultant negative charge flows through the molecule to spring open the epoxide ring. The negative charge can then flow back across the bridgehead by a different route, and the molecule fragments losing nitrogen. It is the energy gain in forming free nitrogen that drives this reaction. The acetylenic ketone produced is easily hydrogenated to produce muscone. Routes such as this are very elegant examples of chemical synthesis,

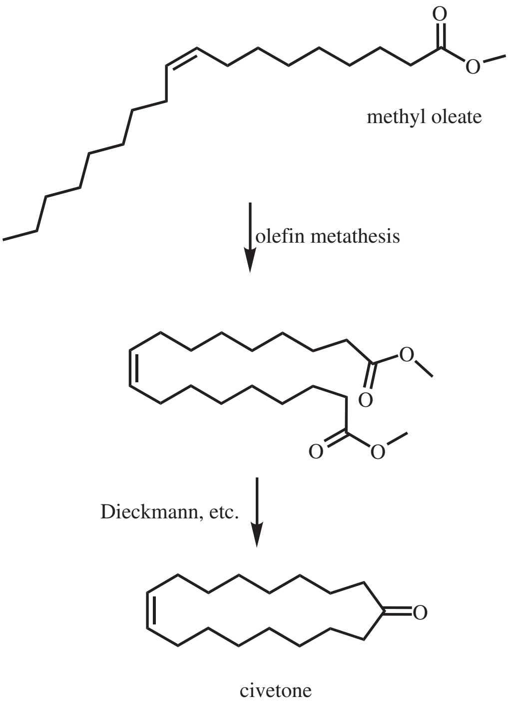


Figure 4.59 *Synthesis of civetone*

but they are multi-step and therefore attract high process costs when operated on an industrial scale.

Olefin metathesis has been used by a number of workers to produce macrocyclic musks. One of the earliest examples is Mol's synthesis of civetone from methyl oleate. This takes advantage of the ideally placed double bond in the starting oleic acid. Unfortunately, the *cis*-geometry of the olefin is lost on metathesis and a mixture of isomers results. Furthermore, almost half of the weight of the starting material is lost as the unwanted 9-octadecene. Dieckmann cyclisation of the metathetical diester, followed by hydrolysis and decarboxylation, gives a mixture of *E*- and *Z*-civetone. The synthesis is shown in Figure 4.59.

It is possible to form rings from long chain di-olefins but, as with esterification of hydroxy acids, high dilution conditions must be employed. An example (Jap. Patent, 2003) is the formation of the lactone analogue of muscone shown in Figure 4.60. This synthesis takes advantage of the ready availability of methyl undecylenate from castor oil.

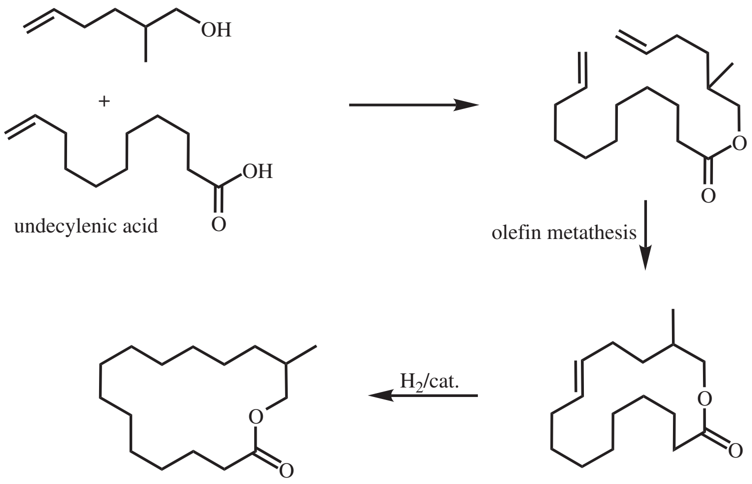


Figure 4.60 *Ring closing metathesis in macrolide formation*

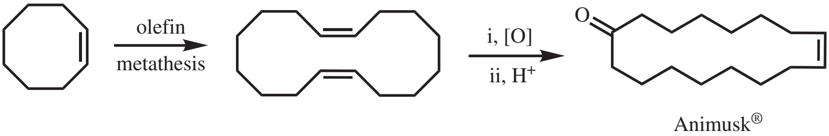


Figure 4.61 *Production of Animusk®*

One elegant use of metathesis, which avoids both loss of material and the need for high dilution, is the synthesis of a novel musk material called Animusk® by the German company Wacker. Cyclooctene is available from dimerisation of butadiene followed by partial hydrogenation. Self-metathesis gives cyclohexadecadiene. Epoxidation of one of the double bonds of the latter, followed by rearrangement of the epoxide, gives Animusk®, as shown in Figure 4.61.

At present, there are still no macrocyclic musks that are available in the same cost bracket as the polycyclic musks although the gap is closing. Consequently, the fragrance industry still carries out a great deal of research into methods of producing macrocyclic musks.

4.4 PERFUME INGREDIENTS DERIVED FROM BENZENE

One of the most important perfume ingredients made from benzene is 2-phenylethanol. The synthesis of this, and related compounds, is shown in Figure 4.62. 2-Phenylethanol is a major component of rose oils and is widely used in perfumery for its blending qualities. In tonnage terms, it is one of the most important of all perfumery ingredients. The original production method involved Friedel–Crafts addition of ethylene oxide to benzene. This is fairly efficient despite some addition of ethylene oxide to the product to give a small amount of polyethoxylated derivatives. The major disadvantage of this route is the safety issue relating to handling of ethylene oxide and benzene. Both reagents can be handled safely, but the engineering required to do so adds to the process costs. Another possibility is to hydrogenate styrene oxide. The production of styrene from benzene is described below. This route gives a very high quality product and the intermediate, styrene oxide, can also be used to produce other fragrance materials. Rearrangement of the epoxide gives phenylacetaldehyde, which has a potent green note. Phenylacetaldehyde is responsible for the characteristic green top-note of narcissus and is used to create a narcissus-like effect in floral perfumes. Addition of alcohols to styrene oxide gives the corresponding acetals of phenylacetaldehyde. The most important of these is the dimethyl acetal, known in the industry by the acronym PADMA. This material has much of the green character of the aldehyde but is more stable chemically. Many esters of 2-phenylethanol are used in perfumery, the acetate, *iso*butyrate and phenylacetate, in particular. These are prepared by esterification of the alcohol (Figure 4.62).

Figure 4.63 shows the process known to the bulk chemicals industry as SMPO, the styrene monomer/propylene oxide process. Styrene is used in polymers, and propylene oxide derivatives have a wide variety of uses including as surfactants and anti-freeze. For the bulk industry, the process is as follows. Addition of ethylene to benzene gives ethylbenzene, which undergoes air oxidation to give the hydroperoxide. Reaction of this with propylene, in the presence of a suitable catalyst, gives styrallyl alcohol and propylene oxide. Styrallyl alcohol is readily dehydrated to styrene. Perfumery interest in this process is fourfold. First, one of the major products, styrene, is a starting material for perfume ingredients, as described above. Second, the other major product, propylene oxide, is also a precursor for a number of fragrance materials and for dipropylene glycol, one of the major solvents used in perfumes. Third, the intermediate, styrallyl alcohol, is the starting material for a number of esters used in perfumery, the acetate in particular. Fourth, but by no means least, the crude styrallyl alcohol contains a small trace of 2-phenylethanol. Since the SMPO process is run on a scale far beyond that of the perfume

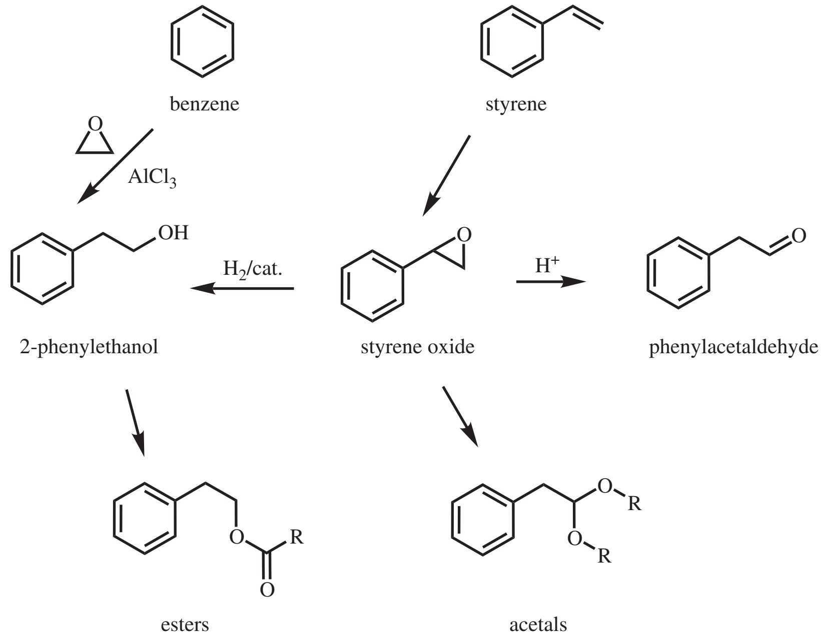


Figure 4.62 *Route to 2-phenylethanol and derivatives*

industry, what is a small trace to the polymer business represents a very significant proportion of the world requirement for 2-phenylethanol as a perfume ingredient. The problem is that of odour quality. It is extremely difficult to purify this by-product 2-phenylethanol to the odour quality of that produced by either of the above routes. The major difficulty lies in removal of traces of 2-ethylphenol which is another trace component in the crude styrallyl alcohol and one which has a very intense odour. However, most of the companies, such as ARCO in the USA and Sumitomo in Japan, who run the SMPO process can produce 2-phenylethanol of a quality which can be used in perfumery (often in collaboration with a perfumery company). The amount of 2-phenylethanol available from this route is dictated by the demand for styrene and propylene oxide, the market value is dictated largely by material from the other two routes and all three run in economic balance.

Figure 4.64 shows the preparation of the hydrocinnamic aldehydes, another family of materials derived from benzene and which possess fresh, white-floral notes reminiscent of muguet (lily of the valley) and cyclamen. One of these, known as Lilial®, Lilistralis® or lily aldehyde, can also be prepared from toluene and that route is also included in this

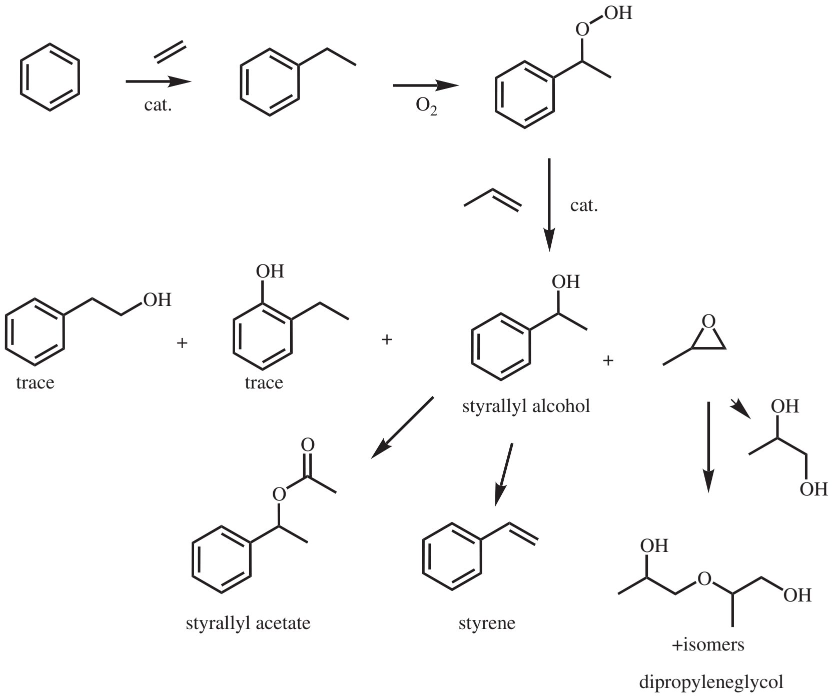


Figure 4.63 *SMPO process*

section, for comparison. Addition of *iso*butene or propylene to benzene gives *tert*-butylbenzene and cumene, respectively. Addition of acrolein or methacrolein diacetates to these gives, after hydrolysis of the intermediate enol ester, the corresponding hydrocinnamaldehydes. It is also possible to prepare the hydrocinnamaldehydes from the substituted toluene by oxidation to the corresponding substituted benzaldehyde, followed by aldol condensation and hydrogenation. The oxidation of the substituted toluenes to the corresponding substituted benzaldehydes can be carried out either by electrochemical oxidation or by chlorination/hydrolysis. This, however, is not the case for oxidation of *p*-cymene (*p*-*iso*propyltoluene), since in this case, the iso-propyl group is more reactive than the methyl group. The substituted benzaldehydes can also be prepared by formylation of the corresponding alkylbenzenes. In Figure 4.61, all of these possibilities are shown for the preparation of Lilial®. The most important route commercially is that which proceeds from toluene through electrochemical oxidation of *tert*-butyltoluene followed by aldol reaction and hydrogenation. Lilial® is an intermediate for

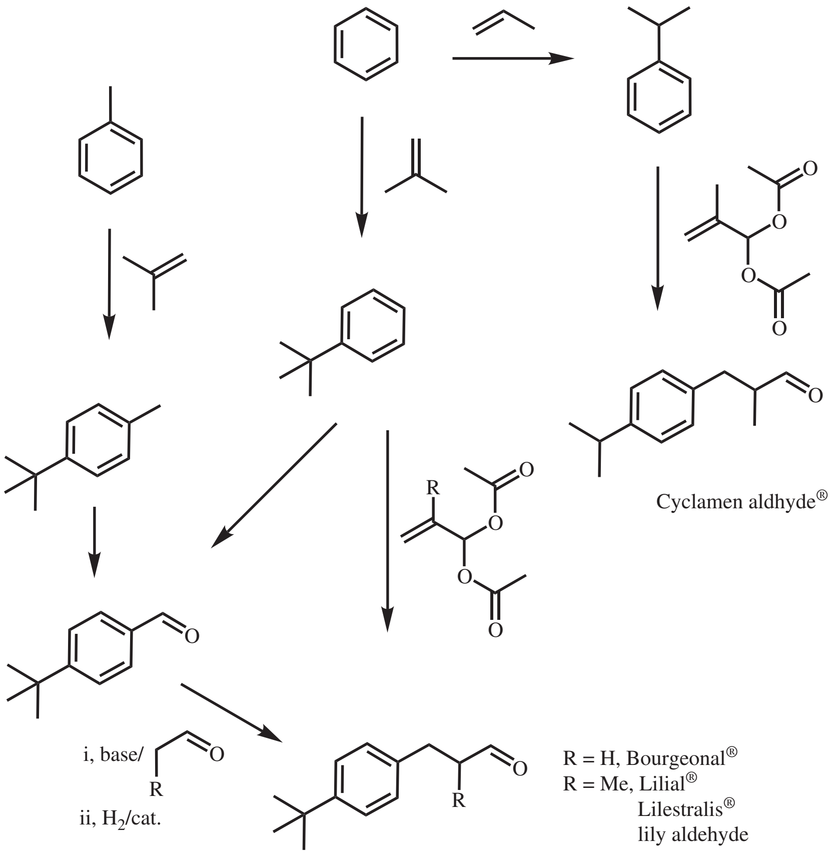


Figure 4.64 *Production of dihydrocinnamaldehydes*

a herbicide and so, some producers gain advantage of scale in their production costs by producing both products. Cumene is produced on a vast scale as a precursor for acetone and phenol, which makes it the most sensible starting material for Cyclamen Aldehyde®. As with so many perfumery materials, this family of aldehydes demonstrates how various synthetic routes are possible and the ones chosen depend on a fine balance of technical, economic and strategic factors.

4.5 PERFUME INGREDIENTS DERIVED FROM TOLUENE

The alkylation of toluene to give hydrocinnamic derivatives is discussed in the previous section alongside the same reaction of benzene.

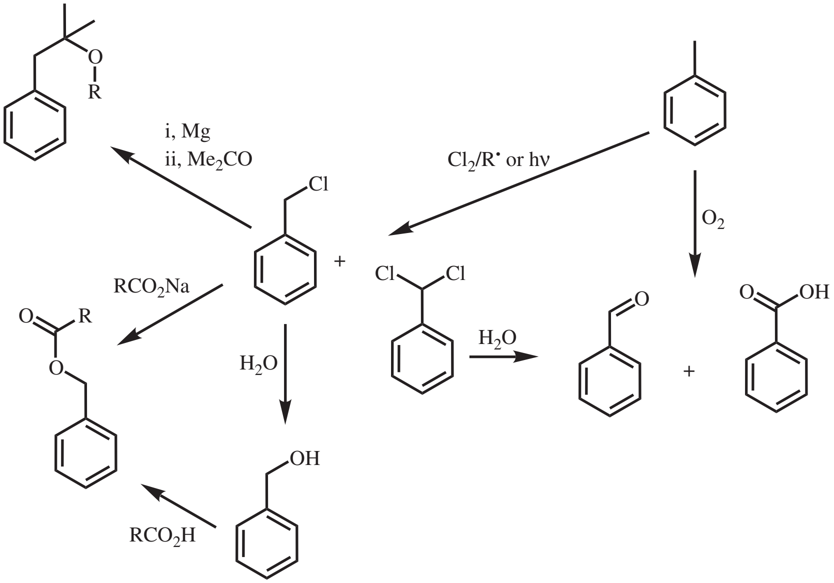


Figure 4.65 *Perfumery materials from toluene*

The tree of products in this section begins with oxidation of toluene as shown in Figure 4.65.

Air oxidation of toluene gives predominantly benzoic acid. This is used in perfumery for the preparation of benzoate esters, benzophenone and various other compounds, but this use is dwarfed by the other industrial uses of benzoic acid. It is used in many different ways, for instance, as a precursor for nylon monomers. Crude benzoic acid contains a small amount of benzaldehyde and this is easily extracted from it. In view of the huge volume of benzoic acid produced, the volume of benzaldehyde recovered from it makes a substantial contribution to that used by the perfumery industry.

Chlorination of toluene under radical conditions (either through the use of an initiator or by photolysis) gives a mixture of mono-, di- and trichlorotoluene. In practice in the perfumery industry, the reaction is run with an excess of toluene present, which means that benzyl chloride is the major product. A little benzal chloride is produced and can be separated and hydrolysed to give benzaldehyde. The major use of benzyl chloride is in the production of benzyl alcohol and its esters. The alcohol is produced by hydrolysis of the chloride. The esters can be prepared by esterification of the alcohol, but it is better economically to prepare them directly from the chloride by reaction with a salt of the corresponding acid. By far the most important of this group of products is benzyl acetate, the major component of jasmine oils. Grignard addition of benzyl chloride to acetone leads to a family of ingredients based on dimethylbenzylcarbinol. The most important member of the family is the acetate, known by the acronym DMBCA.

Although benzaldehyde has an odour which is very characteristic of almonds, it is the chemicals derived from it, rather than its own odour, which make it an important material to the perfumery industry. The major benzaldehyde derivatives are shown in Figure 4.66.

Claisen ester condensation gives cinnamic acid and its esters, the most important of which is methyl cinnamate, followed by benzyl cinnamate.

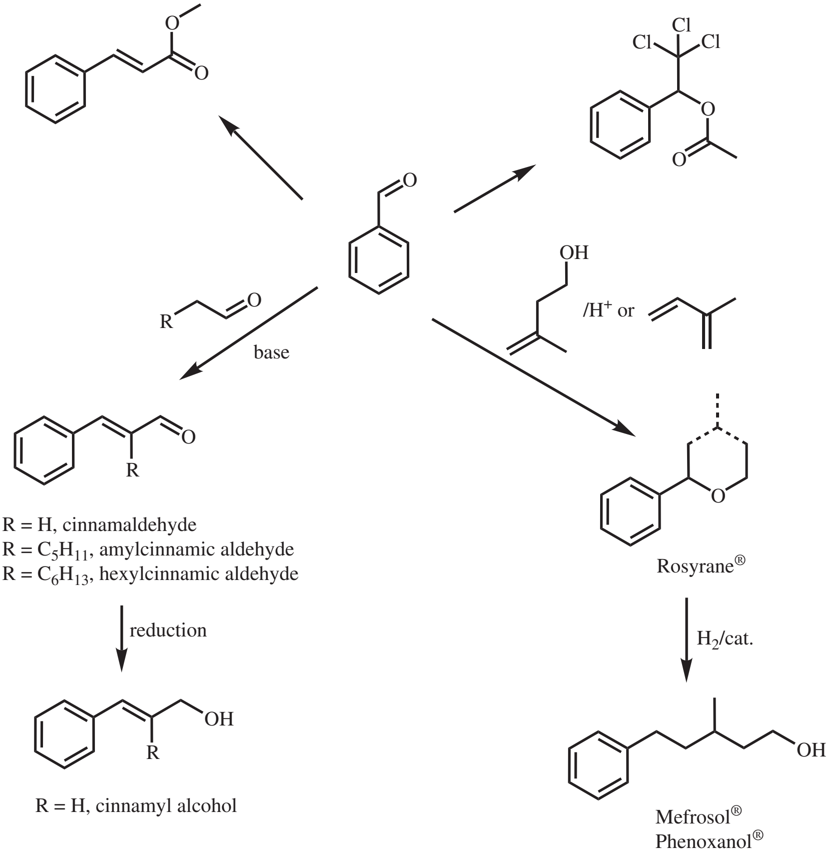


Figure 4.66 *Perfumery materials from benzaldehyde*

Aldol condensation of benzaldehyde with other aldehydes gives the series of α-substituted cinnamaldehydes. The lowest member of the series, cinnamaldehyde, in which R = H, is used to some extent in fragrances but its main use is as the starting material for the corresponding alcohol, cinnamyl alcohol. This is an important component of spicy perfumes where a cinnamon note is required. Its esters, the acetate in particular, are also used for their odours. Much more important are the higher members of the series in which R = *n*-amyl and *n*-hexyl. These two compounds are known as amylcinnamic aldehyde (ACA) and hexylcinnamic aldehyde (HCA), respectively. They possess odours reminiscent of the fatty background note of jasmine although neither are found in jasmine oils. Most synthetic jasmine perfumes use one or both of these compounds as the foundation on which the fragrance is built. They are inexpensive materials and so can be used in large proportions in perfume formulae. They are also very fibre-substantive materials and are therefore of great importance in laundry products such as detergents and fabric conditioners.

Addition of chloroform to benzaldehyde followed by esterification with acetic anhydride gives the trichloro derivative known as rose crystals or, more commonly, by the misnomer, rose acetone. Such misnomers are not uncommon with older fragrance materials. Some are accidental but others were probably intended to deceive competitors in the days before analytical chemistry progressed to the stage where such deception is easily uncovered.

Benzaldehyde undergoes the Prins reaction with *homo*-allylic alcohols to give a variety of perfume ingredients mostly with green, herbaceous odours. These products have very intense odours and so are used at relatively low levels in fragrances. The product obtained from the Prins reaction of benzaldehyde with 3-methylbut-3-ene-1-ol (*iso*-prenol) is known as Rosyrane®. It can also be prepared by hetero-Diels–Alder reaction of benzaldehyde with isoprene using a suitable acidic catalyst. Upon hydrogenation of Rosyrane®, the pyran ring is broken open by hydrogenolysis of the benzylic ether bond giving 3-methyl-5-phenylpentan-1-ol. This material is known under trade names such as Mefrosol® and Phenoxanol®. It has a very pleasant, fresh, white-floral odour.

4.6 PERFUME INGREDIENTS DERIVED FROM PHENOL

Phenol is a material of major commercial importance. One of its earliest uses was as a disinfectant (carbolic acid). Earlier in the twentieth

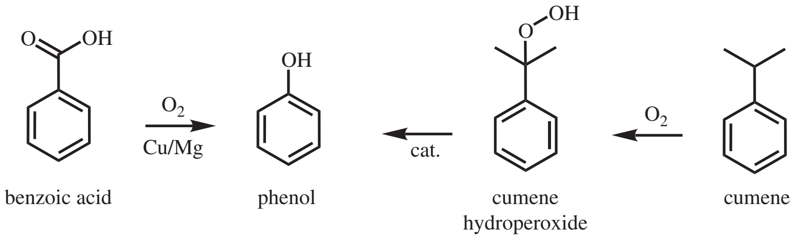


Figure 4.67 *Production of phenol*

century, it became important as a feedstock for resins such as Bakelite, and in the latter part of the century, it also became very important as a precursor for caprolactone and caprolactam and hence polyester and polyamide manufacture. The two major methods for phenol production nowadays are by catalytic oxidation of benzoic acid and catalytic decomposition of cumene hydroperoxide. These are shown in Figure 4.67.

Diphenyl oxide, prepared from phenol, is important in rose and other floral fragrances. Addition of ethylene oxide to phenol gives phenoxyethanol and hence its esters, the most important of which is the *iso*butyrate. Etherification gives materials such as anisole (methyl phenyl ether), estragole (3-(*p*-methoxyphenyl)prop-1-ene, a constituent of tarragon) and anethole (1-(*p*-methoxyphenyl)prop-1-ene, which occurs in, and is strongly characteristic of, aniseed). Anethole and estragole occur in sulfate turpentine. Distillation from this source provides much of the anethole and estragole required, the shortfall in supply being made up by material synthesised from anisole. Addition of *iso*butylene to phenol gives a mixture of *o*- and *p*-*tert*butylphenols. These can be separated and hydrogenated to the corresponding cyclohexanols and then esterified to produce the acetates. The acetates are very important fragrance ingredients and are also used in large quantities. They are known as OTBCHA and PTBCHA, acronyms for *ortho*- and *para-tertiary* butylcyclohexyl acetate, respectively. OTBCHA is also known as Ortholate® and has an apple odour with some fresh woody notes. PTBCHA has a warm sweet, fruity, woody character. Both are mixtures of *cis*- and *trans*-isomers. The *cis*-isomer of PTBCHA is stronger than the *trans*- and has a desirable jasminic/floral character. In consequence, PTBCHA is sold in regular and high *cis*-grades. It is possible to separate the isomers completely but the cost of doing so would be prohibitive (Figure 4.68).

Addition of one carbon unit to phenol in a Friedel–Crafts type reaction gives rise to a family of perfume ingredients of great importance

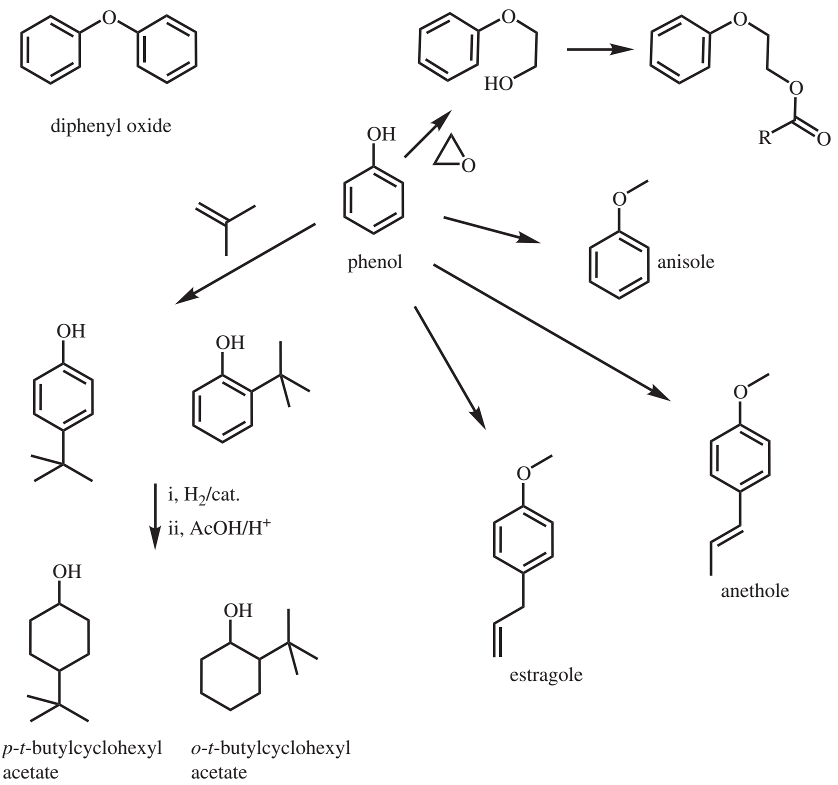


Figure 4.68 *Perfumery materials from phenol*

as shown in Figure 4.69. Carboxylation gives salicylic acid. Acetylation of the phenolic group of salicylic acid gives aspirin and thus the acid is an important commodity chemical for the pharmaceutical industry. The esters of salicylic acid are important to the fragrance industry. Methyl salicylate is the major component of Oil of Wintergreen. Sportsmen and women will readily recognise its odour since it is characteristic of liniments prepared from that oil. The most important salicylates in perfumery are the amyl, hexyl and benzyl derivatives, which are used in very significant quantity. These have persistent, floral, herbaceous odours and make excellent blenders and fixatives for floral perfumes. The odour which we have all come to recognise as characteristic of sun-tan lotion is largely that of these higher salicylate esters.

Addition of formaldehyde to phenol normally produces a resin. However, under controlled, catalytic conditions, it is possible to obtain the hydroxymethyl derivatives in high yield. *o*-Hydroxymethylphenol

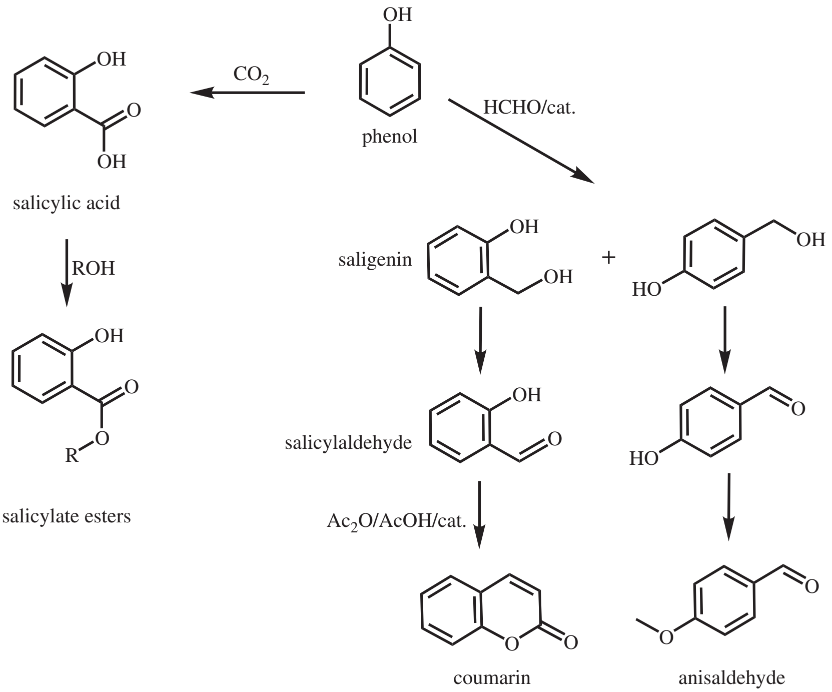


Figure 4.69 *Production of salicylates, coumarin and anisaldehyde*

is known as saligenin and can be oxidised to salicylaldehyde. Similarly, the *p*-isomer can be oxidised to *p*-hydroxybenzaldehyde. Methylation of the latter gives anisaldehyde which has a hawthorn odour and Perkin condensation of the former leads to coumarin. Coumarin is present in newly mown hay and its sweet, hay-like note is used widely in perfumery. Thus, one reaction leads to the precursors for two important fragrance materials. The ratio of *o*- to *p*-substitution can be controlled to some extent by choice of catalyst and conditions, and so the reaction product mixture can be adjusted to suit the demand for the two end products.

Originally, salicylaldehyde was prepared from phenol using chloroform in the Reimer–Tiemann reaction. The Reimer who discovered the reaction is the same person who founded a fragrance company, Haarmann and Reimer, an example of the entrepreneurial approach adopted by many nineteenth century academic chemists.

Oxidation of phenol leads to *o*-dihydroxybenzene, catechol. There are a number of ways of carrying out this oxidation, but the most important commercially is to use hydrogen peroxide as the oxidant with iron salts as catalysts. The monomethyl ether of catechol is known as guaiacol and can be prepared from catechol by partial methylation using dimethyl sulfate or an equivalent reagent. Guaiacol is a precursor for the terpenophenol family of sandalwood chemicals as discussed earlier in the section on terpenes. Formylation of guaiacol gives vanillin, the character impact component of vanilla. The ethyl analogue can be prepared similarly and is known as ethylvanillin. Formylation of the methylene ether of catechol gives methylenedioxybenzaldehyde, which is commonly known as either piperonal or heliotropin. The former name comes from the corresponding acid, piperonylic acid, which is a degradation product of the pungent principles of pepper. The latter, and more common name, is derived from the fact that the odour of the aldehyde is strongly reminiscent of heliotropes, since it is the major fragrant component of those flowers. The vanillic group of compounds are used to give a heavy and long-lasting sweetness to fragrances, a character which is currently much in vogue with perfumes such as Tocade and Angel.

Vanillin and ethylvanillin are not particularly stable chemically. This is not surprising since they possess both an aldehyde and a phenolic group. In functional products, where the pH is not neutral, they will undergo a variety of reactions leading to discoloration. For example, inclusion of vanillin in a white soap will, after a matter of hours, produce a colour close to that of chocolate. There is a marketing phenomenon known as ‘trickle-down’. This is when the odour of a fine fragrance is adapted so that a range of cosmetics, toiletries, soaps, and so on can have the same fragrance to produce a line of products. If the fine fragrance contains vanillin, trickle-down is difficult because of the discoloration issue. One task of the fragrance chemist is to overcome such problems and two solutions to this, developed by chemists at Quest, are shown in Figure 4.70. Protection of the phenolic group of vanillin through the *iso*butyrate ester gives Isobutavan®. Reduction of the aldehyde group of ethylvanillin to a methyl group gives Ultravanil®. (Interestingly, the corresponding methyl ether, obtained by reduction of vanillin, has a strong smoky odour with no trace of vanillic sweetness.) Both of these compounds provide vanillic notes but are much more stable in use than vanillin itself.

Aldol reaction between heliotropin and propionaldehyde, followed by hydrogenation, gives the hydrocinnamaldehyde derivative known as Helional® or Aquanal®. This has a floral note, which is somewhat sweeter than the hydrocinnamaldehydes described in the section on benzene-based materials.

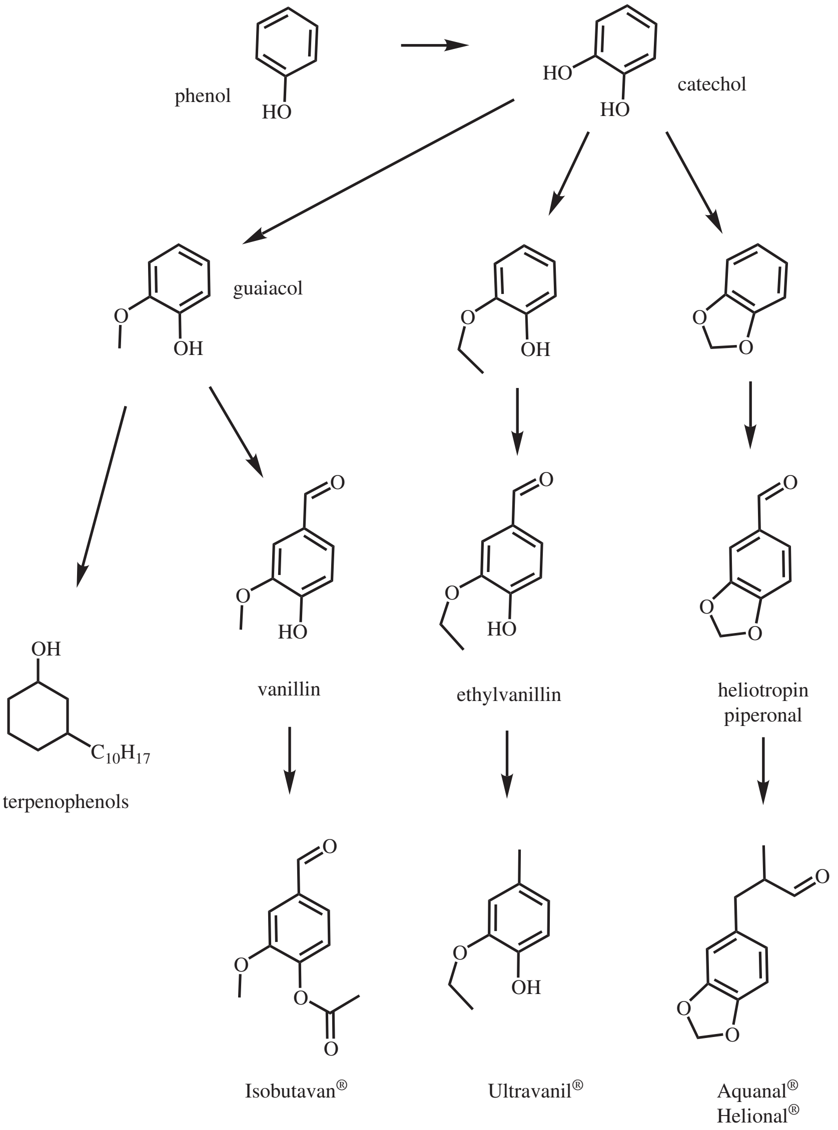


Figure 4.70 *Perfumery materials from catechol*

The catechol-derived materials can also be approached by a different route, using readily available natural products, as shown in Figure 4.71. Clove oil is available at moderate cost and in moderate quantity from several tropical countries such as Indonesia and the Malagasy Republic. The major component of clove oil is eugenol, 3-(3-methoxy-4-hydroxyphenyl)

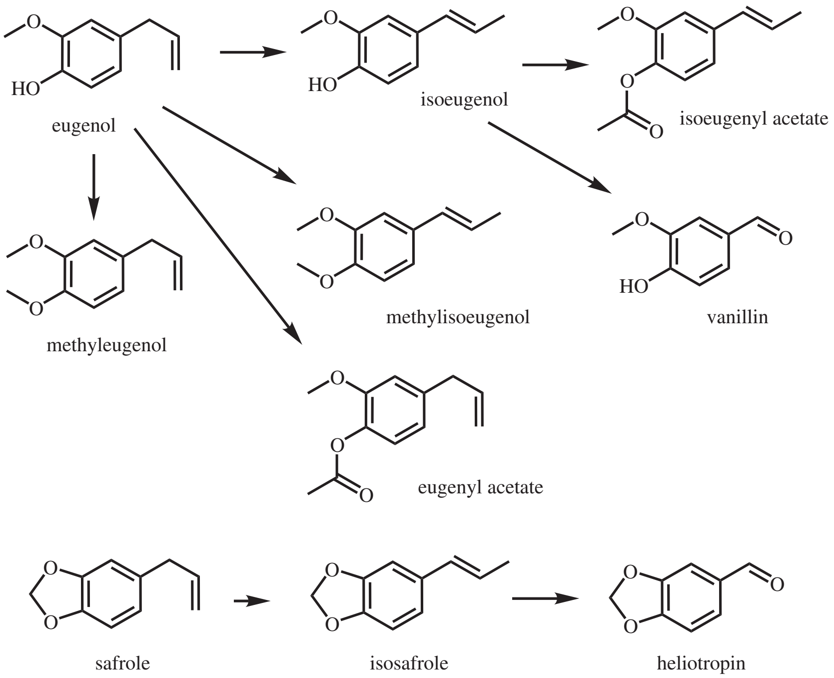


Figure 4.71 *Perfumery materials from eugenol and safrole isoeugenol*

prop-1-ene. This can be isomerised to *iso*-eugenol using basic or metallic catalysts. Ozonolysis of *iso*-eugenol gives vanillin. Similarly, heliotropin can be obtained from safrole, 3-(3-4-methylenedioxyphenyl)prop-1-ene, the major constituent of sassafras oil, which is available from Brazil. The various ethers and esters of these materials shown in Figure 4.71 are used in perfumery. Eugenol itself is, by far, the most important of these, though use of all of the family is declining at present. As well as being present in cloves, eugenol is an important contributor to the odour of carnations and is used in fragrances to that effect. Generally, the catechol route is cheaper than the eugenol/safrole route to vanillin and heliotropin, but local economics may tip the balance in the other direction particularly for heliotropin, which is the more expensive of the two.

4.7 PERFUME INGREDIENTS DERIVED FROM NAPHTHALENE

Acetylation of naphthalene gives methyl naphthyl ketone and sulfonation, followed by alkaline fusion, gives naphthol. The methyl and ethyl ethers of naphthol are prepared from naphthol by reaction with the corresponding alkyl sulfate under basic conditions. These ethers are usually known by the shorter names of yara and nerolin, respectively. Yara, nerolin and methyl naphthyl ketone possess floral odours and are moderately important perfume ingredients. The name nerolin is derived from neroli, a synonym for orange blossom oil.

Oxidation of naphthalene gives phthalic acid. This can also be obtained by air oxidation of *o*-xylene. Phthalic acid is an important feedstock for polymers and plasticiser and so, once again, the fragrance industry is found ‘piggy-backing’ on a larger industry in order to provide inexpensive ingredients. Higher esters of phthalic acid are used as plasticisers and solvents; the ester of most interest to the fragrance industry is the diethyl ester which is used as an odourless solvent for fragrance ingredients (Figure 4.72).

Methyl anthranilate is obtained by the Hoffmann rearrangement of phthalimide, which is easily prepared from phthalic anhydride and ammonia. Methyl anthranilate occurs naturally in many flowers and has a very characteristic and intense, sweet smell. When added to an aldehyde, it forms the corresponding Schiff’s base. If the aldehyde carries an α-hydrogen atom, the Schiff’s base will be in equilibrium with the corresponding enamine. The relative proportions of Schiff’s base and enamine present will depend on the structure of the aldehyde, since the inductive and resonance effects will seek to minimise the total free energy of the molecule. Perfumers refer to the products as Schiff’s bases irrespective of the exact composition. Indeed, Schiff’s bases formed from methyl anthranilate are so important to the industry that the term ‘Schiff’s base’, when used in perfumery, almost invariably implies a Schiff’s base of methyl anthranilate. The importance of these Schiff’s bases to perfumery results from the fact that they increase both the chemical stability and tenacity of the aldehyde component. The Schiff’s bases are less reactive than the free aldehyde and, since the molecular weight is much higher, they are less volatile. Thus, loss of the aldehyde by both chemical reaction and evaporation is slowed down. Moreover, hydrolysis of the Schiff’s base releases both the aldehyde and the methyl anthranilate, both of which have intense odours. Thus, use of a Schiff’s base generates a long-lived fragrance composition of the two ingredients. Originally, the aldehydes used in these Schiff’s bases also had sweet, floral odours and so a harmonious accord was produced. Giorgio is an example of a fragrance, which relies heavily on this type of accord. A new fashion was created when Dior introduced

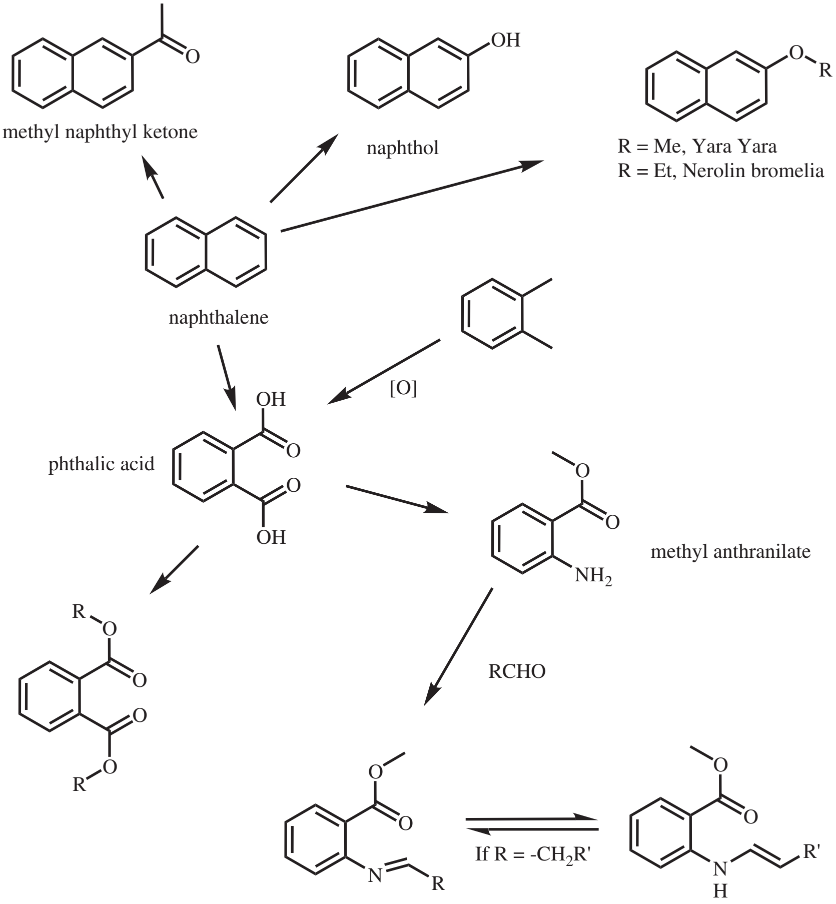
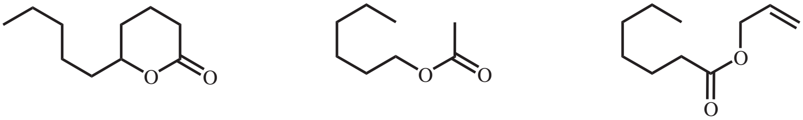


Figure 4.72 *Perfumery materials from naphthalene*

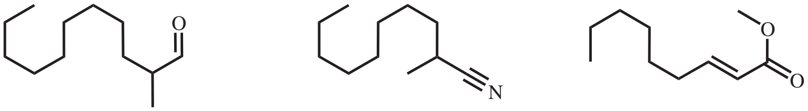
Poison in which the aldehyde component of the Schiff's base has a strident green character, creating a sharp contrast with the heavy sweetness of the methyl anthranilate. The pale yellow colour of methyl anthranilate is darkened when a Schiff's base is formed from it. This is because of the hypsochromic shift in the ultraviolet absorption maximum resulting from the extension of conjugation. This colour has an effect on the colour of any fragrance in which it is incorporated and has to be taken into account if colour would be deleterious in the final product. Many Schiff's bases are available commercially but some perfumers prefer to formulate their fragrances using aldehydes and free methyl anthranilate and allow the Schiff's base to form spontaneously in the perfume.



δ-decalactone
(peach, coconut)

hexyl acetate
(fruity, pear)

allyl heptanoate
(sweet, banana)



aldehyde MNA
(fresh, aldehydic)

Frutonile®
(soft, floral, fruity)

Beauvertate®
(vegetable, earthy)

Figure 4.73 *Some aliphatic perfumery materials*

4.8 PERFUME INGREDIENTS DERIVED FROM ALIPHATIC MATERIALS

A large number of aliphatic fragrance ingredients are used, but few in significant tonnage. This is largely because the materials of use are mostly aldehydes, nitriles and lactones, the majority of which have very intense odours which limit the amount that can be incorporated in a fragrance. A number of volatile esters are also used to give fruity top-notes but, again, these are not used at high levels. Some typical examples, with their odour descriptions, are shown in Figure 4.73. The majority of ingredients of this class can be prepared by straightforward synthetic reactions and functional group interconversions, starting from both natural and petrochemical precursors.

The aliphatic fragrance materials of natural origin are mostly derived from fatty acids and related materials. Because of their biosynthetic pathway (see Chapter 3), those formed directly all have an even number of carbon atoms in the chain. Any with an odd number of carbons in the chain are likely to be breakdown products. Fatty acids are useful precursors for aliphatic fragrance materials. For example, the iso-propyl ester of myristic acid is employed as a solvent. Once a fatty acid is available, appropriate reduction and oxidation reactions open routes to the other members of the series. For instance, octanoic acid can be obtained from coconut oil. Reduction of the acid produces octanol, which can be dehydrogenated to octanal. Octanal is used both as an ingredient in its own right and as a precursor for other materials such as HCA (see Section 4.5).

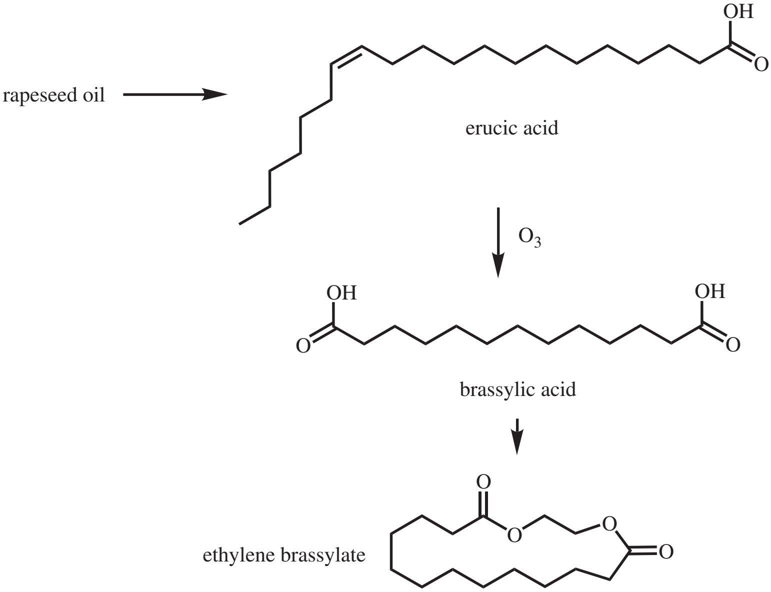


Figure 4.74 *Production of ethylene brassylate*

Two major reactions are used in breaking longer chains to give odd-numbered fragments as fragrance-building blocks. The first is oxidative cleavage of a double bond in an unsaturated fatty acid. Ozonolysis is a convenient method for doing this and is used to provide pelargonic acid (heptanoic acid) and azelaic acid (nonanedioic acid) from oleic acid, the major constituent of olive oil, and brassylic acid (tridecanedioic acid) from the erucic acid (13-docosenoic acid) of rapeseed oil. The formation of brassylic acid and its conversion to the musky ethylene ester (as described earlier under musks) is shown in Figure 4.74.

The other reaction is pyrolytic cleavage (*via* a *retro-ene* reaction) of a fatty acid containing a homoallylic alcohol in the chain. This is used to produce two important feedstocks, heptanal and undecylenic acid, from ricinoleic acid, the major fatty acid component of castor oil. The mechanism of this reaction is shown in Figure 4.75.

Undecylenic acid is important as a starting material for a number of fragrance materials, some of which are shown in Figure 4.76. The two aldehydes are obtained by simple functional-group manipulation. Both are known by trivial, and somewhat confusing, names in the industry; 10-Undecenal as Aldehyde C11 undecylenic or simply Aldehyde C11, and undecanal as Aldehyde C11 undecylic. The fact that the term

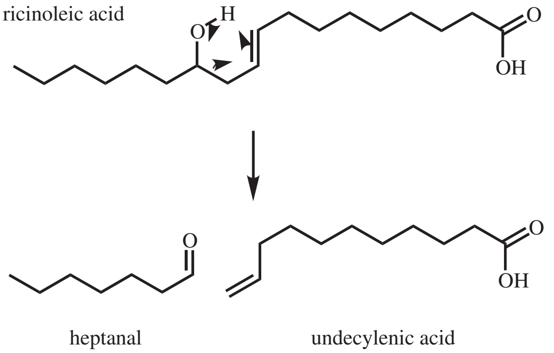


Figure 4.75 *Cracking of ricinoleic acid*

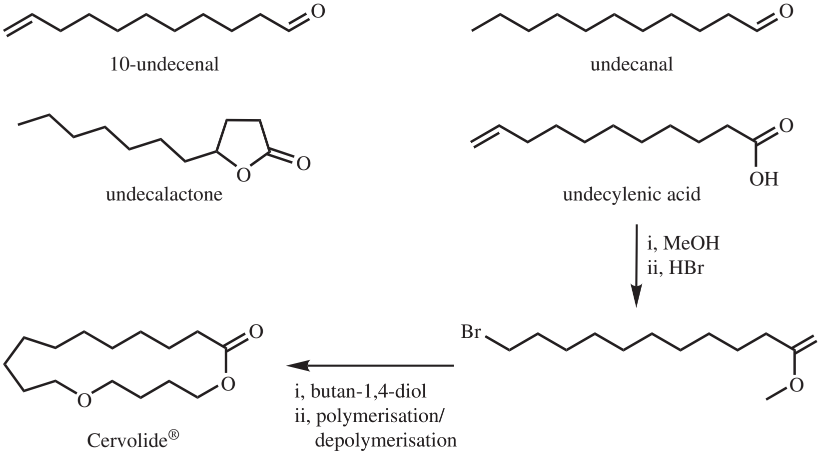


Figure 4.76 *Perfumery materials from undecylenic acid*

Aldehyde C11 refers to the unsaturated aldehyde rather than the saturated one is due to the former having become a standard ingredient before the latter. Treatment of undecylenic acid with a strong acid causes the double bond to migrate along the chain by repeated protonation and deprotonation. Once a carbocation forms on the fourth carbon of the chain, it can be trapped by the carboxylic acid group to form a stable γ-lactone, undecalactone. This material has a very powerful coconut-like odour. Esterification of undecylenic acid followed by anti-Markovnikov addition of hydrogen bromide gives the ω-bromoester. Addition of 1,4-butandiol to this with subsequent polymerisation and depolymerisation (see Section 4.3) gives the musk Cervolide®.

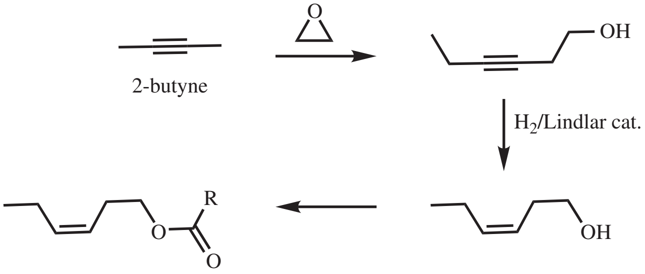


Figure 4.77 *Production of* cis-*3-hexenol and esters*

Synthetic precursors for aliphatic materials mirror the pattern of their naturally derived counterparts in that the commonest units are even in carbon chain length. This is because they are usually derived from ethylene through oligomerisation. Thus, coupling of two ethylene molecules produces a four-carbon chain, three produces six, and so on. In order to obtain an odd number of carbon atoms in the chain, one of the simplest techniques is to add a single carbon to an even chain which can be achieved, for example, by hydroformylation. Hydroformylation also introduces an alcohol function and opens the way for oxidation to aldehydes and acids. Three carbon units are available from propylene as well as by reaction of ethylene with a one-carbon unit.

cis-3-Hexenol occurs in a number of natural sources such as freshly cut grass and strawberries. It possesses a very intense odour, which falls into the odour class known to perfumery as green. Green odours are those that resemble foliage and stems of plants. *cis*-3-Hexenol is very characteristic of cut grass and is used to add a fresh green top-note to fragrances. A number of its esters, such as the acetate and salicylate, are also of use. The synthesis of these materials is shown in Figure 4.77. 2-Butyne is obtained as a by-product stream from a petrochemical process. The hydrogen atoms adjacent to the acetylene bond are acidic enough to be removed by a very strong base, and this allows the triple bond to migrate to the end of the chain. Once there, the terminal hydrogen atom is lost to give the relatively stable acetylide anion and so eventually all of the material is present in this form. The anion reacts with ethylene oxide to give *cis*-3-hexynol which can be converted to *cis*-3-hexenol by hydrogenation over a Lindlar catalyst.

The Prins reaction, the acid-catalysed addition of an aldehyde or ketone to a double bond, is a useful reaction in perfumery chemistry and a number of aliphatic fragrance ingredients are prepared in this way. One example is shown in Figure 4.78. When 1-octene is reacted with formaldehyde, the result is a complex mixture of products of which the pyran shown in Figure 4.78 is the major component. In the case of this pyran, the octene molecule reacted with two molecules of formaldehyde to give an intermediate cation, which is trapped by the acetic acid present in the reaction medium. The product mixture is widely used for its fatty jasminic character and is sold under a variety of trade names such as Jasmopyrane®.

Three other Prins reactions are shown in Figure 4.79. The precursor for two of these is 2-methylpent-1-en-4-ol which gives Gyrane® with

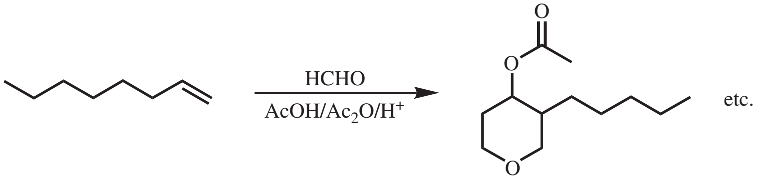


Figure 4.78 *Prins reaction of formaldehyde and octene*

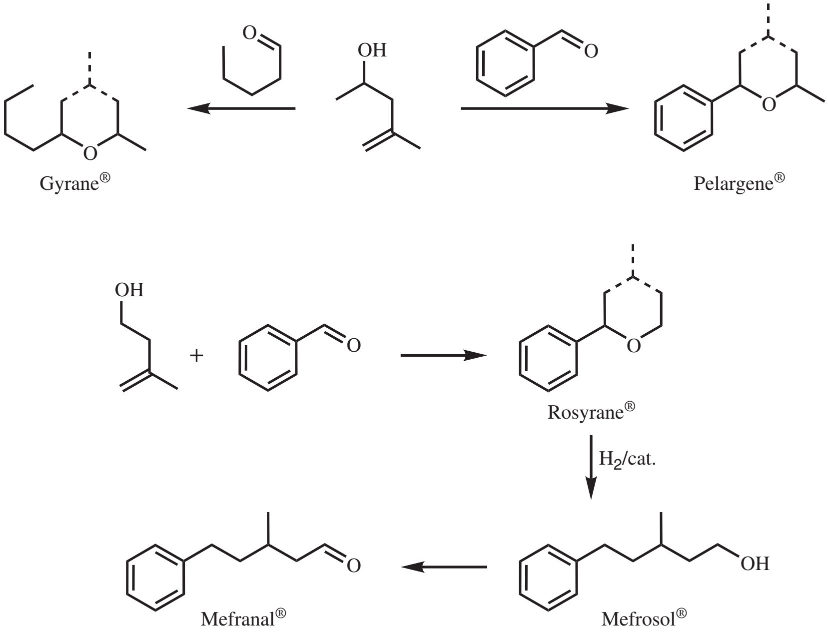


Figure 4.79 *Perfume materials via the Prins reaction*

pentanal and Pelargene® with benzaldehyde. In both cases, the cationic intermediate is trapped by the alcohol of the starting material to form a pyran ring and elimination of a proton can occur in any of three directions, leading to a mixture of isomers. Both products have odours which are rosy and green, Gyrane® is fresh and radiant and Pelargene® resembles crushed leaves. The third reaction between isoprenol and benzaldehyde gives Rosyrane®, which on hydrogenation gives the alcohol known as Mefrosol® or Phenoxanol®. Dehydration of Mefrosol® gives Mefranal®.

The 2-methylpent-1-en-4-ol used as described above is produced from acetone. Acetone readily undergoes a self-aldol reaction in the presence of base to give diacetone alcohol, 2-methylpentan-4-on-2-ol, which can be reduced to hexylene glycol, 2-methylpentan-2,4-diol. Careful dehydration under mild conditions gives the unsaturated Prins precursor. Dehydration under stronger conditions gives 2-methylpenta-1,3-diene, which is the more thermodynamically stable of the two possible diene products. Methylpentadiene is also a useful precursor for fragrance ingredients. In Figure 4.80, it is shown undergoing a Diels–Alder reaction to give Ligustral®, a very intense green ingredient in its own right and also a precursor for Karanal®, a powerful ambergris material.

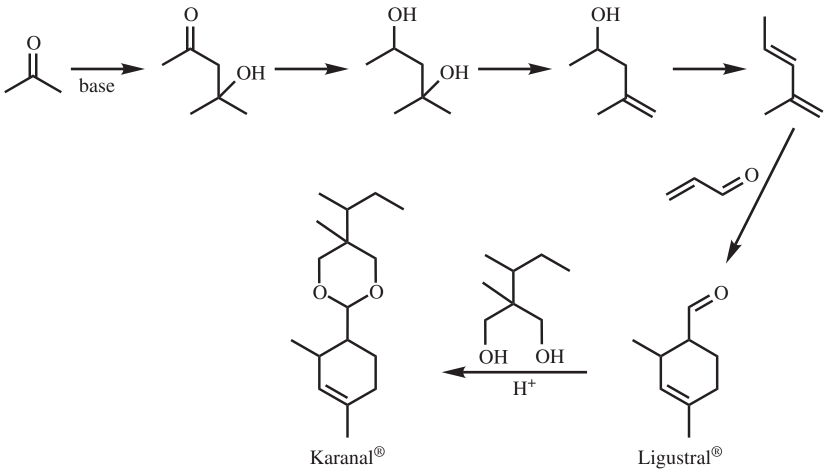


Figure 4.80 *Production of Ligustral® and Karanal®*

4.9 PERFUME INGREDIENTS DERIVED FROM CYCLOPENTANONE

The chemistry of synthetic jasmine materials was given an enormous boost in the 1930s when Nylon 66 was launched as a product. Nylon 66 is a polyamide prepared using adipoyl chloride and hexamethylenetetramine as monomers. The 66 in the name refers to the fact that there are six carbons in each type of unit, which lies between the amide links in the polymer chain. Thus, adipic acid is the key feedstock for Nylon 66 and the introduction of the latter meant that the former became a basic chemical commodity. Pyrolysis of the calcium or barium salt of adipic acid produces cyclopentanone, and so the availability of large quantities of the acid meant that the ketone could also be prepared at low cost.

Cyclopentanone readily undergoes the aldol condensation with a variety of aldehydes to give the 2-alkylidenecyclopentanones. These have jasmine-like odours but are no longer used in perfumery since it was discovered that they have the potential to cause skin sensitisation. The saturated products are safe and are used to give jasminic, fruity, floral odours in fragrances. The most widely used are the *n*-heptyl- (R = pentyl) and *n*-hexyl- (R = butyl) derivatives. These are sold under trade names such as Heptone® and Jasmatone® respectively (Figure 4.81).

The 2-alkylidenecyclopentanones are readily isomerised to the corresponding 2-alkylcyclopent-2-enones by the action of acids or platinum group metals. Michael addition of dimethyl malonate to these, followed by partial hydrolysis and decarboxylation, gives jasmonic acid analogues. The most important of these is methyl dihydrojasmonate, methyl 2-(2-pentylcyclopentan-3-on-1-yl) acetate. This material differs from methyl jasmonate, the natural jasmine component, in that its side chain is saturated whereas the natural material has a *cis*-double bond between the second and third carbon atoms of the chain. Both compounds have similar jasmine odours. The odour is perceived as weak when a fresh sample of material is smelt. However, if the sample is left in a room, the whole room is filled with its delicate floral scent. In perfume compositions, methyl dihydrojasmonate has a blending, fixing and enhancing effect on the other components. These properties have made it one of the most important of fragrance ingredients.

When the 2-alkylcyclopentanones are subjected to the Bayer–Villiger reaction, the resultant lactones are found to have sweet, buttery, peach and coconut odours. These are useful in floral fragrances, but their major use is as butter flavour in margarines.

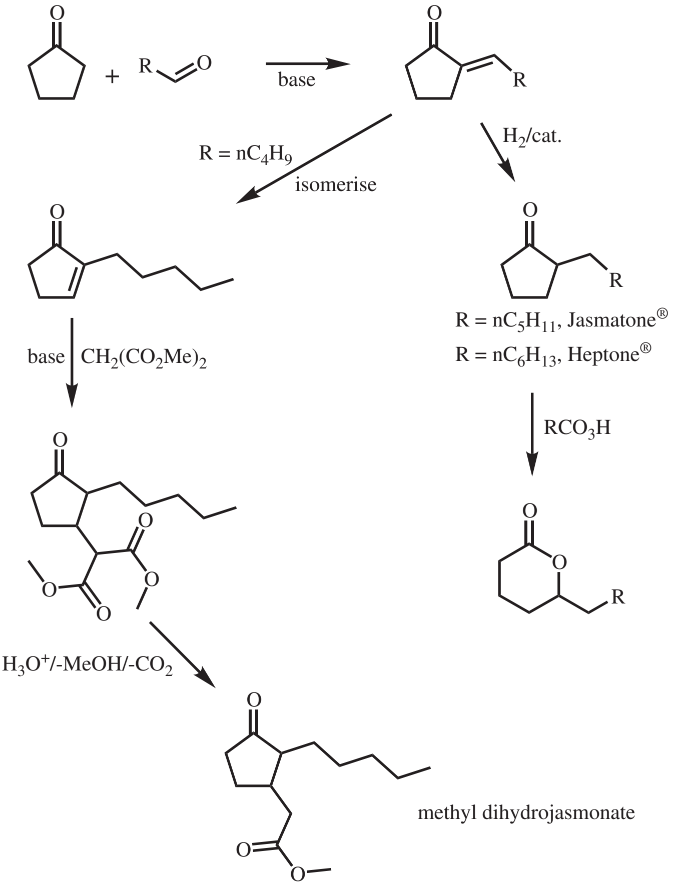


Figure 4.81 *Jasmine ingredients from cyclopentanone*

4.10 PERFUME INGREDIENTS DERIVED FROM DICYCLOPENTADIENE

Dicyclopentadiene is a feedstock for both the fragrance and polymer industries. It forms spontaneously from cyclopentadiene by a Diels–Alder reaction and a *retro*-Diels–Alder reaction can be used to regenerate cyclopentadiene from it. A number of minor fragrance ingredients are produced by Diels –Alder reaction of the monomer with a variety of activated olefins in which the activating group, X, is usually an aldehyde, ketone, ester or nitrile. However, the main fragrance uses stem from the dimer.

The two double bonds of dicyclopentadiene are very different in reactivity and, consequently, selective reactions are easy to achieve. The bond in the bridged ring suffers a high degree of angle strain and will readily undergo any reaction which will result in the two carbon atoms changing from sp2 to sp3 hybridisation since the latter have bond angles much closer to those required by the bridge structure. Thus, for example, carboxylic acids and alcohols will add to that bond under acid catalysis. The product is, of course, a mixture since the regiochemistry of the addition is unaffected by the position of the other double bond. The most important, by far, of the products shown in Figure 4.82 is the product of addition of acetic acid, Jasmacyclene®. This material has a fruity, jasmine-like odour and its use by the fragrance industry runs into thousands of tonnes per annum. Apart from Florocyclene® and Gardocyclene®, the other products shown are all relatively low in tonnage.

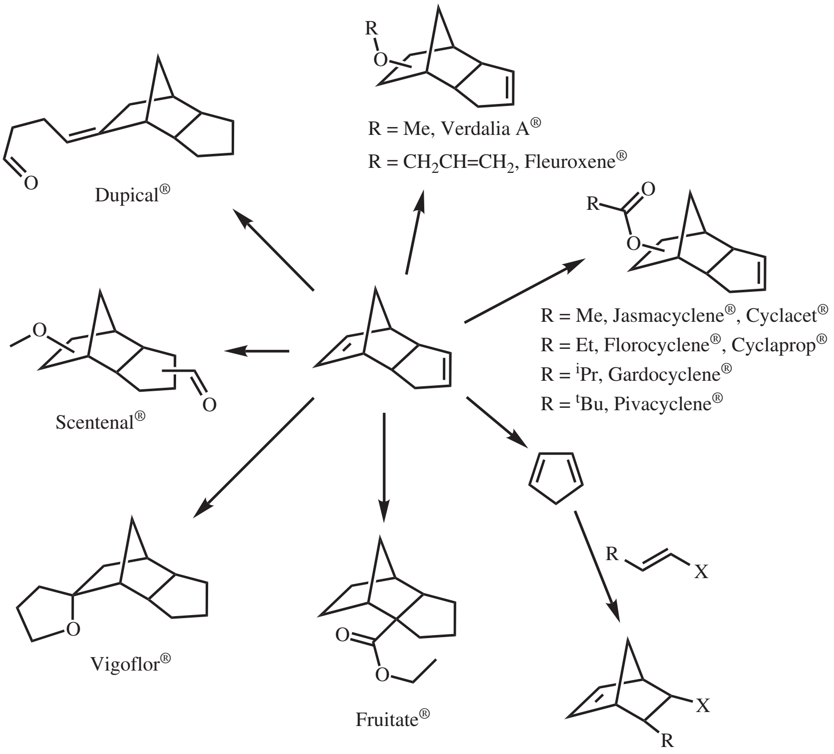


Figure 4.82 *Perfumery materials from dicyclopentadiene*

Dupical® and Scentenal® have fresh, floral, aldehydic odours, the others have varying proportions of floral and fruity notes.

4.11 CONCLUSIONS

The fragrance industry employs a very wide range of chemical processes and often has to deal with complex mixtures of structurally complex materials. The products must be produced at low cost and with minimal environmental impact. They must be safe in use and safe to produce. Minor components in mixtures may play disproportionately large parts in determining the odour of the whole, making quality control of processes a highly skilled occupation. The combinatorial nature of the mechanism of perception of odorants means that, for the foreseeable future, prediction of the odour of any putative molecule will be in terms of probability rather than certainty. The continually changing nature of the consumer-goods market demands a continual supply of novel ingredients to meet the challenges of new products to be perfumed. Initial bioassay (smelling) can be performed by the chemist and thus, feedback is instant; there is no need to go in for lengthy activity testing as is in the pharmaceutical and agrochemical industries. Performance testing and scale up are also faster than is the case with those industries. All of these factors combine to make fragrance chemistry an exciting and challenging environment where scientific, artistic and commercial skills must all be practised together at the highest level of each.

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CHAPTER 5

The Structure of an International Fragrance Company

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Pandora Ltd.

Just as the senses of taste and smell are chemical senses and are integrally connected, the large multinational fragrance suppliers are involved in the manufacture of flavour and perfume. Table 5.1 illustrates a near ten billion dollar industry in the provision of fragrance and flavour compositions, while Table 5.2 details the fifteen billion dollar market for cosmetics and fragrance on a global basis. Companies such as Quest International are the ghostwriters of this industry. Their products, essentially fragrance and flavour concentrates, are provided in bulks of 50, 100 and 200 kg drums, and occasionally in 1 tonne iso-containers to manufacturers of branded products worldwide.

Table 5.1 *World consumption of flavour and fragrance products – 2003 (millions of dollars)*

	United States	*Western Europe*	*Japan*	*China*	*Other*	*Total*	*Percent*
Flavour compositions	1930	2427	1185	801	700	7043	43
Fragrance compositions	1780	1561	201	118	715	4375	27
Essential oils/ natural extracts	620	1293	97	307	450	2767	17
Aroma chemicals	770	706	174	170	276	2096	13
Total	5100	5987	1657	1396	2141	16,281	100%
Percent	31%	37%	10%	9%	13%	100%	

Source: SRI Consulting.

Table 5.2 *World consumption of cosmetics and perfumes – 2003*[a]

	Retail value (billions of dollars)	*Percent*
North America	50.0	32.9
Western Europe	64.0	42.1
Japan	13.6[b]	9.0
Rest of the World	22.1	14.5
Duty-free shops	2.3	1.5
Total	152.0	100.0%

[a] Cosmetics include the following product segments: decorative cosmetics, perfumes and fragrances, hair care, skin care, personal hygiene, oral hygiene and shaving products – all containing fragrance ingredients. The US figure includes soaps.
[b] Excluding soap.
Source: Industry sources; SRI Consulting.

The five largest multinational fragrance houses account for some 70% of the total global fragrance compound sales, having a legacy of a century of experience and a financial strength, which can cope with tens of millions of dollars in expense each year into pure research and development required to keep ahead in the game.

The structure and nature of a fragrance 'house' must reflect its link to the consumer *via* its own multinational clients. It is arguable that the client knows his marketplace best and certainly invests in much market research to ensure this is so, but the fragrance suppliers must understand consumer sensory perception and be alert to the reflection of fashion in terms of odour. The marketers in a fragrance house must be able to anticipate the effects of social trends on fragrance preferences and focus creative resources accordingly, relating brand values to the emotional effects of odour and interpreting market research data to understand precisely the complex influences on purchasing decisions of the consumer. These companies understand the value of the psychology of scent.

Fragrance houses are fed a daily diet of perfumery briefs by manufacturers looking to launch new products or add variants to an existing range. The survival of these houses depends on the successful nurture and assimilation of the information contained in the brief, and on translating it from the written word, or visual image of a pack or advertisement, to a smell. Like the advertising industry, the fragrance industry competes for selection on a list of companies that have the honour of receiving a brief, and then competes again when these companies put forward their creation. In both the industries, there are no prizes for coming second.

The fragrance industry is close to the fashion industry, its trends being olfactive reflections of fashion. Empathy with these trends and the ability to translate them into perfume is highly prized; however, here customer service is also of a high value like in any other industry operating on a global scale. Fragrance suppliers are therefore structured around two main themes, namely:

- *The business-getting chain.* The proactive work on a client's brief with creative teams developed to deliver winning products to the marketplace.
- *The supply chain.* The managed and planned purchasing of raw materials, competitively costed formulae, total quality production techniques and a customer delivery and service department that is second to none.

5.1 THE BUSINESS-GETTING CHAIN

A fragrance house is typically structured in a three-dimensional approach that covers geography, product type and functional skills. The core teams are set up around market product types (the final products into which the fragrances will be incorporated), the main groupings being Fine Fragrance, the prestige personal perfumes, which so readily embody the general image of a fragrance house, Personal Wash (soaps, shower gels, deodorants and shampoos) and Fabrics and Homecare, focusing on laundry and household products such as dish-washing liquids and tablets, multipurpose cleaners and air fresheners.

At the sharp end, marketing and sales personnel (account managers) relate with the client work to interpret the brief. This is fed into the wider team system for response within an agreed timescale. The core team for a response on the brief normally consists of people from Marketing, Perfumery (creative and technical) and Evaluation, while Research and Development may also be involved and technical advice given by the Product Applications Laboratory can also be taken into account. Converting a brief into a business win is dependant on all these disciplines working together, deciphering the need and creating and presenting the fragrant offering. How it is presented will vary tremendously depending on the client; for some the olfactive originality will be the most important criterion, while for others consumer acceptability is the key and so the fragrance will be submitted to a market test.

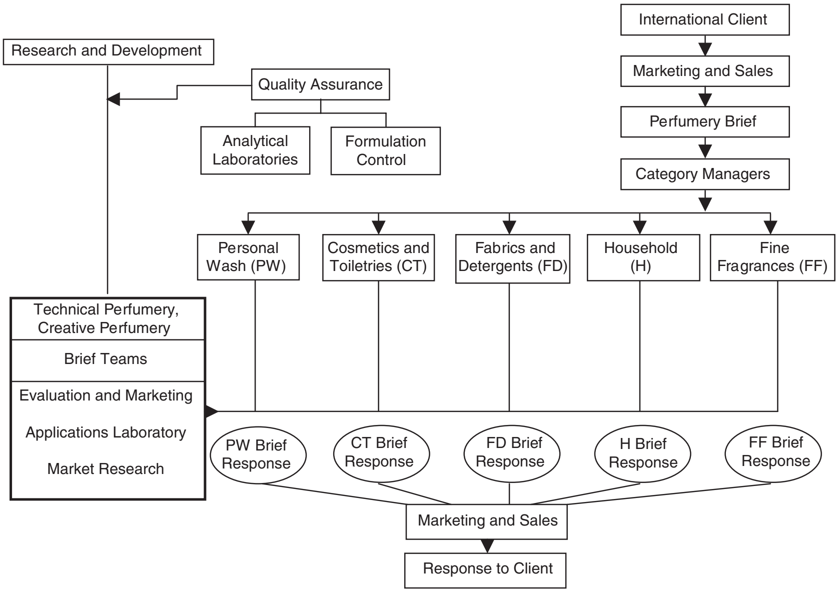

Figure 5.1 *General structure of an international fragrance company around the perfume brief*
(adapted from Curtis and Williams, 1995)

5.2 THE SUPPLY CHAIN

The core areas of the client supply chain – involving the formulation, material collection, production and supply of compound fragrances – are illustrated in Figures 5.1 and 5.2, with the key links between different departments of the company also shown. It should be emphasised that this diagram is not definitive. Each fragrance house displays a shape and structure that best suits its own marketplace, and there is a more complex interplay of communication than the one sketched here.

The scope and breadth of raw material requirement has already been mentioned in Chapter 3. Synthetic aroma chemicals, barring major oil crises, do not normally give problems in terms of stability of supply, relatively stable costs and reliable quality. Natural products are a different story, with the price and quality of an essential oil being very much affected by the climate and weather. The vagaries of a stock market centred on buying and selling futures in crops such as coffee and citrus fruits can multiply the concerns a 1000-fold to cope with the complexities of balancing stock for producing consistent quality finished perfume, blended and supplied on time and within a specified price. It is

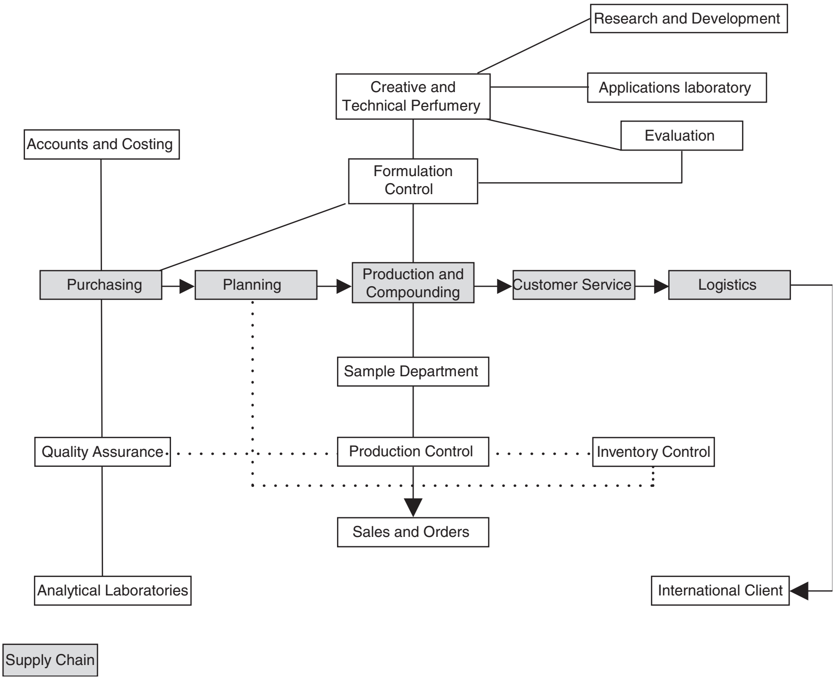

Figure 5.2 *General structure of an international fragrance company around the supply chain*
(adapted from Curtis and Williams, 1995)

notable that a typical fragrance formula, if such a thing exists at all, will consist of several tens, if not hundreds, of ingredients.

The supply chain has two natural facets, which are

– supply of standard orders on a regular, planned basis to clients; and
– supply of a newly won perfume compound into the marketplace for the first time, and initial build-up of supply or demand balance set by the pull of market acceptance.

A fragrance house is like a bespoke tailor, supplying client-specific products, which for multinational clients become essentially their own. There is, however, an ‘off-the-peg’ or ‘fast-shelf’ service for smaller clients, where the resource commitment, pricing demand and fast turnaround of response dictate a different method of interpretation.

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CHAPTER 6

The Perfume Brief

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Chapter 5 illustrates how a fragrance house is structured around its lifeblood – the receipt, response to and winning of client briefings. In this chapter a typical fragrance brief will be created for an imaginary company, Business Scents Ltd wished to launch a fragrance called 'Eve'. Before developing the brief for Eve, it is worthwhile outlining the main considerations of the brief, as this reflects on the chemistry of the final fragrance choice and submission.

Fragrance briefs come in all shapes and sizes, and can be subject to many types of interpretation. Sometimes the client will be very specific about the odour family (the general scent characteristic) that the perfume should be. Other times, the brief will be more open with proposal of the fragrance being left to the supplier. Every brief, however, will specify the product type, the target consumer and any details already known about the package that the fragrance will eventually inhabit. If the fragrance is being created for a range that already exists, then it will need to complement the other products on the market, and the shape, colour of the pack will be known, as will the brand proposition. It is the work of a fragrance house to translate their gut-feel, or verbalise their inner sense of market opportunity into a viable fragrance direction for the product.

Thus, fragrances are created to represent experiences: sometimes the whim of an individual or sometimes a deliberate recreation of a popular aspiration. The 'smell of an Arabian souk' was explored literally by a team of 'perfume hunters', touring a souk in Marrakesh, picking up the wood, spices and incense from such a scene that are the essence Arabie, editing out the inevitable environmental malodours, which may come with such territory. A 'funfair' becomes a melee of candyfloss, chocolate and vanilla, a gustatif combination contrasted with a sharpness indicative of the cordite of the rifle range and the sharp smell of gasoline.

Communication is one of the main challenges faced by the fragrance industry. Fragrance experts, like many experts, have their own language. Clients and consumers speak of scent in different terms. For most people, the ability to describe a smell is limited to 'like' or 'dislike' terms, or at

Table 6.1 *Perfumers' rule of 13 – an aide-memoire*

The 13 'P's that constitute the building bricks of a substantial fragrance brief, with a general outline of the key thoughts linked to them

Product	What is the product formula? What chemical environment will the perfume face? Does the product have colour or any other physical characteristics such as base odour? Any special ingredients?
Positioning	What is the intended market position of the product?
Place	Is the product intended for the national, regional or global marketplace?
Production	What production process will the product undergo, at what time and how will it be dosed?
Package	Can the packaging (*e.g.* aerosol can lining, soap wrapper) be affected by the nature of the fragrance? What colour will it be? Is there a design for the packaging? Will other sensory cues be reinforced by the perfume?
Publicity	On what platform is the product to be promoted? Are there key words, such as smooth, gentle, caring, hardworking, that the perfume will need to evoke?
Pending	What timescale does the brief have? If very short, perhaps a shelf product will suffice, if long term and a significant brief, market research may be feasible and a prerequisite.
Purpose	Is the product new or a range extension? What competition, if any, is it going up against? What is the key objective for the product?
Price	Cost per tonne finished product is a better focus than cost per kilo of product, as it gives flexibility on dosage. Keep within the parameters set (relevant to fragrances for functional products).
Presumption	Ensure you understand the nuances of the country. What does 'green' or 'fresh' mean in the context of the customer's requirements? Never presume anything about anything.
Pugilist	Who are the competitive fragrance houses in the brief? What are their known strengths and weaknesses?
Perfume	Does your final submission meet all the criteria above? If the customer mentions a fine fragrance by name does he or she really mean that particular brand or fragrance direction? Probe. How much fragrance is required for the brief testing? What regulations must the fragrance meet?
Profit	On submission, what margin (profit) suffices so that the winning of the business is worthwhile?

the most association with a place, a feeling or food. The word ‘fresh’ can mean a multitude of different things depending on language or culture. Translation of words and emotions into sensory impact is the service offered by the fragrance industry, and the client brief is the starting point for the understanding to begin.

The creative task is simplified by guiding clients by means of a printed ‘Perfume Brief Document’ to produce an *aide-memoire* against which sales and marketing people will check, marrying smell with market and consumer data to determine the commercial suitability of the scent. A fragrance supplier will receive new briefs every day, most of which are not for high-value fine fragrances, but for more everyday functional products, where the price limit is likely to be one of the major challenges for the perfumer. Table 6.1 and Figure 6.1 give a brief description of the kinds of response and background that are sought, in both list and visual forms. The more information that can be shared between client and supplier, the more chance there is of fragrance fit. For this reason the relationship between manufacturer and supplier is one that has to be based on complete partnership, for the perfumer is trusted with commercially sensitive information.

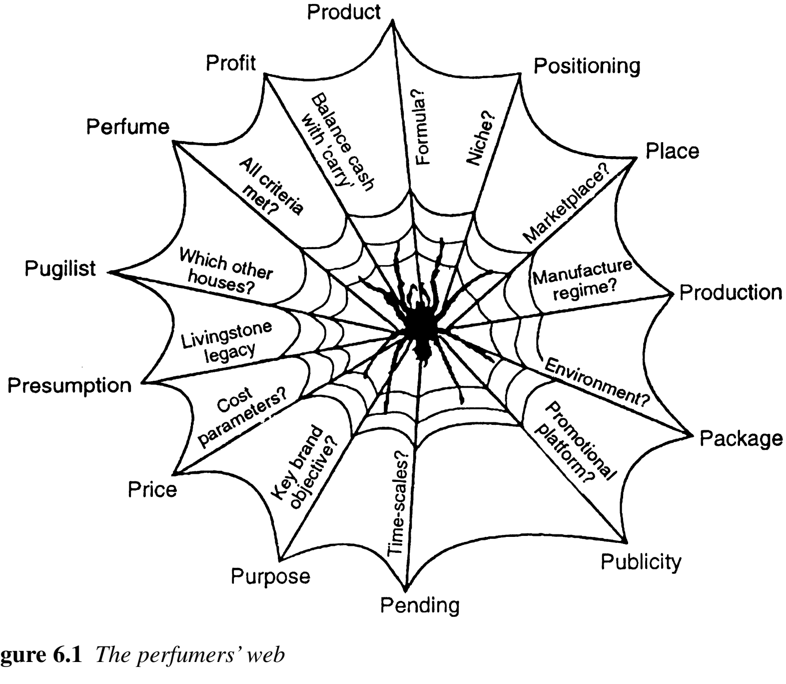

Figure 6.1 *The perfumers’ web*

6.1 BRIEF FOR EVE – PREPARED BY BUSINESS SCENTS LTD

6.1.1 Background

Business Scents Ltd wishes to launch a new female fine fragrance this year that will complement the successful launch of the 'Ninevah' range, launched in 2006, and essentially the hope is that a roll out will occur in Europe and the United States, followed by Asia Pacific, over a 2-year time span. If successful, it is intended to add line extensions to the range, code-named 'Eve,' to include an antiperspirant, soap, shampoo and shower gel. Because of its global market, any fragrance submitted should have 'global appeal' to the core market of females aged between 16 and 35 years, and we look to the fragrance house to also suggest possible names and marketing platforms.

6.1.2 Product Range

- *Alcoholic fine fragrance* – containing 90% denatured cosmetic-grade ethanol. Possibly light coloured – maybe a pastel shade.
- *Soap* – a predominantly vegetable base (80% palm, 20% coconut). Likely to be white, but could be coloured to support the fragrance concept.
- *Antiperspirant* – aerosol form using butane–propane blend as propellant, and packed in lacquer-lined aluminium cans. Should contain an active antiperspirant at an effective dosage (*e.g.* aluminium chlorhydrate).
- *Shampoo* – mild detergent system with built-in conditioners. Suitable for frequent use. Likely to be pearlised and coloured to support the fragrance. Packaging – polyethylene terephthalate (PET) bottle.
- *Shower–bath gel* – clear, high foaming and capable of moisturising the skin. Any advice on novel and useful botanical extracts? Packaging – low-density polyethylene or PET.

6.1.3 General

Product formulation advice is sought from the fragrance houses for products 2–5 to aid with initial stability-testing work and to guide the contract packer(s) with their development.

6.1.4 Fragrance

We seek a novel approach to the fragrance for Eve; perhaps featuring new aroma chemicals or at least an intriguing marketing platform that greets the new age. While Ninevah, our 2001 launch, has been extremely successful, we are averse to a fragrance in the same fragrance area (rose–jasmine floral, woody, moss, oriental) as the brand code-named ‘Eve’ is expected to strike a new direction. Along with a fragrance concept, we are open to thoughts on both name and a broad marketing platform for the perfume.

6.1.5 Timescale

A submission is required (maximum 1 kg) within 6 months of the brief date. Meantime, constant contact with Marketing at Business Scents Ltd is encouraged to determine whether the directions being reviewed are realistic. Interim submissions and dialogue are suggested. We require only one submission per fragrance house, although it is likely that we will develop both the theme and direction of the fragrance at a later stage with the house chosen from this initial brief.

6.1.6 Brief Recipients

There are three recipients for this brief, namely Quest International, Smells ‘R’ Us, and Panaroma.

6.1.7 Price

We do not envisage a cost in excess of US$50 a kilo on a delivered basis to our main manufacturing unit in Holland for the fine fragrance perfume. Modified perfume in the same fragrance direction, but workable in appropriate bases, should cost no more than US$30 a kilo for soap, shampoo, shower gel and antiperspirant formulations.

6.1.8 Production

You are familiar with all our production methods for fine fragrance, soaps, shower gels, shampoos and antiperspirants having had the details of equipment from the ‘Ninevah’ brief. Direct contact with our contract packers is recommended to review or update your knowledge of these methods, and how they impact on perfume dosage.

6.2 TOXICOLOGY

All ingredients used must comply with current RIFM or IFRA standards and conform to COLIPA recommendations.

We look forward to dialogue with your company over the next 3 months, and to your final submission to this exciting project.

CHAPTER 7

Perfume Creation: The Role of the Perfumer

LES SMALL
Consultant Perfumer, Quest International

Business Scents Ltd has briefed Quest to repeat their success of the late 1990s, in which a popular fine fragrance launch was followed by a successful launch of a series of trickle-down products, namely a soap, a shampoo, a shower gel and an antiperspirant. The brief will be known as ‘Project Eve’.

Market research indicates that an exotic tropical, fruity, watery, floral, muguet (lily-of-the-valley) theme would be a likely winner (see Chapter 9).

At the outset a perfumer starts with a blank sheet of paper (or an empty computer screen). From where does he/she get ideas and inspiration? What is currently fashionable? The name of the game is to imagine the odour effect that the Business Scents Ltd product will exhibit in use. How should the alcoholic product smell from the bottle or atomizer? How will it develop on the skin once applied? How long should it last between applications? Once these decisions have been made, the perfumer can start compiling a list of the fragrance raw materials that he wishes to use in order to achieve the desired effect. At first sight there would appear to be hundreds of ingredients to choose from, but in practice there are a number of restricting factors. This concept of freedom of choice of ingredients can be described by employing the analogy of Degrees of Freedom as used in physical chemistry in which a system is constrained by a number of factors. The eight degrees of freedom in perfumery are as follows:

- skin safety;
- environmental safety;

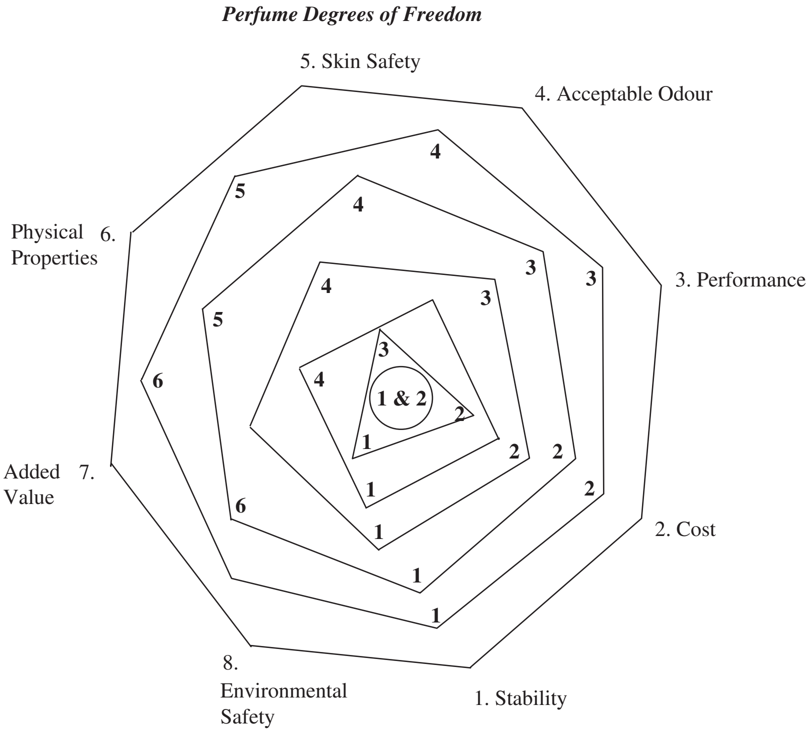

Figure 7.1 *Perfumery degrees of freedom*

- acceptable odour;
- cost;
- stability;
- performance;
- physical properties (*e.g.* solubility, colour); and
- added value (*e.g.* malodour counteractancy, insect repellency).

Figure 7.1 illustrates a series of concentric polygons; it is an attempt to visualise the concept of the perfumery degrees of freedom. The outer octagon represents the system with no constraints, *i.e.* all eight degrees of freedom are available. If a constraint is introduced, for instance *stability*, then the polygon becomes a heptagon and the area contracts. This is analogous to restricting the number of ingredients that can be employed in the creation of the masterpiece! As more constraints are introduced one by one, the polygons become smaller and the number of sides diminish accordingly. Eventually, one arrives at a small area in the centre where nearly all the degrees of freedom have been lost. This is the situation in which relatively few perfume ingredients are available owing to the hostile nature of the medium to be fragranced, for example a limescale remover based on sulfamic acid. Perfume can often provide *added value* to a product mix. For example, it is possible to provide deodorant protection for a soap or deodorant/antiperspirant by combining certain chemical classes of ingredients according to a patented set of rules (Deo Patent Filings).

For an alcoholic fragrance there is almost complete freedom of choice; there is the possibility of acetal formation due to the interaction of the aldehyde and ketone functional groups with alcohol being used as the carrier, but this often has a softening effect on the fragrance and so is not perceived as a disadvantage. There are no other serious chemical constraints on the choice of ingredients, *i.e.* most of the ingredients are *stable* in the aqueous alcoholic product. We shall see later that stability plays a major role in the choice of ingredients for an antiperspirant product.

The *safety* constraint has been defined in the brief and can be checked by the computer or safety officer at any time.

One of the ways in which ingredients can be classified is by their perceived odour properties. The most important facet, as far as the perfumer is concerned, is the odour description. Each perfume company tends to have its own system of classifying odour, but in general there is a set of odour families that tend to be subdivided into odour descriptors. The other important properties are the odour intensity (and how sensitive is the intensity to dilution?) and the longevity of the ingredient on a relevant substrate – in this instance the skin. During the development phase, the relative longevity of initial perfume trials is compared on smelling blotters (thin absorbent paper strips); only towards the end will the perfumer fine-tune with trials on the skin. This is done for the practical reasons of keeping the immediate environment as odour-free as possible and that space on the perfumer's arms is limited!

A search can then be made for ingredients with the desired odour characteristics. For the Business Scents Ltd brief, market research has indicated that exotic, tropical, fruity, watery and floral muguet notes are desirable. This is the *acceptable odour* (hedonic) constraint in our degrees of freedom analogy. An initial trawl through the various databases would indicate several dozen contenders. Many of the tropical, fruity notes will have ester or sulfurous functional grouping, whilst there are several aldehyde groups which exhibit the desired watery muguet character, *e.g.* Lily Aldehyde®, Lyral®, cyclamen aldehyde, Bourgeonal® and hydroxycitronellal. Interestingly, a perfumer can often distinguish the typical odour of certain functional groups. Salicylates tend to have a woody character, which is modified by the length of the attached aliphatic chain or ring structure. Nitriles often smell somewhat metallic. Phenylacetates usually exhibit aspects of honey, *etc*.

The perfumer will probably consult his colleagues in the Natural Products Analysis Department in order to get new ideas for natural tropical, fruity notes (see Chapter 13). They will provide some headspace analyses which should add interesting, novel top-notes to the fragrance creation. In this case we will use a headspace analysis based on a selection of tropical fruits purchased from a market stall in Jakarta, Indonesia. Having checked, with our Buying Department, the commercial availability of the chemicals reported in the headspace analysis, several of those available have been incorporated into a perfume called *Tropical Cocktail*. This has been used as a building block at approximately 2% in the final fragrance mix. Details of the headspace analysis of muguet and broom have also been provided and the decision has been made to use a small quantity of broom absolute in the ‘Project Eve’ fragrance in order to add a natural, fresh florality which would fit well with the watery muguet theme.

After having collected ideas for a set of watery floral ingredients and some exotic fruity ingredients, the perfumer starts to put the ingredients together in groups. The watery ingredients could be blended with other light floral and woody ones. Similarly, an accord of exotic fruity materials may be combined with other suitable odorous ingredients, such as musk, and citrus notes, for example bergamot and grapefruit. These two accords will then be mixed in various ratios until a satisfactory blend is achieved. Other ingredients can be added one by one to this composition in order to modify the effect. For example, touches of camomile oil will add an interesting twist to the fruity top-notes, whilst a small quantity of Amberlyn® will provide subtle amber end-notes. The process is iterative in that a trial formula is made, assessed on a smelling blotter, formula adjusted, reassessed, *etc*. At all times, the overall evaporation profile of the fragrance will be considered together with any *cost* and *skin safety* constraints set by the customer. The ingredients used will have a range of volatilities and intensities and need to be blended carefully together so that no single ingredient dominates the effect of the others. The overall effect, as the perfume evaporates on the warm skin, should be a gradual change from the volatile top-notes of the fragrance through the middle-note theme to the eventual end-notes retained on the skin. The 'shape' of the perfume should be maintained at all times, so for example as soon as a volatile note such as say a fruity ester has evaporated there should be a less volatile fruity note available to maintain the fruity theme in the middle period of the dry-down. Similarly, at the end of the fragrance lifecycle on the skin there should be even less volatile fruity notes (*e.g.* lactones) available. In the same way the green muguet floral notes should keep their shape throughout the evaporation period. For an alcoholic fragrance the top-notes approximately represent the first 15 min of the evaporation, the middle-notes account for the next 3–4 h and constitute the heart of the fragrance, while the end-notes represent the final 5–8 h and give tenacity and depth to the theme.

Once the fragrance has been evaluated on skin and approved by the evaluation panel, the perfumer can start making versions for the other products in the Business Scents Ltd range, namely soap, shampoo, shower and bath gel and antiperspirant.

The first problem to be encountered will almost certainly be that of cost. The client will not be willing to pay the same amount of money for the perfume oil used in the range as for that used in the alcoholic fragrance.

Additionally, each of these products will have associated with them different challenges to the perfumer, who will seek the advice of many of his colleagues within the company. Some of the questions that will be posed are as follows.

7.1 SOAP

If the product is to be a white soap, will any of the ingredients in the perfume formula cause discoloration either immediately or in time? How will the total perfume perform in the soap bar? Will it cover the fatty smell of the base? If not, which ingredients perform best in the dry bar and in use? In order to overcome some of these problems, certain ingredients in the alcoholic fragrance will need to be substituted. For example, to prevent discoloration on storage, the vanillin will be substituted by ethylvanillin, which, because it is more intense, can be dosed at about one-third the vanillin level. Small quantities of Ultravanil® will also be incorporated to boost the vanilla effect without causing discoloration. Indole will be replaced by Indolal® at a slightly higher level, as it gives a less intense animalic jasmine character to the soap. Other ingredients need to be substituted because of their relatively high cost contribution to the perfume formula. However, since many natural oils and absolutes also possess a correspondingly high odour strength and quality, the ratio of the cost to strength/quality should always be considered carefully before removing them. In this case it is difficult to justify the inclusion of the broom absolute in the soap context, so in its place I will add a small quantity of methyl anthranilate (the chemical quantitatively dominating the headspace of the broom). Care should always be taken with anthranilates due to the possible formation of Schiff's bases upon reaction with aldehydes, and extra aldehydes will certainly be added to the soap version of the alcoholic perfume because of their excellent odour performance in covering the fatty smell of common soap bases.

7.2 SHAMPOO

Discoloration and performance issues are important. For example, will the perfume diffuse from the shampoo pack and then again as the hair is being washed in warm water? Will it be substantive on the dry hair? Will it be soluble in the shampoo base? In Chapter 12, the issues relevant to predicting the performance of fragrance chemicals in certain situations are discussed. Molecules with a low relative molecular mass (RMM), for example ethyl hexanoate and limonene, will diffuse most speedily on opening the cap of the shampoo bottle. Furthermore, depending upon their $\log P$ value, they will be more or less 'happy' in the mainly aqueous environment of the shampoo: the higher the $\log P$ value the more hydrophobic the molecules and vice versa. Hence molecules such as limonene with relatively high $\log P$ value ($=4.46$) will diffuse more readily than more polar molecules of similar RMM, such as 2-phenylethanol (phenyl ethyl alcohol or PEA) whose $\log P$ value is 1.52. Other factors complicate the situation and must be taken into consideration. One important factor is the odour intensity of the molecule. A few very potent molecules in the headspace can give a greater odour appreciation than a large number of low-intensity molecules. Another consideration is the active detergent level of the shampoo. This will affect both the appreciation of the fragrance in the headspace above the shampoo and the solubility of the perfume oil in the detergent system. A system with a low concentration of active detergent will have a smaller reservoir of micelles in which to solubilise the perfume mixture. Thus more perfume is available for the headspace because less can be 'dissolved' in the shampoo base.

Substantivity on the hair is another important consideration when creating shampoo fragrances. The consumer expects his or her hair to be perceived as clean and fresh. This impression is closely linked to the longevity of the perfume ingredients on the hair. In this case the molecules with high RMM values will tend to be the ones remaining on the hair after the rinsing and drying process. Again the odour intensity must be sufficiently high for detection by the nose as the number of molecules laid down on the hair surface is relatively small. Chapter 11 can also give clues as to which fragrance molecules are most likely to be retained by the hair protein rather than disappearing down the plughole along with the foam! Molecules with low log P value will tend to stay with the water. In fact, the perfumer will also rely on substantivity data obtained by painstaking, empirical, ingredient studies. Dozens of hair switches are washed, rinsed and then dried following a protocol developed to distinguish between the good, poor or indifferent ingredients when assessed by a panel of perfumers. This information is then added to the perfumer's knowledge base for future use.

So, for the shampoo version, the same changes for avoiding discoloration are made as those for the soap version. However, the percentage weight of citrus oils is increased to boost the limonene content. The fruity notes are also made more dominant, as these are favoured by many shampoo consumers. It will be necessary to check that the watery, floral, tropical and fruity notes are detectable on the hair after the shampooing process.

7.3 SHOWER AND BATH GEL

Here again, issues of discoloration and performance are important. In addition to hair substantivity, retentivity on the body must be considered. In general, the perfume dosage in a shower gel or bath product is double than that in a shampoo so the effect of any potentially discolouring ingredients would be accentuated. The higher perfume level would certainly help fragrance retentivity. However, substantivity on skin is usually less than that on hair for three reasons. First, the skin is warm and hence the rate of perfume vaporisation is higher. Second, the skin is continually secreting other chemicals onto the surface, which can interact with deposited fragrance. Lastly, the skin is less porous in nature than the hair so there are potentially fewer binding sites for the perfume molecules. If the product is destined for use during the bath then diffusion from the bath water becomes important.

7.4 ANTIPERSPIRANT

Here the problems are associated with possible discoloration and stability with the acidic aluminium chlorhydrate (the active ingredient). In Chapter 15, the reasons for the poor stability of Lily Aldehyde®, hydroxycitronellal and cyclamen aldehyde in such a medium have been explained. All three are present in the 'Project Eve' fragrance. In Chapter 10, the empirical stability testing conducted on a range of perfume ingredients in antiperspirant media has been described. This knowledge base is available for screening the 'Project Eve' perfume for possible problem-causing ingredients. Bearing this in mind, it will be necessary to substitute the Lily Aldehyde®, hydroxycitronellal and cyclamen aldehyde in the perfume formula with Florosa® together with a small quantity of Bourgeonal®. Florosa® is very stable in antiperspirant and although Bourgeonal® is not totally stable it is so intense that only traces are required to deliver the desired watery floral effect. Similarly, other relatively unstable ingredients will be substituted by more stable, better performing materials, whilst always maintaining the overall theme or shape of the composition.

So, it can be seen that the role of the perfumer is a blend of an artist and a scientist. The perfumer is continually attempting to bring an imagined odour into being *via* a written perfume formulation in the same way that a composer imagines a symphony and realises it by writing the notes as a score. As in all creative processes, there is a period of reflection prior to starting work. In order to create a pleasant fragrance in a reactive medium, the perfumer must have a full understanding of the odour and physical and chemical properties of the available raw materials. Further discussion on the notion of perfume creativity is outside the scope of this book, but the subject is dealt with more thoroughly in the article entitled 'Perfumery is it art?' in *Manufacturing Chemist* September, 1988.

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CHAPTER 8

Measurement of Fragrance Perception

ANNE CHURCHILL
Quest International

8.1 INTRODUCTION

A wide range of factors affect how we perceive a product and how we choose one product in preference to another. The human race is inherently variable in its sensitivity to different sensory stimuli, to such an extent that while some of us, for instance, can smell a musky odour quite distinctly while others are completely blind or anosmic to it. Above this, the way that each of us responds to a stimulus depends on our previous experiences and associations; so, while one person may find an odour comforting and nostalgic, another person may be completely indifferent to it.

Sensory Analysis and Market Research are two major disciplines that enable us to measure human responses to sensory stimuli. Both rely on well-researched and rigorous techniques and a fundamental understanding of the difficulties involved in working with human subjects, such as those described above.

Sensory Analysis is concerned with quantifying human responses to stimuli. It is a precise, descriptive and measuring technique that characterizes the stimulus. In this case, the particular concern is to evaluate the odour of a perfume, perfume ingredient or perfumed product. This is an important process in enabling the perfumer to understand and quantify the sensory characteristics of the product, as only then can they be manipulated in a controlled way as part of the creative process.

The evaluative and subjective associations made by the consumer can be understood using Market Research techniques. If the market is understood, fragrances can be developed to match or enhance the image of a particular product or market segment. Sensory Analysis is also an important tool in this process. Using powerful statistical techniques, the odour relationships between different products or perfumes can be characterized and quantified, and the results combined with Market Research to enable the subjective associations to be interpreted in terms of odour.

Our sensory tools have for many years been designed to measure perception of the senses in isolation. Recent research has shown that our brains are constantly integrating the information that we receive from all our senses into the multisensory experiences that fill our everyday lives. Recent developments in Sensory Science and Experimental Psychology have focussed on understanding sensory integration and how the perceptions of one sense can very often determine how we experience something in another sense. This integration also means that consumers find it difficult to describe what they perceive using each sense totally segregated. This means that in addition to the more direct measurement techniques of Market Research and Sensory Analysis, we need to investigate more indirect techniques and start to build an understanding of how the senses interact.

Emotion is another aspect of product perception which is difficult to measure using the direct techniques of Sensory Analysis and Market Research; yet, can also strongly influence how a consumer perceives a product. Odours evoke emotional responses, which may take the form of bringing back memories of situations past or simply evoking feelings such as warmth or comfort. Research is currently focussed on understanding how the brain interprets the olfactory messages to convey these emotions, and ways of measuring these responses.

In answering a customer brief such as that from Business Scents Ltd, a perfumer welcomes as much guidance as possible in how to win that brief successfully. The four disciplines mentioned above (Sensory Analysis, Market Research, Statistics and Psychology) together form a powerful analytical and predictive tool, different aspects of which can be used for guidance in perfume creation depending on the requirements of the brief. In the sections that follow in this chapter, a brief outline of the techniques currently used within each of these areas of expertise is given, as are examples of how they could be used to fulfil the Business Scents brief.

8.2 MARKET RESEARCH

Market Research is concerned with understanding a market and the consumers making up the market. It involves the systematic collection, analysis and interpretation of information relevant to marketing decisions.

Although consumers are able to state which perfumes or products they prefer, they are often unable to explain why. One of the key skills in good market research lies in the design of questions that are able to find out as much as possible about the reasons behind the consumer preferences and choices. Techniques used include quantitative methods based on rigorous sampling methods as well as qualitative techniques that probe the complex consumer perceptions and motivations.

Most of us, at one time or another must have been stopped in the street while out shopping and asked to complete a questionnaire, or received a telephone call or a letter through the post, similarly asking us to answer a few questions. This is data collection, the visible part of quantitative market research. Fieldwork normally involves interviewing and completing a questionnaire, often asking respondents to sample products and then answer questions about them, including ratings for items such as liking and propensity to buy. Having collected the data, it is collated, analysed and interpreted in order to present the data in a meaningful way.

Qualitative information is based on ‘understanding’. Much qualitative research is concerned with empathising with the consumer and establishing the meaning that he or she attaches to products or brands. Another focus is motivation; *e.g.*, why does one product rather than another meet consumer needs and what are these needs that are being met? Focus groups are a common approach to collecting qualitative information. A group of six or eight respondents, led by a trained group leader, will discuss or perhaps smell and comment on a range of products. The trained leader is able to probe the discussions in order to elicit more elaborative information and attempts to draw out the respondents’ motivations.

8.3 SENSORY ANALYSIS

Sensory Analysis involves using human subjects as a measuring tool. This presents an immediate problem, as individuals are innately variable, not only as a result of their experiences or expectations, but also as a result of their sensitivity. Thus, each person could genuinely perceive the same product quite differently. It is therefore essential in every sensory test that all variables, except that actually under test, are as carefully controlled as possible to minimize this variability.

A purpose-built sensory panel suite enables testing to be carried out in an environment free of noise or movement, and in rooms that are painted in a neutral colour and fitted with individual booths for each panellist. When testing is carried out, all samples are presented unlabelled so that the panellists do not receive any cues concerning the nature of the product, and the sample appearance is identical; the only variable (as far as possible) is the smell.

Having controlled the environment and the sample, the only other major variable is the panellists. To minimize panellist variability, all panellists are selected for their sensitivity to smell and their short-term odour memory. They undergo 3 months training to be able to carry out discrimination (is there a difference between the two samples?) tests and be able to scale the perceived intensity of an odour in a consistent and reproducible way. A further 3 months training then follows during which panellists either learn to be able to recognise (and name) a range of odour standards and be able to identify them in a complex mixture. Or, they are trained to be able to generate 'consumer' vocabulary to describe products and develop a consensus attribute list that can then be used to profile the samples. These are all highly specialised skills that the average person on the street simply does not possess.

Odour can be described using a number of different dimensions, each of which can be measured using different sensory tests: threshold, intensity and quality (odour profile or qualitative descriptive analysis).

8.3.1 Threshold

Threshold itself can be described at three different levels as follows:

- Detection threshold: Can an odour be detected?
- Recognition threshold: Can an odour be identified?
- Difference threshold: Is there a difference between two odours?

The detection threshold is the lowest stimulus intensity (odour concentration) that the subject can distinguish from an odour-free situation. The subject's response indicates whether the presence of an odour has been perceived or not. Correspondingly, the recognition threshold is the minimum concentration at which an odour can be identified.

Such data provide important fundamental information, such as identifying levels at which an ingredient can be perceived in a product base, or quantitative information describing the activity of odour materials.

8.3.2 Intensity

How strong is an odour? A number of different types of sensory scales are used to measure perceived intensity of odour, two examples of which are given in Figure 8.1. Alternatively, panellists can be trained to use a form of scaling, whereby they allot their own scores to the perceived intensity of odours and score subsequent odours in ratio to one another; this is known as ratio scaling, and it allows a more flexible and arguably more accurate measurement of perceived intensity.

When measuring odour intensity it is important to be aware of Stevens's law, which states that equal changes in stimulus magnitude (S) produce the corresponding change in perceived intensity *(I).* The law can be expressed as a power function, as in equations (1) and (2);

$$I = cSk \tag{1}$$

or

$$\log I = k \log S + \log c \tag{2}$$

i.e. the log of perceived intensity is directly related to the log of the stimulus magnitude.

The increase in perceived intensity with concentration can be represented by a straight line, as shown in Figure 8.2, for odorants A and B. The slope indicates how fast odour intensity rises with concentration and the intercept defines the detection threshold. The following are the two important characteristics of this type of data:

- At higher perfume concentrations, a larger increase in concentration is necessary to give the same change in perceived intensity.
- At different concentrations of two odorants, the rank order of their perceived odour intensity can change. This is true for odorants

Structured scale	
0	*No odour*
1	*Very weak (threshold)*
2	*Weak*
3	*Medium strong*
4	*Strong*
5	*Very strong*

Semi-Structured scale

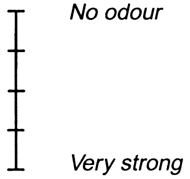

Figure 8.1 *Intensity scales*

A and B in Figure 8.2. At log *Cy,* odorant A is stronger than B; but at log *Cx*, odorant B is perceived as stronger than odorant A.

8.3.3 Quality

What does this odour smell like? The tests used to describe odour quality are known as odour profiling tests. These are the most complex of the sensory tests and, to ensure good quality, accurate and reproducible data are carried out only by highly trained and experienced sensory panellists.

8.3.3.1 Qualitative Descriptive Analysis. Qualitative descriptive analysis (QDA) is a technique developed using trained, in-house panels of assessors. The panellists are presented with the range of products to be assessed and spend their first panel session discussing the properties that they feel are the most important in describing the products and the differences between them. These usually include evaluative attributes, such as refreshing or old fashioned, as well as odour descriptions, such as flowery or medicinal. The panellists agree to a list of attributes and may also agree standards to help them describe the attributes. A series of panel tests is carried out, during which each panellist scores the products on each attribute in the agreed list. The results are averaged across the panel to give a QDA profile of each product, which may be presented as charts or graphs, or analysed in more detail using multivariate techniques (described later).

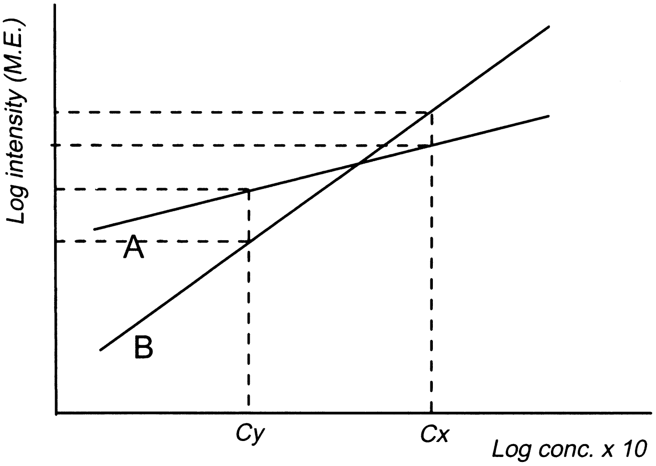

Figure 8.2 *Log perceived intensity vs. log perfume concentration for two odourants*

8.3.3.2 Odour Profiling. Each odour is smelled by the panellist, who then scores the perceived intensity of each odour character that she or he can detect (referring to the set of standard odour references for clarification if necessary), which results in a sensory profile for that odour. A minimum of at least 20 profiles is usually collected for each sample and an average profile is then calculated. A set of typical odour profiles is shown in Figure 8.3. These profiles show the differences in perceived intensity of 13 odour characteristics identified in seven perfume materials, and immediately it is possible to see that although all the materials are floral or muguet in character, one material is far more fruity (cyclamen aldehyde) and another (Mayol®) is far more herbal than the other materials.

Sensory profiling techniques are designed to produce stable and reproducible data, but difficulties arise when trying to compare data obtained from different laboratories. Often the methods of sensory assessment differ and there is no universally accepted odour language or list of odour standards to clarify this problem.

8.3.4 Statistical Techniques

Fragrance profiles are often presented as a series of complex tables or graphs from which patterns, order or exceptions need to be found. Looking at individual profiles it is possible to determine the main odour

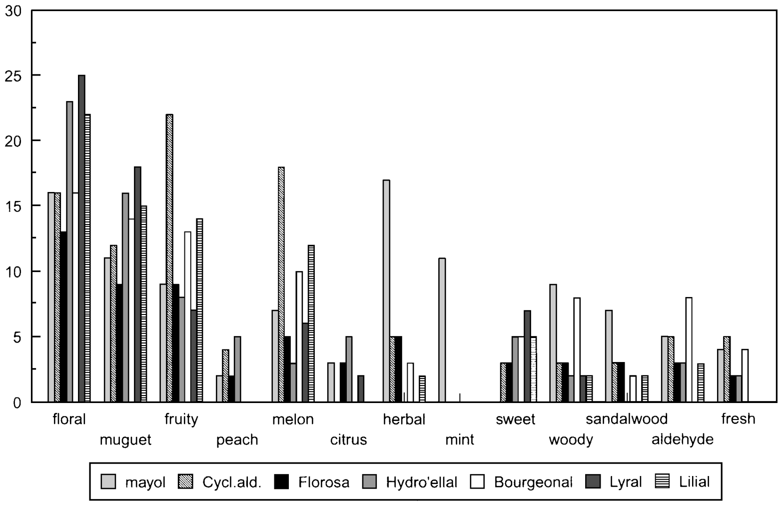

Figure 8.3 *Odour profiles of a selection of muguet perfumery materials*

characteristics. Comparison with the other profiles establishes the main differences between them.

However, this sort of comparison becomes extremely complex when large numbers of perfumes are involved. Multivariate Analysis methods are descriptive procedures that help in this process. These methods are used to model or describe data, such that they can be more easily understood by the researcher and thus simplify data comparison. Sophisticated software now exists that makes this kind of analysis possible without it being necessary to understand fully the mathematical modelling involved in the analysis. However, to interpret and understand the results a basic understanding of the technique is necessary.

8.3.4.1 Multidimensional Scaling. Multidimensional Scaling is just one of the multivariate techniques available. To be asked to take a map of England and measure with a ruler the distance between 20 towns is a fairly straightforward project. Multidimensional scaling does the opposite of this; it takes a set of distances and recreates the map. The ‘distances’ in this case are derived from our sensory panel data by comparing the profiles of every possible pair of samples and deriving a value that represents the overall similarity between each pair. The resulting map places the samples spatially, so that those that are most similar to each other are shown closest together on the map; as samples become progressively more different they are shown progressively further apart.

8.3.4.2 Principal Component Analysis. Principal Component Analysis (PCA) is another technique used; it works differently, but the resulting map can often look very similar. The analysis works using a process of identifying correlations between the different variables used in describing the data (*i.e.* the sensory descriptors used in the profiles). It then searches for the combination of variables that best describes the maximum amount of variation in the data, and draws an axis through the centre of the group of observations, so that the sum of the residual distances is minimized; this is called the first Principal Component. The second Principal Component is then drawn at right angles to the first and explains the maximum amount of the remaining variation.

In addition to depicting the associations among the original variables, PCA can be used to describe the relative ‘locations’ of the measured samples. A plot of principal component scores for a set of products reveals groupings of the samples that may not have been readily apparent from the original data. Figure 8.4 is a multidimensional scaling map derived from the profiling data shown in Figure 8.3. With only seven

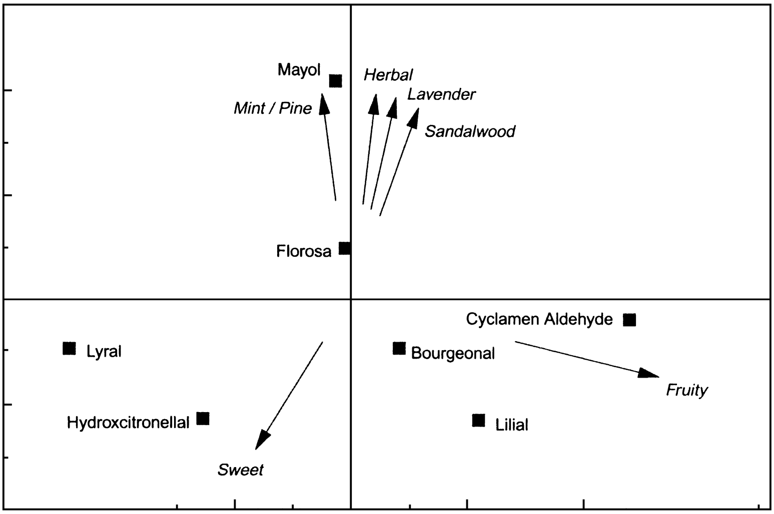

Figure 8.4 *Multidimensional scaling map of muguet perfumery materials*

samples to compare, this is a very simple map; however, it serves to illustrate the kind of information that can be derived from this type of analysis.

The samples are grouped so that those with the most similar odour character are closest together on the map *(e.g.* Lilial®and Bourgeonal®), and those that are most different are furthest apart *(e.g.* cyclamen aldehyde and Lyral®). The arrows indicate the direction of increasing perception of the odour characteristics shown. Only the odour characteristics that significantly correlate with the distribution of the samples across the map are shown; these are the characteristics that are responsible for the systematic differences between the samples.

All the materials were perceived to be floral and muguet in character, so these characteristics are not shown on the map. Cyclamen aldehyde, Lilial® and Bourgeonal® are the most fruity of the samples and are grouped together on the right-hand side of the map, while Lyral®and hydroxycitronellal are the sweetest materials and Mayol® is the most herbal.

8.4 MULTISENSORY APPROACH

We have five senses. Eyes to see, ears to hear, skin to feel and a nose and mouth to smell and taste the world around us. Each of the senses are specialised to provide information about different aspects of our environments. However, there is also a growing understanding that our perception of the world around us relies on an integration of perceptions from all of our senses. This is also relevant in how we perceive and make choices about products. Quest has worked closely with Dr. Charles Spence at Oxford University, who is an international expert in this area, in order to develop an understanding of multisensory perception. This section draws heavily on a review written by Dr. Spence in order to draw together the current thinking in this area (Spence, 2002).

Throughout history, vision has been considered to be the dominant sense. In comparison to other animals, far more of our brains are given over to processing what we see than processing other forms of sensory stimulation. Because of this our other senses can be completely deceived by what we see and several examples of this exist. For example, the perception of the flavour of a food or drink can be altered by what it looks like. In one study, Du Bose and his research team (Du Bose *et al.*, 1980) demonstrated that while most people perceived a cherry-flavoured drink to the taste of cherry when it was coloured cherry-red, many thought it tasted of lime when it was coloured green, and nearly 20% thought it tasted of orange when coloured orange. The pleasure that we get from consuming food and drink can also be enhanced if it looks right. People prefer odours like the smell of fruit if the product is of an appropriate colour (*e.g.* Andre *et al.*, 1970, Christensen, 1983, Zellner *et al.*, 1991).

In humans, hearing is one of the most important senses for social contact and interaction (Hopkins, 1994). Hearing along with vision are our main distance senses. Hearing is the sense that provides us with an excellent early warning system, ideal for alerting us to potential danger by helping to make us aware of things that are obscured visually. But our hearing can also be deceived by what we see. An excellent example of this occurs when we are at the cinema. We perceive the sounds of people speaking in the film to come from the lips of those who are seen on the screen, but in fact the sound comes from the speakers situated elsewhere.

Touch is the first of our senses to develop, and it provides us with our most fundamental means of contact with the world (Gottlieb, 1971, Barnett, 1972). We use touch to share our feelings with others and our first lessons in loving come through the cuddling we receive as a child. Despite the fact that the sense of touch plays an important role in our emotional well being, touch has been something of a neglected sense in recent years. Both hearing and vision can dramatically change what we feel. This is illustrated by the ‘parchment skin illusion’. Jousmäki and Hari (1998) reported that they could make people feel as if their hands were smoother and drier, or rougher and moister simply by manipulating the sounds that the participants heard when they rubbed their hands together (by either boosting or cutting all high frequency sounds above 2 kHz).

This highlights one of the most interesting aspects of sensory interactions. While intuitively we might expect that the only way to change how something feels is by changing its tactile qualities, contemporary research now supports the view that often the most effective way to change how we experience things in one sensory modality is by changing what we perceive in another ‘unattended’ modality.

The two chemical senses, taste and smell, enable us to react to foodstuffs placed in the mouth. We rely on taste and smell to distinguish between edible and inedible, and to advise us about how desirable a food might be. These senses are also the source of much human pleasure and satisfaction.

Smell is the sense most closely linked to the emotional centres of our brain. Although more of our brain is dedicated to vision than to any of the other senses, more of our genes are devoted to the sense of smell (1%) than to the detection of all the other kinds of sensory information. We possess an excellent ability to detect and discriminate odours but we have great difficulty in describing them verbally. Despite this fact, odours have an extraordinary ability to remind us of events and memories of our past, sometimes from many years ago.

The sense of smell is not difficult to deceive. During a television programme about the senses, O’Mahony (1978) told the audience that when they heard a tone they would experience a pleasant country smell. Many viewers wrote in to report that they had perceived the smell of grass or hay. Several people even wrote to complain that they had suffered from attacks of hay fever and sneezing after listening to the tone. The belief that an odour was present was sufficient to trigger these sensations in many people even though the odour was not actually there.

Although the sense of smell is easy to deceive, there is evidence that it can be a defining influence over the more emotional and associative drivers of our choices. This can have confusing influences on our understanding of why we have made specific decisions. An example of this was demonstrated by Laird in 1932, who interviewed 250 housewives to ask about their preferences for a selection of silk stockings. Fifty percent of the women preferred the silk stockings that had a slight narcissus scent over other pairs of stockings that had either a natural, fruity or sachet scent, even though all of the stockings were in fact identical. The women did not attribute their preferences to the smell but to the durability, sheen or weave instead.

In another study, Power (1956) did a market test of shampoos. He reported that the rating of one particular shampoo was changed from the bottom to the top rank out of a range of different products simply by improving its fragrance. As in the case of the stockings, people reported that the shampoo (with the improved fragrance) rinsed better, lathered better and even gave more lustre and shine.

It is surprising that sensory dominance can have such a profound effect on our perceptions. It is even more surprising that the changes in perception due to sensory dominance can be much greater than could be achieved by actual changes (as in the parchment hand illusion).

Much of what we perceive in the world around us is determined by the consequences of sensory dominance. We are not normally aware of the effects that are modulating our perceptions, and this fact has dramatic consequences on our methods of measuring sensory responses to products. Our Market Research and Sensory Analysis techniques are designed to measure peoples' conscious perceptions, but as researchers we also need to be aware of how the other senses influence this perception. The knowledge of the mechanisms of sensory dominance and sensory integration is growing rapidly and offers some exciting possibilities for the future. By developing holistic sensory (involving all the senses) products for the future, we will heighten the total sensory effect and increase the pleasure derived from using a product.

8.5 PSYCHOLOGY OF PERFUME

The process by which olfactory messages are interpreted is not yet fully understood, but it is known that olfactory messages are transmitted from the olfactory bulb along the olfactory nerve directly to the brain, where the path of the message divides into two. One route passes into the olfactory cortex at the front of the brain where identification and differentiation between odours occurs; the other passes into the limbic system at the centre of the brain. The limbic system is believed to be the emotional centre of the brain and it is here that many sensory messages are received and interpreted.

It is believed that this close link between the olfactive sense and the limbic region is the reason for such a close association between smell and emotion. In order to understand this link, researchers have studied the working of the brain. Until recently, few data were available that characterized the nature of odour processing in the brain centres higher than the olfactory bulb. The problem lies in the complexity of the higher brain structures, the lack of understanding of brain mechanisms and the difficulty in detecting and locating brain activity. There is now a variety of imaging techniques that have been developed to investigate the structure and functionality of the brain.

Brain imaging methods were primarily developed for two purposes: first, to visualize structural information about the brain, and second, to measure its functioning. The methods have been developed to facilitate clinical knowledge, diagnosis and treatment and each has its limitations when applied to functional research studies such as olfaction. The chief methods used for visualizing the living brain include standard radiographic methods, contrast radiography, computerized axial tomography, magnetic resonance imaging, positron emission tomography and electroencephalography (EEG).

At Quest, we use spontaneous EEG to measure electrical activity of the brain from the surface of the scalp. A widely available technique, this is also extremely resource-intensive; a typical clinical session may take several hours to complete, including the preparation of the subject, the EEG experiment and collation of the resultant data. The vast amounts of data recorded take time to digitize, summarize and analyse. Rapid software enhancements constantly improve this process, making it more accessible to a larger number of research groups.

In our work, we have found significant differences in the quantitative and topographic changes in brain activity recorded from the scalp following presentation of a range of odour types, and relationships have been found between specific features of the recorded signals and measurable effects of the same stimuli on moods or feelings.

8.6 THE BUSINESS SCENTS BRIEF

The brief from Business Scents Ltd requests submissions for a feminine luxury line that includes a fine fragrance, antiperspirant, shampoo/shower gel and soap. The perfumer has asked for guidelines for the appropriate odour area as soon as possible so that the creative work can begin.

8.6.1 Definition of the Appropriate Odour Area

We already have information available from a project completed recently reviewing the perfumes currently used in toilet-soap bars in Europe.

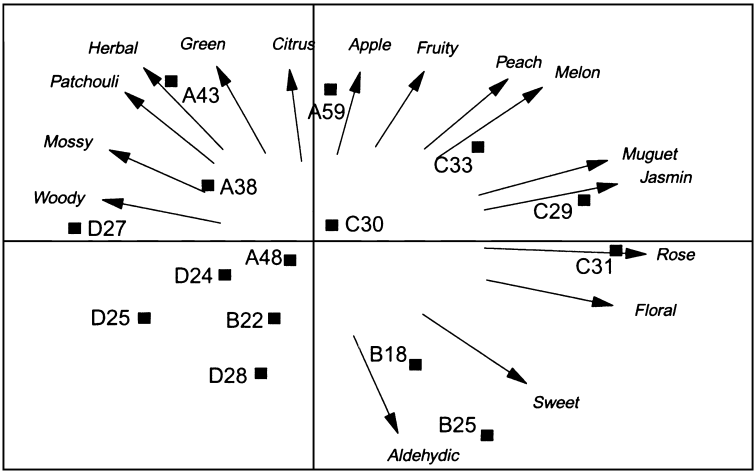

Figure 8.5 *Odour map of European toilet-soap bars*

A range of soaps was profiled by the sensory panel and the results were analysed to give a two-dimensional map, showing the relative similarities in odour character of the perfumes tested (Figure 8.5).

Those fragrances that are closest together on the map are most similar in odour character, whilst those that are furthest apart are most different. So, for instance, fragrances A59 and C33 are relatively similar in odour character, while A59 and D28 are quite different. To interpret the map and describe the nature of the differences between the odours, a correlation analysis is carried out. This analysis enables us to identify the characteristics that are most important in distinguishing between the fragrances, and the direction of increasing perception of each of these characteristics is indicated on the map with an arrow. So we can tell, for instance, that A59 is perceived to be far more fruity than D28.

We used a similar type of analysis to look for correlations between the odour character of the fragrances (defined by their position on the map) and results obtained from large-scale Market Research. The Market Research attributes found to be directly related to the odour perception of the samples are shown on the map in Figure 8.6, with arrows indicating the direction of increasing perception of each attribute.

The map indicates that the fragrances in the herbal, green and citrus areas are totally inappropriate for fragrance development for this brief: they are perceived by the consumer as functional, cleaning, invigorating and refreshing. Neither is the sweet floral, aldehydic area appropriate

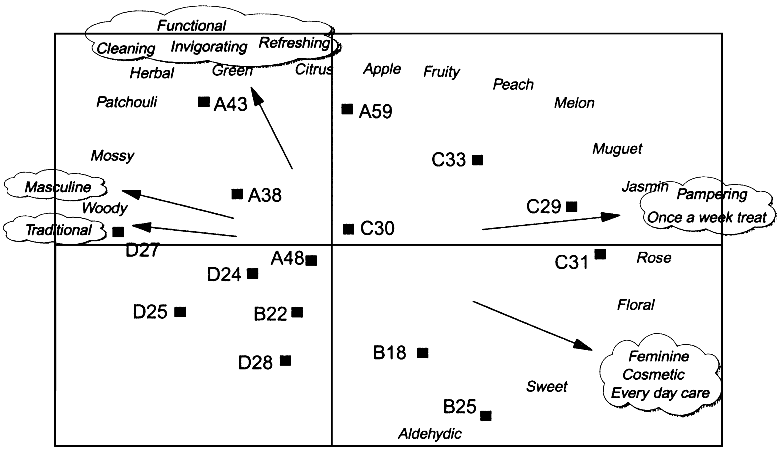

Figure 8.6 *Odour map of European toilet-soap bars with market research overlaid*

as, although it is seen as feminine, is also perceived as cosmetic or suitable for everyday care. The area that the perfumer needs to concentrate on is described as white floral (muguet, jasmine) and fruity (melon, peach), which is perceived to convey the impression of a product that pampers you, and would be used as a once-a-week treat.

8.7 ANALYSIS OF INGREDIENTS TO CONVEY THE APPROPRIATE ODOUR CHARACTERISTICS

In the above sections, the sensory profiling and multidimensional scaling techniques are illustrated using data obtained from the analysis of muguet perfumery materials. From a purely olfactory point of view, the type of odour that best fits the odour described in this brief is cyclamen aldehyde, but with the range of products that need to be considered in this brief, the choice of material is not quite so simple.

The chemistry of the stability of fragrance materials in different bases is discussed in Chapter 9. The olfactory implications of these changes can also be characterized using a sensory test, similar to that carried out with the muguet materials, but placing each at appropriate concentrations (or range of concentrations) in the target-product bases.

8.8 CREATING FRAGRANCES FOR THE FUTURE

As we progress through the twenty-first century, theories are being built and expanded upon that are almost self-fulfilling in creating the trends of the future in fashion, art, family values, *etc.* As fashion designers, social philosophers and economists design our clothes and way of life for the future, so perfume designers seek to create the smells that complement and reinforce this environment. At the same time, a new and exciting smell within the constraints of acceptability gives the designer the advantage of novelty so sought after in the industry.

With the powerful software currently available, it is possible to pull together information from a wide variety of sources to give the perfumer guidelines to help channel or expand creative ideas in a way that is calculated to produce a successful odour type. Traditionally, market research gathers information from consumers in retrospect, using perfumes that have been selected as appropriate for a product and measuring a response to those perfumes, which is inevitably based on experience. For a new trend-setting perfume style, we need to predict or create a popular perfume trend for the future.

A perfume company such as Quest International establishes a perfume and product profile on the basis of large studies on consumer responses (acquired from consumers of different age groups and social profiles) to differentiate perfumes in different products in different countries, over a number of years. At the same time, a profile is built of changing social patterns and how this affects acceptance of different perfume styles. As part of our on-going studies of evaluative responses to odour types, a profile is also built of the feelings or images that are conveyed by different odours.

The availability of evaluative and market research data together with social trends analysis and fashion information, and perfume stability and olfactory performance measures, provides the perfumer with a basis from which to work and add creative skill to develop the new fashion fragrances.

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CHAPTER 9

The Application of Fragrance

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Pertech Associates Ltd.

9.1 THE ROLE OF THE APPLICATIONS DEPARTMENT

An Applications Department within a fragrance house is responsible for 'applying' a fragrance oil or essence to a consumer product. This process in itself can be problematic, since the fragrance may not always be easily incorporated into the product base. Solubility of the fragrance could be a problem, for example, in an aqueous fabric conditioner system, a volatile, silicone-fluid-based antiperspirant or a hair dressing based on a mineral oil. This might manifest itself as a haziness, as floating droplets of fragrance or as complete separation of a clear or milky layer. Dosing of liquid fragrance into talc or powder detergent can prove difficult if perfume dosage levels are above the norm, affecting the powder's flow characteristics or causing lumping.

However, once the perfume has been successfully dosed into the product the job does not stop there. A number of other factors have to be studied to ensure that the perfume remains evenly dispersed within the product and does not cause its physical and chemical characteristics to change significantly over time. The integrity of the product needs to be checked over a period of accelerated storage at different conditions. The latter are often stipulated by the product manufacturer, who wants to ensure that the product's characteristics or activity are unaltered by the fragrance, even if exported to tropical climates or sub-Arctic conditions. However, equally important to the perfumer who is designing a new fragrance, or an evaluator who is selecting a perfume from a repertoire or 'shelf', is to monitor any changes in the fragrance odour once in the product base, for example

- fragrance intensity, that is does it still have a good impact?
- perfume character, that is does the perfume still smell the same or very close to that of a newly made product (allowing for some desirable maturation or ageing of the perfume in the product over a 1- or 2-week period)?

The whole subject of stability testing is discussed in more detail later in this chapter.

Thus, it is the job of the applications chemist to be the 'product expert', who should be able to advise and carry out testing on any potential problems or issues related to the perfume in combination with the product base. This advice may be sought by:

- perfumers and evaluators internally, to guide their choice of perfume types and individual raw materials suitable for a particular product formulation.
- sales personnel, to enable them to ask the right questions of their clients, for example what active ingredient the product might contain or is the type of packaging the client will use likely to cause a problem?
- marketing personnel, of both the supplier and client to help them decide on technical product-concept feasibility and the likely constraints on fragrance creativity.
- the client's technical or R&D department, to discuss the likely test protocols, advise on starting-point product formulations or possible product and perfume interactions to be taken into account during their development work or factory manufacture.

It is also true to say that part of the Applications Department's role is to service any kind of client request for technical information, ranging from where a particular product raw material might be purchased to what sort of natural or functional additives could be added to the product to convey a particular consumer benefit.

To illustrate how an Applications Department works, the approach to the Business Scents Ltd brief given in Chapter 6 is discussed.

9.2 PRODUCT FORMULATIONS

Before new work can begin on sampling or testing any of the perfumer's new creations, the client has asked for suggested starting-point product formulations. Business Scents Ltd does not have the necessary development resource or manufacturing equipment in-house to handle the production of any of the products other than the alcoholic fine fragrance, and will appoint contract manufacturers to do this at a later date. Prototype product samples that contain experimental fragrance creations are thus required for the client to hold initial focus group discussions with target consumers. The applications chemist assigned to the team handling this project therefore needs to be able to produce base formulations within the first few days or weeks, so that the perfumer's initial creations get incorporated and the evaluation process begins. In this case, the client has requested quite conventional, well-known products and suitable examples are available from the bank or repertoire of existing tested and approved (in terms of odour, colour and physical characteristics) formulations. These formulations are sent to Business Scents Ltd and, if they meet their requirements, initial batches of the unfragranced product bases can be prepared in the applications laboratory.

9.2.1 Fine Fragrance

The Business Scents *Eau de Parfum* formulation (Figure 9.1) contains 78% denatured ethanol. The source of this ethanol (*e.g.* synthetic; or

Formulation

Ingredient	*% w/w*
Ethanol (DEB 100)	78.00
Fragrance	12.00
Purified water	8.50
PPG-20 methyl glucose ether[a]	1.00
Benzophenone-2[b]	0.50

Preparation

Blend the fragrance and ethanol, then add the water and remaining ingredients slowly with mixing. Allow to mature at room temperature for up to 10 days and then cool to + 1 °C, followed by filtration. A filter aid, such as magnesium carbonate at 0.2% can be used to remove difficult precipitates. Fill into clean, glass bottles.

Figure 9.1 *Eau de Parfum formulation ([a] Glucam P20® ex Amerchol Corp, Edison, NJ, USA; [b] Uvinul D50® ex BASF AG, Ludwigshafen, Germany)* natural grain starch, sugar beet, or molasses alcohol) can give rise to a different odour in the end product, and thus it is important to know what type of alcohol is likely to be used. Denaturants (which deter people from ingesting the alcohol) are required by legislation in many countries and are also useful to know, although less likely to have a significant effect on the finished product. In this case, a standard 99.7% v/v synthetic grade, containing 0.1% v/v *t*-butanol as a marker and denatured with 10 ppm of Bitrex [INCI name is denatonium benzoate, an extremely bitter substance], is likely to be used by the client. Given the high level of alcohol (and consequent low level of water), there is unlikely to be any need for addition of solubilisers.

The formulation also contains an ultraviolet (UV) radiation absorber, benzophenone-2, to prevent degradation of the fragrance and any dyes by light. Although consumers are encouraged to keep fine fragrances in the dark, the manufacturer needs to protect the product from those customers who insist on storing it on a sunny windowsill! A moisturizing ingredient, which has additional fragrance-fixative properties (PPG-20 methyl glucose ether), is also incorporated in this instance.

As stated in Chapter 7, the perfumer has almost total freedom in creating perfumes for such products and the Applications Department simply needs to check the solubility of the fragrance at a range of temperatures, following the maturation, chilling and filtering process. If the maturation period is sufficient, there should be very little chance of any further solid materials in the fragrance precipitating out over time, although this must be checked. Light stability, even through a UV absorber is being used, must also be checked to ensure that the fragrance does not darken unacceptably or that any dyes added do not fade. It may be that, in this case, a different UV absorber works better or that the perfumer needs to change one or two of the ingredients in the fragrance.

9.2.2 Vegetable Soap

The client has asked for a recommended formulation for a luxurious soap bar, based solely on vegetable fats (Figure 9.2). A high-quality palm-coconut base that is widely available in Europe should be suitable, but it was felt that such a premium product should contain some additional materials to convey ‘added value’. Thus, a powdered cationic polymer is incorporated, which deposits a moisturizing film on skin and helps to promote a rich, creamy lather. Titanium dioxide, a standard additive to any soap, provides greater opacity (and, to some extent, whiteness) to the

Formulation

Ingredient	*% w/w*
Prisavon 9259® soap base[a]	To 100.0
Merquat 2200®[b]	1.00
Fragrance	1.70
Titanium dioxide	0.20
Colourant or white slurry	As required

Preparation
The Merquat®, titanium dioxide, colorant and fragrance are mixed with the soap base, which has been milled once previously. This mix is then milled three further times, followed by plodding, extruding and stamping into bars.

Figure 9.2 *Conditioning vegetable soap formulation ([a] 80 : 20 palm: coconut soap base, ex Uniqema Int., Gouda, The Netherlands; [b] Polyquaternium-7, ex Nalco, Northwich, UK)*

bar and a variety of pigment pastes, which are stable in the alkaline environment (around pH 9–10) and to light, are available to colour the soap any desired shade. A white soap also contains whitener (called a white slurry, consisting of a small amount of blue pigment and a fluorescent whitening agent) because raw soap base is, in fact, a dull, yellowish-cream colour.

What sort of stability issues are there likely to be in such a soap formulation? First, the colour of the fragranced soap is as yet undecided; if it is to be white, then the degree of discolouration, which can be tolerated, is likely to be a lot less than if the bar is coloured to ‘match’ the fragrance type. The disastrous consequences of incorporating too much vanillin into a fragrance for white soap are shown in Figure 9.3. This severe browning reaction begins to occur after a matter of hours and cannot be prevented, since it results from a chemical reaction caused by the high pH of the soap, which is accelerated by exposure to light. For this reason, vanillin is rarely a major ingredient in soap perfumes. It may be used in small amounts in a soap if it is known that the soap is to be dyed with a strong colour. There are alternative materials with vanilla-type odours that can be used instead, such as ethylvanillin (which, although still discolouring, can be used at a lower level), Ultravanil® and Benzoin Hypersoluble P85®. Other perfumery materials that can cause a less radical, but still potentially significant, change in the colour of a white bar include eugenol,

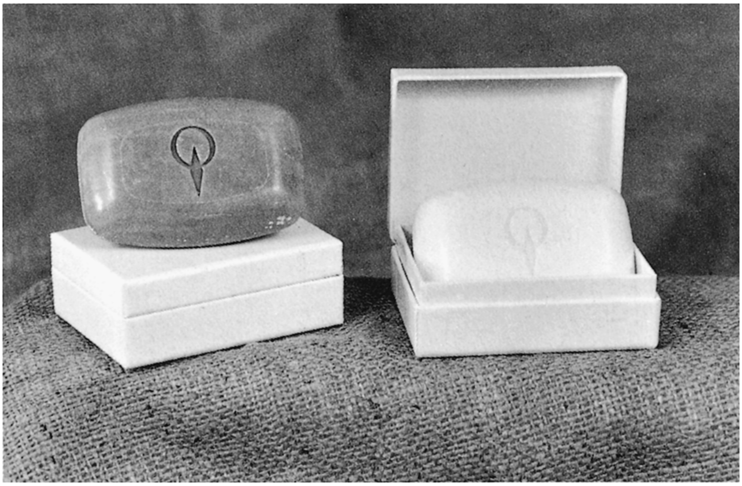

Figure 9.3 *The effect of vanillin on white soap*

isoeugneol, heliotropin, certain mosses, Schiff's bases, citral, indole, *etc*. These also need to be avoided, substituted or used very sparingly and colour changes measured to ensure they stay within specification, even after storage at high temperature (see later).

9.2.3 Aerosol Antiperspirant

The client requires an effective antiperspirant product, which is to be dispensed as an aerosol spray using a propane–butane blend as the propellant gas. Figure 9.4 illustrates the basic components of an aerosol.

The formulation that the applications chemist uses (Figure 9.5) is a powder in volatile silicone-fluid (cyclomethicone) suspension. This type of formulation requires the use of a special valve and actuator system, which allows the powder active (activated aluminium chlorhydrate) to be dispensed without clogging. The antiperspirant active chosen gives good sweat reduction, and has a suitable particle-size distribution, which can be effectively dispensed through the valve and actuator. The cyclomethicone carrier fluid is sufficiently volatile to evaporate from the skin surface and is soluble in the propellant blend, giving a suspension system that is easily dispensed from the can. A high molecular weight silicone gum (diluted in cyclomethicone) is also included to help prevent the formulation of an aerosol 'cloud', which can cause

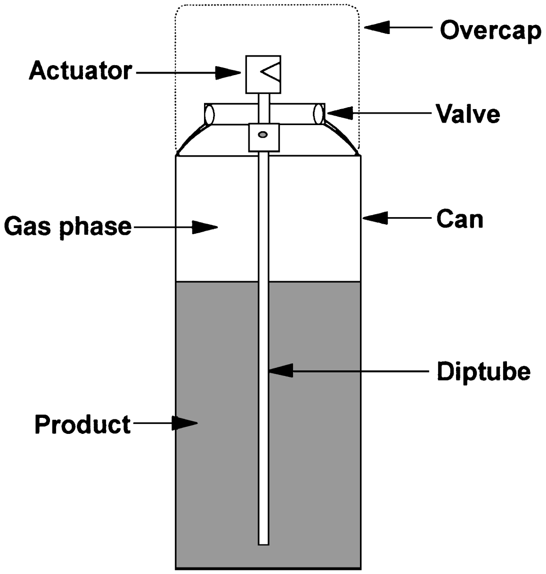

Figure 9.4 *The basic component of an aerosol*

Formulation

Ingredient	*% w/w*
Concentrate	
Isopropyl myristate	10.00
Bentonite	3.65
Volatile silicone-fluid DC 345	48.65
Activated aluminium chlorhydrate powder	36.00
Dow Corning® 1501	0.50
Fragrance	1.20
Aerosol fill	
Concentrate	25.00
Propane–butane propellant, 40 psig	75.00

Preparation

Thoroughly disperse the bentonite and activated aluminium chlorhydrate in the isopropyl myristate and DC 345 fluid, using a high-shear mixer. Add the DC 1501 and the fragrance, mixing well. Fill into internally lacquered aluminium aerosols, as shown above.

Figure 9.5 *Dry aerosol antiperspirant formulation ([a] cyclomethicone, ex Dow Corning Europe, Seneffe, Belgium; [b] cyclomethicone and dimethiconol, ex Dow Corning)* choking or sneezing if inhaled. It also provides a soft, moisturized skin feel, which is important for the image of such a prestigious product.

What sort of problems can an aerosol antiperspirant cause the perfumer and the applications chemist? Empirical testing in a range of antiperspirant bases has identified a number of unstable perfumery ingredients, such as phenolic materials, unsaturated terpene alcohols and their esters (such a linalool, linalyl acetate), unsaturated or reactive aldehydes (such as Lilial® and Ligustral®) some essential oils such as bergamot, lavender and rosemary), citrus oils and spices (such as nutmeg and black pepper) that can undergo chemical reactions in the presence of the acidic antiperspirant active. Such materials should be avoided. Being an aerosol product does in itself create a challenge; traces of metal ions can cause the active or perfume to discolour to pink or brown, which would not be very attractive under the arm or on clothes!

Solubility of the fragrance in the volatile silicone and the propane–butane, although not visible to the consumer, might cause a change in note of the perfume as it is dispensed. The potential for the product to cause can-corrosion must be evaluated. This can be tested by storage testing of the finished product in production specification cans. It is rare for modern anhydrous systems to present major corrosion problems, although all formulations should be checked, especially if they contain significant levels of water.

In any type of product, and especially aerosols, the balance between how the different components of the fragrance are perceived, the so-called odour balance, is altered compared with the neat fragrance oil. Generally, in an aerosol system, the increased surface area afforded by the droplets of the aerosol spray, combined with the highly volatile propellant, tend to give a greater bias towards the more volatile components of the fragrance compound. This causes the initial fragrance perception to be biased towards the top-notes. Such a change in odour balance is especially marked in a product such as the anhydrous antiperspirant, with its high level of propellant. This is why all aerosol fragrances should be evaluated with a formulation and valve system as close as possible to the one used in the finished product. It is also a factor that the perfumer needs to consider when creating the 'Eve' fragrance modifications for this product.

In conclusion, this is probably the most aggressive and potentially complex perfumed product out of the five. It is highly likely that the fine fragrance formulation will need to be significantly modified to be stable in this medium, whilst still giving the overall impression (in use) that it has essentially the same smell.

9.2.4 Shampoo

Figure 9.6 shows the conditioning shampoo formulation selected. It is based on a blend of a high-foaming anionic detergent (sodium lauryl ether sulfate, commonly abbreviated to SLES) and a mild, amphoteric betaine detergent, which lowers the irritation potential of the SLES. A cold-mix opacifying concentrate is added to give an attractive, white, pearly appearance to the product. Conditioning is provided by several keratin substantive materials which deposit a thin protective film on the hair, smoothing down the cuticle and preventing static build-up. A copolymer of wheat protein/silicone glycol is used, since evidence from the manufacturer shows that this helps to shield the hair from environmental damage. Panthenol (commonly called Pro-Vitamin B5 on many leading hair care products) is added to thicken and help repair damaged hair. The final ingredients are preservative (the final choice of which is made by the manufacturer) to prevent microbial spoilage of the product, sodium chloride to thicken the detergent system and, of course, water (which must be deionised and substantially free from bacteria).

The last ingredient, although seemingly innocuous, can cause major problems for the perfumer. In Chapter 7, the perfumer discussed the effect that the polarity, or relative water solubility of the perfumery

Formulation

Ingredient	*% w/w*
Purified water	to 100.00
Sodium lauryl ether sulfate, 28%	41.50
Dehyton K®[a]	8.00
Panthenol	1.00
Crodasone W®[b]	3.00
Preservative	As required
Euperlan PK-3000®[c]	3.00
Fragrance	0.60
Sodium chloride	2.00

Preparation

Add the ingredients to the water in the order given, with efficient stirring. (The level of sodium chloride may require adjustment.)

Figure 9.6 *Frequent-use conditioning shampoo formulation ([a] cocamidopropyl betaine, ex Cognis, Dusseldorf, Germany; [b] hydrolysed wheat protein polysiloxane copolymer, ex Croda Chems, Goole, UK; [c] glycol distearate and laureth-4 and cocamidopropyl betaine, ex Cognis)* ingredient *versus* its molecular mass, has on its odour perceptibility from an aqueous detergent system. The nature, quality and level of the detergent(s) and other materials, such as protein conditioners, can sometimes dramatically affect the perfume headspace over the surface of the product and its in-use odour characteristics. The perfumer thus asks the Applications Department to try various fragrance modifications in the detergent base, to assess on smelling the product straight from the bottle and possibly also during a salon evaluation in which a panel of volunteers has the odour of their hair assessed during washing and immediately after rinsing. Equally important is to monitor the smell on washed and dried hair switches (small samples of untreated virgin hair) for up to 18 hours afterwards, if substantivity or malodour counteractancy is important.

Another problem that the applications chemist may have to contend with is that the perfume may cause the shampoo viscosity to decrease or, more often, increase when added to the unfragranced base. If this is a small change, it can be accommodated by varying the level of thickener. However, if dealing with a client's fully formulated base, this may not be an easy option. In such cases, the perfume formulation needs to be screened for ingredients that are known to affect the viscosity of surfactant systems, such as dipropylene glycol or alcohols, for example citronellol, which often cause a decrease, and diethyl phthalate, isopropyl myristate or terpenes, which can thicken such solutions quite dramatically.

Obviously, the exact formulation of the client's shampoo under consideration may be unknown to the perfumery house and not follow any previously experienced pattern. If so, it may be necessary to resort to single-ingredient testing of perfumery materials to discover which ones can be safely used without problems. Similarly, chemical reactions could take place between certain perfumery raw materials and active ingredients in a shampoo, such as an antidandruff agent like piroctone olamine. Such reactions could lead to discolouration or off-odours in the product, which may only be noticeable after a period of high-temperature storage. As it is a previously tested shampoo base with no problem actives, the formulation given in Figure 9.6 is not expected to give rise to major difficulties but, nevertheless, standard stability testing is initiated when the final fragrances have been selected for submission, to avoid any unpleasant surprises. This is carried out in glass jars, as well as Business Scents Ltd bottles, if available at this stage. Compatibility problems are unlikely to be experienced with polyethylene terephthalate (PET), although it could be affected by citrus and terpene materials. Since the client has specified clear packaging, light testing is also necessary (see later).

Formulation

Ingredient	*% w/w*
Phase A	
Purified water	to 100.00
Sodium lauryl ether sulfate, 28%	54.00
Tegobetain HS®[a]	8.00
Phase B	
Purified water	20.00
Antil 171®[b]	3.00
Preservative	As required
Gluquat 125®[c]	6.00
Necon CPS 100®[d]	2.00
Dow Corning® 193 surfactant[e]	0.70
Phase C	
Fragrance	1.00

Preparation

Blend together the ingredients of phases A and B separately. Slowly add phase B to A, with moderate agitation, until homogeneous. Finally, add the fragrance and mix well.

Figure 9.7 *Shower and bath gel formulation ([a] cocamidopropyl betaine and laureth-4, ex Degussa, Essen, Germany; [b] propylene glycol and PEG-55 propylene glycol oleate, Degussa; [c] lauryl methyl gluceth-10 hydroxypropyl dimonium chloride, ex Noveon Europe, Brussels, Belgium; [d] PEG-15 cocamine phosphate/oleate complex, ex Alzo Int. Inc., NJ, USA; [e] dimethicone copolyol, ex Dow Corning Europe, Seneffe, Belgium)*

9.2.5 Shower and Bath Gel

The basic formulation used for this product is given in Figure 9.7. It is based on a mild blend of a high-foaming SLES, as for the shampoo, plus a high-purity betaine surfactant, which also contains glyceryl laurate to help build viscosity. The active detergent level (18%) is higher than that of the shampoo (14%), because of the greater dilution factor that the shower and bath gel will experience in use. A mild, vegetable-derived humectant which moisturizes and softens the skin, is included, along with a cationic skin conditioner (Necon CPS-10O®) which can be perceived on the skin even after dilution in the large volume of water used in a bath. Additional thickening to the desired gel-like consistency is achieved with a proprietary liquid thickening agent, Antil 171®.

Being an aqueous surfactant system, similar issues to those for shampoo need to be considered. The major difference is that the shower and

Table 9.1 *Botanical extracts with 'relaxing' connotations*

Botanical extract	*Claimed benefit*
Passion flower (*Passiflora incarnata*)	Reported to have analgesic and sedative properties; used in sedative preparations for nervous anxiety
Hawthorn (*Crataegus laevigata*)	Flowering tops are used in sleep-inducing preparations; reduces blood pressure
Valerian root (*Valeriana officinalis*)	Hypotensive; widely used in sleep aid and sedative health foods, herbal teas and medicinal products
Linden (lime tree; *Tilea cordata*)	Sedative, emollient, anti-inflammatory, healing and refreshing; linden tea or 'tilleul' is drunk as a general relaxant

bath gel is designed to be crystal clear and a thick, but still mobile, easily dispensed gel. Thus, the solubility characteristics of the fragrance are critical, as are any significant viscosity variations. The client has asked for recommendations for suitable botanical extracts that could provide a relaxing claim for the product. Such extracts often contain low levels of essential oils, which could add an odour of their own to the base and may reduce the viscosity by virtue of containing propylene glycol as a carrier or solvent. Thus, it is useful to add these ingredients to the finished product base to ensure that the fragrance product still performs well. Table 9.1 gives examples of possible additives that might be recommended.

Both of the detergent products will be coloured with water-soluble dyestuffs to enhance the fragrance concept. Blue, green or peachy-orange are all possible choices for the colour, but unfortunately both blue and red dyes, in particular, are notoriously unstable to light. Thus, as with the shampoo, light-stability testing is needed to ensure that ingredients in the perfume do not destabilise the colour of the product, since it is likely to be packaged in clear or translucent containers.

9.3 STABILITY TESTING

As touched on previously, it is seemingly impossible to predict accurately, in the few short weeks or months of the average product-development process, the chemical and physical changes that might occur after anything up to several years of storage on a consumer's bathroom shelf, or in the dusty corner of a drug store in Timbuktoo. Similarly, the extremes of temperature and/or humidity that a product can be exposed to during transportation or sitting on a quayside in certain parts of the world can be severe, even if only for relatively short periods. Thus, over the years, accelerated test protocols have been developed to provide the manufacturer with a way of predicting likely shelf-life (which is normally a minimum of 2 years) and identifying potential problems before the product reaches the marketplace. The applications chemist has adapted these protocols to highlight any undesirable chemical or physical changes related to the fragrance. Thus, before submission to Business Scents Ltd, the leading contenders for the 'Eve' fragrance and its line extension modifications are subjected to a battery of stability tests, as alluded to previously.

The Arrhenius rate equation forms the basis for the generally accepted theory behind this accelerated testing. This states that for every 10 °C increase in temperature, the rate of reaction doubles. Thus, in theory, the following applies:

$$\begin{aligned} \text{12 months at 20 °C} &= \text{12 weeks at 40 °C} \\ &= \text{6 weeks at 50 °C} \\ &= \text{3 weeks at 60 °C} \end{aligned}$$

In practice, perfumery companies test all products at 0–4, 20 or 25 and 37 °C (or 40 °C) for 12 weeks as a minimum standard. In addition, tests at 45 or 50 °C may be used (except for aerosols, which could explode) if quick results are required, or if it is likely that the product will experience these sort of conditions during its life-cycle. Stability for 4 weeks at 50 °C is considered acceptable in many instances, but signs of instability should be taken as precautionary only, particularly if the samples still appear satisfactory at 37 or 40 °C. This is because certain chemical reactions could occur at these high temperatures that would not happen at ambient temperatures or even at 37 °C. Note that 37 °C became an accepted standard because some cosmetic chemists believed there is a temperature 'barrier', corresponding to the heat of the human body, above which chemical reactions are accelerated beyond that predicted by the Arrhenius equation.

9.3.1 Humidity Testing

Products that are likely to be packaged in permeable materials, such as paper or cardboard, need to be tested in high-humidity conditions. Thus, a common combination is 37 °C/70% relative humidity (RH) or 40 °C/80% RH. This type of testing is most useful for boxed laundry powders (particularly those destined to be sold in tropical climates), but may be important for the 'Eve' soap if it is sold in a wrapper or carton rather than a tightly sealed pack.

9.3.2 Light Testing

A great deal of contention surrounds how best to accelerate the testing of products that will be exposed to daylight, and particularly to strong sunlight. Prior to the development of suitable equipment, samples were placed on a North-facing (so that they were not in direct sunlight) windowsill for however long was available for testing. However, quite variable results are obtained, depending on geographical location and season. Although such tests are often still conducted as a back-up, the availability of UV cabinets has helped to standardise testing. Normally a Xenon arc lamp subjects the samples to UV light in the 300–800 nm wavelength range and can run with a 400 or 1000 W burner fitted. Exposure of 6 h to the 1000 W lamp is usually sufficient to see any changes that are likely to occur in about 3 months of daylight testing. There are, however, considerable disadvantages to this method, including the fact that, despite the presence of a cooling fan, the temperature inside the cabinet can become quite hot, and thus two variables, rather than one, are being tested at once. Also, UV light is not a true reflection of normal daylight and it is possible for some discolouration reactions to occur in the first few hours and then bleach out again before the 6 h duration is complete. Thus, some companies now use light stress chambers, which can be fully temperature controlled (between $+10$ and $+50$ °C) and can be run with simulated daylight bulbs (for real-time testing), UVA or UVB tubes or fluorescent light (to stimulate in-store conditions).

9.3.3 Assessment and Reporting of Results

After 12 weeks of stability testing has been completed (and usually after an interim 4- or 6-week period), all the perfume samples are assessed on odour and physical appearance. At Quest, scales of A–E for perfume character and 1–5 for odour intensity are used. The refrigerated sample (nominally 0°C) is treated as a standard, as perfume degradation is considered to be negligible at this temperature and is therefore rated as A1. The samples from the other conditions are assessed and rated against this standard. Any perfumed sample rated below C3 is regarded as unsatisfactory.

On physical appearance, the samples are checked for discolouration, separation, precipitation, changes in viscosity, *etc.* as appropriate to the product. A viewing cabinet can be useful for looking at the samples under consistent lighting conditions. An unperfumed sample or 'control' is always tested alongside to compare with the perfumed products. An A–D scale is used, in which C or D are considered a failure.

Most products are assessed directly from the bottle or jar, soap wrapper or box. However, some products, the true odour characteristics of which show up better in solution, are dissolved in warm water in an assessment cup. Aerosols are assessed on test pads, with time allowed for the propellant to evaporate. Testing in special, plastic-coated, clear, glass aerosols may be required in cases where solubility or discolouration reactions need to be observed inside the product.

The results for the project 'Eve' stability tests are shown in Table 9.2. These are reported to the perfumer and evaluator assigned to the project,

Table 9.2 *Stability test results for the Eve Project (comments would be given on any significant changes in the odour or physical appearance, and results for the unperfumed controls have been omitted for simplification)*

	Odour				*Physical appearance*				
	0°C	*20°C*	*37°C*	*50°C (4 weeks)*	*0°C*	*20°C*	*37°C*	*50°C (4 weeks)*	*UV 6 h*
'Eve' Eau de Parfum	A1	A1	A/B2	A/B2	A	A	A/B	B	B
Soap Version 1	A1	B2	C3	C3/4	A	B	C	D	D
Soap Version 2	A1	A2	B2	B2	A	A	A/B	B	A/B
Aerosol A/P Version 1	A1	C2	C3		A	B/C	D		
Aerosol A/P Version 2	A1	A1	A2		A	A	A		
Aerosol A/P Version 3	A1	A2	B2		A	A	A/B		
Shampoo/ Shower gel Version 1	A1	B1	B2	B/C2	A	A	A	B	C
Shampoo/ Shower gel Version 2	A1	A1	B1	B1	A	A	B	B/C	A

Odour character: A = no change, excellent, B = very slight modification, C = some modification but acceptable, D = modified, unsatisfactory, E = unrecognisable; Odour intensity: 1 = no change, 2 = slight weakening, 3 = some loss of impact but acceptable, 4 = weak, unsatisfactory, 5 = odourless or extremely weak; Physical appearance: A = no change, B = slight change, C = changed but acceptable, D = unacceptable change, for example discolouration, separation, viscosity change, *etc*.

and then are fed into the stability database on the computer for future reference.

Thus, it can be seen that several of the fragrance modifications created for the line extensions have successfully completed the standard stability tests and the final selection can be made based on additional criteria, such as cost and hedonics. These fragrances can then be submitted to Business Scents Ltd with confidence that they are likely to be stable for at least a year, as long as the bases used are not radically altered. However, it remains the client's and their contract manufacturer's responsibility to carry out their own stability testing in the final product formulations to ensure their consumer safety and acceptability.

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CHAPTER 10

The Safety and Toxicology of Fragrances

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10.1 INTRODUCTION

How many consumers, even the more discerning ones, ever think about the safety of a fine fragrance or aftershave before they buy it? It is likely that they are far more interested in ensuring that the product ‘smells right’ on their skin, and that it blends with and enhances the image they are trying to establish. When ‘Eve’ is launched its image will be the major selling point, not the fact that it is safe for the consumer to use. However, it is certainly not by chance that the consumer does not consciously think about fragrance safety when selecting a product. Their confidence comes from the long history of safe use associated with fragrances, which in turn is the result of the considerable effort that the fragrance industry applies to product safety. Worldwide the fragrance industry is actually ‘self regulating’ as there is little legislation, apart from normal consumer laws, that governs the composition or use of fragrances. This does not mean that the fragrance industry works in a world without law. Legislation on chemicals, intellectual property, occupational health and safety and cosmetics, for example, obviously apply to the fragrance industry.

10.2 SELF REGULATION

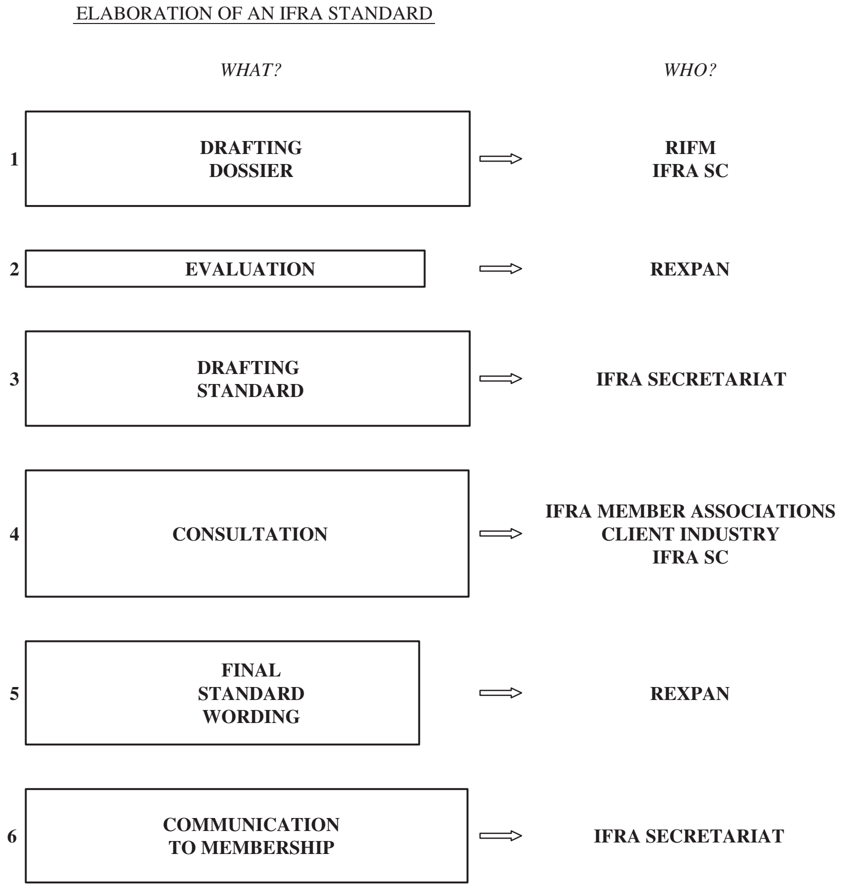
It was realised by the fragrance industry some 40 years ago that this absence of regulations concerning the ingredients that could be used in fragrances, or on the safety of fragrances in consumer products, could expose the consumer to unacceptable risks which would lead to governmental intervention in the industry. To avoid such problems, the industry decided to establish a self-regulatory system involving the two major international fragrance organisations. These are the Research Institute for Fragrance Materials (RIFM) and the International Fragrance Association (IFRA).

RIFM was established in 1966 by the fragrance industry as a non-profit making, independent body whose task was to evaluate the safety of fragrance ingredients. To date, RIFM has tested over 1300 fragrance materials including all of the commonly used ingredients. Once the test results for each material examined have been reviewed and discussed by an independent international panel from academia, which comprises toxicologists, pharmacologists, dermatologists and environmental scientists (the RIFM expert panel, REXPAN), the results are published as peer-reviewed monographs in the journal 'Food and Chemical Toxicology'. RIFM also collates all the information available for an ingredient from the scientific literature and from the aroma chemical manufacturers for inclusion in these monographs. Where they feel the data is insufficient to conduct a complete risk assessment they will undertake a test program within RIFM to fill the data gaps. Should there be a cause for concern about the use of an ingredient this is immediately signalled to the industry through the publication by RIFM of an advisory letter, which is then acted upon by IFRA.

The type of basic tests carried out by RIFM include acute oral toxicity; acute dermal toxicity if the oral toxicity is significant; skin irritation and sensitisation, and phototoxicity if the material adsorbs in the UV range. Where there is a need, much more detailed studies are undertaken, which involve sub-chronic feeding studies, dermal absorption and metabolic fate. Through IFRA, RIFM also collects from the industry consumer exposure data on fragrance ingredients. This ensures that the test data it uses are relevant to the market situation and also provide guidance on the nature of future research. Thus RIFM undertakes a review of its safety data, or instigates further research if the results of these surveys indicate that a particular ingredient is occurring in a wider range of products and/or at higher concentration than when it was first examined.

IFRA was established in 1973 by a number of fragrance trade associations and represents over 100 fragrance manufacturers in 15 countries. With its headquarters in Geneva, IFRA represents the scientific and technical expertise of the industry and is responsible for issuing and up-dating the 'Code of Practice' (IFRA, 1973) upon which the whole self-regulation policy is based. IFRA is funded by these fragrance manufacturing companies, who all agree to abide by the Code of Practice, while they remain members of the association. This Code of Practice has many functions including setting standards for good manufacturing practice within the industry, quality control, labelling and advertising, as well as setting limits on, or prohibiting the use of, certain ingredients.

IFRA and RIFM work closely together and it is only after considerable discussion between RIFM (REXPAN) and the IFRA Scientific Committee that restrictions or prohibitions are imposed. It is REXPAN who makes the final decision on the wording and content of the Standard and then it is the responsibility of the IFRA secretariat to communicate and implement this by way of the Code of Practice and the IFRA website. The flow chart given below shows how this process is undertaken.

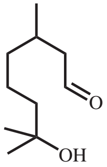


The strict code of practice applied by IFRA not only protects the consumer but also protects the health and wellbeing of those employed within the industry. This is highlighted by a study carried out in 1985 in which it was found there was no increase in mortality from any type of cancer in a group of workers employed in the flavour and fragrance industry, where exposure to a wide range of aroma chemicals is far higher than the consumer would ever encounter (Guberan and Raymond, 1985).

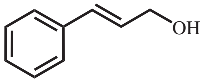
As the fragrance industry does not use toxic, carcinogenic or corrosive substances, why are there use restrictions on some ingredients? The most common cause for such a restriction is the ability of some materials to be skin sensitisers. Unlike skin irritation, which usually disappears soon after the irritant has been removed, skin sensitisation involves the activation of the immune system and reactions can persist for much longer after the initial exposure and can become more severe on subsequent contact.

Skin sensitisation was recognised as a major problem almost at the very outset of ingredient testing and a simple strategy was devised to deal with it. Ingredients were tested in a human predictive-sensitisation patch test at a concentration 10 times greater than the consumer was likely to be exposed to. If this test, which used exaggerated levels under occluded patches as a 'worse case' scenario, proved to be negative then no further action was taken. However, in the early days, if there were signs of sensitisation RIFM would issue an advisory letter and the material would no longer be used in fragrances.

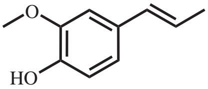
As this approach did not differentiate between weak and strong sensitisers, IFRA subsequently adopted a modified approach. The IFRA technical advisory committee studied the results of the human patch tests, and if necessary asked for further work to be undertaken, to see if a 'no effect' level could be determined for each material. If such a level could be determined, the committee set a guideline value that allowed only one-tenth of the no-effect level to be used in a consumer product. Obviously in some cases this level was below that at which the ingredient made any useful contribution to the fragrance and IFRA recommended a complete ban. However, it did allow many useful ingredients such as hydroxycitronellal (10.1) cinnamic alcohol (10.2) and isoeugenol (10.3) to be used without exposing the consumer to unnecessary risk. An interesting example of this approach is *trans*-2-hexenal (10.4), which has an intense green, fruity, vegetable-like odour. This material was found to be a sensitiser at a level of 0.2% but not at



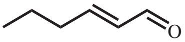
hydroxycitronellal
10.1



cinnamic alcohol
10.2



isoeugenol
10.3



trans-2-hexenal
10.4

0.02%. IFRA thus set a use level of 0.002% (20 ppm) for a consumer product, at which level it can still have a strong influence on a fragrance especially as its odour threshold has been measured at less than 0.1 ppm.

10.3 SAFETY ASSESSMENT

It was realised many years ago that the only difference between a medicine and a poison was the dose administered, as is demonstrated by the tragic consequences of overdosing on paracetamol. It is thus clear that it is not accurate to say a material is safe, as safety cannot be measured in absolute terms. The safety of any material cannot be measured directly but can only be estimated as part of a risk assessment. This type of assessment examines the potential of any material to cause harm and the likelihood of that potential being reached during normal use conditions. If the probability of causing harm under normal use conditions is high, then the material is not to be used, or a different use regime is developed to reduce greatly the chance of a hazardous situation occurring. It has been found that for the rat, the LD_{50} value for sugar is around $33\,g\,kg^{-1}$ of bodyweight (this drops to just $3.5\,g\,kg^{-1}$ bodyweight for table salt). If this result is translated to a 50 kg human, then the quantity of sugar needed to kill half of the recipients would be 1650 g. Thus on adding sugar to tea or coffee (probably 4 g per teaspoon), the acute risk to our health is very small. In this trivial example, the risk assessment shows that there is a safety factor between the applied and possibly harmful dose of over 400 and sugar would be considered safe.

Before a risk assessment for fragrance ingredients can be undertaken, the route of exposure has to be considered. Although LD_{50} values are available for some fragrance ingredients, it is obvious that they are not intended for consumption. Inhalation is the most obvious pathway for examination as fragrances are produced because of their odour. However, it is well known that the human nose is capable of detecting a vast range of materials at levels measured in parts per million and less. For example, if 0.2 mL of a fragrance is applied to the skin (a dab behind each ear) it is detectable to the human nose for several hours after application to anyone close to the wearer. For a fine fragrance which contains 10% of fragrance this means that only 0.02 mL of active ingredients are applied to the skin. If we now say that the fragrance can only be detected if you are within 1 m of the wearer, then 0.02 mL of fragrance diffuses into 8 m^3 of air, equivalent to a concentration of 2.5 ppm (assuming 0.02 mL of fragrance weighs 2×10^4 μg). This assumes that all of the fragrance evaporates immediately. As the fragrance is detectable for some hours after application the actual concentration in the air must be lower than this, and the concentration of any individual ingredient even lower. If any of these ingredients were toxic by inhalation at these levels then they would probably be used as chemical-warfare agents rather than fragrances. As inhalation and ingestion under normal-use conditions are of little consequence for the risk assessment, the role of the fragrance upon the skin must be examined as a route of exposure. In this case, two effects need to be considered. The first is whether the material is irritating when applied to the skin and the second is whether the material can penetrate through the skin and effect the immune system and the organs of the body.

10.4 SKIN IRRITATION

Although unpleasant, skin irritation, which manifests itself as redness (erythema) and/or swelling (oedema), is not a major problem as once the source of irritation is removed the effect diminishes. In fact most fragrance ingredients would only be classed as mild or moderate irritants when in an undiluted form, so the low levels found in consumer products are unlikely to be a serious source of irritation.

10.5 SKIN SENSITISATION

Whereas it used to be thought that the skin was an impermeable barrier to the outside world, it is now known that a large number of chemicals are absorbed to some degree including those used in fragrances (Hotchkiss, 1994). The actual amount that penetrates the skin depends on how much is applied to which area of the body; the area that it is spread over; whether the skin is covered or not and whether the product is rinsed from the skin before absorption can take place. The nature of the product being used can also affect the level of absorption as it has been shown that the alcohol used as carrier in fine fragrances and aftershaves, enhances the penetration of some fragrance ingredients. Not that penetration *per se* is harmful as many angina sufferers will testify. The absorption of glyceryl trinitrate through the skin used to be a rapid way of relieving the pain of this complaint.

For some fragrance ingredients, penetration through the stratum corneum into the epidermis can elicit an immune response leading to allergic contact dermatitis or skin sensitisation. Initially, exposure to a skin sensitiser has no effect, but repeated exposure can induce an allergy, which then can occur on contact with the material at levels below that needed to have an irritant effect. This allergy will usually appear 1–2 days after contact and often becomes more severe over the next 2–3 days. The reaction does not diminish as an irritant reaction does, but may last several days or even weeks after exposure. In severe cases the sufferer will not only need to avoid the cause of the allergy, but also any product, which contains a significant percentage of it.

As part of the 7th amendment to the European Cosmetic Directive (Directive), the Scientific Committee for Consumer Products (SCCP) have proposed that the fragrance ingredients that they see as being the most common cause of allergies should be listed on the label of cosmetic products. This is to act as a warning to those people who know that they have an allergy to one or more of these ingredients and allow them to avoid using them. If any of the 26 fragrance ingredients identified by the SCCP are present above the trigger quantities, they will have to be listed along with the other ingredients on the cosmetic label (Table 10.1). This labelling will be triggered if there is more than 10 ppm of any one of these ingredients present in a 'leave on' skin product and if the level in a 'rinse off' product exceeds 100 ppm. A number of these materials are present in the essential oils used by the fragrance industry and their contribution to the total level of allergen present will have to be included.

These regulations came into force in Europe in March 2005 for any cosmetic product leaving the manufacturer's premises.

For those materials that RIFM has shown to be sensitisers, IFRA has either applied a restriction or banned their use (see Table 10.2). In some cases, IFRA has ruled that only material of a set purity or botanical source can be used in fragrances. For example, IFRA recommends that crude gums of American and Asian styrax should not be used as fragrance ingredients. Only extracts or distillates (resinoids, absolutes and oils), prepared from exudations of *Liquidamber styracifua* L var macrophylla or *Liquidamber orientalis* Mills, can be used and should not exceed a level of 0.6% in a consumer product. For acetylated vetiver oils, IFRA has recommended that they can only be used as fragrance ingredients if they are produced by a method which gives products free from allergens. Such acetylated vetiver oils can be prepared using acetic anhydride

Table 10.1 *Allergen labelling for cosmetic products*

Amyl cinnamic aldehyde*	[Amyl cinnamal]
Amyl cinnamic alcohol*	[Amyl cinnamyl alcohol]
Anisyl alcohol*	[Anise alcohol]
Benzyl alcohol	
Benzyl benzoate	
Benzyl cinnamate	
Benzyl salicylate	
Cinnamic aldehyde	[Cinnamal]
Cinnamic alcohol	[Cinnamyl alcohol]
Citral	
Citronellol	
Coumarin	
Eugenol	
Farnesol	
Geraniol	
Hexyl cinnamaldehyde*	[Hexyl cinnamal]
Hydroxycitronellal*	
Isoeugenol	
Lilial®*	[Butylphenyl methylpropional]
d-Limonene	[Limonene]
Linalool	
Lyral®*	[Hydroxyisohexyl 3-cyclohexne carboxaldehyde]
Methyl heptine carbonate*	[Methyl 2-octynoate]
Methyl ionone alpha iso*	[Alpha-isomethyl ionone]
Oak moss	[*Evernia prunastri*]
Tree moss	[*Evernia furfuracea*]

*not found in nature.

[INCI (International Nomenaclature of Cosmetic Ingredients) name].

Table 10.2 *Examples of fragrance ingredients restricted by IFRA*[*]

Ingredient	*Restriction*	*Reason*	*% allowed on skin*
Acetylated vetiver oil	S	Sensitisation	U
Acetyl ethyl tetramethyltetralin (AETT)	P	Neurotoxicity	0
5-Acetyl-1,2,3,3,6-hexamethyl-indane	R	Phototoxicity	2
Angelica root oil	R	Phototoxicity	0.78
Bergamot oil expressed	R	Phototoxicity	0.4
Bitter orange oil expressed	R	Phototoxicity	1.4
p-tert-Butylphenol	P	Sensitisation and depigmentation	0
Cinnamic alcohol	R	Sensitisation	0.8
Costus root products	P	Sensitisation	0
Cumin oil	R	Phototoxicity	0.4
Cyclamen alcohol	P[†]	Sensitisation	0
Dihydrocoumarin	P	Sensitisation	0
Farnesol	S	Sensitisation	U
Fig leaf absolute	P	Phototoxicity and photosensitisation	0
Grapefruit oil expressed	R	Photosensitisation	4.0
trans-2-heptenal	P	Sensitisation	0
Hexahydrocoumarin	P	Sensitisation	0
trans-2-Hexenal	R	Sensitisation	0.002
Hydroxycitronellal	R	Sensitisation	1.0
Isoeugenol	R	Sensitisation	0.2
Lemon oil cold pressed	R	Phototoxicity	2.0
Lime oil cold pressed	R	Phototoxicity	0.7
Limonene	S	Sensitisation	U
6- and 7-methylcoumarins	P	Photosensitisation	0
Methyl octine carbonate	R	Sensitisation	0.01
Musk ambrette	P	Neurotoxicity and photosensitisation	0
Nookatone	S	Sensitisation	U
Oppoponax	R	Sensitisation	0.60
Pseudoionone	P[†]	Sensitisation	0
Rue oil	R	Phototoxicity	0.78
Safrole, Isosafrole and dihydrosafrole	P[‡]	Chronic toxicity	0
Styrax	R	Sensitisation	0.60

R = restricted. A use limit for consumer products is applied to this material; P = prohibited. This material is banned as a fragrance ingredient; S = specification. There is a defined grade; botanical source or method of production for this material; U = unrestricted. There is no restriction on the use of this material as long as it meets the defined specification.

[*] For more details see the IFRA Code of Practice (IFRA, 1973).

[†] There are exemptions for this material where it occurs as an impurity in another product.

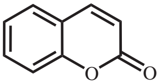
[‡] There are exceptions to this restriction for essential oils containing these ingredients.

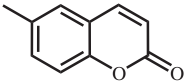
- without a catalyst and at a temperature not exceeding 120 °C,
- with *ortho*-phosphoric acid at room temperature, and
- with sodium acetate in toluene at reflux temperature.

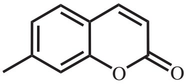
The first two products can be used in their crude form after the usual washing procedures, but may be further purified. In the last case, distillation is necessary to give a suitable product. Table 10.2 gives a list of the materials that are banned or restricted by the guidelines.

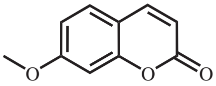
10.6 PHOTOEFFECTS

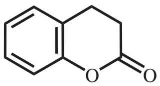
In some instances it is not the material as applied to the skin that causes the allergic reaction, but a combination of the material and exposure to sunlight. In the late 1970s, the cause of an unusual number of cases of dermatitis in people using a sunscreen preparation was traced to the presence of 6-methylcoumarin, which was in the fragrance in the product

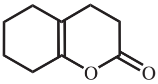

coumarin
10.5


6-methylcoumarin
10.6


7-methylcoumarin
10.7


7-methoxycoumarin
10.8


dihydrocoumarin
10.9


hexahydrocoumarin
10.10

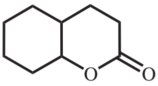

octahydrocounarin
10.11

Table 10.3 *Photosensitising ingredients*

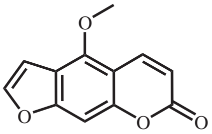
Material	*Sensitiser*	*Photosensitiser*	*Photoirritant*
Coumarin (10.5)	No	No	No
6-Methyl coumarin (10.6)	No	Strong	No
7-Methyl coumarin (10.7)	No	Moderate	No
7-Methoxy coumarin (10.8)	Weak	Moderate	Strong
Dihydrocoumarin (10.9)	Strong	No	No
Hexahydrocoumarin (10.10)	Moderate	No	No
Octahydrocoumarin (10.11)	No	No	No

(Kaidbey and Kligman, 1978). It has since been shown that a number of coumarin derivatives are capable of causing skin sensitisation but only when they are exposed to sunlight (see Table 10.3). As soon as it became apparent that 6- and 7-methylcoumarins and 7-methoxycoumarin were photosensitisers their use was prohibited by IFRA.

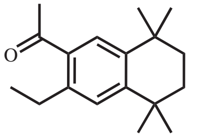
However, some essential oils such as fig leaf absolute and certain citrus peel oils contain natural phototoxic ingredients, usually referred to as furocoumarins (Marzulli and Mailbach, 1970; Fisher and Trama, 1979). For example some grades of Bergamot oil (expressed or cold pressed varieties) are known to contain 5-methoxypsoralen (bergaptene, 10.12), which is a potent phototoxic agent (Zaynoun *et al.*, 1977). The use of these grades of oil, which can contain up to 0.3% of bergaptene, is restricted by IFRA, although the industry now tends to use citrus oils from which the furocoumarins have been removed by distillation or extraction. Fig leaf absolute is banned from use because for even at levels of 0.001%, it can still elicit a photoallergic reaction (Opdyke, 1982).

10.7 NEUROTOXICITY

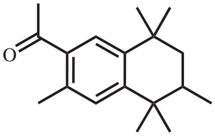
It is obvious that if a fragrance ingredient can penetrate through the skin, then it must have the potential to affect all the systems of the body and not just the immune system. This was found to be true for two materials, both of which exhibited neurotoxic effects. The first material, 6-acetyl-7-ethyl-1,1,4,4-tetramethyltetralin (AETT, 10.13), a synthetic musk was found to cause damage to the nervous system of rats when it was applied dermally at a level of 9 mg kg^{-1} bodyweight per day, over a 26-week period (Spencer *et al.*, 1979; Ford, 1994). Although RIFM established a No Observable Adverse Effect Level (NOAEL), the expert panel decided that there was insufficient evidence to recommend



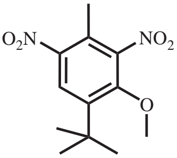
5-methoxypsoralen
10.12



6-acetyl-7-ethyl-1,1,4,4-tetramethyltetralin
10.13



7-acetyl-1,1,3,4,4,6-hexamethyltetralin
10.14



Musk Ambrette
10.15

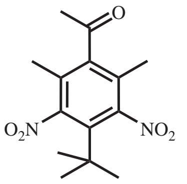
the continued use of this material, and acting on this advice AETT was banned by IFRA. It is interesting that the related and very widely used musk material 7-acetyl-1,1,3,4,4,6-hexamethyltetralin (10.14) does not show any neurotoxic effects. When neurotoxic effects were reported for the second material, Musk Ambrette (10.15), RIFM undertook a research program to enable a comprehensive risk assessment to be made (Spencer *et al.*, 1984).

RIFM first established a dose level that a consumer may be exposed to by normal usage of a wide range of fragranced consumer products that contained Musk Ambrette. This level, which was weighted to the maximum likely exposure, was calculated to be 0.3 $\mathrm{mg\,kg^{-1}}$ bodyweight per day. RIFM also determined that Musk Ambrette had a NOAEL of 10 $\mathrm{mg\,kg^{-1}}$ bodyweight, which gave a safety factor of only 33 (NOAEL/daily exposure). However, skin penetration studies had shown that only 2% of an applied dose of Musk Ambrette was actually absorbed through the skin. Thus the dermally applied daily exposure figure was really 50 times lower than calculated, giving a 50 fold higher safety factor. This evidence would almost certainly allow the industry to continue using Musk Ambrette, but when it was reported to also have photoallergic effects it was banned from use by IFRA (Cronin, 1984). It is interesting to note that other nitrated musk ingredients such as Musk Ketone (10.16) and Musk Xylene (10.17) do not show any evidence of neurotoxic or phototoxic effects (Lovell and Sanders, 1988).

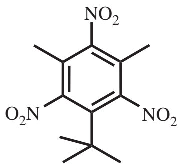
10.8 REPRODUCTIVE EFFECTS

Any chemical including a fragrance ingredient that enters the blood stream could affect the reproductive system and/or the growing foetus leading to a range of problems including infertility and birth defects. Thankfully, there has only been one case of a fragrance ingredient being implicated as causing adverse effects in the offspring of pregnant rats (Mankes *et al*., 1983).

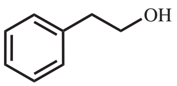
The ingredient was phenylethyl alcohol (PEA, 10.18), which is the major component of rose oil and has been used in perfumery for several centuries. When RIFM examined the results of this study, in which PEA was fed in enormous quantities to pregnant rats by way of a stomach tube (gavage), it concluded that a more appropriate study on dermally applied PEA should be undertaken (Spencer *et al*., 1979). The results of these studies, which included skin penetration and metabolism studies, showed that the PEA that did penetrate the skin was quickly metabolised to phenylacetic acid (PAA, 10.19), a natural component of human blood. It was concluded that the very tiny increase in the PAA level in the blood stream from the use of PEA posed no significant risk to the consumer, and combining the results of other studies a safety factor of many thousands was calculated. The adverse effects, seen in the original study, highlight the difficulties of extrapolating academic animal studies to the actual conditions encountered by the consumer.



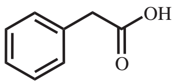
Musk Ketone
10.16



Musk Xylene
10.17



phenylethanol
10.18



phenylacetic acid
10.19

10.9 NATURAL INGREDIENTS

From a recent trend in product labelling and advertising in which 'naturalness' is extolled as a virtue, it may be concluded that natural materials are safer than synthetic ingredients. This is obviously a false conclusion as reference to Tables 10.1 and 10.2 shows a number of natural materials, which are banned or restricted by IFRA, and a number of allergens, which are present in natural essential oils. Both hemlock and botulism are natural but neither could be classed as safe for consumer use. Natural fragrances are no safer than those made with synthetic ingredients and this 'naturalness' concept is not supported or promoted by the fragrance industry. It could well be argued that synthetic materials are safer than natural materials as they are produced to very exact quality standards, whereas the quality of many essential oils varies greatly due to the vagaries of Mother Nature.

10.10 CONCLUSIONS

From the examples quoted above, it should be clear that the use of fragrances poses very little risk to the consumer. Although there have been very few problems associated with fragrance use, RIFM and IFRA continue to examine all the available evidence on both traditional and new fragrance materials to ensure that the consumer, including those who buy Eve, can continue to purchase fragrances without having to worry about their safety.

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CHAPTER 11

Volatility and Substantivity

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Quest International

11.1 PERFUME CREATION AND PHYSICAL CHEMISTRY

The perception of a perfume depends, in the first place, upon the presence of odorant molecules in the air and upon their nature and concentration. Most perfumes start-off life as a liquid, comprising a wide variety of molecules and having a known composition. In general, perfumers do not have a corresponding knowledge of the composition of volatiles in the air above such a mixture, except on those rare occasions where headspace analysis has provided hard analytical data (see Chapter 12). Perfumers therefore have to build up knowledge bases that summarise the olfactory behaviour of hundreds of ingredients under many different circumstances

- in admixture with diverse permutations of other perfume materials (commonly 20–100 different feedstocks, any of which may themselves be complex oils);
- in product forms ranging from aqueous liquids to powders, creams, solids, *etc.*; and
- on substrates such as hair, skin, cotton, *etc.*

Gaining this sort of experience is a lifetime's work for a perfumer, but it is possible to help the process by understanding the factors that govern the physical performance of perfume. One of the challenges for the physical chemist is to be able to predict what happens when a perfume is incorporated into different products; in particular, how does the composition of the perfume headspace alter, and what will be the 'in-use' perfume behaviour (*e.g.* how to maximise a perfume's useful lifetime on skin, or ensure that a laundry perfume not only supplies a pleasant smell in the detergent, but also on fabric after drying).

It is known that as soon as a perfume is introduced into a product matrix, there will be pronounced effects on some ingredients, those for which the 'microenvironmental' interactions have changed significantly. Physicochemical interaction is a consideration regardless of the base, whereas chemical interactions only become really important in consumer products possessing a reactive challenge for fragrance, for example products with low or high pH (*e.g.* below 4, above 9), or redox power (*e.g.* cold wave hair products or laundry bleaches). (Chemical stability issues are elaborated in Chapter 9).

Physical fragrance–base interactions, which are very different to those experienced by fragrance ingredients when in the 'bottle', affect the profile of odorants in the headspace, and may also have consequences for the evaporation characteristics. It is easy to see how complex this situation is, particularly in view of the ever-changing composition of the perfume as it evolves during evaporation. Nevertheless, a number of broad generalisations based on physical properties can aid the perfumer in his selection of ingredients for any particular product and desired performance criteria. Some of the physicochemical properties that underlie ingredient behaviour are highlighted below, and form part of the knowledge base the perfumer brings to bear. In the particular case of the Business Scents Ltd brief, this knowledge can be augmented by studying the fragranced products analytically to quantify actual perfume headspace, and by studying the delivery profiles of the various perfume components to the target substrates, skin and hair. In this way, a broader range of perfume ingredients may be identified that will perform better from the point of view of transport and distribution – after that, it is back to the perfumer.

11.2 PERFUME INGREDIENT VOLATILITY

The term 'volatility' is usually taken to refer to the speed at which a material evaporates. It is not an exactly defined property, and no universally accepted standards are laid down within the scientific literature. As implied above, the 'dry-down' or evaporation behaviour of even an unsophisticated perfume on a simple solid substrate, such as a paper smelling-strip, is complex. Some materials are so volatile that they will be lost much more rapidly than other components (within minutes), while materials at the opposite end of the volatility spectrum may remain for a considerable time (weeks/months). Such differences are naturally of vital interest to the perfumer, and the evaporation rates of perfume ingredients and essential oils from various substrates have been the subject of many studies (see, *e.g.* Appell, 1994). Furthermore, when very different types of material are present in the same mixture (as is the case for the majority of perfumes), there is no doubt that ingredient interactions modify the evaporation behaviour to some extent, and, for materials with intermediate volatility, the constitution of the perfume can govern their behaviour.

An understanding of the inherent tendency of an ingredient to escape into the gas phase is a useful starting point when considering perfume volatility. To a first approximation, the relative molecular mass (RMM) and the boiling point of a perfume ingredient will provide some guidelines to behaviour. For materials whose boiling points are not known, it is generally a sound alternative to look at chromatographic behaviour. For example, the retention time for a material to elute through a gas chromatographic column containing a non-polar phase is often strongly related to boiling point (in fact, such columns are commonly referred to as 'boiling point' columns).

Let us examine the relationship between boiling point and molecular size more closely. Table 11.1 comprises of physicochemical information on a number of materials that are, or have been, used in the fragrance

Table 11.1 *Representative physical properties of perfume ingredients*

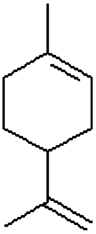
Ingredient	*RMM*	*Boiling point (°C)*	*Vapour pressure (mmHg)*	*sp (MPa$^{1/2}$)*	*Log P*
Benzaldehyde	106.1	178	1.10	21.9	1.50
1,8-Cineole (3)	154.3	176	1.65	16.6	3.22
Cervolide® (2)	256.4	170 (5 mmHg)	N/A	19.0	4.54
Ethyl propanoate	102.1	99	36.5	18.2	1.21
Lilial®	204.2	127 (6 mmHg)	0.0045	18.5	4.22
Limonene (1)	136.2	178	1.40	16.5	4.46
Methyl naphthyl ketone (α-isomer)	170.2	170 (20 mmHg)	0.0014	24.0	3.00
Methyl butanoate	102.1	102	30.2	18.4	1.18
2-Phenylethanol	122.2	218	0.11	23.7	1.36

RMM = relative molecular mass; boiling point is at *ca.* 760 mmHg unless otherwise stated; log P = common logarithm of estimated octanol–water partition coefficient (Rekker, 1977); *sp* = Hildebrand solubility parameter as calculated according to Hoy (Barton, 1985); vapour pressure is at 25 °C; Lilial = 2-methyl-3-(4′-*t*-butylphenyl) propanal; Cervolide = 12-oxacyclohexadecanolide.

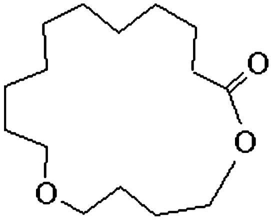
industry. The data were drawn from a number of sources, and some of the parameters (*e.g.* log *P* and *sp*, which are described later) were calculated from specific mathematical models, so that slightly different values may be found in the literature. However, in the context of volatility and substantivity, the emphasis is not on absolute values but rather on understanding and quantifying the differences between molecules.

The RMM of limonene (1), the major terpene in citrus oils, is 136, while its boiling point is *ca.* 178 °C at atmospheric pressure. This is in contrast with Cervolide® (12-oxa-cyclohexadecanolide, a macrocyclic musk; 2), whose RMM is 256 and boiling point is over 290 °C. The majority of perfume ingredients fall between these two extremes, although there are, of course, exceptions. Many odorous materials are more volatile than limonene and find some use in perfumes. Examples of these are methyl butanoate and its isomer ethyl propanoate, with boiling points of 102–99 °C respectively, and both with RMMs of 102. (These ingredients appear in a few perfumes, but are much more widely utilised in the flavour industry.) Odorous materials with RMMs greater than that of Cervolide® are much rarer since there appears to be a natural molecular size limit above which the human nose cannot detect – corresponding to around 300 RMM (*cf.* odorous steroids) – presumably because volatility becomes too small.

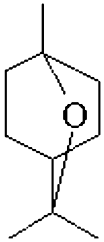
Structures of some perfume ingredients



Limonene
(1)



12-Oxacyclohexadecanolide
(2)



1,8-Cineole
(3)

Perhaps a more direct way to assess volatility is to look at the saturated vapour pressure (SVP) of an ingredient. SVP refers to the equilibrium pressure exerted by a substance in a closed system at a specified temperature (the volume of the system must, of course, be greater than that of the substance). Table 11.1 again quotes representative values. Consider, for example, the volatile material 1,8-cineole (3), which is utilised in many ‘fresh’ perfumes and is also commonly found in toothpaste flavours. This material has a vapour pressure of *ca.*2 mmHg at 25 °C (similar to that of limonene), which in the context of the perfumery world is very high. Most musks have vapour pressures that are 3–5 orders of magnitude smaller than that of cineole. Vapour pressure is directly related to the mass present in the gas phase so, the very fact that musks are perceivable at all to the human olfactory system is a tribute to the impressive 'dynamic range' of olfaction (the sensitivity of canine olfactory systems is even better!).

Equation (11.1) is a useful route for calculating headspace concentrations above a pure substance from vapour pressures; c is the gas-phase concentration in $\mathrm{g\,L^{-1}}$, p^0 the SVP in mmHg and T the temperature in °C. Equation (11.2) is the same equation restated in terms of concentration m in $\mathrm{mol\,L^{-1}}$ at a temperature of 25 °C for cases where the molecular mass is unknown. These equations derive directly from the ideal gas equation.

$$c = 0.01604(p^0)(\mathrm{RMM})/(273.2 + T) \tag{11.1}$$

$$\log_{10}(m) = \log_{10}(p^0) - 4.269 \tag{11.2}$$

So far we have dealt with pure materials. When liquid mixtures are considered, the headspace composition reflects the constitution of the liquid phase. Each component of the mixture is present in the gas phase, but its concentration depends on the nature and concentration of the other components. Clearly, when a pure ingredient is incorporated into a liquid mixture, the 'SVP' mass is no longer present in the headspace (assuming that the ingredient is truly diluted, *i.e.* the system is homogenous with no phase-separated droplets). The SVP still gives a useful guide to the concentration in the headspace, as is evident from Equation (11.3), where p is the partial vapour pressure of the ingredient, x its mole fraction in the liquid and p^0 its SVP. The parameter γ is known as an activity coefficient, and may be considered as an indicator of non-ideal behaviour.

$$p = \gamma\, x\, p^0 \tag{11.3}$$

When γ is unity, the equation reduces to the 'ideal' form known as Raoult's Law, in which the partial pressure of a component above a homogeneous liquid system is directly proportional to its mole fraction. It is very similar to an empirical expression first formulated by Henry for solutes in dilute solution (originally for gases in liquids), in which the solute partial pressure is proportional to concentration (Equation (11.4), where c is the concentration in $\mathrm{g\,L^{-1}}$ and H is referred to as the Henry's

Law constant, though some authors prefer the appellation to refer to the inverse of the function as written here).

$$p = H\,c \tag{11.4}$$

By re-writing Equation (11.4) in terms of mole fraction (and adjusting the dimensions of the constant H), the similarity to Raoult's Law can be seen. In fact, the main difference lies in the choice of standard states for the definition of γ or H, but this is beyond the scope of this chapter. More importantly, we need to understand how and why γ varies from unity. The differences are driven by the interactions that take place between the various components of the mixtures, discussed in the next section.

11.3 PERFUME POLARITY

Mass alone is not the only determinant of volatility. Thus, benzaldehyde has an RMM of 106, close to that of methyl butanoate, yet its boiling point is substantially higher (by 76 °C). Other factors are evidently important, and it becomes useful to deal with the set of molecular properties that collectively contribute to what is known as 'polarity'. Explicit definitions of this term are rare. It relates particularly to the degree to which electronic charge is spread evenly through a molecule or whether certain locations have relatively high concentrations of positive or negative charge. As explained below, we use it to represent the summation of physical interactions at the molecular level that may influence a molecule's free energy and other thermodynamic parameters (and hence also its availability and mobility within any given matrix).

A number of molecular interactions are feasible, but not all are relevant for the types of molecules found in perfumes. For example, ion–ion forces are unlikely to feature in any direct perfume interactions, since fragrance components rarely bear a charge (except for materials, which are influenced by extremes of pH, *e.g.* Schiff's bases are protonated in strong acid, carboxylic acids are substantially anionic above pH 6). The principal physical interactions contributing to overall polarity that need to be considered are ion–dipole, dipole–dipole, dispersion forces ('London' forces) and hydrogen bonding. These may all play a part, depending upon molecular size, the presence of permanent dipoles and the type of functional group present.

As a general guideline, the affinity between a molecule and its microenvironment will usually be higher when the molecule is surrounded by molecules, which are capable of expressing the same types of interaction, for example, limonene dissolved in a hydrocarbon or citronellol dissolved in ethanol. Some specific examples are discussed below, but the effect may be taken as a more general form of the often-expressed chemical adage that 'like dissolves like'. The activity coefficient, γ, introduced in Equation (11.3) provides a useful way of assessing affinity. For any single volatile material, values of γ greater than unity imply partial pressures in excess of that predicted by Raoult's Law, indicating that there is more material in the gas phase than would be expected in an ideal system, and that the material is effectively being 'pushed' out of the system. Conversely, values of γ below unity imply lower partial pressures, less material in the gas phase, slower evaporation and, perhaps, a concomitant increase in the persistence of the material in a system. Besides, certain high-boiling materials are well known to promote the longevity of other materials in a perfume. For the physical chemist, this phenomenon (based on negative deviations from Raoult's Law) is an example of what is termed 'fixation' within the fragrance world, but to the perfumer, the term also conveys harmonious blending of notes throughout a perfume's in-use life.

Table 11.2 contains some data that exemplify the above comments. It cites values of the activity coefficient for an ester (benzyl acetate), an alcohol (heptan-2-ol) and a terpene (limonene) in different environments encompassing a range of polarities: water (highly polar), an aqueous surfactant (as used in shampoos) and a moderately polar solvent used in perfumery (diethyl phthalate, DEP). It can be seen that heptanol and limonene have values of γ in DEP, which are similar and also greater than that of benzyl acetate, but in water limonene has a very large activity coefficient. Limonene is not able to participate in any strong cohesive interactions with water molecules, and the high γ is a consequence

Table 11.2 *Activity coefficients* at 40 °C of perfume ingredients in various media*

Ingredient	*Water*†	*Aqueous SDS*‡	*DEP*§
Benzyl acetate	1750	75	0.3
Heptan-2-ol	2770	57	2.2
Limonene	(>70,000)¶	732	2.3

*Data measurements on an ingredient mixture (taken from Behan and Perring, 1987); †under high dilution conditions (2 ppm); ‡at 0.05% w/w dilution (SDS = sodium dodecyl sulphate at 10% w/w in water); §at 0.05% w/w dilution (DEP = diethyl phthalate); ¶various values appear in the literature; the figure quoted is a minimum.

of this. Heptanol, on the other hand, can participate in hydrogen bonding and exhibits much lower values of γ. Note that in the case of the shampoo, we may be dealing with 'apparent' activity coefficients since the degree of liquid phase homogeneity is not certain (owing to the presence of micelles/emulsion droplets).

To reinforce what this means in practice, Figures 11.1 and 11.2 depict the equilibrium headspace profile (*i.e* the gas-phase concentrations of volatiles) above two systems containing the same perfume ingredients: a neat perfume oil and a cologne (typically these are alcoholic solutions containing 1–3% perfume). Figure 11.1 shows a (partial) headspace chromatogram containing labelled peaks corresponding to three terpene alcohols (dihydromyrcenol, linalool and citronellol) that are frequently used in cologne perfumes. The same three peaks are shown in Figure 11.2, which reveals the headspace profile once the perfume oil has been taken up into aqueous alcohol. It can be seen that the concentrations of the terpene alcohols are relatively reduced in the polar medium (the cologne) compared to non-hydrogen-bonding molecules bearing other functional groups. Similar, but smaller, differences are also present for many of the other materials, and in consequence, differences in odour characteristics may well occur. This level of understanding is useful, but the perfumer needs to know how to select ingredients that will provide superior performance in the target product area. To meet these needs, we need to recognise and ideally measure or calculate the molecular characteristics that govern or mediate activity coefficient behaviour.

It would be very convenient if it were possible to calculate activity coefficients for any molecule in any given environment. Unfortunately, there are few situations where this is so, and rarely do these situations appertain to real products. Thus, for example, we may estimate the activity coefficients of many alkanes and simple derivatives at infinite dilution in water, but the corresponding values for the same materials in a specific shower gel are not readily calculable from first principles. However, a large number of parameters are available in the literature and have been used to answer questions related to physical behaviour.

The pharmaceutical industry has for many years developed mathematical models to explain the biological activity of drugs: these are termed as quantitative structure–activity relationships (QSARs). These techniques may also be applied to the situations described herein, although more correctly we are often more interested in QPARs, where the 'P' stands for 'property' (which may refer to macroscopic properties such as density, melting point or viscosity, or to molecular or sub-molecular

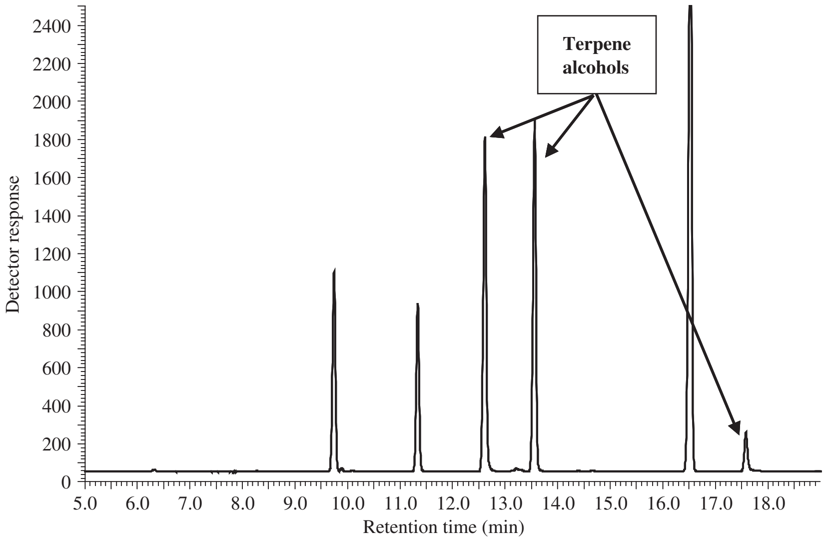


Figure 11.1 *Partial headspace chromatogram of perfume oil*

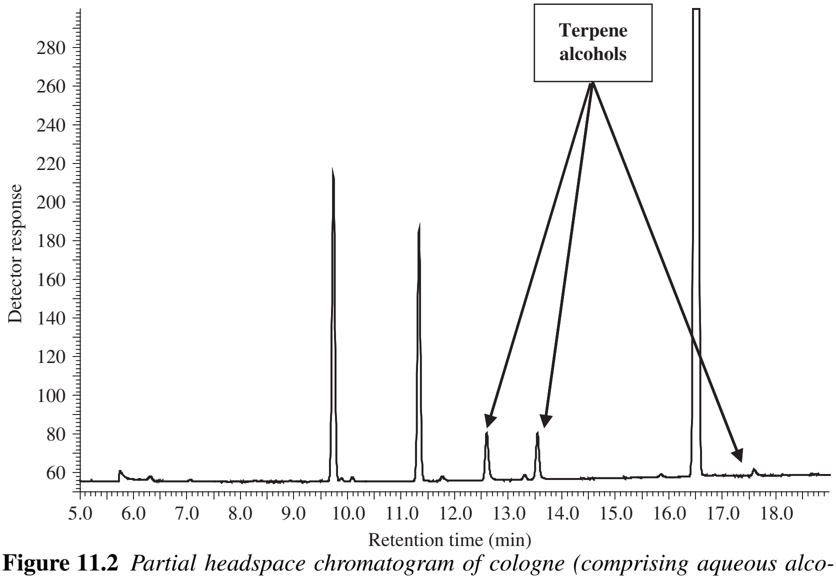


Figure 11.2 *Partial headspace chromatogram of cologne (comprising aqueous alcohol containing 2% of the perfume oil of Figure 11.1)*

properties such as molecular or fragmental volumes or atomic charges). Since these properties are always at least partially dependent upon molecular structure in its broadest sense, the distinction between these approaches is somewhat blurred, particularly when pure structural data are used in the same mathematical model as a melting point! But whatever the semantics, the underlying need remains the same: to understand and predict the effect of changing the structure of a perfume ingredient on its performance, so that we may reasonably estimate, for example, the concentration of a perfume ingredient above a cologne, or its affinity for cotton during a wash cycle. This whole approach is detailed elsewhere in this book (see Chapter 14), so just two parameters found to have widespread application in the perfumery area are discussed here. These parameters are the octanol–water partition coefficient (usually expressed as its common logarithm, log P), and the Hildebrand solubility parameter (sp, often designated δ with or without a subscript). The Hildebrand parameter (Barton, 1985) is defined in Equation (11.5), where ΔH is the molar enthalpy of vaporization, V the molar volume, R the gas constant and T the temperature. The SI unit for sp is MPa$^{0.5}$, but the cgs system equivalent (cal cm^{-3})$^{0.5}$ is commonly seen.

$$sp = [(\Delta H - RT)/V]^{0.5} \tag{11.5}$$

The physical significance of these parameters is discussed in the next section. For now, the key point is that these parameters may feature explicitly in empirical QPARs developed to help ingredient selection or design. Alternatively, they may be used simply as classification variables to help identify ingredients that are likely to exhibit good performance in a specific fragranced product. In this second approach, a number of parameters are investigated for their usefulness in characterising behaviour, for example, a plot of log P *versus* boiling point for a variety of ingredients may lead to the identification of clusters of materials with good performance. Such classification variables may be used qualitatively or quantitatively, depending upon the difficulty of the problem, and the statistical expertise available. The same parameters and approaches find particular use in the development of perfumes exhibiting optimal fragrance longevity, and this is the focus of the next section.

11.4 SUBSTANTIVITY AND RETENTION

We have touched briefly on how reduced vapour pressures can lead to reduced rates of evaporation. This means that physicochemical properties not only influence perfume volatility *per se* but also affect other aspects of fragrance behaviour, such as substantivity and retention. These terms are used within the perfume industry to denote perfume longevity in use, usually with respect to a particular substrate and/or surface (*e.g.* skin, hair, cloth, *etc.*). Perfume substantivity may be effected *via* such means as encapsulation or pro-perfumes (low or no odour precursor perfume molecules), but the thrust of the discussion below is directed towards the inherent substantivity of normal perfumery materials.

To understand perfume behaviour on these surfaces and/or matrices, we must consider the range of attractive or repulsive forces between the perfume components and the surface itself. The situation is complicated by the way in which perfume is delivered to the surface. For example, for a perfume ingredient in a soap bar to be substantive, it must first be efficiently delivered to the skin during washing, then survive rinsing and, finally, be retained for some time on skin. Definitions of *substantivity* and of *retention* are varied, but here *retention* is used to indicate the affinity a perfume has for a substrate when delivered to it, while *substantivity* also includes delivery barriers.

As implied above, the delivery of at least some perfume to a surface is, except perhaps for air fresheners, a key requirement for perfume longevity in use. For certain products such as cologne or deodorant, perfume delivery is very effective since the perfume is applied directly to the target substrate (the skin). Delivery from other products, such as soap or laundry powder, involves perfume transferring from an aqueous detergent solution or dispersion to a substrate or surface. This process has analogies with partitioning of materials between different phases, and, perhaps not surprisingly, we find that the octanol–water partition parameter ($\log P$) may often provide insights into physical behaviour. For example, in dilute wash systems, the deposition of ingredients onto substrates is often moderately or strongly correlated with $\log P$. It is likely that here the $\log P$ values reflect the solubilities of the perfume ingredients. By definition, materials with high $\log P$ such as polycyclic musks ($\log P$ values in the range 4.5–6.5) tend to be hydrophobic ('water-hating') and partition where possible into more lipophilic ('oil-loving') phases. Conversely, materials with low $\log P$ such as 2-phenylethanol ($\log P$ of 1.36) are hydrophilic and either soluble or sparingly soluble in water.

In fact, several well-known models in the literature allow solubilities in water to be estimated reasonably well from knowledge of $\log P$ (and according to the functional groups present). For example, Hansch *et al.* (1968) has published several linear free-energy relationships (LFERs) between molal solubility and log P for various classes of monofunctional molecules. The correlation coefficients for the LFERs (a measure of goodness of fit) were in the range 0.93–0.99, indicating that solubility estimations, at least for some classes of material, are likely to be relatively accurate.

We may conclude from the above that values of log P appear to give some guidance to the tendency of perfume ingredients to move from aqueous systems to (presumably) less polar surfaces (skin, hair, *etc.*). Analogous partition data are available for solvents other than octanol, for example, olive oil, and these may be more pertinent in certain situations. However, log P values based on octanol/water are easily accessible for thousands of substances, and, for most of the compounds found in perfumery, may be estimated reasonably well using one of the mathematical prediction models described in the literature – for example, those due to Rekker (1977) and Leo (1971, 1993). The estimates from the Leo model are commonly referred to as 'Clog P' values, the 'C' standing for *calculated*. A variety of software packages are commercially available for estimating log P values, two of the most popular being those from Daylight Information Systems, Inc. (Clog P) and from Advanced Chemistry Developments, Inc. (ACD log P). In general, estimates from these systems correlate highly, though differences may exist for certain chemical classes. Additionally, it is often true that for many materials partition coefficients determined in different solvent/water systems often correlate strongly with one another.

Sturm and Mansfeld (1978) searched for a quantitative relationship to predict the *residuality* of fragrance during fabric washing and conditioning. They found as expected that the fabric type played a part, but they also discovered that there was a good correlation with gas chromatographic retention times, probably reflecting a dependence on vapour pressure (see above). When log P and other parameters are also considered, it is possible to build up QPARs that represent quite reasonably the connection between substantivity and ingredient properties. A good example in the literature is provided by Escher and Oliveros (1994), who studied the influence of molecular structure on the delivery and substantivity of 15 fragrance components in laundering and drying a range of fabrics. These authors reported a full statistical survey of the key factors determining ingredient behaviour.

In the last decade, a significant number of applications for patents have been filed in the perfume arena that protect perfume formulations designed for longevity and substantivity (see under *Patents* in

References). Such patents can encompass a wide perfume formulation space because the scope of their claim is defined in terms of the parameters described above, rather than by compound name. Particular mention may be made of the patents disclosing 'Enduring Perfumes' and their inverse 'Blooming Perfumes' (assigned to Procter & Gamble), which recognised the importance of log P and boiling points, and pioneered their use in the patent literature.

Unfortunately, the log P of an ingredient does not always suffice to describe behaviour adequately. For example, in concentrated aqueous detergent systems it is probable that perfume partitioning into complex surfactant phases becomes dominant, and knowledge of log P provides only partial understanding. It then becomes necessary to search for other parameters, which may be of more use. As mentioned above, a large number of parameters may be considered, but here we look more closely at just one, the solubility parameter (*sp*, introduced earlier).

The Hildebrand *sp* has its origins in the development of what is known as 'regular solution theory'. As can be seen from Equation (11.5), it is essentially a measure of how much energy is needed to disrupt intermolecular cohesion: the higher the *sp* value the more cohesive the material ('sticky' at the molecular level), and the harder it is to separate into individual molecules in the gas phase. Originally, *sp* was exploited primarily in the paint and polymer industry, but has since been found useful across a number of applications (Barton, 1985), too numerous to discuss here, but dealing with properties such as viscosity, surface adhesion, miscibility and, of course, volatility. As a working rule, different molecules with similar values of *sp* are likely to have significant interaction. This is similar to some of the conclusions made above when discussing polarity, and it also suffers from the same drawback, *viz.* the overall interaction may be complex, deriving from a superposition of mechanisms. It is possible to resolve *sp* into different components reflecting different interactions (*e.g.* hydrogen bonding, dispersion, *etc.*), and these may sometimes be more useful than the overall *sp* value.

The *sp* values of most perfumery ingredients fall between *ca.* 16 MPa$^{1/2}$ (non-polar materials such as terpene hydrocarbons) and *ca.* 25 MPa$^{1/2}$ (polar materials such as alcohols). In general, we expect that materials will have lower activity coefficients in microenvironments characterised by similar values of *sp*. For instance, limonene (*sp* value of 16.5) is expected to be compatible with plastics such as polyethylene and polypropylene (*sp* range typically 16–18), and to exhibit good solubility and retention in these polymers. We would anticipate that other ingredients, such as phenylethanol (*sp* value of 23.7), would have less desirable interactions with these polymers, for example promoting phase separation, crazing, stress cracking, *etc.* On the other hand, in partially hydrolysed polyvinyl acetate (*sp* range typically 22–24), the situation is reversed. The solubility parameter thus finds good practical use for understanding perfume interactions with plastic packaging, as well as for providing a basis for understanding affinities in general.

It is only rarely that we have explicit values of *sp* for a surface or substrate of interest, but this does not impede study and model building from the perfumery perspective. The approaches outlined earlier, together with appropriate parameters capable of 'capturing' the major types of interaction present in a system, may all be used to help build up a picture of the key features in the delivery of perfume to skin, or fabric, *etc.* Once on the target site, the affinity between various perfume ingredients and the site may be quantified analytically, and investigated theoretically. It is important to recall, however, that the single most important property for prediction of substantivity remains, except where partitioning is extremely discriminating, or chemical interaction is possible, the ingredient vapour pressure.

It should be clear from the foregoing that the estimation of perfume substantivity depends upon a number of diverse factors, not least the substrate of interest, the temperature and the presence and nature of perfume-solubilising materials. Under these constraints, it is usual to seek to develop empirical models of substantivity using the regression techniques that have been used extensively in the pharmaceutical industry. A good starting point is given by Equation (11.6).

$$S = \mathrm{A} + \mathrm{B}(gc) + \mathrm{C}\log(\mathrm{polarity}) \tag{11.6}$$

where S is the substantivity or a function thereof; A, B and C are constants; gc is a gas chromatographic index (*e.g.* a Kovats index) and polarity is the octanol–water partition coefficient, *sp*, *etc.*

11.5 CONCLUSIONS

In summary, the volatility and headspace behaviour of perfume components is broadly comprehensible in terms of molecular interactions, both within products such as shampoos and colognes, and on or within substrates such as cloth or hair. However, the extreme complexity of the interactions, and the number of components invariably present, renders it difficult to predict *a priori* headspace compositions in any given situation.

Similar comments also apply to the related phenomena of ingredient or perfume fixation and substantivity. Nevertheless, it is possible to:

- quantify perfume behaviour analytically for any given product and in-use combination; and
- analyse the data obtained from the first stage to identify ingredients that perform well, and to seek (empirical) mathematical models which will explain behaviour for a particular system.

The knowledge and understanding gained in this manner is part of the cycle of learning, perfume creation and performance evaluation that is a fundamental element of modern perfumery.

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CHAPTER 12

Natural Product Analysis in the Fragrance Industry

ROBIN CLERY
Quest International

12.1 INTRODUCTION

In this chapter we will follow the analytical work that might be carried out on some natural products to answer a typical enquiry from creative perfumery. In the second section we will explore the different analytical techniques used in the fragrance industry, concentrating on their application to natural product analysis and the way in which they are used to provide the creative perfumer with information. Many analytical techniques also fulfil other roles in the fragrance industry. These will be mentioned when discussing the techniques individually.

12.2 NATURAL PRODUCT ANALYSIS

The analysis of natural products is a well-established part of the fragrance industry. Historically, essential oil analysis has provided the creative perfumer with the information required to reconstitute the characteristic odour of an oil at a lower cost and has led to the identification of the key components responsible for the odour. An essential oil may contain 300 or more components and there remain many essential oils that have yet to be investigated in detail. Some of these oils may be a source of new odours. The traditional methods of essential oil analysis coupled with the ever-increasing sensitivity of modern equipment still leads to the discovery of fragrant natural chemicals that are new to science.

In Chapter 7, the creative perfumer approached the natural products analysis section for help in finding a natural lead for 'Eve', the new fragrance range for Business Scents Ltd. He is looking for an exciting new, tropical, floral, fruity note to be included in this creation and has requested that some tropical fruit, muguet flowers and broom absolute be investigated. The enquiry can be answered using traditional essential oil analysis or by taking advantage of the recent developments in headspace analysis. The aim, in either case, is to identify a natural product which has the desired fragrance and by extraction and analysis to provide the perfumer with the identity of its key odour ingredients.

12.2.1 The Traditional Approach

The traditional method of essential oil analysis is to extract the plant material by steam distillation or with solvent and then fractionally distil the oil or extract and to isolate individual components by chromatographic techniques for subsequent identification by spectroscopic methods. At each step the odour of the fractions and isolates is assessed and those with the desired characteristics are investigated further. To answer the enquiry about the key odour components of broom absolute, first a sample of the absolute that is of an acceptable odour quality is obtained. The absolute is the alcoholic extract of the concrete, which itself is the solvent extract of the flowers of *Spartium junceum*, Spanish broom, often referred to by its French name Genêt. The odour of any natural extract can vary according to the geographical origin and quality of the plant material, the time of year it is harvested and the extraction method used. If no sample of adequate quality is commercially available then the fresh flowers would be obtained from the plant and the extraction carried out in a laboratory.

The first step towards separating the components of the absolute is fractional distillation. The absolute is distilled in the laboratory under vacuum, with a nitrogen blanket to reduce the risk of thermal degradation of susceptible compounds. The individual fractions collected from the distillation are analysed by gas chromatography–mass spectrometry (GC–MS) to determine their composition; some fractions might contain a single component while others may still be complex mixtures. Each fraction is assessed by a perfumer to determine which retained the most characteristic odour of the original material. The chosen fractions are analysed by GC-sniffing. If any of the individual components of the mixture is attributed with the characteristic odour that the perfumer requires, then work would be directed at isolating and identifying these materials. If, however, the fraction is still too complex it is further fractionated by some form of liquid chromatography, such as flash chromatography, and the sub-fractions are assessed by a perfumer and analysed by GC–MS and GC-sniffing.

If materials are found that have an interesting odour, but which cannot be identified by GC–MS, they are isolated by preparation techniques such as preparative high-performance liquid chromatography (HPLC) or preparative GC and their structures determined by nuclear magnetic resonance (NMR), which, in most cases, is able to provide an adequate identification. As the compositions of the chosen fractions and sub-fractions are determined, the perfumer tries to create an accord, which adequately represents the odour of the original material by reconstructing the fractions from their individual components.

12.2.2 The Headspace Approach

To answer the enquiry about the fragrance of muguet (lily-of-the-valley), it would be most appropriate to use headspace analysis. Some natural materials, especially flowers and fruits, are often not available in sufficient quantity for even a laboratory extraction, while others yield an extract that does not reflect the fragrance of the flower. It is for these flowers that headspace analysis has great advantages. Using the non-destructive headspace-trapping technique and light, portable sampling equipment, the fragrance of any flower, fruit or other natural source can be collected from the field (or greenhouse) and returned to the laboratory for analysis without disturbing the flower or the plant (Figure 12.1).

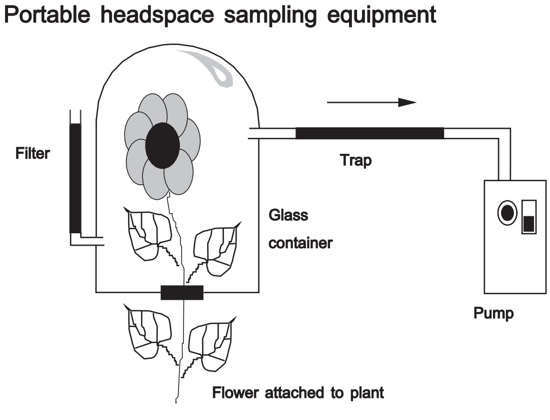
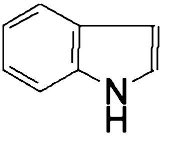


Figure 12.1 *Portable headspace sampling equipment*

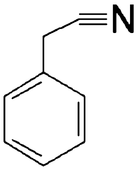
Muguet is a popular garden flower, so finding plants could be as easy as visiting a garden centre or asking friends. Once some suitable flowers have been found, the headspace equipment is taken to the garden, the special glass bell jar carefully set up over the flowers and the headspace collected on several different types of traps. First, about four small thermal desorption traps are used, in which enough sample can be collected in a few minutes, then some larger capacity solvent desorption traps are left in place for about 8 h each. This ensures that a representative sample of the odour is collected and provides appropriate samples for the different analytical techniques to be used.

Back in the laboratory, the thermal desorption traps are analysed by GC–MS and GC–flame ionisation detector (FID) by which means the major components can be quantified and identified. This reveals that the major components of the headspace of muguet are benzyl alcohol, citronellol and citronellyl acetate, and that the minor components with important odours are myrcene, *trans*-ocimene, *trans*-rose oxide, and 6-methylhept-5-en-2-one. Usually, many compounds in the sample cannot be identified definitively by GC–MS. Therefore, the larger traps are desorbed with diethyl ether or a similar solvent, and the resulting solution analysed by GC-sniffing to determine which components had a muguet odour. The solution is also concentrated and analysed by GC–MS to investigate the trace components. The use of nitrogen- and sulfur-specific detectors facilitates the identification of nitrogen- or sulfur-containing compounds, such as indole (1) and benzyl cyanide (2), which have, indeed, been found in muguet.



Indole

(1)



Benzyl cyanide
(Phenyl acetonitrile)

(2)

The results passed on to the perfumer would include the name of the compound, its relative proportion in the headspace sample and an indication of the odour intensity of the material. The perfumer can then select the important odour materials and combine them in an accord to re-create the scent of the flower.

To complete an analysis of fresh, tropical fruit, we took advantage of the Quest office in Indonesia and asked an employee who was travelling to Indonesia to take the equipment, since it is easily portable. With the help of the local perfumers, the equipment was set up to capture the fragrance of the best smelling fruit in a local market. The traps containing the samples were sent back to the laboratory, where the analysis revealed exactly which compounds contributed to the real freshness of the fruit. The GC–MS and GC-sniffing results showed that methyl 3-methylbutanoate, methyl 3-methylpentanoate, methyl 3-methylpent-2-enoate and methyl *trans*-hex-2-enoate are found in these fruits and contribute significantly to their odour. By providing the results of these analyses to the perfumers, we are able to help them understand the construction of the natural fragrances and re-create the original odour more closely.

Modern natural product analysis reveals both the chemical composition of new oils or flower scents and the identity of novel fragrant molecules that may become new perfumery ingredients in the future. It is chemical detective work to solve the mysteries of nature's fragrances that have evolved over the millennia.

12.3 ANALYTICAL TECHNIQUES USED IN THE FRAGRANCE INDUSTRY

12.3.1 Extraction

Steam distillation is the main commercial extraction procedure for the production of essential oils from almost any type of plant material. Solvent extraction is also used commercially and yields a resinoid, concrete or absolute, according to the solvents and the techniques used (Chapter 4). Both steam distillation and solvent extraction are used on a laboratory scale to produce oils and extracts for analysis. Other methods of extraction such as supercritical fluid extraction (SFE), which uses supercritical CO_2 as the extraction solvent, are now being developed and used on both commercial and laboratory scales. The extracts produced by SFE may contain different materials from the steam-distilled oil because of the solvating power of CO_2 and the lower extraction temperature, which reduces thermal degradation. The CO_2 extract may therefore have an odour closer to that of the original material and may contain different fragrant compounds. The choice of extraction procedure depends on the nature and amount of material available, and the qualities desired in the extract. Solvent extraction is better suited to small sample amounts or volatile materials that could be lost during distillation. The solvents used may be single solvents such as pentane or hexane, or mixed solvent systems such as 10% ethyl acetate:90% hexane which is also used on a large scale for extractions in place of benzene, which is no longer used on safety grounds.

Steam distillation is used in the laboratory if it is important to replicate the commercial process or to release all the volatile oils from deep within seeds, dried fruits or woody materials.

12.3.2 Gas–Liquid Chromatography

Usually referred to as simply GC, this is one of the most important and widely used analytical techniques in the fragrance industry, as it is ideally suited to the volatile compounds that are the basis of the industry. It is a means of separating a complex mixture into its components and subsequently quantifying the individual components. As a chromatographic technique, it is based on the partition of analytes between the mobile phase (a gas such as helium, hydrogen or nitrogen) and the liquid stationary phase, which is coated onto the inner wall of a very narrow fused silica column. The time taken for a material to elute from the column (its retention time) is reproducible under identical operating conditions and as the basic physical principles of GC are well established (Ettre and Hinshaw, 1993; Hinshaw and Ettre, 1993) the behaviour of the analytes can be predicted for different operating conditions. The retention time of a compound can be a good guide to its identity by reference to known standards.

The basic design of gas chromatograph can be fitted with a range of specific injectors, columns and detectors to optimise the separation of components and aid their identification. Recent developments in computer control, the use of robotic autosamplers and the trend to couple instruments together for sequential procedures have led to increased automation for routine analytical tasks performed by GCs in research, factory and quality control environments.

12.3.2.1 GC Injection systems. Many specialised injection systems are used with the GC in the modern laboratory to deliver the complete sample to the column without alteration. The conventional injection method is simply to inject a small volume (about 1 μL) of a dilution of the sample using a syringe with a fine needle, which pierces a silicon rubber septum and delivers the sample into a heated chamber where it is vaporised and carried on to the column in the stream of the carrier gas. Some of the carrier gas-containing vaporised samples may be split from the main column flow and vented to reduce the amount of sample delivered to the column. This is known as a ‘split injection’ and is suitable for almost all liquid or soluble materials, even samples that may contain trace amounts of non-volatile contaminants, as these are deposited in the vaporisation chamber of the injector and do not reach the column.

The high temperature of the injector, typically 250 °C, means that this method is not suitable for analytes that are subject to thermal degradation. For these materials an ‘on-column’ method is preferable, in which the solution of the sample is injected directly into the narrow capillary column with a fine needle. On-column injection techniques are also more suitable for extremely dilute samples, as more material is delivered to the column, but are less suitable for dirty samples containing non-volatile contaminants, which accumulate on the column.

One of the most versatile injection systems is the programmable temperature vaporiser (PTV), a design which incorporates a vaporisation chamber with a rapid and accurately controlled heating element, such that the temperature can be programmed to rise following the injection; thus, analytes are swept onto the column without being subjected to temperatures above their boiling point. By electronically controlling the action of the split vent valve, the system can also be used to vent the volatile solvent from the dilute samples at a low temperature and then to deliver the analytes to the column by increasing the temperature. This allows much larger volumes of dilute solutions to be injected, thus increasing the absolute amount of analyte on the column without flooding it with the solvent.

The use of a headspace-injection technique may be preferable if the analytes are contained within a non-volatile or corrosive matrix that cannot be injected directly (*e.g.* perfume in a washing powder). The basic principle of headspace injection is the delivery of a volume of vapour from the space above the sample material to the GC column. This can be achieved in several ways

- a gas-tight syringe;
- a sample loop and a system of valves to fill the loop with vapour then direct it onto the column; and
- an adsorbent on which the volatile materials are trapped, and subsequently desorbed with a solvent or by heating the adsorbent.

Only materials volatile at the sampling temperature are transferred to the GC. Therefore, most headspace injection systems include some means of either gently heating the vial containing the sample or bubbling a gas through the non-volatile liquid to purge the volatiles, which can then be trapped on an adsorbent. The purge and trap method of headspace injection is widely used for analysing very low levels of volatiles in water, whether they are environmental samples or samples of water containing perfume from washing machines or dishwashers. The trapping of natural volatiles on absorbent cartridges is the basis for headspace analysis of flowers and other natural materials (Ter Heide, 1985).

If the target analytes do not readily evaporate from the matrix they are in, then another recently improved technique – direct thermal desorption – is more effective. This is often used for dry, non-volatile matrices like wood, soil, spices or resins. The sample is placed directly in the liner or desorption chamber, which is then flushed with inert carrier gas and heated rapidly to transfer the volatiles to the analytical column.

12.3.2.2 GC Columns. The most common form of GC columns used in the fragrance industry is the wall-coated, open tubular type (WCOT) which are made of fused silica tubing coated with a thin film of stationary phase on the inside and covered with polyamide on the outside for protection. Fused silica columns are usually between 25 and 60 m long, with inner diameters from 0.53 to 0.1 mm. A widely used range of stationary phases is based on phenyl-substituted methyl silicones. The polarity of the phase is determined by the number of phenyl groups, a greater proportion of which increases the polarity of the phase. OV-1 is a common ‘non-polar’ stationary phase composed of dimethyl siloxane, while SE-54 is a more polar phase in which 5% of the dimethyl groups have been substituted with phenyl groups (Figure 12.2). Phases with greater polarity are achieved by using polyethylene glycol (PEG). Most manufacturers retain a similar system of nomenclature for their stationary phases; for example a DB-5 column from one manufacturer is broadly equivalent to HP-5, BPX-5 or ZB-5 from others. All are based on the 5% phenyl 95% dimethyl siloxane polymer although there are small differences in the chromatography achieved on different columns due to the different manufacturing conditions. The range of GC stationary phases is being modified continually and expanded with new materials for specific applications. The so-called ‘low bleed’ phases recently introduced have additional phenyl units in the siloxane chain. These prevent the sequential loss of silicone units from the end of the polymer chain, which causes the column to ‘bleed’ silicones at high temperatures. Cyclodextrin-based stationary phases are now available commercially for the separation of optical isomers, a technique referred to as chiral-GC.

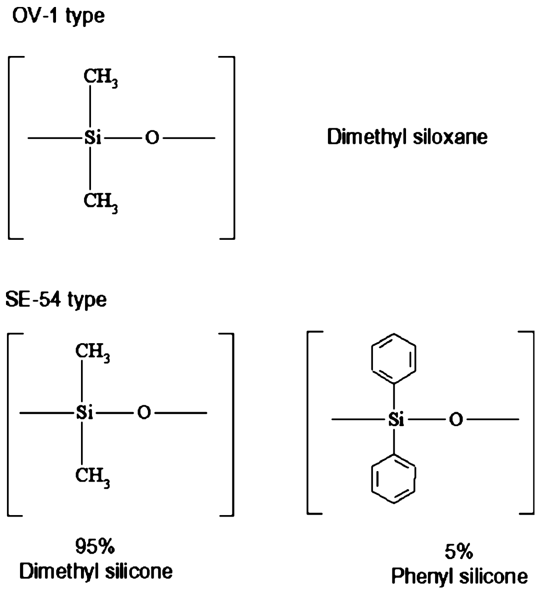


Figure 12.2 *Common GC stationary phases*

12.3.2.3 GC Detectors. The most common detector is the FID. The outlet from the column is directed into a carefully controlled hydrogen flame. The electrical potential across the flame is measured continuously, as this changes when an organic compound elutes from the column and is ionised in the flame. The analogue signal from the detector appears as a series of peaks over the duration of the analysis; which corresponds to each material that elutes from the column. The amplitude of the signal is directly proportional to the amount of material passing through the detector, and therefore the area under a peak on the recorded chromatogram is proportional to the amount of that material in the sample. The data are usually processed by an integrator or computer and the results are commonly expressed as a percentage of the total relative peak area (%RPA), the simplest quantitative measurement of the components in the mixture.

Coupling the column from the GC to a mass spectrometer provides a very powerful combination, GC–MS, which can identify and quantify almost all the compounds in a complex mixture such as an essential oil or perfume by reference to libraries of mass spectra of known compounds. Careful investigation of the mass spectrum can be used deductively to determine a possible structure for an unknown material using fragmentation theories to identify sub-structural components of the molecule. Recent developments in benchtop mass spectrometers have brought a range of specialised MS techniques into the realm of GC–MS machines; techniques such as chemical ionisation and MS–MS are now available, which provide more information on individual sample components allowing better identification of unknown compounds.

GC–Fourier transform infrared (GC–FTIR) spectroscopy is less frequently used than GC–MS, but involves a similar principle in which the outlet from the column is coupled to an infrared spectrophotometer. The technique currently suffers from a lack of library spectra, as the IR spectra taken in the vapour phase can be subtly different from condensed phase spectra or spectra collected using the well-established KBr disc method.

The use of nitrogen- or sulphur-specific detectors for GC enables small quantities of nitrogen- or sulphur-containing molecules to be detected. These often have very powerful odours (Boelens and Gemert, 1994); for example, 2-isobutyl-3-methoxypyrazine from galbanum oil, which has a powerful green note and 1-para menthene-8-thiol, a very powerful natural material with a strong grapefruit odour at concentrations in the ppb range.

GC-sniffing is an adaptation of special importance in the fragrance industry. The effluent from the column is split between a conventional detector and a smelling port that allows the individual components to be smelled by the human nose, which is more sensitive to certain materials than sophisticated detectors (Acree and Barnard, 1994). If the nose belongs to a perfumer, then the odours can be recognised and described immediately. This is particularly useful when trying to establish the odour of a single component in a complex mixture, as GC-sniffing provides information from a few micrograms of sample, which would only otherwise be available if the individual material could be isolated and purified in quantities of several grams (a very time-consuming process requiring relatively large amounts of sample).

Some separations can only be achieved by GC, and if it is necessary to isolate such a material, then preparative GC would be required. The flow from the column is momentarily directed to a cold trap as the desired compound elutes, which then condenses in the trap. The amounts that can be collected in this way are minute but a few hundred micrograms are sufficient for a ^{1}H NMR or IR analysis.

12.3.3 Preparative Liquid Chromatography

Liquid chromatography (Hostettman *et al.*, 1986) in its many forms is a separation technique based on the polarity of the analytes and their partition between the mobile and stationary phases, and is therefore complementary to fractional distillation that separates materials by their boiling point. The usual sequence for fractionating an essential oil or extract is to distil it first and then apply liquid chromatography to the distillation fractions as a further fractionation procedure, rather than an analytical tool. The selectivity of the technique is achieved by choosing a stationary phase, usually from the various activities of silica gel, and varying the polarity of the mobile phase, the solvent, by mixing a non-polar component (such as hexane or pentane) with different amounts of a more polar component (such as diethyl ether, ethyl acetate or chloroform).

In the simplest form of liquid chromatography, the solvent is applied to the top of the column by gravity from a reservoir, a slow but reproducible process. Greater speed and resolution can be achieved, even with relatively large columns containing up to a few kilograms of silica, by using 'flash chromatography', in which air or nitrogen under pressure is used to force the solvent through the column. This is a convenient and reasonably reproducible method for quickly separating fractions of very different polarities. For greater resolution and reproducibility on a smaller scale, the solvent can be pumped through the column at a continuous and controlled rate, a technique called medium-pressure chromatography or preparative high-performance chromatography (Prep HPLC; Verzele and Dewaele, 1986), according to the equipment used. Detectors are not used with gravity or flash systems, but a non-destructive detector, such as a UV spectrophotometric detector or a refractive index detector (relative refractometer), is used with Prep HPLC to monitor the solvent stream and allow accurate collection of fractions as they elute from the column.

Solvent gradients can be used to improve resolution. A simple, stepwise gradient involves sequentially using solvents of increasing polarity and can be applied to any type of column chromatography. Continuous solvent gradients can be generated by modern HPLC pumps, which mix solvents of different polarities to increase gradually the polarity of the mobile phase. Computer-controlled systems equipped with an autosampler and automatic fraction collector are available, which can be programmed to repeat a separation many times and bulk the fractions from each separation. This allows for larger amounts of sample to be processed, while achieving the resolution of a small-scale separation.

Analytical HPLC necessarily includes a detector on the outlet from the column, which responds to the presence of analytes in the solvent stream. Narrow-bore columns and fine particle sizes are used to achieve the best possible resolution. Although analytical HPLC is used in the fragrance industry to investigate the non-volatile fractions of essential oils and product bases, it is much more widely used in other industries (such as the pharmaceutical industry, for which it is the main research tool).

12.4 POSITIVE IDENTIFICATION

Once a fraction or component of an essential oil or an extract has been isolated, its identity needs to be determined. A familiar or common chemical is usually considered to be positively identified, if its retention times on a given GC phase and its mass spectrum match those of a reference material. However, the identification of some materials with similar mass spectra, such as the sesquiterpenoids, requires retention times on two GC phases of different polarity. If the mass spectrum of a chemical is not available in the reference libraries and its structure cannot be conclusively deduced from its mass spectrum, then it must be purified and analysed by other spectroscopic techniques such as NMR, IR or UV spectroscopy. The advantage of NMR over MS is that the spectra produced are usually fully interpretable. A number of different experiments can be carried out using ^{1}H NMR and ^{13}CNMR, which normally generate enough data on the relationship of atoms within the molecule for the molecular structure to be elucidated. However, the exact structure and absolute configuration of a new natural material can only be determined by either complete synthesis or X-ray crystallography. Total synthesis of the material for comparison with the isolate is very time-consuming and may be very complicated for some complex natural materials, and X-ray crystallography is an expensive technique only applicable to crystalline solids. NMR is, therefore, the most revealing analytical technique available for fragrance materials.

12.5 HEADSPACE COLLECTION

The headspace is the air above or around a fragrant substance that contains the volatile compounds. This can be collected for analysis when extraction of the volatiles from the material is not viable. This technique has been extensively developed for the collection and analysis of flower volatiles since many flowers do not yield an extract that reflects the odour of the fresh flower, while others are simply too rare to be available in sufficient quantity for extraction. Many different techniques have been applied to the collection of volatiles from the air above flowers including the use of cold traps, solvent traps, adsorbent materials and capillaries coated with adsorbents (Ter Heide, 1985; Kaiser, 1991). However, the most common method for trapping flower volatiles is the use of adsorbent traps. The traps are small glass tubes containing activated charcoal, Tenax® or a similar porous polymer through which the air is pumped. The volatiles from the air are adsorbed onto the trap, while air and water pass through unretained, thus permitting the volatiles from many litres of air to be concentrated on the adsorbent. The flower is enclosed in a modified bell jar or flask to prevent the volatiles being swept away by air currents and to isolate the flower from any contaminants that may otherwise drift into the sampling area. The traps are inserted into the enclosed space of the bell jar and attached to a small, portable pump until sampling is complete. The traps are then removed, sealed and returned to the laboratory for analysis. The duration and flow rate used for sampling are adjusted to match the expected concentration of volatiles in the air, the capacity of the traps and the intended method of desorption. Traps designed for thermal desorption contain only a few milligrams of adsorbent, and sampling can be completed in a few minutes. In contrast, solvent desorption can be carried out on traps containing any amount of adsorbent from a few hundred milligrams to several grams. The traps have a finite capacity for individual compounds, depending on the amount of absorbent and the dimensions of the trap. Different materials are retained to varying degrees according to their volatility and polarity, and it is therefore important to determine the capacity of the traps to ensure that none of the volatiles are lost during sampling.

Having collected the sample on the adsorbent traps it is important to keep them cool and dark, as the glass and absorbent surfaces are sufficiently reactive to cause degradation of some materials if exposed to light or heat for long periods. However, if adequately protected, they can be stored or transported over long distances.

Samples collected in this way can be desorbed using a solvent which provides a solution for conventional injection on any GC or GC–MS. Thermal desorption of specially designed traps directly onto a GC with the appropriate injection system is more sensitive, and very volatile materials are not obscured by large solvent peaks. However, there is the possibility of thermal degradation of the sample and the entire sample is used at once.

The great advantage of headspace sampling is that it can be carried out relatively easily in the field with simple, portable equipment; this has opened up many exciting possibilities for the natural product chemist who can now analyse the scent of just a single flower. This technique has been successfully applied to a great range of plants and flowers for fragrance analysis, pollination studies and other botanical investigations (Bicchi and Joulain, 1990; Kaiser, 1993; Knudsen et al., 1993;). The sample size can be limiting when it comes to identifying a new material. Although modern instruments are extremely sensitive and only a few nanograms are required for acquisition of a mass spectrum, several hundred micrograms are required for ^{13}C NMR and isolating materials on this scale, even using GC-trapping requires more sample than is usually available by headspace collection.

With the array of extremely sensitive instruments now available, the analytical chemist can identify materials of odour interest that are present at very low levels in natural materials. However, we still rely on traditional techniques and laboratory skills to fractionate samples and isolate new materials for identification.

12.6 THE FUTURE

Over the last 20 years, there has been an ever-increasing consumer interest in natural ingredients. Ethnobotanists have studied the use of plants in traditional cultures like Chinese medicine and the Indian ayurvedic system for many years and now their work is finding application in the cosmetics and fragrance industries in the west. The plants used by rainforest tribes of the Amazon are now being cultivated and extracted on a commercial scale and used in shampoos, toiletries and cosmetics not only as active ingredients but also to impart fragrance. The current interest in natural ingredients looks set to continue, but with ever-increasing legislation on the use and composition of cosmetics and fragrances, it is very important to know the composition of the natural products. So whether it is determining the composition of an essential oil that has recently come to market, or identifying the component responsible for the odour of an exotic flower, or identifying a compound in a natural product for the first time, there is still plenty for an analytical chemist to discover from among the natural materials used in the fragrance industry.

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CHAPTER 13

Chemoreception

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Quest International

13.1 WHY HAVE A SENSE OF SMELL?

The five senses (sight, hearing, taste, smell and touch) developed in living organisms as a means of giving them information about their environment and, indeed, all of the information we have about the world around us comes through them. The senses of smell and taste are referred to as the chemical senses because they give information about the chemistry of the environment. More importantly, they give information about changes in the chemistry of the environment. Any change in the environment could represent either a threat or an opportunity for the organism and so, there is a survival advantage in being able to detect changes, determine whether they represent an opportunity or threat and then take appropriate action. The various survival roles played by these two senses are outlined at the start of Chapter 3. Chemoreception was probably the first of the five senses to appear and was present in very simple, primitive species. The basic mechanism for detection of chemical signals from outside these primitive organisms is probably the basis of the senses of smell and taste, which we and other higher animals possess today. Nature does not discard systems, which work but develops, adapts and refines them. Therefore, this might give us clues about the receptor mechanism since it would probably then have begun evolution as a means by which aquatic organisms could detect water-soluble chemicals. Thus, it will help us to understand our sense of smell if we remember that the basic chemical mechanism is one which originated in the earliest and simplest forms of life and has been adapted to suit the requirements of our bodies.

For most animals, the chemical senses are the ones on which they rely most heavily, it is only some groups of birds and a few primates (chimpanzees, gorillas, orangutans, rhesus macaques and humans) which depend more on sight than on smell and taste. The primates concerned are the only mammals with colour vision, suggesting some sort of evolutionary trade-off between sight and smell. In the course of evolution, humans have lost, and are losing, active olfactory genes at a greater rate than the other primates, probably as a result of our lower dependence on smell for survival.

Over the last decade, many groups around the world have taken up the challenge of understanding olfaction and have already made significant headway. The receptors used to detect odorants are similar to those used to detect hormones and, indeed, to those used in vision. Therefore, any increase in our knowledge of smell and taste receptors could also have benefits in other fields, even though these are more advanced than olfaction at present. Apart from the academic interest, there are obvious commercial reasons for increasing our understanding. For example, my particular interest is in being able to predict the odour of new molecules without having to prepare and evaluate them and thus save time and money in the search for new perfume ingredients.

13.2 MEASURING SMELL

The two primary aspects of odour are character and intensity. Perfumers are also interested in properties such as tenacity and performance but these are derivative properties combining intensity with physical and chemical properties such as volatility, surface recognition/adhesion, chemical stability in the perfumed medium, Raoult's law deviations and so on. In order to study any phenomenon, it is important to be able to measure it. Unfortunately, both odour character and intensity are very difficult to measure. Odour is a phenomenon that exists only in the higher brain and must therefore be measured using psychological techniques. Moreover, it is highly subjective, even to the point where it would appear that each of us has a unique odour perception of the world around us, as will be explained later.

At first it might seem that odour character is easy to measure. One smells a rose and defines the odour as rose. However, as any gardener will tell you, different roses have different scents, so how do we set up a scale of rosiness? This problem increases when evaluating single chemicals, either natural rose components or novel substances, which have a rose character, or parts of the rose character. For example, how do we rate the rose character of the three major chemicals responsible for the natural scent of roses, *viz.* 2-phenylethanol, geraniol and citronellol? The answer is that we cannot. The trained nose will recognise these three chemicals and the contribution they each make to the scent of a rose. The smell of a novel material will then be described in terms such as rose, more like 2-phenylethanol than geraniol and with a hint of carnation. In other words, all of our odour descriptions are associative in that every odour is described in terms of its similarity to other odours. There are no fixed reference points. We tend to classify odours based on their natural sources. For example, we group apple and pear under the heading fruity because both are derived from fruits, and indeed from very similar fruits. However, this can be very misleading. Figure 13.1 shows a series of esters with their fruity scores as determined by a trained sensory panel using amyl acetate as a fruity standard. To the chemist, it is clear that, as steric hindrance around the ester group increases, the fruity character is reduced.

In Figure 13.2, we see a second set of esters, again with a fruity score. In this case, the fruitiness increases with increasing steric congestion around the ester function. The reason for this apparent contradiction with the previous set of results is that the first group of materials are pear

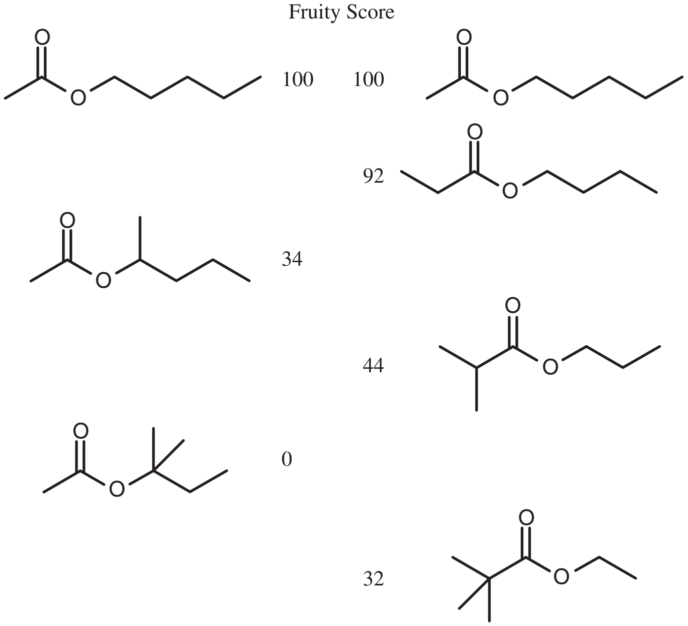


Figure 13.1 *Fruity scores of C7 esters*

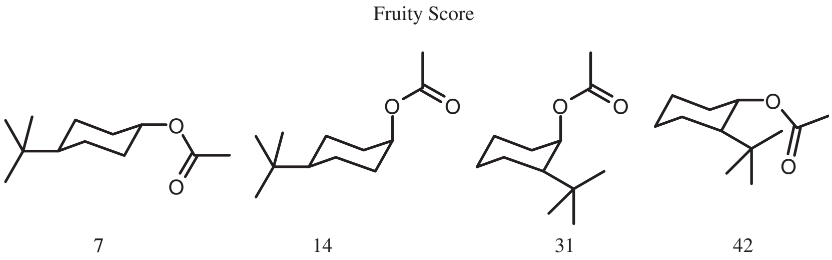
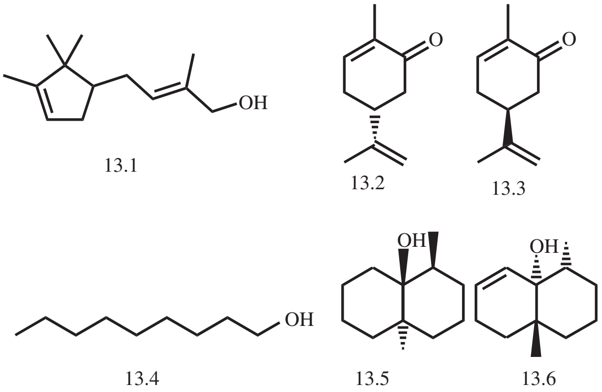


Figure 13.2 *Fruity scores of* t-*butylcyclohexyl acetates*



13.1
13.2
13.3
13.4
13.5
13.6

Figure 13.3 *Some molecules with interesting odour properties*

odorants whereas the second are apple. Thus our grouping of apples and pears as fruit might make sense in general terms but the extrapolation of this classification to their odours is not totally meaningful. Full details of this experiment can be found in the paper by Sell (1986).

Determination of odour character is complicated further by the subjectivity of odour. For example, it is easy to demonstrate that, in blind tests, Bangalol® (**13.1**) is perceived as sandalwood by some people, as musk by others and to a small minority, it is completely odourless (Figure 13.3) (Sell, 2000).

Another complication is that most of our descriptions are of complex mixtures and individual chemical signals are not always additive in a simple predictable arithmetic way. For example, it is known that the odours of the enantiomers of carvone are different. l-Carvone (**13.2**) smells of spearmint whilst d-carvone (**13.3**) smells of caraway. Less well known is the fact that addition of nonanol (**13.4**), an alcohol with an oily smell reminiscent of unperfumed washing up liquid, to l-carvone, will create an odour impression very similar to that of d-carvone. This is a trick of the flavourist's trade and is learnt by experience. Such effects are unpredictable, yet are always a potential issue when smelling mixtures.

Intensity measurements are even more problematical than character assessment. Once again, there are no fixed reference points and, in this case, there are no easy comparisons either. Even at the simplest level, presented with two different concentrations of the same material, how do we decide whether one is twice as strong, two and a half times as strong or a hundred times as strong. Why are we not confused by the statement that 'A large mouse ran up the trunk of a small elephant?' The answer is that our scales for mice and elephants are different and we automatically adjust without even thinking about it. The same happens when we try to estimate the intensity of odour stimuli. We unconsciously adjust our scales to the framework of the experiment in hand.

We also make similar adjustments when thinking about character. Most people will easily distinguish between the enantiomers of carvone and therefore they will be described as different. But how different are they? Thirty per cent of people cannot distinguish between them (Leitereg *et al.*, 1971) but would readily distinguish either of them from hydrogen sulfide.

Because of the difficulty of measuring intensity, people often measure threshold. One method for threshold determination has been described by Acree (1985). However, as described in Chapter 8, the slope of the Stevens' Law plot is as important as the intercept and therefore one compound with a lower threshold than another does not necessarily have a higher intensity at a given concentration. Threshold and intensity also vary from one individual to another and reported figures are usually statistical means. The determination of the threshold of the two enantiomers of geosmin (a potent earthy smelling material) is an example of a piece of high quality work with all the proper statistical controls and balances (Polak and Provasi, 1992).The average result obtained from this study is that the threshold of (−)-geosmin (**13.5**) is 0.0095 ppb in water and that of (+)-geosmin (**13.6**) is 0.078 ppb in water. Thus, the average person will find the threshold of the (−)-isomer to be 11.5 times lower than that of its enantiomer. Yet, on looking at the details of the experiment, it is clear that some individuals will experience very little difference and others will see one threshold as 32 times lower than the other. Moreover, some people will perceive the (+)-isomer as having the lower threshold. The issue of context also extends to input from other senses. For example, addition of a tasteless red dye to white wine will confuse even expert wine tasters because the red colour sets a frame of reference in place in the brain, and this results in the incoming signals being interpreted in a different way from that which would apply with a white reference framework (Morrot *et al*., 2001).

The fact that odour is a higher brain phenomenon also allows for even more complex complications in experimental measurement as demonstrated by the famous Slossen experiment (Slossen, 1889). In this experiment a liquid was poured onto cotton wool at the beginning of a lecture and the audience were asked to indicate when they could smell it. Those in the front row responded quickly and eventually about 70% of the audience believed they could smell it; the liquid was distilled water.

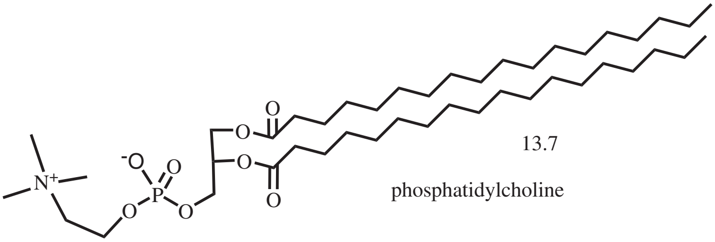
From the above examples, it is clear that a good understanding of sensory science and the techniques employed therein are vital and that experiments reported by people with insufficient understanding of sensory science can lead to very misleading results. Sensory science is described more fully in Chapter 8 and in Neuner-Jehle and Etzweiler's chapter in Müller and Lamparsky's book (Müller and Lamparsky, 1991).

The explanations for all of the above difficulties in odour measurement lie in the combinatorial nature of the mechanism of olfaction and in the neuroprocessing of the signals as we will see later but before we go on to that, it will be helpful to look more closely at the biochemical and physiological background of the sense of smell.

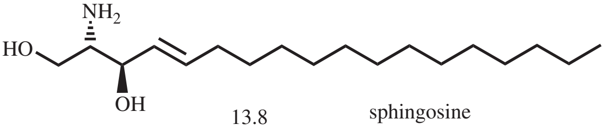
13.3 CELL WALL STRUCTURE

Living organisms are made up of cells. Some simple organisms exist as single cells, complex creatures such as humans contain many millions of cells each performing a specific role in the overall process of life. We can think of cells as tiny chemical factories with chemical reactions occurring inside the cell and chemicals being exchanged between the cell and its environment. The 'solvent' for the intracellular chemistry is water but that is also the major solvent in the world around the cell, and so nature had to devise a system for keeping the cell contents separate from the water in its environment otherwise the cell would be literally washed away and lost in the environment. The solution to the problem was to develop an oily layer called a 'lipid membrane'.

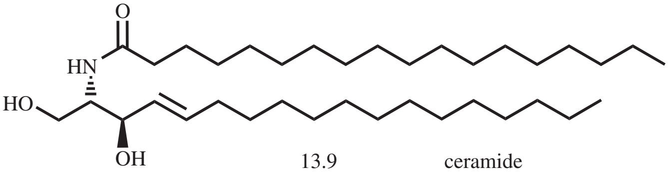
The lipid membrane is made up of a variety of fat-derived chemicals, the most important of which are the phospholipids (or lecithins) and ceramides. Phosphatidylcholine (**13.7**) is a typical phospholipid. The molecular structure is based on glycerol, propan-1,2,3-triol. Two of the alcohol functions are esterified with fatty acids, stearic acid in this case, and the third (one of the primary alcohol functions) with phosphoric acid. The phosphoric acid unit is also esterified with the quaternised amino-alcohol choline. This gives a molecule with two long fatty tails and a polar head group containing both positive and negative charges. Sphingosine (**13.8**) is an amino-diol derived from fatty acid precursors. Formation of an amide link with a fatty acid (stearic in the example shown in Figure 13.4) gives a ceramide (**13.9**) and esterification of this with choline monophosphate gives sphingomyelin (**13.10**). Like phosphatidylcholine, sphingomyelin contains two long fatty chains and a polar



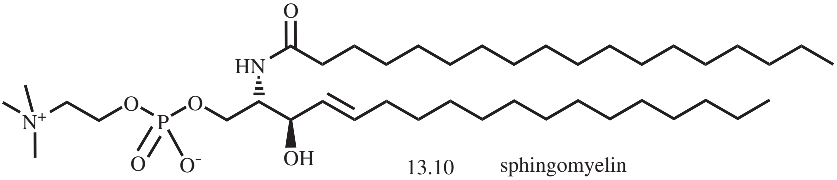
13.7 phosphatidylcholine



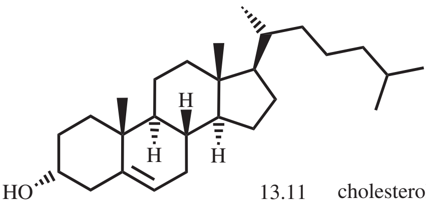
13.8 sphingosine



13.9 ceramide



13.10 sphingomyelin



13.11 cholesterol

Figure 13.4 *Some cell wall components*

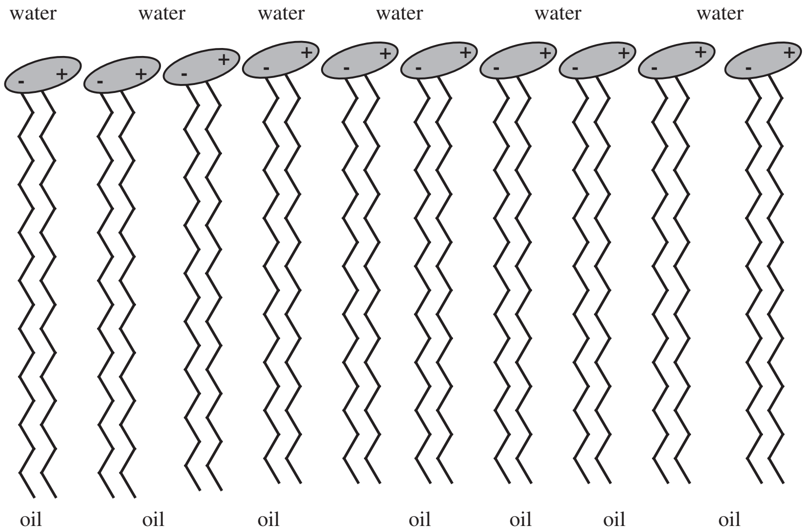


Figure 13.5 *Lipid monolayer*

head group. Normally the fatty chains in phospholipids and ceramides are 16, 18 or 20 carbons long. Sometimes, ceramides contain a 36 carbon chain, that is, one of about twice the normal length. The steroid cholesterol (**13.11**) (which is of terpenoid rather than fatty acid origin) is also present in cell membranes.

When phospholipids or sphingomyelin like materials are added to a two-phase mixture of water and oil, they sit at the interface with the polar head groups in the water layer and the fatty tails in the oil. This is shown in Figure 13.5. The polar head groups are locked together by the hydrophobic/hydrophilic interactions, water forming hydrogen bond bridges between head groups of two different lipid units and also by electrostatic effects of the charges in the head groups. This forms quite a stable monolayer of phospholipid. The monolayer can spread in two dimensions to form a sheet or it can fold into a sphere in three dimensions. Depending on which way it folds, it will either encapsulate an aqueous droplet in an oily continuum or vice versa. Such spheres are known as micelles.

If we substitute the oil phase of Figure 13.5 by the oily face of a second monolayer, the result is a bilayer with water on both sides and an oily phase in the middle. Such a bilayer is shown in Figure 13.6. Each side of the bilayer is basically a monolayer as shown in Figure 13.5 but there are two extra features worth noting. First, in Figure 13.6, one of the

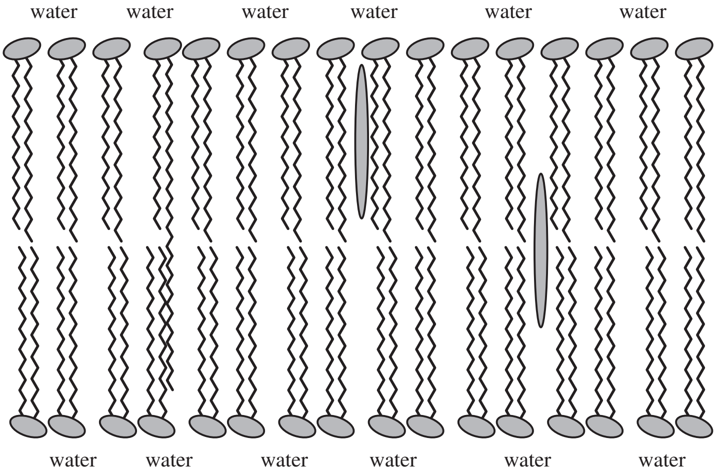


Figure 13.6 *Lipid bilayer*

ceramide units in the upper layer has a double length tail. It can be seen how this crosses over into the opposite monolayer and helps to lock the two together. Second, in Figure 13.6, cholesterol is shown as a large oval disc, viewed end on. The inflexible cholesterol molecule helps to provide rigidity for the whole bilayer by restricting movement of the floppy fatty chains. The bilayer structure thus shown is the basis of the mammalian cell wall. The cell is essentially a micellar structure with a double thickness wall and with aqueous material both inside and outside. The hydrophobic nature of the central part of the bilayer means that the aqueous cell contents are isolated from the aqueous environment. Hydrophobic materials are also unable to cross the cell wall because they are not able to pass through the polar outer surfaces of the bilayer. The cell is therefore totally isolated chemically from its environment.

13.4 PROTEINS INVOLVED IN SIGNAL GENERATION

Having isolated the interior of the cell from its environment, we have protected it from being dispersed in that environment but we have created some problems for it in doing so. The chemical factory of the cell needs to be able to bring feedstocks in from the environment and release waste products out to the environment. A single-cell organism needs to be able to communicate with the outside world and a cell in a multi-cell organism also needs to know what is happening in the rest of the organism. Therefore, some means of crossing the barrier of the cell wall is necessary for both chemicals and information. The rest of this chapter will concentrate on systems for communication between cells and of detecting changes in the environment. The method which nature uses to allow information to pass across the cell wall is to put a protein into the cell wall so that one end of it is inside the cell and the other outside.

One such type of protein is called an ion channel. These proteins sit across the cell wall with a hydrophobic face exposed to the interior of the wall and hydrophilic ends inside the cell and in the surrounding environment. The central core of the protein takes the form of a tube lined with polar groups which can associate with ions, typically Na^+, K^+ and Ca^{2+}, and thus allow them to pass through the tube. There is a gate in the tube which can be open or closed and therefore the flow of ions can be turned on or off as required.

As far as odour is concerned the key receptor proteins belong to the family of 7-transmembrane G-protein coupled receptors (GPCRs). Receptors of this type are used in many different applications in the body to allow communication between cells, for example through the use of hormones as messengers. The olfactory, or smell, receptors have adapted the same basic mechanism to enable animals to learn about the chemistry of their environment. The backbone of each receptor protein passes seven times through the lipid barrier of the cell wall. This means that each one has one end, the amino terminus, and three loops outside the cell and one end, the carboxyl terminus, and three loops inside the cell. The portions of the protein chain which pass through the cell wall, or membrane, coil into α-helices and the seven α-helices come together to form a columnar shape. A schematic representation is shown in Figure 13.7, in which the seven α-helices have been flattened out for ease of visualisation.

It is likely that at least some of the receptor proteins have sugar units attached to their outer surfaces as they are known to be inactivated by the addition of lectins (proteins which bind to sugars). This fact has been used to demonstrate that the enantiomers of carvone are detected by different receptors since the lectin concanavalin A blocks detection of d-carvone, but not the l-isomer (Kirner *et al.*, 2003). Conversely, wheat germ agglutinin, another lectin, demonstrates the reverse pattern of blocking (Deutsch and Apfelbach, 2003).

The portion of the receptor on the inside of the cell is connected to another protein called a G-protein. The key G-protein involved in smell is called transducin, while its counterpart in taste receptor cells is called gustducin. These G-proteins are each actually made up of three different proteins held together as a hetero-trimer. Two other groups of proteins

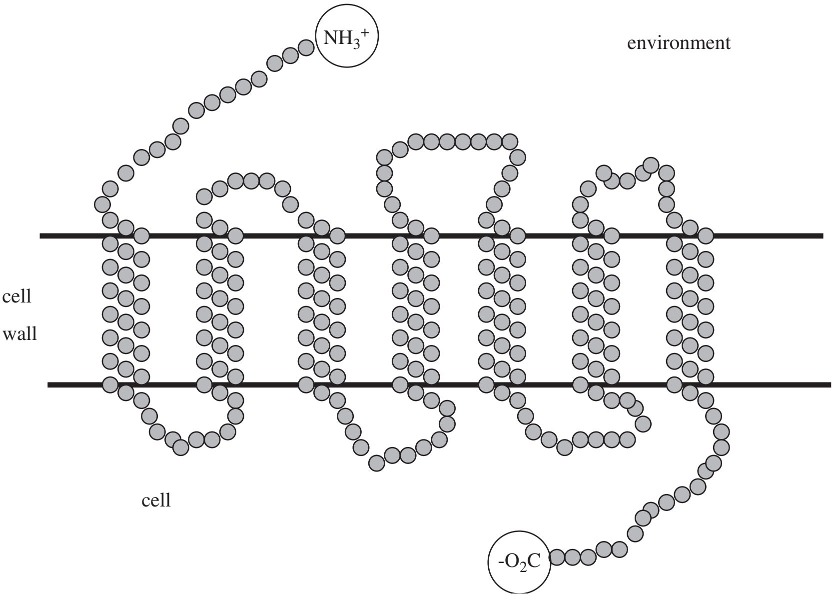


Figure 13.7 *7-Transmembrane G-protein coupled receptor protein*

also play a part in the detection of odour stimuli. Inside the cells, enzymes (adenylyl cyclase and phospholipase C) catalyse chemical reactions which are involved in relaying the initial signal. In the mucus surrounding the olfactory receptor cells, there are proteins called odour-binding proteins (OBPs), because they are known to bind perfume molecules, although their role in olfaction is not fully understood, as will be discussed later. There are also degradative proteins, called cytochromes, in the mucus but these are not part of the detection mechanism.

13.5 ANATOMY OF SMELL

The olfactory receptor cells are located in the olfactory epithelium. This is a greenish–yellowish patch of tissue, several square centimetres in area, which is found on the roof of the nasal cavity, as shown in Figure 13.8. The epithelium is about 100–200 μm thick and is bathed in an aqueous liquid known as the olfactory mucus. The mucus is about 35 μm thick and flows backwards across the epithelium at about 1–6 cm/min. The receptor cells run through the cribriform plate at the base of the skull. At one end (in the epithelium) the cells contain hairs, or cilia, which are 20–200 μm long and which is where the receptor proteins are located.

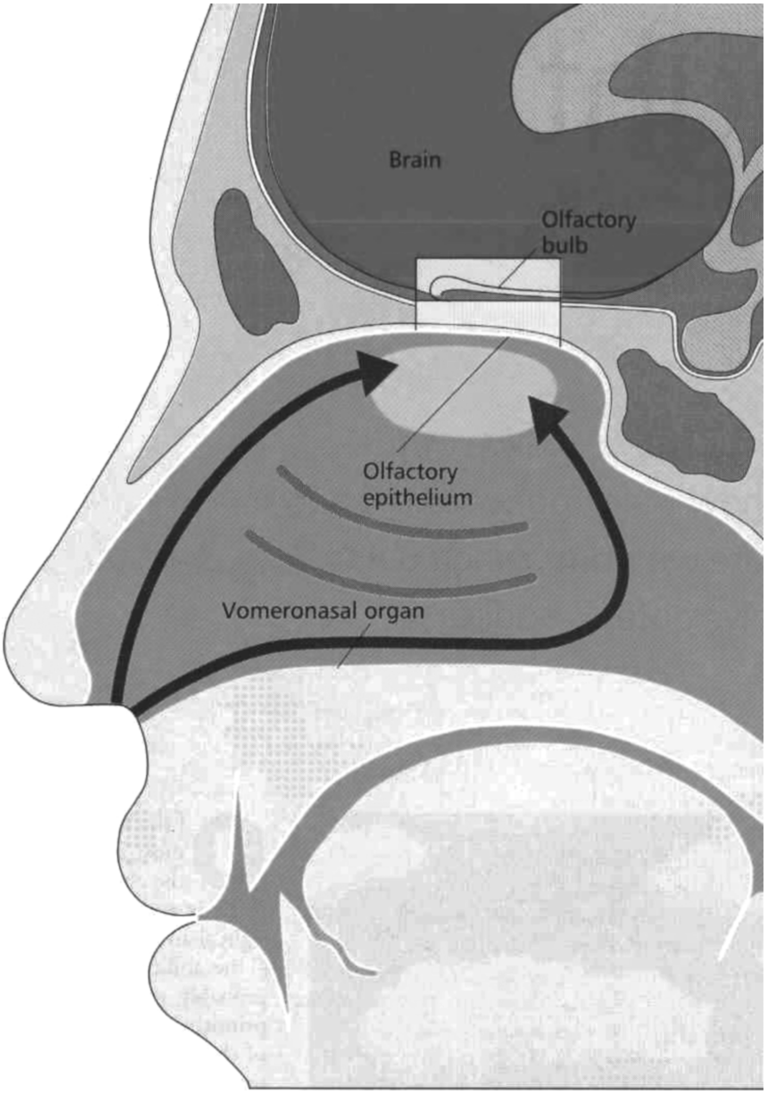


Figure 13.8 *Organs associated with the sense of smell*

At the other end, next to the olfactory bulb, they contain synapses which connect electrochemically to the nerve cells of the olfactory bulb. The latter is part of the brain and this is where signal processing starts. Each receptor cell fires spontaneously at a rate of between 3 and 60 impulses per second. The rate of firing increases when an odorant is present. The neurons from the epithelium converge on areas of the olfactory bulb known as glomeruli. Evidence suggests that all of the signals from each type of receptor cell converge onto a common glomerulus.

About 60% of odorants also trigger receptors in the trigeminal nerve fibres, which run around the entire surface of the nasal cavity. The interaction between the olfactory and trigeminal senses will be discussed later under the section 'Signal Processing'.

There is an ongoing debate about the role of the vomeronasal organ (or Jacobsen's organ) in humans. This organ is very important to many animals such as pigs, snakes and rodents. Snakes use it for detecting food, rodents use it for chemical communication, in other words for the detection of pheromones. In humans it is found on the base of the nasal cavity. It is present in most humans at birth but disappears from most during adolescence. It is not innervated, in other words, it has no direct connection to the brain. Recent experiments suggest that it is not active in humans.

13.6 SIGNAL GENERATION

The process of olfaction begins when odorants from the air in the nose enter the mucus layer around the receptor cell. This air could have reached the nose either by direct inspiration through the nostrils or by diffusion up to the nose from the mouth, as is the case when we smell the volatile components of food in the mouth. In order to have reached the olfactory epithelium by either route, a molecule must be volatile at ambient temperature and pressure. Thus fragrance molecules generally have a molecular weight under 300 Da and are hydrophobic in nature. Therefore, odorant molecules usually have only a limited degree of water solubility. Diffusion through the aqueous layer of the mucus to the receptor cell is possible but the low water solubility of odorants suggests that some assistance might be necessary.

Lipocalins are a family of proteins, which serve to transport small molecules around the body. A sub-set of the lipocalin family is found in the nasal mucosa and its members are known as odour-binding proteins (OBPs). These proteins are known to bind odorants but their role in olfaction is not understood at present. Each OBP is really a complex of two cup-shaped proteins with a 'hinge' formed by the amino acid backbone of one passing through that of the other. Once they have bound a substrate, the cups come together at the rims and so form a spherical hydrophilic unit and an X-ray crystal structure of such an OBP odorant complex has been published (Bianchet *et al.*, 1996). The role of the OBP could be to

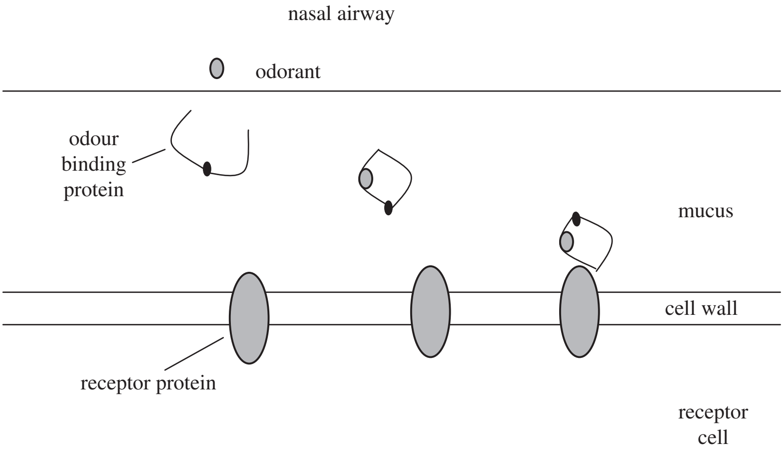
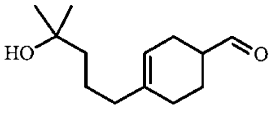


Figure 13.9 *Possible role of odorant binding protein*

remove excessive levels of odorant from the mucus and thus lead to a cleaner, attenuated signal. It could be to transport the odorant across the mucus layer and deliver it to the receptor protein. This putative process is shown schematically in Figure 13.9. Having transported the odorant across the mucus, the OBP might deliver it to the receptor protein or, alternatively, the receptor protein might simply discriminate between the free OBP and one which has been modified by co-ordination with an odorant.

The mechanism by which 'spent' odour signal molecules are removed from the area is also unknown. It could simply be by being carried away in the mucus flow or it could be being trapped by OBPs or by degradation by one of the cytochrome enzymes present in the mucus. It could also be through a combination of these different mechanisms.

The arrival of the odorant or the odorant/OBP complex at the outer surface of the receptor protein induces a change in the latter. It is not possible to see directly what happens at this point. The receptor must be located in the cell wall and therefore we are dealing with single molecule events and these cannot be studied by techniques such as X-ray crystallography. Similarly, the environment is too complex to be amenable to elucidation by NMR. Therefore, ideas on the exact nature of the initial receptor event are based on calculated guesses extrapolated from what we know about other systems. One popular hypothesis is that the binding sites lie inside the cylindrical channel between the seven *trans*-membrane helices of the receptor protein. This would be consistent with hormone receptors and with the optical receptors in which the retinal-derived



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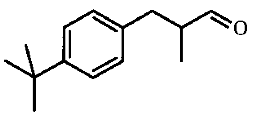
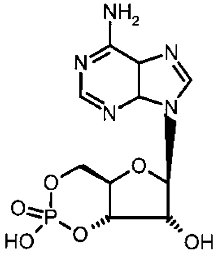


13.13



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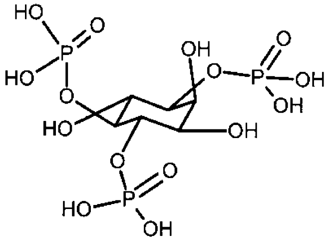

13.15

Figure 13.10 *Muguet ingredients and second messengers*

pigment is held in such a way. Two groups of workers have studied potential binding sites in the rat olfactory receptor protein OR5. Singer and Shepherd (1994) identified a possible binding site for Lyral® (**13.12**) and the other (Bajgrowicz and Broger, 1995) a potential site for Lilial® (**13.13**) (Figure 13.10). The two binding sites were in similar areas of the protein. In both cases, the work involved molecular modelling rather than isolation and characterisation techniques and is therefore circumstantial. However, it is interesting to note that both molecules have similar muguet (lily of the valley) odours.

Whatever happens at the outer face of the receptor protein induces a change in the shape of the inner face. It is thought that one of the seven α-helices is pushed further down into the interior of the cell. This causes the G-protein to break away from the receptor and dissociate into its three constituent parts. One of these fragments serves to switch an enzyme inside the cell from an inactive state into an active one. The two enzymes involved are adenylyl cyclase and phospholipase C. The activated enzyme then carries out a variety of chemical reactions in the cell. One of these reactions is the generation of small molecules known as second messengers.

Adenylyl cyclase generates cyclic adenosine monophosphate (c-AMP) (**13.14**) whereas phospholipase-C produces inositol triphosphate (IP3)

(**13.15**). These second messengers interact with yet another type of protein, the ion channels. They open the gates on the ion channels and allow positively charged ions to flow into the cell, therefore creating an electrical potential between the cell and its environment. This is relieved when the synapse at the other end of the cell, in the olfactory bulb, fires across to the next nerve cell in line and the transduction process is set in motion. The second messengers also set in train a process which deactivates the receptor by phosphorylation, and a dephosphorylation is necessary to make it active again.

Readers wishing to know more detail could consult the excellent reviews by Breer (1994, 2003), Lancet (Lancet and Pace, 1987) and Shepherd (1995).

13.7 THE COMBINATORIAL NATURE OF ODOUR PERCEPTION

There is an obvious survival advantage to being able to detect as wide a range of chemical signals as possible, even molecules which the organism has never encountered before. The mammalian nose has therefore evolved to do this and, for example, synthetic chemists know that they can produce a molecule which never existed before in the universe and still be able to smell it. This tells us that our olfactory receptors are not tuned to specific odours but to physical and chemical properties of odorant molecules. The signal which goes to the brain shows a pattern of firing across a range of receptors and the higher brain interprets this pattern as a phenomenon which we call smell. This combinatorial nature of the sense of smell was postulated by Ernst Polak (1973) and vindicated in 1999 when Linda Buck *et al.* (Malnic *et al.*, 1999) demonstrated that olfactory receptor proteins are broadly tuned so that each receptor type responds to a range of odorant molecules and each odorant molecule interacts with a range of receptors. Her findings were supported the following year by those of Gordon Shepherd *et al.* (Xu *et al.*, 2000).

The gene family, which encodes the receptor proteins is the largest in the genome and actually constitutes about 1% of the latter (Buck and Axel, 1991). Potentially, there are over a thousand different types of receptor proteins. However, not all are expressed in any one species and those that are expressed in one animal are not expressed uniformly across the epithelium. Thus, information about the nature of the odorant could also be gained from knowledge of the intensity of signals arising from different areas of the epithelium. The receptors expressed in fish are from a different part of the 'family tree' of receptors from those expressed by mammals. Intriguingly, the nasal cavity of the frog is divided into four sections rather than the two (left and right) of mammals. One on each side of the frog's head is open to air when the frog breathes and the other two are exposed to water when the frog is submerged. A valve switches between the two states. The receptor proteins expressed in the 'wet' nose are similar to those of fish and those in the 'dry' nose to those of mammals. Tadpoles start life with only the 'wet' nose and the 'dry' nose develops during metamorphosis into frogs.

Most mammals use about 700–800 different types of olfactory receptor proteins in their noses. Humans, chimpanzees, gorillas, orangutans and rhesus macaques use only about half that number. Interestingly, these species are the only animals to possess colour vision and so it would seem that there has been an evolutionary trade-off between smell and colour vision. The rate of loss of olfactory receptor genes is higher in humans than in the other primates, indicating our increased dependence on vision rather than smell (Gilad *et al.*, 2003).

Each human uses only about 350–400 different types of olfactory receptors and there is variation between individuals as to which 350–400 of the 1000 are active genes and which are Ψ-genes (Lancet, 2003). Thus, the subjectivity of olfaction starts at the most basic level in that statistics indicate that each of us probably detects odours with a unique combination of receptors. An example of individual variation is given by Lasker and Teubner (1999). They investigated the ability of subjects to discriminate between stereoisomers in 10 pairs of enantiomers and found that the degree of such ability was time stable for each individual but that there were big differences between individuals.

We each have millions of individual receptor cells in our noses and the receptor proteins in them are not tuned to odour but to physical/chemical properties of the molecules to which they are sensitive. Each type of receptor responds to a variety of odorant molecules and each odorant molecule interacts with a variety of receptor types (to a different degree with each one). This combinatorial mechanism is illustrated in Figures 13.11–13.13. In these figures, we see an array of six receptors interacting with odorants. In Figure 13.11, molecules of odorant A interact with four of the receptor types to send one pattern onwards to the higher brain. In Figure 13.12, molecules of odorant B interact with three of the receptor types to send a different pattern to the brain. The brain can then learn that pattern A means the presence of molecules

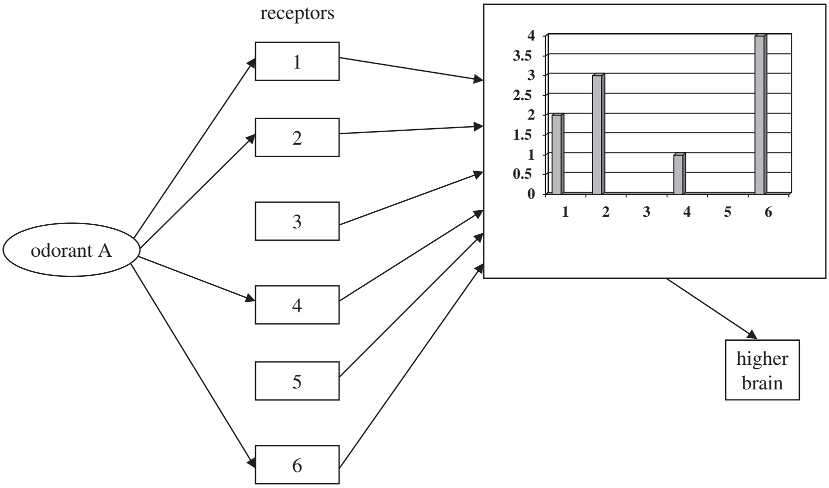

Figure 13.11 *Generation of signal by odorant A*

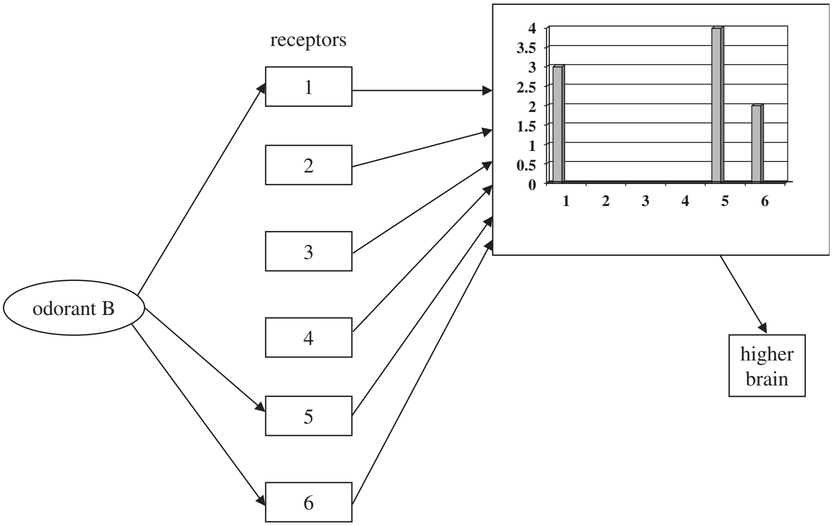

Figure 13.12 *Generation of signal by odorant B*

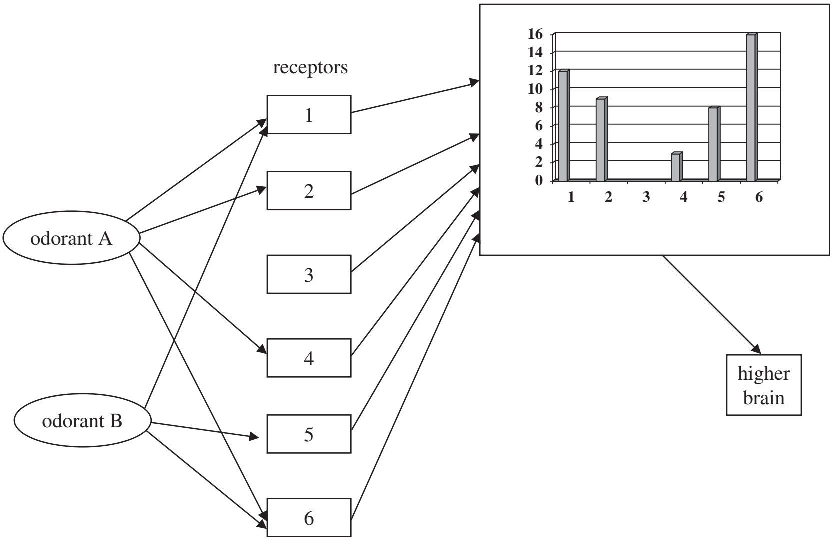

Figure 13.13 *Generation of signal by mixture of odorants A and B*

of odorant A in the nose and pattern B, odorant B. In Figure 13.13, a mixture of molecules of both odorants sends a third pattern to the brain. This pattern is the sum of the two original ones. Initially the brain will interpret this as a new pattern. However, a trained brain will be able to recognise the “A” and “B” patterns within the overall pattern and therefore analyse the odour and identify component parts. Thus our ability to detect and describe something we have never smelt before is easily accounted for. The receptors are not tuned to known molecules and so any molecule entering the epithelium will be sensed. New molecules generate a new pattern and the brain then interprets this by looking for similarities to known patterns (Sell, 2001).

13.8 SIGNAL PROCESSING

The olfactory cells run through the cribriform plate at the base of the skull and feed directly into the olfactory bulb. Neurons from the epithelium converge on areas of the olfactory bulb known as glomeruli. Evidence

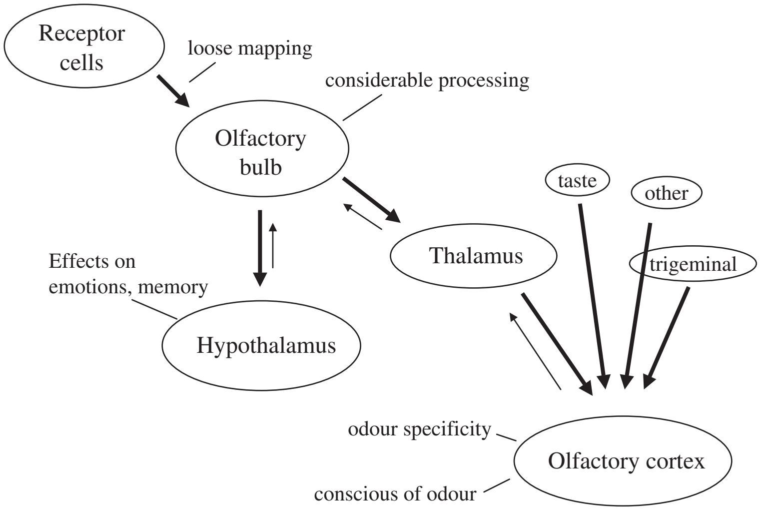

Figure 13.14 *Processed signals are sent to both the thalamus and the hypothalamus*

suggests that all of the signals from one type of receptor converge on a common glomerulus. Signal processing begins in the bulb and processed signals are sent to both the thalamus and the hypothalamus (Figure 13.14). The hypothalamus is the part of the brain that deals with emotion and memory and this direct (and physically short) connection explains why smell has such a fast and effective effect on emotions and memory as these are triggered before the higher parts of the brain are even aware of the fact that an odour has been detected.

About 60% of odorants also trigger receptors in the trigeminal nerve fibres, which run around the entire surface of the nasal cavity. For example, nicotine fires both the olfactory and trigeminal nerves whereas vanillin triggers only the olfactory system. Indeed, it is suggested that our ability to distinguish between the odours of the enantiomers of nicotine is due to the trigeminal rather than the olfactory system (Hummel *et al*., 1992; Thürauf *et al*., 1995).

Odour only exists in the higher brain and is a synthesis of signals from the olfactory pathway, those of the trigeminal system, those from other senses (*e.g.* taste) and so on. There are many gates along the pathway with the potential to shut off or enhance odour signals. All of the various nerve pathways (those due to different smells and those from other channels interact with each other and so we can have odour enhancement or suppression as a result of these various interactions. For instance, any

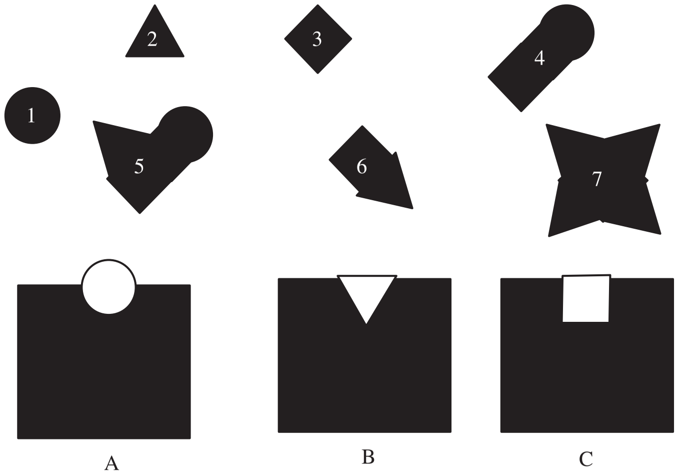

Figure 13.15 *An alternative pictorial representation of the combinatorial mechanism of olfaction. Odorant 1 can trigger receptor A; odorant 2 can trigger receptor B; odorant 3 can trigger receptor C; odorant 4 can trigger receptors A and C; odorant 5 can trigger receptors A and B; Odorant 6 can trigger receptors B and C and odorant 7 can trigger receptor B. Conversely, receptor A can be triggered by odorants 1, 4 and 5; receptor B by odorants 2, 5, 6 and 7; and receptor C by odorants 3, 4 and 6.*

cook will tell you that salt will affect the odour impression from flavours. In this case, the taste signal from the salt receptor is accentuating the smell signal from, for example, a spicy food. Similarly, olfactory fatigue (also known as adaptation) is a result of these higher brain processes. The brain adapts to a continuous signal by closing a gate in the neural pathway. (This is an evolutionary development to divert brain power away from things which do not give useful information.) Basically, the brain is saying, 'If I've been smelling this for the last 2 h and I'm still alive and well, then that odour is not a threat and I don't need to waste effort detecting it.' It is these higher brain interactions that give rise to the difficulties described above in measuring smell.

13.9 IMPLICATIONS FOR ODORANT DESIGN

Synthetic organic chemists working in the field of molecule discovery can take three different approaches to finding new molecules with a desired biological activity. The first is to make molecules on a random basis and screen them for activity. The second is to develop statistical relationships between molecular structure and the desired property (structure–activity relationships or SARs) and use these to guide the search. The third approach is to develop an understanding of the exact mechanism of action of active materials and then use this knowledge to rationally design new molecules. For example, in the pharmaceutical industry, the objective might be to inhibit the activity of a given enzyme. By learning the structure of the enzyme's active site, it is then possible to design molecules with a high chance of binding to that site and blocking it.

It is obvious that the first approach has the lowest chance of success and the third the highest. Good information about the biological target is usually difficult and expensive to obtain and so the usual strategy is to use a combination of all three approaches. Each approach is not used in isolation but information from each is used to improve the others. The more data there are in a statistical model, the better a model it will be. Initially this data comes mostly from the random screening approach, and continued generation of data from random screening will always be of use in refining the SAR. If we think of the mechanism of drug action as a key (the drug) fitting into a lock (the biological target), then we will see that the SAR gives us information about the key whereas biological and biochemical research gives us information about the lock. It is also obvious that information about the key gives us information about the lock and *vice versa*. Thus the statistical and rational approaches are complementary and can be used together to give a better picture of the lock/key interaction.

Discovery chemists in the fragrance industry use the same methods as those of the pharmaceutical industry but in our case, the target property is odour. A detailed account of the techniques employed in the design of novel fragrance ingredients is given in Chapter 15. It is not surprising that workers have postulated theories on how the sense of smell works and tried to correlate these with SAR models. Reviews of the various theories, which have appeared in the past, have been published by Laffort (1994), Rossiter (1996), and in the first edition of this book (Pybus and Sell, 1999). There are two important caveats concerning SARs, which discovery chemists must always remember. First, if the biological mechanism is multi-stage, the SAR will not give information specifically about any one of these stages. Second, SARs are, by their very nature, interpolative and will not predict new structures outside the limits defined by the training set. The latter caveat simply indicates that some level of random screening is worthwhile. The first caveat links to the question, ‘Have advances in our understanding of the mechanism brought us closer to accurate rational design of novel odorants?’

The dream of finding a receptor for any given odour and designing the perfect ligand for it are long dead in fragrance research. We now know that the fit between odorant and receptor is loose and therefore difficult to model. Each receptor type binds to a range of odorants and each odorant binds to a range of receptor types. The pattern of firing is the key at the initial stage but even that is not the whole story as all of the interactions between nerve signals throughout the entire network of processing are also important. Thus to predict, with a high degree of accuracy, the odour of any putative odorant molecule, it would be necessary to know how well it binds with all of the receptor proteins in the subject, how those signals combine and are interpreted at every stage of neuroprocessing and how they interact with all the other signals involved in the overall phenomenon of olfaction. In theory this might be possible one day but the computing power required would be far beyond the economic reach of any machine available today. Furthermore, we also know now that, since each individual has a unique experience of odour, such a rational design would be an individual design.

The pharmaceutical industry builds pharmacophores. These are models defining the requisites for a given activity. They include such features as overall shape and electron distribution, hydrogen-bonding centres, hydrophobic bulk and excluded space (*i.e.* areas of space around the target molecule in which there must be no steric bulk) and ideally these will closely resemble the active site of the enzyme or receptor protein in question. However, the search for an active drug targets a single protein. Olfaction depends on hundreds of target proteins, so is the approach irrelevant for fragrance? No, similar models have been developed and used successfully for fragrance ingredients. For example, the review by Kraft *et al*. (2000) describes olfactophores for a variety of odour types. Any given fragrance molecule will interact to a given degree with each of a range of receptor types and what the olfactophores, or similar models, are doing is to give a statistical average of properties which are required to give a similar profile of receptor binding. Such models are intrinsically statistical in nature and merely estimate the probability of a novel material having the desired odour. For the foreseeable future, it will still be necessary for chemists to synthesise samples of novel materials in order to assess their odour.

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CHAPTER 14

The Search for New Fragrance Ingredients

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Quest International

14.1 INTRODUCTION

The perfumers of today have at their disposal over 3000 fragrance ingredients to choose from when creating a perfume. These include natural oils, nature-identical materials and synthetic compounds (see Section 14.3 for definitions). With such a large number to choose from, one might wonder why there is a need for new fragrance ingredients. In this chapter, the stability of a range of lily of the valley (muguet) odorants is used to illustrate why the introduction of new fragrance ingredients is important. Other reasons for extending the perfumer's palette are also discussed.

Continuing with the muguet theme, I then use examples to explain how a chemist might go about his search for novel materials. The more traditional approaches, such as the analysis of natural products, serendipity and lead optimisation, are illustrated with only one or two examples, since these techniques have been discussed in more detail in Chapters 3 and 12. Instead, I have focused on techniques that have the potential to lead to the discovery of new active compounds by design rather than chance. Over the past 20 years, such approaches have been made easier by the rapid advances in theoretical and computational chemistry and by the introduction of more powerful computers.

The scope of odorant–receptor modelling is discussed. Unfortunately, this modelling work is very speculative and its usefulness is rather limited. This stems from our lack of knowledge of the 3-D structure of olfactory receptors and the combinatorial mechanism of odour perception (see Chapter 13). Instead, the fragrance chemist must turn to structure–odour relationships for the rational design of new fragrance ingredients. Three approaches to the development of structure–activity relationships (SARs) and their use in the fragrance industry are discussed: the Hansch analysis, pattern recognition and the olfactophore approach. Generally, it is considered that the larger the data set, the better the final model is likely to be, provided of course that the data set is of good quality. However, conformational analysis on a limited number of odorants can also be extremely useful and this is illustrated towards the end of the chapter with an account of the discovery of Quests' new captive muguet ingredient, Rossitol®.

14.2 THE NEED

The number of ingredients available to a perfumer creating a fragrance for relatively cheap and hostile products (such as strongly alkaline bleach or acidic antiperspirant) is significantly lower than the number available for use in alcoholic fine fragrances. The perfumers' freedom of choice is considerably restricted by factors such as stability and cost. For example, Figure 14.1 shows the relative organoleptic stability of a range of muguet

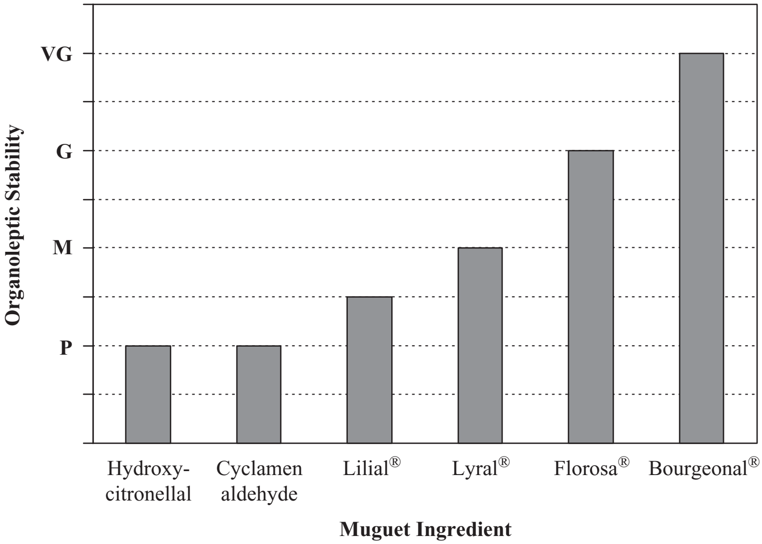
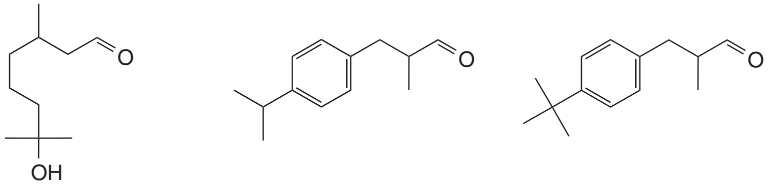


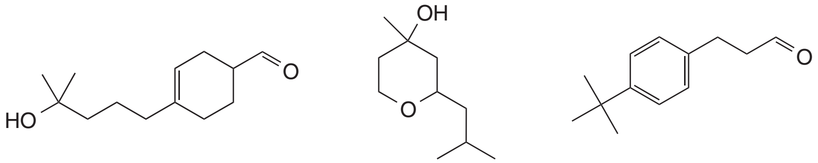
Figure 14.1 *Organoleptic stability of various muguet ingredients in an aerosol antiperspirant after storage at 37 °C for 12 weeks*

ingredients in an aerosol antiperspirant (1–6, Figure 14.2). To determine the organoleptic stability, the ingredient is first dosed into the product base and then stored at a specific temperature (usually 25 or 37 °C) for a given number of weeks (4, 8 or 12). At the end of this period, the odour of the test sample is compared with that of a control and ranked as very good, good, moderate or poor. The control is either a freshly prepared mixture of the fragrance ingredient in the product base or a sample that is as old as the test sample, but which has been stored at a lower temperature to minimise deterioration of the fragrance ingredient. When the odour of the test sample and the control are virtually identical, the fragrance ingredient is said to be organoleptically stable and it is ranked as very good. If either the odour of the fragrance ingredient has virtually disappeared to leave only the odour of the product base or the sample has developed undesirable olfactory notes, the fragrance ingredient is classified as having poor organoleptic stability. In Figure 14.1, the organoleptic stability of the six muguet ingredients is represented by a bar diagram – the taller the bar, the more stable the ingredient. It is clear from this figure that in his creation of a muguet-type fragrance for use in an aerosol antiperspirant, the perfumer is forced to use either Florosa® (5) or Bourgeonal® (6).

The organoleptic stability of an ingredient depends on a combination of two factors: its sensory characteristics and its chemical stability. The latter, of course, will be determined by the nature of the product base.



(1) hydroxycitronellal **(2)** cyclamen aldehyde **(3)** Lilial® (Givaudan)



(4) Lyral® (IFF) **(5)** Florosa® (Quest Int.) **(6)** Bourgeonal® (Quest Int.)

Figure 14.2 *Muguet ingredients used in organoleptic stability work*

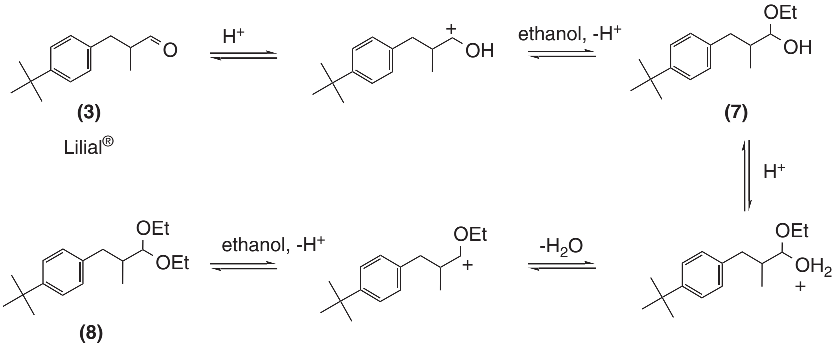
Antiperspirant formulations are acidic because of partial hydrolysis of active antiperspirant agents such as aluminium chlorhydrate (Equation 14.1). It is an inorganic salt that consists essentially of complex aluminium chloride, described empirically as $[Al_2(OH)_5]_n.nCl$. The complex is polymeric and loosely hydrated.

$$[Al_2(OH)_5]^+ + xH_2O \rightleftharpoons [Al_2(OH)_{5+x}] + xH^+ \qquad (14.1)$$

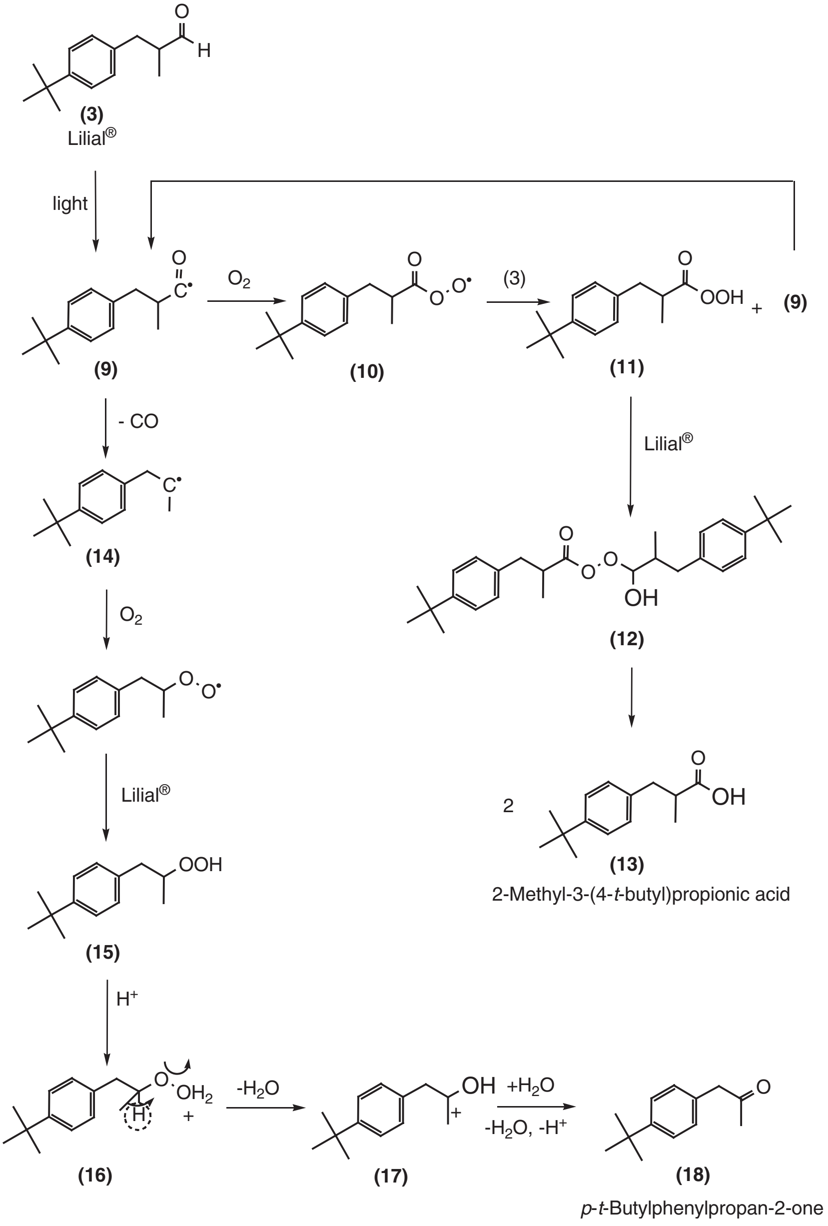
where x is a variable depending on concentration

Florosa® (5) performs well in antiperspirants because it is chemically more stable than the aldehydes. Ether groups are relatively inert and, although possible reactions of the alcohol group will depend upon the specific formulation of the antiperspirant, there is relatively little it could do other than acid-catalysed dehydration, which usually requires fairly high temperatures. Consequently, the level of Florosa® in, for example, an ethanol-based roll-on antiperspirant after 4 weeks of storage at 37 °C remains unchanged. In contrast, the percentage of Lilial® remaining is only 15%. This huge difference is due to the reactivity of the aldehyde functional group. It reacts with ethanol to form the corresponding diethyl acetal (8) and undergoes autoxidation to yield the corresponding carboxylic acid (13). The acetal formation will be acid catalysed and will proceed via the hemiacetal (7) (Scheme 14.1).

Autoxidation is defined as the reaction of organic compounds with oxygen under mild conditions (Scheme 14.2). This chemical transformation is so facile that if, for example, a thin film of Lilial® is placed in a dish overnight, the liquid would be converted into a white crystalline solid, the corresponding carboxylic acid, by the morning. Oxygen itself is too unreactive to be the species that actually abstracts the aldehydic



Scheme 14.1 *Acid-catalysed acetal formation*



Scheme 14.2 *Autoxidation of Lilial®*

hydrogen atom. An initiating process produces a trace of free radical (9), which reacts with oxygen to give the peroxy radical (10). This radical removes a hydrogen atom from a second molecule of Lilial® to form the peracid (11) plus the acyl radical (9). The peracid (11) reacts with a further molecule of Lilial® to form the adduct (12), which then decomposes to two molecules of acid (13). In the case of Lilial®, and other aldehydes which have an alkyl substituent at the α position, there is a second possible autoxidation process. The acyl radical (9) can be decarbonylated to give the secondary carbon radical (14), which can react with oxygen to form the corresponding hydroperoxide (15). Under acidic conditions, the hydroperoxide is protonated (16) and a hydrogen atom migrates to form the alkoxycarbonium-ion intermediate (17), which on hydrolysis yields *p*-*t*-butylphenylpropanone (18). Out of the autoxidation processes, it is formation of the carboxylic acid that predominates with typical ratios of compound 13 to compound 18 being in the order of 20:1.

The initial step of both the above reactions, the formation of an acyl radical, is catalysed by light and metal ions that are capable of a one-electron reduction transition (*e.g.* $Fe^{3+} \rightarrow Fe^{2+}$). Thus, the autoxidation of aldehydes can be greatly slowed down by keeping the compounds in the dark and by very careful purification. However, the most efficient method is the addition of antioxidants, such as phenols and aromatic amines that react preferentially with any radicals that may be present.

Bourgeonal® (6) performs well in antiperspirant for a combination of reasons. Our studies have shown that, despite its structural similarity to Lilial®, it is substantially more stable. After 4 weeks at 37 °C, the level of Bourgeonal® remaining in an ethanol-based roll-on is typically 65%, as opposed to only 15% for Lilial®. Although like Lilial®, Bourgeonal® reacts with ethanol to form the corresponding diethyl acetal, it does not appear to undergo autoxidation. The reasons for this remain unclear. The only structural difference between these two compounds is the absence of a methyl group at the α position of Bourgeonal®. Although this would inhibit autoxidation to the corresponding lower homologue ketone because of the lower stability of primary radicals as opposed to secondary ones (14), one would still expect it to undergo autoxidation to the corresponding carboxylic acid. It is well known that electron-donating groups, such as a benzene nucleus and an ether oxygen atom, facilitate abstraction of the aldehyde hydrogen atom to form the initial acyl radical (9). However, the effect of a methyl group is too small to explain the huge differences observed between autoxidation of Lilial® and Bourgeonal®. The second reason for the good performance of

Bourgeonal® is that it is a very potent material being two–four times stronger than Lilial® at the same concentration (The Givaudan Corp., 1961; Boelens and Wobben, 1980).

Although Florosa® is more stable than the aldehydic muguet materials, it cannot be used as a simple replacement in a formulation. Its floral odour is more rosy and it lacks both the impact and the green and watery notes associated with materials such as Lilial® and Bourgeonal®. Ideally, the perfumer needs a new fragrance ingredient with the chemical stability of Florosa® and the odour properties of Lilial® and Bourgeonal®. This is the challenge for the fragrance chemist. However, even if he were to find a suitable molecule today, it is unlikely that it will be commercially available to the perfumer for another 3–5 years. It takes this long for a new compound to clear all the regulatory, safety, process and production hurdles. Thus, the discovery chemists' role is proactive. Problems that he tackles 'today' will be of enormous value to the perfumery industry of 'tomorrow'. In the meantime, the perfumer working on today's antiperspirant fragrance for the Millennium brief will be guided by olfactory stability data. He will replace the Lilial® in his original formulation with a cheap, stable alternative such as Florosa® and use his skills in creative perfumery to achieve the desired impact and green, watery character (Chapter 7).

The above example illustrates just one of the reasons why there is a continuing need to extend the perfumer's palette. Other advantages that new ingredients might offer over existing aroma chemicals include:

- lower production cost;
- improved biodegradability;
- improved diffusivity;
- improved substantivity (the ability of an ingredient to be delivered to and retained on the substrate to be fragranced, *e.g.* skin, hair or fabric);
- higher odour impact (the more powerful a material and the lower its odour threshold, the less needed in a fragrance composition to have an effect);
- additional functionality (such as deodorancy, insect repellency or antimicrobial activity); and
- uniqueness of odour.

It goes without saying that a new fragrance ingredient must be safe (see Chapter 10). What is not so obvious to people outside the perfumery industry is that for a material to be a commercial success it usually must meet several of the above requirements. This is clearly a demanding task, particularly when some performance criteria are contradictory to each other. For example, a material that exhibits high chemical stability is less likely to be readily biodegradable. To circumvent this problem, high impact materials or better delivery systems are required, which allow less of the fragrance ingredient to be used in the composition. Without doubt, the most important criterion is uniqueness of odour. This allows the perfumers to create something new and trend setting, which cannot be copied by competitors. It is for this reason that novel aroma chemicals are kept 'captive' for a few years, *i.e.* Quest International will only manufacture the compound for internal usage. As Ernst Beaux, the creator of Chanel No. 5, once said:

> *One has to rely on chemists to find new aroma chemicals creating new, original notes. In perfumery, the future lies primarily in the hands of the chemists.*

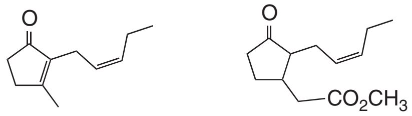
14.3 THE SEARCH

Where does the chemist begin this exciting, yet challenging search for a new fragrance ingredient? Well he has several options, each of which is described below using lily of the valley (muguet) as an example.

14.3.1 Clues from Nature

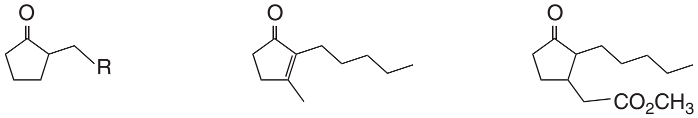
The unprecedented development of analytical techniques from the 1960s through to the present time has enabled the identification of key components in natural products (see Chapter 12) and their subsequent synthesis in the laboratory. Compounds that are prepared by chemical means but are identical in structure to those found in nature are known as nature-identical materials. The synthesis of compounds closely related to these nature-identical materials has led to the discovery of ingredients that are similar in odour but are much easier and cheaper to make than their natural counterparts. A notable example is that of jasmine: the natural absolute costs £3000–5000 per kilogram, the nature-identical materials (19) and (20) cost £300–500 per kilogram and the simpler cyclopentanone derivatives (21–23) £10–50 per kilogram (Figure 14.3).

However, for lily of the valley this approach has not been very successful. Although several groups of workers have studied the composition



(19) Jasmone

(20) Methyl jasmonate

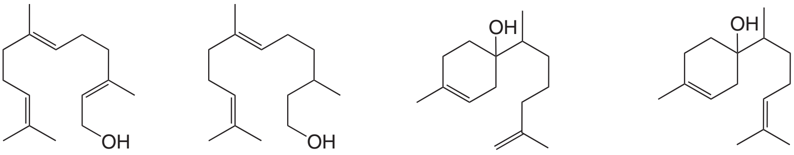


(21)

(22) Dihydrojasmone

(23) Methyl dihydrojasmonate

Figure 14.3 *Compounds with a jasmine odour*



farnesol **(27)**

dihydrofarnesol **(28)**

iso-β-bisabolol **(29)**

β-bisabolol *odourless* **(30)**

Figure 14.4 *Natural compounds with odours reminiscent of lily of the valley*

of the essential oil and concrete of the flowers, none have identified a component that possesses the characteristic odour of the flower (Boelens and Wobben, 1980). The closest match is farnesol (27, Figure 14.4), which has a very mild and delicate sweet-oily odour with a fresh-green note that is reminiscent of certain notes from muguet (Arctander, 1969).

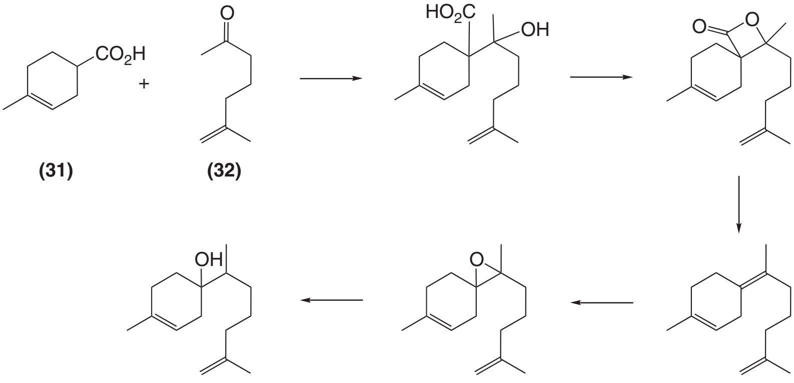
The classical methods like extraction and distillation often yield, particularly in the case of flowers, an extract that does not reflect the odour of the flower. More appropriate are the so-called 'headspace' procedures that attempt to trap the fragrant volatiles of flowers directly. However, these methods produce such small amounts of odour concentrates that the analysis of the constituents has only become possible over the last 15 years with the aid of modern analytical techniques.

In 1993, Surburg *et al.* used two different headspace techniques to isolate volatile constituents of lily of the valley. The first method, dynamic headspace, is described in detail in Chapter 12. In summary, it involves two steps. First the volatile materials released by the plant are trapped by adsorption onto a suitable material (charcoal, tenax or similar macroporous resin). They are then desorbed with a solvent or by heating to produce a concentrate of the volatiles. The second method involves subjecting the flowers to a vacuum; the volatile components distil off together with the water in the plant and are condensed at low temperatures to produce the fragrance concentrate immediately. The concentrate obtained using the vacuum headspace procedure contains higher proportions of higher-boiling, more polar compounds; whereas the low-boiling, less polar components are better represented in the concentrate of the dynamic process. When tested on the smelling blotter, the vacuum product displayed a more typical lily of the valley odour. This was thought to be due to the higher content of compounds like farnesol (27) and dihydrofarnesol(28) (both present at *ca*. 2% in the vacuum product but only at 0.2% in the dynamic product). Brunke *et al.* (1996) have also identified dihydrofarnesol as a fragrant component of lily of the valley using headspace techniques. According to Pelzer *et al.* (1993), dihydrofarnesol is an outstanding lily of the valley fragrance, although its qualities are not apparent on a smelling strip. Its low vapour pressure means that it develops fully only in aerosol form.

Recently, Braun *et al.* (2003) detected a trace component in both East Indian and West Australian sandalwood oils that possessed a strong floral, muguet-like odour. The material was detected by gas chromatography-olfactometry, more commonly referred to as GC-sniffing (see Chapter 12). It was present at such low levels (<0.001%) that its isolation from the oil was impossible. Instead fractional distillation, followed by repeated column chromatography and preparative 2-D GC led to an enriched sample (0.8%), which could be analysed by mass spectroscopy. The mass spectrum of the floral component was very similar to that of β-bisabolol (30), an odourless sesquiterpene alcohol found in both the sandalwood oils. The floral compound was subsequently identified as iso-β-bisabolol (29) based on comparisons of mass spectrum and gas chromatogram data with an authentic sample of iso-β-bisabolol synthesised in five steps from 31 and 32 (Scheme 14.3). Thus, analysis of a sandalwood oil led to the surprising discovery of a new muguet odorant that had not been found in nature or synthesised before.

It is interesting to compare the odour properties and structures of β-bisabolol (30) and iso-β-bisabolol (29). Changing the position of just one double bond converts an odourless molecule into a strong floral material. This phenomenon is quite common in the field of fragrance research. There are many examples of structurally similar compounds that have dramatically different odours. Conversely, we also find examples of very different compounds that have very similar odours. This will be discussed further in the section on SARs and the prediction of odour.

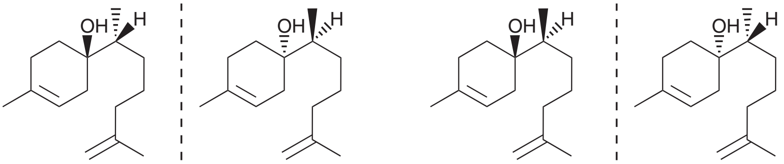
Iso-β-bisabolol (29) is also an example of pronounced odour differences between diastereoisomers and even enantiomers (Figure 14.5). Braun *et al.* (2003) separated the two diastereoisomers (29a and 29b) by HPLC and found that only 29a was odour active. GC smelling on a chiral column revealed that only one of the enantiomers of 29a possessed the strong, floral muguet odour and that the other enantiomer was odourless. The configuration of the active isomer was determined as (6R,7R) based on comparisons of retention time order with enantiomerically pure samples of β-bisabolol (30).



(31) **(32)**

iso-β-bisabolol
(29)

Scheme 14.3 *Synthesis of iso-β-bisabolol*



***(R,R)*-29a**
muguet

***(S,S)*-29a**
odourless

***(R,S)*-29b**
odourless

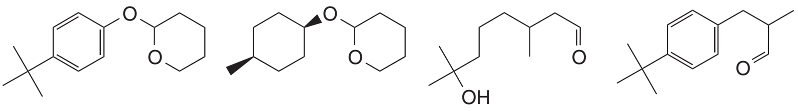
***(S,R)*-29b**
odourless

Figure 14.5 *The four diastereisomers of iso-β-bisabolol*

Olfactory enantiodiscrimination is observed in a number of fragrance ingredients. Further examples from the muguet odour family are mentioned in this chapter. However, for a more detailed account that covers other odour areas, the reader is referred to the review articles of Boelens (1993), Kraft and Fráter (2001) and Brenna *et al.* (2003). The enantioselectivity of odour perception is not surprising since the primary event in olfaction is the interaction of odorants with olfactory receptors, which are themselves chiral (see Chapter 12 for details on mechanism of olfaction). What is surprising is that the cases of high olfactory stereodiscrimination are more an exception and often confined to more rigid molecules. In general, the quantitative and qualitative odour differences of enantiomers are relatively small.

14.3.2 Serendipity

Since the blossom oil of lily of the valley is not commercially available, the perfumers have to rely on synthetic substitutes such as hydroxycitronellal, Lilial® and Bourgeonal® to create their muguet fragrances. The oldest lily of the valley odorants, hydroxycitronellal and cyclamen aldehyde (Winthrop Chem. Corp., 1929), were discovered by chance. As we have seen in the preceeding section, serendipity still continues to play an important role. This is equally true in synthetic organic chemistry. Anselmi *et al.* (1992) synthesised and organoleptically screened a series of 38 tetrahydropyranyl ethers. This class of compounds was chosen because of their ease of synthesis and purification, and because of their stability in alkaline media, conditions under which aldehydes tend to undergo aldol condensations. Two compounds (33 and 34) were described as having a white floral odour reminiscent of hydroxycitronellal and Lilial®. It was postulated that these four molecules were similar in odour because they could adopt very similar conformations (Figure 14.6)



(33) **(34)** hydroxycitronellal **(1)** Lilial® **(3)**

Figure 14.6 *Comparison between the structures of the tetrahydropyranyl ethers of p-tert-butylphenol and cis-4-methylcyclohexanol, and those of hydroxycitronellal and Lilial®. The structures have been drawn with similar orientation of the polar group to better visualise similarities in size and shape of the hydrocarbon region* when interacting with olfactory receptors (see later). In these conformations, the size and shape of the hydrocarbon part and the position of the functional group are similar. In the case of hydroxycitronellal, it was suggested that the main role of the hydroxyl group is to provide the necessary bulk at that end of the molecule and that it is not strictly essential as a functional group.

14.3.3 Synthesis of Analogues

Once a compound with the desired odour properties has been discovered, it is normal practice to prepare a range of analogues. The reasons for this are twofold: first, materials with even better odour characteristics than the parent compound may be identified (*i.e.* lead optimisation) and second, information about the structural requirements for the desired odour characteristics will be acquired. The latter is of particular importance when filing a patent.

Following the discovery of the floral tetrahydropyranyl ethers 33 and 34, Anselmi's group synthesised a much wider range of analogues in order to gain further insight into the relationship between chemical structure and the floral odour of alkoxycycloethers. They investigated the effect of the size of the alkyl substituent, the effect of the position of the alkyl group (limited to a methyl) on the cyclohexane ring, the replacement of the 2-tetrahydropyranyl group by the smaller 2-tetrahydrofuranyl ring (Anselmi *et al.*, 1993) and replacement of the oxygen atoms with either sulfur (Anselmi *et al.*, 1994a) or carbon (Anselmi *et al.*, 1994b).

They found that the position of the methyl group on the cyclohexane ring did not affect the odour appreciably, with all the derivatives having some floral character. However, increasing the size of the substituent at position 4 drastically reduced the odour intensity. Decreasing the ring size of the cycloether group from C6 to C5 did not affect, on average, the odour of the corresponding derivatives, whereas replacement of either one or both oxygen atoms by sulfur resulted in the loss of the floral odour. The sulfur analogues were generally green and fruity, accompanied in some cases by the unpleasant notes often associated with sulfurous materials. The analogues containing only a single oxygen atom, the other being replaced with carbon, were also green and fruity with no floral character.

Another example of analogue synthesis dates back to the 1960s. As mentioned above, the first phenylpropanal to be discovered with a

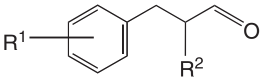


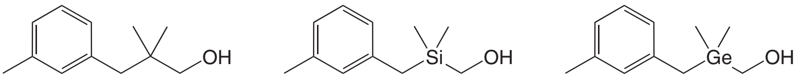
Figure 14.7 *Analogues of cyclamen aldehyde*

white floral odour was cyclamen aldehyde, 3-(4-isopropylphenyl)-2-methylpropanal (2). As the name implies, its odour is reminiscent of wild cyclamen. However, at low concentrations the odour possesses a hydroxycitronellal-like note. Consequently, it was a common practice in the 1960s to replace hydroxycitronellal, which is unstable in alkaline media and irritating to the skin, with cyclamen aldehyde. However, from an odour point of view the substitution was not entirely satisfactory. The discovery of the closely related *t*-butyl analogues (3 and 6 in Figure 14.2) resulted in a certain improvement (Givaudan Corp., 1959; Boelens and Wobben, 1980). Both these compounds have a more pronounced muguet floral odour.

Berends and van der Linde (1967) synthesised and organoleptically assessed 24 analogues of cyclamenaldehyde (Figure 14.7). They varied the nature of the alkyl substituent at the *para* position, altered the position of the alkyl group and replaced one of the α-hydrogen atoms with small alkyl groups. This enabled them to make the following qualitative conclusions about the effect of structure on the odour properties of substituted phenylpropanals.

1) The cyclamen character is most clearly discernible in compounds with a branched alkyl group at the *para* or *meta* position, where the branch is present at the C atom linked to the phenyl ring (*e.g.* $R^1 = CH(CH_3)_2$).
2) Where the branch is at a C atom farther removed from the nucleus, the cyclamen character diminishes and the odour becomes more muguet (*e.g.* $R^1 = -CH_2C(CH_3)_3$).
3) The introduction of an alkyl substituent at the alpha position in relation to the aldehyde group causes a decrease in odour intensity.
4) *Meta* and *para* isomers have similar odour characters and intensity. The *ortho* isomer has a weaker odour of a different type.

More recently, Tacke *et al.* (2002) synthesised silicon and geranium analogues of Majantol® (35), a commercial fragrance ingredient with a muguet-type odour (Figure 14.8). Although sila-majantol (36) possessed the characteristic lily of the valley odour of Majantol®, it lacked



(35) **(36)** **(37)**

Figure 14.8 *Sila- and germa-analogues of Majantol*

its aqueous-aldehydic freshness and was considerably weaker in odour strength. Germa-majantol (37) was weaker still and only possessed a hint of muguet character. The replacement of carbon with silicon or geranium produces analogues that are similar in terms of chemical reactivity and electronic distribution. However, the different covalent radii of carbon, silicon and geranium lead to molecules that are different in size and volatility. Thus, the larger the molecule, the less volatile it is and the weaker its odour strength. The odour character also changes to a certain degree. Similar observations have been seen for other sila-, germa- and even tin-substitutions in fragrance ingredients (for a review see Rossiter, 1996a; Tacke *et al.*, 1999).

14.4 COMPUTER-AIDED DESIGN

14.4.1 Ligand–Olfactory Receptor Modelling

Prior to the early 1980s, the biochemical study of olfaction had been virtually neglected. However, over the past 20 years a great deal of information has been gathered on olfactory receptors and signal transduction, which has provided significant insights into the biochemistry of odour perception (Chapter 13 and Herman, 2002). The first of these was that the olfactory receptors are G-protein-coupled receptors (GPCRs, Buck 1991). These are snake-like proteins that cross the receptor cell membrane seven times (Chapter 13, Figure 13.7). Proteins are chains built of amino acid units. One end of the chain is always an amino group, the other end is always a carboxylic acid. In GPCRs the N-terminal is outside the cell and the C-terminal inside the cell. The crossing of the membrane seven times leads to three extracellular loops and three intracellular loops. The portions of the protein chain that pass through the cell membrane are coiled into α-helices and are referred to as *trans*-membrane (TM) domains. These are grouped in a barrel-like arrangement (Figure14.9). When an odorant molecule interacts with one of these proteins on the extracellular face, it induces a change in shape of the intracellular face. This stimulates an adjacent G-protein, which in turn

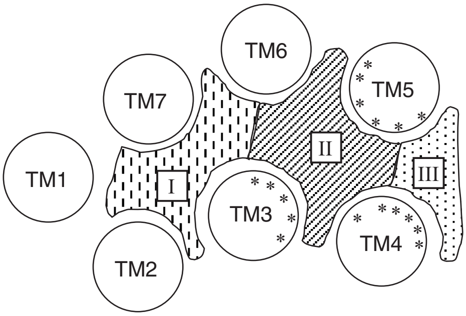


Figure 14.9 *Schematic 2D representation of the OR seven TM segments. The 17 residues constituting proposed areas for odorant binding are marked with an asterisk. Area 1 is a conserved pocket, area II coincides with ligand-binding pockets in other GPCRs and area III is a variable pocket unique to ORs*

leads to a cascade of reactions within the cell that culminates in an electrical signal being sent to the brain.

GPCRs are not unique to odour recognition. They also mediate the sense of vision, taste and pain. In addition, important cell recognition and communication processes often involve GPCRs, making them important targets for drug development. Indeed, it was by analogy to these systems that the olfactory receptors were first discovered.

The prevailing paradigm for GPCRs is that they are narrowly tuned to biological activity (as is the case for most drug–receptor interactions). If this paradigm were to apply to olfactory receptors, then the future for rational odorant design could well be the computerised translation of a gene sequence into a 3-D model of the receptor and the *in silico* high-throughput screening of thousands of molecules (*i.e.* automated molecular docking) to find the few that optimally activate it. These molecules could then be synthesised and evaluated by perfumers to identify the best for development and commercialisation.

However, it has recently emerged that olfactory receptors are tuned to chemical functionality as opposed to odour quality and that they respond in a combinatorial nature (Malnic *et al.*, 1999). A single receptor will respond to multiple odorants and a single odorant will trigger a range of receptors. A single odorant is also likely to trigger a different receptor code at different concentrations. This is consistent with our experience that perceived odour quality of an odorant can, in some circumstances, change at different concentrations. Thus, odour perception depends on a complex set of ligand–receptor interactions. Modelling this for just one odorant would be difficult in itself, for a series of molecules exhibiting similar odour properties it would be virtually impossible. The problem is complicated further by the difficulties associated with modelling GPCRs and our lack of knowledge of the exact nature of the interaction between odorant molecules and olfactory receptors. Thus, the *de novo* design of better performing compounds using receptor–odorant modelling is not an option for today's fragrance chemist. Instead we must turn to structure–odour relationships for the rational design of new and more efficient fragrance ingredients.

Although of no commercial value to the fragrance industry in the foreseeable future, docking experiments of odorant ligands into olfactory receptors and the analysis of protein sequence diversity may be helpful in providing insights into the primary events of odour recognition. The 3-D structures of olfactory receptors are unknown. This lack of structural information is due to the instability of GPCRs outside the membrane and difficulties in crystallisation. Thus, molecular models of the olfactory receptors are built by comparisons either with bacteriorhodopsin (a non-GPCR seven TM helix protein) or more recently with bovine rhodopsin (the only GPCR for which the atomic level structure is known). These are not necessarily good templates. The amino acid sequence homology between GPCRs and bacteriorhodopsin is low ($<30\%$), while the GPCR bovine rhodopsin responds initially to photons and not to organic ligands. This has led to the active development of new receptor-modelling tools based only on the amino acid sequence (Afshar *et al.*, 1998; Vaidehi *et al.*, 2002). This entails automated sequence alignment for a particular family of GPCRs to identify the TM regions and then the building of the TM helices, and subsequently the loops, using specific amino acid rules. For example, highly conserved residues are positioned towards the inside of the α-helical bundle, hydrophobic moments of the helices are pointed outwards towards the membrane and hydrogen-bonding partners and disulfide links between conserved cysteine residues are optimised.

Once molecular models of the receptor are built with a sufficient degree of confidence, the next step is to predict and characterise its binding site for a particular ligand. Because the site is not known, the entire protein (except the intracellular loops) should be scanned to identify likely binding sites and then the relative binding energies of the ligands calculated at these sites. Considering multiple conformations for flexible ligands and receptor side chains adds to the complexity of the search. The protein backbone is usually fixed.

There is evidence that the rat receptor (OR5) responds positively to Lyral and Lilial. Interestingly, both these materials are lily of the valley odorants. Raming *et al.* (1993) expressed the rat receptor protein (OR5) in the baculovirus-Sf9 cell, which showed a twofold increase in the level of inositol triphosphate (IP_3) when stimulated with a mixture of Lyral® and Lilial®. IP_3 is one of the secondary messengers believed to be involved in odour transduction (Breer, 1993). Three years later, measures of direct binding of Lyral® and Lilial® to OR5 were published by Breer and co-workers (Kiefer *et al.*, 1996).

These pioneering studies triggered several groups to carry out docking experiments for Lyral® (Singer and Shepherd, 1994b; Afshar *et al.*, 1998) and Lilial® (Bajgrowicz and Broger, 1995) in the OR5 receptor. Other binding sites that have been predicted include heptanol and hexanol in olfactory receptor S25 (Floriano *et al.*, 2000) and n-heptanal and n-octanal in the mouse and rat receptor I7 (Singer and Shepherd, 1994a; Vaidehi *et al.*, 2002). The results are summarised in Table 14.1.

Table 14.1 *Summary of odorant–receptor docking experiments*

Ligand	*Receptor*	*Putative binding site*	*Reference*
Lyral	OR5	Nine residues of helices 2–7. Tryptophan-278 in helix 7 forms H-bond with aldehyde group, 3 potential residues in helix 5 identified for binding to ligand hydroxy group. Leucine-245 in helix 6 and phenylalanine-104 in helix 3 forms van der Waal's interactions with cyclohexenyl moiety.	Singer and Shepherd (1994b)
Lyral	OR5	Residues in helices 3, 4 and 5. Aspartic acid residue in helix 3 is important for H-bonding to OH of Lyral.	Afshar *et al.* (1998)
Lilial	OR5	Two models obtained. First consists of 11 residues in helices 3, 4, 6 and 7. Threonine-152 in helix 4 forms an H-bond with the aldehyde group. The ligand's phenyl ring is stacked between phenyl rings of phenylalanine-104 (helix 3) and tyrosine-252 (helix 6). The *t*-butyl group finds a match to residues in helices 3, 6 and 7. In second model, orientation of ligand inverted with aldehyde forming H-bond to residues in helix 7.	Bajgrowicz and Broger (1995)
n-Hexanol, heptanol	OR S25	15 residues in helices 3, 5 and 6. Lysine-302 forms H-bond to OH group of ligands.	Floriano *et al.* (2000)
n-Heptanal, n-octanal	Mouse and rat I7	Residues in helices 3, 4 and 6. Lysine-164 forms H-bond to aldehyde group.	Vaidehi *et al.* (2002)

It is noteworthy that the important aspartic acid residue in helix III of OR5 as well as many other side chains identified in Asaf's OR5 model are mutated in OR12. This is consistent with the latter not responding to Lyral®. Vaidehi *et al.* have suggested site-directed mutational studies for the lysine-164 residue in rat and mouse I7, since mutation of this residue should switch receptor specificity towards other functional groups.

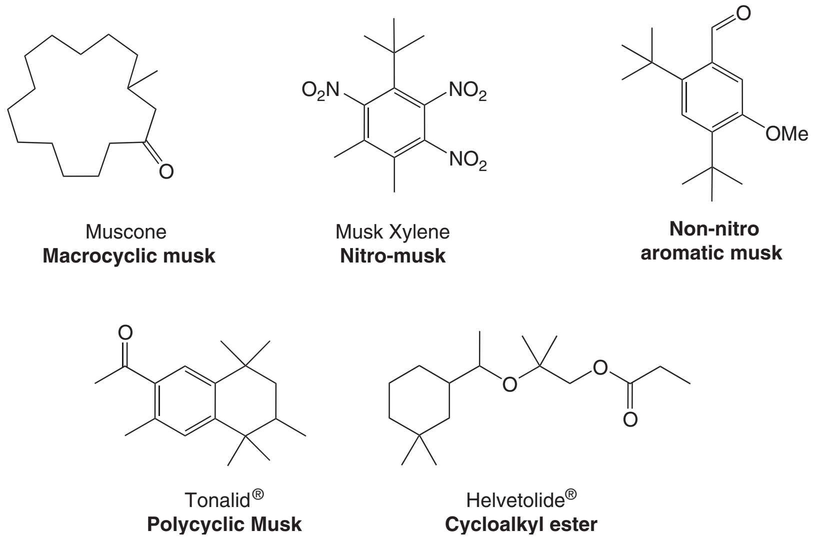
The ability to predict the 3-D structure of GPCRs, coupled with the accumulation of hundreds of olfactory receptor sequences has enabled people to analyse the OR amino acid variability in a structural context. Pilpel and Lancet (1999) studied the amino acid variability for 197 olfactory receptor sequences. Greatest variability was found in TMs 3, 4 and 5, and to a lesser extent in TM 6, with 20 of the 26 variable residues in helices 3–5 located on the predicted inner service of the receptor barrel and 17 of these 20 located in the extracelluar two-thirds of the TM segments. This points towards the location of potential odorant binding sites (Figure 14.9). The variable pocket between residues 3, 4 and 5 (marked II in Figure 14.9) coincides with ligand-binding pockets in many other types of GPCRs. However, the variable residues located in the cleft between TMs 4 and 5 (marked III in Figure 14.9) appear unique to olfactory receptors. The authors propose that this area, partially facing the lipid phase, could be important for the binding of more hydrophobic odorants, which may reach this site via the lipid bilayer.

In summary, independent computational studies point to an odorant-binding pocket composed of residues from TMs 3 to 6, which is located approximately 10–12 Å from the extracellular surface of the receptor. Vaidehi *et al.* (2002) have proposed that after the ligand is bound, the extracellular loop 2 (which links TMs 4 and 5, figure 13.7) may close down over the barrel, perhaps by recognising the exposed side of the ligand or of a part of the TM region that responds to the ligand. This dramatic movement of EC2 may cause helix 3 to move inwards exposing a region of intracellular loop 2 that may trigger the adjacent G-protein.

It is clear that the study of the initial primary receptor events, and all other aspects of human odour recognition including the barely understood role of the brain in decoding olfactory signals, will keep researchers busy for many more years.

14.4.2 Structure–Activity Relationships

The underlying principle behind any SAR is that the molecular structure of an organic compound determines the properties of that compound



Muscone
Macrocyclic musk

Musk Xylene
Nitro-musk

Non-nitro aromatic musk

Tonalid®
Polycyclic Musk

Helvetolide®
Cycloalkyl ester

Figure 14.10 *Structurally different compounds, all possessing musk odours*

whether it is chemical reactivity, physical properties or biological activity. Thus, a set of compounds that exhibit the same type of activity and are detected by the same mechanism must have one or more structural features in common. If such a relationship is found, it can be used to predict the activity of compounds which have not yet been made and as such is a very useful tool in the design of new, potentially active compounds, whether they be pharmacological agents, herbicides, pesticides or chemical communicants (such as odorants and flavorants). Other applications include the investigation of toxicity and the prediction of environmental risk.

In the field of odorants, we find examples of structurally different compounds with similar odour qualities and structurally similar compounds with different odour qualities. A notable example is the well-defined musk odour that can be found within five very different structural families (Figure 14.10). However, within each family there are very tight structural criteria. A very small structural change, such as the addition of one methyl group, can cause complete destruction of the musk odour. This creates a problem for the SAR worker. Some have searched for correlations within a family, while others have tried to speculate on molecular parameters common to all groups.

Another major problem in the application of SAR to odour is the difficulty associated with odour measurement (see Chapters 8 and 12 for details). This difficulty arises from the subjectivity of odour, the dependence of perceived odour strength and character on concentration, and the importance of organoleptic purity as opposed to chemical purity. All these factors can contribute towards discrepancies in odour data from different sources. For example, compounds 33 and 34 (Figure 14.6) were described as rose and jasmine by Quest perfumers but as muguet by the assessors in Anselmi's work (Anselmi *et al.*, 1992). To obtain reproducible odour data, the organoleptic purity of each sample should first be checked by GC-olfactometry and then its odour assessed by an expert panel using a standard glossary of odour descriptors. It should be remembered that without good, precise and reproducible data all subsequent SAR work is a waste of time.

A set of empirical rules, such as the one described for substituted phenylpropanals in Section 14.3.3, is the simplest way of describing a SAR. The Q is put into QSAR by describing the structure of a compound in a quantitative way, *i.e.* by a single numerical value. The simplest example of quantitative descriptors is molecular weight (MW) or the number of atoms present in a molecule. Interestingly, the relationship between the number of atoms and boiling points observed in a series of n-alkanes is one of the first quantitative structure property relationships in organic chemistry (Whitmore, 1937). Today, the number and variety of molecular descriptors that can be calculated and used in the development of SARs are huge. They range from steric properties to electronic effects and include descriptors such as molar volume, molar refractivity, topological indices, geometrical 3-D descriptors, atomic charge, electrostatic potentials and molecular orbital energies to name but a few (for a review, see Livingstone, 2000). Calculated descriptors are preferred over experimental ones since this leads to a QSAR that can be used to predict the activity of compounds, which have not yet been made.

When the compound is described using physical, as opposed to structural, descriptors the relationship becomes a PAR (physical–activity relationship). Commonly used physical properties include the octanol–water partition coefficient ($\log P$), water solubility, melting point, boiling point and vapour pressure. Correlations of this type have been used in the perfumery industry to describe and predict the substantivity and retention of fragrance ingredients, that is the ability of a compound to stick to and remain bound to surfaces such as hair, skin or cloth (see Chapter 11 for more details).

Since the 1980s the rapid development of more sophisticated SAR techniques, particularly in the field of drug design, has had a major impact on analogous research in the field of olfaction. The result is a prolific number of structure–odour relationship publications. Advances in theoretical and computational chemistry, coupled with the introduction of computer graphics, have made conformational analysis easier and allowed the visualisation of molecules in 3-D. This has led to an increase in the number of postulated ‘olfactophores’, which are usually expressed as distance criteria between key structural fragments. Simultaneously, more powerful computers have allowed the rapid calculation of a wide range of molecular descriptors and provided improved data handling. This is reflected in the publication of odour-discriminating models derived using statistical techniques such as pattern recognition and, more recently, neural networks. Three SAR techniques, which have been applied to the study of odour, are described below: the Hansch analysis, pattern recognition and the olfactophore approach. However, for a more detailed account on structure–odour relationships the reader is referred to the reviews of Rossiter (1996a) and Kraft *et al.* (2000).

14.4.2.1 The Hansch Approach. This is one of the most widely used methods for analysing SARs when quantitative biological data are available. It is named after the founder of modern QSAR, Corwin Hansch (Hansch and Fujita, 1964), who suggested that the biological activity of a molecule was a function of its electronic, steric and hydrophobic properties – the latter most often being represented by the partition coefficient (P) between water and octanol (Equation 14.2).

$$\log(\text{activity}) = f(\text{electronic}) + f(\text{steric}) + f(\log P) \tag{14.2}$$

There are two ways of developing a QSAR. The first approach is to set up a model and then test it. So, for example, if we are considering odour intensity we might expect volatility to be important, because the molecules have to become airborne to reach our noses. Log P might also be a relevant parameter because the molecules have to pass through a mucous layer in order to reach the olfactory cilia membrane where the protein receptors are located. When it comes to interaction with the receptor, one might expect hydrogen bonding, electrostatic interactions and shape to be important, and would thus use parameters that describe these properties. The disadvantage of this approach is that you are not searching for the unexpected but only testing your own prejudices. The second approach is to use as many different molecular descriptors as possible and then seek correlations. The parameters can be taken singly or in combinations and may include power terms (*e.g.* x^2, x^3). The main drawbacks with this approach are that it is difficult to limit sensibly the number of parameters, it is difficult to interpret the results, and spurious correlations can be found. To feel confident about the results of a Hansch analysis and to support these results with statistics, the study set must contain at least five well-chosen compounds for every explanatory variable. For example an equation, such as the one above, which has three terms on the right-hand side should be derived from 15 or more compounds.

A set of well-chosen compounds is one that contains sufficient structural variation to permit the uncovering of a relationship between molecular structure and the activity of interest. It is also important that the series of compounds exhibit varied levels of potency. If a SAR is formulated for a set of very similar compounds, both in terms of activity and structure, the correlation is likely to be very high but its ability to predict the activity of materials outside this range very poor.

Odour can be quantified in one of four ways:

1) As a threshold concentration, *e.g.* the minimum concentration of the compound in the air detected by the nose.
2) As a concentration required to produce the same odour strength as a given dilution of a standard compound.
3) As odour intensity ratings, which could in fact be as simple as assigning 0 to an odourless compound, 1 to a compound that has a weak odour, 2 to a compound of moderate intensity and 3 to a very strong smelling compound.
4) As odour similarity (OS) ratings, where the odour quality of one odour type is rated against that of a standard. For example, if a scale of 0–10 were used, a compound which was identical in odour to the standard would be given a score of 10 and one which was completely different a score of 0.

Boelens and Punter (1978) obtained OS ratings for 16 muguet-smelling aldehydes. They derived an equation (Equation 14.3) that related the OS to MW and the Kier connectivity index (X^v). The concept of molecular connectivity was introduced by Randic (1975) and further elaborated by Kier and Hall (1976). It involves the calculation of numerical indices that describe the topology of a molecule. The Kier connectivity index used in Boelens' equation is a measure of the extent of branching.

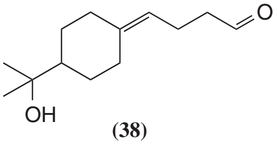

(38)

Figure 14.11 *Boelens' new odorant*

The correlation coefficient, r, is a measure of how well the model describes the observed variation in the data set. A value of 1 would represent a perfect model.

$$\text{OS muguet} = F(\text{MW}) + \text{F}(X^{v}) + \text{C} \tag{14.3}$$

$$n = 16,\ r = 0.92,\ s = 0.90$$

Based on this work, Boelens designed 4-[4-(1-hydroxy-1-methylethyl)-cyclohexylidenyl]butanal (38, Figure 14.11), which, when made, did indeed have excellent olfactive properties. Thus, the activity of compound 27 had been correctly predicted and Boelens' model further substantiated. However, if the prediction had been incorrect it should not, as is quite often the case, be ignored and classified as 'an exception to the rule'. It is important that the reasons for the poor prediction are investigated. Outliers or compounds that exhibit unique biological activity can often provide vital clues about the structural requirements for that biological activity. Thus, the development of a SAR is an iterative process with the information acquired from new materials being used to refine the model.

Boelens has also used this approach to derive QSAR equations for musk, jasmine, fruit and bitter almond odorants (Boelens, 1976; Boelens and Punter, 1978; Boelens *et al.*, 1983). In the case of bitter almond and musk, he concluded that hydrophobic and steric parameters were important. For the jasmine materials, he found that molecular connectivity indices were useful parameters. Molecular connectivity indices were also used by their inventor, Kier *et al.* (1977), to analyse anosmia to fatty acids and the odour similarities of ethereal, floral and benzaldehyde-like odorants. Dearden (1994) also developed a QSAR equation relating the OS of bitter almond odorants to two connectivity indices. Greenberg (1979) found that the odour intensity of a series of homologous compounds was correlated to their hydrophobic properties and not to steric or polar properties while Rossiter (1996b) found that the fruitiness of aliphatic esters was related to steric hindrance of the ester group and either molecular length or log *P*.

14.4.2.2 Pattern Recognition. Pattern recognition deals with the problem of dividing compounds into two or more classes. It is thus well suited to analysing qualitative biological activity data, *i.e.* when of a descriptive nature (*e.g.* odour descriptors) or ranges such as strong, moderate, weak or inactive. Other characteristics of the technique are that it can handle large numbers of compounds of substantial structural diversity. In order to obtain meaningful discrimination between the classes, it is important that the inactive molecules are as close as possible in structure to the active ones.

One of the software systems available for pattern recognition studies is ADAPT (automated data analysis using pattern recognition techniques). The structure of each member of the data set is represented by molecular descriptors. These numerical indices, which encode information about the molecule, fall into four classes: topological, geometrical, electronic and physicochemical. Data are analysed using pattern recognition techniques to develop a classifier that can discriminate between the classes of data.

ADAPT has been developed and used by Jurs in a wide range of SAR applications. In the field of olfaction, these include the correlation of odour intensities for 58 structurally and organoleptically diverse odorants (Edwards and Jurs, 1989), and the investigation of the relationship between molecular structure and musk odour (Jurs and Ham, 1977; Ham and Jurs, 1985; Narvaez *et al.*, 1985). To date, no one has used pattern-recognition techniques in the study of muguet odorants.

There is a range of other statistical techniques that can be used in the formulation of a classification model. Since a detailed description of these is outside the scope of this chapter, those that have been used in the study of odour are listed below:

- Principal component analysis – bitter almond (Zakarya *et al.*, 1993), fruit odour (Rossiter, 1996b).
- Linear discriminant analysis – bitter almond (Zakarya *et al.*, 1993), camphor (Chastrette and Eminet, 1983), musk (Yoshii *et al.*, 1991; Klopman and Ptchelintsev, 1992) and sandalwood (Chastrette *et al.*, 1990).
- Neural networks – musk (Chastrette *et al.*, 1993, 1994; Jain *et al.*, 1994).

14.4.2.3 Conformational Analysis and the Olfactophore Approach. A biophore (pharmacophore for drugs, olfactophore for odorants) is a 3-D arrangement of chemical features in a molecule that is responsible for its biological activity. The first computer program to recognise biophore patterns dates back to 1974. However, it was not until the late 1980s that programmes capable of pharmacophore mapping, prediction and 3-D database searching became commercially available. One of the reasons for this 15-year wait was the need for software capable of rapidly converting 2-D structures to 3-D structures, which then allowed pharmaceutical companies to convert their corporate databases into 3-D databases. An excellent account of the history and evolution of the pharmacophore concept is provided by Güner (2002).

There are two main approaches to the development of a biophore. Where knowledge of the receptor structure is available, ligand docking experiments can be performed to identify the active site. The latter can then be used to search databases, build new compounds or screen candidates. Although useful in drug design, this approach is not applicable to the fragrance industry (see Section 14.4.1). Instead, we use the active analogue approach. It is assumed that for a series of active molecules, which are detected by the same mechanism, there is a common conformation in which key atoms or functional groups are placed at certain relative distances from each other. The identification of a biophore model involves the comparison of all the possible conformations of every molecule in the data set. Since this procedure requires no knowledge of the degree of potency, the olfactophore approach is well suited to qualitative data, for example a group of compounds which all smell very similar. The comparison of conformations is made easier by choosing a rigid active compound as the standard since this molecule will have a limited number of viable conformations that it can adopt. The next step is to take the second most rigid molecule and look for any conformational arrangements that it has in common with the standard. The procedure is repeated for every compound in the data set until, hopefully only a handful of common conformational arrangements are left. All the olfactophore models identified should be explored.

The models can be tested using inactive molecules. These, in theory, should not be able to satisfy the proposed biophore. However, inactivity can be the result of other factors. For example, a molecule that can fulfil the distance geometry requirements may, in fact, be inactive because it cannot reach the site of detection. The latter will be determined by physicochemical properties such as hydrophobicity, solubility and volatility.

In fact, the main criticism of the olfactophore approach is that it does not take into account these types of properties.

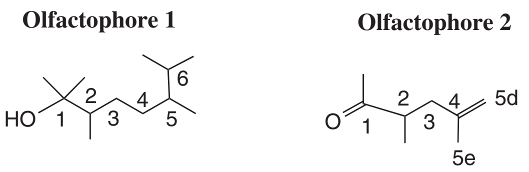
Since the identification of olfactophores is best suited to sets of compounds with a limited amount of conformational flexibility, this technique has mostly been applied to the following odour areas:

- sandalwood (Chastrette *et al.*, 1990; Buchbauer *et al.*, 1994; Aulchenko *et al.*, 1995; Bajgrowicz and Frater, 2000);
- musk (Chastrette and Zakarya, 1988; Bersuker *et al.*, 1991; Kansy *et al.*, 1995);
- ambergris (Bersuker *et al.*, 1985; Dimoglo *et al.*, 1995; Gorbachov and Rossiter, 1999).

Muguet odorants, on the other hand, are not ideal for the olfactophore approach because many of them exhibit a high degree of conformational flexibility and thus have thousands of possible conformational arrangements. To overcome this, Pelzer *et al.* (1993) considered only the energy-minimised forms of the 20 best muguet fragrance ingredients. They found that if the oxygen atom is chosen as a fixed point, the position and substitution pattern of the carbon atoms C-4, C-5 and C-6 play an important role. However, when this model was tested using a further 161 compounds, some of the compounds showed a different substitution pattern. It was concluded that there were two classes of muguet odorants, those that have an OH group and those with a carbonyl group. The two types were shown to have different odour profiles with the carbonyl compounds exhibiting, in addition to the muguet aspect, ‘lime blossom’ notes. The substitution pattern and the distance constraints for the olfactophores of these two classes are shown in Figure 14.12.

Pelzer’s analysis of 73 alcohols produced the following rules for olfactophore 1.

1) C1 is substituted by one to three alkyl groups, ideally three, provided that the hydroxyl group is not overshadowed too strongly by steric hindrance.
2) C2, C5 and to a lesser extent C6 and C7 are substituted by a single alkyl group (ideally methyl). Dimethyl substitution generally has a detrimental effect.
3) Where a double bond is present, it should preferably be at C4 or C6. A double bond between C3 and C4 or, to a lesser extent, between C2 and C3, generally has a negative influence on the odour.



C1-C4 = 3.2 ± 0.3Å
C1-C5 = 4.0 ± 0.3Å
C1-C6 = 5.0 ± 0.3Å
C1-R1 = 4.7 ± 0.4Å
C1-R2 = 6.0 ± 0.4Å

C1-C4 = 3.9 ± 0.3Å
C1-C5d = 4.4 ± 0.3Å
C1-C5e = 5.0 ± 0.3Å

Figure 14.12 *Pelzers' muguet olfactophores*

For olfactophore 2 it was found that

1) An aldehyde is always better than a ketone function.
2) C2 should be substituted by one or two methyl groups, preferably one.
3) C4 should be alkyl substituted, a double bond at C4 is particularly advantageous, and may also be part of an aromatic system.

The use of these models led to the synthesis of a series of interesting new fragrances, the most notable of which was Mugetanol® (39, Figure 14.13). Further investigations of the individual isomers of Mugetanol revealed that the lily of the valley aspect and intensity of the (−)-*cis*-isomer were far superior to the other three. All the other isomers, however, do have a lily of the valley aspect, although distinctly weaker.

Kraft *et al.* (2000) have used the same data set as Pelzer *et al.* (1993), but included all conformers with energies of up to 15 kcal mol^{-1} greater than that of the lowest energy conformer. This is in keeping with the generally accepted theory that the active conformation is not necessarily the lowest energy one (Burgen *et al.*, 1975). In considering higher energy conformers, Kraft *et al.* (2000) generated an olfactophore that mapped well with both fragments of Pelzer's model. It contained a hydrogen-bond donor region (for the OH group), a hydrogen-bond acceptor region (for the carbonyl group) and two hydrophobic regions. The distance criteria between these four regions are shown in Figure 14.14. The distances between the hydrogen-bond donor and the two hydrophobic regions are very similar to those quoted by Pelzer *et al.* (1993) for olfactophore 1 (see C1-R1 and C1-R2 in Figure 14.12). The same is true for distances

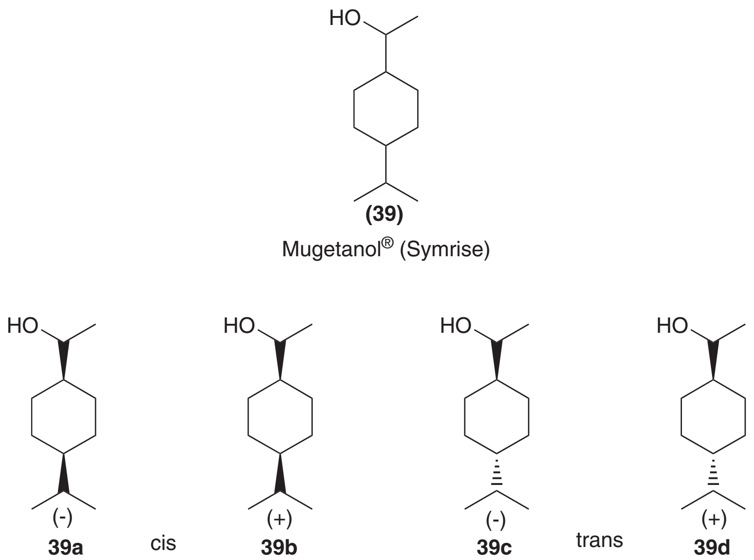
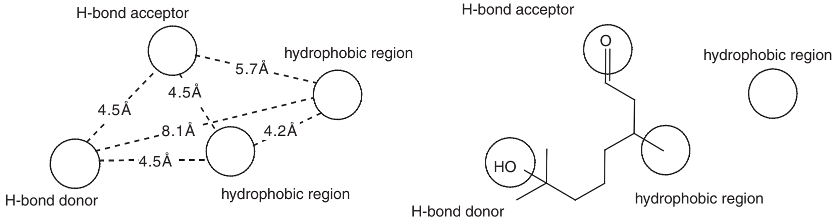


Figure 14.13 *Mugetanol®*



distance criteria between four key regions in Kraft's proposed olfactophore

schematic diagram showing hydroxycitronellal fitting Kraft's proposed olfactophore

Figure 14.14 *Krafts' muguet olfactophore*

between the hydrogen-bond acceptor and two hydrophobic regions (see C1-C4 and C1-5e in olfactophore 2, Figure 14.12). In the case of hydroxycitronellal (1), which is considered by some as the odorant that most closely resembles the natural lily of the valley scent, Kraft *et al.* illustrate how the alcohol and aldehyde group nicely fit the proposed acceptor and donor regions respectively, the 3-methyl group fits the closest hydrophobic region while nothing occupies the second hydrophobic region. For monofunctional muguet odorants, both hydrophobic regions are occupied and either the acceptor or donor region depending on whether the molecule is a carbonyl-containing compound or an alcohol. Kraft *et al.*

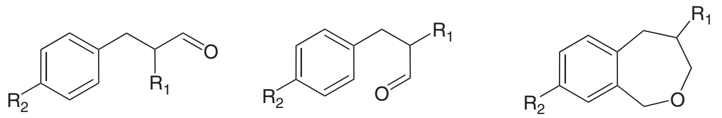
(2000) have also developed olfactophore models for pear, green or galbanum, sandalwood, amber and musk, all using the Catalyst® pharmacophore software from Accelrys (formerly msi).

14.4.3 Conformational Analysis on Small Data Sets

Skouroumounis and Winter (1996) focused their conformational studies on just three substituted phenylpropanals (cyclamen aldehyde (2), Lilial® (3) and Bourgeonal® (6)). Molecular mechanics suggested that there were two low-energy conformations for the aldehyde side chain, either extended or folded. When the side chain possessed alkyl substituents (as in 2 and 3), the folded conformation was of slightly lower energy; but when the side chain was unsubstituted (as in 6), the extended conformation was of lower energy (Figure 14.15). To investigate the relevance of the folded conformation for odour activity, they synthesised a number of 1,3,4,5-tetrahydro-2-benzoxepin derivatives (40) as conformationally restricted analogues of the phenylpropanals. Since none of them possessed the typical lily of the valley odour, they suggested that the extended conformation is more likely to be the 'bioactive' one at the receptor site(s). However, steric factors are not the only pre-requisite for odour. Conversion of an aldehyde group to a cyclic ether will have a dramatic effect on the chemical reactivity and the electrostatic properties of a molecule, and it could be for these reasons that the 1,3,4,5-tetrahydro-2-benzoxepin derivatives do not exhibit the desired muguet odour.

During the course of this work, Skouroumounis and Winter (1996) also synthesised a range of novel phenylpropanals having an additional methyl substituent on the aromatic ring. One of these new aldehydes (41, Figure 14.16) was found to be a good muguet odorant, which the authors claimed was more stable to aerial oxidation than either Lilial (3) or Bourgeonal (6). This statement was based on comparisons of how long the odours of these materials could be perceived on a smelling strip after they had first been dipped; the assumption being the longer lasting the smell, the more stable the aldehyde. However, the longevity of an odour will also depend on the volatility of a molecule (*i.e.* how quickly does it evaporate from the smelling strip) and the odour threshold of the molecule (*i.e.* what is the minimum concentration in air required for the material to be detected). It is far better to substantiate such claims with chemical analysis, such as those described earlier in this chapter.

Yoshii *et al.* (2001) have re-examined the conformers of Lilial® and its corresponding benzoxepin derivative (40a). When the *t*-butyl group and



"extended" conformer

(6) R1 = H, R2 = tBu

"folded" conformer

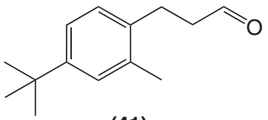
(2) R1 = Me, R2 = iPr
(3) R1 = Me, R2 = tBu

(40)

conformationally restricted analogues which mimic the "folded" conformer

(40a) R1 = H, R2 = t-Bu

Figure 14.15 *Conformational analysis of phenylpropanals and conformationally restricted analogues*

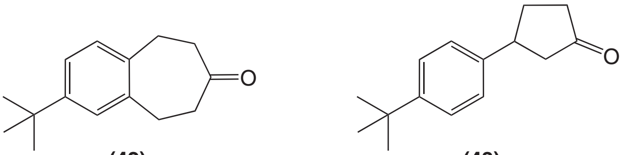


(41)

Figure 14.16 *New substituted phenylpropanal*

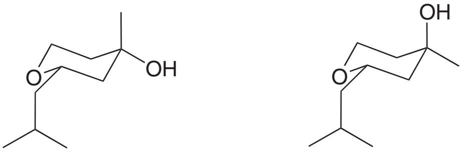
benzene ring were superimposed, no matching conformers for Lilial or benzozepin 40a could be found, *i.e.* the oxygen functionalities were not in similar spatial positions. They thus concluded that it was not surprising that these two materials had different odours. Interestingly, Skouroumounis and Winter (1996) could only obtain a good match for the oxygen functionalities when the planes of the aromatic rings were tilted by an angle of 35° to each other.

Yoshii *et al.* (2001) subsequently extended their conformational analysis to include three other muguet materials: Bourgeonal (6), aldehyde (41) and *cis*-Mugetanol® (39a). Superimposition was again based on the oxygen functionalities, the bulky *t*-butyl or isopropyl groups and the six-membered benzene or cyclohexane ring. The superimposed model was subsequently used to propose candidates that were likely to possess a muguet odour. The top two candidates (42 and 43, Figure 14.17) were synthesised and evaluated by perfumers. Compound 42 was described as having an odour similar to cyclamen aldehyde (2) and Lilial® (3), while 43 was described as lilac, a floral odour very similar to that of lily of the valley. Thus, both compounds possess the predicted target odour, though their intensities are weak and not adequate for commercial perfume materials. This ties in with Pelzer's (1993) observation that ketones are poorer muguet odorants than aldehydes.



(42) **(43)**

Figure 14.17 *Proposed muguet targets based on Yoshii's 3D model*



(5a)
cis-isomer
strong muguet

(5b)
trans-isomer
weak muguet

Figure 14.18 *Odour differences of cis- and trans-Florosa®*

At Quest, we chose Florosa® (5) as the muguet standard for molecular modelling work. The reasons for this were twofold. First, it is relatively rigid and second the two isomers of Florosa® are known to have different organoleptic properties. Sommer and Güntert (1993) have reported that only the *cis*-isomer (5a), in which the hydroxyl and isobutyl groups are both equatorial, is the actual fragrance carrier (Figure 14.18). Our own findings suggest that both isomers have a muguet odour but that the *cis* isomer (5a) is more potent than the *trans* (5b) (Rossiter 1997a). This difference in odour strength was observed in a number of tetrahydropyranols. We postulated that the *trans* isomers could exist in a boat conformation with the OH group intramolecularly hydrogen-bonded to the ether oxygen atom to form a stable six-membered ring (Figure 14.19, Rossiter, 2001). If this were the case, the alcohol group might not be as free to interact with the olfactory receptors thus rendering the *trans* isomers weak or odourless.

We used molecular modelling to investigate whether or not the tetrahydropyran-4-ols could potentially exist in an intramolecularly hydrogen-bonded boat form, and if so whether this was more favoured in the *trans* isomers than in the *cis* (Rossiter, 2001). We found that the energy difference between the chair and boat forms is relatively small for both isomers ($<4\,\text{kcal}\,\text{mol}^{-1}$). Indeed it is less than the estimated thermodynamic strength of a hydrogen bond (Aakeröy and Seddon, 1993; Mascal, 1994), which suggests that both the *cis* and *trans* isomers could, in theory, adopt

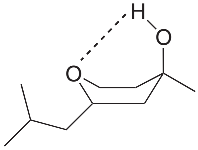
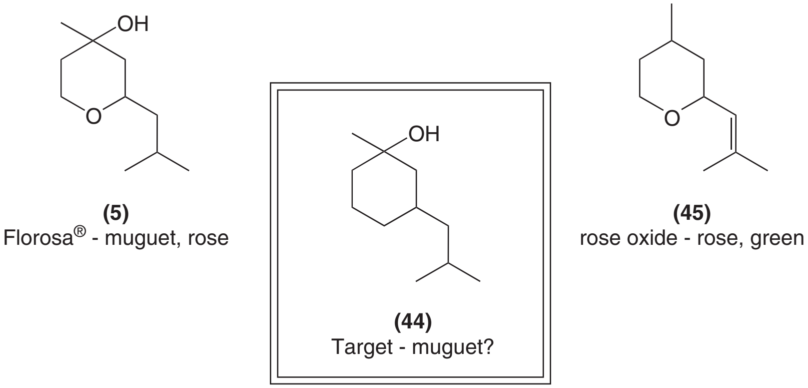


Figure 14.19 *Postulated conformation for the weak-smelling trans-Florosa®*



(5)
Florosa® - muguet, rose

(44)
Target - muguet?

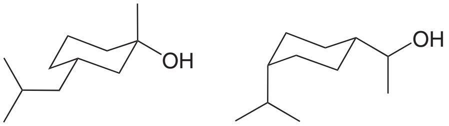
(45)
rose oxide - rose, green

Figure 14.20 *The design of a new target, 1-methyl-3-(2-methyl)cyclohexanol*

an intramolecular hydrogen-bonded boat form. However, perhaps of greater significance is the O–H distance in the chair forms. This is substantially smaller in the *trans* isomers (3.07–3.22 Å) than in the *cis* (4.11–4.30 Å) and well within the criteria for a weak hydrogen bond ($<$3.4 Å). Thus, the attraction between the ether oxygen atom and the hydroxyl hydrogen atom in the *trans* isomers may act as the driving force for conversion of the chair form to a boat or twist boat.

We designed a target molecule that was not capable of intramolecular hydrogen bonding, *i.e.* the ether oxygen was replaced with a methylene. It was anticipated that both isomers of 1-methyl-3-(2-methylpropyl)cyclohexanol (44, Figure 14.20) would have a strong odour. It was felt that the chances of 44 having a pure muguet odour rather than the rosy, muguet character of Florosa® were quite high for the following reasons. Rose oxide (45) has no muguet aspects but is rosy and green. Drawing an analogy with the structure and odour of Florosa® (5), we postulated that the ether oxygen in Florosa® might be responsible for its rose note and the tertiary alcohol for its muguet note.

1-Methyl-3-(2-methylpropyl)cyclohexanol was conveniently prepared in the laboratory in two steps: 1,4-conjugate addition of *iso*-butyl



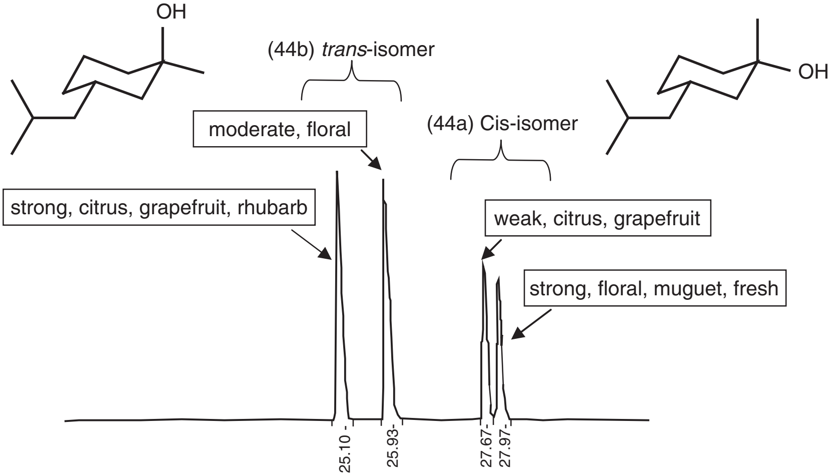
44a **39a**

Figure 14.21 *A comparison of the configurations of cis-Rossitol and cis-Mugetanol®*

magnesium bromide to 2-cyclohexen-1-one in the presence of a copper salt followed by the 1,2-addition of a methyl Grignard reagent. The isomeric mixture obtained using Grignard chemistry typically consisted of 70% *trans*-1-methyl-3-(2-methylpropyl)cyclohexanol and 30% *cis*-1-methyl-3-(2-methylpropyl) cyclohexanol. The mixture possesses an extremely powerful muguet odour with additional citrus and rhubarb notes. Sensory profiling of the individual isomers, which are easily separated by either column chromatography or efficient fractional distillation, and the determination of their relative odour thresholds revealed that both geometric isomers are comparable in odour strength with the *trans* isomer having a more rhubarb, citrus character and the *cis* isomer being more muguet.

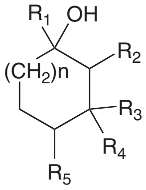
The association of the muguet character with the *cis* isomer (44a) fitted well with our predictions based on Florosa®. The orientation of the alcohol group in relation to the alkyl side chain is also consistent with Pelzer's (1993) postulated active conformation for *cis*-Mugetanol (39a) (Figure 14.21). However, somewhat surprising was the discovery that chirality had a more pronounced effect on the odour character than did the configuration of the molecule. GC olfactometry, using a chiral GC column, revealed that the first enantiomer to elute for both *trans*- and *cis*-1-methyl-3-(2-methylpropyl)cyclohexanol has predominantly a citrus character and that the second isomer is floral (Figure 14.22). The relative strength of the enantiomers governs the overall impression perceived for the two racemic mixtures. The four enantiomers of Mugetanol®, on the other hand, all have a muguet note with the (−)-*cis*-isomer being the more potent (Pelzer *et al.*, 1993). Presumably, chirality is less important in this case because the molecule is more symmetrical, whereas for the 3-alkyl substituted cyclohexanols it appears that the side of the cyclohexane ring to which the substituent is attached is crucial in determining whether the material is citrus or floral in character.

Following the exciting discovery of 1-methyl-3-(2-methylpropyl)cyclohexanol, we synthesised over 25 analogues to investigate the effect of the alkyl substitution pattern and ring size on the odour properties of



Column: CP-Chirasil-Dex CB, dimensions: 25mx0.25mmx0.25Tm;
linear velocity: 17.15cm/sec; temperature programme: 70 to 200°C at 3°C/min;
split flow ratio: 190:1

Figure 14.22 *Chiral GC trace with odour descriptors for the isomeric mixture of 1-methyl-3-(2-methylpropyl)cyclohexanol*



n = 0, 1, 2,3
R1 = H, Me, Et, Pr
R2, R4 and R5 independently are H or Me
R3 = *i*-butyl, *s*-butyl, *t*-butyl, cyclohexyl, *i*-amyl;
the alkyl group can also contain unsaturation.

Figure 14.23 *Structural requirements for a muguet odour in 3-alkylcycloalkanols*

3-alkylcycloalkanols (Rossiter, 2001). For the mixtures, it was found that the floral, citrus character was retained in a number of analogues (as defined in Figure 14.23) but that 1-methyl-3-(2-methylpropyl)cyclohexanol still remained the best. This material has now been commercialised under the trade name of Rossitol® (Rossiter, 1997b, 1998) and is a key captive ingredient within Quest International. Its first use in the fine fragrance market was in *Miracle Homme* (launched by Lancôme, in 2001) where it imparts a translucent freshness.

14.5 SUMMARY

At the beginning of this chapter I explained why there is a continuing need to extend the perfumers' palette. The discovery of new patentable fragrance ingredients which are either cheaper, more stable and more readily biodegradable than existing aroma chemicals or which have a unique odour or additional functionality gives Quests' perfumers a competitive edge.

The limitations of receptor–ligand modelling in the rational design of new odorants have been discussed. SARs and molecular modelling, on the other hand, can provide guidance for the fragrance chemist in his search for new molecules and we have seen four examples where such studies have led to the design of new muguet odorants (Boelens and Punter, 1978; Pelzer *et al.*, 1993; Rossiter, 2001; Yoshii *et al.*, 2001). However, the majority of structure–odour relationships have been developed in hindsight to justify a known outcome and in most cases there are exceptions to the rules. For example, Kraft *et al.* (1999) were surprised to find that transposition of only one methyl group in the hydrophobic prenyl tail of nerol shifted the odour from rose to muguet. They claim that this would not have been predicted on the basis of existing olfactophore models in the muguet family. We too have found examples that contradict Pelzer's models (Rossiter, 1997a) and were amazed to find that chirality had a more pronounced effect on the odour character of Rossitol than did the configuration of the molecule. The prediction of odour intensity as opposed to odour character also remains a significant challenge.

The interpolative nature of SARs means that they cannot be used to predict the activity of compounds outside the scope of the data set. Thus, the discovery of new classes of compounds can only be achieved through serendipity, random screening and chemist's creativity. The use of new feedstocks, chemistry technology and the analysis of natural products continue to be sources of inspiration.

Fragrance research is challenging yet exciting. It is one of the few areas where synthetic chemists can immediately judge for themselves the biological activity of the molecule they have just made. Sometimes it has the odour you are aiming for, other times it may have an unexpected smell that opens avenues for new areas of research. For an inventor of novel fragrance ingredients, the ultimate satisfaction is knowing that perfumers have used your material to create a unique fragrance and to subsequently find this fragranced product on the supermarket shelf.

ACKNOWLEDGEMENTS

Ian Payne, Quest International – for organoleptical stability data of muguet ingredients; Steven Rowlands, Quest International – for analysis of the breakdown products of Lilial® in antiperspirant base; Andy Roche, Reheis – for information on the partial hydrolysis of aluminium chlorohydrate.

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CHAPTER 15

Buying Fragrance Ingredients and Selling Fragrance Compounds

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15.1 BUYING

Perfume is a simple blend of oils, incorporated into a product base. Like many things, the seemingly straightforward is extremely complex. The fragrance industry sources thousands of raw materials and introduces new products to its portfolio every day to meet the bespoke needs of its global clients.

All the major fragrance suppliers work with a large palette of both natural and synthetic ingredients. It is the role of the Procurement Departments to ensure a constant supply of quality ingredients into the fragrance warehouses and compounding factories.

Every perfumer travels the world from the comfort of his laboratory as he mixes essential oils. And though most perfumers, certainly those among the global fragrance suppliers, will not travel to vet the selection of oils, nor recognise instinctively the origin or altitude at which a plantation is sited through smell alone, they recognise the aura and energy of a fragrance oil obtained from the best fragrant flowers. Quest perfumer Francoise Caron is enamoured by the charm of natural raw materials.

> *Each time you chose ginger, narcissus or mimosa, you are perpetrating the tradition and beauty of an ancient craft. At the same time, it is like going on a wonderful journey. Natural materials invoke a change of scenery; they conjure up their country of origin,*

> *the pickers, the atmosphere of the extraction factories and, above all, a wealth of colours and fragrances of breath-taking beauty.*
>
> From *The Book of Perfume* by Elizabeth Barillé

While consistent supply of aroma chemicals is limited only by production capacities, essential oils, extracted from flowers, roots, bark and leaves, have many more variables affecting availability. In many parts of the world the growers are small, traditional farmers, taking their crops to traders, whose bulked up supply is then the source of raw materials for the major fragrance houses. Guaranteeing continuity of quality and supply from such a fragile chain is one of the main challenges for the industry. Add the vagaries of cultural and working differences to changing climates and political, legal, technological, social, economical and environmental changes and you begin to see the complicated jigsaw that the Procurement department work with daily.

Before making the broad points necessary to gain an insight into the inner workings of these areas, a few definitions are required.

15.1.1 Categorisation of Suppliers and Materials

While mechanisms may vary between the top ten fragrance houses, the basics are sound for all, and thus this treatment will suffice to give a good idea of the inner chemistry of the business.

Procurement departments have the twin aims of driving down costs while ensuring quality and reliability of supply. In order to do this, they need to balance three specific elements of its mix of needs: supplier relationships, material nature and material availability.

15.1.1.1 Supplier Relationships

15.1.1.1.1 Strategic. Essentially buyer and seller reside in each other's worlds, and the game is not zero-sum; that is, both sides must see demonstrable growth and profit.

Competition law restricts the interface between competitor companies, yet the largest manufacturers of aroma chemicals are the fragrance suppliers, that is, competitor companies in the marketplace. Each of the global fragrance suppliers manufactures a portfolio of synthetic ingredients – partly to guarantee supply, partly to gain competitive advantage for its own perfumers and partly to ensure originality of fragrance creations. New aroma chemicals are often restricted to use by the company that has invented them for a period, after which they are released in the market and can be bought by the other fragrance houses. This anomaly defines part of the character of the fragrance industry – a small world, in which, of necessity, competitors are each other's suppliers.

15.1.1.1.2 Tactical. A good example of an operational relationship would be computer hardware/software, as communication and knowledge keep a business running just as surely as energy. Companies will have a contract to supply/maintain and renew IT infrastructure on a periodic basis. The relationship between the two businesses is close, however, the functional nature of the product means that the contract can be renegotiated or an alternative supplier brought in. The key to successful tactical relationships is service.

15.1.1.1.3 Transactional. Transactional suppliers would provide sundry items such as stationery, laboratory supplies and work clothing. On a raw material front they would provide ingredients that were not unique or of special quality and where price is open to negotiation. Normally, changes of this type of supplier are frequent, taken that quality and reliability are less likely to be compromised. It is the role of the Procurement department to proactively seek alternative suppliers, vetting new applicants, driving costs down while guaranteeing continuity of supply.

15.1.1.2 Nature of Material

15.1.1.2.1 Custom-made. In the ever-increasingly competitive market of the fragrance industry, global operators seek to reduce costs through shared investment. This leads to a collaborative venture in which two companies benefit from producing a bespoke product. Many of the investments in developing countries have been made through this route; production being lower in cost in certain parts of the world, but standards being driven by the needs of businesses operating to the demands of Western clients. The product is therefore custom-made. The collaboration benefits both parties in a strategic alliance that results in a product that is bespoke and used only by the collaborators.

15.1.1.2.2 Bespoke. Like all businesses, the fragrance industry has diverse needs and is not self-sufficient. Success is often reliant on recognising a good product and seeing a unique application for it. Fragrance delivery systems is a good example here; encapsulate systems used to capture droplets of scent have been used to increase fragrance performance in many product areas such as laundry liquids and powders, shampoos and shower gels. Even laying scent onto floors, effectively trapped in microscopic 'bubble wrap' until walked on, releases scent into the air. In this case the supplier may well be manufacturing for an entirely different industry and will work with the fragrance house to tailor the product to meet the new need, becoming a fragrance special encapsulate.

Drums are another example; steel containers are available off-the-shelf, but the industry is fast moving to plastic (heavy-duty polyethylene, HDPE) because it is lighter, energy efficient and can be used on a recyclable basis. These are obtainable from many suppliers, off-the-shelf. However, many fragrance compounds in concentrated form can be aggressive to plastic, which therefore needs protection. The easiest way to do this is to customise the container by spraying the inside with polytetrafluoroethylene (Teflon™). For fragrance use, it will also have specific valve attachment requirements – different from the norm – thus a standard plastic drum is customised for fragrance use.

15.1.1.2.3 Off-the-shelf. Most sundry items, such as laboratory equipment, stationary supplies, *etc*. are available off-the-shelf. Some raw materials, too, are available off-the-shelf; those that are available in abundance and for which there is room to negotiate terms of supply are lavender, citrus oils and peppermint. Climatic or political changes can alter raw materials from readily available to difficult to find, a fact that will increase price dramatically.

15.1.2 Procurement Organisation

The purchase of raw materials probably accounts for around 80% of a fragrance house's non-labour-related costs, thus the purchasers play a vital role in the efficient organisation of the business. For all non-raw materials, such as logistics, packaging, travel, energy, goods and services, the trend is to outsource much of the procurement process, or at least centralise it, where possible on a global scale, in order to leverage spend.

There is a clear split between natural materials and synthetically derived materials. Natural products are subject to growing seasons, acts of God, swings in supply and demand (witness how much more Vanilla, a natural, and vanillin and a synthesised material, are suddenly required following the launch of a vanilla-flavoured version of Coca Cola) and tend to come from the far-flung corners of our world. Manufactured aroma chemicals are much more predictable in terms of price, availability and quality.

Many essential oils are obtained from a protracted route of middlemen. With Ylang-ylang for instance, the Madagascan hill farmer grows and crops the flowers that are bought by distillation cooperatives, which prepare the oils and sell to the first-world traders, who in turn trade with the fragrance houses. The complex chain is not easy to police in terms of either quality or sustainability. A buyer working in this area needs to be well travelled and plugged into the Internet so as to have an understanding of natural material futures from Florida orange juice to Indonesian patchouli. He needs also to consider long-term security and sustainability of supply.

Speciality aroma chemicals produced by complex synthesis require chemical understanding, since the process by which the ingredient is produced will be a key driver of the ultimate cost of the ingredient. An organic chemist is able to explore new potential routes of synthesis and understand the implications and value of each step in the process, and will often strike up a relationship with the supplier whereby the chemistry of the relationship brings a strategic partnership to life.

One category of aroma chemical is supplied by the petrochemical and coal industry. Termed synthetic ingredients, we deal in semantics. Since petrochemicals and coal are derived from long dead plant matter, they could equally be termed natural. Because man tweaks a spanner here and releases a pressure valve there, they are termed synthetic. If the difference is one of ‘life force’ within the natural plant material, which is absent from the petrochemical route materials, then there is a case for the difference. But it is a moot point in the fragrance industry when either route achieves the desired aroma. The tie to the oil and coal industry though affects price stability and is a key concern to the industry when OPEC flexes its price-rise muscles.

Thus within any Procurement organisation, there tends to be a split of buyers between natural, synthetic, speciality and non-raw material. In the fragrance industry, where the top ten fragrance companies compete to fragrance the manufacturer brands, they are also ingredient suppliers to each other. Another appropriate way of organising is for each senior buyer to work with a handful of key suppliers, thus reflecting the organisation of the industry itself; each manufacturer working with a short list of fragrance houses (who compete aggressively on both a creative and price platform for the privilege). Minor raw materials, those used more sparingly, or less frequently, and non-raw material supplies are given a lower priority and dealt with separately.

Ensuring availability and continuity of supply raw materials is fundamental to a well-organised business. Well in advance of going to market, the Purchasing Department would be advised of the formula for ‘Eve’, and the estimated volume requirement over the first few years. A plan would be drawn up with forecast of need over the months ahead, allowing for launch and seasonal peaks of demand, and appropriate provision made to buy in the raw materials.

15.2 SALES AND MARKETING

Consumers do not buy fragrance; they buy aspiration. A feeling. As explained in a previous chapter, the human psyche explains the intrinsic link between smells and emotion. So do manufacturers buy feelings too?

Not quite. The fragrance suppliers' Sales and Marketing departments have to relate to a diverse set of demands in their relationships with brand managers and procurement specialists in the client companies. Two-thirds of the branded consumer products sold around the world today are manufactured by a handful of global companies such as Unilever, Procter & Gamble, L'Oreal, SCJ, Henkel, Reckitt Benckiser, LvmH, Esteé Lauder and Lancaster Coty. Some names will be familiar, some not. The branded goods they make are bought by consumers all over the world.

Just as the Procurement department of a fragrance house is driven to seek more for less, so the Company's customers have similar objectives in their purchasing organisations. In addition, economies of scale ensure that the negotiation is tough. The fragrance industry today is a very competitive world.

Brand managers are interested in how the consumer will perceive the fragrance as a part of the whole brand positioning. Researchers say that fragrances contained in household and personal products, from shampoo and cosmetics to laundry detergents and household cleaners, are near to synonymous with their perceived quality. Fragrances make clothes smell 'clean', cosmetics 'pretty' and households 'well kept'. Fragrances are key to building consumer confidence in the cleansing abilities of soap or multipurpose cleaners, or the perceived skin improvement brought about by the use of cosmetics. In summary, fragrances enhance our enjoyment of using products of personal hygiene (*e.g.* toilet soap) and hence contribute to our cleanliness and general health.

How the fragrance house works with the client will vary from one company to another. This will depend on the type of product that the fragrance will be incorporated into, and on the personalities and philosophies of the company itself. Some client companies will expect the fragrance house to put forward new fragrance ideas and market concepts; others will issue a very specific briefing document to drive the creative process. For some fine fragrance briefs, the fragrance may be an olfactive interpretation of an individual's brand or idea.

Either way, selling an ethereal product such as a scent requires very specific skills. The sales director will pull together a team to cover all the creative and technical expertise he will need to win the business.

Economic factors are, of course, a big influence on the way that the team will work and the fragrance that will be created as a result. Consumers today expect to see frequent new varieties of familiar products on the shelves of the super market. And they do not expect to see the price they pay, for they increase without justification. A new additive with functional benefit is one way to justify a price increase. More frequently though rationalisation of supply chain or production efficiencies are the only ways remaining for manufacturers to squeeze another point out of the profit margin. So production is increasingly moved to less expensive labour markets, out of the developed West, into developing countries, the industry itself rationalises. The smaller companies are being swallowed up by the larger ones. The manufacturers economise by centralising production, creating one mega plant per region, rather than having lots of smaller ones distributed thoughout. Fragrances are increasingly developed for use across a whole region, or even a product range. This brings its own challenges to the fragrance suppliers, as the number of opportunities to win contracts is reduced, while those that remain are for a greater volume of business.

Asia Pacific is a growing market for the fragrance industry, as a whole generation of people are made aware of Western products through technology and the media. The big players have been present in the East for decades, but the fall of the Communist countries coupled with the rise in Western manufacturing in Eastern countries is bringing increasing wealth to more people. The fragrance industry is racing to understand these consumers. Most market research data have focussed on European and American consumers, but here is a huge population whose needs are relatively unknown. For now it is enough to aspire to Western brands, in the future maybe the needs of Chinese consumers will drive product development in the West.

Of course, the lynchpin part of the organisation to the separate departments of both Procurement and Sales/Marketing is perfumery, along with Formulation Management (FM). Figure 15.1 encapsulates the two ever-turning cycles of the business. In one, Procurement department asks for better forecasts of sales to strengthen its purchasing position. Sales, in turn, demand that perfumery create to a competitive cost, and perfumery/FM look to procurement to provide cheaper products of better and more consistent quality.

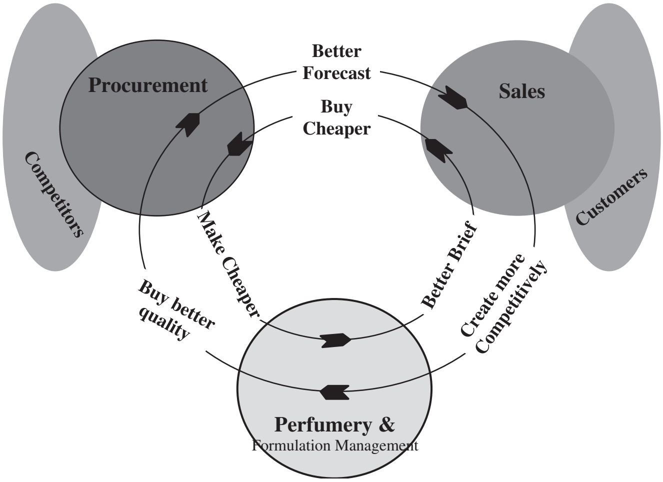

Figure 15.1 *Relationship between procurement, sales and formulation management.*

In the other cycle when Procurement department buys competitively, perfumery have a wider range of material options to respond to the brief and in turn give sales a better choice option to submit to the customer.

Thus, the most complex chemistry the fragrance industry faces is the chemistry of human reactions; the internal chemistry and marketing environment of a fragrance house are seemingly as complex as the aromatic and aliphatic molecules, which make up its products!

CHAPTER 16

The Finale: Brief Submission

LES SMALL
Consultant Perfumer, Quest International

After months of work, the day finally arrives to present the client with the conclusion of the creative and technical work that has resulted from their initial briefing.

The brief issued by Business Scents Ltd for ‘Eve’ gave plenty of scope for creative interpretation and input from Quest specialists to decipher it. Apart from presenting the fragrance itself, it is usual to present ideas for a marketing concept that the fragrance could take in the market.

The composition of the fragrance itself has inspired the way the product relates to the target client, the definition (in this case, a product suitable for women, globally, aged between 16 and 35 years) of the likely consumer and the position of the product as either a stand-alone ‘eau de parfum’ or the linchpin of a product range.

A product aimed at women worldwide must have mass appeal. This was the starting point for considering the marketing platform for ‘Eve’. Searching through extensive databases that contain consumer responses to hundreds of fragrance ingredients and finished perfumes, the marketeers have compiled, for the perfumer’s consideration, lists of ingredients to which a majority of people have reacted positively. The fragrance must be modern, so anything associated with ‘traditional’ or ‘my grandmother’ is rejected; only those liked ingredients linked to current or future trends are retained on the list. Lifestyle trends also provide vital clues. Who is this global female consumer who buys products manufactured by Business Scents Ltd? The marketeers will seek to define her. They imagine a financially independent woman, who lives alone in a city and so has a cosmopolitan outlook and buys sophisticated products. She seeks alternative medicines, is well groomed and fashionable and watches the latest films. She is a fastidious eater and likes to feel good about her contribution to the wellbeing of her family, friends and the environment. Current times have made her worry about the future sometimes. She would have liked to go to Madagascar but watched news of the tsunami with horror and so has decided to postpone the trip.

Now inside the head of this imaginary consumer and brand, with the fragrance selected and perfected by the perfumer, the team venture forth to the client clutching fragrance samples, concept boards and research data to support their interpretation of the brief. The fate of the fragrance is in the hands of the client and possibly depends on future market research. What happens next will vary widely from one manufacturer to another. For some, the submission will be scrutinised by an independent expert 'nose', employed for the purpose of selecting market winners; others will send the submission to consumer panels to test their response, while in other companies one individual will drive the product development and make the fragrance selection.

'Eve' has been created to be the olfactive image of this imaginary consumer. Just as an artist works with colour, light and shade to create an impression of a person or place, so a perfumer works with scent ingredients to produce a sensory experience that relates to an individual's expectations or aspirations.

The first impression of this fragrance is given by the volatile mix of apple and grapefruit and a melange of tropical fruit that whizzes out of the bottle or away from the smelling strip. The character at the heart of the fragrance reflects a strong yet modern female. The scent is predominantly floral, with a twist of exotic spice, and leaves a soft, lingering sense of femininity when dry, which is exuded by a woody amber blend of oils at the base (Figure 16.1).

The excitement of the creative chase is over. Unleashed, the fragrant molecules stream into the air, mixing and rebounding in an irreversible process that is as old as time. It is impossible to put the aroma back in the bottle.

The fragrance industry today faces many challenges – economic, regulatory, social, techncial, political, as well as environmental – but the fact remains that the human relationship with each of the senses remains important, as people increasingly become aware that pleasure can be brought into modern life by returning to the basics: colour, feel, taste, sound and smell.

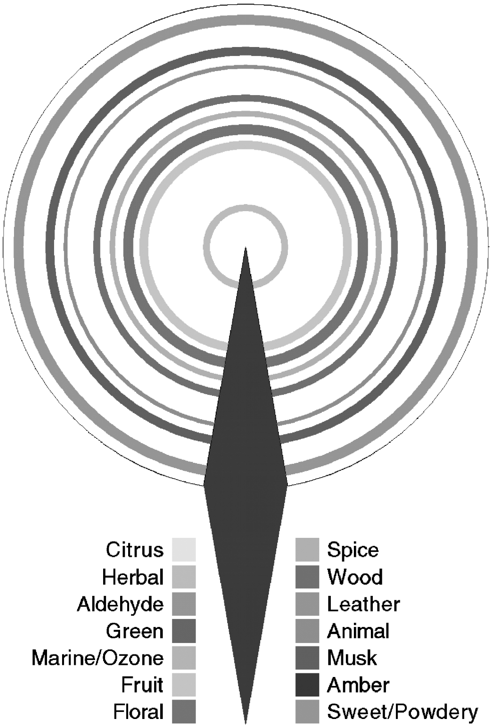

Figure 16.1 *The Lignes de Force of Eve, a graphic description of the fragrance*

Barillé summed up the reason why the fragrance industry will be forever relevant when he wrote, ‘The magic of fragrance lies in the ancestral links between humans and nature and our age old interest in the sweet smelling gifts which express all the beauty of the world.’

APPENDIX I

Some of the more Important Natural Fragrance Materials

EXPRESSED OILS

Bergamot (fruit)
Grapefruit (fruit)
Lemon (fruit)
Lime (fruit)
Mandarin (fruit)
Orange (fruit)
Tangerine (fruit)

DRY DISTILLED OILS

Birch tar (wood)
Cade (wood – juniper)
Gum turpentine (exudate)

TINCTURES

Castoreum (anal glands – beaver)
Civet (anal glands – civet cat)
Vanilla (fruit)

CONCRETES AND RESINOIDS

Benzoin (exudate)
Castoreum (anal glands – beaver)

Civet (anal glands – civet cat)
Elemi (exudate)
Galbanum (exudate)
Jasmine (flower)
Oakmoss (aerial)
Olibanum (exudate)
Orris (rhizome)
Peru balsam (exudate)
Rose (flower)
Styrax (exudate)
Tolu balsam (exudate)
Treemoss (aerial)
Vanilla (fruit)
Ylang ylang (flower)

ABSOLUTES

Castoreum (anal glands – beaver)
Civet (anal glands – civet cat)
Cumin (seed)
Geranium (leaves and branches)
Jasmine (flower)
Oakmoss (aerial)
Orange flower (flower)
Treemoss (aerial)
Ylang ylang (flower)

UNTREATED

Copaiba balsam (exudate)
Labdanum (exudate – boiled to free exudate from plant material)

STEAM DISTILLED OILS

Angelica (root)
Anise (seed)
Ambrette (seed)
Artemisia (aerial parts)
Basil (flowering tops)
Bay (leaf)
Birch leaf

Birch bark
Cabrueva (wood)
Cajeput (leaves and twigs)
Calamus (rhizome)
Camphor (wood)
Cananga (flowers)
Caraway (seeds)
Cassia (leaves)
Cedarwood
  (*Juniperus* type – English, Texan, Chinese – cedrene)
  (*Cedrus* type – Atlas, Himalayan – atlantone)
Cedar leaf (Thuja oil, juniperus)
Celery (seed)
Chamomile (flowers)
Cinnamon bark
Cinnamon leaf
Cistus (ex labdanum exudate)
Citronella (leaves)
Clary sage (flowering tops and leaves)
Clove bud
Clove leaf
Coriander (seeds)
Cornmint (aerial parts)
Cumin (seeds)
Dill (aerial parts)
Elemi oil (exudate)
Eucalyptus (leaves)
Various species, mostly cineole types, *E. citriodora*, citronellal
Fennel (seeds)
Fir needle
Galbanum (exudate)
Geranium (leaves and branches)
Ginger (root)
Guaicwood
Ho (leaf)
Juniper (fruit)
Lavender (aerial parts)(species are lavender, lavandin and spike)
Litsea cubeba (fruit)
Marjoram (leaves and flowers)
Mint (aerial parts) (various species, arvensis, piperita *etc.*)

Neroli (flowers) (orange)
Nutmeg (fruit)
Origanum (aerial)
Orris (rhizomes)
Palmarosa (leaves)
Patchouli (leaf)
Pennyroyal (aerial parts)
Petitgrain (leaves) (orange)
Peru balsam oil (exudate)
Pimento (fruit)
Pine oil (wood)
Rosemary (aerial)
Rose (flower)
Rosewood
Sage (aerial)
Sandalwood (wood)
Sassafras (roots)
Styrax (exudate)
Tarragon (aerial parts)
Thyme (aerial parts)
Ti tree (leaves)
Vetiver (root)
Ylang ylang (flower)

TOP TERPENOIDS

Name	*tpa*[*]	*£/kg*[*]	*Odour type*
Amberlyn + analogues	50	500–750	Ambergris
Bangalol + analogues	30	30	Sandalwood
Carvone	1,500	10	Spearmint
Cedrene derivs	500	20	Cedarwood
Citral	5,000	5	Lemon[†]
Citronellol	3,000	7	Rose
Dihydromyrcene	5,000[¶]	1	
Dihydromyrcenol	4,000	4	Citrus/floral
Geraniol	5,000	4	Rose
Hydroxycitronellal	1,000	8	Muguet
Isobornyl acetate	2,000	1	Pine
Limonene	3,500	1	Orange[§]
Linalool	8,000	4	Floral/wood
Linalyl acetate	2,000	4	Fruity/floral

Continued

Table *Continued*

Name	*tpa**	*£/kg**	*Odour type*
Menthol	12,000	10	Mint/coolant
(Methyl) ionones	5,000	15	Violet
Myrcene	30,000¶	1	Balsamic
Pine oil/Terpineol	30,000	1	Pine
Terpinyl acetate	1,000	3	Herbaceous
Tetrahydrolinalool	2,000	4	Floral

[*] These are approximate figures and are only intended as a guide to show the relative volumes and values.

[†] Citral has little use in fragrance. This tonnage is used for the manufacture of ionones and methylionones.

[‡] This figure relates to the use of orange oil in perfumery. About 1,500 tpa are used in the manufacture of carvone. Total production exceeds 50,000 tpa.

[§] The material used is actually orange terpenes, which is about 80% limonene but the odour comes from minor components.

[¶] A substantial proportion of the total consumption of these materials is used for manufacture of other ingredients.

APPENDIX II

Useful Addresses

The American Society of Perfumers, P.O. Box 1551,West Caldwell, NJ 07004, USA

American Oil Chemists Society (AOCS), 2211 W. Bradley Ave. Champaign, IL, 61821-1827, USA

Aroma Research Institute of America (ARIA), P.O. Box 282 Santa Fe, NM 87504, USA

BP (Chemicals) Building A, Chertsey Road Sunbury on Thames, Middlesex TW16 7LL, UK

The British Fragrance Association, 6 Catherine Street, London WC2B 5JJ, UK

The British Society of Perfumers Glebe Farmhouse, Mears Ashby Road, Wilby, Wellingborough, Northants NN8 2UQ, UK

European Cosmetic Toiletry and Perfumery Association (COLIPA), Rue du Congrès 5-7, B1000, Brussels, Belgium

Comite Francais du Parfum (CEP), 57 Avenue de Villiers, 75017 Paris, France

Cosmetic, Toiletry, and Fragrance Association, 1101 17th Street, NW Suite 300, Washington, DC 20036-4702, USA

Dutch Cosmetic Society, Gebouw Trindenborch, Catharijnesingel 53, 3511 Utrecht, Netherlands

Federation Des Industries De La Parfumerie, 8, Place du Général Catroux, 75017 – Paris, France

FEDAROM, Fédération Française desSyndicats de l'Aromatique, 89, rue du Faubourg St Honoré, 75370 Paris, Cedex 08, France

French Cosmetic Society, 17 Rue Carl-Herbert-92400, Courbevoie, France

German Society of Perfumers, Heinrich-Heine Strasse 25, D-7100 Heilbronn, RFA

Institut Superieur Internacional du Pafum, de la Cosmetiue et de l'Aromatique Alimentaire, 36 Rue de Parc de Clagny, 78000 Versailles, France

International Federation of Cosmetic Societies (IFSCC), GT House, 24/26 Rothesay Road, Luton Beds LU1 1QX, UK

International Fragrance Association, Chemin de la Parfumerie 5, CH-1214 Vernier, Geneva, Switzerland

Italian Federation of Cosmetic, Toiletry and Perfumery Industries (FENAPRO), Corso Venezia 47, Milan 20121, Italy

Italian National Association of Perfumery and Cosmetic Industries, Via Juvara 9, 20129 Milan, Italy

Japan Cosmetic Industry Association, Hatsumei Kaikan 4F, 2-9-14 Toranomon, Minato-ku, Tokyo 105-0001, Japan

LALIQUE SA 11, rue Royale 75008 Paris, France

Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA

Pandora Ltd, 41, Alexandra Corniche, Hythe, Kent, CT21 5RW

Perfumery Education Centre, University of Plymouth Business School, Drake's Circus, Plymouth, Devon PL4 8AA, UK

Pertech Associates Ltd., 32, Linksway, Folkestone, Kent, CT19 5LW

Quest International Fragrance Division HQ, Willesborough Road, Kennington, Ashford, Kent, TN24 0LT, UK

Research Institute for Fragrance Materials, Two University Plaza, Suite 406, Hackensack, NJ 07601, USA

Society of Cosmetic Chemists, 120 Wall Street, Suite 2400, New York, NY 10005-4088

Spanish Perfumer Group, Pau Claris, 107 pral-08009, Barcelona, Spain

Syndicat National des Fabricants de Produits Aromatiques, 48 avenue Riou Blanquet, F - 06130 Grasse, France

The Chartered Institute of Marketing (CIM), Moor Hall, Cookham, Maidenhead, Berks, SL6 9QH, UK

The Cosmetic, Toiletry, and Fragrance Association (CTFA), 1101 17th Street, NW, Suite 300, Washington, DC 20036-4702, USA

The Cosmetic, Toiletry and Perfume Association (CTPA), 35 Dover Street, London, W1X 3RA, UK

The Fragrance Foundation, 145 East 32 Street, New York, NY 10016-6002, USA

The International Federation of Essential Oils and Aroma Trades (IFEAT), Federation House, 6 Catherine Street, London, WC2B 5JJ, UK

The New School of Perfumery Art, 15 Winding way, Verona, NJ 070440, USA

The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge, CB4 0WF, UK

Universite de Montpelier Faculty of Science and technology of Languedoc, Place Eugene Bataillon, 34060 Montpellier, France

Women in Flavor and Fragrance Commerce, P.O. Box 2154, Teaneck, NJ 07666, USA

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*Recommend starting point in each section.
†Titles marked thus are currently available from Allured Publishing Corp. (www.Allured.com)

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