

# CHEMISTRY AND THE SENSE OF SMELL

CHARLES S. SELL

WILEY



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# Contents

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**Preface      vii**

**Acknowledgments      ix**

**Introduction      1**

**1 Why Do We Have a Sense of Smell?      4**

**2 The Mechanism of Olfaction      32**

**3 Analysis and Characterisation of Odour      188**

**4 The Sense of Smell in Our Lives      209**

**5 The Scents of Nature      237**

**6 Manufacture of Fragrance Ingredients      296**

**7 The Design of New Fragrance Ingredients      357**

**8 The Relationship Between Molecular Structure and Odour      388**

**9 Intellectual Challenges in Fragrance Chemistry and the Future      420**

**Glossary      428**

**Index      437**



# Preface

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At the very outset, I must make it clear that this book is a personal perspective on olfaction and the perfume industry. The views expressed in it are mine and not necessarily those of my colleagues, academic contacts or companies or institutions with which I have been associated. The views are those of a chemist and are admittedly biased in favour of fragrance chemists and their art.

At the start of my school life, chemistry was not my favourite subject. However, when I reached the sixth form, I was introduced to organic chemistry and immediately fell in love with the subject. I still have a vivid memory of adding a solution of adipoyl chloride in carbon tetrachloride to one of hexamethylene diamine in water, seeing a film of nylon forming at the interface and then finding that, as I pulled the film out of the mixture, more seemed to grow by magic and, as I drew the film out, it produced a long string of nylon. My interest in the living world drew me to natural products chemistry and the excitement of relating the chemicals I could synthesise in the laboratory to those in living organisms. My time at the Australian National University in Canberra with the late Professor Arthur Birch introduced me to the chemistry of terpenoids, and one of my synthetic targets was a termite trail pheromone, giving rise to my interest in chemical communication. Whilst a post-doctoral researcher at Warwick University working with Professor Bernard Golding, I deepened my understanding of enzymes. My experience in terpenoid chemistry was instrumental in my joining PPL and thus starting a career in fragrance chemistry. Since then, I have worked on analysis of perfume and perfume ingredients, chemical process development and optimisation and also on the discovery of novel fragrance ingredients. The last of these activities led me to speculation about structure/odour relationships and a fascination with the unpredictability of the odour that would be elicited by any new molecular structure. Having spent years struggling with structure/odour relationships in an attempt to understand the sense of smell, I came to the conclusion that I was asking the wrong questions. So I looked to biology to seek the right questions to ask. I was very fortunate to become part of Givaudan and to be involved in TecnoScent, Givaudan's joint venture with ChemCom to explore the olfactory receptors. The study of olfaction has made enormous advances over the last few decades, and the subject of olfactory receptors is a large part of this. We now know the primary structures of all of the human olfactory receptors and the basic principles of how they function in olfactory sensory neurons, two huge steps forward in our understanding which have

both been recognised by the awarding of Nobel Prizes. The olfactory receptors are a vital first stage in the process of olfaction and the key point in the chemistry of the process, before neuroprocessing begins. For this reason, the chapter describing the receptors (Chapter 2) is the largest in the book and considerable space is devoted to providing the context of class A G-protein coupled receptors (GPCRs) in general.

CHARLES S. SELL

*January 2014*

# Acknowledgments

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I would like to thank all of my former colleagues and friends in Givaudan (including PPL, PPF and Quest) and ChemCom and in universities (my teachers, friends and consultants) for their support and encouragement and for their role in developing my interest and thinking in chemistry and fragrance.

My thanks go to Dr. Ton van der Weerd, Dr. Philip Kraft and Stuart Reader for helpful comments on the manuscript, each in his area of expertise. I would also like to thank Dr. Sebastien Patiny for help in producing figures 2.14 and 2.15 and Dr. Philip Kraft for providing figure 8.14.

My wife, Hilary, deserves very special mention and thanks for her patience and tolerance with me during the many hours which I have spent in my study to write this book.



# Introduction

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René Descartes said ‘I think, therefore I am’. The knowledge of one’s own existence is the only certainty which each human has, the rest of what we understand about the universe is comprised of mental models based on input from our senses. Smell is often described as the most mysterious or the least understood of our senses. In the light of the very significant advances in our understanding over the last two decades, I would argue that the latter is not the case. Smell is certainly the oldest of our senses since it is present in even the most primitive living organisms and, throughout evolution, has played a crucial role in survival and development of species. Our understanding of the chemical mechanisms of odour detection in the nose has advanced enormously since Buck and Axel’s discovery in 1991 of the gene family coding for the olfactory receptor proteins. The mysteries of smell revolve around the complexity of the combinatorial detection system and the neuroprocessing that converts the physical input into the mental image which we call smell. Unlike vision where we have three primary colours, each corresponding to a specific wavelength of the electromagnetic spectrum, there are no fixed reference points in odour. When we describe a smell, it is always in relation to other things that elicit a similar mental impression. We might describe one sample as smelling like roses and another as smelling like rotten eggs but neither of these is a fixed reference point. Odour exists as a continuum in a multi-dimensional mental space, and all we can do in describing a new odour is to relate it to known points in that ‘odour space’. Odour classification is merely an attempt to map out regions within that space. Many parts of the brain are involved in converting the chemical stimulus in the nose into the mental odour percept, and some of these brain regions are strongly linked to memory and emotion. Thus an odour can trigger memories or influence emotional states before the subject is consciously aware of smelling it. In humans, the role of smell has extended from a survival tool, giving us information about changes in the chemical environment, through a desire to mask unpleasant odours, into a source of pleasure and artistic expression in the form of perfumery.

The chemistry of fragrance is a fascinating subject because of its breadth and the diversity of other disciplines that impinge upon it. The fragrance industry has

## 2 Introduction

ancient roots. For example, a perfume factory discovered on Crete dates back to 2000 B.C., and Egyptian tomb paintings often portray scenes involving the use of perfumes. In those days, the ingredients of perfumery were extracted from plant and animal sources, and plant extracts still provide many of the key notes in perfumery. Our understanding of how nature produces such an array of intricate chemical structures has grown over the last century and the natural products chemist now works alongside botanists, biochemists and molecular biologists in seeking to further our knowledge of biosynthesis. I never cease to be amazed by the variety of terpenoids that nature makes from a single precursor, isopentenyl pyrophosphate. The modern perfumery industry relies heavily on ingredients synthesised by chemists. The feedstocks include natural extracts such as pinene and petrochemicals such as isobutylene. The complexity of fragrance molecules, the performance and cost constraints of perfumes for household applications and the need to use synthetic routes that do minimal harm to the environment all combine to present a significant challenge for the process chemist. Success in this undertaking requires close collaboration with the chemical engineers who will design the process plant used in manufacture. The first generation of synthetic fragrance ingredients were exact copies of natural counterparts, such as the coumarin, vanillin and heliotropin used in 'Jicky' (1889), but non-nature-identical materials were given a boost in 1921 with the success of Chanel 5 which used small amounts of novel aldehydes to add a unique top note to the rose and jasmine oils in the heart of the fragrance. Designing novel fragrance ingredients is another very significant intellectual challenge and there are many parameters that must be taken into account. It is not sufficient just to produce a pleasing odour, the price must also be acceptable and the substance must be stable to the components of the consumer goods into which perfume is incorporated. These include acids as strong as hydrochloric, bases such as sodium hydroxide and oxidants like sodium hypochlorite and peracetic acid. The material should also be safe to use and should biodegrade easily in sewage treatment plants. Structure/activity relationships are important tools, and these bring the fragrance chemist into contact with mathematicians such as statisticians and computer modellers. Attempts to understand the relationship between molecular structure and odour brings us to the forefront of current scientific research. At least nine Nobel Chemistry Prize winners have mentioned fragrance chemistry in their Nobel Lectures and eight Nobel Prizes have gone to scientists working on the biochemistry and molecular biology of the class of receptor proteins to which the olfactory receptors belong. In 2004, the Nobel Prize for physiology/medicine went to Richard Axel and Linda Buck for their work on identifying the genes responsible for the olfactory receptor proteins which are the basis of our sense of smell. Linda Buck used this discovery to confirm that smell is a combinatorial sense, with each receptor responding to a range of odorants and each odorant stimulating a range of receptors. The 2012 Nobel Prize for chemistry was awarded jointly to Robert Lefkowitz and Brian Kobilka for their work in elucidating the structure and mechanism of action of G-protein coupled receptors, the class to which olfactory receptors belong. Chemists trying to understand the implications of these two great breakthroughs in our understanding of olfaction must be prepared to work at the frontiers between chemistry, molecular

biology, neuroscience and psychology. Albert Einstein said: ‘The most beautiful thing we can experience is the mysterious. It is the source of all true art and science’. We have come a long way in our understanding of fragrance but there is still plenty of mystery to provide us with intellectual challenge and beauty.

The object of this book is to review our current state of knowledge of the chemical aspects of the sense of smell, from the volatile compounds of nature to our man-made odorants that complement them; our understanding of how the nose detects odorants and produces an electrochemical signal which is translated into a mental image; and to touch on the role of this chemical sense in living organisms and in particular in humans and its contribution to our way of life and our well-being. Throughout the book, the emphasis will be on the human sense of smell, but the sense in other species will be included in order to clarify the subject or to provide the context.

# Chapter 1

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## Why Do We Have a Sense of Smell?

### THE EVOLUTION OF OLFACTION

Smell and taste are undoubtedly the oldest of our five senses since even the simplest single-celled organisms possess receptors for detection of small molecules in their environment. For example, Nijland and Burgess have shown that *Bacillus licheniformis* can detect and respond to volatile secretions (ammonia) from other members of the same species (1). One striking example of odour detection by single cells is the human sperm which possesses smell receptors identical to one of those found in the nose, a receptor known as OR1D2, and sperm will actively swim towards the source of any of the odorous molecules, such as Bourgeonal (1.1), that activate this receptor (2). It is presumed that the ovum releases some chemical signal which OR1D2 detects and thus the sperm is led to its target. However, the identity of this chemical signal remains unknown. Even simple organisms, such as the nematode worm *Caenorhabditis elegans*, use the sense of smell for various purposes. For example, they respond to odours by chemotaxis as a way of helping them find food (3) and they also use odorants to control population density (4).

It is easy to imagine how early living cells would gain a survival advantage by developing a mechanism to detect food sources in the primeval environment and to move towards them just as spermatozoa swim toward a source of Bourgeonal (1.1). Having developed such a detection mechanism, the genes coding for the proteins involved would become an important feature of the genome and would undergo development, diversification and sophistication over the course of evolution. Probably because of their evolutionary importance, the genes coding for olfactory receptor (OR) proteins are one of the fastest evolving groups of genes and form the largest gene family in the genome. An interesting recent discovery is that diet and eating habits affect the evolution of taste receptor genes (5). For example, animals such as cats, which are purely carnivorous, have lost functional variants of the sweet receptor. Sea lions and bottle-nosed dolphins were once land animals

but have returned to a marine environment, and members of both species swallow their food whole without tasting it. Sea lions have lost their functional receptors for sweet and umami tastes, and the dolphins have lost these and the bitter receptors also. In all of the examples, the loss is due to mutations in the genes that have made them pseudo-genes. In other words, the genes were there in the ancestors of the species but have been lost owing to changes in diet and habit.

Smell receptors essentially recognise molecules from the environment and thus provide the organism with information about the chemistry of its environment and, more importantly, about changes in that chemistry. In single-celled organisms, the smell/taste receptors are located in the cell wall, in contact with the external environment. As animals became more complex over the course of evolution, specialized taste and smell cells developed and became located in specialised regions of the organisms. Fish have receptors on their skin, therefore in contact with the water which constitutes their environment. In air-breathing animals, the smell organs are located in the nasal cavity. Therefore, odorant molecules reach the olfactory tissue primarily through inhaled air and so must be volatile. For example, in humans the olfactory epithelium (OE) is located at the top of the nasal cavity towards its rear and, thus, under normal conditions, is accessible only to volatile substances. In some species, mice for example, the nose is sometimes placed in physical contact with the scent source (e.g. the murine urine posts which will be described later) and the animal sniffs in such a way that non-volatile materials can be drawn into contact with the sensory neurons. Much of what is commonly considered 'taste' is actually smell. The taste receptors on the tongue sense only sweet (e.g. sucrose), sour (e.g. citric acid), salt (e.g. sodium chloride), bitter (e.g. quinine) and umami (e.g. glutamate); the rest is smell. When odorants are sniffed through the nose, this is referred to as *ortho-nasal olfaction*, whereas the smell of material taken into the mouth and reaching the nose via the airways behind the mouth is known as *retro-nasal olfaction*.

Smell is the most important sense for most animals, the main exceptions being aquatic animals which rely heavily on sound, and diurnal birds and five primates for which vision is the dominant sense. Asian elephants, mice, rats and dogs all have similar olfactory acuity and outperform primates and fur seals (6). Amongst the mammals, only rhesus macaques, chimpanzees, orang-outangs, gorillas and humans rely more on sight than smell. These primates use only about half the number of OR types that other mammals do and are the only mammals with colour vision. Consequently, speculation arose that an evolutionary trade-off between odour and trichromatic vision had occurred. However, an examination and comparison of the olfactory gene repertoires of hominids, old-world monkeys and new-world monkeys led Matsui et al. to conclude that this was not the case (7).

On the other hand, there are many examples of evolutionary pressure affecting the genes for the chemical senses (taste and smell) in the animal kingdom and a few of these will suffice to illustrate this. Viviparous sea snakes do not rely on a terrestrial environment, unlike their oviparous counterparts who lay their eggs on land. The viviparous sea snakes have lost many of their OR genes, whereas

the oviparous species have retained theirs (8). About 4.2 million years ago, giant pandas changed from being carnivores to being herbivores and, at about the same time, lost their umami taste receptors (9). Umami taste is due to glutamate and some nucleotides and is therefore associated with a carnivorous diet. There is therefore speculation that the two phenomena are related, but the fact that the gene is present in herbivores such as the cow and the horse suggests that the loss of the gene might have played a reinforcing role rather than a causative one. A possible alternative explanation for the change of diet has been proposed following an analysis of the panda genome in the context of other species (10).

The mosquito species *Aedes aegypti* and *Anopheles gambiae* belong to the *Culicinae* and *Anophelinae* mosquito clades, respectively. These clades diverged about 150 million years ago, yet there are OR genes that are highly conserved between the two species. Heterologous expression of the genes from both species produced receptors that respond strongly to indole, thus providing evidence of an ancient adaptation that has been preserved because of its life cycle importance (11).

Another interesting example of adaptation involves the response of a local fruit fly to the fruit of the Tahitian tree *Morinda citrifolia*. The fruit of this tree is known as *noni fruit*. It is good for humans but it contains octanoic acid which is toxic to all but one species of fruit flies of the *Drosophila* family. However, *Drosophila sechellia* flies do feed on noni fruit and choose it as a site for egg laying. Fruit flies of the *Drosophila* family have taste organs on their legs and mouthparts. It has been shown that variants in an odour-binding protein (OBP57e) are responsible for this change in food preference and also in courtship behaviour and in determination of whether the OBPs are expressed on the legs or around the mouth. The genes for this OBP are highly variable and allow for rapid evolution and adaptation as evidenced by the altered response of *D. sechellia* to octanoic acid (12).

Mice convey social signals using proteins of the lipocalin family, known as *major urinary proteins* or MUPs. Originally they were restricted in MUP types. But the development of agriculture 20,000 years ago and the resultant closer association of mice with humans, as well as the consequent increased density of murine communities, led to the need for more precise social communication and so the pool of MUP genes has increased. Mice are capable of reproduction at the age of 6 weeks, and so 20,000 years therefore represents a large number of murine generations and easily allows for such evolutionary adaptation (P. Brennan, Personal communication.).

Estimates of the number of olfactory genes per species vary slightly, a typical example (based on the analysis of Zhang and Firestein (13)) is shown in Table 1.1. In vertebrate species, the lowest number of OR genes (14) is found in the puffer

**Table 1.1** Number of Intact Olfactory Genes in Different Species

Species	Chicken	Opossum	Rat	Mouse	Dog	Chimp	Human
Intact genes	554	899	1278	1194	713	353	384

fish (15) and the highest in the cow (2129) (16) (115). For rats and mice, the olfactory genes represent 4.5% of the total genome; for humans the figure is 2%.

Based on the figures in Table 1.1, it is tempting to speculate that the human sense of smell is inferior to that of rats and dogs. However, on examination of the amino acid sequences of OR proteins, we find that the human repertoire of 382 ORs covers all of the chemical space covered by the 1278 receptors of rats. The initial olfactory signal is therefore somewhat less finely tuned in humans but we have an enormous advantage in signal processing because of our very much more powerful brains. So perhaps we do not need the fine detail of input that rodents do because we can make better use of the incoming information and can therefore dispense with an unnecessarily large array of receptor types. Therefore, our sense of smell might be better than we tend to think.

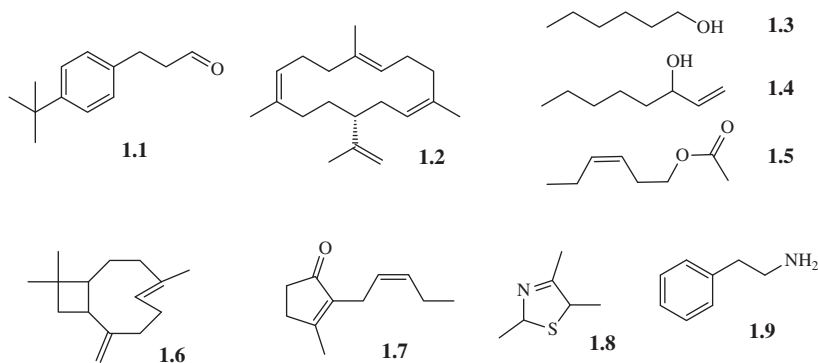
The sense of smell gives organisms (from amoeba to humans) information about the changing chemistry of their environment and thus can alert them to either danger or opportunity. Just as single-celled organisms might use smell/taste to detect amino acids or sugars in their aqueous environment, highly evolved animals use smell to detect the smell of food. For example, lions use smell to detect antelopes in the savannah, monkeys use smell to detect ripe fruit in the rainforest canopy and humans use smell to find the bakery counter at the back of the supermarket. The sense of smell also warns us against the dangers of spoiled food. We quickly learn that the smell of hydrogen sulfide warns us to avoid rotten eggs or meat that has gone bad as a result of bacterial activity. Just as the lion locates the antelope using its sense of smell, the sense of smell can warn the antelope of the approach of the lion. The smell of smoke is a universal warning signal to all mammalian species. It therefore follows from this role in continuously analysing the chemistry of the environment that the sense of smell must be time-based, capable of dealing with complex mixtures of molecules (since natural odours are almost invariably mixtures) and capable of recognising previously unknown molecules. Thus the sense of smell cannot depend on a simple mechanism. The complexity of the sense will be made clear in Chapter 2.

## GOOD FOOD

Taste is used to evaluate food both for its nutritious content and the possible presence of poisons. There are five tastes: sweet identifies carbohydrates for energy; umami identifies essential amino acids; salt ensures the correct electrolyte balance; sour warns against fermentation; and bitter warns against poisons such as alkaloids. The receptors for sweet, bitter and umami are G-protein coupled receptors (GPCRs), as are the ORs. Those for salt and sour are ion channels. In the mouth, there are also neurons containing receptors known as *transient receptor potential channels* (TRPs) which judge temperature, pressure and also poisons. However, much of what is normally referred to by lay people as ‘taste’ or ‘flavour’ is actually smell, and the diversity of odour signals is such that smell has to be sensitive to a much greater range of stimuli than these other senses. For instance, smell is used to

judge quality of food, such as ripeness of fruit by its ester content, and the presence of poisons and bacterial contamination by the presence of amines and thiols. When we smell by sniffing ambient air, the process is known as *ortho-nasal olfaction*, whereas smelling food in the mouth involves air travelling up through the back of mouth and into the rear of the nasal cavity and is thus known as *retro-nasal olfaction*. In his book *Neurogastronomy*, Gordon Shepherd, one of the greatest figures in olfactory neuroscience, suggests that the importance of retro-nasal olfaction helped to shape human evolution (17). This view is supported by the finding that *Homo sapiens* have a larger olfactory bulb and a larger olfactory cortex than did *Homo neanderthalensis*, the only other species to have such a large brain in proportion to overall body size (18). Since neanderthals lost out in competition with *H. sapiens*, we must have had some advantage over them and perhaps the answer does lie in our superior sense of smell compared to theirs.

We all know how the smell of food attracts us. Shoppers are drawn to the smell of freshly baked bread coming from the bakery counter at the back of the supermarket, and it has been shown that blindfolded students can follow a chocolate trail in the same way that a bloodhound will follow a scent trail (19). We also know that the smell of food makes an important contribution to our enjoyment of food, and it also can control our appetite. For example, it has been shown that a complex strawberry flavour gives more feeling of satiety than a simple flavour (20). A line of ants following a food trail is a common sight, and other insects also lay trails between the nest and a food source. For example, the Australian termite species *Nasutitermes exitiosus* lays a trail of the diterpene hydrocarbon neocembrene-A to lead other members of the colony to a newly discovered food source (21) (116). Neocembrene-A (**1.2**) is virtually odourless to humans but the termites are phenomenally sensitive to it. The European grapevine moth *Lobesia botrana* is attracted to grapevines (*Vitis vinifera*) by volatiles produced by the plant. Although it is attracted to individual chemical components such as 1-hexanol (**1.3**), 1-octen-3-ol (**1.4**), (*Z*)-3-hexenyl acetate (**1.5**) and (*E*)- $\beta$ -caryophyllene (**1.6**), the attraction is much more potent when these are present in the ratio found in the plant (22) (Figure 1.1). Similarly, blowflies are attracted to corpses by dimethyl disulfide and 1-butanol (23).



**Figure 1.1** Some chemical signals.

The important role of olfaction in food selection is nicely illustrated by the following example of alteration in odour perception. After mating, the females of the cotton leafworm moth (*Spodoptera littoralis*) change their food preference from lilac flowers (*Syringa vulgaris*) to the leaves of the cotton plant (*Gossypium hirsutum*) which is the best food source for the larvae. This behaviour, which clearly gives the larvae the best survival chance, has been shown to be due to changes in the processing of the olfactory signals in the antennal lobe which is the primary olfactory centre of the insect (24).

Of course, humans represent food for some other species. Smallegange et al. investigated the relative attractiveness to the malarial mosquito *A. gambiae* of fresh human sweat, matured human sweat, used socks and some chemical components of human body odours including ammonia, lactic acid and a blend of these with various fatty acids (25). The skin residues on socks proved the most potent attractant of these. Carlson et al. showed that *A. gambiae* and *D. melanogaster* (a fruit fly) have evolved OR genes covering different parts of odour space. The narrowly tuned receptors of *A. gambiae* respond to volatiles in human sweat, whereas those of *D. melanogaster* respond to volatiles emitted by fruit (26). Cloning the gene for the mosquito's AgOr1 receptor into fruit fly neurons that had been engineered to be otherwise free of ORs resulted in the fruit fly neuron responding to p-cresol, a ligand of AgOr1 and a component of human sweat (27). The silkworm *Bombyx mori* feeds exclusively on mulberry leaves. Tanaka et al. found that the insects were guided to the mulberry by chemotaxis and identified *cis*-jasmone (**1.7**) as the volatile responsible (28). The insects' detection threshold for *cis*-jasmone is 3 pg/l. Tanaka et al. isolated 66 OR genes from the insects, cloned then into *Xenopus* oocytes and showed that one of these receptors, BmOR56, was very selectively tuned to *cis*-jasmone. Of course, it is possible that one species could detect the trail pheromone of another and use it in controlling social behaviour. Thus one species of stingless bee, *Trigona hyalinata*, will avoid food trails left by members of the related species *Trigona spinipes* and thus prevent conflict in competition for food sources (29).

Food source identification can reach subtle levels. For example, the tick *Ixodes hexagonus* is attracted to the smell of sick hedgehogs (*Erinaceus europaeus*) in preference to that of healthy animals (30), and the predatory mite *Neoseiulus baraki* is attracted to those parts of a coconut tree that are infested by the pest *Aceria guerreronis* which is its food source (31). The ladybird, *Coccinella septempunctata*, preys on aphids and will not only detect and respond to the smell of aphids but can also learn to distinguish between the smells of two different cultivars of the same plant and will respond to one that it has already experienced to have been aphid-infested, irrespective of the smell of aphids (32).

## BAD FOOD

The chemical senses provide warnings of dangers. For example, bitter taste in food warns against the possible presence of toxic alkaloids. Bacterial contamination

of food is a clear danger and so something that our senses need to protect us against. Bacterial decomposition of proteins generates a number of characteristic by-products such as ammonia, hydrogen sulfide, methanethiol and dimethyl sulfide. Trimethylamine is responsible for the well-known odour of rotten fish. Lipid oxidation products are another product of bacterial action on food, and so, for example, butyric acid is an indication that milk has gone bad. Since all of these degradation products are volatile, the sense of smell offers an ideal mechanism for their detection and we quickly learn that their odours signal danger. Not only are our detection thresholds for them very low, but the resultant signals are processed faster and more accurately than those of other odours (33).

## NAVIGATION

Smell is also used in navigation by animals. It is well known that salmon return to their natal stream to spawn and that they locate it by smell. Using functional magnetic resonance imaging (fMRI), it is now possible to trace the neural pathway through which this recognition occurs (34). Pigeons also use smell in finding their way back to their home and it has been demonstrated that blocking one nostril results in them taking longer and making more exploratory excursions en route. Interestingly, the effect is greater if it is the right nostril that is blocked (35).

## DANGER SIGNALS

The use of smell to alert animals to danger is well known to humans. In the past, town gas was produced from coal and contained various potentially malodorous thiols which soon became known as a *warning signal* of a leak of highly flammable gas. This association is so strong that cocktails of similar thiols are now added to propane and butane to serve as warnings of leaks. The smell of fire seems to be a strong warning signal for all mammals and it is obvious why it should be so. As will be discussed later, the response of an animal to the odour of a predator is an example of a kairomone, an interspecies semiochemical benefitting the receiver of the signal.

Damage to the skin of one fish has been shown to release a mixture of odorants that trigger the fear reaction in other members of the shoal and therefore drives them to flee from potential predators (36). Madagascan mouse lemurs (*Microcebus murinus* and *M. ravelobensis*) have been shown to distinguish between odours of native predators and other animals and to avoid the former (37). Similarly, rats show innate fear reaction to predator urine but not herbivore urine (38). 3,4-Dehydro-2,4,5-trimethylthiazoline (**1.8**) (also known as 2,5-dihydro-2,4,5-trimethylthiazoline or TMT) is the component in fox urine that elicits the innate fear response of ‘freezing’ in rodents (39). It is detected by a number of receptors in the mouse OE, but only those in certain regions elicit the fear response (40). Deactivation of those receptors prevents the fear response in mice, but these ‘fearless’ mice can still be trained to recognise and respond to

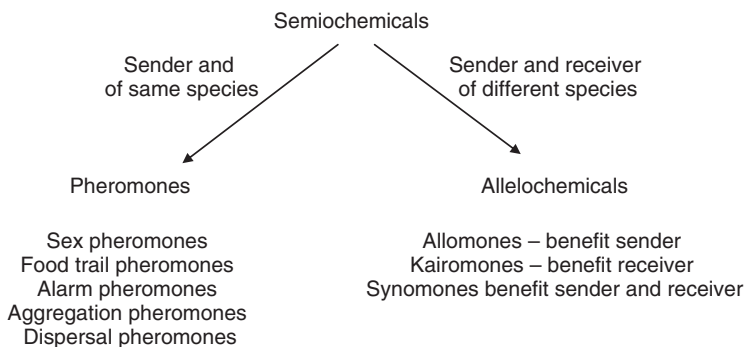
the odour of TMT. This suggests that signals from different regions of the OE of the mouse are processed differently by the brain. The crucial factor in this recognition and response to TMT is that of the pattern of glomerular innervation in the olfactory bulb, as demonstrated by the decreased avoidance behaviour when the targeting of axons is disrupted (41). It has been found that some other odours (even if previously unknown to the rodent) can also disrupt processing of the TMT signal in some (but not all) brain regions (14, 42).

One group of receptors that are involved in detection of nitrogen-containing molecules is the trace amine activated receptors or TAARs. The role of TAAR4 (which responds to TMT) in predator detection has been studied by Liberles et al. (43). They studied the response of TAAR4 to the urine of various species and found that it responded to that of the bobcat and the mountain lion but not to others (including human). The active component was identified as 2-phenylethylamine (**1.9**) which is known to activate a variety of olfactory sensory neurons (OSNs) in mice, both in the OE and vomeronasal organ (VNO). They established that this is present not only in the urine of bobcats and mountain lions but also of lions, jaguars and servals. They confirmed its absence from the urine of humans, cows, pigs, giraffes, moose, squirrels, rats, rabbits and horses. Using the technique of Fendt (44), they found that mice showed a fear response to lion urine and 2-phenylethylamine (**1.9**). When the lion urine was treated with mono-amine oxygenase, the fear response was reduced but not totally eliminated, which led to the conclusion that there are other components in the lion urine that also elicit the fear response in mice.

## CHEMICAL COMMUNICATION

Recognition of the intrinsic smells of food or danger is only part of the story as far as use of olfactory information by animals is concerned. Having developed a means of detecting odorant molecules, plants and animals then evolved the means of communicating with each other through the use of odour. Chemical communication can be used in sexual attraction and behaviour, in social organisation and in defence. When chemical communication is mentioned, the first word that springs to mind is usually 'pheromone'. However, pheromones are only part of the array of chemical messengers, and their exact role is a matter of debate in current scientific circles. Many apparently conflicting results from past experiments on chemical communication have been explained by later work, revealing the unexpected complexity of signalling systems. The chemical signals used by plants and animals are sometimes single chemical entities and sometimes mixtures, either of unrelated substances or of isomeric ratios. In some cases, the exact ratio of components in signal mixtures is crucial, and even relatively small differences from optimum result in failure of the signal to be recognised.

Chemicals used in communication between different organisms are known as *semiochemicals*. Semiochemicals can be used between different members of the same species or between members of different species. Sometimes they benefit



**Figure 1.2** Semiochemical definitions.

the sender of the signal, sometimes its receiver, and sometimes both. Figure 1.2 shows the terms commonly used to describe these various different types of semiochemicals.

The great debate that rages in the field of chemical communication is that of learnt versus innate response to chemical signals. The argument is most intense on the subject of pheromones. Evidence for innate stereotypical response to chemical signals is strongest in insects and other invertebrates. For example, genetic variation in one of the receptors (OR47a) of the fruit fly *D. melanogaster* directly affects the fly's response to the odour of ethyl hexanoate, which is an agonist of that receptor (45). Similarly, 'hard-wired' pheromone-induced behaviour can be found in the common shore crab *Carcinus maenas*, though the structure of the pheromone remains unknown. Male crabs will attempt to mate with stones that have been treated with odours taken from a female, showing that the behaviour is independent of context and input from other senses (46). There are few such clear examples of pheromone-induced behaviour in the case of mammals where learning and context would seem to be much more significant. However, the fact that mice that have been bred in captivity for generations and never exposed to a fox or any other predator will still show the fear response to TMT suggests an innate reaction to that odour.

Part of this discussion, though often not recognised as such, is the question of whether chemicals are produced purely for communication or whether they are produced for other reasons and then a learnt response results in their being adapted for communication by the receiver of the signal. In some cases the answer is obvious, in others it is not so clear, and indeed the real situation could be somewhere between the two. Co-evolution could also contribute to the development of a signalling system in which both sender and receiver adapt so that a chemical that was originally produced for another purpose or merely as a metabolic by-product becomes part of a signalling system. Examples (such as those described below) of a damaged plant 'summoning' help in the form of predators could be considered to be examples of allomones, but the history of how such interplay between species came about

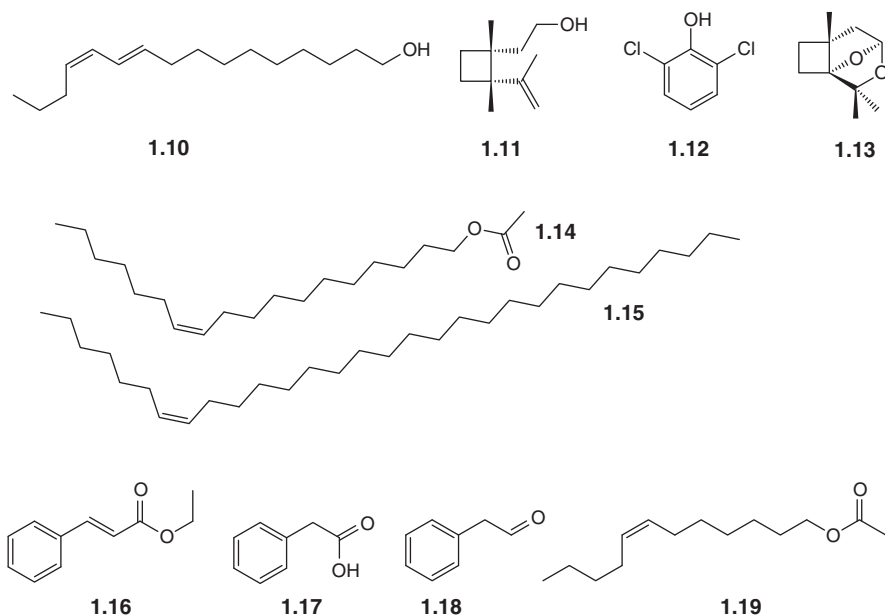
is more difficult to define. Bacterial metabolism produces amines and thiols from proteins and carboxylic acids from lipids. Thus, becoming ill after eating spoiled food would clearly lead to a learnt reaction to smells associated with bacterial contamination, the odour of butyric acid giving warning of sour milk for instance. Markers for good and bad food would therefore fall into the category of kairomones and are probably largely learnt. On the other hand, the trail pheromone laid by *Nasutitermes exitiosus* as described above is clearly an example of an intentional signal. The active component, neocembrene, is not found in the food source and is only produced by the termite when it has identified one. To determine whether the response to the signal is innate or learnt would require careful experimentation with naïve insects.

Karlson and Lüscher defined a pheromone as ‘a substance which is excreted to the outside by an individual and received by a second individual of the same species, in which it releases a specific reaction, for example, a definite behaviour or developmental process (47)’. Wilson and Bossert then suggested classifying pheromones into primer and releaser pheromones, primer pheromones producing neuroendocrine or developmental changes and releaser pheromones eliciting specific behaviour (48). Primer pheromones therefore would tend to fall back into the category of what were originally named *ectohormones* by Bethe. There is evidence that the smell of pups induces changes in the brain of female mice that would lead to the onset of maternal behaviour (49). Such an effect would seem more hormonal than the result of communication.

It is also important to distinguish between pheromones and signature scents. Pheromones are anonymous signals, for which the detector system is hard-wired and no learning is required, the response being innate. For variable signals such as signature scents, the composition is usually complex, pattern recognition is key to interpretation, there is no hard wiring and learning is required. A pheromone is either a single chemical entity or a simple mixture of defined composition and the response to it is innate, whereas signature scents are variable mixtures characteristic of an individual or colony (50). An account of pheromone-induced behaviour will be found in the book by Wyatt (51).

## Insect Pheromones

Examples of compounds that show pheromone activity in the strict sense (innate, stereotypical response with no learning having been involved) are found in insects. Perhaps the best known and most studied is bombykol (**1.10**), the sex attractant of the silkworm *Bombyx mori*. It is released by the female and is a powerful attractant for the male (52). Other sex attractants include grandisol (**1.11**), which is a sex attractant for the male boll weevil *Anthonomus grandis*, and 2,6-dichlorophenol (**1.12**), which is a sex attractant of the Lone Star Tick *Amblyomma americanum* and also a component of disinfectants such as Dettol and TCP. Lineatin (**1.13**) is the aggregation pheromone of the striped ambrosia beetle *Trypodendron lineatum*. This beetle attacks dead and felled Douglas fir trees and uses lineatin to summon others to a newly discovered food source (Figure 1.3).



**Figure 1.3** Some chemical signals used by insects.

11-(Z)-Vaccenyl acetate (**1.14**) is a pheromone that induces male–male aggression in *D. melanogaster* and is detected by the fly's olfactory system. However, another aggression-inducing pheromone, 7-(Z)-tricosane (**1.15**), is detected by their gustatory system. It was found that sensitivity to the latter was required for the former to be effective, but not vice versa, indicating a hierarchical regulation (53).

Insects often synthesise the pheromones themselves, but sometimes they obtain them from food. For example, males of the Oriental fruit moth, *Grapholita molesta*, acquire ethyl cinnamate (**1.16**) from the leaves they feed on whilst larvae, and later use it as a sex pheromone (54).

It would appear that insects process pheromone and food signals differently. After mating, male *Agrotis ipsilon* moths become less sensitive to the female sex pheromone and more sensitive to food-related odours, presumably to enable them to forage more efficiently (55).

However, it has also been found that male *D. melanogaster* flies increase their courtship behaviour when they detect phenylacetic acid (**1.17**) or phenylacetaldehyde (**1.18**), which are food signals. It is possible that this mechanism encourages the insects to breed on good food sources (56). The plasticity of response to pheromones by *D. melanogaster* is illustrated by the fact that males detect rivals by a combination of signals, including olfactory ones, and, on encountering a rival, they increase their mating activity in order to compete more effectively with the rival (57).

In many cases, two sets of signals are used together and achieve a synergistic effect; in other words, the combined signal gives a stronger response than would be expected if the signals were merely additive. An example of this is the combination of the aggregation pheromone of the American palm weevil *Rhynchophorus palmarum* with plant volatiles, which serve as kairomones (58).

(Z)-7-Dodecen-1-yl acetate (**1.19**) is a sex pheromone for several species of moths and butterflies and also plays a role in sexual communication in the Asian elephant. However, because insects and elephants use the same compound, this does not mean that they use it in the same way.

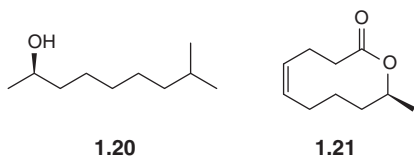
In social insects, the recognition odour of a colony is made up from contributions from every individual. For example, in bees, every member of the hive contributes to the comb odour, and it is this composite odour that is used for distinction between colony members and outsiders. This is a clever trick that allows genetic variation between individuals without destroying the social structure of the colony.

## Vertebrate Pheromones?

Even in non-mammalian vertebrates, the role of ‘pheromones’ becomes less clear than in insects. In journal publications, the term *pheromone* is often used, particularly in the titles of papers, even when the role of the odorant in question is not understood, and therefore caution should always be exercised when reading.

Male budgerigars, *Melopsittacus undulatus*, produce higher levels of octadecanol, nonadecanol and eicosanol in their uropygial glands than do females, and females are attracted to a mixture of these three alkanols when they are present in the right proportions (59). In another example of avian chemical communication, petrels (*Halobaena caerulea* and *H. desolata*) uropygial gland secretions contain range of fatty acid derivatives (including relative alcohols and esters) and their variation is such that they can be used by the birds to determine species, sex and identity of different birds (60). Similarly, the femoral gland secretions of male Spanish rock lizards, *Iberolacerta cyreni*, contain steroids and lipids and females are more attracted to males with high oleic acid content (61). However, this does not necessarily mean that the volatiles produced by males in either example constitute pheromones in the strict sense as defined by Karlson and Lüscher. The odours could merely be signatures that are recognised by the females whose response to them is learnt.

Amphibians such as frogs and newts have four noses, as opposed to the two of most other vertebrates. On each side, they have a ‘wet’ nose and a ‘dry’ nose. The former is used when submerged and the latter when breathing air. They can therefore use water-soluble chemicals such as proteins for communication under water (62) and volatile chemicals for communication through the air. An example of such volatile chemicals is the mixture of (*R*)-8-methyl-2-nonanol (**1.20**) and (*S*)-phoracantholide (**1.21**) produced by males of the Madagascan frog *Mantidactylus multiplicatus*, though their role in communication is not known at present (Figure 1.4) (63).



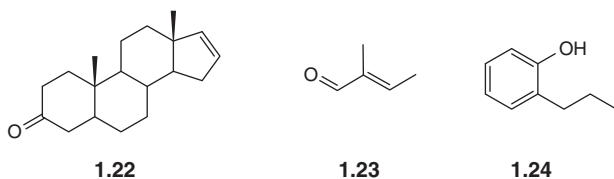
**Figure 1.4** Semiochemicals of *Mantidactylus multiplicatus*.

## Mammalian Pheromones?

In his rigorous analysis of the most significant studies claiming to have identified mammalian pheromones, Dick Doty proposes that mammalian pheromones do not exist (64). In many of the cases concerned, the real situation is complex and many different factors contribute to the behaviour. In other examples, the actual effect is not clear, or control experiments were found to give similar results to those claiming pheromone activity. In some cases, such as the alleged induction of menstrual synchrony in women living closely together (e.g. in hostels), the results are judged by some to be more likely to be a result of aberrations/flaws/omissions in experimental design or in statistical treatment of results. In many instances, the ‘pheromone’ might simply be a signature scent and the response to it a learnt one, analogous to the response of Pavlov’s dogs to the sound of a bell.

A couple of examples of the best known alleged pheromones will serve to illustrate Doty’s thesis.

Perhaps the best known of all is the effect of androstenone (**1.22**) on sows, reported by Melrose et al. in 1971 (65). This steroid is produced by boars and is found in their saliva. When they chomp their jaws, an aerosol containing androstenone is released into the air. The scent of androstenone either from boars or produced synthetically and administered to a sow as an aerosol will cause it to adopt the mating stance (lordosis). So, at first sight, there appears to be evidence for a pheromone effect. However, it is only effective for sexually experienced sows and gives a variable response even within the positive group. Androstenone is not necessary for induction of lordosis, and the sound of the boar grunting can also have the same effect. The activity therefore would seem to be a conditioned response in which the odour cue is learnt and is reinforced by context. This in turn raises the question of whether the androstenone is synthesised for chemical communication at all or whether it is simply a by-product of steroid metabolism that happens to be used in this way (Figure 1.5).



**Figure 1.5** Some chemicals resulting in behavioural responses in mammals.

Response to the rabbit nipple search 'pheromone' was originally thought to be innate because it is displayed by newly born rabbit kits (66). However, the candidate 'pheromone', 2-methyl-2-butenal, (**1.23**) (67) is present in the amniotic fluid and it is now known that mammalian embryos do learn to recognise odours *in utero* and even birds can learn odours *in ovo* and the learning does affect behaviour in later life (68). It is therefore likely that the kits have learnt the odour *in utero* and seek it because of familiarity (69).

As mentioned earlier, (Z)-7-dodecen-1-yl acetate (**1.19**) is a pheromone for over 120 insect species, mostly from the order *Lepidoptera*, and was also found to be used as a sex attractant by female Indian elephants (*Elephas maximus*) who produce it in their urine (70). Male Indian elephants living in the absence of conspecifics in American zoos responded to (Z)-7-dodecen-1-yl acetate (**1.19**), thus giving further credence to the idea that it is a pheromone. However, the degree of response was lower than that to intact urine and a control substance, *o*-propylphenol (**1.24**), elicited the same response. When tested on working elephants in Burma (hence elephants living in close proximity to others), the responses of dominant and subordinate males were different, showing that the response is context-dependent and therefore not a pheromone in the sense defined by Karlson and Lüscher.

In humans, the areas of the body, other than the head, where hair growth is greatest are the armpits and groin regions. The role of hair there is to prevent chafing as the limbs move relative to the torso. The hair is lubricated by secretions containing water, fats and various other chemicals. This provides an ideal location for bacterial action and, consequently, the formation of volatile metabolites. The production of these body odours could therefore be entirely coincidental but their formation does give rise to signature combinations of odorants that can be used for identification and communication.

There are many reports of human beings able to recognise the signature odours of other humans, for example, by the ability to pick out from a variety of T-shirts those that were worn by themselves, those that were worn by a close friend and those that were worn by a stranger. It has also been reported that humans tend to prefer T-shirts that have been worn by people with the most different major histocompatibility complex (MHC) (71). This tends to suggest the role of sweat as a pheromone. Differences in body odour generation and in olfactory sensitivity between the sexes could possibly be used in mate selection and sexual behaviour. Consequently, there has been much research and even more speculation on the subject. Differences in olfactory acuity between the sexes has been studied extensively, and the results are often contradictory. However, more researchers find that women outperform men than vice versa. This could be due to the effect of hormones, or it could be related to many other parameters such as social conditioning. In an attempt to better understand the phenomenon, Doty and Cameron carried out an extensive review of the subject. They concluded that there is no simple relationship between reproductive hormones and olfactory capability and that the interplay of the two is very complex (72).

Most social signals in higher animals are mixtures rather than single chemicals as can be found in insects. Human sweat, for example, contains hundreds, if

not thousands, of individual chemical components and it is the different proportions of these components that allow us to recognise different individuals or to pick our own T-shirt out of a selection of otherwise identical ones that have each been worn by a different person. Fresh human sweat is odourless, and it is bacterial action that produces the characteristic smell. Of course, the exact composition of the complex mixture of odorants that results is the result of both the nature of the human metabolic substrate and the blend of flora on that individual's skin (73, 74). For example, most humans show distinct patterns of composition of axillary sweat components and these can be distinguished by smell, whereas those of monozygotic twins are very similar and not readily distinguishable (75). Furthermore, humans have also been shown to be capable of distinguishing between the body odours of different Western lowland gorillas (*Gorilla gorilla gorilla*) (76). Humans can also distinguish between male and female mouse urine because of differences in the volatile components.

These findings are clear evidence that the human sense of smell is better than Freud would have had us believe. However, as will be described in the next chapter, humans lack the physical organs and brain structures that are involved in detection of putative pheromones in other mammals. Taking this and all of the above into account, it would seem more likely that human odours are signature odours and social markers with a learnt response rather than pheromones in the sense of Karlson and Lüscher.

Of course, the complexity of mammalian odours allows for almost infinite variation from species to species and individual to individual. The exact balance between odorous materials produced directly by the mammal and those produced by microbial action on mammalian substrates enables the resultant signature odour to be used for such purposes as recognition of conspecifics, members of the same or of different social groups, recognition of individuals and determination of sex, reproductive status and social hierarchy. A simple example is the well-known recognition of its own lamb by a mother sheep. After lambing, a ewe's hormones cause it to lick her lamb and, in doing so, she learns the odour of the lamb. Of course, as with most such effects, the odour cue is supported by input from other senses. In this case, it would be learning the visual appearance of the lamb. Female sea lions can also identify their own pups by their smell (77).

As will be further discussed in the next chapter, rodents have four different systems for detection of environmental chemicals. Their VNO contains receptors known as *vomeranasa* receptors, falling into the VR1 and VR2 subtypes. Their ORs are found in the OE. The VR1 receptors are highly sensitive and selective, and the VR2 receptors are highly specific, whereas the ORs are broadly tuned. This makes the VN receptors much more suitable for pheromone detection. Pheromone signals and odours are interpreted in different parts of the brain in hamsters and mice, as are signals from conspecific and heterospecific animals.

An important contribution to the mammalian pheromone debate came from the team of Hurst at Liverpool University. Previous work on murine sex pheromones had given confusing results and left the question of whether such chemicals existed.