Structure-odor relations: a modern perspective

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I. Introduction

This review is intended as an introduction for non-specialists to structure-odor relations (SOR), and as a critique of the field rather than a compendium. The perspective will be that of biology rather than fragrance chemistry. In other words, we are more interested in what SORs tell us about the mechanisms of human olfaction than about the synthetic chemistry of odorants. We believe that the recent advances (see Mombaerts 1999a for review) that followed Buck and Axel's 1991 discovery of odorant receptors will some day make odorant design a rational process . In the meantime, we want to highlight a few salient findings which we feel a successful SOR theory must account for, in the hope that this will help researchers design experiments to elucidate the mystery of primary olfactory reception.

A perennial difficulty of structure-odor relations has been that both structure and odor have proved hard to pin down. Considered as a structure-activity problem, olfaction is several orders of magnitude more complicated than its conventional pharmacological counterparts because there are many more structures and a vast number of odors. There is also an additional problem: as a sensation, olfaction does not seem to enjoy the same status as, say, vision. Most biologists, indeed most people not directly involved with fragrances or flavors seem to think that odor sensation is "subjective" and not necessarily shared by others. It is striking how few experiments in which odorants are applied to biological preparations take into account the perceived odor of the molecules. We hope that biologists will realize that, once a vocabulary is agreed upon, odor is as reliable a sensation as pitch or color.

II .The current state of SORs

Chemists have, by design and by accident, been producing odorants since the dawn of organic chemistry 200 years ago, and a vast database of odorants and their corresponding odor profiles has built up. This seems a good place to state what is perhaps the most surprising fact of SORs: *no two odorants have ever been found to have*

exactly the same odor. Despite figures often mentioned in the literature of "a few thousand", so far as we know, the resolution of the human olfactory system is infinite.

The field of fragrance synthesis, though still small in comparison to, say, pharmaceuticals, is a 8-billion dollar industry dominated by a few large firms: in alphabetical order Dragoco (D), Firmenich (CH), Givaudan-Roure (CH), Haarman & Reimer (D), International Flavors and Fragrances (US), Quest (UK) and Takasago (JN). Each of these firms has a library of tens of thousands of odorants. Understandably, most of this database is proprietary and not available to the scientific community.

Nevertheless, many hundreds have been described in the literature and their SORs have been extensively reviewed, most recently by Rossiter (1996). Most reviews of SORs are collections of disparate facts with no unifying theme save a basic postulate: odor must be related to molecular structure. The search for a predictive theory based on this assumption has been frustrating: Bedoukian (1966) stated that "it is not possible to predict the odor of a substance with any degree of accuracy. McCartney (1968) felt that "the difficulties in the way of uncovering the connection [between structure and odor] have been very great". Hornstein and Teranishi (1967) considered the results of such searches "disappointing". More recently, Frater, Bajgrowicz and Kraft (1998) described the state of SORs as "sorry". Indeed Sell (1999) has recently suggested that there may be no connection at all between structure and odor, and that the wiring from receptor to brain may be arbitrary. The reader interested in getting a feel for the fascinating regularities and irregularities of the structure-odor map is referred to the excellent review by Boelens (1974) and the monograph by Ohloff (1991)

Attempts have been made to accommodate discrepant structure-odor relations by a process known as conformational analysis (Yoshii, Hirono and Moriguchi (1994); This involves exploring the space of conformations adopted by the odorant molecule when deformed away from its energy minimum. The fraction of configuration space allowed depends on the energy arbitrarily assigned to molecular motions. The value of conformational analysis is unclear since it is usually a directed process in which the molecule is bent purposely to resemble another odorant. An example of this is given a by the study of linear musk citronellyl oxalate(Yoshii, Hirono and Moriguchi 1994), whose lowest-energy conformer resembles a macrocyclic musk. At room temperature, however, the linear musk must also also explore a vast range of conformations which resemble dozens of other odorants.

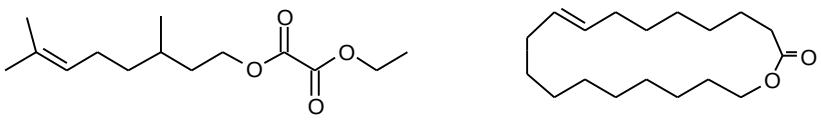
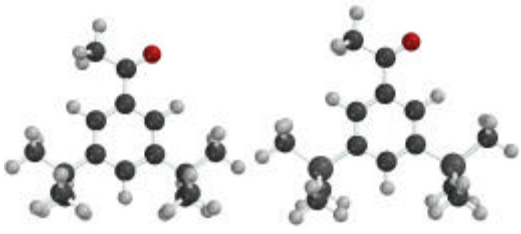


Figure 1 left: Ethyl citronellyl oxalate, a molecule possessing a macrocyclic musk odor but linear in shape. Right: a macrocyclic musk, cyclopentadecanolide. Shape-based theories assume that the linear musk assumes a conformation close to that of the macrocyclic when binding to the receptor, hence the similarity in odor.

The complexity of structure-odor relations, and the fact that the three-dimensional structure of the receptor site is unknown, make it very difficult to apply conventional quantitative structure activity relationships. QSARs have proved very useful in many areas of pharmacology (Balbes et al, 1994, Dearden and James, 1998) . They work best when the structure of the site to which the molecule binds is known exactly from crystallographic measurements. Then the full force of computational chemistry can be brought to bear on designing molecules. Some studies have attempted to calculate both the three dimensional structure of the receptor and its interaction with odorants (Singer, 2000, Floriano et al, 2000). These studies will undoubtedly become increasingly useful as our knowledge of receptor structure increases and modeling techniques become more realistic. In the meantime, most of the work proceeds by examining the structures of the odorants alone. It is not clear how many odorants have been designed using QSAR alone, or even as a principal tool to guide synthesis. Fragrance companies are reluctant to discuss the subject. Perhaps the best indication of this is that new odorant synthesis in the firms still proceeds by trial and error. It is our impression that QSAR has strong competition, particularly from combinatorial chemistry techniques that now make it easier to synthesize large numbers of molecules.

III. What makes an odorant?

The general requirements for an odorant are that it should be volatile, hydrophobic and have a molecular weight less than approximately 300 daltons. Ohloff (1994) has stated that the largest known odorant is a labdane with a molecular weight of 296 . The first two requirements make physical sense, for the molecule has to reach the nose[1] and may need to cross membranes. The size requirement appears to be a biological constraint. To be sure, vapor pressure (volatility) falls rapidly with molecular size, but that cannot be the reason why larger molecules have no smell, since some of the strongest odorants (e.g. some steroids) are large molecules. In addition, the cut-off is very sharp indeed: for example, substitution of the slightly larger silicon atom for a carbon in a benzenoid musk causes it to become odorless (Wrobel and Wannagat, 1982d).



[1] Note,, that some hydrophobic compounds of low volatility can reach the nose from the bloodstream. The garlicky smell of IV thiopental is perceived by anesthesia subjects seconds before they lose consciousness.

Figure 2 Comparison of molecular size between a benzenoid musk (left) derived from acetophenone and its sila counterpart (right) in which the central carbon atom in the t-butyl groups has been replaced with Si. The carbon musk is a strong odorant, the sila musk odorless.

A further indication that the size limit has something to do with the chemoreception mechanism comes from the fact that specific anosmias become more frequent as molecular size increases. At the "ragged edge" of the size limit, subjects become anosmic to large numbers of molecules. An informal poll among perfumers, for example has elicited the fact that most of them are completely anosmic to one or more musks (e.g. Galaxolide® mw 244.38) or, less commonly, ambergris odorants such as Ambrox®, or the larger esters of salicylic acid.

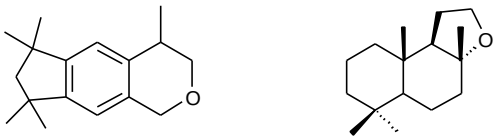


Figure 3 Two molecules which are occasionally odorless to humans, galaxolide (mw 244.38) and Ambrox (mw 236.40)

One can probably infer from this that the receptors cannot accommodate molecules larger than a certain size, and that this size is genetically determined Whissel-Buechy and Amoore (1973) and varies from individual to individual.

III.A Odor descriptors and odor profiles

Odor descriptors are the words that come to mind when smelling a substance. The more generally understood the words are, the more useful they are as descriptors. An untrained observer may for example use "Grandma's linen cupboard" as an accurate descriptor, whereas the professional would be more analytical and say woody (the cupboard) musky (the linen) camphoraceous (the mothballs). Note that these descriptors may be applied to a *single*, pure odorant. Nevertheless, odor description always works by analogy since there is no objective alternative. Odor description seems to have acquired the reputation of being arcane, even fanciful, perhaps in part as a result of the hoopla surrounding fine wines and fragrances.

In practice, it is easy for any observer, after a little training, to use the standard descriptors of fragrance chemistry. Accordingly, almost all the examples in this review are chosen among those commercially available, and we urge the interested reader to obtain some of them and check the odor. Anosmias aside, outright disagreements between observers are, in our experience, rare. One exception is Karanal® (mol) an ambergris odorant which is perceived as animalic by some observers (Charles Sell, personal communication). Another is trans-2-hexenal, perceived as green by some (Arctander 1991) and bitter almond by others (Ohloff,1994).

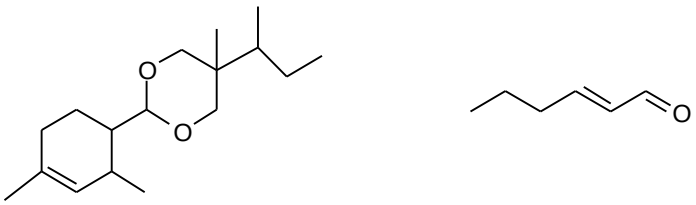


Figure 4 Two molecules whose odor appears to differ between observers. Karanal (left) is a woody-amber to most observers, but smells unpleasantly urinous to some. Trans-2-hexenal (right) is described in the literature either as a green (Arctander 1994) or bitter almonds (Ohloff 1994) odorant. To the authors, it smells of bitter almonds.

The much more common and oft-quoted cases of perceptual disagreements, e.g. phenylacetic acid, are probably due to ambiguity, not difference. Phenylacetic acid smells *both* of honey and of fresh urine. When asked to use either descriptor, subjects will opt for one or the other without hesitation. When asked whether the other descriptor might also apply, however, they will always agree that there is a honey or urine "side" to the smell. This is not so strange when one considers a color analogy. Ask a group whether an appropriate shade of turquoise is blue or green, and you may get half giving each answer. This does not mean they perceive it differently.

The reader wishing to become familiar with odorants and their descriptors can peruse Aldrich's Flavors and Fragrances catalog in which odorants are listed by chemical type and by principal descriptor. Kits of esters and heterocycles are also available from the same firm, which provide an excellent introduction to the raw data of SORs, i.e. structure and odor. It is unfortunate that the vast majority of commercial odorants are not represented in catalogs of chemical suppliers familiar to the biologist. Nevertheless, fragrance firms will on request provide researchers with samples. For those wishing to delve deeper into the subject, Arctander's handbook (Arctander 1994) lists thousands of molecules and their odor profiles, and represents a mine of reliable and largely untapped information on SORs. Unfortunately, the chemical structure drawings in Arctander are antiquated and often unclear, and the book contains no descriptor index. In addition, two companies (Leffingwell and Boelens) offer independent information on fragrances and flavors at www.leffingwell.com and www.xs4all.nl/~bacis.

III.B Some odor categories and their representative molecules, chosen to illustrate structural diversity

Musk: Musk is perhaps the most famous of all odor categories, because of its universal inclusion in fragrance and its exotic origin in the secretions of the musk deer. In fact, because of expense and legislation, musks have been synthetic for a long time. Musk odor descriptors might be "smooth clean, sweet and powdery". The molecules that possess this odor character are exceptionally diverse in structure. Macrocyclic musks contain a 15-18 carbon cycle closed either by a carbonyl or by a lactone and smell similar but fresher and more natural, often with fruity overtones (cyclopentadecanolide, ambrettolide). Nitro musks, discovered originally as a byproduct of explosives chemistry, smell sweeter and are reminiscent of old-fashioned barbershop smells.

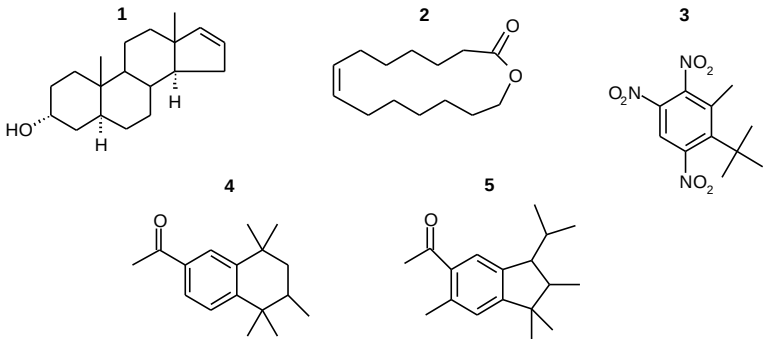


Figure 5: Representatives from five chemical classes which yield musk odors. 1 androst-16-en-3α-ol, a steroid musk. 2: ambrettolide, a macrocyclic musk. 3: Musk Bauer, a nitro musk. 4: Tonalid, a tetralin musk. 4: Traseolide, a indane musk.

Ambergris: Originally derived from concretions spat out by whales and aged in the sun, ambergris odorants smell nothing like natural ambergris tincture, which has a weak animalic marine smell. The smell of ambergris odorants was once aptly described to us by a chemist-perfumer as "glorified isopropanol". Ambergris odorants are of interest to the student of SORs because they provide an interesting combination of very closely related smells with widely different structures: amberketal, timberol, karanal and cedramber are close enough that a perfumer will occasionally mistake them for each other.

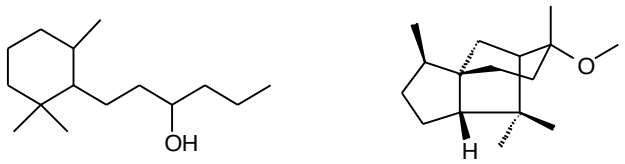


Figure 6 Two ambergris odorants, timberol (left) and cedramber (right)

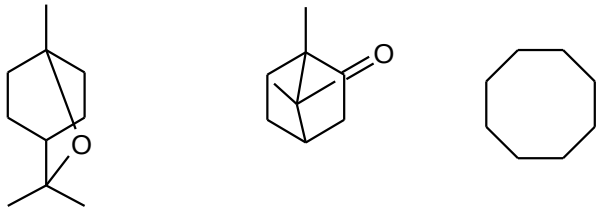


Figure 7 Three camphoraceous odorants: L to R 1,8 cineole, camphor and cyclooctane

Camphoraceous: Camphoraceous (mothball) notes are seldom used in perfumery, but they are of interest of SORs because they formed the basis for one of the early attempts at smell classification by Amoore (1971). Camphor, cyclooctane, cineole are good examples of camphoraceous smells, and smell rather similar to each other.

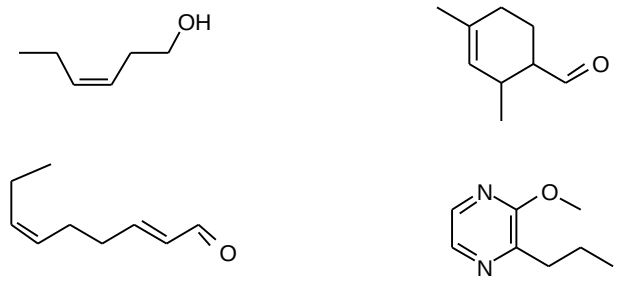


Figure 8 Some examples of green odorants. Clockwise from top left cis-3-hexenol, ligustral, nonadienal and ethylmethoxypyrazine.

Green: Cut grass, fresh green bean notes with a sharp, almost aggressive feel. Diverse compounds possess this descriptor, ranging from classic grassy notes of cis-3-hexenol and ligustral, to the cucumber peel of nonadienal and the bell-pepper green note of some pyrazines.

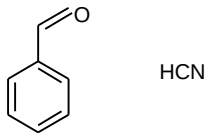


Figure 9. Two bitter almond odorants, benzaldehyde and hydrogen cyanide

Bitter almonds: This easily-recognized category is interesting to students of SORs because it includes a small molecule (HCN) which, however, smells metallic not almond-like to a large fraction of observers (reference). Benzaldehyde, nitrobenzene, trans-2-hexenal (but see above) are good examples.

None of the above: Many other categories such as musty, spicy, aldehydic, lactonic, indolic, marine, etc exist, each with subdivisions. It must be emphasized that the odor categories above are merely convenient descriptors and only cover a very small fraction of odor "space". In fact, particularly when one steps out of perfumery materials proper into smells noticed by chemists in the course of organic and inorganic syntheses the most frequent descriptor appears to be *sui generis,* i.e. a smell associated with nothing in particular.

IV. Plausible theories of odor

Many theories of SORs have been proposed in the past (reviewed in Moncrieff, 1951) but advances in biological understanding, not least the discovery of odorant receptors, have gradually ruled them out. Leaving aside the pessimistic view outlined above, according to which there may be no relationship between structure and odor, there appear to be two possible types of SOR theory left standing. One is based on fragments of molecular shape or **odotopes** (Mori and Shepherd 1994), the other on **molecular vibrations** (Turin, 1996).

IV.A Shape-based theories: Odotopes

Most enzyme-substrate and receptor-ligand binding relies on molecular recognition between protein and ligand. Recognition depends on interactions that can be either attractive or repulsive (Davies and Timms 1998). All attractive chemical interactions are ultimately electrostatic in nature whether they occur between fixed charges, dipoles, induced dipoles or atoms able to form weak electron bonds (e.g. hydrogen bonds). Repulsive interactions can be electrostatic or quantum-mechanical (electron shell exchange repulsion). Almost every change in molecular structure (with some exceptions which will described below) alters the set of surface features capable of forming such attractive or repulsive interactions, and thus affects what we loosely call molecular shape.

The range of known molecular recognition mechanisms in biology is vast. At one extreme might be a vast set of immune-type receptors, each able to bind to a single odorant molecule. At the other end of the spectrum, some binding sites such as those of odorant-binding proteins (Bianchet et al. 1996), albumins (Curry et al. 1999) and cytochromes P_{450} (Lawton and Philpot 1993) are rather non-specific. When odorant receptors were first identified, their large number was taken by some as evidence for immune-like recognition. However, in vivo and, more recently, in vitro studies have shown (refs) that, with one notable exception (ref), receptors respond to more than one odorant, suggesting that they detect the presence not of the whole molecule but of a partial structural feature thereof, hence odotopes.

According to odotope theory the smell of a molecule is then due to the pattern, i.e. the relative excitation of a number N of receptors to which it binds. Even if one assumes that receptors are only on or off, this scheme gives considerable combinatorial room. Consider for instance a molecule having twenty exposed atoms and assume that each odotope involve three of these. A binary (on-off) one-odotope recognition system would then be able to detect 1140 molecules. If odotopes involved four atoms, the number would rise to 4850, etc. Combining odotopes, and adding to this basic scheme a measure of intensity of excitation for each receptor clearly enables it to detect a vast number of odorants. If the large number of odorant receptors is taken to represent odotope categories the combinatorial possibilities become astronomical.

A more sophisticated argument has been made by Lancet et al. (1993). They make plausible assumptions about the number of “subsites” (odotopes) and their variability, combined with calculations of the energetics of binding. Assuming a very

low affinity of 10^{5} M^{-1} for odorant binding, they arrive at the conclusion that in order to recognition 300-1000 receptors are needed, in line with current estimates of receptor number (see section VI for further discussion of this point). Higher affinities, more consistent with olfactory thresholds, lead to greater still receptor numbers.

IV.B Vibration theories

The idea that the nose operates as a vibrational spectroscope was first proposed by Dyson (1938) and later taken up and refined by Wright (1982). What makes it attractive in principle is that vibrational spectra share three properties with human olfaction. 1) No two molecular spectra are exactly alike, particularly in the aptly named "fingerprint region". 2) Many functional groups are easily identified by their specific vibrational frequencies (and by smell, see below). 3) A system utilizing a physical property as basic as vibration will be ready for never-before-smelt molecules, i.e. does not depend on a repertory of existing or expected structures. In that sense, it does not rely on molecular *re*cognition.

Several difficulties beset vibration theories and ultimately caused their demise twenty years ago. 1) Enantiomers, which have identical vibrational spectra in solution, *sometimes* have different odors see Boelens and van Gemert, (1993). Wright countered this by emphasizing that while laboratory spectroscopes were achiral, and thus unable to distinguish between enantiomers, a protein receptor would be intrinsically chiral, and would thus respond differently to enantiomers. A modified version of this argument is described below in section **V.D** 2) No mechanism was ever found for a plausible protein-based spectroscope, infrared optics being obviously out of the question. 3) Wright assumed that receptors were mechanical vibration sensors, and that the receptors in the nose would only be able to feel vibrations excited by thermal motions at body temperature. He then restricted his search for correlations between structure and odor to the region below 600 cm-1. These were somewhat unconvincing, and appeared to have little predictive value.

The situation changed a few years ago with the proposal that electron tunnelling might be a plausible mechanism enabling proteins to act as vibrational spectroscope.

IV.C A biological "spectroscope"

Inelastic electron tunnelling spectroscopy (IETS) is a non-optical form of vibrational spectroscopy (Jaklevic and Lambe, 1966; Hansma, 1982; Adkins and Phillips, 1985). It relies on the interaction between electrons tunnelling across a narrow gap between metallic electrodes. When the gap is empty, tunnelling electrons cross the gap at constant energy and the tunnelling current is proportional to the overlap between filled and empty electronic states in the metals. If a molecule is present in the gap, tunnelling electrons will be scattered by the partial charges on the molecule's constituent atoms, and lose energy to the molecule by exciting one of its vibrational modes. When this happens, electrons can follow an indirect path, first exciting the molecular vibration and then tunnelling to the second metal at a lower energy. The new tunnelling path causes an increase in the conductance of the junction.

Metallic conductors are absent in biology, but electron transfer is ubiquitous. Doing IETS with proteins (figure 1) would involve addition and removal of electrons at well-defined energy levels on either side of an odorant-sized (< 300 daltons) binding site which serves as the tunnelling gap. On one side of the gap, a donor site with occupied donor levels is present, while an acceptor site with empty acceptor levels is on the other side of the tunnelling gap. If there is nothing between the electron source (donor) and sink (acceptor), then for direct tunnelling to occur there must be an (occupied) energy level in the source which matches the energy of an (empty) state in the sink.

If there is a molecule between the electron source and electron sink, and if that molecule vibrates then indirect tunnelling can only if there is an energy level in the source with energy E above that in the sink. In other words, tunnelling occurs only when a molecular vibrational energy E matches the energy difference between the energy level of the donor and the energy level of the acceptor. The receptor then operates as a spectrometer which allows it to detect a single well-defined energy, E . If there are several vibrational modes, which one(s) get excited will depend on the relative strengths of the coupling. That may be expected to depend, among other things on the partial charges on the atoms and the relative orientation of the charge movements with respect to the electron tunnelling path.

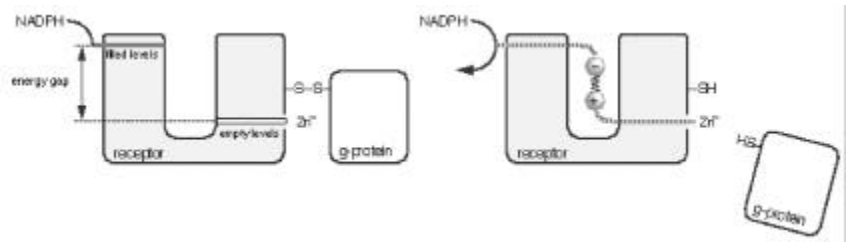


Figure 10 Schematic of the proposed transduction mechanism: the receptor protein accepts electrons from a soluble electron donor (NADPH). When the receptor binding site is empty (top) , electrons are unable to tunnel across the binding site because no empty levels are available at the appropriate energy. The disulfide bridge between the receptor and its associated G-protein remains in the oxidised state. When an odorant (here represented as an elastic dipole) occupies the binding site (bottom), electrons can lose energy during tunnelling by exciting its vibrational mode. This only happens if the energy of the vibrational mode equals the energy gap between the filled and empty levels. Electrons then flow through the protein and reduce the disulfide bridge via a zinc ion, thus releasing the G-protein for further transduction steps.

Unlike conventional IETS, “biological IETS” does not involve scanning of the energy range, which would probably be unfeasible in a biological system. Instead, the range of vibrational energies is covered piecewise by a series of receptors tuned to different energies. The energy range is limited only by the emf (reducing power) of the electron source. An estimate of biological reducing power is 500 mV (1eV = 8086 cm-1) (Frausto da Silva & Williams, 1993), which means that the entire vibrational range to 4000 cm-1 could be sampled. To cover the vibrational spectrum, several receptor classes

would be required, each tuned to a different segment of the vibrational spectrum. A small number might be sufficient, much as three pigments with broad, partially overlapping absorption spectra suffice for color vision. One essential feature of the biological spectrometer is its relatively poor resolution. A biological system must work at ambient or body temperature, i.e. around 300°K. Donor and acceptor levels across the tunnelling gap will therefore have a minimum width of 2kT (˜ 400 cm-1). The range 0 to 4000 cm-1 could thus be covered by 10 or so receptor types. A similar arrangement exists in the other spectral senses, vision and hearing, in which broadly tuned receptors classes cover segments of the complete spectrum.

V Odotopes vs. Vibrations: how they fit the facts

In a field as vast and amorphous as that of SORs, observations can be found to lend support to almost any theory. In what follows, we shall therefore try to stick to observations that are potentially able to *disprove* one or the other of the two contenders.

V.B Smelling chemical groups

A fact that has, in our opinion, received too little attention from olfaction researchers is the ability of humans to detect the presence of functional groups with great reliability (see Klopping 1971 for review). The case of thiols (-SH) is familiar, but other chemical groups such as nitriles (-CN), isonitriles (-NC) oximes (-NOH), nitro groups (NO2), aldehydes (C=O(H)), can be reliably identified once the odor character the functional group character confers is known. When nitriles are used as chemically stable replacement for aldehydes, they impart a metallic character to any smell: cumin nitrile smells like metallic cumin (cuminaldehyde), citronellyl nitrile smells like metallic lemongrass (citronellal), and nonadienylnitrile smells like metallic cucumber (nonadienal). Oximes give a green-camphoraceous character, isonitriles a flat metallic character of great power and unpleasantness, nitro groups a sweet-ethereal character, etc. Remarkably, even *bonds* between atoms can be detected: the acetylenic C-C triple bond of –ynes imparts a isothiocyanate-like mustard-like smell to molecules which is clearly recognizable, for example in acetylene and in methyloctynoate.

V.B.1 Functional groups as odotopes

An odotope theory can explain these regularities only by assuming that the functional group is an odotope. In the older structure-odor literature, this used to be described as electronic factors (as opposed to steric). The idea was that, given that many functional groups were similar in size, the recognition mechanism must somehow be sensitive to the fine structure of the electron distribution (orbital energies, charge density, etc) of the functional group. This seemingly reasonable notion runs into problems on closer examination.

Consider for instance the SH group in, say methanethiol. Alcohols never smell of sulfur, whereas thiols always do. What could make the SH infallibly distinctive as an odotope, as compared to the OH group? Partial charge, bond length, bond angle and atom size are somewhat different between R–SH and R–OH, but it is hard to see how these can be detected with *absolute* reliability by, say, an aminoacid side chain in the

presence of thermal motion. A more distinctive property of sulfur lies in the energy of its lone pair orbitals, as witnessed by the specificity with which it forms complexes with certain metals. If a metal is involved, then it becomes hard to explain that a) dimethyl sulfide has no thiol character and b) that a thioether (-S-) link can often replace a -C=C- with very little change in smell, and no sulfuraceous character (Boelens and van Gemert 1993b)?

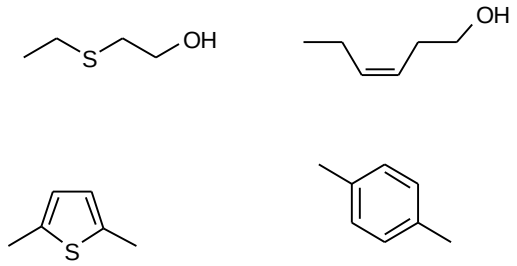


Figure 11 Replacing a C=C bond with a sulfur atom does not change odor character, suggesting that "electronic" properties of sulfur are not sufficient for molecular recognition.

The same problem applies to other smellable functional groups, and can be stated more generally: if functional groups are odotopes, then they are so small as to only be able to form one or two interactions, e.g. hydrogen bonds, etc. with odotope receptors. Their small size will similarly restrict the number of repulsive interactions. Therefore small molecules should bind with various degrees of affinity to many odotope receptors, and small molecules should have similar odors, particularly at high concentrations (Klopping 1971). That is not the case: small molecules like methylnitrile and methylnitrate smell distinctively different at all concentrations. Indeed smaller ones still like ozone, sulfur hexafluoride, carbon disulfide also have this property.

V.B.2 Functional groups and vibrational theory

By contrast, the distinctive smell of functional groups is a natural feature of a vibrational theory. Above 1800 wavenumbers, IR absorption lines are diagnostic of the stretch frequencies of diatomic functional groups. The aldehyde-nitrile replacement rule can be understood from the closeness of their stretch vibration. Similarly, the similarity in smell between acetylenic bonds and isothiocyanates can be explained by their respective stretch frequencies.

The clearest example so far is that of boranes. The terminal B-H bond in boranes has a stretch frequency whose range overlaps with that of thiols. Turin (1996) therefore predicted that boranes should smell sulfuraceous, despite the complete absence of similarity, both structurally and chemically, between boron and sulfur. A comparison between borane and thiol smells is best made using decaborane[2]. Decaborane smells

[2] This experiment requires caution: though stable at room temperature in air, decaborane is reported to be highly toxic, and has a high vapor pressure. It is therefore best to open the container in a fume cupboard, close it again after a few minutes and smell the very small amount of decaborane condensed on the outside of the cap. One of us (LT) has been doing this periodically for some time with no apparent ill effects.

strongly of boiled onion, a typical SH smell. Curiously, its smell was described as "chocolate-like" (chocolate contains some thiols) in papers reporting its synthesis, which may account for the fact that the similarity was not noticed earlier. Other, less stable boranes share this sulfuraceous smell character.

Figure 12 The dependence of the sulfuraceous character on molecular vibrations and atomic partial charges, as predicted by a vibrational theory. Decaborane (left) smells sulfuraceous, and its terminal B-H bonds have a stretch frequency ~ 2500 wavenumbers. In triethylamine-borane (middle), the B-H stretch is shifed to 2300 wavenumbers and the sulfuraceous smell is no longer present. In p-carborane (right) the near-neutral partial charges make the SH bond odorless.

There are three possible non-vibrational interpretations of this finding: 1) Boranes do *not* in fact smell of sulfur 2) the similarity in smell between B-H and S-H, while real, is pure coincidence 3) BH and SH activate the same odotope receptor by some unknown mechanism, despite the difference in shape. In answer to objection 1, we advise the interested reader not to take the authors' word and to smell decaborane observing due precautions. Objection 2 is harder to answer, because the odds against such a thing happening, while large, are impossible to calculate exactly. Predictions are rare in SOR theories, and this is a conspicuously successful one.

Objection 3, by contrast, can be answered rather simply. If terminal BH groups activate the same odotope as SH, then all BH containing compounds should have a sulfuraceous character. This is not the case: as was pointed out to one of us (LT) by RH Biddulph (personal communication), triethylamine-borane does *not* smell sulfuraceous. Remarkably, the vibrational frequency of the BH bond in triethylamine-borane is shifted downwards by 200 wavenumbers. i.e. out of thiol range. Another instance is that of the three isomers of carborane, which smell camphoraceous, though o-carborane has a faint onion-like (sulfuraceous) smell. The reason for this is not yet clear, but their extraordinary chemical stability is consistent with a low polarity of the B-H bond, and this would tend to reduce the intensity of the BH stretch vibration to the point where it may be no longer detectable.

V.B.3 Hindered functional groups

Molecules could in principle be designed to settle the issue of whether functional groups are perceived as odotopes or by their vibrations. Suppose for example that a functional group possessing a distinctive odor was present in a molecule, but buried in such a way as to be inaccessible to molecular recognition. Because tunnelling electrons penetrate the molecule, the vibrational theory would predict that it should still smell, whereas odotope theory would not. The ideal molecule in this respect would include,

say, an SH group within its innards, completely shielded from touch. Such a molecule does not yet exist, and may be impossible to construct given the maximum size requirement for odorants.

Sterically hindered phenols provide a first approximation to this goal. The presence of an OH group on a substituted benzene ring gives the molecule a distinctive "phenolic" odor, which the corresponding benzene does not have. Once again, if one assumes that the OH group is an odotope, then making it less accessible to molecular recognition should silence its smell. This idea is easily tested by comparing the smell of di-*tert*-butyl derivatives of phenol, which are readily available commercially. The results go against the odotope theory. 2,6 di-*tert*-butyl phenol, in which the OH group is strongly hindered smells as phenolic as, say, the 2,4 derivative in which it is more accessible.

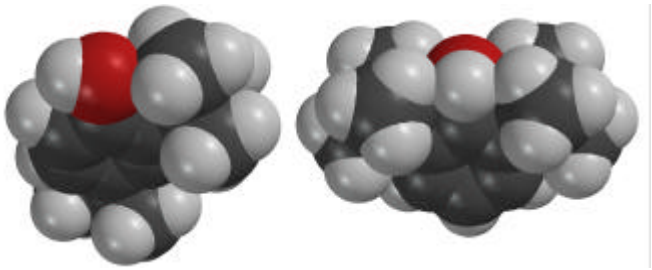


Figure 13 space-filling models of 2,4 (left) and 2,6 di-t-butyl phenols. These two molecules smell equally phenolic, Despite the OH group being accessible in one and sterically hindered in the other

It may be argued that the OH group is insufficiently buried in this molecule, and remains accessible to some molecular interaction. Designing molecules with buried functional groups, for example the trimethylsilyl analogues of phenols and thiophenols could, in principle, settle this question.

V.C Isosteric molecules

A strong test of vibrational vs. odotope theories would be the odor comparison of molecules identical in atom composition, shape, weight, electron distribution all other physical properties but differing only in vibrations. That is of course an unattainable ideal, but one can come quite close, either by element substitution: Ni for Fe inside a metallocene, Si for C, or by isotope substitution (D for H) in a normal odorants.

V.C.1 Metallocenes

Ferrocene and nickelocene have very similar structures and very different smells. Vibrationally, the main difference is in the internal movements of the metal ion between the rings.

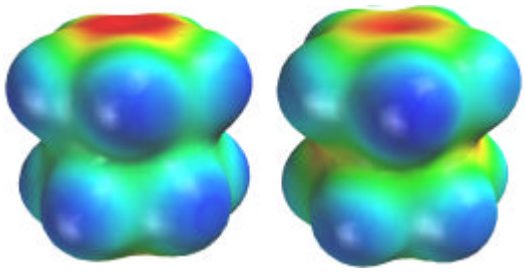


Figure 14 Electron-density maps of ferrocene (left) and nickelocene with electrostatic potential mapped onto the surface. Structure, electron density and potential surfaces calculated by semiempirical methods using Spartan with PM3 parameters. Red is more negative. There are small differences in ring spacing and charge distribution, whereas the odor of these two molecules is radically different: ferrocene smells spicy-camphoraceous, nickelocene smells oily-chemical.

V.C.2 Sila compounds

Silicon (and in some cases Ge and Sn) can replace carbon in odorants (Mundstedt and Wannagat (1985), Wannagat et al. (1985, 1993), Wrobel and Wannagat (1982a-d), 1983). Because of the high polarity and consequent instability of the Si-H group, only C atoms linked to four carbons can be substituted in this fashion. The geometry of Si-C bonds is tetrahedral. Though very similar in overall geometry, *sila* compounds will differ somewhat from the parent carbon compound. The Si-C bond is 1.8 Å long, as compared to 1.5 Å for a typical C-C bond and the Si-C bond is more polar. By contrast, the vibrations of the molecule will be markedly altered by the Si and *Ge* substitution. For example, the Si-C stretch vibration is around 650 wavenumbers instead of 1000.

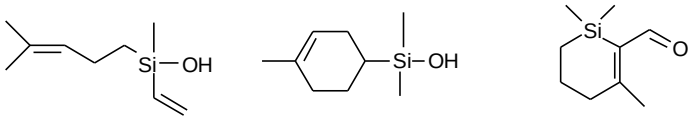


Figure 15: Three representative examples of molecules in which Si replacement for C cause a marked change in odor. Left to tight, sila-linalool, sila terpineol and sila-cyclocitral.

In all cases, there was some change in odor, though it was sometimes subtle and sometimes striking. For example sila substitution in linalool “light and refreshing, floral-woody odor with a citrusy note (Wrobel and Wannagat 1982a) to give sila-linalool makes it “more hyacinth-like, sweeter”. Similarly, sila-terpineol smells more muguet-like and less lilac like than the parent compound, but sila-carvomenthene smells “similar” to the parent carbon compound. Interestingly, though the largest jump in size and other properties occurs between C and Si, Ge derivatives are again different in odor from both. Sila-cyclocitral smells “camphoraceous, sweet earthy with a green tea note”, whereas the parent compound smells “minty, turpentine-like”(Mundstedt and Wannagat, 1985)

In her comprehensive review of SORs, Rossiter (1996) summarized these results by saying “Those examples where the odor of the sila analogue is similar to that of the carbon counterpart are interesting anomalies for [..] vibrational theories. These data could also be interpreted as interesting anomalies for odotope theories. The reader interested in exploring the differences in odor between sila and parent compounds can readily obtain 1,1 dimethyl 1-*sila*cyclohexane (˜cyclopentamethylene dimethylsilane) and its parent compound 1,1 dimethyl cyclohexane from either Aldrich or Lancaster (UK). The difference in smell between the two compounds is striking. The odor profiles, assessed by a professional perfumer, are as follows: 1,1 dimethylcyclohexane, camphoraceous, with a faint sweet fruity, powdery background; 1,1 dimethyl-1-sila cyclohexane: intense, chemical-green note reminiscent of cis-3-hexenol, with a faint camphoraceous background.

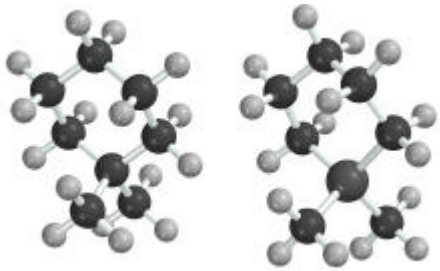


Figure16 The calculated structures of two commercially available compounds with similar shape and very different odors. Left:1,1 dimethylcyclohexane Right:1,1 dimethyl *sila* cyclohexane.

In summary, the results on sila and germa compounds are consistent with both theories. Odotope theory does not adequately account for the very large difference in smell between C and Si, and especially between Si and Ge compounds. Neither theory adequately explains why odor differences should be so marked in some cases and weak in others.

V.C.3 Isotope substitution

Isotope substitution is in principle the best way to make perfectly isosteric compounds differing “only” in molecular vibrations. The “only” in the sentence above illustrates the fact that, as Wade has pointed out in his comprehensive review of isotope effects in biology, there are in fact subtle differences in the physical and chemical properties of isotopes as compared to the parent compound (Wade 1999). Their hydrophobicity will be slightly different because of the small difference in size and polarizability of the electron cloud surrounding the heavier nuclei. In addition the range of conformations that the compound will explore during thermal motion will be different, because the altered masses respond differently to thermal excitations. Nevertheless, these effects are small: isotope separations on chromatography columns require long elution times, and the lowest energy conformation (i.e. molecular shape) will in all cases be unaffected by isotope substitutions. By contrast, effects on molecular vibrations can be large: substitution of D for H reduces the X-H stretch frequencies by a factor of ˜ v2, i.e. for CH for example from 3000 to 2200 wavenumbers.

Effects of isotope substitution (deuterium for hydrogen) on animal olfaction have been known for a long time. Hara (1977) showed that fish could reliably distinguish deuterated glycine from the parent compound. Meloan and collaborators have performed a remarkable series of studies Meloan et al (1988), Kuo (1982), Scriven (1984), Havens (1993), DeCou (1993) in which they showed that insects could distinguish between isotopes. For example cyclohexanone is a powerful cockroach repellent, while deuterated cyclohexanone is inactive.

No human counterpart of these effects was reported until it was reported that deuterated acetophenone could be distinguished from the parent compound by smell (Turin, 1996). These experiments were performed on a gas chromatograph using a smelling port to eliminate the possibility that impurities might be responsible for the smell difference. The difference in smell to trained observers was subtle, but definite.

We have recently found a more striking isotope odor difference in dimethyl sulfide. Arctander describes the odor of dimethyl sulfide as "repulsive, sharp, green, cabbage-like" at high concentrations. Dimethylsulfide-d_6 clearly smells cleaner, more truffle-like without the gassy cabbage-like note of the parent compound. This is a particularly easy experiment to replicate because a) both dimethyl sulfide and dimethylsulfide-d_6 are safe to smell (despite its unpromising descriptors, DMS is a perfumery raw material !) and available at very high purity from Aldrich. b) dimethylsulfide is a very strong odorant so impurities will be unlikely to influence the overall odor. We urge interested readers to do the experiment. The antisymmetric and symmetric C-S stretch vibrations are shifted from 710 and 654 wavenumbers respectively[3] to 670 and 608 wavenumbers.

Finally, one of us (LT) has obtained a sample of deuterated decaborane. Unlike those of thiols, the terminal hydrogens of boranes are not readily exchangeable. This allows one to test whether the stretch frequency of boranes is genuinely necessary to their sulfurous smell. Fully deuterated decaborane (> 90% D) smells distinctively different, more mustard like, pungent and less sulfuraceous than its H counterpart.

In summary, available evidence from isotope experiments appears to be inconsistent with odotope theory, and in broad agreement with vibrational theory. In order for the odotope theory to apply, one would have to postulate additional factors to be involved. For example a very high differential sensitivity of the odotope receptors to small changes in odorant hydrophobicity might account for the results. Alternatively, one might suppose that the fact that different low-lying vibrational states with energies around kT (~ 240) wavenumbers) will be excited in the deuterated odorant will cause its *average* conformation to be slightly different, and thereby cause a difference in odor. There is at present no evidence for either mechanism.

V.D Enantiomers

Most enantiomeric pairs of odorants smell identical, but there are many examples of enantiomer pairs that smell completely different Boelens and Van gemert (1993b) The best known outside fragrance chemistry are R and S carvone: R-carvone smells of mint, S carvone of caraway.

[3] Computed ab initio using a 3-21G* basis set, and corrected to 0.9 of the calculated value

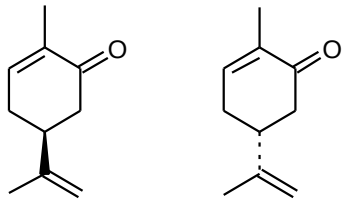


Fig 17 The enantiomers of carvone. (S)-(+) carvone (left) smells of caraway, (R)- (-) carvone (right smells of spearmint.

Differences in smell between enantiomers have in the past been considered strong evidence against vibrational theories of olfaction, because solution IR spectra of enantiomers probed with unpolarized light are of course identical. By contrast, if the IR absorption of a regular solid (crystal) is probed with polarized light, then the spectrum depends on the relative orientation of the molecular dipoles in the crystal to the plane of light polarization.

Turin (1996} has argued that a "biological spectroscope" resembles the latter case, and that the smell of carvone can be explained by a polarization effect. IN a tunnelling mechanism for detection of molecular vibrations the odorant is bound in a fixed orientation in the receptor and is probed by tunnelling electrons which are polarized, i.e. deflected in specific directions by the odorant. Strong dipoles (the C=O group in the case of carvone) will be most likely to show polarization effects. It could be that in mint carvone, the C=O is not detected because it is wrongly oriented. One would then expect that "adding back" the carbonyl vibration by smelling *simultaneously* a small carbonyl-bearing odorant (e.g. acetone, butanone) with mint carvone would change the smell from mint to caraway, which it does [4]. It remains to be seen whether similar experiments can be devised for other enantiomer pairs.

Interestingly, the smell of enantiomers poses problems for odotope theory, though this fact seems to have received no attention. The reason is analogous to the problem with odorless molecules discussed above in section 4.1. Suppose that an odorant is probed by several different odotope receptors. Each of those receptors is likely to be chiral to some extent, because it is near-impossible to engineer a specific protein binding site without chirality. A good indication of this comes from drug-receptor interactions, where drug enantiomers almost always have different actions (Hutt, 1998). Consider now the case of two enantiomers with identical smells. To account for this, odotope theory needs to make one of two assumptions. Either a) the enantiomer binds equally well and with the same affinities to the n (chiral) odotope

[4] The demonstration is easy to perform: mix 3 parts of butanone with 2 parts of mint carvone and smell immediately, because the butanone evaporates rapidly. The mint smell is gone, replaced by a good approximation to caraway.

receptors or b) a completely different set of odotope receptors with opposite chirality are wired in the same fashion to give the same pattern of nerve excitation. Both are rather unlikely.

V.E The evidence from receptor expression studies: patterns of receptor activation

In the last few years, following the early lead of Raming et al. 1993 several receptor expression studies have been published. The advantage of receptor expression is that the response of a single receptor type to different odorants can be assessed directly. The results so far are still somewhat contradictory, and the field is evolving very rapidly. Zhao et al (1998) have expressed receptors in olfactory neurons, and found a broad response spectrum. Their particular receptor subtype was optimally stimulated by heptanal, less so by aldehydes of a shorter or longer chain length, consistent with an odotope-based model. Breer at al (1998) ,Kaluza and Breer (2000) and Touhara et al (1999) also found that different receptors had relatively broad ligand specificity. These results are in agreement with the *in vivo* responses of olfactory receptor neurons (Firestein et al 1993 Duchamp-Viret et al. 1999.

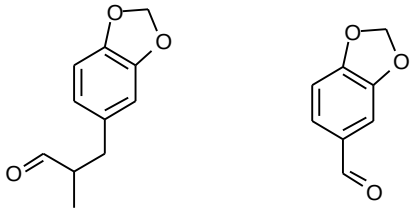


Figure 18 Helional (left) and the related molecule piperonal. A recent study (Wetzel at al 1999) has suggested that helional alone, out of 100 odorants, can activate an olfactory receptor, with even closely related molecules being 1000 times less potent.

By contrast, Krautwurst et al (1998) reported greater specificity in odorant receptor responses, and Wetzel et al (1999) et al reported that a receptor only responded to one odorant (helional) at very low concentrations but not to the closely related molecule piperonal. These remarkable results differ from those reported by other groups, including in vivo studies (see Firestein et al). If confirmed and extended, they would suggest that odorant-receptor interactions are far more specific than has been hitherto supposed.

To date, however, the most comprehensive set of data comes from the elegant work of Malnic et al (1999), in which 14 receptor types were expressed and their responses to a set of 19 odorants compared. Fig 18 illustrates the spectrum of response of the fourteen different receptor types to 19 different odorants. The odorants are arranged in series: carboxylic acids, alcohols, bromo- carboxylic acids and dicarboxylic acids with carbon chains of varying lengths. A remarkable pattern emerges: the matrix of receptor responses to odorants is sufficiently complex that even with this small number of receptors, an odorant in the list can be identified from the pattern of receptors it is

capable of activating. They conclude that “different odorants are recognized by distinct combinations of receptors”, and interpret this data according to an “odotope” model, though they do not use the term and do not specify which odotopes may be involved. The reader is referred to the article by L Buck elsewhere in this volume for a more complete discussion.

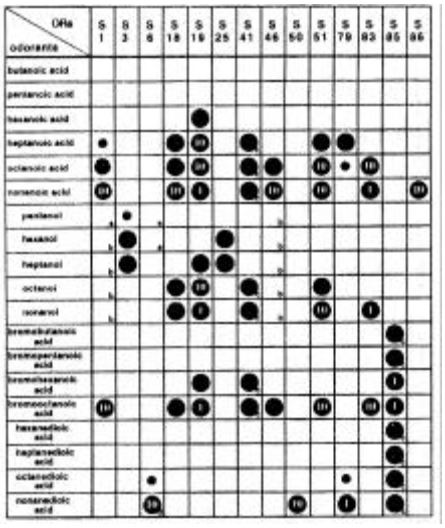


Figure 19 Figure 6 of Malnic et al, showing the pattern of responses (black) circles of different olfactory receptors (columns) to different odorants (rows).

There is, however, another pattern in their data, namely that in each series the number of receptor types that respond increases as one lengthens the carbon chain. This immediately suggests that the spectrum of responses may be related in part to the hydrophobicity of the odorant. The latter can easily and accurately be calculated as logP, where P is the (calculated) partition coefficient between octanol and water. When the number of receptor types activated (a crude measure of the potency of the odorant) is plotted against logP (fig 20) a new pattern is evident. First, the number of receptors activated is roughly proportional to logP for each series of odorants. This suggests that partition into a hydrophobic site, possibly the receptor itself, governs “affinity” within a series. The different series appear to have different efficacies, however: dicarboxylics, while considerably less soluble in octanol than alkanols are clearly more potent.

Such a pattern would be expected from a vibrational mechanism. In this model, the affinity of the odorant for the receptor is governed by logP because the receptor site is hydrophobic. The efficacy, by contrast, is governed by the electron-tunnelling cross-section of the molecule, i.e. its ability to scatter electrons. That in turn is proportional to

$$S = \Sigma\, q^2\, \Delta x^2$$

where q and x are respectively the calculated electrostatic partial charges and atom displacements for each vibrational mode, and the summation is carried out over all vibrational modes. In other words, the larger the charges on the component atoms, and the bigger their displacements the stronger the odorant will be. This makes sense when interpreting figure 20a : the least potent odorants are the alkanols (partial charge largely

on OH), then come the carboxylics (partial charges on the acid group), then the bromocarboxylics (additional charge from the C-Br bond), finally the dicarboxylics (two sets of acid group charges).

The calculated values of S for octanol, octanoic acid, bromooctanoic acid and octanedioic acid are 1.38, 3.50, 3.97 7.48 respectively[5]. When the logs of these values are used to correct the graph in figure left, fig 20 right is obtained. The curves now follow roughly the same linear relationship. This suggests that the data of Malnic et al. are equally consistent with a mechanism invoking odotopes and by a vibrational mechanism involving the physical quantities logP and S.

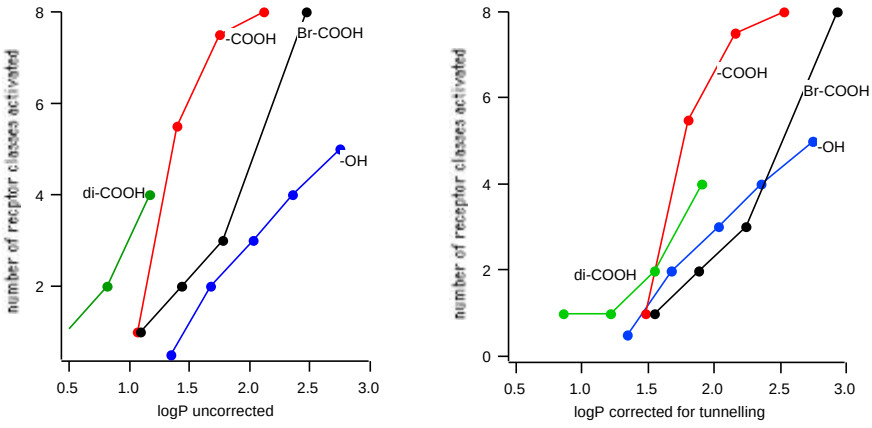


Figure 20 A reanalysis of the published data of Malnic et al on the response of expressed olfactory receptors to a variety of odorants. When the number of different receptor classes activated (ordinate) is plotted against the water-octanol partition coefficient (logP, abscissa), it becomes clear that a determining factor in molecular selectivity is hydrophobicity. When the data is corrected for scattering intensity in a vibrational mechanism (right) , the correlation improves. Weak responses obtained at 100µM (small circles in original figure) were treated as 0.5.

VI Why are there so many receptors?

The large number of receptors sequences found is often taken as evidence in favor of molecular recognition mechanism based on shape. This is not necessarily so. First, if , as seems likely, odorants bind to a specific binding site in the receptor, then the variability needed to accommodate different odorants of mw < 300 daltons should be restricted to a dozen or so neigboring aminoacids which are in direct contact with the odorant (Floriano et al. 2000) . Secondly, a large number of receptors would be more consistent with highly specific responses (one-receptor, one odorant). Most of the evidence points to broadly-tuned receptors, which removes the need for a large number. By way of comparison, several thousand colors can be distinguished using the relative intensity of signals coming from only three types of broadly-tuned retinal cones.

[5] Partial charges (electrostatic fit) and atom displacements were calculated for the lowest homolog in the series using semiempirical mehtods with AM1 parameters (Mac Spartan, Wavefunction, Inc.)

Thirdly, some of the variability seems to be related to the developmental role receptors play in guiding olfactory receptor neurons to the correct place in the bulb (Wang et al, 1998). Olfactory receptor-like proteins have been found in non-olfactory tissues and may serve a general developmental purpose (Dreyer, 1998). It is worth bearing in mind that in higher vertebrates including man some of the olfactory receptor neurons renew themselves throughout life, which may require developmental clues.

A large number of receptors is also expected from a vibrational mechanism. An idealised receptor would have an odorant-binding site that is as unspecific as possible, analogous to the cuvette of an ordinary spectrometer. At a molecular level of course this cannot be achieved, because the cuvette needs to be molecule-sized and will thus always incorporate some element of selectivity and chirality. Conversely, if it were made large enough, say the size of a lipid droplet, in order to accommodate all odorants, it would be too large to allow electron-tunnelling to occur. A biological spectroscope that wishes to accommodate a broad range of odorants therefore needs a large variety of odorant-binding pockets.

Thus, both a shape based and a vibrational theory may require a large number of receptors, and the main difference is in the way the receptors are wired. The arguments set out in Lancet et al. (see section VI.A) which enable them to calculate the number of receptors required to achieve a target affinity for a large set of ligands apply equally well to odotope and vibrational theories. In a shape based theory the receptors are wired by odotope, whereas in a vibrational theory the receptors are wired by spectral class. All receptors binding molecules of different shapes but probing the same part of the vibrational spectrum be expected, say, to project to the same part of the olfactory bulb. There is some evidence that different parts of the rat olfactory epithelium responds to the presence of different functional groups Scott et al, (1996, 1997, but it s not clear whether the differences follow odotopes or vibrations. Not enough is known about the relationship between bulbar responses and either shape or vibration to decide the issue at the moment.

VII The puzzle of odorant intensity

VII.A Odorless molecules

The question of odorant intensity (strong vs. weak) as distinct from odor character (the sum of descriptors) raises issues of unexpected subtlety. Odotope theories implicitly assume that odorant intensity is part of the odor character, i.e. that a molecule can be described legitimately as, say, green, weak, or green, powerful. When traced back to its intellectual roots, this idea originates from pharmacology, where a ligand, irrespective of its affinity for the receptor, may have a low or high efficacy.

This seems reasonable enough, but is actually quite hard to reconcile with odotope theory. For a molecule to be odorless, it would have to be simultaneously odorless to all the odotope receptors that it binds to. While this is possible in principle, it would be more likely to occur with small molecules bearing few odotopes, and therefore binding to few receptors, and gradually less likely as molecular size increases. What is observed is precisely the opposite: with some exceptions about which more below,

odorless molecules appear only as molecular size nears its upper limit, i.e. when the number of possible odotopes available for binding is at its maximum.

An odotope theory modified to account for this might include a size selectivity filter in each receptor, such that the molecule has to fulfill two criteria to be odorant: fit in the filter and bind to the receptor. The difficulty with this ad hoc hypothesis is that it then requires perfect uniformity in the size of the selectivity filters. Were this not the case, one would expect the most frequent outcome to be not an odorless musk but a different smell altogether if only a subset of odotope receptors are still able to smell it. This is not the case, musks are either odorant or odorless to different subjects without change in smell character.

A vibrational theory has the opposite problem, namely that no molecule that has nonzero partial charges on its component atoms should be odorless, since all molecules have a vibrational spectrum. This agrees (Turin, 1996) with the fact that all small molecules are odorous, except for a) The ones with either very weak or zero charge (e.g. hydrogen or oxygen gas) and b) Those where all the vibrations are below kT, since they will be indistinguishable from thermal noise. In a vibrational theory, the odorless character of a molecule can *only* arise from the fact that it does not bind to a receptor.

The problem of odor behavior near the size cutoff also applies to a vibrational theory, but in a less extreme fashion than with odotopes. First of all, there need to be fewer receptor types, ideally only enough to cover the vibrational spectrum piecewise. Turin has estimated their minimum number to be ten. Secondly, the odorant-binding site could in principle be completely nonspecific, since all that is required is that the vibrations of the odorant be probed adequately by the spectroscopic receptor. Indeed, all receptor sites could be identical in structure and differ only in the segment of the spectrum that they probe, which makes it easier to understand why they would all have the same size cutoff. How this might be reconciled with the large number of odorant receptors found has been discussed above.

VII.B Weak and strong odorants

There are few reliable data sets on threshold detection values in the literature van Gemert (2000). The largest data set appears to have been laboriously collected over many years by the fragrance firm Givaudan-Roure. Their *odor-value* chart, which would be of considerable interest to researchers, is (understandably) proprietary. Even when differences in volatility are taken into account, odorants seem to differ in intensity by at least eight orders of magnitude. As was discussed above in section 4.1, odotope and vibrational theories differ crucially in how they account for this fact.

Odotope theories regard odorless, weak and strong odorants respectively as inactive, weak, strong agonists. In other words, in an odotope theory, odorant intensity is in part related to the *efficacy* with which the molecule activates the olfactory receptors, not necessarily just to its *affinity* for the receptors. The difference between affinity and efficacy has been elegantly summed up by Colquhoun (1998): the affinity of a drug for its receptor is “simply the microscopic equilibrium constant for binding to the inactive state”. Efficacy is “the set of all the other equilibrium constants which describe the transduction events that follow the initial binding reaction”. For example, a molecule

which binds best to the active receptor conformation will favor the conformation change from inactive to active (agonist), whereas one that binds tightly to the inactive state will be an antagonist.

In a vibrational theory, efficacy has a different interpretation. All molecules vibrate, and spectral intensities are likely to differ by only a factor of 20 or so between the smallest molecule with the weakest charges (e.g. methane) and a large molecule with large partial charges, (e.g. a nitro musk). To account for the several orders of magnitude in odorant intensity, a vibrational theory must therefore assume that the strongest odorants simply bind most tightly to the receptors, i.e. that efficacy is proportional to affinity. There is, however, a physicochemical difficulty in accounting for the vast range in intensity of odorants simply by assuming that the strong ones bind more tightly. To be sure, odorants differ greatly in polarity, as reflected in their water-octanol partition (logP). LogP varies by 6 orders of magnitude: for example maltol and undecanal, respectively polar and hydrophobic strong odorants have calculated logP values of .07 and 3.20 (Kantola et al. 1991, implemented in Spartan, Wavefunction, inc.)

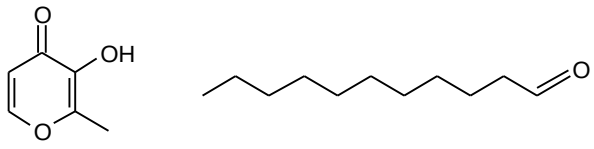


Figure 21 Two extremes of odorant polarity, maltol and undecanal

However, many very strong odorants, such as diacetyl and vanillin are relatively polar. Clearly, some interaction other than hydrophobic partition is required to account for their intensity.

VII.C Structural correlates of odor intensity

It has long been known empirically that certain structural features of molecules tend to make them stronger odorants. Moncrieff (1967) has listed many of these. The clearest correlates seem to be: 1) polar functional groups (OH, C=O, CN, SH, -O-, etc) increase intensity 2) unsaturation generally increases intensity 3) steric shielding of a functional group decreases intensity 4) When two hydrogen bond acceptors are present, the odorant is stronger when they are close to each other (Ohloff bifunctional rule, Ohloff 1994).

Taken together, these rules suggest that odorants may be binding to some ligand that has a high affinity for double bonds and lone pairs. Ohloff's bifunctional rule (see figure below for an example) is particularly interesting, because a) it applies to a large number of structurally unrelated odorants and b) it is consistent with *both* functional groups binding to the same ligand. We propose that a zinc ion coordinated to the receptor protein may function as a ligand for odorants, as was suggested independently by Turin (1996) and more recently by Suslick (2000).

VII.D Zinc binding is a good predictor of odorant intensity

A large number of olfactory receptor sequences have now been published, and new ones appear every month. While many of these sequences may be pseudogenes (Mombaerts, 1999b, Rouquier et al, 2000), it is now possible to form an accurate impression of their relationship to other, known, 7 - TM receptors. Recently, a thorough study by Skoufos (1999) has shown that one of the most conserved regions (Region 3 at the cytoplasmic end of TM helix 6) corresponds to the zinc binding site proposed by Turin 1996 . Interestingly, the histidine that binds the zinc is completely conserved. This is what would be expected if the zinc-binding site were essential to the operation of the receptor, and in particular if it were the odorant-binding site itself. Remarkably, Sheikh et al (1999) have shown that if two histidine zinc-binding sites are *engineered* at the cytoplasmic end of helices 3 and 6, then the presence of zinc prevents receptor activaion in two different types of 7-TNM receptors, suggesting that relative movement of helices 3 and 6 is essential.

Furthermore, there is a good deal of circumstantial evidence linking zinc with olfaction and gustation. Zinc deficiency, either dietary (Alpers, 1994), caused by treatment with histidine (Henkin et al. 1975) , thiocarbamides (Erikssen et al 1975) or captopril (Zumkley et al. 1985) is unique in causing a complete and rapidly reversible anosmia.

Turin (loc cit), pointed out that many strong odorants possessed structural features capable of bidentate binding to a metal ligand. This was recently confirmed (Suslick 2000) by colorimetric measurements of odorant binding to metalloporphyrins. This binding accounts in a straightforward fashion for the fact that a hydrophobic zinc salt, zinc ricinoleate, is a very effective deodorant. There is also a good deal of circumstantial evidence linking zinc with olfaction and gustation. Zinc deficiency, either dietary (Alpers, 1994), caused by treatment with histidine (Henkin et al. 1975) , thiocarbamides (Erikssen et al 1975) or captopril (Zumkley et al. 1985) is unique in causing a complete and rapidly reversible anosmia.

We propose that this notion can be usefully extended by including pi-bonding from double bonds, triple bonds and cyclopropane rings (Bader, 1990) as possible metal ligands. To test this, an unbiased data set is required. Ohloff's (1990) review of strong-weak stereoisomer pairs provides such a set, since it was selected without this theory in mind, and the odor threshold data are reliable. A good example is provided by double bond isomers of lyral (Fig 22). When the three-dimensional structure of these molecules is calculated, and bidentate binding to zinc between the carbonyl oxygen and the double bond is included (Fig 22) , it becomes clear that only the strong isomer (mol) can bind to zinc in this fashion.

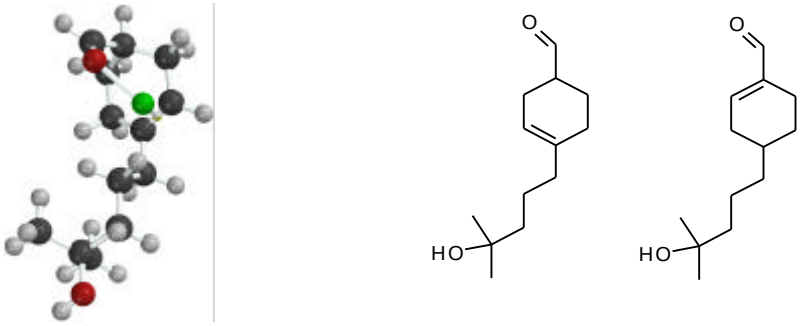


Figure 22 The strong (left) and "odorless" (right) isomers of lyral bound to a zinc ion via the carbonyl oxygen lone pair and the pi-orbital on the double bond. In the weak isomer, the geometry is unfavorable to zinc binding, as reflected by the angle formed by the two bonds to zinc, 102 ° for the strong isomer, 75 ° for the weak one. Structures determined a*b initio* using Spartan software with 3-21G(*) parameters.

The same idea explains intensity differences between many other isomer pairs described by Ohloff. Figure 23 illustrates some of these instances. Examples 1,4 and 6 follow Ohloff's bifunctional rule, the remainder involve a double bond and a functional group.

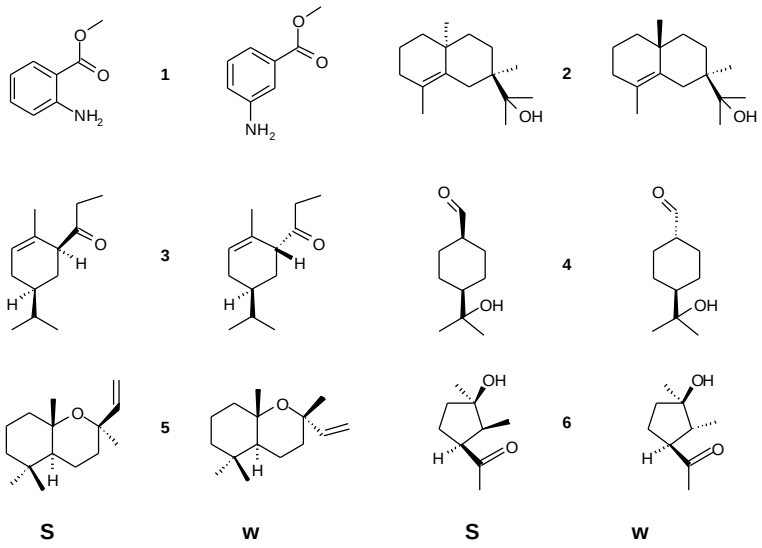


Figure 23 A sample of strong-weak isomer pairs taken from Ohloff (1994). 1-methylanthranilate, 2-eudesmol, 3-neron, 4-p-menthane derivatives, 5-caparrapi oxide, 6- iridanes. In every case, the strong isomer (left) is a bidentate ligand for zinc, whereas the weak isomer has unfavorable geometry for zinc-binding.

There are, however, many exceptions to this rule, namely those molecules for which enantiomers, isomers or diastereomers have different intensities without there being more than one functional group capable of binding to zinc. Examples of this are muscone and Mayol. Clearly, this can have little to do with zinc-binding, and must be due to steric interactions within the receptor site.

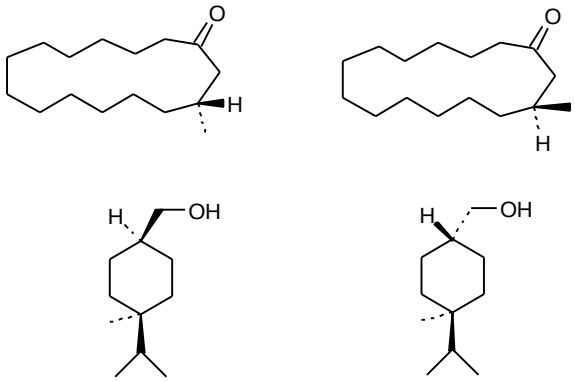


Figure 24 Strong (left)and weak (right) isomers of muscone (top) and mayol (bottom), illustrating that differences in odor intensity cannot always be ascribed to the accessibility of one or more metal coordinating groups.

Many of the best correlations obtained between structure and "odor" are actually done in such a way that what is being tested is the effect of structure on odor intensity rather than on odor character. For example, QSAR studies of musks (Yoshii, 1991, 1992 based on the data of Wood 1968-1970) have been conspicuously successful in predicting odor intensity. If one accepts the notion that a intensity is *not* an odor character, but reflects the ability of the odorant to bind to the receptor, then it becomes clear that what these studies are probing is the size and shape of the binding site.

VIII Summary and conclusions

In summary, it seems fair to say that if the ultimate goal of a theory is predictive power, then both odotopes and vibration still fall short. Neither theory, when faced with a novel molecule, is yet able to predict reliably what its odor character will be. Vibrational theory is conspicuously successful at explaining the fact that we smell functional groups even when sterically hindered, and in accounting for differences in smell between isotopes. Odotope theory explains neither.

By contrast, vibrational theory is intrinsically unable to explain differences in the intensity of different odorants, or which members of a set of related odorants will be odorless. We propose as a working hypothesis to be tested by further experiment, that odor character is determined by molecular vibrations, and odor intensity is determined almost entirely by molecular shape.

We agree with Beets (1957), that while the present theories may be incomplete, "we need not consider the question of whether a relationship exists between structure and odor […] The only question is whether it is simple enough to be detectable with our limited intellectual and technical means".

The fact that after several decades of experimental investigations, the basic mechanism by which odors are detected remains open to question shows that there is much work to be done. At the present rate of discovery, is to be expected that the answer to these questions may come in time for the next edition of this Handbook.

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