Rational odorant design

Luca Turin
Flexitral, Inc, 4313 Walney Rd Chantilly, VA 20151 USA
l.turin@flexitral.com

Introduction:

From its beginnings in the mid-nineteenth century, synthetic organic chemistry has discovered vast numbers of odorant molecules. The advantages of synthesis in cost and purity as opposed to extraction were apparent from the first. Houbigant's *Fougère Royale*[1] (1881), thought to be the first fragrance containing a synthetic material, set a pattern for most great fragrances to follow: a skilful mixture of the natural (oakmoss) and the synthetic (coumarin) respectively giving complexity and power per unit cost. Synthetic coumarin added nothing new to the perfumer's palette, since the natural material was readily available and very pure already, though not cheap. The discovery of the ionones[1], by contrast, went further by replacing fabulously expensive naturals (violet flower absolute) by cheap aroma chemicals. A few years earlier, nitro musks [2] had gone further still and added an entirely new type of smell (the powdery musk) to the perfumer's palette. From then until today, the cheap and the new have been coming out in a continuous stream from the few, and getting fewer, companies involved in odorant discovery. The perfumer's palette now consists of approximately 4000 raw materials, approximately half of which are synthetic. How were they found ? Four types of discovery process account for all but a handful of materials.

1- Grind and Find: Evolution's "four billion year R&D programme" has developed thousands of molecules chiefly intended to be attractive to animals. The fact that we share their tastes (particularly those of pollinating insects) to some extent has been a blessing to the fragrance industry. Analysis of the components of rose oil, for example has yielded hundreds of interesting compounds (the damascones[3,] in addition to its main components, phenylethyl alcohol, eugenol, geraniol and citral .

2- Imitation and Development: Chemists are not bound (or helped) by the rules of biological synthesis, elegant though the latter are. Once a natural molecule is

[1] Fougère Royale can be smelled at the Osmothèque, the perfume museum attached to the ISIPCA school, in Versailles.

identified by painstaking analysis, synthetic organic chemists can design dozens, sometimes hundreds of variations, hoping to find some that are either interesting and cheap (e.g. seco-ionones[4]), or irresistible and expensive (ambrox[5]).

3-Serendipity: Synthetic organic chemistry is an art as well as a science, and the surprises are sometimes more interesting than the intended result. Famously, Baur's nitro musks were originally intended as explosives. But chance only favours the prepared mind, and someone has to notice the serendipitous result. Materials like Osyrol®[6] and Karanal ®[7] are the result of chance discoveries by fragrance chemists followed by recognition and careful analysis.

4- Brute Force: The availability of combinatorial chemistry methods from the pharmaceutical world[8] opens up the possibility of making novel odorants by mass synthesis. The usefulness of this method is limited by the necessity to assay each by a nose, and the difficulty in making sure that the main component, not some impurity, is responsible for the smell. There are a number of cases where trace impurities have proven to be the key odorant material, not the 'named' product, and this has led to the discovery of important compounds. This approach is in its early days.

And lastly,

5- Rational Design: In order to design odorants rationally, one needs to have some understanding of how smell character is written into the molecule. Odour character belongs to biology, i.e. it is a property of the molecule *as perceived* by the nose. Unlike the four previous approaches which relied on chemistry alone, this approach will only be as good as our understanding of human olfaction. Unfortunately, the latter is still sketchy, and as a result very little has been done in this area. Since this is the subject of this article, a brief review of olfactory reception is in order.

Theories of olfaction

For obvious reasons, in its early days understanding of olfaction could not progress faster than that of molecular structure. Early theories of olfaction were mere speculation, and often assumed fanciful physical mechanisms such as rays emitted by odorant molecules, etc. In the late 1920's, one is surprised to find G M Dyson still patiently explaining to an audience of fragrance chemists the notion that there is no action at a distance involved in smell[9]! In 1946, an article by Linus Pauling[10] first hypothesized that shape was

responsible for molecular interactions, and that the recognition of odorants was just one example among many. The idea was taken up by Moncrieff[11] and Amoore[12] two years later and in modified and refined form survives to this day. What has changed in the intervening years is that we have gained a large database of odors and structures, and a vastly better understanding of the ways in which a ligand can interact with a receptor. What has not changed, however, is our ignorance of the exact structure of the receptor, which makes proper modeling of SORs virtually impossible.

Nevertheless**,** Until recently, a poll among specialists as to what gave a molecule its odour would have elicited a uniform answer: "shape"[13,14]. By molecular shape is meant the location in space of the atoms of the molecule. By convention, with some sacrifice of physical realism, this usually means the lowest-energy position of the atoms as determined by a suitable quantum chemistry computational package.

Until 1991, when the receptors were finally discovered[15], studies of the relationship between molecular shape and odour were made without reference to the biological sensor. The discovery that the olfactory receptors were seven-transmembrane helix proteins (7-TM) finally brought this problem into focus. When probed by a biological system, shape now translates into the sum total of all the repulsive and attractive interactions that a molecule feels when bound to a receptor: exchange repulsion plus hydrogen bond donors and acceptors, lone pairs, etc.. The discovery of the receptors was widely expected to confirm the prevailing "shape" theory and to reduce odorant design to a branch of rational drug design. However, several unexpected factors intervened.

The first was the revival[16] of a hitherto discredited contender among olfaction theories: vibration. Dyson[17], and later Wright[18] had argued that there was a better correlation between the odour of a molecule and its vibrational spectrum than between odour and shape. After proving remarkably hardy in the face of opposition, this idea abandoned the field in the late '70 when a) it became clear that some enantiomer pairs have different odours (and identical vibrations when measured by a conventional spectroscope) and b) the proponents of vibrational theories failed to come up with a mechanism by which vibrations might be sensed by a biological system. Turin[16] showed that both problems could be addressed and solved, at least in principle, and put this long-forgotten contender back into the arena.

The second was, in retrospect, that part of the attractiveness of "shape" theories (there are two variations, to be discussed below) lay chiefly in the absence of competition. The effect of this scientific "one-party system" was to discourage a critical look at shape theory itself. The revival of vibration theory, regardless of its intrinsic merits, has led to a critical re-examination of shape

theory in the light of a) new experimental evidence on receptor selectivity b) a large number of discrepant facts which had accumulated over time and c) the growing realisation that since nobody seemed to be designing odorants rationally by shape, the theory could not be *that* good. Turin and Yoshii[19] set out the pros and cons of shape and vibration theories in a recent review. Their arguments, and others which have recently come to light, will now be summarized.

For Shape:

1-Plausibility. Most known molecular recognition mechanisms (including, it now appears, a classic exception: general anaesthesia[20]) require a fit between receptor and agonist, enzyme and substrate, or antibody and antigen. In receptor mechanisms, this fit is not, as it is for enzymes, a prelude to chemistry, but the very event that (sometimes) leads to receptor activation.

2-The nature of the receptors. The fact that olfactory receptors are 7-TM proteins linked to a G-protein cascade suggests that a shape-based mechanism similar to, say, that of other 7-TM types such as adrenergic receptors and rhodopsin[21] is a good place to start.

Against Shape:

1-No predictive ability. Note that I refer here to predictions of odorant *character*, not intensity. As pointed out in a recent review, intensity has everything to do with molecular shape, and some good correlations have been found. Odor character is a different story: despite 150 years of fragrance chemistry and a vast database of odorants, structure-character relations are still in "a sorry state"[14] . Reviews[13] of SORs are essentially catalogues of exceptions. Accordingly, discovery of odorants is still "empirical", i.e. proceeds by trial and error. Claims of odorant discovery by "rational" design based on shape are rarely backed up by disclosure of the complete set of odorants tried before hitting the correct one.

7- Isosteric molecules smell different. There are many examples in the literature of molecules of very similar shapes having very different smells. The most striking is undoubtedly the series of molecules investigated by Wannagat and coworkers[22] in which carbon was replaced by Si, Ge and Sn. Despite possessing very similar geometries, these molecules smell different, and not only between C and Si, but also between Si and Ge or Sn where the size change is a great deal smaller.

2-"Strong" shape looks unlikely. In "strong" shape theories, every odorant fits one receptor with very high affinity and the others little or not at all. This one-odorant, one receptor idea is incompatible with the fact that there are many more (tens of thousands) known odorants, *each of which has a distinctive odor* than receptors (347 according to the latest count[23] , some of which may in fact reside in other tissues). Further, whenever they have been tested, and with only few exceptions so far, odorant receptors appear to respond to a broad spectrum of odorants[24].

3-"Weak" shape appears untestable. "Weak" shape comes in two versions. The first is "odotope" theory, wherein parts of the molecule are felt by the receptors and the odor, like the proverbial elephant described by blind observers, is the sum of the parts. The other is a low-affinity theory in which odorants are "swallowed whole" by the receptors but on average offer a poor fit. Once again the pattern of receptor activation determines odor. But odotopes are "hidden variables" and hard to infer, and receptor structure remains unknown at the kind of resolution needed to make sense of structure-odor relations. Further uncertainty is added by the conformational flexibility of the vast majority of odorant molecules, which makes odotopes literally moving targets, It is hard to think of a conclusive experiment to test these theories. This does not mean that they are false, but weakens in principle the case that can be made in their favour.

4-The chiral receptor problem. Weak and Strong shape theories suffer from a problem due to the chiral nature of both odotopes and the proteins which detect them. Consider an odorant bearing four (generally chiral) odotopes. It will be perceived by four (generally chiral) receptors, and the receptor activation pattern will determine odor character. Now consider the odorant's enantiomer: each of the four odotopes will (generally) give a poor fit to the original receptors, and as a result the enantiomer will be "recognized" by a *different* set of receptors.

One would therefore generally expect enantiomers to have completely *different* smells. This is emphatically not the case. In a compilation of 277 known enantiomer pairs[25] 59% are found to smell similar (one descriptor in common) 5% "identical" (all descriptors in common) 17% different (no descriptors in common) and the remainder 19% are unknown. Even if all the unknowns turned out to have completely different odours, however, *the majority of enantiomer pairs would still smell similar*.

Contrast this with the different picture from conventional pharmacology[26]. There, the difference in activity between eutomer[27] (the correct

enantiomer) and distomer (the incorrect one) is frequently marked. An example are R and S propranolol which differ in potency on the beta-adrenergic receptor by a factor of 130. This case resembles the odorant enantiomer pairs which differ in intensity, though the problem for odorants is raised to the Nth power if odorants activate N different receptors. But frequently, a pattern with no parallel in smell is found: for example the enantiomers quinine and quinidine, have different actions altogether respectively antimalarial and anti-arrythmic, while (+) and (-) dobutamine have opposing actions (agonist vs antagonist) on the same receptor. This brings up a related problem, namely that

5- No odorant antagonists have been found A fundamental feature of shape-based pharmacology, is the existence of antagonists. The mechanism by which receptor antagonists work is thought to be that they bind more tightly to the "off" than to the "on" state of the receptor[28]. Frequently the difference in structure between agonists and antagonists is small, amounting to as little as the replacement of one hydrogen by a fluorine atom. To my knowledge no such molecule has ever been found in the case of odorants. If it existed, it would not be hard to notice. Imagine a molecule derived, from, say, santalol, which had a weak (sandalwood) smell of its own or no smell, but which prevented you from smelling santalol alone for some time thereafter, or when mixed with it. The fact that no such thing has been found in any odour group indicates either that a) there is a problem with the shape idea, b) for some unknown reason the on-off states of smell receptors differ from those of others c) we haven't looked hard enough.

6-We smell functional groups. This puzzling feature of human olfaction has so far defied explanation. The most striking instance, and the best-known, is the -SH (thiol, mercaptan) which imparts to any molecule, *regardless of its shape*, a character appropriately called "sulfuraceous". Less familiar, but equally easy to detect by trained observers, are the smells of nitriles, oximes, isonitriles, isothiocyanates, etc[29]. What makes this remarkable from a molecular recognition point of view is that these functional groups constitute the smallest odotopes, capable of making only one, at most two hydrogen bonds. How this translates into unfailingly accurate detection is hard to understand. For example, the fact that -OH (alcohols) never smell like -SH (thiols) at *any* concentration is hard to reconcile with known molecular recognition mechanisms. Again, the comparison with conventional pharmacology is telling. There, substitutions designed to modify binding to a given receptor are legion. For example, thiobarbiturates,where the purine carbonyl is replaced by a thiocarbonyl[30], and fluorinated benzodiazepines behave similarly to the parent compound. A chemist

colleague humorously summed up this molecular recognition puzzle by saying "It's easy, sulfur is yellow and oxygen red".

For Vibration

1- Functional group recognition. As any IR spectroscopist knows, functional group recognition from their distinctive stretch frequencies is straightforward[31]. The most remarkable instance of this is once again the -SH group which has a "unique" stretch frequency signature around 2550 wavenumbers. A prediction from the vibration theory is that any other group possessing the same vibration frequency should smell sulfuraceous. This is the case: only the B-H stretch vibration of boranes falls in the same range, and boranes, alone among all compounds known to date smell sulfuraceous, as was noticed as early as 1912 by Stock[32], the first to synthesize most of them. There is, of course, very little in common between BH and SH in terms of chemistry. Further, a vibrational theory accounts for *similarities* in smell between molecules. The well-known replacement of nitriles for aldehydes, for example, is understandable by the proximity of their stretch vibrational frequencies.

2-isosteric molecules accounted for. In the cased of the C-Si-Ge-Sn series, for example, the big differences in smell despite similarity in shape are easily accounted for, because almost every molecular vibration will be affected by the large changes in mass.

3- Isotopes smell different (maybe). The ultimate test of a vibrational theory is a pair of molecules differing markedly in vibrations and not at all in shape. This theoretical goal is in fact impossible to attain. Zero-point molecular motion will always differ if two molecules have different vibrations, which means that the average "shape" will differ slightly. Nevertheless, one can come pretty close using isotope substitutions. The substitution of deuterium (mass 2) for hydrogen (mass 1) has the largest relative effect on vibrations involving H atoms, typically the various modes involving CH bonds. The experiment requires that the isotope substitution take place on a) nonexchangeable protons, otherwise D will exchange with H rapidly in the nose b) hydrogens not involved in H bonds, because these will be slightly affected by substitution. Remarkably, there is good evidence that insects and fishes can tell isotopes apart. Recently, a test on human subjects using fully deuterated benzaldehyde showed that subjects could tell it apart from the undeuterated form. Though tantalizing, these experiments suffer from the difficulty of making sure that small amounts of impurities are not affecting the smell. A GC-smelling test of deuterated acetophenone revealed

a (small) difference with normal acetophenone[16]. The ideal would be a combination of GC smelling and large observer set with randomized trials. Such an experiment is planned for the near future.

4-Enantiomers accounted for (differently)

In a vibrational theory, the diversity of receptors is due to them having to accommodate a vast number of unforeseen ligands. Nevertheless, drawing a parallel from color vision, one may expect these receptors to fall in a small number of classes spanning the vibrational spectrum, each class approx 400 wavenumbers in width. Turin[16] has argued that the number of classes is probably less than 10. For enantiomers to smell identical, they would have to stimulate each receptor *class* to the same average extent. In the limit of an infinite number of receptors, therefore, all enantiomers would smell identical because they would always in each class find a receptor to which they bind equally tightly. But humans have 347 olfactory receptor types. Neglecting the possibility that some may not be in the nose at all, assume that each spectrum region is equally represented. That gives ca. 30 receptors *per class* to bind efficiently with the tens of thousands of known odorants. This line of reasoning leads to a surprising conclusion: the smell differences between enantiomers are a manifestation of the imperfection of the system. Turin[16] has suggested a mechanistic explanation of the difference in smell between the enantiomers of carvone. Given that a majority of enantiomers smell similar, the system is doing remarkably well. This idea nevertheless implies the existence of limits on odor prediction (see below)

5-The Chiral Limit.

An advantage of vibrational theory is that it makes no recourse to "hidden variables" like odotopes. Molecules with similar vibrational spectra *as perceived by the nose* should smell similar. Vibrational frequencies can be calculated, and an educated guess can be made of mode intensities perceived by an IETS spectroscope. This approach has been used to predict the smells of existing, well-documented odorants with some success[33]. More recently, a much-expanded version has been used by Flexitral to predict novel odorants successfully (see below).

The discussion in section 4 above about enantiomers, however, raises an interesting problem. Enantiomers must still bind to receptors, and from that standpoint enantiomers are as different from each other as any two molecules can be. Therefore the probability, given a particular molecule with a particular odor, that a structurally *unrelated* one with identical vibrations will smell the same cannot be higher than for enantiomers, i.e. 5% or so., a fraction we call the "chiral limit". This problem will crop up whenever one is trying to improve on a

known odorant and modify its chemical properties without altering its smell. It is probably best in that case to look for structural modifications that change shape as little as possible as well as preserving vibrations.

Against vibration

1-Mechanism novel and unproven. Turin[16] proposed that the receptors detect vibrations by a solid-state mechanism involving inelastic electron tunnelling. While the different parts of the mechanism involve only plausible chemistry and physics, it is fair to say that there is to date no direct evidence in favour (or, for that matter against) this idea. The olfactory receptors are sufficiently different from other 7-TM receptors to make it at least possible that they have diverged to take advantage of this remarkable possibility. Experiments designed to test this idea directly are of course possible, but none have so far been devised.

2-Inability to account for odorant intensity. A feature of a vibrational theory is that to a first approximation, all molecules will have vibrational spectra of comparable intensity. To be sure, larger molecules will have more modes, and groups bearing intense charges may give larger peaks, but intensity differences of no more than a factor of , say, 3 can be accounted for in this fashion. Needless to say, this will not account for the range of odorant intensity which spans at least seven orders of magnitude. As Turin and Yoshii[19] have discussed, vibrational theory is a theory of odor *character* only. This means that odorant intensity is not part of odor character, and can only be due to the more or less tight binding of the odorant to the receptors.

Rational design by shape

If one narrowly defines "rational" to mean quantitative, then very little rational odorant *character* design by shape has been done to date. A recent comprehensive review of fragrance chemistry[14] illustrates odor classes with diagrams of 3D olfactophores (structural features necessary to a given odor type) obtained using Catalyst software. While emphasizing the use of "modern molecular similarity techniques", they nevertheless conclude that the finding of a new odorant is "based almost exclusively on a broad chemical knowledge, experience, fantasy and instinct". While these tools are arguably rational, they are certainly not quantitative. In general, it is hard to assess the success rate of shape-based synthetic programs for odorant discovery, for two reasons. First, successes are more likely to have been reported than failures. Second, even when the research is performed by one of the major manufacturers, it seems that

researchers do not compare the "successful" shape to their extensive (proprietary) databases of molecular structures to see whether counterexamples can be found.

The evidence is therefore fragmentary. Many "rules" for the design of odorants, e.g. the triaxial rule for ambers[5], and steric rules for sandalwoods[34] have been described over the years, but they seem to be of limited validity insofar as counterexamples can always be found[13]. Similarly, structural changes which frequently leave odor "unchanged", such as the isobutenyl-phenyl replacement[34] have been described, but it is not clear how general they are. Turin [2002] has argued that some can be accounted for vibrationally. A celebrated study by Sestanj[4] was among the first to report the successful (qualitatively) rational design of violet odorants (seco-ionones) by excision of half of the cyclohexene ring. A similar qualitative approach has been followed by Rossiter[35] in the design of muguet aromachemicals. This was followed more recently by a comprehensive study of violet odorants[36], in which shape similarity was used to achieve successful predictions of novel violet molecules. The data in the latter study has been re-examined by vibration theory[33], and a reasonable fit of vibration theory to the published odor data was found, suggesting that the correlation between structure and odor may in this case go *via* vibrations.

By contrast, the relationship between odorant *intensity* and shape is well documented[13]. Most early studies took a classical QSAR approach, and used statistical techniques to fit the intensity of the odorants to a variety of molecular descriptors. More recently, greater attention has been paid to biologically plausible receptor-binding mechanisms. In particular, though it clearly does not constitute the whole answer[19], the notion first proposed by Turin[16] that binding to a zinc ion in the receptor is an important factor in odorant intensity is gaining ground[37,38]. Although the 3D structure of the receptor is still unknown, recent studies have given more emphasis than in the past to the involvement of frontier orbitals Recent studies of musks[39], for example, appear to support the idea that odorant intensity depends on the position and prominence of certain electronic features. In view of the remarkable computational power now available on desktop computers, this area is clearly ripe for extensive study.

Rational design by vibration

Few things are more exciting (and, it must be said, scary) to a scientist than a chance to put theory to the test in practical use, all the more so since it is unusual for a theory to be reduced to practice before being validated scientifically. The creation of Flexitral, Inc by private investors early in 2002 afforded me this opportunity. What follows is a progress report on our

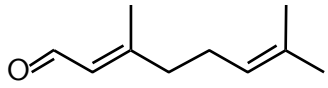
vibrational approach to rational odorant design, as candid as is compatible with the necessity to protect intellectual property. A description of an early attempt at calculating odor character has been given [Turin 2002]. The research effort at Flexitral greatly extended and refined this approach, with equal emphasis on both character and intensity prediction.

Replacement molecules

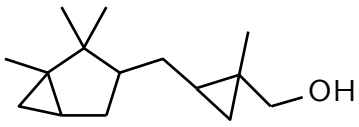
Regardless of their merits and the soundness of the public-health strategy on which they are based, regulatory requirements are a fact of life for the fragrance industry. Recently, a number of staple aroma chemicals among which citral, isoeugenol, lyral, etc. have appeared on a list which signals intent to restrict their use[40]. This is not a tragedy for perfumery: perfumers, like artists in general, are very adept at getting around regulations (think of piano concertos written for the left hand). Nevertheless, there is a pressing need for replacement molecules, particularly to be used in existing (legacy) fragrances.

Acitral ®

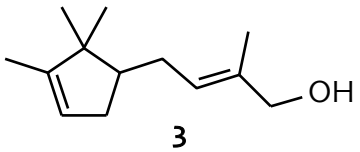
Flexitral began with a conversation with flavourists in Sept 2001 that made us aware of the need for an acid-stable lemon. Citral [1], the main character-impact molecule of lemon oil, is unstable in acid media (e.g. lemonade) and cyclizes quite rapidly (weeks) to unpleasant-smelling compounds. The origin of this instability resides in the tail double bond of citral, and any replacement should seek to replace that feature with another that preserves odor as far as is possible. In the search for possible replacements, we came across the interesting possibility of replacing a double bond with a cyclopropane ring. Givaudan's Javanol® [2], a very powerful and superb-smelling sandalwood material, is derived from [3] by cyclopropanation. Bajgrowicz et al[41] attribute the similarity in smell to the cyclopropane ring being "bioisosteric" to a double bond, though the exact meaning of the word is not defined. To be sure, there are interesting chemical similarities between cyclopropane rings and double bonds, not least the ability to conjugate to neighbouring carbonyls, etc. These similarities have led cyclopropane rings to be informally dubbed "fat olefins".



1



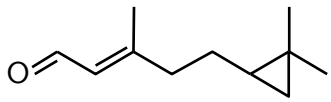
2



3

It seemed at least possible that the preservation of smell character might be due to vibrational, rather than steric or electronic features. Calculations

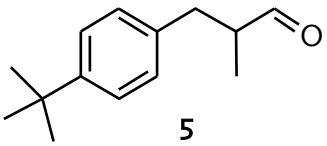
showed that cyclopropanation of the tail double bond would preserve spectra (fig 1). The cis and trans isomers of 5-(2,2-dimethylcyclopropyl)-3-methyl-2-pentenal [4] were synthesized from citral by cyclopropanation of the tail double bond and the reaction product assayed by GC olfactometry. The isomer mixture smelled lemony, though with a drier, slightly more aldehydic, powdery character than citral. The trans isomer (from geranial) appears to have a greener, more intense note than the cis isomer (from neral). In 2M acid solutions, compared to citral which breaks down in minutes, Acitral decays slowly (30% loss after 24 hrs) and gracefully (odorless or pleasant-smelling degradation products). Acitral's acute oral toxicity is low (LD > 2000 mg/kg) and, subject to further regulatory approval, it is presently on its way to commercial use[2].



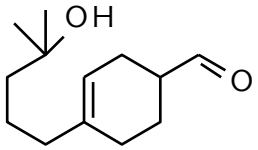
4

Lioral ®

Two prominent materials on the EU list of potential allergens are the lily-of the valley odorants Lilial® [5] and Lyral® [6]. Despite the abundant supply of related odorants, there appears to be a need for novel, powerful muguets with a soft odor profile. Here again, our strategy was to find vibration-conserving



5



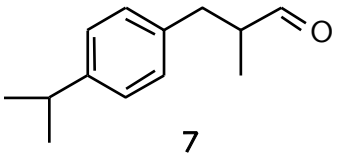
6

replacements. The resemblance in smell in between benzene and thiophene rings in various odorants has been noted many times. Inspired by Boelens' insightful reviews[42], we decided to test the possibility of replacing thiophene for benzene in cyclamenaldehyde[43] [7]. As has been pointed out many times, sulfur containing odorants present unique problems with regard to control of impurities, because these can overwhelm the odor profile when present even in tiny amounts. Thiophene poses fewer problems than most, however, because of its remarkable chemical stability.

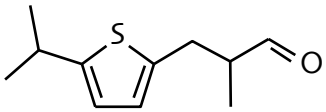
When faced with replacing a six-membered ring with a five-membered one, the question comes up of where to put the substituents. Fortunately in this case there was good agreement between ease of synthesis and computed odor profiles. A computational search through the substituted derivatives revealed

[2] Acitral has just (Oct 2003) obtained GRAS status from FEMA

that 3-(5-isopropylthiophen2-yl)-2-methylpropional [8] was the likeliest candidate and also the easiest to synthesize, given the reactivity of positions 2 and 5 on the thiophene ring. The resulting molecule was named Lioral® (from the Hebrew *li-or*, " light-giver"). It has a classic lily of the valley profile, with a slight cuminic note and is devoid of aggressive "plastic" connotations. In potency it is comparable to Florhydral®.



7



8

2 Prospects for the future

A good theory is, among other things, a labor-saving device. In this respect, vibrational theory is impressive. Using it, Flexitral has developed three novel raw materials in its first six months of operation. In each case, hundreds of molecules were explored computationally but the number of syntheses required to achieve the desired odor profile was less than 5. Since Jasphene was developed, we have, on behalf of clients, gone on to address other requirements of the industry and have come up in a similar fashion with five other products now in various stages of development and certification. It would seem that either we have been blessed with supernatural luck or there is some correspondence between our calculations and odor character.

The human element remains essential, because computational brute force, though vastly cheaper and faster than chemical brute force, is still an inefficient strategy. We improve our calculation tools continuously to broaden the range of structures explored, speed up computation, and achieve greater accuracy in odor character and intensity prediction. But we also try to make them fun to use, because most ideas come from playing. Tasks for the near future include pruning molecular search trees according to ease of synthesis and incorporating other properties such as substantivity and chemical stability in the criteria by which candidate molecules are searched.

Acknowledgements: I thank Jacquelin S Grant CEO, Flexitral, Inc for her valuable advice and constant support.

References:

1- Tiemann F and Kruger P (1893) *Berichte* 26: 2675

2-Baur A (1889) *German Patent* 47 559 May 21

3- Demole, E; Enggist, P; Saeuberli, U.; Stoll, M; Kovats, E (1970) Structure and synthesis of damascenone [2,6,6-trimethyl-1-(trans-crotonoyl)-1,3-cyclohexadiene], odorous constituent of Bulgarian rose oil (Rosa damascena) *Helvetica Chimica Acta*, 53(3), 541-51

4- Sestanj, K. (1962) Synthesis of an acyclic analog of ionone *Croatica Chemica Acta*, 34, 211-17

5- Ohloff, G. Relationship between odor sensation and stereochemistry of decalin ring compounds. *Gustation and Olfaction, Int. Symp.* (1971), 178-83. Editor(s): Ohloff, Guenther. Publisher: Academic, London, Engl.

54:7633-7703

6- Jones, Barry Nicholas; Ansari, Hitzar R.; Jaggers, Brian G.; Janes, John F (1973) Odorous 7-hydroxy-2-methoxy-2,6-dimethyloctane. *Chem. Abstr.* 79:41918e

7- Newman, C P; Rossiter, K J; Sell, C S (1989) Preparation of dioxane derivatives as perfume components *Chem Abstr* 110:13401

8- Nicolaou KC et al., Eds, (2002) *Handbook of Combinatorial Chemistry: Drugs, Catalysts, Materials* (2-Vol. Set) Wiley-VCH;

9- Dyson, G. Malcolm (1928) Some aspects of the vibration theory of odor *Perfumery and Essential Oil Record*, 19, 456-9

10- Pauling, Linus (1946) Analogies between antibodies and simpler molecules *Chemical & Engineering News*, 24, 1064-5

11- Moncrieff, R W (1946) *Chemical Senses* New York John Wiley

12-Amoore, John E (1970). *Molecular Basis of Odor*. Springfield IL: Thomas

13- Rossiter, K. J. (1996) Structure-Odor relationships. *Chemical Reviews* 96: 3201-3240

14- Frater, G. Bajgrowicz, J.A. and Kraft, P. (1998) Fragrance chemistry. *Tetrahedron* 54 7633-7703

15-Buck L and Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell*. 1991 Apr 5;65(1):175-87.

16- Turin L. (1996) A spectroscopic mechanism for primary olfactory reception. *Chem Senses*. Dec;21(6):773-91

17- Dyson, G. Malcolm (1938) The scientific basis of odor. *Chemistry & Industry* 57:647-51

18- Wright, R.H. (1982) *The sense of smell* CRC press, Boca Raton, Florida, USA

19- Turin L and Yoshii F (2003) Structure-Odor Relations: a modern perspective in *Handbook of Olfaction and Gustation* Second Edition Doty R L, Ed. New York Marcel Dekker

20- Franks NP & Lieb WR (1994) Molecular and cellular mechanisms of general anaesthesia. *Nature*. 1994 Feb 17;367(6464):607-14

21- Meng EC and Bourne HR (2001) Receptor activation: what does the rhodopsin structure tell us? *Trends Pharmacol Sci*. 2001 Nov;22(11):587-93. Review.

22- Wannagat, U., Damrath, V, Huch V, Veith, M. and Harder, U. (1993) Sila-Riechstoffe und Riechstofffisostere XII. Geruchsvergleiche homologer organoelementverbindungen der vierten hauptgruppe (C,Si, Ge, Sn). *Journal of Organometallic Chemistry* 443:153-165

23- Zozulya S, Echeverri F, Nguyen T (2001) The human olfactory receptor repertoire

Genome Biol.;2(6):RESEARCH0018. Epub 2001 Jun 01.
24- Malnic, B.,Hirono, J. Sato, T and Buck L.B. (1999) Combinatorial receptor codes for odors. *Cell* 96:713-723
25- http://www.leffingwell.com/chirality/chirality.htm
26- Kennedy I J and Jane D E (2002) Stereochemistry in Drug Design in *Textbook of Drug Design and Discovery*, 3d edn. Pp 53-85
27- Ariens, E. J. (1986). Chirality in bioactive agents and its pitfalls. *Trends Pharmacol Sci 7*, 200-205.
28-Colquhoun, D. (1998) Binding, gating, affinity and efficacy: the interpretation of structure-activity relationships for agonists and of the effects of mutating receptors. *Brit J Pharmacol.* 125:923-947
29- Klopping, H L. (1971) Olfactory theories and the odors of small molecules *Journal of Agricultural and Food Chemistry*, 19(5), 999-1004
30- Goodman LS et al. (2001) *The Pharmacological Basis of Therapeutics*. McGraw Hill.
31- Socrates G (2001) *Infrared and Raman Characteristic Group Frequencies: Tables and Charts*, 3rd Edition New York John Wiley & Sons
32- Stock, A; Massenez, C Boron Hydrides *Berichte*. (1913), 45, 3539-68
33- Turin L. A method for the calculation of odor character from molecular structure. *J Theor Biol.* 2002 Jun 7;216(3):367-85
34- Buchbauer, G; Hillisch, A; Mraz, K; Wolschann, P (1994) Conformational parameters of the sandalwood-odor activity: conformational calculations on sandalwood odor. Part X *Helvetica Chimica Acta*, 77(8), 2286-96
35- Rossiter, K. J. (1997) The design and synthesis of novel muguet materials (Flavours and Fragrances), 21-35
36- Kraft, P Design and synthesis of novel violet odorants with bicyclo[6.4.0]dodecene and bicyclo[5.4.0] undecene skeletons *Synthesis* 1999 4, 695-703
37- Rakow NA, Suslick KS. (2000) A colorimetric sensor array for odour visualization. *Nature*. Aug 17;406(6797):710-3.
38- Wang J, Luthey-Schulten ZA, Suslick KS. Is the olfactory receptor a metalloprotein? *Proc Natl Acad Sci U S A*. 2003 Mar 18;100(6):3035-9.
39- Ishikawa Y, Kishi K Molecular Orbital Approach to Possible Discrimination of Musk Odor Intensity *International Journal of Quantum Chemistry*, 79, 109–119 (2000)
40- EU document SCCNFP/0017/98 Final December 1999 http://europa.eu.int/comm/food/fs/sc/sccp/out98_en.pdf

41- Bajgrowicz, Jerzy A.; Frank, Iris; Frater, Georg; Hennig, Michael Synthesis and structure elucidation of a new potent sandalwood-oil substitute *Helvetica Chimica Acta* (1998), 81(7), 1349-1358
42- Boelens, H (1975) relationships between the chemical structure of compounds and their olfactive properties. *Cosmetics and Perfumery* 89: 1-7
43- Knorr A and Weissenborn A (1929) *US Patent* 1,844,013