Structure/Odor Correlations: the Mechanism of Olfaction and the Design of Novel Fragrance Ingredients

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"One has to rely on chemists to find new aroma chemicals creating new, original notes. In perfumery, the future lies primarily in the hands of chemists."

Perhaps it is not too surprising that this affirmation came from Ernest Beaux, the perfumer who created Chanel 5. Up to the beginning of this century, perfumes were composed almost entirely of natural oils and extracts. In 1889, Jicky set a new trend by using some synthetic ingredients; coumarin, heliotropin and vanillin. However, these were nature identical, their natural counterparts can be found in, for example, tonka beans, heliotrope flowers and vanilla beans respectively. Beaux's use of totally synthetic aldehydes in Chanel 5 in 1921, was a much more significant watershed for the industry and was, in that sense, the beginning of modern perfumery. Since then, fragrance chemists have been engaged in an unending search for new ingredients. In fact, it is only through the use of synthetic ingredients that perfumers can create fragrances which will function well in consumer products such as soaps, detergents and household cleaners and at a price that everyone can afford.

The commercial importance of novel fragrance ingredients adds weight to our scientific curiosity in wishing to understand the relationship between molecular structure and odour. It is widely assumed that, if we could predict more accurately the odour of a putative structure before it was synthesized, it would greatly increase the efficiency, and hence lower the cost, of the search for novel ingredients.

Structure/activity relationships: All physical, chemical and biological properties of a substance are determined by its molecular structure. Therefore, in principle, if one knows exactly how a molecule interacts with a biological system, one could predict the effect it will have on that system. However, our current understanding of the interaction of molecules with biological systems is very far from complete. Indeed, our understanding of the nature of molecules is really only a simple picture and our ability to build effective models of them is limited. As far as biological properties are concerned, even if we were to understand exactly the nature of the receptor/substrate interaction, that would only be part of the picture since transport of the active molecule to the receptor is important. In fact, in some cases (eg anaesthesia), transport is the most important factor.

Therefore, we resort to structure/activity relationships (SARs). Whether we use sophisticated mathematical procedures or "chemists' intuition" to guide our thinking, these SARs are essentially statistical tools. The idea behind structure/activity correlation is to look for features of molecular structure that are held in common by materials with a common biological effect. It is then argued that other materials which also possess the same structural features are more likely to have that biological effect than those which do not.

The most obvious biological property of interest to perfumery is odour. In this paper, I would like to consider the scientific and commercial value of structure/odour relationships (SORs) and their links with theories of olfaction. There are a number of issues in the study of olfaction which the researcher must be aware of if he or she is to avoid falling into a practical or intellectual trap. The issues below should be considered when constructing SORs.

Organoleptic Purity

Consider the first side of the structure/odour equation, which is the structure. Whether or not the original workers were aware of it, the majority of published odour descriptions are of mixtures rather than single compounds. This is because of trace impurities, unresolved peaks on chromatograms, the presence of isomers and so on. In recent years, leading research teams, mostly in the major fragrance houses, have become increasingly aware of this fact

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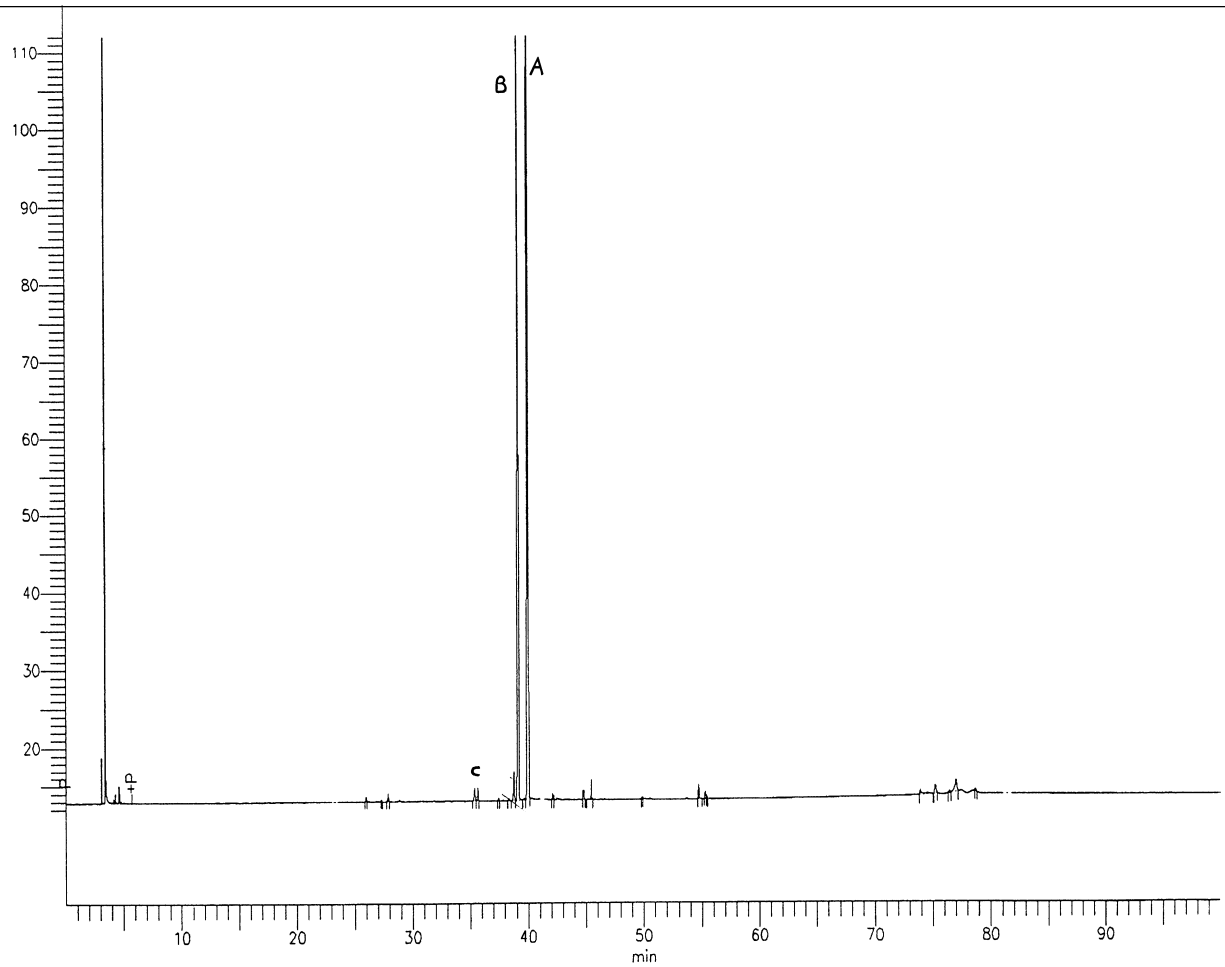

Figure 1. GC trace of a novel fragrance material

and are now using data from organoleptically pure samples for SOR work. However, the majority of historical data must remain suspect until verified using the latest techniques of chiral capillary gc coupled with gc olfactometry.

As an example of the type of problem which arises, Figure 1 shows the gc trace of a novel fragrance material which we prepared. There is one major peak, A, accounting for about 75% of the total material, just ahead of it is peak B which accounts for about 25%. Peak C is barely noticeable initially. This analysis was carried out on a modern high-performance capillary column. Ten or fifteen years ago, peaks A and B would probably not have been resolved. Peak A was the expected reaction product and peak B is due to a double-bond isomer. The mass spectra of the two are essentially identical and the nmr spectra are so close that suspicion would not have been aroused and it would have been concluded that the sample contained a single pure compound. The sample possessed a very strong floral odour, and so the odour would have been attributed to the structure responsible for peak A. However, gc olfactometry showed that the odour of the sample comes entirely from peak C. Peaks A and B are odorless. We have now isolated, characterized and patented the compound responsible for peak C. As it happens, the situation is even more complex than would appear from the figure.

The gc in Figure 1 was run on an achiral column. Compound A exists as *E*- and *Z*-isomers which were not resolved under our conditions, compound B possesses a chiral centre and thus exists in *R*- and S-enantiomeric forms and compound C exists as *cis*- and *trans*-isomers which were only just resolved on our gc. Therefore, in theory, on a more efficient and chiral column, each peak would resolve into two separate peaks

The first issue therefore, is to ensure that the sample under evaluation is organoleptically pure. In other words, the researcher must be confident that the odor described is due solely to the structure in question and that there is no contribution from trace impurities, isomers and the like.

Odor Measurement

Odor has three aspects: character, intensity and tenacity. When we use terms such as rose, spice, wood or musk, we are describing the character of the odor. We usually define the odor character of a material by putting it into a category

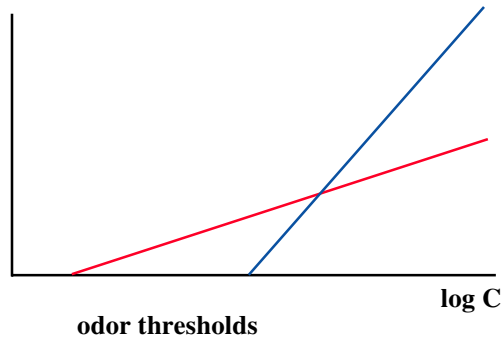

Figure 2. Steven's Law

or by comparing its similarity to other odorous materials. The character may be concentration dependent, and, in most published data, there is no indication that the odor character was evaluated at more than one concentration.

The intensity of an odor is the strength of the signal which we perceive. It is dependent on both transport properties (e.g. volatility, log P) and the receptor/substrate interaction. Intensity cannot be measured instrumentally and must rely on human assessments relative to a standard. Most assessors find this a difficult task to perform with confidence. Training is usually necessary. Care must be taken when measuring intensity because the perceived intensity is related to concentration, but not linearly and not consistently between different materials.

In order to measure intensity, it is necessary to measure concentration. This is much more difficult to do than simply smelling a sample on a perfumer's blotter. Because of this, published data usually omits concentration-dependent measurements. Furthermore, what concentration do we mean? The simplest concentration to measure is that of the odorant in the sample presented for assessment. It is probably more meaningful when we refer to concentration in the inhaled air. However, we must remember that the concentration of odorant at the receptor will depend on both concentration in the inhaled air and transport from that air to the receptor.

Threshold concentration, that is the lowest concentration at which the odor can be detected, is often used as a measure of intensity. This, however, can be misleading. One substance might have a much more intense odor than another at high concentration. However, if the intensity of the first falls off more rapidly on dilution than that of the second, the second may have the lower threshold concentration. This is shown as the Stevens' law plot of Figure 2.[1] Perceived intensity of any odorant varies from individual to individual. Any measurement must be carried out using a large number of individuals. The result should also be reported as a distribution since the spread of values will vary from one compound to another. In some cases, we even see two or more distribution curves for a single compound. In summary, intensities of odorants, or even orders of intensities within a group of odorants, cannot necessarily be compared meaningfully with each other if they have been measured under different conditions.

Tenacity refers to the time over which a material will be perceived after it has been applied to a support, such as paper, cotton, polyester, skin or hair. Obviously this will depend on the total weight applied, the volatility of the material, and interactions between the material and the support. However, because of the low levels of material involved, the measurement is usually by odor rather than by chemical analysis. The measured tenacity is actually a combination of physical tenacity and intensity.

Not surprisingly, the vast majority of published structure/odor relationships (SORs) refer only to character. I would advise any readers who wish to know more about odor measurement to start with the excellent account given by Neuner-Jehle and Etzweiler.[2]

Odor Descriptors are Associative

We do not know what the basic olfactory codons (letters of the olfactory alphabet) are, so we describe odors by association with references. There are two categories of reference standards. The first is the botanical source, which includes rosy, jasminic, herbal and woody. Such systems

lead to numerous problems when it comes to classification of the vast array of odor types. For example, heliotropin is usually classified with vanillin as sweet or powdery. Clove is considered a spice while labdanum is usually classified with ambergris. Lavender is usually classified as herbal. However, all of these are derived from flowers, so why are they not put in the same broad category as rose, jasmine, lilac and so on? Carnation is considered part of the floral category even though the major contributor to its odor is eugenol, the same component that leads to cloves being classified as spicy. In our own work,[3,4] we discovered that classification of apple and pear odors as fruity, led to problems because the structural requirements for each are distinct. Attempts have been made to use similarity measurements against the odor of a selected reference compound.[5] However, this approach is also subject to difficulties in interpretation.

Amoore and others tried to build classification systems based on anosmias.[6] However, this works on the assumption that the codon is odor-based. This is an unproved hypothesis and one which would seem improbable from an evolutionary viewpoint.

We therefore have no good system for the classification of odors and must always remember that our reference points for classification are almost certainly not primary points, but rather composites made up from a combination of different values of the physical and chemical properties; the basic codons. For example, rose might be a combination of signals from four receptors which recognize, respectively; the alcohol function, unsaturation, a specific range of molecular volume and a specific range of polarity.

Odor is Subjective

The subjectivity of odor is manifested at three levels; semantics, hedonics and, most importantly, physiology. The problem of semantics can be easily overcome within any one research group. For instance, at Quest, we train our perfumers, scientists and panelists to work to the same system of odor description and classification. The problem of semantics arises when results from different research groups are considered together because they may, and probably do, use different standards and classification systems. One is therefore always taking a risk when comparing odor data from different sources.

Strictly speaking, hedonic preferences should not affect odor descriptions for SOR purposes. One person will like eugenol because the odor reminds them of apple pies containing cloves. Another may dislike eugenol because of its association with dentistry. Both should be capable of describing it in the same way. However, in my experience, we tend to react more strongly to odors that we dislike than to those we like. Therefore, the person who dislikes eugenol may be more sensitive to eugenol-like nuances in a complex odour.

The physiological origins of subjectivity represent the greatest difficulty for SOR work. It is known that relative

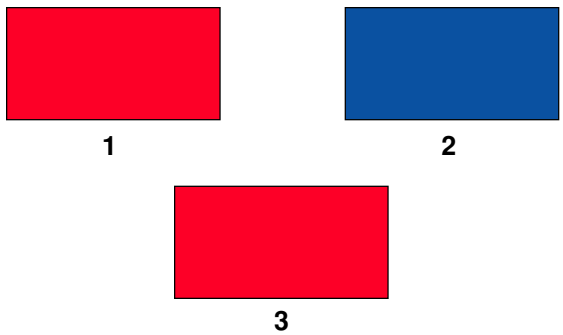

Figure 3. Color analysis

humidity will affect odor perception. So, for a very rigorous study, the humidity of the environment should be controlled. Adaptation (fatigue) and cross-adaptation (one material fatiguing the nose to a different one) to odors is well-known. Precautions must be taken when evaluating samples for study. An odor-free environment is necessary and sufficient time must be allowed between smelling different samples to ensure that cross adaptation is not distorting perception. This is particularly true in the musk, ambergris and sandalwood areas. These odor areas also tend to be the most intensively studied, partly because of the relative rigidity of the molecules. Again, literature data must be treated with caution. In my opinion, as far as SOR studies are concerned, the most serious issue of physiological subjectivity lies in the neurotransmission and interpretation processes in the brain.

An experiment which I have used to demonstrate this on many occasions with different groups of people, is shown in Figures 3-5. When asked to describe the color of rectangle 1 in Figure 3, everyone who is not color blind, replies "red." We do not, and cannot ever know whether the sensations that each one experiences are the same, but all have been trained to describe that sensation as red. Similarly, all will describe rectangle 2 as blue. Having agreed our red and blue standards, I now ask what color best describes rectangle 3 and am invariably given the answer "red."

At this point they are presented with a sample of cyclopentadecanolide and a sample of (+)-(Z)-α-santalol. For the sake of this argument, we will assume that they each can smell both samples and can easily distinguish between them. We now define cyclopentadecanolide as the standard for the musk odour and (+)-(Z)-α-santalol as that for sandalwood. They have now learnt to discriminate between musk and sandalwood in the same way that they discriminate between red and blue. Now I give each a sample of a third compound, Bangalol (2-ethyl-4-[1,1,2-en-5-yl]but-2-en-1-ol), and ask them to compare it with the two standards and describe it as either musk or sandalwood. If odor perception were to work in a similar manner to color vision, there would be a clear and unanimous decision about the classification of Bangalol. However, some people will identify Bangalol as sandalwood while

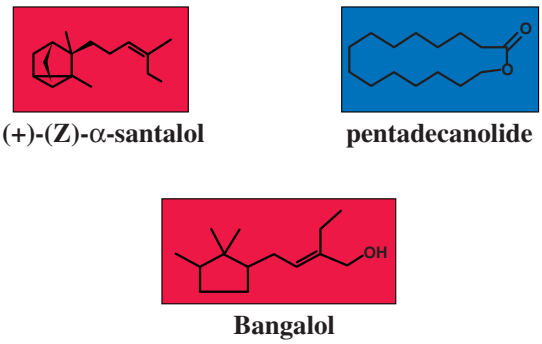

Figure 4. Musk/sandalwood triangle

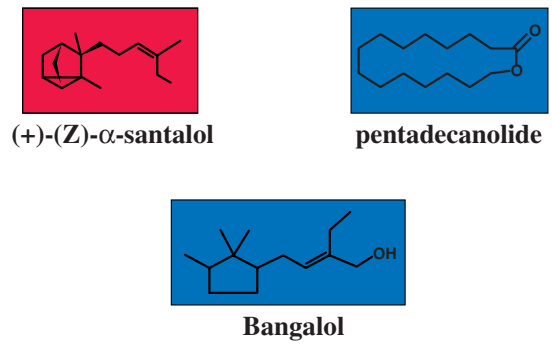

Figure 5. Musk/sandalwood triangle

others will perceive it as musk. In other words, some people see the musk/sandalwood triangle as shown in Figure 4 and some as in Figure 5. The situation is actually even more complex since some people will fail to perceive Bangalol at all (but these might also be among those who fail to perceive one or other or both of the standards) and some will perceive it as urinous. SOR studies almost invariably classify Bangalol as a sandalwood. This may be done because those who consider it to be sandalwood are in the majority, its structure resembles those of other sandalwood ingredients or because of the way it is used in perfumery. Whatever the reason for the classification of Bangalol as sandalwood, its use to relate structure to odor must be questionable. I have used Bangalol as an example in this experiment, but it is not the only compound that will give rise to the effect. There are many, many other compounds that could be used in the same way. Clearly, the phenomenon, known as parosmia, is an intrinsic feature of the sense of smell.

SORs on sandalwood and musk are useful statistical tools in that they indicate a probability that a putative structure will possess the required odour. However, the existence of the above, and similar parosmias must cast doubt on the concept of odor-tuned receptors. This raises the possibility that the phenomenon of odor only occurs higher in the brain as a result of interpretations of signals from receptors that are responding to physical and/or chemical codons. Experimental evidence for this latter hypothesis is now presented in Linda Buck's recent paper.[7] In this paper she demonstrates that smell is a combinatorial sense. Each type of receptor responds to a range of odorants. Each odorant triggers a range of receptor types. Therefore, for each odorant, the brain receives a pattern of signals coming from different receptor types. Recognition of the pattern then creates the sensation of odor. This generates enormous obstacles for anyone trying to design new odorants through understanding of odorant-receptor interaction. For every odor type, it would be necessary to know the primary, secondary and tertiary structure of not just one receptor protein, but of many. It would also be necessary to know how the signals are processed and handled right up into the higher brain. Cracking of such a code will probably be much more expensive than synthesis of test substances, at least for the foreseeable future.

Interpretation of SORs in terms of theories of the initial receptor event in olfaction is fraught with intellectual danger. Additionally, no theory can ever be established beyond doubt; the exception proves (that is, tests) the rule. At present, there are no theories of the primary-receptor event in olfaction which are free from exceptions. Therefore there are no adequate theories.

The Mechanism of Olfaction

Smell, like our other senses, is intriguing. An understanding of how it works would be of immense scientific interest. If that understanding were to help design a new and better generation of fragrance ingredients, it would also be of commercial interest. Therefore, it is not surprising that the search for that understanding is being carried out on all fronts. Some workers have started with observed SORs and then tried to postulate a mechanism based on these. Others have started by proposing a mechanism and then looked for SORs to support it. Because sound biochemical evidence concerning the initial receptor event was very scarce until recently, most of the theories of olfaction to date have relied on one of these two SOR-related approaches. There are two serious intellectual traps that must be avoided if one ventures down this course.

Correlation Does Not Necessarily Mean Causality

There is a good correlation between the weight of apples exported from the state of Washington and the divorce rate in England and Wales. Does this mean that American apples cause English divorces or *vice versa*? Neither! The correlation is purely coincidental.

If something produces two or more effects, then there will be correlations between the effects. It is therefore important to establish which is the causative agent, otherwise one may fall into the trap of mistaking one of the effects for the cause of another. The molecular structure of a substance determines all of its properties. Therefore, it is to be expected that correlations will be found between the properties. The odor of a substance (that is, its effect on the biological system which gives rise to the sensation of odor)

is an effect of its molecular structure. Its water solubility and its spectroscopic properties, for example, are also effects of its structure. Therefore it would not be surprising if correlations were to exist between these properties. However, the existence of such correlations would not necessarily imply a causal relationship.

Discrimination Might Occur at More Than One Point

One common assumption in the study of olfaction is that all of the discrimination occurs at one point, that is when the odorant interacts with the receptor protein. However, there is no proof for this hypothesis. Indeed, there are many possibilities for discrimination at other points:

- the interaction of odorants with odor-binding proteins
- the interaction of odor-binding proteins with receptor proteins
- the patterns of spreading of receptor firings across the epithelium
- in the concomitant input from the trigeminal nerve
- in the handling of the nerve signals in the olfactory bulb and on up in the higher brain

It is notoriously difficult to develop a good SAR for a multi-stage process. Could the difficulties experienced in SOR studies be an indication that, in olfaction, we are dealing with a multi-stage process? What does this mean for those of us who are involved in the search for novel fragrance materials? A great deal has been written on the correlation of odor character with molecular structure and the help which this might lend the discovery chemist in the fragrance industry.[8] However, odor character is only one of the many things required of a fragrance ingredient.

Commercial Considerations

What makes a commercially successful novel fragrance ingredient? There are four main factors to consider, and each can be divided into two or more aspects as shown in Table 1.

The problems involved in distinguishing and measuring the three aspects of odor are discussed above. All that is needed here is a reminder that most SORs consider only odor character and do not specify the concentration at which it was measured.

When a perfumer is shown a novel material for the first time, the response they give to the chemist is usually along the lines of, "Rosy, like geraniol. How does its performance compare with that of geraniol?" By performance, they mean the organoleptic stability in products such as soap, detergent, hypochlorite bleach, peroxide and so on;. the ability of the material to cover the odor of the base and to project a pleasing odor from it; and the way in which it works with other odorants in a perfume formula.

Table 1. The factors that determine the commercial success of novel fragrance ingredients.

Odor	**Performance**
character	in products
intensity	
tenacity	
Availability	**Safety**
raw materials	during manufacture
process	in use
plant	in the environment
price	in perfume formulae

In the next breath, the perfumer will ask, "What do you expect it to cost?" They will assume that, if they are offered the material, they will be free to include it in a formula at any level they choose and submit the resultant perfume against a brief for any volume of business. In other words, the perfumer may establish a requirement for anything from a few kg to thousands of tonnes *per annum*. Thus, a material will only be successful if it can be made available at a competitive price and in any quantity requested. The factors controlling this are listed under availability. Only a chemist who has had some experience in the process side of the fine-chemicals business is in a position to incorporate this thinking into the design of novel odorants.

The other assumption the perfumer will make will be that of safety. I have come to this last, not because it is least important, (on the contrary, it is of utmost importance) but because it is usually the most expensive part of the development process and therefore left to the end. Only when everything else is right, does one embark on the procedures for ensuring that the material will be safe to produce and use.

Determination of the odor character is therefore only a very small part of the selection process for novel fragrance ingredients and is usually the least expensive. The fragrance chemist needs to prepare only a tiny quantity of a substance to know which odor class it belongs to. I find that an experienced fragrance chemist will predict the odor class of a material correctly about 50% of the time, dependant on the odor class concerned. The accuracy will be higher in some areas, such as musk, and lower in others, such as various floral categories. The savings on increasing the accuracy of prediction from 50% to 100% are therefore likely to be relatively small.

Conclusions

Structure/odor correlations have a role to play in assisting the design of novel fragrance ingredients. They are statistical tools that serve as a guide. However, the fragrance chemist must be aware of their limitations and remember that they are only guides and have a limited accuracy. Furthermore, like all tools, they must be treated with care. Good data sets are expensive to obtain. Assurance of organoleptic purity requires expensive equipment and a great deal of operator time and expertise. Odor data is never entirely satisfactory since our standards and classification systems are not optimal and anosmia and parosmia distort results.

Companies must be aware that they must always hold the whole picture in view. When starting a research project aimed at novel fragrance ingredients, it is important to consider not only odor type, but also intensity, tenacity, safety, production cost, security of supply and additional benefits. We look for more accurate predictions of the properties of materials, including SORs, but we also seek continual improvement in the cost of making and screening candidates.

We have found that the instinct of an experienced fragrance chemist is, at present, superior to the best SORs in predicting odor class. This might change, but the savings generated by increasing the reliability of predicting odor type may be less than the cost of producing these superior predictions. However, the experienced fragrance chemist holds in their head, not only a set of SORs for odor-type prediction, but also the knowledge of chemical reactions and processes and feedstock availability that are equally critical in the design of novel materials. These additional fields of knowledge are also stored in computer databases and are helpful in the discovery process. The one thing that computers cannot supply is the creativity of the chemist. I am therefore convinced that chemists will continue to be of vital importance to the industry, just as Ernest Beaux predicted.

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