Rational Odorant Design: Fantasy or Feasibility?

Exploring the two approaches to rational odor design and the possibilities of each

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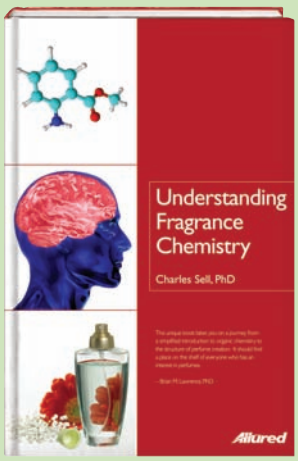
Why do we strive for rational design of odorants?

Since the birth of synthetic organic chemistry in the mid-19th century, fragrance chemists have sought to design and produce fragrance ingredients to supplement those obtained from plant (and formerly animal) sources. The earliest successes were nature-identical materials such as coumarin, heliotropin and vanillin, all of which were used in 1889's *Jicky*. Bertagnini's aldehydes were already known at this time but it was their successful use in 1921 by Ernest Beaux (to provide a new top note character manifested in *Chanel No. 5*) that really stimulated the search for other novel fragrance ingredients.

There are many reasons why this search continues. In addition to originality of odor, cost, security of supply and performance in difficult media (such as laundry detergent), safety and sustainability are becoming increasingly important as plant-derived ingredients are being lost from the perfumer's palette through unsustainable production (e.g., sandalwood, rosewood) or safety issues (e.g., sasafrass, fig leaf).

Screening of potential new ingredients requires time and involves expense in materials and equipment because in order for a material to pass an initial odor screen it is evaluated in a range of consumer products such as soap, laundry detergent, shampoo, conditioner, etc. Obviously, it is desirable to evaluate as few materials as possible and therefore the fragrance chemist will seek to synthesize only those materials with a high chance of passing the screening criteria. This is where rational design comes into play. Rational design uses our knowledge and understanding of fragrance in order to predict molecules with a better than random chance of making the grade as perfumery materials.

Understanding Fragrance Chemistry



For all of those working in the fragrance business and the consumer goods industries it serves, knowledge of chemistry is invaluable in understanding how fragrance is produced, how it works and the factors that control its performance in products. Charles Sell's recently released book, *Understanding Fragrance Chemistry*, concentrates on the aspects of organic chemistry that are of particular importance to the fragrance industry. Topics include: the structure of matter, organic molecules, chemical reactivity, acid/base reactions, oxidation and reduction reactions, perfume structure, chemistry in consumer goods, the biological way we detect odors, how nature makes fragrant molecules, and much more.

Purchase *Understanding Fragrance Chemistry* today by visiting *perfumerflavorist.com/bookstore*.

What must be taken into consideration when designing a novel fragrance ingredient?

The most obvious requirement for a fragrance ingredient is odor. Normally, a pleasant odor character is desired but there are quite a few widely used ingredients (e.g., indole) that few would describe as pleasant, and there are thousands of different pleasant odors that all find use. The same is true with tenacity, intensity and threshold; perfumers use ingredients across the scale for each. So, in terms of odor, there are no right or wrong answers, merely different blends of character, intensity, etc.

In contrast, in the field of safety, wrong answers are definitely possible. Any new substance that could harm production staff during manufacture, the consumer (when in use) or the environment (after use), will not survive the screening process. Substances that do not perform well in perfume formulae or in consumer goods will also be likely to fail in screening. Price is an important consideration because any new ingredient will be in competition with those already on the market and, unless it is more cost-effective than these, it will be unlikely to survive. Secondary benefits are not usually the cause of failure in screening but might well help a borderline material make it through the process to commercialization.

Structure/Activity Relationship (SAR)

There are two basic approaches to rational design of molecules. The first is based on Structure/Activity Relationship (SAR). If the data used in building an SAR has a numerical basis, then the SAR might be described as

a Quantitative Structure/Activity Relationship (QSAR).

The basic principle behind SARs is that the molecular structure of a substance determines its entire properties: physical, chemical and biological. Therefore, if we take a set of molecules with similar activity and find the structural features they have in common and that inactive molecules do not possess, then, by designing new molecules possessing these structural features, we improve the chances of making novel substances with the desired properties.

The most publicized SARs in the fragrance chemistry business are built on odor character. The best example is John Amoore's model for camphoraceous odorants. He proposed that the requirements for a molecule to have such an odor are that the molecule should be hydrophobic and have an ellipsoidal shape with a long axis of 9.5 Å and a short axis of 7.5 Å. This is a simple yet very good model and the author knows of no exception to it. Unfortunately, new camphoraceous odorants are not highly sought after.

As stated earlier, safety is an increasingly important topic, and so fragrance chemists nowadays use SARs on toxicological properties in order to help in the design of new, safer ingredients. Features of molecular structure that are associated with skin sensitization potential or poor biodegradability can therefore be avoided in molecular design.

When it comes to performance of an ingredient, its performance in products can usually be addressed through mechanistic understanding, which is discussed later in the article. Performance in perfume formulae, that is, how well the material blends with other ingredients, is something that a perfumer learns over years of training, but tends to be overlooked by discovery chemists as a target for SARs. Perhaps the reason for this is that performance in formulae is difficult to define and measure.

Secondary benefits such as deo-activity and insect repellency can also be the subject of SAR studies, although odor, safety and performance properties will always take precedence in the fragrance industry.

Availability at the right price and in the right quantity is important for any new fragrance ingredient. Prediction of price and availability will be in the mind of any good discovery chemist and he will use his knowledge of available raw materials and process technology to aim for new ingredients that will fall in the right range. This way of thinking about design can complement an SAR or can serve as a source of inspiration in its own right, but it is unlikely to be classified as an SAR technique per se.

Limitations of SARs: One limitation of SARs is that they are, by their very nature, interpolative. In other words, the predictions obtained from them will always point back towards the types of molecules used in the data set from which the SAR was derived. For example, if given only nitro-musks, then any SAR derived from them would predict more nitro-musks and would not indicate that macrocyclic ketones or lactones should be synthesized. Novel classes of odorants are much more likely to be discovered by serendipity than by use of SARs.

As a general rule, the more steps there are between the molecular structure and the activity in question, the more difficult it will be to find a good SAR, the less reliable that SAR will be and the less correlation there will be between the SAR and the underlying mechanism. At one end of the scale, a simple physicochemical property such as boiling point depends entirely on the molecular structure, and there are very good algorithms for boiling point prediction that mostly give results close to the measured boiling point. At the other end, for biological activities, there are usually a number of steps between structure and effect and thus the SARs become less accurate and less meaningful in mechanistic terms. Odor involves a particularly large number of steps from an airborne molecule to an odor impression in the higher brain (as will be described later in this article) and so it is not surprising that odor SARs are less precise.

Mechanistic Understanding

The other approach to rational design is to develop an understanding of the mechanism by which the desired effect is produced.

Performance of an ingredient in products is usually a simple matter of

chemistry and, in many instances, the fragrance discovery chemist will apply his understanding instinctively in design. For example, when designing new molecules for use at high pH, the chemist will not consider structures containing easily hydrolyzed ester groups. Release from the product matrix to the headspace is less straightforward but an understanding of affinity between molecules and the mathematics of physical chemistry will allow prediction of, by way of example, diffusion of an ingredient from a soap bar to the air around it. Such predictions will be verified by testing during the screening process.

For biological properties such as skin sensitization potential and biodegradability, an understanding of the biological mechanism can greatly assist in the development of SARs. For example, one key step in the chain of events that leads to skin sensitization is the modification of a native skin protein. Thus, it is known that good, "soft" electrophiles are more likely to display this property and chemists can build SARs around that facet of chemical reactivity. Similarly, the β-oxidation pathway is an important route by which bacteria degrade organic molecules and so chemists can look for new molecules that are amenable to it.

Odor Character SARs

As stated earlier, the most talked about SARs in our industry are those for odor character. It is also in this area that there seems to be the greatest confusion as to the boundaries between SARs and mechanistic understanding. A structure/odor relationship is a statistical model based on finding common structural features of molecules that possess a certain odor and that are not possessed by molecules that do not have that odor. John Amoore's camphor model (as discussed previously) is an excellent example and could be used to predict new molecules with a camphoraceous odor. It might give chemists clues about some parts of the overall mechanism of camphor perception, but it does not constitute a full explanation of it. Some SARs are based on individual steps in odor perception, and examples covering the activation of one particular receptor (OR1D2) are discussed later in this article. These SARs might provide useful information about that specific step, but they do not constitute a total explanation of the whole process of odor perception.

From odorant to perceived odor: There are many steps between having an odorant and forming an odor percept in the conscious brain. First, the molecules have to be delivered to the air in the nose. This involves release from the substrate in which they are contained, be that a bar of soap, a perfumer's blotter or just a liquid comprised of other molecules of the same odorant. This is a physicochemical process and the basic principles are well understood. The odorant must then travel from the air in the nose to the receptor. Humans each have two nostrils and two nasal cavities each with their own olfactory epithelium (the tissue containing the receptors). The air flow through each nostril is always different and so the pattern of adsorption of the odorant into the mucus will be different in each epithelium. This gives a dynamic difference in signals coming from each set of receptors and such effects have been shown to aid in odor discrimination. The mucus contains many different chemical species that could affect odorant molecules before they reach the receptors. For instance, it has been shown that enzymes in the nasal mucus (cytochrome P450s) can oxidize odorants before they reach the receptors, meaning that the receptors actually detect metabolites of the odorants as well as the odorants.

Next is the recognition of the odorant molecules by the receptors. Humans use about 350–400 different types of receptors. Each receptor type responds to a variety of odorants and each odorant activates a variety of receptor types. The olfactory receptors (ORs) belong to a family of proteins known as G-protein coupled receptors (GPCRs). Chemists now have crystal structures of several GPCRs and from these they can build computer models of ORs, based on the primary structures of the latter (which are known from the work on the human genome). Biochemists are able to clone olfactory receptors into cells in tissue culture and study their responsiveness to various odorants. Thus, it is now possible to compare computer model predictions of odorant/receptor binding against real results from living cells.

Two recent research publications will serve to illustrate some points about this step in perception.[1,2] Both papers concern the human olfactory receptor OR1D2 (formerly known as hOR17-4) that is found in the nose and in sperm. In the first paper, Doszczak et al. predicted the binding affinity of a number of odorants to the receptor, synthesized the molecules and measured their activity towards the receptor using sperm cells and human kidney cells. They found a good correlation between the predicted binding and the biological activity, thus providing strong evidence that stereo-electronic factors govern the receptor-odorant interaction.[1]

In the second paper, Triller et al. measured the activity of a larger range of odorants with respect to the same receptor, this time cloned into cultured human kidney cells. Odorants that did activate the receptor displayed a wide variety of odor types including muguet, floral, balsamic, fruity, green, anisic and citrus. However, all of these odor types were also displayed by materials that did not activate the receptor. This is clear evidence that there is no simple correlation between activation of a single receptor type and the ultimate odor percept in the brain. In other words, chemists are nearing a point where they will be able to design novel odorants to activate a specific OR, but that will still not allow them to determine in advance the odor character that will be perceived.

The signal combinations that are generated in the olfactory epithelium are coded onto the olfactory bulb—the first part of the brain to start decoding the message from the nose. The signals from each receptor type all converge onto a single point in the bulb, known as a glomerulus. So, for example, all the OR1D2 receptors in the epithelium will send their signals to the same glomerulus. Humans each have two olfactory bulbs, one for each olfactory epithelium. The signals from each nostril are not combined until the next stage of processing.

The number of steps involved in signal processing now increases and many of them run in parallel with feedback from higher in the brain able to magnify, reduce or even eliminate signals coming up from the bulb. Thus, as

expressed by Wilson and Stevenson in their excellent book *Learning to Smell*, "knowledge of the physicochemical features or olfactory bulb maps evoked by those features is insufficient to predict the ultimate olfactory percept."[3] In this book, the authors give a clear picture of the many steps involved in neuroprocessing of the olfactory signal between the olfactory epithelium and the highest parts of the brain, which is where the phenomenon of odor comes into being. They also describe in detail how it has been shown that experience, expectancy and context all play a role in how odors are perceived.

Each human uses his or her own combination of 350–400 receptors from the total pool of about 1,000 and so, even at this most basic level, odor is subjective and we each have our own perception of the odorous world in which we live. Furthermore, since experience, expectancy and context all affect odor perception and are specific to an individual person and a time, then odor perception is certainly very subjective. In order to know exactly how an individual will perceive an odorant molecule, chemists have to know not only the structure of the molecule but also which receptors that individual uses, which of these receptors will respond to the odorant and how strongly, how the receptors code onto his/her olfactory bulbs, how the various parts of his/her brain will handle the signals originating from the bulbs, what his/her previous experience of smelling is, what he/she expects from this odor and the context of the present experience. Chemists are a very long way from knowing enough to be able to predict odor with certainty, based on mechanistic knowledge.

Conclusion

In conclusion, I would argue that rational design has been with us since the earliest days of fragrance chemistry and always will be. Generations of fragrance chemists have used their knowledge of chemical mechanism and SARs (both formal and instinctive) in the design of the new materials that have so enriched perfumery. Anyone who would dismiss the work of fragrance discovery chemists as mere hit-and-miss random synthesis would do the chemists a great injustice and only expose their own ignorance of the complex process of olfaction. What we all must remember is that SARs and mechanistic understanding are not the same thing and both will continue to contribute to rational odor design. For at least the short- to medium-term future, mechanistic understanding will play the greater role in prediction of physical and chemical properties, whereas SARs will be more important when it comes to prediction of biological properties.

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