Biotechnology Ushers in a New Era of Innovation for Perfumers and Flavorists

Recent production milestones demonstrate commercial viability of biosynthesis.

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For a moment, imagine that the constraints of ingredient scarcity and price were no longer issues in the flavor and fragrance industry. Freed from these shackles, what products would the industry create? What fragrances and flavors would be incorporated into which products?

Limitations on supply, whether from diseases like the citrus greening currently ravaging Florida's crops, or overharvesting that has threatened prized species such as sandalwood, have long posed a challenge for the flavor and fragrance industry, and limit its ability to fully leverage some of the most desired aromas and flavors.[1,2]

The Evolution of Chemistry for Flavors and Fragrances

Extracting the aromatic principles of plants dates back to the times of ancient Greece, and was the principle means for creating perfumes through the 1700s in France and other European countries. In the 1800s, chemical analysis of essential oils, the aromatic volatiles of plants, led to the isolation of the molecules responsible for aromas. By the early 20th century, the skills of organic chemistry had joined those of analytical chemistry, enabling scientists to create aromas and flavors in the laboratory.

Despite the progress that has been made, the sesquiterpenes and diterpenes of importance to the industry have complex cyclic structures that can only be reached by lengthy, and hence uneconomical, chemical processes. Therefore, they exist as isolates from an essential oil or as components of an essential oil that has been modified by a simple synthesis.

Sesquiterpenes and diterpenes are found naturally in plants and insects as secondary metabolites (chemical substances that are not involved in the normal growth, development, or reproduction of the plant), but are used to protect the plant against insects, microorganisms and herbivores. The complex cyclic structures of sesquiterpenes and diterpenes are responsible for their properties and functionality.

Terpenes (also terpenoids or isoprenoids) are the most structurally diverse class of biological compounds, with some estimates of their total running as high as several hundred thousand. Sesquiterpenes are a class of terpenes that consist of three isoprene units and have a backbone comprising 15 carbon atoms, while diterpenes are built from four isoprene units and thus contain 20 carbon atoms. Sesquiterpenes and diterpenes generally have three or more chiral centers with very specific arrangements of carbon atoms that are synthesized by enzymes in plants, but unfortunately not in test tubes by chemists.

Using genomics, plant science and genetic engineering techniques, scientists either isolate the gene of interest from its natural source or, if the gene sequence is known, have it synthesized.

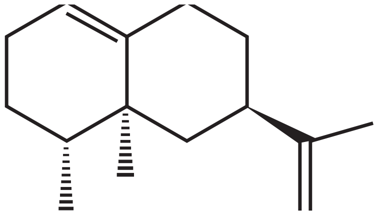
Richard Burlingame, vice president of R&D at Allylix, with a batch of valencene.

In recent years, several platforms for biosynthesis for the production of terpenes have emerged, including fungal strains, yeast and bacteria. And progress in biotechnology may finally be ushering in a new era where sesquiterpenes and diterpenes are readily available, through biosynthesis.

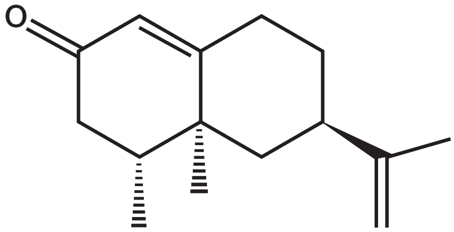
Biosynthetic Technology

Low-cost and high-value terpenes can be produced from yeast fermentation for application in flavors and fragrances. While this type of technology can produce a wide range of specialty chemicals, including pharmaceuticals, the sesquiterpenes valencene (**F-1**) and nootkatone (**F-2**) are being produced in commercial quantities.[3]

F-1. Valencene (FEMA# 3443; CAS# 68773-84-2)



F-2. Nootkatone (FEMA# 3166; CAS# 4674-50-4)



Recent production of large commercial quantities of such materials via biotechnology represents a big milestone. Achieving such quantities involves identification of new sesquiterpene synthetases, enhancement of the enzymes by protein engineering and optimization of the yeast reactions by fermentation engineering.

The first step in developing a terpene biosynthetic process is the isolation of a terpene cyclase gene from its natural source, generally a plant. Using genomics, plant science and genetic engineering techniques, scientists either isolate the gene of interest from its natural source or, if the gene sequence is known, have it synthesized. Inserting a terpene cyclase gene into yeast confers upon the yeast the ability to produce an enzyme that catalyzes the production of a terpene of interest. For example, if a valencene synthase gene is inserted into a yeast strain, expression of that gene results in the production of a valencene synthase enzyme that yeast do not normally produce. When the yeast produce that enzyme, they are able to convert a precursor to valencene. The isolated gene is then further engineered to optimize its expression within the host strain.

Sometimes, a natural gene or enzyme, even after being engineered for optimal expression, will not have high enough activity for economical terpene production. Other times, an enzyme will produce a mixture of products that is not ideal from a commercial perspective. In other words, genes in nature are not always sufficiently active, nor sufficiently specific to the terpene of interest, to make them useful for industrial production. To overcome these challenges, protein engineering can be applied to alter the activity and specificity of the terpene cyclase using discreet changes so that it efficiently produces the high-purity terpene of interest. Using this process, in conjunction with other strain and development technologies, production capacity can be increased by multiples in the hundreds or thousands, from barely detectable to high concentrations in fermentation.

Once the genes are optimized, they are introduced into host strains of yeast that have been optimized for the production of terpenes. Sesquiterpene production can apply yeast host strains that have been engineered to overproduce their 15-carbon biosynthetic precursor, farnesyl pyrophosphate (FPP). FPP is the precursor compound to sesquiterpenes and is converted to products of interest through the action of enzymes known as sesquiterpene synthases. As noted earlier, the introduction of genes for sesquiterpene synthases from plants or fungi into these engineered yeast strains confers upon them the acquired ability to produce sesquiterpenes. What's produced is dependent on the nature and source of the newly introduced gene and enzyme. Thus, the development of new products requires only an incremental investment of effort.

Biosynthetic processes can produce the complex cyclic hydrocarbon structures that underlie the sesquiterpenes and diterpenes of interest to the flavor and fragrance industry. This technology allows for cost-effective and sustainable production of a wide range of sesquiterpenes and diterpenes. The molecules that can be produced through biosynthesis have the potential to fill in many of the gaps that now exist due to the high cost or scarcity of essential oils.

Terpenes produced through [such] platforms are natural, per U.S. and E.U. definitions, in that they are exactly the same as the terpenes that are derived from natural sources. There are no genetically modified organisms in the final product and the final product itself is not a genetically modified organism. For this reason, biotechnology derived terpenes and other products are not required to be labeled as genetically modified organisms (GMO) in the United States and Europe.

Implications for the Flavor and Fragrance Industry

Currently, the biggest restrictions to flavor and fragrance creation, other than regulations, are formula costs. For example, a perfumer has to meet a cost target of around $3/pound for a laundry detergent fragrance, and around $20/pound for a prestige perfume.

If there were no limits imposed by cost and supply, the household product perfumers could use some of the great smelling and long-lasting molecules like patchouli alcohol or β-santalol. They could strive to make long-lasting and natural-smelling fragrances that have a richness and character that cannot be achieved within the current cost structure.

With cost and supply limits removed, prestige fragrance perfumers would be freed to immediately add greater levels of precious essential oils to formulas and make fragrances like the classic *Joy* (Jean Patou), which contains a significant amount of natural ingredients and currently sells for about $350 per ounce at Neiman Marcus. They would use rose absolute, jasmine absolute, the best grades of vetiver oil, the best grades of sandalwood oil, osmanthus absolute, violet absolute, natural vanilla tincture, etc. In addition to naturals, formulators would also have broad access to synthetics, including macrocyclic musks such as ambrettolide, and natural lily of the valley notes such as l-dihydro farnesol. This would provide fragrances that have wonderful natural top notes and dry out to very rich woody and oriental notes. At present, the most popular fragrances are rarely sold at perfume concentration (20%), but rather at cologne concentration, which is 10% perfume oil in alcohol. Few, if any, contain significant amounts of precious essential oils.

After nearly 100 years of hard work, synthetic organic chemists have been able to identify and synthesize most of the top notes that are now used in perfume creation.[4] With respect to top notes, the ones that are not economically available synthetically are the ones that have a chiral center, such as l-citronellol. The class of molecules that are not available by synthetic organic chemistry and that are supplied by essential oils are the sesquiterpenes and diterpenes, which have three to five chiral centers. Supplying these molecules will allow the perfumer to make reasonable renditions of the essential oils that are now not available due to cost.

To summarize, biosynthesis yields natural products that do not require genetically modified organism labeling in the United States and Europe. The platforms can also create novel sesquiterpenes and diterpenes. The method provides a stable and sustainable supply, using abundant and inexpensive agriculturally derived raw materials. Products from biosynthesis technology are also of consistent quality and are not subject to the annual and seasonal variations found in products extracted from plant materials.

While showing promise for decades, important milestones of the viability of biosynthesis to create commercial quantities of complex terpenes have been reached, bringing stable supplies of highly desired fragrances and flavors within economical reach.

For these reasons, the industry stands at the cusp of what will someday be recognized as a major shift in its history, the opening of a door to a new era in which it is possible to create complex ingredients using biosynthesis, and to be free of some of the limitations in supply and price that have long plagued product development and delivery of flavors and fragrances.

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