Learning from nature

High-Impact Aroma Chemistry

The challenges and opportunities of sourcing high-impact aroma chemicals from nature

David Brassington, Oxford Chemicals Ltd., and Robert Edwards, University of Durham

As part of a long-term program to source more natural high-impact aroma chemistries (HIAC), Oxford Chemicals Ltd. (OCL) and the Center for Bioactive Chemistry, Durham University, have been collaborating on the bioproduction of sulfur-containing volatiles. Robert Edwards (Durham University) and David Brassington (OCL) examine some of the technical challenges and opportunities of sourcing HIACs from nature.

Plants as Biofactories for Flavors and Fragrances

Plants already are used extensively as sources of natural materials in the F&F industry, with essential oils being an excellent example. Unlike synthetic sources, naturals contain multiple components and can be unpredictable in terms of quality and quantity. As the continuing increase in oil prices makes plant-based renewables increasingly attractive as alternative feedstocks for biofuel and bulk chemical production, it is timely for the F&F industry to review recent progress in natural product biology and chemistry, and to evaluate future opportunities for harnessing biodiversity for its own applications.

One of the primary objectives in using plant-based feedstocks in any industrial application is to standardize the composition of the starting material as much as possible, ensuring its suitability for downstream processing. For example, in order to derive oil-based products for large-scale extraction, it is desirable to control the chain length and degrees of saturation of plant lipids. This can be achieved by genetic engineering technology. Alternatively, genes encoding specific quality traits can be identified by searching within the naturally diverse genetic pool from which modern crops are derived. Once identified, these useful trait genes can be crossed back into high-yielding modern crops using classical breeding techniques for large-scale production of the new feedstocks. Although such an approach has been applied for polysaccharide and oil-based products, the technology remains underutilized by the F&F industry.

In addition to the benefits of moving to technologies based on renewables, the use of naturals rather than synthetics has obvious advantages in terms of consumer acceptance and also may help to develop new marketing opportunities through scientifically defined health benefits.

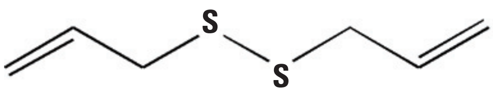
Allium Aroma Chemistry

Among the high-impact aroma chemicals (HIACs), the sulfur-containing materials from *Allium* species (e.g., garlic, onion) illustrate several useful challenges and opportunities in applying biotechnological approaches for HIAC production and the development of new "value-added" markets for these naturals.

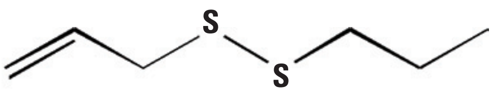
Biological Control of *Allium* HIAC Production

The two best-known groups of *Allium*-derived F&F principles are the allyl or prop-2-enyl sulfides from garlic, and the saturated methyl and propyl sulfides from onions. These form a series of mercaptans, sulfides and trisulfides with distinctive odor characteristics (Figure 1). Some 50 volatile *Allium* sulfides

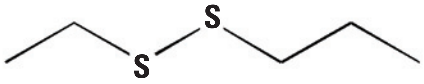
(1) Allyl disulfide (OCL code A0600), (2) Allyl propyl disulfide (OCL code A0960), (3) Ethyl propyl disulfide (OCL code E01282) and (4) Ethyl trisulphide (OCL code E01289); all of these molecules have differing nuances in dilution, offering more than just a basic onion/garlic note to the flavorist **F-1**



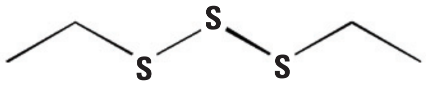
(1)



(2)



(3)



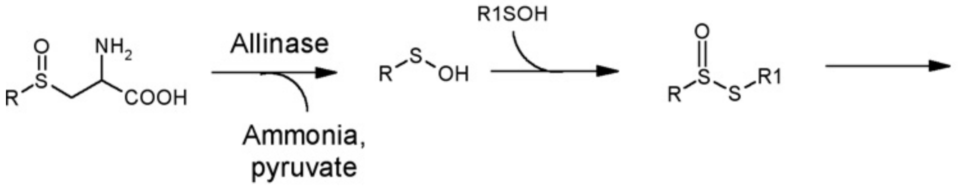
(4)

have been described, arising from six precursor *S*-alk(en)yl-L-cysteine sulfoxides (ACS), of which only four are found in appreciable quantities (Figure 2). These can accumulate up to 1 percent of total bulb fresh weight in harvested plants, such as garlic. Upon tissue damage, the ACSs mix with the plant enzyme alliinase, which releases a reactive sulfenic acid intermediate, along with pyruvate and ammonia (Figure 2). The sulfenic acids then undergo condensation reactions with one another to form either symmetrical or asymmetrical thiosulfinites, which then will readily undergo further reactions and decomposition to form the HIACs shown in Figure 1. Whereas a skilled chemist can partially control the products derived after thiosulfinite formation through selective heating and distillation, all the steps providing the basic substrates for the reactions are determined by the relative rates of ACS turnover by the alliinases in the plant extracts. Because a diverse set of natural HIACs can be generated from a small number of biological precursors, controlling the availability of the ACSs would standardize the biological material prior to distillation. Although attempts to regulate ACS content by controlling plant nutrition and growth have been partially successful, a far more desirable strategy would be to achieve selective and stable production through focused breeding for the respective metabolic traits. When combined with selective downstream processing during extraction, this could be extremely useful in producing specific natural HIACs to order.

Potential for Extending *Allium*-Related Natural HIAC Diversity

Although the large-scale commercial production of these sulfides is restricted to *Allium* species, the ability to produce related compounds is widespread in nature and has been described in *Brassica* species, several tropical plants and basidiomycete fungi (Figure 3). These represent cases in which characteristic garliclike notes have been easily detected, but this probably characterizes only a small fraction of the natural biodiversity of this type of chemistry. An interesting feature of the ACSs is that they are closely related to common plant primary metabolites, so that they have the potential to arise as byproducts of essential pathways in even distantly related species. As potential sources of natural novel HIACs, these alternative biological sources may be of future commercial value when

HIAC formation from ACS precursors F-2



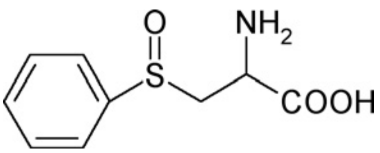
R, R_1 = $—CH_3$, $—CH_2—CH{=}CH_2$, $—CH_2—CH_2—CH_3$, $—CH{=}CH—CH_3$

the principles of selective breeding and molecular genetics are applied to "fishing" for the metabolic traits of interest so as to enhance production in plants.

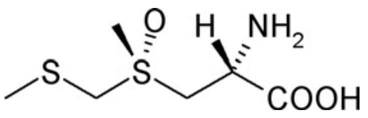
Health Benefits of *Allium* HIACs

The health benefit of the garlic sulfides long has been recognized, but it is only recently that a mechanistic appreciation of the importance of individual compounds as phytoceuticals in the human diet has been understood. When pure *Allium* sulfides were tested, a wide range of biological activities were recorded at the molecular level — including functions as antioxidants, inducers of protective enzymes, inhibition of deleterious enzymes and radical scavenging activity. In turn, these biological activities have health-promoting effects, including reducing the incidence of cancers and cardiovascular disease, as well as counteracting inflammation and infection. Importantly, these effects are compound-specific, and as pharmacologists identify their mechanism of action, there will be an increased demand for specific natural *Allium* HIACs as functional nutraceuticals.

*Allium*like HIAC precursors from unrelated species F-3



S-benzyl cysteine sulfoxide from *Petiveria alliacea* (garlic weed)



S-(methylthiomethyl)cysteine-4-oxide from *Tulbaghia violacea* (society garlic); in *Marasmius* fungi the same compound is made, but with the opposite configuration about the sulfoxide

Plant Biotechnology and F&F Production

Controlling the quality of biological feedstocks using genetics is essential if we are to increase the usage of plant products in the chemical industry. To date, the focus of such approaches has been at either end of the scale of bioproduction — from biomass and biofuel production at one end to improving the yields of small-scale medicinal natural products at the other. As an important sector of the fine chemicals market, the F&F industry also would benefit from adopting this technology.

In conclusion, the global demand for natural products with additional benefits, such as wellness and health, is growing. The main issue facing food and beverage manufacturers is the availability of cost-effective and natural ingredients. This, in turn, challenges F&F companies to deliver natural flavors that support the longer-term needs. This is why the focus on the development of natural HIACs is so important.

Address correspondence to David Brassington, Oxford Chemicals Ltd., North Gare, Seaton Carew, Hartlepool, TS25 2DT, UK; e-mail: david.brassington@oxfordchemicals.com.