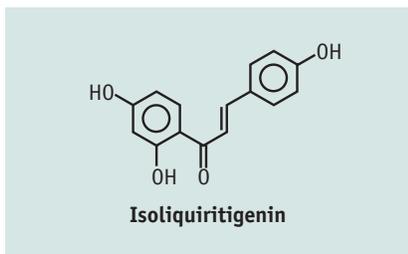


SciTECH Briefs



Researchers have found that isoliquiritigenin, a compound isolated from the tonka bean, might be capable of inhibiting cancer formation.

Natural Cancer Therapy?

For all of the advances in combinatorial synthesis and screening methods, we still often look to Mother Nature for answers to our medical problems. Thus, natural product research continues to flourish as researchers search through the forests, mountains, and oceans for compounds with therapeutic potential. One particularly rich area that is continually being explored is Central and South America, and it is this region where researchers at the University of Illinois at Chicago (www.uic.edu) and the Instituto Nacional de Medicina Tradicional (Lima, Peru) are looking for potential cancer chemopreventive agents.

Recently, the group studied ethyl acetate extracts of the seeds of *Dipteryx odorata*, a tree native to Central and northern South America (*J. Nat. Prod.* **2003**, *66*, 583–587). Historically, extracts of these seeds, known as tonka beans, have been used as flavoring for cigarettes, cocoa, and candies, as well as an ingredient in perfumes and cosmetics. The high fatty acid content of the bean has also led to the use of “tonka butter” as a food source. The researchers, however, were interested in the ability of the extract to induce quinone reductase (QR) in cultured mouse cancer cells. QR and other related drug-metabolizing enzymes are responsible for the detoxification of chemical carcinogens and harmful oxidants, and thus its induction signals compounds that might display cancer chemopreventive effects.

The team purified several known and unknown compounds from the samples

using organic extraction and silica gel chromatography and characterized these compounds with IR and UV spectroscopy as well as NMR spectroscopy and MS. They then tested each of the compounds for the ability to induce QR in cultured cells and identified four effective substances. The researchers subsequently tested three of those compounds in a mouse mammary tissue culture to see whether the compounds could inhibit carcinogen-induced lesion formation.

One of the three compounds, isoliquiritigenin (see figure), showed significant anticancer activity in both assays. These results are consistent with earlier studies of isoliquiritigenin, which showed that it was able to inhibit chemical-induced mouse kidney and colon cancers. Thus, the researchers believe that this compound, which is also a constituent of licorice and shallots, is worthy of further testing for cancer chemopreventive properties.

—Randall C. Willis ◆

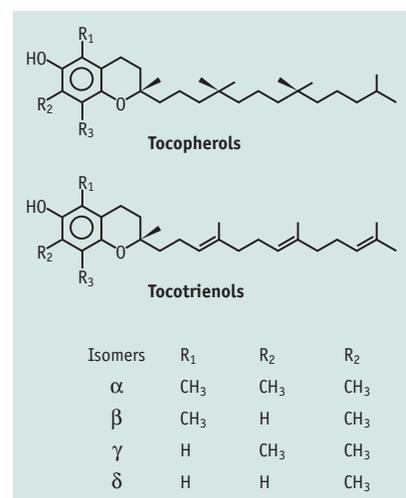
Vetting Vitamin E

Did you eat a bowl of cereal this morning? If you did, vitamin E might well have been among the set of nutrients promised on the side-of-the-box dietary briefing. But what was probably not listed was a breakdown of which of the eight structural varieties of this well-regarded vitamin were included in your breakfast. Gianfranco Panfili and colleagues from Università degli Studi del Molise (www.unimol.it) in Campobasso, Italy, recently developed an HPLC method to quickly and effectively answer this nutritionally important question (*J. Agric. Food Chem.* **2003**, *51*, 3940–3944).

The vitamin E designation can refer to any one of the eight soluble lipid “vitamers”, which include the α -, β -, γ -, and δ -tocopherols and -tocotrienols (see figure). Although the structural differences between these compounds are small, their biological antioxidant activities can vary significantly.

α -Tocopherol has been shown to be the best of the bunch for breaking free-radical-driven chain reactions, but results indicate that α -tocotrienol is appreciably more

efficient as a scavenger of peroxy radicals. At the same time, δ -tocopherol is the most potent cholesterol inhibitor. All this adds up to the need for a reliable analytical technique that is able to isolate, differentiate, and quantify each of the eight molecules to accurately assess the vitamin E-based nutritional value of a food item. A standardized method for this purpose is currently lacking.



Vitamin E vitamers. (Adapted with permission from Panfili, G.; et al. *J. Agric. Food Chem.* **2003**, *51*, 3940–3944.)

Panfili’s group analyzed standard solutions of the eight vitamers as well as their presence in cereal species such as barley, oats, wheat, spelt, and corn using a normal-phase HPLC procedure. In the standard solution measurements, they found the method to give very reproducible results over repeated injections, as long as the column was reactivated after every eight injections with a solution of 10% isopropyl alcohol in *n*-hexane. It also gave linear results over a range of vitamer concentrations, and the calculated detection limits were 0.1–0.3 ng for the different vitamers.

Barley samples, which are known to contain all eight vitamin E varieties, were used to determine an effective standard sample preparation method for vitamin E cereal analysis. Researchers compared the approach of hot saponification followed by *n*-hexane/ethyl acetate extraction, *n*-

hexane/ethyl acetate extraction without saponification, and methanol extraction. The saponification method clearly provided the highest recovery for all of the vitamins and led to cleaner chromatograms.

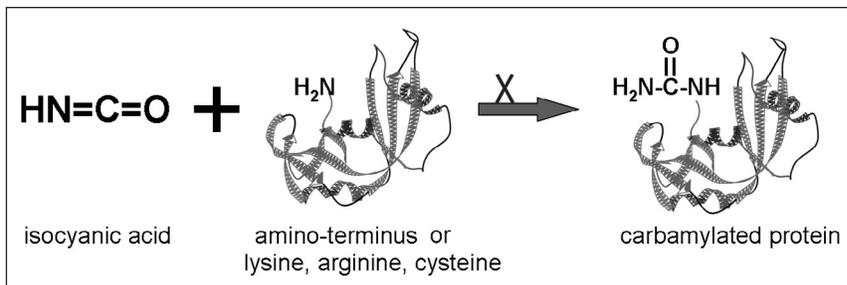
This method was then used for vitamin E analysis of seven different cereal species. It resulted in highly resolved chromatograms for each of them, a typical run lasting about 25 min. The barley was the only sample that contained all of the vitamins, but oats had the greatest amount of α -tocotrienol, and soft wheat topped the list for α -tocopherol and β -tocotrienol.

The researchers say this method might be particularly useful for the analysis of mixtures composed of different cereal species.

—David Filmore ♦

Banishing a Barrier to the Proteome

To deconvolute the molecular complexity of organisms, researchers rely on high-resolution separation techniques. But for this to work, it is critical that the method not modify the molecules in the process. Two-dimensional gel electrophoresis (2-DE) is one of the most prominent techniques used in protein research. But even 2-DE can be swamped by the myriad proteins in crude extracts. Thus, to prepare samples for 2-DE, researchers prefractionate the protein samples using instruments that perform isoelectric focusing (separation on the basis of pI) of the analytes, such as a



Under the right conditions, protein carbamylation, and the obstacle to analysis it causes, can be avoided. (Adapted with permission from McCarthy, J.; et al. *J. Prot. Res.* 2003, 2, 239–242.)

multicompartment electrolyzer (MCE).

To prepare samples for analysis, researchers often add chaotropic agents that disrupt protein complexes and unfold polypeptides. One of the most popular of these agents is urea, but in solution, this compound is in equilibrium with ammonium cyanate, which can react with nucleophiles such as the protein amino terminus, side chain amino groups from lysine and arginine, and the sulfhydryl group of cysteine. The resulting carbamylation generates a protein that is incrementally 43 Da heavier than its unmodified version, blurring the identification process.

To determine the prevalence of carbamylation in protein separations, Ben Herbert and colleagues at Proteome Systems (www.proteomesystems.com) and the University of Verona (www.univr.it) extracted and denatured proteins from *E. coli*. They then fractionated the sample using an MCE and quantified the level of carbamy-

lation using an Axima MALDI-TOF mass spectrometer (Kratos, www.kratos.com) (*J. Prot. Res.* 2003, 2, 239–242).

The researchers found that minimizing the protein extraction time and keeping the samples cold (without allowing the urea to precipitate) reduced the incidence of carbamylation. Furthermore, during MCE separation, the charged products of urea degradation rapidly move to the instrument electrodes, minimizing their exposure to the proteins and therefore the chances of amino and sulfhydryl group modifications. Even after sitting for 48 h in the MCE chamber, no 43-Da increases in protein mass were evident.

The researchers conclude, "By a combination of careful sample preparation and the protective effect of the electric field during electrophoresis, it is possible to banish carbamylation from 2-D [protein] maps forever."

—Randall C. Willis ♦

Science Bits

Biodegrading nerve agents. Scientists from Texas A&M University (www.tamu.edu) and the University of Wisconsin (www.wisc.edu) described an approach for the directed evolution of mutant enzymes specifically enhanced for the catalytic detoxification of organophosphorus nerve agents (*J. Am. Chem. Soc.* 2003, 125, 8990–8991).

Zeolite sorbing. Chemical engineers at the University of Michigan (www.umich.edu) showed that zeolite molecular sieves containing copper or silver ions reduced the amount of sulfur impurities in diesel fuel by a factor of 30 less than the fuel standards to be imposed in the United States in the next few years (*Science (Washington, DC)* 2003, 301, 79–81).

Small sensor. Researchers at Rensselaer Polytechnic Institute (www.rpi.edu) reported the fabrication and successful testing of gas ionization microsensors featuring the electrical breakdown of a range of gases and gas mixtures at carbon nanotube tips (*Nature (London)* 2003, 424, 171–174).

Solventless solution. Scientists at The Hong Kong University of Science and Technology (www.ust.hk) demonstrated a solventless catalytic process that generates polymeric thin films inside microchannels formed via soft lithography. The researchers say it is a less expensive and more environmentally friendly alternative to conventional photolithography (*J. Am. Chem. Soc.* 2003, 125, 9256–9257).

Follow the light. Physicists at the Georgia Institute of Technology (www.gatech.edu) demonstrated a new optical method for controlling the flow of very small volumes of fluids over solid surfaces.

Instead of etched channels, the technique relies on changes in surface tension prompted by light-generated thermal gradients, which opens the possibility for dynamically reprogrammable microfluidic devices (*Phys. Rev. Lett.* 2003, 91, 054501).

Single molecule electronics. Researchers at Columbia University (www.columbia.edu) have used single molecule spectroscopy to measure intramolecular electron transfer in donor-bridge-acceptor systems. According to the scientists, this is the first study of the discrete electron transfer and charge separation fluctuations in such systems on the single-molecule level (*J. Phys. Chem. A* 2003, 107, 6522–6526).