JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

PERSPECTIVE

Research Opportunities for Bioactive Natural Constituents in Agriculture and Food

Prepared for the 50th Anniversary of the Journal of Agricultural and Food Chemistry

RUSSELL J. MOLYNEUX†

Associate Editor, *Journal of Agricultural and Food Chemistry,* Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710

The *Journal of Agricultural and Food Chemistry* recently introduced a new subject matter category titled "Bioactive Constituents" to cover investigations of the composition of natural compounds and their biological activity in crops and foods. It is recognized by the Editors that a number of other journals specialize in various aspects of the chemistry of natural products, but the intent of this classification is to emphasize and stimulate submission of manuscripts in such areas of agricultural and food chemistry that have so far been neglected or under-represented. Selected topics dealing with bioactive constituents are given as representative examples of the types of investigations that would be appropriate to the scope of the Journal.

The playwright William Shakespeare was born and bred in Stratford-upon-Avon, a market town in the heart of the English countryside. As a consequence, his plays are replete with allusions to rural life and agricultural situations. In the tragedy *Romeo and Juliet*, the character Friar Laurence declares

"O mickle is the powerful grace that lies
In plants, herbs, stones, and their true qualities
For naught so vile that on the earth doth live
But to the earth some special good doth give;"

and continues

"Within the infant rind of this weak flower
Poison hath residence, and medicine power
For this, being smelt, with that part cheers each part,
Being tasted, slays all senses with the heart."

The insight of a poet thus expresses concepts that predate the development of modern chemistry, and specifically natural products chemistry, by several centuries. Shakespeare, through Friar Laurence, is recognizing that plants and soils have much ("mickle") within them that extends beyond their macroscopic properties as foodstuffs or as a physical structure for the production of crops, respectively. It is unlikely that he ever conceived that these "true qualities" could exist in isolation from the matrix that encompassed them, but he had the perspicacity to note that even the most insignificant plant possessed some unique properties of use to mankind.

Modern organic chemistry, which was founded primarily upon the study of natural products, led to the isolation and identification of a multitude of such individual compounds and subsequently an understanding of their biological properties (*1*). This has been particularly true for herbal plants, which have been consistently studied for those constituents responsible for their "medicine power", with many journals specifically tailored for the publication of such findings. For agricultural crops, investigations of natural products have been much less tightly focused on individual problem areas, with the singular exception of aroma constituents.

The editorial staff of the *Journal of Agricultural and Food Chemistry* is committed to maintaining the position of the Journal at the forefront of chemical aspects of agricultural and food science. Beginning in 2001 a new category titled "Bioactive Constituents" was introduced to emphasize and stimulate submission of manuscripts dealing with the composition and biological activity of crops and foods. The "Flavors and Aromas" category remains in place, even though it could also be considered to cover bioactive constituents, because it comprises a specialized area of major research effort in its own right.

The Editors recognize that a number of other ACS and non-ACS journals publish and even concentrate on areas dealing with "bioactive constituents". How then should authors, reviewers, and editors decide what aspects of this category are most

† Telephone (510) 559-6043; fax (510) 559-6129; e-mail jafc@pw.usda.gov.

10.1021/jf0207068
Published on Web 10/25/2002

suitable for publication in *JAFC*? A primary consideration (as with all manuscripts submitted to the Journal) should be the target audience. For example, it is unlikely that investigation of the constituents of a minor herbal remedy will generate much interest among the readership of this Journal, whereas investigation of a widely distributed or cultivated plant may ultimately lead to its commercial production. Another condition is that the bioactivity should be adequately documented by appropriate experiments, not merely inferred from anecdotal evidence or from in vitro tests that bear no direct relationship to biological systems. Furthermore, a "bioactivity-directed" strategy with a focus on specific molecules should be employed, rather than random characterization of any and all constituents, irrespective of their significance. Within such constraints there exists great opportunity for investigation of natural constituents in crops and foods and for their potential application to the benefit of mankind. Some possible areas of interest are listed below in an attempt to stimulate more effort on nontraditional topics as well as in certain fields that have not attained their full potential.

Alexipharmics. The public is generally concerned about exposure to environmental toxins, both natural and man-made. Alexipharmics are compounds that have the quality or nature of an antidote to poison, as opposed to constituents that are nutritionally desirable. An extreme example of this is the practice by indigenous groups in the Andes of eating toxic potato species together with clays or soils (geophagy) that adsorb and thus detoxify the alkaloids (*2*). More palatable alternatives as alexipharmics may exist, and if it can be demonstrated that specific components of particular foods have such properties, then these products would have enhanced value in the marketplace. Bioactive constituents of foods and herbs may also affect the metabolism and effectiveness of therapeutic drugs, and such potential interactions need to be carefully delineated to establish whether they may be deleterious or advantageous (*3*).

Allelopathy. The phenomenon of allelopathy, the chemical interaction between plants and microorganisms, has been quite extensively investigated (*4*), but applications in crop production have been limited at best. Extension of investigations beyond identification of the bioactive compounds is needed to determine the stability, distribution, and mode of action of such constituents in the natural environment (*5*) and their use to control competitors of commercial crops either alone or in synergistic combination with reduced levels of synthetic herbicides. Studies in this area could be published in either the "Chemistry of Crop and Animal Protection" or "Bioactive Constituents" category of the Journal, whichever is most appropriate to the particular emphasis of the work.

Cancer Chemoprotection. An association between the presence of specific natural products in certain foodstuffs and a reduced incidence of various types of cancer has been noted; examples are the glucosinolates in cruciferous vegetables (*6*) and limonoids in citrus fruits (*7*). Structure−activity relationships of the glucosinolates and their potential mode of action have been fairly extensively studied, but it is likely that many other bioactive constituents in our foods play a similar role and would benefit from comprehensive investigations. In particular, it should be noted that determination of the bioavailability of individual antitumor compounds is an essential part of such studies. It should also be pointed out that this topic is in distinct contrast to the evaluation of so-called "nutraceuticals" or herbal remedies. In the latter cases the ingestion is voluntary, whereas the chemoprotectants in fruits and vegetables are unavoidably consumed as part of the normal diet, and the challenge is to establish the optimum consumption rate for bioavailability and efficacy.

Control of Fungal and Bacterial Mutation. Mounting evidence suggests that phytochemical constituents such as green tea catechins can suppress mutagenic transformations of bacteria and consequent induction of antibiotic resistance (*8*). Similarly, fungi that biosynthesize toxic contaminants of foodstuffs may be rendered atoxigenic by compounds naturally present in crops. Conversely, fungi that are normally relatively harmless may produce toxins under the influence of other constituents. If such components can be identified, the plants could be bred to contain optimum levels that would reduce or eliminate adverse effects of microorganisms.

Cryptic Natural Toxins. The majority of natural toxins in foods that are currently subjected to regulation and analysis are either naturally present or a consequence of microbiological contamination and are relatively easily anticipated as contaminants. Less evident and rarely investigated are constitutive plant toxicants that may be overlooked because they are consumed by animals and either incorporated indirectly into nonplant food products, concentrated, or biotransformed into toxic metabolites. Certain of these compounds may not be directly toxic, but instead allergenic or photosensitizing, and the cause and effect relationship is therefore obscure. One such example is the occurrence of hepatotoxic alkaloids in honey from bees foraging on plants from a diversity of species (*9*). Ingestion of plant toxins by livestock or wildlife may often be unanticipated, and the potential exists for such compounds to be incorporated into dairy and meat products. It is therefore important to trace the genesis of such compounds as a predictive measure and to establish adequate clearance times before the products can be safely consumed.

Endophytic Fungi. There is increasing evidence to suggest that plants provide an environment for an exceptionally large and diverse assortment of host-specific fungal endophytes (*10*). These microorganisms, which have co-evolved to exist within plant structures, can provide the plants with a competitive advantage, be toxic to herbivorous insects and livestock, or affect their productivity. Although a few such interactions have been investigated by chemical ecologists, it is likely that the role of endophytic metabolites in most agricultural plants is poorly understood and warrants much more rigorous investigation. In addition, studies on the nature of interactions between bioactive root exudates and colonization by fungi of the rhizosphere could be extremely valuable in providing knowledge of their role in nutrient uptake and utilization (*11*).

Molecular Authentication. Flavor and aroma products are frequently authenticated by the presence or absence of specific constituents, but this approach has rarely been applied to bulk commodities. The increasingly international nature of food production and large volumes of imports and exports require that shipments be unequivocally identified with respect to claims of quality, blending, identity as specific cultivars, and points of origin. This approach could also extend to genetically modified organisms; in fact, there is at present very little knowledge as to how introduction of specific genes may alter the matrix of bioactive constituents, for either better or worse. Chemical analysis for specific constituents, in combination with DNA analysis, could provide answers to such questions. It is also important that specific analytical criteria be established for validation of reference compounds chosen as markers and that the identity of potential confounding impurities be determined (*12*).

Molecular Farming. A number of crops are cultivated for the presence of specific bioactive constituents, in particular many spices and flavoring additives such as hops. In most cases the crop is valued on the basis of the amount or relative proportions of active ingredient that a particular variety or cultivar produces. Extension of this concept to the cultivation of nontraditional crops for the production of high-value constituents could provide a significant increase in income to the agricultural sector of the economy, and production of phytochemicals in untransformed and genetically transformed tissue culture is an alternative. Many "weed" species contain natural products that are used in the pharmaceutical industry, but despite this the compounds are often produced by synthetic methods, frequently requiring the use of expensive and environmentally undesirable reagents. There has been a recent trend to synthetic approaches known as "green chemistry", involving relatively benign reagents and minimal formation of side-products, but it seems doubtful that synthesis of structurally complex compounds can compete with the true green chemistry of biosynthesis in a properly cultivated plant source. Furthermore, the energy costs associated with production are eliminated from the synthetic phase and confined to the isolation and purification steps. One example of success in this area is the production of scopolamine from cultivated *Duboisia* species. This has been further extended to production in undifferentiated callus tissues, differentiated shoots, or root cultures, providing opportunities for manipulation of the specific alkaloids produced (*13*). Another example is the anticancer drug, taxol, which can be derived semisynthetically in a few steps from a precursor isolated from leaves of cultivated species of *Taxus* rather than from its original source, the bark of the Pacific yew (*Taxus brevifolia*), a slow-growing tree of very limited distribution (*14*). Structurally complex phytochemicals can also serve as templates on which to build a series of modifications for the exploration of structure–activity relationships.

Phytoanticipins. Phytoalexins, inducible metabolites, formed de novo after invasion by a pathogen or herbivore, either by gene derepression or by activation of latent enzyme systems, have been relatively widely studied, although their promise as tools in agricultural production, as with allelopathic compounds, has not been fully realized. In contrast, the study of phytoanticipins (*15*), constitutive metabolites present in situ, either in the active form or easily generated from a precursor, has been less common. The latter may offer greater potential for manipulation as protective constituents of plants, simply because they are endogenous and there is no delay in the time course of their production after fungal or insect attack. The primary concern should be that they are specifically located in the most appropriate tissue to serve a defensive role.

Phytochemical Communication and Multitrophic Interactions. Evidence is mounting that communication within a given plant species can be mediated by species-specific phytochemicals (*16*), for example, warning of invasion by herbivores. Additional phytochemicals may then be mobilized to defend individuals that have not yet been attacked. As with allelopathy, the phenomenon has not been capitalized upon for agronomic purposes. Similarly, examples of interspecies communication through several trophic levels have been primarily studies of ecological interest. It is probable that extension of the general concept of both inter- and intraspecies phytochemical communication to agricultural crops could yield not only interesting chemistry and bioactivities but also practical benefits to crop production. Further expansion into the analysis of plant constituents in host species by analysis of insects feeding upon them can also provide useful knowledge regarding the localization and transport of phytochemicals within specific plant tissues (*17*).

The above examples represent an idiosyncratic selection of topics that have intrigued the writer. The cited references are not intended to be comprehensive but merely to provide a perception of the diversity of the topics listed. Suggestions of other examples are welcomed and might well provide the basis for a more comprehensive "vision" of the future role of bioactive natural products in agricultural chemistry. It is noteworthy that although the central theme of the above problems is natural products chemistry, a successful attack will require a multidisciplinary approach.

In this regard, it is apparent that the traditional paradigm for natural products studies has shifted drastically over the past two decades from an emphasis on structural elucidation to investigation of mode of action. For all except the most complicated structures, primarily those from marine sources, the determination of structure has turned into a relatively routine exercise due to the extraordinary power of modern spectroscopic techniques, particularly nuclear magnetic resonance spectroscopy and mass spectrometry, and the interface with separation methods (*1*). This may be a desirable or undesirable development, depending upon an individual's point of view. One suspects that much of the challenge of the puzzle of deciphering a structure has vanished, to be replaced instead by less intellectually stimulating questions as to the most economical, or elegant, choice of techniques to use. On the other hand, it is now possible to separate most components of a large suite of compounds present in a given organism and to determine the structures of each, some of which may be present in extraordinarily low amounts yet exhibit exceptional biological potency. In the context of some of the above-mentioned topics this is significant, because a complete understanding of the role of natural products in such phenomena is not likely to be dependent upon a single compound but rather upon a complex interplay of bioactivities. This represents a much greater degree of complexity than traditionally associated with the study of bioactive natural products. However, the sophisticated separation techniques and spectroscopic methods now available are equal to the task; the challenge arises in devising suitable experiments to attack such problems.

Scientists who undertake novel and difficult areas of investigation often face greater barriers to acceptance of their results than those involved in well-established or traditional fields. It is therefore important that such endeavors be supported by the most rigorous standards of chemistry. The ready availability of instrumental techniques can on occasion lead to isolated compounds being subjected to the full power of these techniques only to have their structures established as identical to that of a natural product previously isolated from a different source. The principal of dereplication (*18*, *19*) should be applied to all "novel" isolates and a thorough literature search conducted for structural analogies with known compounds from related organisms at the genus and family level. It is also important not to neglect the "simple" physical techniques such as UV–visible and IR spectroscopy, which may provide the necessary information at a fraction of the time, effort, and expense. Reliance on sophisticated techniques has also led to the omission of information essential for the complete characterization of natural products and deprived other researchers of the ability to compare samples. Melting or boiling points should be determined, in addition to optical rotations when the compound has chiral centers. Most importantly, the elemental composition of new compounds must be determined, either by combustion analysis or by high-resolution mass spectrometry. The guidelines for authors of the Journal specifically address these issues (*20*). The Journal also requires that studies not be fragmented or marginally incremental but rather be original and comprehensive in scope, experimental design, and interpretation (*21*).

Future advances in the application of bioactive natural products to agricultural problems will depend on imaginative exploration of topics that have not traditionally been in the mainstream of subjects published in the Journal. Modern techniques of natural products chemistry, applied with imagination and rigor, are capable of solving the many questions of biology remaining to be answered. If we can achieve such progress, we may fully understand Shakespeare's "*powerful grace that lies in plants, herbs, stones, and their true qualities*" that "*to the earth some special good doth give*".

LITERATURE CITED

(1) Nakanishi, K. An historical perspective of natural products chemistry. In *Comprehensive Natural Products Chemistry*; Barton, D., Nakanishi, K., Meth-Cohn, O., Eds.; Elsevier: Amsterdam, The Netherlands, 1999; Vol. 1, pp xxiii−xl.

(2) Johns, T. Detoxification function of geophagy and domestication of the potato. *J. Chem. Ecol.* **1986**, *12*, 635−646.

(3) Stermitz, F. R.; Lorenz, P.; Tawara, J. N.; Zenewicz, L. A.; Lewis, K. Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1433−1437.

(4) *Allelopathy: Organisms, Processes, and Applications*; Inderjit, Dakshini, K. M. M., Einhellig, F. A., Eds.; ACS Symposium Series 582; American Chemical Society: Washington, DC, 1995.

(5) Bais, H. P.; Walker, T. S.; Stermitz, F. R.; Hufbauer, R. A.; Vivanco, J. M. Enantiomeric-dependent phytotoxic and antimicrobial activity of (±)-catechin. A rhizosecreted racemic mixture from spotted knapweed. *Plant Physiol*. **2002**, *128*, 1173−1179.

(6) Fahey, J. W.; Zalcmann, A. T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5−51.

(7) Manners, G. D.; Hasegawa, S. Squeezing more from *Citrus* fruits. *Chem. Ind*. **1999**, 542−545.

(8) Pillai, S. P.; Pillai, C. A.; Shankel, D. M.; Mitscher, L. A. The ability of certain antimutagenic agents to prevent development of antibiotic resistance. *Mutat. Res*. **2001**, *496*, 61−73.

(9) Edgar, J. A.; Roeder, E.; Molyneux, R. J. Honey from plants containing pyrrolizidine alkaloids: A potential threat to health. *J. Agric. Food Chem*. **2002**, *50*, 2719−2730.

(10) Gusman, J.; Vanhaelen, M. Endophytic fungi: An underexploited source of biologically active secondary metabolites. *Recent Res. Dev. Phytochem*. **2000**, *4*, 187−206.

(11) Tepfer, D.; Goldmann, A.; Pamboukdjian, N.; Maille, M.; Lepingle, A.; Chevalier, D.; Denarie, J.; Rosenberg, C. A plasmid of *Rhizobium meliloti* 41 encodes catabolism of two compounds from root exudate of *Calystegium sepium*. *J. Bacteriol*. **1988**, *170*, 1153−1161.

(12) Pauli, G. F. qNMR−A versatile concept for the validation of natural product reference compounds. *Phytochem. Anal.* **2001**, *12*, 28−42.

(13) Lin, G. D.; Griffin, W. J. Scopolamine content of a *Duboisia* hybrid in callus culture. *Phytochemistry* **1992**, *31*, 4151−4153.

(14) Kingston, D. G. I. Recent advances in the chemistry of taxol. *J. Nat. Prod.* **2000**, *63*, 726−734.

(15) VanEtten, H. D.; Mansfield, J. W.; Bailey, J. A.; Farmer, E. E. Two classes of plant antibiotics: Phytoalexins versus "phytoanticipins". *Plant Cell* **1994**, *6*, 1191−1192.

(16) Karban, R. Communication between sagebrush and wild tobacco in the field. *Biochem. Syst. Ecol.* **2001**, *29*, 995−1005.

(17) Clausen, V.; Frydenvang, K.; Koopmann, R.; Jørgensen, L. B.; Abbiw, D. K.; Ekpe, P.; Jaroszewski, J. W. Plant analysis by butterflies: Occurrence of cyclopentenylglycines in Passifloraceae, Flacourtiaceae, and Turneraceae and discovery of the novel nonproteinogenic amino acid 2-(3′-cyclopentenyl)glycine in *Rinorea*. *J. Nat. Prod*. **2002**, *65*, 542−547.

(18) Cordell, G. A.; Shin, Y. G. Finding the needle in the haystack. The dereplication of natural product extracts. *Pure Appl. Chem.* **1999**, *71*, 1089−1094.

(19) Bradshaw, J.; Butina, D.; Dunn, A. J.; Green, R. H.; Hajek, M.; Jones, M. M.; Lindon, J. C.; Sidebottom, P. J. A rapid and facile method for the dereplication of purified natural products. *J. Nat. Prod*. **2001**, *64*, 1541−1544.

(20) Scope, Policy, and Instructions for Authors. *J. Agric. Food Chem.* **2002**, *50*, 8A−12A.

(21) Ethical Guidelines to Publication of Chemical Research. *J. Agric. Food Chem.* **2002**, *50*, 13A−15A.

Received for review June 25, 2002. Revised manuscript received September 26, 2002. Accepted September 26, 2002.

JF0207068