The rational use of natural products

Plant research is yielding information that may lead to increased use of natural products from microorganisms, and some higher plants, for agrochemical and other purposes (perhaps even helping produce higher value wines). Horace G. Cutler, of the USDA/ARS' Richard B. Russell Center in Athens, Georgia, prepared this review of recent developments at the request of JAOCS *Associate Editor for News W. David Nes, also of the Russell Center.*

When Columbus discovered the West Indies in 1492 (not America; he never set foot on the mainland of either South or North America), he was to put into motion a number of events that would revolutionize the known world of his day. The times were unlike any other in the history of western civilization. The Renaissance was in bloom, art and architecture were flourishing and the marriage of chemistry and medicine was about to be consummated in the context of two very unlikely characters: Frobenius, a publisher who lived in Basel, Switzerland, and, Paracelsus, a genius who practiced medicine and indulged himself with women and drink. These two men knew each other well. Paracelsus saved Frobenius's leg, which was slated for amputation after it had become infected, by using mercury. And, furthermore, he used his new art of formulated mercuric medicines to control syphilis which, it is believed, probably had been brought back to Europe by sailors aboard Columbus's ships. Syphilis has been documented in the skeletal bones of pre-Columbian American Indians. But whatever the source, Paracelsus successfully employed mercury to treat syphilis. Unfortunately, mercury is a heavy metal that can produce nasty side effects in humans and the cure may be as bad as the disease.

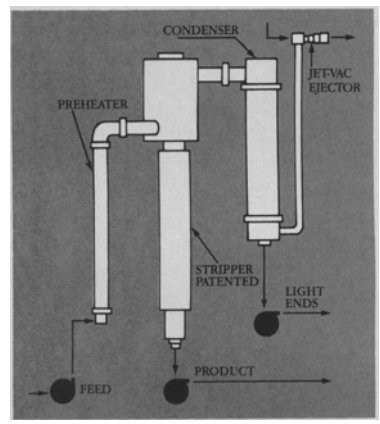
A further development in medicinal chemistry took place in 1910 when Paul Ehrlich introduced arsphenamine "606," Salvarsan, to the world specifically to treat syphilis but, like mercury, arsenic (from which arsphenamine was made) also tends to have toxic properties. However, the compound was not as toxic as the mercury that Paracelsus had employed and arsenic has the unique property of being expelled by the body into the hair where it forms, in the shaft, a bolus of concentrated arsenic. The classic example concerns Napoleon who, in his last exile on remote St. Helena, died of arsenic poisoning—his hair shafts contained knots of arsenic—and the blame was laid on the British whom, it was claimed, had seen to it that Napoleon would cause no further disturbances in Europe. In fact, recent historical research indicates that his attending physician, a Frenchman, gave him arsenic laced wine because the doctor had some good political things going in France and wished to leave his master permanently behind.

The breakthrough for the control of syphilis came with the discovery of penicillin by Florey and Chain in the early 1940s and it was these isomeric antibiotic natural products obtained from *Penicillium notatum* and *P. chrysogenum* that were to change the nature of pharmaceuticals. For the first time in modern history, non-persistent medicinals had come into large-scale use that were either metabolized by the body or excreted into the environment to be biodegraded.

Another significant development in medicine occurred in 1796, when Edward Jenner performed the first successful use of vaccination, using cowpox fluid obtained from the vesicles on the hands of an infected milkmaid to protect an 8-year-old boy against smallpox. Jenner had made the astute observation that people who worked with cows appeared to be immune to the ravages of smallpox.

It is important to realize that, in retrospect, these were two major evolutionary advances in medicinal chemistry. The use of penicillin was a change from the use of toxic, heavy metals, which were difficult to flush from the body, to biodegradable natural prod-

Evaporator Stripper



for continuous refining of . . .

- Vegetable fats & oils
- Animal fats & oils
- Essential oils

Capacity ranges:
100 pounds to thousands of pounds per hour.

ARTISAN®

Artisan Industries Inc.

73 Pond Street • Waltham, Massachusetts 02254-9193
Phone: 617-893-6800 • Telex: 92-3312 • FAX: 617-647-0143

ucts. Jenner's vaccination involved the use of microorganisms to control specific diseases. These are two developmental events which, today for the most part, we take for granted. Yet it is precisely a reiteration of these events that is taking place in agriculture. We are aware that certain agrochemicals may pollute the environment and that even though they may degrade eventually there may be a price to be paid during the time that they persist. Thus, biodegradable natural products of microbial or plant origin and their derivatives may be suitable substitutes for hard pesticides. Another may be the use of microorganisms in either their native state, as with *Bacillus thuringiensis* to control insects, or in the engineered state where certain bits of genetic information that encode for the production of specific biologically active natural products are spliced into microorganisms that can be grown with facility. These, in turn, may be field applied. Since knowledge does not exist in a random state, we shall look at a select number of natural products derived from microorganisms (and some higher plants) and examine their potential use in agriculture and their use in apparently unrelated areas.

Amino acids and 'pseudo-amino acids'

The cyclic oligopeptides of microbial origin are singularly interesting to synthetic chemists, especially those compounds that have biological activity. The fact that they are composed of the natural L-amino and unnatural D-amino acids immediately brings to mind at least two questions. First, what happens if the sequence is changed to all L-amino acids, or D-amino acids and, second, why does the organism bother to make compounds that contain a mixture of L- and D-amino acids? Then, of course, there are the permutations for synthetic substitutions of the L- with the D-species, vice-versa, and scrambling of the sequences so that, theoretically, a data bank could be built for structure-biological activity relationships and, subsequently, the potential for making patentable agrochemicals.

It happened that certain fungal cultures were destroyed by infections of *Bacillus subtilis* (Cohen) and in the case under discussion, an observant scientist noted that his strain of *B. subtilis* was a potent inhibitor of *Monilinia fructicola* (Wint) Honey (1). *M. fructicola*, brown rot of stone fruit, is especially damaging to peaches following harvest and the organism has become resistant to certain fungicides including benomyl and other benzimidazoles (2). When crude preparations of *B. subtilis* were applied to harvested peaches that had been inoculated with *M. fructicola*, a potent antifungal response was noted. It transpired that Iturin A (Fig. 1) (3) and Iturin A-2, A-3 (4) (a mixture) were the responsible agents and it had been reported that these oligopeptides had medicinal value. In plant bioassays, the mixture inhibited the growth of etiolated wheat coleoptiles 48–81% during various stages of purification and control of *M. fructicola* was excellent. A role for the Iturins in agriculture is now clear (2).

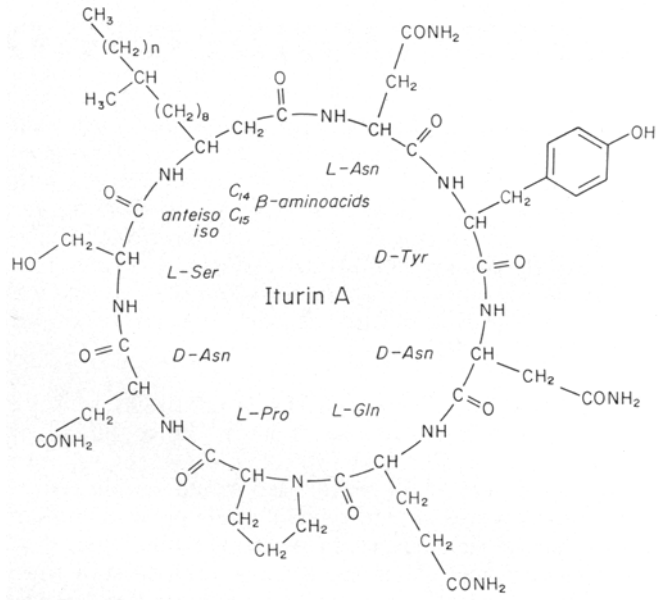


FIG. 1. Iturin A

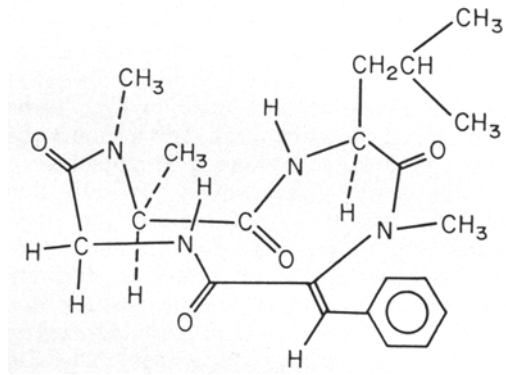


FIG. 2. Tentoxin

However, the structure consists of both D- and L-amino acids and contains L-asparagine, D-tyrosine, D-asparagine, L-glycine, L-proline, D-asparagine, L-serine and the anteiso and iso C_{14} and C_{15} beta-aminoacids, depending upon the particular antibiotic species. Why *B. subtilis* makes 1 mole of L-asparagine and 2 moles of D-asparagine is not clear; neither is the role of these chemical species insofar as biological activity is concerned. The molecule offers further synthetic elaboration because there are four $-CONH_2$ residues and one p-hydroxyl function available for derivatization. But an intriguing question remains: do the hydrolytic fragments of Iturin A, A-2, and A-3 have biological activity? This question becomes more critical upon examination of the next oligopeptide tentoxin.

Tentoxin (Fig. 2) is a cyclic tetrapeptide that contains the amino acids L-leucine, L-N-methylalanine,

glycine, L-N-methyldehydrophenylalanine and is found in *Alternaria tenuis (A. alternata), A. mali* and *A. citri* (5,6). The latter two are found in apple and citrus (Dancy tangerine, *Citrus reticulata* and mandarin cultivars) respectively (7,8). The toxin causes chlorosis in lettuce and mung bean but not in corn or tomato plants. This selectivity suggests that tentoxin may have herbicidal value or that synthetic derivatives and fragments may retain specificity and biological activity. Consequently, both avenues were explored and the products tested in plant systems. The results were surprising. First, N-methylalanine was replaced by proline and while this product, [Pro^1]tentoxin, induced moderate chlorosis in pitted morning glory it elicited intense chlorosis in Barnyard grass and lettuce. In contrast, tentoxin caused intense chlorosis in all three species.

Another synthetic manipulation was the fragmentation of tentoxin to produce a tripeptide which was then elaborated (8). Three of these derivatives exhibited unique and unpredictable responses in bioassays. These were:

(a) tert-butyloxycarbonyl(Boc-Val-N-(C_2H_5) Δ^zPhe-Gly-OMe;
(b) Boc-Leu-N(C_2H_5)Δ^zPhe-Gly-OMe; and
(c) Boc-Leu-N(CH_3)Δ^zPhe-Gly-OMe,

and it is clear that synthetic (c) most readily resembled a natural tentoxin fragment because of the -N(CH_3)Δ^zPhe substitution as opposed to the -N(C_2H_5)Δ^zPhe derivative. In etiolated wheat coleoptile tests, compounds (a) and (b) which contained the C_2H_5 residue significantly ($P<0.010$) inhibited growth at 10^{-3}M but, also, significantly promoted extension at 10^{-4}M, and produced exactly the same response as indole-3-acetic acid. At 10^{-5}M, (a) did not promote growth, but (b) did. Analog (c) inhibited coleoptile extension at 10-3 and 10-4M. Bioassays, using cress seedlings, revealed that (a) and (b) promoted root growth 40 and 10%, respectively. In lettuce seedling assays, (a) produced no response, but (b) promoted lettuce roots 40–50%. As might be anticipated, fragment (c) inhibited cress and lettuce 50% and 60%.

Other research has uncovered the presence of dihydrotentoxin (Fig. 3) in *Altenaria citri* (10): this differs structurally from tentoxin by a singular amino acid. That is, N-methyldehydrophenylalanine is replaced by N-methylphenylalanine and it is precisely the addition of two hydrogen atoms that changes the specific activity of the molecule. Tentoxin induces chlorosis in lettuce seedlings at 0.4~0.8 μg/ml; dihydrotentoxin concentrations have to be at least 100 μg/ml to induce the same response. This observation quickly leads to thoughts about substituting D-amino acids for L- in dihydrotentoxin (all are L-) and this question has been partially answered. As we observed earlier, tentoxin was more active than dihydrotentoxin and it was during the isolation of dihydrotentoxin that the possibility that perhaps dihydrotentoxin was inactive completely and that the activity observed when it was tested on lettuce may have been due to trace amounts of tentoxin. Therefore, by reducing tentoxin with hydrogen, using a palladium catalyst, dihydrotentoxin was produced. All the physical data of the synthetic product matched the authentic natural product with one exception: the values for the optical rotations were totally different. And the synthetic lacked any biological activity. Following digestion with 2N HCl and critical analysis of N-methylphenylalanine it was discovered that the amino acid had a D-configuration as opposed to L-. Thus, the L-leucine, L-N-methylalanine, glycine, D-N-methylphenylalanine derivative has no biological activity. But does there exist in nature a substituted phenyl species of either tentoxin or dihydrotentoxin: can a synthetic product be made that has substitutions at the ortho, meta, or para positions?

There are a handful of other cyclic oligopeptides that have shown high specific activity but the most active of these are the AM toxins I, II, III from *Alternaria mali* (11,12) and these are mentioned because both AM toxins I and III are exceptionally toxic to "Indo" apple cultivars at 0.1 ppb and these metabolites induce necrosis in resistant "Jonathan" cultivars at 1.0 and 10 ppm respectively. Again, as with the cyclic oligopeptides discussed, the possibilities for obtaining biologically active fragments that may have fixed or flexible backbones, thereby altering activity, are numerous.

In considering the oligopeptide fragments it is inevitable that one arrives at the individual components, the amino acids. One of the more significant advances in agriculture in recent times has been the practical use of ethylene to induce ripening in fruits, vegetables and other crops, like tobacco. Ethylene is a natural product and its apparent genesis is from the amino acid methionine (13). The "pseudo-amino acid," 1-aminocyclopropane-1-carboxylic acid (Fig. 4) is a

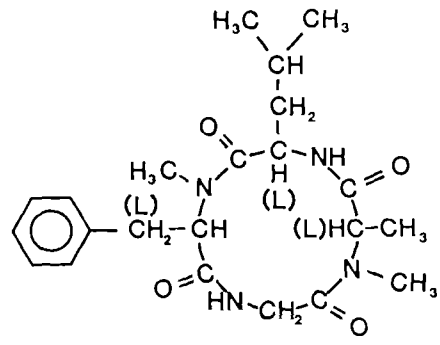


FIG. 3. Dihydrotentoxin

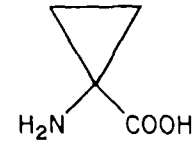


FIG. 4. 1-aminocyclopropane-1-carboxylic acid *a*

curious species because it is a small molecule yet has two functional sites for synthesis, and we know that it is biologically active.

In an apparently unrelated piece of research, a chemist at the University of Georgia was engaged in making synthetic sweeteners for producing low calorie diet drinks and baked goods. The product had to have at least three properties: it had to be several orders of magnitude greater in sweetness than sucrose; there must be no lingering aftertaste; it should withstand baking temperatures and not be degraded. The market product Aspartame (N-L-α-aspartyl-L-phenylalanine-methyl ester) is sweeter than sucrose, but it degrades on heating and also is toxic to phenylketonurics because it contains phenylalanine (Fig. 5). So the materials used by the Georgia group were 1-aminocyclopropane carboxylic acid and aspartic acid. In what must have appeared to be a logical progression of experiments, the carboxyl group was esterified with straight chain and branched chained alcohols so that the most satisfactory product was L-aspartyl-1-aminocyclopropane carboxylate n-propyl ester (Fig. 6) (14). It was this compound that was ~300 times sweeter than sucrose, had no lingering aftertaste, did not degrade at temperatures below 171°C, was not toxic to phenylketonurics and, to date, has shown no toxic effects. The sociological effects of such a discovery are staggering especially in societies with high caloric intake, for those who have difficulty dieting and for those who suffer from diabetes. It is also tempting to speculate that because 1-aminocyclopropane carboxylate occurs naturally there must be oligomeric deviates that have attractive properties. And again, because two functional groups are readily available for synthetic work, many potential derivatives come to mind.

The 1-amino-1-carboxylic acid derivative story does not end there. Over 20 years ago a compound, 1-amino-2-nitrocyclopentane carboxylic acid (Fig. 7), was isolated from culture filtrates of *A. wentii* (15). Early experiments with culture filtrates of *A. wentii* demonstrated that foliar sprays and root zone applications induced yellow strapleaf in chrysanthemum and frenching in tobacco, both responses being, at least, morphological (16). Applications of pure 1-amino-2-nitrocyclopentane carboxylic acid induced exactly the same responses as those observed with the culture filtrate. In attempting to determine the mode of action of the fungal metabolite, it was ascertained that either DL alloisoleucine or DL isoleucine induced yellow strapleaf in chrysanthemum when they were applied to the root zone with two minor differences: 1-amino-2-nitrocyclopentane carboxylic acid, and the persistence of the isoleucine isomers was longer with respect to the strapleaf response. It has, therefore, been postulated that 1-amino-2-nitrocyclopentane carboxylic acid may act by blocking the metabolism of leucine in plants. But the question remains as to how many plant species were challenged with the natural product. Furthermore, it is interesting and perhaps more than coincidental that L-leucine forms part of the backbone of tentoxin and that L-isoleucine occurs in the tetrapeptide phytotoxins cyl-1 and cyl-2 from *Cylindrocladium scoparium* (17–19). At least this suggests that leucine and its congeners may be candidates for further synthetic work.

The ultimate biodegradable herbicide obtained, so far, is the isopropylamine salt of N-(phosphonomethyl) glycine, (Fig. 8), Roundup, and is a molecule based on the simplest amino acid. It is elegant testimony to natural products that such a simple molecule has produced such an outstanding herbicide. And, by way of a cryptic footnote, glycine has a characteristic sweet taste.

While a great deal of emphasis has been given to the effects of oligopeptides and amino acids on plants, at least one example should be given for insects. And that example is a Japanese one that appeared in 1984 (20). At that time, L-alanosine (Fig. 9) was isolated from an unidentified species of *Streptomyces* and when it was fed to fourth-instar larvae of the common army worm, *Leucania separata*, in artificial diet, the effects on ecdysis were dramatic. At diet rates as low as 5 ppm, head capsule removal was inhibited in 50% of the larvae. With rates of 40 ppm, head capsule removal was completely inhibited

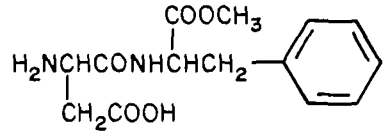


FIG. 5. N-L-(alpha)-Aspartyl-L-phenylalanine 1-methyl ester

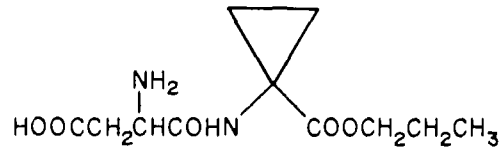


FIG. 6. L-aspartyl-l-aminocyclopropane carboxylate n-propyl ester

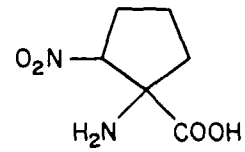


FIG. 7. 1-amino-2-nitrocyclopentanecarboxylic acid

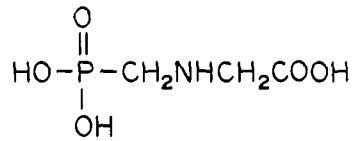


FIG. 8. N-(Phosphonomethyl) glycine

and shedding of the cuticle did not occur. The cabbage armyworm, *Mamestra brassicae,* was also affected the same way. Now it is important to recognize that L-alanosine had originally been isolated from *Streptomyces alanosinicus* for different reasons, in 1966, by Murthy *et al.*, specifically as an antiviral and antitumor agent (21,22). The importance of these divergent, but identical, discoveries gives rise to two observations. First, disciplines fail to communicate with each other and if they do so only by publication, one discipline usually dismisses the work of the other as irrelevant. Second, 23 years have passed since the initial discovery of L-alanosine and 5 years have passed since the extremely important and practical discovery of its insecticidal properties by the Japanese. The molecule contains at least three functional groups that may be used directly for synthetic elaboration (the N=O function may be considered a fourth) and, indeed, the molecule is ripe for synthetic exploitation both as an insecticide and as an antiviral or antitumor agent.

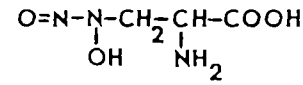


FIG. 9. L-alanosine

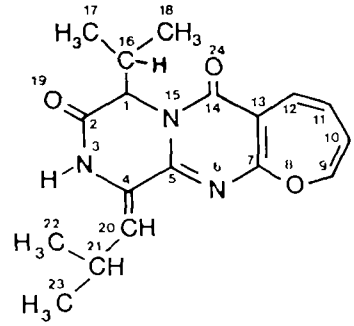


FIG. 10. Cinereain

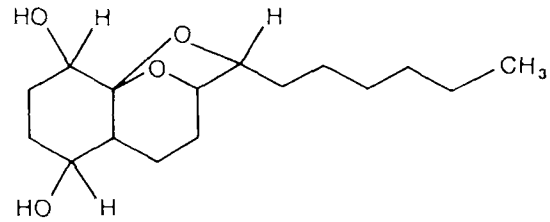


FIG. 11. Koninginin A

Novel natural products

Many examples can be shown to demonstrate that there are novel natural products that have biological activity and potential use in agriculture. Some of them have originated in our laboratory, and we are often asked about the odds of finding novel structures that have activity. It has been our experience that approximately 10% of all accessed microorganisms may produce new compounds or new classes of compounds. This depends largely on the geographical source of the organism and the knowledge to know where to collect. Recently we have discovered two new classes of compounds. They are cinereain (Fig. 10) from *Botrytis cinerea* (23) and koninginin A (Fig. 11) from *Trichoderma koningii* (24). *Botrytis cinerea* is a fungus that attacks fruits and vegetables in the field and in storage. It is best known in Europe and California for its attack on grapes where it produces the "noble rot" and a vintage of rare flavor in Sauternes and related wines. It is said that people who drink the wines fall into two distinct classes: they either like the wine or dislike it intensely. Whichever, the cost is approximately triple to quadruple the normal price and it is difficult to obtain outside the production area. Our strain of *B. cinerea* was obtained from the surface of stored sunflower seed (*Helianthus annuus* L.) and subsequently cultured on solid medium. Cinereain eventually was isolated and its structure determined by single crystal diffraction techniques. The metabolite was inhibitory to the growth of etiolated wheat coleoptiles; at 10^{-3} and 10^{-4}M they were inhibited 100 and 34% respectively. Greenhouse tests were conducted on bean, corn, and tobacco plants but only corn exhibited symptoms of slight necrosis and mild chlorosis. No effects were noted in tests with selected Gram-positive and Gram-negative bacteria. The structure is unique and the oxepine structure is rare among natural products. The compound was not tested in vertebrate systems, neither was it assayed from organoleptic properties. If this compound is present in Sauterne grapes at pressing, and if the compound remains the same during fermentation or is biotransformed, it may be possible to reproduce some of the flavor of "noble rot" wines by artificial means. Unfortunately, only small amounts of cinereain presently exist but direct synthesis may be possible and may give rise to precursors that also possess biological activity.

Another novel structure is koninginin A, which was isolated from *Trichoderma koningii* found growing on the roots and at the soil line of the ornamental *Diffenbachia* sp. Koninginin has moderate activity in the etiolated wheat coleoptile bioassay. It inhibits coleoptiles at 10^{-3}M but there is a series of related compounds that have greater biological activity, and the range and mode of action of the parent compound and its congeners have still to be assessed. It has been specifically included herein so that those who question the presence of a relatively nonlipid slanted article in a lipid journal may take up their writing pads and pens and suggest a biosynthetic pathway.

I should like to mention in passing that several lipids considered as secondary metabolites have now been identified as natural products which control fungal and plant growth, e.g., mevinolin (25), monensin (26), steroidal alkaloids and azasteroids (27) and ce-

rulenin (28). There are still numerous classes of compounds to be discovered and the record will show that many of these will be diverse and will exhibit specific activity in select biological systems.

Conclusion

It becomes increasingly clear, as more and more identical secondary metabolites are isolated from divergent genera of microorganisms, that the genetic code that governs their synthesis has not been deleted during evolution. Finally, it now appears that secondary metabolites will fill at least four niches in the future—that is, as biodegradable agrochemicals, as templates for derivatization as biologically active substances, as markers in biosynthetic pathways and, finally, as probes for genes and genetic expression.

1. Pusey, P.L. and C.L. Wilson *Plant Dis. 753*:58(1984).
2. Gueldner, R.C., C.C. Reilly, P.L. Pusey, C.E. Costello, R.F. Arrendale, R.H. Cox, D.S. Himmelsbach, F.G. Crumley and H.G. Cutler *J. Agric. Food Chem. 411*:33(1985).
3. Peypoux, F., M. Guinaud, G. Michel, L. Delcambe, B. Das and E. Lederer *Biochemistry 3992*:17(1978).
4. Isogai, A., S. Takayama, S. Murakoshi and A. Suzuki *Tetrahedron Lett. 3065*:23(1982).
5. Meyer, W.L., G.E. Templeton, C.I. Grable, R. Jones, L.F. Kuyper, R.B. Lewis, C.W. Sigel and S.H. Woodhead *J. Amer. Chem. Soc. 3802*:97(1975).
6. Okuno, T. *Chem. Abst. 79011*:88(1978).
7. Kohmoto, K., R.P. Scheffer and J.O. Whiteside *Phytopathology 667*:69(1979).
8. Whiteside, J.O. *Plant Dis. Rep. 326*:60(1976).
9. Edwards, J.V., H.G. Cutler, P.S. Zorner, and C.B. Coffman in *Synthesis and Chemistry of Agrochemicals*. Baker, D.R., Fenyes, J.G., Moberg, W.K., Cross, B. Eds. ACS Symposium Series 355, Washington, DC. 1988.
10. Kono, Y., J.M. Gardner and S. Takeuchi *Agric. Biol. Chem. 2401*:50(1986).
11. Ueno, T., T. Nakashima, T. Hayashi and H. Fukami *Agric. Biol. Chem. 2081*:39(1975).
12. Ueno, T., T. Nakashima, Y. Hayashi and H. Fukami *Agric. Biol. Chem. 1115*:39(1975).
13. Yang, S.F. and D.D. Adams in *Biochemistry of Plants (A Comprehensive Treatise)*. Stumpf, P.K., Ed. Academic Press, New York, Vol. 4. 163(1980).
14. Mapelli, C., M.G. Newton, C.E. Ringold and C.H. Stammer *Int. J. Peptide Protein Res. 498*:30(1987).
15. Brian, P.W., G.W. Elson, H.G. Hemming and M. Radley *Nature 998*:207(1965).
16. Woltz, S.S. *Sunshine State Ag. Res. Report* May 10 (1968).
17. Hirota, A., A. Suzuki, H. Suzuki and S. Tamura *Agric. Biol. Chem. 643*:37(1973).
18. Hirota, A., A. Suzuki and S. Tamura *Agric. Biol. Chem. 1185*:37(1973).
19. Takayama, S., A. Isogai, M. Nakata, H. Suzuki and A. Suzuki *Agric. Biol. Chem. 839*:48(1984).
20. Matsumoto, S., S. Sakuda, A. Isogai and A. Suzuki *Agric. Biol. Chem. 827*:48(1984).
21. Murthy, Y.K.S., J.E. Thiemann, C. Coronelli and P. Sensi *Nature 1198*:211(1966).
22. Coronelli, C., C.R. Pasqualucci, G. Tamoni and G.G. Gallo *Il Farmaco, Ed. Sci. 269*:21(1966).
23. Cutler, H.G., J.P. Springer, R.F. Arrendale, B.H. Arison, P.D. Cole and R.G. Roberts *Agric. Biol. Chem. 1725*:52(1988).
24. Cutler, H.G., D.S. Himmelsbach, R.F. Arrendale, P.D. Cole and R.H. Cox *Agric. Biol. Chem.* (in press) 1989.
25. Bach, T.J. and H.K. Lichtenthaler in *Ecology and Metabolism of Plant Lipids*. Fuller, G., Nes, W.D. Eds. ACS Symposium Series 325, Washington, DC. 1987.
26. Agtarap, A., J.W. Chamberlin, M. Pinkerton and L. Steinrauf *J. Am. Chem. Soc. 5737*:89(1967).
27. Nes, W.D., P.K. Hanners, G.A. Bean and G.W. Patterson *Phytopathology 447*:72(1982).
28. Cihlar, R.L. and K.A. Hoberg in *Ecology and Metabolism of Plant Lipids*. Fuller, G., Nes, W.D. Eds. ACS Symposium Series 325, Washington, DC. 1987.

JAOCS CLASSIFIED ADVERTISING

Rates: $40 per column inch. Typesetting is free. AOCS members may run a classified ad for 3 months, free of charge. For display classified advertising ¼ page or larger, see regular *JAOCS* rates. *JAOCS* will provide art and type services for make-up of display ads at a minimal fee.

Payment: Invoices for classified advertising will be mailed 30 days after publication of issue in which the ad appears.

Classified Box Numbers: AOCS will provide a classified box number for confidential responses free of charge. Please specify if you would like this service and supply complete name and address.

Agency Commission: 15% agency commission allowed on display classified advertisements ¼ page size or larger.

Closing Date: Insertion orders and copy for classified advertising is the 3rd of the month prior to issue in which the ad will appear (e.g, January 3rd for publication in the February issue).

Reply to: Myra Barenberg *JAOCS* Classified Advertising P.O. Box 3489 Champaign, IL 61826-3489
Phone: (217) 359-8028 Telex: 4938651 AOCS UI Telefax: (217) 351-8091