that the salinity of the germinating media induced some changes in the composition of lipids in jojoba seeds and these changes are most likely from an indirect effect of salinity on lipid utilization. While we cannot be certain that individual metabolic pathways are being affected differently by increased salinity, if the same general pathway suffers a slower rate of metabolism because of an increase in the free energy of activation, we would expect the pathway to exhibit a greater degree of discrimination among the different substrates.

ACKNOWLEDGMENT

We are grateful to Dr. Anthony H. C. Huang for his suggestions and review of the manuscript and to Ron B. New and Dr. Richard W. Konrat for their assistance in mass spectrometry analyses. Janet Hann and Mitsuo Matsumura are acknowledged for their support in the laboratory work. The Government of Pakistan, Ministry of Education, provided subsistance assistance to S.A.K. during the course of this investigation.

LITERATURE CITED

Davies, A. O.; Harwood, J. L. Effects of Far-red Light on the Labelling of Acyl Lipids During the Germination of Barley (*Hordeum vulgare*). *Ann. Bot.* **1984**, *53*, 141–151.

Duncan, C. C.; Yermanos, D. M.; Kumamoto, J.; Levesque, C. S. Rapid Ethanolysis Procedure for Jojoba Wax Analysis by Gas Liquid Chromatography. *J. Am. Oil Chem. Soc.* **1974**, *51*, 534–536.

Erdei, L.; Stuiver, G. E. C.; Kupier, P. J. C. The Effect of Salinity on Lipid Composition and on Activity of Ca^{2+} and Mg^{2+} Stimulated ATPase in Salt-sensitive and Salt-tolerant *Plantago* Species. *Physiol. Plant.* **1980**, *49*, 315–319.

Gharsalli, M.; Cherif, M. Effect of Sodium Chloride on the Sunflower Plant Lipid. In *Structure, Function and Metabolism of Plant Lipid*; Siegenthaler, P., Eichenberger, W., Eds.; Elsevier Science: Amsterdam, 1984.

Iring, D. W.; Shanon, M. C.; Bredan, V. A.; Mackey, B. E. Salinity Effects on Yield and Oil Quality of High-linoleate and High-oleate Cultivars of Safflower (*Corthamus tinctorius* L.). *J. Agric. Food Chem.* **1988**, *36*, 37–42.

Kayani, S. A.; Naqvi, H. H.; Ting, I. P. Effects of Salinity on Seed Germination, Seedling Growth and Mobilization of Reserves in Jojoba (*Simmondsia chinensis*) Seed. *Crop Sci.* **1990** (in press).

Lees, G. L.; Thompson, J. E. Lipid Composition and Molecular Organization in Plasma Membrane-enriched Fractions from Senescing Cotyledons. *Physiol. Plant.* **1980**, *49*, 215–221.

Miwa, T. K. Jojoba Oil Wax Esters and Derived Fatty Acids and Alcohols Gas Chromatographic Analysis. *J. Am. Oil Chem. Soc.* **1971**, *48*, 259–264.

Moreau, R. A.; Huang, A. H. C. Gluconeogensis from Storage Wax in the Cotyledons of Jojoba Seedlings. *Plant Physiol.* **1977**, 329–333.

Morrison, W. R.; Smith, L. M. Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boronfluoride-methanol. *J. Lipid Res.* **1964**, *5*, 600–608.

Muller, M.; Santarius, K. A. Changes in Chloroplast Membrane Lipids During Adaptation of Barley to Extreme Salinity. *Plant Physiol.* **1978**, *62*, 326–329.

National Academy of Sciences. *Jojoba-New Crop for Arid Lands, New Raw Material for Industry*; National Academy of Sciences: Washington, DC, 1985.

Spencer, G. F.; Plattner, R. D.; Miwa, T. Jojoba Oil Analysis by High Pressure Liquid Chromatography and Gas Chromatography/Mass Spectrometry. *J. Am. Oil. Chem. Soc.* **1977**, *54*, 187–189.

Yermanos, D. M. Composition of Jojoba Seed During Development. *J. Am. Oil Chem. Soc.* **1975**, *52*, 115–117.

Yermanos, D. M.; Duncan, C. C. Quantitative and Qualitative Characteristics of Jojoba Seed. *J. Am. Oil Chem. Soc.* **1976**, *53*, 80–82.

Yermanos, D. M.; Francois, L. E.; Bernstein, L. Soil Salinity Effects on the Composition of Oil and Oil Contents of Safflower Seed. *Argon. J.* **1964**, *56*, 35–37.

Zarrouk, M.; Cherif, A. Effect of Sodium Chloride on Chloroplast Lipid Composition of Olive Tree Leaves (*Olea europea* L.). In *Structure, Function and Metabolism of Plant Lipids*; Siegenthaler, P., Eichenberger, W., Eds.; Elsevier Science: Amsterdam, 1984.

Received for review September 1, 1989. Accepted December 21, 1989.

Registry No. stearic acid, 57-11-4; oleic acid, 112-80-1; docosenoic acid, 28929-01-3; oleyl alcohol, 143-28-2; eicosenol, 115218-60-5; docosenol, 81736-43-8; hexacosenol, 81724-40-5.

Design of Enzyme-Targeted Agrochemicals

Philip Haworth* and Daniel L. Siehl

Sandoz Crop Protection Corporation, 975 California Avenue, Palo Alto, California 94304

The deliberate and considered design of chemical pesticides to inhibit specific enzyme (protein) sites represents the next likely step in the pursuit of novel agrochemicals. The transition from the current procedure of random screening, or analogue synthesis, to enzyme-targeted design will occur through positive cooperativity rather than competition. Possible strategies through which the design of enzyme-targeted agrochemicals can occur are discussed.

The pursuit of novel pesticidal compounds for application in agriculture is now at a critical point that will determine the future of this industry. In almost every case where a pesticide acts through specific enzyme inhi-

0021-8561/90/1438-1271$02.50/0

bition, the compound in question was obtained by accident rather than design. The "accidental" discovery is referred to as random screening (Menn, 1983), and to date this has been the sustaining approach in the agrochemical industry. The concept of designing pesticides, of all kinds, to act at a predetermined and previously characterized site(s) represents the most controversial aspect in the future of novel agrochemical development (Menn, 1983; Menn and Henrick, 1985). The controversy arises not over the desirability of the products of this strategy but rather in the nature of the approach employed to synthesize them.

To assess the potential of targeted agrochemical synthesis, we must first analyze the weakness of the alternative, "random" approach. Reviews of the mechanism of random screening indicate a dramatic rise in the number of compounds required for a successful discovery (Menn, 1983; Menn and Henrick, 1985). In 1956, a commercial product could be expected from screening 1800 compounds; by 1978, the number had risen to as high as 30 000. It must be assumed that this trend has continued in the last 10 years and is reflected in the decreasing number of chemical companies active in pesticide research (Menn, 1983). Despite this discomforting trend, the random approach has worked and the success of this strategy is the major reason we can now consider targeted synthesis (Baillie, 1985; Miller, 1985). For this reason, the transition from random screening to target-directed synthesis can be considered evolutionary rather than revolutionary.

The close relationship between these two approaches is clearly demonstrated when we consider the first step required in any targeted synthesis program. The selection of a target site for any pesticide must give overriding consideration to the "lethality" of that site. In the context of industrial research, the difference between an enzyme inhibitor and a pesticide can be measured in millions of dollars. The most promising target, therefore, is one at which a known pesticide acts. It is no coincidence that discovery of the site of action of atrazine (Arntzen et al., 1983) and subsequent structural characterization of the site (Trebst, 1986) provoked renewed interest in the synthesis of photosynthetic inhibitors (Drabber, 1987). Similarly, the neurotoxic activity of insecticides such as DDT encouraged the synthesis of second-generation pyrethroids (Coats, 1982), e.g., fluvalinate. While this "piggyback" approach to targeted design does have heuristic appeal, it is not always the perfect solution. In many instances, the sins of the father are visited upon the children in the form of resistant pests. In other cases, the products of random screening are so unique that further exploitation of the site is impossible. The postemergent herbicide glyphosate is apparently an excellent example of the unique exploitation of an enzyme site (Steinrücken and Amrhein, 1984).

If a previously exploited enzyme site cannot be guaranteed to give a product, then the next best thing is to exploit another enzyme in the same pathway. Several instances of this approach to selecting a target site can be found in the literature, one obvious example being the compound HOE-704 (Schulz et al., 1988). This herbicidal compound inhibits the second step in the biosynthesis of branched-chain amino acids, the enzyme acetolactate reductoisomerase. The first enzyme in this pathway, acetolactate synthase, is the site of action of the sulfonylurea (LaRossa and Schloss, 1984) and imidazolinone (Shaner et al., 1984) herbicides. This method of target selection does not encounter the same problems as the piggyback approach but often entails extensive biological/biochemical investigation of enzymes not previously characterized in the target pest. It is often the requirement of an extended period of apparently unproductive biochemistry that raises the biggest objections to a target-directed synthesis program.

Having selected a target enzyme, or receptor, and characterized its properties in vitro and, if possible, in vivo, we are faced with the difficult task of chemical synthesis based on a biological/biochemical precedent. The chemist can, in theory, exploit at least three existing lead structures: the substrate(s), the transition state, and the product(s). In the case of many enzymes, natural regulators might also provide a valuable lead structure (Christopherson and Morrison, 1985). The obvious target structure to pursue is that of the transition state, if it can be determined, since this structure will always have the highest affinity for the enzyme active site (Schloss, 1988). The synthesis of such compounds requires detailed information of the mechanistic route of the catalyzed reaction and usually involves complex synthesis of metastable transition-state mimics. Excellent examples of this approach to inhibitor design include targets such as chorismate mutase (Bartlett et al., 1988) and isopentenyl-diphosphate isomerase (Muehlbacher and Poulter, 1988). Yet, despite the sophistication of the science involved, no commercial pesticides have yet been developed from a transition-state design program. However, effective pesticidal transition-state mimics have been obtained by other means. For example, the herbicide gluphosinate (phosphinothricin) is a potent transition-state mimic that inhibits the enzyme glutamine synthetase (Logush et al., 1988). The existence of such compounds does suggest that the synthesis of transition-state analogues will eventually lead to commercial products. Substrate mimics, on the other hand, require less complex biochemical investigation of the target and, in some cases, less complex chemical synthesis. The limitation of substrate analogues often lies in the lack of specificity, since a given compound might often be the substrate of several enzymes. Furthermore, substrate mimics normally require relatively high concentrations at the active site. This last factor is often exacerbated by the subsequent accumulation of the natural substrate during the process of inhibition. The synthesis of irreversible inhibitors (Muehlbacher and Poulter, 1988) will, of course, overcome any accumulation of substrate. The synthesis of substrate analogues is essential in designing receptor ligands, where we can consider the natural ligand (hormone) as a substrate. This approach has been most actively pursued in the area of insecticide design, for example juvenoid analogues (Henrick, 1982), glutamate and GABA antagonists (Eldefrawi et al., 1985), and even neuropeptide analogues (Menn and Borkovec, 1989).

One final approach to target-directed synthesis is the design of suicide substrate compounds that, during the catalytic conversion, covalently attach to the enzyme active site. Such inhibitors often incorporate elements of the substrate, transition state, and product structures and are normally very specific and highly potent irreversible enzyme inhibitor (Waley, 1985). Although few examples of this approach can currently be found in the list of agrochemicals, the literature does contain examples from other fields (Kuo and Jordon, 1983).

At this stage in the evolution of pesticide design, not even the most ardent supporters would suggest that targeted synthesis is ready to replace random screening. However, as the development of molecular modeling and molec-

ular biology improves our ability to probe protein structure–function, the gap between the possibilities and probabilities of this stategy will decrease. The ultimate benefits of this approach will be a much broader range of environmentally safe agrochemicals.

LITERATURE CITED

Arntzen, C. J.; Steinback, K. E.; Vermaas, W. J.; Ohad, I. Molecular Characterization of the Target Site(s) for Herbicides Which Affect Photosynthetic Electron Transport. In *Pesticide Chemistry: Human Welfare and the Environment*; Matsunaka, S., Hutson, D. H., Murphy, S. D., Eds.; Pergamon Press: New York, 1983; Vol. 3.

Baillie, A. C. The Biochemical Mode of Action of Insecticides. In *Approaches to New Leads for Insecticides*; von Keyserlingk, H. C., Jäger, A., von Szezepanski, Ch., Eds.; Springer-Verlag: Berlin, 1985.

Bartlett, P. A.; Nakagawa, Y.; Johnson, C. J.; Reich, S. H.; Luis, A. Chorismate mutase inhibitors: synthesis and evaluation of some potential transition state analogues. *J. Org. Chem.* **1988**, *53*, 3195–3210.

Christopherson, R. I.; Morrison, J. F. Chorismate mutase-prephenate dehydrogenase from *Escherichia coli*: positive cooperativity with substrates and inhibitors. *Biochemistry* **1985**, *24*, 1116–1121.

Coats, J. R. Structure-Activity Relationships in DDT Analogs. In *Insecticide Mode of Action*; Coats, J. R., Ed.; Academic Press: New York, 1982.

Drabber, W. Can Quantitative structural activity analyses and molecular graphics assist in designing new inhibitors of photosystem II. *Z. Naturforsch.* **1987**, *42C*, 713–717.

Eldefrawi, M. E.; Abalis, I. M.; Filbin, M. T.; Eldefrawi, A. T. Glutamate and GABA Receptors of Insect Muscles: Biochemical Identification and Interactions with Insecticides. In *Approaches to New Leads for Insecticides*; von Keyserlingk, H. C., Jäger, A.; von Szezepanski, Ch., Eds.; Springer-Verlag: Berlin, 1985.

Henrick, C. A. Juvenile Hormone Analogs: Structure-Activity Relationships. In *Insecticide Mode of Action*; Coats, J. R., Ed.; Academic Press: New York, 1982.

Kuo, D. J.; Jordan, F. Active site directed irreversible inactivation of brewer's yeast pyruvate decarboxylase by the conjugated substrate analogue (E)-4-(4-chlorophenyl)-2-oxo-3-butenoic acid: development of a suicide substrate. *Biochemistry* **1983**, *22*, 3735–3740.

LaRossa, R. A.; Schloss, J. V. The sulfonylurea herbicide sulfometuron methyl is an extremely potent selective inhibitor of acetolactate synthase in *Salmonella typhimurium*. *J. Biol. Chem.* **1984**, *259*, 8753–8757.

Logush, E. W.; Walker, D. M.; McDonald, J. F.; Leo, G. C.; Franz, J. E. Synthesis of α- and γ-alkyl-substituted phosphinothricins: potent new inhibitors of glutamine synthetase. *J. Org. Chem.* **1988**, *53*, 4069–4074.

Menn, J. J. Present Insecticides and Approaches to Discovery of Environmental Acceptable Chemicals for Pest Management. In *Natural Products for Innovative Pest Management*; Whitehead, D. L., Bowers, W. S., Eds.; Pergamon Press: New York, 1983.

Menn, J. J.; Henrick, C. A. Newer Chemicals for Insect Control. In *Agricultural Chemicals of the Future*; BARC Symposium 8; Hilton, J. L., Ed.; Rowmann and Allenheld: Ottowa, 1985.

Menn, J. J.; Borkovec, A. B. Insect neuropeptides: potential new insect control agents. *J. Agric. Food Chem.* **1989**, *37*, 271–278.

Miller, T. A. Can Mode of Action Studies Guide Chemical Synthesis? In *Approaches to New Leads for Insecticides*; von Keyserlingk, H. C., Jäger, A., von Szczepanki, Ch., Eds.; Springer-Verlag: Berlin, 1985.

Muehlbacher, M.; Poulter, C. D. Isopentyl-diphosphate isomerase: inactivation of the enzyme with active-site-directed irreversible inhibitors and transition-state analogues. *Biochemistry* **1988**, *27*, 7315–7328.

Schloss, J. V. Significance of slow binding enzyme inhibition and its relationship to reaction intermediate analogs. *Acc. Chem. Res.* **1988**, *21*, 348–353.

Schulz, A.; Spönemann, P.; Kocher, H.; Wengenmayer, F. The herbicidally active experimental compound HOE-704 is a potent inhibitor of the enzyme acetolactate reductoisomerase. *FEBS Lett.* **1988**, *230*, 375–378.

Shaner, D. L.; Anderson, P. C.; Stidham, M. A. Imidazolinones: potent inhibitors of acetohydroxyacid synthase. *Plant Physiol.* **1984**, *76*, 545–546.

Steinrücken, H. C.; Amrhein, N. 5-Enoypyruvyl shikimate-3-phosphate synthase of Klebsiella pneumoniae 2. Inhibition by glyphosate [N-(phosphonomethyl)glycine]. *Eur. J. Biochem.* **1984**, *143*, 351–357.

Trebst, A. The topology of the plastoquinone and herbicide binding peptides of photosystem II in the thyalkoid membrane. *Z. Naturforsch.* **1986**, *41C*, 240–245.

Waley, S. G. Kinetics of suicide substrates: practical procedures for determining parameters. *Biochem. J.* **1985**, *227*, 843–849.

Received for review July 11, 1989. Accepted October 22, 1989.