Contemporary Frontiers in Chemical Pesticide Research[1]

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Chemical pesticide research has become a diverse and complex field of science. Rapidly escalating research and development costs, increased difficulties in registration, growing concern for the environment, and a decreasing rate of discovery of chemical pesticides that have both sufficient activity and economic viability to justify commercialization have focused attention on new frontiers which hold promise of discovering safe, cost-effective, selective, and environmentally acceptable pesticide chemicals. Broadly based interdisciplinary research utilizing natural product models, biochemical design, and biorational approaches holds great promise for discovery of better pesticides.

Pesticide research has become such a vast interdisciplinary field of science that a short summary of research in its frontier areas must be, of necessity, subjective and incomplete.

The complexity of pesticide science can only be fully appreciated by considering the diversity of scientific disciplines which together contribute to the advancement of this science. No comprehensive research endeavor in this field can fully succeed today without an intensive interdisciplinary effort which includes chemists, biologists, biochemists, pharmacologists, toxicologists, and other specialists. It is only when such groups are well integrated and motivated to work in concert that the chances of success are increased, may it be in elucidating the mode of action of a pesticide in an academic laboratory or discovery of a new pesticide in an industrial setting.

Since I have spent most of my professional career as an industrial scientist, and in keeping with my experience and scientific interests, I will focus my remarks on research approaches which have led to the discovery of most pesticides known to us today and on those, in my opinion, holding the key of the future.

The approaches considered here are as follows: empirical synthesis/conventional screening, analogue synthesis/SAR optimization, natural product models, biochemical design, and biorational synthesis/innovative screening.

EMPIRICAL AND ANALOGUE SYNTHESIS

Most known pesticides, including chlorinated hydrocarbons, organophosphorus esters (OPs), *N*-methylcarbamates, substituted urea herbicides, thiocarbamates, many of the synthetic pyrethroids, and many others, were discovered via empirical and analogue synthesis in combination with conventional screening and optimization by structure–activity relationships (SAR).

Table I provides a brief chronology of well-known insecticides which were discovered via empirical and guided synthesis programs. A quick survey of the classes and compounds listed in Table I reveals that most insecticides in current use were discovered by these strategies. In some instances, e.g., chlordimeform and diflubenzuron, the discovery emanated from a program aimed at synthesizing new herbicides based on chemical modification of urea herbicides and, in the case of diflubenzuron, evolving the latter from hybridizing the chemistry of the urea herbicides and of dichlobenil (Figure 1). Deletion of two methyl groups from diuron and substitution of a benzoyl carboxy moiety for the nitrile group of dichlobenil yielded a compound, Du-19111, which in combination with innovative biological testing, was shown to be a forerunner of a new selective class of insecticides, which cause the death of insects indirectly by interfering with chitin synthesis and deposition (Verloop and Ferrell, 1977).

This discovery stimulated intensive synthesis programs and new biological testing methods in many industrial research laboratories in the quest of other improved and selective inhibitors of chitin deposition in the insect cuticle. The outgrowth of this effort is summarized in Figure 2, which shows the structures of diflubenzuron (PH 60-40) and three other highly active related compounds: penfluron, EL-494, and Bay Sir-8514.

The discovery that compounds of this class (Figure 2) are insecticidal by virtue of their unique inhibitory action on chitin deposition, (Marx, 1977; Verloop and Ferrell, 1977), ovicidal, and chemosterilant action (Ascher and Nemny, 1974; Wright and Harris, 1976; Oliver et al., 1977) stimulated research into improved in vivo and in vitro assays to direct SAR. In a recent publication Hajjar and Casida (1979) described an elegant in vitro assay using emptied abdomens of the adult milkweed bug, *Oncopeltus fasciatus* (Dallas), which convert ^{14}C-labeled glucose, glucosamine, and *N*-acetylglucosamine to [^{14}C]chitin with yields of 4–5% within 1 h. Addition of diflubenzuron to the incubate resulted in 50% inhibition of [^{14}C]chitin formation at 5.5×10^{-7} M (0.2 μg/g of abdominal wall).

Undoubtedly, assays as described in the foregoing, if they correlate well with in vivo activity, will prove to be of great benefit in SAR optimization and as a rapid guide for directed analogue or de novo synthesis.

As a result of improved screening methods and as a fallout from synthesis of benzoylphenylureas, a new class of pyrazoline insecticides was discovered by Mulder et al. (1975). A lead compound in this series, 3-(4-chlorophenyl)-1-(4-chlorophenylcarbamoyl)-2-pyrazoline (PH 60-41), is shown in Figure 3. Insecticides of this type appear to induce knockdown, cessation of feeding, and convulsions in lepidopterous larvae, presumably acting through central nervous disturbances.

It seems to me that we are now on the threshold of discovering an even greater array of diverse chemical structures than hitherto known, which act on selective target mechanisms in the insect. Much of this is due to our improved knowledge of insecticide pharmacology and insect physiology. As discussed above, it is a matter of

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[1]This article is based on an invitational talk by the author presented at the 178th National Meeting of the American Chemical Society, Washington, DC, Sept 9–14, 1979, on the occasion of being awarded "The Burdick and Jackson International Award for Research in Pesticide Chemistry" by the Pesticide Chemistry Division, American Chemical Society.

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Table I. Chronology of Insecticides Discovered via Empirical and Analogue Synthesis Programs[a]

decade	class of chemicals and names	strategy
1940's	*chlorinated hydrocarbons:* DDT, BHC, aldrin, chlordane, toxaphene	guided synthesis
	OP's: parathion, methyl parathion	guided synthesis
	carbamates: dimetan, dimetilan, isolan	insect repellent synthesis
1950's	carbaryl, malathion, azinphosmethyl, phorate	guided synthesis
	vinyl phosphates	guided synthesis
1960's	fonofos, trichloronate, mexacarbate, bufencarb, carbofuran, aldicarb, methomyl	guided synthesis
	synthetic pyrethroids: resmethrin	from natural product prototype
	formamidines: chlordimeform	herbicide synthesis
1970's	*synthetic pyrethroids:* (2nd generation) cypermethrin, permethrin, decamethrin, fenvalerate	guided synthesis
	new OP's: terbufos, methamidophos, acephate, sulprofos, profenfos	guided synthesis
	new carbamates: bendiocarb, thiofanox	guided synthesis
	benzoylphenyl ureas: diflubenzuron	herbicide synthesis

[a] Adapted in part from Bergman (1979).

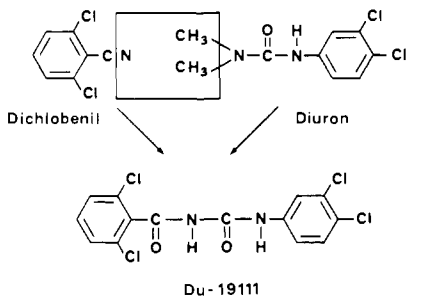

Figure 1. Structures of dichlobenil, diuron, and Du-19111.

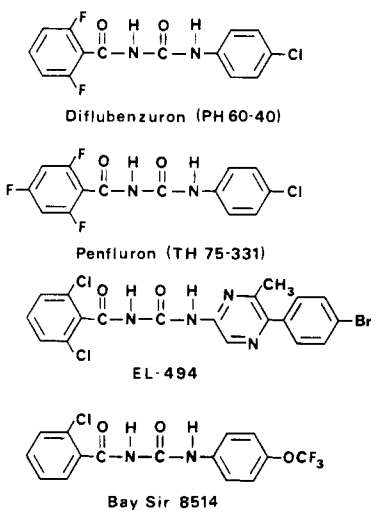

Figure 2. Structures of promising molt disrupting compounds.

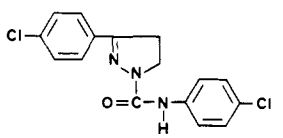

Figure 3. Chemical structure and name of PH 60-41.

record that most of the pesticides in current use were discovered in empirical and/or guided synthesis programs. However, as shown in Table II, the success rate for a commercial pesticide has retrogressed from 1/1800 in 1956 to 1/12000 estimated for 1977 (Gilbert, 1978). According to this progression, we may encounter the astronomical ratio of 1/80000 in 1997. Obviously, it is no longer feasible to pursue this search by the same strategies that worked so well in earlier years. Indeed, the low projected success rate and increasing costs of research and development and regulatory requirements may, according to Gilbert (1978),

Table II. Number of Compounds Passing through Each R&D Stage per Commercial Product[a]

activity	1956	1964	1970	1972	1977 Est.	1997
synthesis and initial bioscreen	1800	3600	8000	10000	12000	?
advanced screening	60	36	80	NA		
field evaluation	6	4	4	NA		
development	2	2	2	NA		
sales	1	1	1	1		

[a] Adapted from Braunholtz (1977) and Gilbert (1978).

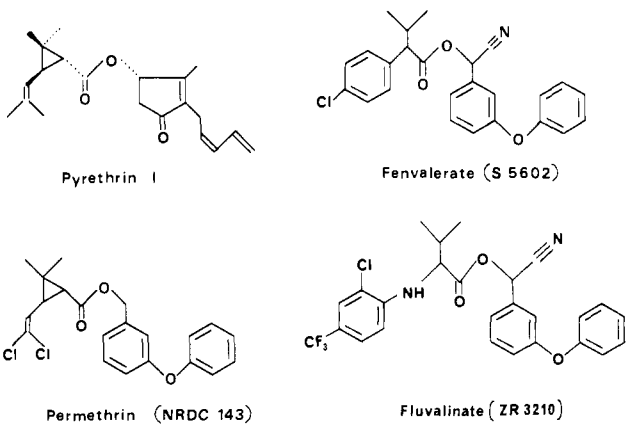

Figure 4. Chemical evolution of pyrethroids.

"tip the scale" against continuing innovative pesticide research.

However, it is my belief that new scientific insights into fundamental life processes in insects and plants coupled with advanced analytical instrumentation offers unparalleled opportunities for more rational research in the quest for discovering selective, effective, and biodegradable pesticides. Let us explore a few of these avenues and illustrate with selected models some opportunities for finding successful compounds at a ratio which increases the economic attractiveness of industrial research in this area.

MODELS FROM NATURAL PRODUCTS

Natural products of plant, animal, or microbial origin provide a vast source of bioactive substances which have been sparingly exploited as synthetic models or as actual pesticides (Casida, 1976; Marini-Bettolo, 1976). The rapid development and application of powerful analytical tools such as MS, NMR, LC, and related techniques have facilitated identification of natural products at an increasing pace.

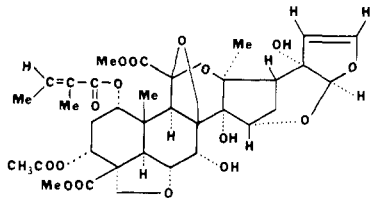

Figure 5. Structure of the insect phagorepellent azadirachtin (Zanno et al., 1975).

Among the best known natural products and ones of great economic importance involve the insecticidal principles found in the extract of pyrethrum flowers, *Chrysanthemum cinerariaefolium*, exploited already in Europe by the 19th century (Gnadinger, 1936; Casida, 1973). Pyrethrin I (Figure 4) proved to be an excellent prototype for synthetic pyrethroids (Elliott, 1977; Elliott and Janes, 1978). Some features of the chemical evolution of synthetic pyrethroids are shown in Figure 4. Systematic synthetic effort led to significant breakthroughs achieved by Elliott and his co-workers (Elliott, 1977), which culminated in the synthesis of permethrin, a potent photostable insecticide. Simultaneously efforts in the Sumitomo Chemical Company Laboratories led to the discovery of fenvalerate which represents a further chemical departure from the pyrethrin template due to the elimination of the cyclopropane ring. Recently, additional chemical alterations carried out in our laboratories resulted in the synthesis of an unusual new series of substituted 3-methyl-2-phenylaminobutanoates. This series, which incorporates a valine moiety, introduces the nitrogen atom into the acid portion of the pyrethroid molecule. Fluvalinate, α-cyano-3-phenoxybenzyl 2-(2-chloro-4-trifluoromethylphenylamino)-3-methylbutanoate represents one of the most active members in this series (Henrick et al., 1980).

Several other active plant principles have been used as insecticides but with more limited success. These include the rotenoids, represented by a complex pentacyclic structure, and the alkaloid nicotine. Neither of these served as useful prototypes for synthetic insecticides for a variety of reasons. Rotenoids failed due to high mammalian and fish toxicity (Matsumura, 1975) and an inflexible structure represented by a pentacyclic ring. Although extensive synthesis and SAR of nicotinoids was carried out (Metcalf, 1955; Yamamoto et al., 1962), little practical success was encountered based on the SAR studies. The Japanese investigators postulated the need for strict structural requirements including the basicity and a fixed distance (4.2 Å) between the two nitrogen atoms in order to bind to the acetylcholine receptor. Apparently, the nicotine model has not been totally shelved. Recently, Soloway and co-workers (1979) reported the synthesis of a highly insecticidal series of nitroketenedimethyl mercaptol substituted diamines. Conceivably, the inspiration for this new series of compounds came from the nicotine model.

Chemical research on bioactive natural product models is especially fruitful when conducted in concert with biologists and ecologists. Several active insect antifeedants have been identified by scientists at the International Centre of Insect Physiology and Ecology in East Africa (Meinwald et al., 1978). Of special interest are the antifeedant sesquiterpenoids warburganal and muzigadial. Despite their deceptively simple chemical configuration, synthesis in this area is very difficult. An even more formidable obstacle to further synthetic modifications presents itself in the structure of the systemic insect phagorepellent, azadirachtin (Figure 5), elegantly identified

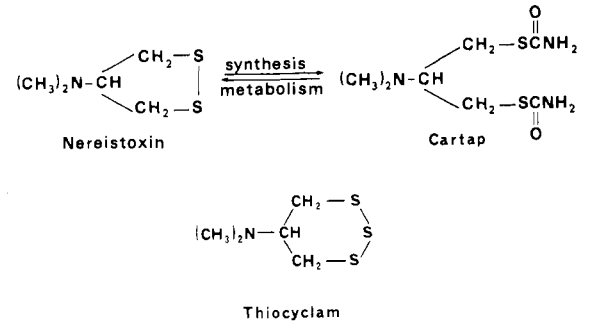

Figure 6. Nereistoxin, its synthetic bisthiocarbamate derivative, cartap and thiocyclam.

by Zanno et al. (1975). This triterpenoid feeding inhibitor is only one of several insect feeding and growth inhibitors present in the neem tree, *Azadirachta indica* A. Juss (Warthen, 1979). A far more useful model was discovered in conjunction with nereistoxin, an insect toxin isolated from the marine annelid, *Lumbriconereis heteropoda* (Konishi, 1972; Sakai and Sato, 1972). Intensive chemical follow-up resulted in the synthesis of the bisthiocarbamate rice insecticide, cartap (Konishi, 1972), and the trithiane insecticide, thiocyclam, *N,N*-dimethyl-1,2,3-trithian-5-amine (Watkins and Weighton, 1975; Berg and Knutti, 1975) (Figure 6). Cartap is converted in vitro and in vivo to nereistoxin (Sakai and Sato, 1972) and similarly in rice paddy water (Tomizawa et al., 1974).

It is very likely that major advances in this frontier area will be forthcoming in the coming years, especially with more intensive search for new pesticidal classes aided by advanced instrumentation and more innovative bioassays.

BIOCHEMICAL DESIGN

Rational design of pesticide chemicals based on metabolic pathways, enzyme function, and mode of action of pesticides may be a goal whose time has come (Corbett, 1974; Hollingworth, 1975; Casida, 1979).

Several early discoveries involving bioactivation reactions of pesticides in insects, mammalian, and plant species have demonstrated the importance of oxidative reactions in the conversion of pesticides to more active forms. Important examples include the oxidation of the thioether moiety of OP insecticides such as demeton, disulfoton, and phorate (Metcalf et al., 1957) and oxidative N-desalkylation of dicrotophos to monocrotophos in animals and plants (Menzer and Casida, 1965). Thiocarbamate herbicides have also been shown to oxidize at the *S*-alkyl moiety to form the corresponding sulfoxides in animals and plants (Hubbell and Casida, 1977). These sulfoxides display increased herbicidal activity compared to the parent thiocarbamates (Casida et al., 1974). Also it has been shown that carbophenothion sulfoxide unexpectedly reduces to the parent carbophenothion in the living rat and in an in vitro preparation containing rat liver enzymes, NADPH and FAD (DeBaun and Menn, 1976), thus demonstrating a bioreversible pathway. Previously sulfoxidation was considered to be an irreversible step leading to lethal synthesis. Metabolic studies with tri-*o*-tolylphosphate (TOCP) led to the discovery of the insecticide salithion (Eto, 1974). In the presence of microsomal enzymes (mfo), TOCP undergoes oxidation and intramolecular transphosphorylation, giving rise to a cyclic saligenin phosphate. The latter served as the prototype for the synthesis of the methoxy ester, salioxon, and its thiono derivative, salithion, a nonneurotoxic, selective insecticide (Eto et al., 1963).

These findings in concert with greater awareness and knowledge of metabolic processes should be useful in de-

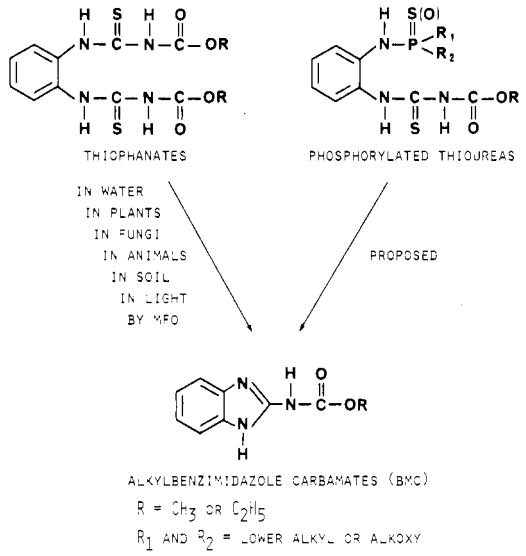

Figure 7. Rearrangement of thiophanates and phosphorylated thioureas to alkylbenzimidazole carbamates (BMC).

signing pesticidal molecules which contain moieties susceptible to bioactivation or detoxification in vivo. The foregoing examples conform also to the definition of prodrugs (Stella, 1975). Accordingly, pesticides undergoing biotransformation prior to exhibiting their pharmacological action should be considered as pro-pesticides. Incorporation of pro-pesticidal features into a molecule could reduce hazard, improve transport to the target, improve residual properties and often change lipophilicity, and enhance penetration through the cuticle of insects and plants.

On the basis of the discovery that benomyl and thiophanate fungicides exert their fungitoxicity through transformation to alkyl benzimidazolecarbamates (BMC) (Kaars-Sijpesteijn, 1977) (Figure 7) and the knowledge that BMC derivatives are potent anthelmintics, certain phosphorylated thioureas were synthesized (Mihailovski and Baker, 1973) and shown to be active anthelmintics (Menn, 1976). Very likely the phosphate moiety serves here as a useful carrier moiety aiding in transport to the target. Cleavage of the OP moiety and loss of S from the thiocarbonyl group results in rearrangement to BMC (Figure 7) (Walter and Voss, 1970). Conceivably other substituents ordinarily considered as toxophores, such as carbamates, may also serve as useful carrier groups for the toxophore moiety.

One of the more intriguing questions involving the mode of action of sulfenimide fungicides characterized by an N–S bond concerns their mode of action. Several of these fungicides contain a trihalomethylthio moiety (Kohn, 1977). Lukens and Sisler (1958) and Lukens (1969) proposed that the fungitoxicity of captan was expressed through the trichloromethylthio moiety which interacts with cellular thiols releasing thiophosgene, the putative toxicant. However, the situation is more complicated since there are several other active sulfenimide fungicides which do not contain a thiophosgene releasing moiety (Kohn, 1977).

Metabolism studies with [*trichloromethyl*-^{14}C]captan reported by DeBaun and co-workers (1974) elucidated the fate of the trichloromethylthio moiety in a mammalian species. On the basis of this work we proposed a scheme for the formation of thiophosgene in vivo by reaction of captan with thiol compounds (Figure 8). Captan was rapidly degraded in the rat by reacting with thiol groups (RSH), and the resultant thiophosgene was rapidly detoxified via three pathways: (1) oxidation and/or hydrolysis to CO_2, (2) condensation with a cysteine moiety of glutathione (GSH) to yield a thiozolidine derivative, and (3) reaction with intestinal sulfite ions to form a sulfonic acid derivative. The last pathway is the most significant detoxification route for orally administered captan, accounting for 35% of the total dose. Since in in vitro experiments this route is circumvented, liberated thiophosgene may have a greater role in altering biochemical processes.

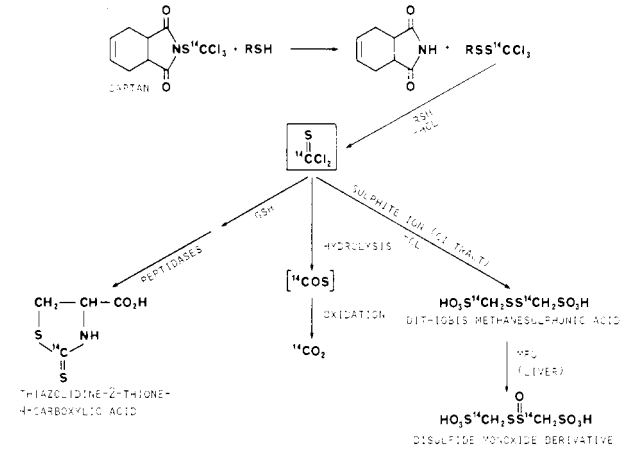

Figure 8. Formation and detoxification of thiophosgene evolved from metabolism of captan in the living rat (adapted from DeBaun et al., 1974).

The foregoing illustrates the importance of metabolic studies not only to assess potential safety of a xenobiotic, but also to help in determining the nature of the toxophore. These studies ultimately assist in the design of new pesticide chemicals.

In conjunction with the discovery and development of pesticides, there has been an ongoing search for compounds which would confer useful selectivity on a pesticide toward nontarget species. In this category are considered herbicide safeners (Pallos and Casida, 1978) and yet to be found practical safeners for hazardous insecticides which may come in contact with nontarget animals and man.

The success encountered in the discovery of safeners which protect corn from injury by thiocarbamate herbicides (Pallos et al., 1978) has led to an intensive search for protectants for other classes of herbicides in several industrial and academic laboratories. Undoubtedly such programs have been aided by the elucidation of the mode of action of the dichloroacetamide antidotes which enhance thiocarbamate sulfoxide detoxification in corn plants, most likely via induction of glutathione (GSH) and glutathione-*S*-transferase (Lay et al., 1975). This model proved to be useful in searching for a safener for the thiocarbamate rice herbicide, molinate, which unexpectedly proved to be highly toxic to Japanese carp, *Cyprinus carpio* var. Yamato Koi (Kawatsu, 1977), but not to the American carp at 50-fold higher concentration in water (Lay et al., 1979). Subsequent metabolism studies with Japanese carp revealed only minor involvement of GSH conjugation in detoxification of molinate sulfoxide (Lay et al., 1979; Lay and Menn, 1979). The latter represents a major detoxification route in the rat (DeBaun et al., 1978). In light of this finding a series of dichloroacetamides were tested as possible safeners for molinate in Japanese carp, either by treating the water or incorporating the compounds in fish food (Lay, 1978). One compound, *N*-ethyl-*N*-benzyldichloroacetamide, when added to water at the rate of 4 ppm or when incorporated in fish food at 0.06%, increased liver GSH approximately 3-fold and 2.5-fold, respectively, over the control and significantly reduced mortality of fish (Lay,

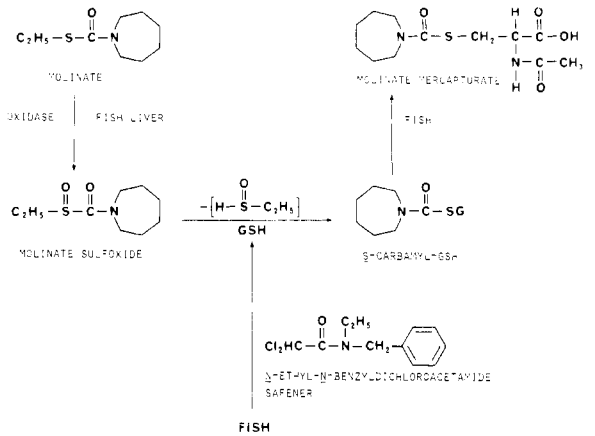

Figure 9. Proposed scheme for the enhancement of molinate sulfoxide detoxification in Japanese carp via elevation of GSH induced by *N*-ethyl-*N*-benzyldichloroacetamide safener.

1978). However, there was no increase in glutathione-*S*-transferase. To explain the possible mechanism of action of the safener in fish, a model based on that shown by Lay et al. (1975) in corn is suggested for the safening effect resulting from the foregoing treatments with *N*-ethyl-*N*-benzyldichloroacetamide (Figure 9). It is proposed that enhancement of GSH in the presence of the safener would increase degradation of molinate sulfoxide via carbamoylation of GSH and formation of *S*-carbamyl-GSH conjugates including molinate mercapturate (Lay and Menn, 1979). The foregoing model, although imperfect and still speculative, suggests a rationale for future search for safeners. Indeed, amelioration of toxicity of other pesticide chemicals detoxified on conjugation with GSH may be achieved in the future by chemicals which enhance the above process in plants and animals. Furthermore, knowledge of key biotransformation reactions and the associated enzymes in the metabolism of xenobiotics, may provide further clues useful in the design of potential safeners and synergists.

BIORATIONAL DESIGN AND INNOVATIVE SCREENING

Biorational design of pesticides is to a larger extent an unconquered frontier. I am sure that with the rapid explosion of new knowledge in the area of molecular biology, biochemistry, and pharmacology significant advances will also be achieved in the biorational design of successful pesticide chemicals. It is still a matter of conjecture as to what is meant by the term "biorational design". Djerassi et al. (1974) first used this term to define "biorational" chemical agents such as pheromones, IGRs, and antijuvenile hormone compounds. One of my colleagues, Clive Henrick (1979), suggested very appropriately that biorational design applies to "chemical follow-up to a biological breakthrough". A suitable example, compatible with this definition, pertains to the discovery of insect growth regulators (IGRs) with juvenile hormone activity which mimic the action of natural juvenile hormones (Slama et al., 1974; Staal, 1975; Menn and Pallos, 1975; Henrick et al., 1976; Siddall, 1976, 1977).

Ground breaking research by Bowers and co-workers (1965), essentially predicting the structure of juvenile hormone JH-III, and the chemical identification of JH-I by Roller et al. (1967) paved the way for the synthesis of potent terpenoid IGRs. In no small measure were these discoveries aided by elegant bioassays designed to assess latent effects of these remarkable compounds, such as morphogenetic, reproductive, and behavioral effects. Figure 10 shows the structures of JH-I, methoprene (Altosid), the first registered commercial IGR; R-20458, an experimental terpenoid IGR; a bisthiolcarbamate IGR (Pallos et al., 1976); and precocene-II, a naturally occurring chromene antijuvenile hormone (Bowers et al., 1976).

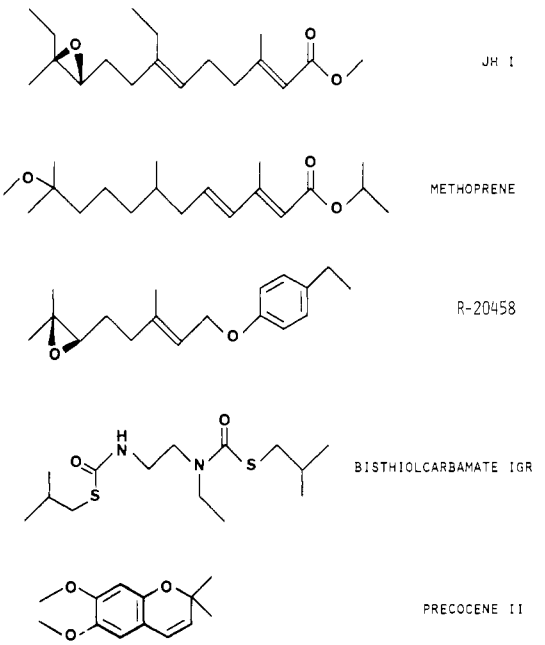

Figure 10. Chemical structure of JH-I, methoprene, R-20458, bisthiolcarbamate-IGR, and precocene-II.

The development of methoprene came as a direct follow-up on the structure of JH-I and early recognition of the activity of exogenous JH-like compounds (Henrick et al., 1973, 1978). The remarkable improvement in bioactivity of methoprene vis-à-vis JH-I resulted from synthetic optimization, replacing the 10,11-epoxide by a tertiary methoxyl group and introducing a conjugated 2,4-dienoic ester system (Siddall, 1977). The IGR R-20458 (Pallos et al., 1971) represents a further chemical departure from JH-I, but it still retains a terpenoid configuration. The bisthiolcarbamate (Figure 10) is a further modification of the JH template yet it retains selective IGR activity (Pallos et al., 1976).

Almost from the beginning of IGR research there was a growing awareness among several investigators that the ultimate, selective insect control chemicals would be substances which antagonize the action of JH (Bowers, 1977). The discovery that the chromenes, precocene-I and -II induced precocious metamorphosis and sterility in a few hemipteran species (Bowers et al., 1976) provided a model for intensive follow-up synthesis in several industrial and academic laboratories. Although little success has been realized thus far, this first anti-JH prototype has paved the road for further research into this most difficult, yet challenging, area of biochemical research.

SUMMATION

The foregoing is a modest effort to highlight a number of newer approaches which hold the promise of translating current and future knowledge into target specific pesticides, conforming to societal and environmental needs.

My career in pesticide research started almost 25 years ago. In this span of time I had the opportunity both as an active participant and as an observer to be part of the many great advances made in the pesticide field during this period.

This was a quarter century of seemingly unlimited opportunities for the discovery and development of a great variety of pesticide chemicals. Perhaps due to the success

of the empirical approach to synthesis and screening in the pesticide field (Corbett, 1974) and the drug field (Burger, 1978), newer approaches were largely neglected. Much new knowledge is currently at our disposal both in the insect and plant sciences. We need to harness and direct this knowledge to improve methods of the past in search of better pesticides. Indeed, in this context the words of Aldous Huxley (1945) are timely: "Facts are ventriloquist's dummies. Sitting on a wise man's knee they may be made to utter words of wisdom; elsewhere, they say nothing, or talk nonsense."

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REVIEW

Vitamin C Contents of Citrus Fruit and Their Products: A Review

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Variability in the vitamin C (ascorbic acid) contents of citrus fruit and their products is influenced by variety, cultural practice, maturity, climate, fresh fruit handling, processing factors, packaging, and storage conditions. Aerobic and anaerobic mechanisms are mainly responsible for the destruction of vitamin C in processed products. The mode of breakdown of vitamin C can best be explained by a first-order reaction but a significant quadratic time effect has been determined by polynominal regression calculations. Plots of log rate (loss of vitamin C) vs. $1/T$ for canned orange juice showed two distinct Arrhenius profiles, whereas canned grapefruit juice showed only one. Retention of vitamin C is greater in canned than bottled juices because of the reducing activity of the tinplate.

It has been known for many centuries that certain fruits and vegetables possess the ability to prevent and cure scurvy. As early as 1564 (Beattie, 1970), citrus fruit were used empirically for the prevention and treatment of this disease. However, it was not until the middle of the 18th century that the role of citrus fruit in fighting scurvy was scientifically demonstrated. In 1752 James Lind, a British Naval physician, published his "Treatise of the Scurvy" with the clinical data to prove that scurvy was due to the lack of an essential food element, now recognized as vitamin C. In a controlled experiment James Lind supplied two oranges and a lemon to seamen with scurvy and found that they were ready for duty in only 6 days. The world voyage of Captain Cook, from 1772 to 1775, also demonstrated that scurvy did not occur if vegetables and fruits (especially oranges and lemons) were included in the seaman's ration (Araujo, 1977). Therefore, from this evidence and as a precaution against this dreaded disease, the British Admiralty in 1795 ordered that every member of the crew be given a ration of lime (or lemon) juice. British sailors, to this day, are often called limeys because of this early association.

A century and more was to pass before definitive efforts were made to isolate and characterize this antiscorbutic factor. Zilva (1927) concentrated an antiscorbutic factor from lemons whereas Szent-Gyorgyi (1928) isolated the same factor (he called it "hexuronic acid") from cow adrenal glands, oranges, and cabbage. Waugh and King (1932) isolated a crystalline antiscorbutic substance from lemon juice and identified it as the same "hexuronic acid" isolated by Szent-Gyorgyi. The earliest official name given to this antiscorbutic factor was cevitamic acid but this name was later dropped in favor of the more common name, ascorbic acid (vitamin C). Haworth and Hirst (1933) and Reichstein et al. (1933) were the first to chemically synthesize vitamin C.

Since citrus fruit and their products are one of the largest suppliers of dietary vitamin C, it is important to know what factors affect vitamin C levels in this important consumer food. Vitamin C levels are influenced by (1) production factors and climate conditions, (2) maturity state and position of fruit on the tree, (3) type of citrus fruit (species and variety), (4) parameters used for processing fruit into different products, (5) type of container for holding the processed product, and (6) handling and storage.

PRODUCTION FACTORS

Citrus trees grow on clay to very sandy soils with properties ranging from fertile to infertile, acid to alkaline pH (5.0-8.5), and good to poor water drainage. The native conditions of the soil are not important to the composition of the fruit, in particular vitamin C, as long as essential nutrients are supplied in adequate amounts, soil pH is maintained between about 5.0 and 7.5, effective control of water supply and drainage is observed, and proper tillage of the soil is practiced (Reuther, 1973).

The supply of essential nutrients to a growing plant is enhanced through fertilization (soil and/or foliar application). Of the 15 elements recognized as essential for citrus growth, namely, carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, iron, zinc, manganese, copper, and molybdenum, only a few have a direct effect on the vitamin C contents of citrus fruit.

Several workers (Hilgeman and Van Horn, 1955; Smith and Rasmussen, 1961; Smith, 1969) have reported an inverse relationship between the quantity of nitrogen applied to grapefruit trees and the amount of vitamin C found in juices of those grapefruit. Reduced levels of vitamin C in

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