MAIN SUBJECT 2

DISCOVERY AND PROPERTIES OF CONVENTIONAL AND NOVEL CHEMICAL CONTROL AGENTS

TOPIC 2A

THE DISCOVERY OF CROP PROTECTION CHEMICALS

CHAIRMEN
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ROLE OF COMPUTERGRAPHICS IN THE DESIGN OF PLANT PROTECTION CHEMICALS

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INTRODUCTION

Let us begin with a fundamental question. What are the research departments of the major drug and crop protection companies trying to do? They are trying to invent small, biologically active molecules whose effects have commercial worth or advantage. In that case, what makes a molecule biologically active? Usually a molecule possesses activity primarily because it binds to an active site on a biological macromolecule, most commonly a three-dimensional protein structure. In the past, the discovery and subsequent development of biological activity has been done in three conceptual ways: (1) large scale empirical screening; (2) close analogue chemistry; (3) the more rational design of biologically active molecules at the molecular level. Although of greater financial potential, the great difficulty with the latter approach has always been our stark inability to answer several crucial questions concerning the binding of a small molecule to its protein receptor. For instance, what do (a) the substrate and (b) the active-site of the receptor actually look like as the one approaches the other. The free energy changes associated with the removal of a molecule from its solvent sheath are to some extent amenable to experimental evaluation, but the exact nature and geometry of the recognition process, collision and chemical binding of a small substrate to a protein is still largely a mystery.

In molecular terms, how one molecule appears to another, whether it be the substrate or the binding site, is really two questions.

Where are the nuclei?

This is not just a question of equilibrium shape as measured by n.m.r., x-ray or neutron spectroscopy, but also concerns what possible shapes the molecule can assume as it interacts with its partner; in general, what flexibility it possesses. Flexibility is clearly a property of both small molecules and the protein binding sites.

Where are the electrons?

This question too can only be studied experimentally for molecules in equilibrium and in a roughly homogeneous environment such as a crystal or in solution. What we really want to know is how the distribution of these electrons around the nuclei determine the likelihood of effective collision and how they then behave during the interaction. Since molecules interact most strongly at their accessible surfaces, it is important to know what these surfaces look like.

Advances in theoretical methods and computer technology mean that both these questions can now be answered using a computer and any number of easily obtained programs. Having obtained the answer to our problem theoretically however, there is a further difficulty. How can these often complex molecular properties be displayed. This is really the crux of the matter for the world's crop protection companies. It is a fact of life that the scientists trained to make molecules will not be influenced by those trained to design them unless the proposed rationale can be seen to be obvious. Computer Molecular Graphics provides this link between a chemist's intuition and the vast array of chemical, physical and biological information. As an example of the use of computer graphics and theoretical methods this paper describes a study in the design of the triazole fungicides, for instance the ICI compounds diclobutrazol ('Vigil') and the new flutriafen, and the Bayer compound triadimefon ('Bayleton'). The Eli Lilly material triarimol ('Elancocide') though not a triazole fungicide is equivalent in its mode of action. This general class of fungicide is now attracting wide commercial interest in both the crop protection and pharmaceutical industries as inhibitors of fungal ergosterol biosynthesis (Fig 1).

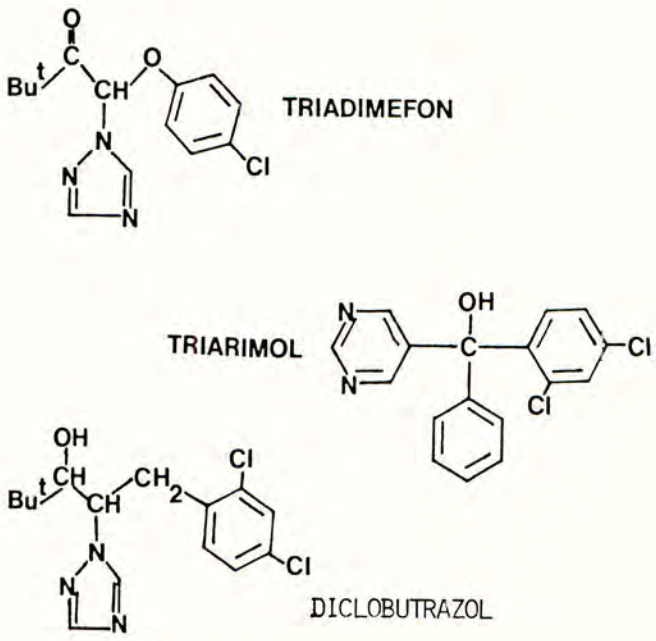

Fig. 1. Triazole fungicides

This design work can be divided into three stages :-

1. Assembly of biochemical, physical and biological information mostly from the literature, but also experiment, to construct a crude two dimensional picture of the site of action of these compounds.

2. A computer graphics facility was then used with available crystal data, molecular orbital and molecular mechanics calculations, infra-red and n.m.r. studies to construct a three dimensional model of the target enzyme active site (a cytochrome P-450) designed specifically to accommodate both the natural substrate (24 methylene 24,25 dihydro-lanosterol) and these known antagonists in their minimum or low energy forms.

3. This model was then used to suggest new structure activity relationships and contribute towards novel fungicide design.

THE QUALITATIVE ENZYME MODEL

It has been shown (Gadher et al 1983) that the triazole fungicides inhibit the 14α-demethylation of 24-methylene 24,25-dihydrolanosterol, the ergosterol precursor. This is a crucial step in ergosterol biosynthesis which has to be completed before a number of other steps can begin more or less in parallel. This inhibition is brought about by the compounds binding to the heme prosthetic group of the cytochrome P-450 oxidase enzyme system which catalyses this transformation. When added to a rat liver cytochrome P-450 preparation, for instance, an unmistakable Type II Soret difference spectrum is produced indicating that the triazole 4-nitrogen coordinates to the heme ferric ion which maintains its ferric (Fe^{3+}) low spin resting state. In so doing the antagonist has to displace the natural sixth liquid of the heme which is probably a water molecular (Griffin and Peterson 1975) or possibly an imidazole group derived from a protein histidine. The other axial ligand, below the heme plane is believed to be a cysteine sulphur as first suggested by Murakami and Mason (Murakami and Mason 1967).

The 14α-demethylation of dihydrolanosterol proceeds in three main stages with the two intermediates - the alcohol, 5α-lanost-8-ene-3β,32-diol, and the aldehyde, 3β-hydroxy-5α-lanost-8-en-32-al, being tightly protein bound. The cytochrome P-450 is the component of the enzyme system required to initiate oxidation of the 14α-methyl group, but not of that responsible for the subsequent oxidation steps required for its elimination as formic acid (Gibbons, Pullinger and Mitropoulos 1979). This initial oxidation also seems to be directly inhibited by the alcohol and aldehyde metabolities.

Consequently in computer modelling the antagonism of these heme binding fungicides it seemed necessary to consider only the first oxidation of the parent lanosterol to the 14-methyl alcohol. It is only in the last few years that a plausible mechanism for this oxidation has been suggested (Sligar, Kennedy and Pearson 1980). On binding the substrate the ferric porphyrin is converted from low to high spin due to the displacement of the high field sixth ligand. Studies with spin labelled substrates have shown that this substrate binding site places the bound molecule very close to the iron (Pirrwitz et al 1982). This complex is then reduced and as Fe^{2+} can then bind molecular oxygen. Further one electron reduction yields a species which is less well defined but corresponds to the hypothetical state [Fe^{3+} O_2^{2-}] which has all the electron equivalents required for

methyl hydroxylation, water production and regeneration of the ferric resting state. This final step, however, requires an effector molecule - a free acylating group, provided in bacterial hydroxylase by the carboxy terminal tryptophan, or the pemultimate glutamine of putidaredoxin. This acyl group is responsible through a peracyl group of generating the final iron-oxene intermediate.

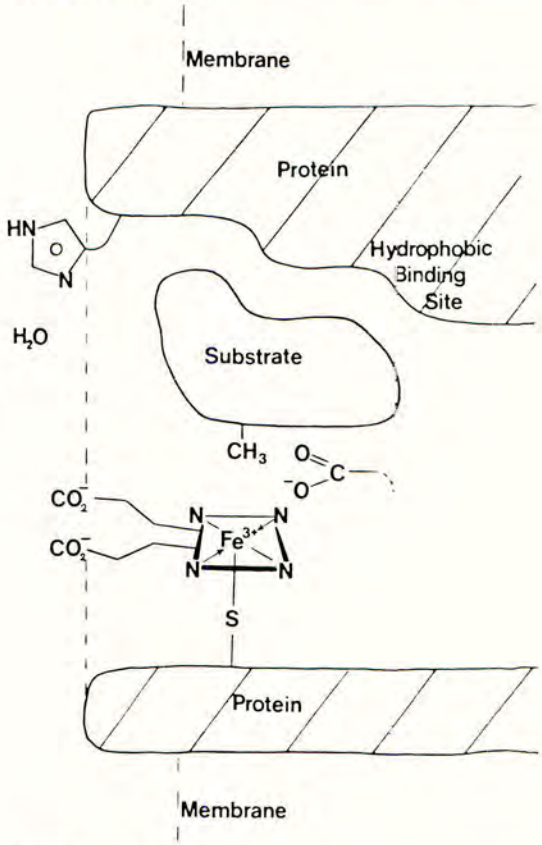

Fig. 2. Model enzyme site

Figure 2 shows the crude two dimensional model of the P-450 active site. In designing an inhibitor for this process there are three central features to consider :-

1. The heme prosthetic group available for complexation.

2. The hydrophobic substrate binding site specific for lanosterol. Indeed a recent paper by Dus (Dus 1982) implicates two binding sites for various cytochrome P-450's - one for substrate and the other for nascent product, and both with activated thiol groups.

3. The occurence of hydrophilic groups in an otherwise grossly hydrophobic environment. The porphyrin propionate side chains as suggested by Peterson et al. (Peterson et al 1978) and the acyl effector group could both intervene between the bound substrate and the plane of the heme. There is also the possibility of hydrogen bonding with the displaced histidine (if present) and also a general polar interaction with the polar interface which exists by virtue of the enzyme sitting in a membrane.

The task now was to locate the natural substrate and the flexible inhibitors in a three dimensional computer model of the enzyme site to examine if interactions with these features could go some way to providing plausible structure/activity relationships.

COMPUTER MODELLING OF ENZYME SITE

The crystal structures of the protoporphyrin IX and the lanosterol nucleus were obtained directly by computer link to the Crystal Structure Search and Retrieval (CSSR) library provided by the SERC on the Edinburgh Dec 10 Computer. The approach and interaction of the lanosterol with the iron-oxene system was then modelled on the graphics screen. Ideally, one might prefer to model some transition state for the reaction of the oxene with the 14-methyl group. However, since the intermediary alcohol could well be an inhibitor for this enzyme, the alcohol ground state geometry was chosen with an iron-oxygen distance of 1.9A° and a carbon-oxygen-iron angle of 130°. These values are those obtained theoretically by Loew for an iron-carbene system (Loew 1980). There are now three single bonds :- iron oxygen, oxygen-carbon and carbon-carbon about which the bound lanosterol can exercise internal rotations. The computer graphics facility could now be used to investigate the possible orientations of the lanosterol relative to the porphyrin ring and calculate sumultaneously, by molecular mechanics, the total internal energy of interaction.

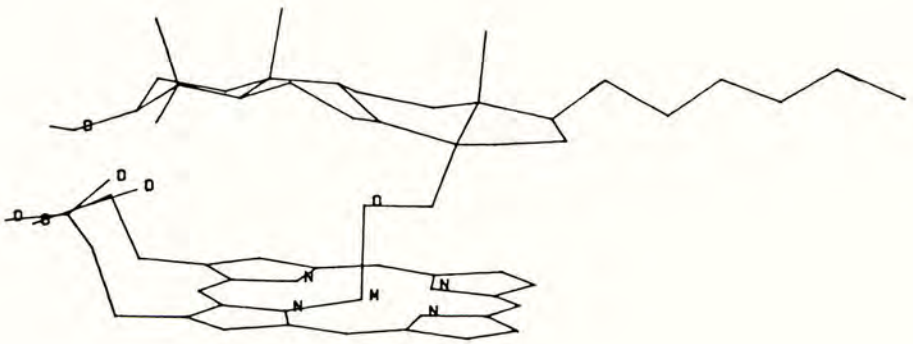

Fig. 3. Lanosterol on P-450 porphyrin ring

Figure 3, for example, places the lanosterol so as the 3β-hydroxyl polar group lies over the propionate side chains. To reduce the complexity of this picture one can now replace the lanosterol structure by a surface canopy to represent the extent of the hydrophobic substrate binding site. There is also the facility to code this surface to signify the electronic properties of the substrates such as their electron density, electrostatic potential, or HOMO/LUMO values. Theoretical work of this type is currently suggesting quite remarkable complementarity of electron properties between bound substrates and protein binding sites. (Weiner 1982).

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THE SHAPES OF BOUND ANTAGONISTS

At the beginning of this study the crystal structures of specimen antagonists were unknown. Theoretically the task of calculating all the low energy shapes for just one molecule of interest is considerable. A complete global minimisation for a typical triazole fungicide eg. diclobutrazol with five axes of rotation, sampled at 30° intervals, involves a quarter of a million individual calculations. Even with a large computer this severely degrades the quality of calculation which can be done at each point. A strategy was used, therefore, which attempted to reduce this number to a manageable level. Firstly, a crude molecular mechanics method based on Van der Waals contacts was used to eliminate from a full conformational search all those shapes which are sterically too high in energy to be considered for further analysis. All the remaining steric minima were then analysed using semi-empirical molecular orbital methods and subject to a single full ab-initio calculation to obtain the absolute minimum energy conformation. The calculated structure for RR-diclobutrazol, for example, agrees very well with the crystal structure as determined by Branch and Nowell (Branch et al. 1983). Good agreement between calculated and x-ray was also observed for the less fungicidally active isomer (RR-) of triadimenol (Spitzer, et al. 1982). The calculated structure for both diclobutrazol and triadimenol (Bayer) also seemed consistent with measured n.m.r. coupling constants.

Hydrogen bonding

In setting up these calculations the hydroxyl proton was placed so as to be unavailable for possible hydrogen bonding with the 2-N of the triazole. Theoretically it is well known that extraordinary lengths have to be undertaken to account for this phenomenon properly, even for simple molecules. It seemed more sensible to calculate the other energy contribution theoretically but to look for the formation of internal hydrogen bonding in a dilution experiment in the infra-red. Intra-molecular hydrogen bonds are not observed in the available crystal structures. Infra-red dilution studies show internal hydrogen bonds in both diclobutrazol diastereoisomers but in neither the active RS-, SR- or the less active RR-, SS- triadimenol. In short, the presence of an intra-molecular hydrogen bond between the hydroxyl group and the triazole 2-nitrogen does not in itself relate directly to activity.

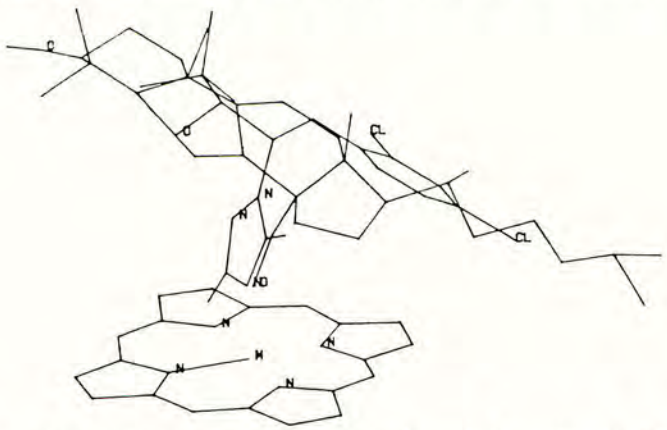

Fig. 4. Comparison of diclobutrazol and lanosterol in P-450 active site.

A COMPARISON OF THE ANTAGONISTS WITH THE NATURAL SUBSTRATE

As an example of the techniques, Figure 4 shows a comparison of the fungicidally active RR- diclobutrazol with the natural substrate lanosterol. The sterol C-32 alcohol is chelated to the iron porphyrin. The three central features of the model cytochrome P-450 can be elucidated. The hydrophobic binding site, the polar region between this hydrophobic region and the heme plane, and a common complexation to the porphyrin iron.

Three features might be noted :

1. The hydrophobic substrate binding site consists of three volumes :-

   a) a region corresponding to the lanosterol A ring which terminates in a polar group, the 3β-hydroxyl. The inhibitor makes no use of this space in the enzyme cleft.

   b) a bulky volume occupied by the inhibitor tertiary butyl group and in part by the sterol 6β-methyl. It might be expected therefore that extension of the t-butyl group other than onto the A ring would reduce activity. In fact, in vitro activity has been shown to be highly sensitive to the size of the this lipophilic moiety.

   c) a deep cavity into which the lanosterol molecule protrudes its side chain and the triazole fungicide projects the benzyl group. This suggests that the benzyl group could be greatly extended, which again agrees with in vitro data. The paraphenyl benzyl compound for example, shows good activity.

2. The antagonist hydroxyl function lies at a distance relative to the heme group which would make it a candidate for hydrogen bonding to either a propionate side chain or an effector acyl group. More generally the polar hydroxyl and the triazole 2-N could mark the interface with polar protein, membrane phospholipid head groups or solution. This agrees very much with the model proposed by Peterson et al. (Peterson et al 1978) for the 5-exo hydroxylation of d and l camphor in mammalain cytochrome P-450, and is also consistent with the relationship they noted from steroid metabolism by cytochrome P-450, between the position hydroxylated and its relation to a polar funtional group.

3. The triazole group binds perpendicularly to the heme group and gauche to the iron-nitrogen bonds in the porphyrin plane.

STRUCTURE AND ACTIVITY

Figure 5 now summarises what the model requires for in vitro anti-14-demethylase activity. A gauche conformation is required between the polar function and the iron chelating group leading to restrictions on the substitution pattern at A, B, C and D. Logically this leads to the possibility of other substitution patterns which will achieve this gauche conformational requirement with groups of the right kind. Substitution at A and B, for example, leaving C and D as hydrogen yields a series of compounds which have all the correct requirements for activity and yet are different in overall appearance.

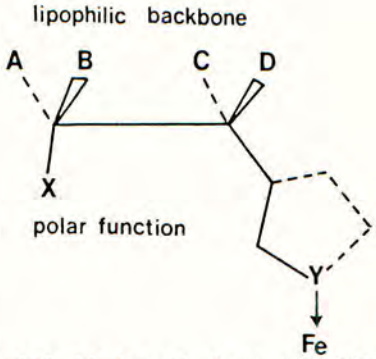

Fig. 5. Model P-450 14-demethylase inhibitor

The new ICI fungicide flutriafen has orthofluorophenyl and parafluorophenyl in these two positions. Theoretical calculations on flutriafen give excellent agreement with a recent crystal structure determination by Kendrick and Owsten at the Polytechnic of North London, which again shows no intra-molecular hydrogen bonding, but a gauche relationship between the hydroxyl function and triazole.

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MODE OF ACTION OF PHOTOSYSTEM II INHIBITORS AND ITS IMPLICATION FOR HERBICIDE DESIGN

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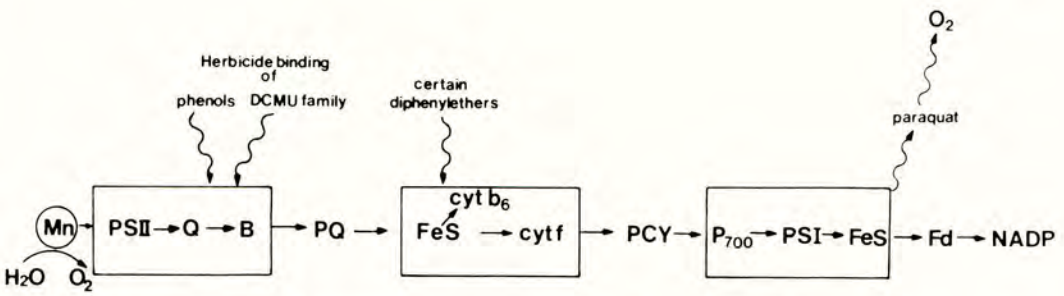
INTRODUCTION

A large number of commercial herbicides are inhibitors of photosynthetic electron flow. Most of these specifically interact with the acceptor side of of photosystem II. Among these compounds are such commercially important herbicides like diuron, atrazine, metribuzin and bromacil (grouped in this report as the DCMU family of herbicides) as well as phenolic herbicides like dinoseb and ioxynil. The study of the mode of action of these inhibitors on photosynthetic electron flow in isolated cell free systems from chloroplasts or algae has greatly stimulated both photosynthesis and herbicide research. This has been reviewed many times, for example by Büchel (1972), Corbett (1974), Moreland (1980), Fedtke (1982) and ourselves (1979, 1983). This review concentrates on new developments: the identification of the receptor proteins in the photosynthetic membrane as the actual target for herbicide interaction with the photosynthetic system, the increasing impact of molecular biology and MO calculations of the essential elements in the chemical structure of the herbicide. This will indicate the present possibilities for rational design of inhibitors fitting optimally into the binding niche on the receptor peptide.

The report will first discuss the present state of the mode of action of the herbicides and then combine the implications from this with further studies by structure activity correlations (QSAR) and the physicochemistry of the essential atoms in the inhibitory compounds.

RESULTS

In photosynthetic electron flow in the thylakoid membrane of the chloroplasts two photosystems in the light oxidize water to oxygen and transport the electrons against the thermodynamic gradient to a reductant with a very electronegative redox potential.

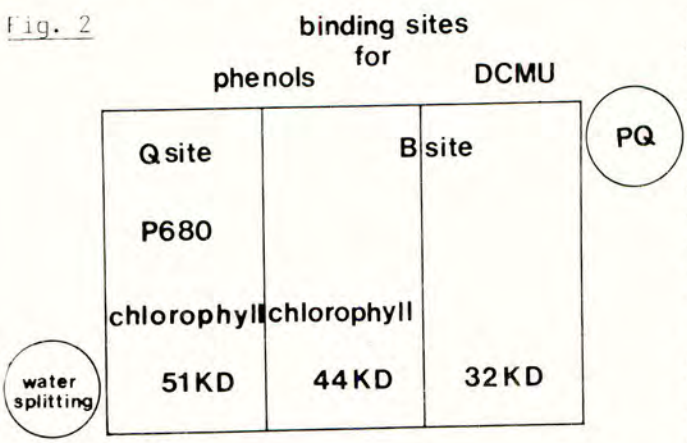


As indicated in Fig. 1 herbicides may interrupt this photosynthetic system

by acting on photosystem I (paraquat) on the cytochrome b/f-complex (certain diphenylethers) or on photosystem II (either phenols or the many herbicidal compounds with a common essential element grouped as the DCMU family). The acceptor side of photosystem II, the target for the many herbicides to be discussed in this report, oxidizes water via a manganese protein complex and funnels single electrons from the light reaction (from the reaction center chlorophyll) via primary acceptors into the two electron carrier plastoquinone. Photosystem II consists of three different integral peptides of molecular weights 51, 44 and 32 kD. The first two of these carry chlorophyll. The latter two are part of the acceptor side, which carries a bound plastosemiquinone and an iron atom as components. This system is now often called B-protein, PS II-plastoquinone oxidoreductase or herbicide binding protein. A number of techniques have identified and correlated these peptides, relevant for the mode of action of herbicides, as reviewed for example at the last international pesticide congress (Arntzen et al. 1983, Oettmeier et al. 1983, Trebst et al. 1983).

Photoaffinity labeling with appropriately substituted radioactive herbicides were instrumental in identifying and assigning the role of the 32 and 44 kD peptides both to photosynthetic function and herbicide action. These studies have established that and which of the membrane peptides of the acceptor side of PS II carry binding sites for the DCMU family as well as for the phenol type family of herbicides. An azido-triazin was used by Gardner (1981) and Arntzen and his colleagues (Pfister et al. 1981) to identify the 32 kD peptide as the receptor for the DCMU type herbicides. Oettmeier et al. (1980, 1982) used an azido derivative of dinoseb to identify the target for the phenol herbicides. Although phenols have multiple effects on chloroplasts (even more in vivo) it became clear that, not the 32 kD but, the 44 kD peptide carries the binding sites for phenol herbicides. Further, evidence from resistant weeds and algae (Pfister and Arntzen 1979, Janatkova and Wildner 1982) with a submembrane system (in particular purified PS II particles, Mullet and Arntzen 1981, Johanningmeier et al. 1983), thermoluminescence (Vass and Demeter 1982) and trypsin digestion of the membrane have supported the notion that two different subunits of the B-protein are involved in herbicide binding - the phenolic compounds acting closer to the reaction center than the DCMU type (see Fig. 2).

Fig. 2



The two subunits must interact very closely, both in functioning in photosynthesis as well as binding of one herbicide to the membrane affects the binding of another herbicide at the same but also at the other subunit. This displacement of a radioactive herbicide by an analogue is actually a convenient way to identify new compounds with the same mode of action - a technique first introduced to this problem by Tischer and Strotmann 1977. The interaction of two peptides is an extension and specification of our earlier scheme for the mode of action of herbicides with PS II: a common binding area for all herbicides with specific but also overlapping binding sites for the individual chemical groups and compounds (Trebst et al. 1979). Recent

evidence suggests regulatory mechanisms in that both membrane phosphorylation (by ATP and a membrane kinase, Shochat 1982) and carboxylation (by bicarbonate, van Rensen and Vermaas 1981) affects functional photosynthetic potency of the B-protein as well as binding capacity for the herbicides. This led to the hypothesis that herbicide binding changes the affinity of plastoquinone binding to the B-protein. This is to say that the mode of action of herbicides consists in preventing the binding of plastoquinone the B-protein where it is reduced in normal function. This does not necessarily mean that the binding sites of PQ and herbicide are identical. It is sufficient to assign the binding sites to the same protein.

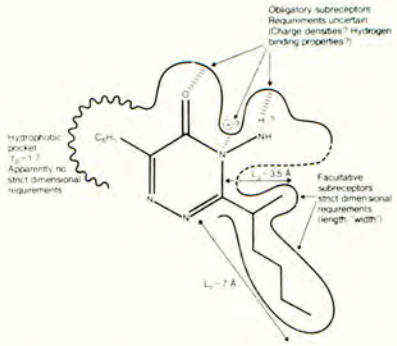
Molecular biology is about to contribute important data. Recently the aminoacid sequence of the 32 kD herbicide binding peptide has been reported (Zurawski et al. 1982) from the DNA sequencing of its gene, located in the chloroplast genom. A prediction of its folding in the membrane from a hydropathy plot of the aminoacid sequence suggests that the 32 kD peptide spans the membrane seven times (Rao et al. 1983). This revises somewhat the earlier designation of the 32 kD peptide as a shielding peptide, although the hydropathy pattern also shows that more hydrophilic amino acids protrude out of the membrane on the matrix than on the inner lumen side.

As mentioned above, herbicide resistant weed proved valuable in assigning the membrane peptides involved in herbicide binding. Recently the aminoacid sequence of the 32 kD peptide of an atrazine resistant Amaranthus species has been completed with the results that just one serine in the chain of the wild type has been changed into a glycin in the mutant (Marx 1983). The comparison in aminoacid sequence of further resistant mutants, easily obtained in algal systems with the cross resistance versus a variety of closely related herbicides will probably shortly enhance considerably our knowledge about the actual amino acid involved in the individual though overlapping binding sites for all herbicides. It is very interesting that in herbicide resistant weeds also the lipid composition of the membrane changes (St. John 1982).

The biochemical characterization of the structure and chemical groups involved in binding of herbicides to its target provides the mirror image of the chemistry of the essential chemical elements in the herbicide itself. The latter have been recognized already very early (see Büchel 1972, Trebst et al. 1979). The many compounds of the DCMU family (ureas, anilides, triazines, triazinones, pyridazinones, uraciles, benzimidazoles etc.) that interact with the 32 kD peptide, share an essential sp2 carbon hybrid attached to a nitrogen and a lipophilic substituent. QSAR studies revealed a dependence of inhibitory potency on lipophilicity (partition coefficient) and a long electronic (sigma) parameters. The phenolic herbicides binding to the 44 kD peptide do not have that essential element of the DCMU family and they follow other structure activity correlations in which steric parameters are of decisive importance (Trebst et al. 1979). Recently an even more detailed fitting of herbicides and receptors has been attempted (Shipman 1980, Trebst et al. 1982, van Assche and Carles 1982). Some time ago we have derived from QSAR studies and CNDO/2 calculations of a set of 3-alkyl-4-amino-6-phenyl-triazin-5-ones a two-dimensional and, therefore, rough model (Draber and Fedtke 1979) of binding to the 32 kD receptor peptide.

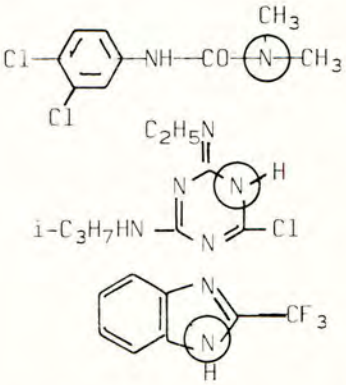
The important feature of this model (Fig. 3) is the positive partial π-charge at the triazinone-ring N-4 atom. This result was repeated recently with refined parameters for a special triazinone (Trebst, Draber and Donner 1983).

Fig. 3



Furthermore, we carried out MO calculations on DCMU, atrazine, and 2-trifluoromethylbenzimidazole. Some results are shown in Fig. 4.

Fig. 4



	pI_{50}	Charge density at the encircled N-atom (CNDO/2) total (non-planar)	π (planar)
DCMU	6,41	-0.209	+0.199
Atrazine	6,4	-0.032	+0.338
2-Trifluoromethylbenzimidazole	3,8	+0.028	+0.369

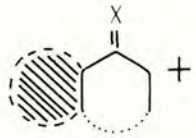
Some explanations are necessary: 1. The energy of the molecules had been minimized by a force-field based electronic model builder prior to performing the MO-calculations. 2. With the two heterocyclic molecules we did not encounter any difficulties to calculate the π-charge density which we consider as significant. DCMU, however, we had to force into a planar conformation which was not obtained by the model builder. 3. The atrazine molecule is written in an unusual way. Only that way yields a positive π-charge on the ring N-1.

Point 3 may look somewhat forced. We do not insist that atrazine has this tautomeric structure in solution. That would contradict all spectroscopic evidence. However, when bound to a proteinaceous membrane (e.g. the 32-kD peptide) it may look different. Already 14 years ago A. Pullman (1969) had carried out a comparison of the then available MO calculation methods (EHT, IEHT, CNDO, PPP) into which she had included the unsubstituted uracil. Her π-charge densities on the two N-atoms correspond very well with ours obtained many years later and they were positive (+0.24 - +0.29). Similar results we could obtain by CNDO/2 calculations of the following classes of molecules which are known to include among them active PS II inhibitors: 1,2,4-triazol-5-ones, 1,3,4-oxadiazol-5-ones, 1,2-pyrazol-5-ones, 4-chloro-1,2-diazin-3-ones, 3-isoxazolo-[4,5-d]pyrimidin-6-ones, and 1,3,5-triazol-2,4-diones. On the other hand, when CNDO/2 calculations are performed of atrazine written in the conventional way as a s-triazine derivative, the π-

charges at the C-atoms vary from 0.14 - 0.18 and those at the N-atoms from -0.21 - -0.24. Though it is unlikely that it will be elucitated soon how atrazine is in reality bound to the protein, it would seem strange if that compound were the only exception of what we consider as a rule.

Consequently we propose the following generalized structure for a PS II inhibitor that binds to the 32 kD polypeptide:

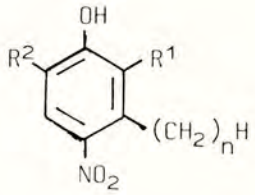
Fig. 5



In this structure the hatched part means a lipophilic group whose volume and steric requirements are not critical. X is in most cases oxygen though there are exceptions (e.g. atrazine, the 2-trifluoromethyl-benzimidazoles). The positive π-charge in α-position to the X=C-group we consider as absolutely necessary. The steric requirements on the right side of the molecule are rather critical.

Regarding the second binding site in the photosynthetic electron chain, the 44 kD polypeptide which binds phenolic inhibitors we had derived for a special set of compounds shown in Fig. 6 a regression equation by using only Verloop's steric parameters (Trebst and Draber 1979; Verloop et al. 1978):

Fig. 6



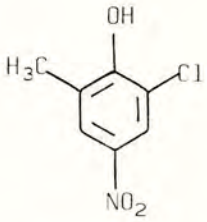
$$pI_{50} = -0.39 + 2.01B_{1-1} + 0.97B_{3-2}$$

$n = 33 \quad r^2 = 0.94 \quad s = 0.25$

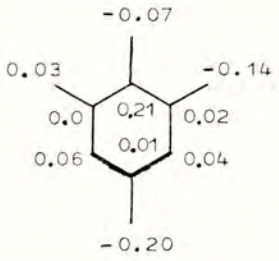
$F = 217 \quad p < 0.0001$

CNDO/2 calculations resulted in an entirely different charge density distribution. An example is shown in Fig. 7:

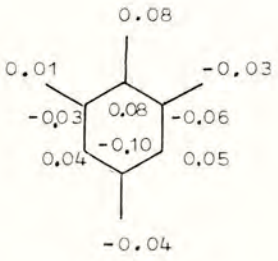
Fig. 7



$pI_{50} = 5.0$



total charge density

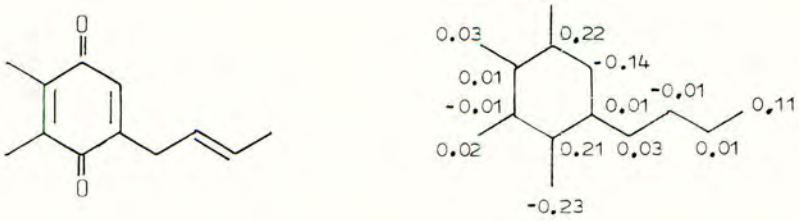


π -charges

It appears to be somewhat premature to generalize the structure and draw a model based just on one QSAR and CNDO calculation. It is evident, however, that the until now available calculations would result in a completely different model from that in Fig. 5.

It has recently been proposed that PS II inhibitors simply act by competing for the binding site of plastoquinone (cf. Fig. 2) at the 32 kD protein. We carried out a CNDO/2 calculation of plastoquinone analogue with a shorter side chain (Fig. 8).

Fig. 8



The net charge distribution does not give any hint whatever that it might electronically correspond to the model in Fig. 5.

We are convinced that the above-mentioned thoughts based on QSARs and MO calculations of PS II inhibitors will soon find its due supplementation in a better understanding of their counterparts, the binding proteins.

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2A—S3

EVOLUTION OF PYRETHROID INSECTICIDES FOR CROP PROTECTION

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ABSTRACT

From the natural insecticide, pyrethrin I, many powerful synthetic insecticides have been discovered. The course of their evolution is traced from pyrethrin I, through the key intermediate esters bioresmethrin and cismethrin (based on 5-benzyl-3-furylmethyl alcohol) to those suitable for agricultural use, such as permethrin, cypermethrin, deltamethrin and fenvalerate. Modern developments include the discovery of compounds more suitable for specific applications.

INTRODUCTION

One strategy by which biologically active compounds have been discovered, exemplified here, depends on recognition of a need, immediate or in the future, for products with particular properties, or combinations of properties. Systematic examination of suitable lead compounds then shows how desirable properties are related to structures, so that further new and improved compounds can be developed.

INSECTICIDES FOR CROP PROTECTION

The insecticides available in the earlier decades of the 20th century could not protect adequately the greatly increasing areas of food and fibre crops grown to supply the needs of the world's expanding population. By the late 1960s residual contact insecticides with the following improved properties were required: high intrinsic potency to insects, low toxicity to mammals and other non-target organisms, and involatility and limited persistence in contact with soils to minimise contamination of the environment.

The natural pyrethrins and closely related synthetic compounds such as allethrin had some of these properties; they were active against a wide range of insect species and of low toxicity to mammals. The potential of natural pyrethrum as a residual contact insecticide had been shown by Potter (1935), who successfully controlled infestations of stored products pests in warehouses with films of it in heavy white oil. In this application two inherent disadvantages of the natural pyrethrins, expense and instability in light, were not important. However, after the war, the now readily available synthetic insecticides discouraged industrial interest in pyrethroids, and the long-term goal of modifying their properties was more appropriately based on work to establish fundamental principles, such as that initiated at Rothamsted by Charles Potter, in 1948. In this, studies of pests of agricultural importance (Elliott et al 1950) showed that the traditional reliance on Musca domestica as a test species could be misleading in establishing structure-activity relationships.

STRUCTURE-ACTIVITY RELATIONSHIPS OF NATURAL AND SYNTHETIC PYRETHROIDS

Staudinger and Ruzicka (1924) investigated the natural pyrethrins in

the period 1910-1916 with the stated intention of synthesising related, possibly simpler, insecticides. They almost completely defined the structures of the acidic components of the esters (as shown for pyrethrin I and pyrethrin II, Figure 1) and with considerable insight prepared, amongst over 120 semi-synthetic derivatives of natural chrysanthemic acid and of pyrethrolone, some insecticidally active esters particularly significant for future developments (see below). By 1946, a combination of degradative and synthetic studies in the United Kingdom and the United States had established the structures now accepted for the natural esters, exemplified (Figure 1) by the two most active components, pyrethrin I and pyrethrin II, and had led to the first synthetic pyrethroid, allethrin (Figure 1) (Schechter et al 1949); Staudinger and Ruzicka (1924) had earlier envisaged the possibility of the simple allyl side chain being adequate to confer activity by their preparation of the chrysanthemate of (A) in a mixture of isomers.

The further stages in elucidating the structural features on which the insecticidal activities of the natural esters depend have been reviewed (Elliott and Janes 1978) and are summarized in the sequence of structures in Figure 1. Unsaturated groups in acidic and alcoholic components appropriately oriented with respect to each other by chiral centres at each end of the ester link are essential for greatest activity. This knowledge was combined with the demonstration, first by Staudinger and Ruzicka (1924) in piperonyl (B) and cuminyl chrysanthemates, later by Barthel (1961) with 6-chloropiperonyl (B) and 2,4-(C) and 3,4-dimethylbenzyl chrysanthemates that simple chrysanthemates were significantly active; alkenylbenzyl chrysanthemates might therefore have enhanced activity. Exploration of this concept led to the active compounds 4-allyl and 2,6-dimethyl-4-allylbenzyl chrysanthemates (D) (Elliott and Janes 1978). Because no simple synthetic route to the Z-pentadienyl system in pyrethrin I was available, the possibility of simulating its function by an aromatic system was examined. The activities of 4-benzyl- and of 2,6-dimethyl-4-benzyl-benzyl chrysanthemates, (F), though less than those of the equivalent 4-allyl compounds (D) suggested that other rings in place of benzyl should be explored. Of the many variations examined, one which showed high activity was 5-benzyl-3-furylmethyl (5B3F), chrysanthemate, isomers of which constitute the commercial insecticides resmethrin and bioresmethrin (5B3F (1RS)-cis,trans- and (1R)-trans-chrysanthemate, respectively). A product with contrasting properties, tetramethrin, the chrysanthemate of (G) (Kato et al 1964) has very rapid knockdown action against houseflies and related pests but inferior killing activity to the wider range of insects to which resmethrin and bioresmethrin are potent.

DEVELOPMENT OF PHOTOSTABLE PYRETHROIDS

Resmethrin and bioresmethrin were not only significantly more active than the natural pyrethrins and allethrin to numerous insect species, but were even less toxic to mammals. This unique combination of properties (LD50, $mg.kg^{-1}$:insects,c. 1; rats,>8000) in structures more accessible by synthesis than the cyclopentenolone esters provided new lead compounds for further developments and the present generation of photostable pyrethroids are related to them, because 5B3F alcohol was found to form significantly active esters from acids whose potential had not been revealed in combination with allethrolone.

The activity of the ethanochrysanthemate (2)(Velluz et al 1969) stimulated a broad investigation (Elliott and Janes 1978) of the influence

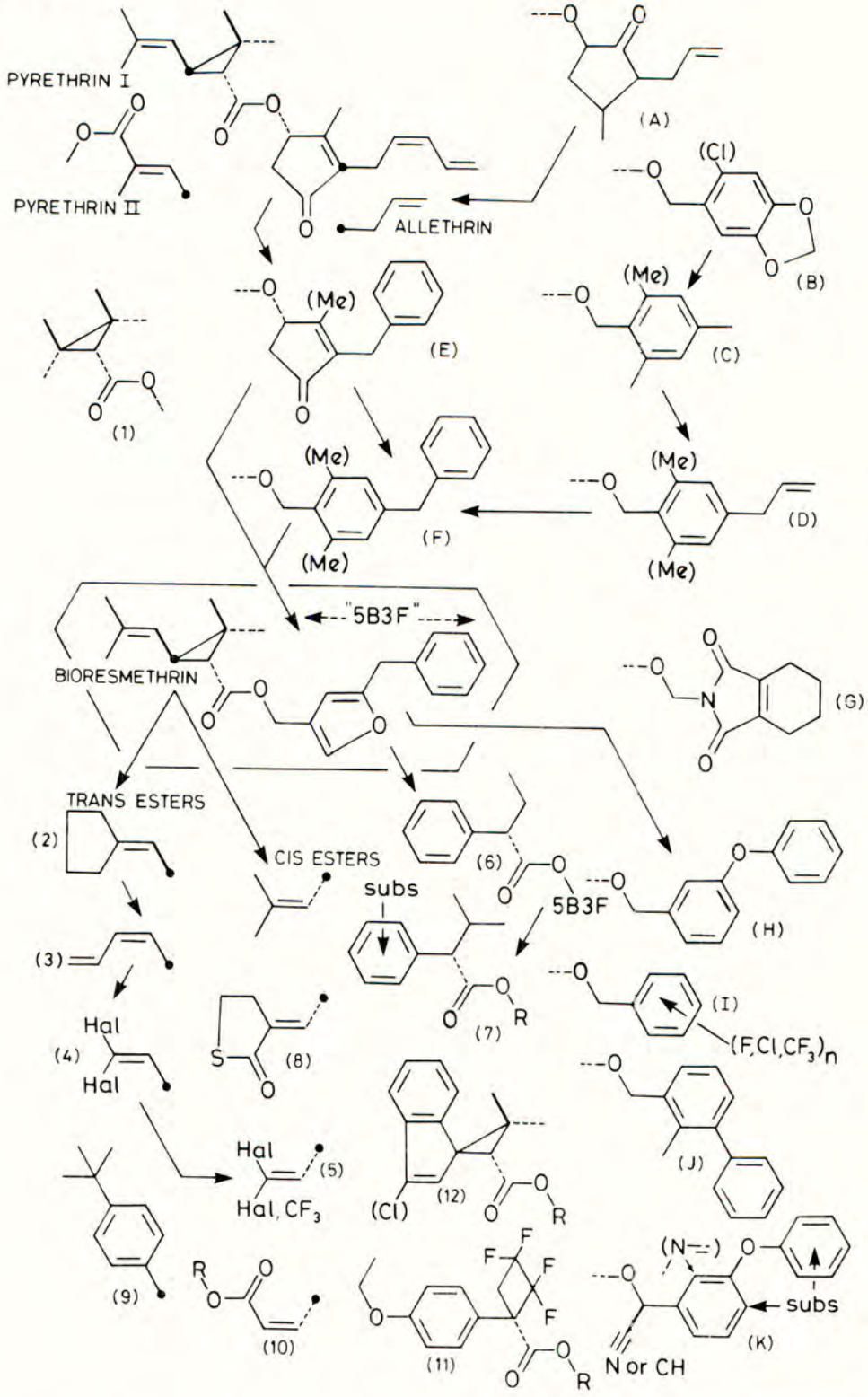

Figure 1. Acid and alcohol components important in the evolution of photostable pyrethroids

of other changes in the side chain of chrysanthemates which revealed the potency of the dienic derivative (3). Other active compounds were the monochloro and dichlorovinyl analogues of chrysanthemic acid and those with dibromo and difluoro substituents (4). Comparable relatives of cis-chrysanthemic esters had unprecedented and unpredicted levels of insecticidal activity. Structural investigations of the origin of the high potency and instability of esters of 5B3F alcohol indicated that some isosteric replacements were possible without great loss of activity, and if these could be at sites of photochemical attack, stability in light might be improved. 3-Benzylbenzyl (in contrast to the 4-benzylbenzyl compounds earlier investigated) and 3-phenoxybenzyl (H) derivatives were stereochemically related to those of 5B3F alcohol, but lacked the photolabile furan ring. Although these two benzyl alcohols gave somewhat less potent chrysanthemates than did the furan alcohol, their combinations with dihalo analogues of chrysanthemic acid were much more potent than would have been predicted from additivity principles. Moreover, in such esters, the photolabile sites identified in resmethrin (Ueda et al 1974) had been replaced and simple practical trials established that photostability in such combinations (for example, (1R)-trans and (1R)-cis permethrin, Figure 2) was greatly enhanced (Elliott and Janes 1978).

5B3F alcohol was also an intermediate in discovery of a second series of photostable esters. Ohno et al (1974) screened its esters with a range of aliphatic and aromatic acids against houseflies and mosquito larvae, having considered that activity of esters with pyrethrolone or allethrolone might be too faint to be detected. The first potent ester was that with ethylphenyl acetic acid (6), from which the more effective esters of isopropyl-substituted phenyl acetates (7) were discovered. The photostable insecticide fenvalerate was obtained when photostable alcohols replaced 5B3F alcohol.

Other structures illustrated on Figure 1 but not specifically discussed were also involved in the evolution described, or are recent extensions of it: (1) Matsui and Kitahara 1967); (8) (Lhoste and Rauch 1976); (9) (Ozawa et al 1978); (10) (Tessier et al 1983); (11) (Holan et al 1983); (12) (Brown and Addor 1979); (E) (Elliott and Janes 1978); (I) (Bull and Searle 1980; Naumann 1979), (J) (Engel et al 1983) and (K) (Matsuo et al 1976; Malhotra et al 1981) summarise developments discussed below with reference to Figure 2.

FURTHER DEVELOPMENT OF PHOTOSTABLE PYRETHROIDS

Other products were derived by further changes in these compounds which could be predicted not to reintroduce centres susceptible to photodegradation or greatly to change overall lipophilic and electronic characteristics (Briggs et al 1983). The potencies of the active isomers in permethrin (Figure 2), the (1R)-trans and (1R)-cis esters, are enhanced by introduction of (S)-α-cyano groups to give (1R)-trans and (1R)-cis-cypermethrin (Elliott et al 1978); the latter very potent isomer can be isolated in a crystalline racemate and thus provided for practical use without optical resolution (Mason and Wood 1980). 4-Fluorosubstituted cypermethrin constitutes the insecticide cyfluthrin (Hammann and Fuchs 1981). The single optical isomer deltamethrin (Elliott and Janes 1978) in which all three optical centres are resolved is produced on a multi-tonne scale in a remarkable commercial process (Martel 1980).

The first photostable pyrethroids were broad spectrum insecticides,

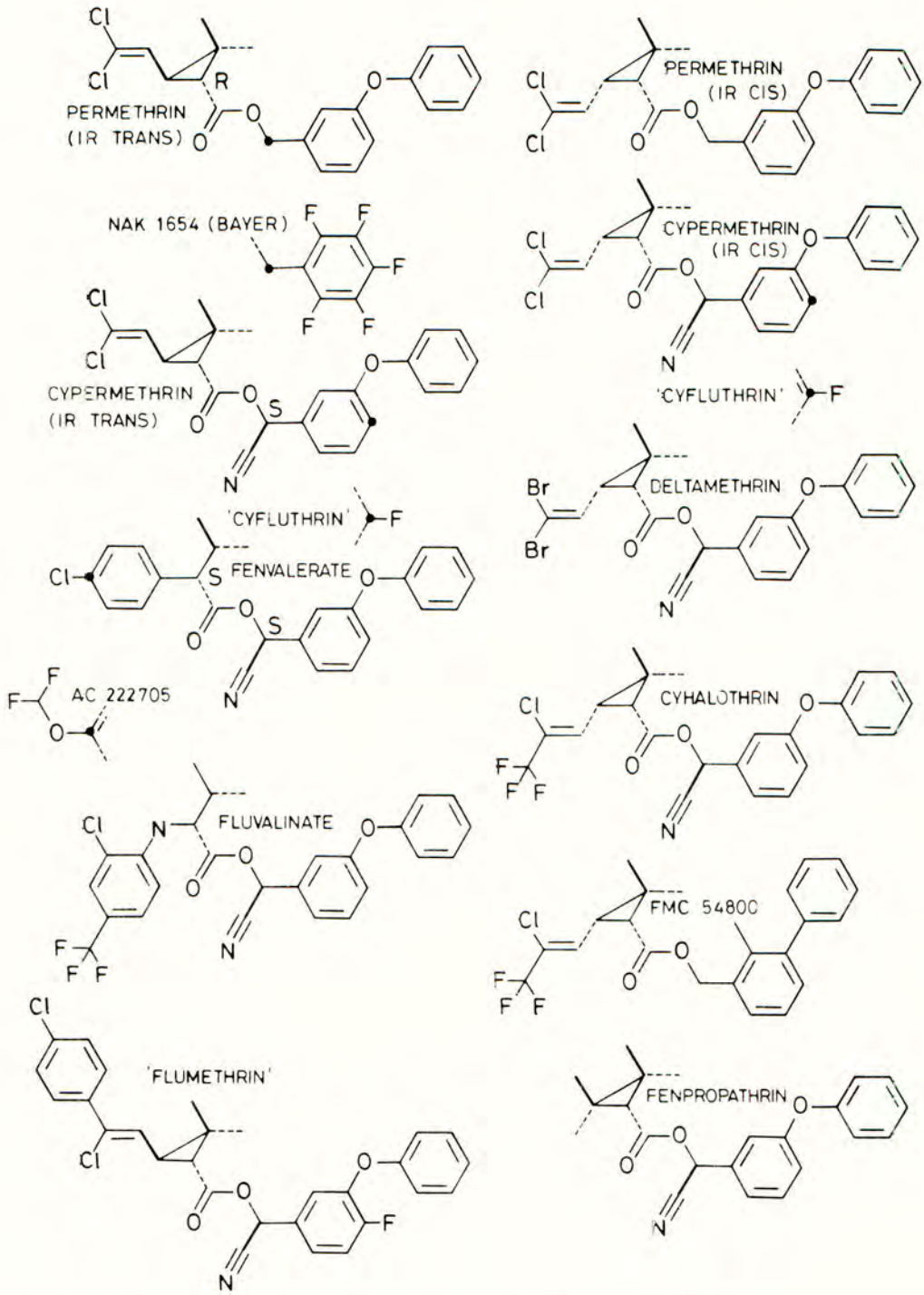

Figure 2. Active isomers of some commercial pyrethroids

with insufficient selectivity between pests and beneficial species for some applications; later structural modifications have produced compounds with high activity against selected pest species. Cyhalothrin, in which the E chlorine in the dihalovinyl side chain of (1R)-cis-cypermethrin is replaced by CF_3 has enhanced activity, particularly against ticks (Stubbs et al 1982). The trans isomer, 'flumethrin', shown (more active than the (1R)-cis) is also very effective against these pests (Stendel and Fuchs 1982); FMC 54800 (Engel et al 1983) and fenpropathrin (Fujita 1981) both show broad-spectrum insecticidal and acaricidal activity. Some fluorinated and chlorinated benzyl esters (Figure 1, I; NAK 1654 (Bayer), Figure 2) have physical properties and activities appropriate for action against soil pests (Briggs et al 1983). Fenvalerate (Ohno et al 1976), AC 222,705 (Whitney and Wettstein 1979) and fluvalinate (Henrick et al 1980) with their varied properties illustrate the range of variations of isopropylphenylacetates possible.

The acidic and alcoholic components in Figure 1 represent only a small proportion of those now known to produce active esters. Moreover, in tests against a wide range of important pests, high activity against a particular species has frequently now been found to be associated with a particular, subtle structural variation, so in the absence of economic constraints, a wide range of species specific pyrethroids could be provided. However, no modification (Tessier et al 1983) has yet raised the general level of insecticidal activity greatly above that established by deltamethrin.

SUMMARY AND CONCLUSIONS

Application of quantitative structure activity relationships in the area of pyrethroids has been limited, because shape and chirality dominate their action and they are very flexible molecules for which quantification of either property for mathematical correlation is difficult. An attempt to relate molecular polarity (measured as P, the partition coefficient) with activity gave a broadly spread series of points with an ill-defined optimum when a range of types of pyrethroids was included (Briggs et al 1976). More restricted studies in which a large part of the molecular structure is unaltered and one or two substituents are varied have been much more successful. For example, in a simple homologous series of acid side chains an optimum polarity was detected (Briggs et al 1976) and in substituted phenyl acid side chains (9), activity correlated strongly with electron donating properties, leading to recognition of the most effective substituent, t-butyl (Ozawa et al 1982). Elsewhere in the molecule, the π values and sizes of over forty substituents on the α-carbon both correlated moderately well with their effects on activity. In a more ambitious study on a large number of benzylchrysanthemates (Nishimura et al 1982) several different correlations were listed, depending in part of the type of activity (knockdown, kill, nervous response) considered.

These results reflect the complexity of the situation. No fully rational approach to developing insecticides related to the natural pyrethrins has been possible, primarily because the precise locations and structures of their sites of action are still unknown, and therefore ab initio design of molecules to interact there is not feasible. However the structural limits within which compounds are active are being defined with increasing precision by empirical synthesis and testing. The success in providing a new group of insecticides based on the natural pyrethrins has depended first on choosing them with their favourable combinations of properties as lead compounds and second on selecting appropriate insects to guide synthesis.

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THE MOLECULAR REQUIREMENTS FOR PHLOEM MOBILITY: AN ACTIVE TRANSPORT HYPOTHESIS

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Introduction

The phloem translocation of a xenobiotic is largely determined by its ability to accumulate behind the phloem membrane. The transport of compounds such as the phenoxy acid and benzoic acid herbicides has been rationalised by the weak acid hypothesis which assumes that acids in the ionised form (A^-) move across membranes much more slowly than in the non-ionised form (HA). The higher pH of the phloem cytoplasm favours the ionised species compared with the cell wall where the non-ionised form is relatively more abundant. However, the weak acid hypothesis does not explain the phloem movement of zwitterions (α amino-isobutyric acid) and cations (1-methylpyridinium ion and paraquat at low concentrations).

Experimental

Two techniques have been used to study the relationship between molecular structure and phloem mobility. Whole plant studies required the application of radiolabelled compound to a small part of a leaf still attached to the growing plant. At an interval of time e.g. 24 hours, the surface residues were removed with nuloidin or cellulose acetate and the plant analysed for radioactivity. In some cases the treated part of the leaf was washed repeatedly with water or buffer solution and then freeze-thawed before further washing. This technique was developed further by cutting thin (0.3 mm) sections of leaf and incubating them in solutions containing radiolabelled compounds. The tissue was subsequently washed repeatedly to obtain an efflux profile.

Results and discussion

There is no doubt that acidic compounds frequently show phloem mobility but it is not always possible to explain this movement in the context of the weak acid hypothesis. For example, while benzoic acid and benzene phosphonic acid are phloem mobile, benzene sulfonic acid is not. This is not because benzene sulfonic acid is incapable of crossing the plasma membrane, as elution analysis of the treated part of the leaf reveals that benzene sulfonic acid is held within the cell until the membrane is broken by freeze-thawing. Some sulfonic acid compounds are capable of translocating in the phloem, for instance the zwitterion 1-methyl-3-sulfopyridinium betaine is more phloem mobile than the 1-methylpyridinium cation. Other acidic compounds may also show poor phloem mobility, 3-carboxyl-1-methylpyridinium betaine is more mobile than 3, 5-dicarboxyl-1-methylpyridinium betaine.

Analysis of the elution profiles using thin leaf slices confirmed that phloem mobile compounds are released very slowly from cells, frequently with a $t_{\frac{1}{2}}$ greater than 2 days. The slow rate of efflux contrasts with the influx where a cell:medium ratio of more than 50:1 can be achieved within one hour for acids (e.g. naphthylacetic acid), zwitterions (e.g. α amino-isobutyric acid) and cations (e.g. 1-methylpyridinium chloride).

The general conclusion is that some compounds show a much more rapid movement into cells than out of them. Phloem mobile compounds show this characteristic but not all compounds which accumulate in cells are phloem mobile. The mechanism by which xenobiotics are transported across cells against the concentration gradient may provide the key to predicting what type of compound will be phloem mobile. The weak acid hypothesis proposes a mechanism based on diffusion, and there is no question that this mechanism exists and that some compounds may accumulate in cells as a result of the combined processes of diffusion and dissociation of ionic groups. However this does not explain the accumulation of zwitterions nor the inability of some acidic compounds to translocate in the phloem. An alternative suggestion is that the transport of xenobiotics either into the cell or into the phloem requires specific carrier molecules located in the membranes. If this is the case the molecular requirements for translocation will be far more subtle than would be expected for a diffusion based mechanism.

Two new lines of evidence suggest that this is the case. First, isomers may have quite different translocation properties, for instance 2-amino-benzoic acid is more phloem mobile than 4-amino-benzoic acid but 4-carboxy-1-methylpyridinium betaine is more phloem mobile than the 2-carboxy isomer. The other evidence comes from the effect of the physiological state of the plant; temperature and light, in particular, have far more effect on phloem mobility than would be expected for a purely physical process, being more characteristic of an active transport mechanism.

The active transport hypothesis of xenobiotic translocation has important implications for the synthesis of phloem mobile pesticides. It increases the range of potentially phloem mobile groups but reduces the chances of achieving mobility by synthesising compounds with particular physico-chemical properties.

2A—R2

RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE AND PHLOEM MOBILITY WITH REFERENCE TO A SERIES OF NAPHTHOXYALKANOIC ACIDS

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Introduction

The design of phloem-mobile insecticides, nematicides and fungicides is a major objective in pesticide research. There is a pressing need for such compounds to control root pests and diseases by foliar application. The aim of this work is to identify some of the chemical requirements for the uptake and translocation of xenobiotics by the phloem.

Materials and Methods

Five to six week old plants of Ricinus communis L. var. Gibsonii (Nichols) were used in all experiments. The lamina of the mature leaf to be treated was enclosed in a transparent perspex box and placed under a 1000w mercury lamp. The solution of a ^{14}C-labelled test compound (100 μM) and ^{3}H-labelled sucrose (50 mM) buffered at pH6 was perfused through the hollow petiole for one hour using a peristaltic pump. The solution was subsequently replaced by one containing unlabelled compounds. Throughout the experiment (6.5h) the phloem exudate was collected from an incision made in the stem bark immediately below the treated leaf. The amounts of ^{14}C and ^{3}H in the exudate were measured by liquid scintillation counting. Exudate required for the assessment of metabolism was taken from a second incision made at the same level, but on the opposite side of the stem. After correcting for metabolism the phloem mobility was expressed as the ratio of the test compound to the applied sucrose in the exudate in six hours. At the end of the experiment the petiole of the treated leaf was extracted and analysed by TLC.

The specific activities of the w-(1-[1-^{14}C]-naphthoxy)alkanoic acids (I,n=1-5) and 2-(1-[1-^{14}C]-naphthoxy)ethanol (II) were approximately 5mCi mmol^{-1} and that of [6,6'(n)-^{3}H]-sucrose was 180 μCi mmol^{-1}.

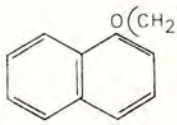
Results and Discussion

The higher members of the series of acids underwent considerable β-oxidation to acids with n=1 or 2. These metabolites were found in the phloem exudate and to a lesser extent in the petiole tissue. In addition, there were significant amounts of polar metabolites (probably conjugates) in the exudate and the petiole. The finding that there was consistently more polar material in the petioles (up to 70%) than in the exudate (up to 20%) suggests that these compounds are taken up and translocated less readily than the free acids.

The results in Table 1 show an apparent correlation between the relative phloem mobilities of the acids and their octanol/water partition coefficients. However, β-oxidation in the petiole may have limited the amounts of the higher homologues available for uptake into the phloem. Furthermore, the substituted ethanol (II) which has a log Po/w similar to that of naphthoxyacetic acid (I, n=1) was not translocated significantly. Clearly a factor other than the partition coefficient is important for phloem mobility and this may be the pK of the compounds. More work is needed to investigate this as the present compounds only cover a narrow pK range (3.5-5.0).

Conclusions

Within this homologous series of naphthoxyalkanoic acids relative phloem mobility decreases as the chain length increases. Although this decrease correlates well with increase in lipophilicity other factors such as metabolism and pK values are probably as important in determining phloem mobility.



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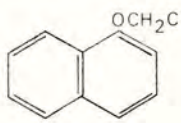

II

TABLE 1

Relative phloem mobility and log $P_{o/w}$ of a group of naphthoxyalkanoic acids and 2-naphthoxyethanol

Compound	I					II
n =	1	2	3	4	5	
Relative mobility	7.8	5.6	2.6	0.3	0.1	< 0.1
Log $P_{o/w}$	2.6	3.0	3.5	4.0	4.5	2.5

OCTANOL/WATER PARTITION COEFFICIENTS AND CHEMICAL UPTAKE BY PLANTS

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Introduction

Uptake of soil-applied chemical by plant roots is necessary for the activity of some pre-emergence herbicides and subsequent translocation to shoots is required for the activity of herbicides that inhibit photosynthesis and for systemic insecticides and fungicides. Most of these systemically active chemicals, or their active metabolites, have octan-1-ol/water partition coefficients (K_{OW}) in the range log K_{OW} -0.5 to 3.5. To investigate further this effect of lipophilicity on uptake and translocation of non-ionised chemicals by plants, we synthesised two series of ^{14}C-labelled compounds, O-methylcarbamoyloximes and substituted phenylureas, and studied their behaviour in barley.

Methods

Plants grown in nutrient solution were transferred to solution containing 5 μM of the compound under test. After 24 or 48 h, the leaves, lower and upper stem sections and roots of the plants were separated, and the chemical within these extracted, purified by tlc and measured by liquid scintillation counting. The results were expressed as the Root Concentration Factor (RCF), Transpiration Stream Concentration Factor (TSCF) and Stem Concentration Factor (SCF), defined as the ratio of concentration in the roots (fresh wt), xylem sap and stem (fresh wt) respectively to that in the external nutrient solution.

Results and discussion

The RCF had a lower limiting value of circa 0.8 for polar chemicals (log $K_{OW} < 1$) consistent with equal concentrations in the water phase within and outside the root. There was no measurable sorption of polar chemicals onto macerated root solids, whereas sorption of more lipophilic materials on macerated roots was logarithmically related to K_{OW} and the RCF for intact plants increased similarly. RCF was related to K_{OW} by

$$\log (RCF - 0.82) = 0.77 \log K_{OW} - 1.52$$

Translocation was most efficient for compounds of intermediate polarity, TSCF approaching 1, the maximum value for passive uptake, at log K_{OW} circa 2. Very polar and very lipophilic compounds were poorly translocated. From data for 17 compounds, an empirical relationship between TSCF and K_{OW} was established

$$TSCF = 0.784 \exp - \left[(\log K_{OW} - 1.78)^2/2.44\right]$$

The TSCF measurements involved the stem and leaves of the plant. When distribution between stem and leaves was examined, the polar chemicals were found at similar concentrations in the stem to those in the xylem sap; thus they did not accumulate in the stem and concentrations in the leaves increased with time. As lipophilicity increased, sorption in the stem increased and so lipophilic chemicals took longer to reach the upper stem and leaves. When maximum concentrations were attained in the stem, SCF and K_{OW} were related by

$$SCF = \left[10^{(0.95 \log K_{OW} - 2.05)+0.82}\right] \; 0.784 \exp - \left[(\log K_{OW} - 1.78)^2/2.44\right]$$

Similar behaviour was observed in other plant species, indicating that root uptake and xylem transport of non-ionised chemicals can be generally predicted from K_{OW} and that apparent differences in translocation between plant species or between chemicals with the same K_{OW} are due to differential rates of metabolism.

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2A—R4

THE DESIGN OF TRIAZOLE FUNGICIDES USING COMPUTER GRAPHICS AND THEORETICAL COMPUTATION

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Background and objectives

The triazole fungicides are now attracting wide commercial interest both as pharmaceutical and crop protection fungicides. Their primary mode of action has been shown to be the inhibition of ergosterol biosynthesis, with the resulting ergosterol deficiency adversely affecting membrane formation and function. This is accomplished through antagonism of the cytochrome P-450 oxidase which catalyses the 14α-demethylation of 24-methylene dihydrolanosterol, the ergosterol precursor.

At the molecular level a contradiction seems to exist in that this cytochrome P-450 whilst exhibiting high selectivity for this particular lanosterol is capable of binding a range of antagonists whose sole structural similarity would appear to be the presence of a nitrogen containing heterocyclic ring and lipophilic backbone groups. Computer graphics has been used to attempt to resolve this contradiction by comparing the molecular shapes of the antagonists with that of the natural substrate and so arrive at an accessible volume for the cytochrome P-450 active site. This, in turn, has implications for novel fungicide design by suggesting compounds which achieve a closer fit to the enzyme active site.

Methods and calculations

A DEC PDP 11/60 minicomputer with GT40 Decgraphics was used driven by the Video Vector Dynamics software package.

Molecular mechanics and semi-empirical molecular orbital calculations were performed on a series of triazole fungicides to determine minimum energy shapes. These calculated conformations gave good agreement with crystal structures where available and seemed consistent with n.m.r. studies. A computer graphics model was then constructed which defined the cytochrome P-450 active site cavity in relation to its porphyrin prosthetic group.

Results and conclusions

The active site model derived from this work has at least three features with which a potential antagonist could interact :-

1. The porphyrin ring available for complexation.
2. A hydrophobic substrate binding site of defined shape giving rise to some lipophilic group specificity.
3. A region of high polarity due possibly to the porphyrin propionate side chain, a catalytic acyl group, polar protein or a solvent/membrane interface.

This agrees very much with the model proposed by Peterson et al for the 5-exo hydroxylation of d and l camphor in mammalian cytochrome P-450. It is also consistent with the relationship they noted in steroid metabolism where the position hydroxylated by cytochrome P-450 is often in a similar position with respect to a polar functional group.

In particular, the position of the polar function in an antagonist may in part explain the relative fungicidal activities of the diasterisomers of diclobutrazol and triadimenol.

Certainly, the binding of many triazole fungicides in this modelled enzyme active site is indicative of many of the observed structure-activity relationships.

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STRUCTURE-ACTIVITY RELATIONSHIPS OF INHIBITORS OF A FUNGAL CHITIN SYNTHETASE

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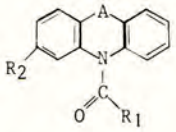
Background and objectives

The inhibition of the sythesis of chitin components of fungal cell walls is an important concept for future pesticides, since selective toxicity to the target organisms can be envisaged. Isoprothiolane, Polyoxins and Nikkomycin are fungicides active in this specific skeletal polysaccharide metabolism (Misato et al. 1979). Recently a new group of rust fungicides (I) was discovered in a screening procedure. To evaluate specificity and structure-activity relationships compounds (I) were subjected to biochemical screening.

(I)



A = CH_2-CH_2, CH= CH

R_1 = lower alkyl, lower alkoxy, phenyl

R_2 = H, Cl

Materials and methods

Compounds (I) were prepared by acylation of 10,11-dihydrodibenz [b,f] azepines and 5H-dibenz [b,f] azepins (Kricka & Ledwith 1974). Enzyme inhibitions were measured with a chitin synthetase from cultures of Coprinus cinereus (Adams & Gooday 1980). The assay for enzyme activity with UDP-(1-^{14}C)-N-acetyl-glucosamine was described by Yuh Nung Jan (1974). The degree of incorporation of ^{14}C-glucosamine into cell walls of yeast was determined by the method of Ritter (1975). By means of regression techniques the influence of lipophilicity, steric and electronic parameters were investigated using the Hansch approach (Martin 1978).

Results and conclusions

The highest inhibition in the C. cinereus assay, I_{50} = 5.6µM, was found with a carbamate (I) (A = -CH_2-CH_2-; R_1 = O-C_3H_7; R_2 = Cl). This value is in the order of magnitude of standards like polyoxin D, I_{50} = 10µM. With about 80 examples a parabolic dependence between log 1/I_{50} and lipophilicity was calculated. The optimum range for octanol/water-log P is 3.5 to 5. An 'in vitro/in vivo' plot between log 1/I_{50} and log 1/W (W = lowest concentration in ppm, which gave > 90% control of Puccinia graminis on wheat) was erratic, although many good 'in vivo' compounds (I) (e.g.: A = CH2-CH2; R_1 = O-CH_2-C≡CH; R_2 = H) had rather good enzyme inhibiting properties. The discrepancies will be discussed based on comparisons of incorporation rates between UDP-N-acetylglucosamine and glucosamine into cell wall chitin and on possible steric effects.

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2A—R6

PYRETHROID SYNERGISM BY ESTERASE INHIBITORS IN COTTON PESTS

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Background and objectives

Esterases, in addition to oxidases, play a major role in pyrethroid detoxification in several species of insects. The toxicity of *cis*-cypermethrin is synergized by profenofos about 20-fold against *Trichoplusia ni* (Ishaaya and Casida, 1980) and about 3-fold against *Spodoptera littoralis* (Ishaaya *et al.*, 1983); phenyl saligenin cyclic phosphonate synergizes the toxicity of *trans*-permethrin over 60-fold against *Chrysopa carnea* larvae (Ishaaya and Casida, 1981). This report summarizes some of our recent studies on pyrethroid synergism by esterase inhibitors against *S. littoralis* and the whitefly *Bemesia tabaci*, two important cotton pests.

Materials and Methods

For laboratory assays, *S. littoralis* larvae (80±5 mg) were fed for 24 h on cotton leaf squares (4 cm^2) or alfalfa leaves treated with esterase or oxidase inhibitors as synergists prior to topical application of pyrethroids (Ishaaya *et al.*, 1983). For field assays, cotton plants were sprayed until runoff with the pyrethroid, synergist or both. Leaf samples were collected at various intervals for residual toxicity determination on *Spodoptera* larvae. Assays with *B. tabaci* were carried out under glasshouse conditions. Cotton seedlings were sprayed with the pyrethroid, synergist or both. Six replicates of 12-15 adults confined in leaf cages were exposed to treated plants at various intervals after application for 24 h mortality determination.

Results and conclusions

Ingestion of 2-4 nmoles/larva of an esterase inhibitor such as profenofos or monocrotophos increased significantly the toxicity of *trans*-permethrin and *cis*-cypermethrin against *S. littoralis* larvae. On the other hand, ingestion of up to 80 nmoles/larva of oxidase inhibitors such as piperonyl butoxide, Niagara 16824 and SV-1 had no effect on the toxicity of these pyrethroids. In a cotton field, addition of monocrotophos to cypermethrin at a ratio of 1:1 synergized the toxicity of cypermethrin against *S. littoralis* and prolonged its activity. The LT_{50} value of 0.001% cypermethrin was ∿ 5 days, vs ∿ 10 days with the mixture. In similar assays profenofos was much less efficient than monocrotophos in synergizing cypermethrin. Monocrotophos synergized very strongly the toxicity of cypermethrin and prolonged its activity against *Bemesia tabaci* adults (Table 1). According to probit-log concentration curves, the toxicity of the mixture was over 30-fold of that of cypermethrin.

TABLE 1

Effect of monocrotophos on the residual toxicity of cypermethrin under glasshouse conditions against *Bemesia tabaci* adults

Days after application	Mortality, % ± SE values		
	Cypermethrin 0.004% a.i.	Monocrotophos 0.004% a.i.	Cypermethrin 0.004% a.i. + Monocrotophos 0.004% a.i.
1	65±5	36±10	98±2
3	35±6	0	93±3*
10	17±7	0	58±4*
14	1	0	25±0*

*Differ significantly at P=0.01 from the sum of mortality obtained with cypermethrin and monocrotophos separately

These results suggest that organophosphorus compounds acting as inhibitors of esterase activity in insects may serve as synergists for pyrethroids against some species. Care must be taken to evaluate the impact of such mixtures on nontarget organisms.

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SINGLET OXYGEN GENERATORS AS POSSIBLE HERBICIDES

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Introduction

Singlet oxygen ($^1O_2$) is generated when certain chemicals such as xanthene dyes are illuminated in the presence of oxygen. Well-known examples are rose bengal and erythrosin. $^1O_2$ generated in biological systems is potentially damaging because it readily reacts with most cellular components including amino acids, nucleic acids and lipids. The action of photosynthetic electron transport inhibitors probably involves $^1O_2$ generated from excited chlorophyll (Pallett and Dodge, 1980), while carotenoid biosynthesis inhibitor herbicides promote plant cell damage by reducing the natural $^1O_2$ quenching system of the chloroplast (Ridley, 1982). Photodynamic damage via $^1O_2$ generators has been demonstrated with bacteria (Bezman et al., 1978), insects (Callaham et al., 1975) and plants (Zweig and Nachtigall, 1975, Percival and Dodge, 1983). We now report further details of the action of rose bengal on plants.

Methods and Results

Six day old flax (Linum usitatissimun) seedlings were sprayed with rose bengal together with 0.5% v/v Tween 20. Detached flax cotyledons or pea (Pisum sativum) leaf discs were floated on rose bengal. Post treatment illumination was provided by fluorescent tubes with a photon flux density of 300μE $m^{-2}s^{-1}$. Phytotoxic symptoms were enhanced by increased concentrations of rose bengal, but limited when the light intensity was reduced. A post application dark period of 24 h was found to enhance subsequent photodynamic effects, and in the case of pigment-loss for example this led to a three fold increase in breakdown. This dark period reduced photodegradation of rose bengal. In all experiments described, the post application dark period was employed.

An early sign of photodynamic damage was the appearance of chlorotic symptoms and both chlorophylls and carotenoids showed approximately 60% breakdown after 24 h illumination. Transmission electron microscopy of treated leaves showed that this was paralleled by membrane destruction initially of the chloroplast and tonoplast. Ethane evolution an indicator of lipid peroxidation and measured by GLC was evident after 12 h illumination and progressively increased. Photosynthetic CO_2 incorporation was inhibited by 60% after 8 h illumination and this was related to a progressive inactivation of the enzyme ribulose bisphosphate carboxylase. In addition to promoting cellular disorganisation, rose bengal promoted the destruction or modification of leaf surface cuticular components as shown by scanning electron microscopy. Similar results to these were obtained with other xanthene dyes eosin Y, erythrosin B, phloxin B and rhodamine B.

Conclusion

These experiments show that plant death is promoted by the application of the photodynamic agent rose bengal. $^1O_2$ generated on illumination probably interacts with cuticular lipids on the leaf surface as well as membrane lipids, enzymes and other components within the leaf cells. These multi-site actions lead to rapid plant death. The herbicidal potential of rose bengal and other $^1O_2$ generators is being further investigated.

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Acknowledgements

This work was supported by a grant from the Agricultural Research Council.

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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS FOR 2-PHENYLMETHYL SULFONYL PYRIDINE-N-OXIDE HERBICIDES

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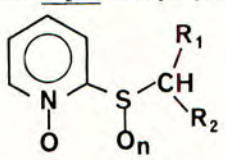
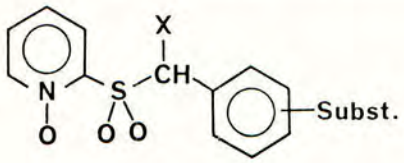
Background and Objectives

Herbicides of the general formula I were recently patented (Plant & Bell 1976, Plant et al 1977) as preemergent control agents for a number of weed species which include switchgrass (Panicum virgatum (L.)), barnyard grass (Echinochloa crus-galli, (L.) Beauv.) and green foxtail (Setaria viridis, (L.) Beauv.) The present study was concerned with quantitatively relating herbicidal activities on the above weed species to the structural features present in subclass II, namely, the effects of phenyl ring substitution and the role of the alpha-methyl group.



I n = 1,2
R_1 = H, CH_3
R_2 = alkyl, aryl



II X = H, CH_3

Materials and Methods

Physicochemical parameters (π , partition coefficient; MR, molar refractivity; F & R, electronic effects) were obtained from several sources (Hansch & Leo 1979, Norrington et al 1975). Using a computer program based on the algorithm of Wooton (Wooton et al 1975), 24 analogs were chosen from subclass II whose physicochemical parameter values simultaneously embodied maximum orthogonality and least inter-parameter correlation.

The preemergent herbicidal activity was determined as ED50 for the selected 24 analogs on the above three weed species by varying applied concentration in a greenhouse assay system scored after 2 weeks. In addition, compounds other than the selected analogs were also assayed in a likewise manner.

Results and Conclusions

Computer-assisted first and second order regression analysis ultimately led to a linear combination of terms which included π , MR, and two indicator variables, Z (denoting the presence of an alpha-methyl group) and H (denoting an ortho substituent capable of hydrogen bonding). For example, the regression equation for green foxtail was: $-\log ED_{50} = 0.43\pi - 0.052\ MR + 0.50\ H + 0.24\ Z$ ($n = 19$, $S = 0.16$, $r^2 = 0.93$). Thus the parameters of greatest significance were π and MR wherein the strong positive π contribution is counterbalanced by the negative MR term. For analogs containing a hydrogen bonding acceptor at the ortho position, e.g. ortho-fluoro, the H term is equal to unity and results in a strong positive contribution. A further positive influence obtained from an alpha-methyl group ($Z = 1$) was found to have marginal statistical significance.

The regression equations were found to account for 79-93% of the herbicidal activity against three weed species. It was further demonstrated that the equations represent the best correlations attainable within the limiting influence of bio-data uncertainty, and served to adequately predict herbicidal activities of analogs outside the selected set.

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THE DESIGN OF A POSTEMERGENCE PHENYLUREA HERBICIDE

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Background and objectives

Upon reviewing the several hundred patents on herbicidal phenylureas we questioned whether the most potent herbicides of this class have already been made and, if not, how they might be identified.

Phenylurea herbicides exert their effect by blocking an electron-transfer step in the oxygen-evolving photochemical reaction of plants known, in isolated chloroplasts, as the Hill reaction. Hansch (1966) developed a two-parameter equation, 1, which describes inhibition of the Hill reaction (PI_{50}) for twelve phenylureas, where π is the lipophilicity substituent constant and σ is the Hammett electronic parameter: $PI_{50} = 0.54\sigma + 1.29\pi + 4.18$ (1). Our approach to the design of phenylurea herbicide was initiated by an examination of equation 1.

Materials and methods

Compilations of Hansch et al. (1973, 1979) were used as sources of π values. The estimation of π by additivity principles was employed in some cases, with the assumption of an increment of $0.5/CH_2$ for π. The free radical parameter, E_R, was obtained from the compilations of Yamamoto and Otsu (1967). The postemergence herbicidal activity was visually rated on a 0-9 scale and converted to an ED_{85} (an estimated dose to cause 85% kill of mild mustard). All ED_{85} data were transposed into molar concentrations, C.

Results and conclusions

A PI_{50} value of 7.4 was predicted from equation 1 of 3-(4-(benzyloxy)-3-chlorophenyl)-1,1-dimethylurea and upon synthesis this compound proved to be a highly active nonselective postemergence herbicide. Twelve analogs bearing substituents in the benzyloxy ring were prepared and evaluated, but their herbicidal activity only correlated weakly with Hill inhibition. A regression and correlation analysis of the herbicidal data afforded best-fit equation 2. This equation, with its negative sign on the dominat E_R term, suggested that the most radical-destabilizing benzyl ring substituents, such as fluorine and hydrogen, should provide the most herbicidally potent compound and this proved correct. We anticipated that by insulating the benzyl group from the ether oxygen the detrimental $-E_R$ radical effect would be minimized. A series of m and p-phenylalkoxyphenyl-1,1-dimethylureas were prepared with an alkoxy group containing from 2 to 5 carbons. These analogs proved to be markedly superior to 3-(4-benzyloxy)3-chloro)-1,1-dimethylurea. The results confirmed the prediction from equation 2 that herbicial activity should be enhanced by eliminating or minimizing the radical stabilising capability of the 3 or 4-benzyloxy substituent on the phenylurea and that, in addition, hydrophobic bonding plays a role in determining the level of observed herbicidal activity.

$$\log(1/C) = 1.3 + 0.42\pi\ (\pm 0.26) - 5.4\ E_R\ (\pm 1.88) \quad (2)$$

where $n = 12$ $r^2 = 0.89$ $s = 0.30$

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2A—R10

A NATURAL HERBICIDE : A NEW APPROACH IN WEED CONTROL

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Background and objectives

In recent years, control of weeds has been achieved to a large extent through massive use of synthetic chemicals. These chemicals, however, have been shown to be variously hazardous to both crops and their consumers (Mathur et al., 1982). Alternatively, use of botanicals is reported to be both safer and effective against many crop pests. Whilst there are a large number of reports on pesticidal activity of plant products and on allelochemicals, little work seems to have been done with respect to their possible use as selective herbicides. Here we report a plant product that may act as a natural herbicide and the mechanism of its selective action.

Materials and methods

Of the many plant species screened, against the test weed Amaranthus spinosus, for seed germinatin inhibitory property, ethanolic extract of Coffea arabica seeds proved promising (Rizvi et al., 1980). Its active principle was isolated by chromatographic techniques and identified as 1,3,7-trimethylxanthine (1,3,7-T) by i.r; u.v; nmr and superimposable i.r spectroscopy (Rizvi et al., 1981). In order to understand the mechanism of its action the effect of 1,3,7-T on amylase activity, water soluble sugars, total starch and protein content of A.spinosus was studied. The utility of 1,3,7-T as a potential herbicide would depend on its general non-toxic effects on the crop plant. Thus, its effects on germination, growth, amylase and nitrate reductase (NR) activities, protein content and yield of Phaseolus mungo, a crop in which the weed is a problem, were investigated.

Results and conclusions

Whilst 1,3,7-T completely inhibited the germination of the test weed at 1200 mg/l, similar treatment of the seeds of P.mungo showed no effect. It also inhibited germination of 7 other noxious weeds at various concentrations. Treatment of 1,3,7-T to seeds of A.spinosus (sub-lethal dose) caused a marked fall (circa 30%) in amylase activity. Kinetic studies with respect to substrate saturation, Km value and also in vitro treatment of the enzyme indicated no effect on its catalytic property. The inhibitory effect of 1,3,7-T on amylase activity could not be counteracted with treatments of GA_3. Analysis revealed that the starch/sugar and insoluble/soluble nitrogen ratios were increased in the seedligs grown from treated seeds. Experiments with respect to growth, amylase and NR activities, ethanol-soluble and insoluble nitrogen, protein content and yield of P.mungo showed no effect.

It is suggested that inhibition of seed germination of A.spinosus is caused by reduced synthesis of amylase and the reduction is not mediated through GA_3. Consequently, the starch/sugar ratio is increased in the seedlings grown from treated seeds. The observed change in the insoluble/soluble nitrogen ratio possibly resulted from an adverse effect on the activity/level of protease, which might have also affected seed germination. The fact that the compound has no effect on growth, amylase and NR activities, various nitrogen fractions and yield, firmly establishes the non-toxicity of 1,3,7-T with respect to P.mungo.

Thus, the compound is a potential selective herbicide. Prospects for use may depend upon, amongst other criteria, cost efficacy and the outcome of studies on mode of application.

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