SYSTEMIC INSECTICIDES

Metabolism and Mode of Action of Schradan

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The metabolism that an insecticide undergoes in plants, insects, or mammals may have an important bearing on its biological action. In the case of octamethylpyrophosphoramide, a biological activation takes place to produce products which are potent inhibitors of cholinesterase and chymotrypsin. The evidence which suggested that this activation involved an oxidation is reviewed. Biological or chemical oxidation may occur at the nitrogen of a dimethylphosphoramide group to produce a new type of functional group, for which the name phosphoramide oxide is proposed. This oxidation increases the enzyme inhibitory activity almost a millionfold by converting the relatively stable pyrophosphoramide to a reactive phosphorylating agent, the monophosphoramide oxide of schradan. Some properties of this new phosphoramide oxide are presented, methods are proposed for its chemical or enzymatic analysis, and the role that it plays in the insecticidal action of schradan is discussed.

SYSTEMICS SYMPOSIUM

SCHRADAN, OCTAMETHYLPYROPHOSPHORAMIDE, holds a unique position among the multitude of chemicals that have been tested as systemic insecticides. Schradan not only first established the practicality of chemotherapeutic treatment for plant pests but is also one of the best systemics available today.

Biochemical studies are essential for the safe and efficient use of insecticides. This is particularly true with systemic insecticides where three biological systems are involved—insects, plants, and mammals. A large number of the newer synthetic organic insecticides are known to be toxic only after biological conversion to form the effective poison. The identity and toxicity of the metabolic intermediates should be established to provide for the safety of animals which might consume the treated plants. Basic research on these biochemical mechanisms also lays the foundations for even more useful chemical developments in the future.

Schradan has received special attention from a biochemical viewpoint since it pioneered the field of systemic insecticides and was the first organophosphate demonstrated to require a metabolic conversion to serve as an effective anticholinesterase agent. A multitude of chemists, entomologists, and toxicologists have studied the mode of action of schradan during the past 4 years. The research team at Wisconsin has investigated the biochemistry of schradan in an attempt to correlate the diversified results of these workers.

Chemical Development

Schrader developed octamethylpyrophosphoramide for insecticidal purposes by replacing the ethoxy groups in tetraethyl pyrophosphate with dimethylamino groups to give the desired stability (*32*). It was developed in the 1939–41 era and patented in German in 1942 (*33*). The name "schradan" has superseded the older designations of Pestox III and OMPA (*18*, *21*).

Schradan can be prepared in the laboratory by heating bis(dimethylamido)-phosphoryl chloride with either ethylbis-(dimethylamido)phosphonate or a suspension of dry sodium bis(dimethylamido)phosphonate. One commercial method involves the direct synthesis from 2 moles of bis(dimethylamido)-phosphoryl chloride in the presence of excess methyldibutylamine to remove the hydrochloric acid formed in the reaction. Another involves direct reaction of dimethylamine with phosphorus trichloride in a bicarbonate buffer solution. The composition of the technical product from the methyldibutylamine method has been studied by Hartley *et al.* (*21*). Table I shows the composition of 85% of the phosphorus compounds in the technical mixture which could be separated from the nontoxic phosphates by extraction from alkaline solution with chloroform.

Octamethylpyrophosphoramide can be obtained in a high state of purity from the technical preparation by appropriate alkaline hydrolysis, distillation, and extraction procedures (*21*). The purified compound is a colorless liquid with a boiling point of 135–137° C. at 1.0 mm. (*27*), a specific gravity of 1.1343 at 25°/4°, and a refractive index of n_D^{25} 1.4612 (*11*). Its empirical formula, $C_8H_{24}N_4O_3P_2$, corresponds to a molecular weight of 286.34 and a phosphorus content of 21.65%. The electron-donor properties of the four-dimethylamino groups make schradan very stable to electrophilic reagents. Acid conditions effect a cleavage of the —P—N— bond with a hydrolysis constant of 3.60×10^{-3} min.$^{-1}$ at 25° C. At 100° C. the pyrophosphate bond is attacked by water with a constant of less than 10^{-5} min.$^{-1}$ and by alkali with a constant of 4.58×10^{-3} min.$^{-1}$ (*22*).

Biological Activation in Mammals

Schradan is very toxic to mammals

Table I. Composition of Technical Schradan

Formula	% by Weight	Relative Degree[a] Alkaline stability	Mammalian toxicity	Syst. insec. effectiveness
$(Me_2N)_2P(O)OP(O)(Me_2N)_2$	40.4	3	1	1
$(Me_2N)_2P(O)OP(Me_2N)(O)OP(O)(Me_2N)_2$	39.1	4	2	1
$[Me_2NP(O)O]_3$	3.2	2	3	2
$Me_2NP(O)$	17.3	1	4	3

[a] Greatest degree of effect designated by 1.

Table II. Effectiveness of Schradan Metabolite as an Enzyme Inhibitor

(Reaction time of inhibitor with enzyme, 24 hours at 5° C. for chymotrypsin and 1 hour at 25° C. for cholinesterase)

Chemical	Molar Concn. for 50% Enzyme Inhibition	
	Rat brain ChE	Cryst. chymotrypsin
Schradan	1.5×10^{-1}	>1.0
Monophosphoramide oxide of schradan	3.6×10^{-7}	2.4×10^{-5}
Tetraethyl pyrophosphate	1.5×10^{-9}	1.0×10^{-5}
Diisopropyl fluorophosphate	$>1.0 \times 10^{-5}$	2.9×10^{-6}

when applied to the skin or administered orally. It has a median lethal dose to laboratory animals of 8 to 35 mg. per kg. (*9, 27, 31*). The probably dangerous dose to man has been estimated at 280 to 560 mg. (*16, 36*). Human patients have received about 25 mg. daily for 3 weeks in the treatment of *Myasthenia gravis* with beneficial effects and no pronounced toxic symptoms (*30*). Animals can build up a definite resistance to schradan, and in certain cases the females are more resistant than the males (*29*). Atropine provides better prophylactic effect with schradan than with most other toxic organophosphates, because schradan induces selective peripheral parasympathetic stimulation without the central nervous system effects which are not antagonized by atropine but are present with most of the organophosphorus cholinesterase inhibitors (*9*).

Certain observations have suggested that schradan is altered in mammals to produce a strong anticholinesterase agent (*9, 14*). Purified schradan is a poor enzyme inhibitor, requiring 1.5×10^{-1} *M* for 50% cholinesterase inhibition and producing no detectable inhibition of chymotrypsin (*1, 2*). Yet after a definite lag period schradan produces the typical symptoms of acetylcholine poisoning evident with other anticholinesterase agents (*9, 14*). It is only about a millionth as effective in the inhibition of mammalian cholinesterase in vitro as are other anticholinesterase drugs with the same level of in vivo toxicity to mammals (*15*). Furthermore, a given amount of schradan in the blood of rabbits has been shown to produce cholinesterase inhibitory activity equivalent to 5000 times this amount of schradan in vitro (*15*). The in vivo activation of schradan appears to occur only in the liver, as demonstrated with partially hepatectomized rats (*7*), with rats with liver damage due to carbon tetrachloride administration (*13*), and by testing slices of various tissues in vitro for their efficiency in converting schradan (9). Liver slices of mice, rats, rabbits, dogs, sheep, swine, and cattle are effective (*3*). Technical schradan preparations contain a weak anticholinesterase agent which is rapidly destroyed by incubation with liver homogenates, but this material is not the same as the liver slice metabolite (*12*).

Studies on the chemical nature of the schradan metabolite were hindered by its instability, very low concentration, and mixture with large amounts of very similar chemical compounds. The active metabolite appeared to be an organophosphate derivative of schradan rather than a material of ionic, proteinaceous, or carbohydrate nature, as it was found to be chloroform-soluble and dialyzable (*15, 19*). The efficiency of enzyme inhibition suggested a high energy type of phosphorylating agent (*15*). This was confirmed when it was found that the active metabolite inhibited the esterase activity of chymotrypsin in the same concentration range as the more toxic phosphoric anhydrides (*1, 3*). The chymotrypsin inhibition was found to be noncompetitive and irreversible (*2*). The liver metabolite served as an acylating agent in producing its enzymatic inhibition, since chymotrypsin was phosphorylated in the process (*2, 5*). The chloroform extractability, instability, and enzymatic inhibition indicated the retention of the pyrophosphate structure intact in the active molecule. Separation of the pyrophosphate materials from liver slices by countercurrent distribution with chloroform and water produced only two distinct peaks, one with a partition coefficient of 7.0 corresponding to schradan and a new one with a coefficient of 1.7 (*2*).

A more complete analysis of these materials was made with a methylene chloride–water distribution as shown in Figure 1 (*2*). The fractions in this distribution study were analyzed for formaldehyde liberated by acid, since an oxidative metabolism might result in the formation of a group capable of liberating formaldehyde. Not only was a formaldehyde-liberating material present but the distribution peak for this material was coincident with the peaks for cholinesterase and chymotrypsin inhibition. This indicated that the active metabolite contained some group which liberates formaldehyde on treatment with acid and that this material was associated with both the chymotrypsin and cholinesterase inhibition. The effect of pH on the partitioning of the metabolite showed that it had weak basic properties (*3*). The metabolite resembled amine oxides in being unstable, showing basic properties, and decomposing to yield an aldehyde and a secondary amine or phosphoramide. Infrared analysis of the metabolite also indicated a similarity to the amine oxides. Thus the mammalian metabolism had converted the dimethylphosphoramide group to a new functional group for which the name "phosphoramide oxide" has been proposed (*2, 3*). The reactions involved in the formation and decomposition of this group are:

As purification of the rat liver metabolite progressed, the ratio of formaldehyde-liberating groups for each pyrophosphate approached 1, a maximum experimental value of 0.96 being attained. The metabolic activation of schradan in mammals thus involved the oxidation of only one dimethylamino group to yield the monophosphoramide oxide of schradan as the major toxic metabolite (*2*).

The phosphoramide oxide of schradan has been isolated from the liver of mammals at the time when the toxic symptoms first appeared (*2*). The maximum stability of the metabolite occurs at about pH 8. It is very sensitive to acid hydrolysis and somewhat less so to alkaline conditions (*2*). This phosphor-

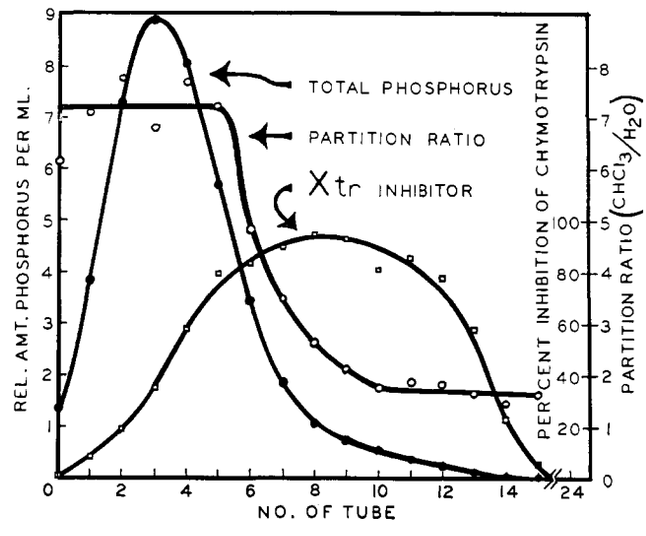


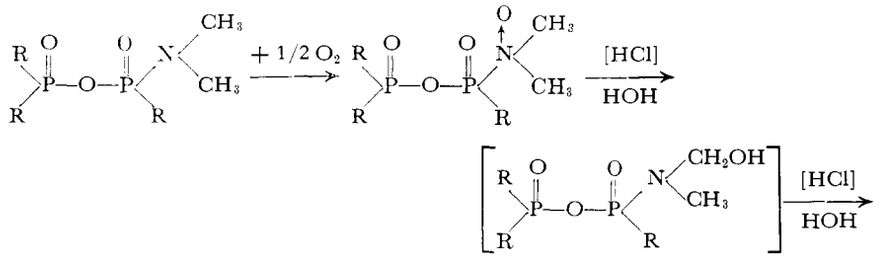
Figure 1. Partial separation of schradan from active metabolite by countercurrent distribution

Methylene chloride and water solvent phases. Phosphorus peak represents schradan plus metabolite; formaldehyde, cholinesterase, and chymotrypsin peaks represent metabolite only

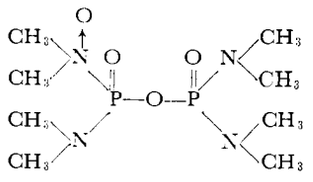
amide oxide inhibits both cholinesterase and chymotrypsin in the same concentration range as do diisopropyl fluorophosphate and tetraethyl pyrophosphate (Table II) (*2*).

Metabolism in Plants

The introduction of a toxic chemical into food crops, as with a systemic insecticide, introduces new problems. The possible mammalian toxicity of the applied chemical and any resultant changes in this chemical or in the plant itself must be evaluated. Hartley (*19*) in England and the Food and Drug Administration of the United States Federal Security Agency (*24*) have been firm in their conviction that a full investigation of the decomposition of schradan in the plant is essential to the safety of the consumer.



$$3\ R{\cdot}HCl + 2\ H_3PO_4 + HCHO + CH_3NH_2{\cdot}HCl$$



Schradan is metabolized by plants to form an anticholinesterase agent. This metabolite can be readily detected by enzymatic assays (*4*) but is not evident to mosquito larvae bioassay (*35*). A nontoxic analog of schradan, orthophosphoric acid tridimethylamide, decomposes at the same rate as schradan in plants (*20, 23*). An oxidative breakdown has been suggested for this triamide with a methyl hydroxymethyl amide as an intermediate (*23*). With schradan, certain metabolites present in very low concentrations, including the anticholinesterase agent (*17*), are soluble in chloroform (*8*) and some can be precipitated by calcium salts from alkaline solutions (*20*). Postulated mechanisms for schradan metabolism in plants have included a biological oxidation of a methyl group (*19*), or of the amide nitrogen (*23*), or the formation of the hydroxymethyl derivative (*19*).

The authors have applied the same isolation and purification procedures used for the mammalian metabolite to the plant metabolite (*6*). The plant metabolite was found to be identical with the animal metabolite in regard to formaldehyde liberation, solvent-partitioning properties, stability, and effectiveness as an enzyme inhibitor (Table III). The plant metabolite that had proved so elusive was the same monophosphoramide oxide of octamethylpyrophosphoramide as produced by mammals (*6*).

Methods of analysis were then developed so that the hazard of this metabolite in plants could be evaluated. Figure 2 shows the sensitivity of the methods for assaying the metabolite (*6*). The total amount of schradan and chloroform-soluble metabolites in plants can be determined by dimethylamine analysis (*17*), or more complete analysis of plants can be achieved through a specific determination of schradan by phosphorus determination after alkaline hydrolysis (*8*) and determination of metabolite based on formaldehyde liberation (*6*).

The phytotoxicity of schradan to plants also appears to be associated with this metabolite. A direct relation occurs between the amount of schradan in the plant, the inactivation of the plant phosphatase enzymes, and the phytotoxicity of the insecticide (*4*). Phosphorus in the soil or nutrient, such as would be provided by a fertilizer, decreases the effectiveness of schradan in treated plants (*4*). This was shown to be due to an effect on absorption through the roots, as no interaction of phosphorus and schradan occurred with seed or foliage application but was very pronounced when absorption through the roots was involved. Orthophosphate itself served as a very good inhibitor of the phosphatase enzymes within the plant and of the extracellular phosphatases secreted by the roots. Isolated roots were able to metabolize schradan to its phosphoramide oxide (*6*) and a schradan metabolite under these conditions inhibited the extracellular root phosphatases (*4*). A definite interaction occurs in plants between schradan absorption, its oxidative metabolism, and the inhibition of the plant phosphatase enzymes in vivo. A partial explanation of the phytotoxicity of schradan may thus be found in its conversion to the phosphoramide oxide, which then inhibits phosphatase or other essential plant enzymes.

Health Hazard

Is the plant metabolite of schradan a serious health hazard to man and domestic animals? Extensive feeding experiments with schradan-treated plants have shown no toxic effects in any cases (*31*). The schradan decomposition products which are not readily extractable with chloroform produce no toxic symptoms at concentrations where their precursor would be lethal (*8*). The chloroform-soluble anticholinesterase agent must still be considered. A new method of approach to this problem was provided with the finding that schradan could be oxidized with permanganate to yield the identical phosphoramide oxide formed by plants and mammals (*5*). Working with the complex oxidation mixtures from permanganate oxidation it was found that the first oxidation product, the monophosphoramide oxide, was the most effective cholinesterase inhibitor that appears in the oxidative degradation scheme for schradan (*5*). Yet oxidation mixtures containing up to 5% of the phosphoramide oxide were no more toxic to white rats than was schradan per se, and only in very exceptional cases would the ratio of metabolite to schradan in the plant be this great (*6*). The stability of the schradan metabolite was found to be very dependent on pH (*2*); its half life in the plant might be on the order of a few hours to 2 or 3 days. Schradan itself has a half life of about 2 weeks in plants (*19*), and the concentration of the metabolite which can be present in the plant is dependent not only on its stability but also on the concentration of its precursor. When an animal consumes a treated plant it takes up both schradan and the phosphoramide oxide. Schradan is rapidly absorbed from the gastrointestinal tract (*9*), but a large proportion of the metabolite might be lost prior to absorption because of its instability. The animal

Table III. Identity of Schradan Metabolites from Plants and Mammals

	Partition Coefficient		
	Schradan	Mammalian metabolite	Plant metabolite
CH_2Cl_2/H_2O	2.0	0.65	0.63
$CHCl_3/H_2O$	6.8	1.58	1.68
CCl_4/H_2O	0.004	0.22	0.22
	Molar Concn., 50% Enzyme Inhibition		
Cholinesterase	1.5×10^{-1}	3.3×10^{-7}	3.5×10^{-7}
Chymotrypsin	>1.0	2.4×10^{-5}	

then converts the absorbed schradan to this same phosphoramide oxide metabolite (*2*). Thus the metabolite (anticholinesterase agent) formed in the plant does not appear to engender a health hazard any greater than schradan itself.

Biochemical Aspects in Insects

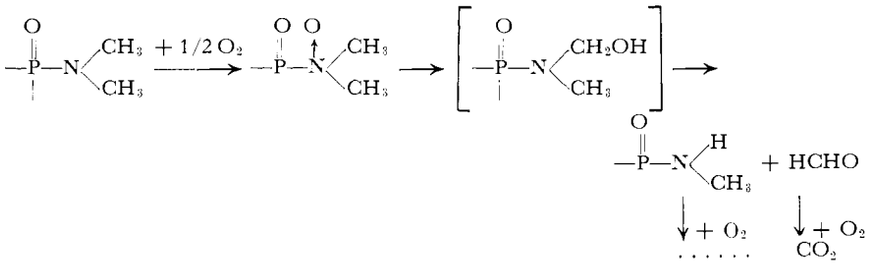
Schradan has very low toxicity to insects by either topical application or injection (*26*). This led DuBois (*9*) to consider the insects incapable of converting schradan to an anticholinesterase agent, a concept later modified by Duspiva (*10*), who considered that only those insects susceptible to the toxic action of schradan contained the enzyme system capable of forming this active inhibitor of cholinesterase. Metcalf (*25*) found that tissues of the cockroach, which is very resistant to schradan, readily form an anticholinesterase agent from schradan.

The chemical nature of the insect metabolite was shown to be identical with that of the other biological metabolites and the active chemical oxidation product (*6*). When the various parts of the cockroach intestine were tested for their in vitro efficiency in metabolizing schradan, it was found that the gastric caecae and the malphigian tubules were the most active tissues (*6*). The difference in susceptibility of insects to the toxic action of schradan did not appear to be due to their ability to form the metabolite nor to detoxify it, as the phosphoramide oxide could readily be isolated from a moderately resistant insect such as the housefly, and the very resistant cockroach, as well as the susceptible aphid (*6*). It appears more probable that the susceptibility variation is due to the sensitivity of the cholinesterase of the individual insect species to the metabolite. This was demonstrated by testing the in vitro inhibition of insect cholinesterase from different sources by the schradan metabolite prepared from liver slices. The cholinesterase from the more resistant insects required from 5 to 50 times the amount of metabolite to effect enzyme inhibition as did that from the more susceptible animals (*6*).

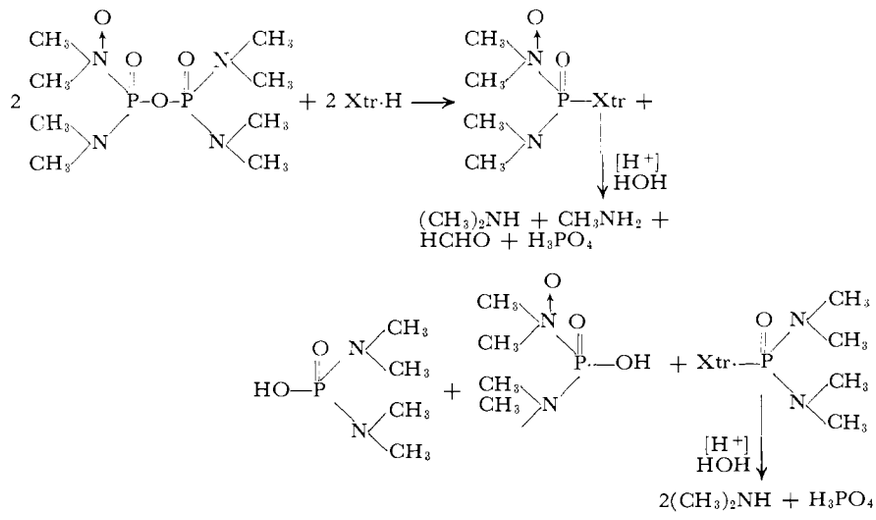
Does the plant or the insect form the effective toxicant when schradan is used as a systemic insecticide? DuBois (*9*) considered the plant to be the important site, while Duspiva (*10*) considered the insect metabolite to be the important factor. With pea plants the amount of schradan, the amount of metabolite, and the insecticidal toxicity are closely correlated (*4*). The problem becomes clarified somewhat with the finding that the plant and insect metabolites are identical in chemical nature (*6*). Partial oxidation enhanced the systemic toxicity of schradan and its *p*-nitrophenyl analog (*6*), indicating that the metabolism step may be the limiting factor in the insecticidal toxicity of schradan. The plant-formed metabolite is probably more important in insect than in mammalian toxicity because of the less drastic pH changes involved in the ingestion and absorption of the metabolite by insects. Yet the slow rate of conversion by the plant makes it likely that the insect contributes the major part of the toxicant.

Chemical Oxidation of Schradan

Numerous attempts have been made to alter schradan chemically and possibly increase its biological activity. Hartley (*19*) noted that schradan was susceptible to attack by chlorine, iodine, and permanganate. The authors have studied the oxidation by neutral permanganate in relation to production of active enzyme inhibitors (*5*). A complex oxidative demethylation occurred with the utilization of 36 equivalents of oxygen per mole of schradan. The first oxidation product formed appeared to be identical with the biological metabolites. The primary steps involved are:



When crystalline chymotrypsin was added to a mixture of these oxidation products where three equivalents of permanganate had been consumed, the enzyme was acylated with 1 mole of phosphorus and was completely inhibited. The acylated enzyme was isolated and chemically analyzed for the organophosphorus residues introduced (*1*, *5*). The analyses agreed with a theoretical value for the reaction with chymotrypsin:

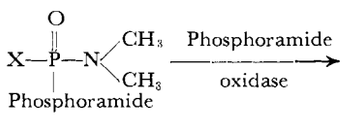


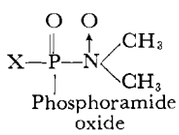
As the permanganate oxidation of schradan progressed, differences appeared in the selectivity of the products for cholinesterase and chymotrypsin. The most efficient cholinesterase inhibitors appeared early in the oxidation scheme and generally paralleled the amount of the phosphoramide oxide present, while the chymotrypsin inhibitors appeared later in the reaction and paralleled the susceptibility of the products to alkaline hydrolysis (*5*). The monophosphoramide oxide appeared to be the best cholinesterase inhibitor formed in the chemical oxidation but was not the most active inhibitor for chymotrypsin. The positive charge of the phosphoramide oxide group may have served to orient the oxide on the cholinesterase molecule prior to reaction with the active site, while electrostatic attraction was not a determining force in the case of chymotrypsin. If this is true, then the reaction product of the monophosphoramide oxide with cholinesterase should contain equimolar formaldehyde-liberating groups and phosphorus instead of the 0.5 mole of formaldehyde per mole of phosphorus obtained with the phosphorylated chymotrypsin.

General Considerations

Certain generalizations can be made from these studies with schradan. Plants, mammals, and insects metabolize schradan to effect a millionfold increase in its

inhibitory activity toward cholinesterase and chymotrypsin. Chemical oxidation produces a similar result. This activation by chemical or enzymatic oxidation appears to be general for dimethylaminophosphoric anhydrides, since in addition to schradan, a variety of other pyrophosphates and anhydrides with fluorine and *p*-nitrophenol undergo this increase in enzyme inhibitory activity (*3*). The oxidation of phosphoramides produces a new functional group which has been designated the phosphoramide oxide group, while the widely distributed enzyme system effecting this conversion has been named a phosphoramide oxidase.





The formation of the phosphoramide oxide produces a positively charged quaternary nitrogen atom from one of the dimethylphosphoramide groups which may be attracted to the negative (anionic) center of the cholinesterase and thus selectively combine with the enzyme. Wilson and Bergmann (*34*) have interpreted the pH dependence of cholinesterase activity in terms of an anionic and esteratic site on the enzyme surface and suggested that the quaternary nitrogen of acetylcholine combines with the anionic site on the enzyme surface. The phosphoramide oxide group also serves to draw electrons from the phosphorus to increase the reactivity of the anhydride bond and so enhance its ability to acylate and further inactivate this physiologically important enzyme. Because cholinesterase is concerned with the enzymic hydrolysis and synthesis of acetylcholine, a biological process that is essential for the metabolic cycle and function of nerve cells (*28*), its inactivation in insects or mammals produces very toxic effects. In contrast, plants do not possess these nerve cells and all the essential enzymes associated with them, so schradan or its metabolite has relatively little toxic action toward most plants compared to insects. This biochemical difference provides a sound basis for the use of systemic insecticides of the type represented by schradan.

Even now, knowledge of the biochemistry and mode of toxic action of schradan is only fragmentary. Yet it clearly illustrates the need for studying the mode of action of agricultural chemicals on all the biological entities with which they may come in contact. A basic and thorough biochemical study is essential for the utmost safety and efficiency in the use of any agricultural chemical.

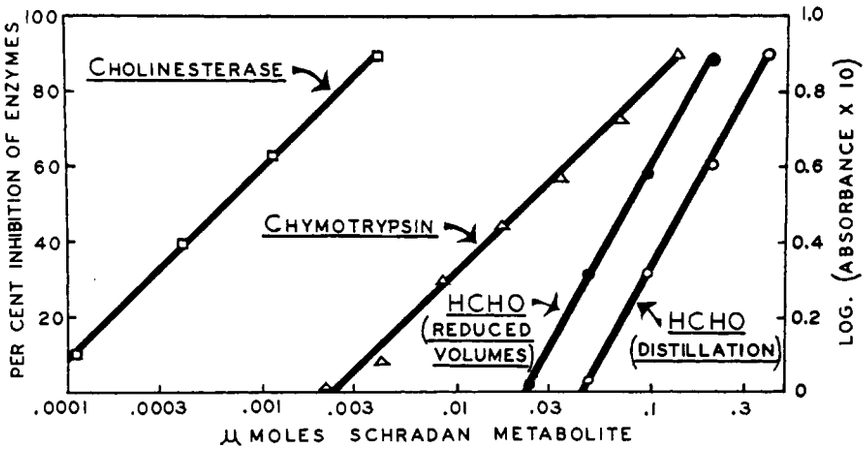


Figure 2. Sensitivity of enzymatic and colorimetric assays for schradan metabolite

Enzymatic assays, manometric and formaldehyde liberation on treotment with acid, colorimetric

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SYSTEMIC INSECTICIDES

Heterocyclic Carbamates Having Systemic Insecticidal Action

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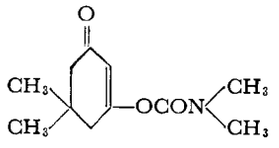
In the search for new insecticides certain heterocyclic dimethyl carbamates were found to have a high degree of systemic as well as a direct insecticidal action. The insecticidal action of 5,5-dimethyldihydroresorcinol dimethylcarbamate, 1-phenyl-3-methylpyrazolyl-(5)-dimethylcarbamate, and 1-isopropyl-3-methylpyrazolyl-(5)-dimethylcarbamate has been investigated. The last-named compound, also known as isolan, has a high degree of toxicity to insects and displays a strong systemic action against aphids. Field tests are under way to determine its practical value.

SYSTEMICS SYMPOSIUM

CERTAIN HETEROCYCLIC DIMETHYL CARBAMATES, like certain organic phosphates, have a high degree of systemic insecticidal action.

Dimetan

Gysin (*2*) reported that phenyl diethyl carbamate and the related 5,5-dimethyldihydroresorcinol diethyl carbamate had good insect-repellent action. However, when the closely related 5,5-dimethyldihydroresorcinol dimethyl carbamate was tested, there was practically no repellent action, but the compound was toxic to flies and some species of aphids. This compound is known under both the code number G-19258 and the common name dimetan. Wiesmann *et al.* (*4*) reported that it was effective against a number of species of aphids in concentrations of 0.02 to 0.04%. It was also translocated in certain plants and had a mild systemic insecticidal action.

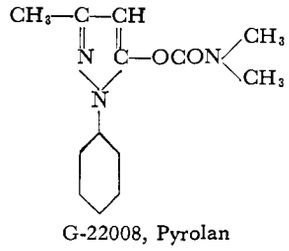


G-19258, Dimetan

None of numerous changes and substitutions in the carbamate or aliphatic portion of the above molecule enhanced insecticidal activity but rather tended to reduce it. For example, if the oxygen atoms of the carbamic acid radical were replaced by one or two sulfur atoms, the insecticidal activity was reduced. Other changes in the molecule resulted in the conclusion that the dimethyl urethanes were the most effective in the series.

Pyrolan

Attention was then directed to enolizable heterocyclic systems. The compound 1-phenyl-3-methylpyrazolyl-(5)-dimethyl carbamate, also known as G-22008 and pyrolan was found to be considerably more toxic to flies and other insects than dimetan, but it was also more toxic to warm-blooded animals. Wiesmann (*3*) reported on the insecticidal properties of pyrolan, and at the time considered it to have possibilities for combating flies in Europe that had developed resistance to DDT insecticides.



G-22008, Pyrolan

This compound was also found to have some systemic action against the green apple aphid (*Aphis pomi*). However, substitutions in the amine radical of the urethane group, additions to the phenyl group, substitutions for the hydrogen in the 4- position of the pyrazolone nucleus, or the replacement of the methyl group in the 3- position resulted in loss of insecticidal activity to a greater or lesser degree.

Isolan

It was surprising therefore to find that replacement of the phenyl nucleus with hydrogen or alkyl radicals resulted in some extremely effective insecticidal compounds. Toxicity to warm-blooded animals was also increased. Compounds of this group had a high degree of contact insecticidal action, but a rather short residual effect. They exhibited strong systemic action, however, with resulting longer residual effect as determined by applications to trunks of trees or stems of plants. The compound 1-isopropyl-3-methylpyrazolyl-(5)-dimethylcarbamate, also known by the code number G-23611 and the common name