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LOW TOXICITY PHOSPHATES

2-Chloro-1-(2,4,5-trichlorophenyl)vinyl Dimethyl Phosphate, a New Insecticide with Low Toxicity to Mammals

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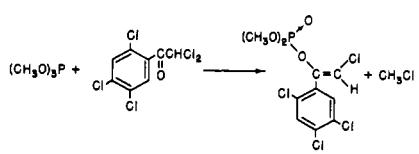
2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate (SD 8447) has shown excellent toxicity to several species of insects in laboratory and field tests but is remarkably safe to laboratory mammals in acute and two-week feeding tests. The poor solubility or partition properties of the compound may limit penetration and translocation in mammals so that amounts of the compound in blood and tissues are low enough to be metabolized without toxic effect.

A MAJOR GOAL of insecticide research, even before the recent public concern over pesticide residues, has been to find compounds which are effective against insects but which are less hazardous to man and other mammals. With this goal in mind, work in the Shell Development Laboratories on insecticidal vinyl phosphates has led to 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate (I) (*9*). This compound is highly toxic to several insect species in both laboratory and field tests but is relatively nontoxic to laboratory test mammals in acute and subacute tests. Compound I is now under development toward commercial use under the code number SD 8447. The present paper presents chemical and biological data for this compound in comparison with data for several of its analogs. An explanation for the outstanding selective toxicity of (I) is suggested from exploratory experiments.

Methods and Results

Preparation and Properties of Compounds. Data on chemical and physical properties of (I) and of four analogs are given in Table I. Compound I was prepared by the reaction given below; the four analogs were obtained in the same way. With chlorine in the 2 and 4 positions of the benzene ring of the ketone intermediate, the reaction is highly selective for the β-isomer (I, II, III), presumably for steric reasons. The β-isomer is arbitrarily designated as the one in which the phosphate group and the largest group on the second vinylic carbon are cis. Only 10% or less of the α-isomer, with the phosphate and chlorine atoms trans on the double bond, were formed and were removed from (I) and (II) by crystallization. By contrast, the α-isomer predominated when the benzene ring was not chlorinated (IV) or with dichloroacetaldehyde as the carbonyl reactant (V); preferential formation of the α-isomer is "normal" in this reaction (*5*). Configurations of the individual isomers and the isomeric content of the five compounds of Table I were assigned from NMR and infrared-absorption spectra following methods used earlier for Phosdrin insecticide (*13*). Both (I) and its ethyl analog (II) are crystalline solids of moderately high melting point which are only very slightly soluble in water. Compound I is also only moderately soluble in hydrocarbons and other common organic solvents; the ethyl analog is more soluble.



I (β-isomer), SD 8447

Compound I and its analogs are, like other organophosphate insecticides, good inhibitors of acetylcholinesterases, although enzyme inhibition studies with (I) and (II) are complicated by the low water solubility of the compounds. Presumably, this inhibition is the principal mechanism of their biological action. Fukuto and Metcalf (*3*) have shown that rates of hydrolysis of substituted phenyl phosphates correlate well with acetylcholinesterase inhibition. However, the data in Table I for the four ethyl esters (II), (III), (IV), and (V) indicate that the reactivity of this group of compounds with cholinesterases does not parallel hydrolysis data at pH 9.1. Secondary interactions between the chlorinated phenyl ring of (II) and (III) with the enzymes increase the inhibition.

Insect and mammalian toxicity data for the same five compounds are given in Table II with similar data for malathion.

Syntheses. The intermediate acetophenones were prepared using the Friedel-Crafts ketone synthesis. Dichloroacetyl chloride (Kay-Fries Co., 99% pure, 88 grams, 0.60 mole) was added in 10 minutes to a stirred slurry of 88 grams, 0.66 mole, of anhydrous, purified, powdered aluminum chloride (Matheson Coleman & Bell) in 109 grams, 0.60 mole of 1,2,4-trichlorobenzene (Matheson Coleman & Bell,

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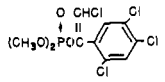
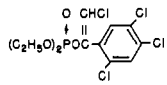
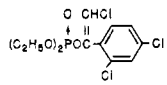
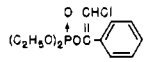
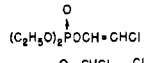
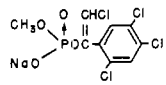
Table I. Physical and Chemical Properties

Number	Structure	somer, %	M.P. ° C., (B.P. ° C./Mm. Hg)	Analysis P, % Calcd.	Analysis P, % Found	Analysis Cl, % Calcd.	Analysis Cl, % Found	Hydrolysis[a]	Cholinesterase, PI_{50} Rat whole blood	Cholinesterase, PI_{50} Human serum	Cholinesterase, PI_{50} Fly head
I		β-100	97-98	8.5	8.4	38.8	38.8	37	4.8	6.7	7.3
II		β-100	80-80.5	7.9	8.3	36.0	36.1	170	5.7	7.6	7.9
III		α-10 β-90	(110/0.001)	8.6	8.7	29.7	29.9	>400	6.5	7.4	7.6
IV		α-55 β-45	(80/0.0006)	10.5	10.7	12.2	12.9	>300		5.8	5.8
V		α-75 β-25	(72/0.06)	14.4	14.6	16.5	17.6	140	5.5	6.7	7.8
VI		β-100	225-230 (dec)	8.3	8.4						

[a] Half-life in hours at 38 ° C. at 2 p.p.m. in 0.01*M* potassium tetraborate buffer (pH 9.1).

Table II. Biological Properties

Compound	Toxicity Index (Standard[a] = 100) Housefly, D	Pea aphid, P	Rice weevil, P	Corn earworm, D	2-Spotted spider mite, P	Acute Oral LD_{50}, Mg./Kg. Male mice	Male rats	Acute Skin LD_{50}, Mg./Kg., Rabbits
I	81	<1	20	54	11	>5000	4000-5000	>2500
II	70	1	16	44	<5	1250-2500	1100	>2500
III	93	2	8	46	5	155	39	1250-2500
IV	0.6	<1	<1	<3	<5	1000-2000	...	...
V	50	4	8	5	32	30.5	7	17.6
VI	0	0	0	0	0	<2000	...	...
Malathion	5	35	21	7	54	2120[b]	1500[b]	>4000[b]

[a] Standard: D = Dieldrin; P = Parathion; higher number indicates higher toxicity. [b] Toxicity from Negherbon Handbook (7).

98%). The mixture was heated slowly to 90° C., held at this temperature for 4 hours, cooled, and poured onto a mixture of ice and a few milliliters of hydrochloric acid. The product was extracted with ether, thoroughly washed, dried, and distilled to give 134 grams, 77% yield, of 2,2,2′,4′,5′-pentachloroacetophenone, b.p. 103–105° C. (0.05 mm.), n_D^{25}1.5957. Anal. found, 33.4% Cl; Calcd., 32.8.

Similarly, *m*-dichlorobenzene gave a 73% yield of 2,2,2′,4′-tetrachloroacetophenone, b.p. 95–98° C. (0.02 mm.), n_D^{25}1.5825.

Compound I was prepared by addition of 23 grams, 0.49 mole, of trimethyl phosphite (Virginia Carolina–97% pure) to 50 grams, 0.17 mole, of the pentachloroacetophenone at 30° to 50° C. in 30 minutes. The mixture was then heated at 110° C. for 30 minutes, cooled, and poured carefully into about 100 ml. ether. After being cooled in ice, the solid was filtered and washed with pentane to give 49 grams (79% yield) of white solid 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate. P^{32}-labeled material was made in the same way using labeled trimethyl phosphite prepared by the method of Potter and Burton (*10*). After purification by chromatography, it was greater than 98% pure radiochemically and had a specific activity of 2 mc. per mm.

The other neutral phosphate esters were prepared similarly except that the liquids were isolated by distillation of the reaction mixture.

The sodium salt (VI) of compound I was prepared by heating 18.7 grams, 0.51 mole, of (I) with 7.5 grams, 0.49 mole, of sodium iodide in 100 ml. of acetone for 2 hours at 25° to 45° C. followed by refluxing for 2 hours. Cooling of the reaction mixture at 4° C. gave 8.6 grams (45%) of (VI).

Solubility, Rate of Hydrolysis, and Cholinesterase Inhibition. Sufficient compound to exceed saturation was added to distilled water, shaken for at least 8 hours at 25° C., and the excess separated by centrifugation if a liquid or filtration if a solid. The solution was made slightly basic with sodium bicarbonate to prevent extraction of phosphorus acids, and an aliquot was extracted immediately with chloroform. The phosphorus in the chloroform was determined using the method of Saliman (*11*). By this method, the solubility of (I) was found to be 11 p.p.m. compared with 5.7 p.p.m. for (II) and 145 p.p.m. for (III). Hydrolysis data were obtained with a solution of 2 p.p.m. of the compound in 0.01*M* potassium tetraborate buffer solution (pH 9.1) at 38° C. Aliquots were removed periodically, and the amount of unhydrolyzed phosphate was determined by the method used for solubility. Results were plotted, and the half-life of hydrolysis was estimated from the early straight portion of the curve; with (I) results were consistent with a first-order reaction for longer than the half-life.

Cholinesterase inhibition values were determined by the electrometric method of Michel (*6*) as modified by Williams and co-workers (*17*).

Insecticidal Tests. Insect test data were obtained by the methods of Corey (*2*). Caged houseflies (*Musca domestica*) in a wind tunnel were sprayed with solutions in deodorized kerosine-acetone. Broad-bean plants were sprayed with aqueous suspensions; pea aphids (*Macro-*

siphum pisi) or corn earworms (*Heliothis zea*) were placed on the sprayed plants. Two-spotted spider mites (*Tetranychus telarius*) on pinto bean plants were sprayed with aqueous suspensions. Rice weevils (*Sitophilus oryzae*) were dipped in aqueous suspensions or solutions of the chemical. The insect toxicity of the compounds was expressed as the toxicity index relative to the standards dieldrin and parathion as described by Sun (*14*). The LC_{50}, in grams per 100 ml., for dieldrin was 0.0053 for houseflies and 0.005 for corn earworms; for parathion the LC_{50} was 0.00037 for pea aphids, 0.015 for two-spotted spider mites, and 0.00075 for rice weevils (*2*).

Acute Toxicity Tests. Nonfasted male Swiss Webster strain mice (19 to 21 grams) and nonfasted male Long-Evans strain rats (95 to 110 grams) were used for the acute oral tests; 10 animals were used at each dose level. Compounds in appropriate solvents were inserted into the esophagus with a syringe using a 20-gage needle. Compound I was used in a mixture of 90% propylene glycol, 10% dimethylacetamide. The animals were observed closely during the first 8 hours after dosage and then checked daily for 14 days. Dermal toxicity was determined with adult albino rabbits (three per group) weighing 1800 to 2300 grams. The undiluted compound was applied to an area of the skin that had been clipped the previous day. The treated area was wrapped with a rubber dam and after 6 hours the wrapping was removed, the test area washed free of compound, and the rabbits were observed for 2 weeks. The LD_{50} values were calculated by the method of moving averages of Weil (*16*).

Subacute Feeding Test. Weanling Long-Evans strain male and female rats (four animals of each sex per group) were fed a finely powdered commercial laboratory ration for a preconditioning period of 5 days. Then compound I was added to the diet at levels of 15, 45, 135, 400, 1200, 3600, and 10,800 p.p.m. for 2 weeks. A control group was also included. All animals were weighed at the beginning of the experiment and after the first and second weeks. Whole blood cholinesterase was determined during the acclimation period and at weekly intervals. At the end of the 2-week study, two males and two females from the control and the 3600- and 10,800-p.p.m. groups were autopsied. No animals died during the feeding, and no gross pathological changes were noted in the sacrificed animals.

Weight gains (Table III) of the male rats fed the 10,800-p.p.m. diet and of the females on the 3600- and 10,800-p.p.m. diets were markedly less than that of the controls and of rats fed lower levels of (I); this effect was primarily due to lower gains in the first week. Whole blood cholinesterase (Table IV) was not inhibited significantly during the 2 weeks at levels less than 400 p.p.m. Inhibition was marginal (72 to 81% of the initial value) at 400 p.p.m. and marked at higher diet levels. In the recovery period on pesticide-free diet, cholinesterase levels of the rats in the 3600-p.p.m. diet returned to normal within 3 to 4 days; those in the 10,800-p.p.m. diet were normal after a week. This recovery of the inhibited cholinesterase was similar to that of other dimethyl phosphates, indicating that the compound was not retained to any great extent in tissues.

Structure *vs.* Activity. In laboratory tests, (I), its ethyl analog (II), and (III) are highly toxic to the housefly and the corn earworm, moderately effective against the rice weevil, and less toxic to the pea aphid and the two-spotted spider mite. Excellent control of houseflies and corn earworms, as well as of other species of insects, has also been demonstrated in field tests. This insect spectrum is quite different from that of malathion and of many other phosphate insecticides which are more effective against the mite and the aphid. Although this spectrum resembles that of the chlorinated hydrocarbon insecticides, a strain of houseflies highly resistant to DDT and cyclodiene insecticides was not resistant to (I). Interesting structural effects among the five analogs are the deactivation of (V), by introduction of a phenyl group to give (IV), and the restoration of activity by introduction of either two or three chlorine atoms in the ring.

Most striking is the great reduction in acute toxicity to mammals from the introduction of the third chlorine in the benzene ring to give (I) and (II). As with many homologous phosphates, the methyl compound is significantly safer to mammals than the ethyl analog, although more toxic to insects. In acute toxicity, (I) appears to be safer than malathion and one of the least hazardous insecticides known. Also, this relative safety was confirmed by the 2-week feeding tests with rats.

Metabolism Studies with Radioactive (I). Single doses of the P^{32} labelled (I) in soybean oil were administered via stomach tube to male and female rats of a Wistar-derived strain. Dosages were 1.0 mg. (2.9 μc.) equivalent to 5.1 mg. per kg. of body weight for the males and 0.8 mg. (2.3 μc.) or 4.9

Table III. Effect of Compound I on Average Growth and Feed Conversion of Male and Female Rats—A Two-Week Study

Compound I Dietary Level, P.P.M.	Week 0–1 Male Gain, g.	1–2 Male Gain, g.	0–2 Male Gain, g.	0–2 Female Gain, g.	1–2 Female Gain, g.	0–2 Female Gain, g.
Control	41	22	63	30	18	48
15	31	34	65	25	27	52
45	41	33	74	29	22	51
135	36	24	60	24	19	43
400	37	26	63	24	17	41
1,200	39	27	66	29	18	47
3,600	36	25	61	20	20	40
10,800	21	25	46	19	18	37

Table IV. Effect of Compound I on Whole Blood Cholinesterase[a] of Rats

Compound I Dietary Level, P.P.M.	Week 0	1	2	2.5[b]	3
			Male		
0	0.93	0.94(101)	0.88(95)	0.88(95)	0.69(75)
15	0.89	0.99(111)	0.83(93)		
45	1.03	0.96(93)	0.83(80)		
135	0.85	0.83(98)	0.76(89)		
400	0.95	0.81(85)	0.73(77)		
1,200	0.91	0.69(76)	0.63(69)		
3,600	0.85	0.56(66)	0.80(94)	0.80(94)	0.83(98)
10,800	0.94	0.35(37)	0.38(40)	0.68(72)	0.72(77)
			Female		
0	1.18	0.92(78)	1.10(93)	1.11(94)	1.05(89)
15	0.90	0.80(89)	0.85(94)		
45	0.98	0.82(84)	0.88(90)		
135	0.93	0.79(85)	0.92(99)		
400	1.06	0.72(68)	0.79(75)		
1,200	0.96	0.67(70)	0.66(69)		
3,600	0.92	0.62(67)	0.52(56)	0.95(103)	0.97(105)
10,800	0.90	0.35(39)	0.35(39)	0.77(86)	0.88(98)

[a] Average determined ΔpH and in parentheses per cent of original cholinesterase activity. [b] Rats changed to a compound-free diet after 2 weeks.

mg. per kg. for the females. The animals were held in metabolism cages and the urine and feces collected separately. Samples were added to a liquid scintillator stock solution and counted in a Packard Model 314-AX liquid scintillation counter, then recounted with an added internal standard of P^{32} labeled (I). The metabolites in the urine were identified by cochromatography on paper with known standards. Table V summarizes the results with two male and two female rats. The P^{32} in the urine was present in metabolites of (I), no intact (I) was detected. Dimethyl hydrogen phosphate, methyl dihydrogen phosphate, and "desmethyl I" (compound VI) were identified. Compounds in the feces were not identified.

Like the other vinyl phosphates, DDVP (*4*), and Phosdrin insecticide (*7*), compound (I) is rapidly metabolized to hydrolysis products of low toxicity to mammals.

Injection and Infusion Studies. Intravenous infusion of saturated saline solutions of (I), (II), or (III) into rabbits for an hour following the general technique of Shellenberger and coworkers (*12*) did not produce symptoms or detectable lowering of whole blood cholinesterase, as expected from solubility and PI_{50} values. Intravenous injection of a solution of (III) in dimethyl sulfoxide into rats gave marginal inhibition of whole blood cholinesterase at 0.1 mg. per kg. and almost complete inhibition after five hours at 1.0 mg. per kg. Similar tests with (I) were even more dramatic. Intravenous injection of 0.26 and 0.48 mg. per kg. in 50% aqueous ethanol had no effect at 2 hours to rats, but caused about 30 and 50% inhibition, respectively, after 24 hours. Subcutaneous injection of 2.68 mg. per kg. in ethanol was without effect at 3 hours, but caused 30% inhibition at 24 hours and 70% at 48 hours. Because of the low solubility of (I) these results were not readily reproduced; when the ethanol concentration in the solution was less than 25%, compound (I) immediately precipitated from solution on addition to blood or other tissue preparations. The slowness of the enzyme reaction rather than the degree of inhibition is the significant conclusion of this work. By contrast, injection of other vinyl phosphates typically gives maximum inhibition within a few minutes. For example, in rabbits, injection of 0.51 to 2.0 mg. per kg. of dimethyl 2,2-dichlorovinyl phosphate (DDVP) caused 50 to 90% inhibition in 10 to 40 minutes followed by recovery of most of the enzyme activity within 3 hours. Similarly, with continuous intravenous infusion of DDVP, the inhibited whole blood cholinesterase began to recover within a few minutes after infusion was stopped (*12*).

Table V. Excretion[a] of Metabolites of P^{32}-Labeled I

Interval, Days	Male Rats				Female Rats			
	1M		2M		3F		4F	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
1.0	73.1[b]	5.5	54.9	11.3	65.9[c]	4.5	63.6	7.4
2.0	78.3	7.5	57.2	13.5	67.8	6.1	69.0	12.2
3.0	79.8	8.7	58.4	15.1	71.7	9.5	70.0	14.1
4.0	80.7	9.5	59.0	16.1	72.5	10.8	70.4	15.2
7.0	81.7	15.4	60.7	24.8	74.0	17.2	71.6	20.1
Total	97.1		85.5		91.2		91.7	
Carcass	8.8		15.5		16.5		8.8	
Grand total	105.9		101.0		107.7		100.5	

[a] Cumulative percentage of administered dose.
[b] Contained 85% dimethyl phosphoric acid, 14% more polar phosphates, and 1% compound VI.
[c] Contained 84% dimethyl phosphoric acid, 16% more polar phosphates.

Discussion and Conclusions

For an insecticidal phosphate to be toxic to both insects and mammals it must move from the locus of application to cholinesterase-active sites—presumably the site of toxic action—in sufficient amount to inhibit the enzyme below the level of activity necessary to sustain life. This movement involves at least the factors of penetration through cuticle or gut wall, transport to both cholinesterase and to metabolic sites, and metabolism. Generally, metabolism can result in either activation or detoxification but with compound (I) and its analogs apparently only detoxification results. Differences between insects and mammals in cholinesterase or in any of the factors involved in movement can result in selective toxicity; more accurately, the balance or net effect of all of the factors in each organism determines the toxicity of a compound. Relative safety to mammals with insecticidal phosphates has probably most often resulted from differences in metabolism; this is especially true with the thionophosphates such as malathion for which activation to the corresponding oxo compound is essential for cholinesterase inhibition. This dependence on selective metabolism as the critical factor for safety to mammals is probably the consequence of two properties of most insecticidal phosphates. First, they are relatively unselective between insect and mammalian cholinesterases. Second, most neutral phosphate esters have good solubility and partitioning properties so that penetration into, and transport within, both insects and mammals usually occurs readily. O'Brien has, indeed, concluded that "Selectivity by absorption differences offers little hope for the development of low-hazard insecticides" (*8*). The authors suggest, however, that for (I), because of its poor solubility, penetration and transport are probably the most critical factors in the safety to mammals. This conclusion results from the following considerations.

First, with respect to the ability of (I) to inhibit cholinesterase, the in vitro values with rat whole blood and human serum for (I) and (II) are similar to those for (V) and other toxic phosphates. Compound I may inhibit rat cholinesterase somewhat less effectively than other compounds but the differences do not appear large enough to account for the much lower toxicity of (I) to rats. The depression of rat cholinesterase in vivo following injection even though slow also supports the conclusion that ability to inhibit cholinesterase is not the limiting factor. This is not meant to imply that death to mammals from large dosages of compound I is the result of cholinesterase inhibition; the critical question is not how (I) kills mammals, but why it does not kill at low dosages.

Second, the rate of metabolism in mammals appears qualitatively to be similar to that of other, much more toxic vinyl phosphates and the products are relatively nontoxic to both insects and mammals. The authors plan to study both the rate and the products of metabolism in more detail, but from present data the metabolism does not appear to be unusual.

Third, the very slow, increasing inhibition of blood cholinesterase in rats following subcutaneous or even intravenous injection of (I) is in sharp contrast to the rapid, short action of DDVP and other vinyl phosphates. Clearly, the latter compounds are readily available to both cholinesterase and metabolic sites, and any material which does not inhibit cholinesterase is rapidly destroyed. Also clear is that compound I is only slowly available for reaction with cholinesterase and for metabolism. Because (I) is poorly soluble in both water and organic solvents, it appears probable that solubility properties limit the availability of (I) to mammals not only after injection but also from oral or dermal application. Metabolism certainly further reduces the concentration of (I) in blood and tissues and contributes to the low toxicity of the compound, but penetration and transport from the site of application appear to be the critical factors. Other causes of low availability of compound (I) have not been

excluded, in particular, inactivation through reversible binding to a protein (*15*).

Turning to the analogs of (I), (V) is highly toxic to mammals both orally and by skin application; obviously, penetration and transport are adequate to deliver sufficient compound to overwhelm the detoxification mechanisms and cholinesterase. compound III is toxic orally but relatively safe dermally, suggesting that cuticular penetration limits the availability of compound III. Compound II is safer than (III), slightly more toxic than (I). As usual for a diethyl analog, compound II is less soluble in water than (I) but more soluble in organic solvents; it is also more stable to hydrolysis and, presumably, to metabolism. Apparently, penetration and transport are also limiting for (II), but detoxification by metabolism is probably not so effective as with (I). Turning to the insect data, obviously (I), (II), and (III) can readily penetrate and move to the cholinesterase-active sites of a number of species of insects because of smaller size or different type of cuticle, tissues, and transport system. The lack of toxicity to aphids and mites might be associated with poor penetration and transport; differences in sensitivity of the different insect cholinesterases have, however, not been excluded.

The authors, therefore, tentatively conclude that the penetration and transport factors are critical in the low toxicity of (I) to mammals, and that poor solubility or partition properties may be the limiting factors. This same situation may apply to other insecticidal phosphates with low mammalian toxicity; the principles obviously have general applicability in the design of safe new insecticides.

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INSECTICIDE SYNTHESIS

The Synthesis and Insecticidal Properties of Some Cholinergic Trisubstituted Acetaldehyde O-(Methylcarbamoyl)oximes

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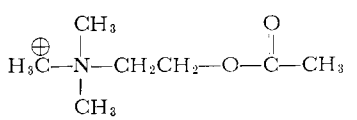
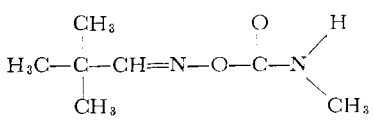
A series of trisubstituted acetaldehyde O-(methylcarbamoyl)oximes, bearing strong structural similarities to acetylcholine, has been shown to possess good insecticidal properties. The most active compounds were those in which one of the substituents was an electronegative group. 2-Methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)-oxime was the most generally effective contact and systemic insecticide.

THE intrinsic insecticidal activity of aryl carbamates generally is correlated with their ability to inhibit acetylcholinesterase (*11*). Among *O*-(methylcarbamoyl)oximes screened for pesticidal activity in these laboratories in recent years, butanone and acetone *O*-(methylcarbamoyl)oximes (I and I*a*, respectively) possessed modest insecticidal properties, although they were only weak inhibitors of fly-head cholinesterase (Table I).

In an effort to improve acetylcholinesterase inhibition, and thus possibly enhance insecticidal activity, *O*-(methylcarbamoyl)oximes were synthesized which structurally resembled acetylcholine (II), thus offering a more exacting fit for the acetylcholinesterase surface. Two model compounds were trimethylacetaldehyde *O*-(methylcarbamoyl)oxime (III) and *tert*-butyl methyl ketone *O*-(methylcarbamoyl)oxime (IV). The *tert*-butyl portion of each of these molecules is isosteric with the trimethylammonium portion of acetylcholine and



II



III

should complement the so-called anionic surface of acetylcholinesterase. In addition, the sum of the interatomic distances from quaternary carbon to carbonyl carbon in III and IV is approximately 5.6 A. while in acetylcholine, the analogous distance from quaternary nitrogen to carbonyl carbon is about 5.9 A. These steric similarities are further confirmed by an examination of molecular models of II and III. *O*-(Methylcarbamoyl)oximes of this type should, therefore, offer considerable opportunity for simultaneous interaction with the anionic and esteratic sites of acetylcholinesterase (*11, 22*).

A marked increase in insecticidal activity, accompanied by an enhanced inhibition of acetylcholinesterase, was