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INSECTICIDE SCREENING

Insecticidal Activity of Alkylthiophenyl N-Methylcarbamates

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The anticholinesterase activity and toxicity to three species of insects were compared for 30 alkylthiophenyl N-methylcarbamates substituted in the ortho-, meta-, and para- positions. Among these compounds, most of them new, are the methyl, propyl, isopropyl, butyl, iso-amyl, allyl, and propargyl phenylthioethers, and several sulfonium salts and oxidation products. Some of the thioether carbamates were highly toxic to insects, and their activities are contrasted with those previously obtained with the corresponding oxygen ethers.

THE interesting biological activities of a series of alkoxyphenyl *N*-methyl-carbamates (7) invited comparison with the related alkylthiophenyl *N*-methyl-carbamates. The latter may be expected to have a somewhat different mode of action because of the ease with which the outer octet of electrons can be expanded to a decet, thus permitting the formation in vivo of stable sulfoxide and sulfone derivatives. The first descriptions of a thioether carbamate, *m*-methylthiophenyl *N*-methylcarbamate, and its methylsulfonium salt were given by Alexander and Cope (7). The latter compound was nearly as toxic to mice as the corresponding quaternary ammonium compound (respective subcutaneous *LD*₅₀ values, 0.37 and 0.27 mg. per kg.). However, the *m*-methylthio-

phenyl *N*-methylcarbamate does not seem to have been tested biologically. Schrader (10) has described the insecticidal activity of 4-methylthiophenyl, 3-methyl-4-methylthiophenyl, and 3,5-dimethyl-4-methylthiophenyl *N*-methylcarbamates (Bayer 37344). Fukuto, Metcalf, and Winton (3) described the anticholinesterase and insecticidal properties of the three isomeric methylthiophenyl *N*-methylcarbamates and of the *d*- and *l*-isomers of 2-(*sec*-butylthio)-phenyl *N*-methyl carbamate.

The present paper reports the results of a systematic examination of the anticholinesterase and insecticidal activities of the ortho-, meta-, and para-isomers of some straight- and branched-chain alkylthiophenyl *N*-methylcarbamates and of the related allylthio- and

propargylthiophenyl *N*-methylcarbamates.

Experimental

Materials and Methods. Most of the compounds investigated were new and were characterized by carbon and hydrogen determination by C. F. Geiger, Ontario, Calif., as shown in Tables I and II and by infrared spectrophotometry. The thioether phenols (Table I) were prepared by treating 2-, 3-, or 4-hydroxy-benzenethiol, prepared by a minor modification of the method of Miller and Read (9), with a slight excess of the appropriate aliphatic bromide or iodide. The *N*-methylcarbamates were prepared from the phenols by treatment with methyl isocyanate in a pressure bottle and were recrystallized from Skellysolve B (hexane fraction) or from benzene.

Table I. Properties of Isomeric Thioether Phenyl N-Methylcarbamates

Substituent	B.P. Phenol, °C.	M.P. Carbamate, °C.	Analysis, % C=	Analysis, % H=	$I_{50}M$ Fly ChE	Relative Affinity	Musca domestica LD50, μg./G. Topical A (alone)	B (1:5 P.B.)[a]	Degree of Synergism, A/B	Culex pipiens 5-fasciatus 1. LC50, P.P.M.
		R = CH₃. Theory, C = 54.80, H = 5.62								
I o-CH₃	73-4/4 mm.	114-16	54.87	5.43	9.0×10^{-7}	222	48.5	14.0	3.4	3.9
II m-CH₃	84-7		Known		7.0×10^{-6}	29	8.5	6.5	1.3	1.5
III -CH₃	113/6 mm.	78-81	54.58	5.59	3.4×10^{-5}	6	26.5	18.5	1.4	4.3
		R = C₃H₇. Theory, C = 58.64, H = 6.71								
IV o-C₃H₇	60-3/0.7 mm.	61-3	58.33	6.32	1.8×10^{-7}	1100	20.0	6.8	2.9	0.18
V m-C₃H₇	102-4/0.5 mm.	42-5	58.54	6.67	1.1×10^{-6}	180	23.5	6.0	3.9	0.096
VI p-C₃H₇	121-8/2.5 mm.	b. 157-9/0.8	59.48	7.00	1.2×10^{-5}	17	32.0	8.0	4.0	0.41
VII o-iso-C₃H₇	83-4/4.6 mm.	65-7	58.15	6.62	1.4×10^{-7}	1420	23.0	12.3	1.9	0.20
VIII m-iso-C₃H₇	86-91/0.5 mm.	64-6	58.30	6.63	1.8×10^{-6}	110	46.5	11.0	4.2	0.13
IX p-iso-C₃H₇	150-2/13 mm.	61-3	59.29	7.03	9.0×10^{-6}	22	700	18.5	39	16.5
		R = C₄H₉. Theory, C = 60.23, H = 7.16								
X o-C₄H₉	72-8/0.4 mm.	62-4	60.36	7.34	1.6×10^{-7}	1250	34.0	7.3	4.6	0.28
XI m-C₄H₉	108-12/0.5 mm.	51-2	59.80	7.14	7.8×10^{-7}	260	25.0	6.3	4.0	0.17
XII p-C₄H₉	110-12/0.5 mm.	59-60	60.78	7.32	3.0×10^{-6}	67	27.0	9.5	2.8	1.35
		R = C₅H₁₁. Theory, C = 61.62, H = 7.55								
XIII o-iso-C₅H₁₁	84-8/0.5 mm.	b. 132-5/1.7 mm.	60.86	7.30	7.4×10^{-7}	270	330	18	18	>10
XIV m-iso-C₅H₁₁	49-55/0.75 mm.	59-61	61.55	7.64	8.8×10^{-7}	230	25	7.5	3.3	1.2
XV p-iso-C₅H₁₁	117-21/0.2 mm.	49-50	61.75	7.92	1.6×10^{-6}	125	105	17.5	6.0	5.4
		R = CH₂=CH—CH₂. Theory, C = 59.17, H = 5.87								
XVI o-CH₂=CHCH₂	56-62/0.6 mm.	65-7	59.51	6.06	2.6×10^{-7}	770	14.0	7.7	1.8	0.16
XVII m-CH₂=CHCH₂	108-10/0.35 mm.	b. 138-9/0.75	60.23	6.00	3.6×10^{-6}	56	75.0	17.5	4.3	0.19
XVIII p-CH₂=CHCH₂	124-7/2.5 mm.	73-6	58.97	5.87	6.8×10^{-6}	29	13.0	10.3	1.2	0.14
		R = CH≡CCH₂. Theory, C = 59.70, H = 5.01								
XIX o-CH≡CCH₂	72-6/0.75 mm.	b. 109-12/0.8 mm.	59.73	4.92	3.4×10^{-7}	590	46.0	13.0	3.5	1.3
XX m-CH≡CCH₂	110-16/0.8 mm.	54-8	59.77	5.02	2.4×10^{-6}	83	12.7	9.5	5.3	0.12
XXI p-CH≡CCH₂	115-17/0.5 mm.	88-90	60.01	4.86	1.7×10^{-5}	12	15.0	11.0	1.4	0.35

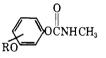[a] Piperonyl butoxide synergist.

The techniques for the determination of the inhibition of fly head cholinesterase and the insecticidal activities to the female housefly (*Musca domestica*), the mosquito larva (*Culex pipiens quinquefasciatus*), and the salt marsh caterpillar larva (*Estigmene acrea*) have been described (6, 7, 8).

Discussion of Results

Anticholinesterase Activity. The molar concentration for 50% inhibition of fly head cholinesterase (I_{50}) for the compounds studied is given in Table I along with the affinity for the enzyme [affinity = I_{50} for phenyl N-methylcarbamate ($2 \times 10^{-4}M$)/I_{50} for substituted phenyl N-methylcarbamate]. A comparision of these data with those previously presented for the related alkoxyphenyl N-methylcarbamates (7, 8) shows that the corresponding thioether carbamates are of substantially higher anticholinesterase activity and that the effects of position isomerism about the phenyl ring are less critical. It would appear that these two differences are due to the larger size of the S atom, van der Waals' radius 1.85 A. as compared to 1.40 A. for the O atom, and the expansibility of the valence shell of the sulfur atom to accommodate a decet

Table II. Biological Activities of Alkoxyphenyl N-Methylcarbamates for Comparison with Table I

	R =	Fly ChE $I_{50}M$	Relative Affinity	Musca domestica LD50, μg./G. Alone	1:5 P.B.[a]	Culex pipiens 5-fasciatus LC50, P.P.M.
XXII	o-CH₃	3.7×10^{-5}	1.4	92.5	18	>10
XXIII	m-CH₃	2.2×10^{-5}	14	90	14.5	10
XXIV	p-CH₃	8.0×10^{-5}	2.0	500	38.5	20
XXV	o-C₃H₇	8.7×10^{-6}	23	105	13.5	2
XXVI	m-C₃H₇	1.6×10^{-5}	12.4	95	15.5	0.9
XXVII	p-C₃H₇[b]	1.1×10^{-4}	1.2	>500	257	>10
XXVIII	o-iso-C₃H₇	6.9×10^{-7}	290	25.5	7	0.3
XXIX	m-iso-C₃H₇	9.2×10^{-6}	22	180	19.5	3
XXX	p-iso-C₃H₇	8.8×10^{-5}	2.3	>500	340	>10
XXXI	o-C₄H₉	1.2×10^{-5}	16.5	175	15.5	5
XXXII	m-C₄H₉	9.4×10^{-6}	21.0	280	17.5	0.3
XXXIII	p-C₄H₉[c]	2.0×10^{-5}	10.0	>500	235	>10
XXXIV	o-CH≡C—CH₂[d]	2.9×10^{-6}	69	6.5	4.6	0.8
XXXV	m-CH≡C—CH₂[d]	4.0×10^{-6}	50	7.5	6.0	0.54
XXXVI	p-CH≡C—CH₂[e]	6.2×10^{-5}	3.1	>500	33.5	>10

[a] Piperonyl butoxide synergist.
[b] New compound m.p. 81-4°. Theory C = 63.14%, H = 7.23%; found C = 62.45%, H = 7.08%.
[c] New compound m.p. 91-2°. Theory C = 64.55%, H = 7.68%; found C = 64.20%, H = 7.88%.
[d] Courtesy of Hercules Powder Co., Wilmington, Del., XXXIV(H-9699), m.p. 84-5°; XXXV(H 9064), m.p. 71-3°.
[e] New compound m.p. 119-22°. Theory C = 64.38%, H = 5.43%; found C = 64.69%, H = 5.27%.

of electrons (as in the oxidation of sulfide to sulfone or in the sulfonium salts) in contrast to the octet of electrons which can be accommodated in the outer shell of oxygen (2).

The alkyl thioether carbamates illustrate the same general relationships of structure to anticholinesterase action as found in the alkyl ether carbamates (8)—i.e., the essentiality of a structure complementary to the active site of the cholinesterase molecule and incorporating the critical distance of about 5A. between the carbonyl atom and the center of the group interacting with the anionic site.

From studies of Fischer-Hirschfelder molecular models and plaster casts of the model of acetylcholine in its extended configuration it appears that maximum fit of the carbamates with the active site of the enzyme occurs when the sulfur atom is in the ortho position of the aromatic ring. This is in agreement with the data of Table I. The most active anticholinesterases were the o-propylthio-(IV), o-isopropylthio-(VII), and o-butylthiophenyl N-methylcarbamates (X). As previously observed with the alkoxyphenyl N-methylcarbamates, the activity in the ortho-substituted compounds increased with chain branching in the order methyl<propyl<butyl<isopropyl<sec-butyl (3). This appears to represent the effect of increasing van der Waals' dispersion forces between the anionic site of cholinesterase and the methyl (or methylene groups) on the substituent of the aromatic ring of the carbamate (8). However, for the thioethers the rate of change of affinity with increasing size of substituent was significantly less than for the oxyethers. For example, in the oxygen series the affinity ratios from methyl to isopropyl to sec-butyl were 5.4 to 290 to 650 and in the sulfur series the values are 222 to 1420 to 1800.

The differences in affinity among ortho-, meta-, and para- isomers decrease from 40- to 100-fold with methyl and propyl to 20-fold with butyl and to only twofold with isoamyl. From observations with molecular models as mentioned above, it appears that the longer butyl and isoamyl chains can bend readily around the large sulfur atom and this allows the ends to interact with the anionic site regardless of the point of attachment to the aromatic ring.

The generally enhanced affinity of the thioether carbamates over the oxyether carbamates (Tables I and II) is especially noteworthy and this difference ranges to as much as 200-fold for comparable isomers. It is difficult to explain this ambiguity except in terms of a specific binding between the sulfur atom of the thioether group and the anionic site of cholinesterase. This enhanced binding may in some way be related to the 3d orbitals of the sulfur atom (2).

Table III. Biological Properties of Other Sulfur-Containing Phenyl N-Methylcarbamates

	Carbamate	M.P. °C.	Analysis, % Theory	Analysis, % Found	I_{50} M Fly ChE	Relative Affinity	Musca domestica LD_{50}, μg./G. A(alone)	Musca domestica LD_{50}, μg./G. B(1:5 P.B.)[a]	Degree of Synergism, A/B	Culex pipiens 5-fasciatus LC_{50}, P.P.M.
XXXVII	p-CH₃SOC₆H₄OC(O)NHCH₃	110–13	C = 50.70 H = 4.93	C = 50.84 H = 5.66	1.6×10^{-5}	12.5	105	15.5	6.8	>10
XXXVIII	p-CH₃SO₂C₆H₄OC(O)NHCH₃	122–24.5	C = 47.16 H = 4.81	C = 47.41 H = 5.23	1.0×10^{-4}	2.0	>500	>500	1.0	>10
XXXIX	CH₃SC₆H₄OC(O)N(CH₃)₂	57–9	C = 57.94 H = 6.32	C = 57.36 H = 6.43	4.5×10^{-4}	0.45	170	57	3.0	>10
XL	4-CH₃S,2-CH₃C₆H₃OC(O)NHCH₃	63–5	C = 56.84 H = 6.63	C = 56.81 H = 6.31	1.75×10^{-5}	11.5	28.0	11.5	2.4	2.0
XLI	4-CH₃S,3-CH₃C₆H₃OC(O)NHCH₃	73–5	C = 56.84 H = 6.63	C = 56.61 H = 6.63	1.3×10^{6}	154	10.7	7.0	1.6	0.45
XLII	2-CH₃S,4-CH₃C₆H₃OC(O)NHCH₃	96–7.5	C = 56.84 H = 6.63	C = 56.58 H = 6.45	1.9×10^{-6}	105	>500	16.5	>30	3.8
XLIII	2-CH₃S,4-CH₃OC(O)NHCH₃	103–5	C = 52.84 H = 5.77	C = 53.16 H = 5.93	1.4×10^{-6}	143	>500	42	>12	3.6
XLIV	3-CH₃S,4-CH₃OC₆H₄OC(O)NHCH₃	160–2	C = 52.84 H = 5.77	C = 53.11 H = 6.16	2.6×10^{-6}	77	>500	330	>1.5	8.1

[a] Piperonyl butoxide synergist.

Insecticidal Activity. The insecticidal activity of the alkylthiophenyl N-methylcarbamates appears to be more complex than the anticholinesterase action. This is apparently due to the action of detoxication systems, as shown by the remarkable uniformity of the toxicity value to the housefly obtained with synergism by piperonyl butoxide (Table I). Piperonyl butoxide is known to block the detoxication mechanism of the carbamates, so that the synergized toxicity is believed to represent more nearly the intrinsic toxicity of the compound (4).

The nature of the aliphatic portion of the thioether has an important bearing upon the relative toxicity of the various isomers to the housefly. Thus for the methyl thioethers, the meta- isomer was most active, for the propyl and isopropyl thioethers the ortho-isomer was most active, for the butyl and isoamyl thioethers, the meta-isomer was most active, and for the allyl thioether the para-isomer was most active. Properties of individual compounds which deserve especial comment include: the outstanding toxicity of m-methylthiophenyl N-methylcarbamate (II) to the housefly, the high toxicity of m-propyl (V) and m-isopropyl thiophenyl N-methylcarbamate (VIII) to the mosquito larva, and the high toxicity of p-allyl (XVIII) and p-propargylthiophenyl N-methylcarbamate (XXI) to the housefly. The m-propargylthiophenyl N-methylcarbamate (XX) was of uniquely high activity to both fly and mosquito larva. The p-methylthiophenyl N,N-dimethylcarbamate (XXXIX, Table III) was about 0.14 as toxic as the monomethylcarbamate (III).

The contrast between the insecticidal activities of the alkylthio- and alkoxyphenyl N-methylcarbamates is particularly interesting (Tables I and II). The greatest variation is found in the para-substituted compounds where p-methylthio- (III), p-propylthio- (VI), p-butylthio- (XII), and p-propargylthio- (XXI) are at least 20 times as toxic to the housefly as the corresponding methoxy- (XXIV), propoxy- (XXVII), butoxy- (XXXIII), and propargyloxy- (XXXVI) phenyl N-methylcarbamates. A similar disparity in toxicity is found to Culex larvae. However, in contrast, the o- (XXXIV) and m-propargyloxyphenyl (XXXV) N-methylcarbamates were the most toxic to the housefly of the compounds investigated and were synergized only slightly, indicating very little enzymatic detoxication compared to their thio analogs (XIX and XX).

The generally increased insecticidal activity of the thioether carbamates over the corresponding alkoxy compounds corresponds to the much higher activity of the former as anticholinesterases. The only suggestion which can be advanced is that the sulfur compounds have a

higher attractivity to the anionic site of cholinesterase through the sulfur atom itself. A possible suggestion may lie in the ability of the thioether compounds to form sulfonium salts which contain a formal positive charge, in vivo within the insect tissues. This suggestion is attractive in view of the high anticholinesterase activity of the methyl sulfonium salt of m-methylthiophenyl N-methylcarbamate (Table IV).

The compounds listed in Table I were evaluated for toxicity to the third instar salt marsh caterpillar. The thioether carbamates were not outstandingly effective against this species. However, compounds II, IV, VII, VIII, XV, XVI, XVIII, and XIX had LC_{50} values of 3 to 10 mg. per 8.5 cm. of cotton leaf disk, or somewhat less than that of the comparable alkoxyethers (7).

Oxidation of Methylthio to Sulfoxide and Sulfone. The effects of the oxidation of the sulfur atom in p-methylthiophenyl N-methylcarbamate to give the p-methylsulfinylphenyl and p-methylsulfonylphenyl N-methylcarbamates are shown in Table III. The sulfoxide (XXXVII) appears just slightly better as an anticholinesterase, but the sulfone (XXXVIII) is less active. Both oxidation products are less toxic to Musca and to Culex larva. These results are in general agreement with those previously obtained with 4-methylthio-3,5-xylenyl N-methylcarbamate and its oxidation products (6), where the sulfoxide was of decreased activity and the sulfone of markedly less activity. This trend is in accord with the known inductive and mesomeric effects of the para-substituents upon the electron density about the carbonyl carbon which are quantitatively represented by the Hammett sigma values: $CH_3S = -0.047$, $CH_3SO = 0.567$, and $CH_3SO_2 = 1.049$. Therefore, only the CH_3S group is electron-donating and may be expected to form the most stable carbamate ester. As was previously concluded (6), the overall steric configuration of the carbamate esters is of such major importance that the electronic effects are less decisive in determining biological activity than they are in the corresponding organophosphorus anticholinesterases. This

would seem to be the predominant reason why the known sequence of in vivo biological oxidations sulfide→ sulfoxide→sulfone does not result in complete inactivity for the alkylthiophenyl N-methylcarbamates. Further study of the biological activity of related sulfoxides and sulfones is clearly warranted.

Effect of Second Ring Substituent on Activity of Methylthiophenyl N-Methylcarbamates. The effect of the incorporation of the 4-methylthio- group in the 3,5-xylenyl N-methylcarbamate has been shown to increase the affinity for ChE about five-fold, the toxicity to Musca two-fold and to Culex larva about 10-fold (6). Additional effects of this kind are shown in Table III, where the methylthio group is combined with methyl or methoxy. The most active compounds were obtained with substitution of 4-CH_3S into the 3-methylphenyl N-methylcarbamate (XLI), which increased the affinity for ChE about 10-fold and the toxicity to Musca about five-fold and to Culex 22-fold. Substitution of 4-CH_3S into the 2-methylphenyl N-methylcarbamate (XL) increased the affinity for ChE eight-fold and the toxicity to Musca about 18-fold and to Culex more than five-fold. Substitution of CH_3S into the 2-position of 4-methylphenyl N-methylcarbamate (XLII) increased the affinity for ChE about 50-fold but had little effect on toxicity. Similar results were obtained with 2-CH_3S substituted into 4-methoxyphenyl N-methylcarbamate (XLIII) which increased the affinity for ChE about 70-fold, increased the toxicity to Culex about six-fold, but had no effect on toxicity to Musca. The 3-methylthio-4-methoxyphenyl N-methylcarbamate (XLIV) had an affinity for ChE about seven-fold that of 3,4-dimethoxyphenyl N-methylcarbamate but like the latter was a poor toxicant (7).

Methyl Sulfonium Salts. The sulfur atoms of the alkyl thioethers readily form methylsulfonium salts when treated with dimethyl sulfate. These are water-soluble and strong electrolytes and are analogous to the quaternary ammonium salts of the prostigmine type which are used medicinally. The formal positive

Table IV. Comparison of Biological Activities of Sulfonium and Ammonium Phenyl N-Methylcarbamates

Phenyl N-Methylcarbamate		I_{50}ChE, M	Affinity	LD₅₀ Musca, μg./G.	LC₅₀ Culex, P.P.M.
XLV	o-Methylthio methosulfate	1.5×10^{-5}	13	>50ᵃ	>10
XLVI	o-Dimethylamino methiodide	1.0×10^{-5}	20	>50	>10
XLVII	m-Methylthio methosulfate	6.5×10^{-7}	310	>50	>10
XLVIII	m-Dimethylamino methiodide	1.8×10^{-8}	11,000	>50	>10
XLIX	p-Methylthio methosulfate	1.1×10^{-5}	18	>50	>10
L	p-Dimethylamino methiodide	3.5×10^{-6}	57	>50	>10

ᵃ Limit of solubility in acetone-water mixture.

charge on the sulfonium salt may be expected to increase the affinity for cholinesterase by coulombic attraction to the negatively charged anionic site. However, as with the quaternary ammonium salts, position isomerism is of overriding importance, as is shown in Table IV. In both the thioether and amine series, the most active quaternary compounds are those with the charged atom in the meta-position of the ring. The attraction of the ammonium nitrogen to the anionic site is considerably stronger than that of the sulfonium sulfur. It is of particular interest to compare the affinities of the sulfonium compounds in Table IV with the uncharged methylthioethers of Table I. It is apparent that quaternization decreases the affinity of the ortho-isomer (XLV) to about 0.05, increases the affinity of the meta-isomer (XLVII) by about 10 times, and increases the affinity of the para-isomer (XLIX) by about 3

times. These affects are qualitatively similar to those observed with quaternization of the uncharged dimethylaminophenyl N-methyl carbamates (8): The ortho-isomer (XLVI) decreased to about 0.2, meta-isomer (XLVIII) increased by about 130-fold, and para-isomer (L) increased about 68-fold.

As has been observed with the quaternary ammonium carbamates (5), the presence of a formal positive charge in the sulfonium carbamates effectively destroys the contact toxicity to *Musca* and *Culex*, presumably because of the inability of the charged molecules to penetrate into the nerve synapse.

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INSECTICIDE METABOLISM

Thin-Layer Chromatography and Cholinesterase Detection of Several Phosphorothiono Insecticides and Their Oxygen Analogs

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Partition thin-layer chromatography techniques have been developed to separate and detect phosphorothionate, phosphorodithioate, and phosphoramidothioate insecticides and their corresponding oxygen analogs. Cellulose layers on chromatoplates are coated with polar and apolar stationary phases and developed with immiscible mobile phase solvents. Different chromatographic systems reverse the order of mobility of the compounds and their oxons. Chromogenic agents detect as little as 0.1 to 0.5 μg. of the compounds tested. A cholinesterase spray method on the intact cellulose layers detects anticholinesterases at the nanogram level or below. The weak cholinesterase inhibitors are also detected by prior conversion to their oxons by suitable oxidation techniques.

ORGANOPHOSPHORUS insecticides containing the thiono sulfur group (P=S) are known to be converted to their corresponding oxygen analogs or oxons (P=O) in biological systems. the latter having greatly enhanced cholinesterase-inhibiting properties (6, 11). Since the oxons are the major anticholinesterase metabolites of this class of compounds and are thought to be primarily responsible for the toxic action, considerable interest has centered on formation and accumulation of these metabolites in animals and plants. The oxons are

generally much more susceptible to hydrolytic degradation than their parent compounds and, probably for that reason, do not usually accumulate to any great extent in biological systems. Sensitive techniques of separation and detection therefore greatly simplify the study of these metabolites. Paper chromatography, combined with direct cholinesterase detection. to detect inhibitors was first reported by Cook (1), who studied activation products of several organophosphorus pesticides. The method was later used to identify anticholinesterases produced in mammals and insects (7, 8, 12).

The present study was concerned with developing thin-layer chromatographic

(TLC) methods for separating several phosphorothionate, $(RO)_2P(S)OX$, phosphorodithioate, $(RO)_2P(S)SX$, and phosphoramidothioate, $(RO)(RNH)P(S)OX$, insecticides from their corresponding oxons and for detecting the inhibitors on the intact plates by the cholinesterase detection method. The advantages of thin-layer chromatography over paper systems are much greater sensitivity and resolution of mixtures, rapid separation, and the ability to separate greater quantities of material, which make this technique preferable in a study of the metabolism of organophosphorus compounds in insects and other organisms.

Several workers have developed TLC methods for organophosphorus insec-

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