steric inhibition may be due to the bulkiness of the enzyme molecule. Electric eel cholinesterase is reported to have a molecular weight of approximately three million (*10*). Since VIII with a nitro group ortho to the phosphorus moiety showed the highest anticholinesterase activity, and since the 1-naphthyl series was generally lower in activity than the 2-naphthyl series, the bulk of the interference may be due to the adjacent second aromatic ring.

Evidence for the enhancement of cholinesterase inhibition by steric effects is also evident in the high activity of diethyl 4-nitro-2-naphthyl phosphate (X). Table III shows that this compound has an unusually high energy of activation and, therefore, less reactive ester linkage. Its high K_t value at 37.5° C. must then be attributed to steric attraction as is evident by high $\Delta S^{\ddagger}$ value. It is possible that the nitro group meta to the diethyl phosphoryloxy moiety facilitates inhibition by binding to the enzyme surface prior to phosphorylation.

Hydrolysis and biological data for a number of diethyl dinitronaphthyl phosphates are also included in Table I. These were considerably less stable to aqueous hydrolysis than the mononitro compounds. The higher reactivity of the P—O—naphthyl bond here is also reflected in their higher rates of cholinesterase inhibition. The data in Table I showed no obvious correlation between hydrolysis and inhibition rates, however. None of the dinitro compounds showed any toxicity to the female housefly. This is to be expected in view of the instability of these compounds in water, and they are probably hydrolyzed before reaching any vital center.

The extremely wide variability in toxicity of the diethyl mononitronaphthyl phosphates was surprising and unexpected. The LD_{50} values in μg. per gram to the female housefly ranged from 2.5 to >500. The data in Table II show that there is no direct correlation between toxicity and anticholinesterase activity. The most toxic compound in the series is the 6-nitro-2-naphthyl phosphate (XII), a moderate cholinesterase inhibitor. The 2-naphthyl phosphates were generally more toxic than the 1-naphthyl phosphates. Although compounds in the 2-naphthyl series were generally better cholinesterase inhibitors than those in the 1-naphthyl series, it is unlikely that the large differences in toxicity can be accounted for by differences in anticholinesterase activity alone. Because of the consistently higher toxicity of compounds in the 2-naphthyl series, these are apparently detoxified at a slower rate than those in the 1-naphthyl series. Terriere *et al.* (*2, 14*) have shown that both naphthalene and 1-naphthol rapidly converted via hydroxylation to a number of metabolites in house flies.

The outstanding toxicity of XII warranted further investigation, and the corresponding phosphorothionate analog was synthesized and examined for toxicity since in many instances the thionate esters show superior insecticidal properties than the corresponding phosphates. *O,O*-Diethyl 6-nitro-2-naphthyl phosphorothioate was slightly less toxic to houseflies than XII with an LD_{50} of 4.5 μg. per gram, but is toxic enough to deserve further attention as a potential insecticide.

Literature Cited

(1) Aldridge, W. N., Davison, A. N., *Biochem. J.* **51,** 62–70 (1952).
(2) Arias, R. O., Terriere, L. C., *J. Econ. Entomol.* **55,** 925–9 (1962).
(3) Berliner, E., Winicov, E. H., *J. Am. Chem. Soc.* **81,** 1630–5 (1959).
(4) Bruice, T. C., Schmir, G. L., *Ibid.*, **79,** 1663–7 (1957).
(5) Bryson, A., *Ibid.*, **82,** 4862–71 (1960).
(6) Dewar, M. J. S., Grisdale, P. J., *Ibid.*, **84,** 3548–53 (1962).
(7) Fukuto, T. R., Metcalf, R. L., *Ibid.*, **81,** 372–7 (1959).
(8) Fukuto, T. R., Metcalf, R. L., J. Agr. Food Chem. **4,** 930–5 (1956).
(9) Hudson, R. F., Green, M., *Ange. Chem. Intern. Ed. Engl.* **2,** 11–20 (1963).
(10) Laidler, K. J., "The Chemical Kinetics of Enzyme Action," Oxford University Press, London, England, 1958.
(11) Metcalf, R. L., March, R. B., *J. Econ. Entomol.* **46,** 288–94 (1953).
(12) Pauling, L., Wheland, G. W., *J. Chem. Physics* **1,** 362–74 (1933).
(13) Schrader, G., British Intelligence Objectives Subcommittee Final Report No. **1808,** 32 (1947).
(14) Terriere, L. C., Boose, R. B., Roubal, W. T. *Biochem. J.* **79,** 620–3 (1961).
(15) Wells, P. R., Ward, E. R., *Chem. Ind.* **1958,** pp. 528–9.

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INSECTICIDE ACTIVITY AND STRUCTURE

Para-Substituted *Meta*-Xylenyl Diethyl Phosphates and *N*-Methylcarbamates as Anticholinesterases and Insecticides

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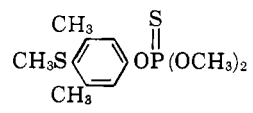
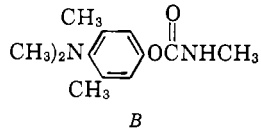
Previous studies from this laboratory have sought to account quantitatively for the wide variation in insecticidal activity exhibited by a variety of monosubstituted phenyl diethyl phosphates. This has been related to the electron density about the reactive phosphorus atom which undergoes a bimolecular reaction with cholinesterase enzyme (*7, 14*). The insecticidal and anticholinesterase activity of the monosubstituted phenyl *N*-methylcarbamates has also been investigated (*12, 13, 16*). However, these carbamates are competitive rather than irreversible inhibitors of cholinesterase, and steric factors play a dominant role in determining their activity. Nevertheless, the electronic properties of the molecule as influenced by various substituent groups have an important effect on activity by influencing the electron density around the carbonyl carbon and thus affecting hydrolytic stability and turnover number with the enzyme.

More recently, the importance of ring alkylation in producing compounds with especially useful and unique insecticidal properties, has been demonstrated with both dialkyl phosphates and *N*-methylcarbamates of various phenols. Examples are *O,O*-dimethyl *O*-4-methylthio - 3,5 - xylenyl phosphorothionate (Compound 37342, Farbenfabriken Bayer) (*A*) and 4-dimethylamino-3,5-xylenyl *N*-methylcarbamate (Zectran, Trademark, Dow Chemical Co.) (*B*).



A



B

Therefore the anticholinesterase activity and insecticidal action of the diethyl phosphates and *N*-methylcarbamates of a series of 4-substituted xylenols were investigated. The influence of the *m*-methyl groups upon the resonance of

A total of 26 para-substituted *meta*-xylenyl diethyl phosphates and *N*-methylcarbamates, most of them new, have been investigated as anticholinesterases and insecticides. The activities of these compounds varied over a considerable range, depending upon the mesomeric effects of the para substituent and the steric interference of the *meta*-methyl groups with the resonance of the para substituent. The biological behavior of these compounds was compared with that of the corresponding para-substituted phenyl esters, and the results are discussed in terms of physical-organic theory.

various para substituents was expected to provide an interesting comparison with the behavior of the para-substituted phenyl compounds of both series previously studied (*7, 12*).

Experimental

Materials and Methods. The new compounds prepared and evaluated in this paper are given in Table I. The various 4-substituted xylenols were obtained from commercial sources or prepared as follows: 4-nitroso-3,5-xylenol, m.p. 179°–80° C. (*2, 3*); 4-nitro-3,5-xylenol, m.p. 103°–6° C. (*1*); 4-cyano-3,5-xylenol, m.p. 177° C. (*22*); 4-thiocyano-3,5-xylenol, m.p. 124°–7° C. (*10*); and 4-methoxy-3,5-xylenol, m.p. 84°–5° C. (*5*). The 4-methylsulfinyl-3,5-xylenol and 4-methylsulfonyl-3,5-xylenol were obtained through the courtesy of Dan MacDougall of the Chemagro Corp.

The diethyl phosphates listed in Table I were prepared as described by Fukuto and Metcalf (*7*) by reacting diethyl phosphorochloridate with the sodium salt of the xylenol. The products were washed with sodium hydroxide solution to remove possible traces of contaminating tetraethyl pyrophosphate and were distilled on the falling-film molecular still.

The *N*-methylcarbamates listed in Table II were prepared by reacting the xylenols with an excess of 50% methyl isocyanate in toluene containing a few drops of triethylamine, followed by heating in a pressure bottle to 100° C. The compounds were recrystallized from Skellysolve B, hexane fraction. Two of these carbamates (XIII) and (XXIII) were obtained from commercial sources as listed and were purified by recrystallizations.

To obtain suitable comparisons between the 4-substituted 3,5-xylenyl diethyl phosphates and the corresponding 4-substituted phenyl diethyl phosphates, *p*-dimethylaminophenyl diethyl phosphate (XXVII) was prepared by the same general method as described above, and its properties are listed in Table III. This table also contains information on the properties of two 4-substituted phenyl *N*-methylcarbamates not previously recorded—the 4-cyano compound (XXVI) and the 4-methylthio compound (XXV). The latter was previously mentioned by Schrader (*19*), who reported the m.p. as 88° C. Microanalyses were by C. F. Geiger, Ontario, Calif.

The methods for the determination of the I_{50} value for the inhibition of housefly-head cholinesterase and for the bimolecular rate constant K_e in liters per mole per minute, have been described previously (*8, 12*). K_e values for some of the 4-substituted phenyl diethyl phosphates, as plotted in Figure 1, are given by Benjamini *et al.* (*4*), and new values have been determined for the following compounds previously described (*7*): phenyl, 2.59×10^1; *p*-chlorophenyl, 2.3×10^3; *p*-tolyl, 2.0×10^1; *p*-methoxyphenyl, 2.95×10^1; and *p*-cyanophenyl, 2.58×10^5 liters per mole per minute. I_{50} values for the 4-substituted phenyl *N*-methylcarbamates as plotted in Figure 2 are taken from Kolbezen *et al.* (*12*) and Metcalf *et al.* (*16*).

The tropical LD_{50} values to the female, susceptible housefly *Musca domestica* and the LC_{50} values for the larvae of the mosquito *Culex pipiens quinquefasciatus* were determined as previously described (*13*). For the carbamates, the LD_{50} to the housefly was also determined for a mixture of the carbamate with five parts by weight of the synergist piperonyl butoxide (Tables II and III). This procedure has been shown by Fukuto *et al.* (*9*) to retard detoxication greatly and to give a better picture of the intrinsic toxicity of the compound.

Table I. Properties of 4-Substituted 3,5-Xylenyl Diethyl Phosphates

	Substituent	Boiling Point, °C.	N_D^{28}	Analysis Calcd.	Analysis Found	Cholinesterase I_{50}	Cholinesterase K_e	LD_{50} Musca, μg./Gram	LC_{50} Culex, P.P.M.
I	CH_3S	140–1/0.2	1.5119	C 51.31 H 6.95	C 51.56 H 7.37	8.2×10^{-6}	6.02×10^3	3.25	0.89
II	CH_3SO	158–62/0.5	1.5112	C 48.74 H 6.61	C 48.96 H 6.61	5.6×10^{-7}	1.51×10^5	5.5	0.62
III	CH_3SO_2	182–4/0.1 m.p. 48°–52°	...	C 46.42 H 6.30	C 45.86 H 6.52	4.6×10^{-8}	1.10×10^6	6.0	3.8
IV	O_2N	127–30/0.1	1.4905	C 47.51 H 5.98	C 48.05 H 6.75	6.8×10^{-8}	6.55×10^5	60	4.45
V	ON	dec.	1.5171	C 50.16 H 6.32	C 50.41 H 7.07	1.8×10^{-8}	6.6×10^6	>500	3.2
VI	CN	130–1/0.5 m.p. 35°–7°		C 55.11 H 6.40	C 55.33 H 7.17	1.8×10^{-7}	2.75×10^5	39.5	3.85
VII	CNS	160/0.5	1.5232	C 49.52 H 5.76	C 48.52 H 5.72	4.6×10^{-6}	7.70×10^5	100	2.3
VIII	CH_3	118–20/0.2	1.4873	C 57.34 H 7.81	C 56.78 H 8.31	$>1.0 \times 10^{-3}$	1.90×10^1	>500	>10
IX	CH_3O	128–130/0.2	1.4823	C 54.15 H 7.34	C 54.54 H 7.95	1.6×10^{-5}	6.84×10^2	>500	>10
X	Cl	121–2/0.2	1.4939	C 49.21 H 6.20	C 49.34 H 6.27	6.0×10^{-6}	1.19×10^4	>500	>10
XI	$(CH_3)_2N$	120–6/0.05	1.4895	C 55.80 H 8.03	C 55.87 H 8.08	8.2×10^{-7}	7.99×10^4	135	>10
XII	H	117–18/0.4	1.4784	C 55.80 H 7.42	C 55.37 H 7.61	4.0×10^{-3}	4.11×10^1	>500	>10

Curve fitting of the points in Figures 1 and 2 was accomplished by the method of least squares using an IBM 1620 computer, programed by M. J. Garber. In calculating these lines, the few points whose position is doubtful because of uncertain sigma values (see Discussion) were eliminated.

Discussion of Results

Xylenyl Diethyl Phosphates. Inspection of the data in Table I shows that the biological activity of the xylenyl diethyl phosphates is markedly affected by the polar character of the para substituent and ranges over approximately 10^3-fold for LD_{50} to the housefly and 10^6-fold for inactivation of fly cholinesterase. As might be predicted from previous studies (*7*, *14*), para substituents with strongly electron-withdrawing properties—i.e., NO_2 (IV), NO (V), CN (VI), CH_3SO (II), and CH_3SO_2 (III)—produce the most active compounds as a consequence of their enhancement of the electrophilic nature of the phosphorus atom. The CH_3S group (I), which is the precursor of the methylsulfinyl and methylsulfonyl groups in biological systems (*4*), is a special case, and its toxicity is due to in vivo oxidation. The relationship between the polar character of the para substituent and the K_e for the reaction with cholinesterase can be quantitatively expressed by the Hammett sigma-rho treatment (*10*) as shown in Figure 1, where:

$$\log k/k_0 = \rho\sigma$$

k_0 and k being the bimolecular rate constants for reaction with cholinesterase of the unsubstituted and substituted reactants. The substituent constant σ defines the magnitude of the electronic effect of the para substituent and is independent of the reaction, while the reaction constant ρ characterizes the nature of the reaction and is constant for all substituents. In Figure 1, ρ is the slope of the line fitted by the method of least squares to all the compounds in Table I except XI, whose position is ambiguous as will be discussed later. The fitted line is in close approximation to most of the points, supporting the now well-validated contention that these phosphate anticholinesterases act by the simple bimolecular reaction with the enzyme where the phosphorus atom makes an electrophilic attack on a nucleophilic center, probably a cyclic nitrogen, at the esteratic site. Figure 1 also contains the points for the comparable series of para-substituted phenyl diethyl phosphates (*7*, *14*), which fall about a line virtually indistinguishable from that of the xylenyl diethyl phosphates. The computed line for the xylenyl esters has the equation $y = 3.301\ (\pm 0.585)\ x - 0.1$ (where $y = \log K_e$ and x = sigma value) and for the phenyl esters $y = 3.970\ (\pm 0.451)\ x - 1.67$, where $y = \log K_e$ for reaction with cholinesterase and x = sigma value for phenyl substituents. Thus there is no statistical difference between the ρ values for the two sets of compounds, and the reaction of the electrophilic phosphorus

Table II. Properties of 4-Substituted 3,5-Xylenyl *N*-Methylcarbamates

	Substituent	Melting Point, °C.	Analysis Calcd.	Analysis Found	Cholinesterase, I_{50}	LD_{50} Musca, µg./Gram Alone	LD_{50} Musca, µg./Gram 1:5 P.B.[a]	LC_{50} Culex, P.P.M.
XIII	CH_3S	118–120	Compound known[b]		1.2×10^{-6}	24.0	12.5	0.23
XIV	CH_3SO	133–4	C 54.75 H 6.27	C 54.85 H 6.17	1.8×10^{-6}	410	16.0	4.7
XV	CH_3SO_2	157–63	C 51.34 H 5.88	C 51.11 H 5.69	2.1×10^{-5}	>500	36.5	>10
XVI	O_2N	107–8	C 53.56 H 5.39	C 53.77 H 5.21	4.2×10^{-6}	>500	23.8	>10
XVII	ON	142–3	C 57.68 H 5.81	C 57.27 H 5.64	8.3×10^{-5}	>500	>500	>10
XVIII	CN	133–5	C 64.68 H 5.92	C 64.82 H 5.53	4.8×10^{-5}	>500	14.3	>10
XIX	CNS	151–3	C 55.91 H 5.12	C 55.64 H 5.12	8.0×10^{-7}	>500	>500	0.50
XX	CH_3	117–19	C 68.37 H 5.28	C 68.20 H 7.26	1.9×10^{-6}	65.0	13.5	0.28
XXI	CH_3O	105–7	C 62.96 H 6.98	C 63.14 H 7.23	1.1×10^{-6}	35.5	7.8	0.29
XXII	Cl	116–17	Compound known[c]		2.1×10^{-6}	>500	31.0	0.89
XXIII	$(CH_3)_2N$	85	Compound known[d]		3.3×10^{-6}	60.0	13.5	0.49
XXIV	H	100.5–102	Compound known[e]		6.0×10^{-6}	60.0	16.5	3.0

[a] Piperonyl butoxide synergist. [b] Bayer 37344. [c] Reference *13*. [d] Zectran, Dow Chem. Co. [e] Reference *12*.

Table III. Properties of Several Phenyl Esters

	Compound	M.P. or B.P., °C	Analysis Calcd.	Analysis Found	Cholinesterase I_{50}	Cholinesterase K_e	LD_{50} Musca, µg./Gram Alone	LD_{50} Musca, µg./Gram 1:5 P.B.[a]	LC_{50} Culex, P.P.M.
XXV	p-$CH_3SC_6H_4OCNHCH_3$ (O)	78–81	C 54.80 H 5.62	C 54.58 H 5.59	3.4×10^{-5}	...	26.5	18.5	>10
XXVI	p-$CNC_6H_4OCNHCH_3$ (O)	128–30	C 61.35 H 4.58	C 61.75 H 4.50	1.7×10^{-4}	...	>500	>500	>10
XXVII	p-$(CH_3)_2NC_6H_4OP(OC_2H_5)_2$ (O)	b.p. 126–8 /0.1 mm.	C 52.74 H 7.38	C 52.75 H 7.60	2.4×10^{-7}	5.39×10^4	22.5		>10

[a] Piperonyl butoxide synergist.

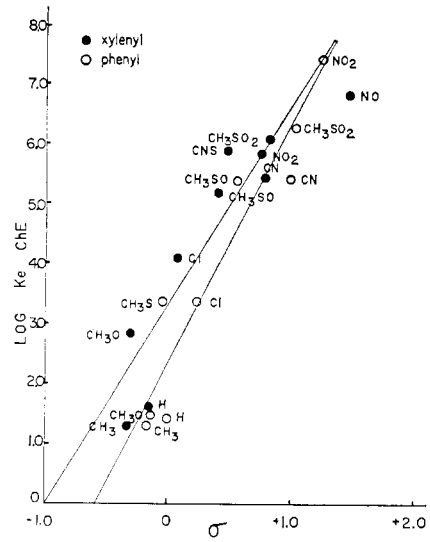


Figure 1. Relationship of log bimolecular rate constant (K_e) for inhibition of fly-head cholinesterase, to sigma value for *p*-substituted phenyl and xylenyl diethyl phosphates.

Plotted lines were calculated by method of least squares

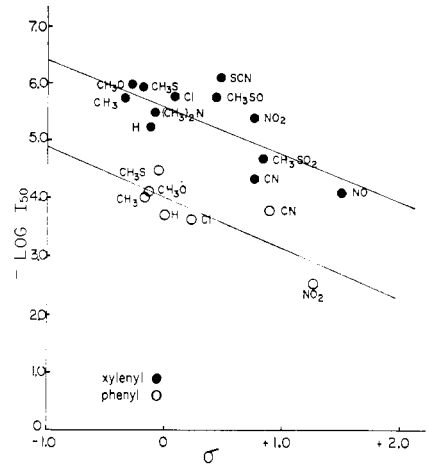


Figure 2. Relationship of $-\log I_{50}$ for inhibition of fly-head cholinesterase to sigma value for *p*-substituted phenyl and xylenyl *N*-methylcarbamates

Plotted lines were calculated by method of least squares

atom with cholinesterase is only slightly influenced by the steric properties of the *m*-methyl groups which are important in the reactivity of competitive inhibitors (*16*). The ρ values for the two series of compounds fall within the same range as that of 4.058 ± 0.178 calculated by Jaffé (*10*) from the data of Aldridge and Davison for the reaction between substituted phenyl diethyl phosphates and erythrocyte cholinesterase. This suggests the essential similarity of various types of cholinesterase.

Resonance Effects and Additive Nature of σ Values. The data in Table I on the multisubstituted xylenyl diethyl phosphates provide an interesting opportunity to investigate the interaction of the para substituents with the neighboring *m*-methyl groups. It is generally believed, from limited data, that sigma values for substituents in the 3-, 4-, and 5-positions of the aromatic system are additive (*10*) or that log $(k/k_0) = \rho \Sigma \sigma$. This, of course, cannot be true if the neighboring substituents undergo resonance or steric interaction. Figure 1 shows that in the xylenyl diethyl phosphates the effect of the *p*-nitro group is relatively much weaker than the effect of this group in the phenyl diethyl phosphates. In the former series, the most active compound is that of the CH_3SO_2 (III) substituent, while in the later series it is the *p*-NO_2 substituent. Wheland *et al.* (*22*) showed that the nitro group in 4-nitro-3,5-xylenol is relatively much less effective in electron-withdrawal (measured $\sigma = 0.77$) than that to be expected by summation of values ($\sigma = 1.17$). These same investigators found no significant difference between the measured and additive sigma values for 4-cyano-3,5-xylenol ($\sigma = 0.79$). These results were ascribed to the necessity for coplanarity between the nitro group and the benzene ring in order for maximum resonance to occur. The presence of the two *m*-methyl groups twists the nitro group out of the plane of the ring by rotation about the carbon-nitrogen bond. With the 4-cyano-3,5-xylenol, however, resonance is not inhibited since the linear cyano group cannot be twisted out of the plane of the ring. Kloosterziel and Backer (*11*) demonstrated that the methylsulfonyl group which exerts a strong negative mesomeric effect through expansion of its octet of electrons to a decet structure, does not require a coplanar configuration with the benzene nucleus to exert maximum resonance. Sigma values for meta-substituted compounds incorporating this group are therefore additive and the measured value for 4-methylsulfonyl-3,5-xylenol was $\sigma = +0.83$. Similar situations exist with the CH_3SO, NO, Cl, CH_3, CH_3O, and CH_3S groups. In summary then, the decreased reactivity of compound IV as compared to III and VI is explainable in terms of resonance inhibition between the 3-, 4-, and 5-substituents and the net effect of this inhibition upon electron-withdrawal from the reactive phosphorus atom.

The data in Figures 1 and 2 were plotted using the observed sigma values given above and for other substituents the additive values between the 3,5-xylenyl group $\sigma = -0.15$ and para substituents as given by Jaffé (*10*) or in more recent treatments by Fickling *et al.* (*6*) *p*-CH_3O ($\sigma = -0.135$), *p*-NO ($\sigma = -1.629$), and Szmant and Suld (*20*) *p*-SCN ($\sigma = +0.60$).

The situation with regard to the substantially reactive 4-dimethylamino-3,5-xylenyl diethyl phosphate (XI) is even more interesting. The dimethylamino-group ($\sigma = -0.600$) is normally strongly electron-donating, and the additive value in the 3,5-xylenyl should be $\sigma = -0.75$, which should result in a most unreactive compound (compare compounds VIII and IX). Taft (*21*) has calculated that in ethyl 3,5-dimethyl-4-dimethylaminobenzoate the steric inhibition of the resonance of the 4-dimethylamino group is nearly complete and the observed $\sigma = -0.11$. However, observed sigma values for the dimethylamino group cover a wide range, indicating that the resonance effects are variable. The theoretical value obtained from Figure 1 is $\sigma = +0.46$. This suggests that at the pH used in the determination of anticholinesterase activity (pH = 7.4) the dimethylamino group must be largely protonated to form the corresponding onium compound ($\sigma = +0.859$). The additive sigma value for this combination with the 3,5-xylenyl group is $\sigma = +0.709$, which approaches the observed value.

Measurement of the dissociation of compound XI showed that the pK_b is 10.5 and that the compound is completely dissociated below pH 8.5.

Insecticidal Activity of Xylenyl Phosphates. The xylenyl dialkyl phosphorothionates offer very interesting possibilities as insecticides. The compound *O,O*-dimethyl 4-methylithio-3,5-xylenyl phosphorothionate was described by Schrader (*19*) as having high insecticidal activity and low mammalian toxicity with an oral LD_{50} to the rat of >1000 mg. per kg. as compared to 10 mg. per kg. for the *O,O*-dimethyl 4-methylthiophenyl phosphorothionate. However, the authors' topical LD_{50} values to the female housefly show 4.3 mg. per kg. for the xylenyl ester as compared to 2.0 for the phenyl ester. This is an extraordinary degree of insecticidal specificity. Schrader (*19*) states that the corresponding *O,O*-dimethyl *O*-4-nitro-3,5-xylenyl phosphorothionate is about 0.001 as active as *O,O*-dimethyl *O*-4-nitrophenyl phosphorothionate (methyl parathion). From previous studies with *O,O*-diethyl *O*-4-methylthiophenyl phosphorothionate (*4*) and with fenthion or *O,O*-dimethyl *O*-4-methylthio-3-methylphenyl phosphorothionate (*15*), the electron-donating methylthio group ($\sigma = -0.047$) undergoes biological oxidation to the methylsulfinyl ($\sigma = +0.567$) and methylsulfonyl groups ($\sigma = +1.049$), which are electron-withdrawing and produce a highly electrophilic phosphorus atom (*17*). This "lethal synthesis" is undoubtedly the explanation of the high insect toxicity of compound I of Table I, which apparently has the optimum

stability to reach the site of action while its less stable oxidation products II and III are subject to destructive hydrolysis while in transport to this site. Compound V with a 4-nitroso group is the most reactive in the series, but apparently is too unstable to be useful as an insecticide. The 4-cyano (VI) and 4-nitro (IV) compounds are also quite active as insecticides. Although the latter substituent has considerably stronger electron-withdrawing properties, the influence of the two adjacent methyl groups upon the mesomerism of the nitro group greatly curtails its activity, as described previously, while the cyano group is not affected in this manner because of its planar orientation. Thus, in general, the authors conclude that for the *O,O*-diethyl *O*-4-substituted 3,5-xylenyl phosphates insecticidal activity and inherent reactivity are closely related, subjected to supplementary biochemical reactions which occur in the environment of the insect.

The notable lack of toxicity of the *p*-thiocyano compound (VII) is probably attributable to its in vivo instability as compounds of this type have been shown to liberate cyanide ion in animal tissues as the result of enzymatic action. The related *O,O*-diethyl *O*-*p*-thiocyanophenyl phosphorothionate is weakly insecticidal (*18*).

Xylenyl *N*-Methylcarbamates. The biological activity of the xylenyl *N*-methylcarbamates given in Table II invites comparison with the data for the xylenyl diethylphosphates shown in Table I. Previous work from this laboratory (*12*, *16*) has shown that the aryl *N*-methylcarbamates are competitive inhibitors of cholinesterase, and recent unpublished kinetic studies have established conclusively that these compounds behave as relatively stable substrates for fly cholinesterase with very low turnover numbers. The authors have shown that steric factors involving the interaction of Van der Waals' forces at the anionic site of cholinesterase greatly influence the magnitude of this competitive inhibition (*16*). Thus, *m*-methylphenyl *N*-methylcarbamate had an affinity for cholinesterase of about 14 times that of the unsubstituted compound. The carbamate with two meta-methyl groups, 3,5-xylenyl-*N*-methylcarbamate (XXIV), had an affinity for fly cholinesterase of 33 times that of the unsubstituted compound and was the most active member of the series of dimethylphenyl (xylenyl) *N*-methylcarbamates (*9*).

Since para substitution of phenyl *N*-methylcarbamates with small groups had little effect on affinity for cholinesterase (*16*), the distribution of activities shown by the para-substituted 3,5-xylenyl *N*-methylcarbamates of Table II must be due to the inductive and mesomeric effects of the substituent upon the electron density at the carbonyl carbon and the consequence of these upon the stability of the carbamate as a substrate for cholinesterase. Table II shows that anti cholinesterase activity and toxicity are substantially affected by the nature of the para substituent. The former varied over a 100-fold and the latter a 25-fold range. Those with strong electron-withdrawing properties—i.e., NO_2 (XVI), NO (XVII), CN (XVIII), and CH_3SO_2 (XV)—produced the least active compounds, while the electron-donating substituents, CH_3 (XX) and CH_3O (XXI), produced the most active compounds. This effect is opposite in character from that described above for the para-substituted 3,5-xylenyl diethyl phosphates, and this is clearly shown when the activity as $-\log I_{50}$ is plotted against the sigma values for the substituent as in Figure 2. The line fitted by the method of least squares has a negative slope and fits the equation $y = -0.832 \pm (0.256)x + 6.41$, as contrasted with the positive slope of 3.301 for the corresponding xylenyl diethyl phosphates. Figure 2 also contains the points for the companion series of para-substituted phenyl *N*-methylcarbamates (*12*, *16*). These clearly fall on a line which is quite distinct from that of the xylenyl *N*-methylcarbamates and whose equation is: $y = -0.889 (\pm 0.287)x + 4.89$. The average of 33-fold greater affinity for cholinesterase shown by the xylenyl *N*-methylcarbamates over the phenyl *N*-methylcarbamates at equivalent sigma values (Figure 2) suggests that steric attraction to the anionic site is considerably more important in determining the anticholinesterase activity of the substituted phenyl *N*-methylcarbamates than inductive or mesomeric effects affecting electron density around the carbonyl carbon. The behavior of the carbamates as shown in Figure 2 is therefore distinctly different from the phosphates in Figure 1 and provides further proof that the anticholinesterase activity of the carbamates is due to competitive inhibition, while that of the phosphates is due to chemical reactivity with the esteratic site.

Insecticidal Activity of Xylenyl Carbamates. Several of the xylenyl *N*-methylcarbamates listed in Table II have practical possibilities as insecticides. 3,5-Xylenyl *N*-methylcarbamate (XXIV) is highly active and was first described by this laboratory in 1954 (*12*). The 4-dimethylamino-3,5-xylenyl *N*-methylcarbamate (XXIII) is a well known commercial insecticide (Zectran, Dow Chemical Co.) and is extremely active against lepidopterous larvae, snails, and many other pests. The compound 4-methylthio-3,5-xylenyl *N*-methylcarbamate (XIII) (Bayer 39007) is being developed on a wide scale as an agricultural insecticide. This compound like the corresponding *O,O*-diethylphosphate (I) is oxidized to the methylsulfinyl (XIV) and methylsulfonyl (XV) derivatives in vivo. However, the toxicity data suggest that such oxidations do not occur rapidly enough to destroy its insecticidal effectiveness. It seems logical that in both I and XIII the adjacent methyl groups decrease the rate of oxidation of the sulfur atom through steric hindrance, over that observed for the unhindered compound (*4*). The other xylenyl carbamates with 4-substituents which are electron-donating—e.g., 4-CH_3 (XX), 4-CH_3O (XXI), and to a lesser extent 4-Cl (XXII)—have interesting insecticidal properties and deserve further investigation.

Attention should be called to the marked enhancement of toxicity to the housefly that occurs when these carbamates are synergized with 5 parts of piperonyl butoxide (Table II). This indicates that the 3,5-xylenyl *N*-methylcarbamates, like most other compounds of this class, are rapidly detoxified by enzymatic action in the body of the housefly (*9*).

Literature Cited

(1) Adams, R., Stewart, H., *J. Am. Chem. Soc.* **63,** 2859 (1941).
(2) Albert, H. E., *Ibid.*, **76,** 4985–8 (1954).
(3) Auers, V. K., Borsche, E., *Ber.* **48,** 1698–1716 (1915).
(4) Benjamini, E., Metcalf, R. L., Fukuto, T. R., *J. Econ. Entomol.* **52,** 94–8 (1959).
(5) Bruice, T. C., Kharasch, N., Winzler, R. J., *J. Org. Chem.* **18,** 83–91 (1953).
(6) Fickling, M. M., Fischer, A., Mann, B. R., Packer, J., Vaughan, J., *J. Am. Chem. Soc.* **81,** 4226–30 (1959).
(7) Fukuto, T. R., Metcalf, R. L., *J. Agr. Food Chem.* **4,** 930–5 (1956).
(8) Fukuto, T. R., Metcalf, R. L., *J. Am. Chem. Soc.* **81,** 372–7 (1959).
(9) Fukuto, T. R., Metcalf, R. L., Winton, M. Y., Roberts, P. A., *J. Econ. Entomol.* **55,** 341–5 (1962).
(10) Jaffé, H. H., *Chem. Rev.* **53,** 191–261 (1953).
(11) Kloosterziel, H., Backer, H., *Rec. Trav. Chim.* **72,** 185–94 (1953).
(12) Kolbezen, M. M., Metcalf, R. L., Fukuto, T. R., J. Agr. Food Chem. **2,** 864–70 (1954).
(13) Metcalf, R. L., Fuertes-Polo, C., Fukuto, T. R., *J. Econ. Entomol.* **56,** 862–4 (1963).
(14) Metcalf, R. L., Fukuto, T. R., *Ibid.*, **55,** 340–1 (1962).
(15) Metcalf, R. L., Fukuto, T. R., *Bull. World Health Organ.* **29,** 219–26 (1963).
(16) Metcalf, R. L., Fukuto, T. R., Winton, M. Y., *J. Econ. Entomol.* **55,** 889–94 (1962).
(17) Meyers, C., Lombardini, G., Bonoli, L., *J. Am. Chem. Soc.* **84,** 4603–4 (1962).
(18) Paikin, D. M., Shabanova, M. P., Gamper, N. M., Efimova, L. F., *Khim. i Primenenie Fosfororgen. Soedin. Akad. Nauk S.S.S.R. Kazansk. Filial, Tr. I Konf.* **1957,** pp. 408–19.

(19) Schrader, G., *Angew. Chemie* **73,** 331–4 (1961).
(20) Szmant, H. H., Suld, G., *J. Am. Chem. Soc.* **78,** 3400–3 (1956).
(21) Taft, R., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., p. 581, Wiley, New York, 1956.
(22) Wheland, G., Brownell, R., Mayo, E., *J. Am. Chem. Soc.* **70,** 2492–5 (1948).

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INSECTICIDE LABELING

A Simple Technique for Tritiation of Aromatic Insecticides

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The tritiating technique of Yavorsky and Gorin has been used to label aromatic insecticides, and the degree of labeling, which varies greatly with compounds, has been tentatively accounted for. The method is simple and requires little space and no expensive reagents or apparatus. The cost per labeled compound is low, on the average $7.00 for 100 to 200 mg. of labeled compound. Activities up to 15.5 mc. per mmole were obtained.

YAVORSKY and Gorin (*11, 12*) have shown that simple aromatic hydrocarbons can be tritiated by direct contact with the tritiating reagent $H_2TPO_4 \cdot BF_3$. Exchange is effected primarily at aromatic sites, although there is some evidence that exchange also takes place very slowly at a tertiary carbon. The method is simple, does not involve the handling of tritium gas, does not produce large quantities of unwanted side-products as in the Wilzbach technique, and requires no elaborate precautions. It uses a relatively inexpensive source of tritium—tritiated water.

The authors felt that this technique might be applicable to the labeling of biologically active compounds providing the method, which was originally applied to 70-gram quantities, could be scaled down sufficiently, and provided that reactive compounds could tolerate the conditions. Accordingly, the tritiation of two chlorinated hydrocarbons and several carbamates and phosphates was attempted in the hope that perhaps some general rule might be formulated by which the degree of labeling for different compounds could be predicted.

Methods

Approximately 180 mg. of phosphoric anhydride (P_2O_5), calculated as a small excess over the theoretical 0.0012 mole —i.e., 171 mg.—were weighed quickly into a 4-dram vial (Kimble Opticlear 60975-L), and the polyethylene top was snapped into place to prevent reaction of moisture from the air with the P_2O_5. The vial was cooled in an ice-water or dry ice–acetone mixture, and 65 μl.(0.0036 mole) of tritiated water (specific activity 1 mc. per μl.) were added with a disposable micropipet. The top was quickly replaced and the vial rotated slowly until the water had taken up all the P_2O_5. When the vial reached room temperature, a $^3/_8$-inch, Teflon-coated magnetic stirrer bar was used to stir the sirupy mixture rapidly for 5 minutes to ensure complete reaction of the water with the P_2O_5. An $^1/_8$-inch air space was left between the vial and the stirrer, otherwise heat from the stirrer influenced the reaction.

While stirring was continued, boron trifluoride was introduced at a rate of one bubble per second for about 7 minutes until the tritiated H_3PO_4 became thoroughly saturated (Figure 1). Although stoichiometrically about 300 bubbles of BF_3 were required, some was lost because of the poor physical conditions of the mixing. The reagent $H_3{}^*PO_4 \cdot BF_3$ is a heavy, colorless liquid. The cap was replaced and stirring continued for about 5 minutes more. Two-hundred milligrams of the compound to be labeled was dissolved in 3 to 5 ml. of cyclohexane (if the compound was apolar) and added to the tritiating reagent. Cyclohexane (Matheson, Coleman and Bell No. 2825) was satisfactory without sodium treatment. The cap was again replaced and stirring continued for 20 hours. Polar compounds were rinsed into the tritiating mixture directly with a few milliliters of cyclohexane, and appeared to dissolve in the reagent mixture; the apolar compounds did not so dissolve.

After the reaction was complete, the contents of the vial were transferred to a 100-ml. separatory funnel. The vial was rinsed two or three times, first with cyclohexane, then with water, and finally with chloroform if the compound was not very soluble in cyclohexane. The rinses were transferred to the separatory funnel; the solvent layer was washed four times with an equal volume of water and dried over anhydrous sodium sulfate for 24 hours. After filtration, the solvent was removed by rotary evaporation. The residue was taken up in a few milliliters of the appropriate solvent for chromatographic separation. If very polar compounds were to be labeled, alternative procedures would be required.

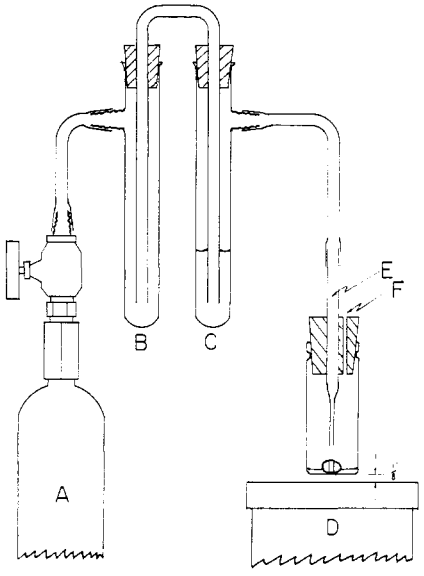

Figure 1. Apparatus for semimicro tritiation

(A) Lecture bottle of BF_3, *(B)* safety trap, *(C)* trap with concd. H_2SO_4 for washing BF_3, *(D)* magnetic stirrer, *(E)* disposable pipet, *(F)* cork slotted to vent excess BF_3

For labeling at elevated temperatures, the stoppered vial was placed in a 75-mm. porcelain evaporating dish containing paraffin oil and a thermometer, and the reaction was carried out over a magnetic stirrer-hot plate combination. A trial was made to calibrate the temperature