Influence of the Sulfur Atom on the Anticholinesterase and Insecticidal Properties of Thioether *N*-Methylcarbamates

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Thirty-three new thioether *N*-methylcarbamates were characterized as inhibitors of housefly cholinesterase and for their toxicity to the housefly and the *Culex* mosquito larva. The activity of the position isomers of the alkylthiophenyl *N*-methylcarbamates is in the order ortho > meta >para. The ortho-isomers show a slight and the para-isomers a well defined maximum of anticholinesterase activity at C_4-C_5. The meta-isomers exhibit alternate peaks of activity. Activity of the para-substituted benzylthiophenyl *N*-methylcarbamates is directly proportional to the electron-withdrawing capacity of the substituent. The isomeric benzothienyl *N*-methylcarbamates are of activity similar to anticholinesterases but are detoxified by mixed-function oxidases at greatly different rates. The results demonstrate the dominant attraction of the sulfur atom to the anionic site of cholinesterase as the major factor in cholinesterase inhibition by these thioether carbamates.

This paper represents a continuation of study of the interaction between phenyl *N*-methylcarbamates and housefly cholinesterase (ChE) and of the susceptibility of these compounds to detoxication by the mixed-function oxidases of the housefly (Metcalf and Fukuto, 1965). Substitution of the phenyl nucleus by a thioether moiety markedly enhanced the affinity of the carbamate for the enzyme and the I_{50} values for C_1-C_4 alkylthiophenyl *N*-methylcarbamates ranged from 0.1 to 0.005 those of the corresponding alkoxyphenyl compounds (Metcalf *et al.*, 1965). Similarly, Durden and Weiden (1969) found that the sulfide sulfur atoms in 2-(methylcarbamoyloxyphenyl)-dithiolanes were responsible for at least a 10-fold increase in anticholinesterase activity, compared to the corresponding dioxolane analogs. This enhanced activity was attributed to the effect of the S atoms in increasing the reversible complexing of the carbamate with cholinesterase.

The causes of this extraordinary effectiveness of the sulfide-containing phenyl *N*-methylcarbamates are poorly understood, although they may relate to the capacity of the S atom to assume a partial positive charge. This should result in additional coulombic attraction of the properly placed S atom to the anionic site of ChE. As an example of the effects of the charged sulfur, the positively charged *m*-methylsulfonium phenyl *N*-methylcarbamate had an I_{50} for fly ChE of 6.5×10^{-7} *M*, compared to 7.0×10^{-6}*M* for the uncharged *m*-methylthiophenyl *N*-methylcarbamate (Metcalf *et al.*, 1965).

The present investigation was planned to yield additional information about the interaction of S-containing carbamates with housefly ChE in regard to a long-chain alkylthiophenyl *N*-methylcarbamates which should provide steric and hydrophobic interactions with the anionic site of ChE and its surrounding lipophilic pool, the polarization of the S atom in substituted benzylthiophenyl *N*-methylcarbamates, and the effects of fusing the S atom into the benzothienyl ring. These structural variations were also evaluated for insecticidal activity and for susceptibility to detoxication by mixed-function oxidases of the housefly.

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MATERIALS AND METHODS

The alkylthiophenols (Table I) were prepared from 2-, 3-, or 4-hydroxybenzenethiol, by minor modifications of the method of Miller and Read (1933) by refluxing a slight excess of the appropriate alkyl halide with the sodium salt of the hydroxybenzenethiol, under nitrogen. The alkyl thioether phenols, whose melting or boiling points are given in Table I, were converted to the *N*-methylcarbamates (Table I) by reaction with a slight excess of methyl isocyanate in toluene or diethyl ether solution containing a few drops of triethylamine catalyst. The crystalline carbamates were recrystallized to constant melting point from hexane, ethanol, ether, or a mixture of benzene and hexane. The liquid carbamates were purified by column chromatography on magnesium silicate (Florisil) and eluted with 3 to 1 ether-hexane and with ether. Purity was confirmed by thin-layer chromatography on silica gel (Absorbosil 1 with 10% calcium sulfate) using ether-hexane (3 to 1), and by infrared spectrometry. Despite repeated attempts, we were unable to prepare *p*-heptyl- or *p*-nonylthiophenyl *N*-methylcarbamates with the proper elemental analyses.

The benzylthiophenols (Table I) were prepared in an analogous way by refluxing the appropriate benzyl chloride with 2-, 3-, or 4-hydroxybenzenethiol in methanolic sodium hydroxide. With the *p*-chlorobenzyl and *p*-nitrobenzyl chlorides there was substantial formation of ether-insoluble, high melting side products which caused difficulties in purification. The *N*-methylcarbamates of Table I were prepared by reaction with methyl isocyanate as described above. Despite repeated efforts, *o*-chlorobenzylthiophenyl *N*-methylcarbamate could not be prepared with a satisfactory elemental analysis.

The 4,4′-dihydroxydiphenyl sulfide (m.p. 148–50°) was prepared by adding sulfur dichloride to phenol in dry toluene at 0° C. The bisphenol was converted to 4,4′-bis-(*N*-methylcarbamyloxy)-diphenyl sulfide by reacting with an excess of methyl isocyanate in diethyl ether. The biscarbamate (m.p. 170–72°) was obtained in 77% yield: theory C = 57.83, H = 4.81; found C = 57.61, H = 5.00.

The 4,4′-dihydroxydiphenyl disulfide (m.p. 148–50°) was prepared in 85% yield by air oxidation of *p*-hydroxybenzenethiol in equimolar quantities of sodium hydroxide in methanol at reflux. The bisphenol was converted to 4,4′-bis-(*N*-methylcarbamyloxy)diphenyl disulfide by treating with an excess of methyl isocyanate in toluene. The carbamate

Table I. Properties of Substituted Phenyl *N*-Methylcarbamates

Substituent	B.P., Phenol, °C.	M.P., Carbamate, °C.	Analysis, % Theory	Found
p-C_2H_5S	142–5/0.4	76–80	C = 56.87	C = 56.69
			H = 6.16	H = 6.27
p-$C_5H_{11}S$	M. 52–6	36–8	C = 61.66	C = 61.50
			H = 7.51	H = 7.22
o-$C_6H_{13}S$	118–20/1.5	48–50	C = 62.74	C = 62.50
			H = 7.86	H = 7.74
m-$C_6H_{13}S$	125–34/0.4	55–7	C = 62.74	C = 62.36
			H = 7.86	H = 8.42
p-$C_6H_{13}S$	M. 58–60	43–5	C = 62.74	C = 62.80
			H = 7.86	H = 7.59
o-$C_7H_{15}S$	130–40/1.4	71–4	C = 64.08	C = 63.69
			H = 8.18	H = 7.91
m-$C_7H_{15}S$	120–30/0.4	72–4	C = 64.08	C = 64.39
			H = 8.8	H = 8.53
o-$C_8H_{17}S$	142–6/1.5	59–62	C = 65.76	C = 65.36
			H = 8.47	H = 8.64
m-C_8-$H_{17}S$	130–5/0.4	78–80	C = 65.76	C = 65.51
			H = 8.47	H = 8.31
p-$C_8H_{17}S$	M. 70–2	46–80	C = 65.76	C = 65.55
			H = 8.47	H = 8.74
o-Iso-$C_8H_{17}S$	120–6/0.4	n_D^{28} 1.5205	C = 65.76	C = 65.65
			H = 8.47	H = 8.56
m-Iso-$C_8H_{17}S$	140–5/0.3	n_D^{28} 1.5222	C = 65.76	C = 63.75
			H = 8.47	H = 7.64
p-Iso-$C_8H_{17}S$	125–35/0.4	n_D^{28} 1.5320	C = 65.76	C = 64.61
			H = 8.47	H = 8.17
o-$C_9H_{19}S$	136–40/0.4	78–80	C = 66.01	C = 66.37
			H = 8.73	H = 8.71
m-$C_9H_{19}S$	155–65/0.4	70–2	C = 66.01	C = 66.44
			H = 8.73	H = 9.23
o-$C_{10}H_{21}S$	140–4/0.4	79–82	C = 66.87	C = 66.81
			H = 8.98	H = 8.90
m-$C_{10}H_{21}S$	160–70/0.4	85–8	C = 66.87	C = 66.70
			H = 8.98	H = 9.08
p-$C_{10}H_{21}S$	M. 83–5	57–9	C = 66.87	C = 66.27
			H = 8.98	H = 9.15
o-$C_6H_5CH_2S$	125–35/0.6	103–4	C = 64.98	C = 64.99
			H = 5.49	H = 5.61
m-$C_6H_5CH_2S$	175–85/0.6	106–8	C = 64.98	C = 65.10
			H = 5.49	H = 5.62
p-$C_6H_5CH_2S$	180–90/0.5 M. 100–2	110–11	C = 64.98	C = 65.20
			H = 5.49	H = 5.64
o-$CH_3C_6H_5CH_2S$	130–40/0.4	124–5	C = 65.98	C = 67.32
			H = 5.84	H = 6.06
m-$CH_3C_6H_5CH_2S$	170–8/0.6	110–12	C = 65.98	C = 67.01
			H = 5.84	H = 6.06
p-$CH_3C_6H_5CH_2S$	M. 105–7	132–3	C = 65.98	C = 66.06
			H = 5.84	H = 6.04
o-$CH_3OC_6H_5CH_2S$	160–70/2	133–5	C = 62.95	C = 63.08
			H = 5.57	H = 5.64
m-$CH_3OC_6H_5CH_2S$	M. 100–1	99–103	C = 62.95	C = 64.06
			H = 5.57	H = 6.04
p-$CH_3OC_6H_5CH_2S$	M. 128–30	122–5	C = 62.95	C = 63.16
			H = 5.57	H = 5.51
m-$ClC_6H_4CH_2S$	150–60/0.6	94–5	C = 57.78	C = 57.03
			H = 4.49	H = 5.32
p-$ClC_6H_4CH_2S$	M. 90–2	122–4	C = 57.78	C = 57.30
			H = 4.49	H = 4.68
o-$NO_2C_6H_4CH_2S$	M. 74–6	117–19	C = 55.90	C = 56.45
			H = 4.35	H = 4.24
m-$NO_2C_6H_4CH_2S$	M. 87–90	102–4	C = 55.90	C = 56.12
			H = 4.35	H = 4.93
p-$NO_2C_6H_4CH_2S$	M. 115–25	144–6	C = 55.90	C = 56.14
			H = 4.35	H = 4.51

(m.p. 179–82°) was obtained in 70% yield: theory C = 52.20, H = 4.67; found C = 52.42, H = 4.88.

The 3-hydroxybenzo-[*b*]-thiophene (m.p. 58–62°) was synthesized by the method of Smiles and McClelland (1921) as modified by Craik and Macbeth (1925). It was converted to 3-benzothienyl *N*-methylcarbamate in the usual way and recrystallized from ethanol (m.p. 125–30°): theory C = 57.95, H = 4.38; found C = 58.32, H = 4.12. Kilsheimer *et al.* (1969) give a melting point of 134°.

The 4-benzothienyl *N*-methylcarbamate (m.p. 119–24°) was purified from the insecticide Mobam by recrystallization in ethanol. Kilsheimer *et al.* (1969) give a melting point of 129°.

The 7-hydroxybenzo-[*b*]-thiophene (m.p. 68.5–70.0°) (Mobil

Table II. Biological Properties of New Alkylthiophenyl *N*-Methylcarbamates

Substituent	$I_{50}M$ Fly ChE	Relative Affinity	*Musca domestica*, LD_{50}, μg./G. A (alone)	B (1:5 PB)	SR Value	*Culex pipiens 5-fasciatus* LC_{50}, P.P.M.
I *p*-C_2H_5S	5.6×10^{-5}	4	20.5	7.3	2.8	4.3
II *p*-$C_5H_{11}S$	1.8×10^{-6}	110	108	30	3.6	2.5
III *o*-$C_6H_{13}S$	2.0×10^{-7}	1000	460	20	2.3	0.54
IV *m*-$C_6H_{13}S$	6.0×10^{-4}	<1.0	>500	500	>1.0	>10
V *p*-$C_6H_{13}S$	4.0×10^{-6}	50	210	50	4.2	3.8
VI *o*-$C_7H_{15}S$	2.1×10^{-7}	954	>500	44	>11	1.0
VII *m*-$C_7H_{15}S$	9.4×10^{-7}	212	>500	143	>3.5	4.3
VIII *o*-$C_8H_{17}S$	4.0×10^{-7}	500	>500	>500	1.0	>10
IX *m*-$C_8H_{17}S$	1.2×10^{-6}	150	>500	>500	1.0	>10
X *p*-$C_8H_{17}S$	1.3×10^{-5}	15	>500	>500	1.0	>10
XI *o*-$C_9H_{19}S$	3.6×10^{-7}	556	>500	>500	1.0	>10
XII *m*-$C_9H_{19}S$	2.9×10^{-7}	690	>500	>500	1.0	>10
XIII *o*-$C_{10}H_{21}S$	5.2×10^{-6}	38	>500	>500	1.0	>10
XIV *m*-$C_{10}H_{21}S$	7.0×10^{-6}	29	>500	>500	1.0	>10
XV *p*-$C_{10}H_{21}S$	$>1\times10^{-4}$	<1.0	>500	500	1.0	>10
XVI *o*-iso-$C_8H_{17}S$	2.4×10^{-7}	833	>500	47	>10	4.6
XVII *m*-iso-$C_8H_{17}S$	1.5×10^{-6}	133	>500	320	>1.5	>10
XVIII *p*-iso-$C_8H_{17}S$	1.3×10^{-5}	15	215	25	8.6	4.2

Table III. Biological Properties of Para-Substituted Benzylthiophenyl *N*-Methylcarbamates

R =	$L_{50}M$ Fly ChE	Relative Affinity	*Musca domestica*, LD_{50} μg./G. A (alone)	B (1:5 PB)	SR Value	*Culex pipiens 5-fasciatus* LC_{50}, P.P.M.
XIX *o*-H	2.2×10^{-7}	909	>500	31	>16	>10
XX *m*-H	7.4×10^{-7}	273	>500	16	>31	7
XXI *p*-H	1.3×10^{-6}	154	>500	13	>39	0.27
XXII *o*-CH_3	2.8×10^{-7}	715	>500	33	>15	>10
XXIII *m*-CH_3	2.0×10^{-6}	100	>500	100	>5	>10
XXIV *p*-CH_3	1.9×10^{-6}	105	>500	125	>4	>10
XXV *o*-CH_3O	6.2×10^{-7}	323	>500	>500	1.0	>10
XXVI *m*-CH_3O	2.0×10^{-6}	100	>500	>500	1.0	8.4
XXVII *p*-CH_3O	4.4×10^{-6}	45.5	>500	>500	1.0	>10
XXVIII *m*-Cl	3.7×10^{-4}	<1.0	>500	>500	1.0	>10
XXIX *p*-Cl	4.3×10^{-7}	465	>500	3	>166	0.52
XXX *o*-NO_2	1.0×10^{-7}	2000	>500	29.5	>17	3
XXXI *m*-NO_2	2.1×10^{-7}	954	>500	25	>20	0.46
XXXII *p*-NO_2	2.0×10^{-7}	1000	>500	25	>20	0.22

Table IV. Biological Properties of Benzothienyl *N*-Methylcarbamates and Carbaryl

N-Methylcarbamate	$L_{50}M$ Fly ChE	Relative Affinity	*Musca domestica*, LD_{50}, μg./G. A (alone)	B (1:5 PB)	SR Value	*Culex pipiens 5-fasciatus* CL_{50}, P.P.M.
XXXIII 3-Benzothienyl	2.4×10^{-6}	83	500	27.5	18	4.60
XXIV 4-Benzothienyl	2.5×10^{-7}	800	18.5	8	2.3	0.58
XXXV 7-Benzothienyl	3.0×10^{-7}	667	120	15	8	1.1

Oil Co.) was converted to 7-benzothienyl *N*-methylcarbamate in the usual way and recrystallized from cyclohexane (m.p. 134–38°). Kilsheimer *et al.* (1969) give a melting point of 139°.

The molar concentration of carbamate reducing the housefly head cholinesterase activity to half its normal velocity (I_{50}) was determined by the manometric method in which inhibitor in acetone solution reacted with enzyme for 15 minutes at 37.5° C. prior to addition of acetylcholine at a final concentration of 0.02*M*, and the CO_2 liberated from 0.025 *M* sodium bicarbonate was measured over an additional 30-minute period. The "relative affinity" for the enzyme was determined by comparison of I_{50} of phenyl *N*-methylcarbamate/I_{50} substituted phenyl *N*-methylcarbamate (Metcalf and Fukuto, 1967).

Topical LD_{50} values for the compounds alone and together with 5 parts of piperonyl butoxide synergist were determined by application of 1-μl. drops of a range of acetone solutions (w./v.) of the compounds to the pronota of 2- to 4-day-old female S_{NAIDM} houseflies. The ratio LD_{50} alone to synergized LD_{50} gives the synergistic ratios (SR values) of Tables II, III, and IV. The LC_{50} values for larvae of *Culex pipiens quinquefasciatus* were determined similarly by adding acetone solutions (w./v.) to 100 ml. of water containing the 4th instar larvae (Metcalf and Fukuto, 1967).

DISCUSSION OF RESULTS

Alkylthiophenyl *N*-Methylcarbamates. The data in Tables I and II indicate the properties of the new isomeric alkylthiophenyl *N*-methylcarbamates prepared in this study, together with their anticholinesterase and insecticidal activity. Comparable data on the isomeric methyl, propyl, isopropyl,

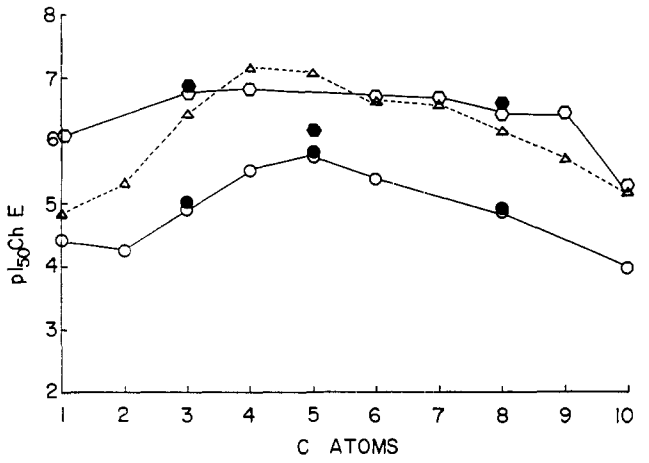


Figure 1. Plot of $-\log I_{50}$ for fly cholinesterase *vs.* number of carbon atoms in side chain of *N*-methylcarbamates

○ *p*-Alkylthiophenyl
◌ *o*-Alkylthiophenyl
△ *m-sec*-Alkylphenyl (Kohn *et al.*, 1965)
●● Isoalkylthiophenyl

butyl, and isopentyl compounds have been presented (Metcalf *et al.*, 1965). The combined data from the two investigations give a picture of the effects of increasing straight- and branched-chain alkyl chains upon the biological activity of these homologs. The relationship between alkyl chain length and anticholinesterase activity is presented graphically in Figure 1.

Position Isomerism and Chain Length. The general order of activity of the several series of compounds over the C_1 to C_{10} range is ortho > meta > para. This is in agreement with previous conclusions (Metcalf *et al.*, 1965) that the optimum complementarity of the alkylthiophenyl *N*-methylcarbamates to the anionic site of ChE occurs when the S atom is in the ortho-position and that in the para-position interaction is much less probable.

The effects of increasing the alkyl chain length from C_1 to C_{10} in the ortho-position are minimal and activity increases only about fivefold to a peak at C_4 and then decreases only slightly to C_9, when it falls abruptly to C_{10}. For the para-isomers, the activity is considerably more dependent upon the alkyl chain length, rising to a well defined maximum at C_5 and then gradually decreasing to C_{10}. The slight variation in the effect of chain length in the ortho-series may reflect the dominant attraction of the optimally located S atom to the negatively charged anionic site of ChE, which minimizes the secondary interactions of the methylene groups. In the para-series the S atom is disadvantageously placed to interact with the negatively charged anionic site and the attraction of the compound may be largely through van der Waals forces and hydrophobic bonding between the alkyl chain and a lipophilic pool at or near the anionic site as envisioned by Belleau and Lacasse (1964) and Hansch and Deutsch (1966). For comparison in Figure 1 we have plotted data from our laboratory and from Kohn *et al.* (1965) on *m-sec*-alkylphenyl *N*-methylcarbamates as inhibitors of fly and bovine erythrocyte ChE. These compounds exhibit a behavior very similar to the *p*-alkylthiophenyl *N*-methylcarbamates, in that activity increases with number of C atoms to C_4 and then decreases with additional chain length at an equivalent rate. Calculation of the change in free energy of binding per CH_2 (Bergmann, 1955):

$$\Delta(\Delta F) = 2.3\ RT\ \Delta pI_{50}$$

gives an average value of about 370 cal. per mole for the *p*-alkylthiophenyl *N*-methylcarbamates and 475 cal. per mole for the *m-sec*-alkylphenyl *N*-methylcarbamates. Both values are within the range of 360 to 950 cal. per mole which is associated with enzyme-substrate interactions through van der Waals dispersion forces and hydrophobic bonding (Webb, 1963). The regular decrease in interaction shown by both series at the C_4-C_5 chain length can be interpreted as suggested by Purcell and Beasley (1968) as reflecting hydrophobic interaction with a lipophilic pool of limited dimensions so that the longer alkyl chains are folded or curled to result in consequent decrease in the binding energy per methylene group.

The meta-isomers represent the most anomalous situation, as the compounds appear to show a series of maximum activities at C_1, C_3, C_7, and C_9. As a tentative explanation, it is suggested that the lipophilic pool contains specific macro- and microstructures which may respond to the peculiarly meta-oriented even and odd-numbered alkyl chains. A similar idea has been advanced by Belleau and Lacasse (1964) for the interaction between quaternary ammonium compounds and the anionic site.

Effects of Branched Chains. The anionic site of ChE has a strong affinity for branched-chain interactions in such compounds as 3-isopropylphenyl *N*-methylcarbamate and 2-isopropoxyphenyl *N*-methylcarbamate, which are substantially more inhibitory than their straight-chain isomers (Metcalf *et al.*, 1962). However, because of the dominant attraction of the S atom to the anionic site, 2-isopropylthiophenyl *N*-methylcarbamate was only slightly more active than 2-propylthiophenyl *N*-methylcarbamate (Metcalf *et al.*, 1965). In the present study, the branched-chain moiety was compared for the isomeric C_3 (isopropyl), C_5 (isoamyl), and C_8 (isooctyl) alkylthiophenyl *N*-methylcarbamates as shown in Figure 1. In the para-series the isoalkyl carbamates were indistinguishable from the corresponding straight-chain compounds and there was marked similarity in the activities of branched- and straight-chain ortho- and meta-compounds. It is concluded that the longer alkyl chains in these compounds make nonspecific interactions with the lipophilic pool surrounding the anionic site in an analogous manner to the straight-chain compounds.

The insecticidal activity of the alkylthiophenyl *N*-methylcarbamates decreased with increasing chain length from *m*-CH_3S (LD_{50} 8.5 μg. per gram to the housefly) to *m*-C_4H_9S (LD_{50} 25.0) (Metcalf *et al.*, 1965). As shown in Table II, the decrease continued for the higher members of the series and none of the straight-chain compounds above C_7 showed any toxicity at 500 μg. per gram except for *p*-isooctyl. With the para-isomers the decrease was abrupt at C_5. Some of the compounds between C_4 and C_7 were readily detoxified, as shown by the high synergistic ratios with piperonyl butoxide synergist (Table II). However, above C_8 synergism was ineffective in demonstrating toxicity. The results with the *Culex* mosquito larvae demonstrated a similar decline in toxicity above C_4, although the C_3-C_5 compounds were the most active, probably because of their greater hydrolytic stability. The longer chain isooctyl compounds were substantially more toxic to both fly and mosquito than their straight-chain analogs.

Benzylthiophenyl *N*-Methylcarbamates. In this series of carbamates, the polarization of the S atom is substantially influenced by the polar effects of the para-substituent of the aromatic ring. With an electron-donating substituent such as *p*-CH_3, the S atom may be expected to bear a partial

negative charge ($-I$ effect) and with an electron-withdrawing substituent such as p-NO_2, the S atom bears a partial positive charge ($-I-M$ effect).

These effects for various substituent groups are quantified by Hammett's sigma values, as shown in Figure 2. Interaction of the S atom of these carbamates with the negatively charged anionic site of ChE should be increased by a partial positive charge and decreased by a partial negative charge. The data in Table III show that the postulated relationship does occur. In all three series the most active compounds were the electron-withdrawing p-NO_2 derivatives and the least active the electron-donating p-CH_3O derivatives. The order of affinity for ChE for the isomeric series is ortho > meta > para or exactly as observed with the alkylthiophenyl *N*-methylcarbamates.

The data from these experiments are plotted in Figure 2, where the $-\log$ of the I_{50} values for ChE are plotted against the σ values for the substituents and the position of the lines determined by the method of least squares. The calculated slopes of the lines were ortho 0.46, meta 0.66, and para 0.82. These slopes correspond to the ρ values for the reaction of the carbamates with ChE and the intercepts of the lines at $\sigma = 0$ show that the reactivity of the ortho-compounds with ChE is approximately six times that of the para compounds and that of the meta-compounds is 1.6 times that of the para-compounds.

The differences between the affinities of the unsubstituted benzylthiophenyl *N*-methylcarbamates and the p-nitro derivatives for each position isomer demonstrate the effects of the partial positive charge on the S atom. These differences are ortho 2.1, meta 3.5, and para 6.5, showing that the better the complementarity of fit of the S atom to the anionic site of ChE (where ortho is optimum) the less the enhancement of affinity by polarization of S. This conclusion is in agreement with results obtained by quaternizing dimethylaminophenyl *N*-methylcarbamates (Metcalf and Fukuto, 1967).

The insecticidal activity of the benzylthiophenyl *N*-methylcarbamates (Table III) was not outstanding. None of the compounds was appreciably toxic to the housefly, although all but the p-methoxy series were synergized substantially with piperonyl butoxide. This is not surprising, as this series of compounds with two aromatic rings and a sulfide sulfur offers substantial opportunity for detoxication by the abundant mixed-function oxidases of the housefly. For the *Culex* mosquito larva the para-substituted compounds were clearly the most effective, suggesting that these might be the most stable in the aquatic environment. For both insects, activity was roughly parallel to the cholinesterase inhibition, although there are obvious exceptions probably determined by selective detoxication.

Benzothienyl *N*-Methylcarbamates. These compounds (Table IV) are isosteres of the naphthyl *N*-methylcarbamates because of the aromatic character imparted to the thienyl ring by the formation of the π molecular orbitals from the conjugation of the p orbitals of the carbon atoms with the $3d$ orbitals of the S atom. They also represent analogs of the alkylthiophenyl *N*-methylcarbamates in which the freely rotatable alkylthio side chain has been fused into the planar thienyl ring. The I_{50} values in Table IV together with those reported by Kilsheimer *et al.* (1969) since this paper was submitted (4-isomer = 7-isomer $3\times10^{-8}M$, 3-isomer 1×10^{-7}, 6-isomer 4×10^{-7}, and 5-isomer 9×10^{-7}) show that the steric orientation of the thienyl ring plays a significant role in the interaction with the ChE molecule. The 5- and 6-benzothienyl *N*-methylcarbamates are sterically analogous to 2-

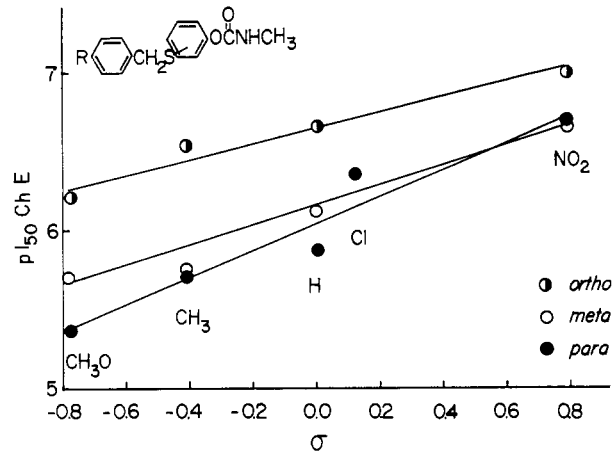
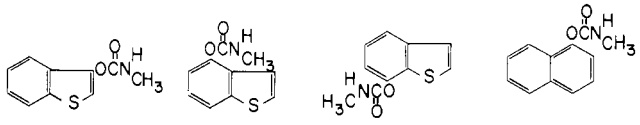


Figure 2. Plot of $-\log I_{50}$ for fly cholinesterase *vs.* sigma values for *o*-, *m*-, and *p*-benzylthiophenyl *N*-methylcarbamates

naphthyl (β-isomers) *N*-methylcarbamate and in these three compounds the 10- to 30-fold reduction in affinity for ChE (over the α-isomers, 4- and 7-benzothienyl and 1-naphthyl) appears to result from the disadvantageous position of the heteronuclear ring which does not interact well with the anionic site (Metcalf and Fukuto, 1965). The anticholinesterase activity in the benzothienyl *N*-methylcarbamates is highest when the carbamoyl moiety is attached to the fully aromatic phenyl ring, as the affinity for ChE of the 7-isomer is 667 and the 4-isomer 800 as compared to 83 for the 3-isomer. The presence of the S atom enhances attraction for ChE from 3.0- to 3.6-fold, as 1-naphthyl *N*-methylcarbamate has an affinity of 222. This enhancement is substantially less than with the alkylthiophenyl *N*-methylcarbamates—e.g., compare 2-methylthiophenyl *N*-methylcarbamate (affinity 222) with 2-ethylphenyl *N*-methylcarbamate (affinity 15).

The insecticidal activities of these compounds (Table IV) are also informative. The 3-benzothienyl derivative resembles carbaryl in its complete lack of toxicity to the housefly and its high synergistic ratio (SR), indicating rapid detoxication (Metcalf *et al.*, 1966). The 7-benzothienyl *N*-methylcarbamate is substantially toxic and has a low SR of 8 for the housefly. The 4-benzothienyl *N*-methylcarbamate is of high toxicity and a very low SR of 2.3. These diversities may be explained in terms of the predominant detoxication of these carbamates in the housefly by hydroxylation of the aromatic rings. In carbaryl the attack by the ·OH hydroxylating radical is largely at the 4- and 5-positions of the naphthalene rings (Dorough and Casida, 1964). The 3-benzothienyl *N*-methylcarbamate is the only isomer studied in which the heteronuclear ring is the fully aromatic phenyl moiety and this compound from the SR values appears to be detoxified about as readily as carbaryl. For the 4-benzothienyl *N*-methylcarbamate, in which detoxication is minimal, the heteronuclear ring is the five-membered thiophene moiety and the sterically analogous point to the 5-hydroxylation of carbaryl is an attack on the S atom, which may result in sulfoxide formation. In the 7-benzothienyl *N*-methylcarbamate in which detoxication is moderate, the sterically analogous position for hydroxylation appears to be the 3-position of the thiophene ring, which is activated for electrophilic attack. These results suggest that an important part of in vivo detoxication in the housefly is through hydroxylation of the heteronuclear ring (opposite to the methylcarbamate group) and that the pseudoaromatic thiophene

ring is less readily attacked by ·OH than the aromatic phenyl ring.



3-Isomer 4-Isomer 7-Isomer Carbaryl

Bis-*N*-methylcarbamylphenyl Sulfides. The 4,4′-bis-(*N*-methylcarbamyloxy)-diphenyl sulfide (I_{50} 2.8 × $10^{-6}M$) and 4,4′-bis-(*N*-methylcarbamyloxy)-diphenyl disulfide (I_{50} 5.2 × 10^{-7} *M*) were nontoxic to the housefly at 500 μg. per gram both alone and together with piperonyl butoxide and to the *Culex* mosquito larva at 10 p.p.m. This suggests that these compounds are very rapidly detoxified and this is supported by the high excitability of the treated flies over the 24- to 48-hour period, indicating some degree of in vivo inhibition of cholinesterase. Further study of the behavior of bis-*N*-methylcarbamates is under way. In comparison, the bis-(*O*,*O*-dimethylthiophosphoryl) ester of 4,4′-bis-dihydroxy-diphenyl sulfide (Abate) has an LD_{50} to the housefly of 105 μg. per gram but an LC_{50} to *Culex* larvae of 0.0016 p.p.m.

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