CARBAMATE INSECTICIDES

Some Structural Relationships of a Group of Simple Alkyl Phenyl *N*-Methylcarbamates to Anticholinesterase Activity

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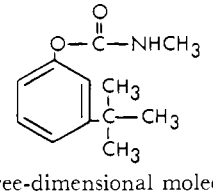
Alkyl phenols with carbon chains from C_3 through C_{10} were synthesized and converted into their respective *N*-methyl carbamates. In vitro cholinesterase determinations were made on all preparations. Inhibition against one particular enzyme was correlated with molecular geometry. In general, meta substitution was superior to ortho and para. Branching on carbon attached to ring increased activity with secondary attachments superior to tertiary. Secondary alkyl groups through C_7 were highly active; maximization of activity occurs between C_3 and C_6. Further branching of the chain was deleterious. Halogen substitution ortho to the carbamate attachment enhanced activity. Some of the simple carbamates were highly insecticidal.

DEVELOPMENT of useful insecticides, particularly those that may be derived from petrochemical sources, is a function of this laboratory. The systematic researches of Metcalf (*7*) and his associates provided a stimulus for the further study of carbamates made from alkyl phenols. Of influence also were certain earlier contributions (*2*, *4*, *5*, *9*, *10*).

This article reports on molecular structures whose geometries simulate acetylcholine. In terms of the experimental procedure, it focuses on configurations that somehow fit the performing centers of a "true" cholinesterase enzyme. By the very nature of the study, phenomena such as detoxication by any of a multitude of mechanisms are omitted, as are important factors relating to absorption through the insect chitin, circulation to points of possible effectiveness, direct action at the synapse receptor centers, or other mechanisms of possible physiological significance.

Stated in its simplest form, *N*-methylcarbamyl esters of certain alkyl phenols provide relatively hydrolytically stable spatial molecular configurations that should compete effectively with acetylcholine for sites on the enzyme protein surface.

At one time, the most interesting and highly inhibitive substance developed by Metcalf and associates was the *N*-methylcarbamate of *m-tert*-butylphenol.



The three-dimensional molecular configuration of this molecule, its hydrolytic stability, and its I_{50} all correlated positively with its insecticidal character.

Experimental

The research procedure may be summarized as an attempt to synthesize substituted phenols of a given configuration, convert these to *N*-methylcarbamates (a large number of other carbamates were also prepared but were always found to be less generally active), and determine the enzyme-inhibitory characteristics of these carbamates using bovine erythrocytes as the enzyme source.

Much of the early literature on alkyl phenols was developed before the days of modern spectroscopy and chromatography. In utilizing these modern tools for the analyses of products derived from procedures described in the earlier literature, we found that in many cases the derived phenols were largely mixtures of isomers which sometimes failed to contain any of the specified configuration. For this reason, many of the syntheses we utilize and describe later are tedious and certainly uneconomical, but provide us with compounds of known configuration. In some cases, technical products useful for structural relationship studies were obtained and quantitative estimates of the isomer content determined. Where the biological activity of minor constituents was already known, the activity of the major constituent could be estimated.

Typical methods include Friedel-Crafts reactions with subsequent purifications, Grignard procedures, and nitrations followed by reduction and diazotization. Catalytic removal of blocking groups was also employed. The methods established no original chemistry and many of the preparations are provided by Moore, Ospenson, and Kohn (*8*).

Cholinesterase Determinations

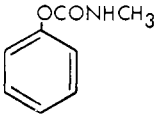
The method of Giang and Hall (*3*) was used with several slight modifications, the most significant of which were the use of purified bovine erythrocyte acetylcholinesterase from Winthrop Laboratories, and the use of a large quantity of premixed enzyme and buffer which were added to each beaker rather than being added separately. This procedure increased the precision of the method and required fewer pipettings.

I_{50} was determined by dissolving the compound in methanol and making serial dilutions in water. One milliliter of each of the water solutions was then incubated with 2 ml. of the enzyme-buffer mixture and the determination completed by a modified Giang and Hall procedure. The per cent inhibitions were calculated and plotted against concentration on a semilog paper with the concentration of the log scale. The curves are S-shaped, with the point of inflection at 50% inhibition. The I_{50} values were then estimated from these plots.

The choice of bovine erythrocytes afforded a large, convenient source of so-called "true" cholinesterase to test the compounds as prepared. Also used, though not extensively reported here, were fly-head cholinesterase and human plasma cholinesterase. The inhibition correlation between enzymes is complex (*1*) and for the sake of simplicity and activity correlation, one single easily available enzyme was chosen for this study.

Results and Discussions

The following discussion is based upon the general formula



The *tert*-butyl derivatives were studied again to confirm the excellence of the meta substitution. Table I shows the ortho substitution to possess fair inhibitory characteristics but it is at least one order inferior to the meta compound. In this table, previously published values for inhibition using fly-head cholinesterase (*7*) are compared with the authors' results with bovine erythrocytes, in micrograms (10^{-6} gram) per milliliter of solution. To convert this to molar values, multiply micrograms per milliliter by 10^{-3}/M.W.

Table I. Carbamates of *tert*-Butyl Phenols

Position of Substituent	I_{50}, μG./Ml. Bovine Erythrocytes	I_{50} Molar fly head (7)	I_{50} Molar bovine erythrocytes
Ortho	1.6	6×10^{-6}	7.7×10^{-6}
Meta	0.11	4×10^{-7}	5.3×10^{-7}
Para	41.0	1.5×10^{-4}	2.0×10^{-4}
Sevin[a]	1.3	...	6.5×10^{-6}

[a] Union Carbide compound: 1-naphthyl *N*-methylcarbamate.

Table II. Carbamates of *M*-Butylphenols

Substituent		I_{50}
—C—C—C—C	(normal)	0.34[a]
—C(—C)—C	(secondary)	0.014
—C(—C)(—C)—C	(tertiary)	0.11

[a] This fair inhibition may have been caused in part by failing to separate completely all of the other isomers.

Table III. Carbamates of Side Chain–Substituted *M*-Propylphenols

Substituent in *n*-Propyl Group	I_{50}
α-Methyl	0.014
β-Methyl	4.0
γ-Methyl (*n*-butyl)	0.34
α-Ethyl	0.009
α,α-Dimethyl	0.035
α,β-Dimethyl	0.11
α-Methyl-β-cyclopropyl	0.04
α,α,β-Trimethyl	0.09

Table IV. Carbamates of Side Chain–Substituted *M*-Butylphenols

Substituent in *n*-Butyl Group	I_{50}
None	0.34
α-Methyl	0.018
α-Ethyl	0.027
α-Propyl	0.25
β-Methyl	2.0
α,α-Dimethyl	0.01

Table V. Carbamates of *M*-*sec*-Alkyl-Substituted Phenols

Total Number of Carbons in Side Chain	I_{50}
3	0.075
4	0.014
5	0.018
6	(0.050) 0.008
7	0.07
8	0.18
9	0.5
10	2.0

Table VI. Carbamates of Meta-Substituted *tert*-Alkyl Phenols

Carbon Skeleton	No. of Carbon Atoms	I_{50}
C(—C)(—C)—C	4	0.11
C(—C)(—C)—C—C	5	0.035
C(—C)(—C—C)—C—C	6	0.01
C(—C—C)(—C)—C—C	7	0.09

To provide structures that would most closely simulate choline, a *tert*-butyl group was used to approximate the closest fit of a carbon analog of the quaternary nitrogen. Both two-dimensional formulas and three-dimensional models (such as Fisher-Hirschfelder) portray this similarity to choline.

Based upon the observed values for the superiority of meta substitution, the *m*-*n*-, *sec*-, and *tert*-butylphenols were prepared and the carbamates from them compared for the inhibitory qualities. Table II shows that the *sec*-butyl is a far more potent inhibitor (× 8 approximately) than the *tert*-butyl.

Although branching is desirable and *sec*-butyl appears superior to *tert*-butyl, not all *sec*-butyl groups are equivalent. In Table III, the data are presented in somewhat different form to include types of branching. The substitutions are considered derivatives of a *n*-propyl alkyl group attached to the ring at the α-position.

The difficult-to-prepare α-ethyl compound appears to be slightly more effective than the highly inhibitory *sec*-butyl compound. It possesses the highest cholinesterase-inhibiting power of any simple alkyl carbamate of which we are aware. The two might more adequately be described as approaching biochemical equivalency. I_{50} (molar bovine) = 4.1×10^{-8} for the α-ethyl homolog.

In Table IV a similar representation is based upon a butyl nucleus attached on α-carbon to the ring. Again, β-substitution of a normal chain provides very little enzyme inhibition. Substitution in the α-position by alkyl groups higher than ethyl results in considerable lessening of activity. The hexyl group provided excellent but inferior inhibition as compared with the similar pentyl configuration.

Tables III and IV show that substitution of the carbon skeleton provides considerable enzyme inhibition as long as the branching group is not too large. In Table V the I_{50} is correlated to the number of carbon atoms for a homologous group of α-methyl–substituted alkyl phenyl carbamates. The simplest member of this group would be isopropyl, followed by *sec*-butyl, *sec*-amyl, etc. In all these cases, the distance in Ångstroms between the point of branching and the carbonyl group should be approximately equivalent. The electrical effects also should not vary significantly. It appears as if the very long tail interferes with the optimum positioning on the enzyme surface. The molecule becomes clumsy and the smaller acetylcholine competes more effectively. In these in vitro studies, the question of hydrophilic, lipophilic differences are eliminated as long as there is sufficient aqueous solubility in the test solutions.

The *m*-isopropyl compound has useful insecticidal activity. All compounds in this homologous series, with an α-methyl substitution, containing between three (isopropyl) and seven (α-methyl hexyl) carbon atoms exhibit areas of insecticidal interest. There is an anomaly in the six-carbon member of this group. One early preparation in small yield, which was not reproducible, gave an abnormally strong inhibition. A second preparation provided a value more nearly consistent with the series. Whether this error was in purification or absolute identification of the isomer or an aberrant I_{50} determination is not known.

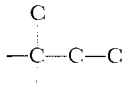
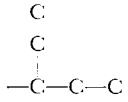
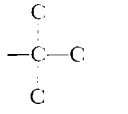
The inhibition appears to maximize between four and six carbons (probably near the four). A similar study of the tertiary substitution on the ring was made. The smallest compound in this series is the *tert*-butyl.

Preparations of these compounds were less unequivocal and small quantities of secondary substitutions were present.

Though it was thought originally that electronegative substitution on the ring would increase the hydrolytic rate and the general instability of the carbamate ester, a considerable number of enzymatically active halogen-substituted *N*-methyl carbamates was found.

Whatever the nonsteric (but practically important) effects may be, the effect of halogen substitution in the six position on the in vitro activity appears in general to enhance and in no case to decrease the

Table VII. Carbamates of 6-Halogenated 3-Alkyl Phenols

3-Substituent	6-Substituent	I_{50}
	H	0.035
	Cl	0.022
	Br	0.07
	H	0.014
	Cl	0.005
	H	0.110
	Cl	0.08

competition of the molecule with acetylcholine for the enzyme site. No such regularity of effect is observed with halogens in other positions.

Further data have been developed and if possible will be systematized in relation to alkyl cresyl and alkyl xylyl carbamates. Other effects such as a restudy of the substitution on the *N*-alkyl group, of unsaturation, and of halogen substitution on the alkyl chain were investigated. In general, these later variations provided little, if any, enhancement of activity and most usually a considerable reduction.

Table VIII is a summary of I_{50} values as against the number of carbon atoms and the nature of the carbon skeleton for the alkyl chain. When one deals with the larger chains and chains of complex structure, the problems of absolute isomer identity and purity become extraordinarily great.

Although there may be errors in some of the assignments, the correlations and the regularities that have been demonstrated presume a general correctness.

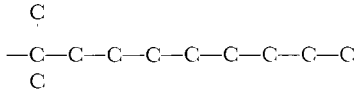
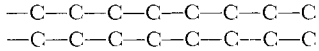
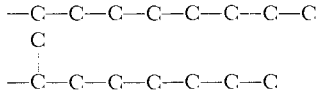
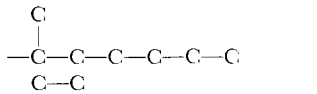
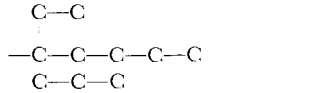
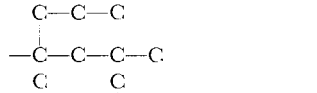
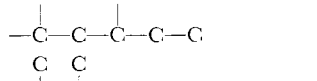
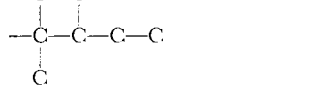
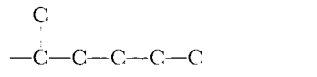
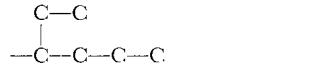
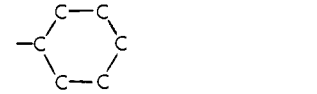
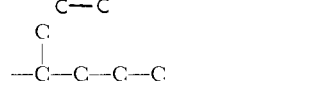
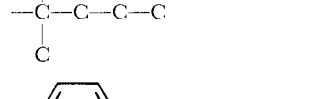
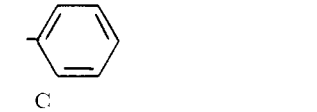
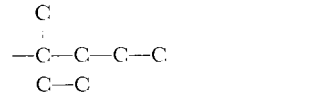
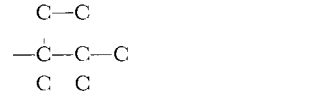
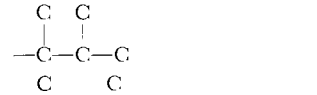
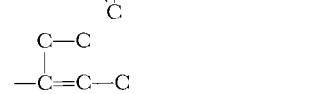
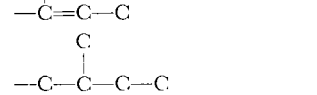
Biological Activity

No group of insecticides provides more interesting biological specificity than these aryl *N*-methylcarbamates In general, all compounds having high inhibiting characteristics provide insecticidal properties on one or more insect species. but not always the same insect.

Many of these compounds show some systemic properties, particularly by soil incorporation methods. Repellancy of leaf feeding insects occurred even where mortality was not high. These biological properties are being investigated further and are beyond the scope of this paper.

Since it was not the objective of these studies merely to inhibit enzymes, Table IX, developed by WHO scientists, is included to show comparison of one of the authors' active compounds for mosquito toxicity with well known insecticides of the carbamate group developed by others.

Table VIII. Carbamates of *M*-Substituted Phenols

Compound Number	No. of Carbon Atoms	Carbon Skeleton Alkyl Group	I_{50}
I	10		2.0
II	9		0.5
III	8		5.0
IV	8		0.18
V	7		0.07
VI	7		0.05
VII	7		0.25
VIII	7		0.10
IX	7		0.09
X	7		>10
XI	6		0.008/0.050
XII	6		0.027
XIII	6		1.8
XIV	6		0.01
XV	6		>10
XVI	5		0.018
XVII	5		0.009
XVIII	5		0.11
XIX	5		0.04
XX	5		0.15
XXI	5		2.0
XXII	5		0.035

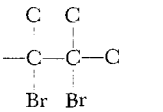
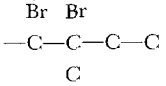
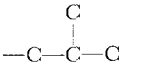
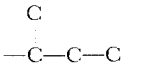
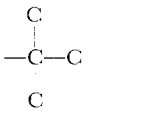
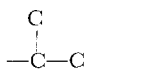
Compound Number	No. of Carbon Atoms	Carbon Skeleton Alkyl Group	I_{50}
XXIII	5		0.6
XXIV	4		0.34
XXV	4		4.0
XXVI	4		0.014
XXVII	4		0.11
XXVIII	3		0.075

Table IX. Biological Activity toward Tropical Mosquitos (6)

Compound, Alkyl Substitution	I_{50}, μG. A. stephensi	A. aegypti
2-Chloro-3-isopropyl phenyl (Hercules)	0.0018	0.0043
3-Isopropylphenyl (Union Carbide)	0.0013	0.0025
2-Isopropoxy phenyl	0.0013	0.0053
3-*sec*-Butyl phenyl XXVI (this paper)	0.0012	0.0023
1-Naphthyl (Sevin, Union Carbide)	0.0037	0.0145
DDT	0.018	0.018

Conclusions

There has been much speculation concerning physiological modes of action of the *N*-methyl phenyl carbamates other than interference with the function of the enzyme acetyl cholinesterase. This study deals only with the competition of synthesized molecules of known geometries with the normal substrate acetylcholine for a place on that enzyme. If these molecules possess modes of activity other than that provided by inhibition measurements, then the total biochemical activity is in some way different and probably greater than that provided through inhibition of AChE alone.

The actual field effectiveness of these substances is of course affected by absorption rates, detoxication in plant and animal, hydrolytic stability, solubilities, sensitivity to visible and ultraviolet radiation, etc. At this time, however, it is impossible to systematize these effects as one can do with the relations of structure to inhibition.

For the simple carbamates containing no hetero atoms on the alkyl groups, the following generalization can be drawn. Compounds of very high inhibiting character can be synthesized with relatively simple structures. Meta-alkyl substitution of the phenol nucleus provides the best opportunities for high activity.

Highest activity is derived from compounds with one α substitution on the alkyl chain. For small chains, α-ethyl substitution appears optimum; for larger chains, α-methyl substitution is optimum. β or other substitution on the side chain is noncontributory or deleterious to activity for the meta isomers. Multiple substitution on the α-position as in *tert*-alkyl groups provides compounds inferior to single substitution (*sec*-alkyl), though the compounds are still highly active. Highly active compounds with *m*-alkyl groups can have their activity enhanced when halogen is substituted on the six position. Too long a chain, even though optimally substituted, decreases the inhibition. All compounds possessing high enzymatic inhibitory properties exhibited areas of high insecticidal potency.

Literature Cited

(1) Dauterman, W. C., O'Brien, R. D., J. AGR. FOOD CHEM. **12,** 318–19 (1964).
(2) Ferguson, G. R., Alexander, C. C., *Ibid.*, **1,** 888–9 (1953).
(3) Giang, P. A., Hall, S. A., *Anal. Chem.* **23,** 1830 (1951).
(4) Grob, H., "Research on Control of Aphids with Chemicals Based on Urethanes and Phosphoric Esters," Third International Congress on Phytopharmacy, Paris, September 1952.
(5) Gysin, H., *Ibid.*, "New Group of Insecticidal Substances."
(6) Hadaway, A. B., Barlow, F., "Toxicity of Some Carbamates to Adult Mosquitoes," Tropical Pesticides Research Unit, Porton.
(7) Metcalf, R. L., "Organic Insecticides," pp. 317–29, Interscience, New York, 1955.
(8) Moore, J. E., Ospenson, J. N., Kohn, G. K., U. S. Patents **3,062,864–3,062,868; 3,066,163; 3,062,707; 3,076,741; 3,110,726** (November 1962–November 1963).
(9) Wiesmann, R., "Research on a New Insecticide Active against Resistant *Musca domestica*," XIIth International Congress of Pure and Applied Chemistry, New York, September 1961.
(10) Wiesmann, R., Gasser, R., Grob, H., *Experientia* **7** (4), 117–20 (1951).

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CARBAMATE INSECTICIDES

Adaptation of Sevin Insecticide (Carbaryl) Residue Method to Various Crops

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SINCE its discovery in 1953, Sevin insecticide (carbaryl) has become established as a broad spectrum pesticide of relatively low mammalian toxicity. This product, chemically 1-naphthyl *N*-methylcarbamate, has been registered for use on approximately 85 field, vegetable, and fruit crops as well as for dermal treatment of most domestic and livestock animals. In addition, it is being investigated for use in oyster beds to control oyster predators. To obtain such broad registration, thousands of samples had to be analyzed to determine residue concentrations in and on the products or certain fractions of products involved. In addition, many related analytical investigations have been performed, including the differentiation between Sevin and 1-naphthol, stability tests in crops and soil, and concentration

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