Preparation and Properties of Phenyl Carbamates with Phosphoryl and Carbamyl Substituents

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We have prepared ortho, meta, and para isomers of phenyl methyl- and dimethyl carbamates whose second ring substituents is a diethyl phosphoryl or diethyl phosphorothionyl or dimethylcarbamyl group. The anticholinesterase activity and the toxicity towards houseflies of these compounds and their intermediates and analogs have been measured. These compounds did not prove to be superior inhibitors or insecticides. A linear relation has been demonstrated between the infrared absorption frequency of the P→O bond of the phosphates and their anticholinesterase activity.

Aromatic phosphates and carbamates are important insecticides today, and may grow to be even more important as the more persistent insecticides are removed from the market. The phosphates and carbamates have in common the fact that they react with acetylcholinesterases, and this reaction is considered to be the basis of their toxic action on insects as well as mammals.

In this paper we describe the preparation and properties of a number of aromatic compounds with two carbamyl substituents or with a carbamyl and phosphoryl (or phosphorothionyl) substituent. There were two hypothetical reasons to postulate that such compounds might have unusual activity. One was that they might react with two functional groups upon the active surface of the enzyme. Another possibility was that statistical factors would improve the probability of phosphorylation or carbamylation of the enzyme. Even if neither of these possibilities could be demonstrated, it was of value to know the role that phosphoryl or carbamyl groups would play as substituents in modifying the activity of other phosphate or carbamate groups. In addition, we have begun in this paper to examine the question of whether charge transfer complex formation plays a role in the interaction between organophosphates and acetylcholinesterase.

MATERIALS AND METHODS

Compounds **I** through **VIII** and **XI** were prepared by a modification of the method of Tokeda Chem. Ind. (1963) using stoichiometric amounts of the appropriate phenol (or its sodium salt, for **XI**) with diethylphosphoryl or diethylphosphorothionyl chloride in tetrahydrofuran (THF) with Na_2CO_3 (**I** through **V**, and **IX**) or K_2CO_3 and catalytic amounts of triethylamine. Purification involved washing with alkali and water of benzene solutions of the compounds (except **III**) and distillation of crude products on a molecular still at between 7.5×10^{-2} and 1.5×10^{-2}mm. Yields were about 50%.

Compound **III** was purified by washing its chloroform solution with water only. After removal of the solvent, the pure compound was obtained.

Compounds **IX** and **X** were made by the method of Metcalf *et al.* (1960) from the *o*- and *m*-hydroxyphenyl methyl carbamates. Compound **XII** was made according to Morel (1899); **XIII** and **XVI** were made according to Einhorn (1898).

Compound **XIV** was prepared by refluxing of stoichiometric amounts (0.1 mol) of dimethylcarbamyl chloride and the sodium salt of resorcinol in THF for 2 hr. From the filtered liquid THF was removed under vacuum on a water bath. The residue was extracted twice with 35 ml of benzene. The combined extracts were washed four times with 5 ml of water each time, then dried over $MgSO_4$, passed through a Florisil column, and eluted with ether. After removing the solvent, an oily material, $n^{20}D$ 1.510, was obtained, which was *m*-hydroxyphenyl dimethyl carbamate sufficiently pure for use as an intermediate in subsequent preparations. For characterization, crystallization was induced over 2 days with occasional rubbing on the glass, and the crystals were washed with ether.

Synthesis of compound **XV** was carried similarly to that of **XIV**, using the sodium salt of hydroquinone and refluxing the reaction mixture for 3 hr under nitrogen. The crystals were filtered off, macerated with a little 1,2-dichloroethane, filtered, and the filter cake (7.0 g) was treated with 30 ml of chloroform and stirred for 30 min at room temperature, then filtered again. This was repeated until the filtrate contained no solute. The combined filtrate was collected and evaporated at room temperature, and the residue washed with a little 1,2-dichloroethane and dried with suction. The product (2.5 g; mp 165–167°C) was *p*-hydroxyphenyl dimethylcarbamate sufficiently pure for carrying out subsequent synthesis. For characterization the product was purified by fractional crystallization from chloroform at room temperature.

Various. Tetracyanoethylene (Aldrich Chemical Co.) was purified by sublimation after trituration with charcoal (mp 201–202°C).

Spectral Measurements. Low-resolution infrared spectra were measured on a Perkin-Elmer infracord spectrophotometer, employing $CHCl_3$ and CCl_4 solutions. High-resolution spectra were measured on a Perkin-Elmer 521 spectrophotometer, using a film between sodium chloride windows. Ultraviolet spectra were measured on Beckman Acta III spectrophotometer.

Toxicity. The toxicity to houseflies was determined by topical application in 1 μl of acetone, using four replicates of 20 insects each (3-day-old females); the standard error of the mean of the LD_{50} was approximately 6%.

Cholinesterase Inhibition. Cholinesterase activity was determined on bovine erythrocyte acetylcholinesterase (Winthrop) by the method of Ellman *et al.* (1961). The pH was 7.6, the temperature 25°C, and the incubation time 10 min. The I_{50} (concentration to inhibit the enzyme 50%) was measured. The standard error of the mean of the I_{50} was approximately 10%.

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Table I. Physical Constants and Analyses of Compounds: $R_1R_2C_6H_4$

No.	R_1	R_2	mp °C	$n^{20}D$	Formula	% C Calcd	% C Found	% H Calcd	% H Found	% N Calcd	% N Found	% P Calcd	% P Found
I	$CH_3NHC(O)O$	*o*-$(C_2H_5O)_2P(O)O$		1.4885	$C_{12}H_{18}NO_6P$	47.5	48.6	5.9	6.2	4.6	4.2		
II	$(CH_3)_2NC(O)O$	*o*-$(C_2H_5O)_2P(O)O$		1.4865	$C_{13}H_{20}NO_6P$	49.2	48.5	6.3	6.6	4.4	4.2		
III		*o*-$(C_2H_5O)_2P(O)O$			$C_{24}H_{34}N_2O_{12}P_2$	47.7	47.5	5.6	5.7	4.6	4.6		
		o-$(C_2H_5O)_2P(S)O$		1.5184	$C_{13}H_{20}NO_5PS$	46.8	46.5	6.0	6.2	4.2	4.1		
IV	$(CH_3)_2NC(O)O$	*m*-$(C_2H_5O)_2P(O)O$		1.4810	$C_{13}H_{20}NO_6P$	49.2	48.9	6.3	6.6	4.4	4.2		
V	$(CH_3)_2NC(O)O$	*m*-$(C_2H_5O)_2P(S)O$		1.5035	$C_{13}H_{20}NO_5PS$	46.8	45.6	6.0	6.3	4.2	4.1		
VI	$(CH_3)_2NC(O)O$	*p*-$(C_2H_5O)_2P(O)O$		1.4910	$C_{13}H_{20}NO_6P$	49.2	49.4	6.3	6.5	4.4	4.2		
VII	$(CH_3)_2NC(O)O$	*p*-$(C_2H_5O)_2P(S)O$		1.4790	$C_{13}H_{20}NO_5PS$	46.8	47.1	6.0	6.1	4.2	4.2		
VIII	$(CH_3)_2NC(O)O$	*o*-$CH_3NHC(O)O$	94–96		$C_{11}H_{14}N_2O_4$	55.4	55.6	5.9	5.9	11.7	11.5		
IX	$(CH_3)_2NC(O)O$	*m*-$CH_3NHC(O)O$	156–158		$C_{11}H_{14}N_2O_4$	55.4	54.5	5.9	5.9	11.7	12.1		
X	$(CH_3)_2NC(O)O$	*o*-$(C_2H_5O)_2P(O)O$		1.4710[a]	$C_{14}H_{22}O_6P_2$	44.0	44.1	6.3	6.3			16.2	16.1
XI	$(C_2H_5O)_2P(O)O$	H		1.4790[b]	$C_{10}H_{15}O_4P$	52.2	52.6	6.5	6.7			13.4	13.0
XII	$(CH_3)_2NC(O)O$	*o*-HO	118–119		$C_9H_{11}NO_3$	59.7	59.5	6.1	6.2	7.7	7.4		
XIII	$(CH_3)_2NC(O)O$	*m*-HO	81–83		$C_9H_{11}NO_3$	59.7	59.7	6.1	6.3	7.7	7.5		
XIV	$(CH_3)_2NC(O)O$	*p*-HO	166–168		$C_9H_{11}NO_3$	59.7	60.5	6.1	6.3	7.7	7.4		
XV[c]	o-HO-C_6H_4-OC(O)NH-$(CH_2)_2$NHC(O)O	*o*-HO	174–176										
XVII[c]	$CH_3NHC(O)O$	H	89–90										
XVIII[c]	$(CH_3)_2NC(O)O$	H	45–47										
XIX[c]	$CH_3NHC(O)O$	3,5-bis-$(CH_3)_2CH$	80–81										

[a] $n^{30}D$ 1.4679 (Tichy *et al.*, 1957). [b] $n^{17}D$ 1.475 (Kenner and Williams, 1955). [c] The compounds are known in the literature.

Table II. Toxicities and Anticholinesterase Activities of Compounds

No.	I_{50} against bovine erythrocyte acetylcholinesterase	Relative I_{50} compared with phenyl dimethyl carbamate	Relative I_{50} compared with diethyl phenyl phosphate	LD_{50} housefly Alone	LD_{50} housefly 1:5 P.B.[a]
I	2.9×10^{-6}	52[a]	379	250	150
II	4.8×10^{-6}	146	229	160	155
III	$>10^{-3}$	<1	<1	>500	
IV	5.5×10^{-5}	13		>500	
V	4.9×10^{-5}	14	22	>500	100
VI	1.9×10^{-5}	37		165	30
VII	1.1×10^{-4}	6	10	>500	50
VIII	7.3×10^{-5}	10		350	
IX	2.6×10^{-4}	3		500	
X	2.8×10^{-4}	3		250	60
XI	$>10^{-3}$		<1	>500	
XII	1.1×10^{-3}			>500	
XIII	$>10^{-3}$	<1		>500	
XIV	$>10^{-3}$	<1		>500	
XV	6.5×10^{-4}	1		>500	
XVI	$>10^{-3}$			>500	
XVII	1.5×10^{-4}			65	
XVIII	7.0×10^{-4}			>500	
XIX	6.2×10^{-7}	242[b]		15	

[a] Value compared with phenyl methyl carbamate. [b] Piperonyl butoxide synergist.

RESULTS

Table I shows the compounds, their analysis, and their physicochemical properties. Table II shows the biological activity of these compounds. It would seem that only the compounds containing a phosphoryl or thiophosphoryl moiety were highly potent (either insecticidally or as anticholinesterase). Nevertheless, it was invariably true that the introduction of a methylcarbamyl or dimethylcarbamyl group into an aromatic organophosphate improved the potency of the compound, the effect being especially marked when the substituents were ortho to each other. By contrast, the introduction of the diethyl phosphoryl group into diethyl phenyl phosphate, or of the methyl carbamate group into a phenyl dimethylcarbamate had a relatively small effect, especially as far as the anticholinesterase activity was concerned. A consequence was that the bis-substituted compounds having phosphate and carbamate groups were good anticholinesterases and had measurable insecticidal effect, whereas the parent unsubstituted carbamate or phosphate had negligible anticholinesterase activity in either case. In spite of these encouraging facts, none of these new compounds was as potent against the insects or the enzyme as the familiar compound **XIX**.

When comparing a group of relatively active compounds, it was possible to show an excellent linear relationship between the infrared P→O stretching frequency and the anticholinesterase activity (Figure 1). The correlation coefficient was −0.84. Fukuto and Metcalf (1956) showed for monosubstituted aromatic phosphates a correlation between anticholinesterase activity and the P–O–C aromatic stretching frequency. The frequencies varied between 1280 and 1250 cm^{-1} (±3 cm^{-1}), in good agreement with the reports that these frequencies fell in the range 1300 to 1250 cm^{-1} (Bellamy and Beecher, 1952; Gore, 1959). As for the C=O, no distinct change was found in its frequency; it was normally in the region of 1730 to 1725 cm^{-1}. This compares fairly well with the report of characteristic infrared absorption spectra for car-

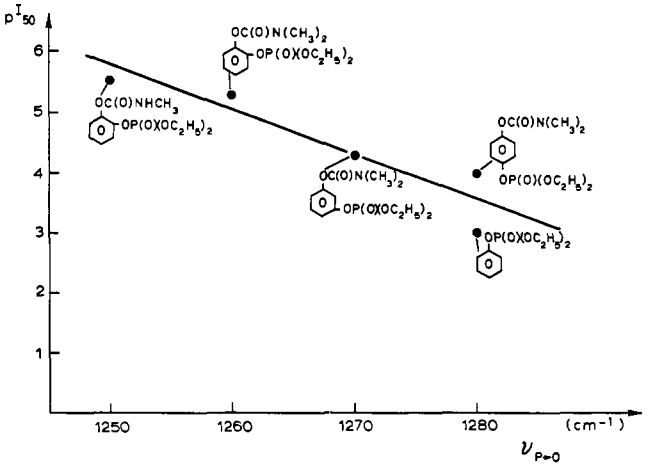

Figure 1. Relationship of pI_{50} and infrared P→O frequency for diethylphosphoryl derivatives of phenyl dimethyl-(methyl-)carbamates

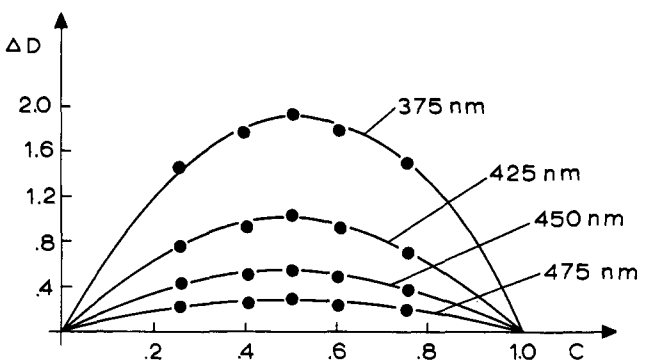

Figure 2. Job's plots for the charge-transfer complex of *m*-dimethylcarbamyloxyphenyl diethyl phosphate with tetracyanoethylene in 1,2-dichloroethane

bamate pesticides by Chen and Benson (1966), who reported a range of 1745 to 1721 cm^{-1}.

A possibility which has never been explored is that these aromatic inhibitors owe some of their potency to an ability to form charge-transfer complexes with the enzyme, prior to phosphorylation or carbamylation. The ability of one of these agents (compound V) to form charge-transfer complexes was measured using tetracyanoethylene as an electron acceptor. A Job's plot (Figure 2) was prepared by plotting the ΔD as a function of the mole fraction of the donor and acceptor (C), in solution of 1,2-dichloroethane. ΔD was defined as the difference between absorption observed in the solution containing acceptor and donor, as compared with solutions containing the individual components measured separately (Bailar, 1956; Vosburgh and Cooper, 1941). The plot shows that complex formation does indeed occur, the stoichiometry being 1:1. It was found that a charge-transfer band of this complex is located at 355 nm in 1,2-dichloroethane solution.

DISCUSSION

Of the ten bis-functional compounds I–X prepared, only compounds V and VI have been reported, in the form of a Japanese patent (Tokeda Chem. Ind., 1963). These compounds were claimed to be useful insecticides, but specific data were not given. Our data suggest that these bis-substituted compounds are not promising insecticides, even in the presence of a five-fold excess of the synergist piperonyl butoxide. The relative ineffectiveness of synergism in aromatic bis-methylcarbamates has previously been noted by Metcalf and Fukuto (1967), who suggest that rapid metabolism of such compounds occurred, even in the presence of the metabolic inhibitors such as piperonyl butoxide.

Is there evidence that bis-functional inhibitors react with two groups on the enzyme? Results of the type reported here cannot provide direct evidence upon mechanism. The probability does not seem great *a priori*, because very precise matching of the two substituents of the inhibitor with the two enzymic groups would be necessary. Furthermore, unless the reactivity of both substituents were identical, it is likely that reaction of one substituent with one enzymic group would predominate, and the existence or nonexistence of a second reaction might well go undetected.

One might hope that the existence of two functional groups would increase the probability that the inhibitor, once bound to the enzyme surface, would be favorably oriented for subsequent phosphorylation or carbamylation. However, the best improvement observed by adding a second functional group was in I, for which the addition of a methylcarbamyl group improved inhibition of the parent diethyl phenyl phosphate 379-fold. Such an effect could well be caused by the electron-withdrawing of the methylcarbamyl group (compare Aldridge and Davison, 1952) without invoking any special mechanism.

Are the mixed phosphates and carbamates acting as phosphorylaters or carbamylaters? Since aromatic phosphorothionates invariably have little anticholinesterase activity, we may assume that the inhibitory activity of the P(S) compounds IV, VI, and VIII are due to their acting as substituted carbamates rather than as substituted phosphates. Consequently one can say that the diethyl phosphorothionyl substituent increases inhibitory activity of the unsubstituted phenyl dimethyl carbamate (XVIII) by 13-fold in the ortho position, 37-fold in the meta position, and ten-fold in the para position. The P(O) bis-functional compounds of this type (*i.e.*, compounds II, V, and VII) are respectively 11-fold, 2.5-fold, and 1.5-fold better inhibitors than their P(S) analogs; one cannot say whether this is because the P(O) act as phosphorylaters or whether carbamylation still occurs, but the P(O) substituent promotes carbamylation more than does a P(S) substituent. One is inclined to favor the first possibility, because the phosphoryl group is more reactive than the carbamyl group, and because of the remarkably close correlation of the P→O stretching frequency with inhibitory activity (Figure 1).

It is clear (Figure 1) that compounds such as V can act as electron donors in the formation of charge-transfer complexes. It remains to be determined whether such complexes contribute in an important way to the reaction between aromatic anticholinesterases and the enzyme surface.

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LITERATURE CITED

Aldridge, W. N., Davison, A. N., *Biochem. J.* **52**, 663 (1952).
Bailar, J. Ch., Ed., The Chemistry of the Coordination Compounds, Reinhold, New York, 1956.
Bellamy, L. J., Beecher, L., *J. Chem. Soc.* 475 (1952).

Chen, J. T., Benson, W. R., *J. Ass. Offic. Agr. Chem.* **49,** 412 (1966).
Einhorn, A., *Ann.* **300,** 135 (1898).
Ellman, G. L., Courtency, K. D., Andres, V. Jr., Featherstone, R. M., *Biochem. Pharmacol.* **7,** 88 (1961).
Fukuto, T. R., Metcalf, R. L., J. AGR. FOOD CHEM. **4,** 930 (1956).
Gore, R. C., *Disc. Farad Soc.* **9,** 138 (1959).
Kenner, G. W., Williams, N. R., *J. Chem. Soc.* 522 (1955).
Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **15,** 1022 (1967).
Metcalf, R. L., Fukuto, T. R., Winton, M. Y., *J. Econ. Entomol.* **53,** 828 (1960).
Morel, A., *Compt. Rend.* **128,** 508 (1899).
Tichy, V., Rattay, V., Janok, J., Valentinova, J., *Chem. Zvesti* **11,** 398 (1957).
Tokeda Chemical Industries, Japan Patent 11 126/62; *Chem. Abstr.* **59,** 9894 (1963).
Vosburgh, W. C., Cooper, G. R., *J. Amer. Chem. Soc.* **63,** 437 (1941).

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Synthesis of Dinitroaniline as Plant Growth Regulators and for Identification of Amines

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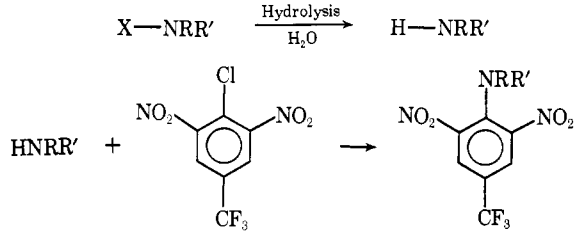
The synthesis of systematic series of 4-substituted amino-3,5-dinitrobenzotrifluorides and 4-substituted 2,6-dinitro-*N*,*N*-di-*n*-propylanilines is reported. The anilines formed by the reaction of 4-substituted 2,6-dinitrochlorobenzenes with amines liberated by the hydrolysis of carbamate, urea, and amide pesticides can be used for the gas chromatographic determination of these pesticides. The preparative procedures developed can be extended to other series of dinitroanilines.

Although the dinitroanilines have been recognized as effective preemergence herbicides for approximately 10 years (Pieczarka *et al.*, 1961, 1962a,b), mode of action studies (Amato *et al.*, 1965; Mann *et al.*, 1968; Standifer *et al.*, 1965; Talbert, 1965; Negi *et al.*, 1968; Shultz *et al.*, 1968; Sawamura and Jackson, 1968a,b) have been limited to the commercially available trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-di-*n*-propyl-*p*-toluidine) and nitralin (4-methylsulfonyl-2,6-dinitro-*N*,*N*-di-*n*-propylaniline). Structure–activity studies (Gentner, 1966, 1970) of the dinitroanilines have also been limited to simple alkyl analogs of trifluralin and nitralin.

Recently, Crosby and Bowers (1968) reported a simple and sensitive procedure for the analysis of pesticides containing the amine function. In this procedure, carbamate, urea, amide, and other amine-containing pesticides are hydrolyzed, and the amine, which is liberated, is analyzed as a dinitroaniline or other similar derivative. The most suitable derivatives were mono- and dinitro-α,α,α-trifluoro-*p*-toluidines; however, the gas chromatographic behavior of only a very limited number



of these derivatives had been studied.

The synthesis of systematic series of 4-substituted amino-3,5-dinitrobenzotrifluorides and 4-substituted 2,6-dinitro-*N*,*N*-di-*n*-propylanilines is presented. The wide variety of steric, electronic, and hydrophobic factors present in these series enable the physical and biochemical properties of the dinitroanilines to be fully investigated.

EXPERIMENTAL

General Procedures for Preparing 4-Substituted 2,6-Dinitrobenzenes. The two general procedures for synthesizing 4-substituted 2,6-dinitrochlorobenzenes (the "direct" nitration of 4-substituted chlorobenzenes and the "indirect" nitration of 4-substituted phenols and subsequent chlorodehydroxylation of the dinitrophenol) are exemplified by the specific preparations below.

4-Chloro-3,5-dinitrobenzotrifluoride. METHOD A. To a mixture of 200 ml of fuming sulfuric acid (30–33%) and 180 ml of red fuming nitric acid at 60°C was added dropwise with stirring 50 g of 4-chloro-3-nitrobenzotrifluoride (Aldrich Chemical Co.) over a period of 30 min. The mixture was kept at 60°C for an additional 30 min and then slowly raised to 100–105°C over a period of 45 min. It was kept at this temperature for 1.5 hr, and after having been cooled to room temperature, was poured over cracked ice and allowed to sit overnight. The mixture was then filtered, and the precipitate was dissolved in ether and rinsed several times with distilled water. The solvent ether was removed at room temperature and the residue recrystallized from ethyl alcohol. The yield of 4-chloro-3,5-dinitrobenzotrifluoride was 74% (45 g), a pale yellow crystalline compound which melted at 56–58°C (Soper, 1966; 53–57°C).

4-*tert*-Butyl-2,6-dinitrochlorobenzene. METHOD B. A solution of 25 g of 4-*tert*-butylphenol (Aldrich Chemical Co.) in 100 ml of glacial acetic acid was added dropwise with stirring over a period of 45 min to a mixture of 50 ml of concentrated nitric acid and 75 ml of glacial acetic acid at 5°C (Dutton *et al.*, 1953a). The reaction mixture was then allowed to warm to room temperature for 30 min. The reaction was then terminated by pouring the mixture over cracked ice. 4-*tert*-Butyl-2,6-dinitrophenol precipitated and was filtered off cold. The crude phenol can be purified as the piperidine salt (recrystallized from 95% ethyl alcohol), and the pure phenol liberated by acidifying the salt in water and extracting

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