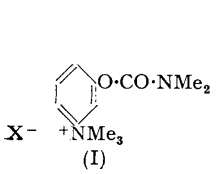
140. *The Synthesis of Neurotropic and Musculotropic Stimulators and Inhibitors. Part V.* Derivatives of Aminophenyl Phosphates as Anticholinesterases.*

By K. J. M. Andrews, F. R. Atherton, F. Bergel, and A. L. Morrison.

A number of (dialkyl phosphato)-*N*-dimethylanilines (Table 2) have been prepared by condensing dimethylaminophenols with the appropriate dialkyl chlorophosphonate. While most of these tertiary bases were stable to heat, the *m*-dimethylamino-homologue readily formed the corresponding betaine (XI). The quaternary salts (Table 3) were found to be potent inhibitors of cholinesterase.

m-(Ethyl diethylaminophosphonato)-*N*-trimethylanilinium methyl sulphate (VII) and *m*-(ethyl diethylaminophosphinato)-*N*-trimethylanilinium methyl sulphate † (VIII) were made by similar methods; they were less active as enzyme antagonists.

The first group of synthetic compounds found to be potent inhibitors of cholinesterase was that of the quaternary salts of *m*-dialkylaminophenyl carbamates (cf. Stedman, *Biochem. J.*, 1926, **20**, 719; Aeschlimann and Reinert, *J. Pharmacol.*, 1931, **43**, 413). One member of this group has found wide clinical application and is known as " Prostigmin " or neostigmine (I).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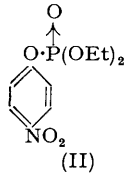
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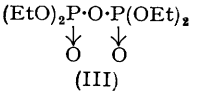
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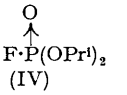
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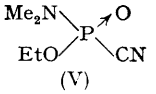
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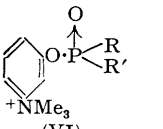

(VI)

In a search for insecticides, Schrader in Germany (B.I.O.S. Final Report, 1947, WO 714) prepared many organic phosphorus compounds which were subsequently shown to be powerful inhibitors of cholinesterase. Most of these compounds were neutral phosphates containing a labile linkage either of an ester [*e.g.*, E 600 (II)] or an anhydride [*e.g.*, tetraethylpyrophosphate (III)] type. Certain fluoro- and cyano-derivatives of phosphorus were also found to be very active, di*iso*propyl fluorophosphonate (IV) (Chapman and Saunders, *J.*, 1948, 1010) and ethyl cyanodimethylaminophosphinate (V) (Holmstedt, *Acta Phys. Scand.*, 1951, **25**, Suppl. 90) being among the most powerful.

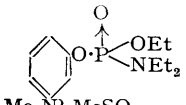
In discussions with Drs. Burgen, Hobbiger, and Keele of the Department of Pharmacology, Middlesex Hospital Medical School, it was agreed that it would be of particular interest to study the anticholinesterase properties of a homologous series of compounds with general formula (VI). Such compounds are obviously closely related to neostigmine and also fulfil the conditions laid down by Schrader as necessary for an insecticide and now known to be equally valid for an active anticholinesterase compound. They would differ from all other known anticholinesterase phosphates in having a quaternary group and this could be expected to influence their physiological properties.

The first compound envisaged was *m*-(ethyl diethylaminophosphonato)-*N*-trimethylanilinium methyl sulphate (VII) which was prepared by condensing *m*-dimethylaminophenol with ethyl chlorodiethylaminophosphinate (prepared according to Michaelis,

* Part IV, *J.*, 1950, 2887. † For nomenclature, see *J.*, 1951, 1868.

Annalen, 1902, **326**, 129) and quaternising the resultant tertiary base with methyl sulphate. This compound and *m*-ethyldiethylaminophosphinato-*N*-trimethylanilinium methyl sulphate (VIII), made in a similar way from chloro-diethylamino-ethylphosphine oxide, were found to be less potent than neostigmine as cholinesterase inhibitors.

Chloro-diethylamino-ethylphosphine oxide was obtained by the reaction of diethylamine with excess of dichloro-ethylphosphine oxide. Compounds of this type with nitrogen, carbon, and halogen atoms attached to the same phosphorus atom have not been reported previously. Dichloro-ethylphosphine oxide was conveniently produced by treating ethylphosphonic acid (Kosolapoff, *J. Amer. Chem. Soc.*, 1945, **67**, 1180) with phosphorus pentachloride. In this and other experiments we have found it expedient to destroy excess of phosphorus pentachloride with sulphur dioxide. Phosphorus oxychloride and thionyl chloride are formed and these can be removed by distillation. This technique, which does not seem to have been applied previously, should prove equally convenient in other instances.



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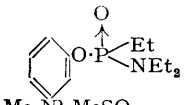

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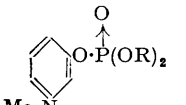
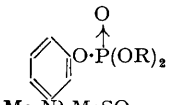


(IX)



(X)

The next groups of compounds investigated were the *m*-(dialkyl phosphato)-*N*-dimethylanilines (IX) and the corresponding *N*-trimethylanilinium methyl sulphates (X).

The tertiary bases (IX; R = Me, Et, Pr^i, Bu^n, and Bu^s) were made by condensing *m*-dimethylaminophenol with the dialkyl chlorophosphonates, which were prepared according to Atherton, Howard, and Todd (*J.*, 1948, 1106) from the corresponding dialkyl phosphites (McCombie, Saunders, and Stacey, *J.*, 1945, 380).

Most of the *m*-(dialkyl phosphato)-*N*-dimethylanilines we have examined are thermally stable under the conditions required to distil them. The dimethyl homologue, however, partly decomposed during distillation to a non-volatile crystalline compound, soluble in alcohol and water but insoluble in ether. These properties, together with the analysis, show the material to be the betaine $Me_3\overset{+}{N}\cdot C_6H_4\cdot O\cdot P(O^-)(=O)OMe$. In order to prepare this betaine in a more nearly quantitative yield, the undistilled ester was heated, whereupon it quickly solidified to a crystalline mass. Crystals of the betaine are deposited in the distilled ester after storage for a few weeks, showing that this rearrangement also occurs slowly at room temperature.

The formation of quaternary salts from tertiary amines and phosphoric esters has been reported by Baddiley, Clark, Michalski, and Todd (*J.*, 1949, 815), who found that, although neutral phosphoric esters containing an arylmethyl group quaternised readily with tertiary bases, triethyl phosphate showed little tendency to react. The facile quaternary salt formation we have observed from a methyl ester may be due both to the superior lability of methyl groups and to the reaction's probably being intramolecular.

The group of compounds represented by (X; R = Me, Et, Pr^i, Bu^n, Bu^s) were prepared by quaternising the tertiary bases with methyl sulphate. As a representative of the *p*-series, *p*-(diethyl phosphato)-*N*-dimethylaniline and its methosulphate were made in a similar manner.

The anticholinesterase activity of these substances *in vitro* against the esterases of red blood-cells and serum, and their activity *in vivo*, have been investigated by Burgen and Hobbiger (*Brit. J. Pharmacol.*, 1951, **6**, 593). From their results, it appears that these " phosphostigmines " are very potent cholinesterase inhibitors, the quaternary derivatives being more potent than the tertiary bases, and the *m*- more potent than the *p*-compounds. Compared with neostigmine, which inhibits 50% of " true " cholinesterase at a molecular concentration of $1{\cdot}14 \times 10^{-7}$ and " pseudo " esterase at $6{\cdot}9 \times 10^{-7}$, the (dimethyl phosphato)trimethylanilinium methyl sulphate achieves the same degree of inhibition at

$7{\cdot}5 \times 10^{-8}$ and $6{\cdot}3 \times 10^{-9}$, respectively. The other interesting indication which emerges from Burgen and Hobbiger's investigations (*loc. cit.*) is that the alkyl groups of the phosphato-residue seem to influence the kinetics governing the action of these substances with the enzyme system, as shown by the different rates of reactivation of the enzyme. In this respect, the diethyl and the di*iso*propyl phosphates bear a noteworthy resemblance to TEPP and DFP which, respectively, carry the same alkyl groups.

Whatever the mechanism of interaction between the cholinesterases and their inhibitors may eventually prove to be, the stability of the phosphates in aqueous solution may have some bearing. Measured by loss of inhibitory power and toxicity, results which we owe to Dr. Hobbiger and Mr. M. W. Parkes, the percentage hydrolysis shows differences in stability between the various dialkyl homologues, as given in Table 3.

Experimental

Table 1. *Dialkyl chlorophosphonates,* $(RO)_2PCl{\rightarrow}O$, *prepared according to Atherton, Howard, and Todd* (*J.*, 1948, 1106) *by the action of sulphuryl chloride on the dialkyl phosphites in* CCl_4 *at* 35°.

R	B.p./mm.	*n*	Yield, %
Me	78—80°/18	1·4115 (22°)	61
Et	84—85°/10	1·4162 (23°)	87
Pr^i	82—84°/10	1·4169 (21°)	82
Bu^n	86—87°/0·5	1·4298 (21°)	85
Bu^s	66—67°/0·7	1·4272 (22°)	80

m-(*Dimethyl Phosphato*)-N-*dimethylaniline.*—*m*-Dimethylaminophenol (6·85 g., 0·05 mole) was dissolved in ethanolic sodium ethoxide (1·25 g., 0·055 mole, in 60 ml. of ethanol) under nitrogen, and dimethyl chlorophosphonate (8 g., 0·055 mole) was run in dropwise during a few minutes. The reaction mixture was heated under reflux for 1 hour and then set aside overnight. The precipitated sodium chloride was filtered off (3·1 g.) and the alcohol was evaporated from the filtrate. A dark oil remained; it was dissolved in carbon tetrachloride, and the solution was extracted three times with 0·5N-sodium hydroxide and three times with water. After filtration of the carbon tetrachloride solution, it was dried (Na_2SO_4) and filtered, and the carbon tetrachloride evaporated *in vacuo*. The residual oily *base* (50%), distilled in a high vacuum, had b. p. $97°/2{\cdot}3 \times 10^{-5}$ mm. A considerable residue was obtained, which was soluble in alcohol and insoluble in ether. Several recrystallisations from alcohol–ether gave the (*methyl phosphato*)-N-*trimethylanilinium betaine*, m. p. 219—221° (Found: N, 5·4; P, 13·1. $C_{10}H_{16}O_4NP$ requires N, 5·7; P, 12·6%) (see also below).

The *diethyl-*, *di*iso*propyl-*, *di*-n-*butyl-*, and *di*-sec.-*butyl-phosphato*-bases were prepared similarly (cf. Table 2), but there was no appreciable residue on distillation.

Table 2. (*Dialkyl phosphato*)-N-*dimethylanilines* (cf. IX).*

Alkyl	B.p./mm.	n_D	Found, % N	Found, % P	Formula	Required, % N	Required, % P
Me	$97°/2{\cdot}3 \times 10^{-5}$	1·5237 (21·5°)	5·8	12·5	$C_{10}H_{16}O_4NP$	5·7	12·6
Et	$106°/10^{-4}$	1·5110 (22°)	5·4	10·6	$C_{12}H_{20}O_4NP$	5·1	11·3
Pr^i	$100—105°/3{\cdot}7 \times 10^{-6}$	1·5000 (21°)	5·0	10·3	$C_{14}H_{24}O_4NP$	4·7	10·3
Bu^n	$138—140°/2{\cdot}3 \times 10^{-6}$	1·4990 (21°)	4·5	9·3	$C_{16}H_{28}O_4NP$	4·25	9·4
Bu^s	$119—124°/2{\cdot}5 \times 10^{-4}$	1·4985 (20°)	4·8	8·2	$C_{16}H_{28}O_4NP$	4·25	9·4
Et †	$122—128°/5 \times 10^{-5}$	1·5098 (20°)	5·5	10·6	$C_{12}H_{20}O_4NP$	5·1	11·3

* All *m*-compounds, except that marked † which is the *p*-isomer.

Table 3. (*Dialkyl phosphato*)-N-*trimethylanilinium Methyl Sulphates* (cf. X).*

Alkyl	M. p.	Found, % N	Found, % P	Formula	Required, % N	Required, % P	Hydrolysis (%) ‡
Me	86—88°	3·8	8·3	$C_{12}H_{22}O_8NSP$	3·8	8·3	83
Et	105—107	4·0	7·2	$C_{14}H_{26}O_8NSP$	3·5	7·8	55
Pr^i	82—84	4·1	7·5	$C_{16}H_{30}O_8NSP$	3·4	7·25	37
Bu^n	98—100	4·4	6·4	$C_{18}H_{34}O_8NSP$	3·1	6·8	—
Bu^s	90—92	3·2	6·0	$C_{18}H_{34}O_8NSP$	3·1	6·8	—
Et †	80—81	3·2	7·8	$C_{14}H_{26}O_8NSP$	3·5	7·8	20

* All *m*-compounds, except that marked † which is the *p*-isomer.
‡ After 500 hours in 1% w/v aqueous solution at 45°.

m-(*Dimethyl phosphato*)-N-*trimethylanilinium Methyl Sulphate.*—To a solution of *m*-dimethylphosphato-*N*-dimethylaniline (1·8 g.) in dry benzene (6 ml.), methyl sulphate (1 ml.) was added and the mixture was set aside for 29 hours at room temperature. The quaternary *salt* crystallised, and further crystallisation occurred on addition of dry ether. It was collected by filtration, dried, and recrystallised twice from alcohol–ethyl acetate–ether, then having m. p. 86—88° (yield, 66%).

The *diethyl-*, *di*iso*propyl-*, *di*-n-*butyl-*, and *di*-sec.-*butyl-phosphato*-homologues were prepared similarly (cf. Table 3).

m-(*Methyl Phosphato*)-N-*trimethylanilinium Betaine.*—*m*-Dimethylaminophenol (13·7 g.) was dissolved in ethanolic sodium ethoxide (2·5 g. of sodium; 100 ml. of dry ethanol), and dimethyl chlorophosphonate (16 g.) was added dropwise, in a nitrogen atmosphere. The reaction mixture was refluxed for 15 minutes and then set aside for 15 hours. The precipitated sodium chloride was filtered off and the solvent was evaporated *in vacuo*. A dark residue remained which was dissolved in carbon tetrachloride and washed twice with water, twice with 0·5N-sodium hydroxide and finally twice with water. After drying of the solution, the carbon tetrachloride was evaporated *in vacuo* and the residue finally stripped at 100°/0·3 mm. A dark oil remained which was heated from 125° to 160° during 30 minutes. It had by then solidified to a buff-coloured crystalline mass, which was recrystallised by dissolution in methanol and addition of ethanol, followed by ether, until the solution was turbid. Crystallisation then took place when the solution was kept in the refrigerator (yield, 6·7 g.; m. p. 213—215°). A further recrystallisation gave colourless crystals (4·3 g.), m. p. 218—220°. From the first mother-liquors a second crop (4·3 g.) was obtained, having m. p. 215—217° (Found: N, 5·8; P, 11·8%).

m-(*Ethyl Diethylaminophosphonato*)-N-*dimethylaniline.*—To ethanolic potassium ethoxide, prepared by adding potassium (1·45 g.) to dry ethanol (80 ml.), was added *m*-dimethylaminophenol (4·1 g.) in a nitrogen atmosphere. When a clear solution had been obtained, ethyl chloro-diethylaminophosphinate (7·44 g.) was added with stirring. There was immediate precipitation of potassium chloride, and the reaction mixture was heated under reflux for 1½ hours, then set aside overnight. The potassium chloride was filtered off and the alcohol evaporated. The residue was dissolved in carbon tetrachloride (50 ml.), and the solution washed three times with N-sodium hydroxide and twice with water. After drying (K_2CO_3) of the carbon tetrachloride solution, the solvent was evaporated *in vacuo*, to give a brown oil which was distilled; the fraction (72%), b. p. 114—118°/10^{-4} mm., was collected. The *base*, redistilled, had n_D^{22} 1·5149 (Found: N, 9·1; P, 10·4. $C_{14}H_{25}O_3N_2P$ requires N, 9·3; P, 10·3%).

The *methosulphate*, prepared in dry benzene, was an oil which could not be induced to crystallise. It was analysed after several precipitations from alcohol with ether, and, finally, stripping at 0·5 mm. (Found: N, 4·9; P, 6·3. $C_{16}H_{31}O_7N_2PS$ requires N, 6·6; P, 7·3%).

Dichloro-ethylphosphine Oxide.—The crude ethylphosphonic acid obtained by hydrolysing dibutyl ethylphosphonate (199·5 g., 0·9 mole) with concentrated hydrochloric acid for 16 hours and evaporating the reaction mixture to dryness, finally under oil-pump vacuum, was dissolved in chloroform (500 ml.). To the stirred solution, phosphorus pentachloride (625 g., 3 moles) was added in portions during ¾ hour. After a further 15 hours' stirring at room temperature, the reaction was completed by 1 hour's refluxing. Excess of phosphorus pentachloride was then destroyed by passing a stream of sulphur dioxide through the ice-cold mixture for several hours. The solution was placed under vacuum to remove dissolved gases and was then evaporated *in vacuo*, and the residue fractionated *in vacuo* through a short column equipped with a stillhead with controllable reflux-ratio. The main fraction (104 g., 78·5%) consisted of *dichloro-ethylphosphine oxide*, b. p. 70—72°/14—15 mm., n_D^{20} 1·4659 (Found: Cl, 48·8. $C_2H_5OCl_2P$ requires Cl^-, 48·3%).

Chloro-ethyl-diethylaminophosphine Oxide.—A solution of diethylamine (36·5 g., 0·5 mole) in light petroleum (150 ml.; b. p. 40—60°) was slowly added during 3 hours to a stirred solution of dichloro-ethylphosphine oxide (49 g., 0·33 mole) in light petroleum (250 ml.; b. p. 40—60°) cooled in ice–salt. After a further hour's stirring at room temperature, the mixture was kept overnight. The precipitated solid was filtered off and washed with light petroleum, the filtrate was evaporated, and the residue distilled in a vacuum. The main fraction (32·3 g.), b. p. 110—124°/10 mm., was redistilled, giving 28·7 g. (63%) of *chloro-ethyl-diethylaminophosphine oxide*, n_D^{20} 1·4669 (Found: N, 8·0; P, 15·7; Cl^-, 18·4. $C_6H_{15}ONClP$ requires N, 7·6; P, 16·9; Cl, 19·4%).

m-(*Ethyl Diethylaminophosphinato*)-N-*dimethylaniline.*—*m*-Dimethylaminophenol (6·85 g.) was added to ethanolic sodium ethoxide (1·42 g. of sodium; 80 ml. of dry ethanol) in a nitrogen atmosphere, and chloro-ethyl-diethylaminophosphine oxide (11·3 g.) was added with stirring.

Procedure as for the preparation of the phosphonato-compound given above gave the *base* (78%), b. p. 132—134°/2·8 × 10^{-4} mm., n_D^{20} 1·5295 (Found : N, 9·8; P, 10·2. $C_{14}H_{25}O_2N_2P$ requires N, 9·9; P, 10·9%).

The authors are indebted to Drs. Hobbiger and Keele for very helpful co-operation, and to Mr. Whiting for technical assistance.

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[*Received, October 18th,* 1951.]