Hydroxypyridine and Hydroxyquinoline Phosphates as Anti-cholinesterases.

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Dialkyl phosphoric esters of hydroxypyridines and hydroxyquinolines have been prepared. The moderate anti-cholinesterase activity of these compounds is enhanced by conversion into the quaternary salts, only one of which was obtained crystalline.

In a previous paper (*J.*, 1952, 780) we described the preparation of phosphoric esters which structurally resembled "Prostigmin." Since the anti-cholinesterase activities of carbamates of 3-hydroxypyridinium salts have been reported on several occasions (Roche Products, B.P. 613,168; Blaschko, Bülbring, and Chou, *Brit. J. Pharmacol.*, 1949, **4**, 29; Wuest and Sakal, *J. Amer. Chem. Soc.*, 1951, **73**, 1210; Casier and Verbecke, *Arch. int. Pharmacodyn.*, 1950, **83**, 452), we have extended our work to the preparation of phosphoric esters of 3-hydroxypyridine and various hydroxyquinolines.

When the potassium or sodium salt of 3-hydroxypyridine reacted with diethyl or di*iso*propyl phosphorochloridate, oils were obtained which could be distilled in a high vacuum; reactions with ethyl *NN*-diethylphosphoramidochloridate and *NNP*-triethylphosphoramidic chloride gave similar products. Relevant data, including toxicities and inhibition of "true" cholinesterase *in vitro*, are given in the Experimental section.

Similarly, 3-, 4-, or 8-hydroxyquinoline, treated with diethyl phosphorochloridate, yielded the corresponding derivatives as distillable oils. Although 3-(di*iso*propoxyphosphinyloxy)quinoline could be made without difficulty, the dimethyl homologue decomposed on attempted high-vacuum distillation into a black resin. By analogy with the behaviour of the *m*-(dimethoxyphosphinyloxy)-*N*-dimethylaniline described in Part V (*loc. cit.*), we believe that betaine may have been formed.

All tertiary bases, with the exception of 3-(diethoxyphosphinyloxy)quinoline, failed to yield crystalline quaternary salts although a wide range of alkyl and aralkyl halides was employed: analyses of liquid methobromides showed them to be substantially the required compounds. When reactions with the halides were attempted above room temperature, the ether-insoluble oils produced contained very little ionisable halogen: this can only be interpreted as the formation of a betaine. Since we had shown that the tertiary bases were stable at those temperatures, this reaction must be represented as an anionic dealkylation similar to those observed by Clark and Todd (*J.*, 1950, 2031).

Crystalline 3-(diethoxyphosphinyloxy)-*N*-methylquinolinium methyl sulphate, desig-

nated Ro 3-0422, showed *in vitro* the highest anti-cholinesterase activity so far recorded in the literature for a phosphorus compound, namely I_{50} (red cell) $= 1{\cdot}5 \times 10^{-10}$. This value is over 300 times the potency of " Prostigmin " against " true " cholinesterase (Hobbiger, *Brit. J. Pharmacol.*, 1954, in the press); the toxicity was correspondingly high (by intravenous route in mice, $LD_{50} = 0{\cdot}023$ mg./kg.).

In view of its pharmacological interest, an investigation of its hydrolysis was carried out. A sample, stored at 25° in an atmosphere of 70% humidity for seven weeks, had lost nearly all its activity ($I_{50} = 2{\cdot}7 \times 10^{-7}$) and gave the same yield of picrate (m. p. 239—241°) as was obtained from an equivalent amount of 3-hydroxy-*N*-methylquinolinium methyl sulphate. Paper chromatography showed, as expected, that breakdown consisted of simple hydrolysis to diethyl hydrogen phosphate and 3-hydroxy-*N*-methylquinolinium methyl sulphate: with solvent systems (upper layers) of ethyl acetate : pyridine : water (100 : 45 : 100) and *tert.*-amyl alcohol : water : formic acid (3 : 3 : 1 and 2 : 2 : 1), aged material gave only two spots, showing R_F values identical with those of authentic samples. Since the products of hydrolysis have little anti-cholinesterase activity, the percentage hydrolysis of solutions of Ro 3-0422 could be estimated from their activity. The following results were obtained with 0·1% solutions:

	I_{50} (red cells)	Hydrolysis (%)		I_{50} (red cells)	Hydrolysis (%)
Fresh solution	$1{\cdot}5 \times 10^{-10}$	—	After 3 weeks at 20°	$3{\cdot}3 \times 10^{-10}$	55
After 3 weeks at 4°...	$2{\cdot}3 \times 10^{-10}$	33	After 3 weeks at 45°	$2{\cdot}5 \times 10^{-8}$	99·4

Experimental

3-(*Diethoxyphosphinyloxy*)*pyridine* (Ro 3-0346).—3-Hydroxypyridine (4·75 g.) was dissolved in a solution of sodium ethoxide prepared from metallic sodium (1·3 g.) and dry ethanol (80 ml.). Diethyl phosphorochloridate (9·75 g.) was added dropwise, with stirring, to the resultant solution during 15 min. The mixture was then boiled for 30 min. and then left overnight. Next day, the sodium chloride was filtered off and the fitrate evaporated in a vacuum. The residual oil was dissolved in carbon tetrachloride (*ca.* 40 ml.) and washed three times with 0·5N-sodium hydroxide and then with water. After being dried (Na_2SO_4), the solvent was evaporated and the residue distilled in a high vacuum, to yield 8·5 g. (73%) of 3-(*diethoxyphosphinyloxy*)*pyridine* as a colourless oil, b. p. 82°/$5{\cdot}8 \times 10^{-6}$ mm., n_D^{22} 1·4752 (Found: N, 6·1; P, 13·1. $C_9H_{14}O_4NP$ requires N, 6·1; P, 13·4%).

The following tertiary bases were prepared similarly:

3-(*Diisopropoxyphosphinyloxy*)*pyridine* (Ro 3-0351) (69%), b. p. 84°/$1{\cdot}5 \times 10^{-4}$ mm., n_D^{22} 1·4687 (Found: N, 5·6; P, 11·9. $C_{11}H_{18}O_4NP$ requires N, 5·4; P, 12·0%).

3-[*Diethylamino*(*ethoxy*)*phosphinyloxy*]*pyridine* (Ro 3-0347) (84%), b. p. 87°/4×10^{-3} mm., n_D^{22} 1·4852 (Found: N, 10·4; P, 11·7. $C_{11}H_{19}O_3N_2P$ requires N, 10·8; P, 12·0%).

3-[*Diethylamino*(*ethyl*)*phosphinyloxy*]*pyridine* (Ro 3-0352) (35%), b. p. 98°/$2{\cdot}8 \times 10^{-4}$ mm., n_D^{22} 1·5030 (Found: N, 11·5; P, 12·0. $C_{11}H_{19}O_2N_2P$ requires N, 11·6; P, 12·8%).

3-(*Diethoxyphosphinyloxy*)*quinoline* (Ro 3-0419) (52%), b. p. 120—124°/$1{\cdot}1 \times 10^{-5}$ mm., n_D^{21} 1·5360 (Found: N, 4·9; P, 10·7. $C_{13}H_{16}O_4NP$ requires N, 5·0; P, 11·0%).

3-(*Diisopropoxyphosphinyloxy*)*quinoline* (Ro 3-0433) (69%), b. p. 120—124°/$2{\cdot}3 \times 10^{-5}$ mm. (Found: N, 4·7; P, 10·0. $C_{15}H_{20}O_4NP$ requires N, 4·5; P, 10·0%).

4-(*Diisopropoxyphosphinyloxy*)*quinoline* (Ro 3-0617) (37%), b. p. 112°/$9{\cdot}2 \times 10^{-5}$ mm., n_D^{23} 1·5212 (Found: N, 4·5; P, 9·6. $C_{15}H_{20}O_4NP$ requires N, 4·5; P, 10·0%).

8-(*Diethoxyphosphinyloxy*)*quinoline* (Ro 3-0417) (57%), b. p. 133—137°/$5{\cdot}2 \times 10^{-5}$ mm., n_D^{21} 1·5447 (Found: N, 5·1; P, 10·3. $C_{13}H_{16}O_4NP$ requires N, 5·0; P, 11·0%).

Ro	I_{50} (Red cells)	LD_{50} (mg./kg. mice, i.v.)
3–0346	5×10^{-7}	0·14; 0·4
,, (Methobromide)	2×10^{-8}	3·2
3–0351	1×10^{-6}	6·0
,, (Methobromide).....	*ca.* 5×10^{-9}	0·33
3–0347	*ca.* 1×10^{-5}	7·7
,, (Methobromide).....	$>1 \times 10^{-5}$	100
3–0352	—	75
,, (Methobromide).....	1×10^{-5}	75
3–0419	$2{\cdot}2 \times 10^{-8}$	1·0
3–0433	$7{\cdot}8 \times 10^{-8}$	—
3–0417	—	37
3–0422	$1{\cdot}5 \times 10^{-10}$	0·023

3-(*Diethoxyphosphinyloxy*)-N-*methylquinolinium Methyl Sulphate* (Ro 3-0422).—Methyl sulphate (0·75 ml.) was added to a solution of 3-(diethoxyphosphinyloxy)quinoline (1·4 g.) in dry benzene (5 ml.), and the mixture was kept at room temperature for 24 hr. The separated oil crystallised on trituration with ether, to give a solid, m. p. 105—107°. Recrystallisation by dissolving it in alcohol and adding dry ether to turbidity gave the *salt*, m. p. 109—110° (yield 72%). Two further crystallisations gave long, colourless needles, m. p. 111—112° (Found: N, 3·9; P, 7·3. $C_{15}H_{22}O_8NPS$ requires N, 3·4; P, 7·6%). The analogous *picrate*, prepared in aqueous solution, had m. p. 135—137° (softened at 108—110°) (Found: C, 46·2; H, 4·0. $C_{20}H_{21}O_{11}N_4P$ requires C, 45·8; H, 4·0%).

3-*Hydroxy*-N-*methylquinolinium Methyl Sulphate*.—Prepared from 3-hydroxyquinoline and methyl sulphate in alcohol at room temperature, this compound had m. p. 176—178° after two recrystallisations from alcohol and gave only one spot on a paper chromatogram (*n*-butanol–water–acetic acid, 10 : 7 : 3; Whatman No. 1 paper) (Found: C, 48·9; H, 5·0. Calc. for $C_{11}H_{13}O_5NS$: C, 48·7; H, 4·8%). The *picrate* prepared in aqueous solution had m. p. 239—241° (Found: C, 49·9; H, 3·1. $C_{16}H_{12}O_8N_4$ requires C, 49·5; H, 3·1%).

Cragoe, Robb, and Bealor (*J. Org. Chem.*, 1953, **18**, 552) give m. p. 163—165° for 3-hydroxy-*N*-methylquinolinium methyl sulphate. We found that when heat was applied in the preparation of this quaternary salt a lower m. p. product was obtained. A paper chromatogram showed the material to be non-homogeneous; the impurity preceded the main spot when the above solvent system was used.

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