RESEARCH PAPERS

THE PHOSPHORYLATION OF ANTI-ADRENERGIC QUATERNARY AMMONIUM SALTS RELATED TO CHOLINE

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Quaternary ammonium salts related to choline with anti-adrenergic properties have been phosphorylated. The resulting betaines were used to investigate a possible means for improving the oral absorption of quaternary ammonium salts. One of these was converted to the α-glycerophosphate for similar study.

THE utility of bretylium for the treatment of hypertension has been criticised because of poor and possibly erratic absorption of oral doses (Dollery, Emslie-Smith and McMichael, 1960). The degree of absorption of oral doses of guanethidine, a drug often compared to bretylium, may be little better than that of bretylium (Dollery, Emslie-Smith and Milne, 1960) but so far there has been no suggestion of an erratic absorption of this drug. Some evidence for uneven absorption of oral bretylium can be found in the results of Duncombe and McCoubrey (1960) and this could arise from the peculiar absorption characteristics of quaternary ammonium salts in general (Levine, Blair and Clark, 1955). They appear to be rapidly absorbed by the intestine for a short time but the rate soon declines to a very low value. The greater part of a dose is not absorbed.

In attempts to overcome this difficulty, analogues of bretylium bearing a choline unit of structure were esterified to give betaines which were anticipated to have pK values more suited to intestinal absorption (Hogben, Tocco, Brodie and Schachter, 1959) yet suffer hydrolysis by tissue enzymes after absorption to regenerate the parent quaternary ammonium salt.

Initial experiments were made with a succinyl derivative (I) (Coker and Copp, 1960) but the pharmacological results were disappointing. Phosphorylcholine is hydrolysed at a moderate rate by non-specific phosphomonoesterases (Roche and Bouchilloux, 1947) and better results were obtained with a weakly acid phosphoric ester betaine (II; R = *o*-chlorobenzyl) (Boura and McCoubrey, 1962). Conversely, the strongly acid diphosphoryl compound (III), given orally, was less readily absorbed than the compound (II; R = *o*-chlorobenzyl) though it was hydrolysed *in vitro* by both acid and alkaline phosphatase from rat liver and intestine respectively. It had moderate activity by the subcutaneous route.

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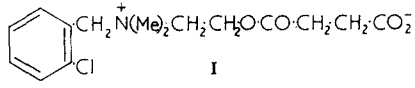
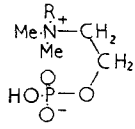


I



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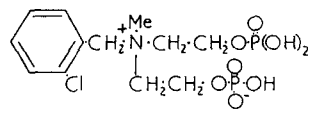


III

Choline can be phosphorylated by various methods (Baer, 1952), the simplest by heating with polyphosphoric acid (Beznák and Chain, 1937). The compounds chosen for phosphorylation gave moderate yields of betaines by this method. During purification, the removal of the excess of phosphoric acid was troublesome. Part of the barium phosphate produced by neutralisation with baryta assumed a colloidal state, though on a few occasions it came down wholly as a heavy precipitate. The conditions for complete precipitation were not discovered. Preliminary boiling to hydrolyse meta- and pyro-phosphoric acids had no influence. In initial experiments the products were difficult to crystallise. Paper chromatography showed that they contained traces of phosphoric acid and when this was removed by passing methanolic solutions down a column of Amberlite IR 45 (OH^- form) they crystallised readily. In this process methanol was used to suppress ionisation of the betaine while allowing the first ionisation of phosphoric acid so that the betaine could pass through the column whilst phosphoric acid was retained.

For the preparation of a ^{14}C-labelled betaine the excess of phosphoric acid was neutralised by ammonia and the bulk of the ammonium phosphate was precipitated by ethanol. Ammonia was then removed by passage down a column of Amberlite IR 120 (H^+) and residual phosphoric acid precipitated by shaking with silver oxide. The soluble silver salt of the betaine was then decomposed by hydrogen sulphide.

Glycerophosphates are not hydrolysed by phosphomonoesterases (Schmidt, Greenbaum, Fallot, Walker and Thannhauser, 1955) though they are readily attacked by phosphodiesterases. It was considered that presentation of an anti-adrenergic agent as a glycerophosphoryl ester betaine, a neutral molecule, might alter the pattern of distribution of the drug, and, in particular, might allow the agent to penetrate into the brain, which contains a phosphodiesterase (Webster, Marples and Thompson, 1957). BW 171C60 (II; R = *o*-chlorobenzyl) was therefore converted into the α-glycerophosphate. The method was not sterospecific and involved preparation of the allyl ester of BW 171C60 and subsequent hydroxylation by alkaline permanganate. Hydroxylation by the method of Woodward, Gunstone and Morris (1957) was unsuccessful and led to loss of the phosphoric ester group, possibly by intramolecular cleavage of a carbonium ion arising by abstraction of bromide ion. The glycerophosphate (BW 564C61) was purified as its cadmium chloride complex by the method used for glycerophosphorylcholine (Tattrie and McArthur, 1955).

Experimental

The dihydroxyphosphinyloxyethylammonium betaines of Table I were prepared in 60–70 per cent yield by the method of Beznák and Chain (1937). The bulk of the barium phosphate was removed by centrifuging and the cloudy supernatant was clarified by filtration through a Sterimat pad. The filtrate was adjusted to pH 4 by 2N sulphuric acid, barium sulphate filtered off and the filtrate evaporated to dryness. The residue was dissolved in 3 parts warm anhydrous methanol and the solution passed through a column (30 × 1 cm.) of Amberlite IR 45 (OH^-) that had been previously washed with water and methanol. The effluent was collected from the first appearance of the betaine and it was washed through by 6 parts of methanol. A small quantity of the betaine remained on the column and could be recovered by further washing. The column could be used repeatedly without regeneration. Evaporation of the effluents gave white residues that crystallised readily from the appropriate solvent (see Table I).

Aqueous solutions of the betaines (1 per cent) had pH approximately 4.

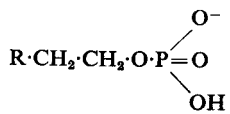
N-o-*Chlorobenzyl*-NN-*di*[2-(*dihydroxyphosphinyloxyethyl*]-N-*methyl*-*ammonium betaine* (BW 293C60) was prepared in 40 per cent yield by phosphorylation of *N-o*-chlorobenzyl-*NN*-di(2-hydroxyethyl)-*N*-methylammonium iodide by the method mentioned above. It crystallised readily from ethanol without need for removal of adsorbed phosphoric acid. The white prisms had m.p. 220–223°. (Found: P, 14·8. $C_{12}H_{20}ClNO_8P_2$ requires P, 15·2 per cent.) An aqueous solution (0·2 per cent) had pH 2·4.

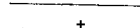
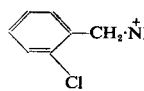
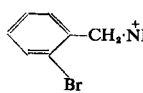
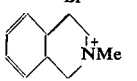
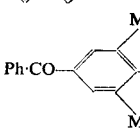
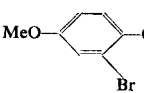
N-o-*Chlorobenzyl*-N-2-*hydroxyethyl*-N-*methyl*-N-[^{14}C]*methylammonium iodide* (BW 329C57). *N-o*-Chlorobenzyl-*N*-2-hydroxyethyl-*N*-methylamine (Coker and Copp, 1960) (211 mg.) was dissolved in ethyl methyl ketone (1 ml.). [^{14}C]Methyl iodide (142 mg.; 1 mc) was condensed on to the frozen solution at −80°. The mixture was allowed to regain room temperature in a stoppered tube, when a crystalline mass formed within 3 hr. After keeping overnight, ethyl acetate (0·5 ml.) was added and after 1 hr. the precipitated solids were centrifuged down, washed with ethyl methyl ketone and finally dried *in vacuo*. The product had m.p. 85–88° (335 mg.; 95 per cent).

N-o-*Chlorobenzyl*-N-2-(*dihydroxyphosphinyloxy*)*ethyl*-N-*methyl*-N-[^{14}C]*methylammonium betaine* (BW 171C60). The above ^{14}C-labelled material (335 mg.; 1 mmole) was heated on a steam-bath with phosphoric acid (90 per cent; 2 g.) under vacuum with a coarse air leak directed to the top of the liquid until the theoretical loss in weight (0·33 g.) had occurred (1 hr.). Phosphorus pentoxide (1 g.) was added and the heating continued for 8 hr. The product was dissolved in water (3 ml.) and warmed gently for 30 min. Aqueous ammonia (30 per cent) was added slowly with cooling until the solution was alkaline. Ammonium phosphates were precipitated by ethanol (75 ml.). The mixture was cooled to 0° and centrifuged and the residue washed with ethanol. The combined supernatants were evaporated to small bulk and passed down a column (10 × 1 cm.) of Amberlite IR 120 (H^+). The effluent was shaken with

TABLE I

2-(DIHYDROXYPHOSPHINYLOXY)ETHYLAMMONIUM BETAINES



R	M.p.	Solvent	Formula	Found			Required		
				C	H	H_2O	C	H	H_2O
	215–216°	ethanol	$C_{11}H_{18}NO_4P \cdot H_2O$	47·1	7·2	6·7	47·6	7·2	6·5
 (*a*)	222–223°	ethanol-ethyl acetate	$C_{11}H_{17}ClNO_4P \cdot H_2O$	41·9	6·0	6·7	42·4	6·1	5·8
 (*b*)	218–219°	ethanol-acetone	$C_{11}H_{17}BrNO_4P \cdot H_2O$	37·1	5·2	5·5	37·1	5·3	5·1
	237–238°	methanol-ether	$C_{12}H_{18}NO_4P \cdot H_2O$	49·1	6·6	5·0	49·8	6·9	6·2
	206–207°	ethanol-ethyl acetate	$C_{21}H_{28}NO_6P \cdot H_2O$	57·7	7·1	4·7	57·4	6·9	4·1
 (*c*)	49–51°	—	$C_{13}H_{21}BrNO_6P \cdot 3H_2O$	34·4	5·7	12·0	34·5	6·0	12·0

(*a*) Cl: Found, 11·0; Required: 11·0 per cent
(*b*) N: Found, 4·0; Required: 3·9 per cent
(*c*) Dried Material—Br: Found, 20·0; Required: 20·1 per cent

fresh silver oxide (from silver nitrate, 0·5 g.) and solids were centrifuged down. The soluble silver salt of the betaine was decomposed by hydrogen sulphide and the mixture filtered and evaporated to dryness. The residue was diluted with unlabelled BW 171C60 (160 mg.) and crystallised from ethanol. Radio-assay of the product (280 mg.) indicated 0·475 mc/mmole (48 per cent). Autoradiographs of chromatograms showed freedom from starting material and one spot at the position occupied by BW 171C60.

N-o-Chlorobenzyl-N-2-[(±)-α-*glyceryloxy(hydroxy)phosphinyloxy*]-*ethyl*-NN-*dimethylammonium betaine* (BW 564C61). BW 171C60 (3 g.) in water (15 ml.) was shaken with fresh silver oxide (from 1·8 g. silver nitrate) for 30 min. and the solids were filtered off. The solution was evaporated to dryness and suspended in ethanol (45 ml.) and allyl bromide (0·9 ml.) added. The mixture was heated at 50° for 1·5 hr., filtered, and the filtrate was evaporated to dryness. The residue was dissolved in water (5 ml.) and passed down a column (10 × 1 cm.) of Amberlite IRA 400 (OH^-). The effluent was collected so long as small portions decolourised bromine water. It was evaporated to dryness and the allyl ester crystallised from acetone-ethanol in white needles, m.p. 96–97° (2·2 g.; 73 per cent). (Found: C, 47·5; H, 6·6; N, 3·8; Loss at 100°, 4·6. $C_{14}H_{22}ClNO_4P{\cdot}H_2O$ requires C, 47·2; H, 6·5; N, 4·0; Loss at 100°, 5·1 per cent).

TABLE II

AVERAGE R_F VALUES FOR THE ABOVE PRODUCTS VISUALISED BY DRAGENDORFF'S REAGENT

Solvent system	BW 329C57	BW 171C60	BW 171C60 allyl ester	BW 564C61
Propanol-ammonia (25 per cent) (6:4) ..	0·84	0·51	—	0·63
s-Butanol-acetic-water (12:5:3)	0·75	0·47	0·73	0·53
Pyridine-n-butanol-ammonia (1:1:1)	0·75	not visualised	0·66	not visualised

The above ester (2·9 g.) was dissolved in water (150 ml.) and 2N sodium carbonate added to give pH 8–9. The solution was cooled to 0° and potassium permanganate (1·8 g.) in water (50 ml.) added with stirring during 15 min. The mixture was adjusted to pH 7 and manganese oxides filtered off. Traces of permanganate in the filtrate were destroyed by bisulphite and the solution was evaporated to dryness. The residue was extracted with hot ethanol and the extract evaporated to small bulk before adding saturated ethanolic cadmium chloride solution. The crystalline precipitate was collected, washed with ethanol, and dried *in vacuo* over phosphorus pentoxide. It melted over the range 112–176°. (Found: C, 24·0; H, 3·9; N, 2·0; P, 4·1; total Cl, 21·2; ionisable Cl, 16·5; loss at 100°, 5·0. $C_{14}H_{23}ClNO_6P{\cdot}1\frac{3}{4}\ CdCl_2{\cdot}2H_2O$ requires C, 24·2; H, 3·7; N, 1·9; P, 4·2; total Cl, 21·8; ionisable Cl, 17·0, loss at 100°, 4·9 per cent).

The product gave a positive chromotropic acid test and was oxidised by periodate. For pharmacological study the product was dissolved in water and shaken with excess silver carbonate at room temperature for

1 hr. Solids were removed and soluble silver precipitated by hydrogen sulphide. The solution was shaken with activated charcoal and filtered, evaporated to dryness and redissolved in butanol. A small amount of solid was removed and the butanol was removed *in vacuo*. The product was a colourless syrup. Yield, 2·25 g. (74 per cent). Chromatograms revealed a trace of BW 329C57 due to hydrolysis.

Chromatographic information is given in Table II.

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