503. *Phospholipids. Part II.* The Stability of Some Derivatives of 2-Aminoethyl Phosphate.*

By D. M. Brown and G. O. Osborne.

Attempts to form 2-aminoethyl dibenzyl phosphate by addition of dibenzyl hydrogen phosphate to ethyleneimine yielded only ethyleneimine polymers. 2-Benzyloxycarboxyamidoethyl diethyl phosphate underwent hydrogenolysis to give a solution containing ethyleneiminium diethyl phosphate, rapid formation of the latter from the intermediate 2-aminoethyl diethyl phosphate being presumed. The mechanism of this conversion, also undergone by 2-aminoethyl diphenyl phosphate, is discussed.

An explanation of the "diazometholysis" reaction of Baer and Maurukas [1] is advanced on the basis of these observations.

Considerable attention has been paid recently to the effect of a neighbouring hydroxyl group on the hydrolysis of di- and tri-alkyl phosphates in relation to nucleic acid chemistry.[2, 3] The increase in rate of hydrolysis due to the vicinal function is dependent on a base- or acid-catalysed internal transesterification, followed by rupture of the readily hydrolysed intermediate cyclic phosphate ester. We are studying the effects of other vicinal functions, and the present experiments deal with the amino-group since the 2-aminoethylphosphoryl group and its derivatives are characteristic of many phospholipids. It was hoped, too, to throw some light on the biological action of the "nitrogen mustards," in view of the suggestion [4] that their cytotoxic effects are due *inter alia* to reaction with the phosphate residues of the nucleic acids.

Although Christensen [5] obtained 2-aminoethyl dihydrogen phosphate by interaction of ethyleneimine and syrupy phosphoric acid, we failed to obtain the corresponding dibenzyl ester by using dibenzyl hydrogen phosphate. Materials containing both phosphorus and nitrogen were formed but these were salt-like and appeared to be the dibenzyl phosphates of ethyleneimine and its polymers. Polymerisation of the imine is acid-catalysed,[6] which may partly explain the non-formation of the 2-aminoethyl ester.

As another route, 2-benzyloxycarboxyamidoethanol was phosphorylated with diethyl phosphorochloridate to yield the oily ester (I) which was readily hydrogenated over 10% palladised charcoal to an oil which gave analytical values only approximating to those required for the amine (II) and had none of the expected properties. In particular this product was water-soluble, but insoluble in most organic solvents, and did not yield the expected derivatives when treated with phenyl *iso*thiocyanate or picric acid. On paper chromatography it behaved as an equimolar mixture of ethyleneimine and diethyl hydrogen phosphate and formed polymeric amines on storage. Paper electrophoresis confirmed the salt-like nature of the products. We thus formulate the hydrogenation product as the diethyl phosphate (III) of ethyleneimine and its polymers. In accord with this, passage of its solution through a cation-exchange resin afforded diethyl hydrogen phosphate, which was characterised as the barium salt. We also find that when the hydrobromide of 2-aminoethyl diphenyl phosphate [7] (II; Ph in place of Et) is basified with sodium carbonate, diphenyl hydrogen phosphate is rapidly formed, together with small amounts of phenyl dihydrogen phosphate; from more concentrated solution an oil

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* Part I, *J.*, 1957, 2034.

[1] Baer and Maurukas, *J. Biol. Chem.*, 1955, **212**, 39.

[2] Brown and Todd, in "The Nucleic Acids," ed. Chargaff and Davidson, Academic Press, New York, 1955, Vol. I., p. 409.

[3] Brown, Magrath, Neilson, and Todd, *Nature*, 1956, **177**, 1124.

[4] For refs. see Ross, *Adv. Cancer Res.*, 1953, **1**, 397.

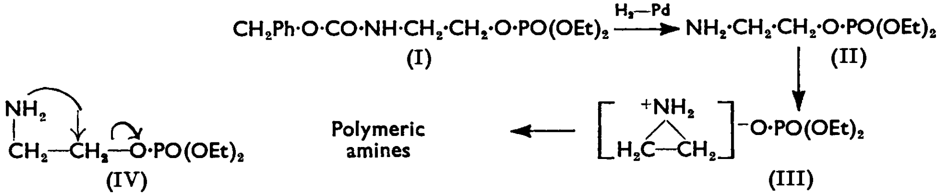
[5] Christensen, *J. Biol. Chem.*, 1940, **135**, 399.

[6] Barb, *J.*, 1955, 2564 and refs. therein cited.

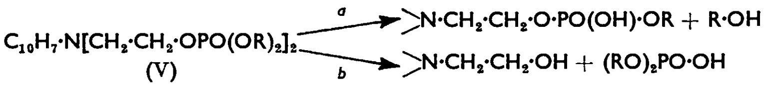
[7] Baer and Stancer, *Canad. J. Chem.*, 1956, **34**, 436.

separates which yields on crystallisation the diphenyl hydrogen phosphate of 2-aminoethyl diphenyl phosphate.

Thus it is evident that in systems of type (II), the vicinal amino-group shows no tendency to attack on phosphorus with formation of a cyclic phosphoramidate, but that the preferred reaction depends on attack on carbon with loss of the phosphate anion by alkyl–oxygen fission. Trialkyl phosphates can *N*-alkylate aromatic amines,[8] whereas claims for their conversion into phosphoramidates by aminolysis are of doubtful validity.[9] Formation of the iminium salt (III), presumably *via* the aminoethyl ester (II) by a process of type (IV) has direct analogies, for example in the chemistry of the " nitrogen mustards." These, essentially derivatives of 2-aminoethyl chloride, exist in aqueous solution in equilibrium with the corresponding iminium chloride, concomitant hydrolysis and dimerisation also occurring.[4] The polymerisations encountered in the present work furnish one explanation for the imperceptibility of the back-reaction (III ⟶ II). Moreover Ross and Davis [10] observe that diethyl and diphenyl hydrogen phosphate have very low



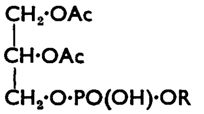
" competition factors " for an *N*-aryldi-2-chloroethylamine in comparison with many other anions including monoalkyl phosphates. They also studied the hydrolysis of two compounds (V; R = Et and Ph) by acid and alkali: except in alkaline hydrolysis of (V; R = Ph) where fission at the phenoxy-linkage (*a*) is exclusive, preferential but not exclusive hydrolysis at the aminoethyl linkage (*b*) occurs. Thus the direction of fission (*b*) of these compounds is essentially the same as that of the ester (II) but intrusion of route (*a*) must depend on the low basicity of the arylamino-group and the greater ease of alkaline hydrolysis of phenyl than of alkyl phosphates.[11] We observe that when the



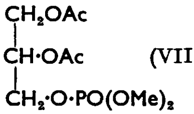
benzyloxycarbonyl derivative (I) is treated with alkali a substance, tentatively identified as 2-aminoethyl ethyl hydrogen phosphate, is formed.

These observations also explain the " diazomethelysis " reaction of Baer and Maurukas.[1] In this, compounds of the cephalin type (VI; Ac = long-chain acyl residue) where R represents a 2-aminoethyl or *O*-serine, but not a choline, residue are converted by exhaustive treatment with diazomethane, into the corresponding diacylglycerol 1-(dimethyl phosphate) (VII). Methylation will yield a phosphotriester which will break down, as

(VI)



$$\begin{array}{l} CH_2{\cdot}OAc \\ | \\ CH{\cdot}OAc \\ | \\ CH_2{\cdot}O{\cdot}PO(OH){\cdot}OR \end{array}$$



$$\begin{array}{l} CH_2OAc \\ | \\ CH{\cdot}OAc \\ | \\ CH_2{\cdot}O{\cdot}PO(OMe)_2 \end{array}$$

(VII)

discussed above, with loss of the residue R as an imine, together, possibly, in the case of (VI; R = serine residue) by β-elimination.[12] Further methylation of the resultant

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[8] Thomas, Billman, and Davis, *J. Amer. Chem. Soc.*, 1946, **68**, 895.

[9] See Kosolapoff, " Organophosphorus Compounds," Wiley, New York, 1950, p. 238.

[10] Ross and Davis, *J.*, 1952, 4296.

[11] Plimmer and Burch, *J.*, 1929, 279.

[12] Riley, Turnbull, and Wilson, *Chem. and Ind.*, 1953, 1181.

monomethyl ester (VI; R = Me) will then yield (VII). Methylation of the cephalin is probably facilitated by partial suppression of zwitterion formation in the non-polar solvent chloroform–ether. 2-Aminoethyl dihydrogen phosphate reacted very incompletely in either suspension with diazomethane, presumably because of the insolubility of the starting material. "Diazomethiolysis" does not take place when R in (VI) is a choline residue. Since the compound is necessarily zwitterionic it is unlikely that the initial methylation could occur, but, even if it did, break down of the phosphotriester would not occur since the nitrogen atom is already in the quaternary state (cf. the stability of choline diphenyl phosphate [13]).

EXPERIMENTAL

The R_F values quoted in paper chromatographic experiments are for the solvent system butan-1-ol–water (86 : 14) on Whatman No. 1 paper.

2-Benzyloxycarboxyamidoethyl Diethyl Phosphate.—Diethyl phosphorochloridate [14] (3·6 g., 1·1 mol.) in chloroform (5 c.c.) was added to 2-benzyloxycarboxyamidoethanol [15] (3·6 g.) and pyridine (1·85 g., 1·1 mol.) in ice-cold dry carbon tetrachloride (20 c.c.). After 1 hr. the solution was allowed to come to room temperature and set aside overnight. Pyridine hydrochloride was removed and the solution washed with sodium hydrogen carbonate solution and water, then dried (Na_2SO_4). Removal of solvents gave an oily *ester* (5·5 g.) which distilled with decomposition. For analysis it was dried at room temperature for 6 hr. at 10^{-4} mm. over phosphoric oxide (Found: C, 51·2; H, 6·5; N, 4·35. $C_{14}H_{22}O_6NP$ requires C, 50·8; H, 6·7; N, 4·2%). The substance had R_F 0·9 when detected with a phosphate spray reagent.

Hydrogenation of 2-Benzyloxycarboxyamidoethyl Diethyl Phosphate.—The above triester (2·32 g.) was hydrogenated in 4 : 1 aqueous ethanol (40 c.c.) at atmospheric pressure over 10% palladised charcoal (0·43 g.) and palladium oxide (0·1 g.). Hydrogen uptake ceased after 1 hr. (145 c.c. absorbed; theor., 163 c.c. without allowance for evolution of carbon dioxide). After filtration the solvent was removed at reduced pressure below 40°, leaving a pale yellow oil (1·3 g.), soluble in water but insoluble in ether. Its infrared spectrum did not show the characteristic doublet in the 3μ region, associated with a primary amino-group. It gave, on paper chromatography, an elongated spot which was positive to the ninhydrin and the phosphate reagent, with R_F 0·4, whereas trialkyl phosphates have, in general, much higher R_F values.

The oil was submitted to electrophoresis on paper (Whatman No. 4) in ammonium acetate buffers of pH 4·0 and 1·5 with a potential drop of 150 v. In each case a spot positive to the phosphate and negative to the ninhydrin reagent migrated towards the anode at the same rate as an authentic specimen of diethyl hydrogen phosphate. A ninhydrin-positive streak (negative to the phosphate spray) migrated towards the cathode.

On paper chromatography of the oil in sodium hydrogen carbonate solution, the phosphate-positive region had R_F 0·2, corresponding to that of sodium diethyl phosphate, while ninhydrin-positive material occupied a streak, R_F 0·3—0·6. In the same solvent system similar ninhydrin-positive streaks were obtained with partially polymerised ethyleneimine; increasing polymerisation corresponded to increasing R_F value.

An aqueous solution of the oil was percolated through a column (10 × 1 cm.) of Dowex-50 resin (H form). The acidic effluent and aqueous washings were neutralised with barium hydroxide solution, and, after evaporation, the barium salt was precipitated as a colourless amorphous powder by addition of ethanol. Its infrared spectrum was identical with that of barium bis(diethyl phosphate). Paper chromatography in the solvent system propan-2-ol–1% aqueous ammonium sulphate (3 : 2) confirmed the identity.

Elution of the column with dilute aqueous ammonia gave material which corresponded on chromatograms to polymerised ethyleneimine. No further attempt was made to characterise it.

Reaction between Ethyleneimine and Dibenzyl Hydrogen Phosphate.—Ethyleneimine [16] and dibenzyl hydrogen phosphate were brought together in chloroform, water, or without solvent at various temperatures, in proportions varying from ten-fold excess of the imine to a two-fold excess of the acid. Chromatography, as above, showed that only polymerisation of the imine

[13] Baer, *J. Amer. Chem. Soc.*, 1947, **69**, 1253.
[14] Mastin, Norman, and Weilmuenster, *J. Amer. Chem. Soc.*, 1945, **67**, 1662.
[15] Rose, *ibid.*, 1947, **69**, 1384.
[16] Wenker, *ibid.*, 1935, **57**, 2328.

occurred. Dibenzyl hydrogen phosphate was always recovered when the products were treated with hydrochloric acid.

Action of Diazomethane on 2-Aminoethyl Dihydrogen Phosphate.—A suspension of the phosphate[17] (0·3 g.) was treated overnight with ethereal diazomethane (2 equiv.), then with a further 4 equiv. during two days. Residual undissolved material was removed by filtration. Paper chromatography of the ether solution in the propan-2-ol–1% aqueous ammonium sulphate solvent system revealed the presence of two substances, both positive to the ninhydrin and phosphate spray reagents. The first (R_F 0·42) corresponded to starting material; the second substance was probably 2-aminoethyl methyl hydrogen phosphate since its R_F value, 0·67, was lower than that expected for a triester. A material (positive to ninhydrin and phosphate reagents) with a closely similar R_F value, probably 2-aminoethyl ethyl hydrogen phosphate, was obtained when 2-benzyloxycarboxyamidoethyl diethyl phosphate was heated with sodium hydroxide solution. In neither experiment could we isolate the pure products.

Action of Sodium Carbonate on the Hydrobromide of 2-Aminoethyl Diphenyl Phosphate.—(a) The hydrobromide (240 mg.) in water (10 c.c.) was treated with N-sodium carbonate (2 c.c.). An emulsion was rapidly formed, from which an oil was deposited. This was extracted into ether, the ether solution dried (Na_2SO_4) and evaporated, and the residual oil crystallised from hot ethanol. It formed needles (31 mg.), m. p. 130°. The m. p. was undepressed on admixture with the *diphenyl hydrogen phosphate* of *2-aminoethyl diphenyl phosphate*, an authentic sample of which separated when solutions of the above hydrobromide and excess of sodium diphenyl phosphate were mixed. After recrystallisation from water it had m. p. 130° (Found: C, 56·7; H, 5·0. $C_{26}H_{27}O_8P_2N$ requires C, 57·4; H, 5·0%).

(b) *Chromatography.* When the hydrobromide was heated in a large excess of N-sodium carbonate at 50° for 10 min., paper chromatography in the propan-2-ol–1% ammonium sulphate system showed the presence of diphenyl hydrogen phosphate as the main phosphorus-containing product (R_F 0·85). Monophenyl dihydrogen phosphate (R_F 0·57) was also present together with a trace of a third substance, R_F 0·75, positive both to the phosphorus and the ninhydrin spray reagent. Confirmatory chromatograms were run in butan-1-ol–water (86 : 14).

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[17] Plimmer and Burch, *Biochem. J.*, 1937, **31**, 398.