XLIII.—*Esters of Phosphoric Acid. Part I. Phosphates of Cetyl Alcohol, Cholesterol, Chloroethyl Alcohol, and Ethylene Glycol.*

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CETYL dihydrogen phosphate (cetylphosphoric acid) has been described by Biehringer (*Ber.*, 1905, **38**, 3974) and cholesteryl phosphates have been prepared by Euler and Bernton (*Ber.*, 1927, **60**, 1720). The latter compounds had not been described when our work was started. Our object has been a study of not only the mono- but also the di- and tri-esters of cetyl alcohol and of cholesterol with phosphoric acid. Both these alcohols occur in nature as esters and their phosphates may likewise be present in animal or vegetable products.

Of the several methods of preparing esters of phosphoric acid the action of syrupy phosphoric acid, or phosphoric oxide, upon the alcohol has yielded the mono- or di-ester. Phosphoryl chloride and the alcohol yield intermediate compounds which are decomposed by water, giving the mono- and di-esters. By means of the sodium alcoholate a tri-ester was prepared by Limpricht (*Annalen*, 1865, **134**, 347). The tri-ester has been obtained from silver alkyl phosphate (Lossen and Kohler, *Annalen*, 1891, **262**, 195) and by the action of phosphorus pentachloride (Winssinger, *Bull. Soc. chim.*, 1888, **48**, 111).

The reaction between ethyl metaphosphate and alcohols was introduced by Langheld (*Ber.*, 1911, **44**, 2076). This method has been specially studied and the products of the reaction are described in Part II (following paper).

It has been necessary to try all the methods above mentioned in order to obtain the mono-, di- and tri-esters of cetyl alcohol and of cholesterol with phosphoric acid.

Carré (*Compt. rend.*, 1904, **138**, 374) prepared the phosphoric esters of ethylene glycol by heating the glycol and phosphoric acid together in molecular proportion. He isolated the mono-ester, but was unable to separate the di- and tri-esters.

Since it was likely that a mixture of esters would always be obtained by the use of glycol, it was considered that a better method would be to start from ethylene chlorohydrin and then to convert the chloroethyl phosphoric esters into the corresponding β-hydroxyethyl compounds. Barium chloroethyl phosphate was prepared by Fischer (*Ber.*, 1920, **53**, 1620) by the action of phosphoryl chloride on glycol.

The three chloroethyl phosphates have been made by us by the action of phosphoryl chloride on ethylene chlorohydrin and they have been converted into the mono- and di-β-hydroxyethyl esters. The trichloroethyl ester was hydrolysed in the process and gave di-β-hydroxyethyl hydrogen phosphate. The monochloroethyl ester was also prepared by the action of ethyl metaphosphate on ethylene chlorohydrin. In order to prepare tri-β-hydroxyethyl phosphate the reaction of ethylene chlorohydrin with trisodium phosphate was attempted, but only the mono-ester resulted. The reaction of phosphoryl chloride with the sodium derivative of glycol was used to obtain more complex esters.

The hydrolysis of the di- and tri-esters by acid and by alkali has also been studied. It was shown by Plimmer (*Biochem. J.*, 1913, **7**, 72) that ethyl dihydrogen phosphate, glycerophosphoric acid, and phytic acid were hydrolysed by acid, but were stable to alkali. Phosphoproteins were found by Plimmer and Scott (J., 1908, **93**, 1699) to be easily hydrolysed by alkali. There appears to be no information about the hydrolysis of the di- and tri-esters.

EXPERIMENTAL.

Barium Monocetyl Phosphate and Monocetyl Dihydrogen Phosphate.—3·5 G. of phosphoryl chloride (1 mol.) were added slowly to a mechanically stirred solution of 5 g. of cetyl alcohol in chloroform. The mixture was warmed under reflux on a water-bath for 2 hours, kept for 24 hours, and then poured into water and thoroughly shaken in order to dissolve the oil. The solution was made neutral to phenolphthalein with barium carbonate and barium hydroxide, the barium phosphate removed, and the filtrate concentrated and treated with an equal volume of alcohol. Crystals (5·5 g.) separated (yield, 60%). On recrystallisation from alcohol and water, pale yellow lustrous crystals of *barium monocetyl phosphate* were obtained (Found: H_2O, 4·4. Found in material dried at 110°: Ba, 29·8; P, 6·3.* $C_{16}H_{33}O_4PBa,H_2O$ requires H_2O, 3·7%. $C_{16}H_{33}O_4PBa$ requires Ba, 30·0; P, 6·8%).

Monocetyl dihydrogen phosphate was formed from the barium salt by the action of dilute sulphuric acid; it was obtained from the filtered concentrated solution. After crystallising from ether, it sintered at 61°, melted at 72°, and corresponded with the compound prepared by Biehringer (*loc. cit.*). The sodium salt, prepared by double decomposition with sodium sulphate, formed colourless crystals soluble in cold water.

Dicetylphosphoryl Chloride and Barium Dicetyl Phosphate.—A mixture of 9 g. of phosphoryl chloride and 25 g. of cetyl alcohol in chloroform was heated under reflux on a water-bath until hydrogen chloride ceased to be evolved. The solution was poured into a large excess of milk of lime and kept for 12 hours. The precipitate was filtered off and washed several times with water. Excess of lime was removed from the filtrate by means of carbon dioxide, and the filtered solution concentrated; 5 g. of a white crystalline powder, which consisted of *calcium monocetyl phosphate*, were obtained on evaporation (Found: Ca, 10·9; P, 8·7. $C_{16}H_{33}O_4PCa$ requires Ca, 11·1; P, 8·5%).

The precipitate was extracted with chloroform, and the chloroform solution evaporated to dryness. *Dicetylphosphoryl chloride* remained as a yellow powder, insoluble in water, soluble with difficulty in alcohol, and very easily soluble in chloroform (yield, 19 g. or 60%) [Found: C, 68·7; H, 11·7; Cl, 6·6; P, 6·0. $(C_{16}H_{33}O)_2POCl$ requires C, 68·1; H, 11·7; Cl, 6·3; P, 5·5%].

When dicetylphosphoryl chloride was boiled under reflux with excess of baryta solution for 10 hours, *barium dicetyl phosphate* formed as a yellowish grease on the surface; it solidified to a hard solid on cooling. It was washed with hot water to remove traces of barium monocetyl phosphate and recrystallised from aqueous alcohol [Found: Ba, 11·7; P, 4·7. $\{(C_{16}H_{33})_2PO_4\}_2Ba$ requires Ba, 11·2; P, 5·05%].

Owing to the insolubility of this salt and the readiness with which it is hydrolysed by acid, dicetyl hydrogen phosphate could not be prepared in a pure state. The acid softened at 64°.

In an attempt to obtain the intermediate cetylphosphoryl

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* All the phosphorus analyses recorded in this paper were made by Neumann's method as modified by Plimmer and Bayliss (*J. Physiol.*, 1906, **33**, 439).

chlorides which are formed by the action of phosphoryl chloride upon the alcohol (compare Wichelhaus, *Annalen*, 1868, Suppl. Bd. **6**, 257), cetyl alcohol and phosphoryl chloride in molecular proportion were heated under reflux on a water-bath for 6 hours, and the product fractionated in a vacuum. No compound could be separated other than cetene, b. p. 153°/15 mm. (compare Dumas and Peligot, *Ann. Chim. Phys.*, 1836, **62**, 8) (Found: C, 85·2; H, 14·8; *M*, ebullioscopic in alcohol, 230. Calc. for $C_{16}H_{32}$: C, 85·7; H, 14·3%; *M*, 224).

Tricetyl Phosphate.—5 G. of cetyl alcohol were heated on a water-bath, and 3 g. of phosphorus pentachloride added very gradually. The yellow waxy product was heated for 2 hours, shaken with several changes of hot water, dried in a vacuum, and crystallised from ligroin, soft, colourless, cubic crystals, m. p. 61°, very easily soluble in chloroform and benzene, being obtained [Found: C, 74·1; H, 12·3; P, 3·95. Calc. for $(C_{16}H_{33})_3PO_4$: C, 74·6; H, 12·85; P, 4·0%].

This compound was also obtained by boiling excess of cetyl alcohol with phosphoric oxide in presence of ether (Cavalier's method, *Ann. Chim. Phys.*, 1899, **18**, 499) in an attempt to prepare dicetyl hydrogen phosphate. To 15 g. of cetyl alcohol, dissolved in ether, 2·5 g. of phosphoric oxide were quickly added. The mixture was heated under reflux until no solid separated on cooling (about 7 days). The ether was removed by evaporation, and the residue crystallised from alcohol, a white crystalline solid, m. p. 61°, being obtained (Found: P, 3·9%). It gave sodium dicetyl phosphate on boiling with dilute caustic soda solution.

Barium Monocholesteryl Phosphate.—4·2 G. (1 mol.) of phosphoryl chloride were slowly added to a mechanically stirred solution of 12 g. of cholesterol in chloroform. After 24 hours the mixture, which had become red, was shaken with a large volume of water and kept for 24 hours. The water was separated and made neutral to phenolphthalein with barium carbonate and baryta. Barium phosphate was filtered off, and the filtrate concentrated and treated with an equal volume of alcohol. A gelatinous precipitate of barium cholesteryl phosphate, barium phosphate, and barium chloride was produced. Repeated crystallisation from aqueous alcohol gave a small yield of *barium monocholesteryl phosphate*, which crystallised in fine white platelets from alcohol and water (Found: H_2O, 10·8; P, 4·6; Ba, 20·15. $C_{27}H_{45}O_4PBa,4H_2O$ requires H_2O, 10·7; P, 4·6; Ba, 20·35%).

Monocholesteryl dihydrogen phosphate was prepared from this salt by precipitating the barium with sulphuric acid. The opalescent filtrate, which could not be clarified even on prolonged boiling, was evaporated to dryness, and the residue crystallised from chloroform [Found : P, 6·5. $(C_{27}H_{45})H_2PO_4$ requires P, 6·6%].

The lead and silver salts are precipitated on addition of lead acetate and silver nitrate to solutions of the barium salt. The copper and sodium salts are soluble in water.

Barium Dicholesteryl Phosphate.—2 G. of phosphoryl chloride were added slowly to 8 g. of cholesterol dissolved in chloroform and the mixture was heated under reflux on a water-bath for 3 hours. The solution was kept for 24 hours and then shaken with a litre of water. The aqueous layer was separated from the cloudy chloroform layer (which contained cholesteryl ether and unchanged cholesterol), made neutral to phenolphthalein with barium carbonate and baryta, and concentrated on a water-bath. On addition of alcohol, barium monocholesteryl phosphate was precipitated. The filtrate was evaporated to dryness, and the residue extracted with 90% alcohol. The crystals which separated from the extract were recrystallised, a very small yield of white minute crystals of *barium dicholesteryl phosphate* being obtained [Found : Ba, 7·5; P, 3·4. $\{(C_{27}H_{45})_2PO_4\}_2Ba$ requires Ba, 7·5; P, 3·4%].

By exactly removing the barium with sulphuric acid and evaporating the filtrate to dryness, *dicholesteryl hydrogen phosphate* was obtained as a syrup which solidified in a vacuum desiccator after several days. The white crystalline magma was insoluble in alcohol but soluble in chloroform [Found : P, 3·7. $(C_{27}H_{45})_2HPO_4$ requires P, 3·7%].

Tricholesteryl Phosphate.—When a mixture of 2 g. of phosphorus pentachloride and 7·6 g. of cholesterol was gently warmed on a water-bath, a vigorous reaction took place and the mixture became liquid. On cooling, it set to a yellow solid, which was crystallised repeatedly from acetone. The ester, however, could not thus be entirely freed from cholesteryl chloride [Found : C, 78·1; H, 10·1; P, 2·4. $(C_{27}H_{45})_3PO_4$ requires C, 80·8; H, 11·2; P, 2·5%].

Investigation of Euler and Bernton's Compounds.—Since the above work was completed * the preparation of mono- and di-cholesteryl phosphates has been described by Euler and Bernton. For comparison with our compounds their experiments have been repeated.

(*a*) 3·86 G. of cholesterol, dissolved in 20 c.c. of pyridine, were cooled in a freezing mixture and poured into 6 g. of phosphoryl chloride in 25 c.c. of pyridine. After 12 hours, the mixture was poured into water and the slimy mass, which was filtered off with great difficulty, was dried in a vacuum desiccator and crystallised from

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* A paper describing these compounds was read at a meeting of the Biochemical Society in June, 1925.

chloroform. The compound had m. p. 195° and contained phosphorus (6·64%) corresponding to monocholesteryl dihydrogen phosphate.

(*b*) Equivalent quantities of phosphoryl chloride and cholesterol were mixed in the reverse order, as also described by the authors, but we did not succeed in isolating a compound corresponding to dicholesteryl hydrogen phosphate. The product of the reaction in our experiments was a voluminous frothy mass from which dicholesteryl ether of m. p. 74° was isolated by extraction with 80% alcohol.

Barium β-Hydroxyethyl Phosphate and Tri-β-chloroethyl Phosphate.—15 G. of phosphoryl chloride were slowly added to 30 g. of ethylene chlorohydrin, which was mechanically stirred, and the mixture kept for 24 hours. It was then shaken with ten times its volume of water. *Tri-β-chloroethyl phosphate* separated as a heavy oil (3 c.c.), which was washed several times with water to remove hydrochloric acid and dried in a vacuum desiccator over caustic potash; it then had *d* 1·39 and b. p. 140°/40 mm. [Found : C, 24·3; H, 4·4; P, 10·8; Cl, 36·9. $(C_2H_4Cl)_3PO_4$ requires C, 25·2; H, 4·3; P, 10·9; Cl, 37·3%].

The aqueous solution was evaporated to dryness in a vacuum, the residue dissolved in water, and the solution made neutral to phenolphthalein with baryta and concentrated. On addition of alcohol, 12 g. of a mixture, probably of barium chloroethyl phosphate with barium chloroethyl pyrophosphate, crystallised. This was dissolved in water, the barium removed with sulphuric acid, and the solution boiled with excess of yellow lead oxide for 24 hours and filtered. Lead was then removed with hydrogen sulphide, and the solution evaporated in a vacuum and neutralised with baryta. The barium β-hydroxyethyl phosphate obtained on evaporation was recrystallised from aqueous alcohol (Found : Ba, 49·3; P, 10·6. Calc. for $HO{\cdot}C_2H_4{\cdot}O{\cdot}PO_3Ba$: Ba, 49·4; P, 11·2%).

Barium Di-β-hydroxyethyl Phosphate.—The above procedure was repeated with 15 g. of phosphoryl chloride and 15 g. of ethylene chlorohydrin, but the mixture was heated on a water-bath for 2 hours. After treatment with water and separation of the oil, the aqueous solution was neutralised with baryta, concentrated, and boiled with excess of lead oxide, and finally the lead salts were converted into barium salts as described above. A mixture of mono- and di-β-hydroxyethyl phosphates was obtained. Separation was effected by crystallisation from water, the *barium* salt of the di-ester being the less soluble [Found : H_2O, 6·7; Ba, 25·55; P, 11·9. $\{(C_2H_4{\cdot}OH)_2PO_4\}_2Ba,2H_2O$ requires H_2O, 6·6; Ba, 25·2; P, 11·4%].

Barium β-Chloroethyl Phosphate.—The barium salts of the chloroethyl phosphates could not be isolated in a pure condition by the above method. Barium chloroethyl phosphate could not be obtained by Fischer's method, possibly owing to the great heat evolved in the reaction, though the reagents were maintained as far as possible at −20°. By substituting ethylene chlorohydrin for glycol a fair yield of the salt was obtained.

8·2 G. of ethylene chlorohydrin in 40 c.c. of pyridine cooled to −20° were added very slowly to 30·6 g. of phosphoryl chloride in 80 c.c. of pyridine kept below −15°. After remaining at this temperature for 2 hours, the mixture was poured into 2 litres of water containing ice, to which 120 c.c. of 25% sulphuric acid had been added. Hydrochloric acid was removed from the solution by means of silver carbonate, and the excess of silver by hydrogen sulphide, and the solution, after being freed from the latter reagent, was made neutral to phenolphthalein with baryta and evaporated in a vacuum until free from pyridine; the excess of barium was then precipitated by means of carbon dioxide. The filtrate from the barium carbonate was concentrated in a vacuum to 200 c.c., alcohol added, and the precipitated *barium chloroethyl phosphate* recrystallised from 50% alcohol (yield, 7 g.) [Found: H_2O, 5·7; Cl, 11·0; P, 9·6; Ba, 43·3. $(C_2H_4Cl)PO_4Ba,H_2O$ requires H_2O, 5·4; Cl, 11·2; P, 9·9; Ba, 43·7%].

The barium salt decomposes at 120°. For the further identification of monochloroethyl dihydrogen phosphate the *brucine* salt was found to be of most value. It is obtained by adding the calculated quantity of brucine to an aqueous solution of monochloroethyl dihydrogen phosphate. The brucine rapidly dissolves and the salt almost immediately begins to crystallise in tufts of fine needles (yield, almost theoretical). The salt is fairly readily soluble in hot water, but almost insoluble in cold [Found: H_2O, 13·5; P, 4·8; Cl, 5·8; N, 4·3; M (for anhydrous salt), ebullioscopic in alcohol, 564. $C_{23}H_{26}O_4N_2,(C_2H_4Cl)H_2PO_4,4H_2O$ requires H_2O, 11·3; P, 4·95; Cl, 5·7; N, 4·5%; M (for anhydrous salt), 554].

Conversion of Brucine β-Chloroethyl Phosphate into Barium β-Hydroxyethyl Phosphate.—The salt (6 g.) was dissolved in hot water, the brucine removed by ammonia, and the filtrate boiled with excess of yellow lead oxide. Lead was removed by hydrogen sulphide, the solution neutralised with baryta and evaporated in a vacuum, and alcohol added. The precipitated barium β-hydroxyethyl phosphate was recrystallised from aqueous alcohol (yield, nearly 2 g.) (Found: Ba, 49·7; P, 11·0%).

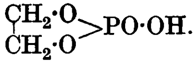
The Action of Ethyl Metaphosphate on Ethylene Chlorohydrin.—This reaction also was tried as a means of preparing barium monochloroethyl phosphate (see Part II).

A mixture of 16 g. of ethylene chlorohydrin and 11 g. of ethyl metaphosphate in chloroform was heated under reflux on a water-bath for 3 hours. The chloroform was then removed by distillation in a vacuum, and the residue dissolved in water and made neutral to phenolphthalein with baryta. The filtrate from barium phosphate was evaporated in a vacuum, and alcohol added very carefully until the solution became turbid. Barium ethyl phosphate separated on standing. After addition of rather more than an equal volume of alcohol to the filtrate, barium β-chloroethyl phosphate slowly separated. The salt was dissolved in water, the above separation repeated, and the barium chloroethyl phosphate recrystallised from 50% alcohol (yield, 3 g.) [Found : H_2O, 5·4. Found in salt dried at 110° : Cl, 12·3; P, 10·6; Ba, 45·8. Calc. for $(C_2H_4Cl)PO_4Ba,H_2O$: H_2O, 5·4. Calc. for $(C_2H_4Cl)PO_4Ba$: Cl, 12·0; P, 10·5; Ba, 46·4%]. It was converted into barium β-hydroxyethyl phosphate by boiling with yellow lead oxide as described above (Found : Ba, 50·8; P, 11·3%).

Formation of Barium Di-β-hydroxyethyl Phosphate from Trichloroethyl Phosphate.—5 G. of trichloroethyl phosphate were boiled with excess of yellow lead oxide and water for 24 hours. Lead was removed from the filtered solution by hydrogen sulphide, and the excess of the latter by evaporation in a vacuum. The solution was then neutralised with baryta and evaporated in a vacuum. Barium di-β-hydroxyethyl phosphate (3 g.) separated and was recrystallised (Found : H_2O, 5·5; Ba, 23·9; P, 11·9%).

Since the trichloroethyl ester suffered hydrolysis by this treatment, its conversion into trihydroxyethyl phosphate was attempted by the action of moist silver oxide. The syrupy trichloroethyl ester, on being left in contact with silver oxide for 8 weeks, was changed into a mobile liquid. This was evaporated in a vacuum. White feathery crystals of silver di-β-hydroxyethyl phosphate were obtained. As this salt was very soluble in water, it was converted into the barium salt by successive treatment with hydrogen sulphide and baryta (Found : Ba, 26·7; P, 12·0%).

Formation of Disodium β-Hydroxyethyl Phosphate from Ethylene Chlorohydrin and Trisodium Phosphate.—This reaction was tried as a means of preparing tri-β-hydroxyethyl phosphate. 10 G. of ethylene chlorohydrin were added to a saturated solution of 20 g. of trisodium phosphate. A sheen, due to minute crystals, appeared almost at once in the solution and developed into a heavy white precipitate. Just sufficient water was added to dissolve the crystals and alcohol was added until the solution was turbid. Tiny glistening platelets of *disodium β-hydroxyethyl phosphate* were first formed and afterwards sodium phosphate crystallised. The crystals were separated by fractional crystallisation from aqueous alcohol and finally disodium β-hydroxyethyl phosphate (11 g.) was obtained in large transparent plates, m. p. 61° [Found: H_2O, 37·1; Na, 15·5; P, 10·4. $(C_2H_4\cdot OH)PO_4Na_2,6H_2O$ requires H_2O, 36·7; Na, 15·6; P, 10·5%].

Formation of Sodium Ethylene Phosphate.—Besides the above esters of ethylene glycol and phosphoric acid, several other esters are theoretically capable of existence, such as  $\left.\begin{array}{l}CH_2\cdot O\\ CH_2\cdot O\end{array}\right>PO\cdot OH$. An attempt was made to prepare a salt of this compound as follows.

The disodium derivative (18 g.) of ethylene glycol (made by heating the glycol with excess of sodium at 170°) was suspended in 500 c.c. of dry chloroform and 28 g. of phosphoryl chloride were slowly added. Much heat was evolved and the apparatus had to be cooled. After the addition the mixture was warmed on a water-bath. Some greasy material, which was probably the excess of the disodium derivative, separated on the surface. The rest was shaken with water and separated from the chloroform, which contained only a trace of trichloroethyl phosphate. The aqueous solution was neutralised with baryta and evaporated in a vacuum. Sodium chloride crystallised. On evaporation of the mother-liquor in a vacuum over sulphuric acid, a colourless syrup resulted containing a mass of regular, truncated, octahedral crystals. These crystals could not be separated, as they formed only when the syrup was too viscid to filter, and it has been so far impossible to find a solvent which did not dissolve both crystals and syrup and prevent crystallisation. The great solubility of the crystals is so different from that of the salts of hydroxyethyl phosphates that it is probable that they are the salt of the above ethylene hydrogen phosphate.

Hydrolysis of the Esters.

Hydrolysis of Barium Diethyl Phosphate and of Triethyl Phosphate.—In view of the fact that ethyl dihydrogen phosphate is not hydrolysed by alkali, the examination of the hydrolysis of the diethyl and triethyl esters necessitates estimation of alcohol, since inorganic phosphoric acid will not be formed. The methods of Haggard (*J. Biol. Chem.*, 1923, **55**, 132) and of Shaffer and Ronzoni (*ibid.*, 1923, **57**, 741) for the estimation of ether in blood and other fluids seemed capable of adaptation to the determination of alcohol.

(1) *Haggard's method.* This consists in the oxidation of the ether, which is removed by a current of air from the liquid, by iodine

pentoxide. The liberated iodine is absorbed in potassium iodide solution and titrated with thiosulphate.

On testing the method with alcohol it was found that the reaction

$$5C_2H_5\cdot OH + 6I_2O_5 = 15H_2O + 10CO_2 + 6I_2$$

takes place at 200°.

The experiment was carried out by bubbling dry air very slowly through the alcohol solution and then through a U tube (in an oil-bath at 200°) containing alternate layers of iodine pentoxide (40 g.) and glass wool. The air current was then passed into potassium iodide solution to absorb the iodine. (Before use, the charged U tube had to be conditioned by heating it at 220° for about 10 hours until iodine was no longer evolved.) Alcohol was completely removed from the solution in 30 minutes, but the apparatus was not capable of dealing with more than 10 mg. of alcohol.

(2) *Shaffer and Ronzoni's method.* In this method the ether is separated by aeration and passed through a series of absorption bottles each containing 25 c.c. of 0·5*N*-potassium dichromate in 50% sulphuric acid. The unreduced dichromate is then estimated by means of potassium iodide and thiosulphate.

It was found that the alcohol could be removed by aeration if the solution were heated on a water-bath. It was oxidised to acetic acid by the dichromate and not further, but at the end of the experiment it was necessary to boil the dichromate for a few minutes to complete the oxidation. The results obtained by this method with pure alcohol were accurate to within 0·5%.

The second method, which is much simpler than the first, was used in the experiments with ethyl and propyl phosphates.

Hydrolysis. 1 G. portions of barium diethyl and of triethyl phosphate were heated under reflux with 200 c.c. of 2*N*-sodium hydroxide on a water-bath for periods ranging from 8 to 360 hours. The solution was then aerated and the alcohol drawn through a train of three absorption bottles each containing 25 c.c. of 0·5*N*-potassium dichromate made up with 50% sulphuric acid. The dichromate portions were mixed, boiled for a few minutes, and made up to a volume of 250 c.c. Quantities of 25 c.c. were treated with excess of potassium iodide solution, and the liberated iodine estimated with thiosulphate.

The acid hydrolysis was carried out in the same way, 200 c.c. of 2*N*-sulphuric acid being used.

In a blank experiment 75 c.c. of the dichromate solution, as used for absorption of the alcohol, were equivalent to 370 c.c. of 0·1*N*-thiosulphate. The results, including those for barium dipropyl phosphate, were as follows :

Alkaline Hydrolysis at 90°.

Phosphate.	Quantity taken (g).	Time (hours).	Alcohol oxidised (as c.c. of 0·1*N*-thiosulphate).	Mols. of alcohol removed.
Barium diethyl	1·0	8	No hydrolysis	0
	1·0	24	,,	0
	1·0	96	,,	0
Barium dipropyl	1·0	48	,,	0
	1·0	96	,,	0
Triethyl	1·0303	15	178	0·79
	1·0303	30	208	0·92

Acid Hydrolysis at 90°.

Phosphate.	Quantity taken (g).	Time (hours).	Alcohol oxidised (as c.c. of 0·1*N*-thiosulphate).	Mols. of alcohol removed.
Barium diethyl	1·0	20	8·2	0·045
	1·0	48	15	0·086
	1·0	144	103	0·53
	1·0	216	148	0·82
	1·0	386	337·5	1·87
Barium dipropyl	1·0	48	44	0·275
	1·0	96	84	0·525
Triethyl	1·0	8	190	0·84
	1·0	48	253	1·12
	1·0	120	315	1·4
	1·0	240	405	1·8
	1·0	360	563	2·5

The dialkyl phosphates are thus not hydrolysed by alkali, and slowly by acid with complete removal of the alcohol only after a long time. Triethyl phosphate was converted into the diethyl phosphate by alkali and almost completely hydrolysed by acid after 360 hours.

Hydrolysis of the Cetyl Phosphates.—The extent of hydrolysis of these esters could be determined by weighing the cetyl alcohol extracted, after cooling, by ether.

Alkaline hydrolysis. 1 G. portions of the barium salts of the mono- and di-cetyl phosphates were boiled under reflux with 200 c.c. of 2*N*-sodium hydroxide: there was no separation of cetyl alcohol.

1 G. of tricetyl phosphate was boiled with 200 c.c. of *N*-sodium hydroxide for 2 hours. A white curdy mass which formed on the surface of the liquid was filtered off; ether then extracted 0·31 g. of cetyl alcohol from the liquid. The aqueous solution on cooling deposited a white flocculent precipitate of *sodium dicetyl phosphate*, which was washed with ether to remove cetyl alcohol [Found: P, 5·3. $(C_{16}H_{33})_2PO_4Na$ requires P, 5·45%].

Acid hydrolysis. 1 G. portions of barium mono- and di-cetyl phosphates were boiled under reflux with 200 c.c. of 2*N*-sulphuric acid for 8 hours in each case. The mono-ester gave 0·509 g. of cetyl alcohol (0·96 mol.), and the di-ester 0·722 g. (1·83 mols.). The results are similar to those obtained with ethyl and propyl phosphates.

Hydrolysis of Phenyl Phosphates.—The barium salts of mono- and di-phenyl hydrogen phosphates were prepared by the action of phosphoryl chloride on phenol. Molecular quantities were heated in an oil-bath at 110° for about 10 hours and the product was fractionated in a vacuum to obtain the corresponding phosphoryl chloride compounds. Monophenylphosphoryl chloride, $OPh{\cdot}POCl_2$, distilled at 240°/ 760 mm., and diphenylphosphoryl chloride, $(OPh)_2POCl$, at 212—215°/21 mm. These compounds were hydrolysed to the phosphoric esters by shaking with water at 60°. The solutions were filtered, and the filtrates evaporated in a vacuum desiccator containing sodium hydroxide.

Triphenyl phosphate was prepared by the action of phosphorus pentachloride on phenol.

The esters were hydrolysed by heating about 1 g. quantities with 200 c.c. of *N*-hydrochloric acid or 200 c.c. of *N*-sodium hydroxide.

The extent of the hydrolysis of these esters was ascertained in the case of the mono-ester by precipitation of the inorganic phosphoric acid as ammonium magnesium phosphate and conversion into magnesium pyrophosphate, and in the case of the di- and tri-esters by steam distillation (after acidification with acetic acid in the alkaline hydrolysis) and estimation of the phenol by bromination in an aliquot part of the distillate, which was made up to 500 c.c. The bromination was effected in a stoppered bottle by adding 10 c.c. of 0·5*N*-potassium bromate and 10 c.c. of 0·5*N*-potassium bromide and 5 c.c. of concentrated hydrochloric acid. After 15 minutes, excess of potassium iodide was added and the iodine liberated was titrated with thiosulphate. 10 C.c. of the bromate solution were equivalent to 32·8 c.c. of 0·1*N*-thiosulphate.

The results of the hydrolysis were as follows :

		Acid Hydrolysis at 85°.		
Ester.	Quantity taken (g.).	Time (hours).	C.c. of thiosulphate.	Mols. of phenol removed.
Triphenyl	1·0222	144	283·3	1·95
Diphenyl	0·9994	24	37·25	0·20
	0·9907	76	141·5	1·01
			$(Mg_2P_2O_7)$	
Monophenyl	1·0059	48	0·2377 g.	0·37
	1·0	168	0·4698 g.	0·73
		Alkaline Hydrolysis at 85°.		
Triphenyl	1·0061	72	268 c.c.	1·9
	1·0058	170	430	3·0
Diphenyl	1·0061	70	280	1·5
	1·0022	170	366	1·98
Monophenyl	1·00	24	194	0·7
	1·00	48	250·5	0·9
	1·00	72	261	0·98

The hydrolysis of mono- and di-phenyl phosphates by alkalis was also examined at 40°, both the inorganic phosphoric acid and the phenol being estimated :

Diphenyl: 1·0037 g. in 24 hours gave 0·16 g. $Mg_2P_2O_7$ and 1·36 mols. phenol.
Monophenyl: 1·0051 g. in 24 hours gave 0·2232 g. $Mg_2P_2O_7$ and 0·35 mol. phenol.

The hydrolysis was thus quite appreciable. This difference between the aliphatic alcohols and phenol may be due to the acidic character of the hydroxyl group conferred upon it by the electronegative nature of the benzene nucleus.

Summary.

Mono-, di-, and tri-cetyl phosphates have been prepared by the action of phosphoryl chloride and pentachloride upon cetyl alcohol. The first two esters were isolated as barium salts.

Mono-, di-, and tri-cholesteryl phosphates have been prepared by similar methods. Tricholesteryl phosphate was not prepared in a pure condition.

Mono- and di-β-chloroethyl phosphates (isolated as barium salts) and trichloroethyl phosphate have been prepared by the action of phosphoryl chloride upon ethylene chlorohydrin.

Monochloroethyl phosphate has also been made by the action of ethyl metaphosphate upon ethylene chlorohydrin.

The barium salts of the mono- and di-chloroethyl phosphates have been converted into the barium salts of mono- and di-β-hydroxyethyl phosphates. Trichloroethyl phosphate was converted into a salt of di-β-hydroxyethyl phosphate.

Disodium β-hydroxyethyl phosphate was formed by the action of ethylene chlorohydrin upon trisodium phosphate.

A very soluble salt, probably of ethylene hydrogen phosphate, is formed by the action of phosphoryl chloride upon the disodium derivative of ethylene glycol. It could not be isolated.

The various esters of phosphoric acid can be completely hydrolysed by dilute acid.

The mono- and di-esters of the aliphatic alcohols with phosphoric acid are not hydrolysed, and the tri-ester is hydrolysed to the di-ester, by dilute alkali.

The mono-, di-, and tri-esters of phenol with phosphoric acid can be completely hydrolysed by dilute alkali.

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