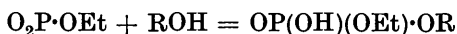


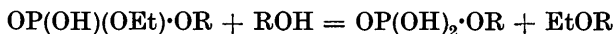
XLIV.—*Esters of Phosphoric Acid. Part II. The Action of Ethyl Metaphosphate on Alcohols, Ammonia, and Some Amino-compounds.*

By ROBERT HENRY ADERS PLIMMER and WILLIAM JOHN NOBBS BURCH.

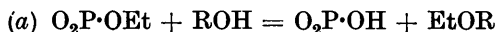
THE action of ethyl metaphosphate on alcohols as a method of preparing esters of phosphoric acid was introduced by Langheld (*Ber.*, 1910, **43**, 1857; 1911, **44**, 2076), who claimed to have made several esters of natural origin but gave no data. The reaction was believed to take place in two stages. The ethyl metaphosphate first reacted with the alcohol by addition :



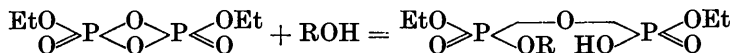
Then, at the temperature of the water-bath, the mono-ester and a mixed ether were produced. These products were believed to result either from the reaction



or from

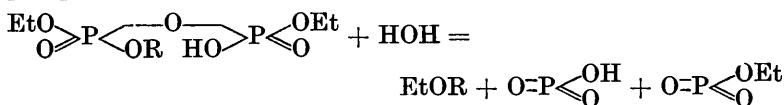


No mixed phosphoric ester was apparently formed, nor were pyrophosphoric esters isolated; possibly they were not formed. In view of the uncertainty of the reaction and for the purpose of preparing certain esters of phosphoric acid, the method has now been tried with propyl alcohol, cetyl alcohol, phenol, and cholesterol, as well as with ethyl alcohol employed by Langheld. We have found that in most cases a mixed ether and sometimes a simple ether is produced and that, except in the case of cholesterol, there is always formed a mixture of the mono- and di-ester of the alcohol taken together with mono- and di-ethyl phosphates. It is difficult to explain the course of the reaction, but it appears that there are several stages. If ethyl metaphosphate has the double formula, as shown by molecular-weight determination, the first stage is probably the addition of the alcohol to form a pyrophosphate ester :



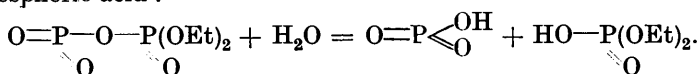
The pyrophosphate ester may be decomposed by the action of

water into a mixed ether, metaphosphoric acid, and ethyl metaphosphate :



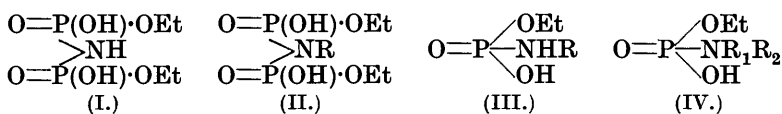
Finally, the metaphosphoric acid reacts with the alcohol to form the mono- and di-esters, $\text{OP}(\text{OH})_2 \cdot \text{OR}$ and $\text{OP}(\text{OR})_2 \cdot \text{OH}$. The simple ether may arise from the action of the alcohol (cetyl) upon the mono-ester (monocetyl).

By these reactions it is impossible to explain the formation of diethyl hydrogen phosphate in all cases. We venture to suggest that ethyl metaphosphate has an alternative formula and that it is decomposed by water into diethyl hydrogen phosphate and metaphosphoric acid :



This would agree with Langheld's observation (*Ber.*, 1910, 43, 1859) that the solution coagulates albumin.

By the action of ethyl metaphosphate on ammonia and various amino-compounds, Langheld also prepared compounds to which he ascribed the formulæ I—IV.



Ammonia and primary amines formed iminopyrophosphates (I and II), secondary amines formed amino-orthophosphates (III and IV). They were decomposed by alkalis or water into ammonia or the amine. In a similar way Strecker and Heuser (*Ber.*, 1924, 57, 1364) prepared compounds of ethyl metaphosphate with hydrazine, hydroxylamine, and phenylhydrazine. They assigned to them the composition ethyl hydrazino-orthophosphate,



ethyl phenylhydrazino-orthophosphate, $\text{O} \cdot \text{P}(\text{OH})(\text{OEt})(\text{NH} \cdot \text{NHPh})$, and ethyl hydroxylamino-orthophosphate, $\text{O} \cdot \text{P}(\text{OH})(\text{OEt})(\text{NH} \cdot \text{OH})$. In all these compounds the nitrogen atom was believed to be directly attached to the phosphorus atom.

On account of the possible relation of these compounds to phosphoproteins in which phosphorus is combined with an amino-acid in a form easily hydrolysed by alkali (Plimmer and Bayliss, *J. Physiol.*, 1906, 33, 439; Plimmer and Scott, *J.*, 1908, 93, 1699) it was thought

that further investigation of these compounds was of importance in the study of the combination of phosphorus in the phosphoproteins. We have found that these compounds after treatment with water give salts of ethyl dihydrogen phosphate with ammonia, hydrazine, or other amino-compounds.

EXPERIMENTAL.

Preparation of Ethyl Metaphosphate.—The ester was prepared as described by Langheld. From 200 g. of phosphoric oxide we obtained, after purification, 100 g. of ethyl metaphosphate as a heavy yellow syrup, which was kept in a vacuum desiccator [Found : C, 21.9; H, 4.6; P, 28.8; *M*, ebullioscopic in chloroform, 216.6, 214.6. Calc. for EtPO_3 : C, 22.2; H, 4.6; P, 28.7%. (EtPO_3)₂ requires *M*, 216].

Ethyl metaphosphate is very soluble in chloroform and glacial acetic acid, moderately easily soluble in acetone, and very slightly soluble in ether, benzene, and ligroin.

Ethyl Alcohol and Ethyl Metaphosphate.—Ten g. of ethyl metaphosphate dissolved in chloroform were added to excess of absolute alcohol and heated under reflux on a water-bath for 2 hours; the mixture then gave no mistiness on addition of ether. The chloroform and excess of alcohol were distilled in a vacuum, the residue was dissolved in water, and the solution made neutral to phenolphthalein with barium carbonate and baryta and warmed on the water-bath. Alcohol was added until the first sign of turbidity appeared. *Barium ethyl phosphate* slowly crystallised in characteristic shining plates. It was recrystallised from aqueous alcohol (yield, 10.9 g.) (Found : H_2O , 5.6; P, 11.1.* $\text{C}_2\text{H}_5\text{PO}_4\text{Ba}, \text{H}_2\text{O}$ requires H_2O , 6.45; P, 11.1%).

The filtrate was divided into two equal parts. One part was evaporated to dryness; alcohol (80%) extracted from the residue *barium diethyl phosphate*, which was crystallised from 80% alcohol (yield, 4.5 g.) [Found : P, 14.0. $\{(\text{C}_2\text{H}_5)_2\text{PO}_4\}_2\text{Ba}$ requires P, 14.0%]. The second part was distilled in a vacuum, but no triethyl phosphate could be obtained.

The original distillate of chloroform and alcohol was not examined for the presence of ethyl ether.

Propyl Alcohol and Ethyl Metaphosphate.—A solution of 12 g. of *n*-propyl alcohol in chloroform was boiled under reflux with 11 g. of ethyl metaphosphate until it gave no mistiness on addition of ether. The chloroform was then distilled off in a vacuum, and the residue dissolved in ether and shaken with baryta solution to convert the

* The phosphorus analyses were made by the method of Neumann as modified by Plimmer and Bayliss (*J. Physiol.*, 1906, 33, 439).

ester into its barium salt. The aqueous solution was evaporated to a small bulk in a vacuum, and alcohol added until the first sign of turbidity appeared; barium ethyl phosphate slowly crystallised (Found: H_2O , 6.0; P, 11.0%). The filtrate was evaporated to dryness in a vacuum; boiling alcohol extracted from the residue *barium di-n-propyl phosphate*, which crystallised on cooling (9 g.) and was recrystallised from hot alcohol [Found: Ba, 27.05; P, 12.4. $\{(\text{C}_3\text{H}_7)_2\text{PO}_4\}_2\text{Ba}$ requires Ba, 27.3; P, 12.45%]. From the remaining solid, 80% alcohol extracted barium diethyl phosphate (11 g.), which was recrystallised (Found: P, 14.0%). The impure *barium dipropyl pyrophosphate* that remained [Found: Ba, 36.7; P, 16.0. $(\text{C}_3\text{H}_7)_2\text{P}_2\text{O}_7\text{Ba}$ requires Ba, 34.5; P, 15.6%] was converted, by warming with dilute baryta solution, into *barium propyl phosphate* (Found: Ba, 49.9; P, 11.2. $\text{C}_3\text{H}_7\text{PO}_4\text{Ba}$ requires Ba, 49.8; P, 11.3%).

On redistilling the chloroform fraction at ordinary pressure, about 2 c.c. of a liquid boiling at 90° were obtained, presumably consisting of dipropyl ether. Ethyl propyl ether was not separable from the chloroform owing to the closeness of their boiling points.

Cetyl Alcohol and Ethyl Metaphosphate.—A solution of 10 g. of cetyl alcohol and 5 g. of ethyl metaphosphate in chloroform was heated under reflux for 2 hours, the chloroform was then evaporated in a vacuum and the residue was dissolved in ether and well shaken with a large volume of water. The aqueous solution was made neutral to phenolphthalein with concentrated baryta solution. From the precipitated barium mono- and di-cetyl phosphates, by repeated extraction with hot water, 2 g. of barium cetyl phosphate were separated; this was recrystallised from water (Found in material dried at 110° : Ba, 30.2; P, 7.0. Calc. for $\text{C}_{16}\text{H}_{33}\text{PO}_4\text{Ba}$: Ba, 30.0; P, 6.8%). The residue of barium dicetyl phosphate was dissolved in alcohol, filtered from barium phosphate, and precipitated, by addition of water, as a gelatinous mass which became a white powder (7 g.) on drying at 110° [Found: Ba, 11.6; P, 5.5. Calc. for $\{(\text{C}_{16}\text{H}_{33})_2\text{PO}_4\}_2\text{Ba}$: Ba, 11.17; P, 5.05%].

The filtrate from the precipitate of the cetyl phosphates was concentrated in a vacuum, and alcohol added; barium ethyl phosphate was precipitated (Found: P, 11.2%). On evaporating the filtrate to dryness and extracting the residue with 80% alcohol, barium diethyl phosphate (3 g.) was obtained; it was recrystallised from alcohol and water (Found: P, 13.7%).

The ethereal solution, fractionated under ordinary pressure, gave two fractions, b. p. 180° and 286° respectively. The former solidified after some time to a white wax, m. p. 20° , which was apparently ethyl cetyl ether (Found: C, 79.1; H, 13.75. Calc. for

$C_2H_5 \cdot O \cdot C_{16}H_{33}$: C, 80.0; H, 14.1%). (Cetyl alcohol melts at 50° and boils at 190° .) The other fraction was a pale yellow solid, m. p. 55° , presumably dicetyl ether.

Phenol and Ethyl Metaphosphate.—Phenol (5 g.) was treated with ethyl metaphosphate (5 g.) as described in the case of propyl alcohol. From the aqueous solution of barium salts, on addition of alcohol, barium ethyl phosphate (Found : P, 11.0%) and *barium phenyl phosphate* (Found in material dried at 110° : Ba, 43.2; P, 10.3. $C_6H_5PO_4Ba$ requires Ba, 44.2; P, 10.0%) were precipitated. They were separated by fractional crystallisation from aqueous alcohol, barium ethyl phosphate being the less soluble.

The filtrate was evaporated to dryness, and the residue extracted with absolute alcohol. Barium diethyl phosphate was insoluble and was recrystallised from dilute alcohol (Found : P, 14.1%). *Barium diphenyl phosphate* was obtained on evaporation of the alcoholic extract; it crystallised from 50% alcohol in fine needles [Found : loss at 60° , 10.15. $\{(C_6H_5)_2PO_4\}_2Ba, 4H_2O$ requires loss, 10.4%. Found in anhydrous salt : Ba, 21.6; P, 9.8. $\{(C_6H_5)_2PO_4\}_2Ba$ requires Ba, 21.4; P, 9.7%]. The chloroform on fractionation gave phenetole, b. p. 176° .

Cholesterol and Ethyl Metaphosphate.—Cholesterol (3.25 g.) was treated with ethyl metaphosphate (5 g.) as in the case of cetyl alcohol (3 hours' heating). The neutralised aqueous solution of barium salts, after addition of alcohol, slowly deposited a mixture of barium ethyl phosphate (Found : P, 11.3%) and *barium cholesteryl phosphate*. They were separated by fractional crystallisation from aqueous alcohol. Only 0.5 g. of barium monocholesteryl phosphate was obtained (Found : loss at 110° , 10.8. $C_{27}H_{45}PO_4Ba, 4H_2O$ requires loss, 10.6%. Found in anhydrous salt : Ba, 22.6; P, 5.1. $C_{27}H_{45}PO_4Ba$ requires Ba, 22.8; P, 5.15%). Barium dicholesteryl phosphate could not be obtained from the filtrate.

On fractionating the chloroform a brown oil (1 c.c.), b. p. $237^\circ/20$ mm., was obtained. This appeared to be *cholesteryl ethyl ether* (Found : C, 84.9; H, 12.1. $C_{27}H_{45} \cdot O \cdot C_2H_5$ requires C, 84.05; H, 12.1%). Dicholesteryl ether was not isolated.

Tyrosine and Ethyl Metaphosphate.—1 G. of tyrosine and 5 g. of ethyl metaphosphate were heated in dry chloroform under reflux on a water-bath for 3 hours. A colourless syrup which floated to the surface was found to contain a high percentage of phosphorus and a very low percentage of nitrogen. It seemed, therefore, that the tyrosine had merely dissolved, since on neutralisation with baryta it was precipitated.

The Product of the Reaction of Ethyl Metaphosphate with Ammonia.—Dry ammonia was passed into a solution of 10 g. of pure

ethyl metaphosphate in chloroform until the reaction was complete. A gelatinous mass was formed. The chloroform was removed by distillation in a vacuum, and the vitreous residue dissolved in dry alcohol and precipitated with dry ether (Found : C, 17.15; H, 6.7; P, 21.6%).

(a) 0.69 G. of the product was dissolved in water, 1 g. of sodium carbonate added, and air aspirated through the solution according to the method of Folin. Ammonia equivalent to 37 c.c. of 0.1*N*-acid was absorbed in excess of standard acid. Hence nitrogen (as ammonia) = 9.1%.

(b) 5 C.c. of a 0.69% solution of the product, when tested for amino-nitrogen by the Van Slyke method with acetic acid (Plimmer, J., 1925, 127, 2651), gave 7.3 c.c. at 18° and 771 mm. after 24 hours. Allowance being made for the nitrogen in the blank determination, the amount of nitrogen = 4.2574 mg. Hence nitrogen = 10.8%.

These analyses and reactions correspond to those of ammonium ethyl hydrogen phosphate $[\text{NH}_4(\text{C}_2\text{H}_5)\text{HPO}_4]$ requires N, 9.7; C, 16.7; H, 7.0; P, 21.7%, and not to those of an iminophosphate (Langheld, *loc. cit.*). A record of only one ammonium salt, $\text{NH}_4(\text{C}_2\text{H}_5)\text{HPO}_4$, could be found (Cavalier, *Ann. Chim. Phys.*, 1899, 18, 449).

Diammonium ethyl phosphate was made from 10 g. of barium ethyl phosphate by adding 4.7 g. of ammonium sulphate in aqueous solution. The barium sulphate was removed, and the filtrate evaporated in a vacuum. A white crystalline salt was obtained which was very soluble in water [Found : N (as ammonia), 17.4; P, 19.1. $(\text{NH}_4)_2\text{C}_2\text{H}_5\text{PO}_4$ requires N, 17.5; P, 19.4%].

Ammonium Ethyl Hydrogen Phosphate.—A mixture of 20 g. of phosphoric oxide and 25 g. of alcohol in ether cooled in ice was left for 12 hours. It was then warmed at 40° for 2 hours, poured into water, and divided into two portions. One portion was partly neutralised with ammonia until the B.D.H. universal indicator used was yellow. The solution was then evaporated to a small bulk and left in a vacuum desiccator. After a time it became a vitreous mass and finally crystallised. The salt was very deliquescent and resembled the salt formed from ethyl metaphosphate and ammonia [Found : N (as ammonia), 9.5; P, 21.7%].

The other portion of the solution was treated with double the quantity of ammonia used above. White flaky crystals of diammonium ethyl phosphate separated [Found : N (as ammonia), 17.25; N (by Kjeldahl's method), 17.1; C, 15.05; H, 8.6; P, 20.4. $(\text{NH}_4)_2\text{C}_2\text{H}_5\text{PO}_4$ requires N, 17.5; C, 15.0; H, 8.1; P, 19.4%].

Ammonium diethyl phosphate was obtained as colourless deliquescent crystals from 1.32 g. of ammonium sulphate and 4.43 g. of

barium diethyl phosphate by removing the barium sulphate and concentrating the filtrate in a vacuum [Found : N (as ammonia), 7.9; P, 17.6. $\text{NH}_4(\text{C}_2\text{H}_5)_2\text{PO}_4$ requires N, 8.2; P, 18.1%].

The Product of the Reaction of Ethyl Metaphosphate with Hydrazine.—This product was prepared by the method of Strecker and Heuser (*loc. cit.*). It consisted of a pale yellow syrup (Found : P, 18.8%) and was tested as follows:—(a) 5 C.c. of a solution containing 0.4136 g. were shaken with benzaldehyde. The benzylideneazine formed gradually became crystalline (0.22 g.); m. p. 91°.

(b) On adding sodium carbonate solution and copper sulphate to a solution of the compound, there was a brisk effervescence of nitrogen and cuprous oxide was precipitated.

(c) On adding excess of copper sulphate to a solution of the compound a fine blue crystalline powder was precipitated [Found : Cu, 21.3; N, 9.8; SO_4 , 66.1. $\text{CuSO}_4(\text{N}_2\text{H}_4)\text{H}_2\text{SO}_4$ requires Cu, 21.7; N, 9.7; SO_4 , 66.4%]. This powder was insoluble in water, and on addition of an alkali cuprous oxide was precipitated and nitrogen evolved. It contained neither phosphorus nor water, and the filtrate contained no hydrazine but the whole of the phosphorus of the compound.

The compound thus appears to be *hydrazine ethyl dihydrogen phosphate*. It reacts with copper sulphate to give *hydrazine copper sulphate* and copper ethyl phosphate :



The Product of the Reaction of Ethyl Metaphosphate with Phenylhydrazine.—This product was prepared by the method described by Strecker and Heuser (*loc. cit.*) and purified by repeated crystallisation from alcohol. It was tested as follows : (a) A solution of the compound in water containing sodium carbonate reduced copper sulphate in the cold.

(b) A solution of the compound was treated with barium carbonate ; barium ethyl phosphate (Found : P, 10.8%) was obtained from the filtrate on addition of alcohol.

(c) The compound was kept for 24 hours with sodium carbonate solution in a stoppered bottle. The liquid then had a distinct odour of phenylhydrazine, and this base was extracted from it with ether and identified. On shaking a solution of the compound in water with benzaldehyde, benzaldehydephenylhydrazone, m. p. 152°, was isolated.

These reactions show clearly that the compound is a salt of phenylhydrazine with ethyl dihydrogen phosphate.

The Product of the Reaction of Ethyl Metaphosphate with Alanine.—Ten g. of alanine were boiled on a water-bath with an excess of ethyl

metaphosphate dissolved in dry chloroform. After 10 hours all the alanine had disappeared and a syrup separated on the surface. This was separated from the chloroform and evaporated in a vacuum. Yield, 16.9 g. The analytical figures (Found: N, 4.15; P, 20.9%) correspond fairly well to those required for alanine ethyl pyrophosphate, *viz.*, N, 4.3; P, 19.1%. The high percentage of phosphorus is probably due to the presence of ethyl dihydrogen phosphate.

The product was tested as follows: (a) Copper carbonate was added to the solution. The filtrate from the excess gave a mixture of the copper salt of alanine and copper ethyl phosphate. Their complete separation was not successful on account of the great solubility of both salts.

(b) Benzoyl chloride (5 g.) was added gradually to 4 g. of the product, the solution being kept alkaline with sodium carbonate. A mixture of benzoic acid and benzoylalanine was obtained. The benzoic acid was separated by solution in ligroin; the residue, crystallised from hot water, consisted of benzoylalanine, m. p. 162°, and contained no trace of phosphorus.

The product thus seems to be a salt of alanine with ethyl trihydrogen pyrophosphate, which becomes a salt of ethyl dihydrogen phosphate on treatment with water.

The Product of the Reaction of Ethyl Metaphosphate with Guanidine.—This reaction with the strongly basic guanidine was studied as a preliminary to the attempted preparation of a phosphorus derivative of arginine and of creatine. The latter combination has recently been described as occurring in muscle by Fiske and Subbarow (*Science*, 1928, 67, 169).

Excess of ethyl metaphosphate dissolved in dry chloroform was boiled under reflux with 5 g. of guanidine carbonate for 20 hours. The guanidine went into solution and a syrup formed on the surface. The syrup was soluble in alcohol, insoluble in ether and chloroform, and very soluble in water. It was dissolved in water, and the solution evaporated to dryness in a vacuum (Found: N, by Kjeldahl's method, 14.2; P, 21.5%). These figures correspond to the figures for *guanidine diethyl pyrophosphate*, which requires N, 14.2; P, 21.4%.

That the compound is a salt was shown by the formation of the yellow crystalline guanidine picrate on addition of a concentrated solution of picric acid. The precipitate was separated from picric acid by washing with ether and had the correct m. p. of 316°. It contained no trace of phosphorus (Found: N, 29.3. Calc. for $\text{CH}_5\text{N}_3, \text{C}_6\text{H}_3\text{O}_7\text{N}_3$: N, 29.1%). This compound again is a salt of ethyl dihydrogen phosphate.

Summary.

By the action of various alcohols on ethyl metaphosphate there has resulted a mixture of esters of phosphoric acid and usually a mixed ether, sometimes a simple ether. The products are more easily reviewed by reference to the following list.

Alcohol taken.	Esters formed.	Ethers.	Alcohol taken.	Esters formed.	Ethers.
Ethyl	Monoethyl Diethyl		Phenol	Monophenyl Diphenyl	ethyl phenyl
Propyl	Monopropyl Dipropyl Monoethyl Diethyl	Dipropyl		Monoethyl Diethyl	
Cetyl	Monocetyl Dicetyl Monoethyl Diethyl	Ethyl cetyl Dicetyl	Chol- esterol	Monochol- esteryl Monoethyl	Ethyl chol- esteryl

The use of ethyl metaphosphate for preparing esters of phosphoric acid, on account of the mixtures produced, is not recommended. It has no advantage over the reaction of alcohol and phosphoric oxide.

The reaction of ethyl metaphosphate with ammonia, hydrazine, alanine, guanidine, and other amino-compounds yields salts of ethyl metaphosphate, which are converted by water into salts of ethyl dihydrogen phosphate, or of diethyl dihydrogen pyrophosphate. It has not been possible to isolate any compound with the composition described by Langheld, or by Strecker and Heuser, in which nitrogen is directly combined with phosphorus.

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