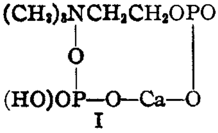
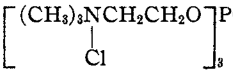
[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Mono and Tricholine Esters of Orthophosphoric Acid[1,2]

BY ERNEST L. JACKSON

Of the three possible simple choline esters of orthophosphoric acid, the triester hitherto has not been reported and only the diester seems to have been obtained pure. Abderhalden, Paffrath and Sickel[3] described crystalline, apparently pure di-(choline bromide) phosphate which they prepared by the reaction of ethyl metaphosphate with choline bromide. The mixed ester, dimethyl mono-(choline chloride) phosphate, was prepared crystalline by Renshaw and Hopkins[4] while Langheld[5] reported the amorphous, impure chloroplatinate of ethyl mono-(choline chloride) phosphate and Abderhalden, Paffrath and Sickel[3] the sirupy, likewise impure ethyl mono-(choline bromide) phosphate. The present paper pertains to the mono and tricholine esters, particularly the preparation of the esters or a suitable derivative in high purity.

By heating choline chloride with a mixture of orthophosphoric acid and phosphorus pentoxide Schmidt[6] obtained a sirup which, by analogy with the reaction of choline chloride with sulfuric acid, he inferred to contain a phosphoric acid ester of choline. The further investigation of this reaction has now led to the preparation of the amorphous calcium salt of the monocholine ester (1)  $(CH_3)_3N(O\!-)CH_2CH_2OPO(OH)(O\!-)\ ;\ (HO)OP\!-\!O\!-\!Ca\!-\!O$ I , which, though free from choline, was contaminated with 3–4% of dicholine phosphate as shown by the isolation of its crystalline chloroaurate. Monocholine phosphate chloroaurate prepared from the calcium salt was crystalline, but contained a little impurity which could not be eliminated. The chloroplatinate, however, could be prepared pure and crystalline from the chloroaurate by removing the gold with finely divided silver[7] and forming the chloroplatinate from the resulting monoester. The pure chloroplatinate is suitable as a source of the monoester for pharmacological study as the platinum is eliminated readily by metallic silver and the monoester is stable in aqueous solution.

Trimethylamine condenses slowly with tri-β-chloroethyl phosphate either in toluene solution or without a solvent, but the products are sirups, probably mixtures consisting mostly of insoluble intermediate condensation products. Pure, crystalline tri(choline chloride) phosphate (II)  $\left[\underset{\displaystyle Cl}{(CH_3)_3\overset{|}{N}}CH_2CH_2O\right]_3PO$ was prepared, however, by carrying out the reaction in anhydrous ethanol solution under selected conditions of temperature and concentration. When heated at 90° for two hours the pure triester, though showing no change in appearance and elementary analysis, acquires a considerably increased physiological activity,[2] evidently due to a partial conversion to a more active compound, perhaps through an intramolecular reaction with neurine chloride as a possible product. The triester yields readily a crystalline chloroaurate.

Experimental

Calcium Phosphato-ethyltrimethylammonium Phosphate (I).—A mixture of 12 g. of pure dry choline chloride, 8.4 g. of 100% orthophosphoric acid and 12 g. of phosphorus pentoxide was heated at 100° under reduced pressure for thirty minutes, the sirup stirred, the stirrer left in the mixture and the heating continued for thirteen hours. The glassy mass was dissolved in 150 cc. of cold water, the solution shaken with excess of calcium carbonate, some activated carbon added, and the solids filtered off and washed with water. The filtrate was neutralized to phenol red with calcium hydroxide solution, the calcium phosphate filtered off after adding some carbon, and the filtrate concentrated immediately under reduced pressure (bath, 60°) to about 100 cc., again neutralized to phenol red with calcium hydroxide solution, some carbon added and filtered off. The concentration was continued to a thin, colorless sirup which was poured slowly into 350 cc. of absolute ethanol, and the precipitated sirup stirred until completely solid and powdered. It was filtered off and washed with absolute ethanol; yield, 16–19 g. The amorphous, hygroscopic calcium salt was precipitated

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[1] Publication authorized by the Surgeon General, U. S. Public Health Service.

[2] The preparation of the esters was undertaken at the suggestion of Dr. Maurice I. Smith in connection with his studies on the pharmacology of the phosphoric acid esters. The physiological action of the compounds, including triethyl and tri-β-chloroethyl phosphates, will be published by him in *Bull. Nat. Inst. of Health*, No. 165 (1935). The investigation was greatly facilitated by the analytical coöperation of Dr. E. Elvove and Mr. C. G. Remsburg of this Institute, to whom we express our thanks.

[3] Abderhalden, Paffrath and Sickel, *Arch. ges. Physiol. (Pflugers)*, **207**, 249 (1925).

[4] Renshaw and Hopkins, THIS JOURNAL, **51**, 953 (1929).

[5] Langheld, *Ber.*, **44**, 2084 (1911).

[6] Schmidt, *Ann.*, **337**, 55 (1904); *cf.* Grün and Limpächer, *Ber.*, **59**, 1350 (1926); **60**, 147 (1927).

[7] *Cf.* Dudley, *Biochem. J.*, **23**, 1071 (1929).

thrice from its solution in 20 cc. of water by ethanol. It was then free from choline,[2] but contained less than 5% of dicholine phosphate. The substance showed no hydrolysis[2] by *N* hydrochloric acid at 100° during fifty minutes.

Anal. Calcd. for $C_5H_{15}O_8NP_2Ca$: C, 18.79; H, 4.74; N, 4.39; P (ionizable), 9.72; P (total), 19.43; Ca, 12.55. Found (dried at 75° *in vacuo*): C, 18.85; H, 4.59; N, 4.17; P (ionizable), 11.65; P (total), 20.40; Ca, 12.73; Cl, 0.00.

Monocholine Phosphate Chloroaurate.—To 10.5 g. of thrice precipitated calcium salt dissolved in 50 cc. of cold 2 *N* hydrochloric acid was added an equimolecular amount of auric chloride in 50 cc. of the same solvent. The precipitate (0.9 g.) was filtered off, washed with cold 2 *N* hydrochloric acid and recrystallized twice from about forty parts of hot *N* hydrochloric acid (clusters of light yellow, short prismatic needles difficultly soluble in water); m. p. 271.5–272° (corr., decomp.). Its composition agreed with that of dicholine phosphate chloroaurate.

Anal. Calcd. for $C_{10}H_{27}O_4N_2PAu_2Cl_8$: C, 12.65; H, 2.87; N, 2.95; P, 3.27; Au, 41.59. Found (dried at 25° *in vacuo* over phosphorus pentoxide): C, 12.78; H, 2.80; N, 3.03; P, 3.27; Au, 41.40.

The principal product was the chloroaurate of monocholine phosphate which was obtained in fractions as homogeneous, long, yellow needles by repeated concentration of the filtrate under reduced pressure (bath, 50°): yield, 14.3 g. or 83%. The crude substance was recrystallized five times from 2 *N* hydrochloric acid, washed with cold water and dried at 80° *in vacuo;* m. p. 174–176° (corr.). The compound, though showing substantially the same analysis after another recrystallization, was somewhat impure. It is hygroscopic and easily soluble in water.

Anal. Calcd. for $C_5H_{15}O_4NPAuCl_4$: C, 11.47; H, 2.89; N, 2.68; P, 5.93; Au, 37.69; Cl, 27.11. Found: C, 12.56; H, 3.10; N, 2.91; P, 6.32; Au, 36.18; Cl, 26.03; Ca, 0.14.

Chloroplatinate.—An aqueous solution (30 cc.) of 2.5 g. of thrice recrystallized monocholine phosphate chloroaurate was shaken for a few minutes with 5 g. of finely divided silver,[7] the solids filtered off and the colorless filtrate concentrated under reduced pressure to a sirup, which was dissolved in 5 cc. of *N* hydrochloric acid and mixed with 33% excess of the theoretical chloroplatinic acid in 5 cc. of water. After adding ethanol to turbidity it was kept at 5° for several hours, the crystals filtered off, washed first with ice-cold 50% ethanol and then with absolute ethanol. A second crop was obtained by concentrating the filtrate under reduced pressure (bath, 50°) to about 5 cc. and adding ethanol to turbidity; yield, 1.7 g. or 92%. One recrystallization by dissolving it in 5 cc. of 0.5 *N* hydrochloric acid at 35–40° and adding 4 cc. of ethanol gave the pure chloroplatinate (1.3 g.) as clusters of orange-colored prismatic needles. For analysis the hygroscopic crystals were dried to constant weight at 25° *in vacuo* over phosphorus pentoxide; m. p. 207–208° (corr., decomp.).

Anal. Calcd. for $C_{10}H_{30}O_8N_2P_2PtCl_6 + 2H_2O$: H_2O, 4.44. Found:[8] H_2O, 4.28. Calcd. for $C_{10}H_{30}O_8N_2P_2PtCl_6$: C, 15.46; H, 3.90; N, 3.61; P, 7.99; Pt, 25.15; Cl, 27.41. Found:[9] C, 15.55, 15.59; H, 4.06, 4.07; N, 3.57, 3.65; P, 8.02, 8.13; Pt, 25.41, 25.67; Cl, 28.03.

Tri-β-chloroethyl Phosphate.—Sixty-four grams of phosphoryl chloride was added during twenty minutes to a mechanically stirred mixture of 106 g. of pure ethylene chlorohydrin and 125 cc. of dry carbon tetrachloride[4] at room temperature. After stirring for thirty minutes it was refluxed gently for sixteen hours, kept at 25° for two days, the carbon tetrachloride and a little ethylene chlorohydrin distilled off through a seven-bulb column (15 mm. at end) and the residue fractionated at 2–3 mm.; yield, 75 g. (60% on the chlorohydrin); b. p. 180–182° (2–3 mm.); d^{20}_{20} 1.428. About 20 g. of product boiling above 215° (2–3 mm.) showed considerable decomposition on attempted distillation. Plimmer and Burch[10] reported about 12% yield, b. p. 140° (40 mm.) and *d* 1.39. The cause of the disagreement in boiling points is not apparent.

Anal. Calcd. for $C_6H_{12}O_4Cl_3P$: C, 25.22; H, 4.24; Cl, 37.26; P, 10.87. Found: C, 25.26; H, 4.39; Cl, 37.27; P, 10.69.

Tri-(choline chloride) Phosphate (Phosphato-tri-(ethyltrimethylammonium chloride)) (II).—A solution of 6.5 g. (0.11 mole) of trimethylamine and 10 g. (0.035 mole) of pure tri-β-chloroethyl phosphate in 12 cc. of anhydrous ethanol[11] was sealed at 0° and then kept at 30–33° for seven days, well-built prisms starting to separate after two to three days. After cooling to 0° the crystals were filtered off, washed with 8 cc. of cold isopropanol and dried at 25° *in vacuo* over calcium chloride; yield, 7.7 g. (48%). The filtrate, diluted with 25 cc. of isopropanol, gave on long standing at 5° about 0.3 g. of crystals. When dissolved in about six parts of absolute ethanol at 60° and decolorized with activated carbon, the triester crystallizes as colorless prismatic needles or elongated prisms in 80–85% yield with little, if any, alcoholysis. It is somewhat hygroscopic, is practically insoluble in benzene, chloroform and ether, difficultly soluble in warm isopropanol, soluble in warm ethanol and readily soluble in cold water to yield a neutral solution from which the chlorine is removed quantitatively by silver nitrate. Its decomposition point (incomplete) of about 245° (uncorr.) is not a suitable criterion of purity. Analyses of thrice recrystallized samples were essentially the same when dried to constant weight at 25° *in vacuo* over phosphorus pentoxide or at 90° *in vacuo*, but the physiological activity[2] was considerably increased by the two hours of heating at 90°.

Anal. Calcd. for $C_{15}H_{39}O_4N_3Cl_3P$: C, 38.90; H, 8.50; N, 9.08; Cl, 22.99; P, 6.70. Found: (dried at 25°) C, 38.68; H, 8.84; N, 8.97; Cl, 22.90; P, 6.85; (dried at 90°) C, 38.85; H, 8.68; N, 8.97; Cl, 22.76; P, 6.71.

Toluene was found unsuitable as a solvent in the prepa-

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(8) Sample previously dried overnight at 25° *in vacuo* over calcium chloride.

(9) Prior to the determination of nitrogen (Kjeldahl) and phosphorus, the sample was digested for two hours with hot concentrated sulfuric acid and the platinum filtered off.

(10) Plimmer and Burch, *J. Chem. Soc.*, 284 (1929).

(11) It is important to use the specified proportions of tri-β-chloroethyl phosphate and ethanol. When 8 cc. of ethanol was used, the separation of a sirup with the crystals of the triester necessitated the interruption of the reaction after three days, removal of the crystals and dilution with ethanol. With 18 cc. the reaction time was considerably greater (twelve days) and the yield of triester was smaller (30%).

ration of the triester. Trimethylamine (6.5 g.) and tri-β-chloroethyl phosphate (10 g.) in 100 cc. of dry toluene, kept sealed at 25–30° for several days or at 78–80° for two days, gave a sirup. A solution of the sirup, prepared at 78–80°, in a mixture of isopropanol and ether yielded at 5° crystals (0.6 g.) which could be recrystallized from isopropanol (needles), but was not investigated further.

Chloroaurate.—The compound was precipitated by adding an aqueous solution (7 cc.) of 1 g. of pure triester to 10% excess of the calculated auric chloride in 10 cc. of water; yield, 2.7 g. It crystallizes from water (difficultly soluble) as clusters of short, yellow prismatic needles melting incompletely at 216° (uncorr.) and to a clear red liquid at 230°.

Anal. Calcd. for $C_{15}H_{39}O_4N_3PAu_3Cl_{12}$: C, 13.11; H, 2.86; N, 3.06; P, 2.26; Au, 43.07. Found (dried at 25° *in vacuo* over phosphorus pentoxide): C, 13.17; H, 2.88; N, 3.07; P, 2.28; Au, 43.20.

Summary

From the products of the phosphorylation of choline chloride with orthophosphoric acid and phosphorus pentoxide the amorphous calcium salt of the monocholine ester of phosphoric acid has been prepared free from choline but containing 3–4% of dicholine phosphate, a new chloroaurate of which is described. Monocholine phosphate chloroaurate prepared from the calcium salt was somewhat impure, but from it could be obtained the pure, crystalline chloroplatinate.

Trimethylamine condenses with tri-β-chloroethyl phosphate under appropriate conditions to give crystalline tri-(choline chloride) phosphate which yields a crystalline chloroaurate. An effect of heat on the pure triester is pointed out.

Improvements have been made in Plimmer and Burch's procedure for the preparation of tri-β-chloroethyl phosphate.

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The Predominant Role of Association in the Dissociation of Simple Straight-Chain Sulfonic Acids in Water. I. Conductivity[1]

BY J. W. MCBAIN AND MARGARET D. BETZ

Among the simplest uni-univalent organic electrolytes are the straight-chain saturated alkyl sulfonic acids, RSO_3H, the higher members of which have been made for the first time and generously placed at our disposal by Prof. C. R. Noller and J. J. Gordon.[2] In a series of communications it will be shown that their behavior at great dilution is that of simple partially dissociated acids, but that in more concentrated solutions, such as from $N/20$ upward, a radical change takes place in their physical chemical properties, such as conductivity, freezing point and electromotive force.

This change is the opposite of that which the interionic attraction theory was set up to explain. It would appear to be due to association in the sense in which this term has so long been used in chemical science, as for example in describing the occurrence of double molecules in the vapor of acetic acid or in solutions of acetic and other acids in benzene. This association is not caused by Coulomb attraction of opposite charges, but it is due to van der Waals cohesive forces. It occurs even in spite of electrostatic repulsion, for it is very marked in the association of ions of like charge.

Since in all the more concentrated solutions the effect of association is so dominant as to submerge the ordinary behavior of interionic attraction, it must surely be a force of universal occurrence and a factor which is seldom negligible. This association must affect not merely ions but also the molecules from which they are derived. Since one of the authors introduced the conceptions of colloidal electrolytes and of neutral and ionic micelles more than twenty years ago,[3] it has been known that the group of colloidal electrolytes embraces more substances than the acids and bases put together.[4] However, it is not sufficiently recognized that colloidal electrolytes appear to be the rule rather than the exception in non-aqueous solutions and that even fairly simple substances in aqueous solution, such as

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(1) Experiments by Miss Betz.

(2) C. R. Noller and J. J. Gordon, THIS JOURNAL, **55**, 1090 (1933); for cetyl sulfonic acid see J. W. McBain and R. C. Williams, *ibid.*, **55**, 2250 (1933); B. Flaschenträger and G. Wannschaff, *Ber.*, **67**, 1121 (1934); R. C. Murray, *J. Chem. Soc.*, 739 (1933).

(3) J. W. McBain and C. S. Salmon, THIS JOURNAL, **42**, 426 (1920); *Proc. Roy. Soc.* (London), **A97**, 44 (1920); J. W. McBain and H. E. Martin, *J. Chem. Soc.*, **105**, 957 (1914); J. W. McBain, *Trans. Faraday Soc.*, **9**, 99 (1913); *Kolloid-Z.*, **12**, 256 (1913); J. W. McBain, M. E. Laing and A. F. Titley, *J. Chem. Soc.*, **115**, 1279 (1919).

(4) Compare the extensive international symposium on colloidal electrolytes of September, 1934, *Trans. Faraday Soc.*, **31**, 1 (1935).