ant had evaporated, stirring of the partially frozen mixture was continued for 15 minutes or more; then the temperature was observed, and a sample of the liquid solution was withdrawn at once using a hypodermic syringe and needle. A small glass wool filter at the end of the needle prevented the inclusion of crystals in the sample. This sample was weighed by difference after being transferred to a stoppered flask containing water. The amount of acid was then determined by titration with standardized 0.1 *N* sodium hydroxide. This procedure gave more nearly reproducible values for the composition of a mixture rich in water than for one rich in acid. Changes in composition of the solution were made by adding one of the components or by changing the amount of the solid phase by melting or by freezing.

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N-Phosphorylated Derivatives of Diethanolamine[1]

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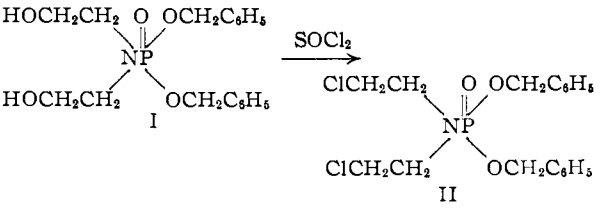
A re-examination[2] of the reported abundance of phosphamidase in malignant tissues as compared to normal tissues[3] has been undertaken with substrates of possible use as chemotherapeutic agents. The substrates prepared for this purpose were N-phosphorylated derivatives of bis-β-chloroethylamine.[2] One approach to the synthesis of compounds of this type appeared to be by way of dibenzyl di-(2-hydroxyethyl)-phosphoramidate (I)[4] which might be partially or totally debenzylated either before or subsequent to chlorination to afford the dibenzyl, monobenzyl and dibasic phosphoric acid derivatives of bis-(β-chloroethyl)-amine.

The dibenzyl di-(2-hydroxyethyl)-phosphoramidate (I) was prepared by reaction of dibenzyl phosphite with two molar equivalents of diethanolamine in carbon tetrachloride.[5] Dibenzyl phosphite was prepared crystalline in over 80% yield by modification of the procedure of Atherton, Oppenshaw and Todd[6] which obviated the hazardous distillation. Pyridine was substituted for dimethylaniline and the distillation time for removal of benzyl chloride from the product was increased. On chlorination with thionyl chloride the dihydroxyphosphoramidate I afforded the corresponding dichlorophosphoramidate II. Hydrogenolysis of this product II over palladium–carbon, however, either when allowed to go to completion, or when terminated after the uptake of one molar equivalent of hydrogen, did not give isolable products. Attempts to prepare the debenzylated dibasic acid, di-(2-hydroxyethyl)-phosphoramidic acid, by other means have been similarly unsuccessful[2] presumably owing to instability of the product.



Partial hydrolysis of dibenzyl di-(2-hydroxyethyl)-phosphoramidate with dilute aqueous alkali resulted in hydrolysis of the P–N bond rather than in ester cleavage. Several attempts were made to isolate benzyl hydrogen di-(2-hydroxyethyl)-phosphoramidate from reactions with 1.5 to 2 molar equivalents of alkali heated to reflux for periods varying from 15 minutes to 1.5 hours. In all cases dibenzylphosphoric acid was obtained in good yield without a trace of the desired product. The preferential hydrolysis of the phosphamide bond in the diester monoamide I is surprising since triesters of phosphoric acid are in general known to be sensitive to alkali.

Several attempts were made to monodebenzylate dibenzyl di-(2-hydroxyethyl)-phosphoramidate (I) with lithium chloride in ethyl cellosolve under various modifications of the conditions which Clark and Todd[7] used to prepare benzyl hydrogen phenylphosphoramidate from dibenzyl phenylphosphoramidate. During the course of the reaction a lithium salt separated in relatively good yield depending upon length of time and heating. This product could not be recrystallized and on analysis the crude material gave a value for nitrogen less than one third of that required for lithium benzyl hydrogen di-(2-hydroxyethyl)-phosphoramidate. Moreover when the crude products were worked up separately or with the reaction mixtures, the only product that could be obtained appeared from the analytical results to be benzylphosphoric acid isolated as the monocyclohexylamine salt.

Experimental[8]

Dibenzyl Phosphite.[6]—To a solution of 88 cc. (138 g.) of phosphorus trichloride in 75 cc. of dry benzene well cooled in ice, a mixture of 158 cc. of dry pyridine and 208 cc. of dry benzyl alcohol was added slowly with vigorous stirring over a period of three hours. After the mixture was stirred for an additional 30 minutes, 104 cc. of benzyl alcohol was added over a period of 20 minutes. The mixture was then allowed to stand at room temperature for 16 hours. The benzene solution was washed in turn with three 500-cc. portions of water, two 500-cc. portions of 5 *N* ammonium hydroxide, two 500-cc. portions of water and finally dried with sodium sulfate. Removal of the benzene under reduced pressure left a light-colored yellow oil from which all traces of benzyl chloride were removed on the steam-bath at 1–3 mm. for 10 hours. The product, a light-yellow oil, crystallized as a solid white mass when stored in the cold; 216.5 g. (82.6%).

Dibenzyl Di-(2-hydroxyethyl)-phosphoramidate (I).—A solution of 41.6 g. of dibenzyl phosphite and 30.4 cc. of diethanolamine in a mixture of 160 cc. of carbon tetrachloride and 160 cc. of chloroform was stirred at room temperature for 19 hours. The reaction mixture became warm during the first hour and turned turbid. When stirring was discontinued, diethanolamine hydrochloride appeared as an orange oil and was separated. The carbon tetrachloride–chloroform solution was shaken with water to remove ex-

(1) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency, and (in part) by a research grant from Mrs. Albert B. Lasker.

(2) O. M. Friedman and A. M. Seligman, THIS JOURNAL, **76**, 655 (1954).

(3) G. Gomori, *Proc. Soc. Exp. Biol. Med.*, **69**, 407 (1948).

(4) The system of nomenclature adopted is in accord with the recommendations of the American Chemical Society Committee on Nomenclature, A.C.S. Official Reports, *Chem. Eng. News*, October (1952).

(5) F. R. Atherton and A. R. Todd, *J. Chem. Soc.*, 674 (1947).

(6) F. R. Atherton, H. T. Oppenshaw and R. A. Todd, *ibid.*, 382 (1945).

(7) V. M. Clark and A. R. Todd, *ibid.*, 2030 (1950).

(8) Microanalyses by S. M. Nagy and colleagues, Microchemical Laboratory, Massachusetts Institute of Technology. All melting points are corrected.

cess diethanolamine and finally concentrated to small volume under reduced pressure. Dilution of the darkly-colored concentrated solution to excess with petroleum ether precipitated the crude product as a yellow-orange oil, 41.5 g. The crude product in benzene was chromatographed through 400 g. of acid-washed alumina. A small amount of material was eluted with benzene and 200 cc. of benzene–acetone (1:1). Pure product was eluted with 500 cc. of acetone–methanol (1:1) and was obtained as a pale yellow oil, 31.8 g., $n^{25}D$ 1.5465.

Anal. Calcd. for $C_{18}H_{24}NPO_5$: C, 59.16; H, 6.62; N, 3.83. Found: C, 59.11; H, 6.73; N, 4.00.

Dibenzyl Di-(2-chloroethyl)-phosphoramidate (II).—A solution of 1.5 cc. (1.77 g.) of the dihydroxyphosphoramidate I, 2 cc. of thionyl chloride and one drop of pyridine in 15 cc. of chloroform was heated under reflux for 15 minutes. The mixture was distilled at reduced pressure to remove excess thionyl chloride and solvent. The residue, 1.83 g., dissolved in benzene was shaken with water. The benzene solution, dried and concentrated to small volume by distillation, was passed over acid-washed alumina from which the product was eluted with benzene as an oil, 0.4 g., $n^{28}D$ 1.5452.

Anal. Calcd. for $C_{18}H_{22}NPO_3Cl_2$: C, 53.74; H, 5.51; N, 3.48; Cl, 17.63. Found: C, 54.16; H, 5.66; N, 3.39; Cl, 18.20.

An additional 0.6 g. of pale yellow oil, probably unchanged starting material, was eluted from the column with acetone, $n^{28}D$ 1.5468.

Hydrolysis of Dibenzyl Di-(2-hydroxyethyl)-phosphoramidate (I) with Base.—A solution of 2 g. of dihydroxyphosphoramidate I in a mixture of 80 cc. of 95% ethanol and 80 cc. of 0.1 *N* sodium hydroxide was refluxed for 30 minutes. Acidification of the reaction mixture after concentration gave an oil that was extracted with ethyl acetate and which crystallized from ether after standing at 5°, 0.7 g., m.p. 77–79°. The m.p. of dibenzylphosphoric acid was variously reported from 78–80°.[9]

Anal. Calcd. for $C_{14}H_{15}PO_4$: neut. equiv., 278.2. Found: neut. equiv., 277.

The cyclohexylamine salt recrystallized from methanol–acetone, m.p. 174–175.5°.

Reaction of Dibenzyl Di-(2-hydroxyethyl)-phosphoramidate (I) with Lithium Chloride.—A solution of 12.5 g. of the dihydroxyphosphoramidate I in 110 cc. of cellosolve, saturated at the boiling point with freshly fused lithium chloride, was heated under reflux for 13.5 hours. Careful acidification of the reaction mixture cooled in ice gave an oil, 8.65 g., that was extracted with ethyl acetate. The cyclohexylamine salt crystallized from methanol–ethyl acetate as long white needles, m.p. 246–248°. The analytical results are in accord with cyclohexylammoniumbenzylphosphoric acid.

Anal. Calcd. for $C_{13}H_{22}NPO_4$: C, 54.33; H, 7.72; N, 4.87. Found: C, 53.29, 54.23; H, 7.66, 7.78; N, 4.68, 4.71.

(9) G. N. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 254.

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Preparation of Salts of Glucuronic Acid

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Methods have been developed for the preparation and isolation of three salts of glucuronic acid which are preferable to published procedures.[1,2] In addition, we have found that the potassium salt of glucuronic acid forms a dihydrate under ordinary conditions rather than one containing 1.5 molecules of water as previously reported.[2]

(1) F. Ehrlich and K. Rehorst, *Ber.*, **58**, 1989 (1925).

(2) F. Ehrlich and K. Rehorst, *ibid.*, **62**, 628 (1929).

Preparation of Sodium and Potassium Salts of Glucuronic Acid.—Preparation of the sodium and potassium salts of glucuronic acid by titration of glucuronolactone with a stoichiometric amount of alkali is accompanied by extensive degradation. We have found that these salts are prepared best by rapid combination of aqueous alkali with glucuronolactone such that the reaction is complete in a few seconds. When the alkali is made up in aqueous alcohol these salts crystallize directly from the reaction medium. Sodium glucuronate crystallizes as a monohydrate and potassium glucuronate as a dihydrate.

Preparation of Ammonium Glucuronate.—The ammonium salt of glucuronic acid cannot be prepared by direct reaction of glucuronolactone with ammonia because amino-amides are formed.[3] However, as reported in the literature,[2] ammonium glucuronate can be prepared by direct reaction of glucuronic acid with aqueous ammonia. The salt crystallizes as a monohydrate from aqueous acetone. We obtain glucuronic acid for this reaction by passing an aqueous solution of the sodium or potassium salt over a cation-exchange resin column.[4]

Experimental

Preparation of Sodium Glucuronate (Monohydrate).—18.5 g. (0.105 mole) of glucuronolactone was stirred rapidly into 60 ml. of 50% (by volume) methanol containing 4.1 g. (0.102 mole) of sodium hydroxide. Crystals formed almost immediately and were removed by filtration after overnight refrigeration. They were washed with 75% (by volume) methanol and then with absolute methanol. The yield was 19 g. (0.081 mole) of colorless crystals dried in air at room temperature.

Anal.[5] Calcd. for $C_6H_9O_7Na\cdot H_2O$: C, 30.8; H, 4.7; Na, 9.8; glucuronic acid, 83; water, 7.7. Found: C, 30.6; H, 5.0; Na, 9.6; glucuronic acid (naphthoresorcinol method), 82; water (Karl Fischer), 8.3.

This salt lost no weight when it was stored under vacuum over anhydrous calcium chloride for 24 hours.

Preparation of Potassium Glucuronate (Dihydrate).—18.5 g. (0.105 mole) of glucuronolactone was stirred rapidly at room temperature into 100 ml. of 30% (by volume) methanol containing 5.8 g. (0.103 mole) of potassium hydroxide. Crystals formed almost immediately and were removed by filtration after overnight refrigeration. They were washed first with 60% methanol and then with absolute methanol. The yield was 24.0 g. (0.09 mole) of colorless crystals dried in air at room temperature.

Anal. Calcd. for $C_6H_9O_7K\cdot 2H_2O$: C, 26.9; H, 4.8; K, 14.6; glucuronic acid, 72; water, 13.4. Found: C, 27.1; H, 4.8; K, 14.6; glucuronic acid, 74; water, 13.2.

This salt lost 7.3% of its weight after 72 hours under vacuum over anhydrous calcium chloride. It returned to its initial weight after overnight standing on the desk top. Thus it appears that the water of crystallization is not tightly bound.

Preparation of Ammonium Glucuronate (Monohydrate).—15.1 g. (0.078 mole) of crystalline glucuronic acid was stirred rapidly into 40 ml. of water containing 1.36 g. (0.080 mole) of ammonia. The glucuronic acid dissolved instantly, and 200 ml. of acetone was added gradually over a one-hour period with continuous agitation. A yield of 14.6 g. (0.065 mole) of crystals was separated by filtration, washed with acetone, and dried at room temperature.

Anal. Calcd. for $C_6H_9O_7NH_4\cdot H_2O$: C, 31.4; H, 6.5; N, 6.1; glucuronic acid, 85; water, 7.9. Found: C, 31.7; H, 6.3; N, 6.0; glucuronic acid, 86; water, 7.8.

(3) H. L. Frush and H. S. Isbell, *J. Res. Natl. Bur. Standards*, **41**, 609 (1948).

(4) Nalcite-HCR, National Aluminate Co., Chicago, Ill.

(5) Microanalyses by G. Stragand, University of Pittsburgh.