[CONTRIBUTION FROM THE HAVEMEYER CHEMICAL LABORATORY, NEW YORK UNIVERSITY]

THE BASIS FOR THE PHYSIOLOGICAL ACTIVITY OF CERTAIN -ONIUM COMPOUNDS.[1] III. CHOLINE DERIVATIVES

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The present paper deals with the syntheses of certain choline derivatives which were prepared with the view, in the main, of obtaining additional material for a study of the relation between structure and chemical, physical and physiological properties of this class of compounds.

Quantitative studies on the stabilities and on certain physical effects of several of these substances as well as a number of other -onium compounds have been made. The results will be published in the near future.

Chloro-acetyl-choline Chloro-acetate, $(CH_3)_3N(OOCCH_2Cl)CH_2CH_2OOCCH_2Cl$.—On account of the extraordinary physiological activity of acetyl-choline salts,[3] it seemed desirable to prepare some of the substituted acetyl derivatives.

The chloro-acetyl derivative was prepared by heating 10 g. of choline chloride in a sealed tube with an excess (13 cc.) of chloro-acetyl chloride at 100°. After two hours' heating, the contents of the tube were repeatedly extracted with dry ether, benzene and petroleum ether, and finally with acetic anhydride. The latter transformed the sirupy liquid into a granular mass. The product was then purified by solution in cold, absolute alcohol and precipitation with dry ether. The thick needle-like crystals so obtained were markedly hygroscopic; m. p. (not sharp), 303° (uncorr.). The product was soluble in alcohols, slightly soluble in acetone and practically insoluble in benzene, petroleum ether, carbon disulfide and chloroform.

Anal. Calcd. for $C_9H_{17}O_4NCl_2$: Cl, 25.91. Found: 26.26, 25.99.

Hunt[4] has found that while this chloro-acetyl-choline has a very intense muscarine action, it is in this respect about one thousandth as active as the non-chlorinated acetyl-choline. Another highly interesting effect of the substitution of the chlorine in the acetyl groups is the change, to a very considerable degree, of the seat of action from the endings of the inhibitory nerve on the heart to a part of the same nerve in the brain. This curious transference of the seat of action of a drug was also noticed by Hunt[5] when a phenyl group had been introduced in the acetyl group.

[1] This problem is being carried on in coöperation with Dr. Reid Hunt of the Harvard Medical School. The physiological data are the basis of another series of papers published elsewhere by him.

[2] In part this paper is constructed from a portion of a thesis presented by John Christie Ware, June, 1922, for the degree of Doctor of Philosophy at New York University.

[3] Hunt has shown that 0.000,000,002,4 mg. of acetyl-choline chloride per kg. of body weight gave a distinct lowering of the blood pressure of a cat [*Am. J. Physiol.*, **45**, 198 (1918)].

[4] Hunt and Renshaw, *J. Pharmacol.*, **25**, 315 (1925).

[5] Hunt and Taveau, *Hygienic Lab. Bull.*, **73**, 28 (1911),

Formocholine (as chloride), $(CH_3)_3N(Cl)CH_2OH$, has been investigated by Hunt and Taveau,[6] and by Dale.[7] The former found it to be distinctly more active than choline in causing a lowering of the blood pressure before atropine (muscarine effect), and about nine times as toxic. Since the acetylation of choline brought about such an extraordinary increase in activity it seemed of interest to see whether the acetylation of the formocholine would give a similar enhancing of the physiological effect. An important reason for desiring to study formocholine derivatives is that, in case these show substantially the same activity as the normal choline, one would then have structurally a somewhat simpler series of compounds to investigate.

No satisfactory method has been presented for the preparation of formocholine, and no record has been found of successful esterification of the product. Formocholine has been prepared previously by the action of silver oxide on iodomethyl-trimethylammonium iodide.[5,8] The iodine is very slowly eliminated and the resulting formocholine base readily undergoes dissociation yielding trimethylamine and formaldehyde. There is also formed some of the less alkylated amines.[8b] The yield of the formocholine, therefore, is very poor indeed.

We have found that the halomethyl acetates condense readily with trimethylamine giving the salts of the desired acetyl-formocholine in excellent yield. It was hoped that this acetyl derivative would prove a good source from which to make formocholine.

Iodomethyl Acetate.—This substance was prepared by a method used by Descudé[9] for the preparation of the corresponding chloro and bromo compounds. It had previously been found that this ester could be prepared in only very small yields by the interaction of methylene iodide and silver acetate.

Ten and two-tenths g. of finely powdered, dry paraformaldehyde was added to 55 g. of acetyl iodide (molecular equivalents) in small portions. A very vigorous reaction took place. After this had subsided the contents of the flask were heated at 100° for one-half hour and then subjected to several fractional vacuum distillations. There was thus obtained a colorless liquid; b. p., 65° (14 mm.); α^{22}, 1.902. The iodomethyl acetate is a distinct lachrymator. It undergoes material decomposition when distilled at atmospheric pressure.

Anal. Calcd. for $C_3H_5O_2I$: I, 63.45. Found: 63.31, 63.22.

Acetyl-formocholine Iodide, $(CH_3)_3NICH_2OOCC_2H_3$.—To a thoroughly cooled absolute alcoholic solution of trimethylamine (about 3.25 g.) in a pressure bottle, there was added slightly more than one molecular proportion (12 g.) of thoroughly cold iodomethyl acetate in an equal volume of absolute alcohol. As soon as the bottle was

[6] Ref. 5, p. 17.

[7] Dale, *J. Pharmacol.*, **6**, 147 (1914).

[8] (a) Hofmann, *Jahresber.*, **1859**, 377. (b) Litterscheid, *Ann.*, **337**, 74 (1904). (c) Erwins, *Biochem. J.*, **8**, 366 (1914).

[9] Descudé, *Bull. soc. chim.*, **27**, 867 (1902).

closed and the materials had mixed, needle-like crystals began to appear. After several hours the crystals were filtered off and washed with a little dry ether. The substance so obtained was practically pure. Two recrystallizations from absolute alcohol did not change the melting point of 152° (corr.). This acetyl derivative was not appreciably hygroscopic.

Anal. Calcd. for $C_6H_{14}NO_2I$: I, 48.99. Found: 49.26, 49.08.

Alkaline hydrolysis of this ester was not successful both on account of the instability of the formocholine base and because the formocholine iodide and the inorganic iodides have much the same solubilities in the solvents that could be used. The acetyl-formocholine chloride was therefore prepared.

Acetyl-formocholine Chloride.—This substance was prepared in a manner similar to that for the iodide. When purified by slow precipitation from its absolute alcohol solution with ether it forms extremely hygroscopic, thin plates. Its chloroplatinate crystallizes in yellow plates.

Anal. Calcd. for $C_6H_{14}NO_2Cl$: Cl, 31.16. Found: 21.05, 21.36.

Hunt[4] has found that the acetyl-formocholine iodide is only from 12 to 24 times as active as formocholine in giving the muscarine effect, whereas he had previously shown[5] that the acetylation of choline enhanced its activity from 60,000 to 100,000 times. This is all the more remarkable since he found formocholine itself to be about eight times as active as the unacetylated choline.

Preparation of Formocholine by the Hydrolysis of Acetyl-formocholine Chloride.—As all attempts to prepare the formocholine by hydrolyzing the ester with alkaline agents had failed, hydrolysis by means of very dilute acid solution was tried.

To 5.5 g. of the ester dissolved in 95% alcohol several drops of concd. hydrochloric acid were added and the product was heated to 56° for three 8-hour periods. The solution was then slowly concentrated. As it cooled, a mass of colorless, striated crystals was formed; yield, about 90%. Samples of these striated crystals 12 × 25 mm. have been obtained. After two recrystallizations from alcohol the product was analyzed.

Anal. Calcd. for $C_4H_{12}ONCl$: Cl, 28.23. Found: 28.10, 28.16.

Arsenic Derivatives of Choline.—Since compounds of the choline type often manifest their effect on certain nerve tissues at a high dilution, it seemed of interest to determine whether this grouping of elements would act as a carrier of other constituents, such as arsenic, to the nerve tissues. We have, therefore, prepared dichloro-arsylethyl-trimethyl-ammonium chloride. This is readily converted by means of alkalies and by a sodium carbonate solution into arsinylethyl-trimethylammonium chloride.

β-Chloro-ethyl-dichloro-arsine.—This was prepared by a method described by Pope.

A stream of pure, dry ethylene was passed through 222.8 g. of arsenic trichloride containing 26.8 g. of powdered aluminum chloride for 16 hours. The materials were thoroughly stirred during the reaction by a mechanical stirrer fitted with a mercury seal. The dark liquid formed was poured onto crushed ice and carbon tetrachloride was immediately added in order to dissolve the oil before extensive hydrolysis could take place. The solution was distilled and the fraction boiling above 70° was fractionated under reduced pressure. The third fractionation yielded 31.3 g.; b. p., 90–93° (30 mm.). From

the carbon tetrachloride originally distilled an additional quantity of the arsine, 2.1 cc., was obtained by fractional distillation. The carbon tetrachloride solution, on standing, passed through an interesting color change from a light pink to a deep indigo blue.

Dichloro-arsylethyl-trimethylammonium Chloride, $(CH_3)_3N—CH_2CH_2.AsCl_2.—$ Cl

Trimethylamine generated from 33 g. of trimethylammonium chloride was adsorbed in 50 cc. of cold toluene. To this thoroughly cold solution 24.4 g. of the arsine in an equal volume of toluene was added. The reaction took place at once with a moderate evolution of heat and the formation of crystals. After the reaction bottle had stood in a pressure cage overnight, the solid matter was filtered off and subjected to a threefold solution in absolute alcohol and precipitation with absolute ether. It was necessary to use thoroughly dry materials, for otherwise the substance separated as an oil. This arsenic derivative of choline crystallized in clusters of hygroscopic needles that had a slightly yellow tint; m. p., 181.1° (corr.). It was very soluble in absolute ethyl alcohol and substantially insoluble in acetone, ether and benzene.

Anal. Calcd. for $C_5H_{13}NCl_3As$: Cl, 39.62. Found: 39.68, 39.62.

Arsinylethyl-trimethylammonium Chloride, $(CH_3)_3N—CH_2—CH_2—AsO.—$ Cl

To one molecular porportion of dichloro-arsylethyl-trimethylammonium chloride dissolved in absolute alcohol were added two proportions of sodium hydroxide dissolved in absolute alcohol. The sodium chloride precipitate was filtered off and ether was added to the filtrate. A very fine, white precipitate formed. This was purified by several recrystallizations from warm absolute alcohol. From this solvent the substance crystallized in small, opaque, scale-like, slightly hygroscopic plates of a delicate cream color; m. p., 194° (corr.).[10]

Anal. Calcd. for $C_5H_{13}NOClAs$: Cl, 16.60; N, 6.56. Found: Cl, 16.68, 16.40; N, 6.30, 6.38.

Preparation of Neurine Bromide.—Neurine in the form of its salts has been the subject of repeated pharmacological investigation, but a satisfactory method for its production in quantity has not been published. The preparations have usually involved the elimination of a hydrohalide from a β-halo-ethyltrimethylammonium salt by means of silver oxide.[11] E. Schmidt[12] has stated that in this method large losses are incurred. He recommends the production of neurine by heating bromocholine bromide in aqueous solutions with barium hydroxide followed by slow evaporation and desiccation over calcium oxide.

It is believed that the method now to be described is distinctly superior,

[10] The trypanoisidal properties and toxicity of this compound have been investigated by Dr. C. N. Myers. It proved to be a striking example of a substance which was more toxic to infected than it was to normal animals. Ten normal rats were injected with amounts of this substance varying from 10 to 50 mg. per kg. of body weight. Those receiving 10 mg. lived and the others died within from four to 24 hours, depending on the amount injected. Sixteen infected animals having from 108,000 to 212,000 trypanosomes per cc. of blood were given from 1 to 12 mg. per kg. of body weight. All of these died within 24 hours with trypanosomes in the blood stream.

[11] (a) Bode, *Ann.*, **267**, 268 (1892). (b) Renshaw, THIS JOURNAL, **34**, 1618 (1912).

[12] Schmidt, *Apoth. Ztg.*, **27**, 682 (1912).

since it can be carried out readily and since a very pure product is obtained in excellent yield.

Fifty g. of powdered bromocholine bromide, covered by about an equal weight of absolute alcohol, was treated with 231 cc. of absolute alcohol containing 0.094 g. per cc. (one molecular equivalent) of potassium hydroxide. The flask was vigorously shaken during the addition of the base and then allowed to stand for an hour. The solution had an odor of trimethylamine but the decomposition was not great. After the potassium bromide had been filtered off, the neurine bromide was precipitated by the addition of 1.5 to 2 volumes of dry ether. The neurine bromide separated in lustrous, thin plates, contaminated with some potassium bromide. It was purified by a three-fold solution in cold alcohol and precipitated with ethyl ether. Petroleum ether may be used. Yields of 89 to 92% have been obtained. It can also be purified by evaporation of the original alcoholic solution under a vacuum and recrystallization from alcohol. The purified product softened at 192° and melted at 194° (corr.) when heated at the rate of 7° per minute.[13]

Action of Bromine on Neurine Bromide.—Bromine is at first absorbed rapidly when added to an alcoholic solution of neurine. After about one-third of the calculated amount has been added the velocity of addition appears to fall off quickly as indicated by the solution taking on a deep red color and having the appearance of containing much free bromine. When the ethyl alcohol solution containing equimolecular quantities of bromine and neurine bromide is evaporated, considerable decomposition occurs and lachrymatory vapors are given off together with a citron-rind odor. From this solution only 34% of dibromoneurine bromide was obtained. Considerable neurine bromide was isolated which had not been acted upon, although a gram-molecular equivalent of bromine had been added. Similar results were obtained by using methyl alcohol as solvent, but in this case approximately 70% of the bromine added.

This interesting retarding effect on the addition of the bromine to the double bond exerted by some product of the reaction was not studied quantitatively. It is suggested, however, that the effect is due to the ready formation of the perbromide of dibromoneurine bromide, and that bromine in this perbromide combination is more active in bringing about substitution than addition reactions. This perbromide was isolated with some difficulty as a crystalline product, and when so obtained it was very hygroscopic and unstable, undergoing complete decomposition within a few days in a stoppered container.

The dibromo compound resulting from this reaction melts with decomposition at 168° (corr.)[14] Bromo-ethenyltrimethylammonium bromide[15] melts sharply at 145° (corr.) when heated at 4° per minute and sharply at 155.5–156° (corr.) when heated at the rate of 7° per minute. This variation in melting point with rapidity of heating is shown by many of these -onium compounds, but none exhibits the effect to the degree that this substance does.

The Action of Phosphorus Oxychloride on Choline Chloride.—Schmidt[16] isolated chlorocholine chloride from a reaction mixture obtained

[13] Hunt has investigated this sample (Ref. 4, p. 342). It is of interest to note that while the substitution of the hydroxy-ethyl group in choline by the ethenyl group brings about an increase in both the muscarine and the (stimulating) nicotine effects, the enhancements of the two actions are not equal, the increase in nicotine-like action being very much greater.

[14] Previously prepared by Bode (Ref. 11a, p. 283).

[15] Previously prepared by Bode (Ref. 11a, p. 278). The introduction of the bromine atom in the neurine brought about substantially no change in activity; see Ref. 4, p. 344.

[16] Schmidt, *Ann.*, **337**, 56 (1904).

by boiling phosphorus oxychloride with choline chloride for four hours. The yield was not given. Since there was a considerable desirability of obtaining a sample of the chlorinated phosphoric acid esters of choline, $Cl_2P(=O)OCH_2CH_2N(CH_3)_3Cl$, for the preparation of the mixed phosphoric acid esters of that substance as well as compounds of the lecithin type, it seemed worth while to investigate this action further.

Ten g. of choline chloride was heated with 30 cc. of recently distilled phosphorus oxychloride until the solution showed the first signs of darkening. The flask containing the product was left overnight connected in series with a sodium hydroxide column to a vacuum pump. The next morning the product had solidified to a crystalline mass. After many failures the following procedure was adopted for purifying the material. It was thoroughly washed with petroleum ether and with anhydrous ethyl ether, dissolved in acetic anhydride and a small amount of ether added. This precipitated slightly colored flocks which were filtered off, and several volumes of anhydrous ether were added in portions to the filtrate. Long, needle-like crystals gradually formed. These were further purified by a twofold solution in acetic anhydride and precipitation with ether. The product so obtained was extremely hygroscopic, giving off hydrogen chloride in contact with air. Water and alcohols dissolved the material with apparent decomposition. It was moderately soluble in acetic anhydride and acetyl chloride and substantially insoluble in carbon disulfide, petroleum ether, ethyl ether and benzene.

Anal. Calcd. for $C_5H_{13}O_2NPCl_3$: N, 5.46. Found: 5.41, 5.60.

It was first thought that this substance was the desired dichloride of the phosphoric acid ester of choline. As it was found impossible, however, to get mixed phosphoric acid ester of the lecithin type from this product, some doubt was cast on that supposition. Subsequently, an analysis for chlorine indicated that but two chlorine atoms were at once precipitable with silver nitrate(calcd. for 2 chlorine atoms: 27.34; found: 28.49). The decomposition products of this substance obtained by treating it with water and cold dilute aqueous alkalies yielded chlorocholine chloride. There seems to be no doubt, then, that the substance isolated is at once a salt and an acid chloride of phosphoric acid, $(CH_3)_3N(OPOCl_2)CH_2CH_2Cl$, a type of compound of which the authors have not found a record. We should have expected such a substance to decompose immediately giving the chloride of the cation. It would appear to be an interesting compound from the point of view of the theories on polar and non-polar valences.

Summary

1. Improved methods for the preparation of neurine and formocholine and the syntheses of certain new choline derivatives are described. Their physiological activities are discussed briefly.

2. The double bond in neurine is incompletely brominated by molecular bromine. This may be due to the formation of a perbromide of the -onium bromide.

3. Arsinylethyl-trimethylammonium chloride is more toxic to animals infected with trypanosomes than to normal animals.

4. A compound which is at once a salt and an acid chloride of phosphoric acid has been isolated.

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A FURTHER STUDY OF THE UTILITY OF ETHYL γ,γ-DIETHOXY-ACETO-ACETATE AS A REAGENT FOR THE SYNTHESIS OF GLYOXALINES

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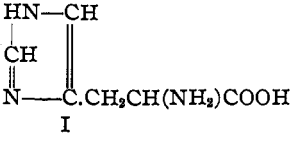
The established therapeutic value of the glyoxaline derivative histamine II, and its relationship structurally to the naturally occurring α-amino acid histidine I, have stimulated a special interest in all possible methods of preparing these two combinations or any of their derivatives synthetically. For the known methods of synthesis thus far developed for



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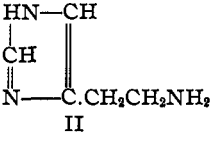
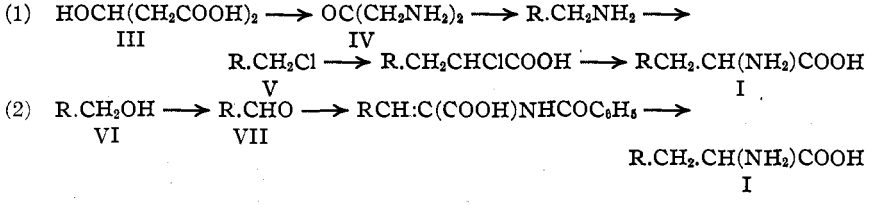


II

the preparation of the amino acid (I) we are indebted to Pyman,[2] who has used citric acid (III) as his starting point and developed successfully two processes for preparing this amino acid (I), the major steps of which are expressed below.[3]



Neither method is productive of the α-amino acid I in large yield, but of the two syntheses the second is apparently the best. Method 1 is singularly dependent for its application on the successful production of chloromethyl-glyoxaline V, while the second synthesis is wholly dependent for success on the availability of the aldehyde derivative of glyoxaline VII. Any methods for obtaining either of these two organic combinations would enhance greatly the practicability of Pyman's two syntheses.

[1] Constructed from a dissertation presented by Edward Wells Rugeley, to the Faculty of the Graduate School of Yale University in partial fulfilment of the requirements for the degree of Doctor of Philosophy, 1923.

[2] Pyman, *J. Chem. Soc.*, **99**, 1386 (1911); **109**, 186 (1916).

[3] R is the glyoxaline nucleus, HN.CH=N—CH=CH.