CXLI.—*Aminoacylcholine Esters. Part I.*
Glycylcholine.

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THE wide distribution of choline in the animal and vegetable kingdoms has led in recent times to a recognition of its importance in biological chemistry. Pharmacologically, this substance and its

derivatives are very interesting. Acetylcholine, for instance, is an extraordinarily potent drug, exhibiting its action on the frog's heart in a dilution of 1 : 1000 millions.

It appeared, therefore, of interest to prepare and examine the properties of the esters of choline with the naturally occurring amino-acids, and this paper deals with the first compound of the series, glycylcholine.

A simple and direct method of obtaining this substance was found. It consisted in mixing glycyll chloride hydrochloride and choline chloride in the form of fine powders and heating at 100° in a vacuum. Hydrogen chloride gas was evolved with formation of glycylcholine, which was isolated as the platinichloride, from which the hydrochloride of glycylcholine chloride was obtained. The yield, considering the nature of the reaction, is fair, the average of a number of preparations being 56 per cent. of the theoretical.

Nothnagel (*Arch. Pharm.*, 1894, **232**, 266), in describing the preparation of acetylcholine, notes that after decomposing the gold salt with hydrogen sulphide, and removing the excess of the latter by heating, the acetylcholine is hydrolysed, for, on adding chloroplatinic acid to the filtrate, choline platinichloride separates out. Accordingly Ewins and others have decomposed acetylcholine platinichloride by adding to its solution in water potassium chloride, evaporating to dryness in a vacuum, and extracting the residue with absolute alcohol, in which acetylcholine chloride is readily soluble.

The first isolation of the hydrochloride of glycylcholine chloride was made by this method. It proved to be very laborious, since the substance is not soluble in absolute alcohol. After evaporating an aqueous solution of the platinichloride with the amount of potassium chloride theoretically required, the glycylcholine chloride hydrochloride was extracted with many washings of hot 98 per cent. alcohol. But this was unnecessary. It was found possible to decompose the platinichloride with hydrogen sulphide, to remove the excess of the latter from the filtrate and to concentrate the solution by distillation in a vacuum at a low temperature, without any perceptible hydrolysis of the glycylcholine occurring. It seems possible that this method may be applicable to acetylcholine, for there does not appear to be any particular reason why the ester linking of glycylcholine should be more stable than that of acetylcholine.

The action of glycyll chloride hydrochloride on free choline was tried, but proved to be complicated, and was not further studied.

Choline is usually described as a colourless syrup, which, being strongly basic, absorbs carbon dioxide from the air, and is thereby transformed into the crystalline carbonate. Griess and Harrow

(*Ber.*, 1885, **18**, 717), however, report that they obtained the free base as hygroscopic crystals; but Guggenheim ("Die Biogenen Amine," p. 68) expresses doubt as to whether their substance was not in reality the carbonate. It is worthy of note that in the course of this work free choline was readily obtained as a solid, crystalline, very hygroscopic mass under conditions precluding the formation of carbonate. A short description of its preparation is appended.

The pharmacological behaviour of glycylocholine and other members of this series of compounds will be discussed elsewhere at a later date.

EXPERIMENTAL.

Glycylocholine Platinichloride,

Glycyl chloride hydrochloride was prepared without difficulty by Fischer's method, a very fine, snow-white powder, which gave a satisfactory analysis, being obtained. It was stored in a vacuum desiccator over phosphoric oxide. Choline chloride was obtained as a fine, white, crystalline powder by dissolving it in a small quantity of absolute alcohol and pouring the solution into a large volume of pure dry ether, at the same time stirring vigorously. The finely divided, precipitated choline chloride was filtered off rapidly and kept in a vacuum desiccator over phosphoric oxide. Equal amounts (0.3 gram) of the two dry powders were mixed in a test-tube, which, after being evacuated with the aid of a water-pump, was half immersed in a boiling-water-bath and heated for about six hours. The powder shrank and developed a faintly yellow tinge. After being allowed to cool, the product was digested with about 5 c.c. of absolute alcohol to decompose any unchanged glycyl chloride hydrochloride and to remove choline chloride, filtered, the residue dissolved in 10 c.c. of 50 per cent. alcohol, and the solution filtered. To the filtrate were added 15 c.c. of a 5 per cent. aqueous solution of chloroplatinic acid. An immediate turbidity was produced and the platinum salt was rapidly deposited in clusters of orange needles. After remaining over-night at about 2°, the platinichloride was filtered off, washed with a little 50 per cent. alcohol, and dried on porous plate in the air: yield 0.68 gram. The salt gave correct figures on analysis, without further purification, and was found to contain 1 mol. of water of crystallisation. It melted at 236—238° (uncorr.) with vigorous decomposition (Found: loss at 122° = 2.83; Pt in anhydrous salt = 34.15. $\text{C}_7\text{H}_{18}\text{O}_2\text{N}_2\text{Cl}_6\text{Pt}, \text{H}_2\text{O}$ requires $\text{H}_2\text{O} = 3.06$. $\text{C}_7\text{H}_{18}\text{O}_2\text{N}_2\text{Cl}_6\text{Pt}$ requires Pt = 34.2 per cent.).

This platinichloride is insoluble in absolute alcohol, but is moderately soluble in water.

Decomposition of Glycylcholine Platinichloride by Hydrogen Sulphide.

The platinichloride (0.1890 gram) was dissolved in 30 c.c. of water at 38°, and hydrogen sulphide was passed through the solution, which was allowed to cool to room temperature, until all the platinum had been removed. After being filtered, the clear, colourless solution was concentrated to about 3 c.c. in a vacuum at 30—35°, made up to 8 c.c. with absolute alcohol, and 5 c.c. of a 5 per cent. aqueous solution of chloroplatinic acid were added; 0.1311 gram of platinichloride was recovered. This melted at 236—238° and gave correct analytical figures for glycylcholine platinichloride (Found: H_2O = 2.55; Pt in anhydrous salt = 34.3 per cent.).

Glycylcholine Chloride Hydrochloride.

The platinichloride (0.453 gram) was dissolved in 70 c.c. of water at 37°, the platinum precipitated by hydrogen sulphide, and the clear filtrate evaporated in a vacuum (water-bath at 30—35°). When the volume was about 4 c.c., alcohol was added and the solution again evaporated in a vacuum. This process was repeated and the solution finally evaporated to dryness. The hydrochloride is a hygroscopic, white, crystalline substance, very soluble in water, from which it crystallises in prismatic needles. It is insoluble in absolute alcohol.

Glycylcholine Aurichloride, $\text{NMe}_3\text{Cl}\cdot\text{C}_2\text{H}_4\text{O}\cdot\text{CO}\cdot\text{CH}_2\cdot\text{NH}_2, \text{HCl}, 2\text{AuCl}_3$.

Glycylcholine platinichloride (0.45 gram) was decomposed by hydrogen sulphide as in the preceding experiment. The filtrate was concentrated in a vacuum to 6 c.c., diluted with 4 c.c. of water, and to the resulting solution (10 c.c.) were added 10 c.c. of an aqueous solution of chloroauric acid. The solution became turbid and fine, golden-yellow needles were rapidly deposited. The yield of the gold salt, which melted and decomposed slightly at 180—184° (uncorr.), was 0.465 gram. It was found to contain no water of crystallisation. It was freely soluble in absolute alcohol, more soluble indeed in this solvent than in water, and could be recrystallised from either solvent. On boiling the aqueous solution, some decomposition was noted.

The best way to recrystallise this salt is to dissolve it in excess of water at 40° and to concentrate the solution at a low temperature in a vacuum until crystallisation occurs. Recrystallised from water in this way, the gold compound separates as glistening, golden platelets having the same melting point and composition as the needles

described above. If heated at 110—120°, the salt slowly loses weight and discolours, obviously suffering slight decomposition.

For analysis, the gold salt was heated in a water-oven at 95° until the weight was constant. Only a minute change in weight of the air-dried material occurred (Found: Au=46.9. $C_7H_{18}O_2N_2Cl_8Au_2$ requires Au=46.9 per cent.).

Glycylcholine chloride hydrochloride forms a *double salt* with mercuric chloride, which crystallises in fine, woolly needles melting at 150—156° (uncorr.). This was prepared by dissolving the hydrochloride obtained from 0.6 gram of the platinichloride in 5 c.c. of water and adding 10 c.c. of a saturated alcohol solution of mercuric chloride. It slowly loses weight on heating at 95°, as does the corresponding salt of choline, owing to the volatility of the mercuric chloride.

Preparation of Crystalline Choline.

Choline chloride (2.5 grams) was dissolved in water and the chlorine removed from the solution by shaking with freshly-precipitated silver oxide. The silver chloride and oxide were filtered off and the solution was concentrated in a vacuum (water-bath at 30—35°). A viscous syrup was obtained, from which a small amount of silver oxide separated. The syrup was dissolved in 70 c.c. of absolute alcohol, and after the suspended silver oxide had been filtered off, the solution was again evaporated in a vacuum. The flask in which the operation was carried out was provided with a capillary bubbling-tube, to which a soda-lime tube was attached, in order to remove carbon dioxide from the air drawn through the liquid. Again a syrup was obtained. Absolute alcohol (70 c.c.) was added and the solution once more evaporated in a vacuum. The viscous syrup obtained was shaken three times with about 50 c.c. of dry ether, the bulk of the ether being removed by means of a pipette. Finally, the residual ether was removed in a vacuum (water-bath heated at 40°). The flask was then transferred to a vacuum desiccator containing phosphoric oxide. After twenty-four hours, a crystalline crust had been formed on the surface of the pasty mass. This was broken up with a glass rod and returned to the desiccator. After remaining for several days, the entire mass became crystalline. About 30 c.c. of dry ether were added and the crystalline material was ground to a coarse powder under the surface of the ether, which was afterwards removed by distillation in a vacuum.

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