90. *Amino-acid and Peptide Esters of Choline as Possible Analogues of the Oxytocic Hormone of the Posterior Lobe of the Pituitary Gland. Part I.*

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A survey of the chemistry of the oxytocic hormone suggests as a plausible hypothesis that it may be a peptide ester of choline, in which one of the amino-acid radicals is cystine, others being possibly tyrosine and arginine. The synthesis of such esters is therefore of interest, and in order to explore the available methods, various acyl and amino-acyl esters of choline and its homologue, methyl-β-hydroxyethyldiethylammonium hydroxide, have been prepared, and the oxytocic activity of some of them tested.

Although the isolation in the pure state of the oxytocic hormone of the posterior lobe of the pituitary gland has not yet been accomplished, our knowledge of its chemistry suggests as one plausible hypothesis that it may be a peptide ester of choline (I), in which one at least of the amino-acid radicals is cystine and others are possibly tyrosine and arginine. The evidence is outlined below.

$$\left[\begin{array}{l}-S\cdot CH_2\cdot CH(NH_2)\cdot CO\cdot NH\cdot CH\cdot CO\cdot NH\cdot CH\cdot CO\cdot NH \ . \ . \ . \ CO\cdot O\cdot CH_2\cdot CH_2\cdot NMe_3\cdot OH \\ \qquad\qquad HO\cdot H_4C_6\cdot CH_2 \qquad\quad [CH_2]_3\cdot NH\cdot C(:NH)\cdot NH_2\end{array}\right]_2$$
(I.)

Du Vigneaud, Sealock, Sifferd, Kamm, and Grote (*J. Biol. Chem.*, 1933, **100**, xciv) isolated tyrosine and cystine from the hydrolysis products of highly active preparations, and Stehle and Fraser (*J. Pharm. Exp. Ther.*, 1935, **55**, 136) obtained the same amino-acids and also arginine from similar sources. Gulland and Randall (*Biochem. J.*, 1935, **29**, 378, 391), Freudenberg, Weiss, and Biller (*Z. physiol. Chem.*, 1935, **233**, 173) and Sealock and du Vigneaud (*J. Pharm. Exp. Ther.*, 1935, **54**, 433) showed that the molecule almost certainly contains a disulphide linkage, and Gulland and Randall (*loc. cit.*, p. 378; *Chem. and Ind.*, 1936, **55**, 442) demonstrated the presence of a redox system of which the potential corresponds to that of the disulphide–sulphydryl system. The last-named authors also found some indication of the possible presence of a second redox system with a different potential. The results obtained by Gulland (*Biochem. J.*, 1933, **27**, 1218) in studying the action of nitrous acid on the hormone are consistent with the presence of amino-, guanidino-, and disulphide groups. The peptide character of the molecule was advocated by Stehle and Fraser (*loc. cit.*) and is supported by the close association of the hormone with proteoses and peptones (Gulland and Lucas, *Biochem. J.*, 1935, **29**, 2208) and by the association of

the as yet unidentified plant and animal enzymes which inactivate the hormone with the proteolytic enzymes (Gulland and Macrae, *Biochem. J.*, 1933, **27**, 1237, 1383).

Electrodialysis (Freeman, Gulland, and Randall, *Biochem. J.*, 1935, **29**, 2211; Das, Ghosh, and Guha, *Z. physiol. Chem.*, 1936, **238**, 131) and electrophoresis (du Vigneaud, Irving, Dyer, and Sealock, *J. Biol. Chem.*, 1938, **123**, 45) demonstrated that the hormone is itself a base or is adsorbed on a basic substance. Freudenberg and Biller (*Naturwiss.*, 1936, **24**, 523) showed that active preparations contain combined choline, and the p_H–stability curve of the hormone (Gaddum, *Biochem. J.*, 1930, **24**, 939) bears some resemblance to that of acetylcholine.

Dialysis experiments proved that the molecular weight of the hormone is relatively small (Smith and McClosky, *J. Pharm. Exp. Ther.*, 1924, **24**, 391; Kamm, *Science*, 1938, **67**, 199; Gulland and Macrae, *Biochem. J.*, 1933, **27**, 1383; Freeman, Gulland, and Randall, *loc. cit.*; Das, Ghosh, and Guha, *loc. cit.*).

In the light of this convergent evidence it was of interest to synthesise esters of choline in which the acyl groups are amino-acid or peptide radicals; moreover, such compounds may prove to be of biological importance in other connections. When this work was begun in 1935 the only example of this type of compound was glycyl choline (Dudley, J., 1921, **119**, 1256), but subsequently Freudenberg and Keller (*Ber.*, 1938, **71**, 329), with the same objective as ours in view, prepared the glycyl, *dl*-alanyl, *dl*-leucyl, and *dl*-valyl esters of choline by the interaction of β-dimethylaminoethanol with the appropriate azido-acyl chloride, conversion into the choline and reduction of the azido-group. The present communication records three other methods of synthesis which we have investigated with a view to determining the most suitable procedure for the preparation of esters of more complex constitution.

(1) The direct combination by heating a mixture of the amino-acyl chloride hydrochloride and choline chloride confirmed Dudley's preparation of glycyl choline, now isolated as the *chloride hydrochloride*, and yielded *glycylglycyl choline chloride hydrochloride*. The method broke down, however, when attempts were made to apply it to the synthesis of leucylglycyl and leucylglycylglycyl choline.

(2) The β-bromoethyl ester of carbobenzyloxycystine, prepared by esterification of bromohydrin with carbobenzyloxycystinyl chloride, and also the *iodoethyl* ester, obtained similarly from iodohydrin, reacted smoothly with trimethylamine to give *carbobenzyloxycystinyl choline bromide* and *iodide* (II) respectively. As an alternative, the iodide was obtained by the interaction of the bromoethyl ester with dimethylamine, followed by conversion of the resulting dimethylaminoethyl ester (III, R = ·CH_2·S·S·CH_2·) into the methiodide.

$$\left[\begin{array}{c}\cdot S\cdot CH_2\cdot CH\cdot CO\cdot O\cdot CH_2\cdot CH_2\cdot NMe_3I\\ |\\ NH\cdot CO\cdot O\cdot CH_2Ph\end{array}\right]_2 \qquad \begin{array}{c}NH\cdot CO\cdot O\cdot CH_2Ph\\ |\\ R\cdot CH\cdot CO\cdot O\cdot CH_2\cdot CH_2\cdot NMe_2\end{array}$$

(II.) (III.)

(3) The procedure considered to be the most generally suitable of the three which we have investigated for the synthesis of amino-acyl and peptidyl esters of choline is the interaction of the carbobenzyloxy-aminoacyl or peptidyl chloride with β-dimethylaminoethanol, the conversion of the resulting ester (III) into the methiodide, and removal of the carbobenzyloxy-group with phosphonium iodide (Harington and Mead, *Biochem. J.*, 1935, **29**, 1602). By these methods were prepared *benzoyl*, *lauryl*, and *dithiodiglycollyl choline iodides*, *phenylalanyl choline iodide hydriodide* (IV), *cysteyl choline iodide hydriodide* (V), the compounds forming the appropriate intermediate synthetic stages, and various related substances obtained by similar reactions in which β-diethylaminoethanol was the starting material in place of the dimethyl derivative.

$$CH_2Ph\cdot CH(NH_3I)\cdot CO\cdot O\cdot CH_2\cdot CH_2\cdot NMe_3I \qquad HS\cdot CH_2\cdot CH(NH_3I)\cdot CO\cdot O\cdot CH_2\cdot CH_2\cdot NMe_3I$$

(IV.) (V.)

Considerable difficulty was experienced in handling many of these compounds on account of their poor powers of crystallisation and their extraordinarily hygroscopic

character; in several cases mere exposure of the solid substance to the atmosphere for a few seconds was sufficient to convert it into a viscous oil.

The oxytocic activity was tested by means of the isolated uterus of the virgin guinea-pig. Neither dithiodiglycollyl nor phenylalanyl choline caused any contraction of the muscle at dilutions up to 1 in 50,000 in the testing bath. Relatively small doses (1 in 200,000) of lauryl choline caused slight contraction, but larger doses seemed to be toxic and to diminish the response to a simultaneous dose of the hormone. Cysteyl choline and cystinyl choline had very slight activity and caused some contraction at dilutions of 1—2 in 100,000, but no estimation of the activity was possible.

Experimental.

Glycyl Choline Chloride Hydrochloride.—A mixture of choline chloride (0·3 g.) and glycyl chloride hydrochloride (0·3 g.) was heated in an evacuated tube for 4 hours at 100°. When cold, the solid was ground with absolute alcohol (5 c.c.), collected, and converted into the chloroplatinate (0·51 g.), m. p. 238°, by the addition of 5% platinic chloride solution (15 c.c.) to a solution in 50% alcohol (10 c.c.). The chloroplatinate in water (80 c.c.) at 37° was decomposed by hydrogen sulphide, and, after being filtered, the solution was concentrated to small volume under reduced pressure, mixed with absolute alcohol, and again evaporated, the process being repeated until crystalline *glycyl choline chloride hydrochloride* (0·15 g.), m. p. 241—242°, separated (Found in material dried at 100° : Cl, 30·1. $C_7H_{17}O_2N_2Cl,HCl$ requires Cl, 30·4%). It formed a flavianate and rufianate insoluble in 50% alcohol, but the latter salt could not be used for the isolation, since decomposition with barium hydroxide also destroyed the choline.

When the condensation of choline chloride and glycyl chloride hydrochloride was carried out as described above for 6 hours and the product was dissolved in water (15 c.c.) and mixed with picrolonic acid (1 g.) in alcohol (15 c.c.), the picrolonate (0·85 g.) separated and was collected and washed. It was decomposed by extraction with ether of a solution in water (50 c.c.) containing 2N-hydrochloric acid (2 c.c.). The aqueous solution was worked up as before to yield glycyl chloride hydrochloride (0·23 g.), m. p. 241°.

Glycylglycyl Choline Chloride Hydrochloride.—A mixture of choline chloride (0·3 g.) and glycylglycyl chloride hydrochloride (0·45 g.) was heated in an evacuated tube for 6 hours at 100°, and the product was dissolved in water (5 c.c.) and mixed with picrolonic acid (1·1 g.) in alcohol (20 c.c.). The picrolonate (0·75 g.) was collected after remaining for 2 hours at 0° and decomposed by extraction with benzene of a solution in warm water (75 c.c.) containing 2N-hydrochloric acid (1·5 c.c.). The aqueous layer was evaporated to dryness under reduced pressure, and the residue repeatedly evaporated with absolute alcohol. *Glycylglycyl choline chloride hydrochloride* (0·2 g.) separated in colourless needles, m. p. 128—130° (Found in material dried at 100° under reduced pressure : Cl, 20·6. $C_9H_{20}O_3N_3Cl,HCl,3H_2O$ requires Cl, 20·6%). When heated at 120° under reduced pressure, this substance lost hydrogen chloride as well as water.

Methyl-β-benzoyloxyethyldiethylammonium Iodide, $Ph{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2MeI$.—When methyl iodide (3·6 g.) was added to β-diethylaminoethyl benzoate (b. p. 173°/28 mm.; hydrochloride, m. p. 125°; *Chem. Zentr.*, 1906, II, 1226) (2·0 g.) in dry benzene (10 c.c.), the iodide separated in crystalline form, and to complete the reaction the mixture was left overnight at 0°; smaller yields were obtained when the mixture was heated under reflux. The *iodide* (2·8 g.) crystallised from alcohol–ether (1 : 1) in colourless rhombic prisms, m. p. 128°, which were soluble in water, acetone and methyl acohol but insoluble in non-ionising solvents (Found in material dried at 100° : N, 3·8; I, 35·0. $C_{14}H_{22}O_2NI$ requires N, 3·8; I, 35·0%).

The *chloride* was prepared by shaking with glass beads a mixture of the iodide (2·0 g.) and freshly prepared dry silver chloride in warm absolute alcohol for 30 minutes. After filtration, the solution was evaporated to dryness below 40°, and the semi-solid residue dissolved in the minimum amount of warm dry alcohol. Ether was added in drops until a precipitate began to separate, and the mixture was cooled thoroughly to effect crystallisation; the procedure was then repeated. The *chloride* (1·5 g.) formed colourless, very hygroscopic, rhombic prisms, m. p. 129° (Found in material dried at 100° : C, 61·9; H, 8·4. $C_{14}H_{22}O_2NCl$ requires C, 61·8; H, 8·1%).

β-*Diethylaminoethyl Laurate.*—Lauryl chloride (5·0 g.) was added gradually to an ice-cold solution of anhydrous β-diethylaminoethanol (2·7 g.) in dry chloroform (20 c.c.), and the solution was washed repeatedly with saturated sodium bicarbonate solution until the washings were free from chloride. The chloroform solution, dried with potassium carbonate, was evaporated below

35°, and the residual oily ester (6·3 g.) was distilled, b. p. 194°/12 mm. It dissolved readily in organic solvents. The *hydrochloride*, prepared by passing dry hydrogen chloride into a solution of the base in light petroleum, crystallised from toluene in colourless needles, m. p. 109°, which dissolved in water and organic solvents except light petroleum (Found in material dried at 80°: C, 64·0; H, 11·6. $C_{18}H_{37}O_2N$,HCl requires C, 64·3; H, 11·3%).

Methyl-β-lauryloxyethyldiethylammonium Iodide, $CH_3{\cdot}[CH_2]_{10}{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2MeI$.—β-Diethylaminoethyl laurate (2·0 g.) and methyl iodide (2·0 g.) were mixed, and next day the crystalline mass was crystallised three times from benzene. The *iodide* (2·5 g.) formed colourless silky needles, m. p. 70° (Found in material dried at 50°: N, 3·5; I, 28·6. $C_{19}H_{40}O_2NI$ requires N, 3·2; I, 28·8%). It dissolved in alcohol and ether but not in light petroleum, and its aqueous solution readily formed a lather.

Benzoyl Choline Iodide.—β-Dimethylaminoethyl benzoate was prepared by the addition in drops during 15 minutes of benzoyl chloride (6·3 g.) to β-dimethylaminoethanol (4·0 g.) (Ingold and Rogers, J., 1935, 722) in dry chloroform (20 c.c.) cooled in ice. After remaining at room temperature for 30 minutes, the mixture was washed repeatedly with saturated aqueous sodium bicarbonate solution until the washings were free from chloride. The chloroform solution was washed with water, dried with potassium carbonate, and evaporated, and the residual ester distilled at 142—144°/20 mm. The hydrochloride, prepared by passing dry hydrogen chloride into a solution of the base in light petroleum, crystallised from absolute alcohol in colourless hygroscopic needles, m. p. 151° (*Chem. Zentr.*, 1907, II, 1464; m. p. 148°).

The colourless crystalline solid which separated when β-dimethylaminoethyl benzoate (1·8 g.) and methyl iodide (5·3 g.) were mixed in dry benzene was collected after 15 minutes, washed with benzene, and crystallised from absolute alcohol. The *iodide* (2·95 g.) formed leaflets, m. p. 243—244° (decomp.), which were readily soluble in water and insoluble in ether and benzene (Found in material dried at 100°: N, 4·3; I, 37·7. $C_{12}H_{18}O_2NI$ requires N, 4·2; I, 37·9%).

The chloride (Fourneau and Page, *Bull. Soc. chim.*, 1914, **15**, 544) was prepared by shaking a mixture of the iodide (1·0 g.) and freshly precipitated, dried silver chloride (0·5 g.) in warm absolute alcohol for 30 minutes in presence of glass beads, and concentrating the filtered solution under reduced pressure below 35°. The chloride (0·7 g.) which separated was recrystallised from ether–alcohol (1 : 1), forming colourless hygroscopic needles, m. p. 206—207° (decomp.), which dissolved readily in water and alcohol but not in acetone (Found in material dried at 100°: N, 5·9; Cl, 14·6. Calc. for $C_{12}H_{18}O_2NCl$: N, 5·8; Cl, 14·6%).

β-Dimethylaminoethyl laurate (2·0 g.), prepared from β-dimethylaminoethanol (0·82 g.) and lauryl chloride (2·0 g.) as described in the case of the diethyl homologue, was a colourless oil, b. p. 193—194°/13 mm. The *hydrochloride*, prepared in the usual way and crystallised from benzene, formed colourless hygroscopic needles, m. p. 143—144° (Found in material dried at 100°: N, 4·6. $C_{16}H_{33}O_2N$,HCl requires N, 4·6%).

Lauryl choline iodide, $Me{\cdot}[CH_2]_{10}{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_3I$ (0·6 g.) (Fourneau and Page, *loc. cit.*), prepared by mixing methyl iodide (1·0 g.) and β-dimethylaminoethyl laurate (0·5 g.) in dry benzene at 0°, crystallised from benzene in colourless needles, m. p. 161—162°, which formed a lather in aqueous solution (Found in material dried at 100°: N, 3·5; I, 30·5. Calc. for $C_{17}H_{36}O_2NI$: N, 3·4; I, 30·7%).

A solution of the chloride, prepared from the iodide (0·2 g.) and fresh, dry silver chloride (0·1 g.) in warm absolute alcohol, was filtered, concentrated under reduced pressure, and mixed with dry ether until crystallisation began. The *chloride* which separated was recrystallised from ether–alcohol, forming long, extremely deliquescent needles, m. p. 54° (Found in material dried at 40°: N, 4·4; Cl, 10·8. $C_{17}H_{36}O_2NCl$ requires N, 4·4; Cl, 11·0%).

Dithiodiglycollyl Choline Iodide, $(\cdot S{\cdot}CH_2{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_3I)_2$.—Finely powdered phosphorus pentachloride (11·5 g.) was added in portions during 30 minutes to a stirred solution of dithiodiglycollic acid (5·0 g.) in dry ether (25 c.c.) cooled in an ice–salt mixture and protected from moisture. The mixture was left in ice for 30 minutes, and the liquid then filtered through sintered glass without exposure to air, and evaporated as completely as possible under reduced pressure at 10°. The residue was thoroughly extracted with three successive portions of dry light petroleum at 10° to remove phosphorus oxychloride, the residual dithiodiglycollyl chloride (3·8 g.) being a pale yellow oil which could be neither crystallised nor distilled. It decomposed rapidly and was therefore prepared immediately before use. It was characterised as the dianilide. A solution of the chloride (1·0 g.) in dry ether (10 c.c.) was dropped into a solution of aniline (1·7 g.; 2 mols.) in dry ether (10 c.c.) cooled in ice. Next day the ether was distilled, and the residue extracted with water to remove aniline hydrochloride. The dianilide (1·49 g.)

crystallised from alcohol in yellow needles, m. p. 164° (Found in material dried at 100°: S, 19·3. Calc. for $C_{16}H_{16}O_2N_2S_2$: S, 19·3%). Beckurts and Frerich (*J. pr. Chem.*, 1902, **66**, 172) record m. p. 160—161°.

Di-(β-dimethylaminoethyl) dithiodiglycollate was prepared as follows: (a) Dithiodiglycollyl chloride (2·0 g.) in dry chloroform (10 c.c.) cooled to 0° was dropped into a stirred solution of β-dimethylaminoethanol (1·7 g.) in dry chloroform (10 c.c.) at 0° during 30 minutes. The mixture separated into two layers, and after remaining at 0° overnight it was extracted with three portions (20 c.c. each) of saturated aqueous ammonium carbonate solution. The chloroform solution was washed with water, dried with sodium sulphate, and freed from solvent below 35° under reduced pressure. The ester (0·7 g.) remained as a brown oil.

(b) A mixture of dithiodiglycollic acid (3·0 g.) and β-dimethylaminoethanol (7·0 g.) in dry tetrachloroethane (30 c.c.) was saturated with dry hydrogen chloride at room temperature and then maintained at 80° for 5·5 hours in a stream of hydrogen chloride. The mixture separated into two layers, and was shaken with successive portions (30 c.c. each) of ammonium carbonate solution until the aqueous layer contained neither chloride nor disulphide (potassium cyanide–sodium nitroprusside test). The tetrachloroethane solution was washed with water, dried, and freed from solvent under reduced pressure. The ester (4·0 g.) remained as an oil.

Attempts to purify the ester failed; it did not crystallise, and decomposed when distilled under reduced pressure; and the picrolonate, flavianate, rufianate, and picrate were oils.

When methyl iodide (2 c.c.) was added to the ester (0·6 g.) in dry benzene (10 c.c.), the di-iodide separated as a yellow viscous oil. The benzene was decanted, and the residue precipitated twice from a concentrated methyl-alcoholic solution by the addition of ether and then dissolved in the minimum quantity of warm methyl alcohol. Anhydrous ether was added cautiously until the solution became cloudy. When the solution was left at 0° for 2 days, the *di-iodide* separated as opaque, yellowish-white, globular masses; recrystallised from alcohol, it formed colourless nodules (0·45 g.), m. p. 156—157°, which dissolved readily in water and methyl alcohol, were less easily soluble in alcohol, and insoluble in ether, benzene, and chloroform (Found in material dried at 100°: S, 10·7; I, 40·9. $C_{14}H_{30}O_4N_2I_2S_2$ requires S, 10·5; I, 41·7%). It gave the qualitative test for the disulphide group with potassium cyanide and sodium nitroprusside.

Methyl-β-glycyloxyethyldiethylammonium Dirufianate,

$$C_{14}H_8O_7S,NH_2\cdot CH_2\cdot CO\cdot O\cdot CH_2\cdot CH_2\cdot NEt_2Me\cdot C_{14}H_7O_7S.$$

—The β-diethylaminoethyl ester of carbobenzyloxyglycine was prepared by dropping β-diethylaminoethanol (1·8 g.) into carbobenzyloxyglycyl chloride (3·4 g.) (Bergmann and Zervas, *Ber.*, 1932, **65**, 1192) in dry chloroform (20 c.c.). After remaining overnight at 0°, the mixture was extracted repeatedly with saturated sodium bicarbonate solution, and the chloroform layer was washed with water, dried, and freed from solvent; the ester (1·5 g.) remained as a gum which did not crystallise and yielded an amorphous picrate, picrolonate, and rufianate. The methiodide (1·1 g.), formed when methyl iodide (1·9 g.) was added to the ester (1·0 g.) in dry benzene, was also a gum, which did not crystallise and yielded an amorphous picrate, flavianate and rufianate.

Phosphonium iodide (0·9 g.) was added to a solution of the methiodide (0·9 g.) in glacial acetic acid (10 c.c.) at 45°, and the mixture was maintained at this temperature while a stream of dry hydrogen was bubbled through it for 10 hours until carbon dioxide ceased to be evolved, as shown by the precipitation of barium carbonate from baryta water. Acetic acid and benzyl iodide were evaporated below 50° under reduced pressure, and the residue was dissolved in warm absolute alcohol. The iodide hydriodide resisted all attempts to crystallise it, and was converted into the *dirufianate*, which separated from alcohol in bright red, irregular crystals, m. p. 259—260° (decomp.) after becoming brown at 230—235° (Found in material dried at 100°: S, 7·7. $C_{37}H_{36}O_{16}N_2S_2$ requires S, 7·7%).

Di-(β-diethylaminoethyl)carbobenzyloxycystine Dimethiodide,

$$[\cdot S\cdot CH_2\cdot CH(NH\cdot CO\cdot O\cdot CH_2Ph)\cdot CO\cdot O\cdot CH_2\cdot CH_2\cdot NEt_2MeI]_2.$$

—The ester, prepared in the usual way from carbobenzyloxycystinyl chloride (2·8 g.) (Bergmann and Zervas, *loc. cit.*) and β-diethylaminoethanol (1·21 g.), was a viscous oil (2·65 g.) which dissolved in organic solvents but could not be crystallised and yielded an amorphous picrate, picrolonate, flavianate, and rufianate.

The *dimethiodide* separated as a cream-coloured solid when methyl iodide (2 c.c.) was added to the ester (2·65 g.) in dry benzene (25 c.c.) and the mixture was left overnight at 0°. It was collected on sintered glass protected from moisture, washed with benzene, precipitated with anhydrous ether from a concentrated solution in dry methyl alcohol, collected in absence of

moisture, and at once transferred to a vacuum desiccator. The dimethiodide (2·26 g.) formed a colourless, very deliquescent powder which at once became sticky on exposure to the atmosphere. When heated, it melted at 67—77°, evolved gas at about 92°, and finally charred at 150° (Found in material dried at 60° : C, 39·2; H, 5·7; N, 4·9; loss at 110° in a vacuum over phosphoric oxide, 8·2. $C_{36}H_{56}O_8N_4I_2S_2,5H_2O$ requires C, 39·8; H, 6·1; N, 5·2; loss, 8·3%). It dissolved readily in water and methyl alcohol, less readily in alcohol, and was insoluble in ether. The dimethopicrate and dimethorufianate were amorphous.

β-*Bromoethyl Carbobenzyloxycystine*, $[\cdot S\cdot CH_2\cdot CH(NH\cdot CO\cdot O\cdot CH_2Ph)\cdot CO\cdot O\cdot CH_2\cdot CH_2Br]_2$.—Carbobenzyloxycystinyl chloride (4·1 g.) was added gradually during 30 minutes to a solution of bromohydrin (1·53 c.c.) and dry pyridine (2·6 c.c.) in dry chloroform (35 c.c.) at 10°. After the mixture had remained at room temperature for 30 minutes, it was boiled for 2 minutes, extracted successively with N/2-hydrochloric acid and N/2-sodium carbonate, washed with dilute sodium chloride solution (to avoid emulsification when water is used), dried, and freed from solvent. The partially crystalline residue (5·1 g.) was extracted repeatedly with dry ether until no more dissolved, and the ether evaporated; the residue crystallised when seeded. Two recrystallisations from a mixture (2 : 1) of benzene and light petroleum (b. p. 60—80°) yielded the *ester* (1·6 g.) as colourless needles, m. p. 86—88°, which were readily soluble in organic solvents (Found in material dried at 75° over phosphoric oxide : C, 43·5; H, 4·4; Br, 22·0. $C_{26}H_{30}O_8N_2Br_2S_2$ requires C, 43·3; H, 4·2; Br, 22·1%).

The β-iodoethyl ester was prepared similarly from iodohydrin.

Carbobenzyloxycystinyl Choline Iodide (II).—(i) A solution of the β-bromoethyl ester of carbobenzyloxycystine (0·9 g.) and dimethylamine (1·0 g.) in dry benzene (5 c.c.) was heated in a sealed tube at 60° for 24 hours. The contents of the tube were evaporated under reduced pressure on the water-bath, the residue dissolved in ether, and the solution extracted with N-hydrochloric acid. The extract was made alkaline with sodium carbonate and extracted with ether, care being taken that the acid and the alkaline solution were kept cool throughout. When dried and distilled, the ethereal solution yielded β-dimethylaminoethyl carbobenzyloxycystine (0·4 g.) as an oil which defied attempts to crystallise it. A solution of the ester (0·35 g.) and methyl iodide (1 c.c.) in dry ether (3·5 c.c.) was left at room temperature for 24 hours. The *di-iodide* (0·43 g.) separated in crystalline state, and when recrystallised from alcohol formed leaflets, m. p. 140—142° (Found in material dried at 75° : C, 41·7; H, 5·4; N, 6·3. $C_{32}H_{48}O_8N_4I_2S_2$ requires C, 41·3; H, 5·1; N, 6·0%).

(ii) A mixture of β-iodoethyl carbobenzyloxycystine (0·9 g.) and a 10% trimethylamine solution in benzene (3 c.c.) was heated in a sealed tube for 3 hours at 60°. The oily residue obtained by removal of the benzene hardened when stirred with ether, and the di-iodide crystallised from alcohol. The dibromide, prepared similarly from the β-bromoethyl ester, was extremely deliquescent and melted unsharply at 235°.

(iii) Carbobenzyloxycystinyl chloride (2·0 g.) was added in portions during 15 minutes to a stirred solution of β-dimethylaminoethanol (0·66 g.) in dry chloroform (30 c.c.) at 0°. After remaining at 0° overnight, the mixture was shaken with 5% ammonium carbonate solution until no more chloride was extracted, and the chloroform layer was washed, dried, and evaporated below 35° under reduced pressure. β-Dimethylaminoethyl carbobenzyloxycystine remained as a gum (2·0 g.) which did not crystallise and yielded an amorphous picrate, picrolonate, flavianate, rufianate, and chloroplatinate.

The dimethiodide separated as a colourless solid when methyl iodide (2·8 g.) was added to the ester (1·6 g.) in dry chloroform (10 c.c.). It was collected without exposure to the atmosphere and purified by adding a concentrated methyl-alcoholic solution to a large excess of dry ether. The *di-iodide* formed extremely hygroscopic leaflets, which sintered at 64°, melted at 70—79° with evolution of gas, and charred at 150° (Found in material dried at 50° : S, 6·7; I, 25·5. $C_{32}H_{48}O_8N_4I_2S_2,2H_2O$ requires S, 6·6; I, 26·1%). It immediately became sticky when exposed to the air, and dissolved readily in methyl alcohol, but sparingly in water, acetone, and alcohol.

Cysteyl Choline Iodide Hydriodide (V).—A mixture of phosphonium iodide (1·0 g.) and carbobenzyloxycystinyl choline iodide (1·0 g.) in acetone (50 c.c.) was maintained at 40° in a current of dry hydrogen under reflux until carbon dioxide ceased to be evolved (10 hours). The resulting solution was concentrated to small volume under reduced pressure at room temperature, and the residue was stirred with ether, purified by repeated precipitation from dry alcoholic solution with dry ether, and finally dried over phosphoric oxide in a vacuum. Air was excluded as far as possible in these processes. *Cysteyl choline iodide hydriodide* (0·65 g.) formed very hygroscopic, cream-coloured flakes, which sintered at 74—75°, melted at 83—85° with evolution of gas, and charred at 150° (Found in material dried at 50° : S, 6·3; N, 4·9. $C_8H_{20}O_2N_2I_2S,3H_2O$

requires S, 6·2; N, 5·4%). The qualitative test for sulphydryl with sodium nitroprusside was positive. The picrate, rufianate, and picrolonate were amorphous.

When oxygen was bubbled through an alcoholic solution of the iodide and the alcohol was evaporated in a desiccator, cystinyl choline iodide hydriodide remained as a glass which gave the qualitative test for the disulphide, but not the sulphydryl, group. It was readily soluble in methyl and ethyl alcohols and in warm water, from which it separated, on cooling, as an oil. It was insoluble in ether, chloroform, and ethyl acetate and did not crystallise from mixtures of these solvents with the alcohols. It is perhaps worthy of note, as a coincidence or otherwise, that solutions of the cysteyl and the cystinyl compound have a marked and persistent cat-like smell, similar to that of flowering currant, and that the evolution of a similar odour characterises all extractions of posterior lobe pituitary powder.

Carbobenzyloxyphenylalanyl Choline Iodide.—Carbobenzyloxyphenylalanyl chloride (1·0 g.) (Bergmann and Zervas, *loc. cit.*) was added gradually to a stirred solution of β-dimethylaminoethanol (0·42 g.) in dry ether (10 c.c.) cooled in ice. Next day the mixture was shaken with 5% ammonium carbonate solution until no more chloride was extracted, and the ethereal solution was washed, dried, and evaporated under reduced pressure. β-Dimethylaminoethyl carbobenzyloxyphenylalanine (0·7 g.) was an oil which did not crystallise.

When methyl iodide (1·5 g.) was added to a solution of the ester (1·0 g.) in dry ether (10 c.c.), the methiodide separated; it was purified by repeated precipitation from dry alcoholic solution with dry ether. The *iodide* was a colourless, very hygroscopic powder which immediately became a gum on exposure to the air (Found in material dried at room temperature: C, 52·0; H, 6·1. $C_{22}H_{29}O_4N_2I$ requires C, 51·5; H, 5·7%). It sintered at 45—48°, melted at 59—62°, evolved gas at 169°, and charred at 190°. The picrate, flavianate, and rufianate were amorphous.

Phenylalanyl Choline Iodide Hydriodide (IV).—A mixture of phosphonium iodide (1·0 g.) and carbobenzyloxyphenylalanyl choline iodide (1·0 g.) in dry acetone (50 c.c.) was maintained at 45° under reflux in a stream of dry hydrogen for 8 hours until carbon dioxide ceased to be evolved. The solution was evaporated to dryness under reduced presssure, and the residue was stirred thoroughly with dry ether and purified by repeated precipitation from dry alcoholic solution with dry ether. The *iodide hydriodide* (0·57 g.) formed a very deliquescent, amorphous solid, which dissolved in water, alcohol, and acetone (Found in material dried at room temperature: C, 36·1; H, 5·6; I, 42·8. $C_{14}H_{23}O_2N_2I,HI,2C_2H_5{\cdot}OH$ requires C, 36·1; H, 6·0; I, 42·4%). It sintered at 40—50°, melted with evolution of gas at 80—83°, and charred at 200°.

The *chloride hydrochloride*, prepared by the action of fresh, dry silver chloride (0·2 g.) on the iodide hydriodide (0·2 g.) in warm alcohol, was an extremely deliquescent, colourless powder (Found in material dried at room temperature: C, 52·2; H, 8·3. $C_{14}H_{23}O_2N_2Cl,HCl$ requires C, 52·0; H, 7·4%).

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