CV.—*Co-ordination Compounds of the Chloroplatinates of Choline and its Esters.*

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ACETYLCHOLINE, a substance possessing intense physiological activity, until recently detected in nature only as a rare product of the vegetable kingdom, has lately been observed for the first time in mammalian tissue by Dale and Dudley (*J. Physiol.*, 1929, **68**, 97), who isolated it from extracts of horse's spleen.

The chemical study underlying its isolation revealed the interesting fact that when mixtures of the chloroplatinates of choline and acetylcholine are crystallised from water a mixed salt, containing one molecule of each base, separates from the solution (Dudley, *Biochem. J.*, 1929, **23**, 1064).

The preparation of this substance, choline-acetylcholine chloroplatinate, from mixtures of the two salts in various proportions, and its constancy on recrystallisation from water, proved it to be a true compound.

In comparison with its component chloroplatinates, not only is the mixed salt considerably less soluble in water but its melting point is higher, and its crystalline form is strikingly different. From water, choline chloroplatinate crystallises in orange-red prismatic (anisotropic) needles, acetylcholine chloroplatinate in fine, yellow, anisotropic needles, and choline-acetylcholine chloroplatinate in isotropic octahedra.

The formation of this mixed salt was ascribed to co-ordination between the hydrogen atom of the hydroxyl group of the choline moiety and the oxygen atom of the acetyl group of the acetylcholine component, thus :

$$\left[\begin{matrix}NMe_3Cl\cdot CH_2\cdot CH_2\cdot O\cdot CO\cdot R\\ NMe_3Cl\cdot CH_2\cdot CH_2\cdot OH\end{matrix}\right]PtCl_4, \text{ where R} = \text{Me.}$$

The work here reported was undertaken primarily to ascertain the effect of lengthening the acyl chain (CO·R) on the capability of the

esters of choline to co-ordinate with choline in the manner indicated. To this end the following acylcholine chloroplatinates were prepared : (1) propionyl, (2) *n*-butyryl, (3) *n*-valeryl, (4) *n*-hexoyl, (5) decoyl (dibutylacetyl).

(1) was the only member of the series which gave a mixed chloroplatinate with properties completely analogous to those of choline-acetylcholine chloroplatinate. It formed isotropic octahedra which withstood recrystallisation from water without change of composition.

(2) also gave a mixed chloroplatinate, which, however, showed signs of instability. It separated, when a hot aqueous solution was cooled, in isotropic crystals, which on standing changed into anisotropic needles. Repeated crystallisation from water caused a slow breakdown of the co-ordination compound, a small proportion of choline chloroplatinate being removed from it at each operation.

(3), (4) and (5) yielded no co-ordination compounds.

An important factor in determining whether or no a co-ordination compound can be isolated from aqueous solutions of mixtures of the chloroplatinates under consideration is, undoubtedly, the relative solubilities of the two salts concerned. Choline chloroplatinate is very soluble in water, and acetylcholine chloroplatinate is less soluble, though the difference is not great. As the acyl chain is lengthened, the solubility of the corresponding chloroplatinate decreases, until eventually an acylcholine (*n*-valerylcholine) is reached whose chloroplatinate is so much less soluble than the choline salt that it crystallises from aqueous mixed solutions in the simple un-co-ordinated state.

An interesting phenomenon, due to the capacity of choline and acetylcholine chloroplatinate to co-ordinate, had been observed in connexion with mixed melting-point determinations on the two salts (Dudley, *loc. cit.*). It was found that, instead of the usual depression, a mixture of the two salts melted and decomposed at a temperature higher than did either of its constituents. The same behaviour was displayed in the case of both of the other acylcholines which yielded co-ordination compounds with choline, whilst the remainder, which did not co-ordinate, behaved normally in mixed melting-point determinations (see Table I, p. 768).

Evidently, during the process of heating the mixture of the chloroplatinate of choline with that of acetyl-, propionyl- or butyryl-choline partial co-ordination occurred, with the result that it melted and decomposed at a temperature higher than the melting point of either constituent, but below that of the pure co-ordination compound.

Recognition of the type of co-ordination here described made it

possible to prepare a pure salt of pyruvylcholine,* which had been unsuccessfully attempted by Le Heux (*Pflüger's Archiv*, 1921, **190**, 284) on account of its physiological interest. It was isolated as a co-ordinated chloroplatinate containing one molecule of pyruvylcholine and one of choline; the introduction of a second CO group had not affected the type of co-ordination. Glycollylcholine also formed an analogous co-ordinated chloroplatinate (R = CH_2·OH), but acetylglycollylcholine (R = CH_2·O·COMe) did not form a co-ordination compound.

It is apparent, therefore, that the power of choline to co-ordinate with its esters, forming mixed chloroplatinates, is limited.

No co-ordination compounds were obtained with any of the following mixtures of the respective chloroplatinates: β-hydroxyethylamine and acetylcholine; β-hydroxyethylamine and β-acetoxyethylamine; choline and neurine. In the last case, only a very small amount of neurine chloroplatinate was available and, as Gulewitsch suggests (*Z. physiol. Chem.*, 1899, **27**, 63) that choline and neurine chloroplatinates form a "loose molecular compound," it would be worth while to re-examine the possibility of this co-ordination with larger quantities of material.

Experimental.

The acylcholines were prepared by heating dry choline chloride with 6—10 times its weight of the various acid chlorides in sealed tubes at 100° for 5—6 hours. Vacuum distillation on a water-bath and treatment of the residue with a small quantity of absolute alcohol for about 30 minutes removed the unchanged acid chloride, and, after the alcohol had been distilled off, the residue was converted into the chloroplatinate either directly or by way of the chloroaurate, as was the more appropriate.

Propionylcholine chloroaurate. The reaction residue from 1·4 g. of choline chloride was dissolved in 20 c.c. of water and a slight excess of 10% aqueous gold chloride was added. The precipitated *chloroaurate* was recrystallised from 360 c.c. of water, forming irregular, anisotropic, yellow needles (2·4 g.), m. p. 131—133° (Found: Au, 39·4. $C_8H_{18}O_2NCl_4Au$ requires Au, 39·5%).

Propionylcholine chloroplatinate. The chloroaurate being more soluble in mixtures of water and alcohol than in either solvent alone, a solution of 1 g. of it in 30 c.c. of alcohol and 10 c.c. of water was shaken with 2 g. of metallic silver (Dudley, *Biochem. J.*, 1929, **23**,

* Matthes (*J. Physiol.*, 1930, **70**, 343) tested the physiological action of this substance and found that pyruvylcholine is about 230 times more powerful than choline in stimulating the isolated intestine of the rabbit. Acetylcholine is about 1000 times more potent than choline in this respect.

1071). The solution of propionylcholine chloride obtained, after filtration, was treated with 20% alcoholic chloroplatinic acid. The *chloroplatinate* produced (0·75 g.) was recrystallised from 7·5 c.c. of water, forming prismatic, reddish-orange, anisotropic needles (0·65 g.), m. p. 244° (decomp.) (Found : Pt, 26·8. $C_{16}H_{36}O_4N_2Cl_6Pt$ requires Pt, 26·8%).

Choline-propionylcholine chloroplatinate. Hot aqueous solutions of 0·35 g. of choline chloroplatinate (1·5 c.c.) and 0·25 g. of propionylcholine chloroplatinate (2·5 c.c.) were mixed. On cooling, the co-ordination *compound* crystallised in orange-yellow isotropic octahedra (0·415 g.). After recrystallisation from 4 c.c. of water, it had m. p. 262° (decomp.); yield, 0·327 g. (Found : Pt, 29·05. $C_{13}H_{32}O_3N_2Cl_6Pt$ requires Pt, 29·0%).

n-*Butyrylcholine chloroplatinate.* The reaction residue from 1 g. of choline chloride was dissolved in 15 c.c. of water and treated with 2 g. of chloroplatinic acid in 5 c.c. of water. After recrystallisation from 20 c.c. of water the *salt* was obtained in anisotropic, orange-yellow needles (1·7 g.), m. p. 244° (decomp.) (Found : Pt, 25·9. $C_{18}H_{40}O_4N_2Cl_6Pt$ requires Pt, 25·8%).

Choline-n-*butyrylcholine chloroplatinate.* To a hot solution of 0·5 g. (1 mol.) of *n*-butyrylcholine chloroplatinate in 5 c.c. of water was added 0·41 g. (1 mol.) of choline chloroplatinate. On cooling, there was an immediate separation of isotropic crystals, resembling in form ammonium chloride, followed by anisotropic needles. The material was redissolved by heating and 0·41 g. of choline chloro platinate in 5 c.c. of hot water was added. The *salt* crystallised as before, orange-yellow isotropic crystals and then anisotropic needles separating from the solution. After standing over-night, the isotropic material had disappeared, the liquid being full of anisotropic needles, which were filtered off and washed with water; m. p. 254° (decomp.) (yield, 0·768 g.) (Found : Pt, 28·45. $C_{14}H_{34}O_3N_2Cl_6Pt$ requires Pt, 28·4%). The substance was twice recrystallised from 10 vols. of water; it separated each time as a mixture of isotropic and anisotropic crystals, the former rapidly changing into the stable anisotropic form in contact with the mother-liquor. After the first recrystallisation the platinum content was 28·1% and after the second 27·9%.

n-*Valerylcholine chloroplatinate.* The reaction residue from 1 g. of choline chloride, dissolved in 10 c.c. of water, gave, on addition of 2 g. of chloroplatinic acid in 10 c.c. of water, a precipitate, which was recrystallised from 30 c.c. of water, fine needles (2·1 g.), m. p. 237° (decomp.), being obtained (Found : Pt, 24·8. $C_{20}H_{44}O_4N_2Cl_6Pt$ requires Pt, 24·8%).

Attempt to make a co-ordination compound. 0·8 G. (2 mols.) of

choline chloroplatinate and 0·5 g. (1 mol.) of *n*-valerylcholine chloroplatinate were dissolved in 10 c.c. of hot water. Unchanged valerylcholine chloroplatinate (0·44 g.) (Pt, 24·9%) was recovered from the cooled solution.

n-*Hexoylcholine chloroplatinate.* The reaction residue from 1·15 g. of choline chloride was dissolved in alcohol. The precipitate obtained by addition of a slight excess of an alcoholic solution of chloroplatinic acid crystallised from 80 c.c. of water in long needles (2·5 g.), m. p. 236—238° (decomp.) (Found: Pt, 24·0. $C_{22}H_{48}O_4N_2Cl_6Pt$ requires Pt, 24·0%).

Attempt to make a co-ordination compound. 0·4 G. (1 mol.) of hexoylcholine chloroplatinate and 0·3 g. (1 mol.) of choline chloroplatinate were dissolved in 10 c.c. of hot water. On cooling, 0·4 g. of unchanged hexoylcholine chloroplatinate (Pt, 24·0%) separated: no co-ordination had occurred.

Decoylcholine chloroplatinate. The reaction residue from 1 g. of choline chloride was precipitated from alcoholic solution with chloroplatinic acid. The *salt* was washed with water and recrystal lised from 25 vols. of 75% alcohol. After two recrystallisations, golden platelets (2 g.) were obtained, m. p. 236° (decomp.) (Found: Pt, 21·1. $C_{30}H_{64}O_4N_2Cl_6Pt$ requires Pt, 21·1%). From water, in which it is very sparingly soluble, the salt crystallises in pale yellow needles.

Hunt and Taveau (*Hyg. Lab. Bull.* No. 73, Washington, 1911) and Le Heux (*Pflüger's Archiv*, 1921, **190**, 280) have prepared the chloroplatinate of propionyl- and of butyryl-choline; they present, however, no adequate characterisation of the salts. Fourneau and Page (*Bull. Soc. chim.*, 1914, **15**, 550) give the m. p. of *n*-butyryl-choline chloroplatinate as 209°; their compound was probably somewhat impure.

Choline-pyruvylcholine chloroplatinate. 0·5 G. of choline chloride and 4 c.c. of freshly prepared pyruvic acid were heated in a sealed tube at 100° for 5 hours. Some decomposition occurred: the contents of the tube were dark brown and considerable pressure was observed on opening the cooled tube. After evaporation in a vacuum at 50° the residual syrup was dissolved in alcohol and an alcoholic solution of chloroplatinic acid was added. The material on crystallisation from 2·5 c.c. of hot water yielded 0·574 g. of isotropic crystals, m. p. 248° (decomp.) (Found: Pt, 28·6. $C_{13}H_{30}O_4N_2Cl_6Pt$ requires Pt, 28·4%). The *chloroplatinate* is apparently dimorphous; on recrystallisation from a small volume of hot water, isotropic crystals are obtained, but slow evaporation of a dilute cold solution results in the deposition of mainly anisotropic needles.

Choline-glycollylcholine chloroplatinate. A mixture of 4 g. of desiccator-dried glycollic acid and 1 g. of choline chloride became pasty in the cold, and after being heated at 100° in a vacuum for 4 hours formed a thick clear liquid which did not solidify on cooling. This was dissolved in alcohol and an alcoholic solution of 2 g. of chloroplatinic acid was added. The material (2·6 g.) obtained was recrystallised from 2 c.c. of water, yielding 1·15 g. of isotropic octahedra, m. p. 244° (decomp.) (Found : Pt, 28·6. $C_{12}H_{30}O_4N_2Cl_6Pt$ requires Pt, 28·9%).

Acetylglycollylcholine chloroaurate. 1 G. of choline chloride was heated in a sealed tube for 5 hours at 100° with 8 c.c. of acetylglycollyl chloride. After removal of the excess of acid chloride in a vacuum at 100°, the syrupy residue was dissolved in water and 10% aqueous gold chloride was added. The *chloroaurate*, after two recrystallisations from 25 vols. of water, formed golden-yellow, irregular plates (2·5 g.), m. p. 158—160° (Found : Au, 36·3. $C_9H_{18}O_4NCl_4Au$ requires Au, 36·3%).

Acetylglycollylcholine chloroplatinate. 1·5 G. of the chloroaurate were suspended in 50 c.c. of 50% alcohol. Although the whole of the salt did not dissolve, decomposition was rapid and complete when it was shaken with 2 g. of metallic silver. After filtration, the solution of the chloride was evaporated in a vacuum, and to the syrupy residue, dissolved in alcohol, was added an alcoholic solution of chloroplatinic acid. The chloroplatinate obtained (1·13 g.), recrystallised from 10 c.c. of water, formed rhombic plates (0·88 g.), m. p. 232° (decomp.) (Found : Pt, 24·0. $C_{18}H_{36}O_8N_2Cl_6Pt$ requires Pt, 23·9%).

TABLE I.

Melting-point determinations of choline chloroplatinate, choline-ester chloroplatinates, and mixtures of the choline salt with each of the choline-ester salts.

	Choline-ester chloroplatinate.	Mixture.	Choline chloroplatinate.
*Acetyl	244°	248°	242°
*Propionyl	244	250	242
*n-Butyryl	244	250	242
n-Valeryl	236	241	242
n-Hexoyl	236	230	242
Decoyl	236	234	242
Acetylglycollyl	232	230	242

* Esters whose chloroplatinates form co-ordination compounds : the melting point of the mixture is higher than that of either component.

In order to ascertain whether co-ordination occurred between the chloroplatinates of choline and acetylglycollylcholine, 0·7 g. (3 mols.)

of the former and 0·3 g. (1 mol.) of the latter salt were dissolved in 7 c.c. of hot water. 0·26 G. of acetylglycollylcholine chloroplatinate crystallised from the solution on cooling: co-ordination had not occurred.

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