LIX.—*The Constitution of Carnitine. Part I. The Synthesis of α-Hydroxy-γ-butyrotrimethylbetaine.*

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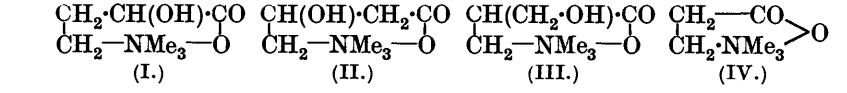
IN spite of several investigations the constitution of carnitine, a constituent of muscular tissue, remains unsettled. Two formulæ (I and II) put forward by Krimberg (*Z. physiol. Chem.*, 1907, **53**, 525) represent carnitine as α(or β)-hydroxy-γ-butyrotrimethylbetaine, of which the latter was preferred because of the appearance of β-hydroxy-*n*-butyric acid in the animal organism (*ibid.*, 1908, **56**, 417). A third formula (III) was subsequently advanced by Engeland (*Ber.*, 1921, **54**, 2208) in which carnitine is represented as β′-hydroxy-β-*iso*butyrotrimethylbetaine.



The evidence in support of (I) is that carnitine on oxidation with calcium permanganate yields β-homobetaine (IV) (Engeland, *Ber.*, 1909, **42**, 2457) and on distillation with aqueous baryta gives trimethylamine and crotonic and succinic acids: the production of these two acids is explained by the simultaneous oxidation and reduction of αγ-dihydroxy-*n*-butyric acid, which might be expected to be produced when trimethylamine is eliminated from the carnitine molecule. In favour of (II) is the fact that carnitine, when heated with sulphuric acid at 130°, loses a molecular proportion of water to yield an unsaturated betaine—"*apo*carnitine"—which is reduced by hydrogen in the presence of palladium to γ-butyrotrimethylbetaine (Engeland, *Ber.*, 1921, **54**, 2208). This point, however, does not appear to be well established, as the following data indicate. The reduction product of *apo*carnitine gave a chloroaurate, m. p. 201—203°. On the other hand, the synthetic γ-butyrotrimethylbetaine prepared by Willstätter's method (*Ber.*, 1902, **35**, 617) by Krimberg (*loc. cit.*) gave a chloroaurate of which the highest value of the m. p. was 182—184°.

There does not appear to be any direct experimental evidence in favour of (III): it was advanced as an alternative when Engeland (*loc. cit.*) found that the α-hydroxy-γ-butyrotrimethylbetaine synthesised by the method of Fischer and Göddertz (*vide infra*) was unaffected by hot sulphuric acid under conditions which bring about the dehydration of carnitine.

Moreover, β-hydroxy-γ-butyrotrimethylbetaine has been synthesised by Rollett (*Z. physiol. Chem.*, 1910, **69**, 60), by Engeland (*Ber.*, 1910, **43**, 2705) and by Tomita (*Z. physiol. Chem.*, 1923, **124**, 253), and Engeland states that the properties of his synthetic betaine differed considerably from those of natural carnitine.

α-Hydroxy-γ-butyrotrimethylbetaine has been synthesised by Fischer and Göddertz (*Ber.*, 1910, **43**, 3278) as follows: α-Bromo-γ-phthalimido-*n*-butyric acid was heated with chalk and water, and the resulting hydroxy-derivative hydrolysed with hydrochloric acid to give γ-amino-α-hydroxy-*n*-butyric acid which, by treatment with alcoholic potassium hydroxide and methyl iodide, yielded α-hydroxy-γ-butyrotrimethylbetaine. The validity of this synthesis has been questioned by Engeland (*Ber.*, 1921, **54**, 2208) on the ground that it was shown by Berlin (*Z. Biol.*, 1907, **53**, 514) that, when phthal-β-bromo-*n*-propylimide is hydrolysed by the method described above, it yields, not only the expected β-aminopropyl alcohol, but also α-aminopropyl alcohol as the result of a molecular rearrangement.

It was therefore decided to undertake the synthesis of α-hydroxy-γ-butyrotrimethylbetaine by an alternative method and to ascer-

tain whether the properties of the product thus obtained agreed with those of (*a*) the synthetic betaine of Fischer and Göddertz, (*b*) natural carnitine.

The outline of the synthesis is as follows : β-Chloropropaldehyde diethylacetal, prepared by the action of alcoholic hydrogen chloride on acraldehyde, was hydrolysed by water, and the resulting β-chloropropaldehyde converted into its potassium bisulphite compound; the latter, by the action of potassium cyanide, gave *γ-chloro-α-hydroxy*-n-*butyronitrile* (V), which in turn gave, by the addition of trimethylamine, the *methochloride* of γ-dimethylamino-α-hydroxy-*n*-butyronitrile (VI). On hydrolysis with hydrochloric acid, this nitrile gave the *hydrochloride* of α-hydroxy-γ-butyrotrimethylbetaine (VII).

CH_2Cl	$CH_2 \cdot NMe_3Cl$	$CH_2 \cdot NMe_3Cl$
CH_2	CH_2	CH_2
$CH(OH) \cdot CN$	$CH(OH) \cdot CN$	$CH(OH) \cdot CO_2H$
(V.)	(VI.)	(VII.)

The properties of the compound thus obtained agree with those given by Fischer and Göddertz for their synthetic betaine; there can be little doubt, therefore, that the betaine obtained by these authors and that described in this communication are both α-hydroxy-γ-butyrotrimethylbetaine, and that, in consequence, the doubts of Engeland are not justified.

Although Fischer and Göddertz (*loc. cit.*) state that the m. p.'s of several derivatives of their synthetic betaine differ considerably from those of corresponding derivatives of natural *l*-carnitine [according to Kutscher (private communication to Engeland, *Ber.*, 1921, **54**, 2208), carnitine can be racemised by a method which was not described, and the chloroaurate of the racemised compound has the same melting point as that of the chloroaurate of the optically active base], it was decided to attempt the resolution of the synthetic α-hydroxy-γ-butyrotrimethylbetaine in order to prepare crystalline derivatives of its optically active modifications, but unfortunately the attempt was not successful. This drawback is, however, not so serious as was at first thought, since it was found that, contrary to the statement of Engeland (*loc. cit.*), the synthetic α-hydroxy-γ-butyrotrimethylbetaine is decomposed by sulphuric acid at temperatures up to 130° with the production of carbon monoxide—a behaviour quite different from that of carnitine with the same reagent. It is thus definitely established that carnitine cannot be α-hydroxy-γ-butyrotrimethylbetaine.

It is hoped to describe in a further communication the synthesis of β′-hydroxy-β-*iso*butyrotrimethylbetaine.

EXPERIMENTAL.

Large quantities of acraldehyde were readily prepared by Moureu's method (*Ann. Chim.*, 1921, **15**, 158).

The method of preparing β-chloropropaldehyde diethylacetal described by Wohl and Emmerich (*Ber.*, 1898, **31**, 1797; 1900, **33**, 2761) having given only about 17% of purified material, the following procedure, which was found after many trials to give the best results, was adopted. Dry hydrogen chloride was passed into a mechanically stirred solution of dry calcium chloride (400 g.) and acraldehyde (1000 g.) in 96% alcohol (2000 g.) at such a rate that saturation was reached in 8—10 hours, the reaction mixture being protected from moisture and being kept cold by means of ice. During the addition of hydrogen chloride, the separation of crystalline hydrated calcium chloride was continuous. Next day the liquid portion, consisting of a small upper layer of aqueous-alcoholic calcium chloride solution superimposed on the crude acetal, was filtered or decanted from the solid calcium chloride, and the latter was washed with ether, which was then added to the liquid portion. This was neutralised by adding it drop by drop to a slight excess of cold, well-agitated 10% sodium hydroxide solution. The lime which had separated during the neutralisation was dissolved by the cautious addition of acetic acid, after which the layer of acetal separated quite readily. The aqueous layer was removed, and the acetal was washed with water, then with very dilute sodium hydroxide solution to render it alkaline once more, and finally with water. After drying with calcium chloride, 1653 g. of acetal, b. p. 72—76°/20 mm., were obtained. Yield 56·4%. On redistillation, the product came over almost completely at 74°/20 mm. and had $d_4^{18\cdot7°}$ 0·9951, $d_4^{22\cdot3°}$ 0·9845, $n_D^{18\cdot7°}$ 1·4206, $n_D^{22\cdot3°}$ 1·4203; $[R_L]_D^{18\cdot7°}$ 42·31, $[R_L]_D^{22\cdot3°}$ 42·84 (calc., 42·58).

When the dry hydrogen chloride was passed more rapidly (during 3—4 hours) into the solution, the yield of acetal fell to 48%.

The *potassium bisulphite compound of β-chloropropaldehyde* was prepared by heating under reflux on a water-bath a mixture of β-chloropropaldehyde diethylacetal (666 g.) and water (72 g.; 2 mols.), with frequent shaking until reaction set in; this became apparent by the coalescence of the two liquid layers and the simultaneous refluxing of alcohol. The quickly cooled reaction liquid was vigorously shaken with a concentrated solution of potassium bisulphite (1·1 mols.), the mixture separated, and the unhydrolysed acetal (about 450 g.) reheated with water. This procedure was repeated four times; the amount of unhydrolysed acetal then amounted to only about 5 g. The potassium bisulphite compound

was precipitated in soft, white crystals on addition of alcohol (Found : K, 18·4. Calc. : K, 18·4%). The corresponding sodium bisulphite compound forms nacreous plates (Found : Na, 11·8. Calc. : Na, 11·7%). For the preparation of γ-chloro-α-hydroxy-*n*-butyronitrile, however, it is unnecessary to isolate the solid bisulphite compound : the solution was used direct.

γ-Chloro-α-hydroxy-n-*butyronitrile* (V) was prepared by adding during ½ hour a cold aqueous solution of potassium cyanide (400 g. of 92%) to a well-stirred solution of the bisulphite compound, kept below 20°. The upper layer of nitrile was separated, the aqueous portion extracted with ether, and the mixture of nitrile and ethereal extract washed with very dilute acid, dried with sodium sulphate and distilled. The *nitrile* distilled at 91·5°/3 mm. (yield, 316 g.; 66%). It is a colourless, odourless, slightly viscous liquid which can be distilled only at low pressures; $d_{4^\circ}^{19\cdot1^\circ}$ 1·0795, $d_{4^\circ}^{23\cdot1^\circ}$ 1·0765, $n_{\mathrm{D}}^{19\cdot1^\circ}$ 1·4399, $n_{\mathrm{D}}^{23\cdot1^\circ}$ 1·4387 (Found : N, 11·7. C_4H_6ONCl requires N, 11·7%).

γ-Chloro-α-cyanopropyl benzoate, prepared by the interaction of γ-chloro-α-hydroxy-*n*-butyronitrile (24 g.), pyridine (17 g.), and benzoyl chloride (29 g.) in ethereal solution, distilled at 147°/3—4 mm. to give a colourless, viscous, highly refractive liquid which soon solidified (yield, 35 g.; 82%). Recrystallised from light petroleum (400 c.c.), it formed small, soft needles, m. p. 59° (Found : N, 6·4. $C_{11}H_{10}O_2NCl$ requires N, 6·3%). The *acetate*, obtained in a similar manner or by the action of boiling acetic anhydride (1 mol.), has b. p. 116—117°/11 mm. and is an odourless, fairly mobile liquid heavier than water; $n_{5790}^{20\cdot1^\circ}$ 1·4342, $n_{5461}^{20\cdot1^\circ}$ 1·4355, $n_{4359}^{20\cdot1^\circ}$ 1·4433 (Found : N, 8·8. $C_6H_8O_2NCl$ requires N, 8·7%).

γ-Dimethylamino-α-hydroxy-n-*butyronitrile Methochloride* (VI).—γ-Chloro-α-hydroxy-*n*-butyronitrile (100 g.) was added to a well-stirred solution of trimethylamine (55 g.) in dry amyl alcohol (200 g.), and the mixture maintained at 35—40°; fine crystals of the *amino-nitrile methochloride* were rapidly deposited. After being stirred for several hours, the mixture was cooled and filtered, and the crystalline material washed with dry ether : the amyl alcohol–ether filtrate deposited a second crop of the methochloride, which was removed and washed. Some uncombined γ-chloro-α-hydroxy-*n*-butyronitrile was recovered after the amyl-alcoholic filtrate had been washed with dilute hydrochloric acid to remove trimethylamine and distilled in a vacuum. The methochloride (yield 75—85%) was recrystallised from amyl alcohol (temperature not above 95°) or precipitated from its solution in absolute alcohol by addition of dry ether and was thus obtained in small, glistening, transparent, very hygroscopic plates, m. p. 137°. It is insoluble in

chloroform, benzene, ether, or acetone (Found : N, 15·6. $C_7H_{15}ON_2Cl$ requires N, 15·6%).

The *chloroaurate*, prepared in the usual manner, crystallised, on slow cooling of its aqueous solution, in long, hairy, yellow needles, m. p. 95° (Found : Au, 40·9. $C_7H_{15}ON_2Cl_4Au$ requires Au, 40·9%). The *chloroplatinate* crystallises from aqueous alcohol in radiating clusters of short, slender, orange needles, m. p. 239—240° (decomp.) (Found : Pt, 28·2. $C_{14}H_{30}O_2N_4Cl_6Pt$ requires Pt, 28·1%).

α-Hydroxy-γ-butyrotrimethylbetaine Hydrochloride (VII).—A mixture of γ-dimethylamino-α-hydroxy-*n*-butyronitrile methochloride (225 g.), water (270 c.c.), and hydrochloric acid (270 c.c.; 2 mols.) was gently boiled for 2 hours, water being added from time to time to keep the volume of the solution constant. The solution was then evaporated until of syrupy consistency and dried in a vacuum until completely solid. The dry material was heated under reflux with alcoholic hydrogen chloride (275 g. of 10%) for 4 hours. After 12 hours, the ammonium chloride was filtered off and washed with a little absolute alcohol, and the combined filtrate and washings were mixed with an equal volume of water and distilled until the temperature was 95°. The residue was diluted with water, and the solution thus obtained was boiled with charcoal and evaporated until crystallisation set in; the crystalline material was filtered off, and the filtrate again evaporated. The process was repeated several times, the total yield of dry hydrochloride being 234 g. (94%). The betaine hydrochloride crystallises from alcohol in glistening prisms which are not excessively soluble in water, from which it may be crystallised—in marked contrast to the difficultly crystallisable *l*-carnitine hydrochloride.

The chloroaurate produced on mixing concentrated aqueous solutions of the betaine hydrochloride and gold chloride was twice recrystallised from water containing a trace of hydrochloric acid, from which it separated in orange-yellow crystals which, after drying at 105°, melted sharply at 173° without decomposition to give a deep orange-red liquid (Found : Au, 39·3; C, 16·6; H, 4·1; N, 2·9. Calc. for $C_7H_{16}O_3NCl_4Au$: Au, 39·3; C, 16·8; H, 3·2; N, 2·8%). The melting point was unaltered by further recrystallisation of the material. Fischer and Göddertz (*loc. cit.*) give m. p. 175—176° (corr.) after sintering. Carnitine chloroaurate has m. p. 153—154°.

A *chloroplatinate* was obtained by mixing equivalent quantities of the betaine hydrochloride and chloroplatinic acid in aqueous solution, evaporating the mixture to a syrupy consistency, and adding warm alcohol until the turbidity thus produced was permanent. On cooling, a small amount of oil, which rapidly solidified,

separated along with orange, crystalline plates. These, after recrystallisation from aqueous alcohol, had m. p. 218° (decomp.) after softening at about 196° (Found : Pt, 24·8. $C_{18}H_{40}O_6N_2Cl_6Pt$ requires Pt, 24·75%). The compound is therefore most probably the chloroplatinate of the ethyl ester of α-hydroxy-γ-butyrotrimethylbetaine produced by the action of ethyl alcohol on the free acid.

By the addition of more alcohol to the filtrate, fine, slender, orange needles were obtained. These began to sinter at about 196° and melted at 210—212° (decomp.) (Found : Pt, 26·3. Calc. for $C_{14}H_{32}O_6N_2Cl_6Pt$: Pt, 26·6%). Fischer and Göddertz (*loc. cit.*) state that the compound prepared by them melts not quite constantly at 216° (corr.) after previous pronounced sintering. They do not, however, give any analytical data. Carnitine chloroplatinate has m. p. 214—218° (decomp.).

In an attempt to effect the resolution of α-hydroxy-γ-butyrotrimethylbetaine the *d*-bromocamphorsulphonate was prepared, but it was a gum which could not be crystallised. The d-*camphorsulphonate* of α-hydroxy-γ-butyrotrimethylbetaine was prepared as follows : The betaine hydrochloride (19·75 g.) and the calculated quantity of the silver salt of Reychler's *d*-camphorsulphonic acid were mixed in aqueous solution, the precipitated silver chloride was removed, the filtrate evaporated, and the resulting syrup kept in a vacuum over sulphuric acid for 2 months. The white, crystalline material obtained separated readily from acetone in tufts of small, slender needles. Repeated crystallisation, however, brought about no alteration in the original rotatory power of the salt.

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