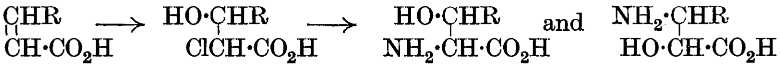
XLVI.—*Hydroxyamino-acids.*

By William John Nobbs Burch.

Dakin has shown (*J. Biol. Chem.*, 1921, **48**, 273) that maleic acid on treatment with chlorine water gives chlorohydroxysuccinic acid, which is converted by the action of ammonia into hydroxyaspartic acid.

Acrylic, crotonic, and tiglic acids behave in the same way under such conditions, giving mixtures of the α- and β-acids:



$$\begin{array}{c}CHR\\ \|\\ CH\cdot CO_2H\end{array} \longrightarrow \begin{array}{c}HO\cdot CHR\\ |\\ ClCH\cdot CO_2H\end{array} \longrightarrow \begin{array}{c}HO\cdot CHR\\ |\\ NH_2\cdot CH\cdot CO_2H\end{array} \text{ and } \begin{array}{c}NH_2\cdot CHR\\ |\\ HO\cdot CH\cdot CO_2H\end{array}$$

Glutaconic acid, however, behaves abnormally, giving *dihydroxyglutaramic acid*, possibly owing to the intermediate formation of a lactam,  $CO_2H\cdot CH_2\cdot \underbrace{CH\cdot CH(OH)\cdot CO}_{NH}$. The conversion of chlorohydroxyglutaric acid into hydroxyglutamic acid by Gabriel's phthalimide process has been unsuccessfully attempted.

EXPERIMENTAL.

Preparation of Glutaconic Acid.—Ethyl sodio-αγ-dicarbethoxyglutaconate (Ingold and Perren, J., 1921, **119**, 1591) was converted into glutaconic acid by a method communicated to the author by Dr. G. A. R. Kon. The tetracarboxylic ester was liberated from the sodium derivative by means of a mineral acid in the presence of ether and, without being dried, the ethereal solution was evaporated. The ester was then heated with alcohol (½ vol.) and 10% hydrochloric acid (3 vols.) until all the oil disappeared and no oil separated on cooling (5 hours). The alcohol was distilled off, and glutaconic acid (m. p. 132°) crystallised on cooling; a little more was obtained by extracting the mother-liquor with ether, drying the extract with sodium sulphate, concentrating it and adding benzene.

Barium α-Chloro-β-hydroxyglutarate.—Glutaconic acid (10 g.) was treated with a large excess of hypochlorous acid (prepared by extracting bleaching powder with water and distilling the extract in a vacuum with the calculated quantity of 5% nitric acid), after 24 hours the mixture was exactly neutralised with baryta and the filtered solution was concentrated in a vacuum, finally over concentrated sulphuric acid; the yellow crystals obtained (16 g.; yield, 66%) were recrystallised from water (Found: Ba, 43·5; Cl, 11·0. $C_5H_5O_5ClBa$ requires Ba, 43·2; Cl, 11·2%).

Ammonium Dihydroxyglutaramate.—Barium α-chloro-β-hydroxyglutaramate was converted into *ammonium dihydroxyglutaramate* by removing the barium as sulphate and adding methyl alcohol saturated with ammonia.

A better yield was obtained as follows: A mixture of 10 g. of glutaconic acid with a large excess of hypochlorous acid was kept for 24 hours and then evaporated to small bulk in a vacuum at 50°. Methyl alcohol saturated with dry ammonia was added in excess, and the mixture left in a stoppered bottle. Rosettes of tiny platelets began to form after 24 hours and the reaction was complete in about a week. The crystals were recrystallised from aqueous alcohol (yield, 6 g. or 43% of the theoretical) [Found: total N (by Kjeldahl's method), 15·65; N (as NH_4), 7·0; C, 34·3; H, 6·7. $C_5H_{12}O_5N_2$ requires total N, 15·55; N (as NH_4), 7·8; C, 34·1; H, 6·8%].

Dihydroxyglutaramic Acid, $CO_2H{\cdot}CH_2{\cdot}CH(OH){\cdot}CH(OH){\cdot}CO{\cdot}NH_2$. —The ammonium salt was treated in aqueous solution with silver oxide, and the product with hydrogen sulphide. The crystalline material obtained did not give the ninhydrin reaction for α-amino-acids, and in an estimation of amino-nitrogen by Van Slyke's method, no nitrogen was obtained after 24 hours by means of acetic acid; the theoretical amount was evolved, however, after 24 hours on treatment with sodium nitrite and concentrated hydrochloric acid (Plimmer, J., 1925, **127**, 2651). On prolonged boiling with baryta, the whole of the nitrogen in the compound was obtained as ammonia. The *acid* reduced ammoniacal silver nitrate and gave a deep blue colour with copper sulphate solution and caustic soda [Found: total N (by Kjeldahl's method), 8·4; N (by Van Slyke's method as above), 8·5; C, 36·5; H, 5·6; *M* (by means of the silver salt), 163. $C_5H_9O_5N$ requires N, 8·6; C, 36·8; H, 5·5%; *M*, 163].

α-Amino-β-hydroxybutyric Acid.—A solution of 4·5 g. of crotonic acid in water was mixed with hypochlorous acid (25% excess) and, after 12 hours, evaporated under diminished pressure. A solution of the residual yellow syrup in methyl alcohol was cooled in ice, saturated with dry ammonia, and heated in an autoclave at 120° for 10 hours. On cooling, no crystals formed. The solution was treated with an excess of baryta, the ammonia removed by aeration and the barium with sulphuric acid, the filtrate boiled with lead oxide to remove chloride, and the lead removed with hydrogen sulphide. The filtrate was treated with silver oxide to ensure complete removal of sulphide and chlorine, filtered, and evaporated in the dark; *silver α-amino-β-hydroxybutyrate* crystallised in thin platelets, which rapidly darkened in light (Found: Ag, 47·2. $C_4H_8O_3NAg$ requires Ag, 47·8%).

The acid was obtained by removing the silver with hydrogen sulphide, and the hydrogen sulphide by aeration. On evaporation in a vacuum, a pale yellow syrup was obtained which slowly crystallised in a vacuum over concentrated sulphuric acid (yield, 2·7 g.; 45% of the theoretical) [Found: total N (Kjeldahl), 11·6; amino-N (Van Slyke), 11·3. Calc. for $C_4H_9O_3N$: N, 11·8%]. The acid is very deliquescent, gives a strong ninhydrin reaction, and forms a pale blue crystalline copper salt and well-defined crystalline salts with cinchonine and quinine (Found for the *cinchonine* salt: N, 10·0. $C_4H_9O_3N,C_{19}H_{22}ON_2$ requires N, 10·2%).

The positive ninhydrin reaction and the fact that the total amino-nitrogen is only obtained after 10 minutes' shaking with sodium nitrite and glacial acetic acid suggest that the product of the above reaction is a mixture of the α- and β-amino-acids.

α-Amino-β-hydroxy-α-methylbutyric Acid,

HO·CHMe·CMe(NH_2)·CO_2H.

—Tiglic acid (5 g.) was treated in the same way as the crotonic acid in the previous experiment. The *silver* salt is a white powder (Found: Ag, 44·6. $C_5H_{10}O_3NAg$ requires Ag, 45·0%). The *acid* was obtained as a yellow syrup which slowly crystallised [Found: N (Kjeldahl), 10·3; amino-N (Van Slyke), 10·1. $C_5H_{11}O_3N$ requires N, 10·5%]. All the preceding statements regarding α-amino-β-hydroxybutyric acid apply also to this acid (Found for the *brucine* salt: N, 7·5. $C_5H_{11}O_3N,C_{23}H_{26}O_4N_2$ requires N, 8·0%).

iso*Serine*.—This was similarly prepared from acrylic acid (Melikow, *Ber.*, 1880, **13**, 1265). It gave the ninhydrin reaction, and the total amino-nitrogen was evolved only after 10 minutes' shaking with glacial acetic acid and sodium nitrite: this suggests that this product also is a mixture of serine and *iso*serine (the α- and β-amino-acids).

Hydroxyaspartic Acid.—Maleic acid was converted in the same way and also by Dakin's method (*loc. cit.*) into hydroxyaspartic acid. The compound obtained by either method gave the ninhydrin reaction, and the whole of the amino-nitrogen was evolved after 5 minutes' shaking with glacial acetic acid and sodium nitrite.

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