STUDIES ON AMINO-ACIDS AND RELATED COMPOUNDS. PART III. ELECTROLYTIC OXIDATION OF ARGININE.

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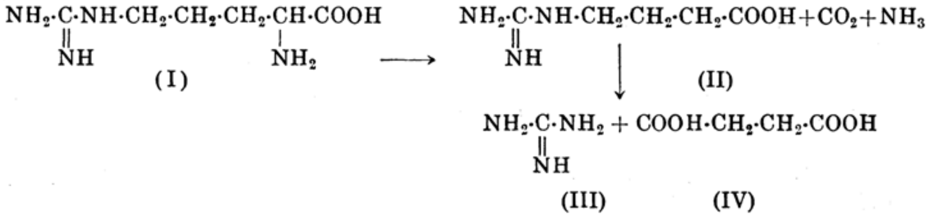
Received March 27th, 1933. Published June 28th, 1933.

In the previous communications the electrolytic oxidations of glutamic acid and its lactam, pyrrolidone-carboxylic acid, were discussed. In the present paper the study on arginine is to be reported, which possesses a guanidine group as its characteristics.

Arginine is considerably unstable towards alkali. When it is boiled with barium hydroxide solution, its guanidine group is easily attacked, yielding urea and ornithine.⁽¹⁾ On the other hand, on treating with an oxidising agent, such as barium permanganate, the guanidine group seems to be rather more stable than the amino-group, and guanidine, succinic acid and guanidine-butyric acid⁽²⁾ are obtained as oxidation products. Agmatine, $H_2N\cdot\underset{\underset{NH}{\|}}{C}\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot NH_2$, the decarboxyl derivative of arginine, also yields above three compounds when it is oxidised with calcium permanganate.⁽³⁾ Fr. Kutscher, the investigator of the above two reactions, has expressed the oxidation of arginine as follows. Arginine (I) is first attacked at its amino-group and is changed to guanidine-butyric acid (II), which yields guanidine (III) and succinic acid (IV) on further oxidation.



$$\underset{(I)}{NH_2\cdot\underset{\underset{NH}{\|}}{C}\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot\underset{\underset{NH_2}{|}}{CH}\cdot COOH} \longrightarrow \underset{(II)}{NH_2\cdot\underset{\underset{NH}{\|}}{C}\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot COOH}+CO_2+NH_3$$

$$\downarrow$$

$$\underset{(III)}{NH_2\cdot\underset{\underset{NH}{\|}}{C}\cdot NH_2} + \underset{(IV)}{COOH\cdot CH_2\cdot CH_2\cdot COOH}$$

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(1) E. Schulze u. E. Winterstein, *Z. physiol. Chem.*, **26** (1898), 1; *Ber.*, **30** (1898), 2879.

(2) E. Benech u. Fr. Kutscher, *Z. physiol. Chem.*, **32** (1901), 278; Fr. Kutscher, *Z. physiol. Chem.*, **32** (1901), 413.

(3) R. Engeland u. Fr. Kutscher, *Ber.*, **43** (1910), 2882; Abderhalden, "Handbuch d. biolog. Arbeitsmethoden," I, 7 (1922), p. 238.

In the case when the β-oxidation is possible, γ-guanidine-butyric acid will be converted into guanidine-acetic acid, of which the methyl derivative is creatine, $H_2N\cdot\underset{\underset{HN}{\|}}{C}\cdot\underset{\underset{CH_3}{|}}{N}\cdot CH_2\cdot COOH$. Thus it is said⁽⁴⁾ that arginine bears close relation to the formation of creatine in the living organism.

An attempt to obtain guanidine and succinic acid from arginine by the electrolytic oxidation has never been reported before. The author separated arginine from the hydrolysate of gelatine and purified it as hydrochloride and then changed it to sulphate. The dilute sulphuric acid solution of the sulphate was oxidised under the same conditions as descrived in the previous papers. The temperature was 25–26°C. during the electrolysis.

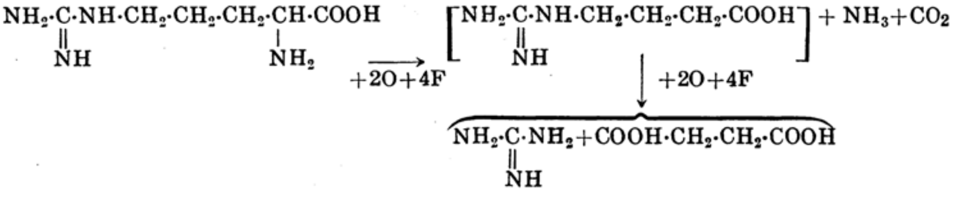
The oxidation has been carried out by the following different ways:—

1) Applying sufficient amount of electricity (10F/mol);

2) Applying insufficient amount of electricity (4F/mol), with the purpose of obtaining the intermediate product, if possible.

The oxidation products were separated almost in the same way as mentioned by Kutscher.⁽⁵⁾ The precipitate formed by the addition of phosphotungstic acid was decomposed by barium hydroxide as usual, and ammonia and guanidine were obtained as volatile and nonvolatile bases respectively. The latter was isolated as picrate in considerable amount. Guanidine-butyric acid, contrary to the author's expectation, could not be isolated even at moderate electric quantity. The filtrate from phosphotungstate always contained succinic acid, which was isolated and its silver salt was analysed. The yield of guanidine was 15% of arginine used in the case of 10F/mol and 32% in the case of 4F/mol. As to the yield of succinic acid it was rather worse, though that acid seemed to be stable towards oxidation. This may be considered as due to the loss caused at the precipitation of phosphotungstates and barium sulphate.

Assuming that guanidine-butyric acid is particularly unstable to anodic oxidation, the mechanism of oxidation in this case may be represented in the same way as Kutscher described.



$$NH_2\cdot\underset{\underset{NH}{\|}}{C}\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot\underset{\underset{NH_2}{|}}{CH}\cdot COOH \xrightarrow[+2O+4F]{} \left[NH_2\cdot\underset{\underset{NH}{\|}}{C}\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot COOH\right] + NH_3 + CO_2$$

$$\downarrow +2O+4F$$

$$\overbrace{NH_2\cdot\underset{\underset{NH}{\|}}{C}\cdot NH_2 + COOH\cdot CH_2\cdot CH_2\cdot COOH}$$

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(4) Dakin, "Oxidations and Reductions in the Animal Body," (1922), p. 82.

(5) loc. cit.

Experimental Part.

d-Arginine hydrochloride was prepared by the method of K. Felix and K. Dirr⁽⁶⁾ and it was recrystallised from alcohol. It melted at 223°C.

Anal.: Subst. = 0.1848; 0.1708 gr.; NH_3 = 35.05, 32.03 c.c. of N./10–H_2SO_4.
Found: N = 26.57, 26.47%.
Calc. for $C_6H_{14}O_2N_4$, HCl (arginine hydrochloride): N = 26.61%.

Four grams of above sample were dissolved in 120 c.c. of N.-H_2SO_4 and sufficient water was added to it. It was distilled under reduced pressure to drive off hydrochloric acid. The above treatments were repeated several times until the residue showed no trace of hydrochloric acid. The solution was made up to 120 c.c. and electrolytically oxidised.

Electrodes: lead peroxide-lead (4×5 cm.); distance of them 2.5 cm.: C.D.: 2 amp./dm^2; electric quantity applied: 4.80 amp. hours. The temperature showed 25°C.

Phosphotungstic acid was added to the oxidised solution and the precipitate formed (A) was separated from the filtrate (B).

Phosphotungstate (A). It was decomposed by barium hydroxide and the solution was distilled at a temperature as low as possible and the distillate (A_1) was received in hydrochloric acid. 0.1 Gr. of hydrochloride of a base was obtained from the distillate. It was found to be ammonia by the analysis of its platinic double chloride.

Anal.: Subst. = 0.1007; Pt = 0.0442 gr. Found: Pt = 43.90%.
Calc. for $(NH_4)_2PtCl_6$: Pt = 43.95%.

After removal of barium exactly, the residue (A_2) was concentrated under reduced pressure, but it was impossible to bring it into crystallisation. The concentrated solution was treated with sodium picrate, and the base was precipitated as the picrate (A_3), which was separated from filtrate (A_4). The picrate (0.8 gr.), recrystallised from alcohol, melted at 315°C. with decomposition coinciding with that of guanidine picrate. It was thoroughly extracted with ether, after acidifying with hydrochloric acid.

Anal.: Picrate = 0.3068; Picric acid obtained = 0.2441 gr.
Found: Picric acid = 79.58%.
Calc. for CN_3H_5, $C_6H_2(NO_2)_3OH$: (picric acid) = 79.45%.

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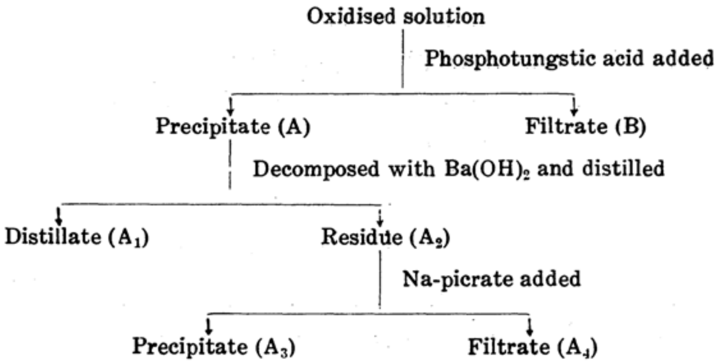
(6) K. Felix und K. Dirr, *Z. physiol. Chem.*, **176** (1928), 38.

A picrolonate was prepared from the above hydrochloric acid solution. It was found to be guanidine picrolonate by its melting point 274°C. An auric double chloride was also prepared and its gold content was determined as follows.

Anal.: Subst. = 0.2680; Au = 0.1315 gr. Found: Au = 49.07%.
Calc. for CN_3H_5, $HAuCl_4$: Au = 49.35%.

Picric acid being removed by extraction with ether, the filtrate (A_4) was treated with phosphotungstic acid as usual, but the attempt to precipitate guanidine-butyric acid as its phosphotungstate was not successful. The small amount of chloroplatinate was got from the above solution, but the analysis of platinum did not coincide with that of the expected compound. It may have been probably that of guanidine.

Anal.: Subst. = 0.0533; Pt = 0.0197 gr. Found: Pt = 36.97%.
Calc. for $(CN_3H_5)_2$, H_2PtCl_6: Pt = 36.96%.



Filtrate (B). The solution, after removing barium with carbon dioxide, was concentrated under reduced pressure and a barium salt was obtained. It was converted into free acid. The free acid, after recrystallisation from water, melted at 183°C. and no lowering of melting point was observed when mixed with a pure sample of succinic acid. Its silver salt was analysed.

Anal.: Subst. = 0.1150; Ag = 0.0747 gr. Found: Ag = 64.95%.
Calc. for $C_4H_4O_4Ag_2$ (silver succinate): Ag = 65.04%.

An acid was again obtained from the filtrate of barium succinate, which was found to be nothing else than succinic acid. The total yield of this acid amounted to 0.25 gr.

The electrolytic oxidation was repeated with different amount of electricity. It was reduced to 4.27F/mol. 10 Gr. of arginine hydrochloride were dissolved in 100 c.c. of water and hydrochloric acid was removed by shaking with 8 gr. of silver sulphate. By adding N.-H_2SO_4, the solution was made up to 300 c.c. (total content of nitrogen was equal to 2.311 gr.) and was electrolysed.

Phosphotungstate (A). Guanidine was isolated as its picrate. It weighed 4.4 gr. After recrystallisation from water, 3.7 gr. of pure sample were obtained. The project to get guanidine-butyric acid even under this condition was a failure.

Filtrate (B). 0.3 Gr. of succinic acid was obtained. It was identified by mixed melting point test.

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