

STUDIES ON AMINO-ACIDS AND RELATED COMPOUNDS.

IX. ELECTROLYTIC OXIDATION OF PROLINE
AND γ -AMINO-BUTYRIC ACID.

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In a previous paper⁽¹⁾ it was reported briefly that the electrolytic oxidation of proline may proceed in a type similar to that of pyrrolidone-carboxylic acid. In this paper the details of the above oxidation and that of γ -amino-butyric acid are reported.

Langheld⁽²⁾ oxidized *dl*-proline with sodium hypochlorite and isolated a certain base having an odour of pyrrolidine. Recently Herbst and Clark⁽³⁾ treated proline with silver oxide, but no definite compound was obtained.

In the present experiment, proline in dilute sulphuric acid was electrolyzed in an undivided cell with a lead peroxide anode and a lead cathode under the following conditions: current quantity about 8F./mol; temperature 35°C.

In the electrolysate, no substance giving aldehydic reaction could be found. By thorough extraction with ether it was divided into two parts. The part soluble in ether was found to consist of a comparatively large amount of succinimide together with a little succinic acid. From the residual part, by treating with barium hydroxide, ammonia as well as pyrrolidine were isolated as volatile bases. The pyrrolidine was confirmed by the analysis of its chloroplatinate and by the formation of characteristic precipitates on addition of a solution of potassium bismuth iodide.

In the solution, from which volatile bases were removed, there was found some amount of amino-nitrogen, which greatly increased when the solution was boiled with barium hydroxide and, in fact, γ -amino-butyric acid was isolated in the form of its chloroplatinate. It may be a proper reasoning to consider that the above increase of amino-nitrogen means an accumulation of γ -amino-butyric acid due to the hydrolysis of pyrrolidone⁽⁴⁾, though, in reality, the base itself was not isolated.

The electrolytic oxidation of γ -amino-butyric acid was carried out just in the same manner as described above and from its electrolysate ammonia, succinic acid, and unchanged amino-acid were isolated.

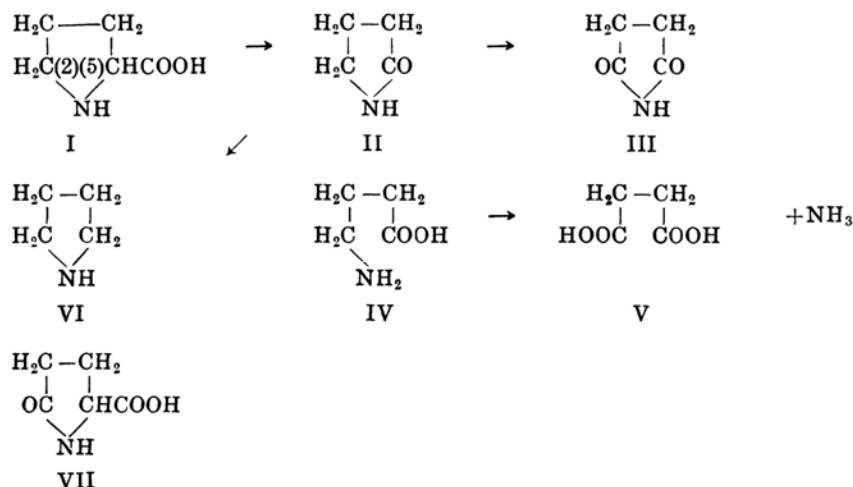
(1) Y. Takayama, this Bulletin, **8** (1933), 213.

(2) K. Langheld, *Ber.*, **42** (1909), 2373.

(3) R. M. Herbst and H. T. Clark, *J. Biol. Chem.*, **104** (1934), 769.

(4) J. Tafel and M. Stern, *Ber.*, **33** (1900), 2230.

Taking the two sets of oxidation into consideration, the mechanism of electrolytic oxidation of proline may be represented as follows:



Proline (I) was first oxidized to pyrrolidone (II) and the latter, on further oxidation, gives succinimide (III) and this course of reaction seems to be a main one, when interpreted from the amount of the final products. γ -Amino-butyric acid (IV) as well as pyrrolidine (VI) are presumed to be derived from pyrrolidone, the former by hydrolysis, the latter by reduction. As to the formation of succinic acid (V) two possible processes can be conceived: hydrolysis of succinimide and oxidation of γ -amino-butyric acid.

It was shown by E. Fischer⁽⁵⁾ that proline can be formed by the reduction of pyrrolidone-carboxylic acid (VII). In the present oxidation, as a matter of fact, not pyrrolidone-carboxylic acid⁽⁶⁾ but γ -amino-butyric acid together with pyrrolidine was obtained, thus, the reverse of the E. Fischer's reaction can not be imagined to occur in this case. It naturally leads to the conclusion that the carbon atom (5) in proline is more readily oxidizable than the carbon atom (2).

Experimental Part.

Electrolytic Oxidation of Proline. Proline (Fraenkel and Landau) m.p. 209–210° after recrystallization from *n*-butyl alcohol.

The above sample (1.151 g., 10 millimols) was dissolved in *N* sulphuric acid (27 c.c.) and electrolyzed under the following conditions: cell the same as described in part VII⁽⁷⁾;

(5) E. Fischer, *Ber.*, **44** (1911), 1332.

(6) Y. Takayama, this Bulletin, **8** (1933), 137.

(7) Y. Takayama, this Bulletin, **8** (1933), 213.

electrodes (2 cm. \times 4 cm.) PbO_2 -Pb; current density 2 amp./dm²; 3.5 volts; current quantity 8 F./mol; temperature 35°C.

The electrolysate of proline (10 millimols \times 2) was thoroughly extracted with ether. Ether was expelled, and the remaining crystalline mass was separated into neutral and acidic parts as usual.

Neutral part (succinimide). Crystals (0.5 g.) obtained were recrystallized from acetone, m.p. 126°. It was found to be succinimide by the determination of nitrogen as well as by the mixed melting point test (Found: N, 14.2. Calc. for $\text{C}_4\text{H}_5\text{O}_2\text{N}$: N, 14.15%).

Acidic part (succinic acid). Crystals (0.2 g.) obtained melted at 183° after recrystallization from water. It was identified as succinic acid by the determination of acid equivalent and mixed melting point test (4.419 mg. of this acid required 5.56 c.c. (0.01346 N NaOH). Calc. for $\text{C}_4\text{H}_6\text{O}_4$: 5.63 c.c.).

Volatile bases (ammonia and pyrrolidine). After removal of the part soluble in ether from the electrolysate, the residual solution was distilled with an excess of barium hydroxide under reduced pressure, the volatile bases being collected in dilute hydrochloric acid. On evaporating up the solution, a very hygroscopic crystalline mass was obtained, which was treated with absolute alcohol, and were separated into soluble and insoluble parts.

The hydrochloride (0.1 g.) insoluble in alcohol was found to be ammonium chloride by the analysis of its chloroplatinate and by the inspection of crystal forms under polarizing microscope (Found: Pt, 43.9. Calc. for $(\text{NH}_4)_2\text{PtCl}_6$: Pt, 43.96%).

On expelling the solvent from the hydrochloride solution very hygroscopic crystals were obtained, which was subjected to steam distillation with sodium hydroxide. The distillate was shaken with an excess of yellow mercuric oxide⁽⁸⁾ and filtered. After twice repeating the above treatments, the solution was distilled with steam. The free base thus obtained gave no reaction of pyrrole, it gave the characteristic crystalline precipitates of pyrrolidine when a solution of potassium bismuth iodide was added. The chloroplatinate of this base crystallized in orange rods (straight extinction), which decomposed at 210°. The result of the analysis coincided with that of chloroplatinate of pyrrolidine (Found: Pt, 35.4. Calc. for $(\text{C}_4\text{H}_9\text{N})_2\text{H}_2\text{PtCl}_6$: Pt, 35.34%).

Separation of γ -Amino-butyric Acid. After removal of part soluble in ether and volatile bases from the electrolysate, the solution was treated with CO_2 , to remove the large bulk of barium. The filtrate was concentrated under reduced pressure, and boiled with an excess of barium hydroxide. The solution, after complete removal of barium, was extracted with ether. From the ethereal extract a small quantity of succinic acid (m.p. 183°) was obtained, which remained unextracted in the previous treatments.

In the residual solution, 1.63 mg. of amino-nitrogen (74.3% of total-nitrogen) was found and it gave an intense ninhydrin reaction. From this solution yellow crystalline chloroplatinate (0.13 g.) was isolated which, after recrystallization, decomposed at 220°. It was identified as γ -amino-butyric acid by the decomposition point and by the comparison of crystalline behaviours under polarizing microscope (straight extinction, sign of elongation: negative) with those of synthetic sample. The hydrochloride of this base was prepared from the chloroplatinate, as usual, and it gave an intense ninhydrin reaction. Here again its behaviours under polarizing microscope coincided with those of synthetic hydrochloride.

To ascertain the nitrogen distribution and the presence of pyrrolidone in the electrolysate, proline (0.707 g.) was electrolyzed (7.9 F./mol) under the same conditions as already

(8) E. Abderhalden, "Handbuch der biologischen Arbeitsmethoden," 1923, I, 7, 347.

described. The volatile bases (NH_3 , pyrrolidine) and barium were removed from the electrolysate, and the amounts of amino-nitrogen as well as total nitrogen in the solution were determined. After boiling with an excess of barium hydroxide for two hours, the amounts of the two forms of nitrogen in it were again determined. The amount of amino-nitrogen (percentage to total nitrogen) reached 93.9% after this treatment, the net increase exceeding 20%.

Oxidation of γ -Amino-butyric Acid. γ -Amino-butyric acid⁽⁹⁾ was prepared by the electrolytic reduction of succinimide. It melted at 203° with decomposition (Found: N, 13.5. Calc. for $\text{C}_4\text{H}_5\text{O}_2\text{N}$: N, 13.59%. Found: Pt, 31.6. Calc. for $(\text{C}_4\text{H}_5\text{O}_2\text{N})_2\text{H}_2\text{PtCl}_6$: Pt, 31.62%).

γ -Amino-butyric acid (1.031 g., 10 millimols) in $\text{N H}_2\text{SO}_4$ (27 c.c.) was electrolyzed, current quantity being 8.25 F./mol, under the same conditions as in the case of proline. Amino-N/total-N in the electrolysate was 30.28%. The electrolysate was extracted thoroughly with ether. Succinic acid (0.2 g.) was obtained from the ethereal extract and it was recrystallized from water, which melted at 183° . The acid equivalent and silver content in its silver salt were determined (29.6 mg. acid required 5.00 c.c. (0.09972 N NaOH). Calc. for $\text{C}_4\text{H}_6\text{O}_4$: 5.02 c.c. Found: Ag, 64.8. Calc. for $\text{C}_4\text{H}_4\text{O}_4\text{Ag}_2$: Ag, 65.04%).

The residue of the ethereal extraction was treated with $\text{Ba}(\text{OH})_2$, and ammonium chloride was isolated as volatile base (Found: Pt, 43.7. Calc. for $(\text{NH}_4)_2\text{PtCl}_6$: Pt, 43.93%). After the removal of succinic acid, ammonia, and barium from the electrolysate, unchanged γ -amino-butyric acid was recovered in crystalline form (m.p. 203°).

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(9) J. Tafel and M. Stern, *Ber.*, **33** (1900), 2224.