Studies on Amino-Acids and Related Compounds. Part X. Electrolytic Oxidation of Aspartic Acid and Malonic Acid.*

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In a previous paper,(1) it was briefly described that the electrolytic oxidation of aspartic acid is rather more complicated in mechanism than that of glutamic acid, although the two compounds are analogous in their structure. In the present paper, the course of oxidation of aspartic acid will be further discussed in its details.

By oxidizing aspartic acid with hydrogen peroxide, Dakin(2) obtained formic acid, acetic acid and acetaldehyde together with a little malonic acid. Langheld(3) obtained acetaldehyde on heating aspartic acid with sodium hypochlorite, and considered malonic acid semialdehyde to be an intermediate product.

In the present experiments, aspartic acid was oxidized with lead peroxide anode in sulphuric acid solution under two different conditions in temperature, one at 35° and the other at about 100°. Malonic acid, which can be presumed to be one of the reaction products in the above oxidation, was also oxidized just in the same way.

In the experiment carried out at 35°, formic acid (10.8 mol%), malonic acid (1.4%), succinic acid (small quantity), ammonia and carbon dioxide were isolated as the oxidation products of aspartic acid. In the same condition, formic acid and carbon dioxide were isolated from malonic acid.

In the case of 100°, the aspect of reaction is somewhat different. Volatile products formed during the electrolysis were slowly distilled off at this temperature and further oxidation of these products was thus prevented. Acetaldehyde (4.1 mol%), formic acid (28.14%), succinic acid (0.36%), ammonia and carbon dioxide were isolated from the oxidation products of aspartic acid. Formic acid (35.2%) together with a little formaldehyde was isolated from those of malonic acid.(4)

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* Translated by the authors from *J. Chem. Soc. Japan*, **56** (1935), 1460.

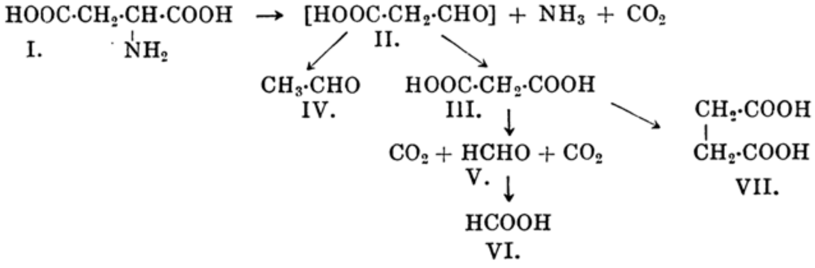
(1) Part VII, this Bulletin, **8** (1933), 215.

(2) H. D. Dakin, *J. Biol. Chem.*, **5** (1909), 409.

(3) K. L. Langheld, *Ber.*, **42** (1909), 2370.

(4) E. Durand, *Chem. Zentr.*, **1903**, II, 968.

From these results, the mechanism of the electrolytic oxidation of aspartic acid and malonic acid may be represented as follows.



The first stage of the oxidation of aspartic acid (I) is doubtless the formation of malonic acid semialdehyde (II). This unstable aldehyde immediately produces malonic acid (III) by further oxidation. Another change occurs without oxidation, and the aldehyde, at the temperature of 100°, losing carbon dioxide,⁽⁵⁾ turns into acetaldehyde (IV). Further oxidation of malonic acid leads to the formation of formic acid (VI) through formaldehyde (V). While the formation of succinic acid (VII), as Fichter⁽⁶⁾ pointed out, is due to the electrolytic oxidation of malonic acid.

Experimental.

I. Electrolytic oxidation of aspartic acid.

Aspartic acid (Kahlbaum) (Found: N, 10.52. Calculated for $C_4H_7O_4N$: N, 10.53%). Apparatus. In the case of 35°, an undivided cell, electrodes (4 cm. × 5 cm.): lead peroxide—lead. In the case of 98–99°, a cylindrical glass vessel (diameter 3 cm., height 12 cm.) provided with a rubber stopper carrying two electrodes, a thermometer and a dropping funnel. A delivery tube connecting to a receiver through a condenser was fused to the cell. During the electrolysis, the volatile oxidation products were distilled into the receiver which was cooled in ice. Electrodes: lead peroxide anode (6 cm. × 7.5 cm.), lead cathode (6 cm. × 4 cm.).

Experiment at 35°. Two cells (each of which contained 6.653 g. or 1/10 mol of aspartic acid in 120 c.c. of N H_2SO_4) were connected in series and immersed in a thermostat of 35°. C.D.: 2 amp./dm.^{2} Current quantities 8.146 F./mol. After the electrolysis the nitrogen distribution (NH_3-N/total N) in the electrolyzate was determined (73.9%) and the main bulk was subjected to steam distillation. In the distillate, no substance giving aldehydic reaction was found.

Volatile acid (*formic acid*). The distillate was titrated with N/10NaOH (106.5 c.c., 10.8 mol%). The resulting salt was converted into lead salt and analysed. Lead formate (Found: Pb, 69.32. Calculated for $(HCO_2)_2Pb$: Pb, 69.71%).

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(5) A. Wohl, *Ber.*, **33** (1900), 2763.

(6) Fr. Fichter and J. Heer, *Helv. Chim. Acta*, **18** (1935), 704.

Non-volatile acids (*malonic and succinic acids*). The residue of the steam distillation was concentrated and extracted with ether. The first part of the ethereal extract yielded a little quantity of crystals on evaporation of the solvent. The crystalline acid, recrystallized from water, melted at 183°. It was identified as succinic acid by the determination of acid equivalent, mixed melting point test and colour reaction of resorcine—sulphuric acid test. The last part of the ethereal extract was evaporated, leaving an oil which slowly crystallized (0.07 g., 1.4 mol% as malonic acid). The crystals, when recrystallized from dilute alcohol, melted at 132–133° and no depression of melting point was observed when mixed with a pure sample of malonic acid. 0.0414 g. of the acid required 7.89 c.c. of N/10NaOH. Calculated for malonic acid ($C_3H_4O_4$): 7.96 c.c. The malonic acid was also identified as barium salt. The salt was dissolved in hot water and reprecipitated with alcohol. Barium malonate⁽⁷⁾ (Found: Ba, 52.93. Calculated for $C_3H_2O_4Ba \cdot H_2O$: Ba, 53.38%).

Volatile base (*ammonia*). The residual solution of above extraction was distilled under reduced pressure with an excess of barium hydroxide. The volatile base was collected in dilute hydrochloric acid. Hydrochloride 1.4 g. Ammonium chloroplatinate (Found: Pt, 43.69. Calculated for $(NH_4)_2PtCl_6$: Pt, 43.96%).

After above treatments, the solution was freed from SO_4'' and $Ba^{\cdot\cdot}$ as usual. Crystalline acid (0.55 g.) was isolated which was identified to be unchanged aspartic acid by the ninhydrin reaction and determination of nitrogen (N, 10.48%).

Experiment at 98–99°. Aspartic acid (3.326 g. × 2, 1/20 mol) was dissolved in N H_2SO_4 (40 c.c. × 2) and electrolyzed in two undivided cells connected in series. The cells were placed in an air thermostat of 98–99°. C.D.: 2 amp./dm.^{2} Current quantities: 8.148 F./mol. The distribution of nitrogen of the electrolyzate: NH_3-N/total N = 73.93%. During the electrolysis, loss of water in the cell caused by distillation was compensated continuously through the dropping funnel.

Volatile substances (*acetaldehyde, formic acid*). Volatile acid, distilled during electrolysis, required 125.1 c.c. of N/10 NaOH; ditto, obtained by steam distillation after electrolysis, 13.9 c.c. of N/10 NaOH; total: 139.0 c.c. of N/10 NaOH, or 28.1 mol% to aspartic acid. The volatile acid was identified to be formic acid by the reaction with mercuric chloride and silver nitrate as well as by the analysis of its lead salt. Lead formate (Found: Pb, 69.47. Calculated for $(CHO_2)_2Pb$: Pb, 69.71%).

Amount of the aldehyde in the distillate was estimated by the titration of the neutralized distillate with bisulphite solution: 2.021 millimol (4.10 mol%) or 0.0890 g. of acetaldehyde. Acetaldehyde-*p*-nitrophenylhydrazone, m.p. 125–126° (Found: N, 23.56. Calculated for $C_8H_9N_3O_2$: N, 23.46%). It showed no depression of melting point when mixed with a specimen of *p*-nitrophenylhydrazone prepared from pure acetaldehyde (Kahlbaum).

Non-volatile acid (*succinic acid*). The residual solution was extracted thoroughly with ether. The ethereal solution was evaporated, leaving crystalline acid which was recrystallized from water. It melted at 183° and was identified as succinic acid by the mixed melting point test. 21 mg. (0.36 mol%) of succinic acid from 1/20 mol aspartic acid (current quantities: 6.17 F./mol; NH_3-N/total N = 41%). The presence of malonic acid could not be confirmed.

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(7) C. Coutelle, *J. prakt. Chem.*, **73** (1906), 73.

Volatile base (ammonia). Hydrochloride 0.9 g. Chloroplatinate (Found: Pt, 43.79. Calculated for $(NH_4)_2PtCl_6$: Pt, 43.96%).

After the removal of the substances described above, unchanged aspartic acid (0.144 g.) was recovered from the residue (Found: N, 10.38. Calculated for $C_4H_7O_4N$: N, 10.53%).

II. **Electrolytic oxidation of Malonic Acid.**

Experiment at 35°. Malonic acid (Kahlbaum, 1.3005 g. × 2, 1/40 mol) was dissolved in N H_2SO_4 (120 c.c. × 2) and electrolyzed in two cells connected in series. C.D.: 2 amp./dm.^{2} Current quantities 3.07 F./mol. The electrolyzate was treated in the same manner as in the case of aspartic acid.

Volatile acid (formic acid). It required 16.70 c.c. of N/10 NaOH, corresponding to 13.4 mol%, and the acid was identified as formic acid by the reaction with silver nitrate and mercuric chloride and also by the crystalline form of the lead salt.

Experiment at 97°. Malonic acid (2.601 g. × 2, 1/20 mol) was dissolved in N H_2SO_4 (40 c.c. × 2) and electrolyzed under the same condition as in the case of aspartic acid. Current quantities: 8.25 F./mol.

Volatile substances (formic acid, formaldehyde). The acid, distilled during the electrolysis (A) required 103.16 c.c. of N/10 NaOH; ditto, obtained by steam distillation after electrolysis (B), 73.00 c.c. of N/10 NaOH; total: 176.16 c.c. of N/10 NaOH, 35.2 mol%. Lead formate (Found: Pb, 70.03% (A), 69.89% (B). Calculated for $(CHO_2)_2Pb$: Pb, 69.71%).

The volatile neutral part of the distillate gave fuchsin reaction and Rimini's reaction, and produced silver mirror. The formaldehyde was determined by the method of G. Romijn: 4.835 mg. (0.32 mol%).

Non-volatile acid (succinic acid). The residue of steam distillation was extracted with ether. The crystals (m.p. 183°, 7.1 mg.) obtained from the ethereal solution were identified as succinic acid by mixed melting point test, as well as by the colour reaction of resorcine-sulphuric acid test.

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