Studies on Amino-Acids and Related Compounds. Part XI. The Formation of Aldehydes by the Electrolytic Oxidation of α-Amino-Acids.*

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In connection with biological oxidation, numerous attempts have been made to elucidate the mechanism of oxidation of α-amino-acids, and several compounds[1] (ketonic acid, imino-acid etc.) have been suggested as the intermediate products of their oxidation.

It was experimentally confirmed that by chemical oxidation amino-acids always produce the next lower aldehydes at the first stage of their oxidation. By electrolytic oxidation of amino-acids in sulphuric acid with platinum anodes, Fichter[2] obtained a little of aldehyde together with other oxidation products, and concluded that the aldehyde is the first oxidation product.

As described in Part VII,[3] when amino-acids were oxidized electrolytically with lead peroxide anode, the aldehyde was found in only a few case, and the author mentioned that aldehyde may be formed but soon further oxidized, and also that the course of reaction may proceed as follows:

$$\begin{array}{ccccc} \mathrm{R{\cdot}CH{\cdot}COOH} & \longrightarrow & \mathrm{R{\cdot}CHO} & + \mathrm{NH_3} + \mathrm{CO_2} \\ \mathrm{NH_2}\ \downarrow & & \downarrow & \\ \text{Melanin} & & \mathrm{R{\cdot}COOH} & \end{array}$$

where R denotes the radicals of aliphatic, aromatic and heterocyclic compounds.

In Part X,[4] it was proved that malonic acid semialdehyde was the first oxidation product of aspartic acid, and the present experiments were undertaken in the same manner to confirm the aldehyde formation from other amino-acids. By removing the oxidation products as quickly as possible from the electrolyzates to prevent further oxidation, aldehydes were successfully obtained in comparatively abundant quantity from four amino-acids.

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* Translated by the authors from *J. Chem. Soc. Japan*, **57** (1936), 449.

(1) Bergel and Bolz, *Z. physiol. Chem.*, **215** (1933), 25; Bergel, *ibid.*, **233** (1934), 66.

(2) Fichter and Schmid, *Helv. Chim. Acta*, **3** (1920), 704.

(3) Takayama, this Bulletin, **8** (1933), 213.

(4) Takayama, this Bulletin, **12** (1937), 338.

On oxidizing glycine, alanine and leucine, Fichter[5] obtained formaldehyde, formic acid, ammonia, amines and carbon dioxide from glycine; acetaldehyde, acetic acid, formaldehyde, formic acid and ammonia, as well as carbon dioxide from alanine; isovaleraldehyde, isovaleric acid, isobutyric acid, acetone and acetic acid from leucine.

In the present experiments, alanine was oxidized in dilute sulphuric acid with lead peroxide anode at 35°, and acetic acid, ammonia and carbon dioxide were isolated, but no aldehyde was found. On the contrary, when alanine was oxidized in the same manner as aspartic acid at about 100°, the volatile oxidation products being distilled off during electrolysis, acetaldehyde (36.5%[6] as *p*-nitrophenylhydrazone) together with acetic acid (17.8%), ammonia and carbon dioxide was obtained.

Even in the experiment at 35°, when the volatile oxidation products were driven into absorption bottles by bubbling air through the cell, other conditions being the same, acetaldehyde could be abundantly separated in the form of *p*-nitrophenylhydrazone and dimedon-derivative. From this fact it was concluded that the absence of the aldehyde in the former experiments was due to further oxidation.

Glycine was oxidized in the same manner and formaldehyde (16.6%) together with formic acid (4.85%) was obtained. Ammonia, methyl-, dimethyl- and trimethyl-amines were isolated as volatile bases. These amines probably resulted from the action of formaldehyde with ammonia.[7] Likewise, valine gave isobutyraldehyde (32.1%) and isobutyric acid (27.5%) on its oxidation.

Fichter[5] mentioned that the electrolytic oxidation of leucine is vigorous and proceeds on even to acetone. This fact was observed also in Part VII.[3] Leucine was oxidized in the same way as alanine at about 100°, and the distillate thus obtained consisted of oily and aqueous layers. The former almost exclusively consisted of isovaleraldehyde (yield 53–56%). The latter contained isovaleric acid (yield 20–22%), but not the lower fatty acids.

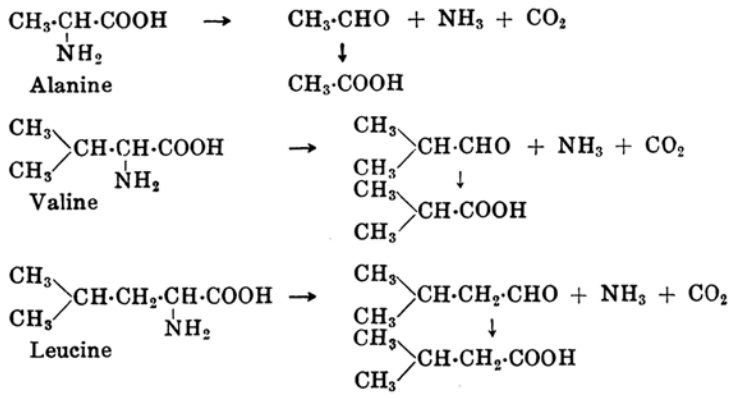
From these results, the mechanism of the electrolytic oxidation described above may be represented as follows:

$$\begin{array}{lcl} CH_2\cdot COOH & \longrightarrow & H\cdot CHO + NH_3 + CO_2 \\ | & & \downarrow \\ NH_2 & & H\cdot COOH \\ \text{Glycine} & & \end{array}$$

(5) Fichter and Kuhn, *Helv. Chim. Acta*, **7** (1924), 164.

(6) The percentages in this paper were expressed in molar per cent.

(7) Plöchl, *Ber.*, **21** (1888), 2117.



Thus, from the experiments on glycine, alanine, valine, leucine and aspartic acid,[4] it may be concluded that the first stage of the electrolytic oxidation of α-amino-acid is the aldehyde formation, and that the general expression described before will represent the course of the electrolytic oxidation of α-amino-acid. Some of these reactions may be applied to industrial purposes.[8]

Experimental.

I. Electrolytic oxidation of alanine.

Experiment at 35°. Alanine (Kahlbaum) (Found: N, 15.83. Calculated for $C_3H_7O_2N$: N, 15.73%). Alanine (8.911 g., 1/10 mol) was dissolved in N H_2SO_4 (120 c.c. × 2) and electrolyzed in two undivided cells connected in series, under following conditions. Cell: similar as described in Part IV.[9] Electrodes (4 cm. × 5 cm.): lead peroxide anode and lead cathode. C.D.: 2 amp./dm.^{2} Current quantities: 7.923 F./mol. The distribution of nitrogen in the electrolyzate after electrolysis: NH_3-N/total N = 96.6%.

During the electrolysis, evolved gas was led to ice-cooled washing bottles containing water to catch both acidic and neutral substances. The aqueous solution in the above bottle gave no aldehydic reaction. The electrolyzate was subjected to steam distillation. The distillate was separated into neutral and acidic parts. The former gave no aldehydic reaction, and the latter was converted into sodium salt.

Volatile acid (*acetic acid*). The sodium salt (4.34 g.) of volatile acid was identified as acetate by acetic ester test and by iodine-lanthanum reaction. Silver acetate (Found: Ag, 64.72. Calculated for $C_2H_3O_2Ag$: Ag, 64.64%).

Volatile base (*ammonia*). After the removal of the volatile acid from the electrolyzate, the residue was distilled with an excess of barium hydroxide under reduced pressure. The volatile base was converted into hydrochloride. It was identified as ammonia. Ammonium chloroplatinate (Found: Pt, 43.68. Calculated for $(NH_4)_2PtCl_6$: Pt, 43.96%).

(8) Japanese patent 110,640.

(9) This Bulletin, **8** (1933), 175.

Only unchanged alanine was recovered from the residue of the above base.

Experiment at 96°. Alanine (2.227 g., 1/40 mol) was dissolved in N H_2SO_4 (40 c.c.) and electrolyzed under the following conditions. Apparatus: the same apparatus as described in Part X.[1] During the electrolysis, the volatile oxidation product was distilled into a receiver which was cooled in ice. Temperature: 96°. Electrodes: lead peroxide anode (6 cm. × 7.5 cm.), lead cathode (6 cm. × 4 cm.). C.D.: 2 amp./dm.^{2} Current quantities: 8.09 F./mol. 3 volts. The distribution of nitrogen in the electrolyzate: NH_3-N/total N = 98.11%. The electrolyzate was treated in the same way as in the case of 35°.

Volatile neutral solution (acetaldehyde). Both the distillate caught in the ice-cooled water and the distillate obtained from the electrolyzate by steam distillation were combined, neutralized and separated into neutral and acidic parts by distillation. Neutral part gave fuchsin-sulphurous acid reaction and silver mirror. *p*-Nitrophenylhydrazone (1.6 g., 36.5%), recrystallized from carbon tetrachloride, gave m.p. 126°. It was identified as acetaldehyde-*p*-nitrophenylhydrazone (m.p. 126°) by mixed melting point test and by nitrogen determination (Found: N, 23.77. Calculated for $C_8H_9N_3O_2$: N, 23.46%).

Volatile acid (acetic acid). The acidic part described above required 43.70 c.c. of N/10 NaOH. That is 17.8% to decomposed alanine. Silver acetate (Found: Ag, 64.72. Calculated for $C_2H_3O_2Ag$: Ag, 64.64%).

Volatile base (ammonia). It was treated in the same manner as in the case of 35°. Hydrochloride 1.1 g. Chloroplatinate (Found: Pt, 43.67. Calculated for $(NH_4)_2PtCl_6$: Pt, 43.96%).

Unchanged alanine (0.05 g.) was recovered from the final residue.

Detection of acetaldehyde. Alanine (1.1375 g., 1/80 mol) was electrolyzed in N H_2SO_4 (120 c.c.) at 35°, bubbling air current between the electrodes in order to expel the volatile substances as quickly as possible. The volatile substances were caught in three washing bottles containing water. C.D.: 2 amp./dm.^{2} Current quantities: 7.203 F./mol. NH_3-N/total N = 47.52%. The solution of volatile substances caught in the washing bottles gave aldehydic reactions and yielded 0.318 g. of acetaldehyde (57.7%, estimated by the bisulphite method). *p*-Nitrophenylhydrazone was precipitated from the solution and recrystallized from dilute alcohol. M.p. 126°. It was identified as acetaldehyde-*p*-nitrophenyhydrazone.

In the same manner, alanine (1/80 mol) was electrolyzed with the current quantities of 6.02 F./mol. The distribution of nitrogen in the electrolyzate: NH_3-N/total N = 47.0%. In this case, the three washing bottles consisted of the one containing water (I) and the others containing dilute alcoholic solution (5%) of dimedon[10] (II, III). Acetaldehyde-*p*-nitrophenylhydrazone (0.04 g.) was obtained from the bottle I, m.p. 120° before and 126° after recrystallization. Acetaldehyde-dimedon was obtained from the bottle II (0.04 g., m.p. 139°) and the bottle III (0.09 g., m.p. 132–134°). The latter was recrystallized from dilute alcohol and it melted at 139°.

II. Electrolytic oxidation of glycine.

Glycine (Kahlbaum) (0.9381 g., 1/80 mol) was dissolved in N H_2SO_4 (30 c.c.) and

(10) Vorländer, *Z. anal. Chem.*, **77** (1929), 241.

electrolyzed under the following conditions. The apparatus is the same as in the case of alanine. Temperature: 99°. Electrodes: lead peroxide anode (2 cm. × 5 cm.), lead cathode (2 cm. × 5 cm.). C.D.: 2 amp./dm.^{2} Current quantities: 6.292 F./mol. The distribution of nitrogen in the electrolyzed solution: NH_3-N/total N = 69.36% (total nitrogen calculated from the glycine).

Volatile acid (*formic acid*). The acid in the distillate driven out during the electrolysis required 4.28 c.c. of 0.1011 N NaOH. The acid in the distillate obtained by the steam distillation required 1.72 c.c. of 0.1011 N NaOH. The total acid amounted to 0.6066 millimol (4.85% to glycine). The acid gave intensely the characteristic reactions of formic acid (the reduction of silver nitrate and mercuric chloride, and the reaction of Hottenroth).

Volatile neutral substance (*formaldehyde*). The above neutralized solution gave the characteristic formaldehyde reaction of fuchsin-sulphurous acid solution containing a little sulphuric acid (Grosse-Bohle). Formaldehyde was estimated by the Romijn's iodine method.[11] Yield 6.2 mg. (16.6% to glycine).

Volatile bases (*ammonia, methylamine, dimethylamine, trimethylamine*).[12] The residue, after the removal of volatile substances by steam distillation, was distilled with an excess of barium hydroxide under reduced pressure. The volatile bases were caught in hydrochloric acid. The very hygroscopic chlorides (1.4 g.) were obtained and treated with absolute alcohol to separate into the soluble and insoluble (0.85 g.) parts. The crystals obtained from the part insoluble in absolute alcohol were identified as ammonium chloride by the behaviour under poralisation microscope and by the analysis of its chloroplatinate (Found: Pt, 43.71. Calculated for $(NH_4)_2PtCl_6$: Pt, 43.96%).

After distilling off alcohol from the part soluble in absolute alcohol, the residual chloride was mixed with sea sand, dried and extracted with hot chloroform. The part insoluble in chloroform was treated with yellow mercuric oxide to make it free from ammonia by the method of François, and then converted into chloroplatinate (0.48 g.), which decomposed at 220°. It was identified as monomethylamine (Found: Pt, 41.56. Calculated for $(CH_3 \cdot NH_2)_2H_2PtCl_6$: Pt, 41.35%). Monomethylamine-picrolonate was obtained, which decomposed at 244°.

The part soluble in chloroform (dimethyl- and trimethyl-amines) was freed from chloroform, dissolved in water and cooled to 0°, from which a small quantity of crystals of trimethylamine periodide was precipitated by adding iodine–potassium iodide solution. The crystals were dissolved in sodium bisulphite solution, and distilled with steam after an addition of sodium hydroxide solution. The distillate containing trimethylamine required 0.30 c.c. of 0.1065 N H_2SO_4. The mother liquor of precipitate of trimethylamine periodide was distilled with steam after an addition of sodium bisulphite and sodium hydroxide. The distillate containing dimethylamine required 2.80 c.c. of 0.1065 N H_2SO_4. The chloroplatinate decomposed at 206° (Found: Pt, 39.57. Calculated for $[(CH_3)_2NH]_2H_2PtCl_6$: Pt, 39.04%).

Only unchanged glycine was recovered from the final residue.

III. Electrolytic oxidation of valine.

dl-Valine (Fränkel, 0.5855 g., 5 millimols) was electrolyzed in N H_2SO_4 (30 c.c.)

(11) G. Romijn, *Z. anal. Chem.*, **36** (1897), 19.
(12) Abderhalden, "Handbuch der Biologischen Arbeitsmethoden," Abt. I, **7**, 349, Berlin (1925); J. Bertheaume, *Compt. rend.*, **150** (1910), 1063, 1251.

at 99°. Electrodes: lead peroxide anode (4 cm. × 7.5 cm.), lead cathode (4 cm. × 4 cm.). C.D.: 2 amp./dm.^{2} Current quantities: 6.209 F./mol. NH_3-N/total N = 89.3%.

Volatile acid (*isobutyric acid*). The acid required 12.24 c.c. of N/10 NaOH. Yield 27.5% to valine.

Volatile neutral substance (*isobutyraldehyde*). Isobutyraldehyde-*p*-nitrophenylhydrazone (0.3328 g., 32.1% to total valine, 36% to decomposed valine), recrystallized from dilute alcohol gave m.p. 132° (Found: N, 20.33. Calculated for $C_{10}H_{13}N_3O_2$: N, 20.29%).

Volatile base (*ammonia*). Hydrochloride (0.2 g.). Ammonium chloroplatinate (Found: Pt, 43.81. Calculated for $(NH_4)_2PtCl_6$: Pt, 43.96%).

IV. Electrolytic oxidation of leucine.

Leucine (Found: N, 10.50. Calculated for $C_6H_{13}O_2N$: N, 10.69%). Cell: glass cylinder (diameter 7 cm., height 23 cm.) with a rubber stopper which carried two electrodes, a gas delivery tube, a thermometer and a dropping funnel. Electrodes: lead peroxide anode (10 cm. × 16 cm.), nickel cathode (10 cm. × 7 cm.).

Leucine (40 g.) was dissolved in N H_2SO_4 (400 c.c.) and electrolyzed at about 100° in the same manner as in the case of aspartic acid. During the electrolysis a solution of leucine (160 g.) in 4N H_2SO_4 (400 c.c.) was dropped into the cell from a dropping funnel. C.D.: 2 amp./dm.^{2} Current quantities: 6.98 F./mol. 3 volts. The distillate obtained during the electrolysis consisted of oily (83 g.) and aqueous layers. The former consisted almost solely of isovaleraldehyde. The latter was an aqueous solution of isovaleric acid (0.0331 equivalent) together with a little isovaleraldehyde. In the same manner, a comparatively large amount of leucine was electrolyzed, and the product was separated into the following four fractions (A, B, C, D).

(A) Acidic part of oily layer. Oil (3 kg.) was shaken with 2% sodium carbonate solution and separated into acidic and neutral parts. The acidic part was extracted with ether and dried over anhydrous sodium sulphate. After expelling off the ether, the residue was fractionated under reduced pressure as described in Table 1.

Table 1.

Fraction	Boiling point at 26 mm.	Distillate (g.)	Isovaleric acid (%) (determined by titration)	Ag % of the silver salt
1	up to 80°	0.9	—	—
2	80–88°	44.7	100.5	51.80
3	88–89°	25.2	100.2	51.78
4	89–91°	4.4	99.4	51.56
5	91°	0.7	99.4	51.51
		Total 75.9		

Calc. for $C_5H_9O_2Ag$: Ag, 51.63%

Anilides were prepared from the fractions 2 and 3, and recrystallized from dilute alcohol. Both melted at 110–111° (isovaleranilide m.p. 110–111°).

(B) Neutral part of oily layer. The neutral part obtained above was dried over anhydrous sodium sulphate, and fractionated as described in Table 2.

Table 2.

Fraction	Boiling point	Distillate (g.)	Specific gravity (23°)
1	75–90°	318	0.7945
2	90–100°	1592	0.7948
3	100–110°	239	0.8076
4	residue	(297)	0.8157

The greater part of this neutral part was aldehyde. Isovaleraldehyde-*p*-nitrophenylhydrazone (0.3 g.) was prepared from the fraction 1 and recrystallized from chloroform. It melted at 110–111°. The same nitrophenylhydrazone was obtained from the fractions 2 and 3; both melted at 110–111°. Isovaleraldehyde-*p*-nitrophenylhydrazone (Found: N, 19.12 (2), 19.14 (3). Calculated for $C_{11}H_{15}N_3O_2$: N, 19.00%).

As the residue (297 g.) in Table 2 contained 3.91% of acid, it was treated with dilute sodium carbonate solution to remove the acid, then dried and fractionated.

Table 3.

Fraction	Boiling point	Distillate (g.)	Fraction	Boiling point	Distillate (g.)
1	84–89°	0.6	6	129–130°	20.0
2	89–92°	2.7	7	130–132°	2.0
3	92–125°	1.6	8	132–135°	2.1
4	125–128°	28.3		residue	7.6
5	128–129°	38.0			Total 102.9

Fractions 1, 2 and 3 contained isovaleraldehyde. Fractions 4, 5 and 6 are now under investigation.

(C) Acidic part of aqueous layer. Aqueous solution (105 litres) was neutralized with sodium carbonate solution and distilled to remove the neutral part. The sodium salt thus obtained was decomposed with dilute sulphuric acid. The acid was dried and fractionated under reduced pressure.

Table 4.

Fraction	Boiling point at 29.4 mm.	Distillate (g.)	Isovaleric acid (%) (determined by titration)	Ag % of the silver salt
1	37–51°	27.7	—	—
2	51–89°	14.3	86.4	51.86
3	89–92°	202.1	101.1	51.94
4	92–95°	34.2	99.6	51.45

Calc. for $C_5H_9O_2Ag$: Ag, 51.63%

Isovaleranilide was prepared. M.p. 110–111°. The acids lower than isovaleric acid were not found.

(D) Neutral part of aqueous layer. The aqueous layer of the distillate (obtained during electrolysis) was neutralized with sodium carbonate solution and extracted with ether. The ethereal extract was dried as usual, and fractionated after removing the ether.

Table 5.

Fraction	Boiling point	Distillate (g.)	Fraction	Boiling point	Distillate (g.)
1	50–75°	2.1	5	105–115°	0.9
2	75–85°	1.7	6	115–125°	1.7
3	85–95°	35.7	7	125–127°	8.1
4	95–105°	1.3		residue	11.3
					Total 62.8

Fraction 3 gave isovaleraldehyde-*p*-nitrophenylhydrazone, m.p. 110–111° (Found: N, 18.98. Calculated for $C_{11}H_{15}N_3O_2$: N, 19.00%). The main portion of this neutral part consisted of isovaleraldehyde.

Volatile base (*ammonia*). Ammonium chloroplatinate (Found: Pt, 43.95. Calculated for $(NH_4)_2PtCl_6$: Pt, 43.96%).

In conclusion, the authors express their sincere thanks to Prof. K. Matsubara for kind inspection of this paper.

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