STUDIES ON AMINO-ACIDS AND RELATED COMPOUNDS. PART I. ELECTROLYTIC OXIDATION OF GLUTAMIC ACID.

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Received March 8th, 1933. Published April 28th, 1933.

Although the mechanism of the oxidative decomposition of some natural amino-acids has been studied to some extent by several investigators,(1) yet we have no satisfactory results in this line. The works published are still imcomplete when viewed from the standpoint of organic chemistry, especially electro-organic chemistry.

With regards to the electrolytic oxidation of amino-acids, C. Neuberg(2) has observed that amino-acids lose ammonia, and are converted into aldehydes with an atom of carbon less, when their dilute aqueous solutions are subjected to electrolysis with platinum electrodes.

Fr. Fichter(3) and his collaborators pointed out that glycine and its allies undergo similar electrolytic oxidation, and their mechanisms are expressed by the following equations, glycine being taken as the type.

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(1) H. D. Dakin, *J. Biol. Chem.*, **1** (1905), 171; **4** (1908), 63; *Biochem. J.*, **10** (1916), 319. K. Langheld, *Ber.*, **42** (1909), 2360. W. Denis, *J. Biol. Chem.*, **9** (1911), 365; **10** (1911), 73. H. Wieland u. E. Bergel, *Ann.*, **439** (1924), 196.

(2) C. Neuberg, *Biochem. Z.*, **17** (1909), 270; **24** (1910), 152.

(3) Fr. Fichter u. M. Schmid, *Helv. Chim. Acta*, **3** (1920), 704; Fr. Fichter u. F. Kuehn, *Helv. Chim. Acta*, **7** (1924), 167.

1) $NH_2CH_2COOH + O + 2F = NH_3 + CH_2O + CO_2$ (Initial reaction)

2) $NH_2CH_2COOH + 3O + 6F = NH_3 + 2CO_2 + H_2O$ (Final stage)

They also mentioned that the electrolytic oxidation of amino-acids may be fairly compared with the oxidative de-amination which these compounds undergo in the living organism, but is far more vigorous and profound.

Though the electrolytic oxidation of other amino-acids would be more complicated than the case above mentioned, the elucidation of the mechanism of these reactions would contribute not only to electro-organic chemistry, but also to biochemistry. From this point of view, a series of researches was instituted, and glutamic acid was selected as the first compound for investigation.

Concerning the oxidation of glutamic acid, several papers have been published. E. Ehrlich⁽⁴⁾ observed that glutamic acid produced succinic acid by alcoholic fermentation. H. D. Dakin⁽⁵⁾ found that succinic acid was a reaction product when glutamic acid was oxidised with hydrogen peroxide. U. Suzuki and Y. Matsuyama⁽⁶⁾ oxidised glutamic acid with nitric acid in the presence of vanadic acid and obtained succinic acid. But no adequate investigation has been reported on the electrolytic oxidation of this amino-acid.

The author has first examined the influence of anode material, current density, and diaphragm on the rate of oxidation of glutamic acid. When the anodic oxidation was carried out in an undivided cell under the same conditions, it was found that lead peroxide gave best current efficiency (considered from the yield of ammonia), out of the three kinds of anode materials, namely, carbon, platinum, and lead peroxide, as the following figures show.

Ammonia-Nitrogen to Total-Nitrogen

Pt: 3.9% C: 4.7% PbO_2: 48.5%.

With regards to current density, 2 amp./dm^2. was found to be most favorable for the formation of both ammonia and succinic acid, and no remarkable difference of the nitrogen distribution was found between the experiments in cells with and without diaphragm.

The Oxidation Products.—*d*-Glutamic acid in N.-sulphuric acid was oxidised at a lead peroxide anode with a current density of 2 amp./dm^2. The oxidised solution was subjected to steam distillation and acetic acid

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(4) E. Ehrlich, *Biochem. Z.*, **18** (1909), 391.

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

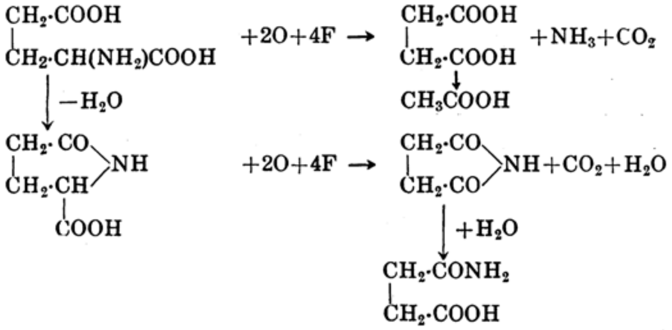
(6) Japanese patent No. 39210.

was obtained from the distillate. Large quantities of ammonium chloride and of succinic acid as well as unchanged glutamic acid were isolated from the residue of the steam treatments.

From the results of the nitrogen distribution in the solution, it seemed that the presence of some imino or amide compound might be possible. In fact, a small quantity of such a compound was isolated and identified as succinamic acid by comparison with the synthetic sample. The presence of this substance may be considered as derived from pyrrolidone-carboxylic acid. The latter acid was first formed from glutamic acid, then it was oxidised to succinimide, which on partial hydrolysis yielded succinamic acid. The formation(7) of pyrrolidone-carboxylic acid in the solution of glutamic acid is quite probable, and it will be discussed fully in the next paper.

Acetic acid obtained from the electrolysate of glutamic acid was speculated as secondary oxidation product of succinic acid. This has been actually proved by the anodic oxidation of pure succinic acid.

Mechanism of the Reaction. The products obtained from glutamic acid in the above oxidation comprise succinic acid, acetic acid, succinamic acid, ammonia and carbon dioxide. Considering the isolation of these substances as the basis, the course of the oxidation can be explained to be as follows:



Experimental Part.

d-Glutamic acid, $N = 9.56\%$ ($C_5H_9O_4N$ requires $N = 9.50\%$), m.p. 209–210°C. $[\alpha]_D^{20} = +31.5°$ (hydrochloride).

Apparatus. Cell: a 250 c.c. cylindrical glass vessel, provided with a rubber stopper, carrying a condenser, a thermometer, and two elec-

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(7) F. W. Foreman, *Biochem. J.*, **8** (1914), 481.

trodes. As for the divided cell, a rectangular glass jar (6.5×4.1×10 cm.) was used, which was bisected vertically, parchment paper being inserted at its joint and tightly braced from outside.

Anode (3×6 cm.) : platinum, carbon, or lead peroxide sheet.
Cathode (3×6 cm.) : lead or carbon sheet.
The distance between the electrodes was 1.7 cm.
Copper coulometer was used for the determination of amount of electricity.

Influence of Anode Material, Current Density and Diaphragm. The experiments were carried out under the following conditions.

(a) *Without a Diaphragm.*—About two grams of glutamic acid was dissolved in 200 c.c. of N.-sulphuric acid and electrolysed at room temperature in the above cylindrical cells which were connected in series. The nitrogen distribution in the solution are summarised in Tables I and II.

Table I.

Influence of Anode Material.

Anode/Cathode	Glutamic acid in gr.	Amp./dm^2.	Amp. hrs.	NH_3-N/Total N (%)
Pt/C	1.989	2.30	1.186	2.18
C/C	1.995	,,	,,	1.97
PbO_2/Pb	1.996	,,	,,	31.70

Table II.

Influence of Anode Material and Current Density.
(NH_2-N was determined by van Slyke's method)

Anode/Cathode	Glutamic acid in gr.	Amp./dm^2.	Amp. hrs.	NH_3-N/Total N (%)	NH_2-N/Total N (%)
Pt/C	2.001	1.89	2.648	3.90	94.7
C/C	2.000	,,	,,	4.70	90.4
PbO_2/Pb	2.100	,,	,,	48.5	32.6
Pt/C	2.000	7.97	2.790	2.20	97.8
C/C	2.000	,,	,,	—	86.6
PbO_2/Pb	2.000	,,	,,	29.7	40.7

Above results clearly show that the best yield of ammonia is obtained in the case when lead peroxide anode was used with current density of 2 amp./dm^2.

(b) *With a Diaphragm.*—Anolyte: 70 c.c. of N.-sulphuric acid containing about 0.7 gr. of glutamic acid. Catholyte: 70 c.c. of N.-sulphuric acid. Anode (3×6 cm.): platinum, carbon, or lead peroxide sheet. Cathode (3×6 cm.): carbon or lead sheet. The results are given in Table III.

Table III.

Influence of Diaphragm.

Anode/Cath.	Glut. acid in gr.	Amp./dm^2.	Amp. hrs.	NH_3-N/Total N (%)			NH_2-N/Total N (%)		
				Anoly.	Cath.	Sum	Anoly.	Cath.	Sum
Pt/C	0.6984	1.86	0.977	2.2	1.5	3.7	88.6	10.2	98.8
C/C	0.6984	,,	,,	2.2	1.3	3.5	87.2	10.6	97.8
PbO_2/Pb	0.6987	,,	,,	40.8	6.1	46.9	23.8	6.3	30.1

The percentage of ammonia obtained in the cases tabulated in Table III are similar to those in Table II, that is to say, no remarkable difference is found between the results of divided and undivided cells.

(c) *Oxidation at a Lead Peroxide Anode.*—About 6 gr. of glutamic acid was dissolved in 160 c.c. of N.-sulphuric acid, and oxidised in undivided cells, with lead peroxide anode at different current densities varying from 2 amp./dm^2. to 13 amp./dm^2. The results are given in Table IV.

Table IV.

Oxidation at a Lead Peroxide Anode.

Glut. acid in gr.	Amp./dm^2.	Amp. hrs.	NH_3-N/Total N (%)	NH_2-N/Total N (%)	Temp.	Volts.
5.987	1.96	7.377	59.3	14.5	—	3.5
6.022	7 38	6.436	62.6*	17.0	35°	4.8
5.997	13.3	6.143	55.4	19.0	50°	6.3

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* In Table II, the better current efficiency is secured in the case of 1.89 amp./dm^2. This contradiction may be due to the difference in concentration of glutamic acid. (The former, 1% solution; the present, 3.8%).

Referring merely to the formation of ammonia, the best result was secured in the case of 7.38 amp./dm^2. By taking the yield of other oxidation products into account, 2 amp./dm^2. was chosen throughout the series of these researches as the most suitable condition. The amount of total-N extremely exceeds the sum of ammonia-N and amino-N. The difference may be (at least partly) ascribed to the presence of succinamic acid.

Isolation of Oxidation Products. Fifty grams of glutamic acid was dissolved in 600 c.c. of N.-sulphuric acid. The solution was placed in two equal rectangular glass vessels connected in series, each of them provided with an ebonite lid carrying a condenser and a thermometer. The solution was electrolysed with a lead peroxide anode (5.6×7.4 cm.) and a lead cathode (5.6×7.4 cm.) under the following conditions: the distance between the anode and the cathode, 3 cm.; C.D., 2 amp./dm^2.; current quantities, 55.8 amp. hours; temperature, 25°C. After the electrolysis, the nitrogen distribution in the solution was determined.

Nitrogen Distribution.

Total-N	98.6%
Ammonia-N (MgO)	87.0
Ammonia-N (30% NaOH)	3.9
Amino-N (van Slyke)	6.2

The ammonia-N (MgO) was determined by distillation over magnesium hydroxide under reduced pressure, and an excess of 30% NaOH was added to the residue. To estimate ammonia-N (NaOH) it was distilled under ordinary pressure, and the distillate was received in N.-sulphuric acid. This nitrogen represents the amount of succinamic acid.

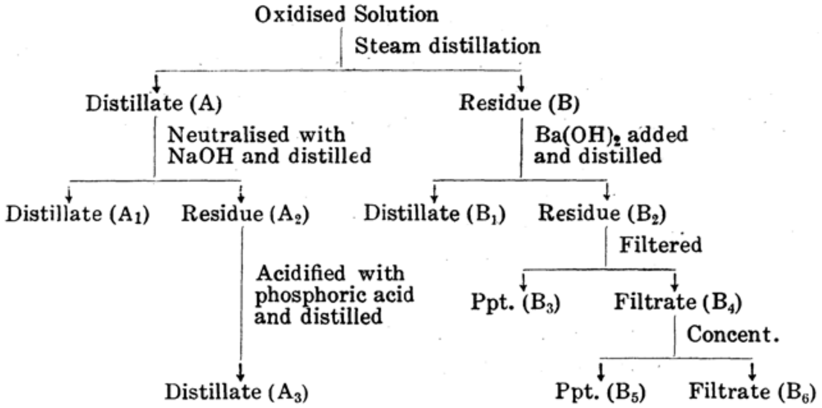
The oxidised solution was first submitted to steam distillation, the distillate (A) was neutralised with sodium hydroxide and redistilled. The first portion of the distillate (A_1) gave no iodoform reaction, and reduced neither Fehling's solution nor Tollens' reagent, but it gave only faint red colouration with fuchsin reagent. The original solution also showed no aldehyde reaction.

Volatile Acid.—Acetic Acid. The neutralised residue (A_2) was evaporated to dryness, an excess of syrupy phosphoric acid added, and it was redistilled. The volatile acid, obtained from the distillate (A_3), was identified as acetic acid by both ferric chloride and acetic ether reactions, and by its silver salt.

Anal.: Subst. = 0.2352; Ag = 0.1521 gr. Found: Ag = 64.66%.
Calc. for $C_2H_3O_2Ag$ (silver acetate): Ag = 64.64%.

The Volatile Base.—Ammonia. After making the residue (B) alkaline with barium hydroxide, it was submitted to distillation under reduced pressure, and a large quantity of ammonia was received in dilute hydrochloric acid (B_1). The ammonium chloride (8 gr.) thus obtained was identified as its platinic double chloride. Other bases were not present.

Anal: Subst. = 0.0983; Pt = 0.0432 gr. Found: Pt = 43.94%.
Calc. for $(NH_4)_2PtCl_6$ (ammonium chloroplatinate): Pt = 43.96%.



Succinic Acid.—The residue (B_2) was filtered. The precipitate (B_3), consisting of barium hydroxide, barium sulphate and organic salts of barium, was treated with sulphuric acid to remove barium, and from the concentrated filtrate free succinic acid crystallised out. The filtrate (B_4) was concentrated and barium succinate (B_5) was obtained which was then converted into free acid as usual. The joined yield was 18 gr. from 50 gr. of glutamic acid. It melted at 183°C. and the melting point showed no lowering when mixed with pure succinic acid.

Anal.: Subst. = 0.1437 gr.; Required N./10-NaOH = 24.27 c.c.
Calc. for $C_4H_6O_4$ (succinic acid): N./10-NaOH = 24.33 c.c.
Subst. = 0.1010; CO_2 = 0.1502, H_2O = 0.0464 gr.
Found: C = 40.56; H = 5.14%.
Calc. for $C_4H_6O_2$ (succinic acid): C = 40.63; H = 5.12%.

The mother liquor (B_6) of barium succinate, free from barium and sulphuric acid, was concentrated when it yielded glutamic acid, its hydrochloride decomposing at 205–206°C.

Succinamic Acid.—Fifty grams of glutamic acid was dissolved in 560 c.c. of N -sulphuric acid and oxidised until one-half of the glutamic acid has been oxidised, the conditions being similar to those in previous cases of oxidation. The current applied amounted to 30.5 amp. hours.

The nitrogen distribution in the oxidised solution was as follows:—

Nitrogen Distribution.

Total-N	97.1%
Ammonia-N (MgO)	43.4
Ammonia-N (NaOH)	9.5
Amino-N (van Slyke)	44.0

The solution was made up to 1000 c.c. by adding water, and to isolate succinamic acid, half of the solution was neutralised with barium carbonate and barium hydroxide. The precipitate thus formed was filtered. The filtrate was concentrated to about 30 c.c. under reduced pressure at 50°C. and barium succinate formed was filtered. The filtered solution was treated with sulphuric acid to remove barium, and an excess of calcium hydroxide was added. Ammonia was driven off by distillation under reduced pressure and glutamic acid was recovered as its calcium salt.

After removing calcium as oxalate, the filtrate was concentrated to a syrup. The syrup was extracted with acetone. The crystals, formed after removal of acetone from the extract, was treated with ether to remove succinic acid, and it was recrystallised from acetone in colourless plate (0.7 gr.), m.p. 154–155°C. It was identified as succinamic acid(8), by the determination of nitrogen, mixed melting point test and conversion into silver salt.

Anal.: Subst. $= 0.1253$ gr.; $NH_3 = 10.65$ c.c. of N./10-H_2SO_4.
Found: $N = 11.91\%$.
Calc. for $C_4H_7O_3N$ (succinamic acid): $N = 11.96\%$.
Anal.: Subst. $= 0.0498$; $Ag = 0.0239$ gr. Found: $Ag = 48.0\%$.
Calc. for $C_4H_6O_3NAg$ (silver succinamate): $Ag = 48.18\%$.

Electrolytic Oxidation of Succinic Acid.—Separation of Acetic Acid. A solution of 20 gr. of succinic acid in 500 c.c. of N.-sulphuric acid was electrolysed with a lead peroxide anode and a lead cathode under the following conditions: anodic current density 2 amp./dm^2., temperature 25°C., current quantity 20.0 amp. hours. The oxidised solution was submitted to steam distillation. The distillate gave neither aldehyde nor

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(8) *Ann.*, **134** (1865), 136; **162** (1872), 174.

ketone reactions. It was converted into sodium salt, dried, treated with phosphoric acid and distilled. Acetic acid was obtained from the distillate. It was identified as its silver salt.

Anal.: Subst. = 0.1359; Ag = 0.0877 gr. Found: Ag = 64.55%.
Calc. for $C_2H_3O_2Ag$ (silver acetate): Ag = 64.64%.

In conclusion, the author expresses his hearty thanks to Prof. K. Matsubara for his kind inspection of this paper.

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