

Studies on Amino-Acids and Related Compounds. XII.* Electrolysis of Phenylalanine and its Derivatives in Nitric Acid.

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In part V⁽¹⁾ it was stated that phenylalanine and tyrosine were readily oxidised at the anode in dilute sulphuric acid and resulted the rupture of benzene nucleus as well as the formation of humin-like substance. In the present paper the results of the electrolysis of phenylalanine and its derivatives in nitric acid are described.

Few reports on the electrolysis of organic compounds in nitric acid were found. Dunbrook and Lowy (1924)⁽²⁾ have obtained *p*-nitrobenzoic acid and *p*-chlorobenzoic acid from *p*-nitrotoluene and *p*-chlorotoluene respectively by the electrolysis in 20% nitric acid. Fichter and Plüss (1932)⁽³⁾ have reported on the formation of nitronaphthalene by the electrolysis of naphthalene in nitric acid.

Although phenylalanine and tyrosine, possessing NH₂-, HO-, and -COOH groups, have a more complicate constitution than nitrotoluene, the present experiments were carried out to find the conditions for preventing the rupture of benzene nucleus even in electrolysis.

Phenylalanine was dissolved in 4–13.7 N nitric acid and electrolysed in an undivided cell with platinum electrodes at 0°–100°C. under the following conditions: 4–10 amp./dm²., 2–7 F./mol.

The results of the electrolysis of phenylalanine in nitric acid depend upon the concentration of nitric acid and the temperature of the cell. In the case of 4–13.7 N nitric acid, a great difference was observed between

* Translated by the authors from *J. Chem. Soc. Japan*, **59** (1938), 1375.

(1) Y. Takayama, this Bulletin, **8** (1933), 178.

(2) R. F. Dunbrook, and A. Lowy, *Trans. Amer. Electrochem. Soc.*, **45** (1924), 81.

(3) Fr. Fichter and E. Plüss, *Helv. chim. Acta*, **15** (1932), 236.

the experiments at 0°–35°, and those at 50°–100°C. The electrolysis at 0°–35°C. proceeded similarly as the electrolysis in dilute sulphuric acid, and the electrolyte gradually assumed a brown colour which was probably due to the formation of melanin-like substance. From the electrolysate no crystalline substance was isolated. On the contrary, at 50°–100°C. the behavior differed markedly from the fact described above. As an example, the result of the experiment under the following conditions: 100°C., 4 amp./dm²., 8 N-NO₃H, will be given. After the application of the electric quantity of about 1 farady per mol, nitrogen dioxide gas began to evolve from the electrolysate. At about 2 faradies per mol, the gas evolution decreased, and yellow crystals of *p*-nitrobenzoic acid gradually deposited. Also in the case of 50° and 60° the same phenomena were observed, but the yields of *p*-nitrobenzoic acid were inferior to the case of 100°C.

Among the concentrations of 4 N, 8 N, and 13.7 N of nitric acid, the one of 8 N gave the best yield in obtaining *p*-nitrobenzoic acid, but it was somewhat less pure than that of 13.7 N. The 4 N one was inferior to 8 N and 13.7 N both in yield and in purity. Accordingly, for the preparation of *p*-nitrobenzoic acid, the use of 8 N nitric acid is recommended. As it will be easily seen from Table 3 and Table 4, the favourable conditions of obtaining *p*-nitrobenzoic acid may be the electric quantity: 3–5 F/mol., and the current density: 4–6 amps./dm.²

From the solution electrolysed at 50°–100°C, *p*-nitrobenzoic acid was separated as the main crystalline compound which was contaminated with a little of benzoic acid and oxalic acid, precipitated as calcium salt from the mother liquor of *p*-nitrobenzoic acid. From the solution electrolysed at 50°C., benzaldehyde was separated along with *p*-nitrobenzoic acid and identified as *p*-nitrophenylhydrazone.

For ascertaining whether the formation of *p*-nitrobenzoic acid from phenylalanine was caused by electrolysis or by merely heating with nitric acid, a solution of phenylalanine in 8 N-NO₃H was kept at 100°C. for the time corresponding to the application of 5 F/mol, but without passing electric current, and it was found that a little of *p*-nitrophenylalanine was obtained, but none of *p*-nitrobenzoic acid. Thus it was confirmed that the formation of *p*-nitrobenzoic acid was caused by the electrolysis.

p-Nitrophenylalanine was electrolysed in the same way as in the case of phenylalanine. *p*-Nitrobenzoic acid was obtained in a good yield and oxalic acid also separated. The electrolysis of benzoic acid as well as *p*-nitrobenzoic acid in nitric acid was performed, but only a small amount of oxalic acid was separated in both cases.

From these facts, the formation of *p*-nitrobenzoic- and benzoic acids from phenylalanine may have taken place as follows:

Phenylalanine changed into *p*-nitrophenylalanine by nitration, and the latter was electrolytically oxidised to *p*-nitrobenzoic acid. Benzoic acid was not an intermediate product in the course of formation of *p*-nitrobenzoic acid, but arose from the part of phenylalanine, unaffected by nitration.

As to the formation of nitronaphthalene by the electrolysis of naphthalene in nitric acid, Fichter and Plüss⁽³⁾ pointed out that it was ascribed to the increase of the total concentration of nitric acid in the anode compartment by electrolysis and to the temperature increase at the anode surface, but not to the increased concentration of nitric acid on

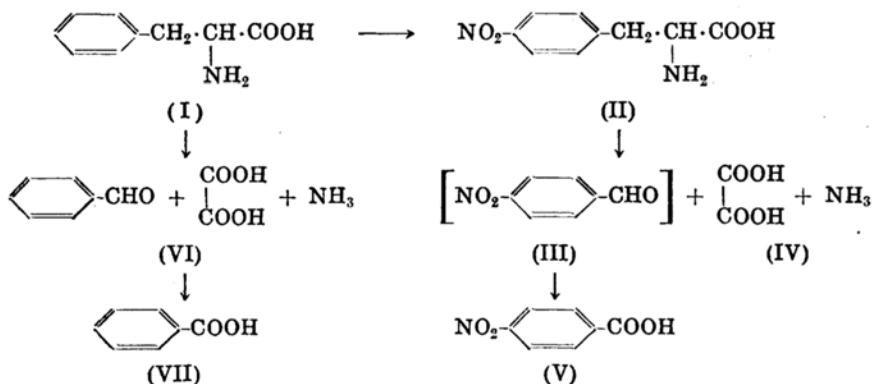
the anode surface. On the contrary, Calhause and Wilson⁽⁴⁾ have concluded from their experiments that the formation of nitronaphthalene by electrolysis was brought about by the highly concentrated film on the anode surface (within diffusion layer).

Though in the present experiments, the mechanism of electrolytical nitration of phenylalanine can not be elucidated, it may be inferred from our experimental results that the nitration of phenylalanine at 100°C. was of partly chemical, and partly electrolytical nature. By the electrolysis of phenylalanine and *p*-nitrophenylalanine in nitric acid, oxalic acid was obtained in yields of 21.8* and 15.5% respectively, while in case of benzoic acid and *p*-nitrobenzoic acid, the yields in oxalic acid was 5.1 and 2.5%, respectively. Thus it may be deduced from these facts that oxalic acid was produced principally by electrolytical oxidation of the side chain.

From the oxidised solution, ammonia was separated only in a small quantity which was probably due to its further decomposition⁽⁵⁾ into nitrogen by the nitrous acid formed during electrolysis.

Phenyllactic acid was electrolysed under the same conditions as phenylalanine, obtaining *p*-nitrobenzoic- and benzoic acids as well as oxalic acid.

Summarizing the facts described above, it may be concluded that the electrolysis of phenylalanine in the nitric acid solution proceeds as follows:



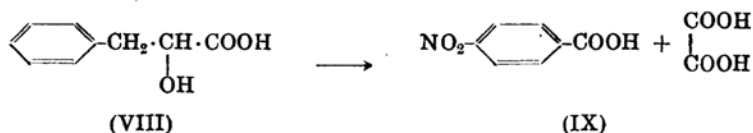
Phenylalanine (I) gave rise to *p*-nitrophenylalanine (II) by nitration, the latter was oxidised to *p*-nitrobenzaldehyde (III), oxalic acid (IV), and ammonia. The aldehyde was easily oxidised to *p*-nitrobenzoic acid (V). This course of reaction seems to be the principal one, considering the amount of the final products. As a side reaction, phenylalanine was oxidised to benzaldehyde (VI), oxalic acid, and ammonia, then the benzaldehyde was oxidised to benzoic acid (VII).

In the same way, phenylacetic acid (VIII) also produced *p*-nitrobenzoic acid (IX) instead of *p*-nitrophenylacetic acid which was expected.

* The percentages in this paper were expressed in molar per cent.

(4) Calhane and Wilson, *Trans. Amer. Electrochem. Soc.*, **63** (1933), 247.

(5) Abbe's Handbuch d. anorg. Chemie, III, (1907), 303.



Experimental.

Natural phenylalanine (N, 8.35. Calc. N, 8.49%) was dissolved in nitric acid and electrolysed in the following apparatus (Fig. 1). Cell: a glass cylinder (A), 14 cm. in height, dia. 3 cm., provided with a glass stopper (B) which carries two electrodes (E). A condenser was fused to the cell.

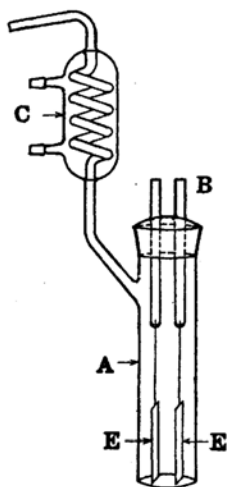


Fig. 1.

Electrode (E): both anode and cathode, platinum plates (2×4 cm.), distance between electrodes: 1 cm.

Electrolytic solution: phenylalanine (20 m. mols) was dissolved in 4–13.7 N NO_3H (30 c.c.).

Current density: 4–10 amps./dm.². Temperature: 0°, 35°, 50°, 60°, 100°C. Electric quantity: 2–7 F/mol. Volt: ca. 1.1 volts (4 amps./dm.²).

Preliminary experiment. (1) *Effect of temperature.* When the electrolysis was undertaken at 0° or 35°C., *p*-nitrobenzoic acid was not obtained, but the colour of the electrolytic solution gradually changed from reddish brown into brown, as the electrolysis proceeded. When the electrolysis was undertaken at above 50°C., *p*-nitrobenzoic acid separated out in yellow crystalline form, and the solution assumed yellow colour. The yields of *p*-nitrobenzoic acid obtained under the different conditions are shown in Tables 1–4.

Table 1.

(C. D.: 4 amps./dm.²).

Expt. No.	Phenylalanine (g.)	Temperature °C.	Conc. of NO_3H (N)	Electric quantities (F/mol.)	<i>p</i> -Nitrobenzoic acid		Melt. point °C.
					(g.)	(%)	
1	3.284	0	6	9	—	—	—
2	3.304	35	4	11	—	—	—
3	5.500	50	8	7 $\left\{ \begin{array}{l} 5\text{F} \\ 2\text{F} \end{array} \right.$	1.4 (oil) 0.45		190
4	3.298	60	8	5	0.91	17.2	190
5	3.297	100	4	7	0.48	14.2	170–183
6	3.332	100	6	7	1.14	33.8	190
7	3.319	100	8	7	1.74	51.4	185

In the above table NH_3 /total-N. of expt. (1) was 20.3%. whereas that of expt. (6) was 1.0%.

(2) *Effect of the concentration of nitric acid.*

Table 2.

(100°, 4 amps./dm².).

Phenyl-alanine (g.)	Conc. of NO_3H (N)	Temp. °C.	Electric quant. (F/mol.)	<i>p</i> -Nitrobenzoic acid		Melt. point. °C.
				(g.)	(%)	
3.297	4	100	7	0.48	14.2	178-183
3.332	6	100	7	1.14	33.8	190
3.319	8	100	7	1.74	51.4	185
3.307	8	100	5	1.32	39.2	190
3.395	13.7	100	7	1.15	33.2	227-229
3.266	13.7	100	5	1.12	33.7	232-234

(3) *Effect of electric quantity.*

Table 3.

(100°, 4 amps./dm²., 13.7 N- NO_3H).

Phenyl-alanine (g.)	Electric quantity (F/mol.)	<i>p</i> -Nitrobenzoic acid		Melt. point. °C.
		(g.)	(%)	
5.50	2	1.80	32.4	231-234
6.60	3	2.25	33.7	231-233
3.27	5	1.12	33.9	232-234
3.31	7	1.15	34.4	227-229

(4) *Effect of current density.*

Table 4.

(100°, 8 N- NO_3H , 5 F/mol.).

Phenyl-alanine (g.)	C. D. (amps./dm ² .)	<i>p</i> -Nitrobenzoic acid		Melt. point. °C.
		(g.)	(%)	
3.31	4	1.32	39.4	190
3.30	6	1.93	57.8	200
5.50	8	1.98	35.6	190-195
3.30	10	0.85	25.5	190

Separation of oxidation products. *p*-Nitrobenzoic acid. Phenyl-alanine (3.207 g., 19.43 m. mol) was dissolved in 8 N- NO_3H (30 c.c.) and

electrolysed under the conditions: 100°C., 4 amps./dm.² In a short time nitrogen dioxide gas began to evolve from the electrolysed solution. After applying the electric quantity of 2 F/mol, crystals began to deposit and increased gradually in quantity. When the electric quantity reached to 5 F/mol, the electrolysate was cooled. The crystals deposited in a large quantity, were filtered. The crystals had an odour of benzaldehyde and melted at 180–188°C. Yield: 1.53 g. The crystals were refluxed with benzene. The part insoluble in benzene gave 0.8 g. and melted at 205–210°C. The yield was 24.6% as *p*-nitrobenzoic acid. It melted at 236–237°C., after it was recrystallised from water (Found: N, 8.47, 8.57. Calc. for C₇H₅O₄N:N, 8.38%; Found: molecular weight, 167.7, 170.6 (Rast). Calc. for C₇H₅O₄N, 167.04).

Ethyl p-nitrobenzoate. A solution of *p*-nitrobenzoic acid (1 g.) in absolute ethyl alcohol (10 c.c.) was saturated with hydrogen chloride and refluxed for four hours. The crystals of the ester obtained amounted to 1.08 g. Yield: 93%. It melted at 57–57.5°C., after it was recrystallised from alcohol (Found: N, 7.08, 7.28. Calc. for C₉H₉NO₄: N, 7.18%).

The part soluble in benzene was evaporated and the residue was sublimed under reduced pressure obtaining 0.2 g., m.p. 120–121°C. It was found to be benzoic acid by the mixed melting point test and by the analysis of its silver salt (Found: Ag, 46.77. Calc. for C₇H₅O₂Ag: Ag, 47.12%).

Oxalic acid. The filtrate of *p*-nitrobenzoic acid was neutralized with sodium hydroxide and calcium oxalate was precipitated by the addition of calcium chloride solution. The precipitate amounted to 0.76 g. (air dried) and its purity was 81.4% determined by the titration with N/10-KMnO₄. It was dissolved in 50 c.c. of dilute hydrochloric acid (1:1) and thoroughly extracted with ether. After evaporating the ethereal solution, the crystalline residue was obtained, 0.4 g. (3.2 m. mols), m.p. 95–98°C. One part of the crystals was recrystallised from water in prisms, m.p. 101–102°C. (5.65 mg. of the acid required 8.93 c.c. of N/10-NaOH. Calc. for C₂H₄O₂·2H₂O: N/10-NaOH, 8.96 c.c.). The other part of the acid was sublimed, and it melted at 185.5°C.

Ammonia. The filtrate of the calcium oxalate was acidified with hydrochloric acid and distilled. From the distillate benzoic acid was obtained, 0.08 g. (0.7 m. mol). To the residue of distillation an excess of magnesium oxide was added and distilled under reduced pressure. The distillate was received in dilute sulphuric acid, 4.03 mg. (NH₃). The sulphate thus obtained was changed into its chloride as usual. By the observation under polarized microscope, it was identified as ammonium chloride: isotropic form, index of refraction $n = 1.641$ (chloronaphthalene-bromonaphthalene), its chloroplatinate: isotropic form.

Electrolyses. (1) Phenylalanine in 13.7 N-NO₃H. Electrolysis was undertaken under the following conditions and the electrolysate was treated in the same manner as in the case of 8 N-NO₃H.

Phenylalanine 3.261 g. (19.75 m. mols), 100°C., 4 amps./dm.², 5 F/mol.

Oxidation products:

p-Nitrobenzoic acid 1.11 g. (6.65 m. mols), m.p. 232–233°C. After

being recrystallised from water it melted at 235°C. Benzoic acid (0.02 g.), m.p. 121–122°C. Oxalic acid, pure calcium oxalate $C_2O_4Ca \cdot H_2O$, 3.92 m. mols, free acid m.p. 101–102°C. Ammonia, 0.54 mg.

(2) *Phenylalanine in 4N-NO₃H*. Electrolysis was undertaken as described above: phenylalanine 20 m. mols, 100°C., 5 amps./dm², 5 F/mol.

Oxidation products: *p*-nitrobenzoic acid, 0.40 g. (2.5 m. mols), m.p. 226–228°C. benzoic acid, 0.12 g. (1 m. mol), m.p. 121–122°C. oxalic acid, 2.3 m. mols, ammonia, 4.6 mg. (0.27 m. mol).

Formation of benzaldehyde and its identification. Phenylalanine (3.3 g., ca. 20 m. mols) was dissolved in 8N-NO₃H (30 c.c.) and submitted to electrolysis under the following conditions: 50°C., 6 amps./dm², 2 F/mol.

During the electrolysis, a reddish brown oily substance separated out from the electrolysate. After cooling the electrolysate, *p*-nitrobenzoic acid contaminated with the oil was separated. The mixture was neutralised with sodium hydroxide and submitted to steam distillation. The upper part of the distillate consisted of an oily layer (0.8 c.c.) with aromatic odour. The oil was dissolved in glacial acetic acid and converted into *p*-nitrophenylhydrazone as usual. After being recrystallised from dilute ethyl alcohol, it melted at 191.5°C., coinciding with benzaldehyde-*p*-nitrophenylhydrazone (m.p. 191–192°). 0.45 g. of *p*-nitrobenzoic acid was obtained from the above-mentioned mixture.

(3) *p-Nitrophenylalanine in nitric acid*. *p*-Nitrophenylalanine $NO_2C_6H_4CH_2CH(NH_2)COOH$, $1.5H_2O$ *p*-Nitrophenylalanine (4.7 g., 20 m. mols) was dissolved in 8N-NO₃H (30 c.c.) and electrolysed: 100°C., 6 amps./dm², 5 F/mol. *p*-Nitrobenzoic acid was also obtained from *p*-nitrophenylalanine as phenylalanine. Yield: 1.14 g. (6.8 m. mols, 34%). It melted at 235–238°C. After being crystallised from water it melted at 236–237°C. From the filtrate oxalic acid was separated as calcium salt, free acid (3.1 m. mols), m.p. 101–102°C. (Found: N/10-KMnO₄, 5.09 c.c. Calc. for $C_2O_4H_2 \cdot 2H_2O$, N/10-KMnO₄, 5.12 c.c.). Ammonia: 1.52 mg. (0.09 m. mol).

(4) *p-Nitrobenzoic acid in nitric acid*. *p*-Nitrobenzoic acid (m.p. 238°C., 0.542 g., 3.25 m. mols) was dissolved in 8N-NO₃H (30 c.c.) and electrolysed: 100°C., 4 amps./dm², 5 F/mol. On cooling 0.51 g. of *p*-nitrobenzoic acid (236–237°C.) was recovered from the electrolysate. Thus *p*-nitrobenzoic acid was attacked only to the extent of 5.9%. Calcium oxalate ($C_2O_4Ca \cdot H_2O$) which separated amounted to 0.0121 g. (0.08 m. mol) and was identified as described above.

(5) *Benzoic acid in nitric acid*. Benzoic acid (m.p. 121–122°C., 2.628 g., 21.5 m. mols,) was dissolved in 8N-NO₃H (30 c.c.) at 100°C. and electrolysed: 4 amps./dm², 5 F/mol. *p*-Nitrobenzoic acid was not obtained, but 1.965 g. (16.1 m. mols) of benzoic acid was recovered on cooling. From the filtrate oxalic acid was separated as calcium salt, 0.175 g. (purity, 92.96%, 1.1 m. mols). It was identified as oxalic acid by the titration with N/10-KMnO₄ and by the mixed melting point test. (Found: N/10-KMnO₄, 17.31 c.c. Calc. for $C_2O_4H_2 \cdot 2H_2O$: N/10-KMnO₄, 17.46 c.c.).

(6) *Oxalic acid in nitric acid.* Oxalic acid (2.51 g., 19.91 m. mols) was dissolved in 8 N- NO_3H (30 c.c.) at 100°C . and electrolysed: 4 amps./ dm^2 , 3 F/mol. Oxalic acid was recovered as described above in the form of calcium salt. The purity of calcium salt was determined by the titration with N/10- KMnO_4 and it was found that the oxalic acid was attacked by the electrolysis to the extent of 95%.

(7) *Phenyllactic acid in nitric acid.* Phenyllactic acid (1.696 g., 10.21 m. mols) was dissolved in 8 N- NO_3H (30 c.c.) and electrolysed: 100°C ., 4 amps./ dm^2 , 5 F/mol. The crystals separated from the electrolysate on cooling, were filtered. Yield: 0.44 g. (2.6 m. mols), 25% as *p*-nitrobenzoic acid. These crystals were boiled with benzene and the part insoluble in benzene melted at $235\text{--}236^\circ$, after being recrystallised from water. It was identified as *p*-nitrobenzoic acid by the mixed melting point test. From the part soluble in benzene, benzoic acid (0.16 g.) was obtained which melted at $122\text{--}123^\circ\text{C}$. after sublimation. Oxalic acid was separated from the filtrate of *p*-nitrobenzoic acid as calcium salt (3.18 m. mols.). The free acid melted at $101\text{--}102^\circ\text{C}$. (Found: N/10- KMnO_4 , 9.62 c.c. Calc. for $\text{C}_2\text{O}_4\text{H}_2 \cdot 2\text{H}_2\text{O}$: N/10- KMnO_4 , 9.58 c.c.). From these experiments it was found that *p*-nitrobenzoic acid could be obtained from both phenylalanine and phenyllactic acid, but the latter was inferior to the former in yield.

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