STUDIES ON AMINO-ACIDS AND RELATED COMPOUNDS. PART VI. ELECTROLYTIC OXIDATION OF IMIDAZOLYL-PROPIONIC ACID.

By Yoshitaro TAKAYAMA.

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In the previous communications,[1] it was stated that pyrrolidone-carboxylic acid was easily oxidised at anode and yielded considerable amount of succinimide. This fact shows that this heterocyclic nucleus is particularly unstable towards anodic oxidation. Some amino-acids possess heterocyclic nuclei, which may undergo more or less individual change by the anodic oxidation. In the present paper, the study on imidazolyl-propionic acid is to be reported. Before trying some experiments on histidine, the above acid was selected, because it has similar structure as histidine, and differs only at one point, the lack of α-amino-group. It seems to be more plain in this case than in the case of histidine itself to investigate the mechanism of oxidation of imidazol nucleus.

As to the oxidation of imidazol derivatives, two reports are cited in the literatures. Wyss[2] oxidised imidazol with chromic acid and potassium permanganate. In the former case, no definite compound was isolated, while in the latter, formic acid was obtained. On the other hand Radziszewski[3] isolated oxamide and oxamic acid (?) when he oxidised imidazol with 3 per cent. solution of hydrogen peroxide.

Little definite information has been given relating to the electrolytic oxidation of heterocyclic compounds. Fr. Fichter and his collaborator[4] have electrolytically oxidised several compounds having pyrazolone nucleus (antipyrine and its allies) and obtained products which were not the same as those obtained by pure chemical methods. But in none of these cases the rupture of pyrazolone nucleus was found to occur.

Imidazolyl-propionic acid was oxidised under the same conditions as in the previous experiments. The temperature was kept at 30°C. by immersing the cell in the thermostat. The electrolysate assumed yellowish tint as the electrolysis proceeded, but the formation of melanin-like substances was not observed. The amount of electrity applied was about 6F./mol.

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(1) Y. Takayama, this Bulletin, **8** (1933), 125, 137.
(2), (3) Br. Radziszewski, *Ber.*, **17** (1884), 1289.
(4) Fr. Fichter and H. D. Montmollin, Helv. *chim. Acta*, **5** (1922), 256.

Nitrogen Distribution in the Electrolysate.

NH_3-N (MgO)	65.8 %
NH_3-N (NaOH)	12.3 %

From this result the presence of amide type compounds can be presumed. The electrolysate was thoroughly extracted with ether. Parts soluble (a) and insoluble (b) in ether were treated separately.

Part Soluble in Ether (a). When ether was expelled there remained a certain amount of crystalline residue, which was separated from a small bulk of the mother liquor. After recrystallisation from water, it melted at 184°C. and was found to be succinic acid by elementary analysis. The further crop* of this acid was obtained from (b) by other treatments. The joined yield of succinic acid amounted to 30.4% of imidazolyl-propionic acid used.

The mother liquor of succinic acid showed fuchsin reaction and silver mirror reaction. *p*-Nitrophenylhydrazone was prepared from it. The hydrazone, after recrystallisation from alcohol, melted at 172–174°C. The aldehyde was found to be succinic acid semialdehyde by the analysis of this hydrazone.

Part Insoluble in Ether (b). An excess of barium hydroxide was added to it and the solution was distilled under reduced pressure until the final distillate showed no reaction of ammonia. The distillate was received in hydrochloric acid, from which ammonia was obtained. It was confirmed by the analysis of its platinic double chloride. The residue was freed from barium, and concentrated under reduced pressure to a small bulk (c), then the estimation of urea was carried out by the following methods.

(1) Xanthydrol Method. The definite volume of the solution (c) was taken and the methyl alcohol solution of xanthydrol (Kahlbaum) $O<\begin{matrix}C_6H_4\\C_6H_4\end{matrix}>CHOH$ was added to it. Urea could be quantitatively precipitated as dixanthyl urea.

(2) Urease Method. Urease was added to the same definite volume. The amount of urea was estimated by the titration of ammonia formed by the enzymatic action of urease.

The results obtained from (1) and (2) fairly coincided with each other and the yield of urea was 1.3% of imidazolyl-propionic acid used. The result of nitrogen distribution shows that the total amount of the

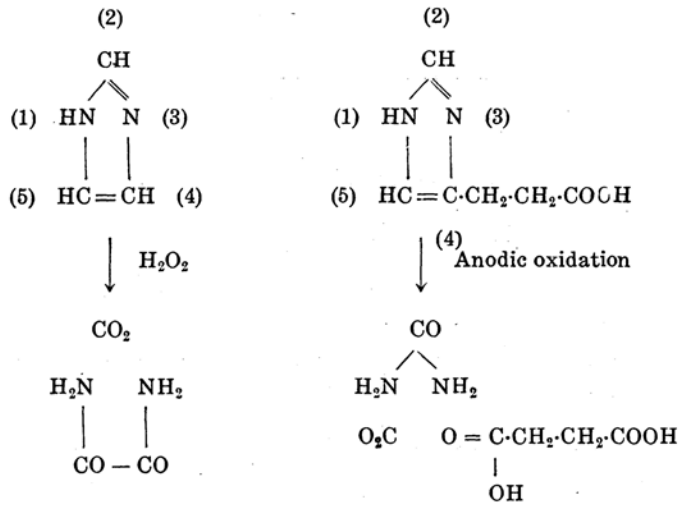
* Though sufficient quantity of ether was used, the extraction of succinic acid was not complete owing to its small solubility in that solvent.

amide compounds amounted to 12.5%. The discrepancy of the results obtained by different determinations may be ascribed to the presence of other compounds of amide type, such as oxamide or oxamic acid. But the project to obatin them from the solution was not successful. The difference is probably caused by the hydrolysis of urea during many treatments for its isolation.

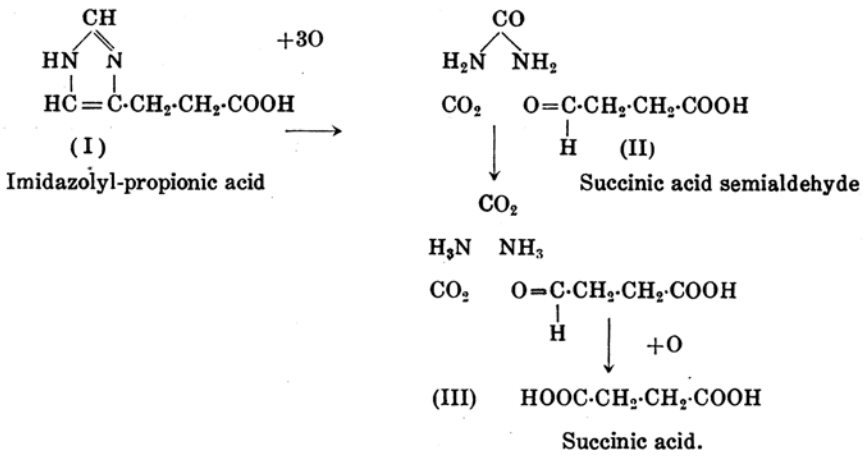
Remaining part of the solution (c) was concentrated to a syrup under reduced pressure and was extracted with hot isobutyl alcohol, and the crystals were obtained from the extract. It was found to be unchanged imidazolyl-propionic acid.

Mechanism of Oxidation of Imidazol Compounds.

As already mentioned[3] oxamide and oxamic acid were isolated by Radziszewski by the oxidation of imidazol. In this case it must be considered that the oxidation of imidazol took place at its carbon (2).



On the contrary, in the above anodic oxidation large amount of succinic acid together with little urea was isolated. This time the oxidation proceeded through another course, imidazol nucleus being attacked at carbon (5). It may be concluded that imidazol nucleus, on oxidation, behaves differently according to the kinds of oxidising agents (and probably to the presence or absence of the side chain). The mechanism of the anodic oxidation of imidazolyl-propionic acid may be represented as follows.



Succinic acid semialdehyde might as well be considered as the product of the secondary cathodic reduction of succinic acid, but the course of formation above cited was justified by the experiments reported in the previous communication.[5]

Experimental Part.

Imidazolyl-propionic acid[6]

Anal.: Subst. = 0.2347 gr. NH_3 = 33.00 c.c. of N./10-H_2SO_4,
Found: H = 19.70%.
Calc. for $C_6H_8O_2N_2$: N = 19.99%.

4.2 Gr. of imidazolyl-propionic acid (ca. 30 millimol) were dissolved in 100 c.c. of 2N.-sulphuric acid and electrolysed under the following conditions. Cell: without diaphragm. C.D. = 2 amp/dm^2. Electrodes (3×7 cm.): lead peroxide—lead. Electric quantity applied: 6.58 F/mol.

Distribution of Nitrogen. The electrolysate was filtered and made up to 125 c.c. 10 c.c. of the solution were taken for the following determinations,

(1) An excess of magnesia was added and distilled under reduced pressure.

(5) Y. Takayama, this Bulletin, **8** (1933), 125: Succinic acid was electrolytically oxidised under the same conditions as above. The electrolysate did not show characteristic reaction of aldehyde.

(6) S. Akabori, *J. Chem. Soc. Japan*, **52** (1931), 849. The sample of imidazolyl-propionic acid was kindly offered by Dr. Akabori and Mr. Numano, to whom the author is very grateful.

NH_3 = 31.68 c.c. of N./10-H_2SO_4	N = 44.2 mg.
total-N in 10 c.c. (calc.)	N = 67.2 mg.
NH_3-N(MgO)/total-N	65.8%.

(2) 30% sodium hydroxide solution was added to the residue and distilled again.

NH_3 = 5.82 c.c. of N./10-H_2SO_4	N = 8.28 mg.
total-N in 10 c.c. (calc.)	N = 67.2 mg.
NH_3-N(NaOH)/total-N	12.3%.

It was previously ascertained that imidazolyl-propionic acid did not yield ammonia when it was boiled with concentrated sodium hydroxide.

Succinic Acid. The oxidised solution was thoroughly extracted with ether. The colourless crystals were obtained from the extract. (0.576 gr.). After recrystallisation from water it melted at 184°C.

Anal.: Subst. = 0.0607; CO_2 = 0.0904; H_2O = 0.0275 gr.
Found: C = 40.61; H = 5.07%.
Calc. for $C_4H_6O_4$ (succinic acid): C = 40.63; H = 5.12%.

The residue of the extraction was evaporated to a syrup under reduced pressure and extracted with acetone. A crystalline residue (0.540 gr.) was obtained from the extract, it was found to be nothing else than succinic acid. The joined yield of succinic acid amounted to 1.116 gr. (9.45 millimol) representing 31.4% of imidazolyl-propionic acid used.

Succinic Acid Semialdehyde. The viscous mother liquor of succinic acid was kept over concentrated sulphuric acid under reduced pressure and crystallised succinic acid was filtered off. Following the description of Carrière,[7] to the filtered solution of *p*-nitrophenylhydrazine (0.5 gr.) in acetic acid (3 gr.) the aldehyde was added. Reddish yellow precipitate formed was collected and drained on porous plate. After repeated recrystallisation from alcohol, with addition of animal charcoal, it melted at 172–174°C.

The melting points of *p*-nitrophenylhydrazone of succinic acid semialdehyde given by many authors are as follows.

Alefeld[8]	175°C.	1909
Langheld[9]	174°C.	1909
Harries[10]	177°C.	1912
Dakin[11]	185–187°C. (when rapidly heated)	1917
Carrière	180–181°C.	1922

(7) Carrière, *Ann. chim.*, [9] **17** (1922), 75.
(8) Alefeld, *Ber.*, **42** (1909), 165, 1426.
(9) Langheld, *Ber.*, **42** (1909), 2371.
(10) Harries, *Ber.*, **45** (1912), 2585.
(11) Dakin, *Biochem. J.*, **11** (1917), 84.

The value obtained by the author (172–174°C.) was lower than any of the above. But the sample was too minute to try further recrystallisation.

Anal.:[12] Subst. = 7.264 mg.; NH_3 = 6.24 c.c. of N./70-H_2SO_4.
Found: N = 17.18%.
Calc. for $C_{10}H_{11}O_4N_3$: N = 17.7%.

Ammonia and Urea. An excess of barium hydroxide was added to the solution (b) and it was distilled under reduced pressure. The distillate was received in hydrochloric acid and the volatile base was collected as hydrochloride. It was found to be ammonia by the analysis of its platinic double chloride.

Anal.: Subst. = 0.1712; Pt = 0.0746 gr. Found: Pt = 43.6%.
Calc. for $(NH_4)_2PtCl_6$: Pt = 43.9%.

The excess of barium was removed exactly and the solution was concentrated to 50 c.c. under reduced pressure. Urea was estimated as follows.

(1) Xanthydrol Method.[13] 5 c.c. out of 50 c.c. (corresponding to 8/100 part of the total volume) were taken and poured into three parts of acetic acid, to which 3 c.c. of 7% methyl alcoholic solution of xanthydrol (Kahlbaum) were added. After the solution was allowed to stand overnight, the flocculent precipitate was collected on a filter. The dixanthyl urea obtained weighed 12.0 mg. corresponding to 1.71 mg. urea.

Anal.: Subst. = 8.169 mg.; NH_3 = 2.60 c.c. of N./70-H_2SO_4
Found: N = 6.36%.
Calc. for $C_{27}H_{20}O_3N_2$: N = 6.66%.

(2) Urease Method.[14] The same volume as in the above method was taken and it was neutralised. A buffer solution of phosphates and 0.1 gr. of urease (Squibb) were added to it. After the solution was kept at 40–50°C. for 15 minutes, an excess of sodium carbonate was added and the rapid current of air was passed through the solution, transferring the formed ammonia into N./70-H_2SO_4 (method of Folin). The same treatment was repeated without adding urease.

(a)	Urease added: N./70-H_2SO_4 6.74 c.c.		N = 1.348 mg.
(b)	Without urease: N./70-H_2SO_4 2.29 c.c.		N = 0.458 mg.
		diff.	N = 0.890 mg.
		calc. for urea	1.91 mg.

(12) As this compound contains nitro and hydrazo groups, it must be previously reduced. Zn and alcohol were chosen as reducing agent. See Hans Meyer's Methodik (1931) p. 426 and Weyl's Methoden, I (1921) p. 52.
(13) Fosse, *Ann. chim.*, (9), **6** (1916), 67.
(14) See Hoppe-Seyler, Thierfelder, „Physiologisch- und pathologisch-chemische Analyse" (1924), p. 702.

The urease used was ascertained beforehand not to contain an appreciable amount of ammonia. Therefore, ammonia formed in (b) may have been produced by the hydrolysis of urea during the concentration of the solution.

Imidazolyl-propionic Acid. The remaining part of the solution was concentrated to a syrup under reduced pressure and was extracted with hot isobutyl alcohol. The solvent was expelled and the residue was redissolved in a small bulk of water. A large amount of acetone was added to it. Colourless crystals were deposited on the wall of the beaker, which melted at 201–202°C. It was found to be imidazolyl-propionic acid by the determination of nitrogen and mixed melting point test.

Anal.: Subst. = 7.883 mg.; NH_3 = 7.90 c.c. of N./70 H_2SO_4.
Found: N = 20.04%.
Calc. for $C_6H_8O_2N_2$: N = 19.99%.

In conclusion, the author wishes to express his sincere thanks to Professor K. Matsubara for his kind encouragement and invaluable advices given to this study, and also to Mr. H. Ôeda for his untired assistance.

Chemical Institute, Faculty of Science,
Imperial University of Tokyo.