STUDIES ON AMINO-ACIDS AND RELATED COMPOUNDS. PART VIII. ELECTROLYTIC OXIDATION OF HISTAMINE AND HISTIDINE.

By **Yoshitaro TAKAYAMA** and **Haruomi ÔEDA.**

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In a previous communication,(1) it was stated that imidazolyl-propionic acid was readily oxidized at the anode and the rupture of imidazol nucleus resulted. In the present paper studies on histamine and histidine are reported and the mechanism of oxidation of these three kindred compounds are discussed. They have the same heterocyclic nucleus, and differ only in the functional grouping attached to the side-chain.

Among various studies on histidine, subjects allied to the present work are treated in the following communications.

In 1907 Knoop(2) reported on the oxidation reactions of histidine and its derivatives and in his study he established the constitution of this amino-acid. He oxidized imidazolyl-lactic acid $C_3H_3N_2\cdot CH_2\cdot CH(OH)\cdot COOH$ (I) with concentrated nitric acid and obtained imidazolyl-glyoxylic acid $C_3H_3N_2\cdot CO\cdot COOH$ (II) which was further oxidized to imidazol-carboxylic acid $C_3H_3N_2\cdot COOH$ (III) by the action of hydrogen peroxide in the presence of acetic acid. On the other hand, oxidation of (I) with barium permanganate yielded imidazolyl-acetic acid $C_3H_3N_2\cdot CH_2\cdot COOH$ (IV). He also stated that histidine $C_3H_3N_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$ itself yielded the same products (II and III) when oxidized with nitric acid, but it was not so readily attacked. In 1909 Langheld(3) found that imidazolyl-acetaldehyde $C_3H_3N_2\cdot CH_2\cdot CHO$ was a reaction product when histidine hydrochloride was oxidized with sodium hypochlorite. In 1916 Dakin(4) obtained cyanomethyl-imidazol $C_3H_3N_2\cdot CH_2\cdot CN$ by oxidizing histidine with toluene-*p*-sulphochloramide.

In none of these papers the rupture of imidazol nucleus is mentioned and no investigation is found on the electrolytic oxidation of this amino-acid.

The authors have first examined the oxidizability of histamine by the method already described(5) (Fig. 1), and compared it with those compounds

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(1) Y. Takayama, this Bulletin, **8** (1933), 189.

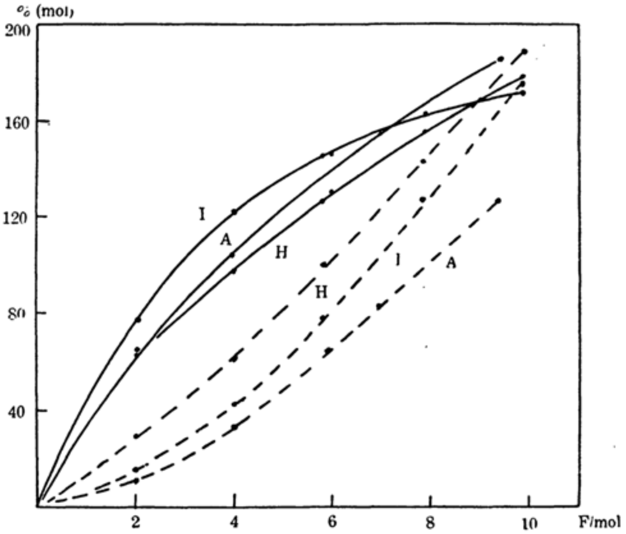
(2) Fr. Knoop, *Beitr. chem. Physiol. Pathol.*, **10** (1907), 111.

(3) K. Langheld, *Ber.*, **42** (1909), 2373.

(4) H. D. Dakin, *Biochem. J.*, **10** (1916), 319.

(5) Y. Takayama, this Bulletin, **8** (1933), 213.

previously dealt with. The curves representing the course of formation of carbon dioxide and ammonia from this amine by electrolysis are of the same type as those of imidazolyl-propionic acid and histidine. From this fact it can be easily expected that the courses of oxidation of these allied compounds do not show marked diversity. But they indicated obvious difference in one point, namely, in the colour of their electrolysates. The electrolysates of imidazolyl - propionic acid and histamine assumed · yellowish tint as the electrolysis proceeded and never turned to black until the end while that of histidine changed to black and considerable amount of a melanin - like substance separated.



NH_3-curves (——), CO_2-curves (- - -)
I = Imidazolyl-propionic acid.
H = Histidine.
A = Histamine.

Fig. 1.

Separation of Oxidation Products of Histamine. Histamine in dilute sulphuric acid solution was electrolyzed with a lead peroxide anode and a lead cathode, conditions being similar to those in the case of imidazolyl-propionic acid, current quantity applied 6 faradays per mol, and the temperature 30°C. The electrolysate was treated with phosphotungstic acid and *the precipitate (A) formed was separated from the filtrate (B).*

Precipitate (A). Ammonia and unchanged histamine were isolated as volatile and non-volatile bases respectively.

Filtrate (B). Urea was estimated in this part by urease method, the amount corresponding to 7.7%.* β-Alanine (8.5%) was also obtained from the concentrated solution on treatment with alcohol and ether and it was confirmed as such by converting it into α-naphthylisocyanate compound.

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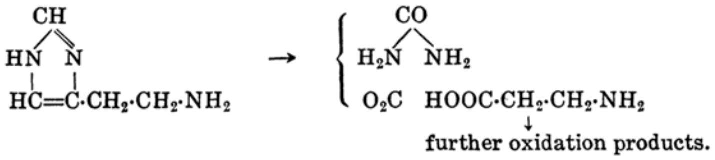
* Small amounts of urea (1.3%), and ammonia (1.0%) were also found in (A), thus total quantity of urea amounting to 9.3% and that of β-alanine 12.0%.

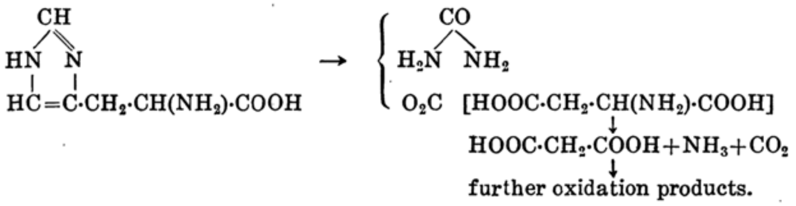
As β-alanine is said to be unstable to alkali,⁽⁶⁾ its amount can be presumed by the estimation of ammonia formed on treatment with caustic alkali. The amount of β-alanine thus found was 11.1%, under the assumption that no other substance yielding ammonia by this treatment was contained.

The Mechanism of the Reaction. The products obtained from histamine in the above oxidation comprise urea (9.0%), β-alanine (8.5%; 12.0%), ammonia, and carbon dioxide. Considering the isolation of these substances as the basis, the course of the oxidation can be explained as follows:



Separation of Oxidation Products of Histidine. Histidine was electrolyzed just in the same manner as above. A melanin-like substance formed during electrolysis was separated after long standing and its nitrogen content was found 13.64%. The electrolysate was treated with phosphotungstic acid as usual. Ammonia and urea (11.1%) together with unchanged histidine were isolated.

Urea was obtained in each of these three cases and it would result from the rupture of imidazol nucleus, and in two previous cases succinic acid semi-aldehyde together with succinic acid and β-alanine were found respectively as the other fragments. In the case of histidine, aspartic acid was expected by analogy to be the first oxidation product, but, in point of fact, it could not be isolated. On the other hand, a small quantity of malonic acid (about 3%)⁽⁷⁾ was found, which may be assumed to be produced by the further oxidation of aspartic acid. The oxidation of histidine may be represented as follows:



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(6) E. Abderhalden and A. Fodor, *Z. physiol. Chem.*, **85** (1913), 112.

(7) Malonic acid is also unstable to oxidation, hence, its poor yield. F. Lieben and E. Molnar, *Monatsh.*, **53** (1929), 7.

The electrolytic oxidation of these kindred compounds, namely, imidazolyl-propionic acid (I), histamine (II) and histidine (III) proceeded through a similar course, imidazol nucleus being attacked and yielding urea. Besides urea, (I) gave succinic acid semi-aldehyde and succinic acid, and (II) gave β-alanine, the same being the case with histidine (III). The latter gave, besides urea, aspartic acid, and this, being too unstable to remain unattacked, was completely oxidized, a part of the product remaining in its intermediate stage as malonic acid.

The formation of a melanin-like substance was restricted to one case, namely, histidine, and it may be concluded that the presence of the cyclic ring together with amino-group in the α-position to the carboxylic group is necessary for its formation.

EXPERIMENTAL PART.

Electrolytic Oxidation of Histamine. Histamine (synthesized)* which was used gave the following analytical result:

Found: N, 37.33. Calc. for $C_5H_9N_3$: N, 37.83%.

The course of formation of carbon dioxide and ammonia during electrolysis was determined after every definite consumption of electricity⁽⁸⁾ (Table 1).

Histamine (4.45g., about 40 milli-mols) was dissolved in 100 c.c. of 2N sulphuric acid and the solution was electrolyzed. Cell: without diaphragm; Electrodes: lead—lead peroxide (3.8 cm. × 5.5 cm.), distance 1.5 cm.; C.D.: 2 amp./dm.^{2}; Electric quantity applied 6 F./mol.

The electrolysate was thoroughly extracted with ether. A small quantity of oil insoluble in water was obtained from the extract, but its quantity was too minute for further investigation.

Table 1.

Current quantity (F./mol)	CO_2/Histamine (mol %)	NH_3/Histamine (mol %)
1.97	9.7	60.4
3.94	34.3	102.7
5.90	67.1	130.2
6.93	82.3	154.8
9.42	126.3	182.7

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* The sample of histamine was kindly offered by Mr. S. Numano, to whom the authors express their thanks.

(8) Cf. Y. Takayama, this Bulletin, **8** (1933), 213.

The residue of the extraction was diluted with 5% sulphuric acid and 20% phosphotungstic acid was added to it.

Precipitate (A). It was decomposed with barium hydroxide, the solution was distilled under reduced pressure at 60°C., and the distillate was received in hydrochloric acid. The hydrochloride of the base was not hygroscopic. It was extracted with cold absolute alcohol. Bases obtained from the extract and the residue were found to be ammonia by the analysis of their platinic double chlorides.

Found for the substance from the extract: Pt, 43.16. Found for the substance from the residue: Pt, 43.72. Calc. for $(NH_4)_2PtCl_6$: Pt, 43.93%.

After removal of barium exactly, the part containing non-volatile base was concentrated under reduced pressure, but it was impossible to bring it into crystallization. The concentrated bulk was treated with picric acid and the base precipitated as picrate. It amounted to 5% and melted at 227°C. after recrystallization from water, coinciding with histamine dipicrate. No other base was obtained from the filtrate of the picrate.

Found: N, 22.28. Calc. for $C_5H_9N_3 \cdot 2C_6H_3O_7N_3$: N, 22.15%.

Estimation of urea (urease method):

Solution taken: 1/20 part.* NH_3 = 3.58 c.c. of N/70 H_2SO_4 = 0.0512 milli-mol.

Calculating into total volume, NH_3 = 1.02 milli-mols, hence, urea = 0.510 milli-mol (1.3%).

Estimation of β-alanine (NH_3** formed on heating at 100°C. for ten minutes with excess of caustic soda):

Solution taken: 1/20 part. NH_3 = 1.46 c.c. of N/70 H_2SO_4 = 0.0209 milli-mol.

Calculating into total volume, NH_3 = 0.42 milli-mol (1.0%).

Filtrate (B). The solution, after removing barium with carbon dioxide and sulphuric acid, was concentrated to 100 c.c. by evaporation under reduced pressure. The amount of urea and β-alanine was determined.

Estimation of urea (urease method):

Solution taken: 1.00 c.c. out of 100 c.c. NH_3 = 4.28 c.c. of N/70 H_2SO = 0.0612 milli-mol.

Calculating into total volume, NH_3 = 6.12 milli-mols, hence, urea = 3.06 milli-mols (7.7%).

Estimation of β-alanine:

Solution taken: 1.00 c.c. out of 100 c.c. NH_3 = 3.07 c.c. of N/70 H_2SO_4 = 0.0439 milli-mol.

Calculating into total volume, NH_3 = 4.39 milli-mols (11.0%).

The remaining part of the solution was concentrated to a syrup, after decolourization with animal charcoal. The syrup was dissolved in dilute alcohol and an excess of ether was added to it until faint turbidity appeared.

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* It was confirmed by the same aeration method that no trace of free ammonia was present in the solution.

** It was ascertained that histamine itself does not yield an appreciable amount of ammonia by this treatment.

The turbidity of the solution soon disappeared, an oil depositing at the bottom, which changed to crystals after long standing. The yield amounted to 0.30 g. (8.5%). The crystalline substance was dissolved in a small bulk of water and recrystallized by adding alcohol. It melted at 198–200°C. coinciding with β-alanine.(9)

Found: N, 16.03. Calc. for $C_3H_7O_2N$: N, 15.73%.

β-Alanine was dissolved in twice its equivalent of N NaOH, diluted with four times of water, and α-naphthyl-isocyanate (1.2 equivalents) was added to the solution. After one hour, an excess of hydrochloric acid was poured, and α-naphthyl-isocyanate compound of β-alanine precipitated. After recrystallization from alcohol it showed effervescence at 196°C. and melted completely at 226–228°C.(10)

Found: N, 11.01. Calc. for $C_{14}H_{14}O_3N_2$: N, 10.86%.

Electrolytic Oxidation of Histidine. Histidine monohydrochloride (Schuhardt) used gave the following analytical results:

Found: N, 20.04; HCl, 16.94. Calc. for $C_6H_9O_2N_3 \cdot HCl \cdot H_2O$: N, 20.05; HCl, 17.39%.

Histidine monohydrochloride (2.95 g., about 15 milli-mols) was dissolved in 100 c.c. of 2N H_2SO_4. Sufficient water was added to the solution, and it was distilled under reduced pressure to drive off hydrochloric acid. This treatment was repeated several times until the distillate showed no trace of hydrochloric acid. The solution was made up to 100 c.c. and was electrolyzed. The current quantity applied was 8 F./mol.

The electrolysate assumed reddish tint (at 1 F./mol), then gradually changed to black, and a considerable amount of a melanin-like substance deposited on the wall of the vessel and its amount increased in the lapse of time even after the termination of electrolysis. After four months, it was separated, well washed, and analyzed after drying over quick lime. (Found: N, 13.64%.) It is amorphous black powder and readily soluble in alkali.

The electrolysate was diluted with 5% sulphuric acid and treated with phosphotungstic acid.

Precipitate (A). Ammonia was obtained as the volatile base.

Found for the chloroplatinate of the base: Pt, 43.87. Calc. for $(NH_4)_2PtCl_6$: Pt, 43.95%.

After removing barium, the part containing non-volatile base was concentrated to a small bulk. A small quantity of crystals was obtained from

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(9) P. Karrer and A. Widmer, *Helvetica Chim. Acta*, **9** (1926), 888.

(10) W. Linneweh, A. W. Keil, and F. A. Hoppe-Seyler, *Z. physiol. Chem.*, **183** (1929), 17.

it, which melted at 258–260°C. with effervescence, after recrystallized from dilute alcohol. It gave positive results in ninhydrine, biuret, and *p*-diazobenzene-sulphonic acid reactions, and its hydrochloride melted at 230–231°C. The base was presumed to be unchanged histidine.

Filtrate (B). After complete separation of barium, the solution was concentrated to 50 c.c. and urea was determined.

Identification of urea (xanthydrol method):

A part of the solution was diluted with glacial acetic acid and the filtered methyl alcoholic solution of xanthydrol (Kahlbaum) was added to it. Urea was precipitated as dixanthyl-urea.

Found: N, 6,74. Calc. for $C_{27}H_{20}O_3N_2$: N, 6.68%.

Estimation of urea (urease method):

Solution taken: 1.00 c.c. out of 50 c.c. $NH_3 = 4.67$ c.c. of N/70 $H_2SO_4 = 0.0667$ milli-mol. Calculating into total volume, $NH_3 = 3.34$ milli-mols, hence, urea $= 1.67$ milli-mols (11.1%).

Malonic acid: Histidine (25 milli-mols) was electrolyzed as described above. The electrolysate was thoroughly extracted with ether. After expelling ether, brown liquid was obtained from the extract and steam was passed through it to drive off volatile acid. The residue was decolourised and titrated with N/10 NaOH. It required 15.8 c.c., amounting to 0.79 milli-mol as a dibasic acid, which corresponds to 3.0%. The neutralized solution was concentrated and a little excess of N $BaCl_2$ was added to it. A barium salt was precipitated from it by adding 60 per cent. alcohol, which was found to be barium malonate⁽¹¹⁾ by the analysis after reprecipitation and drying at 100°C.

Found: Ba, 53.99. Calc. for $C_3H_2O_4Ba{\cdot}H_2O$: Ba, 53.33%.

The barium malonate obtained was dissolved in water and barium was removed as the sulphate. The crystals of the free acid were examined under polarization microscope. It showed oblique extinction, the angle of extinction being 12–13°,⁽¹²⁾ and resembled with a pure specimens of malonic acid under the gypsum-plate.

The present work has been carried out in the Chemical Institute, Faculty of Science, Imperial University of Tokyo, and the authors wish to express their sincere thanks to Prof. K. Matsubara for kind inspection of this paper.

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(11) C. Coulett, *J. prak. Chem.*, **73** (1906), 76.

(12) P. Groth, "Chem. Krystallographie," III (1910), 230.