STUDIES ON AMINO-ACIDS AND RELATED COMPOUNDS. PART VII. ON THE OXIDATION OF VARIOUS AMINO-ACIDS.

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Amino-acids occurring in nature can be represented by the general formula $R(NH_2)\cdot COOH$. R denotes diverse radicals of aliphatic, aromatic and heterocyclic fields, functional group of which varies from acidic, neutral to basic. In addition to these, the two compounds having imino-group, namely proline and oxyproline, are obtained from the hydrolysate of protein.

Many paper[1] have been published on the oxidation of amino-acids by various oxidising agents. But in almost all of these it is discussed merely on the first stage of oxidation (oxidation of α-amino-group), and the changes of the radical R are left untouched.

Recently F. Lieben and E. Molner[2] oxidised many amino-acids with a mixture of potassium bichromate and sulphuric acid and from the results of their oxidation they classified the amino-acids into three groups as follows:

(1) Glycine, leucine, aspartic acid, arginine, cystine, tyrosine and tryptphane.
(2) Alanine, glutamic acid and valine.
(3) Proline, histidine and lysine.

The author reported several studies on the electrolytic oxidation of seven kinds of amino-acids including some of their related compounds, and observed that at the equal consumption of electricity the amounts of decomposed amino-acids widely differed according to their kinds. This difference may be attributed to the relative oxidisability of the group R in the acid. Now the author intends to investigate more scrupulously the oxidation of these compounds by the following experiments.

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(1) A. Strecker, *Ann.*, **123**(1862), 363; K. Langheld, *Ber.*, **42** (1909), 392, 2360; H. D. Dakin, T. B. Cohen, M. Daufresne and J. Kenoyon, *Chem. Abstract*, **10** (1916) 2912; H. Wieland und E. Bergel, *Ann.*, **439** (1924), 196; S. Goldschmidt und W. Beuschel, *Ann.*, **447** (1926), 197.

(2) F. Lieben und E. Molnar, *Monatsh.*, **53-54** (1929), 1–13.

More than ten of the amino-acids having various structure were selected and they were electrolysed under the same conditions. During the course of electrolysis the amounts of carbon dioxide and ammonia produced were measured from time to time. The experiments were carried out with following substances, those with similar structure being taken in pair in each case.

Monoamino-carboxylic acids:

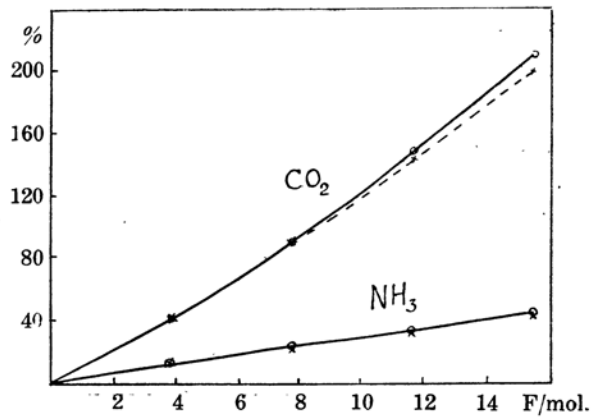
Glycine, alanine, valine, leucine (aliphatic);
Aspartic acid, glutamic acid (aliphatic dicarboxylic);
Tyrosine, phenylalanine (aromatic);
Proline, pyrrolidone-carboxylic acid (heterocyclic);

Diamino-carboxylic acids and others:

Lysine (aliphatic);
Tryptophane, imidazolyl-propionic acid and histidine (heterocyclic).

Electrolytic conditions. 10 Millimols of the sample are dissolved in 30 c.c. of 2N.-sulphuric acid. C.D.: 2 amp./dm^2., electrodes: lead peroxide anode and lead cathode.

Tyrosine and Phenylalanine. (Fig. I). The curves representing the course of formation of carbon dioxide and ammonia from these amino-acids nearly coincide with each other. The curves of tyrosine always lie slightly above those of phenylalanine. The same results were discussed in a previous paper.[3]

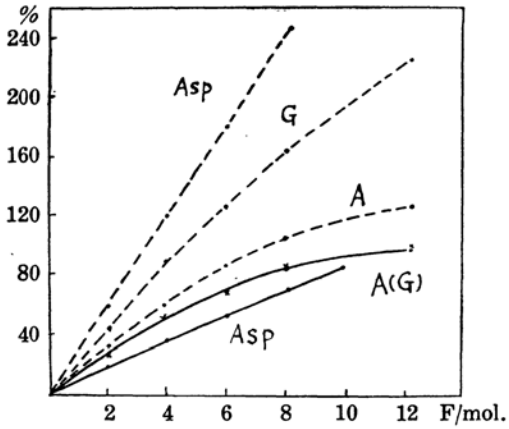


○ Tyrosine. × Phenylalaine.

Fig. I.

(3) Part V. This Bulletin, **8** (1933), 178.

Alanine,[4] Glutamic Acid[5] and Aspartic Acid. (Fig. II). NH_3-curves of the former two are in good coincidence, but some difference is observed between their CO_2-curves. This fact may be explained as follows. α-Oxidation of the two amino-acids proceeds at equal rate, and alanine gives acetic acid while glutamic acid gives succinic acid, and the latter is less stable to oxidation than the former. CO_2 formed in excess in the case of glutamic acid is due to the more readiness to oxidation of succinic acid. NH_3-curve of aspartic acid lies below the above two, and CO_2-curve, on the contrary, lies far above. Aspartic acid gives the highest yield of CO_2, which may be explained by the fact that its oxidation product (malonic acid) is markedly unstable towards oxidation.



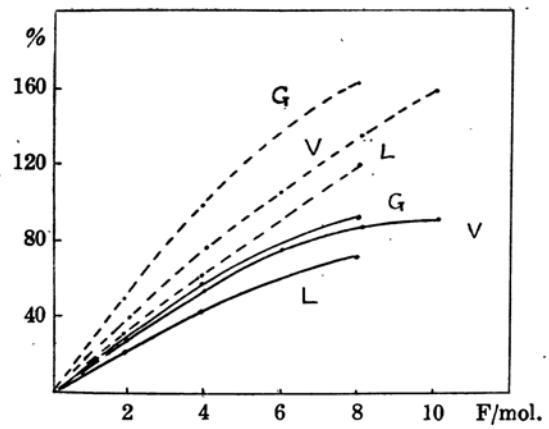
CO_2-curves (----), NH_3-curves (——).
Asp=aspartic acid, G=glutamic acid (×), A=alanine.

Fig. II.

Glycine, Leucine[4] and Valine. (Fig III). NH_3-curves of glycine and valine belong to the same type as that of alanine. In the case of leucine it lies considerably beneath that of alanine. CO_2-curves of these compounds lie above that of alanine. The result shows that the radical R in leucine is less stable to anodic oxidation as Fichter has pointed out.

$$(CH_3)_2CH\cdot CH_2CH(NH_2)COOH \rightarrow (CH_3)_2CH\cdot CH_2\cdot COOH + CO_2 + NH_3$$
$$\downarrow$$
$$(CH_3)_2CH\cdot COOH$$
$$\downarrow$$
$$(CH_3)_2CO \qquad \text{(Fichter)}^{(4)}$$

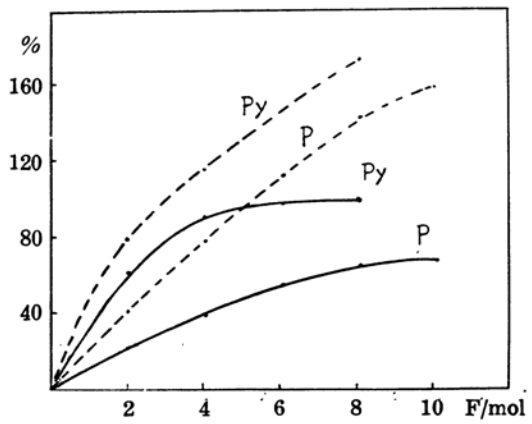
(4) Fr. Fichter und F. Kuhn, *Helv. chim. Acta*, **7** (1924), 167.
(5) Part. I. This Bulletin, **8**(1933), 125.



CO_2-curves (----), NH_3-curves (——).
G=glutamic acid, V=valine, L=leucine.

Fig. III.

Pyrrolidone-Carboxylic Acid[6] **and Proline.** (Fig. IV). NH_3-curve of the former occupies the highest position of those of all amino-acids, and that of proline is apart from it. But the author, in fact, isolated succinimide from the oxidation products of proline—the same product as

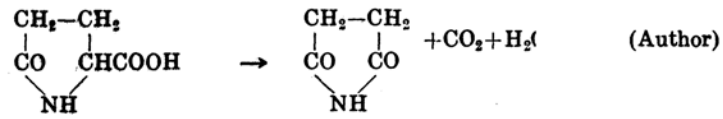


CO_2-curves (----), NH_3-curves (——).
Py=pyrrolidone-carboxylic acid, P=proline.

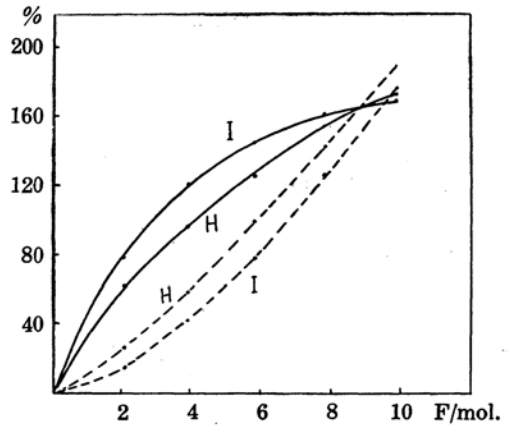
Fig. IV.

(6) Part. II. This Bulletin, **8** (1933), 137.

from pyrrolidone-carboxylic acid. The similar type of oxidation may proceed in these compounds, though somewhat different in rate.

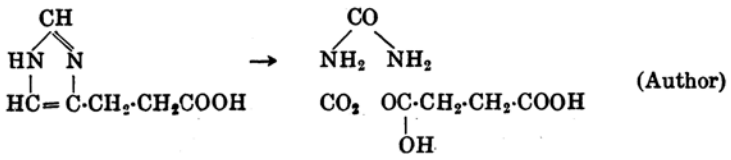


Histidine and Imidazolyl-Propionic Acid.[7] (Fig. V). NH_3-curves and CO_2-curves of these compounds present the similar type. On oxidation, these compounds are easily attacked at their imidazol nucleus, and it is observed that a considerable amount of urea was also formed from histidine. Further works on histidine and proline are now in progress.



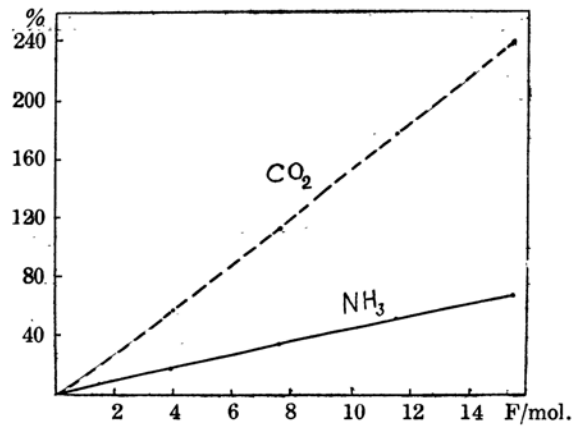
NH_3-curves (——), CO_2-curves (- - - -).
I=imidazolyl-propionic acid, H=histidine.

Fig. V.



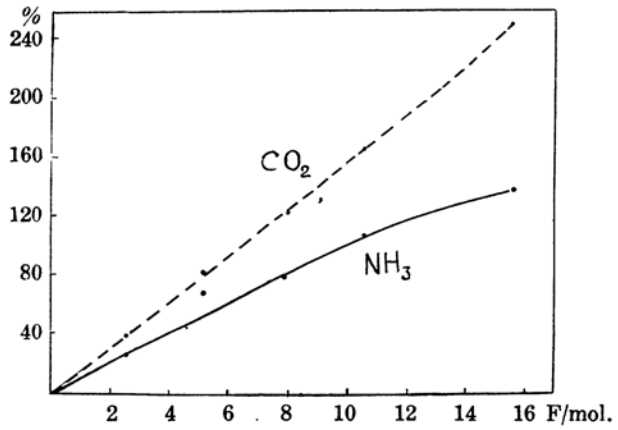
Tryptophane and Lysine. (Fig. VIa, VIb). CO_2-curves of these compounds lie not far away from each other.

(7) Part VI. This Bulletin, 8 (1933), 189.



Tryptophane.

Fig. VIa.



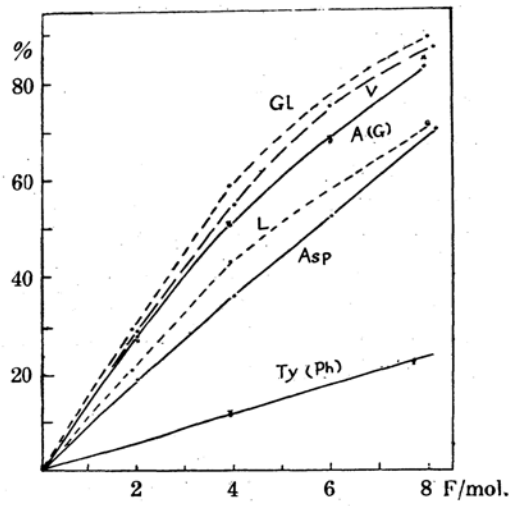
Lysine.

Fig. VIb.

Comparison of the Oxidisability of Monoaminocarboxylic Acids. (Fig. VII). By taking NH_3-curves as a criterion of oxidisability, all amino-acids can be arranged in the following order and divided into three groups.

I) Glycine, valine, glutamic acid and alanine.
II) Leucine and aspartic acid.
III) Tyrosine and phenylalanine.

The members of the first group give curves which are close together, and may be regarded as typical. Curves of the compounds of the third group are completely superposed. Those of the second group are located between the two groups.



Gl=glycine, V=valine, A=alanine, G=glutamic acid (×).
L=leucine, Asp=aspartic acid, Ty=tyrosine, Ph=phenylalnine (×).

Fig. VII.

Colours of the Electrolysates. The electrolysates of some amino-acids are colourless, while those of others are coloured.

(A) Colourless:
Glycine, alanine, valine, leucine, aspartic acid, glutamic acid and pyrrolidone-carboxylic acid.

(B) Coloured, but formation of melanin-like substance is not observed:
Lysine (faint yellow), proline (light brown then colourless).

(C) Dark coloured, and a black melanin (humin) is formed after a certain times:
Tyrosine, phenylalanine (red, then turning to dark brown), tryptophane (dark yellowish brawn), histidine (dark purple).

It is a well known fact that during the hydrolysis of protein a black amorphous powder named humin is formed. According to Roxas[8] following amino-acids have a share in humin formation during the hydrolysis of protein; namely tryptophane, tyrosine, cystine, lysine, arginine, histidine and proline (under a certain condition). Author's results fairly agree with that of Roxas, thus, substances which are classified under (B)

(8) M. L. Roxas, *J. Biol. Chem.*, **27** (1916), 71.

and (C) groups are all those mentioned by Roxas as amino-acids that form humin.

It is cited by many authors that the amino-acids, on oxidation, yield corresponding aldehydes having one atom of carbon less. But in the author's experiments carbonyl compounds are not isolated except in one case, namely imidazolyl-propionic acid. It may be considered that the carbonyl compounds perhaps once formed are soon further oxidised to acids by the vigorous action of lead peroxide anode.

The results of the communications hitherto published may be briefly summarised as follows. Aliphatic α-amino-acids first undergo α-oxidation and produce corresponding aldehydes, which are at once converted into acids. Through further oxidation various products are obtained according to the difference of R-groups. As for aromatic and heterocyclic α-amino-acids, their behaviour on electrolytic oxidation are complicated, and specific groups R are easily attacked. Moreover, these amino-acids produce melanin or humin-like substances.

Experimental Part.

Amino-acids used in this experiment were as follows:

l-Tyrosine, N = 7.76% (calc. for $C_9H_{11}O_3N$, N = 7.74%).
l-Phenylalanine, N = 8.47% (calc. for $C_9H_{11}O_2N$, N = 8.48%).
dl-Alanine (Kahlbaum), N = 15.75% (calc. for $C_3H_7O_2N$, N = 15.73%).
d-Glutamic acid, N = 9.36% (calc. for $C_5H_9O_4N$, N = 9.50%).
Glycine (Merck), N = 18.58% (calc. for $C_2H_5O_2N$, N = 18.67%).
(Kahlbaum) N = 18.66%.
dl-Valine (Eastman), N = 12.27% (calc. for $C_5H_{11}O_2N$, N = 11.96%).
l-Leucine, N = 10.84% (calc. for $C_6H_{13}O_2N$, N = 10.69%).
dl-Aspartic acid (Kahlbaum), N = 10.68% (calc. for $C_4H_7O_4N$, N = 10.53%).
d-Lysine picrate, picric acid = 60.87% (calc. for $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3$, picric acid = 61.06%).
l-Tryptophane (Th. Schuchardt), N = 13.42% (calc. for $C_{11}H_{12}O_2N_2$, N = 13.72%).
l-Pyrrolidone-carboxylic acid, N = 10.82% (calc. for $C_5H_7O_3N$, N = 10.85%).
l-Proline (Fraenkel u. Landau), N = 11.96% (calc. for $C_5H_9O_2N$, N = 12.17%).
β-Imidazolyl-propionic acid, N = 19.80% (calc. for $C_6H_8O_2N_2$, N = 19.99%).
l-Histidine dihydrochloride, N = 18.43% (calc. for $C_6H_9O_2N_3 \cdot 2HCl$, N = 18.42%).

Apparatus is shown in Fig. VIII.

Cell: A cylindrical glass vessel of 60 c.c. capacity, provided with a rubber stopper which carries two electrodes and a rubber stoppered tube (C). The latter (C) is used to pass air after every interruption of electrolysis and at the same time to withdraw the solution with a pipette. To the cell was fused a spiral condenser which serves as delivery tube. It was connected to a bubbler (E) by a piece of rubber tubing.

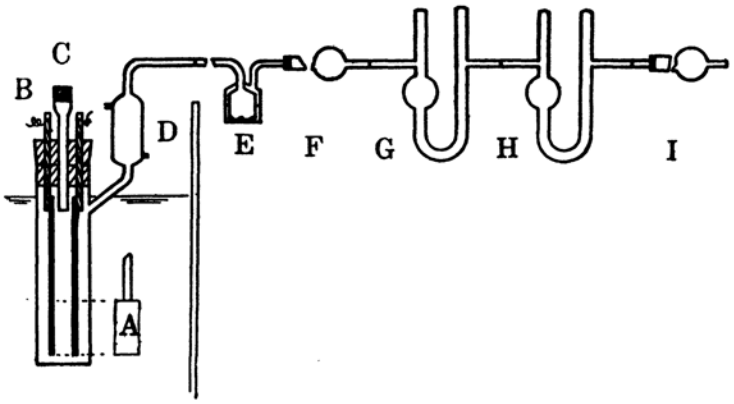


Fig. VIII.

A: Electrodes, lead peroxide anode and lead cathode (2×4 cm.)
B: Brass rods which support electrodes, coated with bakelite varnish.
D: Spiral condenser.
E: Bubbler containing concentrated sulphuric acid.
F: Calcium chloride tube for drying gases.
G and H: Tared absorption tubes for CO_2 containing soda-lime.
I: Calcium chloride guard tube.

Generally ten millimols (in some cases 5 millimols) of amino-acids were dissolved in 30 c.c. of 2N.-sulphuric acid. The solution was electrolysed in the above cell which was kept at a constant temperature (30° or 35°) in a thermostat. Current density was 0.16 amp./2×4 cm. $=2$ amp./dm^2. The amount of electricity was determined by copper coulometer.

After every definite consumption of electricity, i.e. 2F/mol, 4 F/mol and so on, electrolysis was interrupted. Air freed from CO_2 was passed through the apparatus from the tube (C) for about half an hour until all of the CO_2 was replaced by air. Soda-lime tubes were weighed. From the increase of weight of these tubes the amount of carbon dioxide formed was estimated and expressed in molar percentage to amino-acid used. 0.2–0.3 c.c. and 0.3–1.0 c.c. of the solution were drawn out with a pipette from the tube (C) and exactly weighed. In the former total-N and in the latter NH_3-N were determined.* After each set of determinations

* Determination of NH_3 was strictly carried out under the following conditions using micro-Kjeldahl apparatus. 5 c.c. of 30% NaOH were added and steam was passed through the solution for ten minutes.

The amount of water decomposed by electrolysis under this condition is ca. 0.09 c.c. for 1F/mol. Thus the loss of water caused by this effect is neglected.

the volume of the solution was diminished by 0.5–1.3 c.c. The current quantity applied before next determination was reduced to such an extent as 2F/mol is maintained in relation to the original volume of the solution. Correction was made to the amount of CO_2 by the same reason.

Table I. Tyrosine and Phenylalanine.

Solutions: 1.8049 gr. (10.04 millimols) of tyrosine in 30 c.c. of 2N.-H_2SO_4; 1.6498 gr. (9.99 millimols) of phenylalanine in 30 c.c. of 2N.-H_2SO_4; Temp.: 35°C.

Subst. millimols.	Faradays per mol.	CO_2 mol %	NH_3-N mg.	Total-N mg.	NH_3-N/Total-N mol %
I Tyrosine 10.04	1.2093 gr. Cu 3.81	0.1753 gr. 39.9	0.448 mg. in 1 c.c.	1.237 mg. in 0.3 c c.	10.85
I Phenylalanine 9.99	,,	40.1	0.456 1 c.c.	1.240 0.3 c.c.	10.97
II Tyrosine $10.04\times\frac{28.0}{30.0}$	$\frac{3.87}{\text{Total } 7.68}$	$\frac{49.2}{\text{Total } 89.1}$	0.951 1 c.c.	1.262 0.3 c.c.	22.27
II Phenylalanine $9.99\times\frac{28.0}{30.0}$	,,	$\frac{48.8}{88.9}$	0.913 1 c.c.	1.262 0.3 c.c.	21.72
III Tyrosine $10.04\times\frac{26.7}{30.0}$	$\frac{3.95}{11.63}$	$\frac{59.2}{148.3}$	0.726 0.5 c.c.	1.257 0.3 c.c.	34.64
III Phenylalanine $9.99\times\frac{26.7}{30.0}$	,,	$\frac{53.7}{142.6}$	0.688 0.5 c.c.	1.256 0.3 c.c.	32.96
IV Tyrosine $10.04\times\frac{25.9}{30.0}$	$\frac{3.91}{15.54}$	$\frac{60.7}{209.0}$	0.955 0.5 c.c.	1.292 0.3 c.c.	44.34
IV Phenylalanine $9.99\times\frac{25.9}{30.0}$	,,	$\frac{57.0}{199.6}$	0.896 0.5 c.c.	1.264 0.3 c.c.	42.54

Table II. Glutamic Acid and Alanine.

Solutions: 1.4765 gr. (10.04 millimols) of glutamic acid in 30 c.c. of 2N.-H_2SO_4; 0.8917 gr. (10.03 millimols) of alanine in 30 c.c. of 2N.-H_2SO_4; Temp.: 35°C.

Subst. millimols.	Faradays per mol.	CO_2 mol %	NH_3-N mg.	Total-N mg.	NH_3-N/Total-N mol %.
I Glutamic acid 10.04	2.010	42.5	1.111 1 c.c.	0.853 0.2 c.c.	26.0
I Alanine 10.03	,,	32.95	1.231 1 c.c.	0.892 0.2 c.c.	27.6
II Glut. acid $10.04\times\frac{28.8}{30.0}$	3.918	87.53	1.075 0.5 c.c.	0.842 0.2 c.c.	51.0
II Alanine $10.03\times\frac{28.8}{30.0}$	,,	59.91	1.129 0.5 c.c.	0.897 0.2 c.c.	50.4
III Glut. acid $10.04\times\frac{28.1}{30.0}$	5.972	126.8	1.418 0.5 c.c.	0.832 0.2 c.c.	68.1
III Alanine $10.03\times\frac{28.1}{30.0}$	,,	86.11	1.570 0.5 c.c.	0.933 0.2 c.c.	67.3
IV Glut. acid $10.04\times\frac{27.6}{30.0}$	7.950	163.0	1.027 0.3 c.c.	0.808 0.2 c.c.	84.7
IV Alanine $10.03\times\frac{27.6}{30.0}$	,,	104.6	1.168 0.3 c.c.	0.874 0.2 c.c.	82.9
V Glut. acid $10.04\times\frac{27.1}{30.0}$	12.19	224.2	1.145 0.3395 gr.	1.180 0.2220 gr.	97.0
V Alanine $10.03\times\frac{27.1}{30.0}$	,,	125.8	1.348 0.3286 gr.	0.950 0.2211 gr.	95.5

Table III. Valine and Aspartic Acid.

Solutions: 1.1686 gr. (10.03 millimols) of valine in 30 c.c. of 2N.-H_2SO_4; 1.3303 gr. (9.997 millimols) of aspartic acid in 30 c.c. of 2N.-H_2SO_4; Temp.: 35°C.

Subst. millimols.	Faradays per mol.	CO_2 mol %.	NH_3-N mg.	Total-N mg.	NH_3-N/Total-N mol %.
I Valine 10.03	2.014	38.5	1.254 1.032 gr.	1.775 0.4141 gr.	28.4
I Aspartic acid 9.997	,,	59.1	0.860 1.0549 gr.	1.867 0.4296 gr.	18.8
II Valine $10.03\times\frac{28.6}{30.0}$	4.024	75.2	1.231 0.5221 gr.	0.895 0.2067 gr.	54.4
II Aspartic acid $9.997\times\frac{28.6}{30.0}$	,,	119.9	0.831 0.5450 gr.	0.902 0.2111 gr.	35.7
III Valine $10.03\times\frac{27.9}{30.0}$	6.031	106.6	1.632 0.5247 gr.	0.881 0.2130 gr.	75.2
III Aspartic acid $9.997\times\frac{27.9}{30.0}$	,,	179.6	1.155 0.5306 gr.	0.891 0.2128 gr.	52.1
IV Valine $10.03\times\frac{27.2}{30.0}$	8.135	135.8	1.885 0.5135 gr.	0.938 0.2222 gr.	87.0
IV Aspartic acid $9.997\times\frac{27.2}{30.0}$	,,	243.6	1.497 0.5249 gr.	0.856 0.2112 gr.	70.3
V Valine $10.03\times\frac{26.5}{30.0}$	10.17	156.8	2.070 0.5236 gr.	0.948 2.070 gr.	91.2
V Aspartic acid $9.997\times\frac{26.5}{30.0}$	,,	303.3	1.792 0.5210 gr.	0.838 0.2131 gr.	87.2

Table IVa. Glycine and Leucine.

Solutions: 0.7553 gr. (10.07 millimols) of glycine in 30 c.c. of 2N.-H_2SO_4; 1.3126 gr. (10.02 millimols) of leucine in 30 c.c. of 2N.-H_2SO_4; Temp.: 35°C.

Subst. millimols.	Faradays per mol.	CO_2 mol %.	NH_3-N mg.	Total-N mg.	NH_3-N/Total-N mol %.
I Glycine 10.07	1.905	48.8	1.277 1.0497 gr.	0.880 0.2118 gr.	29.2
I Leucine 10.02	,,	30.0	0.878 1.0326 gr.	0.859 0.2075 gr.	20.5
II Glycine $10.07\times\frac{28.8}{30.0}$	3.932	98.6	1.231 0.5289 gr.	0.870 0.2199 gr.	58.9
II Leucine $10.02\times\frac{28.8}{30.0}$	,,	62.3	0.938 0.5345 gr.	0.898 0.2200 gr.	43.0
III Glycine $10.07\times\frac{28.1}{30.0}$	8.06	161.5	1.741 0.5298 gr.	0.796 0.2152 gr.	89.1
III Leucine $10.02\times\frac{28.1}{30.0}$	,,	121.6	1.425 0.5110 gr.	0.839 0.2141 gr.	71.2

Table IVb. Glycine and Leucine.

Solutions: 0.7514 gr. (10.01 millimols) of glycine in 30 c.c. of 2N.-H_2SO_4; 1.3087 gr. (9.982 millimols) of leucine in 30 c.c. of 2N.-H_2SO_4; Temp.: 30°C.

Subst. millimols	Faradays per mol.	CO_2 mol %.	NH_3-N mg.	Total-N mg.	NH_3-N/Total-N mol %.
I Glycine 10.01	2.056	45.25	0.568 0.5434 gr.	1.368 0.3332 gr.	25.5
I Leucine 9.982	,,	24.70	0.3180 0.5315 gr.	1.310 0.3285 gr.	15.0

Table IVb.—(*Concluded*)

Subst. millimols	Faradays per mol	CO_2 mol %.	NH_3-N mg.	Total-N mg.	NH_3-N/Total-N mol %
II Glycine $10.01\times\frac{29.2}{30.0}$	4.094	85.33	1.051 0.5430 gr.	1.250 0.3297 gr.	51.1
II Leucine $9.982\times\frac{29.2}{30.0}$	,,	56.85	0.662 0.5298 gr.	1.323 0.3253 gr.	30.7
III Glycine $10.01\times\frac{28.4}{30.0}$	6.170	120.5	1.266 0.5270 gr.	1.253 0.3257 gr.	60.5
III Leucine $9.982\times\frac{28.4}{30.0}$	,,	87.85	0.957 0.5320 gr.	1.323 0.3290 gr.	44.8
IV Glycine $10.01\times\frac{27.6}{30.0}$	8.16	145.2	0.727 0.2199 gr.	1.296 0.3318 gr.	84.7
IV Leucine $9.982\times\frac{27.6}{30.0}$	,,	118.3	0.518 0.2259 gr.	1.294 0.3266 gr.	57.9
V Glycine $10.01\times\frac{27.1}{30.0}$	10.12	161.1	1.215 0.3289 gr.	0.858 0.2226 gr.	95.7
V Leucine $9.982\times\frac{27.1}{30.0}$	,,	145.7	0.830 0.3210 gr.	0.848 0.2175 gr.	66.3
VI Leucine $9.982\times\frac{26.6}{30.0}$	12.24	171.6	0.881 0.3163 gr.	0.868 0.2235 gr.	71.7

Table V. Pyrrolidone-Carboxylic Acid and Proline.

Solutions: 1.2909 gr. (10.00 millimols) of *l*-pyrrolidone-carboxylic acid in 30 c.c. of 2N.-H_2SO_4; 1.1507 gr. (10.00 millimols) of proline in 30 c.c. of 2N.-H_2SO_4; Temp.: 30°C.

Subst. millimols.	Faradays per mol.	CO_2 mol %.	NH_3-N mg.	Total-N mg.	NH_3-N/Total-N mol %
I Pyrrolidone carb. acid 10.00	2.054	74.3	1.462 0.5568 gr.	1.393 0.3238 gr.	60.9
I Proline 10.00	,,	41.3	0.494 0.5420 gr.	0.425 0.100 gr.	21.4
II Pyrrolidone carb. acid $10.00\times\frac{29.2}{30.0}$	4.067	101.5	1.680 0.4280 gr.	1.380 0.3157 gr.	89.7
II Proline $10.00\times\frac{29.2}{30.0}$	,,	77.6	0.668 0.4341 gr.	1.356 0.3210 gr.	36.5
III Pyrrolidone carb. acid $10.00\times\frac{28.5}{30.0}$	6.099	136.0	1.389 0.3288 gr.	0.986 0.2241 gr.	96.1
III Proline $10.00\times\frac{28.5}{30.0}$	,,	111.5	0.738 0.3256 gr.	0.943 0.2205 gr.	54.8
IV Pyrrolidone carb. acid $10.00\times\frac{28.0}{30.0}$	8.031	173.4	1.396 0.3210 gr.	1.470 0.3296 gr.	97.4
IV Proline $10.00\times\frac{28.0}{30.0}$	8.096	142.9	0.895 0.3266 gr.	0.944 0.2290 gr.	66.4
V Proline $10.00\times\frac{27.5}{30.0}$	10.32	158.5	0.965 0.3321 gr.	0.927 0.2165 gr.	68.0

Table VI. β-Imidazolyl-Propionic Acid and Histidine.

Solutions: 1.4013 gr. (10.00 millimols) of imidazolyl-propionic acid in 30 c.c. of 2N.-H_2SO_4; 10 millimols of histidine in 30 c.c. of 2N.-H_2SO_4; Temp.: 30°C.

Subst. millimols.	Faradays per mol.	CO_2 mol %	NH_3-N mg.	Total-N mg.	NH_3-N/Total-N mol %.
I Histidine 10.00	2.072	24.78	0.624 0.2298 gr.	1.446 0.1130 gr.	63.7
I Imidaz. prop. acid. 10.00	,,	13.93	0.724 0.2244 gr.	0.927 0.1107 gr.	76.8
II Histidine $10.00\times\frac{29.7}{30.0}$	3.95	59.60	0.894 0.2210 gr.	0.1610 0.1263 gr.	95.1
II Imidaz. prop. acid. $10.00\times\frac{29.7}{30.0}$	,,	42.00	1.108 0.2219 gr.	1.00 0.1204 gr.	120.5
III Histidine $10.00\times\frac{29.4}{30.0}$	5.830	99.5	1.148 0.2230 gr.	1.436 0.1155 gr.	124.2
III Imidaz. prop. acid $10.00\times\frac{29.4}{30.0}$	,,	78.63	1.345 0.2215 gr.	0.842 0.100 gr.	144.2
IV Histine $10.00\times\frac{29.1}{30.0}$	7.82	143.0	1.336 0.2072 gr.	1.397 0.1120 gr.	155.4
IV Imidas. prop. acid $10.00\times\frac{29.1}{30.0}$	,,	127.9	1.512 0.2180 gr.	0.981 0.1149 gr.	162.4
V Histidine $10.00\times\frac{28.8}{30.0}$	9.94	189.6	1.615 0.2265 gr.	1.361 0.1123 gr.	176.7
V Imidas. prop. acid $10.00\times\frac{28.8}{30.0}$	,,	175.8	1.588 0.2205 gr.	1.044 0.1230 gr.	169.4

Eleven millimols of histidine dihydrochloride were dissolved in 25 c.c. of 2N.-sulphuric acid and sufficient water was added to it. It was distilled under reduced pressure to drive off hydrochloric acid. The above treatment was repeated several times until the solution showed no trace of hydrochloric acid. The solution was made up to 25 c.c. (total-N 455.9 mg.) from which 23.08-c.c. were taken for electrolysis (corresponding to 10 millimols of histidine).

It was ascertained that imidazolyl-propionic acid did not yield ammonia when it was treated with steam in the presence of conc. sodium hydroxide.

The electrolysate of β-imidazolyl-propionic acid assumed yellowish tint as the electrolysis proceeded, it was transparent throughout the whole course. In the case of histidine, dark purple colouration was already observed after an hour and melanine-like substance began to deposit at 4F/mol and adhered to the electrodes.

Table VII. Tryptophane.

Solutions: 1.0200 gr. (5.00 millimols) of tryptophase in 30 c.c. of 2N.-H_2SO_4; Temp.: 35°C.

Subst. millimols	Faradays per mol	CO_2 mol %.	NH -N mg.	Total-N mg.	NH_3-N/Total-N mol %.
Tryptophane 5	3.88	57.2	0.396 1.094 gr.	0.954 0.2241 gr.	17.0
,, $5\times\frac{28.8}{30.0}$	7.55	113.2	0.715 1.0571 gr.	0.835 0.2087 gr.	33.8
,, $5\times\frac{27.6}{30.0}$	11.45	176.8	0.556 0.5194 gr.	0.949 0.2278 gr.	51.4
,, $5\times\frac{26.9}{30.0}$	15.35	239.6	0.730 0.5244 gr.	0.897 0.2174 gr.	67.4

Table VIII. Lysine.

Solutions: 7.49 millimols of lysine* in 30 c.c. of ca. 2N.-H_2SO_4; Temp.: 35°C.

Subst. millimols.	Faradays per mol	CO_2 mol %.	NH_3-N mg.	Total-N mg.	NH_3-N/Total-N mol %.
Lysine 7.49	2.49	37.6	0.433 0.5 c.c.	0.700 0.1 c.c.	24.6
" $7.49\times\frac{29.4}{30.0}$	5.16	80.1	0.495 0.2 c.c.	1.471 0.2 c.c.	67.2
" $7.49\times\frac{28.4}{30.0}$	7.94	122.2	1.533 0.5 c.c.	1.578 0.2 c.c.	77.8
" $7.49\times\frac{27.7}{30.0}$	10.55	165.0	1.749 0.4 c.c.	1.624 0.2 c.c.	107.8
" $7.49\times\frac{26.7}{30.0}$	15.85	249.8	2.280 0.4 c.c.	1.651 0.2 c.c.	138.2

* 3.75 gr. (10 millimols) of lysine picrate were dissolved in 25 c.c. of 2N.-sulphuric acid. The solution was thoroughly extracted with ether to remove picric acid. By adding 2N.-sulphuric acid the solution was made up to 30 c.c. (content of lysine equals to 7.49 millimols).

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