STUDIES ON AMINO-ACIDS AND RELATED COMPOUNDS. PART V. ELECTROLYTIC OXIDATION OF TYROSINE AND PHENYLALANINE.

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Among various researches on the oxidation of amino-acids, the allied subjects with the present communication are treated in the following ones.

C. Neuberg(1) electrolysed 1-5% solution of several amino-acids with platinum electrodes and observed that the aldehydes having an atom of carbon less were formed from all amino-acids which he dealt with. In the cases of tyrosine and phenylalanine the formation of corresponding aldehydes was assumed from indirect grounds.* The formation of black flocculent precipitates was also observed by him in both of these cases.

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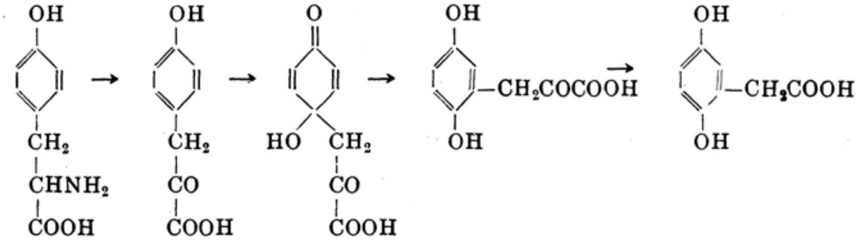
* The electrolysate of tyrosine reduced Fehling's solution and gave a crystalline precipitate with phenylhydrazine or *p*-nitrophenylhydrazine and also showed a purple reaction with ferric chloride. The electrolysate of phenylalanine smelt of phenylacetaldehyde and reduced alkaline silver solution.

(1) C. Neuberg, *Biochem. Z.*, **17** (1909), 270.

W. Denis⁽²⁾ oxidised a small quantity of typrosine with alkaline potassium permanganate and obtained ammonia, carbon dioxide and oxalic acid. He also reported that acetic acid, nitric acid and *p*-hydroxybenzoic acid might be formed.

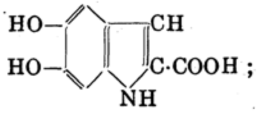
Views⁽³⁾ diverge widely as to the metabolism of these amino-acids in the living organism, but the following scheme is generally accepted. Tyrosine (I) is oxidised up to homogentisic acid (V), through *p*-hydroxyphenylpyruvic acid (II) and two other stages (III, IV).

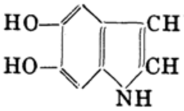


(I) (II) (III) (IV) (V)

In normal organism, homogentisic acid is completely oxidised with the breakdown of benzene nucleus, while in cases of alkaptonuria much of this acid is excreted in urine. Phenylalanine is said, on oxidation in vivo, to be converted into homogentisic acid also.

A tyrosine oxidising enzyme, tyrosinase, produces from tyrosine a pink or red pigment, which on further action changes to a brown or black substance. The latter resembles in all respects with the melanin obtained by usual method. Recently Raper⁽⁴⁾ identified the following intermediate precursors of the black pigment (isolated as their methoxy derivatives) and established a certain definite facts concerning the reaction.





On the other hand, a similar melanin was prepared from tyrosine by a pure chemical method. O. Adler⁽⁵⁾ oxidised tyrosine with hydrogen

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(2) W. Denis, *J. Biol. Chem.*, **10** (1911), 73.

(3) See H. D. Dakin, "Oxidations and reductions in the animal body," (1922), P. 84–87 and H. H. Mitchell and T. S. Hamilton, "The biochemistry of amino-acids," (1929), p. 403–406.

(4) H. S. Raper, *Biochem. J.*, **20** (1926), 735; **21** (1927), 89; **24** (1930), 239.

(5) O. Adler, *Biochem. Z.*, **141** (1923), 304–309.

peroxide and ferric chloride, and obtained a black amorphous powder, which he named tyrosine-melanic acid. On heating it was converted into so-called tyrosine-melanin. K. Kurono(6) isolated the black pigments (soyamelanin, soyamelanic acid) from soy and says that the pigments have the resemblance to Adler's melanins. Prof. Y. Shibata(7) recently published an interesting paper concerning the asymmetric oxidation of dihydroxyphenylalanine by means of metallic complex salts.

As to the electrolytic oxidation of these amino-acids no detailed study has ever been tried before. The author intends to elucidate the mechanism of electrolytic oxidation of these amino-acids by seizing all of the oxidation products. The oxidation was carried out under the same conditions as in the previous communications.

Comparison of Nitrogen Distribution. Dilute sulphuric acid solutions of tyrosine and phenylalanine were electrolysed with lead peroxide anode under the same current conditions. At every definite consumption of electricity (5F/mol), the electrolysis was interrupted and the decrease of amino-nitrogen and amount of ammonia formed were determined. Two couples of curves in Fig. 1 show the courses of reaction.

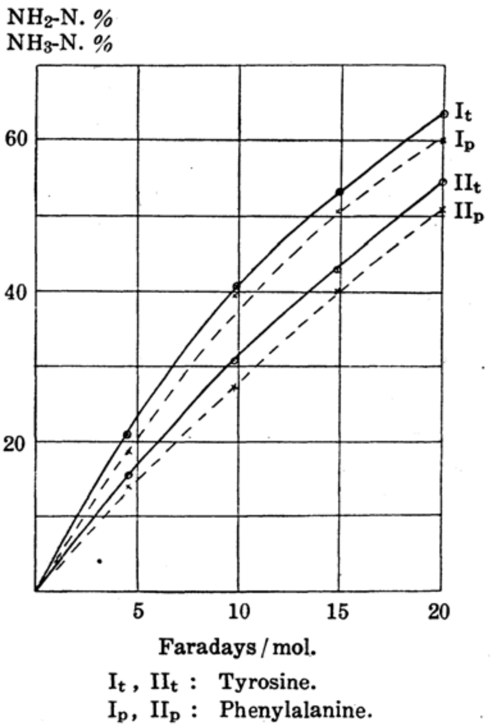

Fig. 1.

From Fig. 1 we may conclude the following two facts. First, if by oxidative deamination all of the freed nitrogen was entirely changed to ammonia, the curves representing decrease of NH$_2$-N (I$_t$ and I$_p$) and increase of NH$_3$-N (II$_t$ and II$_p$) must superpose upon each other. In reality a certain discrepancy is observed, which may partly be ascribed to the formation of melanin and its precursors.

Second, the disparity between I$_t$ and I$_p$ shows that the oxidation of

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(6) K. Kurono and Katsume, *J. Soc. Brewing, Japan,* **21** (1926) No. 2, p. 13; No. 3, p. 8.

(7) Y. Shibata and R. Tsuchida, this Bulletin, **4** (1929), 142.

phenylalanine required much more current quantity than that of tyrosine. This fact agrees with a theory on phenylalanine metabolism remarked by some biochemists—phenylalanine is first converted into tyrosine, preceding to further oxidation.—Though it might be mere coincidence, the last stage of oxidation of these amino-acids do not show marked diversity.

Large amount of tyrosine in 2N.-sulphuric acid solution was electrolysed with lead peroxide anode, current quantity being 15F/mol. The electrolysate assumed yellowish tint, and then changed to brownish black as the electrolysis proceeded. The electrolysate was thoroughly extracted with ether. The part soluble in ether was separated into neutral, phenolic, and acidic parts by usual treatments.

Phenolic part mainly consisted of hydroquinone. From the acidic part of the first extraction a small quantity of *p*-hydroxyphenylacetic acid was isolated. It was identified as such by comparison with the synthetic sample by means of polarising microscope. Succinic acid was also obtained from the acidic part. The part insoluble in ether was concentrated to a small volume and a large amount of tyrosine was recovered. On treating the filtrate of tyrosine with barium hydroxide, ammonia was isolated as the only volatile base. No definite compound was isolated from the residue of the distillation of ammonia.

Volatile Part—The electrolysate was distilled in steam. Small amount of acetic acid, benzoquinone and a liquid of phenolic nature were obtained from the distillate.

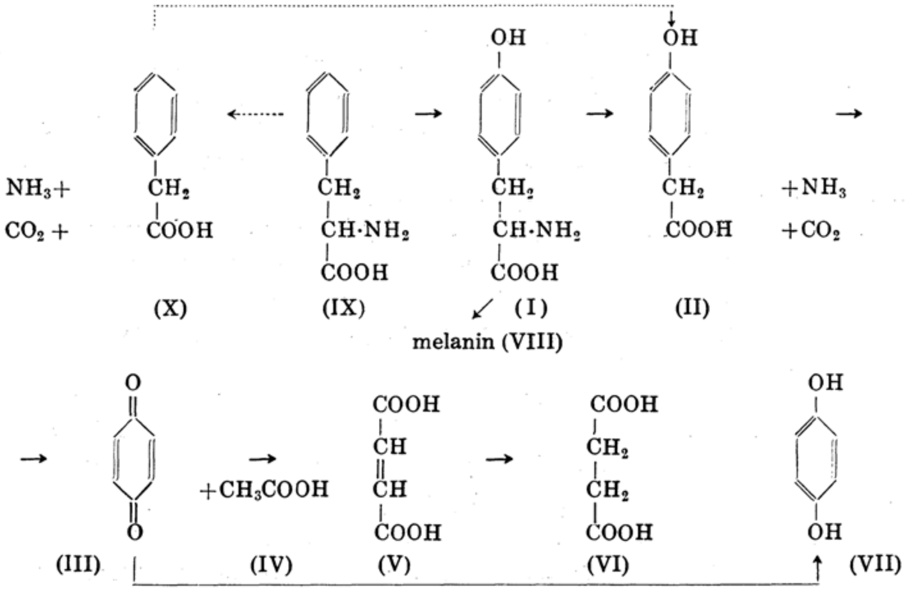
When the electrolysate was allowed to stand for a length of time, a brownish black melanin-like substance was formed. A similar substance was also obtained from other electrolysate, from which the part soluble in ether had been previously removed. The latter sample was washed and dried to a constant weight and was analysed. As already mentioned, three kinds of melanin were isolated by Raper, Adler and Kurono with different treatments. The author's result has nearer approach to that of Adler than to either of the other two.

Melanin from tyrosine electrolysate: $C = 62.44$; $H = 4.28$; $N = 6.25\%$.
Tyrosine melanin (Adler): $C = 60.30$; $H = 2.77$; $N = 12.45\%$.
Tyrosine melanic acid (Adler): $C = 54.40$; $H = 3.46$; $N = 6.54\%$.

Electrolysate of phenylalanine gave the analogous oxidation products. Hydroquinone, succinic acid, acetic acid, ammonia, carbon dioxide and melanin were isolated.

The electrolytic oxidation of typrosine may be represented as follows: The amino-group of tyrosine (I) is first split off and *p*-hydroxyphenylacetic acid (II) as well as ammonia and carbon dioxide are formed. *p*-

Hydroxyphenylacetic acid, on further oxidation, gives quinone (III) and acetic acid (IV). Quinone may be converted into succinic acid (VI) as Kempf⁽⁸⁾ and Fichter⁽⁹⁾ have pointed out. Hydroquinone (VII) is to be formed by the cathodic reduction of quinone.



As to the oxidation of phenylalanine two possible processes can be conceived. First, it (IX) is converted into tyrosine, and then proceeds the course of the oxidation of the latter. Second, the side chain of phenylalanine is first attacked and phenylacetic acid (X) is formed, which is converted into *p*-hydroxyphenylacetic acid (II). In fact, it can not be determined which course the reaction has taken.

Experimental Part.

Sample of tyrosine: $N = 7.71\%$ (calc. 7.74%)

$[\alpha]_D^{20} = -13.0°$ (5 gr. in 100 c.c. of 4% HCl)

$[\alpha]_D^{20} = -\ 8.4°$ (5 gr. in 100 c.c. of 21% HCl)

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(8) R. Kempf, *J. pr. Chem.*, **83** (1911), 329.

(9) Fr. Fichter and E. Schlager, *Helv. chim. Acta*, **10** (1927), 406.

Raper: tyrosine melanin, $N = 8.65\%$.
Kurono: Soya melanin, $C = 56.18$; $H = 2.46$; $N = 7.48\%$.
Soya melanic acid $C = 54.82$; $H = 2.87$; $N = 7.23\%$.

Sample of phenylalanine: N = 8.43% (calc. 8.49%)

$[\alpha]_D^{20} = -32.5°$ (2% solution). Decomposition point 265°C.

Both of them(10) were prepared from the hydrolysate of wheat gluten.

Rate of Oxidation of Tyrosine and Phenylalanine. Sulphuric acid solutions of tyrosine (9.055 gr. ca. 1/20 mol in 120 c.c. of 2N.-H_2SO_4) and of phenylalanine (8.255 gr. ca. 1/20 mol in 120 c.c. of 2N.-H_2SO_4) were electrolysed in undivided cells which were connected in series under the following conditions.

Cells: 200 c.c. capacity glass cylinders. Electrode (4×5 cm.) lead peroxide—lead, C.D.: 2.0 amp./dm^2. Potential diff. between electrodes: 2.5–2.6 volts. Temperature was kept at 35°C. by immersing the cell in a thermostat.

Process of investigation: In the course of electrolysis, at every definite consumption of electricity (5F/mol) a part of the solution was drawn out and the nitrogen distribution was determined as follows:

Total-N in 5 c.c. by Kjeldahl method;
NH_3-N (NaOH) and NH_2-N in 15 c.c. by van Slyke method.

After each set of determinations the volume of the solution is diminished by 20 c.c. The current quantity applied before next determination was reduced to such an extent as 5F/mol is maintained in relation to the original volume of the solution. The four sets of determinations were carried out and the total current quantity applied amounted to 20F/mol. The results are tabulated in Table I and Table II.

Table I.

Faradays per mol.	Substance	Total-N mg.	NH_3-N mg.	NH_2-N mg.
6.449 A.H.	Tyrosine	81.56 (15 c.c.)	12.42 (15 c.c.)	64.86 (15 c.c.)
4.67F/mol	Phenylalanine	82.68 (,,)	12.06 (,,)	67.35 (,,)
6.449 5.663 12.112 A.H.	Tyrosine	69.20 (,,)	20.87 (,,)	41.07 (,,)
9.75F/ ,,	Phenylalanine	69.88 (,,)	19.67 (,,)	42.14 (,,)

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(10) Both of the purified samples were obtained from Mr. R. Nakamura of Suzuki & Co., for whose kindness the author is very grateful.

Table I.—(*Concluded*)

Faradays per mol.	Substance	Total-N mg.	NH_3-N mg.	NH_2-N mg.
12.11 4.71 16.82 A.H.	Tyrosine	57.36 (15 c.c.)	24.45 (15 c.c.)	27.07 (15 c.c.)
14.82F/ ,,	Phenylalanine	59.33 (,,)	23.82 (,,)	29.07 (,,)
16.82 3.85 20.67 A.H.	Tyrosine	46.34 (,,)	25.06 (,,)	16.81 (,,)
20.05F/ ,,	Phenylalanine	49.85 (,,)	25.36 (,,)	20.06 (,,)

Table II.

Faradays per mol.		4.67 F		9.75 F		14.82 F		20.05 F	
Subst.		Ty.	Ph.	Ty.	Ph.	Ty.	Ph.	Ty.	Ph.
$\frac{NH_3\text{-N}}{\text{Total-N}}$	(1)	15.23%	14.58%	30.16%	28.15%	42.62%	40.15%	54.08%	50.87
$\frac{NH_2\text{-N}}{\text{Total-N}}$	(2)	79.52	81.45	59.36	60.30	47.19	48.99	36.27	40.25
NH_2-N decreased	(3)	20.48	18.55	40.64	39.70	52.81	51.01	63.73	59.75
Melanin-N +loss	(4)	5.25	3.97	10.48	11.55	10.19	10.86	9.65	8.85

Ty. = Tyrosine, Ph. = Phenylalanine. (3) = 100%—(2), (4) = (3)—(1).

Separation of Oxidation Products.

90.6 Gr. (0.5 mol) of tyrosine were dissolved in 1200 c.c. of 2N.-sulphuric acid. The solution was placed in two cells of equal size, which were connected in series.

Electrodes (10×12 cm.): lead peroxide anode and lead cathode. C.D.: 2 amp./dm^2., Current quantity applied: 100.8 amp. hrs. for each cell, Temp: 36°C.

Nitrogen Distribution in the Oxidised Solution.

NH_3-N ($MgO+NaOH$)	40.3%
NH_2-N (van Slyke)	41.3%
Melanin-N+loss (difference)	18.4%

(A) Separation of the Part Soluble in Ether. The electrolysate was thoroughly extracted with either. The first extraction showed reddish yellow colour, while the last part was almost colourless. Ether was expelled, 3.1 gr. of crystalline residue remaining. It was separated into three parts, namely phenolic, acidic and neutral as usual.

(a) *Phenolic Part.* After decolourisation with animal charcoal and sulphur dioxide gas, it was recrystallised from water and drained on porous plate. It weighed 1.5 gr. (melting point 155–165°C.). Recrystallisation was repeated until the melting point attained 169°C. It was found to be hydroquinone by elementary analysis and mixed melting point test.

Anal.: Subst. = 0.1026, 0.1020; CO_2 = 0.2468, 0.2453;
H_2O = 0.0496, 0.0532 gr.
Found: C = 65.60, 65.58; H = 5.41, 5.83%.
Calc. for $C_6H_6O_2$ (hydroquinone): C = 65.39; H = 5.49%.

(b) *Acidic Part* (*p-Hydroxyphenylacetic Acid*; *Succinic Acid*). From first part of the extraction a viscous liquid was obtained, which gradually changed to a crystalline mass. On recrystallisation from water it separated out in needles melting at 146–147°C. It was readily soluble in water and showed slight purple black colouration with ferric chloride. No lowering of melting point was observed when mixed with a pure specimen of *p*-hydroxyphenylacetic acid (m.p. 147–148°C.).

Synthesis of p-Hydroxyphenyl-acetic Acid.(11) One gram of *p*-hydroxybenzyl cyanide (Takeda) was hydrolysed by boiling with 5 c.c. of concentrated hydrochloric acid under reflux condenser. After cooling the solution was extracted with ether. Ether was expelled, and then remaining crystals were recrystallised from water. Thus, 0.8 gr. of pure sample melting at 147–148°C. was obtained. Its silver salt was analysed with following result:

Anal.: Subst. = 0.1213; Ag = 0.0505 gr. Found: Ag = 41.64%.
Calc. for $C_8H_7O_3Ag$: Ag = 41.66%.

Crystalline Form and Optical Properties of p-Hydroxyphenyl-acetic Acid. Crystals: rhombic prisms, crystallised from water in needles or rods, sometimes tablets on a slide glass (Fig. 2).

Optical properties: extinction straight; sign of elongation positive, sometimes negative. Crystals always elongated parallel to Y-axis. Z was perpendicular to tablets (elongation positive).

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(11) H. Salkowski, *Ber.*, **17** (1884), 506.

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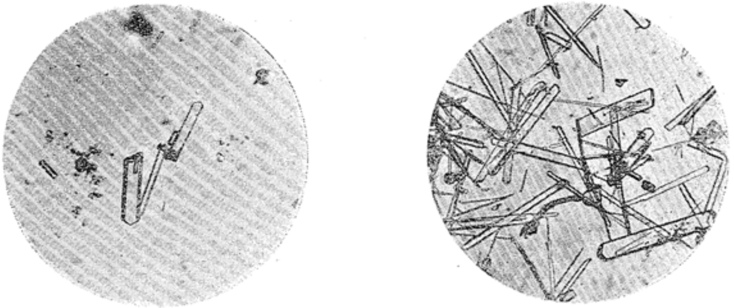

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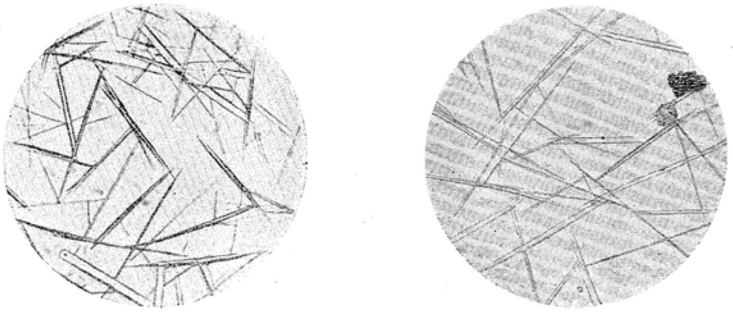

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Fig. 2.'

Index of refraction: $\alpha = 1.547 \pm 0.002$; $\beta = 1.596 \pm 0.001$; $\gamma = 1.639 \pm 0.002$.

Refractive indices were measured by the immersion method: α was measured in the mixture of chlorobenzene and nitrobenzene, β in (bromobenzene-bromoform), and γ in (chloronaphthalene-bromonaphthalene).

Identification: The crystalline substance with m.p. 146–147°C., which was separated from the oxidation products of tyrosine, was identified as *p*-hydroxyphenylacetic acid by immersing in the liquid $n = 1.596$), comparing its other optical properties with the synthetic sample, as well as the mixed melting point test.

Last part of the extraction consisted of nearly pure succinic acid. After recrystallisation from water it (0.2 gr.) melted at 183°C. It was converted into silver salt.

Anal.: Subst. = 0.1915; Ag = 0.1239 gr. Found: Ag = 64.71%.
Calc. for $C_4H_4O_4Ag_2$ (silver succinate): Ag = 65.04%.

(c) *Volatile Part* (*Acetic Acid*; *Benzoquinone*). 90.6 Gr. (1/2 mol) of tyrosine were electrolysed just in the same way as above. The filtered electrolysate was subjected to steam distillation. The total quantity of acid in the distillate was determined by titration, the amount being 0.0448 mol per 1 mol of tyrosine. The distillate was neutralised with sodium carbonate, and phenolic and neutral parts were removed by extraction with ether. The residue was evaporated to dryness. The presence of acetic acid in it was confirmed by acetic ether as well as ferric chloride reactions and the silver salt was prepared, but the amount was too small for analysis. From the above ethereal extract a yellow oil was obtained, from which yellow crystals separated out after a certain time. After being drained on porous plate it melted at 115°C. and showed the same melting point when mixed with a pure sample of benzoquinone. It also gave the iodine reaction, characteristic to quinone, when treated with potassium iodide and chloroform.

(B) **Part Insoluble in Ether.** Barium hydroxide was added to it until neutral to congo red. Barium sulphate was filtered off and the filtrate was boiled with dilute hydrochloric acid. After removing barium the filtrate was concentrated to a syrup. No definite compound was isolated from the syrup (containing 1.44 gr. of nitrogen).

From the filtrate of barium sulphate the crystals (13.3 gr.) were obtained, which were identified as tyrosine by the determination of nitrogen.

Anal.: Subst. = 7.150 mg. NH_3 = 2.65 c.c. of 0.01522 N.-H_2SO_4.
Found: N = 7.90%. Calc. for $C_9H_{11}O_3N$ (tyrosine): N = 7.74%.

(a) *Volatile Base.* The total amount of nitrogen in the mother liquor of tyrosine amounted to 2.338 gr. To a part of it an excess of barium hydroxide was added and distilled under reduced pressure. The distillate was received in hydrochloric acid. Hydrochloride of a base was obtained. It was identified as ammonia by the analysis of its platinic double chloride.

Anal.: Subst. = 0.1924; Pt = 0.0843 gr. Found: Pt = 43.81%.
Calc. for $(NH_4)_2PtCl_6$: Pt = 43.95%.

(b) *Melanin-like Substance.* From the part insoluble in ether brownish precipitate was formed after it was allowed to stand for a month. It was collected on a filter and washed until the washing was free from SO_4'' and dried over lime under reduced pressure. The sample was dried again to a constant weight over calcium chloride at 100°C. and analysed. 0.4 Gr. of the sample was obtained from 90.6 gr. of tyrosine. It was found to be amorphous under polarising microscope and is readily soluble in sodium hydroxide solution and reprecipitated by acid. The sample can be heated in a capillary up to 310°C. without any alteration.

Anal.: Subst. = 0.1003; CO_2 = 0.2296; H_2O = 0.0384 gr.
Subst. = 0.1249; N_2 = 6.82 c.c. (18.4°C., 757.2 mm.).
Found: C = 62.44; H = 4.28; N = 6.25%.

The other sample obtained without separation of the part soluble in ether has darker shade and the content of nitrogen amounted to 4.7%.

Electrolytic Oxidation of Phenylalanine.

82.6 Gr. of phenylalanine were dissolved in 1200 c.c. of 2N.-sulphuric acid and were oxidised in the same way as in the case of tyrosine. The current quantity applied was 202.2 amp. hrs. (15F/mol). The formation of melanin-like substance was also observed in this case.

The investigation of oxidation products was carried out just in the same way as in the case of tyrosine.

(a) **Phenolic Part:** 0.9 Gr. of hydroquinone was isolated; m.p. 169°C.

Anal.: Subst. = 0.0770; CO_2 = 0.1843; H_2O = 0.0380 gr.
Found: C = 65.27; H = 5.52%.
Calc. for $C_6H_6O_2$ (hydroquinone): C = 65.39; H = 5.49%.

(b) **Acidic Part:** 1.6 Gr. of succinic acid were isolated, m.p. 183°C.

Anal.: Subst. = 0.0634; CO_2 = 0.0942; H_2O = 0.0295 gr.
Found: C = 40.52; H = 5.20%.
Calc. for $C_4H_6O_4$ (succinic acid): C = 40.63; H = 5.12%.

(c) **Volatile Part:** The presence of acetic acid was examined by conversion into acetic ether when it gave the other smell, reminding that of ethyl benzoate. But benzoic acid could not be isolated.

(d) **Volatile Base.** Ammonia was isolated.

Anal.: Subst. = 0.2100; Pt = 0.0921 gr. Found: Pt = 43.86%.
Calc. for $(NH_4)_2PtCl_6$, Pt = 43.95%.

Neither *p*-hydroxyphenylacetic acid nor phenylacetic acid were isolated. Further study on this line will be done in near future.

In conclusion, the author wishes to express his sincere thanks to Prof. K. Matsubara for his kind encouragement and invaluable advice given throughout these works.

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