Studies on Amino-Acid and Related Compounds. XIV.* Action of Nitric Acid on Phenylalanine, Tyrosine, and *p*-Hydroxybenzoic Acid.

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In Part XIII[1] it has been briefly described that picric acid was obtained by heating tyrosine with nitric acid under the suitable condition. In the present paper, the behaviour of nitric acid on phenylalanine, tyrosine, and *p*-hydroxybenzoic acid is going to be discussed in detail.

Mörner (1915)[2] has obtained *p*-nitrobenzoic acid, picric acid, and oxalic acid as well as the several other substances by oxidising protein with nitric acid, and he pointed out that the parent substance of nitrobenzoic acid was phenylalanine and those of picric acid were tyrosine and phenylalanine which arose from the protein during the treatment with nitric acid. Mörner[2] has also found that *p*-nitrobenzoic acid was not obtained by heating phenylalanine merely with 60% nitric acid, but that it could be obtained when phenylalanine was mixed with an equal weight of starch and the mixture was kept on standing for 24 hours in 10 times their weight of 60% nitric acid and then heated on a water-bath for 20 hours. In the same way, though without starch, picric acid was obtained also from tyrosine in a small yield of 8.4%.[3]

In the present experiments, phenylalanine was refluxed with 10 times its weight of 8N-NO_3H at 100° for a long time, and neither *p*-nitrobenzoic acid nor oxalic acid was obtained, but only *p*-nitrophenylalanine was isolated. It seemed, therefore, probable that in this case only nitration took place. On the contrary, when phenylalanine was refluxed with nitric acid containing a little of vanadium compound as a catalyser, both nitration and oxidation occurred and *p*-nitrobenzoic acid was obtained in good yield and a little of benzaldehyde as well as benzoic acid was also isolated. In this case, oxalic acid was not isolated, as the acid was completely destroyed by nitric acid in the presence of vanadium compound. In the case of tyrosine, it was refluxed with 8N-NO_3H containing a little of vanadium compound, for 3 hours, and from this solution picric acid (31.4%)[3] and 3,5-dinitro-4-hydroxybenzoic acid (13.2%) were obtained. In this experiment, when the time of refluxing was shortened to 1 hour, the dinitrohydroxybenzoic acid was obtained as a main product, but to which concentrated nitric acid was still more added and refluxed further for 2 hours, and only the picric acid was obtained in good yield (about 45%). In the absence of the catalyser, on heating with 8N-NO_3H tyrosine gave picric and oxalic acids in small yields of 3.5% and of 4.1% respec-

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(1) Part XIII, this Bulletin, **17** (1942), 53.

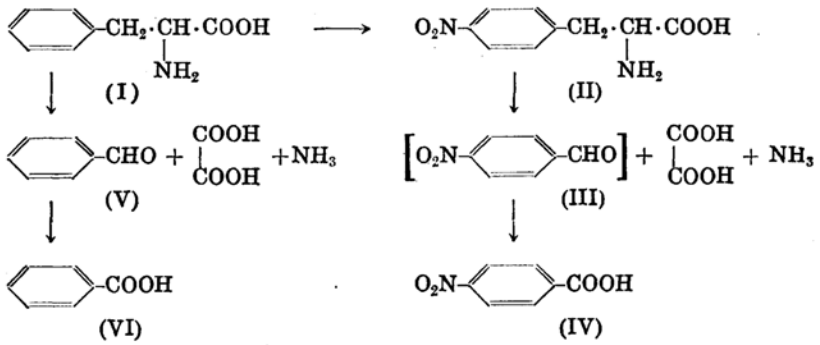
(2) C. Th. Mörner. *Z. physiol. Chem.*, **95** (1915), 265, 287.

(3) The percentages in this paper were expressed in molar per cent.

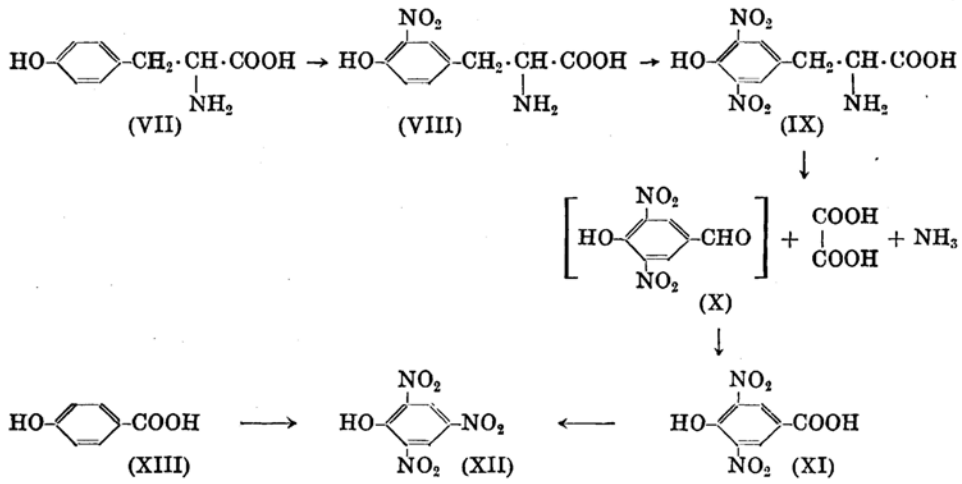
tively. From dinitrohydroxybenzoic acid by merely heating with 8N-NO_3H picric acid was easily obtained in a yield of 95%. Picric acid was also obtained in good yield, by heating dinirotyrosine with nitric acid in the presence of the catalyser, or by merely heating *p*-hydroxybenzoic acid with nitric acid without the catalyser.

From the facts described above, the behaviour of nitric acid on phenylalanine, tyrosine, and *p*-hydroxybenzoic acid may be represented as follows:

Phenylalanine



Tyrosine



Phenylalanine (I) was converted by nitration into *p*-nitrophenylalanine (II) and the latter gave rise to *p*-nitrobenzaldehyde (III), oxalic acid and ammonia by oxidation. This aldehyde was readily oxidised to *p*-nitrobenzoic acid (IV). This course of the reaction may be a main one, infered from the yields of the final products. As a side reaction, phenylalanine was oxidised without nitration to benzaldehyde (V) and the aldehyde was oxidised readily to benzoic acid (VI) in small yield.

Tyrosine (VII) was converted by nitration into mononitrotyrosine (VIII) and then dinitrotyrosine (IX). The latter was oxidised with nitric acid in the presence of the catalyser to 3,5-dinitro-4-hydroxybenzaldehyde (X), oxalic acid, and ammonia. This aldehyde was readily oxidised to 3,5-dinitro-4-hydroxybenzoic acid (XI) and then picric acid (XII) in good yield. Picric acid was also obtained in good yield by merely heating *p*-hydroxybenzoic acid (XIII) with nitric acid. It appeared that the benzene nucleus of tyrosine, in this case, was somewhat attacked by nitric acid.

These methods[4] for preparing *p*-nitrobenzoic and picric acids from phenylalanine and tyrosine respectively, may be applied for industrial purposes, as the crude phenylalanine and tyrosine are separated on the large scale from the by-products of the manufacture of sodium glutamate.

Experimental. *Action of nitric acid on phenylalanine.* Natural phenylalanine (3.325 g., 20.1 m. mols) was dissolved in 8N-NO_3H (30 c.c.) and refluxed at 100° for 8½ hours. From this solution needle crystals (1.4 g.) separated out on adding with sodium hydroxide solution until it gave weak acidic reaction. The crystals melted at 205°, and was submitted to analysis without further purification (Found: N, 11.33%). On concentrating the filtrate the second crops of the crystal (0.85 g.) were also obtained (Found: N, 11.36%). The combined crystals (10.7 m. mols, 53%) were recrystallised from water and analysed (Found: N, 11.67%. Calc. for $NO_2C_6H_4 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \cdot 3/2\, H_2O$: N, 11.81%). The crystals thus obtained was identified as *p*-nitrophenylalanine.

The phenylalanine, therefore, was not oxidised and merely nitrated by the refluxing with nitric acid as described above. As oxalic acid was not found in the filtrate of *p*-nitrophenylalanine, the filtrate was extracted with ether. On distilling off the ether, a little of crystals (0.1 g.) was obtained and recrystallised from water in colourless needles, m.p. 122–123°. It was identified as phenyllactic acid by mixed melting point test. This acid arose from the phenylalanine by the action of nitrous acid formed by oxidation. In the same manner as 8N-NO_3H, phenylalanine was refluxed in 4N-NO_3H, and *p*-nitrophenylalanine (2.89 g.) was obtained (Found: N, 11.56%). It gave somewhat better yield (67.5%) than the case of 8N-NO_3H.

Oxidation of phenylalanine with nitric acid. Natural phenylalanine (10 m. mols) was dissolved in 8N-NO_3H (15 c.c.) containing vanadic acid (0.05 g.) and refluxed for 3 hours.

p-Nitrobenzoic acid. From the above-mentioned oxidised solution, yellow crystals (0.95 g.) separated out on cooling, and melted at 185–200°. The crystals were refluxed with benzene and separated into two parts, insoluble and soluble in benzene. The part insoluble (0.65 g., 39%) in benzene melted at 236°, after recrystallised from water, and it was identified as *p*-nitrobenzoic acid (Found: N, 8.45, 8.25%. Calc. for $NO_2C_6H_4COOH$: N, 8.38%).

Benzoic acid. From the part soluble in benzene, crystals were obtained, on distilling off the benzene. It was sublimed under reduced

(4) Japanese patent 129210; 129211 (picric acid).
134026 (*p*-nitrobenzoic acid).

pressure, yield 0.02 g., m.p. 122–122.5°. It was identified as benzoic acid by mixed melting point test.

Benzaldehyde. The mother liquor of *p*-nitrobenzoic acid was neutralised with sodium hydroxide solution and extracted with ether. From the ethereal solution, an oily substance which had an odour of benzaldehyde was obtained. It was converted into *p*-nitrophenylhydrazone as usual and recrystallised from dilute alcohol in reddish brown thin plates, m.p. 183–185°. It was identified as benzaldehyde by its *p*-nitrophenylhydrazone.

Ammonia. The volatile base was estimated as usual, 0.55 m. mol.

The effect of the concentration of nitric acid and the temperature of heating on the yields of *p*-nitrobenzoic acid from phenylalanine were also studied. Some data are shown in the following table.

Table 1.

Phenylalanine	Concentration of NO_3H	Time of heating	Yields of *p*-nitrobenzoic acid
10 m. mols	8N	3 hours (100°)	0.42 g. (25.2%)
10 m. mols	4N	3 hours (100°)	0.34 g. (20.4%)

Oxidation of tyrosine with nitric acid. Picric acid and dinitrohydroxybenzoic acid. Natural tyrosine (1.81 g., 10.03 m. mols) was dissolved in 8N-NO_3H (15 c.c.) containing vanadic acid (0.1 g.) and refluxed at 110° for 3 hours. After cooling the solution, yellow crystals (0.98 g.) were obtained, and on neutralising the mother liquor with potassium hydroxide, yellow crystals (0.11 g.) of potassium salt were obtained. The former crystals (0.98 g.) were dissolved in potassium hydroxide solution, and the potassium salt (0.73 g.) separated out on cooling. This salt was decomposed with dilute sulphuric acid and extracted with ether. From the ethereal extract, crystals were obtained and melted at 121–122°. The combined yield was 31.4%. It was identified as picric acid by mixed melting point test and by the determination of nitrogen. (Found: N, 18.22, 18.32%. Calc. for $(NO_2)_3 \cdot C_6H_2(OH)$: N, 18.33%). From the mother liquor of potassium salt (0.73 g.), the yellow crystals (0.3 g., 13.2%) separated out in thin plates, on addition of an excess of nitric acid. It melted at 240–242°. It was identified as 3,5-dinitro-4-hydroxybenzoic acid (Found: N, 12.46, 12.49%. Calc. for $(NO_2)_2C_6H_2(OH) \cdot COOH$: N, 12.28%). Oxalic acid was not found in the oxidised solution.

Ammonia. The volatile base (NH_3) was distilled as usual, 2.0 mg. (1.2%).

Tyrosine (10 m. mols) was refluxed in 8N-NO_3H (15 c.c.) containing vanadic acid (0.1 g.) at 110° for 1 hour, then to the solution 13.7N-NO_3H (5 c.c.) was added and refluxed further for 2 hours. From the solution thus obtained, only picric acid was obtained in a yield of 45% without obtaining dinitrohydroxybenzoic acid. Under these conditions, but in the absence of the catalyser, the yields of picric acid, oxalic acid, and ammonia were 3.5%, 4.13%, and 0.3% respectively.

In order to obtain dinitrohydroxybenzoic acid the following procedure may be offered. Tyrosine (10 m. mols) was dissolved in 8N-NO_3H (15 c.c.) which contained vanadic acid (0.1 g.), and refluxed at 100–110° for 1 hour, and the separation of the oxidation products were made as described above. In this case the yield of dinitrohydroxybenzoic acid was 38%. When 4N-NO_3H, instead of 8N-NO_3H, was used and refluxed for 3 hours, the yields of dinitrohydroxybenzoic acid and picric acid were 39.9% and 17.6% respectively. Picric acid was also obtained in good yield (95%) when the dinitrohydroxybenzoic acid was refluxed in 10 times its weight of 8N-NO_3H.

Action of nitric acid on 3,5-*dinitrotyrosine.* Dinitrotyrosine (1.10 g., 3.8 m. mols) was dissolved in 8N-NO_3H (7 c.c.) containing vanadic acid (0.05 g.) and heated at 110° for 3 hours, and picric acid (0.44 g., 1.92 m. mols) was obtained. From the mother liquor potassium picrate (0.04 g., 0.15 m. mol) was also obtained. The combined yield of picric acid ammounted to 54.5% of dinitrotyrosine used. In the above experiment, when the catalyser was omitted, picric acid was obtained only 9.75%, and oxalic acid (35.6%) as well as ammonia (1.1%) was also separated.

Action of nitric acid on p-*hydroxybenzoic acid.* *p*-Hydroxybenzoic acid (10 m. mols) was dissolved in 4N- or 8N-NO_3H (30 c.c.) and refluxed at 100° for 1–3 hours. From the oxidised solution picric acid (70%) was separated as described above, and oxalic acid (11–15%) was also separated as calcium salt by adding $Ca(NO_3)_2$ to the mother liquor.

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