[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

THE CONSTITUTION OF STÄDELER'S TYROSINE SULFONIC ACID

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When proteins are nitrated, or halogenated, one definite point of attack in the molecule is the phenol nucleus of the aromatic amino acid tyrosine combined in the protein. Oswald[2] isolated from the hydrolysis products of several iodinated proteins the amino acid diiodotyrosine, which was shown to be identical with the iodine derivative obtained by direct iodination of tyrosine, and whose constitution was established by Wheeler and Johns.[3] Inouye's[4] observation that one of the hydrolytic products of nitrated silk fibroin is mono-nitrotyrosine was confirmed by the work of Johnson, who also established the constitution[5] of this tyrosine derivative.

Regarding the nature of the products of hydrolysis of sulfonated proteins we have very little knowledge outside of the data contributed by the early work of Mülder[6] and Loew.[7] The sulfonic acids resulting from treatment of proteins with sulfuric acid are usually obtained as amorphous substances with a varying content of sulfur, and it has been assumed that the change brought about by the action of this reagent involves a substitution in the aromatic nucleus of the amino acids, phenylalanine and tyrosine. No sulfonic acid derivative of either of these two amino acids has been identified, however, among the products formed by hydrolysis. In addition, while both of these amino acids have been shown to interact with sulfuric acid with formation of definite sulfonic acids,[8] the constitution of these respective substitution products has not been established with certainty.

We have now repeated the experiments of Städeler, who investigated the action of sulfuric acid on tyrosine in 1860, and have developed a practical method for preparing a definite mono-sulfonic derivative of this amino acid. It is easily obtained in excellent yield, and in a crystalline condition, and this acid obtained by our method is undoubtedly identical with the tyrosine-sulfonic acid originally described by Städeler. We did not obtain

[1] Cheney Brothers Graduate Scholar in Chemistry, 1930–1931.

[2] A. Oswald, *Z. physiol. Chem.*, **70**, 310 (1910); **71**, 200 (1911); **74**, 290 (1911); **75**, 353 (1911).

[3] Wheeler and Johns, *Am. Chem. J.*, **43**, 11 (1910).

[4] Inouye, *Z. physiol. Chem.*, **81**, 82 (1912).

[5] Johnson, THIS JOURNAL, **37**, 2598 (1915); see also Johnson and Kohmann, *ibid.*, **37**, 1863, 2164 (1915); Johnson, Hill and O'Hara, *ibid.*, **37**, 2170 (1915); and Johnson and Hill, *ibid.*, **38**, 1392 (1916).

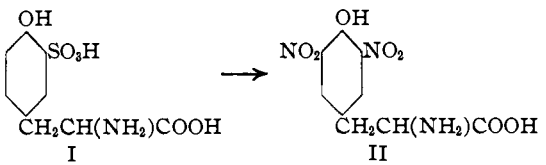
[6] Mülder, *Pogg. Ann.*, **37**, 594 (1836); *Chem. Zentr.*, 242 (1839).

[7] Loew, *J. prakt. Chem.*, [2] **3**, 180 (1871); [2] **5**, 433 (1872).

[8] Städeler, *Ann.*, **116**, 91 (1860); Erlenmeyer and Lipp, *ibid.*, **219**, 209 (1883).

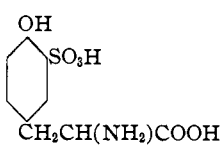
any evidence of the formation of a disulfonic acid derivative of tyrosine corresponding in structure to dinitrotyrosine or diiodotyrosine.

Of the two mono-sulfonic acid derivatives theoretically possible of formation by the action of sulfuric acid on tyrosine, namely, 2- and 3-sulfotyrosines, we now conclude that the acid described by Städeler is to be represented structurally by formula I. This was established by the fact that it interacts smoothly with nitric acid to form 3,5-dinitrotyrosine II with complete substitution of the sulfonic acid group by a nitro radical.



While it would be of interest to establish the presence of this acid I among the products of hydrolysis of a sulfonated protein, we have not made any serious attempts to accomplish such a separation. We are more especially interested at present in the possibility of utilizing tyrosine-3-sulfonic acid as the source of new types of pressor-bases, and for preparing other aromatic derivatives of immediate biochemical interest. The study of this compound is being continued.

Experimental Part

Preparation and Properties of Tyrosine-3-sulfonic Acid.[9]— 

Ten grams of pure crystallized tyrosine is thoroughly mixed with 32.5 g. (six mols) of cold concentrated sulfuric acid. The sulfonation starts immediately on adding tyrosine to the acid and the temperature of the mixture rises quickly to 55°. After the mixture is prepared, it is heated in an oil-bath at a temperature of 95–100° for one hour, and with constant stirring to insure uniformity of reaction. The acid solution is then cooled and poured into 800 cc. of cold water and the sulfuric acid removed quantitatively as barium sulfate by addition of barium hydroxide. After separation of the barium sulfate by filtration, the filtrate and washings are combined and finally concentrated under reduced pressure to a volume of about 70 cc. and this then allowed to stand in an ice chest. A portion of the tyrosine sulfonic acid will crystallize in the form of colorless fine needles. These were separated and the solution concentrated further to 30 cc. in volume and another crop of the pure sulfonic amino acid obtained. The total yield of the sulfonic acid obtainable by crystallization is about 90% of the theoretical. If crystallization is hurried by quick chilling of the solution the tyrosine sulfonic acid always separates in an amorphous condition. Tyrosine sulfonic acid exhibits the characteristic property of forming supersaturated solutions when dissolved in water and consequently the purification of the acid by crystallization is very slow.

Anal. Calcd. for $C_9H_{11}O_6NS$: N, 5.36; S, 12.25. Found: N, 5.33; S, 12.50.

Tyrosine-3-sulfonic acid shows no definite melting point but chars and decomposes

[9] The authors are indebted to the Cheney Brothers, Silk Manufacturers of South Manchester, Connecticut, for the supply of silk fibroin which was used in our research for the preparation of tyrosine.

rapidly when heated above 280°. It is insoluble in cold water, gives an opaque solution when digested with a large volume of boiling water, and also dissolves in warm methyl alcohol. The acid separates from methyl alcohol in an amorphous form when the solvent is evaporated, and it also separates from its aqueous solution in a similar form if the crystallization is hurried. The acid gives a deep violet coloration with ferric chloride solution, and also reacts with Millon's reagent to give a characteristic pink color. The barium, sodium and potassium salts are all amorphous substances which are very soluble in water. Attempts to bring about a reaction with potassium cyanate and phenyl isocyanate were unsuccessful. In other words, the characteristic reactivity of the tyrosine molecule is completely changed by introduction of the sulfonic acid group. The basicity of the α-amino radical is masked by the presence in the molecule of the three negative groupings—OH, SO_3H and COOH.

The Formation of 3,5-Dinitrotyrosine II from Tyrosine-3-sulfonic Acid.—Twenty-two grams of concentrated sulfuric acid is mixed with 3 g. of concentrated nitric acid and the liquid cooled to 5°. Three grams of tyrosine-3-sulfonic acid is then added to this mixed acid in small portions with constant shaking over a period of twenty minutes. The sulfonic acid dissolves completely, giving a light orange-colored solution and the temperature rises to about 25°. After completion of the nitration the acid mixture is poured into 800 cc. of cold water and the sulfuric acid quantitatively removed as barium sulfate. The resulting solution is then made strongly alkaline by addition of ammonia and concentrated to a volume of about 10 cc. under reduced pressure. A brick-red crystalline precipitate of an ammonium salt is obtained which is decomposed by washing with dilute hydrochloric acid. This treatment gave a crystalline substance which was identified as the hydrochloride of 3,5-dinitrotyrosine. It crystallized from hot water in beautiful yellow plates melting with effervescence at 230°. It did not respond to a test for sulfur. The yield of purified acid was about 1.0 g. The identity of this compound was established by comparison with a sample of the hydrochloride of 3,5-dinitrotyrosine prepared by Johnson and Kohmann[5] in 1915. There was no change in melting point when a mixture of the two salts was heated in a capillary tube. It is of interest to note here the stability of this nitration product of tyrosine. The 3,5-dinitrotyrosine hydrochloride prepared by Johnson and Kohmann actually showed no change in melting point after storage in the laboratory for sixteen years.

Anal. Calcd. for $C_9H_9O_7N_3 \cdot HCl$: N, 13.60; Cl, 11.54. Found: N, 13.55; Cl, 11.60.

This formation of dinitrotyrosine is another outstanding example of the easy replacement during nitration of a sulfonic acid group by the nitro radical. This interchange of sulfonic and nitro groups has been observed previously by several investigators.[10]

While tyrosine-3-sulfonic acid interacts smoothly with nitric acid with replacement of the sulfonic radical by a nitro group, an attempt to bring about a corresponding conversion into 3,5-dibromotyrosine[11] by the action of bromine was unsuccessful. This technique of establishing structure of aromatic compounds has been applied with success in other cases by several workers.[12]

[10] Neville and Winther, *Ber.*, **13**, 1946 (1880); Sakellarios, *ibid.*, **55**, 2846 (1922); Schmidt and Glutz, *ibid.*, **2**, 52 (1869); Armstrong, *Z. Chem.*, **14**, 517, 677 (1871); Zincke, *Ann.*, **339**, 202 (1905); Michler and Walden, *Ber.*, **14**, 2176 (1881); Datta and Varma, **41**, 2039 (1919).

[11] Von Gomp and Besanez, *Ann.*, **125**, 281 (1863); Mörner, *Z. physiol. Chem.*, **88**, 124 (1913).

[12] Datta and Bhoumik, THIS JOURNAL, **43**, 303 (1921); Sakellarios, *Ber.*, **55**, 2845 (1922); Sudborough, *J. Chem. Soc.*, 111, 41 (1910); Elgersma, *Rec. trav. chim.*, **48**, 759 (1929); Suter, THIS JOURNAL, **53**, 1112 (1931).

An attempt was also made to establish the constitution of tyrosine-3-sulfonic acid by conversion into a cinnamic acid derivative through deaminization by digestion with methyl iodide and potassium hydroxide. This transformation is not accomplished without complete degradation of the greater part of the sulfonic acid. We did succeed in obtaining a small quantity of the desired cinnamic acid derivative, $HO_3S(OH)C_6H_3CH=CHCOOH$ in the form of its potassium salt, but the quantity formed was so small that this method of operating proved of no practical utility. This potassium salt was purified by crystallization from water. It reacted with concentrated sulfuric acid to give a red colored solution, but did not give color reactions when treated with ferric chloride solution or Millon's reagent. It had no definite melting point.

Anal. Calcd. for $C_{10}H_9O_6SK$: S, 10.80. Found: S, 10.66, 10.9.

Summary

1. When tyrosine is allowed to react with sulfuric acid at 95–100° it is converted smoothly into tyrosine-3-sulfonic acid.

2. The structure of this acid is established by the fact that it is converted into 3,5-dinitrotyrosine by the action of nitric acid.

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SOME OBSERVATIONS CONCERNING THE STRUCTURE OF ISATIDE

BY WARD C. SUMPTER

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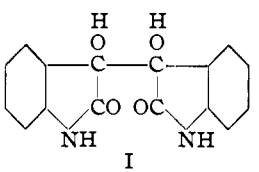
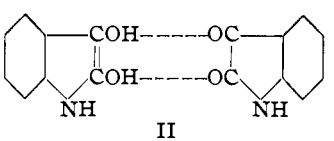
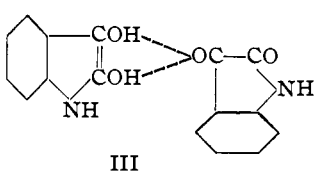
The isatin pinacol formula for isatide, I, proposed by Kohn[1] and by Lefèvre,[2] has been disputed by Heller.[3] Heller regards isatide as being a quinhydrone type of compound, II or III.



I



II



III

Isatide is best prepared by condensing isatin with dioxindole in alcoholic solution in the presence of piperidine. The condensation of a substituted dioxindole with isatin and of a substituted isatin with dioxindole should give rise to two different isatides if the quinhydrone formulation is correct.

[1] Kohn, *Ber.*, **49**, 2514 (1916); Kohn and Klein, *Monatsh.*, **33**, 929 (1912); Kohn and Ostersetzer, *ibid.*, **37**, 25 (1916).

[2] Lefèvre, *Bull. soc. chim.*, **19**, 113 (1916).

[3] Heller, *Ber.*, **49**, 1406 (1916); Heller and Lauth, *ibid.*, **62**, 343 (1929).