1-Methyl-4-(2-methoxy-5-nitrobenzal)-hydantoin (X).—Two grams of sodium were dissolved in 50 cc. of methyl alcohol, and 5 g. of 4-(2-hydroxy-5-nitrobenzal)-hydantoin (XI) and 10 g. of methyl iodide added to the cold sodium methylate solution. This mixture was then heated in a pressure bottle for 6 hours at 100° when a clear solution was obtained. The alcohol was then evaporated and the residue left behind dissolved in a small volume of dilute sodium hydroxide solution. On acidifying the warm solution, and finally cooling this hydantoin separated immediately. It was purified by crystallization from dilute acetic acid and melted at 265° with effervescence. The yield was 6 g.

Calc. for $C_{12}H_{11}O_5N_3$: N, 15.16. Found: N, 15.11.

Reduction of the Nitrohydantoin (X) with Tin and Hydrochloric Acid. Hydrochloride of 1-Methyl-4-(2-methoxy-5-aminobenzyl)-hydantoin (XIII).—The reduction was applied under practically the same conditions as described in the preparation of 4-(2-hydroxy-5-aminobenzyl)-hydantoin (see above). In this case the hydrochloride was extremely soluble in water. It separated from this solvent as a light-colored, crystalline powder, which possessed no definite melting point but gradually decomposed when heated above 175°. It did not give a red color with Millon's reagent.

Calc. for $C_{12}H_{16}O_3N_3Cl$: N, 14.76. Found: N, 14.67.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE SHEFFIELD CHEMICAL LABORATORY OF YALE UNIVERSITY.]

STUDIES ON NITRATED PROTEINS: I. THE DETERMINATION OF THE STRUCTURE OF NITROTYROSINE.[1]

BY TREAT B. JOHNSON AND EDWARD F. KOHMANN.

Received June 11, 1915.

Introduction.

This paper is the first of a projected series of publications from this laboratory, dealing with the chemistry of nitrated proteins. A short, historical review of work already done in this field, previous to the inception of our investigations, has been incorporated. We do not claim to have included here references to all the work done and it is quite probable that some papers have been overlooked. Many of the older journals have been inaccessible to us and consequently we have been obliged to acquire our information regarding some of the earlier developments from the abstract journals. These sources of information are not always reliable. The results obtained in our new researches will be discussed in proper order in subsequent papers. These will have to deal with new data contributing to the present knowledge of the Xanthoproteic and

[1] Part of a dissertation presented by Mr. Edward F. Kohmann to the Faculty of the Graduate School of Yale University, 1915, in candidacy for the degree of Doctor of Philosophy.

Millon's reactions, and also with the study of new organic combinations obtained by hydrolysis of nitrated proteins.

Historical.

I. Definite Nitro-compounds which have been Obtained by Vigorous Treatment of Proteins with Nitric Acid.—While it has been known for a long time that nitric acid will act upon animal and vegetable proteins with production of a yellow color, the study of the reactions involved can be said to have begun, apparently, in the year 1771, when Woulfe[1] made the observation that natural indigo interacts with nitric acid, giving an aqueous solution which will dye silk yellow. Haussmann[2] repeated his work in 1788 and succeeded in isolating a crystalline substance whose constitution was not established. Welter[3] was apparently the first to study the action of nitric acid on protein and obtained, by treatment of silk with nitric acid, a characteristic substance which he called "Amer." He observed that all animal substances, which he examined, were stained yellow by nitric acid. Welter's observations were confirmed by Fourcroy and Vanquelin,[4] who were apparently the first to venture an expression of the constitution of Amer with the following phrase: "hydrocarbure d'azote suroxigéne." Hatschett[5] also investigated the action of nitric acid on proteins and later Chevreul,[6] in 1809, supplemented his work and made a thorough study of "Amer." In 1828 Liebig[7] continued the investigation of previous investigators and prepared again Welter's Amer. He named it "Kohlenstickstoff," and gave precise directions for its preparation. Berzelius[8] designated it as "Pikrinsalpetersäure," while its present name —picric acid— was assigned to it, in 1841, by Dumas.[9] Its constitution was finally established by Laurent[10] in 1842. A second nitro-compound—*p*-nitrobenzoic acid—was obtained, in 1885, by Nencki and Sieber[11] by the action of nitric acid on globin, casein and albumin. As far as the writers are aware, this acid and trinitrophenol (picric acid) are the only two nitro-compounds of known structure which have been obtained by nitration and hydrolysis of proteins with nitric acid.

II. Nitroproteins Formed by Interaction of Nitric Acid with Proteins, and the Nitro-compounds of Definite Constitution, which have been Ob-

[1] *Phil. Trans.*, 1771.
[2] *J. Phys. Chim.*, **32**, 161.
[3] *Ann. chim.*, **29**, 301.
[4] *J. Chem. Physik.*, **2**, 231.
[5] *Phil. Trans.*, 1799.
[6] *Ann. chim.*, **72**, 113 (1809).
[7] *Pogg. Ann.*, **13**, 191 (1828).
[8] Roscoe and Schorlemmer, Vol. 4, p. 110.
[9] *Ann.*, **39**, 350 (1841).
[10] *Ibid.*, **43**, 219 (1842).
[11] *Ber.*, **18**, 394 (1885).

tained from Them by Hydrolysis.—Following the work of Fourcroy and Vanquelin[1] appeared that of Mulder and his co-workers.[2] They described the behavior of nitric acid towards a large number of protein substances and gave to the yellow product obtained the name "xanthoproteic-acid." This is the first time that the term "Xanthoproteic" was used in connection with the action of nitric acid on protein material. Vogel[3] examined the behavior of nitric acid on silk and later Hrusschauer,[4] in 1843, continued the investigation with egg-white. Muhlhauser[5] investigated the behavior of a mixture of nitric and hydrochloric acids on proteins and later, in 1871, Loew[6] contributed his first paper on the action of sulfuric and nitric acids on protein material. The following year he published further on the same subject[7] and described characteristic nitrated products. Günsburg[8] and also Wittich[9] examined the behavior of albumen towards nitric acid, and Johnson[10] and Osborne have independently shown[11] that proteins can enter into ionic reactions with nitric acid. Obermeyer[12] continued the work of Mulder in 1892, being particularly interested in the nature of the aromatic groups of the nitrated protein. He subjected his xanthoprotein to hydrolysis with hydrochloric acid but did not succeed in isolating any characteristic hydrolytic product containing a nitro group. In 1899 appeared the paper of von Fürth,[13] describing the behavior of nitric acid on casein and horn. Characteristic products were isolated, but Samuely[14] considers that his nitration products and those of Loew[1] are different substances.

Habermann and Ehrenfeld[15] investigated the action of nitric acid on certain proteins and succeeded in isolating hydroxyglutaric acid. Following their work no further attention was paid to the problem of nitra-

[1] *Loc. cit.*

[2] *Pogg. Ann.*, **37**, 594; *Centrbl.*, **1836**, 538; *Ann.*, **28**, 73; *Centrbl.*, **1838**, 885; *Natur en Scheik Archief*, **1838**, 87; *Centrbl.*, **1839**, 242; *J. prakt. Chem.*, **20**, 340; *Centrbl.*, **1840**, 515. Van der Pant, *Scheikung Anderzoek*, **2**, 136; *Centrbl.*, **1849**, 342.

[3] *Buchner's neues Repert.*, **8**, 1; *Handwörterbuch der reinen u. angew. Chem.*, **7**, 743 (1859).

[4] *Ann.*, **46**, 348 (1843).

[5] *Ibid.*, **90**, 171 (1854); **101**, 171.

[6] *J. prakt. Chem.*, [2] **3**, 180.

[7] *Ibid.*, [2] **5**, 433 (1872); *Ber.*, **34**, 3560 (1901).

[8] *Wien. Acad. Ber.*, **45**, 643; *Centrbl.*, **1863**, 460.

[9] *J. prakt. Chem.*, **73**, 25.

[10] *J. Chem. Soc.*, **27**, 734 (1874).

[11] *Report Conn. Expt. Station*, **1900**, 399; THIS JOURNAL, **24**, 39 (1902).

[12] *Centrbl. Physiol.*, **6**, 300 (1892); *Centrbl.*, **2**, 529 (1892); *Jahr.*, **1892**, 2113.

[13] "Einwirkung von Salpetersäure auf Eiweissstoff. Habilitationsschrift," Strassburg, 1899.

[14] *Oppenheimer*, Vol. 1, p. 441.

[15] *Z. physiol. Chem.*, **35**, 231 (1902).

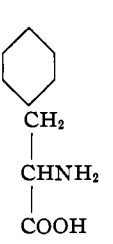
tion until Kossel and Kennaway,[1] in 1911, investigated the behavior of nitric acid towards the protamine—*Clupein.* This underwent nitration smoothly, giving a nitroclupein, which gave on hydrolysis nitroarginine. Later Inouye[2] subjected *nitrofibroin* to hydrolysis with sulfuric acid and isolated from the products of hydrolysis what he believed was nitrotyrosine.[3] Regarding the structure of this acid, we have no definite knowledge. Recently Mörner[4] has contributed an interesting paper in which he has described the isolation of a well-characterized sulfur compound obtained by treating sulfur protein with nitric acid. He isolated methylsulfuric acid, in the form of its barium salt, $(CH_3SO_3)_2Ba.1.5H_2O$, from the hydrolysis liquors of wool, serum, egg protein, casein, ovomucoid, haemoglobin and glutin. Since cystin does not yield this interesting compound with nitric acid, Mörner's observation, therefore, indicates that there are at least two forms of sulfur linkings in sulfur protein.

The Structure of Nitrotyrosine.

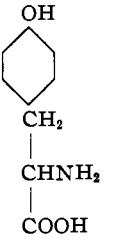
It is evident that before one can hope to formulate definite conclusions regarding the action of nitric acid on proteins, and assign correct structural formulas to the products of hydrolysis containing nitro groups, we must first determine what compounds are formed when the aromatic amino acid constituents of proteins are attacked by this reagent under similar conditions. This involves not only a study of the behavior of nitric acid towards phenylalanine (I), tyrosine (II) and tryptophane (III), but also its action on peptide combinations of these acids and likewise peptides containing these acids in combination with the naturally occurring amino acids of the aliphatic series. The action of nitric acid on tryptophane and peptide combinations containing the above amino acids has never been investigated. Phenylalanine, on the other hand, has been shown by Erlenmeyer and Lipp[5] to interact smoothly with nitric acid giving the corresponding *p*-nitro derivative represented by Formula IV. So far as the writers are aware, however, this amino acid has never been identified among the hydrolytic products of nitrated proteins. It is not improbable that this is the precursor of *p*-nitrobenzoic acid, which was obtained by Nencki and Sieber[6] by treatment of different proteins with strong nitric acid. In the case of tyrosine it has been shown by different investigators that this acid and nitric acid interact smoothly under definite conditions forming *mono*-nitrotyrosine. Inouye[7] likewise obtained what he believed was this same nitro-compound by hydroly-

[1] *Z. physiol. Chem.*, **72**, 486 (1911).
[2] *Ibid.*, **81**, 80 (1912).
[3] Städeler, *Ann.*, **116**, 77 (1860).
[4] *Z. physiol. Chem.*, **93**, 175 (1914).
[5] *Ann.*, **219**, 166 (1883).
[6] *Ber.*, **18**, 394 (1885).
[7] *Z. physiol. Chem.*, **81**, 80 (1912).

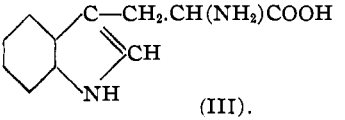
sis of nitrofibroin with sulfuric acid. He was unable to assign a definite structure to his acid because of the fact that it has never been shown in what position tyrosine (II) is attacked by nitric acid. Two isomeric acids might theoretically be formed in this reaction, namely, 3-nitro- and 2-nitrotyrosine represented by Formulas V and VI, respectively. Whether one or both of these two acids are produced has never been established. The primary object of the work described in this paper was the acquirement of experimental data which would enable us to decide beyond doubt the structure of this interesting nitration product.



(I).



(II).



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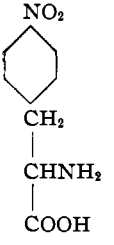
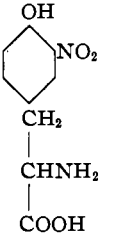
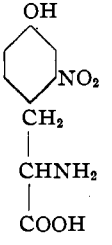


(IV).



(V).



(VI).

That tyrosine will react with nitric acid was apparently first recognized by Warren de la Rue[1] in 1848, who showed that the tyrosine obtained by hydrolysis of cochineal interacts with this reagent giving a new organic acid which, he states, crystallized in the form of needles. No further attention was paid to this observation until 1850, when Strecker[2] reinvestigated this reaction and showed that tyrosine is completely oxidized by vigorous action of strong nitric acid forming oxalic acid. If dilute acid was used, he found that tyrosine was not oxidized, but underwent nitration smoothly, giving him what he called—"salpetersaures Nitrotyrosin." It is interesting to note here that Strecker did not observe the formation of picric acid in this reaction. Strecker described the hydrochloride and barium and silver salts of nitrotyrosine and even prepared the free amino acid by decomposition of its silver salt with hydrogen sulfide. He states that this crystallized in clusters of radiating

[1] *Ann.*, **64**, 35 (1848).

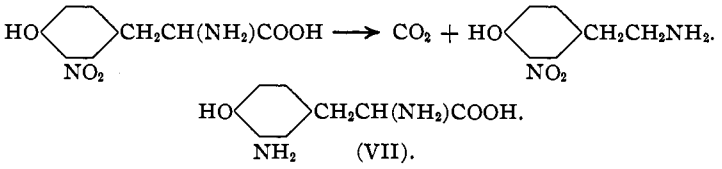
[2] *Ibid.*, **73**, 74 (1850).

needles, which were characterized by the property of giving intensely red solutions when dissolved in aqueous solutions of the alkali metals and ammonia. Strecker did not obtain enough of the amino acid for analysis.

Strecker's observations were confirmed 10 years later by Städeler,[1] who prepared nitrotyrosine according to Strecker's directions. He described the amino acid as a sulfur-yellow substance which had a bitter taste and colored blue litmus red in aqueous solution. He showed that it had both basic and acid properties and described the nitrate, hydrochloride and neutral sulfate of the amino acid. He also tested the stability of the acid on heating and contributed the following statement regarding its behavior under these conditions:

"In stärkerer Hitze tritt schwache Vepuffung ein unter Entwickelung gelbrother Tropfen, die zum Theil zu wenig gefärbten, sternförming gruppierten Krystallen condensiren."

This change was undoubtedly a normal one in which the acid broke down, on heating, with formation of carbon dioxide and the corresponding amine.



(VII).

Beyer,[2] in 1867, prepared nitrotyrosine according to the method of Städeler and subjected it to reduction with tin and hydrochloric acid and obtained the corresponding aminotyrosine. Abderhalden and Massini[3] studied the behavior of this amino acid in alcaptonuric patients. Neuberg[4] considers this acid to have the formula of an ortho derivative represented by Formula VII, but no proof is offered for this. Johnson[5] has stated in a previous paper that nitrotyrosine is very probably a 3-nitro derivative as represented by Formula V.

The first evidence which was produced to indicate that tyrosine undergoes nitration with formation of more than one nitro derivative was published by Casimir Funk, in 1912, in a paper entitled: "The Constitution of Aminotyrosine and the Action of Oxidases on Some Tyrosine Derivatives."[6] He claims to have shown that nitrotyrosine, prepared by Strecker and Städeler's method, is not a definite compound, but a mixture of two isomeric amino acids, represented by Formulas V and VI, respec-

[1] *Ann.*, **116**, 77 (1860).
[2] *Z. Chem.*, **10**, 436 (1867).
[3] *Z. physiol. Chem.*, **66**, 140 (1910); **67**, 404 (1910).
[4] Oppenheimer's *Handbuch der Biochemie*, **4**, II, 363 (1910).
[5] Johnson and Bengis, THIS JOURNAL, **34**, 1054 (1912).
[6] *J. Chem. Soc.*, **101**, 1004 (1912).

tively. Since we have obtained results which do not support Funk's conclusions, it will be necessary, before the discussion of our work, to present briefly here the evidence on which Funk based his conclusions. He reduced his nitrotyrosine to the corresponding aminotyrosine by the action of tin and hydrochloric acid. With the object of converting the latter into 3,4-dihydroxyphenylalanine, he diazotized 10 g. of his diamino acid (melting at 265°) by passing through its aqueous solution the calculated amount of nitrous anhydride and obtained 5 g. of aminotyrosine, having a higher melting point than the original material, namely, 273°. Funk also observed that this differed from the initial acid in stability towards oxidizing agents and concluded, from his observation that it was not changed by the action of specific oxidases (laccase and tyrosinase), that this acid must be considered to be *2-aminotyrosine*, represented by Formula IX. In other words, according to Funk, nitrotyrosine is a mixture of two acids which are transformed on reduction into a mixture of two corresponding aminotyrosines. By the action of nitrous acid on this mixture 3-aminotyrosine (VII) is destroyed, while 2-aminotyrosine (IX) is left unchanged in the solution. Funk apparently did not consider the fact that an aminotyrosine contains two amino groups which are susceptible to attack by nitrous acid and that part of his original amino acid may have been converted into an α-hydroxy acid represented by Formula X or XI. Such a change would be perfectly analogous to that observed by Erlenmeyer and Lipp,[1] who showed that *p*-aminophenylalanine is converted into a mixture of tyrosine and the isomeric α-hydroxy acid, $NH_2C_6H_4CH_2CH(OH)COOH$), by the action of nitrous acid.

Furthermore, Funk states, in the experimental part of his paper, that 0.5 g. of his pure 2-aminotyrosine (IX) gave 0.9 g. of the corresponding tribenzoyl derivative by application of Schotten and Baumann's reaction, which is a yield of 80% of the theoretical. On the other hand, when the same reaction was applied with crude aminotyrosine, he obtained only a very small amount of the same tribenzoyl compound. From these results the following conclusion can therefore be drawn: Ten grams of crude aminotyrosine yield 50% of its weight in 2-aminotyrosine, which is not acted upon by nitrous acid, and from which can be obtained a yield of the tribenzoyl compound corresponding to 80% of the theoretical. The initial aminotyrosine, however, gives only a small amount of the same acyl derivative. These results are somewhat contradictory. Later Funk and Macallum,[2] in a paper entitled: "The Chemical Nature of Substances from Alcoholic Extracts of Various Foodstuffs which Give a Color Reaction with Phosphotungstic and Phosphomolybdic Acids,"

[1] *Loc. cit.*

[2] *Biochem. J.*, **7**, 356 (1913).

stated that both 2-amino- and 3-aminotyrosine react with Folin's[1] uric acid reagent (phosphotungstic acid) and with Folin's polyphenol reagent (phosphomolybdic acid) giving positive tests. Yet they did not mention the source of either compound. They found that nitrotyrosine failed to react with either reagent.

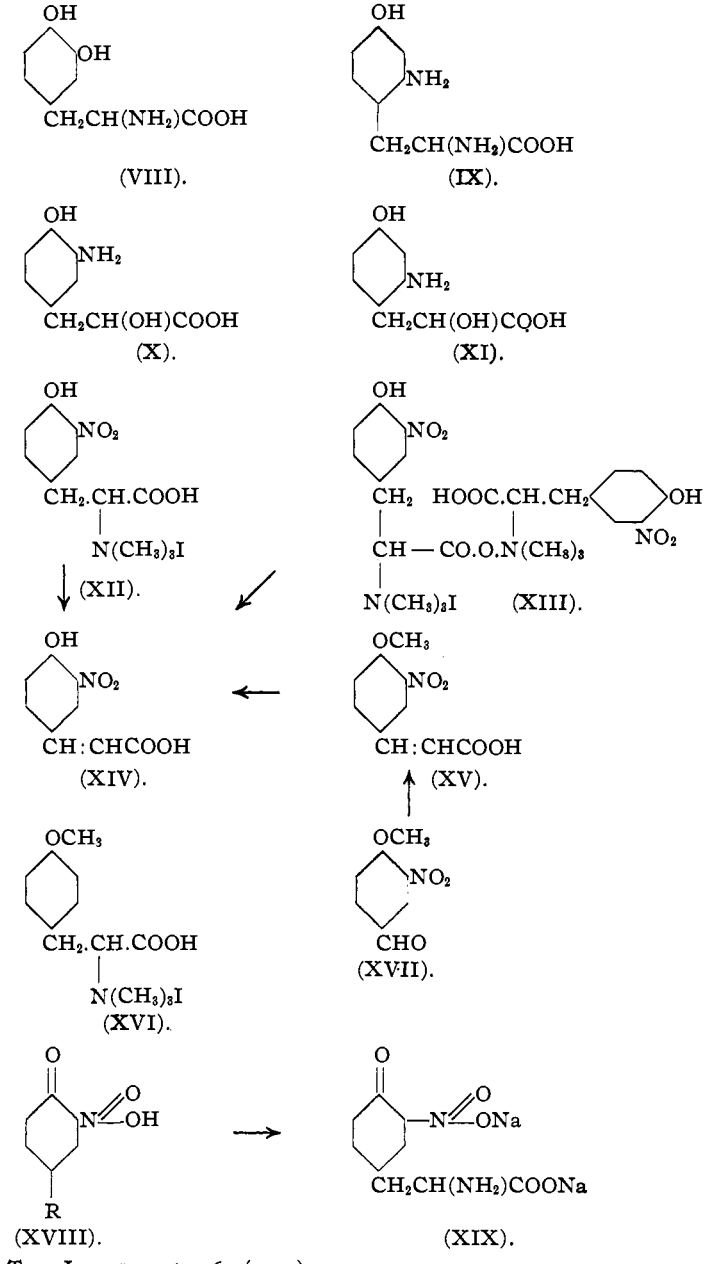
We have now obtained new experimental evidence which proves conclusively that, when tyrosine is subjected to nitration according to the conditions recommended by Strecker and Städeler,[2] it is converted into a mixture of two isomeric nitro acids represented by Formulas V and VI. The chief product of the reaction is not 2-nitrotyrosine (VI) as concluded by Funk, but the isomeric modification (V). In fact, the proportion of the isomer (VI) was so small that it was possible by one reaction only to show that it actually is a product of nitration.

When crude nitrotyrosine is methylated by digestion with methyl iodide in methyl alcohol solution and in the presence of an excess of potassium hydroxide, the hydroxy group remains unchanged and the amino acid is converted into a mixture of two substances—the normal quaternary salt represented by Formula XII, and a secondary product which we have expressed by Formula XIII. The normal salt (XII), however, is the chief product of the reaction. Körner and Menozzi[3] applied the same reaction to tyrosine and obtained the salt (XVI). In the nitrotyrosine, therefore, the nitro group affords a protection for the hydroxyl radicle in such reactions. Apparently we are dealing here with a tautomeric change which involves a rearrangement of the true nitrophenol into its pseudo modification (XVIII) in the presence of alkali, forming the colored salt represented by Formula XIX. Such an assumption is in strict accord with the work of Hantzsch,[4] Hewitt, Johnson and Pope[5] and Meldola and Holley[6] on the structure of the salts of *o*-nitrophenols. That the oxygen ethers of *o*-nitrophenol combinations are easily hydrolyzed in the presence of alkalis, was observed by Miller and Kinkelin,[7] who showed, for example, that the methyl ester of *o*-methoxy-*m*-nitrocinnamic acid is completely saponified by warming with sodium hydroxide and even sodium carbonate in dilute alcohol solution, forming the corresponding *o*-hydroxy-*m*-nitrocinnamic acid.

The quaternary iodide (XII) and the di-quaternary compound (XIII) are both decomposed by vigorous boiling with strong sodium hydroxide solution with evolution of trimethylamine and formation of 3-nitro-4-

[1] *J. Biol. Chem.*, 11, 265 (1912).
[2] *Loc. cit.*
[3] *Gazz. chim. ital.*, 11, 550 (1881).
[4] *Ber.*, **39**, 1073 (1906); **40**, 330 (1907).
[5] *J. Chem. Soc.*, 103, 1626 (1913).
[6] *Ibid.*, 105, 410 (1914).
[7] *Ber.*, **22**, 1705 (1889).

hydroxycinnamic acid (XIV), melting at 223°. A description of the cinnamic acid has recently been given in a paper by Johnson and Kohmann.[1]



[1] THIS JOURNAL, 37, 162 (1915).

We obtained no evidence of the formation of the isomeric 2-nitro-4-hydroxycinnamic acid.

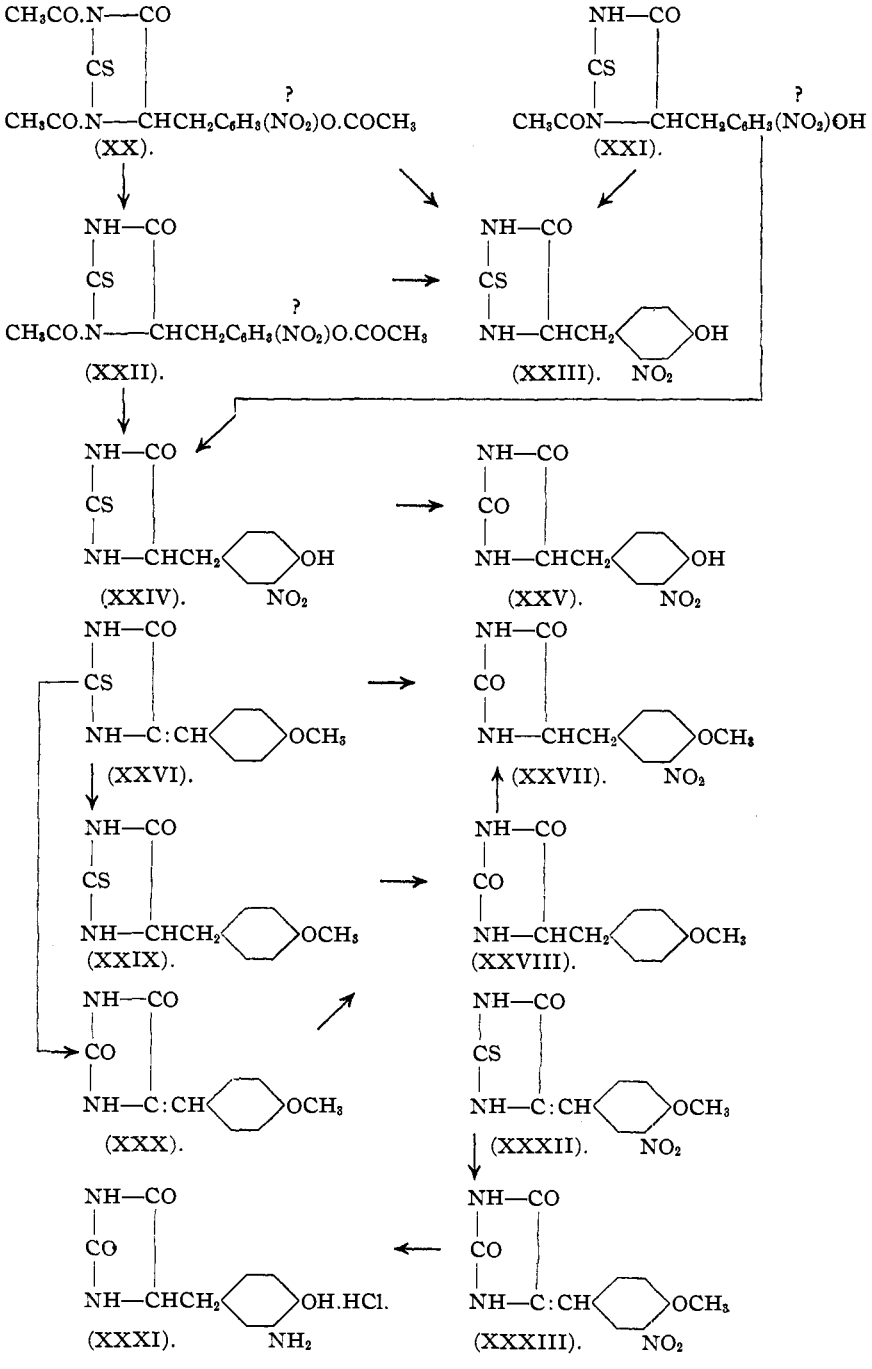
Nitrotyrosine, prepared from tyrosine, interacts smoothly with ammonium thiocyanate in acetic anhydride solution, forming different products, depending apparently on the length of time of heating.

We succeeded in isolating three types of combinations, namely, mono-, di- and triacetyl derivatives, which were all impure and mixtures of isomeric hydantoins. The triacetyl derivative (XX) was easily hydrolyzed by boiling with acetic acid, forming the corresponding diacetyl compound (XXII). That our diacetyl hydantoin (XXII) and monoacetyl compounds (XXI) were mixtures, was established by the fact that both compounds underwent hydrolysis, when digested with hydrochloric acid, giving the two isomeric thiohydantoins, namely, 2-thio-4-(2-nitro-4-hydroxybenzyl)-hydantoin and 2-thio-4-(3-nitro-4-hydroxybenzyl)-hydantoin, represented by Formulas XXIII and XXIV, respectively. Since the hydantoin (XXIV) was obtained in excellent yields in every case and was accompanied by only a small amount of the isomeric thiohydantoin, we conclude that the original nitrotyrosine, from which they were derived, is chiefly a 3-nitro derivative as represented by Formula V. *Tyrosine therefore undergoes nitration with formation of two isomeric nitrotyrosines. That modification, in which the nitro group is substituted in the nucleus, ortho to the hydroxyl group, is the chief product of the reaction.*

The structure of the thiohydantoin (XXIV) was established in the following manner: 2-Thiohydantoin was first condensed with anisic aldehyde with formation of 2-thio-4-anisalhydantoin (XXVI). This was then converted into 4-anisylhydantoin (XXVIII) by two methods. It was either reduced to the thioanisylhydantoin (XXIX) and then desulfurized by digestion with chloroacetic acid, or first desulfurized with formation of (XXX) and the latter finally reduced to the saturated hydantoin (XXVIII). Johnson and Bengis[1] have already shown that this hydantoin (XXVIII) reacts with nitric acid with formation of 4-(3-nitro-4-methoxybenzyl) hydantoin (XXVII). We now find that this hydantoin (XXVII) is easily demethylated by heating with hydrobromic acid in glacial acetic acid solution, giving the same hydantoin (XXV), as is formed by desulfurization of 2-thio-4-(3-nitro-4-hydroxybenzyl)-hydantoin (XXIV) with chloroacetic acid.

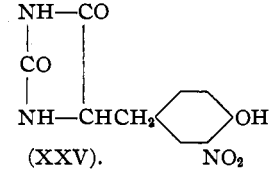
The structure of the hydantoin was also established as follows: The 2-thiohydantoin (XXIV), which was prepared by the action of ammonium thiocyanate on nitrotyrosine, was first desulfurized in the usual manner and the resulting hydantoin (XXV) then reduced to the corresponding aminohydantoin (XXXI). This was isolated in the form of its hydrochloric acid salt. The latter proved to be identical with the hydrochloric acid

[1] THIS JOURNAL, 34, 1056 (1912).

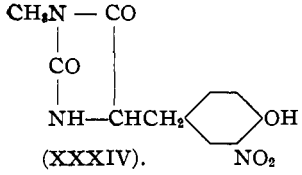

CH3CO.N—CO
CS
CH3CO.N—CHCH2C6H3(NO2)O.COCH3
(XX).
NH—CO
CS
CH3CON—CHCH2C6H3(NO2)OH
(XXI).
NH—CO
CS
CH3CO.N—CHCH2C6H3(NO2)O.COCH3
(XXII).
NH—CO
CS
NH—CHCH2
OH
NO2
(XXIII).
NH—CO
CS
NH—CHCH2
OH
NO2
(XXIV).
NH—CO
CO
NH—CHCH2
OH
NO2
(XXV).
NH—CO
CS
NH—C:CH
OCH3
(XXVI).
NH—CO
CO
NH—CHCH2
OCH3
NO2
(XXVII).
NH—CO
CS
NH—CHCH2
OCH3
(XXIX).
NH—CO
CO
NH—CHCH2
OCH3
(XXVIII).
NH—CO
CO
NH—C:CH
OCH3
(XXX).
NH—CO
CS
NH—C:CH
OCH3
(XXXII).
NO2
NH—CO
CO
NH—CHCH2
OH.HCl.
NH2
(XXXI).
NH—CO
CO
NH—C:CH
OCH3
NO2
(XXXIII).

salt of the hydantoin of aminotyrosine, which was synthesized in the following manner: 2-Thio-3-benzoylhydantoin, which is easily obtained according to the method of Johnson and Nicolet,[1] was condensed with 3-nitroanisic aldehyde (XVII)[2] when 2-thio-4-nitroanisalhydantoin (XXXII) was formed. This was then desulfurized and the resulting hydantoin (XXXIII) reduced with hydriodic acid, and red phosphorus when the hydriodic acid salt of the aminohydantoin was obtained. On digesting this salt with silver chloride it was converted into the corresponding hydrochloride represented by Formula XXXI. The hydantoin and 2-thiohydantoin of 2-nitrotyrosine have not been synthesized.

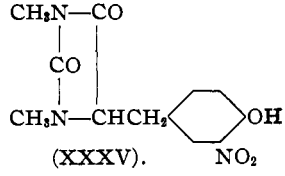
Especially interesting was the behavior of the hydantoin of 3-nitrotyrosine (XXV) on alkylation. It behaved in a similar manner as the free amino acid in that the hydroxyl group was protected against alkylation by the presence of the *ortho* nitro group. When treated with one molecular proportion of methyl iodide the *mono*-allyl derivative (XXXIV) was formed. When an excess of methyl iodide was used the dialkyl derivative (XXXV) was formed. We obtained no evidence of the formation of the methoxy compound (XXXVI).



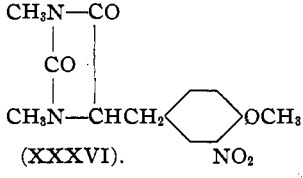
(XXV).



(XXXIV).



(XXXV).



(XXXVI).

Experimental.

Preparation of Tyrosine.—The tyrosine which was used in this investigation was obtained by the hydrolysis of silk noils according to the directions given by Fischer.[3] Noils is a commercial name assigned to a waste product in the silk industry resulting from the combing of silk

[1] THIS JOURNAL, **33**, 1973 (1911).

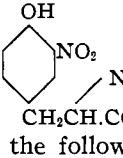
[2] Einhorn and Grabfield, *Ann.*, **243**, 370 (1888).

[3] *Z. physiol. Chem.*, **33**, 181 (1901).

and consists of short fibers of nearly pure *fibroin*.[1] The noils yielded on hydrolysis about 7–8% of their weight in tyrosine. The tyrosine was purified by digesting with glacial acetic acid to remove any leucine present and then recrystallized from boiling water.

Preparation of Nitrotyrosine.—The nitrotyrosine was prepared according to the method described by Städeler.[2] The following procedure was generally followed: Sixteen grams of tyrosine were first suspended in 85 cc. of water and 43 g. of nitric acid (sp. gr. 1.42) were then added slowly while the acid mixture was kept cold by means of an ice bath. The tyrosine completely dissolved and after allowing to stand in an ice bath for 12 hours 19 g. of the nitrate of nitrotyrosine finally separated. After filtering by suction the salt was either first allowed to dry in the air or suspended immediately in 60 cc. of cold water and ammonia added slowly to liberate nitrotyrosine, which generally first separated in an amorphous form. This soon assumed a crystalline condition, however, and was filtered off and washed with cold water. The yield of crude material was about 13 g. This amino acid was used for the following experiments without any further purification.

Inouye[2] states that his nitrotyrosine, which he obtained by hydrolysis of nitrofibroin, gave a red color when warmed with Millon's reagent. It has been our experience that this test is not successful in this case unless special precautions are taken in its application. The color at best is not as strong as that obtained in the case of tyrosine and if too much heat is applied the color is completely destroyed or will not develop. The same considerations hold on applying the test with the hydantoin derivatives of nitrotyrosine. The first effect in general was the production of a yellow color which gradually assumed a red color by gentle warming. This was stronger in some cases than others. If very small amounts were taken the color did not develop. Furthermore, the red color was generally more pronounced when viewed by reflected light.

The Quaternary Salt: Trimethylammonium Iodide of *o*-Nitrotyrosine,  .—In order to obtain this interesting quaternary salt the following procedure was adopted: Five and seven-tenths grams of the crude nitrotyrosine were dissolved in 150 cc. of methyl alcohol, containing in solution 7 g. of pure potassium hydroxide. A

[1] NOTE.—For our supply of silk we are indebted to the Cheney Brothers, silk manufacturers of South Manchester, Connecticut. We take this opportunity to express our appreciation of their interest in our work and willingness to coöperate with us.—T. B. J.

[2] *Loc. cit.*

deep red solution was obtained. Thirty-four grams of methyl iodide were then added and the mixture digested gently, under a reflux condenser, for about 18–19 hours. The reaction was then apparently complete. The excess of alcohol and methyl iodide was evaporated by heating on a water bath and the residue obtained dissolved in about 50 cc. of hot water and the solution decolorized by digestion with bone-coal. On cooling, the quaternary salt began to separate immediately in the form of radiating crystals. After standing for a long time, however, it was observed that a second product likewise crystallized out in the form of short, stout prisms. In order to isolate these two characteristic products an application was made of a fractional crystallization, when there were obtained by cooling to room temperature about 4.9 g. of yellow crystalline material. This was identified as the quaternary derivative of *o*-nitrotyrosine and was purified by crystallization from alcohol, from which it separated, on cooling, in radiating clusters of yellow prisms. After three recrystallizations from alcohol we obtained 3 g. of the salt having a constant melting point of 119° and decomposing at 121°. The salt was dried for analysis by heating at 100°. It contained two molecules of water of crystallization. This could not be determined, however, because the salt slowly underwent decomposition when heated at a temperature sufficiently high to remove the water. It gave Millon's test.

Calc. for $C_{12}H_{17}O_5N_2I.2H_2O$: N, 6.48; I, 29.38.
Found: N, 6.51, 6.48, 6.57, 6.47, 6.43; I, 29.00, 29.43, 29.7, 29.5.

The Diquaternary Salt (XIII).—As stated above, after the separation of the quaternary salt just described, a second crop of crystals was obtained on standing. These were more granular and had a pronounced prismatic habit. It is very slow in separating and generally deposits on the sides and bottom of the beaker. In one experiment four successive crops were filtered off and all found to be identical. This compound, whose structure we have provisionally represented by Formula XIII, crystallizes from hot 95% alcohol in clusters of stout prisms which melt at 220–221° with decomposition. For analysis it was dried at 110°. The salt responded to Millon's test.

Calc. for $C_{24}H_{38}O_{10}N_4I$: N, 8.43; I, 19.1.
Found: N, 8.49, 8.48, 8.43; I, 18.82, 18.87.

In another experiment we obtained from 3.7 g. of recrystallized nitrotyrosine, 3.8 g. of the quaternary salt melting at 119° and 0.9 g. of the diquaternary compound melting at 220–221°. These observations were very uniformly duplicated when nitrotyrosine was exhaustively alkylated with methyl iodide.

Proof of the Structure of *o*-Nitrotyrosine. The Formation of 3-Nitro-4-hydroxycinnamic Acid (XIV) by the Action of Alkali on the Quater-

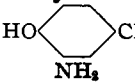
nary Salt (XII).—Two grams of the quaternary salt (XII) were dissolved in 50 cc. of 10% sodium hydroxide solution. The bright red solution was then boiled by immersing the flask in an oil bath. The salt was decomposed by this treatment with evolution of trimethylamine and a bright red sodium salt separated from the hot solution. This was filtered off and examined. It was very soluble in water and insoluble in alcohol. When alcohol was added to its aqueous solution the salt was precipitated in an amorphous condition. In order to obtain the free acid the salt was dissolved in water and the solution acidified with hydrochloric acid, when the above cinnamic acid separated. The yield was 0.75 g. or 78% of a theoretical yield. The acid was insoluble in cold water but slightly soluble in hot, from which it crystallized in long, colorless needles. It was soluble in hot alcohol and crystallized from this solvent in yellow needles arranged in bundles. The acid responded to Millon's test and melted at 223° with decomposition. It was identical with 3-nitro-4-hydroxycinnamic acid, which has recently been described in a paper by Johnson and Kohmann.[1] A mixture of the two acids melted at 223°. It was dried for analysis at 110°.

Calc. for $C_9H_7O_5N$: N, 6.70. Found: N, 6.72, 6.57.

The mother liquor from the red salt was heated in the oil bath for a long time, or until practically no more trimethylamine was evolved. The solution was then cooled and acidified, when a small amount of the cinnamic acid separated. This was separated and the filtrate concentrated and examined further, but we obtained no evidence of the presence of any other acid.

The Formation of 3-Nitro-4-hydroxycinnamic Acid (XIV) by the Action of Alkali on the Diquaternary Derivative (XIII).—By application of the above treatment with 10% sodium hydroxide to the diquaternary salt XIII, 3-nitro-4-hydroxycinnamic acid was also obtained. It melted at 223°. The smoothness of these transformations is well brought out by the following results obtained in one experiment: Sixteen grams of crude nitrotyrosine yielded 10 g. of the quaternary salt and 6 g. of the diquaternary compound. The former yielded on hydrolysis with sodium hydroxide 4.5 g. of the cinnamic acid or 92% of the theoretical yield, while the diquaternary salt gave 3 g. of the unsaturated acid or 79% of the theoretical yield. All the filtrates were again carefully examined, but here again we obtained no evidence of the presence of an isomeric acid.

Hydrochloride of 3-Amino-4-hydroxycinnamic Acid,



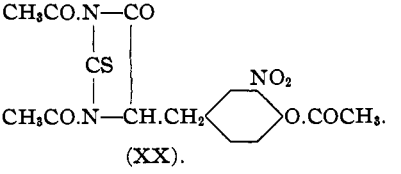
—This salt was obtained by digesting

[1] *Loc. cit.*

the above nitrohydroxycinnamic acid on a steam bath with tin and dilute hydrochloric acid. The nitro-compound underwent reduction smoothly and completely dissolved. After filtering from the excess of tin and cooling, the hydrochloride of the amino acid then separated in the form of colorless needles. The salt was purified by recrystallization from dilute hydrochloric acid. When heated in a capillary tube it began to undergo a change at 225° and at 240° decomposed with effervescence.

Calculated for $C_9H_9O_3N.HCl$: N, 6.51. Found: N, 6.55.

The Action of Nitrotyrosine on Ammonium Thiocyanate in Acetic Anhydride Solution.—Nitrotyrosine exhibits a strong tendency to interact with ammonium thiocyanate and acetic anhydride and various products can be obtained depending upon the conditions employed. In one experiment, twelve grams of crude nitrotyrosine (from tyrosine), 6.3 g. of anhydrous ammonium thiocyanate and 60 cc. of acetic anhydride were mixed in a flask and then heated on a steam bath. On warming gently at first there was an immediate reaction and a clear, orange-colored solution was obtained. Heating was continued for 20 minutes, when the mixture was cooled and finally poured into 400 cc. of cold water. (See below for an examination of this water solution.) A red oil separated which finally solidified. The yield was 14.5 g. This substance was found to be soluble in hot alcohol and in ether, and recrystallized from the former solvent in needles or slender prisms. This solvent, however, was not used for purification. This was accomplished by dissolving the 14.5 g. in 100 cc. of glacial acetic acid and then digesting with a little bone-coal to clarify the solution. On cooling, only 0.2 g. of yellow needles deposited. This product appeared to be a perfectly definite compound and after washing with pure acetic acid was dried for analysis. It melted at 174°. A nitrogen determination agreed with the calculated value for a triacetyl derivative of the thiohydantoin of nitrotyrosine (XX). This is the first



(XX).

case where we have observed the formation of a thiohydantoin containing acetyl groups attached to both nitrogen atoms of the ring, by application of the ammonium thiocyanate reaction. The result obtained by analysis indicated that the hydantoin crystallized with a molecule of acetic acid.

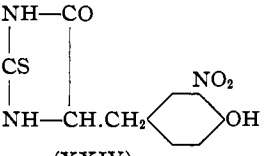
Calc. for $C_{16}H_{15}O_7N_3S.CH_3COOH$: N, 9.27. Found: N, 9.25.

2-Thio-3-acetyl-4-(3-nitro-4-acetoxybenzyl)-hydantoin (XXII).—The acetic acid filtrate left after filtering off the above triacetyl compound was

concentrated to a volume of 20 cc. and cooled when 2.7 g. of this diacetyl compound separated in the form of a fine powder. It was purified by recrystallization from glacial acetic acid and deposited as yellow, rectangular plates which melted at 173–175°. The hydantoin was dried for analysis by heating to constant weight at 110°.

Calc. for $C_{14}H_{13}O_6N_3S$: N, 11.97. Found: N, 11.75.

Having established the presence of these two acetyl derivatives, all the acetic acid filtrates were finally combined and evaporated to dryness on a steam bath. The crystalline residue which was left behind was then digested for one and one-half hours with an excess of concentrated hydrochloric acid and the solution finally evaporated to dryness. The acetyl groups were all removed by this treatment and we obtained the crude thiohydantoin in the form of a yellow powder mixed with a gummy substance which adhered to the sides of the evaporating dish (see below). The yellow powder was identified as *2-thio-4-(3-nitro-4-hydroxybenzyl)-hydantoin* (XXIV).



(XXIV).

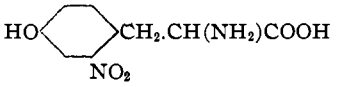
It was purified by crystallization from glacial acetic acid and separated, on cooling, in the form of hexagonal plates which melted at 239–242° with decomposition. The compound has a yellow color and is practically insoluble in water and difficultly soluble in alcohol.

Calc. for $C_{10}H_9O_4N_3S$: N, 15.74. Found: N, 15.60.

2-Thio-4-(2-nitro-4-hydroxybenzyl)-hydantoin (XXIII). — The red, gummy material described in the previous experiment was separated mechanically and dissolved in glacial acetic acid. On cooling this solution rapidly, the hydantoin separated in the form of yellow needles. The yield was very small, being only about 0.1 g. This material was identified as the isomeric hydantoin represented by Formula XXIII, and was formed by action of ammonium thiocyanate on 2-nitrotyrosine which is formed in small amount by the action of nitric acid on tyrosine. The yellow color of the hydantoin is due to the fact that it crystallizes with one molecule of acetic acid. In fact, it readily looses this acid when heated at 110°, giving the plain hydantoin, which is a brick-red compound. The hydantoin has no definite melting point, but begins to char when heated to 270° and gradually undergoes further decomposition when heated above this temperature. An acetic acid determination was made with the following result:

0.0691 g. substance lost at 110° 0.0111 g. CH_3COOH.
Calc. for $C_{10}H_9O_4N_3S.CH_3COOH$: CH_3COOH, 18.3. Found: 16.1.

The identification of this characteristic thiohydantoin was the first evidence that tyrosine interacts with nitric acid with formation of a small amount of *2-nitro-4-hydroxyphenylalanine* (VI). Consequently it was very



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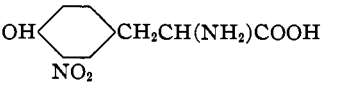

(V).

important to search carefully all filtrates in order to determine whether more of this compound could be obtained for a more thorough examination. We therefore turned our attention to the aqueous solution obtained after pouring the original condensation solution (acetic anhydride + NH_4SCN + crude nitrotyrosine) into cold water. It was first evaporated to dryness and the residue, consisting of a mixture of ammonium thiocyanate, ammonium acetate and thiohydantoins, was then dissolved in an excess of hydrochloric acid. The latter solution was again evaporated to dryness in order to hydrolyze completely any acylhydantoins and the resulting product dissolved in a small volume of glacial acetic acid. To our surprise, on allowing the solution to cool, we obtained a fine crop of yellow needles arranged in rosettes mixed with a small amount of red blocks or stout prisms. The two forms were easily separated mechanically. The yellow material was identified as the *2-thiohydantoin of 2-nitro-4-hydroxyphenylalanine* containing a molecule of acetic acid of crystallization. When heated at 110° it lost its yellow color, became brighter red and melted when heated above 274° with decomposition.

Calc. for $C_{10}H_9O_4N_3S$: N, 15.74. Found: N, 15.9.

The red blocks decomposed at the same temperature as the yellow modification containing acetic acid of crystallization.

In order to be absolutely certain that tyrosine is converted by nitration into a mixture of two isomeric nitro derivatives—2-nitro- and 3-nitrotyrosines—the ammonium thiocyanate reaction was again applied with crude nitrotyrosine. In this experiment 12.7 g. of the nitrotyrosine, 6.5 g. of ammonium thiocyanate and 64 cc. of acetic anhydride were used and the mixture heated as in previous experiments for 20 minutes. After cooling, the reaction mixture was then poured into water when an oil separated which soon solidified. The weight of this material in this case was 16 g. It was very soluble in warm ether, alcohol and glacial acetic acid. Instead of first crystallizing this crude product from glacial acetic acid, as was done in the previous experiment, this time we dissolved it in hot 95% alcohol. On cooling we obtained neither a *triacetyl* nor a *diacetyl* compound as before, but a product deposited in the form of clusters of flat prisms, which melted at 176–178°. The substance apparently underwent a change at this temperature, because if the temperature was held at 176–177° the compound gradually assumed a solid form again

and then melted at 187–188° to a red oil. This compound contained sulfur, was bright yellow in color, and was identified as chiefly *2-thio-3-acetyl-4-(3-nitro-4-hydroxybenzyl)-hydantoin* (XXI). Mixtures of this hydantoin with the triacetyl and diacetyl derivatives represented by Formulas XX and XXII, respectively, melted below 160°. The hydantoin was dried for analysis by heating at 112°.

Calc. for $C_{12}H_{11}O_5N_3S$: N, 13.58. Found: N, 13.25.

When this acetylhydantoin was suspended in strong hydrochloric acid and the mixture heated, it underwent hydrolysis and was converted smoothly into *2-thio-4-(3-nitro-4-hydroxybenzyl)-hydantoin* (XXIV). This was purified by crystallization from hot glacial acetic acid and separated, on cooling, in the form of yellow prisms which decomposed from 239–242°, according to the rate of heating.

After separation of the above acetylhydantoin the alcoholic filtrates were combined and concentrated to a small volume and cooled. At first a few needles deposited which were separated mechanically and discarded. Then we obtained a deposit of rosettes of yellow needles and finally a crop of red prisms. These two latter products were identified as 2-thio-4-(2-nitro-4-hydroxybenzyl)-hydantoin containing acetic acid of crystallization and the free thiohydantoin (XXIII), respectively. The combined yield was 0.5 g. and both forms decomposed at about 270° without further purification. Both forms dissolved in hot glacial acetic acid and also could be recovered by cooling. Rapid cooling favored the formation of the yellow modification and slow cooling the red form. In fact, both forms deposited together by proper regulation of the conditions. When the yellow modification was heated at 112° acetic acid was driven off and the red hydantoin was obtained. Both modifications melted after purification at 273–274° with decomposition.

0.1047 g. hydantoin lost at 112° 0.0195 g. CH_3COOH.
Calc. for $C_{10}H_9O_4N_3S.CH_3COOH$: CH_3COOH, 18.35. Found: 18.6.

Nitrogen determination in pure hydantoin:

Calc. for $C_{10}H_9O_4N_3S$: N, 15.74. Found: N, 15.60, 15.75.

4-(3-Nitro-4-hydroxybenzyl)-hydantoin (XXV).—In order to obtain this hydantoin, 8.7 g. of the corresponding 2-thiohydantoin were suspended in a solution of 30 g. of chloroacetic acid dissolved in 90 cc. of water and the mixture digested for 6.5 hours. After one hour's treatment the thiohydantoin had completely dissolved. After completion of the reaction the acid solution was then cooled, when this new hydantoin separated in the form of rosettes of yellow needles. The yield was 7.7 g. The hydantoin was purified by crystallization from glacial acetic acid, from which it separated as yellow needles melting at 225–226° to a clear oil. This experiment was repeated and a quantitative yield of the hydantoin was obtained.

The hydantoin crystallized with a molecule of acetic acid. This was lost by heating at 110°. The hydantoin gave Millon's test.

0.8850 g. lost at 110° 0.1731 g. of CH_3COOH.
Calc. for $C_{10}H_9O_6N_3.CH_3COOH$: CH_3COOH, 19.3. Found: 19.5.
Calc. for $C_{10}H_9O_6N_3$: N, 16.75. Found: N, 16.66.

This same hydantoin was obtained by demethylation of 4-(3-nitro-4-methoxybenzyl)-hydantoin (XXVII).[1] For example, 3 g. of this hydantoin were heated in a bomb tube at 100° for 3 hours with 50 cc. of glacial acetic acid, which had been saturated at 0° with hydrobromic acid gas. On opening the tube a clear solution was obtained. On allowing this to stand, about 0.5 g. of a difficultly soluble product separated which did not melt at 300°. This was separated and the acid solution evaporated to dryness under diminished pressure. The residue was then dissolved in hot water, separated by crystallization, and finally recrystallized again from acetic acid. It deposited from this solvent in the form of rosettes of needles which melted at 223–4°. A mixture of this with some of the above hydantoin of *o*-nitrotyrosine melted at 223–4°. Therefore, the two compounds were identical. This same experiment was repeated with 3 g. of the methoxyhydantoin and the same result again obtained. The hydantoin crystallized in rosettes of needles and melted at 223–225°. It was identical in every respect with the hydantoin prepared from nitrotyrosine.

Calc. for $C_{10}H_9O_6N_3$: N, 16.75. Found: N, 16.55.

2-Thio-4-(3-nitro-4-methoxybenzal)-hydantoin (XXXII).—In order to obtain this compound 10 g. of 2-thio-3-benzoylhydantoin[2] and 8.2 g. of nitroanisic aldehyde were heated with 16 g. of anhydrous sodium acetate and 50 cc. of glacial acetic acid for 2.5 hours at 155°. On cooling, the fluid completely solidified. This was then thoroughly disintegrated by treatment with 500 cc. of water and the insoluble hydantoin separated by filtration. The yield was 10 g. The hydantoin was very soluble in alcohol and precipitated in a gelatinous condition by dilution with water. If this gelatinous precipitate was redissolved in dilute alcohol and the solution cooled slowly the hydantoin then separated in star-shaped crystals. It crystallized from dilute acetic acid as flat prisms. When heated in a capillary tube the compound began to darken at 240° and then decomposed at 255° with effervescence.

Calc. for $C_{11}H_9O_4N_3S$: N, 15.05. Found: N, 14.95, 14.92.

4-(3-Nitro-4-methoxybenzal)-hydantoin (XXXIII).—This compound has previously been described by Johnson and Bengis.[3] We obtained the same hydantoin by digesting the above 2-thiohydantoin (XXXII) with a

[1] Johnson and Bengis, *Loc. cit.*
[2] Johnson and Nicolet, *Loc. cit.*
[3] *Loc. cit.*

28% solution of choroacetic acid. It crystallized from glacial acetic acid in slender, yellow prisms which partially decomposed at 278° and then effervesced at 284°. From 6 g. of the thio compound we obtained 4.6 g. of the hydantoin. Acetic acid determination:

Calc. for $C_{11}H_9O_5N_3.CH_3COOH$: CH_3COOH, 18.57. Found: 18.48.
Calc. for $C_{11}H_9O_5N_3$: N, 15.97. Found: N, 15.95.

Hydrochloride of 4-(3-Amino-4-hydroxybenzyl)-hydantoin (XXXI).—This salt was obtained by digesting 3 g. of the above benzalhydantoin (XXXIII) with 15 cc. of hydriodic acid and 1.5 g. of red phosphorus. After evaporation of the excess of hydriodic acid the salt was dissolved in water and digested with an excess of silver chloride to precipitate silver iodide and form the hydrochloride. After filtering from silver halides and evaporation of the solution the hydrochloride was crystallized from 20% hydrochloric acid. It separated on cooling as burrs of small prisms which decomposed at about 285°. It crystallized from hydrochloric acid in an anhydrous condition while Johnson and Bengis[1] observed it to crystallize from water with one molecule of water. The same salt was also obtained when the hydantoin of nitrotyrosine (prepared by nitration of tyrosine) was reduced with hydriodic acid and red phosphorus and the resulting hydriodide digested with silver chloride. The hydrochlorides crystallized in the same manner and a mixture of the two behaved as a definite substance when heated in a capillary tube. The melting point was not lowered.

1-Methyl-4-(3-nitro-4-hydroxybenzyl)-hydantoin (XXXIV).—One gram of the hydantoin of *o*-nitrotyrosine (XXV) and one molecular proportion of methyl iodide were dissolve in 30 cc. of methyl alcohol containing 0.22 g. of potassium hydroxide and the mixture heated on the steam bath, with reflux condenser, for 4 hours. On cooling the solution, 0.8 g. of the potassium salt of the unaltered hydantoin separated, showing that practically no reaction took place under these conditions. The experiment was repeated with the same proportions, but heated at 150–155° for two hours in a pressure tube. The alcohol solution was then concentrated and finally diluted with water, when the above hydantoin separated as an oil. This dissolved in hot water and separated, on cooling, as an oil which soon solidified. In crystallized in yellow balls of minute prisms, which melted at 202° to a clear oil. It gave a red color with Millon's reagent.

Calc. for $C_{11}H_{11}O_5N_3$: N, 15.85. Found: N, 15.9.

1,3-Dimethyl-4-(3-nitro-4-hydroxybenzyl)-hydantoin (XXXV).—This was prepared by heating the hydantoin of *o*-tyrosine (XXV) in methyl alcohol solution with 3 molecular proportions of potassium hydroxide and 4 of methyl iodide for 2 hours at 155°. On pouring the alcohol solution

[1] *Loc. cit.*

into cold water the hydantoin separated. It was purified by recrystallization from dilute alcohol and spearated in the form of balls of microscopic crystals. The compound melted from 180–185° and gave a positive test with Millon's reagent. It was dried for analysis at 110°.

Calc. for $C_{12}H_{13}O_5N_3$: N, 15.06. Found: N, 15.22, 15.20.

2-Thio-4-anisalhydantoin (XXVI).—Johnson and O'Brien[1] first prepared this compound by condensing 2-thio-3-benzoylhydantoin with anisic aldehyde and finally hydrolyzing the resulting benzoyl derivative with hydrochloric acid. The same compound was prepared for our work by condensing 2-thiohydantoin with anisic aldehyde in acetic acid solution and in the presence of sodium acetate. From 23 g. of the 2-thiohydantoin we obtained 45 g. of the anisal compound. It was crystallized from glacial acetic acid and melted at 260°.

Calc. for $C_{11}H_{10}O_2N_2S$: N, 11.96. Found: N, 11.95.

2-Thio-4-anisylhydantoin (XXIX).—The hydantoin was prepared by reduction of the above anisalhydantoin (XXVI) with sodium amalgam. The procedure was as follows: Five grams of the unsaturated hydantoin were suspended in a solution of 65 cc. of water and 10 cc. of dilute sodium hydroxide and then 100 g. of 3% sodium amalgam added in small portions at a time. The temperature was kept at 75°. After the amalgam had been added the solution was then filtered and acidified with hydrochloric acid, when the hydantoin separated in a crystalline condition. The yield of crude material was 4.5 g. In order to destroy any hydantoic acid present this material was suspended in hydrochloric acid and the mixture heated on the steam bath. The acid was finally completely evaporated and the hydantoin purified by crystallization from acetic acid. It separated from this solvent in plates and melted at 215°.

Calc. for $C_{11}H_{12}O_2N_2S$: N, 11.87. Found: N, 12.0, 11.92.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF TEXAS.]

THE USE OF CYANIC ACID IN GLACIAL ACETIC ACID SOLUTION, AND IN MIXTURES OF GLACIAL ACETIC ACID WITH OTHER ORGANIC SOLVENTS. DERIVATIVES OF 1-ISOBUTYRIC ACID AMINO-5-DIMETHYLHYDANTOIN.

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Introduction.

As is well known, many reactions in organic chemistry have been found to proceed most smoothly in glacial acetic acid, and this cannot, in every

[1] *Loc. cit.*