was then warmed with an excess of hydrochloric acid to convert any hydantoic acid present into hydantoin and then finally purified by crystallization from alcohol. It melted at 190°.

Calc. for $C_{11}H_{12}O_2N_2S$: N, 11.86. Found: N, 11.80.

NEW HAVEN, CONN.

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

# RESEARCHES ON HYDANTOINS. XXXII. SYNTHESIS OF THE HYDANTOIN OF 2-HYDROXY-5-AMINOPHENYLALANINE.[1]

BY TREAT B. JOHNSON AND WALTER M. SCOTT.

Received June 11, 1915.

The hydantoins of aminophenylalanine derivatives have become of especial interest to us because it now seems very probable that they will find application for the synthesis of α-amino acids of biochemical interest, which it is now practically impossible to prepare easily by other methods. They should be valuable for operations where it is desired to introduce groups into the benzene nucleus by application of the diazo reaction, because the basic influence of the α-amino group of the acid is completely neutralized in such combinations. Furthermore, it has been our experience that the incorporation of the hydantoin nucleus tends to bestow physical properties which are very essential for synthetical work. Such cyclic combinations should be especially valuable for developing methods of synthesizing new, isomeric halogen derivatives of phenylalanine and tyrosine. The iodine derivatives of these two acids are of special physiological interest at the present time. Investigations dealing with methods of synthesis, and other phases of these interesting problems are now in progress in this laboratory.

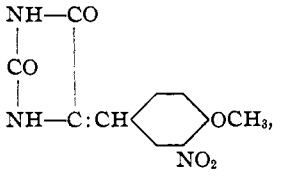
The only aminohydantoin derivatives of phenylalanine or tyrosine which have hitherto been available for synthetical work are the hydantoins of 3-aminotyrosine (III), and its corresponding methyl ether (II), which have been described by Johnson and Bengis[2] in a previous paper from this laboratory. They were prepared by the reduction of 3-nitro-4-methoxybenzalhydantoin (I) with hydriodic acid, and tin and hydrochloric acid, respectively. Johnson and Bengis employed the hydantoin (II), for the synthesis of monobromotyrosine (V).[3] This was accomplished by diazotization of the amino group and by subsequent introduction of bromine into the benzene ring in the usual manner. On subjecting the resulting bromohydantoin to hydrolysis 3-bromo-4-methoxyphenyl-

[1] Part of a dissertation presented by Mr. Walter M. Scott to the Faculty of the Graduate School of Yale University, 1915, in candidacy for the Degree of Doctor of Philosophy.

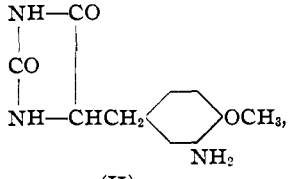
[2] THIS JOURNAL, 34, 1054 (1912).

[3] *Ibid.*, 34, 1061 (1912).

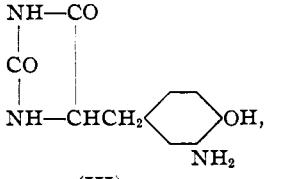
alanine (IV) was formed. This was then converted into monobromotyrosine (V) by warming with hydriodic acid.



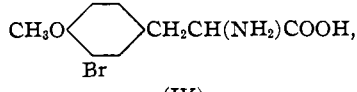
(I).



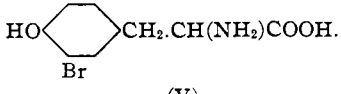
(II).



(III).



(IV).



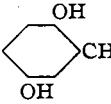
(V).

Isomeric with the hydantoin of aminotyrosine (III), is the hydantoin of 2-hydroxy-5-aminophenylalanine which is represented by Formula XIV, and which was unknown at the time we began our investigation. The starting point for its synthesis was 2-hydroxy-5-nitrobenzaldehyde (VII), which was prepared according to the method described by Miller,[1] namely, by nitration of salicylic aldehyde (VI). The isomeric 2-hydroxy-3-nitrobenzaldehyde, which is also one of the products of this reaction, will be of value for further syntheses. When this nitroaldehyde (VII) was digested with 2-thiohydantoin in acetic acid solution and in the presence of sodium acetate, a normal condensation was easily effected and practically a quantitative yield of 2-thio-4-(2-hydroxy-5-nitrobenzal)-hydantoin (VIII) was obtained. By heating this thio compound with chloroacetic acid in aqueous solution the sulfur was quantitatively replaced by oxygen and the corresponding hydantoin represented by Formula XI was formed. The last step in the synthesis was accomplished by digestion of the nitrohydantoin (XI) with tin and hydrochloric acid. The reduction proceeded normally and after removal of the tin the aminohydantoin (XIV) was isolated in the form of its hydrochloric acid salt. When this hydrochloride was treated in aqueous solution with the required amount of sodium nitrite it was transformed normally into its diazonium derivative. This separated in a crystalline condition and was insoluble in cold water. On warming with water, however, nitrogen was evolved and an excellent

[1] *Ber.*, 20, 1928 (1886).

yield of the hydantoin of 2,5-dihydroxyphenylalanine (XVII) was obtained.

The α-amino acid-2,5-dihydroxyphenylalanine (hydrochinonalanine) (XVI) has never been synthesized, and if known would undoubtedly be an unstable substance. Especial biochemical interest attaches to this compound, however, due to the possibility of its actually functionating as an intermediate product in the formation of homogentisic acid from tyrosine and phenylalanine in cases of alcaptonuria. In fact, Neubauer first gave expression to the possibility of such an intermediate acid being involved in such transformations, when, in his paper entitled—"Über den Abbau der Aminosäuren im gesunden und kranken Organismus," he wrote as follows:[1]

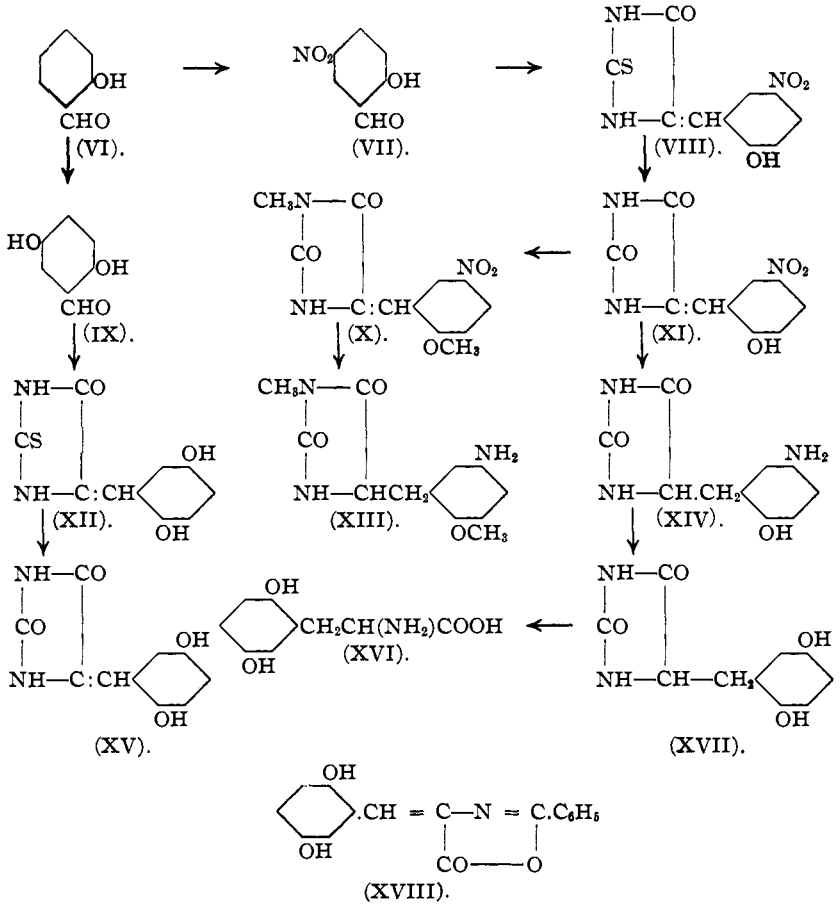
"— es würde da das Phenylalan in zunächst in gewöhnliches *p*-Tyrosin übergehen; es wäre möglich, dass dann sofort die Chinonbildung und die Umlagerung in das Hydrochinonderivat stattfindet; jedenfalls wäre es von Interesse, das noch unbekannte Hydrochinonalanin  $CH_2.CH(NH_2)COOH$, das dabei entstehen müsste, auf sein Verhalten beim Alkaptonuriker zu untersuchen."

Attempts to prepare this α-amino acid (XVI) from its corresponding hydantoin (XVII) have so far been unsuccessful. The hydantoin (XVII) is extremely unstable and it was practically impossible to obtain it in a colorless condition. It was subjected to hydrolysis with barium hydroxide and also sulfuric acid under pressure, but, by both procedures, we obtained only an amorphous product possessing indefinite properties. The substance obtained gave Millon's reaction, but no definite combinations with picric or picrolonic acids, mercury chloride or platinum chloride could be obtained, which would justify a conclusion that we were dealing with a definite compound. It now appears from our experience with the hydantoin that the most feasible way of preparing the amino acid (XVI) will be to synthesize its corresponding dimethyl ether, which should be a stable compound, and then convert this into the amino acid by demethylation under special conditions. Further work on this problem is now in progress.

In connection with our work on methods of preparing the hydantoins of 2,5-dihydroxyphenylalanine (XVII), we also investigated the action of gentisinic aldehyde (IX) on 2-thiohydantoin. These reagents interacted normally when heated together in acetic acid solution and in the presence of sodium acetate, giving the corresponding condensation product represented by Formula XII. On digesting this with chloroacetic acid in aqueous solution it was converted smoothly into the hydantoin (XV). This was a very insoluble compound and possessed no definite melting

[1] *Deutsch. Archiv. f. Klin. Med.*, **95**, 211 (1908).

point. An attempt to convert it into the hydantoin (XVII) by reduction with sodium amalgam was unsuccessful because of its great instability in alkaline solution. The azolactone (XVIII) obtained by condensing gentisinic aldehyde (IX) with hippuric acid was also observed by Neubauer and Flatow[1] to be an extremely unstable compound. The various changes discussed in the previous pages are represented by the following structural formulas:




## Experimental Part.

### Condensation of Gentisinic Aldehyde (IX) with 2-Thiohydantoin.

**2-Thio-4-(2,5-dihydroxybenzal)-hydantoin (XII).**—The gentisinic aldehyde, which was used in this work, was prepared by oxidation of salicylic aldehyde. Neubauer and Flatow[2] accomplished this change

[1] *Z. physiol. Chem.*, **52**, 383 (1907).

[2] *Loc. cit.*

by oxidizing salicylic aldehyde with potassium persulfate. From 62 g. of this phenolic aldehyde they were able to obtain 17 g. of pure gentisinic aldehyde. The potassium salt not being available, we applied Neubauer and Flatow's reaction with sodium persulfate following their method in other details. We found that the aldehyde could be obtained in this manner but the yield was much less than that reported by these investigators. The aldehyde was purified by crystallization from benzene.

The aldehyde was condensed with 2-thiohydantoin by digestion in glacial acetic acid solution in the presence of sodium acetate. After heating for 2 hours at 130–140°, when the reaction was apparently complete, the resulting mixture was then diluted with water and the brown-colored insoluble hydantoin separated by filtration. It was purified by crystallization from dilute acetic acid and separated in prismatic crystals. They did not show a sharp melting point but decomposed at about 270°.

Calc. for $C_{10}H_8O_3N_2S$: N, 11.82. Found: N, 11.87.

**4-(2,5-Dioxybenzal)-hydantoin (XV).** — The above 2-thiohydantoin was converted into this hydantoin by desulfurization with chloroacetic acid. This was accomplished by digesting 3.7 g. of the thiohydantoin with 10 g. of the halogenated acid in 25 cc. of water for 6 hours at 130–140°. This was another case of complete desulfurization without apparent solution of the hydantoin. The hydantoin was purified by repeated washing with hot dilute acetic acid. It showed little tendency to crystallize from the common solvents and decomposed when heated above 300°.

Calc. for $C_{10}H_8O_4N_2$: N, 12.60. Found: N, 12.73.

This hydantoin was extremely unstable in alkaline solution and an attempt to reduce it to the corresponding benzyl derivative with sodium amalgam was unsuccessful.

**2-Hydroxy-5-nitrobenzaldehyde (VII).**—This aldehyde was prepared according to the directions given by Miller.[1] It melts at 126° and, as was shown by Miller, gives on oxidation with chromic acid *m*-nitrosalicylic acid previously described by Hübner.[2]

### Condensation with 2-Thiohydantoin.

**2-Thio-4(2-hydroxy-5-nitrobenzal)-hydantoin (VIII).**—For the preparation of this new hydantoin the following proportions were taken: 15 g. of the nitrosalicylic aldehyde, 9.7 g. of 2-thiohydantoin, 29 g. of fused sodium acetate and 95 cc. of glacial acetic acid. On heating in an oil bath at 140° a clear solution was obtained and after one hour's heating the condensation product began to separate from the hot solution. The heating was continued for 4 hours, when the resulting mixture was thoroughly triturated with water and the insoluble hydantoin finally separated

[1] *Ber.*, **20**, 1928 (1886).

[2] *Ann.*, **195**, 6 (1879).

by filtration. The compound was insoluble in alcohol, benzene, dilute acetic and glacial acetic acid. It was finally purified for analysis by digesting with dilute acetic acid. It did not melt below 300°. The yield of crude hydantoin was 22 g.

Calc. for $C_{10}H_7O_4N_3S$: N, 15.85. Found: N, 15.62.

**4-(2-Hydroxy-5-nitrobenzal)-hydantoin (XI).**—This was prepared in the usual manner by digesting 22 g. of the above thiohydantoin with 48 g. of chloroacetic acid in 150 cc. of water for 4 hours. Here again complete desulfurization was effected without apparent solution of the hydantoin. The hydantoin dissolved with difficulty in hot alcohol and separated on cooling in rosettes of needles, which melted at 286°. The yield was 18 g.

Calc. for $C_{10}H_7O_6N_3$: N, 16.87. Found: N, 16.65, 16.75.

**Reduction of 4-(2-Hydroxy-5-nitrobenzal)-hydantoin with Tin and Hydrochloric Acid.**

**The Hydrochloride of 4-(2-Hydroxy-5-aminobenzyl)-hydantoin (XIV)**—Fourteen grams of the nitrohydantoin were suspended in 250 cc. of concentrated hydrochloric acid with 28 g. of granulated tin. The acid was then raised to boiling and the heating continued until the hydantoin had completely disappeared and dissolved in the acid solution. The solution was then evaporated at 100° to remove the excess of hydrochloric acid, the salt dissolved in water and the tin finally precipitated as sulfide by saturating the solution with hydrogen sulfide. After complete removal of the tin, the solution was then evaporated to dryness and the hydrochloride of the amino hydantoin purified by crystallization from hydrochloric acid. The salt was very soluble and separated from this solvent in colorless corpuscular crystals which decomposed at 242–243° with effervescence leaving a solid which did not melt at 90°. The yield was 11 g.

Calc. for $C_{10}H_{12}O_3N_3Cl$: N, 16.31. Found: N, 16.22.

**4-(2,5-Dihydroxybenzyl)-hydantoin (XVII).**—Practically a quantitative yield of this hydantoin was obtained by diazotization of the above amino hydantoin. The change was effected in the following manner: 11 g. of the hydrochloride of the amino hydantoin were dissolved in 40 cc. of water and the solution cooled to 5°. A solution of 3 g. of sodium nitrite in 10 cc. of water was then added slowly, when there was an immediate reaction with separation of the diazonium salt. This deposited in the form of a light green powder. After allowing to stand for about 30 minutes, this mixture was then heated to about 60° when the diazonium salt began to undergo decomposition with evolution of nitrogen gas. Heating was continued for another half hour when the decomposition was apparently complete and the hydantoin was obtained as a red powder.

This compound gradually underwent oxidation and dissolved in sodium hydroxide forming a dark-colored solution. It dissolved in both hydrochloric and sulfuric acids giving characteristic, purple solutions. The hydantoin was insoluble in alcohol and was finally prepared for analysis. by digesting with alcohol to remove impurities. It did not possess a sharp melting point, but turned dark colored on heating and underwent partial decomposition at about 170° but did not melt below 290°. The yield was 10 g.

Calc. for $C_{10}H_{10}O_4N_2$: N, 12.61. Found: N, 12.34.

**Behavior of 4-(2,5-Dihydroxybenzyl)-hydantoin when Heated with Barium Hydroxide and Sulfuric Acid.**—Our attempts to obtain 2,5-dihydroxyphenylalanine (XVI) from this hydantoin were unsuccessful. The hydantoin is extremely unstable in alkaline and also acid solutions, apparently undergoing oxidation and hydrolysis under both conditions to a dark-colored, amorphous product. For example, in one experiment 2 g. of the hydantoin were digested with 15 g. of barium hydroxide in 75 cc. of water for several days or until the evolution of ammonia had ceased. The barium was then quantitatively removed by precipitation as barium sulfate when we obtained a brown-colored solution containing apparently the desired amino acid. On concentrating this solution, however, it gradually grew darker in color, finally becoming almost black. After complete evaporation a dark brown residue was obtained which was amorphous in character and possessed no definite melting point. The substance contained nitrogen but the analytical values obtained did not indicate that we were dealing here with a definite compound.

We also heated the hydantoin with 30% sulfuric acid at 160° for 6 hours. It was completely decomposed by this treatment with formation of ammonium sulfate. Much black insoluble material was also suspended in the solution when the pressure tube was examined. The acid solution was filtered and the sulfuric acid completely precipitated as barium sulfate. After filtering off the barium sulfate a clear solution was obtained indicating the possibility of isolating here the amino acid. The solution was divided into two equal portions and one acidified with dilute hydrochloric acid. On evaporating, both gradually assumed a dark color owing to oxidation and in both cases only dark-colored, indefinite residues were obtained. The product obtained by evaporation of the aqueous solution gave a positive test with Millon's reagent, but did not give a violet-colored solution when dissolved in a solution of ferric chloride. No precipitates were obtained by adding picric acid, picrolonic and phosphotungstic acids to an aqueous solution of the hydrolytic product. Mixed with the residue obtained by evaporation of the hydrochloric acid solution, was a small amount of ammonium chloride.

**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 $C_{12}H_{11}O_6N_3$: 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.

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[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.