completely dissolves. This is generally accomplished in 1.5–2 hours. After the hydantoin is decomposed the solution is then concentrated to a volume of about 20–25 cc. and cooled, when the benzoic acid will separate. Practically 70% of the benzoic acid will deposit here and can be separated by filtration. After removal of the benzoic acid the filtrate is then concentrated to a thick syrup, by evaporation at 100°, and finally diluted with absolute alcohol. Pure hydantoin will separate immediately in a colorless, granular condition and melting, without further purification, at 217°. This is then separated by filtration and the filtrate combined with its volume of alcohol and the evaporation repeated. A syrup is generally obtained which can then be triturated with alcohol, when more hydantoin will be obtained. From ten grams of the thiohydantoin 3.0 grams of pure hydantoin can easily be obtained. This corresponds to a yield of 70% of the theoretical.

NEW HAVEN, CONN.

[CONTRIBUTIONS FROM THE SHEFFIELD LABORATORY OF YALE UNIVERSITY.]

HYDANTOINS: SYNTHESES OF 3-METHOXY-4-HYDROXY-PHENYLALANINE AND 3,4-DIMETHOXYPHENYLALANINE.

[TWENTY-SIXTH PAPER.]

BY TREAT B. JOHNSON AND ROBERT BENGIS.

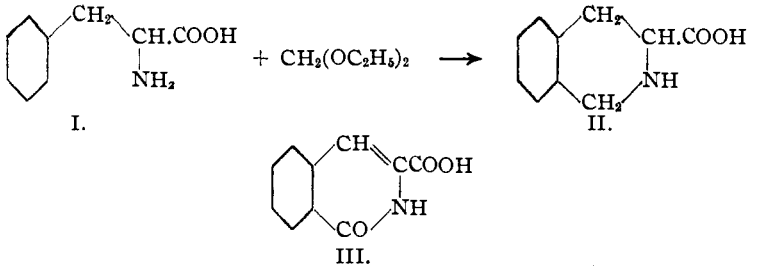
Received July 24, 1913.

The recent and important contributions, by Pictet and his co-workers, to our knowledge of the papaverine and berberine groups of the opium alkaloids have aroused an interest in hydroxy derivatives of the two aromatic α-amino acids, namely, phenylalanine and tyrosine. The naturally occurring bases of these two groups are derivatives of isoquinoline, and the results obtained by Pictet show that there is a genetic relationship between these alkaloids and aromatic α-amino acids. In other words, the evidence is strong, from a chemical standpoint, that the precursors of these alkaloids are amino acids, which result from the decomposition of plant proteins.

Isoquinoline compounds can easily be obtained from the aromatic α-amino acids or their bacterial decomposition products, namely, the corresponding β-amines. Phenylalanine (I) and tyrosine, for example, interact smoothly with methylal and formaldehyde, under proper conditions, with formation of isoquinoline carboxylic acids (II), and Pictet and Spengler[1] express the assumption, in their paper describing these changes, that the isoquinoline alkaloids may be produced in plants by similar condensations. It is of special interest to note here that Beattie[2] actually found 1-oxyisoquinoline-3-carboxylic acid (III) in the form of

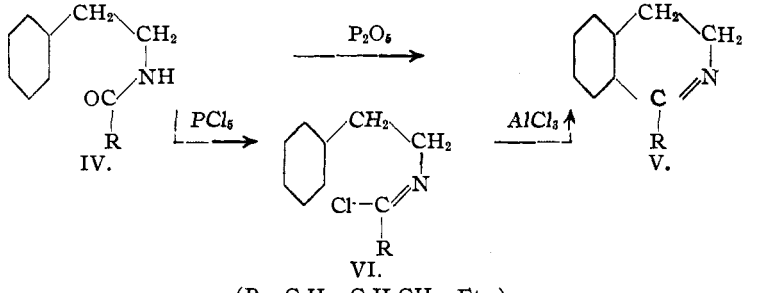
[1] *Ber.*, **44**, 2030. See also Wellisch, *Biochem. Z.*, **49**, 173 (1913).

[2] *Am. Chem. J.*, **40**, 415.



its methyl and ethyl esters, in fasciated plants of the species *Syndesmon thalictroides Hoffing.*

Acyl derivatives (IV) of the aromatic β-amines undergo inner condensations smoothly, by interaction with phosphorus pentoxide, forming isoquinoline compounds (V).[1] The reaction is not only applicable with the plain amines, but Pictet and Gams[2] also showed that it can be applied successfully with aromatic amino alcohols. In fact, by the application of this reaction, Pictet and his co-workers have been able to synthesize the naturally occurring alkaloids, namely, papaverine (XI),[3] laudanosine (XII),[4] berberine (XIV),[5] and oxyberberine (XV).[6] Decker and Kropp[7] effected this transformation of acylamines into isoquinolines by first converting them into imide chlorides (VI), by the action of phosphorus pentachloride, and then closing the chain to the isoquinoline ring (V) by the action of aluminium chloride (Friedel and Craft's reaction). These interesting transformations are represented by the following formulas:



(R = C_6H_5, $C_6H_5CH_2$, Etc.)

The structural relationship between the papaverine and berberine alkaloids is expressed by the formulas below. In this paper is given

[1] Pictet and Kay, *Ber.*, **42**, 1973.

[2] *Ber.*, **43**, 2384.

[3] Pictet and Gams, *Arch. sci. phys. nat.*, **30**, 476; *Compt. rend.*, **149**, 210; *Ber.*, **42**, 2943.

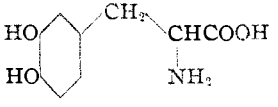
[4] Pictet and Finkelstein, *Compt. rend.*, **148**, 925; *Ber.*, **42**, 1979; Pictet, *Compt. rend.*, **131**, 689.

[5] Pictet and Gams, *Compt. rend.*, **153**, 386; *Ber.*, **44**, 2480.

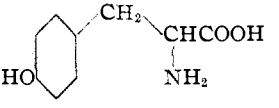
[6] Pictet and Gams, *Compt. rend.*, **152**, 1102; *Ber.*, **44**, 2036.

[7] *Ber.*, **42**, 2075.

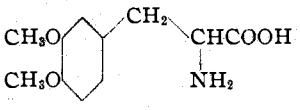
a description of the syntheses and properties of dimethoxyphenylalanine (IX) and the corresponding monomethoxy compound (X). The relationship between these acids and papaverine and laudanosine is apparent by inspection of the formulas of these compounds. The dihydroxy acid (VII) has been synthesized by Funk.[1]



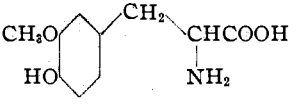
VII.



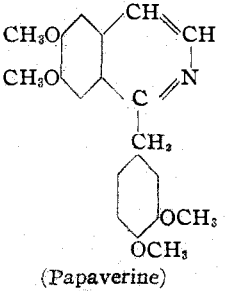
(Tyrosine)
VIII.



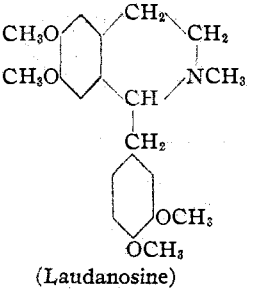
IX.



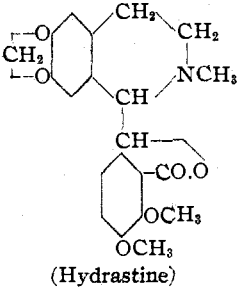
X.



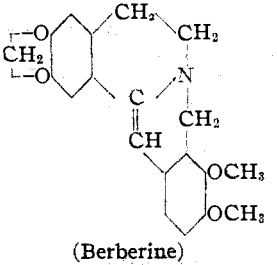
(Papaverine)
XI.



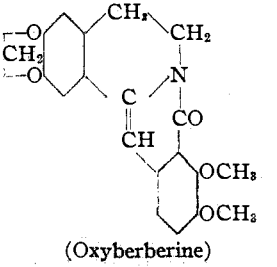
(Laudanosine)
XII.



(Hydrastine)
XIII.



(Berberine)
XIV.



(Oxyberberine)
XV.

These two amino acids (IX) and (X) were obtained by the application of a method for synthesizing α-amino acids, which was developed in this laboratory.[2] This method involves the condensation of an aldehyde with hydantoin, followed by a reduction of the condensation-product to the hydantoin of the corresponding α-amino acid. The amino acid is then formed by hydrolysis of its hydantoin with alkali. The starting points

[1] *J. Chem. Soc.*, 99, 554 (1911).

[2] See addenda to paper by Johnson and Scott, THIS JOURNAL, 35, 1130.

in this work were vanillin (XVI) and methylvanillin or 3,4-dihydroxybenzaldehyde (XVIII).

These two aldehydes condensed smoothly with 2-thiohydantoin[1] in acetic acid solution, and in the presence of sodium acetate, forming the corresponding benzalthiohydantoins (XIX) and (XXI). The yields were excellent, being 91 and 75% of the theoretical, respectively. Vanillin (XVI) also condenses with hydantoin, forming the benzal derivative (XVII), but the yield of this product was much poorer than that obtained when thiohydantoin was used for the condensation. The thiobenzalhydantion (XIX) is converted quantitatively into the oxygen compound (XVII) by desulfurization with chloracetic acid.

Especially interesting was the abnormal behavior of the thiohydantoin (XIX) when reduced with sodium amalgam. The thiohydantoin (XXII) was not obtained as expected, but instead the thiohydantoic acid (XXIII) was formed. On the other hand, the thiohydantoin (XXI) underwent reduction with sodium amalgam without hydrolysis and 3,4-dimethoxybenzylthiohydantoin (XXIV) was formed. The yield was excellent.

Johnson and O'Brien[2] observed that 4-benzal-2-thiohydantoin can be converted into phenylalanine by digestion with tin and hydrochloric acid. The 4-hydroxy-3-methoxybenzalhydantoin (XIX) is likewise desulfurized when reduced with tin and hydrochloric acid. It was converted smoothly by these reagents into 3-methoxy-4-hydroxybenzylhydantoin (XX). This same hydantoin is also formed by reduction of 3-methoxy-4-hydroxybenzalhydantoin (XVII) with tin and hydrochloric acid. It is an interesting fact that the thiohydantoin (XIX) is also converted into the hydantoin (XX) by digestion with stannous chloride in hydrochloric acid solution. 3,4-Dimethoxybenzyl-2-thiohydantoin (XXIV) was transformed quantitatively into its corresponding oxygen derivative (XXV) by digestion with chloroacetic acid.

In order to obtain the amino acids (X) and (IX) their corresponding hydantoins (XX) and (XXV) were subjected to hydrolysis with strong barium hydroxide solution when the hydantoin ring was ruptured and the barium salts of the amino acids were formed. After precipitation of the barium as sulfate, the free acids were then obtained by concentration of their aqueous solutions. A description of these compounds is given in the experimental part of this paper.

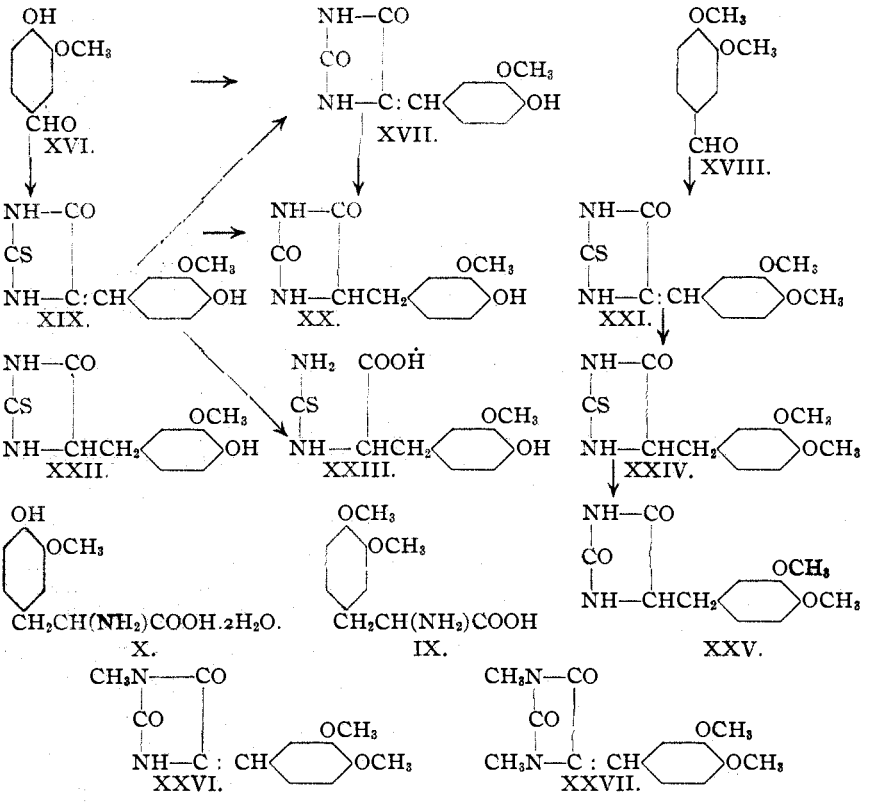
We also investigated the action of methyl iodide on 3-methoxy-4-hydroxybenzalhydantoin (XVII) in the presence of alkali. Two products were isolated, namely, 1-methyl-3,4-dimethoxybenzalhydantoin (XXVI) and 1,3-dimethyl-3,4-dimethoxybenzalhydantoin (XXVII). The hydantoin (XXVII), however, was the chief product of the reaction. We

[1] Johnson and Nicolet, THIS JOURNAL, **33**, 1973.

[2] *J. Biol. Chem.*, **12**, 205.

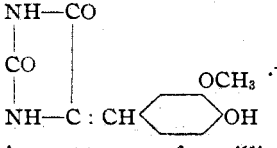
hope to be able to continue the investigation of these interesting methyl derivatives, especially the hydantoin (XXVII).

These various transformations are represented by the following structural formulas.



The investigation of derivatives of the amino acid tyrosine will be continued.

Experimental Part.

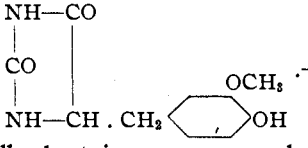
The Condensation of Vanillin with Hydantoin, 4-(3-Methoxy-4-hydroxybenzal)-hydantoin,  .—This new hydantoin was prepared by condensing 29 grams of vanillin with 19.1 grams of hydantoin in the presence of 50 grams of anhydrous sodium acetate and 80 cc. of glacial acetic acid. After heating the mixture in an oil bath at 175–180° for 6.5 hours a clear solution was obtained, which completely solidified on cooling. The reaction product was then disintegrated by digestion

with a large excess of hot water, when the hydantoin was obtained as a yellow powder insoluble in cold water. The hydantoin is soluble in glacial acetic acid and alcohol and is precipitated from both solvents by addition of water. It was purified for analysis by long digestion with water and finally by crystallization from dilute acetic acid. It separated in a granular condition and melted to an oil at 264–265° with slight effervescence. It was dried for analysis at 130°.

Nitrogen determination (Kjeldahl):

Calculated for $C_{11}H_{10}O_4N_2$: N, 11.96; found, 11.87, 11.97.

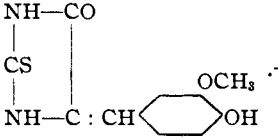
Reduction with Tin and Hydrochloric Acid, 4-(3-Methoxy-4-hydroxybenzyl)-hydantoin,



.—Two and one-tenth grams of the above benzalhydantoin were suspended in 60 cc. of 95% alcohol and, after adding 4.0 grams of granulated tin, the alcohol was saturated with hydrochloric acid gas. After warming for 2 hours on the steam bath the reduction was still incomplete and 4 grams more of tin were added and the reduction continued for 6–8 hours. Finally in order to complete the reduction 4 grams of tin were again added and hydrochloric acid gas conducted into the warm solution until the benzal compound completely dissolved. We obtained a light yellow solution which we evaporated to dryness to remove all excess of acid. The residue was then dissolved in water and the tin precipitated as sulfide by saturating the solution with hydrogen sulfide. After filtering off the tin sulfide, the solution was then concentrated and cooled, when the hydantoin separated in characteristic barrel-shaped prisms. They melted at 194–195° to a yellow oil. The hydantoin was dried for analysis at 125°. Nitrogen determination (Kjelhahl):

Calculated for $C_{11}H_{12}O_4N_2$: N, 11.86; found, 11.97.

The Condensation of Vanillin with 2-Thiohydantoin. 2-Thio-4-(3-methoxy-4-hydroxybenzal)-hydantoin,



.—This new hydantoin was formed by digesting 7.8 grams of 2-thiohydantoin,[1] 10 grams of vanillin and 20 grams of anhydrous sodium acetate with 30 cc. of glacial acetic acid for 5 hours at 157–167°. Within 15 minutes a clear solution was obtained and the condensation-product had begun to separate. After cooling, and thorough disintegration with hot water we obtained 15.5 grams of the crude hydantoin or 91% of the theoretical yield. The

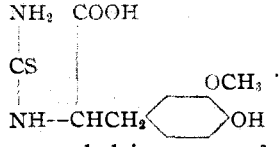
[1] Johnson and Nicolet, *Loc. cit.*

compound was purified by crystallization from dilute alcohol and separated, on cooling, in beautiful, slender, yellow needles, which melted at 232–233° to a yellow oil. In a second experiment the same proportions were taken, but the mixture was heated only 30 minutes at 165°. Within twenty minutes the mixture completely solidified in the hot oil bath. After disintegration with water, we obtained 14 grams of the pure hydantoin melting at 232–233°. Therefore, the reaction is practically complete as soon as the condensation-product has separated from the acid solution and longer heating is unnecessary. This hydantoin dissolves in concentrated sulfuric acid giving a blood-red solution and dissolves in cold sodium hydroxide solution giving a yellow solution, which dyes the skin yellow. It was dried for analysis at 110°. Nitrogen determination (Kjeldahl):

Calculated for $C_{11}H_{10}O_3N_2S$: N, 11.20; found, 11.38.

Desulfurization of 2-Thio-4-(3-methoxy-4-hydroxybenzal)-hydantoin.—Two hundred grams of chloroacetic acid were dissolved in 600 cc. of water and 25 grams of the benzalhydantoin suspended in the solution. This mixture was then heated in an oil at 130–135° for 11 hours, when the solution was filtered hot to remove a trace of unaltered hydantoin. On cooling, 4-(3-methoxy-4-hydroxybenzal)-hydantoin separated in the form of radiating prisms which melted sharply at 265–266° to an oil. The yield was 20.7 grams or 89% of the theoretical yield. The hydantoin did not give a test for sulfur.

Reduction of 2-Thio-4-(3-methoxy-4-hydroxybenzal)-hydantoin with Sodium Amalgam. 3-Methoxy-4-hydroxybenzylthiohydantoic Acid,



—Eight grams of the benzalthiohydantoin were suspended in 50 cc. of water and 3 cc. of dilute sodium hydroxide solution were then added. To this mixture, kept at 80°, were added 90 grams of 3% sodium amalgam in four portions at intervals of one-half hour. Within one hour the thiohydantoin had completely dissolved, giving a pale yellow solution. After heating at 80° for 8 hours the solution was then diluted with water and acidified with hydrochloric acid. No precipitate was obtained, but on standing the thiohydantoic acid finally deposited. More of the same compound was obtained by concentrating the filtrate and cooling. This acid is insoluble in ligroin, slightly soluble in benzene and crystallizes from glacial acetic acid in square plates or tables. It was purified for analysis by crystallization from hot water and separated in long, yellow prisms, which melted at 181–182° with effervescence. The compound was dried for analysis at 115°.

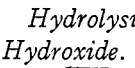
Calculated for $C_{11}H_{14}O_4N_2S$: N, 10.37; found, 10.43, 10.52.

The Conversion of 2-Thio-4-(3-methoxy-4-hydroxybenzal)-hydantoin into 4-(3-Methoxy-4-hydroxybenzyl)-hydantoin.—1. By Reduction with Tin and Hydrochloric Acid.—Five grams of the 2-thiohydantoin and 9.6 grams of granulated tin were suspended in 75 cc. of 95% alcohol and the mixture saturated with hydrochloric acid gas for 2.5 hours. During this treatment the alcohol was warmed on the steam bath. We obtained a light yellow solution. After allowing to stand for 5–6 hours two more molecular proportions of granulated tin were added and hydrochloric acid gas passed into the warm solution for 3 hours. The solution was then filtered, evaporated to dryness at 100° and the residue dissolved in about 400 cc. of hot water. The tin was precipitated as sulfide by saturating the solution with hydrogen sulfide gas. After filtering from the sulfide, the solution was then concentrated to a volume of 10–15 cc. and cooled when the characteristic, barrel-shaped crystals of 4-(3-methoxy-4-hydroxybenzyl)-hydantoin separated and melted without further purification at 190° with decomposition. After crystallizing from hot water, they melted at 193°. A mixture of this with some of the hydantoin described above melted at the same temperature. The compound did not give a test for sulfur and when mixed with some of the thiohydantoic acid, described above, the melting point was lowered to 160–161°. It was dried for analysis at 125°.

Calculated for $C_{11}H_{12}O_4N_2$: N, 11.86; found, 11.45.

2. By Reduction with Stannous Chloride and Hydrochloric Acid.—In this experiment 5 grams of the thiobenzalhydantoin were dissolved in 75 cc. of 95% alcohol and 5.5 grams of crystallized tin chloride added to the solution. After passing hydrochloric acid gas into the solution for 2 hours and then warming for 6 hours at 100°, the alcohol and hydrochloric acid were evaporated. The residue was then dissolved in water, the tin precipitated as usual as tin sulfide and the solution concentrated and cooled. 4-(3-Methoxy-4-hydroxybenzyl)-hydantoin separated and melted at 194–195°. It did not give a test for sulfur and the yield was excellent.

Hydrolysis of 4-(3-Methoxy-4-hydroxybenzyl)-hydantoin with Barium Hydroxide. 3-Methoxy-4-hydroxyphenylalanine,



.—This hydantoin is very stable in the presence of alkali, but undergoes complete hydrolysis if heated with an excess of barium hydroxide for a long time. One gram of the hydantoin and 8.0 grams of the hydroxide were dissolved in 25 cc. of boiling water and the mixture boiled in a Kjeldahl flask for 25 hours. Ammonia was evolved and barium carbonate separated. The barium was removed by adding the required quantity of sulfuric acid and the solution of the amino acid concentrated to a small volume and cooled. The

acid separated in the form of cigar-shaped crystals, which melted at 224° with effervescence. It was purified by crystallization from hot water and separated as slender prisms, which melted at 255–256° with effervescence. The acid dissolves immediately in dilute hydrochloric acid and ammonia, and gives Millon's test. A microphotograph of the crystals of this acid is shown here (Plate I). The yield of the amino acid was 0.6 gram or 65% of the theoretical. The acid contained water of crystallization and was dried, for analysis, in a desiccator over concentrated sulfuric acid. The water was determined by heating at 105–110°.

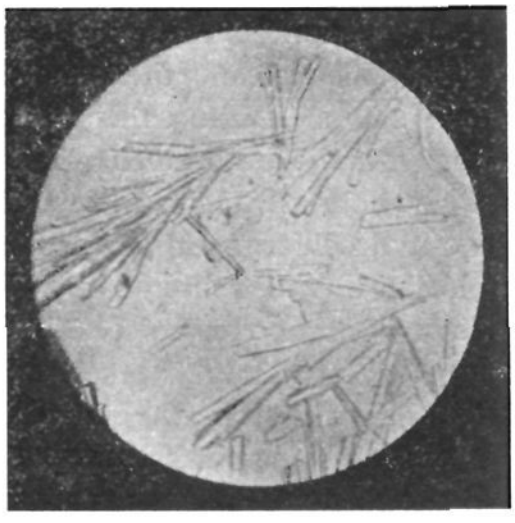


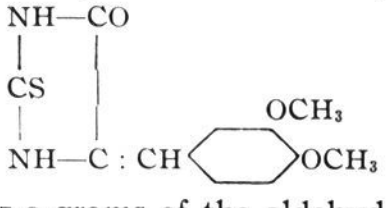
PLATE I.—Crystals of 3-Methoxy-4-hydroxyphenylalanine.

Calculated for $C_{10}H_{13}O_4N.2H_2O$: H_2O, 14.57; found: 14.55.

Nitrogen determination (Kjeldahl) in the anhydrous acid:

Calculated for $C_{10}H_{13}O_4N$: N, 6.63; found: 6.75.

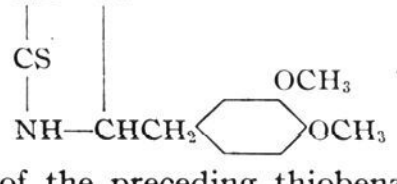
Condensation of 3,4-Dimethoxybenzaldehyde (Methylvanillin) with 2-Thiohydantoin. 2-Thio-4-(3,4-dimethoxybenzal)-hydantoin,



.—This hydantoin was formed by digesting 7.0 grams of the aldehyde, 5.0 grams of thiohydantoin and 15.0 grams of fused sodium acetate with 21 cc. of glacial acetic acid. After heating one hour at 165–170° the solution was cooled and diluted with water, when the hydantoin deposited in prismatic crystals. The yield was 8.3 grams or 75% of the theoretical. The hydantoin was purified by crystallization from 95% alcohol and separated, on cooling, in prisms, which melted at 229–230° to an oil. A mixture of this compound with 2-thiohydantoin (m. 227°) melted at 202–203°. It was dried for analysis at 115°.

Calculated for $C_{12}H_{12}O_3N_2S$: N, 10.60; found: 10.58.

2-Thio-4-(3,4-dimethoxybenzyl)-hydantoin,

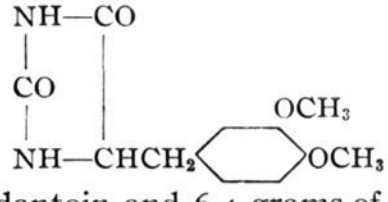


.—This compound was obtained by reduction of the preceding thiobenzalhydantoin with sodium amalgam. Seven grams of the hydantoin were suspended in a mixture of 50 cc. of water and 2 cc. of dilute sodium hydrox-

ide solution. Sixty grams of 3% sodium amalgam were then introduced in ten gram portions, at intervals of 30 minutes, while the solution was heated at 80° on a hot plate. As was observed in other reductions, the solution assumed a light color as the reaction proceeded and after 7 hours the reduction was apparently complete. The solution was then acidified with hydrochloric acid when the hydantoin separated as an oil, which finally solidified. This melted at 102–103° to a turbid oil. This substance was extremely soluble in 95% alcohol and acetic acid. It dissolved in hot water and separated on cooling as an oil, which soon crystallized. The hydantoin contained water of crystallization, which was determined by heating at 110°. The anhydrous material melted at 102–103° to a viscous, turbid oil, which finally became transparent when heated to 120–125°. The yield was 5.8 grams or 77% of the theoretical.

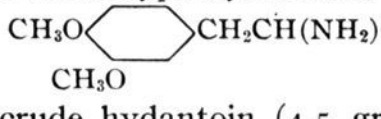
Calculated for $C_{12}H_{14}O_3N_2S.H_2O$: H_2O, 6.38; N, 9.93.
Found: H_2O, 6.24; N, 9.96, 9.91.

Desulfurization of 2-Thio-4-(3,4-dimethoxybenzyl)-hydantoin. 4-(3,4-Dimethoxybenzyl)-hydantoin,



.—Four and eight-tenths grams of the thiohydantoin and 6.4 grams of chloroacetic acid were dissolved in 10 cc. of water and the solution boiled for 5 hours. This mixture was then diluted with alcohol and finally evaporated at 100° when a thick syrup was obtained. This was again dissolved in alcohol and the evaporation repeated when a thick syrup was obtained, which did not solidify. Efforts were made to effect crystallization from different solvents and by allowing to stand in a desiccator over concentrated sulfuric acid. After standing for 12 days it was still an oil and consequently was used for the following experiment:

Hydrolysis of the above Crude Hydantoin with Barium Hydroxide. 3,4-Dimethoxyphenylalanine,



. — The crude hydantoin (4.5 grams) and 23 grams of barium hydroxide were digested with 20 cc. of water until the evolution of ammonia ceased. The mixture was then diluted with 500 cc. of hot water and the barium precipitated by adding the required amount of sulfuric acid. After filtering from barium sulfate, the solution was then

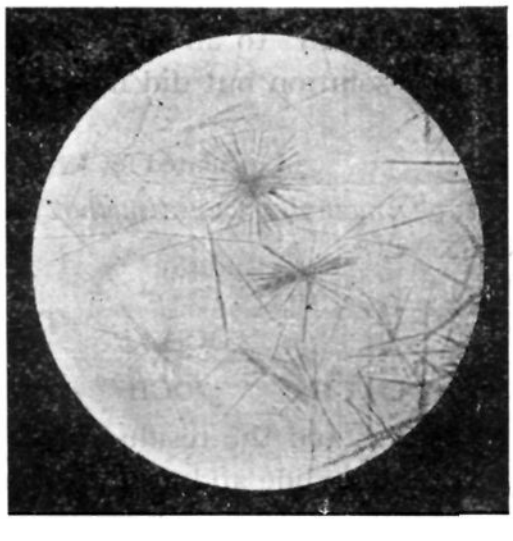
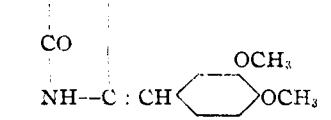


PLATE II.—Crystals of 3,4-Dimethoxyphenylalanine.

concentrated to a volume of 10 cc. and cooled. This new amino acid separated in hair-like crystals, which were grouped in sheaves similar in appearance to that of tyrosine. A microphotograph of the crystals of this acid is shown in Plate II. They melted at 249–250° with effervescence. The acid is very soluble in water and alcohol and does not give Millon's test, showing the absence of phenolic hydroxyl groups. The acid did not contain water of crystallization. It was dried for analysis by heating at 110° for 2.5 hours.

Calculated for $C_{11}H_{15}O_4N$: N, 6.22; found: 6.18.

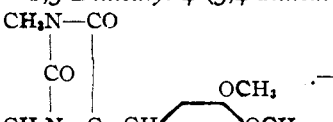
Alkylation of 4-(3-Methoxy-4-hydroxybenzal)-hydantoin with Methyl Iodide. 1-Methyl-4-(3,4-Dimethoxybenzal)-hydantoin,



.—Nineteen and eight-tenths grams of finely pulverized potassium hydroxide (4 molecular proportions) were dissolved in 150 cc. of 95% alcohol. Twenty and seven-tenths grams of the hydantoin were then added, when a bright yellow potassium salt separated immediately. The flask having been connected to a long reflux condenser, 56.3 grams of methyl iodide (4.5 molecular proportions) were added in small portions at a time. A reaction set in immediately, and great precaution had to be taken that the iodide was added slowly, so as to prevent too great evolution of heat and loss of the iodide. After all the iodide was added, the mixture was then heated for 3 hours on the steam bath until neutral to litmus and turmeric. On cooling, the monomethylhydantoin separated and was filtered off. The filtrate was saved for further examination (see below). The crude hydantoin was triturated with cold water to remove traces of inorganic material and finally purified by crystallization from alcohol. It separated in prismatic crystals, which melted at 218° to an oil. The hydantoin dissolved in dilute sodium hydroxide solution but did not give Millon's test. It was dried for analysis at 105°.

Calculated for $C_{12}H_{14}O_4N_2$: N, 10.69; found: 10.76.

1,3-Dimethyl-4-(3,4-dimethoxybenzal)-hydantoin,



.—The alcohol filtrate, above, was evaporated to dryness and the residue triturated first with cold water to remove any potassium iodide and then with a cold dilute solution of sodium hydroxide to remove any unaltered or monomethylhydantoins. We obtained in this manner this dimethyl derivative. It was insoluble in cold water

and soluble in alcohol. It was purified by crystallization from 95% alcohol and separated in distorted prisms, which melted at 122–124°, to an oil. It did not give Millon's test and dissolved in concentrated sulfuric acid, forming a bright red solution. It was dried for analysis at 105°.

Calculated for $C_{14}H_{16}O_4N_2$: N, 10.14; found: 10.13.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY, No. 224.]

STUDIES ON AMYLASES, V. EXPERIMENTS UPON THE PURIFICATION OF THE AMYLASE OF MALT.

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Many of the investigators of malt diastase have interested themselves only in its action, but a number have given more or less attention to the problem of the chemical nature of the enzyme substance. Among these may be mentioned in chronological order, Dubrunfaut,[1] Zulkowski,[2] Loew,[3] Lintner,[4] Hirschfeld,[5] Szilagyi,[6] Egoroff,[7] Osborne,[8] Wroblewski,[9] Seyffert,[10] Sykes and Hussey,[11] Friedenthal,[12] Frankel and Hamburg,[13] Hata,[14] Chrzaszez,[15] Wohl and Glimm,[16] Buroczewski, Krause and Krzemecki,[17] Lyalin,[18] Pribram,[19] Panzer,[20] Van Laer.[21]

Of these investigations that of Osborne is probably the most significant

[1] *Compt. rend.*, **66**, 274–5 (1868), and *Dingler's Polytech. J.*, **187**, 491–501 (1868).

[2] *Wien. Akad.*, **77**, II, 647–55; *Jahr. Thierchem.*, **8**, 356 (1878).

[3] *Pflüger's Arch. ges. Physiol.*, **27**, 203–14 (1882); also **36**, 170 (1885); *J. prakt. Chem.*, [2] **37**, 101–4 (1888).

[4] *J. prakt. Chem.*, [2] **34**, 378–94 (1886); **36**, 481–98 (1887); also *Z. ges. Brauw.*, **1886**, 479, 481; **1888**, 80; and *Woch. Brau.*, **16**, 166 (1899).

[5] *Arch. ges. Physiol.*, **39**, 499–574 (1886); see also reply by Lintner, *Ibid.*, **40**, 311–14 (1887).

[6] *Chem. Ztg.*, **15**, 349–51 (1891).

[7] *J. Rousskago Phys. Chem. Obchtchestva*, **25**, No. 2; *Mon. Sci.*, [4] **8**, II, 741–2 (1894).

[8] THIS JOURNAL, **17**, 587–603 (1895); **18**, 536–42 (1896); also *Ber.*, **31**, 254–9 (1898).

[9] *Ber.*, **30**, 2289–95 (1897); **31**, 1127–30, 1130–36 (1898); *Z. physiol. Chem.*, **24**, 173–223 (1897).

[10] *Z. ges. Brauw.*, **21**, 195–7, 221–3 (1898).

[11] *J. Fed. Inst. Brew.*, **4**, 527 (1898).

[12] His-Engelmann's *Arch. Anat. Physiol., Physiol. Abth.*, **1900**, 181–94.

[13] *Hofmeister's Beitr.*, **8**, 389 (1906).

[14] *Biochem. Z.*, **17**, 156–87 (1909).

[15] *Wochschr. Brau.*, **27** and **29**; also *Z. Spiritusind.*, **34**, 545; *Chem. Abs.*, **6**, 1050 (1912).

[16] *Biochem. Z.*, **27**, 349–75 (1910).

[17] *Bull. intern. acad. sci. Cracovie*, (*A*) **1911**, 369–70; *Chem. Abs.*, **6**, 1757 (1912).

[18] *J. Russ. Phys. Chem. Soc.*, **42**, 624–33; *Chem. Abs.*, **5**, 3833 (1911).

[19] *Biochem. Z.*, **44**, 293–302 (1912).

[20] *Z. physiol. Chem.*, **82**, 276–325, 377–90; **84**, 161–88 (1912–13).

[21] *Bull. Acad. roy. Belg.* (*Classe des sciences*), **1913**, No. 4 (April), 396–451.