Lyman and Reid to require 300 parts of boiling 95% alcohol for its solution, while these mixed esters dissolve in from 9 to 30 parts of alcohol containing much more water.

Since mono-menthyl phthalate melts at 110°, it is surprising that the menthyl *p*-nitrobenzyl ester is an oil.

Summary.

Alcohols may be heated with phthalic anhydride to give acid phthalic esters and the sodium salts of these heated with *p*-nitrobenzyl bromide to give mixed phthalic esters which may be used for identification of the alcohols. The following mixed esters have been made:

Methyl *p*-nitrobenzyl phthalate, $CH_3CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 105.7°;

Ethyl *p*-nitrobenzyl phthalate, $C_2H_5CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 80°;

Propyl *p*-nitrobenzyl phthalate, $C_3H_7CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 53.0°;

Isopropyl *p*-nitrobenzyl phthalate, $C_3H_7CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 74.0°;

Allyl *p*-nitrobenzyl phthalate, $C_3H_5CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 61.5°;

n-Butyl *p*-nitrobenzyl phthalate, $C_4H_9CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 62.0°;

n-Octyl *p*-nitrobenzyl phthalate, $C_8H_{17}CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 41.0°;

Benzyl *p*-nitrobenzyl phthalate, $C_6H_5CH_2CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 83.0°;

Phenylethyl *p*-nitrobenzyl phthalate, $C_6H_5CH_2CH_2CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 84.3°;

Bornyl *p*-nitrobenzyl phthalate, $C_{10}H_{17}CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 100°;

Isobornyl *p*-nitrobenzyl phthalate, $C_{10}H_{17}CO_2.C_6H_4.CO_2CH_2C_6H_4NO_2$, m. 87°.

BALTIMORE, MARYLAND.

[CONTRIBUTION FROM THE SHEFFIELD CHEMICAL LABORATORY OF YALE UNIVERSITY, AND THE CHEMICAL LABORATORY OF MOUNT HOLYOKE COLLEGE.]

RESEARCHES ON HYDANTOINS.

XXXIX. SYNTHESIS OF THE POLYPEPTIDE-HYDANTOIN; TYROSYL-GLYCINE-HYDANTOIN.

BY TREAT B. JOHNSON AND DOROTHY A. HAHN.

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It has been shown in a previous paper from the Sheffield Laboratory[1] that the silk-protein-fibroin undergoes hydrolysis with acids with production of carbon dioxide. The amount that is evolved in this particular

[1] Johnson, *Proc. Nat. Acad. Sci.*, **2**, 69 (1916).

case represents about 1% of the protein taken and with other proteins the quantity has been shown to be much larger. In other words, we are dealing here with a hydrolytic product which plays an important part in the construction of the protein molecule, and the idea has been advanced by the writer that it may result from the breaking down of characteristic peptide combinations, which are linked in the protein molecule in the form of hydantoins. Such combinations of α-amino acids, in which the polypeptide grouping is preserved, are members of a new type of compounds to which we have assigned the class name—*polypeptide-hydantoins*. The first representative of this series to be prepared synthetically was phenylalanylglycine-hydantoin which was described by Johnson and Bates[1] in our second paper on this subject. We have now been able to extend this field of investigation and in this paper will discuss the synthesis of tyrosylglycine-hydantoin (VIII) and also describe several derivatives of this interesting compound. The principal reason why this particular hydantoin was selected for investigation was the fact that the two α-amino acids incorporated in its molecule, *viz.*, glycocoll and tyrosine, constitute a large proportion of the fibroin molecule.

The starting point in our research was 4-anisalhydantoin[2] (III), which, on reduction, is transformed easily and smoothly into the corresponding anisyl derivative (II).[3] Both of these hydantoins undergo alkylation when treated with ethyl chloroacetate in alkaline solution, giving the corresponding acetate derivatives represented by Formulas VI and V, respectively. In both hydantoins the place of substitution is Position 1 of the ring; a fact which is of great interest because the closely related dioxypyrimidines interact under similar conditions giving mixtures. In these latter combinations the molecule is susceptible to attack in two positions and it has been our experience that 1 and 3 substitution products are both formed by alkylation. The hydantoin ester (V) is also formed by reduction of the unsaturated hydantoin (VI).

The polypeptide-hydantoin (VIII) (tyrosylglycine-hydantoin) can be prepared from the saturated and unsaturated hydantoin esters (V) and (VI), or their corresponding acids (I) and (IX). When the hydantoin (VI) or its acid (IX) is used the transformation is easily accomplished by means of hydriodic acid and also by reduction with tin and hydrochloric acid. The ease with which the methoxy grouping is hydrolyzed here is quite remarkable. Ethyl 4-anisylhydantoin-1-acetate (V) undergoes conversion into the polypeptide-hydantoin by digestion with either hydriodic or hydrobromic acids. When hydrobromic acid was used for hydrolysis we were able to isolate an intermediate product which we have proved to be

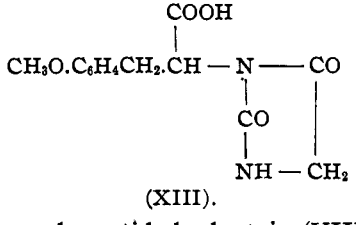
[1] THIS JOURNAL, 38, 1087 (1916).

[2] Wheeler and Hoffmann, *Am. Chem. J.*, 45, 375 (1911).

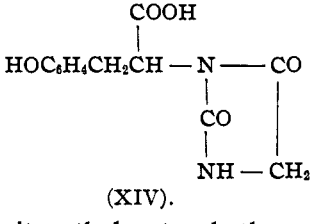
[3] Johnson and Nicolet, *Ibid.*, 47, 474 (1912).

the ethyl ester represented by Formula X. In other words, we are dealing here with a condition where a methoxy group in the benzene nucleus is more easily hydrolyzed by the hydrobromic acid than the ester grouping in the hydantoin. This unique behavior was wholly unexpected and explains why we previously were unable to determine positively the structure of our combinations by testing for the phenol group with Millon's reagent. It has been our experience before that Millon's reagent cannot always be relied upon to distinguish between phenols and their corresponding ethers. Our observations lead to the conclusion that the amino-acid tyrosine may be linked in a protein through its phenol oxygen as well as by a polypeptide linking. Such combinations might easily give tyrosine on hydrolysis with acids and also respond positively to Millon's test for a free phenol hydroxyl group.

The hydantoin acetates (V) and (VI) are both easily hydrolyzed by the action of acids and alkali to their corresponding acids represented by Formulas I and IX, respectively. The hydantoin (IX) is a stable combination in the presence of both hydrolytic agents while the ring in the hydantoin (I) is easily ruptured by the action of alkali giving the dipotassium salt represented by Formula IV. When this salt is decomposed in aqueous solution by acids the dibasic acid (VII) is formed but condenses immediately on warming to the cyclic combination (I). In order to obtain the open chain acid (VII) in a state of purity, it was necessary to decompose its dipotassium salt with hydrochloric acid gas in an anhydrous solvent. We did not detect any tendency for the urea acid (VII) to undergo a molecular condensation with formation of the hydantoin (XIII) isomeric with the hydantoin (I).



(XIII).

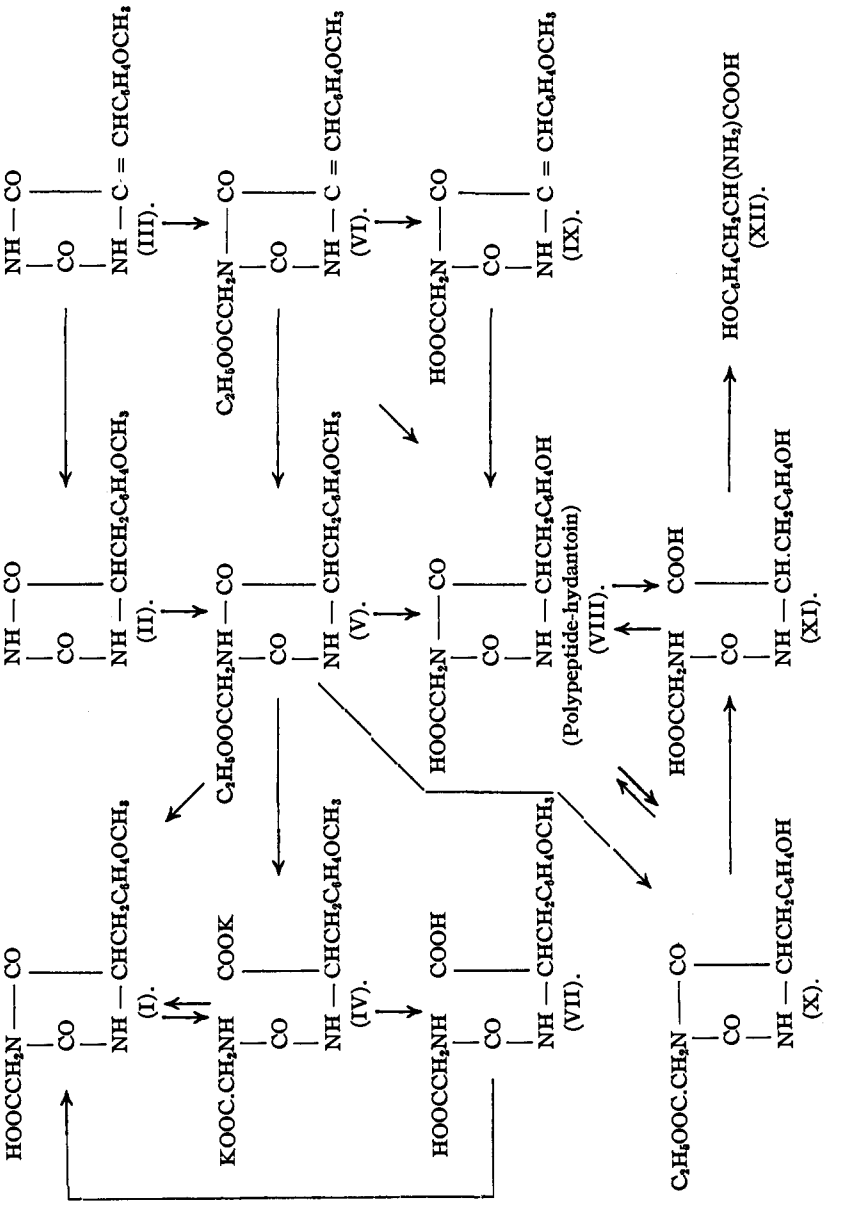


(XIV).

The polypeptide-hydantoin (VIII) and its ethyl ester both undergo hydrolysis by treatment with the required amount of potash, giving the dipotassium salt of the urea (XI). The free acid is easily obtained by decomposing this salt in an anhydrous solvent with dry hydrochloric acid gas. The acid is less stable than the corresponding methoxy derivative represented by Formula VII. It cannot be recrystallized from water, without partial conversion into the polypeptide-hydantoin (VIII). The acid is easily converted into this hydantoin by heating above the temperature of its melting point, and also by allowing it to heat on a hot plate for a long time at 100°. Here also it is important to call attention to the

fact that there was no apparent tendency for this dibasic acid to undergo a molecular condensation with formation of glycyltyrosine-hydantoin represented by Formula XIV.

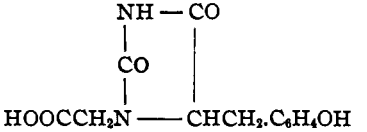
Morel[1] has investigated the action of the isocyanate—$OCNCH_2COO$-



[1] *Compt. rend.*, **143**, 119 (1906).

C_2H_5—on tyrosine in the presence of alkali and has shown that they interact to give a crystalline substance to which he assigned the structure of our urea represented by Formula XI. He states that his product melted at 214°, but he gave no analytical data to support his conclusions. This melting point is only 3° lower than that of our polypeptide-hydantoin (VIII) while the urea acid (XI), according to our experience, melts several degrees higher at 220–224°. In the light of the results obtained by us it seems very probable that Morel was dealing with the polypeptide-hydantoin (VIII) instead of the urea acid as he represented structurally in his paper.

The structure of the polypeptide-hydantoin (VIII), and incidentally that of its derivatives described in this paper, was established by its behavior on intense hydrolysis with hydrochloric acid. The hydantoin was decomposed without formation of ammonia. Carbon dioxide was evolved and a yield of tyrosine was obtained, which corresponded to 80% of the theoretical. The important fact that no ammonia is produced by this hydrolysis is conclusive proof that the acetic acid group occupies Position 1 of the hydantoin ring in the polypeptide (VIII) and not Position 3 as is represented in an isomeric hydantoin represented by Formula XV.



(XV).

Our researches on polypeptide-hydantoins will be continued.

Experimental Part.

4-Anisalhydantoin (III) was prepared from methylene-amino-acetonitrile by converting it into ethyl aminoacetate[1] and treating the latter with potassium cyanate.[2] The resulting ethyl hydantoate was changed into hydantoin by boiling with a 25% solution of hydrochloric acid. The final transformation to anisalhydantoin was effected by condensing this hydantoin with anisic aldehyde according to the method described by Wheeler and Hoffman.[3]

4-Anisylhydantoin (II) was prepared from anisalhydantoin by reduction with tin and hydrochloric acid in alcohol solution. The procedure, outlined by Johnson and Nicolet,[3] was modified slightly. It was found that when one equivalent of tin was incorporated instead of five that somewhat higher per cent. yields ofthe reduction product were obtained. The presence of large quantities of stannic chloride in solution seemed to hinder the separation of the final product. In a series of experiments a yield of

[1] Klages, *Ber.*, 36, 1506 (1903).

[2] *Ber.*, 33, 3419 (1908).

[3] *Loc. cit.*

90% of crude product was obtained, and one recrystallization from 95% alcohol generally gave a substance melting sharply at 182°.

Ethyl 4-Anisalhydantoin-1-acetate (VI).—This was the main product of the reaction when the sodium salt of 4-anisalhydantoin was allowed to interact with ethyl chloroacetate. Thirty grams of anisalhydantoin were suspended in a solution of 3.3 g. of sodium in 300 cc. of 95% alcohol, and the mixture heated on a steam bath until the hydantoin was completely transformed into its sodium salt. This required from 4 to 6 hours. A molecular quantity of finely powdered potassium iodide was then added, and, after a few minutes had been given for it to dissolve, the calculated quantity of ethyl chloroacetate was introduced. The mixture was then heated until the solution was neutral to turmeric paper. It should be noted at this point that the introduction of potassium iodide as a catalyst is necessary for the success of this reaction since negative results were obtained in a series of experiments where no iodide was present. At the conclusion of the reaction the hot solution was filtered from the sodium chloride which had precipitated and the filtrate on cooling solidified to a colorless crystalline product consisting largely of the desired ester. This was filtered, washed with cold alcohol and recrystallized. It was found advisable to recrystallize once from acetic acid and then from alcohol, as the acetic acid was effective in removing traces of color which repeated recrystallizations from alcohol failed to remove. The ester melted at 178.° Additional quantities of this substance were obtained by concentrating the mother liquors. A maximum yield of about 40% of theoretical was obtained in a series of experiments. Analysis:

Calc. for $C_{15}H_{16}O_5N_2$: N, 9.20. Found: N, 8.85, 8.87

The ester was very soluble in hot acetic acid, and soluble in boiling alcohol. It separated from both solvents on cooling in the form of long, colorless needles. It was readily hydrolyzed to the corresponding acid by heating with hydrobromic and concentrated hydrochloric acids. When heated with hydriodic acid it was readily transformed into the polypeptide-hydantoin—*4-hydroxybenzylhydantoin-1-acetic acid* (see below).

Ethyl 4-Anisalhydantoin-1-acetate (V).—This substance may be prepared by either of two methods—namely, the alkylation of 4-anisylhydantoin with ethyl chloroacetate, and the reduction of ethyl 4-anisalhydantoin-1-acetate. The procedure in the first case was analogous to that used in the alkylation of 4-anisalhydantoin; but since anisylhydantoin was completely soluble in a given quantity of hot alcohol while anisalhydantoin remained for the most part undissolved in twice the same relative quantity of that solvent, it was possible in this case to follow the course of the reaction very closely. Twenty-five grams of anisylhydantoin were added to a hot solution of 2.7 g. of sodium in 100 cc. of alcohol.

Solution took place at once and was followed almost immediately by the precipitation of the insoluble sodium salt. To insure a complete reaction the mixture was then boiled for one hour on a steam bath. Twenty grams of potassium iodide were added, and finally 14 g. of ethyl chloroacetate were introduced. In the course of a few minutes of boiling the precipitated sodium salt was observed to disappear completly and for a short period the solution remained clear. Precipitation of sodium chloride finally began and at the end of 15 minutes the reaction was complete. The mixture was filtered while hot and the precipitate washed repeatedly with small quantities of hot alcohol. The combined filtrate and washings on cooling deposited a colorless crystalline substance, which separated in the form of colorless needles, and which, after one crystallization from hot alcohol, melted sharply at 138°. The yield of purified material was 17 g. The mother liquors when concentrated gave mixtures of this substance and unchanged 4-anisylhydantoin. Two grams of the latter were separated in pure condition.

The insoluble residue containing sodium chloride was examined for organic matter, and was found to contain a comparatively large quantity of the sodium salt of 4-anisylhydantoin-1-acetic acid. The mixture was dissolved in about 50 cc. of hot water and on cooling a large part of the sodium salt separated in the form of glistening plates. The salt was decomposed in aqueous solution by hydrochloric acid giving pure 4-anisylhydantoin-1-acetic acid (see below). The filtrate from these crystals gave additional quantities of the same acid when acidified further with concentrated hydrochloric acid. In all 4 g. of 4-anisylhydantoin-1-acetic acid were obtained. The reduction of ethyl 4-anisalhydantoin-1-acetate was accomplished by means of tin and hydrochloric acid in alcoholic solution. All attempts to effect a reduction by means of zinc and acetic acid gave negative results. The procedure was to pass dry hydrogen chloride into a hot alcoholic solution of the hydantoin to which a molecular quantity of tin had been added. The reduction took place slowly but was complete at the end of about 16 hours. During this time the separation of an insoluble substance was observed, but it redissolved as the reaction proceeded. At the end of the reaction the solution was perfectly clear while hot but deposited crystals of ethyl 4-anisylhydantoin-1-acetate on cooling. It melted at 138°. Analysis:

Calc. for $C_{16}H_{18}O_6N_2$: N. 9.15. Found: N, 9.17, 9.09

This ester is soluble in hot water and very soluble in hot alcohol, from both of which it separates on cooling in the form of colorless needles. It was readily hydrolyzed by alkali and hydrochloric acid to the corresponding acid. The effect of heating this ester with hydrobromic acid was remarkable in that the methoxy group of the benzene nucleus was

readily attacked by this reagent. The product which was obtained seemed to depend upon the relative quantities of hydrobromic acid that were used and upon the time involved in heating; and it was possible so to arrange conditions as to get excellent yields of either ethyl 4-hydroxybenzylhydantoin-1-acetate or its corresponding acid—the polypeptide-hydantoin (see below).

4-Anisalhydantoin-1-acetic Acid (IX).—This hydantoin was obtained by the hydrolysis of its ethyl ester. This was effected by heating either with hydrobromic or concentrated hydrochloric acids. Ten grams of the ester were treated with 200 cc. of hydrobromic acid and heated on a water bath for two hours. The product was evaporated to dryness, extracted once or twice with small quantities of hot alcohol to remove any traces of the original ester and finally recrystallized from acetic acid. The yield was quantitative. When hydrochloric acid was used in the hydrolysis the procedure was to suspend the ester in concentrated aqueous acid and to digest on a water bath until the ester was completely transformed. During this process, which required from 36 to 48 hours, it was necessary to saturate the solution repeatedly with hydrogen chloride gas. Both ester and acid are insoluble in concentrated hydrochloric acid but their marked differences in crystalline habit made it possible to detect the end point of the reaction easily. The acid was purified by crystallization from acetic acid. The time involved in the hydrolysis by means of hydrochloric acid makes the operation tedious but the product is colorless, while when hydrobromic acid is used the product is light straw color and it is impossible to remove this color by recrystallization from acetic acid. Analysis:

Calc. for $C_{13}H_{12}O_5N_2$: N, 10.14. Found: N, 10.01, 9.89

This acid is almost insoluble in hot water and alcohol. It is soluble in hot acetic acid from which it separates on cooling in colorless, transparent plates which melt at 271°.

4-Anisylhydantoin-4-acetic Acid (I).—The monopotassium salt of this hydantoin acid was prepared from its corresponding ester by treating the latter in alcohol solution with an equivalent amount of alcoholic potassium hydroxide. The mixture was boiled for one hour and on cooling deposited the potassium salt in the form of glistening plates. The salt is readily soluble in hot alcohol, and was purified by recrystallizing from this solvent. It melts with decomposition at about 260°. Analysis:

Calc. for $C_{13}H_{13}O_5N_2K$: N, 8.8. Found: N, 8.30, 8.32

Potassium 4-anisylhydantoin-1-acetate is readily soluble in cold water. When an aqueous solution of this salt was acidified with hydrochloric acid the free 4-anisylhydantoin-1-acetic acid deposited. The same acid was also obtained conveniently from its ethyl ester by hydrolyzing the latter

in the presence of hydrochloric acid. In this case the procedure was to dissolve the ester in a small quantity of concentrated aqueous hydrochloric acid and to evaporate the solution on a water bath to the point of crystallization. On cooling, the acid separated in the form of hard, colorless prisms. It was purified by recrystallization from water slightly acidified with hydrochloric acid. It melted at 166°. Analysis:

Calc. for $C_{13}H_{14}O_6N_2$: N, 10.07. Found: N, 10.07, 10.08

This hydantoin is very soluble in alcohol and in hot water. It is readily esterified by passing dry hydrogen chloride into its alcoholic solution, when the ethyl ester separates in crystalline form. When heated with hydriodic acid in the presence of red phosphorus the acid is demethylated and transformed into the polypeptide-hydantoin (see below). Attempts were made to prepare the above acid by reduction of 4-anisalhydantoin-1-acetic acid with zinc and acetic acid and also by means of sodium amalgam in alcoholic solution, keeping the solution slightly acid by adding acetic acid from time to time; but in both cases the results were negative. In both cases, however, an interesting observation was made which should be noted here. The acid melting at 271° on continued boiling passed into solution and from this solution on concentration a product was recovered which possessed the same appearance and crystalline habit as the original acid; but which differed in solubility and in melting point. This substance was much more soluble than the acid from which it was derived and showed no tendency to melt at 315°. It dissolved readily in a small quantity of hot concentrated hydrochloric acid, from which solution almost immediately on cooling the acid melting at 271° separated.[1]

The reduction of the double bond in 4-anisalhydantoin-1-acetic acid was finally effected by means of tin and hydrochloric acid, but the change was accompanied by demethylation and the product obtained was 4-hydroxybenzylhydantoin-1-acetic acid (the polypeptide-hydantoin). The procedure was to suspend the substance in a small quantity of concentrated hydrochloric acid, to which a molecular quantity of tin had been added, and to heat on a water bath, charging frequently with hydrogen chloride gas, until solution was complete. The polypeptide-hydantoin separated on cooling, the yield being about 70% of the theoretical.

Symmetrical *p*-Methoxyphenylalanino-glycine Urea (VII).—The dipotassium salt (IV) of this acid was prepared by treating ethyl 4-anisylhydantoin-1-acetate with two equivalents of potassium hydroxide. Four grams of the ester were dissolved in 20 cc. of 95% alcohol and 1.44 g. of potassium hydroxide in 150 cc. of alcohol were added. The mixture was

[1] The investigation of this interesting transformation will be continued by Dr. Hahn. It is probable that we are dealing here with an interesting case of geometrical isomerism. (T. B. J.)

boiled for 3 hours and filtered hot. The residue was then digested several times with small quantities of hot alcohol, in order to remove traces of the monopotassium salt which is always formed during the reaction. The salt was then dried for analysis:

Calc. for $C_{13}H_{14}O_6K_2$: N, 7.52. Found: N, 7.48, 7.60

This salt is insoluble in hot alcohol, but is readily soluble in water. Its aqueous solution when heated with concentrated hydrochloric acid and then cooled, deposited 4-anisylhydantoin-1-acetic acid melting at 166°.

The free acid or symmetrical urea was easily obtained from its dipotassium salt. The latter was suspended in dry benzene and dry hydrochloric acid gas conducted into the mixture for one hour.

The benzene was then decanted through a filter, the residue transferred to the filter and washed first with ether and then repeatedly with cold water. Neither the benzene nor the ethereal filtrates on evaporation left an appreciable residue. The insoluble substance was dried and found to consist of the open chain acid or urea, which melted at 161° with violent effervescence. The yield was quantitative. The urea was purified by crystallization from hot water. Analysis:

Calc. for $C_{13}H_{16}O_6N_2.H_2O$: N, 8.92. Found: N, 9.05, 9.07

This urea was slightly soluble in cold water and very soluble in hot water from which it separated on cooling in the form of large transparent plates. They became opaque when dried on a hot plate. When an aqueous solution of the urea was heated with concentrated hydrochloric acid it passed smoothly into 4-anisylhydantoin-1-acetic acid melting at 166°. A mixture of this hydantoin and the symmetrical urea melted between 120–140°.

The Polypeptide-hydantoin: 4-Hydroxybenzylhydantoin-1-acetic Acid. (VIII).—This compound was prepared in a variety of ways; *viz.*, from ethyl 4-anisalhydantoin-1-acetate by reduction, hydrolysis and demethylation in the presence of hydriodic acid; from the corresponding methoxy acid by reduction and demethylation with hydrogen iodide, or suspended in aqueous solution in the presence of tin and hydrochloric acid; from ethyl 4-anisylhydantoin-1-acetate by the action of hydriodic acid and also of hydrobromic acid; from the corresponding acid by the action of hydrogen iodide; and finally from ethyl 4-hydroxybenzylhydantoin-1-acetate by hydrolysis with concentrated aqueous hydrochloric acid. The most convenient procedure was to start with ethyl 4-anisylhydantoin-1-acetate and to heat it for twenty minutes on a water bath with a small quantity of hydrobromic acid. On the addition of alcohol there was an immediate separation of the demethylated ethyl ester (see below). This was filtered, washed with cold alcohol, and hydrolyzed by boiling for five minutes with concentrated aqueous hydrochloric acid. On cooling

the pure polypeptide-hydantoin separated. When this procedure was modified by adding water instead of alcohol to the solution of ethyl 4-anisylhydantoin-1-acetate in hydrobromic acid, the same product was obtained directly, but it was never so pure. In this case it was necessary to boil the aqueous solution of the substance with animal charcoal in order to remove traces of coloring matter. The polypeptide-hydantoin was purified by recrystallization from hot water from which it separated in hard, compact crystals, showing a characteristic rosette habit of formation. It melted at 217–218°. Analysis:

Calc. for $C_{12}H_{12}O_6N_2$: N, 10.6. Found: N, 10.43, 10.3

This hydantoin is slightly soluble in cold water and very soluble in hot water and alcohol. It could not be recrystallized from alcohol because of the ease with which it underwent esterification. Hydrochloric acid, passed into a saturated alcoholic solution of the acid, precipitated the pure ethyl ester.

Ethyl 4-Hydroxybenzylhydantoin-1-acetate (X).—This ester was prepared by the esterification of its acid; and also by adding alcohol to a solution of ethyl 4-anisylhydantoin-1-acetate in hydrobromic acid according to the procedure already described above. The compound was purified by crystallization from alcohol from which it separates in the form of needles which melt at 195°. The ester is soluble in hot alcohol. It is readily hydrolyzed to the polypeptide-hydantoin when treated with concentrated aqueous hydrochloric acid. Analysis:

Calc. for $C_{14}H_{16}O_6N_2$: N, 9.58. Found: N, 9.59, 9.56

Hydrolysis of the Polypeptide-hydantoin (VIII) with Hydrochloric Acid.—Two grams of the hydantoin and 25 cc. of concentrated hydrochloric acid were heated in a pressure tube at 140–150° for 4 hours. When the tube was opened there was a slight pressure due to the presence of carbon dioxide. The contents of the tube—consisting of a dark reddish brown liquid in which was suspended a mixture of crystalline and humous material—were transferred to an evaporating dish, together with wash water from carefully rinsing the tube. The mixture was evaporated to dryness on a water bath, care being taken to avoid the presence of any vapors of ammonia. A small quantity of the dry residue was then transferred to a test tube and treated with sodium hydroxide until strongly alkaline. Moist turmeric paper failed to reveal the presence of the least trace of ammonia gas when the alkaline solution was warmed.

The residue in the evaporating dish was now digested with a small quantity of hot water, and the dark-colored solution boiled with animal charcoal to remove the coloring matter. Ammonia was then added cautiously, and was accompanied by an immediate discharge of color and the precipitation of a colorless, crystalline compound. This was identified

as tyrosine. In all 1.04 g. of this amino acid were obtained, or a yield of about 80% of the theoretical.

Hydrolysis of the Polypeptide-hydantoin (VIII) and Its Ethyl Ester (X) with Potassium Hydroxide.—Following the procedure of Johnson and Bates,[1] ethyl 4-hydroxybenzylhydantoin-1-acetate was boiled in alcohol solution with 3 molecular proportions of potassium hydroxide. Upon long standing or upon boiling from 2–4 hours an insoluble potassium salt separated in the form of a mesh work of long glistening needles. This substance was not analyzed, as it was so extremely hydroscopic that it seemed hopeless to obtain it in a pure condition. When allowed to stand in contact with its alcohol solution for several days it passed slowly into solution, and on evaporation an oil was obtained which showed no tendency to crystallize. The free polypeptide-hydantoin behaved in every respect like its ester when treated with 3 equivalents of alkali.

Positive results were obtained when two instead of three proportions of alkali were used for hydrolysis. In this case, as in the preceding, the same results were obtained whether the polypeptide-hydantoin or its ester was used in the reaction. The substance was dissolved in alcohol and the base was added in a solution of 60% alcohol. The mixture was boiled for 6 hours and then concentrated to small volume. When a small amount of absolute alcohol was added the dipotassium salt of the corresponding hydantoin acid separated in the form of hard compact crystals. They were worked with hot alcohol, dried and analyzed.

Calc. for $C_{12}H_{12}O_6N_2K_2$: N, 7.82. Found: N, 7.88, 7.94.

The free hydantoic acid on *sym.*-tyrosine-glycine urea (XI) was obtained from its potassium salt by the usual procedure of suspending the substance in dry benzene and passing hydrochloric acid gas into the mixture. The residue obtained at the end of this reaction was washed with ether and then repeatedly with small quantities of water, dried and analyzed.

Calc. for $C_{12}H_{14}O_6N_2$: N, 9.9. Found: N, 9.70.

This hydantoic acid containing a free phenolic rest was found to be much more sensitive than the corresponding methoxy compound; and it was not possible to purify it by recrystallization from hot water. A specimen which melted at 220–224° with violet effervescence yielded, after one crystallization from water, a mixture that melted at 120–140° with slight effervescence. It solidified above this temperature and then melted again at 216–218° (the polypeptide-hydantoin). This mixture (m. 120–140°) when dried on a hot plate at 100° (and even below this temperature) was transformed into the polypeptide-hydantoin melting sharply at 216–218°.

NEW HAVEN, CONN.

[1] *Loc. cit.*