

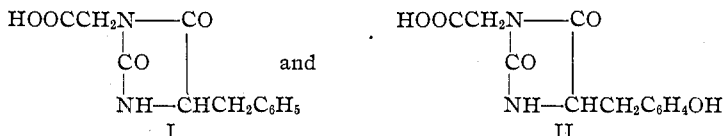
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF MOUNT HOLYOKE COLLEGE]

SYNTHESIS OF POLYPEPTIDE HYDANTOINS. TYROSYL-ALANINE HYDANTOIN

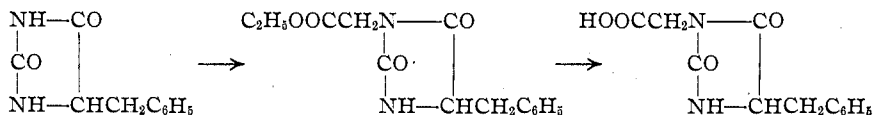
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Treat B. Johnson has shown in an earlier publication² that it is possible to link α -amino acids in such a manner as to produce a cyclic urea combination which hydrolyzes to give α -amino acids and carbon dioxide as the sole products of its decomposition. Compounds of this kind are the representatives of a new class of hydantoin to which the name "polypeptide hydantoin" has been given and for the preparation of which a number of new methods have been developed.³ As yet only two of the many possible examples of this type of substance have been prepared.⁴ These may be regarded as the hydantoin derivatives of the two polypeptides, namely, phenylalanylglycine, $\text{NH}_2\text{CH}(\text{CH}_2\text{C}_6\text{H}_5)\text{CONH}\cdot\text{CH}_2\text{COOH}$, and tyrosyl-glycine, $\text{NH}_2\text{CH}\cdot(\text{CH}_2\text{C}_6\text{H}_4\cdot\text{OH})\text{CONH}\cdot\text{CH}_2\text{COOH}$. They may be represented, respectively, by the following formulas.



Both of these substances were prepared by the alkylation of the corresponding hydantoin with ethyl chloro-acetate and the resulting ester was then converted into the polypeptide hydantoin by saponification.



In each case the structure of the polypeptide hydantoin was established by the fact that the hydantoin molecule breaks down when subjected to intense hydrolysis with hydrochloric acid, *without* the formation of ammonia, the products of decomposition consisting solely of carbon dioxide and the corresponding α -amino acids. In the case of II, for example, 80% of the theoretical amount of tyrosine was isolated. The important fact that no ammonia is formed as a result of this hydrolysis was accepted as furnishing conclusive proof that the acetic acid group occupies Position 1 of the hy-

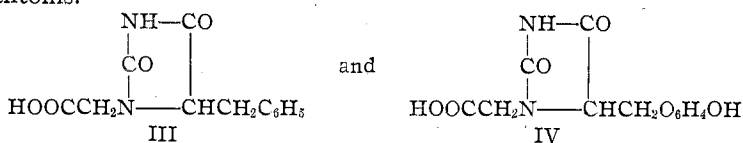
¹ This paper is one of a series undertaken in coöperation with Professor Treat B. Johnson of Yale University.

² *Proc. Nat. Acad. Sci.*, **2**, 69 (1916).

³ *Ibid.*, **2**, 72 (1916).

⁴ (a) Johnson and Bates, *THIS JOURNAL*, **38**, 1087 (1916). (b) Johnson and Hahn, *ibid.*, **39**, 1255 (1917).

dantoin ring and not Position 3 as represented in the corresponding isomeric hydantoin.



This follows from the fact that substances represented by Formulas III and IV would necessarily break down to give ammonia and the corresponding imino acids, $\text{HOOC}\cdot\text{CH}_2\text{NH}\cdot\text{CH}(\text{CH}_2\text{C}_6\text{H}_5)\text{COOH}$, and $\text{HOOC}\cdot\text{CH}_2\text{NHCH}(\text{CH}_2\text{C}_6\text{H}_4\text{OH})\text{COOH}$, when subjected to intense hydrolysis with hydrochloric acid. Aliphatic compounds of the imino acid type have been described and are known to be quite stable but the corresponding aromatic derivatives have not as yet been studied.

A third hydantoin combination, namely, tyrosyl-alanine hydantoin has now been synthesized and the study of this substance and of others closely related to it forms the subject of the present paper. This particular hydantoin was selected as of special interest because of the fact that the two α -amino acids incorporated in its molecule, namely, alanine and tyrosine constitute a large proportion of fibroin, the protein molecule occurring in silk.

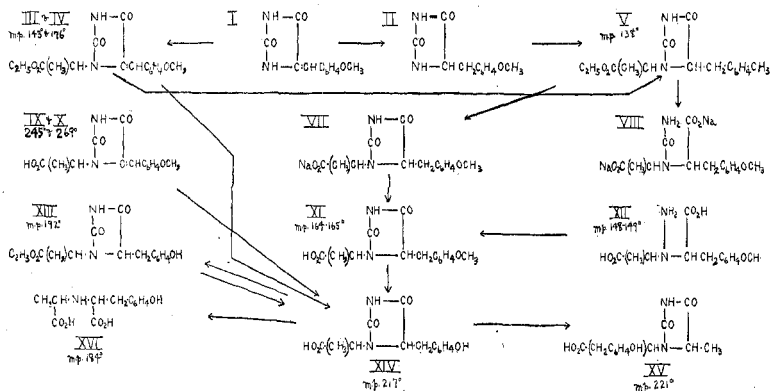
As will be seen by reference to the chart on p. 845 4-anisal hydantoin⁵ (I) was made the starting point in our research. This substance is readily reduced to the corresponding anisyl derivative⁶ (II) and both hydantoin undergo alkylation when treated with ethyl α -bromopropionate in alkaline solution. The two series of alkylated products, one unsaturated and the other saturated, were investigated separately and the conversion of members of the former into members of the latter class served as a convenient method for checking the structural relationships of the various substances which were prepared and studied.

A consideration of the formula of anisal hydantoin (I) shows that if the possibility of substitution on the oxygen atom is disregarded, alkylation will take place on the nitrogen in either the 1 or the 3 position. In the case of the polypeptide combinations previously studied it had been observed that substitution of hydrogen in the N-1 position took place exclusively. It was interesting, therefore, to find that only N-3 derivatives were produced when ethyl α -bromopropionate was used in place of ethyl chloro-acetate in the condensation. In this case isomeric products were formed as a result of the reaction. These substances were found to be esters which melted at 143° and 176° , respectively, (III and IV). Since both were converted into the same hydantoin (XIV) when heated with hydrogen iodide and red phosphorus at low temperatures, they were assumed to possess the same

⁵ Wheeler and Hoffman, *Am. Chem. J.*, **45**, 375 (1911).

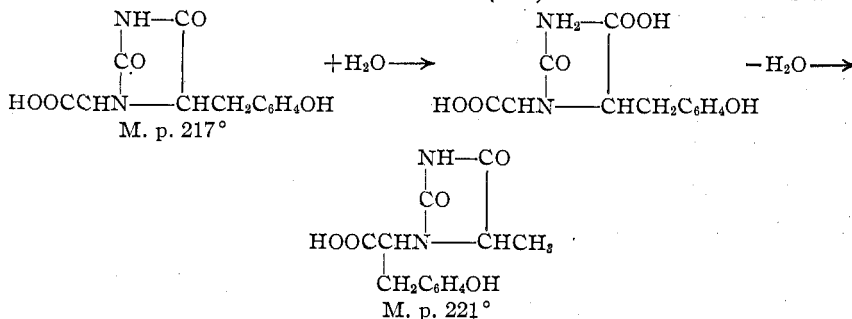
⁶ Johnson and Nicolet, *ibid.*, **47**, 474 (1912).

structural formulas. When hydrolyzed by the action of boiling conc. hydrochloric acid these substances passed into the corresponding isomeric acids which melted at 245° and 269°, respectively (IX and X). The latter were assumed to possess identical structural formulas because of the fact that both were transformed into the same hydantoin (XIV) when heated with hydrogen iodide and red phosphorus at low temperatures. The structure of the hydantoin itself was established by the fact that it decomposed with the evolution of ammonia when subjected to intense hy-



drolisis in the presence of hydrogen iodide and red phosphorus in a bomb tube at a temperature of 180°. The only solid product which was isolated as a result of this decomposition was found to be an acid which melted at 184° and which on analysis corresponded fairly closely to Formula XVI, $\text{CH}_3\text{CH}(\text{COOH})\text{NHCH}(\text{COOH})\text{CH}_2\text{C}_6\text{H}_4\text{OH}$. This substance was obtained in only very small quantities and a thorough study of its properties and derivatives, therefore, has not yet been possible.

Efforts to break down the hydantoin XIV by the action of hydrochloric acid were unsuccessful, but an interesting intramolecular rearrangement was observed. When heated with conc. hydrochloric acid in a bomb tube at 180° the hydantoin which melted at 217° passed quantitatively into an isomeric substance which melted at 221° (XV). Both combinations were



very stable compounds. When mixed in equal quantities and heated, the mass showed a melting point of 206–211°. This phenomenon may be accounted for by assuming that the hydantoin ring opened and closed during the process of heating.

In summarizing the actual procedure employed in producing the above transformations it should be stated that the alkylation of 4-anisal hydantoin with α -ethyl-bromopropionate was never complete. When the mixture was heated for periods of from 3 to 4 hours, the ester melting at 176° was found to be the sole product of the reaction. It was formed, however, only in small quantities. When this ester was separated from the reaction mixture and the reaction then continued for the same period, additional quantities (up to about 36% of the calculated amount) were obtained. When, on the other hand, the mixture was heated continuously for 6 days no trace of the ester melting at 176° was observed and the sole product of the reaction was found to be the isomeric ester melting at 143°. After the reaction mixture was heated for 24 hours it was found to consist of both esters and unacted-upon anisal hydantoin. In trying to account for the disappearance of the ester, melting at 176° as the result of prolonged heating, the possibility of its hydrolysis to the corresponding sodium salt was considered. The evidence here is somewhat conflicting because although it was impossible to isolate such a compound from the reaction mixture, it was found that the pure ester passed quantitatively into its sodium salt when heated with one equivalent of sodium dissolved in absolute alcohol. And as yet no procedure has been discovered by which it is possible to transform what seems to be the *labile* into the *stable* form.

The establishment of well-defined cases of geometrical isomerism in connection with compounds obtained as the result of substitutions in the hydantoin ring is especially interesting in connection with recent speculation in regard to the tautomerism of the hydantoin molecule. Dakin⁷ has attempted to explain the optical inactivity of allantoin by assuming that it is due to the tautomerism of the hydantoin ring which is represented as present in its molecule. He supports his contention by citing the fact that optically active hydantoic acids lose their optical activity when transformed into the corresponding cyclic hydantoin in all cases where the structure of the resulting hydantoin permits of the formation of enol-ketol modifications.⁸ In other words, the mobility of the atoms which go to make up the molecules of tautomeric substances must be regarded as operating against that fixed and definite arrangement of parts which is commonly assumed in order to account for the phenomenon of optical isomerism. Asymmetry of necessity demands a certain rigidity of configuration in the molecule. Now the conception of geometrical isomerism

⁷ Dakin, *J. Chem. Soc.*, 107, 435 (1915).

⁸ Dakin, *J. Biol. Chem.*, 17, 29 (1913); 18, 48 (1914).

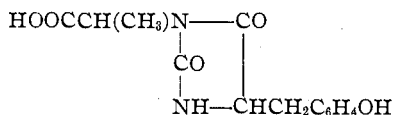
also depends upon the assumption of rigidity in the arrangement of the parts which constitute the molecule. If, then, a condition of tautomerism in the hydantoin molecule may be regarded as interfering with the phenomena of optical isomerism in hydantoin derivatives, it may also be assumed to operate similarly against the phenomena of geometrical isomerism in such substances. That this is not the case is obvious from the occurrence of the two pairs of geometrical isomers which have just been described.

The behavior of the two esters melting at 176° and 143° (III and IV), respectively, in the presence of sodium dissolved in absolute alcohol was rather striking, for while the former passed quantitatively into its sodium salt, the latter appeared to be extremely sensitive and gave a mixture from which it was impossible to separate a pure product. Both esters were found to pass quantitatively into the corresponding isomeric acids when boiled with conc. hydrochloric acid, and both were subsequently readily obtained from their acids by esterification with hydrogen chloride in alcohol solution.

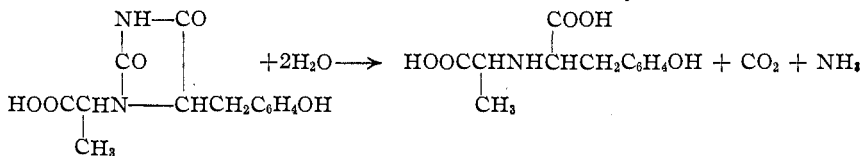
The alkylation of anisyl hydantoin with ethyl α -bromopropionate was also never complete. The resulting mixture was particularly difficult to separate because all of the constituents were extremely soluble. A consideration of the formula of ethyl 4-anisyl-hydantoin-3-propionate (V) shows that there are 2 asymmetric carbon atoms present in its molecule and that, therefore, two isomeric products, representing the meso and racemic modifications, are theoretically possible. Of these only one was separated. This ester was readily hydrolyzed to its corresponding acid by the action of acids and alkali and was also readily obtained from this acid by esterification with hydrogen chloride in alcohol solution. The hydantoin XI may be readily ruptured by the action of alkali, giving the disodium salt represented by VIII. When this salt is decomposed by acids in aqueous solution, the dibasic acid XII is formed, but it is readily condensed to the cyclic combination XI. In order to obtain the open chain acid XII, it is necessary to decompose its disodium salt with hydrochloric acid in an anhydrous solvent. The hydantoin XI was also obtained from IV by the action of sodium amalgam in alcohol. This reaction serves to establish a simple relationship between the unsaturated and saturated series. The fact that ethyl-anisyl-hydantoin-3-propionate and all substances derived from it contain the propionic acid group in the N-3 position was established from the fact that these substances pass readily into the hydantoin XIV when digested with hydriodic acid.

The hydantoin XIV has been prepared from the unsaturated and saturated hydantoin esters III, IV and V and from their corresponding acids IX, X and XI. In every instance the transformation was effected by boiling with conc. hydriodic acid, the reagent functioning in any of its several capacities, namely, to reduce, demethylate, or hydrolyze—as the

case required. The structure of the hydantoin, and incidentally the structure of all the substances which have been described in this paper as related to it, was established by a study of the behavior of this compound when subjected to intense hydrolysis with hydriodic acid. The hydantoin was decomposed with the formation of ammonia, thus showing conclusively that the propionic acid group occupies the N-3 position in the hydantoin ring, as represented in Formula XIV and not the N-1 position as represented in the isomeric hydantoin,



The reaction may be accounted for by supposing that the hydantoin is ruptured under the influence of the reagent with the loss of ammonia and carbon dioxide and the formation of an imido-dicarboxylic acid.



These conclusions show that the alkylation of anisal hydantoin by means of ethyl α -bromopropionate in alkaline solution proceeds quite differently from what has been described as taking place in the presence of ethyl chloro-acetate. In the latter case the reaction resulted in the formation of tyrosylglycine hydantoin, a substance in which the acid residue was represented as occupying the N-1 position in the hydantoin ring. In the reaction under consideration alkylation takes place in the N-3 position, and the resulting tyrosyl-alanine hydantoin must, therefore, be regarded as the first example of an entirely new type of polypeptide hydantoin. This fact is of great interest because it serves to further emphasize the analogy existing between this class of compounds and the closely related dioxypyrimidines. In the case of the latter, alkylation takes place under similar conditions with the formation of mixtures of N-1 and N-3 substitution products, thus showing that in these combinations the molecule is susceptible of attack in both the N-1 and the N-3 positions.⁹

Experimental Part

4-Anisal hydantoin and 4-anisyl hydantoin (I and II) were prepared by the same methods as those which have been described in an earlier paper.^{4b} Both substances were transformed into their respective sodium derivatives when treated with alcohol containing one equivalent of sodium. The time allowed for this reaction was about 12 hours in the case of the former and 2 hours in the case of the latter substance. The reaction never appeared to be complete when anisal hydantoin was used, although

⁹ Johnson and Derby, *Am. Chem. J.*, **40**, 451, 452 (1908).

titrations with standard hydrochloric acid showed the presence of only negligible amounts of sodium in the solution, since the reaction product after alkylation always contained what seemed to be unacted upon anisal hydantoin. It is, of course, impossible to say whether this was formed as the result of hydrolysis of the sodium derivative or whether it represented unacted upon material, although the latter conclusion seemed the more probable. If this assumption is correct it would appear that in some instances an opening of the hydantoin ring occurs and that this is accompanied by the formation of at least small quantities of a disodium derivative. It is impossible to follow the course of this reaction accurately, since both anisal hydantoin and its metallic derivatives are almost insoluble in alcohol.

In the alkylation of anisal hydantoin the procedure was to suspend the sodium derivative in absolute alcohol, add the calculated amount of ethyl α -bromopropionate and boil on a water-bath with a return condenser for various periods of time. The following table serves to show the results which were obtained when approximately equal amounts of the same specimen of sodium derivative (which had been separated and weighed) were treated with the calculated quantity of ester in 200 cc. of absolute alcohol.

Time of the reaction in hours.....	2	2	2	53
G. of sodium derivative.....	20.2	19.8	21.8	19.8
G. of ester, 176°.....	2.4	2.3	2.5	..
G. of ester, 143°.....	5.6

In a subsequent experiment where the sodium derivative was not separated from its reaction mixture before the addition of propionate the following results were obtained.

Time of the reaction in hours...	3	3	12	36	1 week
G. of anisal hydantoin.....	30	50	50	50	50
G. of insoluble residue.....	2.3	13.9	15.2	14.5	22
G. of ester, 176°.....	15.7	18.2	7.7
G. of ester, 143°.....	5.6	12	7

The procedure which was finally worked out as affording the best means for handling the reaction product was to filter cold and then wash with small quantities of cold absolute alcohol. The precipitate was then extracted several times with hot chloroform until nothing more was dissolved. The chloroform on evaporation gave either one or both of the esters as the case might be, and afforded a clean method for separating these substances from all other products which were present in the reaction mixture. The residue left after extraction with chloroform was found to consist of a sodium derivative of anisal hydantoin and when combined with the original filtrate (containing ethyl α -bromopropionate) and heated, yielded additional quantities of ester. When, on the other hand, this residue was boiled with water, solution was always incomplete, a product resembling anisal hydantoin being obtained as a final residue. This same product was obtained when the original precipitate after extraction with chloroform was dissolved in acetic acid. The substance was unchanged when suspended in alcohol and treated with dry hydrogen chloride; it corresponded to anisal hydantoin in melting point and percentage composition but it differed in appearance and in its behavior when treated with dil. aqueous sodium carbonate. It is still under investigation.

The mixture of isomeric esters which was obtained under certain conditions after the evaporation of the chloroform was readily separated by recrystallization from alcohol. The solubilities of the two substances in this solvent are so markedly different that the separation is practically quantitative.

Analyses. Calc. for $C_{16}H_{18}O_6N_2$: N, 8.80. Found (143°): 8.58, 8.64, Found (176°): 8.75, 8.76, 8.78.

The ester melting at 143° is very soluble in hot alcohol, 4 g. dissolving in about 20 cc. It separates from alcohol upon cooling in the form of large, glistening white plates. It is readily purified because while extremely soluble in boiling alcohol, it is only slightly soluble in the cold solvent. For example, 14.3 g. dissolved in 100 cc. of hot alcohol, crystallized out on cooling to give 13.1 g. The substance is readily hydrolyzed by both conc. hydrochloric and hydrobromic acids, and is thus transformed into the corresponding acid which melts at 245° . It was not found possible to prepare this acid from the corresponding sodium salt since the latter did not appear to be formed when the ester was treated in alcohol solution with an equivalent of sodium alcoholate. In fact this ester appears to be so much more sensitive than the isomeric ester melting at 176° that it was found to be much more difficult to work with. For example, when it was boiled with conc. hydrogen iodide in the presence of red phosphorus, the product was a gummy mass from which it was impossible to separate a solid product except by esterifying the mixture. Under these circumstances ethyl 4-hydroxy-benzylhydantoin-3-propionate XIII was formed. This was readily separated and purified and when heated with conc. hydrochloric acid passed into the corresponding polypeptide hydantoin (XIV). When heated for 3 hours at a temperature between 170° and 180° the substance (III) suffered no change.

The ester melting at 176° is soluble in hot alcohol and only slightly soluble in the solvent cold but its relative solubility is markedly different from that of its isomer; only approximately 3 g. will dissolve in 100 cc. of boiling solvent as compared with approximately 14 g. of the ester melting at 143° . In recrystallizing this substance it was, therefore, found convenient to dissolve it in chloroform (100 cc. will dissolve approximately 6.5 g.) and then precipitate by adding alcohol. The substance separates from alcohol and from mixtures of alcohol and chloroform in the form of a felt-like mesh which consists of long, soft, and very fine white needles or plates. The ester is easily transformed into the corresponding acid which melts at 269° when boiled with conc. hydrochloric acid. It is also readily hydrolyzed in the presence of alkali. For example, 3.8 g. of ester suspended in 60 cc. of alcohol was treated with 100 cc. of alcohol which contained 0.3 g. of sodium and the mixture was then heated on a water-bath for 30 minutes. The product consisted of long, coarse, yellow needles which lost their color when dried on a hot plate, and were completely soluble in water. The aqueous solution when acidified with hydrochloric acid gave a heavy white precipitate which melted at about 265° and was identified as the same acid obtained by acid hydrolysis of the ester. When heated with conc. hydrogen iodide in the presence of red phosphorus at a temperature of 130 – 140° the ester melting at 176° passed quantitatively into 4-hydroxybenzylhydantoin-3-propionic acid. Heated alone at 180 – 200° the substance showed a slight tendency to sublime but was otherwise unchanged.

The reduction of the double bond in ethyl 4-anisal-hydantoin-3-propionate, melting at 176° , was effected by the action of sodium amalgam in alcohol. The procedure was as follows. Five g. of ester and 0.5 g. of sodium in 50 g. of mercury were suspended in a solution of 50 cc. of water and 300 cc. of 95% alcohol, and the mixture was boiled for one hour. At the end of that time the clear solution was filtered from the mercury and the mercury washed with a small quantity of hot alcohol. On cooling, a white crystalline solid separated from the filtrate. This was found to consist of almost pure sodium 4-anisal-hydantoin-3-propionate, and the filtrate from it contained the sodium salt of the corresponding saturated acid, which separated in crystalline form upon concentrating the liquors. Both salts in aqueous solution gave their respective acids when acidified with hydrochloric acid. 4-Anisal-hydantoin-3-propionic acid was readily separated from traces of the saturated acid by washing with small quantities of hot water, in which it is insoluble, and then crystallizing from acetic acid. It melted at 269° and was identi-

fied by comparison with an analyzed specimen. 4-Anisyl-hydantoin-3-propionic acid was purified by two recrystallizations from water. It melted at 164–165° and was identified by comparison with an analyzed specimen.

In the alkylation of anisyl hydantoin the procedure was slightly different from that which was employed in the case of anisal hydantoin. The preparation was worked in 10g. portions of 4-anisyl hydantoin in 60 cc. of alcohol. At times the addition of sodium was accompanied by the immediate separation of a solid substance, and at other times under apparently the same conditions the solution remained clear. In any case the mixture was boiled for 2 hours with a return condenser. At the end of that time, a heavy white precipitate having formed, the boiling was stopped and the calculated amount of ethyl α -bromopropionate was added and the boiling continued. The product was examined at the end of 30 minutes and was often found to show a neutral reaction, although sometimes it was not neutral even at the end of several hours of continuous heating. As there was usually a certain amount of insoluble material present at the conclusion of the reaction, the procedure was to filter hot and wash with a small quantity of hot alcohol. The precipitate was found to consist of a mixture of sodium salts, and the filtrate upon cooling usually deposited additional quantities of these salts. The yield of solid product at this point varied between 0.6 g. and 3.5 g. The filtrate upon concentration and the addition of a small quantity of water precipitated a white substance, which after crystallization from alcohol melted at 138° and was found to consist of ethyl 4-anisal-hydantoin-3-propionate. The yield of this substance varied between 2.0 and 3.5 g. It was, however, always contaminated by the presence of unused 4-anisyl hydantoin, and the separation of these two substances, both extremely soluble in alcohol, was a tedious process.

Analyses. Calc. for $C_{16}H_{20}O_6N_2$: N, 8.75. Found: 8.72, 8.65.

This hydantoin is very soluble in hot alcohol, from which it crystallizes upon cooling in a meshwork of long, fine, white needles or plates. It is readily hydrolyzed by alkali or by hydrochloric acid to the corresponding acid. Heated with conc. hydrogen iodide in the presence of red phosphorus it passes quantitatively into 4-hydroxybenzyl-hydantoin-3-propionic acid.

The experiment described in the preparation of ethyl 4-anisyl-hydantoin-3-propionate was repeated 12 times with 10g. portions of 4-anisyl hydantoin, but no method was evolved for increasing the yield of ethyl 4-anisyl-hydantoin-3-propionate. Under what appeared to be identical conditions the products of the reaction varied considerably. The mixture of salts which has been mentioned as always present at the end of the reaction was accumulated and studied. It was found to consist of at least two, and possibly more, substances. Two were separated as the result of fractional precipitation from water and alcohol. One was identified as sodium 4-anisyl-hydantoin-3-propionate in the following ways: (a) its aqueous solution, when acidified with hydrochloric acid, gave a precipitate melting at 164–166°, which was identified by comparison with an analyzed specimen of 4-anisyl-hydantoin-3-propionic acid; (b) when suspended in alcohol saturated with dry hydrogen chloride, it passed quantitatively into its ester, melting at 138°. The second salt was identified as the open-chain derivative of 4-anisyl hydantoin. Its aqueous solution when acidified with hydrochloric acid gave a precipitate melting at 164–168° with a violent evolution of gas. This substance after one crystallization from water melted at 168–169° with evolution of gas.

Analyses. Calc. for $C_{11}H_{14}O_4N_2$: N, 11.76. Found: 11.32, 11.46.

This hydantoin was very soluble in hot water, from which it crystallized upon cooling in the form of long, soft, white needles or plates.

4-Anisal-hydantoin-3-propionic Acid (IX and X).—Two isomeric acids, melting at 245° and 269°, were obtained by the hydrolysis of the low-melting and the high-

melting esters, respectively. The procedure was the same in both cases. For example, 4 g. of ester was added to 50 cc. of conc. hydrochloric acid and the mixture was then boiled for an hour. The ester did not dissolve, but was gradually changed into the acid during the process of boiling. The mixture was filtered at the end of the reaction, and the precipitate washed with water and finally with a little cold alcohol. In the case of both substances the product was practically pure and the yield was quantitative. Hydrolysis of the ester 143° was also brought about by means of hydrobromic acid, the procedure being the same as with the hydrochloric acid, and the yield was again quantitative. Hydrolysis with alkali in the case of the ester 176° resulted in the formation of the corresponding sodium salt. Its aqueous solution, when acidified, precipitated a practically pure acid melting at 267–269°. The yield was quantitative. Both isomeric acids were most conveniently purified by crystallization from hot acetic acid.

Analyses. Calc. for $C_{14}H_{14}O_2N_2$: N, 9.65. Found (245°): 9.43, 9.48. Found (269°): 9.67, 9.71.

The hydantoin melting at 245° is very soluble in hot acetic acid, 3.4 g. dissolving in 40 cc.; it is also soluble in hot alcohol, but is almost insoluble in each solvent when cold. It is slightly ionized by water. It crystallizes from its solutions in the form of hard, white needles or plates. It is readily esterified by passing dry hydrogen chloride into alcohol in which it is partly dissolved and partly suspended. During the passage of the gas the acid slowly dissolves and, on cooling, the ester melting at 143° separates in the form of glistening white plates. When boiled with conc. hydriodic acid in the presence of red phosphorus it dissolves slowly to give 4-hydroxybenzyl-hydantoin-3-propionic acid (XIV).

The hydantoin melting at 269° is soluble in hot acetic acid, 1.7 g. dissolving in 60 cc., and is almost insoluble in the cold solvent. It crystallizes from its solutions in the form of hard, white needles or plates. It is almost insoluble in hot alcohol and, in general, its solubility is relatively less than that of its isomer. It is slightly ionized by water. When suspended in alcohol it is readily esterified by treatment with dry hydrogen chloride. During the process the acid slowly dissolves, and the ester melting at 176° separates upon cooling. When boiled with conc. hydrogen iodide in the presence of red phosphorus it dissolves slowly to give 4-hydroxybenzyl-hydantoin-3-propionic acid.

It should perhaps be noted at this point that the sodium salt of 4-anisal-hydantoin-3-propionic acid, melting at 269°, forms a yellow hydrate. This crystallizes from water in the form of well-defined yellow plates, which when heated or dried in a desiccator become perfectly white. An attempt was made to determine the water of crystallization quantitatively, but the ease with which the hydrate loses its water made this impossible. When air-dried overnight and weighed, and then allowed to stand in a desiccator for 24 hours and weighed again, the loss in weight was roughly equivalent to 4 molecules of water of crystallization.

4-Anisyl-hydantoin-3-propionic Acid (XI).—The monosodium salt of this acid was prepared from the corresponding ester by treating the latter in alcohol solution with an equivalent amount of alcoholic sodium hydroxide. The mixture was boiled for 1 hour and as it cooled the sodium salt separated in crystalline form. The salt was soluble in hot alcohol, and was purified by recrystallization from this solvent.

Analyses. Calc. for $C_{14}H_{15}O_3N_2Na$: N, 8.91. Found: 8.82, 8.86, 8.92.

Sodium 4-anisyl-hydantoin-3-propionate is readily soluble in cold water. When an aqueous solution of this salt was acidified with hydrochloric acid, the free 4-anisyl-hydantoin-3-propionic acid was deposited. The same acid was obtained from ethyl 4-anisyl-hydantoin-3-propionate by hydrolysis in the presence of hydrochloric acid. The procedure was to add 2 g. of ester to 30 cc. of conc. hydrochloric acid and then to connect with a return condenser and boil for 1 hour. Solution was complete at the

end of the first 5 minutes. As the solution cooled the acid separated in the form of hard, white prisms. It was purified by recrystallization from water slightly acidified with hydrochloric acid. It melted at 164–165°.

Analyses. Calc. for $C_{14}H_{16}O_6N_2$: N, 9.58. Found: 9.55, 9.55.

This hydantoin is very soluble in alcohol, soluble in hot water, and only slightly soluble in cold water. It tends to separate from aqueous solutions in the form of an oil if an insufficient amount of water has been used; otherwise, it crystallizes from hot aqueous solutions upon cooling. It is readily esterified by passing dry hydrogen chloride into its alcoholic solution, when the ethyl ester separates in crystalline form. When heated with conc. hydriodic acid in the presence of red phosphorus it is demethylated and transformed into the polypeptide hydantoin (XIV).

The reduction of the double bond in 4-anisal-hydantoin-3-propionate was effected by means of sodium amalgam suspended in alcohol. The procedure used in this case has been described in considering the chemical behavior of ethyl 4-anisal-hydantoin-3-propionate.

Urea of α -Methyl- α -(*p*-methoxybenzyl)diglycolamidic Acid (XII).—The disodium salt (VIII) of this acid was prepared by treating ethyl 4-anisyl-hydantoin-3-propionate with 2 equivalents of sodium. Three g. of ester was dissolved in 80 cc. of 95% alcohol and to the hot solution 0.52 g. sodium in 30 cc. of 95% alcohol was added slowly with constant shaking. Separation of a solid substance began at once, but the mixture was connected with a return condenser and boiled on a water-bath for 4 hours. At the end of that time the hot solution was filtered and the precipitate was washed several times with small quantities of hot alcohol. It was then allowed to dry and was analyzed. It amounted to 3.12 g. and melted at about 240° with decomposition.

Analyses. Calc. for $C_{14}H_{16}O_6N_2Na_2$: Na, 12.99. Found: 13.13, 13.14.

This salt is only very slightly soluble in hot alcohol, but it is readily soluble in cold water. Its aqueous solution when acidified with hydrochloric acid and heated, deposited 4-anisyl-hydantoin-3-propionic acid; m. p., 164–165°.

The free acid or urea was easily obtained from its disodium salt. The latter was suspended in dry benzene and dry hydrogen chloride was conducted into the mixture for 1 hour. The benzene was then decanted through a filter and the pasty mass was washed with several small portions of ether and then with a little aqueous alcohol. It was quickly dissolved in hot water from which it separated upon cooling in the form of large, glistening, white plates. It was further purified by a second recrystallization from water, air-dried overnight and analyzed. The substance melted with a rapid evolution of gas at 148–149°.

Analyses. Calc. for $C_{14}H_{16}O_6N_2$: N, 9.03. Found: 8.49, 8.35.

The urea was very soluble in hot water, 1.5 g. dissolving in 15 cc., and was slightly soluble in cold water. The crystals which were at first clear and transparent became slowly opaque upon standing in the air. When heated with conc. hydrochloric acid the substance passed into the corresponding hydantoin melting at 164–165°.

The Polypeptide-hydantoin, 4-Hydroxybenzyl-hydantoin-3-propionic Acid (XIV).—This compound was prepared in a variety of ways; from both isomeric 4-anisal-hydantoin-3-propionic acids and their corresponding ethyl esters, and from 4-anisyl hydantoin-3-propionic acid and from its ethyl ester. In all instances the procedure was the same. For example, 15 g. of ethyl 4-anisal-hydantoin-3-propionate, melting at 176°, was added to a mixture of 80 cc. of conc. hydriodic acid and 2 g. of red phosphorus and heated for 2 hours at a temperature of 100–110°. At the end of this time the hydriodic acid was distilled at a temperature kept between 170° and 180°. The residue was taken up in about 100 cc. of boiling water and filtered in order to free it from phosphorus. The

filtrate was then concentrated to about 50 cc.; as the solution cooled, 10.2 g. of substance melting at 208–212° separated in crystalline form. This substance was recrystallized twice from water, when it melted at 217° to a clear oil. In some cases crystallization of the hydantoin from its aqueous solution took place spontaneously, and in other cases, where the conditions of the experiment were identical, so far as could be seen, it was impossible to induce crystallization, even after days, without recourse to the use of seed. The introduction of seed into conc. aqueous solutions containing the hydantoin was always effective, but the product which separated in this way sometimes began to melt as low as 110°. The melting point rose rapidly on recrystallization from water, but this was accompanied by loss in the amount of solid product, and the aqueous filtrates when combined and concentrated precipitated an oil which did not solidify after standing for months.

The yield varied from about 30 to 95%. The best yields (90–95%) were obtained by starting with the saturated ester and its acid. Yields between 70 and 80% were obtained in a series of 8 experiments in which 4-anisal-hydantoin-3-propionic acid, melting at 269°, and its ester, melting at 176°, were used. Extremely poor yields were obtained from the isomeric unsaturated acid, melting at 245°, and its ester, melting at 143°. Thus 4 g. of acid when heated with 20 cc. of conc. hydriodic acid and 0.5 g. of red phosphorus gave 1.4 g. of the hydantoin; and the same quantity of ester when treated similarly gave 1.1 g. of the hydantoin. In the first case it was necessary to seed in order to induce crystallization, and in the second case it was necessary to evaporate the aqueous solution to dryness, dissolve the residue in a small quantity of absolute alcohol and saturate with hydrogen chloride. The solution on cooling gave a crystalline precipitate which melted at 187–189°, and which was identified as the ethyl ester of the hydantoin. It was transformed into the latter by digestion with 1:4 hydrochloric acid.

The hydantoin, prepared in these several ways by means of conc. hydriodic acid and red phosphorus, separated from its aqueous solutions in the form of hard, compact crystals, showing a tendency to group themselves in rosetts. These crystals were always more or less discolored, and it was sometimes impossible to remove the discoloration completely by repeated recrystallizations from water.

This same hydantoin was obtained by the hydrolysis of the corresponding ethyl ester. When this was attempted by digestion with conc. hydrochloric acid, the hydantoin separated as a deeply discolored, reddish-brown mass of crystals. This is quite different from what was observed in the case of the hydrolysis of ethyl 4-hydroxybenzyl-hydantoin-1-acetate by means of conc. hydrochloric acid,¹⁰ when the product crystallized out in absolutely pure condition. The apparent sensitiveness of ethyl 4-hydroxybenzyl-hydantoin-3-propionate in the presence of conc. acid, led to the use of dil. acid for purposes of hydrolysis. When 10 g. of ester was boiled with 50 cc. of 1:4 hydrochloric acid, solution took place at the end of 40 minutes. The clear solution was allowed to stand and as it cooled the hydantoin crystallized in the form of almost pure white crystals. The yield was quantitative.

It should be added that aqueous solutions of this hydantoin are very sensitive to the growth of mold and cannot be kept free from mold in summer unless acidified with hydrochloric acid. We hope to make this phenomenon the subject of further study.

Analyses. Calc. for $C_{13}H_{14}O_6N_2$: N, 10.07. Found: 9.72, 9.68.

The results which were obtained in this way corresponded with others which were obtained by titrating solutions of the substances with 0.1 *N* solutions of potassium hydroxide.

The hydantoin is very soluble in cold alcohol and hot water, and crystallizes from

¹⁰ Ref. 4b, p. 1265.

its aqueous solutions upon cooling in the form of hard, compact plates. It is very readily esterified by hydrogen chloride in alcohol solution or even by repeated recrystallization from alcohol.

Polypeptide G.	KOH G.	Calc. %	Found %
0.0975	0.0139	14.12	14.2
0.0106	0.0015	14.12	14.1
0.1085	0.0153	14.12	14.1

Ethyl 4-Hydroxybenzyl-hydantoin-3-propionate (XIII) was prepared by the esterification of the hydantoin (XIV) and was purified by recrystallization from hot alcohol.

Analyses. Calc. for $C_{16}H_{18}O_6N_2$: N, 9.15. Found: 9.07, 9.07, 9.13.

The substance melts at 192° to a clear oil. It is very soluble in hot alcohol and slightly soluble in cold alcohol and in hot water. It crystallizes from its alcohol solutions upon cooling in the form of a mesh of long, soft, white needles or plates. It is readily hydrolyzed to the polypeptide, melting at 217° , on heating with dil. hydrochloric acid.

The Polypeptide-hydantoin, 4-Methylhydantoin-3-hydroxybenzyl-3-propionic Acid (XV) was obtained when the polypeptide, melting at 217° , was heated with conc. hydrochloric acid in a sealed tube. The experiment was undertaken in an effort to open the hydantoin ring by subjecting the substance to intense hydrolysis. In the case of 4-hydroxybenzyl-hydantoin-1-acetic acid¹¹ this was readily induced by heating the substance with conc. hydrochloric acid in a sealed tube at a temperature of 140 – 150° for 4 hours. Similar treatment in the case of the propionic acid derivative failed to effect the breakdown of the ring but resulted in the formation of an isomeric product (XV). This product resembled the original substance in general appearance and properties but melted sharply at 221° . Mixtures of the hydantoin (XIV) melting at 217° and this substance were found to melt between 206° and 211° .

The procedure in carrying out these experiments was as follows. Two g. of 4-hydroxybenzyl-hydantoin-3-propionic acid and 25 cc. of conc. hydrochloric acid were heated in a bomb tube at 155° for 4 hours. The tube was opened at the end of this time when only a very slight pressure of gas was observed. The contents consisted of a white crystalline mass suspended in a colorless acid solution and when removed, diluted with water and filtered, yielded 1.8 g. of substance, melting at 221° . This was recrystallized from hot water.

Analyses. Calc. for $C_{13}H_{14}O_6N_2$: N, 10.07. Found: 9.71, 9.73.

The filtrates from washing and recrystallizing this substance were combined, evaporated to a small volume and tested for ammonia but the results were negative.

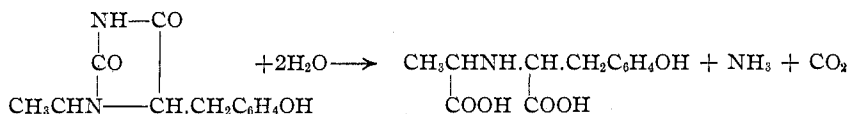
The attempted hydrolysis was repeated under the same conditions but a temperature of 180° . In this case a slight discoloration of the solution was observed accompanied by the formation of traces of humous material. The product again consisted of the substance melting at 221° , and there was no evidence of the formation of even a trace of ammonia.

Hydrolysis of the Hydantoin (XIV) with Concentrated Hydriodic Acid.—The disruption of the hydantoin molecule was finally effected by use of hydrogen iodide, when 2 g. of the hydantoin, 20 cc. of conc. hydriodic acid and 0.5 g. of red phosphorus were heated in a pressure tube at 180° for 6 hours. When the tube was opened a rather considerable pressure of gas was observed. The contents consisted of a small quantity of brown humous material suspended in a colorless solution. There was no trace of the

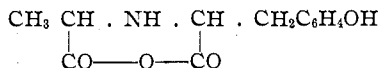
¹¹ Ref. 4b, p. 1264.

crystalline material observed in previous heatings with hydrochloric acid. After dilution with water and subsequent filtration to remove the red phosphorus, the solution 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 residue was then transferred to a test-tube and treated with sodium hydroxide until strongly alkaline. During this process a gas was generated which turned turmeric paper brown and which smelled like ammonia. The main portion of the residue was then digested with a small quantity of water. As the mixture stood it deposited a crystalline product which after one recrystallization from water was perfectly white and melted at 184°.

In subsequent experiments with hydrogen iodide it was possible to curtail the heating from 6 to 2 hours if a temperature of 180–190° was maintained. When, however, the heating was conducted at 140° the contents of the tube consisted of the same white crystalline mass (insoluble in cold hydriodic acid) which had been observed and studied in previous experiments with hydrogen chloride. It was then possible to follow the progress of the decomposition of the hydantoin by means of the complete disappearance of this crystalline product. In all cases where a temperature of 180° was maintained for 2 or more hours, decomposition of the hydantoin took place. This was always accompanied by the formation of ammonia and of the crystalline product which has been described as melting at 184°. The latter was soluble in cold hydriodic acid and in hot water but crystallized from cold water in the form of hard, well-defined plates. Its aqueous solution gave an acid reaction with litmus and the characteristic tyrosine reaction when treated with Millon's reagent. Since, however, the latter reaction took place with greater or less ease in the case of all of the substances which have been described in this series, it served to indicate little more than that a tyrosine residue was present in this substance. The simplest way to account for such a compound is to assume that decomposition of the hydantoin molecule takes place according to the equation



The results of the analysis of the substance were in general agreement with this supposition in that they corresponded closely to the formula calculated for the anhydride of such an acid,



Analyses. Calc. for $\text{C}_{12}\text{H}_{12}\text{NO}_4$: N, 5.95. Found: 5.94, 5.98.

While a thorough study of the properties of this substance has not yet been possible because of the small quantities in which it has been obtained, further work along these lines is now in progress in this Laboratory.

Summary

The alkylation of anisal hydantoin by means of ethyl α -bromopropionate takes place exclusively in the N-3 position and not in the N-1 position as was observed in the case of ethyl chloro-acetate. The product consists of 2 esters which have been found to be geometrical isomers and which may be regarded as representatives of a new type of polypeptide hydantoin. The fact that alkylation may take place in the N-3 as well as in the N-1 position is of importance because it serves to emphasize further the analogy

which exists between the class of polypeptide hydantoins and the closely related class of dioxypyrimidines, since the latter under similar conditions react to give mixtures of N-1 and N-3 substitution products.

The fact that geometrical isomers have been found to exist is of especial importance because it would seem to indicate the possibility of a certain rigidity in the configuration of the hydantoin molecule. Previous to this time the assumption has been made that the mobility of the atoms which go to make up the molecule of tautomeric substances may be regarded as operating against the fixed arrangement of parts which is generally presupposed in order to account for the phenomena of optical and geometrical isomerism.

SOUTH HADLEY, MASSACHUSETTS

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

PREPARATION OF ABSOLUTE ALCOHOL WITH CALCIUM CHLORIDE AND LIME

BY WILLIAM A. NOYES

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Beilstein's "Handbuch der Organischen Chemie" states that absolute alcohol is prepared on a large scale by the use of calcium chloride but I have failed to find details anywhere in the literature. The statement is also made that alcohol combines with calcium chloride and that a high temperature is required to expel the last of the alcohol from the compound formed.

A somewhat extended study of the relations involved has established the following facts.

1. From strong alcohol containing somewhat more than 1 mole of calcium chloride (111 parts) for 1 mole of water (18 parts), alcohol of 99.5% concentration or stronger, may be distilled.

2. On concentration of such a solution a solid alcoholate, not a hydrate, begins to separate when the boiling point reaches 95–100° and there is an equilibrium between the alcoholate and hydrate present. A quite high temperature is required to expel the alcohol from the solid alcoholate.

3. The hydrate of calcium chloride containing 4.5 moles of water (80 parts) for 1 mole of calcium chloride (111 parts) boils at 140° and from such a solution the alcohol may be distilled completely with a strength of 90% or greater. The solution of calcium chloride of this composition is liquid at 140° but solidifies on cooling.

4. Successive distillations of alcohol containing 80 g. of calcium chloride per liter increase the strength of the alcohol approximately as follows, by weight.