[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF MOUNT HOLYOKE COLLEGE]

SYNTHESIS OF THE POLYPEPTIDE-HYDANTOIN: TYROSYL-ALANINE-HYDANTOIN. II

BY DOROTHY A. HAHN AND ELIZABETH GILMAN

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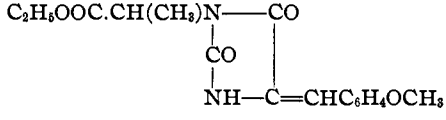
In repeating the work which has been described by Hahn, Kelley and Schaeffer,[1] results have been obtained which necessitate a complete revision of the conclusions then published. It now seems certain that the ethyl α-bromopropionate[2] which was then used in condensation with the sodium derivative of anisalhydantoin contained appreciable quantities of the corresponding acetic ester. Recently a different specimen of ethyl α-bromopropionate[3] was used with the result that an entirely new propionate derivative, m. p. 158–158.5°, was obtained which in turn yielded a quite different series of compounds from those which had previously been described. The new substance[4] has now been identified as ethyl anisalhydantoin-N-1-propionate.



This substance was at first assumed to be a position isomer of the esters melting at 176° and 143° that were obtained in all previous condensations, and it was with a view to establishing these relationships that all three esters were submitted to Dr. Emma P. Carr, of this Laboratory, for spectrographic examination. When results showed that the absorption curves of all three substances were practically identical, it seemed probable that their several relationships could no longer be accounted for on the basis of N-1 and N-3 substitution in the hydantoin ring. Since, however, such deductions depend for accuracy upon the comparison of the absorption curves of a large number of substances of definitely known structure, the development of this phase of the problem necessitated the preparation of additional derivatives of anisalhydantoin. Dr. Carr will

[1] Hahn, Kelley and Schaeffer, THIS JOURNAL, **45**, 843 (1923).

[2] This ester boiled at 158–160° but was prepared from a very impure propionic acid that was obtained from the General Chemical Company in 1918. This acid was rectified and a fraction boiling at 138–142° was used in the preparation of ethyl α-bromopropionate.

[3] This ester also boiled at 158–160° but was prepared from a propionic acid boiling at 139–141°, obtained from C. A. F. Kahlbaum. In both cases the Zelinsky method [*Ber.*, **20**, 2026 (1887)] was used in the preparation of the halogen ester. Acknowledgment is made to Miss Woodward for the preparation of pure ethyl α-bromopropionate.

[4] See Part II of this paper.

publish her results separately but it may be said here that since ethyl 4-anisalhydantoin-N-1-acetate,[5] N-1-methyl-4-anisalhydantoin,[6] and other similar substances gave absorption curves which were practically identical with those of the three esters under investigation, the conclusion seemed inevitable that the latter must all be regarded as representing N-1-substitution products of anisalhydantoin.

A very great difficulty was experienced in attempting to account for the existence of the esters, m. p. 176° and 143°, in view of these facts until the suggestion was made by Dr. Carr that the ester, m. p. 176°, might represent ethyl 4-anisalhydantoin-N-1-acetate and the ester, m. p. 143° (now corrected to 140–142°), mixed crystals of ethyl 4-anisalhydantoin-N-1-propionate and ethyl 4-anisalhydantoin-N-1-acetate. The results of a physicochemical investigation by Dr. Carr showed that this is the case,[7] and the identity of the ester, m. p. 176°, was further established (1) by a mixed melting point with a specimen of ethyl 4-anisalhydantoin-N-1-acetate, prepared by condensing ethyl chloro-acetate with the sodium derivative of anisalhydantoin,[8] and (2) by a reduction of the substance and subsequent hydrolysis[9] of the reduction product to glycine, tyrosine and carbon dioxide.

In the light of these facts it seems necessary to review the conclusions previously published. Thus when ethyl α-bromopropionate which is contaminated by the presence of the corresponding acetate is used in condensations with the sodium derivative of anisalhydantoin, the only product which separates from the mixture during the first 18 hours of boiling is ethyl 4-anisalhydantoin-N-1-acetate. This is due to the fact that its rate of formation is much more rapid than that of the corresponding propionate. Prolonged boiling is accompanied by the gradual disappearance of this substance and finally by its complete replacement by the ester, m. p. 143°. This phenomenon was originally interpreted as due to isomerization, but must now be explained by assuming that ethyl 4-anisalhydantoin-N-1-acetate enters into the formation of mixed crystals, m. p. 140–142°, with the corresponding propionate as rapidly as the latter is formed.

A study of the eutectic mixture, m. p. 140–142°, was complicated by the fact that the substance appears to be perfectly homogeneous and has a characteristic appearance and solubility which are in marked distinction to those of either of the other two esters.[10] It may be recrys-

[5] Johnson and Hahn, THIS JOURNAL, **39**, 1255 (1917).

[6] Johnson and Nicolet, *Am. Chem. J.*, **47**, 469 (1912).

[7] Carr and Dobbrow, THIS JOURNAL, **47**, 2961 (1925).

[8] Ref. 7, p. 2963.

[9] See p. 2952 of this article.

[10] The ester melting at 176° occurred as long, fine needles which separated in a meshwork like asbestos, completely filling the space occupied by the solvent even in the

tallized repeatedly without change and is resolved into its components only by employing solutions of extreme dilution. The transformations of this substance are also very misleading. When hydrolyzed in the presence of dil. hydrochloric acid it yields an acid,[11] m. p. 245°, which is quite different from the acids obtained from the esters melting at 176° and 158–158.5°, respectively, but which also probably represents a eutectic mixture.

In all other transformations the ester, m. p. 143°, gave products identical with those which were obtained from the ester, m. p. 176°, and this seemed to support the assumption that the two substances are geometrical isomers. It may seem astonishing that no solid derivatives of ethyl 4-anisalhydantoin-N-1-propionate, m. p. 158–158.5°, were ever isolated in any of the earlier work, but this is now readily explained in view of the fact that all of the derivatives of ethyl 4-anisalhydantoin-N-1-propionate in which the ethylene linkage has been saturated, exist in two isomeric modifications[12] which even under the best conditions are very difficult to separate and which frequently solidify only after standing for several months.

In conclusion, it may be said that the earlier assumption of N-3-substitution was based upon the fact that ammonia was evolved during the hydrolysis of the polypeptide-hydantoin. The observation has since been made, however, that N-1-derivatives of anisalhydantoin of known structure yield ammonia when subjected to intense hydrolysis. The formation of ammonia in decompositions of this kind cannot, therefore, be made a basis for determining questions of constitution.

Part II

The product which has recently been obtained by condensing ethyl α-bromopropionate with the sodium derivative of anisalhydantoin, m. p. 158–158.5°, may be assumed to have Configuration I[12] on the basis of the following facts. (a) It hydrolyzes in the presence of mineral acid and alkali to give an unsaturated acid II. The latter esterifies in alcohol solution under the action of hydrogen chloride to give the original ester, and is reduced and demethylated by the action of hydrogen iodide to give the same products X and XI that are obtained from the ester by the

case of dilute solutions; 6.5 g. dissolved in 100 cc. of boiling chloroform; 3.5 g. in 100 cc. of boiling alcohol.

The ester, melting at 158–158.5°, formed hard compact prisms on the bottom of the beaker and occupied relatively little space; 35 g. dissolved in 100 cc. of boiling chloroform, 8.0 g. in 100 cc. of boiling alcohol.

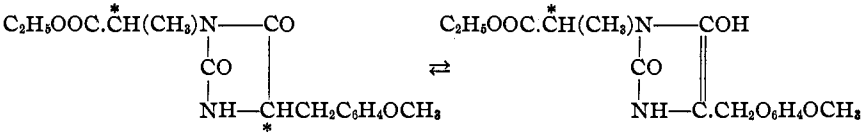
The mixed crystals, m. p. 140–142°, formed large, thin, glistening white plates which separated throughout the space occupied by the solvent. They were extremely soluble in cold chloroform; 16 g. dissolved in 100 cc. of boiling alcohol.

[11] Ref. 1, p. 851–852.

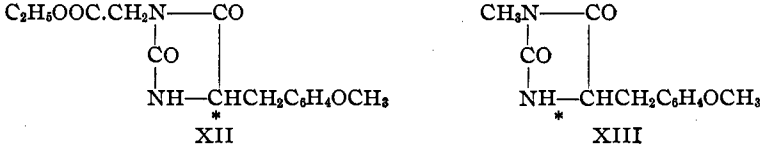
[12] See chart, p. 2945 of this article.

action of the same reagent. (b) It is reduced by the action of hydrogen in the presence of palladium to give a mixture of the isomeric esters IV and V. The saturated esters (IV and V) hydrolyze readily in the presence of dil. hydrochloric acid to give, respectively, the saturated acids VII and VI. The latter have also been prepared by condensing ethyl α-bromopropionate with the sodium derivative of 4-anisalhydantoin and then hydrolyzing the product. The existence of these two well-defined pairs of isomeric products may be explained by assuming that they represent two inactive stereo modifications, since in both cases the reduced molecules contain, respectively, two asymmetric carbon atoms. When these substances are demethylated under the action of hydrogen iodide they yield the same products X and XI that are obtained from ethyl anisalhydantoin-N-1-propionate I under the action of the same reagent.

Any explanation of the existence of isomeric modifications on the basis of enol-keto tautomerism, namely,



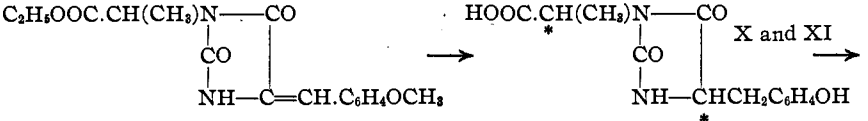
is weakened by the fact that substances such as XII and XIII



XII XIII

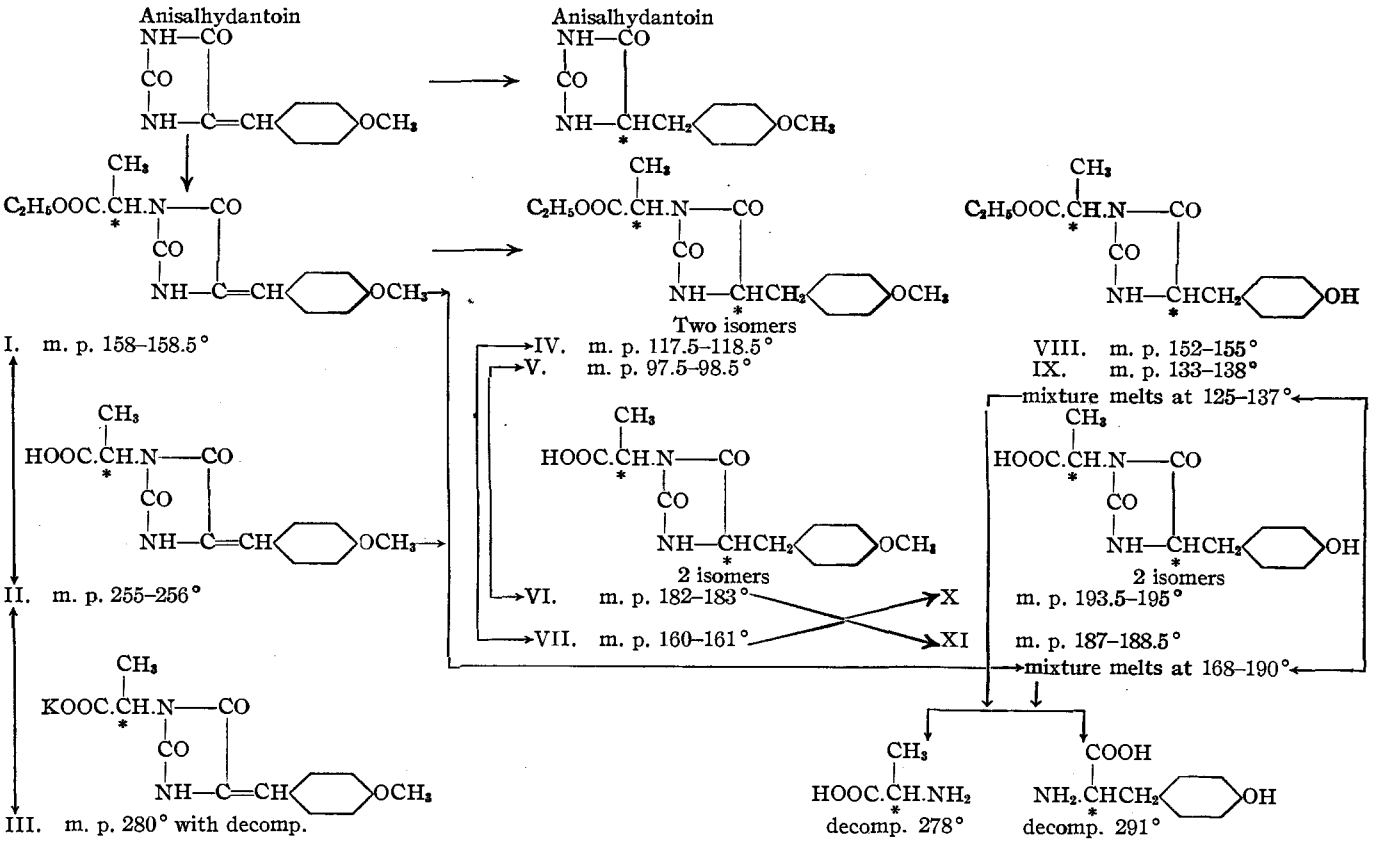
and many others which have been prepared and studied in this Laboratory show no tendency to exist in isomeric modifications.

The proof of the constitution of ethyl 4-anisalhydantoin-N-1-propionate depends, however, primarily upon its transformation into the polypeptide-hydantoins X and XI and upon the fact that these products subsequently hydrolyze under the action of barium hydroxide to give tyrosine, alanine and carbon dioxide.



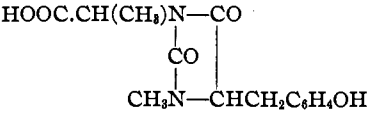
$NH_2CH(COOH)CH.CH_2C_6H_4OH + HOOC.CH(CH_3)NH_2 + CO_2$

Experimental difficulties were presented in the transformation of the ester into the polypeptide-hydantoin by the fact that a homogeneous crystalline product was obtained in 80% yields which although recognized to be a mixture (m. p., 168–190°) could not at first be resolved into its components. It was, therefore, esterified by dissolving it in alcohol


Anisalhydantoin
Anisalhydantoin
Two isomers
I. m. p. 158–158.5°
II. m. p. 255–256°
III. m. p. 280° with decomp.
IV. m. p. 117.5–118.5°
V. m. p. 97.5–98.5°
2 isomers
VI. m. p. 182–183°
VII. m. p. 160–161°
VIII. m. p. 152–155°
IX. m. p. 133–138°
mixture melts at 125–137°
2 isomers
m. p. 193.5–195°
m. p. 187–188.5°
mixture melts at 168–190°
decomp. 278°
decomp. 291°

and saturating the solution with hydrogen chloride. Although this transformation was quantitative, the melting point of the product again proved unsatisfactory, 125–137°. The hydrolytic decomposition of the acid melting at 168–190° and of the ester melting at 125–137° under the action of barium hydroxide was then undertaken, and when it was found that both break down quantitatively into tyrosine, alanine and carbon dioxide, the conclusion seemed inevitable that the mixture must in each case be due to the presence of stereo-isomers. This was found to be true when, after a process of fractional recrystallization extending over several months, the partial resolution of both substances into their respective components, (VIII and IX) and (X and XI) was finally effected. The difficulty which was experienced in the separation of the two modifications appeared to be due in part to a very close similarity in the solubilities of the isomers.

In order to eliminate any possibility that the melting point, 168–190°, of the polypeptide-hydantoin mixture might be due to factors other than the presence of stereo-isomeric modifications as, for example, to traces of impurity such as XIV,



XIV

which might conceivably be formed during the process of demethylation, a more detailed study of the action of hydrogen iodide was undertaken. A determination, according to the Zeisel method, of the methyl iodide evolved when 4-anisalhydantoin-N-1-propionic acid was treated with hydrogen iodide showed, however, that the amount was quantitative and that none of the methyl could have substituted on the nitrogen atom.

Finally, the polypeptide-hydantoins X and XI were obtained separately from the acids VI and VII, respectively, as a result of heating the latter individually with hydrogen iodide at relatively low temperatures. In fact, having once effected the separation of any two modifications it is possible to subject the individual isomers to various chemical transformations (VI → XI; VII → X; VI → V; VII → IV) without obtaining mixtures as long as the reactions do not involve appreciable increases in temperature. When, however, reactions take place at temperatures above 100°, mixtures of isomeric products are invariably formed.

Experimental Part[13]

4-Anisalhydantoin and 4-anisylhydantoin were prepared by methods which have already been described.[5] Both substances were transformed

[13] Acknowledgment is made to Miss Dorothy Bateman and Miss Louise Hersey for their assistance in preparing several of the substances mentioned in this paper.

into their respective sodium derivatives when heated with alcohol which contained one equivalent of the metal.[14] The time allowed for the reaction was about twelve hours in the case of the former and two hours in the case of the latter substance.

Derivatives of Anisalhydantoin

Ethyl 4-Anisalhydantoin-N-1-propionate (I) was prepared by treating the sodium derivative of anisalhydantoin (150 g.) suspended in one liter of absolute alcohol with a molecular quantity of ethyl α-bromopropionate. The mixture was heated continuously on a steam-bath and during the first two or three days the solution was filled with fine, white granules of the sodium derivative which were agitated and held in suspension by the boiling alcohol. After four to six days the solution cleared and a solid cake of yellow material incrusted with white formed at the bottom of the flask. The clearing of the solution was assumed to mark the end of the reaction; the amber-colored supernatant liquid was decanted hot and the residue in the flask was washed with a small quantity of hot alcohol. The residue when dried was found to weigh 84 g. and to consist of a mixture of sodium bromide, a sodium derivative of anisalhydantoin, free anisalhydantoin, and about 7 g. of the desired condensation product. The hot alcoholic solution upon cooling deposited a mass of crystals which when filtered off, washed with hot alcohol and dried, weighed 65 g.; m. p., 149–153°. This precipitate represented the main portion of the condensation product. The mother liquor from these crystals was evaporated to dryness and the residue extracted with chloroform. In this way an additional 8 g. of fairly pure ester was separated from a mixture of anisalhydantoin and sodium bromide.

The ester was freed from traces of anisalhydantoin and other impurities by dissolving it in chloroform. This solution was filtered, concentrated to small volume, diluted with three or four times the original volume of boiling alcohol and set aside to crystallize. In all, 84 g. of anisalhydantoin was recovered, thus making a yield of about 70% of ester from the part entering into the reaction. When pure, the ester melted sharply at 158–158.5°.

Anal. Calcd. for $C_{16}H_{18}N_2O_5$: N, 8.80. Found: 8.81, 8.80.

Ethyl 4-anisalhydantoin-N-1-propionate gives colorless solutions with alcohol, chloroform[15] and acetone (1 part in 15 parts of acetone) from which it separates in the form of hard, transparent prisms. It is readily hydrolyzed in the presence of mineral acid and alkali to the corresponding unsaturated acid and salt, II and III. When reduced catalytically by hydrogen in the presence of palladium it yields the isomeric esters IV and V. Heated with hydrogen iodide at a temperature of 130–140° it is simultaneously reduced, demethylated and hydrolyzed to the isomeric acids X and XI.

4-Anisalhydantoin-N-1-propionic Acid (II) was prepared by hydrolyzing the corresponding ester in the presence of alkali or of mineral acid. A solution of 5 g. of ester in 100 cc. of alcohol to which two equivalents of sodium had been added, was heated in an open beaker on a steam-bath for 15–20 minutes and this when treated with hydrochloric acid, gave an immediate precipitate of 4.5 g. of pure acid; m. p., 255–256°. Since the ester is practically insoluble in hydrochloric acid, both dilute and concentrated, acid hydrolysis was brought about by dissolving 15 g. of ester in 150 cc. of acetic acid and heating for three hours with 17 cc. of concd. hydrochloric acid. The addition of 100 cc. of water caused the precipitation of 14 g. of material, m. p. 235–246°, which was

[14] Ref. 1, p. 848.

[15] For solubilities, see Ref. 10.

first extracted with a small quantity of boiling alcohol to remove any traces of ester and then recrystallized from glacial acetic acid.

Anal. Calcd. for $C_{14}H_{14}O_5N_2$: N, 9.65. Found: 9.65, 9.60.

4-Anisalhydantoin-*N*-1-propionic acid separates from its solutions in clusters of very fine, glistening needles. It is very slightly soluble in boiling alcohol but dissolves readily in boiling glacial acetic acid (6 g. in 80 cc.). Suspended in alcohol and subjected to the action of hydrogen chloride it is slowly esterified to the corresponding ester, I; when heated with hydrogen iodide at a temperature of 130–140° it gives a mixture of X and XI.

POTASSIUM 4-ANISALHYDANTOIN-N-1-PROPIONATE (III) was prepared by dissolving 5 g. of ester, m. p. 158–158.5°, in 50 cc. of 50% aqueous alcohol to which one equivalent of potassium hydroxide had been added, and heating the solution overnight on a steam-bath. When examined in the morning the solution was still faintly alkaline, but the reaction was considered complete and the solution transferred to a beaker, concentrated to small volume, and the salt precipitated by the addition of absolute alcohol. When filtered off, washed with alcohol and dried, 4 g. of faintly yellow, crystalline material was obtained. This became pure white upon one recrystallization from 95% alcohol; m. p. (decomp.), 280°.

Anal. Calcd. for $C_{14}H_{13}O_5N_2K$: N, 8.53. Found: 8.54, 8.60.

Potassium 4-anisalhydantoin-*N*-1-propionate dissolves readily in cold water to give a neutral solution. It is only slightly soluble in cold 95% alcohol, but is fairly soluble in the boiling solvent (4 g. in 200 cc.) from which it crystallizes in clusters of well-defined needles or prisms. It passes quantitatively into the corresponding acid, II, when its aqueous solutions are acidified with hydrochloric acid.

Derivatives Common to 4-Anisalhydantoin and 4-Anisylhydantoin

Ethyl 4-Anisylhydantoin-N-1-propionates, IV and V.—Two isomeric substances corresponding to this formula were obtained by the catalytic reduction of ethyl 4-anisalhydantoin-N-1-propionate. Colloidal palladium (0.42 g. suspended in 5 cc. of water) was introduced into the reduction chamber, a small quantity of 95% alcohol added and the mixture shaken with hydrogen for a few minutes. The addition of 10 g. of ethyl 4-anisalhydantoin-N-1-propionate, m. p. 158–158.5°, dissolved in 200 cc. of hot alcohol was attended by the immediate absorption of hydrogen. The reaction continued in the cold during constant shaking for about 10 hours or until hydrogen was no longer absorbed. After the palladium was removed in the usual way, a clear, colorless solution was obtained which when concentrated to 75 cc. precipitated a crystalline solid, m. p., 112–116°. The filtrate from this on standing gave a second precipitate which was also crystalline and homogeneous, but which melted at 85–90°. During the course of several days, precipitates having the same melting points as the above were deposited successively in almost equal amounts, making a total of about 8 g. After recrystallization from alcohol and from alcohol-water solutions, respectively, substances melting at 117.5–118.5° and 97.5–98.5° were obtained.

Anal. Calcd. for $C_{16}H_{20}O_5N_2$: N, 8.75. Found V (m. p., 97.5–98.5°): 8.70, 8.59; IV (m. p., 117.5–118.5°): 8.74, 8.66.

The higher-melting modification of ethyl 4-anisylhydantoin-N-1-propionate is fairly soluble in boiling alcohol (2.1 g. in 25 cc.) from which it separates in hard, compact, transparent prisms. The lower-melting modification is much more soluble and separates from alcohol-water solutions in clusters of needles which are slightly opaque and which may be readily distinguished from the higher-melting isomer. Both substances hydrolyze readily to give acids when warmed with dil. hydrochloric acid. It is

to be noted that the lower-melting ester is completely transformed into the higher-melting acid, and its isomer into the lower-melting acid (that is, IV → VII, V → VI).

4-Anisylhydantoin-N-1-propionic Acids, VI and VII.—Two isomeric substances which correspond to this formula were obtained by the hydrolysis of the esters V and IV. The procedure was the same in both cases; 2 g. of pure ester dissolved in 30 cc. of 20% hydrochloric acid was warmed in an open beaker on a steam-bath for about an hour. On cooling, practically pure acids separated in quantitative amounts. After one recrystallization from boiling water they melted at 160–161° and 182–183°, respectively.

The same substances were obtained as the result of condensing ethyl α-bromopropionate with the sodium derivative of 4-anisylhydantoin. The primary products of the reaction (consisting of the esters IV and V) were never isolated because of the fact that they form an oily mixture with anisylhydantoin which is very difficult to separate since all three substances have approximately the same solubilities. The oily product was, therefore, digested with concd. hydrochloric acid in order to hydrolyze the esters. The acids which were formed in this way are much more soluble than anisylhydantoin in boiling water and were removed from the solid reaction product by repeated aqueous extractions. The mixture of isomeric acids was finally separated by fractional recrystallization from water, when pure products melting respectively at 160–161° and 182–183° were obtained and identified as the same as the acids derived from the esters IV and V.

Anal. Calcd. for $C_{14}H_{16}O_5N_2$: N, 9.59. Found VI (m. p., 182–183°): 9.02, 9.03; VII (m. p., 160–161°): 8.99, 8.97, 8.92, 9.08.

The low percentage of nitrogen may be explained by assuming the presence of one molecule of water (calcd. for $C_{14}H_{16}O_5N_2$, H_2O: N, 9.03) but no determinations of water of hydration were undertaken because of the lack of material.

The high-melting acid VI, m. p. 182–183°, is fairly soluble in boiling water (2 g. in 50 cc.) from which it separates immediately on cooling in transparent, prismatic needles which resemble thistle. When dissolved in alcohol and treated with hydrogen chloride it passes quantitatively into the low-melting ester V. When heated for one hour at 100° with hydrogen iodide in the presence of red phosphorus it changes quantitatively into the polypeptide-hydantoin XI. The low-melting acid VII, m. p. 160–161°, is much more soluble in boiling water (1 g. in 10 cc.) from which it separates on cooling in large, transparent, diamond-shaped prisms. When dissolved in alcohol and treated with hydrogen chloride it passes quantitatively into the high-melting ester IV. When heated for one hour at 100° with hydrogen iodide in the presence of red phosphorus, it changes quantitatively into the polypeptide-hydantoin X.

4-Hydroxybenzylhydantoin-N-1-propionic Acids (X and XI).—Two isomeric substances which correspond to this formula have been isolated, although they usually form as a mixture which is very difficult to separate. Thus ethyl 4-anisalhydantoin-N-1-propionate (I) and 4-anisalhydantoin-N-1-propionic acid (II) when heated with hydrogen iodide in the presence of red phosphorus react to give a crystalline product; m. p., 168–190°. This melting point remained unchanged even after repeated recrystallizations although the substance appeared to be homogeneous and was found on analysis to correspond to the above formula. For purposes of purification and study, it was then prepared in quantity by heating ethyl 4-anisalhydantoin-N-1-propionate, m. p. 158–158.5°, in 10g. portions with 35 cc. of hydrogen iodide (d., 1.7) and 2 g. of red phosphorus at a temperature of 130–140° for two hours. The product was distilled from a bath kept at 170° and the residue digested with 50 cc. of boiling water and filtered to remove the red phosphorus. The clear, colorless filtrate was concentrated to 15 cc., cooled in a freezing mixture and seeded, when the hydantoin separated in the form of a fine, white crystalline precipitate which appeared to average 7 g.; m. p., 168–190°.

Anal. Calcd. for $C_{18}H_{14}O_5N_2$: N, 10.07. Found: 10.06, 9.92.

The hydantoin is very soluble in alcohol and in hot water (10 g. dissolves in 25 cc.) but is only moderately soluble in cold water. Its aqueous solutions turn blue litmus paper red. It cannot be purified by recrystallization from alcohol because of the ease with which it esterifies. When alcoholic solutions of the hydantoin are saturated with hydrogen chloride it passes quantitatively into a mixture of the corresponding esters, m. p. 125–137°. When heated with barium hydroxide in aqueous solutions it is decomposed into tyrosine, alanine and carbon dioxide.

In order to ascertain whether complete demethylation had taken place under the action of hydrogen iodide, experiments were undertaken for the purpose of determining the exact amount of methyl iodide given off during the reaction. With this end in view, Zeisel's method for methoxyl determination was applied to 4-anisalhydantoin-N-1-propionic acid (II) with the modification that in each experiment a quantity of acetic acid sufficient to keep the substance in solution was added to the reaction mixture;[16] 0.2965 and 0.2998 g. of acid gave 0.2375 and 0.2428 g. of silver iodide, respectively, as compared with 0.2400 and 0.2421 g., calculated. These results show conclusively that complete demethylation must have taken place. The resolution of the mixture into its components was finally effected by a very laborious and protracted process of fractional recrystallization from dilute aqueous solution. At least some of the difficulty which was experienced in separating the two isomers appears to be due to the fact that they differ very slightly in their solubilities in water. Small amounts of two well-differentiated modifications, X and XI, melting at 193.5–195° and 187–188.5°, respectively, were, however, finally separated. Of these the former, X, is the less soluble, and separates from its aqueous solutions in hard, transparent prisms which are colorless when pure but which tend to absorb any foreign coloring matter present in its solutions. The lower-melting and relatively more soluble isomer XI separates from its aqueous solutions in clusters of transparent prisms which may be distinguished by the fact that they rapidly become opaque when exposed to the air.

The isomers X and XI were much more conveniently prepared from the corresponding acids VII and VI. Thus, 1.5 g. of 4-anisylhydantoin-N-1-propionic acid (VII), m. p. 160–161°, when heated for one hour at a temperature of 100° with hydrogen iodide in the presence of red phosphorus was transformed into 1.3 g. of the pure polypeptide-hydantoin X, while the isomeric acid VI gave XI under similar conditions. Pure products were obtained only when the reaction temperature was not allowed to rise above 100°. It is to be noted that demethylation at temperatures of 130–140° resulted in the formation of a mixture; m. p., 168–169°. Thus the same specimen of pure acid, V, gave a single modification of polypeptide-hydantoin, namely, XI, when demethylated at 100°, and gave a mixture of X and XI when temperatures of 130–140° were used.

Anal. Calcd. for $C_{13}H_{14}O_5N_2$: N, 10.07. Found X (m. p., 193.5–195°): 10.15, 10.13; XI (m. p., 187–188.5°): 10.11, 10.15.

Ethyl 4-Hydroxybenzylhydantoin-N-1-propionates, VIII and IX.—Two isomeric substances corresponding to this formula have been isolated; they melt at 133–138° and 152–155°, respectively. In all ordinary preparations, however, these isomers are formed in approximately equal amounts and separate as a crystalline mixture; m. p., 125–137°. This has been obtained (a) by esterifying a mixture of the corresponding isomeric acids X and XI in alcohol under the action of hydrogen chloride and (b) by reducing and demethylating ethyl 4-anisalhydantoin-N-1-propionate I under the action of hydrogen iodide. In both cases it was found that the melting point 125–137° did not improve materially on recrystallizations from alcoholic solutions of ordinary concentrations. In the latter case the procedure was the same as that described previously

[16] Baeyer and Villiger, *Ber.*, **35**, 1199 (1902). Meyer, *Ber.*, **40**, 1437 (1907).

for the preparation of the polypeptide acids X and XI[17] except for the fact that alcohol was added to the aqueous filtrate from the red phosphorus. The solution was evaporated almost to dryness, the residue treated with 100 cc. of alcohol, and this process repeated several times. Under these conditions hydrogen iodide, which is always present, is almost completely removed in the form of ethyl iodide, and the yield of crystalline material is increased, 10g. portions of ethyl 4-anisalhydantoin-N-1-propionate yielding on an average 8 to 9 g. of reduced and demethylated ester; m. p., 125–137°.

Anal. Calcd. for $C_{15}H_{18}O_5N_2$: N, 9.15. Found: 9.07, 9.03, 9.04.

Ethyl 4-hydroxybenzylhydantoin-N-1-propionate is very soluble in boiling alcohol (10 g. in 30 cc.) from which it separates on cooling in the form of small, compact, semi-transparent crystals which seem to become more opaque on exposure to the air. It is readily hydrolyzed into its corresponding acid mixture, m. p. 168–190°, when heated with dil. hydrochloric acid. When heated with barium hydroxide in aqueous alcoholic solutions, it decomposes to give tyrosine, alanine and carbon dioxide.

A partial resolution of the mixture, m. p. 125–137°, into its components was finally effected by fractional recrystallization (extending over a period of five or six weeks) from dil. alcoholic solutions. Two well-differentiated modifications, m. p. 152–155° and 133–138°, were obtained in this way. The former was the less soluble and crystallized from its solutions in the form of single, rectangular, transparent prisms resembling those of the high-melting acid X, while the latter separated in transparent, diamond-shaped prisms which were further distinguished by the fact that, like the crystals of the low-melting acid XI, they became opaque when exposed to the air.

Hydrolysis of the Polypeptide-Hydantoins X and XI and of the Corresponding Ethyl Esters VIII and IX in the Presence of Barium Hydroxide

The specimens of acid and ester which were employed in this hydrolysis consisted in both cases of the mixtures, m. p. 168–190° and 125–137°, respectively. The procedure was the same whether acid or ester was used except for the fact that in the case of the latter enough alcohol was added to the reaction mixture to hold the ester in solution. This alcohol was removed by evaporation at the end of the hydrolysis before proceeding with the separation of the amino acids. Thus 18 g. of ester, m. p. 125–137°, mixed with 120 g. of finely powdered barium hydroxide[18] was suspended in 140 cc. of water, 100 cc. of alcohol was added, and the mixture heated continuously on a steam-bath for four days. During this period the outlet of the condenser was connected with an absorption apparatus containing absolute alcohol and hydrochloric acid, in order to ascertain whether any ammonia or free organic base was given off. At the end of four days 200 cc. of water was added to the reaction flask and the mixture distilled into hydrochloric acid until about 100 cc. of distillate had been collected. When this distillate and the contents of the absorption flask were combined and evaporated, a residue consisting of a very minute quantity of ammonium chloride was obtained. No trace of any organic base was found. The contents of the reaction flask were again diluted with 100 cc. of water and the solid barium hydroxide and barium carbonate present were removed by filtering hot. The residue obtained in this way was extracted several times with hot water in order to free it from traces of organic matter and the extractions were added to the main filtrate. The latter was then treated in either of the following ways.

a. Sulfuric acid was added carefully to the clear filtrate until a point was reached when the addition of a single drop produced no further precipitate in the supernatant liquid. Then by a process of filtering 5 drops and testing first with barium hydrox-

[17] See p. 2949 of this article.

[18] Ref. 6, p. 471.

ide solution, then 5 drops more with sulfuric acid solution, the whole filtrate was brought to a point where neither barium nor sulfate ions were present in excess. The mixture was then filtered hot, and the precipitated barium sulfate extracted with an equal quantity of boiling water. The two filtrates were combined and evaporated to a volume of 30–40 cc. During this process small quantities of tyrosine separated from time to time and were filtered off. The condensed filtrate was then treated with an equal volume of alcohol, when alanine was slowly precipitated. Additional quantities were obtained by concentrating successive filtrates and precipitating with alcohol. The alanine which was obtained in this way was slightly contaminated with tyrosine but was readily freed from this impurity by extraction with water, filtration and reprecipitation with alcohol. A total of 4.2 g. of pure alanine was obtained (calcd. 5.2 g.), a result which under the conditions of the experiment may be regarded as approximately quantitative. The substance was identified by the usual tests and by a mixed-melting-point determination with a speciment of alanine; m. p., 279° with decomposition. The tyrosine formed with alanine as the result of this hydrolysis was for the most part precipitated with barium sulfate and was subsequently separated from this insoluble residue by repeated extractions with dil. hydrochloric acid. When these extractions were combined and the tyrosine was reprecipitated by aqueous ammonia, 60% of the calculated amount was obtained. Complete absence of glycine in the original filtrate from barium sulfate was demonstrated by applying Levene's method for detecting glycine in the presence of alanine by means of the picrate.[19]

b. Somewhat better yields of tyrosine, approximating 70–80% of the calculated amount, were obtained by the following method. The filtrate from barium hydroxide and barium carbonate was saturated with carbon dioxide and then filtered to remove the precipitated barium carbonate. The filtrate and washings from the carbonate were combined, the solution was concentrated and resaturated with carbon dioxide, this process being repeated until the filtrate reached a volume of 30–40 cc. The various precipitates (containing tyrosine as well as barium carbonate) were combined, dissolved in the least possible quantity of hydrochloric acid, and the tyrosine was precipitated by adding aqueous ammonia. The tyrosine was identified by the usual tests and by a mixed-melting-point determination with a specimen of tyrosine; m. p., 295° with decomposition. The alanine which was formed in this hydrolysis was contained in the filtrates from barium carbonate and tyrosine, and was separated by precipitation with sulfuric acid and alcohol according to the method described under (a). The yield of alanine was not as good in this case.

The hydrolysis of the polypeptide-hydantoin, m. p. 217°,[20] conducted under the conditions that have just been described in (b), resulted in the formation of glycine, tyrosine and carbon dioxide. Glycine was separated in the form of glycine ester hydrochloride by evaporating the filtrate from barium carbonate and tyrosine almost to dryness, extracting with absolute alcohol, filtering and saturating the alcohol solution with dry hydrogen chloride. The substance was identified by a mixed-melting-point determination with a specimen of glycine ester hydrochloride, m. p. 144°. No trace of alanine was detected in the filtrate.

Summary

Correction is made of previous work. Four pairs of inactive stereoisomeric modifications of N-1-derivatives of hydantoin are described. It may be noted in the case of IV and V that the corresponding unsaturated compound (I) seems to have no tendency to exist in the form of geomet-

[19] Levene and Van Slyke, *J. Biol. Chem.*, **12**, 285 (1912).

[20] See page 2942 of this article; also Ref. 5, p. 1265; Ref. 1, p. 854.

rical isomers although another type of space isomerism develops simultaneously with the reduction of the double bond.

South Hadley, Massachusetts

[Contribution from the Chemical Laboratory of Mount Holyoke College]

ISOMERIZATION IN THE HYDANTOIN SERIES INDUCED BY THE ACTION OF HYDROGEN CHLORIDE

By Dorothy A. Hahn and Elizabeth Gilman

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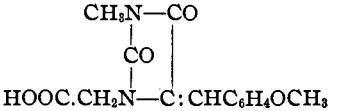
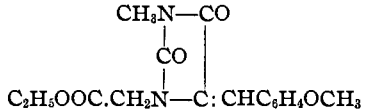
In a recent paper[1] two cases of geometrical isomerism occurring among hydantoin derivatives were described. The substances consisted of two pairs of compounds corresponding, respectively, to Formulas I and II,



I

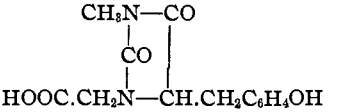
Two isomers; m. p., 167–167.5° and 203–205°



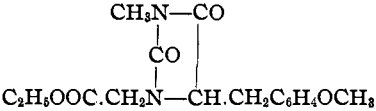
II

Two isomers; m. p., 107–108° and 127–128°

and the conclusion that they represented pairs of geometrical isomers was based upon the fact that all four on treatment with hydrogen iodide gave the same polypeptide-hydantoin (III). Even more convincing evi-



III



IV

dence than the relationship between the two esters, m. p. 107–108° and 127–128°, is one of geometrical isomerism was furnished by the fact that both reduce to a single product (IV) when shaken in alcohol solution in the cold with hydrogen in the presence of colloidal palladium.[2]

The further observation has now been made that isomerization of the lower into the higher-melting ester may be induced by suspending the former in alcohol and saturating the mixture with hydrogen chloride. The reaction is practically quantitative. It is usually attended by a change from a colorless to a colored solution (varying from pale yellow to deep red) and is complete in from eight to forty-eight hours. The product is pure and halogen free after two recrystallizations from alcohol.

Isomerization under these conditions took place so readily and was so complete that the possibility at once suggested itself that other similar hydantoins containing ethylene carbon atoms might be made to isomerize

[1] Hahn and Renfrew, This Journal, **47**, 147 (1925).

[2] Ref. 1, p. 157.