[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF MOUNT HOLYOKE COLLEGE]

SYNTHESIS OF THE POLYPEPTIDE HYDANTOIN GLYCYL N-3-METHYLPHENYLALANINE HYDANTOIN[1]

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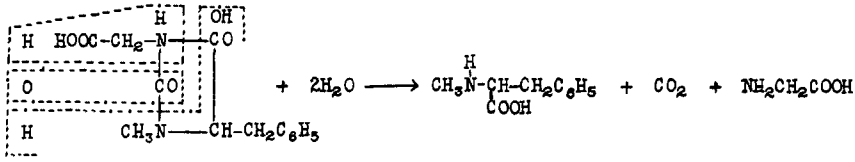
The term polypeptide hydantoin was first applied by T. B. Johnson to cyclic urea combinations of α-amino acids.[2] Such substances may be distinguished from polypeptide combinations by the fact that while the latter undergo hydrolysis to give α-amino acids exclusively, the former give α-amino acids and carbon dioxide. A number of combinations of this type have been synthesized and their properties studied.[3] The present investigation was undertaken with a view to extending the knowledge of this class of compounds and also incidentally to work out a new method of preparation for the amino acid, α-N-methyl-β-phenylpropionic acid. This substance had previously been synthesized by E. Friedmann and S. Gutman[4] from α-bromo-β-phenylpropionic acid. It has now been prepared by decomposing the polypeptide hydantoin (a) N-3-methyl-4-benzyl-hydantoin-N-1-acetic acid and (b) N-1-N-3-dimethyl-4-benzyl-hydantoin under the action of barium hydroxide, as follows.



In a similar manner dimethylbenzylhydantoin (b) hydrolyzes to give α-N-methyl-β-phenylpropionic acid and methylamine. In each case the product was identified by comparison with the same acid obtained by synthesis according to Friedmann and Gutman's method. The preparation and the various chemical relationships of the above respective polypeptide hydantoins will now be considered separately.

The esters of N-3-methyl-4-benzyl-hydantoin-N-1-acetic acid were prepared by starting with ethyl-4-benzalhydantoin-N-1-acetate.[5] In repeating the work of Bates it has been possible to confirm his observation in regard to the existence of two geometrically isomeric forms of benzalhydantoin.[6]

[1] This work is being offered in partial fulfilment of the requirements for the degree of Master of Arts at Mount Holyoke College.

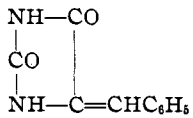
[2] Johnson, *Proc. Nat. Acad. Sci.*, **2**, 69 (1916).

[3] (a) Johnson and Bates, THIS JOURNAL, **38**, 1087 (1916); (b) Johnson and Hahn, *ibid.*, **39**, 1255 (1917); (c) Hahn and Renfrew, *ibid.*, **47**, 147 (1925); (d) Hahn and Gilman, *ibid.*, **47**, 2941 (1925).

[4] Friedmann and Gutman, *Biochem. Z.*, **27**, 491–497 (1910).

[5] Ref. 3 a, p. 1092.

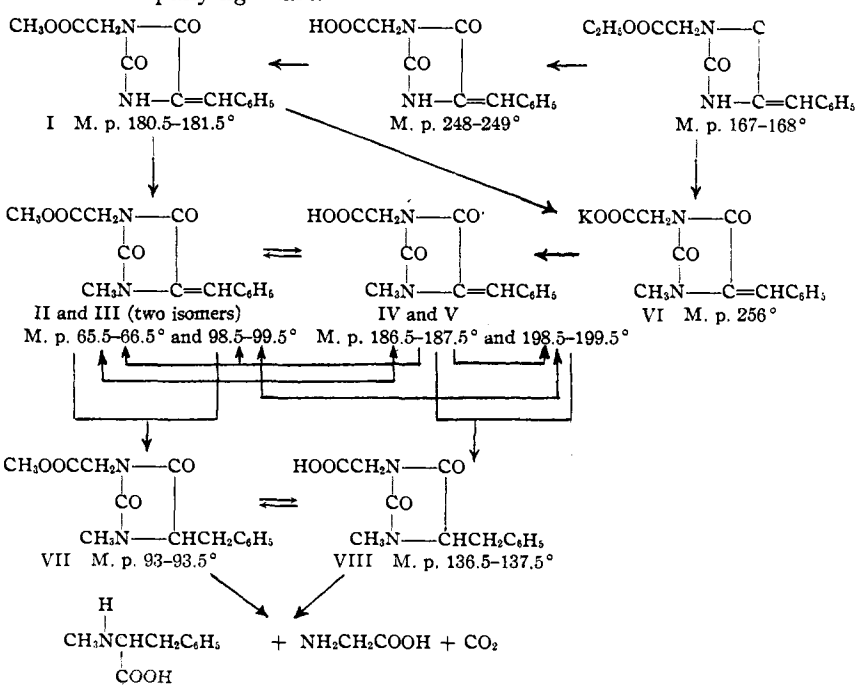
[6] Johnson and Bates, THIS JOURNAL, **37**, 384 (1915).



Curves showing the absorption spectra of these two compounds will be found included in this paper (Fig. 1). The existence of isomeric modifications belonging to the class of unsaturated hydantoins in which neither hydrogen atom nor the nitrogen has been substituted is thus demonstrated and the supposition[3d] is therefore no longer tenable that this kind of isomerism occurs only among unsaturated hydantoins in which the lability of the molecule has been decreased by the substitution of both free hydrogen atoms.

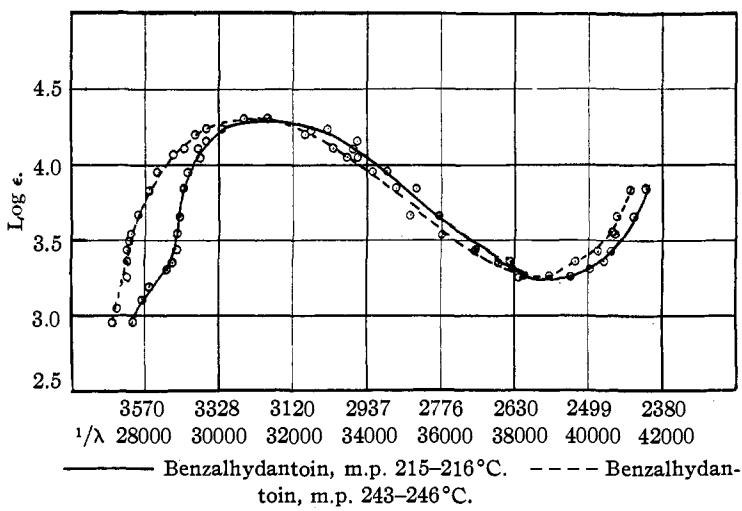
The preparation of methyl-N-3-methyl-4-benzalhydantoin-N-1-acetate, II and III, could not be effected to advantage in the usual way[7] since the yields were very low.

A better method was found to consist in alkylating the N-3-potassium derivative of potassium 4-benzalhydantoin-N-1-acetate. The substance, VI, obtained in this way was formed in good yield and was relatively quite pure. It was, therefore, used as the starting point in the preparation of the great majority of the derivatives of the hydantoins which are shown on the accompanying chart.



[7] Johnson and Nicolet, *Am. Chem. J.*, **47**, 468 (1912).

Potassium-N-3-methyl-4-benzalhydantoin-N-1-acetate, VI, passes into the lower-melting isomer of the corresponding acid, IV, when its aqueous solution is acidified. This acid, IV, isomerizes to give the higher-melting modification, V, when dissolved in aqueous acetic acid and subjected to the action of hydrogen chloride. The same acid, IV, when suspended in alcohol and the solution treated with gaseous hydrogen chloride passes into the corresponding higher-melting ester III. In order to obtain the lower-melting ester, II, it was necessary to esterify potassium N-3-methyl-4-benzalhydantoin-N-1-acetate, VI, by treating it with (a) dimethyl sulfate in an alkaline medium, (b) with methyl iodide in alcohol solution or (c) by treating its silver salt with methyl iodide. The product which



——— Benzalhydantoin, m.p. 215–216°C. – – – – Benzalhydantoin, m.p. 243–246°C.

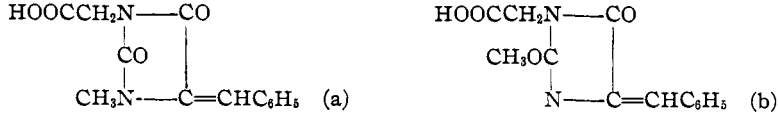
Fig. 1.

was obtained in this way is exceedingly unstable and decomposes on standing with the formation of benzaldehyde. Both esters, II and III, reduce to give the same product, VII, when treated with hydrogen. The corresponding isomeric acids, IV and V, behave similarly, being transformed into the saturated acid, VIII. Other minor interrelationships are indicated on the chart. The constitution of the polypeptide hydantoin VIII, and, therefore, the constitution of all interrelated products, was established by the fact that under the action of barium hydroxide it may be hydrolyzed to give carbon dioxide glycine and α-N-methyl-β-phenylpropionic acid. Curves showing the absorption spectra of I, VII, IV and V will be found in Figs. 2 and 3.[8]

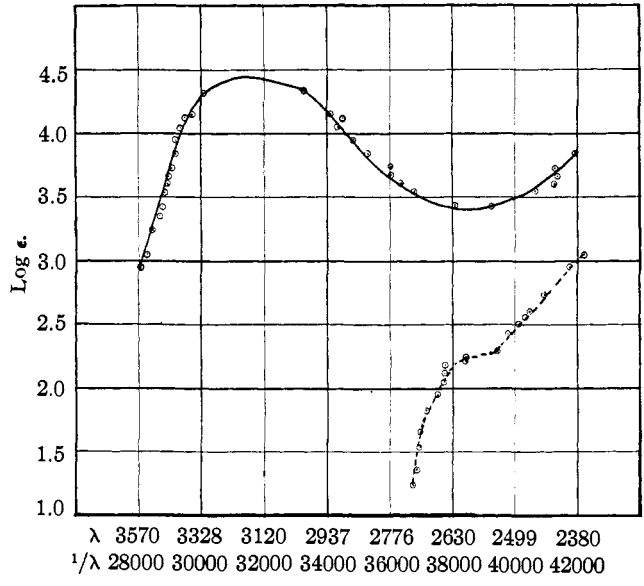
Although the hydrolysis of the polypeptide hydantoin may be said to establish its constitution and the constitution of substances directly

[8] Acknowledgment is made to Dr. Emma P. Carr for advice and assistance in the spectrographic work, which was done with the Hilger Quartz Spectrograph, E 36.

related to it, the possibility, nevertheless, presented itself that tautomeric equilibrium between the forms



might exist and that desmotropic modifications corresponding to these formulas might be assumed to represent the lower- and high-melting isomeric acids, IV and V. In order, therefore, to determine the presence



I ————Methyl-4-benzalhydantoin-N-1-acetate, m.p. 180.5–181.5°C. VII – – – –Methyl-N-3-methyl-4-benzylhydantoin-N-1 acetate, m.p. 93–93.5°C.

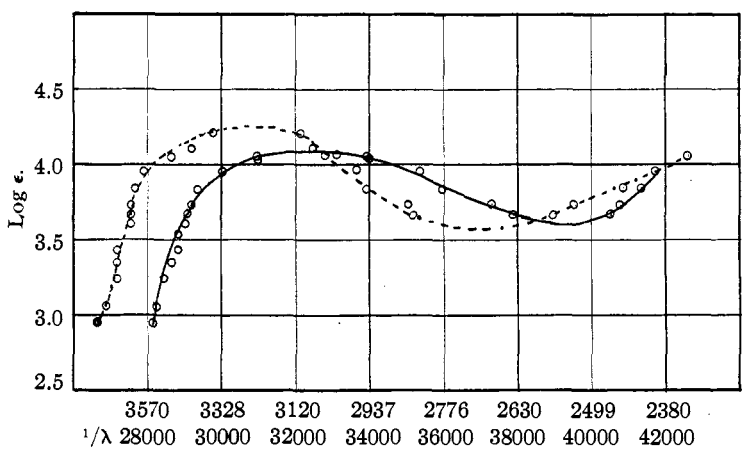
Fig. 2.

of any appreciable quantities of an enol form (b), Zeisel determinations were run on both isomeric acids. The results showed that while traces of methyl were split off from the higher-melting isomer[9] (about 2% of the total methyl), the quantity of the enol modification which might be present at any time would not be sufficient to account for the observed phenomenon of isomerization between the two forms.

In conclusion emphasis must be placed on the fact that in the case of the isomeric esters II and III, the lower-melting modification is by far the more unstable, while in the case of the isomeric acids IV and V the

[9] Goldschmiedt reports numerous instances in which methyl groups have been observed to split off from nitrogen at a low temperature. Compare *Monatsh.*, **27**, 849–878 (1906).

reverse holds true and the lower-melting modification represents the stable form. Such a marked difference in the stability of any two isomeric modifications has never before been observed in this series although a fairly large number of such pairs of compounds have been prepared and studied.[10] In the case of the substances under consideration the lower-melting ester (m. p. 65°) is so unstable that even the purest crystalline specimens, although perfectly dry and apparently free from solvent, slowly change into an oil under the action of light. This change is accompanied by the splitting off of benzaldehyde and the formation of a solid product which is characterized by the fact that it has a relatively high melting point (around 278°) and that it is extremely insoluble in ordinary solvents.



IV ——— N-3-Methyl-4-benzalhydantoin-N-1-acetic acid, m.p. 186.5–187.5°C. V – – – – N-3-Methyl-4-benzalhydantoin-N-1-acetic acid, m.p. 198.5–199.5°C.

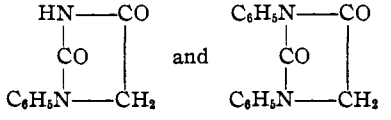
Fig. 3.

The higher-melting isomeric ester does not melt to an oil upon standing but it suffers a change in melting point from 98.5–99.5° to 97–245°. In recrystallizing the mixture which has formed in this way, it is relatively easy to recover the pure ester by filtering its alcoholic solutions from minute quantities of the same insoluble, high-melting products (that is, m. p. 278°). The transformation to this insoluble product is most readily followed in the case of either isomer by allowing a solution of the substance in alcohol or ether to stand in the sunlight or better in a quartz flask under the ultraviolet rays of a mercury lamp. Under these conditions the solution which was originally perfectly clear and transparent begins to show the formation of an extremely light, flocculent precipitate. The formation of the precipitate continues steadily, under the action of ultra-

[10] Ref. 3 d, p. 2953.

violet light, and appears to represent a time reaction. Solutions of the isomeric acid, V, behave similarly but in this case the resulting insoluble product melts even higher, m. p. 292°.

The splitting off of benzaldehyde from the ester, m. p. 65.5°, has been definitely established and in this connection it is interesting to note that the amount of affinity on the methylene carbon atom in the 4-position which may be exercised in holding various substituents in the hydantoin ring, seems to be very decidedly affected by the substitution of hydrogen in the N-3-position. Thus, for example, it has been observed that hydantoins which contain phenyl groups in the N-3 position, namely



will not react with benzaldehyde nor with anisaldehyde,[11] and that in general substitution in this position decreases the reactivity of the methylene hydrogen.[12] It is now found that substituents in the 4-position tend to split off in the case of hydantoin derivatives which also contain substituents in the N-3-position. A more detailed account of the products which result from the decomposition of the isomeric unsaturated acids and esters under the action of light is given in a separate paper (see THIS JOURNAL **49**, 2877 (1927)).

Experimental

Ethyl-4-benzalhydantoin-N-1-acetate was used as the starting point in the present investigation. This substance had been prepared by the alkylation of benzalhydantoin.[11a,5] In repeating this work it has been possible to confirm the discovery by Johnson and Bates[6] of the existence of two isomeric forms of benzalhydantoin. The higher-melting modification, m. p. 246°, could be detected only in handling large quantities of this material since it is present in very small quantities. It is much less soluble in any given solvent than the lower-melting modification, m. p. 215–216.[13] Both substances were analyzed and their absorption curves plotted. It is interesting to note that the absorption spectra of these two substances bear somewhat the same general relationship to each other as has been observed in the case of isomeric modifications of N-1-N-3-di-substituted hydantoins.

The existence of isomeric modifications of ethyl-4-benzalhydantoin-N-1-acetate was not confirmed although large quantities of material were handled and a careful search was made of all residues. In this connection it may be said that the most convenient method for separating ethyl-4-

[11] (a) Wheeler and Hoffman, *Am. Chem. J.*, **45**, 371 (1911) and (b) ref. 3 a, p. 1093.

[12] Johnson and Renfrew (not yet published).

[13] Compare Wheeler and Hoffman, m. p. 220° (ref. 11 a).

benzalhydantoin-N-1-acetate from benzalhydantoin was by extraction with boiling chloroform, in which solvent the latter substance is practically insoluble.

In preliminary experiments undertaken with a view to substituting methyl for hydrogen in the N-3-position by the usual procedure of treating ethyl-4-benzalhydantoin-N-1-acetate first with an equivalent of potassium hydroxide in alcohol solution and then with methyl iodide, it was found that the resulting product consisted of a very bad mixture. In order to eliminate complications which might arise as the result of a possible interchange of ethyl and methyl groups during the course of this reaction, ethyl-4-benzalhydantoin-N-1-acetate was, therefore, transformed into the corresponding methyl ester before alkylation with methyl iodide.

Methyl-4-benzalhydantoin-N-1-acetate, I, was prepared by transforming the corresponding ethyl ester into the potassium salt. One hundred g. dissolved in 1200 cc. of boiling alcohol and treated with 30 g. of potassium hydroxide (1.3 equivalents) in 200 cc. of boiling alcohol, yielded 110 g. of pure salt. This salt was completely soluble in cold water and its aqueous solution when acidified with hydrochloric acid gave quantitative yields of the corresponding acid, m. p. 248–249°.[14] The acid, when suspended in methyl alcohol and the solution saturated with dry hydrogen chloride gas, passed quantitatively into the corresponding methyl ester, m. p. 180.5–181.5° (I).

Anal. Calcd. for $C_{13}H_{12}O_4N_2$: N, 10.77. Found: 10.47, 10.58.

The ester is slightly soluble in cold methyl alcohol (1.3 g. in 200 cc.) but somewhat more soluble in the boiling solvent (1 g. in 40 cc.) from which it separates on cooling in long, white needles which completely fill the space occupied by the solution. Its absorption spectrum corresponds very closely to that of the unsubstituted benzalhydantoin.

Methyl-N-3-methyl-4-benzalhydantoin-N-1-acetate, II and III, m. p. 65.5–66.5° and 98.5–99.5°. Two isomeric esters which correspond to the above melting points have been isolated. Of these the lower-melting modification is the more soluble. A *mixture* of the two isomers was obtained by dissolving 10 g. of methyl-4-benzalhydantoin-N-1-acetate in methyl alcohol and treating the solution first with an equivalent of potassium hydroxide in alcohol solution and then with the calculated quantity of methyl iodide. The solution was heated on a water-bath until neutral to litmus and then set aside to cool. Successive crystalline precipitates were identified as unacted upon methyl-4-benzalhydantoin-N-1-acetate, the corresponding potassium salt, the potassium salt of N-3-methyl-4-benzalhydantoin-N-1-acetate and the corresponding methyl ester, m. p. 65.5–66.5°. In all a maximum yield of about 2 g. of the last named substance was obtained. An oily residue which refused to crystallize was assumed to consist of additional quantities of this ester mixed with its higher-melting isomer, since the oil when taken up in alcohol and subjected to the action of dry hydrogen chloride passed quantitatively into the pure crystalline ester, m. p. 98.5–99.5°. Since a fairly large number of test experiments, operated under different conditions of concentration and employing different quantities of potassium hydroxide, gave more or less identical mixtures which were tedious to separate this method of preparation was abandoned.

A much more convenient method consisted in methylating potassium-4-benzalhydantoin-N-1-acetate instead of either of its corresponding esters. This procedure was suggested by the fact that N-1-N-3-disubstituted hydantoins are very much more soluble than the corresponding N-1-mono-substitution products. Moreover, since potas-

[14] Compare Johnson and Bates, m. p. 258° [THIS JOURNAL, 38, 1094 (1916)].

sium-4-benzalhydantoin-N-1-acetate is almost insoluble in boiling methyl alcohol, it was hoped not only that the course of the reaction might be followed by its gradual disappearance but that the methylated product might be readily separated in pure condition. Both expectations were realized. The potassium N-3-methyl-4-benzalhydantoin-N-1-acetate which was synthesized in this way will be described later. When an aqueous solution of this salt was treated with hydrochloric acid, the lower melting of the two isomeric forms of N-3-methyl-4-benzalhydantoin-N-1-acetic acid, IV (m. p. 186.5–187.5°), was obtained. Using the latter substance as a starting point, it was now possible to obtain either of the two isomeric esters by employing one or the other of the following modes of procedure.

Methyl-N-3-methyl-4-benzalhydantoin-N-1-acetate, II, m. p. 65.5–66.5°, was prepared by esterifying the corresponding lower-melting acid, IV, in alkaline solution under the action of dimethyl sulfate.[15] The fact that the lower-melting ester was formed exclusively under these conditions would seem to strengthen the suspicion that this modification is stable only in neutral and alkaline media. Six grams of acid, m. p. 186.5–187.5°, was added to a solution of 1.2 g. of potassium hydroxide in 40 cc. of methyl alcohol. The resulting clear solution was then treated with the calculated quantity of dimethyl sulfate (6 cc.) which was added slowly through a dropping funnel under constant shaking and cooling in an ice-bath. After standing for two hours under occasional shaking in an ice-bath, solid potassium hydroxide was added in small quantities (total was 2–3 g.) under slight warming until the solution remained neutral. The resulting mixture was treated with enough cold water to dissolve the potassium methyl sulfate which had separated out in the form of fine crystals. The solution of this substance was followed, on the addition of further quantities of water, by the separation of methyl-N-3-methyl-4-benzalhydantoin-N-1-acetate, m. p. 65.5–66.5°. The ester was purified by recrystallization from methyl alcohol.

This ester was also formed when 6.5 g. of N-3-methyl-4-benzalhydantoin-N-1-acetate was suspended in 10 cc. of alcohol and 15 cc. of methyl iodide and heated on a water-bath for twenty hours. The product obtained represented about 20% of the theoretical. It seems probable that the yield could be improved by varying the concentration of the methyl iodide in alcohol since the observation was made that with methyl iodide alone or with very dilute solutions of methyl iodide in alcohol, no reaction took place.

When 2.6 g. of the corresponding silver salt was treated with 10 cc. of methyl iodide diluted with 5 cc. of methyl alcohol, an immediate reaction took place and the ester was deposited in almost pure condition and quantitative yield.

Anal. Calcd. for $C_{14}H_{14}O_4N_2$: N, 10.21. Found: N, 10.16, 10.20.

The ester is very soluble in hot methyl alcohol (2 g. in 5 cc.) from which it separates on cooling in a freezing mixture in the form of rosets composed of fine, white needles or plates. The ester is very unstable and even when pure, dry and apparently free from all traces of solvent decomposes on standing into an oil which smells strongly of benzaldehyde. The period required for this transformation has varied from one week to six months. The ester passes readily and quantitatively into the corresponding higher-melting isomer when dissolved in methyl alcohol and treated with dry hydrogen chloride. It forms a mixture of the corresponding lower-melting acid and the open chain hydantoin acid on saponification with aqueous potassium hydroxide and subsequent acidification of the aqueous solution of the potassium salt. Reduction of the ester, II, to the corresponding saturated ester, VII, took place quantitatively under the action of hydrogen in the presence of palladium.

[15] (a) Compare Ullmann and Wenner, *Ber.*, **33**, 2476 (1900); (b) Hans Meyer, *Ber.*, **37**, 4144 (1904); (c) **40**, 2432 (1907).

Methyl-N-3-methyl-benzalhydantoin-N-1-acetate, III, m. p. 98.5–99.5°, was prepared by dissolving the lower-melting modification of the corresponding acid in methyl alcohol and saturating the solution with dry hydrogen chloride gas. For example, 44 g. of crude acid (m. p. 176–181°) was dissolved in 200 cc. of methyl alcohol and the solution saturated with dry hydrogen chloride. After saturation the mixture was refluxed on a steam-bath and then cooled and resaturated with hydrogen chloride This process was repeated until the crystalline ester separated out from the solution on cooling. In cases where the filtrate from these crystals deposited an oil after concentrating and cooling, the oil was taken up in alcohol, resaturated with hydrogen chloride, etc. In this way a complete transformation of the lower-melting acid, IV, into the higher-melting ester, III, was effected and a theoretical yield of the desired product was obtained. Methyl-N-3-methyl-4-benzalhydantoin-N-1-acetate was also prepared from the higher-melting isomeric acid, V, and from the lower-melting ester, II, by the application of the above general method of procedure.

Anal. Calcd. for $C_{14}H_{14}O_4N_2$: N, 10.21. Found: 10.16, 10.19.

The ester is very soluble in hot methyl alcohol (1 g. in 7 cc.) from which it crystallizes on concentrating the solution or upon precipitation by the addition of water. The purification is most readily effected by recrystallization from concentrated alcohol solutions from which it separates in rosets consisting of pale yellow needles or plates. When reduced by hydrogen in the presence of palladium it passes quantitatively into the corresponding saturated ester, VII. On hydrolysis it passes quantitatively into the corresponding high-melting acid, V.

It was never observed to pass into an oil on standing but did suffer a gradual change in its melting point, from 98–99° to 97–245°. This change resulted from the partial decomposition of the ester into a white substance, m. p. 276–278°, which is insoluble in alcohol and which can, therefore, be separated readily from the undecomposed ester by recrystallizing the latter from alcohol.

Potassium-N-3-methyl-4-benzalhydantoin-N-1-acetate, VI, m. p. 255–256°, was prepared by treating potassium-4-benzalhydantoin-N-1-acetate in 10 g. portions with 1.3 equivalents of potassium hydroxide in alcohol solution. The mixture was heated on a water-bath until the solution was perfectly clear and the transformation to potassium N-3-potassium-benzalhydantoin-N-1-acetate therefore complete. The calculated quantity of methyl iodide was then added and the heating continued at 65–70° for one and one-half hours or until the solution was neutral to litmus. The solution was then concentrated to small volume and the di-potassium derivative precipitated by the addition of ether. If, after concentrating the solution and cooling, the separation of small quantities of a crystalline precipitate was observed, this was removed by filtration before adding the ether, since it always represented traces of unacted upon potassium-4-benzalhydantoin-N-1-acetate. In cases where the methyl alcohol contained traces of acetone, iodoform was precipitated along with the condensation product. The latter was purified by recrystallization from alcohol. Since the purification of the salt when prepared in this way is complicated by the presence of iodoform and also of potassium iodide so that the yield approximated only about 50% of theory, it was found desirable to modify the procedure in the following way. Instead of precipitating the salt by the addition of ether to the concentrated solution of the reaction product, it was diluted with water and hydrochloric acid was added. Under these conditions the corresponding acid was precipitated in yields which vary between 80 and 90% of the theoretical. The acid so obtained reacted with potassium hydroxide in alcohol-water solutions to give the potassium salt in quantitative yields and pure condition. After two recrystallizations from hot methyl alcohol it was heated to constant weight and analyzed.

Anal. Calcd. for: $C_{12}H_{11}O_4N_2K$: N, 9.40. Found: 9.37, 9.32.

Silver-N-3-methyl-4-benzalhydantoin-N-1-acetate was prepared by treating alcohol solutions of the corresponding potassium salt with alcoholic silver nitrate.[16] The precipitated silver salt was dissolved by passing a stream of ammonia gas into the mixture, which was then filtered from silver oxide and the filtrate evaporated over sulfuric acid in a vacuum desiccator. After one recrystallization from boiling water it was analyzed.

Anal. Calcd. for $C_{13}H_{11}O_4N_2Ag$: N, 7.63. Found: 7.67.

The salt is soluble in boiling water (1 g. in 200 cc.) from which it separates on cooling in shining white crystals. It decomposes at 235° with the formation of a silver mirror. It passes into the corresponding acid, IV, when digested with aqueous potassium hydroxide and then acidified.

N-3-methyl-4-benzalhydantoin-N-1-acetic Acid.—Two isomeric forms of this substance, *viz.*, IV and V, m. p. 186.5–187.5° and 198.5–199.5°, were isolated. The lower-melting modification was obtained by treating the condensation product from potassium-4-benzalhydantoin-N-1-acetate with hydrochloric acid in the manner which has just been described. It was also prepared by acidifying aqueous solutions of pure potassium-N-3-methyl-4-benzalhydantoin-N-1-acetate, VI. After recrystallization first from methyl alcohol and then from acetone, it was found to have the following composition.

Anal. Calcd. for $C_{13}H_{12}O_4N_2$: N, 10.77. Found: 10.55, 10.59.

The acid is very soluble in boiling methyl alcohol (1 g. in 10 cc.), acetone (1 g. in 20 cc.) and acetic acid (1 g. in 20 cc.). It separates from these solutions on cooling in the form of small, transparent cubes or prisms. When dissolved in acetic acid, it passes under the action of hydrogen chloride into the higher-melting acid, V, and when dissolved in methyl alcohol and subjected to the same treatment, it passes into the corresponding methyl ester, III. Under the action of dimethyl sulfate in alkaline media it yields the lower-melting ester, II, and with hydrogen iodide it is reduced to the corresponding saturated acid, VIII.

The isomeric acid, V, m. p. 198.5–199.5°, was prepared by dissolving 11 g. of the lower-melting modification, IV, in a mixture of 200 cc. of acetic acid and 1000 cc. of concentrated hydrochloric acid. This solution when evaporated to small volume deposited the substance in pure condition. The acid may also be prepared by starting with higher-melting ester, III. Thus 2 g. of the ester, m. p. 98.5–99.5°, when dissolved in a mixture of 50 cc. of acetic acid and 25 cc. of concentrated hydrochloric acid and heated on a steam-bath in an open beaker for five hours, passed quantitatively into the higher-melting corresponding acid. When purified by recrystallization first from methyl alcohol and then from aqueous acetone, the substance gave abnormally low percentages of nitrogen on analysis. Such specimens when heated in an electric oven at 115° for twenty-four hours suffered a loss in weight which corresponded approximately to the loss of one molecule of water. (Calcd. % loss in weight for 1 mole of H_2O: 6.41. Found: 5.88, 6.03.) Other specimens of the acid which were recrystallized first from glacial acetic acid and then from pure, dry acetone when heated in the above manner suffered a loss in weight corresponding to 0.35 and 0.38% of water present. These specimens after heating gave the following results on analysis.

Anal. Calcd. for $C_{13}H_{14}O_4N_2$: N, 10.77. Found: 10.65, 10.63.

This acid is very soluble in boiling methyl alcohol (1 g. in 6 cc.), acetone (1 g. in 15 cc.) and acetic acid (1 g. in 12 cc.). It esterifies readily to give III when its methyl alcohol solution is treated with dry hydrogen chloride and like the corresponding lower-melting isomer, it passes into the saturated polypeptide hydantoin, VIII, on treatment with hydrogen iodide.

[16] Compare Schaal, *Ber.*, **40**, 4786 (1907).

Zeisel determinations were carried out in the case of both of the unsaturated isomeric acids, IV and V, with a view to ascertaining whether in either case the methyl group had shifted its position in the molecule from nitrogen to oxygen as the result of possible tautomeric rearrangements. The results were as follows: (1) no precipitate in the case of IV; (2) in the case of V, m. p. 196.5–198°, 0.3648 g. of acid gave 0.0085 g. of silver iodide; 0.3598 g. of acid gave 0.0068 g. of silver iodide. These results correspond to 0.15% and 0.12% of methyl, respectively. This may be assumed to be present (a) in combination with oxygen or (b) in loose combination with nitrogen.[17] Since the total percentage of methyl present in the molecule is equal to 5.77%, it follows that 0.5% and 0.2% has been split off in this process.

Methyl-N-3-methyl-4-benzylhydantoin-N-1-acetate, VII, m. p. 93–93.5°, was prepared by reducing the corresponding unsaturated esters, II and III, with hydrogen in the presence of palladium. It was also prepared by esterifying the corresponding acid, VIII, by saturating its methyl alcohol solution with hydrogen chloride. The method followed in reduction was the same as has been previously described in the case of ethyl-4-anisylhydantoin-N-1-propionate;[18] the reaction was quantitative and the resulting product practically pure. The substance was recrystallized from boiling methyl alcohol (1 g. soluble in 2 cc.) from which it separates in the form of large, rectangular prisms.

Anal. Calcd. for $C_{14}H_{16}O_4N_2$: N, 10.14. Found: 9.95, 9.99.

On hydrolysis with potassium hydroxide the ester passes quantitatively into the corresponding acid VIII.

N-3-Methyl-4-benzylhydantoin-N-1-acetic Acid, VIII, m. p. 136.5–137.5°, was prepared by reducing the isomeric unsaturated esters II and III and the acids, IV and V, with hydrogen iodide in the presence of red phosphorus and also from the corresponding saturated methyl ester VII by saponification with potassium hydroxide. In the case of the isomeric unsaturated compounds the procedure was the same in all cases: 2 g. of substance, 0.5 g. of red phosphorus, 20 cc. of acetic acid and 5 cc. of hydrogen iodide (sp. gr. 1.70) were heated for two hours in a flask fitted with a short air condenser on an oil-bath at 120–130°. The solution was then evaporated almost to dryness on a steam-bath, 10 cc. of water added and the solution again evaporated. This process was repeated several times in order to remove traces of hydrogen iodide. The aqueous solution (about 20 cc.) was filtered hot from the red phosphorus and set aside to cool, when on seeding the acid separated in the form of very small, white prisms. Specimens obtained in this way were found in all cases to be identical.

Anal. Calcd. for $C_{13}H_{14}O_4N_2$: N, 10.69. Found: N, 10.65, 10.60.

The acid is soluble in boiling water (1 g. in 8 cc.) from which it is precipitated on cooling in the form of an oil which solidifies on standing. When present in less concentrated aqueous solutions (1 g. in 30 cc.) it separates on seeding in crystalline form. It is very soluble in acetic acid, methyl alcohol and acetone. Dissolved in methyl alcohol and treated with hydrogen chloride it passes readily and quantitatively into the corresponding ester, VII.

Hydrolysis of the Polypeptide Hydantoin N-3-Methyl-4-benzylhydantoin-N-1-acetic Acid, VIII.—The hydrolysis was conducted in the manner described in the case of 4-hydroxy-benzylhydantoin-N-1-propionate.[19] Thus 7.4 g. of acid was added to a flask containing 50 g. of barium hydroxide, 100 cc. of methyl alcohol and 100 cc. of water and heated on a steam-bath for three and one-half days. The product was filtered hot to remove solid barium hydroxide and barium carbonate and the alkaline filtrate *exactly*

[17] Goldschmiedt, *Monatsh.*, **27**, 849–878 (1906).

[18] Ref. 3 d, p. 2948.

[19] Ref. 3 d, p. 2951.

neutralized by the addition of sulfuric acid. The precipitated barium sulfate was filtered hot and extracted several times with small quantities of hot water. The filtrate and aqueous extracts were then combined and evaporated to small volume when successive precipitates of the undecomposed polypeptide hydantoin, VIII (about 3 g.) and α-N-methyl-β-phenylpropionic acid separated. The filtrate from these substances on further concentration was treated with absolute alcohol. The precipitate which formed in this way was suspended in absolute alcohol and the solution saturated with hydrogen chloride, when a crystalline substance was obtained which was identified as glycine ester hydrochloride. The experiment was then repeated and the polypeptide hydantoin digested with barium hydroxide over a longer period (eleven days). Under these conditions the decomposition was found to be complete and quantitative yields of α-N-methyl-β-phenylpropionic acid and glycine hydrochloride were obtained.

The α-N-methyl-β-phenylpropionic acid was identified by comparison with a specimen which was synthesized in the following way. Malonic ester was transformed into benzyl malonic ester[20] and then into bromobenzyl malonic acid, which was purified by recrystallization from toluene.[21] The corresponding monobasic acid (α-bromo-β-phenylpropionic acid) was then prepared according to the method described by Fischer[22] and immediately transformed into the N-methylamino derivative by treatment with methyl amine.[4] The product was purified by crystallization from boiling water when it sublimed at 254°. Its identity was finally established by an analysis.

Dimethylbenzalhydantoin, m. p. 83–84°, was used as the starting point for the preparation of α-N-methyl-β-phenylpropionic acid and was made in the usual way.[7] Benzalhydantoin[11a] was dissolved in a 50% aqueous alcohol solution of potassium hydroxide (1.2 equivalents) and then treated with the calculated quantity of methyl iodide. The product always consisted of a mixture of benzalhydantoin which separated on cooling in almost pure condition (m. p. 200–220°) and a new product (m. p. 70–90°) which separated on concentrating the filtrates. When 1.2 equivalents of potassium hydroxide were used, the yield of the latter substance approximated 30% of the theoretical but on increasing the amount of potassium hydroxide to 2.2 equivalents the yield was increased to 70%. This product was purified by extraction with cold chloroform (in which benzalhydantoin is almost insoluble) and subsequent recrystallization from alcohol. When pure it melted at 83–84°.

Anal. Calcd. for $C_{12}H_{12}O_2N_2$: N, 12.96. Found: N, 12.69, 12.74.

Dimethylbenzalhydantoin is soluble in boiling chloroform (45 g. in 100 cc.), cold 95% alcohol (5 g. in 100 cc.), boiling 95% alcohol (40 g. in 100 cc.) and boiling 50% alcohol (12 g. in 100 cc.). When crystallized from 95% alcohol solution and allowed to cool slowly, it forms large, transparent, rhombic plates. When treated with hydrogen iodide it is readily reduced to the corresponding dimethylbenzylhydantoin.

Dimethylbenzylhydantoin, m. p. 86.5–87°, was prepared by treating dimethylbenzalhydantoin, m. p. 83–84°, with hydrogen iodide (sp. gr. 1.7) in the presence of red phosphorus. The product was obtained in pure condition and in practically theoretical quantities. After three recrystallizations from methyl alcohol, its melting point was 86.5–87°. The fact that its melting point is so nearly the same as that of the corresponding unsaturated compound from which it was prepared led to a doubt as to whether reduction had actually taken place. This doubt was dispelled by melting the two substances together when a mixed melting point of 62–74° was obtained.

Anal. Calcd. for $C_{12}H_{14}O_2N_2$: N, 12.84. Found: N, 12.68, 12.73.

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[20] Conrad, *Ann.*, **204**, 174 (1880).

[21] Fischer, *Ber.*, **37**, 3063 (1904).

[22] Ref. 21, p. 3064.

Dimethylbenzylhydantoin is soluble in hot methyl alcohol (10 g. in 20 cc.) from which it crystallizes in transparent, rectangular prisms.

Hydrolysis of the Polypeptide Hydantoin N-1,3-Dimethylbenzylhydantoin.—The hydrolysis of dimethylbenzylhydantoin was accomplished by boiling 6 g. of the hydantoin with 50 g. of barium hydroxide for four days. The product was filtered from solid barium carbonate and barium hydroxide and then steam distilled into a flask containing hydrochloric acid. The distillate was evaporated to dryness and the residue recrystallized twice from absolute alcohol. When pure it was found to melt at 225–226.5°. This product was shown to consist of the hydrochloride of methylamine, since its melting point was not lowered by melting with a sample of methylamine hydrochloride which had been prepared by neutralizing a 33% solution of methylamine[23] with hydrochloric acid.

The liquid remaining after steam distillation was exactly neutralized with sulfuric acid, filtered from the barium sulfate and concentrated. The crystals which precipitated on cooling were found to be identical with the α-N-methyl-β-phenylpropionic acid prepared from the acetic acid derivative and from malonic ester. Both methylamine hydrochloride and the amino acid were obtained in theoretical yield.

Summary

The amino acid α-N-methyl-β-phenylpropionic acid has been prepared by the hydrolysis of two different polypeptide hydantoins. The phenomenon of isomerization by the action of hydrochloric acid has again been observed in connection with the study of two separate pairs of geometric isomers. The tendency of substituents in the 4-position to split off when the N-3-position is occupied by a group other than hydrogen has been observed. This corresponds with observations already made as to the relative inactivity of methylene hydrogen in the 4-position toward carbonyl oxygen when this hydrogen is present in hydantoin compounds which contain substituents in the N-3-position.

Graphs showing the absorption spectra of isomeric forms of benzalhydantoin and of its N-3-methyl-N-1-acetic acid derivatives serve to confirm the theory that these pairs of isomers are of the geometric variety and also to emphasize the difference in the curves of isomeric substances in which the N-3-position is occupied and in which it is not.

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[23] Purchased from the Eastman Kodak Company, Rochester, New York.