[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

RESEARCHES ON HYDANTOINS. XLVII. SYNTHESIS OF POLYPEPTIDE HYDANTOINS FROM 2-THIOHYDANTOIN-3-ACETIC ACID[1]

BY ALICE G. RENFREW[2] AND TREAT B. JOHNSON

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In the study of new organic principles applicable for internal antisepsis we inaugurated three years ago a new feature by introducing into our researches on germicides an investigation of some phenolic derivatives of hydantoin. Having reliable knowledge of the fact that the hydantoin cycle is a non-toxic organic construction and one which is also very resistant to the action of ferments and bacterial enzymes,[3] we concluded that it would be of special interest to determine whether it would be feasible to utilize the hydantoin nucleus as a vehicle for transporting antiseptic groupings in the body. Johnson and Coghill[4] prepared, in the preliminary work, a series of mono- and di-phenol derivatives of hydantoin with the phenolic grouping $-C_6H_4OH$ substituted on both carbon and nitrogen atoms of the hydantoin cycle. A preliminary bacteriological examination of several of these derivatives has already been reported. None thus far examined exhibited an antiseptic activity comparable to phenol. The new combinations did not prove of practical utility as germicidal agents on account of their insolubility in water. The toxicity of the aromatic phenol group, however, was reduced by coupling the benzene nucleus with the hydantoin.

In continuing this work we sought to incorporate changes in constitution which would be expected to increase the solubility of our compounds and at the same time lead to constructions favorable for pharmaceutical application. We therefore turned our attention to a study of certain simple, phenolic constructions combined with the polypeptide hydantoin nucleus. It was felt that a study of the effect of introducing acid groupings on nitrogen would be beneficial; we therefore undertook the preparation of new combinations of this type. The grouping which we selected to increase the solubility of our phenolic compounds was the acid radical $-H_2CCOOH$. This has been incorporated by substitution in positions

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[2] Constructed from a dissertation presented by Alice Gertrude Renfrew to the Faculty of the Graduate School of Yale University, in June, 1927, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[3] Lewis, *J. Biol. Chem.*, **13**, 347 (1912); **14**, 245 (1913).

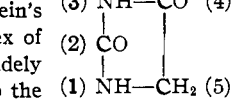
[4] Johnson and Coghill, THIS JOURNAL, **47**, 184 (1925); also Coghill, *ibid.*, **47**, 216 (1925).

1 and 3 of the hydantoin cycle. In this paper we shall confine our discussion to some derivatives of hydantoin-3-acetic acid, I, and its corresponding sulfur analog, II. The results of our study of derivatives of hydantoin-1-acetic acid will be reported in a later paper.[5]

It has been the experience of this Laboratory that aldehyde condensations are more readily effected with 2-thiohydantoins than with the oxygen analogs. Regarding the influence of sulfur in position 4 or in dithiohydantoins on the course of such condensation reactions we have no knowledge. Johnson and his co-workers have made comparative studies of both hydantoin and 2-thiohydantoin in condensations with various aldehydes.[6] The greater activity of the thiohydantoin also characterizes the condensation of hydantoin cycles containing substituents in the 1- and 3-positions of the ring. 1-Phenyl-2-thiohydantoin and 1,3-diphenyl-2-thiohydantoin were condensable with aldehydes without difficulty.[7] Using the corresponding oxygen derivatives, Wheeler and Hoffmann[8] were unsuccessful in effecting similar condensations, although Johnson and Hadley[9] later actually succeeded in condensing 1,3-diphenylhydantoin with benzaldehyde. Thus far no successful condensations of 1-phenylhydantoin with aldehydes have been reported.

The condensations that have been applied successfully with hydantoin-3-acetic acid and its sulfur analog are described in detail in the Experimental Part of this paper, and are illustrated structurally herewith. The three aldehydes used were *p*-anisaldehyde, salicylaldehyde and piperonal. By inspection of the formulas it will be observed that all of these new products have been represented structurally as cyclic methylene condensation reactions. This is an important conclusion of our work as both the 2-thiohydantion-3-acetic acid, II, and its oxygen analog, I, contain two methylene radicals theoretically capable of condensing with aldehydes. In no case, however, have we obtained any evidence that the methylene radical in the acyclic acetic acid group undergoes a condensation reaction with an aldehyde. Also in no aldehyde condensation reaction did we succeed in revealing a single new case of geometric

[5] The system used for numbering the positions of the hydantoin ring, as represented, will be adopted throughout this and following papers. It is in accord with the system of classification in use in Beilstein's "Handbuch der Organische Chemie," and in the subject index of *Chemical Abstracts*. A method of numbering which has been widely used in the past is one in which the numerical assignments to the two nitrogen atoms of the cycle are reversed.

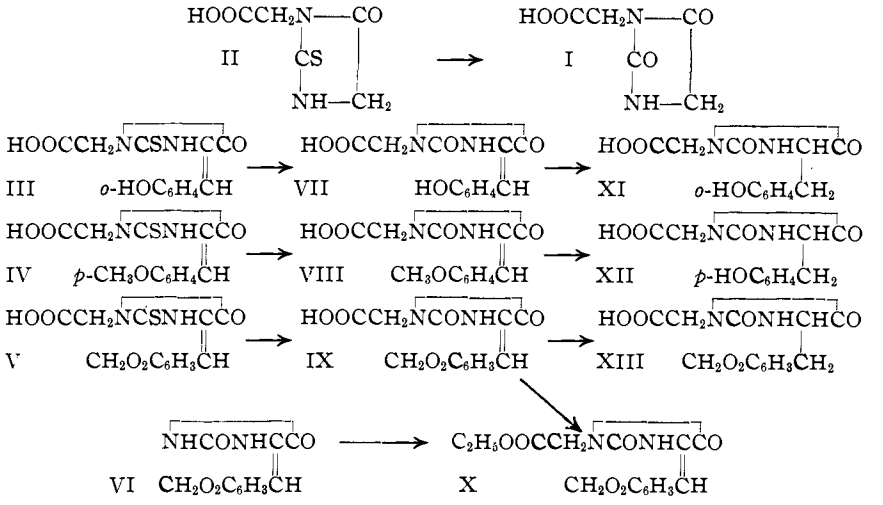


[6] Johnson and Scott, THIS JOURNAL, **37**, 1846 (1915); Johnson, Pfau, and Hodge, *ibid.*, **34**, 1041 (1912); Johnson and Bengis, *ibid.*, **35**, 1606 (1913); Johnson and Wrenshall, *ibid.*, **37**, 2133 (1915).

[7] Wheeler and Brautlecht, *Am. Chem. J.*, **45**, 446 (1911).

[8] Wheeler and Hoffmann, *ibid.*, **45**, 368 (1911).

[9] Johnson and Hadley, THIS JOURNAL, **37**, 171 (1915).



isomerism, despite the fact that the reaction products were carefully studied.

An interesting result worthy of special mention is the fact that most of the condensations applied with the three aldehydes and the hydantoin-3-acetic acids, I and II, in the presence of sodium acetate gave the sodium salts of the condensation products, notwithstanding the fact that all the condensations were carred out in glacal acetic acid solution. The two exceptions were the reaction products resulting from condensation of anisaldehyde and piperonal with 2-thiohydantoin-3-acetic acid. Here the free substituted hydantoin-acetic acids were obtained.

The condensations between 2-thiohydantoin-3-acetic acid, II (or its oxygen analog, I), with aldehydes serve also another useful purpose in that they make it possible to establish conclusively the structure of alkylation products of simple aldehyde-hydantoins when the alkylation method is used in the preparation of polypeptide hydantoin configurations. For example, the structure of the alkylation product X is established by the fact that the same derivative is formed by esterification of the hydantoin IX. It is a common experience that hydantoins of Type VI undergo substitution first in position 3 when subjected to the conditions of an alkylation reaction.

Experimental Part

2-Thiohydantoin-3-acetic acid, $HOOCCH_2\overline{NCSNHCH_2CO}$.—The acid is easily obtained in a yield of 85–90% of the theoretical according to the method of Johnson and Renfrew.[10]

2-Thio-5-*p*-anisalhydantoin-3-acetic Acid, $HOOCCH_2\overline{NCSNHC{=}(CHC_6H_4OCH_3)CO}$.—This hydantoin is formed by digesting thiohydantoin-3-acetic acid (5 g.) with *p*-anisal-

[10] Johnson and Renfrew, THIS JOURNAL, **47**, 240 (1925).

dehyde (5.5 g.) in the presence of 6 g. of anhydrous sodium acetate, 10 cc. of glacial acetic acid and 3 cc. of acetic anhydride. After heating the above mixture in an oil-bath for ten minutes at 250–260°, the fluid changed to a magma of the solid hydantoin. A further 10 cc. of glacial acetic acid was then added and the heating continued for two hours, when the condensation was apparently complete. The hydantoin was separated by filtering the reaction mixture while hot and washing with water and alcohol to remove impurities. A yield of 6.7 g. was obtained, corresponding to 80% of the theoretical. The hydantoin is purified by crystallization from alcohol or acetic acid and melts at 280–282°.

Anal. Calcd. for $C_{13}H_{12}O_4N_2S$: N, 9.59. Found: N, 9.63, 9.67.

5-*p*-Anisalhydantoin-3-acetic acid, $HOOCCH_2\overline{NCONHC{=}(CHC_6H_4OCH_3)CO}$.—This hydantoin-acetic acid has been described previously by Johnson and Hahn,[11] and recently by Granacher and Landolt.[12] The same hydantoin is also obtained by desulfurizing the above 2-thiohydantoin with chloro-acetic acid. A mixture of our product with a specimen of the original hydantoin showed no change in melting point, namely, 269–271°.

In an effort to accumulate further comparative data on the reactivity of the methylene group in nitrogen-substituted hydantoins, this hydantoin was also synthesized by a third method, namely, by condensing anisaldehyde with hydantoin-3-acetic acid. Reaction was brought about by digesting in a mixture of glacial acetic acid and acetic anhydride in the presence of anhydrous sodium acetate. The reaction was complete after refluxing in an oil-bath for three hours. After cooling, the acetic acid solution was diluted with water, when the condensation product separated. This was identified as the sodium salt of anisalhydantoin-3-acetic acid, corresponding in yield to 41% of the theoretical. The salt was initially colorless but after recrystallization from water it separated in green plates which deposited an alkaline residue when burned on platinum foil. This salt was not very soluble in water. These properties characterize the monosodium salt of this hydantoin, which has been studied by Hahn and Burt,[13] thus excluding the possibility of the condensation product being an acyclic hydantoin acid derivative. An excess of hydrochloric acid added to an aqueous solution of the sodium salt produced a heavy precipitate of the corresponding acetic acid. The acid showed a melting point in accordance with that already recorded. With recovery of some salt from filtrates, the total yield of condensation product was 51% of the theoretical. With slight variations in technique this same synthesis has been reported recently by Granacher and Landolt[12] as giving a yield of 57%. This unsaturated hydantoin is reduced smoothly to *p*-hydroxybenzylhydantoin-3-acetic acid when digested with hydriodic acid and red phosphorus.[11]

Salicylalhydantoin-3-Acetic Acid Derivatives

2-Thio-5-salicylalhydantoin-3-acetic Acid, $HOOCCH_2\overline{NCSNHC{=}(CHC_6H_4OH)CO}$.—This compound is easily formed by applying a condensation reaction with salicylic aldehyde and thiohydantoin-3-acetic acid. The reagents required are used in the following proportions: thiohydantoin-acetic acid, 10 g., salicylaldehyde, 10 g., sodium acetate 12 g., and glacial acetic acid, 30 cc. After heating this mixture for ten to fifteen minutes at 150–155°, the liquid became completely saturated with the condensation product and finally completely solidified. Water was then added to bring it to a volume of

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

[12] Granacher and Landolt, *Helv. Chim. Acta*, **10**, 799 (1927).

[13] Hahn and Burt, THIS JOURNAL, **39**, 2468 (1917).

50 cc. and the insoluble condensation product was separated by filtration. This was identified as the sodium salt of 2-thio-salicylalhydantoin-3-acetic acid and we obtained a yield of 14 g. The salt crystallizes from hot water and is converted into its corresponding acid by the action of hydrochloric acid. The yield of hydantoin was 96% of the theoretical. It was purified by crystallization from glacial acetic acid and melted with decomposition at 253–254°.

Anal. Calcd. for $C_{12}H_{10}O_4N_2S$: N, 10.07. Found: N, 10.12, 10.20.

Salicylalhydantoin-3-acetic acid, $HOOCCH_2\overline{NCONHC{=}(CHC_6H_4OH)}CO$.—Ten grams of the above 2-thiohydantoin compound was suspended in a solution containing 30 g. of chloro-acetic acid in 30 cc. of water. After boiling for five hours, the reaction was complete and the hydantoin had separated. This compound is very insoluble in acetic acid, 120 cc. dissolving less than 2 g. of the hydantoin. It crystallized from boiling acetic acid in light yellow, prismatic crystals melting at 273–274° with decomposition.

Anal. Calcd. for $C_{12}H_{10}O_5N_2$: N, 10.68. Found: N, 10.50, 10.56.

Esterification of this hydantoin-acetic acid with ethyl alcohol gives an ester melting at 164° (uncorr.). An attempt was made to prepare this compound by alkylation of salicylalhydantoin[14] with ethyl chloro-acetate, but the reaction does not take place smoothly and a gummy product is formed from which it is very difficult to separate the ethyl ester.

***o*-Hydroxybenzylhydantoin-3-acetic Acid,** $HOOCCH_2\overline{NCONHCH(CH_2C_6H_4OH)}CO$.—This polypeptide hydantoin was first approached by heating salicylalhydantoin-3-acetic acid with hydriodic acid and red phosphorus. This treatment led to the formation of a gummy product which was extremely difficult to purify. It was found that the reduction could be accomplished by using hydriodic acid alone. The unsaturated hydantoin was suspended in five times its weight of hydriodic acid and the mixture carefully heated, but never allowed to boil. After complete solution the mixture was allowed to stand for a few minutes and the acid then diluted with water. Part of the polypeptide hydantoin crystallized directly from this diluted solution; the remainder was obtained by allowing the filtrate to concentrate in a vacuum over sodium hydroxide. The yield of crude reduction product was about 75% of the theoretical. The hydantoin was purified by crystallization from hot water and separated in the form of rosets melting at 189–190°.

Anal. Calcd. for $C_{12}H_{12}O_5N_2$: N, 10.60. Found: N, 10.59, 10.60.

As would be expected this polypeptide hydantoin gives a positive Jaffé picric acid reaction. A red-brown layer develops very rapidly when the test is performed by the modified method of Brand and Sandberg.[15]

2-Thio-5-piperonal-hydantoin-3-acetic Acid, $HOOCCH_2\overline{NCSNHC{=}(CHC_6H_3O_2CH_2)}CO$.—This compound was obtained in a yield of 90% of the theoretical by condensation of piperonal with 2-thiohydantoin-3-acetic acid. The acid, which was the initial reaction product in this case, crystallizes from boiling acetic acid in small, glistening, yellow crystals which melt with decomposition at 291°.

Anal. Calcd. for $C_{13}H_{10}O_5N_2S$: N, 9.02. Found: N, 8.83, 8.97.

5-Piperonalhydantoin-3-acetic acid, $HOOCCH_2\overline{NCONHC{=}(CHC_6H_3O_2CH_2)}CO$.—The above sulfur hydantoin was desulfurized by refluxing for about thirty hours with

[14] Johnson and Scott, THIS JOURNAL, **37**, 1846 (1915).

[15] Brand and Sandberg, *J. Biol. Chem.*, **70**, 381 (1926).

a 50% solution of chloro-acetic acid. The solid never completely dissolved but it gradually became lighter in color and formed hard, compact crystals which settled to the bottom of the flask. The hydantoin separated from acetic acid in the form of lemon-yellow rosets which melted at 275–276°.

Anal. Calcd. for $C_{13}H_{10}O_5N_2$: N, 9.65. Found: N, 9.78, 9.94.

To prove that this compound was a derivative of piperonal-hydantoin rather than a possible condensation product involving a reaction of the methylene radical in the acetic acid group of the hydantoin-3-acetic acid, the acid was esterified with ethyl

$$H_2CO_2C_6H_3CH{=}C(COOH)\overline{NCONHCH_2}CO$$

alcohol. This ester was found to be identical with the hydantoin-ester formed by direct alkylation of the sodium salt of piperonalhydantoin with ethyl chloro-acetate.

Ethyl 5-Piperonal-hydantoin-3-acetate,

$$C_2H_5OOCCH_2\overline{NCONHC{=}(CHC_6H_3O_2CH_2)}CO.$$

—Ten grams of piperonal-hydantoin[8] was suspended in 100 cc. of absolute alcohol containing in solution 1.3 g. of metallic sodium. After digesting for forty-eight hours, two molecular equivalents of ethyl chloro-acetate was added and the digestion continued for two days. The solution was filtered hot and the insoluble residue was triturated with dilute hydrochloric acid, when a small quantity of crystalline material was obtained having the properties of piperonylic acid. From the alcohol solution the above ester separated in the form of prisms melting at 159–160°. It was insoluble in alcohol. When mixed with the ester prepared by esterification of piperonalhydantoin-3-acetic acid, the melting point was not changed.

Anal. Calcd. for $C_{15}H_{14}O_5N_2$: N, 8.80. Found: N, 8.81.

Summary

1. It has been shown that hydantoin-3-acetic acid and its sulfur analog both condense with aldehydes.

2. The cyclic methylene group is the reactive position in the hydantoin.

3. The sulfur hydantoin reacts more favorably with aldehydes than the hydantoin-3-acetic acid.

4. The three aldehydes, anisaldehyde, salicylaldehyde and piperonal gave condensation products which do not exhibit geometric isomerism.

5. The polypeptide hydantoins containing free phenolic groups are being examined to determine their germicidal value and toxicity.

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