negative. It may be concluded, therefore, that neither of these pyrimidines is present in yeast nucleic acid.

3. By application of the acetol test for 5-methylcytosine to the aminopyrimidine fraction of any nucleic acid, it is now possible to detect this substance when present in very minute quantities.

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RESEARCHES ON HYDANTOINS. XLVIII. SYNTHESIS OF POLYPEPTIDE-HYDANTOINS FROM HYDANTOIN-1-ACETIC ACID[1]

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It is a growing belief that heterocyclic combinations containing active methylene groups (—CH_2—) occur in the structure of normal proteins; therefore, any information which increases the present knowledge of the chemistry of such cyclic groupings is of immediate value in the present period of intense interest in protein chemistry. Any consideration of cyclic amide groupings brings up at once the importance of the ureide structure, and the possible presence of polypeptide-hydantoin combinations in protein molecules. As a result of our chemical study of special bacteria like tubercle bacilli, we have learned that organic combinations are present in these unicellular organisms, some of which may function as inhibitors and some as accelerators of cell growth and development. These facts are important and lead one to ask whether there may not be some truth in the recent query of Prentiss,[3] who writes as follows: "Do chemicals exist in the tissues that are poisonous to infecting organisms and can such be isolated and made in sufficient quantities so that they could be used not only in the treatment of these infections, but possibly also in prevention in special instances? The presence of such substances is only hypothetical but the same may be said of numerous others commonly referred to in medical work as vitamines and whose presence we do not doubt." In fact, H. Kossel[4] studied the action of nucleic acid, a common constituent of cellular tissue, on bacteria and actually found that cholera germs and streptococci are readily killed

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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] Prentiss, "The Specific Immunity of the Tissues and Its Bearing on Treatment," *Science*, **62**, 91 (1925).

[4] Kossel, *Nature*, **49**, 240 (1894).

by small quantities of the acid. Anthrax germs were more resistant. He considers that the bactericidal action of lymph cells is due in part at least to the action of nucleic acids. Thus in the consideration of medicinals there may be particular value in the study of structures resembling those functioning in characteristic biochemical products, and it is for such reasons that we emphasize the importance of both acyclic and cyclic peptide linkages.

In our last paper on the chemistry of hydantoins[5] we recorded the behavior toward aldehydes of 2-thiohydantoin-3-acetic acid and its oxygen analog and showed that only one methylene group in this combination is characterized by its pronounced reactivity toward aldehydes. In no case thus far studied have we observed an aldehyde condensation involving the methylene group of the substituent acetic acid radical. In other words, the methylene group —CH_2— is activated by linkage in cyclic combinations as represented by hydantoin and diacipiperazine cycles. Whether corresponding side-chain substitutions in diacipiperazines will interact with aldehydes remains to be determined.

Instances of condensations in which an N-1-substituted hydantoin has been used with an aliphatic group joined to nitrogen adjacent to the methylene carbon are fairly numerous but scattered. In working with such substitution products very inconsistent results have been obtained and the reactivity of the cyclic methylene groups in these combinations has been a debatable question. Since both groupings —$NHCH_2CO$— and =NCH_2-CO— may actually function in amino acids and polypeptides, there is, therefore, a general interest in any reaction that may contribute to a better understanding of the properties inherent in these structures.

According to our present knowledge we are justified in claiming that the methylene group of the hydantoin or thiohydantoin ring is more reactive toward aldehydes than is the methylene radical in acyclic combinations like hippuric acid. This reactivity is decidedly influenced, however, by substitution of aliphatic groups on nitrogen of the hydantoin cycle. Biltz[6] reported practically complete recovery of 1,3-dimethylhydantoin after heating this hydantoin with benzaldehyde in the presence of sodium acetate, acetic acid and acetic anhydride. Working with ethyl hydantoin-1-acetate Johnson and Bates[7] did not succeed in bringing about a condensation with benzaldehyde. No difficulty was encountered in accomplishing condensations by the use of N-1 acyl derivatives like 1-acetylhydantoin[8] and 1-benzoyl-2-thiohydantoin.[9] Erlenmeyer, Jr., also succeeded in condensing creatinine with benzaldehyde in the presence of acetic anhydride, giving

[5] Renfrew and Johnson, THIS JOURNAL, **51**, 254 (1929).

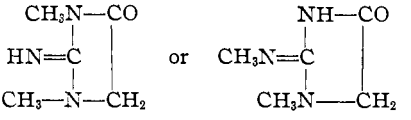
[6] Biltz, *Ber.*, **45**, 1673 (1912).

[7] Johnson and Bates, THIS JOURNAL, **38**, 1087 (1916).

[8] Johnson and Wrenshall, *ibid.*, **37**, 2133 (1915).

[9] Johnson and Kohmann, *ibid.*, **37**, 1863 (1915).

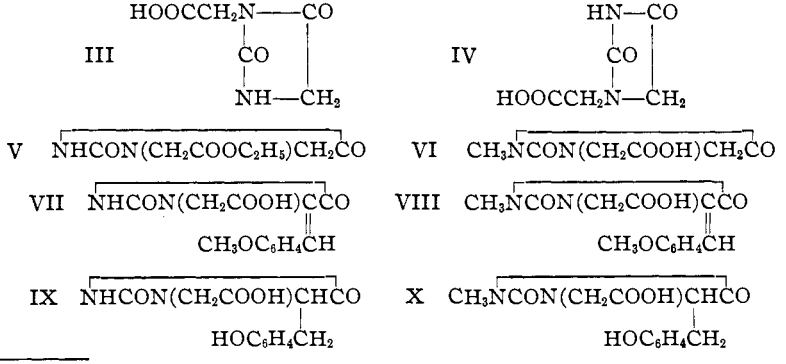
benzalacetylcreatinine.[10] By simple changes in experimental conditions Nicolet and Campbell[11] obtained better yields than those reported by Erlenmeyer, Jr. That creatinine interacts so favorably with aldehydes in acetic anhydride solution is possibly due to the fact that acetylation occurs before condensation, thereby leading to activation of the cyclic methylene group. With substituted glycocyamidine or methylcreatinine like I and II,[12] all attempts by Nicolet and Campbell to bring about condensation



were unsuccessful. Consequently they were unable to obtain any direct evidence as to the structure of the methylation products of creatinine by this method of attack. With 1-methylhydantoin they did succeed in effecting a condensation with benzaldehyde, but only with a yield of 32% of the theoretical. In all the work of Nicolet and Campbell no record is made of the identification of any geometric isomers.

Since the ultimate object of the present research was to develop methods for preparing specific compounds which might exhibit favorable properties as antiseptics, it was important that both N-1 and N-3 acetic acid derivatives of phenolic hydantoins be studied. As stated in our previous paper[5] the introduction of the acetic acid group was expected to increase solubility and lower toxicity of such combinations.

The reactivity of hydantoin-1-acetic acid, IV, toward an aldehyde has now been investigated by us, and a description of the different condensation products resulting from the action of anisaldehyde is given in the Experimental Part of this paper. The reactions that we desire to report at this time are recorded below.



[10] Erlenmeyer, Jr., and Früstück, *Ann.*, **284**, 36 (1895).

[11] Nicolet and Campbell, THIS JOURNAL, **50**, 1155 (1928).

[12] Korndorfer, *Arch. Pharm.*, **242**, 641 (1900); Kunze, *ibid.*, **248**, 578 (1910).

In our research the acid, IV, was used in the form of its ethyl ester, V.[13] When this is condensed with anisaldehyde in the presence of sodium acetate and in acetic acid, the product of reaction is the sodium salt of anisalhydantoin-1-acetic acid, VII. The yield, however, is only 34% of the theoretical and thus far no geometric isomer has been identified. Alkylation of the hydantoin (V) with methyl iodide gives the methylhydantoin represented by Formula VI. When the latter is condensed with anisaldehyde under the usual conditions, two products of reaction result in small yields,[14] namely, the sodium salts of the two geometric isomers of 3-methylanisalhydantoin-1-acetic acid melting at 203–205° and 168–169°, respectively.[15] The results obtained by alkylation with methyl iodide were of special value as they enabled us to decide conclusively that the methylene radical in the N-1-acetic acid group is not the reactive position in ethyl hydantoin-1-acetate, V. Alkylation of anisalhydantoin-1-acetic acid, VII, gave the hydantoin, VIII, which underwent reduction with hydriodic acid, forming 3-methyl-5-hydroxybenzylhydantoin-1-acetic acid, X.[15] This same hydantoin, X, is also formed by the action of hydriodic acid on the two geometric isomers represented by Formula VIII and resulting by condensation of anisaldehyde with the hydantoin, VI. In other words, the reactive methylene group —CH_2— in both N-1- and N-3-hydantoin-acetic acids is the one in cyclic combination. In neither case did we observe any evidence of any other type of condensation reaction.

Experimental Part

Ethyl Hydantoin-1-acetate, V.—The imino-acetonitrile from which this compound was synthesized was prepared according to the method used by Bailey and Snyder[16] and later applied successfully by Johnson and Rinehart.[17] Bailey and Snyder report a 44% yield of the hydantoin ester. In several repetitions of this preparation we have consistently obtained yields varying from 35 to 45%. The ester melts at 85°.

5-*p*-Anisalhydantoin-1-acetic Acid, VII.—The reagents utilized for the preparation of this compound were applied in the following proportions: ethyl hydantoin-1-acetate, 7 g.; anisaldehyde, 6.3 g.; anhydrous sodium acetate, 8 g.; glacial acetic acid, 12 cc., and acetic anhydride, 3 cc. After refluxing this mixture for six hours the resulting solution was diluted with water and the reaction product separated by filtration. This was identified as the sodium salt of anisalhydantoin-1-acetic acid. It was colorless and had no definite melting point, charring badly when heated above 300°. The yield of this salt was about 34% of the theoretical. Apparently all of the hydantoin-1-acetate that reacted with aldehyde underwent saponification in the proccss, as we obtained no

[13] Bailey and Snyder, THIS JOURNAL, **37**, 940 (1915); Jongkess, *Rec. trav. chim.*, **27**, 287 (1908).

[14] It may be predicted from our past experience that the sulfur analog, ethyl 3-methylhydantoin-1-acetate [Bailey and Snyder, THIS JOURNAL, **37**, 940 (1915)], will be found to condense more easily with anisaldehyde.

[15] Hahn and Renfrew, *ibid.*, **47**, 147 (1925).

[16] Bailey and Snyder, *ibid.*, **37**, 935 (1915).

[17] Johnson and Rinehart, *ibid.*, **46**, 768 (1924).

evidence of the formation of ethyl anisalhydantoin-acetate. Allowing for the fact that 55% of anisaldehyde was recovered, the yield of condensation product was 54% of the theoretical.

When this salt was dissolved in water and the solution was acidified with hydrochloric acid, the hydantoin-1-acetic acid separated. This acid is difficultly soluble in alcohol and water and moderately soluble in glacial acetic acid. It melts at 215–216° (uncorr.). The acid crystallizes from ethyl alcohol with one molecule of alcohol of crystallization which it loses at 110°.

Anal. Calcd. for $C_{13}H_{12}O_5N_2 \cdot C_2H_5OH$: N, 8.70. Found: N, 8.88, 9.00.

Anal. (after drying at 110°). Calcd. for $C_{13}H_{12}O_5N_2$: N, 10.14. Found: N, 9.85, 9.90.

In order to increase the yield of this condensation product, we incorporated other condensing agents besides sodium acetate as acetic anhydride and piperidine. With acetic anhydride the unchanged ethyl hydantoin-1-acetate was nearly all recovered after digesting with anisaldehyde for four hours. The ethyl hydantoin-1-acetate was also recovered unaltered when piperidine was employed as a condensing agent.

5-*p*-Hydroxybenzylhydantoin-1-acetic Acid, IX.—This hydantoin was formed when the preceding unsaturated hydantoin was reduced with hydriodic acid. The following proportions were used in the reaction: anisalhydantoin-1-acetic acid, 1.3 g.; hydriodic acid (sp. gr. 1.7) 6.5 cc., and red phosphorus, 0.4 g. The reduction was carried out at 105° for three hours, when methyl iodide was evolved in quantitative yield. After diluting with about 20 cc. of water and filtering from phosphorus, the excess of hydriodic acid was expelled by evaporation; the reduced hydantoin finally separated in a crystalline condition. The hydantoin was purified by crystallization from water and melted at 201°.

Anal. Calcd. for $C_{12}H_{12}O_5N_2$: N, 10.60. Found: N, 10.54, 10.50.

3-Methyl-5-*p*-hydroxybenzylhydantoin-1-acetic Acid, X.—The structure of 5-*p*-hydroxybenzylhydantoin-1-acetic acid and the corresponding anisal precursor was established conclusively as follows. The anisalhydantoin-1-acetic acid was first methylated by the action of methyl iodide in methyl alcohol in the presence of the required proportion of potassium hydroxide. The products of reaction were the methyl ester of 3-methylanisalhydantoin-acetate and the corresponding methylhydantoin-acetic acid. Both of the compounds were reduced by hydriodic acid and red phosphorus to the above methylated hydantoin, 3-methyl-5-*p*-hydroxybenzyl-hydantoin-1-acetic acid. This hydantoin proved to be identical with the methyl-*p*-hydroxybenzylhydantoin-1-acetic acid previously described by Hahn and Renfrew,[15] and there was no lowering of the melting point when a mixture of the two was heated in a capillary tube (167°). The formation of this methylated compound proves conclusively that ethyl hydantoin-1-acetate does not condense with anisaldehyde to form a hydantoin having the constitution XI represented below.

XI $CH_3OC_6H_4CH=C(COOH)—\overline{N}CH_2CONH\overline{C}O$

In other words, the methylene group in the hydantoin cycle is the reactive complex when brought into reaction with aldehydes.

Ethyl 3-Methyl-hydantoin-1-acetate, $CH_3\overline{N}CON(CH_2COOC_2H_5)\overline{CH_2C}O$.—Three grams of ethyl hydantoin-1-acetate dissolved in 15 cc. of methyl alcohol was combined with a solution containing 2.4 equivalents of sodium methylate. This solution, from which an organic salt crystallized as it formed, was refluxed for eight hours and then digested with an excess of methyl iodide. When the reaction was complete, as indicated by the neutrality of the solution, sodium chloride was separated by filtration and the

solution concentrated under diminished pressure. On diluting the concentrated alcohol solution with water and again evaporating to remove alcohol, the ethyl 3-methyl-hydantoin-1-acetate was finally separated by extraction with chloroform. After removing the chloroform the ester was finally purified by crystallization from alcohol. It separated in small, glistening plates melting at 91–92°. The sulfur analog of this ester has been described by Bailey and Snyder.[16]

Anal. Calcd. for $C_8H_{12}O_4N_2$: N, 14.0. Found: N, 14.11, 13.96.

3-Methyl-5-*p*-anisalhydantoin-1-acetic Acid, VIII.—This compound was prepared by condensing 3-methylhydantoin-1-acetic acid with *p*-anisaldehyde in the presence of sodium acetate in glacial acetic acid solution. Here again the product of reaction was a sodium salt which gave the above acid when treated with hydrochloric acid. The high-melting modification of this acid was the first product obtained (melting point 203–205°).[15]

When the crude condensation mixture was extracted with ether in a Soxhlet and the solvent concentrated, a solid residue containing sodium remained behind. This was dissolved in water and on the addition of hydrochloric acid to this salt solution an immediate precipitate was produced. On recrystallization from dilute alcohol it melted at 168–169° and was identified as the labile modification of 3-methyl-5-*p*-anisalhydantoin-1-acetic acid.[15] The yield of either isomer was small.

Interesting results were obtained when the picric acid reaction[18] for a carbonyl group was tried on the above hydantoins. Ethyl hydantoin-1-acetate failed to give the red-brown color characteristic of hydantoin-3-acetic acid, but did give an intense orange-red coloration. On the other hand, both hydroxybenzylhydantoin-1-acetic acid and 3-methyl-*p*-hydroxybenzylhydantoin-1-acetic acid gave negative reactions.

Summary

1. Hydantoin-1-acetic acid condenses with aldehydes with formation of substituted derivatives. These condensations involve a reaction of the cyclic methylene group —CH_2— of the hydantoin cycle.

2. Hydantoin-1-acetic acid is less reactive than its isomer, hydantoin-3-acetic acid.

3. Thus far no evidence has been obtained of the formation of geometric isomers.

4. The biological importance of hydantoin-acetic acids is emphasized, and also their possible structural relationship to proteins.

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[18] Brand and Sandburg, *J. Biol. Chem.*, **70**, 381 (1926).