[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

RESEARCHES ON HYDANTOINS. XLIII. SYNTHESIS OF THE POLYPEPTIDE-HYDANTOIN: "HYDANTOIN-3-ACETIC ACID"[1]

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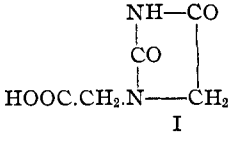
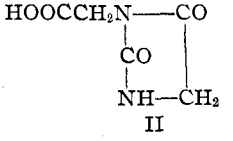
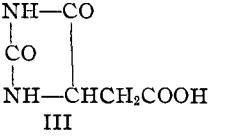
Of the three hydantoin-acetic acids theoretically possible two have already been described in the literature, namely, hydantoin-5-acetic acid[3] III ("malyureidsäure"), and hydantoin-1-acetic acid[4] I. In this paper we shall describe a method of preparing the third representative of this series, namely, hydantoin-3-acetic acid II. This compound is of immediate biological interest as it is the simplest representative of the



I



II



III

polypeptide-hydantoin series.[5] Only two products would be expected to be formed by hydrolysis of this compound, namely, carbon dioxide and glycine.

The starting point of our method of synthesis is ethyl isothiocyanoacetate VI whose preparation has previously been reported by Johnson and Ticknor.[6] This isothiocyanate has also been prepared by Johnson and Hemingway[7] by the action of thiophosgene on ethyl amino-acetate. The mustard oil is the final product of a series of reactions which are expressed by the following equations.

$$2NH_2CH_2COOC_2H_5 + CS_2 = C_2H_5OOC\,CH_2.NH.CSSH.NH_2CH_2COOC_2H_5 \xrightarrow{+Cl.COOC_2H_5}$$

$$C_2H_5OOC.CH_2.NH.CSS.COOC_2H_5 + HCl.NH_2CH_2COOC_2H_5$$

V

$$\downarrow$$

$$C_2H_5OOC.CH_2NCS + C_2H_5OH + COS \qquad \text{VI}$$

Dithiocarbamates of the type represented by Formula V are substances which cannot be purified by distillation and when heated, break down with

[1] This work represents one phase of the research now being carried on in the Sterling Laboratory in coöperation with the National Research Council Subcommittee on "Internal Antisepsis."—T. B. Johnson, Chairman.

[2] Holder of a University Scholarship in 1923–1924.

[3] Guareschi, *Jahresber.*, **1876**, 752; Beilstein's Handbuch, vol. **1**, p. 1383. Grimaux, *Ann. chim. phys.* [5] 11, 402 (1877); *Compt. rend.*, **81**, 325 (1875); *Bull. soc. chim.*, [2] **24**, 337 (1875). Gabriel, *Ann.*, **348**, 89 (1906). Lippich, *Ber.*, **41**, 2972, 2981 (1908). Dakin, *Am. Chem. J.*, **44**, 57 (1908). Johnson and Guest, *ibid.*, **48**, 103 (1912).

[4] Jongkees, *Rec. trav. chim.*, **27**, 287 (1908); *C. A.*, **3**, 421 (1909). Bailey and Snyder, THIS JOURNAL, **37**, 935 (1915).

[5] Johnson and Bates, *ibid.*, **38**, 1087 (1916); *Proc. Nat. Acad. Sci.*, **2**, 69 (1916); *Chem. News*, **113**, 127 (1916). Johnson and Hahn, THIS JOURNAL, **39**, 1255 (1917).

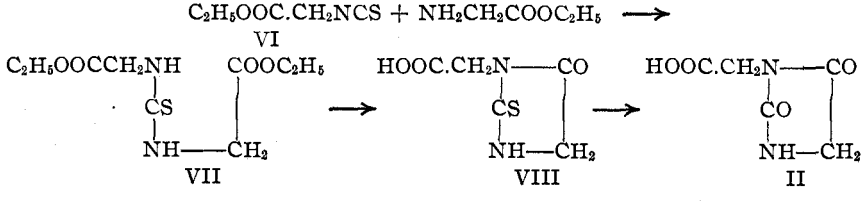
[6] Johnson and Ticknor, *Proc. Nat. Acad. Sci.*, **3**, 303 (1917).

[7] Johnson and Hemingway, THIS JOURNAL, **38**, 1556 (1916).

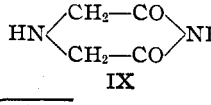
the formation of an isothiocyanate VI, carbonoxysulfide and alcohol. The method has been applied with success for the preparation of several new isothiocyanate combinations, which cannot be obtained in good yields by other methods of synthesis.

A secondary product formed during the destructive distillation of the dithiocarbamate V is the urethan derivative of glycine ethyl ester, $C_2H_5OOC.CH_2NH.COOC_2H_5$. Its formation involves the loss of carbon disulfide from the dithiocarbamate instead of carbonoxysulfide and migration of the carbethoxyl group $—COOC_2H_5$ from sulfur to nitrogen. We are very probably dealing here with a unique molecular rearrangement of the imidoacid-anhydride type and the mechanism of the change will be discussed in a future paper. The formation of a similar sulfur-free product was also observed by Johnson, Hill and Kelsey,[8] who have reported the formation of the urethan derivative, $C_6H_5NHCOCH_2NHCOOC_2H_5$, by destructive distillation of carbethoxyl amino-acetanilide-dithiocarbamate, $C_6H_5NHCOCH_2NHCS_2.COOC_2H_5$.

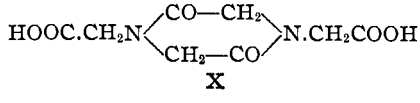
Ethyl isothiocyano-acetate VI reacts smoothly with ethyl amino-acetate to form the symmetrical thio-urea VII. Interaction with glycine leads to the formation of the corresponding mono-ethyl ester, $C_2H_5OOC.CH_2.NH.CS.NH.CH_2.COOH$. This thio-urea VII is converted almost quantitatively by digestion with hydrochloric acid into the 2-thiohydantoin compound VIII. So far as the writers are aware, the isomeric 2-thiohydantoin-1-acetic acid has not been described. The last step of our synthesis, namely, conversion of the 2-thiohydantoin VIII into the polypeptide-hydantoin, hydantoin-3-acetic acid II, is easily accomplished by digesting the thio compound VIII in aqueous solution with chloro-acetic acid



The two hydantoin acetic acids I and II will be utilized as the basis for further new syntheses in our researches on polypeptide-hydantoins and internal antisepsis. We shall also incorporate into our antisepsis work cyclic combinations of the types IX and X. It will be of especial interest to investigate the comparative behavior of methylene groupings in such



IX



X

[8] Johnson, Hill and Kelsey, THIS JOURNAL, **42**, 1711 (1920).

combinations when subjected to experimental conditions favorable for aldehyde-condensation reactions.

Experimental Part

Ethyl Amino-acetate, $NH_2CH_2COOC_2H_5$.—This ester was obtained from the decomposition of the corresponding hydrochloride by means of potassium carbonate, as described by Fischer.[9] The hydrochloride of ethyl amino-acetate was prepared by the esterification of α-hydroformamine cyanide,[10] following the experimental procedure developed by Adams and Marvel.[11]

Ethyl Isothiocyano-acetate, $SCN.CH_2COOC_2H_5$.—Johnson and Ticknor[6] have reported the preparation of this mustard oil from ethyl amino-acetate through the following series of intermediates: diethyl amino-acetate-dithiocarbamate,[12] obtained in the interaction of carbon disulfide with ethyl amino-acetate, is transformed by the action of ethyl chloroformate into carbethoxyl-ethyl amino-acetate-dithiocarbamate; and the latter compound is, then converted into the above isothiocyanate by distillation under diminished pressure. These various changes are expressed by the equations given in the first part of this paper.

Diethyl Amino-acetate-dithiocarbamate, IV.—In all our work the largest quantity of amino-acid ester used in a single experiment was 30 g.[12] The yield of dithiocarbamate was uniformly about 85%. Fourteen g. of carbon disulfide was added slowly to the glycine ester dissolved in 25 cc. of anhydrous ether. Throughout the operation the solution was agitated by a mechanical stirrer. Before the entire quantity of carbon disulfide had been added, glistening white crystals of the dithiocarbamate began to separate. This compound is not a stable salt when exposed to the atmosphere, but can be kept in a vacuum desiccator for 24–48 hours without much discoloration to indicate decomposition.

In successive preparations of the mustard oil from the dithiocarbamate more satisfactory results were always obtained when relatively small quantities of material were used in each experiment.

Carbethoxyl-ethyl Amino-acetate-dithiocarbamate, V.—Thirty-four g. of ethyl chloroformate was added slowly to 68 g. of diethyl amino-acetate-dithiocarbamate suspended in 125 cc. of anhydrous ether and the mixture shaken frequently. Considerable heat was evolved during the reaction and gradually the solid dithiocarbamate was replaced by a more finely divided precipitate of glycine ester hydrochloride, which showed a marked tendency to cake on the walls of the flask. In cases where the dithiocarbamate had been kept long enough to have become somewhat red, the solution was red and the course of the reaction could be followed by noting the disappearance of this color. The reaction mixture was warmed for several hours on the water-bath, then cooled and filtered by suction. The glycine ester hydrochloride was washed with anhydrous ether and the washings were added to the initial filtrate. When the ether had been distilled, the residue of carbethoxyl-ethyl amino-acetate-dithiocarbamate was placed in a distilling flask and subjected to destructive distillation under a pressure of about 10 mm. As the temperature of the oil-bath in which the distilling flask is heated reaches 100–110° an evolution of gas is observed, and as the temperature is raised slowly more vigorous decomposition takes place. At 120–130° the pressure rises finally to about 20–25 mm. and alcohol distils into the receiver. By careful application of heat this destructive-

[9] Fischer, *Ber.*, **34**, 433 (1901).

[10] Johnson and Rinehart, THIS JOURNAL, **46**, 768 (1924).

[11] Adams and Marvel, *Univ. Ill. Bull.*, [6] **49**, 19 (1921); "Organic Chemical Reagents III," John Wiley and Sons, New York, N. Y.

[12] Ref. 9, p. 441.

distillation can be carried on very smoothly and without strong coloration of the reaction product. After the decomposition was complete, the receiver was changed and a fraction of 21 g. of colorless oil was collected, boiling between 105° and 120° (7 mm.). The major portion of this distillate boiled at 104–106°, and on redistilling it we obtained 17 g. of the ethyl isothiocyano-acetate boiling at 104–106° (7 mm.). This agreed in all its properties with the ester previously described by Johnson and Ticknor.[6] On continued distillation of the reaction product we obtained 7–8 g. of a viscous, yellow oil which boiled quite sharply at 134° under 9mm. pressure. This product was identified as the urethan of ethyl amino-acetate.

TABLE I

COMPARATIVE YIELDS OF ISOTHIOCYANATE AND URETHAN

$C_2H_5OOC.CH_2NHCS_2.NH_3.CH_2COOC_2H_5$, g.	30	68	90	
$SCNCH_2COOC_2H_5$, g.	11 (71%)	21 (60%)	20	(43%)
$C_2H_5OOC.CH_2NH.COOC_2H_5$, g.	5	7–8	13	

It is quite apparent from inspection of Table I that the yield of isothiocyanate is not so good when the distillation is conducted with large units. Similar experimental difficulties were noted in the research with carbethoxyl amino-acetanilide-dithiocarbamate.[8]

Ethyl Carbethoxyl-amino-acetate, $C_2H_5OOC.CH_2.NH.COOC_2H_5$.—Twenty-one g. of the high-boiling fraction (134–136°, at 9 mm.) obtained in the preparation of ethyl isothiocyano-acetate was redistilled under 5mm. pressure, when we were able to separate a fraction boiling at 115–117°. This oil gave no test for sulfur and determinations of nitrogen by the Kjeldahl method gave the following results.

Anal. Calcd. for $C_7H_{13}O_4N$: N, 8.0. Found: 7.82, 7.87, 7.96.

This urethan has been described previously by Hantzsch and Metcalf,[13] and also by Fischer and Otto,[14] who prepared it by interaction of ethyl chloroformate with ethyl amino-acetate. They have recorded the following boiling points: 145–146° at 22 mm.; 135° at 16 mm., and 126° at 12mm. pressure.

Diethyl Thioncarbamo-acetate, $C_2H_5OOC.CH_2NH.CS.OC_2H_5$.—Twenty-one g. of ethysl iothiocyano-acetate was heated with 20 cc. of absolute alcohol (3 molecular equivalents) for several hours on a water-bath. When the mixture was distilled under reduced pressure, it was apparent that a complete reaction had not occurred. The fractions collected were therefore combined and heated again for 9 hours at 125°. The excess of alcohol was then removed by distillation and the resulting oil purified by distillation under diminished pressure. The fraction reserved for analysis boiled from 135° to 140° at 10 mm. and gave a strong test for sulfur.

Anal. Calcd. for $C_7H_{13}O_3NS$: N, 7.3. Found: 7.51, 7.55.

There was considerable decomposition during the distillation of this oil and hydrogen sulfide was slowly evolved during the last stages of distillation.

The Action of Ethyl Isothiocyano-acetate on Ethyl Amino-acetate. Preparation of Symmetrical Diethyl Thio-urea-diacetate, $CS(NHCH_2COOC_2H_5)_2$.—Eighteen g. of ethyl isothiocyano-acetate was added in two successive portions to a solution of 15 g. of ethyl amino-acetate in 30 g. of anhydrous ether. The reaction was distinctly exothermic, and the solution was later heated on the water-bath for 40 minutes to complete the reaction. Under the conditions of the experiment crystals did not separate after one to two hours, but the following morning the solution was nearly solid with colorless needle-like crystals. The ether was evaporated in a current of air, leaving a residue melt-

[13] Hantzsch and Metcalf, *Ber.*, **29**, 1680 (1896).

[14] Fischer and Otto, *Ber.*, **36**, 2107 (1903).

ing at 72–80°. This crude material was recrystallized from 50% acetic acid, when 13 g. of diethyl thio-urea-diacetate was obtained melting at 85–87°. This diethyl ester was very soluble in alcohol, not readily soluble in cold water but fairly soluble in hot water.

Anal. Calcd. for $C_9H_{16}O_4N_2S$: N, 11.25. Found: 11.4, 11.5.

An aqueous solution of the diethyl ester when heated with concd. hydrochloric acid was readily changed into 2-thiohydantoin-3-acetic acid, melting at 210–212°. From 6 g. of the ester we obtained 3.8 g. of the 2-thiohydantoin, or a yield of 90%.

Mono-ethyl Thio-urea-diacetate, $C_2H_5OOCCH_2NHCSNHCH_2COOH$.—This thio-urea was obtained when we evaporated the acetic acid filtrate from the recrystallization of the diethyl thio-urea-diacetate as described above. The mono-ethyl ester is readily soluble in alcohol. A specimen recrystallized several times from dil. acetic acid melted at 96°, while a mixture of this ester with the diethyl ester melted at about 70°. From a dilute solution of acetic acid the mono-ethyl ester crystallizes in rosets of long, yellow needles; in very concentrated solutions it forms a mat of light yellow crystals.

Anal. Calcd. for $C_7H_{12}O_4N_2S$: N, 12.72. Found: 12.81, 13.0.

When digested with concd. hydrochloric acid this ester is transformed quantitatively into 2-thiohydantoin-3-acetic acid.

This mono-ethyl thio-urea-diacetate was also formed in the preparation of the thiohydantoin by the action of ethyl isothiocyano-acetate upon ethyl amino-acetate in aqueous solution. The glycine ester was liberated from the corresponding hydrochloride by the action of aqueous-alcoholic potassium hydroxide and the mustard oil added directly to this reaction mixture. Coarse, brown crystals which separated from this solution on standing were extracted with water, recrystallized from acetic acid and identified as the mono-ethyl ester.

2-Thiohydantoin-3-acetic Acid, VIII.—This substituted thiohydantoin was obtained readily and in good yield by the action of hydrochloric acid on either of the thio-ureas described above. As an end-product the thiohydantoin could be obtained fairly pure from the mother liquors after treatment with the hydrochloric acid even when it was difficult to isolate the intermediate products. This hydantoin is readily soluble in alcohol and quite soluble in cold water. In alkali it gives a wine-red solution. The specimen for analysis was recrystallized from glacial acetic acid; from this solvent it separates in small, yellow plates melting at 210–212° to a yellow oil. Impure material often blackens before melting.

Anal. Calcd. for $C_5H_6O_2N_2S$: N, 16.1. Found: 15.81, 16.0.

Hydantoin-3-acetic Acid, II.—Desulfurization of the compound described above was readily accomplished by heating with chloro-acetic acid. Three g. of thiohydantoin-acetic acid was digested on a water-bath for an hour with a solution of 5 g. of chloro-acetic acid in 10 cc. of water. The solution was evaporated to small volume and the crystalline mass extracted with 20 cc. of ether to remove the chloro-acetic and thio-glycolic acids. After recrystallization from alcohol 1.5 g. of hydantoin-3-acetic acid was obtained as a white, crystalline substance melting at 190–191°.

Anal. Calcd. for $C_5H_6O_4N_2$: N, 17.72. Found: 17.56, 17.72.

This polypeptide-hydantoin is soluble in cold water and gives a colorless solution with alkali.

The Action of Ethyl Isothiocyano-acetate on Glycine.—Five g. of glycine was dissolved in 30 cc. of 50% alcohol containing 4 g. of potassium hydroxide. Nine g. of ethyl isothiocyano-acetate was added to this solution, forming an oily layer at the bottom. In a few minutes the solution became pink; this color gradually deepened and a slight heating effect was noticeable. The solution which was now homogeneous in appearance was heated on the steam-bath for an hour. Although some crystals formed after stand-

ing several days, these were not examined. The crude reaction product was evaporated repeatedly with hydrochloric acid. A good yield of 2-thiohydantoin-3-acetic acid was obtained. However, the material was discolored even after recrystallization from acetic acid containing Norite and blackened at 208°, melting at 210–212°.

Summary

1. A practical method for preparing ethyl isothiocyano-acetate is described.

2. Ethyl isothiocyano-acetate interacts smoothly with glycine or its ethyl ester giving the corresponding symmetrically substituted thio-ureas. The latter compounds undergo a cyclic condensation by digestion with hydrochloric acid giving 2-thiohydantoin-3-acetic acid.

3. Desulfurization of this 2-thiohydantoin compound leads to the formation of hydantoin-3-acetic acid, the simplest representative of a polypeptide-hydantoin.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

THE CORRELATION OF SOME AROMATIC TYPES WITH PHYSIOLOGICAL ACTION. LOCAL ANESTHETICS CONTAINING THE FURAN, THIOPHENE AND PYRROLE NUCLEI[1]

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Introduction

Strictly speaking, there are probably no rigidly exclusive aromatic characteristics. Aromatic compounds are accorded generally a formal classification which serves to set them apart from aliphatic compounds, and the basis of such a differentiation is found in a number of essentially distinctive properties. However, such differences from what might be called corresponding aliphatic compounds are largely of degree and not of kind. Notwithstanding, the differentiation is retained. This is so partly for the convenience of such a classification, and partly because the differences, under corresponding conditions, are rather noteworthy when one considers series of compounds in the aggregate.

Aromatic properties are observed with a number of heterocyclic compounds. Some of the more important 5-membered rings are furan (I), thiophene (II) and pyrrole (III). The structural similarities of these types to benzene become apparent when they are written arbitrarily according to the Armstrong-Baeyer centric formulas.[2]

[1] A preliminary report of this work was made at the Spring Meeting of the American Chemical Society held at Birmingham, Alabama, in April, 1922.

[2] Meyer and Jacobson, "Lehrbuch der Organischen Chemie," Vol. II, part 3, section I, pp. 12–17, contains a general critical account of the formulas of these compounds.