

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

THE INTERACTION OF HYDROGEN SULFIDE WITH CERTAIN AMINO AND IMINO ACID NITRILES

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The organic chemist who directs his attention to the study of biochemical changes functioning in the early stages of natural cellular syntheses meets at the start with some very puzzling phenomena of fundamental importance. He is confounded by the problems presented for solution due to the limitations of our present knowledge of the conditions normally effecting and regulating the activity of the simple compounds like carbon dioxide, ammonia, hydrocyanic acid and hydrogen sulfide; and is called upon to interpret puzzling chemical phenomena involving these compounds which are extremely susceptible to mild physical and chemical treatment.

As the science of biochemistry is developed we are continually being introduced to new chemical mechanisms which are apparently regulated or controlled by chemical and physical influences entirely different from those met with in laboratory technique, and in the newer interpretations and postulations made to advance our knowledge we are compelled to conceive of chemical interchanges that would not at first be considered of any biochemical significance. When we stop to consider, however, the chemical significance of some of the remarkable chemical transformations easily accomplished by living cellular organisms we are then forced to admit that the mechanisms involved in such metabolic processes are well worthy of very thoughtful study.

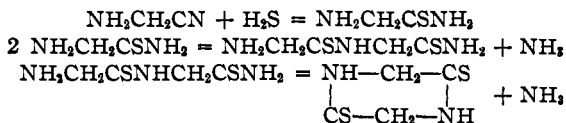
In our preliminary study of the fundamental chemical changes taking place in bacillary growth we have continually been brought into close touch with obscure phenomena characterizing biochemical change, and the observations we have already made in the field of tuberculosis have forcibly suggested to us that simple water soluble substances like ammonia, carbon dioxide and hydrocyanic acid may be of as much fundamental importance in the life of the growing bacterial cell as the more complex colloidal bodies upon which greater emphasis is usually placed by present day investigators.

In consequence of the growing interest in the biochemistry of sulfur in organic combination, it is very desirable that we have an understanding of the chemical behavior of hydrogen sulfide toward nitriles of amino and imino acids. It is believed that amino nitriles, for example, play a far more important role in natural synthetic processes than commonly assumed, and very probably function in the early stages of protein synthesis

¹ Holder of the Metz Research Fellowship in Chemistry, 1926-27.

from simpler substances. It is well known that the —CN group of certain combinations is very susceptible to chemical change when exposed to the action of hydrogen sulfide, and it seems not improbable that hydrogen sulfide may be the first assimilation product of inorganic sulfates, and that certain amino thioamides actually function in the natural synthesis of sulfur proteins. The conception of the thiopolypeptide structure was expressed in a publication by the senior author as early as 1910,² and since that time many facts have been revealed which have confirmed the idea that the —CS·NH— grouping is one of the fundamental constructions functioning in nature.

In 1911 Johnson and Burnham³ contributed the first paper on this subject and reported results obtained when hydrogen sulfide is allowed to interact with the simplest nitrile of an amino acid, namely, amino-acetonitrile. While these reagents do not interact smoothly, they found that the main product of combination was 2,5-dithiopiperazine. Gatewood and Johnson⁴ repeated this work, and in a later publication confirmed the conclusion of the original investigators. Johnson and Burnham considered the dithiopiperazine as the final product of a series of changes and interpreted the course of the reaction according to the following scheme. The nitrile of α -alanine (aminopropionitrile) is known to interact



in a similar manner with hydrogen sulfide to form 3,6-dimethyl-2,5-dithiopiperazine whose physiological action has previously been reported in a publication by Lewis.⁵ Of all the nitriles we have thus far examined in this Laboratory, these are the only two that have been observed to interact with hydrogen sulfide to form dithiopiperazines.

The yield of dithiopiperazine from aminopropionitrile is exceedingly small as this change does not represent the primary reaction in this case, as will be shown below. The experimental evidence thus far accumulated, therefore, does not permit us to draw the conclusion that the formation of dithiopiperazines is a general reaction when nitriles of amino acids are allowed to interact with hydrogen sulfide. Two other characteristic reactions of equal significance have been revealed as a result of our researches in this field.

The action of hydrogen sulfide on four nitriles of amino acids and three imino nitriles has now been studied and their behavior toward this reagent

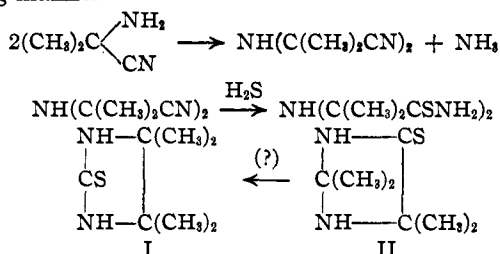
² Johnson and Burnham, *J. Biol. Chem.*, **9**, 331 (1911).

³ Johnson and Burnham, *ibid.*, **9**, 449 (1911).

⁴ Gatewood and Johnson, *THIS JOURNAL*, **48**, 2900 (1926).

⁵ H. B. Lewis, *J. Biol. Chem.*, **14**, 255 (1913).

are recorded in this paper. The most outstanding result of this research has been the discovery of the unique behavior of hydrogen sulfide toward amino-*isobutyronitrile*. These reagents interact to give a definite and well defined crystalline substance whose structure we have not established with absolute certainty. The compound is not a dithiopiperazine, neither is it amino-*isobutyrothio*-amide nor the corresponding thiopolypeptide. It has the empirical formula $C_7H_{14}N_2S$, indicating that two molecules of the nitrile interact with hydrogen sulfide with loss of one carbon and two nitrogen atoms. Provisionally the structure of 2,2,4,4-tetramethyl-5-thio-2-desoxyhydantoin (II) has been assigned to it. So far as we are aware no sulfur cyclic combination of this type has hitherto been described in the literature. The course of the reaction may be formulated in the following manner



That the first step of this change involves the formation of an imino nitrile with loss of ammonia as represented above, is confirmed by the fact that pure iminodi-*isobutyronitrile* interacts with hydrogen sulfide to give the same end product. The possibility of a molecular rearrangement can also be postulated here and the compound be assigned the constitution of a reduced thioglyoxaline as represented by Formula I. This structure is, however, excluded as the compound is soluble in both alkali and acids and undergoes hydrolysis smoothly when heated with hydrochloric acid, giving amino-*isobutyric* acid.

All three compounds, amino-acetonitrile, α -aminopropionitrile, and α -amino-*isobutyronitrile* lose ammonia on heating, their stability increasing, however, with increase in molecular weight. Amino-acetonitrile cannot be distilled without decomposition; aminopropionitrile can be distilled under diminished pressure but on standing it soon loses ammonia, giving the iminonitrile. Amino-*isobutyronitrile* is more stable and decomposes only slightly when distilled at ordinary pressure. The corresponding imino compound is formed only after standing for several days. In other words, these three compounds are sharply differentiated by their degree of stability. In correlation to this difference in degree of stability is the characteristic behavior of each type toward hydrogen sulfide. Amino-acetonitrile interacts with this reagent to form a dithiopiperazine, aminopropionitrile interacts to form predominantly imino-

dipropionitrile, while the amino-*isobutyronitrile* gives a reduced isodiazole combination. As the molecular weight of the nitrile is increased further, not only do we meet with greater stability but also with less reactivity toward hydrogen sulfide. For example, α -aminomethylethylacetonitrile and α -aminodiethylacetonitrile are both very stable, giving no ammonia on distillation, and furthermore failing to react with hydrogen sulfide even when heated under pressure. Possibly steric hindrance in the molecule may be a factor here, influencing the reactivity of these two compounds.

The three imino nitriles examined do not agree in their chemical behavior toward hydrogen sulfide. Iminodiacetonitrile interacts to form three different products. One melts at 124° and is probably the corresponding thio-amide giving, by analysis, the required percentage of nitrogen. The second product formed in this reaction melts at 186° and yields analytical results for nitrogen agreeing with that calculated for 5-thio-2-desoxyhydantoin. The carbon and hydrogen determination, however, gave results altogether too low for this compound. A third product seems to be formed also but it had no definite melting point, was amorphous and could not be obtained in a condition sufficiently pure for an analysis. Iminodipropionitrile was never observed to interact with hydrogen sulfide and in every experiment tried was recovered unaltered. On the other hand, as stated above, the iminodi-*isobutyronitrile* interacts with hydrogen sulfide to give the same product as amino-*isobutyronitrile*, namely, 2,2,4,4-tetramethyl-5-thio-2-desoxyhydantoin (II).

It is a very interesting fact that amino-acetonitrile and iminodiacetonitrile do not react with hydrogen sulfide to form the same sulfur combination, as was observed in the case of amino-*isobutyronitrile* and its corresponding imino derivative.

Experimental Part

The Action of Hydrogen Sulfide on α -Amino-*isobutyronitrile*, 2,2,4,4-Tetramethyl-5-thio-2-desoxyhydantoin.—A solution of 20 g. of the hydrochloride of this nitrile⁶ in 100 cc. of absolute alcohol and 50 cc. of concentrated aqueous ammonia, or 20 g. of the pure nitrile in 75 cc. of absolute alcohol and 25 cc. of concentrated aqueous ammonia, was cooled with ice and salt and saturated with hydrogen sulfide. Under the first condition ammonium chloride separates and is removed by filtration when the reaction is complete. The almost colorless solution is allowed to evaporate spontaneously, when a crystalline product is obtained. This was purified by crystallization from boiling water and melted at 153–155°. The yields obtained were 5.4 and 7.0 g., respectively, and were not increased by using gaseous ammonia in large excess.

Anal. Calcd. for $C_7H_{14}N_2S$: C, 53.1; H, 8.9; N, 17.7 Found: C, 52.7, 53.0; H, 8.6, 8.7; N, 17.8, 17.6.

The reaction product contains sulfur. It is difficultly soluble in cold water and soluble in hot water, alcohol and ether. The hydantoin dissolves readily in both acid

⁶ Gulewitsch and Wasmus, *Ber.*, 39, 1181 (1906); Dubsky, *Ber.*, 49, 1136 (1916).

and alkali solutions and is slowly decomposed with generation of hydrogen sulfide when boiled vigorously with these reagents. The sulfur is extremely firmly bound in this combination. Phosphotungstic acid and sodium nitroprusside tests for sulfur were negative. Also the compound can be refluxed in alcohol with mercuric oxide without loss of sulfur. It forms a mercury salt which crystallizes from ether and melts at 175°. By interaction of this salt with hydrogen sulfide the original hydantoin melting at 155° is regenerated. The hydantoin interacts with chloro-acetic acid giving an oily compound which could not be distilled.

The hydantoin undergoes hydrolysis when digested with concentrated hydrochloric acid, giving the hydrochloride of α -amino-*isobutyric* acid,⁷ melting at 230°. The free amino acid was isolated by treatment of this hydrochloride with silver oxide and hydrogen sulfide successively, and exhibited all the properties of the known acid, possessing a sweet taste and subliming at 280° without decomposition.

Action of Hydrogen Sulfide on α -Aminopropionitrile (Iminodipropionitrile).—A solution of 5 g. of the above nitrile⁸ dissolved in 50 cc. of concentrated alcoholic ammonia was saturated at 0° with hydrogen sulfide and allowed to stand for several hours. The solution was then allowed to evaporate spontaneously in a vacuum desiccator, when a crystalline substance finally separated admixed with some oil which was undistillable. This solid was identified as iminodipropionitrile and after crystallization from ether melted at 67–68°. Several experiments were performed under variable conditions using ammonia in aqueous and alcohol solutions of different concentrations and at various temperatures but in every case the chief product of reaction was the iminonitrile. It is possible that the yellow oil which always accompanies this product was a thio-amide combination but it was so unstable that no method of purification could be devised.

α -Aminomethylethylacetoneitrile and α -aminodiethylacetoneitrile were prepared according to the directions of Biltz and Slotta⁹ with only one change in technique, namely, substitution of ammonium chloride for ammonium sulfate in preparing the nitrile from the corresponding ketones—methyl ethyl and diethyl ketones. Both of these nitriles are very stable and can be kept indefinitely without change. Both compounds revealed themselves as inactive nitriles when exposed to the action of hydrogen sulfide in alcohol-ammonium solution. In all our experiments unaltered nitrile was recovered even when we operated at a temperature as high as 60–70°.

Action of Hydrogen Sulfide on Iminodi-*isobutyronitrile*, 2,2,4,4-Tetramethyl-5-thio-2-desoxyhydantoin (II).—A solution of 8.5 g. of this iminonitrile³ was dissolved in a mixture of 50 cc. of absolute alcohol and 20 cc. of concentrated ammonia and the solution then saturated with hydrogen sulfide at 0°. After allowing it to stand for several hours and then evaporating, we obtained 4.6 g. of the crystalline desoxyhydantoin melting at 153–155°. A mixed melting point and its properties showed that this was identical with the substance obtained by the action of hydrogen sulfide on the α -amino-*isobutyronitrile*.

Several attempts were made to bring about an addition of hydrogen sulfide to iminodipropionitrile, but in no case were we able to obtain evidence of the formation of a thio-amide. The nitrile was recovered unaltered in every experiment tried.

Action of Hydrogen Sulfide on Iminodiacetonitrile.—A solution of 5 g. of this nitrile dissolved in a mixture of 250 cc. of concentrated aqueous ammonia was saturated with hydrogen sulfide and then allowed to stand in a closed flask for several hours. A bright yellow solution resulted. When this was allowed to evaporate at ordinary temperature, about 2.5 g. of a yellow, crystalline substance finally separated. On ex-

⁷ Zelinsky and Stadnikoff, *Ber.*, 39, 1726 (1906).

⁸ Dubsy, *Ber.*, 49, 1048 (1916).

⁹ Biltz and Slotta, *J. prakt. Chem.*, 113, 249 (1926).

amination this was found to be a mixture of two substances which we were able to separate mechanically. The major portion was a deep yellow product, well crystallized and melting at 124°. The second substance began to blacken at 150° in the capillary tube and then melted at 186°. This compound proved to be easily soluble in water and could be freed from the compound melting at 124° by washing with this solvent. Of the purified compound melting at 124° we obtained 2.0 g. and of the higher melting compound 0.3 g. This experiment was repeated several times but without increasing the yield of the compound melting at 186°. The compound melting at 124° is easily purified by crystallization from methyl alcohol, while the 186° product is purified best by dissolving in cold water and then precipitating by dilution with alcohol. Both compounds evolved ammonia when dissolved in cold alkali, and hydrogen sulfide is liberated on acidifying the alkaline solution.

Anal. (Compound melting at 186°, 5-thio-2-desoxyhydantoin.) Calcd. for $C_3H_6N_2S$: N, 27.46. Found: N, 27.48, 27.45. (Compound melting at 124°, imino-diacetothioamide.) Calcd. for $C_4H_8N_2S_2$: N, 25.74. Found: N, 25.51, 25.54.

Summary

1. In our study of the chemical behavior of hydrogen sulfide toward nitriles of amino acids, we have thus far succeeded in revealing the following three characteristic types of reaction. (a) Formation of a dithio-piperazine as is illustrated in the action of hydrogen sulfide on amino-acetonitrile. (b) Molecular condensation with formation of an imino nitrile as is illustrated in the action of hydrogen sulfide on α -amino-propionitrile, giving the corresponding imino compound with loss of ammonia. (c) Formation of a thiodesoxyhydantoin as is illustrated in the action of hydrogen sulfide on amino-*isobutyronitrile*.

2. From the present evidence it is impossible to predict the course of the reaction which will take place when a given nitrile in this series of compounds is exposed to the action of hydrogen sulfide.

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[CONTRIBUTION FROM THE PHYSICAL CHEMICAL LABORATORY OF MCGILL UNIVERSITY]

THE PREPARATION AND PHYSICAL PROPERTIES OF α -, β - AND γ -BUTYLENE AND NORMAL AND ISOBUTANE

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Introduction

A number of the physical properties of hydrocarbons containing two and three carbon atoms have been accurately determined in this Laboratory.¹ The present paper describes an extension of this work to compounds of 4 carbon atoms, namely, the three butylenes and the two butanes. The preparation of these compounds is described and their boiling points, melting points and critical temperatures are tabulated. The vapor pressures, densities and surface tensions were determined over a wide

¹ Maass and Wright, *THIS JOURNAL*, **43**, 1093 (1921).