[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE BUREAU OF DAIRY INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

INTERPRETATION OF THE DEHYDRATION OF ACETYLGLUTAMIC ACID BY MEANS OF GLUTAMYLTHIOHYDANTOIN DERIVATIVES

BY BEN H. NICOLET

RECEIVED NOVEMBER 5, 1929 PUBLISHED MARCH 6, 1930

Bergmann, Stern and Witte[1] have shown that azlactones formed by the simultaneous acetylation and dehydration of the acetyl derivatives of certain amino acids may be coupled with other amino acids to yield acetyl dipeptides. In the case of acetyl-aspartic acid they considered,[2] without appreciable evidence, that the dehydration product was an acetyl-aspartic anhydride, rather than an azlactone. In any case, they isolated the product, and obtained from it by the action of glycine ester in ether solution a substance having the empirical formula of a glycine ester salt of acetylaspartylglycine. No evidence was presented as to which carboxyl group was concerned in the formation of the dipeptide.

The desirability of a new synthesis of glutathione has led to work here on the possibility of obtaining derivatives of the glutathione[2a] type (γ-glutamyldipeptides) by an analogous process. The work has not yet progressed so far as this, but the evidence here given indicates that the desired reactions should take place.

Glutamic acid is so insoluble even in boiling acetic anhydride that it is not practicable to acetylate it directly. On the other hand, acetylglutamic acid (II) is formed in fairly good yield when acetic anhydride in moderate excess is added to a water solution of sodium glutamate.[3] When the solution is sufficiently concentrated, the product separates on acidification and cooling.

On warming with acetic anhydride, acetylglutamic acid dissolves readily and is dehydrated. When the excess acetic anhydride is removed by distillation under reduced pressure, and the residual oil treated with an excess of aniline, a product (VI) results which on condensation with ammonium thiocyanate and acetic anhydride[4] yields 1-acetyl-2-thiohydantoin-5-β-propionanilide (VII) in 34% yield. The dehydration might reasonably have taken place in either of two ways, with the production of the anhy-

[1] Bergmann, Stern and Witte, *Ann.*, **449**, 279 (1926).

[2] Ref. 1, pp. 301–302.

[2a] At the time this paper was written the glutamylcystine structure for glutathione was accepted. Later evidence [Hopkins, *J. Biol. Chem.*, **84**, 269 (1929); Kendall, McKenzie and Mason, *ibid.*, **84**, 657 (1929)] shows that glutathione is a tripeptide. The type of combination, however, remains the same.

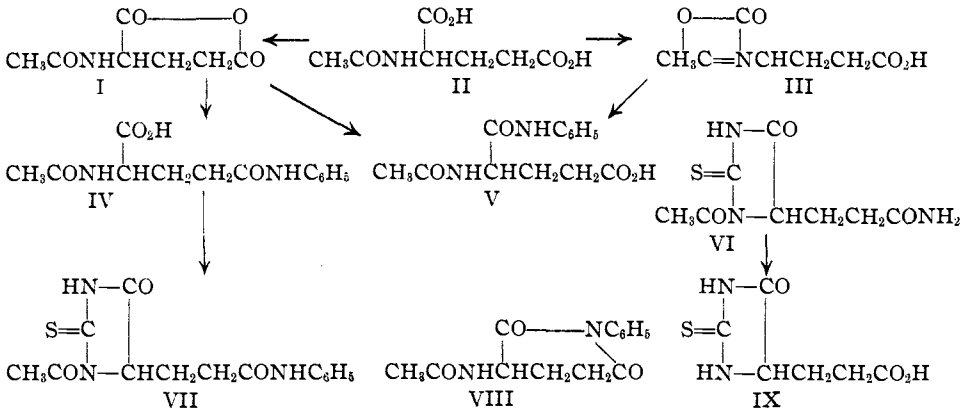
[3] Other amino acids can also be acetylated under these conditions.

[4] Johnson and Nicolet, *Am. Chem. J.*, **49**, 197–204 (1913).

dride I or of the azlactone III. The addition of aniline, in turn, might have yielded either of the two acetylglutamic acid monoanilides (IV and V). The anilide V might have resulted from either the azlactone or the anhydride; it could not have formed a thiohydantoin, and it was not detected. The anilide IV could have been formed only through one of the two possible modes of addition to the true anhydride. The formation of the thiohydantoin VII demonstrates the formation of the anilide IV (and therefore of the anhydride I) and indicates this particular anilide as at least the chief product of the reaction.

TABLE I

FORMULAS AND RELATIONSHIPS OF COMPOUNDS DESCRIBED



When acetylglutamic anhydride, prepared as above, was dissolved in aqueous ammonia, and the solution evaporated to dryness and condensed with thiocyanate as described, the product was 1-acetyl-2-thiohydantoin-5-β-propionamide (VI).[5]

Johnson and Guest[6] have described an indirect synthesis of glutamylthiohydantoin (IX), through pyrrolidonecarboxylic acid. They reported that on attempting to condense glutamic acid directly with thiocyanate solution took place, but the acid was de-aminated. Some such effect might account for the fact that the yield of VII obtained in this work was not larger. It seems, however, more probable that a partial dehydration of the acetylglutamic anilide IV to give the cyclic phenylimide derivative (VIII) (which would not be expected to yield a thiohydantoin) was responsible.

The thiohydantoin VII, dissolved in normal alkali and after half an

[5] Thierfelder, *Z. physiol. Chem.*, **114**, 192 (1921), obtained the hydantoin VI (m. p. 209°) in 22–30% yield from glutamin, and thereby proved the structure of the latter to be $HOOCCH(NH_2)CH_2CH_2CONH_2$. He also converted his VI to IX. His paper was discovered only after the present paper was written.

[6] Johnson and Guest, *Am. Chem. J.*, **47**, 242 (1912).

hour re-acidified, readily lost its acetyl group[7] and formed 2-thiohydantoin-5-β-propionanilide. The hydantoin VI was also easily de-acylated by this method, but the product was too soluble for easy isolation. On further hydrolysis with hydrochloric acid, the final product melted at 122°, and was presumably identical with the glutamylthiohydantoin IX of Johnson and Guest.

Experimental Part

Acetylglutamic Acid (II).[8]—*d*-Glutamic acid (14.7 g., 0.1 mole) was dissolved in 34 cc. (0.2 mole) of 6 *M* sodium hydroxide. To the cold solution was added 15 cc. of acetic anhydride, and the mixture shaken under the tap until the anhydride dissolved. The solution was then acidified with 17 cc. of 12 *M* hydrochloric acid and left in the ice box for some time to crystallize. The crude product (about 10 g.) was recrystallized from a little water to remove sodium chloride. A further quantity was obtained when the mother liquors were evaporated to dryness (at 15–20 mm.), the large residue of salt extracted with alcohol, the extract evaporated and the residue purified from water as described. The total yield was then about 75%. It was also found feasible to isolate the product by repeated extraction of the aqueous salt solution with *iso*-amyl alcohol. Extraction with ether is not effective.

Pure acetylglutamic acid (m. p. 193–194°) is readily soluble in water and alcohol and, on warming, in acetic anhydride.

Anal. (Micro-Kjeldahl). Calcd. for $C_7H_{11}O_5N$: N, 7.41. Found: N, 7.61.

Acetylglutamic Anhydride (I).—Acetylglutamic acid was heated for three minutes to boiling, or for thirty minutes at 100°, with 10 parts of acetic anhydride, and the solution evaporated at 20–30 mm. pressure. The anhydride remained as a viscous oil, readily soluble in organic solvents, except benzene, and only slowly soluble in water with formation of acetylglutamic acid.

1-Acetyl-2-thiohydantoin-5-β-propionanilide (VII).—Acetylglutamic acid (0.01 mole) was converted to the anhydride, and the excess acetic anhydride removed as completely as possible. Twice the calculated amount of aniline was then added, and the mixture, which became warm, was allowed to stand for an hour. Dry ammonium thiocyanate (1.0 g.) and acetic anhydride (8 cc.) were then added, and the mixture heated for one and one-half hours on the steam-bath. It was then cooled and poured into water, from which the solid product presently separated. A further small quantity was obtained from the mother liquors; the total yield was 0.9 g., or 34%. The pure substance, m. p. 197°, is moderately soluble in hot glacial acetic acid, rather difficultly soluble even in hot alcohol, and nearly insoluble in water.

Anal. (Micro-Kjeldahl). Calcd. for $C_{14}H_{15}O_3N_3S$: N, 13.77. Found: N, 14.0.

2-Thiohydantoin-5-β-propionanilide.—The acetyl derivative (0.2 g.) was dissolved in 10 cc. of molar sodium hydroxide. The solution, after standing for half an hour at room temperature, was acidified with hydrochloric acid and the bulky precipitate crystallized from alcohol. The pure substance darkened slowly above 200°, but melted at 216° with only slight decomposition.

Anal. (Micro-Kjeldahl). Calcd. for $C_{12}H_{13}O_2N_3S$: N, 15.95. Found: N, 15.90.

1-Acetyl-2-thiohydantoin-5-β-propionamide (VI).—Acetylglutamic anhydride was

[7] Schlack and Kumpf, *Z. physiol. Chem.*, **154,** 131 (1926), have called attention to this easy method for the de-acylation of 1-acylthiohydantoins, which seems to be quite general.

[8] All the substances described in this paper are derivatives of *d*-glutamic acid.

prepared as described above and treated with an excess of 6 *M* ammonium hydroxide solution. The anhydride rapidly dissolved. The solution was evaporated to dryness under reduced pressure and the residue condensed with ammonium thiocyanate and acetic anhydride as described for the corresponding anilide. A little water (5 cc.) was added to destroy the excess acetic anhydride and the solution was again evaporated nearly to dryness under reduced pressure. On addition of water, the product separated. It was difficultly soluble in cold water, and could be conveniently crystallized from hot water, in which its solubility was about 1%; m. p. 208–209°.

Anal. (Micro-Kjeldahl). Calcd. for $C_8H_{11}O_3N_3S$: N, 18.35. Found: N, 18.51.

Summary

1. Some new thiohydantoin derivatives of glutamic acid are described. Their formation is considered to show that the dehydration of acetylglutamic acid by acetic anhydride forms the true acetylglutamic anhydride, and not an azlactone.

2. When this anhydride reacts with amines, amide formation takes place at the carboxyl group most distant from the amino group. It is planned to extend the application of this reaction to the synthesis of γ-glutamyldipeptides.

BELTSVILLE, MARYLAND

[CONTRIBUTION FROM THE COLLEGE OF PHARMACY AND THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MICHIGAN]

A GENERAL METHOD FOR THE DETERMINATION OF HALOGENS IN ORGANIC COMPOUNDS[1]

BY J. J. THOMPSON AND U. O. OAKDALE

RECEIVED NOVEMBER 6, 1929 PUBLISHED MARCH 6, 1930

Halogen in an organic compound is determined, usually, by the Carius[2] or by the Lemp and Broderson[3] modification of the Parr[4] and Pringsheim[5] methods.

The Carius procedure suffers from a number of disadvantages, namely, the use of a sealed tube the preparation of which requires considerable skill, the use of a bomb furnace in which to heat the tube and the danger of glass splinters falling into the tube when the latter is opened. It requires a number of hours to perform an analysis and it is not always an easy matter to determine when the sample is completely oxidized.

The disadvantageous features of the Lemp and Broderson process are the possibility of incomplete fusion, the danger of rapid oxidation when

[1] This investigation was made possible by a grant from the Faculty Research Fund of the University of Michigan.

[2] Carius, *Ann.*, **136**, 129 (1865).

[3] Lemp and Broderson, THIS JOURNAL, **39**, 2069 (1917).

[4] Parr, *ibid.*, **30**, 764 (1908).

[5] Pringsheim, *Am. Chem. J.*, **41**, 386 (1904).