CCCVII. THE CARBAMIDO-ACID AND HYDANTOIN OF ARGININE.

By WILLIAM ROBERT BOON AND WILLIAM ROBSON.

*From the Department of Physiology, University of London
King's College.*

(Received October 1st, 1935.)

LIPPICH [1906; 1914] AND DAKIN [1918] demonstrated the usefulness of the carbamido-acids and hydantoins for the identification of the corresponding α -amino-acids and Dakin [1918; 1920] elaborated methods for the isolation of proline and hydroxyproline, in the form of their hydantoins, from mixtures of amino-acids. More recently Boyd [1933] showed that the carbamido-compounds could be utilised for isolating in excellent yield certain α -amino-acids from protein hydrolysates.

Reference to the literature, however, shows that whilst our knowledge of the carbamido-acids and hydantoins derived from the mono- and di-carboxylic α -amino-acids is fairly complete, that regarding the corresponding derivatives of the basic α -amino-acids is very limited. Apart from 5- δ -carbamidobutyl-hydantoin and 5- δ -aminobutylhydantoin, recently shown by Hoppe-Seyler [1933] to be derivatives of lysine, and citrulline, claimed by Wada [1930] to be α -amino- δ -carbamidovaleric acid, only the phenylcarbamido- and phenylhydantoin derivatives of ornithine [Herzoy, 1902; Sørensen, 1903; 1905] and of lysine [Herzoy, 1902] are known.

It appeared to us of interest therefore to attempt the preparation of the carbamido-acid and hydantoin derived from arginine, a quantity of which we possessed in the form of its monohydrochloride.

When an aqueous solution of arginine monohydrochloride was heated with potassium cyanate on the water-bath for about an hour and then allowed to cool, a heavy crystalline mass gradually separated. The mother-liquor was alkaline to litmus and our first impression was that this was due to the hydrolysis of the excess of cyanate employed. The crystals which had separated were collected, washed several times with small quantities of distilled water and finally recrystallised from hot water.

A cold saturated aqueous solution of the new compound, which possesses in a marked degree the property of forming supersaturated solutions, was definitely alkaline to litmus, its p_H being in the region of 11. Moreover, it did not appear to be more soluble in cold aqueous sodium hydroxide than in cold water, nor was carbon dioxide evolved when its hot aqueous solution was treated with sodium carbonate. The apparent inactivity of the carboxyl group of the original arginine molecule has led us to regard the formula of the new carbamido compound as

Of the two formulae, the former is more probably the correct one in view of the fact that the compound is stable in hot aqueous solution whilst triacetylarginine, shown by Bergmann and Koster [1926] to have a piperidone nucleus, is decomposed, even by cold water, into acetylurea and acetylnornithine.

The carbamido-compound is naturally very soluble in dilute mineral acids. On evaporating a solution of the compound in hydrochloric acid on the steam-bath to small bulk, there separated on standing for several days large prismatic crystals consisting of arginine hydantoin hydrochloride. Recrystallisation of the hydantoin may be effected either by dissolving it in a small bulk of hot concentrated hydrochloric acid and allowing the solution to cool, or by addition of alcohol to a highly concentrated aqueous solution. Analyses of the product so obtained indicate that it contains one molecule of water of crystallisation, which it loses slowly on heating at 60°.

Picturing the structure of the hydantoin thus derived from arginine as

it may be regarded as the mother substance of α - δ -bisguanidino-*n*-valeric acid anhydride

synthesised by Zervas and Bergmann [1928] and shown by them to agree in every respect with the so-called arginylarginine originally obtained by Fischer and Suzuki [1905] by treating arginine methyl ester with a concentrated solution of hydrogen chloride in methyl alcohol.

EXPERIMENTAL.

Carbamido-arginine. Arginine monohydrochloride (10 g.=1 mol.), prepared from gelatin by the method of Cox [1928] was dissolved in water (80 ml.) and to the solution was added solid potassium cyanate (10 g.=1.25 mol.) with shaking. The mixture was heated on a water-bath for 1 hour and was then set aside to cool overnight. Next morning a heavy crystalline precipitate of the carbamido-acid had separated. The crystals were collected at the pump and recrystallised from the minimum amount of boiling water. The product weighed 8.7 g. (84% of theory) and melted at 172°. Dried at 110° *in vacuo*, it gave on analysis N, 32.0%. $\text{C}_7\text{H}_{15}\text{O}_3\text{N}_5$ requires N, 32.2%.

Arginine hydantoin. 5 g. of carbamido-arginine as prepared above were dissolved in 20 ml. of hydrochloric acid (2*N*) and the solution placed on the boiling water-bath and evaporated to a thick syrup. The syrup was set aside and after some 6 days large flat prisms gradually separated. These were collected and recrystallised by dissolving them either in (i) a few ml. of hydrochloric acid and evaporating the solution almost to dryness, after which crystallisation slowly occurred on standing, or (ii) a few ml. of water and evaporating the solution almost

to dryness; on the addition of a large excess of alcohol, the hydrochloride separated as an aggregate of tiny prisms. The yield was almost quantitative. The product melted sharply at 100°. When dried at 60° for a week, it gave on analysis N, 29.2; Cl, 15.2%. $C_7H_{14}O_2N_5Cl$ requires N, 29.7; Cl, 15.1%.

REFERENCES.

- Bergmann and Koster (1926). *Z. physiol. Chem.* **159**, 179.
Boyd (1933). *Biochem. J.* **27**, 1838.
Cox (1928). *J. Biol. Chem.* **78**, 475.
Dakin (1918). *Biochem. J.* **12**, 290.
—— (1920). *J. Biol. Chem.* **44**, 499.
Fischer and Suzuki (1905). *Ber. deutsch. chem. Ges.* **38**, 4173.
Herzoy (1902). *Z. physiol. Chem.* **34**, 525.
Hoppe-Seyler (1933). *Z. physiol. Chem.* **214**, 267.
Lippich (1906). *Ber. deutsch. chem. Ges.* **39**, 2953.
—— (1914). *Z. physiol. Chem.* **90**, 145.
Sørensen (1903). *Compt. Rend. Lab. Carlsberg*, **6**, 1.
—— (1905). *Compt. Rend. Lab. Carlsberg*, **6**, 126.
Wada (1930). *Biochem. Z.* **224**, 427.
Zervas and Bergmann (1928). *Ber. deutsch. chem. Ges.* **61**, 1195.