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A SYNTHESIS OF HOMOARGININE

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The only amino acid containing the guanidine group that has been isolated from the proteins is arginine or δ-guanidoornithine. The possibility that other homologues of arginine may exist was suggested at one time by Kossel[1] and also by Winterstein and Küng.[2] The supposition largely rested on the anomalous behavior of nitrate protamine and on the presence of non-crystallizable syrups in the arginine fraction of several protein hydrolysates. It seems highly probable at this time, however, that the syrups may have been composed of breakdown as well as racemic products of arginine.

Many attempts have nevertheless been made to prepare homologues of arginine, notably by Winterstein and Küng[2], Heckel[3], and Steib[4]. The early methods simply employed the cyanamide reaction with lysine or with α-β-diaminopropionic acid, whereby non-crystallizable mixtures of mono- and diguanido acids resulted.

The action of *O*-methylisourea on the peptide lysylglutamic acid has been studied and a monoguanidine-substituted derivative has been obtained.[5] Examination of the product indicated that it was predominantly an ε derivative but its properties were such that some α substitution was suspected. The problem resolved itself therefore into the finding of a method whereby the α amino group could be masked, leaving the ω amino group free for the preparation of the guanido derivative. The first successful attempt in this direction had been accomplished by Steib[4] who prepared the α-toluolsulfonyl derivative of ε-benzoyllysine. Treatment of this molecule with baryta removed the ε benzoyl radical; in the following step the ε guanido group was prepared, and finally the α toluolsulfonyl group was removed by means of hot hydriodic acid. A very low yield of the hygroscopic nitrate of ε-guanidolysine was obtained in this manner.

In view of the improved procedures now available for the masking of

[1] KOSSEL, *Z. physiol. Chem.*, **84**, 1–10 (1913).
[2] WINTERSTEIN AND KÜNG, *ibid.*, **59**, 141–164 (1909).
[3] HECKEL, *Monatsh.*, **29**, 779–785 (1908).
[4] STEIB, *Z. physiol. Chem.*, **155**, 292–305 (1926).
[5] GREENSTEIN, *J. Biol. Chem.*, **109**, 529–540, 541–544 (1935).

groups, it was thought of interest to prepare the ϵ-guanidolysine (homoarginine) in amounts sufficient to be of use in further studies. The method of Bergmann, Zervas, and Ross[6] for the preparation of ϵ-carbobenzoxy-*l*-lysine provided the starting point for the synthesis. A satisfactory yield of the crystalline nonhygroscopic sulfate of *dl*-homoarginine was obtained by the following series of reactions:

dl-Lysine → Dicarbobenzoxylysine → Dicarbobenzoxylysyl chloride → ϵ-Carbobenzoxy-α-Carboxylysine anhydride → ϵ-Carbobenzoxylysine → ϵ-Carbobenzoxy-α-benzoyllysine → α-Benzoyllysine → ϵ-Guanido-α-benzoyllysine → *dl*-Homoarginine

The homoarginine was treated with arginase but no action whatever of the enzyme on this substrate was detectable under conditions by which sixty to seventy per cent. of arginine was split. This confirms the observation by Steib[4]. The great specificity of arginase is thus revealed, the action of the enzyme evidently requiring a specific distance between the guanidine group which it attacks and the α amino (or α hydroxyl) and carboxyl groups which it needs as probable points of attachment.

EXPERIMENTAL

ϵ-Carbobenzoxy-dl-*lysine.*—The preparation of this substance followed substantially the methods used by Bergmann, Zervas, and Ross for the *l*-lysine derivative.[6] *dl*-Lysine was synthesized from cyclohexanone oxime along the lines recommended by Eck and Marvel.[7] The racemic dicarbobenzoxylysine crystallized in tiny square prisms from ethyl acetate-petroleum ether, and melted at 104°. It was converted to the acid chloride in dry, chilled, chloroform solution with phosphorous pentachloride. Evaporation *in vacuo* at 50° converted the substance into the carboxylic anhydride (m.p. 92°). Treatment with acetone-hydrogen chloride yielded carbobenzoxy-*dl*-lysine hydrochloride, which after neutralization with ammonia gave the free acid (m. p. 263°).*

Anal. Calc'd for $C_{14}H_{20}N_2O_4$: N, 10.0. Found: N, 10.0.

α Benzoyl-dl-*lysine.*—Twenty-four grams of ϵ-carbobenzoxy-*dl*-lysine was dissolved in 160 cc. 1*N* sodium hydroxide solution and treated at 10° with 18 g. of benzoyl chloride and 150 cc. of 1*N* sodium hydroxide solution. On acidification with 5*N* hydrochloric acid an oil separated, and rapidly hardened on cooling. This product, ϵ-carbobenzoxy-α-benzoyllysine, was dried, pulverized, and extracted several times with ligroïn to remove adhering benzoic acid. It was then dissolved in a methyl alcohol-water mixture; the solution was treated with 60 cc. of 5*N* hydrochloric acid and catalytically hydrogenated in the presence of palladium. When the reaction was at an end, the filtrate from the catalyst was evaporated *in vacuo*, and the aqueous solution was shaken with ether to remove some benzoic acid. After neutralization with ammonia, scratching of the sides of the flask induced the appearance of a white

[6] BERGMAN, ZERVAS, AND ROSS, *ibid.*, **111**, 245–260 (1935).

[7] ECK AND MARVEL, *ibid.*, **106**, 387–391 (1934).

* During evaporation of the acetone-hydrogen chloride mixture, an oil separates which can be removed by ether extraction and subsequently discarded.

precipitate of α-benzoyl-*dl*-lysine. The substance was filtered off, washed well with ice-water, and dried; yield 17 g.; m.p. 211°.

Anal. Calc'd for $C_{13}H_{18}N_2O_3$: N, 11.2. Found: N, 11.1.

ε-*Guanido-α-benzoyl*-dl-*lysine.*—The hydrochloride of 0-methyl isourea[5,8] (40.32 g.) was dissolved in 200 cc. of methanol, and the solution was treated at 0° with 86.9 cc. 4.2*N* sodium methylate solution. The sodium chloride was separated by filtration through infusorial earth, and to the clear filtrate 17 g. of α-benzoyllysine was added, together with enough water to bring the substance into solution. A slight amount of warming was also necessary. The solution was filtered, and allowed to stand at room temperature. Crystallization of long needles began after fifteen minutes. After twenty-four hours the crystalline mass was collected by filtration, washed with ice-water, and recrystallized from hot water. This time the guanidine derivative crystallized in long prisms. It was collected by filtration, washed, and dried. The yield was 12.8 g.; m.p. 273°.

Anal. Calc'd for $C_{14}H_{20}N_4O_3$: N, 19.2. Found: N, 19.1.

ε-*Guanido* dl-*lysine* (*homoarginine*) *sulfate.*—Twelve grams of the benzoyl derivative was dissolved in 120 ccm. of 5*N* hydrochloric acid, and the solution was boiled under reflux for three hours. After cooling, the benzoic acid was extracted with ether, and the aqueous solution was evaporated to a syrup *in vacuo*. Inasmuch as the syrup failed to crystallize it was taken up in water and shaken with a slight excess of silver sulfate. The silver chloride was removed by filtration, and the filtrate was treated with hydrogen sulfide. On evaporation *in vacuo* a syrup again was obtained. It was dissolved in a little water, and treated with a large volume of absolute alcohol. A white oil separated; this solidified on standing in the ice chest for a week. The procedure of dissolving in water and precipitating with alcohol was repeated twice more, after which a crystalline solid in the form of prisms was finally obtained. The crystalline sulfate was non-hygroscopic and amounted to 7.1 gm. The substance decomposed with foaming at 127° after preliminary softening at 112°. It crystallized with 1½ molecules of water.

Anal. Calc'd for $(C_7H_{16}N_4O_2)_2 \cdot H_2SO_4 + 1\frac{1}{2}\ H_2O$: N, 22.3; SO_4, 19.1.
Found: N, 22.0; SO_4, 19.0.

Homoarginine sulfate, on drying for four hours at 56° under 4 mm. pressure, lost 5.2 per cent. in weight; calculated for 1.5 moles of crystal water, 5.3 per cent. The substance gives a positive Sakaguchi reaction. It was further characterized by the formation of its benzilidene derivative.

Benzilidene homoarginine.—Six-tenths gram of homoargine sulfate was dissolved in 10 cc. of water, was treated with enough 1*N* sodium hydroxide solution to bring the pH of the solution to about 9, and then shaken with 0.5 cc. of benzaldehyde. Scratching induced crystallization of the benzilidene derivative in the form of small prisms. After chilling, the product was collected by filtration, washed twice with ice-water, then with a methanol-ether mixture, and finally with dry ether. The yield was 0.4 gm.; m.p. 248° with decomposition.

Anal. Calc'd for $C_{14}H_{20}N_4O_2$: N, 20.3. Found: N, 19.9.

Behavior of homoarginine with arginase.—One-fourth gram of neutralized homoarginine sulfate was incubated at 40° and at pH 8 with an arginase solution prepared from fresh rat liver. A few drops of Manganese chloride solution was added. Simultaneously, a solution containing 0.1 g. of arginine base with an equivalent amount of sulfuric acid was similarly treated. After one hour the solutions were boiled,

[8] KAPFHAMMER AND MÜLLER, *Z. physiol. Chem.*, **225**, 1–12 (1934).

filtered, and treated with urease. After a half-hour's incubation at 40°, the solutions were made up to volume, and aliquots of each were directly Nesslerized. A splitting of 60 to 70 per cent. of arginine was revealed in this way. No trace of any attack by arginase on the homoarginine was evident.

SUMMARY

1. *dl*-Homoarginine (ε-guanidolysine) has been synthesized from *dl*-lysine.

2. The substance is not attacked by arginase.