Synthesis of ε-Pepides of Lysine

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In the course of study of the arrangement of the amino acids surrounding the lysine residue in Bacitracin A, the syntheses of ε-(tosyl-DL-isoleucyl)-L-lysine and the corresponding glycyl and L-leucyl derivatives were reported.[1]

The crystalline *N*-tosylamino acid chlorides were stable enough in alkaline solution to be suitable for coupling with the copper complex of lysine. However, the yields were not always satisfactory and the problem of purification was solved only by countercurrent distribution.

The mixed carboxylic-carbonic anhydride procedure[2] has been found to be a simple procedure for the acylation of the lysine copper complex with carbobenzoxy amino acids. The carbobenzoxy dipeptide copper complex thus obtained was decomposed with hydrogen sulfide and dipeptides were then recrystallized from water. The corresponding tosyl derivatives are very soluble in water; however, both the carbobenzoxy and tosyl derivatives appear to be practically insoluble in organic solvents.

Removal of the carbobenzoxy group by catalytic hydrogenation in the presence of acetic acid yielded the desired ε-peptides in crystalline form. Cleavage of the carbobenzoxy group with hydrogen bromide[3] led to the formation of a hygroscopic dihydrobromide. The dihydrochloride and sulfate salts were also found to be hygroscopic.

In order to demonstrate that no isomeric α-peptide had been formed, carbobenzoxyglycyl-L-lysine was treated with 2,4-dinitrofluorobenzene[4] and the DNP derivative was completely hydrolyzed. Only α-DNP-L-lysine could be detected by electrophoresis.[5]

EXPERIMENTAL

ε-(Carbobenzoxyglycyl)-L-lysine. Carbobenzoxyglycine (2.09 g., 0.1 mole) was dissolved in 20 ml. tetrahydrofuran in the presence of 1.01 g. (0.01 mole) triethylamine. The solution was cooled to −10°C. and 1.13 g. (0.01 mole) ethyl chlorocarbonate was added. Five minutes later a solution of the copper complex of lysine was added with vigorous shaking.

The copper complex was prepared in accordance with the directions of Neuberger and Sanger.[6] Thus 1.5 g. (8.2 mmoles, theor. 0.01 mole) L-lysine monohydrochloride was used and the water solution of the complex (10 ml.) was diluted by addition of 5 ml. tetrahydrofuran and 5 ml. 2*N*-NaOH.

The dipeptide complex formed during the coupling was precipitated by addition of 200–300 ml. of water to yield 3 g. The complex was suspended in 100 ml. of water and hydrogen sulfide bubbled into the mixture for 3 hr. while heating. The copper sulfide was filtered and the desired product was twice recrystallized from hot water to yield 1.6 g. (50%), m.p. 250–252° (decomp.), $[\alpha]_D^{16}$ +8.7° (c, 1.03 in glacial acetic acid). The product was found electrophoretically homogenous, thus giving only a single ninhydrin-positive spot (neutral) in acetate-pyridine buffer *p*H: 5.6. Paper chromatography[7] revealed only one spot, R_f 0.56.

Anal. Calcd. for $C_{16}H_{23}O_5N_3$: C, 56.95; H, 6.8. Found: C, 56.56; H, 6.8.

ε-Glycyl-L-lysine monoacetate. ε-(Carbobenzoxyglycyl)-L-lysine (0.84 g., 2.5 mmoles) was hydrogenated in the presence of palladium black.[8] The compound which at the beginning was partly in solution (acetic acid) dissolved as the hydrogenation proceeded. At the end the catalyst was filtered off, washed several times with 50% acetic acid, and the combined filtrates evaporated *in vacuo* at 35°. To the residue 5 ml. of water was added and the solution reconcentrated. This process was repeated four times and the peptide isolated by addition of acetone, yield 0.5 g. (76%), m.p. 198–200° (decomp.), $[\alpha]_D^{16}$ +85° (c, 1.06 in water); R_f 0.20.

Anal. Calcd. for $C_8H_{17}O_3N_3 \cdot CH_3CO_2H$: C, 45.60; H, 8.03; N, 15.95. Found: C, 45.14; H, 8.12; N, 15.76.

ε-(L-Phenylalanyl)-L-lysine. Carbobenzoxy-L-phenylalanine (1.49 g., 0.005 mole) was coupled with the lysine copper complex in the manner indicated for the preparation of the carbobenzoxyglycyl derivative. The dipeptide complex was washed with 10% sodium carbonate and finally with water, then decomposed with hydrogen sulfide in the presence of acetic acid (10 ml. for 100 ml. water). The dipeptide derivative was recrystallized from 1% acetic acid, yield 1.1 g., m.p. 216–218°, R_f 0.8.

The above acetate salt (1.07 g., 2.5 mmoles) was dissolved in acetic acid and hydrogenated in the presence of palladium black.[8] The catalyst was filtered and the filtrate was evaporated *in vacuo* at 35–40°. Water was added and the solution again concentrated. This process was repeated several times. The peptide was obtained by addition of acetone, yield 0.55 g., m.p. 250–251° (decomp.), $[\alpha]_D^{13}$ +47.1° (c, 1.06 in water), R_f 0.44.

Anal. Calcd. for $C_{15}H_{23}O_3N_3 \cdot {}^1/_2H_2O$: C, 59.58; H, 8.00; N, 13.89. Found: C, 59.93; H, 8.03; N, 13.85.

ε-(Dicarbobenzoxy-L-lysyl)-L-lysine sulfate salt. This compound was prepared in a manner similar to that used in the preparation of the carbobenzoxyglycyl derivative. When 1.9 g. (0.005 mole) dicarbobenzoxy-L-lysine[9] was coupled, 3 g. of dipeptide copper complex was obtained. After decomposition of the complex with hydrogen sulfide in the presence of acetic acid, the dipeptide derivative was separated from the copper sulfide and then dissolved in 50% ethanol containing one equivalent of sulfuric acid. Upon standing in the refrigerator the desired product precipitated, yield 1.5 g. (50%), m.p. 156–158° (after recrystallization from water), R_f 0.87.

Anal. Calcd. for $C_{28}H_{38}O_7N_4 \cdot H_2SO_4$: C, 52.48; H, 6.29; N, 8.74. Found: C, 52.17; H, 6.47; N, 8.50.

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