SOME ε-AMINO DERIVATIVES OF LYSINE

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The presence of peptide crosslinks has been demonstrated in insoluble fibrin[1–3] and the medulla of guinea pig hair[4] by isolation of the peptide, ε-(γ-glutamyl)lysine, from enzyme digests. We have used similar methods to establish the presence of peptide crosslinks between the side-chains of lysyl residues and those of aspartyl and glutamyl residues in wool that has been treated with carbodiimides or heated.[5] This communication describes the preparation of authentic samples of ε-(γ-glutamyl)-lysine and ε-(β-aspartyl)lysine for comparison with peptides isolated from enzyme digests of treated wool.

ε-(γ-Glutamyl)lysine has been synthesized previously by two unambiguous routes.[6,7] The present synthesis, which entails reaction of benzyloxycarbonylglutamic anhydride with lysine copper complex, is much shorter than previous ones and provides pure samples of both ε-(γ-L-glutamyl)-L-lysine and ε-(α-L-glutamyl)-L-lysine in overall yields of 4 and 13% respectively. The two isomers were distinguished by the resistance of the former to hydrolysis by leucine aminopeptidase;[6] the latter was hydrolysed completely to glutamic acid and lysine.

The unambiguous synthesis of ε-(β-L-aspartyl)-L-lysine was carried out by coupling *N*-benzyloxycarbonylaspartic acid α-benzyl ester with the copper complex of lysine, followed by removal of the protecting groups. It is slightly shorter than the synthesis by Shields[8] who used a salt of N^{α}-benzyloxycarbonyllysine benzyl ester instead of lysine copper complex.

Di(*p*-nitrophenyl) sebacate has been claimed to react with the ε-amino groups of the lysyl residues of wool mainly in a bifunctional manner.[9] Enzymic digestion of wool treated in this way would therefore be expected to yield *N*,*N*′-di(5-amino-5-carboxypentyl)sebacamide with little of the monofunctional reaction product,

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[1] Pisano, J. J., Finlayson, J. S., and Peyton, M. P., *Science*, 1968, **160**, 892.

[2] Matacic, S., and Loewy, A. G., *Biochem. biophys. Res. Commun.*, 1968, **30**, 356.

[3] Lorand, L., Downey, J., Gotoh, T., Jacobsen, A., and Tokura, S., *Biochem. biophys. Res. Commun.*, 1968, **31**, 222.

[4] Harding, H. W. J., and Rogers, G. E., *Proc. Aust. biochem. Soc.*, 1970, **3**, 56.

[5] Caldwell, J. B., Holt, L. A., and Milligan, B., *J. appl. Polym. Sci.*, 1970, in press (Proc. 4th Wool Text. Res. Conf.).

[6] Kornguth, M. L., Neidle, A., and Waelsch, H., *Biochemistry*, 1963, **2**, 740.

[7] Zahn, H., and Paetzold, W., *Chem. Ber.*, 1963, **96**, 2566.

[8] Shields, J. E., *Biochemistry*, 1966, **5**, 1041.

[9] Zahn, H., Rouette, H.-K., and Schade, F., *Proc. 3rd Int. Wool Text. Res. Conf.*, 1965, Vol. II, 495.

N-(5-amino-5-carboxypentyl)sebacamic acid. This communication also describes the synthesis of these two ε-lysine derivatives. Approximately equal amounts were found in enzyme digests of wool that had been treated with di(*p*-nitrophenyl)sebacate.[5]

Experimental

ε-(α-L-*Glutamyl*)-L-*lysine*

Solutions of *N*-benzyloxycarbonyl-L-glutamic acid (16·8 g) and dicyclohexylcarbodiimide (12·5 g) in dioxan (50 ml) were mixed at 20°. After 1 hr the dicyclohexylurea was filtered off and washed with dioxan (50 ml). The combined filtrate and washings were added over 1 hr to a solution of L-lysine copper complex hydrochloride (10·7 g) in water (100 ml) and dioxan (50 ml) at 0°, the pH of the mixture being maintained at *c.* 7·5 by addition of 2M NaOH. After stirring for a further 1 hr the mixture was partly evaporated to remove dioxan, adjusted to pH 3·5, and extracted with ethyl acetate. The aqueous layer was then saturated with hydrogen sulphide, heated to boiling, and filtered hot. The ε-[α-(benzyloxycarbonylglutamyl)]lysine, which separated on cooling, was filtered off. The mother liquors were used subsequently for the preparation of ε-(γ-glutamyl)lysine (see below). The crude ε-[α-(*benzyloxycarbonylglutamyl*)]*lysine* (3·1 g, 13% based on *N*-benzyloxycarbonylglutamic acid), after recrystallization from water, had m.p. 228–229°, $[\alpha]_D^{30}$ −4·2 (*c*, 1·0 in 0·5M HCl) (Found: C, 54·8; H, 6·7; N, 10·0. $C_{19}H_{27}N_3O_7,\frac{1}{2}H_2O$ requires C, 54·5; H, 6·7; N, 10·0%). The benzyloxycarbonyl group was removed by treatment with 6M hydrogen bromide in acetic acid at 20° for 30 min and the peptide hydrobromide was precipitated by adding anhydrous ether. An aqueous solution of the hydrobromide was passed through a column of Zeocarb 225 ion-exchange resin (H^+ form); subsequent elution with 2M NH_4OH gave the free peptide, which crystallized from a concentrated aqueous solution on addition of acetone. ε-(α-L-Glutamyl)-L-lysine, which was obtained in 80% yield from the benzyloxycarbonyl derivative, had $[\alpha]_D^{30}$ +27·0° (*c*, 1·0 in H_2O) (lit.[6] +26·5°) (Found: C, 47·8; H, 7·7; N, 14·8. Calc. for $C_{11}H_{21}N_3O_5$: C, 48·0; H, 7·7; N, 15·3%). Paper electrophoresis at pH 2·3 and 3·7 revealed no ninhydrin-positive contaminants.

ε-(γ-L-*Glutamyl*)-L-*lysine*

The mother liquors from the preceding preparation were treated with benzyloxycarbonyl chloride (9 ml) at 25°, the pH of the mixture being maintained at 9 by adding 2M NaOH. After 1 hr unchanged benzyloxycarbonyl chloride was removed by extraction with ether and the aqueous layer was then acidified. Extraction with ethyl acetate yielded the crude bis-benzyloxycarbonyl derivative of ε-(γ-glutamyl)lysine. The benzyloxycarbonyl groups were cleaved by treatment with hydrogen bromide in acetic acid and the resulting hydrobromide converted into the free peptide as described previously. It was crystallized once from water and then from 1M NH_4OH by adding ethanol giving ε-(γ-L-glutamyl)-L-lysine (0·74 g, 4% based on *N*-benzyloxycarbonylglutamic acid), $[\alpha]_D^{30}$ +34·3° (*c*, 1·0 in 0·1M HCl) (Found: C, 47·7; H, 7·6; N, 15·2. Calc. for $C_{11}H_{21}N_3O_5$: C, 48·0; H, 7·7; N, 15·3%). Paper electrophoresis revealed no ninhydrin-positive contaminants.

ε-(β-L-*Aspartyl*)-L-*lysine*

Ethyl chloroformate (0·28 ml) was added dropwise with stirring to a solution of *N*-benzyloxycarbonyl-L-aspartic acid α-benzyl ester[10] (1·02 g) and triethylamine (0·40 ml) in anhydrous dimethylformamide at −15°. After 15 min a solution of L-lysine copper complex hydrochloride (0·67 g) and triethylamine (0·44 ml) in water (10 ml) was rapidly added with vigorous stirring. After a further 15 min at −10° the mixture was kept at room temperature for 2 hr. The mixture was then poured into cold water (400 ml) and the resulting copper complex (1·32 g) was filtered off and washed with water.

A portion of the copper complex (0·52 g) was shaken with 2M HCl (10 ml) for 5 min and a solution of disodium diaminoethanetetraacetate (0·1M, 20 ml) added. The solution was then

[10] Bryant, P. M., Moore, R. H., Pimlott, P. J., and Young, G. T., *J. chem. Soc.*, 1959, 3868.

adjusted to pH 7 by addition of 2M NaOH and the product filtered off and washed with ice-water. Recrystallization from a large volume of water gave ε-[β-(N-*benzyloxycarbonyl*-O$^{\alpha}$-*benzylaspartyl*)]-*lysine* (0·30 g, 60%), m.p. 225–226°, $[\alpha]_D^{30}$ +5·4° (*c*, 1·0 in 0·5M Na_2CO_3) (Found: C, 59·5; H, 6·3; N, 8·5. $C_{25}H_{31}N_3O_7,H_2O$ requires C, 59·6; H, 6·6; N, 8·3%).

The protecting groups were removed by hydrogenation in 50% aqueous n-propanol (40 ml) using palladium on charcoal as catalyst. After 3 hr the catalyst was filtered off and the filtrate evaporated to *c.* 5 ml. Addition of ethanol (30 ml) gave ε-(β-L-aspartyl)-L-lysine (0·14 g, 90%), which had $[\alpha]_D^{30}$ +32·5° (*c*, 0·7 in 0·1M HCl) (lit.[8] $[\alpha]_D^{23}$ +15°) (Found: C, 45·6; H, 7·5; N, 15·8. Calc. for $C_{10}H_{19}N_3O_5$: C, 46·0; H, 7·3; N, 16·1%). Paper electrophoresis failed to reveal any ninhydrin-positive impurities.

N,N′-*Di*(*5-amino-5-carboxypentyl*)*sebacamide*

Sebacoyl chloride (2·0 ml) was added dropwise with stirring to a solution of L-lysine copper complex hydrochloride (3·5 g) in 50% aqueous dioxan (40 ml) at 5°, the pH being maintained at *c.* 9·5 by simultaneous addition of 2M NaOH. After 1·5 hr the dioxan was evaporated under reduced pressure and the remaining aqueous solution cooled. The copper complex of the product was then filtered off, washed with ice-water, acetone, and ether, and then dried. This complex (3·0 g, 43%) was added in portions to a boiling solution of disodium diaminoethanetetraacetate (4·5 g) in water (120 ml). The solution was cooled and the product filtered off, washed with cold water, and then dried. The *product* (1·2 g, 15% based on lysine copper complex hydrochloride) was purified by precipitation from hot 0·2M Na_2CO_3 solution by acidification. The amorphous powder had m.p. >350° (Found: C, 54·1; H, 9·1; N, 11·3. $C_{22}H_{42}N_4O_6,1\frac{1}{2}H_2O$ requires C, 54·4; H, 9·3; N, 11·5%).

N-(*5-Amino-5-carboxypentyl*)*sebacamic Acid*

A solution of sebacic anhydride[11] (1·4 g) in warm dioxan (30 ml) was added dropwise to a vigorously stirred solution of lysine copper complex hydrochloride (2·5 g) in water (60 ml) at 20°. The pH was maintained at 10 by adding 1M NaOH. After 2 hr the pH was adjusted to 7 and the resulting copper complex filtered off, washed with water and hot dioxan, and then dried. A solution of the complex (2·55 g) in 1M HCl (20 ml) was saturated with hydrogen sulphide, warmed to 50°, filtered, cooled, and then neutralized with 3N NaOH. The precipitate was filtered off and recrystallized from 0·2N HCl, giving the *product* (1·2 g, 48% based on sebacic anhydride), m.p. 220–222° (Found: C, 57·7; H, 9·1; N, 8·3. $C_{16}H_{30}N_2O_5$ requires C, 58·2; H, 9·2; N, 8·5%). This product was separated from *N*,*N*′-di(5-amino-5-carboxypentyl)sebacamide by high-voltage electrophoresis at pH 9; neither product contained ninhydrin-positive contaminants.

[11] Voerman, G. L., *Recl Trav. chim. Pays-Bas Belg.*, 1904, **23**, 265.