THE USE OF SEQUESTERING AGENTS IN THE PREPARATION OF ϵ-ACYL-L-LYSINE AND δ-ACYL-L-ORNITHINE DERIVATIVES*

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In peptide syntheses involving lysine it is frequently necessary to mask the ϵ-amino function of this amino acid with a suitable acyl protecting group. The preferential ϵ-acylation of lysine is usually accomplished by treatment of an aqueous solution of the amino acid cupric complex with the appropriate acid chloride, followed by removal of the copper as the insoluble sulphide using gaseous hydrogen sulphide. In this process the cupric ion forms a stable chelate complex with the α-amino and carboxyl groups, thereby preventing acylation of the former. The ϵ-benzyloxycarbonyl,[1] ϵ-*p*-nitrobenzyloxycarbonyl,[2] ϵ-benzoyl,[3] ϵ-*p*-toluenesulphonyl,[4] and ϵ-formyl[5] derivatives of L-lysine have been prepared by various modifications of this general method.

The use of hydrogen sulphide for decomposition of the ϵ-acyl cupric complexes is often unsatisfactory, particularly in larger scale preparations, owing to the low solubility of some ϵ-acyl-L-lysine derivatives in aqueous solution. Moreover, the procedure may involve prolonged boiling of the amino acid derivative with cupric sulphide, with the possibility of some racemization. For preparative purposes it has now been found that removal of the copper can be effected more conveniently by means of the sequestering agent ethylenediaminetetraacetic acid (EDTA), or, in certain circumstances, a chelating resin. In this connection it is noteworthy that Zahn and Pätzold[6] have similarly used potassium cyanide to sequester cupric ions in the synthesis of an ϵ-peptide derivative of lysine. Both potassium cyanide and ethylenediamine were also examined as sequestering agents in the present work, but were not as generally satisfactory as EDTA.

For the preparation of ϵ-acyl-L-lysine derivatives which are sparingly soluble in cold water the cupric complex is dissolved in hot EDTA solution, whereupon the product crystallizes out on cooling, and the metal remains in solution as the EDTA complex (Method A). Alternatively, for preparing larger quantities of very slightly soluble ϵ-acyl compounds, the complex is dissolved in an acidic EDTA solution, which is then neutralized to precipitate the product (Method B). As would be expected the δ-acyl derivatives of L-ornithine can also be prepared in the same way. The

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[5] Hofmann, K., Stutz, E., Spükler, G., Yajima, H., and Schwartz, E. T., *J. Amer. Chem. Soc.*, 1960, **82**, 3727.

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compounds which have been obtained using EDTA to decompose the cupric complexes are collected in Table 1, which includes two hitherto unreported *p*-bromobenzenesulphonyl derivatives.

In the case of acyl derivatives which are soluble in water, such as ε-formyl-L-lysine, a different procedure is adopted. An acidic solution of the cupric complex is treated with a chelating resin, and the ε-acylamino acid eluted with water (Method C). Citrulline[7] (δ-ureido-L-ornithine) has also been prepared by the resin method (Table 1).

TABLE 1

ε-ACYL-L-LYSINES AND δ-ACYL-L-ORNITHINES

Rotations at concentration 2·0 in 2N HCl unless otherwise indicated

Compound	Method	Overall Yield (%)	Yield from Complex (%)	Melting Point	$[\alpha]_D$	Ref.
ε-Benzyloxycarbonyl-L-lysine	B	85	95	278–280°	17·3	1
ε-*p*-Toluenesulphonyl-L-lysine	A	78	97	238 dec.	13·4	4
ε-Benzoyl-L-lysine	A	72	96	253–255	24·5	3
ε-*p*-Nitrobenzyloxycarbonyl-L-lysine	A	66	90	239–240	15·5	2
ε-*p*-Bromobenzenesulphonyl-L-lysine*	A	84	96	264–265	14·8	—
ε-Formyl-L-lysine	C	59	97	228 dec.	15·6†	5
δ-Benzyloxycarbonyl-L-ornithine	B	86	94	254–255	17·8	8
δ-*p*-Bromobenzenesulphonyl-L-ornithine‡	A	88	92	254–256	17·9§	—
Citrulline	C	73	96	228–230	19·5	7

* Found: C, 39·8; H, 4·8; Br, 21·7. Calc. for $C_{12}H_{17}BrN_2O_4S$: C, 39·5; H, 4·7; Br, 21·9%.

† Concentration 1·3 in saturated $NaHCO_3$.

‡ Found: C, 37·6; H, 4·3; N, 8·2. Calc. for $C_{11}H_{15}BrN_2O_4S$: C, 37·6; H, 4·3; N, 8·0%.

§ Concentration 1·0.

In all cases the yields obtained by both processes have exceeded 90% based on the amount of copper complex, and the products are optically pure. The use of sequestering agents should be particularly useful for the introduction of ε-substituents which would be attacked chemically under the rather vigorous conditions of the hydrogen sulphide procedure.

Experimental

The microanalyses were carried out by the Australian Microanalytical Service, Melbourne. Melting points are uncorrected.

The copper complexes of known ε-acyl-L-lysine and δ-acyl-L-ornithines were prepared as described in the literature, and the new *p*-bromobenzenesulphonyl derivatives by the method of Roeske *et al.*[4] Three experimental procedures were used for the decomposition of the complexes, according to the solubility of the product.

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Method A.—The cupric complex (0·005 mole) was dissolved in boiling EDTA solution (0·1N; 100 ml). On cooling the acylamino acid crystallized out; it was collected, washed with water and ethanol, and dried.

Method B.—The complex (0·005 mole) was dissolved in 2N HCl (50 ml), and EDTA solution (0·1N; 100 ml) added. The solution was then neutralized with 2N NaOH, and cooled. The precipitated product was treated as in Method A.

Method C.—A solution of the complex (0·0005 mole) in 0·5N HCl (2 ml) was applied to a column of Chelex 100 chelating resin (1 by 10 cm; H^+ form), and the product eluted with water. The eluate was neutralized with 2N lithium hydroxide, and evaporated to small volume (*c.* 2 ml) *in vacuo*. Addition of ethanol (10 ml) precipitated the product, which was collected after storage overnight at 4°.

Method A is suitable for most small scale preparations. On a larger scale with more sparingly soluble acyl derivatives prohibitive volumes of solution would be necessary, and in these cases Method B is used. Method C is only required for water-soluble products. Details of the various derivatives prepared are given in Table 1.