The Removal of Copper from the Copper Complex of ω-Acylamino Acid with EDTA*

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ω-Acyl derivatives of basic amino acids can be synthesized most conveniently by the acylation of the copper complexes of the amino acid, followed by the removal of copper by hydrogen sulfide. This method is, however, rather troublesome for the synthesis of lysine or ornithine derivatives (e. g. ε-carbobenzoxylysine or δ-carbobenzoxy-ornithine) when water is used as a solvent, because their final products are only slightly soluble in water or are strongly adsorbed on cupric sulfide. This difficulty can be avoided by using such a solvent as boiling 70—80% acetic acid[1] or dilute hydrochloric acid[2], which dissolves the product. However, it might be desirable to avoid the hydrogen sulfide process for the benefit of subsequent catalytic reduction. For this reason, Zahn[3] has used potassium cyanide in 20% acetic acid to remove copper from the copper complex of ε-lysine peptide. Although we applied this method to the preparation of ε-carbobenzoxy-lysine, we could not obtain any successful results because of the low solubility of the product in this solvent. In principle, a reagent which has a stronger affinity to copper than that of amino acid, may also be useful. Therefore, we intended to use EDTA (ethylenediaminetetraacetic acid), which is well known to form a water-soluble copper chelate, as a copper-removing reagent.

Thus, we were successful in obtaining a ω-acyl derivative, e. g., γ-carbobenzoxy-L-α, γ-diaminobutyric acid or ε-tosyl-L-lysine, from its copper complex by merely boiling it with EDTA in water or aqueous ethanol, from which the product separated as white precipitates in the cold. A ω-acyl derivative, such as ε-carbobenzoxy-L-lysine, which is very slightly soluble even in hot water, was also easily prepared by boiling the aqueous suspension of a copper derivative with EDTA, followed by the recrystallization of the raw product from 50% acetic

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[1] J. Noguchi, T. Hayakawa and M. Hiraoka, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **82**, 604 (1961).

[2] B. C. Barrass and D. T. Elmore, *J. Chem. Soc.*, **1957**, 3134.

[3] H. Zahn and W. Pätzold, *Chem. Ber.*, **96**, 2566 (1963).

Table I. Comparison of EDTA and H_2S methods

Amino acid	ω-Acyl group†	Method	Copper complex of ω-acyl deriv., g.	EDTA g.	Product			
					g.	%	M. p., °C	$[\alpha]_D^{31}$
L-Lysine	Tos	EDTA	10	6	8	88	237—240	+14.2° (c=3.2, N HCl)
	Tos	H_2S⁴⁾	26	—	20	85	238—240	+14.4° (c=3.2, N HCl)
L-Lysine	Z	EDTA	15	9	12	89	251—254	+14.4° (c=1.6, N HCl)
	Z	H_2S⁵⁾	61	—	53	96	253—254	+14.1° (c=1.6, N HCl)
L-α,γ-Diaminobutyric acid	Z	EDTA	5	3.5	3.5	79	237	
	Z	H_2S²⁾	5	—	3.3	75	233—235	

† Tos=*p*-toluenesulfonyl-; Z=carbobenzoxy-

4) R. Roeske, F. H. C. Stewart, R. J. Stedman and V. duVigneaud, *J. Am. Chem. Soc.*, **78**, 5883 (1956).

5) F. Sanger and A. Neuberger, *Biochem. J.*, **37**, 515 (1943).

acid containing some EDTA, thus removing the trace of copper.

The yields are comparable to those obtained by the hydrogen sulfide method, as is shown in Table I.

Experimental

The copper complexes of the ω-acyl amino acids were prepared by acylating the copper complexes of the amino acids by the usual method.

EDTA was used as the disodium salt.**

ε-Tosyl-L-lysine.—The copper complex of ε-tosyl-L-lysine was suspended in water or 50% ethanol containing EDTA, the mixture was then boiled until the complex had dissolved completely. When the resulting blue colored solution had cooled, colorless needles were separated from it. The crystals were washed with water and recrystallized from 50% ethanol. For further purification, they were dissolved in dilute hydrochloric acid, decolorized, and neutralized with alkali, and the resulting white precipitates were recrystallized from 50% ethanol.

γ-Carbobenzoxy-L-α, γ-diaminobutyric Acid. — The copper complex was treated as above.

ε-Carbobenzoxy-L-lysine.—The copper complex of ε-carbobenzoxy-L-lysine was suspended in an aqueous EDTA solution and boiled until the violet color of the complex had almost disappeared. After the mixture had cooled, a white product was filtered off, washed thoroughly with water, and recrystallized from 50% acetic acid containing some EDTA in order to remove the trace of unreacted copper. The product was then recrystallized from the same solvent. For further purification, it was treated as above.

The results are summarized in Table I in comparison with those obtained by the hydrogen sulfide method.***

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** Clewat AM (technical grade, kindly supplied by the Teikoku Chem. Ind., Ltd.) or Dotite 2Na (analytical grade, purchased from Wako Pure Chemical Ind., Ltd.) was used. The grade of reagent had no influence on the results.

*** According to the private communication from S. Sakakibara, Institute for Protein Research, Osaka University, this method was also applied successfully in the case of ornithine.