SHORT COMMUNICATIONS

A Novel Deacylation of the γ-Oxoacyl Amino Acid and Peptide

By Fumio SAKIYAMA, Michiyo MORISHITA, Tsuneo SOWA and Kozo NARITA

Institute for Protein Research, Osaka University, Kita-ku, Osaka

(Received September 7, 1965)

Several attempts to effect the cleavage of a specified peptide bond by a chemical reagent have been reported, and some of them are widely utilized for the sequential analysis of protein.[1] For the tyrosyl and tryptophyl bond cleavage, the NBS[2] method has successfully been used. Recently Shaltiel and Patchornik[3] effected an histidyl bond cleavage by using the same principle.

The present aughors have exploited another principle to cleave the histidyl and the tryptophyl bonds. This principle is based, first, on the formation of the γ-oxo group which can be derived from the above residues by the proper reactions: namely, Bamberger's degradation[4] and the subsequent mild hydrolysis of the γ-benzamino group for the histidine residue and peroxide or ozone oxidation[5] for the tryptophan residue. Secondly, the elimination of the γ-oxoacyl group by tetrahydropyridazone formation with hydrazine leads to an exposure of the amino group, which is bonded with the carboxyl side of the amino acid residue in question.

N-Laevuloyl-, *N*-(3-benzoyl-propionyl)- and *N*, *N'*-dibenzoyl-γ-oxoornithyl-L-phenylalanine and *N*-(3-benzoylpropionyl)-L-leucylglycine have been prepared as model compounds for the reaction of the second step.[6] The deacylation of *N*-laevuloyl-L-phenylalanine was performed by the following procedure. *N*-Laevuloyl-L-phenylalanine dicyclohexylamine salt (m. p., 155—156°, 20 μmol.) was heated to a boil in 2 ml. of a 2.0 M acetate buffer (pH 3.6) containing hydrazine (100 μmol.). An aliquot was examined by paper chromatography,[7] and the amino acid released was determined by densitometry after Cd-ninhydrin colorization[8] (Fig. 1). The phenylalanine, quantitatively released after half an hour, was identified as both free amino acid and the DNP[9] derivative by paper and thin-layer chromatography.[10] The cyclization product obtained from the laevuloyl group and hydrazine was shown to be identical with 6-methyl-2, 3, 4, 5-tetrahydropyridaz-3-one[11] by paper and thin layer chromatography[12] and by infrared spectroscopy.

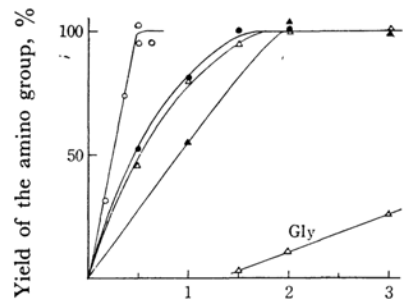

Fig. 1. The release of the amino group from the γ-oxoacyl amino acid and peptide.
○: *N*-laevuloyl-L-phenylalanine
●: *N*-(3-benzoylpropionyl)-L-phenylalanine
△: *N*-(3-benzoylpropionyl)-L-leucylglycine
▲: *N*-(*N*, *N'*-dibenzoyl-γ-oxoornithyl)-L-phenylalanine
Determination of the amino group was performed after paper chromatographic separation of the reaction mixture.

The rate of the release of the amino group depends mainly on the nature of the γ-oxoacyl group, as is shown in Fig. 1. The *N*, *N'*-dibenzoyl-γ-oxoorinthyl group, a descendant of the histidyl residue, was most resistant to cyclization under the present conditions. Prolonged heating caused an appreciable hydrolysis of the peptide bond.

Deacylation from the acetoacetyl derivative of amino acid and peptide can be accomplished by the reaction with phenylhydrazine.[13] A detailed description of this study will be presented elsewhere.

1) Cf. B. Witkop, *Adv. Protein Chem.*, **16**, 221 (1961).
2) NBS, *N*-Bromosuccinimde.
3) S. Shaltiel and A. Patchornik, *J. Am. Chem. Soc.*, **85**, 2799 (1963).
4) E. Bamberger and A. Berle, *Ann.*, **273**, 342 (1893).
5) B. Witkop, *Ann.*, **556**, 103 (1944); A. Previero, E. Scoffone, P. Pajetta and C. A. Benassi, *Gazz. chim. Ital.*, **93**, 841 (1963); A. Previero, M. A. Colletti and L. Galzigna, *Biochem. Biophys. Res. Comm.*, **16**, 195 (1964).
6) The details of the preparation of these new γ-oxoacyl derivatives will be presented later.
7) Solvent system: butan-1-ol-acetic acid-water, 4:1:1 by volume.
8) R. C. Canfield and C. B. Anfinsen, "The Proteins, Vol. I, Ed. by H. Neurath, Academic Press, New York. (1963), p. 318.
9) DNP: 2, 4-Dinitrophenyl. Solvent system, 2-methyl butan-2-ol saturated with 0.1 M phthalate buffer (pH 6.0).
10) Solvent system for phenylalanine, see Ref. 7.
11) L. Wolff, *Ann.*, **394**, 98 (1912).
12) The Ehrlich reagent was used for detection of the pyridazone derivatives after chromatography. Solvent system, see Ref. 7.
13) F. D'Angeli, F. Filia and E. Scoffone, *Tetrahedron Letters*, **1965**, 605.