ACTION OF N-BROMOSUCCINIMIDE ON THE ANTIBIOTIC POLYPEPTIDE A-128-OP

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In an investigation of the structure of natural polypeptides, the specific chemical cleavage of the peptide bonds at tryptophan residues with the aid of N-bromosuccinimide (N-BS) is used not infrequently [1]. The present paper gives the results of an investigation of peptides obtained by the action of N-BS on the acid of the antibiotic A-128-OP, which includes a number of amino acids of nonprotein nature: dehydrotryptophan (ΔTrp), β-methyl tryptophan (β-Me-Trp), cis- and trans-3-hydroxyprolines (c- and t-3-Hyp), β-hydroxyleucine (β-HyLe), and others [2]. The A-128-OP acid is formed in the mild alkaline hydrolysis of the cyclolactone bond of antibiotic A-128-OP and is an 11-membered peptide with cis-3-hydroxyproline at the C-end and aspartic acid at the N-end, the aspartic acid acylating the serine following it by its β-COOH group [2, 3].

Since there is no information in the literature on the action of N-BS on peptides containing dehydrotryptophan, we first investigated the possibility of oxidative cleavage on the following model peptides: N-Bz-Δ-Trp-Gly-OH, N-Bz-Δ-Trp-Pro-OH, N-Bz-Δ-Trp-Trp-OH [4].

With the use of a fivefold excess of N-BS in 70% acetic acid, the glycine and proline were split off to the extent of 35-40%; the third peptide only underwent modification without cleavage of the peptide bond.

The oxidative cleavage of the A-128-OP acid was performed with a tenfold excess of N-BS in 70% CH_3 · COOH at 20°C for 30 min. The mixture of peptides formed was separated by preparative electrophoresis in 1 N CH_3COOH, and the peptides were additionally purified by TLC on silica gel in the butan-1-ol – CH_3COOH – H_2O (4 : 1 : 5) system. For each of the peptides isolated we determined the qualitative and quantitative amino acid compositions, the N- and C-terminal amino acids, their electrophoretic mobilities at pH 2.4, 4.2, and 6.5, their UV spectra, and, in some cases, their NMR spectra. Ninhydrin-negative peptides were treated with o-phenylenediamine (pH 5.0, 100°C, 1 h) to split out derivatives of pyruvic acid [5]. On the basis of the analytical results given in the present paper and previously [3], we determined the amino acid sequence for the following peptides of those that were isolated:

Peptide (I): Δ-Abu → Thr → Ala → Gly-t-3-Hyp-NH_2 [Δ-Abu represents dehydroaminobutyric acid (β-methyldehydroalanine, see [6])].

Peptide (II): A-128-OP acid in which both tryptophan residues had been completely oxidized by the N-BS.

Peptide (III): Asp
|
→ Ser-NH_2.

Peptide (IV): Asp
|
→ Sep → Δ-Abu → Thr → Ala → Gly-t-3-Hyp-NH_2.

Peptide (V): βHyLe → c-3-Hyp-OH.

Peptide (VI): Δ-Trp → βMeTrp.

The amide groups in peptides (I), (III), and (IV) may appear as the result of the cleavage of the peptide bonds at the amino groups of dehydroamino acids [5]: dehydrotryptophan [peptides (I) and (IV)] and α,β-dehydroaminobutyric acid [peptide (III)]. Peptide (V) contains cis-3-hydroxyproline, which is the C-terminal amino acid in the A-128-OP acid [2] and, consequently, the amino group of the β-hydroxyleucine must be acylated by a β-methyltryptophan residue.

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Thus, the analysis of the peptides isolated from A-128-OP acids after the action of N-BS that has been performed gives grounds for assuming that the A-128-OP acid has the following amino-acid sequence: Asp |→ Ser → → Δ- Abu → Thr → Ala → Gly → t-3-Hyp → Δ- Trp → βMeTrp → βHyLe → c-3-Hyp-OH, which is somewhat refined as compared with that given previously [3]. Details of the experiment on the cleavage of the A-128-OP acid will be published later.

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POLYPHENOLS OF *Myricaria alopecuroides*

III. HYDROLYZABLE TANNING SUBSTANCES

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Continuing an investigation of the polyphenols of Myricaria alopecuroides Schrenk., family Tamaricaceae Lindl. [1], by acid hydrolysis of the combined ellagotannins (5% HCl, 100°C) followed by extraction with ether and ethyl acetate and chromatography of the ethyl acetate extract on Sephadex LH-20 we have obtained two cleavage acids.

Acid 1, $C_{14}H_{10}O_{10} \cdot 2H_2O$ (the results of elementary analysis correspond to the calculated figures), mp (decomp.) 277°C, R_f 0.65 in BAW (40:12.5:29; system 1) and 0.65 in 15% CH_3COOH (FN-3: system 2). It forms a deep blue coloration with 1% ferrous ammonium alum (FAA). The reaction for bound ellagic acid is negative [2]. With concentrated H_2SO_4 it gives a positive reaction for the formation of xanthone [3]. The IR spectrum shows the absorption bands of hydroxy groups, a carbonyl group, a benzene ring, and an ether bond (3300, 3000-2600, 1650, 1535, 1610, 1035 cm^{-1}).

The methylation of acid 1 with an ethereal solution of diazomethane gave a heptamethyl derivative, mp 113°C, $C_{21}H_{24}O_{10}$, mol. wt. 436 (mass spectrometrically). The IR spectrum has the absorption bands of methoxy groups, an ester bond, a benzene ring, and an ether bond (2930, 1730, 1600, 1100 cm^{-1}). The NMR spectrum has the signals of three aromatic protons in the form of a superposed singlet and doublet at δ 7.22 ppm and a doublet at 6.71 ppm (J = 2 Hz), and also the signals of the protons of seven methoxy groups at 3.84 ppm (12H) and 3.83 ppm (9H).

The hydrolysis of the heptamethyl derivative with 2 N NaOH gave a pentamethyl ether, mp 247°C. Titration of the pentamethyl ether showed the presence of two carboxy groups.

On the basis of results obtained, the acid 1 was identified as dehydrodigallic acid [4].

Acid 2, $C_{21}H_{10}O_5 \cdot 2H_2O$ (the result of elementary analysis corresponded to the calculated figures), mp (decomp.) 281-283°C, R_f 0.48 in system 1 and 0.60 in system 2. With a 1% solution of FAA it formed a deep blue coloration. The reaction for bound ellagic acid was negative [2] and with concentrated H_2SO_4 it gave a positive reaction for the formation of xanthone [3]. IR spectrum: 3400, 3000-2600, 1690, 1610, 1050 cm^{-1}.

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