ACTION OF N-BROMOSUCCINIMIDE ON SOME PEPTIDES CONTAINING DEHYDROTRYPTOPHAN

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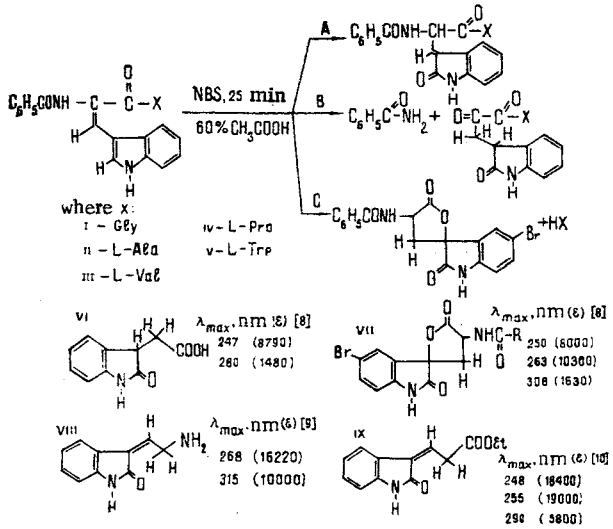
α, β-Dehydrotryptophan (Δ-Trp) is present in a number of polypeptide antibiotics of the telomycin group [1-3] and in products of the metabolism of Aspergillus amstelodami [4, 5]. Its presence is responsible for some peculiar physicochemical properties of these compounds and for difficulties encountered in investigations of their structure.

In order to determine the possibility of the chemical cleavage of peptides containing Δ-Trp, we have studied the action of N-bromosuccinimide (NBS) on a number of model dipeptides containing dehydrotryptophan (Scheme) synthesized in our laboratory [6, 7]. As is well known, NBS is used for the specific chemical cleavage of the peptide bond at the COOH group of tryptophan [8]. During the reaction various products of the oxidation of the pyrrole ring of tryptophan with characteristic absorption bands in the UV spectrum are formed (see VI and VII).



The completeness of the oxidation of the tryptophan residues was monitored from the decrease in the optical activity in the 278-285 nm region. However, under the action of NBS on the Δ-Trp peptides (I-IV) (60% CH_3COOH, 20°C, 25 min) we observed not a decrease but an increase in the absorption in this region of the spectrum with the appearance of two new absorption bands at 255-260 nm ($\varepsilon = 1.8\text{-}2.1 \cdot 10^4$) and 310-315 nm ($\varepsilon = 0.3\text{-}0.5 \cdot 10^4$), which did not change after the addition of 2.5-3.0 equivalents of NBS per mole of peptide (Fig. 1). At the same time, in the 255-268 nm region the tryptophan oxidation products (VI) and (VII) have values of ε 1.5-2.2 times greater, and in the 308-310 nm region compound (VII) has a value of ε 2-3 times smaller than for the oxidation products of the peptides (I-V).

By TLC on SiO_2 in a mixture of the products of the reaction of the peptides (I-IV) with NBS we detected several substances, including benzamide and small amounts of the corresponding C-terminal amino acids. In the peptide (V), containing a L-tryptophan residue, we detected benzamide but no cleavage of the peptide bond

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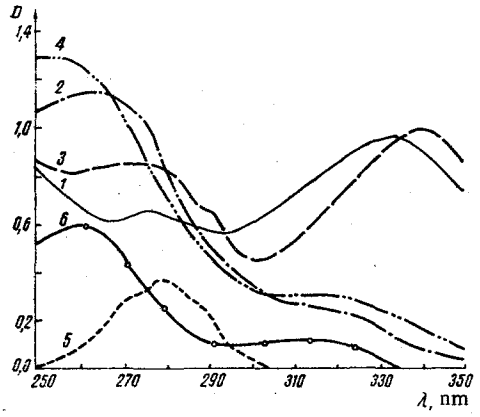

Fig. 1. UV spectra in 60% CH_3COOH (l = 1 cm, c $6 \cdot 10^{-5}$ M): 1) peptide (IV); 2) peptide (IV) + NBS; 3) peptide (V); 4) peptide (V) + NBS; 5) Cbs-Trp; 6) Cbz-Trp-NBS (the UV spectra of peptides (I-III) treated with NBS have a similar form).

between the amino acids was observed. The UV spectrum of peptide (V) also differed from the UV spectra of peptides (I-IV), primarily by the fact that the optical density in the 275-285 nm region fell somewhat as NBS was added. However, after the addition of 3.5-4.0 equivalents of NBS it has practically the same abosrption bands and also the same extinction coefficients at 254 nm ($\varepsilon = 2.3 \cdot 10^4$) and 315 nm ($\varepsilon = 0.54 \cdot 10^4$) as peptides (I-IV).

The appearance of benzamide as the result of the action of NBS on peptides (I-V) and of free amino acids in the action of NBS on peptides (I-V), and also the somewhat higher values of ε in the 250-268 nm and 310-315 nm regions, which are characteristic for isatin derivatives of types (VIII and IX) (see Scheme and Fig. 1) permit the assumption of three of a number of possible directions of the oxidation of Δ-Trp peptides with N-bromosuccinimide (see Scheme). Route A is predominating for peptides (I-IV); reaction C scarcely takes place in the case of peptide (V), and peptides (I-IV) undergo cleavage by route C to the extent of 8-18% with the formation of a compound of type (VIII) and a free C-terminal amino acid. The yield of benzamide (see reaction B) does not exceed 5-10% for all the peptides.

Thus, on working with Δ-Trp peptides it must be borne in mind that NBS causes cleavage of the peptide both at the COOH group of a Δ-Trp residue attached to aliphatic amino acids and also at the $NH-C_\alpha$ bond of the Δ-Trp residue with the liberation of the amide of the amino acid acylating the NH_2 group of the dehydrotryptophan, and also an increase in the value of ε in the 250-268 nm and 310-315 nm regions that is not characteristic for tryptophan peptides and which can be explained by the formation during the reaction of the corresponding alkylideneoxindoles of types (VIII) and (IX).

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