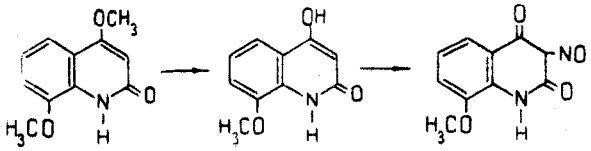
A direct comparison of the substances has shown that norrobustinine is identical with 4-hydroxy-8-methoxycarbostyryl [4]. A mixture of the nitroso derivatives of these substances gave no depression of the melting point. Thus, robustinine has the structure 4, 8-dimethoxycarbostyryl (I).



I II III

REFERENCES

1. I. M. Fakhrutdinova, G. P. Sidyakin, and S. Yu. Yunusov, KhPS [Chemistry of Natural Compounds], 107, 1965.

2. M. F. Grundon and N. J. McCorkindale, J. Chem. Soc., 2177, 1957; E. A. Clarke and M. F. Grundon, J. Chem. Soc., 806, 1964.

3. N. J. McCorkindale, Tetrah., **14**, 223, 1961.

4. B. Berinzaghi, A. Murazabal, R. Labriola, and V. Deulofeu, Jr. Org. Ch., **10**, 181, 1945.

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AMINOETHYLATED PEPSIN

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In the investigation of the primary structure of proteins, hydrolysis with trypsin is first used, this specifically breaking the peptide bonds formed by the carboxy groups of arginine or lysine. Where the content of these amino acids in the protein is low, it is desirable to modify its molecule in such a way that new points for the attack of trypsin appear in the peptide chain. When the thiol groups appearing on reduction of the disulfide bonds are blocked with ethyleneimine by Raftery and Cole's method [1], the cysteine residues are converted into S-(β-aminoethyl) cysteine residues. S-(β-Aminoethyl) cysteine is an isoster of lysine, since its sidechain differs from the side chain of lysine only by a sulfur atom in place of a CH_2 group. This similarity leads to the situation that the peptide bonds formed by the carboxy group of S-(β-aminoethyl) cysteine are hydrolyzed by trypsin, as well as those formed by lysine and arginine.

The molecule of porcine pepsin is characterized by a very low content of basic amino acids (out of 340 amino acid residues, there are only two arginine residues and one lysine residue). In order to increase the number of peptide bonds capable of hydrolysis by pepsin, we have carried out the aminoethylation of pepsin whose disulfide bonds had been preliminarily cleared by reduction with mercaptoethanol. 207 mg of porcine pepsin inactivated with phenol was dissolved in 15 ml of 8 M aqueous urea, with the addition of concentrated LiOH to pH 8.5. 0.42 ml of mercaptoethanol was added to the pepsin solution, and the pH was brought to 8.5 with 1 N LiOH. Argon was passed through the reaction mixture for 5 min, and the solution was left in a closed vessel in an atmosphere of argon at 37° C. After 4 hr, 3.1 ml of ethyleneimine was added to the mixture. Aminoethylation was carried out in an autotitrator for 30 min in a current of argon, the pH being maintained at 8.6 with a 4 M solution of urea in 3 N hydrochloric acid. After the end of the reaction, the reduced aminoethylated pepsin (aminoethylpepsin) was precipitated with a fivefold volume of cold ethanol–1 N hydrochloric acid (39:1), and the precipitate was separated off by centrifuging and was washed with cold alcohol, then it was suspended in 50 ml of water and dissolved at pH 10.3 by the addition of concentrated LiOH, and the aminoethylpepsin was precipitated by the addition of 6 N hydrochloric acid to pH 5.5. The mixture was left at 4° C for 16 hr, after which it was centrifuged, and the precipitate was washed with water, suspended in 30 ml of water, and lyophilized. The yield of aminoethylpepsin was 179 mg. By means of an amino acid analyzer, 1 mole of aminoethylpepsin was found to contain 5.5–5.9 moles of S-(β-aminoethyl)cysteine (taking into account the degree of destruction of this amino acid on alkaline hydrolysis).

The aminoethylpepsin was subjected to triptic hydrolysis at pH 10. A determination of the N-terminal amino acids by dinitrophenylation showed that during the trypsin decomposition a considerable rise in the number of N-terminal amino acids took place (in comparison with the hydrolysis of carboxymethylpepsin [2]). The fractionation of the triptic hydrolyzate of aminoethylpepsin on Sephadex G-50 (4 M urea in triethylammonium carbonate buffer; pH 10) showed a marked decrease in the size of the peak corresponding to the fraction with the high-molecular-weight fragments and an increase in the low-molecular-weight fractions (as compared with the hydrolyzate of carboxymethylpepsin). Trypsin can be used

to decompose aminoethylpepsin into smaller peptides than those that are formed by the hydrolysis of carboxymethylpepsin.

REFERENCES

1. M. A. Raftery and R. D. Cole, Biochim., Biophys. Res. Com., **10**, 467, 1963.
2. V. M. Stepanov and R. A. Matveeva, KhPS [Chemistry of Natural Compounds], 128, 1965.

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