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PHOSPHORUS CONTENT OF HUMAN PEPSIN AND GASTRICSIN

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We have carried out the determination of phosphorus (after mineralization) of samples of human pepsin and gastricsin purified on anion-exchange cellulose [2] by Filipowicz's method [1]. The purity of the samples of pepsin and gastricsin was shown by the results of a determination of the N-terminal amino acids. In the preparation of human pepsin, the only N-terminal amino acid was valine, and in the sample of gastricsin it was serine. To calculate the number of phosphorus atoms in the gastricsin molecule we used Tang and Tang's results [3] on the molecular weight of gastricsin (36 000). It was found that the gastricsin molecule contains one atom of phosphorus while human pepsin contains no phosphorus. At the same time, these enzymes possess similar catalytic activity [2, 4].

Consequently, the phosphate residue is not essential for the activity of the enzymes of the pepsin group [5-7].

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SYNTHESIS OF PEPTIDES ON A RESIN BY THE MIXED ANHYDRIDE METHOD

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At the present time we have shown that to create a peptide bond in the solid-phase method of peptide synthesis it is possible to use the mixed anhydride method with the readily accessible alkyl esters of chlorocarbonic acid [2].

As a model synthesis we used the tripeptide H-Gly-L-Phe-L-Ala-OH (I) [3].

The addition of Cbzo-L-Ala to the chloromethylated resin, the splitting off of the Cbzo protective group by 10% hydrochloric acid in glacial acetic acid, the production of the free amino group, washing, and the determination of the amount of L-Ala added to the resin were carried out by the general method [1]. Thus, 1.02 g of Cbzo-L-Ala was added to 7 g of resin.

To build up the peptide chain further, 4.78 g of Cbzo-L-Phe in 60 ml of methylene chloride was treated at −10° C with 1.81 g of triethylamine, then 5 ml of isobutyl chlorocarbonate was added and the mixture was stirred for 10 min at −10° C. The resulting solution of the mixed anhydride was added to the carefully dried resin bearing the L-alanine in a reaction vessel [4]. The reaction mixture was kept at −10° C for another 30 min and was then left overnight at room temperature.

The subsequent splitting off of the Cbzo protective group by means of 10% hydrochloric acid in glacial acetic acid and neutralization of the dipeptide hydrochloride was carried out in the usual way [1], after which the resin present in the reaction vessel was again dried at the water and oil pumps for 1 hr. In the subsequent condensation of Cbzo-Gly (3.2 g) with the dipeptide attached to the resin, operations similar to those described above for the synthesis of the dipeptide were carried out. To detach the tripeptide from the resin, the latter was twice treated with 3 ml of 2 N caustic soda in 60 ml of ethanol for 30 min. The resulting alkaline solution was made weakly acidic with 20% citric acid. The precipitate of sodium citrate which separated was filtered off and the solution was evaporated in vacuum to dryness. The residue was purified by reprecipitation from methanol with ethyl acetate, and the precipitate was filtered off, washed with alcohol, and dried in vacuum over phosphorus pentoxide.

This gave 1.5 g of chromatographically pure Cbzo-Gly-L-Phe-L-Ala-OH (72%, calculated on the first amino acid added to the resin); mp 86° C, $[\alpha]_D^{20}$ −7.5° (c.2; water).

After removal of the Cbzo protective group (CF_3COOH, 3 min) [5], the trifluoroacetate salt of the peptide (I) obtained was purified by reprecipitation from ethanol with ether. Weight 1.08 g (83%); $[\alpha]_D^{20}$ −22.1° (c 2; water).

The tripeptide (I) was obtained by treating the trifluoroacetate with the calculated amount of triethylamine and subsequent reprecipitation from water with ethanol; it had mp 104° C, $[\alpha]_D^{20}$ −5.5° (c 2; 1 N HCl), which agrees satisfactorily with literature data [3].

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