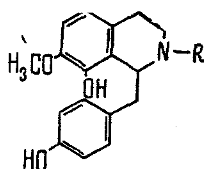

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SIMPLIFICATION OF THE METHOD OF ACTIVATING POLYSACCHARIDE SUPPORTS WITH CYANOGEN BROMIDE

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UDC 543.544

The method of activating polysaccharide supports by treatment with cyanogen bromide in an alkaline medium in order to obtain proteins or ligands for the biospecific (affinity) isolation of enzymes has come into wide use in laboratory and industrial practice [1, 2]. When using this method under laboratory conditions, the greatest difficulties arise in the preparation of crystalline cyanogen bromide, since the difficultly accessible cyanides of alkali metals are necessary [3, 4].

We have found that the difficulties connected with the use of cyanides and the preparation of crystalline cyanogen bromide can be circumvented if, in the first place, instead of cyanides the more accessible alkali metal thiocyanates are used [5]. The bromination of thiocyanates corresponds to the following equation:

$$SCN^- + 4Br_2 + 4H_2O \rightarrow CNBr + SO_4^{-2} + 8H^+ + 7Br^-$$

and the yields of cyanogen bromide determined by the iodometric titration of the solution formed as the result of the reaction amount to ~50%. In the second place, the distillation of the cyanogen bromide and the subsequent manipulations with it (storage, taking weighed samples, dissolution) can be excluded, since the solution of cyanogen bromide formed in its production can be used for activation. Since this solution still contains a considerable amount of acid in addition to cyanogen bromide, the activation process takes place in this case with a high consumption of alkali. Thus, a solution of cyanogen bromide obtained by the following method has been used to activate 30 ml of Sepharose gel swollen in water.

With cooling and stirring, a solution of 2 g of potassium thiocyanate [ch.d.a. ("pure for analysis") grade] in 15 ml of water was added dropwise to a mixture of 4 ml of bromine and 1 ml of water. The resulting solution of cyanogen bromide (~10 mM) with a pale yellow color and a slight deposit of salts, was combined with a suspension of Sepharose (30 ml of gel in 70 ml of water), and with cooling and stirring the mixture was brought to pH 10-11 with a 10 M solution of NaOH and was kept under these conditions for 15-20 min by the addition of a 4 M solution of NaOH. Then the suspension of activated gel was filtered off on

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a glass filter, washed with a 0.2 M solution of $NaHCO_3$, and used for the addition of the ligand.

To Sepharose 4B activated by this method we added peptide proteinase inhibitors (from the results of an amino acid analysis the amount of ligand was 1-5 μmole per ml of gel), and on Sepharose 6B we immobilized the soybean trypsin inhibitor in an amount of 1-2 mg of active (4-5 mg of total) protein, and heparin in an amount of 4-5 mg of heparin per 1 ml of gel.

The availability of the reagents and the simplicity in use of the proposed variant of the activation of polysaccharide supports makes it convenient for medical-biological laboratories and also, apparently, for student practical work.

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A NEW AMINO ACID FROM THE ANTIBIOTIC VANCOMYCIN

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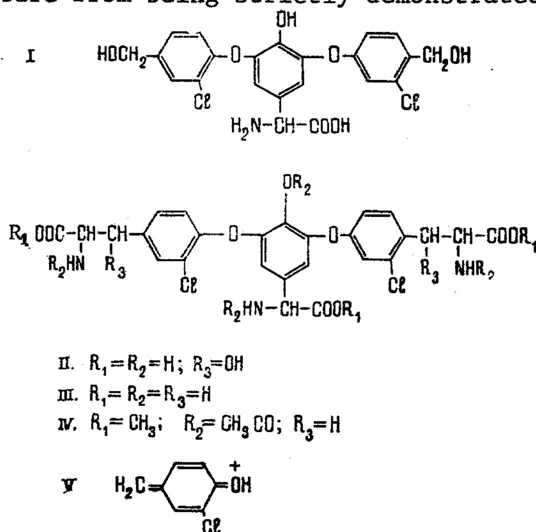
Williams et al. [1] have previously detected in a reductive alkaline hydrolysate of the glycopeptide antibiotic vancomycin a new monoamino triphenoxy monocarboxylic amino acid (I). On the basis of an analysis of the products of alkaline and oxidative cleavage of vancomycin, NMR spectroscopy, and x-ray structural analysis of the antibiotic, Williams et al. [2-4] came to the conclusion that the aglycone of vancomycin contained, in addition to N-methylleucine and actinoidinic and aspartic acids, a residue of a hypothetic trinuclear amino acid (II) which was later called vancomycinic acid [5]. However, the acid (II) was not isolated in the free state because of decomposition under the conditions of hydrolysis, and this prevented its structure from being strictly demonstrated.



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