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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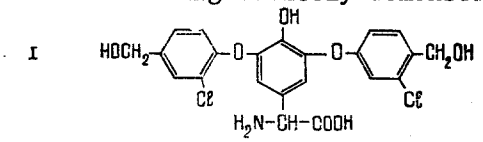
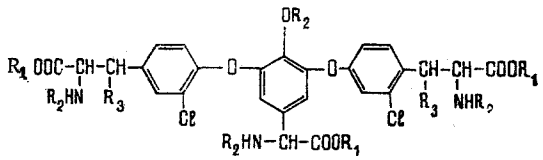
Williams et al. [1] have previously detected in a reductive alkaline hydrolysate of the glycopeptide antibiotic vancomycin a new monoamino triphenoxy monocarboxylic amino acid (I). On the basis of an analysis of the products of alkaline and oxidative cleavage of vancomycin, NMR spectroscopy, and x-ray 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.

I



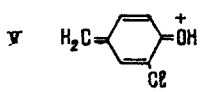


II. $R_1=R_2=H$; $R_3=OH$

III. $R_1=R_2=R_3=H$

IV. $R_1=CH_3$; $R_2=CH_3CO$; $R_3=H$

V



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To investigate the structure of the amino acid (II) we have performed the reductive HI/P hydrolysis of vancomycin by a method developed by ourselves previously [6], in the course of which the β-hydroxy group of the serine residues of amino acid (II) should be replaced by hydrogen. By TLC and ion-exchange chromatography, from the HI/P hydrolysate of vancomycin we isolated a new amino acid — m,m'-di[p-(2-amino-2-carboxyethyl)-m-chlorophenoxy]-p-hydroxyphenylglycine (III), i.e., dideoxyvancomycinic acid. The main properties of amino acid (III) are given below.

UV spectrum, $\lambda_{max}^{70\% C_2H_5OH}$: 233, 276, 284 nm; λ_{min} 261 nm.

Number of free NH_2 groups, 3; number of free COOH groups, 3.

Electrophoretic mobility in relation to ε-DNP-lysine 1.2 in the electrolyte HCOOH—CH_3COOH—H_2O (28:20:52).

TLC, Silufol UV_{254}, R_f 0.14 in the n-butanol—CH_3COOH—H_2O (4:1:5) system; TLC in microcrystalline cellulose, R_f 0.43 in the n-butanol—pyridine—CH_3COOH—H_2O (45:9:36:30) system; R_f 0.68 in the tert-amyl alcohol—CH_3COOH—H_2O (2:1:1) system.

IR spectrum of derivative (IV) of amino acid (III), ν_{max}^{film}: 3375 m (ArOH), 2930 s, 2855 m, 760 s, 720 s (—CH_2—), 2960 w, 1422 s (—CH_3), 1750 s(C=O), 1730 m, 1715 w, 1226 w, 1130 m (ArO—Ar), 1666 s, 1555 w (—CONH—), 1380 s (—CH—), 1246 m, 1050 s, 1020 m (C—O—C), 1226 m, 1615 s, 1580 s, 1170 m (Ar).

Mass spectrum of derivative (IV) of amino acid (III), m/e (% of the maximum peak):

806(1), 804(3), 802(4) M+, 775(2), 773(5), 771(8), 765(1), 763(4), 761(6), 747(4), 745(9), 743(10), 733(7), 731(26), 729(39), 722(3), 720(13), 718(18), 706(3), 704(10), 702(13), 697(7), 695(7), 687(8), 686(5), 674(5), 672(6), 670(11), 664(2), 662(8), 660(12), 604(3), 602(7), 600(10), 574(7), 558(6), 531(7), 472(9), 470(9), 331(9), 303(10), 271(13), 239(15), 214(38), 212(83), 143(44), 141(100).

The UV spectra were taken on a Unicam SP-800 instrumented (United Kingdom), the IR spectra on a UR-20 instrument (GDR), and the mass spectra on a LKB-9000 instrument (Sweden) at 70 eV and 50-190°C. The numbers of NH_2 and COOH groups were determined by the method of partial substitution [7-9].

In the mass-spectrometric analysis of derivative (IV), three peaks of molecular ions were observed with m/e 802, 804, and 806, which coincided completely with the calculated molecular weights of derivative (IV) of amino acid (III) containing two chlorine atoms. The subsequent fragmentation of the molecular ion took place with the stepwise elimination of CH_3OH, $CH_2{=}C{=}O$, and $-COOCH_3$ molecules and by the subsequent cleavage of the molecule at the phenoxy bond, and it corresponded completely to formula (IV). The strongest ion proved to be that with m/e 141, to which fragment (V) with one chlorine atom corresponds. The structure of the amino acid (IV) was confirmed by the features of the IR spectra.

On comparing the analytical results obtained for amino acid (III) and its derivative (IV) with those on the structure of fragment (I) [1] of this amino acid, we came to the conclusion that we are the first to have obtained from the antibiotic vancomycin the dichloro triamino triphenoxy tricarboxylic amino acid (III), which is a product of the reductive dehydroxylation of amino acid (II) forming a component part of the native antibiotic.

Thus, the new amino acid (III) is the dichloro analog of amino acid "E" which we have obtained previously from ristomycin A [6].

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AN INVESTIGATION OF COTTON-SEED GLOBULINS.

XXII. HEMAGGLUTINATING ACTIVITY OF THE 7S GLOBULIN

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We have previously reported the structure of the 7S globulin from cotton seeds [1]. Since many plant proteins are hemagglutinins, i.e., they are capable of agglutinating erythrocytes [2, 3], we have attempted to detect such activity in the 7S globulin.

For the agglutination reaction we used a suspension of rabbit erythrocytes and the soybean agglutinin obtained by a method described by Lis and Sharon [4], and also an extract of clover [5]. The active protein mentioned and the 7S globulin under investigation and its subunits were dissolved in a 0.9% solution of sodium chloride with an initial concentration of 4-5 mg/ml followed by twofold dilution to a concentration of 0.005 mg/ml. To 1 ml of solution was added 1 ml of a suspension of erythrocytes with $D_{620} = 1$; then the mixture was shaken and it was then kept at 37°C, with shaking after 0.5 and 1.5 h, and was left at 4-7°C for 16-18 h. The occurrence of agglutination was estimated visually [6]. A precipitate of erythrocytes in the form of a button or ring showed the absence of an agglutination reaction, while uniform distribution at the bottom of the tube showed a positive result. We found that the active proteins mentioned above and the 7S globulin agglutinate erythrocytes in concentrations not lower than 0.03 mg/ml, while both subunits do so in concentrations of not less than 0.005 mg/ml. Treatment of the erythrocytes with trypsin did not affect the agglutinating activity of the 7S globulin and its subunits.

On the basis of the results obtained, it has been shown that the 7S globulin of the cotton seeds is a phytohemagglutinin.

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