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THE STRUCTURE OF MINOR COMPONENTS OF VIRGINIAMYCIN S.

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During the purification of virginiamycin S* three additional minor components were observed on thin-layer chromatography on silicagel in the system chloroform-methanol, 95:5, viz. S_1 (Rf 0.6), S_2 (Rf 0.35), S_3 (Rf 0.5) besides virginiamycin S (Rf 0.8). These minor components were not present during the original isolation of virginiamycin S (1). Their production probably is due to the use of mutant strains of Streptomyces virginiae or to modifications of the fermentation medium.

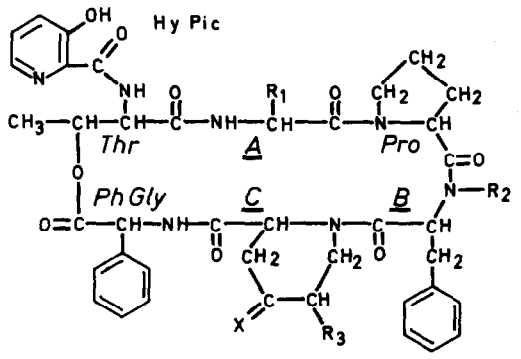
The different components of virginiamycin S were isolated by column chromatography on silicagel with a mixture of chloroform and methanol. Final purification of virginiamycin S_2 and S_3 was achieved by preparative thin-layer chromatography in the same solvent mixture.

Virginiamycin S_1 is a white crystalline product, m.p. 241-242°, $[\alpha]_D^{23}$ - 34.5 (EtOH) having a molecular formula $C_{42}H_{47}N_7O_{10}$, in agreement with the elemental analysis and the molecular ion, m/e 809, observed in the mass spectrum. The U.V. spectrum was almost identical with that of virginiamycin S, which proves that the same chromophore, viz. 3-hydroxypicolinic acid, was present (2). Hydrolysis of this product in 6N hydrochloric acid and paper-chromatography in the system n-BuOH-HOAc-H_2O, 4:1:5, indicated the presence of the amino acids threonine, 4-oxopipecolic acid, alanine, proline, phenylglycine and N-methylphenylalanine. By treatment of a paperchromatogram with D-amino acid oxidase, it also could be shown that alanine had the D-configuration. The interpretation of the mass spectrum further confirmed that α-aminobutyric acid (A in formula I) of virginiamycin S was replaced by alanine in the S_1 component. The structure of virginiamycin S_1 is I $R_1 = R_2 = CH_3$, $R_3 = H$, X = O.

Chromatographic examination of the hydrolysate of virginiamycin S_2 indicated the presence of threonine, proline, α-aminobutyric acid, phenylglycine, phenylalanine and 4-hydroxypipecolic acid. The latter amino acid could be identified as allo-4-hydroxypipecolic acid (OH-COOH cis) by chromatographic comparison with an authentic sample in the system tert-amylalcohol-2,4-lutidine-water, 178:178:114 (3). Further evidence for structure I, $R_1 = C_2H_5$, $R_2 = R_3 = H$, X = HOH, was obtained from the fragment ions observed in the mass spectrum of virginiamycin S_2.

In the hydrolysate of virginiamycin S_3, all amino acids present in virginiamycin S could be

* Staphylomycin® is the trade name of s.a. R.I.T., Genval, Belgium, for the antibiotic virginiamycin.



detected except 4-oxopipecolic acid. In a two-dimensional paperchromatogram in the systems n-butanol-acetic acid-water, 4:1:5 and phenol-water, four additional weak spots, one of which had a yellow colour, were observed after spraying with ninhydrin reagent. The mass spectrum of virginiamycin S_3 indicated that the molecule contained an additional hydroxy group, which was located in the 4-oxopipecolic acid fragment. It has been shown recently that a closely related peptide antibiotic, ostreogrycin B_3, contained 5-hydroxy-4-oxopipecolic acid (4). It is probable that the yellow spot on the chromatogram is due to this amino acid, and that the other additional spots are due to decomposition products of this amino acid. The structure I, $R_1 = C_2H_5$, $R_2 = CH_3$, $R_3 = OH$, $X = O$ is proposed for virginiamycin S_3.

The foregoing data show that virginiamycin S_1 is identical with ostreogrycin B_1 (5,6), vernamycin B γ (7), and pristinamycin IC (8), and that virginiamycin S_3 is identical with ostreogrycin B_3 (4) except for the replacement of N-methylphenylalanine by p-dimethylamino-N-methylphenylalanine. Virginiamycin S_2 does not correspond to ostreogrycin B_2 (6), vernamycin B β (7) and pristinamycin IB (8), because the last-mentioned products contain p-methylamino-N-methylphenylalanine and 4-oxopipecolic acid.

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References

1. H.Vanderhaeghe, P.Van Dijck, G.Parmentier and P.De Somer, Antibiotics & Chemotherapy, 7; 606 (1957).
2. H.Vanderhaeghe and G.Parmentier, J. Am. Chem. Soc., 82, 4414 (1960).
3. G.Parmentier and H.Vanderhaeghe, J. Chromatogr., 4, 228 (1960).
4. B.R.Cox, F.W.Eastwood, B.K.Snell and Lord Todd, Chem. Commun., 1623 (1970).
5. F.W.Eastwood, B.K.Snell and Sir Alexander Todd, J. Chem. Soc., 2286 (1960).
6. Lord Todd, Ind. Chim. Belg., 12, 1433 (1962).
7. M.Bodanszky and M.A.Ondetti, Antimicrobial Agents and Chemotherapy, p. 360 (1963).
8. J.Preud'homme, P.Tarridec and A.Belloc, Bull. Soc. Chim., 585 (1968).