NOTES

THE ABSOLUTE CONFIGURATION OF CYTOVARICIN: ISOLATION OF METHYL β-D-CYMAROSIDE BY METHANOLYSIS

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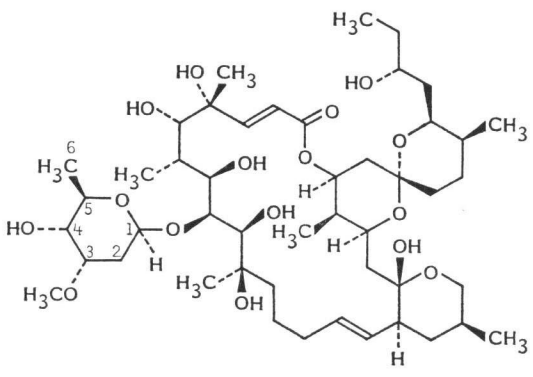
We have reported the isolation[1] and the single-crystal X-ray analysis[2] of a novel neutral macrolide antibiotic cytovaricin, from the culture of a *Streptomyces* sp. In this paper we report the isolation of methyl-β-D-cymaroside as a methanolysis product of cytovaricin, which confirms the absolute configuration of cytovaricin as that shown in Fig. 1.

Cytovaricin (200 mg) was dissolved in 0.4 ml of 0.25% methanolic hydrogen chloride and left overnight at room temperature. Four products (one major and three minor) were revealed by spraying the TLC chromatogram of the methanolysis product with anisaldehyde-sulfuric acid. The reaction mixture was neutralized with dilute ammonium hydroxide and concentrated *in vacuo* to dryness. The residue was chromatographed on a silica gel column with the solvent system, ethyl acetate - benzene (1: 1). The fraction containing the major product was further purified by preparative HPLC [Nucleosil $5C_8$, methanol - water (23: 77)]. The pure product (12 mg) was obtained by distribution between ether and saturated aqueous sodium chloride.

Fig. 1. The absolute structure of cytovaricin.



^{1}H NMR data (400 MHz) with assignment are as follows. δ ($CDCl_3$) 1.32 (3 H, d, $J_{5,6}$=6.2 Hz, H-6), 1.56 (1 H, ddd, $J_{2a,2e}$=14.2 Hz, $J_{2a,1}$= 9.5 Hz, $J_{2a,3}$=2.7 Hz, H-2a), 2.26 (1 H, ddd, $J_{2e,2a}$=14.2 Hz, $J_{2e,3}$=3.7 Hz, $J_{1,2e}$=2.2 Hz, H-2e), 3.23 (1 H, dd, $J_{4,5}$=9.5 Hz, $J_{4,3}$=3.7 Hz, H-4), 3.43 (3 H, s, 1-OCH_3 or 3-OCH_3), 3.48 (3 H, s, 1-OCH_3 or 3-OCH_3), 3.59 ~ 3.64 (2 H, m, H-3 and H-5), 4.57 (1 H, dd, $J_{1,2a}$=9.5 Hz, $J_{1,2e}$= 2.2 Hz, H-1). The data are in agreement with those of methyl 2,6-dideoxy-3-*O*-methyl-β-*ribo*-hexopyranoside (methyl β-cymaroside). In addition side-by-side comparison with the synthetic sample by TLC and HPLC showed that both were identical. Finally, the observed optical rotation was $[\alpha]_D^{20}$ −8.1° (*c* 1.1, methanol), which is in good agreement with that of synthetic methyl β-D-cymaroside [$[\alpha]_D^{20}$ −8.9° (*c* 0.68, methanol)] measured in our hands*.

The above data show that the sugar moiety of cytovaricin is D-cymarose, thus establish the absolute configuration of cytovaricin as shown in Fig. 1.

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References

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* Optical rotation of methyl β-D-cymaroside was reported to be $[\alpha]_D^{20}$ +7° (*c* 1, methanol)[3], which should be corrected.