STRUCTURAL FEATURE OF ANTIBIOTIC A-396-I

Sir:

We have previously reported[1] the isolation of a new water-soluble basic antibiotic A-396-I from *Streptoverticillium eurocidicus* A-396-I, which also produces in nearly equal amounts hygromycin B. The molecular formula $C_{19\pm1}H_{35\pm2}O_{13}N_3$ and the IR spectrum indicated close similarity of this antibiotic to destomycin A[2,3,4] and hygromycin B[5,6].

The antibiotic A-396-I (100 mg) was hydrolyzed with 4 N HCl at 105°C for 10 hours. The hydrolysate was passed through an XE-64 (Na^+) column and eluted with 14 % ammonium hydroxide. The eluate, exhibiting positive ninhydrin reaction, was concentrated to dryness to give colorless crystals (16 mg). By paper chromatographic examination [solvent 1: *n*-propanol - pyridine - acetic acid - water (15 : 10 : 3 : 12); solvent 2: *n*-butanol - acetic acid - water (4 : 1 : 2) the compound showed somewhat solwer mobility and a different ninhydrin color in comparison with a sample of (+) N-methyl-2-deoxystreptamine {$[\alpha]_D^{23}$ +38.6°±0.9° (*c* 0.889, H_2O), prepared from a hydrolysate of hygromycin B}. However, quite similar mobility and color to those of an authentic specimen of 2-deoxystreptamine (Rfs 0.40 and 0.20 in the solvents 1 and 2) was observed. Confirmation of the identity of this compound with 2-deoxystreptamine was made by direct comparison of the NMR spectra and GLC using the authentic specimen. Samples were trimethylsilylated with 25 % bis-(trimethylsilyl)-acetamide in pyridine by heating at 75°C for 20 minutes, and analyzed by a Perkin-Elmer Gas Chromatograph Model 881, on a 6 ft glass column packed with 3.0 % SE-30 coated Chromosorb P, carrier gas: N_2, temperature programming: 120~250°C (4°C/minutes). Both samples gave a peak with retention time of 15.0 minutes.

A ninhydrin-negative effluent fraction from the chromatography on the resin was treated with 0.1 N sodium hydroxide for 20 hours at room temperature and gave a ninhydrin-positive tests. This was thought to mean a conversion of destomic acid lactam, contained in this fraction, to its acid form[3]. The material was then adsorbed on a Dowex 50 (NH_4^+) column and eluted with 4 % ammonium hydroxide. Lyophilization of the eluate gave a colorless powder (12 mg). Comparison of the compound with a sample of destomic acid (prepared from a hydrolysate of hygromycin B) by paper chromatography and an amino acid analysis indicated that the compound was in fact destomic acid. Rfs 0.35 and 0.16 were obtained in the paper chromatograms with solvents 1 and 2, respectively. Amino acid analysis was carried out by an analyzer JLC-3BC, using Aminex resin column (0.9×75 cm), 0.2 M sodium citrate buffer pH 4.25, flow rate 0.79 ml/minute and temperature 50°C. A single peak of retention time of 230 minutes was obtained using a mixture of the tested and reference samples.

Another portion of A-396-I (20 mg) was hydrolyzed with 0.5 N H_2SO_4 at 100°C for 2 hours. The hydrolysate was successively passed through a Dowex 50 (H^+) and then Dowex I (OH^-) columns. Lyophilization of the effluent gave a colorless powder (1.5 mg). Paper chromatography of the compound and of an authentic sample of D-talose gave the same Rf values [ammoniacal silver nitrate (Rfs 0.60 and 0.30 in the solvents 1 and 2)]. GLC carried out under the same condition as above gave a major peak with a retention time of 12.2 minutes. Thus, the presence of talose in the hydrolysate of A-396-I was proved.

The NMR spectra of A-396-I and hygromycin B hydrochlorides measured in D_2O (Figs. 1 and 2) are quite similar except that a signal (-NCH_3, 2.81 ppm, 3H) given in hydgromycin B, was not observed in A-396-I; an anomeric proton (5.29 ppm, J=3 cps) was observed in the spectra of the both antibiotics.

A-396-I was peracetylated with acetic anhydride in pyridine and subjected to mass spectrometry, and the spectrum compared with that of peracetylated hygromycin B. In the spectrum of peracetylated A-396-I, molecular ion peak was not observed. However, many peaks corresponding to the fragment ion peaks in the peracetylated

Fig. 1. NMR spectrum of A-396-I hydrochloride (D_2O)

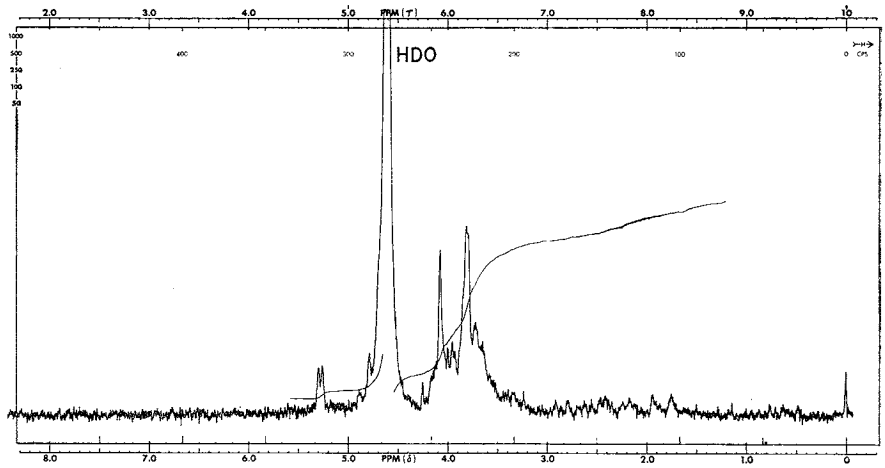
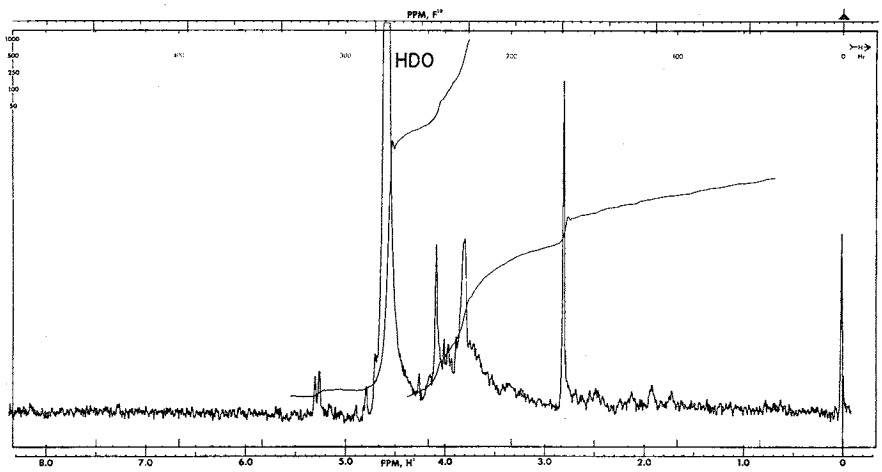


Fig. 2. NMR spectrum of hygromycin B hydrochloride (D_2O)



hygromycin B with difference of mass number 14 were regularly observed; 916 (930)* ($M-CH_3CO_2$), 915 (929) ($M-CH_3CO_2H$), 856 (870) ($M-CH_3CO_2H-CH_3CO_2$), 855 (869) ($M-2CH_3CO_2H$), 813 (827) ($M-CH_3CO_2H-CH_3 \cdot CO_2-CH_3CO$), 796 (810) ($M-2CH_3CO_2H-CH_3 \cdot CO_2$), 771 (785) ($M-2CH_3CO_2-2CH_3CO$), 736 (750) ($M-3CH_3CO_2H-CH_3CO_2$), 711 (725) ($M-CH_3CO_2H-2CH_3CO_2-2CH_3CO$), 669 (683) ($M-3CH_3CO_2-3CH_3CO$), 651 (665) ($M-2CH_3CO_2H-2CH_3CO_2-2CH_3CO$), 609 (623) ($M-CH_3CO_2H-3CH_3CO_2-3CH_3CO$). A few common peaks in both spectra were also observed in the lower mass number region: 586 ($\underline{B}-CH_3 \cdot CO_2H$), 544 ($\underline{A}-2CH_3CO_2$), 543 ($\underline{A}-CH_3CO_2H-CH_3CO_2$), 526 ($\underline{B}-2CH_3CO_2H$), 483 ($\underline{A}-2CH_3 \cdot CO_2H-CH_3CO_2$), 358 ($[\underline{C}+O, H]-CH_3CO_2H$), 298 ($[\underline{C}+O, H]-2CH_3CO_2H$). These tentative assignments (described in parentheses) are considered to be reasonable, if the assumed structure of peracetylated A-396-I is as shown in Fig. 3. Thus, the sequence of the three moieties of A-396-I is suggested to be the same one as in hygromycin B.

The partial structure of hygromycin B has been elucidated by WILEY *et al*[6]. Elucidation of the structure of destomycin A has been completed by KONDO *et al*[4]. More recently NEUSS *et al.*[7] have shown that the structural difference between both antibiotics consists in the presence in hygromycin B of (+)N-methyl-2-deoxystreptamine in the

* Peaks observed with peracetylated hygromycin B.

locus of (−)N-methyl-2-deoxystreptamine in destomycin A. From the above data, the structure of A-396-I is strongly suggested in relation to the established structures of the related antibiotics, except for its stereochemistry.

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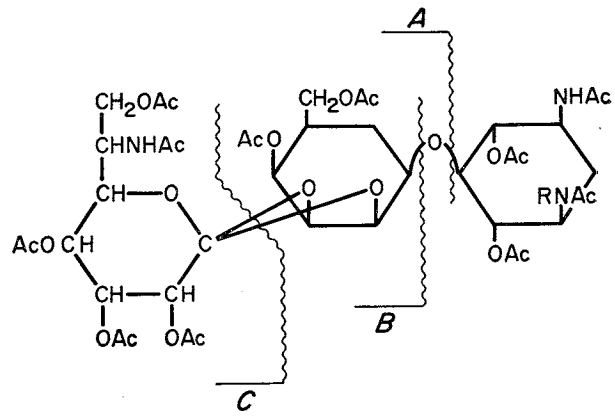
Fig. 3



Peracetylated A-396-I, R=H (M^+=975)
Peracetylated hygromycin B, R=CH_3 (M^+=989)

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