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THE STRUCTURE OF A NEW NUCLEOSIDE ANTIBIOTIC, CAPURAMYCIN

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Summary: The structure of capuramycin has been determined to be an uracil nucleoside with a caprolactam substituent as shown in Fig. 5 by NMR spectral analysis, chemical degradation and X-ray analysis.

During the course of our screening program for new antibiotics, it was found that Streptomyces griseus 446-S3 produced a new nucleoside antibiotic which we have named capuramycin (I). This antibiotic was isolated from the fermentation broth by adsorption on HP-20 followed by silica gel ($CHCl_3$:MeOH = 50:1) and Toyopearl HW-40F (MeOH) column chromatography. It is active against Streptococcus pneumoniae and Mycobacterium smegmatis ATCC 607[1]. In this note we report the structural elucidation of I based on ^{13}C- and ^{1}H-NMR spectral analysis, chemical degradation and X-ray analysis.

The physicochemical properties of I are as follows; white amorphous powder, $C_{23}H_{31}O_{12}N_5$, SI-MS; (m/z) 570 $(M+H)^+$, 592 $(M+Na)^+$, 608 $(M+K)^+$, Anal. found, C; 48.59, H; 5.79, O; 33.27, N; 12.37 %, Calcd., C; 48.50, H; 5.49, O; 33.71, N; 12.30 %, mp. 173-176°C, $[\alpha]_D^{25}$ +99° (c 0.5, H_2O), pKa' 9.1, UV λmax (MeOH) 214nm (ε 16200) and 257 (sh, 9800). The IR spectrum of I (KBr) showed the presence of -OH, -NH and amide functions (3400, 1680, 1515 cm^{-1}) and the absence of ester or carboxylic acid residues. I was positive to potassium permanganate and Molish, but negative to ninhydrin, anthrone, $FeCl_3$ and Sakaguchi reactions. Complete acid hydrolysis of I (6N HCl, 100°C, 16hrs) gave L-lysine and uracil.

The ^{13}C-NMR spectrum[2] of I taken in CD_3OD revealed the following functional groups; 4 X CH_2- (δ_C 29.0-42.5), 1 x N-CH- (53.4), 1 x OCH_3 (58.7), 6 x OCH- (63.5-83.5), 1 x N-CH-O (90.4), 1 x O-CH-O (101.3), 3 x -CH= (102.9-141.9), 1 x =C- (144.3), and 5 x N-C=O (152.5-176.3).

A detailed analysis of the COSY and ^{13}C-^{1}H COSY NMR spectra of I taken in CD_3OD showed the partial structures a, b, and c in Fig. 1. The relationships between protons and carbons not indicated by these methods were established by

Fig. 1.

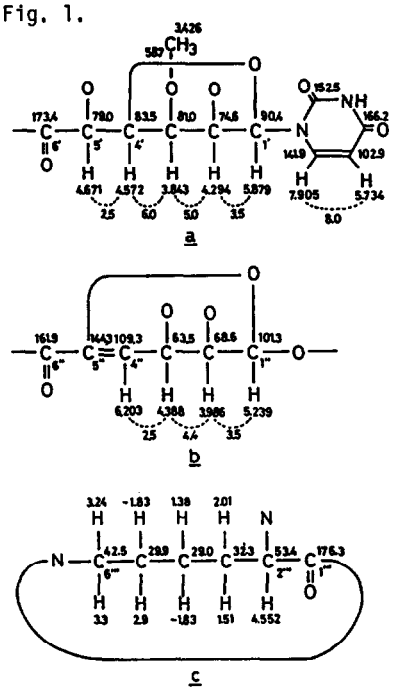
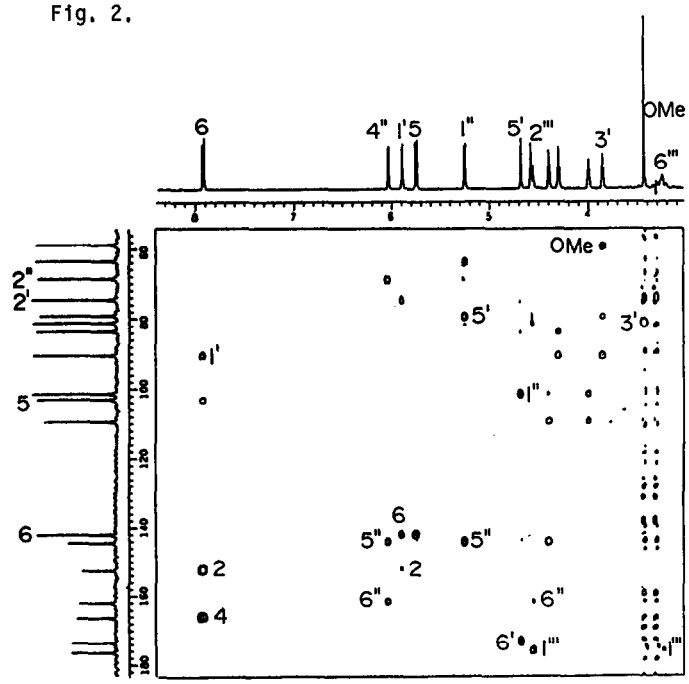


Fig. 2.



the HMBC technique reported by Bax et al[3]. Thus, the position of OCH_3 (δ_H 3.426) on C-3' was established by the cross peak observed between H-3' (δ_H 3.843) and OMe (δ_C 58.7) as shown in the HMBC spectrum of I (Fig. 2). The linkage of an amide carbonyl group to C-5' was confirmed by the cross peak between H-5' (δ_H 4.671) and C-6' (δ_C 173.4). In a similar way, the following connectivities proved by analyzing the same HMBC spectrum; H-1' to C-2 and C-6, H-1" to C-5" via an oxygen, H-3" to C-5", H-4" to C-5" and C-6", H-2"' to C-1"', and H-6"' to C-1"'. Although the linkage between C-1' and C-4' through an oxygen could not be obtained by this technique due to the very small coupling constants between H-1' and C-4', and H-4' and C-1', their relationship was established by X-ray analysis (vide infra).

The information on the linkage between these three fragments a-c was also obtained from the analysis of the HMBC spectrum. For example, H-5' (δ_H 4.671) and H-1" (δ_H 5.239) showed a cross peak with C-1" (δ_C 101.3) and C-5' (δ_C 79.0), respectively, to result in the linkage of fragment a and fragment b. The cross peak between H-2"' (δ_H 4.552) and C-6" (δ_C 161.9) attached fragment c to fragment b. Since it was suggested by IR and tiration data that there existed no ester or free carboxylic function in I (vide supra), the carbonyl function of C-6' must be assigned to an amide residue establishing the planar structure of I (see Fig. 5).

The stereochemistry of I was established as follows.

Mild acid treatment (0.5N HCl, 100°C, 2hrs) of I gave, inter alia, II as the nucleoside component. The physicochemical properties of II are as follows; $C_{11}H_{14}O_8N_2$, SI-MS data of IV; (m/z) 325 (M+Na), ^{1}H-NMR spectral data taken in

D_2O; δ_H 5.89 (1H, d, J=8.0 Hz, H-5), 8.04 (1H, d, J=8.0 Hz, H-6), 5.88 (1H, d, J=4.5 Hz, H-1'), 4.48 (1H, dd, J=4.5, 5.0 Hz, H-2'), 3.99 (1H, dd, J=5.0, 5.0 Hz, H-3'), 4.50 (1H, dd, J=2.2, 5.0 Hz, H-4'), 4.21 (1H, d, J=2.2 Hz, H-5'), 3.48 (3H, s, 3'-OMe), ^{13}C-NMR spectral data taken in D_2O; δ_C C-2 152.1, C-4 166.6, C-5 102.9, C-6 142.3, C-1' 89.7, C-2' 73.4, C-3' 79.9, C-4' 84.1, C-5' 72.0, C-6' 177.4, CH_3O- 58.8. The magnitude of the coupling constant of the anomeric proton ($J_{1',2'}$=4.5 Hz) and the ^{13}C-chemical shift of C-4' (84.1 ppm) of II suggested the presence of a furanose ring.

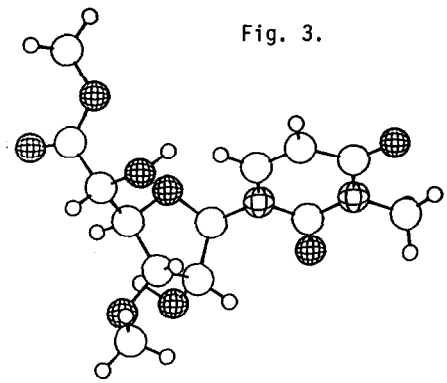


Fig. 3.

The structure of II including its relative stereochemistry was determined by X-ray analysis[4] of its ^{3}N-methyl methyl ester derivative prepared by treatment with diazomethane in MeOH. Its absolute stereochemistry was determined to be as shown in Fig. 3 by its CD spectral data [$[\theta]^{25}_{268}$ +9480 pk, $[\theta]^{25}_{239}$ -4170 tr, (c 0.01, H_2O)] which were very similar to those of uracil polyoxin C[5] [$[\theta]^{25}_{269}$ +12879 pk, $[\theta]^{25}_{235}$ -4545 tr, (c 0.01, H_2O)].

The stereochemistry of the hexuronic acid moiety was determined by analysis of a dihydro derivative of I. Catalytic hydrogenation of I (over 10% Pd-C in MeOH) gave a major dihydro derivative, III, $C_{23}H_{33}O_{12}N_5$, SI-MS; (m/z) 572 $(M+H)^+$, 594 $(M+Na)^+$, UV λmax (H_2O) 262 nm (ε 8540), mp. 182°C, $[\alpha]^{25}_D$ +58°(c 0.5, H_2O). Detailed analysis of the 400 MHz ^{1}H-NMR spectrum of III revealed, in addition to the structures described above, the presence of the partial structure shown in Fig. 4. The ^{13}C- and ^{1}H-NMR data proved the 4-deoxyhexuronic acid structure and the chemical shift of C-1" (δ_C 100.6 and δ_H 5.13, respectively) suggested that this residue took a pyranose form.

Fig. 4.

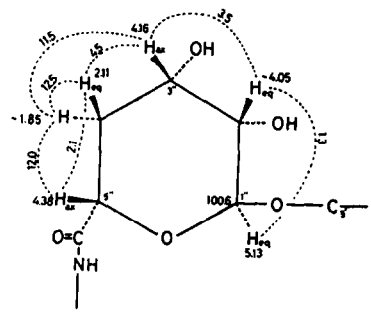
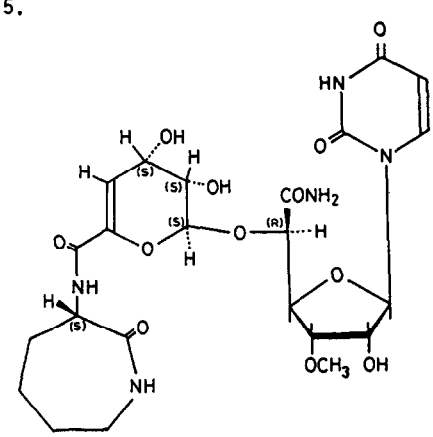


Fig. 5.



The stereochemistry of this sugar residue was established to be as shown in Fig. 4. The coupling constants between H-4"a and H-5" ($J_{4"a,5"}$=12.0 Hz), and H-3" and H-4a" ($J_{3",4"a}$=11.5 Hz) indicated diaxial relationships of these three protons. The stereochemistry of H-2" was proved to be equatorial by the coupling constant between H-2" and H-3" ($J_{2",3"}$=3.5 Hz). The configuration of H-1" was determined by NOE experiments to be equatorial as follows. On irradiating at H-1", NOEs were observed with H-2"eq and the methine proton (H-5') of the other sugar unit but not with H-5"ax, while irradiation at H-5"ax enhanced the signal intensity of H-3"ax without affecting that of H-1".

The absolute stereochemistry of this pyranose ring portion was established by cupra ammonium method[6]. The obtained value, [M]CuAm 436 nm = +2057, showed clearly that the vicinal hydroxy groups on C-2" and C-3" are in an anticlockwise relationship. Consequently the asymmetric centers at C-1", C-2" and C-3" were established to possess S configurations.

Thus, the complete absolute structure of I has been established as shown in Fig. 5. As far as we know, capuramycin is the first natural product with a caprolactam substituent.

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2) ^{13}C- and ^{1}H-NMR spectra were obtained on a JEOL GX-400 spectrometer operating at 100 MHz and 400 MHz, respectively. Chemical shifts are expressed in ppm using TMS or DSS as internal standard.

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4) The ^{3}N-methyl methyl ester of II crystallized in the monoclinic space group $P2_1$ with a=7.540(3), b=7.135(2), and c=13.782(9) A and =92.56(4)°. One molecule of composition $C_{13}H_{18}O_8N_2$ formed the unit. After correction for Lorentz, polarization, and background effects, 916 (91%) of the 1104 reflections measured were judged observed. Block diagonal least squares refinements with anisotropic nonhydrogen atoms and isotropic hydrogens have converged to a conventional crystallographic residual of 0.0812 for the observed data. Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Center.

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