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THE STRUCTURE OF A NOVEL NUCLEOSIDE ANTIBIOTIC, DAPIRAMICIN A

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Summary: Based on ^{1}H- and ^{13}C-NMR spectral data including deuterium induced upfield shift, the structure of dapiramicin A, a novel nucleoside antibiotic has been determined as shown in Fig. 5.

Dapiramicin A (formerly called SF-1917)[1] is an antibiotic produced by Micromonospora sp. SF-1917 which is effective in the treatment of sheath blight disease in the green house test. We wish to report herein the structural elucidation of dapiramicin A accomplished mainly based on ^{1}H- and ^{13}C-NMR spectral analyses.

The physicochemical properties of dapiramicin A (I) are as follows; colorless needles, $C_{21}H_{29}N_5O_{10}$, FD-MS: m/z 511 (M^+), Anal. found, C;47.50, H;5.46, N;12.81, O;32.17%, calcd. for $C_{21}H_{29}N_5O_{10}\cdot H_2O$, C;47.63, H;5.90, N;13.22, O;33.24%, mp. 220-222°C, $[\alpha]_D^{20}$ +117 (c 0.5, MeOH), UV λmax(MeOH) 227nm(ε40200)and 289(ε8640). The IR spectrum of I (KBr) showed the characteristic absorption due to a nitrile group (2235 cm^{-1}) in addition to strong -OH and -NH bands (3405 cm^{-1}).

The 100 MHz ^{13}C-NMR spectrum[2] of I taken in CD_3OD revealed the following groups; 1 X CH_3 (δ_C 18.1), 2 X OCH_3 (54.3, 60.8), 1 X CH_2O (62.1), 8 X CHO- (67.6-87.4), 1 X N-CH-O (77.2), 1 X O-CH-O (104.9), 1 X CN (116.6), 5 X =C- (84.8-164.8) and 1 X -CH= (131.2). The 400 MHz ^{1}H-NMR spectrum[2] of I taken in CD_3OD is summarized as follows, δ_H 1.30 (3H, d, J=6.0 Hz, H-6'), 3.12 (1H, dd, J=9.2, 9.8 Hz, H-4"), 3.18 (1H, dd, J=9.0, 9.2 Hz, H-4'), 3.25 (1H, dd, J=7.8, 9.2 Hz, H-2"), 3.35 (1H, m, H-5"), 3.49 (1H, t, J=9.2 Hz H-3"), 3.57 (3H, s, OCH_3), 3.69 (1H, dd, J=5.5, 12.0 Hz, H-6"a), 3.8-3.85 (3H, m, H-2', 3' and 5'), 3.86 (1H, dd, J=2.2, 12.0 Hz, H-6"b), 4.09 (3H, s, OCH_3), 4.37 (1H, d, J=7.8 Hz, H-1"), 5.77 (1H, d, J=4.8 Hz, H-1') and 7.60 (1H, s, H-8). These spectral data suggest that I is a novel disaccaharide nucleoside possessing a nitrile group on the chromophore.

Treatment of I with acetic anhydride/pyridine gave a hexa-acetate (II)[3], $C_{33}H_{41}N_5O_{16}$, EI-MS:m/z 763 (M^+), Anal. found, C;51.83, H;5.29, N;9.08, calcd., C;51.90, H;5.37, N;9.17. mp. 223-225°C, $[\alpha]_D^{20}$ +74.6 (c 0.5, $CHCl_3$), ^{1}H-NMR (δ_H in $CDCl_3$) 6 X CH_3CO- (δ_C 2.01, 2.02, 2.05, 2.07, 2.14 and 2.97).

On mild acid treatment (AcOH, 100°C, 2 hrs), II was converted to pentaacetyl epidapiramicin

A (III)[4], $C_{31}H_{39}N_5O_{15}$, EI-MS: m/z 721 (M^+), mp. 190-194°C, $[\alpha]_D^{20}$ -15.4°(c 0.5, $CHCl_3$), in which the sign of optical rotation was reversed. The ^{1}H-NMR spectrum of this compound revealed that the acetyl signal observed at a very low field (δ_H 2.97) in II disappeared and that epimerization at C-1' took place to give a β-anomer (δ_H; H-1' 5.46, H-2' 5.00, NH 6.33, $J_{H-1',NH}$=9.5 Hz, $J_{H-1',H-2'}$=9.5Hz. cf. $J_{H-1',H-2'}$ of II = 4.8 Hz). In a similar way, I underwent complete epimerization to give biologically inactive epidapiramicin A, $C_{21}H_{29}N_5O_{10}.H_2O$, FD-MS:m/z 511 ($M^+$), mp. 222-224°C, $[\alpha]_D^{20}$ +14.4° (c 0.5, AcOH) under the same experimental condition.

Analysis of the 400 MHz ^{1}H-NMR spectrum of II in $CDCl_3$ revealed the structure of its sugar moiety as shown in Fig. 1.

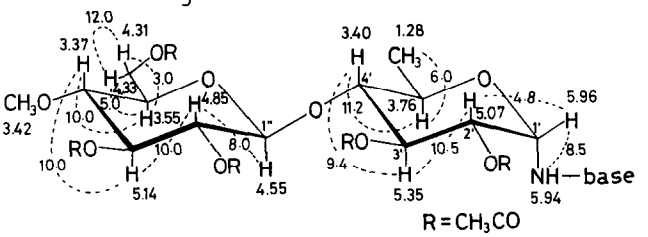


Fig. 1

The large coupling constants observed with H-2' to H-5' and H-1" to H-5" (8.0-11.2 Hz) clearly indicated the presence of 6-deoxy-glucopyranosylamine and 4-O-methylgluco-pyranosyl moieties in II. The coupling between H-1' and NH proton established the linkage of the anomeric carbon C-1' and the chromophore through a nitrogen atom (vide infra). Consequently, 4-O-methylglucopyranose must be connected to C-4' of 6-deoxyglucopyranosylamine by β-glycosidic linkage. The downfield chemical shifts of C-4' (δ_C 81.8) and C-4" (δ_C 77.3) are in agreement with this structure.

These partial structures including absolute stereochemistries of the sugar moieties were further confirmed by chemical degradation as follows. Acid hydrolysis of I (740 mg) and treatment with HCl/MeOH and subsequently with Ac_2O/pyridine followed by purification by silicagel column chromatography (hexane:AcOEt = 9:1-4:1) gave three sugar derivatives (IV, 109 mg, V 48 mg, and VI 198 mg). The component IV eluted first from the column, was identified with methyl 2,3,4-tri-O-acetyl-6-deoxy-α-D-glucopyranoside. $C_{13}H_{20}O_8$, Anal. found, C;51.17, H;6.68. calcd., C;51.31, H;6.63. mp. 74-76°C, $[\alpha]_D^{15}$ +140.7° (c 1, $CHCl_3$). lit.[5] mp. 76-77°C, $[\alpha]_D^{15}$ +153° (c 1.6, $CHCl_3$).

The second component V, was determined to be methyl 2,3,4-tri-O-acetyl-6-deoxy-β-D-gluco-pyranoside by ^{1}H-NMR spectral analysis and was not characterized further.

The third compound VI, which was proved to be an anomeric mixture of methyl 2,3,6-tri-O-acetyl-4-O-methylglucoside by ^{1}H-NMR spectral analysis could not be separated into each anomer by chromatographic procedures. Therefore, it was hydrolyzed with 1N H_2SO_4 (100°C, 10 hrs) to give after an appropriate work up an anomeric mixture of 4-O-methyl-D-glucose, $C_7H_{14}O_6$. EI-MS:m/z 176 (M^+-H_2O), FD-MS:m/z 217 (M^++Na), Anal. found, C;39.51, H;7.72 calcd. for $C_7H_{14}O_6.H_2O$ C;39.62, H;7.60. $[\alpha]_D^{20}$ +80.5° (c 1, MeOH), lit.[6] $[\alpha]_D^{20}$ +80°(c 1.3, MeOH).

Thus, the sugar moiety of I has been established to be 4-(4-O-methyl-β-D-glucopyranosyl)-6-deoxy-α-D-glucopyranosylamine as shown in Fig. 1.

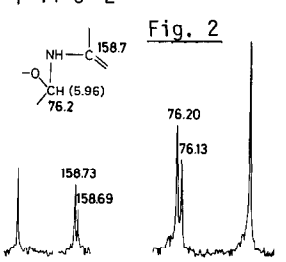


Fig. 2

The linkage of the sugar and chromophore moieties of I was proved by making use of the deuterium induced upfield shifts.[7] Thus, in the ^{13}C-NMR spectrum of II taken in $CDCl_3$ added with one drop of a 1:1 mixture of CD_3OD and CD_3OH, the anomeric carbon (C-1') and an sp^2 carbon (C-2) in

the chromophore moiety appeared as doublets at δ_C 76.20 and 76.13, and δ_C 158.73 and 158.69 (Fig. 2), respectively, due to slow exchange rate between -NH- and -ND-, while the other ^{13}C signals remained unchanged. Therefore, these two carbons must be connected through the nitrogen atom in the glucopyranosylamine residue (-O-$C_{1'}$-NH-C_{2}=). Since $^{13}C\{^1H\}$ long range selective proton decoupling experiments (LSPD)[8] irradiating the proton on this nitrogen (δ_H 5.94) failed to collapse any carbon signals in the chromophore unit except for C-2, both the substituents on C-2 which are three bonds away from the irradiated proton, must be heteroatoms, namely nitrogen atoms in this case. The ^{13}C-chemical shift of C-2 (δ_C 158.9) is in agreement with a cyclic guanidine residue (cf. C-2 of guanosine 154.5[9]).

Fig. 3

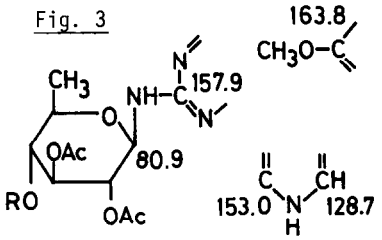
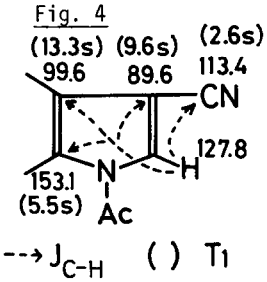


Fig. 4

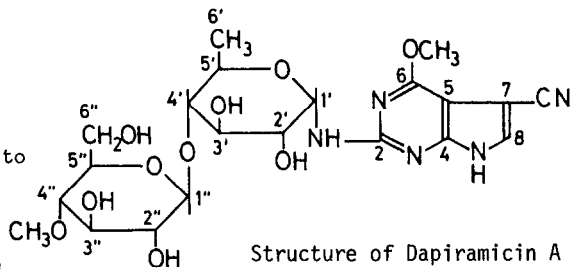


In the case of pentaacetyl epidapiramicin A, III, deuterium induced upfield shifts[7] were observed with four carbons at δ_C 80.9, 157.9, 153.1 (-C=) and 128.7 (-CH=). The first two are straightforwardly assigned to the O-$C_{1'}$-NH-C_{2}= unit, and the remaining two are due to =C-NH-CH=. The latter nitrogen atom was acetylated in II. These relationships and remaining carbons in the chromophore are summarized in Fig. 3. The location of a methoxy carbon on a quaternary sp^2 carbon at δ_C 163.4 was established by LSPD experiment[8]. The characteristic signals at δ_C 113.4 and 84.9 were assigned to a nitrile carbon and the adjacent carbon respectively, based on comparison to toyocamycin.[10]

Further structural information on the chromophore unit was obtained based on longitudinal relaxation times (T_1)[11] and ^{13}C-^1H long range coupling of II as shown in Fig. 4.

Thus, the nitrile carbon at δ_C 113.4 showed very short relaxation time (2.6 sec) as compared to the remaining quaternary sp^2 carbons (5.5-13.3 sec). Therefore the partial structure including the nitrile and C-7 carbons must be placed at the next position to H-8. Long range couplings observed between H-8 and C-5, C-7 and -CN established the fused pyrrole structure (Fig. 4). Since C-5 appearing at a higher field (δ_C 99.6) can not be combined to a heteroatom, it must be connected to $=C_6$-O-CH_3. Thus, the structure of the base moiety and therefore the total structure of I has been determined as shown in Fig. 5.

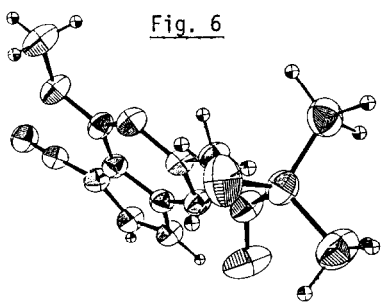
Fig. 5



Structure of Dapiramicin A

The structure of this base moiety was finally confirmed by an X-ray analysis of its pivalyl derivative (VII), $C_{13}H_{15}N_5O_2$[12], as shown in Fig. 6. The compound VII was prepared by treatment with pivalyl chloride/Et_3N in DMF of free

Fig. 6



base (VIII)[13] which had been obtained by acid hydrolysis (0.5N HCl, 90°C, 2 hrs) of I followed by purification using Amberlite XT-2. Crystal data are as follows; orthorhombic, space group: $P2_1/c$, Z=4, lattice constants; a=17.388, b=14.510, c=5.476, cosβ=-0.0432. Final results[14] at R=0.06 gave the structure illustrated in Fig. 6.

It is interesting to note that VIII is closely related in its structure to pre Qo base of hypermodified nucleoside found in t-RNA[15].

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References and footnotes

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

3) ^{13}C-NMR spectral data of II are as follows; δ_C C-2;158.7, C-4;153.1, C-5;99.6, C-6;163.8, C-7;89.6, C-8;127.8, 6-OMe;54.3, 7-CN;113.4, C-1';76.2, C-2';69.6, C-3';69.7, C-4';81.8, C-5';66.5, C-6';17.4, C-1";100.8, C-2";72.0, C-3";74.9, C-4";77.3, C-5";72.8, C-6";62.7, 4'-OMe;60.1.

4) ^{13}C-NMR spectral data of III are as follows; δ_C C-2;157.9, C-4;153.0, C-5;98.3, C-6;163.8, C-7;84.9, C-8;128.7, 6-OMe;53.8, 7-CN;115.1, C-1';80.9, C-2';71.2, C-3';72.7, C-4';81.5, C-5';72.6, C-6';17.1, C-1";100.6, C-2";72.2, C-3";75.0, C-4";77.3, C-5";72.8, C-6";62.7, 4"-OMe;60.0.

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12) VII: $C_{13}H_{15}N_5O_2$, mp >300°C, EI-MS:m/e 273(M^+), Anal. found, C;57.00, H;5.55, N;25.10, calcd. C;57.13, H;5.53, N;25.63. νmax(KBr) 2230 cm^{-1} (-CN), ^{1}H-NMR (in $CDCl_3$), δ_H 1.38 (9H, s), 4.14 (3H, s), 7.69 (1H, s).

13) VIII was characterized as its hydrochloride. $C_8H_7N_5O.HCl$, mp >300°C, EI-MS:m/z 189 (M^+), Anal found, C;41.34, H;3.44, N;29.97, Cl;15.20, calcd. C;42.85, H;3.12, N;31.04, Cl;15.71. νmax 2230 cm^{-1} (-CN), λmax 225 nm (ε18500) and 292 nm (ε4760).

14) Final crystallographic coordinates have been deposited with the Cambridge Crystallographic Data Centre.

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