THE STRUCTURE OF NEW OLIGOSACCHARIDE ANTIBIOTICS, 13-384 COMPONENTS 1 AND 5

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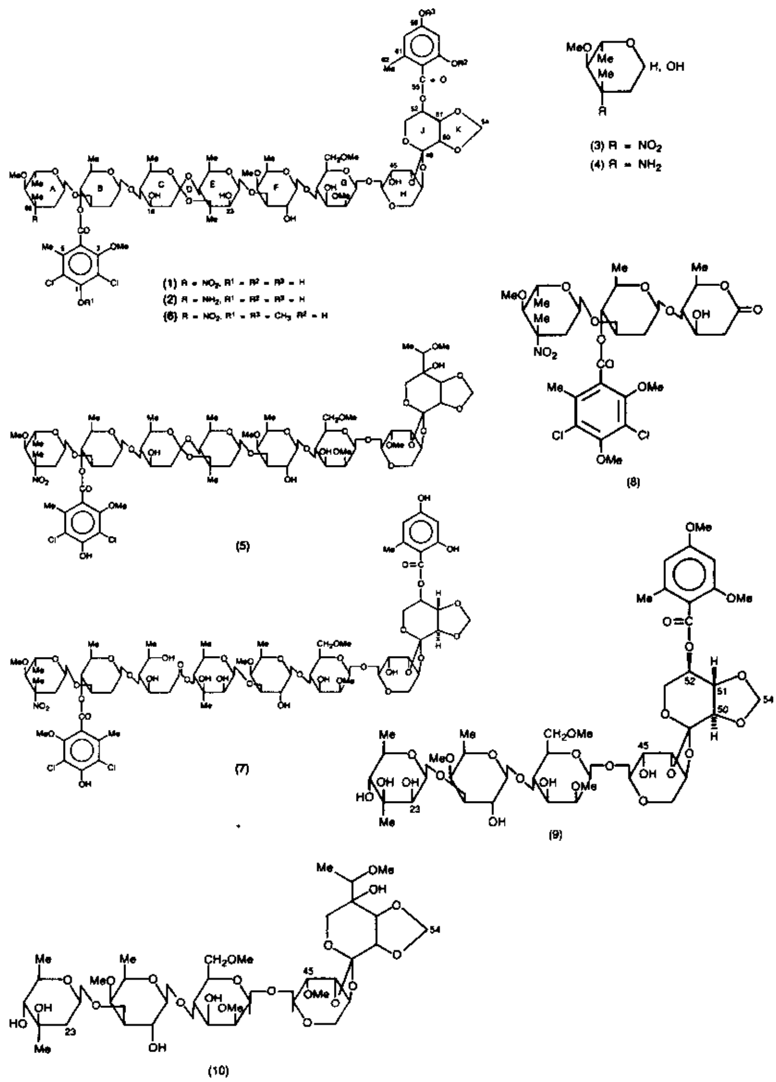
Abstract - Structures of 13-384 antibiotics have been deduced based on chemical degradation and spectroscopic evidence.

Antibiotics 13-384 components 1 (1) and 5 (2) are produced[1] by Micromonospora Carbonacea. They are highly active against gram positive bacteria and in particular they are active against methicillin resistant Staphylococci. Both of these compounds belong to the group of oligosaccharide antibiotics[2] as characterized by the presence of two ortho ester carbon atoms, aromatic esters and several sugar residues. Like everninomicins compound (1) shows the presence of a nitro group at ν max 1540 cm^{-1} and compound (2) lacks this absorption. Catalytic hydrogenation of compound (1) yields compound (2) thus establishing that the two compounds differ in their oxidation level and it will be evident in this communication that compound (1) possesses evernitrose moiety (3) and compound (2) contains 3-desnitro-3-amino-evernitrose residue (4). The ^{1}H-nmr spectrum of (1) (see attached spectrum with a few relevant assignments) indicated the presence of five methyl doublets, two tertiary methyls, two aromatic methyls and five methoxyl groups. The ^{13}C-nmr spectrum indicated two ortho ester carbons at δ 119.2 and 120.4 and when compared[8] with everninomicin D (5) it showed an extra aromatic ester carbonyl carbon at δ 167.0, and six extra aromatic carbons at δ 161.7, 96.3, 158.5, 115.2, 138.7 and 106.8 corresponding to the aromatic residue at C_{52} in (1). Methylation of (1) with diazomethane yielded the dimethyl derivative (6) in which one of the phenolic hydroxyl groups escaped methylation.

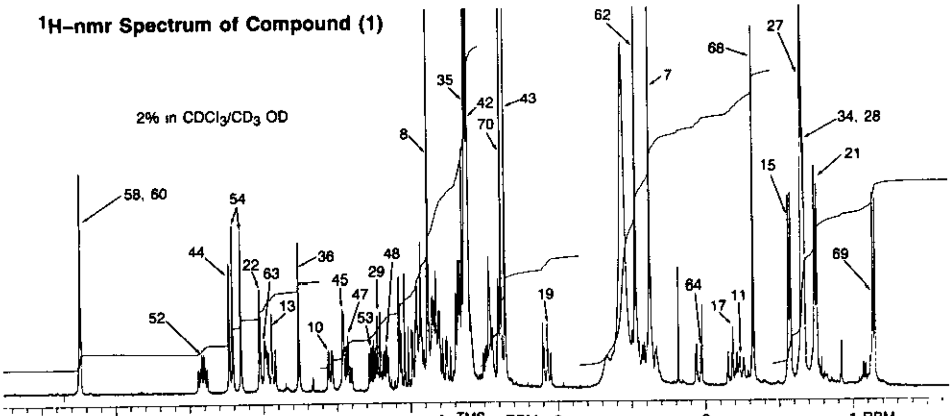
Compound (1) is an amorphous solid, $C_{70}H_{97}NO_{38}Cl_2$, ν max 1540 cm^{-1} (nitro), $[\alpha]_D^{26}$ -47.2^{o} (methanol). Molecular weight of (1) is established to be 1629 based on FAB (fast atom bombardment) mass spectrometry. In an earlier publication[3] we have disclosed the use of high resolution FAB mass spectrometry for the determination of molecular weight and fragmentation pattern of oligosaccharide antibiotics. We, therefore, compared the FAB mass spectra of (1) and everninomicin D (5) (see Figure 1) and concluded that a) the two antibiotics have identical composition and sequence of A, B and C rings; b) the E ring has an extra hydroxyl group in (1); c) the H ring in (1) has a hydroxyl group compared to a methoxyl group in (5) and the substitution pattern at C_{52} in the two compounds are different.

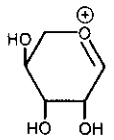
Accurate mass measurement indicated that the group attached at C_{52} in compound (1) to be $C_8H_7O_4$ (m/z 167) and based on ^{1}H-nmr spectra, as will be discussed later, it appeared to be a 2,4-dihydroxy-6-methylbenzoyloxy residue. Results of the high resolution FAB mass spectral data of some important ions of (1) are summarized in Figure 1. As has been reported earlier, these compounds fragment at the center ortho ester generating two sets of fragment ions which are easy to recognize; one set shows chlorine isotopes and the other set of ions contains no chlorine. We observed peaks for $(M+H)^+$ and $(M+Na)^+$ of moderate intensity using glycerol and thioglycerol as a matrix and dimethyl sulphoxide as solvent. However, to obtain the accurate composition of (1), $C_{70}H_{97}NO_{38}Cl_2$, we have added the accurate mass of the chlorine containing fragment m/z 696.1874 (calculated for $C_{29}H_{40}NO_{14}Cl_2$; m/z 696.1826) and the other fragment at m/z 935.3386 (calculated for $C_{41}H_{59}O_{24}$ m/z 935.3396) and subtracted two mass units because each of the above fragments arises by the transfer of a hydrogen atom. Using similar argument we have established the composition of (2) to be $C_{70}H_{99}NO_{36}Cl_2$. As one would expect in chemical degradation experiments, (1) behaves very similarly to everninomicin D[4] (5). Thus when a methylene chloride solution of (1) is stirred with 0.1N HCl, it yields (7) which when treated with diazomethane undergoes cleavage to yield the lactone (8) and compound (9). The lactone (8) is found to be identical with the one obtained from everninomicins B[5], C[6] and D[4]. Single and two dimensional 400 MHz nmr data of (8) show the C_{18} hydroxyl to have equatorial stereochemistry, which is the opposite of what we reported in an earlier work[4], based on an assumption, from ir data, of a boat conformation for the lactone ring in (8).

In compound (1) strong NOESY connectivity among the 17, 19 and 21 protons, plus the two diaxial couplings ($J_{18,19} = J_{19,20} = 8$ Hz) demonstrate a chair conformation and arabino stereochemistry for ring C. A recent publication[9] has also suggested revision of H_{18} stereochemistry. Compound (9) is an amorphous solid $C_{43}H_{64}O_{25}$ (m/z 980.3832), $[\alpha]_D^{26}$ -46.9^o (chloroform), $\lambda^{\text{trifluoroethanol}}$ 242 nm (22,231), 261 (11099), 278 nm (5223), 294 nm (2970), ν max 1735 cm^{-1} (ester). ^{1}H-Nmr spectrum of (9) shows the presence of two methyl doublets (δ1.31, 1.33), a tertiary methyl (δ1.2), an aromatic methyl (δ2.5), five methoxyl groups, four anomeric hydrogens (δ5.31, 4.27, 4.96, 4.78), two aromatic hydrogens appear at (δ6.33) and H_{52} appears at (δ5.43) as a doublet of a doublet and the large coupling constants ($J_{51,52} = J_{52,53a} = 9.7$ Hz; $J_{52,53e} = 5.8$ Hz) would suggest H_{52} to be in an axial orientation.

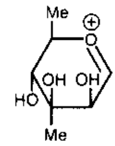


^{1}H-nmr Spectrum of Compound (1)

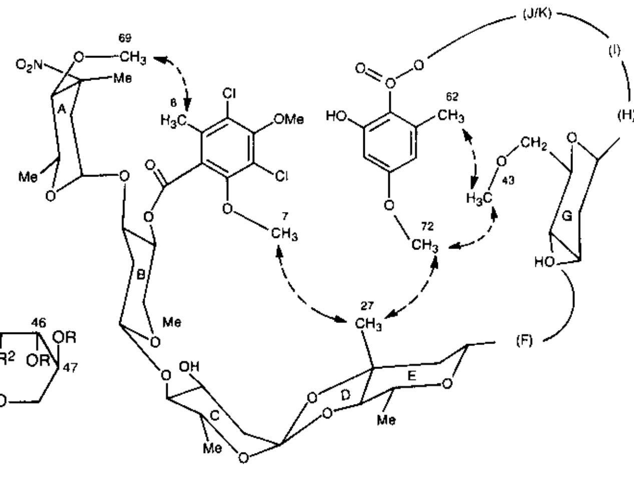




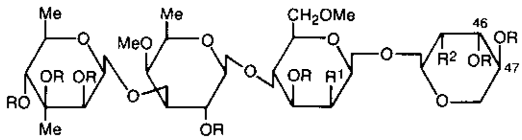
(15)



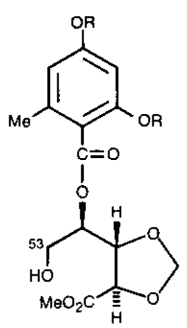
(16)



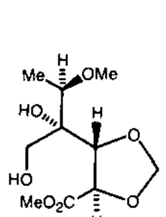
(18)



(11) R = H; R^1 = OMe, R^2 = OH
(13) R = Me, R^1 = R^2 = OMe
(17) R = H, R^1 = R^2 = OMe

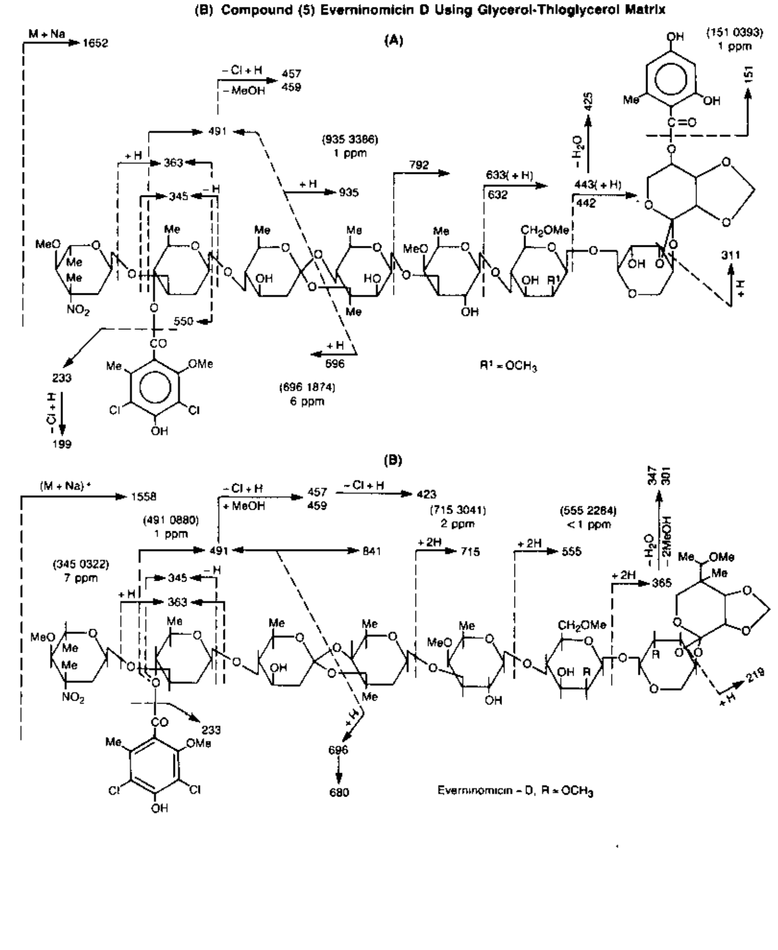


(12) R = CH_3
(19) R = H



(14)

Figure 1. FAB Mass Spectral Data:
(A) Compound (1)-13384 Component 1 and
(B) Compound (5) Everninomicin D Using Glycerol-Thioglycerol Matrix



In compound (9), C_{23} appears at δ 71.1 compared to δ 45.8 for olgose[4,7] (10), indicating a hydroxyl substitution at that carbon. Also in compound (9) C_{45} appears at δ 72.5 compared to δ 80.5 in (10). This indicates that the C_{45} methoxyl group in compound (10) which appears at δ 58.4 is replaced by a hydroxyl substituent at that position in compound (9).

The ^{1}H-nmr spectra of (9) and (10) show the expected similarities and differences. For example, characteristic singlets for H_{54} are present in both the compounds at δ 5.14 and 5.22. In compound (9) H_{52} appears at δ 5.43 as a multiplet and two dimensional nmr connectivity experiments demonstrate its axial-axial couplings with H_{51} and H_{53}. As in (10), H_{50} and H_{51} in (9) are both axial. Hydrolysis of (9) in methanol and p-toluenesulphonic acid yields (11) and (12).

The mass spectral data of (11) shows ions corresponding to (15) at m/z 133 and (16) at m/z 161. When the ^{1}H-nmr and ^{13}C-nmr spectra of (11) are compared with evertetrose B (17)[7] striking similarities become apparent. The difference between the two compounds is the absence of the signal for the C_{45} O-methyl group in (11). This is consistent with all the observations made so far in nmr and ms of (1) and (9). Thus compound (11) must be 45-des-O-methylevertetrose B. To verify the structure further and also to assign the absolute stereochemistry of (11) it is methylated with methyl iodide in the presence of sodium hydride and dimethyl sulphoxide. Permethylated compound (13) thus obtained is found to be identical in all respects [nmr, mass spectra, $[\alpha]_D$ -61.8 ($CHCl_3$)] when compared with permethylated evertetrose B[5]. The structure and absolute stereochemistry of evertetrose B (17) have been rigidly established by us in an earlier publication[7].

The structure of compound (12), oil, $C_{17}H_{22}O_9$ (m/z 370.1255; calc. 370.1264) has been assigned based on nmr and high resolution mass spectral data and correlation with the data published on olgose side chain (14)[4]. Upon methanolysis of compound (1), compound (19) was isolated which on treatment with diazomethane yielded (12). Compound (19), $C_{15}H_{18}O_9$ (m/z 342.0942) showed an aromatic methyl at δ 2.50 (d; $J_{60,62}$ = 1 Hz), two meta coupled aromatic protons at δ 6.23 (H_{58}; $J_{58,60}$ = 2 Hz) and δ 6.19 (H_{60}; $J_{58,60}$ = 2 Hz and $J_{60,62}$ = 1 Hz), a carbomethoxyl group at δ 3.80, two methylene dioxy protons H_{54} at δ 5.03 and 5.24 (J = 0 Hz), H_{50} at δ 4.51 ($J_{50,51}$ = 5 Hz) and H_{51} at δ 4.47 ($J_{51,52}$ = 5 Hz). ^{13}C-nmr of (19) and (12) also verify the structures assigned to these compounds.

Compounds (11) and (12) are derived by methanolysis of (9) and as we have argued in an earlier publication[4] during the structural elucidation of olgose (10), we concluded that (9) is derived by the loss of methanol between the $-CO_2Me$ and C_{53} hydroxyl group in (12) and the hydroxyls at C_{46}, C_{47} in (11) forming an ortho ester linkage in (9). This, as in the case of

olgose, would explain the presence of $-CO_2Me$ in (12) and an ortho ester in (9).
As mentioned, mild acidic hydrolysis of (1) yields (7) which undergoes cleavage with diazomethane to yield (8) and (9). This sequence of reactions parallels chemical degradation of everninomicins[2]. It has been pointed out already that (1) has two ortho ester carbons out of which one belongs to compound (9). The second ortho ester is opened to a hydroxy ester in (7) under acidic conditions and during the diazomethane reaction the newly formed hydroxy ester moiety is cleaved to give (8) in which the phenolic hydroxyl group is methylated and compound (9) in which two phenolic hydroxyl groups are methylated. In (7), a new ester carbonyl group is formed and it lacks the presence of one of the ortho ester carbons of (1). We propose, therefore, that the combination of this ester carbonyl group at C_{16} with the hydroxyl groups at C_{20} and C_{24} of (7) results in the formation of the second ortho ester carbon in the structure of (1). Upon reduction of (1) to give (2), the only changes in the ^{13}C-nmr spectrum are the shift of the C_{65} resonance from δ 90.2 to δ 54.7 and the C-65 methyl resonance from δ 19.5 to δ 20.7. This is consistent with (2) having a structure differing from that of (1) only in that the C-65 nitro group is replaced with an amino group. Thus, based on all the above observations antibiotics 13-384 components 1 and 5 are assigned structures (1) and (2), respectively. In a subsequent publication we shall discuss the details for arriving at the solution conformation (18) for compound (1) based on NOESY experiments.

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