STUDIES ON THE IONOPHOROUS ANTIBIOTICS. XXI[1]
STRUCTURAL ELUCIDATION OF A NEW POLYETHER ANTIBIOTIC 6016 BY APPLICATION OF THE EMPIRICAL RULES IN ^{13}C-NMR SPECTROSCOPY

Sir:

In the previous paper[1], we explained the empirical rules for structural studies of polyether antibiotics by ^{13}C-nmr spectroscopy. Their application in the structural determination of a new antibiotic 6016 is now presented.

A new antibiotic 6016, $C_{46}H_{77}O_{16}Na$, mp. 192 ~ 195°C (dec.), $[\alpha]_D^{20}$ −42.5° (*c* 1, MeOH), $\nu_{max}^{CHCl_3}$ 3450, 3240, 2950 and 1650 cm^{-1}, is produced by *Streptomyces* sp. 6016[2] and active against Gram-positive bacteria, mycobacteria, fungi and yeast.

Its ^{13}C-nmr spectrum (Fig. 1, sodium salt, in $CDCl_3$, TMS as internal standard) shows the presence of a carboxylate (178.4 ppm), three (hemi)ketals (107.9, 99.2 and 96.6 ppm), three methoxys (58.8, 56.7 and 55.8 ppm) and an anomeric carbon (98.9 ppm). These spectral data indicated that the antibiotic possesses the same basic carbon skeleton[1],* common to carriomycin[5], septamycin[6] and A204A[7] and in fact, by application of the empirical rules we have succeeded in the structural determination of 6016 in the following way. The chemical shift values listed in Fig. 2 are bases of the partial structures connected by dotted lines.

Signals Appearing at 45 ~ 47 ppm

The most striking feature of the ^{13}C-nmr spectrum of 6016 is the absence of signals between 40 ~ 55 ppm, the region being specific to the C-2 methine carbons[1]. This phenomenon has never been observed in other polyether antibiotics reported so far. Since removal of the methyl from the C-2 methine does not considerably affect its chemical shift (*cf.* C-2 methylene in lysocellin[8] 45.9 ppm), the only reasonable explanation for this spectral feature can be given by placing a hydroxy function on C-2. In agreement with this assumption, an AB-quartet

Fig. 1. ^{13}C-Nmr spectrum of 6016 Na salt in $CDCl_3$.
The suffix M represents either a methyl or methoxy group on the numbered carbon.
The carboxylic acid (C-1) at 178.4 ppm is not shown in this figure.

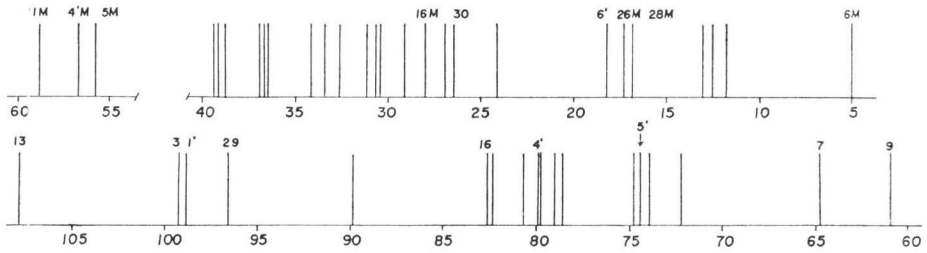
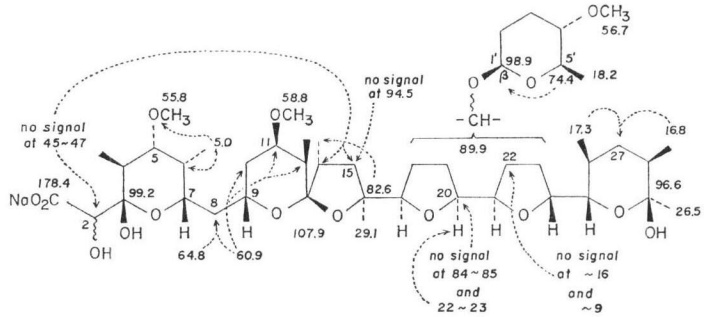


Fig. 2. The structure of 6016 obtained by analysis of its ^{13}C-nmr spectral data.
The chemical shift values are bases of the partial structures connected by dotted lines.



* X-206[3] and alborixin[4], members of another subgroup of the polyether antibiotics with a different framework, are easily distinguished by the absence of methoxy and sugar groups.

at 3.84 (exchanged with D_2O) and 3.92 ppm, $J=5.9$ Hz, in the 270 MHz ^{1}H-nmr spectrum of 6016 sodium salt changed to a sharp singlet at 5.11 ppm in 6016 monoacetate sodium salt.

Absence of a methoxy at C-15 and 27 was also inferred by the same characteristic spectral appearance[1].

A-Ring

The chemical shift of C-7 (64.8 ppm) can be compared with that of carriomycin (64.0 ppm) and clearly shows the absence of both a methyl at C-8 and an oxygen function at C-6. It follows, therefore, that the absolute configuration of A-ring is represented as shown in Fig. 2.

The substitution pattern of C-6 is also corroborated by a signal assignable to the axial methyl at C-6 (5.0 ppm).

B and C-Rings

The oxymethine (C-9, 60.9 ppm) and ketal (C-13, 107.9 ppm) signals strongly suggested the structures of B and C-rings to be identical with those of nigericin[1] (C-9, 60.4, C-13, 107.7 ppm). The chemical shift values of C-16 (82.6 ppm) and the methyl on it (28.0 ppm) are also in agreement with the presence of a methyl at C-14.

No oxymethine signal at 94.5 ppm proved C-15 to be a methylene, this conclusion having been obtained by the absence of a signal at 40~55 ppm (*vide supra*).

D and E-Rings

The absence of a methyl at C-20 was supported by no oxyquaternary carbon signal at 84~86 ppm as well as no methyl resonance at 22~23 ppm. The lack of a methyl signal at ~16 and ~9 ppm excluded the mutalomycin (CH_3 at C-22) or lonomycin (CH_3 at C-22 and OCH_3 at C-23) type structure for E ring.

F-Ring

A pair of methyl signals at 16.8 and 17.2 ppm showed the absence of a methoxy at C-27. This conclusion was also supported by the chemical shift of the hemiketal carbon C-29 (96.6 ppm). Therefore, the structure of the F-ring is identical with that of carriomycin, mutalomycin and septamycin[1].

4′-O-Methylamicetose

The presence of 4′-O-methylamicetose was proved by the signals assignable to C-1′ (98.9), C-4′ (79.9), C-5′ (74.4), C-6′ (18.2) and 4′-OCH_3 (56.7 ppm). The anomeric configuration was determined to be β based on the chemical shift of C-5′. Although the attachment of the sugar to a methine was inferred from the chemical shift of the anomeric carbon, the exact position on the main framework could not be determined unambiguously by ^{13}C-nmr spectroscopy. This problem was solved by the analysis of ^{1}H-nmr spectral data (*vide infra*).

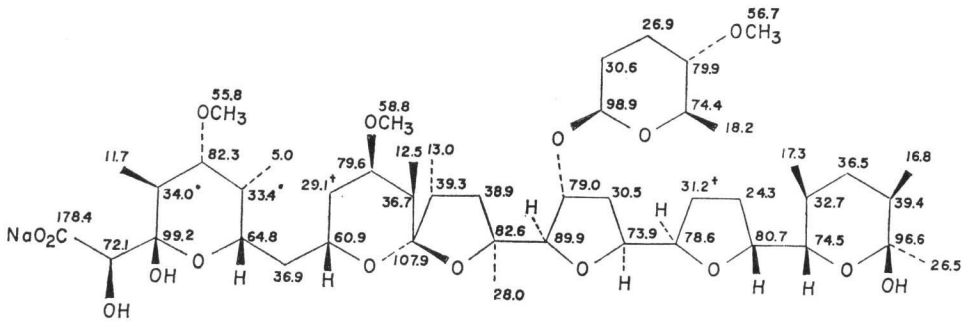
Methoxy Signals

The resonances at 55.8, 58.8 and 56.7 ppm were assigned to methoxy functions at C-5, C-11 and C-4′, respectively, and clearly revealed their absence at C-6, C-15 and C-27. These results are compatible with already established partial structures of the A to F-rings (*vide supra*).

The Position of 4′-O-Methylamicetose on the Main Carbon Skeleton

Now that the structures of A, B, C and F-rings have been established without doubt, the sugar must be placed on either the D or E-ring. Five remaining oxymethine signals to be assigned to C-17, 20, 21, 24 and the sugar bearing carbon were observed at 73.9, 78.6, 79.0, 80.7 and 89.9 ppm. Detailed comparison of these signals with

Fig. 3. The assignment of ^{13}C-nmr spectral data of 6016.
The assignments of carbons marked with ·(C-4 and 6) and +(C-10 and 22) may be exchanged.



those of carriomycin (C-17, 83.2, C-20, 78.9, C-21, 79.0 and C-24, 80.3 ppm)* suggested the sugar may be located on the D-ring. However, the lack of knowledge about the substitution effects on D-ring** prevented the position of the sugar from being determined unambiguously by ^{13}C-nmr spectroscopy.

This problem has been overcome by ^{1}H-nmr spectroscopy as follows: A sharp doublet at 3.61 ppm in the ^{1}H-nmr spectrum of the antibiotic was proved to be on the carbon at 89.9 ppm by selective proton decoupling. Moreover, this proton was coupled to an oxymethine at ~4.4 ppm. These spectral feature can only be accommodated by a D-ring with the sugar at C-18. This structure resulted in a large upfield shift of C-20 (78.9 in carriomycin→73.9 ppm in 6016) which may be rationalized by the stereochemistry with the sugar substituent and the hydrogen at C-20 on the same plane of D-ring.

Thus, the structure of 6016 has been determined as shown in Fig. 3, only leaving the stereochemistry of C-2 to be proved R by an X-ray analysis[9].

The ^{13}C-nmr spectral data of signals which were not used for the structural determination of the antibiotic are of course in good agreement with the proposed structure.

It is interesting to note that very recently K-41 has been reported to be the first polyether antibiotic with a hydroxy group on C-2[10].

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* Detailed assignment of carriomycin will be published elsewhere.

** It should be noted that none of the polyether antibiotics belong to the group under consideration possess a substituent on the D ring except for a methyl at C-20.

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