STUDIES ON THE IONOPHOROUS ANTIBIOTICS. XXVII[1] THE STRUCTURES OF TM-531B (4′-*O*-DEMETHYLDIANEMYCIN) AND TM-531C(3′-HYDROXYDIANEMYCIN), NEW POLYETHER ANTIBIOTICS CONTAINING SUGARS OTHER THAN 4-*O*-METHYL AMICETOSE

Sir:

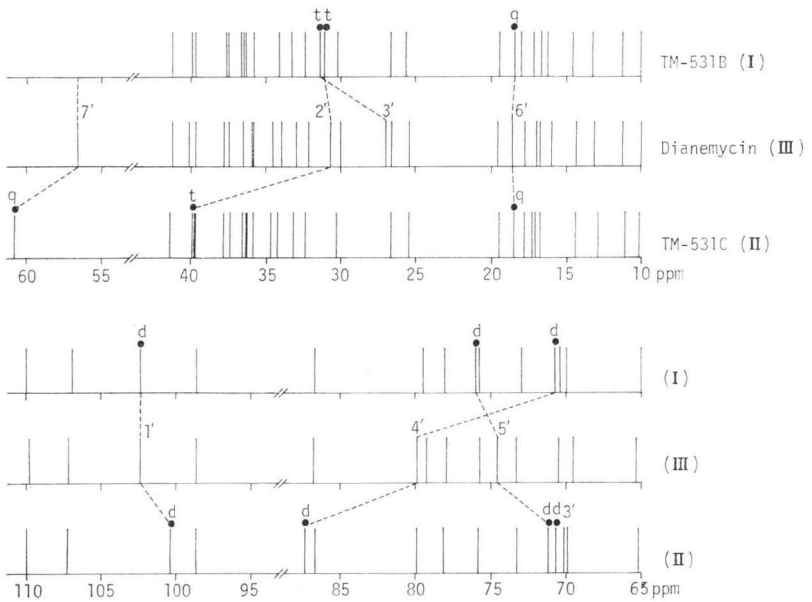
Polyether antibiotics are of considerable interest because of their significant activities against coccidia[2] and toxoplasma[3] as well as the mode of action of ion trapping across biological and artificial membranes[4]. Some of them are widely used as commercially available agents and/or biological tools.

In course of our screening program for these new substances, two polyether antibiotics TM-531B (**I**) and TM-531C (**II**) have been isolated as their sodium salts from the fermentation broth of *Streptomyces hygroscopicus* TM-531, a producing organism of leuseramycin[5] and dianemycin[6]. Their physicochemical properties revealed that both **I** and **II** are closely related congeners of dianemycin (**III**).

This communication deals with their structural elucidation mainly based on the use of ^{13}C NMR spectroscopy.

TM-531B (**I**) (m.p. 252.1~253.2°C, $[\alpha]_D^{26}+37.8°$ (*c* 0.5, CH_3OH), *Anal.* Found: C, 62.99; H, 8.71 and Na, 2.63%, Calcd. for $C_{46}H_{75}O_{14}Na$: C, 63.16; H, 8.58 and Na, 2.63%, M^+ *m/z* 874) is similar to dianemycin (**III**) in its physicochemical properties. The UV absorption at 232 nm and the IR band at 1655 cm^{-1} indicated the presence in **I** of the same α, β unsaturated ketone system as in **III**. Elementary analysis revealed that **I** contains one less carbon and two less hydrogen atoms than **III**. Furthermore, the absence of a methoxy group in **I** was suggested by its ^1H NMR spectrum as well as by the molecular ion peak (*m/z* 874) smaller than that of **III** by 14 mass units. In order to obtain further structural information, the ^{13}C NMR spectrum of **I** was compared with that of **III** (see Fig. 1 and Table 1). We have al-

Fig. 1. ^{13}C NMR spectra of TM-531B, TM-531C and dianemycin.



(I) 207.0(ketone), 184.3(carbonyl), 145.6(-CH=), 133.9(-C=)
(II) 207.0(ketone), 184.2(carbonyl), 145.4(-CH=), 133.9(-C=)
(III) 206.2(ketone), 183.8(carbonyl), 144.9(-CH=), 133.6(-C=)

* Resonances marked with ● are due to the sugar moieties.
** q: Quartet (methyl). t: Triplet (methylene). d: Doublet (methine).
*** The unambiguous assignments of oxygenated methines in the sugar moieties of **I** and **II** were given by selective proton decoupling.

Table 1. ^{13}C NMR chemical shifts of sodium salts of TM-531B, TM-531C and dianemycin taken in $CDCl_3$ at 25.05 MHz.

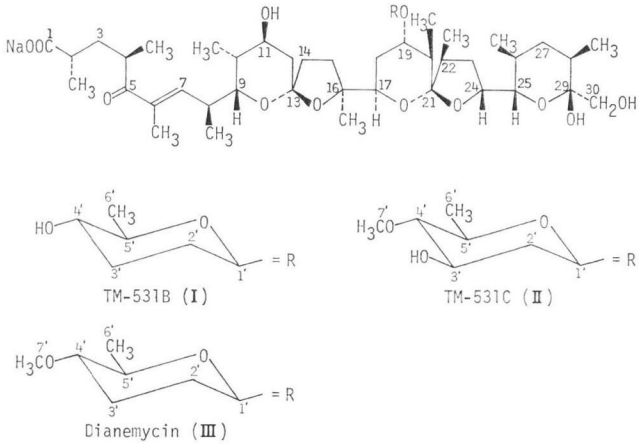
No.	TM-531B (I) Chemical shift (ppm)	TM-531C (II) Chemical shift (ppm)	Dianemycin (III) Chemical shift (ppm)	No.	TM-531B (I) Chemical shift (ppm)	TM-531C (II) Chemical shift (ppm)	Dianemycin (III) Chemical shift (ppm)
C- 1	184.3	184.2	183.8	C-25	73.2	73.1	73.2
C- 2	39.7	39.7	40.2	C-26	33.2	33.1	32.9
C- 3	41.6	41.5	41.5	C-27	36.6	36.5	36.5
C- 4	37.5	37.4	37.5	C-28	35.7	35.7	35.9
C- 5	207.0	207.0	206.2	C-29	98.8	98.7	98.5
C- 6	133.9	133.9	133.6	C-30	65.2	65.1	65.3
C- 7	145.6	145.4	144.9	C-31	16.6	16.6	16.7
C- 8	34.9	34.8	34.6	C-32	17.8	17.8	17.7
C- 9	70.0	69.9	69.6	C-33	16.2	16.1	16.1
C-10	36.3	36.3	35.9	C-34	13.0	12.9	13.1
C-11	70.3	70.2	70.4	C-35	26.7	26.6	26.6
C-12	34.1	34.1	34.0	C-36	10.0	10.0	10.0
C-13	107.2	107.1	106.9	C-37	14.5	14.5	14.4
C-14	39.6	39.7	39.7	C-38	11.2	11.2	11.2
C-15	32.3	32.3	32.2	C-39	17.1	17.1	16.9
C-16	86.9	86.8	86.6	C-40	19.3	19.3	19.5
C-17	75.9	75.8	75.7	C- 1′	102.6	100.4	102.4
C-18	37.6	37.6	37.8	C- 2′	31.1	39.7	30.6
C-19	79.5	79.8	79.2	C- 3′	31.3	70.8	27.0
C-20	37.6	37.6	37.8	C- 4′	70.8	87.3	79.9
C-21	110.0	110.0	109.8	C- 5′	76.1	71.2	74.5
C-22	36.3	36.3	35.9	C- 6′	18.3	18.3	18.5
C-23	30.2	30.1	29.9	C- 7′		60.9	56.7
C-24	78.2	78.1	77.9				

ready accomplished the complete assignments of the ^{13}C NMR spectrum of **III** on the one-to-one basis by the aid of, especially, biosynthetic methods and selective proton decoupling as well as comparison to structurally related compounds[7]. As depicted in Fig. 1, the ^{13}C NMR spectrum of **I** showed close similarity to that of **III** except for four signals due to the sugar moiety; (1) the disappearance of the methoxy resonance at 56.7 ppm, (2) the upfield shift by 9.1 ppm of the C-4′ peak (70.8 ppm in **I** and 79.9 ppm in **III**), and (3) the downfield shift by 1.6 ppm of the C-5′ signal (76.1 ppm in **I** and 74.5 ppm in **III**) and by 4.3 ppm of the C-3′ resonance (31.3 ppm in **I** and 27.0 ppm in **III**). It is noted that the anomeric, C-2′ methylene and C-6′ methyl carbon resonances due to the sugar moiety of **I** were observed in the same region as the corresponding peaks of **III**. These spectral features are reasonably explained by the structural change at C-4′, *i.e.* replacement of the methoxy group by a hydroxyl function (removal of methylation shift[8]). Taking into consideration of a negligible γ-effect on the C-2′ methylene carbon, the new substituent must be equatorially introduced at C-4′, resulting in the structure of amicetose.

This structure was confirmed by 400 MHz ^1H NMR spectroscopy.

Both the ^1H NMR spectra of **I** and **III** gave similar features except for the disappearance of the methoxy signal in **I**. ANTEUNIS *et al.*[9] have extensively investigated the solution conformation of **III** by 300M Hz ^1H NMR spectroscopy. Application of their results enabled the H-1′ and H-6′ resonances to be easily found at δ_H 4.52 (1H, dd, J=2.3, 9.7 Hz) and δ_H 1.27 (3H, d, J= 6.4 Hz), respectively. These proton resonances allowed the aforementioned assignments of C-1′

Fig. 2. Structures of TM-531B, TM-531C and dianemycin.



and C-6′ in the ^{13}C NMR spectrum of **I** to be confirmed by selective proton decoupling experiments.

Spin decoupling experiment (in $CDCl_3$ added with one drop of pyridine-d_5) irradiating at H-6′ collapsed the doublet of quartet centered at δ_H 3.27, assignable to H-5′, to a sharp doublet ($J_{4',5'}$=8.8 Hz). The large coupling constant indicated that H-5′ and H-4′ are in a *trans*-diaxial relationship. Successive irradiation at H-5′ showed the multiplet for H-4′ at $\delta_H \sim 3.25$ to be coupled with two proton at δ_H 1.48 and δ_H 2.07 by the coupling constants of *ca.* 12 and 2.5 Hz, respectively. Therefore, the hydroxyl group must be equatorially attached at C-4′.

Thus, the structure of **I** has been determined 4′-*O*-demethyldianemycin as shown in Fig 2.

TM-531C (**II**) (m.p, 190.4~190.6°C, $[\alpha]_D^{26}$+ 27.4° (*c* 0.5, CH_3OH), *Anal.* Found: C, 62.18; H, 8.60 and Na, 2.54%, Calcd. for $C_{47}H_{77}O_{15}Na$: C, 62.39; H, 8.52 and Na, 2.54%, M^+ *m/z* 904) is also similar to **III** in its physicochemical properties except for the presence of one more oxygen atom than **III**. Their structural differences were determined by analyzing their ^{13}C NMR spectra.

As shown in Fig. 1, the signals due to the aglycones of **II** and **III** were observed in the same region, suggesting that the two antibiotics have the identical aglycone. However, it was noted that the following remarkable differences between them could be found with the peaks corresponding to the sugar moiety of **II***.

(1) The methoxycarbon signal in **II** was shifted downfield by *ca.* 4.2 ppm.

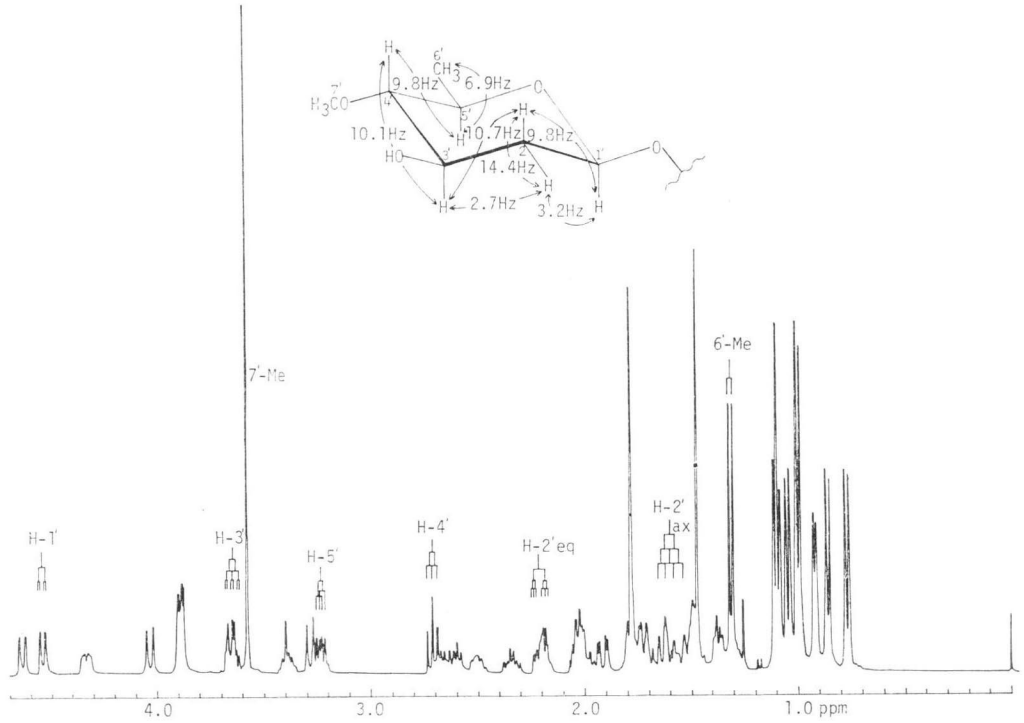
(2) One of the methylene peaks at 27.0 and 30.6 ppm in **III** disappeared and the other moved to 39.7 ppm in **II**.

(3) Three resonances assignable to oxygenated methines in **II** were observed at 70.8, 71.2 and 87.3 ppm while two signals appeared at 74.5 and 79.9 ppm in **III**. These data suggested strongly that a hydroxyl group was introduced to 4-*O*-methyl amicetose in **III**. From slight shift of the anomeric carbon signal in **II**, it was indicated that the OH and OMe groups are substituted at C-3′ and C-4′. In addition, the absence of the γ-effect on the anomeric carbon in **II** revealed that the substituent at C-3′ to be equatorially situated. The methylene carbon signal assignable to C-2′ in **II** is expected to move downfield by *ca.* 8 ppm[11] due to the effect of an equatorially attached group at C-3′. This effect would be counteracted by the γ-effect ($\gamma_{ax}-\gamma_{eq}=\sim 4$ ppm) of the substituent at C-4′ if it were present axially at C-4′. However, since the C-2′ methylene resonance was observed at 39.7 ppm, the substituent at C-4′ must be equatorially attached.

Now, the remaining problem was to assign the position of the methoxy substituent to C-3′ or C-4′. It is reported[12,13] that in inositols and pyranoses an equatorial methoxycarbon nuclei which is situated between two equatorially attached groups absorbs near 60 ppm due to δ-shielding effect whereas an analogous carbon flanked by one axial and one equatorial groups

* The resonances due to the sugar moiety of **II** could be easily distinguished from those of the aglycone by their longer relaxation times (T_1)[10]. Details will be published elsewhere.

Fig. 3. The ^1H NMR spectrum of TM-531C sodium salt measured in $CDCl_3$ at 400 MHz.



or by only one equatorial substituent resonates near 58 ppm. By the application of these rules, the OMe group resonating at 60.4 ppm was concluded to be equatorially situated at C-4′.

The structure of **II** was also confirmed by 400 MHz ^1H NMR spectroscopy.

As shown in Fig. 3, the ^1H NMR spectrum of **II** is very well separated. Based on the assignments of the ^1H NMR spectrum of **III**[9], the H-1′ and H-6′ were straightfowardly ascribed to signals at δ_H 4.54 and δ_H 1.30, respectively.

Extensive proton decoupling revealed the following relationships.

H-2′eq (δ_H 2.20, $J_{1',2'eq}$=3.2 Hz, $J_{2'eq,2'ax}$=14.4 Hz, $J_{2'eq,3'}$=2.7 Hz), H-2′ax (δ_H 1.58, $J_{1',2'ax}$=9.8 Hz, $J_{2'ax,3'}$=10.7 Hz), H-3′ (δ_H 3.63, $J_{3',4'}$=10.1 Hz), H-4′ (δ_H 2.72, $J_{4',5'}$=9.8 Hz). H-5′ (δ_H 3.22, $J_{5',6'}$=6.9 Hz)

These spectral data clearly show that all substituents other than hydrogens are equatorially orientated on the sugar moiety.

As far as we know, it is the first time that 2,6-dideoxy-4-*O*-methyl glucose was found in nature.

Thus, the structure of TM-531C (**II**) has been elucidated as 3′-hydroxydianemycin as shown in Fig. 2. Although the absolute structures of **I** and **II** remain to be clarified, it seems most reasonable to assume that they have the same configuration as dianemycin (**III**) because they have similar optical rotation values.

TM-531B and TM-531C are the first polyether antibiotics which contain sugars other than 4-*O*-methyl amicetose.

Their physicochemical and biological properties will be published elsewhere in detail.

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