STUDIES ON THE IONOPHOROUS ANTIBIOTICS. XXV†

THE ASSIGNMENTS OF THE ^{13}C-NMR SPECTRA OF DIANEMYCIN AND LENOREMYCIN

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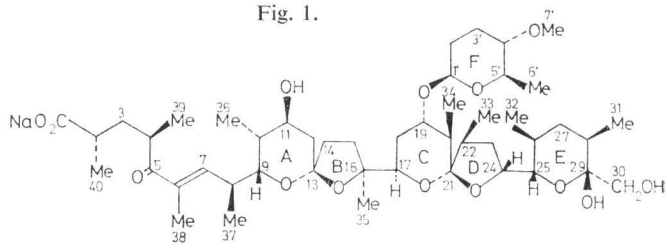
All the resonances observed in the ^{13}C-NMR spectra of polyether antibiotics, dianemycin and lenoremycin (Ro 21-6150) have been assigned by the aid of selective proton decoupling experiments, T_1 value measurements and biosynthetic methods as well as comparison to model compounds such as monensin, nigericin, etheromycin and carriomycin.

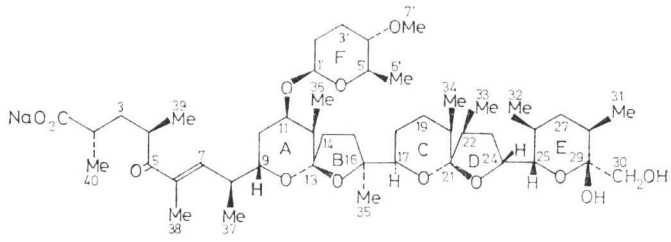
The structures and absolute configuration of several metal salts of the polyether antibiotics dianemycin[1] and lenoremycin (Ro 21-6150)[2] (Fig. 1) have been determined by X-ray analysis. Thus, the Na, K and Tl salts of dianemycin and, more recently, the Ag salt of lenoremycin have been investigated by STEINRAUF *et al.*[3] and BLOUNT *et al.*[4]

Similar to the nigericin family[5,6], these antibiotics show activities against coccidia, Gram-positive bacteria, mycobacteria and fungi[1,2,7]. Although there are structurally some distinct features from the nigericin family (see Fig. 2), dianemycin and lenoremycin show similar biological activities, *i.e,* transport of monovalent cations across biological and artificial membranes[8,9]. Hence the mode of ion trapping action in solution is of considerable interest in view of the structure-biological activity relationships of the naturally occuring ionophores. The assignments of the ^{13}C-NMR spectra of dianemycin and lenoremycin are important and necessary not only to analyze conformations, but also to

Fig. 1.



Dianemycin (**1**)



Lenoremycin (**2**)

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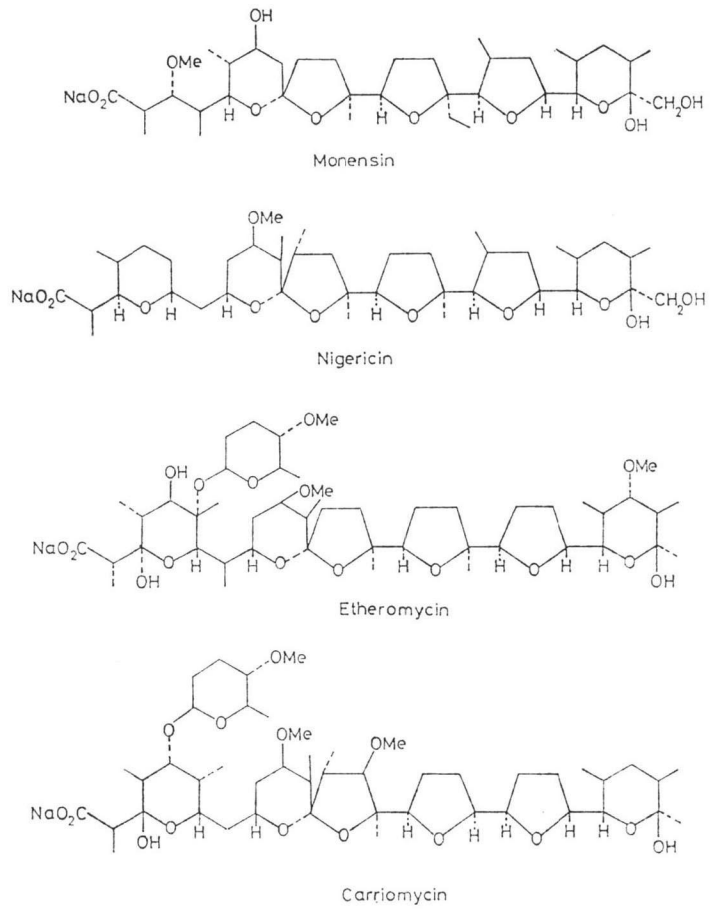
† For part XXIV, see ref. (11).

supply a convenient and rapid method for structural determination of their related antibiotics to be isolated in future[10,11]. Since such kind of activities of polyether antibiotics seem to be associated with their dynamic structures in solution, we have undertaken to analyze solution conformations of dianemycin and lenoremycin by ^{13}C-NMR spectroscopy.

In recent years, ^{13}C-NMR spectroscopy has been recognized to be a powerful and promising analytical tool for complex molecules because of the exact reflection of structural environment to the chemical shifts of the ^{13}C-NMR spectra. For example, we have established empirical rules for the structural determination of the nigericin family, including the stereochemistry, by the use of ^{13}C-NMR spectroscopy[12].

Fig. 2.



^{13}C-NMR Spectra of Dianemycin Sodium Salt (1) and Lenoremycin Sodium Salt (2)

In agreement with the established structures of dianemycin sodium salt (**1**) and lenoremycin sodium salt (**2**), the ^{13}C-NMR spectra of both **1** and **2** measured in chloroform-d_1 showed 47 resonances.

Based on multiplicity information, spin-lattice relaxation time T_1 and chemical shift trend, these signals are divided into the following functional groups: in **1**, eleven methyls at 10.0~26.6 ppm, nine methylenes at 25.4~41.5 ppm, eight methines at 32.9~40.2 ppm, a methoxyl at 56.7 ppm, a hydroxymethyl at 65.3 ppm, eight oxymethines at 69.6~79.9 ppm, a quaternary oxycarbon at 86.6 ppm, three (hemi)ketal carbons at 98.5~109.8 ppm, an anomeric carbon at 102.4 ppm, two olefinic carbons at 133.6 and 144.9 ppm, a carboxylate at 183.8 ppm and a ketone at 206.2 ppm, whereas in **2**, eleven methyls at 11.2~27.0 ppm, ten methylenes at 17.5~41.2 ppm, eight methines at 35.1~41.2 ppm, a methoxyl at 56.7 ppm, a hydroxymethyl at 64.1 ppm, seven oxymethines at 68.0~80.9 ppm, a quaternary oxycarbon at 85.8 ppm, an anomeric carbon at 102.6 ppm, three (hemi)ketal carbons at 98.5~111.1 ppm, two olefinic carbons at 134.0 and 146.2 ppm, a carboxylate at 181.1 ppm and a ketone at 207.4 ppm.

The total assignments of **1** and **2**, and T_1 values of **1** and **2** are summarized in Tables 1 and 2. In addition, the chemical shifts of dianemycin free acid are also contained in Table 1.

Table 1.

Dianemycin sodium salt (1)					Dianemycin free acid	Dianemycin sodium salt (1)					Dianemycin free acid
No.	Functionality	Chemical shift (ppm)	T_1 Value (sec)	^{13}C-Enriched	Chemical shift (ppm)	No.	Functionality	Chemical shift (ppm)	T_1 Value (sec)	^{13}C-Enriched	Chemical shift (ppm)
C 1	carbonyl	183.8	a)	1-P	179.3	C23	$-CH_2-$	29.9	0.09	1-A	29.9
C 2	$-\overset{\vert}{C}H-$	40.2	0.23		37.5	C24	–CHO–	77.9	0.22		77.2
C 3	$-CH_2-$	41.5	0.08	1-P	40.4	C25	–CHO–	73.2	0.21	1-P	73.9
C 4	$-\overset{\vert}{C}H-$	37.5	0.22		36.6	C26	$-\overset{\vert}{C}H-$	32.9	0.23		32.9
C 5	ketone	206.2	a)	1-P	204.8	C27	$-\overset{\vert}{C}H_2-$	36.5	0.11	1-P	37.3
C 6	$-\overset{\vert}{C}=$	133.6	a)		134.2	C28	–CH–	35.9	0.21[b]		34.8
C 7	=CH–	144.9	0.21	1-P	144.9	C29	$\overset{O}{O}\rangle C\langle$	98.5	a)	1-A	98.2
C 8	$-\overset{\vert}{C}H-$	37.8	0.19		37.8	C30	$-CH_2OH$	65.3	0.08		65.9
C 9	–CHO–	69.6	0.23	1-P	69.8	C31	$-CH_3$	16.7	0.36		17.3
C10	$-\overset{\vert}{C}H-$	35.9	0.21[b]		36.6	C32	$-CH_3$	17.7	0.55		17.8
C11	–CHO–	70.4	0.22	1-A	70.6	C33	$-CH_3$	16.1	0.46		16.3
C12	$-CH_2-$	34.0	0.13		33.7	C34	$-CH_3$	13.1	0.59		13.4
C13	$\overset{O}{O}\rangle C\langle$	106.9	a)	1-A	107.1	C35	$-CH_3$	26.6	0.39		27.0
C14	$-CH_2-$	39.7	0.09		39.5	C36	$-CH_3$	10.0	0.66		10.4
C15	$-CH_2-$	32.2	0.08	1-P	32.7	C37	$-CH_3$	14.4	0.36		14.7
C16	$\rangle C\langle_O$	86.6	a)		86.9	C38	$-CH_3$	11.2	1.11		11.3
C17	–CHO–	75.7	0.32	1-A	75.7	C39	$-CH_3$	16.9	0.62		16.3
C18	$-CH_2-$	25.4	0.07		25.2	C40	$-CH_3$	19.5	0.41		18.4
C19	–CHO–	79.2	0.22	1-P	80.1	C 1′	$\overset{O}{O}\rangle CH-$	102.4	0.44		102.2
C20	$-\overset{\vert}{C}H-$	34.6	0.20		34.0	C 2′	$-CH_2-$	30.6	0.25		30.8
C21	$\overset{O}{O}\rangle C\langle$	109.8	a)	1-P	109.6	C 3′	$-CH_2-$	27.0	0.16		27.1
C22	$-\overset{\vert}{C}H-$	35.9	0.21[b]		36.1	C 4′	–CHO–	79.9	0.48		80.2
						C 5′	–CHO–	74.5	0.48		74.5
						C 6′	$-CH_3$	18.5	0.84		18.4
						C 7′	$-OCH_3$	56.7	a)		56.8

a) not determined. b) average T_1 value. Due to the overlapping of these signals, individual T_1 value could not be obtained.
c) 1-P and 1-A represent 1-^{13}C sodium propionate and 1-^{13}C sodium acetate, respectively.

Table 2. Lenoremycin sodium salt (**2**).

No.	Functionality	Chemical shift (ppm)	T_1 Value (sec)	No.	Functionality	Chemical shift (ppm)	T_1 Value (sec)
C 1	carbonyl	181.3	a)	C25	–CHO–	73.1	0.35
C 2	–CH–	39.5	0.36	C26	–CH–	33.0	0.38
C 3	$-CH_2-$	41.2	0.28[b]	C27	$-CH_2-$	36.3	0.18
C 4	–CH–	37.3	0.38	C28	–CH–	36.4	0.29
C 5	ketone	207.4	a)	C29	$\overset{O}{O}\rangle C\langle$	98.5	a)
C 6	–C=	134.0	a)	C30	$-CH_2OH$	64.1	0.17
C 7	=CH–	146.2	a)	C31	$-CH_3$	17.0	0.65
C 8	–CH–	41.2	0.28[b]	C32	$-CH_3$	17.8	0.68
C 9	–CHO–	68.0	0.35	C33	$-CH_3$	15.3	0.33
C10	$-CH_2-$	28.1	0.21	C34	$-CH_3$	13.9	0.65[b]
C11	–CHO–	73.1	0.35	C35	$-CH_3$	27.0	0.48
C12	–CH–	36.4	0.29	C36	$-CH_3$	13.9	0.65[b]
C13	$\overset{O}{O}\rangle C\langle$	108.9	a)	C37	$-CH_3$	14.6	0.80
C14	$-CH_2-$	35.6	0.18	C38	$-CH_3$	11.2	1.39
C15	$-CH_2-$	32.2	0.17	C39	$-CH_3$	17.1	0.65
C16	$\rangle C\langle_O$	85.8	a)	C40	$-CH_3$	20.3	0.54
C17	–CHO–	80.9	0.40	C 1′	$\overset{O}{O}\rangle CH-$	102.6	0.44
C18	$-CH_2-$	17.5	0.21	C 2′	$-CH_2-$	30.0	0.29
C19	$-CH_2-$	26.1	0.19	C 3′	$-CH_2-$	27.5	0.20
C20	–CH–	39.6	0.33	C 4′	–CHO–	79.4	0.37
C21	$\overset{O}{O}\rangle C\langle$	111.1	a)	C 5′	–CHO–	76.1	0.48
C22	–CH–	35.1	0.32	C 6′	$-CH_3$	18.3	0.69
C23	$-CH_2-$	29.8	0.18	C 7′	$-OCH_3$	56.7	a)
C24	–CHO–	79.4	0.37				

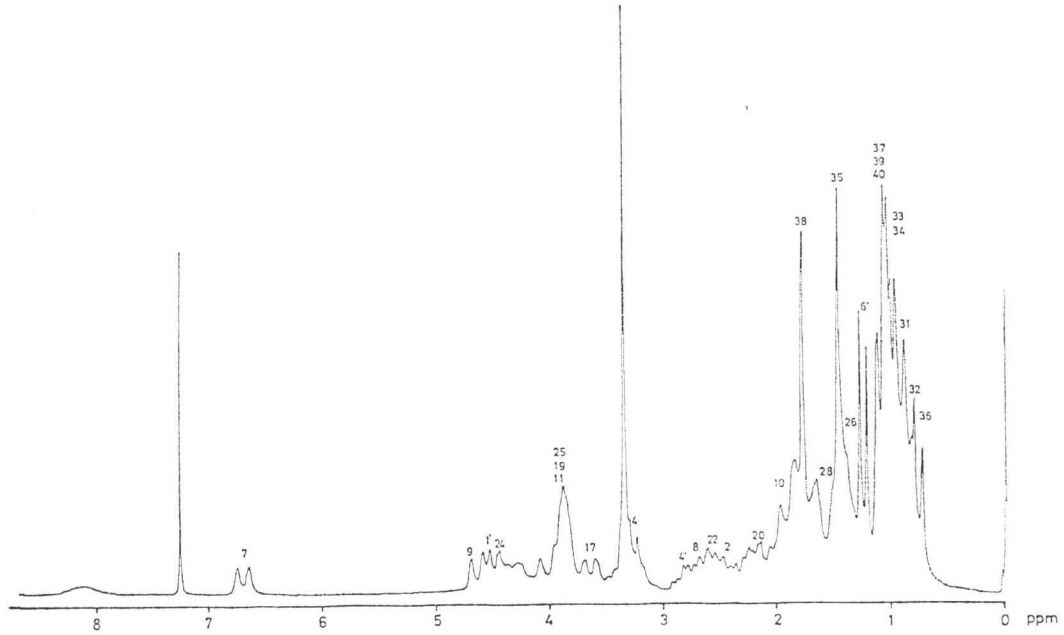
a) not determined. b) average T_1 value. Due to the overlapping of these signals, individual T_1 value could not be obtained.

Selective Proton Decoupling

The assignments of signals in the ^{13}C-NMR spectra are usually facilitated by the selective proton decoupling technique which necessitates unequivocal assignments of proton peaks to be irradiated in the ^{1}H-NMR spectra. This requirement, however, cannot be easily met with complex compounds such as **1** and **2** in the 100 MHz ^{1}H-NMR spectra.

ANTEUNIS *et al.* have extensively investigated the solution conformation of dianemycin and lenoremycin by 300 MHz ^{1}H-NMR spectrometry[13]. Application of their results enabled selective proton decoupling of the same compounds to be carried out even by a spectrometer operating at 100 MHz (with regard to ^{1}H-NMR) to some extent (see 100 MHz ^{1}H-NMR spectrum of **1** in Fig. 3). For example,

Fig. 3. 100 MHz ^{1}H-NMR spectrum of dianemycin Na. (**1**).



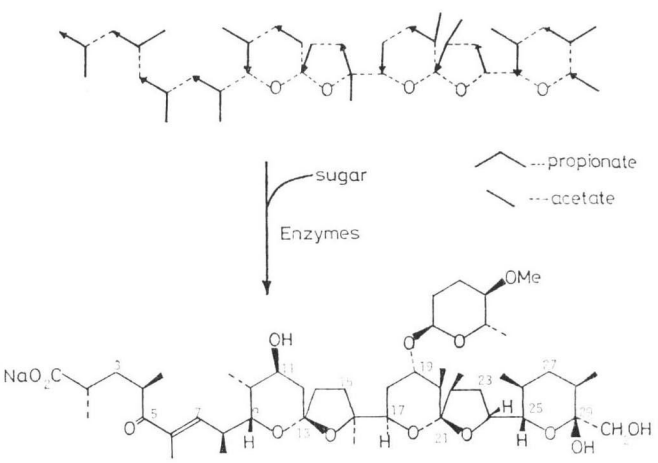
selective irradiation of H-9 (δ H 4.63), which is well separated from the other signals even in the 100 MHz spectrum, collapsed a doublet centered at 69.6 ppm to a sharp singlet. Thus, the peak can be unambiguously attributed to C9.

This selective proton decoupling technique was exploited for the assignments of methine and methyl signals in **1** and **2**.

Biosynthetic Hypothesis

Known biosynthetic studies on other polyether antibiotics[14~20] suggest the polyketide origin of **1** as depicted in Fig. 4. Thus, in **1** labeled with 1-^{13}C sodium propionate, Cl, C3, C5, C7, C9, C15, C19, C21, C25 and C27 would be enhanced, whereas C11, C13, C17, C23 and C29 would be labeled with 1-^{13}C sodium acetate. In accord with this expectation, ten and five enriched signals were observed in the ^{13}C-NMR spectra of **1** labeled with 1-^{13}C sodium propionate and 1-^{13}C sodium acetate, respectively.

Fig. 4. Biosynthesis of dianemycin.



By this method and comparison

of the ^{13}C-NMR spectra of **1** and **2** to those of model compounds, methylene and (hemi)ketal carbon absorptions were unambiguously assigned.

Comparison with Structurally Related Compounds

Comparison of the structures of **1** and **2** reveals the following structural differences between them, (i) the disposition and configuration of a methyl substituent on A-ring, *i.e.* axial at C10 in **1** and equatorial at C12 in **2**, (ii) the location of a sugar moiety at C19 in **1** and C11 in **2** as shown in Fig. 1. These structural differences between **1** and **2** give expedients for assignments of carbons around C10, C11, C12 and C19.

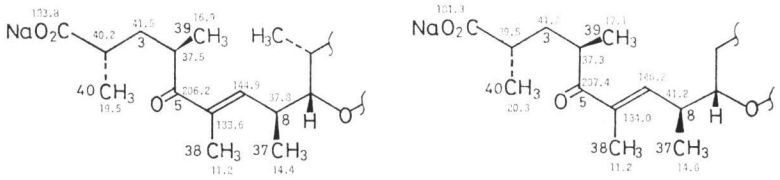
In consideration of structural similarity to **1** and **2**, monensin[21], nigericin[22] and etheromycin[23] were also used as appropriate model compounds (see Fig. 2).

Assignments of the Region C1 ~ C8

Dianemycin Sodium Salt (**1**)

The peaks at 183.8, 206.2, 133.6 and 144.9 ppm can be easily assigned to C1 (carbonyl), C5 (ketone), C6 and C7 (olefinic), respectively, by their chemical shifts and single frequency off resonance decoupling.

Fig. 5. C1 ~ C8.



Dianemycin (**1**) Lenoremycin (**2**)

Selective proton decoupling of H-2 (δ H 3.39) and H-4 (δ H 2.50) caused the doublets centered at 37.5 and 40.2 ppm to sharp singlets, respectively. However, the assignments of these two protons made by ANTEUNIS *et al.*[13] were tentative and may be exchanged. Therefore, we distinguished C2 from C4 by comparing ^{13}C-NMR spectral data of **1** and its free acid. Since the signal at 40.2 ppm in **1** moved upfield by *ca.* 3.0 ppm in dianemycin free acid, it could be assigned* to C2 leaving the signal at 37.5 ppm due to C4. This result required the ANTEUNIS's assignments of H-2 and H-4, including 39 and 40 methyl protons to be exchanged.

The selective proton decoupling spectra irradiating at H-8 (δ H 2.67) and 38 methyl protons (δ H 1.75) proved that the lines at 37.8 and 11.2 ppm must emerge from C8 and C38, respectively. Due to the similar chemical shifts of methyl protons at C37, C39 and C40 (δ H 1.05 ~ 1.10), it is impossible to discriminate between them through this technique. Since the resonance of a carbon β to a carboxylic acid would shift downfield by *ca.* 1.0 ppm upon dissociation of the carboxylic acid[24], the absorption at 19.5 ppm was ascribed to C40 by comparison with the ^{13}C-NMR spectrum of the free acid of **1**, the chemical shift of the corresponding carbon being 18.4 ppm. The other methyl signals except for the peaks at 14.4 and 16.7 ppm could be assigned and distinguished by comparison with model com-

* It is observed that in polyether antibiotics examined all the signal at α-position to a carboxylic acid shift upfield by *ca.* 3.0 ~ 4.0 ppm due to dissociation of the carbonyl carbon from Na^+ to H^+.

pounds and selective proton decoupling experiments as explained later; hence, the resonances at 14.4 and 16.7 ppm are tentatively attributed to C39 and C40, respectively. Since no reliable evidence to distinguish them is in our hand, these assignments may be exchanged.

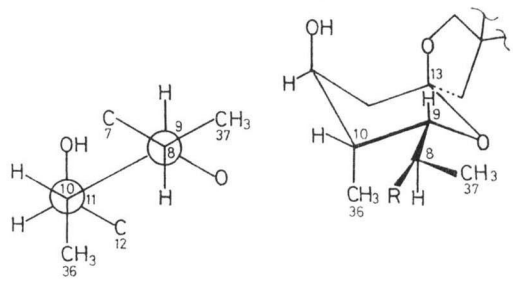
The ^{13}C-NMR spectrum of **1** labeled with 1-^{13}C sodium propionate showed three enriched methylene signals at 41.5, 32.2 and 36.5 ppm, which must be ascribed to C3, C15 or C27. C15 and C27 are assigned by comparison with monensin and nigericin as shown later. By elimination the signal at 41.5 ppm is due to C3.

Lenoremycin Sodium Salt (**2**)

In **2** the assignments for the region C1~C8 were obtained by comparison to **1** and selective proton decoupling as depicted in Fig. 5.

The signal for C8 in **2** shifts downfield by *ca.* 3.0 ppm to that for C8 in **1**. ANTEUNIS *et al.* have revealed that dianemycin (**1**) and lenoremycin (**2**) hold a tennis-ball like conformation in solution[13]. According to their investigation, the partial structure around C8 of **1** is depicted in Fig. 6 by NEWMAN projection. H-8 is disposed *syn*-axial to the methyl substituent at C10, resulting in the more shielding of C8 in **1** (γ-effect). On the other hand, there is no such steric interaction in **2** because of the absence of a substituent at C10.

Fig. 6. Dianemycin.



Assignments of A and B Rings

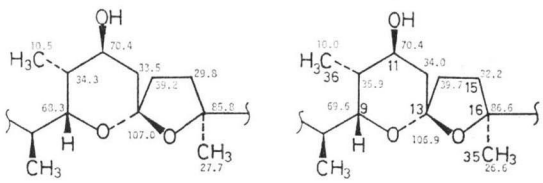
Dianemycin Sodium Salt (**1**)

Since C16 is the only quaternary oxycarbon in **1**, the line at 86.6 ppm can be assigned to C16.

The selective proton decoupling spectra irradiating at H-9 (δ H 4.63), H-10 (δ H 1.92) and 36 methyl protons (δ H 0.76) indicated that the signals at 69.6, 35.9 and 10.0 ppm can be attributed to C9, C10 and C36, respectively. The resonance for C11 could not be discriminated from those for C19 and C25 by selective proton decoupling experiments alone because of the similar chemical shifts of H-11, H-19 and H-25 (δ H 3.88~3.90). However, since only C11 was labeled with 1-^{13}C sodium acetate and C25 was assigned by comparison with nigericin (see later), their distinction can be made with ease. The downfield shift by *ca.* 3.0 ppm of the line at 70.4 ppm on acetylation supported this conclusion.

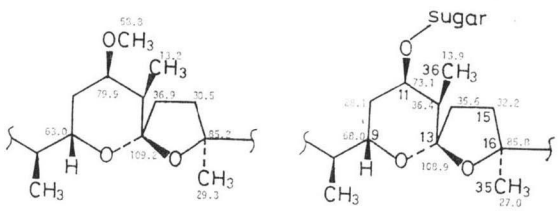
The two (hemi)ketal signals at 106.9 and 98.8 ppm enhanced by 1-^{13}C sodium acetate must be due to C13 and C29 based on biosyn-

Fig. 7a. A and B rings.



Monensin Dianemycin (**1**)

Fig. 7b. A and B rings.



Etheromycin Lenoremycin (**2**)

thetic hypothesis, and their distinction could be obtained by comparison of **1** with monensin and nigericin as shown in Fig. 7a.

In the ^{13}C-NMR spectrum labeled with 1-^{13}C sodium propionate were observed three enriched methylenes at 41.5, 32.2 and 36.5 ppm which must be assigned biosynthetically to C3, C15 and C27 as explained previously. The line at 32.2 ppm is ascribed to C15 based on comparison with monensin. Similarly, of three methylenes C12, C14 and C18 which were not labeled with either 1-^{13}C sodium propionate or 1-^{13}C sodium acetate, and which, therefore, were expected to derive from 2-^{13}C sodium acetate (experiment not carried out), C12 and C14 can be found at 34.0 and 39.7 ppm, respectively on the basis of comparison with monensin.

Since C35 exists in a sterically congested environment[3,18], its T_1 value is shorter than those of the other methyls (see Table 1). In addition, the peak for C35 would resonate at a low field region due to the binding to a quaternary carbon. Thus, the resonance at 26.6 ppm showing a shorter T_1 value is assigned to C35. Selective decoupling at δ H 1.46 and comparison with monensin also confirmed this conclusion.

The chemical shifts of C9~C16 are in excellent agreement with those of corresponding carbons of monensin as shown in Fig. 7a.

Lenoremycin Sodium Salt (**2**)

The assignments for the region C9~C16 were given by selective proton decoupling and comparison to etheromycin[25].

The selective proton decoupling spectra irradiating at H-9 (δ H 4.59), H-11 (δ H 3.60), H-12 (δ H 1.87) and 36 methyl protons (δ H 1.15) indicated that the peaks at 68.0, 73.1, 36.4 and 13.9 ppm are unambiguously attributed to C9, C11, C12 and C36, respectively.

The assignments for methylenes and a ketal C13 were obtained by comparable evidences with etheromycin; the lines at 28.1, 108.9, 35.6 and 32.2 ppm being due to C10, C13, C14 and C15, respectively.

The resonance at 85.8 ppm could be easily identified with the sole quaternary oxycarbon C16.

T_1 value of **2** (see Table 2) and comparison with **1** enabled to assign the peak at 27.0 ppm to C35.

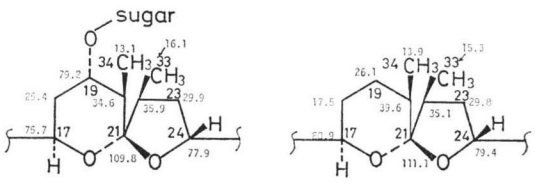
The assignments of A and B rings of **2** is shown in Fig. 7b.

Assignments of C and D Rings

Dianemycin Sodium Salt (**1**)

By selective proton decoupling expriments irradiating at H-17 (δ H 3.66), H-20 (δ H 2.18), H-22 (δ H 2.60) and H-24 (δ H 4.21) the resonances at 75.7, 34.6, 35.9 and 77.9 ppm could be assigned to C17, C20, C22 and C24, respectively. Of two oxymethines enriched by 1-^{13}C sodium acetate (C11 and C17), C11 has been attributed based on selective proton decoupling and acetylation shift (*vide supra*). Thus, the assignment of C17 could be also obtained by elimination. The assignment of C24 was furthermore corroborated by the biosynthetic experiment; C24 being

Fig. 8. C and D rings.



Dianemycin (**1**) Lenoremycin (**2**)

the only oxymethine in the aglycon moiety not labeled by either 1-^{13}C sodium propionate or 1-^{13}C sodium acetate. Since H-11, H-19 and H-25 resonate at the same region (see Fig. 3, δ H 3.88~3.90), it is impossible to discriminate between C11, C19 and C25 through the selective proton decoupling technique. The assignment of C11 had been obtained on the basis of the biosynthetic result as explained previously. The distinction between C19 and C25 could be made by comparison of **1** with nigericin (see Fig. 9); the signals at 79.2 and 73.2 ppm being ascribed to C19 and C25, respectively.

The peak at 29.9 ppm is unequivocally attributed to C23, since it is the only methylene carbon enriched by 1-^{13}C sodium acetate. Comparison of the other methylene carbons in **1** to model compounds and biosynthetic experiments using ^{13}C enriched precursors leave the only methylene signal at 25.4 ppm to be ascribed to C18, because two remaining methylene carbon peaks in the amicetose moiety can be distinguished by longer T_1 values (see Table 1).

C33 and C34 could be discriminated from the rest of the remaining methyl lines by selective proton decoupling and comparison with model compounds. However, due to the overlapping of their methyl proton resonances (δ H 0.99), distinction between C33 and C34 (16.1 and 13.1 ppm) cannot be made by the selective proton decoupling technique. On the other hand, in **2**, the absorptions for C33 (15.3 ppm) and C34 (13.9 ppm) were discriminated by selective proton decoupling experiments (*vide infra*). Taking into account of the γ-effect of the sugar on C34 in **1**, the line at 13.1 ppm which shifted upfield by 0.8 ppm can be assigned to C34. By elimination the resonance at 16.1 ppm is attributed to C33.

Two (hemi)ketal carbon peaks for C21 and C29 remain to be distinguished. The line at 109.8 ppm for C21 enriched by 1-^{13}C sodium propionate was discriminated from C29 which originated from the carbonyl carbon of acetic acid. Thus, the assignments of the region C17~C24 could be unequivocally obtained as depicted in Fig. 8.

Lenoremycin Sodium Salt (**2**)

The assignments of C17~C24 acquired by comparison to **1** and carriomycin[26], and the selective proton decoupling technique are summarized in Fig. 8. The signals for C20, C22, C24 and C33 were unambiguously assigned by selective decoupling irradiation of H-20 (δ H 2.00), H-22 (δ H 2.60), H-24 (δ H 4.40) and C33 methyl protons (δ H 0.99) as shown in Fig. 8.

Since H-17 and H-4′ of the amicetose moiety resonate at the same region (δ H 3.30), the signals at 80.9 and 79.4 ppm could be attributed to C17 and C4′, respectively, by comparison with carriomycin. The assignments of C23 and C21 were made based on comparison to the chemical shifts of the corresponding carbons in **1**; the signals at 29.8 and 111.1 ppm being due to C23 and C21, respectively.

Since no substituent at C19 is present, the methylene C18 would absorb at a higher field region than in **1**. The ^{13}C-NMR spectrum of **2** exhibits two methylene signals at 17.5 and 26.1 ppm for C18 and C19. Taking the β-effect[27] by the sugar substituent to C18 in **1** into consideration, the peaks at 17.5 and 26.1 ppm are assigned to C18 and C19, respectively.

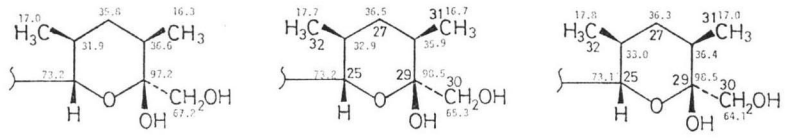
The line at 13.9 ppm was ascribed to C34 by comparison to **1** as explained previously.

Assignments of E Ring

Dianemycin Sodium Salt (**1**)

The signal at 65.3 ppm can be unambiguously identified with a hydroxylmethyl carbon at C30 by single frequency off resonance decoupling and chemical shift trend.

Fig. 9. E ring.



Nigericin Dianemycin (**1**) Lenoremycin (**2**)

The peak due to a hemiketal carbon C29 can be found at 98.5 ppm by comparison to nigericin and its chemical shift; the biosynthetic labeling of this carbon by 1-^{13}C sodium acetate being compatible with this assignment (*vide supra*).

Selective proton decoupling at H-26 (δ H 1.30), H-28 (δ H 1.50), 31 methyl protons (δ H 0.91) and 32 methyl protons (δ H 0.86) caused each absorption centered at 32.9, 35.7, 16.7 and 17.7 ppm collapsed to sharp singlets, respectively. Thus, the signals for C26, C28, C31 and C32 could be unequivocally assigned.

The line at 73.2 ppm had been attributed to C25 by comparison to nigericin as shown previously.

Since C27 originates from a propionate unit, the high intensity methylene peak at 36.5 ppm in the ^{13}C-NMR spectrum labeled with 1-^{13}C sodium propionate must emerge from C27 by analogy of monensin and nigericin.

Lenoremycin Sodium Salt (**2**)

The assignments for E ring of **2** were obtained by comparison of **2** with **1** and nigericin, and confirmed by selective proton decoupling as shown in Fig. 9.

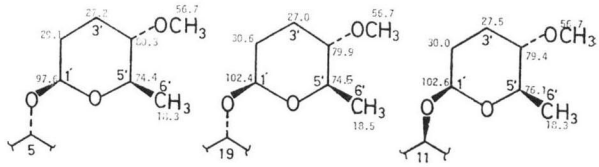
The results on E ring both in **1** and **2** are very consistent with our empirical rules[12].

Assignments of Amicetose

Dianemycin Sodium Salt (**1**)

The signals for the anomeric carbon C1′ and the methoxyl carbon C7′ can be easily found at 102.4 and 56.7 ppm by single off resonance decoupling and chemical shifts.

Fig. 10. Amicetose.



Carriomycin Dianemycin Lenoremycin

By comparison with carriomycin and antibiotic 6016[10], the methylene peaks at 27.0 and 30.6 ppm with longer relaxation time T_1 than those in the aglycon moiety[28] were ascribed to C3′ and C2′, respectively.

The absorptions for C4′, C5′ and C6′ were assigned by comparison to carriomycin[26] and antibiotic 6016[10] as well as by selective proton decoupling irradiating at H-4′ (δ H 2.82), H-5′ (δ H 3.30) and 6′ methyl protons (δ H 1.25); the resonances at 79.9, 74.5 and 18.5 ppm being attributable to C4′, C5′ and C7′, respectively as shown in Fig. 10.

Lenoremycin Sodium Salt (**2**)

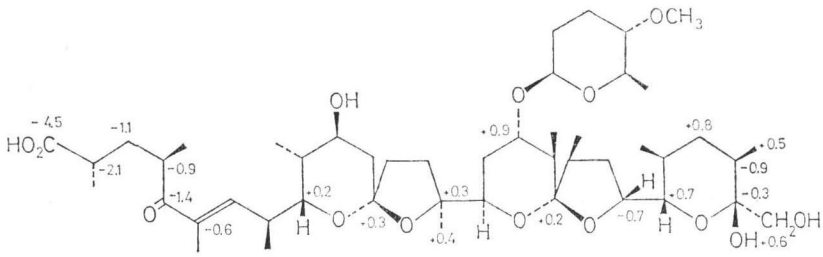
In the same way, the assignments for the amicetose moiety in **2** are given as depicted in Fig. 10.

Assignments of Dianemycin Free Acid

By the same methods used for the sodium salt, the assignments for the free acid of dianemycin were obtained.

A little differences of chemical shifts between them are observed at the region C1 ~ C6 and E ring as shown in Fig. 10. The other signals absorb at the same region as in the sodium salt. These slight variations of the chemical shifts indicate that the region C1 ~ C6 and E ring may participate in the outcome of ion trapping. From this result, it is assumed that the free acid also hold a tennis ball like form as does the sodium salt.

Fig. 11. Dianemycin free acid.



Experimental

^{13}C-NMR

The ^{13}C-NMR spectra of dianemycin sodium salt (**1**), its ^{13}C labeled dianemycin, dianemycin free acid and lenoremycin sodium salt (**2**) were run on a JEOL FX-100 spectrometer (25.05 MHz). Detail experimental conditions were as follows; spectral width 6 KHz, data points 16K, pulse width 9 μsec, repetition 1.46 sec., power level for selective ^{1}H decoupling, $\gamma H_2/2\pi = 850$ Hz. Samples were dissolved in $CDCl_3$ solution at a concentration of 120 mg/0.3 ml and not degassed.

The measurments of T_1 values were obtained by the inversion recovery method.

Dianemycin labeled with ^{13}C-enriched substrates

All fermentations were run at 30°C on a rotary shaker in 500 ml Erlenmeyer flasks containing 100 ml of the following medium.

Two ml of the spore suspension of *Streptomyces hygroscopicus* TM-531[11] were added to a medium containing oat meal 2%, glucose 1%, NaCl 0.3%, $CaCO_3$ 0.2%, meat extract 0.3%, $Fe_2(SO_4)_3$ 0.03%, $MnCl_2$ 0.03%. After 4 days incubation, 2 ml of the resulting culture were used to inoculate second stage flasks of the same medium. After 32 hours of incubation, ^{13}C enriched substrates were separately added (1-^{13}C sodium propionate, 30 mg in 100 ml medium and 1-^{13}C sodium acetate, 80 mg in 100 ml medium) to the flasks and incubation was continued for further 2 days. Two flasks were used for each experiment. To the filtered mycelium was added an equal volume of benzene, and the antibiotic was extracted with the solvent three times.

The solvent extract was evaporated *in vacuo* to give a solid which was purified by preparative TLC using the system benzene - acetone (2: 1) to give pure samples of dianemycin sodium salt labeled with 1-^{13}C sodium propionate or 1-^{13}C sodium acetate (95 mg/200 ml fermentation broth).

Dianemycin diacetate

A solution of acetic anhydride (0.5 ml) in dry pyridine (2 ml) was added to a stirred solution of dianemycin (120 mg) in dry pyridine (5 ml), and the mixture was stirred at ambient temperature for 30 hours. The reaction mixture was poured into water and extracted with $CHCl_3$. The extract was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was chromatographed on preparative TLC using the system benzene - acetone (2: 1) to give dianemycin diacetate. Its physicochemical properties are as follows; IR ($CHCl_3$) 1720, 1630 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.00 (s, 3H, $-COCH_3$), 2.08 (s, 3H,

$-COCH_3$), 3.37 (s, 3H, $-OCH_3$); Anal. Calcd. for $C_{51}H_{82}O_{15}$: C, 65.52; H, 8.78%: Found. C, 65.41; H, 8.82%.

The ^{13}C-NMR spectral data are as follows; Cl 178.6, C2 37.5, C3 39.7, C4 37.3, C5 205.1, C6 134.2, C7 144.5, C8 37.5, C9 70.5, C10 34.6, C11 73.2, C12 32.7, C13 107.1, C14 39.1, C15 32.3, C16 88.0, C17 76.1, C18 25.4, C19 80.1, C20 33.6, C21 109.5, C22 36.4, C23 31.9, C24 78.2, C25 75.5, C26 33.6, C27 37.5, C28 33.6, C29 97.2, C30 67.0, C31 17.3, C32 18.0, C33 16.1, C34 13.2, C35 26.4, C36 10.8, C37 15.4, C38 11.4, C39 16.7, C40 18.4, C1′ 102.1, C2′ 30.7, C3′ 28.1, C4′ 80.2, C5′ 74.5, C6′ 18.4, C7′ 56.8, $-COCH_3$ 21.6 and 20.8, $-COCH_3$ 171.7 and 170.5 ppm.

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References

1) HAMILL, R. L.; M. M. HOHEN, G. E. PITTENGER, J. CHAMBERLIN & M. GORMAN: Dianemycin, an antibiotic of the group affecting ion transport. J. Antibiotics 22: 161～164, 1969
2) LIU, C.-M.; R. EVANS, Jr., L. FERN, T. HERMANN, E. JENKINS, M. LIU, N. J. PALLERONI, B. L. PROSSER, L. H. SELLO, A. STEMPEL, B. TABENKIN, J. W. WESTLEY & P. A. MILLER: Studies on a new polyether antibiotic, Ro 21-6150. J. Antibiotics 29: 21～28, 1976
3) CZERWINSKI, E. W. & L. K. STEINRAUF: Structure of the antibiotic dianemycin. Biochem. Biophys. Res. Commun. 45: 1284～1287, 1971
4) BLOUNT, J. F.; R. H. EVANS, Jr., C.-M. LIU, T. HERMAN & J. W. WESTLEY: X-Ray structure of Ro 21-6150, a polyether antibiotic related to dianemycin. Chem. Commun. 1975: 853～855, 1975
5) HARNED, R. L.; P. H. HIDY, C. J. CORUM & K. L. JONES: Nigericin, a new crystalline antibiotics from an unidentified *Streptomyces*. Antibiot. & Chemoth. 1: 594～596, 1951
6) WESTLEY, J. W.: A proposed numbering system for polyether antibiotics. J. Antibiotics 29: 584～586, 1976
7) WESTLEY, J. W.: Polyether antibiotics: Versatile carboxylic acid ionophores produced by *Streptomyces*. Adv. Appl. Microbiol. 22: 177～223, 1977
8) PRESSMAN, B. C.: Ionophorous antibiotics as model for biological transport. Fed. Proc. 27: 1283～1288, 1968
9) MIYAZAKI, Y.; H. KINASHI, N. ŌTAKE, M. MITANI & T. YAMANISHI: Chemical modification and structure-activity correlation of salinomycin. Agr. Biol. Chem. 40: 1633～1640, 1976
10) SETO, H.; H. NAKAYAMA, T. OGITA, K. FURIHATA, K. MIZOUE & N. ŌTAKE: Studies on the ionophorous antibiotics. XXI. Structural elucidation of a new polyether antibiotic 6016 by application of the empirical rules in ^{13}C-NMR spectroscopy. J. Antibiotics 32: 244～246, 1979
11) MIZUTANI, T.; M. YAMAGISHI, H. HARA, A. KAWASHIMA, S. ŌMURA, M. ŌZEKI, K. MIZOUE, H. SETO & N. ŌTAKE: Studies on the ionophorous antibiotics. XXIV. Leuseramycin, a new polyether antibiotic produced by *Streptomyces hygroscopicus*. 33: 137～143, 1980
12) SETO, H.; K. MIZOUE, H. NAKAYAMA, K. FURIHATA, N. ŌTAKE & H. YONEHARA: Studies on the ionophorous antibiotics. XX. Some empirical rules for structural elucidation of polyether antibiotics by ^{13}C-NMR spectroscopy. J. Antibiotics 32: 239～243, 1979
13) ANTEUNIS, M. J. O.; N. A. RODIOS & G. VERHEGGE: Solution conformation of dianemycin, its sodium salt and of lenoremycin-Na^+. Bull. Soc. Chim. Belg. 86: 609～632, 1977
14) WESTLEY, J. W.; D. L. PRUESS & R. G. PITCHER: Incorporation of ^{13}C-butyrate into antibiotic X-537A (lasalocid). Chem. Commun. 1972: 161～162, 1972
15) WESTLEY, J. W.; R. H. EVANS, Jr., G. HARVEY, R. G. PITCHER, D. L. PRUESS, A. STEMPLE & J. BERGER: Biosynthesis of lasalocid. Incorporation of ^{13}C and ^{14}C labeled substrates into lasalocid A. J. Antibiotics 27: 288～297, 1974
16) DAY, L. E.; J. W. CHAMBERLIN, E. Z. GORDEE, S. CHEN, M. GORMAN, R. L. HAMILL, T. NESS, R. E. WEEKS & R. STROSHANE: Biosynthesis of monensin. Antimicr. Agents & Chemoth. 4: 410～414, 1973

17) DORMAN, D. E.; J. W. PASHAL, W. M. NAKATSUKASA, L. L. HUCKSTEP & N. NEUSS: The use of ^{13}C-NMR spectroscopy in biosynthetic studies. II. Biosynthesis of narasin, a new polyether ionophore from fermentation of *Streptomyces aureofaciences*. Helv. Chim. Acta 59: 2625~2634, 1976
18) SETO, H.; Y. MIYAZAKI, K. FUGITA & N. ŌTAKE: Studies on the ionophorous antibiotics. X. The assignment of the ^{13}C-NMR spectrum of salinomycin. Tetrahed. Lett. 1977: 2417~2420, 1977
19) ŌTAKE, N.; H. SETO & M. KOENUMA: The assignment of the ^{13}C-nmr spectrum of lysocellin and its biosynthesis. Agr. Biol. Chem. 42: 1879~1886, 1978
20) MIZOUE, K.; H. SETO & N. ŌTAKE: The assignment of the ^{13}C-NMR spectrum of lonomycin A and its biosynthesis. in preparation
21) AGTARAP, A. & J. W. CHAMBERLIN: Monensin, a new biologically active compound. IV. Chemistry. Antimicr. Agents & Chemoth. -1967: 359~362, 1968
22) STEINRAUF, L. K.; M. PINKERTON & J. W. CHAMBERLIN: The structure of nigericin. Biochem. Biophys. Res. Commun. 33: 29~31, 1968
23) KUSAKABE, Y.; C. NAGATSU, H. AKUTSU, C. HIROSE, M. MIYAMOTO & A. SEINO: The studies on a new antibiotic C20-12. Abstract Papers of Annual Meeting of the Agri. Chem. Soc., Japan p. 83, Sapporo, 1975
24) LEVY, G. C. & G. L. NELSON: Carbon-13 Nuclear Magnetic Resonance for Organic Chemists. Willey-Interscience, New York, 1973
25) SETO, H. & N. ŌTAKE: Submitted for publication.
26) NAKAYAMA, H.; H. SETO & N. ŌTAKE: in preparation
27) STOTHERS, J. B.: Carbon-13 NMR Spectroscopy. Academic Press, New York, 1972
28) ALLERHAND, A. & D. DODDRELL: Strategies in the application of partially relaxed FOURIER transform nuclear magnetic resonance spectroscopy in assignments of carbon-13 resonances of complex molecules. Stachylose. J. Am. Chem. Soc. 93: 2777~2781, 1971