THE ^{13}C-NMR SPECTRA OF POLYETHER ANTIBIOTICS AND SOME EMPIRICAL RULES FOR STRUCTURAL STUDIES OF POLYETHER ANTIBIOTICS

Haruo Seto* and Noboru Ōtake

Institute of Applied Microbiology, the University of Tokyo, Bunkyo-ku, Tokyo, Japan

Dedicated to Prof. Kyosuke Tsuda on the occasion of his 75th birthday

Abstract -- The ^{13}C-nmr spectral assignments of a subgroup of the polyether antibiotics including lonomycin, mutalomycin, carriomycin, etheromycin, A204A, septamycin, nigericin and monensin are summarized. Based on these spectral data, some empirical rules for structural elucidation of polyether antibiotics have been established and their application to a new antibiotics is presented.

INTRODUCTION

The polyether antibiotics characterized as a class of ionophores possessing several cyclic ether systems are mainly produced by the Streptomyces genus[1]. Due to structural complexity, difficulty to obtain degradation products useful for structural studies, and extensively overlapping ^{1}H-nmr spectra, X-ray analysis has been the only practical method for structural elucidation of these compounds. Until a few years ago, on the other hand, ^{13}C-nmr spectroscopy which has played an important role in natural products chemistry was not a method of choice for structural studies of polyether antibiotics apparently due to the difficulty in analyzing very complicated ^{13}C-nmr spectra. This obstacle has been overcome in recent years by the aid of biosynthetic labeling method[2] as well as Anteunis' extensive ^{1}H-nmr works[3-9] which enable unambiguous assignments of the ^{13}C signals to be made through selective proton decoupling. As a result, enough ^{13}C-nmr spectral data have accumulated to establish the relationships between specific structures and chemical shifts of relevant carbons in the ^{13}C-nmr spectra of polyether antibiotics[10]. Thus, ^{13}C-nmr spectroscopy which allows to observe almost all carbon resonances without overlapping is now becoming a very attractive methodology for researchers working in the field of poyether antibiotics.

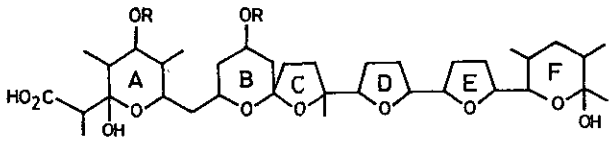
In this review, the ^{13}C assignments of a group of the polyethers will be presented together with

their application to structural studies. These data will be useful for analyzing solution conformations of the polyether antibiotics, their interaction with metal cations and structures of new antibiotics to be isolated in future.

CLASSIFICATION OF POLYETHER ANTIBIOTICS

The polyether antibiotics are divided into five major groups depending on the characteristic partial structures which are reflected in the chemical shifts of ^{13}C-nmr spectra. Representatives of each group are shown in Fig. 1. In this review, special emphasis will be given to the explanation of ^{13}C-nmr spectra of lonomycin-type polyether antibiotics. The ^{13}C-nmr spectral data reported for the members of other groups include lasalocid[11], salinomycin[12], narasin[13], noboritomycin[14], X-14766A[15], lysocellin[16], dianemycin[17], lenoremycin[17](A-130A)[18], leuseramycin[19], A23187[20] and ionomycin[21].

There exists the following carbon skeleton common to the all members of the lonomycin-type antibiotics with the exception of monensin[22] and laidlomycin[23]. The antibiotics of this group possess

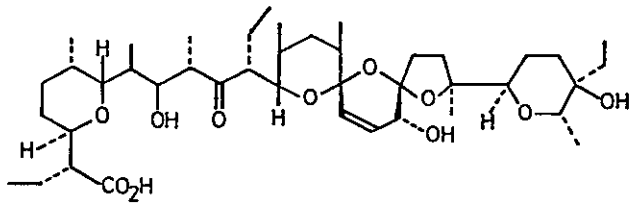


methoxy carbons appearing at 50-62 ppm, more frequently at 55-60 ppm except for laidlomycin. The number of the methoxy function ranges from one (mutalomycin)[24] to five (K-41A and K-41B)[25]. This groups is further divided into the following subgroups. The members of goups 1 and 2 contain three (hemi)ketal carbons whereas those of groups 3 and 4 possess two (hemi)ketal functions.

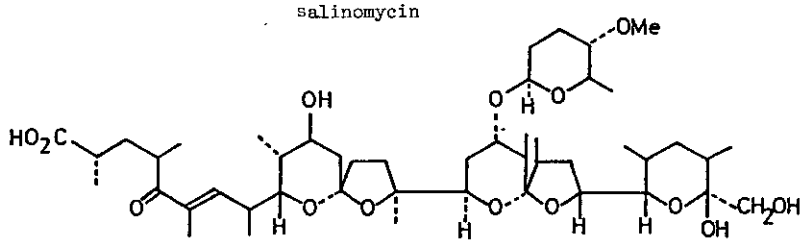
1) Lonomycin[26] and mutalomycin[24] without a deoxysugar (Fig. 5).
2) Carriomycin[27], A204A[28], etheromycin[4], septamycin[29](A28695A)[30], A28695B[30], K-41A[25] and K-41B with one or two deoxysugars (Fig. 9). The resonance of an acetal signal (95-103 ppm) due to O-methylamicetose is found only in this group.
3) Nigericin[32] and grisorixin[33] (Fig. 11) lacking a hemiketal function at C3 which appears at 99-100 ppm in group 1.
4) Monensin[22] and laidlomycin[23] (Fig. 12) without A-ring common to the other members of groups 1 to 3.

TECHNIQUES USED FOR ^{13}C ASSIGNMENTS OF POLYETHER ANTIBIOTICS

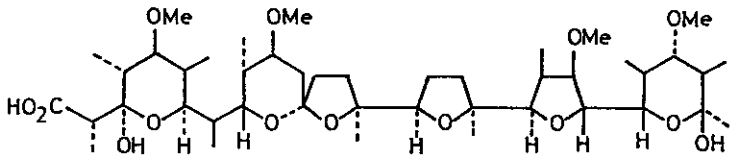
One of the structural features of most polyether antibiotics is the repetition of similar partial structures in the molecules which results in complicated ^{13}C-nmr spectra with many methylene and methine signals congested in a narrow region. Therefore, in addition to commonly employed



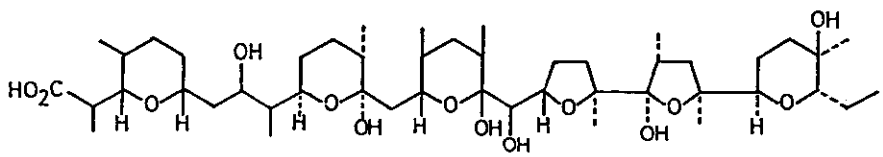
salinomycin



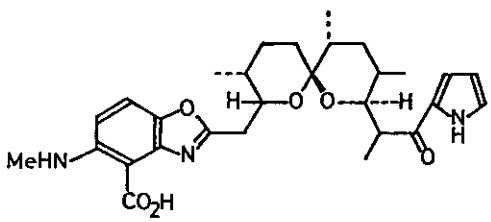
dianemycin



lonomycin



X-206



A23187

Fig. 1. Representative members of various groups of the polyether antibiotics.

techniques such as single frequency off resonance decoupling (sford) and selective proton decouping, partially relaxed Fourier transform (PRFT) nmr techniques and biosynthetic methods are required to obtain total assignments of ^{13}C-nmr spectra of polyether antibiotics. Some explanation of these methods will be given in the following.

A. Differentiation of methyl, methylene, methine and quaternary carbon signals

The first step to analyze ^{13}C-nmr spectra is to discriminate methyl, methylene, methine and quaternary carbons. This can be accomplished mostly by sford or sometimes by weak noise off resonance decoupling which enables to detect non-protonated carbons[34]. In case of polyehter antibiotics, however, sford is not necessarily a useful technique to identify methylenes and methines (and occasionally tert-methyls) in the region of ca. 25-40 ppm due to extensive overlapping of signals under the sford conditions (see Fig. 2e). Higher order coupling as well as unequivalent chemical shifts of some methylene protons make the situation even worse[34].

This problem can be solved in most case by the PRFT nmr technique[35]. In this method, a 90° monitor pulse is applied to the sample under investigation after some interval time following a 180° pulse which is utilized to invert ^{13}C signals. This pulse sequence is repeated until satisfactory signal-to-noise ratio is obtained. During the waiting time between the two pulses, ^{13}C signals begin to recover to the original state according to their longitudinal relaxation times T_1[36]. Since the relaxation time T_1 usually increases in the order of $CH_2 < CH \leqq CH_3 < -C-$,

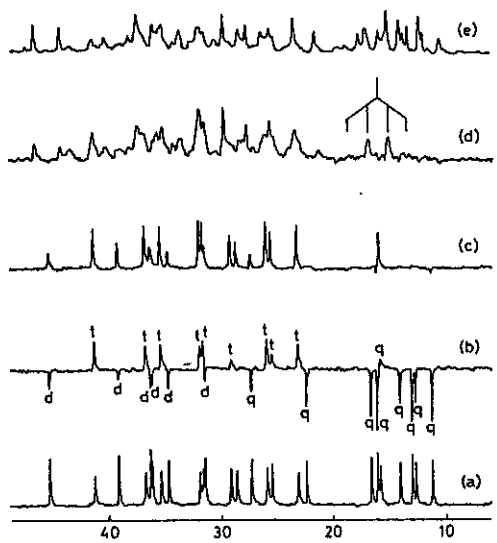


Fig. 2. ^{13}C-Nmr spectra of nigericin sodium salt in $CDCl_3$. (a) proton noise decoupled, (b) PRFT spectrum (waiting time, 0.2 sec), (c) PRFT spectrum (waiting time, 0.35 sec), (d) off-resonance decoupled under PRFT condition (waiting time 0.35 sec) and (e) off-resonance decoupled. q, t and d in (b) represent methyl, methylene and methine, respectively.

these signals can be distinguished from each other by setting an appropriate waiting time. For example, in the PRFT spectrum of nigericin sodium salt[37] (Fig. 2b, waiting time 0.20 sec) methylene resonances appear as positive signals while the methine and methyl peaks remain negative. It should be noted that the discrimination of these signals can be hardly made by sford (Fig. 2e). Although time-consuming, one can obtain more detailed information by measuring relaxation time T_1 which further enables to distinguish carbon resonances due to the deoxysugar showing longer T_1 values from those to the aglycone with much shorter T_1 values in glycosidic polyether antibiotics (group 2, see later). Detailed explanation was given by Wehrli[38] to the use of ^{13}C spin-relaxation data in organic structure assingments.

In special cases, observation of sford spectra under a PRFT condition (Fig. 2d) or selective excitation condition[39] may be necessary to analyze overlapping signals. The latter technique facilitates to observe signals in a very narrow range selectively; thus, preventing undesirable overlapping of unnecessary peaks.

INEPT[40], a new technique introduced very recently which is not yet applied to the nmr studies of the polyether antibiotics, will become the most powerful tool to distinguish methyl, methylene, methine and quaternary carbons in very near future.

B. Selective proton decoupling

This method is widely utilized in the assignments of ^{13}C-nmr spectra. The prerequisites for successful selective proton decoupling are well separation and detailed assignments of ^1H signals to be irradiated in the ^1H-nmr spectra. As explained later, most ^{13}C-nmr works were made by using spectrometers operating at 25 MHz (100 MHz with regard to ^1H resonance frequency). Under such experimental conditions, these requirements can be hardly satisfied due to overlapping of ^1H signals (Fig. 3).

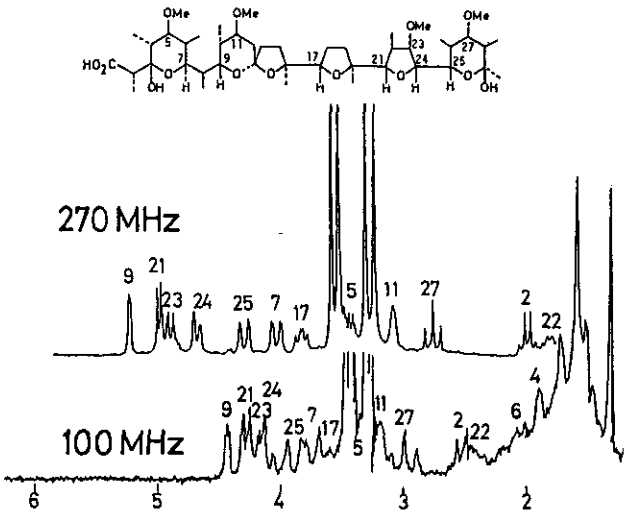


Fig. 3. ^1H-Nmr spectra of lonomycin A sodium salt taken at 100 and 270 MHz in $CDCl_3$.

On the other hand, by taking advantage of better separation obtained by nmr instruments operating at a higher magnetic field, Anteunis et al. made almost complete analysis of ^{1}H signals excepting for some methylenes of the following antibiotics; carriomycin[5], etheromycin[4], lonomycin[6], septamycin[7], nigericin[8] and monensin[9].

Thus, the assignments of ^{13}C resonances of these antibiotics were made possible by selective proton decoupling. For example, based on their work about lonomycin A, signals due to H2, H5, H7, H9, H11, H17, H22, H25 and H27 could be assigned as shown in Fig. 3 and the carbons appended to them were unambiguously identified by selective proton decoupling experiments. Although the protons combining to C21, C23 and C24 are not well separated from each other, it can be seen from the Figure that these carbons could be distinguished as a set from the remaining carbons by this technique. Rigorous assignments of C21, C23 and C24 were obtained by the use of other methods such as comparison to structurally related compounds or biosynthetic method. Useful explanation on selective proton decoupling experiments was given in detail by Johnson[41].

C. Biosynthetic labeling method

It has been well recognized that ^{13}C-nmr spectroscopy in combination with the use of ^{13}C labeled precursors is a very useful tool to investigate the biosynthesis of natural products[42]. Utilization of ^{13}C-^{13}C couplings has made this technique more attractive not only for studying reaction mechanisms such as rearrangement involved in the biosynthetic process, but also for its power in making assignments of ^{13}C signals of complex molecules[2].

Biosynthetic studies reported for other groups of the polyether antibiotics such as lasalocid[11], salinomycin[12], narasin[13], lysocellin[16] and dianemycin[17] by ^{13}C-nmr spectroscopy have revealed that these metabolites are derived from lower fatty acids such as acetic acid, propionic acid and butyric acid. Polyketide origin of monensin was also confirmed by ^{14}C tracers[43].

Taking account of structural similarities between the polyether antibiotics, it can be reasonably assumed that all the members of this group would also be built up from the same biosynthetic precursors. This hypothesis enables one to choose a proper precursor to increase selectively signal intensities of desired carbons, thereby facilitate to make easier and reliable ^{13}C assignments. An application of this technique for analyzing ^{13}C chemical shifts of lonomycin A is explained in the following.

Based on the hypothesis, lonomycin A is expected to derive from five acetate and ten propionate units as shown in Fig. 4. Therefore, use of $CH_3{}^{13}COOH$ will enable to distinguish three oxymethines C11, C17 and C23, and two (hemi)ketals C13 and C29 from the remaining carbons. Furthermore, the carbons adjacent to these functional groups can also be identified by $^{13}CH_3{}^{13}COOH$ which causes ^{13}C-^{13}C couplings to occur between C11-C12, C13-C14, C17-C18, C23-C24 and C29-C30. It should be noticed in this case that the specific partial structures of $O\text{-}C(O)\text{-}CH_2$, O-CH-CH-O and $O\text{-}C(O)\text{-}CH_3$

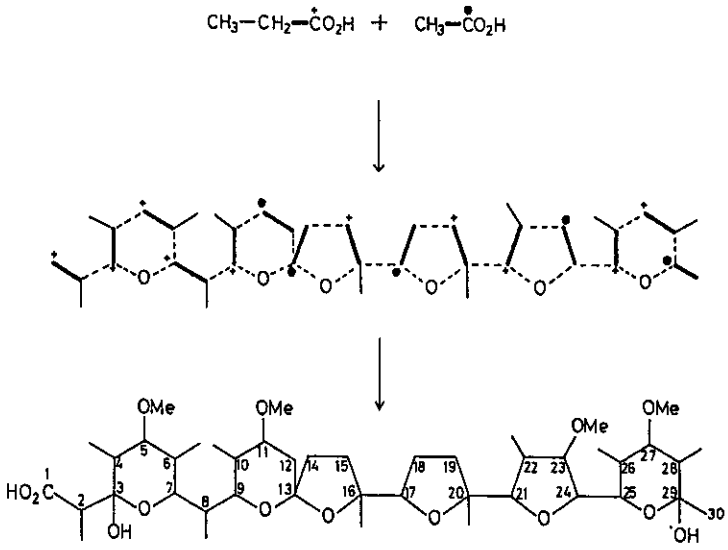


Fig. 4. Biosynthetic pathway of lonomycin A.

are only found at C13-C14, C23-C24 and C29-C30 to give conclusive evidences for the unambiguous assignments of these carbons.

Distinction of the two combinations between an oxymethine and a methylene, i.e. C11-C12 and C17-C18 can be made with ease by selective proton decoupling experiments irradiating at the H11 or H17 proton. Although the H11 and H17 signals are overlapped by other signals in the 100 MHz ^{1}H-nmr spectrum as shown in Fig. 3, separation of these two signals is large enough for selective proton decoupling to be successfully carried out at 25 MHz.

By analogy, it will be easily understood from Fig. 4 that $CH_3CH_2{}^{13}COOH$ and $CH_3{}^{13}CH_2{}^{13}COOH$ can be a great help for signal assignments of the remaining carbons, C1-C2, C3-C4, C5-C6, C7-C8, C9-C10, C15-C16, C19-C20, C21-C22, C25-C26 and C27-C28.

As mentioned earlier, the assignments of methylene carbon signals by selective proton decoupling are frequently distressed by severe overlapping and unequivalent chemical shifts of methylene protons to be irradiated. Biosynthetic labeling, on the other hand, will supply a reliable method to obtain unambiguous assignments of methylene carbons.

In addition to the antibiotics mentioned earlier, this technique has been also used for analyzing ^{13}C-nmr spectra of 6016 and nigericin and the results are contained in Tables 1 and 3.

^{13}C-NMR SPECTRA OF POLYETHER ANTIBIOTICS

For convenient comparison of the ^{13}C-nmr spectral data of polyether antibiotics, the numbering systems proposed by Westley[45] and Westley et al.[46] are employed in this review with a modification

that alkyl or methoxy substituents are referred to the numbers of the carbons to which they attach. This makes it easier to correlate the chemical shifts of methyl, ethyl or methoxy carbons in similar environments, but with different numbers by the proposed systems.

Most ^{13}C-nmr works described in this article were made at 25 MHz in $CDCl_3$ or C_6D_6 solution using tetramethylsilane as internal standard. It should be kept in mind that ^{13}C chemical shifts are somewhat dependent on sample concentration, temperature and solvents. Dorman et al.[13] described that even small amount of water contained in $CDCl_3$ affected the ^{13}C chemical shift of narasin, a polyether antibiotic belonging to another group. Therefore, care must be taken when one compares experimental data with literature values.

Group 1: lonomycin and mutalomycin (see Table 1 and Fig. 5)

The complete assignment of lonomycin A sodium salt was obtained by biosynthetic labeling as described above[44]. The results (Table 1) were exploited to establish "some empirical rules for the structural elucidation of polyether antibiotics"[10] which will be explained later.

Precise selective proton decoupling and comparison to mutalomycin also facilitated to assign methyl and methine resonances which were not enriched by ^{13}C labeled precursors. The ^{1}H-nmr study of lonomycin had been reported by Rodios and Anteunis[6].

Lallemand et al.[47] carried out selective proton decoupling of emericid (identical with lonomycin A) using a spectrometer operating at 62.9 MHz to obtain the almost identical results more easily. Some methylene and methoxy signals, however, remain unassigned. More recently, they reported the advanced assignments of the antibiotic by a new simulation technique[48].

^{13}C-Nmr spectral analysis of mutalomycin was accomplished based on the established chemical shifts of lonomycin carbon signals[44]. During this study, it was found that the configuration of C4 of mutalomycin reported earlier must be revised as shown in Fig. 5.[49]

[The structures of lonomycins B and C]

Lonomycins B (B) and C (C) were isolated from the fermentation broth of Streptomyces ribosidificus as minor components of lonomycin A (A)[50]. B was easily converted to A in organic solvents such as acetone and ethyl acetate at room temperature, whereas the reverse reaction under the same condition was almost negligible. This phenomenon together with the identical mass spectra of the both compounds implies that these two antibiotics are interconvertible through inversion at a ketal or hemiketal function.

Seto et al.[51] determined the position in question by ^{13}C-nmr spectroscopy. As shown in Fig. 6, the ^{13}C-nmr spectra of A and B are very similar suggesting for the most part that the two antibiotics are identical. However, the following remarkable differences between them were observed at around C3 hemiketal carbon.

Table 1. ^{13}C Chemical shifts of sodium salts of lonomycin[1], mutalomycin[1], etheromycin[2], A204A[2,3], carriomycin[2], septamycin[2,4] and 6016[2] in $CDCl_3$.

carbon	functionality	lonomycin	mutalomycin	etheromycin	A204A	carriomycin	septamycin	6016
1	COOH	181.5[a]	181.2	180.7	180.3	180.4	180.2	178.4[b]
2	CH	46.0	47.1	45.1	45.5	46.0	45.3	72.1
3	O-C-OH	100.4[a]	99.6	99.5	99.3	100.1	99.4	99.2[a]
4	CH	35.4	35.0	40.2[+]	41.1	35.2[+]	39.5	34.0[+]
5	CH-O	82.2[a]	82.0	76.6	79.5	77.6	88.5	82.3[a]
6	CH or C-O	31.1	31.1	82.7	77.3	34.6[+]	80.0	33.4[+]
7	CH-O	70.8[a]	71.5	71.4	64.1	64.0	67.3	64.8[b]
8	CH or CH_2	37.7	36.6	39.7[+]	32.7	37.3	32.4	36.9
9	CH-O	63.3[a]	64.1	63.0	61.2	61.2	61.4	60.9[b]
10	CH or CH_2	33.7	40.2	32.5*	31.2*	31.3*	31.1*	31.2*
11	CH-O	82.0[b]	70.3	79.9	79.3	79.4	79.5	79.6[a]
12	CH_2 or CH	34.1[c]	33.4[+]	39.0[+]	36.8	36.7	36.7	36.7
13	O-C-O	107.1[b]	106.8	109.2	106.5	106.4	106.4	107.9[a]
14	CH_2 or CH	39.4[c]	39.0	36.9	46.0[+]	45.9	46.0	39.3
15	CH_2 or CH-O	33.5[a]	33.3[+]	32.9	94.4	94.4	94.5	38.9[a]
16	C-O	84.2	84.3	85.2	83.1	82.9	83.0	82.6
17	CH-O	81.4[b]	81.6	83.0	83.2	83.2	83.2	89.9[b]
18	CH_2	25.9[c]	26.9	24.4	22.9	23.0	23.0	79.0
19	CH_2	30.4[a]	30.7	30.5	25.5	25.6	25.6	30.5[b]
20	C-O or CH-O	85.8	83.8	86.1	78.7	78.9	78.9	73.9
21	CH-O	84.3[a]	86.4	84.0	79.1	79.0	79.1	78.6[b]
22	CH or CH_2	36.1	34.3	29.8*	29.1*	29.1*	29.1*	29.1*
23	CH_2 or CH-O	80.5[b]	32.3	24.4	24.1	24.1	24.1	24.3[b]
24	CH-O	79.8[c]	78.8	80.7	80.0	80.3	80.2	80.7
25	CH-O	73.8[a]	73.4	73.6	73.7	75.3	75.2	74.5[a]
26	CH	37.7	33.1	39.3	39.2	32.7	32.7	32.7
27	CH_2 or CH-O	84.0[a]	36.4	84.6	84.4	36.8	36.8	36.5[a]
28	CH	46.6	40.2	46.3	46.2[+]	39.6	40.6	39.4
29	O-C-O	98.8[b]	96.5	98.4	98.1	96.6	96.6	96.6[b]
30	CH_3	26.5[c]	25.7	26.6	26.5	26.4	26.4	26.5

continued on the next page

continued from the previous page

carbon	functionality	lonomycin	mutalomycin	etheromycin	A204A	carriomycin	septamycin	6016
2-Me		11.5	11.7	11.0	11.5	11.5	11.5	---
4-Me		11.1	11.1	11.8	13.1	11.7	11.9	11.7
6-Me		4.1	4.1	8.1	12.6	5.5	9.9	5.0
8-Me		10.2	10.0	11.8	---	---	---	---
10-Me		12.0	10.6	---	---	---	---	---
12-Me		---	---	13.2	12.5	12.6	12.6	12.5
14-Me		---	---	---	11.5	11.6	11.5	13.0
16-Me		29.2	29.0	29.2	28.4	28.4	28.4	28.0
20-Me		22.3	23.2	22.6	---	---	---	---
22-Me		9.0	16.2	---	---	---	---	---
26-Me		13.8	17.6	13.6	13.5	17.3	17.3	17.3
28-Me		12.5	17.0	12.4	12.5	16.9	16.9	16.8
5-OMe		56.0	55.8	---	---	---	61.5	55.8
6-OMe		---	---	---	49.5	---	---	---
11-OMe		58.6	---	58.8	58.8	58.8	58.7	58.8
15-OMe		---	---	---	60.0	60.0	60.0	---
23-OMe		57.3	---	---	---	---	---	---
27-OMe		59.9	59.6	59.7	---	---	---	---
deoxysugar at				C-6	C-5	C-5	C-6	C-18
1'				95.2	98.3	97.6	96.3	98.9
2'				28.7	29.8	30.8	31.8	30.6
3'				27.3	23.3	27.2	27.6	26.9
4'				79.9	81.2	80.3	80.0	79.9
5'				74.7	68.2	74.4	74.1	74.4
6'				18.2	18.5	18.3	18.5	18.2
4'-OMe				56.9	56.2	56.7	56.6	56.7

+, * Assignments may be interchanged.

[a] enriched by $CH_3CH_2{}^{13}COOH$, [b] enriched by $CH_3{}^{13}COOH$, [c] enriched by ${}^{13}CH_3COOH$.

1) see ref. 44. 2) see ref. 37 and 10. 3) see ref. 25. 4) see ref. 30.

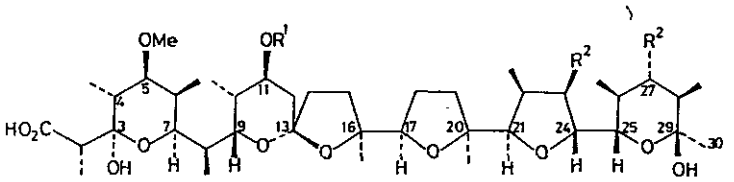


Fig. 5. The structures of lonomycin A (R^1=CH_3, R^2=OCH_3) and mutalomycin (R^1=R^2=H)

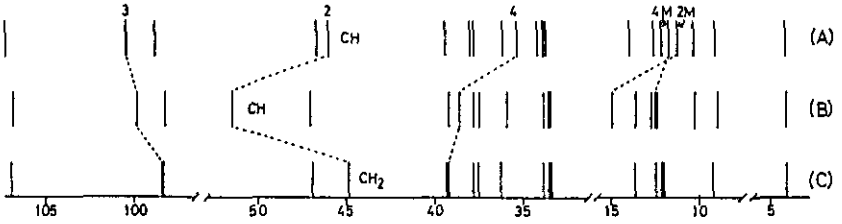


Fig. 6. Pertinent region of the ^{13}C-nmr spectra of lonomycins A, B and C in $CDCl_3$.

(1) The C2 signal at 46.0 ppm in A was shifted downfield by 6.0 ppm.

(2) The absorption of C4 at 35.4 ppm also suffered downfield shift by 3.0 ppm in B.

(3) The methyl resonance CH_3(C2) at 11.1 ppm in A was replaced by a methyl peak at 14.9 ppm in B.

(4) A methyl signal due to CH_3(C4) at 11.6 ppm moved to 12.7 ppm in B.

(5) Slight shift was observed with the carboxyl (C1) and hemiketal (C3) signals.

The structure of A given by an X-ray analysis[26] shows that the relationship of H2 and CH_3(C4) and of H4 and CH_3(C2) are syn axial as shown in Fig. 7. Therefore, it is reasonably assumed that the γ-effect acts strongly on C2, C4, CH_3(C2) and CH_3(C4) in A. The downfield shift of these carbons in B can only be reconciled with the configurational change at C3 as shown in Fig. 7. Thus, C2 and its substituents in B are oriented not to interfere sterically with H4 and CH_3(C4) resulting in

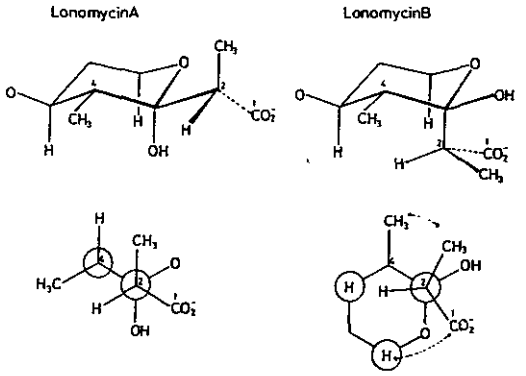


Fig. 7. The stereochemical relatioship around C3 in lonomycins A and B.

the lack of the γ-effect. The similarity of the chemical shifts of the carbons adjacent to the ketal (C13) and hemiketal (C29) functions in both the compounds indicated the structural identity of the remaining part of A and B.

C is similar to A in its physicochemical properties, however, it contains one less carbon and two less hydrogen atoms than A. The ^{13}C-nmr spectrum of C showed close similarity to that of A except for (1) the disappearance of the methyl resonance CH_3(C2) present in A, (2) the downfield shift by 3.0 ppm of the C4 signal and (3) the upfield shift of the C3 peak by 2.0 ppm. In addition, the signal at 46.5 ppm corresponding to C2 in A was shown to be a methylene by the sford spectrum of C. These chemical shift changes are reasonably accounted for by the disappearance of β- or γ-effect[52, 53] on C3 and C4 exerted by the methyl substituent CH_3(C2) in A. Thus, the structures of B and C are illustrated as shown in Fig. 8.

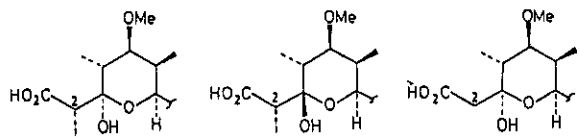


Fig. 8. The partial structures of lonomycins A, B and C. The remaining part of lonomycins B and C are identical with that of lonomycin A.

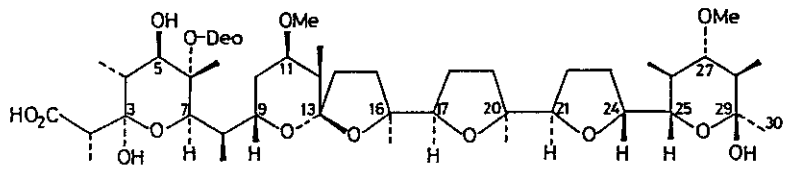
Group 2: etheromycin, A204A, carriomycin, septamycin, 6016, K-41A and K-41B (see Tables 1 and 2 and Fig. 9)

This group contains the largest number of polyether antibiotics which are very close to each other in their structures. Carriomycin[27], septamycin[29], A204A[28], 6016[31], K-41A and K-41B[25] can be distinguished by the positions of methoxy and/or deoxysugar substituents. Furthermore, the latter three compounds are characterized by the presence of a hydroxy function at C2. These structural similarities made the comparison of the ^{13}C chemical shift data within the group a very useful method to achieve total assignments of the ^{13}C-nmr spectra.

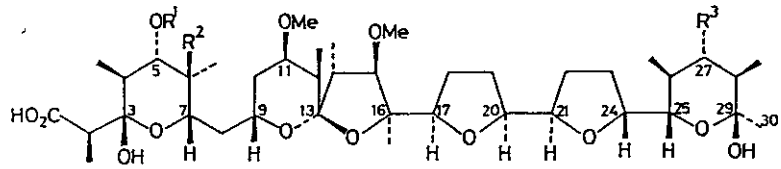
In addition, selective proton decoupling was also effective to achieve ^{13}C assignments of etheromycin[10,37], A204A[10,37], carriomycin[10, 37] and septamycin[10,37] (A28695A)[30] as summarized in Table 1. Again, extensive ^{1}H-nmr analytical works by the Anteunis' group on etheromycin[4], A204A[3], carriomycin[5], and septamycin[7] were the basis for the selective proton decoupling experiments.

As explained later, the structural determination of 6016[54] was made by using "empirical rules"[10]. During this process, about half of the signals of 6016 were analyzed. Its total assignment has been obtained by selective proton decoupling and biosynthetic method, the results of which are also contained in Table 1.

The ^{13}C signals of K-41A sodium salt obtained in C_6D_6 were assigned by making reference to the

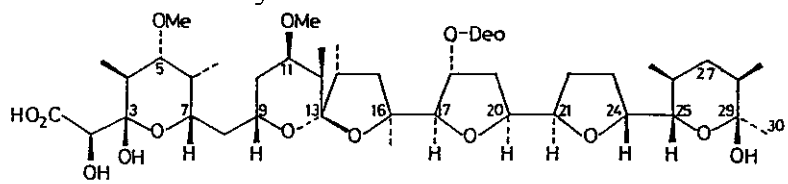


etheromycin

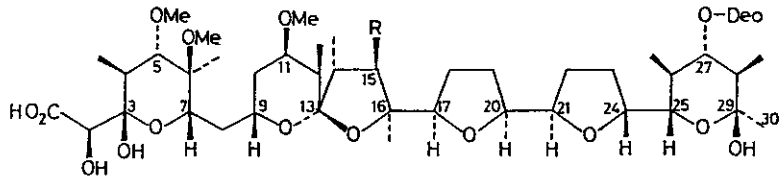


carriomycin (R^1=Deo, R^2=R^3=H), A204A (R^1=epi-Deo, R^2=R^3=OCH_3)

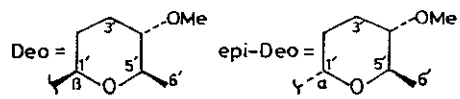
septamycin (R^1=OCH_3, R^2=Deo, R^3=H)

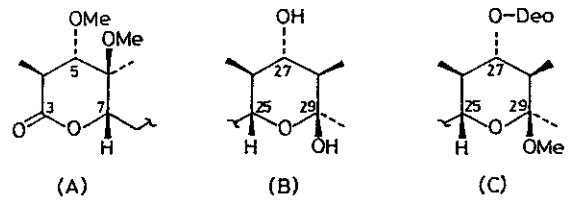


6016



K-41A (R=OCH_3) and K-41B (R=O-Deo)





(A) (B) (C)

Fig. 9. The structures of etheromycin, carriomycin, A204A, septamycin, 6016, K-41A, K-41B and derivatives of K-41A, (A), (B) and (C). The remaining parts of (A), (B) and (C) are identical with that of K-41A.

Table 2. ^{13}C Chemical shifts of sodium salts of K41A and K41B[1] in C_6D_6.

carbon	functionality	K41A	K41B
1	COOH	179.8	179.7
2	CH-OH	72.5	72.4
3	O-C-OH	99.8	99.7
4	CH	39.4	39.3
5	CH-O	86.9	86.9
6	C-O	78.8	78.8
7	CH-O	67.6	67.6
8[a]	CH_2	33.3	33.3
9	CH-O	61.9	62.0
10[a]	CH_2	31.3	31.4
11[b]	CH-O	79.9	79.9
12	CH	37.0	37.2
13	O-C-O	107.2	106.6**
14	CH	46.4	46.5
15	CH-O	94.9	93.3**
16	C-O	83.7	84.3**
17	CH-O	83.8	84.3
18[a]	CH_2	25.9	25.8
19[a]	CH_2	23.3	23.4
20[b]	CH-O	79.6	79.6
21[b]	CH-O	79.4	79.3
22[a]	CH_2	29.2	29.2
23[a]	CH_2	24.3	24.3
24[b]	CH-O	81.1	81.1
25	CH-O	74.6	74.6
26	CH	39.8	39.8
27	CH-O	82.9	83.0
28	CH	48.2	48.2
29	O-C-OH	98.9	98.8
30	CH_3	27.2	27.1

carbon	functionality	K41A	K41B
4-Me[c]		12.5	12.5
6-Me[c]		11.0	10.9
12-Me		12.6	12.6
14-Me		11.7	11.1
16-Me		28.7	26.3**
26-Me		13.8	13.8
28-Me		13.2	13.2
5-OMe		60.8	60.8
6-OMe		50.9	50.9
11-OMe		59.4	59.5
15-OMe		59.9*	---
sugar at C-27			
1'		103.0	103.0
2'		31.0	31.0
3'		27.4	27.4
4'		80.6	80.6
5'		74.7	74.7
6'		18.8	18.8
4'-OMe		56.2	56.2
sugar at C-15			
1"		---	103.8*
2"		---	31.0*
3"		---	27.1*
4"		---	80.2
5"		---	74.9*
6"		---	18.5*
4"-OMe		---	56.4*

[a-d] Tentatively assigned and may be interchanged.

* Signals observed only in K-41B.

** These slightly shifted signals supported the structure of K-41B.

1) see ref. 25.

^{13}C spectra of septamycin sodium salt, A204A sodium salt, K-41A potassium salt and three derivatives (A, B and C in Fig. 9) of K-41A[25]. The spectrum of A confirmed the C1-C4, and C7 signal assignments in K-41A sodium salt, and the spectrum of C sodium salt verified the C29 and C30 signal assignments. The signals due to the deoxysugar moiety as well as C25-C28, and CH_3(C26) and CH_3(C28) in K-41A were easily assigned by comparison with those of septamycin and B sodium salt in which signals due to the deoxysugar at C27 disappeared. The total assignments of K-41A together with its minor component, K-41B, produced by Streptomyces hygroscopicus are summarized in Table 2. The structural determination of K-41B was accomplished by comparison to K-41A. As seen from the Table, the ^{13}C signals of K-41B correspond well to those of K-41A, but K-41B has seven additionl signals (* in Table 2) assignable to the second deoxysugar moiety. Since K-41B lacks the CH_3O(C15) signal, the second deoxysugar was situated at C15 as shown in Fig. 9[25].

Dorman et al. determined the structure of A28695B[30], a minor component of A28695A (septamycin) based on the established ^{13}C-nmr spectral assignment of A28695A. The downfield shifts of the C26 (+5.4 ppm) and C28 (+2.6 ppm) signals (β-effects) and the upfield shifts of the C25 (-4.8 ppm), CH_3(C26) (-3.7 ppm) and CH_3(C28) (-3.3 ppm) signals (steric γ-effects) from A28695A to A28695B were reasonably explained in terms of the structure (Fig. 10). The downfield shift of C29 signal was attributed to the hydrogen bonding between 27-OH and 29-OH.

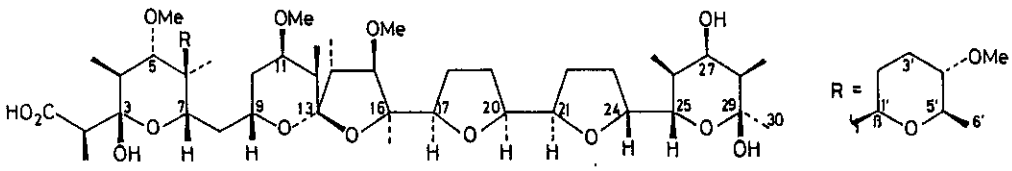


Fig. 10. The structure of A28695B

Group 3: nigericin and grisorixin (see Table 3 and Fig. 11)

Nigericin [32] and grisorixin[33] are closely related to mutalomycin[24] in the right half side structure and to 6016 in the substitution patterns of B and C rings. However, these two antibiotics can be discriminated from the groups 1 and 2 by the disappearance of hemiketal signals at 99-100 ppm in the ^{13}C-nmr spectra. The ^{13}C chemical shift of nigericin sodium salt (see Table 3) were assigned by selective proton decoupling based on ^{1}H-nmr analysis by Rodios and Anteunis[8]. Ambiguities with regards to the chemical shifts of some methylene carbons were eliminated by the aid of biosynthetic method using $CH_3{}^{13}COOH$, $^{13}CH_3{}^{13}COOH$ and $CH_3CH_2{}^{13}COOH$[37].

Based on comparison to nigericin, the assignments of ^{13}C signals of grisorixin were also accomplished[37] as shown in Table 3.

Table 3. ^{13}C Chemical shifts of sodium salts of nigericin, grisorixin, monensin and laidlomycin in $CDCl_3$.[1]

carbon	functionality	nigericin	grisorixin	monensin	laidlomycin	carbon	nigericin	grisorixin	monensin	laidlomycin
1	COOH	183.9[a]	180.9	181.2	179.9	2-Me	14.4[#]	14.1[#]	16.8[#]	16.4[#]
2	CH	45.9	45.8	45.0	43.3	4-Me	11.6	11.9	11.0	10.8
3	CH-O	73.2[a]	73.4	83.0	75.6	10-Me	---	---	10.5	10.0
4	CH	29.0	28.9	37.5	40.2	12-Me	13.0	12.8	---	---
5	CH_2	26.4[b]	26.4	---	---	14-Me	13.4	13.3	---	---
6	CH_2	23.4[c]	23.4	---	---	16-Me	27.7	27.9	27.4	27.4
7	CH-O	68.4[b]	68.0	---	---	20-Me	22.8	22.4	8.1	23.7
8	CH_2	35.8[c]	35.5	---	---	22-Me	16.2[#]	16.5[#]	14.5	13.9
9	CH-O	60.4[b]	60.3	68.3	68.1	26-Me	17.0	17.0	16.8[#]	16.5[#]
10	CH_2/CH	32.3[c]	32.5	34.3	34.4	28-Me	16.4[#]	17.5[#]	16.0	15.8
11	CH-O	79.5[a]	79.3	70.4	70.4					
12	CH/CH_2	36.6[+]	36.7	33.3[+]	33.2[+]	20-CH_2	---	---	30.6	---
13	O-C-O	107.7[a]	107.4	107.0	106.8					
14	CH/CH_2	39.6	39.5	39.2	39.1	3-OMe	---	---	57.8	---
15	CH_2	41.7[a]	41.8	33.5[+]	33.0[+]	11-OMe	59.5	59.3	---	---
16	C-O	82.4[*]	82.2[*]	85.8[*]	85.0[*]					
17	CH-O	81.5[b]	81.7	82.5	81.3	CH_3	---	---	---	9.1
18	CH_2	25.9[c]	25.6	27.3	27.2	CH_2	---	---	---	27.7
19	CH_2	29.6[a]	29.7	29.8	30.1	COO	---	---	---	173.4
20	C-O	84.8[*]	85.0[*]	85.2[*]	83.7[*]					
21	CH-O	85.3[a]	85.8	84.9	86.2					
22	CH	35.2	35.1	34.8	35.1					
23	CH_2	32.1[b]	32.6	33.35	32.8[+]					
24	CH-O	76.5[c]	75.9	76.4	76.4					
25	CH-O	76.9[a]	77.8	74.5	74.4					
26	CH	31.9	31.9	31.8	31.6					
27	CH_2	37.2[a]	36.9	35.7	35.5					
28	CH	36.8[+]	40.3	36.5	36.3					
29	O-C-OH	97.2[b]	96.6	98.3	98.0					
30	CH_3/CH_2OH	67.2[c]	25.7	64.9	64.7					

+,*,# Assignments may be interchanged.

[a] enriched by $CH_3CH_2{}^{13}COOH$.

[b] enriched by $CH_3{}^{13}COOH$.

[c] enriched by $^{13}CH_3COOH$.

1) see ref. 37.

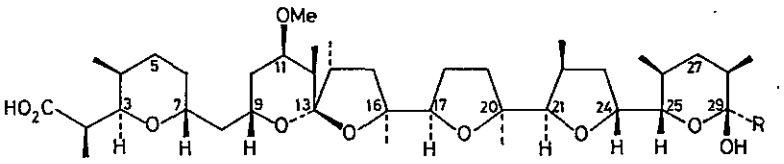


Fig. 11. The structures of nigericin (R=CH_2OH) and grisorixin (R=CH_3)

Group 4: monensin and laidlomycin (see Table 3 and Fig. 12)

The ^{13}C-*nmr spectrum of monensin*[22] *was analyzed partly by selective proton decoupling.* Its structural similarities to mutalomycin (with regard to B and C rings) and nigericin (with regard to E and F rings) were utilized to advance further assignments of the remaining resonances as shown in Table 3.[37] However, the partial structures specific to monensin, i.e. the presence of an ethyl group at C20 and a linear structure (C1 to C4) left some ambiguity in the assignments of relevant carbon signals. The ^1H-nmr spectral analysis of monensin had been made by Anteunis[9]. The methoxy function at C3 and ethyl substituent at C20 in monensin are replaced by propionyl and methyl groups, respectively, in laidlomycin. Taking advantage of these structural differences, the assignment of the ^{13}C-nmr spectrum of laidlomycin was made straightforwardly as summarized in Table 3.[37]

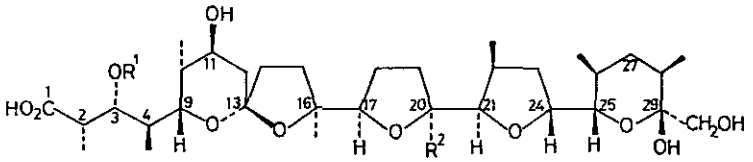


Fig. 12. The structures of monensin (R^1=CH_3, R^2=CH_2CH_3) and laidlomycin (R^1=$COCH_2CH_3$, R^2=CH_3). The numbering system common to the other groups is employed to make comparison easier. Note that carbons 5 to 8 are not present in this system.

^{13}C CHEMICAL SHIFT TRENDS OF POLYETHER ANTIBIOTICS

Some empirical rules for structural elucidation of polyether antibiotics by ^{13}C-nmr spectroscopy

As a result of extensive studies on the assignments of the ^{13}C-nmr spectra of the polyether antibiotics under discussion, Seto et al.[10] established some empirical rules for structural determination of antibiotics possessing the basic carbon skeleton common to groups 1 to 3 (Fig. 13). The rules can also be applied to compounds with similar partial structures such as monensin and dianemycin.

As shown in the following, in extracting the empirical rules from the established assignments, were utilized only signals appearing in the characteristic regions or ones easily distinguishable from the other signals by several ^{13}C-nmr techniques.

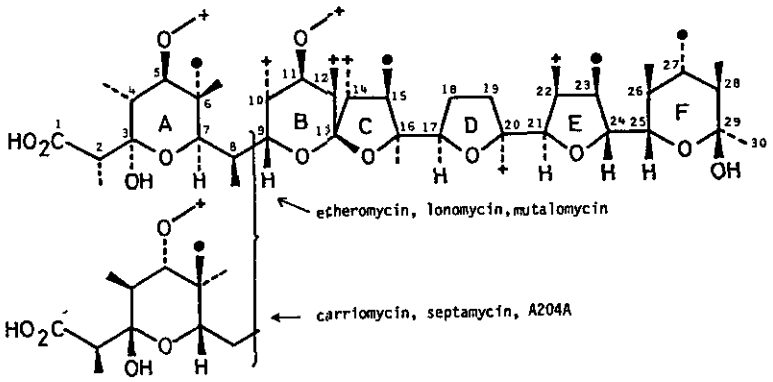
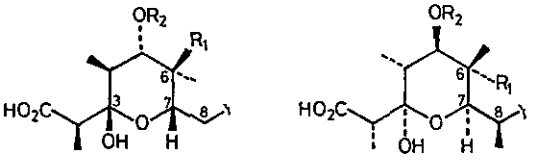


Fig. 13. The basic carbon skeleton common to etheromycin, lonomycin, mutalomycin, carriomycin, septamycin, A204A and nigericin.

(A-ring)

The absolute configuration of the A-ring of carriomycin, septamycin and A204A is opposite to that of lonomycin, mutalomycin and etheromycin as shown. Its stereochemistry is related to the presence



carriomycin (R_1=H, R_2=sugar)
septamycin (R_1=sugar, R_2=CH_3)
A204A (R_1=OCH_3, R_2=sugar)
C-7: 64.0~68.4

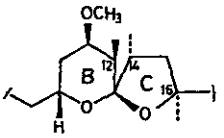
etheromycin (R_1=sugar, R_2=H)
lonomycin and
mutalomycin (R_1=H, R_2=CH_3)
C-7: 70.8~71.5

of a methyl at C8. This methyl causes a downfield shift of the C7 signal(carriomycin, septamycin and A204A).

The presence of a substituent other than the methyl at C6 can be detected by the characteristic chemical shifts of the axial methyl at C6. A methoxy or sugar substituent at C6 causes large downfield shift of this methyl carbon (mutalomycin, lonomycin and carriomycin 4.1-5.5 ppm compared with etheromycin, septamycin and A204A 8.1-12.6 ppm). The signal due to the hemiketal carbon C3 appearing at 99.2-100.4 ppm in the members 1 and 2 is absent in nigericin and grisorixin which have no hydroxy function at C3.

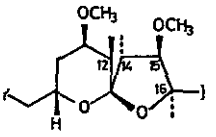
(B and C rings)

Three structures have been reported for the B-ring. The differences are due to the presence of a methyl at C8 and the position of a methyl at C10 or C12. As shown in the figure, a methyl at C8 causes a downfield shift of C9 by ca. 3 ppm. Among the members with a methyl at C8, etheromycin can be distinguished from lonomycin and mutalomycin by the chemical shift of C13.



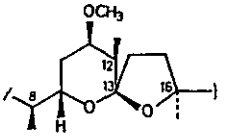
nigericin

C-9: 60.4
C-13: 107.7
C-16: 82.4
CH_3(16): 27.7



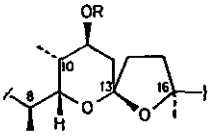
carriomycin, septamycin, A204A

C-9: 61.2-61.4
C-13: 106.4-106.5
C-16: 82.9-83.1
CH_3(16): 28.4



etheromycin

C-9: 63.0
C-13: 109.2
C-16: 85.2
CH_3(16): 29.2



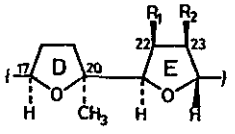
lonomycin (R=CH_3), mutalomycin (R=H)

C-9: 63.3-64.1
C-13: 106.8-107.0
C-16: 84.2-84.3
CH_3(16): 29.0-29.2

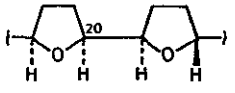
Two kinds of structures exist for the C-ring. One has a methyl substituent at C14 and the other has not. This difference can be detected by the chemical shift of C16. In compounds with a methyl at C14, C16 is observed at rather higher field than those lacking the substituent at C14. The methoxy group at C15 in carriomycin, septamycin and A204A can be detected by the very characteristic oxymethine signal at 94.5 ppm due to C15. The value can be compared with that of the corresponding carbon C25 in noboritomycin[14].

(D and E-rings)

There are two kinds of structures for the D-ring. One has a methyl at C20 and the other has not. The latter group includes carriomycin, septamycin and A204A. Since the methyl at C20 resonates at a characteristic region (22.3-23.3 ppm), its presence can be revealed very easily.



etheromycin (R_1, R_2=H)
lonomycin (R_1=CH_3, R_2=OCH_3)
mutalomycin (R_1=CH_3, R_2=H)
C-20: 83.8-86.1
CH_3(20): 22.3-23.3



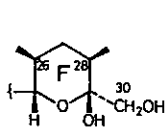
carriomycin
septamycin
A204A
C-20: 78.8-78.9

The structure of the E-ring can be conveniently established by the characteristic resonances of a methyl at C22 (mutalomycin 16.2 and lonomycin 9.0 ppm) and a methoxy at C23 (lonomycin 57.3 ppm). There exists no substituent on the E-ring for compounds without a methyl at C20 such as carriomycin and septamycin.

(F-ring)

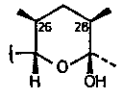
Three kinds of structures are known for the F-ring, the differences being due to the presence of a methoxy at C27 and a hydroxy at C30. Two methyls at C26 and C28 move to higher field due to the γ-effect of the methoxy at C27. Since the chemical shift values of these methyls unsubstituted at C27, such as carriomycin, mutalomycin and septamycin, are characteristic among the methyl resonances, distinction of the nigericin and carriomycin types from the etheromycin type is very straightforward. The chemical shifts of the hemiketal carbon C29 are also useful to detect the methoxy at C27.

Distinction of the nigericin type with a hydroxy substituent at C30 from the other compounds can be made by the characteristic hydroxy methyl signal at 65-67 ppm.



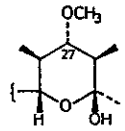
nigericin

C-29: 97.2
C-30: 67.2
CH_3(26) 17.0
CH_3(28). 16.4



carriomycin
mutalomycin
septamycin

C-29: 96.5-96.6
C-30: 25.7-26.4
CH_3(26): ~17.5
CH_3(28): ~17.0

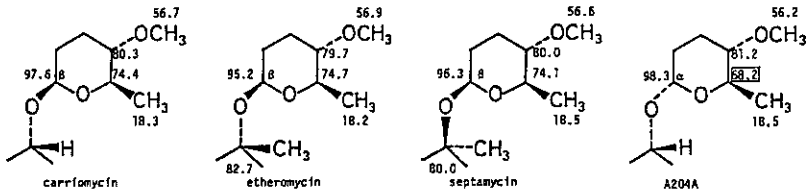


etheromycin
lonomycin
A204A

C-29: 98.1-98.8
C-30: 26.5-26.6
CH_3(26): ~13.5
CH_3(28): ~12.5

(4'-O-methylamicetose)

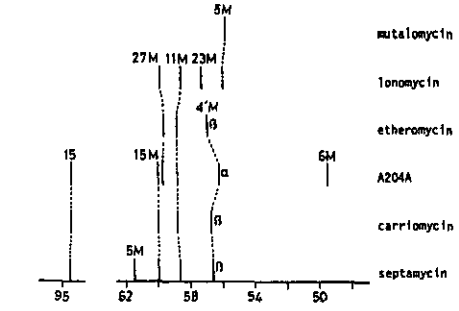
The sugar found in polyether antibiotics is almost always 4'-O-methylamicetose*.

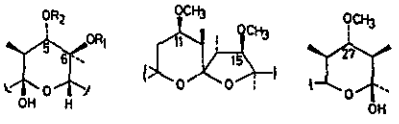


Advantageously, the oxymethine carbons in the sugar moiety can be detected by taking PRFT spectra. Since the chemical shift of an anomeric carbon is affected by the anomeric configuration as well as by the environment of the carbon to which the sugar is attached[56], it is much better to utilize the chemical shift of C5' for obtaining information about the anomeric configuration. The chemical shift of C5' in A204A clearly shows the anomeric configuration to be α. The upfield shift of this carbon is due to the γ-effect of the axial oxygen at C1'. Anomeric carbons attached to a quaternary carbon appear at considerably higher field than those combined to a methine carbon.

* Very recently, two dianemycin like antibiotics, TM-531B and TM-531C have been reported, in which 4'-O-methylamicetose is replaced by amicetose and 2',6'-dideoxy-4'-O-methylglucose, respectively[55].

(methoxy signals)





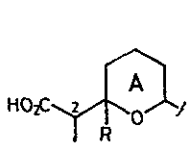
A204A (R_1=CH_3, R_2=sugar)
septamycin (R_1=sugar, R_2=CH_3)

The most characteristic in this region is the methoxy signal (49.5 ppm) linked to the quaternary carbon C6 in A204A. Another signal showing discernible chemical shift (61.5 ppm) in septamycin is assigned to a methoxy carbon at C5 which is connected to the quaternary oxycarbon C6. Thus, the chemical shifts of these methoxy carbons are very useful to know the substitution pattern at C6.

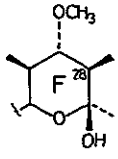
The chemical shifts of the methoxy carbons at C15 and C27 are very close. However, as previously explained, the methoxy at C15 is always accompanied by a very characteristic signal at 94.5 ppm.

(signals appearing at 45-47 ppm)

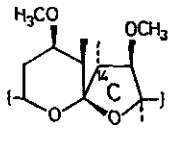
This region is usually specific to the methine carbon (C2) adjacent to the terminal carboxylic acid. In addition, two different methine signals may sometimes be observed. One of them is ascribed to the C28 methine with a methoxy substituent at C27. In this case, two methyls at C26 and C28 do not resonate at ca. 17 ppm and the C29 hemiketal carbon appears at much lower field (98-99 ppm). The other is due to the methine signal of C14 which is accompanied by a characteristic oxymethine signal at 94.5 ppm assignable to C15.



most polyethers
(R=H or OH)



etheromycin
lonomycin
A204A
C-29: 98-99



carriomycin
septamycin
A204A
C-15: 94.5

(Application of the "empirical rules" for the structural elucidation of 6016)

The usefulness of the empirical rules has been exemplified by their application to the structural determination of a new antibiotic 6016[54]. The antibiotic 6016, $C_{46}H_{77}O_{16}Na$, is produced by Streptomyces sp. and active against gram positive bacteria, mycobacteria, fungi and yest.

Its ^{13}C-nmr spectrum (Fig. 14) 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 common to carriomycin, septamycin and A204A and in fact, the structural determination of the antibiotic could be accomplished by application of the empirical rules as shown in Fig. 15.

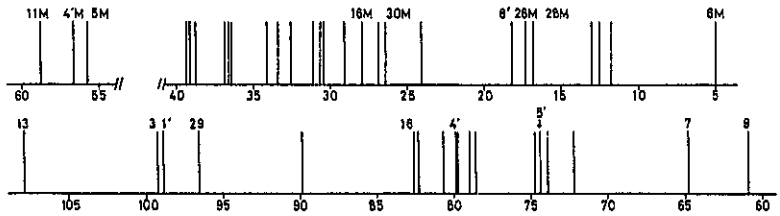


Fig. 14. ^{13}C-Nmr spectrum of 6016 sodium salt in $CDCl_3$. The suffix M represents either a methyl or methoxy group on the numbered carbon. The carboxylic acid is not shown in this Fig.

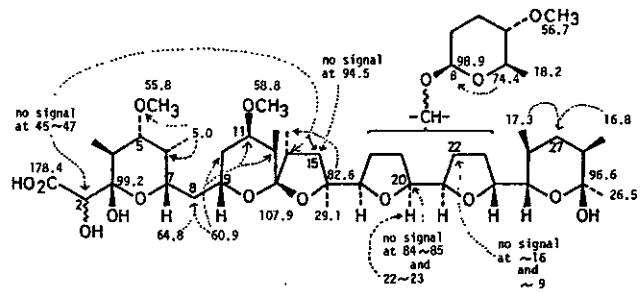


Fig. 15. The structure of 6016 obtained by analysis of its ^{13}C-nmr spectral data. The chemical shift values are basis of the partial structures connected by dotted lines.

^{13}C Chemical shift data supporting for the structures of the A, B, C and F-rings are given in the figure and are connected by dotted lines to the relevant carbons. It should be noted that only carbon signals numbered in Fig. 15 were utilized to structural determination of 6016.

Some comments may be necessary for the carbinol carbon C2 and the position of 4'-O-methylamicetose.

Carbinol carbon C2

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 C2 methine carbons as explained previously. Since removal of the methyl from the C2 methine does not considerably affect its chemical shift (cf. C2 methylene in lysocellin 45.9 ppm[16]), the only reasonable explanation for this spectral feature can be given by placing a hydroxy function on C2.

The position of the deoxysugar on the main framework

Since the structures (and ^{13}C assignments) of A, B, C and F-rings have been established without doublt by the empirical rules (for ^{13}C assignments of these rings, see Table 1), the sugar must be placed on either the D or E-ring (see Fig. 15). Its position was determined 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 by selective proton decoupling to be on the carbon at 89.9 ppm which is not contained in A, B, C or F-ring. Moreover, this proton was coupled to an oxymethine at ca. 4.4 ppm. This spectral feature can only be accomodated by a D-ring with the sugar at C18. This structure resulted in a large upfield shift of C20 (78.9 in carriomycin → 73.9 ppm in 6016) which may be rationalized by the stereochemistry with the sugar substituent and the hydrogen at C20 on the same plane of the D-ring (due to the strong γ-effect). Thus, the structure of 6016 has been determined as shown in Fig. 9. The stereochemistry of C2 has been proved to be R by X-ray analysis[31].

CONCLUSION

^{13}C-Nmr spectroscopy is well known to be a very useful tool for structural elucidation of natural products. This is also true with the polyether antibiotics, especially when good crystals suitable for X-ray analysis are not obtainable.

In addition to structural investigation, this methodology will further enable to study important problems associated with the mechanism of action of the polyether antibiotics such as interaction with metal cations in solution. Subtle conformational changes of the antibiotics caused by complex formation will be amply reflected in their ^{13}C-nmr spectra. Therefore, detailed analysis of the spectral change will give valuable information which can be hardly obtained by other techniques. The basic requirement for ^{13}C-nmr spectroscopy to be fully powerful in such kind of works, i.e. complete assignments of the ^{13}C-nmr spectra of the polyether antibiotics, has been almost satisfied by extensive works as explained in this review and accumulated ^{13}C chemical shift data will become vital to physical, chemical and biochemical studies of the polyether antibiotics in future.

References

1. J. W. Westley, Adv. Appl. Microbiol., 1977, 22, 177.
2. H. Seto, T. Satō and H. Yonehara, J. Amer. Chem. Soc., 1973, 95, 8461.
3. M. J. O. Anteunis and G. Verhegge, Bull. Soc. Chim. Belg., 1977, 86, 353.
4. M. J. O. Anteunis and N. A. Rodios, ibid., 1978, 87, 753.
5. N. A. Rodios and M. J. O. Anteunis, ibid., 1978, 87, 437.
6. N. A. Rodios and M. J. O. Anteunis, ibid., 1978, 87, 437, 1979, 88, 37.

7. N. A. Rodios and M. J. O. Anteunis, J. Antibiotics, 1978, 31, 294.

8. N. A. Rodios and M. J. O. Anteunis, Bull. Soc. Chim. Berg., 1977, 86, 917.

9. M. J. O. Anteunis, ibid., 1977, 86, 367.

10. H. Seto, K. Mizoue, H. Nakayama, K. Furihata, N. Ōtake and H. Yonehara, J. Antibiotics, 1979, 32, 239.

11. J. W. Westley, R. H. Evans, G. Harvey, R. G. Pitcher, D. L. Pruess, A. Stempel and J. Berger, ibid., 1974, 27, 288.

12. H. Seto, Y. Miyazaki, K. Fujita and N. Ōtake, Tetrahedron Lett., 1977, 2417.

13. D. E. Dorman, J. W. Pascal, W. M. Nakatsukasa, L. L. Huchstep and N. Neuss, Helv. Chim, Acta, 1976, 59, 2625.

14. C. Keller-Juslen, H. D. King, H. R. Loosli and A. von Wartburg, J. Antibiotics, 1978, 31, 820.

15. J. W. Westley, R. H. Evans, Jr., L. H. Sello, N. Troupe, C. Liu, J. F. Blount, R. G. Pitcher, T. H. Williams and P. A. Miller, ibid., 1981, 34, 139.

16. N. Ōtake, H. Seto and M. Koenuma, Agr. Biol. Chem., 1978, 42, 1879.

17. K. Mizoue, H. Seto, T. Mizutani, M. Yamagishi, A. Kawashima, S. Ōmura, M. Ōzeki and N. Ōtake, J. Antibiotics, 1980, 33, 144.

18. N. Tsuji, Y. Terui, K. Nagashima, K. Tori and L. F. Johnson, ibid., 1980, 33, 94.

19. T. Mizutani, M. Yamagishi, H. Hara, A. Kawashima, S. Ōmura, M. Ōzeki, K. Mizoue, H. Seto and N. Ōtake, ibid., 1980, 33, 137.

20. C. M. Deber and D. R. Pfeiffer, Biochem., 1976, 15, 132.

21. B. K. Toeplitz, A. I. Cohen, P. T. Funke, W. L. Parker and J. Z. Gougoutas, J. Amer. Chem. Soc., 1979, 101, 3344.

22. M. Pinkerton and L. K. Steinrauf, J. Mol. Biol., 1970, 49, 533.

23. F. Kitame, K. Utsushikawa, T. Kohama, T. Saito, M. Kikuchi and N. Ishida, J. Antibiotics, 1974, 27, 884.

24. T. Fehr, H. D. King and M. Kuhn, ibid., 1977, 30, 903.

25. N. Tsuji, K. Nagashima, Y. Terui and K. Tori, ibid., 1979, 32, 169.

26. N. Ōtake, M. Koenuma, H. Miyamae, S. Sato and Y. Saito, J.C.S. Perkin II, 1977, 494.

27. H. Nakayama, N. Ōtake, H. Miyamae, S. Sato and Y. Saito, ibid., 1979, 293.

28. N. D. Jones, M. O. Chaney, J. W. Chamberlin, R. L. Hamill and S. Chen, J. Amer. Chem. Soc., 1973, 95, 3399.

29. T. J. Petcher and H. P. Weber, J. Chem. Soc. Chem. Comm., 1974, 697.

30. D. E. Dorman, R. L. Hamill, J. L. Occolowitz, Y. Terui, K. Tori and N. Tsuji, J. Antibiotics, 1980, 33, 252.

31. N. Ōtake, T. Ogita, H. Nakayama, S. Sato and Y. Saito, J.C.S. Chem. Comm., 1978, 875.

32. L. K. Steinrauf, M. Pinkerton and J. W. Chamberlin, Biochem. Biophys. Res. Comm., 1968, 33, 29.

33. P. Gachon, A. Kergomard and H. Veschamber, J. Chem. Soc. Chem. Comm., 1970, 1421.

34. K. Roth, Org. Mag. Res., 1977, 10, 56.

35. K. Nakanishi, R. Crouch, T. Miura, X. Dominguez, A. Zamudio and R. Villanreal, J. Amer. Chem. Soc., 1974, 96, 609.

36. J. R. Lyerla, Jr. and G. C. Levy, 'Topics in carbon-13 nmr spectroscopy', Vol.1. G. C. Levy (Ed.), John Wiley and Sons, New York, 1974, pp. 81-148.

37. H. Seto, K. Mizoue and N. Ōtake, unpublished data.

38. F. W. Wehrli, 'Topics in carbon-13 nmr spectroscopy' Vol.2. G. C. Levy (Ed.), John Wiley and Sons, New York, 1976, pp. 53-77.

39. G. E. Martin, J. A. Matson, J. C. Turley and A. J. Weinheimer, J. Amer. Chem. Soc., 1979, 101, 1888.

40. D. M. Doddrell and D. T. Pegg, J. Amer. Chem. Soc., 1980, 102, 6388.

41. L. F. Johnson, 'Topics in carbon-13 nmr spectroscopy', Vol. 3. G. C. Levy (Ed.), John Wiley and Sons, New York, 1979, pp. 2-16.

42. M. Tanabe, 'Biosynthesis', T. A. Geissman (Ed.), The Chemical Society, London, 1974, 2, pp. 241-299, 1975, 3, pp. 247-285, 1976, 4, pp. 204-265.

43. L. E. Day, J. W. Chamberlin, E. Z. Gordee, S. Chen, M. Gorman, R. L. Hamill, N. Neuss, R. E. Weeks and R. Stroshane, Antimicrobial Agents and Chemotherapy, 1973, 4, 410.

44. H. Seto, K. Mizoue and N. Ōtake, J. Antibiotics, 1980, 33, 979.

45. J. W. Westley, ibid., 1977, 29, 584.

46. J. W. Westley, H. Seto and N. Ōtake, ibid., 1979, 32, 959.

47. J. Y. Lallemand, C. Le Cocq, V. Michon, C. Derouet and R. C. Rao, C. R. Acad. Sc. Paris, 1979, 288C, 383.

48. J. C. Beloeil, C. LeCocq, V. Michon and J. Y. Lallemand, Tetrahedron, 1981, 37, 1943.

49. T. Fehr, C. Keller-Juslen, H. R. Loosli, M. Kuhn and A. von Wartburg, J. Antibiotics, 1979, 32, 535.

50. T. Mizutani, M. Yamagishi, H. Hara, S. Ōmura, M. Ōzeki, K. Mizoue, H. Seto and N. Ōtake, ibid., 1980, 33, 1224.

51. H. Seto, K. Mizoue and N. Ōtake, ibid., 1978, 31, 929.

52. J. B. Stothers, 'Carbon-13 NMR spectrocopy', Academic Press, New York and London, 1972, pp. 55-101, 269-278.

53. G. C. Levy and G. L. Nelson, 'Carbon-13 nuclear magnetic resonance for organic chemists', Wiley-Interscience, New York, 1972, pp. 38-58.

54. H. Seto, H. Nakayama, T. Ogita, K. Furihata, K. Mizoue and N. Ōtake, J. Antibiotics, 1979, 32, 244.

55. T. Mizutani, M. Yamagishi, K. Mizoue, A. Kawashima, S. Omura, M. Ozeki, H. Seto and N. Ōtake, ibid., in press.

56. S. Seo, Y. Tomita, K. Tori and Y. Yoshimura, J. Amer. Chem. Soc., 1978, 100, 3331.

Received, 7th September, 1981