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THE ASSIGNMENTS OF THE ^{13}C-NMR SPECTRA OF LONOMYCIN A AND MUTALOMYCIN†

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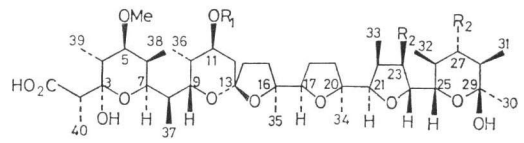
All resonances observed in the ^{13}C-nmr spectra of the polyether antibiotics lonomycin A and mutalomycin have been assigned by the aid of biosynthetic method, selective proton decoupling as well as comparison with structurally related compounds.

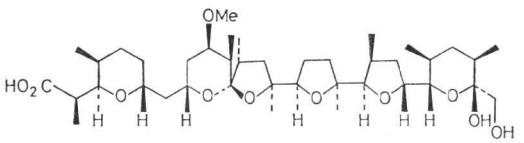
Lonomycin A[2], a member of the polyether antibiotic family, was isolated from the cultured broth of *Streptomyces ribosidificus* TM-481[3] and the absolute configuration of its T1 salt has been determined by an X-ray analysis[4] as shown in Fig. 1.

In common with other polyether antibiotics[5], this compound is effective against coccidia, Gram-positive bacteria and mycobacteria[3] and can transport monovalent cations across biological and artificial membranes[6]. Hence the mode of ion trapping action in solution of lonomycin A is of considerable interest in view of the structure-biological activity relationship of the naturally occurring ionophores. Since such kind of activities of the polyether antibiotics seem to be associated with their dynamic structures in solution, we have undertaken to analyze their solution conformation by the use of ^{13}C-nmr spectroscopy which is well known to reflect structural environments of complex molecules to the chemical shift of the ^{13}C-nmr spectra. Complete assignment of the ^{13}C-nmr spectrum of lonomycin A is important not only for this purpose but also to supply a rapid and convenient method for structural determinations of related antibiotics. In fact, the structures of lonomycins B and C have been determined by ^{13}C-nmr spectroscopy[2]. We wish to report herein the assignments of the ^{13}C-nmr spectra of lonomycin A sodium salt (I) and its structural analog, mutalomycin sodium salt (II)[7,8] (see Fig. 1).

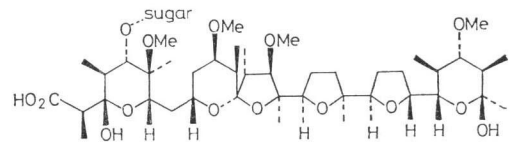
Fig. 1. The structures of lonomycin A (I), mutalomycin (II), nigericin (III) and A204A.



(I) Lonomycin A (R_1=Me, R_2=OMe)
(II) Mutalomycin (R_1=R_2=H)



(III) Nigericin



A204A

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The results of this work have been exploited to establish some empirical rules for structural elucidation of polyether antibiotics[9].

Assignment of the ^{13}C-nmr Spectrum of Lonomycin A

The ^{13}C-nmr spectrum of lonomycin A sodium salt (I) taken in $CDCl_3$ solution showed 44 resonances. Based on multiplicity information, spin lattice relaxation time T_1 and chemical shift trends[10],

Table 1. ^{13}C-Nmr spectral data of lonomycin A (I) and mutalomycin (II) sodium salts.

Carbon	Functionality	Lonomycin A			Mutalomycin
		Chemical shift (ppm)	T_1 value (sec)	Enriched by	Chemical shift (ppm)
1	COO^-	181.5	a	1–P[b]	181.2
2	CH	46.0	0.21		47.1
3	O–C–O	100.4	a	1–P	99.6
4	CH	35.4	0.22		35.0
5	CH–O	82.2	0.21	1–P	82.0
6	CH	31.1	0.20		31.1
7	CH–O	70.8	0.21	1–P	71.5
8	CH	37.7	0.22		36.6
9	CH–O	63.3	0.19	1–P	64.1
10	CH	33.7	c		40.2
11	CH–O	82.0	0.21	1–A	70.3
12	CH_2	34.1	0.11	2–A	33.4[d]
13	O–C–O	107.1	a	1–A	106.8
14	CH_2	39.4	0.08	2–A	39.0
15	CH_2	33.5	c	1–P	33.3[d]
16	C–O	84.2	a		84.3
17	CH–O	81.4	0.21	1–A	81.6
18	CH_2	25.9	0.11	2–A	26.9
19	CH_2	30.4	0.14	1–P	30.7
20	C–O	85.8	a		83.8
21	CH–O	84.3	0.21	1–P	86.4
22	CH	36.1	0.21		34.3
23	CH–O or CH_2	80.5	0.22	1–A	32.3
24	CH–O	79.8	0.18	2–A	78.8
25	CH–O	73.8	0.19	1–P	73.4
26	CH	37.7	0.22		33.1
27	CH–O or CH_2	84.0	0.19	1–P	36.4
28	CH	46.6	0.23		40.2
29	O–C–O	98.8	a	1–A	96.5
30	CH_3	26.5	0.33	2–A	25.7
31	CH_3	12.5	0.52	3–P	17.0
32	CH_3	13.8	0.59	3–P	17.6
33	CH_3	9.0	0.23	3–P	16.2
34	CH_3	22.3	0.46	3–P	23.2
35	CH_3	29.2	0.16	3–P	29.0
36	CH_3	12.0	0.33	3–P	10.6
37	CH_3	10.2	0.47	3–P	10.0
38	CH_3	4.1	0.44	3–P	4.1
39	CH_3	11.1	0.54	3–P	11.1
40	CH_3	11.5	0.38	3–P	11.7
5–OMe		56.0	0.64		55.8
11–OMe		58.6	0.54		
23–OMe		57.3	0.54		
27–OMe		59.9	0.64		

[a]: Not determined.

[b]: 1–P, 3–P, 1–A and 2–A represent $CH_3CH_2{}^{13}COOH$, $^{13}CH_3CH_2COOH$, $CH_3{}^{13}COOH$ and $^{13}CH_3COOH$, respectively.

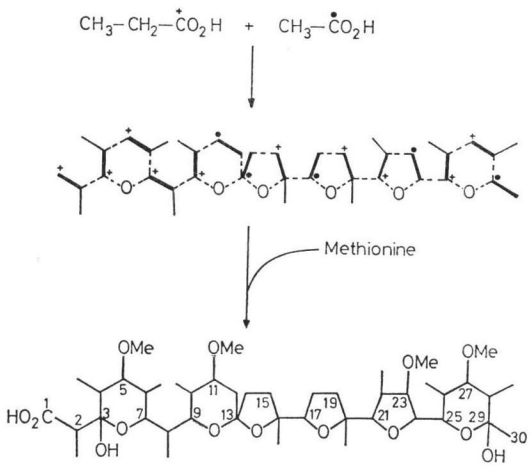
[c]: Due to overlapping of other signals, correct values could not be obtained.

[d]: Assignment may be exchanged.

these signals are classified into one carboxylic acid, three (hemi)ketal carbons, two quaternary oxycarbons, ten oxymethines, eight methines, five methylenes, eleven methyls and four methoxy carbons (Table 1).

Since there are too many carbons in similar environments and no degradation products useful for this investigation could be obtained at a practical yield, we first attempted to label carbons of **I** specifically with ^{13}C-enriched sodium acetate and sodium propionate in order to assist the assignment of the ^{13}C-nmr spectrum of **I**. Based on known biosynthetic studies on polyether antibiotics such as lasalocid[11], salinomycin[12], monensin[13], and lysocellin[14], it is reasonably assumed that **I** would originate from five acetic acid and ten propionic acid molecules with the introduction of four O-methyl groups from methionine (Fig. 2).

Fig. 2. Biosynthetic pathway of lonomycin A.



Lonomycin A

Carbons Labeled with Acetic Acid

According to the above biosynthetic hypothesis, five carbons labeled with [1-^{13}C] sodium acetate must be C11, C13, C17, C23 and C29 as shown in Fig. 2. As expected, five carbon signals at 107.1, 98.8, 82.0, 81.4 and 80.5 ppm were enriched *ca.* two-fold in the ^{13}C-nmr spectrum of **I** isolated from the culture broth of *S. ribosidificus* fed with [1-^{13}C] sodium acetate as a precursor.

Furthermore, in the ^{13}C-nmr spectrum of **I** labeled with [1, 2-$^{13}C_2$] sodium acetate were observed five pairs of ^{13}C-^{13}C couplings which are, based on their chemical shifts and magnitude of coupling constants, classified as summarized in Table 2. Among the coupling pairs which must be ascribed to C11-C12, C13-C14, C17-C18, C23-C24 or C29-C30, the groups (iii), (iv) and (v) are straightforwardly attributed to C13-C14, C23-C24 and C29-C30, respectively, because of their unique partial structures. Therefore, the signals at 107.1, 39.4, 80.5, 79.8, 98.8 and 26.5 ppm were unambiguously assigned to C13, C14, C23, C24, C29 and C30, respectively. The overlapping of some carbon signals and almost identical ^{13}C-^{13}C constants (J_{c-c}=34–36 Hz) of C11, C12, C17 and C18 prevented the identification of

Table 2. Relationship of ^{13}C–^{13}C couplings observed with lonomycin A sodium salt (**I**) labeled with $^{13}CH_3{}^{13}CO_2Na$

Functionality	Pair	Chemical shifts (ppm)	Coupling constants (Hz)	Assignment[b]
–CHO—CH_2–	i	82.0*–34.1	*ca.* 36[a]–*ca.* 36[a]	C11–C12
–CHO—CH_2–	ii	81.4*–25.9	*ca.* 36[a]–*ca.* 34[a]	C17–C18
–O–C(O)–CH_2–	iii	107.1*–39.4	42.1–*ca.* 44[a]	C13–C14
–CHO—CHO–	iv	80.5*–79.8	A_2-type	C23–C24
–O–C(O)–CH_3	v	98.8*–26.5	47.3–47.3	C29–C30

* Labeled by $CH_3{}^{13}CO_2Na$.

[a] Due to the overlapping of other signals, correct values could not be obtained.

[b] For detail see in the text.

their counterparts in the groups (i) and (ii); this problem has been overcome by ^{13}C-{^{13}C} homonuclear spin decouping. Thus, irradiation of methylene signals at 34.1 and 25.9 ppm collapsed oxymethine resonances at 82.0 and 81.4 ppm to a singlet, respectively, revealing the vicinal relationship of these two carbons.

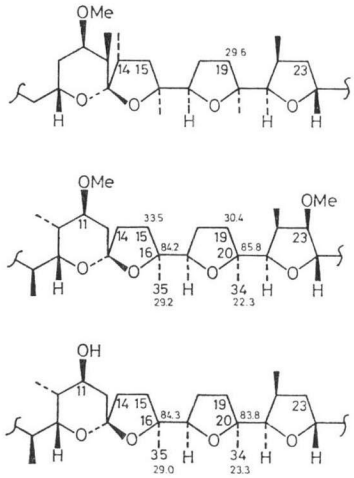
The distinction of the groups (i) and (ii) was accomplished by a selective proton decoupilng experiment (Fig. 4) irradiating at H-11 (3.20 ppm) which enables one to assign the resonance at 82.0 ppm to C11. By elimination, the line at 81.4 ppm is attributed to C17 and in turn, the peaks at 34.1 and 25.9 ppm to C12 and C18, respectively. Selective proton decoupling experiments were carried out based on the ^1H-nmr assignment by ANTEUNIS[15] throughout this work.

Carbons Labeled with Propionic Acid

The ^{13}C-nmr spectrum of **I** labeled with [1-^{13}C] sodium propionate showed ten enhanced signals (about six fold) at 181.5, 100.4, 84.3, 84.0, 82.2, 73.8, 70.8, 63.3, 33.5 and 30.4 ppm which must be assigned to C1, C3, C5, C7, C9, C15, C19, C21, C25 or C27 (see Fig. 2). The line at 181.5 ppm is easily ascribed to C1 (carboxylic acid) by its chemical shift and the peak at 100.4 ppm is identified with C3, since it is the only hemiketal carbon enhanced by [1-^{13}C] sodium propionate.

Two enriched methylene signals at 30.4 and 33.5 ppm which must emerge from C15 and C19 are distinguished by comparison with nigericin (**III**)[16] (Fig. 1). The structural similarities around C19 in these two antibiotics as depicted in Fig. 3 indicated that the methylene peak C19 would resonate at the same region, while the peak due to C15 would shift downfield in **III** due to the β-effect[10] by the methyl substituent at C14. The methylene signal equivalent to the enriched line at 30.4 ppm in **I** was found at 29.6 ppm in **III***, while there was no corresponding methylene peak in **III** around 33 ppm. Thus, the resonances at 30.4 and 33.5 ppm in **I** were assigned to C19 and C15, respectively.

Fig. 3. Comparison of partial structures of lonomycin A (**I**), mutalomycin (**II**) and nigericin (**III**).

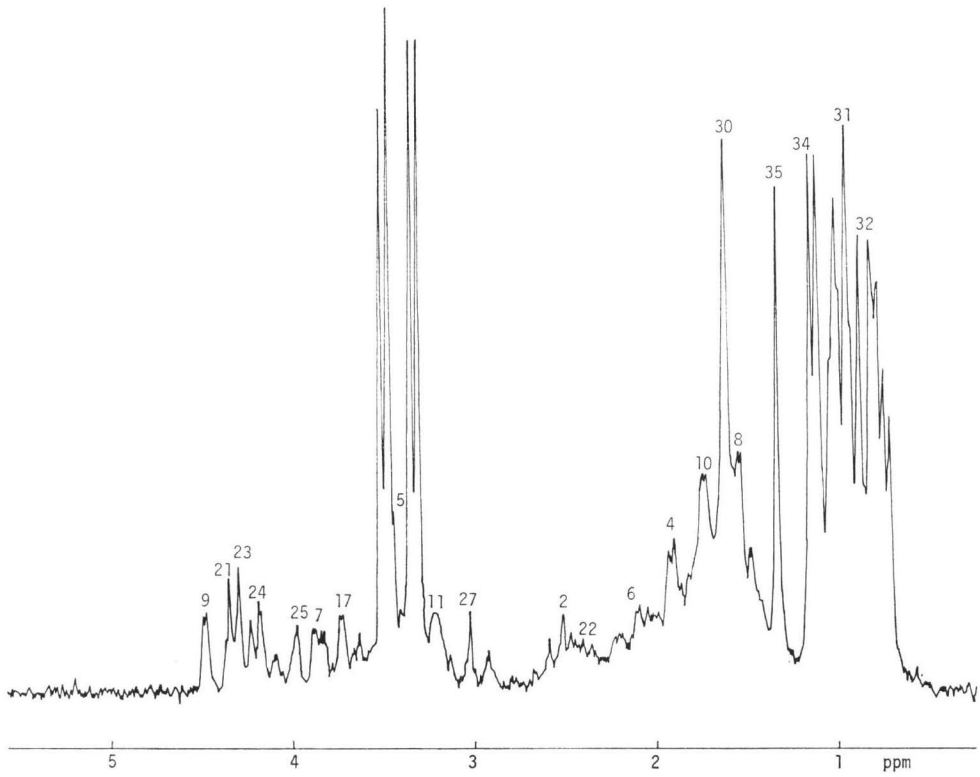


The established assignments of C11, C17, C23 and C24 (*vide supra*) enabled one to neglect resonances of their appended protons appearing in the range of 3.2~4.3 ppm (see Fig. 4) during selective proton decoupling experiments aiming to differentiate oxymethine carbons originating from [1-^{13}C] propionic acid. The selective proton decoupled ^{13}C-nmr spectra irradiating at H-5 (3.41 ppm), H-7 (3.74), H-9 (4.44), H-21 (4.29), H-25 (3.90) and H-27 (3.00) revealed successfully the signals at 82.2, 70.8, 63.3, 84.3, 73.8 and 84.0 ppm to be assigned to C5, C7, C9, C21, C25 and C27, respectively.

The ten carbons C2, C4, C6, C8, C10, C16, C20, C22, C26 and C28 are expected to derive from [2-^{13}C] propionic acid. Although a labeling experiment with the precursor was not carried out, these carbon signals can be differentiated from those labeled by [1-^{13}C] or [2-^{13}C] acetic acid or [1-^{13}C] pro-

* In the ^{13}C-nmr spectrum of nigericin (**III**) labeled with [1-^{13}C] sodium propionate, three methylene signals at 29.6, 37.2 and 41.7 ppm were enhanced which must be assigned to C15, C19 or C27 based on biosynthetic assumption. Details will be published elsewhere.

Fig. 4. 100 MHz ^{1}H-nmr spectrum of Ionomycin A sodium salt taken in $CDCl_3$.



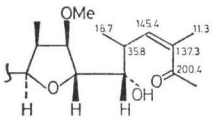
pionic acid by elimination.

The signals at 84.2 and 85.8 ppm are easily identified with the quaternary oxycarbons C16 and C20 by single frequency off resonance decoupling. The corresponding carbons (C16 and C20) absorb at 84.3 and 83.8 ppm in **II**. The structural differences between these two antibiotics (see Fig. 3), *i.e.*, introduction of a methoxy substituent at C23 which is only three bonds away from C20, and methylation of the oxygen function at C11 which is separated by five bonds from C16, both in **I**, would result in the different chemical shifts of C20 among these two compounds with the chemical shifts of C16 unchanged. Thus, the peak at 84.2 ppm in **I** and 84.3 ppm in **II** were assigned to C16. By elimination, the resonance at 85.8 ppm was ascribed to C20 in **I**, the corresponding carbon signal of **II** being present at 83.8 ppm.

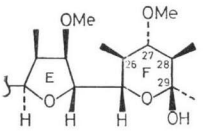
All non-oxygenated methine carbons C2, C4, C6, C8, C10, C22, C26 and C28 originating from [2-^{13}C] sodium propionate could not be differentiated by the biosynthetic method using a singly labeled precursor. Since the proton signals due to H-2, H-4, H-6 and H-22 are well separated from the remaining methine signals as shown in Fig. 4, their appended carbons were differentiated easily by selective proton decoupling experiments as follows: C2 (46.0 ppm), C4 (35.4), C6 (31.1) and C22 (36.1). The appearance of H-8, H-10, H-26 and H-28 signals in the narrow region (1.44~1.69 ppm) prevented assignment of the peaks due to C8, C10, C26 and C28 by the same technique. However, these difficulties were solved by comparison of **I** with structurally related compounds such as **II**, A204A[17] (Fig. 1) and oxolonomycin (Fig. 5).

Fig. 5. Partial structures of oxolonomycin and lonomycin A (**I**).

The remaining part of oxolonomycin is identical with that of lonomycin A (**I**).

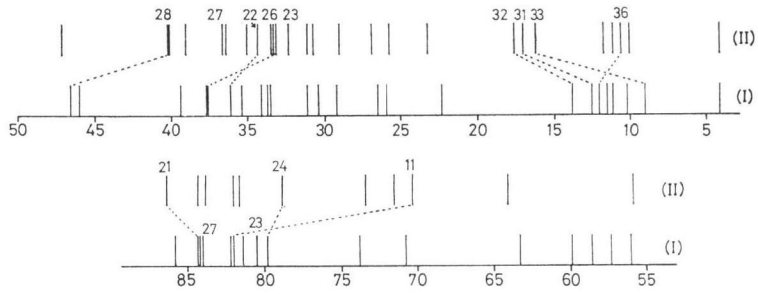


Oxolonomycin



Lonomycin A (**I**)

Fig. 6. Comparison of the ^{13}C-nmr spectra of lonomycin A (**I**) and mutalomycin (**II**).



Due to the β-effect[10] by the OMe function at C27, the signals of C26 and C28 in **I** would shift to lower field than in **II** (Fig. 6). Fortunately, the good separation of H-26 and H-28 in A204A obtained even in the 100 MHz ^{1}H-nmr spectrum permitted assignment of the signals at 39.2 and 46.2 ppm to C26 and C28, respectively, by selective proton decoupling experiments. The corresponding methine signals in **I** were found at 37.7 and 46.6 ppm. These assignments were corroborated by the ^{13}C-nmr spectral data of oxolonomycin, an alkaline degradation product of **I** with the cleaved F-ring (see Fig. 5). Since this structural change was accompanied by the disappearance of the signals at 37.7 and 46.6 ppm these two must be ascribed to C26 and C28. The corresponding absorptions in oxolonomycin shifted to 35.8 and 137.3 ppm. These results leave only two methine signals at 33.7 and 37.7 ppm which were assigned to C8 and C10, respectively, by selective proton decoupling of H-8 (1.57 ppm) and H-10 (1.67).

Methyl Carbon Signals

Although most methyl resonances could be distinguished by the multiplicity information and T_1 values, it was impossible to recognize the signal at 29.2 ppm as a methyl carbon by these techniques due to its shorter T_1 value and overlapping of other signals in the off resonance decoupled spectrum. The labeling experiment gave a conclusive evidence, the peak in question being enhanced *ca.* 6-fold in the ^{13}C-nmr spectrum of **I** labeled with [3-^{13}C] sodium acetate.

Since tertiary methyl carbons resonate at a lower field region than secondary methyl carbons, C30, C34 and C35 are easily differentiated from the rest by their chemical shifts. Among these, the assignment of C30 (26.5 ppm) has been established by the labelling experiment using [1,2-$^{13}C_2$] sodium acetate (*vide supra*). The differentiation between C34 and C35 in **I** (22.3 and 29.2 ppm) and **II** (23.3 and 29.0 ppm) were made based on the same reason as applied for the assignments for two oxyquaternary carbons C16 and C20 (*vide supra*, Fig. 3). The introduction of a methoxy substituent at C23 in **I** would cause the difference of the chemical shifts of C34 between **I** and **II** without affecting the chemical shift of C35. Thus, the signal at 29.2 ppm in **I** is assigned to C35 and that at 22.3 ppm to C34 by elimination. Comparison of the ^{13}C-nmr spectra of **I** and **II** (see Fig. 6) shows that three methyl signals in **II** (16.2, 17.0 and 17.6 ppm) were shifted upfield in **I** (9.0, 12.5 and 13.8 ppm) due to the γ-effect by the methoxy substituent on C23 and C27. Therefore, these resonances must be ascribed

to C31, C32 or C33. Since the signal at 9.0 ppm is characteristic only to **I** among polyether antibiotics with the methoxylated F-ring, such as etheromycin (CP 38295)[18] and A204A[17], it must be assigned to C33. The considerable upfield shift of this carbon signal is caused by the strong γ-effect due to the small dihedral angle[10] between C33 and the methoxy substituent at C23. The two remaining signals at 12.5 and 13.8 ppm were ascribed to C31 and C32, respectively, by selective proton decoupling of H-31 (1.09 ppm) and H-32 (0.99). The appearance of C31 at a higher field than C32 may be rationalized by the γ-effect by the two substituents at C-29. The most upfield signal (4.1 ppm) in **I** must be ascribed to C38 which is strongly shielded because of its axial orientation. The assignment of the corresponding carbon (C38, 5.5 ppm) in carriomycin[19] has been established[20] by selective proton decoupling. The resonance at 12.0 ppm in **I** is ascribed to C36 since it moves upfield by 1.4 ppm in **II** probably by the effect of demethylation at C11.

With the established assignments of the above-mentioned methyl resonances, it became possible to differentiate three remaining methyl carbons by selective proton decoupling; the resonances at 10.2, 11.1 and 11.5 ppm were assigned to C37, C39 and C40, respectively.

Methoxy Carbon Signals

The lines due to four methoxys in **I**, which were expected to derive from the methyl group of methionine could not be discriminated by the biosynthetic method. By comparison of **I** with **II** which possesses only one methoxy group at C5, and to oxolonomycin which lacks the methoxy carbon at C27, the lines at 56.0 and 59.9 ppm were attributed to 5-OMe and 27-OMe, respectively. These assignments are supported by the chemical shift trends that the methoxy carbon nuclei flanked by two equatorial substituents absorb at lower field (near 60 ppm) than the carbons flanked by one axial and one equatorial group (near 58 ppm) in inositol[21] and pyranoses[22].

The absolute structure of **I** determined by an X-ray analysis[4] revealed that the oxygen atom of 11-OMe participates in the formation of a metal complex. Since the signal at 58.6 ppm in **I** shifted upfield in the free acid form by 1.4 ppm, this signal is ascribed to 11-OMe. By elimination, the peak at 57.3 ppm which remained unchanged in the free acid form is assigned to 23-OMe.

Thus, all the ^{13}C resonances of lonomycin A sodium salt have been accomplished as shown in Table 1.

Assignment of the ^{13}C-nmr Spectrum of Mutalomycin

Most ^{13}C signals of **II** could be easily assigned by direct comparison of the ^{13}C-nmr spectra of **I** and **II**. However, the structural differences between them: *i.e.*, replacement of the hydroxy group at C11 in **II** by a methoxy substituent in **I** and the lack of two methoxy functions at C23 and C27 in **II**, would affect the chemical shifts of carbons closely situated to C11, C23 and C27 in **II**.

The ^{13}C-nmr spectra of both the compounds are compared in Fig. 6. Taking into account the magnitude of methylation shift (*ca.* 10 ppm[23]), the signal at 70.3 ppm is attributable to C11. The upfield shift of C24 by 1.0 ppm and the downfield shift of C21 by 2.1 ppm in **II** are reasonably explained in terms of β- and γ-effect[10], respectively, due to the methoxy substituent at C23 in **I**. These assignments were confirmed by selective proton decoupling irradiating at H7 (3.77 ppm)*, H21 (4.12 ppm) and H24 (4.40 ppm).

* The ^1H-nmr spectrum of mutalomycin Na salt was analyzed based on comparison with ANTEUNIS' work on lonomycin A[15].

Two new methylene signals at 32.3 and 36.4 ppm in **II** are attributed to C23 and C27, respectively based on comparison with nigericin[16] (**III**) which possesses the F-ring having no methoxy substituent. The structural similarity around C27 in these two antibiotics suggests that the signal for C27 in **II** will absorb at the same region as in **III**. Since methylene resonances attributable to C27 appeared at 37.2 or 41.7 ppm in **III** (see footnote in page 982), the signal at 36.4 ppm in **II** is ascribed to C27 and the rest at 32.3 ppm to C23. The assignment of C23 in **III** (32.1 ppm) which was confirmed by a biosynthetic experiment is in good agreement with this conclusion.

Due to the β-effect by a methoxy substituent, the methine resonances for C22, C26 and C28 were expected to move higher field on going from **I** to **II**. The assignments of the signals at 33.1 and 40.2 ppm to C26 and C28 in **II** were accomplished by making reference to carriomycin, in which these two carbons absorbing at 32.7 and 39.6 ppm had been firmly analyzed by selective proton decoupling[20]. The only remaining methine at 34.3 ppm in **II** is ascribed by elimination to C22 which is observed at 36.1 ppm in **I**.

As shown in Fig. 6, three methyl signals due to C31, C32 and C33 in **II** have moved considerably to lower field due to the disappearance of the γ-effect by methoxy groups[10]. The resonances at 17.0 and 17.6 ppm are attributed to C31 and C32, respectively, based on comparison with carriomycin, in which the corresponding carbons were observed at 16.9 and 17.3 ppm. The remaining methyl signal at 16.0 ppm is therefore assigned to C33. The total assignment of **II** is summarized in Table 1.

Thus, we have unambiguously assigned all the signals of the ^{13}C-nmr spectra of **I** and **II** on the one-to-one basis by the aid of, especially, biosynthetic methods, selective proton decoupling as well as comparison to structurally related compounds. Recently, LALLEMAND *et al.*[24] have reported the assignment of the ^{13}C-nmr spectrum of emericid[25] (identical with lonomycin A) by selective proton decoupling technique and comparison to model compounds. However, their assignments of the methylene carbons C12, C15 and C18, and four methoxy signals remain to be established. On the other hand, we could have removed these ambiguities by the biosynthetic method. Therefore, this technique together with ^{13}C-{^{13}C} spin decoupling have been proved to be a useful method for analyzing ^{13}C-nmr spectra of very complicated molecules such as polyether antibiotics. The analysis of ^{13}C-nmr spectra of other polyether antibiotics using this technique is now under way.

Experimental

^{13}C-Nmr

^{13}C-Nmr spectra were taken on a JEOL FX-100 spectrometer operating at 25.05 MHz. Detailed experimental conditions were as follows; spectral width 5 KHz except for oxolonomycin sodium salt (6 KHz), data points 16K, pulse width 9 μsec, repetition time 1.46 sec, power level for selective {^{1}H}-^{13}C decoupling, $\gamma H_2/2\pi$=850 Hz. Samples were dissolved in $CDCl_3$ at a concentration of 120 mg/0.3 ml for unlabeled samples and 30 mg/0.3 ml for ^{13}C enriched compounds. The measurements of T_1 values were made by the inversion recovery method.

Lonomycin A 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 ribosidificus* TM-481 were added to a medium containing oatmeal 2%, glucose 1%, meat extract 0.3%, NaCl 0.3%, $Fe_2(SO_4)_3$ 0.04% and $MnCl_2$ 0.04%. After 4 days incubation, 2 ml of the resulting culture were used to inoculate second stage flasks containing the same medium. After 24 hours of incubation, ^{13}C-enriched substrates were separately added ([1-^{13}C] and [3-^{13}C] sodium propionate, 30 mg in 100 ml medium and [1-^{13}C] and [1, 2-$^{13}C_2$] sodium acetate, 50 mg in 100 ml medium) to the flasks and incuba-

tion was continued for a further 2 days. Two flasks were used for each experiment.

After adjusted to pH 9.0, an equal volume of acetone was added to the broth. The solvent extract was filtered and evaporated *in vacuo*, and the antibiotic was extracted with benzene three times. The solution was concentrated *in vacuo* to give a solid which was purified by preparative TLC using the system benzene - acetone (2: 1). The yield of **I** labeled with ^{13}C-precursors was *ca.* 30 mg/200 ml fermentation broth.

Lonomycin A free acid

Lonomycin A sodium salt (150 mg) was dissolved in 50 ml ethyl acetate and the solution was washed with acidic water at pH 3.0 once and then with water twice. After drying over Na_2SO_4, the solvent layer was concentrated *in vacuo* to give lonomycin A free acid (120 mg). Its physico-chemical properties are as follows: Anal. calcd. for $C_{44}H_{76}O_{14}$: C, 63.77; H, 9.18%. found, C, 63.74; H, 9.24%. Mass (*m/e*) 766 ($M^+ - CO_2 - H_2O$). IR (KBr tablet) 1705 cm^{-1}. ^{1}H-nmr ($CDCl_3$) δ_H 3.28 (s, 3H, $-OCH_3$), 3.33 (s, 3H, $-OCH_3$), 3.34 (s, 3H, $-OCH_3$), 3.45 (s, 3H, $-OCH_3$).

The ^{13}C-nmr spectral data are as follows: C1, 173.8; C2, 46.0; C3, 99.8; C4, 35.5; C5, 81.7; C6, 31.1; C7, 72.6; C8, 32.3; C9, 64.8; C10, 37.1; C11, 82.1; C12, 33.1; C13, 106.5; C14, 39.6; C15, 35.0; C16, 85.5; C17, 81.0; C18, 26.7; C19, 31.5; C20, 83.6; C21, 85.0; C22, 36.5; C23, 81.0; C24, 78.7; C25, 73.5; C26, 38.8; C27, 85.0; C28, 46.5; C29, 98.4; C30, 26.7; C31, 12.5; C32, 13.0; C33, 8.3; C34, 23.4; C35, 27.6; C36, 10.4; C37, 11.8; C38, 4.1; C39, 11.2; C40, 11.2; C41, 55.9; C42, 56.2; C43, 55.9; C44, 59.7 ppm.

Oxolonomycin sodium salt

To an aqueous acetone (90%, 50 ml) solution containing lonomycin A free acid (200 mg) was added 1 N NaOH solution (10 ml). After 48 hours, oxolonomycin sodium salt crystallized out as needles (134 mg). Its physico-chemical properties are as follows; Anal. calcd. for $C_{43}H_{71}O_{13}Na$: C, 63.06, H, 8.74, Na, 2.81%. found, C, 62.95, H, 8.72, Na, 2.90%. UV λ_{max}: 231 nm ($E_{1\%}^{MeOH}$ 170). IR (KBr tablet) 1663, 1590 cm^{-1}. ^{1}H-nmr ($CDCl_3$) 3.22 (s, 3H, $-OCH_3$), 3.28 (s, 3H, $-OCH_3$), 3.43 (s, 3H, $-OCH_3$), 6.75 (dd, J=2, 10 Hz, 1H, olefinic). Mass (*m/e*) 768 ($M^+ - CH_3OH - H_2O$). The ^{13}C-nmr spectral data are as follows: C1, 181.4; C2, 44.2; C3, 100.3; C4, 35.6; C5, 81.9; C6, 31.0; C7, 71.0; C8, 33.5; C9, 63.2; C10, 37.4; C11, 82.0; C12, 34.0; C13, 106.3; C14, 39.1; C15, 30.0; C16, 85.4; C17, 81.2; C18, 25.6; C19, 33.5; C20, 84.5; C21, 85.0; C22, 36.3; C23, 80.8; C24, 80.0; C25, 74.5; C26, 35.8; C27, 145.4; C28, 137.3; C29, 200.4; C30, 26.8; C31, 10.8; C32, 16.5; C33, 8.0; C34, 23.0; C35, 28.8; C36, 10.2; C37, 11.6; C38, 4.0; C39, 11.6; C40, 11.2; C41, 55.8; C42, 56.6; C43, 57.0 ppm. The physico-chemical and biological properties will be published elsewhere in detail.

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