STUDIES ON THE IONOPHOROUS ANTIBIOTICS. XVII.[1] THE STRUCTURES OF LONOMYCINS B AND C

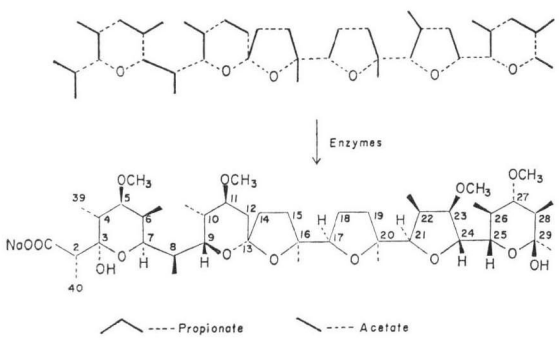
Sir:

Lonomycin A (**I**)* is a member of the family of polyether antibiotics produced by *Streptomyces ribosidificus* TM-481.[2] It is active against Gram-positive bacteria, fungi and yeasts, and is a promising anticoccidial agent. The absolute configuration of **I**[3] has been determined by an X-ray analysis (Fig. 1).

As a result of further screening for concurrent minor components of the antibiotic, two congeners of lonomycin A, *i.e.* lonomycins B (**II**) and C (**III**) have been isolated as their sodium salts from the fermentation broth of the same producing organism.[4] This communication deals with the structural elucidation of **II** and **III** mainly by ^{13}C- and ^{1}H-nmr spectroscopies.

II (m.p. 181~182°C, Anal. found; C, 62.16; H, 8.81 and Na, 2.68%. calcd. for $C_{44}H_{75}O_{14}Na$: C, 62.10; H, 8.88 and Na, 2.70%) has a molecular formula identical with that of **I** and was easily converted to **I** in organic solvents such as acetone, ethyl acetate and chloroform at room temperature (*ca.* 60% conversion after 2 days), whereas the reverse reaction under the same condition was almost negligible. Refluxing of **I** in a chloroform solution for 2 days resulted in the formation of small amount of **II**. Therefore, the equilibrium between **I** and **II** is in favor of dominant formation of **I**. This phenomenon under such mild conditions together with the identical mass spectra[4] of **I** and **II** implies strongly that these two components are interconvertible through inversion at a ketal or hemiketal function.**

Fig. 1. Biogenesis of lonomycin A.



At this point, ^{13}C-nmr spectroscopy was a method of choice to allocate the position involved in the interconversion, since it is a very useful method to detect slight stereochemical changes even in complicated molecules.[5]

Although we have succeeded in the complete assignment of the ^{13}C-nmr spectrum*** of **I**, only partial assignment vital to this structural work will be given here. In the ^{13}C-nmr spectrum of **I**, the carbonyl signal (C-1) was observed at 180.1 ppm, and one ketal and two hemiketal resonances were at around 100 ppm. Oxygenated quaternary and methin carbon signals were found at 63~86 ppm, peaks of methins and methylenes and of methyls were at around 26~47, and 4~29 ppm, respectively.

Since there are too many carbons in **I** situated in similar environments and no degradation products useful for this investigation were obtainable at practically high yield, difficulties in the complete assignment were overcome by comparison of **I** with structurally related polyether antibiotics such as nigericin,[6] mutalomycin[7] and carriomycin,[8] and by biosynthetic enrichments of peak intensities with sodium propionates and acetates labeled by carbon-13 at various positions. Known biosynthetic results on other polyether antibiotics[9~13] suggested that these carbon-13 labeled precursors would increase peak heights of carbon resonances selectively as illustrated in Fig. 1.

In the ^{13}C-nmr spectrum of **I** labeled with sodium [1-^{13}C] propionate, a peak (100.4 ppm) in the (hemi)ketal region was enriched by 6 times, whereas the rest (hemi)ketal resonances were not labeled. Therefore, the peak was assigned to C-3. C-13 (107.1 ppm) and C-29 (98.8 ppm) were unambiguously assigned based on biosynthetic results using sodium [1,2-^{13}C]-acetate ($J_{C13-C14}=42$ Hz and $J_{C29-C30}=47$ Hz) as well as on comparison with model compounds.

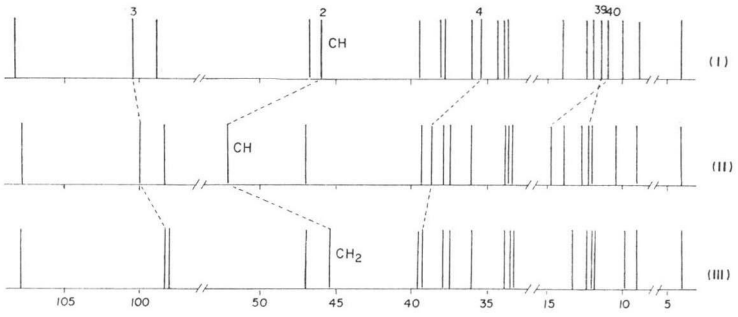
* According to the isolation of minor components, the antibiotic previously called lonomycin is hereafter designated lonomycin A.

** Since only two compounds were recognized in the reaction mixture, involvement in the interconversion of two (hemi)ketal functions was excluded.

*** Detail assignment will be published elsewhere. ^{13}C-nmr and ^{1}H-nmr spectra were obtained as reported previously.[5] ^{1}H-nmr spectra (270 MHz) were taken on a Bruker WH-270 spectrometer.

The C-2 resonance, which was expected to appear at the region characteristic to the α-carbons of the carboxylic acids (*ca.* 40~55 ppm), was distinguished from C-28 (46.6 ppm) by selective proton irradiation at H-2 (δ_H 2.55 ppm). A long range selective proton decoupling experiment[14] irradiating at the same proton collapsed a quartet of doublets centered at 11.1 ppm to a sharp quartet. Thus, this methyl resonance was assigned to C-40. These partial assignments facilitated to reveal the structural differences between **I**, and **II** and **III**.

Fig. 2. Pertinent region of the ^{13}C-nmr spectra of lonomycins.



As shown in Fig. 2, the ^{13}C-nmr spectra of **I** and **II** are very similar suggesting for the most part that the two antibiotics are identical. However, it should be noted that the following remarkable differences between them were observed at around the C-3 hemiketal carbon.

(1) The C-2 signal at 46.0 ppm in **I** was shifted downfield by 6.0 ppm.

(2) An absorption at 35.4 ppm which must be assigned to C-4 (*vide infra*) also suffered downfield shift by 3.0 ppm in **II**.

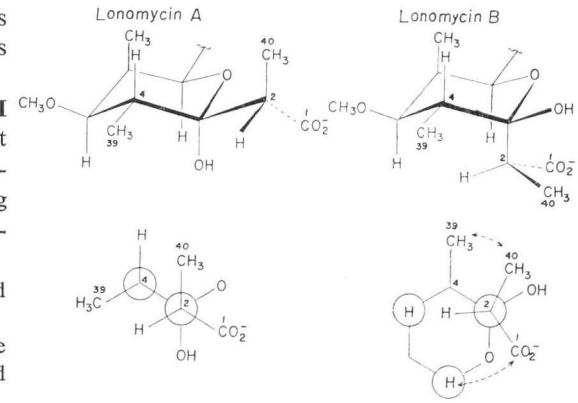
(3) The methyl resonance (C-40) at 11.1 ppm in **I** was replaced by a methyl peak at 14.9 ppm in **II**.

(4) A methyl signal assigned to C-39 (*vide infra*) at 11.6 ppm moved to 12.7 ppm in **II**.

(5) Slight shift was observed with the carboxyl (C-1) (not shown in Fig. 2) and hemiketal (C-3) signals.

The structure of **I** given by an X-ray analysis shows that the relationships of H-2 and CH_3 (C-39) and of H-4 and CH_3(C-40) are *syn* axial as shown in Fig. 3. Conformational analysis on other polyether antibiotics using ^{1}H-nmr spectroscopy (270 MHz) by ANTEUNIS *et al.*[15] suggests that this conformation of **I** would also hold in the chloroform solution. Therefore, it is reasonably assumed that the γ-effect acted strongly on C-2, 4, 39 and 40 in **I**. The downfield shift of these carbons in **II** can be reconciled only by the configurational change at C-3 as shown in Fig. 3. Thus, C-2 and its substituents in **II** are oriented not to interfere sterically with H-4 and C-39 resulting in the lack of the γ-effect. The similarity of the chemical shifts in **I** and **II** of

Fig. 3. The stereochemical relationship around C-3 in lonomycins A and B.



the carbons adjacent to the ketal (C-13) and hemiketal (C-29) functions also indicated the structural difference between **I** and **II** to exist at C-3. The structure of **II** is further corroborated by the very facile conversion of **II** into **I** (*vide supra*) which is promoted by the energetically unfavorable configuration at C-3 in **II** (Fig. 3). Therefore, **II** is the epimer of **I** at the C-3 position.

Having established the structure of **II**, the methyl (11.6 ppm) and methin (35.4 ppm) resonances described above were assigned to C-39 and C-4, respectively.*

Lonomycin C **III** (m.p. 186~187°C, Anal. found; C, 61.87; H, 8.86 and Na, 2.78%. calcd.

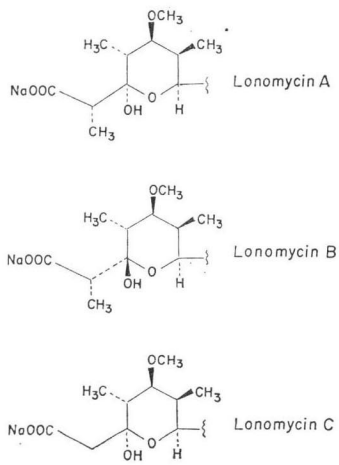
* Without knowing the structure and chemical shift data of **II**, we could not assign the C-39 and C-4 resonances of one-to-one basis. However, information obtained theretofore had confined methyl (10.2, 11.6 and 12.0 ppm) and methin (35.4 and 33.7 ppm) signals assignable to C-39 and C-4, respectively.

for $C_{43}H_{73}O_{14}Na$: C, 61.72; H, 8.74 and Na, 2.75%) is similar to **I** in its physicochemical properties, however it contains one less carbon and two less hydrogen atoms than **I**. As shown in Fig. 2, the ^{13}C-nmr spectrum of **III** showed close similarity to that of **I** except for (1) the disappearance of the methyl resonance (C-40) present in **I**, (2) the downfield shift by 3.0 ppm of the C-4 signal and (3) the upfield shift of the C-3 peak by 2.0 ppm. In addition, the signal at 46.5 ppm corresponding to C-2 in **I** was shown to be a methylene by the off-resonance spectrum of **III**. These chemical shift changes are reasonably accounted for by the disappearance of β- or γ-effect on C-3 and C-4 exerted by the methyl substituent (C-40) in **I**.

A confirmative evidence of this structure was obtained further by ^{1}H-nmr spectrum (270 MHz) which revealed the presence of three singlet methyls and seven doublet methyls in **I**. However, a methyl doublet at 1.04 ppm, which was coupled to a quartet at δ_H 2.55 ppm (H-2) in **I**, was not observed in the ^{1}H-nmr spectrum of **III**. Therefore, it was concluded that the methyl at C-2 was absent in **III**. This ^{1}H-nmr result is consistent with the conclusion obtained by ^{13}C-nmr spectroscopy.

The remaining problem is the stereochemistry of C-3 in **III**. As mentioned above, the difference between **I** and **II** is due to the configurational change at C-3 which was reflected in the ORD spectra of **I** and **II**; the former showed a positive plain curve which was opposite to that of the latter. This result indicated that the ORD spectra of lonomycins are mainly affected by the configuration at C-3 with the contribution by C-2 being negligible. Since the ^{13}C-nmr spectrum of **III** is quite similar to that of **I** except for a few changes described above, it could be assumed that the overall conformations of the two antibiotics are almost identical. Therefore, **III** showing a positive ORD curve identical with that of **I** has the same configuration at C-3 leading to the structure as shown in Fig. 4.

Fig. 4. The partial structures of lonomycins.



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