Communications to the editor

STUDIES ON THE IONOPHOROUS ANTIBIOTICS. XX[1] SOME EMPIRICAL RULES FOR STRUCTURAL ELUCIDATION OF POLYETHER ANTIBIOTICS BY ^{13}C-NMR SPECTROSCOPY

Sir:

The polyether antibiotics characterized as possessing several cyclic ether systems are mainly produced by the *Streptomyces* genus[2]. Due to structural complexity, easier preparation of heavy metal containing derivatives and difficulty to obtain degradation products useful for structural studies, X-ray analysis has been the only practical method for structural elucidation of these compounds.

However, in view of the power in the structural studies of complex molecules, ^{13}C-nmr spectroscopy is expected to become a reliable and useful method in the structural elucidation of these antibiotics if the relationships between specific structures and chemical shifts of relevant carbons in the ^{13}C-nmr spectra can be established.

Fig. 1. Basic carbon skeleton common to etheromycin, lonomycin, mutalomycin, carriomycin, septamycin, A204A and nigericin.
+=H or CH_3
●=H, OH, OCH_3 or sugar

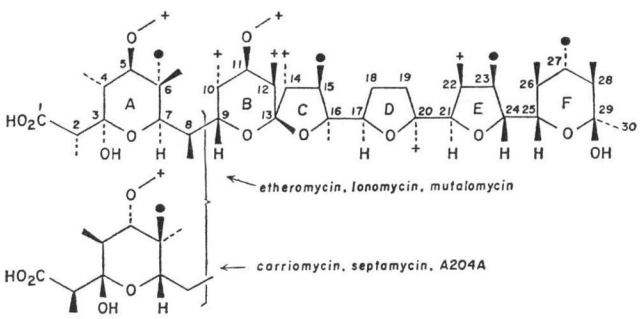
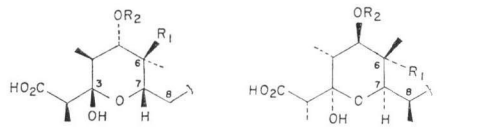


Fig. 2. A-ring.



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

As a result of extensive studies on the assignments* of the ^{13}C-nmr spectra** of polyether antibiotics including lonomycin[3], mutalomycin[4], carriomycin[5], septamycin[6], A204A[7], etheromycin (CP-38295)[8] and nigericin[9], we found some empirical rules very useful for structural elucidation of antibiotics possessing the basic carbon skeleton shown in Fig. 1.***

In this paper these rules will be described in detail. The assignments of the ^{13}C-nmr spectra of polyether antibiotics with a somewhat different carbon skeleton such as salinomycin[10], lasalocid[11] and lysocellin[12] have been reported.

In extracting empirical rules from the established assignments, we decided to utilize only signals appearing in the characteristic regions or ones easily distinguishable from other signals by several ^{13}C-nmr techniques. Therefore, methylene and methine signals in the very crowded region (22~40 ppm) were not taken into consideration. Of course, detailed analysis of these signals must be carried out at the final stage to confirm the proposed structure.

A-Ring

It should be noted that the absolute configuration of the A-ring of carriomycin, septamycin and A204A is opposite to that of lono-

* Detail assignments of these antibiotics will be reported elsewhere. The complete assignment of the ^{13}C-nmr spectrum of lonomycin has been presented at The Meeting of The Agricultural Chemical Society of Japan held at Nagoya, April, 1978.

** All ^{13}C-nmr spectral data used in this work were collected in $CDCl_3$ solution (TMS as internal standard) employing the sodium salts of polyether antibiotics.

*** The structure of A-ring in nigericin is different from the other members cited here.

mycin, mutalomycin* and etheromycin, and that the stereochemistry of the A-ring is related to the presence of a methyl at C-8. Therefore, for structural determinations of polyether antibiotics by ^{13}C-nmr spectroscopy, it is very important to know the substitution pattern at C-8. The introduction of a methyl group at C-8 causes a downfield shift of the C-7 signal (carriomycin, septamycin and A204A 64.0~67.3 ppm compared with etheromycin, lonomycin and mutalomycin 70.8~71.5 ppm).

The presence of a substituent other than the methyl at C-6 can be detected by the characteristic chemical shift of the axial methyl at C-6. A methoxy or sugar substituent at C-6 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 largest shift was observed in the case of A204A in which the substituent at C-6 is a methoxy.

The signal due to the hemiketal carbon C-3 appearing at 99.2~100.4 ppm in most members of this group is absent in nigericin which has no hydroxy function at C-3.

B and C-rings

Three structures have been reported for the B-ring. The differences are due to the presence of a methyl at C-8 (etheromycin, lonomycin and mutalomycin) and the position of a methyl at C-10 (lonomycin and mutalomycin) or C-12 (nigericin, carriomycin, septamycin, A204A and etheromycin). As shown in the figure, a methyl at C-8 causes a downfield shift of C-9 by *ca.* 3 ppm.

Except for etheromycin, the presence of the methyl at C-8 is related to the lonomycin type with a methyl at C-10. Distinction of etheromycin from the latter can be made by the chemical shift of C-13. Since the C-ring structures are identical in both types, the chemical shift of C-13 seems to be dependent on the presence of a methyl at C-12 (etheromycin 109.2, lonomycin and mutalomycin 106.8~107.0 ppm).

Two kinds of structures exist for the C-ring. One has a methyl substituent at C-14 and the other has not. This difference can be detected by the chemical shifts of C-16.* In compounds with a methyl at C-14 such as nigericin, carriomycin, septamycin and A204A, C-16 are observed at rather higher field (82.4~83.1 ppm) than in etheromycin, lonomycin and mutalomycin (84.2~85.2 ppm) which lack the substituent at C-14. The methoxy substituent at C-15 in carriomycin, septamycin and A204A can be detected by the very characteristic oxymethine signal at 94.5 ppm due to C-15. The resonance due to a methyl carbon at C-16 may also be of use to detect the presence of a methyl at C-14.

Fig. 3. B- and C-rings.

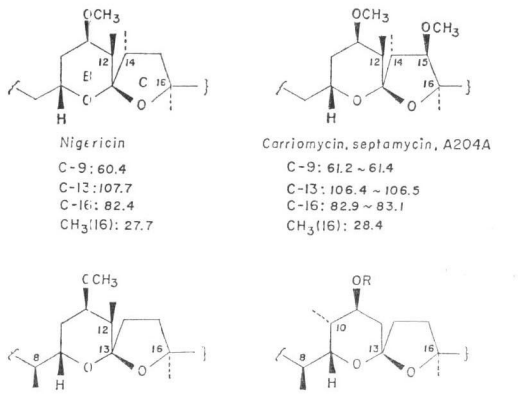
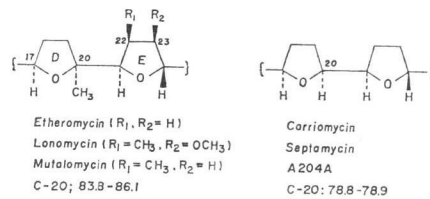


Fig. 4. D- and E-rings.



* The configuration of C-4 in mutalomycin originally reported to be R[4] has been revised to be the same as in lonomycin (A. VON WARTBURG and M. KUHN, private communication).

* It should be noted that the substitution patterns of the D-ring (C-17, 18 and 19) are completely identical in all compounds examined so far. This rule, which may not hold for compounds with different substituents on the D-ring, has been shown to be still applicable for antibiotics with a sugar substituent at C-18 as evidenced by antibiotic 6016 (see the following paper).

D and E-Rings

There are two kinds of structures for the D-ring. One has a methyl at C-20 and the other has not. The latter group includes carriomycin, septamycin and A204A. Since the methyl at C-20 resonates at a characteristic region (22.3 ~ 23.3 ppm), its presence can be revealed very easily. The chemical shift of the quaternary carbon C-20 is affected by not only the methyl but also the methoxy on the E-ring. The effects of these substituents seem to be opposite in sign. Namely, the methyl at C-22 causes an upfield shift of C-20 (etheromycin 86.1 compared to mutalomycin 83.8 ppm), whereas the methoxy at C-23 causes downfield shift (lonomycin 85.8 ppm).

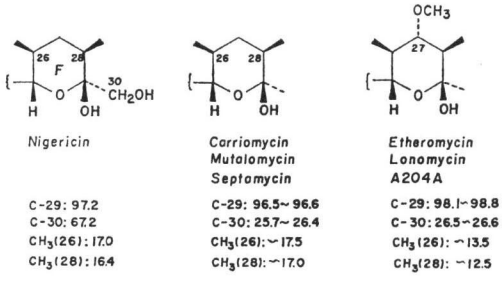
The structure of the E-ring can be conveniently established by the characteristic resonances of a methyl at C-22 (mutalomycin 16.2 and lonomycin 9.0 ppm) and a methoxy at C-23 (lonomycin 57.3 ppm).

There exists no substituent on the E-ring for compounds without a methyl at C-20 such as carriomycin, septamycin and A204A. The chemical shift of the C-20 methine in these compounds (78.8 ~ 78.9 ppm) is not so characteristic.

F-Ring

Three kinds of structures are known for the F-ring, the differences being due to the presence of a methoxy at C-27 and a hydroxy at C-30.

Fig. 5. F-ring.



Two methyls at C-26 and 28 move to higher field due to the γ-effect of the methoxy at C-27. Since the chemical shift values of these methyls unsubstituted at C-27, such as in 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 C-29 are also useful to detect the methoxy at C-27. A methyl signal due to amicetose present in carriomycin, septamycin, etheromycin and A204A can be recognized by its discernible chemical shift (18.5 ppm).

Distinction of the nigericin type with a hydroxy substituent at C-30 from the other can be made by the characteristic hydroxy methyl signal at 65 ~ 67 ppm.

4'-O-Methylamicetose

The sugar found in polyether antibiotics is 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,[13] it is much better to utilize the chemical shift of C-5' for obtaining information about the anomeric configuration. The chemical shift of C-5' 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 C-1'. It should be noticed that anomeric carbons attached to a quarternary carbon appear at considerably higher field (etheromycin 95.2 ppm and septamycin 96.3 ppm) than those combined to a methine carbon (carriomycin 97.6 ppm and A204A 98.3 ppm).

Methoxy Signals

Valuable structural information can also be obtained by analyzing these signals. The most characteristic in this region is the methoxy signal

Fig. 6. G-ring (4'-O-Methylamicetose).

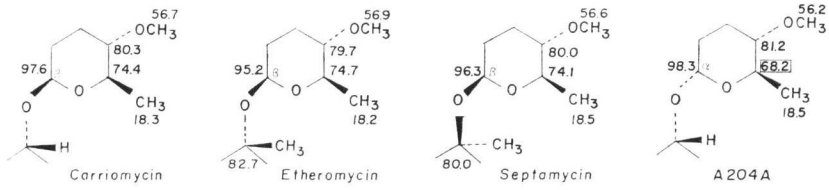
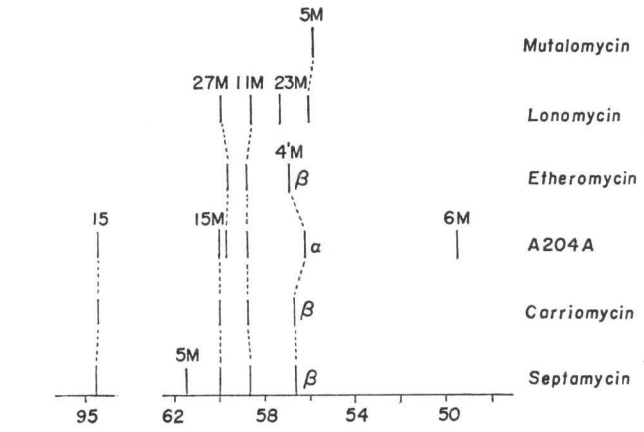
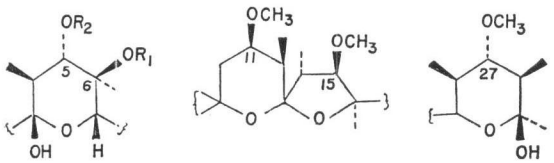


Fig. 7. Methoxy signals

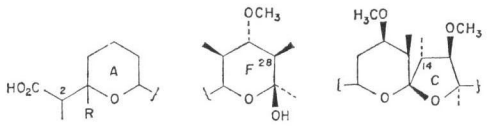




A204A ($R_1 = CH_3$, R_2 = sugar)

Septamycin (R_1 = sugar, $R_2 = CH_3$)

Fig. 8. Signals appearing at 45~47 ppm



Most polyethers (R= H or OH)

Etheromycin
Lonomycin
A204A
C29: 98-99

Carriomycin
Septamycin
A204A
C15: 94.5

(49.5 ppm) linked to the quaternary carbon C-6 in A204A. Another signal showing discernible chemical shift (61.5 ppm) in septamycin is assigned to a methoxy carbon at C-5 which is connected to the quaternary oxycarbon C-6. Thus, the chemical shifts of these methoxy carbons are very useful to know the substitution pattern at C-6.

The chemical shifts of the methoxy carbons at C-15 and 27 are very close. However, as previously explained, the methoxy at C-15 is always accompanied by a very characteristic signal at 94.5 ppm. The chemical shift of a methoxy carbon in the sugar moiety may be of value in distinguishing the anomeric configuration (56.6~56.9 ppm in β- and 56.2 in α-configuration). However, the difference may be too small to draw a definitive conclusion.

Signals Appearing at 45~47 ppm

This region is usually specific to the methine carbon (C-2) adjacent to the terminal carboxylic acid. In addition, two different methine signals may sometimes be observed. One of them is ascribed to the C-28 methine with a methoxy substituent at C-27. In this case, two methyls at C-26 and 28 do not resonate at ~17 ppm and the C-29 hemiketal carbon appears at much lower field (98~99 ppm) as explained previously. The other is due to the methine signal of C-14 which is accompanied by a characteristic oxymethine signal at 94.5 ppm assignable to C-15. Therefore, these two structures can be revealed and distinguished from each other by analyzing signals at 94~99 ppm.

These empirical rules are expected to be useful in structural elucidation of polyether antibiotics having the basic carbon skeleton as shown in Fig. 1. Their application will be described in the following paper.

Acknowledgment

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HARUO SETO
KAZUTOSHI MIZOUE
HIROSHI NAKAYAMA
KAZUO FURIHATA
NOBORU ŌTAKE
HIROSHI YONEHARA

Institute of Applied Microbiology,
The University of Tokyo
1-1-1 Yayoi, Bunkyo-ku, Tokyo, Japan 113

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