PROPOSED STRUCTURE OF ANGOLAMYCIN (SHINCOMYCIN A) BY MASS SPECTROMETRY

Sir:

The basic macrolide antibiotic, angolamycin was isolated from *Streptomyces eurythermus* n. sp. by CORBAZ *et al.* in 1955[1]. They described angolamycin (m.p. 134~136 °C, $[\alpha]_D^{21}$ −64°) as a carbomycin-type macrolide antibiotic from spectroscopic data (UV, λ_{max}^{EtOH} 240 nm (log ε 4.16). IR, ν_{max}^{nujol} 1711, 1686, 1623 cm^{-1}), pKa value (pK_{MCS} 6.74) and the proposed molecular formula ($C_{50\pm1}H_{89\pm2}NO_{18}$). Later, three sugars, L-mycarose, D-mycinose and D-amgolosamine were isolated by acid hydrolysis of angolamycin[2]. This information characterized angolamycin as a trisugar-containing macrolide with a 16-membered lactone ring, however, no report of the complete structure has appeared.

Recently the structure elucidation of 16-membered macrolide antibiotics by mass spectrometry was introduced in our laboratory. This method is based on the direct determination of the structures of aglycone- and sugar-parts, and their sequence analysis, by investigating mass spectrometric fragmentation patterns of the acetate and the trideuteroacetate derived from an original macrolide. Hitherto, this method was successfully adapted to confirm the structures of YL-704 A_1 and B_1[3a] and their minor components C_1, C_2 and W_1[3b] as well as other typical 16-membered macrolides, such as leucomycins, carbomycins, spiramycins and cirramycins[4].

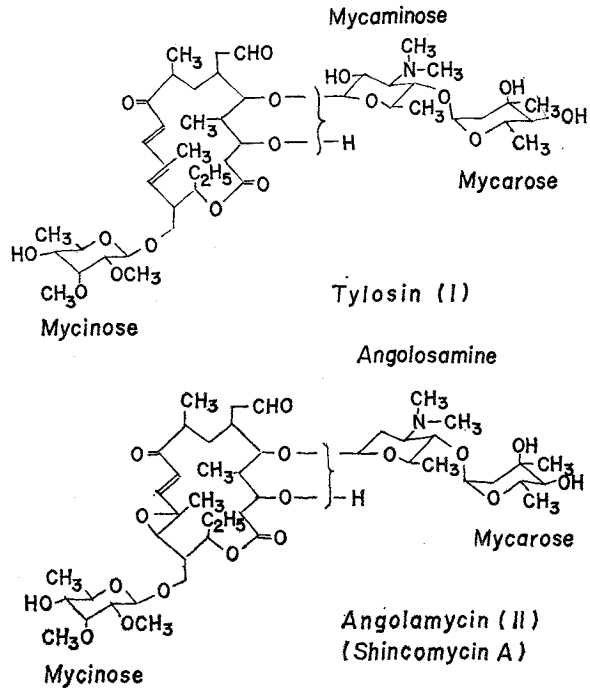
The structure of tylosin ($C_{46}H_{77}NO_{17}$, I) which contains a complex 16-membered aglycone and three sugar moieties was elucidated by MORIN *et al.* in 1970[5]. Considering the chemical resemblance between angolamycin and tylosin, mass spectra of acetyl derivatives of tylosin were first investigated.

In accordance with the structure I, the mass spectra of tetraacetyl tylosin ($C_{54}H_{85}NO_{21}$, m.p. 120~122°C) and tetrakis-(trideuteroacetyl) tylosin ($C_{54}H_{73}D_{12}NO_{21}$, m.p. 121~122°C) showed molecular ions at *m/e* 1083 and *m/e* 1095, respectively. Furthermore, as in the cases of the previously examined antibiotics[3,4] their fragmentation patterns were completely rational as summarized in a diagnostic fragmentation diagram depicted in Scheme 1.

Next, the mass spectra of the acetyl angolamycin derivatives were examined. Triacetyl angolamycin (m.p. 124~125°C, δ 2.07, 2.12 and 2.25 ppm, $CO\underline{CH_3}$ and tris (trideuteroacetyl) angolamycin (m.p. 124~125°C) gave the molecular ions at *m/e* 1041 and *m/e* 1050 corresponding to $C_{52}H_{83}NO_{20}$ and $C_{52}H_{74}D_9NO_{20}$, respectively, and, as a result, the molecular formula of angolamycin was determined to be $C_{46}H_{77}NO_{17}$.

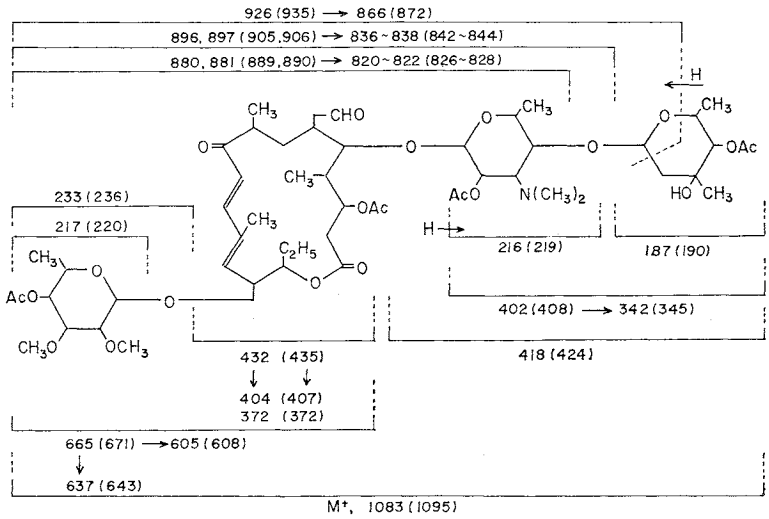
In addition, most of the predominant fragment peaks of triacetyl angolamycin were shifted by three or six mass units from the corresponding peaks of tris (trideuteroacetyl)-derivative, as shown in the diagnostic fragmentation diagram illustrated in Scheme 2. Moreover, some fragments ascribed to the sugar parts of triacetyl angolamycin were identical with those of tetraacetyl tylosin.

Consequently, it has become apparent that angolamycin is a new 16-membered macrolide with the same molecular formula as tylosin



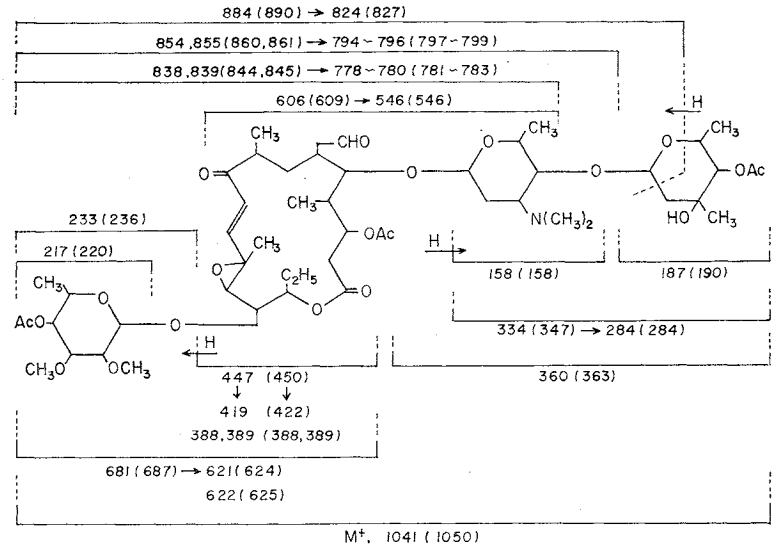
Scheme 1. Diagnostic fragmentations of tetraacetyl tylosin*

* The arabic numbers are *m/e* in the peracetate, and the parenthesized ones show *m/e* in the corresponding deuterium-labeled acetate.



Scheme 2. Diagnostic fragmentations of triacetyl angolamycin*

* The arabic numbers are *m/e* in the peracetate, and the parenthesized ones show *m/e* in the corresponding deuterium-labeled acetate.



(I), but with the different structure (II). The structure of angolamycin (II) proposed by mass spectrometry was compatible with the spectral and chemical data mentioned above, together with the following NMR signals: δ 0.88 ($CH_2\underline{CH_3}$), 1.41 ($\underset{O}{\swarrow\!\!\!\swarrow}\underline{CH_3}$), 2.25 ($N(\underline{CH_3})_2$), 3.55 and 3.61 ($O\underline{CH_3}$), 6.44 (J, 16 Hz, $\underline{CH}$=CH), 6.64 (J, 16 Hz, CH=$\underline{CH}$) and 9.70 ($\underline{CH}O$) ppm.

However, it was not enough to support the attachment of the two sugar residues to one or the other -OH residue of the aglycone by mass spectrometry. Although the whole structures of tylosin and angolamycin were established insufficiently, the diagnostic-fragmentation patterns were described as the location of two sugar residues at 5-position in aglycone, considering the chemical degradation product (mycinsoyl anhydro nortylonolide) reported by MORIN *et al*[5].

In closing, we add that shincomycin A[6],

another basic macrolide antibiotic which had been reported to be similar to angolamycin, was completely identical to angolamycin by the present procedure.

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AKIO KINUMAKI
MAKOTO SUZUKI

Microbial Chemistry Research Laboratory,
Tanabe Seiyaku Co., Ltd.,
Toda, Saitama, Japan

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