NOTE

THE CONSTITUTION OF LAIDLOMYCIN, A NEW ANTIMYCOPLASMAL ANTIBIOTIC

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The production, isolation and biological properties of laidlomycin, $C_{37}H_{62}O_{12}$(M^+; 698), m.p. 151~153°, $[\alpha]_D^{22}$+51.3°($CHCl_3$), obtained from culture filtrates of a strain of *Streptomyces eurocidicus* var. *asterocidicus* were previously reported by us.[1] Chemical structure of this antibiotic had not been elucidated at that time.

The present communication deals with this issue, and structure **I** (Fig. 4), is proposed on the basis of the following evidence.

Considerable experimental difficulties were anticipated in determining the chemical structure of antibiotics of this class by conventional chemical means. However, because of certain similar physicochemical properties of laidlomycin and monensin B (**II**), mass spectrometric measurements* were found to be particularly useful. Since the alkali metal salts of laidlomycin are sufficiently volatile, the sodium salt of laidlomycin, $C_{37}H_{61}O_{12}Na$ (M^+; 720.4085, MH^+; 721), m.p. 277~279°, $[\alpha]_D^{22}$ + 78.9° ($CHCl_3$), was used for mass spectrometric analysis. The mass spectral fragmentation behavior of this sodium salt (LDM-Na) was shown to be very similar to that of monensin's sodium salt,[2,3] especially to the sodium salt of monensin B (**II**) (MNB-Na) (Fig. 1). In the spectrum of LDM-Na, ions appear at *m/e* 705 ($C_{36}H_{58}O_{12}Na$), 703 ($C_{37}H_{60}O_{11}Na$), 689 ($C_{36}H_{58}O_{11}Na$), 647 ($C_{34}H_{56}O_{10}Na$) and 603 ($C_{33}H_{56}O_8Na$). These fragments can be ex-

Fig. 1. Mass spectra of laidlomycin sodium salt and monensin B sodium salt.

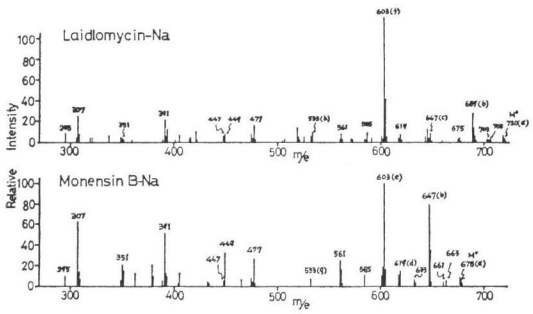
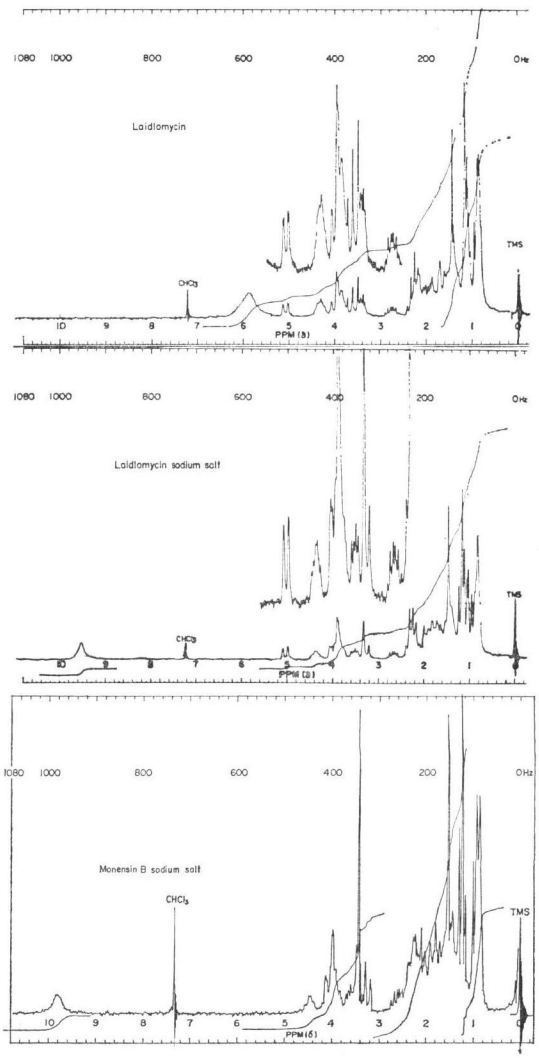


Fig. 2. NMR spectra of laidlomycin, laidlomycin sodium salt and monensin B sodium salt.



* Mass spectrum was obtained with HITACHI RMU-7 mass spectrometer, JMS-01-SG high resolution mass spectrometer and chemical ionization mass spectrometry with isobutane; NMR spectra were determined in $CDCl_3$ (at 100 MHz, TMS as internal reference); IR spectra were obtained in KBr.

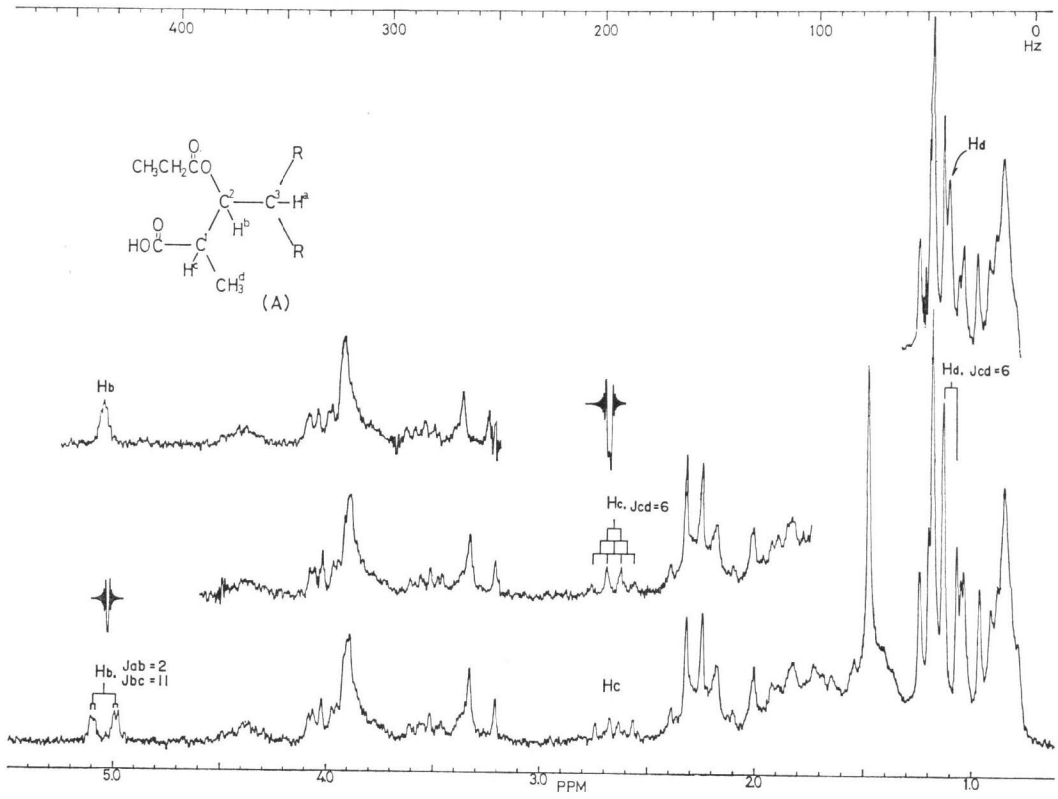
plained as resulting from the loss of CH_3, OH, CH_3O and $C_3H_5O_2$ from *m/e* 720. The LDM-Na and MNB-Na exhibit identical fragmentation patterns in the region below *m/e* 600 and, in the region above *m/e* 600, the appropriate 42-mass unit shifts are seen.

Table 1 summarizes the results from high resolution analysis of the molecular ions and the major fragment ions in the spectra of LDM-Na and MNB-Na. The molecular ions of both antibiotics are accompanied by $[M^+-1]$ ions of greater intensity. LDM-Na was found to differ from MNB-Na by 42 atomic-mass units, which may correspond to C_2H_2O. The base peaks in the spectra of LDM-Na and MNB-Na are at *m/e* 603 [M−75−42] *f* and

Table 1. High resolution analysis of the major ions in the mass spectra of the sodium salts of laidlomycin and monensin B

Fragments	Ions (*m/e*)	
	Laidlomycin	Monensin B
(*a*) M^+	720.4085 $C_{37}H_{61}O_{12}Na$	678.3942 $C_{35}H_{59}O_{11}Na$
(*b*) M^+-31	689.3915 $C_{36}H_{58}O_{11}Na$	647.3744 $C_{34}H_{56}O_{10}Na$
(*c*) $M^+-(CH_3CH_2COO)$	647.3796 $C_{34}H_{56}O_{10}Na$	——
(*d*) M^+-59	619.3723 $C_{33}H_{56}O_9Na$	619.3788 $C_{33}H_{56}O_9Na$
(*e*) $M^+-(CO_2+CH_3O)$	——	603.3892 $C_{33}H_{56}O_8Na$
(*f*) $M^+-(CO_2+CH_3CH_2COO)$	603.3861 $C_{33}H_{56}O_8Na$	——
(*g*) $(e)-C_5H_{10}$	——	533.3021 $C_{28}H_{46}O_8Na$
(*h*) $(f)-C_5H_{10}$	533.3094 $C_{28}H_{46}O_8Na$	——

Fig. 3. NMR spectrum of laidlomycin sodium salt.



at *m/e* 603 [M−75] *e*, which correspond to [M−$C_2H_3O_3$] minus 42 and [M−$C_2H_3O_3$].

Other common peaks were found at *h* or *g* in Fig. 1, corresponding to loss of the hydrocarbon side chain plus a hydrogen atom from *f* or *e*, respectively.[3] In the spectra of LDM-Na and MNB-Na fragment *h* at *m/e* 533 was equal to fragment *g*.

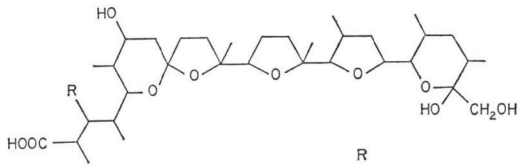
Therefore, it seems probable that the difference of 42 mass units can be explained by its presence in the side chain of the laidlomycin molecule.

The NMR* spectrum of laidlomycin (Fig. 2) is lacking the signal corresponding to the methoxyl group in the side chain of monensins.[2] In the NMR spectrum of LDM-Na (Fig. 3), the double doublet (1H, J=2 and 11 Hz) centered at 5.04 ppm, the multiplet (1H) at 2.7 ppm, and the doublet (3H, J=6 Hz) at 1.9 ppm can be assigned to the H^b, H^c and C-1 methyl protons, respectively. These assignments are supported by the fact that irradiation at 5.04 ppm converted the multiplet at 2.7 ppm into a quartet (J=6 Hz). Furthermore, irradiation at 2.7 ppm converted the C-1 methyl doublet at 1.09 ppm into a singlet and the double doublet at 5.04 ppm (H^b) into a broad singlet which showed further coupling with H^a and other protons.

These observations support partial structure (*A*) (Fig. 3) for laidlomycin. Furthermore, it is clear that laidlomycin has an additional ester group when compared with monensin B on the basis of the following three observations; (1) it shows an absorption band at 1725 cm^{-1} in its IR spectrum,[1] (2) the double doublet signals in its NMR spectrum centered at 5.04 ppm (J=2, 11 Hz), correspond to a hydrogen on a carbon bearing an ester group (Fig. 2), and (3) it gives propionic acid when treated with 5% alcoholic potassium hydroxide for 5 hours at 70°C (data are not shown, detected by gas chromatography).

Thus the 42 mass units difference between laidlomycin and monensin B can be explained by the presence of a propionic ester moiety in laidlomycin in place of the methoxyl group in the monensin B molecule. These results suggest that the structure of laidlomycin must be **I**.

Fig. 4. Structures of laidlomycin and monensin B.



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