Tetrahedron Vol 28, pp. 2839 to 2848 Pergamon Press 1972. Printed in Great Britain

CHEMISTRY OF LEUCOMYCIN-X[1]

CONFORMATIONAL STUDIES ON LEUCOMYCIN

S. ŌMURA,*[1,*2] A. NAKAGAWA,*[1] N. YAGISAWA,*[1] Y. SUZUKI,*[1] and T. HATA*[1] *[2]

School of Pharmacy, Kitasato University*[1] and Kitasato Institute*[2]
5-9-1, Shirokane, Minato-ku, Tokyo, Japan

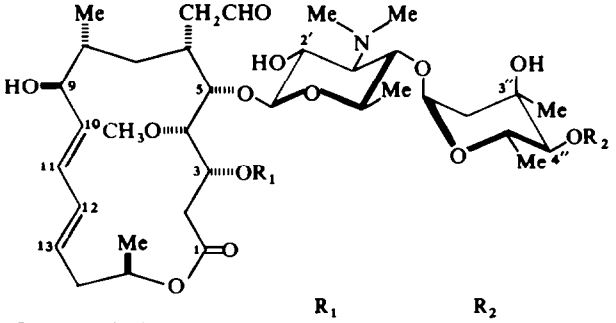
(*Received in Japan* 10 *November* 1971; *Received in the UK for publication* 13 *January* 1971)

Abstract—The conformation of leucomycin, a macrolide antibiotic with a 16-membered ring lactone, was examined in solution by IR, NMR and CD spectral analysis. The IR reveals that the five hydroxyls are all involved in intramolecular H-bonding. The NMR of the 16-membered ring lactone forming the aglycone shows that the acetyl-carbonyl at C-3, the allylic proton at C-11 and the aldehyde proton are in close proximity on the lactone ring. CD studies suggest that the conformation of the 16-membered ring lactone, especially around the lactone, is mobile and solvent dependent.

NOW THAT THE STRUCTURE of leucomycin,[2,3] a macrolide antibiotic having a 16-membered ring lactone, has been clarified, its conformational analysis will provide a valuable index for its chemical modification in examining the relationship between structure and biological activity, and is important in the study of its action mechanism. Conformational analysis[4–8] of erythromycin, a similar antibiotic with a 14-membered ring lactone, has been reported but that of a 16-membered ring lactone has not been clarified. The conformational analysis of leucomycin was carried out, with reference to its IR,[9] NMR, and CD[10] data, and the previously reported absolute structure[11] of demycarosyl-isoleucomycin-A_3 (XV) and X-ray crystal structural data.[12]

IR *spectral studies*

The presence of intramolecular H-bonding was examined in the IR absorption of the OH groups distributed almost uniformly in the molecule. Leucomycin possesses the components shown in Fig. 1. IR spectra of the components U (III) and V (IV), and



		R_1	R_2
Leucomycin-A_1	(I)	H	$COCH_2CH(Me)_2$
Leucomycin-A_3	(II)	COMe	$COCH_2CH(Me)_2$
Leucomycin-U	(III)	COMe	H
Leucomycin-V	(IV)	H	H

FIG. 1. Structures of leucomycin-A_1(I), A_3(II), U(III), and V(IV)

their derivatives in dilute CCl_4 were measured, and the differential spectra of each OH was examined.

Leucomycin-V (IV) has five OH groups; one each at 3″ and 4″ on the mycarose moiety, one at 2′ on mycaminose, and one each at 3 and 9 on the lactone.

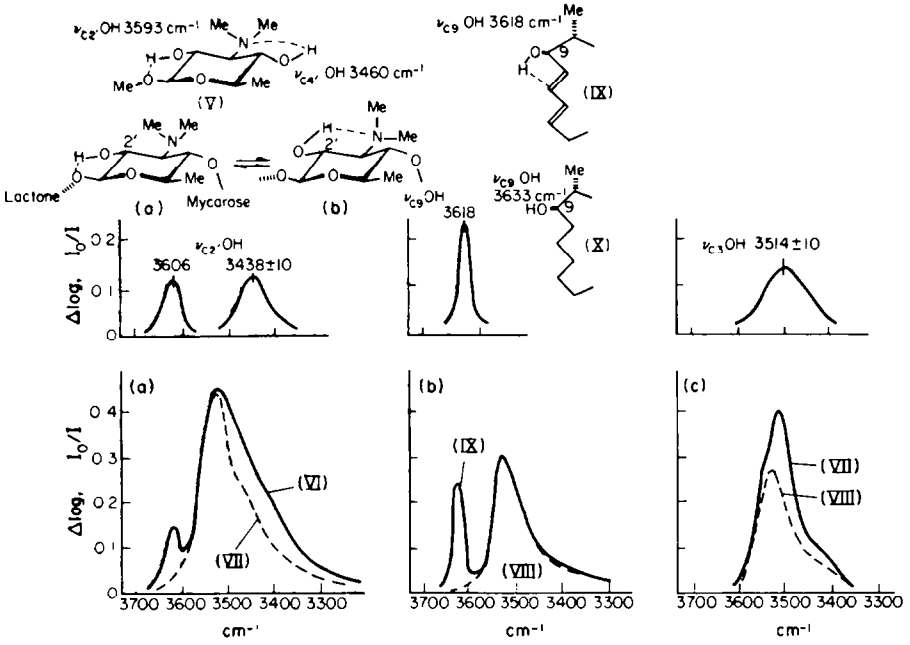


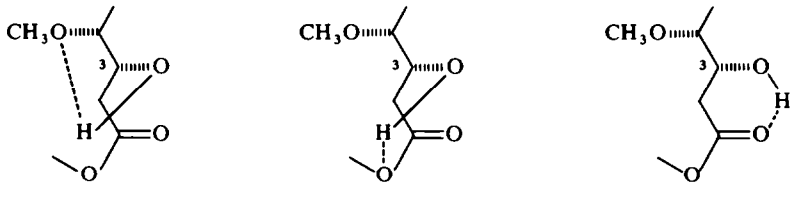
FIG. 2. ν_{O-H} Absorptions of compounds, V, VI, VII, VIII, IX, and X

Firstly, absorption of the equatorial secondary OH at 2′ on the mycaminose in II was examined by comparison with the IR spectrum of β-methylmycaminoside[13] (V) as a model compound, shown in Fig. 2a. The IR spectrum of V has two OH absorptions at 3593 and 3460 cm^{-1}. The former indicates intramolecular H-bonding between the equatorial OH at 2′ and the oxygen of the OMe, and the latter between the equatorial OH at 4′ and the N atom in the dimethylamino group.[14] On the other hand, two OH absorptions of 2′ at 3606 and 3438 ± 10 cm^{-1} were observed from the differential spectra of 9-monoacetyl-leucomycin-A_1 (VI) ($1{\cdot}5 \times 10^{-3}$ mole, CCl_4) and 9,2′-diacetyl-leucomycin-A_1 (VII) ($1{\cdot}5 \times 10^{-3}$ mole, CCl_4). The data indicates that the OH at 2′ on mycaminose is present as two conformers, the absorption at 3606 cm^{-1} (a) showing the OH forming an intramolecular H-bond with ethereal oxygen of the glycoside bond and that at 3438 ± 10 cm^{-1} (b), that with the N atom in dimethylamino group.

With respect to the OH in the 9-position of the lactone, the stretching vibration of OH was calculated as 3618 cm^{-1} from the differential spectrum of triacetyl-leucomycin-A_1 (VIII) ($1{\cdot}5 \times 10^{-3}$ mole, CCl_4) obtained by acetylation of the OH groups in the 3 and 9-positions on the lactone and that in 2′ on mycaminose and 2′-monoacetyl-leucomycin-A_3 (IX) ($1{\cdot}5 \times 10^{-3}$ mole, CCl_4). When IX was catalytically reduced

over platinum to tetrahydro-2'- monoacetyl-leucomycin-A_3 (X); a conjugated diene, the absorption of the OH shifted to a higher wave-number and was observed at 3633 cm^{-1} (Fig. 2b). This revealed that the OH in the 9-position formed an intramolecular H-bond with the π-electrons[15] of the conjugated diene, with absorption at 3618 cm^{-1}.

With respect to the OH at the 3-position on the lactone, the differential spectra of VII, which possesses an OH in the 3-position, and of VIII, in which the OH in 3-position has been acetylated, indicated the OH absorption at 3514 ± 10 cm^{-1} as shown in Fig. 2c. This suggested three possible structures, A, B, and C for formation of H-bonding.



In order to distinguish between the three types, the IR spectra of mycaroside derivatives were measured. For the possibility of structure A, the IR spectrum of acetyl-α-O-methyl-mycaroside (XIa)* indicated an OH forming a H-bond with oxygen in OMe at 3533 cm^{-1} and a free ester CO at 1743 cm^{-1} as shown in Fig 3a. On the other hand, the spectrum of acetyl-β-O-methylmycaroside (XIb)* showed a H-bonded OH absorption at 3604 cm^{-1} and an acetoxyl CO[16,17] in which alcoholic oxygen contributed to intramolecular H-bonding as the proton acceptor, at 1753 cm^{-1}. Since the OH at the 3-position of the lactone has an absorption at 3514 ± 10

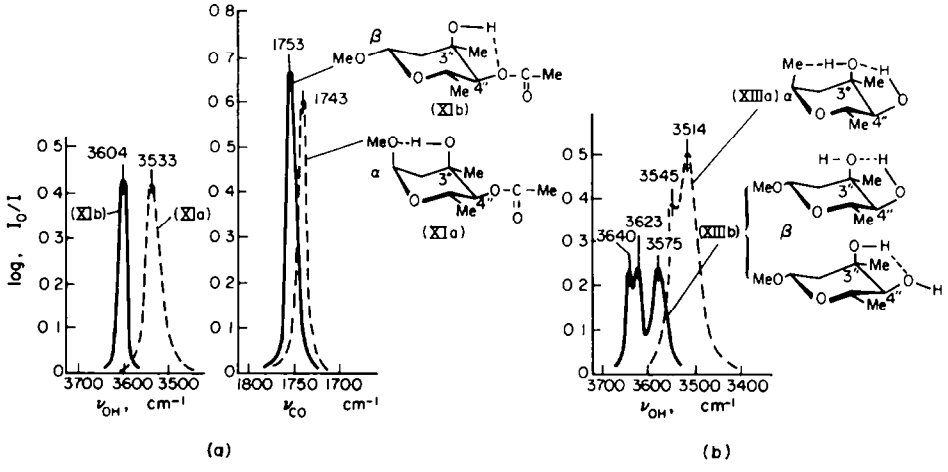


FIG 3. $\nu_{O\ H}$ and ν_{CO} absorptions of XIa and XIb, and ν_{O-H} absorptions of XIIIa and XIIIb

* In order to allow comparison with the structures of the derivatives and components of leucomycin, model compounds, XIa, XIb, XIIIa, and XIIIb were numbered as the 3''-position at tert. hydroxyl and 4''-at sec. hydroxyl or acetoxyl group as shown in Fig 3a and 3b.

cm^{-1}, and considering the values in XIa and XIb, the possibility of structures A and B can be ruled out. In order to examine the possibility of structure C, a comparison was made between the absorption of a CO in components III and IV, and isovaleryl-aldehyde (XII) as a model compound. As shown in Fig 4, IV possesses the lactone CO and the aldehyde CO, and its IR spectrum exhibited absorptions at 1729 and 1716 cm^{-1}. Since the spectrum of XII showed a CO absorption at 1734 cm^{-1}, the absorption at 1729 cm^{-1} in IV was assigned to the aldehyde-CO and that at 1716 cm^{-1} to the lactone-CO. The spectrum of III, in which the 3-position on the lactone ring has been acetylated, showed aldehyde absorption at 1730 cm^{-1}, an acetyl group in the 3-position at 1741 cm^{-1}, and a free lactone-CO at 1754 cm^{-1}. Therefore in IV, the lactone-CO forms a H-bond with the OH in the 3-position, as shown by structure C, and the absorption at 1754 cm^{-1} in III has shifted to a lower wave number by *ca.* 38 cm^{-1}.

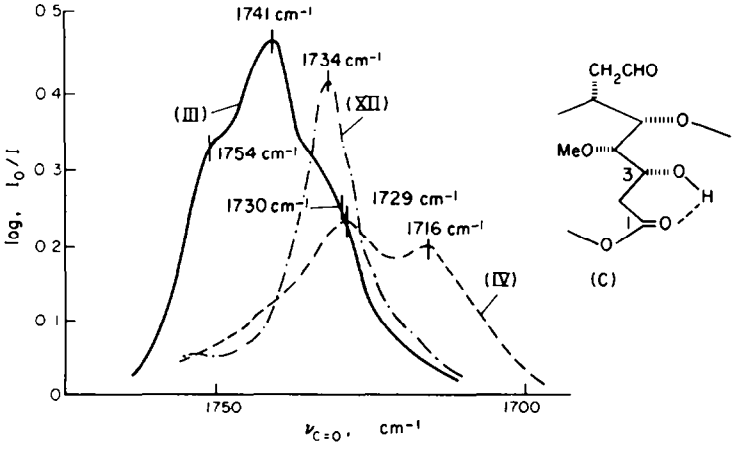


FIG 4. ν_{O-H} absorptions of isovalerylaldehyde (XII), leucomycin-U (III) and -V (IV)

Compound VIII shows OH absorption at 3513 cm^{-1}, and whether the OH at 3″-position forms an intramolecular H-bond or not, the OH absorptions were compared with those of α- (XIIIa)* and β-methylmycaroside (XIIIb),* shown in Fig 3b as model compounds. Absorptions indicating H-bonding was observed in XIIIa at 3514 cm^{-1} for an OH at 3″ and the oxygen in the OMe group, and at 3545 cm^{-1} for the equatorial secondary OH at 4″ and the oxygen of the OH in the 3″ position. These facts revealed that the OH at 3″ on mycarose in IV was forming a H-bond with the ethereal oxygen in glycoside bonding, with absorption at 3513 cm^{-1}, and that, with absorption at 3530 ± 10 cm^{-1} between the secondary OH at 4″ with the oxygen of the OH in the 3″ position.[18] The foregoing evidence reveals that the OH groups present in leucomycin form intramolecular H-bonds as shown in Fig 5.

Comparisons were also made between the hydrogen accepting ability of various oxygens during formation of an intramolecular H-bond from the wave number shifts, $\Delta\nu_{OH}$ on the IR spectra of various derivatives of mycarose. As shown in Scheme 1, this hydrogen-accepting ability was the largest in the oxygen of the OH forming

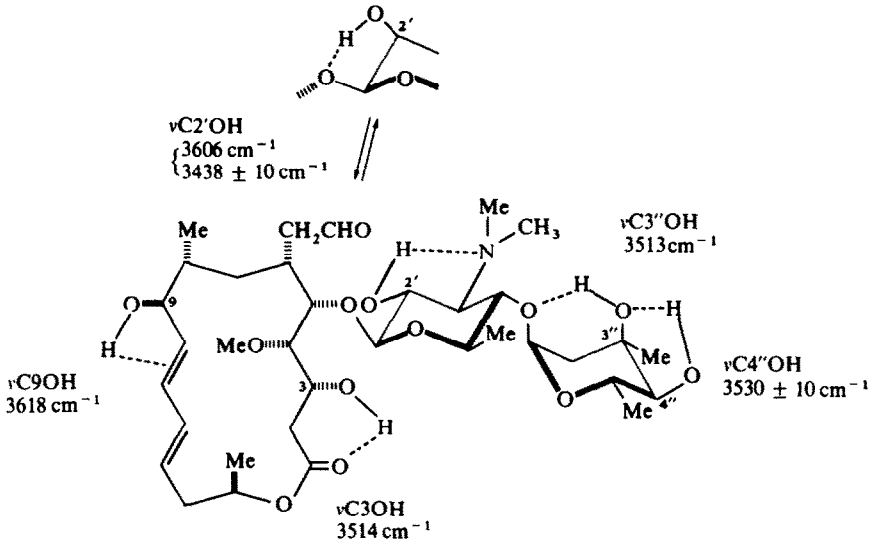


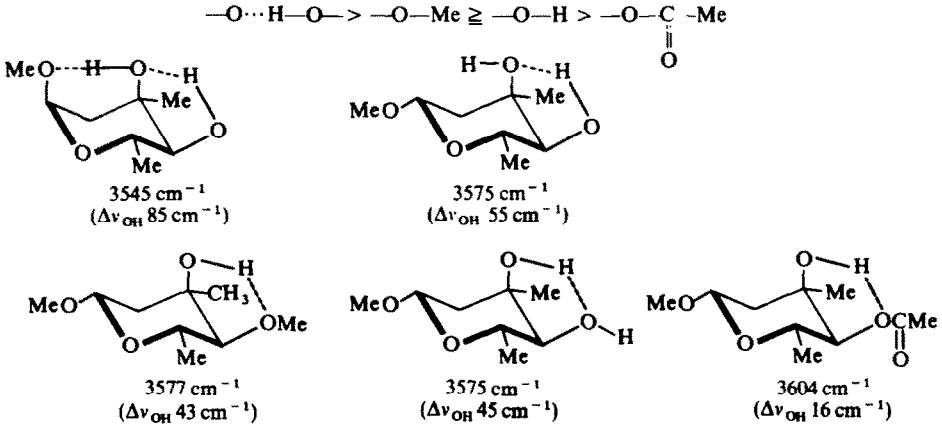
FIG 5. Hydrogen bonds on leucomycin-V (IV) in CCl_4

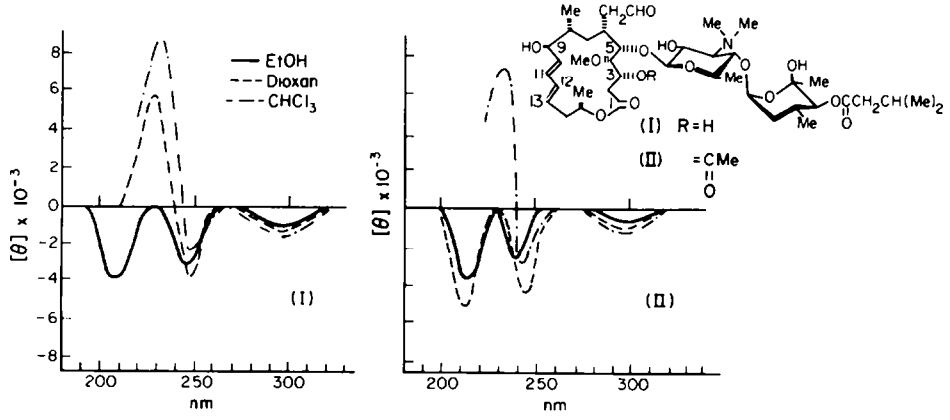
a H-bond, oxygen in the OMe and free OH groups was about the same, and that the oxygen in the base of O-acetyl group was much lower.

CD *and* NMR *studies*

The 16-membered ring lactone in leucomycin contains photoactive chromophores like conjugated dienes, lactone CO, and an aldehyde CO. In order to examine their environment, the CD spectra in various solvents and low-temperature CD in EtOH were measured.

Components I and II were measured in EtOH, dioxan, and $CHCl_3$, and results are shown in Fig 6. A marked solvent effect was observed in both I and II, which





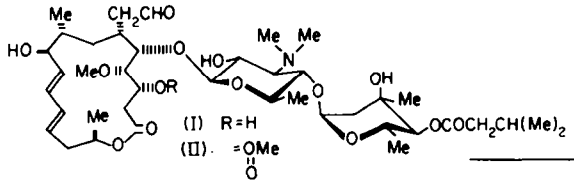
EtOH	208 nm (θ) − 3970	246 −3050	296 −1040
Dioxan	228 nm (θ) + 5610	248 −4060	296 −1520
$CHCl_3$	232 nm (θ) + 8600	249 −2610	296 −1700

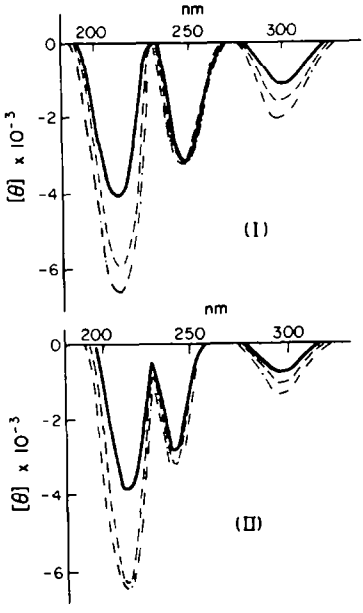
EtOH	215 nm (θ) − 3800	240 −2750	296 −740
Dioxan	215 nm (θ) − 5500	246 −4950	296 −1570
$CHCl_3$	235 nm (θ) + 7300	248 −3610	296 −1830

FIG 6. Circular dichroism curves of leucomycin-A_1(I) and-A_3(II) in various solvents

showed changes in ellipticity, and inversion of the sign of the Cotton effect. In both components, the absorption at 210–230 nm was assigned to the lactone CO,[20,21] that at 240–259 nm to chirality of the conjugated diene[22] since this absorption disappears in the tetrahydro compound (XIV), and the absorption at 296 nm showing a negative Cotton effect was assigned to the aldehyde CO on the lactone ring.[23] In polar EtOH, both I and II showed almost similar CD curves. In $CHCl_3$, the absorption due to the lactone CO is shifted to a longer wave length, molecular ellipticity is increased, and the sign of Cotton effect shows inversion from negative to positive This suggests that the conformation around the lactone part in both I and II differs in EtOH and in $CHCl_3$. In EtOH, the absorption due to the $n \rightarrow \pi^*$ transition of the lactone CO is considered to be under the effect of intermolecular H-bonding with solvent.[24] and a blue shift of 22 nm is observed in I and that of 20 nm in II. The CD curve of the lactone CO in dioxan shows a positive Cotton effect at 228 nm ([θ]-5500) in II, indicating that I and II have different conformations in the same solvent. Reversion of the Cotton effect sign merely by the difference in OH (I) and OAc (II) in 3-position of the lactone ring seems to suggest, together with changes in the Cotton effect accompanying changes in the solvent and in temperature, that the conformation of the lactone ring is rather flexible.

The CD curves of I and II were measured in EtOH at temperatures of 25°, −45°, and −80°. As shown in Fig 7, there were no changes in the sign of Cotton effect due to low temperature in both I and II, but an increase in the molecular ellipticity of the lactone and aldehyde CO was observed. This was considered to be due to the increas-





Temp.	lactone	conjugated diene	aldehyde
25°	208 nm −3970[θ]	246 nm −3050[θ]	296 nm −1040[θ]
−45	212 −5806	246 −3110	296 −1680
−80	212 −6640	246 −3150	296 −2140

solvent : EtOH
Temp.
——— : 25°
- - - - - : −45°(−20°)
- · - · - : −80°

Temp.	lactone	conjugated diene	aldehyde
25°	216 nm −3800[θ]	240 nm −2750[θ]	296 nm −740[θ]
−20	—	—	296
−45	216 −6430	242 −3260	−1170 —
−80	216 −6610	242 −3300	296 −1310

FIG 7. CD curves of leucomycin-A_1(I) and -A_3(II) at low temperature

ing population of the most stable conformer of the solvated molecule, becoming greatest at −80°.

The allyl system possesses a *trans-trans* configuration from the values of the coupling constants, $J_{8,9} = 4{\cdot}2$, $J_{9,10} = 8{\cdot}9$, $J_{10,11} = 15{\cdot}4$, $J_{11,12} = 10{\cdot}0$, and $J_{12,13} = 15{\cdot}2$ Hz in its 100-MHz NMR spectrum.[2] The bonding angle between C-11 and C-12 was calculated to be 140–150° by application of the Karplus equation to the value $J_{11,12} = 10{\cdot}0$ Hz, and a clockwise or anticlockwise conformation with this bonding angle was considered. From the fact that the conjugated diene shows a negative Cotton effect in the CD curve of I and II in EtOH at 246 nm ([θ] −3050 in I) 240 nm ([θ] −2750 in II), and by application of the helicity rule,[22] conformation at C-9 to C-13 was found to be a non-planer anticlockwise type of transoid diene, as indicated by a in Fig 8. This conformation is also deduced from the behavior of I and II in the CD curve in dioxan and $CHCl_3$.

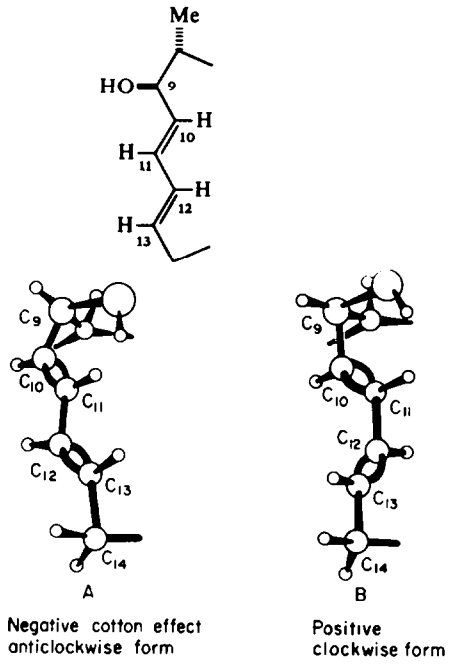


FIG 8. Conformation of conjugated diene

From the results of CD curves, the most mobile part of the lactone ring seems to be the lactone CO and the surrounding groups. This is dependent on the solvent and temperature, and it is difficult to carry out its conformational analysis from the CD curve.

In NMR spectra of I and II, a shift in the chemical shifts of aldehyde proton and C-11 proton is observed in II, in which the OH at 3-position in the lactone ring has been acetylated. In $CDCl_3$, the signal for the aldehyde proton appears at 9·73 ppm and that of C-11 proton at 6·66 ppm in II, while they are observed respectively at 9·87 and 6·2–6·3 in I as shown in Fig 9. When the OH at the 3-position is acetylated, the aldehyde proton shifts to higher field (0·14 ppm) and the C-11 proton to lower field (0·28–0·18 ppm). Similar shifts were observed in the NMR spectra measured in MeOD-d_3.

This suggests that the acetyl CO at the 3-position and the aldehyde proton and C-11 proton are in close proximity on the lactone ring, and both protons are under the magnetic anisotropy effect of the acetyl CO. Comparison of erythromycin and leucomycin suggests that the stable conformation of erythromycin[4–8] might be due to the steric effect of Me groups at 2, 4, 6, 8, 10, and 12 positions which tends to fix the movement of the ring. There is little steric effect of this kind in leucomycin.

With respect to the conformation of the leucomycin molecule, reference was made to the X-ray crystal structure[12] of the aglycone of demycarosyl-isoleucomycin A_3 (XV) which is different from that of II in the OH position as a result of an allyl rearrangement[11] from C-9 to C-13. Considering the CD, IR and NMR data, the preferable conformation of II in EtOH is shown in Fig 10. and is consistent with the X-ray crystal structure of XV, excluding the allyl system. Especially, the fact that the hydro-

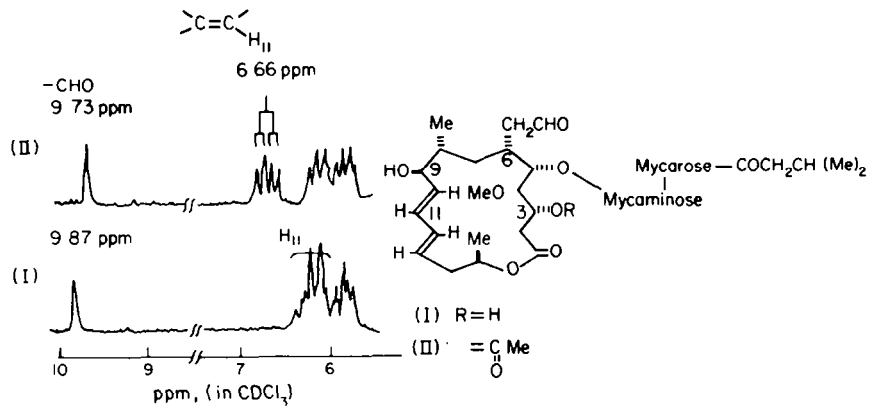


FIG 9. NMR spectra of leucomycin-A_1(I) and -A_3(II)

phylic groups like the aldehyde group, lactone CO, and the OH at the C-9 position in the lactone ring, are all situated above the ring might play an important role in the appearance of the biological activity of leucomycins.

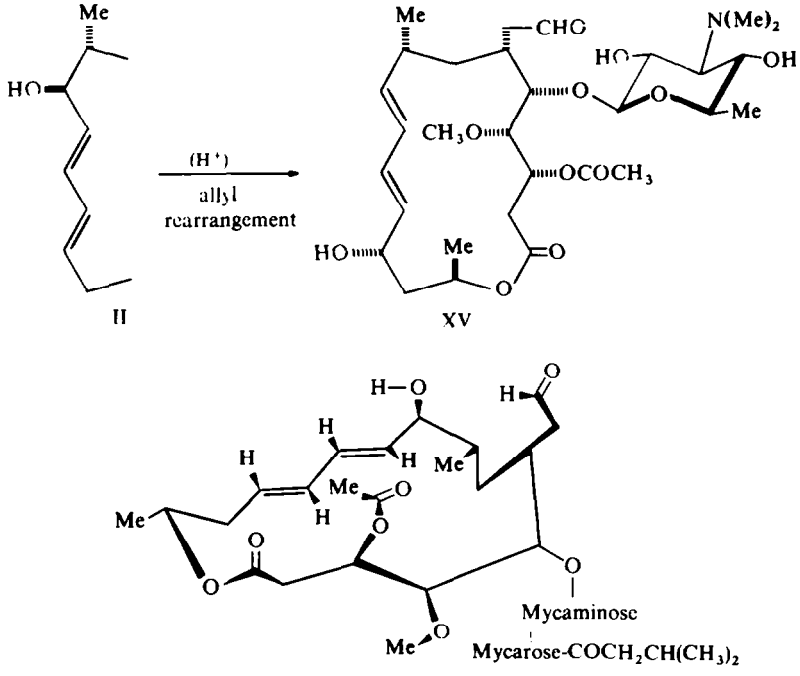


FIG 10. Preferable conformation of aglycone moiety in leucomycin-A_3(II) in EtOH

EXPERIMENTAL

IR spectra were recorded on JASCO DS-403G spectrometer in NaCl 20 mm path length cells in CCl_4. CD curves were measured with a JASCO ORD UV-5 spectropolarimeter fitted with a CD attachment.

Samples were measured with cells of path lengths between 0·1–1·0 mm in purified $CHCl_3$, dioxan, and EtOH at various temperatures. NMR spectra were recorded on Hitachi H-60 (60 MHz) and Varian HA-100 (100 MHz) spectrometers in $CDCl_3$ and methanol-d_4 containing TMS.

The preparations and properties of each compounds, VI, VII, VIII, IX, X, XIa, XIb, XIIIa, XIIIb, and XIV will be published shortly. Compound XII was a commercial product, which was purified by distillation *in vacuo*.

LITERATURE

[1] Part IX. S. Ōmura, Y. Hironaka, and T. Hata, *J. Antibiotics* **23**, 511 (1970)
[2] S. Ōmura, H. Ogura, and T. Hata, *Tetrahedron Letters* 1267 (1967)
[3] S. Ōmura, M. Katagiri, and T. Hata, *J. Antibiotics* **21**, 272 (1968)
[4] P. V. Demarco, *Tetrahedron Letters* 383 (1969)
[5] P. V. Demarco, *J. Antibiotics* **22**, 327 (1969)
[6] T. J. Perun and R. S. Egan, *Tetrahedron Letters* 387 (1969)
[7] T. J. Perun, R. S. Egan, and J. R. Martin, *Ibid.* 4501 (1969)
[8] L. A. Mitscher, B. J. Slater, T. J. Perun, P. N. Jones, and J. R. Martin, *Ibid.* 4505 (1969)
[9] S. Ōmura, A. Nakagawa, N. Yagisawa, and T. Hata, Paper presented at the 91st Annual Meeting of the Pharmaceutical Society of Japan, Sapporo (1970)
[10] S. Ōmura, A. Nakagawa, Y. Suzuki, and T. Hata, Paper presented at the 92nd Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka (1971)
[11] S. Ōmura, A. Nakagawa, M. Katagiri, T. Hata, M. Hiramatsu, T. Kimura, and K. Naya, *Chem. Pharm. Bull.* (Tokyo) **18**, 1501 (1970)
[12] M. Hiramatsu, A. Furusaki, T. Noda, K. Naya, Y. Tomiie, I. Nitta, T. Watanabe, T. Tabe, J. Abe, S. Ōmura, and T. Hata, *Bull. Chem. Soc. Japan* **43**, 1966 (1970)
[13] S. Koto, S. Zen, S. Ōmura, and T. Hata, *Ibid.* (in press)
[14] H. H. Freedman, *J. Am. Chem. Soc.* **83**, 2900 (1961)
[15] J. Grunday and L. J. Morris, *Spectrochimica Acta* **20**, 695 (1964)
[16] H. B. Henbest and B. L. Lovell, *Chem. and Ind.* **21**, 278 (1956)
[17] H. B. Henbest and B. L. Lovell, *J. Chem. Soc.* 1965 (1957)
[18] F. Korte, U. Calussen and G. Snatzbe, *Tetrahedron* **20**, 1477 (1964)
[19] T. J. Perun, R. S. Egan, P. H. Jones, J. R. Martil, L. A. Mitscher, and B. J. Slater, *Antimicrobial Agents and Chemotherapy* 116 (1969)
[20] A. F. Beecham, *Tetrahedron Letters* 3591 (1968)
[21] O. Korver, *Tetrahedron* **26**, 2391 (1970)
[22] A. Moscowitz, E. Charney, U. Weiss, and H. Ziffer, *J. Am. Chem. Soc.* **83**, 4661 (1961)
[23] C. Djerassi and L. E. Geller, *Ibid.* **81**, 2789 (1959)
[24] M. Ito, K. Inuzuka, and S. Imanishi, *Ibid.* **82**, 1317 (1960)
[25] K. M. Wellman, P. H. A. Laur, W. S. Briggs, A. Moscowitz, and C. Djerassi, *Ibid.* **87**, 66 (1965)