A NEW BICYCLO LACTONE FROM LEUCOMYCIN-A_3 BY ALKALI TREATMENT

Sir:

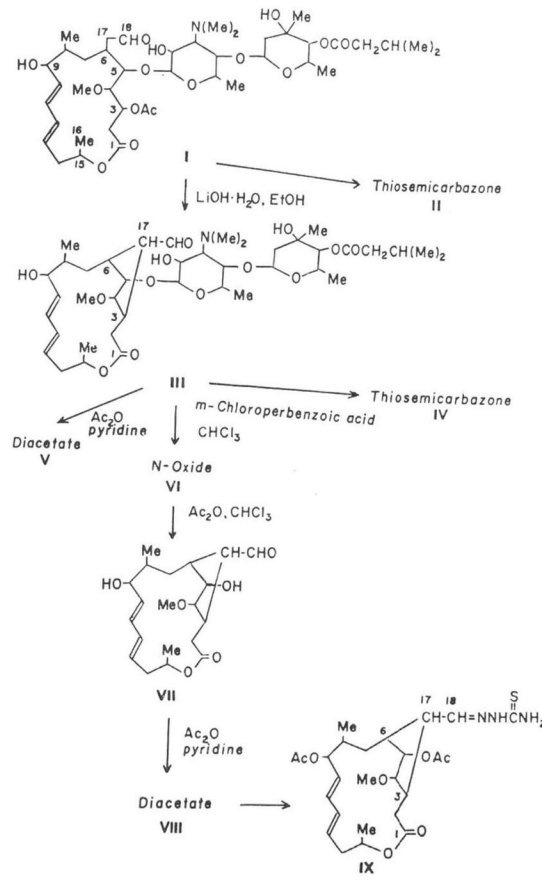
During studies on the chemical conversion, structure and biological activity of leucomycin, we have reported isolation of the aglycone leucomycinol[1], conversion of basic macrolide to neutral macrolide[2], and position isomers of the carbonyl-hydroxyl groups on the lactone ring[3]. The present report describes a compound possessing a bicyclo lactone skeleton obtained by alkali treatment of leucomycin-A_3* (**I**). This new compound was found to have interesting properties with respect to chemical structure and the correlation between structure and biological activity.

Refluxing **I** with one equivalent of $LiOH \cdot H_2O$ in ethanol for 2.5 hours and purification of the reaction product by silica gel chromatography afforded a condensate (**III**) in *ca.* 70 % yield. The nmr spectrum of the **III**, $[\alpha]_D^{20}$ −11.6° (*c* 0.5, EtOH), UV λ_{max}^{EtOH} 234.5 nm (ε 28,850), showed a signal at δ 9.80 (not a clear doublet) for CHO group, and the signal at δ 2.26 for the OAc group at 3-position of the lactone ring in **I** had disappeared.

III was converted to its thiosemicarbazone and subjected to thin-layer chromatography (TLC) on silica gel, developed with benzene-acetone (2:1). Two spots at Rf 0.43 and 0.40 appeared and the main product (**IV**) (Rf 0.40) mp 153~155°C (needles), $[\alpha]_D^{20}$ −71° (*c* 0.5, EtOH), UV λ_{max}^{EtOH} 235 nm (ε 32,500), 275 nm (ε 26,360) was isolated. The nmr spectrum ($CD_3COCD_3+D_2O$) of thiosemicarbazone of leucomycin A_3 (**II**) exhibited a triplet (J=6.0 Hz) signal at δ 7.62 due to −CH=N− proton but that of **IV** showed a doublet (J=6.4 Hz) at δ 7.57 for −CH=N− proton indicating that the group adjacent to CHO in **III** must be a secondary carbon. This fact reveals that the active methylene group adjacent to CHO function in **I** must have undergone a chemical change during alkali treatment.

In order to elucidate the structure of the lactone portion in **III**, the POLONOVSKI reaction, used for isolation of the aglycone from **I**, was applied to isolate the aglycone from **III**. **III** was converted to its N-oxide (**VI**) ($[\alpha]_D^{20}$ −20° (*c* 0.5, EtOH)) by reaction with *m*-chloroperbenzoic acid in chloroform, the N-oxide was refluxed with acetic anhydride in chloroform for 1 hour, and the reaction product was hydrolyzed with $NaHCO_3$ solution. The aglycone moiety (**VII**) was obtained in 15 % yield. **VII** was transformed to its diacetate (**VIII**), $[\alpha]_D^{20}$ −66° (*c* 0.5, EtOH) by acetylation with acetic anhydride in pyridine. The mass spectrum of **VIII** had a molecular ion peak, *m/e* 450 (450.2343; Calcd. for $C_{24}H_{34}O_8$: 450.2253), and the molecular weight of **VII** was determined as 366 ($C_{20}H_{30}O_6$). The nmr spectrum (CD_3COCD_3) of **VIII** showed doublet signals at δ 1.20 and 0.90 respectively for C(15)-Me and C(8)-Me, at δ 2.01 for two OAc groups, and double doublet at δ 3.36 for the proton at C_4.

Chart 1.

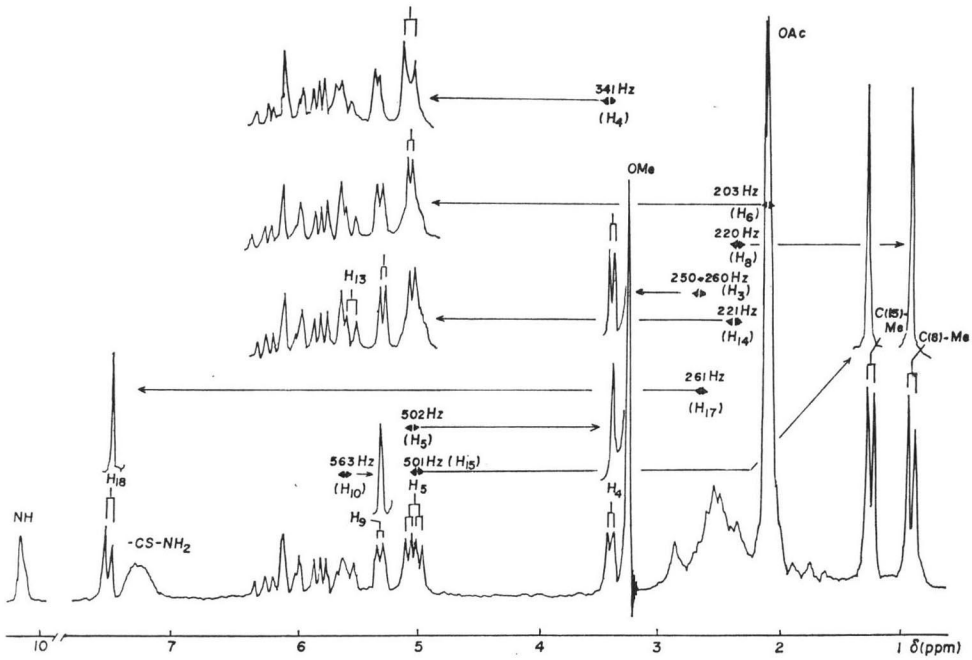


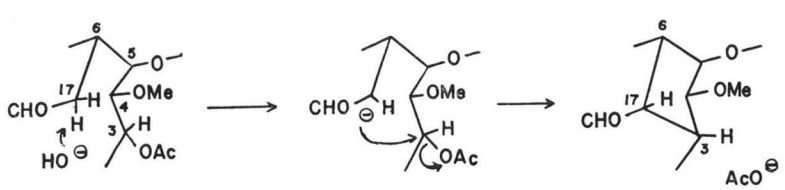
* Identity of leucomycin-A_3 and josamycin has already become apparent[4] and the commercial josamycin was purified by silica gel chromatography (benzene-acetone, 5:1) for use as a sample in the present work.

Signals at δ 4.8~6.3 for four olefinic protons at C_{10} to C_{13}, two protons at C_5 and C_9, and one proton of C_{15} were recognized. The signal for CHO group at δ 9.72 was observed as a double doublet. This fact suggested the possibility that **VII** and **VIII** are mixtures.

VIII was converted to its thiosemicarbazone and subjected to TLC, developed with benzene-acetone (4:1), giving two spots at Rf 0.46 and 0.33. Thiosemicarbazone (**IX**) was isolated as the main product (Rf 0.33). The nmr spectrum (CD_3COCD_3) of **IX**, $[\alpha]_D^{20}$ −71.2° (*c* 0.5, EtOH), UV λ_{max}^{EtOH} 231 nm (ε 26,950), 275 nm (ε 20,040); mass spectrum: *m/e* 523 (M^+) ($C_{24}H_{34}O_7 \cdot NNHCSNH_2$), as shown in Fig. 1, exhibited a signal at δ 7.57 (J=6.2 Hz) for -CH=N-, which gave a singlet by decoupling with methine of 261 Hz. The proton at C_4 was observed as a broad doublet ($J_{4,5}$=5.4 Hz) at δ 3.39, and the proton at C-5 as a double doublet ($J_{4,5}$=5.4, $J_{5,6}$=9.0 Hz) at δ 5.02 and this proton gave a doublet each by decoupling with the protons at C_4 and C_6. The proton at C_9 appeared as a broad doublet ($J_{8,9}$=4.8 Hz) at δ 5.30 and gave a singlet by decoupling with C_{10} proton (563 Hz). The fact that the mass spectrum of **VIII** had *m/e* 450 (M^+) and that the nmr spectrum of **IX** showed the C_4 proton as a broad doublet at δ 3.39 which gave a sharp doublet by decoupling with the signal (250 ~ 260 Hz) corresponding to the methine at C-3 position and a doublet for -CH=N- indicate that C-3 position was deacetylated and condensed with the carbon at C-17 to form a five-membered ring. Consequently, as shown in Chart 1, **III** possesses a bicyclo lactone structure, and the structure of the aglycone moiety (**VII**) is 3,6-(formylmethano)-4-methoxy-8-methyl-9-hydroxy-10, 12-hexadecadien-15-olide. The nmr spectral analysis[5] of I suggests that the acetyl group at C-3 and CHO group are conformationally in close proximity. By taking this into consideration, the mech-

Fig. 1. NMR spectrum of **IX** (100 MHz, CD_3COCD_3)





anism of the alkali reaction reported herein may be expressed as follows:

III is entirely devoid of antibacterial activity and this is thought to be due to the fact that the CHO groups which is one of the most important groups in the 16-membered ring macrolides for activity[3] has been fixed by the formation of a five-membered ring.

Satoshi Ōmura
Akira Nakagawa
Kazuhiro Suzuki
Toju Hata

Kitasato University and
Kitasato Institute
Minato-ku, Tokyo, Japan

(Received February 9, 1974)

References

1) Ōmura, S.; A. Nakagawa, K. Suzuki, T. Hata, A. Jakubowski & M. Tishler: Isolation and structure of leuconolide-A_3 5, 18-hemiacetal and 9-dehydro 18-dihydroleuconolide-A_3. J. Antibiotics 27: 147~149, 1974
2) Nakagawa, A.; K. Suzuki, K. Iwasaki, T. Hata & S. Ōmura: Chemistry of leucomycins. XI. Chemical transformation of a basic macrolide to a neutral macrolide. Chem. Pharm. Bull. (Tokyo): 1974 (in press)
3) Ōmura, S.; M. Tishler, A. Nakagawa, Y. Hironaka & T. Hata: Relationship of structures and microbiological activities of the 16-membered macrolides. J. Med. Chem. 15: 1011~1015, 1972
4) Ōmura, S.; Y. Hironaka & T. Hata: Chemistry of leucomycin. IX Identification of leucomycin A_3 with josamycin. J. Antibiotics 23: 511~513, 1970
5) Ōmura, S.; A. Nakagawa, N. Yagisawa, Y. Suzuki & T. Hata: Chemistry of leucomycins. X. Conformational studies of leucomycin. Tetrahedron 28: 2839~2848, 1972