# ISOLATION AND STRUCTURE OF LEUCONOLIDE-$A_3$ 5, 18-HEMIACETAL AND 9-DEHYDRO-18-DIHYDROLEUCONOLIDE-$A_3$

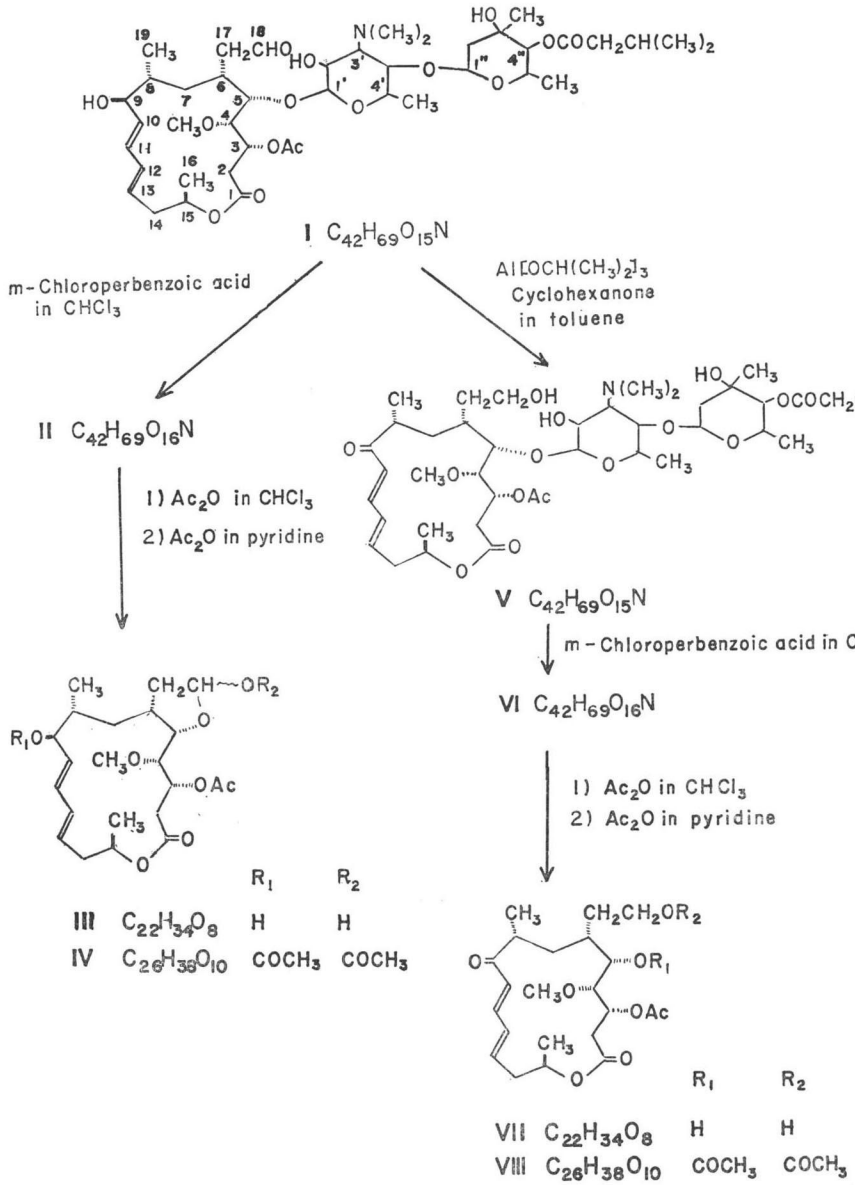
Sir:

Isolation of the aglycone from 14-membered macrolides has already been reported[1~4] but that from 16-membered macrolides has not been reported, except for the modified aglycone from tylosin.[5] Isolation of the lactone moiety from leucomycin,[6~8] a 16-membered macrolide, is of chemical interest and also adds to the correlation between the structure and biological activity in this series.[9,10] Here, we describe and prove the structures of leuconolide-$A_3$ 5, 18-hemiacetal and 9-dehydro-18-dihydro-leuconolide-$A_3$, modified 16-membered lactone aglycones.

Treatment of leucomycin-$A_3$ (**I**) with *m*-chloroperbenzoic acid in chloroform gave leucomycin-$A_3$ N-oxide (**II**), $[\alpha]_D^{18}$ $-19.0°$ (*c* 0.5, EtOH) in a high yield. **II** was refluxed with acetic anhydride in chloroform for 1.5 hours in order to chemically modify the $N(Me)_2 \rightarrow O$ group at 3′-position in its mycaminose moiety.[11] From this reaction product, leuconolide-$A_3$ 5, 18-hemiacetal (**III**), an aglycone moiety formed by liberation of the mycaminose and isovalerylmycarose moiety from **I** was isolated in 10% yield. UV spectrum of **III** (colorless fine needles from benzene, mp $96 \sim 97°C$ $[\alpha]_D^{20}+16.4°$ (*c* 0.5, EtOH) exhibited absorption maximum at 232 nm ($\varepsilon$ 25,560 in EtOH) based on the conjugated double bond between C-10 and C-13. In the nmr spectrum of **III**, signals for 3′-$N(Me)_2$, anomeric proton at 1′-position of mycaminose, anomeric proton at 1″ and isovaleryl group at 4″-position in mycarose, all seen in **I**, have disappeared, indicating that **III** is the aglycone moiety. The nmr spectrum of **III** showed signals at $\delta$ 5.4~6.7 for C(10), C(11), C(12), and C(13) olefinic protons, at $\delta$ 2.10 for 3-acetoxyl in the lactone ring, and at $\delta$ 0.94 (d, $J_{8,19}=6.9$ Hz) and $\delta$ 1.21 (d, $J_{15,16}=6.0$ Hz) for *sec*-Me groups at C-8 and C-15, respectively. The assignment of the methyl groups was proved by the fact that both methyl groups appeared as singlets by decoupling of signals at around $\delta$ 2.0 and $\delta$ 5.0 (m) for the protons at the base of methyl groups.

Acetylation of **III** with acetic anhydride and pyridine gave diacetyl leuconolide-$A_3$ 5, 18-hemiacetal (**IV**), $[\alpha]_D^{20}+33.4°$ (*c* 0.5, EtOH), UV $\lambda_{max}^{EtOH}$ 232 nm ($\varepsilon$ 17,340). The mass spectrum of **IV** gave the molecular ion peak at *m/e* 510 (510.2467; Calcd. for $C_{26}H_{38}O_{10}$: 510.2465) (*Anal.* Calcd. for $C_{26}H_{38}O_{10}$: C 61.16; H 7.45. Found: C 60.96; H 7.67). Therefore, the molecular weight of **III** was determined as 426 (*Anal.* Calcd. for $C_{22}H_{34}O_8$: C 61.97; H 7.98. Found: C 62.01; H 8.12). In the nmr spectrum of **IV**, the signal corresponding to the proton at the base of OMe group at $\delta$ 3.10 (d, $J_{4,5}=9.5$ Hz) became a singlet by decoupling of the absorption at $\delta$ 4.00 (dd, $J_{4,5}=9.5$ Hz, $J_{5,6}=3.9$ Hz). Also, the formation of hemiacetal ring between CHO at C-18 and OH at 5-position was suggested from the fact that CHO proton seen in **I** disappeared in **III** and **IV**, and two OAc groups were introduced in **IV** in the nmr spectrum. Presence of a five-membered hemiacetal was proved from the fact that the C(18)-proton signal appeared at $\delta$ 6.30 (dd, $J_{17,18}=4.8$, 5.4 Hz) and this became a singlet by decoupling of the signal corresponding to the methylene at 17-position at around $\delta$ 2.1. The glycosidic linkage of aglycone and mycaminose moiety was cleavaged simultaneously, forming hemiacetal ring between CHO at C-18 and OH at 5-position. The CD curve of **III** in EtOH showed a negative COTTON effect based on the lactone ester at 215 nm ($[\theta]=-8.47\times10^3$) and on conjugated diene at 245 nm ($[\theta]=-2.42\times10^2$), suggesting that it has a conformation similar to **I**.[12]

From the correlation between the structure and biological activities of 16-membered ring macrolides, the functional group important for the appearance of activity in the aglycone moiety appears to be the -CHO group at 18-position or, in its place, C=O at 9-position. In order to obtain a lactone ring with a C=O at the 9-position, **I** was treated with aluminum isopropoxide and cyclohexanone in toluene. 9-Dehydro-18-dihydroleucomycin-$A_3$ (**V**) ($[\alpha]_D^{20}$ $-67.0°$ (*c* 0.55, EtOH)) thereby obtained showed maximum absorption at 280 nm ($\varepsilon$ 26,000 in EtOH) in its uv spectrum, and absorptions at 1685, 1640, and 1598 $cm^{-1}$ in its ir spectrum for $\alpha, \beta, \gamma, \delta$-unsaturated ketone. In its nmr spectrum, the proton signal for




-CHO at $\delta$ 9.8 in **I** disappeared. Since **V** was acetylated with acetic anhydride in pyridine to give diacetylated compound, **V** was confirmed as shown the stated structure. Reaction of **V** with *m*-chloroperbenzoic acid gave 9-dehydro-18-dihydroleucomycin-$A_3$ *N*-oxide (**VI**) ($[\alpha]_D^{18}$ −26.2° (*c* 0.5, EtOH)). Similar to **II**, reaction of **VI** with acetic anhydride in chloroform afforded 9-dehydro-18-dihydroleuconolide-$A_3$ (**VII**), $[\alpha]_D^{20}$ +54.2° (*c* 0.5, EtOH), UV $\lambda_{max}^{EtOH}$ 279 nm ($\varepsilon$ 10,060). Acetylation of **VII** with acetic anhydride and pyridine gave diacetyl 9-dehydro-18-dihydroleuconolide-$A_3$ (**VIII**), $[\alpha]_D^{20}$ +9.2° (*c* 0.5, EtOH). The mass spectrum of **VIII** gave the molecular ion peak at *m/e* 510 (510.2471; Calcd. for $C_{26}H_{38}O_{10}$: 510.2465) (*Anal.* Calcd. for $C_{26}H_{38}O_{10}$: C 61.16; H 7.45. Found: C 61.09; H 7.76), and the molecular weight of **VII** was determined as 426 (*Anal.* Calcd. for $C_{22}H_{34}O_8$: C 61.97: H 7.98. Found: C 62.16; H 8.03). The nmr spectrum of **VII** exhibited signals at $\delta$ 5.03 (d, $J_{2,3}$=10.0 Hz) for the proton at the base of 3-OAc and at $\delta$ 3.10 (d, $J_{3,4}$=8.8 Hz) for the proton at the

base of 4-OMe, and a signal at $\delta$ 2.52 for OH at 5- and 1 8-positions which disappeared by treatment with deuterium oxide. In the nmr spectrum of **VIII**, the signal at $\delta$ 2.52 for OH in **VII** is no longer present and a new signal at $\delta$ 5.16 (d, $J_{4,5}=10.0$ Hz) is observed for the proton at the base of 5-OAc. The latter was proved by its becoming a singlet by decoupling of the proton at the base of OMe at $\delta$ 3.10.

This is a noble method of an isolation of the intact lactone ring moiety from 16-membered macrolides, though an example of separation of aglycone from tylosin has been reported by MORIN[5] in which drastic hydrolysis with strong acid resulting in the modification of lactone ring were contained and the yield was very low. A great interest was entertained for the presence or absence of antibacterial activity but the antibacterial activities (minimum inhibitory concentration by the agar dilution method) of **III** and **VII** against gram-positive and gram-negative bacteria were more than 100 $\mu$g/ml.

SATOSHI ŌMURA
AKIRA NAKAGAWA
KAZUHIRO SUZUKI
TOJU HATA

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

ANN JAKUBOWSKI
MAX TISHLER

Wesleyan University
Middletown, Conn. 06457 U.S.A.

(Received October 29, 1973)

## References

1) HUNG, P.P.; C.L. MARKS & P.L. TARDREW: The biosynthesis and metabolism of erythromycins by *Streptomyces erythreus*. J. Biol. Chem. 240: 1322~1326, 1965
2) MARTIN, J.R. & W. ROSENBROOK: Studies on the biosynthesis of the erythromycins. II. Isolation and structure of a biosynthetic intermediate, 6-deoxyerythronolide B. Biochemistry 6: 435~439, 1967
3) JONES, P.H. & E.K. ROWLEY: Chemical modification of erythromycin antibiotics. I. 3'-De (dimethylamino) erythromycin A and B. J. Org. Chem. 33: 665~670, 1968
4) JONES, P.H.; K.S. LYEN & W.E. GRUNDY: Chemical modifications of erythromycin antibiotics. II. Synthesis of 4'-hydroxyerythromycin A. Antimicr. Agents & Chemoth.-1968: 123~130, 1969
5) MORIN, R.B.; M. GORMAN, R.L. HAMILL & P.V. DEMARCO: The structure of tylosin. Tetrahedron Letters 1970-54: 4737 ~ 4740, 1970
6) ŌMURA, S.; M. KATAGIRI, H. OGURA & T. HATA: The chemistry of leucomycins. III. Structure and stereochemistry of leucomycin $A_3$. Chem. Pharm. Bull. 16: 1181~1186, 1968
7) ŌMURA, S.; M. KATAGIRI & T. HATA: The chemistry of leucomycins. VI. Structures of leucomycin $A_4$, $A_5$, $A_6$, $A_7$, $A_8$, and $A_9$. J. Antibiotics 21: 272~278, 1968
8) HIRAMATSU, M.; A. FURUSAKI, T. NODA, K. NAYA, Y. TOMIIE, I. NITTA, T. WATANABE, T. TAKE & J. ABE: The crystal and molecular structure of demycarosyl leucomycin $A_3$ hydrobromide. Bull. Chem. Soc. Japan 40: 2982, 1967
9) ŌMURA, S.; M. KATAGIRI, I. UMEZAWA, K. KOMIYAMA, T. MAEKAWA, K. SEKIKAWA, A. MATSUMAE & T. HATA: Structure-biological activities relationships among leucomycins and their derivatives. J. Antibiotics 21: 532~538, 1968
10) 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
11 CAVE, A.; C. KAN-FAN, P. POTIER & J. LEMEN: Modification de la réaction de POLONOVSKI action de l'anhydride trifluoroacétique sur un aminoxyde. Tetrahedron 23: 4681~4689, 1967
12) ŌMURA, S.; A. NAKAGAWA, N. YAGISAWA Y. SUZUKI & T. HATA: Chemistry of leucomycin. X. Conformational studies of leucomycin. Tetrahedron 28: 2839~2848, 1972