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Chemistry of Leucomycins. XII.[1] Application of the Modified Polonovski Reaction to Leucomycin-A_3 N-Oxide

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An aglycone moiety of leucomycin-A_3 (**1**), leuconolide-A_3-5,18-hemiacetal (**3**), was isolated by the application of a modified Polonovski-reaction to leucomycin-A_3 N-oxide (**2**). Two neutral macrolides, 2′-O-acetyl-3′-N-desdimethylamino-3′-oxo-leucomycin-A_3 (**5**) and 2′-O-acetyl-3′-N-desmethyl-N-acetyl leucomycin-A_3 (**8**) in which the mycaminose moiety of **1** was modified, were also isolated from the same reaction. In a similar manner, an aglycone, 9-dehydro-18-dihydro-leuconolide-A_3 (**12**) was obtained from 9-dehydro-18-dihydro-leucomycin-A_3 (**10**). While the latter as an isomer of **1**, differing with respect to the position of carbonyl group on the lactone ring, has significant antimicrobial activity neither its aglycone **12** nor the aglycone of leucomycin-A_3 show antimicrobial activity.

16-Membered macrolide antibiotics possess a macrocyclic lactone to which sugar groupings are attached. Most of them, as illustrated by leucomycin-A_3 (**1**), contain an amino sugar mycaminose and a neutral sugar mycarose combined as a disaccharide and bonded to the 16-membered lactone through a glycosidic linkage. With few exceptions, nearly all the work on the isolation, structure chemistry and structure-activity relation associated with the 16-membered macrolide antibiotics has been carried out in Japan.[3]

In the course of studying the chemistry of the 16-membered macrolides and the structure-biological activity which began six years ago,[4] we directed considerable attention to the preparation of the aglycones having the original lactone structures. As in the case of several 14-membered macrolide antibiotics where the corresponding aglycones had already been prepared and studied,[5,6] we also felt it important to have on hand the 16-membered aglycones for studies of their biological activities and biosynthesis. This paper covers our work on this objective using in the main the readily available leucomycin-A_3 as our substrate.

Initially, we had examined acid hydrolysis of leucomycin-A_3 under various conditions. We were unable to obtain the aglycone in these experiments because of the stability of the antibiotic under mild conditions of acidity. Under more drastic conditions where the glycosidic linkage was ruptured, degradation of the lactone ring moiety occurred. This is not surprising in view of reports on acid hydrolysis of other 16-membered macrolides, namely, tylosin[7] and the antibiotic B-58941[8] where fragmentation or marked alteration of the aglycone structures occurred. We therefore chose to investigate the application of the Polonovski

1) Part XI: A. Nakagawa, K. Suzuki, K. Iwasaki, T. Hata, and S. Ōmura, *Chem. Pharm. Bull.* (Tokyo), **22**, 1426 (1974).
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3) For a review of this work, see S. Ōmura and A. Nakagawa, *J. Antibiotics*, **28**, 401 (1975).
4) S. Ōmura, M. Katagiri, A. Nakagawa, H. Yamada, I. Umezawa, K. Komiyama, and T. Hata, *Progr. Antimicrob. Anticancer Chemother. Proc. 6th Int. Congr. Chemother.*, **2**, 1043 (1969).
5) P.H. Jones and E.K. Rowley, *J. Org. Chem.*, **33**, 665 (1968).
6) P.H. Jones, K.S. Lyen, and W.E. Grundy, *Antimicrob. Agents & Chemother.*, 123, **1968** (1969).
7) R.B. Morin, M. Gorman, R.L. Jamill, and P.V. Demarco, *Tetrahedron Letters*, **1970**, 4737.
8) T. Suzuki, E. Mizuno, and N. Sugita, *Chemistry Letters*, **1973**, 793.

reaction[9,10] to leucomycin-A_3 N-oxide (2). We now wish to summarize our experiences with this reaction.

The N-oxide of leucomycin-A_3 (2) was prepared in high yield by reaction of the antibiotic with *m*-chloroperbenzoic acid in chloroform.[11] The structure of 2 was confirmed by the shift to lower field of the N-methyl signal in the nuclear magnetic resonance (NMR) spectrum. The N-methyl groups of leucomycin-A_3 itself appear at δ 2.6 whereas in the spectrum of the N-oxide they are at δ 3.35. Compound (2), the N-oxide, in chloroform and acetic anhydride, was refluxed for one hour and then hydrolyzed with aqueous sodium bicarbonate. The crude product was then subjected to silica gel column chromatography using the solvent system benzene–acetone (15: 1 to 4: 1). Three compounds, 3, 5 and 8, were isolated as the major products.

Compound (3), $C_{22}H_{34}O_8$, obtained in 10% yield, showed a maximum absorption due to the conjugated diene at 232 nm in its ultraviolet spectrum. The NMR spectrum of 3 showed no signals for the dimethylamino group, the anomeric proton at the 1′-position of the mycaminose moiety, the anomeric proton at 1″ and the isovaleryl group at 4″ position in the mycarose moiety. These absorptions were clearly visible in the spectrum of 1 and their absence from 3 suggested that it was the aglycone, brought about by cleavage between the lactone and sugar moieties. The NMR spectrum of 3 showed signals at δ 5.4—6.7 for the C(10)—C(13) olefinic protons, at δ 2.10 for the acetoxy group at C-3 on the lactone ring, and at δ 0.94 (d, $J_{8,19}$=6.9 Hz) and δ 1.21 (d, $J_{15,16}$=6.0 Hz) for the secondary-methyl groups at

Table I. Chemical Shift and Coupling Constant Values of 9,18-O,O-Diacetyl leuconolide-A_3-5,18-hemiacetal (4)

Protons	H_{2a}	H_{2b}	H_3	H_4	H_5	H_6	H_{7a}	H_{7b}	$-^{17}CH_2-$	H_{18}	C_8–OAc	C_3–OAc	C_4–OMe
Coupling Constant (Hz)	14.4	11.0	0	9.9	3.9		?	?		5.4			
	~0												
Chem. shift (ppm)	1.8~2.5	2.87	5.08	3.10	4.00	*ca.* 2.15	0.8~1.3		*ca.* 2.13	6.30	2.00	2.10	3.52
Spliting	d	dd	d	d	dd	m	m	m	?	t	s	s	s
Spin Decoupling		irr. → s d ← irr.		s ← irr. irr. → d	d ← irr.				irr. → s				

Protons	C_8–CH_3	H_8	H_9	H_{10}	H_{11}	H_{12}	H_{13}	H_{14a}	H_{14b}	H_{15}	C_{15}–CH_3	C_9–OAc
Coupling Constant (Hz)	6.9	4.0	10.0	15.4	15.4	15.2	9.0			6.0		
							4.0					
								?				
Chem. shift (ppm)	0.95	*ca.* 2.0	5.28	*ca.* 5.6	6.61	6.16	*ca.* 5.7	1.8~2.4		5.0	1.21	2.00
Spliting	d	m	dd	dd	dd	dd	m	b	b	b	d	s
Spin Decoupling		irr. → d										

abbreviations: ↑—↑ =coupling, s=singlet, d=doublet, t=triplet, m=multiplet, ? =overlaped

9) M. Polonovski and M. Polonovski, *Bull. Soc. Chim. France*, **41**, 1190 (1972).
10) A. Cave, C. Kan-Fan, P. Potier, and J. LeMen, *Tetrahedron Letters*, **1967**, 4681.
11) S. Ōmura, A. Nakagawa, K. Suzuki, T. Hata, A. Jakubowski, and M. Tishler, *J. Antibiotics*, **27**, 147 (1974).

C-8 and C-15, respectively. Assignment of the two methyl groups was confirmed by decoupling the doublets to singlets upon irradiation of their respective methines about δ 2.0 and δ 5.0.

Acetylation of **3** with acetic anhydride in pyridine gave the diacetyl compound (**4**). The mass spectrograph of **4** gave M^+ ion peak at *m/e* 510 ($C_{26}H_{38}O_{10}$) and fragmentation peaks at *m/e* 451 ($M^+-C_2H_3O_2$) and *m/e* 391 ($M^+-C_4H_6O_4$) thus, establishing the molecular weight of compound (**3**) as 426 ($C_{22}H_{34}O_8$). In the NMR spectrum of **4** (Fig. 1), the signal corresponding to the proton at the base of the methoxyl group at δ 3.10 ($J_{4,5}$=9.9 Hz) became a singlet by decoupling the absorption for the proton on C-5 at δ 4.00 (dd, $J_{4,5}$=9.9 Hz, $J_{5,6}$=3.7 Hz). Formation of a hemiacetal ring between the aldehyde at C-18 and the hydroxyl at C-5 was suggested by the facts that the aldehyde proton seen in **1** was not present in **3** and **4** and that the latter now contained three acetoxyl groups. The presence of the hemiacetal ring was also established with compound (**4**) by the fact that the proton signal at the 18-position, appearing at δ 6.30 (dd, $J_{17,18}$=5.4 Hz), became a singlet by decoupling it from the signal at about δ 2.1 which corresponds to the methylene at the 17-position. From this information, the structure of aglycone **4** was established as 9,18-O,O-diacetyl-leuconolide-A_3-5,18-hemiacetal.[11] The chemical and coupling constant values in the NMR spectrum of **4** are listed in Table I. The circular dichroism (CD) curve of **3** in ethanol, Table I, showed a negative Cotton effect for the lactone ester at 210 nm ($[\theta]=-6.14\times10^4$) and the conjugated diene at 244 nm ($[\theta]=-2.2\times10^4$) suggesting it has a conformation[12] similar to **1**.

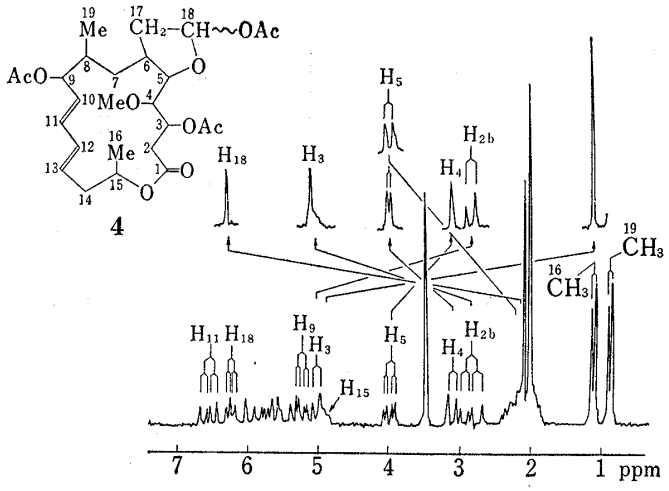

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Fig. 1. NMR Spectrum of 9,18-O,O-Diacetyl-leuconolide-A_3-5,18-hemiacetal (**4**)
(100 MHz, $CDCl_3$)

The second and the major product obtained from the modified Polonovski reaction on leucomycin-A_3 N-oxide was compound (**5**). It was obtained in 30% yield[13] and contained no nitrogen. On treatment with acetic anhydride and pyridine an additional acetyl group was introduced in position 9 to give compound (**6**), $C_{44}H_{66}O_{18}$, the mass spectrograph of which exhibited a fragmentation peak at *m/e* 451 due to the aglycone, and one at *m/e* 229 due to the isovaleryl mycarose moiety, indicating that only the mycaminose moiety had been modified. A more complete structure determination of **5** was accomplished by comparing its NMR spectrum (Fig. 2) to that of **1**. (The assignment of each individual proton on **1** was previously made by interpreting the 251 MHz and 100 MHz NMR spectra of **1**, α,β-isovaleryl mycarose, α,β-methyl mycaminoside[14] and aglycone **3**. Of special importance were the signals observed at δ 2.7—3.7 in **1**, assigned to the protons at the 2′-, 3′-, 4′-, and 5′-positions on the mycaminose moiety). From this comparison, it was determined that the positions of the absorptions for each of the protons attached to the lactone and isovaleryl mycarose moiety of **5** are identical with those observed for **1**. On the other hand, however, significant changes in the mycaminose portion of compound (**5**) were observed. The signal for the dimethylamino group observed at δ 2.56 in **1** has disappeared completely in **5**. In addition, the spectrum of **5** shows the

12) S. Ōmura, A. Nakagawa, N. Yagisawa, Y. Suzuki, and T. Hata, *Tetrahedron*, **28**, 2839 (1972).
13) A. Nakagawa, K. Suzuki, K. Iwasaki, T. Hata, and S. Ōmura, *Chem. Pharm. Bull.* (Tokyo), **22**, 426 (1974).
14) S. Ōmura, M. Katagiri, and T. Hata, *J. Antibiotics*, **21**, 272 (1968).

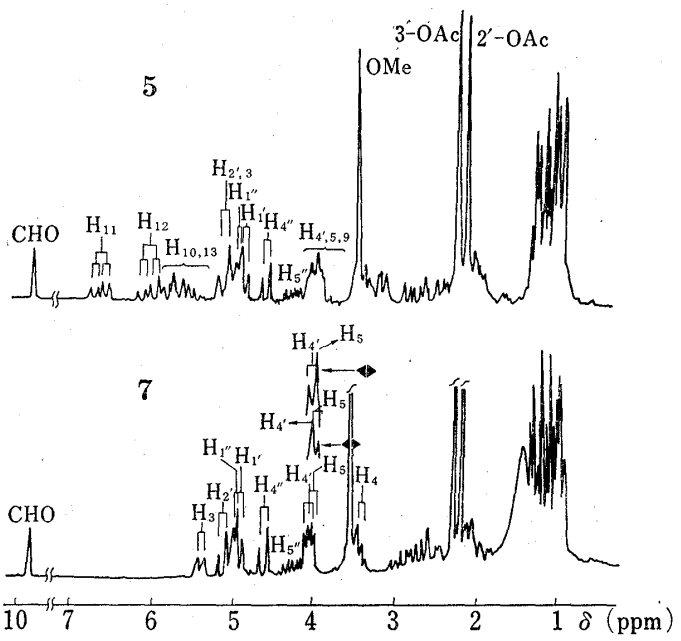


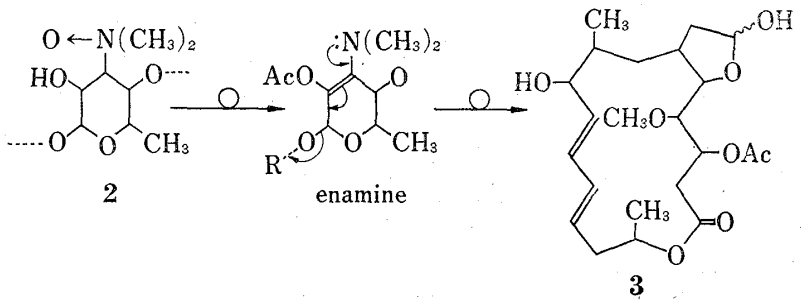
Fig. 2. NMR Spectra of 2'-O-Acetyl 3'-desdimethylamino-3'-oxo-leucomycin-A_3 (**5**) and Its Tetrahydride (**7**)

appearance of a new acetoxyl group at δ 2.1, and each proton at the 1'-, 2'-, 3', and 4'-positions of the mycaminose moiety have either shifted or disappeared entirely. For example, the anomeric proton at the 1'-position, which was observed at δ 4.3 in **1** and the proton at the base of the C-2' hydroxyl group appearing as a doublet at δ 5.0 ($J_{1',2'}$=8.0 Hz) were both shifted to lower field in **5**, where they appear at δ 4.9 and 5.2, respectively. Catalytic hydrogenation of compound **5** with PtO_2 in ethanol to the tetrahydro material **7** ($C_{42}H_{68}O_{17}$) permitted assignment of the three protons overlapping about δ 4.0. The proton at the base of the hydroxyl group at C-9, appearing in **1** as a doublet of doublets at δ 4.0, is shifted to higher field in the spectrum of **7**. This clarifies the absorption at δ 4.0 and facilitates the assignment of the two residual overlapping doublets to the protons at C-5 of the lactone ring and the proton on C-4' of the modified mycaminose. These assignments were further verified when the signal for the proton at C-5 collapsed into a singlet by decoupling the proton at the base of the methoxyl group at C-4 and likewise the doublet at δ 4.1 became a singlet when the proton at the 5'-position was irradiated at δ 3.5.

A comparison of the ^{13}C-NMR off resonance decoupled spectra of **5** and **1** revealed two new carbonyl absorptions in **5**: one at δ 170.0 assigned to the ester at C-2' and the other at δ 197.6 assigned to a ketone at C-3'. Based on the information obtained from the mass spectrum and NMR data, compound (**5**) is identified as 2'-O-acetyl-3'-desdimethylamino-3'-oxo-leucomycin-A_3 in which the dimethylamino group at C-3' has been converted to a ketone and the hydroxyl at C-2' has been acetylated. Final verification is the fragmentation peak in the mass spectrum of **5** for this sugar, having an acyloin skeleton, at *m/e* 186.

This is the first example of the chemical conversion of a basic 16-membered macrolide into a neutral molecule with the basic structure of the macrolide still intact.[13]

Girota and Wendler in a later publication[15] reported the preparation of our compound (**6**) by treating the N-oxide of leucomycin-A_3 with acetic anhydride and pyridine. They also isolated from their reaction mixture a second major product which they established as an



15) N.N. Girota and N.L. Wendler, *Tetrahedron Letters*, **1975**, 227.

enamine formed by introduction of a double bond between positions 2′ and 3′ of the mycaminose moiety. The enamine is readily convertible into our aglycone lactone (**3**) and is believed to be the intermediate in its formation. We were unable to isolate the enamine from our reaction mixture.

The third product isolated in 15% yield from the modified Polonovski reaction with leucomycin-A_3 N-oxide was compound (**8**), $C_{45}H_{17}O_{16}N$. While **8** contained nitrogen, the signal for the dimethylamino group appearing at δ 2.52 in **1**, was missing in its NMR spectrum. Additional acetoxyl groups are observed at δ 2.0—2.3. The mass spectrograph of **8** and its monoacetate **9**, obtained by acetylation of **8** with sodium acetate and acetic anhydride, exhibited fragmentation peaks at *m/e* 409 and 331 for the former and *m/e* 451, 423 and 391 for the latter. Both compounds also gave peaks at *m/e* 229 and 85 for the mycaminose. These data again suggested that the mycaminose has undergone a chemical transformation. Further evidence for the altered mycaminose moiety is that **8** does not form a hydrochloride salt and does not yield a N-methyl-N-formyl derivative as does **1** on oxidation with chromic oxide in pyridine following the procedure of A. Cavé and coworkers.[16,17] The possibility of an N-methyl-N-acetyl mycaminose moiety in **8** was confirmed by the appearance of a fragmentation peak for the modified mycaminose at *m/e* 244 in the mass spectrum. Thus, all the data collected for **8** suggests the most likely structure to be 2′-O-acetyl-3′-N-desmethyl-3′-N-acetyl-leucomycin-A_3.

In the studies on the correlation between structure and microbial activity of the 16-membered macrolide antibiotics, it was found that 9-dehydro-18-dihydro-leucomycin-A_3 (**10**) possessed significant activity when compared with that of **I**. Therefore, it was of interest to isolate the aglycone of **10**. Compound (**10**) was prepared in one step from **1** by refluxing a toluene solution of it for one hour with cyclohexanone and aluminum isopropoxide rather than the two-step procedure previously described,[18] which involved reduction of the aldehyde group with sodium borohydride followed by MnO_2 oxidation of the hydroxyl group at C-9. The ultraviolet (UV) spectrum of **10** exhibited a maximum absorption for the $\alpha,\beta,\gamma,\delta$-unsaturated ketone at 280 nm. An NMR spectrum of the acetylated derivative exhibited two acetoxyl groups verifying reduction of the C-18 aldehyde to an alcohol group. Mechanistically, such a transformation would be expected to occur because the aldehyde group in **1** is in close proximity to the hydroxyl group on C-9[12] and can act as a proton acceptor.

Reaction of **10** with *m*-chloroperbenzoic acid gave 9-dehydro-18-dihydro-leucomycin-A_3 N-oxide (**11**). Treatment of the latter with acetic anhydride in chloroform, in a manner similar to that previously described for the preparation **3**, afforded 9-dehydro-18-dihydro-leuconolide-A_3 (**12**) which with acetic anhydride and pyridine gave 5,18-O,O-diacetyl-9-dehydro-18-dihydro-leuconolide-A_3 (**13**). The mass spectrum of **13** gave M^+ ion peak at *m/e* 510 ($C_{26}H_{38}O_{10}$) which was in agreement with the calculated value. Therefore, the molecular formula of **12** was established as $C_{22}H_{34}O_8$. The NMR spectrum of **12** showed absorptions at δ 5.03 (d, $J_{2,3}$=10.0 Hz) for the proton at the base of the acetoxyl, at

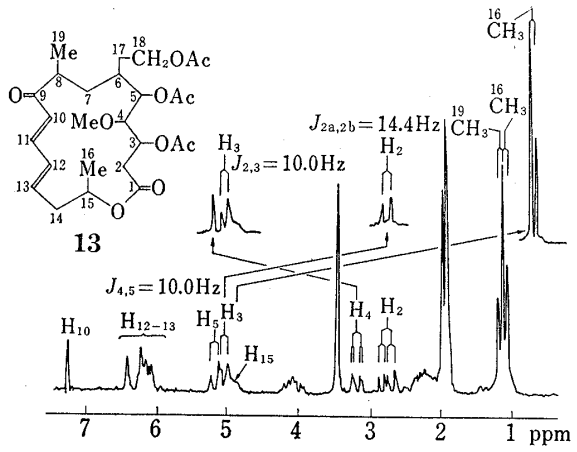


Fig. 3. NMR Spectrum of 5,18-O,O-Diacetyl-9-dehy-dro-leuconolide-A_3 (**13**)
(90 MHz, $CDCl_3$)

16) A. Cavé, C. Kan-Fan, P. Potier, J. LeMen, and M. -M. Janot, *Tetrahedron*, **23**, 4691 (1967).
17) A.A. Jakubowski, M.A. Thesis, Wesleyan University, 1974, P.87.
18) S. Ōmura, M. Tishler, A. Nakagawa, Y. Hironaka, and T. Hata, *J. Med. Chem.*, **15**, 1011 (1972).

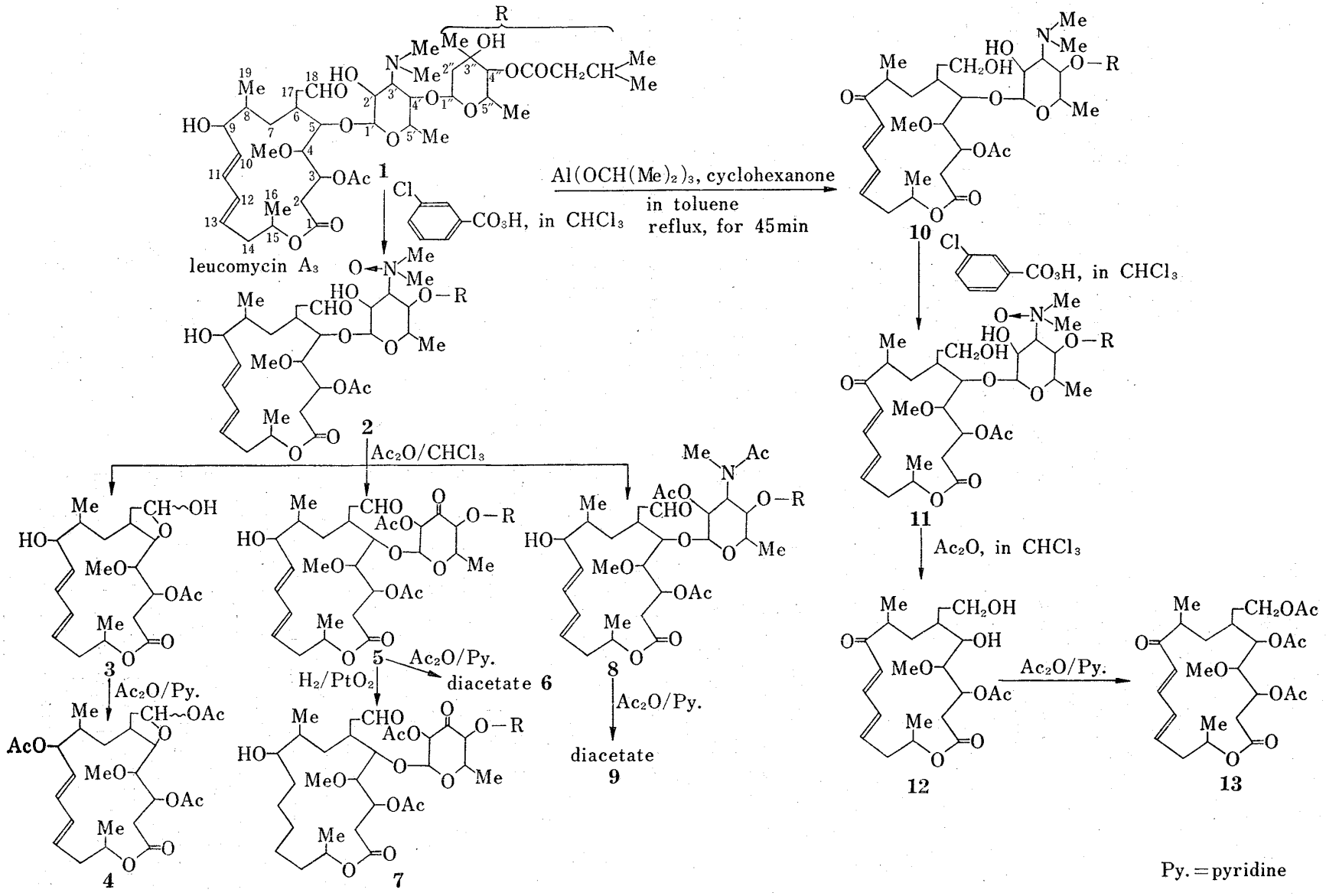


Chart 1

C-3 at δ 3.10 (d, $J_{4,5}$=8.8 Hz) for the proton at the base of the methoxyl at C-4, and at δ 2.52 for the hydroxyl protons at C-5 and C-18 which disappeared upon treatment with deuterium oxide. In the NMR spectrum of **13**, the signal seen at δ 2.52 for the hydroxyl in **12** is no longer present and a new signal at δ 5.16 (d, $J_{4,5}$=10.0 Hz) is observed for the proton at the base of the acetoxyl at the 5-position. Confirmation of this assignment was obtained by decoupling the proton on C-5 from the proton at the base of the C-4 methoxyl which appeared at δ 3.10.

Since this is the first instance that a 16-membered aglycone, which retained the original lactone structure, was isolated, it was of special interest to test for antibacterial activity. Like the 14-membered macrolide aglycones, compounds (**3** and **12**) showed no activity against gram positive and gram negative bacteria at 200 μg/ml.

Experimental

Leucomycin-A_3 N-Oxide (2)——To a solution of leucomycin-A_3 (**1**) (22 g, 2.7×10^{-2} mol) in $CHCl_3$ (350 ml) was added dropwise with stirring at 5° a solution of *m*-chloroperbenzoic acid (5 g, 2.9×10^{-2} mol) in $CHCl_3$ (110 ml). After the addition was complete, the reaction mixture was allowed to stand for 2 hours at room temperature. Excess peracid in the reaction solution was decomposed by the addition of 10% Na_2CO_3 aqueous solution (100 ml×2) and the $CHCl_3$ layer was washed with 5% $NaHCO_3$ aqueous solution (100 ml), and then with H_2O (100 ml). After drying the organic layer over anhyd. Na_2SO_4, the $CHCl_3$ layer was concentrated to about 30 ml and ethyl ether (450 ml) was added to it precipitating a white powder, **2** (20 g). mp 135—136°, $[\alpha]_D^{18}$ −19.0° (*c*=0.5, EtOH). *Anal.* Calcd. for $C_{41}H_{67}O_{16}N$: C, 59.33; H, 8.12; N, 1.69. Found: C, 59.25; H, 8.18; N, 1.91. NMR ($CDCl_3$): δ 0.92—1.28 (CH_3'S), δ 2.24 ($OCCH_3$), δ 3.32 (-OCH_3), δ 3.35 ((CH_3)$_2$N→O), 5.58—7.00 (C=C).

Leuconolide-A_3-5,18-hemiacetal (3)——To a solution of **2** (10 g, 1.2×10^{-2} mol) in $CHCl_3$ (100 ml) was added freshly distilled Ac_2O (7.5 ml, 7.3×10^{-2} mol) and the solution was gently refluxed for 1.5 hours. After completion of the reaction, cold water (300 ml) was added to the reaction mixture and the $CHCl_3$ layer was separated. The aqueous layer was extracted again with 280 ml of $CHCl_3$ and the combined $CHCl_3$ extracts was washed with aqueous sat. $NaHCO_3$ solution and H_2O, successively. After drying the organic layer over anhyd. Na_2SO_4, it was concentrated under a vacuo to dryness. The powder obtained was chromatographed over silica gel G using benzene–acetone (5: 1 to 2: 1) as a developer. The eluates yielded a white powder 3 (960 mg) in yield 10%. Aglycone 3 was crystallized from benzene–*n*-hexane to give colorless needles. mp 96—97°, $[\alpha]_D^{20}$ +16.4° (*c*=0.5, ErOH), UV λ_{max}^{EtOH} nm (ε) 232 (25 560), NMR ($CDCl_3$): δ 0.95 (3H, d., $J_{8,19}$=6.9 Hz), δ 1.21 (3H, d, $J_{15,16}$=6.0 Hz), δ 2.10 (3H, s.), δ 5.06 (1H, d.d., $J_{4,5}$=9.5 Hz, $J_{5,6}$=3.9 Hz). *Anal.* Calcd. for $C_{22}H_{34}O_8$: C, 61.97; H, 7.98. Found: C, 62.01; H, 8.12.

9,18-O,O-Diacetyl-leuconolide-A_3-5,18-hemiacetal (4)——Compound (**3**) (200 mg) was dissolved in Ac_2O (1.4 ml) and pyridine (2 ml). The solution was allowed to stand for 24 hours and then poured into ice water and after neutralization with $NaHCO_3$, extracted with EtOAc. The extract was dried over anhyd. Na_2SO_4, filtered, and the filtrate was concentrated under a vacuo to dryness. The powder (160 mg) was chromatographed on silica gel G using benzene–acetone (10: 1) and yielded a white powder **4** (110 mg); mp 84—85°, $[\alpha]_D^{20}$ +33.4° (*c*=0.5, EtOH). UV λ_{max}^{EtOH} nm (ε) 232 (17 340), NMR ($CDCl_3$): δ 5.08, (1H, d., $J_{2,3}$=11.0 Hz), δ 5.28, (1H, d. d., $J_{8,9}$=4.0 Hz, $J_{9,10}$=10.0 Hz), δ 6.30 (1H, t., $J_{17,18}$=6.30 Hz), Mass Spectrum: M^+ *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.

2′-O-Acetyl-3′-desdimethylamino-3′-oxo-leucomycin-A_3 (5)——Compound (**5**) was obtained from the silica gel chromatography after **3** was separated with benzene-acetone 5: 1 to 2: 1 and collecting the fraction using benzene–acetone 8: 1 to 5: 1 as the developer. The eluates gave compound (**5**) in 30% yield. Compound (**5**) was crystallized from ethyl ether to give colorless needles. mp 129—130°, $[\alpha]_D^{20}$ −104.2° (*c*=0.5, EtOH). UV λ_{max}^{EtOH} nm (ε) 232 (10760). *Anal.* Calcd. for $C_{42}H_{64}O_{17}$: C, 60.00; H, 7.62. Found: C, 60.22; H, 7.92. NMR ($CDCl_3$): δ 2.09 (3H, s.), δ 4.60 (1H, d.d., $J_{4'',5''}$=10.0 Hz), δ 3.44 (3H, s.), δ 5.15 (1H, d., $J_{1',2'}$= 8.0 Hz), δ 9.80. ^{13}C-NMR ($CDCl_3$, TMS as internal standard) δ 9.70 α-anomeric carbon on mycarose, δ 103.7 β-anomeric carbon on mycaminose, δ 127—136 olefinic carbons at C(10)-C(13), δ 168—174 four ester CO carbons, δ 197.3 ketone CO carbon at C-3′, δ 201.1 CHO carbon.

9,2′-O,O-Diacetyl-3′-desdimethylamino-3′-oxo-leucomycin-A_3 (6)——Compound (**5**) (120 mg) was acetylated with Ac_2O (0.3 ml) and pyridine (1.2 ml) in the same way as **3** was converted into **4**. The reaction mixture was purified by silica gel G chromatography using benzene–acetone (10: 1) to give a white powder **6** (100 mg). $[\alpha]_D^{20}$ −106.4° (*c*=0.5, EtOH). NMR ($CDCl_3$): δ 1.98, (-$OCOCH_3$). Mass Spectrum *m/e*: 85 (isovaleryl), 229 (isovaleryl mycarose), 451, 423, 391 (aglycone). *Anal.* Calcd. for $C_{44}H_{64}O_{18}$: C, 59.86; H, 7.48. Found: C, 59.55; H, 7.68.

Tetrahydro-2′-O-acetyl-3′-desdimethylamino-3′-oxo-leucomycin-A_3 (7)——Compound (**5**) (500 mg) was hydrogenated over PtO_2 in EtOH. The theoretical amount of H_2 was taken up in 2 hours giving tetrahydride **7** (490 mg). $[\alpha]_D^{20}$ −81.8° (c=0.5, EtOH), UV: end absorption. NMR ($CDCl_3$): δ 4.01 (1H, d., $J_{5,6}$=8.0 Hz), δ 4.06 (d., $J_{1',2'}$=8.0 Hz), δ 5.15 (1H, d., $J_{1',2'}$=8.0 Hz), δ 5.38 (1H, d., $J_{2,3}$=8.0 Hz).

2′-O-Acetyl-3′-N-desmethyl-3′-N-acetyl-leucomycin-A_3 (8)——Compound (**8**) was obtained from the silica gel column chromatography after separating **3** and **5** using benzene–acetone 10: 1 to 8: 1 as developer. The eluates gave a white powder **8** in 15% yield. *Anal.* Calcd. for $C_{45}H_{71}O_{16}N$: C, 61.29; H, 8.05; N, 1.58. Found: C, 61.31; H, 8.01; N, 1.57. $[\alpha]_D^{20}$ −80.0° (c=0.5 EtOH). UV λ_{max}^{EtOH} nm (ε): 232 (21600). Mass Spectrum m/e: 85 (isovaleryl), 229 (isovaleryl mycarose), 227, 244 (modified mycaminose), 379 and 472 (modified dissaccharide), 409, 391, and 331 (aglycone).

9,2′-O,O-Diacetyl-3′-N-desmethyl-3′-N-acetyl-leucomycin-A_3 (9)——Compound (**8**) (150 mg) was acetylated with Ac_2O and AcONa (100 mg) in AcOH by refluxing for 1 hour. The reaction mixture was poured on ice-water and extracted with ethyl acetate. The extract was evaporated *in vacuo*. The powdered residue was chromatographed over silica gel G using benzene–acetone to give a white powder **9** (120 mg). $[\alpha]_D^{20}$ −60.7° (c=0.5, EtOH). Mass Spectrum m/e: 85 (isovaleryl), 169 (3-O-acetyl mycarose), 229 (isovaleryl mycarose), 227 and 244 (modified mycaminose), 412 and 512 (modified dissaccharide), 451, 423 and 391 (aglycone).

9-Dehydro-18-dihydro-leucomycin-A_3 (10)——To a solution of **1** (10 g, 1.2×10^{-2} mol.) in absolute toluene (80 ml) was added a solution of $Al[OCH(CH_3)_2]_3$ (7 g, 3.4×10^{-2} mol.) in absolute toluene (20 ml). The solution was gently refluxed for 45 min. and after cooling at room temperature, ice water (200 ml) was added to it. The reaction mixture was extracted with EtOAc (150 ml×3) and the extract was dried over anhyd. Na_2SO_4 and the filtrate was concentrated under a vacuo to dryness. The brown powder (9.4 g) was then chromatographed over silica gel G using benzene–acetone (10: 1 to 6: 1) and yielded a white powder **10** (6.8 g). $[\alpha]_D^{20}$ −67.0° (c=0.55, EtOH), UV λ_{max}^{EtOH} nm (ε): 280 (26000), IR ($CHCl_3$): 1740, 1640, 1598 cm^{-1} (α, β, γ, δ unsat. CO, ester CO). *Anal.* Calcd. for $C_{46}H_{73}O_{17}N$: C, 60.57; H, 8.07; N, 1.54. Found: C, 60.64; H, 8.12; N, 1.50.

9-Dehydro-18-dihydro-leuconolide-A_3 N-Oxide (11)——A mixture of **10** (4.5 g) and *m*-chloroperbenzoic acid (1 g) in $CHCl_3$ (200 ml) was stirred at room temperature for 2 hours. The reaction mixture was worked up using the same procedure described for **2** and gave a white precipitate **11** (4.0 g). mp 122—123°, *Anal.* Calcd. for $C_{42}H_{69}O_{16}N$: C, 59.83; H, 8.17; N, 1.88. Found: C, 59.47; H, 8.09; N, 1.90.

9-Dehydro-18-dihydro-leuconolide-A_3 (12)——A solution of **11** (3.3 g, 4×10^{-3} mol.) and Ac_2O (2.5 ml, 2.4×10^{-2} mol.) in $CHCl_3$ (50 ml) was refluxed for 40 min. The solution was then worked up by the same procedure described for **3**, and yielded a crude powder (3.2 g). The powder was purified by chromatography on silica gel G using benzene–acetone (5: 1 to 3: 1). The eluate gave a white powder **12** (290 mg) in yield 9%. $[\alpha]_D^{20}$ +54.2 (c=0.5, EtOH), UV λ_{max}^{EtOH} nm (ε): 279 (10060). NMR ($CDCl_3$): δ 1.20 (3H, d., $J_{8,19}$=8.0 Hz), δ 1.25 (3H, d., $J_{15,16}$=6.0 Hz), δ 2.53 (2H, s.), δ 3.2 (1H, d., $J_{4,5}$=9.5 Hz), δ 5.03 (1H, d., $J_{2,3}$=10.0 Hz). IR ($CHCl_3$): 1742, 1685, 1638, 1598 cm^{-1} (α, β, γ, δ unsat CO and ester CO). *Anal.* Calcd. for $C_{22}H_{34}O_8$: C, 61.97; H, 7.98. Found: C, 62.16; H, 8.03.

5,18-O,O-Diacetyl-9-dehydro-18-dihydro-leuconolide-A_3 (13)——Compound (**12**) (100 mg) was acetylated with Ac_2O (0.1 ml) and pyridine (0.3 ml) and gave a white powder **13** (85 mg). mp 95—97°, $[\alpha]_D^{18}$ +9.2° (c=0.5, EtOH), UV λ_{max}^{EtOH} nm (ε): 280 (17340). NMR ($CDCl_3$): 2.8 (1H, d. d., $J_{2b,3}$=10.0 Hz, $J_{2a,2b}$=14.4 Hz), δ 5.03 (1H, d., $J_{2b,3}$=10.0 Hz), δ 5.16 (1H, d., $J_{4,5}$=9.5 Hz). Mass Spectrum m/e: 510 (M^+, 510.2471; Calcd. for $C_{26}H_{38}O_{10}$: 510.2465).