CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 16 No. 7 July 1968

[Chem. Pharm. Bull. 16(7)1167—1173(1968)]

UDC 615.33.011.5 : 547.458.02

The Chemistry of Leucomycins. II.[1] Glycosidic Linkages of Mycaminose and Mycarose on Leucomycin A_3[2]

Satoshi Ōmura,[3a,b] Michiko Katagiri,[3a] Haruo Ogura,[3b] and Toju Hata[3a,b]

Kitasato Institute[3a] and College of Pharmaceutical Sciences, Kitasato University[3b]

(Received April 26, 1967)

It was previously reported that leucomycin A_3 is a macrolide antibiotic and composed of a lactone containing an aldehyde, O-acetyl, and O-methyl groups, mycaminose, and 4-O-isovaleryl mycarose. The present paper is concerned with the position of the aldehyde group and linkage of the two sugars, and it was found that mycaminose is linked with the aldehyde group at the γ-position through a β-glycosidic linkage, and isovalerylmycarose is linked with mycaminose at the 4-position through an α-glycosidic linkage.

As described in the previous report,[1,4] leucomycin A_3, $C_{42}H_{69}O_{15}N$, is a macrolide antibiotic composed of mycaminose, 4-O-isovalerylmycarose and the macrolactone containing groups such as O-acetyl, O-methyl, $-CH_2CHO$, and $\alpha,\beta,\gamma,\delta$-unsaturated alcohol or ether. In the present paper, we will discuss the position of $-CH_2CHO$ group in relation to the mycaminosidic linkage and also the stereochemistry of the mycarosidic linkage in leucomycin A_3 (I).

In order to prove the position of $-CH_2CHO$ group as adjacent to the mycarosidic linkage, the method of Woodward[5] used in the case of magnamycin was tried. As already reported,[1] the aldehyde group in I is converted to the methyldemycarosylleucomycin A_3 dimethylacetal (II) by methanolysis of I with methanol containing 1% hydrochloric acid. Upon catalytic hydrogenation over palladium charcoal in ethanol, II absorbs 2 molar equivalents of hydrogen and gives methyltetrahydrodemycarosylleucomycin A_3 dimethylacetal (III) $C_{33}H_{61}O_{12}N$. Following reduction with lithium aluminum hydride, III yields methyldeacetyloctahydrodemycarosylleucomycin A_3 (IV), $C_{29}H_{57}O_{10}N$, which, when oxidized with hydrogen peroxide and the crude oxidation product subjected to lactonization by heating with a dilute hydrochloric acid to take off mycaminose, yields the five-membered lactone,[5,6]

1) Part I: *Chem. Pharm. Bull.* (Tokyo), **15**, 358 (1967).
2) Abstract of papers, the 10th Symposium on the Chemistry of Natural Products, 1966, p. 86.
3) Location: *a,b*) *Shiba Shirogane Sankocho, Minato-ku, Tokyo.*
4) S. Ōmura, H. Ogura, and T. Hata, *Tetrahedron Letters*, **1967**, 609.
5) R.B. Woodward, *Angew. Chem.*, **69**, 50 (1957).
6) L.J. Bellamy, "The Infra-red Spectra of Complex Molecules," the 2nd Ed., Methuen & Co., Ltd., London, 1958, p. 186.

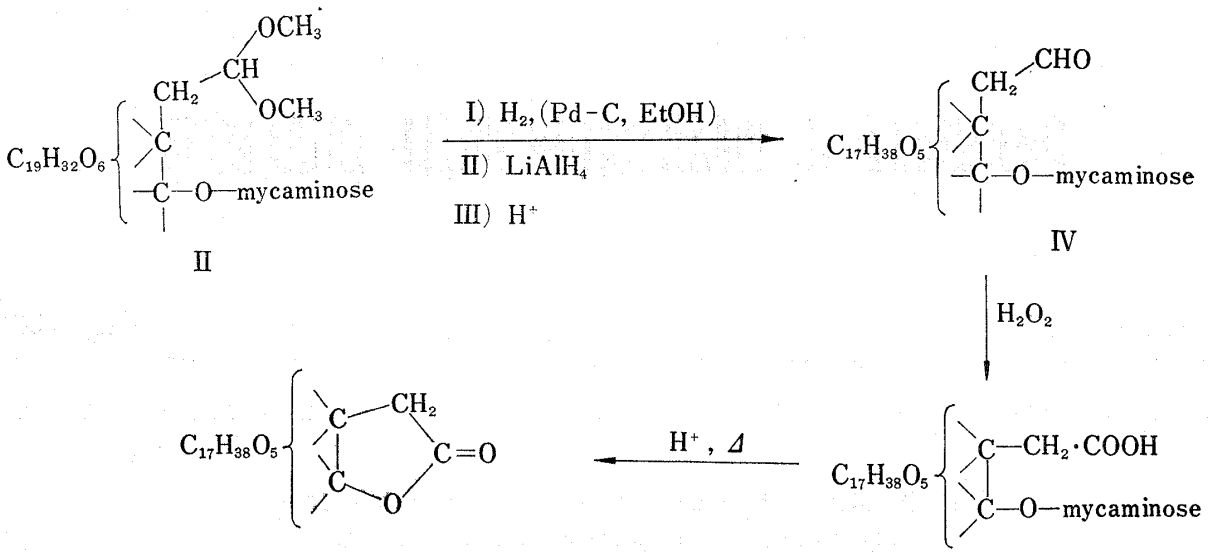

Chart 1

confirmed by the infrared absorption peak at 1775 cm^{-1}. This observation indicates that mycaminose is attached to the γ-position with the aldehyde group.

The pK_a' value of I is 6.70, whereas the demycarosyl derivative (II)[1] has a higher pK_a' value of 7.81. The pK_a' value of diacetylleucomycin-A_3 (VI)[1] is 5.69, lower than that of I, but the pK_a' value of the acetate (VII)[1] of II is 5.40, showing much lower value than that of II. These data are summarized in Table I and would be explained as follows. The hydroxyl group adjacent to the dimethylamino group of mycaminose in I is free, but when it is acetylated, the electron-attracting acetyl group reduces the basicity of the dimethylamino group. The hydroxyl groups on both sides of the dimethylamino group become free *i.e.*, demycarosyl derivative should have an increased basicity than the mycarosyl derivative, whereas acetylation of both hydroxyl groups results in the most weak basicity[5] in these compounds. On the other hand, in the NMR spectra of these compounds, there is a parallel relationship among the signals of six protons of the dimethylamino group and the pK_a' values (Table I).

Table I. pK_a' and NMR of Leucomycin A_3 and Its Related Compounds

Compounds	pK_a'	Chemical shift of $-N(CH_3)_2$ (ppm in $CDCl_3$)
Leucomycin A_3	6.70	2.49
Diacetylleucomycin A_3	5.69	2.40
Demycarosylleucomycin A_3	7.81	2.60
Acetyldemycarosylleucomycin A_3	5.40	2.28

There are two positions available, either 2′ or 4′, for introducing mycarosyl linkage adjacent to the dimethylamino group in mycaminose. The position of the mycarose residue can be fixed at C-4′ from the following experiments. Leucomycin A_3 is methylated with methyl iodide and silver oxide to the O,O-dimethylleucomycin A_3 methiodide (VIII), which is hydrolyzed with hydrochloric acid. After treatment on Amberlite IR 120 (H^+), resulting O-methylmycaminose methohalide (IX) is oxidized with periodic acid to an aldehyde and which is identified with acetaldehyde as its 2,4-dinitrophenylhydrazone.

In order to examine the glycosidic linkages of mycarose as well as mycaminose, signals in the anomer proton region of nuclear magnetic resonance (NMR) spectra of I (Fig. 1) and

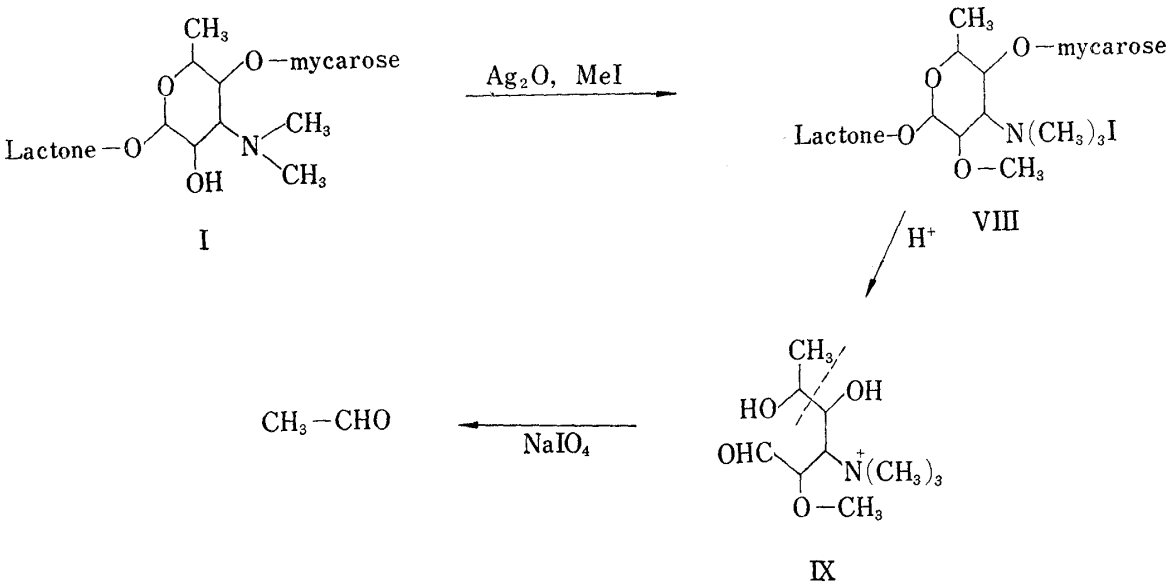

Chart 2

related compounds are compared. The α-isomer of methylmycaminoside[7] shows absorption of the anomer proton at 4.60 ppm (1H, J=4.0 cps) and that of the β-isomer at 4.12 ppm (1H, J=9.0 cps). From the comparison of these NMR spectra of I with those of α- and β-methylmycaminoside, a doublet at 4.30 ppm (1H, J=7.4 cps) is attributed to the anomer proton of mycaminose, and from the J value (axial-axial coupling) it is clear that mycaminose (3,6-dideoxy-3-dimethylamino-D-glucopyranose) is linked with the lactone through β-configuration. It has already been reported[8,9] that mycaminosidic linkage of magnamycin is in β-configuration from the NMR spectral studies (4.48 ppm J=7.1 cps). Although methyl 4-O-isovalerylmycaroside[1] obtained by the methanolysis of I is a mixture of the α-isomer and the β-isomer, which can be separated by chromatography on silicic acid and then by distillation *in vacuo*. The NMR spectra of these isomers are shown in Fig. 2.

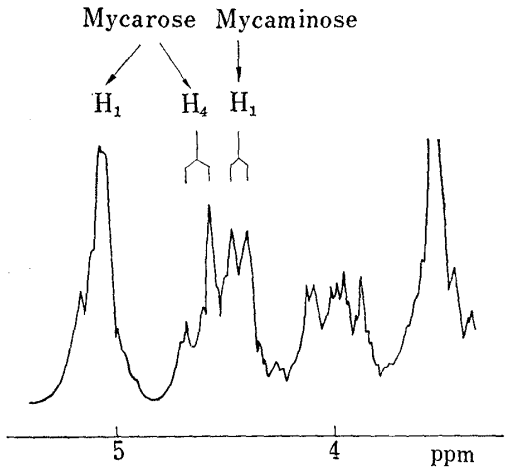

Fig. 1. NMR Spectrum of Leucomycin A_3 ($CDCl_3$, 100 Mc)

Mycarose is reported to be 2,6-dideoxy-3-C-methyl-L-ribohexose,[10] and Xa and Xb can be considered to assume the stable conformations. This conception is supported by the observation that the signal at 4.62 ppm (1H, J=10 cps) seen in the NMR sepctra (Fig. 2) of both Xa and Xb is due to the C-4 proton, which is in axial-axial coupling with C-5 proton. The signal of C-4 proton appears at 3.00 ppm (1H, doublet, J=10 cps) in methylmycaroside, and it is considered that the electronegativity of an acyl group caused the shift to a lower magnetic field. In this case, the signal of C-4 proton of Xa and Xb show peaks at 4.78 ppm (1H, broad triplet) and 4.75 ppm (1H, double doublet, $J_{1,2a}$=8.7 cps, $J_{1,2e}$=2.5 cps), respectively. The axial proton of a pyranoside sugar shows an absorption peak in a higher

7) F.A. Hochstein and P.P. Regna, *J. Am. Chem. Soc.*, **77**, 3353 (1955).
8) A.C. Richardson, *J. Chem. Soc.*, **1962**, 2758. A.B. Foster, T.D. Inch, J. Lehmann, M. Stacey, J.M. Webber, *ibid.*, **1962**, 2116. W. Hofheinz and H. Grisebach, *Z. Naturforsch.*, **17b**, 355 (1962).
9) W. Hofheinz and H. Grisebach, *Ber.*, **96**, 2867 (1963).
10) D.M. Lemal, P.D. Pacht, and R.B. Woodward, *Tetrahedron*, **18**, 1275 (1962).

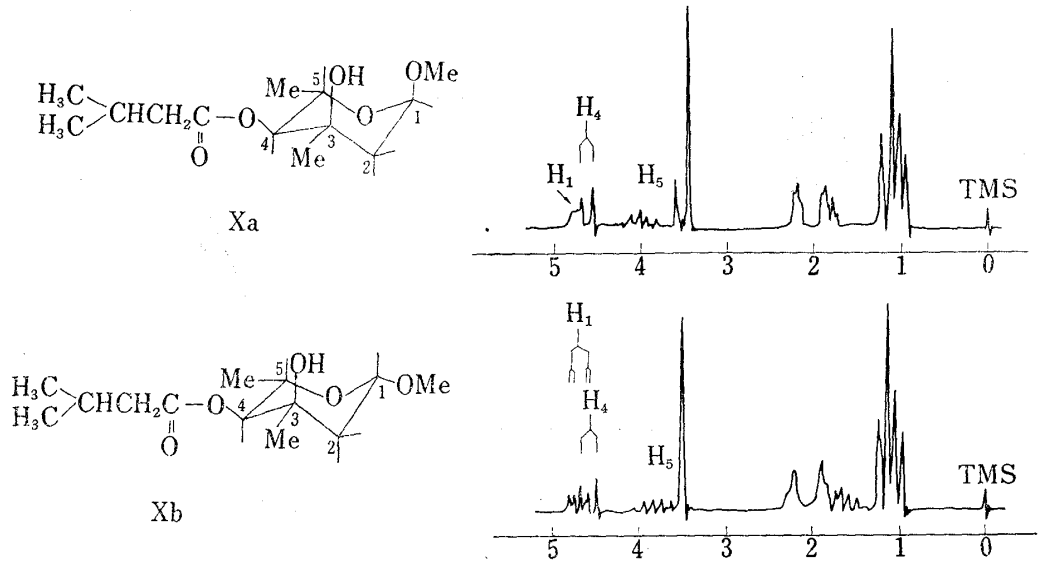

Fig. 2. NMR Spectra of α-(Xa) and β-Methyl-4-O-isovalerylmycaroside (Xb) (60 Mc/s, CCl_4)

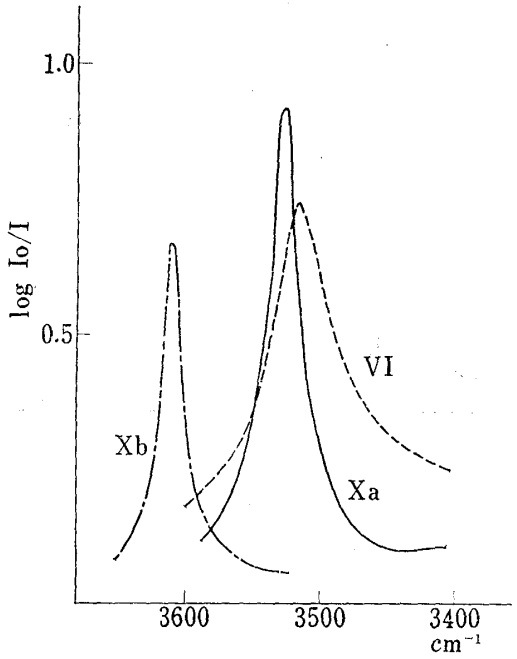

Fig. 3. IR Spectra of α-Methyl 4-O-isovalerylmycroside (Xa), β-Methyl 4-O-isovalerylmycaroside (Xb) and Diacetylleucomycin A_3 (VI) (0.004 mole, CCl_4)

magnetic field than the equatorial proton,[11] and the reason of the signals of C-4 protons of Xa and Xb is considered to be due to the fact that the anomer proton in Xb is affected by the axial OH at C-3, and the signal is shifted to a lower magnetic field.[12] The absorption at 4.60 ppm (1H, J=10.5 cps) of I, as shown in Fig. 1, is assigned to C-4 proton of mycarose on comparison of the NMR spectra of Xa and Xb (Fig. 2). From this J value, the mycarose moiety in I must have the confirmation shown as Xa or Xb. The absorption which vanishes at 5.0—5.1 ppm in the NMR spectrum of I is assumed to be due to the anomer proton of mycarose, but is not proved, as it overlaps with other absorption bands.

Formation of intramolecular hydrogen bond is possible between the tertiary hydroxyl group at C-3 and oxygen of the methoxyl group of the glycosidic linkage in Xa but it is impossible in Xb, because the O-methyl group is in an equatorial position. Furthermore, hydrogen bond formation between the tertiary hydroxyl group and the oxygen of the ether or the carbonyl group of the ester is regarded impossible both in Xa and Xb.[13] The remaining hydroxyl group in the diacetyl derivative (VI) is the tertiary hydroxyl group of mycarose.[4]

11) R.U. Lemieux, R.K. Kullnig, H.J. Bernstein, and W.G. Schneider: *J. Am. Chem. Soc.*, **80**, 6098 (1958). M. Miyamoto, Y. Kawamatsu, M. Shinohara, K. Nakanishi, Y. Nakadaira, and N.S. Bhacca, *Tetrahedron Letters*, **1964**, 2371. S. Hanessian and T.H. Haskell, *ibid.*, **1964**, 2451.

12) T.J. Flautt and W.F. Erman, *J. Am. Chem. Soc.*, **85**, 3212 (1963). T. Okuda and T. Yoshida, *Tetrahedron Letters*, **1965**, 2137.

13) N. Mori, S. Ōmura, and Y. Tsuzuki, Abstract of Papers, the 17th Annual Meeting of the Chemical Society of Japan, 1965, p. 96.

Infrared spectra of Xa, Xb, and VI were measured in a dilute solution of carbon tetrachloride (Fig. 3). Xb shows an absorption peak at 3615 cm^{-1} corresponding to the stretching band of the free hydroxyl group, while Xa shows a hydroxyl absorption at 3530 cm^{-1}, indicating the formation of a hydrogen bond. On the other hand, VI has a peak at 3515 cm^{-1} similar to that of Xa, but at a lower wave number than Xa, and this is probably due to the larger proton-accepting property of the ether-oxygen in VI. It is concluded that mycarose has an α-glycosidic linkage in I, and this conclusion is also supported by the observation on the molecular rotation of I, II, Xa, and Xb. As shown in Table II, the value (−475) calculated by assuming the α-glycoside linkage agree well with experimental data (−458).

Table II. Molecular Rotation of Related to Leucomycin A_3 Compounds

Compound	$[M]_D^{25}$ (in $CHCl_3$)	
	Found	Calcd.
Xa	−351.0 (=a)	
Xb	+ 35.1 (=b)	
II	−124.0 (=c)	
I	−458.0 (=d)	α- d=a+c=−475.0 β- d=b+c=− 88.9

As a summary of the above results, the partial structure of leucomycin A_3 (I) is proposed in Fig. 4.

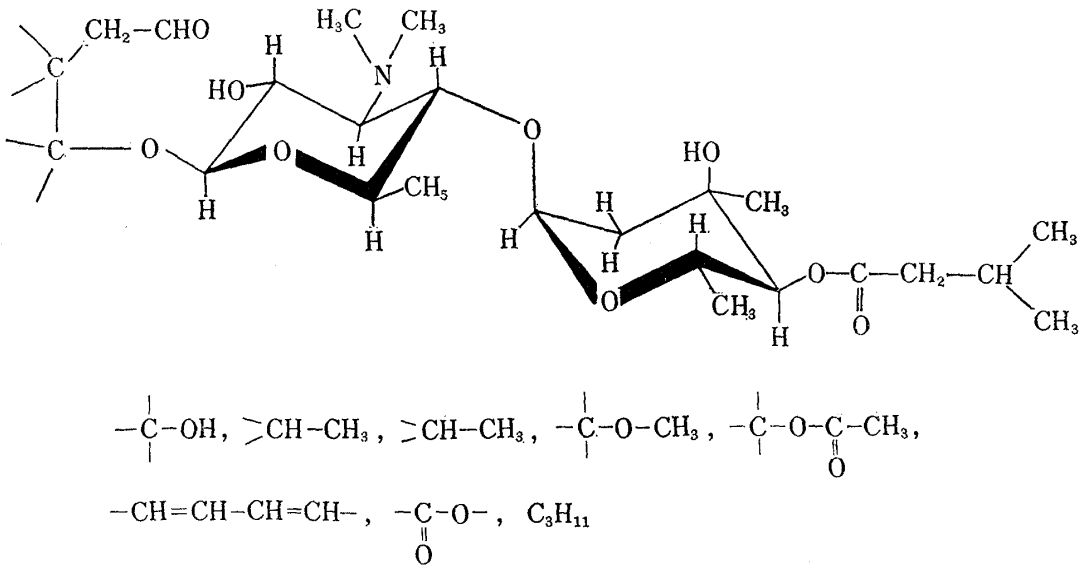

Fig. 4. Partial Structure of Leucomycin A_3

The infrared spectrometric method was applied to the determination of mycarosidic linkage in spiramycin[14] and magnamycin,[14,15] and it was found that both compounds have the α-mycarosidic linkage. The infrared data of Xa, Xb, VI, acetylspiramycin, and acetylmagnamycin are summarized in Table III.

14) M.E. Kuehe and B.W. Benson, *J. Am. Chem. Soc.*, **87**, 4660 (1965).
15) R.B. Woodward, L.S. Weder, and P.C. Dutta: *J. Am. Chem. Soc.*, **87**, 4662 (1965).

Table III. IR Data of Diacetylleucomycin A_3 and Its Related Compounds (0.004 mole, CCl_4)

Compound	ν_{OH}, cm^{-1} (log I_0/I)	
α-Methyl 4-O-isovalerylmycaroside (Xa)		3530(0.95)
β-Methyl 4-O-isovalerylmycaroside (Xb)	3615(0.75)	
Diacetylleucomycin A_3 (V)		3515(0.75)
Acetylmagnamycin		3518(0.78)
Acetylspiramycin-A		3515(0.80)

Experimental[16]

Methyltetrahydrodemycarosylleucomycin A_3 Dimethyl-acetal (III)——A solution of 2.55 g of methyldemycarosylleucomycin A_3 dimethylacetal (II) in 75 ml of EtOH was subjected to catalytic hydrogenation using 0.80 g of 5% Pd-C. Two moles of H_2 was absorbed in 5 hr. The filtrate was concentrated under a reduced pressure, the residue was dissolved in a minimum amount of ether, and triturated with petr. ether to obtain 2.94 g (92%) of III as a fine white powder, $[\alpha]_D^{20}$ +2.5° (c=1.4). *Anal.* Calcd. for $C_{33}H_{61}O_{12}N$: C, 59.71; H, 9.26; N, 2.11. Found: C, 60.20; H, 9.40; N, 2.19.

Acetate of III——III (500 mg) was acetylated with pyridine-Ac_2O. After purification by chromatography on silicic acid with benzene containing 10% of Me_2CO, the acetate was recrystallized from CCl_4 as needles, mp 120—121°, $[\alpha]_D^{25}$ −29.0° (c=1.4). *Anal.* Calcd. for $C_{37}H_{65}O_{14}N$: C, 59.42; H, 8.76; N, 1.87. Found: C, 60.50; H, 8.65; N, 2.01.

Methyldeacetyloctahydrodemycarosylleucomycin A_3 (IV)——A solution of 1.2 g of III in 50 ml of dry dioxan was added dropwise into a solution of 0.30 g of $LiAlH_4$ in 20 ml of dioxan over a period of 0.5 hr. The mixture was stirred for 2 hr at 60°, cooled, and neutralized with 10% AcOH. The solution was adjusted to pH 2 with 1N HCl, and after 4 hr, the solvent was distilled off under a reduced pressure. The residue was dissolved in 10 ml of H_2O, pH of the solution was made 8, and extracted three 40 ml portions of $CHCl_3$. The dried $CHCl_3$ solution was evaporated, under a reduced pressure and left 0.8 g (75%) of IV as a white powder, which showed one spot on thin-layer chromatogram. $[\alpha]_D^{25}$ −5.6° (c=1.3). *Anal.* Calcd. for $C_{29}H_{57}O_{10}N$: C, 60.09; H, 9.90; N, 2.41. Found: C, 60.30; H, 9.70; N, 2.50.

Oxidation of IV——To a solution of 500 mg of IV in 9 ml of 1N NaOH was added 2 ml of 30% H_2O_2, and the resulting solution was left at room temprature for 15 hr. After excess H_2O_2 was decomposed with MnO_2, the solution was made to alkaline and extracted with $CHCl_3$ (practically no extract was obtained). The aqueous layer was acidified to pH 2 with HCl, refluxed for 2 hr, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with a small amount of an aqueous $NaHCO_3$, dried, and evaporated. There was obtained 200 mg of an oily product, IR cm^{-1}: $\gamma_{C=O}$ 1770.

O,O-Dimethylleucomycin A_3 Methiodide (VIII)——To a solution of 4 g of I in 40 ml of MeI at 45°, freshly prepared Ag_2O was added at a rate of 2 g per 0.5 hr with stirring, and progress of the reaction was examined by thin-layer chromatography. The reaction was complete when a total of 10 g of Ag_2O was added. The reaction mixture was filtered and the remaining solid was washed several times with 20 ml each of $CHCl_3$. The filtrate and washings were combined and evaporated to obtain 2.5 g of a viscous yellow substance (VIII). This showed substantially one spot on thin-layer chromatogram and was used for the next reaction without further purification.

Acid Hydrolysis of VIII——VIII (1.7 g) was dissolved in 30 ml of MeOH and H_2O was added to make the total volume about 50 ml. The pH was adjusted to 2, the solution was refluxed in an oil bath for 1 hr, and extracted with $CHCl_3$. The aqueous layer was neutralized and the basic material was adsorbed on a column of 10 ml of Amberlite IR 120 (H^+). After washing the column with 100 ml of H_2O, the base was eluted with 2N HCl, and evaporated under a reduced pressure. The residue was extracted with MeOH and 400 mg of O-methylmycaminose methochloride (IX) was obtained as a viscous yellowish liquid.

Oxidation of IX with Sodium Periodatde——To a solution of IX (300 mg) in 15 ml of H_2O was added 10 ml of H_2O containing 500 mg of $NaIO_4$. The mixture was left at room temperature for 20 hr, and then distilled on an oil bath. To the distillate was added 1% 2,4-dinitrophenylhydrazine hydrochloride solution, and the precipitate obtained was recrystallized from MeOH to yield 150 mg of yellow needles, mp 164°. It was compared with an authentic sample of acetaldehyde 2,4-dinitrophenylhydrazone by mixed mp, IR, and thin-layer chromatography.

16) NMR spectra were measured with either Hitachi H-60 spectrometer at 60 Mc or Varian HA-100 spectrometer at 100 Mc. Infrared spectra were measured in CCl_4 with a Perkin Elmer Model 21 spectrometer using a 3 cm cell and a LiF prism. Unless otherwise stated rotations were measured in $CHCl_3$.

α- and β-Methyl 4-O-Isovalerylmycaroside (Xa and Xb)——Three grams of methyl 4-O-isovalerylmycaroside[4] obtained by the methanolysis of I, was chromatographed on 20×45 cm column of silicic acid and eluted with benzene–Me_2CO (10 : 1). The α-isomer eluted first, followed by the β-isomer. These components were confirmed by thin-layer chromatography and the products were dilstilled *in vacuo*. α-Isomer (Xa): bp 115—116° (2mmHg), $[\alpha]_D^{25}$ −135° (c=1.33), n_D^{25} 1.4476. *Anal.* Calcd. for $C_{13}H_{24}O_5$: C, 60.02; H, 9.18. Found: C, 60.08; H, 9.18. β-Isomer (Xb): bp 117—118.5° (0.7 mmHg), $[\alpha]_D^{25}$ +13.5° (c=1.33), n_D^{25} 1.4518. *Anal.* Calcd. for $C_{13}H_{24}O_5$: C, 60.02; H, 9.18. Found: C, 60.05; H, 9.20.

Acetylmagnamycin——In the same manner as reported by Wagner, *et al.*,[17] 1.0 g of magnamycin was acetylated with pyridine–Ac_2O and gave acetylmagnamycin, mp 145—148°, $[\alpha]_D^{25}$ −80° (c=1.3). *Anal.* Calcd. for $C_{44}H_{69}O_{17}N$: C, 59.78; H, 7.87; N, 1.59. Found: C, 60.01; H, 7.95; N, 1.70.

Acetylspiramycin-A——In the same manner as reported by Paul, *et al*,[18] 1.0 g spiramycin was acetylated with pyridine–Ac_2O gave acetylspiramycin A, $[\alpha]_D^{25}$ −90.5° (c=1.5, EtOH). *Anal.* Calcd. for $C_{49}H_{80}O_{17}N_2$: C, 60.73; H, 8.32; N, 2.89. Found: C, 61.01; H, 8.41; N, 2.90.

Acknowledgement The authors express their gratitude to Dr. S. Saheki of the Goverment Chemical Industrial Reserach Institute, Tokyo, for measuring IR, and to Dr. Y. Tsuzuki and Dr. N. Mori of the Tokyo College of Science for their kind advices. The authors are also grateful to Dr. F.A. Hochstein, Pfizer Co., Inc., for the kind supply of magnamycins.

17) R.L. Wagner, **F.A.** Hochstein, K. Murai, M. Messina, and P.P. Regna, *J. Am. Chem. Soc.*, 75, 4684 (1953).

18) R. Paul and S. Tchelitcheff, *Bull. Soc. Chim. France*, 189 (1965).