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The Chemistry of Leucomycins. II.¹⁾ Glycosidic Linkages of Mycaminose and Mycarose on Leucomycin A₃²⁾

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It was previously reported that leucomycin A₃ 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 isovalerylmicarose is linked with mycaminose at the 4-position through an α -glycosidic linkage.

As described in the previous report,^{1,4)} leucomycin A₃, C₄₂H₈₉O₁₅N, is a macrolide antibiotic composed of mycaminose, 4-O-isovalerylmicarose and the macrolactone containing groups such as O-acetyl, O-methyl, -CH₂CHO, and $\alpha,\beta,\gamma,\delta$ -unsaturated alcohol or ether. In the present paper, we will discuss the position of -CH₂CHO group in relation to the mycaminosidic linkage and also the stereochemistry of the mycarosidic linkage in leucomycin A₃ (I).

In order to prove the position of -CH₂CHO group as adjacent to the mycarosidic linkage, the method of Woodward⁵⁾ used in the case of magnamycin was tried. As already reported,¹⁾ the aldehyde group in I is converted to the methylde-mycarosylleucomycin A₃ 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 methyltetrahydrode-mycarosylleucomycin A₃ dimethylacetal (III) C₃₃H₆₁O₁₂N. Following reduction with lithium aluminum hydride, III yields methylde-acetyloctahydrode-mycarosylleucomycin A₃ (IV), C₂₉H₅₇O₁₀N, 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 Sankochō, 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.

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

The $\text{p}K_a'$ value of I is 6.70, whereas the demycarosyl derivative (II)¹⁾ has a higher $\text{p}K_a'$ value of 7.81. The $\text{p}K_a'$ value of diacetylleucomycin- A_3 (VI)¹⁾ is 5.69, lower than that of I, but the $\text{p}K_a'$ 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 mycaminoses 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 basicity⁵⁾ 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 $\text{p}K_a'$ values (Table I).

TABLE I. $\text{p}K_a'$ and NMR of Leucomycin A_3 and Its Related Compounds

Compounds	$\text{p}K_a'$	Chemical shift of $-\text{N}(\text{CH}_3)_2$ (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 mycaminoses. 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-methylmycaminoses 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 mycaminoses, signals in the anomer proton region of nuclear magnetic resonance (NMR) spectra of I (Fig. 1) and

related compounds are compared. The α -isomer of methylmycaminoside⁷⁾ 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 mycaminoside, and from the J value (axial-axial coupling) it is clear that mycaminoside (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-isovalerylmycaroside¹⁾ 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.

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 spectra (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, $J_{1,2b}=2.5$ cps), respectively. The axial proton of a pyranoside sugar shows an absorption peak in a higher

Fig. 1. NMR Spectrum of Leucomycin A₃ (CDCl₃, 100 Mc)

- 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. Omura, 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 A₃ Compounds

Compound	$[\text{M}]_D^{25}$ (in CHCl_3)	
	Found	Calcd.
Xa	-351.0 (=a)	
Xb	$+35.1$ (=b)	
II	-124.0 (=c)	
I	-458.0 (=d)	$\left\{ \begin{array}{l} \alpha\text{-d} = a + c = -475.0 \\ \beta\text{-d} = b + c = -88.9 \end{array} \right.$

As a summary of the above results, the partial structure of leucomycin A₃ (I) is proposed in Fig. 4.

Fig. 4. Partial Structure of Leucomycin A₃

The infrared spectrometric method was applied to the determination of mycarosidic linkage in spiramycin¹⁴⁾ and magnamycin,^{14,15)} and it was found that both compounds have the α -mycarosidic linkage. The infrared data of Xa, Xb, VI, acetylsiramycin, 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 A₃ and Its Related Compounds (0.004 mole, CCl₄)

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)

Experimental¹⁶⁾

Methyltetrahydrodemycarosylleucomycin A₃ Dimethyl-acetal (III)—A solution of 2.55 g of methylde-mycarosylleucomycin A₃ dimethylacetal (II) in 75 ml of EtOH was subjected to catalytic hydrogenation using 0.80 g of 5% Pd-C. Two moles of H₂ 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, $[\alpha]_D^{25} +2.5^\circ$ ($c=1.4$). *Anal.* Calcd. for C₃₃H₆₁O₁₂N: 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-Ac₂O. After purification by chromatography on silicic acid with benzene containing 10% of Me₂CO, the acetate was recrystallized from CCl₄ as needles, mp 120–121°, $[\alpha]_D^{25} -29.0^\circ$ ($c=1.4$). *Anal.* Calcd. for C₃₇H₆₅O₁₄N: C, 59.42; H, 8.76; N, 1.87. Found: C, 60.50; H, 8.65; N, 2.01.

Methyldeacetyloctahydrodemycarosylleucomycin A₃ (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 LiAlH₄ 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 H₂O, pH of the solution was made 8, and extracted three 40 ml portions of CHCl₃. The dried CHCl₃ 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. $[\alpha]_D^{25} -5.6^\circ$ ($c=1.3$). *Anal.* Calcd. for C₂₉H₅₇O₁₀N: 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% H₂O₂, and the resulting solution was left at room temperature for 15 hr. After excess H₂O₂ was decomposed with MnO₂, the solution was made to alkaline and extracted with CHCl₃ (practically no extract was obtained). The aqueous layer was acidified to pH 2 with HCl, refluxed for 2 hr, and extracted with CHCl₃. The CHCl₃ layer was washed with a small amount of an aqueous NaHCO₃, dried, and evaporated. There was obtained 200 mg of an oily product, IR cm⁻¹: $\nu_{C=O}$ 1770.

O,O-Dimethylleucomycin A₃ Methiodide (VIII)—To a solution of 4 g of I in 40 ml of MeI at 45°, freshly prepared Ag₂O 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 Ag₂O was added. The reaction mixture was filtered and the remaining solid was washed several times with 20 ml each of CHCl₃. 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 H₂O 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 CHCl₃. 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 H₂O, the base was eluted with 2N HCl, and evaporated under a reduced pressure. The residue was extracted with MeOH and 400 mg of O-methylmycamino methochloride (IX) was obtained as a viscous yellowish liquid.

Oxidation of IX with Sodium Periodate—To a solution of IX (300 mg) in 15 ml of H₂O was added 10 ml of H₂O containing 500 mg of NaIO₄. 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 CCl₄ with a Perkin Elmer Model 21 spectrometer using a 3 cm cell and a LiF prism. Unless otherwise stated rotations were measured in CHCl₃.

α - and β -Methyl 4-O-Isovalerylmycaroside (Xa and Xb)—Three grams of methyl 4-O-isovalerylmycaroside⁴⁾ obtained by the methanolysis of I, was chromatographed on 20 $\times$ 45 cm column of silicic acid and eluted with benzene-Me₂CO (10 : 1). The α -isomer eluted first, followed by the β -isomer. These components were confirmed by thin-layer chromatography and the products were distilled *in vacuo*. α -Isomer (Xa): bp 115–116° (2mmHg), $[\alpha]_D^{25} -135^\circ$ ($c=1.33$), n_D^{25} 1.4476. *Anal.* Calcd. for C₁₃H₂₄O₅: C, 60.02; H, 9.18. Found: C, 60.08; H, 9.18. β -Isomer (Xb): bp 117–118.5° (0.7 mmHg), $[\alpha]_D^{25} +13.5^\circ$ ($c=1.33$), n_D^{25} 1.4518. *Anal.* Calcd. for C₁₃H₂₄O₅: 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-Ac₂O and gave acetylmagnamycin, mp 145–148°, $[\alpha]_D^{25} -80^\circ$ ($c=1.3$). *Anal.* Calcd. for C₄₄H₆₉O₁₇N: 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-Ac₂O gave acetylspiramycin A, $[\alpha]_D^{25} -90.5^\circ$ ($c=1.5$, EtOH). *Anal.* Calcd. for C₄₉H₈₀O₁₇N₂: C, 60.73; H, 8.32; N, 2.89. Found: C, 61.01; H, 8.41; N, 2.90.

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