MYCINAMICINS, NEW MACROLIDE ANTIBIOTICS VIII. CHEMICAL DEGRADATION AND ABSOLUTE CONFIGURATION OF MYCINAMICINS

KENJI KINOSHITA, SHUZO SATOI and MITSUO HAYASHI*

Research Center, Toyo Jozo Co., Ltd., Ohito, Shizuoka, 410-23, Japan

KEN-ICHI HARADA and MAKOTO SUZUKI

Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya, 468, Japan

KAZUMI NAKATSU

Department of Chemistry, Faculty of Science, Kwansei Gakuin University Uegahara, Nishinomiya, Hyogo, 662, Japan

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Mycinamicins, a new family of basic 16-membered macrolide antibiotics were produced by *Micromonospora griseorubida* sp. nov. They consist of at least seven components, named as mycinamicin I (**1**), II (**2**), III (**3**), IV (**4**), V (**5**), VI (**6**) and VII (**7**), and possess strong antibacterial activity against Gram-positive bacteria[1~4]. In an earlier study, the structures and stereochemistry of mycinamicins were elucidated on the basis of spectroscopic data, a few chemical degradations and an X-ray crystal structure analysis of mycinolide IV (**8**), which was an aglycone portion of **4**[5]. Recently, we obtained the dedesosaminyl derivative (**10**) of **1** by degradative experiments[6]. In the present paper, we report the structural elucidation of acetyl derivative (**12**) of **10** by X-ray analysis, and hence propose the absolute stereochemistry of mycinamicins (Fig. 1).

Treatment of **1** with *m*-chloroperbenzoic acid in chloroform gave its *N*-oxide in a high yield. Upon subsequent treatment of the *N*-oxide with acetic anhydride in chloroform at reflux for 5 hours, compounds **9**, **10** and **11** were obtained. Compound **10** was acetylated with acetic anhydride in pyridine to yield quantitatively the 5,4″-diacetate (**12**). Compound **12** afforded the suitable crystals for single-crystal X-ray structural study by crystallization from methanol. The elemental analysis and ^{1}H NMR spectrum suggested that the molecule crystallizes as a 1:1 methanol solvate. To protect the crystal from decomposition due to vaporization of the labile solvent of crystallization, the specimen used for the analysis was sealed in capillary tube containing a small amount of the mother liquor at one end. The X-ray photographs showed the crystals to be orthorhombic with space group $P2_12_12_1$. Intensities of 2,458 reflections within $2\theta=45°$ were collected from a crystal of dimensions $0.32\times0.52\times0.58$ mm on a Rigaku automatic four-circle diffractometer with MoKα ($\lambda=0.71069$ Å) radition and the $\omega-2\theta$ scan technique. Of those, 2,051 reflections with $|Fo|>3\sigma(F)$ were used for the structure determination. The crystal data are given in Table 1.

The structure was solved by direct methods using the program MULTAN 78[7], and refined by the block-diagonal least-squares method with anisotropic temperature factors for all non-

Table 1. Crystal data for **12**.

$C_{33}H_{50}O_{12}\cdot CH_3OH$	MW 670.79
Orthorhombic $P2_12_12_1$, $z=4$	Dcal=1.174 g cm^{-3}
$a=13.630$ (1) $b=23.231$ (2)	$c=11.987$ (1) Å
$U=3795.6$ (6) Å	

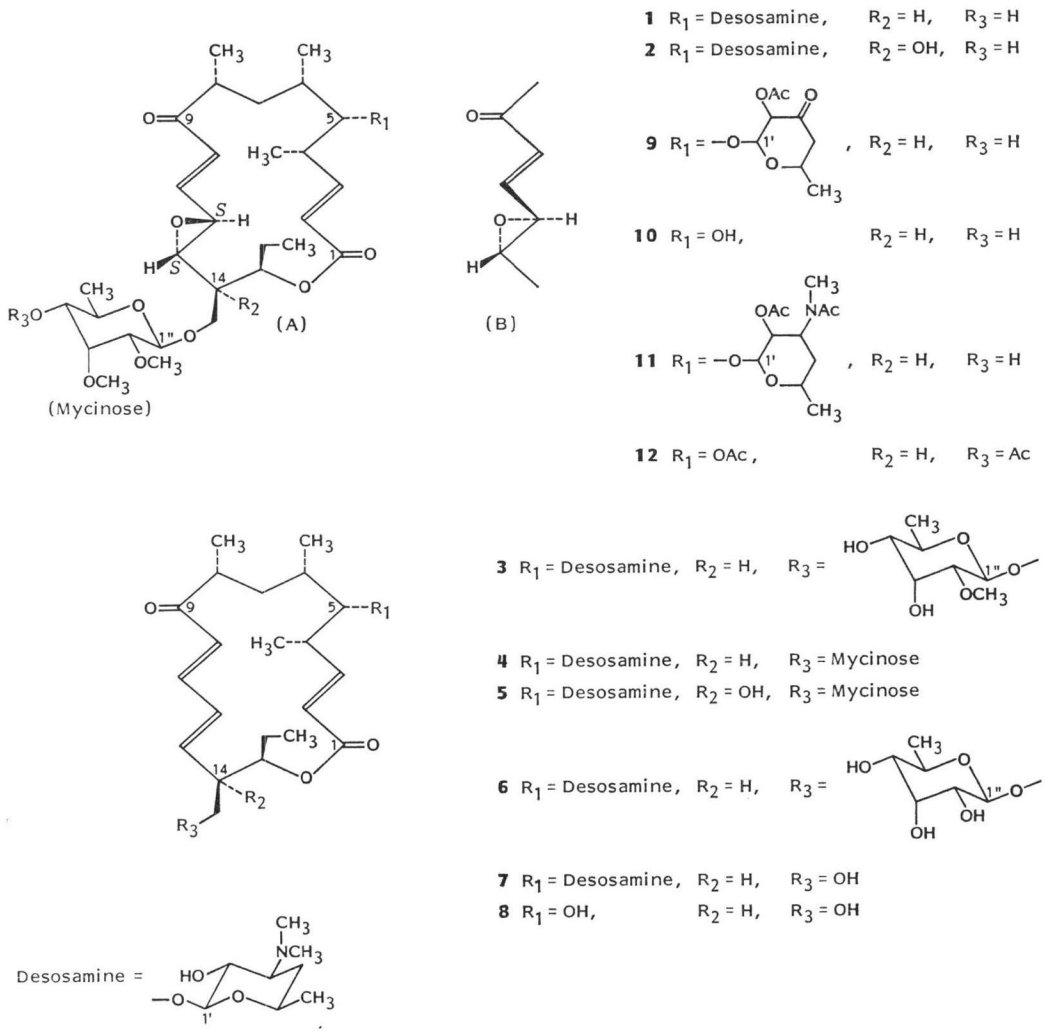
Table 2. The conformation of the 16-membered ring.

Bond	Torsion angle[a]		
	12 (°)	**8** (°)	Δ (°)[b]
O(1)–C(1)–C(2)–C(3)	−176	−177	1
C(1)–C(2)–C(3)–C(4)	171	177	6
C(2)–C(3)–C(4)–C(5)	135	144	9
C(3)–C(4)–C(5)–C(6)	−56	−62	6
C(4)–C(5)–C(6)–C(7)	−62	−69	7
C(5)–C(6)–C(7)–C(8)	176	180	4
C(6)–C(7)–C(8)–C(9)	−66	−56	10
C(7)–C(8)–C(9)–C(10)	−62	−52	10
C(8)–C(9)–C(10)–C(11)	−178	169	13
C(9)–C(10)–C(11)–C(12)	−174	−175	1
C(10)–C(11)–C(12)–C(13)	149	163	14
C(11)–C(12)–C(13)–C(14)	−151	−170	19
C(12)–C(13)–C(14)–C(15)	94	94	0
C(13)–C(14)–C(15)–O(1)	−65	−62	3
C(14)–C(15)–O(1)–C(1)	127	112	15
C(15)–O(1)–C(1)–C(2)	−175	−167	8

[a] The positive sign indicates that the angle is measured in the right way, while the negative sign denotes the measurement in the other way.

[b] Differences between **12** and **8**.

Fig. 1. Absolute configuration of mycinamicins.



hydrogen atoms. The position of the solvent molecule was not definitely determined in the difference electron density map obtained at this refined stage. The final R-factor was 0.149. The molecular structure and the stereoscopic drawing of **12** are shown in Figs. 2 and 3, respectively.

A comparison of the conformations of **8** and **12** is made in Table 2, which contains the torsion angles of the 16 bonds in macrocyclic lactone rings. Aside from the structural difference between –C(12)=C(13)– in **8** and –C(12)–C(13)– in **12**, the torsion angles and hence the overall conformations of the 16-membered rings are very similar. Thus, the mycinose substituent has no effect on the conformation of the 16-membered lactone ring[5].

Since methyl β-D-mycinoside was obtained from methanolysis of **1**[8], we propose, on the basis of the relative stereochemistry obtained from the X-ray analysis, that the configurations at C-4, C-5, C-6, C-8, C-12, C-13, C-14 and C-15 in the aglycone of **12** are *S*, *S*, *S*, *R*, *S*, *S*, *S* and *R*, respectively. Therefore, the absolute configuration of the mycinamicins is established as shown in Fig. 1 (A). In particular the stereochemistry of C-12 and C-13 can be also expressed more precisely by the structure shown in Fig. 1 (B), which is consistent with those of maridomycin[9], rosaramicin[10] and acumycin[11].

Fig. 2. A stereoscopic drawing of **12**.

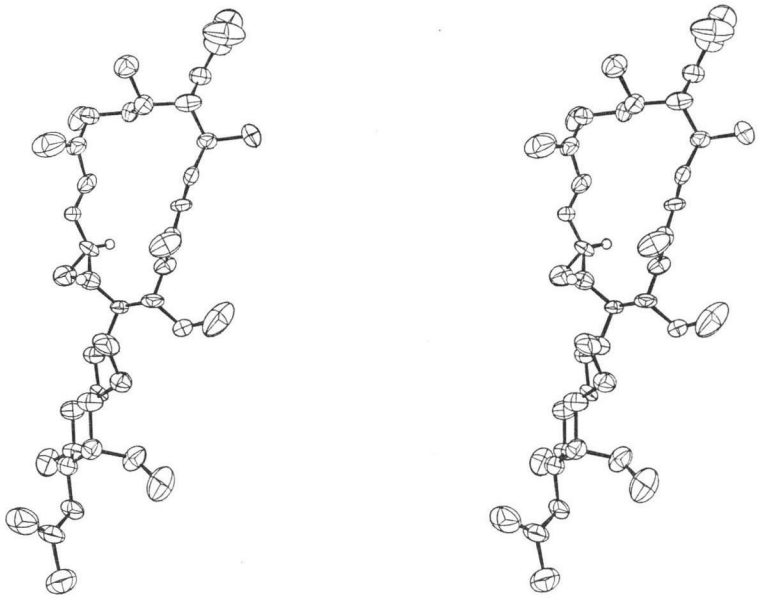
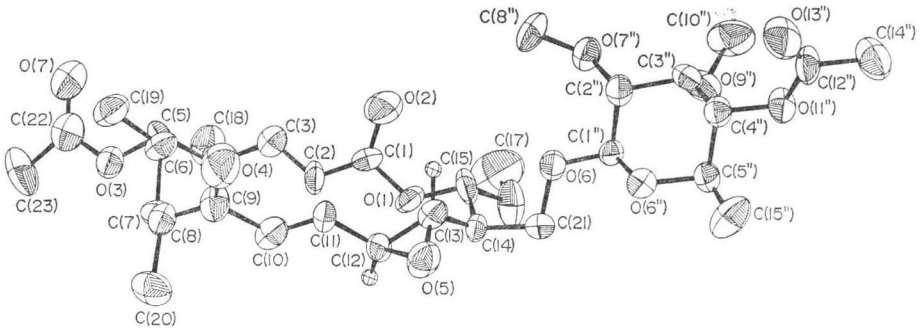


Fig. 3. The molecular structure of **12** viewed along the horizontal plane of the macro ring.



Experimental

Melting points were determined on a Yanaco micro-melting point apparatus and were uncorrected. The IR spectra were taken with a Hitachi 260-50 spectrophotometer. The UV spectra were recorded on a Hitachi 323 spectrometer. The optical rotation at 589 nm was measured with a Jasco automatic polarimeter DIP-180. The NMR spectra were obtained with a Jeol JNM-FX100 spectrometer at 99.55 (^{1}H) and 25.00 (^{13}C) MHz with TMS as an internal reference. The mass spectra were taken with a Jeol JMS-D300 spectrometer. The thin-layer chromatogram (TLC) was obtained on a glass plate coated with Kieselgel-GF_{254}. Column chromatography was carried out using silica gel (Merck, Kieselgel 60).

Mycinamicin I *N*-Oxide

To a solution of mycinamicin I (**1**, 5 g) in $CHCl_3$ (100 ml) was added dropwise with stirring at 5°C a solution of *m*-chloroperbenzoic acid (purity 70%, 1.69 g) in $CHCl_3$ (30 ml). After the addition was complete, the reaction mixture was allowed to stand in the dark for 2 hours at room temperature. Excess peracid in the reaction solution was decomposed with 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 anhydrous Na_2SO_4, the $CHCl_3$ layer was concentrated *in vacuo* to about 10 ml and ethyl ether (100 ml)

was added to give a white powder, mycinamicin I *N*-oxide (2.4 g). MP 110~115°C. $[\alpha]_D^{26}$ −45.9° (*c* 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε) 217 (4.36), 240 (sh, 4.09). IR (KBr) cm^{-1} 3440, 1705, 1690, 1650, 1620. ^1H NMR (CD_3COCD_3) δ 3.16 (6H, s, 3′-$N(CH_3)_2\rightarrow O$), 3.52 (3H, s, 2″-OCH_3), 3.55 (3H, s, 3″-OCH_3), 4.37 (1H, d, *J*=7.0 Hz, 1′-H), 4.57 (1H, d, *J*=8.0 Hz, 1″-H), 5.36 (1H, m, 15-H), 6.09 (1H, d, *J*=15.5 Hz, 2-H), 6.29 (1H, dd, *J*=15.5, 10.0 Hz, 11-H), 6.63 (1H, dd, *J*=15.5, 10.5 Hz, 3-H), 6.99 (1H, d, *J*=15.5 Hz, 10-H). CIMS (*i*-C_4H_{10}) *m/z* 712 (MH^+-H_2O), 696, 538, 424, 381, 363, 347, 345, 176, 175, 174, 158, 143.

Degradative Reaction of Mycinamicin I *N*-Oxide

To a solution of mycinamicin I *N*-oxide (2 g) in $CHCl_3$ (25 ml) was added acetic anhydride (1.0 ml) and the solution was refluxed for 5 hours. After completion of the reaction, cold water (100 ml) was added to the reaction mixture and the $CHCl_3$ layer was separated. The aqueous layer was extracted again with $CHCl_3$ (100 ml) and the combined $CHCl_3$ extracts were washed with aqueous saturated $NaHCO_3$ solution (100 ml ×2) and H_2O (100 ml ×2), successively. After drying the organic layer over anhydrous Na_2SO_4, it was evaporated *in vacuo*. The brown residue was chromatographed over silica gel and elution with benzene - EtOAc - MeOH (170: 30: 2) afforded 2′-*O*-acetyl-3′-dedimethylamino-3′-oxomycinamicin I (**9**, 80 mg) as powder. MP 103~105°C. $[\alpha]_D^{21}$ −54.6° (*c* 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε) 215 (4.54), 240 (sh, 4.26). IR (KBr) cm^{-1} 3480, 1755, 1720, 1695, 1660, 1630. ^1H NMR (CD_3COCD_3) δ 2.12 (3H, s, 2′-$OCOCH_3$), 3.53 (3H, s, 2″-OCH_3), 3.55 (3H, s, 3″-OCH_3), 4.57 (1H, d, *J*=8.0 Hz, 1″-H), 4.81 (1H, d, *J*=8.0 Hz, 1′-H), 5.02 (1H, d, *J*=8.0 Hz, 2′-H), 5.37 (1H, m, 15-H), 6.12 (1H, d, *J*=15.5 Hz, 2-H), 6.32 (1H, dd, *J*=15.5, 9.5 Hz, 11-H), 6.65 (1H, dd, *J*=15.5, 10.5 Hz, 3-H), 6.96 (1H, d, *J*=15.5 Hz, 10-H). CIMS (*i*-C_4H_{10}) *m/z* 725 (MH^+), 583, 555, 537, 409, 391, 381, 365, 363, 347, 345, 175, 171, 143, 129.

Further elution with the same solvent gave 5-dedesosaminylmycinamicin I (**10**, 210 mg) as powder. MP 105~108°C. $[\alpha]_D^{21}$ −56.1° (*c* 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε) 217.5 (4.30), 240 (sh, 4.05). IR (KBr) cm^{-1} 3510, 3420, 1715, 1685, 1645, 1620. ^1H NMR (CD_3COCD_3) δ 3.53 (3H, s, 2″-OCH_3), 3.55 (3H, s, 3″-OCH_3), 4.58 (1H, d, *J*=8.0 Hz, 1″-H), 5.38 (1H, ddd, *J*=3.0, 8.0, 11.0 Hz, 15-H), 6.09 (1H, d, *J*=15.5 Hz, 2-H), 6.30 (1H, dd, *J*=15.5, 9.5 Hz, 11-H), 6.62 (1H, dd, *J*=15.5, 10.5 Hz, 3-H), 6.98 (1H, d, *J*=15.5 Hz, 10-H). CIMS (*i*-C_4H_{10}) *m/z* 555 (MH^+), 537, 381, 365, 363, 347, 345, 175.

After separating **9** and **10**, further elution with the same solvent afforded 2′-*O*-acetyl-3′-*N*-demethyl-3′-*N*-acetylmycinamicin I (**11**, 700 mg) as powder. MP 127~130°C. $[\alpha]_D^{21}$ −49.9° (*c* 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε) 213 (4.42), 240 (sh, 4.09). IR (KBr) cm^{-1} 3480, 1745, 1720, 1695, 1660, 1650, 1635. ^1H NMR (CD_3COCD_3) δ 1.96 (3H, s, 2′-$OCOCH_3$ or 3′-*N*-$COCH_3$), 1.97 (3H, s, 3′-N-$COCH_3$ or 2′-$OCOCH_3$), 2.87 (3H, s, 3′-N-CH_3), 3.52 (3H, s, 2″-OCH_3), 3.55 (3H, s, 3″-OCH_3), 4.57 (1H, d, *J*=8.0 Hz, 1″-H), 4.65 (1H, d, *J*=7.0 Hz, 1′-H), 6.09 (1H, d, *J*=15.5 Hz, 2-H), 6.29 (1H, dd, *J*=15.5, 9.5 Hz, 11-H), 6.64 (1H, dd, *J*=15.5, 10.5 Hz, 3-H), 6.95 (1H, d, *J*=15.5 Hz, 10-H). CIMS (*i*-C_4H_{10}) *m/z* 782 (MH^+), 766, 555, 381, 363, 345, 228, 175.

5-Dedesosaminyl-5,4″-*O*,*O*-diacetylmycinamicin I (**12**)

10 (300 mg) was dissolved in pyridine (1.0 ml) and acetic anhydride (1.0 ml) was added to the solution. The mixture was allowed to stand at room temperature overnight and then ice water added. The precipitate was collected by filtration and crystallized from methanol to give **12** (250 mg) as colorless prisms (*Anal* Calcd for $C_{33}H_{50}O_{12}\cdot CH_3OH$: C 60.88, H 8.11. Found: C 60.89, H 8.28) and was subsequently analyzed by X-ray crystallography. After drying *in vacuo* on P_2O_5, spectral data were measured. MP 198~199°C. $[\alpha]_D^{23}$ −10.2° (*c* 1.0, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε) 213 (4.37), 243 (4.09). IR (KBr) cm^{-1} 1745, 1725, 1695, 1660, 1630, 1235. ^1H NMR (CD_3COCD_3) δ 2.07 (3H, s, 4″-$OCOCH_3$), 2.08 (3H, s, 5-$OCOCH_3$), 3.49 (3H, s, 3″-OCH_3), 3.55 (3H, s, 2″-OCH_3), 4.63 (1H, d, *J*=8.0 Hz, 1″-H), 4.86 (1H, br d, *J*=11.0 Hz, 5-H), 5.38 (1H, ddd, *J*=3.0, 8.0, 11.0 Hz, 15-H), 6.19 (1H, d, *J*=15.5 Hz, 2-H), 6.32 (1H, dd, *J*=15.5, 9.5 Hz, 11-H), 6.69 (1H, dd, *J*=15.5, 10.5 Hz, 3-H), 7.01 (1H, d, *J*=15.5 Hz, 10-H). CIMS (*i*-C_4H_{10}) *m/z* 639 (MH^+), 621, 579, 423, 405, 387, 363, 347, 345, 217. *Anal* Calcd for $C_{33}H_{50}O_{12}$: C 62.05, H 7.89. Found: C 61.81, H 8.16.

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