MYCINAMICINS, NEW MACROLIDE ANTIBIOTICS. IV

STRUCTURE OF MYCINAMICIN III

MITSUO HAYASHI, MASARU OHNO, SEIJI KATSUMATA
and SHUZO SATOI

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

KEN-ICHI HARADA, MOTOHIRO TAKEDA and MAKOTO SUZUKI

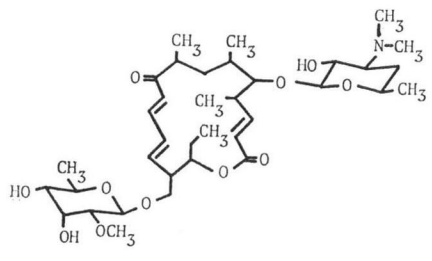
Faculty of Pharmacy, Meijo University
Tempaku-ku, Nagoya, 468, Japan

(Received for publication December 22, 1980)

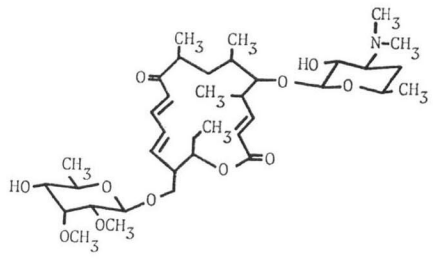
The structure of mycinamicin III coproduced with other mycinamicins in submerged fermentation of *Micromonospora griseorubida* sp. nov. has been elucidated by its spectral properties and chemical degradation studies. Mycinamicin III is 3''-O-demethyl mycinamicin IV.

Mycinamicins, a new family of basic 16-membered macrolide antibiotics with novel skeletons, were obtained from *Micromonospora griseorubida* sp. nov., and named as mycinamicin I, II, III, IV and V, respectively. The isolation of these antibiotics and structures of mycinamicin I, II, IV and V have been reported in the previous papers[1,2]. This paper deals with the structural elucidation of mycinamicin III.

Mycinamicin III (**1**) was isolated as white amorphous powder, mp. 113~115°C, $[\alpha]_D^{25}$ −2.3° (*c* 1.0, MeOH). The composition of **1**, $C_{36}H_{59}NO_{11}$, was established by elemental analysis and high resolution mass spectrum (M^+, *m/z* 681.4096, Δ 1.1 mu.). The IR spectrum suggested the presence of hydroxy (3460 cm^{-1}), α, β-unsaturated lactone (1710, 1645 cm^{-1}) and $\alpha, \beta, \gamma, \delta$-unsaturated ketone (1675, 1625, 1590 cm^{-1}). Two absorption maxima on the UV spectrum at 215 nm (log ε 4.32) and 281.5 nm (log ε 4.33) in methanol showed the existence of an α, β-unsaturated lactone and $\alpha, \beta, \gamma, \delta$-unsaturated ketone chromophores in **1**, respectively. The ^{1}H-NMR spectrum of **1** in CD_3COCD_3 (Fig. 1) revealed the presence of six C-methyl (0.86~1.29 ppm, 18H), one N-dimethyl (2.29 ppm, 6H, s), one O-methyl (3.45 ppm, 3H, s), two anomeric (4.29 ppm, 1H, d, J=7.3 Hz; 4.61 ppm 1H, d, J=7.8 Hz) and six olefinic (5.84~7.17 ppm, 6H, m) protons. The ^{13}C-NMR chemical shifts of **1** were

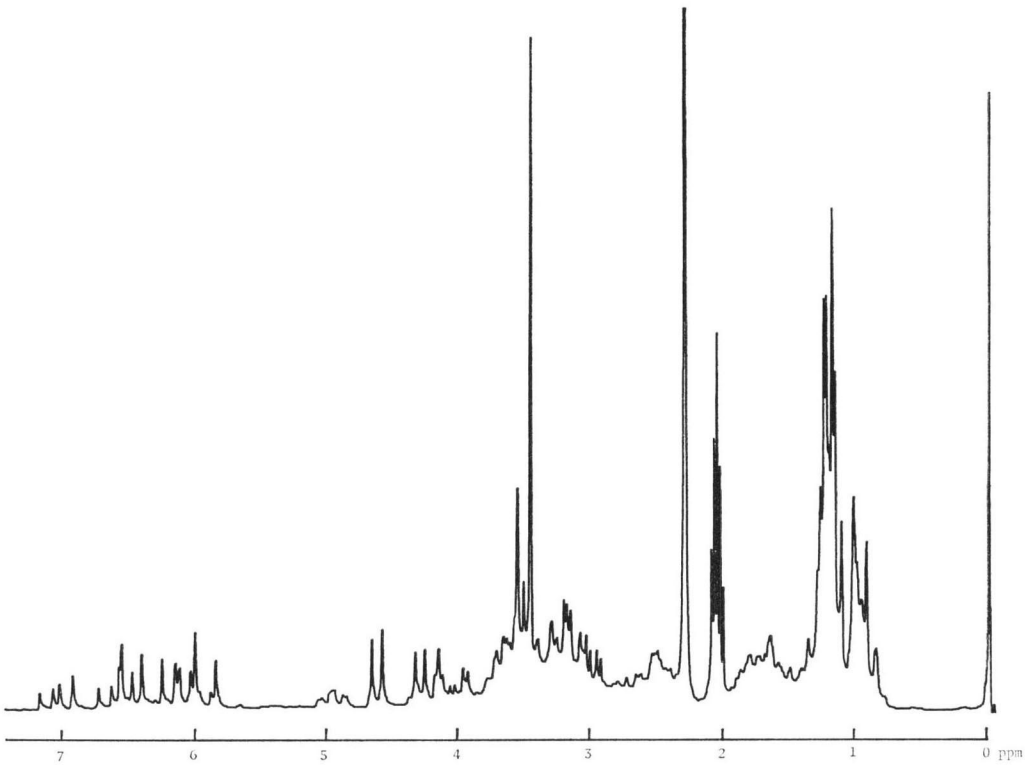


1



2

Fig. 1. ^{1}H-NMR spectrum of mycinamicin III (**1**).



shown in Table 1 together with those of mycinamicin IV (**2**), and their data were very similar with each other. The structural distinction between **1** and **2** was the lack of one O-methyl group in **1**.

The chemical ionization (CI) mass spectrum of **1** was also closely similar to that of **2** using isobutane as the reagent gas (Fig. 2). The diagnostic ions showed the presence of desosamine[3] (*m/z* 158, 174 and 176) and same aglycone part as **2** (*m/z* 347 and 365)[4]. However, the protonated molecular ion (*m/z* 682) and the ion derived from neutral sugar (*m/z* 161) in the CI mass spectrum of **1** appeared at 14 u. lower than the corresponding ions of **2**. In consequence, **1** was assumed to have a demethylated mycinose as neutral sugar in place of mycinose in **2**.

On the methanolysis of **1** with Amberlist 15 in methanol, a neutral methyl glycoside (**3**) was obtained as colorless needles, mp. 98°C, $[\alpha]_D^{27}$ −81.3° (*c* 0.15, MeOH), $C_8H_{16}O_5$ (M.W. 192). The CI mass spectrum of **3** using ammonia showed the characteristic ions, *m/z* 210 ($M\cdot NH_4^+$), 178 ($M\cdot NH_4^+ -32$) and 161 ($M\cdot NH_4^+ -32-17$). Furthermore, acetylation of **3** with acetic anhydride in pyridine gave diacetate (**4**).

The ^{1}H-NMR chemical shifts of **3** and **4** were shown in Table 2. The large coupling constant (J=7.6 Hz) of H-1 displayed that **3** was β-anomer. The signal at δ 1.32 (3H, d, J=6.1 Hz) was assigned to a 6-CH_3 group. Therefore, **3** was considered to be methyl 6-deoxy-mono-O-methyl-β-hexopyranoside. The down-field shifts of H-3 and H-4 in **4** showed that O-methyl group was attached to C-2. The coupling constants of H-2, 3, 4 and 5 indicated that the substituents at C-2, 3, 4 and 5 were equatorially, axially, equatorially and equatorially disposed, respectively. Accordingly, these results established that **3** was methyl 6-deoxy-2-O-methyl-β-*allo*-hexopyranoside.

Table 1. ^{13}C-NMR chemical shifts for **1** and **2** in $CDCl_3$.

Carbon	Mycinamicin III (**1**)		Mycinamicin IV (**2**)	
	Chemical shift δ (ppm)	Multiplicity	Chemical shift δ (ppm)	Multiplicity
1	166.1	s	166.1	s
2	120.9	d	120.9	d
3	151.7	d	151.6	d
4	41.3	d	41.3	d
5	87.8	d	87.9	d
6	34.1	d	34.1	d
7	32.6	t	32.6	t
8	44.9	d	44.9	d
9	203.7	s	203.4	s
10	123.2	d	123.2	d
11	141.8	d	141.7	d
12	133.0	d	133.0	d
13	141.3	d	141.3	d
14	49.2	d	49.2	d
15	73.7	d	73.7*	d
16	25.2	t	25.3	t
17	9.7	q	9.6	q
4 CH_3	19.4	q	19.4	q
6 CH_3	17.4	q	17.4	q
8 CH_3	17.7	q	17.8	q
14 CH_2	68.7	t	68.6	t
1′	104.9	d	104.9	d
2′	70.4	d	70.4	d
3′	65.8	d	65.8	d
4′	28.4	t	28.3	t
5′	69.5	d	69.5	d
6′	21.2	q	21.2	q
$N(CH_3)_2$	40.2	q	40.2	q
1″	100.8	d	101.0	d
2″	80.1	d	81.9	d
3″	69.8	d	79.9	d
4″	72.8	d	72.7	d
5″	69.8	d	70.5	d
6″	17.7	q	17.8	q
2″ OCH_3	59.4	q	59.7	q
3″ OCH_3	—	—	61.7	q

* The previously assigned signal[2] due to carbon 15 at 72.7 ppm is actually at 73.7 ppm.

A naturally occurring 6-deoxy-2-O-methyl-*allo*-D-hexose was isolated first by MÜHLRADT *et al.* and it was named D-javose[5,6]. HOFFMANN *et al.* reported the synthesis of methyl β-D-javoside[7], and **3** is completely identical with methyl β-D-javoside by comparing mp, $[\alpha]_D$ and ^{1}H-NMR spectrum in the literature[7].

Mild acid hydrolysis of **1**, followed by silica gel column chromatography and recrystallization from hexane - acetone afforded two degradation products, **5** and **6**. Compound **5** [mp. 242~243°C,

Fig. 2. CI mass spectra of mycinamicin III (**1**) and mycinamicin IV (**2**).

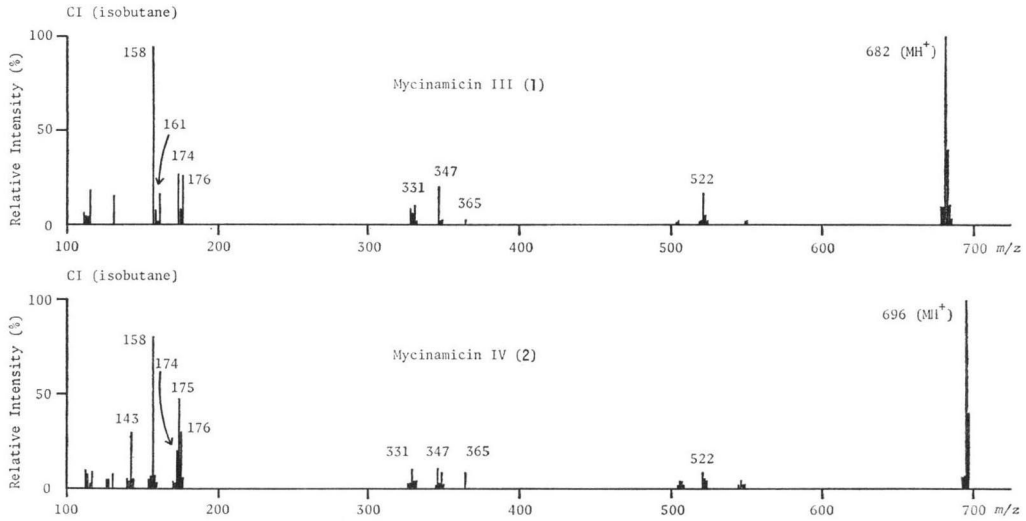
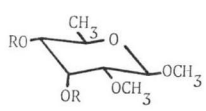


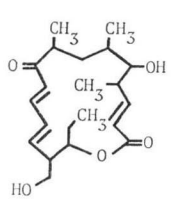
Table 2. ^{1}H-NMR chemical shifts of **3** and **4**.

Compound	Chemical shifts (δ)* (first-order couplings. Hz, in parentheses)									
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5	H-6 ($J_{5,6}$)	3-OH	4-OH ($J_{4,OH}$)	OMe	OAc
3	4.51 d	3.06 dd	4.24 b. t	3.24 ddd	3.69 dq	1.32 d	2.57 b. s	2.37 d	3.52 s	
	(7.6)	(3.2)	(3.2)	(9.3)		(6.1)		(10.3)	3.53 s	
4	4.58 d	3.19 dd	5.72 t	4.55 dd	3.90 dq	1.21 d			3.40 s	2.01 s
	(7.8)	(2.9)	(2.9)	(9.8)		(6.1)			3.55 s	2.13 s

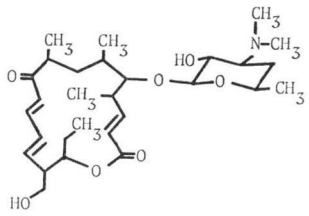
* Signal multiplicities: s, singlet; b. s, broad singlet; d, doublet; t, triplet; b. t, broad triplet; q, quartet.



3 R=H
4 R=Ac



5



6

$[\alpha]_D^{27}$ +23.8° (*c* 1.0, MeOH)] was identical with mycinolide IV and compound **6** [mp. 244~245°C, $[\alpha]_D^{27}$ +49.2° (*c* 0.5, DMSO)] was identical with demycinosyl-mycinamicin IV in their all respects[8].

Therefore, mycinamicin III is finally established as 3″-O-demethyl mycinamicin IV (**1**).

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 were measured with a JASCO auto-

matic 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 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 III (**1**)

Mycinamicin III was obtained as white amorphous powder, melted at 113~115°C, $[\alpha]_D^{25}$ −2.3° (*c* 1.0, MeOH). IR (KBr): 3460, 1710, 1675, 1645, 1625, 1590, 1165, 1070, 1040 cm^{-1}. NMR (Fig. 1). CIMS (Fig. 2). High resolution analysis of mass spectrometry (*m/z*): Calcd. for $C_{36}H_{59}NO_{11}$: 681.4085. Found: 681.4096. *Anal.* Calcd. for $C_{36}H_{59}NO_{11}$: C, 63.41; H, 8.72; N, 2.05%. Found: C, 63.25; H, 9.01; N, 2.10%.

Methanolysis of **1**

1 (800 mg) was dissolved in MeOH (10 ml) and Amberlist 15 (7 ml) was added. The solution was refluxed for 5 hours and then was filtered. The filtrate was evaporated *in vacuo*. The residue (460 mg) was chromatographed over silica gel (40 g) and eluted with benzene - acetone (4: 1) to afford a neutral methyl glycoside (75 mg). Further purification was done by distillation, followed by recrystallization from hexane - acetone to give **3** as colorless needles, mp. 98°C, $[\alpha]_D^{27}$ −81.3° (*c* 0.15, MeOH). IR (KBr): 3455, 3380, 3300, 1205, 1160, 1145, 1075, 1055, 1035 cm^{-1}. ^{1}H-NMR (Table 2). CIMS (NH_3) *m/z*: 210 ($M \cdot NH_4^+$), 178, 161. *Anal.* Calcd. for $C_8H_{16}O_5$: C, 49.99; H, 8.39%. Found: C, 50.19; H, 8.44%.

Acetylation of **3**

3 (10 mg) was dissolved in 0.2 ml pyridine and 0.2 ml acetic anhydride was added to the solution, the mixture was allowed to stand at room temperature overnight. The reaction mixture was poured into a mixture of ice and N hydrochloric acid, extracted with ethyl acetate, washed with saturated $NaHCO_3$ solution and water. The extract was concentrated *in vacuo* to give an oily substance. IR (Film): 1750, 1740, 1240, 1220, 1150, 1105, 1080, 1050 cm^{-1}. ^{1}H-NMR (Table 2). CIMS (NH_3) *m/z*: 294 ($M \cdot NH_4^+$), 277 (MH^+), 262 ($M \cdot NH_4^+$ −32), 245 (MH^+ −32).

Mild acid hydrolysis of **1**

To 3 g of **1**, 0.2 N hydrochloric acid was added until pH 2 and heated at 90°C for 4 days. The reaction mixture was adjusted to pH 9 with 10% Na_2CO_3 solution and extracted with $CHCl_3$. The extract was washed with saturated NaCl solution, dried over Na_2SO_4 and concentrated *in vacuo*. The residue (1.7 g) were chromatographed over silica gel (50 g) and eluted with $CHCl_3$ - MeOH - 28% NH_4OH (500: 10: 1) to afford **5** and **6**. **5** was recrystallized from hexane - acetone as colorless prisms (200 mg); mp. 242~243°C, $[\alpha]_D^{27}$ +23.8° (*c* 1.0, MeOH). *Anal.* Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85%. Found: C, 69.41; H, 8.97%. **6** was recrystallized from hexane - acetone as colorless needles (700 mg); mp. 244~245°C, $[\alpha]_D^{27}$ +49.2° (*c* 0.5, DMSO). *Anal.* Calcd. for $C_{29}H_{47}NO_7$: C, 66.77; H, 9.08; N, 2.68%. Found: C, 66.49; H, 9.19; N, 2.46%.

Acknowledgement

The authors wish to thank Mr. K. OHTA, Mrs. N. MATSUSHITA and Miss H. OHARA of this laboratories for microanalyses and spectral measurements.

References

1) SATOI, S.; N. MUTO, M. HAYASHI, T. FUJII & M. OTANI: Mycinamicins, new macrolide antibiotics. I. Taxonomy, production, isolation, characterization and properties. J. Antibiotics 33: 364~376, 1980
2) HAYASHI, M.; M. OHNO & S. SATOI: Structures of mycinamicins. J. C. S., Chem. Commun. 1980: 119~121, 1980
3) SUZUKI, M.; K. I. HARADA, N. TAKEDA & A. TATEMATSU: Chemical ionization mass spectra of new macrolide antibiotics, M-4365 A_2 and G_2. Heterocycles 8: 199~205, 1977

4) SUZUKI, M.; K. I. HARADA, N. TAKEDA, A. TATEMATSU, M. OHNO, H. OHARA, S. SATOI & M. HAYASHI: Chemical ionization mass spectra of new macrolide antibiotics, mycinamicins. J. Antibiotics, in preparation
5) MÜHLRADT, P.; EK. WEISS & T. REICHSTEIN: Die Cardenolide der Samen von *Antiaris toxicaria* LESCH. Isolierungen und Identifizierungen. Helv. Chim. Acta 47: 2164~2186, 1964
6) MÜHLRADT, P.; EK. WEISS & T. REICHSTEIN: Die Cardenolide der Samen von *Antiaris toxicaria* LESCH. 2. Strukturermittlungen. Ann. Chem. 685: 253~261, 1965
7) HOFFMANN, ST.; EK. WEISS & T. REICHSTEIN: Synthese von 2-O-Methyl-6-desoxy-D-allose(=D-Javose) und 4-O-Methyl-6-desoxy-D-allose. Helv. Chim. Acta 49: 2209~2218, 1966
8) HAYASHI, M.; M. OHNO, K. KINOSHITA, S. SATOI, M. SUZUKI & K. HARADA: Mycinamicins, new macrolide antibiotics. III. Isolation and structures of mycinamicin aglycones, mycinolide IV and V. J. Antibiotics 34: 346~349, 1981