MYCINAMICINS, NEW MACROLIDE ANTIBIOTICS

XIII. ISOLATION AND STRUCTURES OF NOVEL FERMENTATION PRODUCTS FROM *Micromonospora griseorubida* (FERM BP-705)

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(Received for publication June 3, 1991)

Novel 16-membered macrolide antibiotics, mycinamicins IX, XII, XIII, XIV, XV, XVI, XVII and XVIII have been isolated from the culture filtrate of *Micromonospora griseorubida* (FERM BP-705). Fermentation, isolation, structure determination and biosynthetic consideration of these mycinamicin analogs are described.

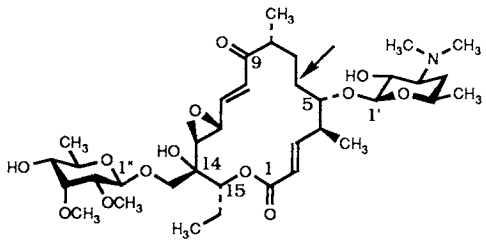
The mycinamicins are 16-membered macrolide antibiotics produced by *Micromonospora griseorubida* (FERM BP-705), which have strong antibacterial activity against Gram-positive bacteria[1]. The complex consists of ten components; mycinamicins I (**1**), II (**2**), III (**3**), IV (**4**), V (**5**), VI (**6**), VII (**7**), VIII (**8**), X (**10**) and XI (**11**)[1~6]. The first intermediate of the biosynthesis of mycinamicins aglycon is protomycinolide IV (**19**)[7], which is assembled from three acetates and five propionates, as shown by labeling studies with radioactive precursors[8]. Recently, we reported on the isolation and chemical structure of mycinonic acids, considered to be biosynthetic intermediates of chain elongation into protomycinolide IV (**19**)[9,10], and proposed biosynthetic pathway before the formation of the lactone **19**. Moreover, we reported the biosynthetic pathway from **19** to **2** from an analysis of bioconversion studies[11]. During the successive search for a new macrolide antibiotic, which is more polar component rather than **2**, we have discovered novel minor components designated mycinamicins IX (**9**), XII (**12**), XIII (**13**), XIV (**14**), XV (**15**), XVI (**16**), XVII (**17**) and XVIII (**18**) from the fermentation broth of mycinamicin-producing strains of *M. griseorubida* (FERM BP-705). Compound **9** was identical with compound V (a minor component of AR-5 from *Micromonospora* sp.) which was isolated by Schering-Plough Corporation's researchers[12]. These compounds are interesting new fermentation products in the biosynthesis of the mycinamicins. In this report we describe the isolation and structural elucidation of these new compounds and discuss their possible roles in mycinamicin biosynthesis.

Results and Discussion

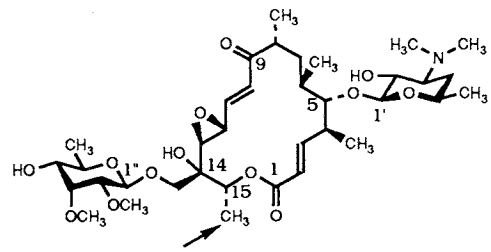
Structure Determination

The physico-chemical properties of compounds **9**, **12**, **13**, **14**, **15**, **16**, **17** and **18** are given in Table 1. The molecular formulas of these compounds were established by HRCI-MS or HRFAB-MS. The ^{13}C NMR spectral data for these mycinamicins are shown in Table 2. The assignments were made on the basis of the ^{1}H-^{13}C chemical shifts correlated with the 2D NMR experiments.

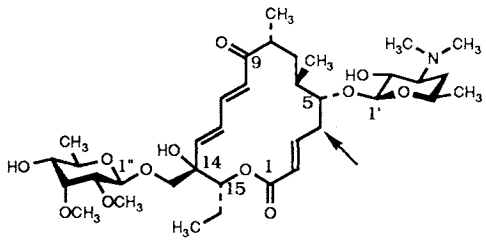
Fig. 1. Structures of mycinamicins IX (**9**), XII (**12**), XIII (**13**), XIV (**14**), XV (**15**), XVI (**16**), XVII (**17**) and XVIII (**18**).



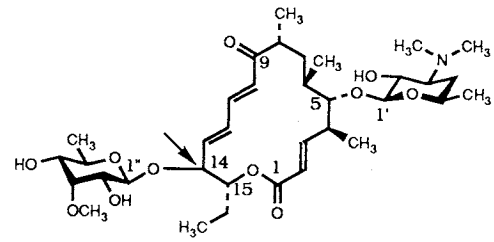
Mycinamicin XII (**12**)



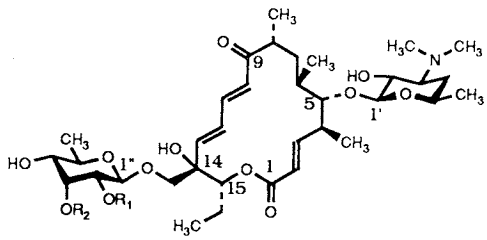
Mycinamicin XIII (**13**)



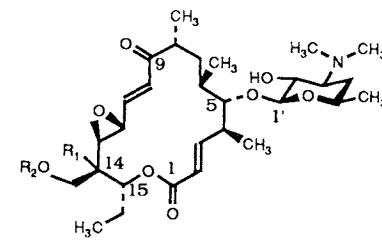
Mycinamicin XIV (**14**)



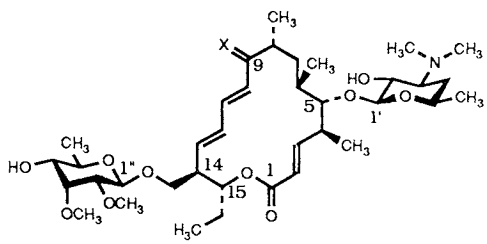
Mycinamicin XVII (**17**)



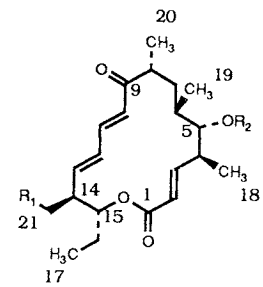
Mycinamicin IX (**9**) $R_1=CH_3$ $R_2=H$
Mycinamicin XV (**15**) $R_1=H$ $R_2=H$
Mycinamicin V (**5**) $R_1=CH_3$ $R_2=CH_3$



Mycinamicin XVIII (**18**) $R_1=H$ $R_2=H$
Mycinamicin I (**1**) $R_1=H$ $R_2=$Mycinose
Mycinamicin II (**2**) $R_1=OH$ $R_2=$Mycinose



Mycinamicin XVI (**16**) X=H, OH
Mycinamicin IV (**4**) X=O



Protomycinolide IV (**19**) $R_1=H$ $R_2=H$
Mycinamicin VII (**7**) $R_1=OH$ $R_2=$Desosamine
Mycinamicin VIII (**8**) $R_1=H$ $R_2=$Desosamine

Table 1. Physico-chemical properties of mycinamicins IX (**9**), XII (**12**), XIII (**13**), XIV (**14**), XV (**15**), XVI (**16**), XVII (**17**) and XVIII (**18**).

Compound	**9**	**12**	**13**	**14**
Formula	$C_{36}H_{59}NO_{12}$	$C_{36}H_{59}NO_{13}$	$C_{36}H_{59}NO_{13}$	$C_{36}H_{59}NO_{12}$
HRCI-MS (m/z, $(M+H)^+$)	698.4113[a]	714.4081	714.4078	698.4113
Calcd:	698.4116	714.4065	714.4065	698.4116
$[\alpha]$ (c, MeOH)	+14.5° (1.00)	−27.3° (0.55)	−27.7° (0.48)	−7.5° (0.37)
UV λ_{max}^{MeOH} nm (ε)	215 (4.35), 280 (4.34)	215 (4.34), 240 (4.04)	217 (4.30), 243 (4.00)	215 (4.30), 280 (4.29)
IR (KBr) cm^{-1}	3450, 1720, 1680, 1645, 1635	3460, 1720, 1695, 1655, 1630	3460, 1715, 1680, 1655, 1635, 1595	3460, 1720, 1680, 1655, 1635, 1595
HPLC Rt (minutes)	3.65	3.72	4.18	5.52
TLC Rf[b]	3.65	0.40	0.38	0.34

Compound	**15**	**16**	**17**	**18**
Formula	$C_{35}H_{57}NO_{12}$	$C_{37}H_{63}NO_{11}$	$C_{35}H_{57}NO_{11}$	$C_{29}H_{49}NO_8$
HRCI-MS (m/z, $(M+H)^+$)	684.3970[a]	698.4484[a]	668.4006	538.3314[a]
Calcd:	684.3959	698.4489	668.4010	538.3380
$[\alpha]$ (c, MeOH)	—	−25.8° (1.00)	—	—
UV λ_{max}^{MeOH} nm (ε)	215 (4.32), 280 (4.30)	218 (4.51), 232 (4.37)	215 (4.28), 280 (4.25)	217 (4.38), 240 (4.19)
IR (KBr) cm^{-1}	3450, 1710, 1680, 1650, 1595	3450, 1715, 1660	3450, 1715, 1695, 1655, 1625	3450, 1710, 1690, 1655, 1625
HPLC Rt (minutes)	3.07	58.13	5.72	4.55
TLC Rf[b]	0.12	0.36	0.42	0.55

—: Insufficient material available.

[a] HRFAB-MS, $(M+H)^+$.

[b] Solvent system: $CHCl_3$-MeOH-28% NH_4OH (150:10:1).

Structure of Mycinamicin IX (**9**)

The protonated molecular ion (m/z 698) appeared at 14 mass units lower than the corresponding ion of **5** (m/z 712) in the CI-MS. The UV spectrum suggested the presence of α,β-unsaturated lactone (215 nm) and $\alpha,\beta,\gamma,\delta$-unsaturated ketone (280 nm). The structure was established by comparing the ^{13}C NMR spectral data of **5** and **9**. This data of **14** are very similar to that for **5**. However, the lack of the 3″-OCH_3 signal at δ_C 61.7 (q) in **5** was observed. Accordingly, the compound **9** was identified as 3″-*O*-demethyl-mycinamicin IX.

Structure of Mycinamicin XII (**12**)

The UV spectrum suggested the presence of α,β-unsaturated lactone (215 nm) and γ,δ-epoxy-α,β-unsaturated ketone (240 nm). The protonated molecular ion (m/z 714) appeared at 14 mass units lower than the corresponding ion of **2** (m/z 728) in the CI-MS. The ^1H NMR spectrum ($CDCl_3$) of **12** was very similar to that of **2**. However, the 19-CH_3 signal observed at δ_H 1.01 (3H, d) in **2** disappeared, while a new methylene signal appeared at δ_H 1.05 (1H, m) and 1.66 (1H, m), in **12**. The structure was further confirmed by comparing the ^{13}C NMR spectral data of **2** and **12**. The 19-CH_3 carbon of **2** at δ_C 17.6 (q) disappeared and instead the 6-CH_2 carbon appeared at upfield of δ_C 32.2 (t) in **12**. From these results, the compound **12** was identified as 19-normycinamicin II.

Structure of Mycinamicin XIII (**13**)

The protonated molecular ion (m/z 714) appeared at 14 mass units lower than the corresponding ion

Table 2. ^{13}C NMR chemical shifts ($CDCl_3$: δ[a]) of mycinamicins IX (**9**), XII (**12**), XIII (**13**), XIV (**14**), XV (**15**), XVI (**16**), XVII (**17**), XVIII (**18**), I (**1**), II (**2**), IV (**4**) and V (**5**).

Carbon	**9**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**1**	**2**	**4**	**5**
1	166.4 (s)	166.1	165.4	166.1	166.6	166.8	165.9	165.7	166.7 (s)	165.9	166.1	166.3
2	121.1 (d)	120.2	120.3	123.1	120.7	120.9	120.5	120.1	120.1 (d)	120.0	120.9	120.7
3	151.9 (d)	151.8	151.7	145.2	152.1	151.7	152.2	151.9	151.5 (d)	151.9	151.6	151.8
4	41.3 (d)	41.0	42.0	32.9 (t)	41.3	40.0	41.3	42.0	41.9 (d)	42.0	41.3	41.3
5	87.9 (d)	82.5	87.3	80.9	87.7	85.8	87.8	87.5	87.5 (d)	87.5	87.9	87.7
6	34.4 (d)	32.2 (t)	34.2	33.5	34.1	35.2	34.1	34.3	34.2 (d)	34.4	34.1	34.1
7	33.2 (t)	25.8	32.0	32.5	32.7	33.5	29.7	32.0	32.1 (t)	32.0	32.6	32.7
8	44.8 (d)	48.3	44.7	44.9	44.8	37.6	44.8	44.7	44.7 (d)	44.6	44.9	44.8
9	203.4 (s)	201.5	201.0	204.0	204.4	76.5 (d)	203.7	201.0	200.8 (s)	200.8	203.4	203.8
10	124.6 (d)	126.8	126.1	124.4	124.0	133.1	124.2	126.0	125.6 (d)	126.2	123.2	123.8
11	141.3 (d)	142.9	143.2	141.4	141.5	128.7	141.0	143.5	143.7 (d)	143.0	141.7	141.4
12	130.6 (d)	54.1	53.9	130.5	130.9	132.9	130.7	59.5	59.6 (d)	60.4	133.0	130.5
13	143.3 (d)	60.5	60.4	143.5	143.5	131.0	140.9	58.5	59.0 (d)	54.0	141.3	143.5
14	77.6 (s)	72.5	72.8	77.5	77.7	48.8 (d)	83.9 (d)	48.8 (d)	47.5 (d)	73.1 (s)	49.2 (d)	77.4 (s)
15	76.1 (d)	74.7	69.5	75.9	75.8	74.5	74.0	71.9	72.4 (d)	74.2	72.7	75.8
16	21.6 (t)	21.4	14.0 (q)	21.5	21.5	25.4	24.5	25.0	24.7 (t)	21.2	25.3	21.4
17	10.4 (q)	10.1	—	10.4	10.4	9.7	9.7	8.9	8.9 (q)	10.1	9.6	10.4
18	19.5 (q)	18.2	18.6	—	19.5	18.9	19.4	18.8	18.9 (q)	19.0	19.4	19.5
19	17.4 (q)	—	17.0	17.8	17.4	17.1	17.4	17.1	17.1 (q)	17.1	17.4	17.4
20	17.6 (q)	17.1	17.5	16.7	17.5	19.3	17.8	17.4	17.5 (q)	17.4	17.8	17.6
21	74.7 (t)	73.2	72.5	75.2	75.7	69.6	—	61.5	67.1 (t)	72.6	68.6	75.2
1′	105.2 (d)	105.9	105.0	102.4	104.8	104.9	104.8	105.1	105.5 (d)	105.0	104.9	104.8
2′	70.3 (d)	70.1	70.4	69.7	70.4	70.4	70.3	70.4	70.3 (d)	70.3	70.4	70.4
3′	66.2 (d)	65.7	65.9	65.8	65.8	65.7	65.9	65.9	65.8 (d)	65.8	65.8	65.8
4′	28.9 (t)	28.4	28.3	28.6	28.3	28.5	28.5	28.3	28.5 (t)	28.5	28.3	28.4
5′	69.5 (d)	69.6	69.5	69.6	69.4	69.5	69.5	69.6	69.4 (d)	69.4	69.5	69.4
6′	21.2 (q)	21.3	21.1	21.2	21.1	21.2	21.1	21.2	21.2 (q)	21.2	21.2	21.2
$N(CH_3)_2$	40.4 (q)	40.3	40.2	40.3	40.2	40.3	40.3	40.3	40.1 (q)	40.2	40.2	40.2
1″	101.4 (d)	101.4	101.3	101.7	101.7	101.1	101.3		100.9 (d)	101.2	101.0	101.6
2″	80.3 (d)	82.0	82.0	81.8	71.0	81.8	72.9		81.9 (d)	81.9	81.9	81.7
3″	70.9 (d)	79.2	79.2	79.2	70.4	79.9	80.6		79.3 (d)	79.3	79.9	79.2
4″	72.9 (d)	72.8	72.8	72.5	72.4	72.7	72.8		72.7 (d)	72.6	72.7	72.5
5″	70.9 (d)	70.9	70.8	70.9	70.7	70.6	70.9		70.6 (d)	70.7	70.5	70.7
6″	17.6 (q)	17.7	17.6	17.6	17.6	17.8	17.7		17.8 (q)	17.7	17.8	17.6
2″-OCH_3	58.9 (q)	59.4	59.3	59.2		59.8	—		59.0 (q)	59.3	59.7	59.1
3″-OCH_3		61.7 (q)	61.7	61.8		61.7	62.1		61.6 (q)	61.6	61.7	61.7

[a] 100 MHz ^{13}C NMR spectrum in $CDCl_3$ with solvent reference at 77.02 ppm. Assignments were made on the basis of ^1H-^{13}C chemical shift correlated 2D NMR.

of **2** (*m/z* 728) in the CI-MS. The presence of α,β-unsaturated lactone and γ,δ-epoxy-α,β-unsaturated ketone were suggested by the UV absorption maximum at 217 and 243 nm. In the ^{1}H NMR spectrum the 17-CH_3 signal observed at δ_H 0.89 (3H, t) in **2** disappeared, while a new methyl signal appeared at δ_H 1.00 (3H, d) in **13**. The ^{13}C NMR spectrum also supported these data. It was further observed by DEPT experiments that the triplet of 16-CH_2 in **2** is absent in the spectrum of **13** and is replaced by a quartet signal at δ_C 14.0 (q), thus indicating the loss of the 17-CH_3. Accordingly, the compound **13** was identified as 17-normycinamicin II.

Structure of Mycinamicin XIV (**14**)

The UV spectrum suggested the presence of α,β-unsaturated lactone (215 nm) and $\alpha,\beta,\gamma,\delta$-unsaturated ketone (280 nm). The compound **14** gave a CI-MS spectrum with the protonated molecular ion at *m/z* 698 and fragment ions at *m/z* 158 (desosamine) and 175 (mycinose) suggesting that it corresponds to mycinamicin V (**5**) lacking a methyl group (14 mass units) in the aglycon. The structure was further confirmed by comparing the ^{1}H and ^{13}C NMR spectral data of **5** and **14**. These data of **14** are very similar to those for compound **5**. However, the 18-CH_3 signal observed at δ_H 1.22 (3H, d) in **5** disappeared, while a new methylene signal appeared at δ_H 2.57 (2H, m) in **14**. The structure was further confirmed by comparing the ^{13}C NMR spectral data of **5** and **14**. The 18-CH_3 carbon of **5** at δ_C 18.2 (q) disappeared and instead the 4-CH_2 carbon appeared at upfield of δ_C 32.9 (t) in **14**. Therefore the compound **14** was identified as 18-normycinamicin V.

Structure of Mycinamicin XV (**15**)

The protonated molecular ion (*m/z* 684) appeared at 28 mass units lower than the corresponding ion of **5** (*m/z* 712) in the CI-MS. The UV spectrum suggested the presence of α,β-unsaturated lactone and $\alpha,\beta,\gamma,\delta$-unsaturated ketone. In the ^{1}H and ^{13}C NMR spectra, the signals of olefine and *N*-dimethyl were observed, but signals of two *O*-methyl groups which were characteristic for mycinose were not recognized. The result suggested the lack of two *O*-methyl carbon in **15**. Accordingly, the compound **15** was identified as 2″,3″-*O*-didemethyl-mycinamicin V.

Structure of Mycinamicin XVI (**16**)

The UV spectrum suggested the presence of α,β-unsaturated lactone (218 nm) and $\alpha,\beta,\gamma,\delta$-diene (232 nm). The IR spectrum showed hydroxyl group (3460 cm^{-1}) and α,β-unsaturated lactone (1715, 1655 cm^{-1}), but the absorption of $\alpha,\beta,\gamma,\delta$-unsaturated ketone moiety which were characteristic for mycinamicin IV (**4**) was not observed in **16**. In the CI-MS spectrum of **16**, the protonated molecular ion (*m/z* 698) appeared at two mass units upper than the corresponding ion of **4** (*m/z* 696). In the ^{1}H NMR spectrum, the olefin proton signals were observed at δ_H 5.74 (1H, d, 2-H), 6.75 (1H, dd, 3-H), 5.75 (1H, dd, 10-H), 6.17 (1H, dd, 11-H), 6.00 (1H, dd, 12-H) and 5.55 (1H, dd, 13-H) and a mutiplet at δ_H 3.92 (1H, m, 9-H) which are not observed in the spectrum of **4** was observed in **16**, indicating dihydrogenation of the 9-ketone carbonyl moiety of **4**. The structure was further confirmed by comparing the ^{13}C NMR spectral data of **4** and **16**. The 9-ketone carbonyl carbon of **4** at δ_C 203.6 (s) disappeared, while a new hydroxyl methine signal appeared at upfield of δ_C 76.5 (d) in **16**. Thus, the compound **16** was determined to be 9,9-dihydro-mycinamicin IV.

Structure of Mycinamicin XVII (**17**)

The protonated molecular ion (*m/z* 668) appeared at 28 mass units lower than the corresponding ion of **4** (*m/z* 696) in the CI-MS. The UV spectrum suggested the presence of α,β-unsaturated lactone (215 nm) and $\alpha,\beta,\gamma,\delta$-unsaturated ketone (280 nm). The structure was established by comparing the ^1H and ^{13}C NMR spectral data of **4** and **17**. The lack of the 2″-OCH_3 signal at δ_H 3.51 (3H, s) in **4** and the 21-CH_2 signal at δ_H 4.04 (1H, dd) and 3.52 (1H, dd) were observed in the ^1H NMR spectrum. In the ^{13}C NMR spectrum, the 21-CH_2 carbon of **4** at δ_C 68.6 (t) disappeared, and the 14-CH carbon of **4** at δ_C 49.2 (d) appeared at downfield of δ_C 83.9 (d) in **17**. These spectra of **17** showed that the neutral sugar (2″-*O*-demethyl-mycinose) was directly attached to the 14-CH carbon instead of the 21-CH_2 carbon of **4**. From these results, the compound **17** was concluded to be 2″-*O*-demethyl-21-normycinamicin IV.

Structure of Mycinamicin XVIII (**18**)

The presence of α,β-unsaturated lactone and γ,δ-epoxy-α,β-unsaturated ketone suggested by the UV absorption maximum at 217 and 240 nm. The compound **18** gives the CI-MS spectrum with the protonated molecular ion and *m/z* 538 suggesting the demycinosyl analog of **1**. The presence of desosamine was shown by the fragment ion at *m/z* 158, and by the characteristic $N(CH_3)_2$ signal at δ_H 2.32 in its ^1H NMR spectrum. Absence of the neutral sugar was indicated by the lack of typical fragment ion at *m/z* 175, and also by the absence of the corresponding signals in the ^1H NMR spectrum (*e.g.* no anomeric proton and two OCH_3 signals at δ_H 4.57 (1H, d, 1″-H), 3.51 (3H, s, 2″-OCH_3) and 3.60 (3H, s, 3″-OCH_3)). Thus, the compound **18** was determined to be 21-*O*-demycinosylmycinamicin I. As final proof, compound **18** was prepared from mycinamicin VII (**7**) according to the epoxydation with *m*-chloroperbenzoic acid in chloroform.

Biosynthetic Consideration

In summary, we isolated eight minor components of mycinamicins from the fermentation broth of *M. griseorubida* and their structures were confirmed in this study. The physico-chemical properties and NMR spectra of mycinamicins XII (**12**), XIII (**13**), XIV (**14**) and XVII (**17**) clearly indicated that the structures of these antibiotics differed from the other mycinamicins by the lack of a *C*-methyl group in the aglycon. These normycinamicins are very interesting with respect to the biosynthesis of macrolide antibiotics. Mycinamicin aglycon is formed by three acetates and five propionates. If an acetate instead of propionate is incorporated as the chain assembly unit, the biosynthesis of these normycinamicins may be easily rationalized within the polyketide chain elongation process in the mycinamicin biosynthesis.

From the bioconversion study (Table 3), mycinamicins IX (**9**), XV (**15**) and XVIII (**18**) were efficiently converted to mycinamicins I (**1**) or II (**2**) by the macrolide-non-producing mutant C-34-10 of *M. griseorubida* (FERM BP-705). These results

Table 3. Bioconversion pattern of mycinamicin II (**2**)-like compounds.

Compound	Bioconversion efficiencies (%)[a]		
	1	**2**	**5**
Mycinamicin I (**1**)	66.1	3.7	—[b]
Mycinamicin II (**2**)	—	92.8	—
Mycinamicin III (**3**)	7.8	61.9	—
Mycinamicin IV (**4**)	13.6	83.0	—
Mycinamicin V (**5**)	—	70.2	8.6
Mycinamicin VI (**6**)	10.7	66.8	—
Mycinamicin VII (**7**)	9.4	54.0	—
Mycinamicin VIII (**8**)	8.8	55.8	—
Mycinamicin IX (**9**)	—	67.6	—
Mycinamicin XV (**15**)	—	36.7	—
Mycinamicin XVIII (**18**)	25.2	3.4	—

[a] The percentages were based upon recovered mycinamicin II-like compound.
—: Not detected.

Table 4. Antibacterial spectra of mycinamicins IX (**9**), XII (**12**), XIII (**13**), XIV (**14**), XV (**15**), XVI (**16**), XVII (**17**) and XVIII (**18**).

Test organism	MIC (μg/ml)							
	9	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Staphylococcus aureus FDA 209P JC-1	0.78	0.39	0.10	0.39	6.25	0.20	3.13	1.56
S. aureus MS353	1.56	0.78	0.20	0.39	6.25	0.39	3.13	1.56
S. epidermidis sp-al-1	0.78	0.39	0.20	0.20	12.5	0.39	3.13	1.56
Streptococcus pyogenes N.Y. 5	0.78	0.20	0.05	0.20	3.13	0.20	0.78	1.56
Micrococcus luteus ATCC 9341	0.20	0.10	0.10	0.39	1.56	0.05	0.20	0.39
Corynebacterium diphtheriae P.W. 8	3.13	3.13	0.39	3.13	1.56	6.25	3.13	0.39
Bacillus subtilis ATCC 6633	1.56	3.13	0.39	0.78	25	6.25	0.39	1.56
Escherichia coli NIHJ JC-2	>100	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa IAM 1095	>100	>100	>100	>100	>100	>100	>100	>100

suggested that these compounds **9**, **15** and **18** were biosynthetic precursors for mycinamicins. Although not established experimentally, the structure elucidation suggested that mycinamicin XVI (**16**) might be a shunt metabolite.

Antimicrobial Activity

The antibacterial activity (MIC) of mycinamicins IX (**9**), XII (**12**), XIII (**13**), XIV (**14**), XV (**15**), XVI (**16**), XVII (**17**) and XVIII (**18**) is shown in Table 4.

Experimental

General Procedure

The IR spectra were taken with a Hitachi 260-50 IR spectrophotometer. The UV spectra were recorded on a Shimadzu UV-365 spectometer. The NMR spectra were obtained with a Jeol JNM-GSX400 spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C) with TMS as an internal reference. The mass spectra were taken with a Jeol JMS-SX102 spectrometer. Analytical HPLC was carried out with a Shimadzu LC-6A system, a YMC-gel ODS 5 μm, stainless steel column (Yamamura Chemical Institute, Ltd., Kyoto), 150 mm × 4 mm i.d. Flow rate of mobile phase (0.1 M NaH_2PO_4-methanol-acetonitrile, 55:31:14) was 0.8 ml/minute and operated at 40°C.

Fermentation

The fermentation of the mycinamicin-producing strain *M. griseorubida* (FERM BP-705) was carried out at 27°C for 7 days under aeration at a rate of 20 liters per minute and agitation at 300 rpm in a 30-liter jar fermenter containing 20 liters of production medium (dextrin 7.0%, glucose 0.5%, cotton meal 2.5%, soybean meal 0.5%, $CaCO_3$ 0.5%, $MgSO_4 \cdot 7H_2O$ 0.4%, K_2HPO_4 0.1%, $CoCl_2 \cdot 6H_2O$ 0.0002%, adjusted to pH 7.0). The medium was inoculated with 5.0% of its volume of a seed culture prepared as follows. The organism was first cultured for 2 days at 30°C on a rotatory shaker in a 150-ml Erlenmeyer flask containing 20 ml of a seed medium (dextrin 1.0%, glucose, 1.0%, Casamino acids 2.5%, yeast extract 0.5%, $CaCO_3$ 0.1%, adjusted to pH 7.0) and the culture (1.0%) was then inoculated into 1 liter of the seed medium in a 5-liter round flask and cultured for 2 days at 30°C on a rotary shaker.

Isolation and Purification

The culture filtrate (17 liters) of mycinamicin-producing strains of *M. griseorubida* (FERM BP-705) was extracted at pH 9.0 with equal volumes of EtOAc. The mycinamicins in the organic extract were transferred to a dilute hydrochloric acid solution (pH 3.0). The acidic aqueous layer was extracted with

$CHCl_3$ at pH 9.0 and this organic extract was concentrated to afford the mycinamicins as a crude powder (*ca.* 9.6 g). The crude mycinamicin complex was dissolved in a small amount of $CHCl_3$ and subjected to silica gel column chromatography. The elution was monitored by TLC on silica gel $60GF_{254}$ plate using $CHCl_3$ - MeOH - 28% ammonia (150 : 10 : 1) system and conc sulfuric acid for detection. The column was eluted sequentialy with $CHCl_3$ - MeOH - 28% ammonia (500 : 10 : 1) for mycinamicin VIII (**8**), $CHCl_3$ - MeOH - 28% ammonia (300 : 10 : 1) for mycinamicins I (**1**)~VII (**7**), and $CHCl_3$ - MeOH - 28% ammonia (100 : 10 : 1) for mycinamicins IX (**9**), XII (**12**), XIII (**13**), XIV (**14**), XV (**15**), XVI (**16**), XVII (**17**) and XVIII (**18**). However, the separation of these compounds was difficult on account of their similar mobilities but was purified by preparative HPLC (YMC-gel ODS S-5, 300 mm × 20 mm i.d.) using 0.1 M NaH_2PO_4 (pH 2.5, adjusted with 20% H_3PO_4) - methanol (6 : 4) as solvent system with detection at 220 nm. Fractions (20 ml) were collected at a flow rate of 10 ml/minute. Individual fractions were assayed by analytical HPLC. Their each fractions were collected and combined, and the MeOH was removed *in vacuo*. The aqueous solutions were extracted with EtOAc at pH 9.0. The EtOAc extract was dried (Na_2SO_4) and concd *in vacuo*. The yield of the eight components **9**, **12**, **13**, **14**, **15**, **16**, **17** and **18** from 17 liters of the culture filtrate was 12 mg, 15 mg, 8 mg, 17 mg, 11 mg, 8 mg, 11 mg and 9 mg, respectively.

Preparation of Mycinamicin XVIII (**18**) from Mycinamicin VII (**7**)

To a solution of mycinamicin VII (**7**, 1 g) in $CHCl_3$ (20 ml) was added dropwise with stirring at 5°C a solution of *m*-chloroperbenzoic acid (purity 70%, 870 mg) in $CHCl_3$ (15 ml). After the addition was complete, the reaction mixture was allowed to stand in the dark for overnight at room temperature and then EtOH (20 ml) and sodium hydrosulfite (934 mg) were added at 5°C. Excess peracid in the reaction solution was decomposed with 10% Na_2SO_4 aqueous solution (30 ml × 2) and the $CHCl_3$ layer was washed with 5% $NaHCO_3$ aqueous solution (30 ml), and then with H_2O (30 ml). After drying the organic layer over anhydrous Na_2SO_4, the $CHCl_3$ layer was concd to dryness *in vacuo*. The crude residue was chromatographed over silica gel and elution with $CHCl_3$ - MeOH - 28% ammonia (500 : 10 : 1) afforded 430 mg as powder. Physico-chemical properties and NMR spectra are identical with those of **18**, isolated from the culture filtrate of mycinamicin-producing strain.

Bioconversion of Mycinamicins IX (**9**), XV (**15**) and XVIII (**18**)

M. griseorubida (FERM BP-705) mutant C-34-10, which produces potential intermediate for formation of protomycinolide IV (**19**), could make mycinamicin II (**2**) if provided with protomycinolide IV (**19**) or other macrolide intermediates of mycinamicins[11]. Mycinamicins IX (**9**), XV (**15**) and XVIII (**18**) were separately added to the 48-hour-old culture of mutant C-34-10 at 100 μg/ml and cultivation was continued for an additional 120 hours. After mycelia were removed, the filtrate were analyzed by HPLC.

Acknowledgments

The authors wish to thank Mr. H. AONO for spectral measurements, Mr. S. YAMAJI for the antimicrobial spectra.

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