Communications to the Editor

MYCINAMICINS, NEW MACROLIDE ANTIBIOTICS

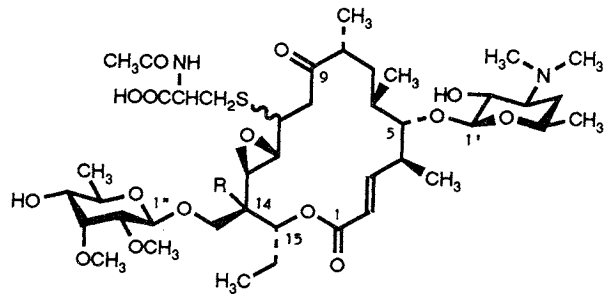
XII. ISOLATION AND STRUCTURAL ELUCIDATION OF MYCINAMICINS X AND XI

Sir:

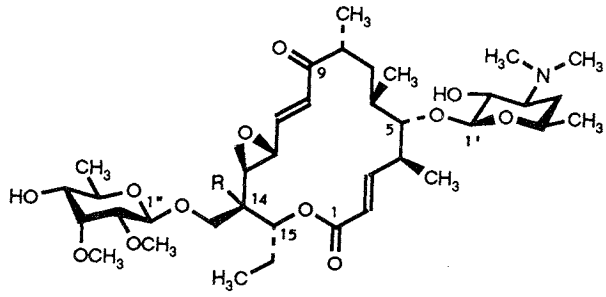
The mycinamicins are 16-membered macrolide antibiotics produced by *Micromonospora griseorubida*, which have strong antibacterial activity against Gram-positive bacteria[1~5]. In the course of search for new antibiotics from the culture filtrates of mycinamicin-producing strains, we have discovered four new minor components designated mycinamicins X (**1**), X′ (**2**), XI (**3**) and XI′ (**4**). From physico-chemical properties, spectroscopic data and chemical degradation studies, these compounds were shown to be analogs of mycinamicins I (**5**) and II (**6**), whose structures are different from compounds **5** and **6** at the C-11 position. In this paper, we describe the isolation and structural elucidation of these compounds.

The culture filtrates (200 ml) of mycinamicin-producing strains of *M. griseorubida* were extracted with $CHCl_3$ at pH 7.5. The organic layer contained mycinamicins. The aqueous layer was adjusted to pH 3.0 with 1 N hydrochloric acid. After this solution was adsorbed on Diaion HP-20 resin (100 ml), the resin was washed with water, and then with 30% aq MeOH. The active components were eluted with 80% aq MeOH. The eluate was concentrated *in vacuo* to give a crude powder. The crude residue was purified by preparative HPLC (YMC-gel ODS I-40/64, 300 mm × 20 mm i.d.) using 0.1 M NaH_2PO_4-MeOH (6:4) and (5:5) as solvent system with detection on 220 nm. Fractions (20 ml) were collected at a flow rate of 10 ml/minute. Individual fractions were assayed by analytical HPLC. Each fraction was collected and combined, and the

Fig. 1. Structures of mycinamicins X (**1**), X′ (**2**), XI (**3**), XI′ (**4**), I (**5**) and II (**6**).



Mycinamicin X (**1**) R = H
Mycinamicin X′ (**2**) R = H
Mycinamicin XI (**3**) R = OH
Mycinamicin XI′ (**4**) R = OH



Mycinamicin I (**5**) R = H
Mycinamicin II (**6**) R = OH

Table 1. Physico-chemical properties of mycinamicins X (**1**), X′ (**2**), XI (**3**), and XI′ (**4**).

	1	**2**	**3**	**4**
Formula	$C_{42}H_{70}N_2O_{15}S$	$C_{42}H_{70}N_2O_{15}S$	$C_{42}H_{70}N_2O_{16}S$	$C_{42}H_{70}N_2O_{16}S$
MW	875.08	875.08	891.08	891.08
MP (°C)	141～143	149～151	155～157	165～167
$[\alpha]_D$ (*c* 1.0, MeOH)	−56.1°	−48.6°	−44.2°	−30.2°
UV λ_{max}^{MeOH} nm (log ε)	212 (4.18)	212 (4.19)	212 (4.23)	212 (4.19)
IR ν_{max} (KBr disk) cm^{-1}	3410, 1710, 1655, 1605	3400, 1705, 1655, 1600	3415, 1710, 1655, 1605	3400, 1710, 1655, 1600

Table 2. ^{13}C NMR chemical shifts (CD_3OD; δ) for mycinamicins X (**1**), X′ (**2**), XI (**3**), XI′ (**4**), I (**5**) and II (**6**).

Carbon	**1**	**2**	**5**	**3**	**4**	**6**
1	168.3 (s)	168.5	168.2	167.8	168.1	168.1
2	122.7 (d)	122.8	122.4	122.0	122.0	121.8
3	153.9 (d)	154.1	153.7	153.8	153.9	153.4
4	42.1 (d)	43.7	43.5	41.9	43.2	43.1
5	87.7 (d)	88.7	88.4	87.1	88.4	88.1
6	37.4 (d)	36.5	36.3	36.8	36.2	36.1
7	32.4 (t)	32.4	32.4	31.9	31.9	32.2
8	46.9 (d)	48.0	46.8	46.3	47.4	46.4
9	214.1 (s)	214.3	203.9	213.5	213.4	203.7
10	44.0 (t)	41.1 (t)	128.5 (d)	43.2 (t)	43.2 (t)	127.9 (d)
11	42.6 (d)	47.8 (d)	145.6 (d)	41.6 (d)	40.5 (d)	145.3 (d)
12	56.8 (d)	57.1	61.0	58.6	61.6	55.5
13	63.2 (d)	61.4	60.6	58.8	63.5	63.0
14	46.6 (d)	48.1 (d)	49.7 (d)	74.4 (s)	74.7 (s)	74.4 (s)
15	75.7 (d)	75.5	74.9	77.3	77.0	76.2
16	27.1 (t)	26.5	26.4	23.1	22.9	22.0
17	10.8 (q)	9.8	10.4	11.2	11.0	10.8
18	18.8 (q)	19.0	20.3	18.6	18.6	19.9
19	18.5 (q)	18.7	18.6	18.0	18.4	18.0
20	18.1 (q)	18.7	18.6	17.6	18.3	18.0
21	69.2 (t)	69.0	68.9	72.9	72.2	72.3
1′	106.5 (d)	106.4	107.0	105.9	105.7	106.6
2′	70.3 (d)	70.3	70.8	69.7	69.6	72.5
3′	67.7 (d)	67.8	66.7	67.3	67.4	66.2
4′	35.2 (t)	34.0	33.7	34.5	33.2	33.2
5′	71.4 (d)	71.6	72.8	70.7	70.7	70.4
6′	22.1 (q)	22.0	22.3	21.6	21.5	21.8
$N(CH_3)_2$	41.0 (q)	41.1	41.7	40.5	40.5	41.2
1″	102.7 (d)	103.0	102.9	102.7	102.6	102.6
2″	83.6 (d)	83.8	83.5	83.1	83.2	83.1
3″	82.1 (d)	82.3	82.1	81.4	81.6	81.6
4″	75.4 (d)	75.3	75.3	74.8	74.9	74.8
5″	71.9 (d)	71.9	71.7	71.5	71.4	71.4
6″	19.0 (q)	19.5	19.0	18.4	19.3	18.5
2″-OCH_3	60.1 (q)	60.3	60.3	59.6	59.9	59.8
3″-OCH_3	62.9 (q)	62.9	62.9	62.4	62.4	62.4
1‴	37.0 (t)	37.6		36.8	37.2	
2‴	56.8 (d)	57.1		56.3	56.3	
3‴	177.6 (s)	177.7		177.0	176.6	
4‴	173.5 (s)	173.5		173.0	173.1	
5‴	23.9 (q)	24.0		23.4	23.6	

MeOH was removed *in vacuo*. The aqueous residues were adsorbed on Diaion HP-20 resins, respectively. Then the resin was washed with water, and eluted with MeOH. The eluates were concentrated *in vacuo*. Each component was obtained as a white amorphous powder. The yield of four components **1**, **2**, **3** and **4** from 200 ml of the culture filtrate was 42 mg, 12 mg, 35 mg and 18 mg, respectively.

The physico-chemical properties of mycinamicins X (**1**), X′ (**2**), XI (**3**) and XI′ (**4**) are summarized in Table 1. The ^{13}C NMR spectral data for these compounds are shown in Table 2.

The molecular formula of mycinamicin X (**1**) was determined to be $C_{42}H_{70}N_2O_{15}S$ based on its FAB-MS spectrum ($(M+H)^+$, *m/z* 875) and elemental analysis (Calcd: C 56.48, H 8.12, N 3.13, S 3.58. Found: C 56.51, H 8.23, N 3.11, S 3.44). The UV spectrum suggested the presence of an α,β-unsaturated lactone (212 nm). The IR spectrum also showed the presence of an α,β-unsaturated lactone (1710, 1655 cm^{-1}) and hydroxyl function (3410 cm^{-1}). In the FAB-MS spectrum of **1**, the protonated molecular ion (*m/z* 875) appeared at 163 mass units higher than the corresponding ion of mycinamicin I (**5**) (*m/z* 712). In the ^1H NMR of **1**, comparison of those data with those of **5** indicated an additional signal at δ_H 2.04 (3H, s) and the saturation of a double bond at C-10 and C-11. By a comparison of the ^{13}C NMR spectra between **1** and **5**, it was found that the signal of the olefinic carbon (δ_C 128.5 and 145.6) in **5** was missing and signals at δ_C 23.9 (q), 37.0 (t), 56.8 (d), 173.5 (s) and 177.6 (s) were newly observed in **1**. On treatment with 1 N NaOH (pH 10, 60°C), compound **1** afforded mycinamicin I (**5**) quantitatively. From these data, the presence of an *N*-acetyl-L-cysteine moiety was suggested. In fact, the alkaline treatment and the hydrolysis (1 N HCl, 100°C, overnight) of **1** gave L-cysteine. These data and the chemical shift of C-10 (δ_C 44.0 (t)) and C-11 (δ_C 42.6 (d)) revealed that the sulfer atom in *N*-acetyl-L-cysteine was connected to C-11. The physico-chemical properties and NMR spectral data of mycinamicin X′ (**2**) were quite similar to those of compound **1**, which accounts for the existence of diastereoisomeric forms. This was confirmed by synthesis by treatment of **5** with *N*-acetyl-L-cysteine (5 equiv) and $NaHCO_3$ (5 equiv) in 80% aq acetone at 50°C for 2 hours to give two products identical in all respects with **1** and **2**.

The molecular formula of mycinamicin XI (**3**) was determined to be $C_{42}H_{70}N_2O_{16}S$ based on its FAB-MS spectrum ($(M+H)^+$, *m/z* 891) and elemental analysis (Calcd: C 55.49, H 8.14, N 3.08, S 3.51. Found: C 55.42, H 8.14, N 2.97, S 3.22). The UV and IR spectral data suggested the presence of an α,β-unsaturated lactone. In the FAB-MS spectrum of **3**, the protonated molecular ion (*m/z* 891) appeared at 163 mass units higher than the corresponding ion of mycinamicin II (**6**) (*m/z* 728). The ^1H NMR spectrum of **3** showed an *N*-acetyl proton at δ_H 2.00 (3H, s) in addition to the protons of **6**, except for the olefinic protons at C-10 and C-11. By a comparison of the ^{13}C NMR spectra between **3** and **6**, it was found that the signal of the olefinic carbon (δ_C 127.9 and 145.3) in **6** was missing and signals at δ_C 23.4 (q), 36.8 (t), 56.3 (d), 173.0 (s) and 177.0 (s) were newly observed in **3**. The ^1H and ^{13}C NMR spectra of **3** were quite similar to those of **1** with the exception of the chemical shift at C-14. On treatment with 1 N NaOH (pH 10, 60°C), compound **3** afforded mycinamicin II (**6**) quantitatively. These data and the chemical shift of C-10 (δ_C 43.2 (t)) and C-11 (δ_C 41.6 (d)) revealed that the sulfer atom in *N*-acetyl-L-cysteine was connected to C-11. Comparison of these data with those of mycinamicin XI′ (**4**) indicated the existence of diastereoisomeric forms. This was confirmed by synthesis by treatment of **6** with *N*-acetyl-L-cysteine

Table 3. Antibacterial spectra of mycinamicins X (**1**), XI (**3**), I (**5**) and II (**6**).

Test organism	MIC (μg/ml)			
	1	**5**	**3**	**6**
Staphylococcus aureus FDA 209P JC-1	1.56	0.10	3.13	0.20
S. aureus MS353	1.56	0.10	6.25	0.20
S. epidermidis sp-al-1	1.56	0.10	1.56	0.10
Streptococcus pyogenes N.Y. 5	3.13	0.20	1.56	0.10
Micrococcus luteus ATCC 9341	0.78	<0.05	0.39	<0.05
Corynebacterium diphtheriae P.W. 8	12.5	1.56	3.13	0.39
Bacillus subtilis ATCC 6633	6.25	0.39	12.5	0.78
Escherichia coli NIHJ JC-2	>100	>100	>100	>100
Pseudomonas aeruginosa PA01	>100	>100	>100	>100

(5 equiv) and $NaHCO_3$ (5 equiv) in 80% aq acetone at 50°C for 2 hours to give two products identical in all respects with **3** and **4**.

In conclusion, we have elucidated the structures of four new macrolide antibiotics, mycinamicins X (**1**), X′ (**2**), XI (**3**) and XI′ (**4**) by spectroscopic comparison with mycinamicins and chemical derivation from mycinamicins.

Mycinamicins X (**1**) and XI (**3**) exhibited antibacterial activity against Gram-positive bacteria. As shown in Table 3, the antibacterial activities of compounds **1** and **3** were much less than those of **5** and **6**.

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(Received May 14, 1991)

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