COMMUNICATIONS TO THE EDITOR

Amicenomycins A and B, New Antibiotics from *Streptomyces* sp. MJ384-46F6

Sir:

In the course of our screening program for novel antibiotics, we found that a strain of *Streptomyces* sp. MJ384-46F6 isolated from a soil sample collected in Numazu-shi, Shizuoka prefecture, Japan, produced new antibiotics, amicenomycins A and B (Fig. 1). In this paper we report the production, isolation, physico-chemical properties, structural studies and biological properties of amicenomycins A and B.

The producing strain was maintained on asparagine-glucose agar slant medium consisting of asparagine 0.05%, K_2HPO_4 0.05%, glucose 1.0% and agar 2.0%. A slant culture of the producing organism was inoculated into twenty 500-ml Erlenmyer flasks each containing 110 ml of a medium consisting of potato starch 2.0%, glucose 2.0%, soybean meal 2.0%, yeast extract 0.5%, NaCl 0.25%, $CaCO_3$ 0.3%, $ZnSO_4 \cdot 7H_2O$ 0.005%, $CuSO_4 \cdot 5H_2O$ 0.0005%, $MnCl_2 \cdot 4H_2O$ 0.0005%, and a drop of silicon oil (adjusted to pH 7.4 before sterilization). The inoculated flasks were incubated at 27°C for 96 hours on a rotary shaker. The cultured broth (2 liters) was centrifuged to separate supernatant and mycelium cake. The supernatant was extracted with ethylacetate. The ethylacetate layer was concentrated to dryness and then applied to a Sephadex LH-20 column (MeOH). The active fraction was concentrated to dryness and finally purified by silica gel column chromatography. Development of the column with toluene-acetone 1:1 and 1:2 gave amicenomycin A (125 mg) and amicenomycin B (65 mg) respectively.

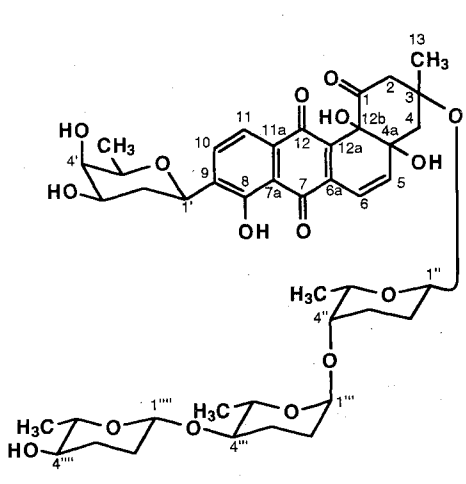
The physico-chemical properties of amicenomycins A and B are summarized in Table 1. The molecular formulae for amicenomycins A and B were determined by HRFAB-MS.

The ^1H and ^{13}C NMR spectra (shown in Table 2) of amicenomycin A are closely related to those of kerriamycin B[1] (= urdamycin A[2]) which is an isotetracenone antibiotic[3] consisting of one unit of aquayamycin[4] as an aglycone, one unit of D-olivose[5] and two units of L-rhodinose[6]. However, amicenomycin A (molecular formula: $C_{43}H_{56}O_{16}$) contains one more methylene group and one less oxymethine group than kerriamycin B (molecular formula: $C_{43}H_{56}O_{17}$).

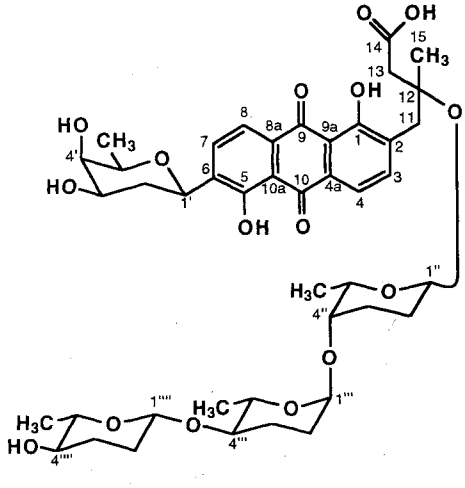
Mild acid hydrolysis of amicenomycin A gave one unit of aglycone and three units of sugar. The aglycone was extracted with ethylacetate and purified by silica gel column chromatography. The MS, UV and ^{13}C NMR spectra of the aglycone are closely similar to those of aquayamycin. The HMQC and HMBC spectra of the aglycone affirm the identity in planar structure. However, the ^1H NMR spectrum shows that 4'-H of amicenomycin A is equatorial instead of axial as observed in that of aquayamycin.

The sugars from the hydrolysate of amicenomycin A were purified by silica gel column chromatography. They were identified to be two units of L-amicetose[7] and one unit of L-rhodinose by the TLC comparison with the authentic samples obtained from antibiotics, MA144-M1 and N1[8] (aclacinomycin analogues) and by the comparison of their optical rotations with those of the published data. Thus amicenomycin A has three units of trideoxyhexose (L-amicetose (2 units) and L-rhodinose), whereas kerriamycin B has one unit of dideoxyhexose (D-olivose) and two units of trideoxyhexose (L-rhodi-

Fig. 1. Structures of amicenomycins A and B.



amicenomycin A



amicenomycin B

Table 1. Physico-chemical properties of amicenomycins A and B.

	amicenomycin A	amicenomycin B
Appearance	Orange powder	Brown powder
MP	150-151°C (dec)	140-143°C (dec)
$[\alpha]_D^{23}$	-12.5° (*c* 0.08, MeOH)	+100.0° (*c* 0.016, MeOH)
Molecular formula	$C_{43}H_{56}O_{16}$	$C_{43}H_{56}O_{16}$
HRFAB-MS (negative)		
Calcd:	828.3568	828.3568
Found:	828.3560	828 3550
UV λmax nm,(log ε) in MeOH	220 (4.71), 320 (3.97), 435 (4.00)	230 (4.58), 255 (4.40), 296 (3.95), 428 (4.02), 448 (4.02)
0.1N NaOH-90% MeOH	230 (4.63), 282 (4.32), 323 (4.19), 570 (3.98)	231 (4.53), 259 (4.40), 296 (3.95), 428 (3.91), 448 (3.91)
IR ν max(KBr) cm^{-1}	3428, 2936, 1728, 1642, 1566, 1437, 1362, 1289, 1264, 1169, 1127, 1063, 1017, 982, 789, 771	3437, 2934, 2361, 2344, 1626, 1584, 1433, 1375, 1314, 1289, 1260, 1169, 1127, 1071, 984, 810
TLC Rf *	0.33	0.23

* Silica gel TLC: $CHCl_3$-MeOH, 10:1.

Table 2. ^{13}C and ^1H NMR assignments of amicenomycin A in CD_3OD.

Position	δC	δH	Position	δC	δH
1	206.2		1"	96.6	4.73 (dd, 8.6, 2.0)
2	52.3	2.85 (s)	2"	32.7	2.20 (m)
3	80.2				1.48 (m)
4	46.7	2.18 (d, 14.7)	3"	26.6	2.10 (m)
		1.95 (d, 14.7)			1.70 (m)
4a	77.2		4"	68.1	3.55 (br. s)
5	147.5	6.39 (d, 9.8)	5"	68.0	4.07 (dq, 6.2, 1.4)
6	117.7	6.84 (d, 9.8)	6"	17.6	1.16 (d 6.2)
6a	139.6				
7	189.7		1"'	96.9	5.00 (d, 2.4)
7a	115.2		2"'	24.7	2.02 (m)
8	158.2				1.46 (m)
9	132.0		3"'	31.2	2.10 (m)
10	135.1	7.98 (d, 8.0)			1.45 (m)
11	120.1	7.58 (d, 8.0)	4"'	81.0	3.05 (ddd, 9.7, 9.2, 4.2)
11a	139.7		5"'	75.5	3.28 (dq, 9.7, 6.3)
12	183.9		6"'	18.6	1.08 (d, 6.3)
12a	139.8				
12b	78.7		1""	104.4	4.46 (dd, 8.2, 1.8)
13	25.2	1.41 (s)	2""	32.1	1.77 (m)
1'	72.3	4.86 *			1.42 (m)
2'	32.2	2.18 (dd, 12.0, 4.4)	3""	31.9	1.95 (m)
		1.63 (ddd, 12.0, 12.0, 12.0)			1.40 (m)
3'	76.0	3.93 (ddd, 12.0, 4.4, 2.4)	4""	72.0	3.06 (ddd, 9.8, 8.8, 4.4)
4'	71.4	3.71 (d, 2.4))	5""	77.1	3.22 (dq, 9.8, 6.6)
5'	76.3	3.75 (q, 6.2)	6""	18.6	1.19 (d, 6.6)
6'	17.7	1.34 (d, 6.2)			

^{13}C and ^1H NMR spectra were recorded at 100 MHz and 400 MHz, respectively.
Chemical shifts in ppm from TMS as an internal standard.
Multiplicity and *J* value (Hz) are in parentheses.
* Obscured by the solvent signal.

Table 3. ^{13}C and ^{1}H NMR assignments of amicenomycin B in CD_3OD.

Position	δC	δH	Position	δC	δH
1	162.5		1"	97.5	4.82 * (br. d, 6.9 **)
2	136.4		2"	32.6	1.67 (m)
3	141.1	7.82 (d, 7.2)			1.52 (m)
4	119.4	7.67 (d, 7.2)	3"	26.7	2.12 (m)
4a	132.9				1.70 (m)
5	159.8		4"	68.1	3.56 (br. s)
6	139.9	7.99 (d, 8.1)	5"	68.2	4.09 (dq, 6.0, 1.2)
7	134.9	7.76 (d, 8.1)	6"	17.6	1.18 (d 6.0)
8	120.2				
8a	133.2		1'''	97.0	5.02 (d, 2.4)
9	189.3		2'''	24.8	2.05 (m)
9a	116.6				1.53 (m)
10	189.4		3'''	31.4	2.18 (m)
10a	116.6				1.55 (m)
11	39.4	3.36 (d, 13.2)	4'''	81.2	3.17 (m)
		3.02 (d, 13.2)	5'''	75.8	3.37 (dq, 9.0, 6.2)
12	79.5		6'''	18.8	1.23 (d, 6.2)
13	46.9	2.65 (d, 14.4)			
		2.78 (d, 14.4)	1''''	104.4	4.54 (dd, 8.0, 1.6)
14	175.3		2''''	32.2	1.83 (m)
15	23.3	1.36 (s)			1.46 (m)
1'	73.0	4.88 *	3''''	32.0	1.98 (m)
2'	32.3	2.22 (dd, 11.8, 4.4)			1.46 (m)
		1.66 (ddd, 11.8, 11.8, 11.8)	4''''	72.2	3.09 (m)
3'	76.2	3.95 (ddd, 11.8, 4.4, 2.4)	5''''	77.2	3.27 (dq, 8.8, 6.4)
4'	71.6	3.73 (d, 2.4)	6''''	18.7	1.19 (d, 6.4)
5'	76.3	3.78 (q, 6.8)			
6'	17.8	1.37 (d, 6.8)			

^{13}C and ^{1}H NMR spectra were recorded at 100 MHz and 400 MHz, respectively.
Chemical shifts in ppm from TMS as an internal standard.
Multiplicity and *J* value (Hz) are in parentheses.
* Obscured by the solvent signal.
** Observable at 40°C.

Table 4. The antimicrobial activities of amicenomycins A and B.

Test organisms	MIC (μg/ml)	
	amicenomycin A	amicenomycin B
Staphylococcus aureus FDA209P	6.25	>100
S.aureus Smith	6.25	>100
S.aureus MS9610	12.5	>100
S.aureus No.5 (MRSA)	6.25	>100
S.aureus No.17 (MRSA)	12.5	>100
Micrococcus luteus FDA16	12.5	>100
M. luteus IFO3333	6.25	>100
M. luteus PCI1001	6.25	>100
Bacillus anthracis	12.5	50
B. subtilis NRRL B-558	50	>100
B. subtilis PCI219	25	>100
B. cereus ATCC 10702	25	50
Corynebacterium bovis 1810	50	>100
Escherichia coli NIHJ	>100	>100
Shigella dysenteriae JS11910	>100	>100
Salmonella typhi T-63	>100	>100
Pseudomonas aeruginosa A3	>100	>50
Klebsiella pneumoniae PCT602	>100	>100
Mycobacterium smegmatis ATCC607	>100	>100

Mueller-Hinton agar (Difco) 37°C.

nose). Connectivities between the sugar moieties, and between the sugar moieties and the aglycone were determined by the HMBC spectroscopy. The anomeric configurations of the sugar moieties were established by the coupling constants of the anomeric proton. These data show that a trisaccharide, β-L-amicetosyl-α-L-amicetosyl-β-L-rhodinose is glycosidically connected to C-3 carbon of the aglycone, whereas a monosaccharide and a disaccharide are connected to C-12b carbon and C-3′ carbon, respectively, of the aglycone in kerriamycin B.

The structure of amicenomycin B was elucidated with various NMR experiments including HMQC and HMBC (the ^{1}H and ^{13}C NMR data are shown in Table 3). It is closely similar to the structure of amicenomycin A. While C-1, C-2, C-3, C-4, C-4a and C-12b form a cyclohexanone ring in amicenomycin A, C-14 (corresponding to C-1 in amicenomycin A) does not attach to C-1 (corresponding to C-12b in amicenomycin A) in amicenomycin B. The structural relationship of these compounds is similar to that of vineomycins A_1[9] and B_2[10]. Considering the biosynthetic study of vineomycins A_1 and B_2[11], it is likely that amicenomycin B is formed by the bond cleavage between C-1 and C-12b of amicenomycin A.

Thus the structures of amicenomycins A and B were determined as shown in Fig. 1. The absolute stereochemistry of these antibiotics remains to be studied.

The antimicrobial activities of amicenomycins A and B are shown in Table 4 by agar dilution method. The acute toxicity (LD_{50}, ip) of amicenomycins A and B in mice were estimated to be >100.0 mg/kg and $17.5 \sim 35.0$ mg/kg, respectively.

Acknowledgments

The authors are grateful to Dr. O. JODO, Mercian Corporation, for supplying the samples of MA144-M1 and N1.

NAOTO KAWAMURA
RYUICHI SAWA
YOSHIKAZU TAKAHASHI
TSUTOMU SAWA
NAOKO KINOSHITA
HIROSHI NAGANAWA
MASA HAMADA
TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received July 19, 1995)

References

1) HAYAKAWA, Y.; K. ADACHI, T. IWAKIRI, K. IMAMURA, K. FRIHATA, H. SETO & N. ŌTAKE: Kerriamycins A, B and C, new isotetracenone antibiotics. Agric. Biol. Chem. 51: 1397～1405, 1987
2) ZEECK, A.; J. ROHR, G. M. SHELDRICK, P. G. JONES & E. F. PAULUS: Structure of a new antibiotic and cytotoxic indicator substance, urdamycin A. J. Chem. Research(s) 104～105, 1986
3) HAYAKAWA, Y.; T. IWAKIRI, K. IMAMURA, H. SETO & N. ŌTAKE: Studies on the isotetracenone antibiotics. I. Capoamycin, a new antitumor antibiotic. J. Antibiotics 38: 957～959, 1985
4) SEZAKI, M.; S. KONDO, K. MAEDA, H. UMEZAWA & M. OHNO: The structure of aquayamycin. Tetrahedron 26: 5171～5190, 1970
5) BERLIN, YU. A.; G. V. BORISOVA, S. E. ESIPOV, M. N. KOLOSOV & V. A. KRIVORUCHKO: Olivomycin and related antibiotics. XIII. Structures of olivose and oliose. Khim. Prir. Soedin. 5: 109～115, 1969
6) BROCKMANN, H. & T. WAEHNELDT: Rhodinose, eine Tridesoxyhexose. Naturwissenschaften 50: 43, 1963
7) STEVENS, C. L.; P. BLUMBERGS & D. L. WOOD: Stereochemical identification and synthesis of amicetose and the stereochemical identification of rhodinose and the sugar from streptolydigin. J. Am. Chem. Soc. 86: 3592～3594, 1964
8) OKI, T.; I. KITAMURA, Y. MATSUZAWA, N. SHIBAMOTO, T. OGASAWARA, A. YOSHIMOTO, T. INUI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Antitumor anthracycline antibiotics, aclacinomycin A and analogues. II. Structural determination. J. Antibiotics 32: 801～819, 1979
9) IMAMURA, N.; K. KAKINUMA, N. IKEKAWA, H. TANAKA & S. ŌMURA: Identification of the aglycone part of vineomycin A_1 with aquayamycin. Chem. Pharm. Bull. 29: 1788～1790, 1981
10) IMAMURA, N.; K. KAKINUMA, N. IKEKAWA, H. TANAKA & S. ŌMURA: The structure of vineomycin B_2. J. Antibiotics 34: 1517～1518, 1981
11) IMAMURA, N.; K. KAKINUMA, N. IKEKAWA, H. TANAKA & S. ŌMURA: Biosynthesis of vineomycin A_1 and B_2. J. Antibiotics 35: 602～608, 1982