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Antimycins A_{10}～A_{16}, Seven New Antimycin Antibiotics Produced by *Streptomyces* spp. SPA-10191 and SPA-8893

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Abstract Seven new antimycin antibiotics, named antimycins A_{10}, A_{11}, A_{12}, A_{13}, A_{14}, A_{15} and A_{16}, were isolated from the fermentation broth of strains of *Streptomyces* spp. SPA-10191 and SPA-8893, along with known antimycins A_1, A_2, A_3 and A_4. The structures of the new antimycins were determined by spectral analyses, including 2D NMR techniques. These compounds exhibited antifungal activity against *Candida utilis*.

Keywords antimycin, antifungal activity, new antibiotic, *Streptomyces*

Introduction

Antimycins are antifungal antibiotics composed of acyl and alkyl side chains and a nine-membered dilactone ring that is linked *via* amide bond to 3-formamidosalicylic acid. They were first isolated from a *Streptomyces* strain in 1949 [1]. The isolation of antimycins A_1 to A_9 have been reported so far, and each of antimycins A_1～A_8 is a mixture of two isomers containing a closely related alkylacyl group [1～3], whereas recently reported antimycin A_9 is an aromatic acyl analogue [4] (Fig. 1). Antimycins are known to inhibit specifically the electron transfer activity of ubiquinol-cytochrome *c* oxidoreductase [5]. Another function of antimycins reported is that they directly inhibit the activity of Bcl-2-related proteins, especially Bcl-x_L, which is an important regulator of cell death and survival [6].

In our screening for biologically active compounds from microbial sources, two strains of *Streptomyces* spp. SPA-10191 and SPA-8893 were found to produce new antimycin antibiotics, named antimycins A_{10}, A_{11}, A_{12}, A_{13}, A_{14}, A_{15} and A_{16} (**1**～**7**), together with known antimycins A_1, A_2, A_3 and A_4. In this paper we report the taxonomy of the producing strains, fermentation, isolation, structure elucidation, and antifungal activity of **1**～**7**.

Materials and Methods

General

UV spectra were recorded on a Hitachi U-2000 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter in a 10 cm cell. FAB-MS spectra were obtained on a JEOL JMS-SX102A spectrometer. NMR spectra were recorded on a JEOL JNM α-500 spectrometer and the chemical shifts are given in ppm referred to $CDCl_3$ as 7.25 ppm (^1H) and 77.0 ppm (^{13}C).

Microorganism

The producing strains SPA-10191 and SPA-8893 were isolated from soil samples collected in Kyoto and Osaka prefectures, Japan, respectively. Both strains have been deposited at the International Patent Organism Depositary,

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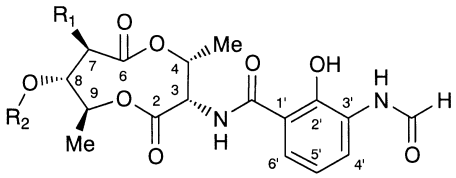

		MW	R_1	R_2 (component a)	R_2 (component b)
Antimycin A_{10ab}	(**1**)	562	4-Methylhexyl	2-Methylbutyryl	Isovaleryl
Antimycin A_{11}	(**2**)	534	Butyl	Isohexanoyl	
Antimycin A_{12}	(**3**)	548	Isopentyl	Isohexanoyl	
Antimycin A_{13}	(**4**)	548	Butyl	4-Methylhexanoyl	
Antimycin A_{14}	(**5**)	562	Isopentyl	4-Methylhexanoyl	
Antimycin A_{15}	(**6**)	562	Hexyl	Isohexanoyl	
Antimycin A_{16}	(**7**)	576	Hexyl	4-Methylhexanoyl	
Antimycin A_{1ab}		548	Hexyl	2-Methylbutyryl	Isovaleryl
Antimycin A_{2ab}		534	Hexyl	Isobutyryl	Butyryl
Antimycin A_{3ab}		520	Butyl	2-Methylbutyryl	Isovaleryl
Antimycin A_{4ab}		506	Butyl	Isobutyryl	Butyryl
Antimycin A_{5ab}		492	Ethyl	2-Methylbutyryl	Isovaleryl
Antimycin A_{6ab}		478	Ethyl	Isobutyryl	Butyryl
Antimycin A_{7ab}		520	Isopentyl	Isobutyryl	Butyryl
Antimycin A_{8ab}		534	Isopentyl	2-Methylbutyryl	Isovaleryl
Antimycin A_9		554	Butyl	Phenylacetyl	

Fig. 1 Structures of antimycins A_{10} (**1**), A_{11} (**2**), A_{12} (**3**), A_{13} (**4**), A_{14} (**5**), A_{15} (**6**), A_{16} (**7**) and known antimycins A_1~A_9.

the National Institute of Advanced Industrial Science and Technology, Japan under the accession numbers FERM P-19028 and FERM P-19027, respectively.

Taxonomy

Taxonomic studies of the strains SPA-10191 and SPA-8893 were performed according to the method of Shirling and Gottlieb [7] after growing on various agar media at 27°C for 14 days. Fine morphological structures were observed using a Hitachi S-800 scanning electron microscope. Color names were determined by using the Color Tone Manual [8]. Cell wall analysis was performed by the method of Staneck and Roberts [9].

Fermentation

A slant culture of each strain was inoculated into a 500-ml Sakaguchi flask containing 75 ml of liquid medium composed of glucose 2%, dextrin 2%, soybean flour 1.5%, yeast extract 0.3%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2%, pH 7.0, and cultured for 4 days at 27°C with reciprocal shaking at 130 rpm. A volume of 6 ml of the seed culture was transferred into 2-liter Sakaguchi flasks containing 300 ml of the same medium, and cultured at 27°C with reciprocal shaking at 115 rpm. The cultivation time of the strains SPA-10191 and SPA-8893 was 8 and 6 days, respectively.

Antifungal Assay

The antifungal activity was measured by the paper-disk method with *Candida utilis* NBRC10707. Test compounds were absorbed by paper disks (6 mm diameter) and placed on the assay plates. The fungus was cultivated in Sabouraud's agar at 30°C. After incubation for 48 hours, zones of inhibition (mm in diameter) were recorded. Antimycin A_3 (Calbiochem) was used as a positive control.

Results

Taxonomy

Strains SPA-10191 and SPA-8893 formed well-branched substrate mycelia without fragmentation on agar media. Aerial mycelia were abundant on yeast extract - malt extract, oatmeal and inorganic salts - starch agar, but scant on glycerol - asparagine agar for both strains (Table 1). The color of aerial mycelium of the strain SPA-10191 was yellow, and that of reverse side of colony was reddish or grayish yellow. The spore chains were *Rectiflexibiles* and consisted of more than 50 spores per chain (Fig. 2a). The spore was oval and 0.5~0.6×0.8~1.0 μm in size with a smooth surface. In the case of the strain SPA-8893, the color of aerial mycelium was gray, and that of reverse side of colony was grayish yellow. The spore chains were *Retinaculiaperti* and consisted of 20~50 spores per chain (Fig. 2b). The spore was oval and 0.6~0.8×0.9~1.2 μm in size with a hairy surface. The physiological characteristics and carbohydrate utilization of both strains are summerized

Table 1 Cultural characteristic of strains SPA-10191 and SPA-8893

Medium		SPA-10191	SPA-8893
Yeast extract - malt extract agar (ISP No.2)	G:	Good	Good
	AM:	Good, yellow	Good, gray
	RS:	Reddish yellow	Grayish yellow
	SP:	None	None
Oatmeal agar (ISP No.3)	G:	Good	Good
	AM:	Good, yellow	Good, gray
	RS:	Reddish yellow	Grayish yellow
	SP:	None	None
Inorganic salts - starch agar (ISP No.4)	G:	Good	Good
	AM:	Good, yellow	Good, gray
	RS:	Reddish yellow	Grayish yellow
	SP:	None	None
Glycerol - asparagine agar (ISP No.5)	G:	Good	Good
	AM:	Scant, yellow	Scant, gray
	RS:	Grayish yellow	Grayish yellow
	SP:	None	None

Abbreviation. G: growth, AM: aerial mycelium, RS: reverse side color, SP: soluble pigment.

a) Strain SPA-10191 b) Strain SPA-8893

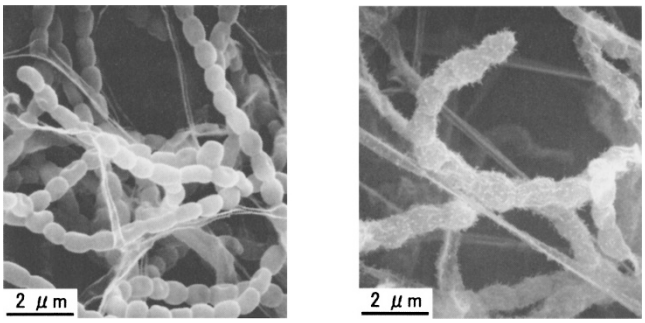

Fig. 2 Scanning electron micrograph of strains SPA-10191 and SPA-8893 grown on ISP medium No. 3 at 27°C for 14 days.

Table 2 Physiological characteristic of strains SPA-10191 and SPA-8893

Characteristic	SPA-10191	SPA-8893
Production of melanoid pigment		
Peptone - yeast extract - iron agar (ISP No. 6)	−	−
Tyrosine agar (ISP No.7)	−	−
Carbohydrate utilization		
L-Arabinose	+	+
D-Fructose	+	+
D-Glucose	+	+
Inositol	±	+
D-Mannitol	+	+
Raffinose	±	−
L-Rhamnose	±	+
Sucrose	±	−
D-Xylose	+	+

in Table 2. The whole-cell hydrolysates of both strains contained LL-diaminopimelic acid. From the above characteristics, the strains SPA-10191 and SPA-8893 were identified as members of the genus *Streptomyces*, and named *Streptomyces* spp. SPA-10191 and SPA-8893.

Isolation

The fermentation broth (3 liters) of strain SPA-10191 was centrifuged at 9,000 *g* for 10 minutes at 20°C. The cell cake was extracted with 2 liters of acetone and concentrated under reduced pressure to yield 1.5 g of oily material. The material was dissolved in 30 ml of ethyl acetate - methanol (50 : 50) and applied to a silica gel column, and the column was eluted with the same solvent. The fractions containing antimycins (0.6 g) were applied to a column of Toyopearl HW-40F (Tosoh) and eluted with methanol. The fractions containing antimycins (0.16 g) were pooled and injected into preparative HPLC equipped with Wakopak Wakosil-II5C18HG-Prep columns (30×100+30×250 mm). The elution was performed with 1% aqueous formic acid - methanol (20 : 80 to 0 : 100 in 130 minutes) at a flow rate of

Table 3 Physico-chemical properties of **1**～**4**

	1	**2**	**3**	**4**
Appearance	Pale yellow solid	Pale yellow solid	Pale yellow solid	Pale yellow solid
Molecular formula	$C_{29}H_{42}N_2O_9$	$C_{27}H_{38}N_2O_9$	$C_{28}H_{40}N_2O_9$	$C_{28}H_{40}N_2O_9$
FAB-MS (*m/z*)	563 $(M+H)^+$	535 $(M+H)^+$	549 $(M+H)^+$	549 $(M+H)^+$
HRFAB-MS (*m/z*)				
Found:	563.2952 $(M+H)^+$	535.2661 $(M+H)^+$	571.2601 $(M+Na)^+$	571.2599 $(M+Na)^+$
Calcd.:	563.2969	535.2656	571.2631	571.2631
UV λ_{max} nm	228 (26,800),	228 (28,700),	228 (28,500),	228 (26,800),
(ε, MeOH)	321 (5,000)	322 (5,000)	321 (4,900)	321 (4,700)
IR ν_{max} (KBr) cm^{-1}	3355, 2960, 1743, 1684, 1643, 1529, 1361, 1142	3340, 2958, 1733, 1689, 1635, 1533, 1369, 1151	3342, 2956, 1739, 1685, 1637, 1529, 1365, 1147	3342, 2958, 1733, 1685, 1635, 1525, 1375, 1149
$[\alpha]_D^{25}$ (MeOH)	+88.5 (*c* 0.03)	+96.7 (*c* 0.03)	+72.2 (*c* 0.18)	+73.3 (*c* 0.17)
Solubility				
Soluble:	$CHCl_3$, MeOH	$CHCl_3$, MeOH	$CHCl_3$, MeOH	$CHCl_3$, MeOH
Insoluble:	H_2O, *n*-Hexane	H_2O, *n*-Hexane	H_2O, *n*-Hexane	H_2O, *n*-Hexane

Table 4 Physico-chemical properties of **5**～**7**

	5	**6**	**7**
Appearance	Pale yellow solid	Pale yellow solid	Pale yellow solid
Molecular formula	$C_{29}H_{42}N_2O_9$	$C_{29}H_{42}N_2O_9$	$C_{30}H_{44}N_2O_9$
FAB-MS (*m/z*)	563 $(M+H)^+$	563 $(M+H)^+$	577 $(M+H)^+$
HRFAB-MS (*m/z*)			
Found:	563.2989 $(M+H)^+$	563.2972 $(M+H)^+$	577.3154 $(M+H)^+$
Calcd.:	563.2969	563.2969	577.3125
UV λ_{max} nm (ε, MeOH)	228 (28,100), 321 (4,900)	228 (27,400), 321 (4,800)	228 (29,100), 321 (5,100)
IR ν_{max} (KBr) cm^{-1}	3338, 2958, 1736, 1684, 1637, 1529, 1367, 1149	3336, 2956, 1736, 1684, 1637, 1533, 1367, 1151	3340, 2958, 1736, 1684, 1637, 1527, 1375, 1149
$[\alpha]_D^{25}$ (MeOH)	+75.0 (*c* 0.07)	+76.7 (*c* 0.15)	+78.8 (*c* 0.08)
Solubility			
Soluble:	$CHCl_3$, MeOH	$CHCl_3$, MeOH	$CHCl_3$, MeOH
Insoluble:	H_2O, *n*-Hexane	H_2O, *n*-Hexane	H_2O, *n*-Hexane

20 ml/minute and detection of UV absorption at 240 nm. Antimycins A_4 (4.3 mg), A_3 (1.7 mg), A_2 (2.0 mg) and A_1 (1.7 mg) and compound **1** (1.3 mg) were eluted at 36.0, 44.0, 48.0, 58.0 and 64.0 minutes, respectively.

Isolation procedures for **2**～**7** from the fermentation broth (3 liters) of strain SPA-8893 were the same as described above for **1**. Compounds **2** (3.5 mg), **3** (9.0 mg), **4** (8.6 mg), **5** (6.0 mg), **6** (7.8 mg) and **7** (5.2 mg) were eluted at 47.0, 53.5, 56.6, 63.8, 67.3 and 76.1 minutes, respectively, in the final HPLC purification, together with antimycins A_1 (13.0 mg), A_2 (5.0 mg), A_3 (6.5 mg) and A_4 (2.6 mg).

Structure Elucidation

The physico-chemical properties of **1**～**7** are summarized in Tables 3 and 4. Their UV and IR spectra showed almost the same absorption spectra respectively, and the UV spectrum (λ_{max} 228 and 321 nm in **1**) and IR absorption bands (ν_{max} 1643, 1684 and 1743 cm^{-1} for amide and ester in **1**) suggested that they were very similar to those of other

Table 5 ^{1}H and ^{13}C NMR chemical shifts of **1** in $CDCl_3$

No.	^{13}C	^{1}H (J=Hz)	No.	^{13}C	^{1}H (J=Hz)
2	170.07		1″	28.64	1.66 (m), 1.23 (m)
3	53.67	5.27 (t, 7.6)	2″	24.46	1.23 (m)
4	70.91	5.72 (q, 7.0)	3″	36.09	1.23 (m)
6	172.93		4″	34.03	1.23 (m)
7	50.12	2.52 (m)	5″′	29.46	1.23 (m)
8	75.29	5.08 (t, 9.7)	6″	11.37	0.81 (t, 6.4)
9	74.90	4.98 (m)	7″	18.99	0.80 (d, 5.8)
4-Me	14.97	1.30 (d, 6.7)	Component a		
9-Me	17.85	1.27 (d, 6.4)	1‴	175.19	
1′	112.54		2‴	41.26	2.40 (m)
2′	150.64		3‴	26.46	1.71 (m), 1.48 (m)
3′	127.41		4‴	11.71	0.93 (t, 7.3)
4′	124.84	8.53 (d, 7.9)	5‴	16.74	1.17 (d, 6.4)
5′	119.00	6.91 (t, 7.8)	Component b		
6′	120.09	7.22 (d, 8.1)	1‴	171.44	
1′-CONH	169.39		2‴	43.23	2.24 (d, 6.7)
1′-CONH		7.05 (d, 6.4)	3‴	25.48	2.13 (m)
2′-OH		12.60 (s)	4‴	22.42	0.97 (d, 6.4)
3′-NHCHO		7.92 (br s)	5‴	22.42	0.97 (d, 6.4)
3′-NHCHO	159.08	8.49 (s)			

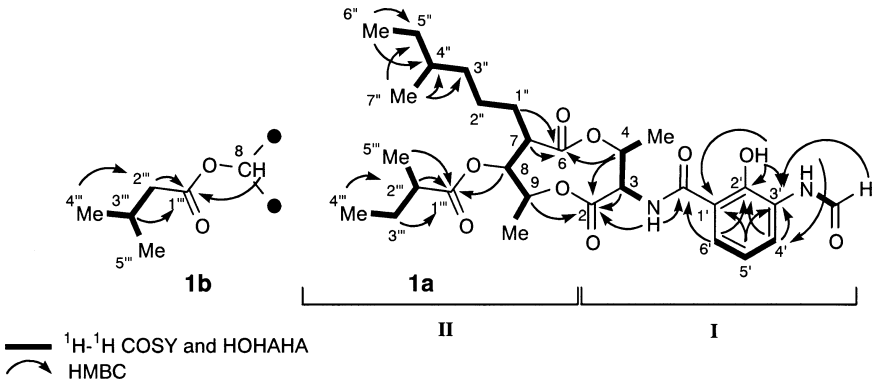

Fig. 3 ^{1}H-^{1}H COSY, HOHAHA and HMBC correlations observed in **1a** and **1b**.

antimycin antibiotics. The molecular formula of **1** established by HRFAB-MS as $C_{29}H_{42}N_2O_9$ was different from those of any known antimycins. The ^{1}H and ^{13}C NMR spectral data of **1** are summarized in Table 5. The NMR data of **1** revealed a mixture of two related compounds **1a** and **1b**, differing in the presence of 2-methylbutyryl or isovaleryl groups in a ratio of 85 : 15. These mixtures were inseparable by HPLC purification. Analysis of the COSY, HOHAHA and HMBC spectra of **1** revealed two partial structures **I** and **II** (Fig. 3). The partial structure **I**, which was assigned on the basis of ^{1}H and ^{13}C chemical shifts and the HMBC correlations, was the same as those observed for known antimycins. The partial structure **II** was identified through the HOHAHA spin network observed from the methyl groups (H_3-6″, H_3-7″, H_3-4‴ and H_3-5‴) and HMBC correlations of H-7 and H_2-1″ with C-6 and of H-8, H-2‴, H_2-3‴ and H_3-5‴ with C-1‴ in **1a** and of H-8, H_2-2‴ and H-3‴ with C-1‴ in **1b**. These data indicated that the 7-alkyl side chain is 4-methylhexyl group and the 8-*O*-acyl group is 2-methylbutyryl group in **1a** and isovaleryl group in **1b**. The HMBC correlations of H-4 with C-6 and of H-9 with C-2 revealed the connection of **I** with **II** to the formation of

Table 6 ^{13}C NMR chemical shifts of **2**∼**7** in $CDCl_3$

No.	**2**	**3**	**4**	**5**	**6**	**7**
2	169.37	169.81	169.81	169.81	169.81	170.08
3	53.67	53.73	53.71	53.73	53.73	53.66
4	70.93	70.96	70.93	70.97	70.93	70.91
6	172.94	172.64	172.66	172.65	172.66	172.94
7	50.11	50.38	50.17	50.39	50.21	50.14
8	75.45	75.49	75.41	75.50	75.46	75.44
9	74.88	74.88	74.86	74.88	74.88	74.88
4-Me	14.98	15.15	15.16	15.16	15.16	14.97
9-Me	17.83	18.01	17.99	18.01	18.01	17.83
1′	112.58	112.46	112.42	112.45	112.45	112.58
2′	150.63	150.42	150.39	150.42	150.42	150.62
3′	127.42	127.27	127.26	127.27	127.27	127.42
4′	124.82	124.68	124.65	124.68	124.68	124.82
5′	118.98	118.86	118.84	118.86	118.86	118.97
6′	120.14	119.99	119.97	119.97	119.98	120.13
1′-CONH	169.37	169.10	169.07	169.11	169.10	169.36
3′-NHCHO	159.08	158.81	158.83	158.77	158.81	159.08
1″	28.14	26.47	28.28	26.51	28.56	28.45
2″	22.41	36.19	22.56	36.19	22.64	22.47
3″	29.20	27.96	29.33	27.97	27.16	27.03
4″	13.76	22.76	13.96	22.81	31.60	31.46
5″		22.69		22.71	29.08	29.08
6″					14.07	13.98
1‴	172.60	172.32	172.42	172.38	172.31	172.67
2‴	32.25	32.40	32.12	32.16	32.39	32.01
3‴	33.68	33.87	31.58	31.65	33.80	31.46
4‴	27.62	27.77	34.06	34.08	27.77	33.95
5‴	22.14	22.28	29.21	29.22	22.31	28.95
6‴	22.14	22.28	11.42	11.40	22.31	11.21
7‴			18.88	18.89		18.72

the 9-membered dilactone. Taken together, the structure of **1** was determined to be a mixture of two related compounds **1a** and **1b**, and both are new 7-alkyl side chain analogues of antimycins as shown in Fig. 1.

Compound **2** had the molecular formula $C_{27}H_{38}N_2O_9$, as established by HRFAB-MS. The ^1H and ^{13}C NMR spectral data of **2**∼**7** are summarized in Tables 6 and 7. On the basis of the ^1H and ^{13}C NMR spectra, **2** was different from **1a** by one methyl and one methine group. Analysis of the COSY, HOHAHA and HMBC revealed that the 4-methylhexyl and 2-methylbutyryl groups in **1a** were replaced with butyl and isohexanoyl groups in **2**, respectively. The molecular formula of **3** and **4** were established as $C_{28}H_{40}N_2O_9$ by HRFAB-MS, differing from that of **2** by the addition of a CH_2 unit. Comparison of the NMR spectra with **2** revealed that the 7-alkyl side chain was replaced with the isopentyl group in **3** and the 8-*O*-acyl group was replaced with the 4-methylhexanoyl group in **4**. The structures of compounds of **5**∼**7** were determined in a similar manner to those of **2**∼**4**. The structures of **5** and **7** differed from **4** only at the 7-alkyl side chain, which were replaced with isopentyl (**5**) and hexyl (**7**) groups. The structure of **6** differed from **3** only at the 7-alkyl side chain as shown in Fig. 1. These results indicated that compounds **2**∼**7** are new 8-*O*-acyl analogues of antimycins.

The optical rotations of **1**∼**7** are similar to those of known antimycins. The ^1H and ^{13}C chemical shifts of the 9-membered dilactone moiety in **1**∼**7** are almost identical to known antimycins. From these results, since known antimycins A_1, A_2, A_3 and A_4 were isolated from the same fermentation broth, **1**∼**7** are considered to possess the same 9-membered dilactone configuration as known antimycins.

Table 7 ^{1}H NMR chemical shifts of **2**∼**7** in $CDCl_3$

No.	2	3	4	5	6	7
3	5.28 (t, 7.6)	5.30 (t, 7.5)	5.30 (t, 7.6)	5.28 (t, 7.6)	5.27 (t, 7.6)	5.27 (t, 7.6)
4	5.72 (q, 7.0)	5.74 (q, 7.3)	5.74 (q, 7.1)	5.72 (q, 7.1)	5.72 (q, 7.0)	5.72 (q, 7.0)
7	2.50 (m)	2.51 (m)	2.52 (m)	2.50 (m)	2.52 (m)	2.52 (m)
8	5.07 (t, 10.1)	5.07 (t, 10.0)	5.07 (t, 9.9)	5.08 (t, 9.9)	5.07 (t, 9.8)	5.07 (t, 9.8)
9	4.97 (m)	5.00 (m)	4.99 (m)	4.99 (m)	4.96 (m)	4.96 (m)
4-Me	1.29 (d, 6.7)	1.30 (d, 6.7)	1.30 (d, 6.7)	1.30 (d, 6.7)	1.30 (d, 6.7)	1.30 (d, 6.7)
9-Me	1.27 (d, 6.4)	1.27 (d, 6.4)	1.27 (d, 6.4)	1.27 (d, 6.4)	1.27 (d, 6.4)	1.27 (d, 6.4)
4′	8.52 (d, 7.4)	8.54 (d, 7.7)	8.54 (d, 7.1)	8.53 (d, 7.1)	8.52 (d, 7.9)	8.52 (d, 7.9)
5′	6.90 (t, 7.9)	6.92 (t, 8.0)	6.92 (t, 8.1)	6.90 (t, 8.1)	6.89 (t, 8.1)	6.89 (t, 8.1)
6′	7.23 (d, 7.7)	7.24 (d, 8.0)	7.24 (d, 7.1)	7.23 (d, 7.1)	7.23 (d, 8.0)	7.23 (d, 8.0)
1′-CONH	7.07 (d, 7.7)	7.08 (d, 7.5)	7.08 (d, 7.6)	7.06 (d, 7.6)	7.07 (d, 7.7)	7.07 (d, 7.7)
2′-OH	12.59 (s)	12.62 (s)	12.62 (s)	12.61 (s)	12.59 (s)	12.59 (s)
3′-NHCHO	7.97 (br s)	7.97 (br s)	7.99 (br s)	7.93 (br s)	7.98 (br s)	7.98 (br s)
3′-NHCHO	8.49 (s)	8.51 (s)	8.51 (s)	8.49 (s)	8.49 (s)	8.49 (s)
1″	1.67 (m),	1.66 (m),	1.68 (m),	1.66 (m),	1.69 (m),	1.69 (m),
	1.23 (m)	1.37 (m)	1.23 (m)	1.37 (m)	1.23 (m)	1.23 (m)
2″	1.23 (m)	1.25 (m)	1.23 (m)	1.15 (m)	1.23 (m)	1.23 (m)
3″	1.23 (m)	1.48 (m)	1.23 (m)	1.46 (m)	1.23 (m)	1.23 (m)
4″	0.85 (t, 7.0)	0.83 (d, 6.7)	0.85 (t, 7.1)	0.83 (d, 6.7)	1.23 (m)	1.23 (m)
5″		0.83 (d, 6.7)		0.83 (d, 6.7)	1.23 (m)	1.23 (m)
6″					0.87 (t, 7.1)	0.86 (t, 7.1)
2‴	2.34 (t, 7.5)	2.37 (t, 7.5)	2.36 (m)	2.36 (m)	2.34 (m)	2.34 (m)
3‴	1.52 (m)	1.52 (m)	1.64 (m),	1.64 (m),	1.55 (m)	1.64 (m),
			1.47 (m)	1.46 (m)		1.45 (m)
4‴	1.56 (m)	1.56 (m)	1.30 (m)	1.38 (m)	1.56 (m)	1.30 (m)
5‴	0.90 (d, 6.5)	0.90 (d, 6.5)	1.23 (m)	1.28 (m)	0.92 (d, 6.4)	1.23 (m)
6‴	0.90 (d, 6.5)	0.90 (d, 6.5)	0.87 (t, 7.0)	0.87 (t, 7.0)	0.92 (d, 6.4)	0.87 (t, 7.0)
7‴			0.86 (d, 6.2)	0.86 (d, 6.2)		0.86 (d, 6.2)

Antifungal Activity

Compounds **1**∼**7** were evaluated for antifungal activity against *Candida utilis* using a paper-disk assay. The diameter of inhibition zone of **1**∼**6** were 8.0∼9.0 and 10.0∼12.0 mm at 0.02 and 0.2 μg/disk, respectively (Table 8). Compound **7** weakly inhibited the growth and the diameter of inhibition zone was 7.0 and 8.0 mm at 0.02 and 0.2 μg/disk, respectively, whereas that of antimycin A_3 as a positive control was 12.0 and 15.0 mm at 0.02 and 0.2 μg/disk, respectively.

Table 8 Antifungal activity of **1**∼**7** and antimycin A_3 against *Candida utilis* NBRC10707

Antimycins	Diameter of inhibitory zone (mm)	
	0.02 μg/disk	0.2 μg/disk
A_{10} (**1**)	8	10
A_{11} (**2**)	9	12
A_{12} (**3**)	9	11
A_{13} (**4**)	8	10
A_{14} (**5**)	8	10
A_{15} (**6**)	8	10
A_{16} (**7**)	7	8
A_3[a)]	12	15

[a)] Positive control.

Discussion

We have isolated seven new antimycin antibiotics **1**∼**7** from the fermentation broths of two *Streptomyces* sp. strains. Compound **1** is a mixture of two isomers containing 2-methylbutyryl and isovaleryl groups, as shown in antimycins A_1, A_3, A_5, and A_8 (Fig. 1). Compound **1** is the

first antimycin antibiotic containing a C_7 7-alkyl side chain, and compounds **2**～**7** are the first examples of antimycins containing C_6 or C_7 8-*O*-alkylacyl group. Compounds **1**, **4** and **6** are closely related to antimycin A_0, but the structure of the latter compound was not clearly elucidated [10]. The order of the antifungal potency was found to be antimycin A_3>**2**≧**3**≧**1**, **4**, **5**, **6**>**7**. This activity order suggested that there are inverse relationships between the antifungal activity and the length of the 7-alkyl and 8-*O*-acyl side chains.

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