J. Antibiot. 58(6): 409–411, 2005

NOTE

THE JOURNAL OF ANTIBIOTICS

New 24-Membered Macrolides SPA-6952A and B Produced by *Streptomyces* sp.

Nobuo Hosotani, Kazuo Kumagai, Hiroyuki Nakagawa, Takuro Shimatani, Ikutaro Saji

Received: April 13, 2005 / Accepted: May 26, 2005

Abstract Two new 24-membered macrolides, SPA-6952A and B, were isolated from the fermentation broth of *Streptomyces* sp. SPA-6952. The structures of the new macrolides were determined by spectral analyses, including 2D NMR techniques. These compounds exhibited cytotoxic activity against human promyelocytic leukemia HL-60 cells.

Keywords macrolide, cytotoxic activity, antimicrobial activity, *Streptomyces*

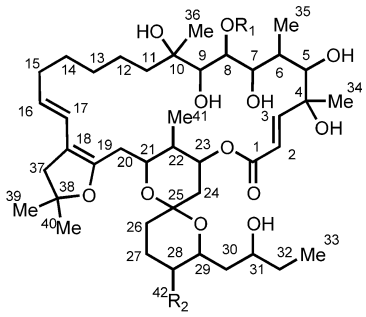
In screening for biologically active compounds from microbial sources, two new 24-membered macrolides, SPA-6952A (**1**) and B (**2**) (Fig. 1), were isolated from the fermentation broth of *Streptomyces* sp. SPA-6952. In this paper we report the fermentation, isolation, structure elucidation, and biological activity of **1** and **2**.

The actinomycete strain SPA-6952 was isolated from a soil sample collected in Osaka Prefecture, Japan, and identified as a *Streptomyces* sp. A slant culture of the strain SPA-6952 was inoculated into a 500-ml Sakaguchi flask containing 75 ml of liquid medium composed of glucose 1.5%, soluble starch 1.5%, cottonseed flour 1.2%, yeast extract 0.05%, KCl 0.2%, $MgSO_4 \cdot 7H_2O$ 0.007%, $CaCO_3$ 0.2%, pH 7.2, 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 for 7 days at 27°C with reciprocal shaking at 115 rpm.

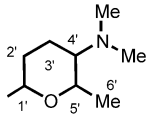
The fermentation broth (7.2 liters) was centrifuged at 9,000 *g* for 10 minutes at 20°C. The supernatant and mycelial cake were extracted with 7.2 liters of 1-butanol and 3 liters of acetone, respectively. Both extracts were concentrated under reduced pressure and combined to yield 8.5 g of brown oil. The oily residue was applied to a column of Toyopearl HW-40F (Tosoh) and eluted with methanol. The fractions containing **1** and **2** were separated into I (1.3 g) and II (4.2 g), respectively, after analysis by HPLC. The fraction I was subjected to preparative HPLC equipped with Wakopak Wakosil-II5C18HG-Prep columns (30×100+30×250 mm). The elution was performed with a 1% aqueous formic acid - methanol gradient (30 : 70 to 0 : 100 in 40 minutes) at a flow rate of 20 ml/minute and detection of UV absorption at 225 nm. The fraction eluted at 24.0 minutes was further purified by preparative HPLC equipped with Wakopak Wakosil-II5C18RS columns (20×50+20×250 mm) using a 1% aqueous formic acid - methanol gradient (45 : 55 to 40 : 60 in 60 minutes) at a flow rate of 7 ml/minute and detection of UV absorption at 225 nm. Compound **1** (5.9 mg) was eluted at 18.0 minutes and obtained as a colorless solid. Similarly, compound **2** was purified from the fraction II by using preparative HPLC twice, first using a gradient of 1% aqueous formic acid - methanol (30 : 70 to 0 : 100 in 45 minutes) at a flow rate of 30 ml/minute on a Wakosil-II5C18HG-Prep columns (50×100+50×250 mm) and secondly a gradient

N. Hosotani (Corresponding author)**, K. Kumagai, T. Shimatani, I. Saji**: Exploratory Research Group, Research Division, Sumitomo Pharmaceuticals Co., Ltd., 4-2-1 Takatsukasa, Takarazuka, Hyogo 665-0051, Japan, E-mail: hosotani@sumitomopharm.co.jp

H. Nakagawa: Genomic Science Laboratories, Research Division, Sumitomo Pharmaceuticals Co., Ltd., 4-2-1 Takatsukasa, Takarazuka, Hyogo 665-0051, Japan



Amino sugar =



	R_1	R_2
SPA-6952A (**1**)	Amino sugar	H
SPA-6952B (**2**)	H	H
Dunaimycin D3S	Amino sugar	Me

Fig. 1 Structures of SPA-6952A (**1**), SPA-6952B (**2**) and dunaimycin D3S.

Table 1 Physico-chemical properties of **1** and **2**

	1	**2**
Appearance	Colorless solid	Colorless solid
Molecular formula	$C_{49}H_{83}NO_{13}$	$C_{41}H_{68}O_{12}$
FAB-MS (*m/z*)	895 $(M+H)^+$	753 $(M+H)^+$
HRFAB-MS (*m/z*)		
Found:	894.5966 $(M+H)^+$	752.4724 $(M)^+$
Calcd.:	894.5943	752.4711
UV λ_{max} nm (ε, MeOH)	257 (11,300)	257 (12,900)
IR ν_{max} (KBr) cm^{-1}	3392, 2933, 1716, 1653, 1458, 1369,	3394, 2931, 1716, 1653, 1458, 1369,
$[\alpha]_D^{25}$ (MeOH)	−48.3 (*c* 0.12)	−23.8 (*c* 0.08)
Solubility		
Soluble:	DMSO, MeOH	DMSO, MeOH
Insoluble:	H_2O, *n*-Hexane	H_2O, *n*-Hexane

of 1% aqueous formic acid - methanol (40 : 60 to 0 : 100 in 72 minutes) at a flow rate of 7 ml/minute on a Wakosil-II5C18RS columns (20×50+20×250 mm). Finally, compound **2** (4.0 mg) was eluted at 35.0 minutes and obtained as a colorless solid.

The physico-chemical properties of **1** and **2** are summarized in Table 1. Both **1** and **2** are soluble in DMSO and MeOH, while they are insoluble in H_2O and *n*-Hexane. Their UV and IR spectra had almost the same absorption maxima, and the UV spectrum (λ_{max} 257 nm) and IR absorption band (ν_{max} 1716 cm^{-1}) suggested the existence of an α,β-unsaturated ester. The presence of a conjugated diene was also indicated by the IR spectrum (ν_{max} 1653 cm^{-1}). The molecular formula of **1** was determined as $C_{49}H_{83}NO_{13}$ by HRFAB-MS. The ^1H and ^{13}C NMR (Tables 2 and 3) and DEPT spectral data of **1** indicated that **1** contained ten methyl groups, fifteen methylene groups, three methine groups, ten oxymethine groups and four quaternary carbons bearing oxygen, as well as a conjugated diene and an α,β-unsaturated ester. Detailed analysis of the COSY, HOHAHA and HMBC spectra of **1** revealed that **1** was a spiroketal 24-membered macrolide with an amino sugar and a conjugated diene fused to the dihydrofuran moiety (Fig. 2). The linkage of the amino sugar to the C-8 position of the aglycone was established by HMBC correlations of H-8 with C-1′ and of H-1′ with C-8. These data were similar to those of dunaimycin D3S [1]. Compound **1** contained ten methyl groups, whereas dunaimycin D3S had eleven methyls. Comparisons of the NMR data between **1** and dunaimycin D3S indicated that **1** differed from dunaimycin D3S by the absence of a methyl substituent at C-28 in the spiroketal ring system. From these results along with unambiguous HMBC correlations of each of ten methyl groups in **1**, compound **1** was

Table 2 ^{13}C NMR chemical shifts of **1** and **2** in MeOH-d_4 (125 MHz)

No.	**1**	**2**	No.	**1**	**2**
1	167.3	167.1	25	99.1	99.2
2	120.3	120.5	26	35.7	35.6
3	153.4	153.1	27	20.1	20.1
4	76.7	76.47	28	32.2	32.3
5	79.6	80.1	29	68.4	68.0
6	37.7	37.6	30	44.3	44.3
7	77.2	78.6	31	70.8	70.8
8	80.3	71.8	32	31.9	31.9
9	75.7	76.52	33	10.6	10.7
10	75.5	76.1	34	27.1	26.8
11	40.7	39.9	35	7.9	8.0
12	24.2	23.7	36	22.8	22.7
13	30.3	30.8	37	44.6	44.6
14	29.9	29.9	38	84.9	84.9
15	33.4	33.2	39	28.9	28.9
16	126.8	127.1	40	28.8	28.8
17	125.0	124.6	41	5.2	5.3
18	111.6	111.8	1′	99.1	
19	149.1	149.0	2′	27.9	
20	29.4	29.3	3′	18.9	
21	67.7	68.3	4′	64.3	
22	34.4	34.3	5′	68.1	
23	72.7	72.4	6′	15.3	
24	36.8	36.7	4′-NMe_2	42.7	

Chemical shifts are given in ppm referenced to MeOH-d_4 as 49.0 ppm.

Table 3 ^{1}H NMR chemical shifts of **1** and **2** in MeOH-d_4 (500 MHz)

No.	1	2
2	6.02 (1H, d, 15.4)	6.02 (1H, d, 15.8)
3	6.98 (1H, d, 15.4)	6.99 (1H, d, 15.8)
5	3.80 (1H, d, 4.0)	3.76 (1H, d, 4.0)
6	1.86 (1H, m)	1.86 (1H, m)
7	3.80 (1H, t, 5.4)	3.76 (1H, d, 4.0)
8	3.88 (1H, d, 8.0)	3.72 (1H, d, 7.3)
9	3.19 (1H, br s)	3.20 (1H, br s)
11	1.50 (1H, m), 1.44 (1H, m)	1.50 (1H, m), 1.44 (1H, m)
12	1.38 (1H, m), 1.25 (1H, m)	1.38 (1H, m), 1.25 (1H, m)
13	1.28 (2H, m)	1.28 (2H, m)
14	1.38 (1H, m), 1.20 (1H, m)	1.38 (1H, m), 1.20 (1H, m)
15	2.20 (1H, m), 2.02 (1H, m)	2.20 (1H, m), 2.02 (1H, m)
16	5.24 (1H, m)	5.24 (1H, m)
17	6.14 (1H, d, 15.5)	6.16 (1H, d, 15.5)
20	2.70 (1H, dd, 13.7, 10.6), 2.27 (1H, m)	2.69 (1H, dd, 13.7, 10.6), 2.26 (1H, m)
21	4.10 (1H, ddd, 11.3, 4.3, 1.8)	4.09 (1H, ddd, 9.4, 3.0, 1.8)
22	2.11 (1H, m)	2.02 (1H, m)
23	5.17 (1H, m)	5.20 (1H, m)
24	1.71 (2H, m)	1.71 (2H, m)
26	1.45 (1H, m), 1.34 (1H, m)	1.45 (1H, m), 1.34 (1H, m)
27	1.60 (2H, m)	1.60 (2H, m)
28	1.58 (1H, m), 1.25 (1H, m)	1.58 (1H, m), 1.25 (1H, m)
29	4.03 (1H, dt, 10.4, 2.4)	4.05 (1H, br t, 10.3)
30	1.58 (1H, m), 1.30 (1H, m)	1.58 (1H, m), 1.43 (1H, m)
31	3.75 (1H, m)	3.76 (1H, m)
32	1.58 (1H, m), 1.42 (1H, m)	1.66 (1H, m), 1.42 (1H, m)
33	0.99 (3H, t, 7.3)	0.99 (3H, t, 7.3)
34	1.33 (3H, s)	1.32 (3H, s)
35	0.97 (3H, d, 7.0)	0.93 (3H, d, 7.0)
36	1.10 (3H, s)	1.18 (3H, s)
37	2.55 (1H, d, 14.4), 2.49 (1H, dd, 14.4, 2.2)	2.54 (1H, d, 14.0), 2.48 (1H, dd, 14.0, 2.2)
39	1.38 (3H, s)	1.37 (3H, s)
40	1.35 (3H, s)	1.35 (3H, s)
41	0.85 (3H,d, 6.7)	0.85 (3H, d, 7.0)
1′	5.09 (1H,dd, 7.0, 4.0)	
2′	2.00 (1H, m), 1.65 (1H, m)	
3′	2.16 (1H, m), 1.86 (1H, m)	
4′	3.16 (1H, m)	
5′	4.57 (1H, m)	
6′	1.35 (3H, d, 7.0)	
4′-NMe$_2$	2.74 (6H, s)	

Chemical shifts are given in ppm referenced to MeOH-d_4 as 3.31 ppm. The coupling constants, *J*, are given in Hz.

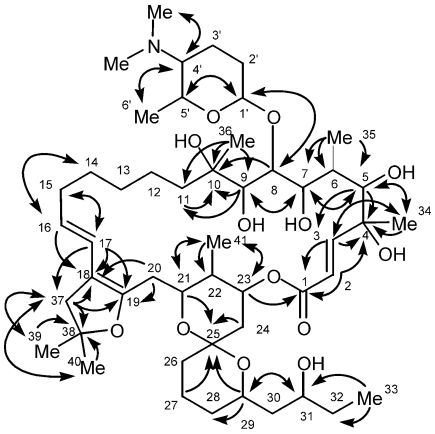


Fig. 2 Key long-range ^{13}C-^{1}H correlations observed in HMBC spectrum of **1**.

determined to be a new 28-demethylated analogue of dunaimycin D3S, as shown in Fig. 1.

The molecular formula of **2** was established as $C_{41}H_{68}O_{12}$ by HRFAB-MS. Comparisons of the molecular formula and the NMR data between **1** and **2** indicated that **2** was an aglycone of **1**, and determined to be a new 28-demethylated aglycone of dunaimycin D3S as shown in Fig. 1. Compounds **1** and **2** also bear close resemblance to ossamycin [2] and NK154183A and B [3], which have been reported from *Streptomyces* spp.

Compounds **1** and **2** exhibited cytotoxic activity against human promyelocytic leukemia HL-60 cells with IC_{50} values of 0.5 and 0.4 μg/ml, respectively. In antimicrobial assay, compounds **1** and **2** were active against *Bacillus subtilis* ATCC 6633 and *Aspergillus niger* JCM 10254 with the same MIC value of 50 μg/ml, but showed no inhibition against *Candida utilis* NBRC 10707 (MIC>100 μg/ml).

Acknowledgements We wish to thank Mr. Tetsuya Nishide for his excellent assistance in the fermentation and isolation.

References

1. Hochlowski JE, Mullally MM, Brill GM, Whittern DN, Buko AM, Hill P, JB. McAlpine JP. Dunaimycins, a new complex of spiroketal 24-membered macrolides with immunosuppressive activity. II. Isolation and elucidation of structures. J Antibiot 44: 1318–1330 (1991)
2. Kirst HA, Mynderse JS, Martin JW, Baker PJ, Paschal JW, Rios Steiner JL, Lobkovsky E, Clardy J. Structure of the spiroketal-macrolide ossamycin. J Antibiot 49: 162–167 (1996)
3. Tsuchiya K, Kimura C, Nishikawa K, Harada T, Nishikiori T, Tatsuta K. NK154183A and B, antitumor substances produced by *Streptomyces* sp. J Antibiot 49: 1281–1283 (1996)