

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

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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%, MgSO₄·7H₂O 0.007%, CaCO₃ 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

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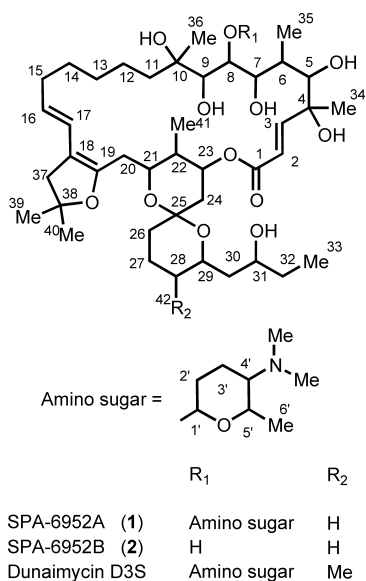


Fig. 1 Structures of SPA-6952A (**1**), SPA-6952B (**2**) and dunaimycin D3S.

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 (<i>m/z</i>)	895 (M+H) ⁺	753 (M+H) ⁺
HRFAB-MS (<i>m/z</i>)		
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,
[α] _D ²⁵ (MeOH)	-48.3 (<i>c</i> 0.12)	-23.8 (<i>c</i> 0.08)
Solubility		
Soluble:	DMSO, MeOH	DMSO, MeOH
Insoluble:	H ₂ O, <i>n</i> -Hexane	H ₂ O, <i>n</i> -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 H₂O 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}

Table 2 ¹³C 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	

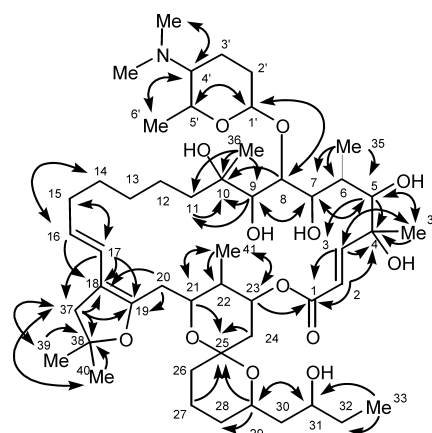
Chemical shifts are given in ppm referenced to MeOH-*d*₄ as 49.0 ppm.

1653 cm⁻¹). The molecular formula of **1** was determined as C₄₉H₈₃NO₁₃ by HRFAB-MS. The ¹H and ¹³C 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 3 ^1H NMR chemical shifts of **1** and **2** in $\text{MeOH-}d_4$ (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 $\text{MeOH-}d_4$ as 3.31 ppm.
The coupling constants, J , are given in Hz.

**Fig. 2** Key long-range $^{13}\text{C-}^1\text{H}$ 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 $\text{C}_{41}\text{H}_{68}\text{O}_{12}$ 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 $\mu\text{g/ml}$, 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 $\mu\text{g/ml}$, but showed no inhibition against *Candida utilis* NBRC 10707 ($\text{MIC} > 100 \mu\text{g/ml}$).

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References

- 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)
- 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)
- 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)