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(*Z*)-Sarcodictyin A, a New Highly Cytotoxic Diterpenoid from the Soft Coral *Bellonella albiflora*

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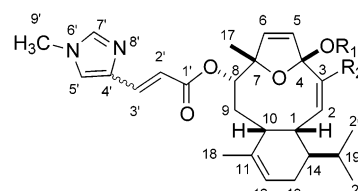
A new cytotoxic diterpene, (*Z*)-sarcodictyin A, was isolated from the Japanese soft coral *Bellonella albiflora*.¹ Its structure was elucidated on the basis of 1D and 2D NMR analysis and chemical degradation. (*Z*)-Sarcodictyin A showed strong cytotoxicity against HeLa human cervix cells.

Sarcodictyin A (**2**) was first isolated from the Mediterranean stolonifer *Sarcodictyon roseum*² and then from the South African soft coral *Eleutherobia aurea* along with two glycosidated congeners, eleuthosides A and B.³ Eleutherobin (**3**), a desacetyl congener of the eleuthosides, was isolated as a potent cytotoxic compound from an Australian *Eleutherobia* sp. It was found to stabilize microtubules by competing with the paclitaxel binding site on microtubule polymers.⁴ Evaluation of the biological activities of this class of compounds using synthetic compounds⁵ led to a common pharmacophore for the microtubule stabilizing activity of the sarcodictyins, eleutherobins, paclitaxel, epothilones, and discodermolide.⁶

In our ongoing program of searching for potential cancer chemotherapeutics from Japanese marine invertebrates, the soft coral *Bellonella albiflora* from southern Japan showed potent cytotoxicity against HeLa human cervix cell lines. Bioassay-guided isolation yielded (*Z*)-sarcodictyin (**1**), along with five known compounds, sarcodictyins A (**2**) and B (**3**), eleutherobin (**4**), (*Z*)-eleutherobin (**5**), and eleutherobin aglycon (**6**). In this paper, the isolation, structure elucidation, and biological activity of this new compound (**1**) are described.

The EtOH extract of the frozen samples (1.5 kg, wet weight) was concentrated and partitioned between H₂O and Et₂O, and the aqueous layer was further extracted with *n*-BuOH. The combined Et₂O and *n*-BuOH layers were subjected to the modified Kupchan procedure⁷ to yield *n*-hexane, CHCl₃, and 60% MeOH layers. The CHCl₃ layer showed potent cytotoxicity and was successively separated by ODS flash chromatography, gel filtration, and ODS HPLC to yield (*Z*)-sarcodictyin A (**1**; 1.0 mg, 6.7 × 10⁻⁵% yield based on wet weight), along with sarcodictyins A (**2**; 14.0 mg, 9.3 × 10⁻⁴%) and B (**3**; 7.4 mg, 4.9 × 10⁻⁴%), eleutherobin (**4**; 6.8 mg, 4.5 × 10⁻⁴%), (*Z*)-eleutherobin (**5**; 0.3 mg, 2.0 × 10⁻⁵%), and eleutherobin aglycon (**6**; 2.5 mg, 1.7 × 10⁻⁴%).

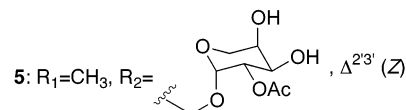
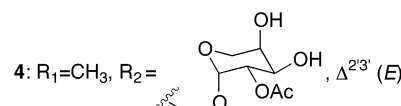
The molecular formula of (*Z*)-sarcodictyin A (**1**) was established as C₂₈H₃₆N₂O₆ by HRFABMS. The ¹H NMR spectrum of **1** was almost superimposable on that of sarcodictyin A (**2**), except for the olefinic protons in the



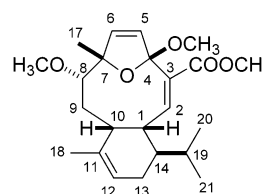
1: R₁=H, R₂=COOCH₃, Δ^{2'3'} (*Z*)

2: R₁=H, R₂=COOCH₃, Δ^{2'3'} (*E*)

3: R₁=H, R₂=COOC₂H₅, Δ^{2'3'} (*E*)



6: R₁=CH₃, R₂=CH₂OH, Δ^{2'3'} (*E*)



urocanyl moiety. These latter protons resonated at δ 5.75 (*J* = 12.7 Hz, H2') and 6.92 (*J* = 12.7 Hz, H3'), while those of **2** were at δ 6.33 (*J* = 15.5 Hz, H2') and 7.50 (*J* = 15.5 Hz, H3'). The coupling constant of 12.7 Hz between H2' and H3' in **1** indicated its *Z*-geometry. Similar upfield shifts of the comparable olefinic protons were also seen in (*Z*)-eleutherobin.⁸

Analysis of the ROESY spectrum established the relative stereochemistry of (*Z*)-sarcodictyin A (**1**). ROESY cross-

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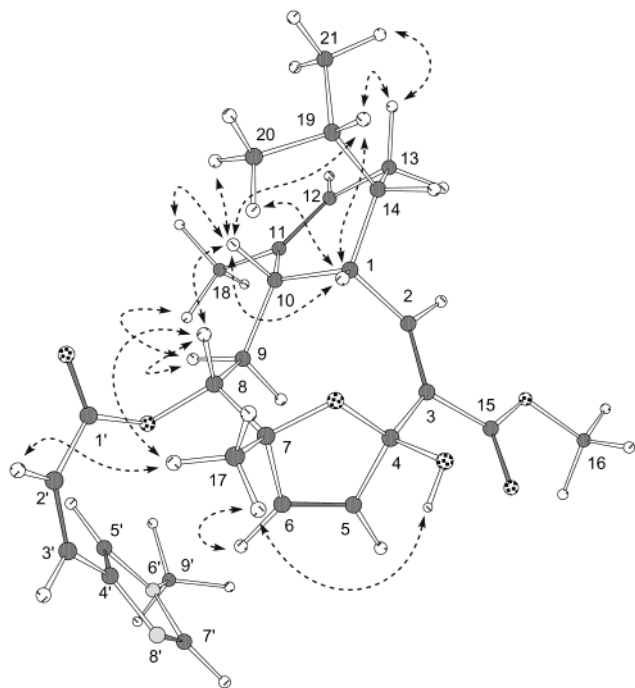


Figure 1. Selected NOEs of (*Z*)-sarcodictyin A (1).

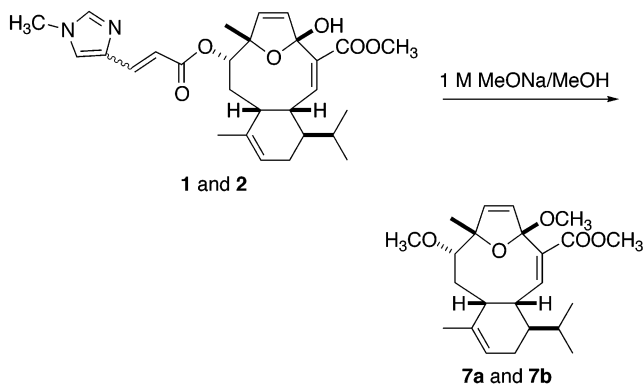


Figure 2. Conversion of **1** and **2** to **7a** and **7b**.

peaks between H₃-20 (δ 0.95–0.97)/H-1 (δ 4.17) and H-10 (δ 4.17) demonstrated that the isopropyl group, H-1, and H-10 were on the same face of the molecule. A strong ROESY correlation from CH₃-17 to 4-OH (δ 6.58) indicated their *cis*-relationship. ROESY cross-peaks between H-8 (δ 4.63), H-10 (δ 4.17), and H-17 (δ 1.34) correlated both relative stereochemistries above, thus confirming that (*Z*)-sarcodictyin A (**1**) had the same relative stereochemistry of the ring portion as that of sarcodictyin A (**2**).

Optical rotations of (*Z*)-sarcodictyin A (**1**) and sarcodictyin A (**2**) showed the opposite signs ($[\alpha]_D^{+40}$ for **1** and -15.2° for **2**).^{2a} Similar inversions of signs were seen for sarcodictyins C (-16.5° , EtOH, c 0.085) and E ($+15.6^\circ$, MeOH, c 0.42),^{2b} suggesting the same absolute stereochemistry for **1** and **2**. To confirm the absolute stereochemistry, (*Z*)-sarcodictyin A (**1**) and sarcodictyin A (**2**) were subjected to transesterification with MeONa/MeOH, in which the urocanyl moieties were removed; **1** and **2** were thus converted to **7a** and **7b** (Figure 2). Although the hydroxyl groups of **7a** and **7b** were unexpectedly methylated, FABMS and ¹H NMR data indicated they had identical planar structures. The CD spectra for **7a** and **7b** both showed negative Cotton effects (231 nm/205 nm), which indicated that they had the same absolute stereochemistry (Figure 3).

(*Z*)-Sarcodictyin A (**1**) was cytotoxic against HeLa cells with an IC₅₀ value of 90 ng/mL. The known compounds **2**–**6** showed cytotoxicity against HeLa cells with IC₅₀'s of 17–90 ng/mL. (*Z*)-Sarcodictyin A (**1**) showed cytotoxicity against HeLa cells comparable to that of sarcodictyin A (**2**), thereby indicating the geometry of the double bond was not important for activity. This is also the case for eleutherobin (**4**, IC₅₀ value 17 ng/mL) and (*Z*)-eleutherobin (**5**, IC₅₀ value 17 ng/mL).⁸ Altering the C-3 substituent from a methoxycarbonyl group in **2** to an ethoxycarbonyl group in sarcodictyin B (**3**, IC₅₀ value 90 ng/mL) has little effect. Eleutherobin aglycon (**6**) is considerably less active than **4** and **5** (IC₅₀ values; **6** = 90 ng/mL, **4/5** = 17 ng/mL), so the loss of sugar decreases potency in the eleutherobin series.^{5c,8}

Experimental Section

General Experimental Procedures. Optical rotation was measured on a JASCO DIP-1000 digital polarimeter. UV spectra were recorded on a Shimadzu BioSpec-1600. CD spectra were recorded in MeOH using a JASCO J-820 spectropolarimeter. NMR spectra were recorded on a JEOL A600 NMR spectrometer operating at 600 MHz for ¹H and 150 MHz for ¹³C. ¹H and ¹³C NMR chemical shifts were referred to DMSO-*d*₆ (δ_H 2.49 and δ_C 39.5). FAB mass spectra were measured on a JEOL JMS700 tandem mass spectrometer using NBA with NaCl as a matrix.

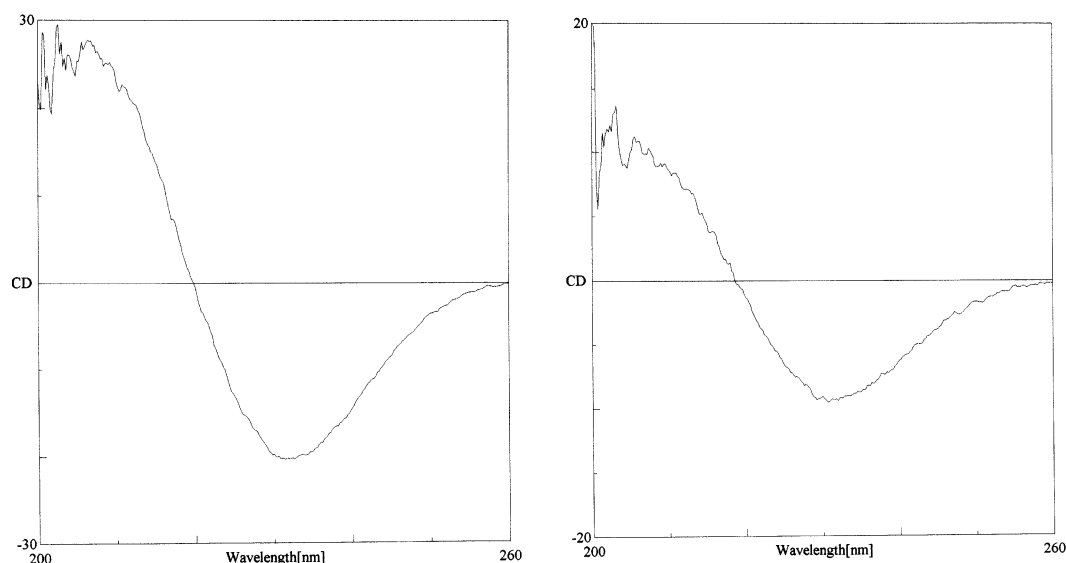
Animal Material. The animal specimens were collected by hand using scuba off Shishi-jima Island in the Amakusa Islands (32°17' N; 130°12' E) in July 1999. They were kept frozen at -20°C until processed.

Isolation. Frozen animals (1.5 kg) were exhaustively extracted with EtOH (3 \times 3 L), and the combined extract was concentrated and partitioned between H₂O and Et₂O. The aqueous layer was further extracted with *n*-BuOH, which was combined with the Et₂O layer. The combined organic layers were subjected to the modified Kupchan procedure:⁷ the layers were first partitioned between *n*-hexane and MeOH/H₂O (90:10), then the MeOH/H₂O (90:10) layer was diluted with H₂O to make MeOH/H₂O (60:40), which was extracted with CHCl₃. The CHCl₃ layer, showing potent cytotoxicity, was separated by ODS flash chromatography using MeOH/H₂O (5:5 and 7:3), MeCN/H₂O (7:3 and 9:1), MeOH, and CHCl₃/MeOH/H₂O (70:30:5). Fractions eluted with MeOH/H₂O (7:3) and MeCN/H₂O (7:3) were combined, concentrated, and gel filtered on a Sephadex LH-20 column (3 \times 90 cm) using MeOH. Cytotoxic fractions were collected and separated by reversed-phase HPLC [COSMOSIL 5C₁₈ ARII, 20 \times 250 mm] using a linear gradient elution from MeOH/H₂O (60:40) to MeOH. The cytotoxic fraction was further purified by reversed-phase HPLC [COSMOSIL 5C₁₈ ARII, 10 \times 250 mm] using MeCN/H₂O (50:50) to afford pure compounds **1** (1.0 mg), **2** (14.0 mg), **3** (6.8 mg), **4** (0.3 mg), **5** (7.4 mg), and **6** (2.5 mg).

(*Z*)-Sarcodictyin A (1**):** white powder; $[\alpha]_D^{+40}$ (c 0.1, EtOH); UV (MeOH) λ_{max} 208 (ϵ 16000), 217 (ϵ 14000), 296 (ϵ 9900) nm; ¹H and ¹³C NMR, see Table 1; FABMS m/z 497 [$M + H$]⁺, 519 [$M + Na$]⁺; HRFABMS m/z 497.2635 (calcd for C₂₈H₃₇N₂O₆ 497.2651).

Transesterification of **1 and **2**.** A portion (0.3 mg) of **1** was dissolved in 0.3 mL of 1 M MeONa/MeOH, and the solution was stirred for 6 h at room temperature. The reaction mixture was applied onto a SiO₂ short column (1 \times 3 cm) and eluted with Et₂O. After evaporating Et₂O, the resulting material was dissolved in MeOH and separated by ODS HPLC [COSMOSIL 5C₁₈-ARI, 10 \times 250 mm] using MeOH as the solvent to give **7a**. Sarcodictyin A was similarly treated to furnish **7b**.

7a: colorless solid; CD (MeOH) $\Delta\epsilon$ – (231 nm), + (205 nm); ¹H NMR (CD₃OD, 600 MHz) δ 4.17 (1H, m, H-1), 6.73 (1H, d, J = 9.6, H-2), 6.37 (1H, d, J = 5.8, H-5), 6.23 (1H, d, J = 5.8, H-6), 3.60 (1H, d, J = 7.7, H-8), 1.76 (1H, dd, J = 1.9, 14.6, H-9a), 1.24 (1H, m, H-9b), 2.44 (1H, bd, J = 12.3, H-10), 5.34 (1H, bs, H-12), 2.38 1H, (bd, J = 18.8, H-13a), 2.09 (1H, bd, J

**Figure 3.** CD spectra of **7a** (right) and **7b** (left).**Table 1.** NMR Spectral Data for (*Z*)-Sarcodictyin A (**1**) in DMSO-*d*₆

C#	¹³ C	¹ H mult., <i>J</i> (Hz)	HMBC	ROSEY
1	34.0	4.17 m		H-8, H-9b, H-10, H-20, H-21
2	142.6	6.44 s	C-4, C-14, C-15	H-10, H-13a, H-13b, H-14, H-16, H-19
3	134.3			
4	114.0			
5	133.5	6.43 d, 5.4	C-4, C-6, C-7	H-6, H-9b
6	132.3	6.09 d, 5.4	C-5, C-6, C-7	H-5, H-9b, H-17
7	88.5			
8	80.4	4.63 d, 7.3	C-5, C-7, C-9, C-10, C-17	H-1, H-9b, H-10, H-20, H-21
9	31.1	1.57 d, 3.5	C-7, C-8, C-10, C-11	H-14
		1.25 m	C-7, C-8, C-10	H-1, H-5, H-6, H-8
10	38.5	2.55 m		H-1, H-2, H-8, H-18, H-20, H-21
11	133.3			
12	121.2	5.31 m		H-13a, H-13b, H-14, H-18
13	23.8	2.30 m		H-2, H-12, H-14
		2.04 m		H-2, H-12, H-20, H-21
14	41.5	1.22 m		H-2, H-12, H-13a
15	166.7			
16	52.7	3.63 s	C-15	H-2
17	25.3	1.34 s	C-6, C-7, C-8	H-6, H-8
18	20.3	1.47 s	C-10, C-11, C-12	H-10, H-12, OH
19	28.5	1.25 m		H-2
20	21.9	0.97 d, 6.9	C-14, C-19, C-21	H-1, H-8, H-10, H-13a
21	21.8	0.95 d, 6.5	C-14, C-19, C-20	H-1, H-8, H-10, H-13a
1'	165.4			
2'	112.3	5.75 d, 12.7	C-4'	H-3'
3'	138.9	6.92 d, 12.7	C-1', C-5'	H-2'
4'	136.1			
5'	125.9	8.22 s	C-4'	H-9'
7'	138.4	7.65 s	C-4'	H-9'
9'	33.5	3.70 s	C-5', C-7'	H-5', H-7'
OH		6.58 s	C-3, C-4	H-17

= 20.0, H-13b), 1.32 (1H, m, H-14), 3.70 (3H, s, H-16), 1.51 (3H, s, H-17), 1.60 (3H, s, H-18), 1.56 (1H, d sept, *J* = 10.0, 6.5, H-19), 0.97 (3H, d, *J* = 6.5, H-20), 0.95 (3H, d, *J* = 6.9, H-21), 3.19 (3H, s, OMe-4 or -8); (CDCl₃, 600 MHz) δ 4.13 (1H, ddd, *J* = 2.7, 3.8, 9.2, H-1), 6.70 (1H, d, *J* = 10.0, H-2), 6.46 (1H, d, *J* = 5.8, H-5), 6.20 (1H, d, *J* = 5.8, H-6), 3.70 (1H, d, *J* = 8.5, H-8), 1.70 (1H, dd, *J* = 2.3, 15.0, H-9a), 1.3 (1H, m, H-9b), 2.43 (1H, bd, *J* = 12.7, H-10), 5.32 (1H, bs, H-12), 2.40 (1H, bd, *J* = 21.2, H-13a), 2.03 (1H, bd, *J* = 20.0, H-13b), 1.40 (1H, m, H-14), 3.71 (3H, s, H-16), 1.56 (3H, s, H-17), 1.58 (3H, s, H-18), 1.53 (1H, d sept, *J* = 9.5, 6.7, H-19), 0.932 (3H, d, *J* = 6.5, H-20), 0.927 (3H, d, *J* = 6.5, H-21), 3.48 (3H, s, OMe-4 or -8), 3.23 (3H, s, OMe-4 or -8); FABMS *m/z* 391 [M + H]⁺, 413 [M + Na]⁺.

7b: colorless solid; CD (MeOH) Δε – (231 nm), + (205 nm); ¹H NMR (CD₃OD, 600 MHz) δ 4.17 (1H, m, H-1), 6.73 (1H, d, *J* = 10.0, H-2), 6.37 (1H, d, *J* = 6.2, H-5), 6.23 (1H, d, *J* = 6.2,

H-6), 3.59 (1H, d, *J* = 7.7, H-8), 1.76 (1H, dd, *J* = 1.9, 14.6, H-9a), 1.24 (1H, m, H-9b), 2.44 (1H, bd, *J* = 12.3, H-10), 5.34 (1H, bs, H-12), 2.38 (1H, bd, *J* = 19.2, H-13a), 2.09 (1H, bd, *J* = 19.6, H-13b), 1.31 (1H, m, H-14), 3.69 (3H, s, H-16), 1.53 (3H, s, H-17), 1.60 (3H, s, H-18), 1.56 (1H, d sept, *J* = 10.0, 6.5, H-19), 0.97 (3H, d, *J* = 6.9, H-20), 0.95 (3H, d, *J* = 6.9, H-21), 3.19 (3H, s, OMe-4 or -8); (CDCl₃, 600 MHz) δ 4.13 (1H, ddd, *J* = 3.5, 4.2, 10.4, H-1), 6.70 (1H, d, *J* = 10.0, H-2), 6.46 (1H, d, *J* = 5.8, H-5), 6.20 (1H, d, *J* = 5.8, H-6), 3.70 (1H, d, *J* = 8.5, H-8), 1.70 (1H, dd, *J* = 1.6, 14.9, H-9a), 1.3 (1H, m, H-9b), 2.43 (1H, bd, *J* = 12.7, H-10), 5.32 (1H, bs, H-12), 2.38 (1H, bd, *J* = 21.5, H-13a), 2.03 (1H, bd, *J* = 20.4, H-13b), 1.40 (1H, m, H-14), 3.71 (3H, s, H-16), 1.56 (3H, s, H-17), 1.58 (3H, s, H-18), 1.53 (1H, d sept, *J* = 10.0, 6.2, H-19), 0.931 (3H, d, *J* = 6.2, H-20), 0.926 (3H, d, *J* = 6.5, H-21), 3.48 (3H, s, OMe-4 or -8), 3.23 (3H, s, OMe-4 or -8); FABMS *m/z* 391 [M + H]⁺, 413 [M + Na]⁺.

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