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Sinulobatins A ~ D, New Amphilectane-type Diterpenoids from the Japanese Soft Coral *Sinularia nanolobata* [1]

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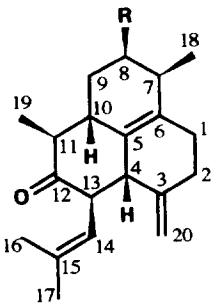
Abstract: Four new amphilectane-type diterpenoids, sinulobatins A (**1**), B (**2**), C (**3**) and D (**4**) were isolated from the Japanese soft coral *Sinularia nanolobata*. Their structures were determined based on spectroscopic evidence and single crystal X-ray analysis. The absolute stereochemistry of **1** was elucidated on the basis of its CD spectrum. These compounds exhibited cytotoxicity against L1210 cells and KB cells *in vitro* with IC_{50} values of 3.0 - 7.7 μg/mL. © 1997 Elsevier Science Ltd.

INTRODUCTION

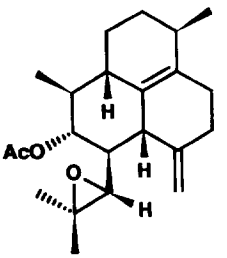
The soft corals have been shown to contain a fairly large variety of secondary metabolites mainly diterpenoids, sesquiterpenoids, and steroids. Many of these constituents are structurally unique and exhibit interesting biological activities, e.g., ichthyotoxicity, toxicity to brine shrimp, cytotoxicity, antimicrobial and antiinflammatory activities.[2] Recently, a variety of diterpenoids were isolated from the Japanese soft corals.[3] We have also reported the structures of two hemolytic diterpenoids,[4] one ichthyotoxic diterpenoid,[5] and two diterpenoids that displayed toxicity to brine shrimp,[6] which were isolated from some Japanese soft corals. In the course of our continuing research on the biologically active constituents from Japanese soft corals, we found that the *n*-hexane soluble part of the MeOH/$CHCl_3$ = 1:1 extracts of *Sinularia nanolobata* (genus: *Sinularia*, family: Alcyoniidae, order: Alcyonacea) showed cytotoxicity against L1210 and KB cells *in vitro*. The bioassay-directed fractionation of the extracts has led to the isolation of four new compounds, designated sinulobatins A (**1**), B (**2**), C (**3**) and D (**4**). In this paper, we report the isolation and structure determination of these compounds on the basis of their spectral properties. The absolute configuration and the cytotoxic activity of these compounds are also discussed.

RESULTS AND DISCUSSION

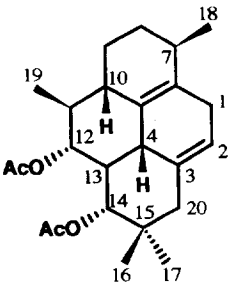
S. nanolobata was collected along the Nichinan Coast, Miyazaki Prefecture, Japan, in December, 1994. Freshly collected animals were stored frozen and subsequently extracted with MeOH followed by a MeOH/$CHCl_3$ = 1:1 mixture. The MeOH and MeOH/$CHCl_3$ mixed solutions were combined and concentrated *in vacuo* to an aqueous suspension followed by extraction with *n*-hexane. The *n*-hexane extract from the whole bodies of *S. nanolobata* (26.0 kg) showed significant cytotoxicity against L1210



sinulobatin A (**1**) : R = OAc
sinulobatin B (**2**) : R = H



sinulobatin C (**3**)



sinulobatin D (**4**)

and KB cells (against L1210 cell with IC_{50} 13.9 μg/ml; against KB cell with IC_{50} 11.7 μg/ml). The crude extract was fractionated by gel-filtration on Sephadex LH-20, silica gel column chromatography, reverse phase column chromatography, and reverse phase HPLC (ODS column) to give sinulobatins A (**1**), B (**2**), C (**3**) and D (**4**).

Sinulobatin A (**1**) was isolated as colorless prisms, m.p. 126.0 - 128.0°C, $[\alpha]_D^{25}$ +115.6° (*c* 0.43, $CHCl_3$). The molecular formula of $C_{22}H_{30}O_3$ was determined by HR positive FAB mass spectrometry (*m/z* 343.2276, $[M + H]^+$, Δ +0.3 mmu) in combination with the ^{13}C-NMR data. The IR spectrum of **1** showed prominent peaks due to double bond (3040, 1660 and 900 cm^{-1}), carbonyl (1715 cm^{-1}), and ester (1735 and 1220 cm^{-1}) groups. The ^1H-NMR spectrum of **1** contained two secondary methyls [δ 1.10 (3H, d, J = 6.4 Hz), 1.00 (3H, d, J = 7.0 Hz)], two vinyl methyls [δ 1.75 (3H, d, J = 1.4 Hz), 1.44 (3H, d, J = 1.4 Hz)], an acetylmethyl [δ 2.06 (3H, s)], an oxymethine [δ 5.11 (1H, m)], an exocyclyc methylene [δ 4.78, 4.56 (1H each, d, J = 1.7 Hz)], and an olefinic proton [δ 5.13 (1H, dq, J = 1.4, 10.0 Hz)]. The ^{13}C-NMR spectrum of **1** suggested the presence of three methylene carbons [δ 27.8, 28.8, 32.1], five methine carbons [δ 38.1, 43.7, 50.9, 51.4, 55.3], four quaternary olefinic carbons [δ 130.3, 132.2, 134.8, 145.0], one ester carbon [δ170.9], and one carbonyl carbon [δ 210.7] (Table 2). These data were confirmed by a HSQC spectrum. Based on these data and the molecular formula, **1** was considered to be a tricyclic C_{22} diterpenoid possessing acetoxyl, exocyclic methylene, trisubstituted olefin, and ketone groups. These facts and the ^1H-^1H COSY spectrum of **1** clearly implied the presence of five partial structures (**a**) ~ (**e**) (Fig. 1). Connectivities of these partial structures were determined by a detailed analysis of the HMBC spectrum of **1**. In particular, HMBC correlations of δ_H 2.28 (H-1) to δ_C 132.2 (C-6), δ_H 2.52 (H-4) to δ_C 130.3 (C-5), δ_H 3.24 (H-13) to δ_C 210.7 (C-12), δ_H 1.00 (H-18) to δ_C 132.2 (C-6), δ_H 1.10 (H-19) to δ_C 210.7 (C-12), δ_H 4.56 (H-20) to δ_C 28.8 (C-2) and δ_C 51.4 (C-4), and δ_H 4.78 (H-20) to δ_C 51.4 (C-4) suggested the connectivity of the partial structure (**a**) ~ (**e**) as shown in Figure 1. Thus **1** was elucidated to be an amphilectane-type diterpenoid.[7] The relative stereochemistry of **1** was revealed by NOE correlations as shown in Figure 2 and J values for the ^1H-NMR spectrum, that is, $J_{4\text{-}13}$ = 12.4 Hz, $J_{10\text{-}11}$ = 14.3 Hz, and $J_{13\text{-}14}$ = 10.0 Hz. Furthermore, the structure was confirmed by means of a single crystal X-ray analysis. Figure 3 shows the ORTEP perspective view of **1**. The absolute configuration of **1** was established by studying its CD spectrum which showed a positive Cotton effect at 294 nm (Δε + 6.3) due to an n→π* transition of the arbonyl group. By applying the

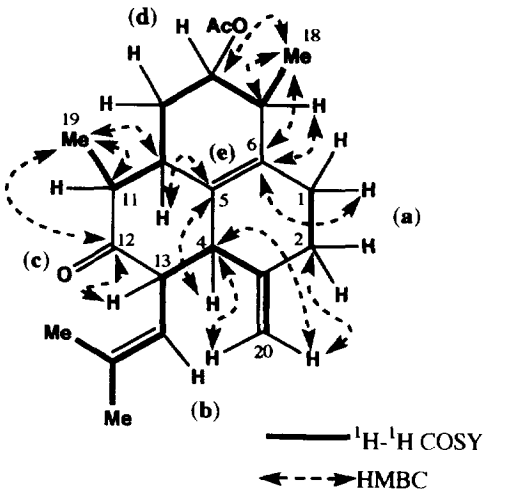


Figure 1. Partial structures (**a**) ~ (**e**) and HMBC correlations of sinulobatin A (**1**)

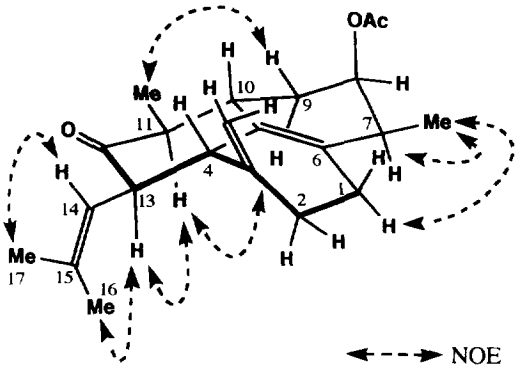


Figure 2. NOESY correlations for sinulobatin A (**1**)

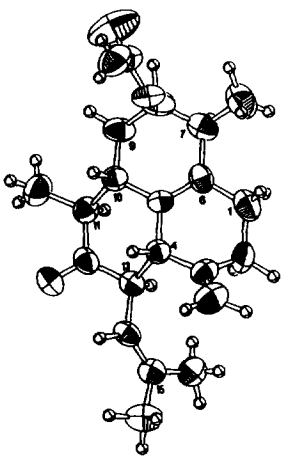


Figure 3. An Ortep view of sinularin A (**1**)

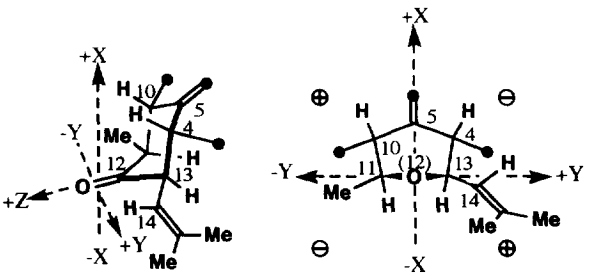


Figure 4. Octant projective view and back octant of sinularin A (**1**)

octant rule[8] to the cyclohexanone system, it could be seen that the side chain at C-13 lay in the back-lower-right octant as shown in Figure 4. These facts suggested that the absolute stereochemistry of C-13 was ascertained to be R, and therefore, the absolute configuration of **1** as 4S, 7S, 8R, 10S, 11S, and 13R was established. Thus, the structure and absolute configuration of sinulobatin A was elucidated as **1** in Figure 2.

Sinulobatin B (**2**) was isolated as a colorless amorphous material, $[\alpha]_D^{25}$ +105.2° (*c* 0.10, $CHCl_3$). The HR positive FAB mass spectrometry (*m/z* 285.2213, $[M+H]^+$, Δ -0.6 mmu) and the ^{13}C-NMR data of **2** indicated a molecular formula of $C_{20}H_{28}O$. **2** showed prominent signals due to

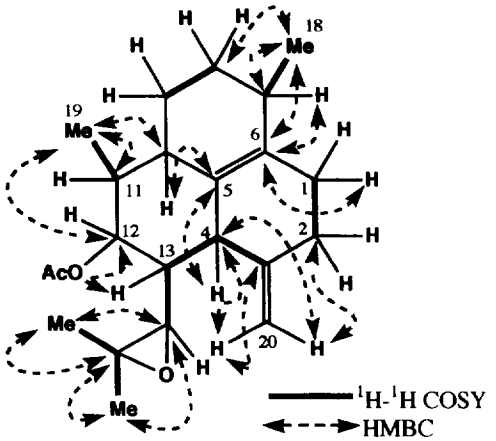


Figure 5. ^{1}H-^{1}H COSY and HMBC correlations of sinulobatin C (**3**)

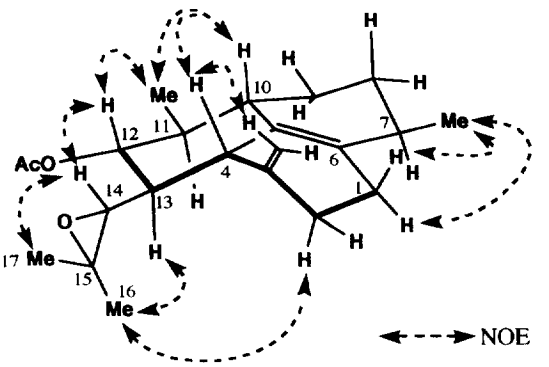


Figure 6. NOESY correlations for sinulobatin C (**3**)

double bonds (3020, 1650 and 890 cm^{-1}) and a carbonyl (1710 cm^{-1}) in the IR spectrum. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1** except for the lack of a signal ascribable to an acetoxyl group at C-8 (Tables 1 and 2). This fact suggested that **2** was the deacetoxylate of **1**, and this was confirmed by detailed analysis of the ^{1}H-^{1}H COSY and HMBC spectra in the same way as in **1**. Thus the structure of sinulobatin B was elucidated as **2**.

Sinulobatin C (**3**), a colorless amorphous material, $[\alpha]_D^{25}$ +98.2° (*c* 0.23, $CHCl_3$), was analyzed for $C_{22}H_{32}O_3$ based on HR positive FAB mass (*m/z* 367.2274, $[M+Na]^+$, Δ +2.4 mmu) and the ^{13}C-NMR spectral data. The IR spectrum of **3** showed prominent signals due to double bonds (3010, 1655 and 900 cm^{-1}) and an epoxy group (3000 and 1260 cm^{-1}). The ^{1}H and ^{13}C-NMR spectra of **3** were similar to those of **1** except for the lack of the signal ascribable to a carbonyl group at C-12, a trisubstituted double bond at C14-C15 and the presence of an epoxy [9] and acetoxyl groups (Tables 1 and 2). The position of the epoxy group in **3** was suggested by a detailed analysis of the ^{1}H and ^{13}C-NMR spectra of **3** (Table 1). Namely, the signals due to H-16 and H-17 in **3** shifted to an upper field (Table 1), and the HMBC correlations of H-16 and H-17 to C-14 and C-15, respectively, were observed as shown in Figure 5. This fact indicated that the epoxy group was located at the C14-C15 positions of **3**. Whereas, the position of the acetoxyl group was also revealed using the 2D NMR spectrum as shown in Figure 5. The relative stereochemistry of **3** was also confirmed on the basis of the NOE correlations as shown in Figure 6 and *J* values in the ^{1}H-NMR spectrum, that is, $J_{4\text{-}13}$ = 13.9 Hz and $J_{13\text{-}14}$ = 9.6 Hz. Thus the structure and relative stereochemistry of sinulobatin C was elucidated as **3** in Figure 6.

Sinulobatin D (**4**) was isolated as a colorless amorphous material, $[\alpha]_D^{26}$ +30.6° (*c* 0.07, $CHCl_3$). On the basis of HR positive FAB mass spectrometry (*m/z* 409.2342, $[M+Na]^+$, Δ −1.3 mmu) and the ^{13}C-NMR data, a molecular formula of $C_{24}H_{34}O_4$ was determined. The signal due to an ester group (1730 and 1260 cm^{-1}) was also recognized in the IR spectrum of **4**. The ^{1}H-NMR spectrum of **4** contained two tertiary methyls [δ 0.82, 0.84 (3H each, s)], two secondary methyls [δ 0.83 (3H, d,

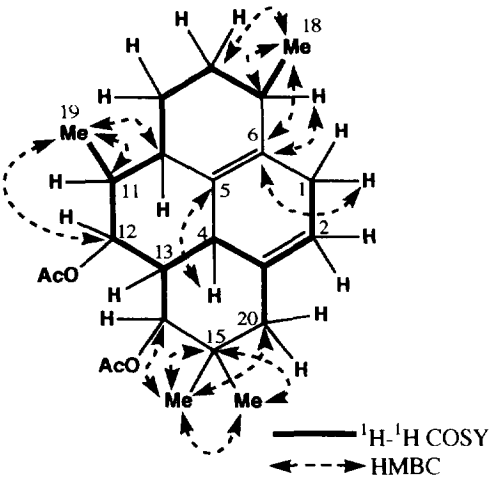


Figure 7. ^{1}H-^{1}H COSY and HMBC correlations of sinulobatin D (**4**)

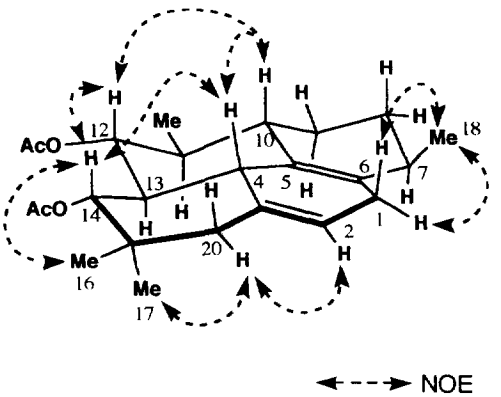


Figure 8. NOESY correlations for sinulobatin D (**4**)

J = 5.9 Hz), 1.01 (3H, d, J = 6.9 Hz)], two acetylmethyls [δ 2.04, 2.05 (3H each, s)], two oxymethines [δ 4.80 (1H, t, J = 9.9 Hz), 5.00 (1H, d, J = 10.6 Hz)], and one olefinic proton [δ 5.42 (1H, dd, J = 3.4, 6.3 Hz)]. The ^{13}C-NMR spectrum of **4** suggested the presence of four methyl carbons [δ 15.3, 19.2, 20.0, 28.3], four methylene carbons [δ 25.0, 29.4, 47.3 × 2], five methine carbons [δ 33.0, 43.2, 43.6, 46.1 × 2], two oxymethine carbons [δ 79.8, 81.2], four olefinic carbons [δ 119.3, 130.3, 131.9, 137.8], and two ester carbons [δ170.9 × 2] (Table 2). By considering these data and the degree of unsaturations of **4** and comparisons with the ^{1}H and ^{13}C-NMR data of sinulobatins A (**1**), B (**2**), and C (**3**), it was evident that the C-5 through C-12 portion of **4** was essentially identical to the comparable part of **3**, and that **4** possessed four rings of these systems. These facts were confirmed by the ^{1}H-^{1}H COSY and HMBC spectra of **4** and these spectra suggested that the fourth ring was constructed between C15 and C20 as shown in Figure 7. The position of the second acetoxyl group was also revealed based on the HMBC spectrum (Figure 7). The relative stereochemistry of **4** was also, in the same manner as sinulobatins A (**1**), B (**2**), and C (**3**), confirmed on the basis of the NOE correlations as shown in Figure 8 and J values from the ^{1}H-NMR spectrum, that is, $J_{11\text{-}12}$ = 9.9 Hz and $J_{13\text{-}14}$ = 10.6 Hz. Thus the structure of sinulobatin D was elucidated as **4** in Figure 8.

Sinulobatins A (**1**), B (**2**), and C (**3**) were tested for their cytotoxic activity against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells in vitro by using an MTT assay.[10] Compounds **1**, **2**, and **3** exhibited cytotoxity against L1210 cells with IC_{50} values of 3.0, 4.8, and 3.2 μg/mL, respectively, and against KB cells with IC_{50} values of 5.1, 7.7, and 4.5 μg/mL, respectively.

The amphilectane type diterpenoids have been isolated from a blue coral,[9] the gorgonian corals,[11] and the marine sponges.[7,12] However, compounds **1**, **2**, **3**, and **4**, isolated in this time, are the first amphilectane type diterpenoids isolated from the alcyonacean corals.

Table 1. ^{1}H-NMR Data of sinulobatins A (**1**), B (**2**), C (**3**), and D (**4**) (500 MHz, in $CDCl_3$)

Position	1	2	3	4
1	2.01 (1H, dt, 5.0, 14.9) 2.28 (1H, m)	2.17 (1H, m) 2.24 (1H, m)	2.03 (1H, m) 2.25 (1H, m)	2.50 (1H, dd, 6.3, 22.2) 2.79 (1H, dd, 3.4, 22.2)
2	2.20 (1H, m) 2.25 (1H, m)	2.24 (2H, m)	2.02 (1H, m) 2.05 (1H, m)	5.42 (1H, dd, 3.4, 6.3)
3	-	-	-	-
4	2.52 (1H, d, 12.4)	2.51 (1H, d, 12.1)	2.52 (1H, d, 13.9)	2.40 (1H, m)
5	-	-	-	-
6	-	-	-	-
7	2.49 (1H, dq, 5.1, 7.0)	2.15 (1H, m)	2.03 (1H, m)	2.05 (1H, m)
8	5.11(1H, m)	1.17 (1H, m) 1.80 (1H, ddt, 3.0 5.6, 12.9)	2.20 (1H, m) 1.73 (1H, m)	1.25 (1H, m) 1.77 (1H, m)
9	1.68 (1H, m) 2.06 (1H, m)	1.36 (1H, dddd, 3.0 7.3, 10.9, 14.6) 2.02 (1H, ddt, 6.7, 14.6)	1.19 (1H, m) 1.99 (1H, m)	1.38 (1H, m) 1.96 (1H, m)
10	2.03 (1H, dt, 4.1, 14.3)	1.94 (1H, dt, 6.7, 11.0)	1.75 (1H, m)	2.05 (1H, m)
11	2.41 (1H, dq, 6.4, 14.3)	2.29 (1H, dq, 6.4, 11.0)	1.22 (1H, m)	1.94 (1H, m)
12	-	-	4.95 (1H, t, 10.2)	4.80 (1H, t, 9.9)
13	3.24 (1H, dd, 10.0, 12.4)	3.27 (1H, ddd, 1.1, 10.7, 12.1)	1.25 (1H, m)	1.88 (1H, m)
14	5.13 (1H, dq, 1.4, 10.0)	5.14 (1H, dt, 1.4, 10.7)	2.49 (1H, d, 9.6)	5.00 (1H, d, 10.6)
15	-	-	-	-
16	1.44 (3H, d, 1.4)	1.44 (3H, d, 1.1)	1.07 (3H, s)	0.82 (3H, s)
17	1.75 (3H, d, 1.4)	1.75 (3H, d, 1.1)	1.17 (3H, s)	0.84 (3H, s)
18	1.00 (3H, d, 7.0)	1.00 (3H, d, 7.1)	0.95 (3H, d, 6.9)	1.01 (3H, d, 6.9)
19	1.10 (3H, d, 6.4)	1.09 (3H, d, 6.4)	0.92 (3H, d, 6.3)	0.83 (3H, d, 5.9)
20	4.56 (1H, d, 1.7) 4.78 (1H, d, 1.7)	4.53 (1H, d, 1.8) 4.75 (1H, t, 1.6)	4.67 (1H, d, 2.0) 4.84 (1H, d, 1.7)	1.87 (1H, m) 2.02 (1H, m)
8-OAc	2.06 (3H, s)	-	-	-
12-OAc	-	-	2.12 (3H, s)	2.04 (3H, s)
14-OAc	-	-	-	2.05 (3H, s)

Spectra were aquired at 23°C. Chemical shifts were given in ppm with respected to TMS as the internal standard.
All assignments were based on ^{1}H-^{1}H COSY, HSQC and HMBC experiments.

Table 2. ^{13}C-NMR Data of sinulobatins A (**1**), B (**2**), C (**3**), and D (**4**) (125 MHz, in $CDCl_3$)

Position	1	2	3	4
1	32.1	31.8	30.6	47.3
2	28.8	29.2	31.6	119.3
3	145.0	145.0	147.3	137.8
4	51.4	52.0	47.0	43.6
5	130.3	130.5	130.3	130.3
6	132.2	135.6	134.8	131.9
7	38.1	35.1	35.0	33.0
8	70.1	29.9	30.6	29.4
9	27.8	27.4	27.1	25.0
10	43.7	45.4	42.4	43.2
11	50.9	51.7	43.7	46.1
12	210.7	210.0	79.0	79.8
13	55.3	54.8	46.4	46.1
14	120.9	121.2	67.2	81.2
15	134.8	135.6	57.2	37.5
16	18.4	19.2	12.0	20.0
17	25.8	25.9	24.5	28.3
18	12.1	19.3	19.1	15.3
19	13.2	11.9	14.9	19.2
20	110.6	110.0	109.3	47.3
8-OAc	21.3, 170.9	-	-	-
12-OAc	-	-	21.4, 169.5	21.4, 170.9
14-OAc	-	-	-	21.6, 170.9

Spectra were aquired at 23°C. Chemical shifts were given in δ(ppm) with respected to TMS as the internal standard. All assignments were aided by DEPT, HSQC and HMBC experiments.

EXPERIMENTAL SECTION

General Experimental Procedures.

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Spectra were recorded using the following instruments: specific optical rotations, JASCO DIP-370 digital polarimeter; IR, JASCO IR-700 infrared spectrophotometer. ^{1}H-NMR spectra were measured at 500 MHz with a Varian Unity-500 and at 600 MHz with a Varian Unity-600 spectrometer. ^{13}C-NMR spectra were measured at 125 MHz with a Varian Unity-500 and at 67.8 MHz with a JEOL GX-270 spectrometer. Chemical shifts were given on a δ (ppm) scale with TMS as the internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). FAB MS and HR positive FAB MS spectra were recorded using a JEOL DX-300 (6 kV, xenon atom beam), and the spectra were measured in CHCl3/*m*-NBA (*m*-nitrobenzyl alcohol) solution. X-ray crystallographic measurements were made on a Rigaku RASA-5R automatic single crystal X-ray instrument. Column chromatography was performed with Sephadex LH-20 (Pharmcia), Kieselgel 60 (No. 7734, Merck), and Cosmosil 5C18 (Nacalai Tesque). Flash chromatography was performed on a Bullcraft Compressor E-5305-T (KISO POWER TOOL) with LiChroprep RP-8 (9362-1M, Cica Merck). HPLC was conducted with a JASCO BIP-I model and a RID-300 RI detector with a Wakosil 5C18 (ODS) column (Wako). Normal and reverse phase TLCs were performed with Kieselgel 60 F_{254} (No. 5715, Merck) and RP-8 F_{254} (No. 13725, Merck), respectively.

Collection, Extraction, and Isolation.

The soft coral *Sinularia nanolobata* (26.0 kg wet weight) was collected by hand using SCUBA at a depth of 2 - 3 m on the coral reef near Nichinan City, Miyazaki Prefecture, Japan, in December, 1994 and identified as *Sinularia nanolobata* Verseveldt, 1977 by Mr. Yukimitsu Imahara. A voucher specimen (No. WMNH-94-INV-4) is presently on deposit at the Wakayama Prefectural Museum of Natural History (Wakayama, Japan). Freshly collected animals were stored frozen and subsequently homogenized with MeOH (30 L) and left at room temperature for a few hours. After filtration, the residue was further extracted with a MeOH/CHCl3 = 1:1 mixture (30 L). The MeOH and MeOH/CHCl3 mixed solutions were combined and concentrated *in vacuo* to an aqueous suspension (8 L) which was then extracted with *n*-hexane (2.7 L × 3). The obtained organic layer was then successively dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue (195.5 g), which showed cytotoxicity, was combined and permeated through a Sephadex LH-20 column, and further eluted with MeOH/CHCl3 (1:1) to yield five fractions: fraction 1 (0.7 g), 2 (17.2 g), 3 (124.1 g), 4 (52.0 g) and 5 (2.2g). The fourth fraction was chromatographed on a silica gel column. Elution was performed with *n*-hexane-AcOEt (98:2→0:100) to yield thirteen frations (fractions 6-18). Fraction 7 (3.3 g) was chromatographed on silica gel (BW-300) using a *n*-hexane-AcOEt (99:1→0:100) as the eluent to give five fractions (fractions 19-23). Fraction 20 was further chromatgraphed on reverse phase flash column chromatograph (LiChroprep RP-8) using a 90% MeOH-H_2O to give sinulobatin B (**2**, 17.9 mg). Fraction 9 (179.6 mg) was chromatographed on silica gel using *n*-hexane-AcOEt (95:5) to yield seven fractions (fractions 24-30). Fraction 29 (59.2 mg) was then chromatographed on a Cosmosil 5C18 using 85% MeOH-H_2O as the eluent to give five fractions

(fractions 31-35). Whereas, fraction 10 (1.6 g) was also chromatographed on a Cosmosil 5C18 using MeOH-H_2O (85%→95%) as the eluent to give six fractions (fractions 36-41). It was proved that fraction 32 (36.0 mg) and fraction 37 (173.8 mg) were identical and both were pure based on TLC and HPLC analysis. Thus, these fractions gave sinulobatin A (**1**, 209.8 mg). Fraction 39 (100.8 mg) was chromatographed on silica gel (BW-300) with *n*-hexane-AcOEt (9:1) to give nine fractions (fractions 42-50). Fraction 34 (3.8 mg) and fraction 45 (59.5 mg) were combined and subjected to reverse phase HPLC (85% MeOH-H_2O) to give sinulobatin C (**3**, 35.2 mg). Fraction 40 (99.5 mg) was chromatographed on silica gel (BW-300) with *n*-hexane-AcOEt (95:5) to give twelve fractions (fractions 51-62). Fraction 53 (6.6 mg) was subjected to reverse phase HPLC (85% MeOH-H_2O) to give sinulobatin D (**4**, 1.6 mg).

Cytotoxic Activity.

Cytotoxic activity in vitro was determined using murine lymphoma L1210 cells and human epidermoid carcinoma KB cells. Roswell Park Memorial Institute Medium 1640 supplemented with 10% FBS, and penicillin-streptomycin was used as the cell culture medium. L1210 or KB cells ($1x10^4$ cells/ml) were cultured in a CO_2 gas incubator at 37°C for 72 hr in 200 μl of medium with a 96-well microplate containing various concentrations of the test compound. Their viability, estimated using the MTT assay,[10] was compared to that of control cells incubated in the identical medium without the compound. The cytotoxicity was evaluated as IC_{50} (μg/ml).

Sinulobatin A (1): Colorless prisms (*n*-hexane); m.p. 126.0 - 128.0°C; $[\alpha]_D^{25}$ +115.6° (*c* 0.43, $CHCl_3$); EIMS (m/z) 342 $[M]^+$, 327, 299; HR positive FABMS found, 343.2276, calcd. for $C_{22}H_{31}O_3$ $[M+H]^+$ 343.2273 ; IR ($CHCl_3$) ν_{max} cm^{-1} 3040, 1735, 1715, 1660, 1220, 900 ; ^{1}H-NMR and ^{13}C-NMR, see Tables 1 and 2.

X-ray Crystal Structure Analysis of 1. Crystal data: $C_{22}H_{30}O_3$, orthorhombic, space group $P2_12_12_1$, No. 19, a = 13.744(4) Å, b = 17.035(7) Å, c = 8.440(4) Å (from 25 orientation reflections, $20 < 2\theta < 55$), V = 1976(1) $Å^3$, Z = 4, Dcalcd. = 1.151 g/cm^3 (CuKα radiation, λ = 1.54178 Å). Intensity data were recorded using a Rigaku AFC5R diffractometer {Cu-Kα radiation, ϖ−2θ scans, 2θmax = 120.1, scan width $(0.92 + 0.30 \tan\theta)°$}. The intensities of three standard reflections remeasured every 1732 reflections during data collection to monitor crystal stability indicated that significant deterioration occurred (overall intensity loss = 6.87 %). From a total of 1732 measurements, those 979 reflections with $I > 3.00\sigma(I)$ were retained for the analysis. Lorentz-polarization corrections were applied. The crystal structure was solved by direct methods (MITHRIL).[13] The non-hydrogen atoms were anisotropically refined. The final cycle of full-matrix least-squares refinement was based on 979 observed reflections and 346 variable parameters and converged with unweighted and weighted agreement factors of : R = 0.043, Rw = 0.044, and GOF = 1.42. Crystallographic calculations were performed on a Micro-VAX 3200 using TEXSAN Structure Analysis Software.[14] In the least-squares iterations, $\Sigma\omega(|Fo|-|Fc|)2$, $\omega = 4Fo^2/\sigma^2(Fo^2)$ was minimized.

Sinulobatin B (2): Colorless amorphous, $[\alpha]_D^{25}$ +105.2° (*c* 0.10, $CHCl_3$); FABMS (m/z) 284 $[M]^+$; HR positive FABMS found, 285.2213, calcd. for $C_{20}H_{29}O$ $[M+H]^+$ 285.2219; IR ($CHCl_3$) ν_{max} cm^{-1} 3020, 2950, 1710, 1650, 1235, 890; ^{1}H-NMR and ^{13}C-NMR, see Tables 1 and 2.

Sinulobatin C (3): Colorless amorphous, $[\alpha]_D^{25}$ +98.2° (*c* 0.23, $CHCl_3$); FABMS (m/z) 367 $[M+Na]^+$; HR positive FABMS found, 367.2274, calcd. for $C_{22}H_{32}O_3Na$ $[M+Na]^+$ 367.2250; IR ($CHCl_3$) ν_{max} cm^{-1} 3010, 3000, 2950, 1735, 1655, 1260, 900; ^{1}H-NMR and ^{13}C-NMR, see Tables 1 and 2.

Sinulobatin D (4): Colorless amorphous, $[\alpha]_D^{26}$ +30.6° (*c* 0.07, $CHCl_3$); FABMS (m/z) 409 $[M+Na]^+$; HR positive FABMS found, 409.2342 calcd. for $C_{24}H_{34}O_4Na$ $[M+Na]^+$ 409.2355; IR ($CHCl_3$) ν_{max} cm^{-1} 3010, 1730, 1260, 900; ^{1}H-NMR and ^{13}C-NMR, see Tables 1 and 2.

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