New Sesterterpenes from the Antarctic Sponge *Suberites* sp.

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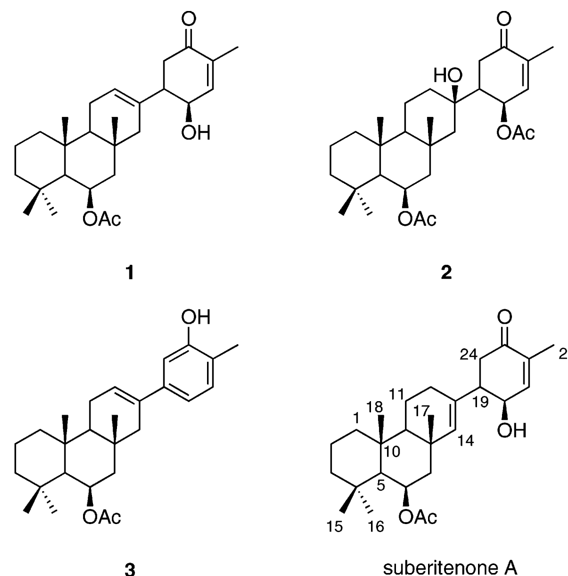
Suberitenones C and D and suberiphenol, three new sesterterpenes of the suberitane class, were isolated from the sponge *Suberites* sp. collected from Antarctica. The structures of these compounds were determined on the basis of combined spectral and chemical analyses.

During the course of our search for bioactive secondary metabolites from marine invertebrates, we reported the isolation of suberitenones A and B, sesterterpenes of an unusual structural class from the Antarctic sponge *Suberites* sp.[1] Suberitenone B displayed moderate inhibition of CETP (cholesteryl ester transfer protein), which plays a key role in the transfer of cholesteryl ester and triglyceride between HDL (high-density lipoproteins) and LDL (low-density lipoproteins).[2−4] More recently, caminatal, a sesterterpene aldehyde with a new carbon skeleton which is related to that of suberitenones, was isolated from another Antarctic sponge, *Suberites caminatus*.[5]

In our continuing search for novel marine metabolites, we recently re-encountered the sponge *Suberites* sp. off the shore of King George Island, Antarctica. Guided by the results of the ^{1}H NMR analyses, the organic extracts from this animal were separated employing solvent partitioning followed by silica vacuum flash chromatography and HPLC to afford several sesterterpenes including suberitenones A and B as the major metabolites. We describe herein the structures of suberitenones C and D and suberiphenol, the new minor constituents of the *Suberites* sp.

Suberitenone C (**1**) was isolated as an amorphous solid, which analyzed for $C_{27}H_{40}O_4$ on the basis of combined HRFABMS and ^{13}C NMR spectrometry. The NMR data of this compound were highly compatible with those of suberitenone A. However, carbon signals of the C ring in the ^{13}C NMR data were significantly shifted between these compounds. The most noticeable differences in the ^{13}C NMR data were the shifts of the olefinic carbons from δ 138.9 (CH, C-14) and 131. 3 (C, C-13) in suberitenone A to those at δ 135.3 (C) and 123.3 (CH) in **1**. Corresponding changes were also observed in the ^{1}H NMR spectra, in which the signal of the olefinic proton at δ 5.13 (1H, s, H-14) in suberitenone A was replaced by a new signal at δ 5.53 (1H, br s). A direct spin-coupling of the newly appearing proton with the methylene protons at δ 2.10 (2H, m) was unobservable in the ^{1}H COSY data of suberitenone A. These spectral differences were accommodated by the shift of a trisubstituted double bond from $\Delta^{13,14}$ to $\Delta^{12,13}$ in **1** that was ascertained by a combination of ^{1}H COSY, TOCSY, *g*HSQC, and *g*HMBC experiments (Table 1). The crucial evidence supporting this interpretation was provided by the TOCSY data in which a proton-spin correlation was observed between the olefinic proton and the H-9 methine proton at δ 1.35 (1H, dd, $J = 11.2$, 5.9 Hz). Furthermore, long-range correlation between the H-17 methyl proton and the olefinic methine carbon, a conspicuous one in the *g*HMBC data of suberitenone A, disappeared in **1**. The stereochemistry of this compound was assigned to be identical to that of suberitenone A on the basis of biogenetic considerations and key ROESY cross-peaks; H-5/H-9, H-5/H-16, H-6/H-7(α and β), H-6/H-16, H-14(α and β)/H-20, H-19/H-24α(δ 2.19), OAc/H-15, OAc/H-16. Thus, the structure of suberitenone C was determined to be a trans-olefinic derivative of suberitenone A.



The molecular formula of suberitenone D (**2**) was assigned as $C_{29}H_{44}O_6$ by combined HRFABMS and ^{13}C NMR analyses. The NMR data of this compound were very similar to those of suberitenone B, one of the major constituents of the crude extract. In addition to the signals for a newly added acetoxyl group {δ_C 169.7 (C), 21.2 (CH_3), δ_H 2.10 (3H, s)}, the only significant differences in the NMR data were the shifts of signals of an oxymethine group {δ_C 67.6 (CH), δ_H 5.50 (1H, dd, $J = 5.9$, 3.4 Hz)}, suggesting that **2** was an acetoxyl derivative of suberitenone B. The location of the acetoxyl group was assigned at C-20 on the

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Table 1. ^1H and ^{13}C NMR Assignments for Compounds **1**−**3**[a]

position	1 δ_H	1 δ_C	1 HMBC	2 δ_H	2 δ_C	3 δ_H	3 δ_C
1	1.70, m; 0.96, m	42.7 CH_2	C-3, C-9	1.76, br d (13.7) 0.83, ddd (13.7, 13.7, 3.4)	41.9 CH_2	1.70, br dd (13.2, 2.9) 0.93, ddd (13.2, 12.2, 3.9)	42.1 CH_2
2	1.73, m; 1.44, m	19.2 CH_2		1.71, m; 1.46, m	18.6 CH_2	1.72, m; 1.47, m	18.5 CH_2
3	1.38, m; 1.23, m	44.6 CH_2		1.36, m; 1.16, m	44.3 CH_2	1.38, m; 1.18 ddd (13.2, 12.2, 2.9)	43.9 CH_2
4		34.5 C			34.0 C		33.9 C
5	1.19, d (2.1)	56.8 CH	C-4, C-10, C-15, C-16, C-18	1.01, d (2.4)	56.7 CH	1.10, d (2.0)	56.4 CH
6	5.49, ddd (3.4, 2.4, 2.4)	70.5 CH	C-7, C-8, carbonyl	5.45, ddd (3.4, 2.4, 2.4)	70.5 CH	5.53, br d (2.0)	70.1 CH
7	1.92, dd (14.2, 2.4) 1.44, dd (14.2, 3.4)	46.8 CH_2	C-5, C-6, C-9, C-17	1.92, dd (14.7, 2.4) 1.20, dd (14.7, 3.4)	46.8 CH_2	2.05, dd (13.7, 2.5) 1.46, dd (13.7, 3.4)	46.2 CH_2
8		32.9 C			34.4 C		32.2 C
9	1.35, dd (11.2, 5.9)	53.7 CH	C-8, C-10, C-11, C-17, C-18	0.87, dd (11.7, 2.0)	58.9 CH	1.37, dd (11.2, 5.4)	52.7 CH
10		37.7 C			37.2 C		37.0 C
11	2.10, m (2H)	23.3 CH_2		1.67, dddd (13.2, 13.2, 11.7, 3.4); 1.58, m	16.9 CH_2	2.23, m[b]; 2.10, m[b]	23.1 CH_2
12	5.53, br s	123.3 CH		2.02, m; 1.13, m	37.5 CH_2	6.06, dt (5.4, 2.5)	123.4 CH
13		135.3 C			72.7 C		133.3 C
14	1.86, d (16.5); 1.77, d (16.5)	49.0 CH_2	C-8, C-9, C-12, C-13, C-17	1.35, dd (13.7, 2.9) 1.16, d (13.7)	53.9 CH_2	2.14, m[b]; 2.12, m[b]	48.3 CH_2
15	0.95, s	33.5 CH_3	C-3, C-4, C-5, C-16	0.91, s	32.9 CH_3	0.96, s	33.3 CH_3
16	1.04, s	23.6 CH_3	C-3, C-4, C-5, C-15	1.00, s	23.0 CH_3	1.01, s	23.2 CH_3
17	1.07, s	21.1 CH_3	C-7, C-8, C-9, C-14	1.31, s	22.8 CH_3	1.07, s	20.7 CH_3
18	1.30, s	16.9 CH_3	C-1, C-5, C-9, C-10	1.18, s	17.2 CH_3	1.28, s	16.3 CH_3
19	2.58, br dd (13.1, 3.4)	46.6 CH	C-13, C-23	1.97, ddd (13.7, 3.4, 3.4)	47.8 CH		141.7 C
20	4.34, br d (5.4)	65.5 CH	C-21, C-22	5.50, dd (5.9, 3.4)	67.6 CH	6.84, dd (7.8, 2.0)	117.3 CH
21	6.79, dq (5.4, 1.5)	144.9 CH	C-19, C-23, C-25	6.80, dq (5.9, 1.5)	137.6 CH	7.04, d (7.8)	130.7 CH
22		135.9 C			139.1 C		121.9 C
23		200.0 C			199.9 C		153.6 C
24	2.80, dd (17.1, 13.1) 2.19, dd (17.1, 3.4)	38.1 CH_2	C-19, C-20, C-23	2.82, dd (17.1, 13.7) 2.57, dd (17.1, 3.4)	34.0 CH_2	6.76, d (2.0)	111.5 CH
25	1.71, br s	15.6 CH_3	C-21, C-22, C-23	1.80, d (1.5)	15.6 CH_3	2.22, s	15.4 CH_3
6-OAc		170.4 C			170.4 C		170.6 C
	2.01, s	21.7 CH_3	carbonyl	2.04, s	21.8 CH_3	2.06, s	21.9 CH_3
20-OAc					169.7 C		
				2.10, s	21.2 CH_3		

[a] NMR data for **1** were obtained in acetone-d_6, while those of other compounds were obtained in chloroform-*d* solutions. Assignments were aided by a combination of ^1H COSY, TOCSY, *g*HSQC, and *g*HMBC experiments. [b] Due to the overlapping with other signals, splitting patterns were not accurately measured.

basis of combined 2-D NMR experiments including *g*HMBC data, which revealed a long-range correlation between the acetoxyl carbon and the H-20 proton at δ 5.50 (Table 1). The structure of **2** was confirmed by chemical transformation. Treatment with acetic anhydride in pyridine converted suberitenone B to an acetoxylated product whose spectral data were identical to those of **2**. Thus, the structure of suberitenone D (**2**) was defined as the C-20 acetoxyl derivative of suberitenone B.

A closely related metabolite, suberiphenol (**3**), was isolated as an amorphous solid, which analyzed for $C_{27}H_{38}O_3$ by combined ^{13}C NMR and HRFABMS analyses. The spectral data of this compound were highly compatible with those of suberitenone C (**1**). Detailed examination of the NMR data revealed that **3** possessed A−C rings identical to those of **1**. The most noticeable difference in the ^{13}C NMR data was the replacement of carbon signals (C19−C24) of ring D, a cyclohexenone, of **1** with those in the region δ 155−110 in **3** (Table 1). Chemical shifts of the corresponding protons at δ 7.04, 6.84, and 6.76 in the ^1H NMR data as well as their AMX coupling pattern revealed the presence of a 2,5-disubstituted phenolic moiety in the molecule. The presence of an aromatic ring was further supported by the downfield shift of the H-25 methyl protons from δ 1.71 (br s) in **1** to δ 2.22 (s) in **3** in the ^1H NMR data, which was characteristic of a benzylic methyl group. This interpretation was confirmed by a *g*HMBC experiment in which several long-range correlations were observed between the newly appearing downfield protons and benzylic methyl protons at δ 2.22 with neighboring carbons. The attachment of the phenolic moiety at C-13 in ring C was also secured by the long-range correlations of H-12 and H-14 at δ 6.06 and 2.12, respectively, with C-19 at δ 142.7. Thus, the structure of suberiphenol was determined to be an aromatized derivative of suberiteneone C.

In our measurement of bioactivities, the new compounds were neither cytotoxic (MTT method, LC_{50} > 100 μg/mL against the human leukemia K562 cell-line) nor antimicrobial (paper-disk method, $D_{i.z.}$ < 3 mm at the concentration of 25 μg against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp.).

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher-Jones apparatus and are uncorrected. Optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. UV spectra were obtained in methanol using a Milton-Roy spectrophotometer. IR spectra were recorded on a Mattson Galaxy spectrophotometer. NMR spectra were recorded in acetone-d_6 and chloroform-*d* solutions containing Me_4Si as internal standard, on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectra were provided by the Korea Basic Science Institute, Taejeon, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. The dark brown specimens of *Suberites* sp. (sample number 00A-4) were collected by scuba at 20−30 m depth off the coast of Weaver Peninsula, King George Island, in December 2000. Morphological features of this animal were identical to those previously described.[1] The voucher specimen is currently on deposit at KORDI under the curatorship of H.-S.L.

Extraction and Isolation. The fresh collection was immediately frozen using dry ice and kept at −25 °C until investigated chemically. The specimens were defrosted (wet wt 0.9 kg), macerated, and repeatedly extracted with MeOH (2 L × 2) and CH_2Cl_2 (2 L × 2). The combined crude extract (ca. 120 g) was partitioned between *n*-BuOH and H_2O, then the former layer (23.4 g) re-partitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer (7.7 g) was subjected to silica vacuum flash chromatography using gradient mixtures of *n*-hexane and EtOAc as eluents. The fraction (390 mg) eluted with 40% EtOAc in hexane was separated by semipreparative silica HPLC (YMC silica column, 1 cm × 25 cm, 20% EtOAc in hexane) to yield 306.4 mg of suberitenone A and a mixture of **1** and **2** as a single peak. Separation of these compounds was accomplished by reversed-phase HPLC (YMC ODS-AM column, 1 cm × 25 cm, 20% aqueous MeOH) to afford 6.1 and 3.7 mg of **1** and **2**, respectively.

The fraction eluted with 20% EtOAc in hexane (310 mg) was separated by silica HPLC (10% EtOAc in hexane), then purified by reversed-phase HPLC (10% aqueous MeOH) to yield 1.9 mg of **3**. The fractions eluted with 50 and 60% EtOAc in hexane from silica vacuum flash chromatography were combined (210 mg) and separated by silica HPLC (YMC silica column, 40% EtOAc in hexane) to yield 56.9 mg of suberitenone B as an amorphous solid.

Suberitenone C (1): amorphous solid, mp 89−92 °C; $[\alpha]^{25}_{D}$ −18.0° (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 (4.15) nm; IR (KBr) ν_{max} 3400 (br), 2925, 1735, 1675, 1570, 1420, 1250 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 451.2821 $[M + Na]^+$ (calcd for $C_{27}H_{40}O_4Na$, 451.2824).

Suberitenone D (2): amorphous solid, mp 102−104 °C; $[\alpha]^{25}_{D}$ −67.5° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (3.99) nm; IR (KBr) ν_{max} 3450 (br), 2920, 1735, 1680, 1570, 1365, 1245, 1020 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 511.3036 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_6Na$, 511.3033).

Suberiphenol (3): amorphous solid, mp 93−94 °C; $[\alpha]^{25}_{D}$ −9.3° (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.14), 251 (3.91), 291 (3.32) nm; IR (KBr) ν_{max} 3400 (br), 2925, 1730, 1705, 1635, 1580, 1395, 1270, 1245 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 433.2724 $[M + Na]^+$ (calcd for $C_{27}H_{38}O_3Na$, 433.2722).

Acetylation of Suberitenone B. To a stirred solution of suberitenone B (3.2 mg) in dry pyridine (0.5 mL) was added acetic anhydride (0.2 mL). After stirring the mixture for 2 h at room temperature, the solvent and excess acetic anhydride were removed under reduced pressure. The residue was redissolved with 10% EtOAc in *n*-hexane and filtered through a silica Cepak column to afford the mono-acetylated product (2.8 mg). The ^1H and ^{13}C NMR data of this compound were identical to those obtained for the natural suberitenone D (**2**).

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