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Suberitane network, a taxonomical marker for Antarctic sponges of the genus *Suberites*? Novel sesterterpenes from *Suberites caminatus*

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Abstract—Two novel sesterterpenes **1** and **2** have been isolated from the Antarctic sponge *Suberites caminatus*. Their structures and relative stereochemistries were determined by spectroscopic methods. Compound **1** features a novel structural type of suberitane network, and an epimer of compound **2** seems to be the biogenetic precursor of **1** and suberitenones A and B. The exclusive prevalence of suberitane-derived sesterterpene metabolites in species of *Suberites* suggests that this skeleton may be a chemical marker of the genus.

In contrast to other Antarctic sponges,[1] the genus *Suberites* appears to be a specialized factory producing a single type of isoprenic skeletal class of metabolites: sesterterpenes. Collections from King George Island[2] and from McMurdo Sound[3] have provided suberitenones A and B, sesterterpenes with a tetracarbocyclic skeleton. These sesterterpenes have defensive properties toward a major Antarctic spongivore, the sea star *Perknaster fuscus*.[3] Also, suberitenone B shows properties as an inhibitor of the cholesteryl ester transfer protein (CETP).[2]

Due to our interest in benthic Antarctic organisms,[4] from an extract of *Suberites caminatus* we have recently described caminatal, a minor metabolite with an unprecedented carbon backbone, caminatane, biogenetically derived from the suberitane skeleton by the oxidative rupture of a ring bond.[1] This finding prompted us to gather *S. caminatus* off King George Island (South Shetlands, Antarctica), in an area closer to the previous collection. In this paper we report on the isolation of two new suberitane-related sesterterpenes, **1** and **2**, and the known[2] suberitenone B, **3**, from this species.

Compound **1** is a pentacyclic structure sharing with suberitenones the three carbocyclic rings unit fused in a perhydrophenanthrene fashion. The remaining cyclohexanone and oxane rings are held together by an oxaspiro carbon, featuring a novel structural type of the suberitane framework. An epimer of compound **2** could be a biogenetic precursor of **1** and suberitenones A and B.

From the crude extract of *S. caminatus* the sesterterpenes **1**–**3** were isolated after flash chromatography followed by successive gel filtration and HPLC.[5]

Oxaspirosuberitenone **1**[6] was a colorless oil. Its EIMS spectrum showed a peak at m/z 446 $[M]^+$ that corresponds to the empirical formula $C_{27}H_{42}O_5$ (HREIMS). The ^{13}C NMR spectrum of **1**, Table 1, indicated the presence of 27 carbons in the molecule whose multiplicities were determined by DEPT spectral data: six methyl groups, eight methylenes, seven methines (three geminal to oxygen), four sp^3 quaternary carbons, one

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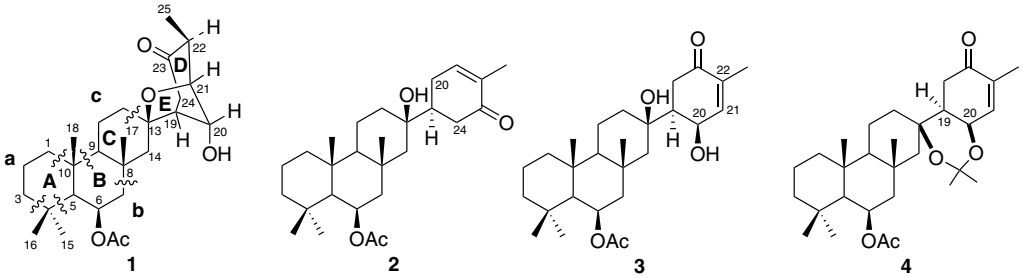

Table 1. ^{1}H and ^{13}C NMR data of compounds **1**–**3** and HMBC of **1** [500 MHz, δ ppm, (J) Hz, $CDCl_3$]

#	1			2		3	
	δ_H	δ_C	HMBC	δ_H	δ_C	δ_H	δ_C
1	0.77 d (4.0)	42.3		0.88 m	42.3	0.84 m	41.8
	1.70 m			1.68 m		1.75 m	
2	1.40 m	18.9		1.45 m	18.8	1.43 m	18.5
	1.70 m			1.70 m		1.72 m	
3	1.18 m	44.7		1.15 m	44.7	1.12 m	44.2
	1.35 m			1.35 m		1.35 m	
4		34.4			34.4		34.0
5	0.98 m	57.2	C-9, C-10, C-15, C-16, C-18	0.88 m	57.2	1.02 d (2.4)	56.7
6	5.44 ddd (2.7, 2.7, 2.7)	71.1	C-8, C-10	5.46 ddd (2.7, 2.7, 2.7)	70.8	5.45 br d (2.9)	70.7
7	α: 1.19 m	47.1	C-5, C-6, C-8, C-9, C-14,	1.29 m	47.3	1.24 dd (3.6, 14.4)	46.9
	β: 1.85 dd (2.6, 14.7)		C-17	1.92 dd (2.5, 14.8)		1.94 dd (2.7, 14.4)	
8		35.3			35.5		34.2
9	0.82 dd (2.2, 15.7)	59.1		1.04 m	59.6	0.88 m	58.8
10		37.5	C-1, C-8, C-17, C-18		37.5		37.1
11	1.45 m	18.9		1.30 m	18.8	1.58 m	16.9
	1.70 m			1.68 m		1.68 m	
12	1.39 m	38.2		1.30 m	38.2	1.11 m	37.9
	1.49 m			2.25 m		2.02 m	
13		85.1			73.0		73.5
14	α: 0.98 m	56.4	C-7, C-9, C-12, C-13, C-19	1.00 m	54.8	1.07 d (13.5)	53.6
	β: 2.30 m			1.88 dd (2.5, 13.6)		1.85 dd (2.7, 13.5)	
15	0.90 s	33.3	C-3, C-4, C-5, C-16	0.91 s	33.3	0.90 s	32.9
16	0.99 s	23.4	C-3, C-4, C-5, C-15	0.98 s	23.4	0.99 s	23.0
17	1.28 s	22.2	C-7, C-8, C-9, C-14	1.17 s	22.2	1.33 s	22.5
18	1.17 s	17.6	C-1, C-5, C-9, C-10	1.12 s	17.7	1.18 s	17.2
19	2.15 m	50.6		2.50 m	40.7	1.72 m	48.0
20	4.40 s	80.9	C-13, C-19, C-21, C-22,	2.50 m	26.9	4.65 dd (2.9, 5.5)	64.3
			C-24	2.40 m			
21	4.09 br s	85.3	C-13, C-20, C-22, C-23	6.75 m	145.8	6.67 dq (1.5, 5.5)	141.6
22	2.25 m	50.6	C-23, C-25		135.4		137.3
23		211.6			200.8		200.9
24	β: 2.35 dd (3.6, 18.0)	43.8	C-13, C-19, C-20, C-22,	2.39 m	39.3	2.50 dd (3.0, 16.8)	32.9
	α: 2.64 dd (2.9, 18.0)		C-23	2.58 dd (2.7, 15.4)		2.81 dd (13.4, 16.8)	
25	1.18 d (7.4)	12.8	C-21, C-22, C-23	1.77 d (1.6)	15.9	1.79 br s	15.6
26		170.8			170.6		170.7
27	2.02 s	22.2	C-26	2.02 s	21.9	2.04 s	21.9

ketone, and one carboxylic carbon. The ^{1}H NMR spectrum, Table 1, showed signals for three protons geminal to oxygen at δ 5.44 (ddd, $J = 2.7, 2.7, 2.7$ Hz), δ 4.40 (s) and δ 4.09 (br s), one acetate methyl group at δ 2.02 (s) and upfield signals for five methyl groups at δ 1.28 (s), δ 1.18 (d, $J = 7.4$ Hz), δ 1.17 (s), δ 0.99 (s), δ 0.90 (s).

From the ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC experiments and from the comparison of the ^{1}H and ^{13}C NMR data with those of suberitenone B, **3**, rings **A**–**C** were confirmed. COSY measurement established the corresponding spin system of fragments **a**–**c**, and the connectivities of these fragments, as depicted in **1**, were aided by HMBC experiments. Compounds **1** and **3** possess an identical molecular formula and, therefore, the same unsaturation degree, thus the absence of the C-21–C-22 double bond suggested the presence of a new ring in **1**. The sp^3 carbons C-21 and C-22 in compound **1** suggested that a new ring **E** was formed by conjugated addition of the hydroxylic group at C-13 to the α,β-unsaturated ketone of ring **D** of suberitenone B. This gave place to an oxa-bicyclo[3.2.1]octane moiety, where the oxaspiro fashion of the linkage between both tricyclic and cyclohexanone ring moieties of the molecule was secured by the following HMBC: H_2-14/C-13, C-19;

H_2-24/C-13, C-19, and H-21/C-13. Thus, the overall planar structure of a sesterterpene for **1** with the requisite seven degrees of unsaturation can be suggested.

The relative configuration of the chiral centers of **1** was determined on the basis of 2D NOESY experiments and spectroscopic data. Upfield chemical shifts of the protons of Me-17 and Me-18 indicated a *trans–anti–trans* fusion of **A/B/C** rings,[2,7] (Fig. 1). The NOE effects observed between H_3-18 and H_3-16/H_3-17, and between H_3-15 and H-5/H-6, placed Me-16, Me-17, Me-18, and the acetate group on the same side of the molecule, thus indicating that the corresponding substituents on rings **A**–**C** possess the same relative 5**S*, 6**R*, 8**S*, 9**S*, 10**R* stereochemistry as in suberitone B, **3**.

The 13**R* stereochemistry of the oxaspiro was established by the NOEs observed between H_3-17 and H-14β, and between H-14α and H-19, indicating that C-19 and the oxygen at C-13 must be in α and β dispositions, respectively. The ^{1}H NMR spectrum of **1** showed a singlet for H-20 and a broad singlet for H-21. The energy-minimized conformation of **1**, deduced by molecular mechanics, is shown in Figure 1. In this conformer, the H-20/H-19 and H-21/H-20 dihedral angles were predicted to be 77.5° and 78.2°, respectively, which is in concordance with the observed value around 0 Hz for both $J_{\text{H-20/H-19}}$ and $J_{\text{H-21/H-20}}$. The NOE effects between H-19 and H_2-24/H-20; and between H-22 and H-20/H-21 are compatible with the observed *J*-coupling and established the relative configuration 19**S*, 20**S*, 21**R*, 22**S* of the chiral centers of ring **D**, and thus the overall stereochemistry of **1** as shown in Figure 1.

19-Episuberitenone **2**[8] was isolated as a colorless oil. The EIMS spectrum showed a peak at *m*/*z* 430 that corresponds to the molecular formula $C_{27}H_{42}O_4$ $[M]^+$ (HREIMS), indicating that **2** has one oxygen atom less than suberitenone B. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** and **3** (Table 1) showed strong similarities, the most significative difference being the replacement of a methine geminal to oxygen ($\delta_{\text{C-20}}$ 64.3, $\delta_{\text{H-20}}$ 4.65 dd) of suberitenone B by upfield protons of a methylene ($\delta_{\text{C-20}}$ 26.9, $\delta_{\text{H-20}}$ 2.50 m and 2.40 m) in **2**. These data, that are consistent with the absence in **2** of the hydroxyl group at C-20 of suberitenone B, were confirmed by COSY and HMBC experiments.

Comparison of the ^{1}H chemical shifts of compounds **2** and **3** were used to establish the fusion of rings **A**–**C**, whereas 2D NOESY experiments (Fig. 2) allowed us to assign the relative stereochemistry of all chiral centers of **2** except C-13. The NOE effect observed between H-6 and H_3-15 and both protons of the methylene H_2-7 indicated that the acetate group at C-6 must be on the same face of the molecule as the methyl groups Me-16, Me-17, and Me-18. Thus partial relative configurations 5**S*, 6**R*, 8**S*, 9**S*, 10**R* were established. As a 13**R* stereochemistry of suberitenone B was assessed by extensive NOE experiments on its acetal derivative **4**,[2] the almost identical chemical shifts of the respective C-13 of **2**: δ_C 73.0 and **3**: δ_C 73.5 allowed us to assign the same 13**R* configuration as in **3** (Fig. 2). However, strong differences around 7 ppm in C-19 (**2**: δ_C 40.7; **3**: δ_C 48.0) suggested changes in its configuration. The NOEs observed between an unoverlapped proton of H_2-14 and the downfield proton of H_2-24, and between an unoverlapped proton of H_2-12 and one proton of H_2-20 suggested an *S* configuration for C-19, and enabled us to establish the whole stereochemistry of **2** as depicted in Figure 2.

It is not unreasonable to query whether the oxaspiro ring system of **1** is derived biosynthetically or by intramolecular Michael-type addition of the hydroxylic group at C-13 during experimental work. However, when a sample of suberitenone B, isolated in this study, was stirred in silica gel and chloroform at room temperature for 48 h no changes were observed in the starting material. Moreover, the exposure of **3** to a variety of reaction conditions[2] to give the acetal **4** and also different C-20 ester derivatives, elapsed without detection of **1**. All this suggested that **1** is a naturally occurring metabolite.

19-Episuberitenone **2** could be biogenetically derived from the oxidation of the previously proposed[1]

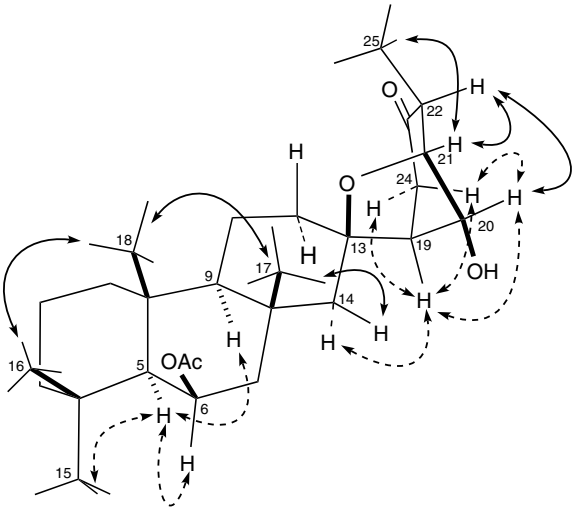

Figure 1. Selected NOEs of compound **1**.

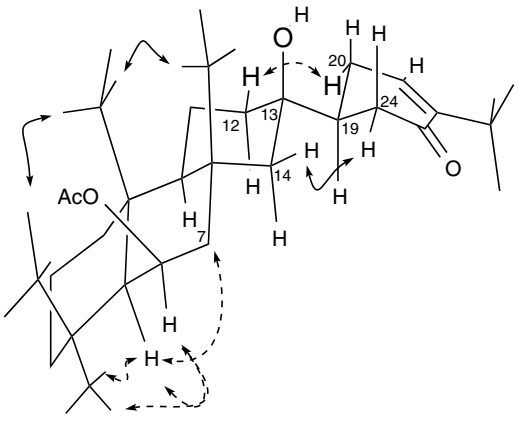

Figure 2. Selected NOEs of compound **2**.

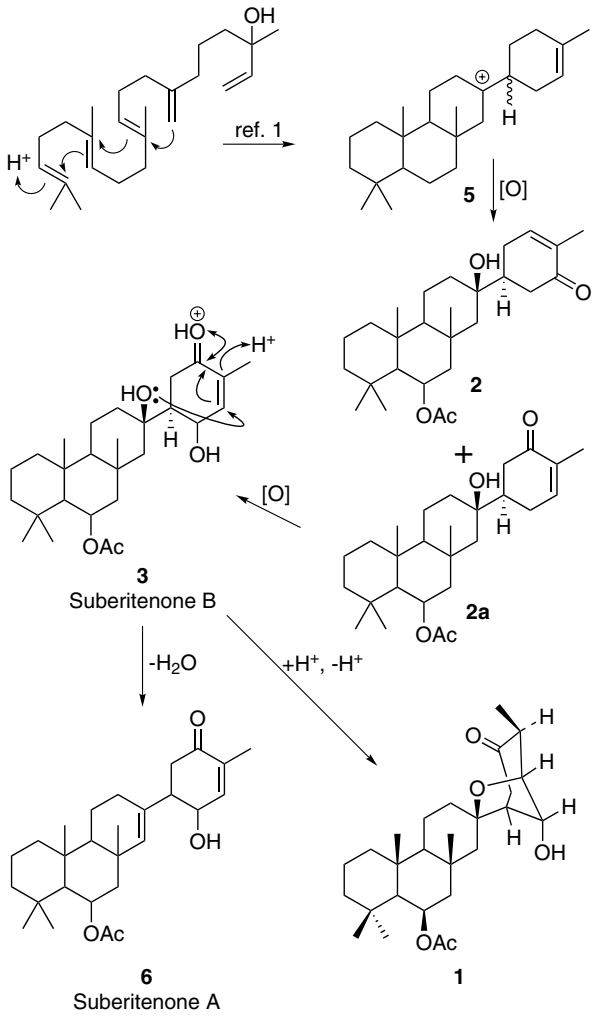

Figure 3. Possible biogenesis of compounds **1**–**3** and **6**.

tetracyclic intermediate **5**. Subsequent oxidation at the allylic position of the enone of **2a** will produce suberitenone B, **3** and, by dehydration of **3**, suberitenone A, **6**. Acid-catalyzed intramolecular attack of the hydroxyl group at C-13 to the conjugated position of enone **3** will transform suberitenone B into the pentacyclic oxaspirosuberitenone **1** (Fig. 3). Until now all suberitane-derived sesterterpenes showed the same C-19 configuration. Compound **2** is the first suberitane-derived metabolite epimeric at C-19. This finding supports **5** as discrete intermediate in the proposed biogenetic pathway.

Irrespective of the location[2,3] of *Suberites* species, similar sesterterpenes related to the suberitane skeleton were found, suggesting that these compounds are de novo synthesized. Since no other skeletal class of isoprene-derived metabolites has been found, the suberitane skeleton may be a chemical marker and a helpful tool for biological studies because of the well recognized complexity of the taxonomical classification of marine sponges.

Secondary metabolites are thought to enhance the fitness of the producing species.[9] The exclusive prevalence of suberitane-derived sesterpene metabolites in species of *Suberites* implies that this invertebrate and its associated microorganisms provide a wealth of material for biosynthetic studies that can reveal (mevalonate or mevalonate-independent pathway) much about the symbiotic or the de novo origin of these metabolites, even enhancing our understanding of how prokariotic and eukariotic cells make lasting alliances.[10]

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5. *Suberites caminatus* was collected by SCUBA diving at King George Island (South Shetlands, Antarctica) at −25 m. Wet sample (1.5 kg) was extracted with acetone at room temperature. After partition with AcOEt–H_2O, the organic phase was concentrated to give a dark residue (3.3 g) and the extract was chromatographed by flash chromatography on silica gel. The fraction eluted with hexane–EtOAc (80:20) (64.6 mg) was further separated by gel filtration to give a fraction (9.5 mg) which was purified by HPLC (Jaigel-sil column 20 × 250 mm, flow 4.5 mL/min, hexane–EtOAc (70:30)) to give compound **1** (1.5 mg). From the fraction eluted with hexane–EtOAc (70:30) (232.6 mg) by flash chromatography, compound **2** (2.1 mg) was isolated after gel filtration chromatography (11.3 mg), and further HPLC (Jaigel-sil column 20 × 250 mm, flow 4.5 mL/min, hexane–AcOEt (70:30)).
6. Colorless oil; $[\alpha]_D^{25}$ +140 (*c* 0.093, $CHCl_3$); IR (film) ν_{max} 3409, 1735, 1638 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m/z* 446 $[M]^+$ (7), 356 $[M-AcOH]^+$ (49), 181 (100); HREIMS $[M]^+$ 446.2992 (calcd for $C_{27}H_{42}O_5$, 446.3032).
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8. Colorless oil; $[\alpha]_D^{25}$ +100 (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 3387, 1728, 1657, 1635 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m/z* 430 $[M]^+$ (5), 412 $[M-H_2O]^+$ (60), 352 $[M-H_2O-AcOH]^+$ (100), 337 (44), 243 (62); HREIMS $[M]^+$ 430.3055 (calcd for $C_{27}H_{42}O_4$, 430.3083), 412.2923 (calcd for $C_{27}H_{40}O_3$, 412.2977), 352.2712 (calcd for $C_{25}H_{36}O$, 352.2766).
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