



Structure determination of Suberitenone B by Two-dimensional NMR techniques

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Abstract: Suberitenone B, sesterterpenoids of an unprecedented skeletal class, has been isolated from the Antarctic sponge *Suberites* sp. Structure of this compound has been determined by combined spectral and chemical studies.

Sponges (phylum Porifera) are recognized as a very prolific source of both biologically active and structurally unique secondary metabolites.¹ However, chemical investigation of these animals has been mainly focused on the tropical and temperate ones, while sponges habitating cold waters have attracted much less attention. In our search for novel bioactive substances from marine organisms, we collected specimens of the brown encrusting sponge (*Suberites* sp.) along the offshore of Weaver Peninsula, King George Island, Antarctica.² In this paper, we report the isolation and structure elucidation of suberitenone B, sesterterpenoids of an unprecedented skeletal class (Table. 1).

Freshly collected specimens were immediately frozen and stored in a freezer until chemically investigated. The defrosted animals were extracted with MeOH and dichloromethane. The combined extract was partitioned between 10% aqueous MeOH and hexane. Each layer was completely dried and subjected to silica vacuum

flash chromatography using sequential mixtures of hexane and EtOAc as eluants. Silica HPLC with the 30-50% EtOAc gave pure suberitenone B (Fig. 1).

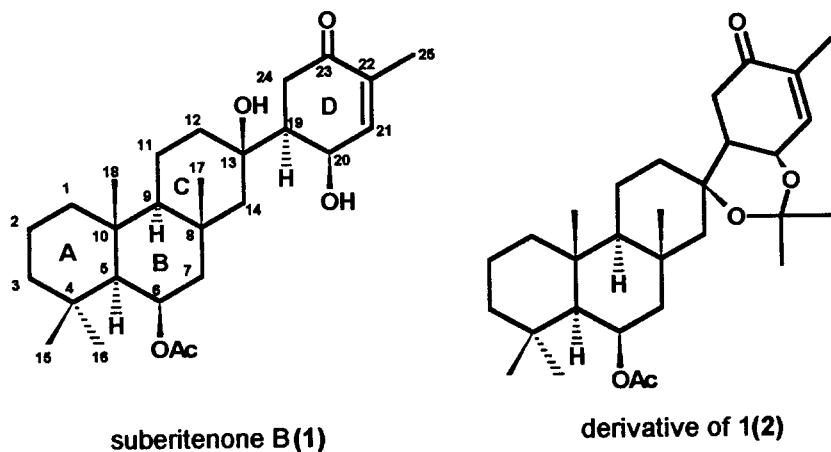


Fig. 1. Structures of suberitenone B (1) and its cyclic ketal derivative.

Suberitenone B (1) was isolated as a white solid of composition $C_{27}H_{42}O_5$ as determined by high-resolution mass and ^{13}C NMR spectroscopic methods. The presence of an α, β -unsaturated ketone was readily recognized by a carbon signal at 200.87 ppm in the ^{13}C spectrum and a strong absorption band at 1670 cm^{-1} in the IR spectrum. And examination of the 1H , ^{13}C , and IR spectra revealed that the remaining four oxygens consisted of as an ester and two hydroxyl group. Combination of the 1H , ^{13}C , 1H COSY, and HMQC experiments determined partial structures a-b (Fig. 2).

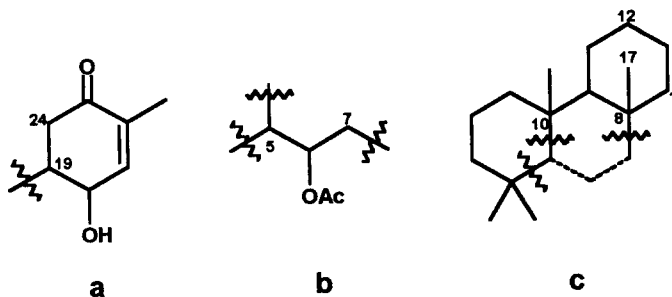


Fig. 2. Partial structures of suberitenone B (1).

Table 1. ^1H and ^{13}C NMR Assignments for Suberitenone B^a

No	Type	Carbon ^b	Proton ^c
1	CH ₂	41.83	1.75 (m), 0.84 (m)
2	CH ₂	18.53	1.72 (m), 1.43 (m)
3	CH ₂	44.23	1.35 (m), 1.12 (m)
4	C	34.01	
5	CH	56.66	1.02 (d, 2.4)
6	CH	70.69	5.45 (brd, 2.9)
7	CH ₂	46.90	1.94 (dd, 14.4, 2.7), 1.24 (dd, 14.4, 3.6)
8	C	34.24	
9	CH	58.76	0.88 (m)
10	C	37.14	
11	CH ₂	16.89	1.68 (m), 1.58 (m)
12	CH ₂	37.87	2.02 (m), 1.11 (m)
13	C	73.46	
14	CH ₂	53.55	1.85 (dd, 13.5, 2.7), 1.07 (d, 13.5)
15	CH ₃	32.92	0.90 (s)
16	CH ₃	23.04	0.99 (s)
17	CH ₃	22.54	1.33 (s)
18	CH ₃	17.19	1.18 (s)
19	CH	47.99	1.72 (s)
20	CH	65.25	4.65 (dd, 5.5, 2.9)
21	CH	141.55	6.67 (dd, 5.5, 1.5)
22	C	137.27	
23	C	200.87	
24	CH ₂	32.89	2.81 (dd, 16.8, 13.4), 2.50 (dd, 16.8, 3.0)
25	CH ₃	15.61	1.79 (brs)
OAc		170.67	
		21.87	2.04 (s)

^a Assignments were aided by ^1H COSY, HMQC and HMBC experiments.

^b 125 MHz, CDCl_3 . ^c 500 MHz, CDCl_3 .

However, structure of the remaining hydrocarbon part was unable to be determined directly by NMR methods because of the overlapping of upfield signals in the ^1H spectrum. Comparison of spectral data with known compounds suggested that **1** possesses conjunctured cyclohexane moieties with bridgehead methyl groups.³ Chemical shifts of the quaternary carbons and methine carbons were very similar to those of terpenoids possessing conjunctured cyclohexane rings. Consideration of the molecular formula revealed that the hydrocarbon part must be c. Confirmation of the

partial structures and determination of their connectivities were aided by HMBC experiments (Fig. 3). Long range correlations between the β -acetoxy carbon at 56.66 ppm (C-8) and methyl protons at 0.90 ppm (H-15) and 0.99 ppm (H-16) and a correlation between the quaternary carbon at 37.14 ppm (C-10) and the α -acetoxy proton at 5.49 ppm (H-6) confirmed the presence of **c** in compound **1**. Also, connection of **b** with **c** was determined by these couplings. This interpretation was further supported by a correlation between the other β -acetoxy carbon at 46.90 ppm (C-7) and methyl protons at 1.33 ppm (H-17). Thus, **b** was assigned to be connected to all of the three quaternary centers of **c**. Finally, the quaternary carbon with hydroxyl group at 73.46 ppm (C-13) shows the connection of **c** with **a** which was also supported by the correlation between the H-14 and H-20 protons and H-12 and H-24 protons in NOESY experiment. The structure of suberitenone B was unambiguously determined as a sesterterpenoid. To the best of our knowledge, the carbon skeleton of suberitenone B is an unprecedented one.⁴

Suberitenone B possesses several asymmetric carbon centers. The *trans*, *trans* orientations for the A/B and B/C ring junctions were determined by comparison of NMR data with related terpenoids.³ The small couplings ($J = 3.9, 2.4, \text{ and } 2.0 \text{ Hz}$) of H-6 proton with adjacent ones revealed that the attachment of the C-6 acetoxy group to ring B was axial. The remaining problem was the stereochemistry of the C-19, C-20 in ring D and the C-13 in ring C. Analysis of the vicinal coupling constants between the H-19 methine proton and H-24 methylene protons ($J_{19,24} = 13.4 \text{ and } 3.0 \text{ Hz}$) revealed that the H-19 proton is pseudoaxial on the cyclohexenone ring. The very small coupling constant between the H-19 and H-29 protons fixed the H-20 proton in a pseudoequatorial position. Assignments of the stereochemistry of C-13 was approached by NOESY experiments in which correlations were found between the H-12 β and one of the H-24 protons and between the H-14 β and H-20 protons.

However, NOESY results were insufficient to determine the stereochemistry of these carbon centers since two configurations were possible for 1 at C-19 (Fig. 4).

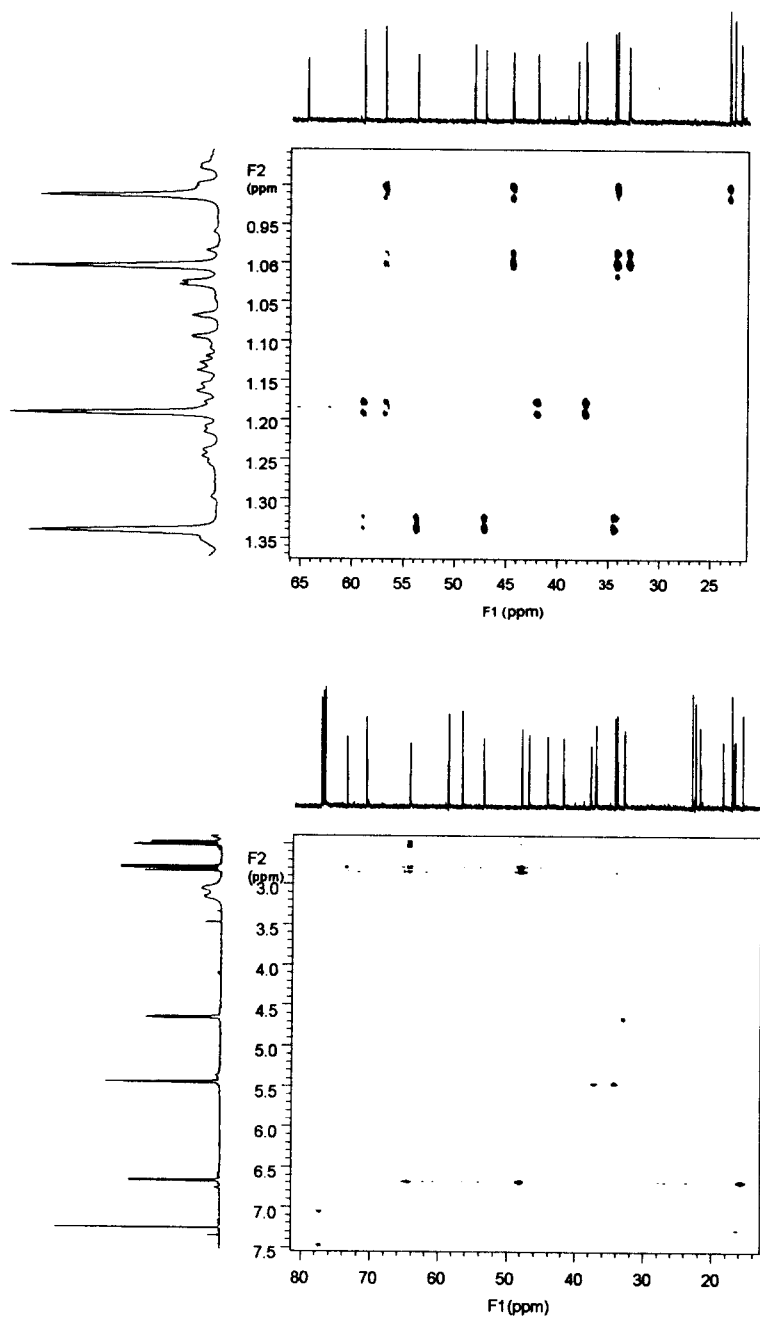


Fig. 3. Partial HMBC spectrum of suberitenone B (1)

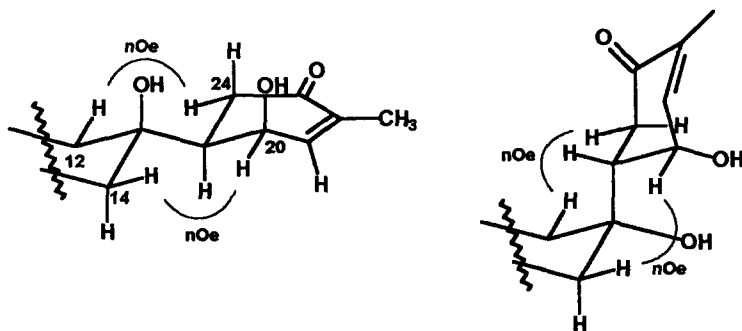


Fig. 4. Possible configurations of suberitenone B from the results of NOESY experiment.

This problem was solved by ROESY experiments on a synthetic derivative **2**, a cyclic ketal (Fig. 1). The key correlations were those between the ketal methyl protons and H-14 β , H-17, and H-20. Thus, the relative configurations of asymmetric centers of **1** were assigned as 5*S*^{*}, 6*R*^{*}, 8*S*^{*}, 9*S*^{*}, 10*R*^{*}, 13*R*^{*}, and 20*R*^{*}.

REFERENCES

- (a) N. Fusetani, S. Matsunaga, *Chem. Rev.* **93**, 1793 (1993).
 (b) D. J. Faulkner, *Nat. Prod. Rep.* **12**, 223 (1995).
- Taxonomical identification of the sponge specimens was kindly provided by professor Patricia R. Bergquist, School of Biological Science, University of Auckland, New Zealand.
- (a) S. De Rosa, R. Puliti, A. Crispino, A. De Giulio, C. A. Mattia, and L. Mazzarella, *J. Nat. Prod.* **57**, 256 (1994).
 (b) R. Lal, R. C. Cambie, C. E. F. Rickard, and P. R. Bergquist, *Tetrahedron Lett.* **35**, 2603 (1994).

(c) H. He, P. Kulanthaivel, and B. J. Baker, *Tetrahedron Lett.* **35**, 7189 (1994).

4. We propose the name “suberitane” for this new carbon skeleton.