

A Cytotoxic Sesquiterpene Alkaloid from the South China Sea Gorgonian *Subergorgia suberosa*

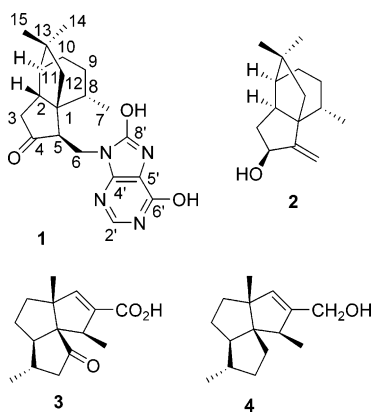
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A new sesquiterpene alkaloid, 6-(9'-purine-6',8'-diolyl)-2 β -suberosanone (**1**), together with three known sesquiterpenes, suberosenol A (**2**), subergorgiac acid (**3**), and subergorgiol (**4**), was isolated from the EtOH/CH₂Cl₂ extracts of the South China Sea gorgonian *Subergorgia suberosa*. The structure of **1** was determined through spectroscopic methods. Compound **1** showed moderate cytotoxicity against the human breast carcinoma MDA-MB-231 cell line with an IC₅₀ of 8.87 μ g/mL.

Previous studies on the chemical constituents of *Subergorgia suberosa* have led to the isolation of several sesquiterpenes^{1–5} and several 9,11-secosteroids.^{6–8} Some of these sesquiterpenes showed cytotoxicity toward several cancer cell lines.^{3–5} During the course of further searching for novel active compounds from gorgonians,^{9,10} we undertook the investigation of the South China Sea gorgonian *S. suberosa*. A new suberosane-type sesquiterpene alkaloid, 6-(9'-purine-6',8'-diolyl)-2 β -suberosanone (**1**), together with three known sesquiterpenes, suberosenol A (**2**),¹¹ subergorgiac acid (**3**),¹ and subergorgiol (**4**),⁴ was isolated from the EtOH/CH₂Cl₂ extracts of *S. suberosa*. In the cytotoxicity assays, we observed that **1** showed moderate cytotoxicity against the human breast carcinoma MDA-MB-231 cell line with an IC₅₀ of 8.87 μ g/mL and potential cytotoxicity toward the MCF cell line at a concentration of 50 μ M. This paper deals with the isolation, structural elucidation, and cytotoxic activity of **1**.



Compound **1** had the molecular formula C₂₀H₂₆N₄O₃ as deduced from NMR spectra and HRESIMS. Thus, 10 degrees of unsaturation was determined for the molecule of **1**. Its UV spectrum exhibited maximum absorption at 212 and 264 nm (aromatic group), while the IR spectrum showed absorption bands for hydroxyls (3500, 3115 cm⁻¹), carbonyl groups (1740, 1710 cm⁻¹), and an aromatic ring

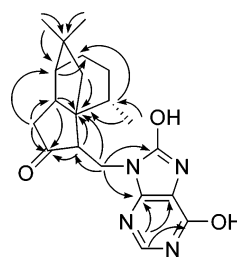


Figure 1. Key HMBC correlations of **1**.

(1670, 1600 cm⁻¹). The ¹³C NMR spectrum showed the presence of 15 basic skeleton carbons, including three methyls (δ_C 16.7, 27.0, 34.4), five methylenes (δ_C 40.8, 41.3, 27.0, 27.9, 48.5), four methines (δ_C 44.0, 52.4, 36.6, 49.7), two quaternary carbons (δ_C 56.7, 39.7), and a ketone carbon (δ_C 216.5, s), along with five low-field carbons [δ_C 108.1 (s), 140.6 (d), 150.2 (s), 151.9 (s), 155.8 (s)]. The ¹H NMR spectrum displayed three methyl groups at δ_H 0.80 (3H, d, J = 7.0 Hz), 1.14 (3H, s), 1.16 (3H, s) and an olefin proton at δ_H 7.91 (1H, s). These NMR spectral data showed similarity with those of suberosanone¹¹ and suberosenone⁵ with the exception of five additional low-field carbons. On the basis of the above data, **1** should be a suberosanone-type sesquiterpene linked with a five-carbon aromatic group.

The five low-field carbon signals [δ_C 108.1 (s), 140.6 (d), 150.2 (s), 151.9 (s), 155.8 (s)] with only one corresponding proton (δ_H 7.91, 1H, s) of **1** were similar to those of 3,7,9-tri-Me-6,8-purinediol that had been found in the South China Sea gorgonian *Echinogorgia pseudossapo*¹² and other analogues.¹³ When the measuring solvent was changed from CDCl₃ to pyridine-*d*₅, two additional signals [δ_H 12.9, 13.7 (each 1H, s, OH)] appeared in the ¹H NMR spectrum. According to the above NMR spectral data, the correlations of δ_H 7.91 (1H, s) with δ_C 150.2 (s), 108.1 (s), and 155.8 (s) in the HMBC spectrum (Figure 1) and the molecular formula of C₂₀H₂₆N₄O₃, the five-carbon aromatic group should be 6',8'-purinediol. In the HMBC spectrum, correlations of δ_H 4.46 (1H, dd, J = 4.6, 14.1 Hz, H-6a), 4.25 (1H, dd, J = 9.0, 14.1 Hz, H-6b) with δ_C 150.2 (s, C-4'), 151.9 (s, C-8') suggested the link of the 6',8'-purinediol moiety with the suberosanone moiety by a C(6)–N(9') bond. The relative stereochemistry of **1** was deduced from a 2D NOE experiment. In the NOESY spectrum of **1** (Figure 2), correlations of H-2 (δ_H 2.40, 1H, overlap) with Me-15 (δ_H

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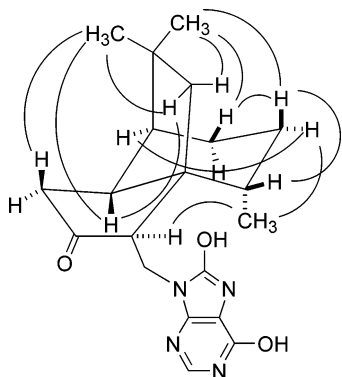


Figure 2. Selective NOE correlations of **1**.

1.16, 3H, s) and H-12a (δ_{H} 1.65, 1H, d, $J = 14.7$ Hz) indicated that H-2 and Me-15 had a β -orientation, because the C-12 methylene was the β -substituent at C-1. NOE correlations of H-9a (δ_{H} 1.19, 1H, m) with Me-7 (δ_{H} 0.80, 3H, d, $J = 7.0$ Hz) and H-11 (δ_{H} 1.77, 1H, m), and H-5 with Me-7, and no NOE correlation of H-11 with H-12 [δ_{H} 1.69, 1.65 (each 1H, d, $J = 14.7$ Hz)], suggested the α -orientation of H-5, H-11, and Me-7. On the basis of the above data, the structure of **1** was elucidated as shown. The H-2 β and positive rotation $\{[\alpha]_{\text{D}}^{20} + 28^{\circ}$ (c 0.2, CHCl_3) $\}$ of **1** were different from the H-2 α and negative rotation of suberosanone $\{[\alpha]_{\text{D}}^{25} - 60^{\circ}$ (c 0.1, CHCl_3) $\}$ and other suberosanone-type sesquiterpenes.¹¹

The cytotoxicity of compound **1** toward the MDA-MB-231 and MCF cancer cell lines was evaluated quantitatively and qualitatively, respectively. It was found that compound **1** showed moderate cytotoxicity against the human breast carcinoma MDA-MB-231 cell line with an IC_{50} of 8.87 $\mu\text{g}/\text{mL}$ and potential cytotoxicity toward the MCF cell line at a concentration of 50 μM .

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were measured with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^1H , ^{13}C NMR and 2D NMR spectra were recorded on a Bruker AV-500 MHz NMR spectrometer with TMS as internal standard. MS spectral data were obtained on an LCQ^{DECA} XP HPLC/MSⁿ spectrometer for ESIMS. Si gel (200–300 mesh) for column chromatography and GF₂₅₄ for TLC were obtained from the Qindao Marine Chemical Factory, Qindao, People's Republic of China.

Animal Material. The South China Sea gorgonian coral *S. suberosa* (3.5 kg, wet weight) was collected in Sanya, Hainan Province, China, in October 2003 and identified by Prof. R. L. Zou, the South China Sea Institute of Oceanology, Academia Sinica. A voucher specimen (No. 0312) was deposited in the South China Sea Institute of Oceanology, Academia Sinica, Guangzhou, China.

Extraction and Isolation. The frozen specimen was extracted with EtOH/ CH_2Cl_2 (2:1) three times at room temperature, and the solution was evaporated in vacuo. The residue was suspended in H_2O and extracted with CHCl_3 three

times. The CHCl_3 layer was concentrated in vacuo to afford 40 g of residue. The CHCl_3 extract was subjected to column chromatography (CC) on silica, using $\text{CHCl}_3/\text{Me}_2\text{CO}$ (from 10:0 to 0:10) as eluent. By combining the fractions with TLC (GF₂₅₄) monitoring, eight fractions were obtained. Fraction 2 was subjected to CC on silica gel, eluted with petroleum ether/EtOAc (from 10:0 to 10:1), to afford **2** (6 mg). Fraction 4 was subjected to CC on silica gel, eluted with petroleum ether/EtOAc (from 10:1 to 8:2), to yield **3** (48 mg) and **4** (5 mg). Fraction 6 was chromatographed over Sephadex LH-20 eluting with $\text{CHCl}_3/\text{MeOH}$ (1:1), then subjected to CC on silica gel, eluted with $\text{CHCl}_3/\text{MeOH}$ (from 12:1 to 10:2), to yield **1** (8 mg).

6-(9'-Purine-6',8'-diolyl)-2 β -suberosanone (1**):** white powder; $[\alpha]_{\text{D}}^{20} + 28^{\circ}$ (c 0.2, CHCl_3); UV (MeOH) λ_{max} 212, 264 nm; IR (KBr) 3500, 3115, 1740, 1710, 1670, 1600 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 2.40 (1H, overlap, H-2 β), 2.47 (1H, m, H-3 β), 2.41 (1H, overlap, H-3 α), 3.32 (1H, dd, $J = 4.4$, 8.3 Hz, H-5 α), 4.46 (1H, dd, $J = 4.4$ Hz, 14.1, H-6a), 4.25 (1H, dd, $J = 8.3$, 14.1 Hz, H-6b), 0.80 (3H, d, $J = 7.0$ Hz, Me-7), 1.54 (1H, m, H-8 α), 1.87 (1H, m, H-9 β), 1.16–1.19 (1H, overlap, H-9 α), 1.61 (1H, m, H-10 β), 1.53 (1H, m, H-10 α), 1.77 (1H, m, H-11 α), 1.69 (1H, d, $J = 14.7$ Hz, H-12 β), 1.65 (1H, d, $J = 14.7$ Hz, H-12 α), 1.14 (3H, s, Me-14), 1.16 (3H, s, Me-15), 7.91 (1H, s, H-2'), ^{13}C NMR (125 MHz, CDCl_3) δ 56.7 (C, C-1), 44.0 (CH, C-2), 40.8 (CH₂, C-3), 216.5 (C, C-4), 52.4 (CH, C-5), 41.3 (CH₂, C-6), 16.7 (C, C-7), 36.6 (CH, C-8), 27.0 (CH₂, C-9), 27.9 (CH₂, C-10), 49.7 (CH, C-11), 48.5 (CH₂, C-12), 39.7 (C, C-13), 27.0 (CH₃, C-14), 34.4 (CH₃, C-15), 140.6 (CH, C-2'), 150.2 (C, C-4'), 108.1 (C, C-5'), 155.8 (C, C-6'), 151.9 (C, C-8'); HRESIMS m/z 369.1920 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{20}\text{H}_{25}\text{N}_4\text{O}_3$ 369.1926).

Biological Assays. Human breast carcinoma MDA-MB-231 and MCF cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD). Cytotoxicity assays were measured by MTT methods as described previously.¹⁴

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References and Notes

- Groweiss, A.; Fenical, W.; He, C. H.; Clardy, J.; Wu, Z. D.; Yiao, Z. N.; Long, K. H. *Tetrahedron Lett.* **1985**, *26*, 2379–2382.
- Parameswaran, P. S.; Naik, C. G.; Kamat, S. Y.; Puar, M. S.; Das, P.; Hegde, V. R. *J. Nat. Prod.* **1998**, *61*, 832–834.
- Wang, G. H.; Ahmed, A. F.; Sheu, J. H.; Duh, C. Y.; Shen, Y. C.; Wang, L. T. *J. Nat. Prod.* **2002**, *65*, 887–891.
- Wang, G. H.; Ahmed, A. F.; Kuo, Y. H.; Sheu, J. H. *J. Nat. Prod.* **2002**, *65*, 1033–1036.
- Bokesch, H. R.; McKee, T. C.; Cardellina, J. H.; Boyd, M. R. *Tetrahedron Lett.* **1996**, *37*, 3259–3262.
- Anjaneyulu, A. S. R.; Rao, K.; Venkateswara, G. *Indian J. Chem.* **1997**, *36B*, 418–423.
- Aknin, M.; Costantino, V.; Mangoni, A. *Steroids* **1998**, *63*, 575–578.
- Subrahmanyam, C.; Kumar, S. R.; Reddy, G. D. *Indian J. Chem.* **2003**, *42B*, 219–220.
- Qi, S. H.; Zhang, S.; Huang, H.; Huang, J. S.; Xiao, Z. H. *J. Nat. Prod.* **2004**, *67*, 1907–1910.
- Qi, S. H.; Zhang, S.; Xiao, Z. H.; Huang, J. S. *Magn. Reson. Chem.* **2005**, *43*, 266–268.
- Sheu, J. H.; Hung, K. C.; Wang, G. H.; Duh, C. Y. *J. Nat. Prod.* **2000**, *63*, 1603–1607.
- Su, J. Y.; Long, K. H.; Jian, Z. G. *Acta Sci. Nat. Univ. Sunyatseni* **1984**, *1*, 97–101.
- Yu, D. Q.; Yang, J. D. *Analytical Chemistry Book*, Vol. VII; Chemical Industry Press: Beijing, 1999; p 743.
- Bednarski, P. J. *Biochem. Pharmacol.* **1992**, *43*, 2609–2620.

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