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

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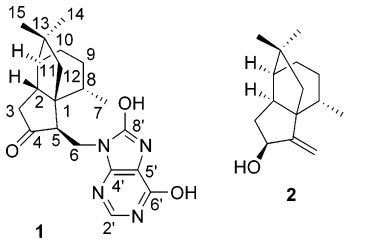
Received March 29, 2005

A new sesquiterpene alkaloid, 6-(9′-purine-6′,8′-diolyl)-2β-suberosanone (**1**), together with three known sesquiterpenes, suberosenol A (**2**), subergorgic acid (**3**), and subergorgiol (**4**), was isolated from the EtOH/CH_2Cl_2 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 IC_{50} of 8.87 μg/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)-2β-suberosanone (**1**), together with three known sesquiterpenes, suberosenol A (**2**),[11] subergorgic acid (**3**),[1] and subergorgiol (**4**),[4] was isolated from the EtOH/CH_2Cl_2 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 IC_{50} of 8.87 μg/mL and potential cytotoxicity toward the MCF cell line at a concentration of 50 μM. This paper deals with the isolation, structural elucidation, and cytotoxic activity of **1**.



Compound **1** had the molecular formula $C_{20}H_{26}N_4O_3$ 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 (1670, 1600 cm^{-1}). The ^{13}C 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 suberosanone[11] and suberosenone[5] 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.

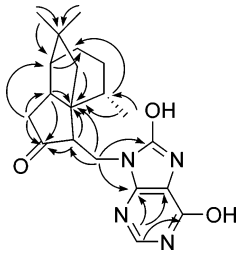


Figure 1. Key HMBC correlations of **1**.

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 $CDCl_3$ 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 $C_{20}H_{26}N_4O_3$, 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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10.1021/np050105l CCC: $30.25
Published on Web 07/20/2005

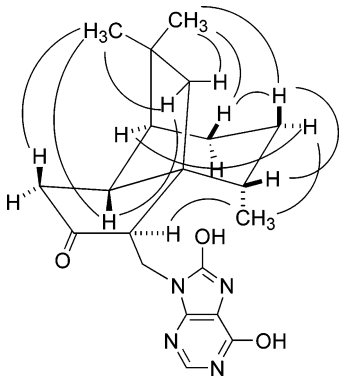

Figure 2. Selective NOE correlations of **1**.

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-2β and positive rotation {$[\alpha]^{20}_D$ +28° (c 0.2, $CHCl_3$)} of **1** were different from the H-2α and negative rotation of suberosanone {$[\alpha]^{25}_D$ −60° (c 0.1, $CHCl_3$)} and other suberosane-type sequiterpenes.[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 μg/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. ^{1}H, ^{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/MS^n spectrometer for ESIMS. Si gel (200−300 mesh) for column chromatography and GF_{254} 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 $CHCl_3$/Me_2CO (from 10:0 to 0:10) as eluent. By combining the fractions with TLC (GF_{254}) 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 $CHCl_3$/MeOH (1:1), then subjected to CC on silica gel, eluted with $CHCl_3$/MeOH (from 12:1 to 10:2), to yield **1** (8 mg).

6-(9′-Purine-6′,8′-diolyl)-2β-suberosanone (1): white powder; $[\alpha]^{20}_D$ +28° (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} 212, 264 nm; IR (KBr) 3500, 3115, 1740, 1710, 1670, 1600 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.40 (1H, overlap, H-2β), 2.47 (1H, m, H-3β), 2.41 (1H, overlap, H-3α), 3.32 (1H, dd, J = 4.4, 8.3 Hz, H-5α), 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-8α), 1.87 (1H, m, H-9β), 1.16−1.19 (1H, overlap, H-9α), 1.61 (1H, m, H-10β), 1.53 (1H, m, H-10α), 1.77 (1H, m, H-11α), 1.69 (1H, d, J = 14.7 Hz, H-12β), 1.65 (1H, d, J = 14.7 Hz, H-12α), 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 (CH_2, C-3), 216.5 (C, C-4), 52.4 (CH, C-5), 41.3 (CH_2, C-6), 16.7 (C, C-7), 36.6 (CH, C-8), 27.0 (CH_2, C-9), 27.9 (CH_2, C-10), 49.7 (CH, C-11), 48.5 (CH_2, C-12), 39.7 (C, C-13), 27.0 (CH_3, C-14), 34.4 (CH_3, 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 [M − H]$^-$ (calcd for $C_{20}H_{25}N_4O_3$ 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]

Acknowledgment. The authors are grateful to the Knowledge Innovation Program of Chinese Academy of Science (grant: KZCX3-SW-216) and Guangdong Natural Science Foundation (2003)11 for financial support.

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NP050105L