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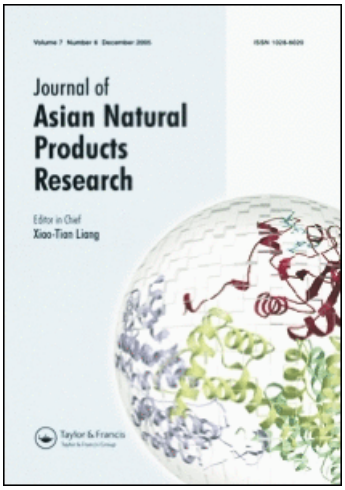


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# Norisoprenoids from the marine sponge *Spheciospongia* sp.

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Chemical examination of a marine sponge *Spheciospongia* sp. collected from South China Sea resulted in the isolation of five norisoprenoid derivatives (**1**–**5**), of which two new compounds were designated with trivial names of spheciospongones A (**1**) and B (**2**). Their structures were determined on the basis of extensive 1D and 2D NMR, and MS spectroscopic data analysis in association with circular dichroism. Norisoprenoids were found from the sponge genus *Spheciospongia* for the first time, and were suggested to be the chemical marks for chemical taxonomy.

**Keywords:** marine sponge; *Spheciospongia* sp.; norisoprenoids; spheciospongones A and B; structural elucidation

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## 1. Introduction

The marine sponge of the genus *Spheciospongia* (Clionidae) consisted of 40 species, and part of the species inhabit in shallow waters of tropical oceans. The genus *Spheciospongia* was reported to contain rich fatty acids and steroids [1,2]. Previous chemical investigation revealed that *Spheciospongia vesparia* contained unique furanose-rich glycosphingolipids [3], while the Philippine *Spheciospongia* sp. produced the PKCζ inhibitory active sterol sulfates [4], along with polyoxygenated sterols [5]. In the course of investigating the chemical diversity of marine organisms growing in South China Sea, an unidentified species of the marine sponge *Spheciospongia* sp. was collected. Extensive column chromatography of its EtOAc extract led to the isolation of five apocarotenoids including two new $C_{13}$-norisoprenoids namely spheciospongones A (**1**) and B (**2**) (Figure 1).

## 2. Results and discussion

Compound **1** was obtained as a colorless oil, and its molecular formula was established as $C_{13}H_{20}O_4$ based on HR-ESI-MS at *m/z* 263.1253 $[M+Na]^+$ and NMR spectral data. The $^1$H NMR spectrum exhibited the resonances for four methyl singlets at $\delta$ 0.84 (3H, s), 1.04 (3H, s), 1.38 (3H, s), and 2.29 (3H, s), four aliphatic protons resonated at the range of $\delta$ 1.60–2.17, a hydroxymethine at $\delta$ 4.26 (1H, ddt), and an olefinic proton at $\delta$ 5.56 (1H, s). The $^{13}$C NMR spectrum exhibited 13 carbon signals involving four methyls, two methylenes, one oxymethine, two olefinic, and a ketone. The HMQC spectrum assigned all protons and their corresponding carbons in the molecule (Table 1). The COSY correlation from the hydroxymethine at $\delta$ 4.26 (1H, H-3) to the methylene protons at $\delta$ 1.66 (1H, ddd, $J = 1.7$, 4.5, 12.5 Hz, H-2a) and 1.60 (1H, dd, $J = 11.6$, 12.5 Hz,

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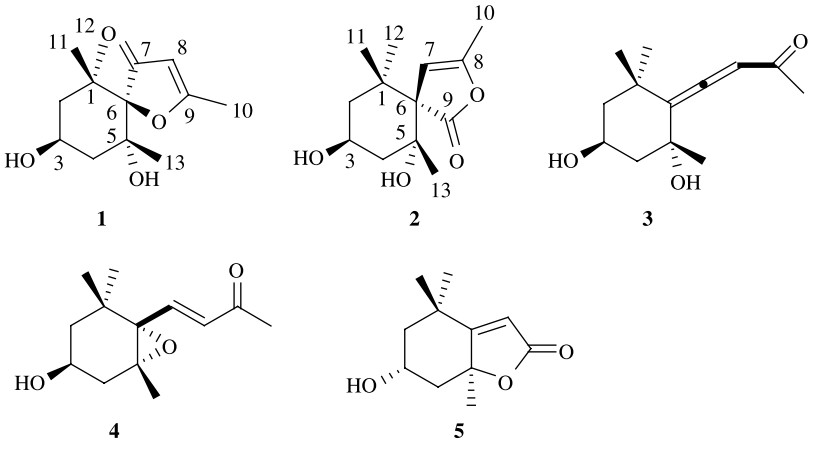


Figure 1. Structures of compounds **1**–**5**.

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H-2b) along with the methylene protons at $\delta$ 2.07 (1H, ddd, $J = 1.7$, 4.5, 13.0 Hz, H-4a) and 1.56 (1H, dd, $J = 11.5$, 13.0 Hz, H-4b) established a moiety of $CH_2$-$CHOHCH_2$. The HMBC correlations from dimethyl protons at $\delta$ 0.84 (3H, s) and 1.38 (3H, s) to C-1 ($\delta$ 39.2), C-2 ($\delta$ 45.8), and C-6 ($\delta$ 92.5), in association with the correlation of a methyl singlet at $\delta$ 1.04 (3H, s, Me-13) to C-6, C-5 ($\delta$ 75.4), and C-4 ($\delta$ 44.7), disclosed a substructure of 1,1,5-trimethyl-3,5-dihydroxycyclohexane. Further HMBC correlations from the olefinic methyl protons at $\delta$ 2.29 (s, C-10) to C-8 ($\delta$ 107.2) and C-9 ($\delta$ 190.3), and from the olefinic proton at $\delta$ 5.56 (1H, s) to C-6, C-7 ($\delta$ 207.6), C-9, and C-10 ($\delta$ 16.6, q) enabled the formation of a 7-oxo-9-methyl-6,7-dihydrofuran ring to be located at C-6 in a spiro form. Taking three sets of unsaturation as accounted for a cyclohexane and a propenone out of four in the molecule, the remaining one set of unsaturation also supported the existence of a 6,9-epoxide group. The relative configurations of **1** were determined on

Table 1. $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectral data of **1** and **2** ($CDCl_3$).

| No. | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$, Hz) | $\delta_C$ | $\delta_H$ ($J$, Hz) |
| 1 | 39.2 | | 37.9 | |
| 2 | 45.8 | 1.66 (ddd, 1.7, 4.5, 12.5), 1.60 (dd, 11.6, 12.5) | 44.2 | 2.03 (dd, 12.1, 12.6), 1.66 (ddd, 2.1, 4.3, 12.6) |
| 3 | 64.3 | 4.26 (ddt, 11.5, 11.6, 4.5) | 64.1 | 4.14 (ddt, 11.6, 12.1, 4.3) |
| 4 | 44.7 | 2.07 (ddd, 1.7, 4.5, 13.0), 1.56 (dd, 11.5, 13.0) | 43.9 | 2.14 (dd, 11.6, 13.0), 1.96 (ddd, 2.1, 4.3, 13.0) |
| 5 | 75.4 | | 75.3 | |
| 6 | 92.5 | | 63.1 | |
| 7 | 207.6 | | 103.5 | 5.22 (s) |
| 8 | 107.2 | 5.56 (s) | 152.9 | |
| 9 | 190.3 | | 178.3 | |
| 10 | 16.6 | 2.29 (s) | 13.8 | 2.09 (s) |
| 11 | 26.3 | 0.84 (s) | 28.1 | 0.87 (s) |
| 12 | 22.1 | 1.38 (s) | 26.1 | 1.23 (s) |
| 13 | 25.8 | 1.04 (s) | 28.3 | 1.18 (s) |

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the basis of the coupling constants and ROESY experiments. The coupling constants $J_{H\text{-}3/H\text{-}2b} = 11.6$ Hz and $J_{H\text{-}3/H\text{-}4b} = 11.5$ Hz disclosed an axial orientation of H-3 when the cyclohexane adopts a 'chair form'. The NOE correlations from H-3 to H-2a, H-4a, and $H_3$-12 ($\delta$ 1.38, s); $H_3$-11 ($\delta$ 0.84, s) to $H_2$-2; and $H_3$-13 ($\delta$ 1.04, s) to $H_2$-4 (Figure 1), and between $H_3$-13 and $H_3$-11 clarified H-3 and $H_3$-12 being α-face in opposite to $H_3$-11 and $H_3$-13. The chemical shift of $H_3$-12 shifted to more downfield than that of $H_3$-11, indicating $H_3$-12 to be located at the deshielded zone of the ketone group. This finding suggested the ketone group of the five-membered ring to be α-oriented. A weak NOE correlation of $H_3$-11 and $H_3$-13 to $H_3$-10 further supported the stereochemistry assignment (Figure 2). Based on the circular dichroism (CD) rule as reported by Gawronski and others [6–8], the absolute configuration of the stereogenic center at the γ-position of a butenolide ring is correlated with the sign of the Cotton effect (CE) of $n-\pi^*$ (235–300 nm) and $\pi-\pi^*$ (200–220 nm) transitions. Accordingly, the right-handed (*P*) helicity of the R—C(γ)—C═C bond system (where R is an alkyl or alkoxy group) gives rise to a negative $n-\pi^*$ and a positive $\pi-\pi^*$ CE, whereas the opposite sign pattern is for the left-handed (*M*) helicity of the bond system. Thus, the negative CE ($\Delta\varepsilon_{265\,nm}$ −1.022) for the $n-\pi^*$ transition of **1** was in agreement with 6*R* by using the right-handed (*P*) helicity (Figure 3). Molecular modeling of **1** using a Gaussian-03 package (B3LYP/6-31G(d) level) for minimizing energy calculation suggested the most stable conformation of **1** to be 3*S*, 5*R*, and 6*R*, which was in agreement with the CD and NOESY results.

The molecular formula of **2** was established to be $C_{13}H_{20}O_4$ by the pseudomolecular ion peak at *m/z* 263.1253 $[M+Na]^+$ in the HR-ESI-MS and NMR spectral data. IR absorptions at 3338, 1783, 1720, and 1688 $cm^{-1}$ suggested the presence of hydroxyl, olefinic, and lactone groups. The $^1H$ and $^{13}C$ NMR spectral data of **2** (Table 1) were very similar to those of **1**, as evident from the presence of four methyl singlets at $\delta$ 0.87, 1.18, 1.23, and 2.09, four aliphatic multiplets ranging between $\delta$ 1.66 and 2.14 for two methylene groups $H_2$-2 and $H_2$-4, a hydroxymethine at $\delta$ 4.14 (1H, ddt, H-3), and an olefinic singlet at $\delta$ 5.22. The $^{13}C$ NMR and DEPT spectra of **2** displayed 13 carbon signals involving a carbonyl carbon at $\delta$ 178.3 (s, C-9) and two olefinic carbons at $\delta$ 103.5 (d, C-7) and 152.9 (s, C-8), which occupied two sets of unsaturation. Thus, the structure of **2** was supposed to possess two aliphatic rings.

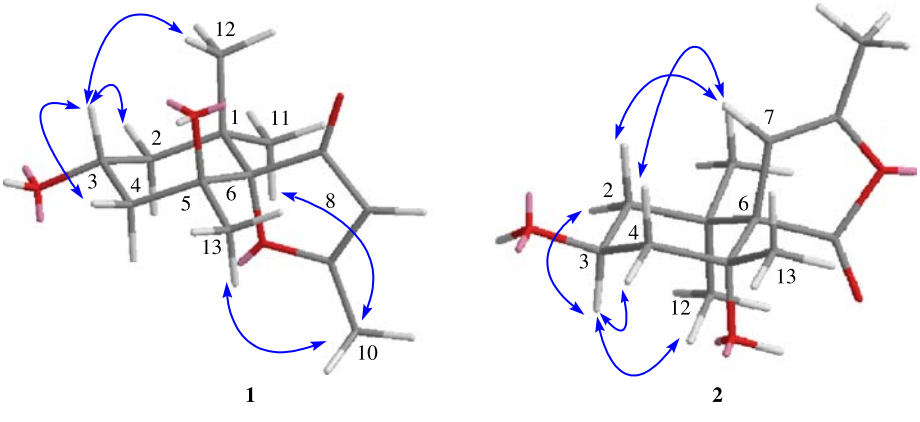


Figure 2. Key NOESY correlations of **1** and **2**.

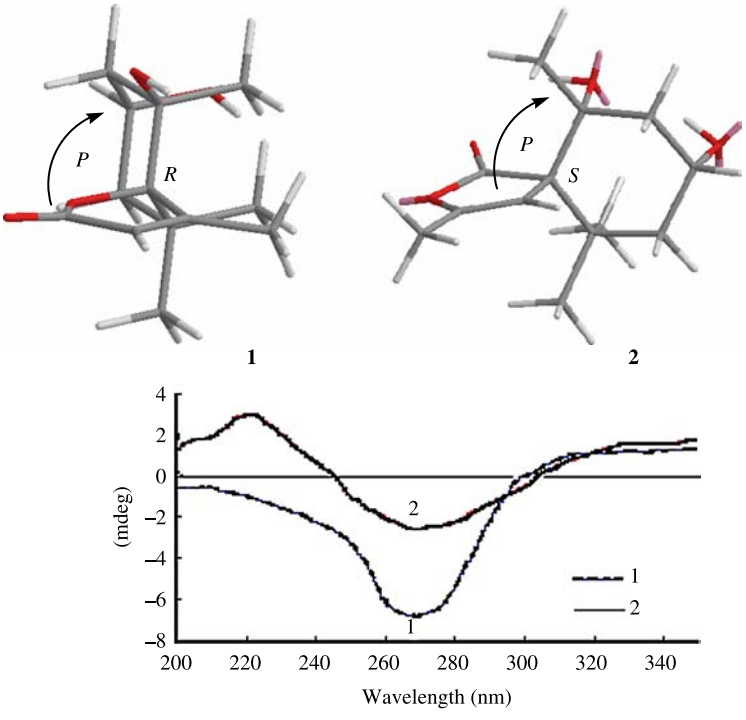


Figure 3. CD spectra of **1** and **2** and the handed helicity rule.

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The COSY and HMBC data analysis revealed that **2** possessed the same substructure of 1,1,5-trimethyl-3,5-dihydroxycyclohexane as in the case of **1**. However, C-6 of **2** shifted to far upfield at $\delta$ 63.1 (s) compared to that of **1**. The HMBC correlation from the olefinic proton at $\delta$ 5.22 (s) to C-10 ($\delta$ 13.8, q), C-6, and C-9, in association with the unsaturation of the molecule, enabled the establishment of a γ-lactone which was located at C-6 in a spiro form (Figure 1). The axial orientation of H-3 was recognized by the $J$ values ($J_{\text{H-3/H-2a}} = 12.1$ Hz, $J_{\text{H-3/H-4a}} = 11.6$ Hz), and it showed the same face with $H_3$-12 due to their NOE relationship. The NOE interaction of H-7 ($\delta$ 5.22, s) to H-2b, H-4b, $H_3$-11, and $H_3$-13 suggested β-orientation of H-7. The absolute configuration of C-6 in **2** was determined to be *S* based on the negative CE ($\Delta\varepsilon_{283\,\text{nm}}$ −0.088) for the $n-\pi^*$ transition and the positive CE ($\Delta\varepsilon_{217\,\text{nm}}$ +0.464) for the $\pi-\pi^*$ transition when applying the right-handed helicity rule (Figure 3). Accordingly, the chiral centers of C-3 and C-5 were determined to be 3*S* and 5*R*.

Compounds **3**–**5** were identical to known norisoprenoids grasshopper ketone (**3**) [9], 3β-hydroxy-5α,6α-epoxy-7-megastigmene-9-one (**4**) [10], and loliolide (**5**) [11–12] based on the comparison of their $^1$H and $^{13}$C NMR, and MS spectral data with those reported in the literature.

## 3. Experimental

### 3.1 General experimental procedures

Optical rotations were measured by a JASCO DIP-370 polarimeter. IR spectra were recorded on a Perkin-Elmer Nicol FT-50X spectrometer in KBr pellets, while UV spectra were detected by a SHIMADZU LC-20AD spectrometer coupled with SPD-M20A. NMR spectra were recorded using a Bruker Avance DRX-500 NMR spectrometer ($^1$H at 500 MHz,

$^{13}C$ at 125 MHz). HR-ESI-MS were measured by a Bruker Daltonics APEX@-FT-ICR-EIMS mass spectrometer in *m/z*. CD spectra were recorded on a Jasco J-810 CD spectropolarimeter. Column chromatography was performed on silica gel G (200–300 mesh; Qingdao Haiyang Chemical Factory, Qingdao, China) and reversed-phase silica gel (Chromatorex $C_{18}$, 40–75 μm; Fuji Silysia Chemical Ltd, Aichi, Japan). Sephadex™ LH-20 was purchased from Amersham Biosciences (Uppsala, Sweden). Silica gel used for TLC and LC was purchased from Qingdao Marine Chemistry Co. Ltd (Qingdao, China). The chemical and reagents used for chromatography were provided by Beijing Chemical Factory (Beijing, China).

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### 3.2 Animal material

The marine sponge *Spheciospongia* sp. was collected off the inner coral reef at a depth of 15 m, near the coastline of southern Sanya, Hainan Island, China. The sample was frozen immediately after collection and kept frozen until extraction. The species was identified by Dr Nicole J. de Voogd (National Museum of Natural History (Naturalis), The Netherlands). A voucher specimen (No. HSG-02) has been deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

### 3.3 Extraction and isolation

The sponge was homogenized and then extracted with EtOH. The extract was concentrated *in vacuo* to afford the residue (253 g), which was successively partitioned between $H_2O$ and petroleum ether, EtOAc, *n*-BuOH. The EtOAc fraction (3.9 g) was chromatographed over silica gel (5.2 × 40 cm) eluting with a gradient of acetone in $CHCl_3$ (acetone–$CHCl_3$ 1:20–1:2, 4 ml/min) to yield 12 fractions ($F_1$–$F_{12}$) as detected by TLC. $F_3$ (270 mg, acetone–$CHCl_3$ 1:10) was chromatographed on a Sephadex LH-20 column ($H_2O$–MeOH 1:9, 3 × 120 cm, 1.5 ml/min) to give five subfractions ($SF_1$–$SF_5$). $F_3$ (38 mg) was subjected to an ODS column (MeOH–$H_2O$ 7:3, 1.8 × 26 cm, 0.8 ml/min) to yield **2** (1.3 mg), **4** (0.9 mg), and **5** (0.9 mg). $F_4$ (152 mg, acetone–$CHCl_3$ 1:8) was applied to a Sephadex LH-20 column ($H_2O$–MeOH 1:9, 3 × 120 cm, 1.5 ml/min) to afford **1** (2.1 mg) and **3** (5.6 mg).

#### 3.3.1 Compound *1*

A colorless oil (MeOH); $[\alpha]_D^{25}$ −20.2 ($c = 4.2$, MeOH); UV (MeOH) $\lambda_{max}$ (nm): 266, 205; IR $\nu_{max}$ (KBr, $cm^{-1}$): 3272, 2923, 2852, 1727, 1662, 1595, 1462, 1380, 1344, 1160; CD (MeOH) $\Delta\varepsilon_{265\,nm}$ −1.022; $^1H$ and $^{13}C$ NMR spectral data, see Table 1; HR-ESI-MS *m/z* 263.1253 $[M+Na]^+$ (calcd for $C_{13}H_{20}O_4Na$, 263.1254).

#### 3.3.2 Compound *2*

A colorless oil (MeOH); $[\alpha]_D^{25}$ −5.8 ($c = 2.6$, MeOH); UV (MeOH) $\lambda_{max}$ (nm): 226, 201; IR $\nu_{max}$ (KBr $cm^{-1}$): 3338, 2925, 2855, 1783, 1720, 1688, 1460, 1375, 1288, 1133, 1039; CD (MeOH) $\Delta\varepsilon_{283\,nm}$ −0.088, $\Delta\varepsilon_{217\,nm}$ +0.464; $^1H$ and $^{13}C$ NMR spectral data, see Table 1; HR-ESI-MS *m/z*: 263.1253 $[M+Na]^+$ (calcd for $C_{13}H_{20}O_4Na$, 263.1254).

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