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海绵 *Spongia* sp.中一个新的 D 环为吡啶环的降海绵烷型二萜

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摘要 在对一种 *Spongia* 属海绵的化学成分研究中, 分离得到一个新的降海绵烷型二萜 dinorspongiapyridine (**1**)和四个已知化合物 **2**～**5**. 通过核磁共振波谱、单晶 X-ray 衍射以及对比文献的方法, 确定了这些化合物的结构及其绝对构型. 化合物 **1** 是首次发现的 D 环为吡啶环的 3,4-裂环-3,19-双降海绵烷型二萜类化合物, 并对其细胞毒活性进行了测试. 此外, 对化合物 **1** 的生物合成途径也进行了探讨.

关键词 海绵; *Spongia*; 降海绵烷型二萜; 吡啶环

A New Dinorspongian Diterpene with Pyridyl D-Ring from the Marine Sponge *Spongia* sp.

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Abstract Chemical investigation of the marine sponge *Spongia* sp. led to a new dinorspongian diterpene dinorspongiapyridine (**1**) together with four known compounds **2**～**5**. Compound **1** was the first instance of 3,4-seco-3,19-dinorspongian diterpene bearing a rare pyridyl D-ring system. The structure with absolute configuration of **1** was elucidated by the methods of NMR and X-ray diffraction. In addition, the cytotoxic activities against several cancer cell lines of **1** were evaluated. The plausible biogenetic pathway of **1** was also proposed.

Keywords sponge; *Spongia*; dinorspongian diterpene; pyridyl ring

1 Introduction

Marine organism was a rich source of bioactive molecules with diverse structures.[1] Spongian diterpenes were the special metabolites isolated from the marine sponges of the orders Dictyoceratida and Dendroceratida as well as the sponge predator nudibranchs.[2] Usually, these compounds featured a 6/6/6/5 tetracyclic skeleton (A/B/C/D ring system), containing a furan, γ-lactone or γ-lactam ring in D-ring,[3] some of which displayed cytotoxic,[4] antimicrobial[5] and anti-inflammatory[6] activities. Notably, only five spongian diterpenes with rare pyridyl D-ring[7] had been isolated so far. Condensation with glycine might be the key process in the pyrigyl D-ring biosynthesis. The unique structures and biological activities of spongian diterpenes had engaged the interest of chemists and biologists.

In our previous chemical investigation of the marine sponge *Spongia* sp., a series of novel spongian diterpenes with unprecedented skeletons had been isolated.[8] In the course of our continue study of this sponge, a new dinorspongian diterpene, dinorspongiapyridine (**1**), together

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with four known alkaloids **2**～**5** had been isolated from the marine sponge *Spongia* sp. (Figure 1). Compound **1** was the first example of 3,4-seco-3,19-dinorspongian diterpene bearing a rare pyridyl D-ring system. Herein, the details of the isolation, structural elucidation and biological evaluation of **1** were described. In addition, the cytotoxic activity as well as plausible biosynthetic pathway of **1** were also discussed.

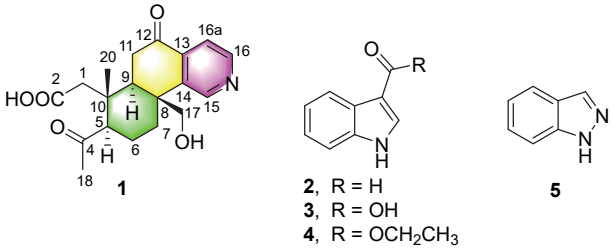


Figure 1 Chemical structures of **1**～**5**

2 Results and discussion

Dinorspongiapyridine (**1**) was isolated as colorless blocks (CH_3OH), m.p. 353～354 ℃, $[\alpha]_D^{25}$ −34.5 (*c* 0.52, CH_3OH). The molecular formula of **1** was deduced as $C_{19}H_{23}NO_5$ by the HR-ESI-MS (*m/z* 346.1651 $[M+H]^+$, calcd for $C_{19}H_{24}NO_5$ 346.1649). The analysis of ^1H NMR and ^{13}C NMR spectra supposed that **1** possessed 19 carbons, including three carbonyls (δ_C 214.1, 199.5 and 175.6), five aromatic carbons [δ_H 8.70 (s), 8.58 (d, *J*=2.1 Hz) and 7.79 (d, *J*=2.1 Hz); δ_C 149.7, 148.6, 145.7, 139.3 and 120.2], two quaternary carbons (δ_C 43.2 and 40.3), two methines (δ_H 3.24 and 2.49; δ_C 56.6 and 46.8), five methenes [δ_H 4.00 (d, *J*=11.1 Hz), 3.81 (d, *J*=11.1 Hz), 3.10 (d, *J*=14.1 Hz), 3.03 (d, *J*=14.1 Hz), 2.79, 2.46, 2.33, 2.00, 1.92 and 1.59; δ_C 65.8, 44.4, 36.1, 33.4 and 22.8] and two methyls [δ_H 2.28 (s) and 1.23 (s); δ_C 31.8 and 19.1]. The above data were similar to those of dinorspongian A,[8b] which suggested that **1** might also be a dinorspongian diterpene. The ^1H NMR and ^{13}C NMR data of **1** were shown in Table 1.

In the ^1H-^1H COSY spectrum, three spin coupling systems were observed (Figure 2). The HMBC correlations from H-6a to C-10, from H-9 to C-5/C-7/C-12/C-14/C-17/C-20, from H-11 to C-10/C-12, from H-17 to C-14 as well as from H-20 to C-5 revealed the B/C ring systems in the spongian diterpenes skeleton. Moreover, the HMBC correlations from H-1 to C-2, from H-5 to C-4, from H-9 to C-1, from H-18 to C-4/C-5 and from H-20 to C-1, indicated that the fragments of acetic acid and acetyl group were attached at C-10 and C-5, respectively. Combining the molecular formula information, a set of HMBC correlations between H-15 and C-8/C-13, between H-16 and C-13/C-15 as well as between H-16a and C-12/C-14, suggested that the D-ring was a pyridyl ring unit fused at the C-13—C-14 bond of the C-ring. Thus, the planar structure of **1** was elucidated (Figure 2).

Table 1 ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) data of **1** in CD_3OD (δ, *J* in Hz)[a]

No.	δ_H	δ_C
1	a, 2.46 b, 2.33	44.4
2	—	175.6
4	—	214.1
5	3.24	56.6
6	a, 2.04～2.00 (m) b, 2.00～1.84 (m)	22.8
7	a, 2.76 (dt, *J*=13.5, 3.3 Hz) b, 1.59 (td, *J*=13.5, 5.1 Hz)	33.4
8	—	43.2
9	2.49 (dd, *J*=14.1, 3.6 Hz)	46.8
10	—	40.3
11	a, 3.10 (dd, *J*=18.3, 14.1 Hz) b, 2.47	36.1
12	—	199.5
13	—	139.3
14	—	145.7
15	8.70 (s)	149.7
16	8.58 (d, *J*=2.1 Hz)	148.6
16	7.79 (d, *J*=2.1 Hz)	120.2
17	a, 4.00 (d, *J*=11.1 Hz) b, 3.81 (d, *J*=11.1 Hz)	65.8
18	2.28 (s)	31.8
20	1.23 (s)	19.1

[a] Overlapped signals are reported without designating multiplicity.

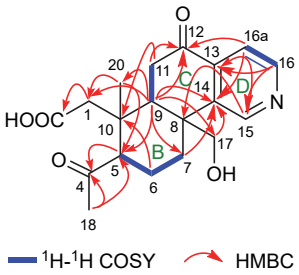


Figure 2 Key ^1H-^1H COSY and HMBC correlations of **1**

In the NOESY spectrum of **1**, the correlations between H-20 and H-17/H-18 indicated that these protons had the same orientation. Meanwhile, the NOE correlations between H-5 and H-1b/H-9 suggested that these protons were in the opposite orientation to those mentioned above (Figure 3). Thus, the relative configuration of **1** was elucidated. The absolute configuration of **1** was determined by X-ray diffraction analysis (Figure 4). The refinement on Cu Kα

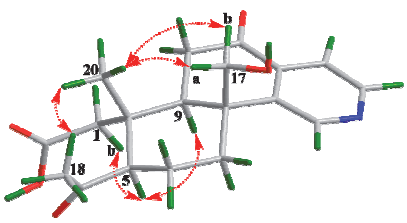


Figure 3 Key NOE correlations of **1**

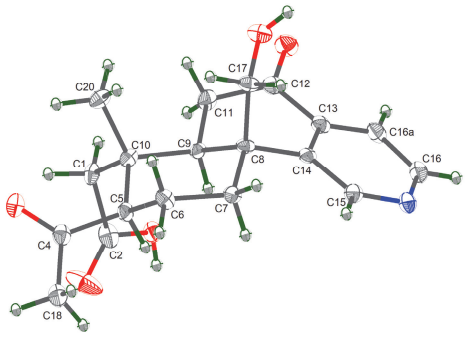
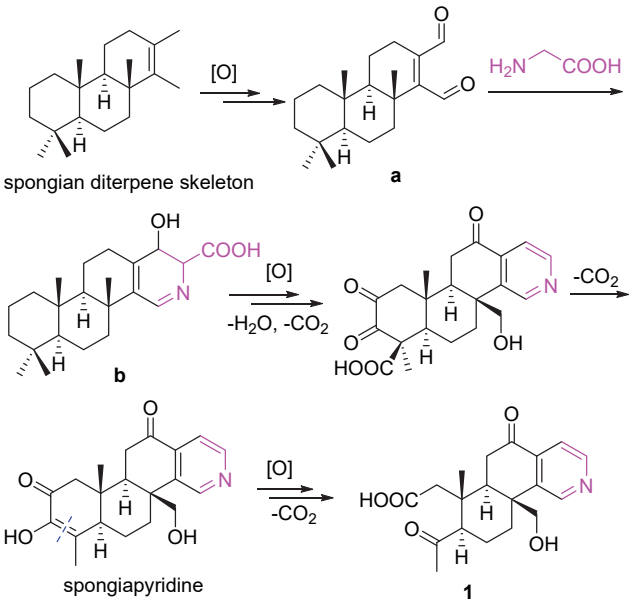


Figure 4 X-ray structure of **1**

data in a good Flack parameter of 0.00(4) supported the absolute configuration of **1** as 5*R*,8*S*,9*R*,10*R*.

A plausible biosynthetic pathway of **1** was also proposed. Basing on the basic skeleton of a spongian diterpene, the intermediate **a** was obtained by oxidation, which was condensed with glycine to yield intermediate **b**. Then, **b** went through dehydration, oxidation and decarboxylation to generate the known compound spongiapyridine.[7a] Finally, **1** was formed by oxidation and decarboxylation of spongiapyridine[8b] (Scheme 1).



Scheme 1 Plausible biogenetic pathway of **1**

The four known compounds were identified as 1*H*-indole-3-carbaldehyde,[9] 1*H*-indole-3-carboxylic acid,[10] 1*H*-indole-3-carboxylic acid acetate[11] and indazole[12] by comparing the spectroscopic data with those of the reported literatures. Cytotoxicity of **1** was evaluated against K562, A549, Hela, HT29, SK-BR-3 and Huh7 cancer cell lines. However, **1** showed no anti-proliferative effects on these carcinoma cells with a concentration of 50 μmol/L.

3 Conclusions

A new dinorspongian diterpene dinorspongiapyridine (**1**), together with four known compounds **2**～**5**, were isolated from the marine sponge *Spongia* sp.. Compound **1** was the first instance of 3,4-seco-3,19-dinorspongian diterpene bearing a rare pyridyl D-ring system. The plausible biogenetic pathway of **1** was proposed, which suggested that the rare pyridyl D-ring was formed by the basic skeleton of a spongian diterpene condensed with glycine. In addition, the cytotoxic activities against several cancer cell lines of **1** were evaluated, which exhibited no anti-proliferative effects.

4 Experimental section

4.1 General experimental procedures

UV spectrum were measured in CH_3OH on a Shimadzu UV-3600 Plus spectrometer. Termo Nicolet iS 50 FT-IR apparatus was used to detect IR spectrum. HR-ESI-MS data were carried out on an Agilent 6210 LC-ESI-Q/TOF mass spectrometer. The 1D and 2D NMR data were acquired by Bruker AV 300 NMR spectrometers. JASCO P-2000 Digital Polarimeter was used for the measurement of optical rotations. Preparative HPLC was performed on Beijing Tongchuang apparatus equipped with C_{18} reversed-phase column (Cosmosil C_{18}-MS-II column, 5 μm, 20 mm×250 mm). Column chromatography was conducted with silica gel (300～400 mesh, Qingdao Haiyang Chemical Co., Qingdao, China) and Sephadex LH-20 (GE Healthcare, American). Thin-layer chromatography (TLC) was performed on silica gel plates (GF-254, Jiangyou Silica Gel Development, Inc., Yantai, China).

4.2 Material

The sponge *Spongia* sp. was collected from Zhanjiang, Guangdong Province, P. R. China, in 2016. It was identified by Prof. Shi-Hai Xu (Jinan University). A voucher specimen (No. 2016-05A) was deposited in the Department of Chemistry, College of Chemistry and Materials Science, Jinan University, Guangzhou, P. R. China.

4.3 Extraction and isolation

The sponge *Spongia* sp. (wet 8 kg) was extracted with 95% ethanol. The crude extract was sequentially partitioned with petroleum ether, chloroform and *n*-butanol. The *n*-butanol extract (15.0 g) fractionated with a silica gel column using a $CHCl_3/CH_3OH$ gradient system and was separated into 10 (Frs. 1～10). 4 fractions (Frs. 2-1～2-4) was attained from the Fr. 2 (830.0 mg) by silica gel column eluted with $CHCl_3/CH_3OH$. Fr. 2-2 (230.2 mg) was applied to Sephadex LH-20 column with CH_2Cl_2/CH_3OH (V∶V=1∶1) to afford 4 fractions (Frs. 2-2-1～2-2-4). Compounds **2** (2.2 mg) and **4** (2.5 mg) were purified from fraction Fr. 2-2-1 (126.4 mg) by semipreparative HPLC using methanol/water (V∶V=35∶65) as mobile phase at a flow rate of 6.0 mL/min and with a C18 reversed-phase column. Meanwhile, **5** (2.3 mg) was obtained from Fr. 2-2-4 (98.3 mg) by preparative HPLC using methanol/wate (V∶V=40∶60) at a flow rate of 6 mL/min. Fr. 4 (1.8 g) submitted to silica gel chromatographic column of $CHCl_3/CH_3OH$ gradient system to give 4 fractions (Frs. 4-1～4-4). Com-

pound **3** (3.1 mg) was isolated from Fr. 4-1 (88.6 mg) by preparative HPLC [6 mL/min, $V(CH_3OH)$ ∶ $V(H_2O)$ = 40 ∶ 60]. Fr. 5 (1.5 g) yielded 3 fractions (Frs. 5-1～Fr. 5-3) by using silica gel column eluted with $CHCl_3/CH_3OH$. Fr. 5-1 (384.2 mg) was submitted to a Sephadex LH-20 column chromatographic column by binary mixtures of $CHCl_3/CH_3OH$ (V ∶ V = 1 ∶ 1) give 4 subfractions (Frs. 5-1-1～5-1-4). Compound **1** (2.1 mg) was purified from fraction Fr. 5-1-2 (110.4 mg) by using methanol/water (V ∶ V = 32 ∶ 68) as mobile phase at semipreparative HPLC with a flow rate of 6.0 mL/min.

Dinorspongiapyridine (**1**): Colorless blocks (CH_3OH), m.p. 353～354 ℃; $[\alpha]_D^{25}$ −34.5 (*c* 0.52, CH_3OH); UV (CH_3OH) λ_{max}: 215 nm; ^1H NMR and ^{13}C NMR data see Table 1; IR ν_{max}: 3391, 1692, 1562, 1358, 1416, 1041, 626 cm^{-1}; HR-ESI-MS calcd for $C_{19}H_{24}NO_5$ $[M+H]^+$ 346.1649, found 346.1651.

Supporting Information The X-ray crystallographic analysis as well as the cytotoxicity activity method.. HR-ESI-MS, IR, UV, 1D, and 2D NMR spectra of compound **1**. The Supporting information is available free of charge via the Internet at http://sioc- journal.cn/.

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