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An unusual spinaceamine-bearing pregnane from a soft coral *Scleronephthya* sp. inhibits the migration of tumor cells

Wei Cheng [a,d], Zhen Liu [a,d], Yang Yu [a], Leen van Ofwegen [b], Peter Proksch [c], Siwang Yu [a,*], Wenhan Lin [a,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, PR China
[b] National Museum of Natural History Naturalis, 2300 RA Leiden, The Netherlands
[c] Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine University, 40225 Duesseldorf, Germany

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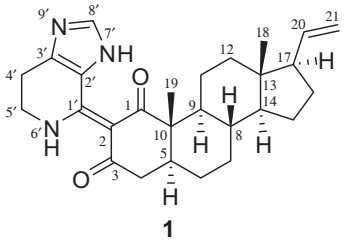
ABSTRACT

An unprecedented spinaceamine-bearing pregnane namely scleronine (**1**) was isolated from a Chinese soft coral *Scleronephthya* sp. Its structure was determined on the basis of 1D and 2D NMR spectroscopic analyses in association with the HRESIMS data, while the absolute configurations were deduced by the single-crystal X-ray diffraction analysis. In addition, a dehydrogenated analogue (**3**) was synthesized through six steps with pregna-1,20-dien-3-one (**2**) as a precursor. The significantly inhibitory effects of **1** and **3** against the migration of tumor cells A549 and B16 accompanying the down-regulation of key genes (TGFβ, TNFα, IL-1β, and IL-6) were observed. These findings suggested that both **1** and **3** are potential for therapeutic usage aiming at cancer metastasis inhibition.

Metastasis is the main leading cause of death for majority of patients who suffered cancers, while statistically as much as 90% death is due to the development of metastases.[1–3] Metastasis encompasses multiple cellular processes, and cancer cells require some functional proteins and pathways to spread from the primary tumor through migration, invading neighbor tissues and the vasculature, and circulation to establish metastatic foci. Mechanically, several signaling pathways such as Wnt/β-catenin, transforming growth factor beta (TGFβ) receptor, epidermal growth factor receptor (EGFR), and others were involved in the pathogenesis.[4,5] Due to the systemic nature and the resistance of disseminated tumor cells to existing therapeutic agents, metastatic cancers are largely incurable. Thus, development of new therapeutics aiming to prevent tumor metastasis is urgently needed. Natural products have been proved to be the major classes of anticancer drugs due to their highly diverse and architecturally complex. Several natural products have been shown to possess abilities to suppress epithelial to mesenchymal transition (EMT) in lung cancer cells.[6] Among the potential nature-derived compounds, the marine natural products such as ecteinascidin 770 (from marine tunicate) was demonstrated to have anoikis sensitizing activity by decreasing the anti-apoptotic protein MCL-1 protein with nontoxic concentrations in non-small cell lung cancer cells,[7,8] while renieramycin M (from marine blue sponge) was reported as a potential anti-metastatic agent by sensitizing anoikis-resistant lung cancer cells by suppressing survival proteins p-ERK and p-AKT, along with anti-apoptotic proteins Bcl-2 and MCL-1.[9] In the course of our investigation for the chemical diversity and the bioactive compounds from marine benthic organisms, the EtOAc extract of a soft coral *Scleronephthya* sp. was found to induce the dauer phenotype of transforming growth factor β (TGFβ) over-expressed *Caenorhabditis elegans* mutants. Chromatographic separation of the active fraction using semipreparative HPLC column led to the isolation of scleronine (**1**) and pregna-1, 20-dien-3-one (**2**).[10]



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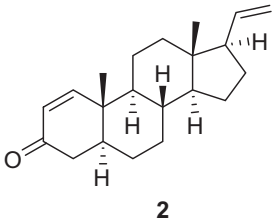

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* Corresponding authors.
E-mail address: whlin@bjmu.edu.cn (W. Lin).
[d] Equal contribution.

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Scleronine (**1**) was obtained as pale yellow crystals. The HRESIMS (*m/z* 434.2796 $[M+H]^+$) and NMR data were in accordance with a molecular formula of $C_{27}H_{35}N_3O_2$, requiring 12 degrees of unsaturation. The ^1H NMR spectrum exhibited the resonances including two methyl singlets at δ_H 0.58 (3H, s, H_3-18) and 1.03 (3H, s, H_3-19), three terminal vinyl resonances at δ_H 5.76 (1H, ddd, J = 16.1, 11.2, 7.5 Hz, H-20), 4.98 (1H, brd, J = 11.2 Hz, H-21a), 4.97 (1H, brd, J = 16.1 Hz, H-21b), and an olefinic proton at δ_H 7.83 (1H, brs, H-8′). The APT spectrum provided a total of 27 carbon resonances, including two methyl groups, six methylene carbons, ten methine carbons, six quaternary carbons, in addition to two ketones and seven olefinic carbons (Table 1). Analyses of 1D and 2D NMR (COSY, HMQC, and HMBC) data revealed that the basic nucleus of **1** is in agreement with a pregnane-type steroid, structurally related to pregna-1,20-dien-3-one (**2**), which was isolated as a main component from the same fraction. However, the NMR resonances for ring A of both compounds were significant distinction. The HMBC correlation between H_3-19 and C-1 (δ_C 204.8, s) indicated that C-1 was located by a ketone group. Additional HMBC correlations from H_2-4 (δ_H 2.33, m) to C-2 (δ_C 104.5, s) and C-3 (δ_C 195.9, s) (Fig. 1A) clarified the location of an additional ketone at C-3, while the quaternary C-2 was assumed to be resided by an olefinic bond. The remaining NMR resonances were attributed to a spinaceamine unit,[11] as evident from the COSY correlations of H_2-5′ (δ_H 3.60, 3.68, m) coupled to H_2-4′ (δ_H 2.80, t, J = 7.7 Hz) and NH (δ_H 12.54, br), in addition to the HMBC interactions of H-8′ (δ_H 7.83 brs) and H_2-4′ with C-2′ (δ_C 120.4, s) and C-3′ (δ_C 146.0, s) and between H_2-5′ and C-1′ (δ_C 157.0, s) (Fig. 1B). On the basis of the molecular unsaturation, the connection of spinaceamine unit with the pregnane nucleus through an olefinic bond across C-2 and C-1′ was conducted. This assignment was supported by the HMBC correlations from NH (δ_H 12.54, br) to the olefinic carbons C-1″ and C-2. The relative configurations of the stereogenic centers in the pregnane nucleus were in agreement with those of **2**, based on the similar NOE interactions of both compounds (Fig. 2). The absolute configurations of **1** were finally determined by the X-ray diffraction of a single crystal using the Flack parameter of 0.3 (8) (Fig. 3).[12] Thus the geometry of the olefinic bridge across C-2/C-1′ was determined to be 2*E*, while the absolute configurations of the stereogenic centers in the pregnane nucleus were assigned as 5*S*, 8*S*, 9*S*, 10*S*, 13*R*, 14*S*, and 17*R*, respectively.

In the biogenetic consideration, compound **2** was postulated to be a precursor to generate **1**. Compound **2** was converted to a dione intermediate possibly by the induction of an enzyme namely Michael hydratase-alcohol dehydrogenase (MhyADH).[13] Enzymatic condensation of the dione pregnane with oxospinaceamine, which was depicted to be derived from natural spinaceamine,[14,15] resulted in the generation of **1** (Fig. 4).

In addition, compound **2** was selected as a precursor for total synthesis of the analogue of **1**. Firstly, an nucleophilic addition of *p*-toluenethiol to **2** yielded a C-1 substituted sulfide **2a** (yield 97%).[16] Dehydrogenation of **2a** by chloreal under nitrogen protection resulted in the production of unsaturated sulfide **2b**, which followed by the conversion of phenylthioether to methoxylated analogue **2c** in sodium methoxide solution. Conversion of **2c** to a dione derivative **2d** was accomplished by the acidic induction in HCl/THF.[17] Subsequently, condensation of **2d** with 3,5-diazaindol-*N*-oxide in Ac_2O yielded compound **3** (Fig. 5).[18] Selective hydrogenation of the double bond C-2/C-1′ of **3** to form **1** is in progress.

Compound **3** was determined as 4′,5′-dehydroscleronine based on the diagnostic 2D NMR data in association with the HRESIMS data. The geometry of $\Delta^{1',2}$ in **3** was determined as *E*, by comparing the experimental ECD data of **3** with those calculated for the model molecules with both *E* and *Z* geometry at the B3LYP/6-311++G(2d, p) level in the gas phase using the B3LYP/6-31G(d)-optimized geometries after conformational searches via MMFF94S force field.[19,20] Based on the Kirk enone helicity rule,[21] the negative n→π CE (Cotton effect) at *ca* 300 nm and positive π→π CE at *ca* 260 nm reflected the *M* helicity of *cis*-enone. The same signs of

Table 1
^1H and ^{13}C NMR data of **1**.

No.	δ_C (ppm)	δ_H (ppm, J in Hz)
1	204.8, C	
2	104.5, C	
3	195.9, C	
4	42.4, CH_2	2.33, m
5	38.3, CH	1.69, m
6	27.8, CH_2	1.45, m
		1.28, m
7	30.7, CH_2	1.59, m
		0.87, m
8	36.5, CH	1.31, m
9	48.2, CH	1.30, m
10	47.4, C	
11	23.3, CH_2	2.37, m
		1.24, m
12	38.0, CH_2	1.60, m
		1.15, m
13	43.8, C	
14	55.9, CH	1.12, m
15	24.9, CH_2	1.64, m
		1.17, m
16	26.9, CH_2	1.72, m
		1.53, m
17	55.3, CH	1.98, q (7.5)
18	13.7, C	0.58, s
19	12.6, C	1.03, s
20	140.1, CH	5.76, ddd (16.1, 11.2, 7.5)
21	115.3, CH_2	4.98, brd (11.2)
		4.97, brd (16.1)
1′	157.0, C	
2′	120.4, C	
3′	146.0, C	
4′	22.2, CH_2	2.80, t (7.7)
5′	41.0, CH_2	3.68, m
		3.60, m
6′		12.50, brs
8′	138.9, CH	7.83, brs

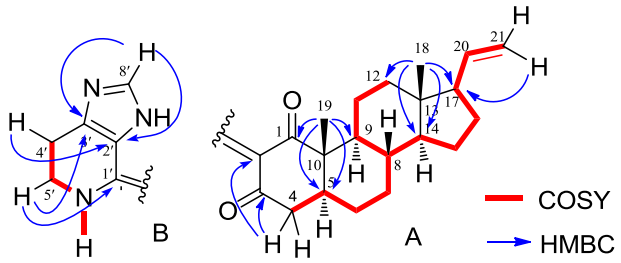

Fig. 1. Key COSY and HMBC correlations of **1**.

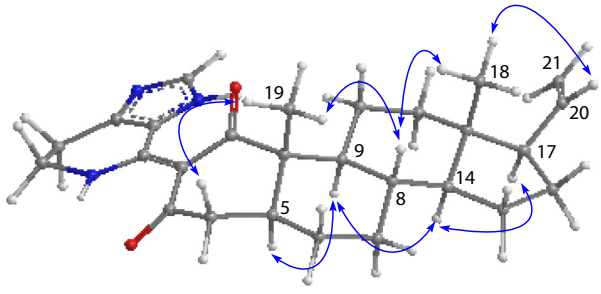

Fig. 2. Key NOE interactions of pregnane nucleus in **1**.