SHORT REPORT

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A New Angular Naphthopyrone from Crinoid *Colobometra perspinosa*

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Abstract: A chemical investigation was carried out on a crinoid *Colobometra perspinosa* collected from Hengchun Peninsula in the South China Sea, which led to the isolation of five angular naphthopyrones (**1**–**5**), including one new metabolite, 8-hydroxy-5,6,10-trimethoxy-2-pentyl-4*H*-naphtho[1,2-b]pyran-4-one (**1**). Their structures were assigned based on spectroscopic methods, including UV, HRESIMS, 1D- and 2D-NMR spectra. The anti-inflammatory activity of the isolated compounds was evaluated and compound **5** was found to inhibit the accumulation of the pro-inflammatory iNOS protein in LPS-stimulated RAW264.7 macrophages.

Keywords: : angular naphthopyrone; crinoid; *Colobometra perspinosa*; iNOS.

1. Animal Source

Specimens of *C. perspinosa* were collected in May of 2016 by scuba divers at a depth of 10-15 m, at the coast of the Hengchun Peninsula in the South China Sea. The specimens were immediately frozen and a voucher specimen (NMMBA-SI-2016-1) was preserved in the National Museum of Marine, Pingtung, Taiwan

2. Previous Studies

In the past 50 years, several angular naphthopyrones were isolated from the crinoids of *Comantheria perplexa* [1], *Comantheria rotula* [2], *Comantheria briareus* [3], *Comanthus parvicirrus* [4-8], as well as an unidentified crinoid of the family Comasteridae [9].

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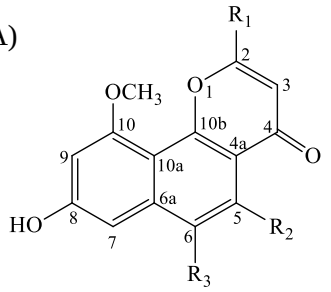
3. Present Study

C. perspinosa (wet/dry weight = 300/120 g) were sliced and extracted with a mixture of methanol:dichloromethane (1:1). The extract was partitioned between ethyl acetate (EtOAc) and H_2O. The EtOAc layer (2.22 g) was applied on silica gel column and eluted with the gradients solvent of *n*-hexane:EtOAc:acetone (from 100% *n*-hexane to 100% acetone) to furnish 15 sub-fractions. Among them, the sub-fraction 13 was further purified by NP-HPLC, using a solvent mixture of *n*-hexane:acetone:EtOAc (6:4:1) to give compound **1** (3.4 mg) (Figure 1).

(A)



1 : R_1 = pentyl; R_2 = OCH_3; R_3 = OCH_3
2 : R_1 = methyl; R_2 = OH; R_3 = OCH_3
3 : R_1 = propyl; R_2 = OCH_3; R_3 = H
4 : R_1 = propyl; R_2 = OH; R_3 = H
5 : R_1 = propyl; R_2 = OCH_3; R_3 = OCH_3
6 : R_1 = methyl; R_2 = OCH_3; R_3 = OCH_3

(B)

Figure 1. (A) The structures of compounds **1**–**6**; (B) *Colobometra perspinosa* in its natural habitat

8-Hydroxy-5,6,10-trimethoxy-2-pentyl-4*H*-naphtho[1,2-b]pyran-4-one (**1**) was isolated as a green powder that showed a sodiated adduct ion peak at *m/z* 395.1465 $(M + Na)^+$ in its HRESIMS spectrum, accounted for the molecular formula, $C_{21}H_{24}O_6$ (Calcd. for $C_{21}H_{24}O_6Na$, 395.1465) (degrees of unsaturation = 10). The IR spectrum showed absorption bands attributed to hydroxy (3368 cm^{-1}) and α,β-unsaturated ketonic (1645 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra showed 21 carbon signals including one methyl, four sp^3 methylenes, three methoxy groups (δ_C 56.1, C-18; 61.2, C-16; 61.8, C-17), three sp^2 methines (δ_C 97.7, C-7; 99.3, C-9; 110.6, C-3), and ten quaternary sp^2 carbons, one of which was a carbonyl (δ_C 177.7, C-4). The ^1H NMR spectrum showed signals for one singlet methyl (δ_H 0.92, 3H, t, J = 7.5 Hz, H_3-15), four pairs of methylene protons (δ_H 1.38, 2H, m, H_2-14; 1.41, 2H, m, H_2-13; 1.85, 2H, td, J = 15.0, 7.5 Hz, H_2-12; 2.72, 2H, t, J = 7.5 Hz, H_2-11), three methoxy groups (δ_H 3.93, 3H, s, H_3-16; 3.98, 3H, s, H_3-17; 4.00, 3H, s, H_3-18), and three aromatic protons (δ_H 6.49, 1H, s, H-3; 6.78, 1H, d, J = 2.5 Hz, H-9; 7.20, 1H, d, J = 2.5 Hz, H-7), which were observed in the ^1H NMR spectrum (Table 1). The structural units of **1** were determined using the COSY spectrum, then two separated coupling systems of H-7/H-9 (by meta coupling) and H_2-11/H_2-12/H_2-13/H_2-14/H_3-15 were subsequently identified. The HMBC correlation between the methoxy group resonances at H_3-16/C-5; H_3-17/C-6; H_3-18/C-10, and H-7/C-6, C-6a, C-8, C-9; H-9/C-7, C-8, C-10 established the presence of the methoxy group at the aromatic rings. The presence of a pentyl group on C-2 was supported by the HMBC correlation between H-3/C-2, C-11, and H_2-11/C-2, C-3 (Figure 2). The UV differences between the linear and angular naphthopyrones are well documented and the absorptions at 238, 273, and 361 nm of **1** indicated an angular naphthopyrone [4].

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

Position	δ_H [a]	δ_C, [b] type
2		168.3, C[c]
3	6.49 s	110.6, CH
4		177.7, C
4a		113.3, C
5		145.9, C
6		143.8, C
6a		159.5, C
7	7.20 d (2.5)	97.7, CH
8		107.7, C
9	6.78 d (2.5)	99.3, CH
10		159.9, C
10a		135.4, C
10b		154.0, C
11	2.72 t (7.5)	34.0, CH_2
12	1.85 td (7.5, 15.0)	26.1, CH_2
13	1.41 m	31.1, CH_2
14	1.38 m	22.4, CH_2
15	0.92 t (7.5)	13.9, CH_3
16	3.96 s	61.2, CH_3
17	3.98 s	61.8, CH_3
18	4.00 s	56.1, CH_3

[a]Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C.
[b]Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.
[c]Multiplicity deduced by DEPT spectra.

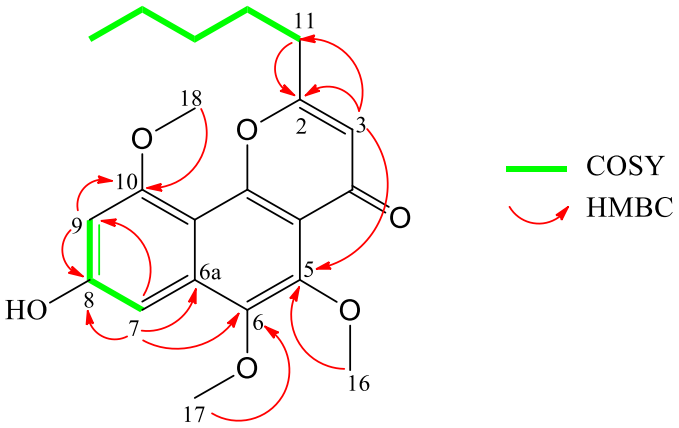


Figure 2. The ^{1}H-^{1}H-COSY and HMBC correlations for **1**

The previously studied ^{13}C NMR were similar to the angular naphthopyrone aromatic ring of compound **1** (Table 2) [8-9]. The known compounds **2**-**5** were identified as compound **2** [9], compound **3** [9], comaparvin [4], and 6-methoxycomaparvin-5-methyl ether [8] by comparing their spectroscopic data with the reported literature. Angular naphthopyrones commonly possess a methyl and a propyl group at the C-2 position. Compound **1** is the first identified compound with a pentyl group at C-2.

Table 2. Key ^{13}C NMR data for compounds **1**, **5**, and **6**

	1	**5**	**6**
Position	**δ_C[a], type**	**δ_C[b], type**	**δ_C,[c]**
4a	113.3, C	114.6, C	113.3
5	145,9, C	146.9, C	146.0
6	143.8, C	135.2, C	142.6
6a	159.5, C	158.0, C	134.2
7	97.7, CH	97.4, CH	96.3
8	107.7, C	108.4, C	159.7
9	99.3, CH	99.0, CH	99.6
10	159.9, C	160.1, C	159.3
10a	135.4, C	143.6, C	106.6
10b	154.0, C	153.0, C	152.7

[a]Spectra recorded at 125 MHz in $CDCl_3$ at 25 °C. [b]Spectra recorded at 100 MHz in $CDCl_3$ [8]. [c]Spectra recorded at 125 MHz in DMSO-d_6 [9].

The *in vitro* anti-inflammatory activities of compounds **1**–**5** were measured by examining the inhibition of LPS (lipopolysaccharide) induced upregulation of iNOS (inducible nitric oxide synthetase) and COX-2 (cyclooxygenase-2) proteins in macrophages using Western blotting analysis. RAW264.7 cells were obtained from the American Type Culture Collection (ATCC TIB-71, Manassas, VA, USA) [10]. In comparison with the cells stimulated with LPS alone, the group of macrophages treated with **5** (10 μM) showed that compound **5** exhibited a potent anti-inflammatory effect with 83.74% iNOS inhibition (Figure 3 and Table 3).

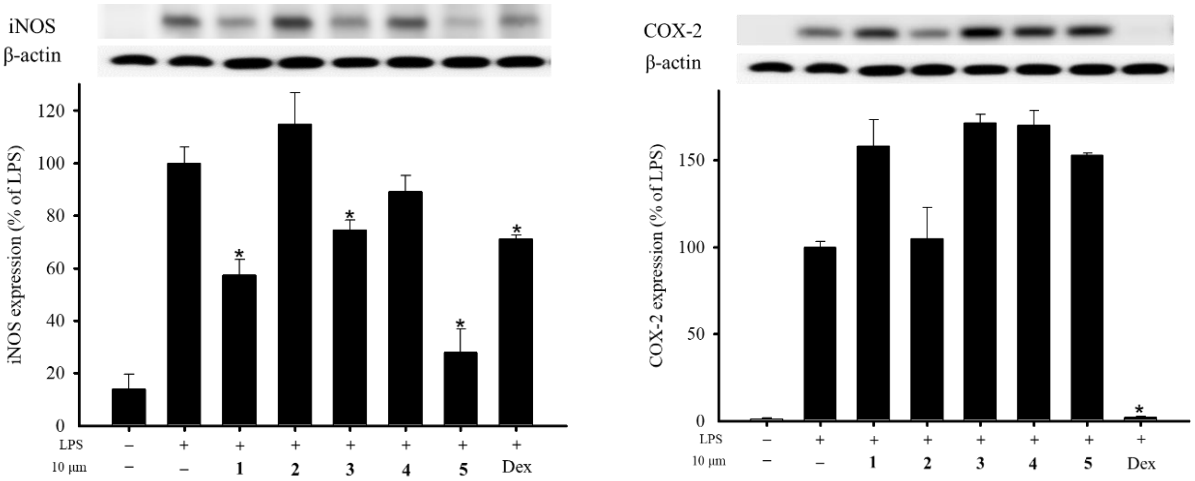


Figure 3. Effect of compounds **1**–**5** (10 μM) on pro-inflammatory iNOS and COX-2 protein expressions in the LPS-stimulated murine macrophage cell line RAW264.7 by Western blotting analysis. Data were normalized to those of cells treated with LPS alone, and the cells treated with dexamethasone (10 μM) were used as a positive control. Data are expressed as the mean ± SEM (n = 3). Significantly different from the cells treated with LPS ($p < 0.05$).

Table 3. Effects of compounds **1-5** on LPS-induced iNOS and COX-2 protein expressions in macrophages

	iNOS	**COX-2**	***β*-actin**
Control	13.96 ± 5.76	1.06 ± 1.16	105.75 ± 4.03
LPS	100.00 ± 6.09	100.00 ± 5.86	100.00 ± 0.50
1	57.26 ± 6.14	158.19 ± 26.29	124.13 ± 7.01
2	114.87 ± 11.95	104.80 ± 31.14	117.88 ± 12.39
3	74.59 ± 3.68	171.37 ± 8.76	127.43 ± 4.49
4	89.16 ± 6.06	170.09 ± 14.63	120.17 ± 16.76
5	27.95 ± 8.75	152.81 ± 2.13	103.67 ± 18.26
Dex[a]	71.13 ± 1.39	2.22 ± 0.69	130.04 ± 8.07

[a]Dexamethasone (DEX, 10 μM) was used as a positive control.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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