Bioorganic & Medicinal Chemistry Letters 24 (2014) 228–232

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

New *anti*-inflammatory cembranoid diterpenoids from the Vietnamese soft coral *Lobophytum crassum*

Nguyen Phuong Thao [a,b], Bui Thi Thuy Luyen [a,b], Nguyen Thi Thanh Ngan [b], Seok Bean Song [b], Nguyen Xuan Cuong [a], Nguyen Hoai Nam [a], Phan Van Kiem [a], Young Ho Kim [b,*], Chau Van Minh [a,*]

[a] Institute of Marine Biochemistry (IMBC), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Nghiado, Caugiay, Hanoi, Viet Nam
[b] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea

ARTICLE INFO

Article history:
Received 13 October 2013
Revised 12 November 2013
Accepted 14 November 2013
Available online 22 November 2013

Keywords:
Lobophytum crassum
Soft coral
Cembranoid
iNOS
COX-2
anti-Inflammatory

ABSTRACT

Four new cembranoid diterpenes lobocrasols A–D (**1**–**4**), were isolated from the methanol extract of the soft coral *Lobophytum crassum*. Their structures were elucidated by spectroscopic analysis and by comparison of the spectroscopic data with those of similar compounds previously reported in literature. The *anti*-inflammatory effects of isolated compounds were evaluated using NF-κB luciferase and reverse transcription polymerase chain reaction (RT-PCR). Compounds **1** and **2** significantly inhibited TNFα-induced NF-κB transcriptional activity in HepG2 cells in a dose-dependent manner, with IC_{50} values of 6.30 ± 0.42 and 6.63 ± 0.11 μM, respectively. Furthermore, the transcriptional inhibition of these compounds was confirmed by a decrease in cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) gene expression levels in HepG2 cells.

Nuclear transcription factor-κB (NF-κB) represents a family of Rel domain-containing proteins including RelA (p65), NF-κB1 (p50, p105), NF-κB2 (p52, p100), RelB, and c-Rel. The activation of NF-κB has been linked to multiple pathophysiological conditions such as cancer, arthritis, asthma, inflammatory bowel disease, and other inflammatory conditions.[1,2] The induction of several pro-inflammatory mediators occurs as resulted by increase of iNOS and COX-2 activities.[3,4] Therefore, suppression of iNOS and COX-2 activities is important for preventing inflammation in organs.[5] NF-κB and the signaling pathways that regulate many physiological processes (including the innate and adaptive immune responses, cell death, and inflammation) have become the focus of intense drug discovery and development efforts.[6,7] An increasing number of marine products have been found to display *anti*-inflammatory effects, such as microcolin A, scytonemin, malyngamides F acetate, phycocyanin, have been isolated from sponges, tunicates, algae, and other organisms.[8]

Soft corals belonging to the genus *Lobophytum* (class Coelenterata, subclass Octocorallia, and family Alcyonaceae) are a rich source of steroids and terpenoids. Most of isolated diterpenes are cembranoid compounds,[9] which are often found in high concentrations (up to 5% dry weight) in soft corals and have possible chemical defense roles against predators such as fish as well as microorganisms and other corals.[10,11] Many cembranoids exhibit various biological activities, such as acetylcholinesterase- and HIV-inhibition,[11,12] as well as antitumor,[13] antimicrobial,[14] cytotoxicity,[13,15] and *anti*-inflammatory properties.[16,17]

Our previous investigations of *Lobophytum crassum* yielded steroidal constituents with *anti*-inflammatory activity.[18] To discover additional novel bioactive substances in the Vietnamese marine invertebrates,[18–21] we carried out further studies on the chemical constituents and biological activities of the soft coral *L. crassum*. In this Letter, we report the isolation, structure determination, and *anti*-inflammatory activity of four new cembranoids, lobocrasols A–D (**1**–**4**), from this soft coral.

The sample of *L. crassum* collected in Con Co, Quangtri, Vietnam in May 2013 and identified by Professor Do Cong Thung (the Institute of Marine Environment and Resources, VAST). A voucher specimen (LC0513) was deposited at the Institute of Marine Biochemistry (IMBC), VAST. A MeOH extract (75.0 g) of the soft coral *L. crassum* was suspended in H_2O and successively extracted with *n*-hexane and CH_2Cl_2. The CH_2Cl_2 fraction was subjected to multiple separation steps over silica gel and YMC RP-18 column chromatography (CC) to afford new compounds **1**–**4**.[22]

Compound **1**[23] was obtained as a colorless oil with the molecular formula, $C_{20}H_{32}O_5$, determined by Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS) at *m*/*z* 353.23282 $[M+H]^+$. The IR spectrum of **1** suggested the presence of hydroxy

* Corresponding authors. Tel.: +82 42 821 5933; fax: +82 42 823 6566 (Y.H.K.); tel.: +84 4 37917053; fax: +84 4 37917054 (C.V.M.).
E-mail addresses: yhk@cnu.ac.kr (Y.H. Kim), cvminh@vast.ac.vn (C.V. Minh).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.11.033

(3441 cm^{-1}) and epoxy (1250 and 909 cm^{-1}) groups. The NMR spectroscopic features indicated that **1** is a cembranoid diterpene, which are typical constituents of soft coral species. The ^{13}C NMR spectrum of **1** displayed twenty carbon signals, and DEPT experiments indicated the presence of four methyl, six methylene, five methine, and five quaternary carbons (see Table 1); including two trisubstituted olefins [δ_C 124.54 (CH, C-3), 142.92 (C, C-4), 123.84 (CH, C-11), 135.28 (C, C-12)], four oxygen-bearing carbons [δ_C 77.68 (CH, C-2), 74.81 (CH, C-7), 75.65 (C, C-8), 98.42 (CH, C-16)], and one epoxy functionality [δ_C 71.24 (C, C-1) and 68.26 (C, C-15)]. The NMR spectroscopic data of **1** were similar to those of laevigatol A,[24] except that the signals of C-7/C-8 epoxide ring in laevigatol A occurred as two hydroxy groups in **1** [δ_C 74.81 (C-7)/δ_H 3.39 (1H, m, H-7) and δ_C 75.65 (C-8)]. The NMR data of **1** were assigned by comparison with those of laevigatol A, and by HMBC analysis. The methyl protons H-19 (δ_H 1.27) exhibited HMBC correlations with C-7 (δ_C 74.81)/C-8 (δ_C 75.65)/C-9 (δ_C 38.16), confirming the locations of two additional hydroxy groups at C-7 and C-8. Detailed analysis of other correlations in the HMBC spectrum further characterized the planar structure of compound **1** (see Fig. 2).

The relative stereochemistries at C-1, C-2, C-7, C-8, C-15, and C-16 of **1** were assigned by comparison of ^1H and ^{13}C NMR data with those of similar reported compounds and further supported by NOESY data. The β-orientation of the C-1/C-15 epoxy group, H-2, and hydroxy group at C-16 were assigned by agreement of ^{13}C NMR data for C-1 (δ_C 71.24), C-2 (δ_C 77.68), C-15 (δ_C 68.26), C-16 (δ_C 98.42), and C-17 (δ_C 11.34) of compound **1** with the corresponding data of laevigatol A[24] at δ_C 71.3 (C-1), 78.1 (C-2), 69.0 (C-15), 99.2 (C-16), and 11.8 (C-17), respectively. This was further confirmed by a correlation of H-2 (δ_H 4.84) with H-18 (δ_H 1.86) and those of H-17 (δ_H 1.45) with H-3 (δ_H 5.33) and H-16 (δ_C 5.26) in the NOESY (see Fig. 2). The ^{13}C NMR chemical shifts at C-7 (δ_C 74.81), C-8 (δ_C 75.65), and C-19 (δ_C 25.77) of **1** were similar to those of (+)-7β,8β-dihydroxydeepoxysarcophytoxide,[25] sinumaximol A, and sinumaximol H[21] suggesting for β-orientation of both hydroxy groups at C-7 and C-8, which was further supported by a NOESY correlation of H-3 (δ_H 5.33) with H-5 (δ_H 2.23) and those of H-7 (δ_H 3.39) with H-5 (δ_H 2.23) and H-19 (δ_H 1.27). Thus, the structure of diterpenoid **1** was established and termed lobocrasol A.

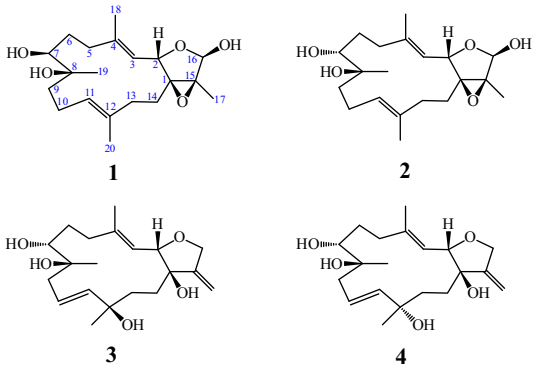

Figure 1. Structures of compounds **1**–**4** from the soft coral *L. crassum*.

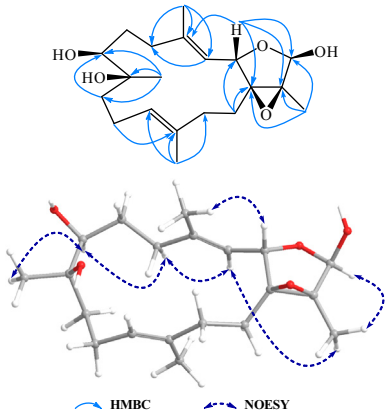

Figure 2. Key HMBC and NOESY correlations of **1**.

Table 1
NMR spectroscopic data of compounds **1** and **2**

C	**1**		**2**	
	δ_C[a,b]	δ_C[a,c] mult. (*J* in Hz)	δ_C[a,b]	δ_C[a,c] mult. (*J* in Hz)
1	71.24	—	71.19	—
2	77.68	4.84 d (10.5)	77.50	4.83 d (11.0)
3	124.54	5.33 d (10.5)	125.28	5.38 d (11.0)
4	142.92	—	139.22	—
5	34.71	2.23 m	34.95	2.00 m/2.40 m
6	31.32	1.61 m/1.68 m	26.52	1.50 m/1.82 m
7	74.81	3.39 m	72.87	3.43 t (9.0)
8	75.65	—	75.83	—
9	38.16	1.68 m	37.00	1.55 m/1.83 m
10	23.23	2.10 m/2.22 m	23.84	2.02 m/2.21 m
11	123.84	5.11 t (7.5)	124.62	4.81[d]
12	135.28	—	134.84	—
13	33.75	1.95 m/2.22 m	35.40	1.92 m/2.20 m
14	24.70	1.56 m/2.00 m	26.27	1.65 m/1.73 m
15	68.26	—	68.21	—
16	98.42	5.26 br s	98.30	5.20 d (4.0)
17	11.34	1.45 s	11.20	1.42 s
18	18.23	1.86 d (1.0)	15.82	1.80 s
19	25.77	1.27 s	24.33	1.15 s
20	16.35	1.60 s	15.00	1.55 s
7-OH			—	3.06 d (9.0)
8-OH			—	2.62 s
16-OH			—	5.26 br s

Assignments were confirmed by HSQC, HMBC, and NOESY experiments.
[a] Measured in $CDCl_3$.
[b] 125 MHz.
[c] 500 MHz.
[d] Overlapped signal.

The FTICRMS of lobocrasol B (**2**)[23] exhibited a pseudo-molecular ion peak at *m/z* 353.23272 $[M+H]^+$, confirming its molecular formula as $C_{20}H_{32}O_5$. The ^1H and ^{13}C NMR data of **2** were similar to those of **1** (see Table 1), apart from significantly different chemical shifts for the oxymethine group at C-7 and the methylene carbon at C-6, thereby suggesting a different configuration at C-7 and/or C-8 in **2** relative to that in **1**. The ^{13}C NMR chemical shifts at C-6 (δ_C 26.52), C-7 (δ_C 72.87), C-8 (δ_C 75.83), and C-19 (δ_C 24.33) of **2** were similar to those of 7α,8β-dihydroxydeepoxysarcophine at δ_C 26.7 (C-6), 72.5 (C-7), 75.4 (C-8), and 24.2 (C-19)[26] and different from those of **1** (see Table 1) and 7β,8α-dihydroxydeepoxysarcophine at δ_C 27.8 (C-6), 72.3 (C-7), 78.0 (C-8), and 26.4 (C-19),[26] thereby indicating a 7α,8β-dihydroxyl configuration in **2**. The relative configurations at C-1, C-2, C-15, and C-16 of **2** were identical to those of **1** based on agreement of ^1H and ^{13}C NMR data between these two compounds and NOESY data. Thus, the gross structure of lobocrasol B (**2**) was elucidated as a C-7 epimer of lobocrasol A (**1**).

Compound **3**[23] was obtained as a colorless oil. The FTICRMS spectrum exhibited a pseudo-molecular ion peak at *m/z* 353.23246 $[M+H]^+$, which is consistent with the molecular formula $C_{20}H_{32}O_5$. The ^{13}C NMR spectrum of **3** showed twenty carbon atom signals, which were identified using DEPT and HSQC spectra as three methyl, seven methylene, five methine, and five quaternary carbons. The NMR spectroscopic data of **3** (see Table 2) were similar to those of sinumaximol G, except for the presence of an oxygenated quaternary carbon, a 1,1-disubstituted double bond, and