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A New Sterol from the Soft Coral *Lobophytum crassum*

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The nuclear factor kappa B (NF-κB) is the 'family name' of a group of proteins, all of which possess the Rel homology domain (~300 amino acids in length) at the N-terminus. In mammalian cells, there are five NF-κB subunits, which are divided into two groups. The first group consists of RelA (also known as p65 or NF-κB3), c-Rel and RelB. The second group includes larger proteins p105 and p100, which are processed to produce the mature p50 (also known as NF-κB1) and p52 (also known as NF-κB2) proteins, respectively.[1] NF-κB was first described in 1986 as a nuclear transcription factor required for immunoglobulin kappa light chain transcription in B-cells. Since then it has been demonstrated that NF-κB is constitutively expressed in all cell types and plays a central role as a regulator of the cellular stress response. The NF-κB-mediated signaling pathway has been considered both pro-inflammatory and *anti*-apoptotic in character, and therefore, has been implicated in the pathogenesis of a wide variety of diseases, including inflammatory disorders and tumor development.[2]

In continuation of our investigation into the *anti*-inflammatory effects of Vietnamese *Lobophytum* soft corals,[3] this paper focuses on the isolation and structural elucidation of four sterols (Fig. 1) from a methanol extract of the soft coral *L. crassum*; their inhibitory effects on NF-κB activation are also described.

Using various chromatographic separations, four sterols were isolated from the methanol extract of freeze-dried bodies of the soft coral *L. crassum* including one new compound, crassumsterol (**1**). The known compounds were elucidated as (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methylcholest-6-en-3β-ol (**2**),[4] ergosterol peroxide (**3**),[5] and 3β-hydroxyandrost-5-en-17-one (**4**)[6] by analyzing the NMR and MS data in comparison with the literature values.

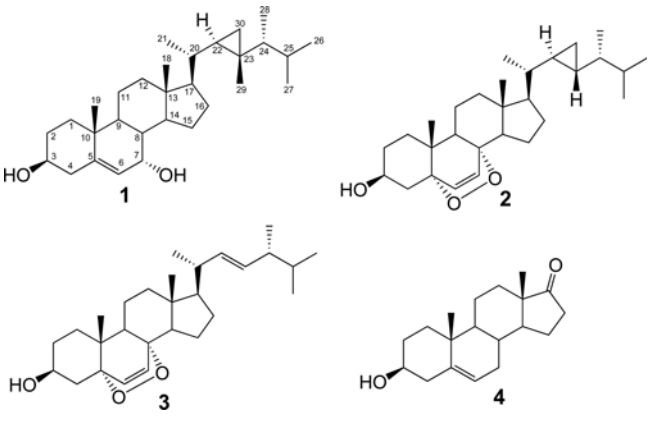

Figure 1. Structures of compounds **1**-**4**.

Crassumsterol **1** was obtained as a white powder. Its molecular formula, $C_{30}H_{50}O_2$, was defined by a pseudo-molecular ion peak at *m/z* 443.38876 $[M+H]^+$ (calcd for $C_{30}H_{51}O_2$, 443.38890) using Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS). The ^{1}H NMR spectrum revealed three singlet methyl [δ_H 0.79 (H-18), 1.10 (H-19), and 1.03 (H-29)] and three doublet (J = 7.0 Hz) methyl [δ_H 0.98 (H-26), 1.08 (H-27), and 1.06 (H-28)] signals, which were typical for three tertiary and three secondary methyl groups, respectively. A seventh methyl signal appeared as a broad singlet at δ_H 1.14, which was overlapped with a methine multiplet of H-20, and four high-field protons at δ_H 0.30 (1H, m, H-22), 0.36 (1H, m, H-24), 0.00 (1H, dd, J = 4.0, 6.0 Hz, H_β-30), and 0.58 (1H, dd, J = 4.0, 9.0 Hz, H_α-30), is characteristic of a gorgosterol-type side chain possessing a cyclopropane ring.[7] An uncommon feature of the ^{1}H NMR spectrum of **1** recorded in $CDCl_3$ was the Me-21 signal, which appeared as a broad singlet (δ_H 1.14) rather than the doublet that is expected to couple to H-20. This feature has been attributed to the fact that Me-21 and H-20 are accidentally isochronous in compounds possessing a gorgosterol-type side chain.[8] In addition, one olefinic and two oxymethine protons were identified by signals at δ_H 5.73 (1H, dd, J = 1.5, 5.0 Hz, H-6), 3.79 (1H, m, H-3), and 3.98 (1H, br s, H-7), respectively. The ^{13}C NMR spectrum of **1** showed 30 carbon signals including 7 methyls, 8 methylenes, 11 methines, and 4 quaternary carbons, as detected by distortionless enhancement by polarization transfer (DEPT) experiments. The signals at δ_C 11.6 (C-18), 18.2 (C-19), 21.1 (C-21), 21.5 (C-26), 22.2 (C-27), 15.4 (C-28), and 14.3 (C-29) were confirmed as the seven methyl groups. Moreover, two oxymethine groups [δ_C 71.3 (C-3) and 65.3 (C-7)] and a tri-substituted double bond [δ_C 146.2 (C, C-5)/123.8 (CH, C-6)] were observed. All carbons were assigned to relevant protons by a heteronuclear single quantum coherence (HSQC) experiment and the results are summarized in Table 1.

The ^{13}C NMR spectral data of **1** were similar to those of

Table 1. The NMR ($CDCl_3$) spectroscopic data of compound **1**

C	δ_C	δ_H mult. (*J* in Hz)	$^a\delta_C$	$^b\delta_C$
1	37.0	1.22 m/1.98 m	31.2	37.6
2	31.3	1.52 m/1.97 m	32.0	32.4
3	71.3	3.79 m	71.0	71.0
4	42.0	2.41 m/2.47 ddd (2.0, 5.0, 6.5)	43.3	43.4
5	146.2	-	139.2	144.9
6	123.8	5.73 dd (1.5, 5.0)	121.6	125.5
7	65.3	3.98 br s	27.0	64.8
8	37.6	1.58 m	35.2	38.4
9	42.3	1.34 m	57.6	42.7
10	37.4	-	43.0	37.7
11	20.7	1.61 m/1.66 m	69.3	21.2
12	39.2	1.31 m/2.17 m	46.9	39.8
13	41.6	-	42.8	42.3
14	49.3	1.55 m	49.4	50.2
15	24.5	1.25 m/1.84 m	24.1	24.7
16	28.3	1.47 m/2.22 m	28.3	28.7
17	57.7	1.52 m	50.8	56.2
18	11.6	0.79 s	12.1	12.0
19	18.2	1.10 s	22.1	18.5
20	35.4	1.14 m	34.5	36.5
21	21.1	1.14 br s	21.1	19.2
22	32.2	0.30 m	27.8	34.0
23	25.8	-	25.8	30.8
24	50.8	0.36 m	49.5	39.3
25	32.0	1.69 m	31.5	31.7
26	21.5	0.98 d (7.0)	21.4	20.7
27	22.2	1.08 d (7.0)	21.9	17.7
28	15.4	1.06 d (7.0)	15.4	15.6
29	14.3	1.03 s	14.3	
30	21.3	β 0.00 dd (4.0, 6.0) α 0.58 dd (4.0, 9.0)	21.2	

$^a\delta_C$ of gorgost-5-ene-3β,11α-diol.[9] $^b\delta_C$ of (24*S*)-ergost-5-ene-3β,7α-diol.[10] Assignments were confirmed by HSQC, HMBC, and NOESY experiments.

gorgost-5-ene-3β,11α-diol[9] (Table 1), except for the significant difference of the data for the double bond and one oxymethine. The cross peaks between H-19 (δ_H 1.10) and C-1 (δ_C 37.0)/C-5 (δ_C 146.2)/C-9 (δ_C 42.3)/C-10 (δ_C 37.4), H-4 (δ_H 2.41/2.47) and C-2 (δ_C 31.3)/C-3 (δ_C 71.3)/C-6 (δ_C 123.8)/C-10 (δ_C 37.4), H-6 (δ_H 5.73) and C-8 (δ_C 37.6)/C-10 (δ_C 37.4), and H-7 (δ_H 3.98) and C-5 (δ_C 146.2)/C-9 (δ_C 42.3) in the heteronuclear multiple-bond correlation (HMBC) spectrum confirmed the positions of the hydroxyl groups at C-3 and C-7, and the double bond at C-5/C-6 (Fig. 2). The proton signals of H-3 at δ_H 3.79 (1H, m) and H-7 at δ_H 3.98 (1H, br s) are representative of H_α-3 and H_β-7, respectively. In addition, the α-orientation of the OH group at C-7 was further confirmed by the ^{13}C NMR chemical shift for C-7 at δ_C 65.3, which agreed with that of (24*S*)-ergost-5-ene-3β,7α-diol[10] at δ_C 64.8; but was quite different from that of (24*S*)-ergost-5-ene-3β,7β-diol[11] at δ_C 73.3. The relative configuration of compound **1** was also confirmed by nuclear Overhauser effect spectroscopy (NOESY; Fig. 2). Thus, **1** was elucidated as gorgost-5-ene-3β,7α-diol, and named crassumsterol.

The *anti*-inflammatory activity of isolated compounds was evaluated by the inhibitory effect on TNFα-induced NF-κB transcriptional activation in Hep-G2 cells.[12] Among isolated compounds, **2** and **3** had a significant inhibitory effect on NF-κB activation with IC_{50} values of 3.90 and 7.05 μM, respectively, whereas **1** and **4** were inactive (Fig. 3). This observation was consistent with previous investigations since these two compounds were reported to have significant *anti*-inflammatory[13-15] and cytotoxic[4,13] effects. The activation of NF-κB has been linked to multiple pathophysiological conditions and induction of numerous pro-inflammatory mediators occurs as a result of increased inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) activities.[16,17] Thus, the effects of compounds **2** and **3** on the expression of these genes in TNFα-stimulated Hep-G2 cells were evaluated using reverse transcriptase polymerase chain reaction (RT-

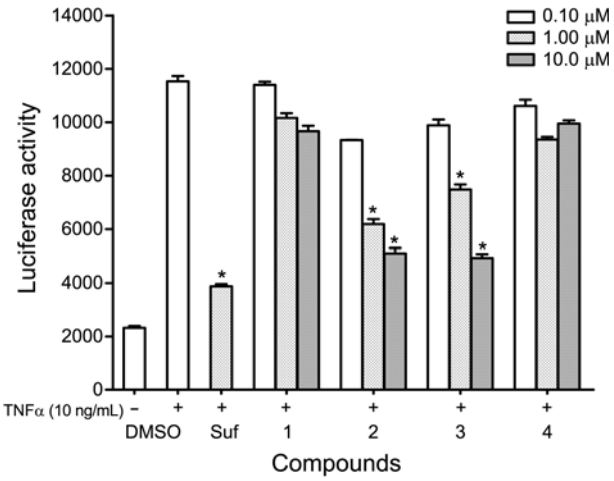

Figure 3. Effects of compounds **1**-**4** on the TNFα-induced NF-κB luciferase reporter activity in Hep-G2 cells. The values are mean ± SD (n = 3). *Signicantly different from TNFα-stimulated group ($p < 0.05$). Suf: Sulfasalazine (1.0 μM) was used as a positive control.

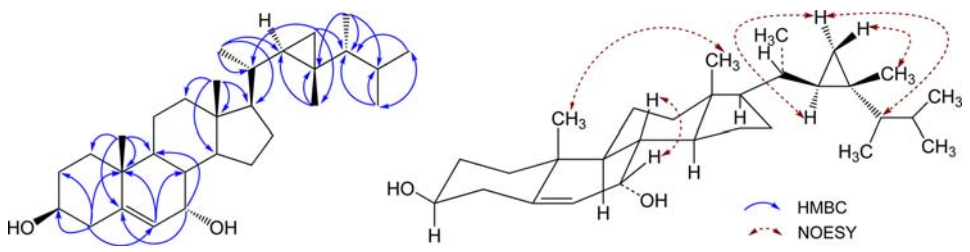

Figure 2. Key HMBC and NOESY correlations of compound **1**.

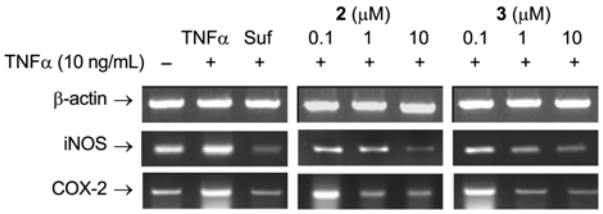

Figure 4. Effects of compounds **2** and **3** on iNOS and COX-2 mRNA expression in Hep-G2 cells. Suf: Sulfasalazine (1.0 μM) was used as a positive control.

PCR) analysis. Consistent with their inhibitory activities towards NF-κB, these two compounds signicantly inhibited the mRNA expression of COX-2 and iNOS in a dose-dependent manner (Fig. 4). Consideration of the structures of **1-4** suggested that the peroxide bridge might play an important role for the *anti*-inflammatory activity of these compounds.

Experimental

General Procedures. Optical rotations were determined on a JASCO P-2000 polarimeter (Hachioji, Tokyo, Japan). The electrospray ionization (ESI) mass spectra were obtained using an Agilent 1200 LC-MSD Ion Trap spectrometer (Agilent Technologies, Palo Alto, CA). The high resolution mass spectra were gained using a Varian 910 FT-ICR mass spectrometer (Varian, CA, USA). The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker, Billerica, MA, USA) and TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck, Darmstadt, Germany) and YMC RP-18 resins (30-50 μm, Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F_{254S} plates (1.15685.0001, Merck, Darmstadt, Germany) and compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 5 minutes.

Marine Materials. The samples of *L. crassum* were collected in Conco, Quang Tri province, Vietnam, during February 2011 and identified by Prof. Do Cong Thung from Institute of Marine Environment and Resources, Vietnam Academy of Science and Technology (VAST). Voucher specimens (No. NCCB M-15) were deposited at the Institute of Marine Biochemistry and the Institute of Marine Environment and Resources, VAST.

Extraction and Isolation. Freeze-dried bodies of *L. crassum* (0.75 kg) were exhaustively extracted three times (each 1 h) with hot MeOH (50 °C) under ultrasonic conditions to obtain 50 g MeOH residue. The residue was suspended in water (2 L) and partitioned with $CHCl_3$ (3 × 2 L), yielding $CHCl_3$ extract (C, 26.0 g), and a water layer (W). The $CHCl_3$ extract (C, 26.0 g) was separated into five fractions, C1-C5, by a silica gel column chromatography (CC) using gradient elution of *n*-hexane-acetone (100:1-1:1, v/v). Fraction C3 (2.1 g) was further separated by YMC CC using methanol-acetone-water (2.5:1:1.1, v/v/v) to obtain six smaller fractions, C3A-C3F. Fraction C3C (0.24 g) yielded crassumsterol (**1**, 5.1 mg) after subjection to silica gel CC with *n*-hexane-acetone (1:1, v/v), followed by YMC RP-18 CC, with acetone-water (1.2:1, v/v) as an eluent. (22*R*,23*R*,24*R*)-5α,8α-Epidioxy-22,23-methylene-24-methylcholest-6-en-3β-ol (**2**, 9.5 mg) and ergosterol peroxide (**3**, 8.2 mg) were purified from fraction C3D (0.55 g) by YMC RP-18 CC using methanol-acetone-water (3:1:1, v/v/v) as an eluent. Fraction C3C (0.39 g) was further separated by silica gel CC, eluted with chloroform-ethyl acetate (30:1, v/v) to give 3β-hydroxyandrost-5-en-17-one (**4**, 11.3 mg).

Crassumsterol 1: amorphous white powder, $[\alpha]_D^{25}$ −15 (*c* 0.25, $CHCl_3$); FTICRMS *m/z* 443.38876 $[M+H]^+$ (calcd for $C_{30}H_{51}O_2$, 443.38890); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) are given in Table 1.

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