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New 19-oxygenated and 4-methylated steroids from the Formosan soft coral *Nephthea chabroli*

Shi-Yie Cheng[a], Ya-Chiang Huang[a], Zhi-Hong Wen[a,b], Chi-Hsin Hsu[a,b], Shang-Kwei Wang[c], Chang-Feng Dai[d], Chang-Yih Duh[a,b,*]

[a] Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung, Taiwan
[b] Center of Asia-Pacific Marine Research, National Sun Yat-sen University, Kaohsiung, Taiwan
[c] Department of Microbiology, Kaohsiung Medical University, Kaohsiung, Taiwan
[d] Institute of Oceanography, National Taiwan University, Taipei, Taiwan

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ABSTRACT

Chemical investigation of the Formosan soft coral *Nephthea chabroli* resulted in the isolation of four new 19-oxygenated steroids, nebrosteroids I–L (**1**–**4**), together with a new 4α-methylated steroid, nebrosteroid M (**5**). The molecular structures of these isolated metabolites were elucidated on the basis of extensive spectroscopic analysis and by comparison of the data with those of related metabolites. Compounds **1**–**5** were evaluated for anti-inflammatory activity using RAW 264.7 macrophages.

1. Introduction

Soft corals belonging to the genus *Nephthea* are an established source of a prodigious array of terpenoids and steroids [1–21]. Previous bioassay results on these secondary metabolites have been shown to exhibit diverse biological properties such as insecticidal [2], cytotoxic [3–11], and anti-inflammatory activities [21]. In the course of our ongoing efforts towards the identification of bioactive substances from marine sources, chemical investigation of the Formosan soft coral *Nephthea chabroli* Audouin (Nephtheidae) was undertaken. We have previously isolated some steroids from the acetone extracts of the organism [21]. Our continuing chemical examination on the chemical constituents of this soft coral *N. chabroli* has now led to the isolation of four new 19-oxygenated steroids, nebrosteroids I–L (**1**–**4**) (Fig. 1), and a new 4α-methylated steroid, nebrosteroid M (**5**) (Fig. 1). The details of the isolation and the structural elucidation of these isolated metabolites are discussed herein. Compounds **1**–**5** were evaluated for their ability to inhibit the expression of the pro-inflammatory inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages.

2. Experimental

2.1. General procedures

Optical rotations were determined with a JASCO P1020 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-4100 infrared spectrophotometer. The NMR spectra were recorded on Bruker Avance 300 NMR/Varian MR 400 NMR/Varian Unity INOVA 500 FT-NMR spectrometers (300/400/500 MHz for ^{1}H and 75/100/125 MHz for ^{13}C), using $CDCl_3$ with TMS as an internal standard. Chemical shifts are expressed in δ (ppm) and coupling constants in Hz. ESIMS were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. Si gel 60 (Merck, 230–400 mesh) and LiChroprep RP-18 (Merck, 40–63 μm) were used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) and precoated RP-18 F_{254s} plates (Merck) were used. High-performance liquid chromatography (HPLC) was carried out using a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 220 nm and a semi-preparative reversed-phase column (Merck, Hibar Purospher RP-18e, 5 μm, 250 mm × 10 mm).

* Corresponding author at: Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan. Tel.: +886 7 5252000x5036; fax: +886 7 5255020.
E-mail address: yihduh@mail.nsysu.edu.tw (C.-Y. Duh).

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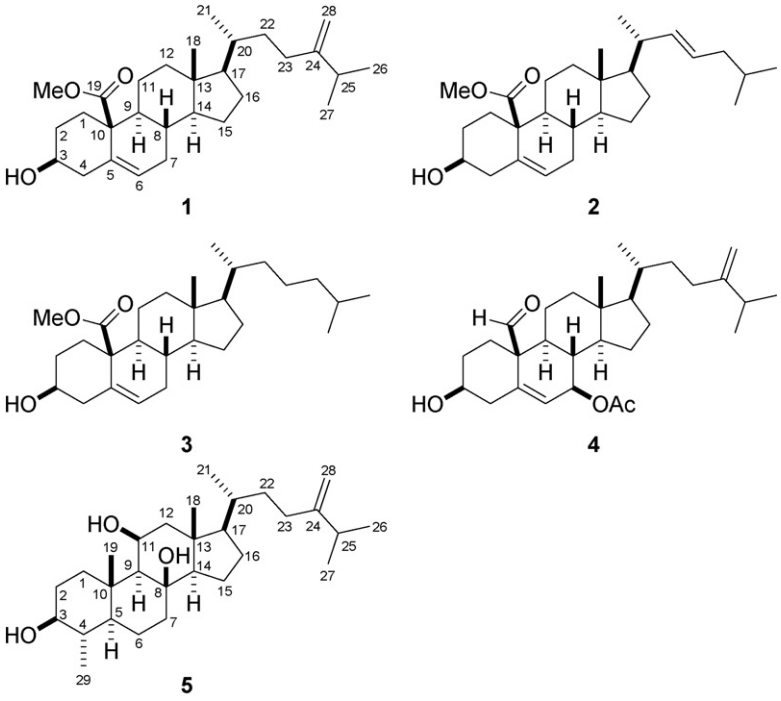

Fig. 1. Structures of **1**–**5**.

2.2. Animal material

The soft coral *N. chabroli* was collected by hand, using scuba diving equipment, from an inner coral reef of the Tsau-Lou-Cho Island of Taiwan at a depth of around 3–4 m, in October 2006. The sample was immediately frozen after collection and stored in a freezer for 4 weeks until extraction took place. The specimen was identified by Prof. C.-F. Dai and a voucher specimen (SL-03) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

2.3. Extraction and isolation

The freeze-dried soft coral (1.0 kg) was chopped into small pieces and extracted with acetone (2.0 L × 3) for 24 h at room temperature. The combined extracts were concentrated *in vacuo* (under 30 °C) to obtain a dry crude extract, which was suspended in water and extracted with EtOAc. The EtOAc phase was evaporated to dryness *in vacuo* to give a brown residue (100 g). The EtOAc residue was subjected to silica gel column chromatography using a stepwise gradient mixture of *n*-hexane–EtOAc–MeOH as eluent and separated into 19 fractions on the basis of TLC and ^{1}H NMR analysis. Fraction 11 (6.7 g) eluted with *n*-hexane–EtOAc (2:1) was chromatographed on Si gel column using *n*-hexane–EtOAc mixtures of increasing polarity for elution, to afford 10 subfractions. The subfraction 11-3 (284 mg), eluted with *n*-hexane–EtOAc (2:1), was fractionated over Sephadex LH-20 (100% acetone) to produce a mixture (56 mg) that was further purified by HPLC (RP-18) using 95% MeOH in H_2O to give **1** (5 mg) and **2** (1 mg). In turn, the subfraction 11-4 (160 mg) was applied to Sephadex LH-20 (100% acetone) to afford a mixture (75 mg) that was further separated by HPLC (RP-18) using 95% MeOH in H_2O to provide **3** (1 mg). Similarly, the subfraction 11-5 (136 mg), eluted with *n*-hexane–EtOAc (1:1), was subjected to a RP-18 column and eluted with 85% MeOH in H_2O to afford a mixture (34 mg) that was further purified by HPLC (RP-18) using 90% MeOH in H_2O to finally give **4** (2 mg). In addition, fraction 12 (4.0 g), derived from the *n*-hexane–EtOAc (1:1) elution, was subjected to Si gel column chromatography and eluted with *n*-hexane–EtOAc (1:2) to afford another subfraction (284 mg). This subfraction was fractionated over Sephadex LH-20 (100% acetone) to produce a mixture (83 mg) that was further separated on HPLC (RP-18), with 90% MeOH in H_2O as the mobile phase, to obtain **5** (5 mg).

2.3.1. Nebrosteroid I (**1**)

White amorphous powder: $[\alpha]_D^{25}$ +33.4 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3421, 2933, 2867, 1723, 1457, 1376, 1209, 1060 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS, *m*/*z* 465 $[M+Na]^+$; HRESIMS, *m*/*z* 465.3342 $[M+Na]^+$ (calcd for $C_{29}H_{46}O_3Na$, 465.3344).

2.3.2. Nebrosteroid J (**2**)

White amorphous powder: $[\alpha]_D^{25}$ +35.1 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3336, 2928, 2855, 1722, 1459, 1375, 1209, 1169 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS, *m*/*z* 451 $[M+Na]^+$; HRESIMS, *m*/*z* 451.3189 $[M+Na]^+$ (calcd for $C_{28}H_{44}O_3Na$, 451.3188).

2.3.3. Nebrosteroid K (**3**)

White amorphous powder: $[\alpha]_D^{25}$ +36.3 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3308, 2927, 2854, 1723, 1457, 1375, 1209, 1060 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS, *m*/*z* 453 $[M+Na]^+$; HRESIMS, *m*/*z* 453.3342 $[M+Na]^+$ (calcd for $C_{28}H_{46}O_3Na$, 453.3344).

2.3.4. Nebrosteroid L (**4**)

White amorphous powder: $[\alpha]_D^{25}$ +19.0 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3304, 2948, 2846, 1735, 1716, 1656, 1447, 1378, 1262, 1049, 957 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS, *m*/*z* 493 $[M+Na]^+$; HRESIMS, *m*/*z* 493.3293 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3294).

2.3.5. Nebrosteroid M (**5**)

White amorphous powder: $[\alpha]_D^{25}$ +30.8 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3335, 2929, 2870, 1643, 1454, 1376, 1172, 1045, 1011 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS, *m*/*z* 469 $[M+Na]^+$; HRESIMS, *m*/*z* 469.3659 $[M+Na]^+$ (calcd for $C_{29}H_{50}O_3Na$, 469.3657).

Table 1
^{1}H NMR spectroscopic data for compounds **1**–**5** in $CDCl_3$.

H	**1**[a]	**2**[b]	**3**[c]	**4**[a]	**5**[b]
1	α: 0.98 m β: 2.61 dt (11.8, 3.2)[d]	α: 1.01 m β: 2.61 dt (13.2, 3.6)	α: 1.01 m β: 2.60 dt (13.5, 3.5)	α: 1.07 m β: 2.59 br d (13.7)	α: 1.00 m β: 1.92 m
2	α: 1.95 m; β: 1.51 m	α: 1.93 m; β: 1.48 m	α: 1.94 m; β: 1.51 m	α: 1.99 m; β: 1.45 m	α: 1.79 m; β: 1.61 m
3	3.55 m	3.55 m	3.55 m	3.56 m	3.07 td (10.4, 5.2)
4	α: 2.48 m; β: 1.78 m	α: 2.48 m; β: 1.78 m	α: 2.48 m; β: 1.80 m	α: 2.48 m; β: 1.80 m	1.42 m
5					0.71 m
6	5.66 br d (5.4)	5.66 br d (5.2)	5.66 br d (5.5)	5.67 br s	α: 1.47 m; β: 1.53 m
7	α: 2.07 m; β: 1.56 m	α: 2.04 m; β: 1.55 m	α: 2.08 m; β: 1.56 m	5.12 d (8.8)	α: 1.19 m; β: 1.72 m
8	1.73 m	1.73 m	1.73 m	1.89 m	
9	1.03 m	1.03 m	1.04 m	1.36 m	0.90 m
11	α: 1.69 m; β: 1.28 m	α: 1.66 m; β: 1.26 m	α: 1.68 m; β: 1.32 m	α: 1.76 m; β: 1.33 m	4.45 br s
12	α: 1.11 m; β: 2.03 m	α: 1.13 m; β: 1.97 m	α: 1.13 m; β: 2.00 m	α: 1.12 m; β: 2.05 m	α: 1.38 m; β: 2.32 m
14	0.97 m	0.94 m	0.95 m	1.09 m	1.23 m
15	α: 1.58 m; β: 1.07 m	α: 1.55 m; β: 1.07 m	α: 1.57 m; β: 1.13 m	α: 1.48 m; β: 1.31 m	α: 1.57 m; β: 1.48 m
16	α: 1.87 m; β: 1.26 m	α: 1.83 m; β: 1.22 m	α: 1.83 m; β: 1.24 m	α: 1.83 m; β: 1.28 m	α: 1.84 m; β: 1.29 m
17	1.11 m	1.11 m	1.09 m	1.14 m	1.05 m
18	0.62 s	0.63 s	0.62 s	0.64 s	1.12 s
19				9.71 s	1.35 s
20	1.38 m	2.01 m	1.37 m	1.42 m	1.44 m
21	0.93 d (6.5)	0.99 d (6.4)	0.89 d (6.5)	0.92 d (6.5)	0.93 d (6.4)
22	1.56 m; 1.15 m	5.19 dd (15.2, 8.0)	1.32 m; 0.99 m	1.52 m; 1.16 m	1.50 m; 1.09 m
23	2.09 m; 1.91 m	5.28 dt (15.2, 6.8)	1.32 m; 1.05 m	2.12 m; 1.87 m	2.09 m; 1.89 m
24		1.81 m	1.12 m		
25	2.23 heptet (6.6)	1.55 m	1.54 m	2.22 heptet (6.7)	2.22 heptet (6.8)
26	1.02 d (6.6)	0.86 d (6.4)	0.86 d (6.5)	1.02 d (6.7)	1.02 d (6.8)
27	1.02 d (6.6)	0.86 d (6.4)	0.86 d (6.5)	1.02 d (6.7)	1.02 d (6.8)
28	4.71 s; 4.65 s			4.71 s; 4.65 s	4.72 s; 4.65 s
29					0.99 d (6.4)
OMe	3.71 s	3.71 s	3.72 s		
OAc				2.07 s	

[a] Spectra recorded at 300 MHz. Assigned by DEPT, COSY, HSQC, and HMBC experiments.
[b] Spectra recorded at 400 MHz. Assigned by DEPT, COSY, HSQC, and HMBC experiments.
[c] Spectra recorded at 500 MHz. Assigned by DEPT, COSY, HSQC, and HMBC experiments.
[d] *J* values (in Hz) in parentheses.

2.4. In vitro anti-inflammatory assay

Murine RAW 264.7 macrophages were obtained from the American Type Culture Collection (ATCC, No. TIB-71) and cultured in Dulbecco's modified essential medium (DMEM) containing 10% heat-inactivated fetal bovine serum, at 37 °C in a humidified 5% CO_2–95% air incubator under standard conditions. The in vitro anti-inflammatory assay was carried out according to the procedure described previously [23]. For statistical analysis, all the data were analyzed by a one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls post hoc test for multiple comparisons. A significant difference was defined as a *P*-value of <0.05.

3. Results and discussion

The sliced bodies of the soft coral *N. chabroli* were exhaustively extracted with 100% acetone. The acetone extract was concentrated and the residue was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was concentrated and the residue was chromatographed on Si gel CC. The fractions containing steroids were selected after the ^{1}H NMR spectroscopic data revealed the presence of various steroids, and these fractions were further purified by a series of reversed-phase C_{18} HPLC to obtain compounds **1**–**5** (see Section 2).

Nebrosteroid I (**1**) was isolated as a white amorphous powder. HRESIMS of **1** exhibited a pseudo molecular ion peak at *m*/*z* 465.3342 $[M+Na]^+$ (calcd for 465.3344) and established a molecular formula of $C_{29}H_{46}O_3$, indicating seven degrees of unsaturation. The ^{1}H NMR signal at δ_H 3.55 (m, 1H) (Table 1) and the IR absorption at 3421 cm^{-1}, as well as the observation of one oxygen-bearing carbon resonance (δ_C 71.0) in the ^{13}C NMR spectrum, revealed the presence of one hydroxyl group. In addition, the ^{1}H NMR signal at δ_H 3.71 (*s*, 3H) and the IR absorption at 1723 cm^{-1}, together with the presence of one methoxy carbon group (δ_C 51.7) and one carboxylate carbon group (δ_C 174.3) in the ^{13}C NMR spectrum, revealed the presence of one methylcarboxylate group. Furthermore, one tri-substituted double bond (δ_C 124.7 and 134.8), and one terminal double bond (δ_C 105.9 and 156.8) were observed in the ^{13}C NMR and the DEPT spectra of **1**. The above functionalities accounted for three of the seven degrees of unsaturation, suggesting a tetracyclic skeleton for **1**.

The long-range ^{1}H–^{13}C correlations (Fig. 2) observed from H_2-1 to C-19, and H-9 to C-19 indicated a methylcarboxylate group at C-10. The COSY correlations from H_2-1 to H_2-4 through H_2-2 and H-3, led to the assignment of the secondary hydroxyl group at C-3. Moreover, interpretation of the ^{1}H–^{1}H COSY spectrum led to three partial structures (I–III). The connections among these partial structures were further established by the HMBC correlations (Fig. 2). The location of the tri-substituted double bond at the C-5/C-6 was clarified by analysis of the HMBC correlations from H_2-4 to C-5, H_2-6 to C-4/C-10 and H_2-1 to C-5. The HMBC correlations from H_2-28 to C-23/C25 and from H-25/H_3-26/H_3-27 to C-24 revealed the position of the terminal double bond at C-24/C-28. The NOESY correlations (Fig. 3) observed between H-3 and H-2α and H-4α indicated the β orientation of the hydroxyl group at C-3. The methylcarboxylate at

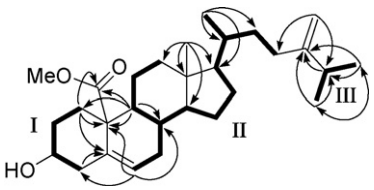

Fig. 2. ^{1}H–^{1}H COSY (▬) and key HMBC (→) correlations of **1**.

Table 2
^{13}C NMR data for compounds **1**–**5** in $CDCl_3$.

C	**1**[a]	**2**[b]	**3**[c]	**4**[a]	**5**[b]
1	33.8 t[d]	33.8 t	34.1 t	30.2 t	37.5 t
2	33.1 t	33.1 t	33.4 t	32.5 t	30.2 t
3	71.0 d	71.0 d	71.3 d	70.2 d	76.5 d
4	44.2 t	44.2 t	44.5 t	42.8 t	38.2 d
5	134.8 s	134.8 s	135.0 s	136.5 s	52.3 d
6	124.7 d	124.9 d	125.0 d	127.2 d	19.2 t
7	30.9 t	30.9 t	31.2 t	74.2 d	39.9 t
8	32.1 d	32.1 d	32.3 d	37.4 d	75.5 s
9	48.8 d	48.8 d	49.1 d	47.5 d	57.6 d
10	50.8 s	50.8 s	51.1 s	53.2 s	36.7 s
11	23.4 t	23.4 t	23.7 t	22.1 t	69.8 d
12	39.6 t	39.5 t	39.8 t	39.3 t	49.3 t
13	42.4 s	42.3 s	42.6 s	43.0 s	42.0 s
14	56.4 d	56.5 d	56.6 d	55.3 d	60.3 d
15	24.1 t	24.1 t	24.3 t	25.0 t	20.0 t
16	28.2 t	28.6 t	28.4 t	28.3 t	27.4 t
17	55.9 d	55.8 d	56.3 d	55.1 d	58.1 d
18	11.8 q	12.0 q	12.1 q	11.7 q	15.5 q
19	174.3 s	174.1 s	174.6 s	204.0 d	15.6 q
20	35.7 d	40.1 d	36.0 d	35.5 d	35.1 d
21	18.7 q	20.8 q	19.0 q	18.7 q	18.3 q
22	34.6 t	138.0 d	36.4 t	34.5 t	34.2 t
23	30.9 t	126.3 d	24.0 t	30.9 t	30.9 t
24	156.8 s	41.9 t	39.7 t	156.7 s	156.7 s
25	33.8 d	28.5 d	28.3 d	33.7 d	33.8 d
26	21.8 q	22.2 q	22.8 q	21.8 q	21.8 q
27	22.0 q	22.3 q	23.1 q	22.0 q	22.0 q
28	105.9 t			106.0 t	106.0 t
29					15.3 q
OMe	51.7 q	51.7 q	52.0 q		
OAc				171.2 s	
				21.6 q	

[a] Spectra recorded at 75 MHz.
[b] Spectra recorded at 100 MHz.
[c] Spectra recorded at 125 MHz.
[d] Attached protons were determined by DEPT experiments.

C-10 was deduced to be in the β orientation based on H-8 correlating to 19-OMe in the NOESY spectrum. The NOESY correlations observed between H-15β and Me-18, H-16β and Me-18, H-4α and H-6, H-7α and H-6, H-9 and H-7α, Me-18 and H-8, H-7α and H-14, H-7α and H-15α, Me-18 and H-20, Me-21 and H-12β in **1** confirmed the relative configurations for each ring junction and chiral center. According to the aforementioned findings and other detailed NOESY correlations (Fig. 3), the structure of **1** was unmistakably elucidated as methyl 3β-hydroxyergosta-5,24(28)-dien-19-oate.

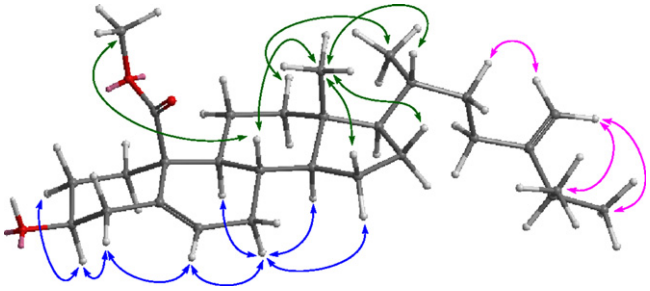

Fig. 3. Selected NOE correlations for **1**.

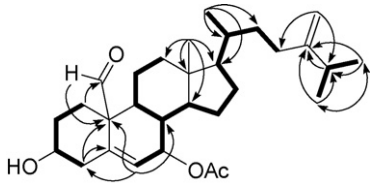

Fig. 4. ^{1}H–^{1}H COSY (▬) and key HMBC (→) correlations of **4**.

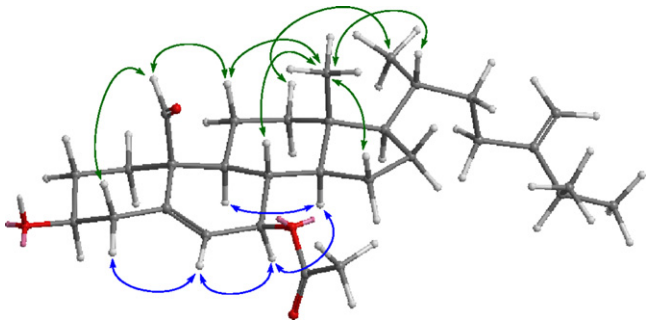

Fig. 5. Selected NOE correlations for **4**.

The NMR spectroscopic data (Tables 1 and 2) of **2** and **3** were analogous to those of **1**, except for the NMR signals due to the side chain. The NMR spectroscopic data of **2** showed the absence of sp^2 methylene C-28 and the presence of an additional 22*E* double bond [δ_H 5.19 (1H, dd, J = 15.2, 8.0 Hz) and 5.28 (1H, dt, J = 15.2, 6.8 Hz); δ_C 138.0 (CH) and 126.3 (CH)]. The NMR spectroscopic data of **3** were also found to be quite similar to those of **2**, but without a double bond in C-22. Compound **3** was already described as a reaction product. ^{13}C NMR spectral data of **3** were not reported and ^{1}H spectral data were not completely assigned in the literature [22].

The positive HRESIMS of nebrosteroid L (**4**) exhibited a pseudo molecular ion peak at m/z 493.3293 $[M+Na]^+$, corresponding to the molecular formula of $C_{30}H_{46}O_4$ and eight degrees of unsaturation. The NMR spectroscopic data (Tables 1 and 2) showed some similarity to those of 24-methylenecholest-5-en-3β,7β,19-triol 7-acetate [24] except for the hydroxymethyl at C-10 was replaced by a formyl [δ_H 9.71 (1H, s); δ_C 204.0 (CH)] in **4**. The HMBC correlations (Fig. 4) from H-19 to C-10 and from H_2-1 to C-19 helped ascertain this assignment. The carbaldehyde group at C-10 was deduced to be in the β orientation based on H-11β (δ_H 1.33 m) correlating to H-19 (δ_H 9.71) in the NOESY spectrum. From the above observations and other detailed NOESY correlations (Fig. 5), the structure of **4** was determined as 7β-acetoxy-3β-hydroxyergosta-5,24(28)-dien-19-al.

The molecular formula of 4α-methyl-5α-ergost-24(28)-en-3β,8β,11β-triol (**5**) was determined to be $C_{29}H_{50}O_3$ from the

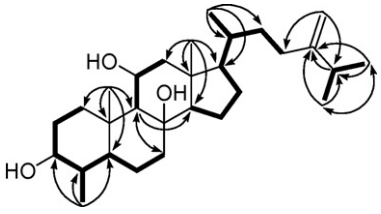

Fig. 6. ^{1}H–^{1}H COSY (▬) and key HMBC (→) correlations of **5**.

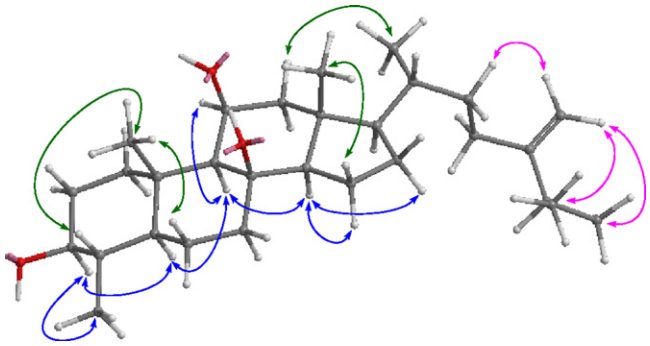

Fig. 7. Selected NOE correlations for **5**.

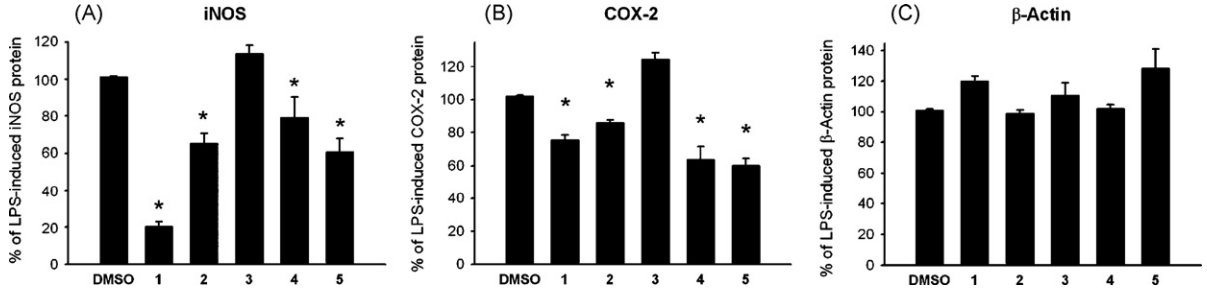

Fig. 8. Effect of compounds **1**–**5** at 10 μM on the LPS-induced pro-inflammatory iNOS and COX-2 proteins expression of RAW 264.7 macrophage cells by immunoblot analysis. (A) Immunoblot of iNOS; (B) immunoblot of COX-2; (C) immunoblot of β-actin. The values are mean ± S.E.M. ($n = 5$). The relative intensity of the LPS alone stimulated group was taken as 100%. *Significantly different from LPS-stimulated (control) group (*$P < 0.05$).

$[M+Na]^+$ ion peak at m/z 469.3659 in HRESIMS, accounting for five degrees of unsaturation. The presence of two oxymethines and one oxygen-bearing quaternary carbon were identified by the ^{1}H NMR [δ_H 3.07 (1H, td, J = 10.4, 5.2 Hz) and 4.45 (1H, br s)] and ^{13}C NMR [δ_C 76.5 (d), 75.5 (s), and 69.8 (d)] spectra, as well as from the IR absorption at 3335 cm^{-1}. Analyses of the ^{13}C NMR, DEPT and HMQC spectroscopic data led to the assignment of six methyls, ten methylenes, nine methines, and four quaternary carbons in the molecule. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) were indicative of a steroid pattern, and closely related to an 4α-methylated steroid, which was characterized by the HMBC correlations (Fig. 6) from Me-29 to C-3, C-4, and C-5. A comparison of the NMR spectroscopic data revealed that the structure of **5** was very compatible to a previously characterized steroid, nebrosteroid A [21], with the exception of a sp^3-methylene carbon of **5** instead of a ketone carbon at C-23 in the latter. The stereochemistry of **5** assigned by the NOESY spectrum was compatible with those of the nebrosteroid A as determined by a computer-generated perspective model using MM2 force field calculations (Fig. 7), in which the close contacts of atoms calculated in space were consistent with the NOESY correlations. The NOESY correlations observed between H_3-19 and H-4, H_3-19 and H-6β, H_3-18 and H-20, H-3 and H-5, H-3 and H_3-29, H-9 and H-5, H-9 and H-14, and H-20 and H-12β in **5** confirmed the same configuration as nebrosteroid A at each ring junction and chiral center. Consequently, the structure of nebrosteroid M (**5**) was definitively established.

As shown in Fig. 8, the in vitro anti-inflammatory activity of compounds **1**–**5** was tested using LPS-stimulated cells. Stimulation of RAW 264.7 cells with LPS resulted in up-regulation of the pro-inflammatory iNOS and COX-2 proteins. Compounds **1**, **2**, **4**, and **5** reduced the levels of iNOS to 20.2 ± 2.6%, 65.2 ± 5.5%, 79.2 ± 11.3%, and 61.0 ± 6.9%, respectively, and of COX-2 to 75.3 ± 3.3%, 86.0 ± 1.7%, 63.8 ± 7.7%, and 60.1 ± 4.6%, respectively, in comparison to those of the control groups. Compound **3** did not inhibit either iNOS or COX-2 proteins expression. All compounds did not affect β-actin protein expression at a concentration of 10 μM. Under the same experimental conditions, 10 μM CAPE (caffeic acid phenethyl ester) reduced the levels of iNOS and COX-2 proteins to 1.5 ± 2.1% and 70.2 ± 11.5%, respectively, relative to the control cells stimulated with LPS.

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