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New diterpenoids and the bioactivity of *Erythrophleum fordii*

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ABSTRACT

A phytochemical investigation of the leaves of *Erythrophleum fordii* Oliv. has led to the isolation of three new cassaine-type diterpenoids, erythrofordin A (**1**), erythrofordin B (**2**) and erythrofordin C (**3**), as well as a norcassaine diterpenoid with a novel skeleton, norerythrofordin A (**4**), and 27 known compounds (**5**–**31**). The structures of **1**–**4** were elucidated on the basis of spectroscopic analysis. Selected compounds from this plant were examined for anti-inflammatory activity. Taraxerol (**16**) displayed potent NO-reducing activity in microglial cells, and gallic acid (**27**) exhibited excellent DPPH radical-scavenging effects.

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1. Introduction

Erythrophleum fordii Oliver (Leguminosae) is widely distributed in China, Taiwan, and Vietnam. It is used as a Chinese traditional medicine, primarily for invigoration and promotion of blood circulation.[1] In prior studies on the genus, *Erythrophleum*, cassaine diterpenoid amines and amides were found as the main constituents,[2–4] with minor amounts of cassaine acids also present.[5] To our knowledge, few chemical and pharmacological investigations have been reported on *E. fordii*. We report herein the isolation and structure elucidation of compounds from this species, including three new cassaine-type diterpenoids, erythrofordins A–C (**1**–**3**), a norcassaine diterpenoid with a novel skeleton, norerythrofordin A (**4**), and 27 known compounds, including 13 triterpenoids (**5**–**17**), five steroids (**18**–**22**) and miscellaneous structure types (**23**–**31**) (see Table 1).

2. Chemistry

Air-dried, powdered whole plants of *E. fordii* were extracted with MeOH at room temperature. The solvent was evaporated and then the resulting residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ fraction was chromatographed on a silica gel column using $CHCl_3$/MeOH gradients to furnish three new cassaine-type diterpenoids (**1**–**3**), a norcassaine diterpenoid with a novel skeleton (**4**), and 27 known compounds (**5**–**31**).

Erythrofordin A (**1**) was obtained as colorless needle, mp 138–139 °C. HRFABMS of **1** exhibited a pseudomolecular ion peak at m/z 395.2068, consistent with the molecular formula $C_{21}H_{30}O_7$. IR absorption bands at 3381, 1726, 1709, and 1642 cm^{-1} were assignable to hydroxyl and carbonyl functions. The ^1H NMR spectrum showed three methyl groups at δ 0.67 (3 H, s), 1.18 (3 H, d, J = 8.7 Hz) and 1.37 (3 H, s), a methoxy group at δ 3.76 (3 H, s), and an olefinic proton at δ 5.77 (1 H, s). The ^{13}C NMR spectrum revealed three carbonyl signals at δ 207.8, 174.2 and 169.5, and a pair of olefinic carbons at δ 167.7 and 112.6, which indicated the presence of a cassaine-type diterpenoid with a β-carbomethoxy group at C-4.[6,7] Evidence for the 6-keto-7β-hydroxy group was provided by the H-7α signal at δ 3.93 (1 H, d, J = 10.6 Hz) and the H-5 signal at δ 2.34 (1 H, s),[4] which was confirmed by HMBC correlations of H-5 (δ 2.34) with C-6 (δ 207.8) and H-7 (δ 3.93) with C-8 (δ 50.8) in the HMBC spectrum. Another oxygen-substituted methine signal appeared at δ 3.36 (1 H, dd, J = 11.6, 4.2 Hz), which correlated with a carbon resonance at δ 78.1 in the HMQC spectrum. From these data, we postulated the presence of a hydroxyl group at C-3, which was supported by correlations in the HMBC spectrum between the proton at δ 1.37 (3 H, s, H-18) and the carbon at δ 78.1 (C-3). The position of a β-carbomethoxy moiety was also confirmed by HMBC correlations between both H-5 (δ 2.34) and H-18 (δ 1.37) with C-19 (δ 174.2). The α,β-conjugated acid moiety in **1** was found to be linked to C-13, based on HMBC correlations between the H-15 proton (δ 5.77) and the C-16 (δ 169.5) and C-12 (δ 23.7) carbons. The

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Table 1
Effects of tested compounds on NADPH oxidase (NOX) and nitric oxide synthase (NOS) activity in murine microglial cells[a]

Compound	% inhibition in NOX (at 50 μM)	IC_{50} (μM) in NOS
4	8.6 ± 0.8	>50
5	0.0 ± 3.3	>50
6	9.2 ± 3.0	>50
10	4.2 ± 5.7	>50
11	10.8 ± 4.4	>50
12	19.2 ± 6.6	>50
13	6.6 ± 5.6	>50
16	11.6 ± 7.8	24.2 ± 2.8
17	5.2 ± 9.7	46.5 ± 3.5
18	17.1 ± 9.1	>50
19	8.7 ± 0.7	>50
20	1.6 ± 3.9	>50
24	6.0 ± 2.3	>50
25	16.3 ± 8.4	>50
26	8.8 ± 0.2	>50
27	21.5 ± 5.9	>50
29	25.2 ± 4.2	>50
30	16.5 ± 1.5	>50
DPI	85.1 ± 2.9	ND
L-NAME	ND	25.8 ± 2.5

[a] NOX and NOS activities were measured as ROS and NO production, respectively, in the presence of 0.1–50 μM of drugs. DPI (diphenyleneiodonium, a NOX inhibitor) and L-NAME (a NOS inhibitor) were included as positive control. Data were calculated as %inhibition or 50% inhibitory concentration (IC_{50}) and expressed as means ± SEM from 3–6 experiments performed on different days using cells from different passage. $^{*}P < 0.05$ as compared with relative positive control (DPI or L-NAME), respectively. "ND", value not determined.

relative configuration of **1** was assigned on the basis of a NOESY experiment. NOE correlations were found between H-3 (δ 3.36) and H_3-18 (δ 1.37), H_3-18 (δ 1.37) and H-5 (δ 2.34), and H-5 (δ 2.34) and H-7 (δ 3.93), indicating that these groups are located on the α-face. The double bond at C-13 and C-15 was assigned as the *Z*-form, which was confirmed by NOE correlation between H-15 (δ 5.77) and H-14 (δ 2.85). Thus, the structure of **1** was proposed as shown, and this compound has been named erythrofordin A (**1**).

Erythrofordin B (**2**) was isolated as optically colorless syrup. The molecular formula of **2** was determined as $C_{23}H_{32}O_8$ from the protonated molecular ion peak at *m/z* 437.2175 $[M+H]^+$ in the HRFABMS. The IR spectrum displayed absorptions at 3379, 1716, and 1740 cm^{-1}, due to hydroxyl and carbonyl groups. Comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1** revealed these compounds to be similar, with the major difference being the appearance of an additional acetoxy group (δ_H 1.95 and δ_C 177.5, 22.2) in **2**. This acetoxy group was positioned at C-7, based on HMBC correlation between δ_H 4.03 (1 H, d, $J = 9.7$ Hz, H-7) and δ_C 177.5 (C-12), as well as NOE correlation between δ_H 1.95 (H-23) and 1.17 (H-17). Assignment of the relative stereochemistry of **2** was also accomplished based on data from a NOESY spectrum and confirmed H-3, H_3-18, H-5, H-7, and H-17 to be on the α-face. However, the C13–C15 double bond of **2** has an *E*- rather than *Z*-configuration, which was determined by NOE correlation between H-15 (δ 5.60) and H-12 (δ 2.15). From the above spectroscopic data, the structure of **2** was fully determined, and this compound has been named erythrofordin B (**2**).

Erythrofordin C (**3**) was obtained as optically colorless syrup and had the molecular formula $C_{21}H_{30}O_7$ as established by HRFABMS. IR absorption bands were observed at 3418, 1702, and 1645 cm^{-1}, attributed to hydroxyl and carbonyl groups. The ^1H and ^{13}C NMR data showed the presence of characteristic signals for three methyl groups, a methoxy group, an olefinic proton and seven corresponding carbon signals at δ 211.5, 178.2, 176.6, 153.2, 121.7, 76.5, and 79.1, which suggested that, like **1**, **3** is also a cassaine-type diterpenoid with a β-carbomethoxy group at C-4. Compared with **1**, the H-5 proton signal was shifted upfield to δ 1.45 (1 H, d, $J = 12.2$ Hz) and coupled with the proton at δ 4.50 (1 H, dd, $J = 12.2$, 0.9 Hz, H-6). Thus, the hydroxyl group was assigned at C-6 and the carbonyl group at C-7. These assignments were further supported by HMBC correlations of H-5 (δ 1.45) with C-6 (δ 76.5) and H-6 (δ 4.50) with C-7 (δ 211.5) in the HMBC spectrum of **3**. The relative stereochemistry of **3** was determined from NOESY correlations between H-3 (δ 3.22), H-5 (δ 1.45) and H-18 (δ 1.63), indicating H-3 and H-18 are α-configured. In addition, H-20 (δ 1.01), H-6 (δ 4.50), and H-8 (δ 2.47) also showed NOESY correlations, which suggests that H-6 and H-8 are β-configured. The double bond at C-13 and C-15 is in the *Z*-form, as indicated by NOE correlations between H-15 (δ 5.71) and H-14 (δ 2.87). Thus, the structure of **3** was fully determined, and the compound was named erythrofordin C.

Norerythrofordin A (**4**) was isolated as optically colorless needle, mp 250–251 °C. The HREIMS of **4** showed a peak at *m/z* 336.1993 corresponding to the molecular formula $C_{19}H_{28}O_5$ and only 19 carbon signals were observed in the ^{13}C NMR spectrum. IR absorption bands at 3408, 1707, and 1645 cm^{-1} were assignable to hydroxyl and carbonyl functions. The ^1H and ^{13}C NMR spectra of **4** and **1** were similar, except for the absence of signals for a carbomethoxy group in of **4**. The presence of H-4 in **4** was confirmed by the presence of a doublet at δ 0.84 (3 H, d, $J = 6.0$ Hz, H-18), and further identified by HMBC correlations of H-18 (δ 0.84) with C-3 (δ 73.9), C-4 (δ 33.4) and C-5 (δ 59.2) in the HMBC spectrum. In the assignment of the stereochemistry of **4**, NOESY crosspeaks for H-3 with H-18, H-18 with H-5, H-5 with H-7, and H-7 with H-9 showed that the corresponding groups are on the α-face. NOESY correlation of H-15/H-14 supported the conclusion that the C13–C15 double bond is in the *Z*-form. Consequently, the structure of **4** was unambiguously established, and the compound has been named norerythrofordin A. In our search of previous publications about cassaine-type diterpenoids, **4** was found to be a new skeleton with only 19 carbons. A plausible biogenetic pathway to norerythrofordin A (**4**) would occur through decarboxylation of erythrofordin A (**1**).

In addition, 27 known compounds were identified by comparison of their physical and spectroscopic data with those of corresponding authentic samples or literature values. These compounds are triterpenoids **5**–**17** [β-amyrinone (**5**),[8] β-amyrin acetate (**6**),[9] cycloartanol (**7**),[10] cycloart-25-en-3-one (**8**),[11] 3,11-dioxoolean-12-ene-30-oic acid (**9**),[12] friedelin (**10**),[13] glutin-5-en-3-β-*O*-acetate (**11**),[14] glutinol (**12**),[15] glutinone (**13**),[16] lupenone (**14**),[17] squalene (**15**),[18] taraxerol (**16**),[17] and taraxerone (**17**)],[17] steroids **18**–**22** [5-stigmasten-3β,7β-diol (**18**),[19] 5-α-stigmasta-7,22-diene-3β-ol (**19**),[20] stigmast-4-ene-3β,6β-diol (**20**),[21] β-sitosterol (**21**),[22] and stigmasterol (**22**)],[22] as well as **23**–**31** [loliolide (**23**),[23] afzelin (**24**),[24] blumenol (**25**),[17] echinulin (**26**),[25] gallic acid (**27**),[26] lutein (**28**),[27] (3*S*,5*R*,6*R*,7*E*,9*R*)-megastigman-7-ene-3,5,6,9-tetraol (**29**),[28] D-pinitol (**30**),[29] and D-(+)-sucrose (**31**)].[30]

3. Anti-inflammatory activities

3.1. Effects of tested compounds on NADPH oxidase (NOX) and nitric oxide synthase (NOS) activity in murine microglial cells

Activated microglial cells play deleterious roles in mediating central nerve system (CNS) inflammatory responses by producing enormous amounts of NO and ROS through induction of inducible nitric oxide synthase (iNOS) and activation of NOX, which results in neuronal damage by NO, ROS, and the more toxic metabolite peroxynitrite ($ONOO^-$).[31–33] We also reported that drugs with antioxidative and NO-reducing activity can prevent stroke-induced brain damage.[34] Therefore, inhibiting NO or ROS production is a useful strategy for treating inflammatory disorders, such as cardiovascular diseases and neurodegenerative disorders.[32,35] The anti-inflammatory potentials of **4**–**6**, **10**–**13**, **16**–**20**, **24**–**27**, **29** and **30** were evaluated by examining their effects on LPS-induced iNOS-dependent NO production and NOX-dependent ROS production in microglial cells. Only taraxerol (**16**) showed effectively inhibited NOS activity with an IC_{50} value of 24.2 μM. Its potency was comparable to that of L-NAME (IC_{50} 25.8 μM), a specific NOS inhibitor. NOX is the major ROS-producing enzyme in activated inflammatory cells.[36] We previously reported that drugs with anti-inflammatory activity also show potent NOX-inhibitory action.[37,38] Therefore, we also evaluated the above compounds on NOX activity in lysates of microglial cells. Our data suggest that gallic acid (**27**) and (3*S*,5*R*,6*R*,7*E*,9*R*)-megastigman-7-ene-3,5,6,9-tetraol (**29**) (maximum inhibition of NOX activity at 50 μM ranging from 21% to 25%) are ordinary inhibitors of NOX, as compared to the specific NOX inhibitor DPI (IC_{50} 16 μM). Moreover, compounds **11**, **12**, **16**, **18**, **25**, and **30** showed only slight (10.8–19.2%) inhibition of NOX activity.

3.2. Measurement of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity

The free radical-scavenging capacities of **4**–**6**, **10**–**13**, **16**–**20**, **24**–**27**, **29** and **30** were examined in a cell-free DPPH solution. Only gallic acid (**27**) showed activity in this assay, with 85.6% maximum radical-scavenging capacity at 50 μM, as compared to 85.7% for the positive control trolox (data not shown).

4. Conclusion

Four new compounds were isolated and identified from *E. fordii*, together with 27 known compounds. Bioassay results indicated that taraxerol (**16**) displays potent NO-reducing activity in microglial cells and gallic acid (**27**) exhibits excellent DPPH radical-scavenging effects.

5. Experimental

5.1. General experimental procedures

Melting points were determined using Yanagimoto MP-S3 micro melting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Hitachi U-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FT-IR Prestige-21 spectrophotometer. ^1H- and ^{13}C-NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on Bruker AVANCE-300, 500 and AMX-400 spectrometers, using tetramethylsilane (TMS) as internal standard. Standard pulse sequences and parameters were used for the NMR experiments and all chemical shifts are reported in parts per million (ppm, δ). FABMS were obtained on a JEOL JMS-700 spectrometer, and EIMS were obtained on a VG-70-250S spectrometer. Column chromatography was performed on silica gel (70–230 mesh, 230–400 mesh). Fractions were monitored by TLC (Merck precoated Si gel 60 F254 plates), using UV light. TLC was conducted on precoated Kieselgel 60 F 254 plates (Merck) and the spots were detected either by examining the plates under a UV lamp or by treating the plates with a 10% methanolic solution of *p*-anisaldehyde acid followed by heating at 110 °C.

5.2. Plant material

The whole plant of *E. fordii* (Leguminosae) was collected near Hanoi in Vietnam on Nov 12 in 2004. The plant material was identified and authenticated by Assoc. Prof. Dr. Vu Xuan Phuong, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology. A voucher specimen (EF04021) has been deposited in the herbarium of the Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hanoi, Vietnam.

5.3. Extraction and Isolation

The air-dried and powdered whole plant of *E. fordii* (10 kg) was extracted with MeOH (6 × 20 L) at room temperature. The filtrate was concentrated under reduced pressure to obtain a dark crude extract (890 g), which was suspended in H_2O, then partitioned with $CHCl_3$ to afford $CHCl_3$-(340 g) and H_2O-soluble portions (480 g), respectively.

The $CHCl_3$-soluble residue (340 g) was chromatographed on a silica gel column eluting with stepwise gradient mixtures of $CHCl_3$–MeOH (19:1 to 0:1) to afford six fractions (1–6). Subfraction 1 was further subjected to column chromatography over silica gel using a stepwise gradient of *n*-hexane-acetone (3:2 – 0:1) and purified by repeated column chromatography to afford **5** (4.1 mg), **6** (4.1 mg), **8** (1.1 mg), **10** (47.8 mg), **11** (20.7 mg), **12** (24.9 mg), **13** (14.3 mg), **14** (0.9 mg), **15** (1.2 mg), **16** (30.1 mg), **17** (27.6 mg), **19** (34.7 mg), **21** (51.0 mg), and **22** (49.0 mg). Fraction 2 was subjected to silica gel column chromatography using *n*-hexane–acetone (3:2–0:1) stepwise gradient mixtures as eluents to afford **7** (0.6 mg), **18** (23.8 mg), **20** (10.2 mg), **23** (0.7 mg), **26** (2.3 mg), and **28** (14.3 mg). Purification of fraction 3 by silica gel column chromatography with *n*-hexane-acetone (2:1 – 0:1) stepwise gradient mixtures as eluents followed by preparative thin-layer chromatography afforded **1** (0.8 mg), **2** (0.7 mg), **3** (0.6 mg), **4** (1.2 mg), and **25** (0.3 mg). Further purification of fraction 4 by column chromatography over silica gel with mixed eluents of diisopropyl ether–MeOH (9:1 – 0:1) and repeated column chromatography and thin-layer chromatography gave compounds **27** (54.8 mg), **24** (1.1 mg), and **29** (0.3 mg). Fraction 5 was further subjected to column chromatography over silica gel using a stepwise gradient of diisopropyl ether–MeOH (9:1 – 0:1) and preparative thin-layer chromatography purification to afford **30** (45.2 mg), and **31** (1.0 mg). Subfraction 6 was further subjected to column chromatography over silica gel using a stepwise gradient of acetone–MeOH (2:1 – 0:1) to afford **9** (0.8 mg).

5.3.1. Erythrofordin A (1)

Colorless powder; mp 138–139 °C; α_{D}^{25} −32.3 (*c* 0.05, CH_3OH); UV (MeOH) λ_{max} (log ε) 219 (3.98) nm; IR (KBr) ν_{max} 3381 (OH), 1726 (CO), 1709 (CO), 1642 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.77 (1 H, d, *J* = 1.4 Hz, H-15), 3.93 (1 H, d, *J* = 10.6 Hz, H-7), 3.77 (1 H, br s, D_2O-exchangeable, OH-3), 3.76 (3 H, s, H-21), 3.48 (1 H, br s, D_2O-exchangeable, OH-7), 3.36 (1 H, dd, *J* = 11.6, 4.2 Hz, H-3), 2.85 (1 H, s, m, H-14), 2.34 (1 H, s, H-5), 2.32 (1 H, m, H-12), 2.28 (1 H, m, H-2), 2.10 (1 H, m, H-12), 2.01 (1 H, m, H-10), 1.93 (1 H, m, H-11), 1.91 (1 H, m, H-6), 1.88 (1 H, m, H-2), 1.74 (1 H, m, H-11), 1.72 (1 H, m, H-1), 1.69 (1 H, m, H-8), 1.65 (1 H, m, H-9), 1.55 (1 H, m, H-2), 1.51 (1 H, m, H-1), 1.37 (3 H, s, H-18), 1.18 (3 H, d, *J* = 8.8 Hz, H-17), 0.67 (3 H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.8 (C-6), 174.2 (C-19), 169.5 (C-16), 167.7 (C-13), 112.6 (C-15), 78.1 (C-3), 75.8 (C-7), 64.4 (C-5), 51.9 (C-21), 50.8 (C-8), 47.8 (C-4), 46.1 (C-9), 43.2 (C-10), 40.5 (C-14), 37.2 (C-1), 27.6 (C-11), 26.3 (C-2), 25.6 (C-18), 23.7 (C-12), 14.5 (C-20), 13.8 (C-17); FABMS *m/z* 395 $[M + H]^+$; HRFABMS *m/z* 395.2068 $[M + H]^+$ (calcd for $C_{21}H_{31}O_7$, 395.2070).

5.3.2. Erythrofordin B (2)

Colorless syrup; α_{D}^{25} −36.6 (*c* 0.01, CH_3OH); UV (MeOH) λ_{max} (log ε) 217 (4.23) nm; IR (KBr) ν_{max} 3379 (OH), 1716 (CO), 1640 (CO) cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 5.60 (1 H, s, H-15), 4.26 (1 H, m, H-14), 4.03 (1 H, d, *J* = 9.7 Hz, H-7), 3.66 (3 H, s, H-21), 3.41 (1 H, dd, $J = 11.7$, 4.2 Hz, H-3), 2.58 (1 H, s, H-5), 2.47 (1 H, td, $J = 13.3$, 3.7 Hz, H-12α), 2.18 (1 H, m, H-2β), 2.15 (1 H, m, H-12β), 1.95 (3 H, s, H-23), 1.95 (1 H, m, H-11), 1.79 (1 H, m, H-1β), 1.77 (1 H, m, H-8), 1.76 (1 H, m, H-11), 1.75 (1 H, m, H-9), 1.62 (1 H, dd, $J = 13.2$, 3.6 Hz, H-2α), 1.41 (1 H, m, H-1α), 1.27 (3 H, s, H-18), 1.17 (3 H, d, $J = 7.0$ Hz, H-17), 0.95 (3 H, s, H-20); ^{13}C NMR (CD_3OD, 75 MHz) δ 210.6 (C-6), 177.5 (C-22), 175.8 (C-19), 171.2 (C-16), 164.3 (C-13), 115.9 (C-15), 78.4 (C-3), 77.2 (C-7), 65.3 (C-5), 51.9 (C-21), 51.3 (C-8), 47.6 (C-9), 46.6 (C-4), 43.4 (C-10), 37.8 (C-1), 32.9 (C-12), 32.4 (C-14), 28.1 (C-11), 28.0 (C-2), 26.2 (C-18), 22.2 (C-23), 14.9 (C-20), 13.3 (C-17); FABMS m/z 437 $[M + H]^+$; HRFABMS m/z 437.2175 $[M + H]^+$ (calcd for $C_{23}H_{33}O_8$, 437.2177).

5.3.3. Erythrofordin C (3)

Colorless syrup; α_{D}^{25} −148.0 (c 0.3, CH_3OH); UV (MeOH) λ_{max} ($\log\varepsilon$) 228 (4.25) nm; IR (KBr) ν_{max} 3418 (OH), 1702 (CO), 1645 (CO) cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 5.71 (1 H, d, $J = 1.1$ Hz, H-15), 4.50 (1 H, dd, $J = 12.2$, 0.9 Hz, H-6), 3.71 (3 H, s, H-21), 3.45 (1 H, d, $J = 10.8$ Hz, H-12α), 3.22 (1 H, dd, $J = 12.2$, 4.3 Hz, H-3), 2.87 (1 H, s, m, H-14), 2.47 (1 H, d, $J = 14.3$ Hz, H-8), 2.12 (1 H, m, H-2β), 2.01 (1 H, m, H-12β), 1.93 (1 H, m, H-1β), 1.90 (2 H, m, H-2 and 11β), 1.75 (1 H, m, H-11α), 1.67 (1 H, dd, $J = 12.4$, 3.0 Hz, H-9), 1.63 (3 H, s, H-18), 1.45 (1 H, d, $J = 12.2$ Hz, H-5), 1.28 (1 H, m, H-1α), 1.09 (3 H, d, $J = 6.9$ Hz, H-17), 1.01 (3 H, s, H-20); ^{13}C NMR (CD_3OD, 125 MHz) δ 211.5 (C-7), 178.2 (C-19), 176.6 (C-16), 153.2 (C-13), 121.7 (C-15), 79.1 (C-3), 76.5 (C-6), 59.2 (C-5), 53.2 (C-8), 51.8 (C-4 and C-21), 47.9 (C-9), 40.4 (C-14), 38.7 (C-1 and C-10), 28.6 (C-11), 28.4 (C-2), 26.9 (C-19), 24.9 (C-12), 15.3 (C-17), 14.1 (C-20); FABMS m/z 395 $[M + H]^+$; HRFABMS m/z 395.2068 $[M + H]^+$ (calcd for $C_{21}H_{31}O_7$, 395.2070).

5.3.4. Norerythrofordin A (4)

Colorless powder; mp 250–251 °C; α_{D}^{25} −60.6 (c 0.04, CH_3OH); UV (MeOH) λ_{max} ($\log\varepsilon$) 223 (4.18) nm; IR (KBr) ν_{max} 3408 (OH), 1707 (CO), 1645 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.95 (1 H, s, 16-COOH), 5.57 (1 H, s, H-15), 4.84 (1 H, d, $J = 5.9$ Hz, D_2O-exchangeable, OH-7), 4.45 (1 H, d, $J = 5.7$ Hz, D_2O-exchangeable, OH-3), 3.96 (1 H, m, H-7), 3.65 (1 H, m, H-12β), 2.91 (1 H, m, H-3), 2.67 (1 H, m, H-14), 2.17 (1 H, d, $J = 4.6$ Hz, H-5), 1.93 (1 H, m, H-12α), 1.90 (1 H, m, H-11), 1.88 (1 H, m, H-9), 1.76 (1 H, m, H-1α), 1.65 (1 H, m, H-2α), 1.62 (1 H, m, H-4), 1.58 (1 H, m, H-8), 1.28 (2 H, m, H-1β and H-2β), 1.08 (3 H, d, $J = 6.8$ Hz, H-17), 0.84 (3 H, d, $J = 6.0$ Hz, H-18), 0.51 (3 H, s, H-19); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 210.2 (C-6), 167.6 (C-16), 165.1 (C-13), 113.4 (C-15), 75.2 (C-7), 73.9 (C-3), 59.2 (C-5), 51.1 (C-8), 43.0 (C-9), 41.7 (C-10), 39.2 (C-14), 36.2 (C-1), 33.4 (C-4), 30.2 (C-2), 26.2 (C-11), 23.4 (C-12), 16.2 (C-18), 13.3 (C-17 and C-19); EIMS m/z 336 $[M]^+$; HREIMS m/z 336.1933 $[M]^+$ (calcd for $C_{19}H_{28}O_5$, 336.1937).

5.4. Anti-inflammatory activities

5.4.1. Measurement of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity

DPPH radical-scavenging capacity assay was performed as in our previous report.[37]

5.4.2. Microglial cell culture and measurements of nitric oxide (NO)

The murine microglial cell line (BV2) was cultured and production of NO was measured by the methods as described in our prior report.[34]

5.4.3. Measurement of NADPH oxidase (NOX) activity

NADPH oxidase activity was measured as described previously.[34]

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