Anti-inflammatory norhopanes from the root bark of *Fagaropsis angolensis* (Engl.) H.M.Gardner

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ABSTRACT

Two new norhopane derivatives namely 3β,6β,22-trihydroxy-7β,11α-di-[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23)-ene (**1**) and 3β,6β,22-trihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23)-ene (**2**) together with two previously reported compounds, including a norhopane, 3β,6β,11α-trihydroxy-7β-[(4-hydroxybenzoyl)oxy]-24-norhopa-4(23),17(21)-diene (**3**) and a norneohopane, (21αH)-24-norneohopa-4(23), 22(29)-diene-3β,6β,7β-triol 7-caffeate (**4**) were isolated from the root bark of *Fagaropsis angolensis*. Elucidation of their structures was achieved by spectroscopic (NMR, IR and UV) and spectrometric (HRESIMS) data and by comparison of these data with those of related compounds in the literature. Compounds **1–4** were evaluated for their anti-inflammatory activity by measuring the levels of cytokines, IL-1β, IL-2, GM-CSF and TNF-α in lipopolysaccharide (LPS) stimulated peripheral blood mononuclear cells (PBMC). All tested compounds decreased secretion of IL-1β and TNF-α. Compounds **2** and **4** caused significant decrease of the production of IL-2, GM-CSF and TNF-α compared to the reference drug, ibuprofen. These findings showed that the root barks of *F. angolensis* are rich source of norhopane derivatives and further provide a scientific rationale of using this plant in Kenyan folk medicine to relieve pain.

1. Introduction

Fagaropsis angolensis (Engl.) H.M.Gardner is a deciduous tree that belongs to the Rutaceae family [1]. It occurs in dry, evergreen forests, throughout tropical regions of East and Central Africa [2,3]. The leaf and root decoctions of this plant are used in Kenyan folk medicine in the management of malaria, back joint aches and cancer [4,5]. The bark is used for treatment of chest and gut ailments including pneumonia, amoebiasis and diarrhea [6,7]. It is important to note that despite its ethno-medicinal application to alleviate pain, surprisingly *F. angolensis* has not been investigated for either its anti-inflammatory or analgesic properties hence the motivation to carry out the current study [8]. The crude methanolic extracts of *F. angolensis* have been reported to exhibit antiplasmodial, selective antimicrobial [9,10] and antiproliferative activities [11]. Previous phytochemical investigations of plants from the genus *Fagaropsis* led to the characterization of interesting family of

secondary metabolites including limonoids, alkaloids, sterols and their ester derivatives as soluble constituents [12–14].

The four compounds (**1–4**) isolated from *F. angolensis* displayed norhopane and norneohopane type skeleton, which constitute a group of pentacyclic triterpenoids, abundant in the plant kingdom [15]. They are widely distributed in nature occurring in bacteria, cyanobacteria as well as higher plants and have been reported to exhibit anticancer, antiparasitic, insecticidal and larvicidal activities [16–19]. A series of rare oxidized hopanes demethylated at C-4 of ring A (24-norhopane) has been reported to occur in terrestrial higher plants [20,21]. The norneohopane carbon skeleton differs from that of norhopane in that it bears a methyl group on C-17 instead of C-18 [22].

To determine the anti-inflammatory activity of compounds **1–4**, lipopolysaccharide (LPS)-stimulated cytokine release peripheral blood mononuclear cells (PBMCs) was measured through levels of cytokines, IL-1β, IL-2, GM-CSF and TNF-α. Herein, we report the isolation,

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structure elucidation and anti-inflammatory activity of three norhopanes (**1–3**) and one norneohopane (**4**) two of which were new from the roots bark of *F. angolensis*.

2. Experimental

2.1. General experimental procedures

NMR spectra were performed on Bruker Avance III spectrometer at 600 MHz (¹H) and 150 MHz (¹³C) using standard pulse sequences and referenced to residual solvent signals. High-resolution mass spectra (ESI-HRMS) were carried out on a LTQ Orbitrap spectrometer (Thermo Scientific, USA) equipped with a HESI-II source. The spectrometer was operated in positive mode with a nominal mass resolving power of 60,000 at *m/z* 400 with a scan rate of 1 Hz under following parameters: spray voltage 6 kV, capillary temperature 300 °C, tube lens 100 V. Ar served as collision gas and N_2 was used as sheath gas (66 arbitrary units) and auxiliary gas (8 arbitrary units). IR analysis were recorded on a bruker Tensor 27 FT-IR Spectrometer using a diffuse reflection apparatus (cricket, Harrick Scientific). For column chromatography, Silica gel 60 and Sephadex LH–20 (25–100 μm, Amersham Biosciences) were used. Specific rotation was recorded on Kruss Optronic Polarimeter P8000-T. TLC was carried out on pre-coated silica gel 60 plates (0.25 mm; Merck, Darmstadt, Germany). Compounds were visualized under UV light and further by spraying with H_2SO_4–H_2O (0.5:9.5, *v*/v).

2.2. Plant material

The roots bark of *Fagaropsis angolensis* were collected at Mrima Hill forest, Coastal Region of Kenya in May 2018 and identified by Mr. Patrick Mutiso, a taxonomist at the School of Biological Sciences (SBS), University of Nairobi herbarium, where a voucher specimen is preserved under number NNA 2018/007.

2.3. Extraction and isolation

The air-dried root barks of *Fagaropsis angolensis* (4.25 kg) were grinded, then exhaustively extracted with CH_3OH/CH_2Cl_2 (50% *v*/v) at room temperature for 48 h. The solvent was removed under reduced pressure using a rotary evaporator (R-100 Büchi, Switzerland) to yield a brown crude extract (170.6 g). Separation was carried out using column chromatography on silica gel as the stationary matrix with 10% ethyl acetate (EtOAc) in *n*-hexane as the mobile phase initially. Elution was done in order of increasing polarity of the solvent system by increasing the polar solvent, from 10%, 20%, 30%, 40%, 50% EtOAc up to neat EtOAc and then using mixtures of 10%, 20%, 30% and 40% of MeOH in EtOAc. This resulted to 505 fractions of 100 mL each, which were combined based on their TLC profiles to only 8 fractions (Fr. 18A-Fr. 18H). Fraction Fr.18E of the main column afforded pink amorphous powder which were filtered *in vacuo* using a büchner funnel and washed repeatedly with 20% EtOAc in *n*-hexane to give compound **3** (12.0 mg). Fraction Fr. 18H was further subjected to column chromatography using silica gel as the stationary phase eluting with gradients of 10% up to 40% MeOH in CH_2Cl_2 to give a semi-pure fraction (90.0 mg). This minor fraction was purified further through chromatography using a chromatotron, eluting with 5% of CH_3OH in CH_2Cl_2 to afford compound **1** (2.3 mg). Fractions Fr. 18F and Fr. 18G were further combined and loaded onto a column packed with silica gel, eluting with gradients of EtOAc in *n*-hexane in increasing polarity from 5% to 100% to afford compounds **2** (6.2 mg) and **4** (4.6 mg).

2.4. 3β,6β,22-Trihydroxy-7β,11α-di[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23)-ene (1)

White amorphous solid; [α]$_D^{21}$ – 19.0 (*c* 0.03, MeOH); LC-UV [MeOH and H_2O (0.1% formic acid)]λ$_{max}$: 259 nm; IR (neat) ν$_{max}$:

Table 1
¹³C and ¹H NMR data of compound 1–2 (150 and 600 MHz, CD₃OD), δ in ppm, *J* in Hz.

Position	1			2		
	δ$_C$	δ$_H$	HMBC	δ$_C$	δ$_H$	HMBC
1	44.6	2.34 *m*	–	41.6	1.83 *m*	–
		1.42 *m*			1.17 *m*	
2	36.7	1.74 *m*	–	33.1	1.90 *m*	–
		1.33 *m*			1.50 *m*	
3	73.6	3.94 *m*	C-1, 2, 4, 5	74.0	3.95 *m*	C-4
4	151.1	–		151.0	–	
5	51.0	1.89 *m*	–	51.5	1.75 *m*	
6	71.6	4.35 *dd* (3.8, 1.9)	C-7, 8, 10	71.9	4.39 *dd* (3.8, 2.0)	C-4, 5, 7, 8, 10
7	76.3	5.21 *d* (3.8)	C-7′, 26	77.1	5.19 *d* (3.8)	C-7′, 8, 14, 26
8	50.0	–	–	48.0	–	–
9	53.0	2.17 *d* (11.3)	C-1, 8, 10, 11, 25, 26	50.6	1.62 *m*	–
10	43.6	–		39.7	–	–
11	74.2	5.77 *td* (11.3, 5.5)	C-7″, 9	22.5	1.77 *m* 1.61 *m*	–
12	33.5	1.84 *m*	–	25.1	1.72 *m* 1.50 *m*	–
13	49.5	1.79 *m*	C-14, 27, 28	49.6	1.58 *m*	–
14	45.7	–	–	45.0	–	–
15	40.5	1.66 *m* 1.04 *m*	–	36.9	1.70 *m* 1.01 *m*	–
16	26.0	1.66 *m* 1.31 *m*	–	24.5	1.73 *m* 1.51 *m*	–
17	52.9	1.08 *m*	–	52.8	1.04 *m*	–
18	47.6	–	–	46.0	–	–
19	40.8	1.45 *m* 1.05 *m*	–	41.0	1.50 *m* 1.01 *m*	–
20	25.7	1.72 *m* 1.38 *m*	–	25.6	1.70 *m* 1.50 *m*	–
21	51.9	1.72 *m*	–	52.0	1.75 *m*	–
22	73.9	–	–	73.9	–	–
23	106.2	5.42 *d* (1.8) 5.21 *m*	C-3, 4, 5	105.8	5.36 *m* 5.22 *m*	C-3, 4, 5
25	16.8	1.13 *s*	C-1, 10	16.6	1.06 *s*	C-1, 9, 10
26	13.8	1.83 *s*	C-7, 8, 9, 14	13.0	1.70 *s*	C-8, 7, 9, 14
27	18.3	1.20 *s*	C-8, 13, 14, 15	17.9	1.10 *s*	C-8, 13, 14, 15
28	15.5	0.75 *s*	C-13, 17, 18, 19	15.8	0.74 *s*	C-13, 17, 18, 19
29	29.7	1.14 *s*	C-21, 22, 30	29.7	1.14 *s*	C-21, 22, 30
30	26.0	1.12 *s*	C-21, 22, 29	26.1	1.12 *s*	C-21, 22, 29
7-O-(4-OHBz)						
7′	167.5	–	–	167.7	–	–
1′	123.1	–	–	123.2	–	–
2′,6′	133.1	7.97 *d* (8.8)	C-2′/6′, 4′, 7′	133.1	7.96 *d* (8.8)	C-3′/5′, 2′/6′, 4′, 7′
3′,5′	116.3	6.87 *d* (8.8)	C-1′, 3′/5′, 4′	116.1	6.86 *d* (8.8)	C-3′/5′, 1′, 4′
4′	163.5	–	–	163.5	–	–
11-O-(4-OHBz)			–	–	–	–
7″	167.2	–	–	–	–	–
1″	122.8	–	–	–	–	–
2″,6″	132.9	7.91 *d* (8.8)	C-2″/6″, 4″, 7″	–	–	–
3″,5″	116.1	6.87 *d* (8.8)	C-1″, 3″/5″, 4″	–	–	–
4″	163.6	–	–	–	–	–

3411, 2971, 1690, 1607, 1271, 1164, 1066, 773, 669 cm^{-1}; HRESIMS: m/z 739.3812 [M + Na]$^+$ (calcd for C$_{43}$H$_{56}$O$_9$Na, 739.3822); ^{1}H NMR (CD$_3$OD, 600 MHz) and ^{13}C NMR (CD$_3$OD, 150 MHz) data, see Table 1 and Figs. S1–S12, see Supporting Information.

2.5. 3β,6β,22-Trihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23)-ene (2)

White amorphous solid; [α]$_D^{21}$ + 23.1 (c 0.13, MeOH); LC-UV [MeOH and H$_2$O (0.1% formic acid)] λ$_{max}$: 230 nm. IR (neat) ν$_{max}$: 3411, 2970, 1691, 1607, 1274, 1065, 771, 669 cm^{-1}; HRESIMS: m/z 603.3658 [M + Na]$^+$ (calcd for C$_{36}$H$_{52}$O$_6$Na, 603.3662). ^{1}H NMR (CD$_3$OD, 600 MHz) and ^{13}C NMR (CD$_3$OD, 150 MHz) data, see Table 1 and Figs. S13–S23, see Supporting Information.

2.6. Anti-inflammatory assay

The anti-inflammatory tests were performed by Pharmacelsus, Saarbrücken, Germany. The PBMCs were isolated from blood ethically collected from four healthy donors with the ethnicity Caucasian (male, 41 years old), African-American (male, 31 years old), African American-Hispanic (male, 29 years old) and Caucasian (male, 32 years old). The pure compounds were dissolved in dimethyl sulfoxide (DMSO) to achieve 20 mM stock solutions. Ibuprofen was also dissolved as 20 mM stock solution in DMSO. Lipopolysaccharide (LPS) was dissolved in cell culture medium at a concentration of 1 mg/mL. The pure compounds and the positive control ibuprofen were used in a concentration of 100 μM. The final concentration of DMSO in all samples was 0.5% and all samples were co-incubated with 10 μg/mL LPS. The PBMCs are the main source of cytokines within the circulating blood. Due to the small amounts of cytokines released by PBMC into the supernatant, a bead-based assay (ProcartaPlex, Luminex) was used to quantify the four cytokines in parallel within a 50 μL sample using appropriate calibration standards. Human cryopreserved PBMC were thawed according to the manufacturer's instructions. Four vials of cells from different donors were pooled. Cells were washed, resuspended in RPMI 1640 containing 10% FBS, plated in 96-well round bottom plates at 100,000 PBMC/well and exposed to the test items at the concentrations specified above. Therefore, dilutions of test items were prepared in a 96-well plate and transferred to the PBMC containing wells. The cells were incubated for 24 h at 37 °C and 5% CO$_2$. Then, plates were centrifuged for 3 min at 350 gyrations without brake and cell-free supernatant was collected and forwarded to cytokine bead-array assay. The later was conducted according to manufacturer's instructions and read in a MagPix reader. For the dose-response relationship, absolute concentrations were calculated by the MagPix software using two separate calibration series as provided by the manufacturer. As negative control, cells were incubated only with cell culture medium. As a positive control for inflammation, cells were incubated with 10 μg/mL LPS and as positive control for anti-inflammation, cells were co-incubated with 10 μg/mL LPS and 100 μM ibuprofen.

3. Results and discussion

Compound **1** (Fig. 1) was obtained as white amorphous solid with [α]$_D^{21}$ = −19.0 (c. 0.03, MeOH). Its positive mode HR-ESI mass spectrum showed a molecular adduct ion [M + Na]$^+$ at m/z 739.3812 corresponding to the molecular formula C$_{43}$H$_{56}$O$_9$ (calcd for C$_{43}$H$_{56}$O$_9$Na, 739.3822) (Fig. S1, Supporting information). Its UV spectrum (Fig. S2, Supporting information) showed absorption maximum at 259 nm while its FT-IR spectrum (Fig. S3, Supporting information) revealed absorption bands at 3411 cm^{-1} for hydroxyl groups and 1690 cm^{-1} for ester carbonyl groups. The ^{1}H and ^{13}C NMR data of **1** (Table 1) showed resonances of two *para*-disubstituted hydroxybenzene moieties each bearing an ester carbonyl at δ$_C$ 167.5 (C-

7′) and 167.2 (C-7″). The ^{1}H and ^{13}C NMR spectra (Figs. S4–S5, Supporting information) showed a set of signals with AA'BB′ spin system with resonances at δ$_H$/δ$_C$ 7.97/133.1 (2H, d, J = 8.8 Hz, H-2′/6′), 7.91/132.9 (2H, d, J = 8.8 Hz, H-2″/6″), 6.87/116.3 (2H, d, J = 8.8 Hz, H-3′/5′) and 6.87/116.1 (2H, d, J = 8.8 Hz, H-3″/5″). Furthermore, ^{1}H and ^{13}C NMR spectra exhibited signals for six tertiary methyls at δ$_H$/δ$_C$ 1.13 (3H, s, H-25)/16.8, 1.83 (3H, s, H-26)/13.8, 1.20 (3H, s, H-27)/18.3, 0.75 (3H, s, H-28)/15.5, 1.14 (3H, s, H-29)/29.7 and 1.12 (3H, s, H-30)/26.0 together with resonances for one exomethylene at δ$_H$/δ$_C$ 5.42 (1H, d, J = 1.8 Hz, H-23α)/5.21 (1H, m, H-23β)/106.2 (C-23) and four oxymethines at δ$_H$/δ$_C$ 3.94 (1H, m)/73.6 (C-3), 4.35 (1H, dd, J = 3.8, 1.9 Hz)/71.6 (C-6), 5.21 (1H, d, J = 3.8 Hz)/76.3 (C-7) and 5.77 (1H, td, J = 11.3, 5.5 Hz)/74.2 (C-11) consistent with 24 norhopane type triterpenoid. These dataset were similar to those reported for 3β,6β-dihydroxy-7β,11α-di[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23),22(29)-diene [18], except for an additional oxidized tertiary carbon at δ$_C$ 73.9 (C-22) in compound **1** in place of an olefinic group in the same position. Thus, **1** was suggested to be a 24-norhopane derivative with an isopropanoyl moiety at C-22 and an olefinic group at C-4/C-23. The placement of the first 4-hydroxylbenzoyloxy group at C-7 was confirmed from HMBC connectivities of H-7 (δ$_H$ 5.21) with the carbonyl carbon at C-7′ (δ$_C$ 167.5) and the tertiary methyl carbon at C-26 (δ$_C$ 13.8). This was further supported from the COSY spectrum (Fig. S7, Supporting information) which showed $J_{1H,1H}$ correlation of the downfield shifted resonance of H-7 (δ$_C$ 5.21, d, J = 3.8 Hz) with the oxymethine proton resonance at H-6 (δ$_H$ 4.35, dd, J = 3.8, 1.9 Hz). Similarly, the second 4-hydroxybenzoyloxy group was placed at C-11 based on the HMBC correlations (Fig. S12, Supporting information) of H-11 (δ$_H$ 5.77) with the carbonyl at C-7″ (δ$_C$ 167.2) together with the methine carbon at C-9 (δ$_C$ 53.0). The COSY spectrum (Fig. S7, Supporting information) showed the $J_{1H,1H}$ correlation of H-11 (δ$_H$ 5.77, td, J = 11.3, 5.5 Hz) with the methine proton at H-9 (δ$_H$ 2.17, d, J = 11.3 Hz) and methylene protons CH$_2$–12 (δ$_H$ 1.84, m) further supporting the C-11 position of this 4-hydroxybenzoyloxy moiety. In addition, the placement of the hydroxyl group at C-3, C-6 and C-22 in **1** was confirmed from the HMBC cross peaks between H-3 (δ$_H$ 3.94) with C-1 (δ$_C$ 44.6), C-2 (δ$_C$ 36.7), C-4 (δ$_C$ 151.1), C-5 (δ$_C$ 51.0); H-6 (δ$_H$ 4.35) with C-7 (δ$_C$ 76.3), C-8 (δ$_C$ 50.0), C-10 (δ$_C$ 43.6) and between H-29/H-30 (δ$_H$ 1.14/1.12) with C-21 (δ$_C$ 51.9) and C-22 (δ$_C$ 73.9), respectively [18,23].

The relative stereochemistry of **1** was established using NOESY spectrum (Fig. 2, Figs. S8–S10, Supporting information), coupling constants between aliphatic protons and the biosynthetical pathway of hopane triterpenoids. Hopane-type triterpene are biosynthesized from an all-chair cyclisation of the squalene precursor [24]. The NOESY spectrum of **1**, showed cross peaks between H-21 (δ$_H$ 1.72) with Me-28 (δ$_H$ 0.75) thus confirming the assignment of H-21 to be in α-orientation. Furthermore, the NOESY spectrum showed other relevant correlations between Me-28 (δ$_H$ 0.75) and Me-27 (δ$_H$ 1.20); Me-27 (δ$_H$ 1.20) and H-7 (δ$_H$ 5.21) confirming the β–configuration of 7-(4-hydroxybenzoyl)oxy group; between H-7 (δ$_H$ 5.21) and H-5 (δ$_H$ 1.89), H-5 (δ$_H$ 1.89) and H-6 (δ$_H$ 4.35) supporting the β-orientation of 6-OH group. The α–configuration of the 11-(4-hydroxybenzoyl)oxy group was similarly confirmed from the NOESY connectivities of H-11 (δ$_H$ 5.77) with Me-25 (δ$_H$ 1.13) and Me-26 (δ$_H$ 1.83). In addition, from the COSY spectrum (Fig. S7, Supporting information) two equatorial-axial correlations, influenced by the axial position of the hydroxyl at C-6, were observed between H-6 and H-5 and between H-6 and H-7 ($J_{6,7}$ = 3.8 Hz) in support of the α-orientation of H-6. Likewise, protons H-9 and H-11 were deduced to have *peri*-planar orientation based on the observed coupling constants between H-9 and H-11 ($J_{9,11}$ = 11.3 Hz) and supported by NOESY interaction from H-11 (δ$_H$, 5.77) to the β-disposed Me-25 (δ$_H$, 1.13) protons. The other stereocenters were set as expected from the biogenesis of hopane molecules. Based on these spectroscopic and spectrometric data and literature values of related compounds [18,20], **1** was newly characterized as 3β,6β,22-trihydroxy-7β,11α-di[(4-
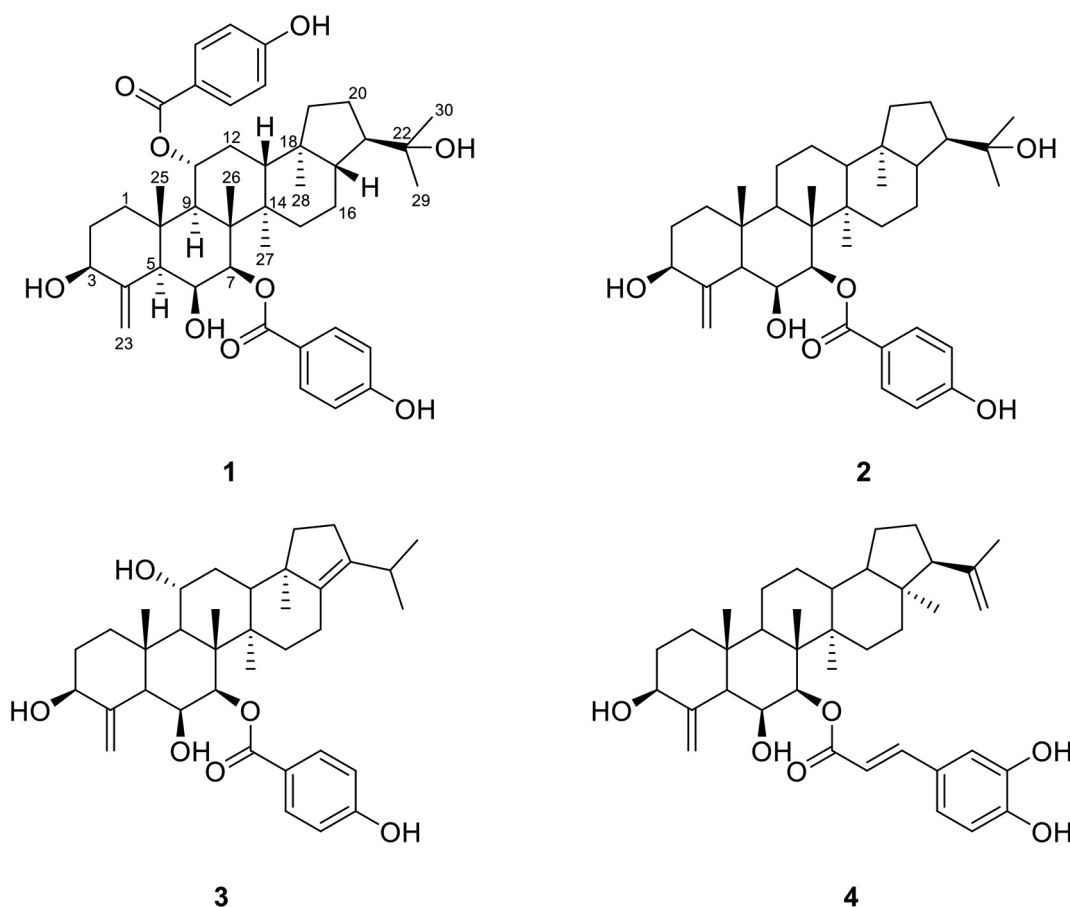
Fig. 1. Chemical structures of compounds **1–4** isolated from *Fagaropsis angolensis* (Engl.) H.M.Gardner.

hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23)-ene.

Compound **2** (Fig. 1) was isolated as a white amorphous solid with $[α]_D^{21} = + 23.1$, (c. 0.13, MeOH). Its molecular formula $C_{36}H_{52}O_6$ was determined from its positive mode HR-ESI mass spectrum which showed a molecular adduct ion $[M + Na]^+$ at *m/z* 603.3658 (calcd for $C_{36}H_{52}O_6Na$, 603.3662) (Fig. S13, Supporting information). Its UV spectrum (Fig. S14, Supporting information) indicated absorption maximum at 230 nm and its FT-IR spectrum (Fig. S15, Supporting information) showed absorption bands at 3411 cm^{-1} for hydroxy stretching and 1691 cm^{-1} for ester carbonyl groups. The ^{1}H and ^{13}C NMR data of **2** (Table 1, Figs. S16–S17, Supporting information) was similar to that of **1** except that **2** displayed only one 4-hydroxybenzoyloxy moiety placed at C-7 of the 24-norhopane skeleton. This was confirmed from the ^{1}H NMR spectrum which showed the disappearance of the downfield shifted oxymethine resonance at δ$_H$ 5.77 (H−11) and one set of resonances displaying an AA'BB' spin system for 7-(4-hydroxybenzoyl)oxy group in **2**. Furthermore, the ^{1}H NMR spectrum (Fig. S16, Supporting information) of **2** showed the appearance of methylene resonances that were significantly upfield shifted at δ$_H$ 1.77 and 1.61 assigned to CH$_2$–11. The corresponding ^{13}C NMR resonances displayed a similar pattern supporting the proposed assignment for **2**. The HMBC spectrum (Fig. S23, Supporting information) showed corrections between H-7 (δ$_H$ 5.19) and C-7′ (δ$_C$ 167.7) highlighting the position of the 4-hydroxybenzoyloxy group on the hopane skeleton. The NOESY correlations observed in **2** (Fig. 2), were identical to those displayed in **1** confirming that the configurations of atoms in the two compounds from *F. angolensis* were conserved probably due to their similar biosynthetic pathway. Complete assignment of **2** was established using ^{1}H and ^{13}C NMR coupled with COSY, HSQC and HMBC spectra (Figs. S16–S23, Supporting information) most of which were similar to those of **1**. Based on these spectral data and comparison with

data of previously isolated compounds **2** was identified as 3β,6β,22-trihydroxy-7β-[(4-hydroxybenzoyl)oxy]-21αH-24-norhopa-4(23)-ene (**2**).

The two known compounds were identified as 3β,6β,11α-trihydroxy-7β-[(4-hydroxybenzoyl)oxy]-24-norhopa-4(23),17(21)-diene (**3**) and (21αH)-24-norneohopa-4(23),22(29)-diene-3β,6β,7β-triol 7-caffeate (**4**) (Fig. 1) by comparison of their respective spectra data to those in the literature [18,20].

The anti-inflammatory activities of the isolated compounds were evaluated by measuring the levels of pro-inflammatory cytokines IL-1β, IL-2, GM-CSF and TNF-α in LPS stimulated PBMCs. The assay is based on the fact that when inflammation occurs, many cytokines are produced and released from PBMCs as part of the immune response. This situation can be replicated *in vitro* by incubating PBMCs with bacterial LPS, a major structural component of the outer wall of gram-negative bacteria, and considered to be a potent initiator of inflammatory responses [25]. Ibuprofen was used as the reference anti-inflammatory drug. As shown in Table 2, after incubation of PBMCs with LPS the release of the cytokines IL-1β, GM-CSF and TNF-α were increased compared to the medium control. When cells were co-incubated with LPS and ibuprofen, the release of the pro-inflammatory cytokines were decreased from 21.97–77.40% in comparison with LPS control. Ibuprofen did not show an effect on the release of IL-2. As shown in Table 3 and Fig. 3, all tested compounds showed decreased release of IL-1β from 66.02–99.71% compared to LPS control. Compounds **2–4** resulted to a decreased production of IL-2 (43.46–72.61%) while **1** showed an increased release of the cytokine (111.94%) in comparison with the LPS control. All the tested items inhibited the production of GM-CSF except **1** which increased GM-CSF secretion to 162.24% in comparison with LPS control. The production of TNF-α was significantly reduced by all test items compared to the LPS control (16.09–46.17%). The decrease
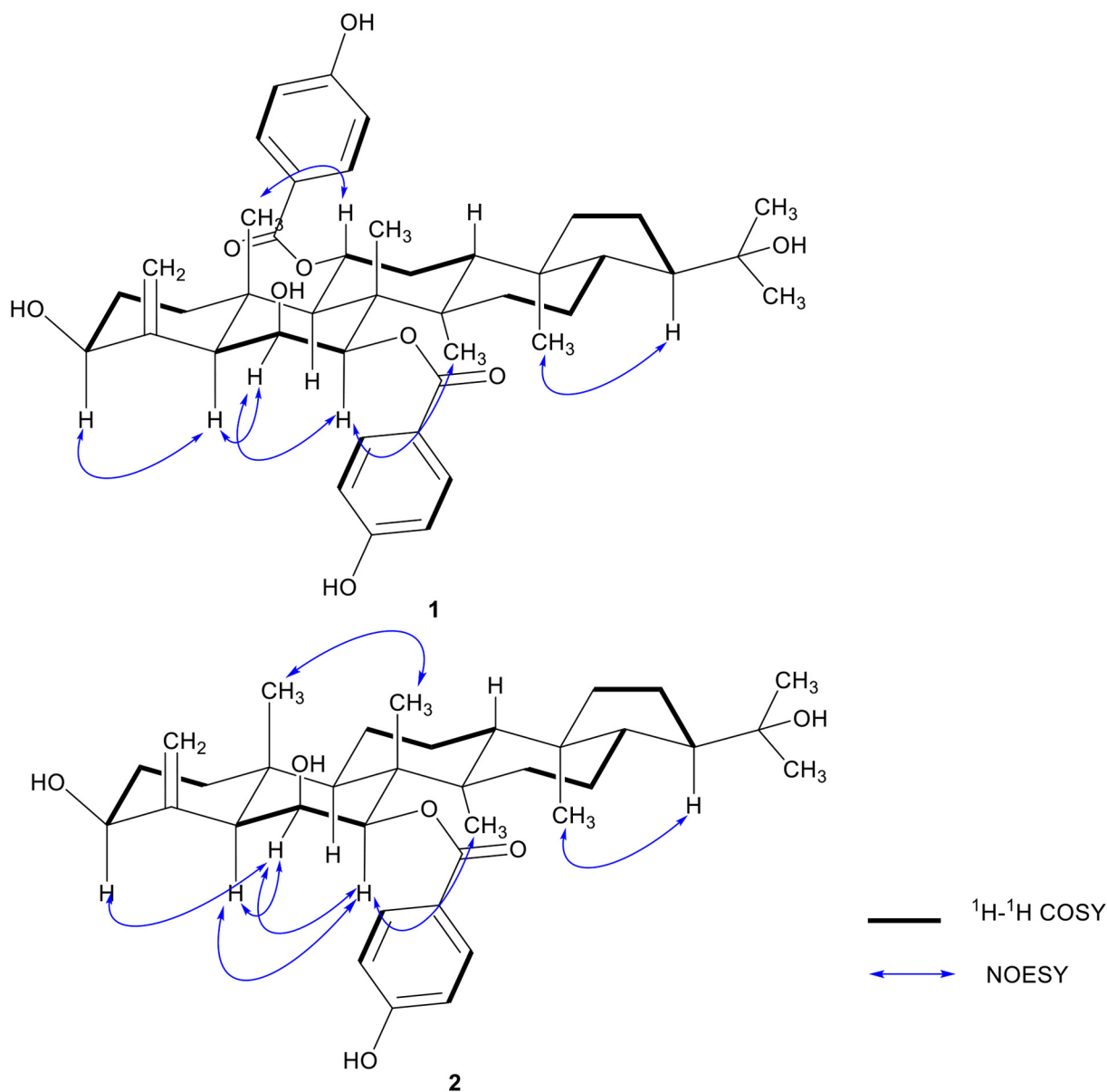
Fig. 2. $^1H-^1H$ COSY and NOESY spectrum for compound **1** and **2**.

Table 2
Results of controls (mean ± SD, n = 3).

Controls		Cytokine release [pg/mL]			
		IL-1β	IL-2	GM-CSF	TNF-α
Medium	Mean	568.68	229.25	56.33	334.79
	SD	26.22	14.03	8.87	19.97
LPS	Mean	9080.11	70.45	108.06	1815.02
	SD	712.46	7.28	5.24	271.69
Ibuprofen	Mean	1995.27	70.45	54.25	1404.79
	SD	287.26	7.28	12.69	357.71
Ibuprofen	% of LPS control	21.97	100.00	50.21	77.40

Table 3
Percentage of Cytokine Release compared to LPS Control.

Cytokine release [% of LPS control]				
Compound (100 μM)	IL-1β	IL-2	GM-CSF	TNF-α
Ibuprofen	21.97	100.00	50.21	77.40
1	99.71	111.94	162.	46.17
2	35.93	43.46	36.15	16.09
3	67.73	72.61	73.00	21.92
4	66.02	58.04	10.14	21.72

was in the similar range than it was for the standard drug, ibuprofen (77.40%).

Based on the anti-inflammatory results (Table 3 and Fig. 3) for the three related norhopanes, compound **2** (16.09–43.46% of the LPS control) displayed the highest inhibition potencies against all cytokines (IL-1β, IL-2, GM-CSF and TNF-α) followed by **3** (21.92–73.00% of the LPS control) and lastly **1** (46.17–162.24% of the LPS control). The structures for **1** and **2** are similar except for the presence of an additional *para*-hydroxybenzoate group at C-11 in **1.** The presence of this additional moiety seemed to contribute to substantial decrease in cytokine inhibition activity of **1** compared to **2**. However, it is not clear whether the it is the positioning of the hydroxyl group at C-11 in **3** *versus* C-22 in **1** or the presence of an olefinic group at C-17/C-21 that is contributing to the reduction in anti-inflammatory activity in **3** (21.92–73.00% of the LPS control) compared to **1.**
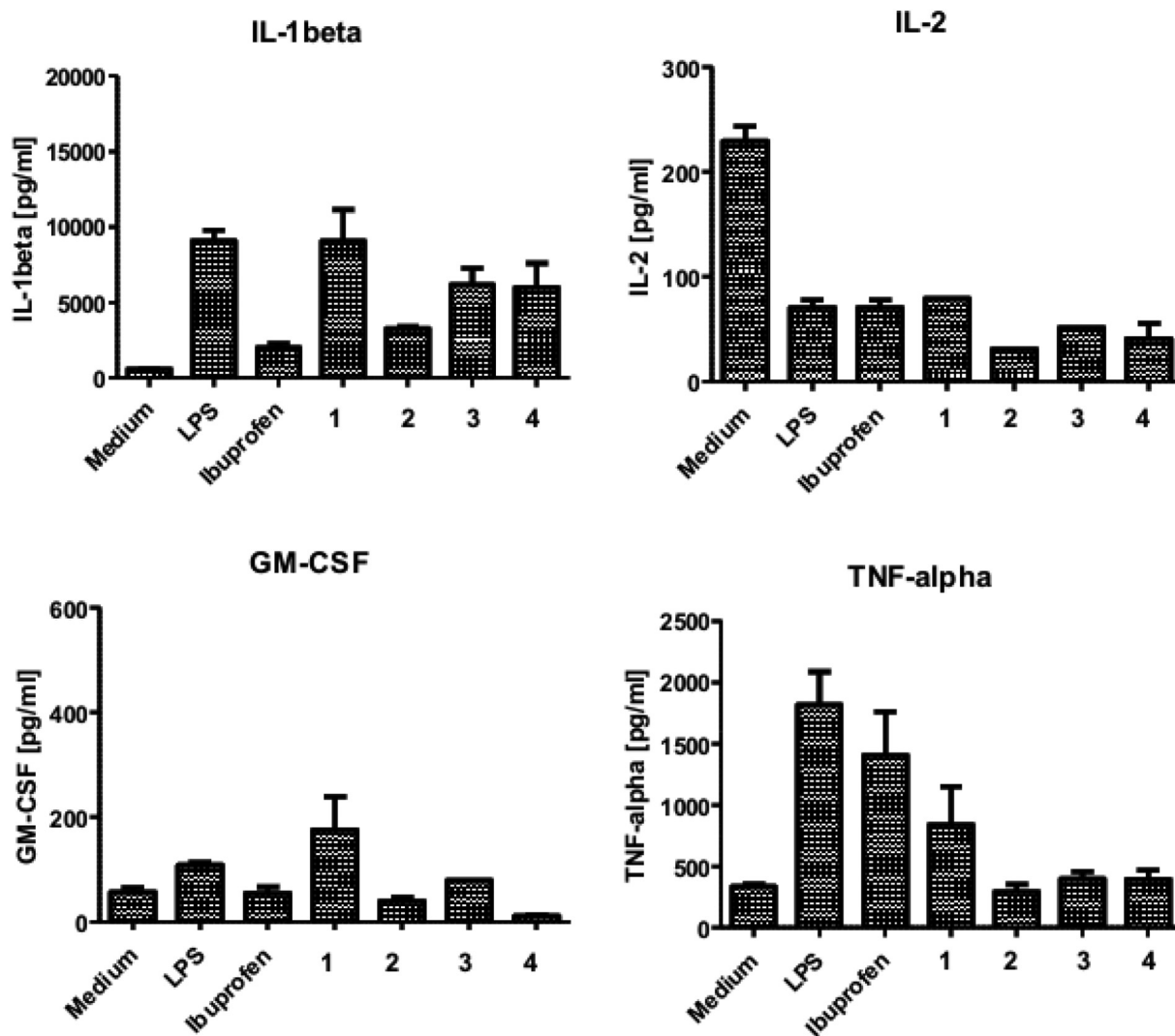
IL-1 beta

IL-2

GM-CSF

TNF-alpha

Fig. 3. Results of cytokine release of PBMCs after incubation with test items ($n = 3$).

4. Conclusion

Phytochemical investigation of a methanol-dichloromethane (1:1) roots bark extract of *F. angolensis* led to the identification of two new norhopane triterpenoids (**1–2**) along with a previously reported norhopane (**3**) and a norneohopane (**4**). All individual compounds exhibited anti-inflammatory effect against the tested cytokines in comparison with the LPS control. Compounds **2** and **4** caused significant decrease of the production of IL-2, GM-CSF and TNF-α compared to the reference drug ibuprofen. In order to carry out comprehensive structure-activity relationship studies these compounds could be re-isolated and modified in order to obtain analogues probably with improved anti-inflammatory activities.

Declaration of Competing Interest

The authors have no potential conflict of interest.

Appendix A. Supplementary data

Supplementary material: The spectroscopic (NMR, IR and UV) and spectrometric (HRESIMS) data of compounds **1** and **2** can be found in the supporting information. Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2020.104690.

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