Five New Triterpene Glycosides from *Lysimachia foenum-graecum* and Evaluation of their Effect on the Arachidonic Acid Metabolizing Enzyme

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Original Paper

Abstract

Five new oleanane-type triterpene saponins, named foenumosides A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**), were isolated from the aerial parts of *Lysimachia foenum-graecum* Hance. Their structures were identified on the basis of 1D and 2D NMR techniques, including H-H COSY, HMQC, HMBC, HMQC-TOCSY, ROESY experiments as well as chemical methods. We have evaluated the cytotoxity of **1** – **5** against rat and human polymorphonuclear leukocytes and the effect of **5** on the arachidonic acid metabolizing enzyme. All compounds showed a high degree of toxicity except for compound **5**, while **5** notably reduced the production of leukotriene B_4 (LTB_4) from rat peritoneal leukocytes with an IC_{50} value of 74 μM without inhibiting human elastase. Compound **5** also reduced the production of 12-HHTrE and 12-HETE by 14% and 50% as a measurement for cyclooxygenase-1 and 12-lipoxygenase inhibition at 100 μM.

Key words
Lysimachia foenum-graecum · saponin · triterpene glycosides · arachidonic acid

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Introduction

Lysimachia foenum-graecum Hance (Primulaceae) distributed mainly in Guanxi and Yunnan Provinces of China, has been commonly used as perfume plant and pest repellent. In Chinese folk medicine, the plant has also been used for the treatment of cold and headache [1]. It has been reported that some plants of the genus *Lysimachia* contained saponins and flavones [2], [3], [4], [5], [6]. However, no extensive investigation has been reported on chemical constitutents of *L. foenum-graecum* except for the essential oil. In our recent study, five new oleanane-type triterpene saponins, foenumosides A – E (**1** – **5**), were isolated from the aerial parts of *L. foenum-graecum*. Their structures were identified mainly by 1D and 2D NMR techniques. In this paper, we describe the isolation and structural elucidation of these five new triterpene glycosides as well as the effect on the arachidonic acid metabolizing enzyme of compound **5.**

Materials and Methods

General

Optical rotations were taken on a SEPA-300 polarimeter. IR was obtained on a Bio-Rad FTS-135 infrared spectrometer with KBr pellets. MS were performed on a VG Autospec-3000 spectrometer at 70 eV. ^{1}H-NMR, ^{13}C-NMR and 2D-NMR were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard at 25 °C. Semi-preparative HPLC was performed on

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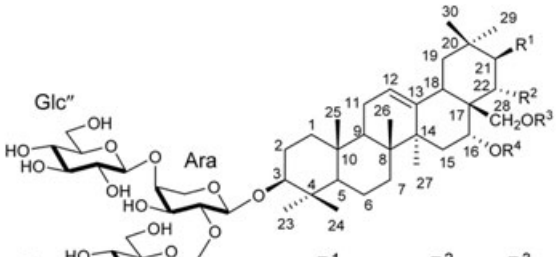
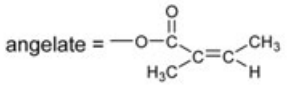


	R^1	R^2	R^3	R^4
1	angelate	angelate	H	H
2	angelate	OH	Ac	H
3	angelate	OH	H	Ac
4	H	angelate	H	H
5	angelate	angelate	Glc	H



an Agilent 1100 liquid chromatograph with a Zorbax SB-C18, 9.4 mm × 25 cm column, a flow rate of 3 mL/min and detection with a UV detector at 210 nm. GC was run on Fisons MD 800 instrument. Silica gel for TLC and column chromatography was obtained from Qingdao Marine Chemical Inc., China.

Plant material

The aerial parts of *L. foenum-graecum* were collected in the Yunnan Province, China, in August 2001, and were identified by Professor Zhong-Wen Lin. A voucher specimen (KIB No. 2001-08-03 Lin) has been deposited in the Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The aerial parts of *L. foenum-graecum* (7.0 kg) were extracted with 70% Me_2CO (3 × 25 L) at room temperature and filtered. The filtrate was concentrated and partitioned with EtOAc (6 L) and *n*-BuOH (6 L). The *n*-BuOH part was evaporated under vacuum to afford 230 g of residue, which was subjected to silica gel column chromatography (∅ 9 × 120 cm) eluted with a $CHCl_3$-MeOH (1 : 0 – 0 : 1, each 30 L) gradient system (saturated with H_2O) to yield fractions I – VIII. Fraction III (20 g) was applied to a silica gel column (∅ 7 × 100 cm) using $CHCl_3$-MeOH-H_2O (8 : 2 : 0.1, 20 L) for elution followed by a reversed-phase column (Rp-18, ∅ 3.5 × 60 cm) using 60% MeOH (4 L) as eluent and then by semi-preparative HPLC (CH_3CN-H_2O-THF, 38 : 57 : 5.0, 2 L) to yield **2** (32 mg), **3** (59 mg), **4** (78 mg). Fraction IV (15 g) was chromatographed (∅ 5 × 50 cm) on MPLC ($CHCl_3$-MeOH-H_2O, 10 : 3 : 0.1, 10 L) followed by semi-preparative HPLC (CH_3CN-H_2O-THF, 32 : 63 : 5.0, 2 L) to give **1** (132 mg) and **5** (84 mg).

Isolates

Foenumoside A (**1**): white powder; $[\alpha]_D^{23.3}$: –10.71° (*c* 0.14, MeOH); UV (MeOH): λ_{max} (log ε) = 212 nm (4.36); IR (KBr): ν_{max} = 3427, 2928, 1704, 1643, 1455, 1387, 1241, 1165, 1075 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5): δ = 1.18 (3H, s, Me-23), 1.04 (3H, s, Me-24), 0.83 (3H, s, Me-25), 0.86 (3H, s, Me-26), 1.81 (3H, s, Me-27), 1.10 (3H, s, Me-29), 1.33 (3H, s, Me-30), 3.16 (1H, dd-like, H-3), 0.70 (1H, d, *J* = 11.3 Hz, H-5), 1.66 (1H, m, H-9), 5.43 (1H, brs, H-12), 4.51 (1H, brs, H-16), 3.11 (1H, dd-like, H-18), 6.69 (1H, d, *J* = 10.1 Hz, H-21), 6.31 (1H, d, *J* = 10.1 Hz, H-22), 3.41 (1H, d, *J* = 10.8 Hz, H-28a), 3.66 (1H, d, *J* = 10.8 Hz, H-28b), 5.97 (1H, qq-like, H-3′), 2.07 (3H, d, *J* = 7.0 Hz, Me-4′), 2.01 (3H, s, Me-5′), 5.91 (1H, qq-like, H-3″), 2.04 (3H, d, *J* = 7.0 Hz, Me-4″), 1.90 (3H, s, Me-5″); ^{1}H- and ^{13}C-NMR data see Tables **1**, **2** and **3**; FAB-MS (negative): *m/z* = 1255 $[M-1]^-$, 1110, 948, 809, 742, 643, 513, 421, 325, 205, 159, 99; HR-ESI-MS (negative): *m/z* = 1255.6500 (calcd. for $C_{63}H_{99}O_{25}$ $[M-1]^-$: 1255.6475).

Foenumoside B (**2**): white powder, $[\alpha]_D^{24.0}$: –1.25° (*c* 0.13, MeOH); UV (MeOH): λ_{max} (log ε) = 204 nm (4.14); IR (KBr): ν_{max} = 3432, 2927, 1716, 1634, 1456, 1386, 1239, 1163, 1074, 1041 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5): δ = 1.16 (3H, s, Me-23), 1.01 (3H, s, Me-24), 0.82 (3H, s, Me-25), 0.97 (3H, s, Me-26), 1.79 (3H, s, Me-27), 1.11 (3H, s, Me-29), 1.30 (3H, s, Me-30), 3.12 (1H, dd-like, H-3), 0.68 (1H, d, *J* = 11.3 Hz, H-5), 1.62 (1H, m, H-9), 5.46 (1H, brs, H-12), 4.72 (1H, brs, H-16), 2.84 (1H, dd-like, H-18), 6.49 (1H, d, *J* = 10.1 Hz, H-21), 4.48 (1H, d, *J* = 10.1 Hz, H-22), 4.22 (1H, brs, H-28), 5.89 (1H, qq-like, H-3′), 2.03 (3H, d, *J* = 8.8 Hz, H-4′), 1.97 (1H, s, H-5′), 1.98 (3H, s, $COCH_3$); ^{1}H- and ^{13}C-NMR data see Tables **1**, **2** and **3**; FAB-MS (negative): *m/z* = 1215 $[M-1]^-$, 1069, 907, 809, 663, 599, 511, 421, 325, 233, 159, 99; HR-ESI-MS (negative): *m/z* = 1215.6127 (calcd. for $C_{60}H_{95}O_{25}$ $[M-1]^-$: 1215.6162).

Foenumoside C (**3**): white powder, $[\alpha]_D^{23.9}$: –23.03° (*c* 0.15, MeOH); UV (MeOH): λ_{max} (log ε) = 207 nm (4.15); IR (KBr): ν_{max} = 3430, 2930, 1716, 1640, 1455, 1379, 1254, 1161, 1070, 1043 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5): δ = 1.16 (3H, s, Me-23), 1.01 (3H, s, Me-24), 0.76 (3H, s, Me-25), 0.73 (3H, s, Me-26), 1.47 (3H, s, Me-27), 1.13 (3H, s, Me-29), 1.29 (3H, s, Me-30), 3.07 (1H, dd, *J* = 11.3, 3.7 Hz, H-3), 0.62 (1H, d, *J* = 11.3 Hz, H-5), 1.60 (1H, m, H-9), 5.34 (1H, brs, H-12), 5.90 (1H, d, *J* = 8.8 Hz, H-16), 2.95 (1H, dd-like, H-18), 5.97 (1H, d, *J* = 10.1, H-21), 4.78 (1H, d, *J* = 10.1 Hz, H-22), 3.65 (1H, d, *J* = 10.3 Hz, H-28a), 3.91 (1H, d, *J* = 10.3 Hz, H-28b), 5.87 (1H, qq-like, H-3′), 2.00 (3H, d, *J* = 7.0 Hz, H-4′), 1.92 (3H, s, H-5′), 2.5 (3H, s, $COCH_3$); ^{1}H- and ^{13}C-NMR data see Tables **1**, **2** and **3**; FAB-MS (negative): *m/z* = 1215 $[M-1]^-$, 1158, 1069, 907, 809, 741, 599, 339, 205, 159, 99; HR-ESI-MS (negative): *m/z* = 1215.6149 (calcd. for $C_{60}H_{95}O_{25}$ $[M-1]^-$: 1215.6162).

Foenumoside D (**4**): white powder, $[\alpha]_D^{23.7}$: -1.2° (*c* 0.14, MeOH); UV (MeOH): λ_{max} (log ε) = 207 nm (4.17); IR (KBr): ν_{max} = 3425, 2929, 1692, 1641, 1457, 1386, 1241, 1160, 1074 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5): δ = 1.15 (3H, s, Me-23), 1.01 (3H, s, Me-24), 0.81 (3H, s, Me-25), 0.86 (3H, s, Me-26), 1.84 (3H, s, Me-27), 1.04 (3H, s, Me-29), 1.28 (3H, s, Me-30), 3.12 (1H, dd, *J* = 11.5, 3.8 Hz, H-3), 0.62 (1H, d, *J* = 11.3 Hz, H-5), 1.68 (1H, m, H-9), 5.38 (1H, brs, H-12), 4.69 (1H, dd-like, H-16), 3.09 (1H, brs, H-18), 2.82 (1H, overlap, H-21a), 2.04 (1H, overlap, H-21b), 6.21 (1H, dd, *J* = 11.6, 6.3 Hz, H-22), 3.55 (1H, d, *J* = 10.1 Hz, H-28a), 3.69 (1H, d, *J* = 10.1 Hz, H-28b), 5.91 (1H, qq-like, H-3′), 2.08 (3H, d, *J* = 7.3 Hz, Me-4′), 1.19 (3H, s, Me-5′); ^{1}H- and ^{13}C-NMR data see Tables **1**, **2** and **3**; FAB-MS (negative): *m/z* = 1157 [M – 1], 1075, 1011, 995, 929, 849, 809, 599, 527, 449, 379, 325, 233, 159, 99; HR-ESI-MS (negative): *m/z* = 1157.6132 (calcd. for $C_{58}H_{93}O_{23}$ $[M-1]^-$: 1157.6107).

Foenumoside E (**5**): white powder, $[\alpha]_D^{22.8}$: –3.65° (*c* 0.14, MeOH); UV (MeOH): λ_{max} (log ε) = 211 nm (4.34); IR (KBr): ν_{max} = 3432,

Table **1** ^{13}C-NMR data (δ values) for the aglycone of compounds **1** – **5**

C	1	2	3	4	5	C	1	2	3	4	5
1	38.9	38.9	38.8	38.9	38.9	23	28.1	28.1	28.0	28.1	28.1
2	26.5	26.5	26.4	26.5	26.4	24	16.7	16.7	16.8	16.7	16.7
3	89.1	89.1	89.0	89.1	89.1	25	15.9	15.7	15.6	15.7	15.8
4	39.6	39.6	39.5	39.6	39.5	26	16.9	17.1	16.5	16.9	16.9
5	55.8	55.8	55.8	55.8	55.7	27	27.6	27.5	27.0	27.6	27.6
6	18.5	18.5	18.3	18.5	18.5	28	63.7	66.5	64.7	63.6	73.2
7	33.1	33.2	33.0	33.1	33.0	29	29.6	29.9	30.1	33.6	29.5
8	40.1	40.1	40.0	40.1	40.2	30	20.4	20.3	20.2	25.3	20.5
9	47.0	47.0	46.9	47.0	46.9	Angeloyl moiety					
10	36.8	36.9	36.8	36.8	36.8	1′	167.7	168.6	168.3	168.1	167.7
11	23.9	24.0	23.8	23.9	23.9	2′	129.0	129.5	129.2	129.5	128.9
12	124.0	124.1	124.5	123.8	124.5	3′	137.1	136.2	136.9	136.7	137.3
13	142.7	142.8	141.8	143.7	142.1	4′	15.8	16.0	16.0	15.9	15.9
14	41.7	41.9	41.4	41.6	41.6	5′	21.1	20.8	21.2	21.1	21.0
15	34.9	34.7	30.9	35.1	34.8	1″	168.2				168.2
16	68.7	67.7	71.6	70.2	68.1	2″	129.1				129.1
17	48.1	47.2	47.3	44.9	46.9	3″	137.2				137.8
18	40.2	40.6	39.8	40.9	40.9	4″	15.9				15.9
19	47.3	47.3	47.6	47.5	46.9	5″	20.9				20.8
20	36.4	36.2	36.1	32.1	36.3	Acetyl moiety					
21	78.8	81.2	80.5	41.8	78.2	1″		170.8	170.1		
22	73.7	71.1	70.6	73.0	74.7	2″		21.1	22.3		

^{13}C-NMR data were obtained in pyridine-d_5, 400 MHz, 25 °C; chemical shifts (δ) are given in parts per million.

2927, 1706, 1639, 1458, 1388, 1358, 1243, 1163, 1077, 1042 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5): δ = 1.17 (3H, s, Me-23), 1.03 (3H, s, Me-24), 0.85 (3H, s, Me-25), 1.03 (3H, s, Me-26), 1.79 (3H, s, Me-27), 1.07 (3H, s, Me-29), 1.28 (3H, s, Me-30), 3.13 (1H, dd-like, H-3), 0.68 (1H, d, J = 11.1 Hz, H-5), 1.68 (1H, dd-like, H-9), 5.42 (1H, brs, H-12), 4.72 (1H, brs, H-16), 2.87 (1H, dd-like, H-18), 6.67 (1H, d, J = 10.1 Hz, H-21), 6.21 (1H, d, J = 10.1 Hz, H-22), 3.41 (1H, d, J = 9.3 Hz, H-28a), 4.04 (1H, d, J = 9.3 Hz, H-28b), 5.94 (1H, qq-like, H-3′), 2.05 (3H, brs, Me-4′), 1.98 (3H, s, Me-5′), 5.89 (1H, qq-like, H-3″), 2.05 (3H, brs, Me-4″), 1.81 (3H, s, Me-5″); ^{1}H- and ^{13}C-NMR data see Tables **1**, **2** and **3**; FAB-MS (negative): m/z = 1417 [M – 1]$^-$, 1336, 1256, 1220, 1110, 886, 748, 599, 465, 279, 183, 99; HR-ESI-MS (negative): m/z = 1417.6977 (calcd. for $C_{69}H_{109}O_{30}$ [M – 1]$^-$: 1417.7003).

Acid hydrolysis and GC analysis

Compounds **1** – **5** (each 5 mg) in 1 M HCl-dioxane (1 : 1, 5 mL) were heated at 95 °C for approximately 6 h. The reaction mixture was extracted with $CHCl_3$ three times. Evaporation of the aqueous layer provided a sugar residue, which was dissolved in 5 mL pyridine and treated with 0.5 mL trimethylchlorosilane (TMCS, Fluka) at room temperature for 30 min. The reaction mixture was evaporated to dryness under vacuum. The mixture of trimethylsilated derivatives of the monosaccharides was dissolved in 1 mL ethyl ether and then directly subjected to GC analysis on an MD 800 instrument. GC: AC-5 capillary column (∅ 0.25 mm × 30 m); column temperature: 180 – 260 °C, rate 5 °C/min; column head pressure: 12 Pa; carrier gas: He. The sugar units were determined by comparing the retention times to authentic sugar. R_f (s) = Glc (690), Ara (426), Rha (432). The sugars glucose, arabinose and rhamnose were detected in **1** – **5**.

Pharmacological activity

The cytotoxicity to rat and human polymorphonuclear leukocytes (PMNL) was tested using the MTT method [7]. The effect on leukotriene B_4 production from rat polymorphonuclear leukocytes was assayed as reported previously [8]. The inhibitory activity on cyclooxygenase-1 (COX-1) and 12-LOX was measured using the methods described in the literature [9]. All compounds were assayed as possible inhibitors of human elastase according to the methods in the literature [10].

Results and Discussion

Foenumoside A (**1**) was obtained as a white powder. Its molecular formula $C_{63}H_{100}O_{25}$ was deduced from the quasimolecular ion peak [M – 1]$^-$ at m/z = 1255 in the negative FAB-MS as well as the analysis of NMR data. Briefly, analysis of the NMR data (see Tables **1**, **2** and **3**) indicated that **1** was a saponin containing a triterpene aglycone and four monosaccharides. The ^{13}C-NMR spectrum of **1** showed 63 carbon signals, from which 23 were assigned to four monosaccharide units, 30 to triterpene aglycone part, the remaining 10 to two angeloyl moieties. The seven tertiary methyl groups and one trisubstituted olefinic proton (δ_H = 5.43, brs) in the ^{1}H-NMR spectrum, together with the corresponding ^{13}C NMR signals, suggested an olean-12-ene skeleton. Detailed comparison between the ^{13}C-NMR data for **1** and those reported in the literature [11], [12] suggested that the aglycone of **1** was [3β,16α,21β(Z),22α(Z)]-olean-12-ene-3,16,21,22,28-pentol 21,22-bis(2-methyl-2-butenoate) (21,22-*O*-diangeloylbarringtogenol C) [13].

Table **2** ^{13}C-NMR spectral data for the sugar moieties of **1** – **5**

C	*1*	*2*	*3*	*4*	*5*
3-*O*-tetrasugar chain moiety					
Ara					
1	104.6	104.6	104.6	104.5	104.5*
2	80.9	81.0	80.9	80.9	80.8
3	72.4*	72.4*	72.4*	72.4	72.4*
4	74.9*	74.8	74.9*	74.8	74.9*
5	63.7	63.6	64.6	63.7	63.7
Glc′					
1	105.1	105.5	105.5	105.8	105.5
2	77.3	77.3	77.3	77.4	77.4
3	76.4	76.5	76.4	76.5	76.4
4	71.8	71.7	71.8	71.7	71.7
5	78.1	78.1	78.1	78.1	78.1
6	62.9	62.9	62.9	62.9	62.9
Rha					
1	101.6	101.6	101.6	101.6	101.6
2	72.4*	72.4*	72.4*	72.5	72.4*
3	72.7	72.7	72.7	72.8	72.7
4	74.7	74.7	74.7	74.9*	74.7
5	69.5	69.5	69.4	69.5	69.5
6	19.0	19.0	19.0	19.0	19.0
Glc″					
1	103.1	103.1	103.1	103.1	103.2
2	74.9*	74.9	74.9*	74.9*	74.9*
3	79.5	79.6	79.6	79.6	79.6
4	71.9	71.9	71.9	71.9	71.9
5	78.4	78.4	78.4	78.5	78.5
6	62.7	62.7	62.7	62.6	62.6
Glc‴ at C-28					
1					104.5*
2					75.2
3					78.4
4					71.8
5					78.2
6					63.0

* Overlap with other signals.

[13] C-NMR data of sugar moieties were obtained in pyridine-d_5, 400 MHz; chemical shifts (δ) are given in parts per million. Assignments made by a combination of ROESY, HMQC, COSY, and HMBC, HMQC-TOCSY data.

Table **3** ^{1}H-NMR spectral data for the sugar moieties of **1** – **5**

H	*1*	*2*	*3*	*4*	*5*
3-*O*-tetrasugar chain moiety					
Ara					
1	4.94 (brs)	4.91 (brs)	4.88 (brs)	4.91 (brs)	4.93 (brs)
2	4.55	4.55	4.56	4.57	4.57
3	4.68	4.68*	4.68	4.72	4.68
4	4.26	4.27	4.28*	4.25	4.25
5	4.32, 3.78	4.30*, 3.76	4.34, 3.78	4.32, 3.78	4.32, 3.79
Glc′					
1	5.37 (d, 7.6)	5.37 (d, 8.8)	5.38 (d, 7.6)	5.37 (d, 7.3)	5.37 (d, 7.8)
2	4.04	4.07	4.07	4.06	4.05*
3	4.28	4.25	4.28*	4.29	4.28
4	4.18	4.22*	4.22*	4.22	4.17*
5	4.08	4.30*	4.06	4.04	4.08
6	4.35, 4.24	4.35, 4.22*	4.37, 4.22*	4.38, 4.23	4.35, 4.23
Rha					
1	6.39 (brs)	6.40 (brs)	6.41 (brs)	6.41 (brs)	6.40 (brs)
2	4.51	4.69	4.70	4.51	4.50
3	4.59	4.68*	4.67	4.59	4.61
4	4.52	4.57	4.58	4.52	4.55
5	5.01	5.03	5.04	5.03	5.02
6	1.79 (d, 5.8)	1.77 (d, 5.8)	1.80 (d, 5.7)	1.79 (d, 5.8)	1.79 (d, 5.8)
Glc″					
1	5.25 (d, 7.8)	5.27 (d, 7.5)	5.27 (d, 7.3)	5.24 (d, 7.0)	5.24 (d, 7.5)
2	4.17	4.17	4.16	4.14	4.18
3	4.16	4.14	4.17	4.16	4.17*
4	4.13	4.12	4.13	4.12	4.14*
5	3.80	3.79	3.78	3.80	3.81
6	4.57, 4.48	4.59, 4.49	4.53, 4.48	4.55, 4.48	4.58, 4.47
Glc‴ at C-28					
1					4.71 (d, 8.0)
2					3.99
3					4.26
4					4.14*
5					4.05*
6					4.44, 4.39

* Overlap with other signals.

[1] H-NMR data of sugar moieties were obtained in pyridine-d_5, 400 MHz; chemical shifts (δ) are given in parts per million. Assignments made by a combination of ROESY, HMQC, COSY, and HMBC, HMQC-TOCSY data.

Four monosaccharide units were determined from the HMQC-TOCSY spectrum with the aid of COSY, HMQC and HMBC spectra. Starting from the anomeric proton signal at δ_H = 5.37 (1H, d, J = 7.6 Hz), six correlated carbon signals were observed in the HMQC-TOCSY spectrum and determined in sequence to be at δ_C = 105.1 (C-1), 77.3 (C-2), 76.4 (C-3), 71.8 (C-4), 78.1 (C-5) and 62.9 (C-6), which suggested a glucose unit. Similarly, another glucose unit was identified from analysis of the HMQC-TOCSY spectra. A 6-deoxymonosaccharide was elucidated from the methyl carbon at δ_C = 19.0 and from the corresponding methyl proton doublet at δ_H = 1.79 (J = 5.8 Hz). The HMQC-TOCSY spectrum showed correlations of an aromeric proton of a 6-deoxy sugar to carbon signals at δ_C = 101.6 (C-1) and 72.4 (C-2), and of a methyl proton δ_H = 1.79 (1H, d, J = 5.8 Hz) to carbon signals δ_C = 19.0 (C-6), 69.5 (C-5), 74.7 (C-4) and 72.7 (C-3) therefore indicating a rhamnose unit. The remaining five carbon signals suggested a pentose whose aromeric proton δ_H = 4.94 (1H, brs) was only correlated to four carbon signals at δ_C = 104.6 (C-1), 80.9 (C-2), 72.4 (C-3) and 74.9 (C-4) in HMQC-TOCSY spectrum, implying an arabinose unit. The oxygen-bearing methylene at δ_C = 63.7 was assigned to be at the C-5 position of arabinose based on H-H COSY and HMBC experiments. The above inferences for the monosaccharide unit were also further confirmed by GC analysis of the acid hydrolysate of **1**.

The glycosidic position of the aglycone was determined to be the C-3 position on the basis of HMBC correlation between the aromeric proton of arabinose δ_H = 4.94 (1H, brs) with δ_C = 89.1 (C-3). The connectivity among the monosaccharide units was established with the following HMBC correlations: H-1 (δ_H = 5.37) of inner glucose with C-2 (δ_C = 80.9) of arabinose; H-1 (δ_H = 5.25) of outer glucose with C-4 (δ_C = 74.9) of arabinose; H-1 (δ_H =

6.39) of rhamnose with C-2 (δ_C = 77.3) of inner glucose. The anomeric configurations of two glucoses were determined to be β orientated from the coupling constants of the anomeric protons. Similarly, the aromeric protons of rhamnose and arabinose were found to be in the α orientation. Thus, the complete structure of **1** was elucidated as 3-*O*-{α-rhamnopyranosyl(1→2)-β-glucopyranosyl(1→2)-[β-glucopyranosyl(1→4)]-α-arabinopyranosyl}-21,22-*O*-diangeloylbarringtogenol C, and named foenumoside A.

Foenumoside B (**2**), a white powder, was assigned the molecular formula of $C_{60}H_{96}O_{25}$ from its quasimolecular ion peak at *m/z* = 1215 $[M-1]^-$ in negative FAB-MS as well as the analysis of NMR data (Tables **1**, **2** and **3**). The analysis of ^{13}C-NMR data (see Tables **1**, **2** and **3**) of **2** showed the elimination of an angeloyl and an additional acetyl in **2** when compared to **1**. The downfield shift at δ_C = 66.5 (C-28) indicated that the C-28 position of the aglycone was substituted by an acetoxy, which was confirmed by long-range correlations between the proton signal at δ_H = 4.22 (H_2-28, brs) and the ester carbonyl signal at δ_C = 170.8 and between the acetyl methyl proton signal at δ_H = 1.98 (3H, s) and the carbon signals at δ_C = 66.5 (C-28) and 170.8 (-CO-) in the HMBC spectrum. The hydroxyl substitution at C-22 was determined with HMBC correlations between the proton signal at δ_H = 4.48 (1H, d, *J* = 10.1 Hz) and the carbon signals at δ_C = 67.7 (C-16), 47.2 (C-17), 81.2 (C-21) and 66.5 (C-28). This indicates that an angeloyl at C-22 is absent. The stereochemistry of H-21 and H-22 were established to be α and β by analyzing the ROESY data of **2**. The NMR signals (see Tables **2** and **3**) due to the sugar moiety were superimposable on those of **1** thereby demonstrating that the two compounds share the same sugar-substituted pattern. Therefore, the structure of **2** was identified as 3-*O*-{α-rhamnopyranosyl(1→2)-β-glucopyranosyl(1→2)-[β-glucopyranosyl(1→4)]-α-arabinopyranosyl}-21-*O*-angeloyl-28-*O*-acetylbarringtogenol C, and named foenumoside B.

Foenumoside C (**3**), a white powder, displayed a quasimoecular ion peak at *m/z* = 1215.6149 $[M-1]^-$ (calcd. for $C_{60}H_{95}O_{25}$: 1215.6162) in negative HR-ESI-MS, which is consistent with the molecular formula $C_{60}H_{96}O_{25}$. The comparison of NMR data (see Tables **1**, **2** and **3**) between **2** and **3** revealed great similarity. The only difference was that the C-16 position of **3** was substituted by an acetoxy based on the HMBC correlation between the proton signal at δ_H = 5.90 (H-16) and the carbonyl at δ_C = 170.1 and between the acetyl methyl proton at δ_H = 2.5 and δ_C = 71.6 (C-16). The acetoxy group was substituted at C-28 in **2**. Thus, the structure of **3** was established to be 3-*O*-{α-rhamnopyranosyl(1→2)-β-glucopyranosyl(1→2)-[β-glucopyranosyl(1→4)]-α-arabinopyranosyl}-16-*O*-acetyl-21-*O*-angeloylbarringtogenol C, and named foenumoside C.

Foenumoside D (**4**), a white powder, was assigned to have a molecular weight of 1158 as indicated by a quasimolecular ion peak at *m/z* = 1157 $[M-1]^-$ in the FAB-MS (negative-ion mode). This is in agreement with a molecular formula of $C_{58}H_{94}O_{23}$. The ^{13}C-NMR spectrum (see Tables **1** and **2**) showed 58 carbon signals. A close match of the NMR signals (see Tables **1**, **2** and **3**) between **4** and **1** implied that **4** possessed the similar aglycone and the same tetrasaccharide chain at C-3. Compared to the NMR data of **1**, the NMR spectrum of **4** indicated elimination of an angelate from C-21 or C-22, and replacement of an oxygen-bearing methine with a methylene. A pair of methylene protons at δ_H = 2.82 (1H, overlap) and 2.04 (1H, overlap), directly attached to carbon signal at δ_C = 41.8 from the HMQC experiment, in turn showed HMBC interactions with carbon signals at δ_C = 44.9 (C-17), 73.0 (C-22), 32.1 (C-20) and 25.3 (C-30). This indicated the absence of the angelate at C-21 in **4**. H-22 was β oriented based on the ROESY correlation of H-22 with Me-30 and H-18. It was concluded from the above evidence that the structure of **4** is 3-*O*-{α-rhamnopyranosyl(1→2)-β-glucopyranosyl(1→2)-[β-glucopyranosyl(1→4)]-α-arabinopyranosyl}-21-dehydroxy-22-*O*-angeloylbarringtogenol C, and named foenumoside D.

Foenumoside E (**5**) was obtained as a white powder. The molecular formula of $C_{69}H_{110}O_{30}$ was deduced from the quasimolecular ion peak at *m/z* = 1417 $[M-1]^-$ in negative FAB-MS and by analysis of NMR data. The molecular weight of **5** was 162 mass units more than that of **1**, which implied an additional hexose unit in **5**. The NMR data (see Table **1**) of the aglycone of **5** were superimposable on those of **1** indicating that the two compounds share the same aglycone. In the HMQC-TOCSY spectrum, an additional anomeric proton at δ_H = 4.71 was correlated with six carbons at δ_C = 104.5 (C-1), 75.2 (C-2), 78.4 (C-3), 71.8 (C-4), 78.2 (C-5) and 63.0 (C-6) indicating a glucose unit. This glucose was attached to C-28 of the aglycone based on the HMBC correlation between the anomeric protons at δ_H = 4.71 and δ_C = 73.2 (C-28). The configuration of the anomeric proton of glucose was established to be β based on the coupling constant of the anomeric proton. Thus, the structure of **5** was established as 3-*O*-{α-rhamnopyranosyl(1→2)-β-glucopyranosyl(1→2)-[β-glucopyranosyl(1→4)]-α-arabinopyranosyl}-21,22-*O*-diangeloylbarringtogenol C 28-*O*-glucopyranoside, and named foenumoside E.

Compounds **1**, **2** and **4** showed a high toxicity against both human and rat polymorphonuclear leukocytes (PMNL) at 10 μM or more with a survival coefficient of 50% or less. Compound **3** inhibited the growth of rat cells at all the doses tested; however, it failed to inhibit the growth of human cells at 10 and 50 μM (100% viability). Compound **5** had no cytotoxicity against rat or human PMNL at the assayed doses (10–100 μM). Because of the observed toxicity of compounds **1**, **2**, **3** and **4**, we only chose **5** to test for bioactivity in the arachidonic acid metabolism. The production of leukotriene B_4 (LTB_4) from rat peritoneal leukocytes was notably reduced by **5** at 100 μM (81%). The effect was concentration-dependent, and the IC_{50} value was determined as 74 μM. When tested for inhibitory effects on COX-1 and 12-LOX at 100 μM, **5** reduced the production of 12-HHTrE (COX-1 metabolite) by 14% and the production of 12-HETE (12-LOX metabolite) by 50%. All compounds were assayed as possible inhibitors of human elastase, but none of them inhibited the enzyme release or the activity.

The selective toxicity among the five isolated saponins should be of interest, especially that of compound **5**, which demonstrated low toxicity against rat and human PMNL. In addition, **5** was found to inhibit the activity of 5-LOX and COX-1. This is of possible interest because both enzymes are implicated in the development and metabolism of cancer cell lines [14], [15] and in inflammatory pathologies such as asthma and arthritis.

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