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A triterpenoidal saponin and sphingolipids from *Pteleopsis hylodendron*

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ABSTRACT

From the stem bark of *Pteleopsis hylodendron*, a triterpenoidal saponin bellericagenin {B 3-*O*-[β-D-glucopyranosyl-(1 → 2)-α-D-glucopyranoside] (**1**) (Pteleopsoside)} and two sphingolipids, hylodendroside-I (**2**), and hylodendroside-II (**3**) were isolated, along with a synthetically known compound, {2α, 3β, 23-triacetoxy-19α-hydroxyolean-12-en-28-oic acid (**4**)}. Other known compounds, friedelin (**5**), β-carotene (**6**), lupeol (**7**), sitosterol (**8**), and stigmasterol (**9**), were also obtained. Their structures were deduced with the help of detailed spectroscopic studies.

1. Introduction

Pteleopsis hylodendron (Mildbr.) found in Cameroon (Liben and Satabie, 1983) belongs to the family Combretaceae. This genus comprises 10 species, distributed in the forest regions of West and Central Africa (Irvin, 1961). This plant is highly valued in folk medicine. The aqueous decoction of the stem bark is used to treat sexually transmitted diseases, female sterility, liver and kidney disorders, and dropsy (Ruzicka and Eschenmoser, 1953). The methanolic and ethyl acetate extracts of the stem bark of *P. hylodendron* showed antibacterial and antifungal activities against different pathogenic microorganisms. The ethyl acetate extract of the plant showed antioxidant activity, as reported by us (Atta-ur-Rahman et al., 2001). A systematic study of the stem bark resulted in the isolation and characterization of a new saponin, pteleopsoside (**1**) and two sphingolipids, hylodendroside-I (**2**), and hylodendroside-II (**3**), along with a synthetically known triterpenoid, 2α, 3β, 23-triacetoxy-19α-hydroxyolean-12-en-28-oic acid (**4**) (Kwang et al., 1994). Some known compounds, friedelin (**5**) (Salazar et al., 2000), β-carotene (**6**) (Bjornland et al., 1989), lupeol (**7**) (Monaco and Previteva, 1984), sitosterol (**8**) and stigmasterol (**9**) (Sadikum et al., 1996), were also obtained for the first time from this species.

Sphingolipids form a biologically important class of compounds (Kolter and Sandhoff, 1999), some of which have been reported to exhibit antihepatotoxic (Kim et al., 1997), antitumor and immunostimulatory activities (Natori et al., 1994). These are mainly reported from different fungi (Lourenco et al., 1996) and sponges (Kim et al., 1997; Hattori et al., 1998; Hirsch and Kashman, 1989; Yunker and Scheuer, 1978; Li et al., 1995; Gao et al., 2001a).

2. Results and discussion

Compounds **1–3** were isolated by vacuum liquid chromatography of the ethyl acetate soluble fraction and compound **1** was obtained as a colorless amorphous powder. The molecular ion [M^+] in HR FABMS at *m/z* 845.4457 ($C_{42}H_{68}O_{17}$, Calcd. 845.4534), indicating the presence of nine double bond equivalents, which were accounted for five cyclic rings, one trisubstituted double bond, one carbonyl carbon, and two sugar moieties. The UV spectrum exhibited absorptions at 202 and 214 nm (Scott, 1964). The IR spectrum displayed bands at 3435 (OH), 1647 (C=O), and 1385–1379 cm^{-1} (*gem*-dimethyl) (Pavia et al., 1979). The mass fragments at *m/z* 683, and 521 indicated the presence of two sugar units. The loss of sugar units and a water molecule from [M^+] yielded a peak at *m/z* 502. The base peak at *m/z* 264 may arise by the retro Diels-Alder cleavage of ring C which indicated the presence of an Δ^{12} β-amyrin skeleton (Budzikiewicz et al., 1963).

The ^{1}H NMR spectrum (C_5D_5N, 400 MHz) of compound **1** showed a number of characteristic signals for sugar and triterpene moieties. The anomeric protons appeared at δ 6.32 (*d*, *J* = 2.9 Hz), and 5.91 (*d*, *J* = 7.7 Hz), indicating α and β-configuration of the sugar moieties, respectively. The ^{1}H NMR spectrum of **1** showed five

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3H singlets (δ 1.54, 1.31, 1.10, 1.13 and 1.12), which were due to the C-27, C-29, C-30, C-26, and C-25 methyl protons. This further indicated the triterpenoid nature of the molecule. Two sets of AB doublets, resonating at δ 4.02 and 4.62 (each, *d*, J = 11.4 Hz), and 4.27 and 4.81 (each, *d*, J = 11.4 Hz), were assigned to the C-23 and C-24 hydroxymethylene protons, respectively. Three carbinol methine signals, resonating at δ 4.41 (*ddd*, $J_{1ax.2ax}$ = 14.1 Hz, $J_{2ax.3ax}$ = 9.5 Hz, $J_{2ax.1eq}$ = 4.4 Hz) and 4.32 (*d*, $J_{2ax.3ax}$ = 9.5 Hz) and 3.52 (*d*, $J_{18ax.19eq}$ = 4.4 Hz), were assigned to the C-2, C-3 and C-19 protons, respectively (see Fig. 1).

The broad band decoupled ^{13}C NMR spectrum (C_5D_5N, 100 MHz) of compound **1** showed resonances for 42 carbons, including five methyl, 12 methylene, 17 methine, and eight quaternary carbons. The signal at δ 180.2 was assigned to a carboxyl carbon, while signals at δ 144.3 and 123.2 were due to the olefinic C-13 and C-12, respectively.

The aglycon (bellericagenin B) of **1** is previously very well identified (Mahato et al., 1992). The downfield chemical shift of the C-2′ methine (δ 82.3) indicated that other sugar was attached to C-2′. In the HMBC spectrum, the anomeric C-1″ proton (δ 5.91) exhibited connectivity with C-2′ (δ 82.3), thus supporting a 1 → 2 linkage between the two sugar units (Agarwal, 1989, 1992). Acid hydrolysis of compound **1** yielded D-glucose, which was identified by co-TLC with an authentic sample. The above spectroscopic evidence led to the structure of the compound as bellericagenin B 3-*O*-[β-D-glucopyranosyl-(1 → 2)-α-D-glucopyranoside] (**1**) (Pteleopsoside).

Compound **2** was obtained as a light brown gummy solid. The molecular formula $C_{48}H_{91}NO_9$ was determined by HR FABMS [M+H$^+$], *m/z* 826.6693 (Calcd. for 826.6771). In the positive FABMS, fragment ions were observed at *m/z* 826 [M+H]$^+$, 808 [M+H−H_2O]$^+$, 790 [M+H−2H_2O]$^+$, and 664 [M+H−162]$^+$ were appeared. The IR spectrum showed absorption bands for a secondary amide (1625, 1545 cm^{-1}), C=C (1644 cm^{-1}), and hydroxyl (3434 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR spectral data of **2** indicated the presence of a sugar, an amide linkage, and two long chain aliphatic moieties, suggesting the glycolipid (sphingolipids) nature of the molecule (Table 2).

In the ^{1}H NMR spectrum of compound **2** a number of singlets and multiplets between δ 1.23–2.81 at 1.23 (18 × CH_2), 1.30 (3 × CH_2), 2.32 (1 × CH_2), 1.41 (2 × CH_2), 1.99 (1 × CH_2), two AB doublets at δ 4.51/4.72 (J = 10.9, 5.8/J = 10.9, 4.7 Hz) and a triplet at δ 0.85 (6H, *t*, J = 6.2 Hz) indicated the presences of two long aliphatic chains or a branched aliphatic chain. A downfield doublet at δ 8.56 (*d*, J = 8.5 Hz) was assigned to amide proton. The protons of *trans*-olefinic bonds appeared at δ 5.52 (*dt*, $J_{9,10}$ = 16.3 Hz, $J_{8,9}$ = 4.9 Hz), and 5.48 (*dt*, $J_{9,10}$ = 16.3 Hz, $J_{10,11}$ = 5.1 Hz). A doublet of triplets at δ 5.55 ($J_{12,13}$ = 15.7 Hz, $J_{11,12}$ = 4.8 Hz) and a multiplet at δ 5.61 ($J_{12,13}$ = 15.7 Hz) were assigned to the C-9/C-10 and C-12/C-13 protons. An anomeric signal at δ 4.96 (*d*, $J_{ax.ax}$ = 7.9 Hz) suggested a β-configuration of sugar moiety.

The ^{13}C NMR spectrum of **2** showed signals for 48 carbon atoms, including two methyl, 33 methylene, 12 methine, and one quaternary carbon atoms. This suggested a glycosphingolipid skeleton (Kawai et al., 1986). This was supported by the observations that a proton attached to a nitrogen appeared at δ 8.56 in the ^{1}H NMR spectrum and that a tertiary carbon at δ 52.8, and a quaternary carbon at δ 175.9 were correlated with this nitrogen proton in the HMBC spectrum (Fig. 2). Acidic hydrolysis of compound **2** afforded aglycon and D-glucose, the letter being finally identified as D-glucose by co-TLC with an authentic sample.

The positions of double bonds in the long sphingoid chain were deduced by 2D NMR data. The geometry of the C-9/C-10 and C-12/C-13 alkenyl bonds were found to be *trans* based on vicinal coupling constants ($J_{9,10}$ = 16.3 Hz) and ($J_{12,13}$ = 15.7 Hz). The *trans* configuration of the C-9/C-10 and C-12/C-13 double bonds was also deduced from the chemical shifts of the allylic C-8 and C-14 (δ 35.7 and 33.7, respectively). Typically, the carbons next to a *trans* double bond appear between δ 32 and 33, while those next to a *cis* double bond appears upfield between δ 27 and 28 (Stotuers, 1972; De Haan and Van de Ven, 1973). Four methine carbons resonating at δ 130.4, 130.9, 131.4, and 131.8, further suggested two double bonds in the molecule. The above spectral data revealed that compound **2** is a glycosphingolipid of the C_{18}-sphinga-9,12-dienine type. The structure of **2** was finally established through characteristic fragment ions in EIMS (Scheme 1) and through ^{1}H–^{1}H COSY, HOHAHA, HMQC and HMBC correlations. Key cross-peaks were H-2′ with C-1′, C-3′, C-4′ observed in the HMBC spectrum. Similarly, H-2 exhibited HMBC correlations with C-1, C-3, and C-4. The alkenyl H-9 showed cross-peaks with C-8, C-10, and C-11. While the C-11 methylene protons showed cross-peaks with C-10, C-12, C-13, and C-14 supporting double bonds between C-9/C-10 and C-12/C-13.

The acyl moiety was therefore deduced to be a α-hydroxylated linear acyl chain without any branch, and with an α-amine group at C-2 of the other side chain (Stotuers, 1972). The position of the glucose moiety on the amine half was inferred from the downfield chemical shift of the hydroxymethylene carbon (C-1) at δ 70.4. This was deduced through HMBC correlation between the anomeric proton (δ 4.96) and the C-1 hydroxymethylene carbon.

The relative stereochemistry at C-2 and C-3 were proposed as *2S*, and *3R* (identical to that of D-sphingosine) on the basis of ^{13}C NMR spectral data, since the chemical shifts of C-2 (δ 52.8), and C-3 (δ 75.9) were in agreement with those of the reported natural product, agelasphin-9b (δ 50.1 and 76.4) (Natori et al., 1994).

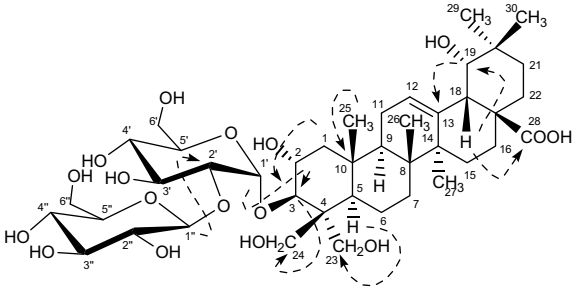


Fig. 1. Key HMBC interactions in compound **1**.

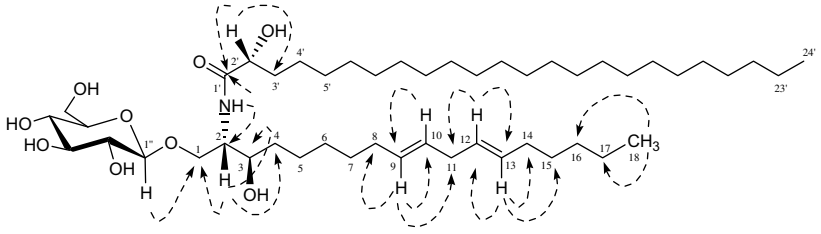
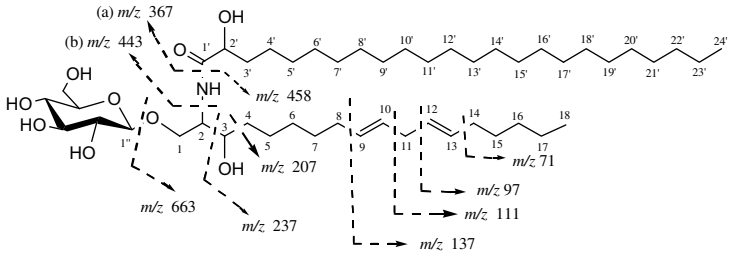


Fig. 2. Key HMBC interactions in compound **2**.



Scheme 1. Mass fragmentation pattern in compound **2**.

The EIMS of the fatty acid methyl ester, obtained by methanolysis of **2**, exhibited a base peak at *m/z* 398 $[M^+]$ and an ion at *m/z* 339 $[M-COOCH_3]^+$. This established the molecular weight of the fatty acid fragment to be 384 $[M-CH_2]^+$. The structure of the acyl moiety was determined to be 2-hydroxytetracosanoyl from the ^1H NMR spectrum of the methyl ester. The lengths of the long chain base (LCB) and the fatty acid were determined by FABMS, which showed fragment ions at *m/z* 367 (a) and 443 (b) and also from the EIMS, which showed a prominent peak at *m/z* 663 due to elimination of a sugar moiety from the $[M^+]$. The mass spectrum supported the base moiety to be C_{18}-sphinga-9, 12-dienine. Based on this evidence, compound **2** was assigned the structure 1-*O*-D-glucopyranosyl-(2*S*,3*R*,9*E*,12*E*)-2-*N*-[(2*R*)-hydroxytetracosanoyl]-octadecasphinga-9, 12-dienine (**2**).

Compound **3**, a light brown gummy solid, was assigned the molecular formula $C_{49}H_{93}NO_{10}$ by HR FABMS, which showed an $[M+H]^+$ peak at *m/z* 856.6798 (Calcd. *m/z* 856.6877). Another main fragment appeared at *m/z* 693 due to the loss of a sugar moiety. The ^1H and ^{13}C NMR spectra of **3** closely resembled those of **2**, except for the integration of the aliphatic methylene protons between δ 1.22–1.29 (*br s*, 18 × CH_2) and the presence of one OH-bearing methine at δ 4.18. The IR spectral data of compound **3** showed absorptions for a secondary amide (1643, 1547 cm^{-1}), C=C (1660 cm^{-1}) and hydroxyl (3310 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectra of **3** (Table 2) were consistent with the presence of a secondary amide group (δ_H 8.59, 1H, *d*, *J* = 9.1 Hz, δ_C 175.7).

The lengths of the long chain base and the fatty acid were determined by FABMS, which showed fragment ions at *m/z* 381 (a) and 459 (b). On the other hand, the EIMS showed a prominent peak at *m/z* 693 due to the elimination of a sugar moiety from the $[M^+]$ ion. The mass fragmentation pattern is presented in Scheme 2. Detailed analysis of the $^1H/^1H$ COSY spectrum of compound **3** indicated connectivities of the H_2-1 was found to be coupled with H-2, which in turn was coupled with H-3 and H-4. The key HMBC interactions are shown in Fig. 3.

The relative stereochemistry at C-2, C-3, and C-4 were deduced to be 2*S*, 3*S*, and 4*R*, respectively, similar to that of D-sphingosine on the basis of the ^{13}C NMR spectral data. Methanolysis of **3** yielded the fatty acyl moiety as methyl pentacosanoate. Thus, on the above spectral observation, the structure of the compound was deduced as 1-*O*-D-glucopyranosyl-(2*S*,3*S*,4*R*,9*E*,12*E*)-2-*N*-[(2*R*)-hydroxypentacosanoyl]octadecasphinga-9,12-dienine (**3**).

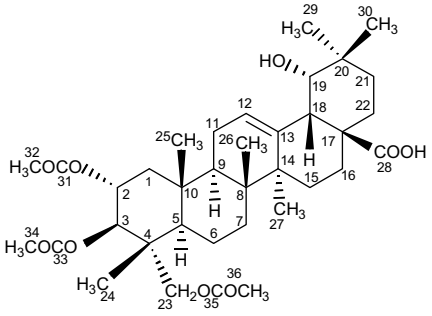
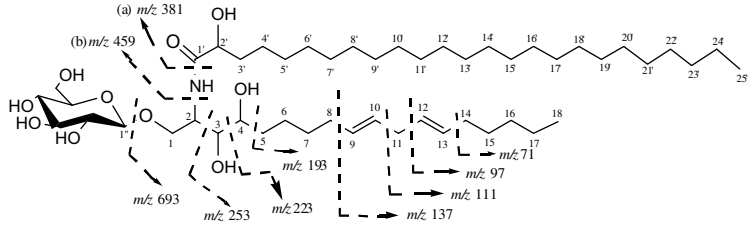


Fig. 4. 2 α, 3 β, 23-Triacetoxy-19α-hydroxyolean-12-en-28-oic acid (**4**).



Scheme 2. Mass fragmentation pattern in compound **3**.

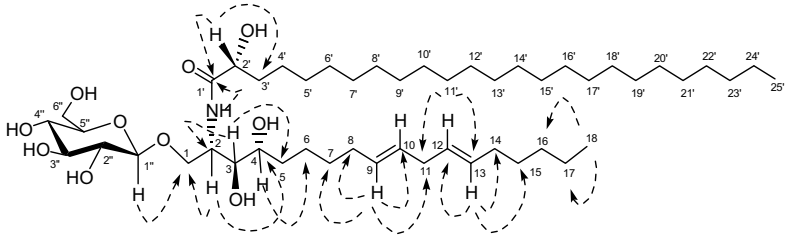


Fig. 3. Key HMBC interactions in compound **3**.

Compound **4** (Fig. 4), $C_{36}H_{54}O_9$ (*m/z* 630.3768), was also isolated as a colorless solid powder from the same extract. Comparison of the spectral data (UV, IR, MS, ^1H and ^{13}C NMR) indicated that compound **4** was a synthetically known compound [2α, 3β, 23-triacetoxy-19α-hydroxyolean-12-en-28-oic acid] and this is the first report of its isolation from any natural source (Kwang et al., 1994).

3. Experimental

3.1. General experimental procedures

Column chromatography (CC): silica gel, 70–230 mesh. Flash chromatography (FC): silica gel 230–400 mesh. TLC: pre-coated silica gel G-25-UV_{254} plates: detection at 254 nm, and by ceric sulphate reagent. Optical rotations: JASCO-DIP-360 digital polarimeter. UV and IR spectra: Hitachi UV-3200 and JASCO-302A spectrophotometer, respectively. ^1H and ^{13}C NMR, COSY, HMQC and HMBC spectra: Bruker spectrometers operating at 500 and 400 MHz; chemical shifts (δ) in ppm and coupling constants in Hz, EI (*m/z*, rel. int. %), FABMS: MAT 311A and JEOL HX 110 with a data system. Optical rotations were measured on Schmidt + Haensch Polartronic D. EI and HR EIMS were measured on Varian mass spectrometers. Thin layer chromatography (TLC) was performed on pre-coated silica gel plates (DC-Alufolien 60 F_{254} of E. Merck) and spots were detected by using ceric sulfate and aniline phthalate reagent as spraying reagents. Vacuum liquid chromatography (petroleum ether:$CHCl_3$ and $CHCl_3$:MeOH) was performed by using silica gel (type 60, Merck).

3.2. Plant material

The stem bark of *P. hylodendron* Mildbr. was collected from the eastern part of Cameroon in August 1997. A voucher specimen (# 5582 SRFCAM) was deposited at the National Herbarium (Yaounde, Cameroon).

3.3. Extraction and isolation

Air-dried and pulverized stem bark of *P. hylodendron* (11 kg) was macerated at room temperature (25 °C) in methanol (30 l) for 24 h and extracted three times. The combined methanolic extracts were concentrated to dryness (2.5 kg). The concentrated brownish methanolic extract (1 kg) was dissolved in distilled water (1 l). It was first defatted with petroleum ether, followed by extraction with $CHCl_3$, EtOAc, and *n*-BuOH. Vacuum liquid chromatography (VLC) (petroleum ether:$CHCl_3$ and $CHCl_3$:MeOH) on silica gel, followed by column chromatography (CC) on silica gel. The column was eluted with $CHCl_3$–MeOH mixtures to afford **1** (10.3 mg) in $CHCl_3$:MeOH (8:2) solvent system. Further elution of the column with a gradient solvent system of CH_3Cl and MeOH (1–25% MeOH/CH_3Cl) yielded compounds **2** (7.8 mg) and **3** (9.7 mg). By elution of the column with $CHCl_3$:MeOH (8.5:1.5), compound **4** (11.1 mg) was obtained.

3.4. Bellericagenin B 3-O-β-D-glucopyranosyl (1 → 2)-O-α-D-glucopyranoside (*1*)

Colorless amorphous powder compound (10.3 mg). $[\alpha]^{25}$ +13.3 (CH_2Cl_2–CH_3OH; *c* = 0.01). IR ν_{max} ($CHCl_3$) cm^{-1}: 3435 (OH), 2944 and 2879 (CH), 1652 (C=C), 1647 (C=O), 1448 (CH_2), 1385–1379 (*gem*-dimethyl). UV λ_{max} (MeOH) (log ε) nm: 202 (3.7), 214 (3.4). ^1H (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) δ: see Table 1. EIMS (probe) 70 eV, *m/z* (rel. int. %): 520 (22) [M−2 × hexoses]$^+$, 502 (15), 458 (39), 442 (18), 411 (9), 408 (17), 248 (77), 255 (11), 264 (100), 222 (9), 201 (94), 131 (12), 119 (28), 27 (21). FABMS (+ve) : 845 $[M+H]^+$. HR FABMS *m/z*: 845.4457, $C_{42}H_{68}O_{17}$ (Calcd. 845.4534).

Table 1
^1H and ^{13}C NMR chemical shifts (δ) assignments of compound **1** in C_5D_5N

Carbon no.	^{13}C NMR	Multiplicity	^1H NMR (*J* = Hz)
1	47.8	CH_2	1.97, 1.59
2	69.7	CH	4.41 (*ddd*, *J* = 14.1, 9.5, 4.4)
3	81.4	CH	4.32 (*d*, *J* = 9.5)
4	47.9	C	–
5	48.6	CH	1.51 *m*
6	19.4	CH_2	1.55, 1.48 *m*
7	33.3	CH_2	2.10, 1.65 *m*
8	40.2	C	–
9	48.2	CH	1.87 *dd*
10	38.3	C	–
11	24.4	CH_2	2.03, 1.30
12	123.2	CH	5.52 (*t*, *J* = 3.2)
13	144.3	C	–
14	42.1	C	–
15	28.9	CH_2	1.78 *m*
16	27.9	CH_2	0.91 *m*
17	46.4	C	–
18	44.8	CH	3.43 (*br s*, $W_{1/2}$ = 4.6)
19	80.1	CH	3.52 (*d*, *J* = 4.4)
20	35.5	C	–
21	28.8	CH_2	1.82, 2.01 *m*
22	33.2	CH_2	0.88, 2.76 *m*
23	64.3	CH_2	4.02, 4.62 (AB *d*, *J* = 11.4)
24	62.8	CH_2	4.27, 4.81 (AB *d*, *J* = 11.4)
25	17.1	CH_3	1.12 *s*
26	17.4	CH_3	1.13 *s*
27	24.8	CH_3	1.54 *s*
28	180.2	C	–
29	30.3	CH_3	1.31 *s*
30	26.3	CH_3	1.10 *s*
α Glc-1′	104.5	CH	6.32 (*d*, *J* = 2.9)
2′	82.3	CH	4.06 *m*
3′	77.9	CH	4.16 *m*
4′	71.3	CH	4.11 *m*
5′	78.1	CH	3.77 *m*
6′	62.6	CH_2	4.28 (*dd*, *J* = 11.5, 5.0); 4.43 (*dd*, *J* = 11.5, 2.5)
β Glc-1″	102.8	CH	5.91 (*d*, *J* = 7.7)
2″	76.9	CH	4.06 *m*
3″	78.3	CH	4.16 *m*
4″	71.2	CH	4.24 *m*
5″	78.2	CH	3.87 *m*
6″	62.2	CH_2	4.37 (*dd*, *J* = 11.5, 4.0); 4.45 (*dd*, *J* = 11.5, 3.1)

3.5. Acid hydrolysis of *1*

Compound **1** (3 mg) was dissolved in MeOH (3 ml) distilled H_2O (3 ml). Then 5% HCl (3 ml) solution was added and the solution was refluxed for 7 h at 60 °C. After cooling, MeOH was evaporated in vacuo. The reaction mixture was extracted thrice with $CHCl_3$. The residue obtained after removal of acid was compared with standard sugar units on silica gel plates (E. Merck Art. No. 5554) using *n*-BuOH-EtOAc-*iso*-PrOH-HOAc-H_2O (7:20:12:7:6) solvent system and the sugar was found to be D-glucose (two units).

3.6. Hylodendroside-I (*2*)

A light brown gummy solid material (7.8 mg). $[\alpha]_D^{25}$ +1.21 (CH_2Cl_2–CH_3OH; *c* = 0.16). No UV absorption between 210 and 600 nm (in MeOH). IR ν_{max} ($CDCl_3$) cm^{-1}: 3434, 1644, 1625, 1545, 1030, 839. ^1H (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) δ: see Table 2. EIMS (probe) 70 eV, *m/z* (rel. int. %): 663 $[M^+-162]$ (8), 443 (5), 367 (44), 227 (21), 237 (13), 207 (18) 137 (54), 124 (51), 111 (46), 97 (25), 84 (22), 71 (14). FABMS: 826 $[M+H]^+$. HR FABMS *m/z*: 826.6693, $C_{48}H_{91}NO_9$ (Calcd. 826.6771).

Table 2
^{1}H and ^{13}C NMR data for compounds **2** and **3** in C_5D_5N

Position	**2**		**3**	
	δ_C	δ_H	δ_C	δ_H
1	70.4	4.51 (*dd*, *J* = 10.9, 5.8) 4.72 (*dd*, *J* = 10.9, 4.7)	70.2	4.53 (*dd*, *J* = 11.4, 6.1) 4.68 (*dd*, *J* = 11.4, 5.9)
2	52.8	5.25 (*m*)	51.8	5.25 (*m*)
3	75.9	4.27 (*m*)	76.2	4.27 (*m*)
4	35.4	2.32 (*t*, *J* = 8.0)	71.4	4.18 (*m*)
5	27.8	1.30 (*br s*)	35.7	2.21 (*t*, *J* = 7.8)
6	27.2	1.30 (*br s*)	28.0	2.11 (*m*)
7	28.3	1.30 (*br s*)	29.1	1.88 (*m*)
8	35.7	2.03 (*m*)	32.9	2.02 (*m*)
9	130.4	5.52 (*dt*, *J* = 16.3, 4.9)	131.7	5.50 (*dt*, *J* = 16.7, 5.0)
10	130.9	5.48 (*dt*, *J* = 16.3, 5.1)	130.2	5.48 (*d*, *J* = 16.7, 4.9)
11	33.2	2.81 (*m*)	33.2	2.81 (*m*)
12	131.4	5.55 (*dt*, *J* = 15.7, 4.8)	130.5	5.52 (*dt*, *J* = 17.4, 4.9)
13	131.8	5.61 (*m*, *J* = 15.7)	132.2	5.61 (*m*, *J* = 17.4)
14	33.7	2.07 (*m*)	34.2	2.01 (*m*)
15	28.9	1.23 (*br s*)	29.4	1.98 (*m*)
16	30.2	1.54	29.9	1.54
17	23.0	1.41	22.9	1.36
18	14.4	0.85 (*t*, *J* = 6.2)	14.3	0.85 (*t*, *J* 8 6.7)
N-H	–	8.56 (*d*, *J* = 8.5)	–	8.59 (*d*, *J* = 9.1)
1′	175.9	–	175.7	–
2′	71.3	4.19 (*dd*, *J* = 7.9, 4.1)	72.5	4.58 (*dd*, *J* = 7.7, 3.5)
3′	34.2	1.99	35.5	2.10
4′	26.3	1.79	25.9	1.78
5′-22′	29.5–30.0	1.23–1.30 (*br s*)	29.3–30.2	1.22–1.29 (*br s*)
23′	23.0	1.41 (*m*)	26.7	1.40 (*m*)
24′	14.4	0.85 (*t*, *J* = 6.2)	22.9	1.36 (*m*)
25′	–	–	14.3	0.85 (*t*, *J* = 6.7)
Glc-1″	105.5	4.96 (*d*, *J* = 7.9)	105.8	4.94 (*d*, *J* = 7.7)
2″	75.2	3.98 (*m*)	75.2	3.98 (*m*)
3″	78.2	3.86 (*m*)	78.8	3.83 (*m*)
4″	72.6	4.59 (*m*)	72.5	4.56 (*m*)
5″	78.4	4.19 (*m*)	78.7	4.19 (*m*)
6″	62.7	4.33 (*dd*, *J* = 12.2, 8.7)	62.8	4.35 (*dd*, *J* = 12.1, 9.3)
4.45 (*dd*, *J* = 12.2, 4.9)		4.48 (*dd*, *J* = 12.1, 5.6)		

3.7. Methanolysis of **2**

Compound **2** (3 mg) was treated with 5% HCl in MeOH (1–2 ml) and refluxed for 12 h. The reaction mixture was extracted with hexane. The concentrated hexane layer was subjected to silica gel column chromatography to yield (2*R*)-hydroxytetracosanoic methyl ester. The aqueous MeOH layer of the hydrolysate was evaporated under reduced pressure to remove the residual HCl. The resulting residue was partitioned between H_2O and EtOAc. The H_2O layer was concentrated and purified on a C-18 reversed-phase column to afford methyl glucopyranoside.

Methanolysis of **2** afforded (2*R*)-hydroxytetracosanoic methyl ester. $[\alpha]_D^{25}$ −1.7 (*c* = 0.03, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.19 (1H, *br s*), 3.66 (3H, *s*, OCH_3), 1.75 (*m*), 2.33 (*br s*, OH), 1.23 (*br s*, $CH_2 \times 15$), 0.86 (3H, *t*, *J* = 6.6 Hz). EIMS (probe) 70 eV, *m/z* (rel. int. %): 398 [M^+] (81), 384 [M^+-CH_2] (53), 339 [$M^+-COOCH_3$] (29). The data were identical to those published earlier (Natori et al., 1994; Liu et al., 1998).

3.8. Hylodendroside-II (**3**)

A light brown gummy solid (9.7 mg). $[\alpha]_D^{25}$ +4.2 (CH_2Cl_2–CH_3OH; *c* = 0.24). No UV absorption between 210 and 600 nm (in MeOH). IR ν_{max} ($CHCl_3$) cm^{-1}: 3310, 1660, 1643, 1547, 1030, 839. ^{1}H (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) δ: see Table 2. EIMS (probe) 70 eV, *m/z* (rel. int. %): 693 [M^+-162] (17), 677 (10), 459 (14), 381 (23), 253 (13), 193 (18) 137 (54), 124 (24), 111 (51), 97 (31), 84 (28), 71 (17). FABMS: 856 [M+H]$^+$. HR FABMS *m/z*: 856.6798, $C_{49}H_{93}NO_{10}$ (Calcd. 856.6877).

3.9. Methanolysis of **3**

Compound **3** (3 mg) was treated with 5% HCl in MeOH (1–2 ml) and refluxed for 12 h. The reaction mixture was extracted with hexane. The conc. hexane layer was passed through silica gel to yield (2*R*)-hydroxytetracosanoic methyl ester. The aqueous MeOH layer of the hydrolysate was evaporated under reduced pressure to remove the residual HCl. The resulting residue was partitioned between H_2O and EtOAc. The H_2O layer was concentrated and purified on a C-18 reverse-phase column to afford methyl glucopyranoside.

Methanolysis of **3** afforded (2*R*)-hydroxypentacosanoic methyl ester; $[\alpha]_D^{23}$ −2.4 (*c* = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.21 (1H, *br s*), 3.62 (3H, *s*, OCH_3), 1.76 (*m*), 2.33 (*br s*, OH), 1.23 (*br s*, $CH_2 \times 14$), 0.87 (3H, *t*, *J* = 6.6 Hz). EIMS (probe) 70 eV, *m/z* (rel. int. %): 412 [M^+] (77), 398 [M^+-CH_2] (36), 339 [$M^+-COOCH_3$] (55). The data was found to be identical to those published earlier (Natori et al., 1994).

3.10. 2α, 3β, 23-Triacetoxy-19α-hydroxyolean-12-en-28-oic acid (**4**)

A colorless amorphous solid compound (11.1 mg). $[\alpha]_D^{25}$ +3.7 ($CDCl_3$; *c* = 0.7), IR ν_{max} ($CHCl_3$) cm^{-1}: 3624 (OH), 2943, 2873 (CH), 1741 (ester carbonyl), 1697 (C=O), 1650 (C=C), 1458 (CH_2), 1371 (CH_3). UV λ_{max} (MeOH) (log ε) nm: 205 (3.3). EIMS (probe) 70 eV, *m/z* (rel. int. %): 630 (14) [M^+], 612 (17), 585 (10), 366 (14), 367 (23), 246 (13), 264 (18), 201 (54), 131 (24), 119 (51), 57 (31). FAB MS: 631 [M+H]$^+$. HR EIMS *m/z*: 630.3768, $C_{36}H_{54}O_9$ (Calcd. 630.3767). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.67, 0.85, 0.93, 0.94, 1.04, and 1.20 (3H, *s*, each), 1.99, 2.01, 2.07 (each 3H, *s*,

AcO × 3), 3.04 (*br s*, $W_{1/2}$ = 4.4 Hz, H-18), 3.31 (1H, *d*, *J* = 4.0 Hz, H-19), 3.7 and 3.5 (1H each, CH_2OAc, *dd*, *J* = 11.7 Hz), 5.12 (1H, *ddd*, $J_{2ax.1ax}$ = 14.7 Hz, $J_{2ax.3ax}$ = 10.6 Hz, $J_{2ax.1eq}$ = 4.4 Hz, H-2) and 5.07 (1H, *d*, $J_{2ax.3ax}$ = 10.6 Hz, H-3), 5.42 (1H, *br s*, H-12). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 184.1 (C-28), 170.8 (C-31), 170.4 (C-33), 170.3 (C-35), 142.7 (C-13), 124.4 (C-12), 81.4 (C-19), 74.7 (C-3), 69.8 (C-2), 65.1 (C-23), 47.8 (C-5), 47.1(C-9), 45.2 (C-17), 43.4 (C-18), 43.2 (C-1), 42.1 (C-4), 41.8 (C-14), 39.1 (C-10), 38.0 (C-8), 34.6 (C-20), 32.4 (C-7), 32.1 (C-22), 27.9 (C-21), 27.2 (C-15), 25.2 (C-29), 24.8 (C-27), 24.3 (C-30), 23.8 (C-16), 23.7 (C-11), 20.8 (C-34), 20.7 (C-32), 20.2 (C-36), 17.9 (C-6), 17.1 (C-25), 16.7 (C-26), 13.8 (C-24).

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