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PHYTOCHEMISTRY

Two saponins from *Pteleopsis hylodendron*

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Abstract

Two new saponins, 2α,3α,19α,23-tetrahydroxyolean-12-en-28-*O*-β-D-galactoside, and sitosterol α(1 → 3) diglucoside, were isolated from the stem barks of *Pteleopsis hylodendron* along with a known triterpenoid, 2α,3β,21β,23-tetrahydroxyolean-12-en-28-oic acid, which was isolated for the first time from this plant. The structures of these compounds were elucidated with the help of spectroscopic studies. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Pteleopsis hylodendron*; Combretaceae; Saponins

1. Introduction

Pteleopsis hylodendron Mildbr. (Combretaceae) is a big tree commonly found in the forest regions of West and Central Africa (Irvine, 1961; Liben, 1983). The genus is represented in Africa by ten species. Only *P. hylodendron* is found in Cameroon (Liben, 1983). The 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 as well as dropsy. We report here the first phytochemical work carried out on *P. hylodendron* which has resulted in the isolation and characterization of two new saponins, 2α,3α,19α,23-tetrahydroxyolean-12-en-28-*O*-β-D-galactoside (**1**) and sitosterol α(1 → 3) diglucoside (**2**), as well as a known triterpenoid, 2α,3β,21β,23-tetrahydroxyolean-12-en-28-oic acid (**3**), from the stem bark of the plant.

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2. Results and discussion

Air dried stem bark of *P. hylodendron* was extracted with methanol and the extract thus obtained after defatting with hexane was further fractionated with chloroform and ethyl acetate. The ethyl acetate fraction on further purification afforded compounds **1**–**3**.

Compound **1** (Fig. 1) was isolated as a white amorphous solid. The UV spectrum of **1** showed only end absorption at 203 nm, indicating the absence of any chromophore. The IR spectrum showed absorption bands at 3452 (OH), 1736 (ester carbonyl) and 1385–1379 (*gem*-dimethyl) cm^{-1}.

The HRFAB MS showed the $[M+1]^+$ ion at m/z 667.3986 corresponding to the molecular formula $C_{36}H_{58}O_{11}$ (calcd. 666.3979) with eight degrees of unsaturation in the molecule, five of which are accounted for by the aglycone cyclic rings, one by the double bond, one by the carbonyl group and one by the sugar moiety. The HREI MS exhibited a peak at m/z 486.3350 [M^+-H_2O-162 ($C_{30}H_{46}O_5$, calcd. 486.3345)], the difference from the $M^+ + 1$ ion in

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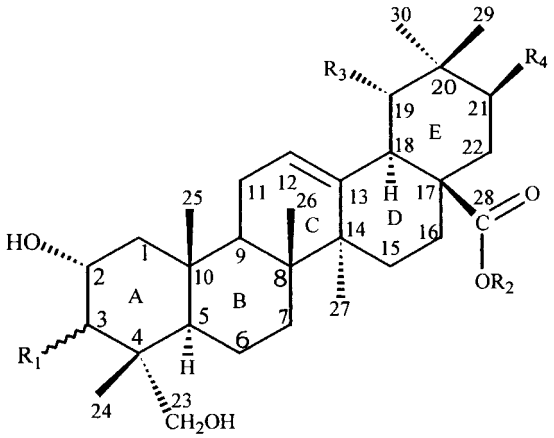

Fig. 1. Compound **1**: R_1: αOH, R_2: galactose, R_3: OH, R_4: H. Compound **3**: R_1: βOH, R_2: H, R_3: H, R_4: OH.

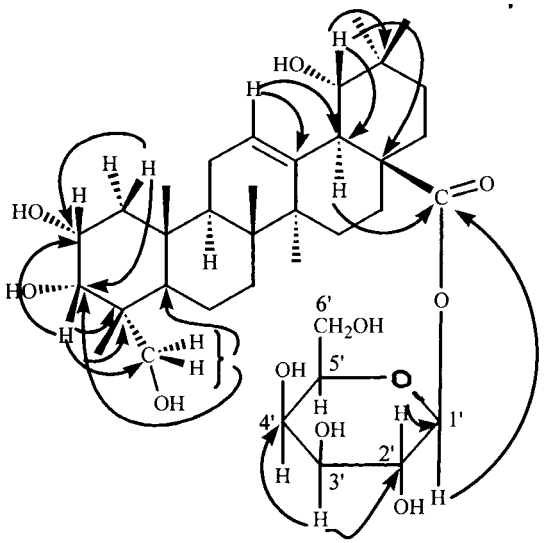

Fig. 2. Important HMBC interactions in Compound **1**.

HRFAB MS indicating the presence of a sugar moiety in the molecule. The other mass fragments were at *m/z* 240.1739 (A/B ring, $[C_{14}H_{24}O_3]^+$) and 222.1630 $[240.1739\text{-}H_2O]^+$. The base peak at *m/z* 264.1720 (D/E ring, $[C_{16}H_{24}O_3]^+$) may arise by retro Diels–Alder cleavage of ring C which indicated the presence of an Δ^{12}-β-amyrin skeleton with one hydroxy and carboxyl groups on rings D, E (Bombardelli, Bonati, Gabetta & Mustich, 1974).

The ^{1}H-NMR spectrum (CD_3OD, 300 MHz) of **1** showed six 3H-singlets for the six methyl groups at δ 1.30, 1.02, 0.94, 0.93, 0.75 and 0.67 which were attributed to H_3-27, H_3-25, H_3-30, H_3-29, H_3-26 and H_3-24, respectively. A set of AB doublets resonating at δ 3.25 (d, $J_{23\alpha,23\beta}=9.0$ Hz) and 3.48 (d, $J_{23\beta,23\alpha}=9.0$ Hz) was assigned to the geminally coupled H_2-23. A set of double doublets at δ 3.26 and 3.82 was due to the H_2-6′ of the sugar moiety. Three carbinol methine protons appeared at δ 3.66 (ddd, $J_{2\beta,1\alpha}=11.0$ Hz, $J_{2\beta,1\beta}=3.9$ Hz, $J_{2\beta,3\beta}=3.0$ Hz), 3.35 (d, $J_{3\beta,2\beta}=3.0$ Hz) and 3.26 (d, $J_{19\beta,18\alpha}=5.8$ Hz) which were assigned H-2, H-3 and H-19, respectively. The axial disposition (β-orientation) of H-2 was deduced from the *trans* diaxial coupling of 11.0 Hz observed between H-2β and H-1α. The other two coupling constants (3.0 and 3.9 Hz) fall in the range of equatorial–equatorial and equatorial–axial couplings, indicating H-3 to be in an equatorial disposition (β-orientation). The axial–equatorial coupling of 5.8 Hz observed between H-19 and H-18 indicated H-19 to be β-oriented, and thus confirmed the α-orientation for the hydroxyl group at C-19. The olefinic H-12 appeared as a multiplet at δ 5.33. The anomeric proton resonated as a doublet at δ 5.37 ($J_{1,2}=8.0$ Hz), the coupling constant indicating the presence of a β-D-conformation of the sugar moiety (Bombardelli et al., 1974).

The ^{13}C-NMR [(CD_3OD, 75 MHz), broad-band decoupled, DEPT] spectra of **1** showed resonances for all thirty six carbons revealing six methyl, ten methylene, twelve methine and eight quaternary carbons. The ^{13}C-NMR spectra also showed one carbonyl signal at δ 178.0, two sp^2 carbons at δ 124.0 and 144.0 for C-12 and C-13, and seven carbinol methine carbons at δ 69.7, 71.2, 74.0, 78.4, 78.5, 78.7 and 82.5 for C-2, C-2′, C-5′, C-3, C-3′, C-4′ and C-19, respectively. Two carbinol methylenes and an anomeric carbon appeared at δ 62.5, 66.8 and 95.8 for C-6′, C-23 and C-1′, respectively (Yang-Hua & Fu-Bao, 1991). The observed chemical shifts of the sugar protons were compared with those reported for the corresponding monosaccharides (Jansson, Kenne & Widmalm, 1989) on the basis of which it was concluded that **1** has triterpenoidal skeleton with one double bond and one sugar moiety.

In the COSY 45° spectrum, H-2 (δ 3.66) showed vicinal couplings with methine H-3 (δ 3.35) and methylene H_2-1 protons (δ 1.90 and 1.52). Strong interactions between H-18 (δ 3.05)/H-19 (δ 3.26), and H-12 (δ 5.33)/H-11 (δ 2.00) were also observed.

Direct one-bond ^{1}H/^{13}C connectivities of each protonated carbon were deduced with the help of HMQC data (Table 1). H-2 (δ 3.66), H-3 (δ 3.35) and H-19 (δ 3.26) were directly connected with C-2 (δ 69.7), C-3 (δ 78.4) and C-19 (δ 82.5), respectively. The two hydroxymethylenes H_2-6′ (δ 3.26, 3.82) and H_2-23 (δ 3.25, 3.48) exhibited interactions with C-6′ (δ 62.5) and C-23 (δ 66.8), respectively. The olefinic H-12 (δ 5.33) and anomeric H-1′ (δ 5.37) were connected with C-12 (δ 124.0) and C-1′ (δ 95.8), respectively.

The HMBC spectrum (Fig. 2) served to place various functionalities at appropriate places through quaternary carbons. H-1 exhibited long-range interactions with C-2 (δ 69.7) and C-3 (δ 78.4), whereas H-3 (δ 3.35) was coupled with C-2 (δ 69.7), C-4 (δ 44.1) and

Table 1
Chemical shift assignments for compounds **1**–**3**, (CD_3OD for **1** and **3,** DMSO for **2**)

Nos.	**1**		**2**		**3**	
	^{1}H δ (J = Hz)	^{13}C (multiplicity)	^{1}H δ (J = Hz)	^{13}C (multiplicity)	^{1}H δ (J = Hz)	^{13}C (multiplicity)
1	1.52, 1.90 (m)	47.8 (CH_2)	1.00, 1.80 (m)	36.8 (CH_2)	1.90 (m)	46.3 (CH_2)
2	3.66 (11.0, 3.9, 3.0, ddd)	69.7 (CH)	1.50, 1.55 (m)	23.8 (CH_2)	3.48 (4.5, 11.0, 13.5, ddd)	67.4 (CH)
3	3.35 (3.0, d)	78.4 (CH)	3.37 (m)	87.8 (CH)	3.10 (9.3, d)	75.7 (CH)
4	–	44.1 (C)	2.30, 2.64 (m)	38.1 (CH_2)	–	42.4 (C)
5	1.30 (m)	48.5 (CH)		140.0 (C)	1.25 (m)	46.2 (CH)
6	1.45 (m)	19.3 (CH_2)	5.32 (m)	121.2 (CH)	1.45 (m)	17.0 (CH_2)
7	1.65 (m)	33.3 (CH_2)	1.90, 1.80 (m)	31.4 (CH_2)	1.65 (m)	32.4 (CH_2)
8	–	40.9 (C)	1.40 (m)	31.3 (CH)	–	40.1 (C)
9	1.85 (m)	49.1 (CH)	0.89 (m)	49.6 (CH)	1.85 (m)	47.7 (CH)
10	–	39.2 (C)	–	39.0 (C)	–	38.6 (C)
11	2.00 (m)	24.9 (CH_2)	1.45, 1.40 (m)	20.6 (CH_2)	2.20 (m)	23.0 (CH_2)
12	5.33 (m)	124.0 (CH)	1.94, 1.93 (m)	39.3 (CH_2)	5.25 (t)	122.2 (CH)
13	–	144.0 (C)	–	41.0 (C)	–	143.3 (C)
14	–	42.8 (C)	0.98 (m)	56.1 (CH)	–	28.5 (C)
15	1.70 (m)	29.4 (CH_2)	1.15, 1.16 (m)	25.5 (CH_2)	1.70 (m)	27.0 (CH_2)
16	0.89 (m)	38.5 (CH_2)	1.23, 1.24 (m)	27.7 (CH_2)	1.53 (m)	34.8 (CH_2)
17	–	47.2 (C)	1.08 (m)	55.4 (CH)	–	44.8 (C)
18	3.05	45.1 (CH)	0.64 (s)	11.6 (CH_3)	2.90 (m)	43.4 (CH)
19	3.26 (5.8, d)	82.5 (CH)	0.94 (s)	18.9 (CH_3)	2.20 (m)	27.7 (CH_2)
20	–	35.9 (C)	1.34 (m)	35.4 (CH)	–	34.9 (C)
21	1.75 (m)	29.6 (CH_2)	0.89 (6.5, d)	18.6 (CH_3)	3.09 (4.4, 4.4, dd)	80.1 (CH)
22	2.35 (m)	35.0 (CH_2)	1.30, 1.29 (m)	33.4 (CH_2)	2.15 (m)	32.1 (CH_2)
23	3.25, 3.48 (9.0, 9.0, dd)	66.8 (CH_2)	1.82, 1.81 (m)	45.1 (CH_2)	3.01,3.30 (10.0, 10.0, dd)	64.1 (CH_3)
24	0.67(s)	13.7 (CH_3)	0.91 (m)	29.2 (CH)	0.91 (s)	13.0 (CH_3)
25	1.02 (s)	17.4 (CH_3)	1.20 (m)	28.7 (CH)	0.86 (s)	16.3 (CH_3)
26	0.75 (s)	17.8 (CH_3)	0.81 (6.5, d)	19.1 (CH_3)	0.56 (s)	16.4 (CH_3)
27	1.30 (s)	25.0 (CH_3)	0.75 (6.5, d)	19.8 (CH_3)	1.24 (s)	24.7 (CH_3)
28	–	178.0 (C)	1.25, 1.26 (m)	22.1 (CH_2)	–	179.2 (C)
29	0.93 (s)	28.6 (CH_3)	0.79 (t)	11.7 (CH_3)	0.83 (s)	28.6 (CH_3)
30	0.94 (s)	25.2 (CH_3)			0.67 (s)	24.3 (CH_3)
Gal 1′	5.37 (8.0, d)	95.8 (CH)				
Gal 2′	3.32 (m)	71.2 (CH)				
Gal 3′	3.39 (m)	78.5 (CH)				
Gal 4′	3.28 (m)	74.0 (CH)				
Gal 5′	3.30 (m)	78.7 (CH)				
Gal 6′	3.26 (dd, 9.8) 3.82 (dd, 10.1)	62.5 (CH_2)				
βGlc 1′			4.30 (8.0, d)	103.9 (CH)		
βGlc 2′			3.05 (m)	73.7 (CH)		
βGlc 3′			3.16 (m)	76.9 (CH)		
βGlc 4′			3.27 (m)	68.3 (CH)		
βGlc 5′			3.17 (m)	77.1 (CH)		
βGlc 6′			3.70, 3.38 (m)	61.1 (CH_2)		
αGlc 1″			4.33 (4.0, d)	100.2 (CH)		
αGlc 2″			3.10 (m)	72.1 (CH)		
αGlc 3″			3.15 (m)	76.0 (CH)		
αGlc 4″			3.08 (m)	70.1 (CH)		
αGlc 5″			3.16 (m)	76.7 (CH)		
αGlc 6″			3.65, 3.42 (m)	60.8 (CH_2)		

C-23 (δ 66.8). H-23 (δ 3.25, 3.48) showed heteronuclear shift interactions with C-3 (δ 78.4), C-4 (δ 44.1) and C-5 (δ 48.5). This established the location of two secondary hydroxyls at C-2 and C-3 of ring A and rendered C-4 to be the most probable site for the hydroxymethylene. Similarly H-12 (δ 5.33) afforded strong long-range interactions with quaternary C-13 (δ 144.0) and C-18 (δ 45.1). H-19 (δ 3.26) showed interactions with C-17 (δ 47.2), C-18 (δ 45.1) and C-20 (δ 35.9), thus confirming the site of third secondary hydroxyl in ring E. The presence of a sugar residue at C-28 was evident from the HMBC spectrum where the anomeric H-1′ (δ 5.37) was found to have connectivity with the C-28 (δ 178.0) carbonyl.

Acid hydrolysis of compound **1** afforded a sugar which was identified as D-galactose by comparison

with an authentic sample. The chemical shift assignments were made on the basis of the information obtained from ^{1}H-NMR, ^{13}C-NMR, COSY-45°, HOHAHA, HMQC, HMBC and comparison of the observed data with those reported for the corresponding monosaccharides (Mahato, Nanday & Kunda, 1992). From the above spectral observations, the compound was deduced to be a triterpenoidal saponin with the structure 2α,3α,19α,23-tetrahydroxyolean-12-ene-28-*O*-β-D-galactoside (**1**).

Compound **2** was isolated as a colorless amorphous solid. The IR spectrum afforded intense absorptions at 3398 (OH), 1472 (C=C) and 1085 (C–O–C) cm^{-1}. The HRFAB MS showed the $[M+1]^{+}$ ion at *m/z* 739.4922 corresponding to the molecular formula $C_{41}H_{70}O_{11}$ (calcd. 738.4918) with seven degrees of unsaturation. Four degrees of unsaturation were fulfilled by the steroidal nucleus, one by the double bond, and two by the sugars. HREI MS exhibited the base peak at *m/z* 396.3743 corresponding to the formula $C_{29}H_{48}$ (calcd. 396.3755). The ion *m/z* 396.3743 in EIMS results from the loss of [M^{+}-341.7314], indicating the presence of two sugar moieties in the molecule.

The ^{1}H-NMR spectrum (CD_3OD, 400 MHz) of **2** contained two 3H-singlets at δ 0.64 and 0.94 for H_3-18 and H_3-19, respectively, three 3H-doublets at δ 0.89 ($J_{21,20}=6.5$ Hz), 0.81 ($J_{26,25}=6.5$ Hz) respectively and 0.75 ($J_{27,25}=6.5$ Hz) for the secondary H_3-21, H_3-26 and H_3-27, respectively, and a 3H-triplet at δ 0.79 ($J_{29,28}=7.0$ Hz) for the primary H_3-29, respectively. The olefinic H-6 appeared at δ 5.32 as a multiplet. Eight downfield signals for the protons geminal to the secondary hydroxyl groups were observed between δ 3.27–3.05. Furthermore four protons of the two hydroxymethylene appeared at δ 3.38/3.70, and 3.42/3.65. The two anomeric H-1′ and H-1″ appeared at δ 4.30 and 4.33 (d, $J_{1',2'}=8.0$ Hz) respectively. The axial–axial coupling constant of 8.0 Hz between C-1′ and C-2′ protons indicated the β-D-conformation of the sugar moiety.

The COSY-45° and HOHAHA experiments revealed two discrete spin systems corresponding to the two sugar units. In glycoside **I**, a spin comprising of seven different types of protons resonating at δ 4.30 (H-1′), 3.05 (H-2′), 3.16 (H-3′), 3.27 (H-4′), 3.17 (H-5′) and 3.38/3.70 (H_2-6′) was unraveled. Another spin system of seven different types of protons resonating at δ 4.33 (H-1″), 3.10 (H-2″), 3.15 (H-3″), 3.08 (H-4″), 3.16 (H-5″) and 3.42/3.65 (H_2-6″) was also traced out which corresponded to the glycoside **II**.

The ^{13}C-NMR (Broad-band decoupled and DEPT) spectra (Iribarren & Pomilio, 1984) of **2** revealed the presence of six methyl, thirteen methylene, nineteen methine and three quaternary carbons. Signals for the thirteen aliphatic oxygen-bearing carbons appeared between δ 60.8 and 103.9. The two anomeric carbons appearing at δ 103.9 and 100.2 were assigned to C-1′ and C-1″ indicating a β-D-conformation for glucose **I** unit and α-D-conformation for glucose **II** unit. This conclusion was made taking into account that the anomeric carbons of the methyl α-D-glucopyranoside appear at δ 100.0 and of methyl β-D-glucopyranoside at δ 103.9 (Pfeffer, Valentine & Parrish, 1979).

The aglycone (sitosterol) of **2** was previously very well established (Rubinstein, Goad, Clague & Mulheim, 1976). The linkage between the two glucose units and their linkage with the aglycone were established with the help of enzymatic hydrolysis. In the HMBC spectrum the anomeric proton (δ 4.33) of glycoside **II** showed interaction with the C-3′ methine carbon resonating at δ 76.9, whereas the other anomeric proton at δ 4.30 of glycoside **I** showed interaction with the methine C-3 at δ 87.8.

Compound **3** (Fig. 1), $C_{30}H_{48}O_6$ (*m/z* 504.3451), was also isolated as a colorless amorphous solid from the same extract. The spectral data (UV, IR, MS, ^{1}H- and ^{13}C-NMR) confirmed the identity of **3** as 2α,3β,21β,23-tetrahydroxyolean-12-ene-28-oic acid previously isolated from *Caccinia glauca* (Tewari, Ayengar & Rangaswami, 1970).

3. Experimental

Vacuum liquid chromatography (hexane:chloroform and chloroform:methanol) was performed using silica gel (type 60, Merck). Thin layer chromatography was carried out using Merck precoated silica gel sheets (60 F_{254}). Column chromatography was performed using silica gel (230–400 mesh). Ceric sulphate spray reagent and UV light were used for the detection of compounds. UV spectra were recorded on a Hitachi UV 3200 spectrophotometer. IR spectra were recorded on a JASCO 302-A spectrophotometer. A JASCO DIP-360 digital polarimeter was used to determine optical rotation values. Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. FAB MS measurements were carried out on a JEOL-HX 110 mass spectrometer. EI MS were recorded on a Varian MAT 311A mass spectrometer. The ^{1}H-NMR spectra were recorded on Bruker AM 400 and 300 MHz spectrometers, respectively, while ^{13}C-NMR spectra was recorded at 100 and 75 MHz on the same instruments.

3.1. Plant material

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

4. Extraction and isolation

The air dried and pulverized stem bark of *P. hylodendron* Mildbr. (11 kg), was macerated at room temperature (25°C) in methanol (30 L) for 24 hours and extracted three times. The combined methanolic extracts were concentrated to dryness (2.5 kg). The concentrated brownish methanolic extract (1.0 kg) was dissolved in distilled water (1 L). It was first defatted with petroleum ether, followed by extraction with chloroform, ethyl acetate and *n*-butanol. These extracts were evaluated for antibacterial and antifungal activities. The ethyl acetate extract (51.5 g) was found to be active against the bacteria *Bacillus cereus*, *Corynebacterium diptheriae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Streptococcus pyogenes*, so it was subjected to vacuum liquid chromatography (VLC) (hexane: chloroform and chloroform: methanol) on silica gel (900 g, 60 PF_{254} Merck) followed by column chromatography (CC) on silica gel (70–230 mesh size). The column was eluted with a chloroform–methanol mixture with increasing amounts of methanol to afford compound **1** (49.8 mg. 4.5×10^{-4}% yield) on elution with $CHCl_3$: MeOH (90:10), compound **2** (30.1 mg, 2.7×10^{-4}% yield) on elution with $CHCl_3$: MeOH (85:15) and compound **3** (79.6 mg, 7.3×10^{-4}% yield) on elution with $CHCl_3$: MeOH (92:8).

2α,3α,19α,23-Tetrahydroxyolean-12-ene-28-O-β-D-galactoside (**1**): $C_{36}H_{58}O_{11}$; white solid, UV (MeOH) (log ϵ): 203.2 (3.6456) nm; $[\alpha]_D^{25} + 30$ (MeOH, c. 0.02); IR ν_{max}^{KBr} cm^{-1}: 3452 (OH), 1736 (C=O) and 1385–1379 (*gem*-dimethyl). HRFAB MS: 667.3986 [M + 1] (calcd. 667.3979). HREI MS: 486.3350 (calcd. 486.3345) [M-$C_6H_{12}O_6$], 264.1720 [$C_{16}H_{24}O_3$], 240.1739 [$C_{14}H_{24}O_3$], 222.1630 [$C_{14}H_{24}O_3$-H_2O] and 220.1822 [$C_{16}H_{24}O_3$-CO_2]. ^{1}H-NMR (300 MHz, CD_3OD): δ Table 1. ^{13}C-NMR (75 MHz, CD_3OD): δ Table 1.

4.1. Acid hydrolysis of *1*

Compound **1** (5 mg) was dissolved in MeOH (5 ml) - distilled H_2O (5 ml). Then 5% HCl (5 ml) solution was added in it and the solutions refluxed for 7 h at 60°C. After cooling, MeOH was evaporated in vacuo. The reaction mixture was extracted thrice with chloroform. The residue obtained after removal of acid was compared with standard sugars on silica gel plates (E. Merck Art. No. 5554) using *n*-BuOH–EtOAc–*iso*–PrOH–HOAc–H_2O (7:20:12:7:6). The TLC was run thrice in the same direction. Spots were visualized with aniline phthalate reagent. The sugar was found to be D-galactose.

4.1.1. β-Sitosteroldiglucoside (*2*)

$C_{41}H_{70}O_{11}$; white solid, IR ν_{max}^{KBr} cm^{-1}: 3398 (OH), 1472 (C=C) and 1085 (C–O–C). HRFAB MS: 739.4922 [M + 1] (calcd. 738.4918). HREI MS: 396.3743 [$C_{29}H_{48}$-H_2O] (calcd. 396.3755), 381.3508 [$C_{29}H_{48}$-H_2O–CH_3], 273.2210 [$C_{29}H_{50}O$-side chain] and 145.0509 [$(MeCO)_3O$]. ^{1}H-NMR (400 MHz, DMSO): δ Table 1. ^{13}C-NMR (100 MHz, DMSO): δ Table 1.

4.2. Enzymatic hydrolysis of *2* with α-glucosidase

Saponin **2** (0.2 mM) mixed with sodium phosphate (50 mM) and sodium chloride (100 mM) (pH 6.8) and was incubated with α-glucosidase (from Brewers yeast) (0.032 U/ml, Sigma 6136) for 1 h at 37°C and the product was extracted with methanol. The hydrolysed product was found to β-sitoglucoside when compared with the standard on TLC (MeOH–$CHCl_3$ 1:9). This indicated that the second sugar unit has an α-glucosidic linkage.

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