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NEW STEROIDAL GLYCOSIDES FROM THE STEM BARK OF *Mimusops elengi*

N. Akhtar,[1] M. Ali,[2*] and M. S. Alam[1]

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*Two new steroidal glycosides (**1** and **2**) have been isolated from the ethanolic extract of the stem bark of* Mimusops elengi *L. and characterized as stigmasta-5,22-dien-3*β*-ol-3*β*-D-glucuropyranosyl-(6′*β*→1″)-D-glucopyranoside (**1**) and* β*-sitosterol-3*β*-(3‴,6‴,7‴-trihydroxynaphthyl-2‴-carboxyl)-4″-glucopyranosyl(1″→4′)-glucopyranoside (**2**) along with the known compounds stigmasta-5-en-3*β*-ol, lup-20(29)-en-3*β*-ol, and stigmasta-5-en-3*β*-D-glucopyranoside. Their structures have been elucidated on the basis of spectral data analysis and chemical reactions.*

Keywords: *Mimusops elengi* L., Sapotaceae, stem bark, steroidal glycosides.

Mimusops elengi L. (Sapotaceae) is an ornamental small tree with sweet scented flowers growing wild throughout the greater part of India [1]. All parts of the plant are prescribed in the indigenous system of medicine as a febrifuge, astringent, purgative, and stimulant [2]. The decoction of the bark is used as a gargle [3]. It exhibited antimicrobial, antiulcer, hypotensive, anti-HIV, and spasmolytic activities [4–8]. Ursolic acid, betulinic acid, the fatty acid ester of α-spinasterol [9], 3β,6β,19α,23-tetrahydroxyurs-12-ene, 1β-hydroxy-3β-hexanoyllup-20(29)-ene-23,28-dioic acid, and bassic acid have been isolated from the bark [10, 11]. This paper deals with the isolation and structural elucidation of two new steroidal glycosides from the stem bark of *Mimusops elengi* L.

Compound **1**, designated as stigmasterol-β-D-glycopyranoside, was obtained as a colorless amorphous powder from chloroform–methanol (19:1) eluants. It gave a positive test for sterol glycosides. Its IR spectrum exhibited absorption bands for hydroxyl groups (3450, 3380, 3260 cm^{-1}), ester group (1738 cm^{-1}), and unsaturation (1614 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular weight was established at *m/z* 750, consistent with the molecular formula of a sterol diglucoside, $C_{41}H_{66}O_{12}$, and confirmed by HR-MS 751.9713 $[M + H]^+$. Its mass spectrum showed important ion fragments appearing at *m/z* 411 [$M - C_{12}H_{19}O_{11}$, diglycoside]$^+$, 271 [$411 - C_{10}H_{19}$, side chain]$^+$, 255 [$271 - Me]^+$, and 239 [$255 - Me]^+$. These fragments suggested that it was a C_{29} sterol possessing one double bond in the carbocyclic ring system and one double bond in the side chain. The mass spectrum indicated the presence of an ethyl group in the side chain, which was placed at C-24 on the basis of biosynthesis analogy as well as similarities in chemical shifts of protons and carbons of the side chain with the related compounds. The ion fragments at *m/z* 339 $[M - 411]^+$ and 179 $[C_6H_{11}O_6]^+$ suggested the glucuronosyl glucoside nature of the carbohydrate moiety. The ^1H NMR spectrum of **1** exhibited two one-proton multiplets at δ 5.07 and 5.28 assigned to vinylic H-22 and H-23. A one-proton broad signal at δ 5.30 was ascribed to vinylic H-6. A one-proton broad multiplet at δ 3.85 with half-width 16.5 Hz was attributed to α-oriented C-3 carbinol proton. Two one-proton doublets at δ 4.95 (J = 7.2 Hz) and 4.88 (J = 7.0 Hz) were attributed to H-1′ and H-1″ anomeric protons, respectively. A two-proton broad signal at δ 3.33 was associated with oxygenated C-6″ methylene protons. The remaining hydroxymethine protons of the glycosidic linkage appeared between δ 4.50–3.35. Two three-proton broad signals at δ 0.66 and 1.19 were assigned to C-18 and C-19 tertiary methyl protons. Four doublets at δ 0.96 (J = 6.1 Hz), 0.86 (J = 8.4 Hz), 0.81 (J = 6.3 Hz), and 0.83 (J = 6.3 Hz), all integrating for three protons each, were due to C-21, C-26, and C-27 secondary and C-29 primary methyl protons, respectively. The presence of all the methyl signals in the range δ 1.19–0.66 suggested the location of these functionalities on the saturated carbons.

1) Department of Chemistry, Faculty of Science; 2) Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University) New Delhi-110 062, India, e-mail: mali_hamdard@yahoo.co.in. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 465–468, July–August, 2010. Original article submitted March 3, 2009.

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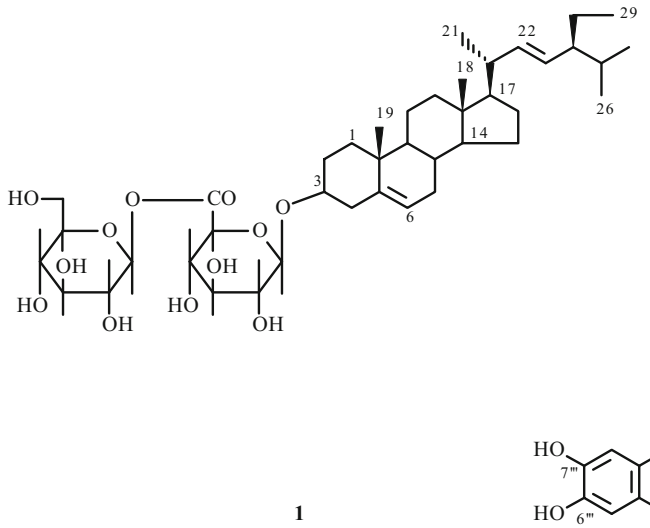
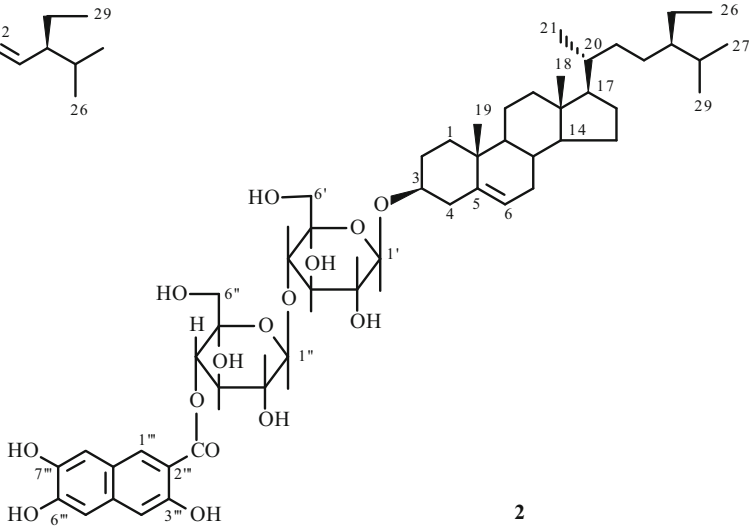
TABLE 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for Compounds **1** and **2**

C atom	δ_H (J/Hz)		δ_C	
	1	**2**	**1**	**2**
1	1.34 m, 2.94 m	1.34 m, 2.26 m	37.43	38.95
2	1.86 m, 1.82 m	1.79 m, 1.73 m	31.75	27.68
3	3.85 br.m ($w_{1/2}$ = 16.5)	3.09 br.m ($w_{1/2}$ = 16.5)	74.94	73.36
4	2.24 m, 1.99 m	2.87 m, 2.22 br.s	43.56	40.03
5	–	–	138.58	141.23
6	5.30 br.s	5.17 m	119.42	121.04
7	2.17 m, 1.99 m	1.67 m, 2.41 m	32.17	31.28
8	1.24 m	1.16 m	33.86	33.58
9	1.54 m	1.51 m	53.82	49.49
10	–	–	35.28	36.72
11	2.17 m, 1.52 m	2.02 m, 1.49 m	22.81	39.76
12	1.07 m, 1.82 m	1.01 m, 1.89 m	38.53	38.28
13	–	–	39.41	41.50
14	1.15 m	1.15 m	54.58	56.05
15	1.22 br.s, 1.55 br.s	1.03 m, 1.63 m	24.98	25.42
16	1.63 m, 1.50 m	1.67 m, 1.34 m	29.71	28.99
17	1.39 m	1.39 m	59.17	55.33
18	0.66 br.s	0.60 br.s	12.17	11.43
19	1.19 br.s	1.11 br.s	20.46	19.56
20	2.53 m	2.02 m	34.52	36.09
21	0.96 d (J =6.1)	0.81d (J = 6.5)	18.93	18.44
22	5.07 m	1.63 m, 1.06 m	125.96	33.25
23	5.28 m	1.29 m, 1.79 m	127.79	35.42
24	2.27 m, 1.99 m	1.29 m	48.01	45.08
25	1.54 m	1.63 m	25.41	29.06
26	0.86 d (J = 8.4)	0.77 d (J = 6.0)	20.93	18.93
27	0.81 d (J = 6.3)	0.71 d (J = 6.3)	22.17	20.47
28	1.26 m, 1.63 m	1.15 m, 1.67 m	23.85	23.73
29	0.83 d (J = 6.3)	0.73 d (J = 5.7)	9.89	11.48
1′	4.95 d (J = 7.2)	4.12 d (J = 7.5)	110.04	107.17
2′	4.08 m	3.38 m	87.13	78.32
3′	3.35m	3.41 m	68.84	70.00
4′	4.06 m	3.85 m	71.62	76.68
5′	4.50 m	3.94 br.s	85.45	79.76
6′	–	3.07 d (J = 8.22), 3.04 d (J = 8.22)	170.85	61.08
1″	4.88 d (J = 7.0)	4.14 d (J = 7.5)	102.48	100.79
2″	4.08 m	3.38 m	76.51	77.02
3″	3.35 m	3.41 m	68.84	73.00
4″	4.06 m	3.38 m	71.69	69.98
5″	4.26 m	3.94 br.s	80.43	78.76
6″	3.33 br.s, 3.33 br.s	3.07 d (J = 8.22), 3.04 d (J = 8.22)	61.98	60.72
1‴	–	7.32 br.s	–	112.21
2‴	–	–	–	140.22
3‴	–	–	–	158.44
4‴	–	7.32 br.s	–	111.35
5‴	–	7.38 br.s	–	110.35
6‴	–	–	–	151.97
7‴	–	–	–	148.10
8‴	–	7.38 br.s	–	110.35
9‴	–	–	–	139.58
10‴	–	–	–	135.94
11‴	–	–	–	173.16

Coupling constants in Hz are given in parentheses.



1



2

The ^{13}C NMR spectrum of **1** showed important signals for the ester carbon at δ 170.85 (C-6′), the vinylic carbon at δ 138.58 (C-5), 119.42 (C-6), 125.96 (C-22), and 127.79 (C-23), the carbinol carbon at δ 74.94 (C-3), anomeric carbons at 110.04 (C-1′) and 102.48 (C-1″), sugar carbons between δ 85.54–61.98, and the remaining methyl, methylene, and methine carbons between δ 59.17–9.89. The existence of the ester signal at δ 170.85 suggested that the anomeric proton of the glucose moiety was esterified with the carboxylic group of the glucuronic acid forming the (1″*β*→6′) linkage. The ^{1}H and ^{13}C NMR values of the steroidal nucleus of **1** were compared with the related stigmasterols [12, 13]. The HMBC spectrum of **1** showed correlations of C-3 with H_2-2, H_2-4, and H-1′; C-6 with H_2-7; and C-22 with H-20, H_3-21, and H-23. Acid hydrolysis of **1** yielded stigmasterol, D-glucose, and D-glucuronic acid (TLC comparable). On the basis of these findings, the structure of **1** has been established as stigmasta-5,22-dien-3*β*-ol-3*β*-D-glucuropyranosyl-(6′*β*→1″)-D-glucopyranoside. This is a new steroidal glycoside isolated for the first time from a natural source.

Compound **2**, named *β*-sitosterol diglucosyl naphthoate, was obtained as a colorless amorphous powder from chloroform–methanol (93:7) eluats. It responded positively to the tests for sterol glycosides. Its IR spectrum exhibited absorption bands for hydroxyl groups (3540, 3400, 3365 cm^{-1}), ester group (1741cm^{-1}), and unsaturation (1610 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular weight was established at 940, consistent with the molecular formula $C_{52}H_{76}O_{15}$ for a carboxynaphthalene substituted diglucoside of *β*-sitosterol, and confirmed by HR-MS at *m/z* 941.1714. Its mass spectrum showed ion fragments generated at *m/z* 413 [M – glycoside]$^+$, 527 [$C_{23}H_{27}O_{14}$; Glu → Glu → $C_{11}H_7O_4$]$^+$, 575 [*β*-sitosterol → glucoside]$^+$, and 365 [$C_{17}H_{17}O_9$; Glu → $C_{11}H_7O_4$]$^+$, indicating that a diglucoside linked with a trihydroxynaphthalenic carboxylate was attached to *β*-sitosterol. The ^{1}H NMR spectrum of **2** showed two broad signals at δ 7.32 and 7.38, both integrating for two protons each, assigned to H-1‴ and H-4‴ and H-5‴ and H-8‴ aromatic protons, respectively. A one-proton multiplet at δ 5.17 was ascribed to vinylic proton H-6. Two one- proton doublets at δ 4.12 (J = 7.5 Hz) and 4.14 (J = 7.5 Hz) were attributed to H-1′ and H-1″ anomeric protons, respectively. A one-proton broad multiplet at δ 3.09 with half-width 16.5 Hz was attributed to the *α*-oriented H-3 carbinol proton. The hydroxymethine proton of the sugar moiety appeared between δ 3.94–3.38. Two doublets at δ 3.07 (J = 8.22 Hz) and 3.04 (J = 8.22 Hz), both integrating for two protons each, were associated with the H_2-6′ and H_2-6″ hydroxymethylene proton. Two three-proton broad signals at δ 0.60 and 1.11 were assigned to C-18 and C-19 tertiary methyl protons of the sterol moiety. Four doublets at δ 0.81 (J = 6.5 Hz), 0.77 (J = 6.0 Hz), 0.71 (J = 6.3 Hz), and 0.73 (J = 5.71 Hz), all integrating for three protons each, were attributed to C-21, C-26, C-27 secondary, and C-29 primary methyl protons, respectively. The remaining methylene and methine protons resonated as multiplets between δ 2.41–1.06. The existence of all the methyl protons in the range δ 1.11–0.50 indicated that all these functionalities were attached on the saturated carbons. The ^{13}C NMR spectrum of **2** exhibited signals for the ester carbon in the deshielded range at δ 173.16, aromatic carbons between δ 158.44–110.35, vinylic carbons at δ 141.23 (C-5) and 121.04 (C-6), and the remaining carbons of the *β*-sitosterol framework from δ 56.05 to 11.48. The ^{1}H and ^{13}C NMR values of the steroidal nucleus were compared with *β*-sitosterol, stigmast-4-en-6*β*-ol-3-one [12, 13], and lawsaritol [14]. The HMBC spectrum of **2** exhibited correlation of C-3 with H_2-2, H_2-4 and H-1′; C-4′ with H-6′ and H-1″; and C-11‴ with H-4″ and H-1‴. Acid hydrolysis of **2**

yielded β-sitosterol and D-glucose (TLC comparable). On the basis of the above-mentioned discussion, the structure of **2** has been elucidated as β-sitosterol-3β-(3‴,6‴,7‴-trihydroxynaphthyl-2‴-carboxyl)-4″-glucopyranosyl(1″→4′)-glucopyranoside. This is a new steroidal glycoside isolated from a natural or synthetic source for the first time. The known compounds have been identified as stigmast-5en-3β-ol (β-sitosterol) [15, 16], lup-20(29)-en-3β-ol(lupeol) [16, 17], and stigmast-5en-3β-ol-β-D-glucopyranoside (β-sitosterol glucoside) [16, 18].

EXPERIMENTAL

General Methods. Melting points were determined on a Perfit melting point apparatus (Ambala, India) and are uncorrected. IR spectra were recorded on KBr discs, using a Bio-Rad FT-IR 5000 spectrometer (FTS 135, Hongkong). UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin–Elmer, Switzerland) in methanol. ^{1}H and ^{13}C NMR spectra were scanned using Bruker Advance DRY 400 spectrospin and Bruker Advance DRY 100 spectrospin instruments (Germany), respectively, in $CDCl_3$ and TMS as an internal standard. FAB-MS spectra were obtained using a JEOL-JMS-DX 303 spectrometer (USA). Column chromatography was performed on silica gel (Qualigens, Mumbai, India) 60–120 mesh. TLC was run on silica gel G (Qualigens, Mumbai, India). Spots were visualized by exposure to iodine vapor, UV radiation, and by spraying reagents.

Plant Material. The barks of *Mimusops elengi* were purchased from the Khari Baoli local market of Delhi and authenticated by Dr. H. B. Singh, taxonomist, NISCAIR, CSIR, New Delhi. A voucher specimen, No. PRL/JH/03/05, was deposited in the Herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction and Isolation. The bark of *Mimusops elengi* was dried in an oven at 45°C for 2–3 days and coarsely powdered. The ground bark (3 kg) was extracted with ethanol in a Soxhlet apparatus. The ethanol extract was concentrated under reduced pressure to yield a dark brown viscous mass (120 g, 4.0%). The extract was dissolved in a minimum amount of methanol and adsorbed on silica gel (60–120 mesh) for preparation of slurry. The air-dried slurry was chromatographed over the silica gel column packed in petroleum ether (60–80°C). The column was eluted with petroleum ether, petroleum ether–chloroform (9:1, 3:1, 1:1, 1:3 v/v), chloroform, chloroform–methanol (99:1, 98:2, 95:5, 9:1, 3:1, 1:1, 1:3 v/v), and methanol, successively, in order of increasing polarity to isolate the following compounds.

Compound 1. Elution of the column with chloroform–methanol (99:1) yielded a colorless amorphous powder of **1**, recrystallized from methanol; 410 mg (0.013%); R_f 0.68 (toluene–ethyl formate–formic acid 10:10:3); mp 255–256°C. UV (MeOH, λ_{max}, nm): 247 (log ε 5.7). IR (KBr, ν_{max}, cm^{-1}): 3450, 3380, 3260, 2920, 2846, 1738, 1614, 1578, 1465, 1354, 1168, 1106, 989. ^{1}H NMR, see Table 1. ^{13}C NMR, see Table 1. +ve FAB-MS *m/z* (rel. int.): 750 $[M]^+$ ($C_{41}H_{66}O_{12}$) (1.1), 411 (15.3), 397 (55.3), 395 (35.0), 379 (10.1), 353 (8.2), 339 (7.9), 271 (11.2), 255 (18.6), 239 (19.2), 215 (20.6), 189 (27.1), 179 (30.8), 161 (46.2), 145 (49.8), 121 (69.1), 95 (100). HR-MS: 751.9713 $[M + H]^+$ (calcd for $C_{41}H_{67}O_{12}$ 751.9702).

Acid Hydrolysis of 1. Compound **1** (20 mg) was dissolved in MeOH and 2N HCl (1:1) and the solution heated up to half volume left. The solution was extracted with EtOAc (3 × 10 mL), washed with water (2 × 10 mL), dried over Na_2SO_4, and evaporated to obtain the stigmasterol, mp 168–169°C, co-TLC comparable. The aqueous phase was concentrated and subjected to paper chromatography using *n*-butanol–ethanol–water (4:1:2.2) as the developing solvent system for standard samples of monosaccharide. The paper was sprayed with aniline hydrogen phthalate and the sugars were identified as D-glucose and D-glucuronic acid.

Compound 2. Elution of the column with chloroform–methanol (93:7) produced a pale yellow amorphous powder of **2**, recrystallized from methanol; 100 mg (0.003%); R_f 0.50 (toluene–ethyl acetate–formic acid 5:4:1); mp 279–280°C (decomp.). UV (MeOH, λ_{max}, nm): 253, 364 (log ε 5.7, 1.1). IR (KBr, ν_{max}, cm^{-1}): 3540, 3400, 3365, 2943, 2846, 1741, 1610, 1490, 1420, 1367, 1097, 1063, 758. ^{1}H NMR, see Table 1. ^{13}C NMR, see Table 1. +ve FAB-MS *m/z* (rel. int.): 940 $[M]^+$ ($C_{52}H_{76}O_{15}$), 575 (5.2), 527 (13.2), 413 (11.6), 397 (100), 381 (12.3), 365 (16.3), 273 (9.8), 255 (9.6), 240 (8.3), 136 (55.3), 119 (12.6), 107 (21.3). HR-MS: 941.1712 $[M + H]^+$ (calcd for $C_{52}H_{76}O_{15}$ 941.1723).

Alkaline Hydrolysis of 2. Compound **2** (20 mg) was dissolved in MeOH and 1 N KOH (1:1) and heated for 1 hour. The solution was extracted with EtOAc (3 × 10 mL), and washed with water (2 × 10 mL), dried over Na_2SO_4, and evaporated to give β-sitosterol, mp 138–139°C, co-TLC comparable. The aqueous phase was neutralized with dil. HCl, concentrated, and analyzed by paper chromatography along with standard samples of monosaccharides. *n*-Butanol–ethanol–water (4:1:2.2) was used as the developing solvent system. The paper was sprayed with aniline hydrogen phthalate. The sugar was identified as D-glucose.

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