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NEW STEROIDAL GLYCOSIDES FROM *MIMUSOPS ELENGI*

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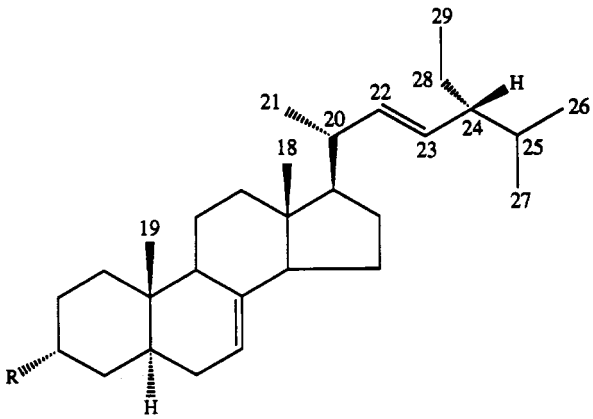
ABSTRACT.—Two new steroidal glycosides [**1** and **2**] have been isolated from *Mimusops elengi* and characterized as the 3-*O*-β-D-glucopyranoside and the 3-*O*-β-D-galactopyranoside of (24*R*)-stigmast-7,22(*E*)-dien-3α-ol, respectively.

The genus *Mimusops* belongs to the family Sapotaceae and comprises 30 species, of which three, including *M. elengi* L., are indigenous to Pakistan. *M. elengi* is an ornamental tree with sweet-scented flowers. The bark and fruit of this plant are used in the treatment of diarrhea and dysentery, and a decoction of the bark is used as a gargle (1,2). The pounded seeds pasted with oil are used for the treatment of obstinate constipation. Pillow stuffing made from the dried flowers induces nasal discharge and relieves headache (2). Previously, several triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids have been reported from this species (2). The current report describes the isolation and structural elucidation of two new steroidal glycosides [**1** and **2**] of the stigmastane series which were obtained from the hexane-soluble fraction of a MeOH extract of *M. elengi* by a combination of various kinds of chromatography.

The glycoside **1** was obtained in pure form through its tetraacetate **1a** (see Experimental) followed by deacetylation with NaOMe/MeOH as colorless crystals, mp 281–282° (dec); $[\alpha]^{20}D$ −15.9°. It gave positive Liebermann-Burchard and Salkowski tests for steroids as well as the Molish's test for glycosides (3). The positive hrfabms exhibited a peak at *m/z* 575.42324 $[M+H]^+$, compatible with the molecular formula $C_{35}H_{58}O_6$ (calcd 575.42331). The ir spectrum showed bands for hydroxyl (3330–3450 cm^{-1}), sugar C-O (1075, 1050, and 1027 cm^{-1}), double-bond (3025–3075, 1640–1650, 970 and 850 cm^{-1}), and *gem*-dimethyl (1363–1378 cm^{-1}) functionalities.

The ^1H-nmr spectrum of **1** exhibited a characteristic signal for an anomeric proton at δ 4.56. The coupling constant (J=7.9 Hz) implied a β-configuration of the sugar residue. The upfield shift of the proton at C-5 of the sugar moiety of the



1 R=β-D-glucopyranosyl
1a R=β-D-glucopyranosyl tetraacetate
2 R=β-D-galactopyranosyl
3 R=OH

tetraacetate derivative also confirmed this observation (4). The ^{1}H-nmr spectrum further showed signals of six methyls [two singlets at δ 0.54 and 0.81, three doublets at δ 0.80, 0.86, and 1.00, each showing a coupling of 6.5–7.0 Hz, and a triplet at δ 0.81 (J=7.2 Hz)]. The olefinic protons were observed at δ 5.15 (1H, m) and δ 5.20 (1H, ddd, J=16, 7.0, and 7.0 Hz) and 5.30 (1H, dd, J=16 and 7.0 Hz).

The broad-band ^{13}C-nmr spectrum of **1** showed the presence of 35 carbon atoms in the molecule, whose multiplicities were determined by DEPT experiments. Out of these, six carbon signals were in the glycosidic region corresponding to a hexose moiety. The remaining 29 carbon signals were attributable to the aglycone.

On acid hydrolysis, **1** yielded D-glucose and a free crystalline sterol [**3**], mp 150–152°; $[\alpha]^{20}_D$ +15° (c=0.4, $CHCl_3$). Its hrms showed a molecular ion peak at m/z 412.3695 corresponding to the formula $C_{29}H_{48}O$ (calcd 412.3704). The ir spectrum showed bands for hydroxyl (3410–3440 cm^{-1}), double bond (3025–3075, 1640–1650, 950, 850 cm^{-1}) and *gem*-dimethyl (1363–1378 cm^{-1}) units. The ^{1}H-nmr spectrum showed olefinic protons at δ 5.18 (1H, m, H-7), δ 5.20 (1H, ddd, J=16, 7.0, and 7.0 Hz) and δ 5.30 (1H, dd, J=16 and 7.0 Hz), a carbinylic proton at δ 3.35 (1H, m, $W_{1/2}$=15.0 Hz), and six methyl groups [two singlets at δ 0.54 and 0.81, three doublets at δ 0.78, 0.85, and 1.03, each showing a coupling of 6.5–7.0 Hz, and a triplet at δ 0.81 (J=7.2 Hz)].

A monounsaturated steroidal nucleus and a monounsaturated $C_{10}H_{19}$ side-chain was inferred from the ms, which showed characteristic peaks at m/z 273 [M−side-chain]$^+$, 271 [M−side-chain−2H]$^+$, 255 [M−side-chain−H_2O]$^+$ (5), 253 [M−side-chain−2H−H_2O]$^+$, and 83. The last-mentioned fragment peak is reported to be characteristic of Δ^{22} sterols (6). This was further confirmed by the ^{13}C-nmr spectrum in which the signals at δ 138.04 and 129.6 showed complete agreement with those of C-22 and C-23 in the analogous data of α-spinasterol and chondrillasterol (7,8). Two further olefinic carbons at δ 117.29 and 139.6 were typical of Δ^7 sterols (9) and this was further authenticated by the characteristic upfield shifts of methyl protons at C-18 and C-19 when compared to stigmasterol (10).

In the ^{1}H-^{1}H one-bond COSY nmr spectrum of the free sterol **3**, the carbinol methine proton at δ 3.35 showed cross-peaks with four other protons limiting the hydroxyl group to either C-2 or C-3. The assignment was made to C-3 on biogenetic grounds and was further supported through characteristic chemical shifts of C-1, C-2, and C-4 in the ^{13}C-nmr spectrum (11). The magnitude of the coupling constant of the carbinolic proton and its half-height line-width value were in confirmation with its β and equatorial configuration. The *R* configuration at C-24 was established by the method of Thompson *et al.* (9) and Itoh *et al.* (10) through comparison of ^{1}H- and ^{13}C-nmr spectra with steroids related to α-spinasterol and chondrillasterol (7,8). Thus, the sterol was identified as the hitherto unreported (24*R*)-stigmast-7,22(*E*)-dien-3α-ol and compound **1** as its 3-*O*-β-D-glucopyranoside.

Compound **2** was also obtained as colorless crystals, mp 291°, $[\alpha]^{20}_D$ −9.4°, and showed the same color reactions as **1**. The positive-ion hrfabms showed a molecular ion peak at m/z 575.42327, consistent with a molecular formula of $C_{35}H_{58}O_6$ (calcd 575.42331). The ir spectrum was very similar to that of **1** and its ^{1}H-nmr spectrum also showed similar resonances, except for signals of the sugar residue. An anomeric proton occurred at δ 4.20, which, on the basis of a coupling constant of 7.8 Hz, indicated that the sugar linkage was in the β configuration. The ^{13}C-nmr signals of the sugar moiety also showed different chemical shifts than analogous data for **1**, but agreed closely with those of galactose.

Acid hydrolysis yielded D-galactose and the same aglycone as **1** (mixed mp, optical rotation, and superimposable ir, ^{1}H- and ^{13}C-nmr spectra). Thus, the structure of compound **2** could be elucidated as (24*R*)-stigmast-7,22(*E*)-dien-3α-ol 3-*O*-β-D-galactopyranoside.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The instruments used were as follows: ^{1}H nmr (500 MHz) and ^{13}C nmr (125 MHz), Bruker AM-500; ms, JEOL HX-110, fabms matrix, glycerol; optical rotations, Jasco DIP-360; ir, Jasco A-302; mplc and flash cc, Si gel Si 60 (230–400 mesh), cc Si gel Si 60 (70–230 mesh); gc Shimadzu 9-A, column 25% Carbowax 20M on Chromosorb W 80/100 mesh, 145°, FI detector. The DEPT and HMQC nmr experiments were performed as reported earlier (12,13). The 2D *J*-resolved spectra (12,13) were recorded in each case to determine exact chemical shifts, multiplicities, and coupling constants.

PLANT MATERIAL.—*Mimusops elengi* was collected from the Karachi region and identified by Prof. M. Qaiser, Department of Botany, University of Karachi. A voucher specimen has been deposited at the Herbarium of the Department of Botany, University of Karachi.

EXTRACTION AND ISOLATION.—The shade-dried plant material (70 kg) was extracted four times with MeOH (100 liters, 96 h each). The residue from the MeOH extract was partitioned between hexane and H_2O. The hexane-soluble fraction was subjected to mplc using various mixtures of hexane, $CHCl_3$, and MeOH as eluents. The residue which eluted with $CHCl_3$ was mainly a mixture of **1** and **2** which could be resolved through cc. Elution with hexane-EtOAc (7:3) gave **1** with some minor impurities which could not be separated through chromatographic techniques. Further elution with hexane-EtOAc (6:4) provided **2** which could be crystallized from MeOH-$CHCl_3$ (1:1) (80 mg). Purification of **1** was achieved through acetylation with pyridine-Ac_2O (1:1) followed by flash cc using hexane/EtOAc as eluent. The head fractions which were eluted with hexane-EtOAc (7:3) crystallized from MeOH-$CHCl_3$ (1:1) to provide the tetraacetate derivative of **1**. Its positive hrfabms gave a $[M+H]^+$ peak at *m/z* 743.4655 consistent with a molecular formula of $C_{43}H_{66}O_{10}$ (calcd 743.46556). Deacetylation with NaOMe in MeOH afforded **1**, which crystallized from MeOH/$CHCl_3$ (100 mg).

(24R)-Stigmast-7,22(E)-dien-3α-ol β-D-*glucopyranoside* [**1**].—Colorless crystals, mp 281–282° (dec); $[\alpha]^{20}$D −15.9° (*c*=0.11, $CHCl_3$/MeOH); ir ν max 3450–3330, 3075–3025, 1650–1640, 1378–1363, 1050, 1027, 970, 850 cm^{-1}; ^{1}H nmr (CD_3OD-$CDCl_3$, 1:1, 500 MHz) aglycone: δ 0.54 (3H, s, Me-18), 0.80 (3H, d, *J*=7.0 Hz, Me-26), 0.81 (3H, s, Me-19), 0.81 (3H, t, *J*=7.2 Hz, Me-29), 0.86 (3H, d, *J*=6.5 Hz, Me-27), 1.00 (3H, d, *J*=6.5 Hz, Me-21), 3.34 (1H, m, $W_{1/2}$=15.0 Hz, H-3β), 5.15 (1H, dist. t, H-7), 5.20 (1H, ddd, *J*=16.0, 7.0, and 7.0 Hz, H-23), 5.30 (1H, dd, *J*=16.0 and 7.0 Hz, H-22); sugar: δ 3.15 (1H, dd, *J*=7.9 and 8.0 Hz, H-2′), 3.27 (1H, t, *J*=8.7 and 9.0 Hz, H-4′), 3.34 (1H, d, *J*=9.0 Hz, H-5′), 3.40 (1H, dd, *J*=8.0 and 8.7 Hz, H-3′), 3.62 (1H, dd, *J*=12.08 and 2.2 Hz, H-6′a), 3.80 (1H, dd, *J*=12.05 and 5.1 Hz, H-6′b), 4.52 (1H, d, *J*=7.9 Hz, H-1′); ^{13}C nmr ($CDCl_3$/CD_3OD, 125 MHz) aglycone: δ 139.2 (C-8), 138.3 (C-22), 129.4 (C-23), 117.1 (C-7), 79.5 (C-3), 56.6 (C-17), 55.8 (C-14), 51.2 (C-24), 49.4 (C-9), 43.0 (C-13), 40.7 (C-5), 40.2 (C-20), 39.5 (C-12), 37.1 (C-1), 34.3 (C-10), 34.1 (C-4), 31.9 (C-25), 29.7 (C-2), 29.5 (C-6), 28.4 (C-16), 25.4 (C-28), 23.0 (C-15), 21.5 (C-11), 21.5 (C-27), 21.0 (C-21), 19.0 (C-26), 13.0 (C-19), 12.9 (C-29), 12.0 (C-18); sugar: δ 99.6 (C-1′), 77.0 (C-5′), 76.4 (C-3′), 75.3 (C-2′), 70.2 (C-4′), 62.3 (C-6′). The assignments were confirmed through HMQC and by comparison with related glucosides (9).

(24R)-Stigmast-7,22(E)-dien-3α-ol β-D-*glucopyranosyl tetraacetate* [**1a**].—Mp 137°; $[\alpha]^{20}$D −7° (*c*=0.2, $CHCl_3$); fabms *m/z* 743 $[M+H]^+$, 413 [*m/z* 743−glucose tetraacetate]$^+$; ir ν max 3060–3025, 1770, 1760, 1750, 1745, 1650, 1640, 1375–1360, 1250, 1050, 1027, 970, 850 cm^{-1}; ^{1}H nmr (CD_3OD-$CDCl_3$, [1:1] 500 MHz) δ 0.54 (3H, s, Me-18), 0.80 (3H, d, *J*=7.0 Hz, Me-26), 0.81 (3H, s, Me-19), 0.81 (3H, t, *J*=7.2 Hz, Me-29), 0.86 (3H, d, *J*=6.5 Hz, Me-27), 1.00 (3H, d, *J*=6.5 Hz, Me-21), 1.98, 2.00, 2.01, 2.02 (4×3H, s, OAc, at 2′, 3′, 4′, and 6′), 3.52 (1H, m, $W_{1/2}$=15.0 Hz, H-3β), 3.65 (1H, m, H-5′), 4.11 (1H, dd, *J*=12.1 and 2.3 Hz, H-6′a), 4.20 (1H, dd, *J*=12.05 and 5.3 Hz, H-6′b), 4.56 (1H, d, *J*=7.9 Hz, H-1′), 4.93 (1H, dd, *J*=9.6 and 8.0 Hz, H-2′), 5.04 (1H, t, *J*=9.5 Hz, H-4′), 5.18 (1H, t, *J*=9.5 Hz, H-3′), 5.15 (1H, dist. t, H-7), 5.20 (1H, ddd, *J*=16.0, 7.0, and 7.0 Hz, H-23), 5.30 (1H, dd, *J*=16.0 and 7.0 Hz, H-22); ^{13}C nmr ($CDCl_3$, 125 MHz) aglycone: δ 139.1 (C-8), 138.3 (C-22), 129.4 (C-23), 117.3 (C-7), 79.7 (C-3), 56.5 (C-17), 55.7 (C-14), 51.2 (C-24), 49.5 (C-9), 43.1 (C-13), 40.6 (C-5), 40.2 (C-20), 39.4 (C-12), 37.2 (C-1), 34.3 (C-4), 34.2 (C-10), 31.9 (C-25), 29.9 (C-2), 29.4 (C-6), 28.5 (C-16), 25.4 (C-28), 23.1 (C-15), 21.6 (C-11), 21.5 (C-27), 21.0 (C-21), 19.0 (C-26), 13.1 (C-19), 12.9 (C-29), 12.1 (C-18); sugar: δ 99.6 (C-1′), 73.9 (C-5′), 72.5 (C-2′), 71.9 (C-3′), 68.7 (C-4′), 62.2 (C-6′), O-CO-Me (173.4, 170.4, 169.4, 169.3), O-CO-*Me* (21.4, 20.9, 20.6, 20.5). The assignments were con-

firmed through HMQC and by comparison with related glucosides (9).

(24R)-Stigmast-7,22(E)-dien-3α-ol β-D-*galactopyranoside* [**2**].—Colorless crystals: mp 291°; $[\alpha]^{20}D$ −9.4° (*c*=0.5, $CHCl_3/CH_3OH$); ir ν max 3435–3405, 3060–3020, 1685–1660, 1370–1358, 950, 850 cm^{-1}; ^1H nmr ($CD_3OD/CDCl_3$, 500 MHz) aglycone: δ 0.45 (3H, s, Me-18), 0.80 (3H, d, *J*=6.5 Hz, Me-26), 0.80 (3H, s, Me-19), 0.81 (3H, t, *J*=7.2 Hz, Me-29), 0.85 (3H, d, *J*=6.5 Hz, Me-27), 1.02 (3H, d, *J*=6.5 Hz, Me-21), 3.36 (1H, m, $W_{1/2}$=11.1 Hz, H-3), 5.20 (1H, dist. t, H-7), 5.23 (1H, ddd, *J*=16.0 and 7.0 Hz, H-23), 5.31 (1H, dd, *J*=16.0 and 7.0 Hz, H-22); sugar: δ 3.81 (1H, dd, *J*=1.53 and 1.65 Hz, H-4′), 4.20 (1H, d, *J*=7.8 Hz, H-1′); ^{13}C nmr ($CD_3OD/CDCl_3$, 125 MHz) aglycone: δ 139.0 (C-8), 137.9 (C-22), 129.0 (C-23), 117.2 (C-7), 78.5 (C-3), 55.8 (C-17), 55.7 (C-14), 51.0 (C-24), 49.2 (C-9), 43.1 (C-13), 40.0 (C-5), 40.6 (C-20), 39.3 (C-12), 36.8 (C-1), 34.1 (C-10), 33.9 (C-4), 31.6 (C-25), 29.7 (C-2), 28.2 (C-16), 25.1 (C-28), 22.8 (C-15), 21.2 (C-11), 21.0 (C-21), 20.7 (C-27), 20.0 (C-6), 19.4 (C-26), 12.6 (C-19), 11.8 (C-29), 11.7 (C-18); sugar: δ 100.8 (C-1′), 76.3 (C-5′), 73.4 (C-3′), 72.2 (C-2′), 69.7 (C-4′), 61.3 (C-6′). The nmr assignments were confirmed through HMQC and by comparison with published data of related galactosides (14).

ACID HYDROLYSIS OF **1** AND **2**.—Compound **1** (70 mg) was hydrolyzed with 2 N HCl and dilute MeOH (9 ml MeOH and 1 ml H_2O) at 100° for 3 h. The solvent was evaporated under reduced pressure. The residue was diluted with H_2O and neutralized with Ag_2CO_3. The reaction mixture was then extracted with EtOAc to furnish the aglycone moiety, while the aqueous layer was evaporated under reduced pressure. The residue obtained showed the presence of D-glucose when compared with an authentic sample of this sugar on tlc. The identity was also confirmed by comparing the retention time of the TMS ether of the glycone with a standard sample (retention time α-anomer 4.1 min and β-anomer 7.8 min). The acid hydrolysis of **2** was carried out exactly as for **1**. The mp, optical rotation, ^{13}C-nmr, and ^1H-nmr spectra of the aglycone were identical to that obtained from **1**. The sugar contained in the aqueous layer was identified as D-galactose by comparison with the authentic sample on tlc. It was also confirmed through the retention time of its TMS ether with a standard (retention time α-anomer 3.8 min, β-anomer 5.2 min).

(24R)-Stigmast-7,22(E)-dien-3α-ol [**3**].—Mp 150–152°; $[\alpha]^{20}D$ +15° (*c*=0.4, $CHCl_3$); hreims *m/z* 412.37046 $[M^+]$ ($C_{29}H_{48}O$), 399 $[M-Me]^+$, 394 $[M-H_2O]^+$. Further peaks at *m/z* 273, 271, 255, 253, and 83 were common to stigmasterol and related steroids (7,8); ir ν max 3440–3410, 3025–3075, 1640–1650, 1363–1378, 950, 850 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.54 (3H, s, Me-18), 0.78 (3H, d, *J*=7.0 Hz, Me-26), 0.81 (3H, t, *J*=7.2 Hz, Me-29), 0.85 (3H, d, *J*=6.5 Hz, Me-27), 1.03 (3H, d, *J*=6.5 Hz, Me-21), 3.35 (1H, m, $W_{1/2}$=15.0 Hz, H-3), 5.18 (1H, m, H-7), 5.21 (1H, ddd, *J*=16.0, 7.0, and 7.0 Hz, H-23), 5.32 (1H, dd, *J*=16.0 and 7.0 Hz, H-22); ^{13}C nmr ($CD_3OD/CDCl_3$, 125 MHz) δ 139.6 (C-8), 138.0 (C-22), 129.6 (C-23), 117.2 (C-7), 74.0 (C-3), 55.8 (C-17), 55.6 (C-14), 51.3 (C-24), 49.0 (C-9), 43.1 (C-13), 40.4 (C-5), 40.2 (C-20), 39.6 (C-12), 37.0 (C-1), 34.4 (C-10), 33.9 (C-4), 31.8 (C-25), 29.4 (C-6), 28.4 (C-16), 27.6 (C-2), 25.5 (C-28), 23.1 (C-15), 21.0 (C-21), 19.2 (C-26), 12.5 (C-19), 12.6 (C-29), 12.0 (C-18). The assignments were confirmed through HMQC and by comparison with related steroids (7,8).

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