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NATURAL DRUGS

NEW ALIPHATIC ESTER, β-SITOSTEROL DIGLUCOSIDE AND VESICARIA BIFLAVONES FROM THE SEEDS OF *RUMEX VESICARIUS* L.

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Abstract: *Rumex vesicarius* L. (Polygonaceae) is an annual, monoecious, glabrous, pale green herb cultivated as a leafy vegetable in south western Asia and northern Africa. Its seeds are prescribed as a refrigerant, laxative, antidote for scorpion venom and to cure dysentery and liver diseases. Phytochemical investigation of a methanolic extract of the seeds of *R. vesicarius* resulted in the isolation of a new aliphatic ester *n*-heptacosanyl *n*-hexanoate (**2**), a steroidal diglucoside stigmasta-5-en-3-ol-3-*O*-β-D-glucopyranosido-(4→1'')-*O*-β-D-glucopyranoside (**3**) and two bioflavonoids characterized as (2a,3a-*trans*)-3a(β),5a,7a,3'a,4'a-pentahydroxyflavanolyl-(8a→2')-5,7,3'-trihydroxy-4'-methoxy-8-*n*-but-3''-enyl-flavanone (**4**) and 5,7,3',4',5'-pentahydroxy-8-(*cis*-1''α,2''β-dihydroxyhept-4''-enyl-7''-oic acid)-flavanoyl-(2'→8a)-5a,7a,3'a,5'a-tetrahydroxy-4'a-methoxyflavanone (**5**) together with stigmasterol (**1**). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

Keywords: *Rumex vesicarius*, Polygonaceae, seeds, aliphatic ester, β-sitosterol diglucoside, biflavonoids, *c*haracterization

Rumex vesicarius L. (Polygonaceae), known as Chukra or Bladder dock, is an annual, monoecious, glabrous, dichotomously branched, succulent pale green herb. It is a native to south western Asia and northern Africa; cultivated as a leafy vegetable in many parts of India (1). It is prescribed to treat asthma, bronchitis, constipation, calculus, dyspepsia, flatulence, hepatic diseases, heart troubles, hiccough, indigestion, nausea pains, spleen diseases, piles, scabies, leucoderma, toothache and tumors (1-4). It possesses diuretic, antiscorbutic, appetizer, astringent, carminative, laxative, stomachic and tonic properties. The leaves are eaten fresh and much appreciated for their acid taste; they can be added to salads and used as an antidote for snake venom. The plant is prescribed to reduce biliary disorders and to control cholesterol levels. The seeds are utilized as a refrigerant, to cure dysentery and as an antidote for scorpion venom. The seed powder is taken orally to treat liver diseases and as a laxative (1-3). The plant contained flavonoids (vitexin, isovitexin, orientin and isorientin), anthraquinones, particularly in roots (emodin and chrysophanol), quinones, carotenoids, vitamins, proteins, lipids, carbohydrates, reducing sugars, phenols, tannins, saponins, triterpenoids and organic acids (5-10). The drug showed antidiarrheal and antidysenteric (10), antimicrobial (9, 11-13), antioxidant (14) and diuretic (15) activities. The present paper describes the isolation and characterization of four new phytoconstituents from the seeds of *R. vesicarius*.

EXPERIMENTAL

General

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infra red spectra were recorded on Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets; ν_{max} values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were scanned on Advance DRX Bruker spectrospin 400 and 100 MHz, respectively, instruments (Karlsruhe, Germany) using TMS as an internal standard. Mass spectra were obtained by effecting FAB ionization at 70 eV on a JEOL-JMS-

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DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualized by exposing to iodine vapors, UV radiation and spraying with ceric sulfate solution.

Plant material

The seeds of *R. vesicarius* were procured from the Khari Baoli market of Delhi and identified by Prof. M.P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen is deposited in the herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy.

Extraction and isolation

The air-dried seeds (2.0 kg) were coarsely powdered, defatted with petroleum ether and extracted with methanol exhaustively in a Soxhlet apparatus. The combined extracts were filtered and concentrated under reduced pressure to get a dark brown viscous mass (125 g, 6.25%). The dried extract was dissolved in minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for preparation of a slurry. It was dried in air and chromatographed over silica gel column (1.6 m × 16 mm × 2 mm) packed in petroleum ether. The column was eluted successively with different solvents in increasing order of polarity in various combinations of chloroform, chloroform-methanol (19.9 : 0.1; 99 : 1; 97 : 3; 19 : 1; 93 : 7; 9 : 1; 17 : 3; 3 : 1; 3 : 2; 2 : 3, v/v) and methanol. The fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of the seeds of *R. vasicarius:*

Stigmasterol (1)

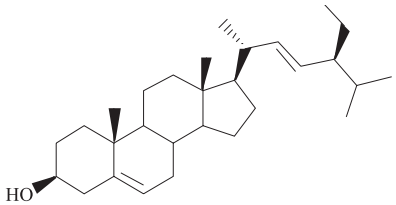
Elution of the column with chloroform gave a colorless, amorphous powder of **1**, recrystallized from acetone, 50 mg (0.0025% yield); R_f: 0.51 (petroleum ether-$CHCl_3$-MeOH, 1 : 4 : 1, v/v/v); co-TLC comparable; m.p. and m.m.p.: 168-170°C; IR (KBr, cm^{-1}): 3480, 2930, 2853, 1640, 1470, 1260, 1180, 1020; ^{1}H NMR ($CDCl_3$, δ, ppm): 5.35 (brs, 1H, H-5), 5.14 (dd, 1H, *J* = 15.3, 8.4 Hz, H-22), 5.02 (dd, 1H, *J* = 8.1, 15.3 Hz, H-23), 3.95 (brm, 1H, $w_{1/2}$ = 18.5 Hz, H-3α), 1.01 (brs, 3H, Me-19), 0.93 (d, 3H, *J* = 6.3 Hz, Me-21), 0.84 (d, 3H, *J* = 6.3 Hz, Me-26), 0.82 (d, 3H, *J* = 6.3 Hz, Me-27), 0.80 (d, 3H, *J* = 6.2 Hz, Me-29), 0.69 (brs, 3H, Me-18); +ve ESI MS *m/z*: 412 $[M]^+$ ($C_{29}H_{48}O$) (8.2).

***n*-Heptacosanyl *n*-hexanoate (2)**

Further elution of the column with chloroform eluants produced colorless crystals of **2**, recrystallized from methanol, 157 mg (0.078% yield); R_f: 0.40 ($CHCl_3$-MeOH, 4 : 1, v/v); m.p.: 70-72°C; IR (KBr, cm^{-1}): 2920, 2852, 1718, 1635, 1465, 1380, 1225, 1165, 785; ^{1}H NMR ($CDCl_3$, δ, ppm): 4.17 (t, 2H, *J* = 6.8 Hz, H_2-1'), 2.31 (t, 2H, *J* = 7.2 Hz, H_2-2), 2.01 (m, 2H, H_2-3), 1.68 (m, 2H, CH_2), 1.25 (brs, 52H, 26 × CH_2), 0.87 (t, 3H, *J* = 6.5 Hz, Me- 27'), 0.83 (t, 3H, *J* = 6.1 Hz, Me-6); ^{13}C NMR ($CDCl_3$, δ, ppm): 173.74 (C-1), 74.09 (C-1'), 39.18 (CH_2), 37.30 (CH_2), 34.77 (CH_2), 34.17 (CH_2), 32.45 (CH_2), 31.95 (CH_2), 30.06 (CH_2), 29.72 (CH_2), 29.68 (10 × CH_2), 29.61 (CH_2), 29.56 (CH_2), 29.39 (CH_2), 29.34 (CH_2), 29.22 (CH_2), 29.18 (CH_2), 28.97 (CH_2), 27.99 (CH_2), 25.34 (CH_2), 25.22 (CH_2), 22.71 (CH_2), 14.19 (C-27'), 14.13 (C-6); +ve ESI MS *m/z*: 494 $[M]^+$ ($C_{33}H_{66}O_2$) (5.1), 395 (11.7).

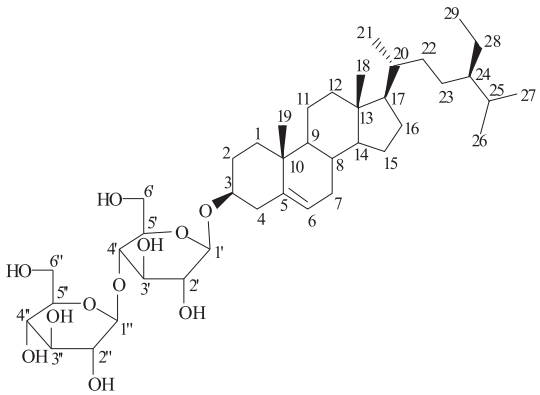
β-Sitosterol diglucoside (3)

Elution of the column with chloroform-methanol (19 : 1, v/v) afforded a colorless, amorphous powder of **3**, recrystallized from methanol, 55 mg (0.003% yield); R_f: 0.67 ($CHCl_3$-MeOH, 1 : 1, v/v); m.p.: 225°C; IR (KBr, cm^{-1}): 3450, 3404, 3380, 2919, 2848, 1645, 1474, 1100, 1080; UV λ_{max} (MeOH): 243 nm (log ε 3.2); ^{1}H NMR (DMSO-d_6, δ, ppm): 5.55 (d, 1H, *J* = 5.1 Hz, H-6), 5.32 (d, 1H, *J* = 7.8 Hz, H-1'), 4.90 (d, 1H, *J* = 7.7 Hz, H-1''), 4.48 (m, 2H, H-5', H-5''), 4.23 (m, 1H, H-2'), 4.13 (m, 1H, H-2''), 4.01 (m, 1H, H-3'), 3.81 (m, 1H, H-3''), 3.54 (brm, 1H, $w_{1/2}$ = 18.3 Hz, H-3α), 3.50 (m, 1H, H-4'), 3.45 (m, 1H, H-4''), 3.18 (brs, 2H, H_2-6)', 3.07 (brs, 2H, H_2-6''), 0.99 (brs, 3H, Me-19), 0.95 (d, 3H, *J* = 6.1 Hz, Me-21), 0.88 (d, 3H, *J* = 5.8 Hz, Me-27), 0.84 (d, 3H, *J* = 6.0 Hz, Me-26), 0.82 (d, 3H, *J* = 6.1 Hz, Me-29), 0.65 (brs, 3H, Me-18); ^{13}C NMR ($CDCl_3$, δ, ppm): 36.84 (C-1), 32.11 (C-2), 73.46 (C-3), 41.49 (C-4), 140.45 (C-5), 121.18 (C-6), 31.35 (C-7), 34.36 (C-8), 49.63 (C-9), 36.18 (C-10), 20.60 (C-11), 39.79 (C-12), 41.49 (C-13), 56.19 (C-14), 24.50 (C-15), 28.73 (C-16), 55.43 (C-17), 13.92 (C-18), 18.68 (C-19), 35.49 (C-20), 22.11 (C-21), 33.37 (C-22), 25.47 (C-23), 45.16 (C-24), 29.07 (C-25), 18.73 (C-26), 19.02 (C-27), 23.86 (C-28), 11.68 (C-29), 103.48 (C-1'), 70.09 (C-2'), 70.58 (C-3'), 68.32 (C-4'), 76.76 (C-5'), 61.09 (C-6'), 100.81 (C-1''), 70.79 (C-2''), 70.16 (C-3''), 65.75 (C-4''), 76.71 (C-5''), 60.08 (C-6''); +ve ESI MS m/z (rel. int.): *m/z* 738 $[M]^+$ ($C_{41}H_{70}O_{11}$) (2.6), 413 (21.3)

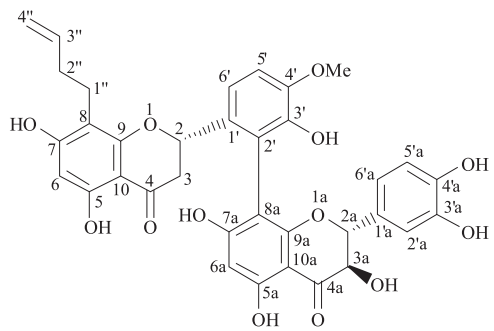


1. Stigmasterol

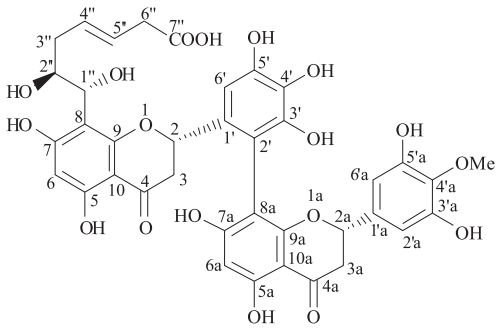
2. *n*-Heptacosanyl-*n*-hexanoate



3. β-Sitosterol diglucoside



4. Vesicariabiflavanone A



5. Vesicariabiflavanone B

Figure 1. Structures of compounds **1-5** isolated from the methanolic extract of the seeds of *Rumex vesicarius* L.

($C_{29}H_{49}O$) (3.7), 398 (11.9), 273 (9.8), 255 (33.7), 240 (25.8), 213 (14.1).

Vesicariabiflavanone A (4)

Further elution of the column with chloroform-methanol (19 : 1, v/v) yielded a yellow, amorphous powder of **4**, recrystallized from methanol, 286 mg (0.0143% yield); R_f: 0.55 ($CHCl_3$-MeOH, 1 : 1, v/v); m.p.: 203-205°C; IR (KBr, cm^{-1}): 3410, 3380, 3310, 2925, 2855, 1705, 1695, 1640, 1596, 1575, 1470, 1380, 1120, 1060; UV λ_{max} (MeOH): 255, 288, 370 nm (log ε 0.8, 5.3, 1.3); ^{1}H NMR ($CDCl_3$, δ, ppm): 6.94 (brs, 1H, H-6), 6.83 (brs, 1H, H-6a), 6.76 (d, 1H, *J* = 7.8 Hz, H-5'), 6.72 (d, 1H, *J* = 7.5 Hz, H-5'a), 6.70 (d, 1H, *J* = 2.5 Hz, H-2'a), 6.68 (d, 1H, *J* = 7.8 Hz, H-6'), 6.65 (dd, 1H, *J* = 2.5, 7.5 Hz, H-6'a), 5.25 (brm, 1H, H-3''), 5.07 (d, 1H, *J* = 5.8 Hz, H_2-4''a), 5.04 (d, 1H, *J* = 5.8 Hz, H_2-4''b), 4.98 (dd, 1H, *J* = 13.1, 2.9 Hz, H-2), 4.95 (d, 1H, *J* = 11.1 Hz, H-2a), 4.48 (d, 1H, *J* = 11.1 Hz, H-3a), 3.31 (brs, 3H, OMe), 3.11 (dd. 1H, *J* = 13.1, 17.2 Hz, H_2-3*ax*), 2.93 (dd, 1H, *J* = 2.9, 17.2 Hz, H_2-3*eq*), 2.73 (brm, 2H, H_2-1''), 2.61 (brm, 2H, H_2-2''); ^{13}C NMR ($CDCl_3$, δ, ppm): 82.57 (C-2), 42.06 (C-3), 196.04 (C-4), 163.97 (C-5), 95.79 (C-6), 166.49 (C-7), 94.62 (C-8), 162.98 (C-9), 101.40 (C-10), 129.47 (C-1'), 114.26 (C-2'), 144.33 (C-3'), 144.73 (C-4'), 114.69 (C-5'), 118.78 (C-6'), 44.53 (C-1''), 39.04 (C-2''), 129.03 (C-3''), 102.47 (C-4''), 80.51 (C-2a), 71.39 (C-3a), 194.90 (C-4a), 163.08 (C-5a), 95.57 (C-6a), 166.10 (C-7a), 92.53 (C-8a), 162.22 (C-9a), 99.70 (C-10a), 129.02 (C-1a'), 113.06 (C-2a'), 144.02 (C-3a'), 144.77 (C-4a'), 114.61 (C-5a'), 117.18 (C-6a'), 55.04 (OMe); +ve ESI MS *m*/*z* (rel. int.): 658 $[M]^+$ ($C_{35}H_{30}O_{13}$) (1.2), 356 $[C_{20}H_{20}O_6]^+$ (2.1), 302 $[C_{15}H_{10}O_7]^+$ (11.2), 152 (14.3).

Vesicariabiflavanone B (5)

Further elution of the column with chloroform-methanol (19 : 1, v/v) furnished a yellow, amorphous powder of **5**, recrystallized from methanol, 519 mg (0.0259% yield); R_f: 0.65 ($CHCl_3$-MeOH, 1 : 1); m.p.: 210°C; IR (KBr, cm^{-1}): 3485, 3404, 3380, 2955, 1695, 1685, 1640, 1550, 1410, 1320, 1250, 1065, 960, 855; UV λ_{max} (MeOH): 242, 291, 366 nm (log ε 1.1, 5.6, 1.3); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.96 (s, 1H, H-6), 6.88 (s, 1H, H-6a), 6.76 (s, 1H, H-6'), 6.71 (d, 1H, *J* = 2.5 Hz, H-2'a), 6.67 (d, 1H, *J* = 2.5 Hz, H- 6'a), 5.76 (m, 1H, $w_{1/2}$ = 8.5 Hz, H-4''), 5.36 (m, 1H, $w_{1/2}$ = 8.3 Hz, H-5''), 5.28 (dd, 1H, *J* = 12.8, 2.9 Hz, H-2), 5.16 (dd, 1H, *J* = 12.6, 2.7 Hz, H-2a), 4.51 (d, 1H, *J* = 6.3 Hz, H-1''), 3.71 (m, 1H, $w_{1/2}$ = 14.7 Hz, H-2''α), 3.15 (brs, 3H, OMe), 3.12 (dd, 1H, *J* = 17.2, 12.8 Hz, H_2-3*ax*), 3.08 (dd, 1H, *J* = 17.3, 12.7 Hz, H_2-3'*ax*), 2.95 (d, 1H, *J* = 15.6 Hz, H_2-6''a), 2.90 (d, 1H, *J* = 15.6 Hz, H_2-6''b), 2.85 (dd, 1H, *J* = 2.9, 17.2 Hz, H_2-2*eq*), 2.80 (dd, 1H, *J* = 2.7, 17.3 Hz, H_2-2*eq*), 2.64 (m, 1H, H_2-3''a), 2.48 (m, 1H, H_2-3''b); ^{13}C NMR ($CDCl_3$, δ, ppm): 78.59 (C-2), 44.92 (C-3), 197.85 (C-4), 164.45 (C-5), 96.18 (C-6), 166.93 (C-7), 95.14 (C-8), 163.63 (C-9), 101.96 (C-10), 130.12 (C-1'), 115.51 (C-2'), 145.32 (C-3'), 145.88 (C-4'), 144.47 (C-5'), 114.47 (C-6'), 83.21 (C-1''), 71.73 (C-2''), 38.21 (C-3''), 128.22 (C-4''), 127.23 (C-5''), 115.32 (C-6''), 188.02 (C-7''), 78.20 (C-2a), 42.19 (C-3a), 196.41 (C-4a), 163.02 (C-5a), 95.96 (C-6a), 166.77 (C-7a), 93.33 (C-8a), 163.48 (C-9a), 100.63 (C-10a), 129.65 (C-1a'), 114.30 (C-2a'), 145.08 (C-3a'), 145.65 (C-4a'), 144.41 (C-5a'), 115.32 (C-6a'), 55.72 (OMe); +ve ESI MS *m*/*z*: 766 $[M]^+$ ($C_{37}H_{34}O_{18}$) (3.1), 317 (5.3), 166 (9.2).

RESULTS AND DISCUSSION

Compound **1** was the known phytoconstituent identified as stigmasterol (Fig. 1).

Compound **2** was obtained as colorless crystals from chloroform eluants. Its IR spectrum displayed important absorption bands for ester group (1718 cm^{-1}) and long aliphatic chain (785 cm^{-1}). Its mass displayed a molecular ion peak at *m*/*z* 494 corresponding to molecular formula of an ester $C_{33}H_{66}O_2$. An ion peak arising at *m*/*z* 395 [$C_{1'}$-O ester fission, $CH_3(CH_2)_{26}O]^+$ suggested that *n*-heptacosanyl moiety was esterified with *n*-hexanoic acid. The ^{1}H NMR spectrum of **2** displayed two two-proton triplets at δ 4.17 (*J* = 6.8 Hz) and 2.31 (*J* = 7.2 Hz) ppm assigned to oxygenated methylene H_2-1' protons and methylene H_2-2 protons adjacent to ester group, respectively. The remaining methylene protons appeared as two-proton multiplets at δ 2.01 and 1.68 ppm and as a broad singlet at δ 1.25 (28 × CH_2) ppm. Two three-proton triplets at δ 0.87 (*J* = 6.5 Hz) and 0.83 (*J* = 6.1 Hz) ppm were accounted to terminal primary C-27 and C-6´ methyl protons, respectively. The ^{13}C NMR of **2** exhibited signals for ester carbon at δ 173.74 ppm (C-1), oxygenated methylene carbon at δ 74.09 ppm (C-1'), other methylene carbon signals between δ 39.18-22.71 ppm and methyl carbons at δ 14.19 (C-27') and 14.13 (C-6) ppm. The HMBC spectrum of **2** showed correlations of H_2-2, H_2-3 and H_2-1' with C-1; H_2-2' and H_2-3' with C-1'; H_2-5 with C-6; and H_2-26' with C-27'. On the basis of these evidences the structure of **2** has been elucidated as *n*-heptacosanyl *n*-hexanoate, a new fatty ester (Fig. 1).

Compound **3**, designated as β-sitosterol diglucoside, was obtained as a colorless, amorphous powder from chloroform-methanol (9 : 1, v/v) eluants. It gave a positive Liebermann Burchard test for sterols and tests for glycosides. Its IR spectrum exhibited absorption bands for hydroxyl groups (3450, 3404, 3380 cm^{-1}) and unsaturation (1645 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **3** was determined at *m*/*z* 738 consistent with a molecular formula of steroidal diglucoside $C_{41}H_{70}O_{11}$. The fragment ions arising at *m*/*z* 413 [M-glycone]$^+$, 398 [413-Me]$^+$, 273 [413-$C_{10}H_{21}$, side chain]$^+$, 255 [273-H_2O]$^+$, 240 [255-Me]$^+$ and 213 [255-ring D fission]$^+$ were characteristic for β-sitosterol aglycone. The ^1H NMR spectrum **3** exhibited three one-proton doublets at δ 5.55 (*J* = 5.1 Hz), 5.32 (*J* = 7.8 Hz) and 4.90 (*J* = 7.7 Hz) ppm ascribable to vinylic H-6 and anomeric H-1' and H-1'' protons, respectively. The other sugar protons appeared between δ 4.48-3.07 ppm. A one-proton broad multiplet at δ 3.54 ppm with half-width of 18.3 Hz was assigned to oxygenated methine H-3α proton. Two three-proton broad singlets at δ 0.99 and 0.65 ppm and four three-proton doublets at δ 0.95 (*J* = 6.1 Hz, Me-21), 0.88 (*J* = 5.8 Hz, Me-27), 0.84 (*J* = 6.0 Hz, Me-26), 0.82 (*J* = 6.1 Hz, Me-29) ppm were associated with tertiary C-19 and C-18, secondary C-21, C-27 and C-26 and primary C-29 methyl protons, all attached to saturated carbons. The ^{13}C NMR spectrum of **3** displayed signals for 41 carbons. The important signals appeared for vinylic carbons (δ 140.45, C-5; 121.18, C-6 ppm), anomeric carbons (δ 103.48 ppm, C-1'; 100.81 ppm, C-1''), oxygenated methine carbon (δ 73.46 ppm, C-3) and hydroxymethylene carbons (δ 61.09 ppm, C-6'; 60.08 ppm, C-6''). The presence of H-4' signal as a multiplet at δ 3.50 ppm in the ^1H NMR spectrum and ^{13}C NMR signal in the deshielded region at δ 68.32 ppm (C- 4') suggested (4'→1'') linkage of the sugar units. The ^1H NMR and ^{13}C NMR spectral data of the steroidal nucleus were compared with other stigmastene-type molecules (16-18). The ^1H-^1H COSY spectrum of **3** showed correlations of H_2-1, H_2-2 and H_2-4 with H-3; H_2-4, H-8 and H_2-7 with H-6; H-3, H-2' and H-5' with H-1'; and H-4' and H-2'' with H-1''. The HMBC spectrum of **3** exhibited interactions of H_2-1, H_2-2 and H_2-4 with C-3; H_2-4, H-6, H_2-7 with C-5; H-3, H-2' and H-5' with C-1'; and H-4', H-2'' and H-5'' with C-1''. Acid hydrolysis of **3** yielded D-glucose and β-sitosterol, co-TLC comparable with the authentic samples. On the basis of these findings the structure of **3** was established as stigmasta-5-en-3-ol-3-*O*-β-D-glucopyranosido-(4'→1'')-*O*-β-D-glucopyranoside (Fig. 1).

Compound **4**, named vesicariabiflavanone A, was obtained as a yellow amorphous powder from chloroform-methanol (19 : 1, v/v) eluants. It gave positive tests of flavonoids and showed UV absorption maxima at 255, 288, 370 nm characteristics of flavanones (19). Its IR spectrum displayed absorption bands for hydroxyl groups (3410, 3380, 3310 cm^{-1}), carbonyl group (1705 cm^{-1}) and unsaturation (1640, 1695 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **4** was determined at *m*/*z* 658 consistent with the molecular formula a biflavonoid $C_{35}H_{30}O_{13}$. The generation of the important fragment peaks at *m*/*z* 356 $[C_{20}H_{20}O_6]^+$ and 302 $[C_{15}H_{10}O_7]^+$ supported biflavonoid nature of the compound. An ion peak at *m*/*z* 152 $[C_8H_8O_3]^+$ arising due to RDA fission further substantiated the presence of a flavanol unit linked to a flavanone of the biflavonoid. The ^1H NMR spectrum of **4** displayed two one-proton singlets at δ 6.94 and 6.83 ppm assigned to aromatic H-6 and H-6a protons, respectively. Three one-proton doublets at δ 6.76 (*J* = 7.8 Hz), 6.72 (*J* = 7.5 Hz), and 6.65 (*J* = 7.5 Hz) ppm were ascribed correspondingly to *ortho*-coupled H-5', H-5'a and H-6' aromatic protons. A one-proton doublet at δ 6.70 (*J* = 2.5 Hz) ppm and a one-proton double doublet at δ 6.68 (*J* = 2.5, 7.8 Hz) ppm were accounted to *meta*-coupled H-2'a and *meta-, ortho*-coupled H-6'a protons, respectively. A one-proton broad multiplet centered at δ 5.25 ppm was attributed to vinylic methine H-3'' proton whereas H_2-4'' vinylic methylene protons appeared as two one-proton broad singlets at δ 5.07 and 5.04 ppm. A one-proton double doublet in the non-aromatic region at δ 4.98 (*J* = 13.1, 2.9 Hz) ppm and a one-proton doublet at δ 4.95 (*J* = 11.1 Hz) ppm were due to the oxygenated methine H-2 and H-2a, respectively. A one-proton doublet at δ 4.48 (*J* = 11.1 Hz) ppm and a three-proton broad singlet at δ 3.31 ppm were accounted to α-oriented H-3a carbinol and methoxy protons, respectively. Two one-proton double doublets at δ 3.11 (*J* = 13.1, 17.2 Hz) ppm and 2.93 (*J* = 2.9, 17.1 Hz) ppm were due to methylene H_2-3*ax* and H_2-3*eq* protons, supporting flavanone ring system of one of the unit of the biflavonoid skeleton (20-22). The methylene protons of the side chain resonated as two-proton multiplets at δ 2.73 (H_2-1'') and 2.61 (H_2-2'') ppm. Its ^{13}C NMR spectrum showed important signals for carbonyl carbons (δ 196.04, C-4; 194.90, C-4a ppm), vinylic carbons of side chain (δ 129.03, C-3''; 102.47, C-4'' ppm), oxygenated methine carbons (δ 82.57, C-2; 80.51, C-2a ppm), hydroxymethine carbon at δ 71.39 (C-3a) ppm and methoxy carbon (δ 55.04 ppm). The substituted aromatic carbons C-8 and C-8a appeared at δ 94.62 and

δ 92.53 ppm, respectively. The ^{1}H-^{1}H COSY spectrum of **4** showed correlations of H-2 with H-3 and H-6'; H-5' with H-6'; H-3'' with H_2-1'', H_2-2'' and H_2-4''; H-2a with H-3a, H-2'a and H-6'a; and H-5'a with H-6'a. In the HMBC spectrum of **4** H-6 interacted with C-5 and C-7; H-2 and H-3 correlated with C-4; H-2, H-5', H-6' interacted with C-1'; H-5' and OMe correlated with C-5'; H_2-1'', H_2-2'' and H_2-4'' interacted with C-3''; H-2a and H-3a interacted with C-4a; and H-2a, H-6'a and H-2'a interacted with C-1'a. On the basis of above discussion, the structure of **4** was elucidated as (2a,3a-*trans*)-3a(β),5a,7a, 3'a,4'a-pentahydroxyflavanolyl-(8a→2')-5,7,3'-trihydroxy-4'-methoxy-8-*n*-but-3''-enyl-flavanone, a new natural biflavonoid (Fig. 1).

Compound **5**, designated vesicariabiflavanone B, was obtained as a yellow amorphous powder from chloroform-methanol (19 : 1, v/v) eluants. Its UV spectrum showed absorption maxima at 242, 291, 366 nm characteristics of flavanones (19). Its IR spectrum displayed absorption bands for hydroxyl groups (3485, 3404, 3380 cm^{-1}), carboxyl group (1695 cm^{-1}), carbonyl function (1685 cm^{-1}) and aromatic ring (1640, 1550, 1065 cm^{-1}). On the basis of its mass and ^{13}C NMR spectra its molecular ion peak was determined at *m*/*z* 766 corresponding to a molecular formula of a biflavanonoid $C_{37}H_{34}O_{18}$. The ion peaks arising at m/z 166 $[C_9H_{10}O_3]^+$ formed due to retro-Diels-Alder fragmentation and at *m*/*z* 317 $[C_{5'} - C_{8a}$ fission, $C_{16}H_{13}O_7]^+$ indicated flavanone nature of the compound possessing a methoxy group in the B ring. The ^{1}H NMR spectrum of **5** displayed three one-proton singlets at δ 6.96, 6.88 and 6.76 ppm assigned to aromatic H-6, H-6a and H-6', two one-proton doublets at δ 6.71 (J = 2,5 Hz) and 6.67 (J = 2.5 Hz) ppm ascribed to *meta*-coupled H-2'a and H-6'a, respectively, two one-proton multiplets at δ 5.76 ($w_{1/2}$ = 8.5 Hz) and 5.36 ($w_{1/2}$ = 8.3 Hz) ppm attributed to *cis*-oriented vinylic H-4'' and H-5'', respectively, two one-proton double doublets at δ 5.28 (J = 12.8, 2.9 Hz), 5.16 (J = 12.6, 2.7 Hz, ppm), and a one-proton doublet at δ 4.51 (J = 6.3 Hz) ppm due to correspondingly oxygenated methine H-2 and H-2a and β-oriented carbinol H-1'', a one-proton multiplet at δ 3.71 ppm with half-width of 14.7 Hz assigned to α-oriented carbinol H-2'' and methoxy protons as a three-proton singlet at δ 3.15 ppm. Four one-proton double doublets at δ 3.12 (J = 17.2, 12.8 Hz), 3.08 (J = 17.3, 12.7 Hz), 2.85 (J = 2.9, 17.2 Hz), 2.80 (J = 2.7, 17.3 Hz) ppm were due to methylene H_2-3 and H_2-3*a* supporting flavanone nature of the molecule (20-22). The remaining methylene of the side chain appeared at δ 2.95, 2.90, 2.64 and 2.48 ppm. Further evidences in support of the structure of compound **5** were drawn from its ^{13}C NMR spectrum which showed important signals for carboxylic carbon (δ 188.02 ppm, C-7''), carbonyl carbons (δ 197.85 ppm, C-4; 196.41 ppm, C-4a), vinylic carbons of side chain (δ 128.22 ppm, C-4''; 127.23 ppm, C-5''), oxygenated methine carbons (δ 78.59 ppm, C-2; 78.20 ppm, C-2a) and methoxy carbon (δ 55.72 ppm). Substituted aromatic carbons C-8, C-8a and C-2' appeared at δ 95.14, 93.33 and 115.51 ppm, respectively. The ^{1}H-^{1}H COSY spectrum of **5** showed correlations of H-2 with H-3; H-1'' with H-2''; H_2-3'', H-4'' and H_2-6 with H-5''; and H-2a with H-3a. The HMBC spectrum of **5** exhibited interactions of H-3 with C-4; H-2 and H-6' with C-1'; H-6 with C-7; H-6a with C-7a; H-3a with C-4a; H-2a, H-2'a and H-6'a with C-1'a. On the basis of above discussion, the structure of **5** was elucidated as 5,7,3',4',5'-pentahydroxy-8-(cis-1''α, 2''β-dihydroxyhept-4''-enyl-7''-oic acid)-flavanoyl-(2'→8a)-5a,7a,3'a,5'a-tetrahydroxy-4'a-methoxyflavanone, a new biflavonoid (Fig. 1).

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