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Research Article

NEW HOMOSESQUITERPENOL AND STIGMASTERYL DIGALACTOSIDE FROM THE STEM BARK OF *TERMINALIA ARJUNA*

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ABSTRACT
Terminalia arjuna (Roxb.) Wight et Arnot. (Combretaceae) is a large tree found throughout India. Its bark is used as a cardioprotective agent in hypertension and ischaemic heart diseases. Phytochemical investigation of the stem bark of *T. arjuna* (Roxb.) procured from Delhi furnished two new phytoconstituents characterized as 4-methyl-4-hydroxymethylene-6*β*-(10-methyl octanyl) cyclohexane (arjunahomosesquiterpenol) and stigmast-5,22-dien-3*β*-ol-3*β*-D-galactofuranosyl-(2′→1″)-*β*-D-galactofuranoside along with a known triterpenic glucoside termiarjunoside I. The structures of all the isolated compounds have been elucidated on the basis of spectral data analysis and chemical reactions.
KEYWORDS: *Terminalia arjuna*, Combretaceae, stem bark, homosesquiterpenol, stigmasteryl digalactoside.

INTRODUCTION

Terminalia arjuna (Roxb.) Wight et Arnot. (Combretaceae) is a deciduous, large tree distributed throughout India. Its stem bark is extensive used in Indian system of medicine as a cardiac tonic with particular efficacy against heart failure, ischaemic cardiomyopathy, artherosclerosis and coronary artery ailments[1,2]. The cardioprotective activities of bark have also been substantiated by various pharmacological evaluation and clinical trials[3]. The plant is also useful in cancer treatment[4]. A number of triterpenoids, e.g. arjunic acid, arjungenin, arjunglyosides, arjunetin, arjunolic acid[5,6]; termiarjunosides[7,8], olean-3*β*,22*β*-diol-12-en-28-oic acid-28*β*-D-glucopyranoside[9], terminolitin[10], flavonoids, e.g. arjunone, arjunolone and luteolin[11], tannins[12], naphthanol glycoside[7], phenolics[13], phytosterols[14] and cardenolide[15,16] have been isolated from the plants, but the issue of active principles and mechanism of therapeutic activity of *T. arjuna* remain to be elucidated. In present study, we have isolated homosesquiterpenol and stigmasteryl digalactoside along with termiarjunoside I from the stem bark of *T. arjuna*.

MATERIALS AND METHODS

General

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded on KBr pellet using a Jasco FT/IR-5000 instrument (FTS 135, Hongkong). The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were scanned on Avance DRX 400, Bruker spectrospin 400 MHz instrument (Rheinstetten, Germany) using $CDCl_3$ as solvent and TMS as internal standard. FAB-MS were measured using JEOL-JMS-DX 303 spectrometer (Peabody, MA, USA). Column (450×4×0.2 cm) chromatography was performed on silica gel (60-120 mesh, Qualigens, Mumbai, India) and thin layer chromatography on silica gel G-coated TLC plates (Merck). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying ceric sulphate solution.

Plant material

The stem bark of *T. arjuna* was procured from local market of Delhi, Khari Baoli and authenticated by Prof. M.P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen No. PRL/JH/08/47 was deposited in the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi, India.

Extraction and isolation

The stem bark of *T. arjuna* was dried at 45 ºC for 3 days and coarsely powdered. The powdered bark (3 kg) was extracted exhaustively with ethanol (95 %) in a Soxhlet apparatus. The ethanolic extract was concentrated under reduced pressure to yield a dark brown, viscous mass (700 g, 23.3 %). The dried extract was dissolved in minimum amount of methanol and adsorbed on silica on silica gel (60-120 mesh) for preparation of slurry. It was air dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1, and 1:3), chloroform and finally the mixture of chloroform and methanol (99:1, 49:1, 19:1, 9:1, 3:1, 1:1) and methanol in the order of increasing polarity to isolate the following compounds:

Arjunahomosesquiterpenol (1)

Elution of the column with petroleum ether-chloroform (3:1) afforded colourless crystals of **1,** recrystallized from acetone, 210 mg (0.0058 % yield, R_f: 0.6 (petroleum ether : chloroform, 9:1); m.p. 152-153 ºC; IR ν_{max} (KBr): 3520, 2877, 2855, 1435, 1260, 986, 795 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.87 (1H, brs, H_2-15a), 3.82 (1H, brs, H_2-15b), 2.57 (1H, m, $w_{1/2}$=15.2 Hz, H-6α), 2.31 (1H, m, $w_{1/2}$=15.5 Hz, H-10α), 1.60 (4H, brs, H_2-5, H_2-7), 1.55 (2H, m, CH_2), 1.29 (4H, m, CH_2), 1.25 (10H, m, 5×CH_2), 1.01 (3H, brs, Me-16), 0.96 (3H, d, *J*=6.5 Hz, Me-17), 0.88 (3H, t, *J*=6.1 Hz, Me-14); ^{13}C NMR ($CDCl_3$): δ 40.26 (C-1), 35.49 (C-2), 38.30 (C-3), 48.93 (C-4), 41.35 (C-5), 47.62 (C-6), 30.67 (C-7), 29.64 (C-8), 29.43 (C-9), 45.16 (C-10), 29.60 (C-11), 29.27 (C-12), 22.73 (C-13), 14.55 (C-14), 63.21 (C-15), 24.81 (C-16), 17.37 (C-17); +ve FAB MS *m/z* (rel. int): 255 $[M+H]^+$ ($C_{17}H_{35}O$) (12.8) 239 (11.3), 225 (10.6), 223 (12.5), 211 (11.2), 197 (12.3), 169 (23.6), 155 (53.6), 141 (29.3), 127 (42.8).

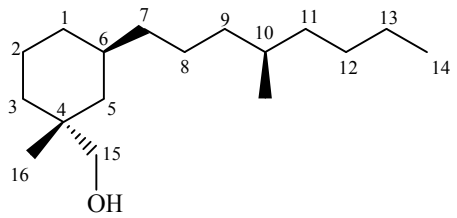
Stigmasteryl digalactoside (2)

Elution of the column with chloroform:methanol (9:1) furnished colourless crystals of **2**, recrystallized from methanol, 330 mg (0.0091 % yield, R_f: 0.7

(chloroform:methnol, 9:1); m.p. 252-253 ºC; IR ν_{max} (KBr): 3510, 3460, 3300, 2950, 2860, 1640, 1375, 1210, 1120 cm^{-1}; ^1H NMR (DMSO-D6): δ 5.31 (1H, brs, H-6), 5.29 (1H, m, H-22), 5.23 (1H, m, H-23), 4.93 (1H, d, *J*=7.8 Hz, H-1″), 4.89 (1H, d, *J*=7.8 Hz, H-1″), 4.28 (1H, dd, *J*=7.8, 6.8 Hz, H-2′), 4.21 (1H, dd, *J*=7.3, 6.8 Hz, H-2″), 4.15 (1H, d, *J*=6.8 Hz, H-3′), 4.00 (1H, d, *J*=6.5 Hz, H-3″), 3.71 (2H, brs, H_2-5′), 3.66 (2H, brs, H_2-5″), 3.58 (1H, brm, $w_{1/2}$=16.5 Hz, H-3α), 3.21 (2H, brs, H_2=6′), 3.14 (2H, brs, H_2-6″), 2.23-1.13 (25H, m, 9×CH_2, 7×CH), 1.09 (3H, brs, Me-19), 0.91 (3H, d, *J*=6.5 Hz, Me-21), 0.84 (3H, d, *J*=6.2 Hz, Me-26), 0.82 (3H, d, *J*=6.3 Hz, Me-27), 0.80 (3H, d, *J*=6.3 Hz, Me-29), 0.67 (3H, brs, Me-18); ^{13}C NMR (DMSO-D_6): δ 37.18 (C-1), 32.35 (C-2), 81.82 (C-3), 42.01 (C-4), 148.69 (C-5), 126.42 (C-6), 30.61 (C-7), 32.90 (C-8), 51.95 (C-9), 36.56 (C-10), 23.94 (C-11), 39.92 (C-12), 42.71 (C-13), 54.83 (C-14), 24.75 (C-15), 28.54 (C-16), 52.46 (C-17), 11.91 (C-18), 21.72 (C-19), 36.57 (C-20), 18.39 (C-21), 127.68 (C-22), 132.16 (C-23), 45.37 (C-24), 29.56 (C-25), 19.33 (C-26), 19.01 (C-27), 22.83 (C-28), 11.81 (C-29), 106.02 (C-1′), 83.65 (C-2′), 72.71 (C-3′), 85.56 (C-4′), 72.73 (C-5′), 60.61 (C-6′), 106.07 (C-1″), 83.19 (C-2″), 73.08 (C-3″), 84.02 (C-4″), 70.32 (C-5″), 61.33 (C-6″); +ve FAB MS *m/z* (rel. int): 737 $[M]^+$ ($C_{41}H_{69}O_{11}$) (11.0) 557 (11.2), 411 (10.1), 396 (9.8), 394 (9.2), 273 (22.6).

Termiarjunoside I (3)

Elution of the column with chloroform-methanol (17:3) gave colourless amorphous powder of **3,** recrystallized from chloroform-methanol (1:1), 335 mg (0.0093 % yield, R_f: 0.55 (chloroform:acetone, 4:1); m.p. 234-237 ºC; +ve FAB MS *m/z* (rel. int): 667 $[M+H]^+$ ($C_{36}H_{59}O_{11}$) (2.3).



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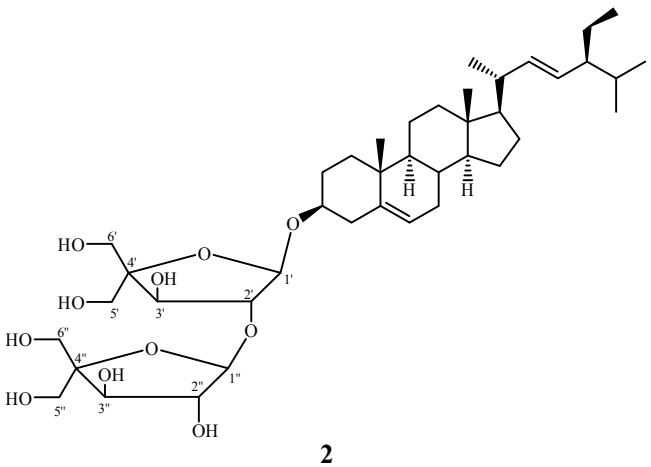

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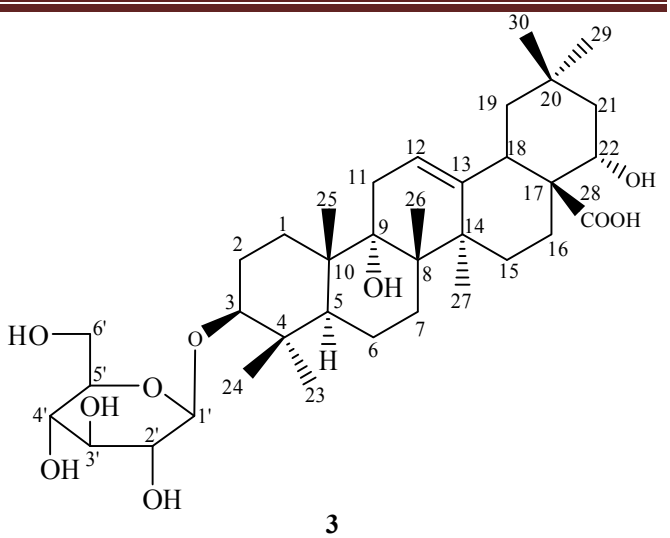

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RESULTS AND DISCUSSION

Compound **1**, named arjunahomosesquiterpenol, was obtained as colourless crystalline product from petroleum ether:chloroform (3:1) eluents. Its IR spectrum demonstrated the presence of a characteristic absorption band for hydroxyl group (3520 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular ion peak was determined at *m/z* 255 $[M+H]^+$ corresponding to structural formula of a homosesquiterpene molecule $C_{17}H_{35}O$. The prominent ion peaks arising at *m/z* 239 $[M-Me]^+$, 225 $[M-C_2H_5]^+$, 211 $[M-C_3H_7]^+$, 197 $[M-C_4H_7]^+$, 169 $[M-C_6H_{13}]$, 155 $[M-C_7H_{15}]^+$, 141 $[M-C_8H_{17}]^+$, 127 $[M-C_9H_{19}]^+$ and 223 $[M-CH_2OH]^+$ suggested that the molecule possessed a C_9-side chain attached to a hydroxyl substituted dimethyl cyclohexane ring. The ^1H NMR spectrum of **1** showed two one-proton broad singlets at δ 3.87 and 3.82 assigned to oxygenated C-15 methylene protons. A broad singlet at δ 1.01, a doublet at δ 0.96 (*J*=6.5 Hz) and a triplet at δ 0.88 (*J*=6.1 Hz), all integrated for three-proton each, were ascribed to tertiary C-16, secondary C-17 and primary C-14 methyl protons, respectively. The remaining methylene and methine protons appeared between δ 2.57-1.25. The ^{13}C NMR spectrum of **1** exhibited a hydroxyl methylene carbon signal at δ 63.21 (C-15), methyl carbons at δ 14.55 (C-14), 24.81 (C-16) and 17.37 (C-17) and other methylene and methine carbons from δ 45.16 to 22.73. The absence of any signal beyond δ 3.87 in the ^1H NMR spectrum and δ 63.21 in the ^{13}C NMR ruled out the existence of a vinylic linkage in the molecule. On the basis of the foregoing account, the structure of **1** has been established as 4-methyl-4-hydroxymethylene-6β-(10-methyl octanyl)-cyclohexane. This is a new homosesquiterpene.

Compound **2**, named stigmasteryl digalactoside, was obtained as colourless crystals from chloroform-methanol (9:1) eluents. It gave positive tests of steroidal glycosides and had disctinct IR absorption bands for hydroxyl groups (3510, 3460, 3300 cm^{-1}) and unsatuaration (1640 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra the molecular ion peak of **2** was determined at *m/z* 737 [M+H] consistent to the molecular formula of a steroidal diglycoside $C_{41}H_{69}O_{11}$. The ion peaks arising at *m/z* 557 $[M-C_6H_{11}O_6]^+$, 411$[M-C_{12}H_{21}O_{10}]^+$, 396 $[411-Me]^+$, 394 $[M-C_{12}H_{22}O_{10}]^+$ and 273 $[411-C_{10}H_{19}$, side chain$]^+$ indicated that the compound was a diglycoside of stigmasterol. ^1H NMR spectrum of **2** showed three one-proton signals as a broad singlet at δ 5.31 and as multiplets at δ 5.29 and 5.23 assigned to vinylic H-5, H-22 and H-23, respectively. A one-proton broad multiplet at δ

3.58 with half-width of 16.5 Hz was ascribed to oxygenated methine H-3α proton. Two one-proton doublets at δ 4.93 (J=7.8 Hz) and 4.89 (J=7.3 Hz) were attributed to anomeric H-1′ and H-1″ protons, respectively. The other sugar proton appeared from δ 4.28 to 3.14. Six three-proton signals as broad singlets at δ 0.67 and 1.09 and as doublets at δ 0.91 (J=6.5 Hz), 0.84 (J=6.2 Hz), 0.82 (J=6.3 Hz) and 0.80 (J=6.3 Hz) were associated with the tertiary C-18 and C-19, secondary C-21, C-26 and C-27 and primary C-29 methyl protons, respectively, all attached to unsaturated carbons. The other methine and methylene protons resonated from δ 2.23 to 1.13. The ^{13}C NMR spectral data of **2** showed signals for vinylic carbons at δ 148.69 (C-5), 126.42 (C-6), 127.68 (C-22) and 132.16 (C-23), oxygenated methine carbon at δ 81.82 (C-3), anomeric carbons at δ 106.02 (C-1′) and 106.07 (C-1″) and other sugar carbons from δ 83.65 to 60.61. The presence of H-1′ at δ 4.28 and H-1″ at δ 4.21 in the deshielded region and carbon signals at δ 83.65 (C-2′), 85.56 (C-4′), 83.19 (C-2″) and 84.02 (C-4″) in the downfield region suggested furanic forms of the sugar units and attachment of the sugar units in (2→1″) linkage. Acid hydrolysis of **2** yielded stigmasterol and galactoside. On the basis of these evidences the structure of new steroid digalactoside has been established as stigmast-5,22-dien-3β-ol-3β-D-galactofuranosyl-(2′→1″)-β-D-galactofuranoside.

Compound **3** was the known compound characterized as olean-1α,3β,9α,22α-tetraol-12-en-28-oic acid-3β-D-glucopyranoside (termiarjunoside I)[17].

CONCLUSION

The homosesquiterpenol and stigmasteryl digalactoside are isolated from the stem bark of *T. arjuna* for the first time which may be useful for therapeutic uses of the stem bark.

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