Diterpenic labdane galactofuranosides from the roots of *Calotropis procera* (Ait.) R. Br.

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Two labdane-type diterpenic galactosides characterized as labdan-18-ol-β-D-galactofuranoside 4 and labdan-3β-ol-11,15-oxide-18,20-dioic acid-3β-D-glucoturanoside 5 have been isolated for the first time from the roots of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae) along with *n*-decanoyl-β-D-glucopyranoside 1, *n*-hexacosanoyl-β-D-glucopyranoside 2 and *n*-octadecanoyl-β-D-glucopyranoside 3. The spectral data of 4 and 5 have been given and structures are established.

**Keywords:** *Calotropis procera* (Ait.) R. Br., Asclepiadaceae, roots, labdanyl galactosides, isolation

*Calotropis procera* (Ait) R. Br. (Asclepiadaceae), known as Apple of Sodom, Milkweed or Swallowwort, is a small, hardy, pubescent, evergreen, erect and compact shrub, up to 4.5 m high, covered with cottony tomentum. It exudes copious milky sap when cut. It grows wild in south eastern Asia including India, Pakistan and Afghanistan, tropical Africa, Indochina, Morocco and Senegal mainly in drier and warm regions up to 1,050 m altitude on coarse, sandy and alkaline soils\(^1\). The root is cylindrical, branched, curved, light, woody and grayish white. It resembles with the root of *Cephaelis ipecacuanha* (Broter) A. Richard (Rubiaceae) in medicinal properties and is substituted for it\(^2\). The roots are alterative, anthelmintic, depurative, diaphoretic, emetic, expectorant, febrifuge and purgative; prescribed to treat anasarca, asthma, ascites, bronchitis, cough, cutaneous diseases, intestinal worms, leprosy and eczema\(^3,4\). The root powder promotes gastric secretion; fresh root is used as tooth brush to cure toothache\(^1\). A root paste mixed with the leaves of *Ocimum sanctum* is taken orally to relieve menorrhoea\(^1\). The phytoconstituents cardenolides\(^5,6\), flavone glycoside\(^7\), pentacyclic triterpenoids\(^8,13\), sterols\(^7,14\), fatty acids\(^5\) and norditerpenyl ester\(^13\) have been reported from the roots. This manuscript describes the isolation and characterization of labdanyl galactosides and acyl glucosides from the roots of *C. procera* collected from the arid region of Rajasthan.

**Results and Discussion**

Compound 1, 2, 3 were the known phytoconstituents characterized as *n*-decanoyl-β-D-glucopyranoside, *n*-hexacosanoyl-β-D-glucopyranoside and *n*-octadecanoyl-β-D-glucopyranoside, respectively, on the basis of spectral data analysis\(^5,6\).

Compound 4, named labdanyl galactoside, was obtained as a colourless crystalline mass from chloroform-methanol (9:1) eluants. Its IR spectrum showed characteristic absorption bands for hydroxyl groups at 3387 and 3260 cm\(^{-1}\). On the basis of FAB mass and \(^{13}\)C NMR spectra, its molecular weight was established at \(m/z\) 457 [M+H]\(^+\) corresponding to a diterpenic glycoside, \(C_{26}H_{49}O_6\). It has three degrees of double bond equivalents. Two of them were adjusted in bicyclic ring system of labdane-type diterpene and the remaining one in the sugar moiety. The prominent ion peaks arising at \(m/z\) 179 \([C_6H_{11}O_3]^+\), 277 \([M – 179, C_{20}H_{37}]^+\) and 293 \([M- C_6H_{13}O_3, C_{20}H_{37} -O]^+\) suggested the attachment of \(C_6\)-sugar with the diterpenic residue. The \(^1\)H NMR spectrum of 4 showed a one-proton doublet at \(\delta\) 5.21 (J = 7.1 Hz) assigned to anomic H-1’ protons. A one-proton double doublet at \(\delta\) 4.60 (J = 7.1, 6.5 Hz), two one-proton multiplets at \(\delta\) 4.55 and 3.38, a one-proton
doublet at δ 3.62 (J = 6.5 Hz) and a two-proton doublet at δ 3.13 (J = 8.5 Hz) were ascribed to the other sugar protons H-2', H-4', H-3', H-5' and H-2'-6', respectively. A two-proton doublet at δ 3.32 (J = 8.5 Hz) was due to oxygenated methylene H-2-18 protons. Three broad signals at δ 1.19, 1.16 and 1.13, three protons each, a three-proton doublet at δ 1.01 (J = 6.0 Hz) and a three-proton triplet at δ 0.76 (J = 6.3 Hz) were associated with tertiary methyl Me-16, Me-17 and Me-19, secondary methyl Me-20 and primary methyl Me-15 protons, respectively, all attached to saturated carbons. The remaining methine and methylene protons appeared from δ 2.49 to 1.40. The 13C NMR spectrum of 4 showed signals for anomic carbons at δ 105.64 (C-1'), other sugar carbons from δ 82.48 to 61.02, oxygenated methylene carbon at δ 62.28 (C-18) and methyl carbons between δ 23.03 – 17.86. The presence of C-2' and C-4' carbons in the deshielded region at δ 82.48 and 77.21, respectively, indicated furanose form of the sugar unit. The 1H-1H COSY spectrum of 4 showed correlations of H-5 with Me-16, Me-17 and H-2-6; H-2-18 with H-2-7, H-9 and H-2-1'; and H-3' with H-1', H-2', H-2-5' and H-2-6'. The HMBC spectrum of 4 exhibited interactions of C-5 with H-2-3, Me-16, Me-17 and H-2-6; C-13 with H-2-12, Me-20, H-2-14 and Me-15; C-1' with H-2-18, H-2- and H-3'; and C-4' with H-2-5', H-2-6' and H-3'. The 1H and 13C NMR spectral values of 4 were compared with the reported labdane-type compounds.17-19 Acid hydrolysis of 4 yield β-D-galactose. On the basis of the foregoing account, the structure of 4 has been elucidated as labdan-18-ol-β-D-galactofuranoside. This is a new labdane-type galactoside isolated from a plant source.

Compound 5, named proceralabdanoside, was obtained as a colourless crystalline mass from chloroform-methanol (9:1) eluants. It effervescences with sodium bicarbonate solution and gave positive tests for glycosides. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3510, 3460, 3365 cm⁻¹), δ-lactone group (1730 cm⁻¹) and carboxylic functions (3280, 3190, 1690 cm⁻¹). On the basis of FAB mass and 13C NMR spectra, the molecular weight of 5 has been established at m/z 545 [M+H]+ consistent with the molecular formula of a diterpenic glycoside, C26H41O2. The ion fragments arising at m/z 498 [M -HCOOH]+ and 452 [498 - HCOOH]+ indicated the presence of two carboxylic functions in the molecule. The ion peaks generated at m/z 143 [C3H6O2COOH]+ and 401 [M - 143]+ supported the existence of one of the carboxylic group in the δ-lactones ring and another one in the bicyclic ring system. The ion peaks appearing at m/z 364 [M – C6H12O6]+, 349 [364 - Me]+, 221 [364 - 143]+, 381 [M – C6H11O5]+ and 180 [C6H12O6]+ suggested that C6 sugar unit was present in the molecule. The 1H NMR spectrum of 5 showed a one-proton doublet at δ 5.27 (J = 7.2 Hz) assigned to anomeric H-1' proton. A one-proton double doublet at δ 4.35 (J = 7.2, 6.8 Hz), two one-proton multiplets at δ 4.05 and 3.41, a one-proton doublet at δ 3.69 (J = 6.8 Hz) and a two-proton doublet at δ 3.17 (J = 9.1 Hz) were ascribed to furanic sugar H-2', H-4', H-5', H-3' and H-2-6' protons, respectively. A one-proton broad multiplet at δ 4.68 with half-width of 18.3 Hz and a one-proton double doublet at δ 4.16 (J = 9.3, 5.2 Hz) were attributed to α-oriented oxygenated methine H-11 and H-3 protons.
respectively. Three broad signals at δ 1.18, 0.98 and 0.95 integrating for three-protons each were due to correspondingly tertiary C-16, C-17 and C-19 methyl protons. The remaining methylene and methine protons appeared between δ 2.81–1.23. The 13C NMR spectrum of 5 exhibited signals for carboxylic carbons at δ 178.93 (C-18) and 177.50 (C-20), δ-lactone carbon at δ 174.95 (C-15), oxygenated methine carbons at δ 70.98 (C-3) and 71.85 (C-11), anomeric carbon at δ 105.36 (C-1’) and other sugar carbons from δ 82.57 to 60.59. The presence of C-2’ and C-4’ carbon signals in the deshielded region at δ 82.57 and 76.78, respectively, suggested furanic form of the sugar unit. The 1H-1H COSY spectrum of 5 showed correlations of H-1’ with H-2’ and H-3; H-5 with H-3, Me-16 and H-2; and H-11 with H-9, H-8, H-2’ and H-13. The HMBC spectrum of 5 exhibited interactions of C-3 with H-1’, H2-2, Me-16 and H-5; C-18 with H-8, H2-7 and H-9; C-20 with H-13, H2-14, and H2-12; and C-15 with H2-14, H-13 and H-11. The 1H and 13C NMR spectral values of 5 were compared with labdane type terpenoids. Acid hydrolysis of 5 yielded D-galactose. On the basis of spectral data analysis and chemical reactions, the structure of 5 has been established as labdan-3β-ol-11,15-olide-16,17-dioic acid-3β-D-galactofuranoside. It is a new labdane type diterpenic glycoside.

Experimental Section

Melting points were measured on a thermo-electrically operated Perfit apparatus and are uncorrected. UV-Vis spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as solvent. IR spectra were recorded in KBr pellet on Shimadzu FTIR-8400 spectrophotometer. 1H NMR (300 MHz) and 13C NMR (75 MHz) spectra were scanned by Bruker spectrospin NMR instrument, using TMS as internal standard. FAB mass spectra were recorded on a Jeol D-300 instrument. For column chromatography, silica gel (60-120 mesh, Merck, Mumbai, India) was used and thin-layer chromatography was performed on silica gel G coated TLC plates (Merck, Mumbai, India).

Plant material

The roots of C. procera was collected from semi-arid area in the neighbourhood of Jaipur, Rajasthan, and identified by Prof. M. P. Sharma, taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen (No. PRL/ JH / 08 / 32) is deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction and isolation

The air-dried roots (2 kg) of C. procera were coarsely powdered and extracted exhaustively in a Soxhlet apparatus with methanol for 72 hr. The methanolic extract was concentrated under reduced pressure to obtain a dark brown viscous mass (210 g). A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The extract (150 g) was dissolved in methanol (250 mL) and adsorbed on silica gel (60-120 mesh) for column chromatography. The slurry 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, 1:1, and 1:3), pure chloroform and finally the mixture of chloroform and methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having same Rf values were combined and crystallized. The isolated compounds were recrystallized to get the pure compounds.

Capryl glucoside, 1

Elution of the column with chloroform–methanol (49 : 1) afforded colourless crystals of 1 which were purified by recrystallization from methanol, 151 mg (0.0075% yield), Rf: 0.59 (MeOH), m.p. 149-50°C; +ve FAB-MS: m/z 335 [M+H]+ (C16H31O6) (1.1), 155 (73.2). Hydrolysis of 1 yielded capric acid and D-glucose.

Palmityl glucoside, 2

Elution of the column with chloroform–methanol (19 : 1) gave colourless mass of 2, purified by preparative TLC, 143 mg (0.0072% yield), Rf: 0.6 (methanol), m.p.135- 36°C; +ve FAB-MS: m/z (rel. int.): 419 [M+H]+ (C26H41O6) (18.3), 255 (22.0), 239 (24.1), 207 (21.0), 179 (25.6), 163 (17.2). Hydrolysis of 2 with ethanolic HCl yielded palmitic acid and D-glucose.

Stearyl glucoside, 3

Elution of the column with chloroform–methanol (93 : 7) afforded colourless crystals of 3, purified by recrystallization from methanol, 135 mg (0.00671%
yield), Rf: 0.5 (methanol), m.p. 151-52°C; +ve FAB-MS: m/z (rel. int.): 447 [M+H]+ (C_{26}H_{41}O_{7}) (10.1), 284 (6.9), 180 (6.8). Hydrolysis of 3 with ethanolic HCl yielded stearic acid and D-glucose.

Labdanyl galactoside, 4
Elution of the column with chloroform-methanol (9:1) furnished colourless crystals of 4, purified by recrystallization from chloroform-methanol (1:1), 145 mg (0.007% yield), m.p. 65-67°C, Rf: 0.90 (chloroform-methanol, 3:1); UV-Vis (methanol): 215 nm (log ε 1.3); IR (KBr): 3387, 3260, 2932, 1612, 1512, 1423, 1265, 1074, 862 cm⁻¹; ¹H NMR (DMSO-d₆): δ 5.27 (1H, d, J = 7.2 Hz, H-1'), 4.68 (1H, brm, w₁/₂ = 18.3 Hz, H-11a), 4.35 (1H, dd, J = 7.2, 6.8 Hz, H-2'), 4.16 (1H, dd, J = 9.3, 5.2 Hz, H-3'), 3.41 (1H, m, H-5'), 3.17 (2H, d, J = 9.1 Hz, H-6'), 2.81 (1H, d, J = 8.9 Hz, H-14α), 2.75 (1H, d, J = 4.5 Hz, H-14β), 2.31 (1H, m, w₁/₂ = 17.6 Hz, H-8a), 2.27 (1H, dd, J = 9.1,4.8 Hz, H-12α), 2.23 (1H, dd, J = 4.8,5.1 Hz, H-12β), 2.19 (2H, m, H-2'), 2.15 (1H, m, H-2α), 2.11 (1H, brm, w₁/₂ = 17.8 Hz, H-13α), 2.08 (1H, m, H-9), 2.01 (1H, m, H-7α), 1.97 (1H, m, H-7β), 1.93 (1H, m, H-1β), 1.86 (1H, m, H-5α), 1.23 (1H, m, H-6α), 1.21 (1H, m, H-6β), 1.18 (3H, brs, Me-16), 0.98 (3H, brs, Me-17), 0.95 (3H, brs, Me-19); ¹³C NMR (DMSO-d₆): δ 38.58 (C-1), 19.50 (C-2), 70.98 (C-3), 39.50 (C-4), 47.96 (C-5), 23.19 (C-6), 44.69 (C-7), 41.85 (C-8), 49.28 (C-9), 39.50 (C-10), 71.85 (C-11), 29.71 (C-12), 35.19 (C-13), 55.94 (C-14), 174.95 (C-15), 22.76 (C-16), 16.42 (C-17), 178.93 (C-18), 21.14 (C-19), 177.50 (C-20), 105.36 (C-1'), 82.57 (C-2'), 63.27 (C-3'), 76.78 (C-4'), 63.31 (C-5'), 60.59 (C-6'); +ve FAB-MS: m/z (rel. int.): 545 [M+H]+ (C_{26}H_{41}O_{7}) (11.3), 498 (22.5), 452 (33.6), 401 (23.5), 381 (26.7), 364 (24.0), 355 (23.8), 349 (33.4), 221 (18.3), 180 (21.9), 143 (18.6).

Acid hydrolysis of 5
Compound 5 (10 mg) was dissolved in ethanol (15 mL), conc. HCl (2 mL) added and the reaction mixture heated for 1 hr on a steam bath. The solution was acidified with dil. HCl to pH 3.0, extracted with chloroform (3 × 5 mL) to separate labdanoic acid and the aqueous solution concentrated. It was run on silica gel TLC with standard sample of D-galactose using n-butanol-acetic acid-water (4:1:5, upper layer) as a developing solvent system, Rf: 0.12, co-TLC comparable.

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References


