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Terpenoid glycosides from the roots of *Calotropis procera* (Ait.) R. Br.

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ABSTRACT

Calotropis procera (Ait.) R.Br. (Asclepiadaceae) is a laticiferous shrub found in tropical and subtropical Asia and Africa. Its roots are prescribed to treat cough, diarrhea, skin diseases, rheumatism and as emetic, expectorant and substituent for ipecacuanha. Two new terpenyl constituents characterized as bisabolan-11,14-diol-14-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside and 2-limonenyloxybenzoyl-1β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranoside along with the known compounds tricapryl glyceride and α-amyrin acetate have been isolated for the first time from the methanolic extract of the roots of C. procera. The structures of these phytoconstituents have been established by analysis of spectral data and chemical reactions.

Key words: *Calotropis procera*, Asclepiadaceae, roots, procerabisabolanyl diglucoside, proceralimonenyl tetraglycosidic salicylate.

INTRODUCTION

Calotropis procera (Ait) R. Br. (Asclepiadaceae), known as Apple of Sodom, Milkweed or Swallow-wort, is a small, hardy, pubescent, evergreen, erect and compact shrub, up to 4.5 m high, covered with cottony tomentum. It exudates 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 course, sandy and alkaline soils. Its growth is luxuriant on rubbish heaps, waste or fallow lands, along roadsides, sea shores and river bank [1]. The root is cylindrical, branched, curved, light, woody and grayish white. It resembles with the root of *Cephaelis ipecacuanha* (Broter) A. Richard

(family Rubiaceae) in action and is substituted for it. The roots are alterative, anthelmintic, depurative, diaphoretic, emetic, expectorant, febrifuge and purgative; used to treat anasarca, asthma, ascites, bronchitis, cough, cutaneous diseases, intestinal worms, leprosy and eczema [2,3]. 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 menorrhalgia [4]. 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 two new terpenyl glycosides from the roots of *C. procera* collected from the arid region of Rajasthan.

MATERIALS AND METHODS

General experimental Procedures

Melting points were measured on a Perfit apparatus and are uncorrected. IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded by Bruker spectrospin NMR instrument using TMS as internal standard. FAB ionization at 70 eV was scanned on a Jeol D-300 instrument (Jeol, USA). For Column chromatography, silica gel (60-120 mesh, Merck, Mumbai, india) was used. Thin-layer chromatography was performed on silica gel G coated TLC plates (Merck, Mumbai, India). Spots were visualized by exposure to iodine vapors, UV radiation and by spraying with ceric sulphate solution.

Plant material

The roots of *C. procera* was collected from waste land 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. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous dark brown mass was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for column chromatography for preparation of slurry. The slurry (200 g) 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, 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 R_f values) were combined and crystallized. The isolated compounds were recrystallized to get the pure compounds. The following compounds were isolated:

Tricapryl glyceride (1)

Elution of the column with petroleum ether yielded a semisolid mass of **1**, purified by preparative TLC, 40 mg (0.036% yield), R_f : 0.70 ($CHCl_3$); UV λ_{max} (MeOH]) : 207 nm (log ε 3.1); IR ν_{max} (KBr) **:** 1742, 1641, 720 cm^{-1}; +ve FAB MS *m/z* (rel. int.) : 555 $[M+H]^+$ ($C_{33}H_{63}O_6$) (14.8), 399 (21.2), 383 (25.6), 171 (22.7), 155 (100). Alkaline hydrolysis of **1** yielded capric acid, mp 31° C, co-TLC comparable.

α-Amyrin acetate (2)

Elution of the column with petroleum ether - chloroform (1 : 9) afforded colourless crystals of **2**, recrystallized from acetone, 25 mg (0.022% yield), R_f : 0.6 (petroleum ether-chloroform, 1:1); m.p.: 225-227º C; IR ν_{max} (KBr) : 1732, 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 5.12 (1H, m, H-12), 4.45 (1H, dd, J = 5.5, 9.0 Hz, H-3α), 2.03 (3H, brs, $COCH_3$), 1.13 (3H, brs, Me-25), 1.06 (3H, brs, Me-23), 1.02 (3H, brs, Me-27), 1.00 (3H, brs, Me-24), 0.97 (3H, d, J = 6.1 Hz,Me-30), 0.94 (3H, d, J = 6.3 Hz, Me-29), 0.91 (3H, brs, Me-28), 0.86 (3H, brs, Me-26); ^{13}C NMR ($CDCl_3$) : δ 80.43 (C-3), 123.82 (C-12), 139.08 (C-13), 170.43 (Ac), 20.92 ($COCH_3$); +ve FAB MS *m/z* (rel. int.) : 469 $[M+H]^+$($C_{32}H_{53}O_2$) (15.3).

Bisabolanyl diglucoside (3)

Elution of the column with chloroform - methanol (4 : 1) afforded colourless crystals of **3**, recrystallized from $CHCl_3$- MeOH (1:1), 40 mg (0.036 % yield), R_f. 0.3 ($CHCl_3$- MeOH, 7:3), m.p. : 115-116 ^{o} C; UV λ_{max} (MeOH) : 206 nm (log ε 3.7); IR ν_{max} (KBr): 3485, 3366, 3290, 2937, 2845, 1626, 1412, 1049, 927, 832 cm^{-1}. ^{1}H NMR (DMSO-d_6): 4.90 (1H, d, J = 7.5 Hz, H-1'), 4.87 (1H, d, J = 7.1 Hz, H-1''), 4.28 (1H, dd, J = 7.5, 7.3 Hz, H-2'), 4.07 (2H, m, H-5', H-5''), 3.82 (1H, m, H-2''), 3.65 – 3.47 (4H, m, H-3'. H-4', H-3'', H-4''), 3.25 (2H, d, J = 6.5 Hz, H_2-14), 3.13 (2H, d, J = 9.1 Hz, H_2-6'), 3.06 (2H, d, J = 6.9 Hz, H_2-6''), 2.73 (1H, m, H-6), 2.66 (1H, m, $w_{1/2=}$8.9 Hz, H-3α), 2.48 – 2.11 (8H, m, H_2-1, H_2-2, H_2-4, H_2-5), 1.66 (1H, m, H-7), 1.50 – 1.42 (6H, m, H_2-8, H_2-9, H_2-10), 1.20 (6H, brs, Me-12, Me-13), 0.79 (3H, d, J = 6.9 Hz, Me-15); ^{13}C NMR (DMSO-d_6): 35.08 (C-1), 29.47 (C-2), 42.33 (C-3), 29.49 (C-4), 26.16 (C-5), 49.53 (C-6), 37.67 (C-7), 25.64 (C-8), 22.56 (C-9), 30.56 (C-10), 74.75 (C-11), 21.38 (C-12), 21.40 (C-13), 62.60 (C-14), 15.01 (C-15), 104.44 (C-1'), 82.91 (C-2'), 72.06 (C-3'), 70.29 (C-4'), 77.69 (C-5'), 61.22 (C-6'), 92.22 (C-1''), 73.26 (C-2''), 72.04 (C-3''), 70.31 (C-4''), 74.72 (C-5''), 61.20 (C-6''); +ve FAB ms *m/z* (rel. int) : 567 $[M+H]^+$ ($C_{27}H_{51}O_{12}$) (21.2), 507 (16.3), 387 (19.2), 341 (15.6), 241 (18.7), 225 (28.1), 179 (27.2), 129 (23.8), 101 (78.9), 73 (31.2).

Hydrolysis of 3

Compound **3** (40 mg) was dissolved in ethanol (10 ml) and conc. HCl (3 ml) added. This mixture was heated on a steam bath for 1 hr. The solvent was concentrated under reduced pressure and chromatographed over silica gel TLC along with standard samples of sugar. The sugar was characterized as D-glucose. R_f 0.12 (n-butanol –AcOH- H_2O, 4:1:5, top layer).

Limonenyloxybenzoyl tetraglycoside (4)

Elution of the column with chloroform - methanol (1 : 1) gave colourless crystals of **4**, recrystallized from methanol, 45 mg (0.040% yield), R_f : 0.5 ($CHCl_3$ - MeOH, 1:3); m.p**. :** 307-

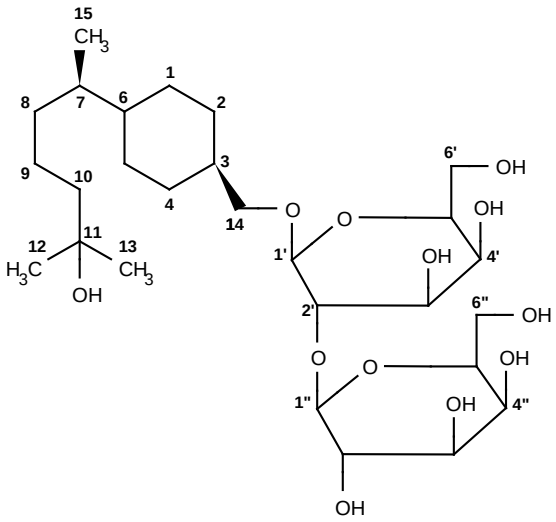
309º C; UV λ_{max} (MeOH) : 206, 276 nm (log ε 4.8, 1.1); IR ν_{max} (KBr) : 3515, 3433, 3260, 3180, 2950, 2864, 1680, 1644, 1507, 1053, 1396, 1031 cm.$^{-1}$. ^{1}H NMR (DMSO-d_6): 7.43 (1H, dd, J = 9.5, 2.3 Hz, H- bz 3), 6.56 (1H, dd, J = 8.9, 2.5 Hz, H- bz 6), 6.35 (1H, m, H- bz 4), 6.18 (1H, m, H- bz 3), 5.15 (1H, d, J = 7.1 Hz, H-1'), 4.93 (1H, d, J = 7.2 Hz, H-1"), 4.84 (2H, d, J = 7.3 Hz, H-1'", H-1""), 4.49 (2H, m, H-5', H-5"), 4.37 (1H, m, H-5'"), 4.22 (1H, m, H-5""), 4.13 (1H, m, H-2'), 4.05 (1H, m, H-2"), 4.02 (1H, m, H-2'"), 3.75 (1H, m, H-2'"), 3.68 – 3.38 (8H, m, H-3' to H-3"", H-4' to H-4""), 3.22 (1H, d, J = 10.2 Hz, H_2-10a), 3.19 (1H, d, J= 10.2 Hz, H_2-10b), 3.11 (2H, d, J = 8.7 Hz, H_2-6'), 3.08 (2H, d, J = 9.3 Hz, H_2-6"), 2.69 (1H, m, H-1), 2.25 (1H, m, H-4), 2.01 (1H, m, H-7), 1.83 – 1.21 (8H, m, H_2-2, H_2-3, H_2-5, H_2-6), 1.07 (3H, d, J = 6.1 Hz, Me-8), 0.91 (3H, d, J = 7.3 Hz, Me-9); ^{13}C NMR (DMSO-d_6): 56.13 (C-1), 29.28 (C-2), 28.90 (C-3), 55.09 (C-4), 29.32 (C-5), 29.35 (C-6), 48.84 (C-7), 24.82 (C-8), 25.13 (C-9), 63.95 (C-10), 109.98 (C-1'), 73.25 (C-2'), 70.41(C-3'), 71.99 (C-4'), 75.29 C-5'), 61.33 (C-6'), 96.99 (C-1"), 73.29 (C-2"), 70.38 (C-3"), 71.75 (C-4"),74.98 (C-5"), 60.95 (C-6"), 93.26 (C-1'"), 72.93 (C-2'"), 70.68 (C-3'"), 71.70 (C-4'"), 76.81 (C-5'"), 177.68 (C-6'"), 92.35 (C-1""), 72.44 (C-2""), 72.05 (C-3""), 70.68 (C-4""), 75.26 (C-5""), 178.22 (C-6""), 151.91 (C-bz 1), 162.36 (C-bz 2), 134.06 (C-bz 3), 124.82 (C-bz 4), 118.65 (C-bz 5), 120.03 (C-bz 6), 174.58 (C-bz 7). +ve FAB MS *m/z* (rel. int.): 953 $[M+H]^+$ ($C_{41}H_{61}O_{25}$) (3.1), 353 (24.8), 193 (39.8), 155 (100), 139 (37.2), 137 (36.0), 121 (16.8), 112 (33.0), 96 (74.8).

RESULTS AND DISCUSSION

Compounds **1** and **2** were the known phytoconstituents characterized as tricapryl glyceride [15] and α-amyrin acetate [16], respectively.

Compound **3**, named bisabolanyl diglucoside, was obtained as a colourless crystalline mass from chloroform-methanol (4:1) eluants. It responded positively to tests of glycosides. Its IR spectrum displayed characteristics absorption bands for hydroxyl groups (3485, 3366, 3290 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular ion peaks of **3** was determined at *m/z* 567 $[M+H]^+$ pointing out to the molecular formula of a sesquiterpenic diglucoside, $C_{27}H_{51}O_{12}$. The ion fragments arising at *m/z* 179 $[C_6H_{11}O_6]^+$, 387 $[M-179]^+$, 341 $[C_6H_{10}O_6 - C_6H_{10}O_5]^+$ and 225 $[M - 341]^+$ indicated that diglucosyl moiety was linked to a bisabolanediol-type sesquiterpene. The ion peaks generating at *m/z* 507 $[M-C_3H_7O, C_{10} - C_{11}$ fission$]^+$, 73 $[C_9 - C_{10}$ fission, $CH_2C(CH_3)_2$ OH$]^+$, 101 $[C_7$-C_8 fission, $(CH_2)_3$ C$(CH_3)_2$ OH$]^+$ and 129 $[C_6 - C_7$ fission, $C_8H_{17}O]^+$ suggested the presence of one of the hydroxyl group at C – 11. The ^{1}H NMR spectrum of **3** exhibited two one-proton doublets at δ 4.90 (J = 7.5 Hz) and 4.87 (J = 7.1 Hz) assigned to anomeric H-1′ and H-1″ protons, respectively. A one-proton double doublet at δ 4.28 (J = 7.5, 7.3 Hz) was attributed to sugar H-2′ proton and its deshielding position suggested the attachment of the second sugar moiety at C-2′. The other sugar protons appeared from δ 4.07 to 3.06. A two - proton doublet at δ 3.25 (J = 6.5 Hz) was attributed to oxygenated C-14 methylene protons. A six-proton broad signal at δ 1.20 was accounted to tertiary C-12 and C-13 methyl protons establishing the existence of the tertiary hydroxyl group at C-11. A three- proton doublet at δ 0.79 (J = 6.9 Hz) was associated with secondary C-15 methyl protons. The remaining methine and methylene protons resonated between δ 2.73 – 1.42. The ^{13}C NMR spectrum of **3** showed 27 carbon signals and the important signals appeared for anomeric carbons at δ 104.44 (C - 1′) and

92.22 (C - 1″), other sugar carbons between δ 82.91 – 61.20, oxygenated methylene carbon at δ 62.60 (C - 14), methyl carbons at δ 21.38 (C-12), 21.40 (C-13) and 15.01 (C-15). The shifting of C-2′ carbon signal in the downfield region at δ 82.91 supported location of another sugar moiety at C-2′. The HMBS spectrum of **3** exhibited correlations of C-11 with Me-12, Me-13 and H_2-10; C-14 with H_2-2, H_2-4 and H-1'; and C-2' with H-1', H-3' and H-1''. Acid hydrolysis of **3** yielded D-glucose. On the basis of the above mentioned discussion, the structure of **3** has been formulated as bisabolan-11,14-diol-14-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside. This is a new sesquiterpenic diglucoside.



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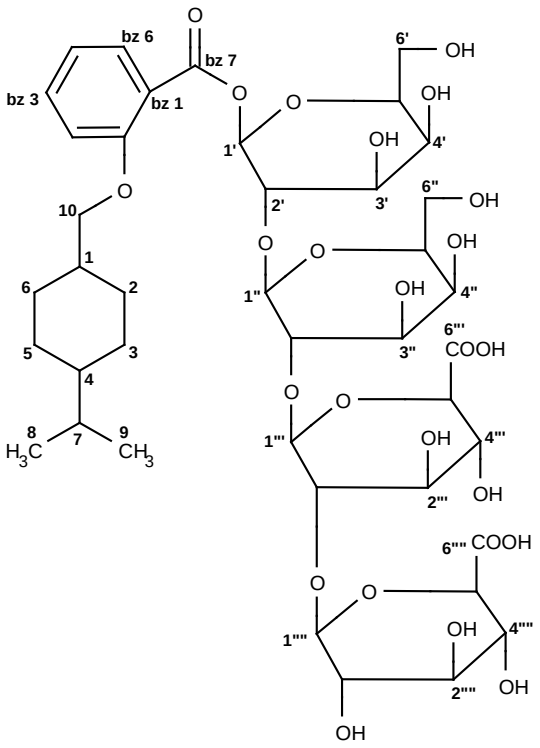
Compound **4,** designated as limonenyloxybenzoyl tetraglycoside, was obtained as a colourless crystalline mass from chloroform – methanol (1:1) eluants. It gave positive tests for glycosides and produced effervescences with sodium bicarbonate solution. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3515, 3433, 3260 cm^{-1}) and carboxylic function (3180, 1680 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular formula of **4** has been established at *m/z* 953 $[M+H]^+$ consistent with the molecular formula of a monoterpenic benzoyl tetraglycoside, $C_{41}H_{61}O_{25}$. The ion fragments arising at *m/z* 193 $[C_6H_9O_7]^+$ and 353 $[C_6H_9O_6\text{-}C_6H_8O_6]^+$ indicated that two glucuronic acid units were attached at the terminal of the glycosidic chain. The ion peaks generating at *m/z* 121 $[C_6H_4(OH)CO]^+$ and 137 $[C_6H_4(OH)COO]^+$ supported the attachment of the salicylate unit in the glycosidic chain. The ion peaks produced at *m/z* 155 $[C_{10}H_9O]^+$, 112 $[155 –C_3H_7]^+$, 139 $[C_{10}H_{19}]^+$ and 96 $[139 – C_3H_7]^+$ suggested the existence of tetrahydrolimonenyl type moiety as an aglycone molecule. The ^1H NMR spectrum of **4** showed two one – proton double doublets at δ 7.43 (J = 9.5, 2.3 Hz) and 6.56 (J = 8.9, 2.5 Hz) and two one-proton multiplets at δ 6.35 and 6.18 assigned to aromatic H-3, H-6, H-4 and H-3 protons, respectively. Two one - proton doublets at δ 5.15 (J = 7.1 Hz) and 4.93 (J = 7.2 Hz) and a one-proton doublet at δ 4.84 (J = 7.3 Hz) were ascribed to anomeric H-1', H-1", H-1'" and H-1"" protons, respectively. The other sugar protons appeared between δ 4.49 – 3.08. Two one- proton doublets at δ 3.22 (J = 10.2) and 3.19 (J = 10.2 Hz) were accounted to oxygenated methylene H_2-10 protons. Two three - proton doublets at δ 1.07 (J = 6.1 Hz) and

0.91 (J = 7.3 Hz) were associated with the secondary methyl H_3-8 and H_3-9 protons, respectively. The remaining methine and methylene protons resonated from δ 2.69 to 1.21. The ^{13}C NMR spectrum of **2 s**howed signals for 41 carbon atoms and the important signals appeared for carboxylic carbons at δ 177.68 (C-6''') and 178.22 (C-6''''), ester carbon at δ 174.58 (bz-7), aromatic carbon from δ 162.36 to 118.64, anomeric carbons at δ 109.98 (C-1'), 96.99 (C-1''), 93.26 (C-1''') and 92.35 (C-1''''), oxygenated methylene carbon at δ 63.95 (C-10), 61.33 (C-6') and 60.95 (C-1''), other sugar carbons from δ 76.81 to 70.38 and methyl carbons at δ 24.82 (C-8) and 25.13 (C-9). The downfield shifting of H-2' proton at δ 4.13, H-2'' at δ 4.05 and H-2''' at δ 4.02 in the ^1H NMR spectrum and C-2' at δ 73.25, C-2'' at δ 73.29 and C-2''' at δ 72.93 in the ^{13}C NMR spectrum suggested the location of the sugar (1→2) chain. The presence of C-1' signal at δ 109.98 in the ^{13}C NMR spectrum suggested its attachment to the benzoyl group. The existence of the other anomeric carbons in the upfield region between δ 96.99-92.35 supported the attachment of the anomeric carbons to the sugar units. The HMBC spectrum of **4** showed correlations of C-bz 2 with H- bz 3, H-bz 4 and H $_2$-1; C-bz 7 with H-bz 6 and H-1'; C-2' with H-1', H-3' and H-1''; C-2'' with H-1'', H-3'' and H-1'''; C-6''' with H-5'''; and C-6'''' with H-5'''. Acid hydrolysis of **4** yielded salicylic acid, D - glucuronic acid and D - glucose. On the basis of spectral data analysis and chemical reactions, the structure of **4** has been elucidated as 2-limonenyloxybenzoyl-1β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranoside. This is a new monoterpenic tetraglycoside.



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CONCLUSION

Phytochemical investigation of the roots of *Calotropis procera* led to the isolation of new sesquiterpenic diglycoside and monoterpenic tetraglycoside. This is the first report of occurring terpenyl glycosides in the roots of *Calotopis* species of arid region and may be used as chromatographic markers for this species.

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