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Aliphatic and phenolic glycosides from the roots of *Calotropis procera* (Ait.) R.Br.

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Abstract: An *n*-butanyl diglucuronoside and a phenolic glycoside characterized as n-butan-1,4-diol-1,4-β-D-diglucuronopyranoside and O-methylresorcinyl-β-D-glucuronopyranosyl-(2→1)-β-D- glucopyranosyl-(2→1)-β-D-glucopyranoside, respectively, have been isolated for the first time from the methanolic extract of the roots of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae) along with the known compounds α-amyrin acetate and β-sitosterol glucoside. The structures of all the phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.
Keywords: *Calotropis procera*, Asclepiadaceae, Roots,butanediol diglucuronoside, methyl resorcinyl triglycoside.

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 menorrhoea[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 new aliphatic and phenolic glycosides from the roots of *C. procera* collected from the arid region of Rajasthan.

Experimental

Melting points were determined on a Perfit melting point apparatus (Ambala, India) and are uncorrected. IR spectra were recorded on KBr discs, using a Bio-Rad FT-IR 5000 spectrometer (FTS 135, Hongkong, China). UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. ^{1}H and ^{13}C NMR spectra were scanned using Bruker Advance DRY 400 spectrospin and Bruker Advance DRY 100 spectrospin

instruments (Germany), respectively, in DMSO-d_6 and TMS as an internal standard. FAB MS spectra were obtained using JEOL-JMS-DX 303 spectrometer (Bruker Daltonics, MA, USA). Column chromatography was performed on silica gel 60-120 mesh. TLC was run on silica gel G (Qualigens, Mumbai, India). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying reagents.

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.

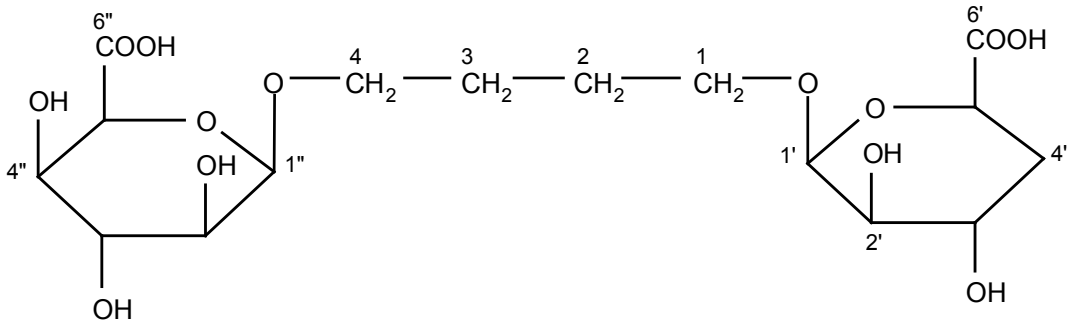
Extraction: The air-dried roots (2 kg) of *C. procera* were coarsely powdered and extracted exhaustively with methanol in a Soxhlet apparatus for 72 hr. The methanolic extract was concentrated under reduced pressure to obtain dark brown viscous mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous dark brown mass was adsorbed on silica gel (60-120 mesh) for column chromatography after being dissolved in little quality of methanol 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 and 1:3) finally with pure chloroform. 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 purified by preparative TLC and recrystallization.

α-Amyrin acetate: Elution of the column with petroleum ether - chloroform (1 : 9) afforded colourless crystals of α-amyrin acetate **(1)**, recrystallized from acetone, 25 mg (0.022% yield), R_f : 0.6 (petroleum ether-chloroform, 1:1); m.p.: 225-227^o C; IR ν_{max} (KBr) : 1732, 1638 cm^{-1}; ^1H 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.) : 468 $[M]^+$ ($C_{32}H_{52}O_2$) (15.3).

β-Sitosterol glucoside: Elution of the column with chloroform-methanol (19:1) furnished colourless amorphous powder of β-sitosterol glucoside (2), recrystallized from methanol, 260 mg (0.013% yield); m.p.: 270-272°; R_f: 0.53 (benzene: chloroform: methanol; 5:4:1). IRν_{max} (KBr**)**: 3450, 2917, 2849, 2383, 1636, 1460, 1074, 795 cm^{-1}; +ve ESI MS *m/z* (*rel. int.*): 576 $[M]^+$ ($C_{35}H_{60}O_6$).

Butanediol diglucuronoside: Elution of the column with chloroform - methanol (4 : 1) gave colourless crystals of butanediol diglucuronoside (3), recrystallized from MeOH, 25 mg (0.022% yield), R_f : 0.8 ($CHCl_3$-MeOH, 1:3), m.p. : 209 – 211 ^{o} C; UV λ_{max} (MeOH) : 212 nm (log ε 3.2); IR ν_{max} (KBr) : 3425, 3388, 3255, 2924, 2854, 1680, 1610, 1404, 1083, 1040 cm^{-1}. ^1H NMR (D_2O) : δ 5.08 (1H d, J=7.2 Hz H-1′), 4.90 (1H, d, J = 7.1 Hz, H-1"), 4.68 (1H, d, J = 6.3 Hz, H=5'), 4.49 (1H, d, J = 8.1 Hz, H-5"), 4.22 (1H, m, H-2'), 4.19 (1H, m, H-2"), 4.01 (1H, m, H-3'), 4.92 (1H, m, H-3"), 3.72 (1H, m, H-4'), 3.64 (1H, m, H-4"), 3.26 (2H, brs, H_2-1), 3.15 (2H, brs, H_2-4), 2.61 (2H, m, H_2-2), 2.37 (2H, m, H_2-3); ^{13}C NMR (D_2O) : δ 64.20 (C-1), 50.42 (C-2), 43.18 (C-3), 64.20 (C-4), 100.12 (C-1'), 74.11 (C-2'), 72.75 (C-3'), 71.41 (C-4'), 75.58 (C-5'), 181.94 (C-6'), 100.08 (C-1"), 74.09 (C-2"), 72.72 (C-3"), 71.38 (C-4"), 75.56 (C-5"), 179.77 (C-6"); +ve FAB MS *m/z* (*rel. int.*) : 442 $[M]^+$ ($C_{16}H_{26}O_{14}$) (11.3), 397 (38.2), 352 (49.6), 334 (18.3), 193 (51.8), 177 (25.3), 149 (29.6), 133 (35.0).

Compound 3 (5 mg) was dissolved in ethanol (5 ml), dil. HCl (2 ml) added and reaction mixture was heated for 1 hour on a steam bath. The solvent was concentrated under reduced pressure to get β-D-glucuronic acid, mp 164-165^{o}C, R_f 0.13 (phenol saturated with H_2O).



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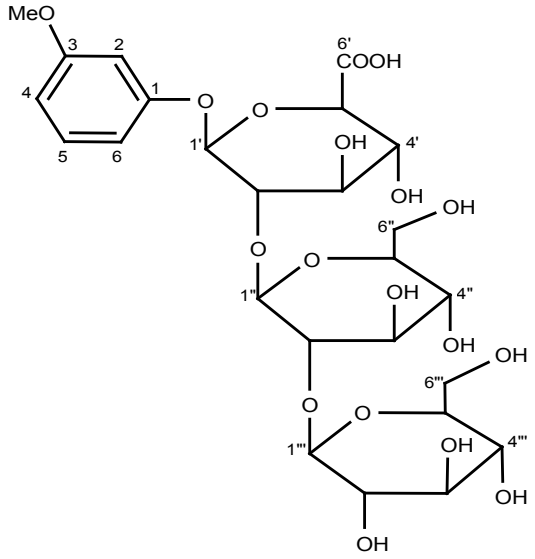
Methyl resorcinyl triglycoside: Elution of the column with $CHCl_3$ - MeOH (3 : 2) furnished colourless crystals of methyl resorcinyl triglycoside (4), recrystallized from MeOH, 51 mg (0.044 % yield), R_f : 0.7 ($CHCl_3$- MeOH, 13:7), m.p. : 160-161^o C; UV λ_{max} (MeOH) : 212, 285 nm (log ε 1.3, 0.7); IR ν_{max} (KBr) : 3562, 3389, 3337, 3280, 2940, 2850, 1690, 1460, 1366, 1210, 1003, 942 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.47 (1H, d, J=3.0 Hz, H-2), 6.57 (1H, m, H-4), 6.22 (1H, m, H-6), 5.59 (1H, m, H-5), 5.13 (1H, d, J= 7.8 Hz, H-1'), 4.89 (2H, m, H-1", H-1'"), 4.75 (1H, m, H-5'), 4.69 (1H, m, H-5"), 4.46 (3H, brs, OMe), 4.31 (1H, m, H-5'"). 4.05 (1H, dd, J=7.8, 7.6 Hz, H-2'), 4.02 (1H, m, H-2"), 3.00 (1H, m, H-2'"), 3.87 (1H, m, H-3'), 3.70 (1H, m, H-3"), 3.64 (1H, m, H-3'"), 3.60 (1H, m, H-4'), 3.57 (1H, m, H-4"), 3.48 (1H, m, H-4'"), 3.19 (1h, d, J=8.1 Hz, H_2-6"a), 3.15 (1H, d, J=8.1 Hz, H_2-6"b), 3.04 (1H, d, J=9.0 Hz, $H_2$6'"a), 3.01 (1H, d, J=9.0 Hz, H_2-6'"b); ^{13}C NMR (DMSO-d_6): δ 162.30 (C-1), 151.81 (C-2), 164.15 (C-3), 124.71 (C-4), 122.06 (C-5), 140.13 (C-6), 108.87 (C-1'), 75.25 (C-2'), 72.84 (C-3'), 71.91 (C-4'), 73.05 (C-5'), 178.16 (C-6'), 96.94 (C-1"), 74.91 (C-2"), 73.15 (C-3"), 70.35 (C-4"), 76.77 (C-5"), 61.24 (C-6"), 92.27 (C-1'"), 72.65 (C-2'"), 72.84 (C-3'"), 70.62 (C-4'"), 72.38 (C-5'"), 61.22 (C-6'"), 56.02 (OMe); +ve ion ESI MS *m/z* (rel. int.) : 624 $[M]^+$ ($C_{25}H_{36}O_{18}$) (2.1), 342 (2.6), 180 (100).

Discussion

Compound **1** and **2** were the known phytoconstituents characterized as α-amyrin acetate and β-sitosterol glucoside, respectively.

Compound **3**, named butanediol diglucuronoside, was obtained as a colourless crystalline mass from chloroform – methanol (4 : 1) eluants. It gave effervescences with sodium bicarbonate solution indicating the presence of carboxylic group in the molecule. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3425, 3388 cm^{-1}) and carboxylic function (3255, 1680 cm^{-1}). On the basis of FAB mass and ^{13} NMR spectra, the molecular weight of 3 has been established at *m/z* 442 corresponding to the molecular formula of an aliphatic diol diglycoside, $C_{16}H_{26}O_{14}$. It indicated four double bond equivalents, two each of them were adjusted in the glycosidic units and carboxylic groups. The ion fragments arising at *m/z* 397 $[M - COOH]^+$, 352 [397-COOH]$^+$ and 334 $[352 - H_2O]^+$ supported the presence of two carboxylic groups in the molecule.



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The ion peaks generating at *m/z* 193 $[C_5H_8O_5 - COOH]^+$, 149 $[193\text{-}CO_2]^+$, 177 $[C_5H_8O_4 - COOH]^+$ and 133 $[177 - CO_2]^+$ suggested the existence of glucuronic acid units in the molecule . The ^{1}H NMR spectrum of 3 exhibited two one-proton doublets at δ 5.08 (J=7.2 Hz) and 4.90 (J=7.1 Hz) assigned to

anoneric H-1' and H-1" proton respectively. Two one-proton doublets at δ 4.68 (J = 6.3Hz) and 4.49 (J = 8.1 Hz) were ascribed to sugar H-5' and H-5", respectively. The other sugar protons appeared from δ 4.22 to 3.64. Two broad signals at δ 3.26 and 3.15 were attributed correspondingly to oxygenated methylene H_2-1 and H_2-4 protons. Two multiplets at δ 2.61 and 2.37 integrating for two protons each were associated with the methylene H_2-2 and H_2-3 protons respectively. The ^{13}C NMR spectrum of 3 showed the presence of sixteen carbon signals and the important for carboxylic carbons at 181.94 (C-6′) and 179.77 (C-6″), anomeric carbons at δ 100.12 (C-1′) and 100.08 (C-1") other sugar carbons from δ 75.58 to 71.38, oxygenated methylene carbons at δ 64.20 (C-1, C-2) and other methylene carbons at δ 50.42 (C-2) and 43.18 (C-3). The absence of any singnal beyond δ 5.08 in the ^{1}H NMR spectrum and between δ 179.77 to 100.12 in the ^{13}C NMR spectrum supported the saturated nature of the molecule. Acid hydrolysis of 3 yielded β-D- glucuronic acid. On the basis of the foregoing evidences, the structure of 3 has been characterized as n-butan-1,4-diol-1,4-β-D diglucuronopyranoside.

Compound 4, named methyl resorcinyl triglycoside, was obtained from chloroform - methanol (3 : 2) eluants. It produced effervescences with sodium bicarbonate solution and gave positive tests for glycosides. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3562, 3389, 3337 cm^{-1}) and carboxylic group (3280, 1690 cm^{-1}). On the basis of ^{1}H and ^{13}C NMR spectra, the molecular weight of 4 was established at m/z 624 corresponding to the molecular formula of a phenolic glycoside, $C_{25}H_{36}O_{18}$. The ion fragments arising at m/z 180 $[C_6H_{12}O_6]^+$ and 342 $[C_6H_{11}O_5$-$C_6H_{10}O_6]^+$ indicated that diglucosidic unit was attached to the terminal of the triglycoside unit. The ^{1}H NMR spectrum of 4 exhibited a one-proton doublet at δ 7.47 (J = 3.0) assigned to meta - coupled aromatic H-2 proton. Three one-proton multiplets at δ 6.57, 5.59 and 6.22 were ascribed to aromatic H-4, H-5 and H-6 protons, respectively. A one-proton doublet at δ 5.13 (J = 7.8 Hz) and a two - proton broad signal at δ 4.89 were attributed to anomeric H-1' and to H-1" and H-1"' protons, respectively. The other sugar proton signals appeared from δ 4.75 to 3.01. A three - proton broad signal at δ 4.46 was accounted to methoxy proton. The ^{13}C NMR spectrum of 4 displayed carbon signals for 25 carbon atoms and the important signals appeared for carboxylic carbon at δ 178.16 (C-6'), aromatic carbons in the range of δ 164.15 - 122.06, methoxy carbon at δ 56.02 anomeric, carbons at δ 109.87 (C-1'), 96.94 (C-1") and 92.27 (C-1"') and hydroxymethylene carbons at δ 61.24 (C-6") and 61.22 (C-6"'). The other sugar carbons resonated from δ 76.77 to 70.35. The presence ^{1}H NMR signals of H-2' and H-2" as a one - proton double doublet at 4.05 (J = 7.8, 7.6 Hz) and as a one-proton multiplet at δ 4.02 and ^{13}C NMR signals for C-2' and C-2" at δ 75.25, and 74.91 respectively, all in deshielded region, suggested (2→1) linkage of the sugar units. Acid hydrolysis of 4 yielded glucuronic acid and D-glucose. On the basis of spectral data analysis and chemical reactions, the structure of 4 has been determined as O-methyl resorcinyl -β-D-glucuronopyranosyl (2→1)-β-D-glucopyranosyl-(2→1)-β-D-glucopyranoside.

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