INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
ISSN 2230 – 8407
Available online www.irjponline.com
Research Article

PHYTOCHEMICAL INVESTIGATION OF THE ROOTS OF *CALOTROPIS PROCERA* (AIT.) R. BR.

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Article Received on: 10/07/11 Revised on: 14/08/11 Approved for publication: 20/09/11

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ABSTRACT
Two new phytoconstituents characterized as n-tetradecanyl n-hexadec-9-enoate (n-tetradecanyl palmitoleate) (**4**) and 18∝H – urs– 12 – en - 3β, 25 - olide (proceraursenolide) (**5**) have been isolated for the first time from the roots of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae) along with the known compound tridecanoyl glycerol (**1**), 1-octadec-9′-enoyl -2,3- dihexadecanoyl glycerol (**2**), tridocosanoyl glycerol (**3**), urs-12-en-3β-olyl acetate (α-amyrin acetate) (**6**) and 18αH-urs-12, 20(30)-dien-3β-yl acetate (**7**). The structures of all these phytoconstituents have been established by spectral data analysis and chemical reactions.
KEYWORDS: *Calotropis procera*, Asclepiadaceae, roots, triterpenoids, palmitoleate ester, glycerides.

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 ester and triterpenic lactone along with glycerides and triterpenic esters from the roots of *C. procera* collected from the arid region of Rajasthan.

MATERIAS 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 in $CDCl_3$, 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).

Plant material

The roots of *C. procera* were 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 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) and 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.

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.*) : 554 $[M]^+$ ($C_{33}H_{62}O_6$) (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.

Oleodipalmityl glyceride (2)

Elution of column with petroleum ether - chloroform (9 : 1) gave colourless semi-solid of **2**, purified by preparative TLC, 45 mg (0.040% yield, R_f : 0.4 ($CHCl_3$- MeOH, 97:3); IR ν_{max} (KBr) : 1721, 1645, 714 cm^{-1}; +ve FAB MS *m/z* (*rel int.*) 832 $[M]^+$ $[C_{53}H_{100}O_6]$ (1.1), 577 (9.8), 338 (14.3), 265 (12.8), 255 (21.8), 239 (23.7). Alkaline hydrolysis of **2** yielded palmitic and oleic acids, co-TLC comparable.

Tribehenyl glyceride (3)

Elution of the columns with petroleum ether - chloroform (1 : 1) afforded pale yellow oily compound **3**, 45 mg (0.040% yield), R_f : 0.8 ($CHCl_3$-MeOH, 99:1); IR ν_{max} (KBr) : 1731, 1640, 725 cm^{-1}; +ve FAB MS *m/z* (*rel. Int.*) : 1058$[M]^+$ ($C_{69}H_{134}O_6$) (1.5), 719 (10.5), 396 (15.3), 380 (9.8), 339 (15.6), 323 (6.5). Alkaline

hydrolysis of 3 produced behenic acid, mp 85-87° C, co-TLC comparable.

n-Tetradecanyl palmitoleate (4)

Further elution of the column with petroleum ether - chloroform (1 : 3) afforded colourless mass of **4**, recrystallized from acetone, 70 mg (0.062 % yield), R_f : 0.9 ($CHCl_3$-MeOH, 97:3); m.p. : 71-72 ^{o} C; UV λ_{max} (MeOH) : 212 nm (log ε 3.9); IR ν_{max} (KBr) : 2917, 2849, 1740, 1642, 1462, 1377, 1255, 1167, 1102, 719 cm^{-1}; ^1H NMR ($CDCl_3$) : δ 5.77 (1H, m, H-9), 5.01 (1H, m, H-10), 4.58 (1H, brs, H_2-1′a), 4.46 (1H, brs, H_2-1′b), 2.31 (2H, m, H_2-2), 2.05 (2H, m, CH_2-8), 2.02 (2H, m, CH_2-11), 1.74 (8H, brs, 4xCH_2), 1.25 (34H, brs, 17xCH_2), 0.87 (3H, t, J = 6.9 Hz, Me-16), 0.85 (3H,t, J = 6.0 Hz, Me-14′); ^{13}C NMR ($CDCl_3$) : δ 172.06 (C-1), 128.51 (C-9), 123.98 (C-10), 63.13 (C-1'), 55.79 (C-2), 43.87 (CH_2), 42.68 (CH_2), 32.21 (CH_2), 31.84 (CH_2), 29.61 (18x CH_2), 22.61 (CH_2), 14.03 (Me-16, Me-14'); +ve FAB MS *m/z* (rel. int.) : 450 $[M]^+$ ($C_{30}H_{58}O_2$) (11.2), 253 (21.8). Alkaline hydrolysis of 4 yielded palmitoleic acid, co-TLC comparable.

Procerausenolide (5)

Elution of the column with petroleum ether - chloroform (1:3) mixture gave colourless amorphous powder of **5**, recrystallized from acetone, 25 mg (0.022% yield), R_f : 0.4 (petroleum ether – $CHCl_3$, 3:1); m.p.: 85-86^o C; UV λ_{max} (MeOH) : 208 nm (log ε 2.1); IR ν_{max} (KBr) : 2955, 2924, 2852, 1728, 1654, 1464, 1369, 1294, 1188, 1097, 990 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.18 (1H, dd, J= 9.9, 8.1 Hz, H-12), 4.51 (1H, dd, J= 8.7, 5.6 Hz, H-3α), 1.06 (3H, brs, Me-23), 1.01 (3H, brs, Me-27), 0.97 (3H, brs, Me-24), 0.95 (3H, d, J=6.5 Hz, Me-30), 0.87 (3H, brs, Me-28), 0.83 (3H, d, J=6.6 Hz, Me-29), 0.79 (3H, brs, Me-26); ^{13}C NMR ($CDCl_3$): δ 37.71 (C-1), 29.09 (C-2), 80.63 (C-3), 38.44 (C-4), 55.26 (C-5), 18.24 (C-6), 32.95 (C-7), 39.62 (C-8), 47.63 (C-9), 43.29 (C-10), 22.45 (C-11), 124.34 (C-12), 138.59 (C-13), 41.53 (C-14), 29.67 (C-15), 25.79 (C-16), 33.16 (C17), 59.06 (C-18), 39.62 (C-19), 39.86 (C-20), 31.23 (C-21), 41.33 (C-22), 28.73 (C-23), 15.69 (C-24), 170.15 (C-25), 17.49 (C-26), 23.65 (C-27), 26.60 (C-28), 16.84 (C-29), 21.37 (C-30). +ve FAB MS *m/z* (rel. int.) : 438 $[M]^+$($C_{30}H_{46}O_2$) (2.5), 423 (5.8), 408 (71.1), 393 (11.2), 220 (25.2), 218 (65.8), 205 (32.4), 203 (51.0), 191 (32.5), 190 (42.8), 188 (25.1), 176 (21.8), 175 (18.2), 173 (18.0), 161 (26.4), 158 (32.3), 146 (34.7), 134 (43.8), 131 (17.6), 119 (28.3).

α-Amyrin acetate (6)

Elution of the column with petroleum ether - chloroform (1 : 9) afforded colourless crystals of **6**, 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).

Proceraursenyl acetate (7)

Elution of the column with chloroform gave colourless crystals of **7**, recrystallized from acetone, 25 mg (0.022% yield), R_f : 0.8 (petroleum ether - $CHCl_3$, 1:3); m.p.: 133 -135^o C; IR ν_{max} (KBr) : 1726, 1640 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.11 (1H, m, H-12), 4.59 (2H, brs, H_2-30), 4.45 (1H, dd, J = 5.6, 9.6 Hz, H-3α), 2.03 (3H, brs, Ac); ^{13}C NMR ($CDCl_3$): δ 8.71 (C-3), 124.03 (C-12), 139.26 (C-13), 154..13 (C-20), 113.90 (C-30), 170.75, 21.19 (Ac); +ve FAB MS *m/z* (rel. int.) : 466 $[M]^+$ ($C_{32}H_{50}O_2$) (23.7).

RESULTS AND DISCUSSION

The compounds **1**, **2**, **3**, **6** and **7** were the known phytoconstituents characterized as tricapryl glyceride, oleodipalmityl glyceride, tribehenyl glyceride, α-amyrin acetate and proceraursenyl acetate, respectively, on the basis of their spectral data analysis. The fatty acid glycerides are reported from the roots of *C. procera* for the first time**.**

Compound **4**, named n-tetradecanyl palmitoleate, was obtained as a colourless crystalline mass from petroleum ether – chloroform (1 : 3) eluants. It decolorized bromine water due to the presence of unstauration. Its IR spectrum showed characteristic absorption bands for ester groups (1740 cm^{-1}), unsaturation (1642 cm^{-1}) and long aliphatic chain (719 cm^{-1}). The FAB mass and ^{13}C NMR spectra of **4** established a molecular ion peak at *m/z* 450 consistent to a molecular formula of fatty acid ester, $C_{30}H_{58}O_2$. The generation of an ion peak at *m/z* 253 $[CH_3(CH_2)_5CH{=}CH(CH_2)_7COO]^+$ suggested that palmitoleic acid was esterified with an aliphatic alcohol. The ^1H NMR spectrum of **4** showed two one-proton multiplets at δ 5.77 and 5.01 assigned to vinylic H-9 and H-10 protons, respectively. Two one- proton broad signals at δ 4.58 and 4.46 were ascribed to oxygenated methylene H_2-1' protons. The remaining methylene protons appeared between δ 2.31-1.25. Two three- proton triplets at δ 0.87 (J = 6.9 Hz) and 0.85 (J = 6.0 Hz) were accounted to primary methyl H_3-16 and H_3-14' protons, respectively. The ^{13}C NMR spectrum of **4** displayed signals for ester carbon at δ 172.06 (C-1), vinylic carbons at δ 128.51 (C-9) and 123.98 (C-10), oxygenated methylene carbon at δ 63.13 (C-1'), other methylene carbons between δ 55.79-22.61 and methyl carbons at δ 14.03 (C-16, C-14'). Alkaline hydrolysis of **4** yielded palmitoleic acid. On the basis of above mentioned evidences, the structure of **4** has been established as n-tetradecanyl n-hexadec-9-enoate. This is a new aliphatic ester.

Compound **5** , named proceraursenolide, was obtained as a colourless amorphous powder from petroleum ether – chloroform (1:3) eluant. It responded positively to Liebermann - Burchard test for triterpenoids. Its IR spectrum showed characteristic absorption bands for ester group (1728 cm^{-1}) and unsaturation (1654 cm^{-1}). On the basis of FAB mass and ^{13}C spectra, its molecular weight was established at *m/z* 438 $[M]^+$ consistent to the molecular formula of a triterpenoid lactone, $C_{30}H_{46}O_2$. It indicated eight double bond equivalents; five of them were adjusted in the pentacyclic carbon framework of a triterpenoid, one in the vinylic linkage and two in the lactone ring. The generation of the ion peaks at *m/z* 423 [M - Me]$^+$, 408 [423 - Me]$^+$ and 393 [408 - Me]$^+$ indicated that free hydroxyl or carboxylic groups were absent in the molecule. The ion fragments arising at *m/z* 220 and 218 due to retro – Diels – Alder fragmentation suggested the existence of the vinylic linkage at C-12. The prominent ion peaks formed at *m/z* 205 [220 - Me]$^+$, 190 [205 - Me]$^+$ and 175 [190 - Me]$^+$ ruled out free hydroxyl group in ring A. The ion peaks appearing at *m/z* 203 [218 - Me]$^+$, 188 [203 - Me]$^+$, 173 [188 - Me]$^+$ and 158 [173 - Me]$^+$ supported the location of the methyl function in rings C, D and E. The prominent ion peak

at *m/z* 134 arose due to expulsion of the C_6H_{12} unit [$C_{17,22}$ - $C_{18,19}$ fission]$^+$ and at *m/z* 119 [134 - Me]$^+$ suggested saturated nature of the ring E. The ion peaks produced at *m/z* 191 [218 - $CHCH_2$]$^+$, 176 [191- Me]$^+$, 161 [176 - Me]$^+$, 146 [161 - Me]$^+$ and 131 [146 - Me]$^+$ supported retro-Diels Alder fragmentation due to the presence of C-12 vinylic linkage .The ^{1}H NMR spectrum of **5** exhibited a one - proton double doublet at δ 5.18 (J = 9.9, 8.1 Hz) assigned to vinylic H-12 proton. A one- proton double doublet at δ 4.51 with coupling interactions of 8.7, 5.6 Hz was attributed to α-oriented oxygenated methine H-3 proton and its shifting in the deshielded region indicated its lactone form. Five three proton broad signals at δ 1.06, 0.97, 1.01 and 0.87 and 0.79 and two three- proton doublets at δ 0.83 (J = 6.6 Hz) and 0.95 (J = 6.5 Hz) were ascribed to tertiary C-23, C-24, C-26, C-27 and C-28 and secondary C-29 and C-30 methyl protons of ursene - type triterponiod, respectively. The remaining methylene and methine protons resonated between δ 2.30 - 1.13. The ^{13}C NMR spectrum of **5** displayed 30 carbon signals and the important signals appeared for lactone carbon at δ 170.15 (C-25), oxygenated methine carbon at δ 80.63 (C-3), vinylic carbons at δ 124.34 (C-12) and 138.59 (C-13) and methyl carbons between δ 28.73 - 15.69. The ^{1}H and ^{13}C NMR spectral data of **5** have been compared with the ursene- type triterpenoids[15,16]. On the basis of these evidences the structure of **5** has been elucidated as 18∝H – urs– 12 – en - 3β, 25 - olide. This is a new triterpenoids lactone.

In conclusion, the phytochemical investigation of the roots collected from the arid region of Rajasthan indicated marked variation in the chemical constituents of the herbal drugs. The earlier reported cardenolides could not be isolated from this plant. The chromatographic finger-printing developed for the plant would not be applicable for the plant grown in arid zone.

ACKNOWLEDGEMENT

The authors are thankful to the Head, SAIF, Central Drug Research Institute, Lucknow for recoding the spectral data of the phytoconstituents.

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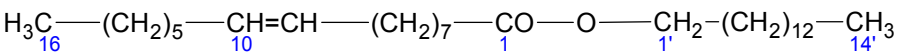

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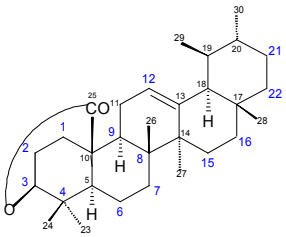

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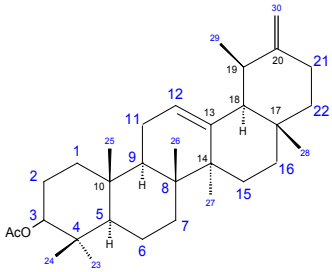

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Source of support: Nil, Conflict of interest: None Declared