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Norditerpenic ester and pentacyclic triterpenoids from root bark of *Calotropis procera* (Ait) R. Br.

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A new norditerpenyl ester, named Calotropterpenyl ester, and two unknown pentacyclic triterpenoids, namely calotropursenyl acetate and calotropfriedelenyl acetate have been isolated from the root bark of *Calotropis procera*. Their structures have been established as 6,10,14-trimethylpentadec-6-enyl-2′, 4′, 8′, 12′, 16′-pentamethyl nonadecane ester, urs-12,19(29)-diene-3β-yl acetate and friedelin-1-ene-3β-yl acetate, respectively, on the basis of spectral data analyses and chemical reactions.

1. Introduction

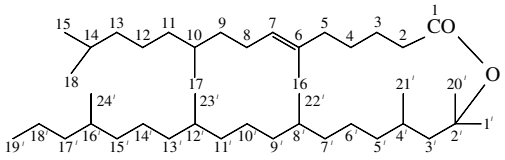
The genus *Calotropis* (Asclepidiaceae) is a glabrous or hairy laticiferous shrub or small tree, commonly known as "The Swallow Wort" or "Milk Weed" and distributed in tropical and subtropical Asia and Africa [1]. Its root bark is used in curing skin diseases, enlargement of abdominal viscera, intestinal worms, cough, ascitas, anaserca, snakebite, toothache, dysentery, syphilis and as a substitute of Ipecacuanha [2]. It contains digitanol [3], calotropin [4], ursane type triterpenes [5, 6], sterols [6] and fatty acids [7]. This paper describes the isolation and characterization of one new norditerpenic ester and two unknown pentacyclic triterpenic esters from the petroleum ether extract of the root bark of *C. procera*.

2. Investigations, results and discussion

Calotropterpenyl ester (**1**) was obtained as a colourless crystalline powder from petroleum ether eluants. Its IR spectrum exhibited absorption bands for the ester group (1730 cm^{-1}) and unsaturation (1625 cm^{-1}). Its MS showed a molecular ion peak at m/z 618 related to a norditerpenic ester formula, $C_{42}H_{82}O_2$. The spectrum had a base peak at m/z 265 due to the elimination of a norditerpenoyl unit containing an olefinic bond. The other important ion peaks appearing at m/z 222 $[265\text{-}C_3H_7]^+$, 283 $[M^+\text{-}265\text{-}C_5H_{11}]^+$, 240 $[M^+\text{-}265\text{-}C_8H_{17}]^+$ and 129 $[M\text{-}265\text{-}C_{16}H_{33}]^+$, 183 $[C_{13}H_{27}]^+$, 113 $[C_8H_{17}]^+$ and 71 $[C_5H_{11}]^+$ suggested that the norditerpenoyl moiety is esterified with a norsesterterpenoxyl unit. The ^1H NMR spectrum of **1** exhibited a one-proton triplet at δ 5.13 (J = 5.1 Hz) assigned to H-7. A three-proton broad singlet at δ 1.80 was due to the C-16 methyl group attached to the C-6 olefinic carbon. Two three-proton broad singlets at δ 1.23 and 1.20 were attributed to C-1′ and C-20′ methyl protons, respectively attached to the oxygenated carbon. Two doublets at δ 0.97 (J = 6.5 Hz) and 0.77 (J = 6.0 Hz), integrated three protons each, were ascribed to C-22′ and C-21′ secondary methyls, respectively. The remaining methyls resonated at δ 0.83 and 0.73. The ^{13}C NMR spectrum of **1** showed 42 carbon signals. The signals at δ 139.62 and 124.3 were assigned to C-6 and C-7 olefinic carbons. The ester carbonyl carbon appeared at δ 171.01. The ^{13}C NMR values were compared with those of acyclic diterpenes [8, 9].

Hydrolysis of **1** yielded a norsesterterpenyl alcohol (**2a**), IR ν_{max} 3445 (OH) cm^{-1}, $[M]^+$ m/z at 354 ($C_{24}H_{50}O$), and a norditerpenoic acid (**1b**) $[M]^+$ m/z at 282 ($C_{18}H_{34}O_2$). On the basis of these evidences the structure of **1** has been elucidated as 6,10,14-trimethylpentadec-6-enyl-2′,4′,8′,12′,16′-pentamethyl nonadecane ester. This contributes the first report of occurrence of a diterpenic ester in a *Calotropis* species.

Compound **2**, named calotropursenyl acetate, responded positively to the Liebermann-Burchard test of triterpenes. Its molecular weight was established as 466 $[M]^+$ relating to the molecular formula $C_{32}H_{50}O_2$ on the basis of MS and ^{13}C NMR data. Its IR spectrum showed the presence of a acetate group (1730 cm^{-1}) and a methylene group (1640, 880 cm^{-1}). The ^1H NMR spectrum of 2 displayed signals for vinylic protons on C-12 (δ 5.20, 1 H) and C-29 (δ 4.66–4.60, 2 H), an equatorial carbinol proton (δ 4.50, dd, J = 9.5, 5.0 Hz) and an acetoxy group (δ 2.05, 3 H), six tertiary methyl (δ 1.03, Me-27, 1.00, Me-23, 0.90, Me-26, 0.86, Me-28; 0.83, Me-25-25; 0.80, Me-24) and a C-30 secondary methyl (δ 0.96, d, J = 6.5 Hz). The ^1H NMR values were compared with those of calotropenyl acetate and other similar triterpenes [5, 10]. The MS of **2** showed important ions associated with amyrins and at m/z 423 $[M\text{-}CH_3CO]$ and 206 $[M\text{-}CH_3COOH]^+$. The significant peaks at m/z 249 and 217 corresponded to the typical retro-Diels-Alder



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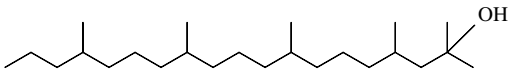
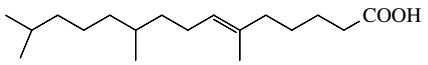
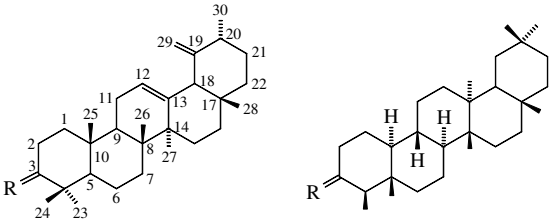


1a



1b



cleavage of the Δ^{12}-pentacyclic skeleton at C_4-C_{11} and C_8-C_{14} bonds with a methylene group attached at C-19. This was confirmed by the peaks appearing at m/z 202 [217-Me]$^+$, 135 [217–82]$^+$. 219 [249-2xMe]$^+$, 189 [249-CH_3COOH]$^+$, 174 [189-Me]$^+$, 159 [174-Me]$^+$ and 82 [$H_2C = CCH(CH_3)CH_2CH_2$]$^+$. These data suggested that the acetate group was present in ring A.

The ^{13}C NMR data were compared with those of calotropenyl acetate [10] and other triterpenes [12]. The spectrum showed the presence of 32 carbon signals. The signals at δ 124.81 (C-12), 140.70 (C-13), 154.60 (C-19) and 107.10 (C-29) were assigned to olefinic carbons. The C-3 methine carbon appeared at δ 81.01. Treatment of **2** with ethanolic potassium hydroxide at reflux temperature afforded calotropursenol (**2a**). The value of the important ion peak at m/z 217 did not alter in the MS of **2a** which supported the location of acetoxy groups in ring A. Further treatment of the free alcohol **2a** with Jones reagent yielded calotropuresonone (**2b**) (IR 1710 cm^{-1}). The ketone **2b** responded positively to the Zimmermann test [13] for 3-oxo triterpenoids. The $NaBH_4$ reduction [10] of **2b** regenerated the parent alcohol **2a**, confirming the equatorial orientation of the hydroxyl group in **2a** and, hence, the acetoxy group in **2**. On the basis of these evidences, the structure of **2** was elucidated as urs-12,19 (29)-dien-3β-yl acetate.

Calotrofriedelenyl acetate (**3**), obtained from benzene fractions, showed a positive liebermann-Burchard test. Its IR spectrum showed characteristic absorption bands for an ester group (1730 cm^{-1}) and unsaturation (1635 cm^{-1}). It exhibited a molecular ion peak at m/z 468 in its MS corresponding to the molecular formula $C_{32}H_{52}O$. The MS of **3** was characteristic of pentacyclic friedelane-type triterpenes in which the rings A, B, C and D were saturated. The major peaks were generated at m/z 408 [M-ACOH]$^+$, 453 [M-Me]$^+$, 205 [C_8-C_{14} and $C_{12}-C_{13}$ fission]$^+$, 202 [M-206-ACOH]$^+$, 219 [M-206-Ac]$^+$, 204 [219-Me]$^+$, 189 [204-Me]$^+$, 248 [M-206-CH_2]$^+$ and 174 [189-Me]$^+$. These data suggested the presence of the acetate group in ring A. The value of the important peak at m/z 205 did not change on deacetylation of compound **3**. The ^{1}H NMR spectrum of **3** showed two vinylic double doublets at δ 6.17 (J = 6.3, 4.5 Hz) and 6.60 (J = 5.5, 4.5 Hz) assigned to H-1 and H-2, respectively. The C-3 carbinol proton appeared as a double doublet at δ 4.20 (J = 9.0, 5.5 Hz). A three-proton broad singlet at δ 2.00 was ascribed to an acetyl group. Seven tertiary methyl signals resonated in between δ 1.20–0.83. A three proton doublet at δ 1.06 J = 6.0 Hz was associated to the C-23 secondary methyl group. The ^{13}C NMR spectrum of **3** displayed 32 carbon signals. The signals at δ 170.90 and 21.34 were assigned to a acetyl group. The C-3 carbinol carbon appeared at δ 80.90. Two downfield signals at δ 139.60 (C-1) and 124.31 (C-2) were ascribed to olefinic carbons. The nature of each carbon was determined by DEPT experiments. The ^{13}C NMR values were compared with friedelin and several of its derivatives [14–16]. Treatment of **3** with ethanolic potassium hydroxide at reflux temperature yielded a free alcohol **3a** which on further treatment with Jones reagent formed the ketone **3b**. The ketone **3b** responded positively to the Zimmermann-test [13] for 3-oxo terpenoids. On the basis of these evidences the structure of **3** was elucidated as friedelin-1-ene-3β-yl acetate.

3. Experimental

3.1. General procedure

M.p.'s were determined on a perfit apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DRX-300-300 MHz instrument in $CDCl_3$ using TMS as internal standard. ^{13}C NMR spectra were screened on a Bruker DRX-300 75.50 MHz instrument in $CDCl_3$. MS were scanned on a Jeol D-300 instrument. CC was carried out using silica gel (60–120 mesh). Homogeneity of the compounds was checked on silica gel G coated TLC plates in solvent systems: (a) C_6H_6-petroleum ether (1 : 1), $C_6H_6-CHCl_3$ (9 : 1) and petroleum ether-EtOAc (9 : 1). Iodine vapours, perchloric acid, ceric sulphate and an UV lamp were used for visualization of TLC spots.

3.2. Plant material

Roots (22 kg of *C. procera* were colected from the South Delhi region and identified by Dr. M. P. Sharma Taxonomist, Department of Botany, Faculty of Science. Jamia Hamdard. A voucher specimen is deposited at the Herbarium of the Department.

3.3. Extraction

The dried and pulverized root bark (2 kg) was extracted with EtOH 95% in a Soxhlet apparatus. The extract was concentrated under reduced pressure to get a dark brown viscous semi-solid mass (185 g). The dried alcoholic extract was dissolved in minimum amount of MeOH and adsorbed on silica gel to form a slurry. The slurry was air-dried and chromatographed over a silica gel column prepared in petroleum ether. The column was eluted with petroleum ether, petroleum ether-$CHCl_3$ (9 : 1, 3 : 1, 1 : 1, 1 : 3 v/v), $CHCl_3$, $CHCl_3$-MeOH (99 : 1, 99 : 2, 95 : 5, 3 : 1, 1 : 1, 1 : 3 v/v) and MeOH to isolate the following compounds:

3.4. Characterisation of the compounds

3.4.1. Calotropterpenylester (1), compounds 1a and 1b

Elution of the column with petroleum ether furnished colourles crystals of **1**, recrystallized from MeOH-Me_2CO (1 : 1), 11.13 g (0.56% yield), R_f 0.75 (petroleum etherbenzene, 1 : 1), m.p. 118–119 °C, [α_D] +3.10 (C 0.9648 in MeOH). UV λ_{max} (MeOH) 241 nm (log ε 5.3), IR ν_{max} 2955, 2885, 1730, 1625, 1460, 1445, 1370, 1245, 1030, 995, 755 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 5.13 (1 H, t, J = 5.1 Hz, H-7), 1.80 (3 H, brs, H_3-16) 1.23 (3 H, brs, H_3-1′), 1.20 (3 H, brs, H_3-20′), 0.97 (3 H, d, J = 6.5 Hz, H_3-22′), 0.83 (9 H, H_3-17, H_3-19′, H_3-23′), 0.77 (3 H, d, J = 6.0 Hz, H_3-21′, 0.73 (9 H, brs, H_3-15, H_3-18, H_3-24′). EIMS m/z (rel. int.) 618 [M]$^+$ ($C_{42}H_{82}O_2$), (22.9) 283 (19.2), 265 (100), 240 (50.2), 222 (30.7), 201 (34.6), 183 (21.9), 181 (28.2), 165 (28.1), 163 (35.8), 129 (19.3), 115 (55.7), 113 (40.2), 97 (54.1), 85 (19.6), 71 (21.3), 57 (52.3), 43 (38.5), ^{13}C NMR ($CDCl_3$); δ 171.01 (c-4), 40.04 (C-2), 31.25 (C-3), 29.68 (C-4), 39.61 (C-5), 139.62 (C-6), 124.3 (C-7), 39.37 (C-8), 28.74 (C-9), 42.08 (C-10), 27.95 (C-11), 26.59 (C-12), 28.11 (C-13), 39.22 (C-14), 16.50 (C-15), 22.42 (C-16), 17.49 (C-17), 16.87 (C-18), 21.41 (C-1′), 81.02 (C-2′), 37.03 (C-3′), 59.11 (C-11′), 33.74 (C-5′), 32.88 (C-6′), 25.81 (C-7′), 55.27 (C-8′), 23.39 (C-9′), 23.60 (C-10′), 36.80 (C-11′), 47.61 (C-21′), 23.22 (C-13′), 38.01 (C-100), 37.71 (C-15′), 44.02 (C-16′), 22.53 (C-17′), 38.51 (C-18′), 18.23 (C-19′), 15.72 (C-20′), 16.00 (C-21′), 16.31 (C-22′), 16.73 (C-23′), 21.38 (C-24′).

Hydrolysis of **1**: Compound **1** (100 mg) was heated with alcoholic 0.1 N KOH solution (10 ml) for 30 min. Water (20 ml) was added and the reaction mixture was extracted with $CHCl_3$ (3 × 10 ml). The organic phase was washed with H_2O (2 × 15 ml), dried (Na_2SO_4) and evaporated to get norsesterterpenyl alcohol (**1a**), m.p. 91–92 °C IR ν_{max} 3445 cm^{-1}, EIMS m/z (rel int.) 354 [M]$^+$ ($C_{24}H_{50}O$) (3.8), 336 (11.2), 281 (7.1), 253 (7.6), 211 (8.3), 183 (9.1), 113 (15.2), 71 (61.9), 43 (100).

After extraction with $CHCl_3$ the mother liquor, was acidified to Congo red and re-extracted with $CHCl_3$. After drying on Na_2SO_4 and evaporation, norditerpenoic acid (**1b**) was obtained, m.p. 101–103 °C, IR ν_{max} 3250, 1690 cm^{-1} EIMS m/z 282 [M]$^+$ ($C_{18}H_{34}O_2$), (9.1), 265 (2.3), 238 (6.5), 181 (21.2), 141 (12.3), 127 (14.5), 113 (21.6), 85 (53.7), 71 (55.1), 57 (67.6), 43 (100).

3.4.2. Calotropursenyl acetate (2), compounds 2a and 2b

Elution of the column with petroleum ether furnished a colourless amorphous mass of **2**, recrystallized from $CHCl_3$–MeOH (1 : 1), 3.5 g (0.018% Yield), R_f 0.80 (petroleum etherbenzene, 1 : 1), m.p. 140–142 °C, $[\alpha]_D^{24}$ − 16.3 (C = 0.55, MeOH), UV λ_{max} 217, 240 nm (log ε 5.5, 5.3), IR ν_{max} 2945, 2860, 1730, 1640, 1465, 1390, 1255, 1010, 880 cm^{-1}.

^{1}H NMR ($CDCl_3$), 5.20 (1 H, m, H-12), 4.66–4.60 (2 H, brs, CH_2-29), 4.50 (1 H, dd, J = 9.5, 3.0 (Hz, H-3α), 2.05 (3 H, brs, $COCH_3$), 1.03 (3 H, brs, Me-27), 1.00 (3 H, brs, Me-23), 0.96 (3 H, d, J = 6.5 Hz, Me-30), 0.90 (3 H, brs, Me-26), 0.86 (3 H, brs, Me-28), 0.83 (3 H, brs, Me-25), 0.80 (3 H, brs, Me-24).

EIMS m/z (rel. int.) 466 $[M]^+$ ($C_{32}H_{50}O_2$) (44.2), 423 (30.7), 406 (9.0), 391 (14.0), 249 (20.3), 219 (43.8), 217 (44.5), 202 (47.6), 189 (79.3), 174 (22.9), 159 (34.8), 135 (61.3), 120 (52.2), 107 (81.1), 95 (89.6), 82 (100), 55 (80.9), 43 (42.7).
^{13}C NMR: δ 38.41 (C-1), 23.65 (C-2), 81.01 (C-3), 37.76 (C-4), 54.42 (C-5), 18.15 (C-6), 34.48 (C-7), 40.89 (C-8), 48.65 (C-9), 37.02 (C-10), 21.43 (C-11), 124.81 (C-12), 140.70 (C-13), 33.98 (C-14), 26.22 (C-15), 26.84 (C-16), 42.00 (C-17), 50.38 (C-18), 154.60 (C-19), 39.34 (C-20), 33.98 (C-21), 38.85 (C-22), 27.90 (C-23), 16.45 (C-24), 15.85 (C-25), 16.28 (C-26), 25.58 (C-27), 28.65 (C-28), 107.10 (C-29), 19.43 (C-30), 171.0 (CO), 21.24 ($COCH_3$).
Hydrolysis of **2**: Compound **2** (100 mg) was refluxed with 5% ethanolic KOH solution (20 ml) for 2 h. The reaction mixture was neutralized with dilute HCl and extracted with $CHCl_3$. The organic phase was washed with H_2O, dried over anh. Na_2SO_4 and evaporated. The residue was crystallized from $CHCl_3$–MeOH (1 : 1) to yield calotropursenol (**2a**) m.p. 152–153 °C, IR ν_{max} 3404, 1610, 895 cm^{-1}, EIMS m/z (rel. int.) 424 $[M]^+$ ($C_{30}H_{48}O$) (7.3), 217 (43.1), 207 (11.2), 189 (51.2).
Oxidation of **2a**: Compound **2** (20 mg) was dissolved in acetone (50 mg) and oxidized with Jones reagent (5 ml) to yield calotropuresenone (**2b**), m.p. 126–127°. IR ν_{max} 1710, 1615, 980 cm^{-1} EIMS m/z (rel. int.) 422 $[M]^+$ ($C_{30}H_{46}O$) (13.1).
Reduction of **2b**: Calotropursenone (**2b**) (10 mg) was dissolved in 2 ml of EtOH, and 5 mg of $NaBH_4$ was then added with stirring (2 h). After dilution with H_2O, the mixture was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with H_2O, dried, and evaporated to obtain **2a**, m.p. 151–53 °C.

3.4.3. Calotropfriedelenyl acetate (**3**)*, compounds* **3a** *and* **3b**

Elution of the column with benzene furnished a colourless amorphous powder of **3**, recrystallized from MeOH 8.2 g (0.42% yield). R_f 0.76 (benzene-Chloroform, 9 : 1) m.p. 120–122 °C, $[\alpha]_D^{25} + 22.1$ (C 0.85, MeOH), UV λ_{max} (MeOH) 220 nm log ε 4.5) IR ν_{max} (KBr) 2940, 2865, 1730, 1635, 1465, 1395, 1250, 1005 cm^{-1}.
^{1}H NMR (300 MHz $CDCl_3$): δ 6.17 (1 H, dd, J = 6.3, 4.5 Hz, H-1), 6.60 (1 H, dd, J = 5.5, 4.5 Hz, H-2), 4.20 (1 H, dd, J = 5.5, 9.0 Hz, H-3α). 2.00 (3 H, brs, $COCH_3$), 1.70 (3 H, brs, Me-27), 1.06 (3 H, d, J = 6.0 Hz, Me-23), 1.00 (3 H, brs, Me-29), 0.87 (3 H, brs, Me-30), 0.90 (6 H, brs, Me-26, Me-28), 0.83 (6 H, brs, Me-24, Me-25).
EIMS m/z (rel. int.): 468 $[M]^+$ ($C_{32}H_{52}O_2$) (25.6), 453 (7.7), 408 (9.1), 248 (13.9), 219 (100), 205 (28.2), 204 (28.2), 202 (71.0) 191 (37.4), 188 (93.0).
^{13}C NMR (75.5 Hz): δ 139.60 (C-1), 124.31 (C-2), 80.90 (C-3), 47.60 (C-4), 37.72 (C-5), 39.56 (C-6), 17.45 (C-7), 55.23 (C-8), 36.45 (C-9), 59.02 (C-10), 33.68 (C-11), 28.69 (C-12), 39.99 (C-13), 36.97 (C-14), 32.82 (C-15), 27.90 (C-16), 29.63 (C-17), 41.49 (C-18), 36.74 (C-19), 28.04 (C-20), 28.69 (C-21), 39.21 (C-22), 15.67 (C-23), 16.53 (C-24), 18.20 (C-25), 17.45 (C-26), 21.20 (C-27), 28.69 (C-28), 31.20 (C-29), 23.55 (C-30), 170.90 (60), 21.34 ($COCH_3$).
Hydrolysis of **3**: The acetate **3** (100 mg) was refluxed with 5% ethanolic KOH (20 ml) for 1 h. The reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ solution, after washing with H_2O and drying over anh. Na_2SO_4, was evaporated to afford calotropfriedelenol (**3a**) which was crystallized from MeOH as an amorphous powder, m.p. 131–132 °C, IR ν_{max} 3420, 1645, 890 cm^{-1}, EIMS m/z (rel. int.) 426 $[M]^+$ $C_{30}H_{50}O$) (11.2). Jones oxidation of **3a**: Compound 3a (15 mg) dissolved in acetone (40 ml) was oxidized with Jones reagent to yield the 3-keto derivative **3b**, m.p. 125–126°, IR ν_{max} 1700 cm^{-1}.

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