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Note

Phytochemical investigation of *Calotropis procera* Ait roots

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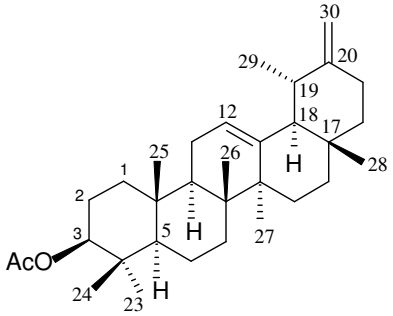
Phytochemical investigation of the roots of *Calotropis procera* Ait. (Asclepiadaceae) yields two new phytoconstituents procerursenyl acetate and proceranol together with the known compounds *N*-dotriacont-6-ene, glyceryl mono-oleolyl-2-phosphate, methyl myrisate, methyl behenate and glyceryl-1,2-dicapriate-3-phosphate. The structures of the new compounds have been identified as urs-18α-*H*-12,20(30)-diene-3β-yl acetate and *n*-triacontan -10β-ol on the basis of spectral data analysis and chemical reactions.

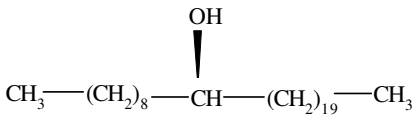
Keywords: *Calotropis procera*, Asclepiadaceae, roots, procerursenyl acetate, proceranol

Calotropis procera R.Br (Asclepidiaceae), commonly known as 'swallow wart' or 'milk weed', is a glabrous or hairy laticiferous shrub or small tree, found in tropical and subtropical Asia and Africa[15]. Traditionally the roots are used to treat diarrhoea, cough, skin diseases, rheumatism, as an expectorent and emetic[11]. Earlier pentacyclic triterpenes[5,4,7,10], alkaloid[8], cardinolides[14], phytosterols[9,12] and triterpenoid saponins[6] have been isolated from the roots. This paper describes the isolation and structure elucidation of two new phytoconstituents together with five known compounds from the roots of *C. procera.*

Procerursenyl acetate **1** was obtained as colourless crystals from pet. ether-chloroform (1:1) eluents. It responded positively to Liebermann-Burchardt test for triterpenes. Its IR spectrum exhibited characteristic absorption bands at 1725 cm^{-1}or ester group and 1640 for unsaturation. Its mass spectrum displayed a molecular ion peak at *m/z* 466, corresponding to triterpinic acetate $C_{32}H_{50}O_2$. It indicated eight double bond equivalents; five of them were adjusted in a pentacyclic carbon framework of ursane-like triterpene, two in the vinylic linkages and one in the ester group. The mass spectrum also displayed fragment ion peaks at *m/z* 250 and *m/z* 216 generated due to retro-Diel's Alder fragmentation of ring C indicating the location of the vinylic linkage at C-12 and acetoxy group in ring A/B. Other ion fragments appeared at *m/z* 190 $(250\text{-AcOH})^+$ and *m/z* 406 $[M\text{-AcOH}]^+$. The acetoxy group was placed at C-3 on the basis of biogenetic consideration. The prominent ion fragments arising at *m/z* 189 $[216\text{-CH=}\ CH_2]^+$, 174 $[189\text{-Me}]^+$, 159 $[174\text{-Me}]^+$, 201 $[216\text{-Me}]^+$, 186 $[201\text{-Me}]^+$, and 171 $[186\text{-Me}]^+$ also indicated the location of the vinylic linkage in ring C at C-12 and another olefinic linkage in ring D/E. The ^{1}H NMR spectrum of **1** displayed a deshielded one-proton multiplet at δ 5.17. Compound **1** displayed a deshielded one-proton mutliplet at δ 5.17 assigned to vinylic *H*-12 proton. Two one-proton broad signals at δ 4.85 and δ 4.73 were associated with CH_2-30 exocyclic methylene protons. A one-proton double doublet at δ 3.24 with coupling interactions of 5.5 and 9.5 Hz was assigned to 3β-carbinol proton. A one - proton doublet δ 2.02 (J = 7.32 Hz) was accounted to 18α protons. The acetyl protons appeared as a three-proton broad signal at δ 2.35. The methyl protons appearing at δ 0.86 (Me-23), 0.71 (Me-24), 0.69 (Me-25), 0.64 (Me-26), 0.75 (Me-27) and 0.79 (Me-28) as broad signals suggested the tertiary nature of these methyl protons. The Me-29 secondary protons appeared as a three-proton doublet at δ 0.82 (J = 6.1 Hz) supporting the ursane type carbon framework of the molecule. The ^{1}H NMR values were compared with those of calotropenyl acetate and other similar triterpenes[3]. The presence of all the methyl signals between δ 0.86-0.64 indicated that these methyl functionalities were located on the saturated carbons. The ^{13}C NMR spectrum of **1** exhibited important carbon resonances



1



2

of ursane-type triterpenes[2,3,13]. It showed signals for ester (δ 170.50, acetate), vinylic (δ 124.12, C-12; 139.37, C-13; 154.24, C-20 and 106.84, C-30) and carbinol (δ 80.75, C-3) carbons. The methyl carbons appeared at δ 25.92 (C-23), 20.84, (C-24), 17.99 (C-25), 25.38 (C-26), 28.44 (C-27), 30.98 (C-28), 16.41 (C-29). On the basis of above discussion the structure of **2** has been formulated as urs-18α*H*-12, 20 (30)-diene-3β-yl acetate. Earlier urs-18α*H*-12,20(30)-diene-3α-yl acetate[2] and urs-18β-*H*-12,20(30)-diene-3yl acetate[1] from the roots of *Calotropis gigantea* have been reported.

Proceranol **2** was obtained as colourless crystals from chloroform-methanol (19:1) eluents. Its IR spectrum displayed characteristic absorption bands for hydroxyl group at 3432 cm^{-1} and long chain aliphatic moiety at 720 cm^{-1}. Its mass spectrum showed a molecular ion peak at *m/z* 438 corresponding to the molecular formula of saturated alcohol, $C_{30}H_{62}O$. The prominent ion fragments generated at *m/z* 127[C_9-C_{10} fission, $C_9H_{19}]^+$, 311[M-127, CH_3 $(CH_2)_{19}$ CH-OH]$^+$, 157[C_{10}-C_{11} fission, CH_3 $(CH_2)_8$ CH-OH]$^+$, and 281[M-157, $C_{20}H_{41}]^+$ indicated the location of the hydroxyl group at C-10. The ^{1}H NMR spectrum of **2** displayed a one-proton broad multiplet centred at δ 4.38 assigned to H-10 carbinol proton placed in α-orientation on the basis of its $w_{1/2}$ = 23.11 Hz. The vicinal methylene protons H_2-9 and H_2-11 resonated as two-proton each multipletes at δ 2.23 and δ 2.01, respectively. The remaining methylene protons appeared at δ 1.47 (2 × CH_2) and δ 1.85 (23 × CH_2). The primary methyl protons appeared as two three-proton triplets at δ 0.81 (*J* = 6.6 Hz) and δ 0.78 (*J* = 6.6 Hz) assigned correspondingly to Me-1 and Me-30 protons. The ^{13}C NMR spectrum of compound **2** displayed signals for carbinol carbon C-10 at δ 82.9 and terminal primary methyl carbon at δ 17.15 (CH_3-1) and δ 14.32 (CH_3-30). The methylene carbons appeared at δ 29.69 (26 × CH_2) and δ 22.13 (CH_2). The absence of any signal beyond δ 4.38 in the ^{1}H NMR spectrum and δ 82.9 in the ^{13}C NMR spectrum supported the saturated nature of the molecule. On the basis of above discussion the structure of **2** was elucidated as *n*-triacontan-10β-ol. This is a new phytoconstituents isolated from a herbal drug for the first time.

Experimental Section

All chemicals used were of analytical grade: petroleum ether, methanol, chloroform, sulphuric acid were purchased from CDH-Chemicals, India. The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on a Win IR FTS 135 instrument (Biorad, USA). ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker spectrospin NMR instrument in $CDCl_3$, using TMS as internal standard, with chemical shift expressed in parts per million (δ) and coupling constant (*J*) in Hertz. Maldi TOF MS were scanned at 70 eV on an ultraflex TOF/TOF instrument (Jeol, USA). Column chromatography was performed on silica gel (Merck, 60-120 mesh) and thin-layer chromatography on silica gel G coated TLC plates (Merck).

The roots of *C. procera* were collected from the local market of Khari Baoli, Delhi and identified by - Dr. M. P. Sharma, taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard. A voucher specimen No. KB/ND/PRL/CP/13 has been deposited in the herbarium of Faculty of Pharmacy, Jamia Hamdard, New Delhi.

The air-dried roots (2 kg) of *C. procera* were coarsely powdered and extracted in a Soxhlet apparatus with methanol for 72 hr. The methanolic extract was concentrated under reduced pressure to give a viscous dark green mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents.

The viscous dark green mass was adsorbed on silica gel (60-120 mesh) for column 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 pet. ether, mixture of pet. ether and chloroform (9:1, 3:1, 1:1 and 1:3), pure chloroform and finally the mixture of chloroform and methanol (99:1, 98:2, 96:4, 95:5, 95:5, 9:1). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same R_f values) were combined and crystallized. The following isolated compounds were recrystallized to get the pure compounds.

***n*-Dotriacont-6-ene**. Elution of column with petroleum ether-chloroform (3:1) (fraction 1-25) afforded colourless crystals of **1**, recrystallised with MeOH: acetone (1:1), 522 mg (0.043% yield). R_f : 0.68 ($CHCl_3$: MeOH: 9:1); m.p. 90-94°C; UV λmax (MeOH): 399.5 nm; ^{1}H NMR ($CDCl_3$): δ 5.30 (1H, m, H-7), 5.05 (1H, m, H-6), 2.21 (2H, m, H_2-5), 1.76 (2H, m, H_2-8), 1.52 (2H, m, CH_2), 1.46 (4H, brs, 2 × CH_2), 1.18 (46H, brs, 23 × CH_2), 0.78 (6H, brs, Me-32, Me-1). +ve Maldi Tof MS: *m/z* 448 $[M]^+$ ($C_{32}H_{64}$) (6.2), 377 (250), 351 (17.1).

Procerursenyl acetate 1. Elution of the column with petroleum ether-chloroform (1:1) (Fraction 26-30) produced colourless crystalline compound **2**, recrystallised from methanol, 5763 mg (0.48% yield). R_f.: 0. 72 ($CHCl_3$: pet. ether: 9:1) m.p. 130-34°C; UV λ max (MeOH): 241 nm (log ε 4.5); IR ν max (KBr): 2921, 2850, 1725, 1640, 1470, 1320, 1210, 1150 and 1015, cm^{-1}; ^{1}H NMR ($CDCl_3$): **Table I**; ^{13}C NMR ($CDCl_3$): **Table I**; +ve Maldi Tof MS: *m/z* 466 $[M]^+$ ($C_{32}H_{50}O_2$), (4.3), 406 (5.6), 250 (21.6), 216 (31.5), 201 (9.8), 189 (25.3), 186 (33.2), 174, 171 (56.7), 159 (22.1), 156 (49.8).

Glyceryl mono-oleolyl-2-phosphate. Elution of the column with petroleum ether-chloroform (1:3) (fractions 31-46) yielded colourless crystalline compound **3**, recrystallised from methanol; 671 mg (0.055% yield). R_f 0.61 ($CHCl_3$); m.p. 220-24°C; UV λmax (MeOH): 241 nm (log ε 2.9); IR ν max (KBr): 3450, 1733, 1652, 1557, 1464, 1370, 1210, 1115, 1005, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.27 (1H, m, H-9'), 5.05 (1H, m, H-10′), 2.24 (2H, m, H-2′), 1.96 (2H, m, H_2 -9′), 1.89 (2H, m, H_2-10′), 1.52 (2H, m, CH_2), 1.18 (20H, brs, 10 × CH_2), 0.80 (3H, t, *J* = 6.9 Hz, Me-18′); ^{13}C NMR ($CDCl_3$): δ 173.13 (C-1'), 129.96 (C-9'), 124.97 (C-10′), 68.86 (C-2), 62.07 (C-1, C -3), 50.63 (C-2), 33.27 (CH_2), 32.14 (CH_2), 31.86 (CH_2), 29.64 (6 × CH_2), 29.27 (CH_2), 29.08 (CH_2), 27.14 (CH_2), 24.23 (CH_2), 14.169 (CH_3-18′). +Ve Maldi Tof MS: *m/z* 436 $[M^+]$ ($C_{21}H_{41}O_7P$) (11.6).

Methyl myrisate. Elution of the column with chloroform(fraction 47-61) furnished colourless crystalline mass of **4**, recrystallised from methanol: acetone (2:1), 864 mg (0.072% yield). R_f 0.82 ($CHCl_3$); m.p. 64-66°C; UV λ Max (MeOH): 242 nm (log ε 2.3); IR ν max (KBr): 2918, 2949, 1735, 1464, 1379, 1262, 1195, 1020, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.36 (3H, brs, OMe), 2.27 (2H, brs, H_2-2), 1.19 (22H, Brs, 11 × CH_2), 0.78 (3H, *J* = 6.1 Hz, Me-14). ^{13}C NMR ($CDCl_3$): δ 173.30 (C-1), 50.28 (OCH_3), 34.01 (CH_2-2), 29.58 (10 × CH_2), 24.87 ((CH_2-13), 21.05 ((CH_2), 13.87 (Me-14). +ve Maldi Tof MS: *m/z* 242 $[M^+]$ ($C_{15}H_{30}O_2$) (93.1).

Methyl behenate. Elution of the column with chloroform -methanol (99:1) (fraction 62-85), afforded colourless crystal of **5**, recrystallised from methanol 356 mg (0.029% yield). R_f. 0.88 ($CHCl_3$: MeOH: 4:1); m.p. 53-54°C; U.V. λ max (MeOH):

Table I — ^{1}H and ^{13}C NMR spectral values of procerursenyl acetate **2**

Position	^{1}H NMR		^{13}C NMR
	α	β	
1	1.51 m	1.40 m	39.36
2	1.93 m	1.86 m	23.43
3	dd (5.5, 9.5)	------	80.75
4	—	—	38.08
5	1.44 m	—	55.12
6	1.40 m	1.36 m	19.42
7	1.22 m	1.36.m	34.52
8	—	—	41.74
9	1.93 m	—	49.74
10	—	—	36.89
11	1.80 brs	1.75 m	21.23
12	5.17 m	—	124.12
13	—	—	139.37
14	—	—	47.08
15	1.12 m	0.98 m	26.77
16	1.10 m	1.17 m	27.86
17	—	—	48.60
18	2.02 d (7.32)	—	58.93
19	1.51 m	—	38.72
20	—	—	154.24
21	1.77 m	1.51m	32.68
22	1.12	1.10 m	38.08
23	0.86 brs	—	25.92
24	0.71 brs	—	20.84
25	0.69 brs	—	17.99
26	0.64 brs	—	25.38
27	0.75 brs	—	28.44
28	0.79 brs	—	30.98
29	0.82d (6.1)	—	16.41
30	4.85 brs	4.73 brs	106.84
AcO	2.35 brs	—	170.50, 21.01

Coupling constants in Hertz are provided in parenthesis

The structures of the known compounds have been identified as *N*-dotriacont-6-ene, glyceryl mono-oleolyl -2-phosphate, methyl myrisate, methyl behenate and glyceryl -1,2-dicapriate-3-phosphate on the basis of spectral data analysis.

242 nm (log ε 3.1); IR ν max (KBr): 2917, 2849, 1733, 1463, 1375, 1262, 1071, 1020, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.41 (3H, brs, OMe), 2.91 (1H, brs, H_2-2a), 2.87 (1H, brs, H_2-2b, 1.22 (38H, brs, 19 × CH_2), 0.85 (3H, t, *J* = 6.1 Hz, Me-22); ^{13}C NMR (CD_3Cl): δ 175.06 (C-1), 50.21 (OMe), 31.80 (CH_2-2), 29.57 (18×CH_2), 22.56 (CH_2), 13.95 (CH_3-22). +ve Maldi Tof MS: *m/z* 354 [M^+]($C_{23}H_{46}O_2$) (12.6).

Proceranol 2. Further, elution of the column with chloroform-methanol (99 : 1) (fraction 86-98) Furnished colorless mass of **2**, recrystallised from methanol 399 mg. (0.033% yield). R_f 0.83($CHCl_3$: MeOH: 4:1); m.p. 210-14°C; UV λmax (MeOH): 330 nm; IR ν max (MeOH): 3432, 2918, 2849, 1463, 1261, 1097, 1015, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.38 (1H, brm, $w^{1/2}$ =23.11 Hz, H-10α), 2.23 (2H, m, H_2-9), 2.01 (2H, m, H_2 -11), 1.47 (4H, brs, 2 × CH_2), 1.85 (46H, brs, 23 × CH_2), 0.81 (3H, t, *J* = 6.6 Hz, Me-1), 0.78 (3H, t, *J* = 6.6 Hz. Me-30); ^{13}C NMR ($CDCl_3$): δ 82.9 (C-10), 29.69 (26 × CH_2), 22.13 (CH_2), 17.15 (CH_3-1), 14.32 (CH_3-30). +ve Maldi Tof MS: *m/z* 438 $[M]^+$ ($C_{30}H_{62}O$) (9.6), 311 (21.6), 281 (18.9), 127 (65.3), 157 (42.5).

Glyceryl-1,2-dicapriate-3-phosphate. Elution of the column with chloroform-methanol (97:3) (fraction 99-112), furnished colourless mass, recrystallised from methanol 369 mg (0.030% yield). R_f. 0.45 ($CHCl_3$**:** MeOH: 5:1); m.p. 230-34°C; UV λmax (MeOH): 242 nm (Log ε 4.1); IR ν max (KBr): 3429, 2919, 2850, 1739, 1725, 1515, 1466, 1268, 1165, 1105, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.04 (1H, m H-2), 3.86 (2H, d, *J* = 7.2 Hz, H_2= 3), 3.42 (2H, brs, H_2-1), 2.27 (2H, brs, H_2-2′), 1.96 (2H, brs, H_2-2″, 1.60 (2H, brs, CH_2), 1.54 (4H, brs, 2 × CH_2), 1.18 (22H, brs, 11 × CH_2), 0.81 (3H, t, *J* = 6.2 Hz, Me-10'), 0.78 (3H, t, *J* = 6.5 Hz, Me-10″; ^{13}C NMR ($CDCl_3$): δ 173.11 (C-1′), 168.91 (C-1″, 73.15 (C-2), 63.27 (C-1), 61.78 (C-3), 52.16 (CH_2), 29.70 (12 × CH_2), 24.89 (CH_2), 21.56 (CH_2), 14.79 (CH_3-10'), 14.55 (CH_3-10). +ve Maldi Tof MS: *m/z* 480 $[M]^+$ ($C_{23}H_{45}O_8P$).

Conclusion

Two new phytoconstituents (procerursenyl acetate, urs-18α*H*-12, 20(30)-diene-3β-yl acetate; proceranol, *n*-triacontan-10β-ol) have been reported from root of *Calotropis procera* as natural products.

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