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Rare Chemical Constituents from *Calotropis gigantea* Roots

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Four new chemical constituents including one naphthalene derivative, named calotropnaphthalene, two terpene derivatives, namely calotropisesquiterpenol and calotropisesterterpenol and an aromatic product designated as calotropbenzofuranone along with a known compound, sucrose, have been isolated from the roots of the *Calotropis gigantea*. The structures of these chemical constituents have been established as 1-methoxy-4-ethyl naphthalene, 6-(2-methyl-2, 3-dihydroxypentyl)-11, 11-dimethyl cyclohex-8-ene-10-one-7-oic isopentenyl ester, 14-(15, 15-dimethyl cyclohexanyl-14, 19, 25-tricyclo)-3,7,11-trihydroxymethylene-tridecane and 8,15-dihydro benzofuranyl-18-hepta-7, 15-dione-16-oic acid, respectively, on the basis of the spectral data analyses and chemical reactions.

Calotropis gigantea (Linn.) Ait. f. (Asclepiadaceae) is a much-branched, hardy, erect and wooly shrub found growing up to an altitude of 900 m throughout India. Its roots resemble ipecac in properties and act as diaphoretic, stimulant, expectorant, carminative and cardiotonic. They are useful in leprosy, eczema, syphilis, elephantiasis, mecurial cachexia, ulceration, cough, asthma, ascites, anacara and stomachal disorders[1]. Some pentacyclic triterpenoids have been reported from the roots of *Calotropis* species[2-5]. The present paper describes the isolation and characterization of four new chemical constituents from the root of this plant.

EXPERIMENTAL

Extraction:

The roots of *C. gigantea* were collected from Dasna (Ghaziabad). The dried and coarsely powdered roots (3 kg) were extracted with ethanol (95%) exhaustively in a Soxhlet apparatus. The extract was dried under vacuum yielding a thick, viscous, dark reddish brown mass (125 g) (4.17%). The dried ethanolic extract was dissolved in minimum amount of MeOH and adsorbed on silica gel (100 g) with constant stirring until completely dried and subjected to silica gel column prepared in petroleum ether (60-80°). The column was eluted successively with petroleum ether, petroleum ether-$CHCl_3$ (9:1, 3:1, 1:1, 1:3 v/v), $CHCl_3$, $CHCl_3$-MeOH (99:1, 95:5, 3:1, 1:1 v/v) and MeOH to isolate the following compounds:

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Compound 1 :

Elution of the column with petroleum ether eluants gave light yellow amorphous powder of 1, re-crystallized from MeOH, 120 mg (0.004%), mp-119-121°. UV λ_{max} (MeOH): 214, 238, 355 nm (log ε 4.5, 6.7, 0.8). IR λ_{max} (KBr) : 1640, 1610, 1525, 1410, 1055, 790 cm^{-1}. ^{1}H-NMR ($CDCl_3$) : δ 8.00 (1H, dd, J=3, 8.5 Hz, H-8), 7.37 (3H, m, H-7, H-6, H-5), 6.50 (1H, m, H-2), 5.80 (1H, m, H-3), 4.23 (1H, brs, H-1), 3.43 (3H, brs, OMe), 2.63 (1H, m, H-4), 2.40 (2H, m, H-9), 0.87 (3H, t, J=4.5 Hz, Me-10). EIMS *m/z* (rel. int.) : 188[M]$^+$ ($C_{13}H_{16}O$) (34.1), 173 (7.2), 162 (4.2), 159 (36.2), 144 (4.6), 142 (4.9), 131 (13.8), 128 (6.8), 120 (1.0), 118 (13.2), 105 (100), 103 (7.1), 89 (5.5), 76 (18.3), 43 (5.0), 31 (91.5). Elemental analysis: found C 83.01, H 8.36; calcd. C 82.97, H 8.51 for $C_{13}H_{16}O$. ^{13}C NMR ($CDCl_3$): δ 77.8 (C-1), 150.8 (C-2), 134.6 (C-3), 29.8 (C-4), 132.1 (C-5), 126.5 (C-6), 133.5 (C-7), 133.6 (C-8), 126.1 (C-9), 116.2 (C-10), 22.6 (C-11), 15.3 (C-12), 51.8 (OCH_3).

Compound 2 :

Elution of the column with the $CHCl_3$-MeOH (95:5) furnished colourless crystalline product 2, recrystallised from MeOH, 203 mg (0.0067%), mp 168-170°, $[\lambda]_D^{35}$-41° (c 0.42, MeOH). UV α_{max} (MeOH): 238 nm (log ε 6.3). IR v_{max} (KBr): 3440, 2915, 2840, 1725, 1710, 1600, 1465, 1370, 1255, 1050, 700 cm^{-1}. ^{1}H-NMR (DMSO-d_6: δ 6.93 (1H, d, J=3.0 Hz, H-9), 5.86 (1H, m, H-8), 5.30 (1H, m, H-2'), 4.86 (2H, brs, H_2-1'), 4.10 (1H, m, H-3), 2.30 (1H, d, J=6.0 Hz, H-7), 2.00 (1H, m, H-6), 1.80 (2H, m, H_2-5), 1.70 (2H, m, H_2-4), 1.67 (6H, brs, Me-4' Me-5'), 1.33 (6H, brs, Me-1, Me-12), 1.26 (3H, brs, Me-14), 0.66 (3H, brs, Me-15). EIMS *m/z* (rel. int.): 352 $[M]^+$ ($C_{20}H_{32}O_5$) (1.0), 293 (1.0), 249 (2.1), 235 (1.8), 117 (1.4), 113 (1.2), 99 (20.9), 89 (13.2), 85(3.9), 71 (10.9), 59 (11.8). Elemental analysis: found C 69.29; H 8.96; calcd. C 68.11, H 9.09 for $C_{20}H_{32}O_5$. ^{13}C NMR (DMSO-d_6) :δ 22.9 (C-1), 81.4 (C-2), 77.8 (C-3), 26.3 (C-4), 21.8 (C-5), 38.2 (C-6), 38.5 (C-7), 150.5 (C-8), 129.7 (C-9), 206.1 (C-10), 44.8 (C-11), 22.9 (C-12), 167.6 (C-13), 26.7 (C-14), 25.6 (C-15), 63.2 (C-1'), 118.6 (C-2'), 131.4 (C-3'), 17.2 (C-4'), 25.5 (C-5').

Acetylation of 2:

Compound 2 (45 mg) was acetylated with a mixture of AC_2O (3 ml) and pyridine (1 ml) overnight at room temperature. The reaction mixture was poured into ice cold water, extracted with $CHCl_3$ (3x10 ml), the organic phase washed with H_2O (2x10 ml), dried (Na_2SO_4) and evaporated to get monoacetyl product (**2a**), mp 105-106°. IR v_{max} (KBr): 3445, 1740, 1725, 1710, 1610, 1465, 1370, 1250, 1055, 910 cm^{-1}.

Alkaline Hydrolysis of 2 :

Compound 2 (25 mg) was heated with 2N ethanolic KOH solution for 2 hr. Water (10 ml) was added, neutralized with $CHCl_3$ (3x5 ml). After usual work-up the sesquiterpenoic acid (**2b**) was obtained, mp 123-124°, different spots on TLC on comparison with the original compound.

Compound 3:

Elution of the column with $CHCl_3$-MeOH (1:1) furnished light yellow amorphous powder of **3**, re-crystallized from MeOH80 mg (0.0026%), mp 131-132°. UV λ_{max} (MeOH): 214, 238 nm (log ε 4.5, 6.3). IR v_{max} (KBr): 3250, 1640, 1585, 1480, 1410, 1360, 1220, 1120, 1065, 1045, 850 cm^{-1}. ^{1}H-NMR (C_5D_5N) : δ 4.70 (2H, brs, CH_2OH), 4.66 (4H, brs, 2xCH_2 OH), 1.86 (2H, m, CH_2), 1.40 (6H, brs, 2xCH, 2xCH_2), 1.20 (4H, m, 2xCH, CH_2), 1.00 (14H, brs, 7xCH_2), 0.68 (6H, brs, 2xCH_3), 0.63 (3H, brs, CH_3), 0.30 (2H, m, CH_2-25). EIMS *m/z* (rel. int.): 396 $[M]^+$ ($C_{25}H_{48}O_3$) (55.4), 339 (5.6), 273 (5.4), 255 (18.1), 201 (10.2), 187 (8.9), 161 (22.1), 159 (22.2), 151 (15.3), 137 (21.6), 123 (23.5), 120 (42.1), 1.09 (35.8), 84 (32.5), 83 (49.6), 73 (24.1), 69 (75.6), 55 (100). Elemental analysis: found C 78.4, H 12.41; calcd. C 77.75, H 12.12 for $C_{25}H_{48}O_3$. ^{13}C NMR (C_5D_5N): δ 15.3 (C-1), 28.1 (C-2, C-4, C-5, C-6, C-8, C-9, C-10, C-12, C-13), 29.7 (C-3, C-7, C-11), 47.1 (C-14), 39.8 (C-15), 32.3 (C-16), 30.2 (C-17), 31.9 (C-18), 20.1 (C-19), 62.1 (C-20, C-21)$^{C-22}$, 19.3 (C-23), 22.1 (C-24), 29.8 (C-25).

Acetylation of 3:

Compound 3 (15 mg) was acetylated with a mixture of Ac_2O (3 ml) and pyridine (1 ml) overnight at room temperature. Next morning water (10 ml) was added and the reaction mixture extracted with $CHCl_3$ (3x10 ml). The organic phase was washed with water (2x10) ml), dried over Na_2SO_4 and evaporated to secure triacetyl product **3a**, mp 125-126°. IR v_{max} (KBr): 1735, 1725, cm^{-1}.

Compound 4:

Elution of the column with the MeOH furnished light yellow amorphous powder of 4, crystallized from MeOH, 115 mg (0.0038%), mp 182-183°. UV λ_{max} (MeOH): 248, 262 nm (log v 5.4, 5.5). IR v_{max} (KBr): 3320, 1785, 1725, 1665, 1590, 1465, 1370, 1355, 1305, 1250, 1235, 1145, 1085, 1025, 985, 905, 835, 780, 755 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.90 (1H,dd, J=3, 9 Hz, H-10), 7.71 (2H, m, H-12, H-13), 6.80 (1H, m, H-11),3.06 (1H, brs, H-8), 2.20 (2H, brs, H_2-6), 1.68 (1H, m, H-5), 1.36 (6H, brs, 3xCH_2), 0.63 (3H, t, J=4 Hz, Me-1). EIMS *m/z* (rel. int.): 290 $[M]^+$ ($C_{16}H_{18}O_5$) (32.6), 264 (8.8), 261 (5.3), 246 (9.1), 233 (11.1), 188 (8.5), 175 (11.9), 161 (8.5), 157 (2.2), 133 (3.8), 129 (6.5), 115 (2.4), 113 (6.0), 104 (25.8), 92 (64.1), 85 (16.3), 76 (15.3), 71 (31.8), 57 (100), 44 (24.3). Elemental analysis: found C 65.91, H 6.46; calcd. C 66.20, H 6.20 for $C_{16}H_{18}O_5$. ^{13}C NMR ($CDCl_3$): δ13.9 (C-1), 22.9 (C-2), 28.3 (C-3, 25.5 (C-4), 34.1 (C-5), 37.1 (C-6), 203.5 (C-7), 27.7 (C-8) 113.8 (C-9), 129.8 (C-10), 122.6 (C-11), 127.8 (C-12), 115.2 (C-13), 155.5 (C-14), 177.9 (C-15), 183.1 (C-16).

Methylation of 4:

Compound **4** (15 mg) was dissolved in solvent ether (10 ml) and ethereal solution of CH_2N_2 added. The reaction mixture was left overnight. Evaporation of the

solvent yielded a gummy product of monomethyl ester (**4a**), re-crystallized from MeOH, mp 171-172°, different sports on TLC from that of the original compound.

RESULTS AND DISCUSSION

Compound **1**, named, calotropnaphthalene, was obtained as light yellow amorphous powder from petroleum ether eluants. Its IR spectrum showed characteristic bands for olefinic linkage at 1640, 1610 cm^{-1}. Its mass spectrum showed a molecular ion peak at *m/z* 188 corresponding to $C_{13}H_{16}O$. It indicated six double bond equivalents. The diagnostically important peaks appeared at *m/z* 173 $[M-Me]^+$, 142 $[175-OMe]^+$, 162 $[M-CH=CH]^+$, 131 $[162-OMe]^+$, 159 $[M-CH_2CH_3]^+$, 144 $[159-Me]^+$, 128 $[159-OMe]^+$ and 76 suggesting dihydronaphthalene type skeleton possessing one methoxy group and one ethyl group. This fact was supported from the ion peaks appearing at *m/z* 118, 103 $[118-Me]^+$, 120, 105 $[120-Me]^+$ and 89 $[120-OMe]^+$.

The ^{1}H-NMR spectrum of 1 displayed four downfield signals at δ 8.00 (dd, J=3, 8.5 Hz) assigned to H-8, 7.37 (3H, m) ascribed to H-7, H-6 and H-5, 6.50 (m) attributed to H-2 and 5.80 (m) associated with H-3. The C-1 carbinol proton appeared as a broad singlet at δ 4.23. A three-proton broad signal at δ 3.43 was accounted to methoxy group. The signals at δ 2.63 (1H, m), 2.40 (2H, m) and 0.87 (3H, t, J=4.5Hz) were due to H-4, H-9 and Me-10. The ^{13}C NMR spectrum of 1 showed eight unsaturated, one carbinol and one methoxy carbons.

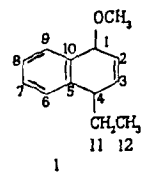
On the basis of these evidences the structure of calotropnaphthalene (**1**) has been established as 1-methoxy-4-ethyl naphthalene. This is a new naphthalene derivative reported for the first time from a natural or synthetic source.

Compound **2**, named calotropisesquiterpenol, was obtained as colourless crystalline product from chloroform-methanol (95:5), eluants. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3440 cm^{-1}), ester group (1725 cm^{-1}), carbonyl group (1710 cm^{-1}) and unsaturation (1600 cm^{-1}. The mass spectrum of **2** exhibited a molecular ion peak at *m/z* 352 consistent with the molecular formula of sesquiterpenic ester $C_{20}H_{32}O_5$. The ion fragments at *m/z* 117, 235, 89 $[117-CH_2CH_2]^+$, 71 $[89-H_2O]^+$, 99 $[117-H_2O]^+$, 59, 293 and 249 $[293-CHOH-CH_2]^+$ indicated the presence of dihydroxyhexyl group attached to the cyclohexenone ring. The ion peaks at *m/z* 113 and 85 suggested the pentenyl ester of the sesquiterpenoic acid.

The ^{1}H-NMR spectrum of **2** displayed three one-proton each vinylic signals at δ 6.93 (d, J=3.0 Hz), 5.86 (m) and 5.30 (m) assigned to H-9, H-8 and H-2.' The carbinol protons appeared as two-proton broad singlet at δ 4.86 (CH_2-1') and as one-proton multiplet at δ 4.10 (H-3). A six-proton broad singlet at δ 1.67 was attributed to C-4' and C-5' methyl group attached to the olefinic carbon. The other methyl protons appeared at δ 1.33 (Me-1, Me-12), 1.26 (Me-14) and 0.66 (Me-15). The ^{13}C NMR spectrum of **2** exhibited the presence of four olefinic, one carbonyl, three carbinyl and one ester carbons. Acetylation of **2** with Ac_2O-pyridine mixture formed a monoacetyl product (**2a**) which still had a free tertiary hydroxyl group. Acid hydrolysis of **2** yielded a sesquiterpenoic acid (**2b**). On the basis of these evidences the structure of calotropisesquiterpenol (**2**) has been established as 6-(2-methyl-2, 3-dihydroxypentyl)-11, 11-dimethyl-cyclohex-8-ene-10-one-7 oic isopentenyl ester. This is a new sesquiterpenic ester and is being reported for the first time from a natural or synthetic source.

Compound **3**, named calotropisesterterpenol, was obtained as light amorphous powder from chloroform-methanol (1:1) eluants. Its IR spectrum showed absorption bands for hydroxyl group (3250 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 396 corresponding to a bicyclic sesterterpenoid triol $C_{25}H_{48}O_3$. The important ion peaks at *m/z* 84, 123, 151 and 137 $[151-CH_2]^+$ indicated that a bicyclic C_9-unit was present at one of the terminal of carbon chain. The existence of three-hydroxymethylene groups at C-20, C-21 and C-22 was inferred from the ion peaks appearing at *m/z* 273, 201, 159 and 73.

The ^{1}H-NMR spectrum of **3** displayed two broad signals at δ 4.70 (2H) and 4.66 (4H) due to the three hydroxymethylene protons. The methyl protons appeared at δ 0.68 (2x*Me*) and 0.63 (*Me*). A two proton multiplet at δ 0.30 was associated with C-25 methylene protons. The remaining methine and methylene protons appeared in between δ 1.86-1.00. The ^{13}C NMR spectrum of **3** the hydroxy methylene carbons appeared at δ 62.1 and the C-1, C-23 and C-24 methyl carbons resonated at δ 15.3, 19.3 and 24.1, respectively. Acetylation of **3** with acetic anhydride and pyridine yielded a triacetyl derivative (**3a**). From the foregoing evidences the structure of calotropisesterterpenol (**3**) has been established as 14-(15, 15-dimethyl cyclohexanyl-14, 19, 25-tricyclo)-3, 7, 11-trihydroxymethylene-tridecane. This is a new



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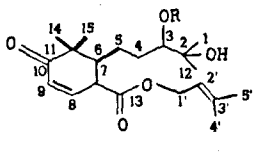
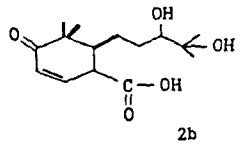
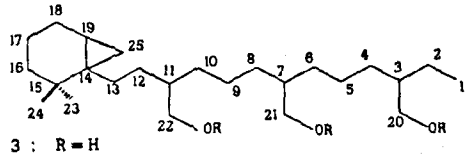


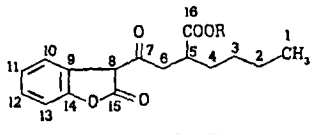
2 : R = H
2a : R = Ac



2b



3 : R = H
3a : R = Ac



4 : R = H
4a : R = CH_3

sesterterpenic alcohol isolated for the first time from a synthetic or natural source.

Compound **4**, named calotropbenzofuranone, was obtained as light yellow amorphous powder from methanol. It showed effervescences with sodium bicarbonate solution. Its IR spectrum exhibited absorption bands for γ-lactone (1785-cm^{-1}), carbonyl group (1725 cm^{-1}), carboxyl group (3320, 1665 cm^{-1}), unsaturation (1590 cm^{-1}), aromatic bands (985, 905, 835 cm^{-1}) and aliphatic chain (780, 755 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/z* 290 consistence with the molecular formula $C_{16}H_{16}O_5$. The fragmentation pattern of **4** suggested that the compound belonged to substituted benzodihydrofuran-type carbon framework. This was supported from the ion peaks appearing at *m/z* 133, 92 and 76 [92-O]$^+$. The presence of carbonyl group at C-7 was deduced from the ion peaks generating at *m/z* 157, 129 [157-CO]$^+$ and 161. The ion fragements at *m/z* 175, 233, 113 [157-CO_2]$^+$, 115 [129-CH_2]$^+$, 85 [129-CO_2]$^+$, 71 [115-CO_2]$^+$ and 246 [M-CO_2]$^+$ supported the presence of the carboxlic group at C-5.

The ^{1}H-NMR spectrum of **4** displayed three down-field signals as a one- proton double doublets at δ 7.90 (1H, J=3, 9 Hz) assigneable to H-10 and two as a two-proton multiplet at δ 7.71 associated with H-12 and H-13 and one as a one-proton multiplet at 6.80 accounted to H-11. A one-proton broad singlet at δ 3.06 was ascribed to C-8 methine proton. The terminal methyl group appeared as a three-proton triplet at δ 0.63 (J=4 Hz). The signals at δ 2.20 (2H), 1.68 (1H) and 1.36 (6H) were assigned to the remaining methine (H-5) and methylene functionalities. The ^{13}C NMR spectrum of **4** showed the presence of six olefinic, one ester, one acid and one carbonyl carbons. Treatment of **4** with diazomethane yielded a monomethyl ester **4a**. These data led to establish the structure of calotropbenzofuranone (**4**) as 8.15 dihydrobenzofuranyl-18-hepta-7, 15-dione-16-oic acid. This is also a new aromatic natural product.

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