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Two new anthraquinones from the roots of *Rubia cordifolia* Linn.

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Two new anthraquinones, named cordifoliol and cordifodiol, have been isolated from the roots of *Rubia cordifolia*. Their structures have been established as 1-hydroxy-3-ethyl-9,10-anthraquinone (**1**) and 1,8-dihydroxy-11, 20 (15-pentyl-naphthaquinonyl) phenanthrene (**2**) on the basis of spectral data analyses and chemical reactions.

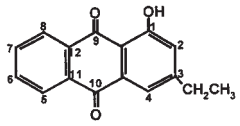
1. Introduction

The genus *Rubia* (Rubiaceae) comprises 500 genera and 600 species. About 76 genera and 274 species are found in India. *Rubia cordifolia* L., known as "Madder or Manjit", has the origin in the eastern Himalayas and mountains of southern and western India [1]. It is an important crude drug used in indigenous systems of medicine [2]. The plant has antioxidant [3], antihaemorrhagic [4], hepatotoxic [5], antineoplastic [6], antiallergenic [7], antibacterial [8] and antiviral [9] properties. It exerts uricolytic activities and promotes disintegration and elimination of urinary stones [10]. The isolation of quinones [11–13], terpenoides [14–16], and cyclic peptides [17, 18] has already been reported. Anthraquinones like mollugin and furomollugin strongly suppressed hepatitis B [19]. Anthraquinones were also found to exhibit cytotoxic activity [20]. This present paper describes the isolation and characterization of two new anthraquinones from a chloroform extract of the roots of *R. cordifolia*.

2. Investigations, results and discussion

Cordifoliol (**1**) was obtained as orange crystals from petroleum ether-chloroform (9 : 1) eluants. Its positive reaction with $FeCl_3$ and IR spectrum (3443 cm^{-1}) indicated its phenolic nature. Its positive reaction with KOH and UV spectrum (248, 291 and 422 nm) suggested the compound's anthraquinonidal nature. The appearance of absorption bands in IR spectrum at 1670 and 1655 cm^{-1} showed the existence of the chelated and unchelated carbonyl group, respectively. The EI-MS of **1** exhibited a molecular ion peak at m/z 252 related to an anthraquinone formula, $C_{16}H_{12}O_3$. It displayed diagnostically important fragment ions for anthraquinonidal moiety at m/z 238 $[M-CH_3]^+$, 224 $[M-CO]^+$, 181 $[224-CH_3-CO]^+$, 120, 104 and 76. The fragment ion at m/z 91 $[120-C_2H_5]^+$ suggested the existence of a hydroxyl and an ethyl group in the same benzene ring, well supported by the ^1H NMR spectrum, which showed six aromatic protons, indicating the presence of two substitutions only. Two meta-coupled, integrated for one proton each, at δ 6.73 (d, J = 2.4 Hz, H-2) and 7.22 (d, J = 2.4 Hz, H-4), indicated the presence of an ethyl group at C-3 position [21]. HMQC and HMBC confirmed the location of the ethyl group at C-3 through two and three bond relations from methylene protons to C-3, C-2 and C-4, from H-4 to C-2, C-13 and C-10, from H-6 to C-7, C-8, C-11, from H-5 to C-7, C-10 and C-12. A_2B_2 type coupled protons [22] at δ 8.28 (2 H, m, H-5 and H-8) and 7.78 (2 H, m, H-6 and H-7) suggested the unsubstituted nature of the other benzene ring. Methyl and methylene protons resonated at δ 0.85 (J = 5.6 Hz) and 1.57, respectively. The ^{13}C NMR data and DEPT experiments confirmed the presence of one methyl, one methylene, six methines and eight quaternary carbons, of which two carbonyl carbons were observed at δ 188.95 and δ 182.38. Based on these findings, the structure of **1** has been established as 1-hydroxy-3-ethyl-9,10-anthraquinone.

Cordifodiol (**2**) was obtained as red crystals from petroleum ether-chloroform (7 : 3) eluants. Its UV spectral data (258, 281 sh, 306 sh, 344, 362 and 436 nm) were characteristic of phenathrene derivatives [23]. The positive reaction with $FeCl_3$ and IR spectrum absorption (3460 cm^{-1}) suggested the compound's phenolic nature. Carbonyl absorption band was observed at (1690 cm^{-1}). The EI-MS of **2** displayed a molecular ion peak at m/z 396 consistent with the molecular formula $C_{26}H_{20}O_4$. A prominent fragment ion at m/z 208 suggested the existence of phenanthrene nucleus. The important ion peaks at m/z 160, 132, 68 and 57 indicated that the compound possesses a phenathrene moiety with a naphthaquinone ring attached to C-11 and C-20 carbon atoms. The position of hydroxyl group was determined by its ^1H NMR spectrum which displayed one singlet, integrated for two protons, at δ 7.76 (H-9 and H-22) and ortho, meta-coupled protons at 8.28 (dd, J = 8.06 and 2.2 Hz, H-2 and H-7) and at 7.58 (d, J = 8.06 Hz, H-3 and H-6), suggesting the position of hydroxyl group at C-1 and C-8, confirmed by HMQC and HMBC through two and three bond relations from H-2 to C-1, C-3, C-4 and C-22, from H-3 to C-2, C-1, C-21 and C-5, from H-3 to C-7, C-4 and C-10, from H-7 to C-6, C-8, C-9 and C-5, from H-22 to C-1, C-4, C-20 and C-2. The spectrum exhibited signals for ortho-coupled protons at δ 8.20 (d, J = 7.6 Hz, H-14), δ 8.80 (d, J = 8.8 Hz, H-17) and δ 7.78 (dd, J = 8.8, 2.2 Hz, H-16), indicating the position of a butyl group at C-15, confirmed by HMQC and HMBC through two and three bond relations



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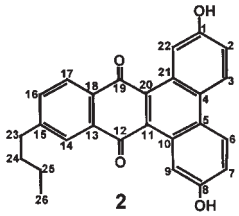

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from methylene protons to C-15 and C-14, from H-14 to C-16, C-18 and C-12, from H-16 to C-15 and C-13, from H-17 to C-19, C-15 and C-13. Three methylene and one-methyl protons resonated between δ 2.54–1.54 and at δ 0.95 (t, J = 5.6 Hz) respectively. The ^{13}C NMR data and DEPT experiments confirmed the presence of one methyl, three methylenes, nine methines and thirteen quaternary carbons, of which two-carbonyl carbon were observed at δ 183.47 and δ 183.02. These observations led to formulate the structure of **2** as 1,8-dihydroxy-11,20(15-pentyl-naphthaquinonyl) phenanthrene.

3. Experimental

3.1. General procedure

M. p.'s were determined on a Perfit apparatus and are uncorrected. IR spectra were recorded in KBr pellets on a Perkin-Elmer 377 instrument. ^1H and ^{13}C NMR spectra were recorded at 600 and 150 MHz (Brucker spectrospin NMR instrument in $CDCl_3$), respectively, using TMS as internal standard. 2D NMR were run at 500 (^1H) and 125 MHz (^{13}C). EIMS spectra were scanned at 70 eV 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 the solvent systems toluene-ethyl acetate (9 : 1) and toluene-ethyl acetate-acetic acid (5 : 2 : 0.5). 5% alcoholic KOH was used for the visualization of the TLC spots.

3.2. Plant material

Roots (2 kg) were procured from Herb indica, Chandigarh, identified and authenticated 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 and isolation

Powdered roots (1.5 kg) were extracted exhaustively with chloroform in a Soxhlet apparatus. The chloroform extract was concentrated under reduced pressure in a Bucchi rotavapour to yield a thick brown mass. The extract was adsorbed on silica gel to form a slurry, which was loaded on a silica gel column packed in petroleum ether. The column was eluted with petroleum ether-chloroform in different proportions to isolate the following compounds.

3.4. Characterization of the compounds

3.4.1. *Cordifoliol* (1)

Elution of the column with petroleum ether-chloroform (9 : 1) afforded orange crystals of **1**, recrystallized from chloroform-methanol (1 : 1), 30 mg, R_f 0.54 (toluene-ethyl acetate, 9 : 1), m.p. 200–201 °C, UV λ_{max} (MeOH) 248 nm (logε 5.6), 291 (log ε 4.2), 422 (log ε 1.8); IR ν_{max} 3443, 2960, 2857, 1670, 1655, 1590, 1421, 1260, 1230, 1172, 1112, 1020 cm^{-1}; ^1H NMR ($CDCl_3$): δ 13.98 (1 H, brs, OH), 8.28 (2 H, m, H-5 and H-8), 7.78 (2 H, m, H-6 and H-7), 7.22 (1 H, d, J = 2.4 Hz, H-4), 6.73 (1 H, d, J = 2.4 Hz, H-2), 1.57 (2 H, brs, CH_2) and δ 0.85 (3 H, t, J = 6.5 Hz, CH_3); EIMS m/z (rel. int.) 252 [M^+] ($C_{16}H_{12}O_3$), (2.3), 238 (100), 224 (2.2), 223 (2.3), 209 (16.4), 181 (54.0), 164 (14.7), 152 (70.0), 150 (24.4), 132 (4.2), 120 (3.1), 105 (30.0), 104 (28.2), 91 (12.2), 76 (70.3); ^{13}C NMR ($CDCl_3$): δ 161.06 (C-1), 133.78 (C-2), 115.22 (C-3), 119.22 (C-4), 133.27 (C-4a), 134.96 (C-5), 126.84 (C-6), 127.78 (C-7), 133.96 (C-8), 134.52 (C-8a), 188.95 (C-9), 137.22 (C-9a), 182.38 (C-10), 131.31 (C-10a), 16.15 (CH_3) 29.67 (CH_2).

3.4.2. *Cordifodiol* (2)

Elution of the column with petroleum ether-chloroform (7 : 3) furnished red crystals of **2**, recrystallized from chloroform-methanol (1 : 1), 25 mg, R_f 0.60 (toluene-ethyl acetate-acetic acid, 5 : 2 : 0.5), m.p. 222–223 °C. UV λ_{max} (MeOH) 234 nm (log ε 5.8), 278 (log ε 4.4), 362 (log ε 2.4), 436 (log ε 1.9); IR $\nu\mu_{max}$ (KBr) 3460, 2960, 2857, 1690, 1590, 1421, 1260, 1230, 1172, 1112, 1020 cm^{-1}; ^1H NMR ($CDCl_3$): δ 12.78 (2 H, s, 2 × OH), 8.80 (1 H, d, J = 8.8 Hz, H-17), 8.28 (2 H, dd, J = 8.06 and 2.2 Hz, H-2 and H-7), 8.20 (1 H, d, J = 7.6 Hz, H-14), 7.78 (1 H, dd, J = 8.8 and 2.2 Hz, H-16), 7.76 (2 H, s, H-9 and H-22), 7.58 (2 H, d, J = 8.16 Hz, H-3 and H-6), 2.54 (2 H, dd, J = 5.4 and 5.2 Hz, H_2-23), 1.54 (4 H, brs, H_2-24 and H_2-25), 0.95 (3 H, t, J = 5.6 Hz H_3-26); EIMS m/z (rel. int.) 396 [M^+] ($C_{26}H_{20}O_4$) (5.2), 255 (2.3), 211 (10.4), 208 (75.7), 188 (3.6), 175 (5.0), 164 (50.0), 160 (20.2), 132 (40.6), 109 (2.5), 96 (10.0), 83 (50.1), 68 (20.3), 57 (12.6); ^{13}C NMR ($CDCl_3$): δ 145.30 (C-1), 127.45 (C-2), 127.17 (C-3), 133.43 (C-4), 131.32 (C-5), 127.15 (C-6), 127.23 (C-7), 145.30 (C-8), 133.92 (C-9), 131.32 (C-10), 133.63 (C-11), 183.02 (C-12), 133.60 (C-13), 134.12 (C-14), 133.43 (C-15) 127.51 (C-16), 134.94 (C-17), 133.92 (C-18), 183.47 (C-19), 133.64 (C-20), 133.43 (C-21), 134.04 (C-22), 31.92 (C-23), 29.68 (C-24), 29.70 (C-25) 21.91 (C-26).

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