

Note

New anthraquinones from *Rubia cordifolia* roots

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Three new anthraquinones namely 1-hydroxy-2, 7-dimethylanthraquinone, 2-hydroxy-6-methylanthraquinone and 2, 6-dihydroxyanthraquinone along with *n*-nonadecane, *n*-heptadecane, 8'-hydroxy-*n*-pentadecanyl decan-4-en-1-oate, *n*-octacosanyl octa-1-oate and 3, 5-di-(*p*-hydroxybenzyl) phenol have been isolated from *Rubia cordifolia* roots and characterized on the basis of spectral data.

Key words: *Rubia cordifolia* Linn., Rubiaceae, anthraquinone

IPC Code: Int. Cl.⁸ A61K

Rubia cordifolia L. (Rubiaceae), known as *Manjista* in Sanskrit, is a very variable, scandant, perennial, climber or creeper that grows in the North-West Himalayas, Nilgiris, Mahabaleshwar and other hilly districts of India¹. A number of anthraquinones and triterpenes have been reported from *R. cordifolia*²⁻⁶. Traditionally, it is considered useful in inflammations, ulcers and skin diseases⁷.

Materials and methods

Mps are uncorrected. FT/IR: Jasco FTIR-5000; UV: Beckman DU-64, MeOH; ¹H NMR (400 MHz): Advance DRY 400, Bruker Spectrospin, CDCl₃; ¹³C NMR (400 MHz): Advance DRY 100, Bruker Spectrospin, CDCl₃ with TMS as an internal standard; MS: JEOL-JMS-DX 303; CC: silica gel (Qualigens), 60-120 mesh; TLC: silica gel G (Qualigens). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying with ceric ammonium sulphate and perchloric acid.

Plant material

The roots of *R. cordifolia* was collected from Khari Baoli and identified by Dr. M. P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard.

Extraction

The air-dried roots (2 kg) of *R. cordifolia* were coarsely powdered and extracted in a Soxhlet

apparatus with methanol for 72 hr. The methanolic extract was concentrated to obtain dark viscous mass (150 g). A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents.

Isolation

The viscous dark green mass (150 g) was adsorbed on silica gel (60-120 mesh) for column after being dissolved in little quantity of methanol for preparation of slurry. The slurry (150 g) was air dried, packed and chromatographed over silica gel column packed in pet. ether. The column was eluted successively with pet. ether, pet. ether-chloroform (90:10, 75:25, 50:50 and 25:75), chloroform, chloroform-methanol (99:1, 98:2, 96:4, 95:5, 97:3, 90:10) which resulted in isolation of compounds **1-8**.

Compound 1

Elution of column with pet. ether (fractions 1-77) yielded colourless amorphous powder of **1**, recrystallized from CHCl₃-MeOH (1:1), 45 mg (yield, 0.003%). *R*_f: 0.34 (*n*-hexane); m.p. 70-72°C; UV (MeOH): 203 nm (log ε 1.2); IR (CCl₄): 2923, 2845, 2342, 1430, 1015, 794 cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 1.23 (30H, br s, 15 × CH₂), 0.86 (3H, t, *J* = 6.1 Hz, Me-17), 0.84 (3H, t, *J* = 6.0 Hz, Me-1); +ve FAB MS (rel. int.): *m/z* [M+1]⁺ 241 (11.1), [M]⁺ 240 (C₁₇H₃₆) (10.2).

Compound 2

Elution of column with pet. ether (fractions 159-183), furnished colourless amorphous powder of **2**, recrystallized from MeOH, 50 mg (yield, 0.003%). *R*_f: 0.31 (*n*-hexane); m.p. 92-94°C; UV (MeOH): 205 nm (log ε 1.2); IR (CCl₄): 3450, 2910, 2851, 1720, 1654, 1650, 1459, 1020, 795 cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 5.13 (1H, m, H-4), 5.19 (1H, m, H-5), 4.15 (1H, br m, *w*_{1/2} = 8.1 Hz, H-8'β), 3.35 (2H, br s, H₂-1'), 2.66 (4H, br s, H₂-2, H₂-3), 2.01 (2H, m, H₂-6), 1.66 (2H, m, H₂-7), 1.56 (2H, m, H₂-9'), 1.46 (10H, br s, 5 × CH₂), 1.23 (12H, br s, 6 × CH₂), 1.16 (4 H, br s, 2 × CH₂), 0.87 (3H, t, *J* = 6.1 Hz, Me-10), 0.83 (3H, t, *J* = 6.2 Hz, Me-15'); +ve FAB MS (rel. int.): *m/z* [M+1]⁺ 397 (60.1), [M]⁺ 396 (C₂₅H₄₈O₃) (18.6), 299 (12.6), 297 (12.5), 153 (24.5), 99 (19.6), 97 (26.1).

Compound 3

Elution of column with pet. ether-CHCl₃ (3:1), (fractions 310-315) yielded colourless amorphous powder of **3**, recrystallized from CHCl₃-MeOH (1:1), 70 mg, (yield 0.005%). *R_f*: 0.46 (pet. ether: chloroform, 3:1); m.p. 84-86°C; UV (MeOH): 203 nm (log ϵ 1.3); IR (CCl₄): 2922, 2857, 1430, 1083, 1077, 1024, 795 cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 1.43 (2H, br s, CH₂), 1.23 (10H, br s, 5 \times CH₂), 1.14 (40 H, br s, 20 \times CH₂), 0.79 (3H, t, *J* = 6.1 Hz, Me), 0.74 (3H, t, *J* = 6.0 Hz, Me); +ve FAB MS (rel. int.): *m/z* [M+1]⁺ 395 (12.3), [M]⁺ 394 (C₂₈H₅₈) (14.1).

Compound 4

Elution of column with pet. ether-CHCl₃ mixture (1:1), (fractions 320-329) furnished brown amorphous powder of **4**, recrystallized from diethyl ether, 35 mg (yield 0.002%). *R_f*: 0.53 (pet. ether-CHCl₃, 1: 9); m.p. 238-240°C; UV (MeOH): 201, 248, 283, 312, 431 nm (log ϵ 3.1, 2.3, 1.2, 4.3, 4.9); IR (CCl₄): 3440, 2923, 2857, 1719, 1702, 1654, 1560, 1510, 1460, 1050, 1018 cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 8.23 (1H, dd, *J* = 8.7, 3.3 Hz, H-6), 8.18 (1H, dd, *J* = 8.1, 3.3 Hz, H-3), 7.93 (1H, d, *J* = 3.3 Hz, H-8), 7.91 (1H, br s, OH), 7.55 (1H, d, *J* = 8.7 Hz, H-5), 7.25 (1H, d, *J* = 8.1 Hz, H-4), 2.50 (6H, br s, Me-11, Me-12); ¹³C NMR (DMSO-*d*₆): δ 152.50 (C-1), 150.77 (C-2), 133.17 (C-3), 118.10 (C-4), 121.80 (C-4a), 187.72 (C-10), 122.12 (C-5a), 133.90 (C-5), 126.43 (C-6), 146.68 (C-7), 130.50 (C-8), 125.71 (C-8a), 181.47 (C-9), 136.06 (C-9a), 31.07 (C-11), 33.67 (C-12); +ve FAB MS (rel. int.): *m/z* [M+1]⁺ 253 (33.1), [M]⁺ 252 (C₁₆H₁₂O₃) (65.2).

Compound 5

Elution of the column with pet. ether-CHCl₃ mixture (1:1), (fractions 330-340) furnished brick red amorphous powder of **5**, recrystallised from MeOH, 70 mg (yield 0.006%). *R_f*: 0.61 (CHCl₃); m.p. 260-262°C; UV (MeOH): 201, 248, 283, 319, 347, 417 nm (log ϵ 4.1, 3.6, 2.8, 3.6, 4.8); IR (KBr): 3480, 2991, 2846, 1703, 1686, 1654, 1580, 1544, 1510, 1077, 1024 cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 8.15 (1H, dd, *J* = 7.32, 3.0 Hz, H-3), 8.11 (1H, dd, *J* = 8.22, 3.1 Hz, H-7), 7.88 (2H, br s, H-1, H-5), 7.61 (1H, d, *J* = 7.32 Hz, H-4), 7.19 (1H, *J* = 8.22 Hz, H-8), 2.08 (3H, br s, Me-11); ¹³C NMR (DMSO-*d*₆): δ 126.43 (C-1), 152.75 (C-2), 132.77 (C-3), 116.16 (C-4), 120.80 (C-4a), 188.72 (C-10), 121.12 (C-5a), 133.99 (C-5), 150.77 (C-6), 126.68 (C-7), 133.50 (C-8), 123.71 (C-8a), 180.47 (C-9), 135.06 (C-9a), 30.67 (C-11); +ve FAB

MS (rel. int.): *m/z* [M+1]⁺ 239 (12.0), [M]⁺ 238 (C₁₅H₁₀O₃) (4.2).

Compound 6

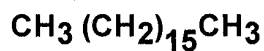
Elution of column with pet. ether-CHCl₃ mixture (1:1), (fractions 341-345) furnished red coloured crystals of **6** (460 mg) recrystallized from MeOH-diethyl ether (1:1) 35 mg (yield 0.002%). *R_f*: 0.23 (CHCl₃-MeOH, 4:1); m.p. 274-75°C; UV (MeOH): 228, 244, 310, 536 nm (log ϵ 3.2, 3.9, 1.7, 1.2); IR (CCl₄): 3490, 2922, 2852, 2361, 1703, 1687, 1655, 1560, 1544, 1544, 1524, 1460, 1078, 1018 cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 8.17 (1H, dd, *J* = 8.4, 2.2 Hz, H-3), 8.13 (1H, dd, *J* = 8.22, 2.1 Hz, H-7), 7.89 (1H, d, *J* = 2.4 Hz, H-1), 7.87 (1H, d, *J* = 2.1 Hz, H-5), 7.62 (1H, d, *J* = 8.4 Hz, H-8), 7.21 (1H, d, *J* = 8.22 Hz, H-4); ¹³C NMR: (DMSO-*d*₆): δ 126.63 (C-1), 150.75 (C-2), 132.72 (C-3), 133.92 (C-4), 120.794 (C-4a), 188.66 (C-10), 121.06 (C-5a), 134.34 (C-5), 152.61 (C-6), 133.47 (C-7), 133.10 (C-8), 126.37 (C-8a), 180.40 (C-9), 135.0 (C-9a); +ve FAB MS (rel. int.): *m/z* [M+1]⁺ 241 (25.2), [M]⁺ 240 (C₁₄H₈O₄) (12.3).

Compound 7

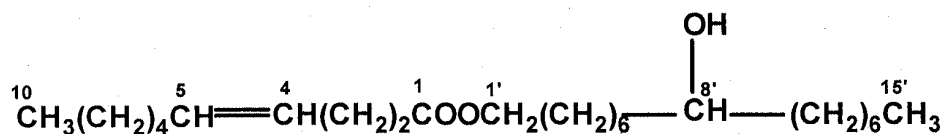
Elution of column with pet. ether-CHCl₃ mixture (1:3), (fractions 356-360) furnished brown coloured crystals of **7**, recrystallized from MeOH 210 mg (yield 0.014 %). *R_f*: 0.67 (CHCl₃); m.p. 90-92°C; UV (MeOH): 202 nm (log ϵ 2.3); IR (CCl₄): 2920, 2852, 1725, 1620, 1480, 1265, 1061, 1024, 791 cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 3.90 (1H, d, *J* = 9.39 Hz, OCH₂-a), 3.86 (1H, d, *J* = 9.39 Hz, OCH₂-b), 2.21 (1H, d, *J* = 7.23 Hz, COCH₂-a), 2.18 (1H, d, *J* = 7.23 Hz, COCH₂-b), 1.99 (2H, m, H₂), 1.51 (2H, m, H₂), 1.25 (60 H, br s, 30 \times CH₂), 0.86 (6H, br s, 2 \times Me); +ve FAB MS (rel. int.): *m/z* [M+1]⁺ 551 (4.1), [M]⁺ 550 (C₃₇H₇₄O₂) (11.3).

Compound 8

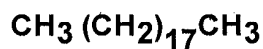
Elution of column with CHCl₃-MeOH mixture (6:4), (fractions 402-413), furnished brown colour crystals of **8**, yielded 35 mg, recrystallized from acetone (0.002% yield). *R_f*: 0.49 (CHCl₃-Ethyl acetate-MeOH, 7:1:2); m.p. 212-14°C; UV (MeOH): 203, 246, 274 nm (log ϵ 3.1, 2.2, 2.1); IR (KBr): 3490, 3360, 2922, 2816, 1655, 1544, 1510, 1072, 1029 cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 8.15 (2H, br s, H-2, H-6), 8.09 (2H, br s, H-17, H-19), 7.90 (6H, br s, H-9, H-10, H-12, H-13, H-16, H-20), 7.67 (2H, m, H-4), 2.50 (4H, br s, 2 \times CH₂); +ve FAB MS (rel. int.): *m/z* [M+1]⁺ 307 (5.2), [M]⁺ 306 (C₂₀H₁₈O₃) (10.1).



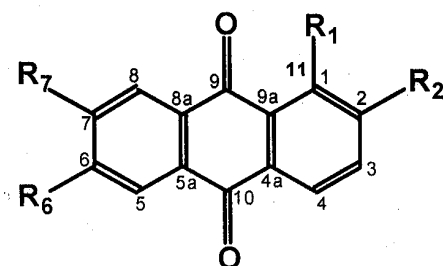
1



2



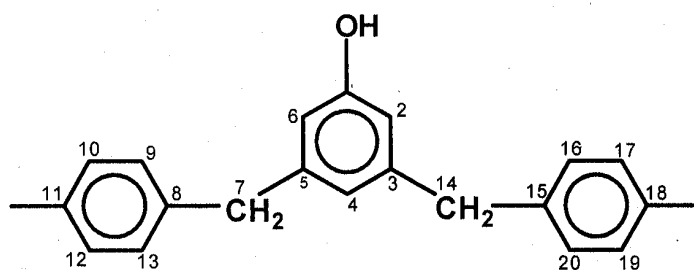
3



	R ₁	R ₂	R ₆	R ₇
4	OH	CH ₃	H	CH ₃
5	H	OH	CH ₃	H
6	H	OH	OH	H



7



8

Results and Discussion

Compound 1

Compound **1** was obtained as a colourless amorphous powder from the pet. ether eluants. It did not respond to tetranitromethane and bromine water indicating saturated nature of the molecule. Its IR

absorption bands at 1015 and 794 cm^{-1} are due to saturated nature of the molecule. Its +ve FAB mass spectrum showed a molecular ion peak at m/z 240 corresponding to $\text{C}_{17}\text{H}_{36}$. Most of the ion peaks were separated by 14 mass units and intensities of the fragments decreased with increasing molecular weight

confirming the presence of the long chain hydrocarbon. The ^1H NMR spectrum of compound **1** displayed two three-proton triplets at δ 0.86 ($J = 6.1$ Hz) and δ 0.84 ($J = 6.0$ Hz) assigned to terminal C-1 and C-17 primary methyl protons, respectively. The methylene protons resonated as broad signal at δ 1.23 ($15 \times \text{CH}_2$). The compound did not react with any oxidizing or acetylating reagents. On the basis of these studies the compound **1** has been identified as *n*-heptadecane.

Compound 2

Compound **2** obtained as amorphous powder from pet. ether responded positively to tetranitromethane and bromine water test indicating olefinic linkage. Its IR spectrum showed absorption bands for hydroxyl group (3450 cm^{-1}), ester group (1720 cm^{-1}), unsaturation (1654 cm^{-1}) and long aliphatic chain (795 cm^{-1}). Its +ve FAB mass spectrum showed a molecular ion peak at m/z 396 corresponding to molecular formula $\text{C}_{25}\text{H}_{48}\text{O}_3$ of a hydroxy ester. It showed one double bond equivalent that was adjusted in an olefinic linkage the position of which at C-4 was confirmed by fragments at m/z 299 and 97 due to C_3 - C_4 fission. Other important fragments at m/z 153 [CO - O fission] and 297 and 99 [C_8 - C_9 fission] supported the presence of hydroxyl group in the compound. More intense clusters of peaks relating to $\text{C}_n\text{H}_{2n-1}$ (e.g., m/z 97, 297 etc.) in comparison to that corresponding to $\text{C}_n\text{H}_{2n+1}$ (e.g., m/z , 99, 299 etc.) indicated the olefinic nature of the compound. The ^1H NMR spectrum of compound **2** exhibited two one-proton multiplets at δ 5.31 and 5.19 assigned to two vinylic protons at C-4 and C-5. A one-proton broad multiplet at δ 4.15 with $w_{1/2} = 8.1\text{ Hz}$ was assigned to β -oriented carbinol proton at C-8'. Methylene protons adjacent to the ester group at C-1' resonated as two-proton broad signal at δ 3.035. Rest of the methylene protons resonated between δ 2.66-1.16. Two three-protons triplets at δ 0.87 ($J = 6.5$ Hz) and 0.83 ($J = 6.2$ Hz) were associated with correspondingly to C-10 and C-15' primary methyl protons. The absence of any signal between δ 3.35 and 2.66 ruled out the location of any carbinol proton in the molecule. On the basis of these information the structure of the compound **2** has been elucidated as 8-hydroxy *n*-pentadecanyl decan-4-en-1-oate.

Compound 3

Compound **3** was obtained as a colourless amorphous powder from pet. ether- CHCl_3 (3:1). The

molecule responded positively with tetranitromethane and bromine water for unsaturation and did not give effervescences with sodium bicarbonate or derivative with 2,4-DNP reagent for carboxylic and carbonyl groups respectively. Its IR absorption showed characteristic absorption band for long aliphatic chain at 795 cm^{-1} . Its +ve FAB mass spectrum showed a molecular ion peak at m/z 394 corresponding to $\text{C}_{28}\text{H}_{58}$. Most of the ion peaks were separated by 14 mass units and intensities of the fragments decreased with increasing molecular weight confirming the presence of the long chain hydrocarbon. The ^1H NMR spectrum of compound **3** displayed two three-proton triplets at δ 0.79 ($J = 6.1$ Hz) and 0.74 ($J = 6.0$ Hz) assigned to terminal primary methyl protons respectively. The methylene protons resonated as broad signals at δ 1.43 ($1 \times \text{CH}_2$), 1.23 ($5 \times \text{CH}_2$) and 1.14 ($20 \times \text{CH}_2$). The data led to identify the compound as *n*-nonadecane.

Compound 4

Compound **4** was obtained as brown amorphous powder from pet. ether- CHCl_3 (1:1). It showed a bright orange-red fluorescence in UV 365 nm, suggesting the presence of anthraquinone moiety. Its IR spectrum displayed characteristic absorption band for hydroxyl group (3440 cm^{-1}), carbonyl group (1654 cm^{-1}) and β -methyl group (1460 cm^{-1}). Its +ve FAB mass spectrum showed a molecular ion peak at m/z 252 corresponding to molecular formula $\text{C}_{16}\text{H}_{12}\text{O}_3$ of an anthraquinone derivative. The ^1H NMR spectrum of compound **4** exhibited two *ortho* and *meta* coupled double doublets at δ 8.23 ($J = 8.7, 3.3$ Hz) and δ 8.18 ($J = 8.1, 3.3$ Hz) assigned to H-6 and H-3 protons respectively. A six-proton broad singlet at δ 2.50 was accounted to Me-11 and Me-12 protons. Three one-proton doublets at δ 7.93 ($J = 3.3$ Hz), 7.55 ($J = 8.7$ Hz) and 7.25 ($J = 8.1$ Hz) were accounted to H-8, H-5 and H-4 protons, respectively. The ^{13}C NMR spectrum of **4** exhibited carbonyl carbons at δ 187.72 (C-10), 181.47 (C-9), aromatic carbon in the range δ 152.50-118.10 and methyl carbons at δ 31.07 (C-11) and 33.67 (C-12). On the basis of these spectral data analysis, the structure of **4** has been established as 1-hydroxy 2, 7-dimethylantraquinone. This anthraquinone derivative is reported for the first time from *R. cordifolia*.

Compound 5

Compound **5** was obtained as red colour crystals from pet. ether- CHCl_3 (1:1). It showed a bright orange

red fluorescence in UV 365 nm, suggesting the presence of anthraquinone moiety. Its IR spectrum displayed characteristic absorption bands for hydroxyl group (3480 cm^{-1}) and carbonyl group (1654 cm^{-1}). Its +ve FAB mass spectrum showed a molecular ion peak at m/z 238 corresponding to molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_3$ of an anthraquinone derivative. The ^1H NMR spectrum of **5** exhibited two one-proton double doublets at δ 8.15 ($J = 7.32, 3.0\text{ Hz}$) and 8.11 ($J = 8.22, 3.1\text{ Hz}$) assigned to *ortho meta*-coupled H-3 and H-7. A two-proton broad signal at δ 7.88 was accounted to H-1 and H-5. Two one-proton doublets at δ 7.61 ($J = 7.32\text{ Hz}$) and 7.19 ($J = 8.22\text{ Hz}$) were ascribed to *ortho*-coupled H-4 and H-8, respectively. A three-proton broad signal at δ 2.08 was attributed to C-11 methyl function attached to the aromatic ring at C-9. The ^{13}C NMR spectrum of **5** exhibited carbonyl carbons at δ 188.72 (C-10), 180.47 (C-9), aromatic carbon in the range δ 152.75-116.16 and methyl carbon at δ 30.67. On the basis of these spectral data the structure of **5** has been formulated as 2-hydroxy-6-methylanthraquinone. This anthraquinone derivative is reported for the first time from *R. cordifolia*.

Compound 6

Compound **6** was obtained as red colour crystals from pet. ether- CHCl_3 (1:1) mixture. It showed orange-red fluorescence in UV 365 nm, suggesting the presence of anthraquinone moiety. Its IR spectrum displayed characteristic absorption bands for hydroxyl group (3490 cm^{-1}) and carbonyl group (1655 cm^{-1}). Its +ve FAB mass spectrum showed a molecular ion peak at m/z 240 corresponding to molecular formula $\text{C}_{14}\text{H}_8\text{O}_4$ of an anthraquinone derivative. The ^1H NMR spectrum of **6** displayed two one-proton doublets, at δ 8.17 ($J = 8.4, 2.2\text{ Hz}$) and δ 8.13 ($J = 8.22, 2.1\text{ Hz}$) assigned to *ortho-meta*-coupled H-3 and H-7. Two doublets at δ 7.62 ($J = 8.4\text{ Hz}$) and 7.21 ($J = 8.22\text{ Hz}$), both integrating for one proton each, were accounted to *ortho*-coupled H-8 and H-4 protons. The ^{13}C NMR spectrum of **6** exhibited carbonyl carbon signals at δ 188.66 (C-6) and 180.40 (C-13) and aromatic carbon signals between δ 152.61-1121.06. On the basis of this information, the structure of **6** has been established as 2, 6-dihydroxyanthraquinone. This anthraquinone derivative is reported for the first time from *R. cordifolia*.

Compound 7

Compound **7**, an aliphatic ester was obtained as colourless amorphous mass from the pet. ether- CHCl_3

(1:3) eluants. The molecule did not respond positively to tetranitromethane and bromine water test for unsaturation. Its IR absorption showed characteristic absorption bands for ester group at 1725 cm^{-1} and long aliphatic chain at 795 cm^{-1} . Its +ve FAB mass spectrum showed a molecular ion peak at m/z 550 corresponding to $\text{C}_{37}\text{H}_{74}\text{O}_2$ of an aliphatic ester. Most of the ion peaks were separated by 14 mass units and intensities of the fragments decreased with increasing molecular weight confirming the presence of the long aliphatic chain. The ^1H NMR spectrum of compound **7** exhibited two doublets, one-proton each, at δ 3.90 ($J = 9.39\text{ Hz}$) and 3.86 ($J = 9.39\text{ Hz}$) assigned to oxygenated methylene proton, respectively. A one-proton each, doublet at δ 2.21 and 2.18 were assigned to carbonyl methylene protons. The remaining methylene protons appeared at δ 1.99 (2H), 1.51 (2H) and 1.25 (60H). A six-proton broad singlet at δ 0.86 was assigned to methyl protons. On the basis of these evidences, the structure of **7** has been determined tentatively as *n*-octacosanyl octa-1-oate.

Compound 8

Compound **8** was obtained as brown coloured crystals from CHCl_3 -MeOH (6:4) eluents. It showed purple colour when treated with FeCl_3 suggesting the presence of phenolic moiety. Its IR spectrum displayed characteristic absorption bands for hydroxyl group ($3490, 3360\text{ cm}^{-1}$) and aromatic nature ($1544, 1510, 1070\text{ cm}^{-1}$) in the molecule. Its +ve FAB mass spectrum showed a molecular ion peak at m/z 306 corresponding to molecular formula $\text{C}_{20}\text{H}_{18}\text{O}_3$. The ^1H NMR spectrum of **8** displayed two two-proton broad singlets at δ 8.15 and 8.09 assigned to H-2, H-6, H-17 and H-19 aromatic methine protons. A six-proton broad signal at δ 7.90 and a one-proton multiplet were accounted to other aromatic methine protons *viz.* H-9, H-10, H-12, H-13, H-16, H-20 and H-4. A four-proton broad singlet at δ 2.50 was assigned to the methylene protons. On the basis of this information, the structure of **8** has been established as 3, 5-di-(*p*-hydroxybenzyl)phenol.

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