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# New compounds from the seeds of *Embelia ribes* Burm

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Phytochemical investigation of the seeds of *Embelia ribes* Burm. resulted in the isolation of three new compounds identified as 3-(4″-hydroxyoctadecanyloxy)-*p*-quinonyl-5-methylene-8-(10-pentanyloxy)-*p*-quinone (embelinol) (**1**), *n*-pentacosanyl-*n*-nonadeca-7′-en-9′-α-ol-1′-oate (embeliaribyl ester) (**2**) and 1,2,4,5-tetrahydroxy 3-undecanyl benzene (embeliol) (**3**) along with the known compound 2,5-dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-dione (embelin) (**4**). Their structures have been established on the basis of spectral data analyses and chemical reactions

## 1. Introduction

*Embelia ribes* Burm. (Myrsinaceae), commonly known as *vidang*, a large scandent shrub having branches with long internodes, grows throughout India, up to 1,500 m elevation in hilly regions (Arya 1997). The seeds contain embelin (2.5–3.1%), quercitol (1.0%), fatty ingredients (5.2%), the alkaloid christembine, a resinoid, volatile oil, fixed oil, colouring matter, tannins and "vidangin" (Chopra et al. 1996). The fruits are credited as bitter, analgesic, vulnerary anthelmintic, to cure bronchitis, to remove bad humour from the body (Kirtikar and Basu 2000), for dental, oral and throat troubles, abdominal discomfort and post-operative gastro-intestinal symptoms (Asolkar et al. 1992). An aqueous extract of berries exhibited resorptive (Asolkar et al. 1992) and anthelmentic actions (Amar Singhe et al. 1993; Paul and Vatsa 1980). This communication describes the isolation and characterisation of three new phytoconstituents from the seeds of *E. ribes*.

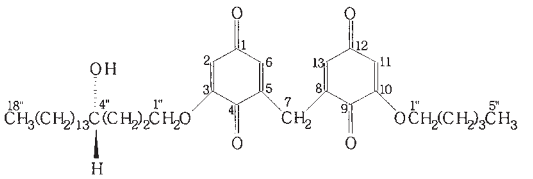
## 2. Investigations, results and discussion

Compound **1**, designated as embelinol, was obtained as a dark green coloured amorphous mass and recrystallised from chloroform-methanol (1 : 1). Its IR spectrum showed absorption bands for hydroxy (3450 $cm^{-1}$) and quinone (1695 $cm^{-1}$) groups. The mass spectrum exhibited a molecular ion peak at m/z 598 corresponding to the dialkylated bisquinone molecule $C_{36}H_{54}O_7$. The prominent ion peaks of m/z 227 [$C_{3''}$—$C_{4''}$ fission, $CH_3(CH_2)_{13}$ CHOH]$^+$, 269 [O—$C_{1''}$ fission, $CH_3(CH_2)_{13}CHOH(CH_2)_3]^+$, 285, 313 [$C_{10}$—O fission]$^+$, 242[313-$(CH_2)_4CH_3]^+$, 226[313-$OC_5H_{11}]^+$ and 391[$C_7$—$C_8$ fission]$^+$ suggested the existence of pentyloxy at one quinone ring, *n*-octadecan-4″-ol-1″-oxy group at another quinone ring and bridging of two quinone rings by a methylene group. The $^1H$ NMR spectrum of **1** displayed a two-proton broad signal at δ 6.62 assigned to H-2 and H-11 and two one proton signals at δ 5.36 and 5.28 ascribed to H-6 and H-13, respectively. A one-proton broad multiplet at δ 4.28 ($w_{½}$ = 4.2 Hz) was ascribed to C-4″ β carbinol proton. Four one-proton doublets at δ 4.21 (J = 6.9 Hz), 4.17 (J = 6.9 Hz), 4.07 (J = 6.8 Hz) and 3.98 (J = 6.8 Hz), were associated with C-1″ and C-1′ oxygenated methylene groups, respectively. Two three-proton triplets at δ 0.88 (J = 6.0 Hz) and 0.85 (J = 6.5 Hz) were accounted to C-18″ and C-5′ primary methyl functionalities, respectively. The remaining methylene groups appeared between δ 2.74–1.25. The $^{13}C$ NMR spectrum of **1** exhibited signals for quinone carbons at δ 181.9 (C-1, C-12) and 178.7 (C-4, C-9). The signals at 151.0 and 148.1 were accounted to C-3 and C-10 oxygenated carbons of the quinone rings. The C-4″ carbinol carbon, and C-1″ and C-1′oxygenated methylene carbons resonated at 68.36, 65.0 and 62.1, respectively. The remaining methylene and methyl carbons appeared between δ 34.1–14.1. On the basis of the foregoing account the structure of **1** has been formulated as 3-(4″-hydroxyoctadecanyloxy)-*p*-quinonyl-5-methylene-8-(10-pentanyloxy)-*p*-quinone. This is an unknown compound reported from a natural or synthetic source for the first time.

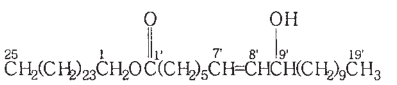
Compound **2**, named embeliaribyl ester, was obtained as colourless amorphous powder from chloroform-methanol (99 : 1) eluants. It responded positively with tetranitromethane and bromine water for unsaturation and demonstrated the presence of hydroxyl group (3495 $cm^{-1}$), ester linkage (1746 $cm^{-1}$), unsaturation (1622 $cm^{-1}$) and long aliphatic chain (741, 710 $cm^{-1}$) in its IR spectrum. It had a molecular ion peak at m/z 662 consistent with a molecular formula $C_{44}H_{86}O_3$. It indicated two double bond equivalents, one each adjusted in olefinic linkage and ester group. The important ion peaks at m/z 367 [$C_1$—O fission, $CH_3$ $(CH_2)_{23}$ $CH_2O]^+$, 295 [M-367]$^+$ and 395 [CO—$C_1$, fission, $CH_3(CH_2)_{23}CH_2OCO]^+$ suggested esterification of *n*-pentacosanol with an acid. The ion peaks at m/z 465 [$C_{6'}$—$C_{7'}$ fission]$^+$, 197 [M-465]$^+$, 171 [$C_{8'}$—$C_{9'}$ fission, HOCH $(CH_2)_9$ $CH_3]^+$ and 491 [M-171]$^+$ supported the location of the vinylic linkage at C-7′ and hydroxyl group at C-9′. The mass spectrum of **2** gave $C_nH_{2n+1}$, $C_nH_{2n}$ and

$C_nH_{2n-1}$ ions with higher abundance for lower fragments. Most of the fragments were separated by 14 mass units and decreased in abundance with increasing molecular weight of long straight chain hydrocarbon. The $^1H$ NMR of **2** displayed a one proton double doublet at δ 5.31 (J = 18.6, 5.2 Hz) and a one-proton broad signal at δ 5.28 assigned to vinyl H-8′ and H-7′, respectively. Two one-proton doublets at δ 4.31 (J = 11.1 Hz) and 4.17 (J = 11.1 Hz) were ascribed to oxygenated $H_2$-1 methylene proton. A one-proton carbinol broad multiplet at δ 3.99 ($w_{1/2}$ = 5.7 Hz) was accounted to H-9′β orientated carbinol proton. Two one-proton doublets at δ 2.46 (J = 6 Hz) and 2.44 (J = 6 Hz) were associated with H-2′ methylene protons adjacent to ester group. A six-proton broad signal at 0.88 was attributed to terminal primary methyl functionalities. The remaining methylene protons resonated at δ 2.76 (2 H) and between δ 2.40- 1.25. The $^{13}C$ NMR spectrum of **2** displayed deshielded carbon signals at δ 173.2, 130.2 and 128.1 assigned correspondingly to the ester carbon C-1′ and vinylic carbons C-8′ and C-7′. The C-9′ carbinol and C-1 oxygenated methylene carbons appeared at δ 68.9 and 62.1, respectively. The remaining methylene and methyl carbons appeared in the range of δ 34.2–14.1. On the basis of spectral data analyses and chemical evidences, the structure of the unknown ester **3** has been elucidated as *n*-pentacosanyl-*n*-nonadeca-7′-en-9′-α-ol-1′-oate. This is a new aliphatic ester isolated from *E. ribes* or any other species for the first time.

Compound **3**, named embeliol, was obtained as a reddish-orange crystalline product from chloroform-methanol (99 : 1) eluants. It gave a green colour with ferric chloride and showed IR absorption bands for hydroxyl groups (3494, 3312 $cm^{-1}$). It had a molecular ion peak at m/z 296 in the mass spectrum corresponding to tetrahydroxy substituted undecanyl benzene $C_{17}H_{28}O_4$. The important ion peaks at m/z 239 $[M-C_4H_9]^+$, 225 $[M-C_5H_{11}]^+$, 211 $[M-C_6H_{13}]^+$, 197$[M-C_7H_{15}]^+$, 183$[M-C_8H_{17}]^+$, 155 $[M-C_{10}H_{11}]^+$, and 141 $[M-C_{11}H_{13}]^+$ supported the attachment of an undecanyl side chain to the benzene ring. The $^1H$ NMR spectrum of **3** displayed a one-proton deshielded broad signal at δ 7.72 assignable to H-6. Two one-proton doublets at δ 2.06 (J = 6.0 Hz) and 2.02 (J = 6.0 Hz) were ascribed to C-7 methylene group attached to the aromatic ring. A three-proton triplet at δ 0.97 (J = 6.5 Hz) was accounted to C-17 primary methyl protons. The remaining methylene protons appeared at δ 1.60 (2 H), 1.47 (2 H), 1.30 (4 H) and 1.26 (10 H). The $^{13}C$ NMR spectrum of **3** exhibited oxygenated aromatic carbons at δ 169.9 (C-1, C-4), and 167.4 (C-2, C-5) in addition to signals at δ 116.1 (C-3) and 102.2 (C-6). An upfield signal at δ 12.6 was accounted to C-17 methyl carbon. The remaining methylene carbons resonated in the range δ 30.2–20.8. On the basis of the foregoing account, the structure of **3** has been formulated as 1,2,4,5-tetrahydroxy-3-undecanyl benzene. This is a new phenolic constituent isolated from a natural or synthetic source for the first time.

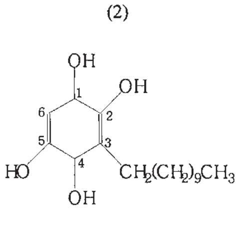


(1)

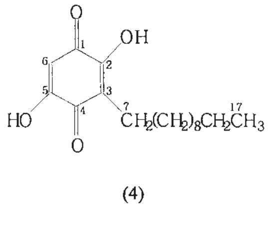


$$\overset{25}{C}H_2(CH_2)_{23}\overset{1}{C}H_2O\overset{\overset{O}{\|}}{\underset{1'}{C}}(CH_2)_5\overset{7'}{C}H{=}\overset{8'}{C}H\overset{\overset{OH}{|}}{\underset{9'}{C}}H(CH_2)_9\overset{19'}{C}H_3$$

(2)



(3)



(4)

Compound **4**, named embelin, was characterised as 2,5-dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-dione on the basis of spectral data analyses, physical constants and co-TLC.

## 3. Experimental

### 3.1. *General procedure*

Melting points were determined on a Perfit melting point apparatus in one end open capillary tubes and are uncorrected. UV spectra were recorded on a Lamda Bio-20 spectrophotometer in methanol; IR spectra on a Bio-Rad FT spectrophotometer using KBr pellets; $^1H$ NMR spectra on an Advance DRY 400 Bruker Spectrospin 400 MHz instrument using $CDCl_3$ as solvent and TMS as an internal standard; $^{13}C$ NMR Spectra on an Advance DRY 400 Bruker Spectrospin 100 MHz with TMS as an internal standard in 5 mm spinning tubes at 27 °C; Mass Spectra (MS) by effecting Electron Impact (EI) ionization at 70 eV on a JEOL-JMS-DX 303 instrument, equipped with direct inlet probe system. Silica gel (60–120 mesh) and silica gel-G were used for the column and TLC, respectively. Homogeneity of column fractions was controlled by TLC and the spots were visualised in UV light, iodine chamber and by spraying the plates with ceric ammonium sulphate.

### 3.2. *Plant material*

The plant material, *E. ribes* berries, was procured from Khari Boali market, Delhi and identified by Dr. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard.

### 3.3. *Extraction and isolation*

The fruits of *E. ribes* were dried in an oven at a temperature below 45 °C for 2–3 days and coarsely powdered. The pulverised seeds (2.0 kg) were extracted exhaustively with ethanol (95%) in a Soxhlet extractor. The ethanolic extract was concentrated under reduced pressure to give a dark brown, viscous syrupy mass (430 g, 21.5%). The extract was dissolved in a minimum amount of methanol and adsorbed on silica gel (60–120 mesh) for preparation of a slurry. It was dried, packed and chromatographed over a silica gel column in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol in the order of increasing polarity to isolate the compounds.

### 3.4. *Characterization of the compounds*

#### 3.4.1. *Embelinol* (**1**)

Elution of the column with $CHCl_3$ afforded the dark green amorphous mass of **1**, which was crystallized from $CHCl_3$–MeOH (1 : 1), 1.10 g (0.055% yield); $R_f$: 0.36 (cyclohexane : ethyl acetate: 9.7 : 3); m.p.: 150–152 °C, UV $\lambda_{max}$: 266 (log ε 6.2); IR $\upsilon_{max}$: 3450, 2955, 2845, 1695, 1650, 1560, 1510, 1475, 1395, 1190 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 6.62 (2 H, brs, H-2, H-11), 5.36 (1 H, brs, H-6), 5.28 (1 H, brs, H-13), 4.28 (1 H, brm, $w_{1/2}$ = 4.2 Hz, H-4″), 4.21 (1 H, d, J = 6.9 Hz, $H_2$-4″), 4.17 (1 H, d, J = 6.9 Hz, Hz-1″ b), 4.07 (1 H, d, J = 6.8 Hz, $H_2$-1′a) 3.98 (1 H, d, J = 6.8 Hz, $H_2$-1′ b), 2.74 (2 H, m, $CH_2$), 2.34 (2 H, m, $CH_2$), 2.03 (2 H, m, $CH_2$), 1.84 (2 H, m, $CH_2$), 1.60 (2 H, brs, $H_2$-7), 1.45 (2 H, brs, $CH_2$), 1.25 (24 H, brs, 12 × $CH_2$), 0.88 (3 H, t, J = 6.0 Hz, M-18″), 0.85 (3 H, t, J = 6.5 Hz, Me-5′); $^{13}C$ NMR ($CDCl_3$): δ 181.9 (C-1, C-12), 178.7 (C-4, C-9), 151.0 (C-3), 148.1 (C-10), 130.2 (C-5), 130.0 (C-8), 128.1 (C-6, C-13), 120.1 (C-20), 118 .4 (C-2, C-11), 68.36 (C-4″), 65.0 (C-1″), 62.1 (C-1′), 34.1 (C-7), 31.9 ($CH_2$), 31.5 ($CH_2$), 29.6 (4 × $CH_2$), 29.3 (3 × $CH_2$), 29.1 (2 × $CH_2$), 28.0 ($CH_2$), 27.2 ($CH_2$), 25.6 ($CH_2$), 24.8 ($CH_2$), 22.7 ($CH_2$), 22.6 ($CH_2$), 22.2 ($CH_2$), 21.7 (C-5′) 14.1 (C-18″); +ve ion FAB MS m/z (rel. int.): 598 $[M]^+$ ($C_{36}H_{54}O_7$) (32.1), 527 (9.7), 511 (10.1), 405 (10.3), 401 (11.3), 391 (13.6), 371 (11.3), 357 (13.6), 337 (41.3), 329 (22.8), 313 (32.1), 285 (31.3), 269 (25.7), 242 (23.1), 227 (26.5), 226 (26.7), 203 (34.8), 197 (21.3), 193 (22.6).

### *3.4.2. Embeliaribyl ester* (**2**)

Elution of the column with $CHCl_3$-MeOH (99 : 1) furnished colourless crystals of **2**, which were recrystallized from $CHCl_3$–MeOH (1 : 1), 230 mg (0.0115% yield); $R_f$: 0.41(benzene : chloroform : methanol: 48 : 50 : 2), m.p.: 158–159 °C; UV $\lambda_{max}$: 224 nm (log ε 5.6); IR $\upsilon_{max}$: 3495, 2942, 2880, 1746, 1622, 1454, 1366, 1245, 1142, 1089, 1007, 741, 710 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 5.31 (1 H, dd, J = 18.6, 5.2 Hz, H-7′), 5.28 (1 H, brs, H-7′), 4.31 (1 H, d, J = 11.1 Hz, $H_2$-1a), 4.17 (1 H, d, J = 11.1 Hz, $H_2$-1b), 3.99 (1 H brm, $w_{1/2}$ = 5.7 Hz, H-9′), 2.76 (2 H, brs, $H_2$-6′), 2.46 (1 H, d, J = 6 Hz, $H_2$-2′a), 2.44 (1 H, d, J = 6 Hz, $H_2$-2′b), 2.40 (2 H, brs, $H_2$ –10′), 2.31 (2 H, brs, $H_2$-2), 2.03 (2 H, brs, $H_2$-5′), 1.60 (2 H, brs, $CH_2$), 1.30 (28 H, brs, 7 × $CH_2$), 1.25 (36 H, brs, 18 × $CH_2$), 0.88 (6 H, brs, 2 × $CH_3$); $^{13}C$ NMR ($CDCl_3$): δ 173.2 (C-1′), 130.2 (C-8′), 128.1 (C-7′), 68.9 (C-9′), 62.1 (C-1), 34.2 (C-2′), 34.0 (C-6′), 31.9 (C-10′), 31.5 (C-2), 29.7 (H × $CH_2$), 29.3 (7 × $CH_2$), 29.1. (9 × $CH_2$), 28.0 ($CH_2$), 27.2 ($CH_2$), 25.6 ($CH_2$), 24.8 ($CH_2$), 22.7 ($CH_2$), 22.6 ($CH_2$), 14.1 (C-19′, C-25); +ve ion FABMS (rel. int.): m/z 662 $[M]^+$ ($C_{44}H_{86}O_3$) (8.2), 491 (9.3), 465 (11.7), 395 (13.6), 381 (11.6), 367 (22.8), 351 (16.3), 323 (13.6), 295 (16.3), 281 (11.3), 253 (12.6), 239 (38.3), 225 (16.1), 211 (16.3), 197 (21.1), 183 (21.6), 171 (28.7), 169 (20.3), 155 (21.3), 141 (26.2), 127 (38.3), 113 (37.1).

### *3.4.3. Embeliol* (**3**)

Elution of the column with $CHCl_3$–MeOH (99 : 1) gave reddish-orange crystals of **3**, which were recrystallized from MeOH 200 mg, (0.01% yield); $R_f$ : 0.40 (benzene : chloroform : ethyl acetate : methanol: 1 : 1 : 0.6 : 0.4); m.p.: 141–143 °C; UV $\lambda_{max}$: 267 nm (log ε 6.3); IR $\upsilon_{max}$ (KBr): 3494, 3312, 2955, 2882, 1618, 1600, 1420, 1383, 1324, 1294, 1223, 1139, 1089, 1014, 981 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 7.72 (1 H, brs, H-6), 2.06 (1 H, d, J = 6.0 Hz, $H_2$-7a), 2.02 (1 H, d, J = 6.0 Hz, $H_2$-7b), 1.60 (2 H, m, $CH_2$), 1.47 (2 H, m, $CH_2$), 1.30 (4 H, brs, 2 × $CH_2$), 1.26 (10 H, brs, 5 × $CH_2$), 0.97 (3 H, t, J = 6.5 Hz, Me-17); $^{13}C$ NMR ($CDCl_3$): 169.9 (C-1, C-4), 167.4 (C-2, C-5), 116.1 (C-3), 102.2 (C-6), 30.2 (C-7), 29.8 (C-8), 28.0 (C-9), 27.9 (C-10), 27.8 (C-11), 27.5 (C-12), 26.5 (C-13), 25.5 (C-14), 21.0 (C-15), 20.8 (C-16), 12.6 (C-17); +ve ion FABMS m/z (rel. int.): 296 $[M]^+$ ($C_{17}H_{28}O_4$) (25.3), 239 (18.7), 225 (11.6), 211(11.7), 197 (14.5), 183 (17.6), 155 (33.7), 141(23.8).

### *3.4.4. Embelin* (**4**)

Elution of the column with $CHCl_3$ yielded orange lustrous crystals of **4**, which were recrystallized from MeOH, 1.02 mg (0.051% yield); $R_f$: 0.33 (chloroform : benzene: 9 : 1); m.p.: 142–143 °C, UV $\lambda_{max}$: 266 nm (log ε 5.9), IR $\upsilon_{max}$: 3350, 2920, 2852, 1699, 1649, 1559, 1502, 1472, 1396, 1189 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ + DMSO $d_6$): δ 7.41 (1 H, brs, H-6), 2.41 (1 H, d, J = 6.0 Hz, H-7a), 2.39 (1 H, d, J = 6.0 Hz, H-7b), 1.42 (4 H, brs, 2 × $CH_2$), 1.25 (14 H, brs, 7 × $CH_2$), 0.87 (3 H, t, J = 6.0 Hz, Me-17); $^{13}C$ NMR ($CDCl_3$): δ 181.2 (C-1), 152.4 (C-2), 178.4 (C-4), 119.1 (C-3), 147.2 (C-5), 117.8 (C-6), 30.9 (C-7), 28.7 (C-8), 28.6 (C-9), 28.5 (C-10), 28.3 (C-11), 27.1 (C-12), 21.7 (C-13), 21.5 (C-14), 21.2 (C-15), 20.9 (C-16), 13.5 (C-17); +ve ion FABMS m/z (rel.int.): 294 $[M]^+$ ($C_{17}H_{26}O_4$) (10.1), 209 (67.3), 167 (18.1), 155 (25.9), 153 (34.7).

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### References

Amar Singhe APG, Sharma RD, Chaturvedi DK, Agarwal (1993) Anthelmintic effect of ayurvedic recipe Kuberakshadi yoga in intestinal worms among children, J Res Edu Indian Med 12: 27–31.

Arya VS (1997) Indian Medicinal Plants, vol 2, Asiatic Publishing House, Delhi, p. 368–371.

Asolkar LV, Kakkar KK, Chakre OJ (1992) Glossary of Indian Medicinal Plants with Active Principles, part I, PID, New Delhi, p. 290–291.

Chopra RN, Nayar SL, Chopra IC (1996) Glossary of Indian Medicinal Plants, NISCOM, New Delhi, p. 106.

Kirtikar KR, Basu BD (2000) Indian Medicinal Plants, vol 2, p. 1477–1480.

Paul R, Vatsa SR (1980) Clinical trial of an ayurvedic herb in worm infestation. Nagarjun 23: 234–235.