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Chemical Constituents From the Roots of *Oenothera biennis* L.

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Abstract

Oenothera biennis L. (Onagraceae), is a biennial herb, native to eastern and central North America. It is cultivated in temperate regions of the world and in Indian gardens. The plant parts are used to treat gastro-intestinal disorders, eczema, whooping cough, asthma, blood disorders, laziness, obesity, piles and boils. The dried root powder was exhaustively extracted with methanol and the extract concentrated to yield a dark brown viscous mass. It was dissolved in small quantity of methanol and adsorbed onto silica gel (60 - 120 mesh) for preparation of a slurry. The air dried slurry was subjected to chromatography over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, chloroform and methanol in order of increasing polarity to isolate the new phytoconstituents characterized as 3,11,15-trimethyl-14β-hydroxy-*n*-hexadeca-7-en-4,18-olide (phyt-7-enyl-14β-ol 4β,18-olide, **2**), 3-methyl-6α,8β-dihydroxy-7-carboxylic acid tetralin-1,9β-olide (**3**), 3,7-dimethyl-11-hydroxymethylene dodec-3α,6α-diol- 11-enyl 2′,3′,6′-benzene triol (**4**), 1,9,10-trimethoxy-3,11-dihydroxy-13-(18,19-dihydroxyprenyl)-anthracene (**5**) and α-D-glucopyranosyl–(4→1′)-α-D-glucopyranosyl -6′-cetoleate (**6**) along with benzoic acid (**1**). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

1 Introduction

Oenothera biennis L., syn., *O. muricata* L., *O. suaveolens* Desf., *Brunyera biennis* Bubani, *Onagra biennis* (L.) Scop. (Onagraceae), known as evening-primrose, evening star and sun drop, is a native to eastern and central North America and grown in Indian gardens[1]. It is a biennial, 30 – 150 cm high herb with lanceolate leaves, erect, stout and softly-hairy stem, oblong and hairy pods with precious seeds. Its roots and leaves are edible.

A tea prepared from the leaves is taken as a dietary aid, stimulant and to treat cough, blood disorders, laziness and obesity. A root paste is applied to subside piles and boils and to improve muscle strength. The leaves, stem bark, flowers and seed oil are used to treat gastro-intestinal disorders, eczema, whooping cough and asthma. Evening primrose oil from the seeds relieves abdominal bloating, acne, allergies, Alzheimer's disease, asthma, attention deficit hyperactivity disorder, autoimmune conditions, breast pain, cardiovascular disease, cirrhosis of the liver, cramps, diabetic neuropathy, depression, dysmenorrhea, eczema, endometriosis, fatigue syndrome, fibrocystic breasts, hot flushes, gastrointestinal symptoms, menstrual hyperchlolesterolemia, hypertension, impotence, female infertility, inflammation, migraine, multiple sclerosis, myalgia, obesity, nerve damage, neurodermatitis, osteoporosis, premenstrual problems, psoriasis, rheumatoid arthritis , rosacea, schizophrenia, tension and whooping cough[2-4].

The seeds are a potential source of unsaturated fatty acids including γ-linoleic (8–14%), ω-6 polyunstaturated fatty, oleic, palmitic and stearic acids[5-8], phytosterols[9, 10] and phenolic compounds[11]. The roots contained aryl, lipid and triterpenic constituents[12], lanosterols, tetralin-1, 9-olide and phytyl lactone[13,14]. The leaves possessed flavonoids[15,16] and andoenothin[17]. The manuscript describes isolation and

characterization of diterpenic and tetraline lactones, dodecenyl benzene triol, prenyl anthracene and an acyl diglucoside as new chemical constituents from the roots of *O. biennis*.

2 Materials and Methods

2.1. General procedures

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer-Rotkreuz, Switzerland) in methanol. Infrared spectra were recorded on Bio-Rad FTIR 5000 spectrophotometer (FTS 135, Kawloon, Hong Hong) using KBr pellets; γmax values are given in cm^{-1}. The ^{1}H and ^{13}C NMR spectra were screened on Advance DRX Bruker spectrospin 400 and 100 MHz, respectively, instruments (Karlesruthe, Germany) using $CDCl_3$ or DMSO-d_6 as a solvent and TMS as an internal standard. Mass spectra were scanned by effecting ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualized by exposing to iodine vapours, UV radiation and spraying with ceric sulphate solution.

2.2. Plant material

The roots of *O. biennis* were collected from Delhi and identified by Dr. M. P. Sharma, Department of Botany, Jamia Hamdard. A voucher specimen has been retained in the Phytochemistry Research Laboratory, Jamia Hamdard.

2. 3. Preparation of extract

The dried roots (1.0 kg) were coarsely powdered, defatted with *n*-hexane and exhaustively extracted in a Soxhlet apparatus with methanol. The methanolic extract was concentrated under reduced pressure to yield a dark brown viscous mass (11.8 g). A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents.

2.4. Isolation of phytoconstituents

The viscous dark brown extract (150 g) was dissolved in small quantity of methanol and adsorbed onto silica gel (60 - 120 mesh) for preparation of a slurry. The slurry was air dried and subjected to chromatography over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3), chloroform and the mixture of chloroform - methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3). Various fractions were collected separately and matched by TLC to check the homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get the following compounds:

*2.5. Benzoic acid (**1**)*

Elution of the column with chloroform furnished colourless crystals of **1,** recrystallized from methanol; 227 mg, m. p.: 221 – 222 ^{o}C, UV λ_{max} (MeOH): 210, 270 nm; IR γ_{max} (KBr): 3368, 2950, 2841, 1702, 1656, 1543, 1433, 1329, 1268, 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 6.99 (2H, m, H-2, H-6), 6.97 (2H, m, H-3, H-5), 6.94 (1H, m, H-4); ^{13}C NMR ($CDCl_3$): δ 145.38 (C-1), 138.61 (C-2, C-6), 120.97 (C-3, C-5), 109.41 (C-4), 169.47 (C-7); +ve ion FAB MS *m/z* (rel. int.): 122 $[M]^+$ ($C_7H_6O_2$) (10.6).

*2.6. Phyt-7-enyl-14β-ol-4β,18-olide (**2**)*

Further elution of the column with chloroform afforded a pale yellow semisolid mass of **2,** 167 mg, UV λ_{max} (MeOH): 213, 229 nm; IR γ_{max} (KBr): 3411, 2922, 2847, 1737, 1646, 1447, 1389, 1258, 1041 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, H-8), 4.06 (1H, brm, $w_{1/2}$ = 17.2 Hz, H-4α), 3.67 (1H, brm, $w_{1/2}$ = 14.2 Hz, H-14α), 2.72 (2H, m, H_2-6), 2.36 (2H, m, H_2-9), 2.23 (1H, m, H-11), 2.05 (1H, m, H-3α), 2.01 (2H, m, H_2 -5), 1.80 – 1.19 (9H, m, H-5, 4 x CH_2), 1.15 (3H, d, J = 6.5 Hz, Me-16), 1.10 (3H, d, J = 6.7 Hz, Me-20), 1.03 (3H, d, J = 6.6 Hz, Me-17), 0.99 (3H, d, J = 6.7 Hz, Me-19), 0.85 (3H, d, J = 6.3 Hz, Me-1); ^{13}C NMR ($CDCl_3$): δ 14.48 (C-1), 22.19 (C-2), 39.51 (C-3), 78.85 (C-4), 58.25 (C-5), 50.04 (C-6), 140.22 (C-7), 120.15 (C-8), 30.51 (C-9), 30.53 (C-10), 42.73 (C-11), 21.23 (C-12), 21.43 (C-13), 68.19 (C-14), 31.49 (C-15), 18.33 (C-16), 16.51 (C-17), 166.42 (C-18), 20.17 (C-19), 18.27 (C-20); FAB MS *m/z* (rel. int.): 324 $[M]^+$ ($C_{20}H_{36}O_3$) (12.7), 281 (14.5), 267 (23.8), 251(4.7), 239 (19.2), 157 (8.3), 129 (6.7), 57 (13.2).

*2.7. 3-Methyl-6α,8β-dihydroxy-7-carboxylic acid tetralin-1,9β-olide (**3**)*

Elution of the column with chloroform – methanol (49 : 1) gave pale yellow crystals of **3,** recrystallized from chloroform – methanol (1: 1); 131 mg, m. p.: 118 - 119 ^{o}C, UV λ_{max} (MeOH): 211, 241 nm; IR γ_{max} (KBr): 3448, 3288, 3236, 2951, 2846, 1765, 1702, 1626, 1547, 1443, 1359, 1247, 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.06 (1H, d, J = 1.5 Hz, H-2), 7.02 (1H, d, J = 1.5 Hz, H-4), 4.23 (1H, d, J = 10.2 Hz, H-9α), 3.36 (1H, d, J = 5.1 Hz, H-6β), 3.32 (1H, dd, J = 5.1, 10.2 Hz, H-8α), 2.79 (1H, dd, J = 10.2, 5.1 Hz, H-7β), 2.33 (3H, s, Me-12); ^{13}C NMR ($CDCl_3$): δ 146.61 (C-1), 110.19 (C-2), 110.12 (C-3), 121.55 (C-4), 139.85 (C-5), 68.52 (C-6), 52.47 (C- 7), 66.72 (C-8), 77.86 (C-9), 121.53 (C-10), 169.17 (C-11), 28.55 (C-12), 179.35 (C-13); FAB MS *m/z* (rel. int.): 264 $[M]^+$ ($C_{13}H_{12}O_6$) (10.4), 246 (15.3) , 219 (8.9), 201 (19.2).

*2.8. 3,7-Dimethyl-11-hydroxymethylene dodec-3α,6α-diol- 11-enyl 2',3',6'-benzene triol (**4**)*

Elution of the column with chloroform - methanol (48 : 1) produced yellow crystals of **4,** recrystallized from chloroform – methanol (1: 1); 208 mg, m. p. : 141 - 143 ^{o}C, UV λ_{max} (MeOH): 213, 273 nm; IR γ_{max} (KBr): 3411, 3283, 3248, 2925, 2856,

1643, 1510, 1456, 1325, 1267, 1176, 1033 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.29 (1H, d, J = 10.8 Hz, H-4′), 7.25 (1H, d, J = 10.8 Hz, H-5′), 7.23 (1H, s, H-12), 3.88 (2H, s, H_2-15), 3.79 (1H, ddd, J = 4.8, 5.1, 5.9 Hz, H-6β), 2.27 (1H, dd, J = 9.3, 9.9 Hz, H_2-10a), 2.16 (1H, dd, J = 9.2, 11.8 Hz, H_2-10b), 2.08 – 1.28 (13H, , 6 x CH_2, CH), 1.25 (3H, s, Me-13), 0.99 (3H, d, J = 6.9 Hz, Me-14), 0.87 (3H, t, J = 6.3 Hz, Me-1); ^{13}C NMR ($CDCl_3$): δ 14.36 (C-1), 22.97 (C-2), 72.10 (C-3), 38.27 (C-4), 32.25 (C-5), 62.42 (C-6), 52.40 (C-7), 41.55 (C-8), 29.97 (C-9), 56.71 (C-10), 125.55 (C-11), 107.75 (C-12), 28.12 (C-13), 19.48 (C-14), 61.17 (C-15), 132.91 (C-1′), 153.41 (C-2′), 167.87 (C-3′), 131.16 (C-4′), 129.21 (C-5′), 166.97 (C-6′); FAB MS *m/z* (rel. int.): 382 $[M]^+$ ($C_{21}H_{34}O_6$) (37.1), 309 (12.7), 281 (5.7), 201 (11.5), 131 (15.4) , 125 (7.8), 101 (12.3), 73 (8.1).

*2.9. 1,9,10-Trimethoxy-3,11-dihydroxy-13-(18,19-dihydroxyprenyl)-anthracene (**5**)*

Elution of the column with chloroform–methanol (19 : 1) yielded yellow crystals of **5**, recrystallized from chloroform – methanol (1: 1); 121 mg, m. p.: 221-223 ^oC, UV λ_{max} (MeOH): 241, 259, 324 nm; IR γ_{max} (KBr): 3315, 2927, 2848, 1638, 1529, 1476, 1418, 1353, 1203, 1095 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.79 (1H, s, H-6), 7.66 (1H, d, J = 2.4 Hz, H-2), 7.61 (1H, s, H-8), 7.19 (1H, d, J = 2.4 Hz, H-4), 4.23 (3H, s, OMe), 4.20 (3H, s, OMe), 4.15 (3H, s, OMe), 3.16 (2H, d, J = 5.6 Hz, H_2-18), 3.11 (2H, d, J = 5.6 Hz, H_2-19), 2.35 (2H, m, H_2-15), 1.75 (2H, m, H_2-16), 1.17 (1H, m, H-17); ^{13}C NMR ($CDCl_3$): δ 158.62 (C-1), 141.29 (C-2), 158.31 (C-3), 120.92 (C-4), 112.45 (C-5), 120.56 (C-6), 112.41 (C-7), 116.74 (C-8), 155.57 (C-9), 155.09 (C-10), 165.02 (C-11), 117.05 (C-12), 145.07 (C-13), 113.25 (C-14), 30.09 (C-15), 23.09 (C-16), 39.86 (C-17), 62.39 (C-18), 62.36 (C-19), 57.45 (OMe), 57.26 (OMe), 57.22 (OMe); FAB MS *m/z* (rel. int.): 402 $[M]^+$ ($C_{22}H_{26}O_7$) (3.1), 387 (35.7), 372 (5.9), 357 (22.5).

*2.10. α-D-glucosyl-(4→1′)-α-D-glucosyl-6′-cetoleate (**6**)*

Elution of the column with chloroform-methanol (9:1) gave a yellow semisolid mass of **6**, purified by preparative TLC (chloroform: methanol, 3:1); 153 mg, UV λ_{max} (MeOH): 217 nm (log ε 4.2); IR λ_{max} (KBr): 3427, 3396, 3312, 2925, 2849, 1725, 1650, 1458, 1379, 1273, 1163, 1075, 723 cm^{-1}; ^1H NMR (MeOD): δ 5.22 (1H, d, J = 3.7 Hz, H-1α), 4.09 (1H, m, H-5), 3.96 (1H, m, H-2), 3.81 (1H, m, H-3), 3.65 (1H, m, H-4), 3.13 (2H, d, J = 11.5 Hz, H_2-6), 5.15 (1H, d, J = 3.9 Hz, H-1′α), 4.06 (1H, m, H-5'), 3.89 (1H, m, H-2'), 3.79 (1H, m, H-3'), 3.51 (1H, m, H-4'), 3.46 (2H, d, J = 9.0 Hz, H_2-6'), 5.30 (2H, m, H-9″, H-10″), 2.32 (2H, m, H_2-2″), 1.85 (2H, m, H_2-8), 1.81 (2H, m, H_2-11″), 1.50 (2H, m, H_2-3″), 1.38 (2H, m, H_2-7″), 1.23 (22H, brs, 11 x CH_2), 0.78 (3H, t, *J*= 6.8 Hz, Me-20″); ^{13}C NMR (DMSO-d_6): δ 103.73 (C-1), 74.59 (C-2), 72.05 (C-3), 71.85 (C-4), 76.99 (C-5), 61.66 (C-6), 103.54 (C-1'), 73.95 (C-2'), 71.95 (C-3'), 70.53 (C-4'), 76.87 (C-5'), 65.34 (C-6'), 173.16 (C-1″), 53.86 (C-2″), 133.40 (C-9″), 129.71 (C-10″), 34.89 – 31.87 (6 x CH_2), 29.97 – 29.31 (5 x CH_2), 27.25 (CH_2), 26.24 (CH_2), 25.14 (CH_2), 22.78 (CH_2), 13.46 (C-20″); +ve FAB MS *m/z* (rel. int.): 634 $[M]^+$ ($C_{32}H_{58}O_{12}$) (14.6), 325 (14.8), 309 (12.1), 179 (8.7), 163 (19.5).

3 Results and Discussions

Compound **1** was the known compound identified as benzoic acid.

Compound **2** showed IR absorption bands for hydroxyl group (3411 cm^{-1}), δ-lactone (1737 cm^{-1}) and unsaturation (1646 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular weight was determined at *m/z* 324 corresponding to a molecular formula of an acyclic diterpenic lactone, $C_{20}H_{36}O_3$. The ion peaks generating at *m/z* 281 [C_{14} – C_{15} fission, M - $C_3H_7]^+$, 251 [C_{13} – C_{14} fission]$^+$, 239 [C_{11} - C_{12} fission]$^+$ and 129 [C_{10} - C_{11} fission]$^+$ suggested location of the hydroxyl group at C-14. The ion fragments produced at *m/z* 57, 267 [C_3 - C_4 fission]$^+$ and 157 [C_8 – C_9 fission]$^+$ indicated the existence of the δ-lactone ring at C-4(18) and the vinylic linkage at C_7 position.

The ^1H NMR spectrum of **2** showed a one - proton multiplet at δ 5.35 assigned to vinylic H-8 proton. A one-proton broad multiplet at δ 4.06 with half width of 17.2 Hz was ascribed to α-oriented oxymethine H-4 proton. A one-proton multiplet at δ 3.67 with half-width of 14.2 Hz was attributed to carbinol H-14α proton. Four three-proton doublets at δ 1.15 (J = 6.5 Hz), 1.10 (J = 6.7 Hz), 1.03 (J = 6.6 Hz) and 0.99 (J = 6.7 H) and a three-proton triplet at δ 0.85 (J = 6.3 Hz) were associated with the secondary C-16, C-20, C-17 and C-19 and primary C-1 methyl protons, respectively, all attached to saturated carbons. The remaining methine and methylene protons appeared from δ 2.72 to 1.19. The ^{13}C NMR spectrum of **2** showed the presence of 20 carbon atoms and the important signals appeared for lactone carbon at δ 166.42 (C-18), oxymethine carbon at δ 78.85 (C-4), carbinol carbon at δ 68.19 (C-14), vinylic carbons at δ 140.22 (C-7) and 120.15 (C-8) and methyl carbons at δ 14.48 (C-1), 18.33 (C-16), 16.51 (C-17), 20.17 (C-19) and 18.27 (C-20). The DEPT spectrum of **2** displayed the presence of five methyls, seven methylene, six methine and two quaternary carbons. The ^1H-^1H COSY spectrum of **2** exhibited correlations of H-4 with H-3, H_2-5 and H_2-6; H-8 with H_2-6 and H_2-9; and H-14 with H_2-13, H-15, Me-16 and Me-20. The HMBC spectrum of **2** showed that H-3 and H_2-5 interacted with C-4; H_2-6 and H-8 interacted with C-7; and H_2-13, H-14, Me-16 and H-20 interacted with C-14. The HSQC experiment showed important interactions between the vinylic proton H-8 at δ 5.35 with the C-8 carbon signal at δ 120.15, carbinol H-14 proton at δ 3.67 with C-14 carbon signal at δ 68.19 and oxymethine H-4 at δ 4.06 with C-4 carbon signal at δ 78.85. On the basis of spectral data analysis, the structure of **2** has been established as 3,11,15-trimethyl-14β-hydroxy-*n*-hexadeca-7-en-4,18-olide (phyt-7-enyl-14β-ol 4β,18-olide), a new diterpenic lactone.

Compound **3** produced effervescences with sodium bicarbonate indicating the presence of a carboxylic function. Its IR spectrum showed absorption bands for hydroxyl groups (3448, 3288 cm^{-1}), carboxylic group (3236, 1702 cm^{-1}), five membered lactone ring (1765 cm^{-1}) and aromatic ring (1626, 1547, 1031 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular formula was determined at *m/z* 264 consistent with a molecular formula of a tetrahydronaphthalene - type lactone $C_{13}H_{12}O_6$. Elimination of a carboxylic group from the molecular ion peak yielded an ion fragment at *m/z* 219. Removal of a water molecule and subsequently the carboxylic group generated the ion fragments at *m/z* 246 and 201, respectively. The ^1H NMR spectrum of **3** showed two one - proton doublets at δ 7.06 (J = 1.5 Hz) and 7.02 (J = 1.5 Hz) assigned to meta-coupled aromatic H-2 and H-4 protons, respectively. Two one-proton doublets at δ 4.23 (J = 10.2 Hz) and 3.36 (J = 5.1 Hz) were attributed correspondingly to oxymethine H-9α and carbinol H-6β protons. Two one-proton double doublets at δ 3.32 (J = 5.1, 10.2 Hz, H-8α) and 2.79 (J = 10.2, 5.1 Hz, H-7β), and a three-proton singlet at δ 2.33 (Me-12) were ascribed to carbinol H-8α, methine H-7β and C-12 methyl protons, respectively. The ^{13}C NMR spectrum of **3** exhibited the presence of 13 carbon signals and the important carbon signals appeared for carboxylic carbon at δ 179.35 (C-13), lactone carbon at δ 169.17 (C-11), carbinol carbons at δ 68.52 (C-6) and 66.72 (C-8), oxymethine carbon at δ 77.86 (C-9), methyl carbon at δ 28.55 and aromatic carbons between δ 146.61 - 110.12. The DEPT spectrum of **3** showed the presence of one methyl and six each of methine and quaternary carbons. The ^1H-^1H COSY spectrum of **3** showed correlations of H-4 with H-2, H-6 and Me-12; and H-8 with H-7 and H-9. The HMBC spectrum of **3** exhibited that H-2 interacted with C-11; Me-12, H-4 and H-2 interacted with C-3; H-7 interacted with C-13; and H-8 and H-9 interacted with C-10. The HSQC experiment showed important correlations between the aromatic proton signals at δ 7.06 (H-2) and 7.02 (H-4) with the respective carbon signals at δ 110.19 (C-2) and 121.55 (C-4); carbinol protons at δ 3.36 (H-6) and 3.32 (H-8) with carbon signals at δ 68.51 (C-6) and 66.72 (C-8), respectively; oxymethine proton at δ 4.23 (H-9) with carbon signal at δ 77.86 (C-9) and methyl H_3-12 proton at δ 2.33 with the carbon signal at δ 28.55 (C-12). On the basis of these evidences the structure of **3** has been elucidated as 3-methyl-6α,8β-dihydroxy-7-carboxylic acid tetralin-1,9β-olide, a new tetralin lactone.

Compound **4** gave positive tests of phenols and showed IR absorption bands for hydroxyl groups (3411, 3283, 3248 cm^{-1}) and aromatic ring (1643, 1510, 1033 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular ion peak was determined at *m/z* 382 consistent with a molecular formula of a sesquiterpenic benzene triol $C_{21}H_{34}O_6$. The ion peaks arising at *m/z* 73 [$C_3 - C_4$ fission, $C_4H_9O]^+$, 309 [M – 73]$^+$, 101 [$C_5 - C_6$ fission, $C_6H_{13}O]^+$, 281 [M – 101]$^+$ and 131 [$C_6 - C_7$ fission, $C_7H_{15}O_2]^+$ suggested existence of the hydroxyl groups at C_3 and C_6 carbons. The ion fragments produced at *m/z* 201 [$C_{10} - C_{11}$ fission, $C_{12}H_{25}O_2]^+$ and 125 [$C_{12} - C_{1'}$ fission, $C_6H_5O_3]^+$ indicated the presence of the C-15 hydroxymethylene group and a vinylic linkage located at C_{11} and a benzene triol ring at C_{12} carbons. The ^1H NMR spectrum of **4** showed two one-proton doublets at δ 7.29 (J = 10.8 Hz) and 7.25 (1H, d, J = 10.8 Hz) assigned to ortho-coupled aromatic H-4′ and H-5′ protons, respectively. A one-proton singlet at δ 7.23 , a two-proton singlet at δ 3.88 and a one-proton triplet doublet at δ 3.79 (J = 4.8, 5.1, 5.9 Hz) were attributed correspondingly to vinylic H-12 linked to the aromatic ring, hydroxymethylene H_{12} protons located on the unsaturated C_{11} carbon and carbinol C-6β proton. Three three-proton signals appearing as a singlet at δ 1.25, as a doublet at δ 0.99 (J = 6.9 Hz) and as a triplet at δ 0.87 (J = 6.3 Hz) were accounted to tertiary C-13, secondary C-14 and primary C-1 methyl protons, respectively, all attached to the saturated carbons. The remaining methine and methylene protons resonated from δ 2.27 to 1.28. The ^{13}C NMR spectrum of **4** displayed the presence of 21 carbon signals and the important signals appeared for benzene carbons between δ 167.87 – 129.21, vinylic carbons at δ 125.55 (C-11) and 107.75 (C-12), carbinol carbons at δ 72.10 (C-3) and 62.42 (C-6), hydroxymethylene carbon at δ 61.17 (C-15) and methyl carbons at δ 14.36 (C-1), 28.12 (C-13) and 19.48 (C-14). The DEPT spectrum of **4** showed the presence of three methyl, seven methylene, five methine and six quaternary carbons. The ^1H-^1H COSY spectrum of **4** showed correlations of Me-13 with H_2-2 and H_2-4; H-6 with H_2-5, H-7, Me-14 and H_2-8; H-12 with H_2-10 and H_2-15; and H-4′ with H-5′. The HMBC spectrum of **4** exhibited that Me-1, H_2-2, H_2-4 and Me-13 interacted with C-3; H_2-5 and H-7 interacted with C-6; H_2-10, H-12 and H_2-15 interacted with C-11; and H-4′ and H-5′ interacted with C-3′.

The HSQC experiment displayed important correlations between the aromatic proton signals at δ 7.29 (H-4′) and 7.25 (H-5′) with the carbon signals at δ 131.16 (C-4′) and 129.21 (C-5′); vinylic proton at δ 7.23 (H-12) with the carbon signal at δ 107.75 (C-12), carbinol proton at δ 3.79 with carbon at δ 62.42 (C-6), hydroxymethylene proton at δ 3.90 (H_2-15) with carbon signal at δ 61.17 (C-15) and methyl proton signals at δ 0.87 (Me-1), 1.25 (Me-13) and 0.99 (Me-14) with their respective carbon signals at δ 14.36 (C-1), 28.12 (C-13) and 19.48 (C-14). On the basis of these spectral data analysis the structure of **4** has been established as 3,7-dimethyl-11-hydroxymethylene dodec-3α,6α-diol- 11-enyl 2′,3′,6′-benzene triol, a phenolic substituted sesquiterpene.

Compound **5** responded positively to phenolic tests and showed UV absorption maxima at 241, 259, 324 nm for aromaticity. Its IR spectrum had absorption bands for hydroxyl groups (3315 cm^{-1}) and aromatic ring (1638, 1529, 1095 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular formula was established at *m/z* 402 consistent with a molecular formula of a prenylated anthracene $C_{22}H_{26}O_7$. The ion peaks arising at *m/z*

387 [M - Me]$^+$, 372 [387 – Me]$^+$and 357 [372 - Me]$^+$ suggested the presence of three methoxy groups in the molecule. The ^{1}H NMR spectrum of **5** showed two one-proton doublets at δ 7.66 (J = 2.4 Hz) and 7.19 (J = 2.4 Hz), and two one –proton singlets at δ 7.79 and 7.61 assigned to aromatic H-2, H-4, H-6 and H-8 protons, respectively, three methoxy protons as three-proton singlets at δ 4.23, 4.20 and 4.15, methylene protons as two-proton multiplets at δ 2.35 and 1.75, a methine proton as a one-proton multiplet at δ 1.17 and hydroxymethylene protons as two-proton doublets at δ 3.16 (J = 5.6 Hz) and 3.11 (J = 5.6 Hz) accounted to H_2-18 and H_2-18 protons, respectively. The ^{13}C NMR spectrum of **5** displayed the presence of 22 carbon signals and the important signals appeared for aromatic carbons between δ 165.02 – 112.41 , methoxy carbons at δ 57.45, 57.26 and 57.22, hydroxymethylene carbons at δ 62.39 (C-18) and 62.36 (C-19), methylene carbons at δ 30.09 (C-15) and 23.09 (C-16) and methine carbon at δ 39.86 (C-17).

The DEPT spectrum of **5** showed the presence of three methoxy, four methylene, five methine and ten quaternary carbons. The ^{1}H-^{1}H COSY spectrum of **5** showed correlations of H-17 with H_2-15, H_2-16, H_2-18 and H_2-19; and H-2 with H-4. The HMBC spectrum of **5** exhibited interactions of H_2-18, H_2-19 and H_2-16 with C-17; H_2-15 with C-13; H-2 and H-4 with C-3; and H-8 with C-9. The HSQC experiment showed important correlations between the aromatic proton signals at δ 7.66 (H-2), 7.19 (H-4), 7.79 (H-6) and 7.61 (H-8) with their respective carbons at δ 141.29 (C-2), 120.92 (C-4), 120.56 (C-6) and 116.74 (C-8); methylene proton signals at δ 2.35 (H_2-15) and 1.75 (H_2-16) with the carbon signals at δ 30.09 (C-15) and 23.09 (C-16), respectively, and hydroxymethylene proton signals at δ 3.16 (H_2-18) and 3.11 (H_2-19) with the corresponding carbon signals at δ 62.39 (C-18) and 62.36 (C-19). On the basis of these spectral data analysis the structure of **5** has been established as 1,9,10-trimethoxy-3,11-dihydroxy-13-(18,19-dihydroxyprenyl)-anthracene, a new anthracene derivative.

Compound **6** gave positive tests for glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3427, 3396, 3312 cm^{-1}), ester function (1725 cm^{-1}) and long chain aliphatic hydrocarbon (723 cm^{-1}). The molecular ion peak of **6** was determined at *m/z* 634 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of a diglycosyl ester, $C_{32}H_{58}O_{12}$. The ion fragments generating at *m/z* 163 $[C_6H_{11}O_5]^+$, 179 $[C_6H_{11}O_6]^+$, 325 $[C_6H_{12}O_6$-$C_6H_{10}O_4]^+$ and 309 [M - 325, $CH_3(CH_2)_9$-CH=CH-$(CH_2)_7COO^+$] suggested that a dihexose unit was esterified with cetoleic acid.

The ^{1}H NMR spectrum of **6** exhibited a two-proton multiplet at δ 5.30 assigned to vinylic H-9″ and H-10″ protons. Two one-proton doublets at δ 5.22 (J = 3.7 Hz) and 5.15 (J = 3.9 Hz) were ascribed to α-oriented anomeric H-1 and H-1′ protons, respectively.

The other sugar protons appeared from δ 4.09 to 3.13. A three-proton triplet at δ 0.78 (J= 6.8 Hz) was accounted to terminal C-20″ primary methyl protons. The remaining methylene protons resonated between δ 2.32 – 1.23. The ^{13}C NMR spectrum of **6** exhibited signals for the ester carbon at δ 173.16 (C-1″), anomeric carbons at δ 103.73 (C-1) and δ 103.54 (C-1'), other sugar carbons in the range from δ 76.99 to 61.66, methylene carbons between δ 53.86 - 22.78 and methy1 carbon at δ 13.46 (C-14″). The shifting of ^{1}H NMR H-4 signal in the deshielded region at δ 3.65 and ^{13}C NMR C-4 signal at δ 71.85 indicated (4→1′) linkage of the sugar units.

The presence of ^{1}H NMR signal for oxymethylene H_2-6' in the deshielded region at δ 3.46 and ^{13}C NMR signals for C-6′ at δ 65.34 suggested the attachment of ester linkage C-6′. The ^{1}H-^{1}H COSY spectrum of **6** displayed correlations of H-1' with H-2', H-3' and H-4; H-5' with H-4', H-3' and H_2-6′; and H-9'' with H_2-8'', H-10″ and H_2-11''. The HMBC spectrum of **6** showed that H-4 and H-2' interacted with C-1′; H_2-6' and H_2-2″ interacted with C-1″; and H_2-8″, H-9'' and H_2-11'' correlated with C-10''.

The HSQC spectrum of **6** exhibited correlations of anomeric protons at δ 5.22 (H-1) and 5.15 (H-1′) and vinylic protons at δ 5.30 (H-9″, H-10″) with their respective ^{13}C NMR signals at δ 103.73 (C-1), 103.54 (C-1'), 133.40 (C-9″) and 129.71 (C-10″). Acid hydrolysis of **6** yielded cetoleic acid and D-glucose (R_f 0.12, *n*-butanol-acetic acid-water, 4:1:5). On the basis of this discussion the structure of **6** has been elucidated as α-D-glucopyranosyl–(4→1′)-α-D-glucopyranosyl -6′-cetoleate, a new ester glycoside.

4 Conclusion

Phytochemical investigation of a methanolic extract of the roots of *O. biennis* yielded benzoic acid, diterpenic and tetraline lactones, dodecenyl benzene triol, prenyl anthracene diol and an acyl diglucoside for the first time. This work has enhanced understanding about the phytoconstituents of the plant. These secondary metabolites can be utilized as effective analytical markers for identity, purity and quality control of this plant in future.

5 Conflicts of Interests

The authors hereby declare that there are no conflicts of interests.

6 Author's contributions

SS and MA carried out isolation, data analysis/interpretation and manuscript preparation. SRM worked on the structural formulae, research conception/design and data acquisition.

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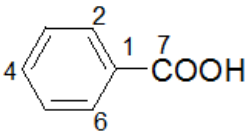
University, New Delhi for recording spectral data of the compounds.

8 Conflict of interest

The authors declared that there are no conflicts of interest.

9 Author's contributions

SS, MA and SRM performed the experimental work and drafted the manuscript.



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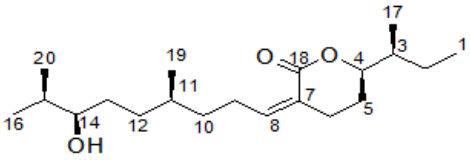
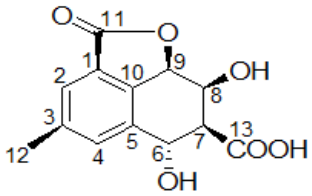
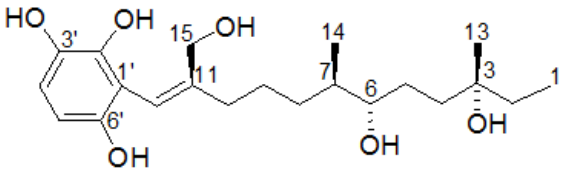


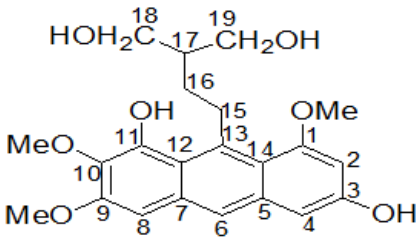
Phyt-7-enyl-14β-ol-4, 18-olide (**2**)



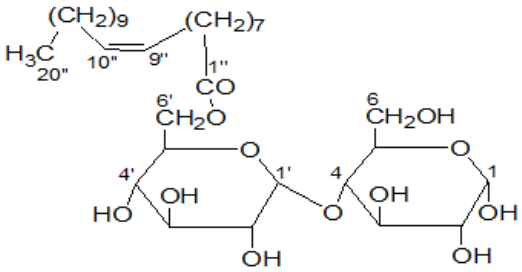
3-Methyl-6α, 8β-diol-7-carboxylic acid tetralin-11, 9β-olide (**3**)



3,7-Dimethyl-3α, 6α-diol-11-hydroxymethylene dodec-11-enyl- 2′, 3′, 6′-benzene triol (**4**)



1, 9, 10-Trimethoxy-3,11-dihydroxy-13-(18,19-dihydroxyprenyl)-anthracene (**5**)



α-D-Glucopyranosyl-(4→1′)-α-D-glucopyranosyl-6′-cetoleate (**6**)

Fig 1: Structures of chemical constituents

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