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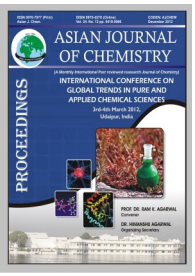

New Chemical Constituents from the *Oenothera biennis* Roots†

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Two new compounds oenotheraphenoxylactone and oenotheraphytyllactone along with four known compounds have been isolated from the roots of *Oenothera biennis*. Their structures have been elucidated with the help of 300 MHz NMR using 1D and 2D spectral methods viz: ^{1}H and ^{13}C NMR, COSY, HSQC, HMBC and DEPT aided by EIMS and IR spectroscopy.

Key Words: *Oenothera biennis*, Onagraceae, Root, New constituents.

INTRODUCTION

Oenothera biennis (Onagraceae) is a genus of herbs and undershrubs; its species are distributed mainly in temperate America with some species occurring in tropics. Some of the species including *O. biennis* have been introduced into the Indian gardens[1,2]. The oil from the seeds of evening primrose is a rich dietary source of γ-linoleic acid required for the formation of prostaglandins and related hormones[3]. The seeds are reported to possess fatty acids[4] and sterols[5] while the leaves contain flavonoids[6,7] and oenothin[8]. The plant is reported to possess antiarthritic[9], antitumor and antithrombic[8,10] properties. Antimicrobial activity and chemical constituents from *O. biennis* roots have been reported[11-13].

In continuation of our study on *O. biennis* roots constituents, we have reported new and known compounds[14]. This paper deals with the isolation and structure elucidation of two new compounds, oenotheraphenoxylactone (**1**) and oenotheraphytyllactone (**2**), on the basis of ^{1}H and ^{13}C NMR, DEPT spectroscopic studies, including 2D-NMR, COSY, HMBC and HSQC from the roots. This is the first report of the two isolated compounds (**1-2**) along with four known compounds, oleanolic acid, gallic acid, β-sitosterol and β-sitosterol-β-D-glucoside from the roots of *O. biennis*. Due to significance of medicinal natural products of these plant roots, the work in this area has already been done. The aim of the present investigation is to report some of the new findings in the form of natural products from *O. biennis* roots.

EXPERIMENTAL

All chemicals used were of analytical grade. Hexane, ethyl acetate, methanol, ethanol, sulphuric acid and vanillin were purchased from Loba Chemie, India. Pre-coated TLC plates (layer thickness 0.25 mm), silica gel for column chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63 μm) were from Merck (Germany). Authentic standards of chemicals were purchased from Sigma-Aldrich (USA). Melting points were determined on an electrochemical engineering melting point apparatus. Optical rotation was measured on a Rudolf autopol model polarimeter. Both ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-300 model spectrometer operating at 300 and 75 MHz, respectively. NMR spectra were obtained in deuterated methanol and mixture of methanol and chloroform using tetramethylsilane (TMS) as an internal standard, EIMS data were recorded on a JEOL SX-102 spectrometer.

Oenothera biennis roots was grown at CIMAP experimental field in Lucknow, India and harvested in May 2007. A voucher specimen of roots [reference code CIMAP-2007(OB)] has been deposited with the department of Process Chemistry and Chemical Engineering. The roots from the harvested plants were separated by cutting and dried at room temperature (25-30 ºC) for 7 d. Dried roots were ground or powdered by machine.

Extraction of roots: The powdered roots of *O. biennis* (11.5 kg) were immersed in MeOH (25 L) for 7 days at room

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temperature and then concentrated in vacuo to yield 300 g of extract.

Separation and isolation of compounds: The entire methanol extract (300 g) was subjected to normal phase column chromatography over silica gel column (60-120 mesh, 8.0 kg, 5.5 cm × 180 cm) to yield 44 fractions (each of 2 L) with the following eluants: fractions 1-2 with *n*-hexane, fractions 3-4 with *n*-hexane:ethyl acetate (9:1), fractions 5-6 with *n*-hexane:ethyl acetate (8:2), fractions 7-8 with *n*-hexane: ethyl acetate (7:3), fractions 9-10 with *n*-hexane:ethyl acetate (6:4), fractions 11-12 with *n*-hexane:ethyl acetate (1:1), fractions 13-14 with *n*-hexane:ethyl aceate (4:6), fractions 15-16 with *n*-hexane: ethyl acetate (3:7), fractions 17-18 with *n*-hexane:ethyl acetate (2:8), fractions 19-20 with *n*-hexane: ethyl acetate (1:9), fractions 21-22 with ethyl acetate, fractions 23-24 with ethyl acetate : methanol (9.5:0.5), fractions 25-26 with ethyl acetate:methanol (9:1), fractions 27-28 with ethyl acetate:methanol (8:2), fractions 29-30 with ethyl acetate: methanol (7:3), fractions 31-32 with ethyl acetate:methanol (6:4), fractions 33-34 with ethyl acetate:methanol (1:1), fractions 35-36 with ethyl acetate:methanol (4:6), fractions 37-38 with ethyl acetate:methanol (3:7), fractions 39-40 with ethyl acet-ate:methanol (2:8), fractions 41-42 with ethyl acetate:methanol (1:9), fractions 43-44 with methanol. All fractions were examined by TLC. Fractions 1-4 were not further separated because of their fatty nature. Further chromatography of the fractions 7-8 (30 g) eluting with dichloromethane:methanol (99:1, V:V) afforded β-sitosterol (3 g), whose identity was confirmed through the comparison of TLC and spectroscopic data[15] with those of an authentic sample. Fractions 17-18 of main column after chromatography was obtained oleanolic acid (2.5 g), whose identity was confirmed through standard and spectroscopic data[16]. Further rechromatography of the fractions 21-22 (19 g) over silica gel (60-120 mesh) eluting with chloroform and methanol yielded good quantity of gallic acid (9 g), whose identity was confirmed with authentic sample from Sigma. Further rechromatography of the fractions 19-20 (15 g) over silica gel (60-120 mesh) eluting with chloroform and methanol yielded 10 fractions (each 250 mL). Fraction 3 was again rechromatographed overLiChroprep RP-18 (ODS silica gel; 40-63 μm: 100 g; each fraction 100 mL) eluting sequentially with methanol containing 80, 60, 40, 20, 10 and 0 % of water to yield two new compounds **1** (30 mg), **2** (15 mg). Fractions 9-10 after rechromatography was obtained β-sitosterol-β-D-glucoside, whose identity was identified through Co-tlc with the previously isolated sterol glycosides from several plants (Fig. 1).

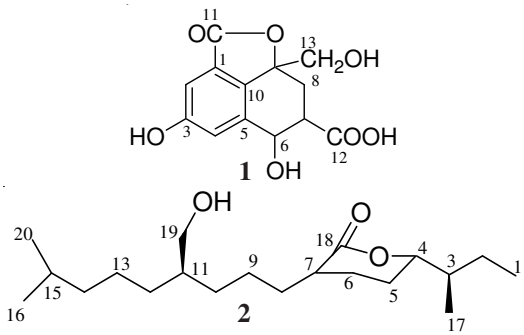

Fig. 1. Chemical structures of compounds **1** and **2**

3, 6-dihydroxy-7-carboxylic acid-9-hydroxymethylene-tetralin-1,9-olide.(1): Yellow semisolid; m.p. 120º; $[\alpha]_D^{22}$ + 21.20 (c 0.3, MeOH); IR (KBr, ν_{max}, cm^{-1}): 3450, 3290, 3234, 2955, 2842, 1764, 1704, 1610, 1543, 1440, 1355, 1245, 1037; ^{1}H NMR (CD_3OD): δ 7.05 (2H, brs, H-2, H-4), 4.23 (1H, d, *J* = 9.9 Hz, H-6α), 3.35 (1H, br s, H_2-13a), 3.31 (1H, br s, H_2-13b), 2.79 (1H, ddd, *J* = 9.9, 17.7, 5.1 Hz, H-7), 2.39 (1H, d, *J* = 17.7 Hz, H_2-8a), 2.33 (1H, d, *J* = 5.1 Hz, H_2-8b); ^{13}C NMR (MeOD): δ 139.87 (C-1), 110.17 (C-2), 146.60 (C-3), 110.09 (C-4), 121.53 (C-5), 68.49 (C-6), 52.44 (C-7), 38.59 (C-8), 66.70 (C-9), 121.51 (C-10), 169.14 (C-11), 179.30 (C-12), 77.86 (C-13); EIMS *m/z* (rel. int.): 280 $[M]^+$ ($C_{13}H_{12}O_7$) (13.6), 249 (6.8), 231 (7.1), 186 (5.4).

3,15-Dimethyl-11-hydroxymethylene-*n*-hexadeca-6-en-4, 18-olide (2): Semisolid; $[\alpha]_D^{22}$ +34.1 (c 0.4, MeOH); IR (KBr, ν_{max} cm^{-1}) : 3401, 2918, 2850, 1735, 1645, 1445, 1383, 1044; ^{1}H NMR ($CDCl_3$ + MeOD): δ 5.31 (1H, m, H-6), 3.85 (1H, br, m, $W_{1/2}$ = 16.5 Hz, H-4α), 3.66 (1H, d, *J* = 7.2 Hz, H_2 -19a), 3.62 (1H, d, *J* = 6.3 Hz, H_2 -19b) 2.70 (2H, m, H_2-5), 2.37 (2H, m, H_2-8), 2.25 (1H, m, H-11), 2.07 (1H, m, H-3), 2.03 (2H, m, H_2-9), 1.81 (1H, m, H-15), 1.73 (1H, m, H-2), 1.62 (2H, m, H_2-10), 1.27 (4H, brs, H_2-10 and H_2-12), 1.22 (2H, m, H_2-13), 1.19 (2H, brs, H_2-14), 1.17 (2H, brs, H_2-2), 1.19 (3H, dd, *J* = 6.9, 6.6 Hz Me-1), 1.04 (3H, d, *J* = 6.5 Hz, Me-17), 0.91 (3H, d, *J* = 7.5 Hz, Me-16), 0.86 (3H, d, *J* = 6.1 Hz, Me-20); ^{13}C NMR ($CDCl_3$ + MeOD): δ 14.55 (C-1), 20.16 (C-2), 39.52 (C-3), 78.89 (C-4), 58.24 (C-5), 120.11 (C-6), 140.16 (C-7), 50.01 (C-8), 30.49 (C-9), 30.52 (C-10), 42.72 (C-11), 22.19 (C-12), 21.41 (C-13), 21.23 (C-14), 31.48 (C-15), 18.35 (C-16), 16.53 (C-17), 166.39 (C-18), 68.16 (C-19),18.27 (C-20); EIMS *m/z* (rel.int.): 324 $[M]^+$ ($C_{20}H_{36}O_3$) (12.6), 281 (14.2), 267 (23.5), 239 (18.9), 129 (4.1), 85 (12.2), 57 (12.7).

RESULTS AND DISCUSSION

Compound **1**, designated as oenotheraphenoxylactone, was obtained as light brown crystalline mass from EtOAc-MeOH (9.8:0.2) eluants. It gave light blue colour with ferric chloride indicating phenolic nature and produced effervescences with sodium bicarbonate due to the presence of free carboxylic function. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3450, 3290 cm^{-1}), carboxylic group (3234, 1704 cm^{-1}), five membered lactone ring (1764 cm^{-1}) and aromatic ring (1610, 1543, 1037 cm^{-1}). On the basis of mass and ^{13}C NMR spectrum its molecular formula was established at *m/z* 280 corresponding to tetrahydronapthalene type lactone $C_{13}H_{12}O_7$. Elimination of hydroxylmethylene group from the molecular ion peak yielded an ion fragment at *m/z* 249 which on subsequent removal of water molecule and carboxylic group generated the ion fragments at *m/z* 231 and 186, respectively (Fig. 2).

The ^{1}H NMR spectrum of **1** showed a two-proton broad signal at δ 7.05 assigned to aromatic H-2 and H-4 protons. A one-proton doublet at δ 4.23 (*J* = 9.9 Hz) was attributed to α-oriented H-6 carbinol proton. Two broad signals at δ 3.35 and 3.31 were ascribed to hydroxymethylene H_2-13 protons. A one-proton doublet of double doublet at δ 2.79 (*J* = 9.9, 13.5, 5.1 Hz) was accounted to α-oriented H-7 methine protons. Two one-proton doublets at δ 2.39 (*J* = 17.7 Hz) and 2.33 (*J* = 5.1

Hz) were associated with the methylene H_2-8 protons. The ^{13}C NMR spectrum of **1** exhibited the presence of 13 carbon signals and the important carbon signals appeared for carboxylic carbon at δ 179.30 (C-12), lactone carbon at δ 169.14 (C-11), hrdroxyl methine carbon at δ 68.49 (C-6), hydroxymethylene carbon at δ 77.86 (C-13), oxygenated quaternary carbon at δ 66.70 (C-9) and aromatic carbons between δ 146.60 - 110.09. The DEPT spectrum of **1** showed the presence of two methylene, four methine and seven quaternary carbons. The HSQC experiment showed important correlations between the aromatic proton signals at δ 7.05 with the carbon signals at δ 110.17 and 110.09; between the carbinol proton at δ 4.23 with carbon signals at δ 68.49; and hydroxymethylene proton at δ 3.35/ 3.31 with the carbon signal at δ 77.86. The ^1H-^1H COSY spectrum of **1** showed correlations of H-6 with H-4, H-7 and H_2-8; H_2-13 with H_2-8. The HMBC spectrum of **1** exhibited that C-11 interacted with H-2; C-4 interacted with H-2 and H-6; C-12 interacted with H-7, H-6 and H_2-6; and C-13 interacted with H_2-8. On the basis of these evidences the structure of **1** has been characterized as 3,6-dihydroxy-7-carboxylic acid-9-hydroxymethylene-tetralin-1, 9-olide.

Compound **2**, named oenotheraphytyllactone, was obtained as a colourless crystalline mass from EtOAc:MeOH (9.8:0.2) eluants. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3410 cm^{-1}), δ-lactone (1735 cm^{-1}) and unsaturation (1645 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular weight has been established at *m/z* 324 consistent with the molecular formula of an acyclic diterpene $C_{20}H_{36}O_3$. It indicated three degrees of double bond equivalents, which were adjusted in the vinylic linkage, carbonyl function and lactone ring system. The important ion peaks arising at *m/z* 281 $[M\text{-}C_3H_7]^+$, 85 $[C_{11}$ - C_{12} fission$]^+$ and 129 $[C_{10}$ - C_{11} fission$]^+$, suggested location of the hydroxyl methylene group at C-11. The ion peaks generating at m/z 57, 267 $[C_3$ - C_4 fission$]^+$ supported the presence of the δ-lactone ring at C-4 (Fig. 2). The ^1H NMR spectrum of **2** showed a one proton multiplet at δ 5.31 assigned to vinylic H-6 proton. A one-proton broad multiplet at δ 3.85 with half width of 16.5 Hz was ascribed to α-oriented H-4 oxygenated methine proton. Two one-proton doublets at δ 3.66 (J = 7.2 Hz) and 3.62 (J = 6.3 Hz) were attributed to hydroxymethylene H_2-19 protons. Three doublets at δ 1.04 (J = 6.5 Hz), 0.91 (J = 7.5 Hz) and 0.86 (J = 6.1 Hz) and a double doublet at δ 1.19 (J = 6.9, 6.6 Hz), all integrated for three protons each, were associated with the secondary C-16, C-17 and C-20 and primary C-1 methyl protons, respectively. The remaining methylene and methine protons resonated between δ 2.70 -1.17. The presence of methyl signals between δ 1.19-0.86 indicated that all these functionalities were attached to the saturated carbons. The ^{13}C NMR spectrum of **2** showed the presence of 20 carbon atoms and the important signals appeared for lactone carbon at δ 166.39 (C-18), oxygenated methine carbon at δ 78.89 (C-4), hydroxymethylene carbon at δ 68.16 (C-19), vinylic carbons at δ 120.11 (C-6) and 140.16 (C-7) and methyl carbons at δ 14.55 (Me-1), 16.53 (Me-17), 18.35 (C-16) and 18.27 (Me-20). The DEPT spectrum of **2** exhibited the presence of four methyls, nine methylene, five methine and two quaternary carbons. The HSQC experiment showed important interactions between the vinylic proton at C-6 at δ 5.31 with the carbon signal at δ 120.11, between carbinol proton at δ 3.85 with carbon signal at δ 78.89 and between hydroxymethylene signal at δ 3.66/ 3.62 with the carbon signal at δ 68.16. The ^1H-^1H COSY spectrum of **2** exhibited correlations of H-4 with H-3, H_2-5 and H-6; H-6 with H_2-5 and H_2-8; and H_2-19 with H-11 and H_2-10. The HMBC spectrum of **2** showed that C-6 interacted with H_2-5 and H_2-8; C-18 interacted with H-6 and H_2-8; C-4 interacted with H-3 and H_2-5 and C-19 interacted with H-11 and H_2-10. On the basis of spectral data analysis, the structure of **2** has been elucidated as **3**, 15-dimethyl-11-hydroxymethylene-*n*-hexadeca-6-en-4,18-olide (Fig. 2).

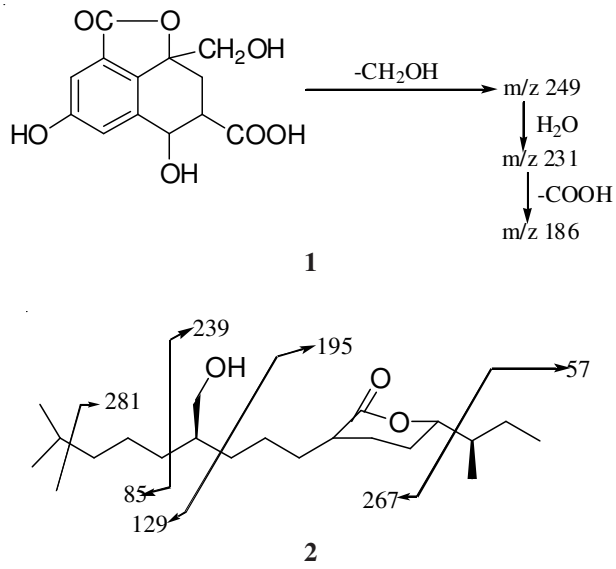

Fig. 2. Fragmentation pattern of compounds **1** and **2**

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