TWO NEW COUMARINS FROM *Euonymus hamiltonianus*

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*Two new coumarins, euonidiol (**1**) and euoniside (**2**), and a known flavone, luteolin 7-methyl ether, were isolated from the aerial parts of the plant* Euonymus hamiltanianus *Wall. All the compounds were characterized on the basis of spectral analysis viz. ^{1}H NMR, ^{13}C NMR, DEPT, IR, UV, ESI-MS, and elemental analysis.*

Key words: *Euonymus hamiltonianus*, euonidiol, euoniside, luteolin 7-methyl ether.

Plants of the Celastraceae family comprise 60 genera and 850 species worldwide [1]. Many of them have been used in traditional medicine [2, 3]. Genus *Euonymus* of the family Celastraceae is reported to be a rich source of sesquiterpene alkaloids [4], sesquiterpene esters [5], sesquiterpene pyridine alkaloids [6], sesquiterpene polyol esters [7], flavonoids, and coumarins [8]. The plant species belonging to this genus has wellknown medicinal activity such as cytotoxic [9], antitumor [10], immunosuppressive [11], insecticidal [12], and insect-antifeedant activity [13], and reverses multi-drug resistance in cancer cells [14]. *Euonymus hamiltonianus* Wall. is a small tree growing wild in Kashmir at altitudes from 3000–4000 meters. To the best of our knowledge, this plant species has not been investigated for its phytochemical characterization so far. Keeping in view the biological importance of *Euonymus* species and our continued interest in the characterization of lead bioactive molecules, we have undertaken a research program to investigate the molecular characterization of *Euonymus hamiltonianus* Wall. The methanolic and ethyl acetate extracts of the aerial parts of the plant lead to the isolation and characterization of two new coumarins, euonidiol (**1**) and euoniside (**2**) and a known flavone, luteolin 7-methyl ether for the first time [15].

Column chromatography of the methanolic extract of the leaf of *Euonymus hamiltonianus* resulted in the isolation of the following two compounds.

Euonidiol (1). Elemental analysis agrees with molecular formula $C_{14}H_{14}O_5$, mp166–167°C. This molecular formula was confirmed by ESI-MS, *m/z* at 285 [M+Na]. The UV spectrum contained λ_{max} at 248, 259, and 314 nm characteristic of courmarins. The IR spectrum revealed characteristic -OH absorption at 3457 cm^{-1}, aromatic C-H absorption at 2973 cm^{-1}, and carbonyl absorption at 1712 cm^{-1}. The NMR spectrum showed eight kinds of protons. Two signals at δ 1.47 and δ 1.52 (each 3H, s) showed the presence of two methyl groups. The signals at δ 5.68 (1H, d, J = 6.0 Hz) showed a benzylic proton simultaneously attached with hydroxyl, and the signal at δ 4.40 (1H, d, J = 6.0 Hz) is due to the proton on the carbon directly attached to the oxygen atom of the dihydrofurano ring. The signals at δ 6.24 and 7.90 (each 1H, d, J = 9.5 Hz) are the diagnostic signals of the coumarin nucleus corresponding to the protons of the α,β-unsaturated carbonyl system. The signals at δ 6.99 and 7.56 (each 1H, d, J = 8.4 Hz) are due to the protons attached to the aromatic ring at position 6 and 5 respectively. Further, the signal at δ 6.99 also indicates that the oxygen atom of the dihydrofuran ring is attached with position 8 rather than position 7 of the coumarin nucleus as in vagnidiol [16]. Had it been at position 7, the signal at δ 6.99 would have been upfield due to the shielding effect of adjacent oxygen. The stereochemistry of the two chiral centers 2′ and 3′ were also confirmed by correlating it with the stereochemistry of vagnidiol as well as from the chemical shift of H-2′ and H-3′ in ^{1}H NMR. Acetylation of **1** using Ac_2O/pyridine resulted in the formation of a monoacetate derivative. However, when DMAP was used in place of pyridine, it resulted in the formation of a diacetate, indicating that one of the -OH is tertiary. ^{13}C NMR and DEPT spectrum of **1** showed 14 carbon signals – two methyls, six methines, and six quaternary carbons. Therefore the structure of **1** is as shown Fig. 1.

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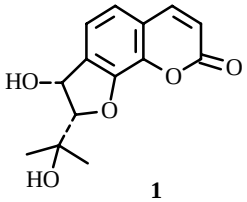

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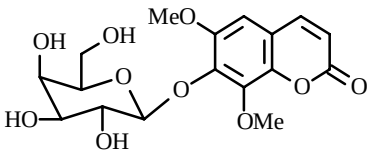

2

Fig. 1.

Euoniside (2). Elemental analysis agrees with molecular $C_{17}H_{20}O_{10}$, mp 198–199°C. This molecular formula was again analyzed by ESI-MS at 407 [M+Na]. The UV spectrum shows λ_{max} at 220, 242, 291, and 302 nm characteristics of coumarins. The IR spectrum revealed -OH absorption at 3572 cm^{-1}, aromatic C-H absorption at 2954 cm^{-1} and carbonyl stretching at 1717 cm^{-1}. The ^{1}H NMR spectrum of **2** showed two signals at δ 3.82 and 3.91 (each 3H, s, OCH_3) corresponding to two methoxyls attached to the aromatic ring at positions 8 and 6 respectively. The two doublets at δ 6.41 and 7.97 (each 1H, d, J = 9.5 Hz) revealed the presence of two protons of the α,β-unsaturated system of the coumarin nucleus and the singlet at δ 7.13 revealed the aromatic proton at position 5, which is highly shielded due to the two methoxyls at positions 6 and 8. The remaining signals at δ 5.15 (1H, d, J = 7.8, H-1′), 4.03, 4.21, 4.16, 4.31 (each 1H, m)), and 3.92 (1H, d, J = 7.7, H-6′) were found to be due to the glycan moiety. The sugar was found to be galactose by hydrolyzing **2** with 1N HCl and comparing the R_f of the hydrolyzed product with that of an authentic sample (galactose). The sugar was further confirmed by matching its ^{1}H NMR values with that of galactose. Therefore the structure of **2** is as shown in Fig. 1.

EXPERIMENTAL

Materials and Methods. Melting points are uncorrected and were determined on a BUCHI melting point apparatus. IR were recorded on a Bruker vector 22 spectrometer as KBr pellets with absorption given in cm^{-1}. UV spectra were scanned in methanol on specord S100. ^{1}H NMR and ^{13}C NMR spectra were run on 200 MHz and 500 MHz Bruker Daltonics instruments respectively using TMS as an internal standard. Mass spectra were recorded by using a Bruker Daltonics electrospray ionization. Column was run using silica gel (60–120 mesh), TLC was run on silica gel G and fluorescent aluminum TLC using solvents pet. ether–ethyl acetate (8:2) and $CHCl_3$–MeOH (9:1). Spots were visualized on TLC under UV light, ferric chloride, cerric ammonium sulfate, and exposure to iodine vapor in an iodine chamber, and also by heating the chromatoplates at 100°C in an oven after spraying with 10% H_2SO_4.

Plant Material. The aerial part of the plant was collected in October 2004 from Ganderbal (Srinagar) Kashmir, Jammu and Kashmir. A voucher specimen (No. 1132/03) was deposited in the herbarium of the institute.

Extraction and Isolation. Air-dried and coarsely powdered (aerial part) plant material (2 kg) was extracted exhaustively with hexane for 48 hrs. The deffated material was dried and extracted with methanol for 62 hours. The methanolic extract was concentrated under reduced pressure to give crude extract, 102 g. The dried methanolic extract (60 g) was dissolved in the minimum amount of methanol and adsorbed on silica gel to form slurry. The air-dried slurry was subjected to silica gel column chromatography. The column was eluted with different percentages of petroleum ether, and ethyl acetate and finally with methanol. The following compounds were isolated.

Euonidiol (1). Elution of column with EtOAc–MeOH (9.5:0.5; v/v) afforded pinkish amorphous powder of **1** (60 mg), mp 166–167° C; $[\alpha]_D^{25}$ +88° (*c* 0.25; MeOH); UV (MeOH, λ_{max}, nm): 248, 259, 314 (log ε 4.02, 3.99, 4.17); IR (KBr, ν, cm^{-1}): 3457 (OH), 2973 (Ar), 1712 (C=O), 1619, 1489, 1399, 1350, 1080, 852, 770; ^{1}H NMR (200 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.24 (1H, d, J = 9.5, H-3), 7.90 (1H, d, J = 9.5, H-4), 7.56 (1H, d, J = 8.4, H-5), 6.99 (1H, d, J = 8.4, H-6), 4.40 (1H, d, J = 6.0, H-2′), 5.68 (1H, d, J = 6.0, H-3′), 1.47 (3H, s, H-5′), 1.52 (3H, s, H-6′); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 163.2 (C-2), 112.4 (C-3), 145.3 (C-4), 131.7 (C-5), 123.0 (C-6), 117.2 (C-7), 160.5 (C-8), 151.4 (C-9), 113.3 (C-10), 71.6 (C-2′), 92.6 (C-3′), 68.6 (C-4′), 26.7 (C-5′), 27.4 (C-6′); ^{13}C NMR (DEPT): 112.4 (C-3), 145.3 (C-4), 131.7 (C-5), 123.2 (C-6), 71.1 (C-2′), 92.5 (C-3′), 26.7 (C-5′), 27.4 (C-6′); ESI-MS: 285 [M+Na].

Euoniside (2). Elution of column with EtOAc–MeOH (7:3; v/v) afforded white amorphous powder of **2**; 40 mg, mp 198–199° C; $[\alpha]_D^{25}$ +212° (*c* 0.40; MeOH); UV (MeOH, λ_{max}, nm); 220, 242, 291, 302 (log ε 4.32, 3.78, 4.17, 4.19); IR (KBr, ν, cm^{-1}): 3572 (OH), 2954 (Ar), 1717 (C=O), 1642, 1573, 1408, 1073, 805, 725; ^{1}H NMR (200 MHz, DMSO-d_6, δ,

ppm, J/Hz): 6.41 (1H, d, J = 9.5, H-3), 7.97 (1H, d, J = 9.5, H-4), 7.13 (1H, s, H-5), 5.15 (1H, d, J = 7.8, H-1′), 4.03 (1H, m, H-2′), 4.21 (1H, m, H-3′), 4.16 (1H, m, H-4′), 4.31 (1H, m, H-5′), 3.92 (1H, d, J = 7.7, H-6′), 3.82 (3H, s, -OCH_3-8), 3.91 (3H, s, -OCH_3-6); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 160.2 (C-2), 115.0 (C-3), 144.9 (C-4), 115.9 (C-5), 142.2 (C-6), 142.7 (C-7), 140.9 (C-8), 149.9 (C-9), 115.2 (C-10), 102.6 (C-1′), 74.6 (C-2′), 77.9 (C-3′), 70.4 (C-4′), 76.9 (C-5′), 61.7 (C-6′), 57.1 (-OCH_3-8), 61.3 (-OCH_3-6); ^{13}C NMR (DEPT-135): 115.3 (C-3), 144.9 (C-4), 115.0 (C-5), 102.6 (C-1′), 74.4 (C-2′), 78.0 (C-3′), 70.4 (C-4′), 77.0 (C-5′), 61.7 (C-6′), 57.1 (OCH_3-8), 61.8 (OCH_3-6); ESI-MS: *m/z* 407.1 [M+Na].

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