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# GLUTINANE TRITERPENES FROM THE STEM BARK OF *Euonymus hamiltonianus*

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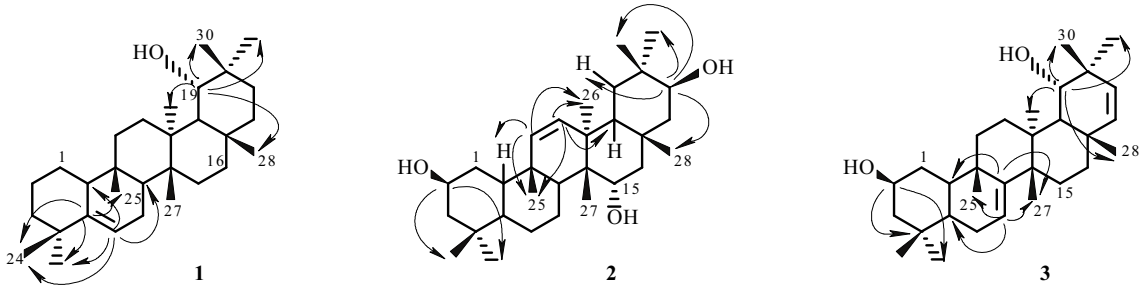
*Three new glutinane-type triterpenes, 19α-glutin-5-en-19-ol (**1**), 2β,15α,21β-glutin-11-ene-2,15,21-triol (**2**), and 2β,19α-glutin-7,21-diene-2,19-diol (**3**), were isolated from the stem bark of* Euonymus hamiltonianus. *Their structures were determined by 1D and 2D NMR along with MS and IR.*

**Key words**: *Euonymus hamiltonianus*, Celastraceace, glutinane type triterpenes.

Plants of the Celastraceae family comprise 60 genera and 850 species worldwide [1]. Many of them have been used in traditional medicine [2, 3]. The 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 well-known medicinal activity such as cytotoxic [9], antitumor [10], immunosuppressive [11], and insecticidal [12] and insect-antifeedant activity [13], and is capable of reversing multidrug resistance in cancer cells [14]. *Euonymus hamiltonianus* Wall. is a small tree growing wild in Kashmir at altitudes from 3000–4000 meters. By continuing the pursuit of active principles of the Celastraceae family, particularly of the genus *Euonymus*, the ethyl acetate extract of the stem bark of *Euonymus hamiltonianus* leads to the isolation and characterization of the three new glutinane-type triterpenes 19$\alpha$-glutin-5-en-19-ol (**1**), 2$\beta$,15$\alpha$,21$\beta$-glutin-11-ene-2,15,21-triol (**2**), and 2$\beta$,19$\alpha$-glutin-7,21-diene-2,19-diol (**3**). In this paper the isolation and characterization of compounds **1–3** are presented.

Column chromatography of the ethyl acetate extract of the stem bark of *Euonymus hamiltonianus* leads to the isolation and characterization of three new pentacyclic glutinane-type triterpenes, 19$\alpha$-glutin-5-en-19-ol (**1**), 2$\beta$,15$\alpha$,21$\beta$, glutin-11-ene-2,15,21-triol (**2**), and 2$\beta$,19$\alpha$-glutin-7,21-diene-2,19-diol (**3**).




**1** **2** **3**

Fig. 1. Significant HMBC correlations of functional carbons of compounds **1–3**.

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TABLE 1. $^{13}C$ NMR (500 MHz, $CDCl_3$, ppm) and HMBC of Compound **1**

| C atom | $\delta_C$ | HMBC | C atom | $\delta_C$ | HMBC |
|---|---|---|---|---|---|
| 1 | 42.1 | Me-25, H-3 | 16 | 24.2 | Me-27, Me-28, H-18 |
| 2 | 38.2 | Me-24, Me-23, H-10 | 17 | 36.1 | Me-26, H-15, H-18, H-19, H-21 |
| 3 | 33.3 | Me-24, Me-23, H-1$\alpha$, H-1$\beta$ | 18 | 39.4 | Me-30, Me-29, Me-28, Me-26, H-1$\alpha$, H-22 |
| 4 | 39.9 | H-2, H-6, H-10 | 19 | 70.5 | Me-30, Me-29, Me-28, Me-26, H-21 |
| 5 | 142.2 | Me-25, Me-24, Me-23, H-7, H-1 | 20 | 32.2 | H-18, H-22 |
| 6 | 120.0 | Me-24, Me-23, H-8, H-10 | 21 | 22.2 | Me-30, Me-29, Me-28, Me-26, H-19 |
| 7 | 55.2 | Me-27, Me-25 | 22 | 25.1 | Me-30, Me-29, H-16, H-18 |
| 8 | 35.1 | Me-27, Me-25, H-10, H-11 | 23 | 28.1 | H-3, H-6, H-10 |
| 9 | 33.1 | H-1, H-7, H-12 | 24 | 18.2 | H-3, H-6, H-10 |
| 10 | 50.1 | Me-25, Me-24, Me-23, H-2, H-6, H-8, H-11 | 25 | 17.1 | H-1$\alpha$, H-1$\beta$, H-7, H-12 |
| 11 | 29.1 | Me-26, Me-25, H-8, H-10 | 26 | 18.5 | H-11, H-19 |
| 12 | 33.3 | Me-27, Me-26, Me-25, H-18 | 27 | 19.1 | H-7, H-8, H-16$\alpha$, H-16$\beta$ |
| 13 | 42.4 | Me-27, H-11, H-15, H-19 | 28 | 20.2 | H-16, H-18, H-22$\alpha$, H-22$\beta$ |
| 14 | 40.4 | Me-26, H-7, H-16, H-18 | 29 | 28.4 | H-18, H-22$\alpha$ |
| 15 | 28.1 | Me-28, Me-27, Me-26, H-18 | 30 | 22.4 | H-18, H-22$\beta$ |

TABLE 2. $^{13}C$ NMR (500 MHz, $CDCl_3$, ppm) and HMBC of Compound **2**

| C atom | $\delta_C$ | HMBC | C atom | $\delta_C$ | HMBC |
|---|---|---|---|---|---|
| 1 | 31.1 | Me-25, H-3, H-5 | 16 | 31.1 | Me-28, Me-27, H-18, H-22$\beta$ |
| 2 | 72.5 | Me-24, Me-23, H-10 | 17 | 36.4 | Me-26, H-15, H-19, H-21 |
| 3 | 29.2 | Me-24, Me-23, H-5 | 18 | 45.8 | Me-30, Me-29, Me-28, Me-26, H-16, H-22 |
| 4 | 37.0 | H-3, H-5, H-10 | 19 | 48.4 | Me-30, Me-29, Me-26, Me-28, H-21 |
| 5 | 55.2 | Me-25, Me-24, Me-23, H-5, H-7$\alpha$ | 20 | 33.3 | H-18, H-22$\beta$ |
| 6 | 18.2 | Me-25, Me-24, H-8, H-10 | 21 | 70.7 | Me-30, Me-29, Me-28, H-19 |
| 7 | 31.9 | Me-27, Me-25, H-5 | 22 | 20.2 | Me-30, Me-29, H-18, H-16 |
| 8 | 41.0 | Me-27, Me-25, H-6, H-11, H-10 | 23 | 27.1 | H-3, H-5, H-10 |
| 9 | 44.2 | Me-27, H-12 | 24 | 19.1 | H-3, H-5, H-10 |
| 10 | 38.2 | Me-25, Me-24, Me-23, H-2, H-6, H-11 | 25 | 18.1 | H-1, H-7, H-11 |
| 11 | 123.2 | Me-26, Me-25, H-8, H-10 | 26 | 20.1 | H-11, H-19 |
| 12 | 142.3 | Me-27, Me-26, Me-25, H-18 | 27 | 19.8 | H-7, H-8, H-16$\beta$ |
| 13 | 42.2 | Me-27, H-15, H-19 | 28 | 20.3 | H-16, H-18, H-22 |
| 14 | 30.2 | Me-26, H-7, H-16, H-11 | 29 | 28.1 | H-18, H-22 |
| 15 | 71.1 | Me-28, Me-27, Me-26, H-18 | 30 | 25.2 | H-18, H-22 |

The IR spectrum of **1** revealed a hydroxyl band stretching at 3429 $cm^{-1}$. Two-dimensional correlation experiments (COSY, HSQC, HMBC, and NOESY) were carried out in order to deduce the double bond and hydroxyl present at the 5 and 19 positions, respectively. The $^{13}C$ experiments showed eight tertiary methyls and ten methylene groups, five methines, and seven quaternary carbons, indicating that compound **1** is a glutinane-type of triterpene. The presence of a tetrasubstituted double bond at $C_5$-$C_6$ was confirmed by analysis of HMBC correlations (Table 1). The resonance frequency of H-19 appears at $\delta$ 3.58, J = 3.8 Hz coupled with an equatorial/axial coupling of two vicinal *cis*-protons. The NOESY showed the correlation of H-19 with the doublet at $\delta$ 1.29 and $\delta$ 1.37, which includes H-21 and H-22, from which the correlation of OH, which is at the $\alpha$ configuration, can be confirmed. The orientation and position of other protons were confirmed by HMBC experiments given in Table 1. By this analysis, compound **1** was determined as 19$\alpha$-glutin-5-en-19-ol.

The IR spectrum of **2** revealed OH absorption at 3430 $cm^{-1}$. The $^1H$ NMR spectrum showed the presence of eight tertiary methyl groups (Table 2). The olefinic protons showed two signals at $\delta$ 5.39, J = 11.2 and $\delta$ 5.50, J = 11.2 Hz, each indicating a *trans* configuration of the protons. The resonance of H-2 at $\delta$ 3.54, m coupled with H-1 and H-3 showed that it is attached to carbon, which is flanked by the OH functionality. The coupling constants $J_{15\beta,16\alpha}$ = 11.2 Hz and $J_{15\beta,6\beta}$ = 5.0 Hz are in agreement with the $\alpha$-position, which is also confirmed by NOE experiments, due to the observed interaction of H-15 and H-18.

TABLE 3. $^{13}C$ NMR (500 MHz, $CDCl_3$, ppm) and HMBC of Compound **3**

| C atom | $\delta_C$ | HMBC | C atom | $\delta_C$ | HMBC |
|---|---|---|---|---|---|
| 1 | 31.2 | Me-25, H-3, H-5 | 16 | 25.3 | Me-28, Me-27, H-18, H-22 |
| 2 | 71.2 | Me-24, Me-23, H-10 | 17 | 35.6 | Me-26, H-19, H-21 |
| 3 | 29.3 | Me-24, Me-23, H-5 | 18 | 45.5 | Me-30, Me-29, Me-28, Me-27, Me-26 |
| 4 | 38.2 | H-2, H-6, H-10 | 19 | 74.1 | Me-30, Me-29, Me-28, Me-26, H-21 |
| 5 | 50.1 | Me-25, Me-24, Me-23, H-1, H-7 | 20 | 33.2 | H-18, H-22 |
| 6 | 38.2 | Me-25, Me-24, Me-23, H-8, H-10 | 21 | 127.1 | Me-30, Me-29, Me-28, H-19 |
| 7 | 125.0 | Me-27, Me-25, H-5 | 22 | 126.3 | Me-30, Me-29, H-16, H-18 |
| 8 | 140.4 | Me-27, Me-25, H-6, H-10, H-11 | 23 | 26.3 | H-2, H-5, H-10 |
| 9 | 43.3 | Me-27, H-12 | 24 | 20.2 | H-2, H-5, H-10 |
| 10 | 37.6 | Me-25, H-2, H-6 | 25 | 19.2 | H-1, H-11 |
| 11 | 30.3 | Me-26, Me-25, H-10 | 26 | 21.2 | H-11, H-19 |
| 12 | 34.3 | Me-27, Me-26, Me-25, H-18 | 27 | 19.2 | H-7, H-16$\beta$ |
| 13 | 41.1 | Me-27, H-16$\beta$, H-19 | 28 | 20.2 | H-15, H-18, H-22 |
| 14 | 41.1 | Me-26, H-16 | 29 | 27.6 | H-18, H-22 |
| 15 | 29.3 | Me-28, Me-27, Me-26, H-18 | 30 | 24.9 | H-18, H-22 |

The resonance of H-21 at δ 3.52, J = 10.5 is a dd due to coupling with H-22$\alpha$ and H-22$\beta$, presenting a 22$\beta$, $J_{21\alpha,22\alpha}$ = 4.0 Hz and $J_{21\alpha,22\beta}$ = 12.0 Hz and suggesting that H-21 is in the $\alpha$-orientation, which is confirmed by NOESY correlation with H-19$\alpha$, H-22$\alpha$, Me-29, and Me-30. The $^{13}C$ NMR chemical shifts of the detected signals for the methyl groups of **2** are approximately in the same order of magnitude as that of **1**. DEPT experiment showed the presence of eight methyls, seven methylene, nine methines, and six quarternary carbons. The downfield chemical shifts were given to those carbons which are attached to hydroxyls at positions C-2 (δ 72.5), C-15 (δ 71.1), and C-21 (δ 70.7). The positions and stereo arrangements were given by HMBC and NOESY experiments. The structure of compound **2** was elucidated to be 2$\beta$,15$\alpha$,21$\beta$, glutin-11-ene-2,15,21-triol.

The IR spectrum of **3** showed a band at 3430 $cm^{-1}$, which corresponds to OH stretching. The $^1H$ NMR and 2D experiments showed the presence of eight methyl groups of the glutinane nucleus (Table 3). The olefinic protons showed two signals at δ 5.16, d, J = 11.2 Hz and 5.19, d, J = 11.2 Hz, indicating *trans* configuration of the protons. HMBC (Fig. 1) experiments show that the double bond was positioned between C-21 and C-22. The olefinic proton at δ 5.41 was positioned at C-7 and the double bond between C-7 and C-8. The resonance of H-2 at δ 3.52, m, coupled with H-1 and H-3, is approximately the same as that of **2**. The doublet at δ 3.51, J = 3.7 Hz was assigned to the proton attached to the carbon, which is hydroxylated. The position and configuration of the hydroxyl is $\alpha$, as confirmed by HMBC and NOESY experiments. The $^{13}C$ NMR and DEPT experiments showed eight methyls, seven methylenes, eight methines, and seven quaternary carbons. By the 2D and HMBC experiments, the structure **3** was assigned to the compound. By these experiments the structure of the compound was determined to be 2$\beta$,19$\alpha$-glutin-7,21-diene-2,19-diol.

## EXPERIMENTAL

**General**. Melting points are uncorrected and were determined on a BUCHI melting point apparatus. Optical rotations were measured on a Perkin–Elmer 241 polarimeter in $CHCl_3$ solution. IR were recorded on a Bruker Vector 22 spectrometer as KBr pellets with absorption given in $cm^{-1}$. $^1H$ NMR and $^{13}C$ NMR were run on a 500 MHz Bruker Daltonics instrument using TMS as internal standard. Mass spectra were recorded by using 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 $CH_2Cl_2$–EtOAc and $CHCl_3$–EtOAc. Spots were visualized on TLC by spraying with cerric ammonium sulfate, with exposure to iodine vapor in an iodine chamber.

**Plant Material**. The stem bark of the plant was collected in October 2004 from Ganderbal (Kashmir), India. A voucher specimen was deposited in the Herbarium of the institute (No. 1021/06).

**Extraction and Isolation**. Air-dried and coarsely powdered (aerial part) plant material (5 kg) was extracted exhaustively with hexane for 28 h. The remaining plant material was dried and extracted with ethyl acetate for 48 h. The ethyl acetate extract was concentrated under reduced pressure to give a crude extract, 124 g. The ethyl acetate extract (124 g) was dissolved

in the minimum amount of chloroform and adsorbed on silica gel to form a slurry. The air-dried slurry was subjected to silica gel chromatography. The column was eluted with different percentages of petroleum ether, dichloromethane, and ethyl acetate. The following compounds were isolated.

**19$\alpha$-Glutin-5-en-19-ol (1)**. White crystalline powder, mp 178–179°C ($CH_2Cl_2$–EtOAc); $[\alpha]_D^{22}$ +119.4° (*c* 0.20, $CHCl_3$); ESI-MS *m/z*: 449 $[M+Na]^+$; IR (KBr, ν, $cm^{-1}$): 3429, 2960, 1596, 1465, 1382, 1242, 1192, 1050, 957, 838; $^1H$ NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.38 (1H, d, J = 3.8, H-6), 3.58 (1H, d, J = 3.8, H-19), 2.32 (2H, m, H-7), 2.30 (2H, m, H-10), 1.65 (1H, d, J = 3.8, H-18), 1.62 (1H, s, H-8), 1.51 (1H, d, J = 12.1, H-12), 1.48 (1H, dd, J = 11.2, 5, H-16), 1.46 (2H, dd, J = 11.2, 5, H-15), 1.37 (2H, d, J = 12.1, H-22), 1.29 (2H, d, J = 12.1, H-21), 1.26 (2H, m, H-1), 1.22 (2H, m, H-2), 1.20 (2H, m, H-3), 1.18 (2H, d, J = 10.5, H-11), 0.94 (3H, s, H-23), 0.92 (3H, s, H-26), 0.89 (3H, s, H-29), 0.88 (3H, s, H-30), 0.86 (3H, s, H-28), 0.85 (3H, s, H-25), 0.83 (3H, s, H-27), 0.71 (3H, s, H-24).

**2$\beta$,15$\alpha$,21$\beta$-Glutin-11-ene-2,15,21-triol (2)**. White amorphous powder, mp 180–181°C ($CHCl_3$–EtOAc); $[\alpha]_D^{22}$ –68.2° (*c* 0.20, $CHCl_3$); ESI-MS *m/z*: 481 $[M+Na]^+$; IR (KBr, ν, $cm^{-1}$): 3421, 2948, 1576, 1460, 1346, 1233, 1132, 1072, 930, 852; $^1H$ NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.50 (1H, d, J = 11.2, H-12), 5.39 (1H, d, J = 11.2, H-11), 3.55 (1H, d, J = 5.8, H-15), 3.54 (1H, m, H-2), 3.52 (1H, dd, J = 10.5, 4.0, H-21), 1.89 (2H, d, J = 4.4, H-19), 1.86 (1H, d, J = 4.4, H-18), 1.62 (1H, m, H-8) 1.58 (2H, m, H-7), 1.28 (2H, m, H-16), 1.13 (2H, m, H-1), 1.20 (2H, m, H-3), 1.19 (2H, dd, J = 12.0, 4.0, H-22), 1.17 (2H, m, H-10), 0.97 (3H, s, H-23), 0.92 (3H, s, H-26), 0.89 (1H, s, H-5), 0.89 (3H, s, H-29), 0.88 (3H, s, H-28), 0.87 (2H, m, H-6), 0.86 (3H, s, H-30), 0.85 (1H, s, H-25), 0.83 (1H, s, H-27), 0.72 (3H, s, H-24).

**2$\beta$,19$\alpha$-Glutin-7,21-diene-2,19-diol (3)**. White amorphous powder, mp 165–166°C ($CH_2Cl_2$–EtOAc); $[\alpha]_D^{22}$ –91.4° (*c* 0.25, $CHCl_3$); ESI-MS *m/z*: 463 $[M+Na]^+$; IR (KBr, ν, $cm^{-1}$): 3430, 2941, 1587, 1457, 1343, 1229, 1119, 1073, 945, 869; $^1H$ NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.41 (1H, dd, J = 11.5, 5.5, H-7), 5.19 (1H, d, J = 11.2, H-22), 5.16 (1H, d, J = 11.2, H-21), 3.53 (1H, m, H-2), 3.51 (1H, d, J =3.7, H-19), 2.34 (2H, m, H-6), 1.81 (2H, dd, J =10.5, 3.6, H-16), 1.78 (2H, dd, J =10.5, 3.6, H-15), 1.63 (1H, d, J = 3.7, H-18), 1.53 (2H, dd, J = 10.6, 5.2, H-12), 1.50 (1H, m, H-10), 1.22 (2H, dd, J = 10.6, 5.2, H-11), 1.21 (2H, m, H-3), 1.12 (2H, m, H-1), 0.96 (3H, s, H-23), 0.92 (3H, s, H-28), 0.91 (3H, s, H-26), 0.91 (3H, s, H-27), 0.89 (1H, m, H-5), 0.89 (3H, s, H-29), 0.87 (3H, s, H-30), 0.85 (3H, s, H-25), 0.75 (3H, s, H-24).

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