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TRITERPENES OF *Euonymus alatus* AND THEIR CYTOTOXIC ACTIVITY

Gui-Hua Tu,[1] Xin-Wei Shi,[1] Yuan Zhao,[2] Cheng-Dong Zheng,[1,2] and Jin-Ming Gao[1*]

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Plants of the Celastraceae family, a well-known kingdom of folk medicine in various countries [1], consist of 98 genera with approximately 1264 species [2]. As one of the largest genera of the family, *Euonymus* comprises nearly 200 species, and more than 100 of them can be found in China. Many compounds with antitumor, antifeedant, insecticide, anti-HIV, and hepatoprotective activities have been isolated from the species of this genus [3]. Among these constituents, triterpenes have always attached the attention of scientists in drug development due to their wide range of bioactivities and various kinds of skeletons.

Euonymus alatus (Thunb.) Sieb., known as "gui-jian yu," "gui-jian chou," and "qian-pi ceng" in Chinese, is distributed in most parts of China [4]. Triterpenes in this herb were also isolated by former workers. Fang et al. obtained *epi*-friedelanol [5], lupeol, and 3β-hydroxy-30-norlupan-20-one [6] in 2007 and 2008, and Liu et al. [7] reported the isolation of five triterpenes in 2009: friedelinol, friedelin, lupenone, betulin, and oleanolic acid. A wide range of biological effects, including antitumor, antidiabetic, antihypertensive, antihyperglycemic [8], and antimicrobial activities were reported in previous studies on *Euonymus alatus* [9, 10]. In the search for bioactive triterpenes, column chromatographic (CC) isolation of the ethyl acetate (EtOAc) fraction of the root bark of *Euonymus alatus* in our study yielded eight triterpenes, and the cytotoxic activity of the chemicals was also determined by MTT assay.

In our study, chromatographic purification of the EtOAc fraction of root bark of *Euonymus alatus* (Thunb.) Sieb. resulted in the isolation of compounds **1**–**8**. Structural assessment based on chemical methods and spectral data of these compounds indicated that the eight triterpenes are 30-hydroxy-3-friedelanone (**1**), 29-hydroxy-3-friedelanone (**2**), friedelin (**3**), wilforlide A (**4**), β-amyrin (**5**), oleana-9(11):12-dien-3-ol (**6**), oleanolic acid (**7**), and 3β,28-dihydroxy-12-ursene (**8**).

Compound **1** was obtained as a white amorphous powder (mp 274–276°C). A molecular ion peak $[M]^+$ at *m/z* 442 observed in the mass spectra showed the molecular formula to be $C_{30}H_{50}O_2$. The ^1H NMR spectrum revealed the presence of seven methyl groups: δ_H 0.86 (3H, d, J = 6.7 Hz, H-23), 0.72 (3H, s, H-24), 0.87 (3H, s, H-25), 1.00 (3H, s, H-26), 1.07 (3H, s, H-27), 1.11 (3H, s, H-28); one of them was attached to a tertiary methyl, while the other six were attached to a quaternary carbon. The signal of the proton connected with the hydroxyl group carbon consisted of two parts: 3.34 (1H, d, J = 10.7 Hz) and 3.42 (1H, d, J = 10.7 Hz), while the signal of the secondary methyl carbon connected with the hydroxyl group appeared at δ_C 71.9, showing that this hydroxyl group was attached to C-30 [11]. The ^1H and ^{13}C NMR ($CDCl_3$) data agreed with those of literature data [11, 12], so compound **1** was identified as 30-hydroxy-3-friedelanone.

Compound **2** was obtained as a white amorphous powder (mp 272–273°C), and the molecular ion peak ($[M]^+$, *m/z* 442) observed in the mass spectra indicated that the molecular formula is $C_{30}H_{50}O_2$. The ^1H and ^{13}C NMR ($CDCl_3$) data agreed with the literature data [11, 12], so compound **2** was identified as 29-hydroxy-3-friedelanone.

Compound **3** was obtained as a white amorphous powder (mp 266–268°C) and had the molecular $C_{30}H_{50}O$ according to the mass spectra ($[M + H]^+$, *m/z* 427). The ^1H and ^{13}C NMR ($CDCl_3$) data were in agreement with the literature [13, 14], so compound **3** was assigned as friedelin.

Compound **4** was isolated as a needlelike crystal (mp 319–322°C) and was determined as $C_{30}H_{50}O_2$ by EI-MS ($[M]^+$, *m/z* 456). The spectral data agreed with the literature [14, 15], and it was assigned as wilforlide A.

1) Research Centre for Natural Medicinal Chemistry, College of Science, Northwest A&F University, Yangling, Shaanxi, 712100, P. R. China, fax: 86 29 87092335, e-mail: jinminggaocn@yahoo.com.cn; 2) College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 575–576, July–August, 2011. Original article submitted March 25, 2010.

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Compound **5** was isolated as a white amorphous powder (mp 197–198°C) and had the molecular formula $C_{30}H_{50}O$ based on the mass spectra ($[M + H]^+$, *m/z* 427). The ^1H and ^{13}C NMR data agreed with the literature [16, 17], so compound **5** was confirmed as β-amyrin.

Compound **6** was also obtained as a white amorphous powder (mp 219–221°C). The ion peak $[M]^+$ at *m/z* 424 ($C_{30}H_{48}O$) and the other peaks at *m/z* 271 $[C_{19}H_{27}O]^+$ and 255 $[C_{19}H_{27}]^+$ observed in the mass spectra indicated that it belongs to the $\Delta^{9,(11)\,:12}$ type triterpenoid [17]. Compound **6** was thus assigned as oleana-9(11):12-dien-3-ol, in agreement with the reported ^1H and ^{13}C NMR data [17].

Compound **7** was obtained as a white amorphous powder (mp 308–310°C), and had an ion peak $[M]^+$ at *m/z* 456. The spectral data were almost the same as those of compound **5**, but a –COOH was at position 28 instead of CH_3. Compared with the spectral data in the literature [13, 16], compound **7** was assigned as oleanolic acid.

Compound **8** was obtained as a white amorphous powder (mp 212–213°C), and the mass spectral data indicated a molecular formula of $C_{30}H_{50}O_2$ ($[M + H]^+$, *m/z* 423). The spectral data of compound **8** were basically in agreement with those reported in the literature [18], so we identified it as 3β,28-dihydroxy-12-ursene.

The triterpenes isolated in our study and reported in previous researches [5–7] support the statement that triterpenes are the most common constituents in the family Celastraceae, and the main skeletons are of oleanane, ursane, friedelane, and lupine types [19]. Among the compounds obtained in this study, the occurrence of 30-hydroxy-3-friedelanone (**1**), 29-hydroxy-3-friedelanone (**2**), β-amyrin (**5**), oleana-9(11):12-dien-3-ol (**6**), and 3β,28-dihydroxy-12-ursene (**8**) in *Euonymus alatus* (Thunb.) Sieb. has been reported for the first time. The remaining compounds friedelin (**3**), wilforlide A (**4**), and oleanolic acid (**7**) have all been isolated in this species before and are very common in the other genera of Celastraceae, indicating the agreement in chemical composition in this family. To the best of our knowledge, oleana-9(11):12-dien-3-ol (**6**) and 3β,28-dihydroxy-12-ursene (**8**) were first isolated from the genus *Euonymus*. The MTT assay showed that the cytotoxic activity (inhibition rate, %) of compounds **1**, **2**, **3**, **5**, and **8** on MDA-MB-435 were 57.38, 19.74, 10.99, 10.07, and 9.27%, respectively, at a concentration of 10 μg/mL.

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