Short communication

Two new diterpenoids from *Leonurus japonicus*

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**A B S T R A C T**

Two new labdane-type diterpenoids, 5-ethoxy-3-(2-((R)-4-hydroxy-2.5.5.8a-tetramethyl-3-oxo-3.5,6.7,8.8a-hexahydropentalen-1-yl)ethyl)furan-2(5H)-one (1), and 5-hydroxy-4-((2-((18.35,7aS)-1-hydroxy-4.4,7a-trimethyl-3-propionyloctahydroisobenzofuran-1-yl)ethyl)furan-2(5H)-one (2), were isolated from the EtOH extract of the herb of *Leonurus japonicus* Houtt., Lamiaceae. Their chemical structures were determined by analysis of 1D, 2D NMR, HRESIMS, and by comparison with known analogs.

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**Introduction**

*Leonurus japonicus* Houtt., Lamiaceae, is an annual or biennial herbaceous plant widely distributed and cultivated in China. The dried herb is used in TCM for the treatment of various diseases, such as menstrual disturbances, dysmenorrhoea, and amenorrhoea (Liu et al., 2014). Recently, phytochemical studies on this plant have been reported (Romero-González et al., 2006; Ye et al., 2014; Fuchino et al., 2013; Chang et al., 2010; Moon, 2010; Xiong et al., 2013a,b; Zhang et al., 2013; Peng et al., 2013). Our previous investigation on two plants of the family Lamiaceae resulted in the isolation of a number of new labdane diterpenes (Wu et al., 2011, 2013). In our research on psychoactive natural products from *L. japonicus*, two diterpenoids were isolated and identified. Their chemical structures for 1 and 2 were shown. Herein, we report the isolation, structural elucidation of these compounds.

**Materials and methods**

NMR spectra were recorded on a Bruker Avance III spectrometer operating at 400 MHz for 1H and 100 MHz for 13C. HRESIMS were measured with Waters LPLC-LCT Premier XE and was controlled by MassLynx 4.1 software. Optical rotations were acquired with a WZZ-2ss automatic polarimeter (Shanghai Shenguang High Strength Bolts Co., Ltd., China). Column chromatography was performed with silica gel (200–300 mesh, Yantai Institute of Chemical Technology, Yantai, China), Sephadex LH-20 (GE Healthcare). HPLC separation was performed on an instrument (LC-3000, Beijing Chuangxintongheng Science & Technology Co., Ltd., Beijing, China) consisting of two pumps and a UV/vis detector with an YMC-ODS-A (150 mm × 20 mm) preparative column packed with C18 (5 μm).

The herb of *L. japonicus* Houtt., Lamiaceae, was purchased from Zhangye, Gansu Province of China in March, 2014. It was identified by one of the authors (Dr. H. Wu). A voucher specimen (Code: hkww-aynu-20140301) was deposited at the Pharmaceutical Research Lab, Anyang Normal University.

The dried and powdered herb of *L. japonicus* (85 kg) was extracted with 95% EtOH (3 × 4001) by percolation at room temperature. The solvent was evaporated under reduced pressure at 45 °C to yield 7.2 kg viscous syrup, which was dissolved in water and extracted with EtOAc to get 3 kg extract. The EtOAc fraction was suspended in 30% EtOH and allowed to pass through a column (30 cm × 100 cm) packed with AB-8 macroporous resin (10 kg), then eluted with 50%, 70%, 90%, and 95% EtOH to obtain fractions A-D. Fraction C (693 g) was separated by silica gel CC over petroleum ether–EtOAc (10:1, 4:1, 2:1, 1:1) to yield subfractions (C1–C4). The further separation of fraction C2 with Sephadex LH-20 (MeOH), and then separated by reversed-phase preparative HPLC (60% acetonitrile in water, at a flow rate of 3 ml per min) to yield pure 1 (9 mg) and 2 (38 mg). In addition, a known compound (+)-sesamin, a lignan, was precipitated from the concentration of the eluate of the Sephadex LH-20 column.

5-Ethoxy-3-(2-((R)-4-hydroxy-2.5.5.8a-tetramethyl-3-oxo-3.5,6.7,8.8a-hexahydropentalen-1-yl)ethyl)furan-2(5H)-one (1): Light yellow oil, [α]D20 = –0.33 (ε = 0.1, MeOH); 1H and 13C NMR, see Table 1, HR-ESIMS m/z 375.2153 [M+H]+ (calcd. 375.2171).
5-Hydroxy-4-(2-((1R,3S,3aS,7aS)-1-hydroxy-4,4,7a-trimethyl-3-propionyloctahydroiso-benzo-furan-1-yl)ethyl)furan-2(5H)-one (2): Colorless oil, [α]D20 = +55.4 (c = 0.2, MeOH); 1H and 13C NMR, see Table 1. HR-ESIMS m/z 367.2113 [M+H]+ (calcd. 367.2121), 389.1928 [M+Na]+ (calcd. 389.1940).

Results and discussion

A 95% ethanol extract of *Leonurus japonicus* Houtt., Lamiaceae, was suspended in water and extracted with EtOAc. The EtOAc fraction was subject to macroporous resin (AB-8) column chromatography (CC), and then to silica gel CC, Sephadex LH-20 CC, and HPLC, affording two new compounds.

Compound 1 was obtained as a yellowish oil. Its molecular formula was determined as C22H30O5 on the basis of HRESIMS ([M+H]+ 375.2153), indicating eight degrees of unsaturation. The 1H and 13C NMR (Table 1) showed the presence of one ethoxy group, and twenty additional carbon resonances, attributed to four tertiary methyl groups, five methylenes, two methines with one sp² carbon (δ 6.88/142.4) and one acetal (δ 5.84/101.6), and nine quaternary carbons (with seven sp² carbons, an α, β-unsaturated ketone as revealed by the low field signals at δC 164.3, 181.5, and a lactone carbonyl at δC 170.9). These data suggested that 1 was a labdanoid-type diterpenoidal compound. These data were comparable to those of leojaponin (Romero-González et al., 2006), with similar resonances for the A and B rings. The 13C NMR resonances at δC 170.9, 142.4, 137.4, 101.6, 65.2, and 14.5 and the 1H NMR resonances at δH 6.88 (1H, s), 5.84 (1H, s), 3.84 (2H), and 1.29 (3H) indicated that 1 possessed a 3-substituted 5-ethoxyfuran-2(5H)-one moiety at C-12 rather than the furan ring of leojaponin. The signal of a hydroxy group was easily deduced, as there was no HMOC correlation between the hydroxyl proton (δH 6.96 s) and any other carbon, and the HMBC spectrum showed the correlations of the hydroxyl proton with three sp² carbons (δC, 143.1, 140.6, and 181.5), and also the HMBC correlations (Fig. 1) of H3-17 with C-7, C-8, and C-9 allowed us to determine the only possible partial structure was C-C(OH)-C(C=O)-C(CH3)2-C, which was identical to those of leojaponin. A comparison of the NMR spectroscopic data of the C-11 to C-16 region of 1 to those reported for the structural analog sibiricine A (Boalino et al., 2004) facilitated assembly of its C-11 to C-16 component. However the only C-15 relative configuration could not be assigned. Thus, the molecular structure of 1 was determined as 5-ethoxy-3-(2-((1R,3S,3aS,7aS)-1-hydroxy-4,4,7a-trimethyl-3-propionyloctahydroiso-benzo-furan-1-yl)ethyl)furan-2(5H)-one. Compound 2 was obtained as a colorless oil, and the molecular formula was determined as C20H30O6 on the basis of HRESIMS ([M+H]+ 375.2113; calcld. 376.2121), indicating eight degrees of unsaturation. The 1H and 13C NMR (Table 1) showed the presence of twenty carbon resonances, attributed to one secondary methyl group (δ 106.9), three tertiary methyl groups (δ 0.79/33.1, 1.07/22.2, 1.09/16.2), six methylenes, four methines with one sp² carbon (δ 5.91/112.9) and two oxygenated (δ 6.19/97.3, 4.40/83.5), and six quaternary carbons. These data were comparable to those of seco-labdane (Rustaiyan et al., 1995), with similar resonances for the A and B rings. The 13C NMR resonances at δC 170.8, 167.1, 112.9, and 97.3 and the 1H NMR resonances at δH 5.91 (1H, d, J = 2.0 Hz), 6.19 (1H, s) indicated that 2 possessed a 3-substituted 5-hydroxyfuran-2(5H)-one moiety, similar to sibiricine A (Boalino et al., 2004) at C-12 rather than the furan ring of seco-labdane. The molecular structure of 2 was confirmed by the HMBC correlations.
between H_{3-20} and C-1/C-5/C-9; H_{3-18} and C-4/C-5/C-19; H_{3-17} and C-7/C-8; H-5 and C-1/C-3/C-4/C-6/C-7/C-9/C-10; H-6 and C-4/C-5/C-7/C-8/C-9/C-10; H-11 and C-9/C-12/C-13; H-12 and C-11/C-13/C-14/C-16; H-14 and C-12/C-13/C-15/C-16; H-16 and C-12/C-13/C-14/C-15 (Fig. 1). The relative configurations at C-5, 6, 9 as assigned via NOE association were the same as the reported those of seco-labdane (Fig. 2). Therefore, the structure of 2 was defined as 5-hydroxy-4-(2-(((1R,3S,5aS,7aS)-1-hydroxy-4,4,7a-trimethyl-3-propionyloctahydroisobenzofuran-1-yl)ethyl)furan-2(5H)-one. The structure of compound 1, bearing an ethoxy group at C-15, must be an artifact of isolation, produced during the extraction procedure with EtOH from the naturally occurring hemicetin. Yan-Ping Shi et al. have reported several labdane-type diterpenoids with ethoxy and methoxy groups from L. heterophyllus, and indeed, they could not find the same compounds when the dry plant was extracted by CHCl_3 and monitored by HPLC analysis (Gong et al., 2012). Therefore, it can be predicted that if protic solvents (such as ROH) were used, corresponding products with alkoxyl groups might be generated. The formation of compound 2 might be easily explained via a retro aldon reaction starting from a similar derivative of 2 (Rustaiyan et al., 1995). In conclusion, the EtOH extract of the herb of L. japonicus was purified by multiple chromatographic methods. Two diterpenoids were isolated and their chemical structures were elucidated, and one of them has an α,β-unsaturated lactone moiety, whose structure is similar to leojaponin (with a cross-conjugated α,β-unsaturated ketone moiety) except for the lack of a furanic ring. O-ethylation is unusual in plants biosynthesis; therefore, compound 1 is an artifact from the extraction process with ethanol. To the best of our knowledge, α,β-unsaturated carbonyl groups function as potent Michael receptors, which can react with nucleophilic reagents, thus this compound might have potent affinity to a series of receptors or enzymes.

Conflicts of interests

All authors declare that there are no conflicts of interests and they affirm that this paper consists of original and unpublished work.

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