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단신 (Notes)

Diterpenoids from *Leonurus japonicus*

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Leonurus japonics 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, especially menstrual disturbances, dysmenorrhea, and amenorrhea.[1] Recently, phytochemical studies on this plant have been reported.[2–10] Our previous investigation on two plants of the family Lamiaceae resulted in the isolation of a number of new labdane-type diterpenes.[11,12] In our continuing research on secondary metabolites from *L. japonicus*, five diterpenoids including two new and three known ones were isolated and identified. The new compounds (*Fig.* 1) were elucidated as 1-((2S)-2-(3-(furan-3-yl)propanoyl)-2,6,6-trimethylcyclohexyl)-2-hydroxybutan-1-one (1), and 1-((1S,3R,3aS)-3-(2-(furan-3-yl)ethyl)-3-methoxy-3a,7,7-trimethyloctahydroisobenzo furan-1-yl)propan-1-one (2). The known compounds were identified as *seco*-labdane (3), 6b-hydroxy-15,16-epoxylabda-8,13(16), 14-trien-7-one (4), and leojaponin (5).

The ethanol extract of the powdered herb of *L. japonicus* was first separated by macroporious resin (D101) and then repeatedly separated by silica gel column chromatography, Sephadex LH-20, and preparative HPLC to give compounds **1**–**5**.

Compound **1** was obtained as a colorless oil, $[\alpha]_D^{20}$ +36.3 (*c* 0.1, MeOH), and was established to have a molecular formula of $C_{20}H_{30}O_4$ by HRESIMS ($[M + H]^+$ *m/z* 335.2178; calcd. 335.2222). The ^1H, ^{13}C NMR and DEPT data (*Table* 1) exhibited resonances for three tertiary methyl groups (δ_H 0.76, 0.87, 0.90; δ_C 29.3, 29.5, 25.6, respectively), two carbonyl groups (δ_C 215.7, 213.2), a monosubstituted furan (δ_H 7.33, 7.23, 6.25; δ_C 110.9, 123.8, 139.2, 142.8), five methylenes (δ_C 37.2, 34.2, 31.4, 26.7, 19.5, 19.1), two methines including one oxygenated (δ_H 3.58 s, 4.03 d J = 8.0 Hz; δ_C 53.8, 80.6), and two quaternary carbons (δ_C 34.5, 49.9). These spectroscopic data indicated **1** to be an 8,9-*seco*-labdane diterpenoid with a hydroxyl group (δ_H 3.44 s). The ^1H NMR data of **1** were similar to those of the lagopsin F[13] and 8,9-secohispanolone[14] except for the absence of the resonances of an acetoxy group and a replacement of carbonyl with a hydroxyl group in lagopsin F. However, carefully analysis of the H-H COSY spectra, the cross peak between the two protons of methines (H-5 and H-7) was not observed, and correlation from H-7 to H_2-8 (δ_H 1.30 m) was obviously detected. It suggested that the hydroxyl group was connected to C-7, rather than C-6 as lagopsin F. The proposed structure of **1** was further confirmed by the HMBC correlations between H_3-20 and C-1/C-5/C-9/C-10; H_3-18 and C-19/C-4/C-5; H_3-17 and C-7/C-8; H-16 and C-13/C-14/C-15; H-15 and C-13/C-14/C-16; H-14 and C-13/C-15/C-16; H_2-12 and C-9/C-11/C-13/C-14/C-16; H_2-11 and C-9/C-12/C-13; H-8 and C-7/C-17; H-5 and C-1/C-3/C-4/ C-6/C-7/C-9/C-10, and H-7 and C-5/C-6/C-8/C-17 (*Fig.* 2). However, the C-7 relative configuration could not be assigned. Therefore, the structure of **1** was identified as 1-((2S)-2-(3-(furan-3-yl)propanoyl)-2,6,6-trimethylcyclo-hexyl)-2-hydroxybutan -1-one, and named secoleojaponol. Compound **1** has not been previously reported.

Compound **2**, a colorless oil, $[\alpha]_D^{20}$ - 3.6 (*c* 0.1, MeOH) was shown to have a formula of $C_{21}H_{32}O_4$ by HRESIMS ($[M + H]^+$ *m/z* 349.2020; calcd. 349.2015). The NMR data (*Table* 1) showed that compound **2** has three tertiary methyl groups (δ_H 0.75, 1.04, 1.12; δ_C 33.6, 22.2, 17.0, respectively) and a methoxy group (δ_H 3.35, s; δ_C 48.8) a propanoyl group [δ_H 1.08 (3H, t, J = 8.0 Hz), 2.65 (2H, q, J = 8.0 Hz); δ_C 7.6,

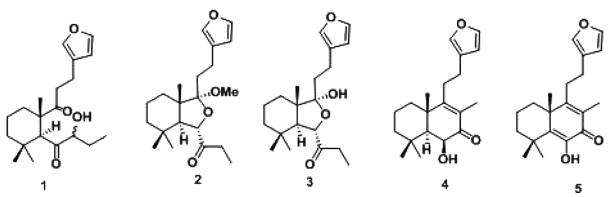

***Figure* 1.** Chemical structures of compounds **1**–**5**.

***Table* 1.** ^{13}C and ^{1}H NMR data of **1** and **2** ($CDCl_3$, *J* in Hz)

No.	1		2	
	δ_C	δ_H	δ_C	δ_H
1	31.4, t	1.94, m	30.7, t	1.89, m; 2.18, m
2	19.1, t	1.50, m	19.3, t	1.54, m
3	34.2, t	1.54, m	41.3, t	1.41, m; 1.10, m
4	34.5, s		32.3, s	
5	53.8, d	3.58, s	54.6, d	2.44, d (12.0)
6	213.2, s		83.3, d	4.24, d (8.0)
7	80.6, s	4.03, d (8.0)	213.5, s	
8	26.7, t	1.30, m	32.4, t	2.65, q (8.0)
9	215.7, s		110.5, s	
10	49.9, s		48.8, s	
11	37.2, t	2.75, m	30.7, t	1.92, m; 2.17, m
12	19.5, t	2.72, m	18.6, t	2.33, m; 2.46, m
13	123.8, s		124.5, s	
14	110.9, d	6.25, s	110.6, d	6.27, s
15	142.8, d	7.33, s	142.9, d	7.37, s
16	139.2, d	7.23, s	138.3, d	7.27, s
17	10.9, q	1.09, t (8.0)	7.6, q	1.08, t (8.0)
18	29.7, q	0.87, s	33.6, q	0.75, s
19	29.3, q	0.76, s	22.2, q	1.04, s
20	25.6, q	0.90, s	17.0, q	1.12, s
OMe			48.8, q	3.35, s

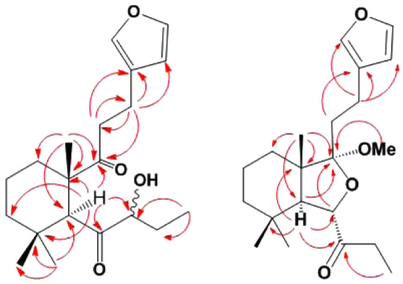

***Figure* 2.** HMBC correlations of compounds **1**–**2**.

30.7, 213.5], a monosubstituted furan (δ_H 7.37, 7.27, 6.27; δ_C 110.6, 124.5, 138.3, 142.9), five methylenes, two methines (one oxygenated). The ^{1}H and ^{13}C NMR data of **2** were very similar to those of the reported data of *seco*-labdane[15] except for an additional methoxyl group. Further 2D NMR experiment revealed that the chemical structure of **2** was in agreement with the hemiacetal hydroxyl group in *seco*-labdane replaced by a methoxyl group (*Fig*. 3), due to the HMBC correlations between between H_3-20 and C-1/C-5/C-9/C-10; H_3-17 and C-7/C-8; H_2-12 and C-9/C-11/C-13/C-14/C-16; H_2-11 and C-9/C-12/C-13; H-5 and C-4/C-6/C-7/C-10, H_3-methoxy and C-9. Therefore, the structure of 2 was elucidated as 1-((1S, 3R, 3aS)-3-(2-(furan-3-yl)ethyl)-3-methoxy-3a,7,7-trimethyloctahydroiso-benzofuran-1-yl)propan-1-

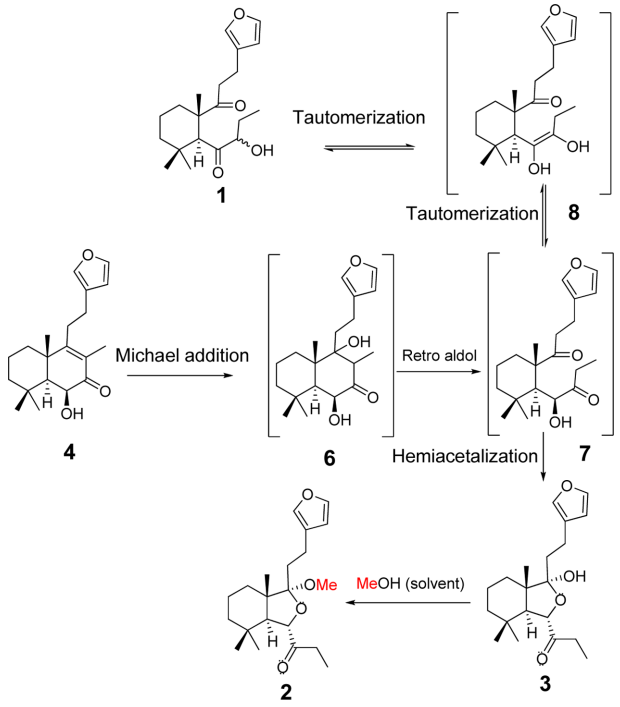

***Figure* 3.** Possible genesis pathways of the isolates.

one, and named methoxylsecolabdane. This compound has not been previously reported.

Compound **3** was isolated as a white gum. ^{1}H and ^{13}C NMR data of **3** were identical with those of reported data of *seco*-labdane.[15] So the chemical structure of compound **3** was elucidated as shown (*Fig*. 1).

Compound **4** was isolated as a white gum. The ^{1}H and ^{13}C NMR data of **4** were identical with those of reported data of 6b-hydroxy-15,16-epoxy-labda-8,13(16),14-trien-7-one.[15] Therefore, the chemical structure of compound **4** was elucidated as shown (*Fig*. 1).

Compound **5** was isolated as a yellowish oil. The ^{1}H and ^{13}C NMR data of 5 were identical with those of reported data of leojaponin.[2] Thus, the chemical structure of compound **5** was elucidated as shown (*Fig*. 1).

Compounds **3** was first isolated from *Galeopsis angustifolia*, and A. Rustraiyan[15] thought that it was formed *via* a retro aldol reaction starting from a derivative **6** of **4** having a tertiary hydroxyl group at C-9 and subsequent formation of the hemiacetal between a newly formed keto group at C-9 and the hydroxyl group at C-6 (*Fig*. 3). Rodriguez[16] observed the thermal cleavage of the single bond between C-8 and C-9 of hispanolone by heating at 190 °C for 10 min under N_2 and without solvent, and Hiroyuki Fuchino[17] also found the degradation of a similar compound in deuterated chloroform at 40 °C over 90 min. In addition Jun Li,[13] Rong-Tao Li,[14]

and Yuji Narukawa[18] also reported this kind of transformation. Considering the diversity of the isolates, compound **1** might be formed by tautomerization of the retro aldol reaction product **7**, and **2** was generated by the reaction of **3** with solvent (MeOH) during the evaporation procedure. In order to confirm the naturally presence of the secondary metabolite in the herb, it is suggested that the raw material should be collected freshly, and dried quickly by lyophilization, and all the extraction and isolation should be kept at a low temperature without using acidic and aprotic solvents.

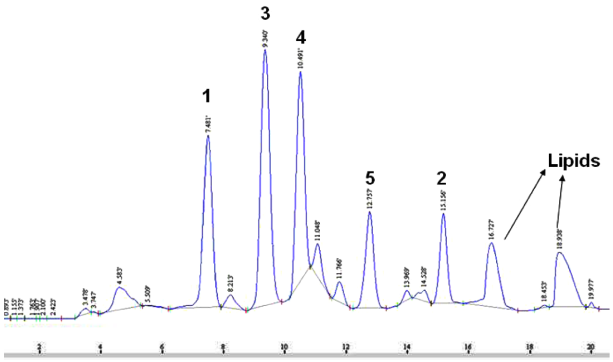

***Figure* 4.** HPLC separation of compounds **1**–**5**.

EXPERIMENTAL

Reagents and Instruments

NMR spectra were recorded on a Bruker Avance III spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. HRESIMS were measured with Waters UPLC-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 (25×1 cm) semi-preparative column packed with C18 (5 μm). All organic solutions used were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Methanol used for HPLC was of HPLC-grade and purchased from Fisher Scientific Company (Fair Lawn, NJ, USA).

Plant Material

The herb of *L. japonicus* 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: hkwu-aynu-20140301) was deposited at the Pharmaceutical Research Lab, Anyang Normal University.

Extraction and Isolation

The dried and powdered herb of *L. japonicus* (85 kg) was extracted with 95% EtOH (3 × 400 L) 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) to get subfraction C1. The further separation of C1 with Sephadex LH-20 (MeOH) to yield a mixture rich in diterpenes (total diterpenoids). The total diterpenoids sample was purified by preparative HPLC. The mobile phase was a linear gradient of methanol (A) and H_2O (B) as follows: methanol-water (methanol: 0–20 min, 85–98%; 20–22 min, 95–85%; 22–27 min, 85%). The flow-rate was 4.0 ml min^{-1}, the injection volume was 200 mL for each run and the effluent was monitored at 215 nm by a UV detector. Peaks were collected manually, and the retention times for the isolates were 7.4 min (**1**, 15 mg), 9.3 min (**3**, 46 mg), 10.4 min (**4**, 35 mg), 12.7 min (**5**, 27 mg), and 15.1 min (**2**, 18 mg) respectively. Identification of the HPLC peak fractions was performed by HRESIMS, ^{1}H and ^{13}C NMR, HMBC and HMQC technologies. The typical HPLC graph was shown in *Fig*. 4.

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Supporting Information. The ^{1}H and ^{13}C NMR, DEPT, HMQC, and HMBC spectra measured in $CDCl_3$ of new compounds **1**–**2** are provided.

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