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Bis-spirolabdane diterpenoids from *Leonurus japonicus* and their anti-platelet aggregative activity

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ABSTRACT

Six bis-spirolabdane diterpenoids along with four known analogues were isolated from the aerial parts of *Leonurus japonicus*. Their structures and absolute configurations were elucidated by spectroscopic analyses, single-crystal X-ray diffraction, and a modified Mosher's method. The inhibitory activity of the compounds against the abnormal increase in platelet aggregation induced by adenosine diphosphate was investigated. Only the (13*R*)-bis-spirolabdane diterpenoids exhibited a significant effect.

1. Introduction

Leonurus japonicus Houtt. (Labiatae) is widely distributed and cultivated in China, particularly in Anhui, Jiangsu, Sichuan, and Zhejiang provinces. The aerial parts of *L. japonicus*, "yi mu cao" in Chinese, are often used to treat various diseases such as blood stasis, menstrual disturbance, dysmenorrhea, and amenorrhea [1,2]. Previous investigations on *L. japonicus* (synonyms *Leonurus heterophyllus*) have led to the isolation of many diterpenoids, particularly the spirolabdane diterpenoids [3–7]. However, their anti-platelet aggregation activities in relation to the traditional use of *L. japonicus* have rarely been reported [8]. In our search for bioactive diterpenoids, the EtOAc extract of *L. japonicus* was phytochemically investigated, affording six new bis-spirolabdane diterpenoids (**1**–**6**) and four known analogues (**7**–**10**) (Fig. 1). Their inhibitory activities against platelet aggregation were found to depend on the absolute configuration of the C-13 position in these compounds.

2. Experimental

2.1. General

Optical rotations were measured using a Perkin-Elmer 341 plus. IR spectra were recorded using a Nicolet 5700 FT-IR microscope instrument. NMR spectra were obtained using a Bruker-AVIIIHD-600 spectrometer with the solvent peaks used as the references. HRESIMS spectra were measured using a Waters Synapt G2 HDMS. Column chromatography (CC) was performed using silica gel (200–300 mesh, Yantai Institute of Chemical Technology, Yantai, China), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical, Co., Japan), and Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden). HPLC separation was performed using an instrument equipped with a Cometro 6000LDS pump, a Cometro 6000PVW UV/VIS detector, and an Ultimate (250 × 10 mm^2) preparative column packed with C18 (5 μm). TLC was performed using glass precoated silica gel GF254 plates (Qingdao Marine Chemical Inc., Qingdao, China).

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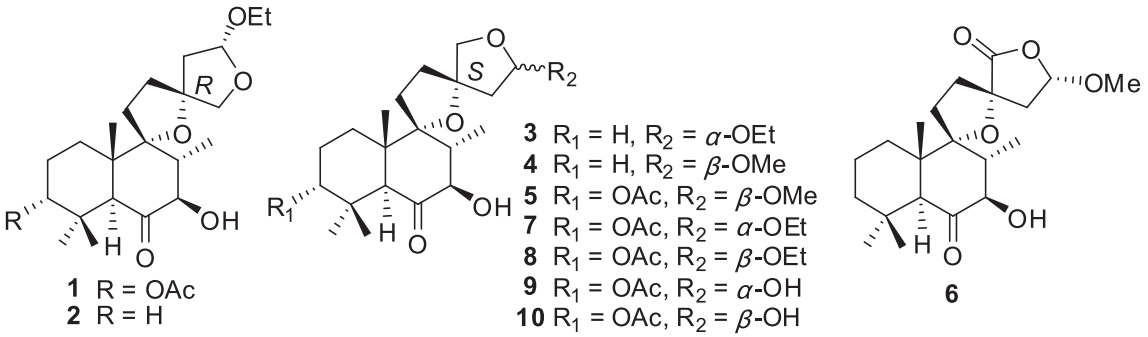

Fig. 1. Chemical structures of compounds **1**–**10**.

2.2. Plant material

The aerial parts of *L. japonicus* were collected in May of 2012 from a field in Wenjiang District, Chengdu City, Sichuan Province, China. The herb was identified by Prof. Min Li (Chengdu University of TCM, Sichuan, China). A voucher specimen (SYMC-0522) was deposited at the School of Pharmacy, Chengdu University of TCM, China.

2.3. Extraction and isolation

The air-dried aerial parts of *L. japonicus* (20 kg) were extracted with 95% EtOH (3 × 160 L) at room temperature for 3 × 72 h. The EtOH extract was evaporated under reduced pressure to afford a dark brown residue (1.2 kg). The residue was suspended in H_2O and successively partitioned into EtOAc (400 g) and *n*-BuOH (160 g) fractions. The EtOAc fraction was subjected to silica gel CC using a gradient elution of petroleum ether/acetone (100:1–0:1, v/v) to afford 19 fractions (F_1–F_{19}) based on TLC analyses. F_8 (8.2 g) was subjected to MCI with a gradient of increasing EtOH (50%–100%) in water to afford five subfractions ($F_{8\text{-}1}$–$F_{8\text{-}5}$). $F_{8\text{-}3}$ (2.1 g) was further separated by RP-MPLC affording seven parts, $F_{8\text{-}3\text{-}1}$–$F_{8\text{-}3\text{-}7}$. $F_{8\text{-}3\text{-}2}$ was purified by RP semipreparative HPLC (73% MeOH in H_2O) to afford mixed crystals **9** and **10** (2.8 mg). $F_{8\text{-}3\text{-}3}$ was purified via PTLC (petroleum ether/acetone, 5:1 v/v) followed by RP semipreparative HPLC (72% MeCN in H_2O) purification to afford **4** (1.5 mg, t_R = 35 min) and **6** (2.4 mg, t_R = 39 min). The separation of $F_{8\text{-}3\text{-}4}$ using Sephadex LH-20 (petroleum ether/$CHCl_3$/MeOH, 5:5:1 v/v/v) and RP semipreparative HPLC (76% MeCN in H_2O) successively afforded compounds **1** (13 mg, t_R = 42 min), **2** (16.5 mg, t_R = 26 min), **3** (9 mg, t_R = 29 min), **5** (1.6 mg, t_R = 47 min), **7** (15 mg, t_R = 55 min), and **8** (22 mg, t_R = 61 min).

(−)-(3*R*,5*S*,7*R*,8*R*,9*R*,10*S*,13*R*,15*R*)-3-Acetoxy-7-hydroxy-15-ethoxy-9,13;15,16-diepoxylabdan-6-one (**1**): Colourless crystals (acetone); $[\alpha]_D^{20}$ − 58.8 (*c* 0.10, MeOH); IR ν_{max} 3456, 2977, 2922, 2881, 1733, 1708, 1373, 1241, 1307, 904, 800 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data see Table 2; and (+)-HRESIMS m/z 461.2520 $[M + Na]^+$ (calcd. for $C_{24}H_{38}O_7Na$, 461.2515).

(−)-(5*S*,7*R*,8*R*,9*R*,10*S*,13*R*,15*R*)-7-Hydroxy-15-ethoxy-9,13;15,16-diepoxylabdan-6-one (**2**): Colourless oil; $[\alpha]_D^{20}$ − 92.2 (*c* 0.15, MeOH); IR ν_{max} 3471, 2978, 2924, 2873, 1707, 1472, 1391, 1368, 1324, 1269, 1111, 1041, 995, 951, 906 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data see Table 2; and (+)-HRESIMS *m/z* 403.2455 $[M + Na]^+$ (calcd. for $C_{22}H_{36}O_5Na$, 403.2460).

(+)-(5*S*,7*R*,8*R*,9*R*,10*S*,13*S*,15*R*)-7-Hydroxy-15-ethoxy-9,13;15,16-diepoxylabdan-6-one (**3**): Colourless oil; $[\alpha]_D^{20}$ + 114.9 (*c* 0.20, MeOH); IR ν_{max} 3466, 2979, 2925, 2872, 1707, 1468, 1391, 1368, 1324, 1256, 1107, 1044, 997, 952, 932 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data see Table 2; and (+)-HRESIMS *m/z* 403.2456 $[M + Na]^+$ (calcd. for $C_{22}H_{36}O_5Na$, 403.2460).

(−)-(5*S*,7*R*,8*R*,9*R*,10*S*,13*S*,15*S*)-7-Hydroxy-15-methoxy-9,13;15,16-diepoxylabdan-6-one (**4**): Colourless oil; $[\alpha]_D^{20}$ − 69.4 (*c* 0.05, MeOH); IR ν_{max} 3471, 2926, 2857, 1708, 1461, 1376, 1204, 1127, 1105, 1040, 992 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data see Table 2; and (+)-HRESIMS *m/z* 389.2310 $[M + Na]^+$ (calcd. for $C_{21}H_{34}O_5Na$, 389.2304).

(−)-(3*R*,5*S*,7*R*,8*R*,9*R*,10*S*,13*S*,15*S*)-3-Acetoxy-7-hydroxy-15-methoxy-9,13;15,16-diepoxylabdan-6-one (**5**): Colourless oil; $[\alpha]_D^{20}$ − 38.3 (*c* 0.04, MeOH); IR ν_{max} 3454, 2976, 2923, 2856, 1733, 1707, 1439, 1371, 1250, 1102, 1039, 989 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data see Table 2; and (+)-HRESIMS *m/z* 447.2366 $[M + Na]^+$ (calcd. for $C_{23}H_{36}O_7Na$, 447.2359).

(+)-(5*S*,7*R*,8*R*,9*R*,10*S*,13*S*,15*R*)-7-Hydroxy-15-methoxy-9,13;15,16-diepoxylabdan-6,16-dione (**6**): Colourless oil; $[\alpha]_D^{20}$ + 45.2 (*c* 0.05, MeOH); IR ν_{max} 3370, 2926, 2852, 1784, 1708, 1461, 1372, 1246, 1093, 1041, 937 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data see Table 2; and (+)-HRESIMS *m/z* 403.2094 $[M + Na]^+$ (calcd. for $C_{21}H_{32}O_6Na$, 403.2097).

2.4. X-ray crystallography data of compound *1*

$C_{24}H_{38}O_7$, M = 438.54, orthorhombic, $P2_12_12_1$, a = 10.8395(3) Å, b = 12.9176(4) Å, c = 17.2745(5) Å, V = 2418.76(12) $Å^3$, Z = 4, T = 293(2) K, μ(CuKα) = 0.713 mm^{-1}, D_{calcd} = 1.204 $g \cdot cm^{-3}$, 21,236 reflections measured (8.548 ≤ 2Θ ≤ 134.616), and 4323 unique (R_{int} = 0.0598, R_{sigma} = 0.0276), which were used in all calculations; final R_1 = 0.0483, wR_2 = 0.1428.

The data were collected using an Xcalibur Atlas Gemini ultra diffractometer with CuKα radiation. The crystal structures were solved by direct methods using the SHELXS-97 programme and refined anisotropically by least-squares method using the SHELXL-97 refinement package [9]. The absolute