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Leojaponic acids A and B, two new homologous terpenoids, isolated from *Leonurus japonicus*

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[ABSTRACT] The present study aimed at isolation and purification of the bioactive terpenoids from the herb of *Leonurus japonicus* by chromatographic separations such as silica gel, sephadex LH-20 and C_{18} reversed phase silica gel, as well as preparative HPLC. As a result, leojaponic acids A (**1**, $C_{17}H_{24}O_4$) and B (**2**, $C_{18}H_{26}O_4$), two homologous terpenoids, together with (−)-loliolide (**3**), 1-(3-ethylphenyl) ethane-1, 2-diol (**4**) and dibutyl phthalate (**5**), were isolated from the EtOH extract of *L. japonicus*. All the chemical structures of the isolates were elucidated on the basis of 1D and 2D NMR analyses. Compounds **1** and **2** were new terpenoids, and Compounds **3** and **4** were isolated and identified for the first time from this plant. In addition, the α-glucosidase and tyrosinase inhibitory activity of the new compounds were evaluated.

[KEY WORDS] *Leonurus japonicus*; Leojaponic acid; Terpenoid

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Introduction

Leonurus japonics Houtt. (Lamiaceae) is an annual or biennial herbaceous plant widely distributed and cultivated in China. The dried herb is used in Traditional Chinese medicine 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 of *Lamiaceae* resulted in the isolation of a number of new labdane diterpenes [11-13]. In our continuous research on secondary metabolites from *L. japonicus*, two terpenoids named as leojaponic acids A (**1**) and B (**2**), together with three different types of compounds (−)-loliolide (**3**), 1-(3-ethylphenyl) ethane-1, 2-diol (**4**) and dibutyl phthalate (**5**) were isolated and identified in the present study (Fig. 1). Interestingly, compounds **1** and **2** belonged to a homologous series with similar formula, varying by a -CH_2- unit, although their chemical structures resembled leojaponin with the same rings [2, 14]; the biogenesis of these compounds was still unknown. Herein, we present the isolation, structure elucidation, and bioactivity of the new compounds.

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Results and Discussion

Compound **1** was obtained as a pale yellow solid and its molecular formula was determined to be $C_{17}H_{24}O_4$ on the basis of the HRESIMS spectrum at *m/z* 291.159 7 $[M - H]^-$ (Calcd. 291.159 6), and which was also confirmed by analysis of 1D and 2D NMR spectra (Table 1). Seventeen carbon signals were observed, including five methylenes, and four tertiary methyl groups (including one overlapped carbon signal) as indicated by ^{1}H NMR and DEPT spectra (δ_H 1.40, 1.40, 1.41, 1.95, all *s*; δ_C 26.7, 26.9, 26.9, 10.2). There were six sp^2 carbons corresponding to two tetrasubstituted olefins (δ_C 141.0, 143.2, 126.8, 167.5), a conjugated ketone and carboxyl group (δ_C 179.6, 181.8). One of the tetrasubstituted alkenyl portions appeared to be part of an α, β-unsaturated ketone as revealed by the low field signals at 167.5, 181.8. It exhibited very similar chemical shifts of the ring A/B carbons to those of leojaponin [13]. In the HMBC spectrum,

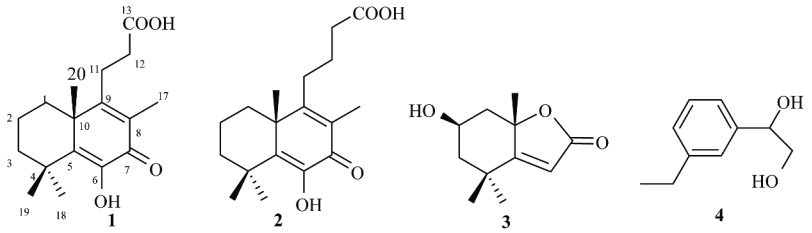

Fig. 1 Structures of compounds 1–4

correlations of H_3-17/C-7, C-8, and C-9; H_3-19 and H_3-20/C-3, C-4, and C-5; H_3-18, H_3-19 and H_3-20/C-5; H_3-20/C-5, C-9, C-10 confirmed the ring A/B structure. The ^{13}C NMR resonance at δ_C 179.6 and the relative polar property of compound 1 (20% MeOH in water as HPLC mobile phase) indicated that this compound must have a -COOH group on the structure, and its connection was determined by the observation of HMBC cross peaks between H_2-11 (δ_H 2.76 m) and carboxyl C-13 (δ_C 179.6) (Fig. 2). These results suggested that the -COOH would be at the end of an ethyl group, which joins the carboxyl group to a decalin skeleton of leojapoin, instead of a furanyl ring. The absolute configuration of the chiral carbon C-10 was proposed as *R* since all the labdanes of *Leonurus* genus up to date studied possess this configuration [2]. Therefore, the chemical structure of Compound **1** was elucidated as 3-(4-hydroxy-2, 5, 5, 8a-tetramethyl-3-oxo-3, 5, 6, 7, 8, 8a-hexahydronaphthalen-1-yl) propanoic acid, a new compound named as leojaponic acid A.

Table 1 ^1H (400 MHz) and ^{13}C NMR (100 MHz) data for compound 1 in MeOD (*J* in Hz)

Position	δ_H (mult., Hz)	δ_C
1	1.52 (m); 2.16 (m)	28.8
2	1.77 (m); 1.94 (m)	16.7
3	1.39 (m); 1.93 (m)	35.9
4		35.3
5		141.0
6		143.2
7		181.8
8		126.8
9		167.5
10		43.9
11	2.67 (m); 2.75 (m)	27.3
12	2.32 (m)	36.9
13		179.6
17	1.95 (s)	10.2
18	1.40 (s)	26.7
19	1.40 (s)	26.9
20	1.41 (s)	26.9

Compound **2** was also obtained as a pale yellow solid and its molecular formula was determined to be $C_{18}H_{26}O_4$ by the

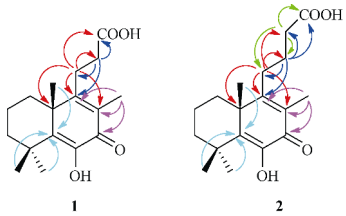

Fig. 2 Key HMBC correlations of compounds 1 and 2

HRESIMS spectrum at *m/z* 305.175 4 $[M - H]^-$ (Calcd. 305.175 3), and which was also confirmed by analysis of 1D and 2D NMR spectra (Table 2). Eighteen carbon signals (one overlaped) were observed including six methylenes, and four tertiary methyl groups as indicated by ^{13}C NMR and DEPT spectra. There were also six sp^2 carbons corresponding to two tetrasubstituted olefins (δ_C 141.0, 143.2, 126.8, 167.8), and the characteristic carbonyl of a cross-conjugated α, β-unsaturated ketone (δ_C 181.8) [2] and carboxyl group (δ_C 180.6) as those of Compound **1**. Considering the molecular

Table 2 ^1H (400 MHz) and ^{13}C NMR (100 MHz) data for compound 2 in MeOD (*J* in Hz)

Position	δ_H (mult., Hz)	δ_C
1	1.52 (m); 2.15 (m)	28.8
2	1.75 (m); 1.89 (m)	16.7
3	1.42 (m); 1.94 (m)	36.9
4		35.3
5		141.0
6		143.2
7		181.9
8		126.8
9		167.8
10		43.9
11	2.46 (m)	30.5
12	1.78 (m)	25.2
13	2.28 (t, J = 6.8)	38.1
14		180.6
17		10.3
18	1.94 (s)	26.7
19	1.39 (s)	27.0
20	1.39 (s)	27.0