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DENSISPICNINS A AND B, TWO UNUSUAL MONOTERPENES FROM *PEDICULARIS DENSISPICA* FRANCH.

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Abstract – Two unusual monoterpenes, densispicnins A (**1**), B (**2**), together with the four known monoterpenes, mussaenoside (**3**), yuheinoside (**4**), mussaenin A (**5**), argyol (**6**), were isolated from the whole plant of *Pedicularis densispica* Franch. The structures of **1** and **2** were elucidated mainly based on spectral data including 1D-, 2D-NMR (HSQC, HMBC, ROESY) and MS experiments.

INTRODUCTION

Genus *Pedicularis* L. comprises about 329 species in China,[1] in which some species have been used to treat diseases.[2] Many compounds were isolated from *Pedicularis,* including iridoids, phenylpropanoids and so on.[3,4,5] Among them, some compounds showed biological activities of antioxidation and antitumour.[6,7,8] In previous paper,[9] we have reported the new iridoid glycosides from *P. dolichocymba*. Herein we report on the isolation and structural elucidation of two new monoterpenes from *P. densispica* Franch, named densispicnins A (**1**) and B (**2**), together with four known monoterpenes mussaenoside (**3**), [10]yuheinoside (**4**),[11] mussaenin A (**5**),[12] argyol (**6**)[13] (Figure 1).

RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless solid. The molecular formula of **1** was determined to be $C_{10}H_{16}O_3$ by positive HR-ESI-MS (calcd. for $[M+Na]^+$: 207.0992; found: 207.0987). The IR spectrum (KBr) showed absorptions for hydroxyl groups (3421 cm^{-1}) and ether functions (1101, 1047 cm^{-1}). The ^{13}C NMR (DEPT) spectra (Table 1) of **1** showed signals for one methyl, four methylenes including two

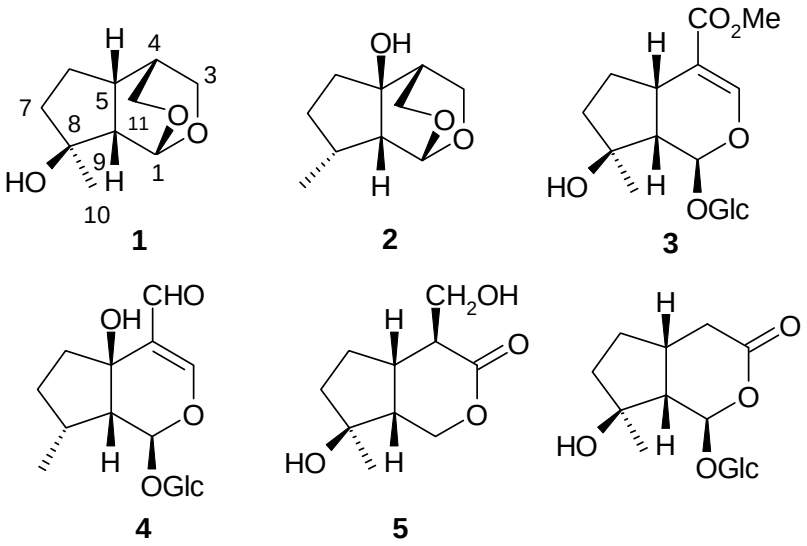


Figure 1 Structures of compounds **1**-**6**

bearing oxygen functions (δ_C 63.7, 68.6), four methines including one bearing oxygen function (δ_C 90.8) and one bearing oxygen function quaternary carbon atom (δ_C 82.0), which were further confirmed in the ^{1}H NMR spectrum. A total of ten carbons were observed, which is characteristic of a monoterpene skeleton. Consideration of the type of iridoids previously isolated from the Genus *Pedicularis* and the MS data, as well as the characteristic NMR spectra, allowed us to deduce the basic skeleton of **1** as a cyclopenta[*c*]pyran monoterpene. The structure of **1** could be determined by its HMBC spectrum (Figure 2), in which long-range correlations were observed between H-1 [δ_H 4.83 (d, J=1.5Hz)] and C-3, C-11, C-9; between H-4 (δ_H 2.72) and C-3, C-11, C-5, C-9; between H-5 (δ_H 1.75) and C-9, C-8; between H-7 (δ_H 1.68, 1.92) and C-8, C-9; between H-9 (δ_H 2.21) and C-1, C-4, C-7, C-8; between H-10 [δ_H 1.41 (s)] and C-7, C-8, C-9. The ROESY correlation of H-5 (δ_H 1.75) with H-9 (δ_H 2.21) suggested that the configuration of H-5 and H-9 were in accordance with those of natural iridoid compounds. However, the stereochemistry at C-8 of **1** could not be determined by ROESY experiment, but considering compound **1** and **3** were isolated from the same extract, the structure of **1** was supposed as shown in Figure 1, which was named densispicnin A.

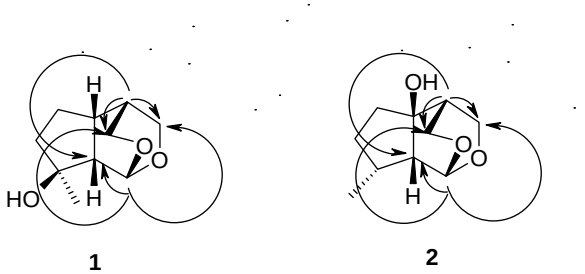


Figure 2 Key HMBC correlations of compounds **1** and **2**

Compound **2** was obtained as a colorless solid. The molecular formula of **2** was determined to be $C_{10}H_{16}O_3$ by positive HR-ESI-MS (calcd. for $[M+Na]^+$: 207.0992; found: 207.0990). The IR spectrum

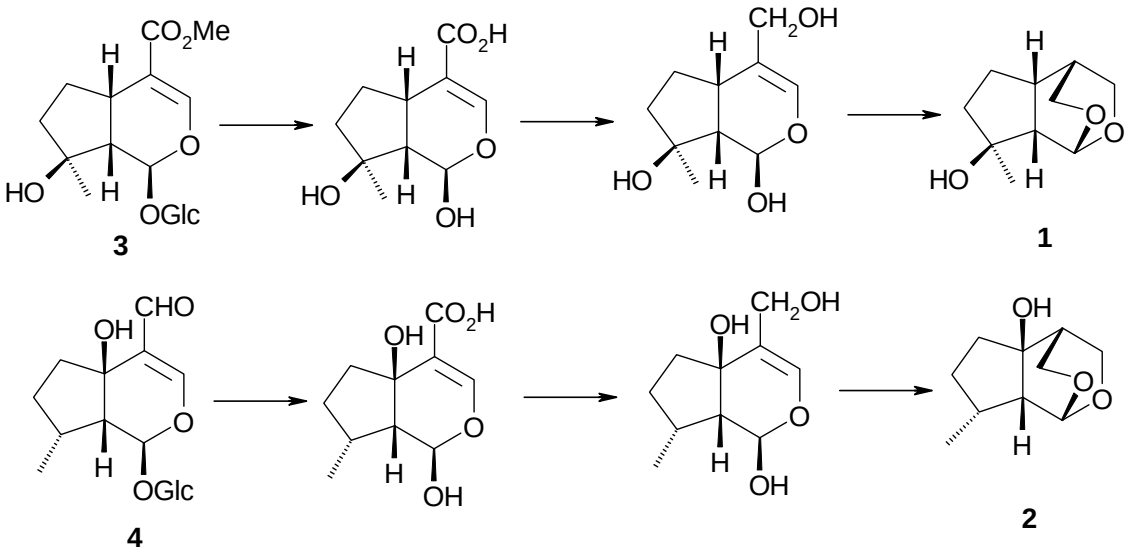
(KBr) showed absorptions for hydroxyl groups (3454 cm^{-1}) and ether functions (1057, 1017cm^{-1}). The ^1H, ^{13}C NMR (DEPT) spectra of **2** (Table 1) showed signals for one methyl, four methylenes including two bearing oxygen functions (δ_C 63.8, 64.8), four methines including one bearing oxygen function (δ_C 91.9) and one bearing oxygen function quaternary carbon atom (δ_C 79.2). Comparsion of The NMR spectra of **1** and **2** , incidating that these two compounds had the same skeleton, as supported by HMBC experiments. The HMBC (Figure 2) spectra of **2** demonstrated the following key correlations: H-1 [δ_H 4.87 (d, J=1.3Hz)] and C-3, C-11, C-9, C-5; H-4 (δ_H 2.41) and C-6, C-9; H-9 [δ_H 2.07 (d, J=8.8 Hz)] and C-1, C-5, C-7; H-10 [δ_H 1.04 (d, J=7.0Hz)] and C-7, C-8, C-9. According to HMQC, ^1H-^1H COSY and HMBC (Figure 2) experiments, the characteristic ^{13}C NMR signals at δ C15.1 was ascribable to C-10 of **2**. This was simliar to δ C15.9 at C-10 of **4**.[11] Therefore, the structure of **2** was supposed as shown in Figure 1, which was named densispicnin B.

Compound **1** and **2** has a rare acetal skeleton. From a biogenetic point view, the precursors of **1** and **2** may be **3** and **4**, respectively, which have been isolated from the same plant. A Plausible biogenetic pathway is shown in Scheme, in which hydrolysis, oxidation, reduction and condensation reaction are probably reponsible for the above transformation.

Table 1 ^1H- (400MHz) and ^{13}C-NMR (100MHz) data of **1** and **2** in $CDCl_3$ (δ in ppm, *J* in Hz)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	4.83(d, 1H, 1.5)	90.8(d)	4.87(d, 1H, 1.3)	91.9(d)
3	3.94(d,1H, 9.0) 4.02(m, 1H)	63.7(t)[a]	3.95(m, 1H) 4.01(dd, 1H, 1.6, 9.3)	64.8(t)[a]
4	2.72(m, 1H)	37.0(d)	2.41(m, 1H)	35.9(d)
5	1.75(m, 1H)	31.2(d)		79.2(s)
6	1.71(m, 2H)	27.4(t)	1.52(m, 1H) 1.86(m, 1H)	34.6(t)
7	1.68(m, 1H) 1.92(m, 1H)	40.6(t)	1.84(m, 1H) 2.21(dd, 1H, 7.7, 12.5)	40.3(t)
8		82.0(s)	1.81(m, 1H)	38.0(d)
9	2.21(m, 1H)	54.9(d)	2.07(d, 1H, 8.8)	55.9(d)
10	1.41(s, 3H)	24.5(q)	1.04(d, 3H, 7.0)	15.1(q)
11	3.87(dd, 1H, 3.3, 8.3) 4.10(dd, 1H, 2.9, 8.4)	68.6(t)[b]	3.92(dd, 1H, 2.5, 8.6) 4.35(dd, 1H, 3.2, 8.4)	63.8(t)[b]

*a and b = assigments can be reversed.



Scheme 1 Possible biosynthetic pathways of compounds **1** and **2** from compounds **3** and **4**

EXPERIMENTAL

General Experimental Procedures: Optical rotations were measured with a Perkin Elmer 241 polarimeter. IR spectrum was obtained on a Bio-Rad FTS-165 spectrophotometer with KBr pellets. UV spectrum was taken on a Spect 50-UV/Vis instrument (Analytic Jena AG). HR-MS were recorded on a Bruker APEX Ⅱ. 1D and 2D-NMR spectra were recorded on a Bruker AM-400 and a DRX-500 spectrometer with TMS as internal standard. Column chromatography was performed over Silica gel (200-300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co., Ltd., Sweden), respectively.

Plant Material: The plant material was collected in Zhong Dian, Yunnan Province of China in August 2004 and identified by Prof. Wang Hong, Kunming Institute of Botany, Chinese Academy of Sciences. The voucher specimen (KUN 0474455) was deposited in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation: The dried and powdered whole plant material (8 kg) of *P. densispica* was extracted three times with 95% EtOH under reflux, the residue was suspended in water and partitioned with petroleum ether, EtOAc, and *n*-BuOH, respectively. The EtOAc portion (52 g) was divided into 4 fractions (Frs 1-4) over silica gel column eluted with $CHCl_3$-MeOH (100:1) followed by increasing concentrations of MeOH. Fr.1 was separated further by column chromatography on silica gel and Sephadex LH-20 to give compounds **1** (8 mg) and **2** (7 mg). Fr.2 was separated by chromatography over MCI and silica gel to afford compounds **5** (32 mg) and **6** (13 mg). Compounds **3** (130 mg) and **4** (104 mg) were obtained from Fr.4 by HPLC (Zorbax ODS-C18, MeOH-H_2O, 2:8).

Densispicnin A (1): colorless solid; $[\alpha]_D^{20}$ -7° (*c* 0.20, MeOH); UV λ_{max}^{MeOH} nm (log ε): 204 (2.20); IRν_{max}^{KBr} cm^{-1}: 3421，2919，2850，1627，1334， 1101，1047; Positive FAB-MS *m/z*: 185 $[M+H]^+$; HR positive ESI-MS *m/z*: $[M+Na]^+$ 207.0987 (Calcd for $C_{10}H_{16}O_3Na$: 207.0992); ^1H and ^{13}C NMR spectral data see Table 1.

Densispicnin B (2): colorless solid; $[\alpha]_D^{20}$-5° (*c* 0.98, MeOH); UV λ_{max}^{MeOH} nm (log ε): 205 (2.02); IRν_{max}^{KBr} cm^{-1}: 3454，2950，2878，1717，1636，1130，1106，1057，1017，838; Positive FAB-MS *m/z*: 185 $[M+H]^+$; HR positive ESI-MS *m/z*: $[M+Na]^+$ 207.0990 (Calcd for $C_{10}H_{16}O_3Na$: 207.0992); ^1H and ^{13}C NMR spectral data see Table 1.

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