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SABINAPERINS A AND B, TWO NEW LIGNANS FROM *JUNIPERUS SABINA*

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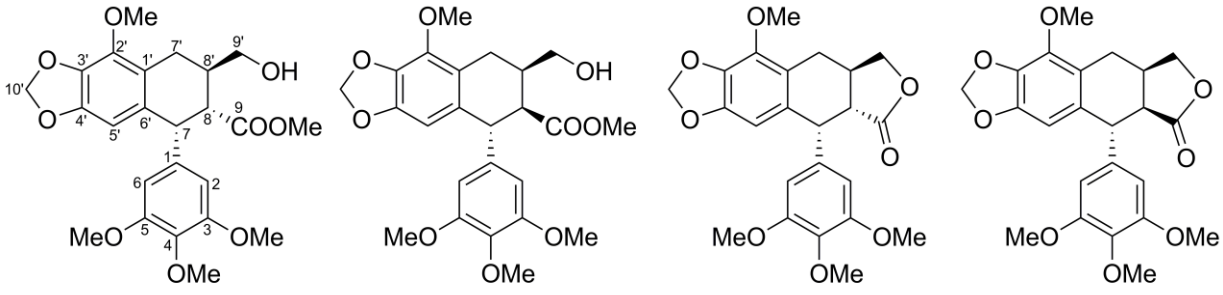
Abstract – Two new lignans, sabinaperins A (**1**) and B (**2**), have been isolated from the aerial part of *Juniperus sabina* L. together with six known lignans. Their structures were established on the basis of 1D and 2D-NMR data analysis.

There are ten species of *Juniperus* (Cupressaceae) which are growing in Kazakhstan[1] and many interesting compounds including sesquiterpenes, diterpenes, and lignans, have been isolated from *Juniperus* species.[2] *Juniperus sabina* L. distributed in the Altay mountains has been used in Kazakh traditional folk medicine for the treatment of various diseases such as nephritis, cystitis, rheumatoid arthritis, lumbago, leg pain, brucellosis, skin disease, and disinfections.[3] In our search for structurally and biologically interesting natural products, two new lignans, sabinaperins A and B (**1** and **2**), were isolated from *J. sabina* together with known related lignans, β-peltatin A methyl ether (**3**),[4] β-peltatin B methyl ether (**4**),[5] 3-*O*-demethylyatein,[6] acetyl epipodophyllotoxin,[4] epipodophyllotoxin ethyl ether,[6] and (-)-dihydrosesamin.[7] In this paper we describe the isolation and structure elucidation of **1** and **2**.



[†]Dedicated to Prof. Dr. Albert Padwa, Emory University on the occasion of the 75th birthday.

Sabinaperin A (**1**), colorless solid, possessed a molecular formula $C_{24}H_{28}O_9$, which was established by HRESITOFMS [*m/z* 483.1641, $(M+Na)^+$, Δ +1.0 mmu]. The IR absorption bands were characteristic of hydroxy (3400 cm^{-1}) and ester carbonyl (1730 cm^{-1}) groups. The ^1H NMR spectrum of **1** showed the presence of five methoxy (δ_H 3.75 × 2, 3.80, 3.64, and 4.05), three aromatic [δ_H 6.13 (2H) and 6.16 (1H)], and methylenedioxy protons [δ_H 5.86 (2H, s)] as well as seven aliphatic protons [δ_H 4.36 (1H), 2.96 (1H), 2.45 (1H), 3.10 (1H), 2.36 (1H), and 3.72 (2H)] (Table 1). The gross structure of **1** was deduced from detailed analyses of two-dimensional NMR data, including ^1H–^1H COSY, HSQC, and HMBC spectra in $CDCl_3$ (Figure 1). The ^1H–^1H COSY and HSQC spectra revealed the presence of a partial structure (C-7–C-8 and C-7'–C-9') as shown in Figure 1. The connectivity between two aromatic rings and this partial structure was revealed by the HMBC correlations of H-7' to C-1' (δ_C 120.5) and H-7 to C-1', C-6' (δ_C 130.8), C-5' (δ_C 103.2), and C-1 (δ_C 137.5), indicating the presence of two C6-C3 units linked together which is characteristic for lignan. In HMBC spectrum, the presence of a 1,3-benzodioxole skeleton was deduced by the cross peaks from H-10' to C-3' and C-4'. The HMBC correlations of 2'-OMe to C-2' confirmed the attachment of a methoxy group at C-2'. The HMBC correlations of 3-OMe and 5-OMe to C-3 and C-5 (δ_C 153.0) and of 4-OMe to C-4 (δ_C 137.1) established the connection between three methoxy units and an aromatic ring. Further analysis of the ^1H, ^{13}C and 2D-NMR data indicated the structure of **1** as methyl 2'-methoxy deoxypodophyllotoxinate.[8] Indeed, except for the appearance of a methoxy signal (δ_H 4.05) in place of an aromatic proton signal and an up-field shift of H-7'a signal (δ_H 2.45), ^1H NMR data of **1** is similar to those of methyl deoxypodophyllotoxinate.[8] The coupling constant of H-7 (d, 5.4 Hz) and H-8 (dd, 11.4, 5.4 Hz) served to establish the orientation of H-7, H-8 and H-8' to be α, α, and β, respectively.[8,9]

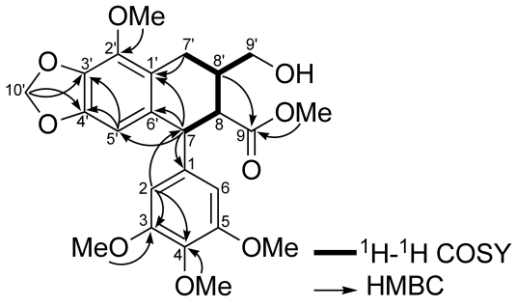


Figure 1. Selected 2D NMR Correlations of Sabinaperins A (**1**) and B (**2**)

Sabinaperin B (**2**), colorless solid, possessed a molecular formula $C_{24}H_{28}O_9$ as established by HRESITOFMS [*m/z* 483.1634, $(M+Na)^+$ Δ +1.0 mmu]. ^1H NMR spectrum of **2** is similar to that of **1** (Table 1), with the coupling constant of H-8 (δ_H 3.06, 1H, 5.5, 3.7 Hz) as the main differences between **1** and **2**, suggesting their diastereomeric relationship. Furthermore, ^1H NMR data of **2** is similar to those

of an epimer at C-8 of methyl deoxypodophyllotoxinate,[8] except for the appearance of a methoxy signal (δ_H 4.03) in place of an aromatic proton signal and an up-field shift of H_2-7' signals (δ_H 2.64 and 2.82), which indicates the structure of **2** as an epimer at C-8 of **1**. As in the case of **1**, the planar structure of **2** was confirmed by analysis of the 2D-NMR data (Figure 1), and the relative structure with *cis* configuration between H-8 and H-8' was deduced from ^{1}H NMR coupling constant (3.7 Hz) of H-7/H-8, and also by a NOESY correlation between H-7 and H-8.

Table 1. ^{1}H and ^{13}C NMR Data for Sabinaperins A and B (**1** and **2**) in $CDCl_3$ at 300K[a]

	1		**2**		
Position	δ_H	δ_C	δ_H	δ_C	HMBC (^{1}H)
1		137.5		140.5	2, 6, 7
2	6.13(1H, s)	106.7	6.25(1H, s)	106.3	6, 7
3		153.0		153.0	2, 6, 3-OMe
4		137.1		137.0	2, 6, 4-OMe
5		153.0		153.0	2, 6, 5-OMe
6	6.13(1H, s)	106.7	6.25(1H, s)	106.3	2, 7
7	4.36(1H, d, 5.4)	48.0	4.36(1H, d, 5.5)	46.1	2, 6, 5', 8
8	2.96(1H, dd, 11.4, 5.4)	48.5	3.06(1H, dd, 5.5, 3.7)	49.0	7', 8'
9		174.5		174.4	7, 8', 9-OMe
3,5-OMe	3.75(6H, s)	56.1	3.77(6H, s)	56.2	
4-OMe	3.80(3H, s)	60.8	3.82(3H, s)	60.8	
CO_2Me	3.64(3H, s)	51.4	3.61(3H, s)	51.8	
1'		120.5		120.3	7, 5', 7'
2'		140.0		140.2	2'-OMe
3'		134.4		134.2	10'
4'		147.4		147.5	10'
5'	6.16(1H, s)	103.2	6.13(1H, s)	103.8	7
6'		130.8		130.2	7, 8
7'a	2.45(1H, dd, 17.0, 11.0)	26.5	2.64(1H, dd, 17.5, 8.0)	24.6	8, 9'
7'b	3.10(1H, dd, 17.0, 5.0)		2.82(1H, dd, 17.5, 6.0)		
8'	2.36(1H, m)	32.4	2.37(1H, m)	35.1	7, 8
9'a	3.72(2H, m)	66.1	3.68(1H, m)	64.3	7'
9'b			3.75(1H, m)		
10'a	5.86(2H, s)	100.7	5.84(1H, s)	100.6	
10'b			5.86(1H, s)		
2'-OMe	4.05(3H, s)	59.3	4.03(3H, s)	59.3	

It is well known that β-peltatin A methyl ether (**3**) is converted irreversibly to β-peltatin B methyl ether (**4**) by alkaline epimerization.[10] Heating each of **1** and **2** in methanol at 60 °C for 12 h gave **4** at a different yield. The conversion rates indicated that **2** is more stable than **1**. However, sabinaperins A and B (**1** and **2**) could not be obtained from **3** and **4** by treatment of acid or base in methanol.[8,10]

The CD spectra of **1** ($[\theta]_{206}$ -20659, $[\theta]_{247}$ +3536, and $[\theta]_{273}$ -1718) and **2** ($[\theta]_{205}$ -38783, $[\theta]_{216}$ +15847, and $[\theta]_{245}$ +6505) showed Cotton effects similar to those of **3** ($[\theta]_{207}$ -124438, $[\theta]_{245}$ +23958, and

$[\theta]_{275}$ -5045) and **4** ($[\theta]_{200}$ -32377, $[\theta]_{214}$ +36318, and $[\theta]_{242}$ +6464), respectively. Additionally, the CD of **4** which was obtained from the conversion of **1** and **2** is identical to **4**. Therefore the absolute configuration of **1** and **2** were determined to be 7*R*, 8*R*, 8'*R* and 7*R*, 8*S*, 8'*R*, respectively.

EXPERIMENTAL

General Experimental Procedures. CD spectra were measured on a JASCO J-820 spectropolarimeter, and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. ^{1}H and 2D NMR spectra were recorded on a JEOL ECA600 and Bruker AV 600 spectrometers, and chemical shifts were referenced to the residual solvent peaks (δ_H 7.26 and δ_C 77.0 for $CDCl_3$). Standard pulse sequences were employed for the 2D NMR experiments. High-resolution ESI MS were obtained on a LTQ Orbitrap XL (Thermo Scientific). HPLC was performed on a CAPCELL PAK C_{18} MG-II, 5 μm (ϕ10 x 250 mm).

Plant Material. The aerial part of *Juniperus sabina* L. was collected in Altay mountain (Xinjiang region, PRC) in 2009. The botanical identification was made by pharmacist Bahargul Konirhan, Institute of Medicine Inspection Department of Altay City, Xinjiang, China.

Extraction and Isolation. The aerial part of *J. sabina* (2 kg) was extracted with 70% EtOH, in which 100g of the extract was dissolved in water and then partitioned with *n*-hexane, $CHCI_3$, and *n*-BuOH successively. The *n*-hexane fraction was subjected to a silica gel column chromatography (elution, hexane/EtOAc 1:0 to 1:0) to obtain 15 fractions and the fraction (868.5 mg) which was eluted by n-hexane/EtOAc (1:1) was further separated by silica gel column (toluene/EtOAc 1:0 to 1:1). Fraction (25.7 mg) eluted by toluene/EtOAc (8:2) obtained from the previous column was separated by ODS column (9:1-1:9, H_2O:MeOH) then further purified by an ODS HPLC column (MeOH/H_2O) to afford sabinaperin A **1** (0.5 mg, 0.00014%) and sabinaperin B **2** (0.5 mg, 0.0002%) as colorless solid together with known related lignans, β-peltatin A methyl ether **3**, β-peltatin B methyl ether **4**, 3-*O*-demethylyatein, acetyl epipodophyllotoxin, epipodophyllotoxin ethyl ether, and (-)-dihydrosesamin.

Sabinaperin A 1, colorless solid, IR (KBr) ν_{max} 3400 and 1730 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); HRESITOFMS *m*/*z* 483.1641 (M+Na; calcd for $C_{24}H_{28}O_9Na$, 483.1631). UV (MeOH) λ_{max} 204 (ε 16000) and 283 (1000) nm; CD (MeOH) $[\theta]_{206}$ -20659, $[\theta]_{247}$ +3536, and $[\theta]_{273}$ -1718.

Sabinaperin B 2, colorless solid, IR (KBr) ν_{max} 3400 and 1730 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); HRESITOFMS *m*/*z* 483.1634 (M+Na; calcd for $C_{24}H_{28}O_9Na$, 483.1631). UV (MeOH) λ_{max} 203 (ε 29700) and 280 (1200) nm; CD (MeOH) $[\theta]_{205}$ -38783, $[\theta]_{216}$ +15847, and $[\theta]_{245}$ +6505.

Chemical Conversion of 1 and 2 into 4. Each of the solution of sabinaperin A (**1**, 0.30 mg) and sabinaperin B (**2**, 0.30 mg) in MeOH (0.2 mL) was kept at 60 °C for 12 h. After evaporation, each residue was applied to a silica gel column (toluene/EtOAc, 4:1) separately to give a compound (0.11 mg

from **1**; 0.20 mg from **2**), whose spectroscopic data were identical to that of β-peltatin B methyl ether (**4**).

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