

HETEROCYCLES, Vol. 84, No. 2, 2012, pp. 1259 - 1263. © 2012 The Japan Institute of Heterocyclic Chemistry
 Received, 7th June, 2011, Accepted, 30th June, 2011, Published online, 14th July, 2011
 DOI: 10.3987/COM-11-S(P)30

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

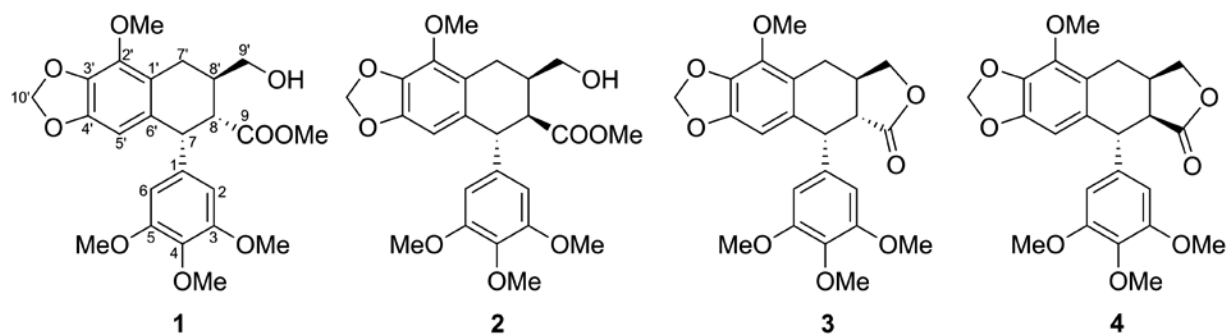
SABINA

Jenis Janar,^{a,b} Alfarius Eko Nugroho,^a Yusuke Hirasawa,^a Bahargul Konirhan,^c and Hiroshi Morita^{a,*}

^aFaculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41 Shinagawa-ku, Tokyo 142-8501, Japan, ^bDepartment of Organic Chemistry and Natural Compound Chemistry, Al-Farabi Kazakh National University, al-Farabi ave.71, Almaty 050038, Kazakhstan, ^cInstitute of Medicine Inspection Department of Altay City, Xinjiang, China

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 Kazakhstan¹ 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*-demethyleatein,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, $(M+Na)^+$, $\Delta +1.0$ 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 \times 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}

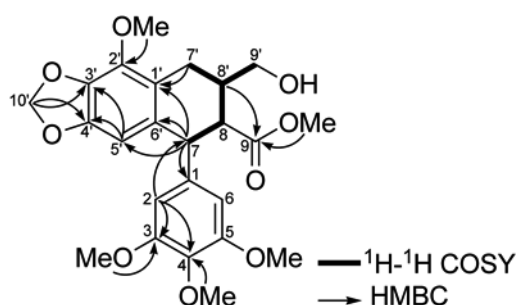


Figure 1. Selected 2D NMR Correlations of Sabinaperins A (**1**) and B (**2**)

Sabinaperin B (**2**), colorless solid, possessed a molecular formula $C_{24}H_{28}O_9$ as established by HRESITOFMS [m/z 483.1634, $(M+Na)^+$ $\Delta +1.0$ 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 H₂-7' 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 ¹H 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. ¹H and ¹³C NMR Data for Sabinaperins A and B (**1** and **2**) in CDCl₃ at 300K^a

Position	1		2		HMBC (¹ H)
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	
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 ₂ Me	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 *7R*, *8R*, *8'R* and *7R*, *8S*, *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. ^1H 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 ($\phi 10 \times 250 \text{ mm}$).

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, CHCl_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*-demethyleatein, acetyl epipodophyllotoxin, epipodophyllotoxin ethyl ether, and (-)-dihydrosesamin.

Sabinaperin A 1, colorless solid, IR (KBr) ν_{max} 3400 and 1730 cm^{-1} ; ^1H and ^{13}C NMR data (Table 1); HRESITOFMS m/z 483.1641 ($\text{M}+\text{Na}$; calcd for $\text{C}_{24}\text{H}_{28}\text{O}_9\text{Na}$, 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} ; ^1H and ^{13}C NMR data (Table 1); HRESITOFMS m/z 483.1634 ($\text{M}+\text{Na}$; calcd for $\text{C}_{24}\text{H}_{28}\text{O}_9\text{Na}$, 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 $^{\circ}\text{C}$ 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**).

ACKNOWLEDGEMENTS

This work was supported by a grant from Tokyo Biochemical Foundation and Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and a grant from the Open Research Center Project.

REFERENCES

1. M. C. Baytenov, *Flora of Kazakhstan*, Almaty, Vol. 1, 1963, 71.
2. (a) R. P. Adams, *Biochem. Syst. Ecol.*, 1999, **27**, 709; (b) M. L. A. Seca and M. S. A. Silva, *Recent Progress in Medicinal Plants*, 2007, **16**, 401; (c) J. D. Pascual, A. S. Feliciano, and J. M. Miguel Del Corral, *Phytochemistry*, 1983, **22**, 300.
3. X. Xu, K. Bahargul, B. Hang, and X. G. Jia, *The Pharmacography of Kazakh Medicinal Plants*, The Ethnic Press, Beijing, 2009, 357.
4. A. S. Feliciano, J. M. M. Del Corral, and M. Gordaliza, *Phytochemistry*, 1989, **28**, 659.
5. A. S. Feliciano, J. M. M. Del Corral, M. Gordaliza, and A. Castro, *Phytochemistry*, 1990, **29**, 1335.
6. A. S. Feliciano, J. M. M. Del Corral, M. Gordaliza, and M. A. Castro, *Phytochemistry*, 1991, **30**, 3483.
7. (a) A. S. Feliciano, J. M. M. Del Corral, M. Gordaliza, and M. A. Castro, *Magn. Reson. Chem.*, 1993, **31**, 868; (b) L. Guangze, T. Xuan, F. Ruihong, F. Juntao, and Zh. Xing, *Nongyaoxue Xuebao*, 2005, **7**, 364.
8. A. S. Feliciano, J. M. M. Del Corral, J. L. Lopez, and Pascual-Teresa, *Phytochemistry*, 1992, **31**, 267.
9. M. Gordaliza, A. Castro, J. M. M. Del Corral, M. L. Lopez-Vazquez, and A. S. Feliciano, *Tetrahedron*, 1997, **53**, 15743.
10. (a) J. L. Harwell, A. W. Schrecker, and G. Y. Greenberg, *J. Am. Chem. Soc.*, 1952, **74**, 6285; (b) A. Akahori, F. Yasuda, M. Ando, K. Mori, and T. Okanishi, *Chem. Pharm. Bull.*, 1972, **20**, 1150; (c) H. F. Stahelin and A. von Wartburg, *Cancer Res.*, 1991, **51**, 5; (d) M. Kuhn and A. von Wartburg, *Experientia*, 1963, **19**, 391; (e) J. Renz, M. Kunhn, and A. von Wartburg, *Justus Liebigs Ann. Chem.*, 1965, **681**, 207.