NEW LIGNANS FROM *Syringa reticulata* Var. *mandshurica*

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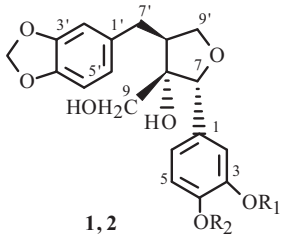
In the search for platelet-activating-factor (PAF) antagonists, two new lignan compounds were isolated from the leaves of Syringa reticulata *Hara var.* mandshurica*. Their structures were elucidated as (7*R*,8*S*, 8′*S*)-3,4,3′,4′-dimethylenedioxy-8,9-dihydroxy-8.8′, 7-*O*-9′-lignan (mandshuricol A) and (7*R*,8*S*,8′*S*)-3′,4′-methylenedioxy-4-methoxy-3,8,9-trihydroxy-8.8′, 7-*O*-9′-lignan (mandshuricol B), Mandshuricol A and B showed antagonistic activity on PAF in the [^{3}H] PAF receptor binding assay with IC_{50} values of 4.8×10^{-5} M and 3.5×10^{-5} M, respectively.*

Keywords: *Syringa reticulata* var. *mandshurica*, lignan, PAF antagonistic activity.

Syringa reticulata Hara var. *mandshurica* is a dwarfish tree found in northeastern and northern China. The leaves of this plant species are used as an antibiotic, to eliminate inflammation, and as an antiviral in folk medicine [1, 2]. During the course of our investigation for platelet-activating-factor (PAF) antagonists from the EtOAc extract of the leaves of *Syringa reticulata* var. *mandshurica*, two new oleaginous compounds, (7*R*,8*S*,8′*S*)-3,4,3′,4′-dimethylenedioxy-8,9-dihydroxy-8.8′, 7-*O*-9′-lignan [mandshuricol A (**1**)] and (7*R*,8*S*, 8′*S*)-3′,4′-methylenedioxy-4-methoxy-3,8,9-trihydroxy-8.8′, 7-*O*-9′-lignan [mandshuricol B (**2**)], were obtained. We now report the isolation and structural elucidation of the two new compounds, as well as the evaluation of their PAF antagonist activity.



1, 2

1: $R_1R_2 = CH_2$; **2:** $R_1 = CH_3$, $R_2 = H$

Mandshuricol A (1) was obtained as a colorless oil and has a molecular ion peak at 372.1203 in its HR-EI-MS, corresponding to the formula $C_{20}H_{20}O_7$. The EI-MS shows characteristic fragmentation patterns at *m/z* 135 and 149 arising from benzyl or tetrahydrofuran ring cleavage [3]. The IR spectrum of compound **1** suggested the presence of hydroxyl group (s) (3465, 1115, and 1090 cm^{-1}), and phenyl ring (s) (1601, 1580, and 870 cm^{-1}). Compound **1** gave a positive Labat test, indicating the presence of one or two dimethylenedioxy groups.

The proton signals at δ 6.78 (1H, dd, J = 8.05, 1.50 Hz, H-6), 6.74 (1H, d, J = 7.92 Hz, H-5), and 6.80 (1H, d, J = 1.52 Hz, H-2) and the proton signals at δ 6.65 (1H, dd, J = 8.00, 1.51 Hz, H-6′), 6.70 (1H, d, J = 7.98 Hz, H-5′), and 6.71 (1H, d, J = 1.52 Hz, H-2′) in the ^{1}H NMR spectrum of compound **1** (Table 1) indicated the presence of two 1,3,4-trisubstituted phenyl rings, while the two 3,4-dimethylenedioxy groups in the structure were indicated by the proton signals at δ 5.90 (2H, s) and 5.88 (2H, s).

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TABLE 1. ^{1}H and ^{13}C NMR Spectral Data of Compounds **1** and **2** (CD_3OD, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		136.2		135.3
2	6.80 (d, J = 1.52)	109.0	6.88 (d, J = 1.85)	110.5
3		149.5		150.8
4		149.2		150.2
5	6.74 (d, J = 7.92)	110.3	6.76 (d, J = 8.18)	111.3
6	6.78 (dd, J = 8.05, 1.50)	121.1	6.80 (dd, J = 8.22, 1.88)	119.6
7	4.75 s	91.3	4.78 s	91.5
8		83.4		83.2
9	3.22 (d, J = 11.30)	67.3	3.24 (d, J = 11.50)	
	3.06 (d, J = 11.30)		3.07 (d, J = 11.50)	67.6
1′		134.6		134.4
2′	6.71 (d, J = 1.52)	108.3	6.71 (d, J = 1.55)	108.1
3′		148.7		148.9
4′		147.6		147.5
5′	6.70 (d, J = 7.99)	109.4	6.69 (d, J = 8.10)	109.2
6′	6.65 (dd, J = 8.00, 1.51)	122.8	6.63 (dd, J = 8.06, 1.53)	122.6
7′	2.94 (dd, J = 13.55, 4.06)	34.4	2.96 (dd, J = 13.02, 4.02)	
	2.56 (dd, J = 13.55, 11.05)		2.56 (dd, J = 13.05, 11.00)	34.7
8′	2.48 m	47.8	2.50 m	47.6
9′	3.99 (dd, J = 8.05, 7.80)	73.9	4.00 (dd, J = 8.02, 7.80)	
	3.74 (dd, J = 8.08, 7.82)		3.75 (dd, J = 8.05, 7.80)	74.5
-OCH_2O-	5.90 s	102.6		
	5.88 s	102.4	5.88 s	102.2
OMe			3.85 s	55.6

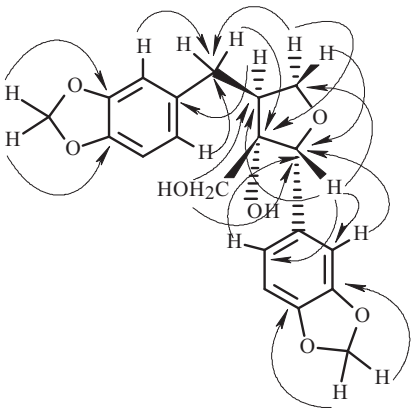

Fig. 1. Key HMBC correlations of compound **1**.

The ^{1}H NMR spectrum also showed three methylene groups with nonequivalent protons; among them, one was attributed to H-9′at δ 3.99 (1H, dd, J = 8.05, 7.80 Hz) and 3.74 (1H, dd, J = 8.08, 7.82 Hz), another was attributed to H-9 at 3.22 (1H, d, J = 11.30 Hz) and 3.06 (1H, d, J = 11.30 Hz), and the third was attributed to H-7′ at 2.94 (1H, dd, J = 13.55, 4.06 Hz) and 2.56 (1H, dd, J = 13.55, 11.05 Hz), which exhibited HMQC correlations with C-9′ at δ 73.9, C-9 at δ 67.3, and C-7′ at δ 34.4, respectively. Additionally, the multiplets at δ 2.48 (1H, m, H-8′) and the singlet at δ 4.75 (1H, s, H-7), showing HMQC correlations with carbon signals at δ 47.8 and 91.3, were attributed to the methine protons at C-8′ and C-7, respectively.

In comparison with the ^{1}H, ^{13}C NMR, and EI-MS spectral data of vladinol B [4], the structure of **1** was proposed to be 3,4,3′,4′-dimethylenedioxy-8,9-dihydroxy-8,8′,7-*O*-9′-lignan. The structure of **1** was also supported by its HMBC spectrum (Fig. 1), which showed the correlations of the dimethylenedioxy proton signals at δ 5.90 to C-3 (δ 149.5) and C-4 (149.2), and dimethylenedioxy proton signals at δ 5.88 to C-3′ (148.7), and C-4′ (147.6). The above data show that compound **1** has the same planar structure as that of dihydropaulownin [5]; however, the differences of optical rotation and ^{1}H NMR spectral data between them suggested that they have a different stereochemistry.

TABLE 2. PAF Binding Inhibition Activity of Compounds Mandshuricol A and B and Ginkgolide B

Concentration, μM	Binding inhibition, %	
	mandshuricol A (**1**)	mandshuricol B (**2**)
100	73.4	83.6
50	59.4	70.7
25	30.1	40.2
12.5	8.1	12.1

Mandshuricol A (**1**): $IC_{50} = 4.8 \times 10^{-5}$ M; mandshuricol B (**2**): $IC_{50} = 3.5 \times 10^{-5}$ M; ginkgolide B: $IC_{50} = 1.5 \times 10^{-7}$ M.

The relative stereochemistry of compound **1** was further established from NOESY interactions. The interaction between H-7 (δ 4.75) and H-7′ (2.94 and 2.56), and that between H-9 (3.22 and 3.06) and H-7′ (2.94 and 2.56) indicated that C-7′, C-9, and H-7 were on the same side of the molecule, while the absence of interaction between H-8′ (δ 2.48) and H-7 (4.75) or H-9 (3.22 and 3.06) suggested that H-8′ and 8-OH were on the other side. The CD spectrum of compound **1** exhibited a positive Cotton effect at λ 247.5 (+5.06) and a negative Cotton effect at λ 215.0 (–8.15), indicating that the two 3,4-dimethylenedioxy phenyl groups were oriented in a clockwise manner. These data as well as the optical rotation ($[\alpha]_D^{25}$ +26.5°) established the structure of **1** as (7*R*,8*S*,8′*S*)-3,4,3′,4′-dimethylenedioxy-8,9-dihydroxy-8,8′, 7-*O*-9′-lignan [4, 6].

Mandshuricol B (2) was obtained as a colorless oil and has a molecular ion peak at 374.1360 in its HR-EI-MS, corresponding to the formula $C_{20}H_{22}O_7$. Compound **2** was similar to **1** in its CD, UV, IR, and NMR spectra, which suggested that it is a derivative of **1**. The ^{1}H NMR spectrum of **2** showed a methoxyl signal at δ 3.85 (3H, s), a dimethylenedioxy signal at δ 5.88, and the signals of two 1,3,4-trisubstituted phenyl rings (δ 6.60–6.80, 6H) (Table 1), indicating that only one of the aryl groups was present as a 3,4-dimethylenedioxy substituent. In the HMBC spectrum of compound **2**, the correlations between H-6 (δ 6.80) and C-4 at δ 150.2 and between H-methoxyl (δ 3.85) and C-3 at δ 150.8 indicated that the methoxyl group was located at C-3, while a phenolic hydroxyl was located at C-4. In addition, the signals of H-9 (δ 3.24, 3.07), H-8′ (δ 2.50), H-9′ (δ 4.00, 3.75), H-2 (δ 6.88), and H-6 (δ 6.80) were correlated to the signal of C-7 (δ 91.5). Thus, the structure of **2** could be assigned 3′,4′-methylenedioxy-4-methoxy-3,8, 9-trihydroxy-8.8′,7-*O*-9′-lignan. This assignment was supported by its EI-MS spectrum, which gave fragment ion peaks at *m/z* 135 and 151, arising from benzyl or tetrahydrofuran ring cleavage. The additional NOESY and optical rotation were similar to those of **1**, indicating that they possess the same stereochemistry. On the basis of the above data, the structure of **2** was determined to be (7*R*,8*S*,8′*S*)-3′,4′-methylenedioxy-4-methoxy-3,8,9-trihydroxy-8.8′,7-*O*-9′-lignan.

Since several lignans, including kadsurenone and magnone A and B, were found to have PAF receptor antagonist activity [6, 7], the [^{3}H]-PAF antagonist activity of **1** and **2** was tested in parallel with ginkgolide B, a well known potent PAF antagonist. As shown in Table 2, compounds **1** and **2** showed similar potency with IC_{50} values of 4.8×10^{-5} M and 3.5×10^{-5} M, respectively. Although the antagonist activities of the new lignans were much weaker than that of ginkgolide B, our results suggested that mandshuricol A (**1**) and B (**2**) might be, at least in part, responsible for the proposed therapeutic effect of the leaves of *Syringa reticulata* Hara var. *mandshurica*.

EXPERIMENTAL

Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin–Elmer model 241 polarimeter. UV spectra were measured on a Shimadzu UV2401 spectrometer. IR spectra were taken on a Perkin–Elmer 983 G spectrometer. The NMR spectra were recorded on a Varian Inova 600 spectrometer in CD_3OD using tetramethylsilane (TMS) as internal standard. HR-EI-MS and EI-MS were determined on a Micromass ZabSpec spectrometer. CD spectra were determined on a JASCO-715 spectrometer. Preparative HPLC was carried out on a column of ODS (250 × 9.4 mm i.d., Agilent Zorbax SB-ODS, Palo Alto, USA) with a Waters 2996 detector; the flow rate was 2 mL/min and the wave length for detection was 230 nm. MPLC was carried out on a column of silica gel H (460 × 26 mm i.d., Buchi Borosilikat 4.6, Flawil, Swiss). Silica gel (200–300 mesh) for column chromatography was obtained from Qingdao Marine Chemical Factory, Qingdao, China. Precoated plates of silica gel GF254 were used for TLC and detected under UV.

Plant Materials. The leaves of *Syringa reticulata* Hara var. *mandshurica* were collected in Heilongjiang province of China in October 2005 and identified by Prof. Jian-wen Wang of our college. A voucher sample is deposited in the Herbarium of the College of Pharmacy, Soochow University.

Extraction and Isolation. The dried plant material (5 kg) was percolated with 150 L of MeOH. The solvent was subsequently dried under reduced pressure to give a residue, which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble fraction was further partitioned between petroleum ether and 90% MeOH (v/v). The 90% MeOH fraction (125 g) was chromatographed over a silica gel column, which was eluted with petroleum ether–EtOAc gradients to afford 20 fractions (F1–F20). Further purification of F8 through MPLC, using petroleum ether–EtOAc (80:20) as eluent, yielded an umber oil (85 mg). Further purification of the umber oil using Sephadex LH-20 chromatography (eluted by $CHCl_3$–MeOH, 1:1) yielded a yellow oil (70 mg). Mandshuricol A (**1**) (58 mg) and mandshuricol B (**2**) (8 mg) were obtained from the yellow oil through RP-HPLC using MeOH–H_2O (80:20) as eluents.

Mandshuricol A (1). Colorless oil; $[\alpha]_D^{25}$ +26.5° (*c* 0.02, MeOH); CD (MeOH, Δε, λ, nm): +5.06 (247.5), –8.15 (215.0); UV (MeOH, λ_{max}, nm) (log ε): 205 (4.55), 218 (3.36), 279 (3.25); IR (KBr, ν_{max} cm^{-1}): 3465, 1601, 1580, 1115, 1090, 870; EI-MS *m/z* (%): 372 (68.5), 151 (22.0), 149 (100.0), 135 (45.0), 121 (23.0); HR-EI-MS *m/z*: 372.1203 ($[M]^+$, calcd 372.1209 for $C_{20}H_{20}O_7$). ^1H and ^{13}C NMR (CD_3OD) spectral data are shown in Table 1.

Mandshuricol B (2). Colorless oil; $[\alpha]_D^{25}$ +24.8° (*c* 0.01, MeOH); CD (MeOH, Δε, λ, nm): +5.10 (246.5), –7.85 (215.0); UV (MeOH, λ_{max}, nm) (log ε): 204 (4.52), 219 (3.38), 280 (3.20); IR (KBr, ν_{max} cm^{-1}): 3625, 3462, 1600, 1580, 1110, 1090, 872; EI-MS *m/z* (%): 374 (70.0), 153 (25.0), 151 (100.0), 135 (47.5), 123 (25.0); HR-EI-MS *m/z*: 374.1360 ($[M]^+$, calcd 374.1366 for $C_{20}H_{22}O_7$). ^1H and ^{13}C NMR (CD_3OD) spectral data are shown in Table 1.

Inhibition of [^{3}H]PAF Binding to Washed Rabbit Platelets. Binding of [^{3}H]PAF to rabbit platelets was carried out according to the methods of [7]. The reaction mixture consisted of 100 μL of platelet suspension (4×10^8 cells/mL), 90 μL of [^{3}H]PAF (0.9 nM, 70,000 dpm) with or without unlabeled PAF (500-fold of [^{3}H]PAF), and 60 μL of sample or control solution (DMSO solution), in a total volume of 500 μL. The reaction mixture was incubated at room temperature for 40 min. The free PAF was separated from bound PAF by filtration of the reaction mixture using a MultiScreen filtration system (DYQ-II, Shanghai Medical equipment Factory) through GF/C filters and counted in the scintillation fluid. The filters were rapidly washed with 2 mL ice-cold buffer and then dried and placed into vials containing 1 mL of the scintillation fluid. Radioactivity was then measured in a liquid scintillation counter (LS6500, Beckman). The difference between total radioactivities of bound [^{3}H]-PAF in the absence and presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. In a set of experiments, [^{3}H]PAF was incubated with different concentrations of PAF receptor antagonists, and the effect of the antagonist on the specific binding was expressed as percentage inhibition of the control. The IC_{50} value was defined as the final concentration of the inhibitor required to block 50% of the specific [^{3}H]PAF binding to rabbit platelet receptors. Assay results are expressed as the mean of three separate experiments.

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