Magnone A and B, Novel Anti-PAF Tetrahydrofuran Lignans from the Flower Buds of *Magnolia fargesii*

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In a search for platelet-activating-factor (PAF) antagonists, two new lignan compounds were isolated from the Chinese crude drug shin-i, the flower buds of *Magnolia fargesii*. Their structures were elucidated as (2*S*,3*R*,4*R*)-tetrahydro-2-(3,4-dimethoxyphenyl)-4-(3,4-dimethoxybenzoyl)-3-(hydroxymethyl)furan (magnone A, **1**) and (2*S*,3*R*,4*R*)-tetrahydro-2-(3,4,5-trimethoxyphenyl)-4-(3,4-dimethoxybenzoyl)-3-(hydroxymethyl)furan (magnone B, **2**). Magnones A and B showed antagonistic activity against PAF in the [^{3}H]PAF receptor binding assay with the IC_{50} values of 3.8×10^{-5} M and 2.7×10^{-5} M, respectively.

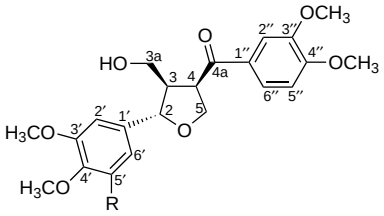
Platelet-activating factor (PAF) is a biologically active phospholipid that is released from sensitized rabbit basophils after antigenic challenge.[1,2] PAF is active at nanomolar concentrations and exerts various pathophysiological activities such as hypotension, increase in vascular permeability, acute inflammation, asthma, cardiac anaphylaxsis, thrombosis, gastrointestinal ulceration, endotoxin shock, allergic skin disease, and the rejection of organ transplantation.[3−5]

In a search for PAF receptor antagonists from natural products, we screened traditional medicines through a PAF receptor binding assay, in which the EtOAc extract of the flower buds of *Magnolia fargesii* Cheng (Magnoliaceae) was found to have PAF antagonistic activity.

The Chinese drug "shin-i" consisting of the dried flower buds of *M. fargesii* have been used to treat nasal empyema with headache, sinusitis, and allergic rhinitis.[6] Pharmacological studies have revealed that this material had uterus-stimulating, hypotensive, antifungal, and skeletal muscle contracting effects.[7,8] In previous reports on chemical investigation of this plant, essential oils, lignans, neolignans and sesquiterpenes have been found.[8−15] Some of these lignans (fargesone A and B, denudatin B, magnolin, liroresinol-B dimethyl ether, pinoresinol dimethyl ether, fargesin, demethoxyaschantin, and aschantin) isolated from this plant showed Ca^{2+} antagonistic activity and PAF antagonist activity.[8,15]

Recently, we reported the isolation of four sesquiterpenes, namely oplopanone, oplodiol, homalomenol A, and 1β,4β,7α-trihydroxyeudesmane from the EtOAc extract of the same plant.[16] In this paper, we describe the isolation and structural elucidation of two new lignans (**1** and **2**) and their PAF antagonistic effect.

Compound **1** revealed UV maxima at 230, 277, and 304 nm and IR absorptions at 3467 and 1658 cm^{-1}, suggesting the presence of a hydroxyl group and a carbonyl group conjugated with an aromatic ring in the



1, R = H
2, R = OCH_3

furanoid lignan skeleton.[17] The EIMS spectrum of **1** exhibited a molecular ion peak at *m*/*z* 402 and a characteristic fragmentation pattern at *m*/*z* 194, 192, 167, 166, and 165 (base ion peak) arising from benzylic and tetrahydrofuran ring cleavages.[18,19] The ^{1}H NMR spectrum of **1** showed four methoxyl groups at δ 3.81, 3.85, 3.88, and 3.89 (each 3H, s), two nonequivalent methylene protons at δ 3.63 (1H, dd, J = 10.9, 5.6 Hz, Ha-3a), 3.72 (1H, dd, J = 10.9, 4.4 Hz, Hb-3a), and six aromatic protons as two ABX systems, one at δ 6.96 (1H, d, J = 1.9 Hz), 6.76 (1H, d, J = 8.3 Hz), 6.88 (1H, dd, J = 8.3, 1.9 Hz) and the other at δ 7.51 (1H, d, J = 2.0 Hz), 6.86 (1H, d, J = 8.3 Hz), 7.54 (1H, dd, J = 8.3, 2.0 Hz), indicating the presence of two 1,3,4-trisubstituted benzene rings. Another salient feature of the ^{1}H NMR spectrum was the presence of signals at δ 4.25 (1H, dd, J = 11.2, 10.7 Hz, Ha-5), 4.13 (1H, obscured by overlapping, Hb-5), and δ 4.63 (1H, d, J = 9.1 Hz, H-2), 2.85 (1H, m, H-3), 4.13 (1H, m, H-4), integrating for the remaining three methine protons. From the above data, the structure of **1** was proposed as a 2,3,4-trisubstituted tetrahydrofuran-type lignan having two veratryl moieties. The ^{13}C NMR and DEPT spectra suggested that the skeleton consisted of 22 carbons: four primary, two secondary, eight tertiary, and seven quarternary (including one ketone, δ 198.01) carbons. The chemical shifts of the protonated carbons of **1** were assigned by combining the HMQC spectrum with the ^{1}H and ^{13}C NMR spectral data as listed in Table 1. In the HMBC spectrum of **1** (Table 2), the correlations between the signals of H-2″, H-6″, H-3, H-4, and H-5 and the signal at δ 198.01 suggested that the carbonyl group must be

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Table 1. ^{1}H and ^{13}C NMR Chemical Shift Assignments of Compound **1** and **2**[a]

carbon	compound 1 δ ^{1}H	compound 1 δ ^{13}C	compound 2 δ ^{1}H	compound 2 δ ^{13}C
2	4.63 d (9.1)	83.76 (d)	4.60 d (8.8)	83.86 (d)
3	2.85 m	52.09 (d)	2.85 m	52.13 (d)
3a	363 dd (10.9, 5.6)	61.43 (t)	3.65 dd (11.0, 4.5)	61.39 (t)
	3.72 dd (10.9, 4.4)		3.72 dd (11.0, 4.5)	
4	4.13 m	49.66 (d)	4.15 m	49.56 (d)
4a		198.01 (s)		197.75 (s)
5	413 m	70.82 (t)	4.11 m	70.88 (t)
	4.25 dd (11.2, 10.7)		4.23 t (11.1)	
1′		132.88 (s)		136.24 (s)
2′	6.96 d (1.9)	109.54 (d)	6.60 s	103.56 (d)
3′		148.89 (s)		153.29 (s)
4′		149.20 (s)		137.59 (s)
5′	6.76 d (8.3)	110.79 (d)		153.29 (s)
6′	6.88 dd (8.3, 1.9)	119.30 (d)	6.60 s	103.56 (d)
1″		129.69 (s)		129.64 (s)
2″	7.51 d (2.0)	110.55 (d)	7.50 d (2.0)	110.52 (d)
3″		149.20 (s)		149.18 (s)
4″		153.65 (s)		153.64 (s)
5″	6.86 d (8.3)	110.08 (d)	6.80 d (8.3)	110.06 (d)
6″	7.54 dd (8.3, 2.0)	123.16 (d)	7.54 dd (8.3, 2.0)	123.14 (d)
OMe	3.81 s	55.88 (q)	3.75 s	55.96 (q)
	3.85 s	55.99 (q)	3.82 s	56.10 (1)
	3.88 s	56.11 (q)	3.82 s	60.77 (q)
	389 s		3.87 s	
			3.89 s	

[a] Assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC.

Table 2. HMBC Correlations of Compound **1** and **2**

carbon	1	2
2	H-3, H_2-3a, H-4, H_2-5, H-2′, H-6′	H-3, H_2-3a, H-4, H_2-5, H-2′, H-6′
3	H-2, H_2-3a, H-4, H_2-5	H-2, H_2-3a, H_2-5
3a	H-2, H-4, H_2-5	H-2, H-3, H-4
4	H-2, H-3, H_2-3a, H_2-5	H-2, H-3, H_2-3a, H_2-5
4a	H-3, H-4, H_2-5, H-2″, H-6″	H-3, H_2-5, H-6″
5		H-4
1′	H-2, H-3, H-2′, H-5′	H-2, H-3
2′	H-2, H-6′	H-2
3′	H-2′, H-5′	H-2′, H-6′
4′	H-2′, H-5′, H-6′	H-2′, H-6′
5′		H-2′, H-6′
6′	H-2, H-2′, H-5′	H-2
1″	H-5″, H-6″	H-2″, H-6″
2″	H-6″	H-6″
3″	H-2″, H-5″	H-2″, H-5″
4″	H-2″, H-5″, H-6″	H-2″, H-5″
6″	H-2″, H-5″	H-2″

located at C-4a. In addition, the signals of H-3, H-3a, H-4, H-5, H-2′, and H-6′ were correlated to the signal of C-2 (δ 83.76). Thus, the structure of **1** could be assigned as tetrahydro-2-(3,4-dimethoxyphenyl)-4-(3,4-dimethoxybenzoyl)-3-hydroxymethylfuran. The relative configuration of **1** was determined as described below.

A literature search revealed that *cis* and *trans* orientation of substituents at C-2 and C-3 would give the signal of H-2 at *ca.* δ 5.5[20−22] and 4.7,[11,23−26] respectively. The H-2 signal of **1** (δ 4.63) agreed well with the assignment of the *trans* configuration. The NOESY spectrum displayed correlations between H-3 and H-4, and H-2 and H-3a but was not observed between H-2 and H-3. This led to the assignment of *trans* and *cis* orientions for H-2/H-3 and H-3/H-4, respectively. These data as well as the optical rotation ($[\alpha]^{25}_D$ +24.7°) established the structure of **1** as (2*S*,3*R*,4*R*)-tetrahydro-2-(3,4-dimethoxyphenyl)-4-(3,4-dimethoxybenzoyl)-3-(hydroxymethyl)furan. This novel compound was named magnone A.

Table 3. PAF Binding Inhibition Activity of Magnone A (**1**), B (**2**), and Ginkgolide B

compound	concentration (μM)	binding inhibition (%)
magnone A (**1**)	100	75.2
	50	56.7
	25	41.6
	12.5	19.4
	$IC_{50} = 3.8 \times 10^{-5}$ M	
magnone B (**2**)	100	81.7
	50	64.3
	25	42.9
	12.5	37.1
	$IC_{50} = 2.7 \times 10^{-5}$ M	
ginkgolide B	$IC_{50} = 1.9 \times 10^{-7}$ M	

Compound **2** was similar to **1** in its CD, UV, IR, and NMR spectra, which thus suggested it to be a derivative of **1**. The ^{1}H NMR spectrum of **2** showed five methoxy signals and five aromatic proton signals, and two of the latter, overlapping at δ 6.60 (2H, s, H-2′ and -6′), indicated that one of the aryl groups was present as a 3,4,5-trimethoxyphenyl substituent. This assignment was supported by the EIMS spectrum, which gave fragment ion peaks at *m*/*z* 195 and 181, arising from benzylic cleavage. The EIMS spectrum also showed a molecular ion peak at *m*/*z* 432, which is 30 amu more than that of **1**. The greater abundance of the peak at *m*/*z* 165 (base ion peak) than that at *m*/*z* 181 gave evidence that the dimethoxyl-substituted aromatic group was a benzoyl rather than a phenyl group.[22,25] The proposed structure of **2** was further confirmed by ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, and HMQC spectra. In the HMBC spectrum of **2**, the correlations clearly resembled those of **1** except for the correlation between the signal at δ 6.60 (H-2′, -6′) and the signal at δ 83.86 (C-2), which suggested the presence of a trimethoxyphenyl substituent at C-2. The methylene proton signal of H-3a displayed a NOE to the methine proton signal of H-2 but not to the methine proton signal of H-4 in **2**. The additional NOEs 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 (2*S*,3*R*,4*R*)-tetrahydro-2-(3,4,5-trimethoxyphenyl)-4-(3,4-dimethoxybenzoyl)-3-(hydroxymethyl)furan. This novel compound was named magnone B.

Since several lignans including kadsurenone, L-652,-731, and 3,7-dioxabicyclo-(3,3,0)-octane skeleton compounds were found to have PAF receptor antagonistic activity,[15,27−29] the [^{3}H]-PAF antagonistic activity of **1** and **2** was tested in parallel with ginkgolide B, a well-known potent PAF antagonist. As shown in Table 3, compound **1** and **2** showed similar potency with IC_{50} values of 3.8×10^{-5} M and 2.7×10^{-5} M, respectively. Although the antagonistic activities of the new lignans were much weaker than that of ginkgolide B, our results suggested that magnone A (**1**) and B (**2**) might be, at least in part, responsible for the proposed therapeutic effect of the flower buds of *M. fargesii*.

Experimental Section

General Experimental Procedures. The melting points were measured by a Electrothermal 9100 and are

uncorrected, and the optical rotation and CD spectra were recorded on a JASCO DIP-370 digital polarimeter and a JASCO J-720 spectropolarimeter, respectively. The IR and UV spectra were recorded using a Magna 550 spectrophotometer in KBr pellet and a Milton Roy Spectronic 3000 Array spectrophotometer in MeOH solution, respectively. The EIMS spectra were obtained on a Hewlett-Packard 5889A. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL Lambda 400 spectrometer, and the chemical shifts were referenced to TMS as the internal standard. Column chromatography was carried out on Kieselgel 60 (Merck No. 9385 and 7729) and LiChroprep RP-18 (Merck, 40−63 μm).

Plant Material. The dried flower buds of *M. fargesii* Cheng were purchased from Il-Shin Pharm. Co. (Taejon, Korea). The voucher specimen is deposited in our laboratory (NDC-052).

Extraction and Isolation. The dried and pulverized flower buds of *M. fargesii* (3 kg) were extracted with MeOH and concentrated under reduced pressure to give a residue (225 g). The residue was partitioned between *n*-hexane (40 g), EtOAc (109 g), *n*-BuOH (20 g), and water. The EtOAc extract was loaded on a silica gel column and eluted with a stepwise solvent gradient of MeOH in $CHCl_3$ to afford 19 subfractions. Subfraction 14 (1.8 g) was rechromatographed on a RP-18 column using MeOH−H_2O (2:1) as an eluent to give 11 fractions. Fraction 5 was further purified by repeated silica gel column chromatography ($CHCl_3$−MeOH 99:1) to give compounds **1** (28 mg, 0.01%) and **2** (84 mg, 0.04%), respectively.

Magnone A (1): colorless crystals from MeOH; mp 149−150 °C; $[\alpha]^{25}_D$ +24.7° (*c* 0.5, MeOH); CD MeOH, $\Delta\epsilon$ (λ, nm), 1.67 (283), 1.17 (253), 0.95 (230), negative tail; UV (MeOH) λ_{max} (log ϵ) 230 (4.37), 277 (4.11), 304 (3.91) nm; IR (KBr) ν_{max} 3467, 1658, 1595, 1589, 1520, 1466, 1438, 1420, 1267, 1157, 1023, 960, 866 cm^{-1}; EIMS *m*/*z* (rel int) 402 $[M]^+$ (22.2), 371 (1.4), 237 (0.9), 219 (11.4), 210 (25.5), 206 (1.4), 194 (19.9), 192 (40.4), 167 (19.6), 166 (22.3), 165 (100), 151 (18.2), 139 (19.0), 137 (19.0), 135 (3.7), 107 (12.5), 105 (5.6), 77 (44.4), 55 (54.4); ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HMBC see Table 2; NOESY 2/3a, 2/5, 2/2′, 2/6′, 3/4, 3/2′, 4/2″, 4/6″, 5″/6″.

Magnone B (2): colorless oil; $[\alpha]^{25}_D$ −2.8° (*c* 1.2, MeOH); CD MeOH, $\Delta\epsilon$ (λ, nm), 0.24 (339), 0.91 (286), 0.41 (253), negative tail; UV (MeOH) λ_{max} (log ϵ) 226 (4.47), 274 (4.16), 303 (3.98) nm; IR (neat) ν_{max} 3510, 1666, 1589, 1512, 1458, 1419, 1265, 1126, 1018, 764 cm^{-1}; EIMS *m*/*z* (rel int) 432 $[M]^+$ (76.3), 418 (2.6), 371 (1.1), 267 (1.7), 249 (15.4), 240 (30.2), 236 (7.8), 224 (31.7), 222 (68.6), 196 (18.6), 195 (28.1), 193 (28.6), 181 (26.5), 169 (20.3), 168 (6.3), 165 (100), 151 (11.0), 137 (21.0), 135 (4.7), 107 (11.4), 105 (5.4), 77 (28.9), 55 (22.7); ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HMBC see Table 2; NOESY 2/3a, 2/5, 2/5′, 3/2′, 4/6″, 5/6″.

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 Valone[30] and Yang *et al.*[31] with some modifications. 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). 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 (Millipore, MA). The filters were rapidly washed with ice-cold buffer and then dried and placed into vials containing 3 mL of the scintillation fluid. Radioactivity was then measured in a liquid scintillation counter (Beckman LS6000TA). 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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