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Sesquiterpene Components from the Flower Buds of *Magnolia fargesii*

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From the Chinese crude drug *shin-i*, the flower buds of *Magnolia fargesii*, four sesquiterpene, oplopanone (**1**), oplodiol (**2**), homalomenol A (**3**) and 1β,4β,7α-trihydroxyeudesmane (**4**) were isolated. These structures were elucidated and the ^{13}C-NMR chemical shifts of these compounds were revised by means of various 2D-NMR techniques.

Key words : *Magnolia fargesii*, sesquiterpene, oplopanone, oplodiol, homalomenol A, 1β,4β, 7α-trihydroxyeudesmane

INTRODUCTION

There are several commercial articles of *shin-i*, and the main source is the dried flower buds of *Magnolia fargesii* Cheng (Magnoliaceae). This drug has a history of safe use and has been used for the treatment of nasal congestion with headache, sinusitis, and allergic rhinitis (Namba 1986). Pharmacological studies have revealed that *shin-i* has uterus-stimulating, hypotensive, antifungal, and skeletal muscle contracting effects (Tang and Eisenbrand 1992; Chen *et al.*, 1988a). In previous reports on chemical investigation of this plant, many kinds of essential oils, lignans, neolignans and sesquiterpenes have been found and pharmacological activities of these lignans from *shin-i*, Ca^{2+} antagonistic activity and PAF antagonistic activity were revealed (Miyazawa *et al.*, 1992; Miyazawa *et al.*, 1994; Miyazawa *et al.*, 1996; Chen *et al.*, 1988b; Kakisawa *et al.*, 1970; Hwang *et al.*, 1990; Pan *et al.*, 1987).

Recently, we have been searching for PAF receptor antagonists from traditional crude drugs. Meanwhile, four sesquiterpens, oplopanone (**1**), oplodiol (**2**), homalomenol A (**3**) and 1β,4β,7α-trihydroxyeudesmane (**4**) were isolated from the EtOAc extracts of the herb besides seven known phenolic lignans with PAF antagonistic activity. We presented here the structural elucidation of the four sesquiterpenes, which were firstly isolated from this plant.

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MATERIALS AND METHODS

General experimental procedures

The melting points were measured by a Electrothermal 9100 and are uncorrected. The EI-MS specta were performed on a Hewlett-Packard 5889A. ^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a Varian Unity 300 and Bruker DRX-300 spectrometer. HMBC data were recorded on a Bruker DMX-600 spectrometer. Chemical shifts were referenced to TMS as the internal standard. CC was carried out on Kieselgel 60 (Merck No. 9385 and 7729) and LiChroprep® RP-18.

Plant material

The dried flower buds of *Magnolia fargesii* Cheng used for this study was purchased from Il-Shin Pharm. Co. (Taejon, Korea) which imported the material from China. The voucher specimen is deposited in our laboratory (NDC-052).

Extraction and isolation

The dried and pulverized flower buds of *Magnolia fargesii* (3 kg) were extracted with MeOH at room temperature for several days. The MeOH extracts were 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, in the usual order.

The EtOAc extract was loaded on a silica gel CC

eluted with a stepwise solvent gradient of MeOH in $CHCl_3$ to afford nineteen subfractions. The subfr. 6 (1.6 g) was rechromatographed on a RP-18 column and eluted by MeOH-H_2O (2:1), to give eleven fractions. The fifth fraction of them was purified by recrystallization to give compound **1** (310 mg). The subfr. 8 (3 g) and 13 (3 g) were further purified by repeated RP-18 (MeOH-H_2O, 3:2) and SiO_2 column chromatography ($CHCl_3$-MeOH, 99:1) to give compound **2** (35 mg), **3** (13 mg) and **4** (85 mg), respectively.

Oplopanone (1)

Needles in MeOH, mp. 87~88°C, EI-MS: *m/z* (rel. int.) 238 $[M]^+$ (22.8), 221 (21.4), 203 (8.3), 195 (8.8), 177 (10.1), 153 (100). ^{1}H-NMR (300 MHz, $CDCl_3$) see Table I; ^{13}C-NMR (75 MHz, $CDCl_3$) see Table I.

Oplodiol (2)

Needles in MeOH, mp. 83~85°C, EI-MS: *m/z* (rel. int.) 238 $[M]^+$ (10), 220 (45), 202 (19), 187 (60), 177 (28), 159 (100). ^{1}H-NMR (300 MHz, $CDCl_3$) see Table II; ^{13}C-NMR (75 MHz, $CDCl_3$) see Table II.

Homalomenol A (3)

Oil, EI-MS: *m/z* (rel. int.) 238 $[M]^+$ (14.8), 220 (87.1), 205 (45.9), 202 (27.2), 187 (39.6), 179 (24.2), 177 (55.7), 162 (51.2), 147 (41.8), 135 (62.6), 123 (100). ^{1}H-NMR (300 MHz, $CDCl_3$) see Table III; ^{13}C-NMR (75 MHz, $CDCl_3$) see Table III.

1β,4β,7α-trihydroxyeudesmane (4)

Amorphous, mp. 135~140°C, EI-MS *m/z* (rel. int.) 239 $[M\text{-}OH]^+$ (0.5), 238 $[M\text{-}H_2O]^+$ (0.2), 213 $[M\text{-}C_3H_7]^+$ (26) 195 $[213\text{-}H_2O]^+$ (100), 177 $[195\text{-}H_2O]^+$ (53), 159 $[177\text{-}H_2O]^+$ (23). ^{1}H-NMR (300 MHz, $CDCl_3$) see Table IV; ^{13}C-NMR (75 MHz) see Table IV.

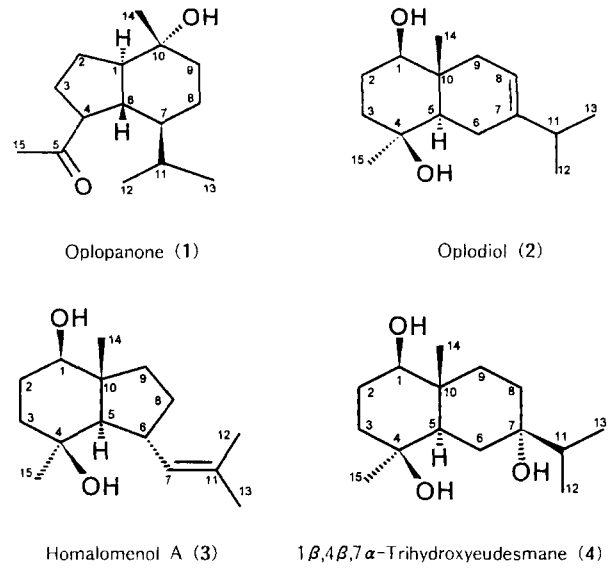

Fig. 1.

RESULTS AND DISCUSSION

Four compounds were isolated from the EtOAc extract of the flower buds of *Magnolia fargesii* by successive silica gel and RP-18 column chromatography.

Compound **1** exhibited molecular ion peak at *m/z* 238 in its EI-MS spectrum. The ^{1}H- and ^{13}C-NMR spectra (Table I) of **1** showed characteristic signals for one tertiary methyl group (δ_H 1.20; δ_C 20.26), one isopropyl group (δ_H 1.45, 0.69, 0.90; δ_C 29.47, 15.55, 21.90) and one acetyl group (δ_H 2.19; δ_C 211.47, 29.47), which were same to those of oplopanone. Oplopa-

Table I. NMR data of oplopanone (**1**) from *Magnolia*

No.	^{13}C-NMR		^{1}H-NMR	
	Herz[a]	**1**[b]	Herz[a]	**1**[b]
1	49.50[c]	56.95		1.45 (1H, m)
2	28.66[d]	25.27		1.42 (1H, m), 1.83 (1H, m)
3	25.31[d]	28.57	2.65 (m)	1.58 (1H, m), 1.97 (1H, m)
4	57.07	55.69		2.65 (1H, ddd, 5.4, 5.7, 10.5 Hz)
5	211.33	211.47		
6	55.81	46.68		1.82 (1H, m)
7	46.77[c]	49.37		1.08 (1H, m)
8	23.07[d]	22.96		1.10 (1H, m), 1.60 (1H, m)
9	42.11	42.00		1.38 (1H, m), 1.80 (1H, m)
10	72.95	72.96		
11	29.45	29.47	0.90 (d, 7 Hz)[c]	1.45 (1H, m)
12	21.94[e]	21.90[c]	0.69 (d, 7 Hz)[c]	0.90 (3H, d, 7.2 Hz)c
13	20.32[e]	15.55[c]	1.20	0.69 (3H, d, 6.9 Hz)c
14	15.63	20.26	2.18	1.20 (3H, s)

[a]Herz, W. and Watanabe, K., *Phytochemistry* **26**, 1457 (1983).
[b]Assignments were carried out by means of DEPT, ^{1}H-^{1}H COSY, HMQC and COLOC.
[c-e]The assignments may be reversed in each column.

Table II. NMR data of oplodiol (**2**) from *Magnolia*

No.	^{13}C-NMR		^{1}H-NMR	
	Herz[a]	**2**[b]	Herz[a]	**2**[b]
1	79.93	79.86	3.31 (dd, 11.0, 4.0)	3.32 (1H, dd, 11.4, 3.9)
2	40.78[c]	26.72		1.92, 1.80 (1H each, m)
3	39.53[c]	39.43		1.74, 1.56 (1H each, m)
4	70.97	70.93		
5	46.34	46.22		1.33 (1H, dd, 11.5, 5.9)
6	26.83[d]	23.02		2.07 (2H, m)
7	141.94	141.87		
8	116.11	116.05	5.34 (brd, 4.5)	5.36 (dd, 3.4, 2.1)
9	23.10[d]	40.67		2.13, 1.92 (1H each, m)
10	37.73	37.64		2.33 (1H, sept, 6.8)
11	35.01	34.95	2.21 (sept, 7.0)	
12	21.97[e]	21.75	1.04 (d, 7.0)	1.06 (3H, d, 6.8)
13	21.25[e]	21.19	1.04 (d, 7.0)	1.06 (3H, d, 6.8)
14	11.72	11.67	0.97	0.99 (3H, s)
15	29.88	29.83	1.19	1.21 (3H, s)

[a]Herz, W. and Watanabe, K., *Phytochemistry* **26**, 1457 (1983).
[b]Assignments were carried out by means of DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC and COLOC.
[c-e]The assignments may be reversed in each column.

none was first isolated from Oplopanax japonicus, and recently, detected from some other species (Herz and Watanabe, 1983; Bohlmann *et al.*, 1984; Maqua *et al.*, 1988; Feliciano *et al.*, 1988). In the ^{1}H- and ^{13}C-NMR of **1**, the chemical shifts were exactly consistented with literature data of oplopanone (Herz and Watanabe, 1983). However almost their signal assignments had mistaken. Therefore, ^{1}H-^{1}H COSY, HMQC, DEPT and COLOC were measured for detailed analyses of ^{1}H- and ^{13}C-NMR data. Each correlation of proton and carbon was measured by HMQC. In COLOC, the long range couplings were observed by the singlet methyl proton signal of C-15 from the methine carbon signal of C-4 and carbonyl carbon signal of C-5, the singlet methyl proton signal of C-14 from the carbon signal of C-10 bearing hydroxyl group and methylene carbon signal of C-9, and two doublet methyl proton signals of C-12 and C-13 from methine carbon of C-11. The remaining proton siganls were assigned by the 1H-1H COSY spectrum. That is, the methine proton signal of C-4 coupled with the methine proton signal of C-6 and methylene proton signal of C-3, the methylene proton signal of C-3 coupled with the methylene proton signal of C-2, the methylene proton signal of C-2 coupled with the methine proton signal of C-1, and the methine proton signal of C-1 coupled with the methine proton signal of C-6. On the basis of these data, the proton and carbon

Table III. NMR data of homalomenol A (**3**) from *Magnolia*

No.	^{13}C-NMR		^{1}H-NMR	
	Sung[a]	**3**[b]	Sung[a]	**3**[b]
1	80.0	79.95	3.36 (dd, 11.0, 4.1)	3.39 (1H, dd, 11.4, 4.2)
2	36.7	27.93		1.78, 1.61 (1H each, m)
3	40.7	40.63		1.62, 1.45 (1H each, m)
4	71.8	71.69		
5	59.1	59.02		1.00 (1H, m)
6	35.0	34.91	2.92 (16 lines)	2.95 (1H, m)
7	132.2	132.12	5.05 (d sp, 9.3, 1.4)	5.08 (brd, 9.5)
8	29.6	29.56		2.05, 1.27 (1H each, m)
9	24.6	38.62		1.59, 1.30 (1H each, m)
10	47.2	47.16		
11	128.7	128.65		
12	30.7	18.08	1.63 m	1.67 (3H, m)
13	25.8	25.72	1.63 m	1.67 (3H, m)
14	14.2	14.12	1.03 (d, 0.9)	1.06 (3H, d, 0.7)
15	18.1	30.67	1.10 s	1.13 (3H, s)

[a]Sung, T. V., Steffan, B., Steglich, W., Klebe, G. and Adam, G., *Phytochemistry*, 31, 3515 (1992).
[b]Assignments were carried out by means of DEPT, ^{1}H-^{1}H COSY, HMQC and COLOC.

Table IV. NMR data of 1β,4β,7α-trihydroxyeudesmane (**4**) from *Magnolia*

No.	^{13}C-NMR			^{1}H-NMR	
	Sung[a]	4[b]	4[c]	Sung[a]	4[b]
1	80.5	79.31	81.03	3.21 (dd, 12.0, 4.0)	3.31 (1H, dd, 11.4, 3.9)
2	40.1	26.73	28.11		1.89, 1.62 (1H each, m)
3	40.6	39.68	40.91		1.71, 1.56 (1H each, m)
4	72.1	71.55	72.59		
5	46.1	44.65	46.49		1.46 (1H, m)
6	29.3	28.94	29.76		1.58, 1.46 (1H each, m)
7	74.8	73.79	75.34		
8	30.1	29.26	30.50		1.58 (1H, m)
9	27.7	34.58	36.16		1.67, 1.38 (1H each, m)
10	35.8	38.84	40.47		
11	40.5	39.16	41.05		1.62 (1H, m)
12	17.4	16.80	17.76	0.94 (d 7.0)	0.95 (3H, d, 6.9)
13	17.5	16.95	17.89	0.94 (d 7.0)	0.95 (3H, d, 6.9)
14	12.2	11.58	12.57	0.96 s	0.99 (3H, s)
15	29.9	29.78	30.31	1.10 s	1.14 (3H, s)

[a]Sung, T. V., Steffan, B., Steglich, W., Klebe, G. and Adam, G., *Phytochemistry*, 31, 3515 (1992). Measured in CD_3OD.
[b]Assignments were carried out by means of DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, COLOC and NOESY. Measured in $CDCl_3$.
[c]Assignments were carried out by means of DEPT. Measured in CD_3OD.

chemical shifts of oplopanone (1) were completely assigned as shown in Table I.

Compound **2** exhibited molecular ion peak at m/z 238 in its EI-MS spectrum. The ^{1}H-NMR spectrum of **2** showed one vinyl proton at δ 5.36 (1H, dd, *J*=3.4, 2.1 Hz), a methine proton attached a hydroxyl function at δ 3.32 (1H, dd, *J*=11.4, 3.9 Hz), an isopropyl group at δ 2.33 (1H, sept, *J*=6.8 Hz) and 1.06 (6H, d, *J*=6.8 Hz), two tertiary methyl proton at δ 1.21 (3H, s) and 0.99 (angular Me, s). Characteristic ^{13}C-NMR signals at δ 141.87 (C), 116.05 (CH), 79.86 (CH), 70.93 (C), 37.64 (C), 29.83 (CH_3) and 11.67 (CH_3) suggested that **2** was oplodiol, a eudesmene skeleton, having a secondary and a tertiary hydroxyl group and a trisustituted double bond. Each correlation of proton and carbon was measured by HMQC. In COLOC, the long range couplings were observed by the singlet methyl proton signal of C-14 from the carbon signal of C-9, the methine proton signal of C-5 from the carbon signals of C-6 and 15, and the singlet methyl proton signal of C-15 from the carbon signals of C-3 and 4. In the HMBC correlations, the methine proton signal bearing hydroxyl group at δ 3.31 was correlated to the carbon signals of C-2, C-3, C-5 and C-14. The olefinic proton signal at δ 5.36 (H-8) was long-range-coupled to the carbon signals of C-6, C-9, C-10 and C-11. These results indicated that the carbon signals at δ 40.67, 26.72 and 23.02 were assigned to C-9, C-2 and C-6, respectively. The stereochemistry of hydroxy group at C-1 was supposed to be equatorial position (β) from the fact that the coupling constants (dd, *J*=11.4 and 3.9 Hz) of H-1 in the ^{1}H-NMR spectrum of 2 indicated its axial position. This was supported by the upfield shift of the angular methyl signal (δ 11.67) of C-14 in the ^{13}C-NMR spectrum of 2 (Nyasse *et al.* 1988). On the basis of these data, the proton and carbon chemical shifts of compound 2 were completely assigned and the structure of 2 was identified as oplodiol which was first isolated from Oplopanax japonicus (Minato and Ishikawa, 1967). Hence, the previous ^{13}C-NMR assignments (Herz and Watanabe, 1983) have to be corrected as those given in Table II.

Compound **3** exhibited molecular ion peak at *m/z* 238 in its EI-MS spectrum. The ^{1}H-NMR spectrum of **3** showed signals for four tertiary methyl groups, two of them shifted downfield to δ 1.67 (6H, m), one methine proton at δ 3.39 (1H, dd, *J*=11.4, 4.2 Hz) and one olefinic proton at δ 5.08 (1H, brd, *J*=9.5 Hz). The ^{13}C-NMR spectrum of **3** indicated the presence of a trisubstituted double bond (δ 132.12, CH and 128.65, C) and two carbon atom carrying hydroxyl group at δ 79.95 (CH) and 71.69 (C), respectively. The ^{1}H-^{1}H COSY spectrum of **3** showed two set of spin-spin coupling systems of protons; the one composed of H-1, H-2 and H-3, and the other composed of H-5, H-6, H-7, H-8, H-9, H-12 and H-13. In particular, the methine proton signal of H-6 was correlated with H-7 and H-8, and the long range couplings were observed between the signal of H-7 and the signals of H-12 and H-13. In COLOC, the long range couplings were observed by the singlet methyl proton signal of C-14 from the carbon signals of C-5 and 10 and the singlet methyl proton signal of C-15 from the carbon signal of C-3. Based on the above spectral data, the structure of 3 was proposed to homalomenol A, which was first isolated from the roots of Homalomena aromatica (Sung *et al.*, 1992). But, in their reported data, the carbon chemical shifts of C-2 and C-9, and C-12 and C-15 were exchanged each other, respectively.

Compound **4** exhibited fragment ion peaks at m/z 238 $[M-H_2O]^+$, 213 $[M-C_3H_7]^+$, 195 $[213-H_2O]^+$, 177 $[195-H_2O]^+$ and 159 $[177-H_2O]^+$, in its EI-MS spectrum. Compared to oplodiol (**2**), compound **4** had a similar ^{1}H-NMR spectrum except the absence of the olefinic proton. Characteristic ^{13}C-NMR signals of δ 79.31 (CH), 71.55 (C), 73.79 (C), 38.84 (C) and 11.58 (CH_3) suggested a eudesmane skeleton with one secondary and two tertiary hydroxy groups. In the HMBC correlations, the methine proton signal (H-11) of isopropyl group was correlated to the carbon signals of C-6, C-7 and C-8, and two methyl proton signals (H-12 and 13) of isopropyl group were correlated to the carbon signals of C-7. The methyl proton signal of H-14 was coupled to the carbon signals of C-1, C-5, C-9 and C-10. The stereochemistry of C-1, C-4 and C-7 was finally confirmed by NOESY spectrum. That is, NOE was observed between H-1 and H-3ax, 5 and 9_{ax}, and between H-14 and H-2_{ax}, 6_{ax} and 8_{ax}. But, NOE was not observed between two methyl proton signals (H-12 and 13) of isopropyl group and H-5 and 9_{ax}. From the above data, the structure of **4** was assigned to 1β,4β,7α-trihydroxyeudesmane, which was also first isolated from the roots of Homalomena aromatica (Sung *et al.*, 1992). But, in their reported data, the carbon chemical shifts of C-2, 9 and 10 had mistaken. The assignments of them were revised as shown in Table IV.

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