Lignans and Sesquiterpenes from *Magnolia praecocissima*

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A new lignan 1 was isolated together with the five known lignans 2—6 and four sesquiterpenes 7—10 from the seeds of *Magnolia praecocissima.* The structure of 1 was elucidated by analysis of spectroscopic data and chemical reaction. Furthermore, the absolute configurations of 1, 2, and 3 were determined by the modified Mosher's method.

Key words *Magnolia praecocissima*; Magnoliaceae; lignan; sesquiterpene; modified Mosher's method

Magnolia species produce a variety of lignans, neolignans, sesquiterpenes, and sesquiterpene lactones. The essential oil of *Magnolia praecocissima* (*M. kobus* var., Japanese name: 'Kobushi'), a valuable Japanese decorative plant, contains many types of terpenes and lignans.[1)] In a previous paper,[2)] we reported the structure and neurotrophic activity of sesquiterpene-neolignans isolated from *Magnolia obovata.* As part of our search for neurotrophic substances in natural products, we have studied the chemical constituents of the seeds of *M. praecocissima.*

The seeds of *M. praecocissima* were extracted with methanol. The methanol extract was subjected repeatedly to column chromatography using silica gel and Sephadex LH-20 to afford a new lignan **1** and the five known lignans, (−)-fargesol (**2**),[3)] magnostellin B (**3**),[4)] sesamine (**4**),[5)] kobusin (**5**),[5)] eudesmin (**6**),[5)] and the four sesquiterpenes oplopanone (**7**),[6)] zingibertriol (**8**),[7)] 6,15α-epoxy-1β,4β-dihydroxyeudesmane (**9**),[8)] and parthenolide (**10**).[9)]

Compound **1** had the molecular formula $C_{24}H_{30}O_8$ determined from high-resolution electron-ionization mass spectroscopy (HR-EI-MS) at 446.1933 $[M]^+$. The IR spectrum of **1** displayed absorption bands attributable to a hydroxyl group (3512 cm^{-1}) and an ester carbonyl group (1748 cm^{-1}). Its ^{1}H-NMR spectral data were found to be very similar to those of (−)-fargesol **2**[3)] except for the presence of a newly appearing acetyl group resonating at δ_H 1.91 and a shift of the H-9′ proton signal at δ_H 3.85 by *ca.* 0.42 ppm lower than that of **2**. In the heteronuclear multiple-bond correlation (HMBC) experiment (Fig. 1), the H-9′ proton signal at δ 3.85 showed a correlation with the ester carbonyl carbon signal (δ_C 170.7), indicating the presence of the acetyl group at C-9′, and other correlations shown in Fig. 1 supported a 8,8′-linked tetrahydrofuran structure.

The acetylation of **1** gave diacetate **1a**, all spectral data of which were in agreement with the diacetate derived from **2**. These results suggested that **1** is 9′-*O*-acetyl-(−)-fargesol. The relative configuration of **2** was proposed to be the all *trans*-relationships of H-7′, H-8′, and H-8.[3)] This was substantiated by the nuclear Overhauser effect spectroscopy (NOESY) experiments with **1** (Fig. 1), and the relative configuration of H-7 was also elucidated as shown in Fig. 1 on the basis of NOE correlations of the H-7 proton signal to H-8 and H-8′, and correlations of the H-8 proton signal to H-2 and H-6. But the absolute configuration of C-7 in **2** had not been clear. To determine the absolute configurations on the C-7 of **1** and **2**, a modified Mosher's method[10)] was applied to

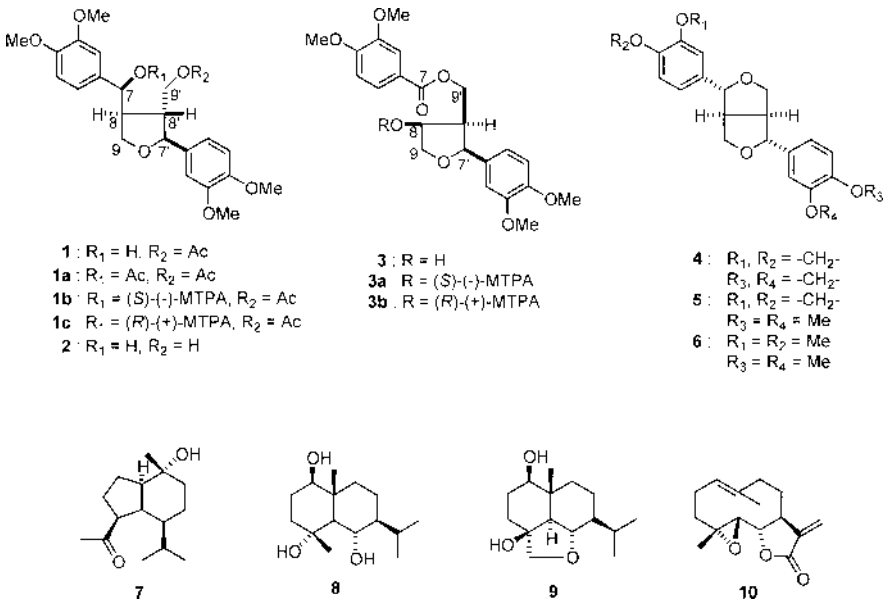

Chart 1

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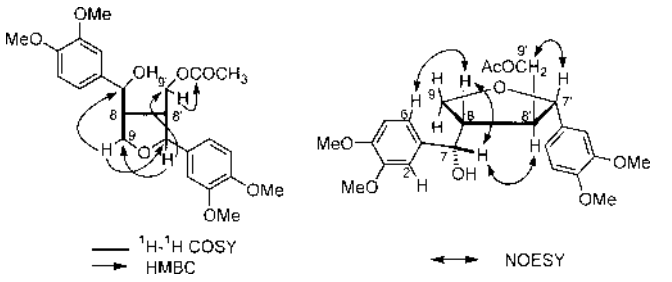

Fig. 1. ^{1}H–^{1}H COSY, HMBC, and NOESY Correlations of Compound **1**

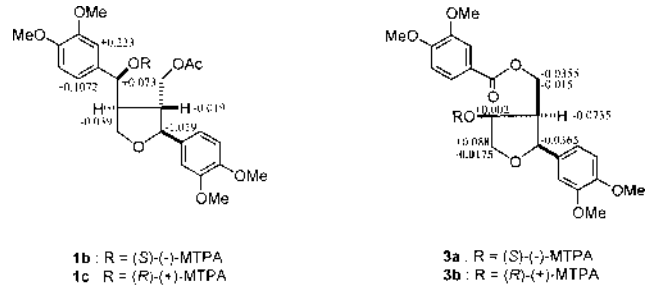

Fig. 2. $\Delta\delta$ ($\delta S-\delta R$) Values Obtained from MTPA Esters of Compounds **1** and **3**

1. Treatment of **1** with (*S*)- and (*R*)-MTPA in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (4-DMAP) afforded the (*S*)-MTPA ester **1b** and the (*R*)-MTPA ester **1c**, respectively. The $\Delta\delta$ values between **1b** and **1c** were calculated as shown in Fig. 2. Thus the absolute configurations on C-7 in **1** and **2** were determined to be *S*. Therefore the structure of **1** was assigned to 9′-*O*-acetyl-(7*S*,8*R*,7′*S*,8′*S*)-(−)-fargesol and **2** was (7*S*,8*R*,7′*S*,8′*S*)-(−)-fargesol.

The spectral data of compound **3** were identical in all respects to those of magnostellin B.[4] All *cis*-relationships of H-7′, H-8′, and H-8 were confirmed by the NOESY experiment. However, its absolute configuration remained unsolved. To determine the absolute configurations on C-8 of **3**, the (*S*)-MTPA ester **3a** and the (*R*)-MTPA ester **3b** were prepared from **3**. The $\Delta\delta$ values between **3a** and **3b** were calculated as shown in Fig. 2. However, the $\Delta\delta$ value for H-9 revealed abnormal data (−0.0175). This irregularity was presumably due to the anisotropy effect of the aromatic ring on the tetrahydrofuran.[11] Therefore this value should be excluded and the absolute configuration of C-8 was determined to be *R*. Thus the structure of **3** was determined to (8*R*,7′*R*,8′*R*)-magnostellin B. This is an example of an exception to the rule of the modified Mosher's method.

In conclusion, we isolated six lignanes and four sesquiterpenes from the seeds of *M. praecosissima*, but we found none of the sesquiterpene-lignans that are characteristic neurotrophic substances found in *M. obovata.*

Experimental

Optical rotations were measured with a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Jasco FT-IR 5300 IR spectrophotometer. NMR spectra were recorded on a JEOL GX-400 and a Varian Unity 600 instrument. MS were recorded on a JEOL AX-500 instrument.

Extraction and Isolation The seeds of *M. praecocissima* were collected in Kyoto University and identified by professor S. Kawano. The seeds were extracted with MeOH, and the extract was condensed under reduced pressure. The MeOH extract (59.7 g) was chromatographed on silica gel eluted with an *n*-hexane–EtOAc gradient to divide it into 1—12 fractions. Fraction 5 was purified by silica gel chromatography [*n*-hexane–EtOAc (3 : 1)] to give **4** (754 mg). Fraction 7 was purified by silica gel chromatography [*n*-hexane–EtOAc (3 : 1)] to give **5** (96 mg) and **9** (7 mg). Fraction 8 gave **6** (2.2 g). Fraction 9 was purified by Sephadex LH-20 [$CHCl_3$–MeOH (4 : 1)] and silica gel chromatography [$CHCl_3$–MeOH (19 : 1)] to give **7** (4 mg). Fraction 10 was purified by Sephadex LH-20 [$CHCl_3$–MeOH (1 : 1)] and silica gel chromatography [$CHCl_3$–MeOH (9 : 1)] to give **1** (24 mg). Fraction 11 was purified by Sephadex LH-20 [$CHCl_3$–MeOH (1 : 1)] and repeated silica gel chromatography [$CHCl_3$–MeOH (49 : 1), $CHCl_3$–MeOH (19 : 1)] to give **3** (9 mg), **8** (2 mg), and **10** (1 mg). Fraction 12 was purified by Sephadex LH-20 [$CHCl_3$–MeOH (1 : 4)] and silica gel chromatography [$CHCl_3$–MeOH (9 : 1)] to give **2** (5 mg).

9′-*O*-Acetyl-(7*R*,8*S*,7*R*,8*S*)-(−)-fargesol (**1**): Colorless oil. $[\alpha]_D^{21.2}$ +35.2° (c=1.20, $CHCl_3$). IR cm^{-1}: 3512, 1748, 1595. EI-MS: *m*/*z* 446 (M^+, 400), 189 (31), 167 (57), 151 (31). HR-EI-MS: *m*/*z* Found: 446.1933; Calcd for $C_{24}H_{30}O_8$: 446.1940. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.91 (3H, s, MeCO), 2.10 (1H, m, 8′-H), 2.50 (1H, m, 8-H), 3.85 (2H, m, 9′-H), 3.86 (3H, s, OMe), 3.87 (3H, s, OMe), 3.88 (3H, s, OMe), 3.90 (3H, s, OMe), 3.98 (1H, dd, J=7.4, 9.3 Hz, 9-H), 4.34 (1H, dd, J=4.1, 9.3 Hz, 9-H), 4.52 (1H, d, J=7.7 Hz, 7′-H), 4.59 (1H, d, J=7.7 Hz, 7-H), 6.81 (2H, br s, 5, 6-H), 6.84 (1H, d, J=8.2 Hz, 5′-H), 6.86 (1H, s, 2-H), 6.88 (1H, dd, J=1.9, 8.2 Hz, 6′-H), 6.92 (1H, d, J=1.9 Hz, 2′-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.7 (MeCO), 49.0 (C-8′), 49.9 (C-8), 55.87 (OMe), 55.90 (OMe), 63.8 (C-9′), 70.1 (C-9), 75.8 (C-7), 83.9 (C-7′), 109.2 (C-2, 2′), 110.9 (C-5, 5′), 118.6 (C-6), 118.9 (C-6′), 133.6 (C-1), 135.1 (C-1′), 148.6 (C-3), 148.8 (C-3′), 149.1 (C-4), 149.2 (C-4′), 170.7 (C=O).

Acetylation of 1 and 2 Acetic anhydride (1 ml) was added to a solution of **1** (9 mg) or **2** (4 mg) in pyridine (1 ml), and the mixture was allowed to stand at room temperature for 16 h. The reaction mixture was poured into water and extracted with EtOAc. After evaporation of solvent, the residue was chromatographed on silica gel (*n*-hexane–EtOAc, 1 : 1) to give the acetate **1a** (5 mg) from **1** and **1a** (2 mg) from **2** as an oil, respectively. The spectral data (^{1}H-NMR, MS, $[\alpha]_D$) were identical in all respects with those of authentic samples.

Preparation of (*S*)-MTPA Ester and (*R*)-MTPA Ester from 1 A solution of **1** (1.6 mg) in CH_2Cl_2 (1 ml) was treated with (*S*)-MTPA (15 mg) in the presence of DCC (15 mg) and 4-DMAP (15 mg), and the reaction mixture were stirred at room temperature for 12 h. The reaction mixture was purified by preparative TLC silica gel with *n*-hexane–AcOEt (1 : 1) to give the (*S*)-MTPA ester **1b** (0.8 mg). The (*R*)-MTPA ester **1c** (0.6 mg) was also obtained from **1** by the same procedure as described above.

(*S*)-MTPA Ester of **1**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.917 (3H, s), 1.946 (1H, m, H-8′), 2.7428 (1H, m, H-8), 3.763 (2H, m, H-9′), 3.8 (2H, m, H-9), 4.495 (1H, d, J=8.1 Hz, H-7′), 5.852 (1H, d, J=9.9 Hz, H-7).

(*R*)-MTPA Ester of **1**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.984 (3H, s), 1.984 (1H, m, H-8′), 2.745 (1H, m, H-8), 3.703 (1H, dd, J=5.5, 11.4 Hz, H-9′), 3.776 (1H, dd, J=4.8, 11.4 Hz, H-9′), 3.998 (1H, m, H-9), 4.127 (1H, dd, J=4.0, 9.2 Hz, H-9), 5.514 (1H, d, J=8.1 Hz, H-7′), 5.779 (1H, d, J=9.9 Hz, H-7).

(7*S*,8*S*,7′*R*,8′*R*)-(−)-Fargesol (**2**): Colorless oil. $[\alpha]_D^{22.0}$ −360° (c=0.1, MeOH).

Magnostellin B (**3**): Colorless oil. $[\alpha]_D^{24.0}$ +33.9° (c=0.41, $CHCl_3$). IR cm^{-1}: 3504, 1709, 1599, 1516, 1464, 1420, 1273, 1136. EI-MS: *m*/*z* 418 (M^+, 23), 236 (50), 219 (12), 205 (22), 192 (100), 165 (46). HR-EI-MS: *m*/*z* Found: 418.1622; Calcd for $C_{22}H_{26}O_8$: 418.1628. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 2.56 (1H, m, H-8′), 3.89 (3H, s, OMe), 3.90 (3H, s, OMe), 3.91 (3H, s, OMe), 3.94 (3H, s, OMe), 4.07 (2H, d, J=4.4 Hz, H-9), 4.42 (1H, dd, J=11.3, 6.0 Hz, H-9′), 4.48 (1H, dd, J=11.3, 6.0 Hz, H-7′), 4.49 (1H, m, H-8), 4.63 (1H, d, J=8.0 Hz, H-7′), 6.82 (1H, d, J=8.2 Hz, H-5′), 6.87 (1H, d, J=8.5 Hz, H-5), 6.95 (1H, dd, J=8.2, 1.9 Hz, H-6′), 7.01 (1H, d, J=1.9 Hz, H-2′), 7.48 (1H, d, J=1.9 Hz, H-2), 7.57 (1H, dd, J=8.5, 1.9 Hz, H-6). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 56.1 (C-8′), 55.91 (OMe), 55.93 (OMe), 56.0 (OMe), 56.1 (OMe), 63.3 (C-9′), 74.7 (C-9), 75.2 (C-8), 83.3 (C-7′), 109.3 (C-2′), 110.2 (C-5), 110.9 (C-5′), 112.0 (C-2), 118.9 (C-6′), 122.0 (C-1), 123.6 (C-6), 133.1 (C-1′), 148.7 (C-4′), 149.0 (C-3′), 149.3 (C-3), 153.4 (C-4), 166.4 (C-7).

Preparation of (*S*)-MTPA Ester and (*R*)-MTPA Ester from 3 A solution of **3** (1.5 mg) in CH_2Cl_2 (1 ml) was treated with (*S*)-MTPA (15 mg) in the presence of DCC (15 mg) and 4-DMAP (15 mg), and the reaction mixture were stirred at room temperature for 12 h. The reaction mixture was purified by preparative TLC silica gel with *n*-hexane–AcOEt (1 : 1) to give the (*S*)-MTPA ester **3a** (1.0 mg). The (*R*)-MTPA ester **3b** (1.0 mg) was also ob-

tained from **3** by the same procedure as described above.

(*S*)-MTPA Ester of Magnostellin B (**3a**): ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 2.770 (1H, m, H-8′), 3.983 (1H, dd, J=1.6, 11.0 Hz, H-9), 4.262 (1H, dd, J=4.4, 11.3 Hz, H-9′), 4.367 (1H, dd, J=5.8, 11.0 Hz, H-9), 4.555 (1H, dd, J=4.4, 11.3 Hz, H-9′), 4.653 (1H, d, J=10.4 Hz, H-7′), 5.848 (1H, dd, J=4.1, 4.1 Hz, H-8).

(*R*)-MTPA Ester of Magnostellin B (**3b**): ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 2.767 (1H, m, H-8′), 4.058 (1H, dd, J=1.6, 11.3 Hz, H-9), 4.122 (1H, dd, J=4.4, 11.3 Hz, H-9′), 4.298 (1H, dd, J=4.1, 11.3 Hz, H-9), 4.529 (1H, dd, J=4.1, 11.3 Hz, H-9′), 4.709 (1H, d, J=10.4 Hz, H-7′), 5.892 (1H, dd, J=4.1, 4.1 Hz, H-8).

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