Lignan and Neolignan Derivatives from *Magnolia denudata*

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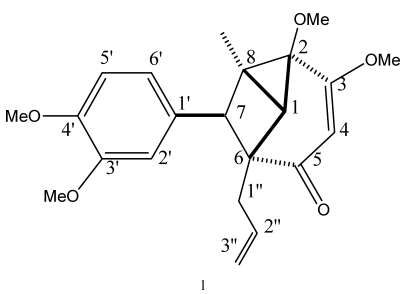
A new tricyclo[4.2.0.0^{2,8}]octane-type neolignan, 6-allyl-7-(3,4-dimethoxyphenyl)- 2,3-dimethoxy-8-methyl-tricyclo[4.2.0.0^{2,8}]oct-3-en-5-one, together with 15 known lignan and neolignan derivatives have been isolated from the flower buds of *Magnolia denudata* DESR. and the structures of these compounds have been elucidated based on the ^{1}H- and ^{13}C-NMR spectra and two-dimensional NMR methods such as HMBC, HMQC, and NOESY.

Key words *Magnolia denudata*; flower bud; Xin Yi; lignan; neolignan

Magnolia denudata, known as "XinYi" in traditional Chinese medicine, has been used for the treatment of headache caused by nasal congestion, sinusitis, and allergic rhinitis. Additionally, it is known as a rich source of lignans and neolignans, which have attracted considerable interest because of their complex structures and notable physiologic effects. Recently, as a result of study of the pharmacologic activities of lignans and neolignans from this plants, Du *et al.* reported antiinflammatory activities.[1)] The anti-platelet-activating factor (PAF) activity of these compounds was also examined.[2)] Kwon *et al.* reported cholesterol acetyltransferase inhibitory activity,[3)] and Kim *et al.* reported that the compounds could be successfully used for the management of allergic diseases and that the pharmacologic effect induced apoptosis of RBL-2H3 cells *via* mitochondria and caspase.[4)]

A number of chemical investigations on the isolation of lignans and neolignans from the leaves, flower, and twigs have been performed. Twenty compounds were isolated from the leaves of *M. denudata*, including 16 lignans, which were classified into six structural types by Du *et al.*[1)] Kuroyanagi *et al.* found 25 lignan and neolignan derivatives from the twigs.[2)] Funayama *et al.* found four lignans isolated from root bark.[5)] Iida *et al*. found denudatins A and B and denudatone, burchellin, veraguensin, and futoenone isolated from the leaves of *M. denudata*.[6)] In connection with our studies on the genera *Magnoliaceae* that has diverse biological activities, we have surveyed the constituents of the flower buds of *M. denudata* to isolate the new neolignan 6-allyl-7-(3,4-dimethoxyphenyl)-2,3-dimethoxy-8-methyl-tricyclo[4.2.0.0^{2,8}]oct-3-en-5-one (**1**), together with 15 known tetrahydrofuran-type and bis-tetrahydrofuran-type lignans and neolignans. The known lignans and neolignans were identified as veragensin (**2**),[2)] galgravin (**3**),[7)] a lignan (**4**),[8)] fargesone B (**5**),[9)] lariciresinol (**6**),[10)] a lignan (**7**),[11)] fargesin (**8**),[12)] (−)-methylpiperitol (**9**),[13)] magnolone (**10**),[14)] (−)-galbacin (**11**),[15)] licarin B (**12**),[16)] acuminatin (**13**),[2)] hancinone (**14**),[2)] burcellin (**15**),[2)] and a neolignan (**16**)[2)] by analysis of their ^{1}H- and ^{13}C-NMR spectra and comparison with published data. This paper deals with the isolation and structural elucidation of the new compound on the basis of two-dimensional NMR spectroscopic data.



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Results and Discussion

The methanolic extract was suspended in H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was repeatedly column chromatographed on silica gel, ODS, and preparative HPLC (ODS) to afford 16 compounds. Fifteen were identified as known compounds by comparison of their physical and spectral data with the reported values and on the basis of the spectroscopic evidence.

Compound **1**, an amorphous powder, $[\alpha]_D$ +67.2° ($CHCl_3$), showed a molecular ion peak at *m*/*z* 370 in the EI-MS, and HR-EI-MS gave the molecular formula $C_{22}H_{26}O_5$. The IR spectrum showed an α,β-unsaturated carbonyl group at 1648 cm^{-1}. The ^{1}H-NMR signals comprised one tertiary methyl at δ 1.63 (3H, s), four methoxyls at δ 3.39, 3.50, 3.79, and 3.80, one allyl group at δ 2.65 (1H, m), 2.90 (1H, m), 5.23 (1H, br d, J=10.4 Hz), 5.26 (1H, br d, J=17.1 Hz), 6.05 (1H, m), two methine protons at δ 2.26 (1H, s), 3.73 (1H, s), and one olefinic proton at δ 4.86 (1H, s), together with three aromatic protons at δ 6.43 (1H, br s), 6.47 (1H, br d, J=8.5 Hz), and 6.67 (1H, d, J=8.5 Hz). On the other hand, the ^{13}C-NMR signals displayed 22 carbon signals composed of the methyl carbon (δ 13.0), four methoxyl carbons (δ 55.6, 55.8, 56.3, 56.7), six aromatic carbons (δ 109.2, 110.8, 118.1, 130.9, 143.9, 148.5), four olefinic carbons (δ 104.3, 118.6, 134.4, 171.5), three quaternary carbons (δ 37.1, 53.7, 67.6), two methine carbons (δ 35.8, 51.2), one methylene carbon (δ 39.5), and one carbonyl carbon (δ 196.2). In the ^{1}H-detected heteronuclear multiple bond correlation (HMBC), the proton signal at δ 2.26 (1H, s) correlated with the quaternary carbons at δ 37.1, 53.7, and δ 67.6, bearing a methyl group, an allyl group, and a methoxyl group, respectively. In addition, this proton is also correlated with a benzylic carbon at δ 51.2 and a methoxy bearing sp^2 carbon at δ 171.5. The proton signal at δ 3.73 (1H, s) correlated with the aromatic carbons (δ 109.2, 118.1, 130.9), allylic carbon at δ 39.5, and with the methine carbon at δ 35.8, the carbonyl carbon at δ 196.2, and two quaternary carbons at δ 37.1 and δ 53.7. Therefore the structure of **1** was suggested to be a [4.2.0.0^{2,8}] skeleton. Furthermore, an

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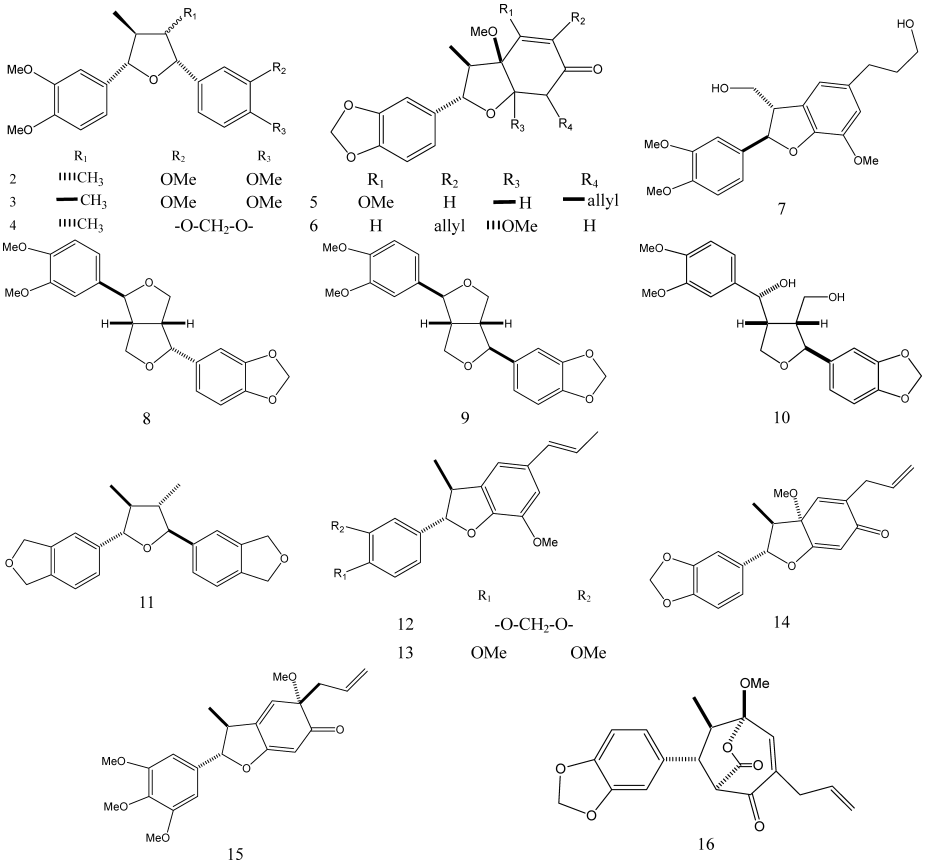

Table 1. NMR Spectral Data for **1** ($CDCl_3$)

Position	δ_C	δ_H	^1H–^1H COSY	HMBC	HMQC
1′	130.9				
2′	109.2	6.43 (1H, br s)		51.2, 118.1, 130.9, 143.9, 148.5	109.2
3′	143.9				
4′	148.5				
5′	110.8	6.67 (1H, d, J=8.5 Hz)	6.47	118.1, 130.9, 143.9, 148.5	110.8
6′	118.1	6.47 (1H, br d, J=8.5 Hz)	6.67	51.2, 109.2, 143.9, 148.5	118.1
1	35.8	2.26 (1H, s)	3.39, 3.50, 3.78, 3.80	13.0, 37.1, 39.5, 51.2, 53.7, 67.6, 130.9, 171.5	35.8
2	67.6				
3	171.5				
4	104.3	4.86 (1H, s)	1.63, 3.50	53.7, 67.6, 171.5, 196.2	104.3
5	196.2				
6	53.7				
7	51.2	3.73 (1H, s)	1.63, 3.39, 6.43	13.0, 35.8, 37.1, 39.5, 53.7, 109.2, 118.1, 130.9, 196.2	51.2
8	37.1				
1″	39.5	2.90 (1H, m)	2.26, 6.05	35.8, 51.2, 53.7, 118.6, 134.4, 196.2	39.5
		2.65 (1H, m)	2.90, 6.05		
2″	134.4	6.05 (1H, m)	2.65, 2.90, 5.26, 5.23	39.5	134.4
3″	118.6	5.26 (1H, br d, J=17.1 Hz)	6.05	39.5	118.6
		5.23 (1H, br d, J=10.4 Hz)			
–OMe (C-3′)	55.6	3.80 (3H, s)	1.63, 3.39, 3.50	143.9	55.6
–OMe (C-4′)	55.8	3.79 (3H, s)		148.5	55.8
–OMe (C-2)	56.7	3.39 (3H, s)	1.63, 2.26	67.6	56.7
–OMe (C-3)	56.3	3.50 (3H, s)	1.63, 2.26, 4.86	171.5	56.3
–Me (C-8)	13.0	1.63 (3H, s)	2.26, 3.73	35.8, 37.1, 51.2, 67.6	13.0

olefinic proton at δ 4.86 (1H, s) correlated with the methoxy-bearing sp^2 carbon at δ 171.5 and a carbonyl carbon at δ 196.2 in the HMBC spectrum. Other protons also showed the HMBC as listed in Table 1, and thus its structure was estimated to be a novel compound constructed with a six-member ring, four-member ring, and three-member ring, that is, the plane structure for **1** was represented as 6-allyl-7-(3,4-dimethoxyphenyl)-2,3-dimethoxy-8-methyl-tricyclo[4.2.0.$0^{2,8}$]oct-3-en-5-one. Moreover, nuclear Overhauser effect enhancement spectroscopy (NOESY, Fig. 1) was observed between H-1 (δ 2.26) and C-2-OCH_3, and between C-8-CH_3 (δ 1.63) and C-2-OCH_3; H-2′,6′ was observed at

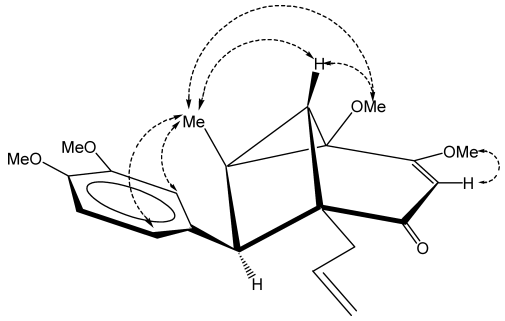

Fig. 1. NOESY of **1**

the benzene ring; H-1 (δ 2.26). Therefore the structure with relative configuration of **1** was deduced as shown in Fig. 1.

Experimental

General Procedures Optical rotations were determined on a JASCO DIP-1000 polarimeter. EI-MS and HR-EI-MS were obtained using a JEOL JMS-DX300 and JMS-DX303HF spectrophotomer, respectively. NMR spectra were measured in $CDCl_3$ on a JEOL α-500 spectrometer (500 MHz) and chemical shifts were referenced to TMS. Column chromatography was carried out on silica gel 60 (230—400 mesh, Merck) and Chromatorex ODS (30—50 μm, Fuji Silysia Chemical Ltd.). Preparative HPLC was carried on an ODS column (Nihon Waters Ltd.) using the MeOH–H_2O solvent system. TLC was performed on precoated silica gel 60 F_{254} (0.2 mm, Merck)

Plant Material The flower buds of *M. denudata* were obtained by Uchida Wakan-Yaku Co. Ltd., Japan, in May 2003. A voucher specimen (MD 52003) was deposited in the Herbarium of the Department of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

Extraction and Isolation The dry flower buds (1.0 kg) of *M. denudata* were extracted with MeOH three times under reflux. The methanolic extract was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble portion (50 g) was repeatedly subjected to silica gel column chromatography with benzene–EtOAc (5 : 1) to afford 10 fractions (frs. 1—10). Fraction 2 (13.4 g) was repeatedly subjected to silica gel column chromatography with benzene–EtOAc (15 : 1—10 : 1) to afford **11** (12 mg), **12** (179 mg), **13** (11 mg), **14** (43 mg), and **16** (8 mg). Fraction 3 (5.9 g) was subjected to silica gel column chromatography with benzene–EtOAc (15 : 1—10 : 1) to afford 10 fractions (frs. 3-1—10). Fraction 3-5 was purified by preparative HPLC (ODS, 70% CH_3OH) to afford **4** (6.1 mg) and **6** (4.6 mg). Fractions 3-6 (455 mg) and 3-7 (943 mg) were repeatedly subjected to silica gel column chromatography with haxane–acetone (1 : 1) and hexane–EtOAc (3 : 1) to afford **5** (34.6 mg) and **8** (26.3 mg). Fractions 3-9 and 3-10 (105 mg) were purified with preparative HPLC (ODS, 70% CH_3OH) to afford **3** (5.1 mg), **2** (17.1 mg), and **1** (27.3 mg). Fraction 7 (2.4 g) was further subjected to silica gel column chromatography with $CHCl_3$–MeOH (200 : 1) to give **9** (6.4 mg). Fraction 9 (6.47 g) was subjected to silica gel column chromatography with benzene–MeOH (50 : 1) and hexane–acetone (3 : 2) to give the two compounds **10** (24 mg) and **7** (23 mg), respectively. Fraction 10 (2.9 g) was subjected to silica gel column chromatography with hexane–acetone (4 : 1—1 : 1) and benzene–EtOAc (3 : 1) to give **15** (67 mg).

Compound **1**: Amorphous powder, $[\alpha]_D$ +67.2° (c=1.68, $CHCl_3$), IR (KBr) ν_{max} cm^{-1}: 2931, 2854, 1648. ^{1}H-NMR (500 Hz, $CDCl_3$) and ^{13}C-NMR (500 Hz, $CDCl_3$): see Table 1. EI-MS *m*/*z*: 370 (M^+), HR-EI-MS *m*/*z*: 370.1780 (M^+) ($C_{22}H_{26}O_5$).

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