Bicyclo[3.2.1]octane and 6-Oxabicyclo[3.2.2]nonane Type Neolignans from *Magnolia denudata*

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From the ethyl acetate soluble fraction of twigs of *Magnolia denudata* (Magnoliaceae), seven new neolignan derivatives, 1—7, were isolated along with eighteen known lignan and neolignan derivatives, 8—25. The structures of the new neolignans were elucidated by means of spectral methods, especially by ^{1}H-NMR and ^{13}C-NMR spectra, and two dimensional NMR methods such as ^{1}H-detected heteronuclear multiple bond connectivity (HMBC), ^{1}H-detected multiple quantum coherence (HMQC) and ^{1}H–^{1}H-correlation spectroscopy (COSY). Compounds 1—4 have novel structures possessing a 6-oxabicyclo[3.2.2]nonane skeleton and compounds 5—8 also have novel structures possessing a bicyclo[3.2.1]octane skeleton. The anti-platelet-activating factor (PAF) activity of these compounds was tested by measurement of inhibition activity against acetyl transferase to *lyso*-PAF.

Key Words *Magnolia denudata*; Magnoliaceae; neolignane; bicyclo[3.2.1]octane; 6-oxa-bicyclo[3.2.2]nonane; anti-platelet-activating factor activity

Platelet-activating factor (PAF) is a potent lipid mediator in inflammation and asthma. A neolignan, kazurenone, isolated from *Piper futokadsura*, was reported as a potent anti-PAF constituent.[1)] Miwa and his coworkers proposed a new method based on inhibition of acetyl transferase activity to *lyso*-PAF for testing anti-PAF activity.[2)] The new method has been applied to many kinds of natural products, two of which, the neolignan derivatives magnolol and honokiol, isolated as main constituents from *Magnolia obovata*, showed potent anti-PAF activity.[2)] So it was promising that lignan and neolignan derivatives were anti-PAF reagents. So, we planned to isolate some lignan and neolignan derivatives and test their anti-PAF activity by the Miwa method. *Magnolia* sp. and *Piper* sp.[3)] plants are well known as sources of lignans and neolignans. Isolation of the constituents of *M. denudata* was carried out by repeated silica gel column chromatography and HPLC using a reversed phase (octadecyl silica (ODS)) column to give new neolignan derivatives, compounds **1**—**7**, along with known lignan and neolignan derivatives, compounds **8**—**25**. The structures of the new compounds were determined by spectral methods such as ^{1}H-NMR, ^{13}C-NMR , ^{1}H-detected heteronuculear multiple bond connectivity (HMBC), nuclear Overhauser effect (NOE) and circular dichroism (CD) spectrum. This paper deals with the structural elucidation of these neolignan derivatives and their anti-PAF activity.

Results and Discussion

The methanol extract of powdered twigs of *M. denudata* was partitioned between EtOAc and water to give the EtOAc soluble fraction. The EtOAc soluble fraction gave seven new neolignan derivatives, named as denudanolides A (**1**), B (**2**), C (**3**), D (**4**), denudadiones A (**5**), B (**6**) and C (**7**) along with known lignan and known neolignan compounds by repeated silica gel column chromatography and HPLC using ODS column. The structures of the known compounds, **9**—**25**, were examined from spectral data and identified with known compounds, denudatin B (**9**),[4)] denudatin A (**10**),[4)] **11**,[5)] kadsurin A (**12**),[6)] **13**,[7)] **14**,[8)] burcellin (**15**),[9)] **16**,[10)] nirandin A (**17**),[11)] licarin B (**18**),[12)] acuminatin (**19**),[13)] veraguensin (**20**),[14)] grandisin (**21**),[15)] yangambin (**22**),[16)] syringresinol (**23**),[17)] isodihydrofutoquinol A (**24**)[18)] and isodihydrofutoquinol B (**25**),[18)] respectively.

Denudanolide A (**1**) gave the molecular formula, $C_{20}H_{20}O_6$, from hi-resolution electron impact (HR-EI)-MS (*m/z* 356.1220 $[M]^+$). The IR spectrum of **1** showed absorption bands due to a lactone carbonyl (1752 cm^{-1}) and an α,β-unsaturated ketone (1681 cm^{-1}). The ^{1}H-NMR spectrum of **1** showed the presence of a methoxyl group [δ 3.56 (s)], a vinyl group [δ 5.80 (ddt, J=16.8, 10.0, 6.8 Hz), 5.18 (md, J=10.0 Hz), 5.15 (md, J=16.8 Hz), 3.10 (dd, J=16.4, 6.8 Hz), 3.01 (dd, J=16.4, 6.8 Hz)], an olefin proton [δ 6.71 (br s)], a doublet methyl group [δ 1.07 (d, J=8.0 Hz)], a methylenedioxy group [δ 5.96 (s)], a singlet proton [δ 3.87 (s), H-1], a doublet proton [δ 2.59 (d, J=8.4 Hz, H-7)], multiplet methine proton [δ 2.93 (dq, J=8.4, 8.0 Hz, H-8)] and 3,4-dioxophenyl group [δ 6.75 (d, J=8.0 Hz, H-5′), 6.64 (d, J=1.6 Hz, H-2′), 6.62 (dd, J=8.0, 1.6 Hz, H-6′)]. The ^{13}C-NMR spectrum of **1** showed the presence of a methoxyl group (δ 50.9), a conjugated carbonyl group (δ 189.3), lactone carbonyl group (δ 166.0), ketal carbon (δ 106.6), a methylenedioxy group (δ 101.4), two double bond (δ 143.6, 140.7, 133.7, 118.5) and a phenyl group (δ 148.5, 147.3, 134.6, 120.7, 108.7, 107.4). These NMR data suggested the planar structure having a bicyclo ring for **1**. The HMBC experiment indicated the presence of a 6-oxabicyclo[3.2.2]-nonane skeleton and the relative position of the functional groups of **1** as shown in Fig. 1. The relative configuration of **1** was elucidated from coupling constants and difference NOE experiments as shown in Fig. 2. The coupling constant between H-1 and H-9 was almost 0 Hz. This indicated that the dihedral angle between H-1 and H-9 was approximately 90°. The Dreiding molecular model of **1** indicated the presence of two conformations in **1** and one of them had the 90° dihedral angle between H-1 and H-9. In the difference NOE experiment, H-1 correlated with H-2′ and H-6′. H-9 correlated with Me-8. OMe at C-5 correlated with H-8. H-8 correlated with H-2′ and H-6′. These NOE experiments indicated the relative configuration of **1** as shown in Fig. 2. The absolute configuration of **1** was determined by the CD spec-

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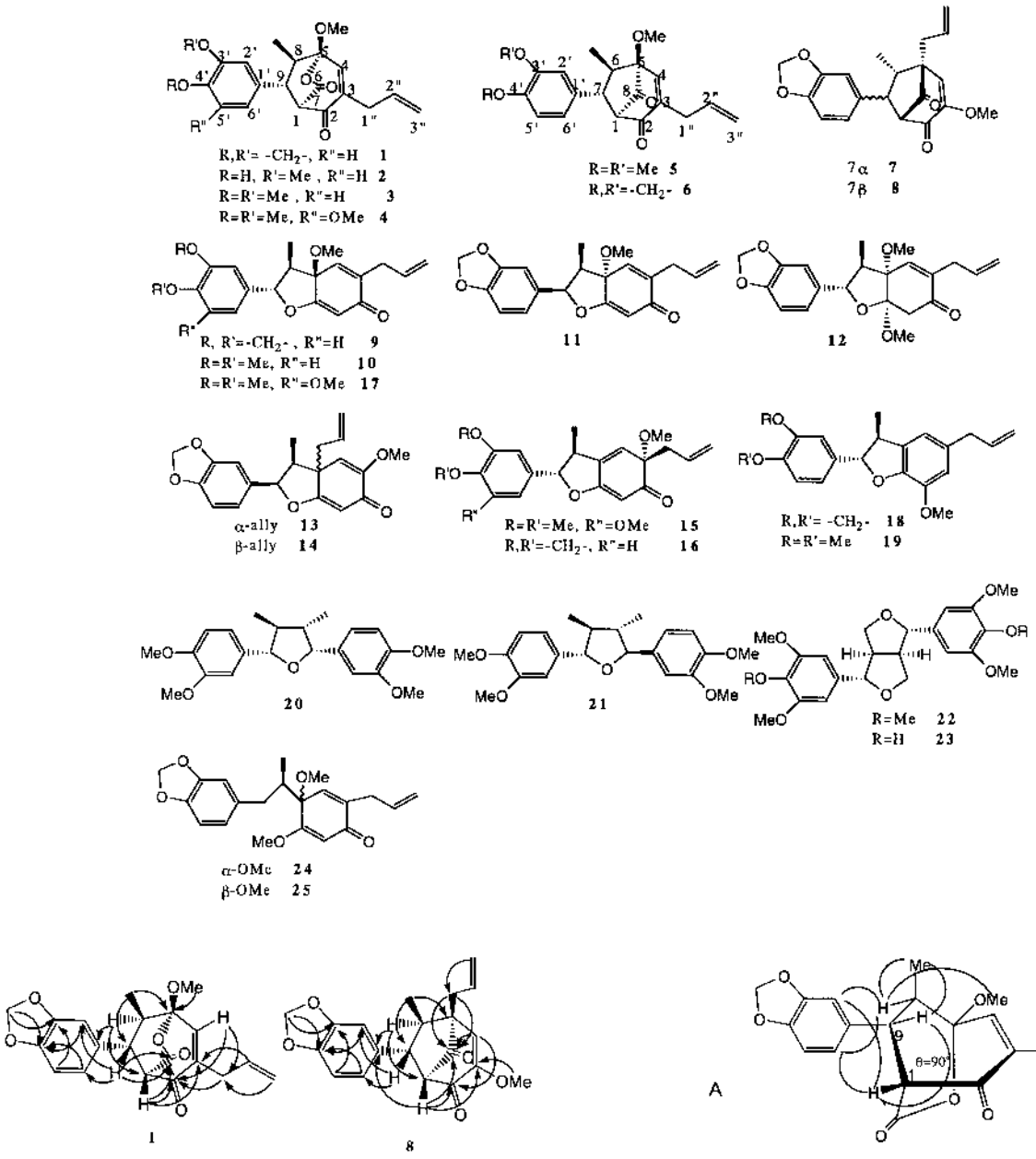

Fig. 1. Significant HMBC Correlations of **1** and **8**

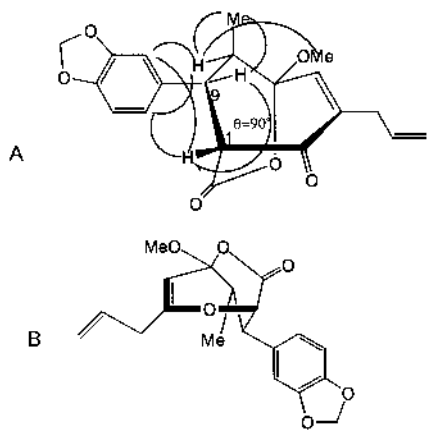

Fig. 2. Difference NOEs and Dihedral Angle between H-1 and H-9 (A) and Octant Projection of **1** (B)

A) Dihedral angle between H-1 and H-9 was predicted to be 90 degrees from the coupling constant of H-1 (J=0 Hz) and was supported from Dreiding model. B) Octant projection of α,β-unsaturated ketone of **1** was deduced from CD data ($[\theta]_{343}$−3465; n→π*, $[\theta]_{238}$+28182; $\pi\rightarrow\pi$*).

trum of **1**, in which CD Cotton effects based on n→π* and $\pi\rightarrow\pi$* transition of the α,β-unsaturated ketone chromophore was observed at 343 nm (−3465) and 238 nm (+28182), respectively . The CD Cotton effects were applied to the empirical rule[19, 20] for an α,β-unsaturated ketone as shown in Fig. 2, which determined the absolute configuration to be (1*R*,5*S*,8*R*,9*R*). Thus, the structure of **1** was elucidated to be (1*R*,5*S*,8*R*,9*R*)-3-allyl-8-methyl-5-methoxy-9-(3,4-methylenedioxyphenyl)-6-oxabicyclo[3.2.2]non-3-en-2,7-dione.

The molecular formula of denudanolide B (**2**) was assumed to be $C_{20}H_{22}O_6$ from the EI-MS (*m/z* 358 $[M]^+$). The ^{13}C-NMR data showed the presence of twenty carbon signals, from which the molecular formula of **2** was determined to be $C_{20}H_{22}O_6$. The ^{1}H-NMR spectrum of **2** was very similar (see Table 1) with those of **1** except the presence of 4-hydroxy-3-methoxyphenyl [δ 3.87 (s)] instead of a 3,4-methylenedioxyphenyl group. These facts were indicated from the ^{13}C-NMR spectrum (see Table 2) and the molecular formula of **2**. The position of the methoxyl group on the phenyl moiety was confirmed by the difference NOE experiment. Irradiation at the methoxyl group (δ 3.87) on the aromatic ring gave a correlation with H-2 [δ 6.62 (d, J=2.0 Hz)]. The CD spec-

Table 1. ^{1}H-NMR Data of 6-Oxabicyclo[3.2.2]nonane and Bicyclo[3.2.1]octane Type Neolignans (400 MHz, in $CDCl_3$)

	1	**2**	**3**	**4**
H-1	3.87 s	3.91 s	3.91s	3.93 s
4	6.71 br s	6.73 br s	6.73 br s	6.74 br s
6				
7				
8	2.39 dq (J=8.4, 8.0)	2.41 dq (J=8.4, 6.8)	2.43 dq (J=8.0, 6.8)	2.45 dq (J=8.8, 6.8)
9	2.59 d (J=8.4)	2.60 d (J=8.4)	2.62 d (J=8.0)	2.66 d (J=8.8)
2′	6.64 d (J=1.6)	6.62 d (J=2.0)	6.65 d (J=2.0)	6.35 s
5′	6.75 d (J=8.0)	6.86 d (J=7.6)	6.81 d (J=8.4)	
6′	6.62 dd (J=8.0, 1.6)	6.66 dd (J=7.6, 2.0)	6.72 dd (J=8.4, 2.0)	6.35 s
1″	3.10 mdd (J=16.4, 6.8)	3.11 mdd (J=16.0, 5.2)	3.11 mdd (J=16.8, 6.8)	3.11 mdd (J=16.0, 6.8)
	3.01 mdd (J=16.4, 6.8)	3.04 mdd (J=16.0, 6.8)	3.05 md (J=16.8, 6.8)	3.05 mdd (J=16.0, 7.2)
2″	5.80 ddt (J=16.8, 10.0, 6.8)	5.81 ddt (J=17.2, 10.4, 6.8)	5.81 ddt (J=17.2, 10.4, 6.8)	5.81 ddt (J=17.2, 10.6, 6.8)
3″	5.18 md (J=10.0)	5.19 md (J=10.4)	5.19 md (J=10.4)	5.19 md (J=10.4)
	5.15 md (J=16.8)	5.16 md (J=17.2)	5.16 md (J=17.2)	5.17 md (J=17.2)
Me	1.07 d (J=8.0)	1.08 d (J=6.8)	1.09 d (J=6.8)	1.11 d (J=6.8)
OMe	3.56 s	3.75 s	3.57 s	3.58 s
		3.87 s	3.86 s	3.84 s×2
			3.87 s	3.83 s
O–CH_2–O	5.96 s			

	5	**6**	**7**	**8**
H-1	3.52 s	3.59 s	3.74 d(J=7.2)	3.54 s
4	7.04 br s	7.03 br s	6.26 s	5.78 s
6	2.46 over lap	2.41 dq (J=8.0, 6.5)	2.53 dq (J=6.0, 7.2)	2.10 quin (J=7.2)
7	2.46 over lap	2.45 d (J=8.0)	3.11 dd (J=6.0, 7.2)	2.50 over lap
8				
9				
2′	6.57 d (J=2.0)	6.58 d (J=2.0)	6.52 d (J=2.0)	6.53 d (J=2.0)
5′	6.79 d (J=8.0)	6.73 d (J=8.0)	6.70 d (J=7.6)	6.72 d (J=8.4)
6′	6.66 dd (J=8.0, 2.0)	6.57 dd (J=8.0, 2.0)	6.54 dd (J=7.6, 1.6)	6.53 dd (J=8.4, 2.0)
1″	3.20 mdd (J=16.4, 7.2)	3.12 mdd (J=16.5, 7.0)	2.61 dd (J=14.8, 7.2)	2.50 over lap
	3.07 mdd (J=16.4, 6.8)	3.07 mdd (J=16.5, 7.0)	2.44 dd (J=14.8, 6.8)	
2″	5.86 ddt (J=16.0, 10.8, 6.8)	5.85 ddt (J=16.5, 10.5, 7.0)	5.95 ddt (J=16.8, 10.4, 7.0)	5.96 ddt (J=17.2, 10.0, 7.6)
3″	5.18 md (J=10.8)	5.18 md (J=10.5)	5.29 md (J=16.8)	5.19 md (J=17.2)
	5.17 md (J=16.0)	5.17 md (J=16.5)	5.27 md (J=10.4)	5.16 md (J=10.0)
Me	1.07 d (J=6.0)	1.06 d (J=6.5)	1.14 d (J=7.2)	1.05 d (J=6.8)
OMe	3.63 s	3.63 s	3.68 s	3.73 s
	3.84 s			
	3.85 s			
O–CH_2–O		5.94 s	5.91 s	5.94 s

trum of **2** was not measured because of a lack of sample amount. Similarities of the ^{1}H-NMR (Table 1) and ^{13}C-NMR data (Table 2) of the 6-oxabicyclo[3.2.2]-nonane part of **2** with those of **1** indicated that **2** had the same relative configuration with that of **1** and the absolute configuration of **2** was assumed to be the same as that of **1**. Thus, the structure of **2** was assumed to be 3-allyl-8-methyl-5-methoxy-9-(4-hydroxy-3-methoxyphenyl)-6-oxabicyclo-[3.2.2]non-3-en-2,7-dione and the absolute configuration of **2** was supposed to be (1*R*,5*S*,8*R*,9*R*).

Denudanolide C (**3**) gave the molecular formula, $C_{21}H_{24}O_6$, from HR-EI-MS (*m/z* 372.1573 $[M]^+$). The IR spectrum of **3** showed the absorption bands due to lactone carbonyl (1750 cm^{-1}) and an α,β-unsaturated ketone (1681 cm^{-1}). The ^{1}H-NMR spectrum of **3** showed similar signal patterns with those of **2** except for the presence of three methoxy groups [δ 3.57 (3H, s), 3.87 (6H, s)]. The ^{13}C-NMR spectrum of **3** also showed the same signal patterns with that of **1** except for two methoxy groups on the aromatic ring. These indicated the presence of a 6-oxabicyclo[3.2.2]nonane skeleton and a 3,4-dimethoxyphenyl group in **3**. The CD spectrum of **3** showed a similar Cotton effect ($[\theta]_{345}-3720$, $[\theta]_{236}+3054$) with those of **1**. From these data, the structure of **1** was determined to be (1*S*,5*R*,8*R*,9*R*)-3-allyl-9-methyl-5-methoxy-8-(3,4-dimethoxyphenyl)-6-oxabicyclo[3.2.2]non-3-en-2,7-dione.

Denudanolide D (**4**) gave the molecular formula $C_{22}H_{26}O_7$ from the HR-EI-MS (*m/z* 402.1704 $[M]^+$). The IR spectrum showed absorption bands due to a lactone carbonyl (1750 cm^{-1}) and an α,β-unsaturated ketone (1681 cm^{-1}). The ^{1}H-NMR spectrum showed the presence of a symmetrical 3,4,5-trimethoxyphenyl group [δ 3.83 (3H,s), 3.84 (6H, s), 6.35 (2H, s)]. The ^{1}H-NMR signal patterns of the other parts were identical with those of **1** (see Table 1). This was also seen in the ^{13}C-NMR data [δ 60.9 (MeO at C-4′), 56.3 (MeO at 3′ and 5′), 136.7 (at C-4′), 153.8 (at C-3′ and 5′), 104.4 (at C-2′ and 6′), 137.7 (at C-1′)]. From these data the structure of **4** was assumed to be a 6-(3,4,5-trimethoxyphenyl)-derivative of **1**. The CD spectrum of **4** also showed a similar Cotton effect ($[\theta]_{347}-4248$, $[\theta]_{248}+15624$) with that of **1**. These facts

Table 2. ^{13}C-NMR Data of 6-Oxabicyclo[3.2.2]nonane and Bicyclo[3.2.1]octane Type Neolignans (100 MHz, in $CDCl_3$)

C-No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	65.4	65.6	66.5	66.3	69.9	69.8	69	70.3
2	189.3	189.4	189.4	189.3	194.4	194.3	189.9	190.2
3	140.7	140.8	140.8	140.9	140.5	140.4	153.7	152.5
4	143.6	143.5	143.5	143.6	147.2	147.2	121.9[a)]	118.0
5	106.6	106.7	106.7	106.6	89.4	89.3	55.0	57.4
6					45.3	45.2	44.8	47.0
7	166	166.3	166.1	166.2	48.8	48.9	49.5	50.2
8	45.3	45.4	45.3	45.3	202.2	202	201.4	202.6
9	46.0	46.0	45.9	46.5				
Me	15.3	15.4	15.4	15.6	13.7	13.5	18.1	14.0
1′	134.6	132.8	133.5	137.7	133.9	135.0	131.6	135.3
2′	107.4	109.2	110.1	104.3	110.1	107.3	108.5[b)]	107.3
3′	147.3	145.3	148.7	153.8	148.5	148.3	147.1	148.3
4′	148.5	147.2	149.7	136.7	149.4	147	148.1	146.9
5′	108.7	114.8	111.7	153.8	111.5	108.5	108.6[b)]	108.6
6′	120.7	120.4	119.6	104.3	119.3	120.5	121.7[a)]	120.6
1″	34.1	34.2	34.2	34.2	32.7	32.7	32.6	35.5
2″	133.7	133.7	143.5	143.6	133.8	133.8	133.3	133.2
3″	118.5	118.5	118.5	118.6	118.1	118.1	119.6	119.1
OMe	50.9	50.9	50.9	51.0	54.0	53.9	55.7	55.7
		56.0	56.0 ×2	56.3×2	56.0			
				60.9	56.1			
O–CH_2–O	101.4					101.3	101.2	101.2

a,*b*) Assignments may be interchangeable.

indicated that the structure of **4** was (1*R*,5*S*,8*R*,9*R*)-3-allyl-8-methyl-5-methoxy-9-(3,4,5-trimethoxyphenyl)-6-oxabicyclo[3.2.2]non-3-en-2,7-dione.

Denudadione A (**5**) gave the molecular formula, $C_{21}H_{24}O_5$, from HR-EI-MS (*m/z* 356.1611 $[M]^+$). The IR spectrum of **5** showed absorption bands due to a strained ketone (1767 cm^{-1}) and an α,β-unsaturated ketone (1681 cm^{-1}). The ^{1}H-NMR spectrum of **5** showed the presence of two methoxyl groups [δ 3.84 (3H, s), 3.85 (3H, s)] on aromatic carbons, a methoxyl group [δ 3.63 (3H, s)] on an aliphatic carbon, an allyl group [δ 5.17 (md, J=16.0 Hz), 5.18 (md, J=10.8 Hz), 5.86 (ddt, J=16.0, 10.8, 6.8 Hz), 3.20 (mdd, J=16.4, 7.2 Hz), 3.07 (mdd, J=16.4, 6.8 Hz)], a doublet methyl group [δ 1.07 (d, J=6.0 Hz)], a 3,4-dioxyphenyl group [δ 6.79 (d, J=8.0 Hz), 6.66 (dd, J=8.0, 2.0 Hz), 6.57 (d, J=2.0 Hz)], a singlet methine proton (δ 3.49) and a singlet olefin proton [δ 7.04 (s)]. The ^{13}C-NMR spectrum of **5** showed the presence of two carbonyl carbons (δ 202.2 and 194.4), a 3,4-dioxyphenyl group (δ 148.5, 149.4, 133.9, 119.3, 111.5, 110.1), two double bonds (δ 140.5, 147.2, 133.8, 118.1), a carbinyl carbon (δ 89.4), a characteristicly lower shifted methine carbon (δ 69.9), two methoxyl carbons on an aromatic ring [δ 56.0 (MeO×2)] and a methoxyl group (δ 54.0) on an aliphatic carbon. From this NMR evidence, the planar structure was assumed to be a bicyclo[3.2.1]octane derivative, and its structure was confirmed by HMBC experiment as shown in Fig. 1. The H-1 proton appeared at δ 3.14 as singlet signal, from which the dihedral angle between H-1 and H-7 of **5** was assumed to be approximately 90°. The Dreiding molecular model of **5** showed the presence of only one conformation having approximately 90° dihedral angle between H-1 and H-9. Relative configuration of **5** was deduced by means of a difference NOE experiment (Fig. 2). The absolute configuration of **5** was assumed from reaction mechanism from bicyclo[3.2.1]octane derivatives into 6-oxabicyclo[3.2.2]-nonane derivatives (**1**—**4**). In the course of the Baeyer-Villiger oxidation of bicyclo[3.2.1]octane derivatives to 6-oxabicyclo[3.2.2]nonane derivatives, the configuration at C-4 was retained, so the absolute configuration of **5** turns out to be (1*R*,5*R*,6*R*,7*R*). The CD spectrum of **5** showed a negative Cotton effect ($[\theta]_{335}$−10252) and a positive Cotton effect ($[\theta]_{270}$+5553). These results were applied to the empirical rule[19,20] to give the same result as the above supposition. From these facts, the structure of **5** was determined to be (1*R*,5*R*,6*R*,7*R*)-3-allyl-6-methyl-5-methoxy-7(3,4-dimethoxyphenyl)-bicyclo[3.2.1]oct-2-en-2,8-dione.

Denudadione B (**6**) gave the molecular formula, $C_{20}H_{20}O_5$, from HR-EI-MS (*m/z* 340.1282 $[M]^+$). The ^{1}H-NMR spectrum of **6** showed a similar signal pattern with that of **5** except for the presence of a methylene dioxyphenyl group [δ 5.94 (s)] instead of the 3,4-dimethoxphenyl group in **5**. The ^{13}C-NMR spectrum also showed the presence of a 3,4-methylenedioxyphenyl group (δ 101.3). The ^{1}H- and ^{13}C-NMR spectra of the bicyclo[3.2.1]octane part of **6** were identical with those of **5** (Tables 1, 2). The CD spectrum ($[\theta]_{332}$−6195, $[\theta]_{262}$−12628) of **6** was identical with that of **5**. From these facts, the structure of **6** was determined to be (1*R*,5*R*,6*R*,7*R*)-3-allyl-6-methyl-5-methoxy-7-(3,4-methylendioxyphenyl)-bicyclo[3.2.1]oct-2-en-2,8-dione.

Compound **8** gave *m/z* 340 $[M]^+$ from EI-MS. The ^{13}C-NMR spectrum gave 20 carbon signals. From these data, the molecular formula of **8** was deduced to be $C_{20}H_{20}O_5$. The ^{1}H-NMR and ^{13}C-NMR spectra of **8** showed the presence of the same groups with those of **5**. But the signal patterns of the ^{1}H-NMR and ^{13}C-NMR spectra of **8** showed a small difference with those of **5**. This was assumed to depend on the difference of the position of the methoxyl group and the allyl group. So we assumed that the position of the methoxyl group and the allyl group should be interchanged. This was confirmed by the HMBC experiment. The relative configuration of **8** was discussed from the coupling constants (the coupling constant between H-1 and H-7 was 0 Hz) and a differ-

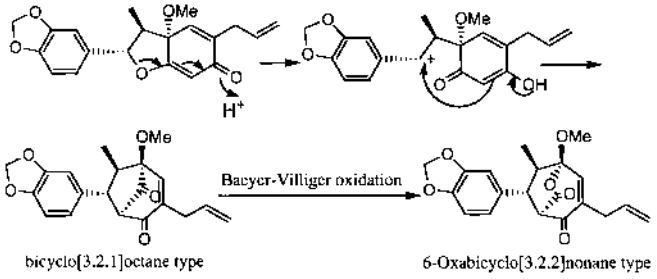

Fig. 3. Biogenesis of Denudanolides from Hydrobenzofurane Type Neolignan

ence NOE experiment. These indicated that **8** was a known compound isolated from *Mezilaurs itauba*[21] including the relative configuration. The racemate of this compound was synthesized as an intermediate to guianin.[22] The CD spectrum of **8** gave a opposite Cotton effect ($[\theta]_{350}+3825$ and $[\theta]_{250}-14025$) with that of **5**. Thus, the absolute stereochemistry of the 6-oxabicyclo[3.2.2]nonane skeleton of **8** was found to be antipodal with that of **5**.

Denudadione C (**7**) gave the molecular formula, $C_{20}H_{20}O_5$, from HR-EI-MS, *m/z* 340.1323 $[M]^+$. The ^{1}H-NMR and ^{13}C-NMR spectra of **7** showed the presence of a 7-(3,4-methylendioxyphenyl) group, a 5-allyl group, a 3-methoxy group and a bicyclo[3.2.1]oct-3-en-2,8-dione skeleton, the same as **8**. But, the H-1 proton appeared as doublet signal (J=7.2 Hz). This indicated that the relative configuration between H-1 and H-7 was different from that of **8**, and **7** was an epimer of **8** at C-7. Thus, the structure of **7** was found to be rel-(1*S*,5*S*,6*S*,7*R*)-5-allyl-6-methyl-3-methoxy-7-(3,4-methylenedioxyphenyl)-bicyclo[3.2.1]oct-3-en-2,8-dione.

Ten compounds, **1**, **9**, **12**—**14**, **17** and **19**—**22**, of the isolated neolignans were tested for anti-PAF activity by Miwa's method as described in the previous papers.[2] Of these tested compound, **1** and **9** showed moderate anti-PAF activity, 50% inhibition at 50 and 100 μg/ml, respectively.

The novel 6-oxabicyclo[3.2.2]nonane type neolignans might be synthesized from hydrobenzofuranoid type neolignanes through a bicyclo[3.2.1]octane type neolignan by protonation at the C-6 carbonyl, followed by skeleton rearrangement and Baeyer-Villiger oxidation as shown in Fig. 3.

Experimental

^{1}H-NMR and ^{13}C-NMR spectra were measured with a 400 MHz instrument (JEOL α-400), chemical shifts are given in δ value (ppm) with tetramethylsilane (TMS) as internal standard. Column chromatographies were carried out on silica gel (Merck). Semi-preparative HPLC was carried out on an ODS column (YMC R-ODS-7) using the CH_3CN–H_2O solvent system. EI-MS and HR-EI-MS were measured on JEOL JMS-AX505W mass spectrometer. IR spectra were obtained with a Perkin Elmer GX FT-IR spectrometer. UV spectra were obtained with a Hitachi U3410 spectrometer. CD spectra were obtained with JASCO J-20A spectroporalimeter. TLC was carried out using precoated Silica gel 60F_{254} plates (Merck).

Plant Source and Isolation Twigs of *Magnolia denudata* were collected at a private garden in Shizuoka city on July 1995. The twigs (1.5 kg) were powdered and extracted with hot MeOH under reflux. The MeOH extract was partitioned between AcOEt and water to give an AcOEt soluble fraction. The AcOEt soluble fraction (29 g) was chromatographed on a silica gel column using a gradient $CHCl_3$–MeOH solvent system to give ten fractions, fr. 1 (900 mg), fr. 2 (100 mg), fr. 3 (240 mg), fr. 4 (760 mg), fr. 5 (720 mg), fr. 6 (2.0 g), fr. 7 (2.1 g), fr. 8 (800 mg), fr. 9 (1.8 g) and fr. 10 (11 g). These fractions were further separated by repeated and precise HPLC using an ODS column and a CH_3CN–water solvent system. Fraction. 2 gave licarin B (**18**) (13 mg). Fraction 4 gave acuminatin (**19**) (10 mg), denudanolide A (**1**) (17 mg), denudadione A (**5**) (12 mg). Fraction 5 gave denudatin B (**9**) (290 mg), denudatin A (**10**) (40 mg), **11** (10 mg), kadsurin A (**12**) (36 mg) and denudadione B (**6**) (14 mg). Fraction 6 gave denudanolide B (**2**) (20 mg), C (**3**) (13 mg), D (**4**) (12 mg), denudadione C (**7**) (12 mg), **8** (5 mg), veraguensin (**20**) (27 mg), grandisin (**21**) (27 mg), isodihydrofutoquinol A (**24**) (7 mg) and isodihydrofutoquinol B (**25**) (12 mg). Fraction 7 gave mirandin-A (**17**) (20 mg) and yangambin (**22**) (50 mg). Fraction 8 gave **13** (150 mg), **14** (12 mg), burcellin (**15**) (11 mg) and **16** (10 mg). Fraction 10 gave syringresinol (**23**) (200 mg).

Denudanolide A (**1**) HR-EI-MS; *m/z* 356.1220 $[M]^+$ (Calcd for $C_{20}H_{20}O_6$; 356.1259), IR ν_{max} (KBr) cm^{-1}; 1752, 1681, 1490, 1249, 1038, CD; $[\theta]_{343}-3465$, $[\theta]_{280}-2541$, $[\theta]_{238}+28182$ (MeOH), ^{1}H-NMR data are shown in Table 1 and ^{13}C-NMR data are shown in Table 2.

Denudanolide B (**2**) EI-MS; *m/z* 358 $[M]^+$ $C_{20}H_{22}O_6$, ^{1}H-NMR data are shown in Table 1 and ^{13}C-NMR data are shown in Table 2.

Denudanolide C (**3**) HR-EI-MS; *m/z* 372.1546 $[M]^+$ (Calcd for $C_{21}H_{24}O_6$; 372.1573), IR ν_{max} (KBr) cm^{-1}; 1750, 1681, 1518, 1263, 1240, 1144, CD; $[\theta]_{345}-3720$, $[\theta]_{236}+30504$ (MeOH), ^{1}H-NMR data was shown in Table 1 and ^{13}C-NMR data was shown in Table 2.

Denudanolide D (**4**) HR-EI-MS; *m/z* 402.1704 $[M]^+$ (Calcd for $C_{22}H_{24}O_7$; 402.1678), IR ν_{max} (KBr) cm^{-1}; 1750, 1681, 1592, 1240, 1129, CD; $[\theta]_{347}-4248$, $[\theta]_{248}+15624$ (MeOH), ^{1}H-NMR data are shown in Table 1 and ^{13}C-NMR data was shown in Table 2.

Denudadione A (**5**) HR-EI-MS; *m/z* 356.1661 $[M]^+$ (Calcd for $C_{21}H_{24}O_5$; 356.1624), IR ν_{max} (KBr) cm^{-1}; 1768, 1681, 1518, 1263, 1144, CD; $[\theta]_{335}-10252$, $[\theta]_{305}+5553$, $[\theta]_{270}-11108$, $[\theta]_{230}+37288$ (MeOH), ^{1}H-NMR data are shown in Table 1 and ^{13}C-NMR data are shown in Table 2.

Denudadione B (**6**) HR-EI-MS; *m/z* 340.1282 $[M]^+$ (Calcd for $C_{20}H_{20}O_5$; 340.1311), IR ν_{max} (KBr) cm^{-1}; 1767, 1681, 1490, 1249, 1038, CD; $[\theta]_{332}-6195$, $[\theta]_{301}+3812$, $[\theta]_{262}-12628$, $[\theta]_{225}+27877$ (MeOH), ^{1}H-NMR data are shown in Table 1 and ^{13}C-NMR data are shown in Table 2.

Denudadione C (**7**) HR-EI-MS; *m/z* 340.1323 $[M]^+$ (Calcd for $C_{20}H_{20}O_5$; 340.1311), ^{1}H-NMR data are shown in Table 1, ^{13}C-NMR data are shown in Table 2.

(1*S*,5*S*,6*S*,7*S*)-5-Allyl-6-methyl-3-methoxy-7(3,4-methylendioxyphenyl)-bicyclo[3.2.1]oct-3-en-2,8-dione (**8**) EI-MS; *m/z* 340 $[M]^+$ $C_{20}H_{20}O_5$, IR ν_{max} (KBr) cm^{-1}; 1671, 1624, 1517, 1268, 1140, CD; $[\theta]_{350}+3825$, $[\theta]_{250}-14205$ (MeOH), ^{1}H-NMR data are shown in Table 1 and ^{13}C-NMR data are shown in Table 2.

Anti-PAF Activity Anti-PAF activity test was carried out according to the novel method for determination of acetyl-CoA : *lyso*-PAF acetyltransferase activity as previously mentioned.[2]

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