J Wood Sci (2008) 54:483–489
DOI 10.1007/s10086-008-0983-3

ORIGINAL ARTICLE

Syamsul Falah · Takeshi Katayama · Toshisada Suzuki

Chemical constituents from *Gmelina arborea* bark and their antioxidant activity

Received: March 24, 2008/ Accepted: August 4, 2008 / Published online: November 3, 2008

Abstract *Gmelina arborea* Roxb. is a fast-growing species and is known to have been used in traditional Indian medicine. Chemical constituents from the bark have not been reported, although some chemical constituents from part of this plant (heartwood, leaf, and root) are known. In this study, the bark meal was successively extracted with acetone and methanol. Fractionation of the acetone extract with *n*-hexane, diethyl ether, and ethyl acetate and subsequent chromatographic separation of the fractions led to the isolation of four compounds. The diethyl ether-soluble fraction yielded tyrosol [2-(4-hydroxyphenyl)ethanol] (**1**); (+)-balanophonin (**2**), an 8-5′ neolignan, with opposite optical rotation to known (−)-balanophonin; and gmelinol (**3**), a known lignan. The ethyl acetate-soluble fraction afforded a new phenylethanoid glycoside to the best of our knowledge, which was identified as (−)-*p*-hydroxyphenylethyl[5‴-*O*-(3,4-dimethoxycinnamoyl)-*β*-D-apiofuranosyl(1‴ → 6′)]-*β*-D-glucopyranoside (**4**). From the methanol extract, two known compounds, 2,6-dimethoxy-*p*-benzoquinone (**5**) and 3,4,5-trimethoxyphenol (**6**), were isolated and identified. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay of the identified compounds indicated that 3,4,5-trimethoxyphenol (**6**) exhibited moderate activity.

Key words *Gmelina arborea* Roxb · Bark extractives · Phenylethanoid glycoside · Antioxidant activity

S. Falah · T. Katayama (✉) · T. Suzuki
Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki-cho, Kagawa 761-0795, Japan
Tel. +81-87-891-3083; Fax +81-87-891-3021
e-mail: katayama@ag.kagawa-u.ac.jp

Part of this report was presented at the 57th and 58th Annual Meetings of the Japan Wood Research Society, Hiroshima and Tsukuba, August 2007 and March 2008, respectively

Introduction

Bark is a portion of the vascular plant body consisting of all tissues external to the vascular cambium. Bark has several functions in the living tree. It protects the tree from attack by microorganisms, insects, and mammals, and from chemicals in the atmosphere.[1] Mechanically, the bark contributes to bending strength of the stems.[2] In the wood processing industry and the pulp and paper industry, bark is a major by-product. It is used in energy production in boiler kilns or furnaces. A tannin-based adhesive from bark can be used as a substitute for synthetic phenolic resin,[3] and can be used to produce particleboard.[4] In some countries, bark has been used as a source of remedies for treatment of many diseases. The most popular bark used as a natural medicine is *Cinchona officinalis* bark, which has been used as an antimalarial agent during the past two centuries. The bark also has potential use as an interesting source of strong antioxidants.[5] Pycnogenol, which is extracted from the bark of *Pinus maritima*, has antioxidant activity.[6]

In this study, bark of *Gmelina arborea* Roxb. was used. This woody plant has been used as cardiotonic in traditional Indian medicine.[7] Its stem bark can be used as an antidiarrhoeal[8] and for treatment of intermittent fevers.[9] Its leaf can be used for stomach disorders,[10] can be given orally for coughs, gonorrhoea, and ulcers, and can be used topically for scorpion stings and snakebites.[11] The crushed roasted fruit and the juice are applied externally to the itch.

Gmelina arborea is a fast-growing tree species.[12] It has been widely planted in southern China, Bangladesh, and Southeast Asian countries, including Myanmar, Thailand, Vietnam, Indonesia, and the Philippines;[13] and less widely in tropical African and Latin American countries.[14] Its wood is suitable for molding, furniture, interior wood working, ship building, plywood,[15] pulp and paper,[16] and can be used for carpentry and fodder.[17] Some chemical constituents from this tree are known. Lignans,[18–22] iridoid glycosides,[23] flavonoids,[24] furanoresorcinol,[25] and an isoxazole alkaloid[26] were isolated from the heartwood. An apiose-containing coumarin glycoside was isolated from the root.[27]

Its leaves contained luteolin (a flavone),[28] alkaloids,[29] and gmelinoside (an acetylated iridoid glycoside).[30] It was reported that lignans from the heartwood of *G. arborea* [(+)-arboreol, (+)-paulownin, (+)-gmelinol, and (+)-epieudesmin] showed antifungal activity.[22] However, chemical constituents from the bark have not been reported. Recently, it was observed that bark and fruit of *G. arborea* protected liver cells from oxidative stress.[31] In this study, we isolated and identified chemical constituents from the bark of *G. arborea* Roxb. and investigated their 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity.

Materials and methods

General experimental procedures

Column chromatography was conducted on a FMI pump system with a Sigma-Aldrich Michel-Miller column of Merck silica gel 60 (230–400 mesh, ASTM). Analytical thin-layer chromatography (TLC) and preparative TLC were performed by using plates precoated with Merck silica gel 60 F_{254} (0.25 and 0.50 mm thickness, respectively). Analytical high-performance liquid chromatography (HPLC) was carried out on a Jasco MD-2010 plus equipped with a Jasco UV-970 intelligent UV/Vis detector (280 nm) and a Shimadzu Chromatopac C-R6A, using a reversed-phase column (Tosoh, TSK-GEL ODS-80TS, 250 × 4.6 mm i.d., stainless steel). Preparative HPLC (flow rate: 2.0 ml/min, detection: UV 280 nm) was done using the same system as above except for the column size (300 × 7.8 mm i.d.). Melting points were measured on a Yanaco MP 52641 micro melting point apparatus and were uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra and two-dimensional NMR spectra [^{1}H-^{1}H correlation spectroscopy (COSY), nuclear Overhauser effect (NOE) spectroscopy (NOESY), ^{1}H detected multiple quantum coherence (HMQC) spectroscopy, and ^{1}H detected multiple bond connectivity (HMBC) spectroscopy] were obtained on a JEOL JNM Alpha 400 FT-NMR spectrometer using tetramethylsilane as an internal standard. Chemical shifts were expressed as δ (in ppm) and coupling constants (J) were recorded in hertz. Electron-impact (EI, 70 eV) and fast-atom bombardment (FAB) mass spectra (MS) were acquired on a JEOL JMS-SX102A mass spectrometer. Fourier-transform infrared (FT-IR) spectra were recorded on a Jasco FT/IR-670 Plus spectrophotometer. UV-visible absorbance was determined on a Shimadzu UV 1600 spectrophotometer. Optical rotations were measured with a Jasco P-1010 polarimeter.

Plant materials

The bark was stripped from *Gmelina arborea* trunk that was collected from Sumedang, West Java, Indonesia, in May 2005. The chipped bark was ground in a Wiley mill and the resulting meal was screened to give 40–80 mesh bark meal. The voucher specimen was deposited in the Department of Forest Product Technology, Winaya Mukti University, Bandung, Indonesia.

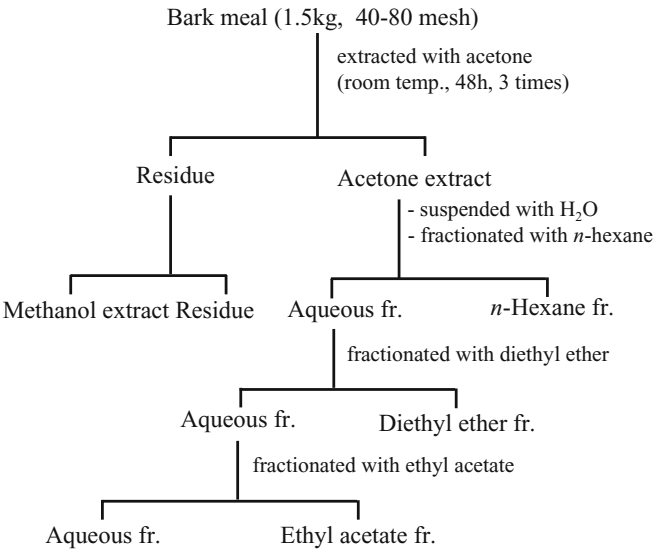

Fig. 1. Procedure for extraction and fractionation of *Gmelina arborea* bark meal

Extraction and fractionation

The bark meal (40–80 mesh, 1500 g air-dried) was extracted three times with acetone (3000 ml) for 48 h at room temperature, and then with methanol (MeOH) similarly to give an acetone extract (12.54 g) and a methanol extract (15.10 g). The acetone extract was reconstituted with 75 ml of acetone and the solution was suspended with the addition of 20 ml of water. The suspension was successively fractionated with *n*-hexane, diethyl ether, and ethyl acetate (EtOAc), to give *n*-hexane-soluble fraction (3.42 g), diethyl ether-soluble fraction (1.82 g), and EtOAc-soluble fraction (1.72 g), respectively. The general scheme of these successive fractionations is shown in Fig. 1.

Isolation and identification of compounds

The diethyl ether-soluble fraction was chromatographed on a silicagel column (*n*-hexane/EtOAc = 100:0 to 0:100 as a stepwise elution, column size 300 × 21 mm i.d.) to give 120 fractions (each 20 ml). On the basis of UV detection (280 nm), these fractions were collected to afford seven major fractions: fr. I (frs. 1–36), fr. II (frs. 37–53), fr. III (frs. 54–74), fr. IV (frs. 75–90), fr. V (frs. 91–102), fr. VI (frs. 103–110), and fr. VII (frs. 111–120). Fr. III was purified by preparative HPLC [MeOH/H_2O = 20:80 (v/v) as eluent] to give compound **1** (16.3 mg). Fr. IV was separated by TLC [*n*-hexane/EtOAc = 1:4 (v/v) as developing solvent] to yield nine fractions (frs. IV-1 to IV-9). Fr. IV-2 (R_f 0.81) was further separated by TLC [*n*-hexane/EtOAc = 1:4 (v/v)] to afford four fractions (frs. IV-2-1 to IV-2-4), and then fr. IV-2-3 (R_f 0.56) was purified by preparative HPLC [MeOH/H_2O = 30:70 (v/v)] to give compound **2** (9.7 mg). Fr. IV-3

(R_f 0.75) was repurified by TLC [*n*-hexane/EtOAc = 1:4 (v/v)] to yield five fractions (frs. IV-3-1 to IV-3-5) and then fr. IV-3-3 (R_f 0.56) was subjected to preparative HPLC [MeOH/H_2O = 45:55 (v/v)] to give compound **3** (0.8 mg).

The ethyl acetate-soluble fraction was chromatographed on a silica gel column [*n*-hexane/EtOAc = 100:0 to 0:100 (v/v)] to give 67 fractions (each 20 ml). These fractions were collected with UV detection (280 nm) to afford 9 major fractions, fr. I′ (frs. 1–10), fr. II′ (frs. 11–15), fr. III′ (frs. 16–20), fr. IV′ (frs. 21–25), fr. V′ (frs. 26–34), fr. VI′ (frs. 35–45), fr. VII′ (frs. 46–53), fr. VIII′ (frs. 54–58), and fr. IX′ (frs. 59–67). Fr. VIII′ was separated by TLC [CH_2Cl_2/MeOH = 85:15 (v/v)] to give 9 fractions (frs. VIII′-1 to VIII′-9). Fr. VIII′-3 (R_f 0.47) was purified by TLC [CH_2Cl_2/MeOH = 80:20 (v/v)] to yield 7 fractions (frs. VIII′-3-1 to VIII′-3-7). Fr. VIII′-3-3 (R_f 0.56) was subjected to preparative HPLC [MeOH/H_2O = 40:60 (v/v)] to give compound **4** (23.4 mg).

The methanol extract was chromatographed on a silica gel column [*n*-hexane/EtOAc = 100:0 to 0:100 (v/v), column size 450 × 51 mm i.d.] to give 150 fractions (each 20 ml). These fractions were collected to afford 12 major fractions, fr. I″ (frs. 1–25), fr. II″ (frs. 26–38), fr. III″ (frs. 39–50), fr. IV″ (frs. 51–63), fr. V″ (frs. 64–73), fr. VI″ (frs. 74–84), fr. VII″ (frs. 85–95), fr. VIII″ (frs. 96–112), fr. IX″ (frs. 113–120), fr. X″ (frs. 121–128), fr. XI″ (frs. 129–138), and fr. XII″ (frs. 139–150). Fr. IV″ was separated by TLC [*n*-hexane/EtOAc = 1:4 (v/v)] to afford 9 fractions (frs. IV″-1 to IV″-9), and fr. IV″-7 (R_f 0.44) was compound **5** (3.7 mg). Fr. V″ was purified by TLC [CH_2Cl_2/MeOH = 85:15 (v/v)] to afford 9 fractions (frs. V″-1 to V″-9). Fr. V″-4 (R_f 0.75) was repurified by TLC [CH_2Cl_2/MeOH = 85:15 (v/v)] to give compound **6** (2.2 mg) (R_f 0.47). The chemical structure of isolated compounds was determined by EI-MS, FAB–MS, and ^{1}H and ^{13}C NMR spectroscopy.

*Compound **1***. White powder. Mp. 89°–92°C (lit. 91°C).[32] IR (KBr): ν_{max} cm^{-1} 3389, 3149, 2879, 2512, 1599, 1513, 1452, 1363, 1233, 1105. EI-MS *m/z* (rel. int.): 138 $[M]^+$ (60), 107 (100), 77 (60). ^{1}H NMR ($CDCl_3$): δ 2.80 (2H, t, J = 6.6 Hz, 7-H_2), 3.82 (2H, br. t, J = 6.6 Hz, 8-H_2), 4.97 (1H, br. s, Ar-4-OH), 6.78 (2H, d, J = 8.3 Hz, equivalent, 3- and 5-H), 7.10 (2H, d, J = 8.5 Hz, equivalent, 2- and 6-H).

*Compound **2***. A pale yellow oil. $[\alpha]_D^{20}$ +33° (*c* 0.090, MeOH). IR (KBr): ν_{max} cm^{-1} 3398, 1663, 1593, 1518, 1332, 1211, 1133. EI-MS *m/z* (rel. int.): 356 $[M]^+$ (21), 338 $[M–H_2O]^+$ (100), 326 $[M–CH_2O]^+$ (56), 165 (32), 152 (28), 151 (18), 137 (22), 115 (27), 77 (39). ^{1}H NMR ($CDCl_3$): δ 3.69 (1H, m, 8-H), 3.88 (3H, s, 3-OCH_3), 3.94 (3H, s, 3′-OCH_3), 3.96–4.00 (2H, partially overlapping, 9-H_2), 5.63 (1H, s, Ar-OH), 5.65 (1H, d, J = 7.1 Hz, 7-H), 6.62 (1H, dd, J = 15.7, 7.7 Hz, 8′-H), 6.90 (3H, br. s, 2-, 5-, and 6-H), 7.05 (1H, br. s, 2′-H), 7.14 (1H, br. s, 6′-H), 7.42 (1H, d, J =15.6 Hz, 7′-H), 9.66 (1H, d, J = 7.8 Hz, 9′-CHO).

*Compound **3***. Colorless powder. EI-MS *m/z* (rel. int.): 402 $[M]^+$ (100), 236 (23), 221 (19), 207 (21), 179 (51), 177 (44), 167 (42), 166 (68), 165 (61), 151 (61), 139 (31), 95 (20). ^{1}H NMR ($CDCl_3$): δ 3.13 (1H, m, 8-H), 3.32 (1H, t, J = 9.4 Hz, 9a-H), 3.72 (1H, d, J = 9.5 Hz, 9′a-H), 3.89 (3H, s, 4-OCH_3), 3.90 (3H, s, 3-OCH_3), 3.91 (3H, s, 4′-OCH_3), 3.92 (3H, s, 3′-OCH_3), 3.98 (1H, t, J = 9.1 Hz, 9b-H), 4.23 (1H, d, J = 9.3 Hz, 9′b-H), 4.61 (1H, s, 7′-H), 5.23 (1H, d, J = 6.3 Hz, 7-H), 6.84 (1H, dd, J = 8.4, 1.3 Hz, 6-H), 6.87 (1H, d, J = 8.3, 5-H), 6.90 (1H, d, J = 1.5 Hz, 2-H), 6.91 (1H, d, J = 8.5 Hz, 5′-H), 6.95 (1H, dd, J = 8.3, 1.7 Hz, 6′-H), 6.97 (1H, d, J = 1.7 Hz, 2′-H).

*Compound **4***. Colorless powder. Mp. 83°–85°C. $[\alpha]_D^{20}$ –38.8° (*c* 0.129, MeOH). IR (KBr): ν_{max} cm^{-1} 3348, 1516, 1262. FAB-MS *m/z*: 645 $[M+Na]^+$, 623 $[M+1]^+$, 622 $[M]^+$. ^{1}H and ^{13}C NMR (CD_3OD) data, see Table 1.

*Compound **5***. Yellow powder. Mp. 218°–220°C. IR (KBr): ν_{max} cm^{-1} 3061, 1696, 1644, 1592, 1441. EI-MS *m/z* (rel. int.): 168 $[M]^+$ (100), 138 (53), 125 (51), 112 (27), 97 (46), 80 (67), 59 (26), 53 (33). ^{1}H NMR ($CDCl_3$): δ 3.82 (6H, s, equivalent, 2- and 6-OCH_3), 5.86 (2H, s, equivalent, 3- and 5-H).

*Compound **6***. Pale brown powder. Mp. 148°–151°C (lit. 147°C).[32] IR (KBr): ν_{max} cm^{-1} 3323, 1645, 1109. EI-MS *m/z* (rel. int.): 184 $[M]^+$ (62), 169 (100), 141 (63), 126 (26), 111 (29). ^{1}H NMR ($CDCl_3$): δ 3.78 (3H, s, 4-OCH_3), 3.82 (6H, s, equivalent, 3- and 5-OCH_3), 4.76 (1H, br. s, Ar-OH), 6.09 (2H, s, equivalent, 2- and 6-H).

Antioxidant activity assay

The antioxidant activity of the isolated compounds were assayed for the effect of scavenging stable DPPH free radicals, according to the literature method[33] with slight modification. The compounds and trolox were dissolved in MeOH to make a 50 ppm solution. Each of the test sample solutions (0, 30, 60, 90, 120, and 150 µl) was added into a mixture (0.9 ml) of 0.4 mM DPPH solution, 20% MeOH aqueous solution, and 0.2 M 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer solution (1:1:1). The resulting mixtures were shaken on a vortex mixer and stood for 20 min, and then the absorbance of the remaining DPPH in the mixture was determined with a UV-visible spectrophotometer at 520 nm. The percentage inhibition of the DPPH radicals by the samples were calculated from the following equation:

$$\text{DPPH radical scavenging (\%)} = (1 - A/A_0) \times 100$$

where A_0 is the absorbance of the mixture without a sample and A is the absorbance of the mixture with a sample after 20 min.

Results and discussion

Bark meal of *Gmelina arborea* was successively extracted with acetone and methanol. The acetone extract was fractionated with *n*-hexane, diethyl ether, and ethyl acetate to give the respective soluble fractions. From the diethyl

Table 1. ^{1}H and ^{13}C nuclear magnetic resonance data of compound **4** and its COSY and HMQC correlations

Position	^{1}H (δ)	^{13}C (δ)	COSY	HMQC (δ)
Acyl moiety				
1	–	128.72	–	–
2	7.19 (d, J = 2.0 Hz)	111.47	H-6	C-2 (111.47)
3	–	150.72	–	–
4	–	152.86	–	–
5	6.96 (d, J = 8.3 Hz)	112.59	H-6	C-5 (112.59)
6	7.15 (dd, J = 8.4, 2.1 Hz)	124.14	H-5, H-2	C-6 (124.14)
7	7.66 (d, J = 16.1 Hz)	146.83	H-8	C-7 (146.83)
8	6.42 (d, J = 15.9 Hz)	116.05	H-7	C-8 (116.04)
9	–	168.69	–	–
3-OCH_3	3.86 (br. s)	56.51	–	3-OCH_3 (56.51)
4-OCH_3	3.85 (br. s)	56.41	–	4-OCH_3 (56.41)
Aglycone				
1′	–	130.71	–	–
2′/6′	7.03 (d, J = 8.5 Hz)	130.92	H-3′/H-5′	C-2′/C-6′ (130.92)
3′/5′	6.67 (d, J = 8.5 Hz)	116.12	H-2′/H-6′	C-3′/C-5′ (116.12)
4′	–	156.72	–	–
7′	2.80 (t, J = 6.8 Hz)	36.38	H-8′a, H-8′b	C-7′ (36.38)
8′a	3.69 (td, J = 7.8, 2.2 Hz)	72.17	H-7′, H-8′b	C-8′ (72.17)
8′b	3.96 (m, overlapping)	–	H-7′, H-8′a	C-8′ (72.17)
Glucose				
1″	4.28 (overlapping)	104.38	H-2″, H-3″	C-1″ (104.38)
2″	3.18 (dd, J = 9.0, 7.8 Hz)	75.00	H-1″, H-3″	C-2″ (75.00)
3″	3.35 (t, J = 8.9 Hz)	78.00	H-1″, H-2″	C-3″ (78.00)
4″	3.27 (t, J = 9.0 Hz)	71.68	H-5″	C-4″ (71.68)
5″	3.41 (m)	76.76	H-4″, H-6″	C-5″ (76.76)
6″a	4.01 (dd, J = 11.1, 2.1 Hz)	68.58	H-6″b	C-6″ (68.58)
6″b	3.63 (dd, J = 11.2, 6.2 Hz)	–	H-6″a, H-5″	C-6″ (68.58)
Apiose				
1‴	5.04 (d, J = 2.2 Hz)	110.60	H-2‴	C-1‴ (110.60)
2‴	3.95 (d, J = 2.2 Hz)	78.98	H-1‴	C-2‴ (78.98)
3‴	–	78.45	–	
4‴a	3.85 (overlapping)	75.04	H-4‴b	C-4‴ (75.04)
4‴b	4.05 (d, J = 9.8 Hz)	–	H-4‴a	C-4‴ (75.04)
5‴	4.28 (overlapping)	67.50	–	C-5‴ (67.50)

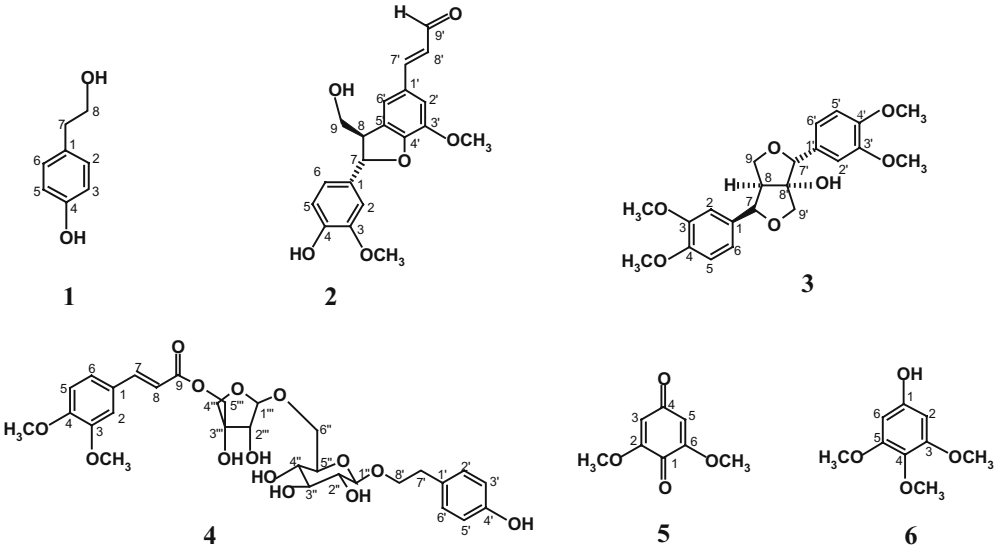

Fig. 2. Chemical constituents isolated from *Gmelina arborea* bark. The absolute configuration of **3** is unknown. The structural formula of (+)-**3** is used for convenience

ether-soluble fraction, compounds **1**, **2**, and **3** (Fig. 2) were obained. Compound **1** was isolated as a white powder. The ^{1}H NMR spectrum of compound **1** showed two doublets at δ 6.78 (2H, J = 8.3 Hz, 3- and 5-H) and δ 7.10 (2H, J = 8.5 Hz, 2- and 6-H) for *para*-substituted symmetrical aromatic ring protons, a broad singlet of Ar-4-OH at δ 4.97, and two triplets for an Ar-CH_2CH_2-O- moiety at δ 2.80 (2H, J = 6.6 Hz, 7-H) and 3.83 (2H, J = 6.6 Hz, 8-H). Both COSY and NOESY (Fig. 3) spectra showed correlations of 2- and 6-Hs with 3- and 5-Hs. EI-MS showed the molecular ion peak at m/z 138 and a fragment ion peak of $[HOC_6H_4CH_2]^+$ at m/z 107. Therefore, compound **1** was identified as 4-(2-

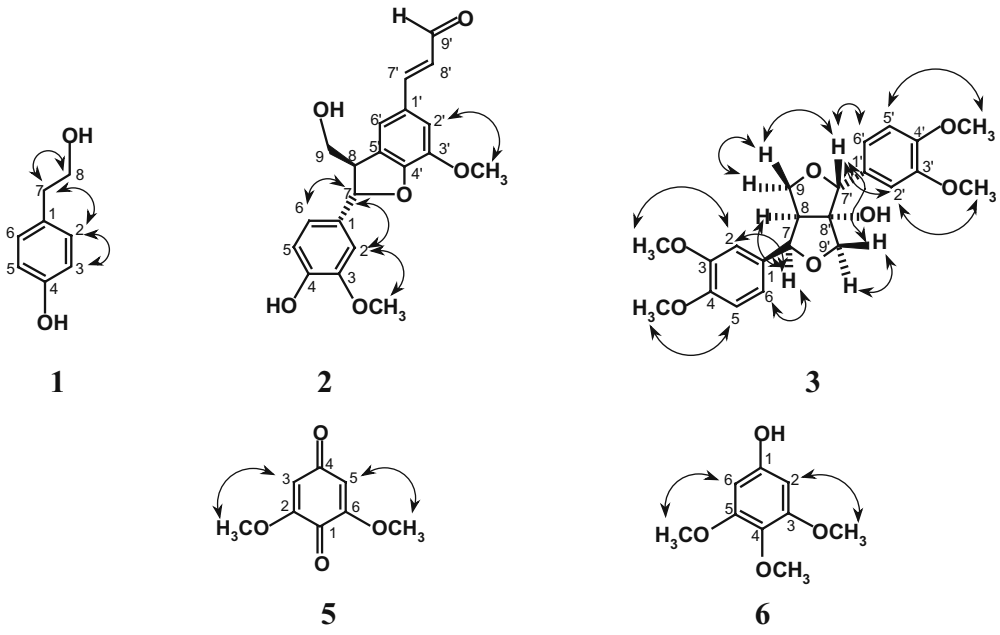

Fig. 3. The NOESY correlations (*arrows*) in the compounds isolated from *Gmelina arborea* bark. The absolute configuration of **3** is unknown. The structural formula of (+)-**3** is used for convenience

hydroxyethyl)phenol [2-(4-hydroxyphenyl)ethanol]. These data were identical with those of tyrosol as a component of olive (*Olea europaea*) oil.[34]

Compound **2** was isolated as pale yellow syrup. The ^{1}H NMR spectrum showed two singlets of aromatic methoxyl groups (δ 3.94 and 3.88); two doublets at δ 9.66 (1H, J = 7.8 Hz, 9′-CHO) and 7.42 (1H, J = 15.6 Hz, 7′-CH=), and a doublet of doublets (δ 6.62, J = 15.7, 7.7 Hz, 8′-CH=) of a *trans*-cinnamaldehyde moiety; a doublet of 7-benzylic methine (δ 5.65, J = 7.1 Hz), a multiplet of 8-benzylic methine (δ 3.69), and a broad singlet of Ar-4-OH (δ 5.63). It suggested the presence of 9-methylene. Signals of aromatic protons at δ 6.90 (Ar-2, 5, and 6-H), 7.05 (Ar-2′-H) and 7.14 (Ar-6′-H) showed a characteristic pattern of a phenylcoumaran-type dilignol (or a benzofuran-type neolignan). The EI-MS data showed the molecular ion peak at *m/z* 356, and the fragment ion peaks of $[M–H_2O]^+$ at 338 and $[M–CH_2O]^+$ at 326. These spectral data and the COSY and NOESY (Fig. 3) correlations indicated that structure of **2** was a coniferaldehyde derivative of (*E*)-dehydrodiconiferyl alcohol. The large proton coupling constant between 7-H and 8-H ($J_{7,8}$ = 7.1 Hz) suggested the dihydrofuran ring has a *trans*-configuration. The ^{1}H NMR and EI-MS data were identical with those of synthetic (+)-(7*S*,8*R*) balanophonin.[35] Although natural (–)-balanophonin was isolated from *Balanophora japonica*,[36] compound **2** was identified as (+)-balanophonin, (+)-(*E*)-(7*S*,8*R*)-4,9-dihydroxy-3,3′-dimethoxy-7,4′-epoxy-8,5′-neolign-7′-en-9′-al, which was isolated from *G. arborea* for the first time.

Compound **3** was isolated as colorless powder. The ^{1}H NMR spectrum showed two pairs of tri-substituted aromatic protons (2, 5, 6-H, and 2′, 5′, 6′-H) and four singlets of aromatic methoxyl groups at δ 3.89, 3.90, 3.91, and 3.92 (each 3H), which indicates that this compound has two veratryl groups. The presence of two doublets (δ 3.72, J = 9.5 Hz, 9′a-H, and δ 4.23, J = 9.3 Hz, 9′b-H) and two apparent triplets (δ 3.32, J = 9.4 Hz, 9a-H, and δ 3.98, J = 9.1 Hz, 9b-H) for nonequivalent geminal methylene protons at C-9′ and C-9, respectively, one (not two) multiplet (δ 3.13, 8-H), one doublet (δ 5.23, J = 6.3 Hz) (benzylic 7-H), and one singlet (not doublet) (benzylic 7′-H) suggested a furanofuran lignan structure without 8′-H. The NOESY correlations (Fig. 3) showed that the 7-H has correlation with 8-H, but not with 9-H or 9′-H, whereas the 7′-H has correlation with two protons of 9-H and 9′-H, which supported the lignan structure with 7α-H (*epi*-form). EI-MS showed the molecular ion peak at *m/z* 402. These data were identical with those of gmelinol (8′-OH), a known lignan isolated from the heartwood of this species.[18,22] Because the amount of **3** was too small to measure the optical rotation with a polarimeter, the absolute configuration of **3** is unknown. To show the relative configuration in Figs. 2 and 3, a structural formula of the (+)-enantiomer is used for convenience.

From the ethyl acetate-soluble fraction, compound **4** was isolated as white powder. The ^{1}H NMR spectrum (Table 1) showed two doublets at δ 6.67 (2H, J = 8.5 Hz, 3′- and 5′-H) and 7.03 (2H, J = 8.5 Hz, 2′- and 6′-H) for *para*-substituted aromatic protons, and a triplet at δ 2.80 (J = 6.8 Hz, 7′-H_2) and two signals at δ 3.69 (1H, td, J = 7.8, 2.2 Hz, 8′-Ha) and 3.96 (1H, m, 8′-Hb) for a –CH_2CH_2– group, which indicates the presence of a 2-(*p*-hydroxyphenyl)ethoxyl moiety. The two signals were assigned as a nonequivalent oxymethylene connected to a stereogenic carbon. Two strong singlets at δ 3.85 (3H) and 3.86 (3H) for aromatic methoxyl groups; two doublets (δ 7.19, 1H, J = 2.0 Hz, and δ 6.96, 1H, J = 8.3 Hz) and a doublet of doublets at δ 7.15 (1H, J = 8.4, 2.1 Hz) for tri-substituted 2, 5, 6-aromatic protons; and two doublets at δ 7.66 (1H, J = 16.1 Hz, 7-CH=) and 6.42 (1H, J = 15.9 Hz, 8-CH=) for alkene protons indicated the presence of a *trans*-3,4-dimethoxycinnamoyl moiety. The other ^{1}H NMR signals located in the range δ 3.18 to 5.04 suggested the

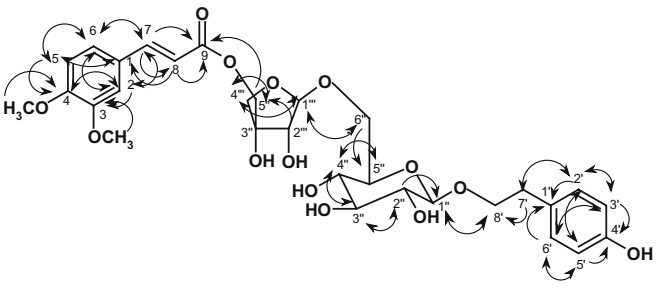

Fig. 4. The HMBC H → C correlations in compound **4**

presence of a saccharide moiety, which was supported by the presence of the 11 ^{13}C NMR peaks (Table 1) in the range δ 67.50 to 110.60, except for δ 72.17 (8′-C), suggesting that the sugar was a disaccharide. The other 17 ^{13}C NMR peaks showed the presence of 1 carboxyl carbon (δ 168.69), 2 methoxyl carbons (δ 56.41 and 56.51), 2 methylene carbons (δ 36.38 and 72.17), 12 aromatic or 2 olefinic carbons [δ 111.47, 112.59, 116.05, 116.12 (2C), 124.14, 128.72, 130.72, 130.92 (2C), 146.83, 150.72, 152.86, and 156.72] indicating the above two aromatic moieties. By comparison of the ^{13}C NMR data of analogue compounds,[37] it was suggested that the disaccharide was composed of a glucose and an apiose. The peaks of anomeric carbons at δ 104.38 and 110.60 were assigned as C-1″ of the glucopyranose and C-1‴ of the apiofuranose, respectively. The ^{1}H NMR signals of the anomeric protons at δ 4.28 (overlapping, 1″-H) and δ 5.04 (1H, d, J = 2.2 Hz, 1‴-H) supported the presence of the disaccharide. The large coupling constant between 2″-H and 1″-H ($J_{2'',1''}$ = 7.8 Hz) indicated that the anomeric center of the glucopyranose group had the β-configuration[36,37] rather than the α-configuration, which gives the smaller proton coupling constant ($J_{2'',1''}$ = ~4 Hz).[38] The apiofuranosyl group was also identified by the presence of a quaternary carbon (C-3‴) and two methylene carbons (C-4‴ and C-5‴). The COSY correlations showed that there was no correlation between C-4‴-H_2 and C-5‴-H_2 which also indicated the presence of the apiofuranosyl group. In the HMBC spectrum (Fig. 4), the proton signals at δ 4.28 (overlapping, 1″-H and 5‴-H) showed three-bond correlations with the carbon peaks at δ 72.17 (C-8′), 75.04 (C-4‴), 78.98 (C-2‴), and 168.69 (C-9), as well as two-bond correlations with those at 75.00 (C-2″). The proton signal at δ 3.69 (8′-H) showed three-bond correlations with the carbon peak at δ 104.38 (C-1″). Consequently, it was found that the *p*-hydroxyphenylethoxyl moiety was linked at C-1″ of the glucose, and that the C-9 of the cinnamoyl moiety was esterified to the 5‴-OH (δ 4.28). The peak of apiofuranosyl C-5‴ at δ 67.50 was shifted downfield, compared with the C-5 peaks (δ 63.8)[39] of a nonacylated apiofuranosyl unit, supporting the position of the ester. The proton signals at δ 5.04 (1‴-H) and 3.63 (6″-H) showed long correlations with C-6″ (δ 68.58) and C-1‴ (δ 110.60), respectively. The former ^{13}C NMR peak of glucopyranosyl at δ 68.58 (C-6″) was conspicuously deshielded, compared with that of a nonacylated glucopyranosyl unit C-6 (δ 60.7).[39] This evidence suggested that C-1‴ of the apiose unit was glycosidically linked

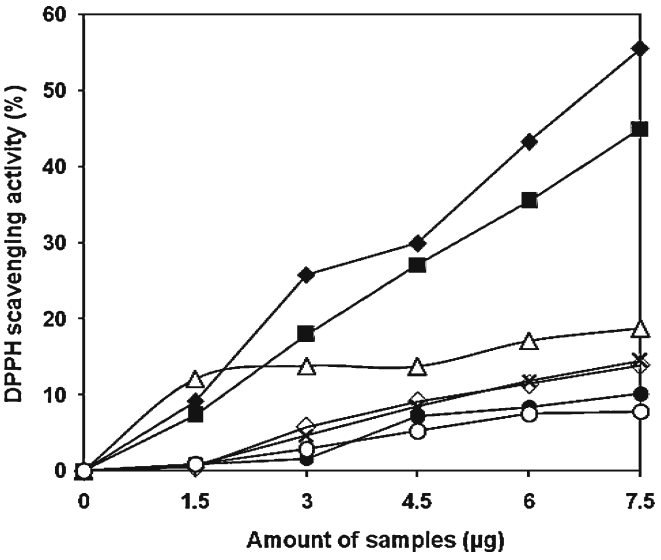

Fig. 5. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the chemical constituents from *Gmelina arborea* bark. *Filled diamonds*, trolox; *filled squares*, 3,4,5-trimethoxyphenol (**6**); *open triangles*, tyrosol (**1**); *crosses*, gmelinol (**3**); *open diamonds*, (+)-balanophonin (**2**); *filled circles*, 2,6-dimethoxy-*p*-benzoquinone (**5**); *open circles*, (−)-*p*-hydroxyphenylethyl[5‴-*O*-(3,4-dimethoxycinnamoyl)-β-D-apiofuranosyl(1‴ → 6″)]-β-D-glucopyranoside(**4**)

to C-6″ of the glucose. The FAB-MS showed its molecular ion peaks and the related ion peaks: $[M+Na]^+$ at *m/z* 645, $[M+1]^+$ at *m/z* 623, and $[M]^+$ at *m/z* 622. Therefore, compound **4** was identified as (−)-*p*-hydroxyphenylethyl[5‴-*O*-(3,4-dimethoxycinnamoyl)-*β*-D-apiofuranosyl (1‴ → 6″)]-*β*-D-glucopyranoside. To our knowledge, this is a new compound.

The methanol extract of *G. arborea* bark afforded two compounds, **5** and **6** (Fig. 2). The former was isolated as yellow powder. The EI-MS showed its molecular ion peak at *m/z* 168. The ^{1}H NMR spectrum showed two singlets at δ 5.86 (2H, CH=) and 3.82 (6H, $-OCH_3$), which have a NOESY correlation with each other (Fig. 3). Compound **5** was identified as 2,6-dimethoxy-*p*-benzoquinone by comparison of data with that of the authentic compound isolated from Tanjung wood (*Mimusops elengi*).[40]

Compound **6** was isolated as pale brown powder. The ^{1}H NMR spectrum showed a singlet of aromatic-H at δ 6.09 (2H), a broad singlet of a phenol at δ 4.76 (1H), and two singlets of aromatic methoxyl groups at δ 3.82 (6H) and 3.78 (3H), indicating a symmetric aromatic structure. The NOESY spectrum (Fig. 3) showed that the aromatic protons had correlations with the aromatic methoxyl groups at δ 3.82. EI-MS data showed its molecular ion peak at *m/z* 184. Compound **6** was identified as 3,4,5-trimethoxyphenol by comparison of the data with that of the authentic compound isolated from Tanjung wood (*M. elengi*).[40]

DPPH free radical scavenging assay of the identified compounds (Fig. 5) indicated that 3,4,5-trimethoxyphenol (**6**) exhibited a moderate antioxidant activity, whereas the others (**1**–**5**) showed weak activity. A previous study of tyrosol (**1**) isolated from *Olea europea* found weak antioxidant activity.[41] (+)-Balanophonin (**2**, an 8-5′ neolig-

nan) and gmelinol (**3**, a lignan) had less effective radical scavenging activity than 3,4,5-trimethoxyphenol (**6**), but stronger than 2,6-dimethoxy-*p*-benzoquinone (**5**) and (−)-*p*-hydroxyphenylethyl[5‴-*O*-(3,4-dimethoxycinnamoyl)-*β*-D-apiofuranosyl(1‴ → 6″)]-*β*-D-glucopyranoside (**4**, a phenylethanoid glycoside). High-level methoxylation of lignans diminishes the DPPH radical scavenging activity due decreased content of the phenolic hydroxyl group.[42] Most quinones exhibit much lower radical scavenging activity than lignans.[43]

Acknowledgments We thank Dr. Fumio Kawamura (Forestry and Forest Products Research Institute, Tsukuba, Japan) for providing us with an authentic sample of gmelinol.

References

1. Laver ML (1991) Bark. In: Lewin M, Goldstein IS (eds) Wood structure and composition. Marcel Dekker, New York, pp 409–433
2. Niklas KJ (1999) The mechanical role of bark. Am J Bot 86: 465–469
3. Dalton LK (1977) Tannin-formaldehyde as adhesives for wood. Aust J Appl Sci 1:54–61
4. Kim S, Lee YK, Kim HJ, Lee HH (2003) Physico-mechanical properties of particleboards bonded with pine and wattle tannin-based adhesives. J Adhes Sci Tech 7:1863–1875
5. Pietarinen SP, Willför SM, Ahotupa MO, Hemming JE, Holmbom BR (2006) Knotwood and bark extracts: strong antioxidants from waste materials. J Wood Sci 52:436–444
6. Packer L, Rimbach G, Virgili F (1999) Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. Free Radical Biol Med 27:704–724
7. Somanadhan B, Varughese G, Palpu P, Sreedharan R, Gudiksen L, Smitt UW, Nyman U (1999) An ethnopharmacological survey for potential angiotensin converting enzyme inhibitors from Indian medicinal plants. J Ethnopharmacol 65:103–112
8. Agunu A, Yusuf S, Andrew GO, Zezi AU, Abdurahman EM (2005) Evaluation of five medicinal plants used in diarrhoea treatment in Nigeria. J Ethnopharmacol 101:27–30
9. Maisch JM (1885) Botanical medicine monographs and sundry on some useful plants of the natural order of Verbenaceae. Am J Pharm 57:7
10. Kala CP (2005) Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. J Ethnobio Ethnomed 1:1–8
11. Sharma HK, Changte L, Dolui AK (2001) Traditional medicinal plants in Mizoram, India. Fitoterapia 72:146–161
12. Haggar JP, Briscoe CB, Butterfield RP (1998) Native species: a resource for the diversification of forestry production in the lowland humid tropics. Forest Ecol Manag 106:195–203
13. Jensen M (1995) Trees commonly cultivated in Southeast Asia illustrated field guide. RAP publication: 1995/38. Food and Agriculture Organization, Bangkok, Thailand
14. Evans J (1982) Plantation forestry in the tropics. Clarendon, Oxford, UK, p 472
15. Wang Z (2004) Cultivation and utilization of *Gmelina arborea* in South Yunnan, China. New Forests 28:201–205
16. Ali F, Sarma TC, Saikia CN (1993) Pulp and paper from certain fast-growing plant species. Bioresource Technol 45:65–67
17. Atmadja S (2005) Community forestry on government lands: forest farming in East Java, Indonesia. Sylvanet 18:2–6
18. Anjaneyulu ASR, Row LR, Subrahmanyam C (1972) A new lignan from *Gmelina arborea* Linn. Tetrahedron Lett 22:2179–2182
19. Anjaneyulu ASR, Rao AM, Rao VK, Row LR, Pelter A, Ward RS (1977) Novel hydroxy lignans from the heartwood of *Gmelina arborea*. Tetrahedron 33:133–143
20. Anjaneyulu ASR, Rao KJ, Rao VK, Row LR, Subrahmanyam C, Pelter A, Ward RS (1975) The structures of lignans from *Gmelina arborea* Linn. Tetrahedron 31:1277–1285
21. Satyanarayana P, Rao K, Ward RS, Pelter A (1986) Arborone and 7-oxo-dihydrogmelinol: two new keto-lignans from *Gmelina arborea*. J Nat Prod 49:1061–1064
22. Kawamura F, Ohara S, Nishida A (2004) Antifungal activity of constituents from the heartwood *Gmelina arborea*: part 1. Sensitive antifungal assay against Basidiomycetes. Holzforschung 58:189–192
23. Kawamura F, Ohara S (2005) Antifungal activity of iridoid glycosides from the heartwood of *Gmelina arborea*. Holzforschung 59:153–155
24. Nair AGR, Subramanian SS (1975) Quercetagetin and other flavones from *Gmelina arborea* and *Gmelina asiatica*. Phytochemistry 14:1135–1136
25. Olatunji G (1999) Furanoresorcinol from the heartwood of *Gmelina arborea*. Cellulose Chem Technol 33:37–39
26. Barik BR, Bhowmik T, Dey AK, Patra A, Chatterjee A, Joy SS (1992) Premnazole an isoxazole alkaloid of *Premna integrifolia* and *Gmelina arborea* with antiinflammatory activity. Fitoterapia 63: 295–299
27. Satyanarayana P, Subrahmanyam P, Kasai R, Tanaka O (1985) An apiose-containing coumarin glycoside from *Gmelina arborea* root. Phytochemistry 24:1862–1863
28. Rao DV, Rao EV, Viswanadham N (1967) Occurrence of luteolin in the leaves of *Gmelina arborea* Linn. Curr Sci 36:71–72
29. Bhattacharjee AK, Das AK (1969) Phytochemical survey of few Mysore plants. Econ Bot 23:274–276
30. Hosny M, Rosazza PN (1998) Gmelinosides, twelve acylated iridoid glycosides from *Gmelina arborea*. J Nat Prod 61:734–742
31. Sinha S, Dixit P, Bargava S, Devasagayam TPA, Ghaskadbi S (2006) Bark and fruit of *Gmelina arborea* protect liver cells from oxidative stress. Pharm Bio 44:237–243
32. Pouchert CJ, Behnke J (1993) The Aldrich library of ^{13}C and ^{1}H FT NMR spectra, edn 1, vol 2. Aldrich, USA
33. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181:1199–1200
34. Giovannini C, Straface E, Modesti D, Coni E, Cantafora A, De Vincenzi M, Malorni W, Masella R (1999) Tyrosol, the major olive oil biophenol, protects against oxidized-LDL-induced injury in Caco-2 Cells. J Nutr 129:1269–1277
35. Yuen MSM, Xue F, Mak TCW, Wong HNC (1998) On the absolute structure of optically active neolignan containing a dihydrobenzo[b]furan skeleton. Tetrahedron 54:12429–12444
36. Haruna M, Koube T, Ito K, Murata H (1982) Balanophonin, a new neolignan from *Balanophora japonica* Makino. Chem Pharm Bull 30:1525–1527
37. Yamasaki T, Masuoka C, Nohara T, Ono M (2007) A new phenylethanoid glycoside from the fruits of *Callicarpa japonica* Thunb. var. *luxurians* Rehds. J Nat Med 61:318–322
38. Hsieh HJ, Giridhar R, Wu WT (2007) Regioselective formation of kojic acid-7-*O*-alpha-D-glucopyranoside by whole cells of mutated *Xanthomonas campestris*. Enzym Microb Tech 40:324–328
39. Tanaka T, Ikeda T, Kaku M, Zhu XH, Okawa M, Yokomizo K, Uyeda M, Nohara T (2004) A new lignan glycoside and phenylethanoid glycoside from *Strobilanthes cusia* Bremek. Chem Pharm Bull 52:1242–1245
40. Katayama T, Maeda M, Suzuki T, Syafii W, Muladi S (2005) Extractives from tanjung wood, akar kuning wood, and gimbul wood and their antioxidant and antifungal activities. Proceeding of the 6th International Wood Science Symposium, Bali, August 2005, pp 329–333
41. Bonilla MP, Salido S, van Beekb TA, Linares-Palomino PJ, Altarejos J, Nogueras M, Sánchez A (2006) Isolation and identification of radical scavengers in olive tree (*Olea europaea*) wood. J Chromatogr A 1112:311–318
42. Eklund PC, Langvik OK, Warna JP, Salmi TO, Willfor SM, Sjoholm RE (2005) Chemical studies on antioxidant mechanisms and free radical scavenging properties of lignan. Org Biomol Chem 3:3336–3347
43. Cai YZ, Sun M, Xing J, Luo Q, Corke H (2006) Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sci 78:2872–2888