http://www.pjbs.org



ISSN 1028-8880

Pakistan Journal of Biological Sciences



Chemical Constituents from Swietenia macrophylla Bark and Their Antioxidant Activity

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Abstract: Chemical constituents of the bark of *Swietenia macrophylla* King (Meliaceae) was investigated not only to develop further bark utilization but also to understand the biochemical function of the bark in the forest environment. A new phenylpropanoid-substituted catechin, namely, swietemacrophyllanin $[(2R^*,3S^*,7"R^*)$ -catechin-8,7"-7,2"-epoxy-(methyl 4",5"-dihydroxyphenylpropanoate)] (1) was isolated from the bark of *S. macrophylla* together with two known compounds, catechin (2) and epicatechin (3). The structure of 1 was elucidated by spectroscopic data and by comparison of the NMR data with those of catiguanins A and B, phenylpropanoid-substituted epicatechins. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the isolated compounds indicated that all of the three compounds have strong activity compared with trolox as a reference. Swietemacrophyllanin (1) had the strongest activity with a 50% inhibitory concentration (IC₅₀) value of 56 μ g mL⁻¹.

Key words: Swietenia macrophylla, bark extractives, phenylpropanoid-substituted catechin, catechin, antioxidant activity

INTRODUCTION

Swietenia macrophylla King (Meliaceae) called Big-Leaf mahogany or Honduras mahogany distributed in Central America and tropical South America (Brown et al., 2003; Lanib, 1966). Mahogany wood has been used for many purposes, such as fine furniture and cabinet making, interior trim, paneling, fancy veneers, musical instruments, boat building, pattern making, turnery and carving. The leaves can be used for dyeing agent (Mahale et al., 2006). The seeds have been used for leishmaniasis and abortion medicine by an Amazonian Bolivian ethnic group (Bourdy et al., 2000) and for treatment of hypertension, diabetes and malaria as a folk medicine in Indonesia (Kadota et al., 1990a). The antimalarial activity of a water extract of S. macrophylla seeds was found against Plasmodium falciparum (Murnigsih et al., 2005). The seeds also have antibabesial (Murnigsih et al., 2005) and antidiarrhoeal activities (Maiti et al., 2007). The bark extract has been used as an astringent for wounds and used occasionally for tanning because of the rich red colour. Because S. macrophylla was well-known for its fast growth and adaptability, it has been afforested for production of the timber as well as regeneration of forests in tropical regions including Indonesia. Although originally Mahogany wood was provided by Swietenia mahagoni (L.) Jacq. which is called Mahogany

Tree or West Indian mahogany and is native to the West Indies and Florida, the species have now become very rare because of over-harvesting.

From S. macrophylla seeds many kinds of tetranortriterpenoids or limonoids, namely, swietenine (Solomon et al., 2003), swietenolide (Connolly et al., 1965), 8,30-epoxy-swietenine acetate (Taylor and Taylor, 1983), swietenolide diacetate (Chan et al., 1976), 3β,6-dihydroxydihydrocarapin augustineolide and (Mootoo et al., 1999), as well as five derivatives of the former three compounds (Kojima et al., 1998) were isolated and identified. The presence of known fatty acids and terpenoids were reported from the seeds (Chan et al., 1976). γ-Himachalene, germacrene D, germacrene A, acid cadina-1,4-diene, hexadecanoic hexadecanoate were analyzed as the essential oil components from the fresh terminal shoots and mature leaves (Soares et al., 2003). However, no chemical constituents from the bark have been reported, although, from S. mahagony bark and wood, five limonoid-type triterpene possessing dior mono-orthoesters (swietenialides A-E) together with 2-hydroxyswietenin (Saad et al., 2003) and cycloeucalenol (a known triterpene) (Amorós-Marín et al., 1959), respectively, were isolated. From the seeds of S. mahagony, tetranortriterpenoids [five swietenins (B-F), three swietemahonins (A-G), acylswietenolides, seven

swietemahonolide, mahonin and secomahoganin] related to swietenine and swietenolide were isolated and identified (Kadota et al., 1990a-c). We have investigated chemical constituents of S. macrophylla bark not only to develop further bark utilization but also to understand the biochemical function of the bark in the forest environment. Bark is a waste material of wood processing industry, whereas it is a potential source of bioactive compounds such as an antioxidant, antifungal, or antitermite. In this paper, we would like to report isolation and identification of a new phenylpropanoid-substituted catechin together with catechin and epicatechin from the bark and their potent antioxidant activity. These compounds are polyphenols or flavan-3-ols that are different class of compounds from those isolated so far.

MATERIALS AND METHODS

General experimental procedures: Low to medium pressure column chromatography was conducted on a FMI pump system with a 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 was done using the same system as above except for the column size (300×7.8 mm i.d.). A melting point was measured on a Yanaco MP 52641 micro melting point apparatus and was uncorrected. 1H and 13C nuclear magnetic resonance (NMR) spectra and two dimensional NMR spectra [¹H-¹H correlation spectroscopy (COSY), nuclear Overhauser effect (NOE) spectroscopy (NOESY), ¹H detected multiple quantum coherence (HMQC) spectroscopy and ¹H detected multiple bond connectivity (HMBC) spectroscopy] were determined on a JEOL JNM Alpha 400 FT-NMR spectrometer (400 MHz) using tetramethylsilane as an internal standard. Chemical shifts and coupling constants (J) were expressed as δ (in ppm) and Hz, respectively. Fast atom bombardment (FAB) mass spectra (MS) were acquired on a JEOL JMS-SX102A mass spectrometer. Fourier-transform infrared (FT-IR) spectra were measured on a Jasco FT/IR-670 Plus spectrophotometer. UV-visible spectra were carried out on a Shimadzu UV 1600 spectrophotometer.

Plant materials: The bark was stripped from *S. macrophylla* trunk, collected from Sumedang, West Java, Indonesia in July 2006. The chipped bark was

ground in a Wiley mill and the resulting meal was screened to give 40-80 mesh bark meal in size. The voucher specimen was deposited in the Department of Forest Product Technology, Winaya Mukti University, Bandung, Indonesia.

Extraction and fractionation: The bark meal (40-80 mesh, 1500 g air dried) was extracted three times with acetone (each 3 L) for 48 h at room temperature to give an acetone extract (276.1 g) and a methanol extract (96.7 g), respectively. The acetone extract was reconstituted with 250 mL of acetone and the solution was suspended by the addition of 50 mL of water. The suspension was successively fractionated with n-hexane, diethyl ether and ethyl acetate, to give n-hexane soluble fraction (3.9 g), diethyl ether soluble fraction (16.1 g) and ethyl acetate soluble fraction (42.9 g), respectively.

Isolation of compounds: The ethyl acetate soluble fraction (20 g) was chromatographed on a silica-gel column (nhexane/EtOAc = 100:0 to 0:100 and then 100% MeOH) by a stepwise elution to afford nine fractions (fr. 1' to 9'). Fr. 5' (2.5 g) was rechromatographed on a silica-gel column $[CH_2Cl_2/MeOH = 80:20, (v/v)]$ to give eight fractions (fr. 5'-1 to 5'-8). Fr. 5'-3 (450 mg) was separated by preparative TLC $[CH_2Cl_2/MeOH = 75:25, (v/v)]$ as a developing solvent] to yield eight fractions (fr. 5'-3-1 to 5'-3-8) and then fr. 5'-3-5 (143.3 mg) was purified by preparative HPLC [flow rate 2.0 mL min⁻¹, detection: UV 255 nm, eluent: MeOH/H₂O (30:70, v/v)] to give compounds 1 (3.5 mg) and 3 (3.9 mg). The diethyl ether soluble fraction (15 g) was chromatographed on a silica-gel column (nhexane/EtOAc = 100.0 to 0.100) to give nine fractions (fr. 1 to 9). Fr.7 (0.7 g) was separated by a Sep-pak column C18 (EtOAc/n-hexane = 20:80 to 0:100, as an eluent) to afford six fractions (fr.7-1 to 7-6). Fr.7-1 (489 mg) was purified by preparative TLC [$CH_2Cl_2/MeOH = 85:15 (v/v)$] to yield eight fractions (fr. 7-1 to 7-8). Fr. 7-1-6 (65 mg) was repurified by preparative HPLC [flow rate 2.0 mL min⁻¹, detection: UV 280 nm, eluent: MeOH/H₂O (30:70, v/v)] to give compound 2 (5.6 mg).

Spectroscopic data

Compound 1: Pale red amorphous solid Mp. 202-204°C. IR (KBr): $v_{max}cm^{-1} 3366$ (OH), 1743 (C=O), 1619 (aromatic C=C), 1524 (aromatic C=C), 1449 (aromatic C=C), 1282 (COOCH₃), 1112 (COO-CH₃). FAB-MS m/z: 505 [M+Na]⁺, 483 [M+H]⁺, 482 [M]⁺. 1 H and 13 C NMR (CD₃OD): (Table 1).

Compound 2: Pale brown amorphous solid. IR (KBr): $v_{max}cm^{-1}$ 3367, 1608, 1521, 1467, 1285, 1145, 818. FAB-MS m/z: 291 [M+H]⁺. ¹H NMR (CD₃OD): δ 2.50 (1H, dd, J = 16.1, 8.1 Hz, Hb-4), 2.84 (1H, dd, J = 16.1, 5.4 Hz, Ha-4),

Table 1: ¹H and ¹³C NMR data of swietemacrophyllanin (1)

Position	¹H (δ)	¹³ C (δ)
2	4.73 (d, J = 7.1 Hz)	82.8
3	4.01 (m)	68.6
4	2.83 (dd, J = 16.3, 5.1 Hz)	28.3
	2.60 (dd, J = 16.4, 7.4 Hz)	
5		156.2
6	6.10 (s)	95.9
7		153.1
8		104.3
4a		103.9
8a		153.1
1'		132.2
2'	6.85 (d, J = 2.0 Hz)	115.3
3'		146.1
4'		146.3
5'	6.77 (d, J = 8.1 Hz)	116.1
6'	6.73 (dd, J = 8.3, 2.0 Hz)	119.7
1"		116.2
2"		146.6
3"	6.45 (s)	104.2
4"		146.3
5"		142.3
6"	6.63 (s)	114.9
7"	4.43 (dd, J = 7.4, 4.8 Hz)	31.4
8"	2.46 (dd, J = 14.2, 7.5 Hz)	45.0
	2.64 (dd, J = 14.3, 4.8 Hz)	
9"-C=O		174.4
-OCH ₃	3.44 (s)	51.9

3.97 (1H, m, H-3), 4.56 (1H, d, J = 7.6 Hz, H-2), 5.85 (1H, s, H-8), 5.92 (1H, br. s, H-6), 6.71 (1H, dd, J = 8.3, 2.0 Hz, H-6'), 6.76 (1H, d, J = 8.1 Hz, H-5'), 6.83 (1H, d, J = 2.0 Hz, H-2'). 13 C NMR (CD₃OD): δ 28.6 (C-4), 68.9 (C-3), 82.9 (C-2), 95.5 (C-6), 96.3 (C-8), 100.9 (C-4a), 115.3 (C-2'), 116.1 (C-5'), 120.1 (C-6'), 132.3 (C-1'), 146.2 (C-3'), 146.3 (C-4'), 156.9 (C-8a), 157.6 (C-5), 157.9 (C-7).

Compound 3: Pale brown amorphous solid. IR (KBr): $v_{max}cm^{-1}3366$, 1606, 1520, 1464, 1283, 1146, 1094, 825. FAB-MS m/z: 329 [M+K]⁺, 313 [M+Na]⁺, 291 [M+H]⁺. ¹H NMR (CD₃OD): δ 2.73 (1H, dd, J = 16.8, 2.7 Hz, H-4a), 2.86 (1H, dd, J = 16.7, 4.0 Hz, H-4b), 4.17 (1H, m, J = 1.5 Hz, H-3), 4.81 (1H, br. s, H-2), 5.91 (1H, d, J = 2.4 Hz, H-8), 5.93 (1H, d, J = 2.4 Hz, H-6), 6.75 (1H, dd, J = 8.1, 2.2 Hz, H-6'), 6.77 (1H, d, J = 8.1 Hz, H-5'), 6.97 (1H, d, J = 2.0 Hz, H-2'). ¹³C NMR (CD₃OD): δ 29.3 (C-4), 67.5 (C-3), 79.9 (C-2), 95.9 (C-6), 96.4 (C-8), 100.1 (C-4a), 115.3 (C-2'), 115.9 (C-5'), 119.4 (C-6'), 132.3 (C-1'), 145.8 (C-4'), 146.0 (C-3'), 157.4 (C-8a), 157.7 (C-5), 158.0 (C-7).

DPPH radical scavenging activity assay: The antioxidant activity of the isolated compounds was assayed on the effect of scavenging the stable DPPH free radicals by the literature method (Blois, 1958) with a slight modification. As a reference compound was used 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), an aqueous tocopherol analogue. The compounds and trolox were dissolved in MeOH to achieve concentrations of 25, 50, 75

and 100 μ g mL⁻¹ and each of the test sample solutions (90 μ L) was added into mixtures 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 mixtures was determined with a visible spectrophotometer at 520 nm. The percentage inhibition of the DPPH free radicals by the samples was calculated from the following equation:

DPPH radical scavenging activity (%) = $(1-A/A_0)\times100$

where, A_{\circ} is the absorbance of the mixture without a sample and A is the absorbance of the mixture with a sample after 20 min. The inhibitory concentration (IC₅₀) value was obtained by interpolation of concentration-DPPH radical scavenging activity curve.

RESULTS AND DISCUSSION

The bark meal of *S. macrophylla* 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 three compounds **1**, **2** and **3**. Compounds **1** and **3** were isolated from the ethyl acetate soluble fraction. Compound **2** was isolated from the diethyl ether soluble fraction (Fig. 1).

Compound 1 was isolated as pale red amorphous solid. Its ¹H NMR spectrum (Table 1) showed one remarkable singlet at δ 3.44 (3H, -OCH₃) with seven aliphatic and six aromatic proton signals. The ¹³C NMR spectrum (Table 1) showed eighteen aromatic carbon peaks indicating the presence of three aromatic rings as well as one ester carbonyl (>C=O) and six aliphatic carbons, one of which is -OCH₃, indicating the presence of -COOCH3 and five methylenes and/or methines. The presence of the ester was also supported by the IR spectrum of 1 that showed strong bands at 1743 cm⁻¹ (>C=O), 1282 cm⁻¹ (CO-OR) and 1112 cm⁻¹ (COO-R). The three aromatic signals in the ¹H NMR spectrum, namely, two doublets at δ 6.77 (1H, J = 8.1 Hz, H-5') and 6.85 (1H, J = 2.0 Hz, H-2') and a doublet of doublets at δ 6.73 (1H, J = 8.3, 2.0 Hz, H-6') as well as the ¹³C NMR peaks at δ 146.1 (C-4'-O-) and 146.3 (C-3'-O-) indicated the presence of a 3',4'-dihydroxyphenyl group (B-ring). The four aliphatic proton signals, a doublet at δ 4.73 (1H, J = 7.1 Hz, H-2), a multiplet at δ 4.01 (1H, H-3) and two doublets of doublets at δ 2.60 (1H, J = 16.4, 7.4 Hz, Hb-4) and 2.83 (1H, J = 16.3, 5.1 Hz, Ha-4) for nonequivalent methylene protons adjacent to an asymmetric carbon (C-3)

Fig. 1: Chemical constituents isolated from Swietenia macrophylla bark

showed the presence of a flavan-3-ol skeleton (C-ring), which was also supported by the 13 C NMR peaks of three aliphatic carbons at δ 82.8 (C-2), 68.6 (C-3) and 28.3 (C-4). The larger proton coupling constant between H-2 and H-3 ($J_{2,3}$ = 7.1 Hz) indicated that the flavan-3-ol has a 2,3-trans-configuration, not a *cis*-configuration which gives the smaller proton coupling constant ($J_{2,3}$ = \sim 2 Hz). Therefore, it was determined that this compound is a derivative of catechin by comparison with the spectroscopic data of 2 and (+)-catechin (Davis *et al.*, 1996) as well as 3 and (-)-epicatechin (Sun *et al.*, 2006; Fan *et al.*, 2004).

On the other hand, the aromatic A-ring is suggested to be a penta-substituted ring with only unsubstituted carbon (C-6-H), because the ¹H and ¹³C NMR showed peaks at δ 6.10 (1H, s, H-6) and δ 95.9 (C-6-H) and 104.3 (<u>C</u>-8-C) together with δ 156.2 (C-5-O), 153.1 (C-7-O), 153.1 (C-8a-O) and 103.9 (C-4a-C), but neither H-8 signal nor C-8-H peak (whose presence could be predicted around δ 96.3 for a catechin or 96.4 for an epicatechin). Beside the catechin skeleton, the presence of six aromatic carbons, two aliphatic carbons and a methyl ester was also shown by the ¹³C NMR, which indicated that 1 consists of a methyl disubstituted-phenylpropanoate moiety (the aromatic B'-ring and the C-7", 8" and 9" side chain). The ¹H NMR spectrum also showed two singlets of aromatic protons at δ 6.45 (1H) and 6.63 (1H) which should be para-orientated and correspond to two ¹³C NMR peaks of aromatic \underline{C} -H at δ 104.2 and 114.9. The peak at δ 116.2 was assigned to aromatic C-1"-C and three peaks of the five peaks at δ 142.3-146.6 were assigned to three C-O of the B'-ring and the remaining two peaks were to two C-O of the B-ring as above. Thus, the B'-ring was determined as tetra-substituted one with C-1"-C, C-2"-O, C-4"-O, C-5"-O, C-3"-H and C-6"-H. Furthermore, the presence of C-7"-H (asymmetric methine) and C-8"-H₂ (nonequivalent methylene) was shown by the ¹H NMR spectrum: a doublet of doublets at δ 4.43 (J = 7.4, 4.8 Hz) and two doublets of doublets at δ 2.46 ($J = 14.2, 7.5 \,\mathrm{Hz}$) and 2.64 (J = 14.3, 4.8 Hz), respectively and by the ¹³C NMR peaks at δ 31.4 (C-7") and 45.0 (C-8"). The ¹³C NMR peak of the C-8 (\delta 104.3) is remarkably shifted to downfield due to deshielding, compared with that of C-8 $(\delta 95.5)$ in **2** (catechin). Therefore, the 1,2,4,5-tetrasubstituted-phenylpropane moiety (B'-ring) is considered to be fused to the 4a,5,7,8,8a-penta-substituted A-ring of the catechin moiety at the C-8 and C-1" position via C-7" and at the C-7 and C-2" position via the C-7 oxygen atom. The FAB-MS of 1 showed its molecular ion peak and the related ion peaks: $[M+Na]^+$ at m/z 505, $[M+H]^+$ at m/z 483 and $[M]^+$ at m/z 482. This molecular weight supported the planar structure formula of 1.

Thus, those data were carefully compared with those of catiguanins A and B, diastereomers of the phenylpropanoid-substituted epicatechins isolated very recently from the bark of *Trichilia catigua* (Meliaceae) (Tang *et al.*, 2007). These are structurally analogous to compound **1**. However, the NMR data showed that there are two differences between them. One is the downfield shift of the C-2 peak (δ 82.8) of **1**, a catechin-type flavan, compared with those of the catiguanins (δ 79.8 for the A and 80.1 for the B), epicatechin-type flavans. The other is the larger proton coupling constant $J_{2,3}$ (= 7.1 Hz) of **1**

than those of the catiguanins (epicatechin-type), whose H-2 signals were appeared at δ 4.92 as broad singlets. The difference between catiguanins A and B is attributed to a difference of configuration at C-7" (C-9 in the literature). The 13 C NMR peaks of **1** at δ 95.9 (C-6), 103.9 (C-4a) and 104.3 (C-8) were consistent with those found in catiguanin A. The NOESY correlations of 1 (Fig. 2) showed that H-6" in the B' ring has correlation with both H-7" and methyl ester protons. This suggested that the methoxycarbonylmethyl group (C8"-C9") at C-7" in 1 is trans-oriented relative to the B-ring or is the β configuration similar to catiguanin A and cinchonain 1a (Foo, 1987). All of the NMR data were assigned as in Table 1. The relative configuration of **1** is $2R^*$, $3S^*$, $7"R^*$. Therefore, compound 1 was identified as a new phenylpropanoid-substituted catechin, (2R*,3S*,7"R*)catechin-8,7"-7,2"-epoxy-(methyl 4",5"-dihydroxyphenylpropanoate), named as swietemacrophyllanin.

Compound **2** was isolated as pale brown amorphous solid. All of the 1 H and 13 C NMR signals of **2** were assigned as in the section spectroscopic data with the aid of two dimentional NMR spectra. FAB-MS showed [M+H] † peak at m/z 291. Those data suggested that **2** was a 5,7,3',4'-tetrahydroxyflavan-3-ol. The larger coupling constant between H-2 and H-3 ($J_{2,3} = 7.6$ Hz) indicated the C-ring has a 2,3-trans-configuration. Therefore, compound **2** was identified as catechin, 2,3-trans-5,7,3',4'-tetrahydroxyflavan-3-ol, by comparison of those data with those of (+)-catechin isolated from green tea (Davis *et al.*, 1996).

Compound 3 was isolated as pale brown amorphous solid. All of the ¹H and ¹³C NMR signals of 3 were assigned as in the section spectroscopic data with the aid of two dimentional NMR spectra. FAB-MS showed $[M+K]^{+}$ peak at m/z 329, $[M+Na]^{+}$ peak at 313 and $[M+H]^{+}$ peak at 291. Those data suggested that 3 was a 5,7,3',4'tetrahydroxyflavan-3-ol. The 2,3-cis-configuration of the C-ring was determined by the smaller coupling constant between H-2 and H-3. The signal of H-2 appeared as a broad singlet at δ 4.81 (Sun et al., 2006; Fan et al., 2004). Therefore, compound 3 was identified as epicatechin, 2,3cis-5,7,3',4'-tetrahydroxyflavan-3-ol, by comparison of those data with those of (-)-epicatechin (Sun et al., 2006; Fan et al., 2004; Davis et al., 1996). Recently, this laboratory isolated 3 from Tanjung wood (Mimusops elengi) (Katayama et al., 2005).

The antioxidant activity of the isolated compounds was assessed by the DPPH free radical scavenging assay as shown in Table 2. All of the three compounds exhibited stronger activity than the trolox. Swietemacrophyllanin (1) scavenged 50% DPPH free radical at the lowest inhibitory concentration (IC₅₀: 56 µg mL⁻¹), which

Fig. 2: NOESY correlations of swietemacrophyllanin (1)

Table 2: DPPH scavenging activity of the compounds isolated from *Swietenia macrophylla* bark with 50% inhibitory concentration [IC_{v0} (µg mL⁻¹)] value

Compounds	$IC_{50} (\mu g m L^{-1})^{\mathbb{A}}$
Swietemacrophyllanin (1)	56
Epicatechin (3)	59
Catechin (2)	70
Trolox	81

A: The values were obtained by interpolation of concentration-DPPH radical scavenging activity curve

indicates that this new compound has the strongest activity of the three and trolox. Potent antioxidant activity of catechin and epicatechin is well-known.

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