Antibacterial Flavanones and Dihydrochalcones from *Macaranga trichocarpa*

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Previously we had isolated two prenylated flavanones and two prenylated dihydrochalcones, macatrichocarpins A – D (**1** – **4**), from the acetone extract of the leaves of *Macaranga trichocarpa*. Re-examination of the fractions containing minor components resulted in the isolation of four more flavonoid derivatives, including two new prenylated dihydrochalcones, oxymacatrichocarpin C (**5**) and isomacatrichocarpin C (**6**). The structures of these compounds were determined by the analysis of UV, NMR, and mass spectral data. The eight isolated flavonoids were tested on eight pathogenic bacteria and found to be mostly moderate antibacterial agents, with the lowest MIC value of 26.5 μM achieved by the flavanone macatrichocarpin A (**1**) against *Bacillus subtilis*.

Key words: Antibacterials, Isomacatrichocarpin C, Oxymacatrichocarpin C, *Macaranga trichocarpa*

Introduction

The genus *Macaranga* (Euphorbiaceae) comprises about 250 species, and their distribution covers the region from Africa and Madagascar in the West to tropical Asia, North Australia, and the Pacific islands in the East (Blattner *et al*., 2001). In recent communications, we have reported the isolation of farnesylated and geranylated flavonols from *M. gigantea* (Tanjung *et al*., 2009), *M. pruinosa* (Syah and Ghisalberti, 2010), and *M. rhizinoides* (Tanjung *et al*., 2010); prenylated dihydroflavonols from *M. lowii* (Agustina *et al*., 2012) and *M. recurvata* (Tanjung *et al*., 2012); phenolic derivatives containing an irregular sesquiterpenyl side chain from *M. pruinosa* (Syah and Ghisalberti, 2010, 2012); as well as prenylated and methylated flavanones and dihydrochalcones, trivially named macatrichocarpins A – D (**1** – **4**), from *M. trichocarpa* (Syah *et al*., 2009). The compounds **1** – **4** were the major components of the acetone extract of the *M. trichocarpa* plant leaves. In continuation of our work on the Indonesian *Macaranga*, the present paper reports the isolation of additional dihydrochalcone derivatives, trivially named oxymacatrichocarpin C (**5**) and isomacatrichocarpin C (**6**) (Fig. 1), as minor components of the extract. Together with these two dihydrochalcones, known flavanone derivatives were also isolated, namely 7-*O*-methylnaringenin (**7**) and 4′,7-di-*O*-methylnaringenin (**8**). Compounds **1** – **8** were evaluated for their antimicrobial properties against eight pathogenic bacteria.

Results and Discussion

Compound **5** was isolated as a pale yellow solid. The HREI mass spectrum of **5** showed a molecular ion at m/z 372.1574, consistent with the molecular formula $C_{21}H_{24}O_6$ (calcd. 372.1573; Δ 0.27 ppm). The UV absorption bands of compound **5** in MeOH (λ_{max} 202, 223, and 286 nm) were typical of a dihydrochalcone chromophore, and were similar to those of macatrichocarpins C (**3**) and D (**4**) (Syah *et al*., 2009). On addition of NaOH and $AlCl_3$, the longest absorption band (λ_{max} 286 nm) was shifted to longer wavelengths (λ_{max} 320 and 368 nm, respectively), indicating that compound **5** contains free and chelated

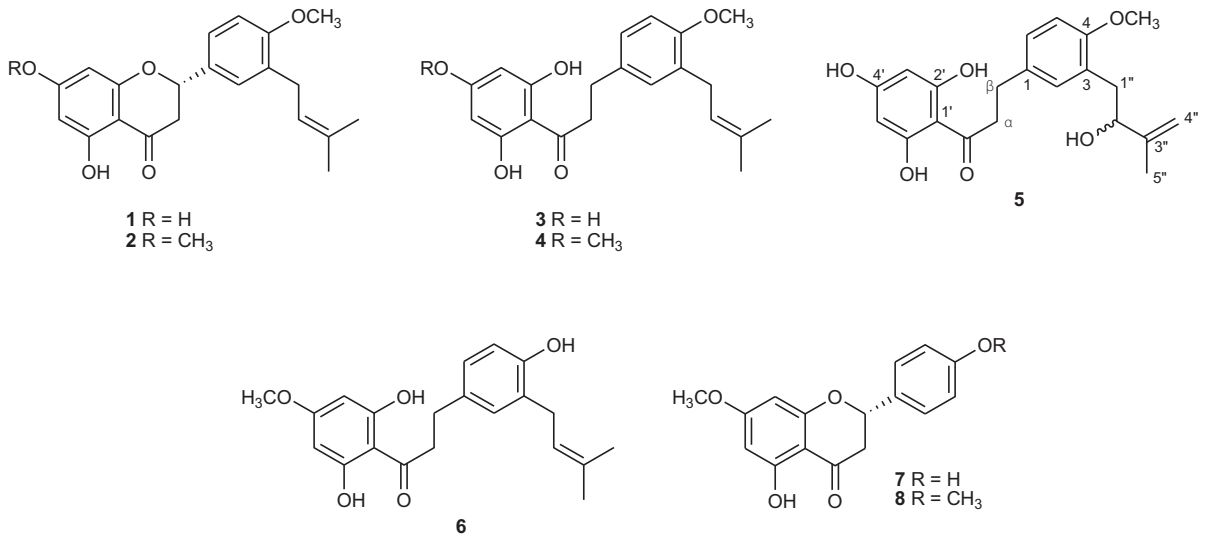

Fig. 1. Chemical structures of the constituents isolated from *Macaranga trichocarpa*.

Table I. NMR data of compounds **5** (in acetone-d_6) and **6** (in $CDCl_3$).

No.	δ_H (multiplicity, J in Hz)		δ_C	
	5	**6**	**5**	**6**
1′	–	–	105.1	104.9
2′/6′	–	–	165.4	163.4
3′/5′	5.92 (s)	5.93 (s)	95.7	94.2
4′	–	–	165.2	165.5
C=O	–	–	205.4	204.9
α	3.33 (t, 8.3)	3.34 (t, 7.5)	46.7	46.0
β	2.87 (t, 8.3)	2.91 (t, 7.5)	30.1	29.9
1	–	–	134.1	133.8
2	7.08 (d, 2.3)	6.96 (d, 2.5)	132.2	130.0
3	–	–	128.1	126.7
4	–	–	156.8	152.4
5	6.81 (d, 8.2)	6.72 (d, 8.5)	111.1	115.6
6	7.05 (dd, 8.2, 2.3)	6.97 (dd, 8.5, 2.5)	127.9	127.3
1″	2.88 (ov), 2.68 (dd, 13.4, 7.9)	3.32 (br d, 7.0)	37.8	29.9
2″	4.27 (br t, 6.8)	5.31 (tm, 7.0)	75.7	121.9
3″	–	–	149.1	134.6
4″	4.79 (m), 4.67 (m)	1.77 (s)	110.3	25.8
5″	1.75 (br m)	1.76 (s)	18.0	17.9
2′/6′-OH	11.78 (br s)	10.26 (br s)		
4′-OH	9.30 (br s)	–		
4-OCH_3	3.78 (s)	3.77 (s)		
4′-OCH_3			55.7	54.4

phenolic OH groups. The ^1H and ^{13}C NMR spectra (Table I) showed a pair of triplets of two hydrogen atoms at δ_H 3.33 and 2.87 ppm, a carbon signal at δ_C 205.4 ppm for a conjugated C=O group, and four oxyaryl carbon signals [δ_C 165.4 (2C), 165.2, 156.8 ppm], suggesting that compound **5** is a dihydrochalcone having the same oxygenation pattern as compounds **3** and **4** (Syah *et al.*, 2009). In fact, the aromatic region of the ^1H NMR spectrum showed signals very similar to those of compounds **3** and **4**, including a broad singlet of two hydrogen atoms of the chelated OH groups (δ_H 11.78 ppm). Compound

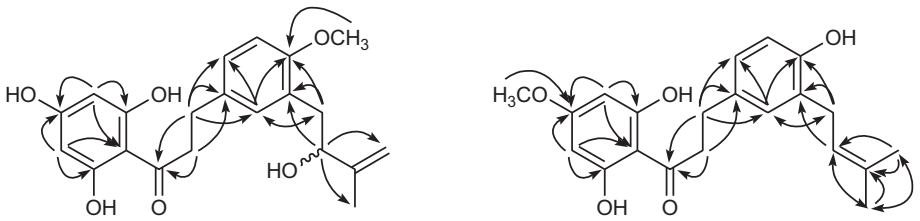

Fig. 2. Selected important HMBC correlations in compounds **5** and **6**.

Table II. MIC values (in μM) of compounds **1**–**8**.

Compound	Class	Gram-(+)		Gram-(−)					
		B. sub	*S. aur*	*E. aer*	*E. coli*	*P. aer*	*S. typ*	*S. dys*	*V. cho*
1	FV	26.5	105.8	423.3	105.8	211.6	211.6	105.8	105.8
2	FV	50.9	101.8	101.8	50.9	50.9	203.6	50.9	50.9
3	DH	105.2	105.2	841.8	105.2	210.4	420.9	210.4	210.4
4	DH	101.2	405.0	50.6	101.2	101.2	202.5	101.2	101.2
5	DH	201.4	402.8	402.8	201.4	201.4	402.8	201.4	201.4
6	DH	52.6	210.4	210.4	105.2	105.2	420.9	105.2	105.2
7	FV	523.9	523.9	523.9	262.0	262.0	523.9	262.0	262.0
8	FV	124.9	249.8	124.9	62.4	62.4	124.9	62.4	62.4
Chloramphenicol		7.2	7.2	29.1	3.6	116.1	29.1	7.2	7.2

FV, flavanone; DH, dihydrochalcone; *B. sub*, *Bacillus subtilis*; *S. aur*, *Staphylococcus aureus*; *E. aer*, *Enterobacter aerogenes*; *E. coli*, *Escherichia coli*; *P. aer*, *Pseudomonas aeruginosa*; *S. typ*, *Salmonella typhi*; *S. dys*, *Shigella dysenteriae*; *V. cho*, *Vibrio cholerae*.

5 also contains a methoxy group (δ_H 3.78 ppm and δ_C 55.7 ppm) and a C_5 side chain (δ_H 2.88 and 2.68 ppm, $-CH_2-$; 4.27 ppm, $-CH-OH$; 4.79 and 4.67 ppm, $=CH_2$; 1.75 ppm, $-CH_3$) in the form of a 2-hydroxy-3-methyl-3-butenyl group. The position of the methoxy signal (δ_H 3.78 ppm) was determined to be at C-4 of ring B, from the observation of a long-range ^{13}C-^{1}H correlation between this signal with an oxyaryl carbon signal at δ_C 156.8 ppm (Fig. 2). This carbon signal also showed the same correlation with the methylene signal (δ_H 2.88 and 2.68 ppm) (Table I). Therefore, oxymacatrichocarpin C was assigned as 1-(2,4,6-trihydroxyphenyl)-3-[4-methoxy-3-(2-hydroxy-3-methylbut-3-enyl)phenyl]propan-1-one (**5**) (Fig. 1). The stereochemistry at C-2″ was not determined.

The UV absorption behaviour of compound **6** suggested that this compound has chromophore and oxygenated functionalities similar to those of compound **5**. The molecular formula $C_{21}H_{24}O_5$ was assigned to compound **6** based on its HRESI-TOF mass spectrum (found, m/z 357.1689 $[M+H]^+$; calcd. 357.1702 $[M+H]^+$; Δ 3.64 ppm), indicating that compound **6** is an isomer of compound **3**. The NMR data of this compound were very close to those of compound **3** (Syah *et al.*, 2009) (Table I), except that in the HMBC spectrum the proton signal of the methoxy group (δ_H 3.77 ppm) was correlated with a deshielded oxyaryl carbon signal at δ_C 165.5 ppm (Fig. 2), assignable to C-4′ of the aromatic ring A. Support for the methoxy group at C-4′ was also obtained from a NOE-1D experiment. Irradiation at the methoxy proton signal increased the signal area of the H-3′/5′ signal (δ_H 5.93 ppm). Thus, compound **6** was assigned as 1-(2,6-dihydroxy-4-methoxyphenyl)-3-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]propan-1-one (isomacatrichocarpin C) (Fig. 1).

The antibacterial properties of compounds **1**–**8** were evaluated against eight pathogenic bacteria, including *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Vibrio cholerae*. The minimum inhibitory concentrations (MIC) were determined by a two-fold microdilution method (CLSI, 2012), with chloramphenicol as positive control. All compounds **1**–**8** exhibited varying degrees of moderate activity against the tested bacteria (Table II). Compounds **2** and **8** exhibited a broad spectrum of activities (MIC 50.9–249.8 μM), while compounds **1** and **3**–**7** were less active. How-

ever, compound **1** exhibited the best antibacterial effect against Gram-positive *Bacillus subtilis* (MIC 26.5 μM). The flavanones were more active than the dihydrochalcone derivatives, and more interestingly, hydrophobicity through methylation and prenylation gave a significant contribution to the antibacterial properties.

These antibacterial properties of the flavanone derivatives are consistent with those reported in the literature. The importance of prenylation in the enhancement of antibacterial properties of flavanones has been indicated by the work of Mitscher *et al.* (1983), in which 8-prenylpinocembrin (MIC 36.9 – 73.9 μM) was more active than pinocembrine (MIC 369.9 μM) itself. Other results also showed that 6- and 8-prenylflavanone derivatives were able to inhibit the growth of *Staphylococcus aureus* at 0.28 μM (Wachter *et al.*, 1999), while triprenylated flavanones with a 4′-hydroxy group in ring B were reported to be even more active than vancomycin against *Streptococcus agalactiae* 11159 (Rukachaisirikul *et al.*, 2007). However, several other groups found that prenylated- or geranylated flavanone derivatives had mostly moderate antibacterial activities (MIC $>$ 45 μM) (Rahman and Gray, 2002; Sohn *et al.*, 2004). In addition, our compounds in the flavanone series had MIC values higher than those reported by Tsuchiya *et al.* (1996) (MIC 7.1 – 35.1 μM), who tested a number of prenylated flavanone derivatives against methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Interestingly, working similarly on MRSA strains, Navratilova *et al.* (2013) also found that various geranylated flavanone derivatives exhibited good antibacterial properties, and *O*-methylation also enhanced the antibacterial activities. In the dihydrochalcone series, it was found that 2-hydroxy- and 2,4,4′,6-tetrahydroxydihydrochalcone were inactive (MIC $>$ 4.4 mM) and very weakly active (MIC 911.4 μM), respectively, against a number of MRSA strains (Osorio *et al.*, 2012). Moreover, a dihydrochalcone isolated from *Piper aduncum*, 2,6-dihydroxy-4-methoxydihydrochalcone, was moderately active against *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Okunade *et al.*, 1997), and a number of similar dihydrochalcones lacking a hydroxy group at ring B were inactive against *Escherichia coli* and *Staphylococcus aureus* (Lavoie *et al.*, 2013). Comparison of the antibacterial properties of these dihydrochalcone derivatives with those of compounds **3** – **6** also demonstrate the importance of oxygenated functionality at ring B, *C*-prenylation, and *O*-methylation for antibacterial activities.

In conclusion, in addition to the previously isolated flavonoids **1** – **4** (Syah *et al.*, 2009), four additional flavonoid derivatives, **5** – **8**, including two new prenylated dihydrochalcones, **5** and **6**, have been isolated from the acetone extract of *M. trichocarpa* collected from Kalimantan Island of Indonesia. These compounds were tested on eight pathogenic bacteria and exhibited moderate to weak antibacterial activity.

Experimental

General experimental procedures

UV spectra were measured with a Varian Cary 100 Conc (Varian Australia, Mulgrave, Victoria, Australia) instrument. ^{1}H and ^{13}C NMR spectra were recorded with an Agilent DD2 system (Agilent Technologies, Santa Clara, CA, USA) operating at 500 (^{1}H) and 125 (^{13}C) MHz using residual and deuterated solvents as reference standards. High-resolution mass spectra were obtained with either a VG Autospec Micromass mass spectrometer (EI mode, 70 eV) (Waters Micromass, Manchester, UK) or an ESI-TOF Waters LCT Premier XE mass spectrometer (Waters Micromass, Milford, MA, USA). Vacuum liquid chromatography (VLC) and column chromatography (CC) were carried out using Merck silica gel 60 GF_{254} and silica gel G60 35 – 70 mesh (Merck, Darmstadt, Germany). For thin-layer chromatography (TLC) analysis, precoated silica gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm thickness) were used.

Plant material

Samples of the leaves of *M. trichocarpa* were collected from Lungkut Layang Village, Timpah District, Center Kalimantan Province, Indonesian Borneo. The plant was identified by Mr. Ismail Rachman, Herbarium Bogoriense, Bogor, Indonesia, and a voucher specimen was deposited in the herbarium.

Extraction and isolation

The dried and powdered leaves of *M. trichocarpa* (1 kg) were macerated with acetone and, after solvent evaporation under reduced pressure, afforded a dark green acetone extract (50 g). Purification of compounds **1** – **4** from fraction 6 and fractions 9 – 11 has been described previously (Syah *et al.*, 2009). Fraction 12 (1 g) was fractionated by radial chromatography with $CHCl_3$/EtOAC mixtures (from 9:1 to 3:2, v/v)

as eluents to give five major subfractions, 12.1 – 12.5. Subfraction 12.2 (200 mg) was purified using the same method with diisopropyl ether/*n*-hexane/EtOAC (5:3:2) and afforded compound **5** (10 mg). Fraction 8 (2 g) was fractionated using VLC eluted with CH_2Cl_2, *n*-hexane/EtOAc (7:3 and 5:5) to give twelve subfractions, 8.1 – 8.12. According to TLC, the components were contained in the subfractions 8.1 and 8.2, (96 mg), and 8.5. Purification of the component in subfractions 8.1 and 8.2 was achieved using radial chromatography and *n*-hexane/diisopropyl ether (8:2) as eluent to give compound **8** (2 mg). Further purification of subfraction 8.5 using Sephadex LH-20 CC, elution with MeOH, and radial chromatography with CH_2Cl_2 as eluent gave 15 subfractions, 8.5.1 – 8.5.15. Using the same method, from subfraction 8.5.2 [*n*-hexane/$CHCl_3$ (2:8)] compounds **6** (8 mg) and **7** (14 mg) were obtained.

Oxymacatrichocarpin C (**5**): Pale yellow solid. – $[\alpha]_D^{25} + 1.5$ (MeOH, *c* 0.6). – UV: λ_{max} (MeOH) (log ε) = 202 (4.43), 223 (4.20), 286 (4.12); (MeOH + NaOH) 203 (4.50), 222 (sh, 4.18), 320 (4.11); (MeOH + $AlCl_3$) 202 (4.47), 220 (4.26), 308 (4.17), 368 (3.46) nm. – ^{1}H NMR (500 MHz, acetone-d_6): see Table I. – ^{13}C NMR (125 MHz, acetone-d_6): see Table I. – HREIMS: found, m/z = 372.1574 $[M]^+$; calcd. for $C_{21}H_{24}O_6$, m/z = 372.1573 $[M]^+$.

Isomacatrichocarpin C (**6**): Pale yellow solid. – UV: λ_{max} (MeOH) (log ε) = 203 (4.51), 223 (4.25), 286 (4.17); (MeOH + NaOH) 202 (4.58), 223 (sh, 4.24), 320 (4.18); (MeOH + $AlCl_3$) 202 (4.52), 220 (4.29), 308 (4.17), 371 (3.36) nm. – ^{1}H NMR (500 MHz, $CDCl_3$): see Table I. – ^{13}C NMR (125 MHz, $CDCl_3$): see Table I. – HRESIMS: found, m/z = 357.1689 $[M+H]^+$; calcd. for $C_{21}H_{24}O_5$, m/z = 357.1702 $[M+H]^+$.

Antibacterial assay

Determination of the minimum inhibition concentration (MIC) using the broth microdilution method was carried out according to the methods suggested by the Clinical and Laboratory Standards Institute (CLSI, 2012). The samples were dissolved in dimethyl sulfoxide (DMSO) to achieve 250 μg/mL in the first well. Two-fold dilution of samples was performed in a 96-wells microplate over the range 1.17 μg/mL to 300 μg/mL. This was achieved by filling all wells with 200 μL of Mueller Hinton broth (MHB) medium. Then 200 μL of each sample (500 μg/mL) were transferred into the first well. Two-fold serial dilution was performed by transferring 200 μL of the mixture in the first well into the next consecutive well until the end of the row. At the last well, 200 μL of the mixtures were discharged, so that the total solution volume in each well was 200 μL. Then 10 μL bacterial suspension were transferred into all wells. The microplate was incubated for 24 h at 37 °C. MIC is defined as the lowest concentration at which no bacterial growth is observed at 600 nm using a universal microplate reader. The test for all samples, positive control, and negative control were performed in triplicate, and the MIC was taken from at least two identical results. Chloramphenicol was used as the positive control.

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