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Flavanones from the Flower of *Macaranga triloba*

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Abstract: *Macaranga triloba* belongs to the family of Euphorbiaceae. Investigation on the dichloromethane extract of flower of *Macaranga triloba* collected at Hulu Terengganu, Malaysia has yielded four flavanone compounds known as 6-prenyl-3'-methoxy-eriodictyol (1), nymphaeol-B (2), nymphaeol-C (3) and 6-farnesyl-3',4',5,7-tetrahydroxyflavanone (4). The structures of these compounds were elucidated based on spectroscopic methods including nuclear magnetic resonance (NMR-1D and 2D), UV, IR as well as mass spectrometry. This is the first report of 6-prenyl-3'-methoxy-eriodictyol(1) and 6-farnesyl-3',4',5,7-tetrahydroxyflavanone (4) from the genus of *Macaranga*.

Key word: Euphorbiaceae · *Macaranga* · Flavonoids · Prenyl · Geranyl · Farnesyl · NMR

INTRODUCTION

The genus *Macaranga* is one of the largest genera of the Euphorbiaceae, with approximately 300 species [1]. *Macaranga triloba* locally known as "Mahang merah" is a tree endemic to Southeast Asia at forest margins and its water extract is used as pain relief for stomach trouble in Java [2]. A previous phytochemical investigation on the leaves of this plant has resulted in the isolation of geranylated flavanones, 2'-hydroxy-macarangaflavanone A and 4',7-dihydroxy-8-methylflavan [3]. A phytochemical study by Jang and co-researchers on the chemical constituents of the leaves of *Macaranga triloba* collected from West Kalimantan, Indonesia has led to the isolation of a new rotenoid, 4,5-dihydro-5'α-hydroxy-4'α-methoxy-6a,12a-dehydro-α-toxicarol, together with 12 known compounds namely, (+)-clovan-2β, 9α-diol, ferulic acid, 3,7,3',4',tetramethylquercetin, 3,7,3'-trimethylquercetin, 3,7-dimethylquercetin, abscisic acid, 1β,6α-dihydroxy-4(15)-eudesmene, 3β-hydroxy-24-ethylcholest-5-en-7-one, loliolide, scopoletin, taraxerol and 3-*epi*-taraxerol [4]. Besides that, the triterpene constituents of the apicuticular wax blooms obtained from the stems on *M. triloba* have been analyzed by GC-MS [5]. This paper reports on the isolation of flavanone compounds from the flower of *Macaranga triloba* which has not been reported before.

Experimental

General Procedures: The ^{1}H-NMR and ^{13}C-NMR were recorded in acetone-D and chloroform-D on Bruker 300 Ultrashield NMR spectrometer measured at 300 and 75 MHz. Chemical shifts are reported in ppm and δ and the coupling constants are given in Hz. Melting point was taken on a hot stage Gallen Kamp melting point apparatus with microscope and was uncorrected. The infrared (IR) was recorded on the Perkin Elmer spectrum one FT-IR spectrometer. The ultraviolet (UV) spectra were recorded on Shimadzu UV-Vis 160i. The mass spectra were measured on Perkin Elmer Clarus 600T spectrometer 70 eV. Vacuum liquid chromatography (VLC) used Silica gel 60, 70-230 mesh ASTM (Merk 1.07747), Radial chromatography used Si-gel 60 PF_{254} (Merck catalog number: 1.07749). Aluminum supported silica gel 60 F_{254} was used for thin layer chromatography, while glass supported silica gel 60 F_{254} was used for preparative thin layer chromatography.

Plant Material: The flowers of *Macaranga triloba* were collected from Pasir Raja, Hulu Terengganu, Malaysia and a voucher specimen (UiTM17/09) was deposited at the Herbarium of Universiti Teknologi MARA, Malaysia.

Extraction and Isolation: The flowers (1.5 kg) of *M. triloba* were air dried, ground and soaked successively with hexane, dichloromethane and methanol for 72 hours.

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The dichloromethane extract (44.41 g) appeared as dark brown gum was subjected to vacuum liquid chromatography (VLC) using gradient elution system of Hex:Ea. Twenty five fractions from three separate fractionation were obtained and spotted on TLC and similar profile were grouped to yield 6 fractions. Fraction 3 was subjected to column chromatography (CC) using gradient elution system of Hex:$CHCl_3$ and Hex:Ea to yield 47 fractions which were pooled to yield 15 fractions. Fraction 5 was subjected to medium pressure liquid chromatography (MPLC) to yield 27 fractions. Fractions 1-6 were combined and subjected to radial chromatography (RC) to yield 1 (21.1 mg) using solvent system of $CHCl_3$:Acetone with ratio 9:1. Fraction 6 was further chromatographed on reverse phase column chromatography (RPCC) to yield 26 fractions using H_2O:MeCN solvent system. Fractions 2-11 were combined and subjected to RC to which compound 2 (326.7 mg) was obtained using $CHCl_3$:Ea solvent system with ratio 9.5:0.5. Fractions 14-19 were group together and subjected to RC using $CHCl_3$:Ea with ratio 9:1 to yield compound 3 (20.6 mg). Compound 4 (58.1 mg) was easily obtained from the first RPCC from fraction 21.

6-Prenyl-3'-methoxy-eriodictyol (1):
yellow amorphous solid. MP: 82-85°C. MS m/z: 370, $C_{21}H_{22}O_6$. UV λ_{max} nm MeOH: 208, 216, 266, 290, 307, 334. IR ν_{max} cm^{-1}: 3364, 2945, 2830, 1706, 1270, 1030.

^{13}CNMR ($CDCl_3$, 75 MHz) δ ppm: 196.7 (C-4), 164.1 (C-7), 162.1 (C-5), 160.2 (C-9), 147.6 (C-5'), 147.0 (C-4'), 131.0 (C-1'), 130.3 (C-3"), 122.8 (C-2"), 119.4 (C-2'), 114.8 (C-3'), 110.1 (C-6'), 107.4 (C-6), 102.4 (C-10), 95.5 (C-8), 76.1 (C-2), 55.4 (-OCH_3), 42.7 (C-3), 25.0 (C-5"), 21.4 (C-1"), 17.0 (C-4").

Nymphaeol-B (2):
yellow amorphous solid. MP: 79-82 °C. MS m/z: 424, $C_{25}H_{28}O_6$. UV λ_{max} nm MeOH: 210, 246, 253, 269, 282, 302, 306. IR ν_{max} cm^{-1} : 3366, 2940, 2830, 1710, 1266.

^{13}CNMR ($CDCl_3$, 75 MHz) δ ppm: 196.7 (C-4), 166.5 (C-7), 164.4 (C-5), 163.8 (C-9), 144.7 (C-4'), 143.2 (C-3'), 134.5 (C-3"), 130.9 (C-8"), 128.9 (C-1'), 126.8 (C-2'), 124.2 (C-7"), 123.3 (C-2"), 117.7 (C-6'), 112.6 (C-5'), 102.2 (C-10), 95.9 (C-8), 95.0 (C-6), 76.3 (C-2), 42.4 (C-3), 39.5 (C-5"), 26.5 (C-6"), 24.9 (C-9"), 24.3 (C-1"), 16.8 (C-10"), 15.5 (C-4").

Nymphaeol-C (3):
light brown gum. MS m/z: 492, $C_{30}H_{36}O_6$. UV λ_{max} nm MeOH: 210, 239, 262, 291, 296, 356

IR ν_{max} cm^{-1} : 3366, 2945, 2834, 1705, 1270.

^{13}CNMR ($CDCl_3$, 75 MHz) δ ppm: 197.1 (C-4), 164.0 (C-7), 162.1 (C-5), 160.6 (C-9), 144.7 (C-4'), 143.2 (C-3'), 134.5 (C-3"), 130.9 (C-8"), 130.3 (C-3"'), 129.2 (C-1'), 126.7 (C-2'), 124.1 (C-7"), 123.4 (C-2"), 122.9 (C-2"'), 117.7 (C-6'), 112.6 (C-5'), 107.4 (C-6), 102.3 (C-10), 95.5 (C-8), 76.3 (C-2), 42.4 (C-3), 39.5 (C-5"), 26.5 (C-6"), 25.0 (C-9"), 24.9 (C-4"'), 24.3 (C-1"), 21.4 (C-1"'), 17.0 (C-10"), 16.8 (C-5"'), 15.4 (C-4").

6-Farnesyl-3',4',5,7-tetrahydroxyflavanone (4):
yellow amorphous solid. MP: 158-162 °C. MS m/z: 492, $C_{30}H_{36}O_6$. UV λ_{max} nm MeOH: 250, 271, 290, 306, 332. IR ν_{max} cm^{-1} : 3350, 2947, 2835, 1706, 1270.

^{13}CNMR ($CDCl_3$, 75 MHz) δ ppm: 196.4 (C-4), 164.0 (C-7), 161.4 (C-9), 161.1 (C-5), 145.5 (C-4'), 145.1 (C-3'), 134.5 (C-3"), 134.0 (C-8"), 130.8 (C-1'), 130.7 (C-13"), 124.4 (C-12"), 124.2 (C-7"), 122.7 (C-2"), 118.3 (C-6'), 115.1 (C-5'), 113.8 (C-2'), 108.2 (C-6), 102.2 (C-10), 94.5 (C-8), 79.0 (C-2), 42.8 (C-3), 39.6 (C-5"), 39.6 (C-10"), 26.6 (C-6"), 26.4 (C-11"), 25.0 (C-14"), 20.7 (C-1"), 16.9 (C-15"), 15.4 (C-4"), 15.3 (C-9"),

RESULTS AND DISCUSSION

Four flavanones were isolated from the flower of *Macaranga triloba* namely 6-prenyl-3'-methoxy-eriodictyol (1) nymphaeol-B (2), nymphaeol-C (3) and 6-farnesyl-3',4',5,7-tetrahydroxyflavanone (4). All compounds are either prenylated, geranylated or farnesylated at C-6 or/and C-2' of the flavanone skeleton. Hydroxyl group present in all compound at C-5 forming hydrogen bond with oxygen of carbonyl group (C-4). Most compounds has OH substituted at C-5, C-7, C-3' and C-4' and only compound 1 has a methoxy substituent at C-3'.

Compound 1 was isolated as amorphous yellow, exhibiting an M^+ at m/z 370, corresponding to molecular weight $C_{21}H_{22}O_6$. The ^{1}H-NMR spectrum of 1 exhibited the signal for a phenolic OH at δ_H 12.25 (s, OH-5), which was chelated to the oxygen of carbonyl group at C-4. Signals at δ_H 5.46 (1H, *dd*, *J* = 12.6, 3.0 Hz), 2.78 (1H, *dd*, *J* = 17.1, 3.0 Hz) and 3.18 (1H, *dd*, *J* = 17.1, 12.6 Hz), were attributed to H-2 and H-3 of a flavanone nucleus. An ABX system could be observed with the presence of signals at δ_H 6.89 (1H, *d*, *J* = 8.1 Hz), 7.03 (1H, *dd*, *J* = 8.1, 1.8 Hz) and 7.22 (1H, *d*, *J* = 1.8 Hz). In addition, a singlet at δ_H 6.05 indicated that ring A is a pentasubstituted ring system. Two olefinic methyl groups appeared as singlet at δ_H 1.62, a methylene (δ_H 3.24) and a vinyl protons (δ_H 5.23) indicates the presence of a prenyl group. In the HMBC spectrum of 1, the methylene signal at δ_H 3.24 (H-1")

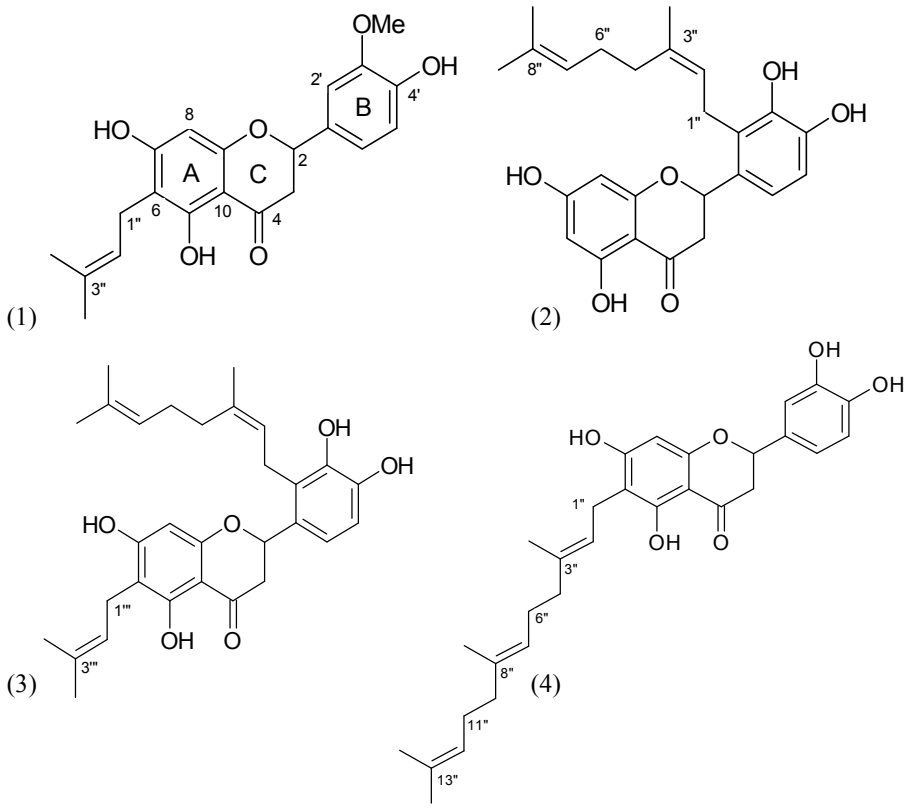

Fig. 1: Flavanones from the flower of *Macaranga triloba*

was observed to be correlated with C-5 (δ_C 162.1) and C-7 (δ_C 164.1), indicating that the prenyl group was attached to C-6 (Fig. 1). Besides, a methoxy signal was observed at δ_H 3.91 (3'-OCH_3). The signals in the ^{1}H and ^{13}C-NMR spectra were assigned based on the ^{1}H-^{1}H COSY, HMQC and HMBC data. The configuration of 1 was determined by comparing the spectral data of 1 (Table 1) with the literature values and was determined to be 6-prenyl-3'-methoxy-eriodictyol. This compound has been isolated from the flowering twigs of *Silphium laciniatum* (Asteraceae) [6], but had never been reported from *Macaranga* genus.

Compound 2 appeared as yellow amorphous solid, with an M^+ at *m/z* 424, corresponding to molecular weight $C_{25}H_{28}O_6$. The ^{1}H-NMR spectrum of 2 exhibited the signal for a phenolic OH at δ_H 12.20 that was similar to compound 1. Two doublets at δ_H 6.82 and 6.97 (*d*, *J* = 8.1 Hz) corresponded to *ortho*-coupled protons in B ring indicating substitution at C-2'. A pair of *meta*-coupled protons appeared as singlet at δ_H 5.96. Similar to compound 1, signals at δ_H 5.62(1H, *dd*, *J* = 13.2, 2.7 Hz), 2.67 (1H, *dd*, *J* = 17.1, 2.7 Hz) and 3.18 (1H, *dd*, *J* = 17.1, 13.2 Hz), were attributed to H-2 and H-3 of the flavanone nucleus. The signals of three olefinic methyl groups (δ_H 1.56, 1.60, 1.70), two methylene protons (δ_H 1.97, 2.05) and two vinyl protons (δ_H 5.07, 5.19) indicated the presence of a geranyl group that based on observation should attached to C-2'. This was supported by the HMBC correlation of 2, where the methylene signal at δ_H 3.56 (H-1") was observed to be correlated with C-1' (δ_C 128.9) and C-3' (δ_C 143.2), indicating the attachment of geranyl group to C-2' (Fig. 1). The spectral data of compound 2 resemble nymphaeol-B which was reported from *Okinawa propolis* [7], *Hernandia hymphaefolia* (presl) Kubitzki [8] and the Taiwanese *propolis* [9].

Compound 3 was isolated as light brown gum, exhibiting an M^+ at *m/z* 492, corresponding to molecular weight $C_{30}H_{36}O_6$. The presence of *ortho*-coupled protons, three olefinic methyl, two methylene and two vinyl protons clearly indicate similarity of B ring with compound 2. However, a singlet at δ_H 6.04 (H-6) indicates substitution at C-6 which was supported by the presence of additional of two olefinic methyl (δ_H 1.58, 1.61), a methylene (δ_H 3.22) and a vinyl protons (δ 5_H20) indicating the substituent as a prenyl group. The HMBC spectrum of 3 confirmed the attachment of a prenyl at C-6

Table 1: ^{1}H NMR Spectral data for compound 1, 2, 3 and 4

Position	(1)	(2)	(3)	(4)
2	5.46, *dd,* (*J* = 3.0 Hz, 12.6 Hz)	5.62, *dd,* (*J* = 2.7 Hz, 13.2 Hz)	5.59, *dd,* (*J* = 2.7 Hz, 13.5 Hz)	5.35, *dd,* (*J* = 3.0 Hz, 12.6 Hz)
3	3.18, *dd,* (*J* = 12.6, 17.1 Hz, H-3a)	2.78, *dd*, (*J* = 3.0, 17.1 Hz, H-3b)	3.18, *dd,* (*J* = 13.2, 17.1 Hz, H-3a)	2.67, *dd*, (*J* = 2.7, 17.1 Hz, H-3b)
	3.16, *dd,* (*J* = 13.5, 17.1 Hz, H-3a)	2.66, *dd*, (*J* = 2.7, 17.1 Hz, H-3b)	3.11, *dd,* (*J* = 12.6, 17.1 Hz, H-3a)	2.71, *dd*, (*J* = 3.0, 17.1 Hz, H-3b)
4	-	-	-	-
5	12.25(-OH, *s*)	12.20 (-OH, *s*)	12.21 (-OH, *s*)	12.47 (-OH, *s*)
6	-	5.96, *s*	-	-
7	-	-	-	-
8	6.05, *s*	5.96, *s*	6.04, *s*	6.04, *s*
9	-	-	-	-
10	-	-	-	-
1’	-	-	-	-
2’	7.22, *d*, (J_m = 1.8 Hz)	-	-	7.04, *s*
3’	-	-	-	-
3’-OCH_3	3.91, *s*	-	-	-
4’	-	-	-	-
5’	6.89, *d*, (J_o = 8.1 Hz)	6.82, *d*,(J_o = 8.1 Hz)	6.84, *d*,(J_o = 8.4 Hz)	6.87, *s*
6’	7.03, *d*,(J_m = 1.8 Hz, J_o = 8.1 Hz)	6.97, *d*, (J_o = 8.1 Hz)	7.07, *d*, (J_o = 8.4 Hz)	6.87, *s*
1”	3.24, *d*, (*J* = 7.2 Hz)	3.56, *d*, (*J* = 6.6 Hz)	3.57, *d*, (*J* = 6.5 Hz)	3.36, *d*, (*J* = 7.5 Hz)
2”	5.23, *tt*, (*J* = 1.2, 7.2 Hz)	5.19, *td*, (*J* = 6.6 Hz)	5.18, *t*, (*J* = 6.5 Hz)	5.28, *td*, (*J* = 6.0 Hz)
3”	-	-	-	-
4”	1.62, *s*	1.70, *s*	1.69, *s*	1.79, *s*
5”	1.62, *s*	1.97, *t*, (*J* = 6.6 Hz)	2.01, *t*, (*J* = 6.9 Hz)	1.95, *m*
6”		2.05, *m*	2.06, *m*	2.00, *m*
7”		5.07, *dt*, (*J* = 1.5, 6.5 Hz)	5.06, *qt*, (*J* = 1.5, 6.5 Hz)	5.12, *dt*, (*J* = 1.2, 5.7 Hz)
8”		-	-	-
9”		1.60, *s*	1.61, *s*	1.58, *s*
10”		1.56, *s*	1.55, *s*	1.95, *m*
1”’/11”			3.22, *d*, (*J* = 6.5 Hz)	2.00, *m*
2”’/12”			5.20, *qt*, (*J* = 1.2, 6.6 Hz)	5.09, *dt*, (*J* = 1.5, 5.4 Hz)
3”’/13”			-	-
4”’/14”			1.61, s	1.65, *s*
5”’/15”			1.58, s	1.58, *s*

with the observed correlations of the methylene at δ_H 3.22 (H-1”’) with C-5 (δ_C 162.1) and C-7 (δ_C 164.0). The spectral data of 3 showed close similarity to nymphaeol-C, a prenylated and geranylated flavanone previously isolated from Okinawa propolis [7], *Hernandia hymphaefolia* (presl) Kubitzki [8] and *Macaranga tanarius* [10].

Compound 4 was obtained as yellow amorphous solid, exhibiting an M^+ at *m/z* 492, corresponding to molecular weight $C_{30}H_{36}O_6$. The ^{1}H-NMR spectrum of 4 was very similar to 1 except for additional signals which indicates the prenyl group at C-6 being replaced by a farnesyl group. The additional signals can be observed with the presence of three olefinic methyl at δ_H 1.58 (6H) and 1.65 (3H), two methylene protons (δ_H 1.95-2.00) and three vinyl protons at δ_H 5.09 and 5.12 clearly indicative of a farnesyl group. This was further supported by the observed correlation of methylene (δ_H 3.36, H-1”) with C-5 (δ_C 161.4) and C-7 (δ_C 164.0), in HMBC spectrum confirming the farnesyl attachment to C-6. Thus compound 4 was determined to be 6-farnesyl-3’,4’,5,7-tetrahydoxyflavanone previously reported from the aerial parts of *Boronia Ramosa* (Rutaceae) [11] and isolated from *Macaranga* genus for the first time.

CONCLUSION

As a conclusion, four flavonoid compounds, 6-prenyl-3’-methoxy-eriodictyol (1), nymphaeol-B (2), nymphaeol-C (3) and 6-farnesyl-3’,4’,5,7-tetrahydroxyflavanone (4) have been successfully isolated from the flower of *M. triloba*.

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