Prenylflavonols from the Leaves of *Macaranga sampsonii*

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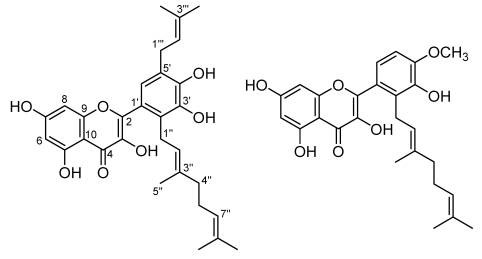
Four novel prenylflavonols, macaranones A—D (1—4), were isolated from the leaves of *Macaranga sampsonii*. Their structures were elucidated on the basis of spectroscopic data. Macaranones C (3) and D (4) represent first two examples of flavonols having an unusual peltogynoid skeleton which is formed from a 2′-geranylflavonol by cyclization between 3-OH and C-1″ of the 2′-geranyl substituent of the flavonol. Compounds 1—4 were evaluated for the cytotoxicity against several human cancer cell lines.

Key words *Macaranga sampsonii*; *Macaranga*; prenylflavonol; flavonoid

Prenylated flavonoids are attracting more and more attention from the scientific community due to their structural uniqueness and interesting biological activities.[1)] These compounds have a relatively narrow distribution in the plant kingdom.[1)] The genus *Macaranga*, one of the largest genera of the Euphorbiaceae family, with no less than 280 species,[2)] has been indicated to be a rich source of prenylated flavonoids, especially geranyl flavonoids.[3—12)] *M. sampsonii* is a small tree native to South China and North Vietnam.[2)] No phytochemical studies were previously reported. Within the scope of our continuous search of bioactive compounds from natural plants in South China, we investigated the leaves of this plant and isolated four novel prenylflavonols, macaranones A—D (**1**—**4**). Macaranones C (**3**) and D (**4**) are the first two examples of flavonols having an unusual peltogynoid skeleton formed from a 2′-geranylflavonol by cyclization between 3-OH and C-1″ of 2′-geranyl group of the flavonol. Here we report the isolation and structural elucidation of these compounds.

Results and Discussion

The ground dry leaves of *M. sampsonii*, after defatting with petroleum ether, were extracted with EtOAc. The resultant EtOAc extract was subjected to repeated column chromatography over silica gel, polyamide, and Sephadex LH-20, and HPLC to afford four new flavanols, macaranones A—D (**1**—**4**).



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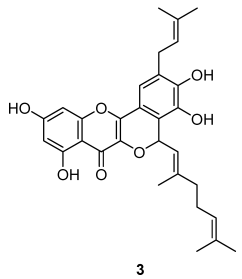

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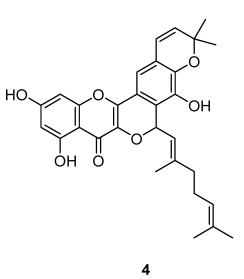

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Macaranone A (**1**), a yellow amorphous solid, had a molecular formula of $C_{30}H_{34}O_7$ as determined from the HR-ESI-MS ion at *m*/*z* 529.2181 $[M+Na]^+$. The ^{13}C-NMR spectrum (Table 1) indicated the presence of 30 carbons, including five methyl groups and a carbonyl carbon [δ 176.2 (C-4)], corresponding to a flavonoid containing an isoprenyl group and a geranyl group. The ^{1}H-NMR (Table 1) revealed the presence of two *meta*-coupled [δ 6.17 (1H, d, J=2.0 Hz, H-6) and 6.25 (1H, d, J=2.0 Hz, H-8)] and an uncoupled [δ 6.65 (1H, s, H-6′)] aromatic protons, four phenolic hydroxy groups [δ 10.69, 8.85, 8.67, and 8.40 (1H each, br s)], and a hydrogen-bonded hydroxy group [δ 12.53 (1H, s, OH-5)], suggesting a 7-substituted 5-hydroxyflavonol with the pentasubstituted B-ring. The spectrum, analyzed in combination of the ^{1}H–^{1}H correlation spectroscopy (COSY) (Fig. 1) and heteronuclear multiple quantum coherence (HMQC) spectra, also indicated the presence of an isoprenyl group [δ 1.64 and 1.66 (each 3H, br s, H_3-4‴ and H_3-5‴), δ 3.24 (2H, d, J=7.2 Hz, H_2-1‴), and δ 5.26 (1H, t, J=7.2 Hz, H-2‴)] and a geranyl group [δ 1.32, 1.45, and 1.55 (each 3H, br s, H_3-5″, H_3-9″, and H_3-10″), δ 1.70 and 1.75 (each 2H, m, H_2-4″ and H_2-6″), δ 3.26 (2H, d, J=7.2 Hz, H_2-1″), and δ 4.96 and 4.93 (each 1H, t, J=7.2 Hz, H-2″ and H-7″)].[13)] The complete structure was deduced from the heteronuclear multiple bond correlation (HMBC) spectrum (key correlations depicted in Fig. 1). HMBC correlations from the hydroxy proton at δ 10.69 to the three carbons at δ 93.3 (C-8), 98.1 (C-6), and 163.7 (C-7) indicated the attachment of the hydroxy group to C-7. Cross-peak between the only proton (δ 6.65) on the B-ring and the quaternary carbon at δ 150.7 (C-2) allowed assignment of the proton to H-6′. The correlations from H-6′ to C-1‴ (δ 28.1), C-1′ (δ 121.6), C-2′ (δ 126.7), and C-4′ (δ 145.0), from H_2-1‴ (δ 3.24) to C-5′ (δ 125.8), C-6′ (δ 121.9), and C-4′, and from H_2-1″ (δ 3.26) to C-1′, C-2′, and C-3′ (δ 143.1) indicated the attachment of the isoprenyl group to C-5′, the geranyl group to C-2′, and two hydroxy groups to C-3′ and C-4′. Therefore, the structure of **1** was assigned as

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Table 1. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR Data for Compounds **1**—**4** in DMSO-d_6

Position	**1**		**2**		**3**		**4**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2		150.7		150.2		148.1		147.6
3		136.3		136.5		140.2		140.3
4		176.2		176.3		174.6		174.6
5		160.9		160.9		161.5		161.5
6	6.17 d (2.0)	98.1	6.18 d (2.0)	98.2	6.16 d (2.0)	98.4	6.17 d (2.0)	98.5
7		163.7		163.8		163.6		163.7
8	6.25 d (2.0)	93.3	6.26 d (2.0)	93.4	6.43 d (2.0)	93.7	6.42 d (2.0)	93.6
9		156.7		156.7		155.7		155.8
10		103.3		103.5		104.4		104.5
1′		121.6		123.3		114.0		115.2
2′		126.7		127.9		121.8		123.0
3′		143.1		144.0		140.7		140.6
4′		145.0		148.6		147.6		143.5
5′		125.8	6.92 d (8.0)	109.0		128.8		120.8
6′	6.65 s	121.9	6.89 d (8.0)	121.2	7.05 s	114.7	7.12 s	111.0
1″	3.26 d (7.2)	25.7	3.30 d (6.8)	25.5	6.21 d (9.2)	68.8	6.15 d (9.6)	68.7
2″	4.96 t (7.2)	122.7	4.98 t (6.8)	122.5	5.38 br d (9.2)	121.1	5.42 d (9.6)	120.8
3″		133.7		134.0		131.1		131.5
4″	1.70 m	39.1	1.71 m	38.9	1.91 m	38.7	1.93 m	38.7
5″	1.32 s	15.7	1.34 br s	15.8	1.88 br s	16.8	1.88 br s	16.7
6″	1.75 m	26.1	1.76 m	26.1	1.94 m	25.4	1.96 m	25.4
7″	4.93 t (7.2)	124.1	4.93 t (6.8)	124.1	4.88 t (6.8)	123.5	4.89 t (7.2)	123.5
8″		130.6		130.6		130.8		130.8
9″	1.45 s	17.4	1.45 br s	17.4	1.41 s	17.4	1.42 s	17.4
10″	1.55 s	25.4	1.55 br s	25.4	1.43 s	25.1	1.44 s	25.1
1‴	3.24 d (7.2)	28.1			3.30 d (7.2)	28.2	6.50 d (10.0)	121.1
2‴	5.26 t (7.2)	122.5			5.28 t (7.2)	122.3	5.83 d (10.0)	131.7
3‴		131.4				131.9		77.4
4‴	1.64 s	17.6			1.70 s	17.8	1.42 s	27.6
5‴	1.66 s	25.5			1.70 s	25.5	1.42	27.8
4′-OCH_3			3.81 s	55.9				
3-OH	8.85 br s		8.93 br s					
5-OH	12.53 s		12.51 s		12.82 s		12.77 s	
7-OH	10.69 br s		11.80 br s		10.81 br s		10.81 br s	
3′-OH	8.40 br s		8.75 br s		9.15 br s		9.19 br s	
4′-OH	8.67 br s				9.15 br s			

shown.

Macaranone B (**2**) was also obtained as a yellow amorphous solid. Its molecular formula was determined as $C_{26}H_{28}O_7$ from the HR-ESI-MS ion at m/z 475.1709 $[M+Na]^+$ and the NMR spectra (^{1}H-, DEPT-, and ^{13}C-NMR). The ^{1}H-NMR spectrum of **2** (Table 1) was similar to that of **1** except that the signals for the isoprenyl group and the singlet for H-6′ in **1** were absent in **2**. Instead, two doublets (J=8.0 Hz) at δ 6.92 and 6.89 for two *ortho*-coupled aromatic protons and a singlet at δ 3.81 for an aromatic methoxy group were present. Interpretation of ^{1}H–^{1}H COSY, HMQC, and HMBC spectra (Fig. 1) led to full assignments of ^{1}H- and ^{13}C-NMR data as shown in Table 1, and unambiguously established the location of these two aromatic protons at C-5′ and C-6′, and the methoxy group at C-4′. Accordingly, the structure of **2** was determined as shown.

Macaranone C (**3**) was establisted as having a molecular formula of $C_{30}H_{32}O_7$, comprising two hydrogens less than **1**. The ^{1}H-NMR spectrum of **3** was very similar to that of **1** but with two significant differences: one of the two signals for benzylic methylenes (H_2-1″ and H_2-1‴) was absent while a methine signal was present at δ 6.21 (1H, d, J=9.2 Hz), and one of three olefinic methine protons in the isoprenoid side chains appeared as a broad doublet at δ 5.38 (J=9.2 Hz) instead of a broad triplet in **1**. By analysis of the ^{1}H–^{1}H COSY (Fig. 1) and HMQC spectra, the protons in the ^{1}H-NMR spectrum and the hydrogen-bearing carbons in the ^{13}C-NMR spectrum were assigned as shown in Table 1, indicating that C-1″ position appeared as an oxymethine group[14] instead of a methylene in **1**. H–C long-range correlations from the

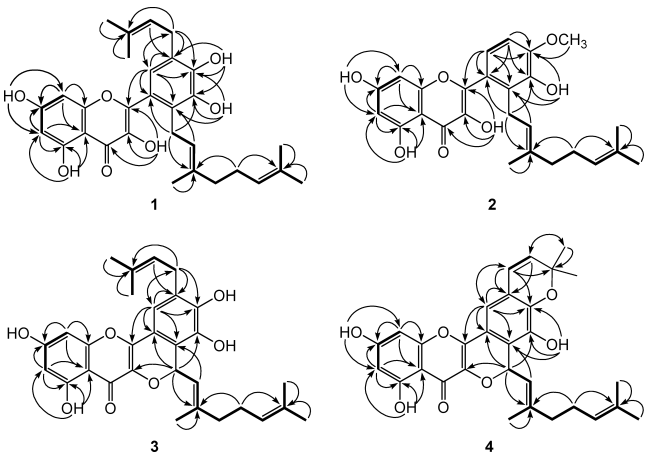

Fig. 1. COSY (Bold Lines) and Key HMBC (Arrows) Correlations of **1**—**4**

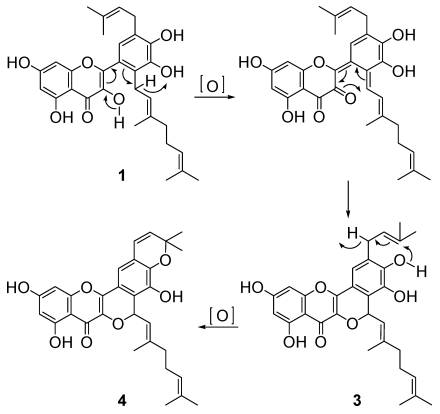

Chart 1. A Plausible Biogenetic Transformation from **1** to **3** and **4**

oxymethine proton at δ 6.21 (H-1″) to C-3 (δ 140.2), C-1′ (δ 114.0), C-2′ (δ 121.8), and C-3′ (δ 140.7) observed in the HMBC spectrum (Fig. 1) revealed the connectivity between C-3 and C-1″ *via* an oxygen bridge to form a pyran ring. Thus, **3** has the structure as shown. This compound, possessing a chiral center at C-1″, was found to exist as a racemic mixture in view of its optical inactivity.

Macaranone D (**4**) was determined to have a formula of $C_{30}H_{30}O_7$, containing two hydrogens less than **3**. Its ^{1}H- and ^{13}C-NMR spectra (Table 1) indicated a structure similar to **3** but with a different C_5-isoprenoid moiety at C-5′. The ^{1}H-NMR signals at δ 6.50 (1H, d, J=10.0 Hz, H-1‴), 5.83 (1H, d, J=10.0 Hz, H-2‴), and 1.42 (6H, s, H_3-4‴ and H_3-5‴), and the corresponding carbon resonances at δ 121.1 (C-1‴), 131.7 (C-2‴), 77.4 (C-3‴), 27.6 (C-4‴), and 27.8 (C-5‴) indicated the presence of a 2,2-dimethylchromene ring.[13] The arrangement of the chromene ring on the flavonol nucleus was achieved by analysis of the HMBC spectrum (Fig. 1). H–C long-range correlations from H-1‴ to C-4′, C-5′, C-6′, and C-3‴, and from H-6′ to C-4′ and C-1‴ indicated the connectivity between C-4′ and C-3‴ *via* an oxygen bridge. Thus, the structure of **4** was assigned as shown. This compound, with no optical activity, was also a racemate.

Compounds **3** and **4** represent the first two examples of flavonols possessing an unusual peltogynoid skeleton which is formed from a 2′-geranylflavonol by cyclization between 3-OH and C-1″ of the 2′-geranyl substituent of the flavonol. They seem to be biogenetically derived from **1** through phenol oxidative cyclization as found for the flavones with dihydrobenzoxanthone and pyranoflavone skeletons[15,16] in consideration of co-occurrence of them with **1**. A route for biogenetic transformation from **1** to **3** and **4** was postulated as shown in Chart 1.

Prenylated flavonoids have been found to exhibit a wide range of bioactivities, including *in vitro* antitumor activity.[1,16,17] Compounds **1**—**4** were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method[18] for the cytotoxicity against human cancer cell lines including lung cancer (A549), pulmonary carcinoma (LAC), gastric carcinoma (SGC-7901), and hepatoma (HepG2) cell lines, but found to be inactive at 20 μg/ml except that **1** exhibited weak activity against HepG2 cell line (IC_{50}= 6.9 μg/ml).

Experimental

General Procedures Optical rotations were obtained on a Perkin-Elmer 343 polarimeter with MeOH as solvent. UV spectra were recorded in MeOH on a Perkin-Elmer Lambda 25 UV–vis spectrophotometer. ^{1}H- (400 MHz), ^{13}C- (100 MHz), and 2D-NMR spectra were recorded on a Bruker DRX-400 in DMSO-d_6 with the residual solvent peak (δ_H 2.49 and δ_C 39.51) as reference. HR-ESI-MS were obtained on a Bruker Bio TOF IIIQ mass spectrometer in positive-ion mode. ESI-MS were collected on an MDS SCIE API 2000 LC/MS/MS instrument. Preparative HPLC was run with a Shimadzu LC-6A pump and a Shimadzu RID-10A refractive index detector using an XTerra prep MS C_{18} column (10 μm, 300×19 mm). For column chromatography, Si gel 60 (100—200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Develosil ODS (10 μm, Nomura Chemical Co. Ltd., Japan), polyamide (Shanghai Hushi Chemical Co. Ltd., China), and Sephadex LH-20 were used.

Plant Material The leaves of *M. sampsonii* were collected from Dinghu Mountain, Zhaoqing, Guangdong, China, in January 2007. A voucher specimen (KUN0188993) has been deposited at the Herbarium of South China Botanical Garden, Chinese Academy of Sciences.

Extraction and Isolation Powder of dry leaves of *M. sampsonii* (1.9 kg) was sequentially extracted three times each with petroleum ether, EtOAc, and 95% EtOH. The EtOAc extract, upon evaporation, yielded a deep brown syrup (26.0 g). This syrup was subjected to Si gel CC eluted with $CHCl_3$–MeOH mixtures of increasing polarity (98 : 2 to 6 : 4) to obtain 11 fractions (I—XI). Fraction I, obtained on elution with $CHCl_3$–MeOH (98 : 2), was chromatographed on a polyamide column using MeOH–H_2O (6 : 4) to obtain six subfractions (I-1—6). Subfraction I-2 was further separated by Sephadex LH-20 CC using MeOH followed by HPLC using 70% MeOH to afford **2** (15 mg). By the same method, subfraction I-3 gave **1** (350) and **3** (56 mg), and subfraction I-4 afforded compound **4** (8 mg).

Macaranone A (**1**): A yellow amorphous solid; UV λ_{max} (MeOH) nm (ε): 209 (19330), 254 (17780), 297 (7580), 352 (9330). ^{1}H- and ^{13}C-NMR, see Table 1; negative ESI-MS *m/z*: 1011 $[2M-H]^-$, 505 $[M-H]^-$; positive ESI-MS *m/z*: 529 $[M+Na]^+$, 507 $[M+H]^+$, 451 $[M-C_4H_7]^+$, 383 $[M-C_9H_{15}]^+$, 355, 329, 327, 153; HR-ESI-MS *m/z*: 529.2181 $[M+Na]^+$ (Calcd for $C_{30}H_{34}O_7Na$, 529.2202).

Macaranone B (**2**): A yellow amorphous solid; UV λ_{max} (MeOH) nm (ε): 211 (18200), 254 (13490), 295 (sh.), 385 (6610); ^{1}H- and ^{13}C-NMR, see Table 1; negative ESI-MS *m/z*: 903 $[2M-H]^-$, 451 $[M-H]^-$, 367 $[M-C_6H_{12}-H]^-$, 299, 179, 151; HR-ESI-MS *m/z*: 475.1709 $[M+Na]^+$ (Calcd for $C_{26}H_{28}O_7Na$, 475.1733).

Macaranone C (**3**): A yellow amorphous solid; $[\alpha]_D^{24}$ ±0° (c=0.8, MeOH); UV λ_{max} (MeOH) nm (ε): 209 (28840), 263 (15850), 295 (sh), 385 (9550); ^{1}H- and ^{13}C-NMR (100 MHz, DMSO-d_6), see Table 1; negative ESI-MS *m/z*: 1007 $[2M-H]^-$, 503 $[M-H]^-$, 433 $[M-C_5H_{10}-H]^-$, 351, 179, 151; HR-ESI-MS *m/z*: 527.2054 $[M+Na]^-$ (Calcd for $C_{30}H_{32}O_7Na$, 527.2046).

Macaranone D (**4**): A yellow amorphous solid; $[\alpha]_D^{24}$ ±0° (c=0.4, MeOH); UV λ_{max} (MeOH) nm (ε): 206 (25120), 256 (7940), 297 (6310), 342 (4470); ^{1}H- and ^{13}C-NMR, see Table 1; negative ESI-MS *m/z*: 1039 $[2M+Cl]^-$, 1003 $[2M-H]^-$, 537 $[M+Cl]^-$, 501 $[M-H]^-$, 349, 179, 151; HR-ESI-MS *m/z*: 525.1860 $[M+Na]^+$ (Calcd for $C_{30}H_{30}O_7Na$, 525.1889).

Cytotoxicity Assay Cytotoxicity was determined by MTT method[16] using human lung cancer (A549), human pulmonary carcinoma (LAC), human gastric carcinoma (SGC-7901), and human hepatoma (HepG2) cells grown in RPMI-1640 medium plus 10% heat-inactivated fetal bovine serum. The assays were performed in 96-well microtiter plates. Serial two-fold dilutions of compounds **1**—**4** were made in dimethyl sulfoxide (DMSO). Then 5 μl of each serial solution was added to 195 μl (about 10000 cells) culture medium in wells. After incubation at 37 °C for 48 h, 10 μl of MTT (5 g/l) was added to each well and incubated for four more hours, and then liquid in the wells was removed. DMSO (200 μl) was added to each well. The absorbance was recorded on a microplate reader (Bio-Rad model 550) at a wavelength of 570 nm. IC_{50} was defined as 50% reduction of absorbance in the control assay which was treated with 2.5% DMSO alone. The IC_{50} values for **1** were determined to be 6.9 (HepG2), and >20.0 (other cell lines) mg/ml; the values for **2**—**4** were all more than 20.0 μg/ml towards all test cell lines.

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