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Diterpenylated and prenylated flavonoids from *Macaranga denticulata*

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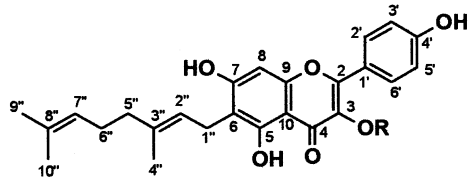
Abstract—An *O*-methylated analogue of macarangin (**1**) and denticulaflavonol (**2**) which possesses a diterpene substituent have been isolated along with other compounds from the leaves of *Macaranga denticulata*. Their detailed 1D and 2D spectral analysis are reported. The radical scavenging properties of compounds **3**–**5** were evaluated. Among the compounds tested, **4** showed a pronounced antioxidant activity (IC_{50} 0.032±0.001 mM). © 2002 Elsevier Science Ltd. All rights reserved.

In our investigation for bioactive constituents from plants, we studied the leaves of *Macaranga denticulata* Muell. Arg. (synonymous name *Macaranga henricorum* Hemsl., Euphorbiaceae), known in Thai as 'Tong Taeb'.[1] The plant is a shrubby tree which grows to about 20 m in height. The stem water decoction has been used traditionally for washing wounds and drunk as tonic by women after child labor.[1] Previously reported constituents from the species of this genus comprise terpenes, steroids, hydrolysable tannins and prenylflavanones from *M. tanarius*,[2–5] bergenin derivatives from *M. peltata*,[6] chromenoflavones from *M. indica*,[7] a hexahydroxanthene derivative and a geranyl flavonol from *M. vedeliana*,[8,9] prenylated flavanones from *M. pleiostemona*,[10] and geranyl stilbenes from *M. schweinfurthii*.[11]

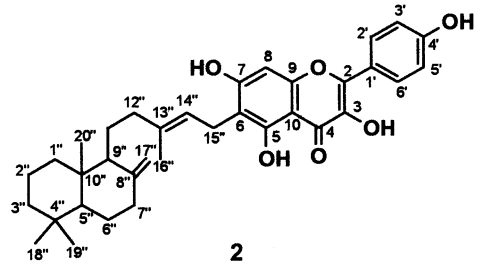
Two flavonoids, one of which is named denticulaflavonol, features the presence of a unique labdane diterpene moiety, have been isolated from the leaves of this plant along with other compounds. To our knowledge, this is the first example of a flavonoid possessing a diterpene substituent. We herein describe the isolation, structural elucidation and radical scavenging properties of the isolates.

The chloroform extract of the dried leaves of *M. denticulata* was purified as described in the experimental section to yield two compounds (**1**, **2**) as well as scopoletin (**3**),[12] macarangin (**4**)[8] and sophoraflavanone B (**5**).[13] The known compounds were identified by comparison of their published spectral data.



1, R = Me

4, R = H



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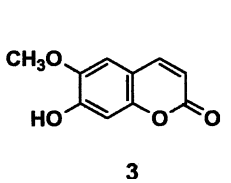

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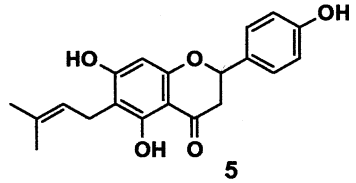

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Compound **1** was obtained as yellow sticky solid. It was assigned the molecular formula $C_{26}H_{28}O_6$ by HR-EIMS. The FT-IR showed absorption bands at ν_{max} 3275, 1658 and 1608 cm^{-1} indicating the presence of a hydroxy, an

Keywords: *Macaranga denticulata*; Euphorbiaceae; diterpene; flavonoid; diterpenylated flavonoid; antioxidant.

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Table 1. ^{1}H and ^{13}C NMR spectral data of **1** (in MeOH-d_4), **2** and **4** (in $CDCl_3$)

No.	**2**			**1**			**4**	
	δ_H[a]	δ_C[b]	HMBC	δ_H[a]	δ_C[b]	HMBC	δ_H[a]	δ_C[b]
2		146.0 (s)			130.6 (s)			145.5 (s)
3	6.59 (s, 3-OH)	135.5 (s)	3-OH/C-2, 3, 4	3.66 (s, 3-OMe)	139.8 (s)[c]	3-OMe/C-3	5.9 (s, 3-OH)	135.4 (s)
4		175.3 (s)			179.9 (s)			175.2 (s)
5	12.1 (s, 5-OH)	158.0 (s)	5-OH/C-5, 6, 10		159.7 (s)		12.1 (s, 5-OH)	157.4 (s)
6		109.4 (s)			112.9 (s)			109.4 (s)
7	6.25 (s, 7-OH)	161.7 (s)	7-OH/C-6, 7, 8		163.7 (s)		6.6 (s, 7-OH)	161.8 (s)
8	6.48 (s)	94.3 (d)	C-6, 9, 10	6.33 (s)	93.9 (d)	C-4, 6, 7, 9, 10	6.45 (s)	94.3 (d)
9		155.0 (s)			156.3 (s)			155.0 (s)
10		103.2 (s)			105.7 (s)			103.5 (s)
1′		123.5 (s)			122.7 (s)			123.4 (s)
2′,6′	8.12 (d, 8.9)	129.6 (d)	C-2, 4′	7.87 (d, 8.9)	131.3 (d)	C-2, 3′, 5′	8.1 (d, 8.5)	129.6 (d)
3′,5′	6.97 (d, 8.8)	115.6 (d)	C-1′, 4′	6.82 (d, 8.9)	116.5 (d)	C-2′, 4′	6.94 (d, 8.3)	115.6 (d)
4′	5.37 (s, 4′-OH)	157.3 (s)	4′-OH/C-3′, 5′		161.6 (s)		5.5 (s, 4′-OH)	157.6 (s)
1″	a 1.74 (m), b 0.97 (ddd, 12.8, 12.8, 3.9)	39.0 (t)	C-3″, 5″, 9″, 20″	3.22 (m)	22.2 (t)	C-5, 7, 2″, 3″	3.48 (d, 7.1)	21.4 (t)
2″	n.o.	n.o.		5.14 (dd, 7.2, 6.3)	123.5 (d)	C-6, 4″, 5″	5.28 (m)	120.9 (d)
3″	a 1.37 m, b 1.15 (ddd, 13.4, 13.2, 4.0)	42.1 (t)	C-1″, 18″, 19″		135.7 (s)			139.8 (s)
4″		n.o.		1.68 (s)	16.3 (q)	C-3″, 5″	1.83 (s)	16.2 (q)
5″	1.05 (dd, 9.9, 2.7)	55.5 (d)	C-1″, 6″, 10″, 18″, 19″, 20″	1.86 (dd, 7.4, 6.8)	40.9 (t)	C-2″, 3″, 4″, 6″	2.10 (m)	39.7 (t)
6″	a 1.67 (m), b 1.27 (m)	24.4 (t)	C-8″	1.95 (dd, 7.6, 7.1)	27.7 (t)	C-3″, 5″, 7″	2.10 (m)	26.3 (t)
7″	a 2.4 (ddd, 12.7, 4.1, 2.3), b 1.90 (ddd, 13.7, 13.3, 4.7)	38.3 (t)	C-5″, 6″, 8″, 17″	4.95 (m)	125.4 (d)	C-9″, 10″	5.05 (m)	123.7 (d)
8″		148.6 (s)			132.0 (s)			132.1 (s)
9″	1.56 (m)	56.0 (d)	C-1″, 8″, 17″, 20″	1.51 (s)	25.8 (q)	C-7″, 10″	1.68 (s)	25.7 (q)
10″		39.6 (s)		1.45 (s)	17.7 (q)	C-7″, 9″	1.59 (s)	17.7 (q)
11″	a 1.47 (m), b 1.43 (m)	19.4 (t)	C-9″, 12″					
12″	a 2.19 (m), b 1.85 (m)	38.5 (t)	C-13″					
13″		140.7 (s)						
14″	5.27 (dd, 7.3, 6.5)	120.6 (d)	C-12″, 16″					
15″	3.49 (d, 7.0)	21.4 (t)	C-5, 6, 7, 13″, 14″					
16″	1.84 (s)	16.4 (q)	C-12″, 13″					
17″	a 4.80 (d, 1.2), b 4.49 (s)	106.2 (t)	C-7″, 8″					
18″	0.86 (s)	33.6 (q)	C-3″, 19″					
19″	0.79 (s)	21.7 (q)	C-3″, 18″					
20″	0.67 (s)	14.5 (q)	C-1″, 5″, 9″					

n.o. signal was not observed, may be obscured by other signals.
[a] Multiplicities and *J*-values in Hz are given in parentheses.
[b] Multiplicities were deduced from DEPT experiments.
[c] Signal of 3-OCH_3 was found at δ_C 60.5 (q).

α,β-unsaturated carbonyl and aromatic groups, respectively. The ^{1}H NMR in MeOH-d_4 exhibiting one set of AB doublets at δ_H 7.87 (2H, d, J=8.9 Hz) and 6.82 (2H, d, J=8.9 Hz) together with the ^{13}C NMR signals at δ_C 161.6 (s), 131.3 (d), 116.5 (d) and 122.7 (s) indicated the *para*-disubstituted phenol ring. The presence of a geranyl group was deduced from the mass spectrum which showed base peak at *m/z* 313 ($[M-CH{=}CMeCH_2CH_2CH{=}C(Me)_2]^+$, 100 %) as well as the ^{1}H NMR showing two olefinic protons at δ_H 5.14 (1H, d, J=7.2, 6.3 Hz) and 4.95 (1H, m), allylic protons at δ_H 3.22 (2H, d, J=3.4, 1.7 Hz), two sets of two proton signals at δ_H 1.95 (dd, J=7.6, 7.1 Hz) and 1.86 (dd, J=7.4, 6.8 Hz) and three methyl group signals at δ_H 1.68 (s), 1.51 (s), and.1.45 (s). The lowfield signal at δ_H 12.98, an indicative evidence of a C-5–OH proton intramolecularly H-bonded to C-4 carbonyl oxygen atom of a flavonoid nucleus, could be detected when deuterated chloroform was used as solvent although the compound is sparingly soluble. A broad signal at δ_H 6.33 assigned to the C-8 H showed $^{3}J_{CH}$ correlations to the signals at δ_C 105.7 (C-10), 112.9 (C-6) as well as $^{2}J_{CH}$ and $^{4}J_{CH}$ correlations to the signals at δ_C 156.3 (C-9), 163.7 (C-7) and 179.9 (C-4), respectively. A geranyl substitution at C-6 was deduced from the important $^{3}J_{CH}$ correlations of the signal at δ_H 3.22 (H-1″) to ^{13}C resonances at δ_C 112.9 (C-6), 123.5 (C-2″), 135.7 (C-3″) 159.7 (C-5) and 163.7 (C-7). This compound showed NMR data very similar to values published for macarangin (**4**), a compound previously isolated from *M. vedeliana*[8] and also isolated in the present study. The only difference observed was a methyl proton signal at δ_H 3.66. The $^{3}J_{CH}$ correlation between the signal at δ_H 3.66 with the signal at δ_C 139.8 (C-3) strongly indicated the presence of an *O*-methyl group at C-3. Compound **1** was proposed to be 3-*O*-methyl-macarangin, 3-*O*-methyl-6-[(*E*)-3″,8″dimethyl-2″,7″-octadienyl]-kaempferol.

Compound **2** was obtained as yellowish sticky mass. The HR-EIMS gave a molecular formula of $C_{35}H_{42}O_6$. The IR spectrum showed the presence of a hydroxyl and a carbonyl groups at ν_{max} 3232 and 1647 cm^{-1}, respectively. The ^{1}H NMR spectrum measured in $CDCl_3$ showing two sets of doublets at δ_H 8.12 (H-2′, 6′) and 6.97 (H-3′, 5′), a one proton singlet at δ_H 6.48 (H-8) and a downfield proton at δ_H 12.1 (C-5–OH) with the absence of the 3-OMe group

signal, as found in compound **1**, indicated the existence of a kaempferol $C_{15}H_9O_6$ unit with the same substitution pattern as for **1**. The remaining $C_{20}H_{31}$ moiety, establishing five double bond equivalents, was recognized as a labdane type diterpene conjugate by the presence of an exocyclic methylene group signals at δ_H 4.80 (1H, d, J=1.2 Hz) and 4.49 (1H, s), a vinylic proton of a trisubstituted double bond at δ_H 5.27 and a downfield methyl group signal at δ_H 1.84 (3H, s) assignable to –C=C–CH_3 in addition to the three shielded quaternary methyl group singlets at δ_H 0.86, 0.79 and 0.67 which have one-bond correlations with the ^{13}C shifts of the methyl groups commonly encountered in the labdanic diterpenes[14] at δ_C 33.6 (C-18″), 21.7 (C-19″) and 14.5 (C-20″) in the HMQC spectrum, respectively. Connection of the labdanyl unit to C-6 was established by the long range ^1H–^{13}C correlations of signals at δ_H 3.49 (assigned for H-15″) to signals at δ_C 109.4 (C-6), 158.0 (C-5) and 161.7 (C-7). The placement of a trisubstituted double bond at C-13″–C-14″ was further deduced by the ^1H–^1H COSY and HMBC correlations particularly of H-15″ to C-13″ (δ_C 140.7) and C-14″ (δ_C 120.6). Detailed interpretation of the ^1H–^1H COSY, HMQC and HMBC spectra, in conjunction with the analysis of ^{13}C shifts of several representatives of the labdane diterpenes reported previously,[14] permitted unambiguous assignments of ^1H and ^{13}C resonances as indicated in Table 1, the key long range ^1H–^{13}C correlations are also shown. Compound **2**, trivially named denticulaflavonol, was identified as 6-[15″-labda-8″(17″),13″(14″)-dienyl]-kaempferol.

Due to the scarcity of pure samples, only compounds **3**–**5** were evaluated for their antioxidant properties using 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radical.[15] Macarangin (**4**) exhibited an IC_{50} 0.032±0.001 mM which is as pronounced as a standard antioxidant, 2,6-di-(*tert*-butyl)-4-methylphenol (BHT), IC_{50} 0.031±0.001 mM. Scopoletin (**3**) also showed rather significant antioxidant property (IC_{50} 0.342±0.026 mM). Since sophoraflavanone B (**5**) showed only 31.8% reduction at 0.759 mM, its IC_{50} was therefore not determined.

1. Experimental

1.1. General

The specific rotations were measured by a Jasco DIP 1020 polarimeter. The IR spectra were obtained on a Perkin–Elmer 1760x FT-IR spectrophotometer. UV spectra were recorded on a Perkin–Elmer Lambda 6 instrument. EI-MS and HR-EIMS spectra were recorded on a Finnigan MAT 90 instrument. ^1H and ^{13}C spectra were obtained with a Bruker AVANCE 400 MHz spectrometer with the solvent signal as internal reference.

1.2. Plant material

Leaves of *M. denticulata* were collected from Trat Province in December 1997. The species was kindly identified by Dr K. Chayamarit of the Royal Forest Herbarium, Bangkok. A voucher specimen (SSMD/1997) is kept at the Chemistry Department, Faculty of Science, Ramkhamhaeng University.

1.3. Extraction and isolation

The dried leaves (1230 g) were ground and extracted successively with *n*-hexane, chloroform and methanol, respectively. The chloroform extract (33.7 g) was separated on silica gel with gradient of *n*-hexane/CH_2Cl_2 (15:85) to CH_2Cl_2/MeOH (3:2) to afford nine fractions. Fraction 8 was purified by column chromatography over a Si gel, with gradient of *n*-hexane/EtOAc (3:1) to EtOAc/MeOH (9:1) to obtain six fractions (fractions 8.1–8.6). Additional Si gel chromatography of fraction 8.3 with *n*-hexane/EtOAc (9:1) afforded 12 subfractions (subfractions 8.3.1–8.3.12). Compounds **1** (20 mg, 0.00163% w/w) and **5** as a sticky gum (31 mg, 0.00252% w/w) were obtained from subfraction 8.3.6 after further column chromatography over Si gel (*n*-hexane/EtOAc 8:2). Fraction 6 was chromatographed over Si gel, eluted with a gradient of CH_2Cl_2 to CH_2Cl_2/MeOH (6:4) and afforded subfractions 6.1–6.5. Subfraction 6.4 was further chromatographed over Si gel using gradient of *n*-hexane/CH_2Cl_2 2:8 to CH_2Cl_2 and yielded subfractions 6.4.1–6.4.3. Subfraction 6.4.1 contained pure compound **3** as a pale yellow solid, mp 193–195°C, (20 mg, 0.00163% w/w). Subfraction 6.4.2 gave compound **2** (3 mg, 0.00024% w/w) after additional column chromatography over Si gel using *n*-hexane/EtOAc 9:1. Subfraction 6.4.3 was purified by Si gel column chromatography using gradient of *n*-hexane/CH_2Cl_2 2:8 to CH_2Cl_2/MeOH 9:1 to yield compound **4** as a yellow solid, mp 140–143°C, (120 mg, 0.00975% w/w).

1.3.1. 3-*O*-Methyl-macarangin (1). Yellow sticky solid; R_f 0.17 [silica gel, *n*-hexane/EtOAc 7:3]; $[\alpha]_D^{26}$=−0.5254 (*c* 0.590, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.50), 214 (4.49), 270 (4.27), 339 (4.23) nm; IR (film) ν_{max}: 3275, 2936, 1658, 1608, 1568, 1471, 1359, 1286, 1233, 1177, 1089, 973, 815 cm^{-1}; ^1H and ^{13}C NMR data (measured in MeOH-d_4) see Table 1; EI-MS (70 eV) *m/z* (rel. int.): 437 ($[M+1]^+$, 1) 371 (21), 365 (11), 351 (18), 345 (21), 344 (61), 340 (16), 325 (15), 314 (20), 313 (100), 299 (10), 281 (5), 270 (13), 253 (6), 242 (9), 203 (10), 165 (8), 121 (55), 105 (11), 91 (18); HR-EIMS *m/z* 436.1894 $[M]^+$ (calcd for $C_{26}H_{28}O_6$, 436.1886, error in ppm=1.83).

1.3.2. Denticulaflavonol (2). Yellow sticky mass; R_f 0.32 [silica gel, *n*-hexane/EtOAc 7:3]; $[\alpha]_D^{26}$=−131.8 (*c* 0.050, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (4.67), 270 (4.13), 329 (4.04) nm; IR (film) ν_{max}: 3232, 2921, 1647, 1542, 1508, 1363, 1180, 1087, 883, 841 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 1; HR-EIMS *m/z* 558.2978 $[M]^+$ (calcd for $C_{35}H_{42}O_6$, 558.2981, error in ppm=0.53).

1.4. Bioassay

Compounds **3**–**5** were tested for radical scavenging properties using DPPH.[15] 50 μL of a solution containing the compound to be tested was added to 5 ml of a 0.004% methanolic solution of DPPH. Absorbance at 517 nm was determined after 30 min at 37°C, and the percent of activity was calculated. IC_{50} is the mean±standard deviation of three assays.

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