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Prenylated flavonoids of the leaves of *Macaranga conifera* with inhibitory activity against cyclooxygenase-2

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Abstract

Two prenylated flavonoid derivatives, 5-hydroxy-4′-methoxy-2″,2″-dimethylpyrano-(7,8:6″,5″)flavanone (**1**) and 5,4′-dihydroxy-[2″-(1-hydroxy-1-methylethyl)dihydrofurano]-(7,8:5″,4″)flavanone (**2**), were isolated from an ethyl acetate-soluble extract of the leaves of *Macaranga conifera* using an in vitro activity-guided fractionation procedure based on the inhibition of cyclooxygenase-2. Also obtained were eight known compounds, 5,7-dihydroxy-4′-methoxy-8-(3-methylbut-2-enyl)flavanone (**3**), lonchocarpol A (**4**), sophoraflavanone B (**5**), 5,7-dihydroxy-4′-methoxy-8-(2-hydroxy-3-methylbut-3-enyl)flavanone (**6**), tomentosanol D (**7**), lupinifolinol (**8**), isolicoflavonol (**9**), and 20-epibryonolic acid (**10**). The structures of compounds **1** and **2** were determined using spectroscopic methods. All isolates were tested for their inhibitory effects against both cyclooxygenases-1 and -2, and selected compounds were evaluated in a mouse mammary organ culture assay.

Keywords: *Macaranga conifera*; Euphorbiaceae; Favonoids; 5-Hydroxy-4′-methoxy-2″,2″-dimethylpyrano-(7,8:6″,5″)flavanone; 5,4′-Dihydroxy-[2″-(1-hydroxy-1-methylethyl)dihydrofurano]-(7,8:5″,4″)flavanone; Lonchocarpol A; Tomentosanol D; Cyclooxygenase-1 and -2; Mouse mammary organ culture assay

1. Introduction

The genus *Macaranga* (Euphorbiaceae) comprises more than 300 species found mainly in the southern part of Asia (Webster, 1994). Local healers apply fresh or dried leaves of certain *Macaranga* species to treat cuts, swellings, sores, bruises and boils (Singh et al., 1984; Nick et al., 1995). Previous phytochemical investigations have been performed on various *Macaranga* species, as exemplified by the isolation of allelopathic prenylflavonones and other constituents from *M. tanarius* (Hui et al., 1975; Tseng et al., 2001), prenylated flavonones from *M. pleiostemona* (Schütz et al., 1995), cytotoxic geranyl stilbenes from *M. schweinfurthii* (Beutler et al., 1998), a cytotoxic prenylated stilbene from *M. mappa* (Van der Kaaden et al., 2001) and chromenoflavones from *M. indica* (Sultana and Ilyas, 1986). Up to the present, no phytochemical or pharmacological investigations have been reported on *Macaranga conifera*.

Several epidemiological studies have demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) have cancer preventive and tumor regressive effects in the human colon (Giardiello et al., 1995; Levy, 1997). Cyclooxygenase-2 (COX-2) is responsible for the biosynthesis of prostaglandins under acute inflammatory conditions (O'Banion et al., 1992; Smith et al., 1994). Thus, the regulation of the COX pathway provides an excellent approach for the discovery of cancer chemopreventive agents (Kelloff et al., 1996; Kawamori et al., 1998; Cuendet and Pezzuto, 2000). As part of our

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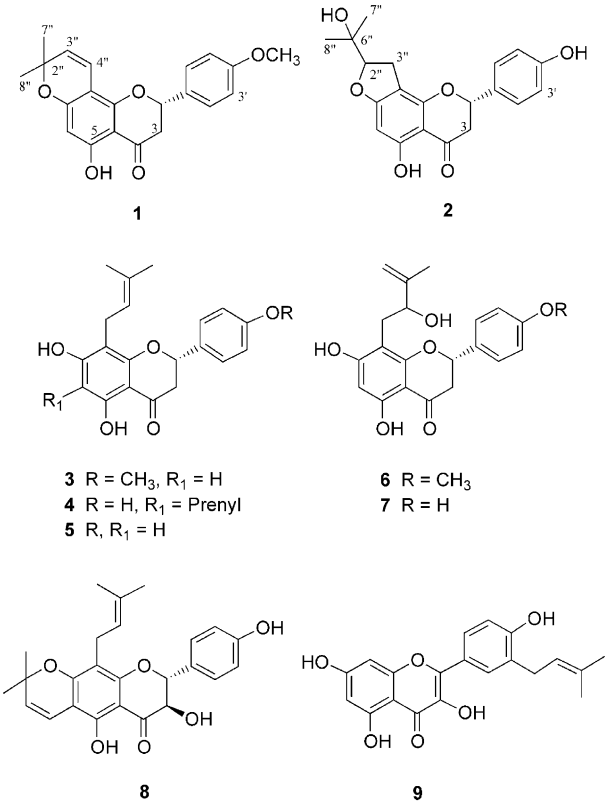
continuing search for novel, plant-derived cancer chemopreventive agents (Kinghorn et al., 1998), an ethyl acetate soluble extract of the leaves of *M. conifera* (Zoll.) Muell. Arg. (Euphorbiaceae) was shown to exhibit significant biological activity in a preliminary in vitro screening against COX-2. Bioassay-guided fractionation of the ethyl acetate-soluble extract, using this in vitro COX-2 inhibition assay, led to the purification of two new prenylated flavonoid derivatives (**1** and **2**) and eight known compounds (**3**–**10**). All isolates were evaluated for their potential cancer chemopreventive properties against both COX-1 and -2. Selected compounds were then chosen for evaluation in a mouse mammary organ culture assay. The structure elucidation of **1** and **2** and the biological evaluation of **1**–**10** are described herein.



2. Results and discussion

The ethyl acetate-soluble fraction of the methanol extract of the leaves of *Macaranga conifera* utilizing a COX-2 inhibition assay to monitor fractionation, led to the isolation and characterization of two new compounds (**1** and **2**), along with eight known compounds, 5,7-dihydroxy-4′-methoxy-8-(3-methylbut-2-enyl)-flavanone (**3**) (Parsons et al., 1993), lonchocarpol A (**4**) (Fomum et al., 1987; Tahara et al., 1994), sophoraflavanone B (**5**) (Komatsu et al., 1978), 5,7-dihydroxy-4-methoxy-8-(2-hydroxy-3-methylbut-3-enyl)flavanone (**6**) (Parsons et al., 1993), tomentosanol D (**7**) (Tanaka et al., 1997), lupinifolinol (**8**) (Smalberger et al., 1974), isolicoflavonol (**9**) (Hatano et al., 1988), and 20-epibryonolic acid (**10**) (Chang et al., 1996). The known compounds were identified by physical (mp, $[\alpha]_D$) and spectroscopic data measurement (MS, ^{1}H NMR, ^{13}C NMR and 2D NMR) and by comparison with published values.

Compound **1** was obtained as a white amorphous powder and gave a protonated molecular ion at m/z 353.1375 $[M+1]^+$ by HREIMS, consistent with an elemental formula of $C_{21}H_{21}O_5$. The ^{1}H NMR spectrum of **1** (Table 1) showed resonances for an ABX system at δ_H 2.78 (1H, *dd*, $J=17.2$, 3.0 Hz), δ_H 3.04 (1H, *dd*, $J=17.2$, 13.0 Hz) and δ_H 5.36 (1H, *dd*, $J=13.0$, 3.0 Hz), which is diagnostic for H-2 and H-3 of a flavanone nucleus (Yakushijin et al., 1980). The doublet resonances at δ_H 5.45 ($J=10.1$ Hz) and δ_H 6.52 ($J=10.1$ Hz), each equivalent to one proton, and the two singlet resonances at δ_H 1.42 (3H) and δ_H 1.44 (3H), were characteristic of the *cis* double bond and *gem*-dimethyl group of a 2,2-dimethylchromene moiety, respectively (Rao and Srimannarayana, 1983). Two *ortho*-coupled doublets centered at δ_H 6.95 (2H, $J=8.7$ Hz) and δ_H 7.38 (2H, $J=8.7$ Hz) were assigned to the protons of a *para*-disubstituted benzene ring (B ring). Also, a singlet at δ_H 12.10 (OH-5), which was strongly hydrogen-bonded to the C-4 carbonyl group, and a singlet at δ_H 3.84 (3H, *s*, OCH_3-4′)

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **1** (in $CDCl_3$) and **2** (in CD_3COCD_3)[a]

	1		**2**	
Position	δ_C	δ_H	δ_C	δ_H
2	78.9	5.36 *dd* (13.0, 3.0)	79.8	5.49 *dd* (12.7, 3.0)
3	43.2	2.78 *dd* (17.2, 3.0)	43.5	2.74 *dd* (17.1, 3.0)
		3.04 *dd* (17.2, 13.0)		3.16 *dd* (17.1, 12.7)
4	196.0		196.9	
5	163.8	12.10 *br s*	165.9	12.45 *br s*
6	97.6	6.00 *s*	91.5	5.88 *s*
7	162.3		169.9	
8	102.0		105.3	
9	156.9		158.2	
10	102.9		103.5	
1′	130.5		130.8	
2′/6′	127.6	7.38 *d* (8.7)	129.0	7.40 *d* (8.6)
4′	160.0		158.7	
3′/5′	114.1	6.95 *d* (8.7)	116.2	6.89 *d* (8.6)
2″	78.1		92.7	4.73 *dd* (9.4, 8.0)
3″	126.4	5.45 *d* (10.1)	27.1	2.96 *m*
4″	115.6	6.52 *d* (10.1)		
6″			71.3	
7″	28.3[b]	1.42 *s*[b]	25.7[b]	1.19 *s*[b]
8″	28.5[b]	1.44 *s*[b]	25.8[b]	1.26 *s*[b]
OCH_3-4′	55.4	3.84 *s*		

[a] TMS was used as internal standard; chemical shifts are shown in the δ scale with J values (Hz) in parentheses. Run at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

[b] Assignments may be interchanged within the same column.

were observed in the ^{1}H NMR spectrum. These data suggested that **1** has a flavanone skeleton with one hydroxyl group, one methoxyl group and a dimethylpyran moiety, and these inferences were confirmed using the DEPT, COSY and HMQC NMR techniques. The positions of the substituents were deduced as occurring at C-5 (hydroxyl), C-4′ (methoxyl), and C-7, C-8 (dimethylchromene ring) using the HMBC NMR technique (see Experimental). The absolute stereochemistry at C-2 of compound **1** was established as *S* based on the observation of a positive Cotton effect at 316 nm (θ +0.26) and a negative Cotton effect at 296 nm (θ −1.00) in its circular dichroism spectrum (Gaffield, 1970). Thus, the structure of the new compound **1** was elucidated as (2*S*)-5-hydroxy-4′-methoxy-2″,2″-dimethylpyrano-(7,8:6″,5″)flavanone.

Compound **2** was obtained as a pale yellow solid and its molecular formula established as $C_{20}H_{20}O_6$ by positive HRESIMS at *m*/*z* 357.1232 ($[M+1]^+$). In the ^{1}H NMR spectrum of **2** (Table 1), three protons at δ_H 2.74 (1H, *dd*, *J* = 17.1, 3.0 Hz), δ_H 3.16 (1H, *dd*, *J* = 17.1, 12.7 Hz) and δ_H 5.49 (1H, *dd*, *J* = 12.7, 3.0 Hz), typically assignable to H-3 and H-2 of a flavanone system, were observed. The ^{1}H NMR spectrum of **2** also showed a downfield resonance at δ_H 12.45, attributed to a chelated hydroxyl proton, while two doublets in the aromatic region (at δ 7.40 and 6.89, each 2H, *J* = 8.6 Hz) suggested the presence of a *para*-substituted aromatic ring. Additionally, four proton signals at δ_H 1.19, 1.26 (each 3H, *s*, *gem*-dimethyl), 2.96 (2H, *m*, methylene protons) and 4.73 (*dd*, *J* = 9.4, 8.0 Hz, oxymethine proton) indicated that compound **2** is based on a flavanone skeleton with a 1-hydroxy-1-methylethyldihydrofuran group (Tahara et al., 1994; Ngadjui et al., 1999). The location of each functional group was confirmed using 2D NMR spectroscopic techniques, suggesting that these two hydroxyl groups were present at C-5 and C-4′, with the dihydrofuran ring attached to C-7 and C-8. The absolute configuration at C-2 was confirmed by a negative Cotton effect in the $\pi \rightarrow \pi^*$ transition region (~290 nm) of the CD spectrum. Thus, the structure of the new compound **2** was elucidated as (2*S*)-5,4′-dihydroxy-[2″-(1-hydroxy-1-methylethyl)dihydrofurano]-(7,8:5″,4″)flavanone.

Isolates **1**–**10** were evaluated for their cyclooxygenase-1 and -2 (COX-1 and -2) inhibitory activity (Table 2). In recent investigations, flavan and stilbenoid derivatives have been found to exhibit significant in vitro inhibitory activity against both COX-1 and COX-2 (Jang et al., 1997; Waffo-Téguo et al., 2001a; Su et al., 2002). Also, prenylation of resveratrol at certain positions increased in vitro inhibitory potency against COX-1 but not against COX-2 (Su et al., 2002). In the present study, in vitro inhibitory potency against both COX-1 and-2 of sophoraflavanone B (**5**; IC_{50} 72.6 and >100 μM, respectively) was increased significantly by prenylation at position C-6 as in lonchocarpol A (**4**; IC_{50} 16.9 and 9.5 μM, respectively). It was also observed that replacement of a methoxyl group at the C-4′ position, as in compound **6** (IC_{50} >100 μM), with a hydroxyl group, as in compound **7** (IC_{50} 27.8 μM), led to an increase of in vitro inhibitory potency against COX-2 in a selective manner. Isolicoflavonol (**9**) showed significant inhibitory activities against both COX-1 (IC_{50} 10.4 μM) and COX-2 (IC_{50} 6.2 μM). However, further investigations are needed to determine whether the inhibitory potency of compound **9** is due to its hydroxyl group at C-3 and/or its prenyl group at C-3′. Compound **9** was recently isolated by our group as a potent aromatase inhibitor from the whole plants of *Broussonetia papyrifera* (Lee et al., 2001). Compounds **4**–**9** were evaluated for their potential to inhibit the development of 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced preneoplastic mammary lesions using a mouse mammary organ culture assay system (Mehta and Moon, 1991), and the results are summarized in Table 2. Lonchocarpol A (**4**), which showed strong inhibitory activities in both COX-1 and -2, also exhibited significant inhibitory activity (86.1% at 10 μg/ml). Therefore, compound **4** is a promising candidate for additional biological evaluation to further define its potential as a cancer chemopreventive agent.

Table 2
Inhibitory activities of compounds **4**–**9** against cyclooxygenase-1 (COX-1) and -2 (COX-2), and in a mouse mammary organ culture (MMOC) model[a]

Compound	COX-1 [IC_{50} (μM)]	COX-2 [IC_{50} (μM)]	MMOC[b] (at 10 μg/ml)
4	16.9	9.5	86.1
5	72.6	>100	37.5
6	126.2	>100	25.0
7	>100	27.8	68.2
8	12.8	28.9	ND[c]
9	10.4	6.2	58.4
trans-Resveratrol[d]	1.1	1.3	87.5

[a] The new compounds **1** and **2** and the known compounds, 5,7-dihydroxy-4′-methoxy-8-(3-methylbut-2-enyl)flavanone (**3**) and 20-epibryonolic acid (**10**) were inactive (IC_{50} >100 μg/ml) in the COX-1 and COX-2 assays.

[b] Inhibition of 7,12-dimethylbenz[*a*]anthracene-induced preneoplastic lesions with mouse mammary organ culture; results are expressed as percent inhibition. On the basis of historical controls, an inhibition of >60% (at 10 μg/ml) is considered significant.

[c] Not determined because the amount of the available compound was insufficient.

[d] *trans*-Resveratrol was used as a positive control.

3. Experimental

3.1. General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. UV

spectra were recorded with a Beckman DU-7 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrometer. NMR experiments were conducted on Bruker DPX-300 and Bruker DRX-500 MHz spectrometers with tetramethylsilane (TMS) as internal standard. EIMS and HREIMS were recorded on a Finnigan MAT 90 instrument operating at 70 eV and ESMS on a Hewlett-Packard 5989B mass spectrometer with a 5998A electrospray interface. MALDI-TOF-MS data were obtained on a Bruker Reflex III TOF mass spectrometer.

TLC analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich, Milwaukee, WI) followed by charring at 110 °C for 5–10 min. Silica gel (Merck 60A, 70–230 or 200–400 mesh ASTM) and Sephadex LH-20 (Sigma) were used for CC. Prep. TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness). All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ) and distilled before use. A YMC-pack ODC-AQ column (5 μm, 25 × 1 cm i.d., YMC Co., Wilmington, NC) and a YMC-guardpack ODC-AQ guard column (5 μm, 5 × 2 cm i.d.) were used for prep. HPLC, along with two Waters 515 HPLC pumps and a Waters 2487 dual λ absorbance detector (Waters, Milford, MA).

3.2. Assay for inhibition of COX activity

Inhibition assays against both COX-1 and -2 were performed as described previously by measuring prostaglandin E_2 (PGE_2) production (Cuendet and Pezzuto, 2000; Waffo-Téguo et al., 2001b). Reaction mixtures were prepared in 100 mM Tris–HCl buffer, pH 8.0, containing 1 μM heme, 500 μM phenol, 300 μM epinephrine, sufficient amounts of COX-1 or COX-2 to generate 150 ng of PGE_2/ml, and various concentrations of test samples. The reaction was initiated by the addition of arachidonic acid (final concentration, 10 μM) and incubated for 10 min at room temp. (final volume, 200 μl). Then, the reaction was terminated by adding 20 μl of the reaction mixture to 180 μl of 27.8 μM indomethacin, and PGE_2 was quantitated by an ELISA method. Samples were diluted to the desired concentration with 100 mM potassium phosphate buffer (pH 7.4) containing 2.34% NaCl, 0.1% bovine serum albumin, 0.01% sodium azide, and 0.9 mM Na_4EDTA. Following transfer to a 96-well plate (Nunc-Immuno Plate Maxisorp, Fisher) coated with a goat anti-mouse IgG (Jackson Immuno Research Laboratories), the tracer (PGE_2-acetylcholinesterase; Cayman Chemical, Ann Arbor, MI) and primary antibody (mouse antiPGE_2; Monsanto, St. Louis, MO) were added. Plates were then incubated at room temperature overnight, reaction mixtures were removed, and wells were washed with a solution of 10 mM potassium phosphate buffer (pH 7.4) containing 0.01% sodium azide and 0.05% Tween 20. Ellman's reagent (200 μl) was added to each well, and the plate was incubated at 37 °C for 3–5 h, until the control wells yielded OD = 0.5–1.0 at 412 nm. A standard curve with PGE_2 (Cayman Chemical, Ann Arbor, MI) was generated from the same plate, which was used to quantify the PGE_2 levels produced in the presence of test samples. Results were expressed as a percentage relative to a control (solvent-treated samples). All determinations were performed in duplicate, and values generally agreed within 10%. Dose–response curves were generated for the calculation of IC_{50} values.

3.3. Mouse mammary organ culture assay

This assay was carried out according to an established protocol (Mehta and Moon, 1991). In brief, BALB/c female mice were pretreated for 9 days with estradiol (1 μg) and progesterone (1 mg). On the tenth day, the mice were sacrificed and the second pair of thoracic mammary glands was dissected on silk and transferred to 60-mm culture dishes containing 5 ml of Waymouth's 752/1 MB medium supplemented with streptomycin, penicillin and L-glutamine. The glands were incubated for 10 days (37 °C, 95% O_2 + 5% CO_2) in the presence of growth-promoting hormones (5 μg insulin, 5 μg of prolactin, 1 μg aldosterone, and 1 μg hydrocortisone per ml of medium). Glands were exposed to 2 μg/ml 7,12-dimethylbenz(*a*)-anthracene (DMBA) between 72 and 96 h. After exposure, glands were rinsed and transferred to new dishes with fresh medium. The fully differentiated glands were then permitted to regress by withdrawing all hormones except insulin for 14 additional days. Test compounds were present in the medium during days 1–10 of culture; mammary glands were scored for incidence of lesions. Compounds were tested at a single concentration of 10 μg/ml, and 15 glands were used for each determination. Results were expressed as percent inhibition (clear glands/glands determination lesions), and no signs of toxicity were visualized with these experimental conditions.

3.4. Plant material

The leaves of *Macaranga conifera* (Zoll.) Muell. Arg. (Euphorbiaceae) were collected in Kintap, South Kalimantan, Indonesia, in May 1994 by K.K., H.H.S.F. and L.B.S.K. Voucher specimens (number K042) have been deposited at the Herbarium Bogoriense, Bogor, Indonesia and at the Field Museum of Natural History, Chicago, IL.

3.5. Extraction and isolation

The dried and milled plant material (1180 g) was extracted with MeOH (3 × 4 l) by maceration. The

extracts were combined and concentrated *in vacuo* at 40 °C. The concentrated extract was suspended in 90% MeOH and then partitioned with petroleum ether (3 × 2 l) to afford a petroleum ether-soluble syrup (D001, 15.9 g) on drying. Next, the aqueous methanol extract was concentrated and suspended in H_2O (2 l) and partitioned again with EtOAc (3 × 2 l) to give an EtOAc-soluble extract (D002, 57.5 g) and an aqueous residue (D003, 78.60 g). The rates of inhibition of cyclooxygenase-2 (COX-2) by D001, D002 and D003 at 10 μg/ml were 37, 77, and 40%, respectively.

Based on the initial biological testing, the EtOAc-soluble extract (D002) was subjected to silica gel chromatography using a $CHCl_3$–MeOH gradient (from 24:1 to 1:1 v/v) to yield 11 pooled fractions (F004–F014). Of these, F007 and F008 showed the most potent COX-2 inhibitory activities (72 and 80% inhibition at 10 μg/ml, respectively), and were combined (19 g). Separation on silica gel with gradient mixtures of $CHCl_3$–Me_2CO (from 19:1 to 100% acetone) afforded fractions F015–F023. Of these, fractions F016 and F019 were the most active (80 and 88% inhibition at 10 μg/ml, respectively). Then, F016 [eluted with $CHCl_3$–Me_2CO (19:1 v/v), 2.7 g] was applied to a silica gel column with petroleum ether–Me_2CO (24:1 → 1:1 v/v) to provide fractions F024–F035. 5,7-Dihydroxy-4′-methoxy-8-(3-methylbut-2-enyl)flavanone (**3**, 90 mg, 0.0076%) was isolated from fraction F030, eluted with petroleum ether–Me_2CO (13:1 v/v), by recrystallization from MeOH. Final purification of fraction F024, eluted by petroleum ether–Me_2CO (24:1 v/v), was carried out by prep. TLC using *n*-hexane–EtOAc (4:1 v/v) as developing solvent, to yield compound **1** (2.0 mg, 0.00017%, $R_f = 0.65$). From fraction F031, eluted by petroleum ether–Me_2CO (11:1 v/v), lonchocarpol A (**4**, 14 mg, 0.00012%) was isolated by prep. TLC using $CHCl_3$–MeOH (19:1 v/v) as the developing solvent. 5,7-Dihydroxy-4′-methoxy-8-(2-hydroxy-3-methylbut-3-enyl)flavanone (**6**, 12 mg, 0.001%) was isolated from fraction F035, eluted by petroleum ether–Me_2CO (4:1 v/v), and purified by recrystallization (petroleum ether/EtOAc).

Purification of active fraction F019 [eluted with $CHCl_3$–Me_2CO (14:1 v/v), 2.4 g] by passage over silica gel with petroleum ether–Me_2CO (14:1 → 3:1 v/v) afforded fractions F043–F054. 20-Epibryonolic acid (**10**, 6 mg, 0.00051%) and lupinifolinol (**8**, 5.3 mg, 0.00045%) were crystallized from F045 (MeOH) and F046 (petroleum ether–EtOAc, 1:1), respectively. Sophoraflavanone B (**5**, 21 mg, 0.0018%) was isolated by prep. TLC using $CHCl_3$–Me_2CO (8:1 v/v) as developing solvent. Fraction F049, eluted with petroleum ether–Me_2CO (7:1 v/v), was passed over a column containing Sephadex LH-20 (Sigma, St. Louis, MO) using MeOH for elution, resulting in the purification of isolicoflavonol (**9**, 34 mg, 0.0029%). Compound **2** (2.3 mg, 0.0002%) and tomentosanol D (**7**, 7.2 mg, 0.00061%) were isolated from subfraction F051, eluted with petroleum ether–Me_2CO (5:1 v/v), by semi-preparative HPLC eluted with mixtures of MeCN, MeOH and 0.1% HOAC (in H_2O) (30:15:55 v/v) (flow rate, 3.2 ml/min; detection wavelength, 210 and 290 nm) at room temp.

*3.5.1. 5-Hydroxy-4′-methoxy-2″,2″-dimethylpyrano-(7,8:6″, 5″)flavanone (**1**)*

Colorless powder, mp 153–155 °C; $[\alpha]_D^{20}$ −48° ($CHCl_3$; *c* 0.1); CD nm (*n*-hexane; 23 °C; rel. [θ]): 223 (+0.85), 246 (+0.47), 278 (−0.79), 296 (−1.00), 316 (+0.26); UV λ_{max} *n*-hexane nm (log ϵ): 205 (4.35), 272 (4.27), 295 (3.71), 357 (3.15); IR ν_{max} NaCl cm^{-1}: 3018, 2925, 2853, 1640, 1517, 1459, 1216, 759; HMBC correlations: H-2/C-2′, C-6′; H-3/C-4, C-2, C-1′; OH-5/C-10, C-5, C-6; H-6/C-10, C-5, C-7, C-8; H-2″ and H-6′/C-2, C-3′, C-4′; H-3′ and H-5′/ C-1′, C-2′, C-4′; OCH_3-4′/C-4′; H-4″/C-7, C-8, C-9, C-2″; H-3″/C-8, C-2″, C-7″, C-8″; H-7″/C-3″, C-2″, C-8″; H-8″/C-3″, C-2″, C-7″; EIMS *m*/*z* (rel. int.): 352 ($[M]^+$ 25), 337 (65), 203 (100), 129 (40); HREIMS *m*/*z*: 353.1375 ($C_{21}H_{20}O_5$, calc. 353.1389 $[M+1]^+$); ^1H and ^{13}C NMR spectral data: Table 1.

*3.5.2. 5,4′- Dihydroxy -[2″- (1-hydroxy-1-methylethyl) dihydrofurano]-(7,8:5″,4″)flavanone (**2**)*

Pale yellow solid; $[\alpha]_D^{20}$ −99° (MeOH; *c* 0.1); CD nm (EtOH; 23 °C; rel. [θ]): 228 (+0.53), 238 (+1.00), 271 (−0.37), 279 (−0.19), 290 (−0.44); UV λ_{max} EtOH nm (log ϵ): 239 (3.94), 298 (4.13), 337 (3.70); IR ν_{max} NaCl cm^{-1}: 3500–3100, 3020, 2974, 2930, 1643, 1608, 1516, 1474, 1383, 1249, 1143, 1090, 754; HMBC correlations: H-2/C-2′, C-6′; H-3/C-2, C-4, C-1′; OH-5/C-10, C-5, C-6; H-6/C-10, C-5, C-7, C-8; H-2′ and H-6′/C-2, C-3′, C-4′; H-3 and H-5′/C-1′, C-2′, C-4′; H-3″/C-7, C-8, C-9, C-2″, C-6″; H-2″/C-7, C-7″, C-8″; H-7″/C-2″, C-6″, C-8″; H-8″/C-2″, C-6″, C-7″; ESIMS *m*/*z* (rel. int.): 357 ($[M+1]^+$ 100), 285 (11), 237 (14), 219 (23), 165 (16), 147 (17); HRESIMS *m*/*z*: 357.1232 ($C_{20}H_{21}O_6$, calc. 357.1186 $[M+1]^+$); ^1H and ^{13}C NMR spectral data: Table 1.

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