

## New phenolic derivatives from *Vernonia mapirensis* Gleason

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### Abstract

Six new phenolic derivatives, including four flavonoids and two benzofuranones, were isolated from the aerial part extracts of *Vernonia mapirensis* Gleason (synonymous *Lepidaploa mapirensis* (Gleason) H. Robinson, *Vernonia trichoclada* Gleason, Asteraceae family), together with four known flavonoids. Their structural elucidation was achieved by extensive spectroscopic methods, 1D- (<sup>1</sup>H, <sup>13</sup>C, <sup>13</sup>C DEPT, TOCSY, ROESY) and 2D-NMR experiments (DQF-COSY, HSQC, HMBC) as well as ESI-MS analysis.

**Keywords:** *Vernonia mapirensis*, Asteraceae, flavonoids, benzofuranones

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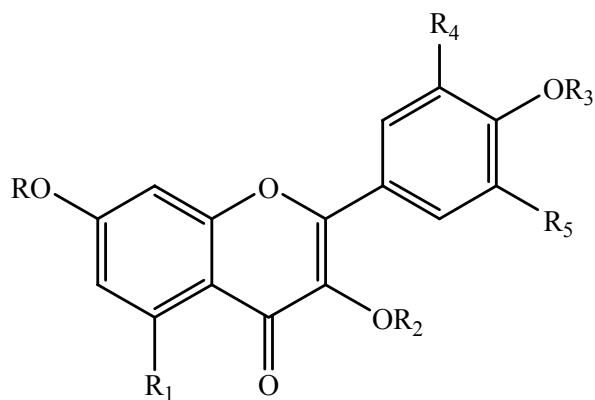
### Introduction

*Vernonia* genus (Asteraceae family) comprises tropical and sub-tropical species widespread through both the hemispheres.<sup>1</sup> Previous phytochemical studies on this genus led to the isolation and characterization of flavonoids, steroidal glycosides, and sesquiterpenes.<sup>2-5</sup> In our continuing studies on the chemistry of *Vernonia* species, we selected *V. mapirensis* Gleason (synonymous *Lepidaploa mapirensis* (Gleason) H. Robinson, *Vernonia trichoclada* Gleason), a species native to Bolivia where is used traditionally for the preparation of anti-inflammatory remedies.

The aim of our work was to carry out the phytochemical investigation of *V. mapirensis* aerial parts and herein we report the isolation and structural characterization of six new phenolic derivatives, including four flavonoids (**1-4**) and two benzofuranones (**5-6**), from the methanol and chloroform-methanol extracts of the title plant, on the basis of extensive spectroscopic and spectrometric analysis (1D-NMR, 2D-NMR, ESI-MS).

## Results and Discussion

Compound **1** was isolated as a yellow amorphous powder. Its molecular formula was established as  $C_{36}H_{36}O_{19}$  by means of ESI-MS ( $[M-H]^-$  peak at  $m/z$  771),  $^{13}C$ ,  $^{13}C$ -DEPT NMR, and elemental analysis. Analysis of 600 MHz NMR spectra suggested a flavonoid skeleton for compound **1**. The  $^1H$ -NMR spectrum (Table 1) indicated a 5,7-dihydroxylated pattern for ring A (two *meta*-coupled doublets at  $\delta$  6.16 and 6.33,  $J = 1.5$  Hz) and a 3',4'-dihydroxylation pattern for ring B (ABX system signals at  $\delta$  6.97, d,  $J = 8.5$  Hz; 7.84, dd,  $J = 8.5, 2.0$  Hz; 7.65, d,  $J = 2.0$  Hz), allowing the aglycon to be recognized as quercetin.<sup>6</sup> The  $^1H$ -NMR spectrum of **1** also showed signals ascribable to sugar moieties and a *p*-coumaroyl residue (Table 1). Two anomeric protons arising from the sugar moieties appeared at  $\delta$  5.26 and 4.88 each (1H, d,  $J = 7.5$  Hz), which correlated respectively with signals at  $\delta$  103.4 and 104.7 ppm in the HSQC spectrum. All the  $^1H$ - and  $^{13}C$ -NMR signals of **1** were assigned using 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments. Complete assignments of proton and carbon chemical shifts of the sugar portion were accomplished by DQF-COSY and 1D-TOCSY experiments and allowed the identification of the sugars as two  $\beta$ -D-glucopyranosyl units, one terminal and one esterified. The configurations of the sugar units were assigned after hydrolysis of **1** with 1 N HCl. The hydrolysate was trimethylsilylated, and GC retention times compared with those of authentic sugar samples prepared in the same manner. The lower field shifts of  $H_2-6'''$  ( $\delta$  4.32 and 4.23) of one glucosyl unit suggested the substitution site of the *p*-coumaroyl moiety. Unequivocal information could be obtained by 2D-NMR spectra; the HMBC experiment indicated correlations between  $\delta$  5.26 (H-1''') and 135.6 (C-3),  $\delta$  4.88 (H-1'') and 148.0 (C-3'),  $\delta$  4.32 and 4.23 ( $H_2-6'''$ ) and 168.5 (COO). Thus, the structure of **1** was determined as quercetin 3-*O*-(6''-*p*-coumaroyl)- $\beta$ -D-glucopyranoside-3'-*O*- $\beta$ -D-glucopyranoside.



<b>1</b>	R = R <sub>3</sub> = R <sub>5</sub> = H	R <sub>1</sub> = OH	R <sub>2</sub> = (6''- <i>p</i> -coumaroyl)glc	R <sub>4</sub> = <i>O</i> -glc
<b>2</b>	R = R <sub>4</sub> = R <sub>5</sub> = H	R <sub>1</sub> = OH	R <sub>2</sub> = (6''- <i>p</i> -coumaroyl)glc	R <sub>3</sub> = Me
<b>3</b>	R = R <sub>2</sub> = Me	R <sub>1</sub> = OMe	R <sub>3</sub> = R <sub>5</sub> = H	R <sub>4</sub> = <i>O</i> -glc
<b>4</b>	R = R <sub>1</sub> = H	R <sub>2</sub> = glc	R <sub>3</sub> = Me	R <sub>4</sub> = R <sub>5</sub> = OH

**Table 1.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of compounds **1-2** ( $\text{CD}_3\text{OD}$ , 600 MHz)<sup>a</sup>

position	1		2		
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	
2		159.0	2	159.3	
3		135.6	3	136.0	
4		179.0	4	179.8	
5		163.5	5	163.5	
6	6.16 d (1.5)	100.0	6	6.50 d (2.0)	99.4
7		166.3	7		165.8
8	6.33 d (1.5)	94.2	8	6.68 d (2.0)	95.0
9		159.0	9		158.9
10		105.8	10		106.0
1'		123.1	1'		122.0
2'	7.65 d (2.0)	117.2	2'	7.95 d (8.0)	129.6
3'		148.0	3'	7.08 d (8.0)	115.7
4'		145.0	4'		159.0
5'	6.97 d (8.5)	118.0	5'	7.08 d (8.0)	115.7
6'	7.84 dd (2.0, 8.5)	123.2	6'	7.95 d (8.0)	129.6
			4'-OMe	3.90 s	58.5
3'-O-Glc 1''	4.88 d (7.5)	104.7	3-O-Glc1''	5.27 d (7.8)	103.5
2''	3.58 dd (7.5, 9.0)	74.8	2''	3.54 dd (7.8, 9.5)	74.0
3''	3.52 t (9.0)	77.3	3''	3.50 t (9.5)	77.8
4''	3.42 t (9.0)	71.2	4''	3.39 t (9.5)	71.6
5''	3.53 m	78.4	5''	3.60 m	75.4
6''a	3.95 dd (5.0, 12.0)	62.4	6''a	4.34 dd (12.0, 4.5)	64.5
6''b	3.79 dd (3.5, 12.0)		6''b	4.24 dd (12.0, 3.0)	
3-O-Glc1'''	5.26 d (7.5)	103.4	<i>p</i> -coumaroyl 1		125.0
2'''	3.56 dd (7.5, 9.0)	73.6	2,6	7.95 d (8.0)	129.7
3'''	3.50 t (9.0)	77.7	3,5	6.83 d (8.0)	116.2
4'''	3.40 t (9.0)	71.8	4		159.5
5'''	3.59 m	75.2	$\alpha$	6.42 d (16.0)	116.1
6'''a	4.32 dd (12.0, 5.0)	64.2	$\beta$	7.65 d (16.0)	146.0
6'''b	4.23 dd (12.0, 3.5)		COO		168.3
<i>p</i> -coumaroyl 1		125.2			
2,6	8.00 d (8.5)	130.0			
3,5	6.90 d (8.5)	116.5			
4		159.0			
$\alpha$	6.40 d (16.0)	116.0			
$\beta$	7.68 d (16.0)	146.2			
COO		168.5			

<sup>a</sup> Coupling pattern and coupling constants ( $J$  in Hertz) are in parentheses.

**Table 2:**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of compounds **3-4** ( $\text{CD}_3\text{OD}$ , 600 MHz)<sup>a</sup>

Position	<b>3</b>		<b>4</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
2		158.2		158.1
3		142.0		134.5
4		179.0		176.0
5		166.0	8.02 d (8.0)	128.3
6	6.51 d (2.0)	97.0	6.95 dd (8.0, 2.0)	116.9
7		162.2		164.7
8	6.85 d (2.0)	93.4	6.93 d (2.0)	103.3
9		158.5		158.3
10		110.0		103.0
1'		122.3		122.0
2'	8.04 d (2.0)	118.3	7.30 s	100.9
3'		148.0		146.8
4'		145.3		141.0
5'	6.97 d (8.5)	118.0		146.8
6'	7.83 dd (2.0, 8.5)	126.5	7.30 s	100.9
3-OMe	3.80 s	60.0		
5-OMe	3.95 s	56.5		
7-OMe	3.92 s	56.3		
4'-OMe			3.90 s	56.7
3'-O-Glc 1''	4.88 d (7.5)	104.7		
2''	3.58 dd (7.5, 9.0)	74.8		
3''	3.52 t (9.0)	77.3		
4''	3.42 t (9.0)	71.2		
5''	3.53 m	78.4		
6''a	3.95 dd (12.0, 5.0)	62.4		
6''b	3.79 dd (12.0, 3.5)			
3-O-Glc1''			5.31 d (7.5)	102.9
2''			3.52 dd (7.5, 9.5)	74.0
3''			3.47 t (9.5)	78.1
4''			3.39 t (9.5)	71.3
5''			3.56 m	77.7
6''a			3.90 dd (12.0, 5.0)	62.9
6''b			3.87 dd (12.0, 3.5)	

<sup>a</sup> Coupling pattern and coupling constants ( $J$  in Hertz) are in parentheses.

To compound **2** was assigned the molecular formula  $\text{C}_{31}\text{H}_{28}\text{O}_{13}$  by ESI-MS ( $[\text{M}-\text{H}]^-$  peak at  $m/z$  607),  $^{13}\text{C}$  NMR (Table 1), and  $^{13}\text{C}$  DEPT data. The  $^1\text{H}$  NMR of **2** (Table 1) was very similar to **1** except for signals of ring B of the aglycon moiety that in **2** were typical of a kaempferol 4'-methyl ether.<sup>6</sup> The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR exhibited signals which can be ascribed to a kaempferol, *p*-coumaroyl, and methoxyl moieties along with those of one anomeric proton identified with the help

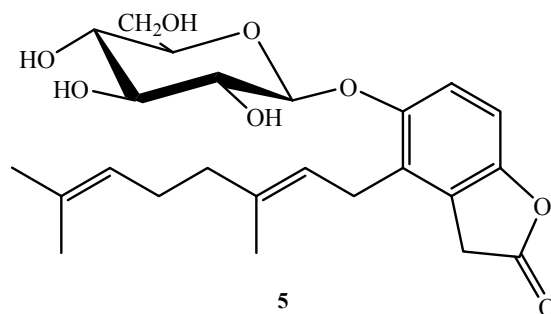
of DQF-COSY and 1D-TOCSY as one glucose ( $\delta$  5.27,  $J = 7.8$  Hz). The configuration of the glucose unit was determined as reported for compound **1**. The assignments of all protonated carbons were accomplished by interpretation of the HSQC NMR spectrum while HMBC experiment correlations indicated connections between  $\delta$  5.27 (H-1'') and 136.0 (C-3),  $\delta$  4.24 and 4.34 (H<sub>2</sub>-6'') and 168.3 (COO),  $\delta$  3.90 (OMe) and 159.0 (C-4'). From these results, the structure of **2** was concluded to be kaempferol 4'-methyl ether 3-*O*-(6''-*p*-coumaroyl)- $\beta$ -D-glucopyranoside.

The molecular formula C<sub>24</sub>H<sub>26</sub>O<sub>12</sub> for compound **3** was determined by ESI-MS ([M+H]<sup>+</sup> at  $m/z$  507), <sup>13</sup>C, <sup>13</sup>C-DEPT NMR analyses and was supported also by elemental analysis. Its <sup>1</sup>H- and <sup>13</sup>C-NMR spectra (see Table 2) indicated that it was a quercetin 3,5,7-trimethyl ether derivative.<sup>7</sup> Its <sup>1</sup>H-NMR spectrum further displayed signals for one sugar residues that were easily clarified with the help of 1D-TOCSY and DQF-COSY experiments, leading to the identification of one  $\beta$ -D-glucopyranosyl residue. The configuration of the glucose unit was determined as reported for compound **1**. HMBC correlations [ $\delta$  4.88 (H-1'') and 148.0 ppm (C-3')] established the substitution sites of the glucose moiety and the three methoxyl groups, allowing compound **3** to be identified as quercetin 3,5,7-trimethyl ether 3'-*O*- $\beta$ -D-glucopyranoside.

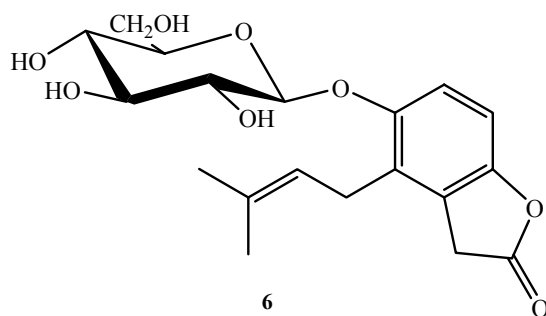
Compound **4** was assigned the molecular formula C<sub>22</sub>H<sub>22</sub>O<sub>12</sub> ([M-H]<sup>-</sup> peak at  $m/z$  477). Analysis of its MS, <sup>13</sup>C, and <sup>13</sup>C DEPT NMR data indicated that it was a flavonoid with 16 carbon atoms assigned to the aglycon and 6 carbons to the sugar moieties. The 600 MHz <sup>1</sup>H NMR spectrum indicated an unusual 7-hydroxylated pattern for ring A (ABX system signals at  $\delta$  6.93, d,  $J = 2.0$  Hz; 6.95, dd,  $J = 8.0, 2.0$  Hz; 8.02, d,  $J = 8.0$  Hz) and a 3',4',5'-trihydroxylation pattern for ring B (two-proton singlet at  $\delta$  7.30), allowing the aglycon to be recognized as 3,7,3',4',5'-pentahydroxyflavone or robinetin.<sup>8,9</sup> Except for the aglycon signals, the <sup>1</sup>H NMR spectrum of **4** revealed the presence of one-proton doublet at  $\delta$  5.31 ( $J = 7.5$  Hz) representative of one anomeric proton and of a three-proton singlet at  $\delta$  3.90 attributable to a methoxyl group. Selected 1D TOCSY data, when compared with those obtained from the <sup>13</sup>C NMR and HSQC experiments, allowed the identification of the sugar as glucose with a  $\beta$ -configuration. The relative positions of the  $\beta$ -D-glucopyranose and methoxyl units were established from the HMBC correlations [ $\delta$  5.31 (H-1'') with 134.5 ppm (C-3) and  $\delta$  3.90 (OMe) with 141.0 ppm (C-4')]. Compound **4** was therefore identified as 3,7,3',5'-tetrahydroxy-4'-methoxyflavone 3-*O*- $\beta$ -D-glucopyranoside.

Compound **5** displayed the molecular formula C<sub>24</sub>H<sub>32</sub>O<sub>8</sub>. Its ESI-MS spectrum revealed a molecular ion at  $m/z$  447, together with ion at  $m/z$  285, corresponding to the loss of one hexose unit. In the <sup>1</sup>H NMR spectrum of **5** (Table 3), two *ortho*-coupled aromatic protons (each d,  $\delta$  6.95 and 6.66,  $J = 8.5$  Hz) and an isolated methylene group (s,  $\delta$  3.56) were evident, suggesting the presence of an *ortho*-disubstituted 2(3*H*)-benzofuranone.<sup>10</sup> Moreover the <sup>1</sup>H NMR data showed signals for three tertiary methyl ( $\delta$  1.82, 1.63, and 1.58), two olefinic protons ( $\delta$  5.11 and 5.09), and three methylene groups ( $\delta$  3.62 and 3.54, 2.09 and 1.99). A 1D-TOCSY subspectrum obtained by irradiating signal at  $\delta$  5.11 showed a set of coupled protons at  $\delta$  3.62 and 3.54 (CH<sub>2</sub>) and  $\delta$  1.82 (CH<sub>3</sub>), while irradiating signal at  $\delta$  5.09 the set of coupled protons at  $\delta$  2.09 and 1.99 (both CH<sub>2</sub>) and  $\delta$  1.63 and 1.58 (both CH<sub>3</sub>) was observed. Analysis of the correlated <sup>13</sup>C NMR signals in the HSQC spectrum and DQF-COSY led to the identification of a geranyl side chain. One sugar residue, easily recognizable as  $\beta$ -D-glucopyranose by 1D-TOCSY and DQF-COSY experiments, was also present. The D configuration of the glucose residue was established as reported for compound **1**. All carbon and proton signals of the molecule **5** were assigned from the HSQC and HMBC experiments: long-range correlations between  $\delta$  3.56 (H<sub>2</sub>-3) and 132.6 (C-4), 153.0 (C-7a),

and 180.8 (C-2), between  $\delta$  3.62 and 3.54 (H<sub>2</sub>-1') and 126.0 (C-3a), 132.6 (C-4), 135.2 (C-3'), and 150.8 (C-5), and between  $\delta$  4.79 (H-1'') and 150.8 ppm (C-5), restricted the location of the geranyl side chain and glucose moiety to C-4 and C-5, respectively. Thus, the new compound **5** was characterized as 4-geranyl-5-*O*- $\beta$ -D-glucopyranosyl-2(3*H*)-benzofuranone. This is the first report of a 2(3*H*)-benzofuranone from a *Vernonia* species. The aglycon of compound **5** was previously identified from *Mimulus clevelandii*.<sup>10</sup>



Compound **6** gave molecular formula C<sub>19</sub>H<sub>24</sub>O<sub>8</sub> as determined from <sup>13</sup>C, <sup>13</sup>C DEPT NMR, and ESI-MS analyses ([M-H]<sup>-</sup> peak at *m/z* 379). The analysis of the NMR data of **6** and the comparison with those of **5** revealed signals completely superimposable, except for those of the side chain, consisting in five carbon skeleton in **6** instead of ten in **5**. Particularly, in the <sup>1</sup>H NMR spectrum of **6** were evident signals for two tertiary methyl ( $\delta$  1.81 and 1.66), one olefinic protons ( $\delta$  5.13), and one methylene group ( $\delta$  3.56) ascribable to an emiterpene unit. The structure elucidation of **6** was achieved by 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments allowing to identify **6** as 4-isoprenyl-5-*O*- $\beta$ -D-glucopyranosyl-2(3*H*)-benzofuranone.



Four known flavonoids, quercetin, rutin, acacetin 7-*O*-rutinoside, and quercetin 3-*O*-(6''-caffeoyl)- $\beta$ -D-glucopyranoside were identified by means of 1D- and 2D-NMR spectroscopy, ESI-MS analysis, and by comparison of their data with those reported in the literature.<sup>6,11,12</sup>

**Table 3.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of compounds **5-6** ( $\text{CD}_3\text{OD}$ , 600 MHz)<sup>a</sup>

position	<b>5</b>		<b>6</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
2		180.8		181.2
3	3.56 s	38.4	3.58 s	38.4
3a		126.0		126.0
4		132.6		132.0
5		150.8		150.0
6	6.95 d (8.5)	116.4	6.69 d (8.0)	115.9
7	6.66 d (8.5)	115.6	6.97 d (8.0)	116.8
7a		153.0		153.0
1'a	3.54 dd (15.0, 7.0)	26.7	3.52 dd (14.8, 7.0)	27.0
1'b	3.62 dd (15.0, 7.0)		3.62 dd (14.8, 7.0)	
2'	5.11 m	125.1	5.13 br t (7.0)	125.3
3'		135.2		135.2
4'	1.82 s	16.7	1.81 s	24.0
5'	1.99 t (7.5)	41.0	1.66 s	17.5
6'	2.09 dd (7.5, 1.5)	27.8		
7'	5.09 m	125.6		
8'		132.1		
9'	1.63 s	25.9		
10'	1.58 s	17.8		
<i>O</i> -Glc 1''	4.79 d (7.5)	104.0	4.81 d (7.5)	104.7
2''	3.46 dd (7.5, 9.0)	75.2	3.51 dd (7.5, 9.0)	75.9
3''	3.44 t (9.0)	78.2	3.38 t (9.0)	78.8
4''	3.38 t (9.0)	71.5	3.28 t (9.0)	72.0
5''	3.36 m	78.2	3.49 m	78.8
6''a	3.88 dd (12.0, 5.0)	62.7	3.91 dd (12.0, 5.0)	63.0
6''b	3.71 dd (12.0, 3.5)		3.74 dd (12.0, 3.5)	

<sup>a</sup> Coupling pattern and coupling constants ( $J$  in Hertz) are in parentheses.

## Experimental Section

**General Procedures.** Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 10 cm microcell. Elemental analysis was obtained from a Carlo Erba 1106 elemental analyzer. UV spectra were recorded on a Perkin-Elmer-Lambda 12 spectrophotometer. A Bruker DRX-600 NMR spectrometer using the UXNMR software package was used for NMR experiments. ESIMS (positive and negative mode) were obtained using a Finnigan LC-Q Advantage Termoquest spectrometer, equipped with Xcalibur software. TLC was performed on precoated Kieselgel 60 F<sub>254</sub> plates (Merck, Darmstadt, Germany); compounds were detected by spraying with  $\text{Ce}(\text{SO}_4)_2/\text{H}_2\text{SO}_4$  (Sigma-Aldrich, St. Louis, Mo, USA) and NTS (Naturstoffe reagent)-PEG (Polyethylene glycol 4000) solutions. Column chromatography was

performed over Sephadex LH-20 (Pharmacia); reversed-phase (RP) HPLC separations were conducted on a Waters 515 pumping system equipped with a Waters R401 refractive index detector and Waters U6K injector, using a C<sub>18</sub>  $\mu$ -Bondapak column (30 cm x 7.8 mm) and a mobile phase consisting of MeOH-H<sub>2</sub>O mixtures at a flow rate of 2 mL/min. GC analyses were performed using a Dani GC 1000 instrument.

**Plant material.** The aerial parts of *Vernonia mapirensis* Gleason were collected in Cotapata, Bolivia, in 2001. A voucher specimen (Michel R de y Morale L. No. 2991 HNB) is deposited at the Herbario Nacional de Bolivia.

**Extraction and isolation.** The dried powdered leaves of *V. mapirensis* (500 g) were extracted with *n*-hexane, CHCl<sub>3</sub>, CHCl<sub>3</sub>-MeOH (9:1), and MeOH, by Accelerated Solvent Extraction (ASE), to give 19.5, 18.0, 13.0, and 18.5 g of the respective residues. The methanol extract (18.5 g) was partitioned between *n*-BuOH and H<sub>2</sub>O to give a *n*-BuOH soluble portion (3.6 g) which was chromatographed over a Sephadex LH-20 column (100 cm x 3 cm) with MeOH as eluent. A total of 50 fractions were collected (8 mL each) and combined according to TLC analysis [silica 60 F<sub>254</sub> gel-coated glass sheets with *n*-BuOH-AcOH-H<sub>2</sub>O (60:15:25) and CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (40:9:1)] to give five pooled fractions (A-E) together with pure quercetin 3-*O*-(6''-caffeoyl)- $\beta$ -D-glucopyranoside and quercetin. Fraction C (270 mg) was purified by RP-HPLC on a C<sub>18</sub>  $\mu$ -Bondapak column (30 cm x 7.8 mm, flow rate 2.0 mL min<sup>-1</sup>) using MeOH-H<sub>2</sub>O (4.7:5.3) to give rutin (20 mg, *t*<sub>R</sub> = 16 min), compounds **1** (10 mg, *t*<sub>R</sub> = 19 min), and **2** (6 mg, *t*<sub>R</sub> = 29 min). Fraction D (150 mg) was purified by RP-HPLC on a C<sub>18</sub>  $\mu$ -Bondapak column (30 cm x 7.8 mm, flow rate 2.0 mL min<sup>-1</sup>) using MeOH-H<sub>2</sub>O (5.5:4.5) to yield compounds **3** (12 mg, *t*<sub>R</sub> = 29 min), **4** (7 mg, *t*<sub>R</sub> = 33 min), and acacetin 7-*O*-rutinoside (4 mg, *t*<sub>R</sub> = 40 min). A portion of the CHCl<sub>3</sub>-MeOH residue (4.0 g) was chromatographed on Sephadex LH-20 using MeOH as eluent; fractions of 8 mL were collected and grouped into nine major fractions (A-I) by TLC results on silica 60 F<sub>254</sub> gel-coated glass sheets with *n*-BuOH-AcOH-H<sub>2</sub>O (60:15:25) and CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (40:9:1). Fraction D (100 mg) was purified by RP-HPLC on a C<sub>18</sub>  $\mu$ -Bondapak column (30 cm x 7.8 mm, flow rate 2.0 mL min<sup>-1</sup>) using MeOH-H<sub>2</sub>O (2:3) as eluent, to yield pure compounds **6** (4 mg, *t*<sub>R</sub> = 9 min) and **5** (15 mg, *t*<sub>R</sub> = 12 min). Fraction E was purified by RP-HPLC on a C<sub>18</sub>  $\mu$ -Bondapak column (30 cm x 7.8 mm, flow rate 2.0 mL min<sup>-1</sup>) using MeOH-H<sub>2</sub>O (1:1) as eluent to obtain rutin (20 mg, *t*<sub>R</sub> = 12 min).

**Quercetin 3-*O*-(6''-*p*-coumaroyl)- $\beta$ -D-glucopyranoside-3'-*O*- $\beta$ -D-glucopyranoside (**1**).** Yellow amorphous powder, [ $\alpha$ ]<sub>D</sub>: -21° (*c* 0.1, MeOH), <sup>1</sup>H and <sup>13</sup>C NMR (600 MHz, CD<sub>3</sub>OD): see Table 1, ESIMS: *m/z* 771 [M-H], Anal. Calcd for C<sub>36</sub>H<sub>36</sub>O<sub>19</sub>: C, 55.96; H, 4.70. Found C, 56.00; H 4.72.

**Kaempferol 4'-methyl ether 3-*O*-(6''-*p*-coumaroyl)- $\beta$ -D-glucopyranoside (**2**).** Yellow amorphous powder, [ $\alpha$ ]<sub>D</sub>: -44° (*c* 0.1, MeOH), <sup>1</sup>H and <sup>13</sup>C NMR (600 MHz, CD<sub>3</sub>OD): see Table 1, ESIMS: *m/z* 607 [M-H], Anal. Calcd for C<sub>31</sub>H<sub>28</sub>O<sub>13</sub>: C, 61.18; H, 4.64. Found C, 61.14; H 4.67.

**Quercetin 3,5,7-trimethyl ether 3'-*O*- $\beta$ -D-glucopyranoside (**3**).** Yellow amorphous powder, [ $\alpha$ ]<sub>D</sub>: +32° (*c* 0.1, MeOH), <sup>1</sup>H and <sup>13</sup>C NMR (600 MHz, CD<sub>3</sub>OD): see Table 2, ESIMS: *m/z* 507 [M+H]<sup>+</sup>, 345 [M+H-162]<sup>+</sup>, 330 [M+H-162-15]<sup>+</sup>, Anal. Calcd for C<sub>24</sub>H<sub>26</sub>O<sub>12</sub>: C, 56.92; H, 5.17. Found C, 57.00; H 5.13.

**3,7,3',5'-Tetrahydroxy-4'-methoxyflavone 3-*O*- $\beta$ -D-glucopyranoside (**4**).** Orange amorphous powder, [ $\alpha$ ]<sub>D</sub>: +36° (*c* 0.1, MeOH), <sup>1</sup>H and <sup>13</sup>C NMR (600 MHz, CD<sub>3</sub>OD): see Table 2, ESIMS: *m/z* 477 [M-H], Anal. Calcd for C<sub>22</sub>H<sub>22</sub>O<sub>12</sub>: C, 55.23; H, 4.64. Found C, 55.20; H 4.61.



**4-Geranyl-5-O- $\beta$ -D-glucopyranosyl-2(3H)-benzofuranone (5).** Amorphous powder, UV/Vis  $\lambda_{\text{max}}$  (MeOH) nm (log  $\epsilon$ ): 218 (4.09), 295 (3.50),  $^1\text{H}$  and  $^{13}\text{C}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ): see Table 3, ESIMS:  $m/z$  447 [M-H] $^-$ , 285 [M-H-162] $^-$ , Anal. Calcd for  $\text{C}_{24}\text{H}_{32}\text{O}_8$ : C, 64.27; H, 7.19. Found C, 64.24; H 7.20.

**4-Isoprenyl-5-O- $\beta$ -D-glucopyranosyl-2(3H)-benzofuranone (6).** Amorphous powder, UV/Vis  $\lambda_{\text{max}}$  (MeOH) nm (log  $\epsilon$ ): 216 (4.15), 290 (3.71),  $^1\text{H}$  and  $^{13}\text{C}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ): see Table 3, ESIMS:  $m/z$  379 [M-H] $^-$ , 217 [M-H-162] $^-$ , Anal. Calcd for  $\text{C}_{19}\text{H}_{24}\text{O}_8$ : C, 59.99; H, 6.36. Found C, 60.05; H 6.37.

**Acid hydrolysis of compounds 1-6.** A solution of each compound (1-6, 2.0 mg each) in 1 N HCl (1 mL) was stirred at 80 °C in a stoppered reaction vial for 4 h. After cooling, the solution was evaporated under a stream of  $\text{N}_2$ . Each residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.2 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution, the residue was partitioned between water and  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  layer was analyzed by GC using an L-CP-Chirasil-Val column (0.32 mm x 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. Peaks of the hydrolysate were detected by comparison with retention times of authentic sample of D-glucose (Sigma Aldrich) after treatment with 1-(trimethylsilyl)imidazole in pyridine.

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