Antioxidant Flavone Glycosides from the Leaves of *Fargesia robusta*

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The aqueous methanolic leaf extract of *Fargesia robusta* var. *Pingwu* was evaluated in vitro for its antioxidant capacity using the TEAC and ORAC assays. C-Glycosyl flavones, farobin A (**1**) and farobin B (**2**), together with three known compounds, tricin-5-*O*-glucopyranoside (**3**), 2″-*O*-α-rhamnosyl-6-*C*-(6-deoxy-*ribo*-hexos-3-ulosyl)luteolin (**4**), and luteolin-6-*C*-glucopyranoside (homoorientin) (**5**), were isolated from the hydroalcoholic extract of the leaves of *F. robusta*. The structures of the compounds were determined by spectroscopic analyses including UV, 1D and 2D NMR, and MS. Compounds **1**, **4**, and **5** exhibited potent antioxidant activity in the TEAC assay, while compounds **1**, **3**, and **5** showed the highest antioxidant capacity in the ORAC assay.

The formation of reactive oxygen species (ROS) is a natural consequence of aerobic metabolism and is an integral part of tissue oxygen homeostasis maintenance.[1] When present in high concentrations, these compounds can damage cellular proteins and lipids and form carcinogenic DNA adducts. Although ROS play crucial roles in normal physiological processes, such as the apoptotic elimination of damaged cells, aberrant production or regulation of ROS activity has been demonstrated to contribute to the development of some prevalent diseases, including cancer and cardiovascular disease.[2] The purpose of antioxidants is to prevent ROS concentrations from reaching harmful intracellular levels.[2] Polyphenols, like flavonoids, have antioxidant properties and may react directly with reactive oxygen or nitrogen species. However, there is emerging evidence that the protective effect of flavonoids against oxidative stress is mediated not only by direct radical scavenging. Myhrstad et al.[3] showed that relatively low concentrations of flavonoids stimulated transcription of a critical gene for glutathione synthesis in cells. Glutathione, a thiol-containing tripeptide, is the major contributor to the redox state of the cell.[4] Several activities of flavonoids have been proposed in this respect, including the prooxidant properties of some flavonoids. In this way, it is possible that repeated mild cellular oxidative stress induced by flavonoids boosts cellular antioxidant defense systems and in the long term shifts these defense systems to a higher steady state, thereby preventing disease development or reducing the impact of oxidative stress when disease occurs.[5]

Bamboos are members of the subfamily Bambusoideae within the grass family Poaceae. Bamboos are distributed all over the world, but major species richness is found in Asia-Pacific and South America.[6] They are broadly divided into two tribes, i.e., woody bamboos and herbaceous bamboos, with nearly 1500 species.[7,8] *Fargesia robusta* 'Pingwu', *Phyllostachys nigra,* and *Sasa veitchii*, the three species investigated in this study, all belong to the woody bamboos. *F. robusta* and *P. nigra* originate from China, and *S. veitchi* originates from Japan.

Specific bamboo species have been used in Asian traditional medicine for their antipyretic, hemostatic, and detoxifying properties.[9−13] In Korea, the juice and leaves of *P. nigra* var. *Henonis* are used to treat respiratory diseases and acute infections. In China, a syrup made from the culms is used as a cough remedy and to loosen phlegm.[14] The leaves of Sasa-species, which are called "Kuma-saza" in Japanese, have been used to treat burns and diuretic problems.[15] In addition to its use in Asian folk medicine, the dry leaves of *S. veitchii* are used as an antioxidant food supplement in Japan,[16] and an extract of *P. nigra* 'Henonis' (antioxidant of bamboo leaves, AOB) was approved by the Chinese Food Additive Standardization Committee as a novel kind of natural antioxidant.[17] The potential health benefits from bamboo and bamboo products may arise from the presence of phytochemicals with potent antioxidant capacity. Bamboo species belonging to the genus *Fargesia* have no history of traditional medicinal use. However, *Fargesia* species are important food sources of the giant panda.[18]

In this paper we report the isolation and structure elucidation of the main antioxidant compounds of *F. robusta* 'Pingwu', a perennial bamboo species found in China that has not yet been phytochemically investigated. Its antioxidant capacity was found to be superior to those of *S. veitchii* and *P. nigra*, for which the main flavonoid-type compounds have been described before.[16,19]

This study is part of an investigation into several morphological heterogeneous bamboo species from different genera with the aim to characterize and compare their secondary metabolite profile in relation to a range of in vitro bioactivities.

The antioxidant capacity of an aqueous methanolic leaf extract (MeOH−H_2O (1:1)) of *F. robusta* 'Pingwu' was determined by applying the TEAC and ORAC assays. For comparison, the corresponding leaf extracts of *P. nigra* and *S. veitchii*, two species used in Asian traditional medicine and food industry, were also evaluated. The activity was expressed as μmol Trolox/100 g dry weight of the leaves. In Table 1, the antioxidant capacity of the crude extracts is presented. The ORAC value reflects the capacity of scavenging peroxy radicals by hydrogen atom transfer, an essential step in the termination of radical chain reactions involved in lipid oxidation. The TEAC assay reflects the ability to undergo single-electron transfer. It measures the reducing ability of the substrate (antioxidant). Reducing antioxidants are useful in the neutralization of water-soluble oxidants such as peroxynitrite and hypochlorite.[20] As these assays are based on different mechanisms, the results obtained give a more complete description of the antioxidant properties of the investigated extracts and compounds. In both assays, the aqueous methanolic extract from the leaves of *F. robusta* 'Pingwu' showed the highest antioxidant capacity (Table 1). Despite the fact that *F. robusta* 'Pingwu' has no current application as antioxidant food supplement, in contrast to *P. nigra* and *S. veitchii*, it is clearly an important source of antioxidants relative to the other two species.

To characterize the main antioxidant compounds, the leaf extract of *F. robusta* 'Pingwu' was fractionated using semipreparative chromatography. Six fractions (A−F) were collected. The elution sequence of the compounds differed from the analytical method (Figure 1). Each fraction was evaluated for its antioxidant capacity

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Table 1. Antioxidant Capacity of the Aqueous Methanolic Leaf Extracts of *P. nigra, F. robusta* 'Pingwu', and *S. veitchii* (μmol Trolox/100 g DW), of the Semipreparative Fractions (μmol Trolox/g DW), and of Compounds **1**−**5** (μmol Trolox/μmol compound), Using the TEAC and ORAC Assays[a]

	TEAC	ORAC
P. nigra	3343 ± 78	32257 ± 1805
F. robusta 'Pingwu'	10392 ± 197	84569 ± 5105
S. veitchii	5119 ± 350	64630 ± 1797
A	50 ± 16	1846 ± 148
B	343 ± 54	3295 ± 43
C	297 ± 35	2714 ± 200
D	151 ± 19	3254 ± 198
E	247 ± 19	1947 ± 50
F	448 ± 18	3999 ± 82
1	0.332 ± 0.013	26.8 ± 0.60
2	0.075 ± 0.007	8.3 ± 1.46
3	0.063 ± 0.006	18.2 ± 1.28
4	0.646 ± 0.037	5.7 ± 0.08
5	1.56 ± 0.124	66.5 ± 3.85
quercetin	3.99 ± 0.155	8.7 ± 0.23

[a] The data are means ± SD of triplicates of a single sample.

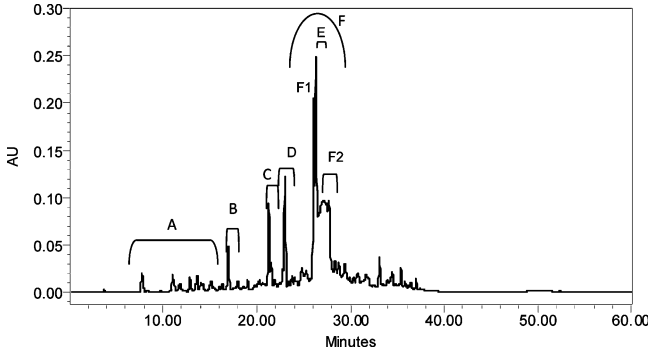


Figure 1. Chromatogram (350 nm) of the aqueous methanolic extract of *F. robusta* 'Pingwu' with indication of the different fractions.

in the ORAC and TEAC assays. The antioxidant values, normalized for dry weight of the fractions, are presented in Table 1. From these results it is evident that several compounds contribute to the antioxidant capacity. On the basis of their UV spectra, the compounds present in fractions B−F were characterized as flavones, whereas fraction A contained mainly phenolic acids.

The less abundant phenolic acids (fraction A) were not further characterized. The main compound in fraction B, homoorientin (**5**) (luteolin-6-C-β-D-glucopyranoside) (t_R 17.1 min), was identified by comparing its UV spectrum and retention time with those of an authentic standard. Its identity was confirmed through coelution with this standard. The main compounds present in fractions C, D, E, and F were purified for identification. After large-scale extraction and defatting with *n*-hexane, the aqueous methanolic leaf extract was fractionated through Sephadex LH-20 column chromatography. The obtained fractions were analyzed with HPLC-DAD. Fractions B and D were found in the first fraction (S1). A second fraction (S2) contained mainly fractions C, E, and F2, while F1 was found in a third fraction (S3). Sephadex fractions were further purified by semipreparative chromatography using subsequent elution with aqueous MeOH and aqueous MeCN. Purification of S2 resulted in the isolation of compounds **1** (t_R 21.1 min) and **4** (t_R 26.3 min). Further purification of fraction S3 resulted in the isolation of compound **2** (t_R 26.1 min). Compound **3** (t_R 22.9 min) was isolated from S1.

The TOF-MS of compound **1** gave a molecular ion at *m/z* 579.1683 ($[M + H]^+$), which corresponds to a molecular formula of $C_{27}H_{31}O_{14}$. The fragment ion at *m/z* 417 ($[M + H - 162]^+$) indicated loss of an *O*-glycosidic hexose. The absorption maxima

Table 2. Concentration (%) of the Isolated Compounds in the Fresh Leaves of *F. robusta* 'Pingwu', *P. nigra*, and *S. veitchii*

species	**1**	**2**	**3**	**4**	**5**
F. robusta 'Pingwu'	0.11	0.14	0.49	0.48	0.03
P. nigra			0.08		0.17
S. veitchii			0.36		

at 257, 269sh, and 347 nm (MeOH−H_2O) are attributed to a flavone skeleton and are similar to those of homoorientin. The ^{1}H NMR spectrum confirmed the presence of a C-6-substituted luteolin derivative, as indicated by the flavone protons at δ 6.72 (1H, s, H-3), 6.87 (1H, d, H-5′), 6.88 (1H, s, H-8), and 7.39 (2H, m, H-2′ and H-6′). Two anomeric protons appeared at δ 5.27 (1H, dd, J = 12.0, 2.4 Hz) and 4.83 (1H, d, 6.6 Hz), which correlated with signals at δ 65.4 and 102.8 in the HMQC spectrum. These data indicated that one sugar moiety was connected through a C-linkage [δ_C 65.4 (C-1″)] and the other through an O-linkage [δ_C 102.8 (C-1‴)]. The coupling constants of the two anomeric protons indicated that each sugar moiety was connected to the flavone through a β-linkage. The HMBC correlation between δ 5.27 (H-1") and 163.8 (C-7) and the downfield shift of C-6 (δ 113.6) suggested that the C-linked sugar was attached to the C-6 position of the flavone.

In the COSY spectrum, the anomeric proton at δ_H 5.27 (H-1″) was coupled to two nonequivalent geminal protons at δ 2.85 (t, 12.0 Hz) and 1.25 (d, 14.0 Hz), assigned to H-2″ax and H-2″eq, respectively. Further observation of cross-peaks including δ 2.85/1.25, 2.85/3.83, 3.83/3.22, and 3.87/1.06 permitted the assignment of residual H-3″, H-4″, H-5″, and H-6″, respectively. The large coupling constant (12.0 Hz) between H-1″ and H-2″ indicated the axial position of H-1. H-3″ appeared as a broadened doublet and coupled with H-4″ (J = 3.0 Hz). The small coupling constant of H-4″ (J = 3.0 Hz) indicated its equatorial position. The orientation of H-5″ was elucidated to be axial because of the NOE correlations with H-1″. The NMR data of the sugar unit at C-6 of compound **1** were comparable to previously reported data,[21−23] suggesting a 6-*C*-boivinopyranosyl moiety. The slight differences in chemical shifts of the boivinose unit, in comparison to those reported by Wang et al.,[22] may be explained by the influence of a 7-*O*-glucopyranoside moiety on the electron density in compound **1**. The use of DMSO-d_6 in this study is responsible for the observed differences in chemical shifts compared to the boivinosyl moiety in methanol-d_4, reported by Suzuki et al.[21,24,25]

The structure of the boivinose unit was further substantiated by HSQC data indicating the presence of a methylene carbon at δ_C 29.7 (C-2″), connected to δ_H 2.85 (H-2″ax) and δ_H 1.25 (H-2″eq). The HMBC spectrum showed a connection between δ_C 65.4 (C-1″) and δ_H 1.25 (H-2″). The chemical shifts of the C-linked sugar unit for compound **1** (Table 3) are similar to those of the corresponding apigenin analogue recorded in DMSO-d_6,[23] confirming its structure.

Acid hydrolysis confirmed the presence of an O-linked sugar. The HMBC correlation between δ_H 4.83 (H-1‴) and δ_C 163.8 (C-7) revealed that the O-linked sugar was attached to C-7 of the flavone skeleton, which was also confirmed by an NOE correlation between H-1‴ and H-8 (δ 6.88) and a downfield shift of H-8. ^{1}H NMR, HMQC, and COSY data indicated that this sugar was glucose. Nevertheless, enzymatic hydrolysis with β-glucosidase did not cause a decomposition of **1**. This can be explained by the steric effects of the boivinose unit at C-6, which may prevent the enzyme from approaching the site of hydrolysis.

Consequently, this compound was identified as luteolin-6-*C*-β-boivinopyranosyl-7-*O*-β-glucopyranoside. Although flavonoid C-glycosides are commonly found in higher plants, flavonoids bearing a boivinopyranoside such as **1** are rare. This is the first report of luteolin-6-*C*-β-boivinopyranosyl-7-*O*-β-glucopyranoside, named farobin A.

The molecular ion of compound **2**, $[M + H]^+$ at *m/z* 579.1714, also corresponds to a molecular formula of $C_{27}H_{31}O_{14}$. A fragment

Table 3. ^{1}H and ^{13}C NMR Data (DMSO-d_6, 300 MHz for ^{1}H and 75 MHz for ^{13}C) for Compounds **1**−**4**[a]

	1		**2**		**3**		**4**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	165.2		164.0		161.7		164.3	
3	103.8	6.72, 1H, s	104.4	6.83, 1H, s	107.0	6.83, 1H, s	104.3	6.66, 1H, s
4	182.8		182.3		177.8		182.7	
5	157.0		158.2		159.3		162.0	
6	113.6		111.4		105.2	6.80, 1H, br s	104.0	
7	163.8		163.6		161.7		163.0	
8	95.4	6.88, 1H, br s	95.5	6.55, 1H, s	99.3	6.80, 1H, br s	93.7	6.49, 1H, s
9	157.0		156.9		159.1		157.4	
10	105.8		104.2		108.5		108.2	
1′	121.8		125.4		121.1		121.9	
2′	114.3	7.39, 1H, br s	114.3	7.51, 1H, m	104.7	7.28, 1H, br s	113.9	7.41, 1H, m
3′	146.6		147.7		148.9		146.4	
4′	151.0		149.2		140.0		150.3	
5′	116.8	6.87, 1H, d (8.4)	116.7	7.24, 1H, d (9.0)	148.9		117.6	7.39, 1H, d (8.4)
6′	119.8	7.39, 1H, m	119.0	7.51, 1H, m	104.7	7.28, 1H, br s	119.6	7.41, 1H, m
C-3′-5′-O-Me					57.0	3.88, 6H, s		
1″	65.4	5.27, 1H, dd (12.0, 2.4)	67.3	5.30, 1H, dd (11.7 and 2.7)	105.2	4.69, 1H, d (6.6)	74.0	4.82, 1H, d (7.5)
2″	29.7	2.85, 1H, t (12.0) ax 1.25, 1H, d (14.0) eq	31.8	2.25, 1H, t (11.9) ax 1.47, 1H, d (14.1) eq	74.3	3.31, 1H, m	76.1	5.25, 1H, d (10.2)
3″	67.9	3.83, 1H, d (3.0)	67.5	3.84, 1H, d (3.6)	76.3	3.29, 1H, m	206.7	
4″	70.5	3.22, 1H, d (3.0)	70.5	3.21, 1H, d (3.6)	70.3	3.19, 1H, t (9.0)	78.7	3.88, 1H, d (9.9)
5″	70.8	3.87, 1H, q-like (6.6)	71.2	3.99, 1H, q (6.3)	78.2	3.48, 1H, m	78.8	3,38, 1H, m
6″	17.9	1.06, 1H, d (6.6)	17.9	1.12, 1H, d (6.6)	61.5	3.54, 1H, m and 3.74, 1H, d (11.1)	19.6	1.28, 1H, d (6.0)
1‴	102.8	4.83, 1H, d (6.6)	101.9	4.87, 1H, d (6.9)			99.8	4.62, 1H, br s
2‴	74.4	3.35, 1H, d (7.5)	73.9	3.32, 1H, m			70.8	3.68, 1H, br s
3‴	75.8	3.31, 1H, t-like (9.0)	76.5	3.29, 1H, m			70.8	3.06, 1H, m
4‴	69.9	3.21, 1H, m	69.5	3.18, 1H, m			71.8	2,94, 1H, t (9.0)
5‴	78.1	3.48, 1H, m	78.0	3.47, 1H, m			69.5	2,38, 1H, m
6‴	61.5	3.51, 1H, m and 3.77, 1H, m	61.4	3.70, 1H, d (11.4) and 3.55, 1H, m			18.3	0.79, 1H, d (5.7)
							18.0	0.65, 1H, d (6.0)

[a] Values in parentheses indicate coupling constants in Hz.

ion at *m/z* 417 $[M - (162) + H]^+$ was observed, indicating the presence of an *O*-glycosidic hexose moiety. The UV spectrum showed absorption maxima at 272 and 338 nm ($MeOH-H_2O$), characteristic for a flavone. Besides the characteristic signals for a flavone skeleton at δ 6.83 (1H, s) and 6.55 (1H, s) attributed respectively to H-3 and H-8, the ^{1}H NMR spectrum showed also a 3′,4′-substitution pattern at δ 7.51 (2H, m) and 7.24 (1H, d, 9.0 Hz), attributed respectively to H-2′, H-6′ and H-5′. Two anomeric protons appeared at δ 5.30 (1H, dd, J = 11.7, 2.7 Hz) and 4.87 (1H, d, J = 6.9 Hz), which correlated, respectively, with signals at δ 67.3 and 101.9 in the HMQC spectrum. This data indicated that one sugar moiety was connected through a C-linkage [δ_C 67.3 (C-1″)] and the other through an O-linkage [δ_C 101.9 (C-1‴)]. The coupling constants of the two anomeric protons indicated that each sugar moiety was connected to the flavone through a β-linkage. HMQC and COSY spectra confirmed the presence of a glucose moiety. Although sugar carbon signals in the ^{13}C NMR spectrum were similar to those of **1**, an NOE correlation between H-1‴ at δ_H 4.87 and H-5′ at δ_H 7.24 and an HMBC correlation between H-1‴ at δ_H 4.87 and C-4′ at δ_C 149.2 indicated that the glucose moiety was attached to C-4′ of the flavone skeleton.

The HSQC spectrum confirmed the presence of a methylene carbon at δ_C 31.8, connected to H-2″ax at δ_H 2.25 and H-2″eq at δ_H 1.47. The HMBC spectrum showed a connection between C-1″ at δ_C 67.3 and H-2″ at δ_H 2.25. The COSY spectrum showed a coupling between H-1″ (δ 5.30, J = 2.7, 11.7 Hz) and two nonequivalent geminal protons at δ 1.47 (H-2″eq) and 2.25 (H-2″ax). According to the above data and literature values,[21−23] the structure of this C-linked sugar is β-boivinose. The difference in chemical shifts of the methylene protons compared with compound **1** (Table 3) can be explained by the presence of the glucose moiety at C-7 in compound **1**. Consequently, **2** was identified as luteolin-6-*C*-β-boivinopyranosyl-4′-*O*-β-glucopyranoside, a new flavone glycoside named farobin B, which is a regioisomer of **1**. Furthermore, farobin B is an epimer of 5,7,3′-trihydroxy-6-*C*-β-D-digitoxopyranosyl-4′-*O*-β-D-glucopyranosylflavonoside, present in *Pleioblastus argenteastriatus,*[26] another bamboo species. This compound bears a digitoxopyranose at C-6, whereas this sugar is boivinose in farobin B.

Compound **3** was identified as tricin-5-*O*-β-D-glucopyranoside. This compound was identified by comparing experimental and reported[27] spectroscopic data. Tricin and its glycosides are widely distributed in plants belonging to the Poaceae family.[28]

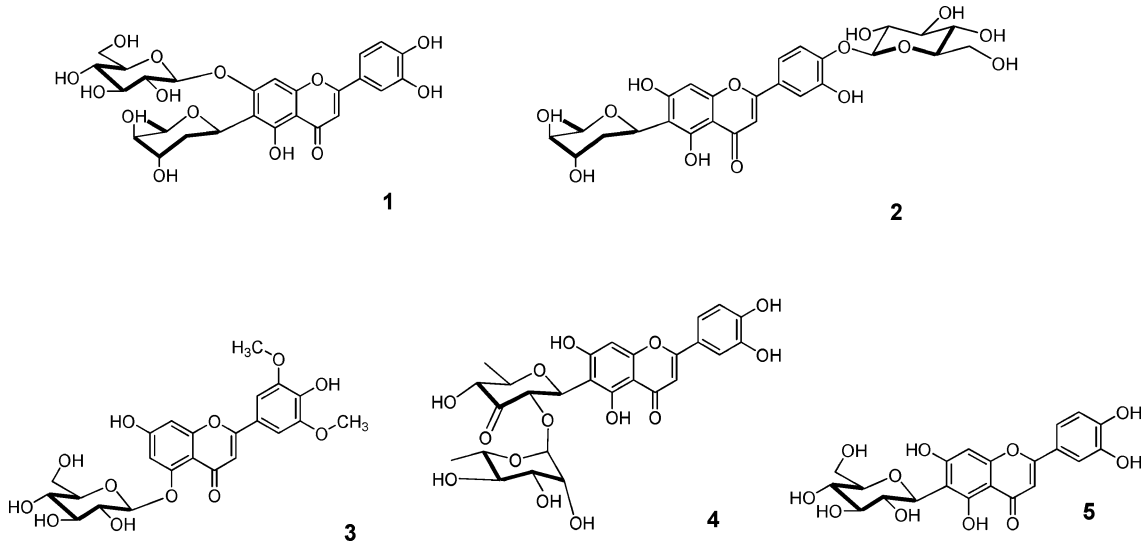
Compound **4** was characterized as 2″-*O*-α-rhamnosyl-6-*C*-(6-deoxy-*ribo*-hexos-3-ulosyl)luteolin (cassiaoccidentalin B) by comparison of experimental and reported[29] spectroscopic data.

A group of inseparable compounds (F2), showing relatively high antioxidant capacity, were also collected from the Sephadex column. On the basis of UV and NMR data of this fraction, it could be established that the major compounds in this fractions belong to the class of keto-sugar bearing luteolin derivatives.

For each identified compound a standard curve was made. On the basis of extraction yield and dry weight of the leaves, the concentration in the fresh leaves was calculated. For comparison, the concentrations of the identified compounds, if present, were also calculated in the leaves of *P. nigra* and *S. veitchii* (Table 2). From Table 2 it may be concluded that there are strong qualitative differences in composition between the investigated bamboo species, which is reflected in their antioxidant capacities (Table 1).

The in vitro antioxidant capacity of the five compounds isolated from *F. robusta* 'Pingwu' was evaluated using the ORAC and TEAC assays (Table 1). For comparison, we evaluated the antioxidant capacity of quercetin, a known antioxidant. Homoorientin (**5**) was the most potent antioxidant present in *F. robusta* 'Pingwu'. It showed lower TEAC but higher ORAC values than quercetin. The antioxidant capacities of compound **2** and **3** in the TEAC assay were negligible. This confirms the importance of the number of hydroxy groups and particularly the presence of a B-ring catechol group for antioxidant capacity.[30] On the other hand, this correlation was not found in the ORAC assay, as can be seen from

Chart 1



the relatively low ORAC value of **4** in comparison with **5**. These two compounds differ only in the kind of sugar attached to C-6 of the flavone. The flavonol quercetin, possessing an additional hydroxy group at C-3, also had a lower ORAC value than **5**. Similar observations were made in a study of Roy et al.,[31] where the number of hydroxy groups of catechins poorly correlated with the observed ORAC values. It is possible that structural features other than the number of hydroxy groups play an important role in the scavenging of peroxy radicals.

Experimental Section

General Experimental Procedures. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Varian Mercury 300 spectrometer using the solvents DMSO-d_6 and TMS (Sigma-Aldrich, Bornem, Belgium) as internal standard. Trifluoroacetic acid-*d* (99.5 atom %D) was purchased from Sigma-Aldrich (Bornem, Belgium) and used for a downfield displacement of the H_2O signal in the ^{1}H NMR spectrum. Mass spectra and exact MS were performed on a Waters LCT Premier XE orthogonal acceleration time of flight mass spectrometer. HPLC analyses were performed by HPLC-UV using a Waters 2695 Alliance separations module and 996 photodiode array detector (Waters, Milford, MA). HPLC solvents were purchased from Biosolve (Valkenswaard, The Netherlands). Semipreparative chromatography was performed on a Gilson instrument with 506 C system interface, Gilson 322 pump system, and 156 UV/vis detector, using an Alltima C_{18} column (250 × 22 mm, 10 μm). Sephadex-LH-20 (25−100 μm, Sigma-Aldrich, Bornem, Belgium) was used for flash column chromatography. Celite 545 was bought from Acros Organics (Geel, Belgium). A multilabel counter (Wallac 1420, PerkinElmer, Turku, Finland) was used for the TEAC and ORAC assay using an optical excitation filter of 720 nm (TEAC) and an excitation/emission filter of 485/535 nm (ORAC). Potassium peroxidosulfate, fluorescein sodium salt, 2,2′-azobis(2-methylpropionamidine) dihydrochloride, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid were purchased from Sigma-Aldrich (Bornem, Belgium). Homoorientin was obtained from Extrasynthese (Genay, France). Quercetin dihydrate (99%) and β-glucosidase were purchased from Sigma-Aldrich (Bornem, Belgium).

Plant Material. *Fargesia robusta* 'Pingwu', *Phyllostachys nigra*, and *Sasa veitchii* were in vitro propagated and kindly provided by Oprins Plant NV. A voucher specimen was deposited in the Herbarium of the Ghent University Botanical Garden, Faculty of Sciences, Ghent University, under accession numbers PG 11274 for *F. robusta* 'Pingwu', PG 11286 for *P. nigra*, and PG 11289 for *S. veitchii*.

HPLC Analysis. The chromatographic profiles of the semipreparative fractions and Sephadex fractions and the purified compounds were analyzed by HPLC. HPLC analyses were performed on a Varian Omnisphere C_{18} column (i.d.: 4.6 mm, length: 250 mm). The mobile phase consisted of 0.025% HCOOH in H_2O (solvent A), 0.025% HCOOH in CH_3CN (solvent B), and 0.025% HCOOH in MeOH (solvent D). The elution program was 0−3 min isocratic at 85% A (7.5% B and D); 3−8 min linear gradient from 85 to 76% A; 8−11 min isocratic at 76% A; 11−18 min linear gradient from 76 to 66% A; 18−28 min linear gradient from 66 to 56% A; 28−36 min linear gradient from 56 to 19% A; 36−42 min from 19 to 5% A; 42−50 min isocratic at 5% A; 50−57 min linear gradient from 5 to 85% A; 57−60 min isocratic at 85% A. The flow rate was 0.7 mL/min and the temperature 35 °C.

Bioactivity-Guided Fractionation. Freeze-dried leaves were successively extracted three times with MeOH−H_2O (1:1, v/v) at 40 °C in a sonication bath (Bandelin Sonorex, Berlin, Germany) for 30 min. After removing the solvent under reduced pressure and freeze-drying, the extract was defatted through solvent−solvent extraction (*n*-hexane−H_2O−MeOH (20:17:3, v/v/v)). The defatted and dried extract was fractionated using semipreparative chromatography. The extract was passed through a reversed-phase C_{18} column, using 40% MeOH in H_2O. Six fractions (A, B, C, D, E, and F) were collected and analyzed with HPLC. Each fraction was subjected to the TEAC and ORAC assay.

Isolation and Identification. Fresh leaves (400 g) were harvested and freeze-dried. The milled leaves were successively extracted three times with 4 L of MeOH−H_2O (1:1, v/v) at 40 °C in a sonication bath (Bandelin Sonorex) for 30 min. After removing the solvent under reduced pressure and freeze-drying, the extract was defatted through solvent−solvent extraction (*n*-hexane−H_2O−MeOH (20:17:3, v/v/v)). The defatted and dried extract was adsorbed on Celite and applied on a Sephadex LH-20 column. Fractionation was carried out by elution with solvents of increasing strength (respectively, 100% H_2O, 10%, 30%, 50%, 70%, and 90% MeOH in H_2O) to yield three bioactive fractions (10%, 30%, and 50% MeOH; S1, S2, and S3, respectively). The fractions were analyzed with HPLC-DAD. Two consecutive cycles of isocratic semipreparative HPLC (by subsequent elution with MeOH−H_2O and CH_3CN−H_2O, respectively) resulted in the isolation of luteolin-6-*C*-β-boivinopyranosyl-7-*O*-β-glucopyranoside (**1**) (15 mg), luteolin-6-*C*-β-boivinopyranosyl-4′-*O*-β-glucopyranoside (**2**) (15 mg), tricin-5-*O*-β-D-glucopyranoside (**3**) (25 mg), and 2″-*O*-α-rhamnosyl-6-*C*-(6-deoxy-*ribo*-hexos-3-ulosyl)luteolin (**4**) (35 mg). Each subfraction and purified compound were analyzed with HPLC.

Farobin A (1): yellow powder; t_R = 21.2 min; UV λ_{max} (MeOH−H_2O) 257, 269sh, and 347; ^{1}H and ^{13}C NMR, Table 3; positive ESIMS, 33 V, *m/z* (rel int) 579.1683 $[M + H]^+$ (33), 417.1163 $[M - (glc) + H]^+$ (93), calcd mass for $C_{27}H_{31}O_{14}$ 579.1714.

Farobin B (2): yellow powder, t_R = 26.1 min; UV λ_{max} (MeOH−H_2O) 272, 337; ^{1}H and ^{13}C NMR, Table 3; positive ESIMS, 33 V, *m/z* (rel int) 579.1714 $[M + H]^+$ (35), 417.1180 $[M - (glc) + H]^+$ (17), calcd mass for $C_{27}H_{31}O_{14}$ 579.1714.

TEAC Assay. The antioxidant capacity was determined by the TEAC assay using the protocol described by Re et al.[32] The assay was adapted to be conducted in a 96-well microplate. ABTS was dissolved in H_2O to a 7 μM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 μM potassium persulfate and kept in the dark at room temperature for 12−16 h before

use. The resulting $ABTS^{\bullet+}$ solution was diluted with MeOH to an absorbance of 0.70 (±0.02) at 720 nm. After addition of 200 μL of diluted $ABTS^{\bullet+}$ solution to 20 μL of the test sample, the microplate was shaken and the absorbance was measured after 6 min. A blank (MeOH) was measured with each assay. For each session of measurements, a standard curve of Trolox was plotted (10−50 μM). Trolox solutions were prepared in MeOH. All measurements were performed in triplicate. The percentage reduction of $ABTS^{\bullet+}$ was plotted against substrate concentration. The antioxidant capacity of the bamboo extracts was expressed as Trolox equivalents (TE, μmol Trolox/100 g DW or μmol compound).

ORAC Assay. The ORAC assay was adapted from the protocols proposed by Ou et al.[33] Sodium fluorescein was dissolved in phosphate buffer solution (PBS) (75 mM, pH 7.4) to obtain a stock solution of 4.8 mM. The working solution (48 nM) was prepared by subsequent dilution in PBS. A 10 mL solution of AAPH (2,2-azinobis(2-amidinopropane) dihydrochloride) was prepared at a concentration of 64 mM. For each session of measurements, a standard curve of Trolox was plotted (3−40 μM). Trolox solutions were prepared in PBS. All measurements were performed in triplicate. A blank (PBS) was run with each assay. Sample (25 μL) was mixed with sodium fluorescein (150 μL) and incubated for 10 min at 37 °C. AAPH (25 μL) solution was then added to the mixture, and the microplate was shaken. The fluorescence ($\lambda_{excitation} = 485$ nm; $\lambda_{emission} = 535$ nm) was registered 60 times with a delay of 60 s between repeats. The quantitation of the antioxidant capacity was based on the calculation of the area under the curve. The capacity was expressed as Trolox equivalents (TE, μmol Trolox/100 g DW or μmol compound).

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Supporting Information Available: ^{1}H and ^{13}C NMR data of the new compounds **1** and **2**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Castro, L.; Freeman, B. *Nutrition* **2001**, *17*, 161–165.
(2) Seifried, H. E.; Anderson, D. E.; Fisher, E. I.; Milner, J. A. *J. Nutr. Biochem.* **2007**, *18*, 567–579.
(3) Myhrstad, M. C. W.; Carlsen, H.; Nordstrom, O.; Blomhoff, R.; Moskaug, J. O. *Free Radical Biol. Med.* **2002**, *32*, 386–393.
(4) Thomas, J. A.; Poland, B.; Honzatko, R. *Arch. Biochem. Biophys.* **1995**, *319*, 1–9.
(5) Moskaug, J. O.; Carlsen, H.; Myhrstad, M. C. W.; Blomhoff, R. *Am. J. Clin. Nutr.* **2005**, *81*, 277S–283S.
(6) Bystriakova, N.; Kapos, V.; Lysenko, I.; Stapleton, C. M. A. *Biodiversity Conserv.* **2003**, *12*, 1833–1841.
(7) Zhang, W. C.; Clark, L. G. Phylogeny and classification of the Bambusoideae (Poaceae). In *Grasses: Systematics and Evolution*; Jacobs, S. W. L. E., J. E., Ed.; CSIRO Publishing: Collingwood, 2000; pp 35−42.
(8) Ohrnberger, D. *The Bamboos of the World*; Elsevier: Amsterdam, 1999.
(9) Bae, K. *The Medicinal Plants of Korea*; Kyo-Hak Publishing Company: Seoul, 2000; pp 565−567.
(10) Kim, T. J. *Korean Resources Plant*; Seoul National University Press: Seoul, 1996; Vol. V, pp 37−42.
(11) Okabe, S. T., K.; Takagi, K.; Shibata, M. *Jpn. J. Pharmacol.* **1975**, *25*, 608–609.
(12) Yook, C. S. *Colored Medicinal Plants of Korea*; Academy Book Publishing Company: Seoul, 1993; pp 88−91.
(13) Namba, T. *The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Pictures, Vol. II*; Hoikusha Publishing Company: Osaka, 1993; pp 92−95.
(14) Perry, L. M. *Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses*; The MIT Press: Cambridge, 1980; p 169.
(15) Akamatsu, K. *Wakanyaku*; Ishiyaku Shuppan Co. Ltd.: Tokyo, 1970; p 649.
(16) Nakajima, Y.; Yun, Y. S.; Kunugi, A. *Tetrahedron* **2003**, *59*, 8011–8015.
(17) Ministry of Health. *Technical Standards for Testing and Assessment of Health Food*; Beijing, 2003; p 203.
(18) Keski-Saari, S.; Ossipov, V.; Julkunen-Tiitto, R.; Jia, J. B.; Danell, K.; Veteli, T.; Zhang, G. Q.; Xiong, Y. W.; Niemela, P. *Biochem. Syst. Ecol.* **2008**, *36*, 758–765.
(19) Jung, S. H.; Lee, J. M.; Lee, H. J.; Kim, C. Y.; Lee, E. H.; Um, L. H. *Biol. Pharm. Bull.* **2007**, *30*, 1569–1572.
(20) Huang, D. J.; Ou, B. X.; Prior, R. L. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856.
(21) Suzuki, R.; Okada, Y.; Okuyama, T. *J. Nat. Prod.* **2003**, *66*, 564–565.
(22) Wang, G. J.; Chen, Y. M.; Wang, T. M.; Lee, C. K.; Chen, K. J.; Lee, T. H. *J. Ethnopharmacol.* **2008**, *118*, 71–78.
(23) Di, Z.; Jie, Y.; Xue-Tao, D.; Mao-Xiang, L.; Qiang, W. *Chin. J. Nat. Med.* **2009**, *7*, 184–186.
(24) Abraham, R. J. B. J. J.; Griffiths, L.; Koniotou, R. *Magn. Reson. Chem.* **2005**, *43*, 611–624.
(25) Pauli, G. F. *J. Nat. Prod.* **2000**, *63*, 834–838.
(26) Guo, X. F.; Yue, Y. D.; Tang, F.; Wang, J.; Yao, X. *J. Asian Nat. Prod. Res.* **2008**, *10*, 903–907.
(27) Adjei-Afriyie, F.; Kim, C. S.; Takemura, M.; Ishikawa, M.; Horiike, M. *Biosci., Biotechnol., Biochem.* **2000**, *64*, 443–446.
(28) Harborne, J. B.; Hall, E. *Phytochemistry* **1964**, *3*, 421–428.
(29) Hatano, T.; Mizuta, S.; Ito, H.; Yoshida, T. *Phytochemistry* **1999**, *52*, 1379–1383.
(30) Heim, K. E.; Tagliaferro, A. R.; Bobilya, D. J. *J. Nutr. Biochem.* **2002**, *13*, 572–584.
(31) Roy, M. K.; Koide, M.; Rao, T. P.; Okubo, T.; Ogasawara, Y.; Juneja, L. R. *Int. J. Food Sci. Nutr.* **2010**, 109–124.
(32) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.
(33) Ou, B. X.; Hampsch-Woodill, M.; Prior, R. L. *J. Agric. Food Chem.* **2001**, *49*, 4619–4626.

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