FULL PAPER

Baeckeins J and K, Two Novel *C*-Methylated Biflavonoids from the Roots of *Baeckea frutescens* and Their Cytoprotective Activities

by **Bei-Xi Jia***[a][b], **Ao Jia**[a], **Chao-Jie Li**[a], **Hui-Qin Zhao**[a], **Feng-Xiao Ren**[c], **Hong-Min Liu***[a], and **Qiang Wang***[b]

[a]) Department of Pharmacognosy, College of Pharmacy, Zhengzhou University, No. 100 of Science Road, Zhengzhou 450001, P. R. China (phone: +86-371-67781891; fax: +86-371-67781908; e-mail: jiabeixi_2003_123@163.com (*B.-X. J.*)) (phone: +86-371-67781905; fax: +86-371-67781908; e-mail: liuhm@zzu.edu.cn (*H.-M. L.*))

[b]) State Key Laboratory of Natural Medicines, China Pharmaceutical University, No. 24 Tongjia Lane, Nanjing 210009, P. R. China (phone: +86-25-83271390; fax: +86-25-85301528; e-mail: qwang49@163.com)

[c]) Henan Taifeng Pharmaceutical Science and Technology Group Co., Ltd., Zhengzhou 450001, P. R. China

Baeckea frutescens is an aromatic shrub used as ornamentals and as food flavor spices in the southern part of P. R. China. Two novel *C*-methylated biflavonoids named baeckeins J (**1**) and K (**2**) were isolated from the roots of *B. frutescens*, which possessed the unique carbon skeleton conjugated of a flavonol and one isoflavanonol molecule *via* the linkages of C(2)–C(8*) and C(3)–O–C(7*). The structures of compounds **1** and **2** were elucidated by analysis of 1D- and 2D-NMR, and HR-ESI-MS spectral data, and the absolute configuration for chiral C-atoms C(2) and C(3) were assigned by CD spectrometry combined with quantum chemical calculations. In the bioassay, baeckeins J and K exhibited strong cytoprotective effects on H_2O_2-induced oxidative cell death in PC12 cells.

Keywords: Myrtaceae, *Baeckea frutescens*, *C*-Methylated biflavonoid, Baeckeins J and K, Cytoprotective activity.

Introduction

Baeckea frutescens L. (Myrtaceae), an aromatic low-growing shrub, is used as ornamentals and as food flavor spices. During recent years, *B. frutescens* has been used as a daily health tea in the southern part of P. R. China, receiving considerable attention. Previous studies on this plant indicated the presence of essential oil [1][2], sesquiterpenes [3], phloroglucinols [4], chromones [5][6], flavonoids [7 – 10], and their derivatives. Preliminary phytochemical investigations of this plant in our laboratory have reported eleven *C*-methylated flavonoids and biflavonoids [11 – 14]. It is known that *C*-methylated flavonoids are not widely distributed, but have occurred in multiple genera of the Myrtaceae family [15 – 18]. The existing literature suggested that *C*-methylated flavonoids might be distinctive of Myrtaceae, providing the significance of plant chemotaxonomy. As part of our ongoing search for structurally interesting and bioactive *C*-methylated flavonoids, two novel *C*-methylated biflavonoids named baeckeins J (**1**) and K (**2**) were isolated from the title plant (*Fig. 1*). In this article, the isolation and structural elucidation of these isolates are present. 1D- (including ^1H and ^{13}C) and 2D-nuclear magnetic resonance (NMR, including HSQC, HMBC, and NOESY) and high-resolution electrospray ionization mass (HR-ESI-MS) spectral data were extensively applied to characterize their structures and to establish the $^1H/^{13}C$-resonance assignments. Circular dichroism (CD) spectrometry combined with quantum chemical calculations were employed to assign the absolute configurations.

Results and Discussion

Baeckein J (**1**) was obtained as yellow amorphous powder. Its molecular formula was determined as $C_{32}H_{22}O_{14}$ by the positive HR-ESI-MS (*m/z* 631.1082 $[M + H]^+$, calculated 631.1082; *Fig. S6*), indicating 22 degrees of unsaturation. The IR spectrum showed absorption bands for OH group (3425 cm^{-1}), C=O group (1637 cm^{-1}), and aromatic functionalities (1516 and 1441 cm^{-1}; *Fig. S7*). The UV spectrum (λ_{max} 242, 258, 308, and 377 nm) and the positive result for the Mg-HCl reaction suggested **1** to be a flavonoid.

The ^{13}C-NMR spectrum (*Table*) of **1** displayed 32 C-atom signals, including two C=O groups (δ(C) 185.6 (C(4)) and 176.0 (C(4*))), two aromatic Me groups (δ(C) 6.9 (Me–C(6)) and 7.4 (Me–(6*))), seven tertiary C-atoms (CH) (δ(C) 119.0, 118.5, 116.0, 115.5, 115.3, 114.8, and 94.4), and 21 quaternary C-atom, suggesting a carbon skeleton of biflavonoid (*Fig. S2*). Among these signals, three characteristic C-atoms (δ(C) 176.0 (C(4*)), 147.8 (C(2*)), and 135.7 (C(3*))) indicated the presence of a flavonol moiety. The ^1H-NMR spectra (*Table*) for **1**

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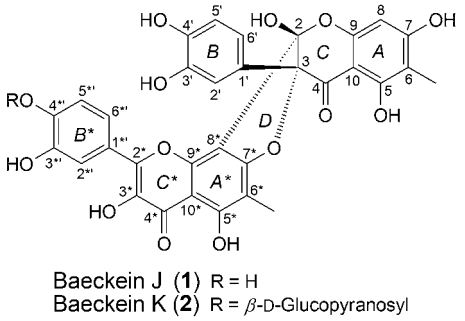

Fig. 1. Structures of compounds **1** and **2**.

showed signals for two sets of typical *ABX* coupling systems (δ(H) 6.73 (1 H, *d*, *J* = 8.7, H–C(2′)), 6.78 (1 H, *d*, *J* = 2.0, H–C(5′)), and 6.64 (1 H, *dd*, *J* = 8.7, 2.0, H–C(6′))) and (δ(H) 6.55 (1 H, *d*, *J* = 8.7, H–C(2*′)), 7.82 (1 H, *d*, *J* = 2.0, H–C(5*′)), and 6.07 (1 H, *dd*, *J* = 8.7, 2.0, H–C(6*′))), corresponding to the 3′,4′-dihydroxy-substituted *B* ring of flavonoids (rings *B* and *B**). Two aromatic Me signals (δ(H) 1.90 (3 H, *s*, Me–C(6)) and 2.07 (3 H, *s*, Me–C(6*))) and one aromatic H-atom (δ(H) 6.03 (1 H, *s*, H–C(8))) were also present (*Fig. S1*). Taken together, these above 1D-NMR data suggested that **1** was composed of two molecules of flavonoids with certain structural characteristics of the reported compound 6-*C*-methylquercetin [9][19].

Analysis of the 1D-NMR and HSQC spectra (*Fig. S1 – S3*) of **1** made the assignments of one flavonoid molecule (the Segment I), referring to rings *A**, *B**, and *C**, which were confirmed by HMBC correlations (*Fig. 2* and *Table S1*) from Me–C(6*) (δ(H) 2.07) to (δ(C) 163.4 (C(5*)), 102.8 (C(6*)), and 160.6 (C(7*))), from H–C(2*′) (δ(H) 6.55) to (δ(C) 147.8 (C(2*)), 121.5 (C(1*′)), and 144.6 (C(3*′))), from H–C(5*′) (δ(H) 7.82) to (δ(C) 147.8 (C(2*)), 121.5 (C(1*′)), 144.6 (C(3*′)), 146.7 (C(4*′)), and 119.0 (C(6*′))), and from H–C(6*′) (δ(H) 6.07) to (δ(C) 147.8 (C(2*)), 146.7 (C(4*′)), and 116.0 (C(5*′))) (*Fig. S4*). Compared with the literature values of compound 6-*C*-methylquercetin [9][19], the segment I was further identified, but the absence of an H–C(8*) signal indicated a substituted C(8*), suggesting one available binding position for the other flavonoid molecule (Segment II).

Inspection of the rest signals for segment II revealed some structural characteristics of segment I, referring to a ring *A* of 6-*C*-methylquercetin and a 3′,4′-dihydroxy-substituted ring *B*. The major differences between the two segments were in the ring *C*. In the ^{13}C-NMR spectrum of **1**, the C=O C-atom (δ(C) 185.6 (C(4))) and two quaternary C-atoms (δ(C) 103.0 (C(2)) and 92.3 (C(3))) revealed that the carbon skeleton of ring *C* in the segment II should be a dihydropyrone ring, different from the pyrone ring *C* of 6-*C*-methylquercetin, and new substituents must be bound to the C-atoms C(2) and C(3), suggesting two available binding positions for the segment

Table. ^{1}H- and ^{13}C-NMR data of compounds **1** and **2**. δ in ppm, *J* in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(2)		103.0		103.0
C(3)		92.3		92.3
C(4)		185.6		185.7
C(5)		161.3		161.3
C(6)		104.3		104.4
Me(6)	1.90 (*s*)	6.9	1.91 (*s*)	7.4
C(7)		167.1		166.9
H–C(8)	6.03 (*s*)	94.4	6.04 (*s*)	94.3
C(9)		159.7		159.7
C(10)		98.5		98.6
C(1′)		125.0		124.9
H–C(2′)	6.73 (*d*, *J* = 8.5)	114.8	6.73 (*d*, *J* = 8.5)	114.8
C(3′)		144.7		144.7
C(4′)		145.7		145.7
H–C(5′)	6.78 (*d*, *J* = 2.0)	115.3	6.79 (*d*, *J* = 2.0)	115.6
H–C(6′)	6.64 (*dd*, *J* = 8.5, 2.0)	118.5	6.68 (*dd*, *J* = 8.5, 2.0)	118.3
C(2*)		147.8		146.1
C(3*)		135.7		136.6
C(4*)		176.0		176.3
C(5*)		163.4		163.6
C(6*)		102.8		102.9
Me(6*)	2.07 (*s*)	7.4	2.08 (*s*)	7.4
C(7*)		160.6		160.6
C(8*)		104.4		104.5
C(9*)		149.1		149.1
C(10*)		105.9		106.0
C(1*′)		121.5		124.7
H–C(2*′)	6.55 (*d*, *J* = 8.5)	115.5	6.93 (*d*, *J* = 8.5)	115.7
C(3*′)		144.6		145.8
C(4*′)		146.7		146.8
H–C(5*′)	7.82 (*d*, *J* = 2.0)	116.0	7.62 (*d*, *J* = 2.0)	115.4
H–C(6*′)	6.07 (*dd*, *J* = 8.5, 2.0)	119.0	6.39 (*dd*, *J* = 8.5, 2.0)	118.9
Glc(1″)			4.77 (*d*, *J* = 6.9)	101.4
H–C(2″)			3.31 – 3.32 (*m*)	73.2
H–C(3″)			3.39 – 3.45 (*m*)	77.0
H–C(4″)			3.17 – 3.18 (*m*)	69.6
H–C(5″)			3.23 – 3.24 (*m*)	75.9
H–C(6″)			3.57 (*dd*, *J* = 11.1, 5.1), 3.83 (*dd*, *J* = 11.0, 2.0)	60.6

I. The distinctive chemical shifts (δ(C) 103.0 (C(2)), 92.3 (C(3)), and 185.6 (C(4))) suggested a bond of C(3)–C(1′) between rings *B* and *C*, indicating a isoflavanonol structure, which was confirmed by the evident cross-peaks from H–C(2′), H–C(5′), and H–C(6′) to C(3) (instead of C(2)) in the HMBC spectrum. Also the segment II, referring to rings *A*, *B*, *C*, and *D* was confirmed by HMBCs (*Fig. 2* and *Table S1*) from Me–C(6) to (δ(C) 161.3 (C(5)), 104.3 (C(6)), 167.1 (C(7)), 94.4 (C(8)), and 98.5 (C(10))), from H–C(8) to (δ(C) 185.6 (C(4)), 104.3 (C(6)), 167.1 (C(7)), 159.7 (C(9)), and 98.5 (C(10))), from H–C(2′) to (δ(C) 125.0 (C(1′)), 144.7 (C(3′)), 145.7 (C(4′)), 115.3 (C(5′)), and 118.5 (C(6′))), from H–C(5′) to

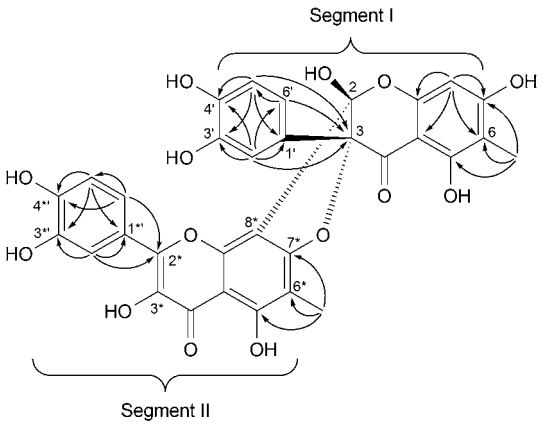

Fig. 2. Selected HMBC correlations for compound **1**.

(δ(C) 92.3 (C(3)), 125.0 (C(1′)), 144.7 (C(3′)), 145.7 (C(4′)), and 118.5 (C(6′))), and from H–C(6′) to (δ(C) 92.3 (C(3)), 145.7 (C(4′)), and 115.3 (C(5′))) (*Fig. S4*).

The above comprehensive spectral analysis indicated that C-atoms C(2), C(3), and C(8*) provided three available binding positions for the segments I and II. Comparative evaluation of the chemical shifts for C(2), C(3), and C(8*) revealed that the C(2) was bound to the aromatic C-atom C(8*) and the C(3) should be bound to another O-atom. Accordingly, the C(2)–C(8*) bond and the C(3)–O–C(7*) linkage were deduced from these evidences together with the molecular weight and the remaining degrees of unsaturation. It was interesting that the optimal connections of C(2)–C(8*) and C(3)–O–C(7*) between the two segments formed a furan ring *D*. And the unusual furan ring *D* was verified by comparison of the experimental NMR data with the values simulated by the ACD Labs software and the related NMR data of known compounds baeckeins F – I [14]. As a result, the planar structure for **1** was determined.

In the molecule of **1**, the C-atoms of C(2) and C(3) provided two chiral centers, and there were four relative configurations labeled as AC-1 with (2*S*,3*S*), AC-2 with (2*R*,3*S*), AC-3 with (2*R*,3*R*), and AC-4 with (2*S*,3*R*). The CD spectrum (*Fig. 3*) for **1** showing positive *Cotton* effects (CEs) at 218 and 307 nm, and negative CEs at 238, 282, 335, and 379 nm, but these spectral data including the nuclear *Overhauser* effect (NOE) increments in our NOESY experiment were not sufficient enough to determine the absolute configuration for C(2) and C(3). In this case, quantum chemical CD calculations were employed [12 – 14]. All geometries were optimized by B3LYP functional with 6-31G* basis-set, and ωB97XD functional with 6-311G* basis-set for heavy atoms and 6-31G* for H-atoms, respectively, was employed to conduct time-dependent density functional theory (TD-DFT) calculations. Based on the resulting electronic excitation energies and rotatory strengths, the Multiwfn 3.3.7 program in combination with Origin software was employed to obtain these electronic circular dichroism (ECD) spectra. The calculated CD spectrum (*Fig. 3* and *Fig. S15*) for the configuration of AC-3 displayed diagnostic negative CEs and positive CEs, which exhibited good agreements with the experimental CD, and allowed the assignments of absolute configurations of **1** as (2*R*,3*R*). Based on these

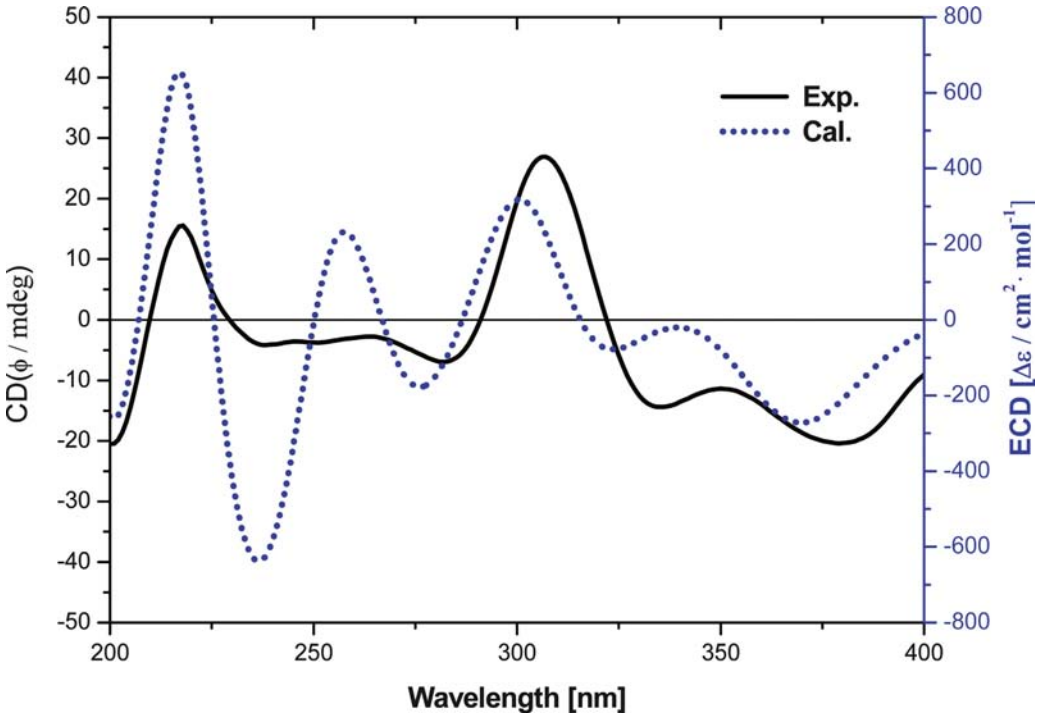

Fig. 3. Experimental CD and calculated CD spectrum for compound **1**.

above results, the structure of baeckein J (**1**) was unambiguously established.

Baeckein K (**2**) was isolated as yellow amorphous solid and gave the *quasi*-molecular ion peak $[M + H]^+$ at *m*/*z* 793.1607 (calc. 793.1611 for $C_{38}H_{33}O_{19}$) in the HR-ESI-MS (*Fig. S13*), suggesting a molecular formula of $C_{38}H_{32}O_{19}$. Its IR spectrum showed absorption bands for OH group (3396 cm^{-1}), C=O group (1637 cm^{-1}), and aromatic functionalities (1508 and 1442 cm^{-1}; *Fig. S14*). The UV spectra (λ_{max} 240, 307 and 372 nm) and positive results for the Mg-HCl reaction and *Molish* reagent, and the chemical formula revealed **2** to be a biflavonoid glycoside. The ^{13}C-NMR spectrum for **2** (*Table*) displayed a group of signals (δ(C) 101.4, 77.0, 75.9, 73.2, 69.6, and 60.6) belonging to a hexosyl unit, and 32 C-atom signals attributable to a carbon skeleton of biflavonoid, among which were two C=O groups (δ(C) 185.7 (C(4)) and 176.3 (C(4*))), two aromatic Me groups (δ(C) 6.9 (Me(6)) and 7.2 (Me(6*))), seven CH (δ(C) 118.9, 118.3, 115.7, 115.6, 115.4, 114.8, and 94.3), and 21 quaternary C-atoms (*Fig. S9*). The ^{1}H-NMR spectrum (*Table*) for **2** showed two typical *ABX* coupling systems (δ(H) 6.73 (1 H, *d*, *J* = 8.7, H–C(2′)), 6.79 (1 H, *d*, *J* = 2.0, H–C(5′)), and 6.68 (1 H, *dd*, *J* = 8.7, 2.0, H–C(6′))) and (δ(H) 6.93 (1 H, *d*, *J* = 8.7, H–C(2*′)), 7.62 (1 H, *d*, *J* = 2.0, H–C(5*′)), and 6.39 (1 H, *dd*, *J* = 8.7, 2.0, H–C(6*′))), two aromatic Me signals (δ(H) 1.91 (3 H, *s*, Me–C(6)) and 2.08 (3 H, *s*, Me-C(6*))), one aromatic H-atom (δ(H) 6.04 (1 H, *s*, H–C(8))), and a series of signals in the range of about δ(H) 5.0 – 3.0 related to a sugar moiety (*Fig. S8*). A comparison of the 1D-NMR data for **2** with those of **1** suggested that **1** and **2** had the same carbon skeleton and functional groups, except for the signals assignable to a hexosyl moiety. The planar structure for the aglycone part of **2**, including segments I and II, was the same as that of **1**, which was confirmed by the HMBC experiment (*Fig. 4* and *Fig. S11*). The sugar moiety was identified as the D-glucose by both thin-layer chromatography (TLC) and gas chromatography (GC) analysis after the acid hydrolysis experiment for **2** [20][21]. The large ^3J(1,2) coupling constant of the anomeric H-atom (δ(H) 4.77 (1 H, *d*, *J* = 7.0, H–C(1″)) revealed the β-configuration for the glucose moiety, and the location of the glucose residue being at C(4*′) was confirmed by the HMBC cross-peak from H–C(1*″) to C(4*′) (δ(C) 161.6) and the NOE correlation (*Fig. 4*) between H–C(1″) and H–C(2*′) (δ(H) 7.50 (1 H, *s*)) (*Fig. S12*). Furthermore, the β-D-glucose moiety was verified by the related data in the literature [9]. The absolute configuration for the quaternary C-atoms of C(2) and C(3) in **2** was finally assigned as (2*R*,3*R*) by comparison of the characterized shapes in computed CD with those in the experimental CD spectrum (*Fig. 5*), which displayed positive CEs at 213 and 306 nm, and negative CEs at 231, 252, 285, 335 and 378 nm.

The cytoprotective activities for compounds **1** and **2** were investigated. The cytotoxicity against PC12 cells was first tested and both **1** and **2** displaying no cytotoxic effects (IC_{50} > 100 μM), and then the cytoprotective evaluation was carried out using the PC12 cells stressed by H_2O_2. When the cells survival (%) for the blank (the group without any treatment) and the control (the H_2O_2-treated group) were 99.9% and 40.0%, the cytoprotective effects (%; the relative cytoprotective effect to the control) for the tested compounds **1** and **2** (10 μM) were 54.8% and 60.2%, respectively, which showed potent cytoprotective effects.

Conclusions

Following the reported compounds baeckeins A – I, another two novel *C*-methylated biflavonoids baeckeins J and K were obtained from the roots of *B. frutescens*. From a chemical point of view, the unusual carbon skeleton of baeckein J could be regarded as the first conjugate of a flavonol (Segment I) and one isoflavanonol molecule (Segment II) *via* the linkages of C(2)–C(8*) and C(3)–O–C(7*), while baeckein K was the corresponding glucopyranoside. The absolute configurations for C(2) and C(3) were determined by quantum chemical CD calculations

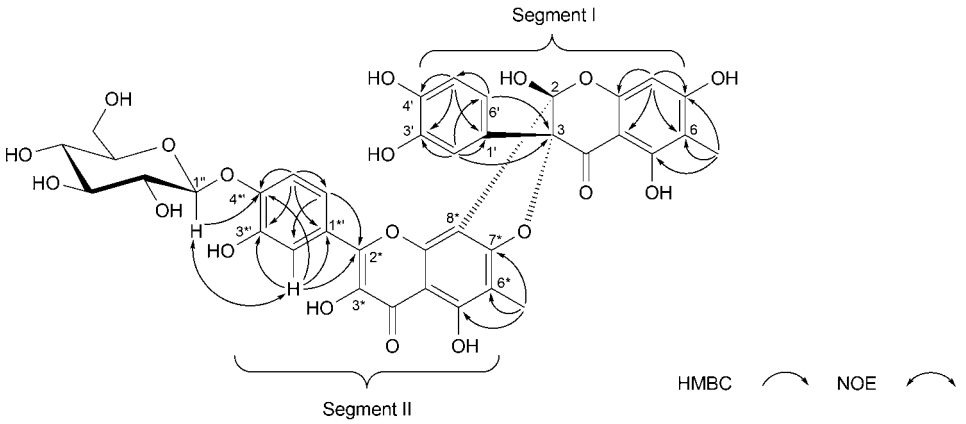

Fig. 4. Selected HMBC and NOE correlations for compound **2**.

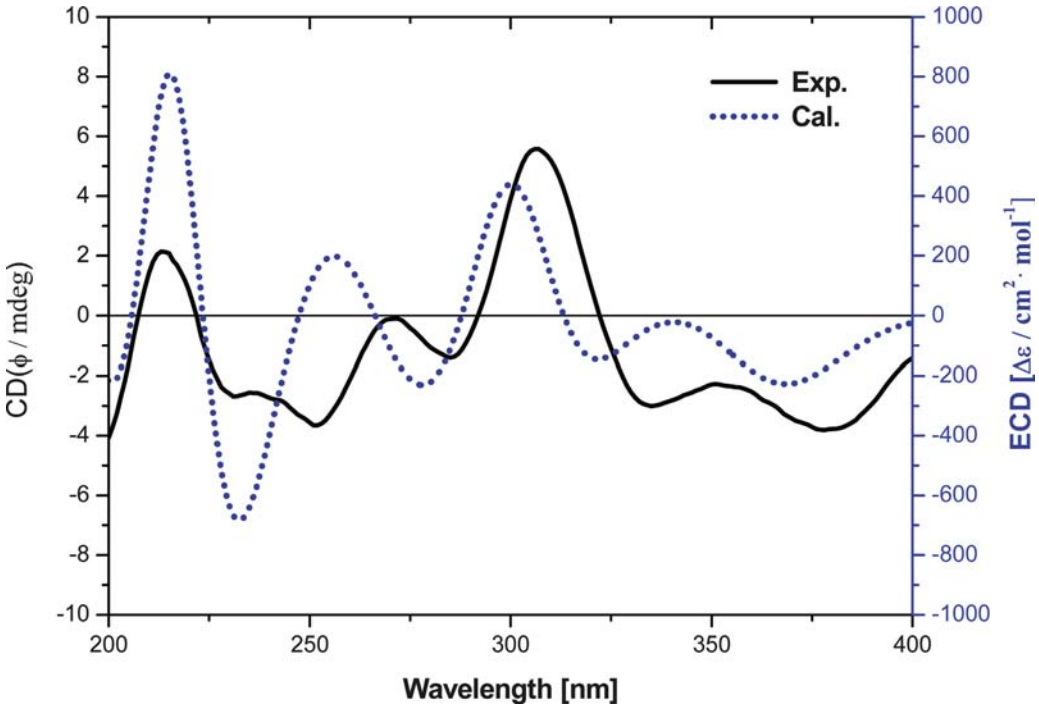

Fig. 5. Experimental CD and calculated CD spectrum for compound **2**.

(TD-DFT). The bioassay for baeckeins J and K suggested that *C*-methylated flavonoids might be good cytoprotective agents. The present studies have provided additional phytochemical and bioactive information for resource development and utilization of *B. frutescens* with the food and health purposes.

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Supporting Information

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/hcla.201600013.

Experimental Part

General

Column chromatography (CC): silica gel (SiO_2; 200 – 300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, P. R. China), *ODS* (40 – 65 μm; *Fuji*, Toyama-Pref., Japan) and *Sephadex LH-20* (20 – 100 μm; *Pharmacia*, Uppsala, Sweden). GC: *Agilent 6890 Plus* gas chromatography (*Agilent Tech.*, California, USA) with an FID detector and a *DB-5* capillary column (30 m × 0.25 mm, i.d.). Optical rotations: *JASCO P-1020* polarimeter (*JASCO*, Tokyo, Japan). UV Spectra: *Shimadzu-UV-2450* UV/VIS spectrophotometer (*Shimadzu*, Tokyo, Japan); λ_{max} (log ε) in nm. CD Spectra: *JASCO J-810* circular dichroism spectrometer (*JASCO*); λ_{max} ($\Delta\varepsilon$) in nm. IR Spectra: *Nicolet Impact-410* spectrometer (*Nicolet*, Wisconsin, USA); KBr disks; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AV-500* NMR instrument (*Bruker*, Karlsruhe, Germany); in (D_6)DMSO; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Agilent 6530 LC-Q-TOF/MS* instrument (*Agilent Tech.*, Santa Clara, CA, USA).

Plant Material

The roots of *B. frutescens* were collected from Nanning, Guangxi Province, P. R. China, and identified by Prof. *Qiang Wang*, China Pharmaceutical University. A voucher specimen (No. GS001) has been deposited in the Department of Chinese Materia Medica Analysis, China Pharmaceutical University, Nanjing, P. R. China.

Extraction and Isolation

Air-dried and crushed roots of *B. frutescens* (10.0 kg) were extracted with 90% EtOH (30.0 l × 3) at 80 °C for 3 h and concentrated in vacuum to give 600.0 g of extract, which was suspended in H_2O (5.0 l) and partitioned with petroleum ether (PE) (60 – 90 °C, 5.0 l × 3), AcOEt (5.0 l × 10), and BuOH (5.0 l × 10) successively. The

AcOEt soluble part (203.6 g) was first subjected to a SiO_2 CC (200 – 300 mesh, 2.0 kg, 120 × 10.0 cm, i.d.) and eluted with the gradient solvent of $CHCl_3$/MeOH (1:0 → 1:1, *v/v*) to yield eight fractions (*Frs. 1 – 8*) on the basis of TLC analysis. TLC was performed on precoated SiO_2 GF_{254} plates and spots were visualized under the UV light (254 or 365 nm) or detected by spraying the $AlCl_3$/EtOH (1%) soln. A part of *Fr. 6* (2.1 g) was submitted to a *Sephadex LH-20* column (120 × 2.0 cm, i.d.) and eluted with $CHCl_3$/MeOH (1:1, *v/v*) to afford six subfractions (*Frs. 6-1 – 6-6*), of which subfraction *Fr. 6-6* (300.0 mg) was then subjected to repeated CC (*Sephadex LH-20*, 120 × 2.0 cm, i.d., MeOH) to obtain compound **1** (30.0 mg) and *Fr. 6-4* (160.0 mg) was repeatedly chromatographed over an *ODS* column (60 × 2.5 cm, i.d., MeOH/H_2O, 50:50, *v/v*) to give purified compound **2** (30.0 mg).

Baeckein J (= **(7a*S*,13a*S*)-2,7a-Bis(3,4-dihydroxyphenyl)-7a,13a-dihydro-3,5,9,11,13a-pentahydroxy-6,10-dimethyl-4*H*,8*H*-pyrano[2′,3′:4,5]benzofuro[3,2-*b*][1]benzopyran-4,8-dione**; **1**). Yellow amorphous powder. $[\alpha]_D^{24.5} = -564.4$ (c = 0.10, MeOH). UV (MeOH): 242 (2.80), 258 (2.93), 308 (3.06), 377 (2.62). IR (KBr): 3425, 1637, 1516, 1441, 1325, 1289, 1165, 1100, 987, 960, 917, 867, 800, 730, 623, 599, 490, 434. CD (MeCN): 218 (+15.56), 238 (−4.18), 282 (−6.95), 307 (+26.91), 335 (−14.42), 379 (−20.39). ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (pos.): 631.1082 ($[M + H]^+$, $C_{32}H_{23}O_{14}^+$; calc. 631.1082).

Baeckein K (= **(7a*S*,13a*S*)-7a-(3,4-dihydroxyphenyl)-2-[4-(*β*-D-glucopyranosyloxy)-3-hydroxyphenyl]-7a,13a-dihydro-3,5,9,11,13a-pentahydroxy-6,10-dimethyl-4*H*,8*H*-pyrano[2′,3′:4,5]benzofuro[3,2-*b*][1]benzopyran-4,8-dione**; **2**). Yellow amorphous solid. $[\alpha]_D^{24.4} = -127$ (c = 0.10, MeOH). UV (MeOH): 240 (2.98), 307 (3.10), 372 (2.74). IR (KBr). 3396, 1637, 1508, 1442, 1327, 1296, 1201, 1167, 1099, 1071, 916, 866, 800, 731, 604, 493. CD (MeCN): 213 (+2.14), 231 (−2.69), 252 (−3.66), 285 (−1.40), 306 (+5.58), 335 (−3.01), 378 (−3.81). ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (pos.): 793.1607 ($[M + H]^+$, $C_{38}H_{33}O_{19}^+$; calc. 793.1611).

Acid Hydrolysis and GC Analysis

Compound **2** (2.0 mg) was hydrolyzed with 10% HCl/dioxane (1:1, *v/v*, 5.0 ml) at 80 °C for 4.0 h. The mixture was diluted with H_2O and extracted with AcOEt (5.0 ml × 3). The sugar component in the H_2O layer was evaporated under reduced pressure, and then, the obtained sugar residue was divided into two halves. One half was identified by SiO_2 TLC with $CHCl_3$/MeOH/H_2O (8:5:1) by comparison with the authentic sample [20], and the other half was dissolved in dry pyridine (1.0 ml), and L-cysteine methyl ester hydrochloride (2.0 mg) was added, followed by heating at 60 °C for 2.0 h. Finally, the resulting soln. was extracted with cyclohexane and H_2O, and the combined org. phase was analyzed by GC. The temp. for injection and detector were 250 and 280 °C, resp. The initial temp. of 180 °C was maintained for 5.0 min and then raised to 260 °C at the rate of 8 °C/min, and He was used as the carrier gas [21]. The standard D-glucose (*Sigma–Aldrich*, St. Louis, MO, USA) was subjected to the same reaction and GC analysis under the same conditions.

Computational Details

All geometries were optimized by B3LYP functional with 6-31G* basis-set [22 – 24]. ωB97XD functional with 6-311G* basis-set [25][26] for heavy atoms and 6-31G* for H-atoms was employed to conduct TD-DFT calculations. Based on the resulting electronic excitation energies and rotatory strengths, Multiwfn 3.3.7 program [27] in combination with Origin software was employed to obtain the ECD spectra. Polarized continuum mode (PCM) implicit solvent model was used to represent MeCN solvent environment. All calculations were performed by *Gaussian* 09 program, revision D.01 [28].

Cytoprotective Activity

PC12 cells, a rat pheochromocytoma cell line, were routinely cultured in *Dulbecco*'s Modified *Eagle* Medium (DMEM; *Sigma–Aldrich*) containing 10% fetal bovine serum, 100 units/ml of penicillin, 100 μg/ml streptomycin, and 3% L-glutamine. Cells were fed with fresh medium every 2 or 3 d and maintained in collagen-coated T75 tissue culture flasks in a humidified atmosphere of 5% CO_2 at 37 °C. When cells became 80% confluent, they were harvested by dislodging them from the flask surface with a flow of medium from a pipette, and dispersing them through a 22-gauge needle. The dispersed PC12 cells were then seeded on collagen-coated 96-well plates at a density of 2×10^4 cells/well. After 24 h of subculture, the cells in 96-well plates were treated with the test compounds **1** and **2**. For the study of cytotoxicity, treated cells were incubated for 48 h at 37 °C. For cytoprotective assessments, 0.4 mM H_2O_2 was concomitantly added and cells were incubated for 2 h at 37 °C. Then the treatment medium was removed and the cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method. Cells were incubated for 5 h at 37 °C with MTT (0.5 μM) and dissolved in fresh complete medium, in which metabolically active cells reduced the dye to purple formazan. Finally, the crystals were dissolved with DMSO and the absorbance was measured at 570 nm on a microplate reader (*Tecan Sunrise*, Männedorf, Switzerland). In cytoprotective assay, the H_2O_2-treated group was used for the control and the group without any treatment was used for the blank. Each concentration of the compounds was tested in three parallel wells. The relative cytoprotection to the control was calculated with the following method [29]: cytoprotection (%) = (Abs. of sample − Abs. of control)/Abs. of blank × 100.

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