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C-dideoxyhexosyl flavones from the stems and leaves of *Passiflora edulis* Sims

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ABSTRACT

The stems and leaves of *Passiflora edulis* Sims, are used as a folk medicine for treating both anxiety and nervousness in American countries. Phytochemical investigation of the *n*-butanol (*n*-BuOH) fraction of this plant led to the isolation of four new 2,6-dideoxyhexose-*C*-glycosyl flavones, including luteolin-8-*C*-β-digitoxopyranosyl-4′-*O*-β-D-glucopyranoside (**1**), apigenin-8-*C*-β-digitoxopyranoside (**2**), apigenin-8-*C*-β-boivinopyranoside (**3**) and luteolin-8-*C*-β-boivinopyranoside (**4**), together with five known compounds (**5**–**9**). The structures of these compounds were elucidated by extensive spectroscopic methods. All compounds were evaluated for their neurite outgrowth enhancing activities and the results indicated that luteolin (**7**) enhanced NGF-induced neurite outgrowth in PC12 cells at 50.0 μM.

1. Introduction

The genus *Passiflora* comprises approximately 500 species, which are mainly distributed in the warm and tropical regions of America and a few of them in Southeast Asia and Australia (Hickey & King, 1988). *Passiflora edulis* is usually called the passion fruit and there are two types of this fruit: the purple form (*P. edulis* Sims) and the yellow form (*P. edulis var. flavicarpa* Degenerer) (Chen & Lu, 1994), which are currently being cultivated in many countries for commercial purpose as edible fruits due to their health benefits. Their leaf extracts have long been used in traditional folk medicines as a remedy for many neurogenic diseases in Europe and America. Earlier studies have shown the neuropharmacological activities of *P. edulis*, including its anxiolytic, anticonvulsant and sedative activities (Barbosa et al., 2008; Coleta et al., 2006; Deng, Zhou, Bai, Li, & Li, 2010; Li et al., 2011; Sena, Zucolotto, Reginatto, Schenkel, & De Lima, 2009). There have also been a few reports referring to its anti-inflammatory (Farid et al., 2010; Montanher, Zucolotto, Schenkel, & Fröde, 2007; Zucolotto et al., 2009), antibacterial (Kannan, Parimala, & Jayakar, 2011), antioxidant (Rudnicki et al., 2007; Sunitha & Devaki, 2009) and blood pressure attenuating activities (Zibadi et al., 2007) etc.

A good number of *Passiflora* species have been studied with respect to their flavonoid composition. Previous studies have concluded that they are a rich source of *C*-glycosyl flavones. In *P. edulis*, orientin 2″-rhamnoside (Coleta et al., 2006), 6-*C*-chinovoside and 6-*C*-fucoside of luteolin were identified (Mareck, Herrmann, Galensa, & Wray, 1991). The uncommon *C*-deoxyhexosyl derivatives of luteolin and apigenin have been reported for the first time from the leaf extract of *P. edulis* by a high-performance liquid chromatography-diode array detection-tandem mass spectrometry (HPLC-DAD–MS/MS) method (Ferreres et al., 2007). However, to the best of our knowledge, 2,6-dideoxysugar moiety bearing in flavones are rarely reported from *Passiflora* species up to now. The present paper reports the isolation and structural elucidation of new dideoxyhexosyl-*C*-glycoside flavones (Fig. 1), as well as their neurite outgrowth enhancing activities involved in the stems and leaves of *P. edulis* Sims.

2. Materials and methods

2.1. General

Optical rotations were measured on a Jasco P-1020 (Jasco International Co., Ltd., Tokyo, Japan) automatic digital polarimeter. UV spectra were taken on a Shimadzu UV-2401PC (Shimadzu, Kyoto, Japan) spectrophotometer. IR spectra were obtained on a Bruker Tensor 27 FT-IR (Bruker Optics GmbH, Ettlingen, Germany) spectrometer with KBr pellets. NMR spectra were acquired on Bruker AV-400 or DRX-500 or Bruker AVANCE III-600 (Bruker BioSpin GmbH, Rheinstetten, Germany) instruments, using tetramethylsilane (TMS) as an internal standard. ESIMS (including HRESIMS) were recorded on an API QSTAR Pulsar i (MDS Sciqaszex, Concord, Ontario, Canada) mass spectrometer. Silica gel (200-300 mesh; Qingdao Marine Chemical Inc., Qingdao, China), MCI gel CHP 20P (75–150 μm; Mitsubishi Chemical Corp., Tokyo, Japan),

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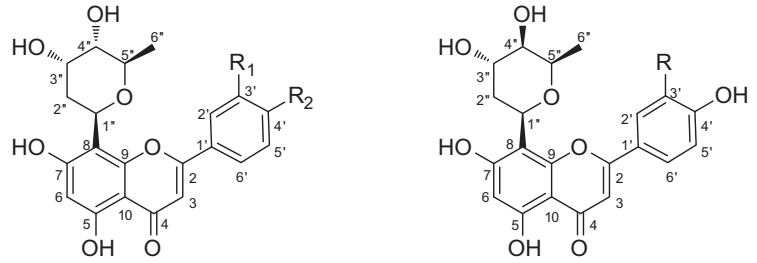

Fig. 1. Chemical structures of compounds **1**–**5**.

Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), and LiChroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany) were used for normal pressure column chromatography (CC). Fractions monitored by TLC, and spots were visualised with UV light at 254 nm.

2.2. Plant material

The stems and leaves of *P. edulis* Sims were collected at the botanical garden of Kunming, Yunnan Province, People's Republic of China, in June 2009, and identified by Dr. En-De Liu, Kunming Institute of Botany, CAS. A voucher specimen (KUM 0368034) was deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

2.3. Extraction and isolation

The air-dried stems and leaves of *P. edulis* Sims (5.7 kg) were powdered and extracted five times with 70% aq. acetone (20 L × 5, 1 d, each) at room temperature. After removing the solvent under reduced pressure using a rotary evaporator at 50 °C, the resulting gum was dried to yield a solid residue (752 g). A portion of crude extract (700 g) was suspended in distilled water and successively partitioned with ethyl acetate (yield: 250 g) and *n*-butanol (yield: 210 g). The *n*-BuOH solube extract (204 g) was subjected to silica gel column (9 × 120 cm, 2.0 kg) and eluted with a $CHCl_3$–MeOH (95:5, 90:10, 85:15, 10:20, 75:25, 70:30, 60:40; each 20 L, v/v) gradient, eight fractions were collected on the basis of their TLC characteristics as follows: **A** (6.8 g, brown solid), **B** (4.5 g, brown solid), **C** (17.6 g, brown solid), **D** (12.8 g, brown solid), **E** (8.9 g, brown solid), **F** (52.8 g, white solid), **G** (20.3 g, brown solid), **H** (25.6 g, black solid). Fraction **B** (4.5 g) was further separated by silica gel column (3 × 25 cm, 50 g) and eluted with petroleum ether (PE)–acetone (80:20, 70:30, 500 mL each, v/v) to give three fractions ($\mathbf{B}_1$–$\mathbf{B}_3$). Fraction $\mathbf{B}_1$ (0.3 g) was further purified by Sephadex LH-20 column (1.8 × 125 cm, 55 g) eluted with $CHCl_3$–MeOH (1:1, 250 mL, v/v) to yield compound **6** (12 mg). Fraction **C** (17.6 g) was submitted to MCI gel CHP 20P column (6 × 40 cm, 600 mL) eluted with gradient aqueous MeOH to afford 2 portions: $\mathbf{C}_1$, MeOH–H_2O (50:50, 1.5 L, 60:40, 1 L, v/v); $\mathbf{C}_2$, MeOH–H_2O (80:20, 1.2 L, 90:10, 600 mL, v/v). Fraction $\mathbf{C}_2$ (5.2 g) was purified by RP-18 gel column (3.5 × 30 cm, 120 g) and eluted with MeOH–H_2O (60: 40, 250 mL, v/v) and then further purified by Sephadex LH-20 gel column (1.8 × 125 cm, 56 g) with MeOH (250 mL, v/v) to yield compound **7** (8 mg). Fraction **D** (12.8 g) was submitted to MCI gel CHP 20P CC (6 × 40 cm, 600 mL) eluted with gradient MeOH–H_2O (30:70, 50:50, 80:20, each 1.2 L, v/v) to obtain four fractions ($\mathbf{D}_1$–$\mathbf{D}_4$). Fraction $\mathbf{D}_1$ (2.1 g) and $\mathbf{D}_2$ (4.3 g) were gel filtrated by Sephadex LH-20 CC (3.0 × 125 cm, 140 g) (MeOH) to obtain $\mathbf{D}_{1-1}$–$\mathbf{D}_{1-4}$ and $\mathbf{D}_{2-1}$–$\mathbf{D}_{2-3}$, respectively. D_{1-3} (0.2 g) was separated by silica gel (1.3 × 25 cm, 10 g), and eluted with PE–acetone–HCOOH (60:40:0.1, 250 mL, v/v) to yield compounds **2** (13 mg) and **4** (26 mg). $\mathbf{D}_{2-2}$ (0.35 g) was separated by silica gel (1.5 × 40 cm 20 g) with $CHCl_3$–MeOH–HCOOH (90:10:0.1, 300 mL, v/v) to yield compounds **3** (3 mg) and **5** (42 mg). Fraction **E** (8.9 g) was chromatographyed by silica gel column (3.5 × 40 cm, 100 g), eluted with $CHCl_3$–MeOH–H_2O (14:3:1, 7:3:1, lower layer, each 1 L, v/v) to obtain 5 fractions ($\mathbf{E}_1$–$\mathbf{E}_5$). Fraction $\mathbf{E}_2$ (2.3 g) was subjected to Sephadex LH-20 column (3.0 × 125 cm, 135 g) (MeOH) to obtain **8** (17 mg). Fraction **G** (20.3 g) was further separated by silica gel (6 × 30 cm,, 200 g) using EtOAC–MeOH (95:5, 90:10, 80:20; each 2 L, v/v) to obtain four fractions (G_1–G_4). Fraction G_3 (5.6 g) was submitted to MCI gel CHP 20P column eluted with gradient aqueous methanol to afford three fractions (G_{3-1}–G_{3-3}). G_{3-2} (2.4 g) further purified by RP-18 gel column (3.0 × 30 cm, 80 g) (MeOH–H_2O, 40:60, 60:40, 1 L, each, v/v), and then by Sephadex LH-20 gel (3.0 × 125 cm, 145 g) (MeOH–H_2O, 90:10, 500 mL, v/v) to obtain compounds **1** (112 mg) and **9** (132 mg). All the compounds had a degree of purity >90%.

2.4. Cell culture and evaluation of neurite outgrowth

Neurite Outgrowth-Promoting Activity. The neurotrophic activities of the test compounds were examined according to an assay using PC12 cells as reported (Marcotullio et al., 2006). Briefly, PC12 cells were maintained in F12 medium supplemented with 12.5% horse serum (HS), and 2.5% foetal bovine serum (FBS), and incubated at 5% CO_2 and 37 °C. Test compounds were dissolved in DMSO. For the neurite outgrowth-promoting activity bioassay, PC12 cells were seeded at a density of 2×10^4 cells/mL in 48-well plate coated with poly-L-lysine. After 24 h, the medium was changed to that containing 10 μM of each test compounds plus 10 ng/mL nerve growth factor (NGF), or various concentrations of NGF (50 ng/mL for the positive control, 10 ng/mL for the negative control). The final concentration of DMSO was 0.05%, and the same concentration of DMSO was added into the negative control. After 72 h incubation, the neurite outgrowth was assessed under a phasecontrast microscope. Neurite processes with a length equal to or greater than the diameter of the neuron cell body were scored as neurite bearing cells. The ratio of the neurite-bearing cells to total cells (with at least 100 cells examined/view area; 5 viewing area/well) was determined and expressed as a percentage.

3. Results and discussion

3.1. Structure elucidation of compounds

Compound **1** had a molecular formula of $C_{27}H_{30}O_{14}$ deduced from the negative HRESIMS (*m/z* 577.1539, $[M-H]^-$). The fragment

ion signal at *m/z* 415 ([M−H−162]$^-$) indicated the loss of a glycosyl hexose residue. The absorption maxima at 210, 248, 272, and 334 nm in the UV spectrum were attributed to a flavone skeleton. The ^{1}H NMR spectrum (Table 1) of **1** exhibited an ABX-system at δ_H 7.58 (1H, d, J = 2.0 Hz, H-2′), 7.55 (1H, dd, J = 8.4, 2.0 Hz, H-6′) and 7.30 (1H, d, J = 8.4 Hz, H-5′), two singlets corresponding to H-3 and H-6 (or H-8). Two anomeric proton signals at δ_H 5.64 (1H, dd, J = 12.0, 1.8 Hz) and 4.92 (1H, d, J = 7.8 Hz) correlated with the carbon signals at δ_C 68.7 and 103.3 in the HSQC spectrum, respectively, indicating that one sugar moiety may connected through a *C*-linkage [δ_C 68.7 (C-1″)] (Tai et al., 2011) and the other one via an *O*-linkage [δ_C 103.3 (C-1‴)]. A sugar moiety was due to the distinct anomeric signal at δ_H 4.92 and oxythine protons in the range δ_H 3.42–4.92 in the ^{1}H NMR spectra. The HSQC and ^{1}H–^{1}H COSY spectra (Fig. 2) permitted the assignments of H-2‴ (δ_H 3.56), H-3‴ (δ_H 3.50), H-4‴ (δ_H 3.42), H-5‴ (δ_H 3.50) and H-6‴ (δ_H 3.91 and δ_H 3.73), respectively. In the ROESY spectrum, the correlations of H-1‴ ↔ H-5‴, H-1‴ ↔ H-3‴ and H-4‴ ↔ H-6‴ were observed. The ^{13}C NMR spectrum displayed four oxygenated methine carbons at δ_C 78.4 (d), 77.5 (d), 74.7 (d), 71.2 (d), one oxygenated methylene carbon at δ_C 62.5 (t), and one anomeric carbon at δ_C 103.3 (d), indicating the presence of a glucopyranosyl unit (Table 1). The HMBC correlations from the protons at δ_H 4.92 (H-1‴), δ_H 7.55 (H-6') and δ_H 7.58 (H-2') to the carbon at δ_C 150.1 (C-4′) (Fig. 2) and the ROESY of H-1‴ ↔ H-5' indicated that the *O*-linked sugar moiety was attached to C-4′ of the flavone skeleton.

The structure of the *C*-linked sugar unit was based on deduction from detailed analysis of 1D and 2D-NMR spectra. In the ^{1}H–^{1}H COSY spectrum (Fig. 2), H-1″/H-2″/H-3″/H-4″/H-5″/H-6″ were observed. The HMBC (Fig. 2) correlations from the proton at δ_H 5.64 (H-1″) to the carbons at δ_C 38.4 (C-2″), 68.8 (C-3″) and 74.6 (C-5″), from the proton at δ_H 1.92 (H-2″eq) to the carbon at δ_C 74.4 (C-4″), from the protons at δ_H 1.37 (H-6″) to the carbons at δ_C 74.6 (C-5″) and 74.4 (C-4″) were observed. The anomeric proton at δ_H 5.64 (H-1″) coupled to two nonequivalent protons at δ_H 2.34 (ddd, 13.9, 12.0, 1.6 Hz) and 1.92 (ddd, 13.9, 3.5, 1.8 Hz), assigned to H-2″ax and H-2″eq. The coupling constant (J = 12.0 Hz) between H-1″ and H-2″ indicated the axial position of H-1″; H-3″ appeared as a broadened singlet which indicated its equatorial position; H-4″ appeared as a broadened doublet and the coupling constant with H-5″ (J = 9.6 Hz), indicating the axial position of H-4″ and H-5″, respectively. This can be confirmed from the ROESY correlations of H-1″ ↔ H-5″ and H-4″ ↔ H-2″ax (Fig. 3). Therefore, the structure of the *C*-linked sugar unit for compound **1** was final determined to be a digitoxose unit. From a comparison of the ^{13}C shift of the A-ring (C-5 to C-10) with those in the literatures for orientin, homoorientin, luteolin-6-*C*-chinovoside and luteolin-6-*C*-fucoside (Burns, Ellis, & March, 2007; Mareck et al., 1991), the sugar must be bound to C-8 of the luteolin, which was further confirmed by the HMBC correlations from the proton at δ_H 5.64 (H-1″) to the carbons at δ_C 156.4 (C-9) and 163.9 (C-7). The coupling constants of the two anomeric protons indicated that each sugar moiety was connected to the flavone via a β-linkage.

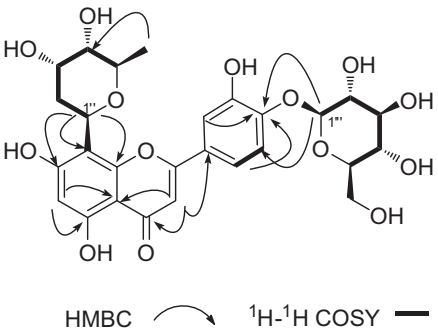

Fig. 2. Key HMBC and ^{1}H–^{1}H COSY correlations of **1**.

Table 1
^{1}H NMR and ^{13}C NMR Data for compounds **1**, **2**, **5** (δ in ppm).

No.	**1**		**2**		**5**	
	δ_C (mult)	δ_H (mult, J in Hz)	δ_C (mult)	δ_H (mult, J in Hz)	δ_C (mult)	δ_H (mult, J in Hz)
2	165.3 (s)		165.5 (s)		165.0 (s)	
3	104.7 (d)	6.63 (s)	103.5 (d)	6.95 (s)	103.6 (d)	6.95 (s)
4	184.0 (s)		183.1 (s)		183.1 (s)	
5	162.2 (s)		161.9 (s)	13.85 (s, OH-5)	161.9 (s)	13.90 (s, OH-5)
6	100.3 (d)	6.21 (s)	99.8 (d)	6.73 (s)	99.7 (d)	6.70 (s)
7	163.9 (s)		163.1 (s)	–	163.0 (s)	–
8	106.8 (s)	–	106.7 (s)	–	107.0 (s)	–
9	156.4 (s)	–	155.5 (s)	–	155.8 (s)	–
10	105.6 (s)	–	105.4 (s)	–	105.4 (s)	–
1′	127.2 (s)	–	122.6 (s)	–	123.3 (s)	–
2′	115.2 (d)	7.58 (d, 2.0)	129.4 (d)	8.31 (d, 8.6)	115.6 (d)	8.24 (d, 1.8)
3′	148.8 (s)	–	116.9 (d)	7.30 (d, 8.6)	148.0 (s)	–
4′	150.1 (s)	–	162.9 (s)	–	151.9 (s)	–
5′	117.9 (d)	7.30 (d, 8.4)	116.9 (d)	7.30 (d, 8.6)	116.7 (d)	7.33 (d, 8.3)
6′	120.0 (d)	7.55 (dd, 8.4, 2.0)	129.4 (d)	8.31 (d, 8.6)	120.0 (d)	7.92 (dd, 8.3, 1.8)
1″	68.7 (d)	5.64 (dd, 12.0, 1.8)	68.2 (d)	6.31 (br d, 12.0, 2.0)	68.2 (d)	6.34 (br d, 11.6)
2″	38.4 (t)	2.34 (ddd, 13.9, 12.0, 1.6) 1.92 (ddd, 13.9, 3.5, 1.8)	38.5 (t)	2.68 (ddd, 14.0, 12.0, 2.0) 2.43 (ddd, 14.0, 2.5, 2.0)	38.4 (t)	2.74 (br dd, 13.6, 11.6) 2.25 (br d, 13.6)
3″	68.8 (d)	4.13 (br s)	68.6 (d)	4.59 (br s)	68.3 (d)	4.58 (br s)
4″	74.4 (d)	3.51 (br d, 9.6)	74.3 (d)	3.91 (br d, 9.5)	74.3 (d)	4.06 (br d, 9.4)
5″	74.6 (d)	3.98 (dq, 9.6, 6.2)	74.3 (d)	4.52 (dq, 9.5, 6.2)	74.3 (d)	4.51 (dq, 9.4, 6.0)
6″	18.8 (q)	1.37 (d, 6.2)	19.2 (q)	1.37 (d, 6.2)	19.2 (q)	1.32 (d, 6.0)
1‴	103.3 (d)	4.92 (d, 7.8)				
2‴	74.7 (d)	3.56 (m)				
3‴	77.5 (d)	3.50 (m)				
4‴	71.2 (d)	3.42 (m)				
5‴	78.4 (d)	3.50 (m)				
6‴	62.5 (t)	3.91 (dd, 12.0, 5.0) 3.73 (dd, 12.0, 8.0)				

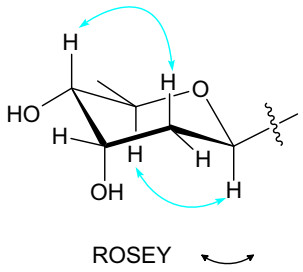

Fig. 3. Key ROESY correlations of sugar moiety of **1**.

Consequently, this compound was identified as luteolin-8-*C*-β-digitoxopyranosyl-4′-*O*-β-D-glucopyranoside. Although flavonoid *C*-glycosides are commonly found in plants, flavonoids bearing a digitoxopyranosyl residue such as **1** are not common.

Compound **2** was determined to have a molecular formula of $C_{21}H_{20}O_8$ with the molecular ion at *m/z* 399.1075 ($[M-H]^-$) in the negative HRESIMS spectrum. Compared with **1**, the spectroscopic properties were similar (Table 1) except that one 4′-*O*-substituted glucose moiety was omitted, the other B-ring with a single ortho substituent exhibited by the signals at δ_H 8.31 (2H, dd, J = 8.6 Hz) and 7.30 (2H, dd, J = 8.6 Hz). The correlations from the anomeric proton at δ_H 6.31 (1H, dd, J = 12.0, 2.0 Hz) to the carbon at δ_C 163.1 (C-7), 155.5 (C-9) and 106.7 (C-8) in the HMBC spectrum, indicated the digitoxose moiety was attached at C-8 position of apigenin. Others assignments listed were based on HSQC and HMBC correlations. Therefore, the structure of compound **2** was identified as apigenin-8-*C*-β-digitoxopyranoside.

Compound **3** gave a molecular ion at *m/z* 399.1089 ($[M-H]^-$) in the HRESIMS spectrum, which corresponds to a molecular formula of $C_{21}H_{20}O_8$. Compared to compound **2**, their spectroscopic properties were similar (Table 2). These two compounds differed only in the sugar unit attached to C-8 of apigenin. The location of the sugar moiety was determined by the correlation of OH-5 ↔ H-6 in the ROESY spectrum, and the HMBC correlations (Fig. 4) of the proton δ_H 6.36 (H-1″) to δ_C 154.5 (C-9) and 163.8 (C-7) in **3**. The ^1H–^1H COSY spectrum showed correlations of H-1″/H-2″/H-3″/H-4″/H-5″/H-6″. The HSQC spectrum suggested the presence of a methylene carbon at δ_C 33.9, connected to H-2″ax [δ_H 3.16 (ddd, 13.5, 12.0, 2.0 Hz)] and H-2″eq [δ_H 2.18 (br d, 13.5 Hz)].The large coupling constant (dd, J = 12.0 Hz) between H-1″ and H-2″ indicated that the axial position of H-1″; the orientation of H-5″ was elucidated to be axial from the ROESY correlation of H-1″ ↔ H-5″ (Fig. 5); H-3″ and H-4″ appeared as a broadened singlet, indicating that they were at equatorial position. The NMR data indicated that the sugar unit for **3** was boivinopyranosyl residue (Hoyweghen Van, Karalic, Calenbergh Van, Deforce, & Heyerick, 2010). Based on the above evidences, compound **3** was identified as apigenin-8-*C*-β-boivinopyranoside.

Compound **4** gave a negative molecular ion at *m/z* 415.1031 ($[M-H]^-$) in the HRESIMS spectrum, which corresponds to a molecular formula of $C_{21}H_{20}O_9$. The NMR data of **4** (Table 2) showed the presence of a sugar moiety was attached to luteolin. An ABX-system [δ_H 8.27 (1H, s), 7.99 (1H, d, J = 8.2 Hz) and 7.32 (1H, d, J = 8.2 Hz)] exhibited in ^1H NMR spectrum. The boivinose unit assignments listed were based on 2D-NMR spectra, and the position was the same as compound **3** which were determined by the observable correlations of the proton at δ_H 6.31 (H-1″, 1H, dd, J = 12.0, 2.0 Hz) to the carbon at δ_C 163.4 (C-7), 154.5 (C-9) and 107.1 (C-8) in the HMBC spectrum. Then, it was identified as luteolin-8-*C*-β-boivinopyranoside.

Compound **5** gave a negative molecular ion at *m/z* 415.1034 ($[M-H]^-$) in the HRESIMS spectrum, which corresponds to a molecular formula of $C_{21}H_{20}O_9$. The NMR data of **5** (Table 1) were similar to those of **2**. The distinct was that **5** exhibited an ABX system [8.24 (1H, d, J = 1.8 Hz), 7.92 (1H, dd, J = 8.3, 1.8 Hz) and 7.33 (1H, d, J = 8.3 Hz)] instead of AA′BB′ system [8.31 (2H, dd, J = 8.6 Hz) and 7.30 (2H, dd, J = 8.6 Hz)] of **2** in ^1H NMR spectrum. The location of the digitoxose moiety was also confirmed by the HMBC correlations of δ_H 6.34 (H-1″, 1H, br d, J = 11.6 Hz) to δ_C 163.0 (C-7), 155.8 (C-9) and 107.0 (C-8); δ_H 13.9 (OH-5) to δ_C 99.7 (d, C-6), 105.4 (C-10) and 161.9 (C-5) were observed. This compound was identified as luteolin-8-*C*-β-digitoxopyranoside (Kannan et al., 2011). Its spectroscopic data were reported for the first time.

From the NMR and MS data and corresponding with those form literatures, 4 known compounds were identified as chrysin (**6**) (Agrawal & Schneider, 1983), luteolin (**7**) (Wagner, Chari, & Sonnenbichler, 1976), chrysin-7-*O*-β-D-glucopyranoside (**8**) (Wen

Table 2
^1H NMR and ^{13}C NMR Data for compounds **3**–**4** (δ in ppm).

No.	**3**		**4**	
	δ_C (mult)	δ_H (mult, J in Hz)	δ_C (mult)	δ_H (mult, J in Hz)
2	165.1 (s)	–	164.8 (s)	–
3	103.8 (d)	7.01 (s)	103.5 (d)	6.98 (s)
4	183.6 (s)	–	183.0 (s)	–
5	162.4 (s)	13.92 (s, OH-5)	161.9 (s)	13.86 (s, OH-5)
6	100.5 (d)	6.77 (s)	100.1 (d)	6.74 (s)
7	163.8 (s)	–	163.4 (s)	–
8	107.9 (s)	–	107.1 (s)	–
9	154.5 (s)	–	154.5 (s)	–
10	105.7 (s)	–	105.1 (s)	–
1′	122.8 (s)	–	122.9 (s)	–
2′	130.2 (d)	8.46 (d, 8.7)	115.0 (d)	8.27 (br s)
3′	117.4 (s)	7.31 (d, 8.7)	147.6 (s)	–
4′	163.3 (s)	–	151.7 (s)	–
5′	117.4 (d)	7.31 (d, 8.7)	117.0 (d)	7.32 (d, 8.2)
6′	130.2 (d)	8.46 (d, 8.7)	120.1 (d)	7.99 (br d, 8.2)
1″	69.4 (d)	6.36 (dd, 12.0, 2.4)	69.3 (d)	6.31 (dd, 12.0, 2.0)
2″	34.3 (t)	3.16 (ddd, 13.8, 12.0, 2.4) 2.18 (ddd, 13.8, 2.4, 2.4)	33.9 (t)	3.06 (ddd, 13.5, 12.0, 2.0) 2.18 (br d, 13.5)
3″	69.1 (d)	4.70 (br s)	68.4 (d)	4.67 (br s)
4″	71.1 (d)	4.02 (br s)	70.8 (d)	4.01 (br s)
5″	73.3 (d)	4.75 (br q, 6.5)	72.6 (d)	4.67 (br q, 6.2)
6″	18.3 (q)	1.59 (d, 6.5)	17.7 (q)	1.57 (d, 6.2)

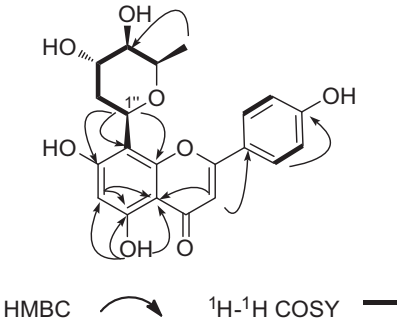

Fig. 4. Key HMBC and COSY correlations of **3**.

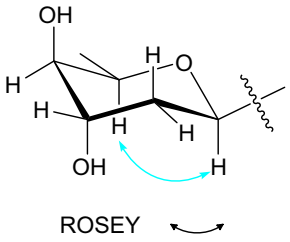

Fig. 5. Key ROESY correlation of sugar moiety of **3**.

et al., 2011) and chrysin-7-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranoside (**9**) (Montoro, Carbone, De Simone, Pizza, & De Tommasi, 001).

3.2. Characteristic data of compounds(1–5)

Luteolin-8-*C*-β-digitoxopyranosyl-4′-*O*-β-D-glucopyranoside (**1**): yellow amorphous powder; $[\alpha]_D^{25.6}$: −11.4 (*c* 0.18, MeOH); UV (MeOH): λ_{max} (log ε) 210 (4.51), 248 (4.15), 272 (4.25), 334 (4.20) nm; IR (KBr): υ_{max} 3423, 2960, 2875, 1726, 1658, 1615, 1505, 1357, 1073 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Table 1; HRESIMS *m/z* 577.1539 $[M-H]^-$ (calcd for $C_{27}H_{29}O_{14}$, 577.1557).

Apigenin-8-*C*-β-digitoxopyranoside (**2**): yellow amorphous powder; $[\alpha]_D^{25.7}$:+67.3 (*c* 0.12, MeOH); UV (MeOH): λ_{max} (log ε) 212 (4.49), 271 (4.26), 332 (4.49) nm; IR (KBr): υ_{max} 3527, 3440, 2965, 2927, 1659, 1612, 1511, 1382, 1083 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data: Table 1; HRESIMS *m/z* 399.1075 $[M-H]^-$ (calcd for $C_{21}H_{19}O_8$, 399.1079).

Apigenin-8-*C*-β-boivinopyranoside (**3**): yellow amorphous powder; $[\alpha]_D^{25.8}$:+56.9 (*c* 0.19, MeOH). UV (MeOH): λ_{max} (log ε) 212 (4.34), 271 (4.10), 332 (4.13) nm; IR (KBr): υ_{max} 3424, 3264, 2960, 2930, 1658, 1610, 1512, 1384, 1067 cm^{-1}; ^{1}H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) data: see Table 1; HRESIMS *m/z* 399.1089 $[M-H]^-$ (calcd for $C_{21}H_{19}O_8$, 399.1079).

Luteolin-8-*C*-β-boivinopyranoside (**4**): yellow amorphous powder; $[\alpha]_D^{25.8}$:+49.8 (*c* 0.13, MeOH). UV (MeOH): λ_{max} (log ε) 210 (4.50), 257 (4.17), 269 (4.16), 295 (3.94), 350 (4.23) nm; IR (KBr): υ_{max} 3494, 3420, 2975, 2923, 1659, 1608, 1489, 1361, 1043 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data: see Table 1. HRESIMS *m/z* 415.1031 $[M-H]^-$ (calcd for $C_{21}H_{19}O_9$, 415.1029).

Luteolin-8-*C*-β-digitoxopyranoside (**5**): yellow amorphous powder; $[\alpha]_D^{25.7}$:+87.8 (*c* 0.15, MeOH); UV (MeOH): λ_{max} (log ε) 209 (4.56), 257 (4.25), 269 (4.23), 349 (4.49) nm; IR (KBr): υ_{max} 3421, 3263, 2967, 2927, 1660, 1608, 1490, 1354, 1078 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) data: see Table 1; HRESIMS *m/z* 415.1034 $[M-H]^-$ (calcd for $C_{21}H_{19}O_9$, 415.1029).

3.3. Neurite outgrowth in PC12 cells

For the purpose of evaluating the effect of neuroprotective effects of this species, the isolated flavonoids **1**–**9** were evaluated for the enhancing activity on NGF-induced neurite outgrowth in PC12 cells. The result indicated that the proportion of the NGF (10 ng/mL)-induced neurite-bearing cells were enhanced by **7** (luteolin) at the concentration of 50 μM. Others showed no activity on 10 ng/mL NGF-induced neurite outgrowth in PC12 cells at 50.0 μM.

4. Conclusions

Phytochemical study of *P. edulis* Sims has resulted in four new *C*-dideoxyhexosyl flavones (**1**–**4**), together with five known flavones (**5**–**9**). As far as we know, this is the first report of *C*-dideoxyhexosyl flavones isolated from *Passiflorae*. Take into consideration that this plant is used both for medicine and juice material provided, it is clearly important for the revealing of its chemical constituents. In this study, luteolin (**7**) enhanced NGF-induced neurite outgrowth in PC12 cells at 50.0 μM. Further studies should also be carried out to reveal more ingredients and other pharmaceutical functions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.07.101.

References

Agrawal, P. K., & Schneider, H. J. (1983). Deprotonation-induced carbon-^{13}C NMR shifts in phenols and flavonoids. *Tetrahedron Letter, 24*, 177–180.

Barbosa, P. R., Valvassori, S. S., Kappel, V. D., Bordignon, C. L., Jr., Martins, M. R., Gavioli, E. C., et al. (2008). The aqueous extracts of Passiflora alata and *Passiflora edulis* reduce anxiety-related behaviors without affecting memory process in rats. *Journal of Medicinal Food, 11*, 282–288.

Burns, D. C., Ellis, D. A., & March, R. E. (2007). A predictive tool for assessing ^{13}C NMR chemical shifts of flavonoids. *Magnetic Resonance in Chemistry, 45*, 835–845.

Chen, X. J., & Lu, L. X. (1994). Karyotype analysis of two major cultivated forms of *Passiflora* L. *Journal of Fujian Agricultural University, 23*, 24.

Coleta, M., Batista, M. T., Campos, M. G., Carvalho, R., Cotrim, M. D., Lima, T. C. M. D., et al. (2006). Neuropharmacological evaluation of the putative anxiolytic effects of *Passiflora edulis* Sims, its sub-fractions and flavonoid constituents. *Phytotherapy Research, 20*, 1067–1073.

Deng, J., Zhou, Y., Bai, M., Li, H., & Li, L. (2010). Anxiolytic and sedative activities of *Passiflora edulis* f. *flavicarpa*. *Journal of Ethnopharmacology, 128*, 148–153.

Farid, R., Rezaieyazdi, Z., Mirfeizi, Z., Hatef, M. R., Mirheidari, M., Mansouri, H., et al. (2010). Oral intake of purple passion fruit peel extract reduces pain and stiffness and improves physical function in adult patients with knee osteoarthritis. *Nutrition Research, 30*, 601–606.

Ferreres, F., Sousa, C., Valentão, P., Andrade, P. B., Seabra, R. M., & Gil-Izquierdo, A. (2007). New *C*-deoxyhexosyl flavones and antioxidant properties of *Passiflora edulis* leaf extract. *Journal of Agricultural and Food Chemistry, 55*, 10187–10193.

Hickey, M., & King, C. (1988). 100 families of flowering plants. In S. M. Walters (Ed.), *Passiflora juss* (pp. 130–133). Cambridge: Cambridge University Press.

Hoyweghen Van, L., Karalic, I., Calenbergh Van, S., Deforce, D., & Heyerick, A. (2010). Antioxidant flavone glycosides from the leaves of *Fargesia robusta*. *Journal of Natural Products, 73*, 1573–1577.

Kannan, S., Parimala, D. B., & Jayakar, B. (2011). Antibacterial evaluation of the methanolic extract of *Passiflora edulis*. *Hygeia: Journal of Drugs and Medicines, 3*, 46–49.

Li, H. W., Zhou, P., Yang, Q. Q., Shen, Y., Deng, J., Li, L., et al. (2011). Comparative studies on anxiolytic activities and flavonoid compositions of *Passiflora edulis* '*edulis*' and *Passiflora edulis* '*flavicarpa*'. *Journal of Ethnopharmacology, 133*, 1085–1090.

Marcotullio, M. C., Pagiott, R., Maltese, F., Obara, Y., Hoshino, T., Nakahata, N., et al. (2006). Neurite outgrowth activity of cyathane diterpenes from Sarcodon cyrneus, cyrneines A and B. *Planta Medica, 72*, 819–823.

Mareck, U., Herrmann, K., Galensa, R., & Wray, V. (1991). The 6-*C*-chinovoside and 6-*C*-fucoside of luteolin from *Passiflora edulis*. *Phytochemistry, 30*, 3486–3487.

Montanher, A. B., Zucolotto, S. M., Schenkel, E. P., & Fröde, T. S. (2007). Evidence of anti-inflammatory effects of *Passiflora edulis* in an inflammation model. *Journal of Ethnopharmacology, 109*, 281–288.

Montoro, P., Carbone, V., De Simone, F., Pizza, C., & De Tommasi, N. (2001). Studies on the constituents of *Cyclanthera pedata* fruits: Isolation and structure elucidation of new flavonoid glycosides and their antioxidant activity. *Journal of Agricultural and Food Chemistry, 49*, 5156–5160.

Rudnicki, M., De Roberto, O. M., Pereira, T. V., Reginatto, F. H., Dal-Pizzol, F., & Fonseca Moreira, J. C. (2007). Antioxidant and antiglycation properties of *Passiflora alata* and *Passiflora edulis* extracts. *Food Chemistry, 100*, 719–724.

Sena, L. M., Zucolotto, S. M., Reginatto, F. H., Schenkel, E. P., & De Lima, L. T. C. M. (2009). Neuropharmacological activity of the pericarp of *Passiflora edulis flavicarpa* degener: Putative involvement of *C*-glycosyl flavonoids. *Expermental Biology and Medicine, 234*, 967–975.

Sunitha, M., & Devaki, K. (2009). Antioxidant activity of *Passiflora edulis* Sims leaves. *Indian Journal of Pharmaceutical Sciences, 71*, 310–311.

Tai, Z. G., Cai, L., Dai, L., DONG, L. H., Wang, M. F., Yang, Y. B., Cao, O. E., & Ding, Z. T. (2011). Antioxidant activity and chemical constituents of edible flower of *Sophora viciifolia*. *Food Chemistry, 126*, 1648–1654.

Wagner, H., Chari, V. M., & Sonnenbichler, J. (1976). Carbon-^{13}C NMR spectra of naturally occuring flavonoids. *Tetrahedron Letter, 17*, 1799–1802.

Wen, J. B., Zhang, Q. W., Yin, Z. Q., Li, Y. P., Han, X. W., & Ye, W. C. (2011). Flavonoids from the seeds of *Oroxylum indicum* (L.) Vent. *Chinese Pharmaceutical Journal, 46*, 170–173.

Zibadi, S., Farid, R., Moriguchi, S., Lu, Y., Foo, L. Y., Tehrani, P. M., et al. (2007). Oral administration of purple passion fruit peel extract attenuates blood pressure in female spontaneously hypertensive rats and humans. *Nutrition Research, 27*, 408–416.

Zucolotto, S. M., Goulart, S., Montanher, A. B., Reginatto, F. H., Schenkel, E. P., & Fröde, T. S. (2009). Bioassay-guided isolation of anti-inflammatory *C*-glucosyl flavones from *Passiflora edulis*. *Planta Medica, 75*, 1221–1226.