Bioactive Phenylethanoid Glycosides from *Buddleia lindleyana*

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Nine phenylethanoid glycosides were isolated from *Buddleia lindleyana*. On the basis of spectral analyses, their structures were elucidated to be acteoside (**1**), echinacoside (**2**), citanoside A (**3**), leucosceptoside A (**4**), leucosceptoside B (**5**), pedicularioside A (**6**), isoacteoside (**7**), arenariside (**8**), and a new compound named buddleoside A (**9**). The eight known compounds were isolated from this plant for the first time. The neuroprotective effects of compounds **1** (acteoside), **5**, **6**, **8** and **9** on the 1-methyl-4-phenylpyridinium ion (MPP^+)-induced cell death in mesencenphalic neurons were investigated. Mesencenphalic neurons treated with MPP^+ underwent cell death as determined by MTT assay. Simultaneous treatment with the phenylethanoid glycosides markedly attenuated MPP^+-induced cytotoxicity.

Key words: Buddleia lindleyana, Phenylethanoid Glycosides

Introduction

Buddleia lindleyana is a plant of the family *Buddleiaceae*, which has been widely used in southwest region of China as anti-inflammatory medicine for treating swelling, pain of lungs and skin wounds. Many plants of this family have been reported to possess anti-inflammatory, antibacterial and cytotoxic properties [1 – 4] which are usually attributed to flavonoids, sesquiterpenes and phenylethanoid glycosides. Studies on the chemical constituents and bioactivities of *Buddleia lindleyana* have rarely been reported. Here we report the isolation and characterization of nine phenylethanoid glycosides from the ethanol extract (95%) of *Buddleia lindleyana*. On the basis of spectral analyses, their structures were elucidated to be acteoside (**1**), echinacoside (**2**), citanoside A (**3**), leucosceptoside A (**4**), leucosceptoside B (**5**), pedicularioside A (**6**), isoacteoside (**7**), arenariside (**8**)**,** and a new compound named buddleoside A (**9**). The known eight compounds were isolated from this plant for the first time. The neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) has been employed to recreate a Parkinson's disease-like model in both rodents and primates. MPP^+ (1-methyl-4-phenylpyridinium ion) is an active metabolite of MPTP on dopaminergic neurons. Previous studies have shown that MPP^+ can elicit apoptosis in embryonic dopaminergic mesencenphalic neurons [5]. Acteoside and tubuloside B of phenylethanoid glycosides inhibit apoptosis by the MPP^+ [6, 7]. However, it is not clear whether other phenylethanoid glycosides affect MPP^+-induced cell death or not. In our study, we investigated the effect of compounds **1** (acteoside), **5**, **6**, **8** and **9** on the MPP^+-induced cell death in mesencenphalic neurons and found that compounds **1** (acteoside), **5**, **6**, **8** and **9** improved cell viability and had the anti-cell death activity induced by 1-methyl-4-phenylpyridinium ion (MPP^+) in the primary cultured mesencenphalic neurons.

Results and Discussion

The powders of *Buddleia lindleyana* were extracted with 95% EtOH. The extract was suspended in water and extracted successively with petroleum ether, EtOAc, *n*-BuOH. The *n*-BuOH soluble part was separated by a combination of D_{101} macroporous resin, silica gel, RP-18 silica gel and Sephadex LH-20 column chromatographies to obtain compounds **1** – **9**.

Compound **9**, a pale-yellow amorphous powder, has the molecular formula $C_{44}H_{52}O_{22}$ as revealed by HR-FAB-MS *m/z*: 955.2857 $[M+Na]^+$. The ^1H NMR spectrum of **9** exhibited three sets of aromatic protons ascribable to the ABX systems, two sets of *trans*-olefin protons, along with a methoxy group, and the signals

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No.	Compound **9** δ_c	Compound **9** δ_H	Angoroside C δ_c	Angoroside A δ_c
Fer. 1	127.7		127.7	
2	111.9	7.07 (d, 2.0)	111.9	
3	149.8		149.5	
4	150.7		151.0	
5	117.2	6.81 (d, 8.0)	116.6	
6	124.0	6.96 (dd, 8.0, 2.0)	124.5	
7	148.1	7.60 (d, 16.0)	148.2	
8	115.6	6.43 (d, 16.0)	115.1	
9	168.5		168.4	
OCH_3	56.5	3.8 (s)	56.5	
Caf. 1	127.7			127.7
2	115.3	7.06 (d, 2.0)		114.7
3	146.8			146.9
4	149.4			149.9
5	116.3	6.79 (d, 8.0)		116.4
6	123.3	6.92 (dd, 8.0,2.0)	123.3	
7	147.0	7.58 (d, 16.0)		148.3
8	114.7	6.30 (d, 16.0)		115.3
9	168.1			168.4
Agy.1	131.8		133.0	131.6
2	116.6	6.69 (d, 2.0)	112.9	116.6
3	146.1		147.6	146.3
4	144.6		147.4	144.7
5	116.5	6.67 (d, 8.0)	117.2	117.2
6	121.3	6.53 (dd, 8.0, 2.0)	121.3	121.4
7	36.4	2.68 (t)	36.7	36.7
8	71.9	3.98, 3.70 (m)	72.3	72.4
OCH_3			56.5	
glc1	104.0	4.31 (d, 8.0)	105.2	105.1
2	76.1		76.2	76.1
3	81.5		81.6	81.7
4	70.4	4.80	70.6	70.5
5	75.2		75.0	75.0
6	69.1		69.1	69.1
rha1	103.0	5.14 (br.s)	103.1	103.1
2	72.3		72.4	72.4
3	72.3		72.2	72.4
4	73.8		74.1	74.1
5	70.6		70.6	70.5
6	18.4	1.08 (d, 6.0)	18.5	18.6
arb1	102.8	4.51 (d, 7.0)	104.2	104.2
2	72.6	5.10 (t, 7.0)	72.5	72.1
3	72.4	3.73 (dd)	73.9	73.9
4	70.7	3.86 (br s)	69.6	69.6
5	66.7	3.87, 3.53	66.9	66.8

Table 1. The NMR data of compound **9** (CD_3OD).

Fer. = Feruloyl, Caf. = Cafferoyl, Agy. = Agylcone.

Table 2. The key HMBC data of compound **9** (CD_3OD).

Proton	^{1}H NMR	Carbon	^{13}C NMR
Glc 1-H	4.31	Aglycone 8-C	71.9
Rha 1-H	5.14	Glc 3-C	81.5
glc 4-H	4.80	Caffeoyl 9-C	168.1
Ara1-H	4.51	Glc 6-C	69.1
Feruloyl 2-H	7.60	Feruloyl 2-C	111.9
Ara 2-H	5.10	Feruloyl 9-C	168.5

Table 3. Protection effect of phenylethanoid glycosides on MPP^+-induced cell death.

Group	Concentration (μg·ml^{-1})	Optical density ($\bar{x} \pm$ SD)
Control		1.14 ± 0.02
MPP^+		0.78 ± 0.02
MPP^+ + EGF (100 ng/ml)		$0.87 \pm 0.02^*$
MPP^+ + compound **5**	40	$1.06 \pm 0.10^*$
MPP^+ + compound **1**	40	$1.40 \pm 0.06^*$
MPP^+ + compound **6**	40	$1.47 \pm 0.03^*$
MPP^+ + compound **8**	40	$1.41 \pm 0.02^*$
MPP^+ + compound **9**	40	$1.62 \pm 0.08^*$

Significance: $P < 0.05$ *vs* MPP^+ group; n = 3; EGF was used as the positive control.

of ethylol group (see Table 1). All these proton signals revealed the presences of a feruloyl moiety, a caffeoyl moiety and a 3, 4-dihydroxyphenylethyl moiety.

Acidic hydrolysis of **9** showed the presence of glucose, arabinose and rhamnose which were identified by co-TLC with authentic samples (see Experimental Section). The ^{13}C NMR spectrum gave three anomeric carbon signals at $\delta = 104.0$, 103.0, 102.8. In the ^{1}H NMR, three anomeric proton signals at δ4.31 (d, $J = 8.0$ Hz, 1H, 1-glc-H), 5.14 (br.s, 1H, 1-rha-H) and 4.51 (d, $J = 7.0$ Hz, 1H, 1-arab-H), and one methyl signal $\delta = 1.08$ (d, $J = 6.0$ Hz, 1H, CH_3-rha) were observed, which suggested that the glucose moiety had β-configuration and that the arabinose and rhamnose units had α-configurations. A comparison of the ^{13}C NMR data of **9** with those of angoroside A [8] indicated that the signals for the phenethyl moiety, caffeoyl moiety, β-D-glucose, α-L-rhamnose and arabinose were in good agreement, except that two upfield shifts were observed for C-1 (-1.4 ppm) and C-3 (-1.5 ppm) of arabinose in **9**. One extra group of signals due to the feruloyl moiety appeared in **9** (see Table 1). Furthermore the ^{13}C NMR data of the feruloyl moiety were also consistent with those of angoroside C [9]. Connection of the feruloyl moiety was determined by HMBC experiments, which revealed the cross peaks (see Table 2 and Fig. 1). Thus the structure of **9** was established as 2-(3, 4-dihydroxyphenyl)-ethyl-*O*-[α-L-rhamnopyranosyl-($1 \rightarrow 3$)-4'-*O*-cafferoyl-(2"-*O*-feruloyl)-*O*-α-L-arabinopyranosyl-($1 \rightarrow 6$)]-O-β-D-glucopyranoside (see Fig. 1). MALDI-TOF-MS gave the quasi molecular ion peaks at *m/z* 955 $[M+Na]^+$ and 971 $[M+K]^+$,

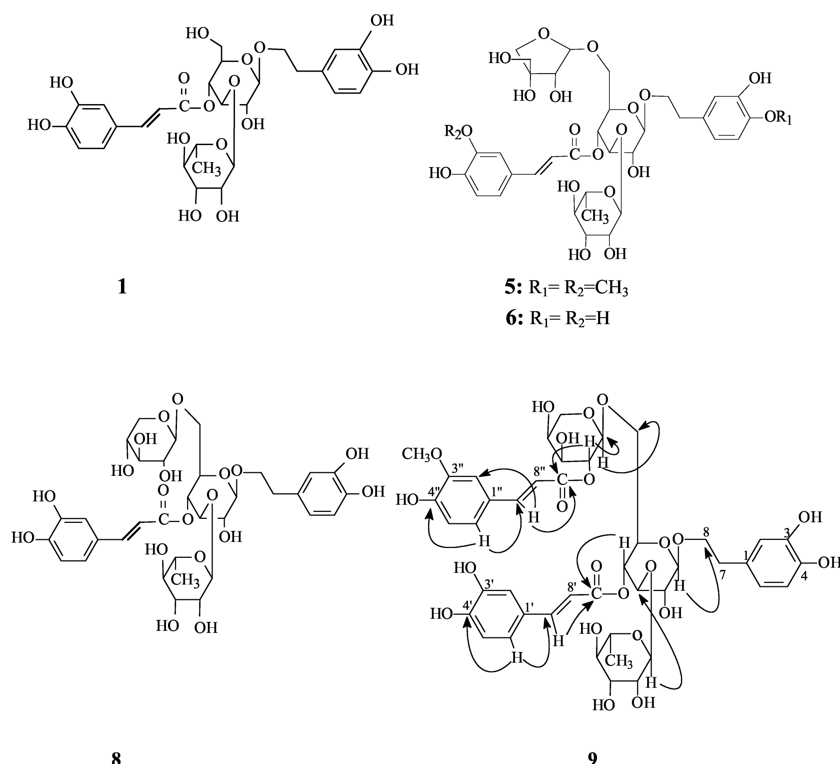

Fig. 1. The structures of **1**, **5**, **6**, **8**, **9** and key correlations of **9** in the HMBC spectrum.

supporting the structure of **9**. To our knowledge, it is a new compound named buddleoside A.

The structures of the known compounds (**1**–**8**) were identified on the basis of physical and chemical properties, spectral analyses, and comparison with the literature [10–14]. In order to investigate the biological activity of phenylethanoid glycosides obtained from *Buddleia lindleyana*, the MPP^+-induced neurotoxicity in dopaminergic neurons had been employed as an etiology of PD(Parkinson's disease) [15] for screening neuro-protective agents. An apoptotic model induced by MPP^+ in the cultured mesencephalic neurons of the ED14-16 rat was established by the method used by Mochizuki [5]. According to this test, five phenylethanoid glycosides exhibit remarkable neuroprotective effects (see Table 3), Among those compounds, compound **9** prevents MPP^+-induced cell death most potently.

Experimental Section

General experimental procedures

Melting points were determined on an X4 apparatus and are uncorrected. Optical rotations were taken with an AA-10R Automatic Polarimeter (Optical Activity Ltd.). ^{1}H and ^{13}C NMR, 1D-TOCSY, H-H COSY, HSQC and HMBC were recorded with a Bruker AM-500 instrument. HR-FAB-MS was taken on a KYKY-ZHP-5# spectrometer. MALDI-TOF-MS was taken on a BIFLEX-II spectrometer. Silica gel was purchased from Marine Chemical Factory in Qingdao. Sephadex LH-20 and Rp-18 (Chemical Reagent Factory, Tianjin) were used.

Plant material

The plant was collected in Jingzhai, Guangxi Autonomous Region, China, and identified by Prof. Hubiao Chen, Department of Natural Medicines, Peking University, and a specimen was deposited in the Department of Natural Medicines, Peking University.

Extraction and isolation

The material (13.5 kg) was extracted with 95% EtOH. The extract was suspended in water and partitioned with petroleum ether, EtOAc and *n*-BuOH. The EtOAc soluble extract (57 g) was separated by column chromatography on silica gel eluted with $CHCl_3$ –Me_2CO (99: 1 → 20: 80) to afford 26 fractions. Fr. 25 was fractionated on silica gel column chromatography eluted with $CHCl_3$–MeOH (5 : 1) to give 12 subfractions. Subfr. 10 was chromatographed over Sephadex LH–20 with 80% MeOH-H_2O as eluent to yield

compound **1** (40 mg). The *n*-BuOH souble extract (300 g) was fractionated by CC on silica gel, using $CHCl_3$-MeOH-H_2O (65 : 35 : 10, lower layer) as eluent to give 14 subfractions. Subfr. 1 was chromatographed on a Rp-18 silica gel column (30% MeOH–H_2O) to yield compound **2** (30 mg). Fr. 3 – 5 were separated by the combination of Rp–18 silica gel column (30% MeOH–H_2O), Sephadex LH–20 with 50% MeOH–H_2O and PTLC [$CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer)] to afford compound **3** (30 mg), **4** (40 mg), **6** (70 mg), **8** (50 mg), **9** (50 mg), respectively. Subfr. 9 – 13 were also fractionated by repeating the above procedure to obtain compound **5** (300 mg), **7** (5 mg), respectively.

Acidic hydrolysis of compound **9** *on TLC plate*

A sample (1 mg) was dissolved in 1 ml of MeOH and loaded on a TLC plate. The plate was suspended over a solution of 6 ml of 10 N HCl at a temperature of 60 °C, for 30 min. After hydrolysis, HCl absorbed by the silica gel on the plate was evaporated. Then the plate was chromatographed using $CHCl_3$–MeOH–H_2O (6 : 4 : 1) as a development system and visualized by spraying with phenylamine–*ortho*–benzenedicarboxylic acid reagent, following by heating. This method was used for identifying the sugars by comparison with authentic samples.

Identification

Buddleoside **9**: a pale-yellow amorphous powder, M. p. 215 – 218 °C, $[\alpha]_D^{25}$ – 126.3 (MeOH, 0.07), HR-FAB-MS show a quasi molecular ion peak at *m/z*: 955.2857 $[M+Na]^+$, calcd. 955.2866. MALDI-TOF-MS also gave the ion peak at *m/z* 955 $[M+Na]^+$ and 971 $[M+K]^+$. ^1H and ^{13}C NMR data (500 MHz, CD_3OD) see Table 1.

Assay for anti-apoptosis activity of Buddleia lindleyana

Cell cultures were prepared according to the method used by Mochizuki [5]. The animals were equally divided into 4 group: a control group, an MPP^+ model group, a positive drug group and five phenylethanoid glycosides groups. The cell viability was determined using a modified MTT [16] assay. In brief, mesencephalic neurons were seeded in 96-well plates at a density of 1×10^4 cells per well. The cultures were grown for 4 days, and then medium was changed to that containing 40 μg/ml concentration of five phenylethanoid glycosides. After 6 h, 100 μm MPP^+ was added. After incubation for up to 48 h later, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg/ml in DMEM) was added to the 96-well plates and the cells were allowed to incubate for 4 h at 37 °C. After the medium had been removed, the cell and dye crystals were solubilized by adding 200 μl of DMSO, and the absorption was measured at 570 nm (540 nm as a reference) with a model 550 microplate reader (Bio-Rad). The absorption was larger and cell viability was higher. As a positive control, 100 ng/ml of epidermal growth factor (EGF) was used.

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[1] T. Yoshida, J. Nobuhara, N. Fujii, T. Okuda, Chem. Pharm. Bull. **26**, 2543 (1978).
[2] P. J. Houtghon, H. Hikino, Planta Med. **55**, 123 (1989).
[3] T. Kosaku, B. H. Jeffrey, G. W. Peter, Phytochemistry **32**, 421 (1993).
[4] T. Yoshida, J. Nobuhara, N. Fujii, T. Okuda, Chem. Pharm. Bull. **26**, 2535 (1978).
[5] H. Mochizuki, N. Nakamura, Y. Mizuno, Neurosci. Lett. **170**, 191 (1994).
[6] Xiaoping Pu, Zhihong Song, Yahyun Li, Pengfei Tu, Huinong Li, Planta. Med. **69**, 65 (2003).
[7] Guoqing Sheng, Xiaoping Pu, Lei Li, Penfei Tu, Changling Li, Planta. Med. **68**, 966 (2002).
[8] I. Calis, G. A. Gross, O. Sticher, Phytochemistry **26**, 2057 (1987).
[9] I. Calis, G. A. Gross, O. Sticker, Phytochemistry **27**, 1465 (1988).
[10] H. Kobayashi, H. Oguchi, N. Takizawa, M. Toshio, A. Veno, Chem. Pharm. Bull. **35**, 3309 (1987).
[11] H. Kobayashi, H. Karasawa, T. Migase, S. Fukushima, Chem. Pharm. Bull. **32**, 3009 (1984).
[12] M. Toshi, A. Koizumi, A. Ueno, T. Noro, M. Kuroyanagi, S. Furushima, Chem. Pharm. Bull. **30**, 2732 (1982).
[13] Liu Zimin, Jia Zhongjian, Phytochemistry **30**, 1341 (1991).
[14] C. Andary, G. Privat, J. Nat. Prod. **48**, 778 (1985).
[15] J. W. Langston, P. Ballard, J. W. Tetrud, I. Irwin, Science **219**, 979 (1983).
[16] T. Yamamoto, K. Yuyama, K. T. Nakamura, Eur. J. Pharmacol. **397**, 25 (2000).