Phytochemistry 69 (2008) 2200–2204

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Chemical constituents of *Aeschynanthus bracteatus* and their weak anti-inflammatory activities

Su-Mei Li [a,b,c], Xian-Wen Yang [b], Yun-Heng Shen [b], Lin Feng [d], Yue-Hu Wang [a], Hua-Wu Zeng [b], Xiao-Hua Liu [b], Jun-Mian Tian [b], Ya-Na Shi [a], Chun-Lin Long [a,e,*], Wei-Dong Zhang [b,*]

[a] Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Heilongtan, Kunming 650204, PR China
[b] Department of Natural Product Chemistry, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, PR China
[c] Graduate School of Chinese Academy of Sciences, Beijing 100039, PR China
[d] Department of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, PR China
[e] College of Life and Environment Sciences, Central University for Nationalities, Beijing 100081, PR China

ARTICLE INFO

Article history:
Received 9 November 2007
Received in revised form 2 April 2008
Available online 28 June 2008

Keywords:
Aeschynanthus bracteatus
Gesneriaceae
Phenylethanoids
Aeschynanthosides A–D
RAW 264.7 macrophages
Anti-inflammatory
NO

ABSTRACT

Chemical examination of the EtOAc extract from the aerial parts of *Aeschynanthus bracteatus* led to isolation of four phenylethanol glycosides, aeschynanthosides A–D (**1**–**4**), and 55 known constituents, including 8 phenylethanoids, 23 phenols, 5 lignans, 7 flavonoids, 9 terpenoids, and 4 others. Their structures were elucidated mainly by detailed spectroscopic studies and comparison with published data. All 59 compounds were isolated for the first time from an *Aeschynanthus* species. The isolates were also tested for inhibitory activities against LPS-induced NO production in RAW 264.7 macrophages. Aeschynanthoside D (**4**) and naringenin (**41**), within the concentration arrange tested (50–100 μg/mL), showed very weak dose-dependent effects with the inhibition rate of 24.2%, 35.4%, 66.0%, and 9.5%, 40.1%, 65.0%, respectively, relative to positive controls.

1. Introduction

The Yao people are the most extensively distributed ethnic group in the South of China. For thousands of years, they have been known for their significant resistance to disease and for their familiarity with medicinal herbs (Li and Long, 2000; Liu, 2002; Liu and Ding, 1995; Long and Li, 2004). Moreover, their medicinal baths are thought to play an important role on preventing and curing common diseases among Yao communities (Li et al., 2006). In 2005, we surveyed the herbal plants used for the medicinal baths by Red-headed Yao people in Jinping city of Yunnan Province, SW China (Li et al., 2006).

Aeschynanthus bracteatus Wall. ex A. DC. (Gesneriaceae) is an adnascent shrub growing on the stem of large trees in the tropical rain forest of Yunnan Province, China (Wang, 1990; Wu and Raven, 1998). It has long been used to treat rheumatoid arthritis and postpartum convalescence in medicinal baths (Li et al., 2006). Previous studies have showed that the EtOH extract of *A. bracteatus* possessed significant anti-inflammatory activities *in vitro*. Thus this research study was carried out to investigate systematically the chemical constituents of *A. bracteatus*, which led to the isolation of 4 new and 55 known compounds. This paper deals with the isolation and structure elucidation of new compounds and inhibitory activities of 13 compounds against LPS-induced NO production in macrophages.

2. Results

The EtOAc-soluble fraction of the EtOH extract of the whole plants of *A. bracteatus* was subjected to silica gel, RP-18, and Sephadex LH-20 column chromatographic purification, as well as repeated prep. TLC to yield 4 new phenylethanoids and 55 known compounds (Fig. 1).

Compound **1** had a molecular formula $C_{30}H_{36}O_{16}$, as evidenced by the positive HRESIMS at *m/z* 675.1889 $[M + Na]^+$. The IR spectrum indicated the presence of hydroxyl (3409 cm^{-1}), ester carbonyl (1707 cm^{-1}), and aromatic ring (1604, 1529, 1446 cm^{-1}) moieties. The ^1H and ^{13}C NMR spectroscopic data of **1** (Table 1) suggested the presence of four fragments, including a phenylethanol, a

* Corresponding authors. Address: Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Heilongtan, Kunming 650204, PR China. Tel.: +86 871 5223233; fax: +86 871 5216201 (C.-L. Long); tel./fax: +86 21 25070386. (W.-D. Zhang).
E-mail addresses: long@mail.kib.ac.cn (C.-L. Long), wdzhangy@hotmail.com (W.-D. Zhang).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.05.012

Chemical

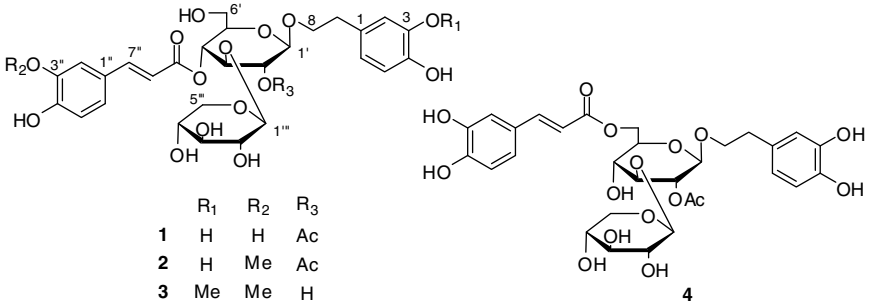


Fig. 1. Chemical structures of aeschynanthosides A-D (**1-4**).

Table 1
^{1}H and ^{13}C NMR spectroscopic data for aeschynanthosides A–D (**1**–**4**) in CD_3OD

No.	1		2		3		4	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1		131.7 *s*		131.8 *s*		131.6 *s*		131.7 *s*
2	6.66 (*d*, 1.8)	117.2 *d*	6.64 (*d*, 2.1)	117.2 *d*	6.68 (*d*. 1.8)	113.8 *d*	6.70 (*d*, 0.9)	117.2 *d*
3		145.9 *s*		146.0 *s*		148.8 *s*		145.9 *s*
4		144.4 *s*		144.5 *s*		145.8 *s*		144.5 *s*
5	6.69 (*d*, 8.1)	116.3 *d*	6.67 (*d*, 8.1)	116.3 *d*	6.81 (*d*, 8.1)	115.5 *d*	6.79 (*d*, 7.8)	116.3 *d*
6	6.52 (*dd*, 8.1, 1.8)	121.3 *d*	6.52 (*dd*, 8.1,2.1)	121.3 *d*	7.08 (*dd*, 8.1, 1.8)	122.4 *d*	6.49 (*dd*, 7.8, 0.9)	121.4 *d*
7	2.68 (*t*, 6.1)	36.3 *t*	2.69 (*t*, 6.3)	36.3 *t*	2.86 (*t*, 7.0)	36.7 *t*	2.67 (*t*, 6.5)	36.4 *t*
8	3.64 *m*; 4.05 (*dt*, 10.2, 6.1)	71.9 *t*	3.64 *m*; 4.07 (*dt*, 9.6, 6.3)	71.8 *t*	3.62 *m*; 4.07 (*dt*, 9.9, 7.0)	72.1 *t*	3.60 *m*; 3.97 (*dt*, 9.6, 6.5)	72.0 *t*
1′	4.53 (*d*, 8.4)	101.9 *d*	4.53 (*d*, 7.8)	101.9 *d*	4.52 (*d*, 7.5)	103.9 *d*	4.48 (*d*, 8.1)	102.1 *d*
2′	4.91 *m*	74.2 *d*	4.89 *m*	74.2 *d*	4.88 *m*	74.9 *d*	4.98 *m*	73.7 *d*
3′	3.98 (*t*, 9.6)	82.2 *d*	3.99 (*t*, 9.6)	82.2 *d*	3.80 (*t*, 9.3)	85.4 *d*	3.70 (*t*, 9.3)	84.8 *d*
4′	4.99 (*t*, 9.6)	70.6 *d*	4.99 (*t*, 9.3)	70.7 *d*	4.93 (*t*, 9.3)	70.9 *d*	3.47 (*t*, 9.3)	70.2 *d*
5′	3.58 *m*	75.8 *d*	3.58 *m*	75.9 *d*	3.60 *m*	75.9 *d*	3.59 *m*	75.1 *d*
6′	3.60 *m*; 3.68 (*d*, 12.0)	62.0 *t*	3.58 *m*, 3.69 (*d*, 10.2)	62.1 *t*	3.56 *m*, 3.64 *m*	62.4 *d*	4.34 *m*, 4.50 (*d*, 12.0)	64.4 *t*
1″		127.7 *s*		127.7 *s*		127.7 *s*		127.6 *s*
2″	7.08 (*d*, 1.8)	115.2 *d*	7.18 (*d*, 1.8)	111.7 *d*	7.20 (*d*, 1.8)	111.7 *d*	7.06 *s*	115.2 *d*
3″		146.8 *s*		149.4 *s*		149.4 *s*		146.8 *s*
4″		149.6 *s*		150.7 *s*		150.7 *s*		149.7 *s*
5″	6.80 (*d*, 8.4)	116.6 *d*	6.82 (*d*, 8.1)	116.6 *d*	6.73 (*d*, 7.8)	116.6 *d*	6.65 (*d*, 7.8)	116.6 *d*
6″	6.94 (*dd*, 8.4, 1.8)	123.0 *d*	7.07 (*dd*, 8.1, 1.8)	124.1 *d*	7.00 (*dd*, 7.8, 1.8)	124.1 *d*	6.90 (*d*, 7.8)	123.2 *d*
7″	7.56 (*d*, 15.9)	147.3 *d*	7.63 (*d*, 15.8)	147.1 *d*	7.64 (*d*, 15.9)	147.2 *d*	7.57 (*d* 15.9)	147.4 *d*
8″	6.25 (*d*, 15.9)	115.2 *d*	6.35 (*d*, 15.8)	115.5 *d*	6.67 (*d*, 15.9)	116.0 *d*	6.28 (*d* 15.9)	114.8 *d*
9″		168.4 *s*		168.3 *s*		168.4 *s*		169.1 *s*
1‴	4.21 (*d*, 7.2)	106.3 *d*	4.21 (*d*, 7.8)	106.4 *d*	4.40 (*d*, 7.8)	106.9 *d*	4.28 (*d*, 7.5)	105.8 *d*
2‴	3.03 (*t*, 8.1)	74.6 *d*	3.03 *m*	74.7 *d*	3.08 *m*	75.7 *d*	3.19 *m*	74.4 *d*
3‴	3.23 (*t*, 9.0)	77.6 *d*	3.20 (*t*, 9.0)	77.7 *d*	3.28 (*t*, 9.0)	77.5 *d*	3.32 *m*	77.6 *d*
4‴	3.31 *m*	71.0 *d*	3.30 *m*	71.0 *d*	3.32 *m*	71.0 *d*	3.51 *m*	70.9 *d*
5‴	3.11 (*d*, 10.8); 3.62 *m*	67.0 *t*	3.06 *m*; 3.62 *m*	67.0 *t*	3.08 *m*; 3.64 *m*	67.3 *t*	3.24 *m*; 3.90 (*d*, 9.9)	67.0 *t*
3-OMe					3.84 *s*	56.5 *q*		
3″-OMe			3.88 *s*	56.5 *q*	3.89 *s*	56.4 *q*		
2′-OAc		172.3 *s*		172.2 *s*				172.3 *s*
	1.93 *s*	21.2 *q*	1.98 *s*	21.2 *q*			1.91 *s*	21.2 *q*

[a] Recorded at 600 MHz, *J* in Hz within parenthesis.
[b] Recorded at 75 MHz.

caffeoyl, a glucose, and xylose unit. These data were very similar to those of conandroside (Jensen, 1996). In addition, the signals of an acetyl group were also observed [δ_H 1.93 (3H, s); δ_C 172.3 (s), 21.2 (q)]. Since the significant upfield shift of C-1′ from δ_C 103.7 to 101.9, C-2′ from δ_C 75.6 to 74.2 and C-3′ from δ_C 85.2 to 82.2, compared with conandroside, the acetyl group was presumed to be attached to the C-2′ position. This was confirmed by analysis of HMBC correlations from H-2′ at δ_H 4.91 of the glucosyl moiety to the ester carbonyl at C-2′ (δ_C 172.3) of the acetyl group (Fig. 2). Thus, compound **1** was determined as 2′-*O*-acetyl-conandroside, and named aeschynanthoside A.

Compound **2** was found to possess the molecular formula $C_{31}H_{38}O_{16}$ from the positive HRESIMS at *m/z* 689.2037 $[M + Na]^+$. Its physical and spectroscopic data were almost the same as those

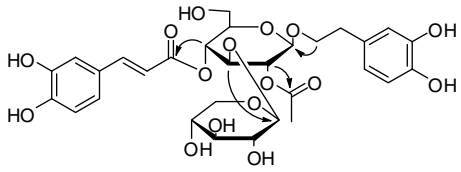


Fig. 2. Key HMBC correlations of aeschynanthoside A (**1**).

of **1**. Close comparison of the ^{1}H and ^{13}C NMR spectroscopic data of these two compounds indicated the following differences: a feruloyl group instead of a caffeoyl moiety was located at C-4′ in compound **2**. The HMBC correlations from 3″-OMe at δ_H 3.88 to C-3″ (δ_C

149.4) and the H-6″ at δ_H 7.07 to C-4″ (δ_C 150.7), but not the C-3″of the feruloyl group, also supported this conclusion. Therefore, compound **2** was assigned as 2′-*O*-acetyl-3″-*O*-methyl-conandroside, and named aeschynanthoside B.

Compound **3** had a molecular formula of $C_{30}H_{36}O_{16}$ as determined from the negative HRESIMS at *m/z* 637.2136 $[M-H]^-$. Comparison of **3** with **2** showed they had almost the same physical and NMR spectroscopic data. However, differences were found in the ^{13}C NMR spectrum: another methoxy signal at δ_C 56.5 (q) was evident, while there was no acetyl group compared to **2**. The HMBC correlations from 3-OMe at δ_H 3.84 to C-3 (δ_C 148.8) and H-6 at δ_H 7.08 to C-4 (δ_C 145.8), but not C-3 of an homovanillyl moiety, indicated that compound **3** was 3,3″-*O*-methyl-conandroside, and named aeschynanthoside C.

Compound **4** had the same molecular formula $C_{30}H_{36}O_{16}$ as **1**, as shown by the negative HRESIMS at *m/z* 651.1926 $[M-H]^-$, Moreover, its UV, IR, ^1H, and ^{13}C NMR spectroscopic data were also almost the same as **1**. However, comparison of the ^{13}C NMR spectrum of these two compounds showed that there were small different shifts: downfield from 62.0 ppm in **1** to 64.4 ppm in **4** at C-6′, which implied that the caffeoyl moiety should be located at C-6′ in **4** instead of C-4′ in **1**. This was confirmed by the HMBC correlations of H_2-6′ (δ_H 4.34, 4.50; each for 1H) to C-9″ (δ_C 169.1). Based on the above evidence, compound **4** was deduced to be 2′-*O*-acetyl-3′-*O*-*β*-D-glucopyranosyl-calceolarioside B, and named aeschynanthoside D.

Nitric oxide (NO) is a relevant target of inflammatory process (Calixto et al., 2003). The inhibition of NO release may be considered as a therapeutic agent in the inflammatory diseases (Li et al., 2006). As such, thirteen compounds, representing five main types of compounds in relatively high amounts, were selected to test the inhibitory abilities against LPS-induced NO production in RAW264.7 macrophages. Macrophages released low levels of NO (1.12 ± 0.28 μM). However, it increased up to 6.35 ± 0.18 μM after stimulated by LPS for 18 h. Aminoguanidine, a known positive control for nitric oxide synthase (NOS) inhibitor, reduced significantly the NO production (50.4% at 25 μM). Among the 13 tested isolates in the concentration range of 50, 75, and 100 μg/mL, aeschynantoside D (**4**) and naringenin (**41**), exhibited inhibitory activities against the increase of NO production with the inhibition rates of 24.2%, 35.4%, 66.2%, and 9.5%, 40.1%, 65.3%, respectively (Table 2).

Table 2
Effect of 13 compounds against lipopolysaccharide (LPS)-induced NO production in RAW264.7 macrophages ($n = 4$, means ± SD)

Groups	Dose (μg/mL)	NO (μM)	Inhibition rate (%)
Cells alone		1.12 ± 0.28	
LPS[a]	1	6.35 ± 0.18	100
Aminoguanidine[b]	3.4	3.15 ± 0.25[d]	50.4
Aeschynanthoside D (**4**)	100	2.15 ± 0.34[d]	66.2
	75	4.10 ± 0.32[d]	35.4
	50	4.81 ± 0.39[d]	24.2
Naringenin (**41**)	100	2.20 ± 0.33[d]	65.3
	75	3.80 ± 0.21[d]	40.1
	50	5.75 ± 0.53	9.5
Vanillin (**17**)	100	4.74 ± 0.55[d]	25.4
	75	5.41 ± 0.20[d]	14.8
	50	7.98 ± 0.42	NA[e]
Calceolarioside B (**7**)	100	5.40 ± 0.30[d]	14.9
	75	6.51 ± 0.22	NA[e]
	50	8.38 ± 0.73	NA[e]
Calceolarioside E (**8**)	100	5.68 ± 0.18[d]	10.5
	75	6.82 ± 0.06	NA[e]
	50	7.44 ± 0.19	NA[e]
Ocs[c]	100	–	NA[e]
	75	–	NA[e]
	50	–	NA[e]

[a] Negative control.
[b] Positive control.
[c] Other compounds, including aeschynanthoside A (**1**), aeschynanthoside B (**2**), cinnamic acid (**34**), dehydrodiconiferyl alcohol 9′-glucopyranoside (**39**), hemiphloin (**43**), ormocarpin (**47**), nigaichigoside F1 (**54**), and suavissimoside F1 (**56**).
[d] Statistical significance from LPS at $p < 0.01$.
[e] No activity observed.

3. Conclusions

Fifty-nine compounds were the first report on chemical constituents from the plants of *Aeschynanthus* genus. The anti-inflammatory activities of some compounds isolated from *A. bracteatus* were coincident with the traditional usages of this plant to treat rheumatoid arthritis. The anti-inflammatory activities were partially owed to the inhibitory effects of aeschynanthoside D (**4**) and naringenin (**41**) on NO production.

4. Experimental

4.1. General experimental procedures

NMR spectra were recorded on a Bruker Avance 600 or Avance 300 NMR spectrometer in CD_3OD with TMS as internal standard. ESIMS were acquired on an Agilent LC/MSD Trap XCT mass spectrometer, whereas HRESIMS were measured using a Q-TOF micro mass spectrometer (Waters, USA). Optical rotations were recorded using a Perkin–Elmer 341 polarimeter, whereas UV spectra were obtained by Shimadzu UV-2550 UV–vis spectrophotometer. IR spectra were recorded on a Bruker Vector 22 spectrometer spectrometer with KBr pellets. Materials for CC were silica gel (100–200 mesh; Huiyou Silical Gel Development Co. Ltd. Yantai, PR China), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-GEL ODS-A (50 μm; YMC, MA, USA). Prep. TLC (0.4–0.5 mm) was conducted with glass precoated silica gel GF_{254} (Yantai). Compounds were visualized by exposure to UV at 254 nm.

4.2. Plant material

The aerial part of *A. bracteatus* was collected from Jinping County, Yunnan Province, China, and identified by Prof. Heng Li (Department of Biogeography and Biodiversity, Kunming Institute of Botany). A voucher specimen (No. 99007) is deposited at the Herbarium of Kunming Institute of Botany.

4.3. Extraction and isolation

The dried and powdered aerial part of *A. bracteatus* (6.5 kg) was extracted with 95% EtOH for 3 × 2 h under room temperature. The combined extracts were concentrated to a small volume *in vacuo* and then partitioned with petroleum ether and EtOAc successively. The EtOAc extract (80 g) was subjected to silica gel CC eluted with a $CHCl_3$-MeOH gradient (100–0%) to give 6 fractions (Fr.1–Fr.6). Fraction Fr.6 was subjected repeatedly to Sephadex LH-20 and ODS CC. Final purification afforded **1** (108.1 mg), **3** (1.2 mg), and **4** (82.7 mg) by repeated prep. TLC (EtOAc–MeOH–H_2O: 20.0–3.4–2.7), and **2** (9.6 mg) by prep. TLC ($CHCH_3$–MeOH–H_2O: 7.0–3.0–0.5). Other 55 known compounds were obtained from all of the 6 fractions, they were 8 phenylethanoids: calceolarioside A (**5**, 22.3 mg) (Nicoletti et al., 1986), calceolarioside B (**6**, 71.8 mg) (Nicoletti et al., 1986), calceolarioside E (**7**, 184.8 mg) (Damtoft et al., 1993), 3,4-dihydroxyphenethyl glucoside (**8**, 6.0 mg) (Greca

et al., 1998), 3,4-dihydroxyphenylethanol-8-*O*-[β-D-apiofuranosyl-(1-2)]-β-D-glucopyranoside (**9**, 25.4 mg) (Zheng et al., 2004), verbascoside (**10**, 7.8 mg) (Andary et al., 1982), isonuomioside A (**11**, 109.2 mg) (Kasai et al., 1991), sanangoside (**12**, 18.1 mg) (Jensen, 1996); 23 phenols: koaburaside (**13**, 9.0 mg) (Chung et al., 1997), coniferyl alcohol (**14**, 16.8 mg), sinapinaldehyde (**15**, 5.0 mg) (Yahara et al., 1991), (*R*)-2-hydroxy-1(4-hydroxy-3-methoxyphenyl)propan-1-one (**16**, 15.0 mg) (Demir et al., 2002), vanillin (**17**, 62.5 mg), syringaldehyde (**18**, 6.0 mg) (Yang, 2006), coniferaldehyde (**19**, 6.0 mg) (Sy and Brown, 1999), tyrosal (**20**, 8.1 mg), l-(3,4-dimethoxyphenyl)-1,3-propanediol (**21**, 4.3 mg) (Pearl and Gratzl, 1962), 3,4′-dihydroxypropiophenone (**22**, 4.0 mg), 1-(2-hydroxy-5-methoxyphenyl)-1,2-propanediol (**23**, 8.5 mg), *C*-veratroylglycol (**24**, 21.5 mg) (Kijjoa et al., 1995), 2,4′-dihydroxyacetophenone (**25**, 11.0 mg), piceol (**26**, 11.0 mg) (Yang, 2006), 2-methoxy-4-(3-methoxy-l-propenyl)-phenol (**27**, 4.0 mg) (Naito et al., 1992), syringenin (**28**, 2.8 mg), 1,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)propan-2-one (**29**, 5.7 mg), ω-hydroxypropioguaiacone (**30**, 3.3 mg) (Yang, 2006), veratric acid (**31**, 8.4 mg), ferulic acid (**32**, 25.6 mg) (Yang, 2006), ethylparaben (**33**, 6.0 mg), cinnamic acid (**34**, 38.5 mg), 4-hydroxybenzoic acid (**35**, 13.6 mg) (Yang, 2006); 5 lignans: icariol A2 (**36**, 10.0 mg) (Yamauchi et al., 2007), balanophonin (**37**, 35.3 mg) (Haruna et al., 1982), guaiacylglycerol-β-ferulic acid ether (**38**, 2.9 mg) (Ichikawa et al., 2003), (7S, 8R) dehydrodiconiferyl alcohol 9′-glucopyranoside (**39**, 17.1 mg) (Jiang et al., 2001), alaschanioside C (**40**, 4.1 mg) (Gao and Jia, 1995); 7 flavonoids: naringenin (**41**, 24.0 mg) (Wilcox et al., 1999), evofolin B (**42**, 5.4 mg) (Wu et al., 1995), hemiphloin (**43**, 117.6 mg) (Lorente et al., 1982), prunin (**44**, 26.4 mg), corymboside (**45**, 18.3 mg) (Besson et al., 1979), pyrroside B (**46**, 10.2 mg) (Kazuyuki et al., 1988), ormocarpin (**47**, 25.8 mg) (Nyandat et al., 1990); 9 terpenoids: grasshopper ketone (**48**, 6.2 mg) (Meinwald et al., 1968), 9-hydroxylinalool (**49**, 17.1 mg), 4-epipinfaensin (**50**, 4.6 mg) (Terreaux et al., 1996), paradrymonoside (**51**, 6.6 mg) (Terreaux et al., 1996), epidihydrophaseic acid (**52**, 4.5 mg) (Champavier et al., 1999), trachelosperogenin A1 (**53**, 7.0 mg) (Abe and Yamauchi, 1987), nigaichigoside F1 (**54**, 89.1 mg) (Seto et al., 1984), nigaichigoside F2 (**55**, 8.3 mg) (Seto et al., 1984), suavissimoside F1 (**56**, 70.7 mg) (Abe and Yamauchi, 1987); and 3 others: 7-octene-1,6-diol (**57**, 5.1 mg) (Kanchanapoom et al., 2001), dianthoside (**58**, 7.8 mg) (Looker and Fisher, 1985), 6′-*O*-β-D-apiofuranosyl-dianthoside (**59**, 10.5 mg). Detailed procedures for extraction and isolation of all these 59 compounds see Scheme 1.

4.4. Aeschynanthoside A (*1*)

Brown gum; $[\alpha]_D^{19}$ −55.2 (*c* 0.50, CH_3OH); UV λ_{max}^{Meoh} nm (log ε): 213 (4.32), 232 (4.04), 247 (3.94), 292 (3.98), 329 (4.06); IR ν_{max}^{KBr} cm^{-1}: 3408, 2928, 1743, 1707, 1604, 1529, 1446, 1409, 1373, 1247, 1119, 1043, 815, 596 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 4.2; ESIMS (positive) *m/z* 675 $[M + Na]^+$; HRESIMS (positive) *m/z* 675.1889 $[M + Na]^+$ (calcd. for $C_{30}H_{36}O_{16}Na$, 675.1901).

4.5. Aeschynanthoside B (*2*)

Brown gum; $[\alpha]_D^{19}$ −49.4 (*c* 0.50, CH_3OH); UV λ_{max}^{Meoh} nm (log ε): 218 (4.34), 234 (4.15), 248 (3.98), 294 (4.07), 325 (4.19); IR ν_{max}^{KBr} cm^{-1}: 3408, 2931, 1707, 1598, 1516, 1374, 1352, 1248, 1158, 1125, 1042, 817, 671 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 4.2; ESIMS (positive) *m/z* 689 $[M + Na]^+$; HRESIMS (positive) *m/z* 689.2037 $[M + Na]^+$ (calcd. for $C_{31}H_{38}O_{16}Na$, 689.2058).

4.6. Aeschynanthoside C (*3*)

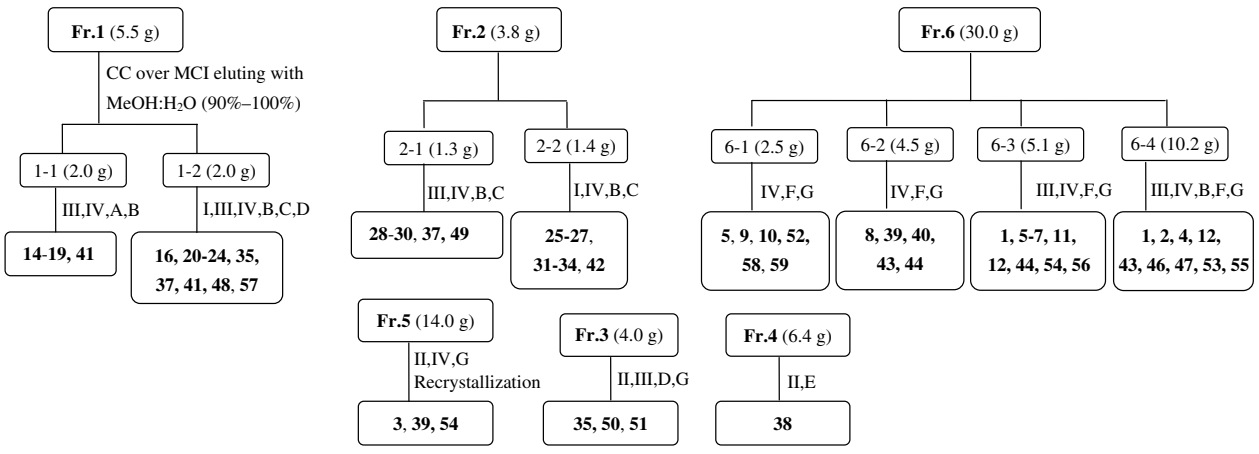
Brown gum; $[\alpha]_D^{19}$ −16.0 (c 0.50, CH3OH); UV λ_{max}^{Meoh} nm (log ε): 212 (4.34), 237 (4.19), 244 (3.92), 293 (4.05), 323 (4.16); IR ν_{max}^{KBr} cm^{-1}: 3407, 2933, 1705, 1602, 1519, 1371, 1359, 1245, 1153, 1129, 1041, 819, 677 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESIMS (positive) *m/z* 661 $[M + Na]^+$; HRESIMS (negative) *m/z* 637.2136 $[M - H]^-$ (calcd. for $C_{30}H_{37}O_{15}$, 637.2132).

4.7. Aeschynanthoside D (*4*)

Brown gum; $[\alpha]_D^{19}$ −15.9 (*c* 0.50, CH_3OH); UV λ_{max}^{Meoh} nm (log ε): 216 (4.21), 230 (4.07), 251 (3.95), 299 (4.11), 327 (4.20); IR ν_{max}^{KBr} cm^{-1}: 3411, 2941, 1710, 1612, 1510, 1370, 1347, 1240, 1163, 1120, 1047, 823, 668 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESIMS (positive) *m/z* 675 $[M + Na]^+$; HRESIMS (negative) *m/z* 651.1926 $[M - H]^-$ (calcd. for $C_{30}H_{35}O_{16}$, 651.1925).

4.8. Assay for inhibitory ability against LPS-induced NO production in RAW 264.7 macrophages

RAW 264.7 macrophages were seeded in 24-well plates (10^5 cells/well). The cells were co-incubated with drugs and LPS (1 μg/ml) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW 264.7 macrophage



Scheme 1. Flow chart for the isolation of chemical constituents from *Aeschynanthus bracteatus*. [I: CC over ODS eluted with gradient MeOH–H_2O (5–90%); II: CC over ODS eluted with gradient MeOH-H_2O (0–100%); III: CC over LH_{20} eluted with $CHCl_3$-MeOH (1:1); IV: CC over LH_{20} eluted with MeOH; A: prep.TLC ($CHCl_3$–MeOH, 50:1); B: prep.TLC (petrolum ether–EtOAc, 1:1); C: prep.TLC ($CHCl_3$–MeOH, 20:1); D: prep.TLC (EtOAc–MeOH, 50:1); E: prep.TLC ($CHCl_3$–MeOH, 5:1); F: prep.TLC ($CHCl_3$–MeOH–H_2O, 7.0:3.0:0.5); G: prep.TLC (EtOAc–MeOH–H_2O, 20.0:3.4:2.7)].

supernatants with Griess reagent. Aliquots of supernatants (100 μL) were incubated, in sequence, with 50 μL 1% sulphanilamide and 50 μL 0.1% naphthyl ethylene diamine in 2.5% phosphoric acid solution. The absorbance at 570 nm was read using a microtiter plate reader. Results are expressed as means ± SD. Statistical analysis was performed using Student's *t*-test, and $p < 0.05$ was considered significant.

Acknowledgements

We acknowledge the Department of Instrumental Analysis of the Second Military Medical University for measuring UV, IR, NMR spectra, and the Shanghai Institute of Organic Chemistry for measuring the optical rotations. We wish to thank Guangwan Hu and Lvliu Zou who helped in collection of plant material. Thanks are also expressed to Professor Heng Li, a botanist at Kunming Institute of Botany, who helped in identification of the plants collected from the fields. This research was supported by the Ministry of Science and Technology of China (Nos. 2004DKA30430, 2005DKA21006), and the Ministry of Education of China through its 111 Project (B08044, CUN 985-3-3).

References

Abe, F., Yamauchi, T., 1987. Trachelosperosides, glycosides of 19α-hydroxyursane-type triterpenoids from *Trachelospermum asiaticum* (Trachelospermum. III). Chem. Pharm. Bull. 35, 1748–1753.

Andary, C., Wylde, R., Laffite, C., Privat, G., Winternitz, F., 1982. Structures of verbascoside and orobanchoside, caffeic acid sugar esters from *Orobanche rapum-genistae*. Phytochemistry 21, 1123–1127.

Besson, E., Dombris, A., Raynaud, J., Chopin, J., 1979. Corymboside, nouvelle di-c-glycosylflavone des racines de *Carlina corymbosa*. Phytochemistry, 18.

Calixto, J.B., Otuki, M.F., Santos, A.R., 2003. Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor kappa B (NF-kappaB). Planta Med. 69, 973–983.

Champavier, Y., Comte, G., Vercauteren, J., Allais, D.P., Chulia, A.J., 1999. Norterpenoid and sesquiterpenoid glucosides from *Juniperus phoenicea* and *Galega officinalis*. Phytochemistry 50, 1219–1223.

Chung, M.I., Lai, M.H., Yen, M.H., Wu, R.R., Lin, C.N., 1997. Phenolics from *Hypericum geminiflorum*. Phytochemistry 44, 943–947.

Damtoft, S., Franzyk, H., Jensen, S.R., Nielsen, B.J., 1993. Iridoids and verbascosides in *Retzia*. Phytochemistry 34, 239–243.

Demir, A.S., Sesenoglu, O., Eren, E., Hosrik, B., Pohl, M., Janzen, E., Kolter, D., Feldmann, R., Dunkelmann, P., Muller, M., 2002. Enantioselective synthesis of α-hydroxy ketones via benzaldehyde lyase-catalyzed C–C bond formation reaction. Adv. Synth. Catal. 344, 96–103.

Gao, J.J., Jia, Z.J., 1995. Lignan, iridoid and phenylpropanoid glycosides from *Pedicularis alaschanica* Maxim (Scrophulariaceae). Indian J. Chem. 34B, 466–468.

Greca, M.D., Ferrara, M., Fiorentino, A., Monaco, P., Previtera, L., 1998. Antialgal compounds from *Zantedeschia aethiopica*. Phytochemistry 49, 1299–1304.

Haruna, M., Koube, T., Ito, K., Murata, H., 1982. Balanophonin, a new neo-lignan from *Balanophora japonica* Makino. Chem. Pharm. Bull. 30, 1525–1527.

Ichikawa, M., Ryu, K., Yoshida, J., Ide, N., Kodera, Y., Sasaoka, T., Rosen, R.T., 2003. Identification of six phenylpropanoids from garlic skin as major antioxidants. J Agric Food Chem 51, 7313–7317.

Jensen, S.R., 1996. Caffeoyl phenylethanoid glycosides in *Sanango racemosum* and in the Gesneriaceae. Phytochemistry 43, 777.

Jiang, Z.H., Tanaka, T., Sakamoto, M., Jiang, T., Kouno, I., 2001. Studies on a medicinal parasitic plant: lignans from the stems of *Cynomorium songaricum*. Chem. Pharm. Bull. 49, 1036–1038.

Kanchanapoom, T., Kasai, Ryoji., Picheansoonthon, C., Yamasaki, K., 2001. Megastigmane, aliphatic alcohol and benzoxazinoid glycosides from *Acanthus ebracteatus*. Phytochemistry 58, 811–817.

Kasai, R., Ogawa, K., Ohtani, K., Ding, J.K., Chen, P.Q., Pei, C.J., Tanaka, O., 1991. Phenolic glycosides from Nuo-Mi-Xang-Cao, a Chinese acanthaceous Herb. Chem. Pharm. Bull. 39, 927–929.

Kazuyuki, H., Toshiko, S., Yasuhisa, S., Nobutoshi, T., Takao, M., Chen, C.M., 1988. Chemical and chemotaxonomical studies of filices. LXXIV.: The novel flavanone glycosides of *Pyrrosia linearfolia* (Hook.) Ching. Yakugaku Zasshi 108, 417–421.

Kijjoa, A., Pinto, M.M.M., Anantachoke, C., Gedris, T.E., Herz, W., 1995. Dolabranes from *Endospermum diadenum*. Phytochemistry 40, 191–193.

Li, R., Long, C.L., 2000. Studies on Yao ethnomedicine in Jinping of Yunnan. Chin. Pharm. J. 35, 775–779.

Li, S.M., Long, C.L., Liu, F.Y., Lee, S.W., Guo, Q., Li, R., Liu, Y.H., 2006. Herbs for medicinal baths among the traditional Yao communities of China. J. Ethnopharmacol. 108, 59–67.

Liu, Y.H., 2002. Research of Yao Nationality Medicine in Hunan. Hunan Science & Technology Press, Changsha.

Liu, Y.H., Ding, F., 1995. Summarization of Yao nationality and medicine. Hunan Zhong Yi Yao Za Zhi (Hunan J. Tradition. Chin. Med.) 5, 24–25.

Long, C.L., Li, R., 2004. Ethnobotanical studies on medicinal plants used by the Red-headed Yao People in Jinping, Yunnan Province, China. J. Ethnopharmacol. 90, 389–395.

Looker, J.H., Fisher, M.S., 1985. Synthesis and characterization of the naturally occurring monocyclic γ-pyrone glucosides. J. Carbohydr. Chem. 4, 381–392.

Lorente, F.T., Ferreres, F., Barberan, F.A.T., 1982. 6-C-glucosylnaringenin from flowers of Acacia retinoide. Phytochemistry 21, 1461–1462.

Meinwald, J., Erickson, K., Hartshorn, M., Meinwald, Y.C., Eisner, T., 1968. Defensive mechanisms of arthropods. XXIII. An allenic sesquiterpenoid from the grasshopper *Romalea microptera*. Tetrahedron Lett. 25, 2959–2962.

Naito, T., Niitsu, K., Ikeya, Y., Okada, M., Mitsuhashi, 1992. A phthalide and 2-farnesyl-6-methyl benzoquinone from *Ligusticum chuangxiong*. Phytochemistry 31, 1787–1789.

Nicoletti, M., Galeffi, C., Messana, I., Garbarino, J.A., Gambaro, V., Nyandat, E., Marini-Bettolo, G.B., 1986. New phenylpropanoid glucosides from *Calceolaria hypericina*. Gazz. Chim. Ital. 116, 431–433.

Nyandat, E., Hassanali, A., Vicente, Y.D., Multari, G., Galeffi, C., 1990. The 7,7″-β-diglucoside of (2S,3R)-chamaejasmin from *Ormocarpum kirkii*. Phytochemistry 29, 2361–2364.

Pearl, I.A., Gratzl, J., 1962. Studies on lignin and related products. XVI. Synthesis of lignin model compounds of the phenylglycerol β-ether and related series. J. Org. Chem. 27, 2111–2114.

Seto, T., Tanaka, T., Tanaka, O., Naruhashi, N., 1984. β-Glucosyl esters of 19α-hydroxyursolic acid derivatives in leaves of *Rubus species*. Phytochemistry 23, 2829–2834.

Sy, L.K., Brown, G.D., 1999. Coniferaldehyde derivatives from tissue culture of *Artemisia annua* and *Tanacetum parthenium*. Phytochemistry 50, 781–785.

Terreaux, C., Maillard, M.P., Gupta, M.P., Hostetimann, K., 1996. Triterpenes and triterpene glycosides from *Paradrymonia macrophylla*. Phytochemistry 42, 495–499.

Wang, W.C., 1990. Flora Reipublicae Popularis Sinicae. Science Press, Beijing.

Wilcox, L.J., Borradaile, N.M., Huff, M.W., 1999. Antiatherogenic properties of naringenin, a citrus flavonoid. Cardiovasc. Drug Rev. 17, 160–178.

Wu, T.S., Yeh, J.H., Wu, P.L., 1995. The heart wood constituents of *Tetradium glabrifolium*. Phytochemistry 40, 121–124.

Wu, Z.Y., Raven, P., 1998. Flora of China. Science Press and Missouri Botanical Garden, Beijing and St. Louis.

Yahara, S., Nishiyori, T., Kohda, A., Nohara, T., Nishioka, I., 1991. Isolation and characterization of phenolic compounds from *Magnolia cortex* produced in China. Chem. Pharm. Bull. 39, 2024–2036.

Yamauchi, H., Kakuda, R., Yaoita, Y., Machida, K., Kikuchi, M., 2007. Two new glycosides from the whole plants of *Glechoma hederacea* L. Chem. Pharm. Bull. 55, 346–347.

Yang, X.W., 2006. Chemical constituents and neuroprotective activities of *Tarenna attenuata* and *Baccaurear ramiflora*. Ph.D dissertation. Kunming Institute of Botany, Chinese Academy of Sciences, Kunming.

Zheng, X.K., Liu, Y.B., Li, J., Feng, W.S., 2004. One new phenylethanoid glycoside from *Corallodiscus flabellata*. Acta Pharm. Sinica 39, 716–718.