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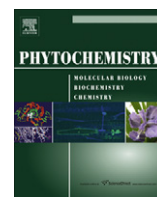
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## Phytochemistry

journal homepage: [www.elsevier.com/locate/phytochem](http://www.elsevier.com/locate/phytochem)Chemical constituents of *Aeschynanthus bracteatus* and their weak anti-inflammatory activitiesSu-Mei Li<sup>a,b,c</sup>, Xian-Wen Yang<sup>b</sup>, Yun-Heng Shen<sup>b</sup>, Lin Feng<sup>d</sup>, Yue-Hu Wang<sup>a</sup>, Hua-Wu Zeng<sup>b</sup>, Xiao-Hua Liu<sup>b</sup>, Jun-Mian Tian<sup>b</sup>, Ya-Na Shi<sup>a</sup>, Chun-Lin Long<sup>a,e,\*</sup>, Wei-Dong Zhang<sup>b,\*</sup><sup>a</sup> Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Heilongtan, Kunming 650204, PR China<sup>b</sup> Department of Natural Product Chemistry, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, PR China<sup>c</sup> Graduate School of Chinese Academy of Sciences, Beijing 100039, PR China<sup>d</sup> Department of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, PR China<sup>e</sup> 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 range 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.

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## 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 post-

partum 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<sub>30</sub>H<sub>36</sub>O<sub>16</sub>, as evidenced by the positive HRESIMS at *m/z* 675.1889 [M + Na]<sup>+</sup>. The IR spectrum indicated the presence of hydroxyl (3409 cm<sup>−1</sup>), ester carbonyl (1707 cm<sup>−1</sup>), and aromatic ring (1604, 1529, 1446 cm<sup>−1</sup>) moieties. The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of **1** (Table 1) suggested the presence of four fragments, including a phenylethanol, a

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## Chemical

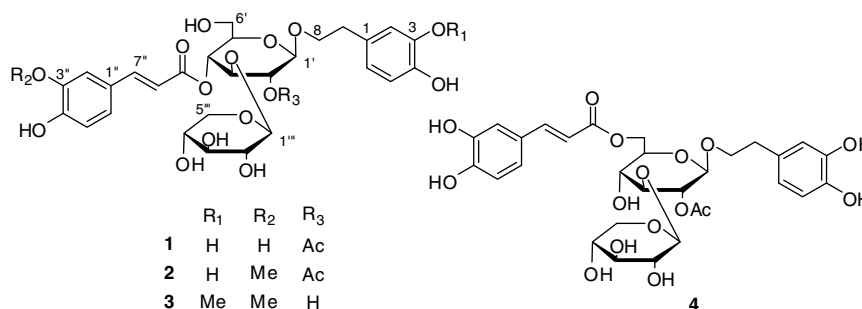


Fig. 1. Chemical structures of aescynanthosides A-D (1–4).

Table 1

<sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data for aescynanthosides A–D (1–4) in CD<sub>3</sub>OD

No.	1		2		3		4	
	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$
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

<sup>a</sup> Recorded at 600 MHz, *J* in Hz within parenthesis.<sup>b</sup> 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 [ $\delta_{\text{H}}$  1.93 (3H, s);  $\delta_{\text{C}}$  172.3 (s), 21.2 (q)]. Since the significant upfield shift of C-1' from  $\delta_{\text{C}}$  103.7 to 101.9, C-2' from  $\delta_{\text{C}}$  75.6 to 74.2 and C-3' from  $\delta_{\text{C}}$  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  $\delta_{\text{H}}$  4.91 of the glucosyl moiety to the ester carbonyl at C-2' ( $\delta_{\text{C}}$  172.3) of the acetyl group (Fig. 2). Thus, compound 1 was determined as 2'-O-acetyl-conandroside, and named aescynanthoside A.

Compound 2 was found to possess the molecular formula C<sub>31</sub>H<sub>38</sub>O<sub>16</sub> from the positive HRESIMS at *m/z* 689.2037 [M + Na]<sup>+</sup>. Its physical and spectroscopic data were almost the same as those

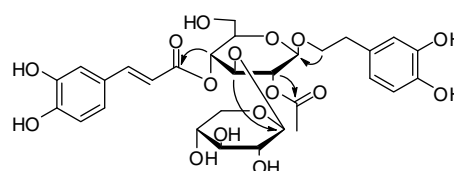


Fig. 2. Key HMBC correlations of aescynanthoside A (1).

of 1. Close comparison of the <sup>1</sup>H and <sup>13</sup>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  $\delta_{\text{H}}$  3.88 to C-3'' ( $\delta_{\text{C}}$

149.4) and the H-6'' at  $\delta_{\text{H}}$  7.07 to C-4'' ( $\delta_{\text{C}}$  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 aescynanthoside B.

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

Compound **4** had the same molecular formula  $\text{C}_{30}\text{H}_{36}\text{O}_{16}$  as **1**, as shown by the negative HRESIMS at  $m/z$  651.1926  $[\text{M} - \text{H}]^-$ . Moreover, its UV, IR,  $^1\text{H}$ , and  $^{13}\text{C}$  NMR spectroscopic data were also almost the same as **1**. However, comparison of the  $^{13}\text{C}$  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<sub>2</sub>-6' ( $\delta_{\text{H}}$  4.34, 4.50; each for 1H) to C-9'' ( $\delta_{\text{C}}$  169.1). Based on the above evidence, compound **4** was deduced to be 2'-O-acetyl-3'-O- $\beta$ -D-glucopyranosyl-calceolarioside B, and named aescynanthoside 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

( $1.12 \pm 0.28 \mu\text{M}$ ). However, it increased up to  $6.35 \pm 0.18 \mu\text{M}$  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  $25 \mu\text{M}$ ). Among the 13 tested isolates in the concentration range of 50, 75, and  $100 \mu\text{g/mL}$ , aescynanthoside 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).

### 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 aescynanthoside 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  $\text{CD}_3\text{OD}$  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  $\mu\text{m}$ ; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-GEL ODS-A (50  $\mu\text{m}$ ; YMC, MA, USA). Prep. TLC (0.4–0.5 mm) was conducted with glass precoated silica gel GF<sub>254</sub> (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  $3 \times 2$  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  $\text{CHCl}_3$ -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- $\text{H}_2\text{O}$ : 20.0–3.4–2.7), and **2** (9.6 mg) by prep. TLC ( $\text{CHCH}_3$ -MeOH- $\text{H}_2\text{O}$ : 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

**Table 2**

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

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

<sup>a</sup> Negative control.

<sup>b</sup> Positive control.

<sup>c</sup> Other compounds, including aescynanthoside A (**1**), aescynanthoside B (**2**), cinnamic acid (**34**), dehydroniconiferyl alcohol 9'-glucopyranoside (**39**), hemiphloin (**43**), ormocarpin (**47**), nigaichigoside F1 (**54**), and suavisissimoside F1 (**56**).

<sup>d</sup> Statistical significance from LPS at  $p < 0.01$ .

<sup>e</sup> No activity observed.



et al., 1998), 3,4-dihydroxyphenylethanol-8-O-[ $\beta$ -D-apiofuranosyl-(1-2)]- $\beta$ -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), 1-(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-1-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),  $\omega$ -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- $\beta$ -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), alaskanioside 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), pyrrorside 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), paradymonoside (**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- $\beta$ -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<sub>3</sub>OH); UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 213 (4.32), 232 (4.04), 247 (3.94), 292 (3.98), 329 (4.06); IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3408, 2928, 1743, 1707, 1604, 1529, 1446, 1409, 1373, 1247, 1119, 1043, 815, 596 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 4.2; ESIMS (positive) *m/z* 675 [M + Na]<sup>+</sup>; HRESIMS (positive) *m/z* 675.1889 [M + Na]<sup>+</sup> (calcd. for C<sub>30</sub>H<sub>36</sub>O<sub>16</sub>Na, 675.1901).

#### 4.5. Aeschynanthoside B (**2**)

Brown gum;  $[\alpha]_D^{19}$  -49.4 (c 0.50, CH<sub>3</sub>OH); UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 218 (4.34), 234 (4.15), 248 (3.98), 294 (4.07), 325 (4.19); IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3408, 2931, 1707, 1598, 1516, 1374, 1352, 1248, 1158, 1125, 1042, 817, 671 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 4.2; ESIMS (positive) *m/z* 689 [M + Na]<sup>+</sup>; HRESIMS (positive) *m/z* 689.2037 [M + Na]<sup>+</sup> (calcd. for C<sub>31</sub>H<sub>38</sub>O<sub>16</sub>Na, 689.2058).

#### 4.6. Aeschynanthoside C (**3**)

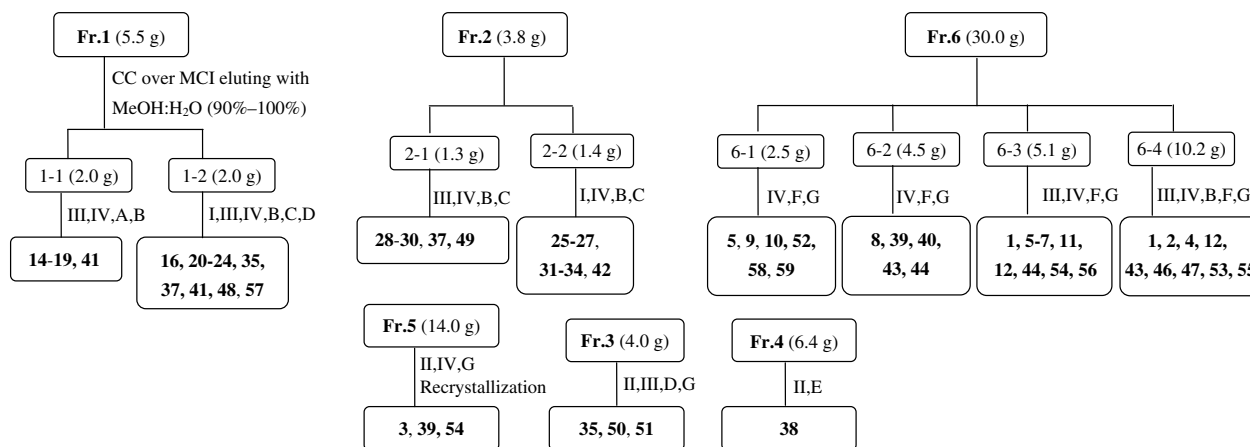
Brown gum;  $[\alpha]_D^{19}$  -16.0 (c 0.50, CH<sub>3</sub>OH); UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 212 (4.34), 237 (4.19), 244 (3.92), 293 (4.05), 323 (4.16); IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3407, 2933, 1705, 1602, 1519, 1371, 1359, 1245, 1153, 1129, 1041, 819, 677 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; ESIMS (positive) *m/z* 661 [M + Na]<sup>+</sup>; HRESIMS (negative) *m/z* 637.2136 [M - H]<sup>-</sup> (calcd. for C<sub>30</sub>H<sub>37</sub>O<sub>15</sub>, 637.2132).

#### 4.7. Aeschynanthoside D (**4**)

Brown gum;  $[\alpha]_D^{19}$  -15.9 (c 0.50, CH<sub>3</sub>OH); UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 216 (4.21), 230 (4.07), 251 (3.95), 299 (4.11), 327 (4.20); IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3411, 2941, 1710, 1612, 1510, 1370, 1347, 1240, 1163, 1120, 1047, 823, 668 cm<sup>-1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; ESIMS (positive) *m/z* 675 [M + Na]<sup>+</sup>; HRESIMS (negative) *m/z* 651.1926 [M - H]<sup>-</sup> (calcd. for C<sub>30</sub>H<sub>35</sub>O<sub>16</sub>, 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<sup>5</sup> cells/well). The cells were co-incubated with drugs and LPS (1  $\mu$ g/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<sub>2</sub>O (5–90%); II: CC over ODS eluted with gradient MeOH–H<sub>2</sub>O (0–100%); III: CC over LH<sub>20</sub> eluted with CHCl<sub>3</sub>–MeOH (1:1); IV: CC over LH<sub>20</sub> eluted with MeOH; A: prep.TLC (CHCl<sub>3</sub>–MeOH, 50:1); B: prep.TLC (petroleum ether–EtOAc, 1:1); C: prep.TLC (CHCl<sub>3</sub>–MeOH, 20:1); D: prep.TLC (EtOAc–MeOH, 50:1); E: prep.TLC (CHCl<sub>3</sub>–MeOH, 5:1); F: prep.TLC (CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O, 7.0:3.0:0.5); G: prep.TLC (EtOAc–MeOH–H<sub>2</sub>O, 20.0:3.4:2.7)].

supernatants with Griess reagent. Aliquots of supernatants (100  $\mu$ L) were incubated, in sequence, with 50  $\mu$ L 1% sulphanilamide and 50  $\mu$ L 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  $\pm$  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).

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