



## Chemical constituents from *Glochidion assamicum*



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### ABSTRACT

Phytochemical investigation of aerial parts of *Glochidion assamicum* Hook. f. led to the isolation of one new triterpenoid saponin, 3-O-[β-glucopyranosyl-(1→3)-α-arabinopyranosyl]-gymnestrogenin (assamicoside A, **7**), and ten known compounds (**1–6**, **8–11**). Their structures were identified by spectroscopic analysis and by comparison of their spectral data with those reported in the literature. The chemotaxonomic analysis is in agreement with previous work on the constituents of other *Glochidion* species. A spirostane-type C<sub>27</sub> steroid (**4**) isolated from *G. assamicum* for the first time can be used to differentiate this species from other *Glochidion*.

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## 1. Subject and source

Plants of the genus *Glochidion* (family Euphorbiaceae), widely distributed in China, are commonly used as folk medicines in the treatment of influenza, dysentery, impaludism, rheumatoid arthritis, and dyspepsia (Xiao et al., 2007). In recent years, some lupane-type triterpenes from species of *Glochidion* have been found to possess antitumour-promoting and cytotoxic activities (Xiao et al., 2007). *Glochidion assamicum* Hook. f. is a small tree growing mainly in Yunnan, Guizhou, Guangxi, Guangdong, Hainan and Taiwan Provinces of China. The aerial parts of this plant were collected from Xishuangbanna of Yunnan Province in March 2005. A voucher specimen of the plant (H20050313002) was identified by Prof. Deding Tao and deposited in the herbarium of the Kunming Institute of Botany, Yunnan, P.R. China. (Figs. 1 and 2)

## 2. Previous work

The genus *Glochidion* (Euphorbiaceae) consists of nearly 200 species of which 28 are in China (Li and Michael, 2008). Previously, a limited number of *Glochidion* species have been investigated phytochemically, indicating the occurrence of several types of phytochemicals, such as triterpenoids from *Glochidion hohenackeri* (Ganguly et al., 1966), *Glochidion wrightii* (Hui and Fung, 1969; Zhang et al., 2012), *Glochidion dasyphyllum*, *Glochidion zeylanicum*, and *Glochidion macrophyllum* (Hui et al., 1970), *Glochidion venulatum* (Ahmad and Zaman, 1973), *Glochidion eriocarpum* (Hui and Li, 1976; Kiem et al., 2009),

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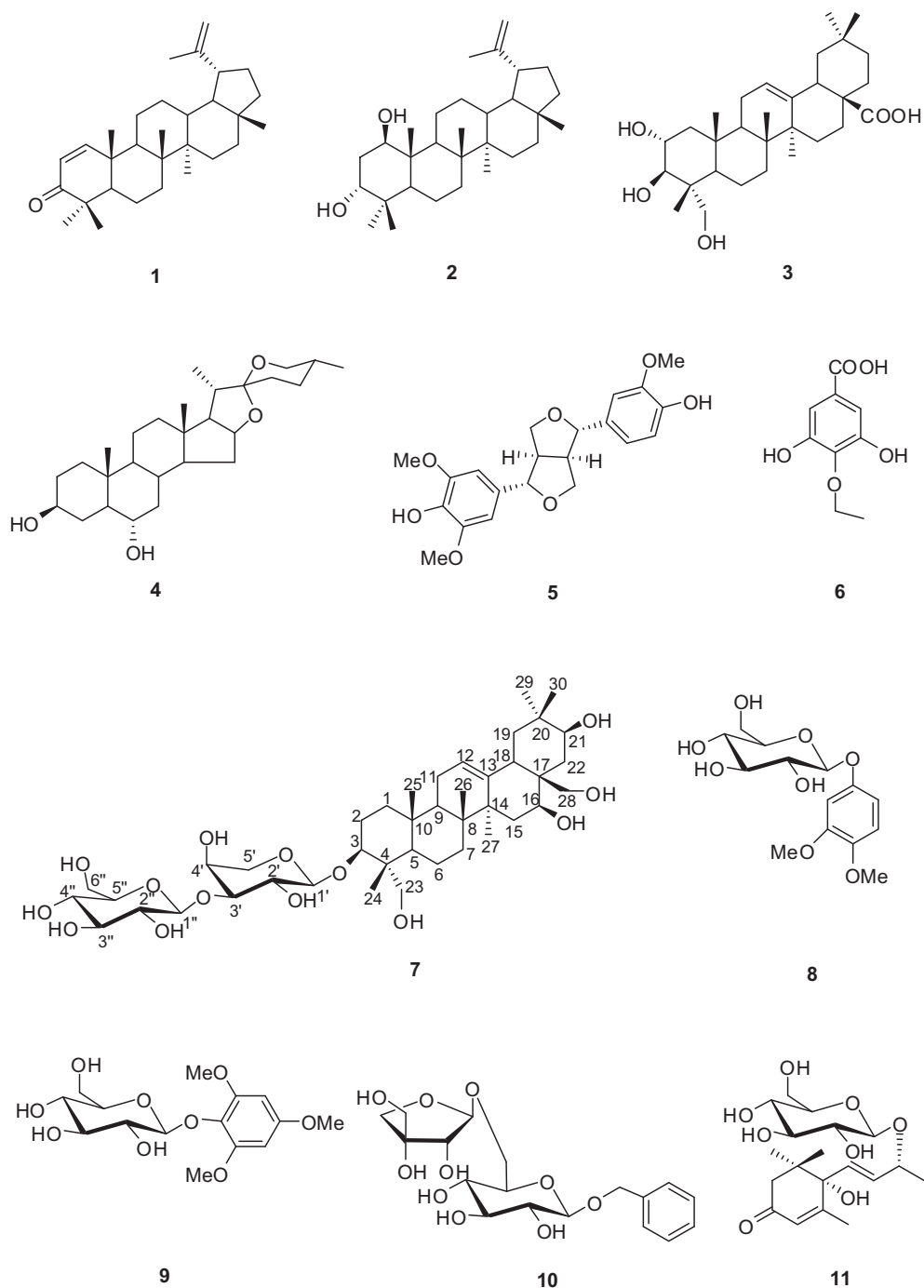


Fig. 1. Structures of compounds 1–11 isolated from *Glochidion assamicum*.

*Glochidion macrophyllum* (Hui and Li, 1978), *Glochidion puberum* (Hui and Li, 1978; Zhang et al., 2008, 2011), *Glochidion heyneanum* (Srivastava and Kulshreshtha, 1986, 1988), *Glochidion coccineum* (Xiao et al., 2008), and *G. assamicum* (Liu et al., 2011); megastigmane glycosides from *G. zeylanicum* (Otsuka et al., 2003a, 2003b); flavanol glycosides from *G. zeylanicum* (Otsuka et al., 2001), and *Glochidion obliquum* (Thang et al., 2011); lignans from *Glochidion obovatum* (Takeda et al., 1998), *G. zeylanicum* (Otsuka et al., 2000a), *Glochidion rubrum* (Cai et al., 2009) and *G. wrightii* (Zhang et al., 2012); bisabolane-type

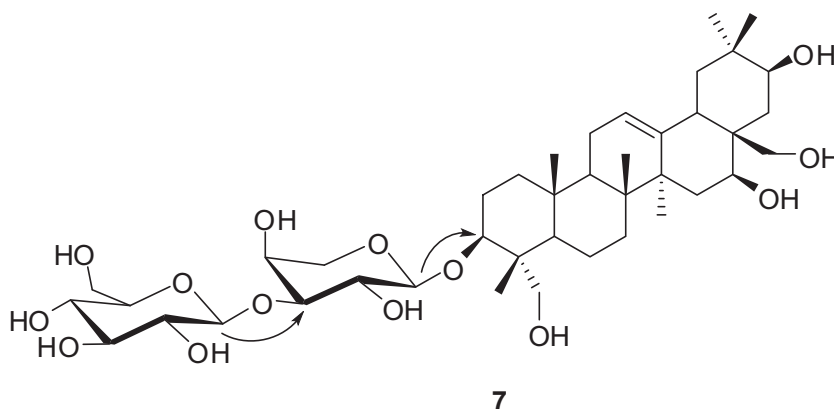


Fig. 2. Selected HMBC correlations of **7**.

sesquiterpenoids from *G. coccineum* (Xiao et al., 2007); steroids from *G. dasyphyllum*, *G. zeylanicum*, and *G. macrophyllum* (Hui et al., 1970), *G. venulatum* (Ahmad and Zaman, 1973), and *G. wrightii* (Zhang et al., 2012); and butenolides from *Glochidion acuminatum* (Otsuka et al., 1998, 2004) and *G. zeylanicum* (Otsuka et al., 2000b). Herein, we report the isolation and characterization of the chemical constituents including a new triterpenoid saponin (**7**) from the aerial parts of *G. assamicum*.

### 3. Present study

The air-dried and powdered aerial parts (14 kg) of *G. assamicum* were extracted with 95% ethanol for three times and each for 3 h. The extract was suspended in H<sub>2</sub>O (2.5 L) and then extracted with EtOAc for four times (each for 2.5 L). The EtOAc extract (200 g) was subjected to column chromatography (CC) over silica gel using gradient petroleum ether–EtOAc (10:0→1:1) to yield eleven fractions (Fr.1–Fr.11). Fr. 2 was repeated CC on silica gel to give glochidone (**1**, 847 mg) (Ganguly et al., 1966). Fr. 10 was repeated CC on silica gel and Sephadex LH-20 to give glochidiol (**2**, 9 mg) (Roshan et al., 1980), arjunolic acid (**3**, 9 mg) (Kundu and Mahato, 1993), chlorogenic acid (**4**, 40 mg) (Mimaki et al., 2000; Subramanian and Nair, 1970), medioresinol (**5**, 5 mg) (Abe and Yamauchi, 1988), and 4-*O*-ethylgallic acid (**6**, 631 mg) (Mehta and Dubey, 1988). The aqueous layer was evaporated and was purified by CC (D101, elution with H<sub>2</sub>O and acetone successively) to give two fractions. The acetone fraction was subjected to repeated CC on silica gel, ODS and Sephadex LH-20 to give 3-*O*-[β-glucopyranosyl-(1→3)-α-arabinopyranosyl]-gymnestrogenin (**7**, 38 mg), 3,4-dimethoxyphenyl-β-D-glucopyranoside (**8**, 18 mg) (Pan and Lundgren, 1995), 2,4,6-trimethoxyphenol-1-*O*-β-D-glucopyranoside (**9**, 25 mg) (Wang et al., 2006), icaraside F<sub>2</sub> (**10**, 5 mg) (Miyase et al., 1988), and corchoionoside C (**11**, 5 mg) (Yoshikawa et al., 1997).

Structures of known compounds (**1–6**, and **8–11**) were established conclusively by MS and extensive <sup>1</sup>H, <sup>13</sup>C and DEPT NMR spectra analysis and comparison with literature data.

Compound **7** (α<sub>D</sub><sup>28.6</sup> = 24.17, *c* = 0.015, CH<sub>3</sub>OH) was isolated as white solid. The molecular formula was determined as C<sub>41</sub>H<sub>68</sub>O<sub>14</sub> from negative HR-ESI-MS ([M – H]<sup>–</sup> at *m/z* 783.4529, calc. 783.4530 for C<sub>41</sub>H<sub>67</sub>O<sub>14</sub>). Comparison of the <sup>1</sup>H and <sup>13</sup>C NMR data of **7** (see Table 1) with those of glochidioside (Srivastava and Kulshreshtha, 1986) and gymnemic acid VII (Yoshikawa et al., 1989), indicated that they all contained the same aglycone (gymnestrogenin). Furthermore, the presence of two anomeric protons at δ<sub>H</sub> 4.35 (d, *J* = 7.5) and 4.55 (d, *J* = 7.5) suggested that **7** contained two sugar moieties. The two sugar moieties were assigned as arabinopyranose (δ<sub>C</sub> 106.1, 72.0, 84.2, 69.5, 66.9) and glucopyranose (δ<sub>C</sub> 105.4, 75.2, 77.6, 71.0, 77.8, 62.2) by comparison of <sup>13</sup>C NMR data of the sugar units with literature (Zhang et al., 2008; Kiem et al., 2009). The coupling constants of the two anomeric protons, H-1' (δ<sub>H</sub> 4.35, d, *J* = 7.5 Hz) and H-1'' (δ<sub>H</sub> 4.55, d, *J* = 7.5 Hz), determined the configurations of arabinose and glucose as α and β, respectively (Tian et al., 2006). The arabinose moiety directly attached at C-3 (δ<sub>C</sub> 83.3) position of the aglycone was confirmed by the HMBC correlation between H-1' and C-3. The downfield shift of C-3' (δ<sub>C</sub> 84.2) of the arabinose suggested that the glucose was at C-3', which was also supported by the HMBC correlation between H-1'' and C-3'. Thus, the compound **7**, named as assamicoside A, was established as 3-*O*-[β-glucopyranosyl-(1→3)-α-arabinopyranosyl]-gymnestrogenin.

### 4. Chemotaxonomic significance

The aerial parts of *G. assamicum* contain lupane-type triterpenes (**1**, **2**), oleanane-type triterpenes (**3**, **7**), megastigmane glycosides (**11**), lignans (**5**), and phenolic glycosides (**8**, **9** and **10**), in agreement with previous work on the constituents of other species of *Glochidion* (summarized in Table 2). Lupane-type triterpenes, such as glochidone, glochidiol, and glochidonol,

**Table 1**<sup>1</sup>H (500 MHz) and <sup>13</sup>C (125 MHz) NMR data for compound **7**<sup>a, b</sup> (in methanol-*d*<sub>4</sub>).

No.	$\delta_{\text{H}}$	$\delta_{\text{C}}$	No.	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1	1.63, m 0.97, m	39.6	22	2.30, m 1.38, m	33.7
2	1.87, m 1.76, m	26.3	23	3.63, m 3.30, m	65.0
3	3.60, m	83.3	24	0.73, s (3H)	13.4
4		43.8	25	1.01, s (3H)	16.6
5	1.23, m	48.2	26	1.02, s (3H)	17.4
6	1.52, m 1.40, m	18.8	27	1.24, s (3H)	27.4
7	1.71, m 1.33, m	33.2	28	3.77, d (10.5) 3.28, m	67.9
8		41.0	29	0.95, s (3H)	29.7
9	1.61, m	48.1	30	0.88, s (3H)	17.6
10		37.4	1'	4.35, d (7.5)	106.1
11	1.94, m (2H)	24.7	2'	3.69, m	72.0
12	5.27, brs	124.4	3'	3.65, m	84.2
13		143.3	4'	4.04, brs	69.5
14		44.6	5'	3.84, m 3.56, m	66.9
15	1.81, m 1.36, m	36.5	1''	4.55, d (7.5)	105.4
16	4.21, dd, (11.5, 4.5)	68.4	2''	3.30, m	75.2
17		44.1	3''	3.38, m	77.6
18	2.26, m	44.2	4''	3.34, m	71.0
19	1.81, m 1.16, dd (14.0, 4.5)	48.3	5''	3.30, m	77.8
20		37.1	6''	3.84, m 3.69, m	62.2
21	3.55, m	73.9			

<sup>a</sup> Assignments were made by a combination of 1D and 2D-NMR techniques (<sup>1</sup>H–<sup>1</sup>H COSY, HSQC, and HMBC).<sup>b</sup> All proton signals integrate to <sup>1</sup>H, unless otherwise indicated.**Table 2**Phytochemical investigation of thirteen species of *Glochidion*.

	Lupane-type triterpenes	Oleanane-type triterpenes	Megastigmane glycosides	Lignans	Phenolic glycosides	Flavanol glycosides	Steroids	Butenolides
<i>G. acuminatum</i> <sup>a</sup>					+			+
<i>G. assamicum</i> <sup>b</sup>	+		+	+	+			
<i>G. coccineum</i> <sup>a</sup>	+	+						
<i>G. dasphyllum</i> <sup>a</sup>	+						+	
<i>G. eriocarpum</i> <sup>a</sup>	+	+						
<i>G. heyneanum</i> <sup>a</sup>	+	+						
<i>G. hohenackeri</i> <sup>a</sup>	+							
<i>G. obliquum</i> <sup>a</sup>		+				+	+	
<i>G. obovatum</i> <sup>a</sup>			+	+	+			
<i>G. puberum</i> <sup>a</sup>	+	+						
<i>G. rubrum</i> <sup>a</sup>				+	+			
<i>G. venulatum</i> <sup>a</sup>	+							
<i>G. wrightii</i> <sup>a</sup>	+	+		+			+	+
<i>G. zeylanicum</i> <sup>a</sup>	+		+	+		+		+

<sup>a</sup> Previously reported investigation.<sup>b</sup> Present investigation.

may be useful chemotaxonomic markers in plants belonging to the genus *Glochidion*. In the present study we report, for the first time, a spirostane-type C<sub>27</sub> steroid (chlorogenin, **4**) from *Glochidion*, although the compound have been isolated from *Agave americana* (Subramanian and Nair, 1970). In addition, this steroid can be used to differentiate *G. assamicum* from other *Glochidion* species.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.bse.2012.12.025>.

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