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Cytotoxic triterpenoid saponins from *Aesculus glabra* Willd.

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ABSTRACT

Twenty-four acylated polyhydroxyoleanene saponins were isolated from the seeds of *Aesculus glabra*. Sixteen of them, namely aesculiosides G1–G16 (**1**–**16**), were determined as compounds by spectroscopic and chemical analysis. The structural features of all 24 saponins are: (1) arabinofuranosyl units affixed to C-3 of the glucuronopyranosyl unit in the trisaccharide chain; (2) no 24-OH substitution; (3) C-2 sugar moiety substitution of the 3-*O*-glucuronopyranosyl unit is either glucopyranosyl or galactopyranosyl. The features of these isolated saponin structures provide more evidence for chemical taxonomy within the genus *Aesculus*. The cytotoxicity of the aesculiosides (**1**–**16**) were tested against A549 and PC-3 cancer cell lines with GI_{50} from 5.4 to >25 μM.

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1. Introduction

Aesculus glabra Willd. (Hippocastanaceae or Sapindaceae) is native to the midwest United States and the extended southeast area. The species is the state tree of Ohio, USA and is commonly known as the Ohio buckeye or American buckeye (Harris, 2003; Little, 1980). The seeds and roots of *A. glabra* have been used for stunning fish by Native Americans. The leaves and buds of *A. glabra* have been shown to be toxic to the central nervous system in cattle (Casteel et al., 1992). In regards to its medicinal use, the Cherokee pounded *A. glabra* seeds to make poultices for soothing aches and pains (Harris, 2003).

Of the 12 species of *Aesculus* L., six species have been extensively investigated in chemical constituents, *Aesculus hippocastanum* L. (Komissarenko et al., 1994; Konoshima and Lee, 1986; Yoshikawa et al., 1994, 1996, 1998), *Aesculus chinensis* Bunge (Guo and Yang, 2004; Wei et al., 2004; Yang and Guo, 2007; Zhang et al., 1999; Zhao et al., 2001), *Aesculus pavia* L. (Schrutka-Rechtenstamm et al., 1988; Zhang and Li, 2007; Zhang et al., 2006), *Aesculus assamica* Griffith (Liu et al., 2005a,b, 2006, 2008), *Aesculus turbinata* Blume (Kimura et al., 2006, 2004; Ogawa et al., 2008; Yang and Zhao, 2008; Yang et al., 2000, 2008; Zhao and Yang, 1999), and *Aesculus indica* (Camb.) Hook. (Sati and Rana, 1987a,b; Singh et al., 1986, 1987, 1989; Srijayanta et al., 1999). To date, more than 210 compounds have been isolated and identified primarily from the fruits or seeds of *Aesculus* sp., with polyhydroxylated triterpenoid saponins as the major active principles (Zhang et al., 2010). However, chemical investigation of *A. glabra* is limited. There is only one available report on the identification of sapogenins from enzymatic and acid hydrolysis products of the saponin fraction of *A. glabra* seeds (Aurada et al., 1984).

Our ongoing project involved with anticancer saponins from Native American plants has led to the isolation of diversified triterpenoid saponins (Wang et al., 2010; Zhang and Li, 2007; Zhang et al., 2006, 2010). From fruits of *A. pavia*, 31 triterpenoid saponins were isolated (Zhang and Li, 2007; Zhang et al., 2006). Also shown was that some of the acylated polyhydroxyoleanene saponins are cytotoxic against various cell lines as well as displaying Topo I inhibition activity (Wang et al., 2010; Zhang and Li, 2007). Herein is described the isolation, structural elucidation, and cytotoxicity of 24 triterpenoid saponins, including 16 new compounds from the seeds of *A. glabra*.

2. Results and discussion

The investigation of the *n*-butanol-soluble partition of *A. glabra* seed extracts led to the isolation of 16 new triterpenoid saponins, along with eight known saponins. Through comparison of NMR and MS data with reference data, the eight known saponins were identified as aesculioside II_a (Zhang et al., 2006), aesculioside II_f (Zhang and Li, 2007), aesculioside II_g (Zhang and Li, 2007), aesculioside II_j (Zhang and Li, 2007), aesculioside II_k (Zhang and Li, 2007), aesculioside III_f (Zhang and Li, 2007), xanifolia-Y_3 (Chan et al., 2008), and xanifolia-Y_{10} (Chan et al., 2008), respectively.

Aesculioside G1 (**1**) gave an $[M-H]^-$ pseudo-molecular ion at *m/z* 1099.5373 in the negative HR-ESI-MS, corresponding to a molecular formula of $C_{54}H_{84}O_{23}$. The NMR data of **1** displayed

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Table 1a
^{1}H NMR spectroscopic data for compounds **1–8** (600 MHz in pyridine-d_5).

Proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	0.87 *m* 1.41 *m*	0.87 *m* 1.41 *m*	0.87 *m* 1.40 *m*	0.86 *m* 1.40 *m*	0.90 *m* 1.43 *m*	0.88 *m* 1.43 *m*	0.88 *m* 1.44 *m*	0.87 *m* 1.41 *m*
2	1.82 *m* 2.15 *m*	1.83 *m* 2.14 *m*	1.85 *m* 2.19 *m*	1.84 *m* 2.18 *m*	1.84 *m* 2.19 *m*	1.84 *m* 2.17 *m*	1.85 *m* 2.19 *m*	1.86 *m* 2.22 *m*
3	3.25 (*dd*, 11.5, 3.8)	3.26 (*dd*, 11.8, 3.8)	3.28 (*dd*, 10.8, 3.1)	3.28 (*dd*, 11.0, 3.2)	3.25 (*dd*, 11.5, 4.1)	3.27 (*dd*, 11.5, 4.3)	3.27 *br s*	3.28 (*dd*, 10.9, 3.3)
5	0.83 *m*	0.83 *m*	0.78 *m*	0.77 *m*	0.84 *m*	0.83 *m*	0.82 *m*	0.78 *m*
6	1.58 *m* 1.61 *m*	1.40 *m* 1.60 *m*	1.32 *m* 1.52 *m*	1.32 *m* 1.52 *m*	1.38 *m* 1.61 *m*	1.39 *m* 1.61 *m*	1.38 *m* 1.59 *m*	1.31 *m* 1.51 *m*
7	2.07 *m* 2.15 *m*	2.08 *m* 2.18 *m*	1.31 *m* 1.60 *m*	1.30 *m* 1.59 *m*	2.07 *m* 2.14 *m*	2.07 *m* 2.16 *m*	2.06 *m* 2.16 *m*	1.30 *m* 1.58 *m*
9	1.71 *m*	1.71 *m*	1.72 *m*	1.72 *m*	1.73 *m*	1.72 *m*	1.72 *m*	1.73 *m*
11	1.90 *m*	1.90 *m*	1.89 *m*	1.88 *m*	1.93 *m*	1.93 *m*	1.94 *m*	1.84 *m*
12	5.55 *br s*	5.54 *br s*	5.47 *br s*	5.45 *br s*	5.47 *br s*	5.47 *br s*	5.53 *br s*	5.44 *br s*
15	4.28 (*d*, 4.0)	4.28 (*d*, 3.8)	1.67, *m* 1.94 (1H, *d*, 11.3)	1.66, *m* 1.93 (1H, *d*, 9.9)	4.30 (*d*, 3.1)	4.24 (*d*, 4.3)	4.20 (*d*, 2.8)	1.62, *m* 1.88 (1H, *d*, 11.9)
16	4.68 (*d*, 4.0)	4.66 (*d*, 3.8)	4.77 (*m*)	4.75 (*m*)	4.78 (*d*, 3.1)	4.46 (*d*, 4.3)	4.45 (*d*, 2.8)	4.51 (*m*)
18	2.84 (*dd*, 13.5, 3.2)	2.82 (*dd*, 13.5, 3.4)	2.87 (*dd*, 13.6, 3.2)	2.85 (*dd*, 13.4, 2.9)	2.90 (*dd*, 12.1, 2.7)	3.12 *s*	3.11 *s*	3.15 (*dd*, 13.2, 2.9)
19	1.41 *m* 3.10 (*t*, 13.5)	1.41 *m* 3.09 (*t*, 13.5)	1.42 *m* 3.12 (*t*, 13.6)	1.41 *m* 3.11 (*t*, 13.4)	1.41 *m* 3.07 (*t*, 12.1)	1.45 *m* 3.12 *s*	1.45 *m* 3.12 *s*	1.45 *m* 3.12 (*t*, 13.2)
21	6.48 (*d*, 9.8)	6.52 (*d*, 10.1)	6.48 (*d*, 10.2)	6.50 (*d*, 10.0)	6.49 (*d*, 10.0)	6.70 (*d*, 10.1)	6.77 (*d*, 10.0)	6.73 (*d*, 10.2)
22	4.49 (*d*, 9.8)	4.47 (*d*, 10.1)	4.54 (*d*, 10.2)	4.48 (*d*, 10.0)	4.42 (*d*, 10.0)	6.39 (*d*, 10.1)	6.30 (*d*, 10.0)	6.34 (*d*, 10.2)
23	1.28 *s*	1.29 *s*	1.31 *s*	1.31 *s*	1.21 *s*	1.22 *s*	1.20 *s*	1.33 *s*
24	1.17 *s*	1.17 *s*	1.18 *s*	1.18 *s*	1.12 *s*	1.13 *s*	1.14 *s*	1.19 *s*
25	0.86 *s*	0.86 *s*	0.85 *s*	0.84 *s*	0.85 *s*	0.86 *s*	0.85 *s*	0.84 *s*
26	1.09 *s*	1.09 *s*	1.00 *s*	1.00 *s*	1.02 *s*	1.03 *s*	1.02 *s*	0.87 *s*
27	1.86 *s*	1.85 *s*	1.85 *s*	1.84 *s*	1.87 *s*	1.88 *s*	1.87 *s*	1.87 *s*
28	4.38 (2H, *m*)	4.35 (2H, *m*)	4.28 (*d*, 9.6) 4.32 (*d*, 9.6)	4.25 (*d*, 9.9) 4.29 (*d*, 9.9)	3.76 (*d*, 10.4) 4.03 (*d*, 10.4)	3.54 (*d*, 10.7) 3.80 (*d*, 10.7)	3.47 (*d*, 10.8) 3.76 (*d*, 10.8)	3.41 (*d*, 10.5) 3.67 (*d*, 10.5)
29	1.13 *s*	1.14 *s*	1.13 *s*	1.12 *s*	1.12 *s*	1.14 *s*	1.14 *s*	1.14 *s*
30	1.32 *s*	1.30 *s*	1.33 *s*	1.32 *s*	1.32 *s*	1.32 *s*	1.34 *s*	1.38 *s*
C_{21}	Tig	Ang	Tig	Ang	Ang	Tig	Ang	Tig
3	7.04 (*q*, 7.0)	5.91 (*q*, 7.2)	7.03 (*q*, 7.2)	5.90 (*q*, 7.2)	5.91 (*q*, 7.0)	7.11 (*q*, 7.3)	5.92 (*q*, 7.1)	7.07 (*q*, 7.2)
4	1.61 (*d*, 7.0)	2.06 (*d*, 7.2)	1.62 (*d*, 7.2)	2.07 (*d*, 7.2)	2.07 (*d*, 7.0)	1.64 (*d*, 7.3)	2.10 (*d*, 7.1)	1.63 (*d*, 7.2)
5	1.87 *s*	1.97 *s*	1.87 *s*	2.01 *s*	1.98 *s*	1.96 *s*	2.00 *s*	1.94 *s*
C_{22} or, C_{28}	Ac	Ac	Ac	Ac		Ang	Tig	Tig
2	2.00 *s*	1.99 *s*	2.04 *s*	1.99 *s*				
3		–				5.78 (*q*, 7.0)	6.81 (*q*, 7.2)	6.99 (*q*, 7.2)
4		–	–	–		1.97 (*d*, 7.0)	1.34 (*d*, 7.2)	1.46 (*d*, 7.2)
5	–	–	–	–		1.76 *s*	1.76 *s*	1.84 *s*
C_3	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*
1	4.99 (*d*, 7.4)	5.00 (*d*, 7.4)	4.90 (*d*, 7.4)	4.90 (*d*, 7.2)	4.92 (*d*, 7.9)	5.00 (*d*, 7.6)	5.00 (*d*, 7.2)	4.92 (*d*, 7.6)
2	4.40 (*dd*, 8.2, 7.4)	4.40 (*dd*, 9.1, 7.4)	4.40 (*dd*, 8.5, 7.4)	4.40 (*dd*, 9.2, 7.2)	4.41 (*dd*, 9.3, 7.9)	4.42 (*dd*, 9.1, 7.6)	4.40 (*dd*, 8.8, 7.2)	4.42 (*dd*, 9.2, 7.6)
3	4.21 (*t*, 8.2)	4.21 (*t*, 9.1)	4.23 (*t*, 8.5)	4.21 (*t*, 9.2)	4.29 (*t*, 9.3)	4.23 (*t*, 9.1)	4.27 (*t*, 8.8)	4.23 (*t*, 9.2)
4	4.43 (*t*, 8.2)	4.43 (*t*, 9.1)	4.54 (*t*, 8.5)	4.43 (*t*, 9.2)	4.42 (*t*, 9.3)	4.42 (*t*, 9.1)	4.36 (*t*, 8.8)	4.50 (*t*, 9.2)
5	4.60 (*d*, 8.2)	4.52 (*d*, 9.1)	4.48 (*d*, 8.5)	4.52 (*d*, 9.2)	4.53 (*d*, 9.3)	4.51 (*d*, 9.1)	4.47 (*d*, 8.8)	4.51 (*d*, 9.2)
$C_{2'}$	Gal-*p*	Gal-*p*	Gal-*p*	Gal-*p*	Glc-*p*	Gal-*p*	Gal-*p*	Gal-*p*
1	5.34 (*d*, 7.8)	5.33 (*d*, 7.5)	5.35 (*d*, 7.6)	5.35 (*d*, 7.2)	5.48 (*d*, 7.4)	5.35 (*d*, 7.6)	5.33 (*d*, 7.3)	5.36 (*d*, 7.5)
2	4.46 (*dd*, 9.3, 7.8)	4.46 (*dd*, 8.8, 7.5)	4.47 (*dd*, 8.5, 7.6)	4.46 (*dd*, 8.9, 7.2)	4.07 (*dd*, 9.5, 7.4)	4.47 (*dd*, 8.2, 7.6)	4.51 (*dd*, 9.5, 7.3)	4.48 (*dd*, 9.2, 7.5)
3	4.11 (*dd*, 9.3, 3.6)	4.11 (*dd*, 8.8, 3.1)	4.12 (*dd*, 8.5, 3.3)	4.11 (*dd*, 8.9, 4.0)	4.25 (*t*, 9.5)	4.12 (*dd*, 8.2, 3.4)	4.10 (*dd*, 9.8, 3.8)	4.12 (*dd*, 9.2, 3.4)
4	4.57 (*d*, 3.6)	4.57 (*d*, 3.1)	4.58 (*d*, 3.3)	4.57 (*d*, 4.0)	4.13 (*dd*, 9.5, 9.1)	4.58 (*d*, 3.4)	4.57 (*d*, 3.8)	4.59 (*d*, 3.4)
5	3.96 *m*	3.96 *m*	3.97 *m*	3.96 *m*	3.92 *m*	3.97 *m*	3.96 *m*	3.98 *m*
6	4.45 (*dd*, 11.5, 4.5) 4.53 (*dd*, 11.5, 7.7)	4.45 (*dd*, 11.5, 3.4) 4.53 (*dd*, 11.5, 7.8)	4.47 (*dd*, 11.1, 4.2) 4.55 (*dd*, 11.1, 7.7)	4.45 (*dd*, 11.8, 3.5) 4.53 (*dd*, 11.8, 7.5)	4.36 *m* 4.56 (*dd*, 12.0, 2.3)	4.46 (*dd*, 11.2, 4.0) 4.54 (*dd*, 11.2, 7.5)	4.45 (*dd*, 11.5, 3.2) 4.52 (*dd*, 11.5, 7.2)	4.56 (*dd*, 11.5, 3.4) 4.48 (*dd*, 11.5, 7.5)
$C_{3'}$	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*
1	6.05 *br s*	6.04 *br s*	6.08 *br s*	6.08 *br s*	6.13 *br s*	6.07 *br s*	6.07 *br s*	6.08 *br s*
2	4.94 *m*	4.94 *m*	4.96 *m*	4.94 *m*	4.95 *m*	4.95 *m*	4.94 *m*	4.97 *m*
3	4.79 *br d*, 5.5	4.79 *br d*, 5.0	4.79 *br d*, 5.4	4.79 *br d*, 5.3	4.78 *br d*, 5.0	4.79 *br d*, 5.5	4.79 *br d*, 5.4	4.81 *br d*, 5.2
4	4.84 (*td*, 5.5, 3.4)	4.84 (*td*, 5.0, 3.3)	4.86 (*td*, 5.4, 3.3)	4.84 (*td*, 5.2, 3.3)	4.87 (*td*, 5.0, 3.3)	4.86 (*td*, 5.5, 3.6)	4.88 (*td*, 5.4, 3.2)	4.86 (*td*, 5.2, 3.3)
5	4.16 (*dd*, 11.0, 3.1) 4.31 (*dd*, 11.0, 5.5)	4.16 (*dd*, 11.5, 3.3) 4.31 (*dd*, 11.5, 5.0)	4.17 (*dd*, 11.8, 3.3) 4.33 (*dd*, 11.8, 5.4)	4.16 (*dd*, 11.1, 3.1) 4.31 (*dd*, 11.1, 5.3)	4.17 (*dd*, 11.5, 3.3) 4.32 (*dd*, 11.5, 4.8)	4.17 (*dd*, 11.4, 3.6) 4.32 (*dd*, 11.4, 5.2)	4.15 (*dd*, 11.7, 3.5) 4.34 (*dd*, 11.7, 5.8)	4.17 (*dd*, 11.0, 3.3) 4.34 (*dd*, 11.0, 5.3)

Table 1b
^{1}H NMR spectroscopic data for compounds **9**–**16** (600 MHz in pyridine-d_5).

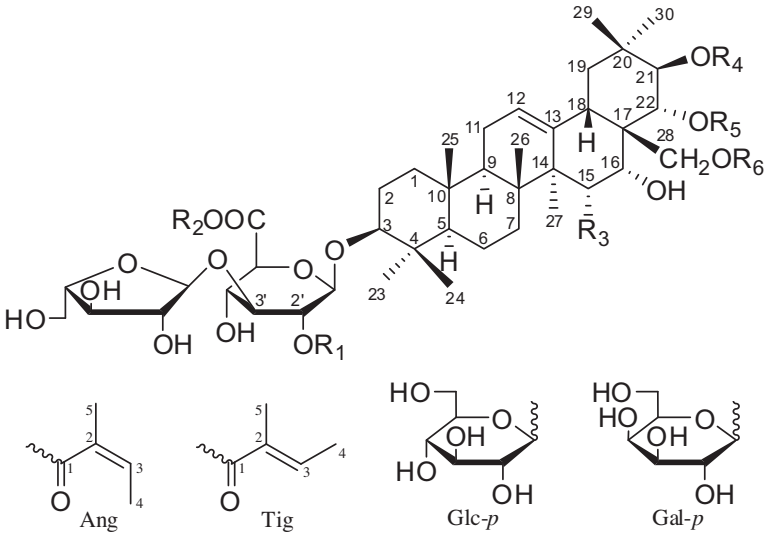
Proton	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	0.87 *m*	0.85 *m*	0.88 *m*	0.87 *m*	0.88 *m*	0.86 *m*	0.85 *m*	0.87 *m*
	1.41 *m*	1.40 *m*	1.44 *m*	1.44 *m*	1.43 *m*	1.41 *m*	1.41 *m*	1.40 *m*
2	1.84 *m*	1.84 *m*	1.82 *m*	1.83 *m*	1.82 *m*	1.85 *m*	1.86 *m*	1.85 *m*
	2.18 *m*	2.19 *m*	2.09 *m*	2.06 *m*	2.18 *m*	2.15 *m*	2.19 *m*	2.18 *m*
3	3.28 (*dd*, 11.8, 4.1)	3.28 (*dd*, 11.5, 3.9)	3.27 (*dd*, 14.3, 4.3)	3.27 (*dd*, 12.5, 3.8)	3.28 (*dd*, 11.1, 3.7)	3.28 (*dd*, 11.5, 3.5)	3.29 (*dd*, 11.5, 3.7)	3.34 (*dd*, 11.7, 3.8)
5	0.78 *m*	0.77 *m*	0.77 *m*	0.77 *m*	0.84 *m*	0.83 *m*	0.78 *m*	0.77 *m*
6	1.31 *m*	1.31 *m*	1.31 *m*	1.32 *m*	1.41 *m*	1.41 *m*	1.33 *m*	1.33 *m*
	1.51 *m*	1.53 *m*	1.53 *m*	1.51 *m*	1.63 *m*	1.62 *m*	1.53 *m*	1.53 *m*
7	1.31 *m*	1.30 *m*	1.30 *m*	1.30 *m*	2.08 *m*	2.06 *m*	1.63 *m*	1.63 *m*
	1.59 *m*	1.58 *m*	1.57 *m*	1.58 *m*	2.19 *m*	2.18 *m*	1.88 *m*	1.88 *m*
9	1.73 *m*	1.73 *m*	1.73 *m*	1.73 *m*	1.72 *m*	1.71 *m*	1.73 *m*	1.74 *m*
11	1.85 *m*	1.90 *m*	1.92 *m*	1.85 *m*	1.90 *m*	1.84 *m*	1.83 *m*	1.84 *m*
12	5.44 *br s*	5.44 *br s*	5.44 *br s*	5.44 *br s*	5.52 *br s*	5.50 *br s*	5.42 *br s*	5.41 *br s*
15	1.62, *m*	1.62, *m*	1.62, *m*	1.62, *m*	4.20 (*d*, 3.7)	4.19 (*d*, 3.3)	1.64, *m*	1.63, *m*
	1.89 (1H, *d*, 12.0)	1.87 (1H, *d*, 10.5)	1.87 (1H, *d*, 10.7)	1.89 (1H, *d*, 11.2)			1.88 (1H, *d*, 11.0)	1.88 (1H, *d*, 10.8)
16	4.52 *m*	4.50 *m*	4.49 *m*	4.51 *m*	4.40 (*d*, 3.7)	4.39 (*d*, 3.3)	4.47 *m*	4.46 *m*
18	3.14 *m*	3.14 *m*	3.14 (*t like*, 14.3)	3.13 (*t like*, 14.5)	3.08 *s*	3.02 *s*	3.10 *m*	3.09 *m*
19	1.45 *m*	1.45 *m*	1.46 (*t*, 14.3)	1.45 (*t*, 14.5)	1.44 *m*	1.42 *m*	1.43 *m*	1.43 *m*
	3.11 *m*	3.14 *m*	3.15 (*t*, 14.3)	3.13 (*t*, 14.5)	3.07 *s*	3.02 *s*	3.10 *m*	3.10 *m*
21	6.69 (*d*, 10.0)	6.77 (*d*, 10.2)	6.77(*d*, 10.2)	6.72(*d*, 10.1)	6.59(*d*, 10.0)	6.60(*d*, 10.1)	6.62(*d*, 10.1)	6.64(*d*, 10.2)
22	6.39 (*d*, 10.0)	6.29 (*d*, 10.2)	6.30(*d*, 10.2)	6.34(*d*, 10.1)	6.26(*d*, 10.0)	6.20(*d*, 10.1)	6.28(*d*, 10.1)	6.23(*d*, 10.2)
23	1.33 *s*	1.32 *s*	1.33 *s*	1.32 *s*	1.22 *s*	1.21 *s*	1.25 *s*	1.25 *s*
24	1.19 *s*	1.19 *s*	1.18 *s*	1.18 *s*	1.14 *s*	1.13 *s*	1.14 *s*	1.14 *s*
25	0.84 *s*	0.84 *s*	0.85 *s*	0.84 *s*	0.86 *s*	0.86 *s*	0.84 *s*	0.83 *s*
26	0.87 *s*	0.87 *s*	0.87 *s*	0.88 *s*	1.03 *s*	1.02 *s*	0.87 *s*	0.87 *s*
27	1.87 *s*	1.86 *s*	1.85 *s*	1.85 *s*	1.86 *s*	1.86 *s*	1.86 *s*	1.85 *s*
28	3.45 (*d*, 10.6)	3.40 (*d*, 10.3)	3.40(*d*, 10.2)	3.44(*d*, 10.7)	3.46(*d*, 10.2)	3.45(*d*, 10.4)	3.41(*d*, 10.2)	3.40(*d*, 10.1)
	3.69 (*d*, 10.6)	3.66 (*d*, 10.3)	3.67(*d*, 10.2)	3.68(*d*, 10.7)	3.73(*d*, 10.2)	3.70(*d*, 10.4)	3.64(*d*, 10.2)	3.64(*d*, 10.1)
29	1.14 *s*	1.13 *s*	1.14 *s*	1.13 *s*	1.13 *s*	1.11 *s*	1.13 *s*	1.11 *s*
30	1.37 *s*	1.35 *s*	1.35 *s*	1.36 *s*	1.34 *s*	1.31 *s*	1.35 *s*	1.33 *s*
C_{21}	Tig	Ang	Ang	Ang	Tig	Ang	Tig	Ang
3	7.09 (*q*, 7.2)	5.93 (*q*, 7.2)	5.93(*q*, 7.0)	5.93(*q*, 7.1)	7.12(*q*, 7.1)	7.12(*q*, 7.2)	7.13(*q*, 7.2)	5.99(*q*, 7.2)
4	1.65 (*d*, 7.2)	2.07 (*d*, 7.2)	2.07(*d*, 7.0)	2.11(*d*, 7.1)	1.67(*d*, 7.1)	2.11(*d*, 7.2)	1.67(*d*, 7.2)	2.12(*d*, 7.2)
5	1.96 *s*	2.01 *s*	2.01	2.03	1.98	1.78	1.98	1.94
C_{22} or C_{28}	Ang	Tig	Tig	Ang	Ac	Ac	Ac	Ac
2			–	–	1.78 *s*	2.02 *s*	1.92 *s*	1.92 *s*
3	5.88 (*q*, 7.0)	6.99 (*q*, 7.1)	6.97(*q*, 7.0)	5.99(*q*, 7.2)				
4	2.04 (*d*, 7.0)	1.46 (*d*, 7.1)	1.47(*d*, 7.0)	2.07(*d*, 7.2)				
5	1.90 *s*	1.84 *s*	1.86 *s*	1.91 *s*				
C_3	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*
1	4.93 (*d*, 7.3)	4.92 (*d*, 7.2)	4.92 (*d*, 7.4)	4.93 (*d*, 7.6)	4.92 (*d*, 7.4)	4.92 (*d*, 7.2)	4.92 (*d*, 7.4)	4.93 (*d*, 7.7)
2	4.41 (*dd*, 9.2, 7.3)	4.40 (*dd*, 8.7, 7.2)	4.36 (*dd*, 9.2, 7.4)	4.37 (*dd*, 9.1, 7.6)	4.42 (*dd*, 8.4, 7.4)	4.41 (*dd*, 8.6, 7.2)	4.41 (*dd*, 9.0, 7.4)	4.41 (*dd*, 8.5, 7.7)
3	4.23 (*t*, 9.2)	4.22 (*t*, 8.7)	4.17 (*t*, 9.3)	4.18 (*t*, 9.1)	4.28 (*t*, 8.4)	4.27 (*t*, 8.6)	4.25 (*t*, 9.0)	4.25 (*t*, 8.5)
4	4.46 (*t*, 9.2)	4.46 (*t*, 8.7)	4.30 (*t*, 9.3)	4.30 (*t*, 9.1)	4.43 (*t*, 8.4)	4.44 (*t*, 8.6)	4.46 (*t*, 9.0)	4.44 (*t*, 8.5)
5	4.51 (*d*, 9.2)	4.50 (*d*, 8.7)	4.42 (*d*, 9.3)	4.43 (*d*, 9.1)	4.53 (*d*, 8.4)	4.53 (*d*, 8.6)	4.51 (*d*, 9.0)	4.53 (*d*, 8.5)
OCH_3			3.76 *s*	3.76 *s*				
$C_{2'}$	Gal-*p*	Gal-*p*	Gal-*p*	Gal-*p*	Glc-*p*	Glc-*p*	Glc-*p*	Glc-*p*
1	5.36 (*d*, 7.6)	5.35 (*d*, 7.5)	5.35 (*d*, 7.4)	5.36 (*d*, 7.6)	5.49 (*d*, 7.4)	5.49 (*d*, 7.6)	5.50 (*d*, 7.5)	5.36 (*d*, 7.5)
2	4.48 (*dd*, 8.5, 7.6)	4.48 (*dd*, 9.8, 7.5)	4.47 (*dd*, 8.6, 7.4)	4.49 (*dd*, 9.2, 7.6)	4.08 (*dd*, 9.5, 7.4)	4.07 (*dd*, 9.2, 7.6)	4.09 (*dd*, 8.5, 7.5)	4.09 (*dd*, 8.5, 7.5)
3	4.12 (*dd*, 8.5,3.3)	4.12 (*dd*, 9.8, 3.7)	4.12 (*dd*, 8.6, 3.5)	4.13 (*dd*, 9.2, 3.8)	4.25 (*t*, 9.5)	4.24 (*t*, 9.2)	4.26 (*t*, 8.8)	4.25 (*t*, 9.2)
4	4.59 (*d*, 3.3)	4.59 (*d*, 3.7)	4.59 (*d*, 3.5)	4.60 (*d*, 3.8)	4.14 (*dd*, 9.5, 9.0)	4.12 (*dd*, 9.2, 9.1)	4.15 (*dd*, 9.5, 8.8)	4.15 (*dd*, 9.2, 9.0)
5	3.98 *m*	3.98 *m*	3.98 *m*	3.99 *m*	3.93 *m*	3.92 *m*	3.94 *m*	3.93 *m*
6	4.48 (*dd*, 11.4, 3.9)	4.55 (*dd*, 11.5, 4.2)	4.47 (*dd*, 10.9, 4.5)	4.47 (*dd*, 11.8, 3.8)	4.37 *m*	4.36 *m*	4.38 *m*	4.36 *m*
	4.56 (*dd*, 11.4, 7.7)	4.47 (*dd*, 11.5, 7.9)	4.56 (*dd*, 10.9, 7.1)	4.56 (*dd*, 11.8, 3.8)	4.56 (*dd*, 11.7, 3.2)	4.55 (*dd*, 12.0, 2.5)	4.58 (*dd*, 11.6, 3.1)	4.58 (*dd*, 11.4, 2.8)
$C_{3'}$	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*
1	6.07 *br s*	6.07 *br s*	6.02 *br s*	6.03 *br s*	6.12 *br s*	6.10 *br s*	6.12 *br s*	6.03 *br s*
2	4.97 *m*	4.97 *m*	4.97 *m*	4.97 *m*	4.96 *m*	4.94 *m*	4.97 *m*	4.97 *m*
3	4.81 *br d*, 5.1	4.81 *br d*, 5.5	4.81 *br d*, 5.2	4.82 *br d*, 5.0	4.80 *br d*, 5.4	4.79 *br d*, 5.6	4.80 *br d*, 5.3	4.82 *br d*, 5.4
4	4.86 (*td*, 5.1, 3.7)	4.86 (*td*, 5.5, 3.6)	4.82 (*td*, 5.2, 3.5)	4.82 (*td*, 5.0, 3.5)	4.86 (*td*, 5.4, 3.6)	4.84 (*td*, 5.6, 3.3)	4.87 (*td*, 5.3, 3.7)	4.82 (*td*, 5.4, 3.6)
5	4.17 (*dd*, 11.0, 3.7)	4.17 (*dd*, 11.3, 3.6)	4.15 (*dd*, 11.5, 3.5)	4.17 (*dd*, 11.2, 3.5)	4.16 (*dd*, 11.2, 3.6)	4.16 (*dd*, 11.1, 3.3)	4.18 (*dd*, 11.4, 3.7)	4.17 (*dd*, 11.4, 3.6)
	4.34 (*dd*, 11.0, 5.1)	4.33 (*dd*, 11.3, 5.5)	4.31 (*dd*, 11.5, 5.2)	4.32 (*dd*, 11.2, 5.0)	4.33 (*dd*, 11.2, 5.4)	4.31 (*dd*, 11.1, 5.6)	4.34 (*dd*, 11.4, 5.3)	4.32 (*dd*, 11.4, 5.4)

Table 2a
^{13}C NMR Spectroscopic Data for compounds **1**–**8** (150 MHz in pyridine-d_5).

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	8
1	39.1	39.0	39.0	38.9	39.0	39.0	39.0	38.9
2	26.7	26.7	26.7	26.7	26.7	26.7	26.6	26.7
3	89.8	89.8	89.9	89.9	89.7	89.8	89.7	89.9
4	39.3	39.6	40.3	40.4	40.0	39.8	39.6	39.9
5	55.6	55.6	55.9	55.9	55.6	55.6	55.5	55.9
6	18.8	18.9	18.5	18.5	18.9	18.9	18.8	18.5
7	36.7	36.7	33.2	33.2	36.8	36.8	36.7	33.2
8	41.5	41.3	40.9	40.9	41.7	41.8	41.3	41.3
9	47.3	47.3	47.1	47.1	47.2	47.2	47.2	47.0
10	37.0	37.0	37.3	37.1	37.3	37.3	37.0	37.3
11	24.0	24.1	24.0	23.9	24.1	24.1	24.0	23.9
12	125.6	125.6	124.1	124.1	124.9	125.5	125.4	124.0
13	143.9	143.8	143.1	143.0	144.7	144.7	144.6	143.1
14	48.2	48.5	42.1	42.1	48.8	48.7	48.5	41.7
15	67.6	67.6	34.8	34.7	67.5	67.7	67.6	35.0
16	72.3	72.3	67.8	67.7	72.5	73.7	73.4	68.7
17	47.7	47.8	47.8	47.6	48.3	48.1	47.8	48.3
18	41.4	41.4	40.7	41.4	41.3	41.1	41.0	40.2
19	47.0	47.0	47.4	47.4	47.5	47.0	46.9	47.4
20	36.6	36.6	36.5	36.4	36.4	37.1	36.8	36.4
21	81.5	81.1	81.7	81.3	81.5	79.1	78.6	79.3
22	71.2	71.0	71.3	71.3	71.8	73.5	73.7	74.2
23	28.0	28.0	28.1	28.0	27.8	28.0	27.9	28.0
24	16.8	16.9	16.7	16.7	16.9	16.9	16.8	16.7
25	15.8	15.8	15.8	15.8	15.9	15.8	15.7	15.8
26	16.8	16.8	17.0	17.0	17.6	17.7	17.6	16.9
27	21.2	21.2	27.5	27.5	21.2	21.3	21.2	27.6
28	65.8	65.9	66.6	65.9	65.4	63.2	62.9	63.7
29	29.8	29.8	29.9	29.8	29.8	29.7	29.5	29.7
30	20.1	20.2	20.2	20.3	20.4	20.4	20.1	20.2
C_{21}	Tig	Ang	Tig	Ang	Ang	Tig	Ang	Tig
1	168.7	168.0	169.0	168.9	169.0	168.2	168.1	168.8
2	130.1	129.9	130.0	129.9	129.9	129.7	129.2	129.8
3	136.4	136.2	136.3	136.2	136.1	137.0	136.6	136.8
4	14.2	15.9	14.2	15.9	15.9	14.2	16.1	14.2
5	12.5	20.7	12.5	20.8	21.1	12.5	20.9	12.5
C_{22} or C_{28}	Ac	Ac	Ac	Ac		Ang	Tig	Tig
1	170.9	170.8	171.0	171.0		168.6	168.3	170.9
2	20.8	21.1	20.8	21.1		129.9	129.6	129.9
3						136.1	137.1	137.0
4						15.6	13.9	14.1
5						20.8	12.3	12.4
C_3	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*
1	105.2	105.4	105.1	105.2	105.1	105.5	105.2	105.2
2	78.7	78.7	78.9	78.7	78.9	78.8	78.7	78.8
3	86.3	86.3	86.2	86.3	85.6	86.3	86.3	86.3
4	71.7	71.8	71.4	71.8	71.8	71.9	71.7	71.7
5	77.4	77.3	77.2	77.3	77.2	77.3	77.0	77.2
6	172.5	172.4	172.1	172.3	173.0	172.6	172.7	172.8
$C_{2'}$	Gal-*p*	Gal-*p*	Gal-*p*	Gal-*p*	Glc-*p*	Gal-*p*	Gal-*p*	Gal-*p*
1	104.8	104.9	104.8	104.8	104.0	104.8	104.8	104.8
2	73.3	73.3	73.6	73.3	76.2	73.6	73.4	73.3
3	75.1	75.1	75.2	75.1	78.4	75.2	75.0	75.2
4	69.8	69.8	69.8	69.8	72.6	69.8	69.8	69.9
5	76.7	76.7	76.7	76.7	77.9	76.7	76.8	76.7
6	61.9	61.9	62.0	61.9	63.6	62.0	61.9	61.9
$C_{3'}$	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*
1	111.0	111.1	111.1	111.1	110.9	111.1	111.0	111.2
2	83.4	83.4	83.6	83.4	83.4	83.4	83.5	83.6
3	77.8	77.8	77.8	77.8	77.8	77.8	77.7	77.7
4	85.7	85.8	85.6	85.8	85.7	85.8	85.7	85.6
6	62.5	62.5	62.5	62.5	62.5	62.5	62.6	62.4

typical characteristics of a polyhydroxy-substituted olean-12-ene-type aglycon. The seven tertiary methyl signals of the aglycone were observed at δ_H 0.86, 1.09, 1.13, 1.17, 1.28, 1.32, 1.86 and the Δ12,13 olefinic resonances at δ_H 5.55 and δ_C 125.6 and 143.9, respectively (Tables 1a, and 2a). The low-field shifts of H-15 and H-16 indicated they were oxygen bearing. This was confirmed by the low-field shift of H_3-27, characteristic of a 16-α-hydroxyolean-ane (Herlt et al., 2002). The low-field shift of H-7 (>2 ppm) and the relatively high-field shift of C-27 were characteristic of a H-15-hydroxyl substitution (Fu et al., 2006; Tang et al., 2004; Zhang et al., 2006). By comparing NMR data of **1** with reference data, a set of correlated signals at C-21 and C-22 (δ_H 6.48, 4.49 and δ_C 81.5, 71.2) indicated an acylated group at C-21 and a hydroxyl group at C-22 (Table 2a). The H-21 and H-22 are in a diaxial orientation from their coupling constants (J = 9.8 Hz) (Matsushita et al., 2004; Tang et al., 2004). Thus, the aglycone of **1** was identified as

3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene (R1-barrigenol) (Zhang et al., 2006). This was also confirmed by the acid hydrolysis followed by alkaline hydrolysis of **1**, which afforded R1-barrigenol according to the NMR data (D'Acquarica et al., 2002). The ^{1}H NMR spectra of **1** showed tigloyl signals at δ_H 7.04 (1H, *q*, *J* = 7.0), 1.61 (3H, *d*, *J* = 7.0), and 1.87 (3H, *s*) as well as an acetyl resonance at δ_H 2.00 (3H, *s*). The ^{13}C NMR data of the methyl signals at δ_C 12.5, 14.2 for tigloyl and δ_C 20.8 for acetyl was also in agreement with the elucidation. The resonance at δ_C 168.7 was assigned as the carboxyl of the tigloyl group by the HMBC spectra. From the HMBC correlation of δ_C 168.7 and H-21 signal at δ_H 6.48, the connectivity between tigloyl and C-21 was achieved. The characteristic signals of H_2-28 of oleanane-type triterpenoid at around δ_H 3.4 and 3.6 (1H each, *d*, *J* ≈ 10 Hz) and C-28 at δ_C 63 disappeared. Low field shifts of both H_2-28 and C-28 implied an acetyl substituted at this position (Matsushita et al., 2004; Zhang and Li, 2007). HMBC correlation of carboxyl resonance at δ_C 170.9 with H_2-28 at δ_H 4.38 further confirmed the 28-acetyl group substitution. Signals of three anomeric protons at δ_H 4.99 (1H, *d*, *J* = 7.8 Hz), 5.34 (1H, *d*, *J* = 7.6 Hz), 6.05 (1H, *br s*), which were correlated with carbons at δ_C 105.2, 104.8, and 111.0, respectively, established the presence of three monosaccharide residues. The acid hydrolysis of **1** afforded D-glucuronic acid, D-galactose and L-arabinose (confirmed by the optical rotation data of each isolated sugar). By taking advantage of HSQC, DQF-COSY, HMBC and 2D-TOCSY, the complete assignments of ^{1}H NMR and ^{13}C NMR signals of the D-glucuronopyranosyl acid, D-galactopyranosyl and L-arabinofuranosyl were achieved. The HMBC correlation of anomeric protons at δ_H 4.99 (1H, *d*, *J* = 7.4 Hz) with the downfield shift signal of C-3 at δ_C 89.8 suggested the linkage of a β-configuration D-glucuronic acid to C-3. The pattern of ^{13}C NMR data of GlcA-*p* (δ_C 105.2 (C-1′), 78.7 (C-2′), 86.3 (C-3′), 71.7 (C-4′), 77.4 (C-5′), 172.5 (C-6′)) indicated that the sugar moieties D-galactopyranosyl and L-arabinofuranosyl attach to C-2′ and C-3′. The attachment of D-galactopyranosyl and L-arabinofuranosyl to C-2′ and C-3′ of D-glucuronopyranosyl acid were also confirmed by HMBC experimental data (δ_H 5.34 and 6.05 correlated with δ_C 78.7(C-2′) and 86.3(C-3′), respectively). The D-galactopyranosyl was determined to be in β-pyranose form according to its ^{13}C NMR spectroscopic data (δ_C 104.8 (C-1′), 73.3 (C-2′), 75.1 (C-3′), 69.8 (C-4′), 76.7 (C-5′), 61.9(C-6′)) and ^3J (H-1″,



	R_1	R_2	R_3	R_4	R_5	R_6
1	Gal-*p*	H	OH	Tig	H	Ac
2	Gal-*p*	H	OH	Ang	H	Ac
3	Gal-*p*	H	H	Tig	H	Ac
4	Gal-*p*	H	H	Ang	H	Ac
5	Glc-*p*	H	OH	Ang	H	H
6	Gal-*p*	H	OH	Tig	Ang	H
7	Gal-*p*	H	OH	Ang	Tig	H
8	Gal-*p*	H	H	Tig	Tig	H
9	Gal-*p*	H	H	Tig	Ang	H
10	Gal-*p*	H	H	Ang	Tig	H
11	Gal-*p*	CH_3	H	Ang	Tig	H
12	Gal-*p*	CH_3	H	Ang	Ang	H
13	Glc-*p*	H	OH	Tig	Ac	H
14	Glc-*p*	H	OH	Ang	Ac	H
15	Glc-*p*	H	H	Tig	Ac	H
16	Glc-*p*	H	H	Ang	Ac	H

Fig. 1. The structures of 16 new saponins from *Aesculus glabra*.

H-2″) coupling constant (J = 7.8 Hz). The α-arabinofuranosyl signals were fully assigned by the HSQC and DQF-COSY spectra, which is also in agreement with previous reference data (Zhang and Li, 2007). By an additional NOESY experiment of **1**, the stereochemistry of the aglycone and sugar moieties were further confirmed. Based on the above evidence, the structure of the new saponin **1** was elucidated as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-tigloyl-28-acetyloxy-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene (Fig. 1).

The molecular formula of aesculioside G2 (**2**) was deduced as $C_{54}H_{84}O_{23}$ from its negative HR-ESI-MS spectra, which displayed an $[M-H]^-$ ion at m/z 1099.5361. Careful comparison of ^1H NMR, ^{13}C NMR and HSQC spectra suggested that **1** and **2** shared both the same aglycon and sugar chain. The C-28 acetyl substitution of **2** was confirmed by HMBC experiment. The characteristic olefinic quartet signal of a tigloyl moiety at δ_H 7.04 disappeared in the ^1H NMR spectra. The emerging quartet resonance at δ_H 5.91 indicated an angeloyl group, while the HSQC correlation signals of δ_H 2.06 with δ_C 15.9 and δ_H 1.97 with δ_C 20.7 in **2** confirmed the presence of an angeloyl moiety. The angeloyl substitution was established at C-21 by HMBC correlation of a carboxy carbon at δ_C 168.0 with H-21 at δ_H 6.52. Structure **2** was thus assigned as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-angeloyl-28-acetyloxy-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

Aesculioside G3 (**3**) was assigned a molecular formula of $C_{54}H_{84}O_{22}$ from the negative HR-ESI-MS ion peak at m/z 1083.5378 ($[M-H]^-$). The 16 mass units less than **1** and **2** suggested the absence of a hydroxyl group. The signal of H_3-27 (δ_H 1.85) remaining at low field region indicated the same 16-hydroxyl substitution as **1** and **2**. However, the chemical shift change of C-27 from around δ_C 21 to δ_C 27.5 implied that there was no 15-OH in **3**. The C-15 and H-15 signals appeared at δ_C 34.8 and δ_H 1.67, 1.94 through analysis of COSY, HMBC and HSQC correlations. Meanwhile, C-7 and C-16 resonances, which shifted to a lower field at δ_C 33.2 and 67.8 as a result of the absence of 15-OH further confirmed this deduction. The aglycone of **3** was then identified as 3β,16α,21β,22α,28-pentahydroxyolean-12-ene (barringtogenol C) (Takao and Lee, 1986). The NMR spectroscopic data of the three monosaccharides in **3** were found to be superimposable to those in **1** and **2**. The DQF-COSY and HMBC spectra confirmed the same trisaccharide chain as **1** and **2**, [β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl, linked to the 3-β position of the barringtogenol C aglycon. The tigloyl group exhibited a quartet signal at δ_H 7.03 (1H, J = 7.2 Hz), a doublet at δ_H 1.62 (3H, J = 7.2 Hz) and a singlet at δ_H 1.87 (3H) in the ^1H NMR spectra. A set of acetyl resonances were found at δ_H 2.04 (3H) and δ_C 171.0, 20.8. The HMBC correlations (δ_C 169.0 with δ_H 6.48, δ_C 171.0 with δ_H 4.28, 4.32) confirmed the linkage of the tigloyl and acetyl group to C-21 and C-28. From the above evidence, aesculioside G3 (**3**) was determined as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-tigloyl-28-acetyloxy-3β,16α, 21β,22α,28-pentahydroxyolean-12-ene.

Aesculioside G4 (**4**) was determined as having the same molecular formula as **3** ($C_{54}H_{84}O_{22}$) on the basis of its $[M-H]^-$ ion peak at m/z 1083.5378 in the HR-ESI-MS. Detailed comparison of NMR data of **4** with those of **3** revealed that both shared a common aglycone, barringtogenol C, and the trisaccharide moiety. The ^1H NMR spectra of **4** showed a characteristic angeloyl signal at δ_H 5.90 (1H, *q*, J = 7.2 Hz) and an acetyl resonance at δ_H 1.99 (3H, *s*). The angeloyl and acetyl groups were established as C-21 and C-28 substitutions, respectively, by their HMBC correlations of carboxyl carbons with the corresponding C-21 and C-28 hydrogens. The full assignments were supported by DQF-COSY, HSQC, and HMBC spectral an alysis. Therefore, structure **4** was determined as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-angeloyl-28-acetyloxy-3β,16α,21β,22α,28-pentahydroxyolean-12-ene.

The molecular formula of aesculioside G5 (**5**) was assigned as $C_{52}H_{82}O_{22}$ according to its $[M-H]^-$ ion peak at m/z 1057.5229 in the negative HR-ESI-MS spectra. Extensive analysis on the NMR data suggested that the aglycone of **5** is R1-barrigenol. The only acyl group in **5** was identified as angeloyl by its characteristic signals at δ_H 5.91 (1H, q, J = 7.0 Hz), 2.07 (1H, d, J = 7.0 Hz), 1.98 (3H, s), and δ_C 136.1, 15.9, 21.1. The linkage of the angeloyl group was established at C-21 by HMBC experiments, in which the carboxyl C-1 of the angeloyl group at δ_C 169.0 was correlated with H-21 of the R1-barrigenol aglycone (δ_H 6.49) and the angeloyl H-3 (δ_H 5.91). In comparing the HSQC spectrum of **5** with those of aesculioside II_a, the signal differences were found at the oligosaccharide region. Acid hydrolysis products of **5** yielded D-glucuronic acid, D-glucose and L-arabinose (also confirmed by the optical rotation of each isolated sugar). The resonances of β-D-glucuronopyranosyl acid and α-L-arabinofuranosyl were easily assigned by HSQC, COSY and HMBC spectra, and the rest of sugar signals were elucidated as β-D-glucopyranosyl (Matsushita et al., 2004). The linkage of the β-D-glucopyranosyl moiety to C-2 of the β-D-glucuronopyranosyl moiety was confirmed by HMBC experiment. Structure of **5** was thus assigned as 3-*O*-[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-angeloyl-3β,15α,16α, 21β,22α,28-hexahydroxyolean-12-ene.

Aesculioside G6 (**6**) and aesculioside G7 (**7**) had the same molecular formula of $C_{57}H_{88}O_{23}$, which were deduced from their HR-ESI-MS spectra ($[M-H]^-$ m/z 1139.5638 and 1139.5654, respectively). NMR analysis of **6** and **7** suggested that both compounds included an aglycone, a trisaccharide, an angeloyl, and a tigloyl, moiety, respectively. The aglycones of **6** and **7** were identified with same structure as R1-barrigenol according to their NMR spectra. The trisaccharide signals of **6** and **7** were superimposable to those of other compounds with a chain of [β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl. The tigloyl moiety of **6** was determined to be present at C-21 by HMBC correlation of the C-1 carboxyl of tigloyl at δ_C 168.2 with both H-22 of the R1-barrigenol aglycone (δ_H 6.70) and the tigloyl H-3 (δ_H 7.11). HMBC correlation of δ_C 168.6 with δ_H 6.39 (H-22 of the R1-barrigenol aglycone) and δ_H 5.78 (H-3 of the angeloyl) confirmed the linkage of angeloyl to C-22 position in **6**. In **7**, the HMBC correlations (δ_C 168.1 of angeloyl to δ_H 6.77 (H-21) and 5.92 (H-3 Ang), and δ_C 168.3 of tigloyl to δ_H 6.30 (H-22) and 6.81 (H-3 tig)) established that the angeloyl is at C-21 and the tigloyl is at C-22. It should be noted here that all the ^1H NMR signals of the angeloyl/tigloyl groups upfield shift about δ_H 0.2–0.3 ppm if angeloyl/tigloyl group is at C-22 instead of C-21, while no significant changes could be found in the angeloyl/tigloyl ^{13}C NMR resonances if angeloyl/tigloyl group at either C-21 or C-22 (Wang et al., 2010; Zhang and Li, 2007; Zhang et al., 2006, 2010). This can be considered an important evidence to establish the substitution position of an angeloyl/tigloyl group at C-21 or C-22. Aesculioside G6 (**6**) and aesculioside G7 (**7**) were then elucidated as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-tigloyl-22β-angeloyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene and 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-angeloyl-22β-tigloyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene, respectively.

Aesculioside G8 (**8**) was assigned a molecular formula of $C_{57}H_{88}O_{22}$ as deduced from its HR-ESI-MS data ($[M-H]^-$ m/z, 1123.5640, $C_{57}H_{87}O_{22}$). The aglycone was elucidated as barringtogenol C according to NMR data analysis. The oligosaccharides were identified as glucuronopyranosyl acid, galactopyranosyl and arabinofuranosyl moieties, respectively, by chemical degradation (see Section 4) and their connectivity was established as galactopyranosyl affixing to C-2 and arabinofuranosyl to C-3 of the glucuronopyranosyl by a combination of ^1H, ^{13}C, DQF-COSY, HSQC, and

Table 2b
^{13}C NMR Spectroscopic Data for compounds **9**–**16** (150 MHz in pyridine-d_5).

Carbon	**9**	**10**	**11**	**12**	**13**	**14**	**15**	16
1	38.9	38.8	38.8	38.8	39.0	38.9	38.9	38.9
2	26.7	26.7	26.6	26.6	26.7	26.7	26.7	26.8
3	89.9	89.9	90.1	90.1	89.8	89.8	89.9	89.8
4	40.0	40.1	40.0	40.1	39.3	39.2	39.1	39.2
5	55.9	55.9	55.9	55.9	55.6	55.6	55.8	55.9
6	18.6	18.5	18.5	18.5	18.9	18.9	18.6	18.6
7	33.7	33.2	33.2	33.1	36.7	36.8	34.7	34.8
8	40.5	40.4	40.3	40.4	41.8	41.3	40.1	40.0
9	47.0	47.0	47.1	47.1	47.2	47.2	47.1	47.1
10	37.3	36.6	36.6	36.6	37.3	37.0	37.1	37.0
11	24.0	23.9	23.9	23.8	24.0	24.0	23.9	23.9
12	124.1	124.1	124.1	124.1	125.5	125.4	124.0	124.0
13	143.1	143.1	143.5	143.1	144.7	144.0	143.2	143.1
14	42.0	42.0	42.1	42.0	48.6	48.6	42.0	42.1
15	34.9	34.9	35.0	34.9	67.6	67.6	34.7	34.8
16	68.8	68.6	68.6	68.8	72.7	72.7	68.2	68.2
17	48.3	48.7	48.7	48.7	48.1	48.1	48.3	48.6
18	40.2	40.2	40.1	40.2	41.0	41.0	40.2	40.3
19	47.4	47.3	47.3	47.3	47.0	46.9	47.3	47.4
20	36.4	37.1	37.2	37.1	37.2	37.0	36.8	36.8
21	79.3	78.9	78.8	78.9	79.4	78.9	79.5	79.1
22	73.7	74.1	74.1	73.8	74.1	74.1	74.4	74.5
23	28.1	28.0	28.0	28.0	27.8	27.8	28.0	28.0
24	16.7	16.7	16.7	16.7	16.9	16.9	16.7	16.9
25	15.8	15.8	15.6	15.8	15.8	15.8	15.8	15.7
26	16.9	16.9	16.9	16.9	17.6	17.7	16.9	17.1
27	27.6	27.7	27.7	27.7	21.2	21.2	27.5	27.5
28	63.8	63.6	63.6	63.7	63.2	63.3	63.9	64.0
29	29.7	29.5	29.5	29.7	29.5	29.5	29.7	29.6
30	20.2	20.3	20.3	20.3	20.1	20.2	20.2	20.3
C_{21}	Tig	Ang	Ang	Ang	Tig	Ang	Tig	Ang
1	168.8	168.2	168.2	168.2	168.6	168.6	168.4	168.5
2	129.8	129.5	129.5	129.5	130.1	130.1	129.8	129.9
3	136.9	136.8	136.7	137.2	137.0	137.0	136.9	137.3
4	14.2	15.8	15.8	15.9	14.2	15.9	14.2	15.9
5	12.5	21.1	21.1	21.1	12.5	20.8	12.5	21.0
C_{22} or C_{28}	Ang	Tig	Tig	Ang	Ac	Ac	Ac	Ac
1	170.9	168.7	168.6	168.6	171.3	171.3	170.8	170.5
2	129.9	129.5	129.5	129.5	20.8	21.1	20.9	20.9
3	136.6	137.0	137.2	137.3				
4	15.8	14.1	14.1	15.9				
5	21.0	12.4	12.4	20.9				
C_3	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*	GlcA-*p*
1	105.3	105.1	105.1	105.1	105.1	105.1	105.3	105.3
2	78.9	78.8	78.7	78.8	78.7	78.7	78.8	78.9
3	86.3	86.3	86.1	86.1	85.8	85.8	86.3	86.4
4	71.9	71.9	71.5	71.5	71.9	71.8	71.9	71.9
5	77.2	77.1	76.4	76.4	77.3	77.3	77.3	77.4
6	172.5	172.9	169.7	169.7	172.3	172.6	172.5	172.5
OCH_3			52.3	52.3				
$C_{2'}$	Gal-*p*	Gal-*p*	Gal-*p*	Gal-*p*	Glc-*p*	Glc-*p*	Glc-*p*	Glc-*p*
1	104.8	104.8	104.8	104.8	104.0	104.0	104.1	104.1
2	73.5	73.6	73.5	73.5	76.2	76.2	76.2	76.3
3	75.2	75.2	75.2	75.2	78.4	78.4	78.4	78.6
4	69.9	69.9	69.9	69.8	72.6	72.6	72.7	72.8
5	76.8	76.7	76.8	76.8	77.9	78.1	78.1	78.1
6	62.0	62.0	61.9	61.9	63.6	63.6	63.6	63.7
$C_{3'}$	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*	Ara-*f*
1	111.1	111.1	111.1	111.2	110.9	110.9	110.9	111.0
2	83.6	83.6	83.6	83.6	83.4	83.4	83.6	83.6
3	77.8	77.8	77.7	77.7	77.8	77.8	77.8	77.9
4	85.6	85.6	85.6	85.6	85.8	85.8	85.6	85.7
6	62.5	62.5	62.5	62.4	62.5	62.5	62.5	62.2

HMBC spectroscopic analyses. The two quartets at δ_H 7.07 and 6.99 indicated two tigloyl groups in **8**. The two sets of ^{1}H and ^{13}C NMR tigloyl signals were fully assigned and the HMBC correlations of their carboxyl groups to H-21 or H-22 showed that the two tigloyls are at C-21 and C-22, respectively. Structure **8** was then determined as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-tigloyl-22β-tigloyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene.

Both aesculioside G9 (**9**) and aesculioside G10 (**10**) were determined as $C_{57}H_{88}O_{22}$ on the basis of their HR-ESI-MS spectra ($[M-H]^-$ *m/z*, 1123.5700 and 1123.5702) as well as their ^{13}C NMR data (Table 2b). NMR spectroscopic analysis suggested the same

Table 3
Cytotoxic activities of compounds **1**–**16** against A549 and PC-3 cell lines (GI_{50}).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Doxorubicin
A549 (μM)	18.12	22.81	22.66	16.52	>25	10.39	11.23	10.35	0.41
PC-3 (μM)	16.03	17.39	15.25	19.59	>25	8.99	5.42	8.96	0.32
	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**	
A549 (μM)	9.69	10.21	9.06	1.53	14.51	14.34	9.53	14.72	0.41
PC-3 (μM)	7.1	9.16	8.93	8.64	10.56	7.88	8.23	12.38	0.32

barringtogenol C aglycon and trisaccharide as found in **3**, **4** and **8**. Two acyl groups, tigloyl and angeloyl, were identified in both **9** and **10**. In **9**, the ^{1}H NMR data patterns of the tigloyl (δ_H 1.65 (H-4, 3H, *d*, *J* = 7.2 Hz) and 1.96 (H-5, 3H, *s*)) and angeloyl (δ_H 2.04 (H-4, 3H, *d*, *J* = 7.0 Hz) and 1.90 (H-5, 3H, *s*)) indicated tigloyl at C-21 and angeloyl at C-22 as previously discussed. The HMBC experiment further confirmed this deduction (δ_C 168.8 of tigloyl to δ_H 6.69 and 7.09, δ_C 170.9 of angeloyl to δ_H 6.39 and 5.88) (Table 2b). In **10**, an angeloyl group at C-21 and a tigloyl group at C-22 were established by the upfield shift of tigloyl signals, downfield shift of angeloyl and similar HMBC analysis. Therefore, structure **9** was elucidated as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-angeloyl-22β-tigloyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene. Accordingly, the structure of **10** was deduced as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-tigloyl-22β-angeloyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene.

The molecular formula of aesculioside G11 (**11**) was deduced as $C_{58}H_{90}O_{22}$ by its HR-ESI-MS spectral data ($[M-H]^-$ *m/z*, 1137.5827). The molecular weight, 14 mass units more than aesculioside G10 (**10**), suggested a methoxyl substitution of **10**. Extensive analysis of NMR data of **11** on its aglycone, as well as its 21 and 22 acylated side-chains, established the partial structure of 3β,16α,21β,22α,28-pentahydroxyolean-12-ene aglycone linked with 21-angeloyl and 22-tigloyl groups, which is same as that observed for aesculioside G10 (**10**). Further comparison of NMR data of **11** showed that it was almost superimposable to aesculioside G10 (**10**) except for slight differences in sugar signal regions. By the aid of DQF-COSY, HSQC, and 2D-TOCSY and HMBC, the signals of D-glucuronopyranosyl acid, D-galactopyranosyl, and L-arabinofuranosyl in the trisaccharide chain were unambiguously assigned. In the data set for D-glucuronopyranosyl acid, C-4 and C-5 signals were found downfield shifted (δ_C 71.5 and 76.4) and H-4 and H-5 was found upfield shifted (δ_H 4.30 and 4.42) (Fu et al., 2004). This indicated the substitution at C-6 of GlcA. A methoxyl group singlet at δ_H 3.76 (δ_C 52.3) was then identified and established as the substituent at the C-6 carboxyl of D-glucuronic acid by its HMBC correlation. Consequently, the structure of **11** was elucidated as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-[6-*O*-methyl]-β-D-glucuronopyranosyl-21β-tigloyl-22β-angeloyl-3β,16α, 21β,22α,28-pentahydroxyolean-12-ene.

The molecular formula of aesculioside G12 (**12**) was established as $C_{58}H_{90}O_{22}$ from the HR-ESI-MS spectral data ($[M-H]^-$ *m/z*, 1137.5840). The NMR data of **12** was in good resemblance with those of xanifolia-Y_{10} (Chan et al., 2008). The same structure of 3β,16α,21β,22α,28-pentahydroxyolean-12-ene aglycone linked with two angeloyl groups at C-21 and C-22 as in xanifolia-Y_{10} was confirmed by analysis of HSQC and HMBC correlations. Being 14 mass units more than xanifolia-Y_{10} and the presence of an additional methoxyl signal at δ_H 3.76 and δ_C 52.3 suggested that **12** is a methoxylated product of xanifolia-Y_{10}. Comparison of the D-glucuronic acid signals of **12** with those of xanifolia-Y_{10} showed that C-4 and C-5 (δ_C 71.5 and 76.4) in **12** were downfield shifted and H-4 (δ_H 4.30) was upfield shifted. This pattern implied methyl esterification of C-6 carbonyl of GlcA, which is same as aesculioside G11 (**11**). Further HMBC experiment confirmed the elucidation. Thus, **12** was determined as 3-*O*-[β-D-galactopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-[6-*O*-methyl]-β-D-glucuronopyranosyl-21β-angeloyl-22β-angeloyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene.

Aesculioside G13 (**13**) and aesculioside G14 (**14**) were determined to have the same molecular formula $C_{54}H_{84}O_{23}$ based on their HR-ESI-MS data ($[M-H]^-$ *m/z* 1099.5341 and 1099.5294). The characteristic chemical shifts of H_3-27 and C-27 (δ_H 1.86 and δ_C 21.2 for **13**, and δ_H 1.86 and δ_C 21.2 for **14**) suggested hydroxylation at C-15 and C-16 of the triterpenoid aglycon. The acylation of C-21 and C-22 was deduced from the NMR data (**13**, δ_H 6.59 and 6.26, *J* = 10.0 Hz, and δ_C 79.4 and 74.1; **14**, δ_H 6.60 and 6.20, *J* = 10.1 Hz, and δ_C 78.9 and 74.1). The quartet at δ_H 7.12 which correlated with δ_C 137.0 in HSQC spectrum indicated a tigloyl group in **13**. The two methyl groups in the tigloyl moiety were found in resonance at δ_H 1.67, 1.98 and δ_C 14.2, 12.5. The tigloyl unit together with another acetyl unit was established at C-21 and C-22, respectively, by their HMBC correlations of the carbonyl signal with H-21 and H-22. The trisaccharide chain consisted of D-glucuronic acid, D-glucose, and L-arabinose as determined by acid hydrolysis products. The complete assignment of the NMR signals was fulfilled by a combination of DQF-COSY, 2D-TOCSY, and HSQC spectroscopic analysis. The linkages of β-D-glucopyranosyl and α-L-arabinofuranosyl were determined at the C-2 and C-3 of the β-D-glucuronopyranosyl unit, respectively, by the HMBC correlations. Compared with the NMR data of the same sugar units in our previous reports on aesculioside IIe from *A. pavia*, significant chemical shift changes can be found with the NMR data of the β-D-glucopyranosyl unit (Zhang and Li, 2007; Zhang et al., 2006). C-4 and C-6 in the D-glucose ^{13}C NMR data of **13** shifted about 2–3 ppm. H-4 and H-5 were also found downfield and upfield shifted, respectively (both by about 0.3–0.4 ppm). These changes can be attributed to the effects of C-24 substitution (Matsushita et al., 2004; Zhang and Li, 2007; Zhang et al., 2006). All triterpenoid saponins isolated from *A. pavia* with the same sugar chain of 3-*O*-[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl had hydroxyl substitutions at C-24. Thus, the structure of **13** was established as 3-*O*-[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-tigloyl-22β-acetyl-3β,15α,16α,21β,22α, 28-hexahydroxyolean-12-ene. With similar analysis and elucidation, **14** was determined as 3-*O*-[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-angeloyl-22β-acetyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

Aesculioside G15 (**15**) and aesculioside G16 (**16**) had the same molecular formula of $C_{54}H_{84}O_{22}$ as determined by negative HR-ESI-MS ($[M-H]^-$ *m/z* 1083.5329 and 1083.5365). Their NMR spectroscopic data were found superimposable to aesculiosides II_j and II_k with the exception of differences in the sugar signal region with glucose in place of galactose (Zhang and Li, 2007). Based on the results of acid hydrolysis and NMR spectroscopic data analysis as used for elucidating aesculiosides G13 (**13**) and G14 (**14**), the trisaccharide structure 3-*O*-[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl was established. The structures of aesculioside G15 (**15**) and aesculioside G16 (**16**) were thus assigned

as 3-*O*-[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-tigloyl-22β-acetyl-3β,16α,21β,22α, 28-pentahydroxyolean-12-ene and 3-*O*-[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl-(1→3)-β-D-glucuronopyranosyl-21β-angeloyl-22β-acetyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene, respectively.

Compounds **1**–**16** were tested for their cytotoxicity against non-small cell lung tumor cells (A549) and human prostate cancer cell line (PC-3) with doxorubicin serving as a positive control. As shown in Table 3, the GI_{50} of **1**–**16** ranged from 5.4 to >25 μM. These results basically support previous reports that compounds with angeloyl/tigloyl groups at both C-21 and C-22 had better activity than those with only one angeloyl/tigloyl group at C-21 or C-22 (Chan, 2007; Chan et al., 2008; Wang et al., 2010). No influence of 6-*O*-methylation at β-D-glucuronopyranosyl on the cytotoxicity has been observed in this study.

3. Conclusion

There are a total of 210 compounds, including 99 triterpenoids and triterpenoid saponins that have been identified from the genus *Aesculus* (Zhang et al., 2010). Current phytochemical investigations on *A. glabra* have suggested that the *Aesculus* genus is a good source of cytotoxic structurally diverse saponins that exhibit cytotoxic effects. The series of saponins from *A. glabra* had the same trisaccharide chain as those isolated from *A. pavia*, which is an arabinofuranosyl unit affixed to C-3 of the glucuronopyranosyl unit and an galactopyranosyl/glucopyranosyl unit affixed to C-2. Our previous hypothesis on the chemotaxonomic value of an arabinofuranosyl substitution at glucuronopyranosyl C-3 as a biomarker for North American *Aesculus* species was confirmed with the trisaccharide chain structural feature (Zhang and Li, 2007; Zhang et al., 2006). Two other important structural features of triterpenoid saponins in *A. glabra* are: (1) no saponins with a 24-OH have been found in *A. glabra*; (2) the C-2 of the glucuronopyranosyl unit could be either glucopyranosyl or galactopyranosyl. Previous studies have shown that the C-2 of the glucuronopyranosyl unit is normally galactopyranosyl if there is no hydroxyl at C-24, but is a glucopyranosyl or xylopyranosyl unit if a hydroxyl is present at C-24 (Zhang et al., 2010). More discovery of new saponins from the genus *Aesculus* may not only provide more structure activity data of acylated polyhydroxy saponins, but may also contribute to enhancing our understanding of the taxonomy and evolution of the genus *Aesculus*.

4. Experimental

4.1. General experimental procedures

NMR experiments were performed using either a Bruker DRX600 or JEOL ECS 400 spectrometers, with spectroscopic data referenced to the solvent used. HR-mass spectra were acquired using a PE SCIEX QSTAR LC/MS/MS and a Waters Q-Tof Premier mass spectrometer. Optical rotation values were measured on a JASCO P-1010 polarimeter. Octadecyl-functionalized silica gel (ODS, Aldrich) was used for open column chromatography (CC). HPLC analysis was performed on an Agilent 1100 HPLC system with an Agilent 1100 diode array detector or an Agilent 1100 refractive index detector using Agilent ODS columns (Zorbax SB-C18, 4.6 × 250 mm, 5 μm, column A; Zorbax SB-C18, 4.6 × 250 mm, 3.5 μm, column B) or a SUPEICOGEL CA column (column C, 300 × 7.8 mm, Supelco). Preparative HPLC was performed with an Acuflow Series III pump connected to an Acutect 500 UV/VIS detector using an Alltima C18 column (column D, 250 × 22 mm, 10 μm, Alltech).

4.2. Plant material

Seeds of *A. glabra* were purchased from F.W. Schumacher Co., Inc. (Sandwich, MA) and were identified by Shiyou Li. A voucher specimen (Tex-Nac-FWS-2009) was deposited at the National Center for Pharmaceutical Crops at Stephen F. Austin State University, USA.

4.3. Extraction and isolation

A total of 1 kg air-dried plant material were ground to a coarse powder with a Thomas Model 4 Wiley Mill. The ground material were placed into a 100 × 800 mm column and percolated with EtOH–H_2O (8 L, 95:5, v/v) at room temperature for 24 h. The extracts were concentrated under reduced pressure to give a residue (105 g). This was then suspended in H_2O (1 L) and partitioned successively with EtOAc (3×1 L) and *n*-BuOH (3×1 L). The *n*-butanol fraction (35 g) was applied to an open ODS column, and successively eluted with 1L of MeOH–H_2O (35:65, 65:35, 80:20) and MeOH, respectively. A Spectra/Chrom CF-2 Fraction Collector (Houston, TX, USA) was used to collect 50 mL elution for each fraction. Fractions were then dried with Savant SpeedVac System AES 2010 (Thermo Fisher Scientific) and dissolved in DMSO for the further analysis and separation. Based on results from HPLC analysis (column A, CH_3CN:H_2O with 0.5% HOAc (45:55, v/v), 0.7 mL/min, detected at 205 nm), fractions 30 and 31 were applied on preparative HPLC (column D, CH_3CN:H_2O with 0.5% HOAc (40:60, v/v), 3 mL/min, detected at 205 nm) to furnish three subfractions. From subfraction I, **5** (t_R 41 min, 9 mg) and aesculioside II_a (t_R 42 min, 17 mg) were obtained (column B, CH_3CN:H_2O with 0.5% HOAc (37:63, v/v), 0.7 mL/min, detected at 205 nm). **13** (t_R 31 min, 5 mg) and aesculioside II_f (t_R 32 min, 19 mg) were isolated from subfraction II (column B, CH_3CN:H_2O (40:60, v/v), 0.7 mL/min). Subfraction III afforded **14** (t_R 34 min, 12 mg) and aesculioside II_g (t_R 36 min, 14 mg) (column B, CH_3CN:H_2O (40:60, v/v), 0.7 mL/min). Fractions 34 and 35 were combined and gave **1** (t_R 67 min, 32 mg) and **2** (t_R 73 min, 27 mg) from preparative HPLC (column D, CH_3CN:H_2O with 0.5% HOAc (40:60, v/v), 3 mL/min, detected at 205 nm). Fraction 46 was isolated by analytical HPLC to yield **15** and aesculioside II_j (t_R 39 min, 5 mg; t_R 40 min, 2 mg) (column B, CH_3CN:H_2O with 0.5% HOAc (40:60, v/v), 0.7 mL/min). Fraction 47 afforded **16** and aesculioside II_k (t_R 42 min, 13 mg; t_R 44 min, 10 mg) using same HPLC method as that used for fraction 46. Preparative HPLC of fraction 57 (column D, CH_3CN:H_2O with 0.5% HOAc (48:52, v/v), 3 mL/min, detected at 205 nm) successively yielded **3** (26 mg, t_R 47 min), **4** (65 mg, t_R 52 min), aesculioside III_f (15 mg, t_R 56 min), **6** (62 mg, t_R 62 min), and **7** (10 mg, t_R 68 min). Fractions 59 and 60 were isolated to afford compounds xanifolia-Y_3 (180 mg) using preparative HPLC (column D, t_R 72 min with CH_3CN:H_2O with 0.5% HOAc (48:52, v/v) or t_R 45 min with CH_3CN:H_2O with 0.5% HOAc (55:45, v/v), 3 mL/min). Compounds **8** (3 mg), **9** (2 mg), and **10** (8 mg) were purified from fractions 61 and 62 by preparative HPLC (column D, CH_3CN:H_2O with 0.5% HOAc (55:45, v/v), 3 mL/min, detected at 205 nm, t_R 50, 55 and 63 min, respectively). Xanifolia-Y_{10} (100 mg, t_R 67 min) was separated from fraction 63 (column D, CH_3CN:H_2O with 0.5% HOAc (55:45, v/v), 3 mL/min). Using preparative HPLC, fractions 69–71 afforded compounds **11** (3 mg, t_R 58 min) and **12** (16 mg, t_R 63 min) (column D, CH_3CN:H_2O with 0.5% HOAc (65:35, v/v), 3 mL/min).

4.4. Aesculioside G1 (*1*)

White amorphous powder; $[\alpha]_D^{20}$ +2.3 (c 0.340, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1a and 2a; HRESIMS: *m/z* 1099.5373 $[M-H]^-$ (calcd. for $C_{54}H_{83}O_{23}$, 1099.5325).

4.5. Aesculioside G2 (**2**)

White amorphous powder; $[\alpha]_D^{20}$ −1.8 (c 0.363, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1a and 2a; HRESIMS: *m/z* 1099.5361 $[M-H]^-$ (calcd. for $C_{54}H_{83}O_{23}$, 1099.5325).

4.6. Aesculioside G3 (**3**)

White amorphous powder; $[\alpha]_D^{20}$ +7.4 (c 0.310, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1a and 2a; HRESIMS: *m/z* 1083.5378 $[M-H]^-$ (calcd. for $C_{54}H_{83}O_{22}$, 1083.5376).

4.7. Aesculioside G4 (**4**)

White amorphous powder; $[\alpha]_D^{20}$ −1.3 (c 0.651, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1a and 2a; HRESIMS: *m/z* 1083.5382 $[M-H]^-$ (calcd. for $C_{54}H_{83}O_{22}$, 1083.5376).

4.8. Aesculioside G5 (**5**)

White amorphous powder; $[\alpha]_D^{20}$ −8.0 (c 0.163, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1a and 2a; HRESIMS: *m/z* 1057.5229 $[M-H]^-$ (calcd. for $C_{52}H_{81}O_{22}$, 1057.5220).

4.9. Aesculioside G6 (**6**)

White amorphous powder; $[\alpha]_D^{20}$ −21.6 (c 0.554, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1a and 2a; HRESIMS: *m/z* 1139.5638 $[M-H]^-$ (calcd. for $C_{57}H_{87}O_{23}$, 1139.5638).

4.10. Aesculioside G7 (**7**)

White amorphous powder; $[\alpha]_D^{20}$ −18.2 (c 0.121, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1a and 2a; HRESIMS: *m/z* 1139.5654 $[M-H]^-$ (calcd. for $C_{57}H_{87}O_{23}$, 1139.5638).

4.11. Aesculioside G8 (**8**)

White amorphous powder; $[\alpha]_D^{20}$ −7.9 (c 0.076, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1a and 2a; HRESIMS: *m/z* 1123.5640 $[M-H]^-$ (calcd. for $C_{57}H_{87}O_{22}$, 1123.5689).

4.12. Aesculioside G9 (**9**)

White amorphous powder; $[\alpha]_D^{20}$ −15.2 (c 0.062, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1b and 2b; HRESIMS: *m/z* 1123.5700 $[M-H]^-$ (calcd. for $C_{57}H_{87}O_{22}$, 1123.5689).

4.13. Aesculioside G10 (**10**)

White amorphous powder; $[\alpha]_D^{20}$ −15.3 (c 0.247, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1b and 2b; HRESIMS: *m/z* 1123.5702 $[M-H]^-$ (calcd. for $C_{57}H_{87}O_{22}$, 1123.5689).

4.14. Aesculioside G11 (**11**)

White amorphous powder; $[\alpha]_D^{20}$ −22.3 (c 0.102, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1b and 2b; HRESIMS: *m/z* 1137.5827 $[M-H]^-$ (calcd. for $C_{58}H_{89}O_{22}$, 1137.5846).

4.15. Aesculioside G12 (**12**)

White amorphous powder; $[\alpha]_D^{20}$ −19.5 (c 0.237, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1b and 2b; HRESIMS: *m/z* 1137.5840 $[M-H]^-$ (calcd. for $C_{58}H_{89}O_{22}$, 1137.5846).

4.16. Aesculioside G13 (**13**)

White amorphous powder; $[\alpha]_D^{20}$ −15.7 (c 0.240, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1b and 2b; HRESIMS: *m/z* 1099.5341 $[M-H]^-$ (calcd. for $C_{54}H_{83}O_{23}$, 1099.5325).

4.17. Aesculioside G14 (**14**)

White amorphous powder; $[\alpha]_D^{20}$ −14.5 (c 0.286, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1b and 2a; HRESIMS: *m/z* 1099.5294 $[M-H]^-$ (calcd. for $C_{54}H_{83}O_{23}$, 1099.5325).

4.18. Aesculioside G15 (**15**)

White amorphous powder; $[\alpha]_D^{20}$ −26.9 (c 0.121, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1b and 2b; HRESIMS: *m/z* 1083.5329 $[M-H]^-$ (calcd. for $C_{54}H_{83}O_{22}$, 1083.5376).

4.19. Aesculioside G16 (**16**)

White amorphous powder; $[\alpha]_D^{20}$ −27.9 (c 0.073, MeOH); For ^1H NMR and ^{13}C spectroscopic data, see Tables 1b and 2b; HRESIMS: *m/z* 1083.5365 $[M-H]^-$ (calcd. for $C_{54}H_{83}O_{22}$, 1083.5376).

4.20. Acid hydrolysis and alkaline hydrolysis (Zhang et al., 2006)

Compounds **1** (11.8 mg), **5** (5.5 mg), **13** (3.1) and **15** (2.6 mg) were respectively incubated in a solution of 3 mL 1 M HCl in dioxane/water (1:1, v/v), this being maintained at 80 °C for 2 h. The solutions were individually evaporated to remove dioxane and extracted with $CHCl_3$–MeOH (7:3) (3×3 mL). The upper aqueous layers containing monosaccharides were neutralized using an ion-exchange resin (Amberlite MB-3) column, and then lyophilized to give a sugar mixture. Sugar mixtures were developed by TLC with a solvent system, MeCOEt-iso-PrOH–Me_2CO–H_2O (20:10:7:6). Monosaccharides from **1**, **5**, **13** and **15** were identified with authentic sugar samples. After preparative TLC of the sugar mixture from **1** and **5**, the optical rotation of each purified sugar was measured.

The lower layer partitioned from **1** hydrolyzed products extraction was washed with H_2O and concentrated to give an amorphous powder (5.6 mg). The powder was heated in 0.8 M NaOH (3 mL) at 80 °C for 4 h. The reaction mixture was neutralized with 1 M HCl and extracted with EtOAc (3 mL × 3). The organic layers were combined and then evaporated to dryness under a vacuum. The residue was subjected to HPLC purification affording R1-barrigenol (2.3 mg), this being identified by co-HPLC analysis with an authentic sample and comparison of NMR data with reference.

4.21. Cytotoxicity assays

The A549 and PC-3 cell lines were a kind gift from Peiying Yang of The University of Texas MD Anderson Cancer Center. The cytotoxicity assay procedure using WST-8 as a measurement of cell growth and viability was performed as previously described (Wang et al., 2010). Doxorubicin was used as a positive control in all assays.

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