Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 68 (2007) 2075–2086
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Cytotoxic triterpenoid saponins from the fruits of *Aesculus pavia* L.

Zhizhen Zhang, Shiyou Li *

National Center for Pharmaceutical Crops, Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, Texas 75962-6109, USA

Received 30 November 2006; received in revised form 26 February 2007
Available online 27 June 2007

Abstract

Continued chemical investigation on the fruits of North American *Aesculus pavia* L. resulted in the isolation and identification of 13 polyhydroxyoleanene pentacyclic triterpenoid saponins, named aesculiosides IIe–IIk (**1**–**7**), and IIIa–IIIf (**8**–**13**), together with 18 known compounds: aesculiosides Ia–Ie (**14**–**18**), IIa–IId (**19**–**22**), IVa–IVc (**23**–**25**), 3-*O*-[β-D-galactopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene (**26**), 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,16α,21β,22α,24β,28-hexahydroxyolean-12-ene (**27**), 3-*O*-[β-D-galactopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene (**28**), R_1-barrigenol (**29**), scopolin (**30**), and 5-methoxyscopolin (**31**). The structures of these compounds were elucidated by spectroscopic and chemical analyses. Compounds **14**–**22** and **26**–**28** were tested *in vitro* for their activity against 59 cell lines from nine different human cancers including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast. It was found that compounds with two-acyl groups at C-21 and C-22 had cytotoxic activity for all cell lines tested with GI_{50} 0.175–8.71 μM, while compounds without acyl groups at C-21 and C-22 had weak or no cytotoxic activity. These results suggest that the acyl groups at C-21 and C-22 are essential for their activity.

Keywords: *Aesculus pavia* L.; Hippocastanaceae; Cytotoxic activity; Triterpenoid saponins; Aesculiosides

1. Introduction

Aesculus pavia L., known as "red buckeyes" or "scarlet buckeye", is a shrubby or small tree species naturally distributed in the southeastern United States (Little, 1980). Previous work on this plant indicated the presence of carotenoids (Neamtu and Bodea, 1974) and sapogenins (Schrutka-Rechtenstamm et al., 1988). We recently isolated and identified 12 new polyhydroxyoleanene pentacyclic triterpenoid saponins aesculiosides Ia–Ie, IIa–IId, IVa–IVc from a plant located in Nacogdoches, Texas (Zhang et al., 2006). From a plant of different provence growing in the same location, we surprisedly isolated 13 new triterpenoid saponins named aesculiosides IIe–IIk (**1**–**7**) and IIa–IIIf (**8**–**13**). In this paper, we report the isolation and structure elucidation of these new compounds and the cytotoxic activity of some saponins.

2. Results and discussion

The column fraction Aes-P from an ethanol extract of the fruits of *A. pavia* L. was separated by a low-pressure column of ODS to give four fractions Aes-I, Aes-II, Aes-III, and Aes-IV. Further separation of Aes-I, Aes-II, Aes-III and Aes-IV was achieved by preparative MPLC and HPLC using a preparative ODS column and an analytical ODS column to give compounds **1**–**31**, respectively (see Section 4).

Known compounds **14**–**18**, **30**, and **31** were obtained from fraction Aes-I, and identified by NMR and MS analysis as aesculiolisides Ia–Ie (**14**–**18**) (Zhang et al., 2006), scopolin (**30**) (Zhang et al., 2005), and 5-methoxy-

* Corresponding author. Tel.: +1 936 468 2071; fax: +1 936 468 7058.
E-mail address: lis@sfasu.edu (S. Li).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.05.020

scopolin (**31**) (Wagner and Bladt, 1975). While known compounds **23**–**29** were obtained from fraction Aes-IV and identified by NMR and MS analysis as aesculiolisides IVa–IVc (**23**–**25**) (Zhang et al., 2006), 3-*O*-[β-D-galactopyranosyl (1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,15α,16α, 21β,22α, 28-hexahydroxyolean-12-ene (**26**) (Chan et al., 2005), 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,16α,21β, 22α,24β,28-hexahydroxyolean-12-ene (**27**) (Voutquenne et al., 2005; Zhang et al., 2006), 3-*O*-[β-D-galactopyranosyl-(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene (**28**) (Voutquenne et al., 2005; Zhang et al., 2006), and R_1-barrigenol (**29**) (Zhang et al., 2006), respectively.

Compounds **1**–**7** and **19**–**22** were obtained from fraction Aes-II as colorless powders. Compounds **19**–**22** were identified by NMR and MS analysis and comparison with reference data as known saponins, aesculiosides IIa–IId (**19**–**22**) (Zhang et al., 2006).

Each of the compounds **1**–**7** contained either a tigeloyl or an angeloyl group at C-21 and an acetyl group at C-22, as indicated by the characteristic NMR signals of these groups (Tables 1 and 3) (Yoshikawa et al., 1996, 1998; Zhang et al., 1999, 2006). The downfield chemical shifts at δ 6.59–6.62 (1H, *d*, 10.0–10.1 Hz) and δ 6.22–6.27 (1H, *d*, 10.0–10.1 Hz) were assigned to H-21 and H-22,

Table 1
^{13}C NMR spectroscopic data for the aglycone moieties of **1**–**13** (150 MHz in pyridine-d_5)[a]

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	38.4	38.8	38.8	38.4	38.4	38.4	38.6	38.3	38.5	38.7	38.3	38.5	38.6
2	26.5	26.6	26.6	26.4	26.5	26.5	26.3	26.5	26.5	26.5	26.3	26.4	26.5
3	91.3	89.8	89.9	91.2	91.5	89.7	89.7	91.0	91.2	89.6	91.0	91.1	89.6
4	43.5	39.6	39.6	43.6	43.6	39.8	39.7	43.5	43.6	39.9	43.5	43.6	39.7
5	55.6	55.5	55.6	55.8	56.0	55.9	55.6	55.9	56.0	55.6	55.7	55.8	55.8
6	18.4	18.7	18.8	18.3	18.4	18.3	18.1	18.3	18.5	18.6	18.4	18.6	18.5
7	36.4	36.6	36.6	32.9	33.1	32.9	33.0	33.0	36.5	36.6	36.5	36.5	36.6
8	40.2	40.8	40.9	40.1	39.9	40.0	40.0	39.6	40.5	40.0	39.9	40.0	40.6
9	46.9	47.1	47.1	46.7	46.7	46.8	46.8	46.8	47.1	47.0	46.9	46.9	46.9
10	36.6	36.9	36.9	36.5	36.6	36.7	36.7	36.5	36.6	36.7	36.5	36.7	36.8
11	23.7	23.9	23.9	23.7	24.0	23.4	23.6	23.6	23.5	23.6	23.7	23.7	23.6
12	124.9	125.3	125.4	123.6	123.5	123.5	123.5	123.7	125.3	125.3	124.9	125.2	125.1
13	143.5	143.5	143.6	143.2	142.8	142.9	142.8	142.8	143.5	143.6	143.0	143.1	143.5
14	47.5	47.7	47.7	41.6	41.6	41.5	41.6	41.8	47.5	47.6	47.5	47.6	47.6
15	67.5	67.4	67.5	34.4	34.6	34.2	34.3	34.5	67.6	67.5	67.4	67.5	67.6
16	72.7	73.4	72.8	67.8	68.0	68.0	67.9	67.9	73.2	73.1	73.3	73.3	73.6
17	48.1	48.1	48.3	48.1	48.0	47.9	47.8	48.1	48.1	48.0	48.0	47.9	48.1
18	40.6	41.0	41.4	40.2	40.1	40.1	40.1	40.2	40.7	40.6	40.6	40.6	40.9
19	46.6	46.8	46.8	47.2	47.1	47.0	47.0	47.3	46.9	46.9	46.7	46.7	46.8
20	36.3	36.3	36.1	36.3	36.3	36.2	36.2	36.2	36.2	36.2	36.2	36.2	36.3
21	78.9	79.2	78.9	79.1	78.9	79.0	78.9	80.9	79.0	79.0	78.9	78.9	79.0
22	73.6	73.4	73.7	74.0	74.3	74.1	74.0	70.9	73.3	73.4	73.6	73.4	73.8
23	21.9	27.9	27.9	22.0	22.2	27.7	27.7	22.0	22.1	27.9	22.0	22.0	27.8
24	63.0	16.7	16.8	63.1	63.2	16.5	16.5	63.1	63.6	16.7	63.1	63.2	16.8
25	15.4	15.7	15.7	15.5	15.5	15.5	15.5	15.5	15.8	15.6	15.5	15.5	15.7
26	17.1	17.5	17.5	16.5	16.6	16.8	16.8	16.6	17.5	17.4	17.1	17.0	17.4
27	20.9	21.0	20.6	27.2	27.4	27.1	27.2	27.1	20.9	20.9	20.7	20.8	20.8
28	62.8	63.4	63.2	63.5	63.8	63.4	63.6	66.3	63.3	63.1	62.9	63.0	63.0
29	29.3	29.4	29.4	29.5	29.4	29.4	29.4	29.6	29.7	29.5	29.3	29.3	29.3
30	19.9	20.0	20.1	19.9	20.2	19.9	20.0	19.9	20.1	19.9	19.9	19.9	19.9
C_{21}	Tig	Tig	Ang	Tig	Ang	Tig	Ang	Ang	Ang	Tig	Tig	Tig	Tig
1	168.0	168.0	167.9	168.1	167.8	167.9	168.0	168.0	167.9	168.2	168.2	168.0	167.9
2	129.4	129.3	129.1	129.2	128.9	129.0	129.0	129.6	129.7	129.9	129.8	129.7	129.8
3	137.0	137.0	137.2	136.8	137.1	136.8	137.0	136.9	137.0	136.9	136.8	136.8	137.0
4	14.0	14.2	15.8	13.9	15.9	13.9	15.6	15.6	15.6	14.1	13.8	13.9	14.0
5	12.3	12.4	21.0	12.2	21.0	12.3	20.9	20.6	20.6	12.3	12.1	12.2	12.3
C_{22} or C_{28}	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Mp	Mp	Tig	Ang	Tig
1	170.8	170.8	170.7	170.8	170.9	170.6	170.6	170.7	176.9	177.0	168.5	168.3	168.3
2	20.7	20.7	20.9	20.8	20.8	20.6	20.7	20.7	34.6	34.7	129.3	129.5	129.5
3									19.2	19.3	137.0	136.9	137.1
4									19.5	19.5	13.6	15.3	13.9
5											11.9	20.4	12.2

[a] Assignments were based on COSY, HMQC, and HMBC experiments.

Table 2
^{13}C NMR spectroscopic data for the sugar moieties of **1**–**13** (150 MHz in pyridine-d_5)[a]

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	12	**13**
GlcA-*p*													
1	104.5	105.1	105.4	104.5	104.8	104.2	104.8	104.5	105.0	104.9	104.2	104.2	104.6
2	78.0	78.1	78.0	78.0	78.2	78.2	78.2	78.2	78.4	78.4	78.1	78.2	78.5
3	86.2	86.2	86.3	86.3	86.6	86.1	86.1	86.1	86.3	86.2	86.3	86.2	86.3
4	69.5	69.7	69.7	71.6	71.7	69.5	69.5	69.5	69.6	69.7	69.5	69.5	69.6
5	77.1	77.1	77.2	77.1	77.3	77.3	78.5	76.7	77.3	77.3	77.2	77.1	77.2
6	171.9	172.0	171.9	172.0	171.9	171.9	171.9	171.9	169.1	169.0	171.9	171.9	171.9
	Glc-*p*	Gal-*p*	Gal-*p*	Glc-*p*	Glc-*p*	Gal-*p*	Gal-*p*	Glc-*p*	Glc-*p*	Gal-*p*	Glc-*p*	Glc-*p*	Gal-*p*
1	103.7	104.7	104.7	103.6	103.9	104.3	104.5	103.5	103.7	104.6	103.5	104.0	104.4
2	75.0	73.9	73.4	75.2	75.5	73.2	73.3	75.3	75.5	73.2	75.3	75.3	73.5
3	78.0	75.0	75.1	78.1	78.0	75.0	75.0	78.2	78.4	75.0	78.2	78.2	75.0
4	69.5	69.7	69.7	69.5	69.5	69.7	69.5	69.5	69.6	69.7	69.5	69.5	69.5
5	78.2	76.6	76.6	78.2	78.3	76.4	76.4	78.1	78.6	76.5	78.2	78.3	76.6
6	61.2	61.8	61.8	61.2	61.3	61.9	61.9	61.5	61.6	61.9	61.7	61.7	62.0
Ara-*f*													
1	111.2	111.0	111.0	110.9	111.2	110.6	110.8	110.9	110.3	110.1	110.0	110.8	110.7
2	83.2	83.5	83.5	83.4	83.6	83.2	83.3	83.1	83.6	83.6	83.5	83.6	83.5
3	77.5	77.5	77.7	77.5	77.5	77.5	77.6	77.9	77.8	77.9	77.6	77.6	77.9
4	85.0	85.4	85.4	85.2	85.4	85.2	85.2	85.1	85.5	85.4	85.2	85.2	85.6
5	62.3	62.3	62.4	62.3	62.3	62.5	62.3	62.7	63.0	62.6	62.4	62.5	62.3

[a] Assignments were based on COSY, HMQC, and HMBC experiments.

respectively, based on the HMBC correlations. The angeloyl or tigenoyl group at C-21 and the acetyl group at C-22 were established by HMBC correlations of H-21 of the aglycone with C-1 of the angeloyl or tigenoyl group, and H-22 of the aglycone with C-1 of the acetyl group. Full assignments of the ^{13}C and ^{1}H NMR signals were achieved by a combination of ^{1}H, ^{13}C, COSY, HMQC, and HMBC spectroscopic analyses.

Aesculioside II$_e$ (**1**) was assigned a molecular formula of $C_{54}H_{84}O_{24}$, as deduced from the main $[M + Na]^+$ ion *m/z* at 1139.5248 in the positive HRESIMS as well as from its NMR spectroscopic data. The NMR data (Tables 1–4) of **1** were characteristic of a polyhydroxyoleanene triterpenoid glycoside. After detailed NMR spectra interpretation, the aglycone was identified as 3β,15α,16α,21β,22α,24β,28-heptahydroxyolean-12-ene (**1a**) (Zhang et al., 2006). The stereochemistry of the aglycone was confirmed by a NOESY experiment (Fig. 1) (Tang et al., 2004). Compound **1** exhibited three sugar anomeric protons at δ 4.80 (1H, *d*, $J = 7.8$ Hz), 5.47 (1H, *d*, $J = 7.6$ Hz), 6.06 (1H, *br s*) (Table 4) and three protonated carbons at δ 104.5, 103.7, 111.2 (Table 2) in HMQC spectrum. The ^{13}C NMR spectrum of **1** displayed 54 carbon signals, from which 30 were assigned to the aglycone part, 17 to the trisaccharide moiety, five to a tigeloyl group, and the remaining two to an acetyl group. Alkaline hydrolysis of **1** liberated prosapogenin that was identified as aesculioliside I$_b$ (**15**) (Zhang et al., 2006) by co-HPLC analysis with the authentic sample. Acid hydrolysis of **1** produced three monosaccharide sugars (D-glucuronic acid (GlcA), D-glucose (Glu), and L-arabinose (Ara)), which were identified by co-TLC analysis and by measurement of optical rotation after separation by preparative TLC (Zhang et al., 2006). The ^{1}H and ^{13}C NMR spectra of **1** displayed characteristic signals (Tables 1 and 3) of a tigeloyl group (Yoshikawa et al., 1996, 1998; Zhang et al., 1999) and an acetyl group (δ_C 170.8, 20.7 and δ_H 2.00) (Tables 1 and 3). As observed in the HMBC spectrum, the long-range correlations of H-21 (δ 6.59, 1H, *d*, 10.0 Hz) of the aglycone with C-1 (δ 168.0) of the tigeloyl unit, and H-22 (δ 6.27, 1H, *d*, 10.0 Hz) of the aglycone with C-1 of the acetyl unit established that the tigeloyl and acetyl groups were attached to C-21 and C-22, respectively. The downfield chemical shifts of H-21 and H-22 also indicated that C-21 and C-22 were the acyl positions. The identity of the monosaccharides and the sequence of the trisaccharide chain were determined by a combination of ^{1}H–^{1}H COSY, HMQC, HMBC, and ROESY experiments, and by comparison with reference data (Zhang et al., 2006). After the assignments of the protons and protonated carbons were established (Tables 2 and 4), the three sugar units were identified as glucuronic acid, glucose and arabinose (Yoshikawa et al., 1996, 1998; Zhang et al., 1999, 2006). From their ^{13}C NMR spectroscopic data and $^{3}J_{H1,H2}$ coupling constants (Tables 2 and 4), the glucuronic acid and glucose units were determined to be in the pyranose form with a β-anomeric configuration, and arabinose to be in the α-furanose form (Zhang et al., 2006). The whole sugar sequence of **1** was deduced from the HMBC and ROESY information as described in Fig. 1. The structure of aesculioside II$_e$ (**1**) was established as 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-tigeloyl-22-*O*-acetyl-3β,15α,16α,21β,22α,24β,28-heptahydroxyolean-12-ene.

Aesculioside II$_f$ (**2**) gave a $[M + Na]^+$ ion *m/z* at 1123.5299 in the positive HRESIMS, 16 mass units lower

Table 3
^{1}H NMR spectroscopic data for the aglycone moieties of **1**–**7** (600 MHz in pyridine-d_5)[a]

Proton	1	2	3	4	5	6	7
1	0.85, 1.43	0.81, 1.40	0.81, 1.42	0.82, 1.32	0.78, 1.40	0.82, 1.42	0.84, 1.41
2	2.16 (2H, *m*)	2.12 (2H, *m*)	2.18 (2H, *m*)	2.28 (2H, *m*)	2.29 (2H, *m*)	2.12 (2H, *m*)	2.14 (2H, *m*)
3	3.45 (1H, *dd*-like)	3.27 (1H, *dd*-like)	3.29 (1H, *dd*-like)	3.44 (1H, *dd*-like)	3.44 (1H, *dd*-like)	3.30 (1H, *dd*-like)	3.28 (1H, *dd*-like)
5	0.90	0.82	0.82	0.84	0.86	0.80	0.80
6	1.60, 1.62	1.59, 1.62	1.57, 1.59	1.51, 1.56	1.53, 1.55	1.50, 1.57	1.51, 1.53
7	2.01, 2.08	2.05, 2.12	2.00, 2.07	1.26, 1.53	1.28, 1.53	1.30, 1.58	1.30, 1.57
9	1.67	1.70	1.66	1.72	1.67	1.72	1.72
11	1.90, 1.95	1.80, 1.90	1.73, 1.87	1.72, 1.90	1.64, 1.87	1.72, 1.90	1.77, 1.88
12	5.52 (1H, *br s*)	5.51 (1H, *br s*)	5.51 (1H, *br s*)	5.40 (1H, *br s*)	5.39 (1H, *br s*)	5.44 (1H, *br s*)	5.41 (1H, *br s*)
15	4.20	4.19	4.21	1.61, 1.87	1.61, 1.90	1.62, 1.88	1.64, 1.85
16	4.45	4.45	4.52	4.46	4.47	4.47	4.47
18	3.08	3.05	3.03	3.07	3.04	3.09	3.07
19	1.43, 2.09	1.46, 3.06	1.43, 3.07	1.40, 3.08	1.42, 3.10	1.50, 3.10	1.42, 3.10
21	6.59 (1H, *d*, 10.0 Hz)	6.57 (1H, *d*, 10.0 Hz)	6.61 (1H, *d*, 10.1 Hz)	6.59 (1H, *d*, 10.0 Hz)	6.62 (1H, *d*, 10.1 Hz)	6.60 (1H, *d*, 10.0 Hz)	6.62 (1H, *d*, 10.0 Hz)
22	6.27 (1H, *d*, 10.0 Hz)	6.25 (1H, *d*, 10.0 Hz)	6.22 (1H, *d*, 10.1 Hz)	6.26 (1H, *d*, 10.0 Hz)	6.22 (1H, *d*, 10.1 Hz)	6.27 (1H, *d*, 10.0 Hz)	6.22 (1H, *d*, 10.0 Hz)
23	1.31 (3H, *s*)	1.29 (3H, *s*)	1.30 (3H, *s*)	1.35 (3H, *s*)	1.36 (3H, *s*)	1.29 (3H, *s*)	1.31 (3H, *s*)
24	3.32 (1H, *d*, 10.0 Hz), 4.26	1.19 (3H, *s*)	1.19 (3H, *s*)	3.31 (1H, *d*, 10.5 Hz), 4.25	3.32 (1H, *d*, 10.6 Hz), 4.31	1.18 (3H, *s*)	1.19 (3H, *s*)
25	0.87 (3H, *s*)	0.85 (3H, *s*)	0.86 (3H, *s*)	0.65 (3H, *s*)	0.65 (3H, *s*)	0.84 (3H, *s*)	0.83 (3H, *s*)
26	1.03 (3H, *s*)	1.01 (3H, *s*)	1.03 (3H, *s*)	0.80 (3H, *s*)	0.81 (3H, *s*)	0.87 (3H, *s*)	0.86 (3H, *s*)
27	1.86 (3H, *s*)	1.85 (3H, *s*)	1.86 (3H, *s*)	1.83 (3H, *s*)	1.83 (3H, *s*)	1.85 (3H, *s*)	1.84 (3H, *s*)
28	3.47, 3.72 (each, 1H, *d*, 10.0 Hz)	3.45, 3.65 (each, 1H, *d*, 10.0 Hz)	3.46, 3.71 (each, 1H, *d*, 10.9 Hz)	3.39, 3.65 (each, 1H, *d*, 10.8 Hz)	3.39, 3.66 (each, 1H, *d*, 10.6 Hz)	3.40, 3.64 (each, 1H, *d*, 10.6 Hz)	3.39, 3.65 (each, 1H, *d*, 10.3 Hz)
29	1.14 (3H, *s*)	1.12 (3H, *s*)	1.12 (3H, *s*)	1.11 (3H, *s*)	1.10 (3H, *s*)	1.12 (3H, *s*)	1.11 (3H, *s*)
30	1.36 (3H, *s*)	1.34 (3H, *s*)	1.33 (3H, *s*)	1.34 (3H, *s*)	1.32 (3H, *s*)	1.36 (3H, *s*)	1.33 (3H, *s*)
C_{21}	Tig	Tig	Ang	Tig	Ang	Tig	Ang
3	7.13 (1H, *q*, 7.0 Hz)	7.11 (1H, *q*, 7.0 Hz)	5.98 (1H, *q*, 7.2 Hz)	7.11 (1H, *q*, 7.0 Hz)	5.98 (1H, *q*, 7.2 Hz)	7.0 (1H, *q*, 7.0 Hz)	5.99 (1H, *q*, 7.1 Hz)
4	1.67 (3H, *d*, 7.0 Hz)	1.66 (3H, *d*, 7.0 Hz)	2.13 (3H, *d*, 7.2Hz)	1.66 (3H, *q*, 7.0 Hz)	2.12 (3H, *d*, 7.2Hz)	1.66 (3H, *d*, 7.0 Hz)	2.11 (3H, *d*, 7.1 Hz)
5	1.99 (3H, *s*)	1.97 (3H, *s*)	2.04 (3H, *s*)	1.97 (3H, *s*)	2.04 (3H, *s*)	1.99 (3H, *s*)	2.04 (3H, *s*)
C_{22}	Ac	Ac	Ac	Ac	Ac	Ac	Ac
2	2.00 (3H, *s*)	2.03 (3H, *s*)	1.99 (3H, *s*)	1.92 (3H, *s*)	1.94 (3H, *s*)	1.92 (3H, *s*)	1.94 (3H, *s*)

[a] Assignments were based on COSY, HMQC, and HMBC experiments. Due to severe overlapping in the ^{1}H spectrum only detectable relative *J* (Hz) are reported.

than that of **1**, implying the absence of an oxygen-bearing function in **2**. The ^{13}C NMR spectrum of **2** also showed 54 signals as **1**, of which 30 were assigned to the aglycone, 17 to the oligosaccharide moiety, and the remaining seven to two other groups identified from the NMR spectroscopic data as tigeloyl (Yoshikawa et al., 1996, 1998; Zhang et al., 1999) and acetyl groups (Tables 1 and 3). Detailed NMR spectroscopic analysis indicated that the aglycone of **2** was somewhat different from that of **1** based on the different chemical shifts of C-4, C-23, and C-24 (Table 1), due to the substituent at C-24. The –CH_2OH group at C-24 in **1** was replaced by a methyl group in **2**, thus the aglycone of **2** was assigned as R_1-barrigenol (**2a**). The presence of three sugar units in **2** was indicated by the anomeric protons at δ 4.84 (1H, *d*, $J = 7.2$ Hz), 5.34 (1H, *d*, $J = 7.2$ Hz), and 6.06 (1H, *br s*), and the protonated carbons at δ 105.1, 104.7, and 111.0 observed in the HMQC spectrum. By NMR analysis, it was concluded that the glucose in **1** was replaced in **2** by a galactose, based on the differences of their ^{13}C NMR chemical shifts (Table 2) (Yoshikawa et al., 1996, 1998; Zhang et al., 1999, 2006). The linkage of the trisaccharide moiety **2** were also deduced from the HMBC and NOESY information as depicted for **1**. The structure of aesculioside II_f (**2**) was established as 3-*O*-[β-D-galactopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-tigeloyl-22-*O*-acetyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

Aesculioside II_g (**3**) had the same molecular formula of $C_{54}H_{84}O_{23}$ as **2** deduced from the $[M + Na]^+$ ion *m*/*z* at 1123.5295 in the positive HRESIMS. An extensive NMR spectroscopic study suggested that **3** and **2** differ structurally only in the acyl substitute at C-21. The characteristic NMR signals at δ_C 14.2 (C-4), 12.4 (C-5) and δ_H 7.11 (1H, *q*, $J = 7.0$ Hz, H-3), 1.66 (3H, *q*, $J = 7.0$ Hz, H-4) of the tigeloyl group in **2** were replaced

by the characteristic NMR resonances at δ_C 15.8 (C-4), 21.0 (C-5) and δ_H 5.98 (1H, *q*, $J = 7.2$ Hz, H-3), 2.13 (3H, *q*, $J = 7.2$ Hz, H-4) of an angeloyl group in **3**, indicating **3** had an angeloyl substitute at C-21. Accordingly, the structure of aesculioside II_g (**3**) was established as 3-*O*-[β-D-galactopyranosyl(1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-acetyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

Aesculiosides II_h (**4**) and II_i (**5**) had the same molecular formula of $C_{54}H_{84}O_{23}$ as deduced from their HRESIMS and NMR spectroscopic data. The NMR spectral resonances due to the aglycone and the oligosaccharide moiety of **4** were very similar to those of **5**, suggesting that **4** and **5** share the same aglycone, which was assigned as protoaescigenin **4a** (Yoshikawa et al., 1996, 1998; Zhang et al., 1999, 2006) by NMR analysis, and the same trisaccharide chain. Alkaline hydrolysis of **4** and **5** both produced the prosapogenin **17**, identified by co-HPLC analysis with the authentic sample as 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-3β,16α,21β,22α,24β,28-hexahydroxyolean-12-ene (aesculioliside I_d). Compounds **4** and **5** are structurally different only in the acyl substitute at C-21, similar to the difference between **2** and **3**. The acyl substituents at C-21 in **4** and **5** were identified as tigeloyl and angeloyl, respectively, based on their characteristic NMR data (Tables 1 and 3) (Yoshikawa et al., 1996, 1998; Zhang et al., 1999). Therefore, the structure of aesculiosides II_h (**4**) and II_i (**5**) were established as 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-tigeloyl-22-*O*-acetyl-3β,16α,21β,22α,24β,28-hexahydroxyolean-12-ene, and 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-acetyl-3β,16α,21β,22α,24β,28-hexahydroxyolean-12-ene, respectively.

The same molecular formula of $C_{54}H_{84}O_{22}$ for aesculiosides II_j (**6**) and II_k (**7**) were determined by their HRESIMS and ^{13}C NMR data. The NMR data (Tables 1–4) of **6** and **7** were also characteristic of the polyhydroxyoleanene triterpenoid glycosides with the same aglycone **6a** and a same trisaccharide unit as well as two-acyl groups. Alkaline hydrolysis of **6** and **7** produced the prosapogenin **16**, identified by co-HPLC analysis with the authentic sample as 3-*O*-[β-D-galactopyranosyl(1 → 2)]- α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene (aesculioliside I_c). A detailed NMR produced spectral analysis indicated that compounds **6** and **7** differ structurally only in the acyl substitute at C-21 in the same way as the differences between **4** and **5**, that is, **6** has a tigeloyl group at C-21, and **7** has an angeloyl group at C-21. Thus, the structures of aesculiosides II_j (**6**) and II_k (**7**) were established as 3-*O*-[β-D-galactopyranosyl-(1 → 2)]-α-L-arabinofuranosyl-(1 → 3)-β-D-glucuronopyr-anosyl-21-*O*-tigeloyl-22-*O*-acetyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene, and 3-*O*-[β-D-galactopyranosyl-(1 → 2)]-α-L-arabinofuranosyl-(1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-acetyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene, respectively.

Compounds **8**–**13** were obtained from the fraction Aes-III as colorless powders. Aesculioside III_a (**8**) had the same molecular formula of $C_{54}H_{84}O_{23}$ as **5**. Alkaline hydrolysis of **8** also produced compound **17** as **5**, indicating that the two saponins share a same prosapogenin, that is 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl-

Table 4
^1H NMR spectroscopic data for the sugar moieties of **1**–**7** (600 MHz in pyridine-d_5)[a]

Proton	1	2	3	4	5	6	7
GlcA-*P*							
1	4.80 (1H, *d*, 7.8 Hz)	4.84 (1H, *d*, 7.2 Hz)	4.87 (1H, *d*, 7.2 Hz)	4.90 (1H, *d*, 7.6 Hz)	4.92 (1H, *d*, 7.4 Hz)	4.85 (1H, *d*, 7.6 Hz)	4.87 (1H, *d*, 7.6 Hz)
2	4.25	4.24	4.23	4.26	4.25	4.23	4.24
3	4.26	4.27	4.25	4.27	4.27	4.25	4.25
4	4.50	4.57	4.59	4.48	4.52	4.59	4.61
5	4.57	4.53	4.43	4.57	4.58	4.58	4.46
	Glc-*p*	Gal-*p*	Gal-*p*	Glc-*p*	Glc-*p*	Gal-*p*	Gal-*p*
1	5.47 (1H, *d*, 7.6 Hz)	5.34 (1H, *d*, 7.2 Hz)	5.35 (1H, *d*, 7.8 Hz)	5.50 (1H, *d*, 7.6 Hz)	5.52 (1H, *d*, 7.6 Hz)	5.31 (1H, *d*, 7.2 Hz)	5.34 (1H, *d*, 7.6 Hz)
2	4.06	4.41	4.48	4.06	4.07	4.40	4.53
3	4.25	4.11	4.07	4.26	4.29	4.09	4.12
4	4.57	4.57	4.59	4.58	4.59	4.59	4.62
5	3.64	3.96	3.97	3.65	3.63	3.95	3.62
6	4.30, 4.40	4.40, 4.55	4.46, 4.52	4.29, 4.36	4.35, 4.49	4.40, 4.44	4.48, 4.57
Ara-*f*							
1	6.06 (1H, *br s*)	6.06 (1H, *br s*)	6.07 (1H, *br s*)	6.08 (1H, *br s*)	6.09 (1H, *br s*)	6.01 (1H, *br s*)	6.05 (1H, *br s*)
2	4.84	4.99	4.91	4.99	5.01	4.92	4.96
3	4.60	4.80	4.79	4.79	4.82	4.64	4.77
4	4.77	4.86	4.82	4.87	4.87	4.90	4.87
5	4.19, 4.32	4.17, 4.31	4.12, 4.27	4.17, 4.30	4.17, 4.33	4.17, 4.40	4.18, 4.36

[a] Assignments were based on COSY, HMQC, and HMBC experiments. Due to severe overlapping in the ^1H spectrum only detectable relative *J* (Hz) are reported.

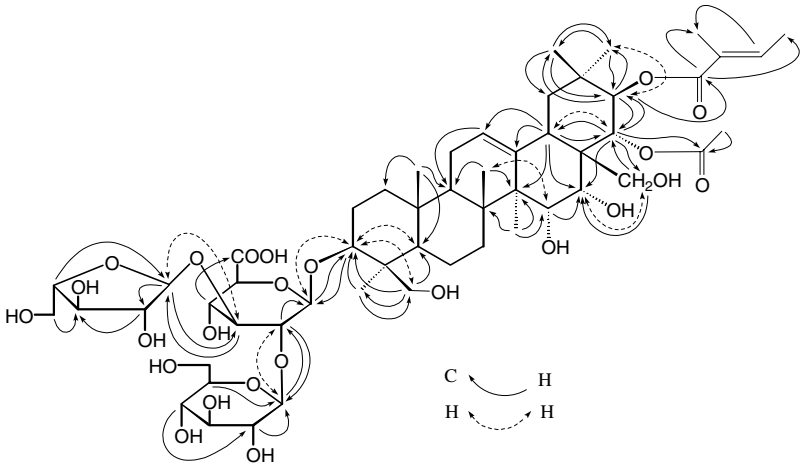


Fig. 1. Key HMBC and NOE correlations of aesculioside II_e (**1**).

(1 → 3)-β-D-glucuronopyranosyl-3β,16α,21β, 22α,24β,28-hexahydroxyolean-12-ene (aesculioliside I_d). Detailed NMR spectroscopic analyses indicated that compounds **8** and **5** are structurally different in the position of their acetyl groups. Based on long-range correlations in the HMBC spectrum of **8**, the carbon signals at δ 80.9 and 66.3 were assigned to C-21 and C-28, and the proton resonances at δ 6.49 (1H, d, 9.8 Hz) and 4.28, 4.30 (each 1H, d, 10.3 Hz) to H-21 and H-28. The HMBC correlations of H-21 with C-1 (δ 168.0) of the angeloyl group and H-28 with C-1 (δ 170.7) of the acetyl group established C-21 and C-28 were the positions of the acylation. Therefore, the structure of aesculioside III_a (**8**) was determined as 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-28-*O*-acetyl-3β,16α,21β,22α,24β,28-hexahydroxy-olean-12-ene.

The HRESIMS of **9** displayed an extensive $[M + Na]^+$ ion *m*/*z* at 1167.5538, corresponding to the molecular formula $C_{56}H_{88}O_{24}$. Comparison of NMR spectroscopic data of **9** and **1** indicated that both compounds had the same aglycone **1a** and the same trisaccharide unit. However, compound **9** had two different acyl groups, which were characterized as angeloyl (Zhang et al., 1999) and 2-methypropanoyl (Matsushita et al., 2004), respectively, on the basis of their NMR data (Tables 1 and 5) and by comparison with the literature data (Matsushita et al., 2004; Zhang et al., 1999). As described in Fig. 2, the linkage of the woacyl groups of angeloyl at C-21 and 2-methypropanoyl at 22 were established by long-range correlations. Thus, the structure of aesculioside III_b (**9**) was determined as 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-2-methypropanoyl-3β,15α,16α,21β,22α,24β,28-heptahydroxyolean-12-ene.

Aesculioside III_c (**10**) had a $[M + Na]^+$ ion *m*/*z* at 1151.4027, corresponding to the molecular formula $C_{56}H_{88}O_{23}$, one oxygen atom less than **9**. Detailed comparison of NMR data of **10** with those of **9** indicated that **10** is structurally different from **9** in three substituents at C-21, C-24 of the aglycone, and C-2 of the Glc A. The angeloyl group at C-21, $-CH_2OH$ at C-24 and the glucose residue at C-2 of the Glc A in **9** were replaced in **10** by a tigeloyl group, $-CH_3$ and galactose, respectively, as indicated by their NMR data (Tables 1, 2, 5, 6) as previously discussed. The full structure assignment was confirmed by COSY, HMQC and HMBC correlations. Therefore, the structure of aesculioside III_c (**10**) was determined as 3-*O*-[β-D-galactopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-tigeloyl-22-*O*-2-methypropanoyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

Aesculiosides III_d (**11**) and III_e (**12**) had the same molecular formula of $C_{57}H_{88}O_{24}$ as deduced from their HRESIMS and ^{13}C NMR data. NMR data analysis of **11** and **12** indicated that **11** and **12** had a common aglycone (**1a**), a common trisaccharide chain as well as a common tigeloyl at C-21 as those of **1**. Alkaline hydrolysis of **11** and **12** gave the same prosapogenin (**15**) as **1**. Further NMR spectroscopic comparison of **1**, **11**, and **12** indicated that these three compounds are structurally different only in the acyl group at C-22. In the NMR spectra of **11**, the signals at δ 170.8 and 20.6 for the acetyl group in **1** were absent, instead of the characteristic NMR signals of a tigeloyl group signals at δ_C 13.6 (C-4), 11.9 (C-5) and δ_H 6.81(1H, q, $J = 7.0$ Hz, H-3), 1.34 (3H, q, $J = 7.0$ Hz, H-4) (Tables 1 and 5) (Yoshikawa et al., 1996, 1998; Zhang et al., 1999). Similarly, the NMR spectra of **12** displayed characteristic NMR signals of an angeloyl group signals at δ_C 15.3 (C-4), 20.4 (C-5) and δ_H 5.76 (1H, q, $J = 7.0$ Hz, H-3), 1.95 (3H, q, $J = 7.0$ Hz, H-4) (Tables 1 and 5) (Yoshikawa et al., 1996, 1998; Zhang et al., 1999). Consequently, the structure of aesculiosides III_d (**11**) and III_e (**12**) were established as 3-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-tigeloyl-22-*O*-tigeloyl-3β, 15α,16α, 21β, 22α,24β, 28-heptahydroxyolean-12-ene, and 3-*O*-[β-D-glucopyrano-

Table 5
^{1}H NMR spectroscopic data for the aglycone moieties of **8**–**13** (600 MHz in pyridine-d_5)[a]

Proton	8	9	10	11	12	13
1	0.80, 1.37	0.83, 1.42	0.84, 1.45	0.81, 1.43	0.82, 1.30	0.83, 1.43
2	2.15 (2H, *m*)	2.13 (2H, *m*)	2.13 (2H, *m*)	2.43 (2H, *m*)	2.41 (2H, *m*)	2.43 (2H, *m*)
3	3.42 (1H, *dd*-like)	3.42 (1H, *dd*-like)	3.23 (1H, *dd*-like)	3.41 (1H, *dd*-like)	3.42 (1H, *dd*-like)	3.30 (1H, *dd*-like)
5	0.85	0.85	0.85	0.90	0.86	0.82
6	1.50, 1.53	1.51, 1.56	1.53, 1.61	1.56, 1.59	1.60, 1.62	1.57, 1.60
7	1.26, 1.53	2.05, 2.17	2.08, 2.18	2.00, 2.12	1.93, 2.11	2.02, 2.12
9	1.67	1.69	1.72	1.67	1.65	1.70
11	1.74, 1.88	1.73, 1.88	1.75, 1.89	1.75, 1.89	1.75, 1.86	1.81, 1.95
12	5.44 (1H, *br s*)	5.53 (1H, *br s*)	5.55 (1H, *br s*)	5.55 (1H, *br s*)	5.54 (1H, *br s*)	5.57 (1H, *br s*)
15	1.64, 1.88	4.23	4.24	4.23	4.23	4.25
16	4.75	4.46	4.47	4.47	4.46	4.47
18	3.12	3.07	3.09	3.12	3.10	3.13
19	1.39, 3.14	1.43, 3.10	1.47, 3.10	1.46, 3.14	1.47, 3.12	1.48, 3.15
21	6.49 (1H, *d*, 9.8 Hz)	6.67 (1H, *d*, 10.0 Hz)	6.65 (1H, *d*, 10.2 Hz)	6.73 (1H, *d*, 10.2 Hz)	6.69 (1H, *d*, 10.1 Hz)	6.76 (1H, *d*, 10.0 Hz)
22	4.48 (1H, *d*, 9.8 Hz)	6.25 (1H, *d*, 10.0 Hz)	6.29 (1H, *d*, 10.2 Hz)	6.34 (1H, *d*, 10.2 Hz)	6.39 (1H, *d*, 10.1 Hz)	6.36 (1H, *d*, 10.2 Hz)
23	1.32 (3H, *s*)	1.34 (3H, *s*)	1.30 (3H, *s*)	1.29 (3H, *s*)	1.30 (3H, *s*)	1.27 (3H, *s*)
24	3.29 (1H, *d*, 10.0 Hz), 4.30	3.33 (1H, *d*, 10.0 Hz), 4.34	1.21 (3H, *s*)	3.31 (1H, *d*, 10.0 Hz), 4.34	3.31 (1H, *d*, 10.0 Hz), 4.34	1.18 (3H, *s*)
25	0.66 (3H, *s*)	0.70 (3H, *s*)	0.88 (3H, *s*)	0.68 (3H, *s*)	0.68 (3H, *s*)	0.87 (3H, *s*)
26	0.93 (3H, *s*)	0.99 (3H, *s*)	1.05 (3H, *s*)	0.97 (3H, *s*)	0.97 (3H, *s*)	1.04 (3H, *s*)
27	1.82 (3H, *s*)	1.87 (3H, *s*)	1.88 (3H, *s*)	1.87 (3H, *s*)	1.87 (3H, *s*)	1.88 (3H, *s*)
28	4.28, 4.30 (each, 1H, *d*, 10.3 Hz)	3.50, 4.75 (each, 1H, *d*, 10.0 Hz)	3.49, 4.76 (each, 1H, *d*, 10.2 Hz)	3.48, 3.75 (each, 1H, *d*, 10.8 Hz)	3.54, 3.77 (each, 1H, *d*, 10.3 Hz)	3.50, 3.77 (each, 1H, *d*, 10.6 Hz)
29	1.13 (3H, *s*)	1.14 (3H, *s*)	1.15 (3H, *s*)	1.15 (3H, *s*)	1.15 (3H, *s*)	1.17 (3H, *s*)
30	1.32 (3H, *s*)	1.36 (3H, *s*)	1.36 (3H, *s*)	1.37 (3H, *s*)	1.38 (3H, *s*)	1.39 (3H, *s*)
C_{21}	Ang	Ang	Tig	Tig	Tig	Tig
3	5.92 (1H, *q*, 7.0 Hz)	6.08 (1H, *q*, 7.0 Hz)	7.12 (1H, *q*, 7.0 Hz)	7.06 (1H, *q*, 7.0 Hz)	7.10 (1H, *q*, 7.0 Hz)	7.07 (1H, *q*, 7.0 Hz)
4	2.06 (3H, *d*, 7.0 Hz)	2.17 (3H, *d*, 7.0 Hz)	1.69 (3H, *d*, 7.0 Hz)	1.62 (3H, *d*, 7.0 Hz)	1.65 (3H, *d*, 7.0 Hz)	1.63 (3H, *d*, 7.0 Hz)
5	2.01 (3H, *s*)	2.07 (3H, *s*)	1.99 (3H, *s*)	1.93 (3H, *s*)	1.96 (3H, *s*)	1.94 (3H, *s*)
C_{22} or C_{28}	Ac	Mp	Mp	Tig	Ang	Tig
2	1.99 (3H, *s*)	2.35 (1H, *m*)	2.36 (1H, *m*)			
3		1.05 (3H, *t*, 7.0 Hz)	1.03 (3H, *t*, 7.0 Hz)	6.81 (1H, *q*, 7.0 Hz)	5.76 (1H, *q*, 7.0 Hz)	6.81 (1H, *q*, 7.0 Hz)
4		1.01 (3H, *t*, 7.0 Hz)	1.01 (3H, *t*, 7.0 Hz)	1.34 (3H, *d*, 7.0 Hz)	1.95 (3H, *d*, 7.0 Hz)	1.34 (3H, *d*, 7.0 Hz)
5				1.76 (3H, *s*)	1.76 (3H, *s*)	1.94 (3H, *s*)

[a] Assignments were based on COSY, HMQC, and HMBC experiments. Due to severe overlapping in the ^{1}H spectrum only detectable relative *J* (Hz) are reported.

syl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-tigeloyl-22-*O*-angeloyl-3β,15α,16α,21β,22α,24β,28-heptahydroxyolean-12-ene, respectively.

Aesculioside III$_f$ (**13**) was assigned a molecular formula of $C_{57}H_{88}O_{23}$ deduced from HRESIMS data. A detailed NMR spectroscopic study suggested that compounds **13**

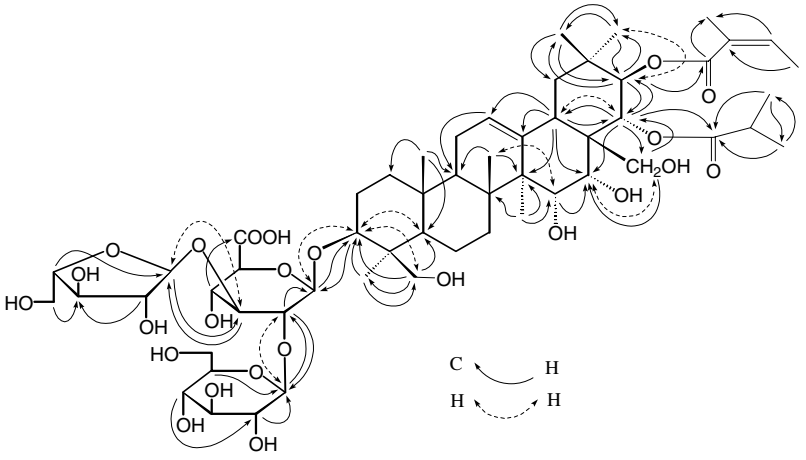


Fig. 2. Key HMBC and NOE correlations of aesculioside III$_b$ (**9**).

Table 6
^{1}H NMR spectroscopic data for the sugar moieties of **8**–**13** (600 MHz in pyridine-d_5)[a]

Proton	8	9	10	11	12	13
GlcA-*p*						
1	4.80 (1H, *d*, 7.6 Hz)	4.78 (1H, *d*, 7.5Hz)	4.85 (1H, *d*, 7.5Hz)	4.82 (1H, *d*, 7.6 Hz)	4.86 (1H, *d*, 7.5 Hz)	4.86 (1H, *d*, 7.5 Hz)
2	4.25	4.25	4.28	4.25	4.25	4.26
3	4.27	4.27	4.29	4.27	4.27	4.27
4	4.58	4.58	4.57	4.53	4.54	4.6
5	4.40	4.40	4.40	4.40	4.40	4.40
	Glc-*p*	Glc-*p*	Gal-*p*	Glc-*p*	Glc-*p*	Gal-*p*
1	5.47 (1H, *d*, 7.6 Hz)	5.47 (1H, *d*, 7.6 Hz)	5.35 (1H, *d*, 7.6 Hz)	5.43 (1H, *d*, 7.5 Hz)	5.42 (1H, *d*, 7.5 Hz)	5.30 (1H, *d*, 7.5 Hz)
2	4.06	4.06	4.48	4.04	4.03	4.50
3	4.28	4.26	4.11	4.27	4.25	4.09
4	4.50	4.58	4.57	4.53	4.54	4.56
5	3.65	3.60	3.96	3.62	3.63	3.94
6	4.33, 4.48	4359, 4.48	4.49, 4.53	4.34, 4.46	4.35, 4.46	4.47, 4.52
Ara-*f*						
1	6.11 (1H, *br s*)	6.10 (1H, *br s*)	6.09 (1H, *br s*)	6.04 (1H, *br s*)	6.04 (1H, *br s*)	6.04 (1H, *br s*)
2	4.98	4.97	4.98	4.95	4.95	5.01
3	4.71	4.79	4.71	4.70	4.73	4.65
4	4.92	4.93	4.93	4.90	4.89	4.97
5	4.17, 4.35	4.17, 4.37	4.17, 4.40	4.19, 4.34	4.18, 4.31	4.17, 4.35

[a] Assignments were based on COSY, HMQC, and HMBC experiments.

and **2** shared a same aglycone (**2a**) and a same trisaccharide moiety, but differed only in the acyl substituent at C-22. The acyl substituent at C-22 in **13** was determined as tigeloyl on the basis of its characteristic NMR data (Tables 1 and 5) (Yoshikawa et al., 1996, 1998; Zhang et al., 1999). Accordingly, the structure of aesculioside III$_f$ (**13**) was established as 3-*O*-[β-D-galactopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 3)-β-D-glucuronopyranosyl-21-*O*-tigeloyl-22-*O*-tigeloyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

The compounds **14**–**22** and **26**–**28** from the fruits of *A. pavia* were *in vitro* screened for cytotoxicity against non-small cell lung cancer (NCI-H460), CNS cancer (SF-268), and breast cancer (MCF7). Five positive compounds (**21**, **22**, **26**, **27**, and **28**) were further evaluated against 59 cell lines from nine different human cancers including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast. Compounds **26**–**28** with two-acyl groups at C-21 and C-22 showed activity with GI$_{50}$ 0.175–8.71 μM against almost all tumor cell lines tested (Table 7). Compounds **21** and **22** with only one acyl group at C-21 also showed activity with GI50 3.48 μM to >25 μM against the cell lines tested (Table 7).

3. Conclusions

Our previous chemical investigation on this plant showed that the structures of saponins isolated from North American *A. pavia* are different from those isolated from Eurasian *A. hippocastanum* and *A. chinensis* in their oligosaccharide moieties, suggesting a different chemotaxonomic feature between North American *A. pavia* and Eurasian *Aesculus* species (Zhang et al., 2006). Current chemical investigation on this plant collected in different location further supported this conclusion. It is worthwhile to mention that compounds **23**–**28** are the main compounds in the saponin fraction based on our previous and current investigations. Interestingly, compounds **26**–**28** with two-acyl groups at C-21 and C-22 showed activity against almost all tumor cell lines tested. But, compounds **14**–**18** without acyl group at C-21 and C-22 showed no or weak activity. These results suggest that the acyl groups at C-21 and C-22 are important for the activity of these triterpenoid saponins.

4. Experimental

4.1. General experimental procedures

NMR experiments were performed on a Bruker 600 MHz NMR instrument. NMR data were reported as δ (ppm) values and referenced to the solvent used. HRESIMS were measured on a PE SCIEX QSTAR LC/MS/MS spectrometer. Octadecyl-functionalized silica gel (Aldrich) was used for low-pressure chromatography. HPLC analysis was performed on a Hewlett Packard Series 1100 with a HP 1100 diode array detector using a Hypersil ODS column (column A, 250 × 4.6 mm, 5 μm, Supelco; detector: 208 nm; flow rate: 0.5 mL/min; solvent A: CH_3CN–H_2O, 30:70 + 0.1% AcOH; solvent B: CH_3CN–H_2O, 40:60 + 0.1% AcOH; solvent C: CH_3CN–H_2O, 42:58 + 0.1% acetic acid). Preparative HPLC was performed with an Acuflow Series III pump connected with an Acutect 500 UV/VIS detector using an Econosil ODS column (column B, 250 × 22 mm, 10 μm, Alltech; detector: 208 nm; flow rate: 3.0 mL/min; solvents A, B, and C).

Table 7
Cytotoxicity data of compounds **21**, **22** and **26**–**28** (GI_{50}, μM)[a]

Compounds (NSC#)	**21** (D736862)	**22** (D736863)	**26** (D736859)	**27** (D736857)	**28** (D736858)
Leukemia					
CCRF-CEM	–	20	3.44	0.175	2.53
HL-60 (TB)	5.06	7.40	5.71	0.961	1.50
K-562	4.96	14.1	6.65	3.64	5.99
MOLT-4	–	18.4	–	–	–
RPMI-8226	–	>25	4.73	2.43	2.51
SR	3.60	3.71	0.866	0.333	0.649
Non-small cell lung cancer					
A549/ATCC	4.69	4.65	4.27	4.22	6.59
EKVX	4.99	>25	4.24	3.78	5.71
Hop-62	5.09	10.8	3.98	2.76	4.32
Hop-92	–	>25	3.32	1.52	2.84
NCI-H226	5.59	>25	4.86	5.72	6.63
NCI-H23	5.12	10.1	4.59	4.68	4.37
NCI-H322M	4.47	20.1	4.03	4.89	7.98
NCI-H460	4.52	>25	4.54	3.99	4.37
NCI-H522	4.52	3.55	4.53	4.55	5.40
Colon cancer					
COLO 205	3.96	5.50	3.28	2.42	3.56
HCC-2998	5.24	6.64	–	2.56	8.71
HCT-116	6.84	>25	4.04	2.75	3.63
HCT-15	9.05	>25	2.43	5.51	7.49
HT29	4.97	>25	3.80	1.94	3.46
KM12	4.26	22.6	3.74	3.56	5.61
SW-620	4.45	>25	2.77	4.62	6.61
CNS cancer					
SF-268	4.72	>25	4.22	2.76	3.48
SF-295	4.83	>25	3.44	4.63	4.83
SF-539	6.35	7.72	4.05	2.64	3.18
SNB-19	3.71	>25	2.68	2.19	2.97
SNB-75	5.25	>25	4.62	3.75	3.27
U251	4.39	>25	4.74	6.42	8.54
Melanoma					
LOX IMVI	4.44	>25	3.44	4.63	6.16
MALME-3M	5.15	>25	4.25	5.70	8.18
M14	4.67	>25	4.14	3.28	3.31
SK-MEL-2	4.19	>25	4.35	4.47	4.56
SK-MEL-28	4.68	>25	5.34	4.40	4.15
SK-MEL-5	5.43	>25	3.48	4.58	4.78
UACC-257	4.41	–	4.62	5.22	5.77
UACC-62	4.86	>25	4.10	5.03	5.75
Ovarian cancer					
IGROV1	4.27	19.4	3.36	4.57	5.29
OVCAR-3	5.26	>25	4.51	4.89	6.43
OVCAR-4	4.96	7.90	5.46	3.67	3.32
OVCAR-5	4.17	7.41	5.07	4.04	4.94
OVCAR-8	4.57	>25	3.35	2.62	4.55
SK-OV-3	3.76	>25	4.01	4.70	4.70
Renal cancer					
786-0	4.42	>25	5.30	3.02	3.98
A498	4.35	5.38	2.20	6.14	5.11
ACHN	3.86	>25	3.50	4.96	4.66
CAK1-1	4.43	14.9	5.05	5.64	6.90
RXF-393	4.72	–	2.68	–	3.57
SN12C	4.35	10.9	3.60	3.68	4.00
TK-10	4.68	>25	4.36	5.14	6.71
UO-31	3.75	4.09	2.92	3.37	3.84
Prostate cancer					
PC-3	–	6.73	4.27	1.72	3.43
DU-145	5.75	>25	4.07	4.51	4.44

(*continued on next page*)

Table 7 (*continued*)

Compounds (NSC#)	**21** (D736862)	**22** (D736863)	**26** (D736859)	**27** (D736857)	**28** (D736858)
Breast cancer					
MCF7	4.80	>25	4.75	4.21	4.70
NCI/ADR-RES	–	>25	–	–	–
MDA-MB-231/ATCC	3.64	11.5	2.57	1.78	3.34
HS 578T	3.48	23.3	6.14	2.82	1.68
MDA-MB-435	6.02	>25	3.84	1.94	1.41
BT-549	7.83	7.05	–	–	–
T-47D	4.29	>25	4.62	3.56	4.31

[a] GI_{50} (concentration at 50% growth inhibition) values are identical to the IC_{50} (the half maximal inhibitory concentration) values.

D-Glucose, D-galactose, L-arabinose and D-glucuronic acid were purchased from Aldrich.

4.2. Plant material

Fruits of *A. pavia* L. were collected from a single plant, in Nacogdoches, Texas, on October 6, 2004, and were identified by Shiyou Li. A voucher specimen (CMPR-AP2004106) was deposited at the National Center for Pharmaceutical Crops of Stephen F. Austin State University, USA. This plant was different in location from the one previously investigated (CMPR-2003101) (Zhang et al., 2006).

4.3. Extraction and isolation

Air-dried fruits (450 g) were ground to a coarse powder and percolated five times with EtOH–H_2O (95:5, %). EtOH–H_2O solubles were then concentrated under vacuum to give an extract (50.0 g). Part of this extract (30.0 g) was suspended in H_2O and partitioned successively with $CHCl_3$ and *n*-BuOH. The *n*-butanol-soluble fraction (16.0 g) was applied to a column of Diaion HP-20 eluting with MeOH–H_2O (3:7) and MeOH–H_2O (80–20). The latter was concentrated to afford a fraction (coded Aes-P), and then subjected to ODS cc eluting with a MeOH–H_2O gradient (4:6; 1:1, 6:4, and 8:2) to give four fractions: Aes-I (MeOH–H_2O (4:6) elution), Aes-II (MeOH–H_2O (1:1) elution), Aes-III (MeOH–H_2O (6:4) elution), and Aes-IV (MeOH–H_2O (8:2) elution). Fraction Aes-I was subjected to additional ODS cc eluting with MeOH–H_2O (35:65) to furnish Aes-Ia and Aes-Ib. Aes-Ib was separated by preparative MPLC (column B and solvent A) to give **14** (15.6 mg, t_R 48.1 min), **15** (5.8 mg, t_R 42.6 min), **16** (17.5 mg, t_R 68.4 min), **17** (11.6 mg, t_R 57.6 min), and **18** (2.5 mg, t_R 80.3 min). Compounds **30** and **31** were isolated from Aes-Ia by Sephadex LH-20 cc eluting with MeOH. Fraction Aes-II was separated by preparative MPLC (column B and solvent B) to give five subfractions: Sf-IIa (t_R 49.3 min), Sf-IIb (t_R 55.6 min), Sf-IIc (t_R 60.9 min), Sf-IId (t_R 74.5. min), and Sf-IIe (t_R 79.6 min). These five subfractions (Sf IIa–IIe) were further separated by HPLC (column A and solvent B) to give compounds **1**–**7** and **19**–**22**, of which **1** (20.2 mg, t_R 16.8 min) and **19** (9.8 mg, t_R 16.8 min) were from Sf-IIa; **2** (13.0 mg, t_R 18.6 min) and **20** (10.0 mg, t_R 20.3 min) from Sf-IIb; **3** (9.7 mg, t_R 20.9 min) and **21** (10.5 mg, t_R 22.9 min) from Sf-IIc; **4** (10.1 mg, t_R 24.7 min) and **22** (2.6 mg, t_R 26.6 min) from Sf-IId; **5** (10.6 mg, t_R 28.9 min), **6** (10.1 mg, t_R 29.1 min) and **7** (6.5 mg, t_R 31.4 min) from Sf-IIe. Similarly, the fraction Aes-III was separated by preparative MPLC (column B and solvent C) to afford Aes-IIIa (4.3 mg, t_R 70.0 min), **11** (3.0 mg, t_R 78.5 min), **12** (2.0 mg, t_R 84.0 min), and **13** (2.5 mg, t_R 98.0 min). Ase-IIIa was further isolated by HPLC (column A and solvent C) to yield **8** (1.3 mg, t_R 26.0 min), **9** (1.2 mg, t_R 27.9 min), and **10** (1.0 mg, t_R 29.2 min). Compounds **23**–**29** were obtained from fraction Aes-IV using the same purified methods as our previous publication (Zhang et al., 2006).

4.4. Aesculioside II_e (**1**)

Colorless powder from CH_3CN–H_2O, t_R 16.8 min (column A, solvent B). HRESIMS: *m/z* 1139.5248 $[M + Na]^+$ (calcd. for $C_{54}H_{84}NaO_{24}$, 1139.5250). For ^{13}C and ^1H NMR spectroscopic data, see Tables 1–4.

4.5. Aesculioside II_f (**2**)

Colorless powder from CH_3CN–H_2O, t_R 18.6 min (column A, solvent B). HRESIMS: *m/z* 1123.5299 $[M + Na]^+$ (calcd. for $C_{54}H_{84}NaO_{23}$, 1123.5301). For ^{13}C and ^1H NMR spectroscopic data, see Tables 1–4.

4.6. Aesculioside II_g (**3**)

Colorless powder from CH_3CN–H_2O, t_R 20.3 min (column A, solvent B). HRESIMS: *m/z* 1123.5295 $[M + Na]^+$ (calcd. for $C_{54}H_{84}NaO_{23}$, 1123.5301). For ^{13}C and ^1H NMR spectroscopic data, see Tables 1–4.

4.7. Aesculioside II_h (**4**)

Colorless powder from CH_3CN–H_2O, t_R 24.7 min (column A, solvent B). HRESIMS: *m/z* 1123.5295 $[M + Na]^+$

(calcd. for $C_{54}H_{84}NaO_{23}$, 1123.5301). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4.

4.8. Aesculioside II_i (5)

Colorless powder from CH_3CN–H_2O, t_R 28.9 min (column A, solvent B). HRESIMS: *m/z* 1123.5299 $[M + Na]^+$ (calcd. for $C_{54}H_{84}NaO_{23}$, 1123.5301), 1139.5164 $[M + K]^+$ (calcd. for $C_{54}H_{84}KO_{23}$, 1139.5041). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4.

4.9. Aesculioside II_j (6)

Colorless powder from CH_3CN–H_2O, t_R 28.9 min (column A, solvent B). HRESIMS: *m/* 1107.5347 $[M + Na]^+$ (calcd. for $C_{54}H_{84}NaO_{22}$, 1107.5352), 1123.5190 $[M + K]^+$ (calcd. for $C_{54}H_{84}KO_{22}$, 1123.5091). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4.

4.10. Aesculioside II_k (7)

Colorless powder from CH_3CN–H_2O, t_R 31.4 min (column A, solvent B). HRESIMS: *m/* 1107.5348 $[M + Na]^+$ (calcd. for $C_{54}H_{84}NaO_{22}$, 1107.5352), 1123.5226 $[M + K]^+$ (calcd. for $C_{54}H_{84}KO_{22}$, 1123.5091). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4.

4.11. Aesculioside III_a (8)

Colorless powder from CH_3CN–H_2O, t_R 26.0 min (column A, solvent C). HRESIMS: *m/z* 1123.5306 $[M + Na]^+$ (calcd. for $C_{54}H_{84}NaO_{23}$, 1123.5301), 1139.5218 $[M + K]^+$ (calcd. for $C_{54}H_{84}KO_{23}$, 1139.5041). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5, and 6.

4.12. Aesculioside III_b (9)

Colorless powder from CH_3CN–H_2O, t_R 27.9 min (column A, solvent C). HRESIMS: *m/z* 1167.5538 $[M + Na]^+$ (calcd. for $C_{56}H_{88}NaO_{24}$, 1167.5563). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5, and 6.

4.13. Aesculioside III_c (10)

Colorless powder from CH_3CN–H_2O, t_R 29.2 min (column A, solvent C). HRESIMS: *m/z* 1151.4027 $[M + Na]^+$ (calcd. for $C_{56}H_{88}NaO_{23}$, 1151.5614). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5, and 6.

4.14. Aesculioside III_d (11)

Colorless powder from CH_3CN–H_2O, t_R 32.2 min (column A, solvent C). HRESIMS: *m/z* 1179.5568 $[M + Na]^+$ (calcd. for $C_{57}H_{88}NaO_{24}$, 1179.5563), 1195.5281 $[M + K]^+$ (calcd. for $C_{57}H_{88}KO_{24}$, 1195.5303). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5, and 6.

4.15. Aesculioside III_e (12)

Colorless powder from CH_3CN–H_2O, t_R 33.7 min (column A, solvent C). HRESIMS: *m/z* 1179.5578 $[M + Na]^+$ (calcd. for $C_{57}H_{88}NaO_{24}$, 1179.5563), 1195.5400 $[M + K]^+$ (calcd. for $C_{57}H_{88}KO_{24}$, 1195.5303). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5, 6.

4.16. Aesculioside III_f (13)

Colorless powder from CH_3CN–H_2O, t_R 35.5 min (column A, solvent C). HRESIMS: *m/z* 1163.5603 $[M + Na]^+$ (calcd. for $C_{57}H_{88}NaO_{23}$, 1163.5614), 1179.5311 $[M + K]^+$ (calcd. for $C_{57}H_{88}KO_{23}$, 1179.5354). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5, and 6.

4.17. Acid hydrolysis of compound 1

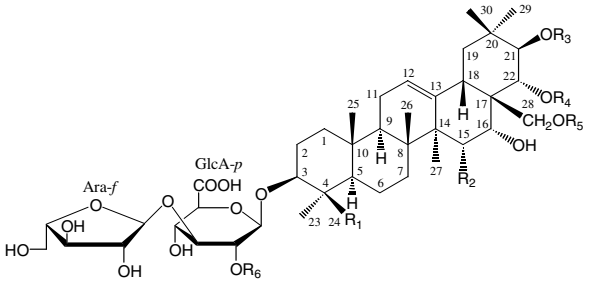
Compound **1** (15 mg) was refluxed with 1 mL 1 M HCl (dioxane–H_2O, 1:1) at 80 °C for 2 h. After the dioxane was removed, the solution was extracted with $CHCl_3$–MeOH (7:3, 1 mL × 3). The monosaccharide portion was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, and then freeze-dried. Three monosaccharides were identified using authentic samples by TLC in MeCOEt–*iso*-PrOH–Me_2CO–H_2O (20:10:7:6) as glucose, arabinose, and glucuronic acid. After preparative TLC of the sugar mixture in this solvent, the optical rotation of each purified sugar was measured (Zhang et al., 2006).

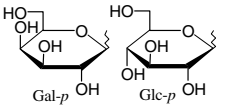
4.18. Alkaline hydrolysis of compounds 1, 4–7, and 11–13

Compound **1** (3.0 mg) was refluxed with 1 mL 0.8 M NaOH at 80 °C for 4 h. After cooling, the reaction mixture was neutralized with 1 M HCl and then extracted with *n*-BuOH (2 mL × 3). The organic layers were combined and then evaporated to dryness under a vacuum. The residue was subjected to HPLC purification affording a prosapogenin (**15**, 2.3 mg), which was identified by co-HPLC analysis with an authentic sample. By the same method, **4** and **5** afforded **17**, **6** and **7** afforded **16**, **11** and **12** afforded **15**, and **13** afforded **14**.

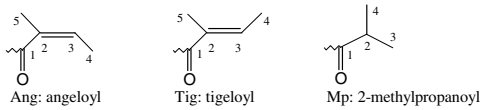
4.19. Cytotoxicity assay

Cytotoxicity of compounds **14**–**22** and **26**–**28** was assayed by National Cancer Institute (NCI) using the Methodology of The In Vitro Cancer Screen (National Cancer Institute, 2007).

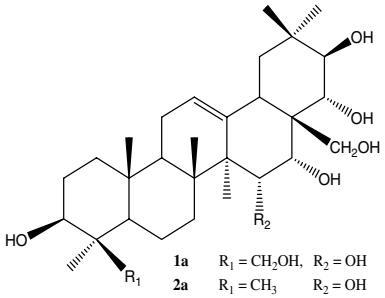




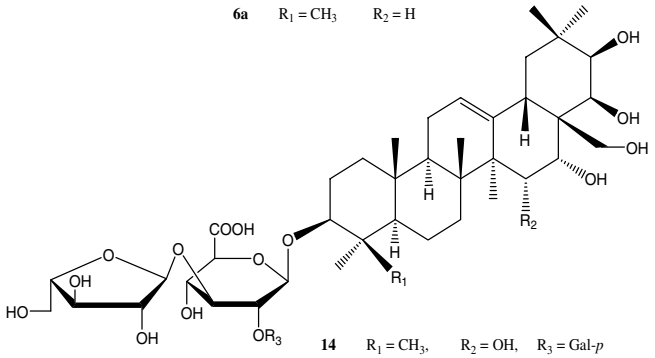
GlcA-*p*: β-D-glucuronopyranosyl acid
Gal-*p*: β-D-galactopyranosyl
Glc-*p*: β-D-glucopyranosyl
Ara-*f*: α-L-arabinofuranosyl



	R_1	R_2	R_3	R_4	R_5	R_6
1	CH_2OH	OH	Tig	Ac	H	Glc-*p*
2	CH_3	OH	Tig	Ac	H	Gal-*p*
3	CH_3	OH	Ang	Ac	H	Gal-*p*
4	CH_2OH	H	Tig	Ac	H	Glc-*p*
5	CH_2OH	H	Ang	Ac	H	Glc-*p*
6	CH_3	H	Tig	Ac	H	Gal-*p*
7	CH_3	H	Ang	Ac	H	Gal-*p*
8	CH_2OH	H	Ang	H	Ac	Glc-*p*
9	CH_2OH	OH	Ang	Mp	H	Glc-*p*
10	CH_3	OH	Tig	Mp	H	Gal-*p*
11	CH_2OH	OH	Tig	Tig	H	Glc-*p*
12	CH_2OH	OH	Tig	Ang	H	Glc-*p*
13	CH_3	OH	Tig	Tig	H	Gal-*p*



1a $R_1 = CH_2OH$, $R_2 = OH$
2a $R_1 = CH_3$, $R_2 = OH$
4a $R_1 = CH_2OH$, $R_2 = H$
6a $R_1 = CH_3$, $R_2 = H$



14 $R_1 = CH_3$, $R_2 = OH$, R_3 = Gal-*p*
15 $R_1 = CH_2OH$ $R_2 = OH$, R_3 = Glc-*p*
16 $R_1 = CH_3$, $R_2 = H$, R_3 = Gal-*p*
17 $R_1 = CH_2OH$ $R_2 = H$, R_3 = Glc-*p*

Acknowledgements

This study is partially funded by the US CDC Grant (R01 CI000315-01). The authors thank: Camptotech Inc.'s cytotoxicity testing at the National Cancer Institute (NCI); The Keck/IMD NMR Center, which is funded by the W.M. Keck Foundation and the University of Houston; Dr. Youlin Xia for NMR analysis assistance; Dr. Shane Tichy for HRESI-MS analysis, and Stacy Ownby, Dr. Wei Yuan, Dr. Ping Wang, and Dr. Wanli Zhang for manuscript preparation.

References

Chan, P.K., Mak, M.S., Wang, Y., 2005. US Pat. Appl. Publ. 103pp., Ser. No. 117,745, Application: US 2005-131551 20050517.

Little, E.L., 1980. Field Guide to North American Trees. Alfred A. Knopf, New York.

Matsushita, A., Sasaki, Y., Warashina, T., Miyase, T., Noguchi, H., Vander Velde, D., 2004. Hydrocotylosides I–VII, new oleanane saponins from *Hydrocotyle sibthorpioides*. J. Nat. Prod. 67, 384–388.

National Cancer Institute, 2007. DTP Human Tumor Cell Line Screen. <http://dtp.nci.nih.gov/branches/btb/ivclsp.html>.

Neamtu, G., Bodea, C., 1974. Chemotaxonomic investigations on higher plants VII. Carotenoid pigments in some species of *Aesculus* genus. Studii si Cercetari de Biochimie 17, 41–46.

Schrutka-Rechtenstamm, R., Robien, W., Jurenitsch, J., 1988. Structure of the sapogenins of seeds from *Aesculus pavia* L.. Pharmazie 43, 208–210.

Tang, M.J., Shen, D.D., Hu, Y.C., Gao, S., Yu, S.S., 2004. Cytotoxic triterpenoid saponins from *Symplocos chinensis*. J. Nat. Prod. 67, 1969–1974.

Wagner, H., Bladt, S., 1975. Phytochemistry 14, 2061–2064.

Voutquenne, L., Guinot, P., Froissard, C., Thoison, O., Litaudon, M., Lavaud, C., 2005. Haemolytic acylated triterpenoid saponins from *Harpullia austro-caledonica*. Phytochemistry 66, 825–835.

Yoshikawa, M., Murakami, T., Matsuda, H., Yamahara, J., Murakami, N., Kitagawa, I., 1996. Bioactive saponins and glycosides. III. Horse chestnut. (1): The structures, inhibitory effects on ethanol absorption, and hypoglycemic activity of escins Ia, Ib, IIa, IIb, and IIIa from the seeds of *Aesculus hippocastanum* L.. Chem. Pharm. Bull. 44, 1454–1464.

Yoshikawa, M., Murakami, T., Yamahara, J., Matsuda, H., 1998. Bioactive saponins and glycosides. XII. Horse chestnut. (2): structures of escins IIIb, IV, V, and VI and isoescins Ia, Ib, and V, acylated polyhydroxyoleanene triterpene oligoglycosides, from the seeds of horse chestnut tree (*Aesculus hippocastanum* L., Hippocastanaceae). Chem. Pharm. Bull. 46, 1764–1769.

Zhang, Z.Z., Koike, K., Jia, Z.H., Nikaido, T., Guo, D.A., Zheng, J.H., 1999. New saponins from the seeds of *Aesculus chinensis*. Chem. Pharm. Bull. 47, 1515–1520.

Zhang, Z.Z., Li, S.Y., Zhang, S.M., 2005. Six new triterpenoid saponins from the root and stem bark of *Cephalanthus occidentalis* L.. Planta Med. 70, 355–361.

Zhang, Z.Z., Zhang, S.M., Li, S.Y., 2006. Triterpenoid saponins from the fruits of *Aesculus pavia*. Phytochemistry 67, 784–794.