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Triterpenoid saponins from the fruits of *Aesculus pavia*

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Abstract

The compounds, named aesculiosides Ia–Ie, IIa–IId, and IVa–IVc, were isolated from an ethanol extract of the fruits of North American *Aesculus pavia*, along with two known compounds. Their structures were characterized as polyhydroxyoleanene pentacyclic triterpenoid saponins by spectroscopic and chemical analyses. These saponins were divided into three elution zones by chromatography according to the polarity because of the acyl substitution at C-21 and C-22 of the aglycone saponins moiety. These are structurally different from those isolated from Eurasian *Aesculus hippocastanum* and *Aesculus chinensis* in their oligosaccharide moieties.

Keywords: *Aesculus pavia*; Hippocastanaceae; Saponins; Aesculiosides

1. Introduction

The genus *Aesculus* (family Hippocastanaceae) consists of 13 species of deciduous trees and shrubs, and is distributed in the northern hemisphere, primarily in eastern Asia and eastern North America, with one species native to Europe, and two to western North America. *Aesculus* species are often used as ornamentals for their showy flowers and large foliage. There are two Eurasian species commonly used in traditional medicine: *Aesculus hippocastanum* (Common horse chestnut) and *Aesculus chinensis* (Chinese horse chestnut). In Europe, the bark and leaves of *A. hippocastanum* have been employed as an astringent to treat diarrhea and hemorrhoids (Bisset, 1994). Aescin, a saponin mixture from the seeds of this species, has been shown to have a clinically significant activity in chronic venous insufficiency, hemorrhoids and post-operative edema (Sirtori, 2001). In mainland China, the seeds of *A. chinensis* have been used as a stomachic and analgesic in the treatment of distention and pain in chest and abdomen, and in the treatment of malaria and dysentery (Jiangsu New Medical College, 1977). Tablets made from the seeds are also used for treating heart diseases (Institute of Materia Medica, Chinese Academy of Medical Sciences, 1984). The chemical constituents of both *A. hippocastanum* (Yoshikawa et al., 1996, 1998) and *A. chinensis* (Wei et al., 2004; Yang et al., 1999; Zhao et al., 2001; Zhao and Yang, 2003; Zhang et al., 1999a) have been extensively investigated.

North American *Aesculus* species are often poisonous, and the seeds and roots of these species were formerly used to stun fish by native Americans (Harris, 2003; Little, 1980). *Aesculus pavia* L., known as "red buckeye" 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). Our interests in identification of saponin constituents from the *Aesculus* genus (Zhang et al., 1999a) and the American native plants (Zhang et al., 2005) prompted us to conduct a detailed chemical investigation of the saponins occurring as a very complex mixture from this source. In this paper, we

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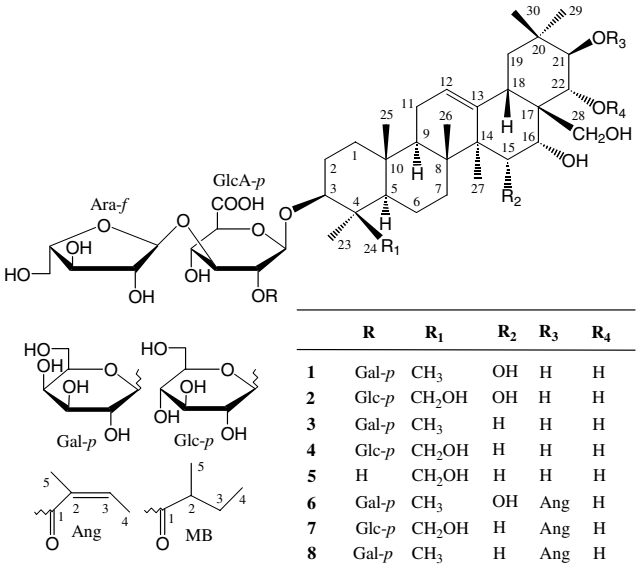
describe the isolation and structure elucidation of 12 saponins, named aesculiosides I_a–I_e (**1**–**5**), II_a–II_d (**6**–**9**), and IV_a–IV_c (**10**, **12**, **14**), respectively.



GlcA-*p*: *β*-D-glucuronopyranosyl acid
Gal-*p*: *β*-D-galactopyranosyl
Glc-*p*: *β*-D-glucopyranosyl
Ara-*f*: *β*-L-arabinofuranosyl
Ang: angeloyl
MB: 2-methylbutanoyl

	R	R_1	R_2	R_3	R_4
1	Gal-*p*	CH_3	OH	H	H
2	Glc-*p*	CH_2OH	OH	H	H
3	Gal-*p*	CH_3	H	H	H
4	Glc-*p*	CH_2OH	H	H	H
5	H	CH_2OH	H	H	H
6	Gal-*p*	CH_3	OH	Ang	H
7	Glc-*p*	CH_2OH	H	Ang	H
8	Gal-*p*	CH_3	H	Ang	H
9	H	CH_2OH	H	Ang	H
10	Gal-*p*	CH_3	OH	Ang	MB
11	Glc-*p*	CH_2OH	H	Ang	Ang
12	Glc-*p*	CH_2OH	H	Ang	MB
13	Gal-p	CH_3	H	Ang	Ang
14	Gal-p	CH_3	H	Ang	MB

2. Results and discussion

The column fraction Aes-P from an ethanol extract of the fruits of *A. pavia* was separated by a low-pressure column of ODS to give four elution zones I (Aes-I), II (Aes-II), III (Aes-III), and IV (Aes-IV) that were divided according to decreasing polarity because of the acyl substitution at C-21 and C-22 of the aglycone: zone I contained the most polar compounds without acylation at C-21 and C-22; zone II included the medium polar compounds with acylation at C-21 only; zone IV (Aes-IV) is characterized by the presence of less polar compounds with acylation at both C-21 and C-22. Zone IV represents the major by weight of the fraction Aes-P. These three different elution zones I, II, and IV were further separated by preparative MPLC and HPLC using a preparative ODS column and an analytical ODS column, respectively, to yield saponins **1**–**14** (see Section 4).

Compounds **1**–**5** were obtained from zone I as amorphous powders with adequate solubility in a mixture of methanol and water. Their IR spectra showed absorption bands due to hydroxyl (3416–3423 cm^{-1}) and carboxylic (1715–1720 cm^{-1}) groups, respectively.

Aesculioside Ia (**1**) was assigned a molecular formula of $C_{47}H_{76}O_{21}$, as deduced from the $[M + Na]^+$ ion m/z at 999.4835 and the $[M + K]^+$ ion m/z at 1015.4519 in the positive HRESIMS, as well as from its NMR spectroscopic data. The NMR data (Tables 1–3) of **1** were characteristic of a polyhydroxyoleanene triterpenoid glycoside with three sugar units. Detailed NMR spectroscopic data analysis indicated that the aglycone of **1** is 3β,15α,16α,21β, 22α,28-hexahydroxyolean-12-ene (R1-barrigenol, **1a**) (D'Acquarica et al., 2002; Konoshima and Lee, 1986; Tang et al., 2005). The stereochemistry of the aglycone was confirmed by a NOESY experiment and from the vicinal coupling constants of the key protons (Fig. 1 and Table 3). Acid hydrolysis of **1** afforded the aglycon, R1-barrigenol (**1a**), identified by NMR and MS analysis and comparison with reference data (Konoshima and Lee, 1986; Tang et al., 2005), and three monosaccharide sugars, D-galactose, L-arabinose and D-glucuronic acid, identified by co-TLC analysis and by measurement of optical rotation after separation by preparative TLC (Voutquenne et al., 2005). In the ^{13}C NMR spectrum of **1**, the glucuronic acid (Yoshikawa et al., 1998, 1996; Zhang et al., 1999a) resonated at δ 104.5 (C-1), 78.3 (C-2), 85.3 (C-3), 71.5 (C-4), 76.4 (C-5), and 171.8 (C-6), and the galactose (Yoshikawa et al., 1996; Zhang et al., 1999a) at δ 104.1 (C-1), 73.0 (C-2), 74.3 (C-3), 69.3 (C-4), 76.1 (C-5), and 61.4 (C-6), respectively. The glucuronic acid and galactose units were determined to be in the pyranose form with an β-anomeric configuration from their ^{13}C NMR spectroscopic data and ^3JH1, H2 coupling constants (Table 2). The α-furanose form of arabinose was confirmed (D'Acquarica et al., 2003; Tang et al., 2005) from the carbon signals at δ 110.3 (C-1), 82.9 (C-2), 77.4 (C-3), 84.8 (C-4), and 62.0 (C-5), which correlated with δ 5.99 (1H, *br s*, H-1), 4.84 (H-2), 4.60 (H-3), 4.77 (H-4), 4.16 (H-5a), and 4.33 (H-5b), respectively, in the HSQC spectrum. The attachment of GlcA unit to C-3 of the aglycone, suggested by the downfield chemical shift δ 89.6 (C-3), was confirmed by the HMBC correlations. These were observed with H-3 of the aglycone and C-1 of GlcA, as well as H-1 (δ 4.81, 1H, *d*, 7.8 Hz) of GlcA with C-3 of the aglycone, whereas the attachment of galactose and arabinose to C-2 and C-3 of GlcA were established by the following HMBC correlations: H-2 (δ 4.34) of GlcA with C-1 of Gal, H-1 (δ 5.23, 1H, *d*, 7.2 Hz) of Gal with C-2 of GlcA, H-3 (δ 4.23) of GlcA with C-1 of Ara, and H-1 of Ara with C-3 of GlcA. The sugar chain of **1** was also deduced from the following NOE correlations observed in the NOESY spectrum: H-3 of aglycone with H-1 of GlcA, H-2 of GlcA with H-1 of Gal, and H-3 of GlcA with H-1 of Ara (Fig. 1). Full assignments of the ^{13}C and ^1H NMR spectroscopic signals of **1** were carried out using COSY, HSQC, and HMBC correlations (Fig. 1). The structure of **1** was established as 3-*O*-[β-D-galactopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-α-D-glucuronopyranosyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

Aesculioside I_b (**2**) gave a $[M + Na]^+$ ion m/z at 1015.4701 and a $[M + K]^+$ ion m/z at 1031.4438 in the positive HRESIMS, 16 mass units higher than that of **1**, implying the presence of an additional oxygen-bearing

Table 1
^{13}C NMR spectroscopic data for the aglycone moieties of **1**–**10**, **12**, and **14** (150 MHz in pyridine d_5)[a]

Carbon	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**	**7**	**8**	**9**	**10**	**12**	**14**
1	38.3	38.3	38.4	38.3	38.3	38.5	38.3	38.3	38.3	38.4	38.3	38.5
2	26.3	26.2	26.2	26.2	26.2	26.3	26.3	26.4	26.4	26.5	26.3	26.3
3	89.6	91.1	89.7	91.2	88.5	89.5	91.2	89.7	88.6	89.6	91.2	89.8
4	39.5	43.6	39.6	43.6	43.6	39.6	43.7	39.6	43.6	39.6	43.5	39.6
5	55.1	55.3	55.1	55.5	55.5	55.3	55.6	55.5	55.4	55.5	55.7	55.6
6	18.3	18.3	18.2	18.2	18.3	18.2	18.3	18.2	18.3	18.5	18.2	18.2
7	36.2	36.3	32.9	32.6	32.9	36.3	32.9	32.8	33.0	36.4	32.9	32.9
8	40.1	40.2	40.2	40.1	40.2	40.3	40.1	40.2	40.1	40.3	40.2	40.1
9	46.8	46.7	46.7	46.5	46.7	46.7	46.6	46.6	46.8	46.8	46.6	46.7
10	37.1	37.2	36.6	36.5	36.6	37.1	36.5	36.6	36.5	37.3	36.5	36.6
11	23.6	23.6	23.7	23.5	23.6	23.6	23.6	23.6	23.6	23.8	23.6	23.6
12	124.1	124.1	123.0	123.0	123.0	124.6	123.5	123.6	123.5	125.3	123.7	123.8
13	143.2	143.1	143.2	143.2	143.1	143.1	143.2	143.1	143.2	143.1	143.2	143.2
14	47.3	47.4	41.7	41.6	41.6	47.6	41.6	41.6	41.7	47.5	41.7	41.6
15	66.8	66.8	34.1	33.6	34.0	67.0	34.3	34.2	34.2	67.3	34.5	34.5
16	71.8	71.8	67.5	66.5	67.5	73.3	67.5	67.6	67.5	73.4	68.0	67.9
17	48.1	48.1	48.1	48.1	48.1	48.0	48.1	48.1	48.0	48.1	48.1	48.2
18	41.2	41.3	40.9	40.5	40.8	40.8	40.1	40.2	40.1	40.8	40.0	40.0
19	47.3	47.3	47.6	47.3	47.3	47.0	47.3	47.3	47.3	46.6	47.0	469
20	36.3	36.3	36.3	36.3	36.3	36.3	36.3	36.4	36.3	36.4	36.4	36.4
21	77.8	77.9	77.9	77.9	77.9	81.4	81.5	81.5	81.5	78.3	78.5	78.5
22	76.3	76.4	76.7	76.5	76.5	72.8	72,8	72.8	72.8	73.0	73.3	73.3
23	27.5	22.1	27.5	21.9	22.1	27.6	22.0	27.6	22.1	27.6	22.1	27.6
24	16.5	62.9	16.5	62.9	62.8	16.5	62.9	16.6	62.9	16.5	63.0	16.6
25	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5
26	16.8	16.8	16.4	16.3	16.3	17.2	16.8	16.8	16.8	17.1	16.7	16.7
27	20.6	20.6	27.0	26.9	27.1	20.8	27.1	27.1	27.1	20.8	27.1	27.1
28	66.8	66.8	68.0	66.8	67.7	65.6	65.5	656	65.5	62.8	63.4	63.3
29	29.3	29.4	29.6	29.4	29.5	29.3	29.3	29.3	29.3	29.3	29.3	29.4
30	19.0	18.9	19.0	18.9	19.0	19.9	19.9	20.0	20.0	19.9	19.9	19.9
C_{21}						Ang	Ang	Ang	Ang	Ang	Ang	Ang
1						167.9	167.9	167.8	167.8	167.9	167.9	167.9
2						129.8	129.9	129.9	129.9	129.7	129.7	129.7
3						136.0	136.1	136.0	136.0	138.5	138.4	138.5
4						15.7	15.7	15.7	15.7	15.7	15.7	15.7
5						20.7	20.8	20.8	20.7	20.7	20.7	20.8
C_{22}										MB	MB	MB
1										176.0	176.0	176.0
2										41.5	41.5	41.5
3										26.6	26.5	26.5
4										11.6	11.6	11.6
5										17.0	16.9	16.9

[a] Assignments were based on COSY, HSQC, and HMBC experiments.
[b] Data were recorded in pyridine-d_5/MeOH-d_4 (5:1).

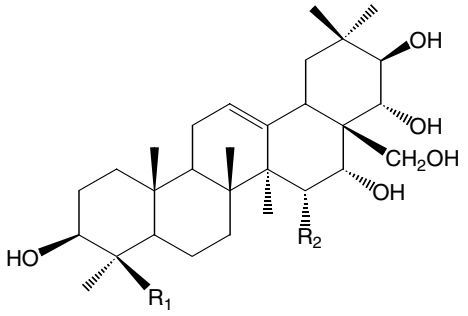
function in **2**. The ^{13}C NMR spectroscopic data (Table 1) for the aglycones of **2** and **1** were very close, except for those of C-4, C-23, and C-24. In the ^{13}C NMR spectrum of **1**, the signals at δ 39.5 (*s*, C-4), 27.5 (*q*, C-23), and 16.5 (*q*, C-24) were replaced in **2** by signals at δ 43.6 (*s*), 22.1 (*q*), and 62.9 (*t*), respectively, indicating the presence of the additional oxygen-bearing function at C-24. Interpretation of 1D and 2D NMR spectra allowed the aglycone of **2** to be identified as 3β,15α,16α, 21β,22α,24β,28-heptahydroxyolean-12-ene (**2a**). The anomeric protons at δ 4.84 (1H, *d*, $J = 7.8$ Hz, GlcA), 5.42 (1H, *d*, $J = 8.4$ Hz, Glc), and 6.01 (1H, *br s*, Ara), correlating with the anomeric carbons at δ 104.3, 103.3, and 110.5 observed in the HSQC spectrum showed that **2** also contains three monosaccharide moieties as in **1**. By NMR spectroscopic analysis, it was concluded that galactose in **1**was replaced in **2** by a glucose. The two hexoses could be distinguished by the ^{13}C NMR chemical shifts at C-2 and C-3 (Table 2, Yoshikawa et al., 1996; Zhang et al., 1999a,b; Voutquenne et al., 2005). Three monosaccharides in the acid hydrolysate of **2** were identified as D-glucose, L-arabinose and D-glucuronic acid by the same method as described for **1**. The sugar chain of **2** was also established by the HMBC and NOESY information as depicted for **1**. The structure of **2** was thus assigned as 3-*O*-[β-D-glucopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-α-D-glucuronopyranosyl-3β,15α, 16α,21β,22α,24β,28-heptahydroxyolean-12-ene.

Table 2
^{13}C and ^{1}H NMR spectroscopic data for the sugar moieties of **1**, **2**, and **5** [in pyridine-d_5/MeOH-d_4 (5:1)][a]

Carbon	1	2	5	Proton	1	2	5
GlcA-*p*				GlcA-*p*			
1	104.3	104.3	105.2	1	4.81 (1H, *d*, 7.8 Hz)	4.84 (1H, *d*, 7.8 Hz)	4.92 (1H, *d*, 7.8 Hz)
2	78.3	77.5	75.0	2	4.34 (1H, m)	4.26 (1H, m)	3.99 (1H, m)
3	85.3	85.9	79.6	3	4.23 (1H, m)	4.21 (1H, m)	4.48 (1H, m)
4	71.5	71.5	71.8	4	4.30 (1H, m)	4.38 (1H, m)	4.13 (1H, m)
5	76.4	76.5	78.9	5	4.35 (1H, m)	4.44 (1H, m)	4.62 (1H, m)
6	171.8	171.9	171.9				
	Gal-*p*	Glc-*p*			Gal-*p*	Gle-*p*	
1	104.1	103.3		1	5.23 (1H, *d*, 7.2 Hz)	5.42 (1H, *d*, 8.4 Hz)	
2	73.0	75.0		2	4.37 (1H, m)	3.95 (1H, m)	
3	74.3	77.6		3	4.07 (1H, m)	4.16 (1H, m)	
4	69.3	69.2		4	4.47 (1H, m)	4.45 (1H, m)	
5	76.1	77.8		5	3.97 (1H, m)	3.59 (1H, m)	
6	61.4	61.2		6	4.45 (1H, m), 4.55 (1H, m)	4.30 (1H, m), 4.38 (1H, m)	
Ara-*f*							
1	110.3	110.5	108.9	1	5.99 (1H, *brs)*	6.01 (1H, *brs*)	6.30 (1H, 6rs)
2	82.9	82.9	81.5	2	4.84 (1H, m)	4.90 (1H, m)	4.79
3	77.4	77.4	78.4	3	4.60 (1H, m)	4.69 (1H, m)	4.73
4	84.8	84.9	87.3	4	4.77 (1H, m)	4.79 (1H, m)	5.02
5	62.0	62.1	62.3	5	4.16 (1H, m), 4.33 (1H, m)	4.09 (1H, m). 4.30 (1H, m)	4.25 (1H, m), 4.29 (1H, m)

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

Aesculioside I_c (**3**) displayed a $[M + Na]^+$ ion *m*/*z* at 983.4802 and a $[M + K]^+$ ion *m*/*z* at 999.4573 in the positive HRESIMS, corresponding to a molecular formula of $C_{47}H_{76}O_{20}$, and has one less oxygen atom when compared with **1**. The spectroscopic NMR data for the sugar part of **3** bore a close resemblance to those of **1**, revealing that **3** has a common sugar substitution pattern to **1**, that is, β-D-galactopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranoside. The presence of the furanose form of arabinose was confirmed by its characteristic NMR spectroscopic data (D'Acquarica et al., 2003; Tang et al., 2005) that are different from those of the pyranose form of arabinose in barringtoside C (Pal et al., 1994). The aglycone of **3** was somewhat different from that of **1**, based on the different chemical shifts of C-14, C-15, and C-16 (Table 1), due to the substituent at C-15. NMR spectroscopic data analysis showed the hydroxyl group at C-15 in **1** was absent in **3**. Thus, the aglycone of **3** was identified as 3β,16α,21β,22α,28-pentahydroxyolean-12-ene (barringtogenol-C, **3a**) (Konoshima and Lee, 1986; Pal et al., 1994). Acid hydrolysis of **3** afforded the aglycone, barringtogenol-C, identified by NMR and MS data analysis and comparison with reference data (Konoshima and Lee, 1986); the three monosaccharides sugars, D-galactose, L-arabinose and D-glucuronic acid, were also identified by the same method as depicted for **1**. From above evidence, the structure of **3** was established as 3-*O*-[β-D-galactopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene.



1a $R_1 = CH_3$, $R_2 = OH$
2a $R_1 = CH_2OH$, $R_2 = OH$
3a $R_1 = CH_3$, $R_2 = H$
4a $R_1 = CH_2OH$, $R_2 = H$

Aesculioside I_d (**4**) exhibited an intense $[M + Na]^+$ peak at *m*/*z* 999.4732 and a $[M + K]^+$ peak at *m*/*z* 1015.4466 in the positive HRESIMS, 16 mass units less than that of **2**, suggesting **4** is a derivative of **2** with the absence of a hydroxyl group. The NMR spectroscopic data of the sugar part in **4** were almost superimposable on those of the sugar part in **2**, thus **4** and **2** share the same sugar substitution pattern of β-D-glucopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranoside. Extensive NMR spectroscopic data analysis showed that the structural difference for the aglycones of **4** and **2** was only at C-15, in a similar manner to the aglycones of **3** and **1.** Thus, the

Table 3
^{1}H NMR spectroscopic data for the aglycone moieties of **1**–**6** (600 MHz in pyridine-d_5)[a]

Proton	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**
1	0.87, 1.43	0.87, 1.40	0.86, 1.48	0.78. 1.34	0.94, 1.51	0.86,1.45
2	1.86, 2.15 (1H, *m*)	1.89, 2.28 (1H, *m*)	1.89, 2.16 (1H, *m*)	1.89, 2.29 (1H, *m*)	1.97, 2.36 (1H, *m*)	1.86, 2.23 (1H, *m*)
3	3.25 (1H, *dd*-like)	3.41 (1H, *dd*-like)	3.26 (1H, *dd*-like)	3.42 (1H, *dd*-like)	3.62 (1H, *dd*-like)	3.33 (1H, *dd*-like)
5	0.79	0.90	0.76	0.87	0.91	0.80
6	1.31, 1.52	1.26, 1.54	1.30, 1.52	1.16, 1.48	1.28,163	1.31,1.51
7	2.00, 2.04	1.96, 2.06	1.31, 1.63	1.25, 1.58	1.31, 1.60	2.00, 2.05
9	1.68	1.63	1.73	1.69	1.73	1.76
11	1.86, 1.90	1.74, 1.85	1.84, 1.90	1.72, 1.84	1.75, 1.88	1.86,1.92
12	5.45 (1H, *br s*)	5.45 (1H, *br s*)	5.38 (1H, *br s*)	5.34 (1H, *br s*)	5.38 (1H, *br s*)	5.49 (1H, *br s*)
15	4.33	4.36	1.67, 2.07 (1H, *d* 10.8 Hz),1	1.63, 2.00 (1H, *d*, 12.0 Hz)	1.64, 2.04 (1H, *d*, 11.4 Hz)	4.30
16	4.79 (1H, *d*,4.2 Hz)	4.83 (1H, *d*,4.2 Hz)	5.03 (1H, *m*)	5.03 (1H, *m*)	4.94 (1H, *m*)	4.47
18	2.70 (1H, *d*,10,2 Hz)	2.72 (1H, *d*,10,2 Hz)	2.80 (1H, *d*,10,8 Hz)	2.70 (1H, *d*,12,6 Hz)	2.74 (1H, *d*,12,6 Hz)	2.87 (1H, *d*,12.8 Hz)
19	1.34,2.92 (1H, *t*, 10.2 Hz)	1.36,2.92 (1H, *t*, 10.2 Hz)	1.45, 3.03 (1H, *t*, 10.8 Hz)	1.42,3.00 (1H, *t*, 12.6 Hz)	1.38, 2.96 (1H, *t*, 12.6 Hz)	1.39,3.01 (1H, *t*, 12.8 Hz)
21	4.68 (1H, *d* 9.6 Hz	4.72 (1H, *d*, 9.6 Hz)	4.81 (1H, *d*, 9.6 Hz	4.67 (1H, *d* 9.6 Hz)	4.72 (1H, *d* 9.6 Hz)	6.45 (1H, *d* 9.6 Hz)
22	4.49 (1H, *d* 9.6 Hz	4.51 (1H, *d* 9.6 Hz	4.64 (1H, *d* 9.6 Hz	4.50 (1H, *d* 9.6 Hz	4.54 (1H, *d* 9.6 Hz	4.80 (1H, *d* 9.6 Hz
23	1.26 (3H, *s*)	1.29 (3H, *s*)	1.32 (3H, *s*)	1.28 (3H, *s*)	1.46 (3H, *s*)	1.31 (3H, *s*)
24	1.11 (3H, *s*)	3.26 (1H, *d*,12.0 Hz), 4.30	1.17 (3H, *s*)	3.25 (1H, *d*, 10.8 Hz), 4.29	3.60 (1H, *d*, 10.2 Hz), 4.16	1.22 (3H, *s*)
25	0.84 (3H, *s*)	0.67 (3H, *s*)	0.83 (3H, *s*)	0.65 (3H, *s*)	0.80 (3H, *s*)	0.87 (3H, *s*)
26	1.01 (3H, *s*)	0.96 (3H, *s*)	0.91 (3H, *s*)	0.82 (3H, *s*)	0.88 (3H, *s*)	1.02 (3H, *s*)
27	1.78 (3H, *s*)	1.79 (3H, *s*)	1.86 (3H, *s*)	1.77 (3H, *s*)	1.85(3H, *s*)	1.89 (3H, *s*)
28	3.66, 4.02(each, 1H, *d*, 12.0 Hz)	3.69, 3.99 (each, 1H, *d*, 11.4 Hz)	3.73, 4.02 (each, 1H, *d*, 12.0 Hz)	3.70, 4.00 (each, 1H, *d*, 10.8 Hz)	3.67, 3.90 (each, 1H, *d*, 10.8 Hz)	3.75, 4.00 (each, 1H, *d*, 10.2 Hz)
29	1.26 (3H, *s*)	1.28 (3H, *s*)	1.32 (3H, *s*)	1.26 (3H, *s*)	1.30 (3H, *s*)	1.09 (3H, *s*)
30	1.30 (3H, *s*)	1.32 (3H, *s*)	1.38 (3H, *s*)	1.31 (3H, *s*)	1.34 (3H, *s*)	1.31 (3H, *s*)
C_{21}						Ang
3						5.92 (1H, q, 6.6 Hz)
4						2.06 (1H, *d*, 6.6 Hz)
5						1.99 (3H, s)

[a] Assignments were based on COSY, HSQC, and HMBC experiments, and due to severe in the ^{1}H spectrum., only detectable relative *J*(Hz) are reported.
[b] Data were recorded in pyridine-d_5/MeOH-d_4 (5:1).

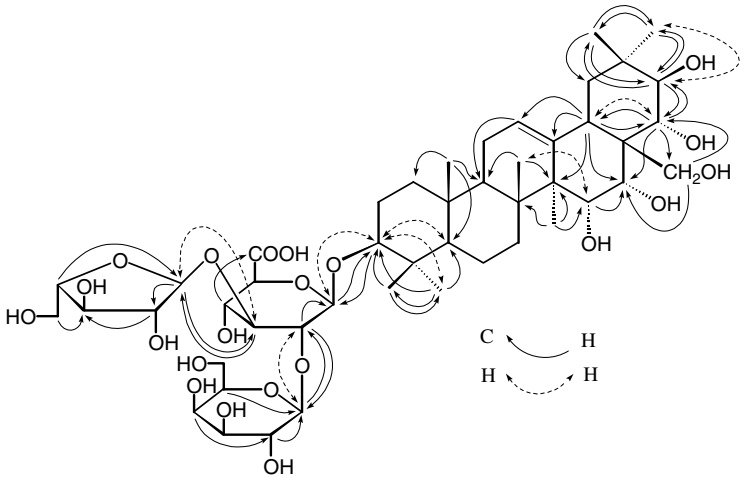


Fig. 1. Key HMBC and NOE correlations of aesculioside I_a (**1**).

aglycone of **4** was identified as 3β,16α,21β,22α, 24β, 28-hexahydroxyolean-12-ene (protoaescigenin, **4a**) (Yoshikawa et al., 1998, 1996; Zhang et al., 1999a). Therefore, the structure of **4** was established 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.

Aesculioside I_e (**5**) was assigned a molecular formula of $C_{41}H_{66}O_{16}$, as deduced from its HRESIMS data. The NMR spectroscopic resonances due to the aglycone of **5** were very similar to those of **4**, verifying that compounds **5** and **4** share the same aglycone (protoaescigenin, **4a**). In the HSQC spectrum, NMR data showed that **5** contains two monosaccharides, which were identified as β-D-glucuronopyranosyl and α-L-arabinofuranosyl residues (Table 2). The disaccharide moiety attached at C-3 of the aglycone was established from the following HMBC correlations: H-3 (δ 3.62, 1H, *dd*-like) of the aglycone with C-1 (δ 105.2) of GlcA, H-1 (δ 4.92, 1H, *d*, 7.8 Hz) of GlcA with C-3 (δ 88.5) of the aglycone, H-3 (δ 4.48) of GlcA with C-1 (δ 108.9) of Ara, and H-1 (δ 6.30, 1H, *br s*) of Ara with C-3 (δ 79.6) of GlcA. Accordingly, the structure of **5** was established as 3-*O* − α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-3β,16α,21β,22α,24β,28-hexahydroxyolean-12-ene.

Aesculiosides II_a-II_b (**6**–**9**) were obtained from zone II as amorphous powders with good solubility in methanol and pyridine. The molecular formulae of compounds **6**($C_{52}H_{82}O_{22}$), **7**($C_{52}H_{82}O_{22}$), **8**($C_{52}H_{82}O_{21}$), and **9**($C_{46}H_{72}O_{17}$) were deduced from their HRESIMS data (see Section 4)). The IR spectra of **6**–**9** exhibited absorption bands at 3417–3426 cm^{-1} for hydroxyl groups, 1716–1720 cm^{-1} for carboxylic groups, and 1650–1656 cm^{-1} for α, β-unsaturated carbonyl esters. Each of compounds **6**–**9** contained an angeloyl group, indicated by the characteristic NMR signals (Tables 1, 3, and 4; Yoshikawa et al., 1998, 1996; Zhang et al., 1999a). The downfield chemical shift (δ 6.45–6.50, 1H, *d*, 9.6 Hz) was assigned to H-21 based on the HMBC correlations of H-21 with C-29 and 30. The HMBC correlation between H-21 and C-1 of the angeloyl group indicated the position of the angeloyl residue at C-21. Alkaline hydrolysis of compounds **6**–**9** liberated **1**, **4**, **3**, and **5**, respectively, which were identified by co-HPLC analysis with their authentic samples. Full assignments of the ^{13}C and ^{1}H NMR signals of compounds **6**–**9** were carried out using COSY, HSQC, and HMBC correlations. Thus, the structures of the new compounds **6**–**9** were established as 3-*O*-[β-D-galactopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-3β, 15α,16α,21β,22α,28-hexahydroxyolean-12-ene (aesculioside II_a, **6**), 3-*O*-[β-D-glucopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-3β, 16α,21β,22α,24β,28-hexahydroxyolean-12-ene (aesculioside II_b, **7**), 3-*O*-[β-D-galactopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene (aesculioside II_c, **8**), and 3-*O*-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-3β,16α,21β,22α,24β, 28-hexahydroxyolean-12-ene (aesculioside II_d, **9**).

Compounds **10**–**14** were obtained from zone IV as amorphous powders with good solubility in methanol and pyridine. The IR spectra of **10**–**14** displayed similar absorption bands to **6**–**9**. Each of the compounds **10**–**14** was acylated at both C-21 and C-22, as indicated by the downfield values of H-21 (δ 6.68–6.72, 1H, *d*, 9.6–10.2 Hz) and H-22 (δ 6.25–6.35, 1H, *d*, 9.6–10.2 Hz). Full assignments of the ^{13}C and ^{1}H NMR signals were carried out using COSY, HSQC, and HMBC correlations. **11** and **13** were known saponins, and identified as 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, and 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, respectively, by NMR and MS analysis and comparison with reference data (Voutquenne et al., 2005).

Table 4
^{1}H NMR spectroscopic data for the aglycone moieties of **7**–**10**, **12**, and **14** (600 MHz in pyridine-d_5)[a]

Proton	**7**	**8**	**9**	**10**	**12**	**14**
1	0.84, 1.37	0.88, 1.43	0.94, 1.45	0.85, 1.43	0.80, 1.43	0.87, 1.41
2	1.90 2.39 (1H, *m*)	1.88, 2.17 (1H, *m*)	1.92, 2.19 (1H, *m*)	1.90, 2.16 (1H, *m*)	1.92, 2.40 (1H, *m*)	1.86, 2.21 (1H, *m*)
3	3.45 (1H, *dd*-like)	3.28 (1H, *dd*-like)	3.63 (1H, *dd*-like)	3.27(1H, *dd*-like)	3.44(1H, *dd*-like)	3.29 (1H, *dd*-like)
5	0.87	0.79	0.91	0.83	0.88	0.77
6	1.27, 1.54	1.31, 1.51	1.27, 1.53	1.41, 1.60	1.53, 1.63	1.52, 1.64
7	1.27, 1.65	1.29, 1.65	1.33, 1.69	2.10, 2.13	1.28, 1.53	1.31, 1.64
9	1.73	1.74	1.77	1.73	1.75	1.74
11	1.73, 1.86	1.80, 1.90	1.78, 1.95	1.83, 1.92	1.74, 1.89	1.80,1.89
12	5.37 (1H, *br s*)	5.39 (1H, *br s*)	5.39 (1H, *br s*)	5.53 (1H, *br s*)	5.41 (1H, *br s*)	5.42 (1H, *br s*)
15	1.67, 1.90	1.68, 1.90	1.75, 1.91	4.23 (1H, *m*)	1.70, 1.92	1.66, 1.91
16	4.88 (1H, *m*)	4.88 (1H, *m*)	4.85 (1H, *m*)	4.48 (1H, *m*)	4.53 (1H, *m*)	4.52 (1H, *m*)
18	2.93 (1H, *d* 10.8 Hz)	2.93 (1H, *d* 13.8 Hz)	2.95 (1H, *d* 12.0 Hz)	3.09 (1H, *m*)	3.13 (1H, *m*)	3.13 (1H, *m*)
19	1.39, 3.0 (1H, *t*, 10.8 Hz)	1.40, 3.08 (1H, *t*, 13.8 Hz)	1.43, 3.12 (1H, *t*, 12.0 Hz)	1.45, 3.11 (1H, *m*)	1.43, 3.11 (1H, *m*)	1.43, 3.11 (1H, *m*)
21	6.43 (1H, *d* 9.6 Hz)	6.46 (1H, *d* 9.6 Hz)	6.50 (1H, *d* 9.6 Hz)	6.68 (1H, *d* 9.6 Hz)	6.63 (1H, *d* 10.2 Hz)	6.69 (1H, *d* 10.2 Hz)
22	4.80 (1H, *d* 9.6 Hz)	4.81 (1H, *d* 9.6 Hz)	4.71 (1H, *d* 9.6 Hz)	6.25 (1H, *d* 9.6 Hz)	6.28 (1H, *d* 10.2 Hz)	6.26 (1H, *d* 10.2 Hz)
23	1.33 (3H, *s*)	1.32 (3H, *s*)	1.54 (3H, *s*)	1.30 (3H, *s*)	1.36 (3H, *s*)	1.34 (3H, *s*)
24	3.32 (1H, *m*),4.36	1.20 (3H, *s*)	3.69 (1H, *d*,10.2 Hz),4.24	1.20(3H, *s*)	3.33 (1H, *d*, 10.8 Hz),4.33	1.20 (1H.*s*)
25	0.66 (3H, *s*)	0.84 (3H, *s*)	0.82 (3H, *s*)	0.87 (3H, *s*)	0.66 (3H, *s*)	0.84 (3H, *s*)
26	0.81 (3H, *s*)	0.87 (3H, *s*)	0.88 (3H, *s*)	1.03 (3H, *s*)	0.82 (3H, *s*)	0.88 (3H, *s*)
27	1.85 (3H, *s*)	1.86 (3H, *s*)	1.90 (3H, *s*)	1.87 (3H, *s*)	1.85 (3H, *s*)	1.86 (3H, *s*)
28	3.67, 3.9 (each, 1H, *d*, 9.6 Hz)	3.69, 3.98 (each, 1H, *d*, 9.6 Hz)	3.71, 3.97 (each, 1H, *d*, 9.6 Hz)	3.49, 3.77 (each, 1H, *d*, 9.6 Hz)	3.42, 3.67 (each, 1H, *d*, 9.6 Hz)	3.42, 3.68 (each, 1H, *d*, 9.6 Hz)
29	1.11 (3H, *s*)	1.13 (3H, *s*)	1.15 (3H, *s*)	1.13 (3H, *s*)	1.11 (3H, *s*)	1.12 (3H, *s*)
30	1.33 (3H, *s*)	1.34 (3H, *s*)	1.35 (3H, *s*)	1.34 (3H, *s*)	1.34 (3H, *s*)	1.34 (3H, *s*)
C_{21}	Ang	Ang	Ang	Ang	Ang	Ang
3	5.92 (1H,q, 6.6 Hz)	5.92 (1H,q, 7.2 Hz)	5.92 (1H,q, 6.6 Hz)	6.08 (1H,q, 7.2 Hz)	6.10 (1H,q, 7.2 Hz)	6.09 (1H, q, 6.6 Hz)
4	2.06 (1H, *d*, 6.6 Hz)	2.07 (1H, *d*, 7.2 Hz)	2.08 (1H, *d*, 6.6 Hz)	2.19 (1H, *d*, 7.2 Hz)	2.19 (1H, *d*, 7.2 Hz)	2.19 (1H, *d*, 6.6 Hz)
5	1.99 (3H, *s*)	2.00 (1H, *s*)	2.01 (3H, *s*)	2.07 (1H, *s*)	2.07 (1H, *s*)	2.08 (1H, *s*)
C_{22}				MB	MB	MB
2				2.16 (1H, *m*)	2.29 (1H, *m*)	2.29 (1H, *m*)
3				1.26, 1.73	1.33, 1.74	1.33, 1.74
4				0.77 (3H, *t*, 7.2 Hz)	0.84 (3H, *t*, 7.2 Hz)	0.85 (3H, *t*, 7.2 Hz)
5				0.99 (3H, *d*, 7.2 Hz)	1.12 (3H, *d*, 7.2 Hz)	1.13 (3H, *d*, 7.2 Hz)

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

Aesculioside IV_a (**10**) was assigned a molecular formula of $C_{57}H_{90}O_{23}$, as deduced from its HRESIMS and ^{13}C NMR spectroscopic data. Alkaline hydrolysis of **10** afforded a prosapogenin, identified as **1** by co-HPLC analysis with an authentic sample. The ^{13}C NMR spectrum of **10** showed 57 signals, of which 30 were assigned to the aglycone, 17 to the oligosaccharide moiety, and the remaining ten to two other groups identified from the NMR spectroscopic data as angeloyl (Tables 1 and 4; Yoshikawa et al., 1998, 1996; Zhang et al., 1999a) and 2-methylbutanoyl moieties (Tables 1 and 4; Guo and Kenne, 2000; Matsushita et al., 2004; Nord and Kenne, 2000). As observed in the HMBC spectrum, the long-range correlations of H-21 (δ 6.68, 1H, *d*, 9.6 Hz) of the aglycone with C-1 (δ 167.9) of the angeloyl unit, and H-22 (δ 6.25, 1H, *d*, 9.6 Hz) of the aglycone with C-1 (δ 176.0) of the 2-methylbutanoyl unit, established that the angeloyl and 2-methylbutanoyl ester groups were attached to C-21 and C-22, respectively. The structure of the new saponin **10** was thus elucidated as 3-*O*-[β-D-galactopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-2-methylbutanoyl-3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene.

The molecular formula of aesculioside IVb (**12**) ($C_{57}H_{90}O_{23}$) was deduced from its HRESIMS and ^{13}C NMR spectroscopic data. The NMR data for compounds **12** and **11** were almost superimposable, except for the signals of the acyl substituent at C-22, revealing that **12** and **11** share a common aglycone and sugar substitution pattern, but differed only in the acyl group at C-22. Alkaline hydrolysis of **12** furnished the prosapogenin, identified as **4** by co-HPLC analysis with an authentic sample. The acyl substituent at C-22 in **12** was identified as 2-methylbutanoyl, based on its characteristic NMR signals, as previously discussed for **10**. A HMBC correlation of H-22 (δ 6.28, 1H, *d*, 10.2 Hz) of the aglycone with C-1 (δ 176.0) of the 2-methylbutanoyl unit confirmed that the 2-methylbutanoyl unit was linked at C-22. Consequently, the structure of the new saponin **12** was established as 3-*O*-[β-D-glucopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-2-methylbutanoyl-3β,16α,21β,22α,24β,28-hexahydroxyolean-12-ene.

Aesculioside IVc (**14**) was assigned the molecular formulae of $C_{57}H_{90}O_{22}$, as determined by HRESIMS. Alkaline hydrolysis of **14** furnished a prosapogenin, identified as **3** by co-HPLC analysis with an authentic sample. A detailed NMR spectroscopic study suggested that compounds **14** and **13** differ structurally only in the acyl substituent at C-22 similar to the differences between compounds **12** and **11**. The NMR signals of the acyl substituent at C-22 of **14** were very close to those of **12**, indicating the acyl substituent at C-22 was 2-methylbutanoyl in **14**. Accordingly, the structure of the new saponin **14** was established as 3-*O*-[β-D-galactopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-2-methylbutanoyl-3β,16α,21β,22α,28- pentahydroxyolean-12-ene.

3. Conclusions

Interestingly, saponins **1**–**14** have an oligosaccharide chain at C-3 of the aglycone with an α-arabinofuranosyl unit affixed to C-3 of the glucuronic acid and are structurally different from saponins isolated from *A. hippocastanum* and *A. chinensis*. Those saponins isolated from the two Eurasian species have a trisaccharide chain at C-3 of the aglycone with a β-glucopyranosyl unit attached to C-4 of the glucuronic acid. This significant chemical difference indicates a different chemotaxonomic feature and may be the basis for possible different medicinal uses between *A. pavia* and Eurasian *Aesculus* species. This result also supports a conclusion drawn from phenotypic and DNA sequence analyses that eastern North American species of *Aesculus* represent a different evolutionary lineage diverged early from their Eurasian counterparts (Forest et al., 2001; Hardin, 1957; Xiang et al., 1998).

4. Experimental

4.1. General experimental procedures

Optical rotations were determined on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on an ATI Mattson Genesis Series FT-IR spectrometer. NMR experiments were performed on Varian Unity Plus 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 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, 50:50 + 0.1% AcOH). Preparative MPLC 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). D-glucose, D-galactose, L-arabinose and D-glucuronic acid were purchased from Aldrich.

4.2. Plant material

The fruits of *A. pavia* were collected from a single plant in Nacogdoches, Texas in October 2003, and were identified by Dr. Shiyou Li. A voucher specimen (CMPR-2003101) is deposited at the National Center for Pharmaceutical Crops of Stephen F. Austin State University, USA.

4.3. Extraction and isolation

The fresh fruits were minced and air-dried. The air-dried fruits (350 g) were ground to a coarse powder and percolated five times with 95% ethanol. The alcohol extract (46.0 g) was concentrated, suspended in water, and then partitioned successively with $CHCl_3$ (500 mL × 3 times) and *n*-BuOH (500 mL × 3 times). The *n*-BuOH-soluble fraction (20.0 g) was applied to a column of Diaion HP-20 eluting with H_2O, H_2O-MeOH (2:3; 1:4) and MeOH. The MeOH–H_2O (4:1) elution was dried to afford a fraction (coded Aes-P). This fraction was applied to an ODS column eluting with MeOH–H_2O (2:3; 1:1; 3:2; 4:1) to give four fractions, Aes-I (MeOH–H_2O, 2:3), Aes-II (MeOH–H_2O, 1:1), Aes-III (MeOH–H_2O, 3:2), and Aes-IV (MeOH–H_2O, 4:1). Fraction Aes-I was separated by preparative MPLC (column B and solvent A) to give **1** (15.6 mg, t_R 48.1 min), **2** (5.8 mg, t_R 42.6 min), **3** (17.5 mg, t_R 68.4 min), **4** (11.6 mg, t_R 57.6 min), and **5** (2.5 mg, t_R 80.3 min). Similarly, fraction Aes-II was separated by preparative MPLC (column B and solvent B) to give **6** (9.8 mg, t_R 48.1 min), **7** (10.0 mg, t_R 55.1 min), **8** (10.5 mg, t_R 61.1 min), and **9** (2.6 mg, t_R 74.2 min); and part of fraction Aes-IV was separated by preparative MPLC (column B and solvent C) to give **10** (9.8 mg, t_R 51.2 min), Aes-IV_a (15.5 mg, t_R 66.1 min), and Aes-IV_b (20.1 mg, t_R 78.1 min). Compounds **11** (6.3 mg, t_R 24.2 min) and **12** (3.3 mg, t_R 25.6 min) were further separated from Aes-IV_a by HPLC (column A and solvent C), while **13** (7.1 mg, t_R 28.8 min) and **14** (4.2 mg, t_R 30.3 min) were separated from Aes-IV_b by HPLC using the same conditions as compounds **11** and **12**.

4.4. Aesculioside I_a (*1*)

Colorless powder from MeOH–H_2O, $[\alpha]_D^{25}$ −18.5 (MeOH–H_2O, 60:40; *c* 0.2), t_R 15.9 min (column A, solvent A). IR ν_{Max}^{KBr} cm^{-1}: 3416, 2933, 1716, 1456, and 1069. HRESIMS: *m/z* 999.4835 $[M + Na]^+$ (calcd. for $C_{47}H_{76}NaO_{21}$, 999.4777), 1015.4519 $[M + K]^+$ (calcd. for $C_{47}H_{76}KO_{21}$, 1015.4516). For ^{13}C and ^1H NMR spectroscopic data, see Tables 1–3.

4.5. Aesculioside I_b (*2*)

Colorless powder from MeOH–H_2O, $[\alpha]_D^{25}$ −17.6 (MeOH–H_2O, 60:40; *c* 0.2), t_R 14.2 min (column A, solvent A). IR ν_{Max}^{KBr} cm^{-1}: 3419, 2939, 1718, 1453, and 1071. HRESIMS: *m/z* 1015.4701 $[M + Na]^+$ (calcd. for $C_{47}H_{76}NaO_{22}$, 1015.4726), 1031.4438 $[M + K]^+$ (calcd. for $C_{47}H_{76}KO_{22}$, 1031.4465). For ^{13}C and ^1H NMR spectroscopic data, see Tables 1–3.

4.6. Aesculioside I_c (*3*)

Colorless powder from MeOH–H_2O, $[\alpha]_D^{25}$ −19.3 (MeOH–H_2O, 60:40; *c* 0.2), t_R 20.5 min (column A, solvent A). IR ν_{Max}^{KBr} cm^{-1}: 3423, 2942, 1720, 1450, and 1073. HRESIMS: *m/z* 983.4802 $[M + Na]^+$ (calcd. for $C_{47}H_{76}NaO_{20}$, 983.4828), 999.4573 $[M + K]^+$ (calcd. for $C_{47}H_{76}KO_{20}$, 999.4567). For ^{13}C and ^1H NMR spectroscopic data of the aglycone of **3,** see Tables 1 and 3.

4.7. Aesculioside I_d (*4*)

Colorless powder from MeOH–H_2O, $[\alpha]_D^{25}$ −20.6 (MeOH–H_2O, 60:40; *c* 0.2), t_R 18.1 min (column A, solvent A). IR ν_{Max}^{KBr} cm^{-1}: 3421, 2937, 1715, 1455, and 1068. HRESIMS: *m/z* 999.4732 $[M + Na]^+$ (calcd. for $C_{47}H_{76}NaO_{21}$, 999.4777), 1015.4466 $[M + K]^+$ (calcd. for $C_{47}H_{76}KO_{21}$, 1015.4516). For ^{13}C and ^1H NMR spectroscopic data of the aglycone of **4,** see Tables 1 and 3.

4.8. Aesculioside I_e (*5*)

Colorless powder from MeOH–H_2O, $[\alpha]_D^{25}$ −18.6 (MeOH–H_2O, 60:40; *c* 0.2), t_R 22.6 min (column A, solvent A). IR ν_{Max}^{KBr} cm^{-1}: 3423, 2941, 1720, 1452, and 1070. HRESIMS: *m/z* 837.4267 $[M + Na]^+$ (calcd. for $C_{41}H_{66}NaO_{16}$, 837.4249), 853.3997 $[M + K]^+$ (calcd. for $C_{41}H_{66}KO_{16}$, 853.3988). For ^{13}C and ^1H NMR spectroscopic data, see Tables 1–3.

4.9. Aesculioside II_a (*6*)

Colorless powder from MeOH, $[\alpha]_D^{25}$ −23.5 (MeOH; *c* 0.2), t_R 16.1 min (column A, solvent B). IR ν_{Max}^{KBr} cm^{-1}: 3417, 2951, 1717, 1650, 1457, and 1070. HRESIMS: *m/z* 1081.5173 $[M + Na]^+$ (calcd. for $C_{52}H_{82}NaO_{22}$, 1081.5195), 1097.4894 $[M + K]^+$ (calcd. for $C_{52}H_{82}KO_{22}$, 1097.4935). For ^{13}C and ^1H NMR spectroscopic data of the aglycone of **6,** see Tables 1 and 3.

4.10. Aesculioside II_b (*7*)

Colorless powder from MeOH, $[\alpha]_D^{25}$ −25.6 (MeOH; *c* 0.2), t_R 20.3 min (column A, solvent B). IR ν_{Max}^{KBr} cm^{-1}: 3420, 2948, 1720, 1656, 1451, and 1073. HRESIMS: *m/z* 1081.5258 $[M + Na]^+$ (calcd. for $C_{52}H_{82}NaO_{22}$, 1081.5195), 1097.4975 $[M + K]^+$ (calcd. for $C_{52}H_{82}KO_{22}$, 1097.4935). For ^{13}C and ^1H NMR spectroscopic data of the aglycone of **7,** see Tables 1 and 4.

4.11. Aesculioside II_c (*8*)

Colorless powder from MeOH, $[\alpha]_D^{25}$ −27.3 (MeOH; *c* 0.2), t_R 22.9 min (column A, solvent B). IR ν_{Max}^{KBr} cm^{-1}: 3425, 2943, 1716, 1654, 1455, and 1070. HRESIMS: *m/z* 1065.5202 $[M + Na]^+$ (calcd. for $C_{52}H_{82}NaO_{21}$, 1065.5246), 1081.4907$[M + K]^+$ (calcd. for $C_{52}H_{82}KO_{21}$, 1081.4986). For ^{13}C and ^1H NMR spectroscopic data of the aglycone of **8,** see Tables 1 and 4.

4.12. Aesculioside II_d (9)

Colorless powder from MeOH, $[\alpha]_D^{25}$ −25.1 (MeOH;*c* 0.2), t_R 26.8 min (column A, solvent B). IR ν_{Max}^{KBr} cm^{-1}: 3426, 2941, 1719, 1656, 1458, and 1075. HRESIMS: *m/z* 919.4689 $[M + Na]^+$ (calcd. for $C_{46}H_{72}NaO_{17}$, 919.4667), 935.4380 $[M + K]^+$ (calcd. for $C_{46}H_{72}KO_{17}$, 935.4407). For ^{13}C and ^1H NMR spectroscopic data of the aglycone of **9,** see Tables 1 and 4.

4.13. Aesculioside IV_a (10)

Colorless powder from MeOH, $[\alpha]_D^{25}$ −35.1 (MeOH;*c* 0.2), t_R 19.7 min (column A, solvent C). IR ν_{Max}^{KBr} cm^{-1}: 3421, 2938, 1709, 1650, 1453, and 1069. HRESIMS: *m/z* 1165.5747 $[M + Na]^+$ (calcd. for $C_{57}H_{90}NaO_{23}$, 1165.5771), 1181.5530 $[M + K]^+$ (calcd. for $C_{57}H_{90}KO_{23}$, 1181.5510). For ^{13}C and ^1H NMR spectroscopic data of the aglycone of **10,** see Tables 1 and 4.

4.14. Aesculioside IV_b (12)

Colorless powder from MeOH, $[\alpha]_D^{25}$ −36.2 (MeOH; *c* 0.2), t_R 25.6 min (column A, solvent C). IR ν_{Max}^{KBr} cm^{-1}: 3423, 2945, 1716, 1655, 1456, and 1073. HRESIMS: *m/z* 1165.5750 $[M + Na]^+$ (calcd. for $C_{57}H_{90}NaO_{23}$, 1165.5771), 1181.5498 $[M + K]^+$ (calcd. for $C_{57}H_{90}KO_{23}$, 1181.5510). For ^{13}C and ^1H NMR spectroscopic data of the aglycone of **12,** see Tables 1 and 4.

4.15. Aesculioside IV_c (14)

Colorless powder from MeOH, $[\alpha]_D^{25}$ −37.3 (MeOH;*c* 0.2), t_R 30.3 min (column A, solvent C). IR ν_{Max}^{KBr} cm^{-1}: 3428, 2939, 1721, 1655, 1450, and 1073. HRESIMS *m/z* 1149.5759 $[M + Na]^+$ (calcd. for $C_{57}H_{90}NaO_{22}$, 1149.5821), 1165.5520 $[M + K]^+$ (calcd. for $C_{57}H_{90}KO_{22}$, 1165.5561). For ^{13}C and ^1H NMR spectroscopic data of the aglycone of **14,** see Tables 1 and 4.

4.16. Acid hydrolysis of compounds 1, 2, and 3

Compound **1** was refluxed with 1 mL 1 M HCl (dioxane–H_2O, 1: 1) at 80 °C for 2 h. After dioxane was removed, the solution was extracted with $CHCl_3$-MeOH (7:3, 1 mL × 3). The extraction was washed with H_2O and then concentrated to give an amorphous powder (**1a**). The monosaccharide portion was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, and then freeze-dried. Three monosaccharides were identified with authentic samples by TLC in MeCOEt-*iso*-PrOH-Me_2CO-H_2O (20:10:7:6) as galactose, arabinose and glucuronic acid. After preparative TLC of the sugar mixture in this solvent, the optical rotation of each purified sugar was measured. By the same method, compound **3** afforded **3a**, and the monosaccharides in **3** were to be D-galactose, L-arabinose and D-glucuronic acid, and the monosaccharides in **2** to be D-glucose and L-arabinose, and D-glucuronic acid.

4.17. Alkaline hydrolysis of compounds 6–10, 12, 14

Compound **6** (3.0 mg) was refluxed with 0.8 M NaOH (1 mL) 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 (**1**, 2.3 mg), identified by co-HPLC analysis with an authentic sample. By the same method, **7–10, 12, 14** afforded the prosapogenins **4**, **3**, **5**, **1**, **4**, and **3**, respectively, identified by HPLC analysis with their authentic samples.

4.18. Compounds 11, 13, 1a and 3a

For the physicochemical properties and spectroscopic data of the known compounds **11**, **13**, **1a** and **3a** see references as cited in Section 2.

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