New Saponins from the Seeds of *Aesculus chinensis*

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Eight new acylated polyhydroxyoleanene triterpenoidal saponins, aesculiosides A—H (1—8), along with four known ones, have been isolated from the seeds of *Aesculus chinensis*. On the basis of extensive NMR studies, the structures of the new compounds were determined to be 21-*O*-tigloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl acid (1), 21-*O*-angeloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl acid (2), 21,22-*O*-ditigloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl acid (3), 21-*O*-tigloyl-22-*O*-angeloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl acid (4), 21,22-*O*-ditigloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-methyl β-D-glucuronopyranosate (5), 21-*O*-tigloyl-22-*O*-angeloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-methyl β-D-glucuronopyranosate (6), 21-*O*-tigloyl-28-*O*-acetylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-methyl β-D-glucuronopyranosate (7) and 21-*O*-angeloyl-28-*O*-acetylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-methyl β-D-glucuronopyranosate (8).

Key words aesculioside A—H; triterpenoidal saponin; *Aesculus chinensis*; Hippocastanaceae

Aesculus chinensis BGE (Hippocastanaceae) is a tall tree widely distributed in Hebei, Henan and Shanxi provinces of P. R. China. Its seeds, called "Sha Luo Zi" in traditional Chinese medicine, have long been used as a stomachic and analgesic in the treatment of distension and pain in chest and abdomen, and also in the treatment of malaria and dysentery.[1)] Tablets made from the extract of the seeds are used for treating heart diseases.[2)] In Europe, the bark and leaves of *A. hippocastanum* (horse-chestnut) have been employed in folk medicine as an astringent in diarrhoea and haemorrhoids.[3)] The saponin mixture, called escin because of its seeds, has been used in therapy of peripheral vascular disorders and in the cosmetic field for prevention and treatment of cellulitis because of its antiinflammatory, antiedamatous and capillary-protective properties.[4)] Recently, twelve saponins were isolated from the seeds of *A. hippocastanum*[5)] and the antiinflammatory activity of some compounds and their structure–activity relationships were also reported.[6)] However, up to now there has been no chemical study on *A. chinensis*. The medicinal value attached to *A. chinensis* prompted us to conduct a chemical investigation on the seeds of this plant. From the saponin fractions, four pairs of new geometrical isomer triterpenoidal glycosides, named as aesculiosides A—H, together with two pairs of known ones, were obtained. In this contribution, we describe their isolation and structural elucidation by means of extensive NMR studies, including distortionless enhancement by polarization transfer (DEPT), double-quantum filtered ^{1}H–^{1}H correlated spectroscopy (DQF-COSY), homonuclear Hartmann–Hahn (HOHAHA), ^{1}H–^{13}C heteronuclear correlation (HETCOR), heteronuclear single-quantum correlation (HSQC), heteronuclear multiple-bond connectivity (HMBC) and rotating-frame Overhauser enhancement spectroscopy (ROESY) experiments.

Results and Discussion

Aesculioside A (**1**) had a molecular formula of $C_{53}H_{84}O_{23}$ determined by its ^{13}C-NMR data and the $[M+Na]^+$ ion at *m/z* 1111 and $[M+K]^+$ ion at *m/z* 1127 in the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) (positive ion mode). The IR showed absorption at 1716 and 1655 cm^{-1} assignable to the carbonyl group and the α,β-unsaturated carbonyl ester. Acid hydrolysis of **1** afforded **1a** (Chart 2) identified as 21-*O*-tigloylprotoaescigenin based on the NMR and MS data and the monosaccharide of the acidic hydrolysate of **1** was identified as glucose based on the gas-liquid chromatography (GLC) analysis. The glucuronic acid was detected by co-TLC analysis (both compound **1** and the authentic sugar were subjected to the TLC plate and then hydrolyzed under HCl vapor at 65 °C for 1 h, developing solvent: $CHCl_3$–MeOH–H_2O, 10 : 5 : 1). The NMR spectra of **1** were characteristic of an acylated polyhydroxyoleanene triterpenoidal glycoside with three monosaccharides. The ^{13}C-NMR data (Table 1) and the ^{1}H-NMR data (shown in Experimental) indicated the aglycone moiety was protoaescigenin.[5,7)] In the ROESY spectrum, the strong NOE between H-21 (δ 6.43, 1H, d) and H_3-29 (δ 1.11, 3H, s) demonstrated that the H-21 was α-configuration, while the β-orientation of H-22 was indicated by the coupling constant between H-21 and H-22 (J=10.2 Hz) and the NOE between H-22 (δ 4.84, 1H, d) and H_β-18 (δ 2.94). The ^{1}H-NMR spectrum of **1** displayed three anomeric proton doublets at δ 4.92 (d, J=7.8 Hz), 5.22 (d, J=7.8 Hz) and 5.62 (d, J=7.8 Hz). These correlated to three anomeric carbons at δ 104.6, 104.7 and 104.3, respectively, in the HETCOR spectrum. The identification and the full assignments of the proton and carbon signals for the sugar moieties were achieved by a combination of DQF-COSY, HOHAHA, HETCOR, HMBC and ROESY experiments as described in our previous papers.[8)] Accordingly, the three monosaccharides were determined to be one β-D-glucuronopyranosyl acid and two β-D-glucopyranoses, and the assignments of the protons and protonated carbons were established as listed in Tables 2 and 3. The sequence of the trisaccharide unit was unambiguously determined by the following significant cross peaks in the

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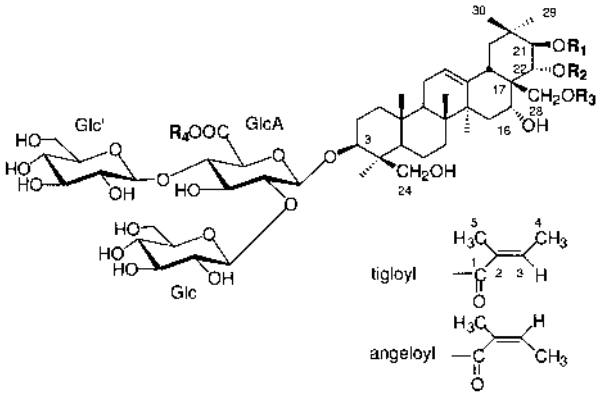
HMBC experiment: H-1 (δ 5.62) of Glc with C-2 (δ 79.7) of GlcA and H-1 (δ 5.22) of Glc′ with C-4 (δ 81.7) of GlcA. As observed in the HMBC spectrum, a long-range correlation between H-1 (δ 4.92) of GlcA and C-3 (δ 91.1) of the aglycone revealed the trisaccharide chain was linked to the C-3 position. The key NOEs of H-1 (δ 5.22) of Glc′ with H-4 (δ 4.60) of GlcA, H-1 (δ 5.62) of Glc with H-2 (δ 4.30) of GlcA and H-1 (δ 4.92) of GlcA with H-3 (δ 3.41) of the aglycone strongly supported the trisaccharide structure deduced above.



1	R_1 = tigloyl	R_2 = H	R_3 = H	R_4 = H
2	R_1 = angeloyl	R_2 = H	R_3 = H	R_4 = H
3	R_1 = tigloyl	R_2 = tigloyl	R_3 = H	R_4 = H
4	R_1 = tigloyl	R_2 = angeloyl	R_3 = H	R_4 = H
5	R_1 = tigloyl	R_2 = tigloyl	R_3 = H	R_4 = CH_3
6	R_1 = tigloyl	R_2 = angeloyl	R_3 = H	R_4 = CH_3
7	R_1 = tigloyl	R_2 = H	R_3 = Ac	R_4 = CH_3
8	R_1 = angeloyl	R_2 = H	R_3 = Ac	R_4 = CH_3
9	R_1 = tigloyl	R_2 = Ac	R_3 = H	R_4 = H
10	R_1 = angeloyl	R_2 = Ac	R_3 = H	R_4 = H
11	R_1 = tigloyl	R_2 = H	R_3 = Ac	R_4 = H
12	R_1 = angeloyl	R_2 = H	R_3 = Ac	R_4 = H

Chart 1

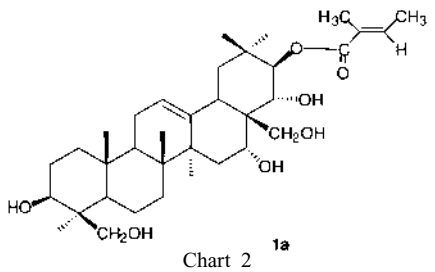


Chart 2

The ^{13}C-NMR spectrum of **1** showed 53 signals, of which 30 were assigned to the triterpenoid moiety, 18 to the three monosaccharides, and the remaining 5 corresponded to a tigloyl group[5] identified by extensive NMR analysis. HMBC relationship of C-1 (δ 168.6) of the tigloyl group with H-21 (δ 6.43) indicated C-21 was the acylated position. On the basis of the above evidence, the structure of **1** was determined as 21-*O*-tigloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl acid.

Aesculioside B (**2**) displayed the same MADIL-TOF MS peaks at *m/z* 1111 $[M+Na]^+$ and 1127 $[M+K]^+$ as compound **1**. The NMR data for compounds **1** and **2** were almost superimposable, except for some signals of the acyl substituent at C-21, suggesting **1** and **2** shared a common aglycone and sugar-substitution pattern but differed in the acyl group at C-21. In compound **2**, the angeloyl group, a geometrical isomer of tigloyl group, was indicated by the characteristic NMR signals at δ 5.89 (1H, dq, H-3), δ 2.05 (3H, dd, H_3-4), δ 15.9 (C-4) and δ 21.1 (C-5) as compared with their counterparts at δ 7.01 (1H, dq, H-3), δ 1.60 (3H, dd, H-4), δ 14.2 (C-4) and δ 12.4 (C-5) in compound **1**. Consequently, the structure of aesculioside B (**2**) was elucidated as 21-*O*-angeloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl acid.

Aesculiosides C (**3**) and D (**4**) had the same molecular formula of $C_{58}H_{90}O_{24}$ deduced from their ^{13}C-NMR data and MALDI-TOF MS information. ^{1}H- and ^{13}C-NMR spectra indicated that compounds **3** and **4** possessed an identical trisaccharide chain and aglycone as **1** but differed in the acyl groups substituted at the E-ring. Detailed NMR analysis suggested that both compounds had the same acyl group (tigloyl) at C-21 as that in **1**. The ^{13}C-NMR spectrum of **4** displayed five additional carbon signals at δ 168.3, 136.6, 129.2, 15.7 and 20.8. This combined with the two characteristic proton signals at δ 5.84 (1H, dq) and 2.02 (1H, dd) manifested that the other acyl group in **4** was angeloyl. HMBC correlation of H-21 (δ 6.74, 1H, d) of the aglycone with C-1 (δ 167.9) of the tigloyl group and H-22 (δ 6.37, 1H, d) of the aglycone with C-1 (δ 168.3) of the angeloyl group provided definitive evidence that the tigloyl group and

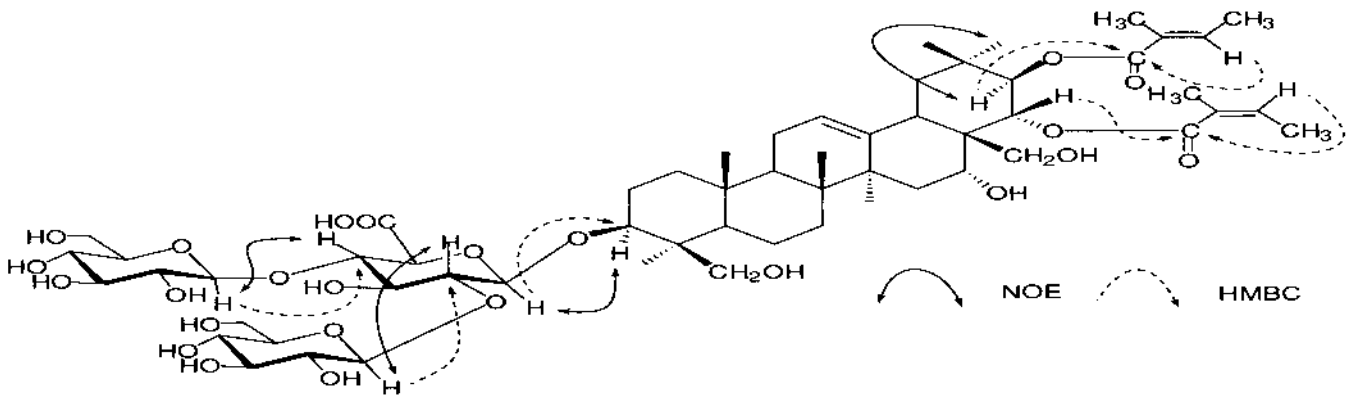


Chart 3. Some Key NOEs and Long-Range Correlations Observed in ROESY and HMBC Experiments of Compound **4**

Table 1. ^{13}C-NMR Data for the Aglycone Moieties (125 MHz in Pyridine-d_5)[a)]

	1	2	3	4	5	6	7	8	9	10	11	12
1	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5	38.5
2	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6	26.6
3	91.1	91.1	91.1	91.1	91.1	91.2	91.2	91.2	91.1	91.2	91.1	91.1
4	43.7	43.7	43.7	43.7	43.8	43.7	43.7	43.7	43.7	43.7	43.7	43.7
5	56.1	56.1	56.1	56.1	56.2	56.1	56.1	56.1	56.1	56.1	56.1	56.1
6	18.5	18.5	18.5	18.5	18.6	18.5	18.7	18.5	18.5	18.5	18.5	18.5
7	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2
8	39.9	39.9	40.0	40.0	40.0	40.0	39.9	39.9	39.9	39.9	39.9	39.9
9	46.8	46.7	46.7	46.7	46.8	46.7	46.8	46.8	46.7	46.8	46.7	46.8
10	36.4	36.4	36.5	36.6	36.5	36.6	36.5	36.4	36.5	36.4	36.5	36.4
11	24.0	24.0	24.0	24.0	24.1	24.0	24.0	24.1	24.0	24.1	24.0	24.1
12	123.1	123.0	123.0	123.0	123.1	123.0	123.8	123.8	123.0	123.0	123.0	123.0
13	143.6	143.5	142.8	142.8	142.9	142.8	142.8	142.7	142.9	142.7	142.9	142.7
14	41.8	41.8	41.7	41.7	41.7	41.7	41.8	41.8	41.7	41.8	41.7	41.8
15	34.4	34.4	34.9	34.9	34.9	34.8	34.6	34.6	34.6	34.6	34.6	34.6
16	67.9	67.9	68.5	68.6	68.5	68.5	67.6	67.6	68.1	67.9	67.6	67.6
17	48.2	48.2	48.3	48.0	48.3	48.0	47.1	47.1	47.9	48.0	47.1	47.1
18	40.4	40.4	40.3	40.1	40.1	40.1	40.6	40.6	40.1	40.5	40.1	40.5
19	47.9	47.9	47.3	47.3	47.3	47.3	47.3	47.3	47.2	47.3	47.3	47.3
20	36.4	36.2	36.3	36.4	36.4	36.4	36.4	36.1	36.4	36.1	36.4	36.1
21	82.0	81.7	79.2	79.2	79.2	79.2	81.6	81.2	79.4	79.3	81.2	81.2
22	72.9	73.0	74.0	73.7	74.1	73.6	71.7	71.1	74.3	74.0	71.1	71.1
23	22.5	22.5	22.5	22.5	22.5	22.5	22.4	22.4	22.5	22.5	22.5	22.5
24	63.3	63.3	63.3	63.3	63.3	63.2	63.2	63.2	63.3	63.3	63.3	63.3
25	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6
26	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.9	16.7	16.7	16.7	16.7
27	27.4	27.4	27.5	27.5	27.5	27.5	27.4	27.4	27.4	27.4	27.4	27.4
28	65.9	65.9	63.4	63.6	63.5	63.6	66.4	66.4	63.7	63.6	66.4	66.4
29	29.9	29.9	29.6	29.6	29.6	29.6	29.9	29.8	29.5	29.5	29.5	29.8
30	20.3	20.4	20.1	20.2	20.2	20.2	20.1	20.2	20.1	20.2	20.1	20.2
C_{21}	Tig	Ang	Tig	Tig	Tig	Tig	Tig	Ang	Tig	Ang	Tig	Ang
1	168.6	168.7	168.1	167.9	168.1	167.9	168.5	168.6	168.1	168.6	168.1	168.6
2	129.9	129.6	129.9	129.5	129.5	129.5	129.8	129.5	129.5	129.5	129.5	129.5
3	135.9	136.1	136.7	136.8	136.6	136.8	136.3	136.2	136.9	136.1	136.9	136.2
4	14.2	15.9	14.2	14.2	14.2	14.2	14.2	15.9	14.2	15.9	14.2	15.9
5	12.4	21.1	12.4	12.4	12.4	12.4	12.4	21.0	12.4	21.0	12.4	21.0
C_{22} or C_{28}			Tig	Ang	Tig	Ang	Ac	Ac	Ac	Ac	Ac	Ac
1			168.4	168.3	168.5	168.3	170.7	170.7	171.0	170.7	171.0	170.7
2			129.2	129.2	129.3	129.2	20.7	21.0	20.9	20.8	20.9	20.7
3			137.2	136.6	137.1	136.6						
4			14.0	15.7	14.0	15.7						
5			12.3	20.8	12.3	20.8						

a) The assignment was based upon DEPT, DQF-COSY, HOHAHA, HETCOR or HSQC, ROESY and HMBC experiments.

the angeloyl group were linked to C-21 and C-22, respectively. The downfield shifts of H-21 and H-22 also indicated they were the acylated positions. Therefore, compound **4** was defined to be 21-*O*-tigloyl-22-*O*-angeloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl acid. Some key nuclear Overhauser effects (NOEs) and long-range correlations observed in ROESY and HMBC experiments of compounds **4** are depicted in Chart 3.

A detailed NMR study suggested that compounds **3** and **4** differed only in the substituent at C-22 of the aglycone. The characteristic NMR signals of the substituent at C-22 in **3** resonated at δ 6.93 (1H, dq, H-3), δ 1.43 (3H, dd, H_3-4), δ 14.0 (C-4) and δ 12.3 (C-5), instead of their counterparts at δ 5.84 (1H, dq, H-3), δ 2.02 (3H, dd, H_3-4), δ 15.7 (C-4) and δ 20.8 (C-5) in **4**, indicating the substituent at C-22 in **3** was a tigloyl group. Accordingly, compound **3** was deduced as 21,22-*O*-ditigloyl-protoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl acid.

Aesculiosides E (**5**) and F (**6**) were obtained in very small quantity. They have the same molecular formula of $C_{59}H_{92}O_{24}$, as determined by the MADIL-TOF MS data and the ^{13}C-NMR information. A detailed NMR analysis makes it possible to conclude that compound **5** had the same aglycone and the same substituents at C-21, C-22 as well as a similar trisaccharide chain at C-3 of the aglycone as compound **3**. As compared with compound **3**, the NMR spectra of **5** showed an additional methoxyl group (δ_C 52.7, δ_H 3.92) and the upfield shifts of C-6 (δ 169.5, Δ 2.4) and C-5 (δ 75.0, Δ 0.8) of the GlcA revealed the methoxyl group was connected to C-6 of the GlcA. Thus, compound **5** was determined as 21,22-*O*-ditigloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-methyl β-D-glucuronopyranosate. Proceeding in the same way, compound **6** was defined as 21-*O*-tigloyl-22-*O*-angeloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-methyl β-D-glucuronopyranosate.

Aesculiosides G (**7**) and H (**8**) showed the same MALAI-TOF MS peak at *m/z* 1167 $[M+Na]^+$ and 1183 $[M+K]^+$,

Table 2. ^{13}C-NMR Data for the Sugar Moieties (125 MHz in Pyridine-d_5)[a)]

	1	2	3	4	5	6	7	8	9	10	11	12
GlcA												
1	104.6	104.6	104.6	104.6	104.7	104.7	104.7	104.7	104.6	104.6	104.6	104.6
2	79.7	79.7	79.7	79.7	79.5	79.5	79.4	79.4	79.7	79.7	79.7	79.7
3	76.5	76.5	76.5	76.5	76.2	76.4	76.2	76.2	76.4	76.4	76.4	76.4
4	81.7	81.8	81.8	81.8	81.8	81.7	81.8	81.8	81.7	81.7	81.7	81.7
5	75.7	75.5	75.8	75.8	75.0	75.0	74.9	74.9	75.7	75.7	75.7	75.7
6	171.9	172.0	171.9	171.9	169.5	169.5	169.5	169.5	172.0	171.9	171.9	171.9
OCH_3					52.7	52.7	52.7	52.7				
Glc												
1	104.3	104.3	104.3	104.3	104.3	104.3	104.2	104.3	104.3	104.3	104.3	104.3
2	75.7	75.7	75.8	75.8	75.7	75.7	75.7	75.7	75.7	75.7	75.7	75.7
3	78.1[b)]	78.0[b)]	78.1[b)]	78.1[b)]	78.1[b)]	78.0[b)]	78.1[b)]	78.1[b)]	78.0[b)]	78.0[b)]	78.0[b)]	78.0[b)]
4	69.8	69.7	69.7	69.7	69.8	69.8	69.7	69.7	69.8	69.7	69.7	69.7
5	78.3	78.4	78.4	78.4	78.4	78.3	78.4	78.4	78.3	78.4	78.4	78.4
6	61.6	61.6	61.6	61.6	61.6	61.6	61.6	61.6	61.6	61.6	61.6	61.6
Glc′												
1	104.7	104.7	104.7	104.7	105.1	105.1	105.2	105.1	104.6	104.7	104.7	104.7
2	74.9	74.9	74.9	74.9	74.5	74.5	74.5	74.5	74.9	74.9	74.9	74.9
3	78.1[b)]	78.1[b)]	78.2[b)]	78.2[b)]	78.2[b)]	78.1[b)]	78.2[b)]	78.2[b)]	78.1[b)]	78.1[b)]	78.1[b)]	78.1[b)]
4	71.5	71.5	71.5	71.5	71.5	71.5	71.5	71.5	71.5	71.5	71.5	71.5
5	78.5	78.5	78.5	78.5	78.5	78.5	78.5	78.5	78.5	78.5	78.5	78.5
6	62.4	62.4	62.4	62.4	62.4	62.4	62.3	62.3	62.4	62.4	62.4	62.4

a) The assignment was based upon DEPT, DQF-COSY, HOHAHA, HETCOR or HSQC, ROESY and HMBC experiments. *b*) Data with the same labels in each column may be interchangeable.

Table 3. ^{1}H-NMR Data for the Sugar Moieties (500 MHz in Pyridine-d_5)[a)]

	1	2	3	4	5	6	7	8
C_3-GlcA								
1	4.92 d (7.8)	4.92 d (7.7)	4.93 d (7.7)	4.93 d (7.8)	4.90 d (7.6)	4.89 d (7.5)	4.87 d (7.7)	4.87 d (7.5)
2	4.30	4.29	4.30	4.31	4.25	4.26	4.22	4.24
3	4.36	4.35	4.39	4.38	4.32	4.33	4.29	4.30
4	4.60	4.59	4.61	4.60	4.45	4.46	4.43	4.44
5	4.63	4.62	4.63	4.62	4.53	4.54	4.52	4.54
OCH_3					3.92	3.92	3.92	3.92
Glc								
1	5.62 d (7.8)	5.63 d (7.7)	5.64 d (7.7)	5.63 d (7.6)	5.62 d (7.8)	5.63 d (7.9)	5.60 d (7.7)	5.61 d (7.7)
2	4.10	4.10	4.10	4.12	4.07	4.08	4.06	4.06
3	4.19	4.18	4.20	4.20	4.17	4.18	4.16	4.17
4	4.50	4.49	4.50	4.50	4.47	4.48	4.46	4.49
5	3.70	3.69	3.70	3.69	3.70	3.71	3.68	3.69
6	4.36, 4.46	4.34, 4.44	4.34, 4.45	4.35, 4.45	4.35, 4.45	4.36, 4.46	4.32, 4.43	4.36, 4.47
Glc′								
1	5.22 d (7.8)	5.23 d (7.7)	5.24 d (7.7)	5.23 d (7.8)	5.02 d (7.8)	5.03 d (7.9)	5.00 d (7.7)	5.01 d (7.7)
2	4.04	4.03	4.03	4.04	3.97	3.98	3.94	3.97
3	4.19	4.18	4.20	4.20	4.17	4.18	4.16	4.17
4	4.20	4.19	4.20	4.20	4.18	4.19	4.17	4.18
5	4.00	4.00	4.01	4.01	3.98	4.00	3.96	4.00
6	4.26, 4.48	4.27, 4.47	4.27, 4.48	4.28, 4.50	4.29, 4.49	4.30, 4.51	4.26, 4.49	4.27, 4.50

a) The assignment was based upon DEPT, DQF-COSY, HOHAHA, HETCOR or HSQC, ROESY and HMBC experiments.

corresponding to the molecular formula of $C_{56}H_{88}O_{24}$. The NMR data of **7** and **8** bore a close resemblance to those of compounds **11** and **12**, respectively, except for the carbon signals at C-5 and C-6 of the GlcA. In the NMR spectra, both compounds **7** and **8** displayed a methoxyl group signal at δ_C 52.7 and δ_H 3.92 (3H, s). The methoxyl group linked to C-6 of the GlcA was indicated by the upfield shifts of C-6 (δ 169.5, Δ 2.4) and C-5 (δ 74.9, Δ 0.8) of the GlcA as compared with compounds **11** and **12**. Consequently, compounds **7** and **8** were respectively elucidated as 21-*O*-tigloyl-28-*O*-acetylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-methyl β-D-glucuronopyranosate, and 21-*O*-angeloyl-28-*O*-acetylprotoaescigenin 3-*O*-[β-D-glucopyranosyl-(1→2)][β-D-glucopyranosyl-(1→4)]-methyl β-D-glucuronopyranosate.

Compounds **9**, **10** and **11**, **12** were identified as escins Ia, Ib and isoescins Ia, Ib,[5)] respectively, based on their physicochemical properties and spectral data. The ^{13}C-NMR data are shown in Tables 1 and 2. The four saponins were isolated and identified from the title plant for the first time and they are the main compounds in the saponin fraction.

It is worthwhile to mention that C-28, though not directly acylated by any acyl groups, also experienced an upfield shift to 5 ppm from the influence of the acylation at C-21 and C-

22. In the case of desacylescin compounds, the C-28 resonated at δ 68.3—68.6.[5a] However, for those compounds being acylated at C-21 only (aesculiosides A and B), the C-28 was observed at δ 65.9, while for the diacyl compounds (aesculiosides C—F and escins Ia, Ib), much stronger shielding effects were noticed and C-28 were found around δ 63.4—63.7 (see Table 1). By carefully examining the energy-minimized model, we found that C-28 were quite near in space to these carbonyl groups and positioned under them. Such phenomena, therefore, may be attributed to the anistropic shielding effect from the α,β-unsaturated carbonyl groups at C-21 and C-22 of the E-ring.

Experimental

General Melting points were measured with a Yanaco microscope and are uncorrected. Optical rotations were performed with a JASCO DIP-370 digital polarimeter. IR spectra were carried out on a JASCO 300E FTIR spectrometer. MALDI-TOF MS was conducted using a Perseptive Biosystems Voyager DE-STR mass spectrometer. The ^{1}H- and ^{13}C-NMR were recorded on a JEOL α-500 spectrometer in pyridine-d_5 solution and chemical shifts were expressed in δ (ppm) with reference to tetramethylsilane (TMS). Diaion HP-20 (Mitsubishi Chemical), silica gel (Silica gel 60, Merck), and octadecyl silica (ODS) (Chromatorex, 100—200 mesh, Fujisylisia) were used for open column chromatography. Preparative medium-performance liquid chromatography (MPLC) and high-performance liquid chromatography (HPLC) was performed using an ODS column (SSC-ODS, 40—60 μm, detector: UV 210 nm) and an ODS column (PEGASIL ODS-2, Senshu Pak, 20 mm i.d.$\times$150 mm, detector: UV 210 nm), respectively. GLC: Shimadzu GC-7A, Column: Silicone OV-17 on Uniport HP (80—100 mesh), 3 mm i.d.$\times$2.1 m; column temperature, 160 °C; carrier gas, N_2, flow rate 30 ml/min.

Plant Material The seeds of *A. chinensis* Bge were purchased from a market in Beijing, P. R. China in January 1998, and were identified by Professor Junhua Zheng. A voucher specimen was deposited in the Division for Pharmacognostical Biotechnology, School of Pharmaceutical Sciences, Beijing Medical University, Beijing, P. R. China.

Extraction and Isolation The powdered seeds (3.0 kg) of *A. chinensis* were refluxed with EtOH three times for 2 h. The EtOH extract was concentrated (340 g), suspended in water and then applied to a column of Diaion HP-20 (1500 ml). The column was eluted with H_2O and 30, 50, 70, 100% MeOH to give two saponin containing fractions, 50% MeOH part (32 g) and 70% MeOH part (120 g). The 70% MeOH part (20 g) was chromatographed over silica gel and open ODS columns to yield three saponin fractions of A (0.5 g), B (15.0 g) and C (1.0 g). Fraction A, part of fraction B (1.5 g) and fraction C was respectively subjected to MPLC (MeOH : H_2O) and HPLC (CH_3CN : 0.06% TFA) purification to afford **1** (12 mg) and **2** (13 mg), **9** (120 mg) and **10** (150 mg), **11** (200 mg) and **12** (160 mg), respectively. In the same way, 50% MeOH part was applied to silica gel and open ODS columns to give fractions A (1.25 g), B (1.53 g), C (0.6 g), D (3.0 g) and E (22.5 g). Further MPLC and HPLC purification of fractions B and D afforded **3** (10 mg), **4** (9 mg), **5** (5 mg), **6** (5 mg), **7** (18 mg), and **8** (15 mg), respectively.

Aesculioside A (**1**): An amorphous solid from MeOH; mp 254—255 °C (dec.); $[\alpha]_D^{25}$ −23° (c=0.12, MeOH); IR ν_{max}^{KBr}: 3414, 2927, 2825, 1716, 1655, 1248, 1074 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.43 (1H, d, J=10.2 Hz, H-21), 5.38 (1H, br s, H-12), 4.86 (1H, br s, H-16), 4.84 (1H, d, J=10.2 Hz, H-22), 4.28 (1H, d, J=9.7 Hz, H_2-24), 3.96 (1H, d, J=9.9 Hz, H_2-28), 3.68 (1H, d, J=9.9 Hz, H_2-28), 3.41 (1H, m, H-3), 3.33 (1H, d, J=9.7 Hz, H_2-24), 2.94 (1H, m, H-18), 1.83, 1.34, 1.33, 1.11, 0.81, 0.67 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-tigloyl δ 7.01 (1H, dq, J=7.1, 1.5 Hz, H-3), 1.60 (3H, dd, J=7.1, 1.1 Hz, H_3-4), 1.87 (3H, s, H_3-5), other NMR data see Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) m/z 1111 [M+Na]$^+$, 1127 [M+K]$^+$.

Aesculioside B (**2**): An amorphous solid from MeOH; mp 236—237 °C (dec.); $[\alpha]_D^{25}$ −32° (c=0.12, MeOH); IR ν_{max}^{KBr}: 3414, 2927, 2866, 1715, 1654, 1259, 1074 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.46 (1H, d, J=10.1 Hz, H-21), 5.37 (1H, br s, H-12), 4.87 (1H, br s, H-16), 4.80 (1H, d, J=10.1 Hz, H-22), 4.28 (1H, d, J=10.3 Hz, H_2-24), 3.94 (1H, d, J=10.4 Hz, H_2-28), 3.66 (1H, d, J=10.4 Hz, H_2-28), 3.40 (1H, m, H-3), 3.33 (1H, d, J=10.3 Hz, H_2-24), 2.95 (1H, m, H-18), 1.83, 1.33, 1.33, 1.11, 0.80, 0.66 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-angeloyl δ 5.89 (1H, dq, J=7.1, 1.5 Hz, H-3), 2.05 (3H, dd, J=7.1, 1.5 Hz, H_3-4), 1.99 (3H, s, H-5), other NMR data are shown in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) m/z 1111 [M+Na]$^+$, 1127 [M+K]$^+$.

Aesculioside C (**3**): An amorphous solid from MeOH; mp 249—250 °C (dec.); $[\alpha]_D^{25}$ −42° (c=0.11, MeOH); IR ν_{max}^{KBr}: 3420, 2926, 2867, 1733, 1698, 1654, 1274, 1073 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.73 (1H, d, J=10.1 Hz, H-21), 6.33 (1H, d, J=10.1 Hz, H-22), 5.42 (1H, br s, H-12), 4.50 (1H, br s, H-16), 4.25 (1H, d, J=10.5 Hz, H_2-24), 3.63 (1H, d, J=11.9 Hz, H_2-28), 3.42 (1H, m, H-3), 3.40 (1H, d, J=11.9 Hz, H_2-28), 3.34 (1H, d, J=10.5 Hz, H_2-24), 3.11 (1H, m, H-18), 1.83, 1.36, 1.35, 1.13, 0.80, 0.66 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-tigloyl δ 7.05 (1H, dq, J=7.1, 1.5 Hz, H-3), 1.61 (3H, dd, J=7.1, 1.1 Hz, H_3-4), 1.92 (3H, s, H-5), C_{22}-tigloyl δ 6.93 (1H, dq, J=7.1, 1.5 Hz, H-3), 1.43 (3H, dd, J=7.1, 1.1 Hz, H_3-4), 1.85 (3H, s, H_3-5), other NMR data are listed in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) m/z 1193 [M+Na]$^+$, 1209 [M+K]$^+$.

Aesculioside D (**4**): An amorphous solid from MeOH; mp 225—226 °C (dec.); $[\alpha]_D^{25}$ −52° (c=0.10, MeOH); IR ν_{max}^{KBr}: 3431, 2926, 2873, 1700, 1672, 1654, 1268, 1071 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.74 (1H, d, J=10.1 Hz, H-21), 6.37 (1H, d, J=10.1 Hz, H-22), 5.42 (1H, br s, H-12), 4.53 (1H, br s, H-16), 4.28 (1H, d, J=10.6 Hz, H_2-24), 3.66 (1H, d, J=10.5 Hz, H_2-28), 3.44 (1H, d, J=10.5 Hz, H_2-28), 3.42 (1H, m, H-3), 3.34 (1H, d, J=10.6 Hz, H_2-24), 3.11 (1H, m, H-18), 1.85, 1.35, 1.33, 1.12, 0.81, 0.67 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-tigloyl δ 7.07 (1H, dq, J=7.2, 1.5 Hz, H-3), 1.62 (3H, dd, J=7.1, 1.1 Hz, H_3-4), 1.95 (3H, s, H-5), C_{22}-angeloyl δ 5.84 (1H, dq, J=7.2, 1.5 Hz, H-3), 2.02 (3H, dd, J=7.2, 1.4 Hz, H_3-4), 1.89 (3H, s, H_3-5), other NMR data are given in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) m/z 1193 [M+Na]$^+$, 1209 [M+K]$^+$.

Aesculioside E (**5**): An amorphous solid from MeOH; mp 217—218 °C (dec.); $[\alpha]_D^{25}$ −20° (c=0.40, MeOH); IR ν_{max}^{KBr}: 3407, 2929, 2867, 1716, 1681, 1653, 1273, 1143, 1073 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.72 (1H, d, J=10.1 Hz, H-21), 6.32 (1H, d, J=10.1 Hz, H-22), 5.43 (1H, br s, H-12), 4.53 (1H, br s, H-16), 4.28 (1H, d, J=11.2 Hz, H_2-24), 3.65 (1H, d, J=10.7 Hz, H_2-28), 3.42 (1H, m, H-3), 3.40 (1H, d, J =10.7 Hz, H_2-28), 3.34 (1H, d, J=11.2 Hz, H_2-24), 3.12 (1H, m, H-18), 1.83, 1.36, 1.34, 1.13, 0.81, 0.68 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-tigloyl δ 7.05 (1H, dq, J=7.1, 1.4 Hz, H-3), 1.61 (3H, dd, J=7.1, 1.1 Hz, H_3-4), 1.92 (3H, s, H_3-5), C_{22}-tigloyl δ 6.93 (1H, dq, J=7.2, 1.4 Hz, H-3), 1.44 (3H, dd, J=7.0, 1.1 Hz, H_3-4), 1.83 (3H, s, H_3-5), other NMR data see Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) m/z 1207 [M+Na]$^+$, 1213 [M+K]$^+$.

Aesculioside F (**6**): An amorphous solid from MeOH; mp 231—232 °C (dec.); $[\alpha]_D^{25}$ −23° (c=0.38, MeOH); IR ν_{max}^{KBr}: 3414, 2928, 2875, 1725, 1680, 1646, 1267, 1145, 1073 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.68 (1H, d, J=10.1 Hz, H-21), 6.38 (1H, d, J=10.1 Hz, H-22), 5.43 (1H, br s, H-12), 4.51 (1H, br s, H-16), 4.27 (1H, d, J=11.2 Hz, H_2-24), 3.66 (1H, d, J=10.8 Hz, H_2-28), 3.44 (1H, d, J=10.8 Hz, H_2-28), 3.40 (1H, m, H-3), 3.33 (1H, d, J=11.2 Hz, H_2-24), 3.12 (1H, m, H-18), 1.84, 1.37, 1.33, 1.13, 0.81, 0.68 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-tigloyl δ 7.07 (1H, dq, J=7.2, 1.5 Hz, H-3), 1.63 (3H, dd, J=7.0, 1.1 Hz, H_3-4), 1.95 (3H, s, H_3-5), C_{22}-angeloyl δ 5.84 (1H, dq, J=7.2, 1.5 Hz, H-3), 2.02 (3H, dd, J=7.1, 1.5 Hz, H_3-4), 1.89 (3H, s, H_3-5), other NMR data are shown in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) m/z 1207 [M+Na]$^+$, 1213 [M+K]$^+$.

Aesculioside G (**7**): An amorphous solid from MeOH; mp 239—240 °C (dec.); $[\alpha]_D^{25}$ −18° (c=0.12, MeOH); IR ν_{max}^{KBr}: 3411, 2924, 2858, 1751, 1680, 1655, 1266, 1072 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.45 (1H, d, J=9.9 Hz, H-21), 5.46 (1H, br s, H-12), 4.76 (1H, br s, H-16), 4.52 (1H, br s, H-22), 4.29 (2H, m, H_2-28), 4.23 (1H, d, J=11.2 Hz, H_2-24), 3.35 (1H, m, H-3), 3.30 (1H, d, J=11.2 Hz, H_2-24), 2.84 (1H, m, H-18), 1.83, 1.34, 1.31, 1.11, 0.96, 0.68 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-tigloyl δ 7.02 (1H, dq, J=7.1, 1.5 Hz, H-3), 1.61 (3H, dd, J=7.1, 1.1 Hz, H_3-4), 1.87 (3H, s, H_3-5), C_{28}-acetyl δ 2.04 (3H, s, H_3-2), other NMR data are listed in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) m/z 1167 [M+Na]$^+$, 1183 [M+K]$^+$.

Aesculioside H (**8**): An amorphous solid from MeOH; mp 230—231 °C (dec.); $[\alpha]_D^{25}$ −17° (c=0.18, MeOH); IR ν_{max}^{KBr}: 3402, 2924, 2849, 1742, 1695, 1656, 1264, 1069 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.49 (1H, d, J=10.8 Hz, H-21), 5.45 (1H, br s, H-12), 4.75 (1H, br s, H-16), 4.52 (1H, br s, H-22), 4.30 (2H, m, H_2-28), 4.24(1H, d, J =11.6 Hz, H_2-24), 3.35 (1H, m, H-3), 3.31 (1H, d, J=11.6 Hz, H_2-24), 2.83 (1H, m, H-18), 1.80, 1.31, 1.31, 1.13, 0.94, 0.68 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-angeloyl δ 5.90 (1H, dq, J=7.1, 1.5 Hz, H-3), 2.05 (3H, dd, J=7.1, 1.1 Hz, H_3-4), 1.99 (3H, s, H_3-5), C_{28}-acetyl δ 2.04 (3H, s, H_3-2), other NMR data are given in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) m/z 1167 [M+Na]$^+$, 1183 [M+K]$^+$.

Escin Ia (**9**): An amorphous solid from MeOH; mp 205—206 °C (dec.); $[\alpha]_D^{25}$ −18° (*c*=0.12, MeOH); IR ν_{max}^{KBr}: 3404, 2925, 2849, 1715, 1680, 1269, 1087 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.58 (1H, d, *J*=10.9 Hz, H-21), 6.26 (1H, d, *J*=10.9 Hz, H-22), 5.62 (1H, d, *J*=7.8 Hz, Glc-H-1), 5.39 (1H, br s, H-12), 5.20 (1H, d, *J*=7.8 Hz, Glc′-H-1), 4.92 (1H, d, *J*=7.6 Hz, GlcA-H-1), 4.60 (1H, br s, H-16), 4.26(1H, d, *J* =10.6 Hz, H_2-24), 3.66 (1H, d, *J*=10.9 Hz, H_2-28), 3.41 (1H, m, H-3), 3.38 (1H, d, *J*=10.9 Hz, H_2-28), 3.32 (1H, d, *J*=10.6 Hz, H_2-24), 3.13 (1H, m, H-18), 1.83, 1.31, 1.31, 1.10, 0.80, 0.67 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-tigloyl δ 7.10 (1H, dq, *J*=7.1, 1.5 Hz, H-3), 1.60 (3H, dd, *J*=7.1, 1.1 Hz, H_3-4), 1.91 (3H, s, H_3-5), C_{22}-acetyl δ 1.98 (3H, s, H_3-2), other NMR data are given in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) *m/z* 1153 $[M+Na]^+$, 1169 $[M+K]^+$.

Escin Ib (**10**): An amorphous solid from MeOH; mp 187—188 °C (dec.); $[\alpha]_D^{25}$ −13° (*c*=0.11, MeOH); IR ν_{max}^{KBr}: 3404, 2925, 1726, 1681, 1269, 1087 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.56 (1H, d, *J*=10.6 Hz, H-21), 6.25 (1H, d, *J*=10.6 Hz, H-22), 5.60 (1H, d, *J*=7.6 Hz, Glc-H-1), 5.40 (1H, br s, H-12), 5.21 (1H, d, *J*=7.6 Hz, Glc′-H-1), 4.91 (1H, d, *J*=7.7 Hz, GlcA-H-1), 4.65 (1H, br s, H-16), 4.24 (1H, d, *J*=11.5 Hz, H_2-24), 3.68 (1H, d, *J*=10.6 Hz, H_2-28), 3.35 (1H, m, H-3), 3.65 (1H, d, *J* =10.6 Hz, H_2-28), 3.31 (1H, d, *J*=11.5 Hz, H_2-24), 3.03 (1H, m, H-18), 1.80, 1.31, 1.31, 1.13, 0.94, 0.68 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-angeloyl δ 5.90 (1H, dq, *J*=7.1, 1.5 Hz, H-3), 2.05 (3H, dd, *J*=7.1, 1.4 Hz, H_3-4), 1.99 (3H, s, H_3-5), C_{22}-acetyl δ 2.04 (3H, s, H_3-2), other NMR data are given in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) *m/z* 1153 $[M+Na]^+$, 1169 $[M+K]^+$.

Isoescin Ia (**11**): An amorphous solid from MeOH; mp 197—198 °C (dec.); $[\alpha]_D^{25}$ −12° (*c*=0.13, MeOH); IR ν_{max}^{KBr}: 3416, 2926, 1723, 1686, 1268, 1079 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.46 (1H, d, *J*=10.7 Hz, H-21), 5.61 (1H, d, *J*=7.8 Hz, Glc-H-1), 5.46 (1H, br s, H-12), 5.23 (1H, d, *J*=7.7 Hz, Glc′-H-1), 4.92 (1H, d, *J*=7.5 Hz, GlcA-H-1), 4.73 (1H, br s, H-16), 4.56 (1H, br s, H-22), 4.32 (2H, m, H_2-28), 4.26(1H, d, *J*=10.9 Hz, H_2-24), 3.36 (1H, m, H-3), 3.33 (1H, d, *J*=10.9 Hz, H_2-24), 2.84 (1H, m, H-18), 1.83, 1.32, 1.30, 1.12, 0.93, 0.69 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-tigloyl δ 7.05 (1H, dq, *J*=7.1, 1.5 Hz, H-3), 1.62 (3H, dd, *J*=7.1, 1.1 Hz, H_3-4), 1.88 (3H, s, H_3-5), C_{28}-acetyl δ 2.03 (3H, s, H_3-2), other NMR data are given in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) *m/z* 1153 $[M+Na]^+$, 1169 $[M+K]^+$.

Isoescin Ib (**12**): An amorphous solid from MeOH; mp 215—216 °C (dec.); $[\alpha]_D^{25}$ −16° (*c*=0.10, MeOH); IR ν_{max}^{KBr}: 3426, 2925, 1711, 1695, 1246, 1037 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz): aglycone δ 6.48 (1H, d, *J*=10.1 Hz, H-21), 5.61 (1H, d, *J*=7.3 Hz, Glc-H-1), 5.44 (1H, br s, H-12), 5.22 (1H, d, *J*=7.5 Hz, Glc′-H-1), 4.91 (1H, d, *J*=7.5 Hz, GlcA-H-1), 4.75 (1H, br s, H-16), 4.60 (1H, br s, H-22), 4.30 (2H, m, H_2-28), 4.25 (1H, d, *J*=11.3 Hz, H_2-24), 3.36 (1H, m, H-3), 3.32 (1H, d, *J*=11.3 Hz, H_2-24), 2.81 (1H, m, H-18), 1.81, 1.33, 1.30, 1.19, 0.96, 0.67 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-angeloyl δ 5.90 (1H, dq, *J*=7.1, 1.5 Hz, H-3), 2.05 (3H, dd, *J*=7.1, 1.5 Hz, H_3-4), 1.99 (3H, s, H_3-5), C_{28}-acetyl δ 2.00 (3H, s, H_3-2), other NMR data are given in Tables 1, 2 and 3; MALDI-TOF MS (positive ion mode) *m/z* 1153 $[M+Na]^+$, 1169 $[M+K]^+$.

Acidic Hydrolysis of Aesculioside A (1) Aesculioside A (**1**, 10 mg) was heated in 1 ml 1 M HCl (dioxane–H_2O, 1 : 1) at 80 °C for 2 h in a water bath. After dioxane was removed, the solution was extracted with EtOAc (1 ml×3). The extraction was washed with H_2O and then evaporated to dryness in a vacuum. The residue was subjected to HPLC purification (90% MeOH–H_2O) to furnish **1a** (4 mg). The monosaccharide portion was neutralized by passing through an exchange resin (Amberlite MB-3) column, concentrated (dried overnight) and then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 h. After the excess reagent was decomposed with water, the reaction product was extracted with hexane (1 ml×2 times). The TMSi derivatives of the monosaccharide were identified as glucose by co-GLC analysis with standard monosacchride. The glucuronic acid was detected by co-TLC analysis with authentic sample (developing solvent: $CHCl_3$–MeOH–H_2O, 10 : 5 : 1).

Aglycone **1a**: An amorphous solid from MeOH; mp 244—246 °C (dec.); $[\alpha]_D^{25}$ +33.9° (*c*=0.39, MeOH); IR ν_{max}^{KBr}: 3470, 3396, 2942, 1696, 1449, 1264, 1158, 1034 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 400 MHz): aglycone δ 6.45 (1H, d, *J*=9.9 Hz, H-21), 5.44 (1H, br s, H-12), 4.88 (1H, br s, H-16), 4.87 (1H, d, *J*=9.9 Hz, H-22), 4.53 (1H, d, *J*=9.9 Hz, H_2-24), 3.99 (1H, d, *J*=10.0 Hz, H_2-28), 3.72 (1H, d, *J*=10.0 Hz, H_2-28), 3.71 (1H, d, *J*=9.9 Hz, H_2-24), 3.69 (1H, m, H-3), 3.00 (1H, dd, *J*=14.2, 3.4 Hz, H-18), 1.84, 1.59, 1.36, 1.13, 0.94, 0.91 (each 3H, s, H_3-27, 30, 23, 29, 26, 25); C_{21}-tigloyl δ 7.02 (1H, dq, *J*=7.0, 1.4 Hz, H-3), 1.61 (3H, dd, *J*=7.0, 0.9 Hz, H_3-4), 1.87 (3H, s, H_3-5); MALDI-TOF MS (positive ion mode) *m/z* 611 $[M+Na]^+$, 627 $[M+K]^+$.

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