Three New Triterpene Saponins from the Seeds of *Aesculus chinensis*

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Three new triterpenoid saponins were isolated from the seeds of *Aesculus chinensis*, and characterized as 22-tigloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid (escin IVg, 1), 22-angeloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid (escin IVh, 2) and 16-angeloyl-21-acetylprotoaescigenin 3-*O*-[β-D-glucopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid (escin VIb, 3), together with two known compounds, escin IIIa (4) and desacylescin I (5). Their structures were established on the basis of spectroscopic and chemical evidence.

Key words *Aesculus chinensis*; Hippocastanaceae; escin IVg; escin IVh; escin VIb

Escin,[1] an anti-inflammatory and anti-edemigenous principle from the seeds of the Chinese horse chestnut tree (*Aesculus chinensis* Bge., family Hippocastanaceae) consists of a series of analogous pentacyclic triterpenoid oligoglycosides. During our work on the saponin constituents from the seeds, we have isolated and identified escins Ia, Ib, IVc, IVd, IVe and IVf, together with isoescins Ia and Ib. And it was found for the first time that some of them possessed anti-human immunodeficiency virus (HIV)-1 protease activity.[2] In this paper, we report the isolation and the structure elucidation of five additional saponins, including three new ones.

Results and Discussion

The crude saponin was obtained from a 90% ethanolic extract of the seeds of *A. chinensis* as described previously.[2] After isolation of eight saponins, the remaining fraction was subjected to preparative high-performance liquid chromatography (HPLC) repeatedly affording five saponins. Among them, escin IIIa (**4**) and desacylescin I (=aesculuside B, **5**) were identified by comparison of the spectral data with those reported.[3,4]

Compound **1** was isolated as a white amorphous powder. Matrix-assisted laser desorption ionization-time of flight-mass spectrum (MALDI-TOF-MS) showed quasimolecular ion peaks at *m/z* 1111 $[M+Na]^+$ and 1127 $[M+K]^+$. High-resolution (HR) negative-ion SI (secondary ion)-MS revealed its molecular formula to be $C_{53}H_{84}O_{23}$. The infrared (IR) spectrum of **1** showed absorption bands at 1710 and 1604 cm^{-1}, characteristic of α,β-unsaturated ester, and broad bands at 3414 and 1076 cm^{-1}. Alkaline hydrolysis of **1** with 1% MeONa liberated desacylescin I (**5**), which was identified by direct comparison with an authentic sample on HPLC. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra showed signals due to the protoaescigenin skeleton [^{1}H-NMR: six quarternary methyl groups at δ 0.62, 0.77, 1.26, 1.31, 1.40, 1.82, and one broad singlet at δ 5.42 (H-12)], a trisaccharide moiety [^{1}H-NMR: anomeric protons at δ 5.55 (d, J=8.0 Hz, H-1″), 5.21 (d, J=8.0 Hz, H-1‴), 4.77 (d, J=8.0 Hz, H-1′); ^{13}C-NMR: anomeric carbons at δ 104.2 (C-1′, C-1‴), 104.1 (C-1″)] and a tigloyl group [^{1}H-NMR: δ 6.98 (H-3⁗), 1.80 (Me-5⁗), 1.44 (Me-4⁗); ^{13}C-NMR: δ 169.3 (C-1⁗), 129.4 (C-2⁗), 136.7 (C-3⁗), 13.9 (C-4⁗), 12.2 (C-5⁗)]. The attachment of a tigloyl group at C-22 was deduced from the heteronuclear multiple-bond coherence (HMBC) experiment, which showed a long-range correlation between H-22β (δ 6.16) and a carbonyl carbon (δ 169.3) of a tigloyl group. Furthermore, the trisaccharidic structure was characterized by HMBC correlations between the following pairs: C-3 (δ 90.9) and H-1′, C-2′ (δ 79.5) and H-1″ and C-4′ (δ 82.7) and H-1‴. Comparison of the ^{13}C-NMR spectral data due to the sugar moiety with those of **5** revealed the identical structure. On the basis of the above evidence, the structure of **1** was determined as 22-tigloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid, and named escin IVg.

Compound **2** was purified as a white amorphous powder. MALDI-TOF-MS showed a quasimolecular ion peak at *m/z* 1111 $[M+Na]^+$, while its molecular formula was also suggested to be $C_{53}H_{84}O_{23}$ by HR negative-ion SI-MS, identical with that of escin IVg (**1**). Likewise, the IR spectrum showed absorption bands ascribable to an α,β-unsaturated ester. Alkaline hydrolysis of **2** yielded **5**. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1**. The only difference between them was the presence of signals due to an angeloyl group [^{1}H-NMR: δ 5.85 (H-3⁗), 1.94 (Me-5⁗), 2.03 (Me-4⁗); ^{13}C-NMR: δ 169.4 (C-1⁗), 129.3 (C-2⁗), 136.5 (C-3⁗), 15.8 (C-4⁗), 20.8 (C-5⁗)] instead of those of a tigloyl group. The HMBC experiment confirmed the same acylation position and the identical trisaccharide moiety. Therefore, **2** was established as 22-angeloylprotoaescigenin 3-*O*-[β-D-glucopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid, and named escin IVh. Escins IVg and IVh are a pair of geometrical isomers.

Compound **3** was obtained as a white amorphous powder. MALDI-TOF-MS showed the quasimolecular ion peak at *m/z* 1153 $[M+Na]^+$. Its high-resolution positive-ion MALDI-TOF-MS revealed its molecular formula to be $C_{55}H_{86}O_{24}$. It suggested that **3** has the same molecular composition as previously isolated escin Ia, which has been determined by a single-crystal X-ray analysis.[2] The ^{1}H- and ^{13}C-NMR spectra exhibited signals assignable to the protoaescigenin skeleton, an angeloyl group [^{1}H-NMR: δ 5.87 (H-3⁗), 1.90 (Me-5⁗), 1.98 (Me-4⁗); ^{13}C-NMR: δ 168.3 (C-1⁗), 129.2 (C-2⁗), 136.0 (C-3⁗), 15.9 (C-4⁗), 21.0 (C-5⁗)], an acetyl group [^{1}H-NMR: δ 2.50 (Me–OAc); ^{13}C-NMR: δ 170.0 (C-1⁗),

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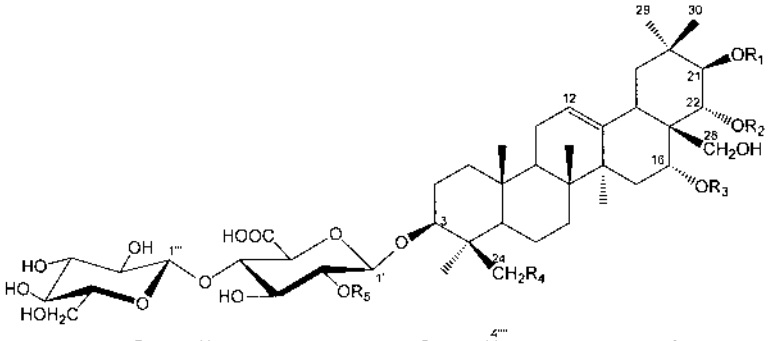

	R1	R2	R3	R4	R5
1	H	Tig	H	OH	Glc
2	H	Ang	H	OH	Glc
3	Ac	H	Ang	OH	Glc
4	Tig	Ac	H	H	Gal
5	H	H	H	OH	Glc

Table 1. ^{13}C-NMR Spectral Data for Escins IVg (**1**), IVh (**2**), and VIb (**3**) (δ Values)[a)]

C	1	2	3	C	1	2	3
1	38.3	38.3	38.4	29	30.1	30.1	30.0
2	26.2	26.2	26.4	30	19.2	19.2	20.1
3	90.9	90.9	91.2	1′	104.2	104.2	104.7
4	43.4	43.4	43.6	2′	79.5	79.6	79.4
5	55.9	55.9	56.0	3′	76.5	76.4	76.7
6	18.4	18.3	18.4	4′	82.7	82.7	82.4
7	33.0	33.0	33.1	5′	75.4	75.5	75.8
8	39.7	39.7	39.9	6′	175.0	175.1	172.9
9	46.5	46.5	46.7	1″	104.1	103.9	104.1
10	36.2	36.2	36.3	2″	75.4	75.5	75.6
11	23.9	23.9	23.9	3″	78.1	78.2	78.2
12	123.2	123.0	123.2	4″	69.6	69.6	69.8
13	143.1	143.1	141.8	5″	77.4	77.4	78.0
14	40.2	40.2	41.3	6″	61.4	61.4	61.6
15	34.6	34.6	30.9	1‴	104.2	104.3	104.7
16	68.9	69.0	71.5	2‴	74.8	74.8	73.9
17	48.0	47.7	47.6	3‴	77.9	78.2	78.4
18	41.4	41.4	39.9	4‴	71.2	71.1	71.5
19	47.5	47.5	47.2	5‴	77.8	77.8	77.9
20	36.8	36.9	36.0	6‴	61.9	61.9	62.2
21	76.5	76.6	79.8	1⁗	169.3	169.4	168.3
22	77.4	77.1	71.0	2⁗	129.4	129.3	129.2
23	22.3	22.3	22.4	3⁗	136.7	136.5	136.0
24	63.1	63.1	63.2	4⁗	13.9	15.8	15.9
25	15.4	15.4	15.5	5⁗	12.2	20.8	21.0
26	16.5	16.5	16.6	1‴′			170.0
27	27.3	27.4	27.0	2‴′			22.2
28	63.9	64.1	64.8				

a) Measured in pyridine-d_5.

22.2 (C-2⁗)] and a trisaccharide moiety [^{1}H-NMR: δ4.79 (d, J=7.5 Hz, H-1′), 5.33 (d, J=7.5 Hz, H-1‴) and 5.60 (d, J=7.5 Hz, H-1″); ^{13}C-NMR: δ104.7 (C-1′, C-1‴) and 104.1 (C-1″)]. Compared with the protoaescigenin skeleton of **5**, however, C-16 shifted to the downfield by 3.8 ppm, while C-15 shifted to the upfield by 3.2 ppm. HMBC correlation between signals of H-16β (δ5.90) and a carbonyl carbon (δ168.3) of an angeloyl group indicated that the angeloyl group is attached to C-16. An acetyl group was determined to attach to C-21 on the basis of the deshielding of 1.3 ppm for C-21 and the shielding of 6.0 ppm for C-22, relative to those of **5**. Moreover, a correlation between H-21 (δ5.95) and Me–OAc (δ2.50) in the nuclear Overhouser effect spectrometry (NOESY) spectrum also inferred the structure. The sugar moiety of **3** was determined to be identical with that of **5** after alkaline hydrolysis. Consequently, the structure of **3** was concluded to be 16-angeloyl-21-acetylprotoaescigenin 3-*O*-[β-D-glucopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid, and named escin VIb. It was the first C-16 acylated compound from escins.

It should be noted that since all the above compounds were detected by HPLC analysis of the ethanolic extract, they are not artifacts produced during the isolation process. Recently, Zhang *et al.*[5)] reported eight new triterpene saponins, aesculiosides A—H from the seeds of the same plant, but their structures were different in acylated positions from the saponins isolated at the present time.

Experimental

General IR: KBr discs, Perkin-Elmer 983 model; NMR: Varian INOVA-500 Spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C including ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H-detected multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC) and NOESY. Chemical shifts are given in δ relative to TMS as internal standard. MALDI-TOF-MS: BIFLEX III (Bruker). HR-SI-MS: APEX II FT-ICRMS (Bruker Daltonics). For preparative HPLC (pump: P2000; detector: UV3000 and software: PC1000. Thermo Separation Products, U.S.A.), an octadecyl sil (ODS) column [10 μ C_{18} (2), 250×21.2 mm, Phenomenex, U.S.A.] column was used. For HPLC analysis, an ODS column [LUNA 5 μ C_{18} (2), 250×4.60 mm, Phenomenex, U.S.A.] was used.

Plant Material The seeds of *Aesculus chinensis* Bge. were collected at Lueyang County, Shaanxi Province, P. R. China, in September 1997. The voucher specimens are deposited at the National Laboratory of Natural and

Biomimetic Drugs, Peking University.

Extraction and Isolation of Compounds The seeds of *A. chinensis* were extracted as described previously.[2] Further chromatography of the 70% EtOH fraction on prep. HPLC [MeCN–0.5% AcOH aq. (7 : 3 and 6 : 4, v/v)] yielded escins IVg (**1**, 32 mg), IVh (**2**, 35 mg), VIb (**3**, 41 mg), IIIa (**4**, 50 mg) and desacylescin I (**5**, 75 mg).

Alkaline Hydrolysis of Saponins 1—3 The saponin (10 mg of **1**, **2** or **3**, respectively) was added to a MeOH solution (10 ml) of MeONa (1 mg). The mixture was stirred at room temperature for 8 h and then neutralized with Dowex 50W×8 (H^+ form). After removal of the solvent *in vacuo*, the residue was dissolved in MeOH (1 ml) and analyzed by HPLC [solvent, MeOH–1% aqueous phosphoric acid (7 : 3); flow rate, 0.5 ml/min; wavelength, 220 nm].

Escin IVg (**1**): White amorphous powder. $[\alpha]_D^{25} = -25.0°$ (MeOH; $c=1.00$). IR (KBr) ν_{max}: 3423, 2922, 1710, 1604, 1407, 1273, 1076, 1040, 617 cm^{-1}. MALDI-TOF-MS *m/z* 1111 $[M+Na]^+$, 1127 $[M+K]^+$. HR-SI-MS (negative-ion mode) *m/z*: 1087.5291, Calcd for $C_{53}H_{83}O_{23}$ $[M-H]^-$: 1087.5331. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.62 (3H, s, H_3-25), 0.65 (1H, d, $J=12.5$ Hz, H-5), 0.77 (3H, s, H_3-26), 1.21 (2H, m, H_2-7), 1.26 (3H, s, H_3-23), 1.31 (3H, s, H_3-29), 1.40 (3H, s, H_3-30), 1.44 (1H, d, $J=7.0$ Hz, H_3-4‴), 1.55 (1H, d, $J=14.5$ Hz, H-9), 1.80 (3H, s, H_3-5‴), 1.82 (3H, s, H_3-27), 3.02 (1H, t, $J=13.5$ Hz, H_b-19), 3.11 (1H, d, $J=13.5$ Hz, H-18), 3.28 (1H, d, $J=11.5$ Hz, H_a-24), 3.30 (1H, dd-like, H-3α), 3.34 (1H, d, $J=11.0$ Hz, H_a-28), 3.60 (1H, d, $J=11.0$ Hz, H_b-28), 4.21(1H, d, $J=11.5$ Hz, H_b-24), 4.47 (1H, br s, H-16β), 4.77 (1H, d, $J=8.0$ Hz, H-1′), 5.07 (1H, d, $J=9.5$ Hz, H-21α), 5.21 (1H, d, $J=8.0$ Hz, H-1‴), 5.42 (1H, br s, H-12), 5.55 (1H, d, $J=8.0$ Hz, H-1″), 6.16 (1H, d, $J=9.5$ Hz, H-22β), 6.99 (1H, dq-like, H-3‴). ^{13}C-NMR spectral data are given in Table 1.

Escin IVh (**2**): White amorphous powder. $[\alpha]_D^{25} = -60°$ (MeOH; $c=1.05$). IR (KBr) ν_{max}: 3409, 2922, 1713, 1609, 1411, 1242, 1164, 1074, 1040, 612 cm^{-1}. MALDI-TOF-MS *m/z* 1111 $[M+Na]^+$. HR-SI-MS (negative ion mode) *m/z*: 1087.5308, Calcd for $C_{53}H_{83}O_{23}$ $[M-H]^-$: 1087.5331. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.60 (3H, s, H_3-25), 0.77 (3H, s, H_3-26), 1.24 (3H, s, H_3-23), 1.27 (3H, s, H_3-29), 1.34 (1H, m, H_a-19), 1.38 (3H, s, H_3-30), 1.56 (1H, d, $J=14.5$ Hz, H-9), 1.80 (3H, s, H_3-27), 1.94 (3H, s, H_3-5‴), 2.03 (1H, d, $J=6.0$ Hz, H_3-4‴), 3.02 (1H, t, $J=11.0$ Hz, H_b-19), 3.07 (1H, d, $J=11.0$ Hz, H-18), 3.27 (1H, d-like, H_a-24), 3.28 (1H, br s, H-3α), 3.38 (1H, d, $J=9.5$ Hz, H_a-28), 3.61 (1H, d, $J=9.5$ Hz, H_b-28), 4.40 (1H, d, $J=11.5$ Hz, H_b-24), 4.48 (1H, br s, H-16β), 4.73 (1H, d, $J=8.0$ Hz, H-1′), 5.02 (1H, d, $J=9.0$ Hz, H-21α), 5.21 (1H, d, $J=8.0$ Hz, H-1‴), 5.42 (1H, br s, H-12), 5.51 (1H, d, $J=8.0$ Hz, H-1″), 5.85 (1H, dq-like, H-3‴), 6.16 (1H, d, $J=9.0$ Hz, H-22β). ^{13}C-NMR spectral data are given in Table 1.

Escin VIb (**3**): White amorphous powder. $[\alpha]_D^{25} = -55°$ (MeOH; $c=1.00$). IR (KBr) ν_{max}: 3408, 2923, 1711, 1570, 1421, 1260, 1075, 1044, 659 cm^{-1}. MALDI-TOF-MS *m/z* 1153 $[M+Na]^+$. HR MALDI-TOF-MS (positive ion mode) *m/z*: 1153.5432, Calcd for $C_{55}H_{86}O_{24}Na$ $[M+Na]^+$: 1153.5401. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.65 (3H, s, H_3-25), 0.65 (3H, s, H_3-26), 1.10 (3H, s, H_3-29), 1.22 (1H, d, $J=13.5$ Hz, H_a-19), 1.26 (3H, s, H_3-23), 1.26 (3H, s, H_3-30), 1.47(3H, s, H_3-27), 1.90 (3H, s, H_3-5‴), 1.98 (3H, d, $J=7.0$ Hz, H_3-4‴), 2.50 (3H, s, OAc), 2.69 (1H, t, $J=10.0$ Hz, H_b-19), 2.94 (1H, d, $J=13.0$ Hz, H-18), 3.24 (1H, dd-like, H-3α), 4.51 (1H, d, $J=10.0$ Hz, H-22β), 4.79 (1H, d, $J=7.5$ Hz, H-1′), 5.33 (1H, d, $J=7.5$ Hz, H-1‴), 5.60 (1H, d, $J=7.5$ Hz, H-1″), 5.57 (1H, br s, H-12), 5.87 (1H, dq-like, H-3‴), 5.90 (1H, d, $J=4.5$ Hz, H-16β), 5.95 (1H, d, $J=10.0$ Hz, H-21α). ^{13}C-NMR spectral data are given in Table 1.

Escin IIIa (**4**): White amorphous powder. $[\alpha]_D^{25} = -53.3°$ (MeOH; $c=0.90$). IR (KBr) ν_{max}: 3423, 2922, 1709, 1609, 1382, 1272, 1074, 600 cm^{-1}. MALDI-TOF-MS *m/z*: 1137 $[M+Na]^+$.

Desacylescin I (**5**): Colorless fine crystals, mp 260—262 °C. $[\alpha]_D^{25} = -33.9°$ (MeOH; $c=1.15$). IR (KBr) ν_{max}: 3400, 2940, 2328, 1725, 1607, 1413, 1376, 1260, 1159, 1074, 1029, 904, 858, 798, 638 cm^{-1}. MALDI-TOF-MS *m/z* 1029 $[M+Na]^+$, 1045 $[M+K]^+$.

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