Three Escin-Like Triterpene Saponins: Assamicins VI, VII, and VIII from the Seeds of *Aesculus assamica* GRIFF

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Three new escin-like triterpene saponins, assamicins VI (**1**), VII (**2**), and VIII (**3**), were isolated from the seeds of *A. assamica*, together with a known saponin, isoescin Ib (**4**). Their structures were established as 28-*O*-acetyl-21-*O*-(3,4-di-*O*-angeloyl-6-deoxy-β-glucopyranosyl)-3-*O*-{*O*-β-glucopyranosyl-(1 → 2)-O-[β-glucopyranosyl-(1 → 4)]-β-glucopyranuronosyl}protoaescigenin (**1**), 21-*O*-angeloyl-3-*O*-{*O*-α-rhamnopyranosyl-(1 → 2)-*O*-[β-glucopyranosyl-(1 → 3)]-β-glucopyranuronosyl}protoaescigenin (**2**), and 21-*O*-angeloyl-3-*O*-{*O*-[β-glucopyranosyl-(1 → 3)]-β-glucopyranuronosyl}protoaescigenin (**3**) on the basis of spectroscopic analysis (protoaescigenin = (3β,4β,16α,21β,22α)-olean-12-ene-3,16,21,22,23,28-hexol; angelic acid = (2*Z*)-2-methylbut-2-enoic acid).

Introduction. – A number of escin-like saponins have been isolated from plants of the Aesculus genus, of which various biological activities have been studied and reported, such as anti-inflammatory and hypoglycemic activities, inhibitory effects on ethanol absorption, as well as anti-HIV-1 protease activities [1 – 14]. As part of our research on saponin constituents from the seeds of *Aesculus assamica* GRIFF, we have already reported the isolation and structure elucidation of five new escin-like triterpenoid saponins including assamicins I, II, III, IV, and V, as well as the known compounds protoaescigenin, 21-*O*-angeloylprotoaescigenin, 21-*O*-angeloyl-3-*O*-{*O*-β-glucopyranosyl-(1 → 2)-*O*-[β-glucopyranosyl-(1 → 4)]-β-glucopyranuronosyl}protoaescigenin, and isofraxoside [12 – 14] (protoaescigenin = (3β,4β,16α,21β,22α)-olean-12-ene-3,16,21,22,23,28-hexol; angelic acid = (2*Z*)-2-methylbut-2-enoic acid). Among the isolates, assamicins I and II were found to show insulin-like hypoglycemic activity [12].

Our continuing search now led to the isolation of three new escin-like triterpene saponins, assamicins VI (**1**), VII (**2**), and VIII (**3**), which were obtained from the seeds of *A. assamica*, together with a known saponin, isoescin Ib (**4**) [5], which was isolated for the first time from this plant (*Figure*). Herein, we report the structure elucidation of the new compounds.

Results and Discussion. – The saponin fraction of the MeOH extract of the seeds of *Aesculus assamica* GRIFF, collected in Yunan Province, China, as described previously

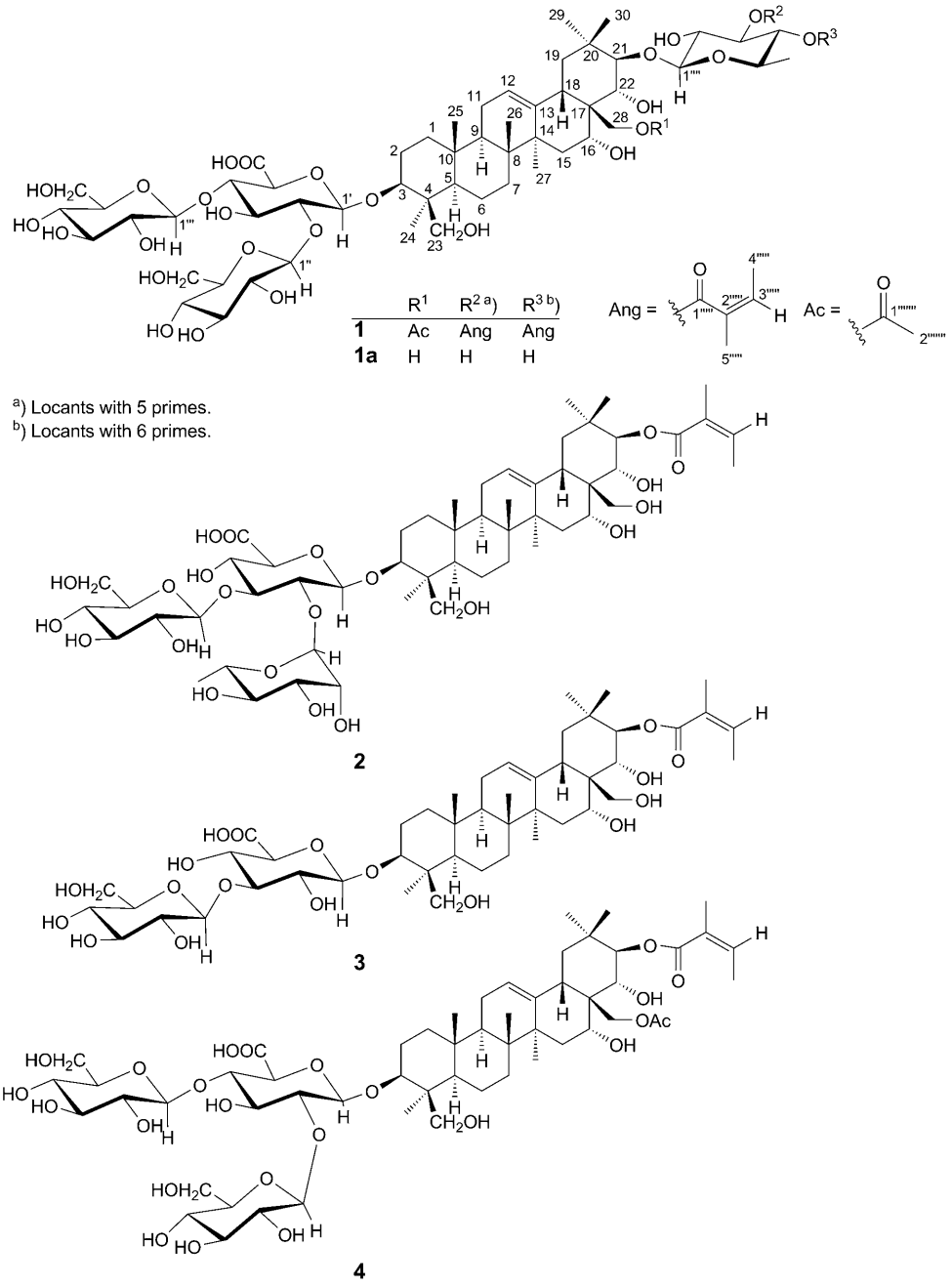

Figure. *Structures of* **1**–**4** *isolated from* Aesculus assamica GRIFF

[13], was purified by *LH-20*-column chromatography and HPLC to afford compounds **1**–**4**.

Compound **1** was obtained as an optically active white amorphous powder. Its IR spectrum showed absorption bands due to CO, ester, and olefin functions at 1720, 1716, 1650, and 1640 cm^{-1}, and two broad absorption bands at 3420 and 1060 cm^{-1} suggested the existence of an oligoglycoside structure. Positive- and negative-ion-mode ESI-MS of **1** showed the quasimolecular ion peaks at *m/z* 1381 ($[M+Na]^+$) and 1357 ($[M-H]^-$), respectively, and the positive-ion-mode HR-FAB-MS revealed the molecular formula $C_{66}H_{102}O_{29}$ for **1**. The fragmentation patterns in the ESI-MS (pos.) of **1** indicated the loss of hexose units (*m/z* 1219 ($[M+Na-162]^+$), 1057 ($[M+Na-162-162]^+$), 881 ($[M+Na-162-162-176]^+$), and 571 ($[M+Na-162-162-176-310]^+$)). The ^{1}H- and ^{13}C-NMR signals of **1** (*Table 1*) showed a close resemblance to those of assamicin III previously isolated from this plant [13]. Alkaline hydrolysis of **1** with 10% aqueous KOH solution/50% aqueous dioxane 1:1 produced the same deacylated saponin **1a** as previously obtained from assamicin III by the same treatment [13]. Previously, we reported that acid hydrolysis of **1a** gave 6-deoxyglucose (6-deoxy-Glc), glucose (Glc), glucuronic acid (GlcA), and protoaescigenin, as identified by GLC and TLC analysis in comparison with authentic compounds [13]. The ^{1}H- and ^{13}C-NMR signals were assigned by a combination of DEPT, COSY, TOCSY, HMQC, HMBC, and NOESY experiments (*Table 1*). On the basis of the spectral evidences, the structure of compound **1** was determined to be 28-*O*-acetyl-21-*O*-(3,4-di-*O*-angeloyl-6-deoxy-β-glucopyranosyl)-3-*O*-{*O*-β-glucopyranosyl-(1 → 2)-*O*-[β-glucopyranosyl-(1 → 4)]-β-glucopyranuronosyl}protoaescigenin, and designated as assamicin VI (**1**).

The ^{1}H-NMR spectrum of **1** included signals due to the protoaescigenin skeleton at δ(H) 0.68 (Me(25)), 0.92 (Me(26)), 1.24 (Me(24)), 1.47 (Me(29)), 1.30 (Me(30)), and 5.43 (broad, H–C(12)), to the four β-anomeric protons at δ(H) 4.82 (*d*, *J* = 8.0, H–C(1′) of GlcA), 5.48 (*d*, *J* = 7.6, H–C(1″) of Glc), 5.18 (*d*, *J* = 7.6, H–C(1‴) of Glc), and 4.92 (*d*, *J* = 8.0, H–C(1⁗) of 6-deoxy-Glc) together with anomeric C-atoms at δ(C) 104.6 (C(1′) of GlcA), 104.6 (C(1″) of Glc), 104.6 (C(1‴) of Glc), and 105.7 (C(1⁗) of 6-deoxy-Glc)], to an AcO group at δ(H) 2.10 (*s*) and δ(C) 170.7 (C=O), and to two angeloyl groups (Ang) at δ(H) 1.87 (Me(5⁗)), 1.90 (Me(5⁗′)), 1.95 (*d*, *J* = 8.0, Me (4⁗)), 1.97 (*d*, *J* = 8.0, Me (4⁗′)), 5.89 (*q*, *J* = 8.0, H–C(3⁗)), 5.98 (*q*, *J* = 8.0, H–C(3⁗′)), and δ(C) 128.3 (C(2⁗′)), 127.7(C(2⁗)), 137.7 (C(3⁗)), 138.9 (C(3⁗′)), 167.4 (C(1⁗′)), 166.8 (C(1⁗)). The HMBC correlation of H–C(6⁗)/ C(4⁗) and C(5⁗) indicated the presence of 6-deoxy-β-glucose, which was also confirmed by comparison with NMR data of assamicin II [12] and III [13]. The position of the sugar moieties at C(3) and C(21) and the interlinking among sugar moieties were deduced from HMBC experiments, in which H–C(1′), H–C(1″), H–C(1‴), and H–C(1⁗) were correlated with δ(C) 91.8 (C(3)), 78.5 (C(2′)), 82.0 (C(4′)), and 91.8 (C(21)). The positions of the acetyl and angeloyl groups were also deduced from HMBC cross-peaks, *i.e.*, CH_2(28)/δ(C) 170.7 (C=O of Ac), H–C(3⁗)/δ(C) 167.4 (C=O of Ang), and H–C(4⁗)/δ(C) 166.8 (C=O of Ang). In the NOESY plot, the correlations H–C(5)/Me(24), CH_2(23)/Me(25), Me(25)/Me(26), H–C(16)/H–C(22), and CH_2(28) and H–C(18)/Me(27), Me(29)/H–C(21), and Me(30)/H–C(22) were observed, which confirmed that the relative configuration of **1** is the same as that of assamicin II and III.

Compound **2** was obtained as an optically active white amorphous powder. In its IR spectrum, absorption bands due to OH, CO, ester, and olefin functions were observed at 3415, 2920, 1712, 1650, 1380, 1242, 1150, and 1050 cm^{-1}. The HR-FAB-MS revealed the molecular formula $C_{53}H_{84}O_{22}$ for **2**. In the positive-ion-mode ESI-MS of **2**, a quasimolecular-ion peaks was observed at *m/z* 1095 ($[M+Na]^+$), together with three

Table 1. *^{1}H- and ^{13}C-NMR* (500 and 100 MHz, resp.) *Data of* **1** *in (D_5)Pyridine.* δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
CH_2(1)	0.73–0.75 (*m*), 1.30–1.34 (*m*)	38.5	H–C(4′)	4.46–4.50 (*m*)	82.0
CH_2(2)	1.78–1.80 (*m*), 2.10–2.14 (*m*)	26.5	H–C(5′)	4.28–4.30 (*m*)	76.2
H–C(3)	3.32–3.34 (*m*)	91.8	C(6′)	–	170.7
C(4)	–	43.8	β-Glc:		
H–C(5)	0.78–0.82 (*m*)	56.2	H–C(1″)	5.48 (*d*, J = 7.6)	104.6
CH_2(6)	1.22–1.24 (*m*), 1.44–1.46 (*m*)	18.5	H–C(2″)	4.47–4.49 (*m*)	73.5
CH_2(7)	1.18–1.20 (*m*), 1.48–1.50 (*m*)	33.2	H–C(3″)	3.98–4.02 (*m*)	78.4
C(8)	–	40.0	H–C(4″)	4.36–4.40 (*m*)	70.9
H–C(9)	1.62–1.66 (*m*)	46.7	H–C(5″)	4.26–4.32 (*m*)	78.0
C(10)	–	36.4	CH_2(6″)	4.38–4.42 (*m*), 4.49 (*d*, J = 7.2)	62.4
CH_2(11)	1.80–1.82 (*m*)	24.0	β-Glc:		
H–C(12)	5.43 (br. *s*)	122.9	H–C(1‴)	5.18 (*d*, J = 7.6)	104.6
C(13)	–	142.9	H–C(2‴)	4.00–4.04 (*m*)	75.0
C(14)	–	41.8	H–C(3‴)	3.98–4.02 (*m*)	78.4
CH_2(15)	1.58–1.62 (*m*), 1.88–1.91 (*m*)	34.7	H–C(4‴)	4.38–4.40 (*m*)	71.5
H–C(16)	4.85 (br. *s*)	67.9	H–C(5‴)	3.98–4.02 (*m*)	78.4
C(17)	–	46.9	CH_2(6‴)	4.38–4.42 (*m*), 4.49 (*d*, J = 7.2)	62.4
H–C(18)	2.78–2.82 (*m*)	40.5	6-deoxy-β-Glc:		
CH_2(19)	1.36–1.38 (*m*), 3.08 (*t*, J = 13.5)	47.7	H–C(1⁗)	4.92 (*d*, J = 8.0)	105.7
C(20)	–	37.1	H–C(2⁗)	4.12 (*t*, J = 8.8)	73.2
H–C(21)	4.80 (*d*, J = 9.2)	91.8	H–C(3⁗)	3.78 (*t*, J = 9.2)	75.4
H–C(22)	4.47–4.49 (*m*)	73.5	H–C(4⁗)	5.26 (*t*, J = 9.2)	73.9
CH_2(23)	3.33 (*m*), 4.18–4.22 (*m*)	63.4	H–C(5⁗)	3.66–3.70 (*m*)	70.2
Me(24)	1.24 (*s*)	22.5	Me(6‴)	1.21 (*d*, J = 6.0)	17.7
Me(25)	0.68 (*s*)	15.6	AngO–C(3‴):		
Me(26)	0.92 (*s*)	16.8	C(1⁗)		167.4
Me(27)	1.82 (*s*)	27.4	C(2⁗)		128.3
CH_2(28)	4.25–4.27 (*m*); 4.35 (*d*, J = 11.2)	66.5	H–C(3⁗)	5.89 (*q*-like)	137.7
Me(29)	1.47 (*s*)	29.8	Me(4⁗)	1.95 (*d*, J = 8.0)	15.8
Me(30)	1.30 (*s*)	20.1	Me(5⁗)	1.87 (*s*)	20.5
AcO–C(28):			AngO–C(4‴):		
C(1‴′)	–	170.7	C(1‴′)	–	166.8
C(2‴′)	2.10 (*s*)	20.8	C(2‴′)		127.7
β-GlcA:			H–C(3‴′)	5.98 (*q*-like)	138.9
H–C(1′)	4.82 (*d*, J = 8.0)	104.6	Me(4‴′)	1.97 (*d*, J = 8.0)	15.8
H–C(2′)	4.38–4.40 (*m*)	78.5	Me(5‴′)	1.90 (*s*)	20.6
H–C(3′)	4.26–4.29 (*m*)	76.9			

fragment ions at 949 ($[M + Na - 146]^+$), 787 ($[M + Na - 146 - 162]^+$), and 611 ($[M + Na - 146 - 162 - 176]^+$), indicating the sequential losses of three hexose units. The acid hydrolysis of **2** liberated the aglycone and sugars: rhamnose (Rha), glucuronic acid, glucose, and 21-*O*-angeloylprotoaescigenin were identified by GLC and TLC comparison with authentic compounds. The ^{1}H- and ^{13}C-NMR data of **2** (*Table 2*) were similar to those of the known compound aesculioside B, revealing the presence of one angeloyl group, three sugar units, and a protoaescigenin moiety [8]. The position of angeloyl group, the sugar moiety, and the interlinkage pattern among sugars were clarified by an HMBC experiment (correlations H–C(21)/168.6 (C(1) of Ang),

H–C(3)/C(1′), H–C(1″) of Rha/C(2′) of GlcA, and H–C(1‴) of Glc/C(3′)). The ^{1}H- and ^{13}C-NMR signals were assigned by analysis of the DEPT, COSY, TOCSY, HMQC, HMBC, and NOESY data (*Table 2*). The relative configuration in the sapogenin of **2** was determined to be the same as in **1** according to the NOESY data. The configurations at the anomeric C-atoms were determined to be β for Glc and GlcA, and α for Rha from the coupling constant of the anomeric protons and comparison with literature data [15]. The structure of compound **2** was established as 21-*O*-angeloyl-3-*O*-{*O*-α-rhamnopyranosyl-(1 → 2)-*O*-[β-glucopyranosyl-(1 → 3)]-β-glucopyranuronosyl}protoaescigenin, and designated as assamicin VII (**2**).

Table 2. *^{1}H- and ^{13}C-NMR* (500 and 100 MHz, resp.) *Data for* **2** *in (D_5)Pyridine.* δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
CH_2(1)	0.78–0.80 (*m*), 1.28–1.31(*m*)	38.4	β-GlcA		
CH_2(2)	1.78–1.82 (*m*), 2.19–2.21 (*m*)	26.3	H–C(1′)	4.77 (*d*, J = 7.2)	104.7
H–C(3)	3.34–3.37 (*m*)	90.7	H–C(2′)	4.36–4.38 (*m*)	77.4
C(4)	–	43.7	H–C(3′)	4.31–4.33 (*m*)	84.5
H–C(5)	0.80–0.82 (*m*)	55.8	H–C(4′)	4.33–4.36 (*m*)	73.0
CH_2(6)	1.45–1.47 (*m*), 1.64–1.67 (*m*)	18.2	H–C(5′)	4.45–4.47 (*m*)	77.4
CH_2(7)	1.20–1.22 (*m*), 1.50–1.52 (*m*)	33.1	C(6′)	–	170.4
C(8)	–	39.7	α-Rha:		
H–C(9)	1.63–1.65 (*m*)	46.6	H–C(1″)	6.15 (br. *s*)	103.2
C(10)	–	36.2	H–C(2″)	4.88–4.90 (*m*)	72.2
CH_2(11)	1.59–1.62 (*m*), 1.78–1.80 (*m*)	23.8	H–C(3″)	4.52–4.54 (*m*)	72.6
H–C(12)	5.38 (br. *s*)	123.4	H–C(4″)	4.34–4.36 (*m*)	73.7
C(13)	–	143.2	H–C(5″)	5.00–5.02 (*m*)	70.1
C(14)	–	41.6	Me(6″)	1.64 (*d*, J = 7.0)	18.2
CH_2(15)	1.58–1.60 (*m*), 1.92–1.95 (*m*)	34.2	β-Glc:		
H–C(16)	4.85 (br. *s*)	67.6	H–C(1‴)	5.24 (*d*, J = 7.0)	103.7
C(17)	–	47.6	H–C(2‴)	4.34–4.36 (*m*)	71.7
H–C(18)	2.84–2.87 (*m*)	40.3	H–C(3‴)	3.82–3.85 (*m*)	75.1
CH_2(19)	1.35–1.37 (*m*), 3.00 (*t*, J = 13.5)	47.9	H–C(4‴)	4.36–4.38 (*m*)	70.5
C(20)	–	35.8	H–C(5‴)	3.80–3.82 (*m*)	76.6
H–C(21)	6.40 (*d*, J = 10.0)	81.4	CH_2(6‴)	4.33–4.36 (*m*), 4.42–4.44 (*m*)	61.5
H–C(22)	4.75 (*d*, J = 10.0)	72.9	AngO–C(21):		
CH_2(23)	3.23 (*d*, J = 11.5), 4.18–4.22 (*m*)	63.1	C(1⁗)	–	168.6
Me(24)	1.17 (*s*)	22.2	C(2⁗)	–	129.3
Me(25)	0.60 (*s*)	15.6	H–C(3⁗)	5.87 (*q*-like)	135.5
Me(26)	0.74 (*s*)	16.5	Me(4⁗)	2.00 (*d*, J = 7.2)	15.7
Me(27)	1.79 (*s*)	27.2	Me(5⁗)	1.92 (*s*)	20.8
CH_2(28)	3.61 (*d*, J = 10.0), 3.89 (*d*, J = 10.0)	66.0			
Me(29)	1.03 (*s*)	29.6			
Me(30)	1.26 (*s*)	20.1			

Compound **3** was obtained as an optically active amorphous white powder. HR-FAB-MS revealed the molecular formula of **3** to be $C_{47}H_{74}O_{18}$. In the positive-ion-mode ESI-MS of **3**, a quasimolecular-ion peak was observed at m/z 949 ($[M + Na]^+$), together with two fragment ions at 787 ($[M + Na - 162]^+$) and 611 ($[M + Na - 162 - 176]^+$), indicating the sequential losses of two hexose units. The acid hydrolysis of

compound **3** liberated the aglycone and sugars: glucuronic acid, glucose, and 21-*O*-angeloylprotoaescigenin were identified by GLC and TLC comparison with authentic compounds. The ^{1}H- and ^{13}C-NMR signals of **3** (*Table 3*) indicated the presence of a protoaescigenin moiety. The ^{1}H- and ^{13}C-NMR signals were assigned by analysis of the DEPT, COSY, TOCSY, HMQC, HMBC, and NOESY data (*Table 3*). The relative configuration of **3** was determined by a NOESY experiment as described above for **1** and **2**. From these analyses, the structure of compound **3** was established as 21-*O*-angeloyl-3-*O*-[*O*-β-glucopyranosyl-(1 → 3)-β-glucopyranuronosyl]protoaescigenin, and designated as assamicin VIII (**3**).

Table 3. *^{1}H- and ^{13}C-NMR* (500 and 100 MHz, resp.) *Data for* **3** *in (D_5)Pyridine.* δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
CH_2(1)	0.80–0.82 (*m*), 1.38–1.40 (*m*)	39.5	Me(27)	1.86 (*s*)	28.2
CH_2(2)	1.90–1.92 (*m*), 2.10–2.12 (*m*)	27.6	CH_2(28)	3.66 (*d*, *J* = 10.0), 3.94 (*d*, *J* = 10.0)	65.7
H–C(3)	3.50–3.53 (*m*)	90.0	Me(29)	1.10 (*s*)	30.7
C(4)	–	42.8	Me(30)	1.32 (*s*)	21.1
H–C(5)	0.88–0.90 (*m*)	57.0	β-GlcA:		
CH_2(6)	1.28–1.30 (*m*), 1.62–1.64 (*m*)	19.7	H–C(1′)	5.08 (*d*, *J* = 7.0)	106.7
CH_2(7)	1.22–1.24 (*m*), 1.54–1.56 (*m*)	34.3	H–C(2′)	4.08–4.10 (*m*)	75.7
C(8)	–	40.9	H–C(3′)	4.48–4.50 (*m*)	84.5
H–C(9)	1.69–1.71 (*m*)	45.3	H–C(4′)	4.10–1.13 (*m*)	72.6
C(10)	–	37.0	H–C(5′)	4.23–4.26 (*m*)	77.4
CH_2(11)	1.70–1.72 (*m*), 1.84–1.86 (*m*)	25.0	C(6)	–	172.0
H–C(12)	5.38 (br. *s*)	123.4	β-Glc:		
C(13)	–	144.4	H–C(1″)	5.20 (*d*, *J* = 7.0)	105.8
C(14)	–	41.4	H–C(2″)	4.06–4.08 (*m*)	75.9
CH_2(15)	1.57–1.60 (*m*), 1.92–1.95 (*m*)	35.3	H–C(3″)	4.20–4.22 (*m*)	78.8
H–C(16)	4.85 (br. *s*)	67.0	H–C(4″)	4.34–4.36 (*m*)	74.8
C(17)	–	49.1	H–C(5″)	3.96–3.98 (*m*)	79.4
H–C(18)	2.94–2.96 (*m*)	40.9	CH_2(6″)	4.18–4.20 (*m*), 4.50–4.52 (*m*)	63.4
CH_2(19)	1.38–1.40 (*m*), 3.10 (*t*, *J* = 13.5)	47.7	AngO–C(21):		
C(20)	–	37.4	C(1‴)	–	168.8
H–C(21)	6.43 (*d*, *J* = 10.0)	82.6	C(2‴)	–	130.5
H–C(22)	4.79 (*d*, *J* = 10.0)	73.7	H–C(3‴)	5.90 (*q*-like)	135.5
CH_2(23)	3.51–3.54 (*m*), 4.29–4.31 (*m*)	64.1	Me(4‴)	2.02 (*d*, *J* = 7.2)	16.6
Me(24)	1.47 (*s*)	24.1	Me(5‴)	1.96 (*s*)	21.7
Me(25)	0.76 (*s*)	16.4			
Me(26)	0.83 (*s*)	17.7			

The presence of the protoaescigenin moiety in **3** was established by the six Me signals at δ(H) 0.76, 0.83, 1.10, 1.32, 1.47, and 1.86 (Me (24), Me (26), Me (29), Me (30), Me (24), Me (27)), an oxygenated CH_2 and two oxygenated CH groups (δ(H) 3.66 and 3.94 (2*d*, *J* = 10.0, CH_2(28)), 6.43 (*d*, *J* = 10.0, H–C(21)), 4.79 (*d*, *J* = 10.0, H–C(22))), an olefinic H-atom at δ(H) 5.38 (br. *s*, H–C(12)). Moreover, the signals of two glycosyl moieties were present at δ(H) 5.08 (*d*, *J* = 7.0, H–C(1′) of GlcA) and 5.20 (*d*, *J* = 7.0, H–C(1″) of Glc), besides those of an angeloyl group at δ(H) 1.96 (*s*, Me), 2.02 (*d*, *J* = 7.2, Me), and 5.90 (*qd*-like, =CH). In the HMBC spectrum, the long-range correlations were observed between H–C(21) and δ(C) 168.8 (C(1) of Ang), between H–C(1′) of GlcA and C(3), and between H–C(1″) of Glc and C(3′) of GlcA.

In our bioactivity screening, compounds **1**–**4** showed no cytotoxicity on K562 and HL-60 cell lines at the concentration of 100 μg/ml.

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Experimental Part

General. GC: *HP-5890-II* spectrometer, with a *SE30* capillary column (12 m, 0.22 mm i.d.) and a H_2 flame-ionization detector (FID, 270°); column temp. 170→250°, heating rate 5°/min; carrier gas N_2 (30 ml/min). TLC: SiO_2 *60* F_{254}; detection by spraying with 10% H_2SO_4 soln. and heating. Column chromatography (CC): *Diaion HP-20* (*Mitsubishi Kasei*), *LH-20* (*Amersham Biosciences*), and *ODS* (*Lobar*, 40–63 μm; *Merck*). Prep. HPLC: *ODS* column (*C-18*, 250 × 20 mm; *Shimadzu Pak*; detector: RID). Optical rotations: *P-1020* digital polarimeter (*Jasco Corporation*). IR Spectra: *Shimadzu- FT/IR-8400* spectrometer; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra (incl. 2D-NMR): *Bruker-AV-500* spectrometer; at 500 MHz for ^1H and 100 MHz for ^{13}C; chemical shifts δ in ppm rel. to Me_4Si as an internal standard, coupling constants *J* in Hz. MS: *Jeol JMS-DX302* spectrometer for FAB and HR, and *Bruker-Esquire-2000* spectrometer for ESI; in *m*/*z*.

Plant Material. The seeds of *Aesculus assamica* were collected in Yunnan Province in 2000 and identified by Prof. *Qishing Sun* of the Shenyang Pharmaceutical University. A voucher specimen (YXS-200-12) was deposited with the Department of Natural Product Chemistry of the Shenyang Pharmaceutical University, P. R. China.

Extraction and Isolation. As reported previously [13], the saponin-rich fraction obtained by CC (*HP-20* resin, 50% EtOH eluate) from the H_2O-soluble extract of seeds was subjected to CC (*ODS*, 30% MeOH, 50% MeOH, 70% MeOH, 100% MeOH) to give 10 subfractions. The fourth subfraction (70% MeOH eluate, 0.5 g) was resubjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) and finally purified by prep. HPLC (MeCN/0.1% aq. CF_3COOH soln. 65:35; flow rate 3.0 ml/min): **1** (6.2 mg), **2** (8.5 mg), **3** (10.5 mg), and **4** (15.2 mg).

Assamicin VI (=*(3β,4β,16α,21β,22α)-28-(Acetyloxy)-21-{[6-deoxy-2,4-*O*-bis[(2Z)-2-methyl-1-oxobut-2-en-1-yl]-β-glucopyranosyl}oxy}-16,22,23-trihydroxyolean-12-en-3-yl* O*-β-Glucopyranosyl-(1→2)]-*O*-[β-glucopyranosyl-(1→4)]-β-glucopyranosiduronic Acid*; **1**): Amorphous powder. $[\alpha]_D^{26} = -41.6$ ($c = 0.5$, MeOH). IR (KBr): 3420, 2932, 1720, 1716, 1650, 1640, 1060. ^1H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 1381 ($[M+Na]^+$), 1219 ($[M+Na-162]^+$), 1057 ($[M+Na-162-162]^+$), ([881 $[M+Na-162-162-176]^+$), 571 ($[M+Na-162-162-176-310]^+$). ESI-MS (neg.): 1357 ($[M-H]^-$). HR-FAB-MS (pos.): 1381.6383 ($[M+Na]^+$, $C_{66}H_{102}NaO_{29}^+$; calc. 1381.6405).

Assamicin VII (=*(3β,4β,16α,21β,22α)-16,22,23,28-Tetrahydroxy-21-{[(2Z)-2-methyl-1-oxobut-2-en-1-yl]oxy}olean-12-en-3-yl* O*-6-Deoxy-α-mannopyranosyl-(1→2)*-O*-[β-glucopyranosyl-(1→4)]-β-glucopyranosiduronic Acid*; **2**): Amorphous powder. $[\alpha]_D^{26} = -22.8$ ($c = 0.5$, MeOH). IR (KBr): 3415, 2920, 1712, 1650, 1380, 1242, 1150, 1050. ^1H- and ^{13}C-NMR: *Table 2*. ESI-MS (pos.): 1095 ($[M+Na]^+$), 949 ($[M+Na-146]^+$), 787 ($[M+Na-146-162]^+$), 611 ($[M+Na-146-162-176]^+$). ESI-MS (neg.): 1071 ($[M-H]^-$). HR-FAB-MS (pos.): 1095.5335 ($[M+Na]^+$, $C_{53}H_{84}NaO_{22}^+$; calc. 1095.5352).

Assamicin VIII (=*(3β,14β,16α,21β,22α)-16,22,23,28-Tetrahydroxy-21-{[(2Z)-2-methyl-1-oxobut-2-en-1-yl]oxy}olean-12-en-3-yl 3-*O*-β-Glucopyranosylglucopyranosiduronic Acid*; **3**): Amorphous powder. $[\alpha]_D^{26} = -20.8$ ($c = 0.5$, MeOH). IR (KBr): 3412, 1724, 1718, 1680, 1655, 1050. ^1H- and ^{13}C-NMR: *Table 3*. ESI-MS (pos.): 949 ($[M+Na]^+$), 1219 ($[M+Na-162]^+$), 787 ($[M+Na-162]^+$), 611 ($[M+Na-162-176]^+$). ESI-MS (neg.): 925 ($[M-H]^-$). HR-FAB-MS (pos.): 949.4786 ($[M+Na]^+$, $C_{47}H_{74}NaO_{18}^+$; calc. 949.4773).

Alkaline Hydrolysis of **1**. A soln. of saponin **1** (4 mg) in 10% aq. KOH soln./50% aq. dioxane soln. 1:1 (*v*/*v*, 2 ml) was stirred at 37° for 1 h. The mixture was neutralized with 10% aq. AcOH. After evaporation of the solvent from the filtrate, the residue was dissolved in H_2O (5 ml) and extracted with AcOEt/BuOH 1:1. The org. solvents were evaporated, and the residue was purified by prep. TLC (SiO_2,

$CHCl_3$/MeOH/H_2O 70:30:5) to give the deacylated saponin **1a** (*ca.* 2 mg) that was identified by ESI-MS: 1157 ($[M+Na]^+$), 995 ($[M+Na-162]^+$), 833 ($[M+Na-162-162]^+$), 657 ($[M+Na-162-162-176]^+$), 511 ($[M+Na-162-162-176-146]^+$), as reported previously [13].

Acid Hydrolysis of **1a**, **2**, *and* **3**. Compounds **1a** (2 mg), **2** (4 mg), and **3** (4 mg) were each dissolved in H_2O (1 ml) and treated with 20% aq. H_2SO_4 soln. (1 ml). The mixture was heated under reflux for 4 h, then neutralized with sat. $NaHCO_3$ soln., and extracted with AcOEt (3×). TLC ($CHCl_3$/MeOH 95:5; R_f 0.35) analysis of the AcOEt extracts showed the presence of protoaescigenin in **1a** and of 21-*O*-angeloylprotoaescigenin in both **2** and **3** by comparison with authentic compounds. The compounds of the aq. phase were subjected to GLC analysis of their trimethylsilyated derivatives (6-deoxyglucose (t_R 4.18 min), glucose (t_R 7.35 min), rhamnose (t_R 4.48 min), and glucuronic acid (t_R 10.51 min), as described previously [13][15].

Bioassay. The cytotoxicity assay with cultured K562 and HL-60 cell lines were carried out as reported previously [13].

REFERENCES

[1] B. Singh, P. K. Agrawal, R. S. Thakur, *J. Nat. Prod*. **1987**, *50*, 781.
[2] M. Yoshikawa, E. Harada, T. Murakami, H. Matsuda, N. Wariishi, J. Yamahara, N. Murakami, I. Kitagawa, *Chem. Pharm. Bull*. **1994**, *42*, 1357.
[3] M. Yoshikawa, T. Murakami, H. Matsuda, J. Yamahara, N. Murakami, I. Kitagawa, *Chem. Pharm. Bull*. **1996**, *44*, 1454.
[4] H. Matsuda, Y. Li, T. Murakami, K. Ninomiya, J. Yamahara, M. Yoshikawa, *Biol. Pharm. Bull.* **1997**, *20*, 1092.
[5] M. Yoshikawa, T. Murakami, J. Yamahara, H. Matsuda, *Chem. Pharm. Bull.* **1998**, *46*, 1764.
[6] X.-W. Yang, J. Zhao, Y.-X. Cui, X.-H. Liu, C.-M. Ma, M. Hattori, L.-H. Zhang, *J. Nat. Prod.* **1999**, *62*, 1510.
[7] J. Zhao, X. W. Yang, Y. X. Cui, X. H. Liu, S. Y. Liu, H. Y. Zhi, J. Y. Chen, F. B. Zhu, Y. L. Xue, D. B. Liu, *Chin. Chem. Lett.* **1999**, *10*, 291.
[8] Z. Zhang, K. Koike, Z. Jia, T. Nikaido, D. Guo, J. Zheng, *Chem. Pharm. Bull.* **1999**, *47*, 1515.
[9] J. Zhao, X.-W. Yang, M. Hattori, *Chem. Pharm. Bull.* **2001**, *49*, 626.
[10] J. Zhao, X.-W. Yang, *J. Asian Nat. Prod. Res.* **2003**, *5*, 197.
[11] F. Wei, L.-Y. Ma, W.-T. Jin, S.-C. Ma, G.-Z. Han, I.-A. Khan, R.-C. Lin, *Chem. Pharm. Bull.* **2004**, *52*, 1246.
[12] T. Sakurai, T. Nishimura, N. Otake, X. S. Yao, K. Abe, M. Zeida, H. Nagasawa, S. Sakuda, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 807.
[13] H. Liu, X. Zhang, H. Gao, N. Wang, S. Jin, B. Cai, X. Yao, G. Cai, *Chem. Pharm. Bull.* **2005**, *53*, 1310.
[14] H. W. Liu, X. S. Yao, N. L. Wang, G. P. Cai, *Chin. Chem. Lett.* **2006**, *17*, 211.
[15] H. Liu, Z. Xiong, F. Li, G. Qu, H. Kobayashi, X. Yao, *Chem. Pharm. Bull.* **2003**, *51*, 1089.

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