

Bioactive Saponins and Glycosides. XII.¹⁾ Horse Chestnut. (2): Structures of Escins IIIb, IV, V, and VI and Isoescins Ia, Ib, and V, Acylated Polyhydroxyoleanene Triterpene Oligoglycosides, from the Seeds of Horse Chestnut Tree (*Aesculus hippocastanum* L., Hippocastanaceae)

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Received July 2, 1998; accepted August 21, 1998

New acylated polyhydroxyoleanene triterpene oligoglycosides, escins IIIb, IV, V, and VI and isoescins Ia, Ib, and V, were isolated from the seeds of horse chestnut tree (*Aesculus hippocastanum* L.). Their structures were elucidated on the basis of chemical and physicochemical evidence.

Key words escin; isoescin; horse chestnut; *Aesculus hippocastanum*; saponin; Hippocastanaceae

In the course of our characterization studies on bioactive saponin constituents in medicinal foodstuffs²⁾ and natural medicines,^{1,3)} the saponin mixture "escin" from the seeds of horse chestnut tree (*Aesculus hippocastanum* L., Hippocastanaceae) was found to show inhibitory effects on the increase of serum glucose levels in glucose-loaded rats and on ethanol absorption in rats. We have hitherto isolated five acylated polyhydroxyoleanene triterpene oligoglycosides with hypoglycemic and ethanol absorption inhibitory activities, escins Ia (1), Ib (2), IIa (3), IIb (4), and IIIa (6), from the saponin mixture and reported their structures.⁴⁾ Furthermore, we have reported the structure requirements of escins for their inhibitory activity on the increase of serum glucose levels in glucose-loaded rats and their mode of action.⁵⁾ Recently, we have also reported the antiinflammatory activity of escins Ia, Ib, IIa, and IIb and the structure-activity relationships.⁶⁾ As a continuation of our study on the saponin constituents in the seeds of horse chestnut tree, we have isolated seven new acylated polyhydroxyoleanene triterpene oligoglycosides called escins IIIb (8), IV (9), V (10), and VI (11) and isoescins Ia (12), Ib (13), and V (14). In this paper, we describe the structure elucidation of these new escins and isoescins (8—14).

Escin IIIb (8) was isolated as colorless fine crystals of mp 194.1—196.5 °C from CHCl₃–MeOH. The IR spectrum of 8

showed absorption bands ascribable to carboxyl, ester, and olefin functions at 1736, 1721, 1655, 1649, and 1636 cm⁻¹ and broad absorption bands at 3425 and 1075 cm⁻¹ suggestive of an oligoglycosidic structure. In the negative-ion FAB-MS of 8, a quasimolecular ion peak was observed at *m/z* 1113 (M–H)⁻, while its positive-ion FAB-MS showed quasimolecular ion peaks at *m/z* 1137 (M+Na)⁺ and *m/z* 1159 (M+2Na–H)⁺. High-resolution MS analysis of quasimolecular ion peaks in the positive-ion FAB-MS revealed the molecular formula of 8 to be C₅₅H₈₆O₂₃. Alkaline hydrolysis of 8 with 10% aqueous potassium hydroxide in 50% aqueous dioxane (1 : 1) liberated desacylescins III (7)⁴⁾ together with acetic acid and angelic acid. The organic acids were derived to their *p*-nitrobenzyl esters,⁷⁾ which were identified by HPLC analysis.

The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Table 1) spectra of 8, which were assigned with the aid of various NMR experiments,⁸⁾ showed signals ascribable to the desacylescins III moiety [δ 3.38, 3.62 (both d, *J*=10.2 Hz, 28-H₂), 3.39 (dd-like, 3-H), 4.62 (br s, 16-H), 4.97 (d, *J*=6.3 Hz, 1'-H), 5.15 (d, *J*=7.6 Hz, 1'''-H), 5.20 (d, *J*=7.6 Hz, 1''-H), 6.17 (d, *J*=10.2 Hz, 22-H), 6.60 (d, *J*=10.2 Hz, 21-H)] together with an acetyl group [δ 1.93 (s)] and an angeloyl group [δ 2.02 (s, 5'''-H₃), 2.10 (d, *J*=7.3 Hz, 4'''-H₃), 5.97 (dq-like, 3'''-H)]. The carbon and proton signals in the ¹H- and ¹³C-NMR data

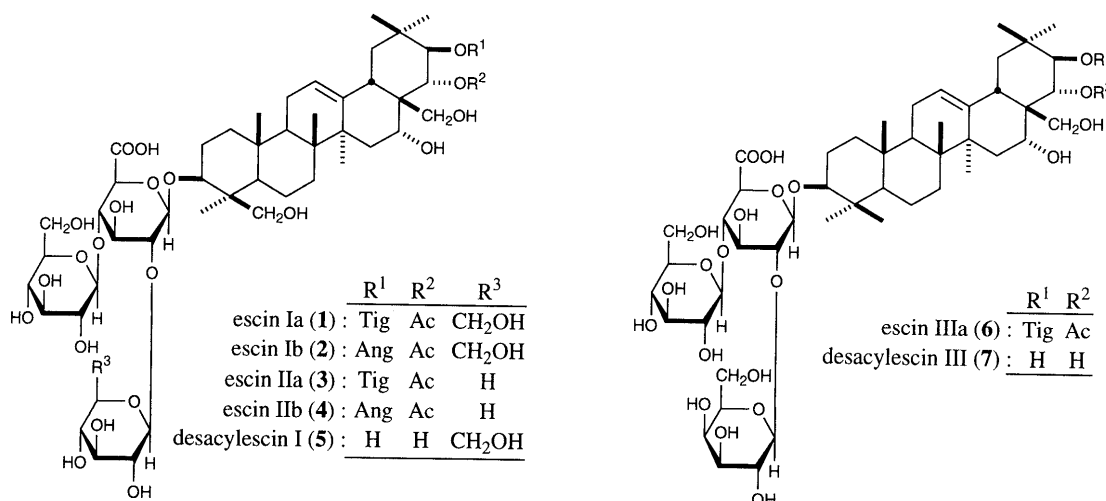


Chart 1

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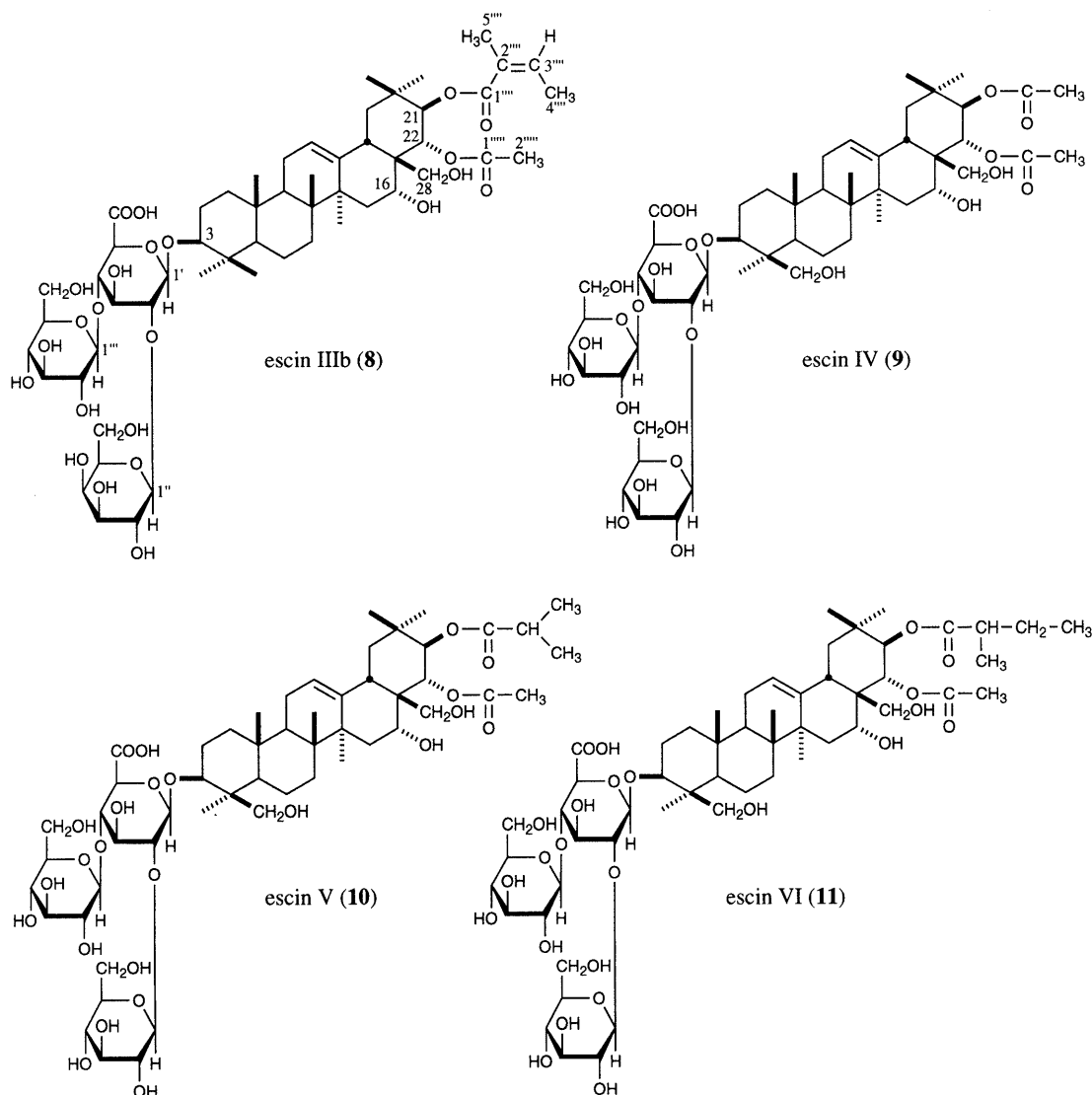


Chart 2

of **8** were found to be superimposable on those of escin IIIa (**6**), except for the signals due to the angeloyl group of **8**. The linkage positions of the acetyl and angeloyl groups were characterized by a heteronuclear multiple bond connectivity (HMBC) experiment on **8**. Namely, the HMBC experiment showed long-range correlations between the 21-proton and the carbonyl carbon (C-1''') of the angeloyl group and between the 22-proton and the carbonyl carbon (C-1''') of the acetyl group. Furthermore, comparison of the ^{13}C -NMR data of **8** with those for desacylescins III (**7**)⁴ revealed acylation shifts around the 21- and 22-protons in **8**. On the basis of the above mentioned evidence, the structure of escin IIIb was determined to be expressed as 21-*O*-angeloyl-22-*O*-acetyl-barringtonol C 3-*O*-[β -D-galactopyranosyl(1 $\rightarrow$ 2)][β -D-glucopyranosyl(1 $\rightarrow$ 4)]- β -D-glucopyranosiduronic acid (**8**).

Escin IV (**9**) was also isolated as colorless fine crystals of mp 226.9–228.1 °C from CHCl_3 –MeOH and its IR spectrum showed absorption bands due to hydroxyl, ester, and carboxyl at 3421, 1737, 1719, and 1074 cm^{-1} . The molecular formula $\text{C}_{52}\text{H}_{82}\text{O}_{24}$ was determined from its negative- and positive-ion FAB-MS [m/z 1089 ($\text{M}-\text{H})^-$, m/z 1113 ($\text{M}+\text{Na})^+$, and m/z 1135 ($\text{M}+2\text{Na}-\text{H})^+$] and by high-resolution FAB-MS measurement. Alkaline hydrolysis of **9** liber-

ated desacylescins I (**5**)⁴ and acetic acid. The ^1H -NMR (pyridine- d_5) and ^{13}C -NMR (Table 1) spectra⁸ of **9** showed the presence of two acetyl groups [δ 1.98, 2.12 (both s, 2''', 2''-H₃)] together with two methine protons on carbons bearing an acetyl group [δ 6.14 (d, $J=9.9$ Hz, 22-H), 6.45 (d, $J=9.9$ Hz, 21-H)]. In an HMBC experiment, long-range correlations were observed between the 21-proton and the carbonyl carbon (δ_{C} 171.1) of the 21-acetyl group and between the 22-proton and the carbonyl carbon (δ_{C} 170.9) of the 22-acetyl group. Additionally, acetylation shifts were observed around the 21- and the 22-protons in **9**. Consequently, the structure of escin IV was elucidated as 21,22-di-*O*-acetylprotoaescigenin 3-*O*-[β -D-glucopyranosyl(1 $\rightarrow$ 2)][β -D-glucopyranosyl(1 $\rightarrow$ 4)]- β -D-glucopyranosiduronic acid (**9**).

Escin V (**10**) and isoescins V (**14**) were isolated as colorless fine crystals of mp 215.8–217.1 °C and 198.8–200.7 °C, respectively. Their IR spectra were similar to each other and showed absorption bands due to hydroxyl, carboxyl, and ester functions. Escin V (**10**) and isoescins V (**14**) were found to have the same molecular formula $\text{C}_{54}\text{H}_{86}\text{O}_{24}$, which was obtained from the quasimolecular ion peak in their negative- and positive-ion FAB-MS at m/z 1117 ($\text{M}-\text{H})^-$, m/z 1141 ($\text{M}+\text{Na})^+$, and m/z 1163 ($\text{M}+2\text{Na}-\text{H})^+$ and by high-resolu-

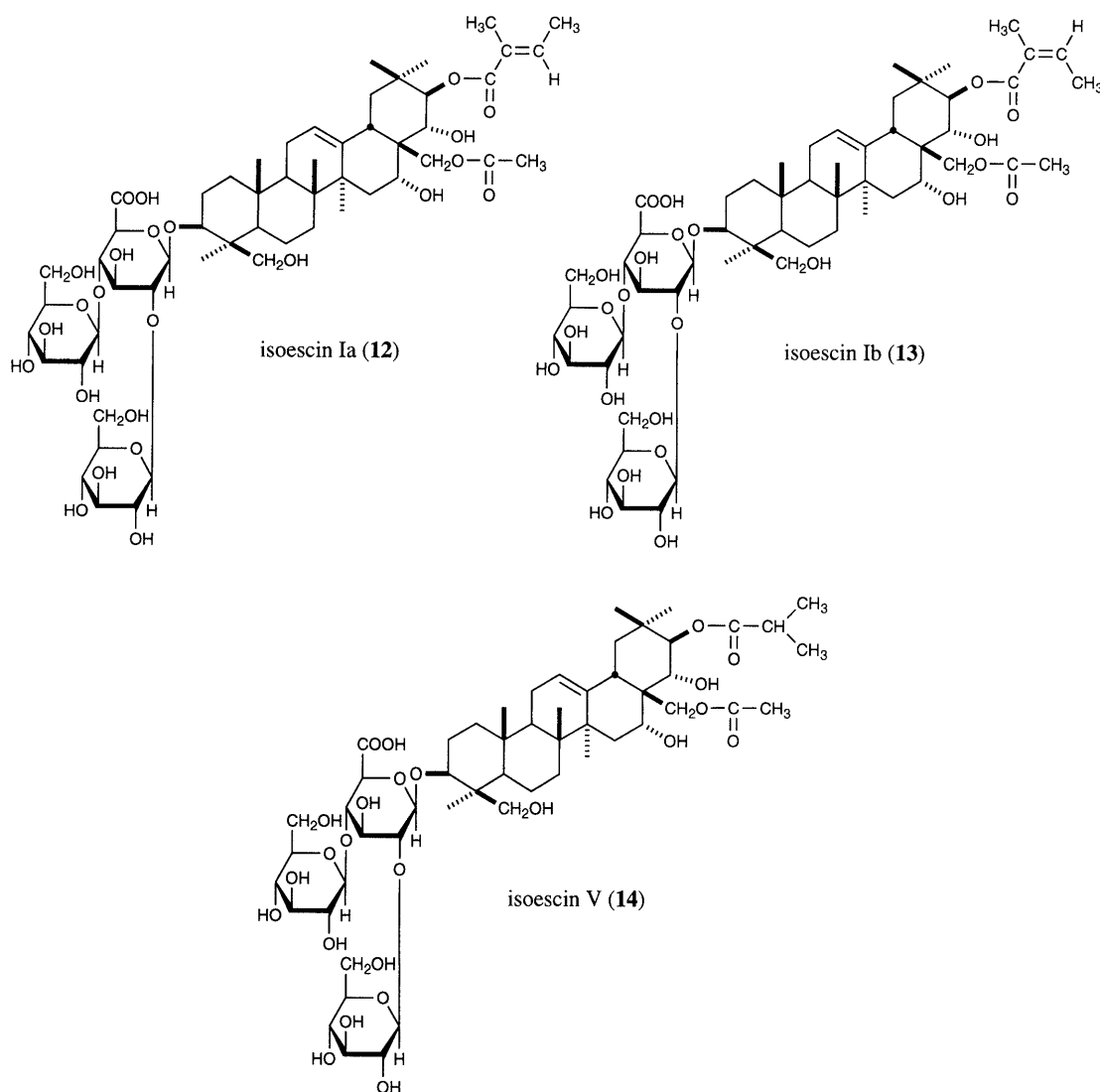


Chart 3

tion MS measurement. Alkaline hydrolysis of **10** and **14** liberated **5**⁴⁾ and two organic acids (acetic acid and isobutyric acid).

The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Table 1) spectra⁸⁾ of **10** showed the presence of the 21,22-di-*O*-acylated structure of **5** [δ 3.30, 4.25 (both m, 24-H₂), 3.33, 3.71 (both m, 28-H₂), 3.41 (dd-like, 3-H), 4.42 (m, 16-H), 4.91 (d, J =7.6 Hz, 1'-H), 5.18 (d, J =7.9 Hz, 1''-H), 5.57 (d, J =7.3 Hz, 1'''-H), 6.14 (d, J =10.9 Hz, 22-H), 6.46 (d, J =10.9 Hz, 21-H)] together with an acetyl group [δ 1.95 (s, 2'''-H₃)] and an isobutyryl group [δ 1.24 (s, 3'''', 4'''-H₃)]. The positions of the two acyl groups were characterized by an HMBC experiment on **10**, which showed long-range correlations between the 21-proton and the carbonyl carbon of the isobutyryl group and between the 22-proton and the carbonyl carbon of the acetyl group. On the other hand, the ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Table 1) spectra⁸⁾ of **14** showed the presence of an acetyl group [δ 1.98 (s, 2'''-H₃)], an isobutyryl group [δ 1.20, 1.25 (both d, J =7.3 Hz, 3'''', 4'''-H₃)], and the 21,28-di-*O*-acylated structure of **5** [δ 3.33, 4.31 (both m, 24-H₂), 3.43 (dd-like, 3-H), 4.24 (m, 28-H₂), 4.45 (d-like, 22-H), 4.67 (br s, 16-H), 4.90 (d, J =7.6 Hz, 1'-H), 5.18 (d, J =7.6 Hz, 1''-H), 5.57 (d, J =7.6 Hz, 1'''-H), 6.32 (d, J =9.9 Hz,

21-H)]. In an HMBC experiment on **14**, long-range correlations were observed between the 21-proton and the carbonyl carbon of isobutyryl group and between the 28-protons and the carbonyl carbon of the acetyl group. Furthermore, acylation shifts in the ¹³C-NMR spectra of **10** and **14** were observed around the 21 and the 22-position in **10** or around the 21 and the 28-positions in **14**. Finally, acid treatment of **10** with *p*-toluenesulfonic acid in acetonitrile–water (1:1) at 60 °C yielded **14** to give a mixture of **10** and **14** (*ca.* 1:1).⁹⁾ Consequently, the structures of escin V and isoescin V were determined as 21-*O*-isobutyryl-22-*O*-acetylprotoaescigenin 3-*O*-[β -D-glucopyranosyl (1 $\rightarrow$ 2)] [β -D-glucopyranosyl(1 $\rightarrow$ 4)]- β -D-glucopyranosiduronic acid (**10**) and 21-*O*-isobutyryl-28-*O*-acetylprotoaescigenin 3-*O*-[β -D-glucopyranosyl(1 $\rightarrow$ 2)] [β -D-glucopyranosyl(1 $\rightarrow$ 4)]- β -D-glucopyranosiduronic acid (**14**), respectively.

Escin VI (**11**), obtained as colorless fine crystals of mp 220.5–222.3 °C, showed absorption bands due to hydroxyl, ester, and carboxyl functions in its IR spectrum. The molecular formula C₅₅H₈₈O₂₄ was determined from the quasimolecular ion peaks [m/z 1131 (M–H)[–], m/z 1155 (M+Na)⁺, m/z 1177 (M+2Na–H)⁺] in the negative- and positive-ion FAB-MS and by high-resolution MS measurement. Alkaline hy-

Table 1. ^{13}C -NMR Data for Escins IIIb (8), IV (9), V (10), and VI (11) and Isoescins Ia (12), Ib (13), and V (14)

	8	9	10	11	12	13	14
C-1	38.9	38.6	38.6	38.6	38.6	38.6	38.6
C-2	26.6	26.6	26.6	26.5	26.6	26.5	26.6
C-3	89.3	91.2	91.2	91.1	91.2	91.2	91.2
C-4	39.6	43.8	43.8	43.8	43.8	43.7	43.8
C-5	55.8	56.2	56.2	56.2	56.2	56.2	56.3
C-6	18.5	18.6	18.6	18.6	18.6	18.5	18.6
C-7	33.2	33.3	33.3	33.3	33.3	33.2	33.3
C-8	40.1	40.0	40.0	40.0	40.0	39.9	40.0
C-9	47.0	46.8	46.8	46.8	46.8	46.8	46.8
C-10	36.8	36.4	36.4	36.4	36.4	36.4	36.5
C-11	23.9	24.1	24.1	24.0	24.1	24.0	24.2
C-12	122.7	122.8	122.7	122.7	122.7	122.8	122.8
C-13	142.9	142.9	142.9	142.9	142.8	142.7	143.0
C-14	41.8	41.7	41.7	41.7	41.9	41.8	41.9
C-15	34.7	34.7	34.6	34.6	34.6	34.6	34.6
C-16	68.1	68.1	68.1	68.0	67.7	67.6	67.7
C-17	48.1	48.0	48.1	48.1	47.2	47.1	47.1
C-18	40.1	40.2	40.2	40.2	40.6	40.5	40.6
C-19	47.3	47.3	47.3	47.2	47.4	47.3	47.4
C-20	36.3	36.2	36.4	36.3	36.3	36.0	36.2
C-21	79.0	79.5	78.9	79.9	81.7	81.5	81.2
C-22	74.6	74.5	74.3	74.4	71.5	71.4	71.3
C-23	28.1	22.5	22.5	22.5	22.6	22.5	22.6
C-24	16.8	63.3	63.4	63.3	63.3	63.2	63.3
C-25	15.9	15.6	15.6	15.6	15.6	15.6	15.6
C-26	17.0	16.8	16.8	16.7	16.9	16.9	16.9
C-27	27.5	27.4	27.4	27.4	27.4	27.3	27.4
C-28	64.0	64.0	64.0	64.0	66.5	66.5	66.5
C-29	29.5	29.4	29.5	29.6	29.8	29.7	29.8
C-30	20.3	20.0	20.1	20.1	20.1	20.1	20.0
3-O- β -D-glucopyranosiduronic acid moiety							
C-1'	105.1	104.6	104.7	104.6	104.6	104.5	104.6
C-2'	82.3	79.9	79.9	79.9	79.9	79.9	79.9
C-3'	75.9	76.5	76.4	76.5	76.5	76.4	76.5
C-4'	81.7	81.6	81.6	81.6	81.5	81.3	81.6
C-5'	75.4	75.7	75.7	75.7	75.7	75.6	75.8
C-6'	172.0	171.8	171.8	171.8	171.8	171.7	171.8
2'-O- β -D-galactopyranosyl or glucopyranosyl moiety							
C-1''	106.6	104.4	104.4	104.4	104.4	104.3	104.4
C-2''	74.6	75.7	75.7	75.7	75.7	75.6	75.8
C-3''	74.8	78.2	78.2	78.1	78.2	78.1	78.1
C-4''	69.6	69.9	69.8	69.8	69.8	69.8	69.8
C-5''	76.9	78.4	78.5	78.4	78.5	78.3	78.5
C-6''	61.5	61.6	61.6	61.6	61.6	61.5	61.6
4'-O- β -D-glucopyranosyl moiety							
C-1'''	104.6	104.6	104.7	104.6	104.6	104.5	104.6
C-2'''	74.8	74.9	74.8	74.8	74.8	74.7	74.9
C-3'''	78.1	78.2	78.1	78.0	78.1	78.0	78.1
C-4'''	71.6	71.6	71.6	71.6	71.6	71.5	71.6
C-5'''	78.4	78.4	78.5	78.4	78.5	78.3	78.5
C-6'''	62.6	62.5	62.5	62.4	62.5	62.4	62.5
21-O-acyl moiety							
C-1''''	167.8	171.1	176.7	176.3	168.6	168.5	177.3
C-2''''	129.1	21.0	34.9	41.9	129.9	129.5	34.8
C-3''''	137.0		19.5	27.1	136.3	137.0	19.7
C-4''''	15.7		19.3	12.0	14.1	15.8	19.2
C-5''''	21.0			17.0	12.4	20.9	
22- or 28-O-acetyl moiety							
C-1'''''	171.0	170.9	171.0	171.0	170.6	170.5	170.6
C-2'''''	20.9	20.9	21.0	21.0	20.7	20.6	20.6

(pyridine- d_5 , 68 MHz)

drololysis of **11** liberated **5**⁴⁾ together with acetic acid and 2-methylbutyric acid. The ^1H -NMR (pyridine- d_5) and ^{13}C -NMR (Table 1) spectra⁸⁾ of **11** showed signals due to an acetyl group [δ 1.96 (s, 2''''-H₃)] and a 2-methylbutyryl

group [δ 0.95 (t, $J=7.0$ Hz, 4''''-H₃), 1.23 (d, $J=7.0$ Hz, 5''''-H₃), 1.79 (m, 3''''-H₂), 2.50 (m, 2''''-H)] together with the 21,22-di-O-acylated structure of **5** [δ 3.33, 4.27 (both m, 24-H₂), 3.38, 3.60 (both m, 28-H₂), 3.42 (dd-like, 3-H), 4.43 (m, 16-H), 4.91 (d, $J=7.3$ Hz, 1'-H), 5.18 (d, $J=7.6$ Hz, 1''-H), 5.57 (d, $J=7.6$ Hz, 1'''-H), 6.15 (d, $J=10.9$ Hz, 22-H), 6.48 (d, $J=10.9$ Hz, 21-H)]. An HMBC experiment on **11** showed long-range correlations between the 21-proton and the carbonyl carbon of the 2-methylbutyryl group and between the 22-proton and the carbonyl carbon of the acetyl group. Observation of acetylation shifts around the 21- and the 22-positions in the ^{13}C -NMR data of **11** allowed us to confirm the structure of escin VI as 21-O-2-methylbutyryl-22-O-acetylprotoaescigenin 3-O-[β -D-glucopyranosyl(1 $\rightarrow$ 2)] [β -D-glucopyranosyl(1 $\rightarrow$ 4)]- β -D-glucopyranosiduronic acid (**11**).

Isoescins Ia (**12**) and Ib (**13**) were also isolated as colorless fine crystals of mp 193.6–195.5 °C and 223.2–225.5 °C, respectively. Isoescins Ia (**12**) and Ib (**13**) were found to have the same molecular formula C₅₅H₈₆O₂₄, which was elucidated from the quasimolecular ion peaks [m/z 1129 (M-H)]⁻ in the negative-ion FAB-MS and by high-resolution MS measurement. Alkaline hydrolysis of **12** and **13** furnished **5**⁴⁾ and two organic acids. Namely, acetic acid and tiglic acid were obtained from **12**, while acetic acid and angelic acid were obtained from **13**.

The ^1H -NMR (pyridine- d_5) and ^{13}C -NMR (Table 1) spectra⁸⁾ of **12** and **13** showed signals due to two acyl groups and the 21,28-di-O-acylated structure of **5** [**12**: δ 4.25 (m, 28-H₂), 6.38 (d, $J=9.9$ Hz, 21-H); **13**: δ 4.27 (m, 28-H₂), 6.42 (d, $J=9.6$ Hz, 21-H)]. The carbon and proton signals in the ^1H - and ^{13}C -NMR data of **12** and **13** were very similar to those of escins Ia (**1**)⁴⁾ and Ib (**2**)⁴⁾ respectively, except for the signals due to the 28-acetoxyl group of **12** and **13**. Long-range correlations were observed between the 21-proton and the carbonyl carbon of tigloyl or angeloyl group and between the 28-proton and the carbonyl carbon of acetyl carbon in the HMBC experiment on **12** and **13**. In addition, acetylation shifts were observed around the 21- and the 28-positions in the ^{13}C -NMR data of **12** and **13**. Acid treatment of **1** and **2** provided **12** and **13**, respectively, to yield a mixture of **1** and **12** and a mixture of **2** and **13**.⁹⁾ Consequently, the structures of isoescins Ia and Ib were determined to be 21-O-tigloyl-28-O-acetylprotoaescigenin 3-O-[β -D-glucopyranosyl(1 $\rightarrow$ 2)] [β -D-glucopyranosyl(1 $\rightarrow$ 4)]- β -D-glucopyranosiduronic acid (**12**) and 21-O-angeloyl-28-O-acetylprotoaescigenin 3-O-[β -D-glucopyranosyl(1 $\rightarrow$ 2)] [β -D-glucopyranosyl(1 $\rightarrow$ 4)]- β -D-glucopyranosiduronic acid (**13**), respectively.

Experimental

The following instruments were used to obtain physical data: melting points, Yanagimoto micro-melting point apparatus MP-500D (values are uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter ($l=5$ cm); IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^1H -NMR spectra, JEOL EX-270 (270 MHz) and JNM LA-500 (500 MHz) spectrometer; ^{13}C -NMR spectra, JEOL EX-270 (68 MHz) and JNM LA-500 (125 MHz) spectrometer with tetramethylsilane as an internal standard.

The following experimental conditions were used for chromatography: ordinary-phase silica-gel column chromatography, Silica-gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reversed-phase silica-gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, pre-coated TLC plates with Silica-gel 60F₂₅₄ (Merck, 0.25 mm) (ordinary phase) and Silica-gel RP-18 60F₂₅₄ (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, pre-coated TLC plates

with Silica-gel RP-18 60WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with 1% Ce(SO₄)₂–10% aqueous H₂SO₄ and heating.

Isolation of Escins IIb (1), IV (2), V (3), and VI (4) and Isoescins Ia (5), Ib (6), and V (7) from the Seeds of Horse Chestnut An earlier saponin fraction (4.5 g, 1.69%), obtained from the seeds of horse chestnut tree (10 kg, powder, Maruzen Pharmaceutical Co., Ltd., Hiroshima) as reported previously,^{4b)} was subjected to repeated HPLC [YMC-Pack R&D ODS-5 (20×250 mm, i.d.), 1) MeOH–1% aqueous AcOH (7:3, v/v); 2) CH₃CN–1% aqueous AcOH (2:3, v/v)] separation to give escins IIb (6, 24 mg, 0.007%), IV (7, 96 mg, 0.027%), V (8, 44 mg, 0.012%), and VI (9, 38 mg, 0.011%) and isoescins Ia (10, 64 mg, 0.018%), Ib (11, 66 mg, 0.019%), and V (12, 16 mg, 0.004%).

Escin IIb (8): Colorless fine crystals from CHCl₃–MeOH, mp 194.1–196.5 °C, $[\alpha]_D^{25} -8.1^\circ$ ($c=0.1$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₅₅H₈₅O₂₃Na (M+Na)⁺: 1137.5458. Found: 1137.5481. Calcd for C₅₅H₈₅O₂₃Na₂ (M+2Na–H)⁺: 1159.5277. Found: 1159.5359. IR (KBr): 3425, 1736, 1721, 1655, 1649, 1636, 1076 cm^{–1}. ¹H-NMR (pyridine-*d*₅) δ : 0.85, 0.89, 1.29, 1.31, 1.83, 1.93, 2.02 (3H each, all s, 25, 26, 23, 30, 27, 2''', 5'''–H₃), 1.09 (6H, s, 24, 29–H₃), 2.10 (3H, d, $J=7.3$ Hz, 4'''–H₃), 3.05 (1H, m, 18–H), 3.38, 3.62 (1H each, both d, $J=10.2$ Hz, 28–H₂), 3.39 (1H, dd-like, 3–H), 4.62 (1H, br s, 16–H), 4.97 (1H, d, $J=6.3$ Hz, 1'–H), 5.15 (1H, d, $J=7.6$ Hz, 1''–H), 5.20 (1H, d, $J=7.6$ Hz, 1'''–H), 5.40 (1H, br s, 12–H), 5.97 (1H, dq-like, 3'''–H), 6.17 (1H, d, $J=10.2$ Hz, 22–H), 6.60 (1H, d, $J=10.2$ Hz, 21–H). ¹³C-NMR (pyridine-*d*₅) δ : given in Table 1. Negative-ion FAB-MS: m/z 1113 (M–H)[–]. Positive-ion FAB-MS: m/z 1137 (M+Na)⁺, 1159 (M+2Na–H)⁺.

Escin IV (9): Colorless fine crystals from CHCl₃–MeOH, mp 226.9–228.1 °C, $[\alpha]_D^{25} -15.8^\circ$ ($c=0.1$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₅₂H₈₂O₂₄Na (M+Na)⁺: 1113.5094. Found: 1113.5072. IR (KBr): 3421, 1737, 1719, 1655, 1638, 1074 cm^{–1}. ¹H-NMR (pyridine-*d*₅) δ : 0.68, 0.81, 1.06, 1.27, 1.34, 1.80, 1.98, 2.12 (3H each, all s, 25, 26, 29, 30, 23, 27, 2''', 2'''–H₃), 3.03 (1H, m, 18–H), 3.36, 3.60 (1H each, both m, 28–H₂), 3.36, 4.31 (1H each, both m, 24–H₂), 3.42 (1H, dd-like, 3–H), 4.43 (1H, m, 16–H), 4.91 (1H, d, $J=7.6$ Hz, 1'–H), 5.18 (1H, d, $J=7.6$ Hz, 1''–H), 5.38 (1H, br s, 12–H), 5.57 (1H, d, $J=7.6$ Hz, 1'''–H), 6.14 (1H, d, $J=9.9$ Hz, 22–H), 6.45 (1H, d, $J=9.9$ Hz, 21–H). ¹³C-NMR (pyridine-*d*₅) δ : given in Table 1. Negative-ion FAB-MS: m/z 1089 (M–H)[–]. Positive-ion FAB-MS: m/z 1113 (M+Na)⁺, 1135 (M+2Na–H)⁺.

Escin V (10): Colorless fine crystals from CHCl₃–MeOH, mp 215.8–217.1 °C, $[\alpha]_D^{25} -12.9^\circ$ ($c=0.2$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₅₄H₈₆O₂₄Na (M+Na)⁺: 1141.5407. Found: 1141.5410. IR (KBr): 3453, 1736, 1719, 1655, 1638, 1075 cm^{–1}. ¹H-NMR (pyridine-*d*₅) δ : 0.69, 0.81, 1.05, 1.27, 1.34, 1.80, 1.95 (3H each, all s, 25, 26, 29, 30, 23, 27, 2''', 2'''–H₃), 1.24 (6H, d, $J=7.3$ Hz, 3''', 4'''–H₃), 2.67 (1H, m, 2'''–H), 3.00 (1H, m, 18–H), 3.30, 4.25 (1H each, both m, 24–H₂), 3.33, 3.71 (1H each, both m, 28–H₂), 3.41 (1H, dd-like, 3–H), 4.42 (1H, m, 16–H), 4.91 (1H, d, $J=7.6$ Hz, 1'–H), 5.18 (1H, d, $J=7.9$ Hz, 1''–H), 5.39 (1H, br s, 12–H), 5.57 (1H, d, $J=7.3$ Hz, 1'''–H), 6.14 (1H, d, $J=10.9$ Hz, 22–H), 6.46 (1H, d, $J=10.9$ Hz, 21–H). ¹³C-NMR (pyridine-*d*₅) δ : given in Table 1. Negative-ion FAB-MS: m/z 1117 (M–H)[–]. Positive-ion FAB-MS: m/z 1141 (M+Na)⁺, 1163 (M+2Na–H)⁺.

Escin VI (11): Colorless fine crystals from CHCl₃–MeOH, mp 220.5–222.3 °C, $[\alpha]_D^{25} -19.8^\circ$ ($c=0.1$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₅₅H₈₈O₂₄Na (M+Na)⁺: 1155.5563. Found: 1155.5587. Calcd for C₅₅H₈₇O₂₄Na₂ (M+2Na–H)⁺: 1177.5383. Found: 1177.5482. IR (KBr): 3424, 1736, 1719, 1655, 1639, 1075 cm^{–1}. ¹H-NMR (pyridine-*d*₅) δ : 0.69, 0.82, 1.07, 1.28, 1.39, 1.80, 1.96 (3H each, all s, 25, 26, 29, 30, 23, 27, 2''', 2'''–H₃), 0.95 (3H, t, $J=7.0$ Hz, 4'''–H₃), 1.23 (3H, d, $J=7.0$ Hz, 5'''–H₃), 1.79 (2H, m, 3'''–H₂), 2.50 (1H, m, 2'''–H), 3.00 (1H, m, 18–H), 3.33, 4.27 (1H each, both m, 24–H₂), 3.38, 3.60 (1H each, both m, 28–H₂), 3.42 (1H, dd-like, 3–H), 4.43 (1H, m, 16–H), 4.91 (1H, d, $J=7.3$ Hz, 1'–H), 5.18 (1H, d, $J=7.6$ Hz, 1''–H), 5.39 (1H, br s, 12–H), 5.57 (1H, d, $J=7.6$ Hz, 1'''–H), 6.15 (1H, d, $J=10.9$ Hz, 22–H), 6.48 (1H, d, $J=10.9$ Hz, 21–H). ¹³C-NMR (pyridine-*d*₅) δ : given in Table 1. Negative-ion FAB-MS: m/z 1131 (M–H)[–]. Positive-ion FAB-MS: m/z 1155 (M+Na)⁺, 1177 (M+2Na–H)⁺.

Isoescin Ia (12): Colorless fine crystals from CHCl₃–MeOH, mp 193.6–195.5 °C, $[\alpha]_D^{25} -4.0^\circ$ ($c=0.1$, MeOH). High-resolution negative-ion FAB-MS: Calcd for C₅₅H₈₅O₂₄ (M–H)[–]: 1129.5431. Found: 1129.5386. IR (KBr): 3432, 1738, 1719, 1655, 1649, 1638, 1076 cm^{–1}. ¹H-NMR (pyridine-*d*₅) δ : 0.70, 0.94, 1.09, 1.30, 1.33, 1.79, 1.86, 2.02 (3H each, all s, 25, 26, 29, 30, 23, 27, 5''', 2'''–H₃), 1.61 (3H, d, $J=6.9$ Hz, 4'''–H₃), 2.82 (1H, dd-like, 18–H), 3.32, 4.30 (1H each, both m, 24–H₂), 3.42 (1H, dd-like, 3–H), 4.25 (2H, m, 28–H₂), 4.47 (1H, d-like, 22–H), 4.72 (1H, br s, 16–H), 4.89 (1H, d, $J=7.6$ Hz, 1'–H), 5.18 (1H, d, $J=7.9$ Hz, 1''–H), 5.44 (1H, br s, 12–

H), 5.54 (1H, d, $J=7.6$ Hz, 1'–H), 6.38 (1H, d, $J=9.9$ Hz, 21–H), 7.00 (1H, dq-like, 3'''–H). ¹³C-NMR (pyridine-*d*₅) δ : given in Table 1. Negative-ion FAB-MS: m/z 1129 (M–H)[–].

Isoescin Ib (13): Colorless fine crystals from CHCl₃–MeOH, mp 223.2–225.5 °C, $[\alpha]_D^{25} -4.3^\circ$ ($c=0.1$, MeOH). High-resolution negative-ion FAB-MS: Calcd for C₅₅H₈₅O₂₄ (M–H)[–]: 1129.5431. Found: 1129.5324. IR (KBr): 3432, 1734, 1719, 1655, 1647, 1638, 1076 cm^{–1}. ¹H-NMR (pyridine-*d*₅) δ : 0.69, 0.94, 1.10, 1.29, 1.33, 1.79 (3H each, all s, 25, 26, 29, 30, 23, 27–H₃), 1.99 (6H, s, 5''', 2'''–H₃), 2.04 (3H, d, $J=7.5$ Hz, 4'''–H₃), 2.81 (1H, dd-like, 18–H), 3.33, 4.32 (1H each, both m, 24–H₂), 3.41 (1H, dd-like, 3–H), 4.27 (2H, m, 28–H₂), 4.48 (1H, d-like, 22–H), 4.71 (1H, br s, 16–H), 4.89 (1H, d, $J=7.6$ Hz, 1'–H), 5.15 (1H, d, $J=7.3$ Hz, 1''–H), 5.44 (1H, br s, 12–H), 5.54 (1H, d, $J=7.6$ Hz, 1'''–H), 5.90 (1H, dq-like, 3'''–H), 6.42 (1H, d, $J=9.6$ Hz, 21–H). ¹³C-NMR (pyridine-*d*₅) δ : given in Table 1. Negative-ion FAB-MS: m/z 1129 (M–H)[–].

Isoescin V (14): Colorless fine crystals from CHCl₃–MeOH, mp 198.8–200.7 °C, $[\alpha]_D^{25} -5.3^\circ$ ($c=0.1$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₅₄H₈₆O₂₄Na (M+Na)⁺: 1141.5407. Found: 1141.5374. Calcd for C₅₄H₈₅O₂₄Na₂ (M+2Na–H)⁺: 1163.5226. Found: 1163.5306. IR (KBr): 3425, 1736, 1719, 1655, 1638, 1075 cm^{–1}. ¹H-NMR (pyridine-*d*₅) δ : 0.69, 0.94, 1.09, 1.28, 1.33, 1.79, 1.98 (3H each, all s, 25, 26, 29, 30, 23, 27, 2''', 2'''–H₃), 1.20, 1.25 (3H each, both d, $J=7.3$ Hz, 3''', 4'''–H₃), 2.64 (1H, m, 2'''–H), 2.80 (1H, dd-like, 18–H), 3.33, 4.31 (1H each, both m, 24–H₂), 3.43 (1H, dd-like, 3–H), 4.24 (2H, m, 28–H₂), 4.45 (1H, d-like, 22–H), 4.67 (1H, br s, 16–H), 4.90 (1H, d, $J=7.6$ Hz, 1'–H), 5.18 (1H, d, $J=7.6$ Hz, 1''–H), 5.43 (1H, br s, 12–H), 5.57 (1H, d, $J=7.6$ Hz, 1'''–H), 6.32 (1H, d, $J=9.9$ Hz, 21–H). ¹³C-NMR (pyridine-*d*₅) δ : given in Table 1. Negative-FAB-MS: m/z 1117 (M–H)[–]. Positive-FAB-MS: m/z 1141 (M+Na)⁺, 1163 (M+2Na–H)⁺.

Alkaline Hydrolysis of Escins IIb (8), IV (9), V (10), and VI (11) and Isoescins Ia (12), Ib (13), and V (14)

A solution of escins (8, 9, 10, 11, 12, 13, or 14, 20 mg each) in 10% aqueous KOH–50% aqueous dioxane (1:1, v/v, 2 ml) was stirred at 37 °C for 1 h. The reaction mixture was neutralized with Dowex HCR W×2 (H⁺ form) and the resin was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by silica gel column chromatography [2 g, CHCl₃–MeOH–H₂O (65:35:10, lower layer)] to give desacylescins III (7, 14.0 mg from 8) or desacylescins I (5, 14.1 mg from 9, 13.9 mg from 10, 13.5 mg from 11, 14.3 mg from 12, 14.5 mg from 13, and 13.8 mg from 14) and an organic acid fraction. A solution of the organic acid fraction (1 mg each) in dichloroethane (2 ml) was treated with *p*-nitrobenzyl-*N,N'*-diisopropylurea (10 mg) and the reaction mixture was subjected to HPLC analysis [column: TSK-gel ODS-Prep (250×4.6 mm i.d.), solvent: MeOH–H₂O (70:30, v/v), flow rate: 1 ml/min] to identify *p*-nitrobenzylacetate [*t*_R (8.0 min) from 8–14], *p*-nitrobenzylangelate [*t*_R (18.0 min) from 8 and 13], *p*-nitrobenzyltiglate [*t*_R (17.0 min) from 12], *p*-nitrobenzylisobutyrate [*t*_R (13.2 min) from 10 and 14], and *p*-nitrobenzyl-2-methylbutyrate [*t*_R (19.5 min) from 11].

Acid Treatment of Escins Ia (1), Ib (2), and V (8) A solution of escins (1, 2, 3, each 50 mg) in CH₃CN–H₂O (1:1, v/v, 1 ml) was treated with *p*-TsOH·H₂O (50 mg) and the reaction mixture was stirred at 60 °C for 3 h. The reaction mixture was neutralized with saturated aqueous NaHCO₃. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by HPLC [MeOH–1% aqueous trifluoroacetic acid (TFA) (75:25, v/v)] to give isoescins [12 (21.5 mg), 13 (25.0 mg), 14 (23.9 mg)] and recovered starting escins [1 (26.1 mg), 2 (24.9 mg), 10 (24.8 mg)]. Isoescins, thus obtained, were identical with authentic samples by HPLC, $[\alpha]_D$, and ¹H- and ¹³C-NMR spectra comparisons.

Acknowledgments The authors are grateful to the Ministry of Education, Science, Sports and Culture of Japan for a Grant-in-Aid for Scientific Research (C) (Grant No. 09672177) and for Encouragement of Young Scientists (Grant No. 09771932).

References and Notes

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- 8) The ^1H - and ^{13}C -NMR data of **8**—**14** were assigned with the aid of homo- and hetero-correlation spectroscopy (^1H - ^1H , ^1H - ^{13}C COSY), homo- and hetero-nuclear Hartmann-Hahn spectroscopy (^1H - ^1H , ^1H - ^{13}C HOHAHA), distortionless enhancement polarization transfer (DEPT) and HMBC experiments.
- 9) Escins (**1**, **2**, **10**) were found to be converted to the corresponding isoescins (**12**, **13**, **14**) by weak acid treatment. Therefore these isoescins may be secondary products formed by acyl migration during the isolation product.