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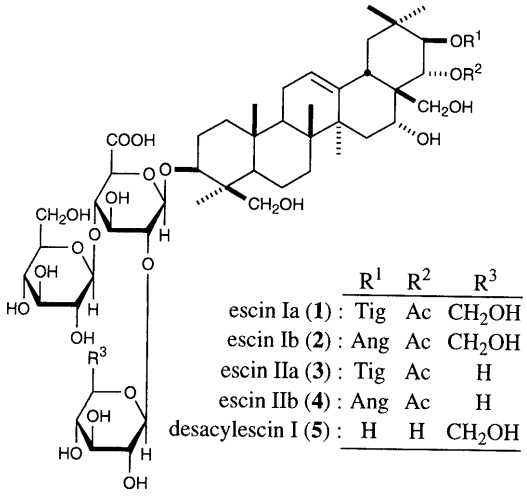
New acylated polyhydroxyoleanene triterpene oligoglycosides, escins IIIb, IV, V, and VI and isoescins Ia, Ib, and V, were isolated from the seeds of horse chestnut tree (*Aesculus hippocastanum* L.). Their structures were elucidated on the basis of chemical and physicochemical evidence.

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

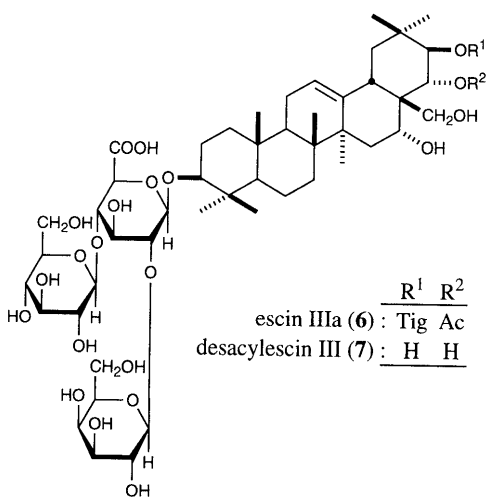
In the course of our characterization studies on bioactive saponin constituents in medicinal foodstuffs[2] and natural medicines,[1,3] the saponin mixture "escin" from the seeds of horse chestnut tree (*Aesculus hippocastanum* L., Hippocastanaceae) was found to show inhibitory effects on the increase 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 $CHCl_3$–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 $C_{55}H_{86}O_{23}$. Alkaline hydrolysis of **8** with 10% aqueous potassium hydroxide in 50% aqueous dioxane (1 : 1) liberated desacylescin 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 ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **8**, which were assigned with the aid of various NMR experiments,[8] showed signals ascribable to the desacylescin III moiety [δ 3.38, 3.62 (both d, J=10.2 Hz, 28-H_2), 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⁗-H_3), 2.10 (d, J=7.3 Hz, 4⁗-H_3), 5.97 (dq-like, 3⁗-H)]. The carbon and proton signals in the ^{1}H- and ^{13}C-NMR data



	R^1	R^2	R^3
escin Ia (**1**) :	Tig	Ac	CH_2OH
escin Ib (**2**) :	Ang	Ac	CH_2OH
escin IIa (**3**) :	Tig	Ac	H
escin IIb (**4**) :	Ang	Ac	H
desacylescin I (**5**) :	H	H	CH_2OH



	R^1	R^2
escin IIIa (**6**) :	Tig	Ac
desacylescin III (**7**) :	H	H

Chart 1

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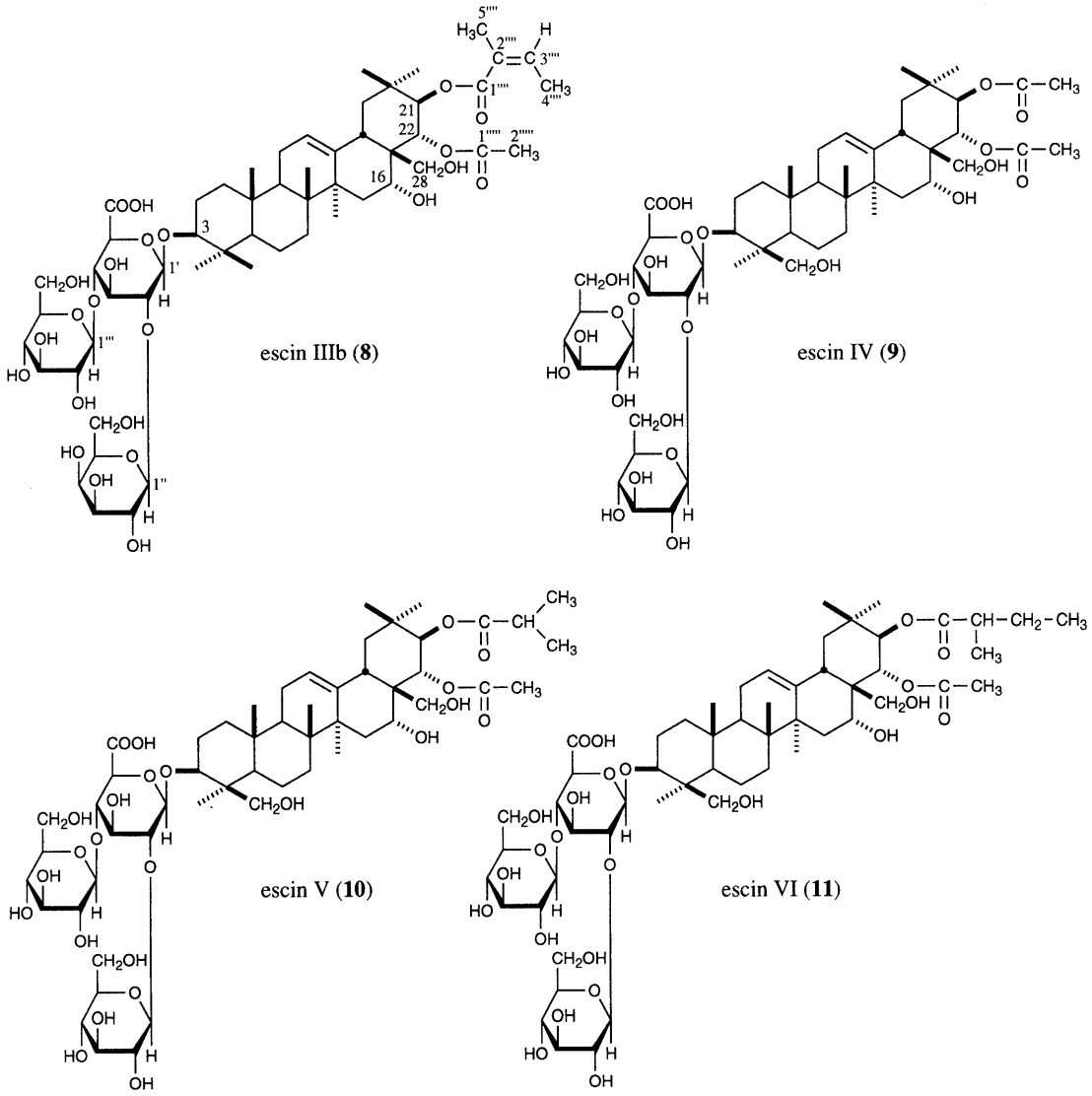

Chart 2

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 desacylescin 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*-acetylbarringtogenol C 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→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 $C_{52}H_{82}O_{24}$ was determined from its negative- and positive-ion FAB-MS [*m/z* 1089 $(M-H)^-$, *m/z* 1113 $(M+Na)^+$, and *m/z* 1135 $(M+2Na-H)^+$] and by high-resolution FAB-MS measurement. Alkaline hydrolysis of **9** liberated desacylescin I (**5**)[4] and acetic acid. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **9** showed the presence of two acetyl groups [δ 1.98, 2.12 (both s, 2$''''$, 2$''''$-H_3)] 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 (δc 171.1) of the 21-acetyl group and between the 22-proton and the carbonyl carbon (δc 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→2)][β-D-glucopyranosyl(1→4)]-β-D-glucopyranosiduronic acid (**9**).

Escin V (**10**) and isoescin 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 isoescin V (**14**) were found to have the same molecular formula $C_{54}H_{86}O_{24}$, which was obtained from the quasimolecular ion peak in their negative- and positive-ion FAB-MS at *m/z* 1117 $(M-H)^-$, *m/z* 1141 $(M+Na)^+$, and *m/z* 1163 $(M+2Na-H)^+$ and by high-resolu-

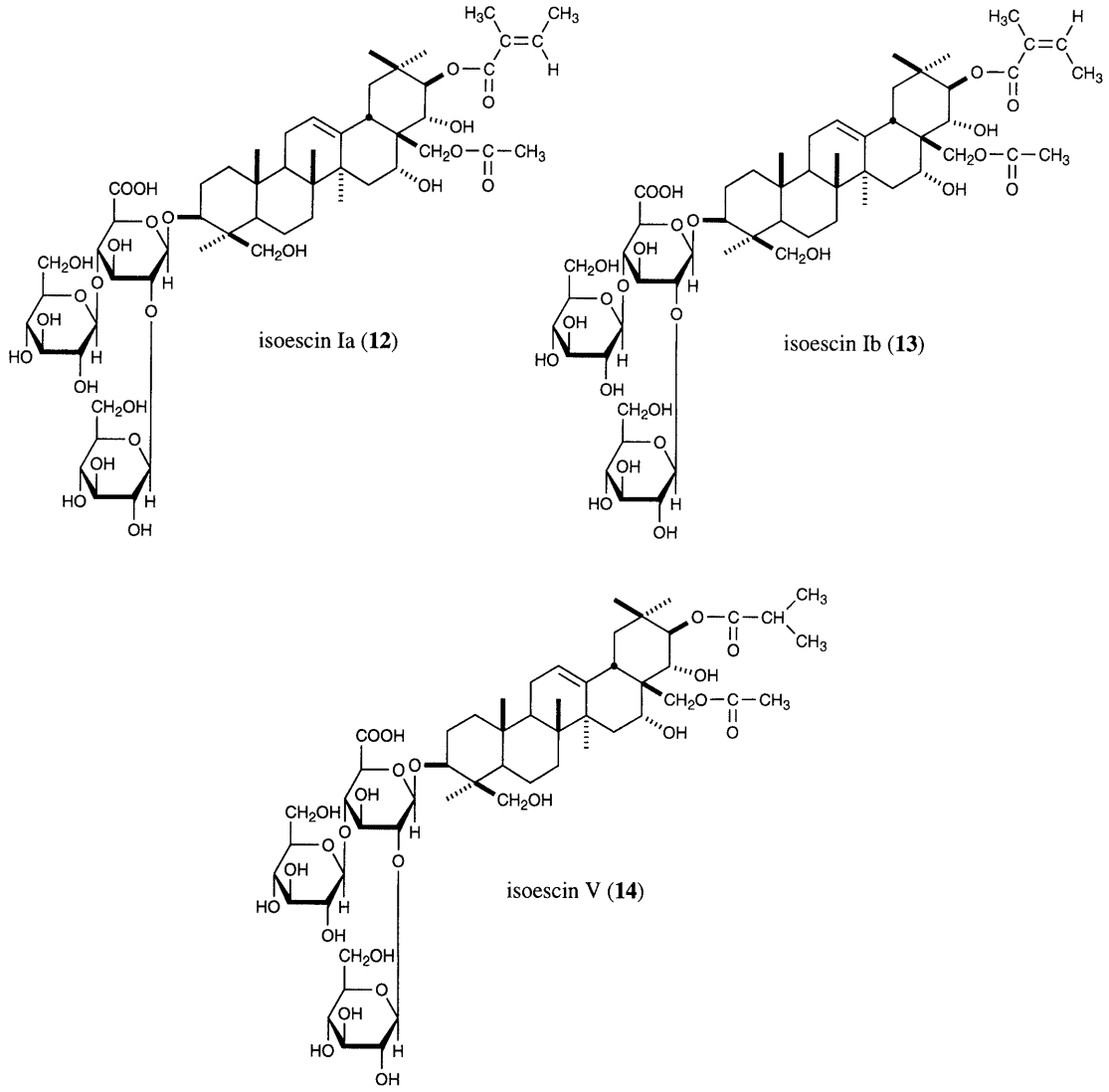

Chart 3

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

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **10** showed the presence of the 21,22-di-*O*-acylated structure of **5** [δ 3.30, 4.25 (both m, 24-H_2), 3.33, 3.71 (both m, 28-H_2), 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⁗-H_3)] and an isobutyryl group [δ 1.24 (s, 3⁗, 4⁗-H_3)]. 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 ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **14** showed the presence of an acetyl group [δ 1.98 (s, 2⁗-H_3)], an isobutyryl group [δ 1.20, 1.25 (both d, J=7.3 Hz, 3⁗, 4⁗-H_3)], and the 21,28-di-*O*-acylated structure of **5** [δ 3.33, 4.31 (both m, 24-H_2), 3.43 (dd-like, 3-H), 4.24 (m, 28-H_2), 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 ^{13}C-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*-toluenesulfolic 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→2)] [β-D-glucopyranosyl(1→4)]-β-D-glucopyranosiduronic acid (**10**) and 21-*O*-isobutyryl-28-*O*-acetylprotoaescigenin 3-*O*-[β-D-glucopyranosyl(1→2)] [β-D-glucopyranosyl(1→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 $C_{55}H_{88}O_{24}$ 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)

drolysis of **11** liberated **5**[4] together with acetic acid and 2-methylbutyric acid. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **11** showed signals due to an acetyl group [δ 1.96 (s, 2⁗-H$_3$)] and a 2-methylbutyryl group [δ 0.95 (t, J=7.0 Hz, 4⁗-H$_3$), 1.23 (d, J=7.0 Hz, 5⁗-H$_3$), 1.79 (m, 3⁗-H$_2$), 2.50 (m, 2⁗-H)] together with the 21,22-di-*O*-acylated structure of **5** [δ 3.33, 4.27 (both m, 24-H$_2$), 3.38, 3.60 (both m, 28-H$_2$), 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→2)] [β-D-glucopyranosyl(1→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 $C_{55}H_{86}O_{24}$, 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 ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] 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-H$_2$), 6.38 (d, J=9.9 Hz, 21-H); **13**: δ 4.27 (m, 28-H$_2$), 6.42 (d, J=9.6 Hz, 21-H)]. The carbon and proton signals in the ^{1}H- 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→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid (**12**) and 21-*O*-angeloyl-28-*O*-acetylprotoaescigenin 3-*O*-[β-D-glucopyranosyl(1→2)] [β-D-glucopyranosyl(1→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; ^{1}H-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 60F$_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica-gel RP-18 60F$_{254}$ (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(SO$_4$)$_2$–10% aqueous H_2SO_4 and heating.

Isolation of Escins IIIb (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) CH_3CN–1% aqueous AcOH (2 : 3, v/v)] separation to give escins IIIb (**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 IIIb (**8**): Colorless fine crystals from $CHCl_3$–MeOH, mp 194.1—196.5 °C, $[\alpha]_D^{26}$ −8.1° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{55}H_{86}O_{23}Na$ $(M+Na)^+$: 1137.5458. Found: 1137.5481. Calcd for $C_{55}H_{85}O_{23}Na_2$ $(M+2Na-H)^+$: 1159.5277. Found 1159.5359. IR (KBr): 3425, 1736, 1721, 1655, 1649, 1636, 1076 cm^{-1}. ^{1}H-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″″-H$_3$), 1.09 (6H, s, 24, 29-H$_3$), 2.10 (3H, d, J=7.3 Hz, 4″″-H$_3$), 3.05 (1H, m, 18-H), 3.38, 3.62 (1H each, both d, J=10.2 Hz, 28-H$_2$), 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). ^{13}C-NMR (pyridine-d_5) δc: 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 $CHCl_3$–MeOH, mp 226.9—228.1 °C, $[\alpha]_D^{26}$ −15.8° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{82}O_{24}Na$ $(M+Na)^+$: 1113.5094. Found: 1113.5072. IR (KBr): 3421, 1737, 1719, 1655, 1638, 1074 cm^{-1}. ^{1}H-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″″-H$_3$), 3.03 (1H, m, 18-H), 3.36, 3.60 (1H each, both m, 28-H$_2$), 3.36, 4.31 (1H each, both m, 24-H$_2$), 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). ^{13}C-NMR (pyridine-d_5) δc: 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 $CHCl_3$–MeOH, mp 215.8—217.1 °C, $[\alpha]_D^{24}$ −12.9° (c=0.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{54}H_{86}O_{24}Na$ $(M+Na)^+$: 1141.5407. Found: 1141.5410. IR (KBr): 3453, 1736, 1719, 1655, 1638, 1075 cm^{-1}. ^{1}H-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″″-H$_3$), 1.24 (6H, d, J=7.3 Hz, 3″″, 4″″-H$_3$), 2.67 (1H, m, 2″″-H), 3.00 (1H, m, 18-H), 3.30, 4.25 (1H each, both m, 24-H$_2$), 3.33, 3.71 (1H each, both m, 28-H$_2$), 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). ^{13}C-NMR (pyridine-d_5) δc: 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 $CHCl_3$–MeOH, mp 220.5—222.3 °C, $[\alpha]_D^{26}$ −19.8° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{55}H_{88}O_{24}Na$ $(M+Na)^+$: 1155.5563. Found: 1155.5587. Calcd for $C_{55}H_{87}O_{24}Na_2$ $(M+2Na-H)^+$: 1177.5383. Found: 1177.5482. IR (KBr): 3424, 1736, 1719, 1655, 1639, 1075 cm^{-1}. ^{1}H-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″″-H$_3$), 0.95 (3H, t, J=7.0 Hz, 4″″-H$_3$), 1.23 (3H, d, J=7.0 Hz, 5″″-H$_3$), 1.79 (2H, m, 3″″-H$_2$), 2.50 (1H, m, 2″″-H), 3.00 (1H, m, 18-H), 3.33, 4.27 (1H each, both m, 24-H$_2$), 3.38, 3.60 (1H each, both m, 28-H$_2$), 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). ^{13}C-NMR (pyridine-d_5) δc: 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 $CHCl_3$–MeOH, mp 193.6—195.5 °C, $[\alpha]_D^{25}$ −4.0° (c=0.1, MeOH). High-resolution negative-ion FAB-MS: Calcd for $C_{55}H_{85}O_{24}$ $(M-H)^-$: 1129.5431. Found: 1129.5386. IR (KBr): 3432, 1738, 1719, 1655, 1649, 1638, 1076 cm^{-1}. ^{1}H-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″″-H$_3$), 1.61 (3H, d, J=6.9 Hz, 4″″-H$_3$), 2.82 (1H, dd-like, 18-H), 3.32, 4.30 (1H each, both m, 24-H$_2$), 3.42 (1H, dd-like, 3-H), 4.25 (2H, m, 28-H$_2$), 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). ^{13}C-NMR (pyridine-d_5) δc: given in Table 1. Negative-ion FAB-MS: m/z 1129 $(M-H)^-$.

Isoescin Ib (**13**): Colorless fine crystals from $CHCl_3$–MeOH, mp 223.2—225.5 °C, $[\alpha]_D^{25}$ −4.3° (c=0.1, MeOH). High-resolution negative-ion FAB-MS: Calcd for $C_{55}H_{85}O_{24}$ $(M-H)^-$: 1129.5431. Found: 1129.5324. IR (KBr): 3432, 1734, 1719, 1655, 1647, 1638, 1076 cm^{-1}. ^{1}H-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-H$_3$), 1.99 (6H, s, 5″″, 2″″″-H$_3$), 2.04 (3H, d, J=7.5 Hz, 4″″-H$_3$), 2.81 (1H, dd-like, 18-H), 3.33, 4.32 (1H each, both m, 24-H$_2$), 3.41 (1H, dd-like, 3-H), 4.27 (2H, m, 28-H$_2$), 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). ^{13}C-NMR (pyridine-d_5) δc: given in Table 1. Negative-ion FAB-MS: m/z 1129 $(M-H)^-$.

Isoescin V (**14**): Colorless fine crystals from $CHCl_3$–MeOH, mp 198.8—200.7 °C, $[\alpha]_D^{26}$ −5.3° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{54}H_{86}O_{24}Na$ $(M+Na)^+$: 1141.5407. Found: 1141.5374. Calcd for $C_{54}H_{85}O_{24}Na_2$ $(M+2Na-H)^+$: 1163.5226. Found: 1163.5306. IR (KBr): 3425, 1736, 1719, 1655, 1638, 1075 cm^{-1}. ^{1}H-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″″″-H$_3$), 1.20, 1.25 (3H each, both d, J=7.3 Hz, 3″″, 4″″-H$_3$), 2.64 (1H, m, 2″″-H), 2.80 (1H, dd-like, 18-H), 3.33, 4.31 (1H each, both m, 24-H$_2$), 3.43 (1H, dd-like, 3-H), 4.24 (2H, m, 28-H$_2$), 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). ^{13}C-NMR (pyridine-d_5) δc: 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 IIIb (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, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer)] to give desacylescin III (**7**, 14.0 mg from **8**) or desacylescin 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*′-diisopropylisourea (10 mg) and the reaction mixture was subjected to HPLC analysis [column: TSK-gel ODS-Prep (250×4.6 mm i.d.), solvent: MeOH–H_2O (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 CH_3CN–H_2O (1 : 1, v/v, 1 ml) was treated with *p*-TsOH·H_2O (50 mg) and the reaction mixture was stirred at 60 °C for 3 h. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$. 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 ^{1}H- and ^{13}C-NMR spectra comparisons.

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References and Notes

1) Part XI: Yoshikawa M., Murakami T., Yashiro K., Yamahara J., Matsuda H., Saijoh R., Tanaka O., *Chem. Pharm. Bull.*, **46**, 647—654 (1998).
2) *a*) Yoshikawa M., Murakami T., Komatsu H., Yamahara J., Matsuda H., *Heterocycles*, **47**, 397—405 (1998); *b*) Yoshikawa M., Murakami T., Komatsu H., Matsuda H., *Chem. Pharm. Bull.*, **46**, 812—816 (1998); *c*) Yoshikawa M., Murakami T., Shimada H., Fukada N., Matsuda H., Sashida Y., Yamahara J., *Heterocycles*, **48**, 869—873 (1998).
3) *a*) Matsuda H., Murakami T., Ninomiya K., Inadzuki M., Yoshikawa

M., *Bioorg. Med. Chem. Lett.*, **7**, 2193—2198 (1997); *b*) Yoshikawa M., Murakami T., Hirano K., Inadzuki M., Ninomiya K., Matsuda H., *Tetrahedron Lett.*, **38**, 7395—7398 (1997); *c*) Matsuda H., Li Y., Murakami T., Matsumura N., Yamahara J., Yoshikawa M., *Chem. Pharm. Bull.*, **46**, 1339—1403 (1998).

4) Yoshikawa M., Murakami T., Matsuda H., Yamahara J., Murakami N., Kitagawa I., *Chem. Pharm. Bull.*, **44**, 1454—1464 (1996).

5) Matsuda H., Murakami T., Li Y., Yamahara J., Yoshikawa M., *Bioorg. Med. Chem.*, **6**, 1019—1023 (1998).

6) *a*) Matsuda H., Li Y., Murakami T., Ninomiya K., Araki N., Yoshikawa M., Yamahara J., *Bioorg. Med. Chem. Lett.*, **7**, 1611—1616 (1997); *b*) Matsuda H., Li Y., Murakami T., Ninomiya K., Yamahara J., Yoshikawa M., *Biol. Pharm. Bull.*, **20**, 1092—1095 (1997).

7) Yoshikawa K., Nakagawa M., Yamamoto R., Arihara S., Matsuura K., *Chem. Pharm. Bull.*, **40**, 1779—1782 (1992).

8) The ^{1}H- and ^{13}C-NMR data of **8**—**14** were assigned with the aid of homo- and hetero-correlation spectroscopy (^{1}H-^{1}H, ^{1}H-^{13}C COSY), homo- and hetero-nuclear Hartmann-Hahn spectroscopy (^{1}H-^{1}H, ^{1}H-^{13}C HOHAHA), distortionlesss 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.