Bioactive Saponins and Glycosides. III.[1] Horse Chestnut. (1): The Structures, Inhibitory Effects on Ethanol Absorption, and Hypoglycemic Activity of Escins Ia, Ib, IIa, IIb, and IIIa from the Seeds of *Aesculus hippocastanum* L.

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Received February 21, 1996; accepted April 8, 1996

Five bioactive triterpene oligoglycosides named escins Ia, Ib, IIa, IIb, and IIIa were isolated from the seeds of horse chestnut tree, *Aesculus hippocastanum* L. (Hippocastanaceae). The chemical structures of escins Ia, Ib, IIa, IIb, and IIIa were determined on the basis of chemical and physicochemical evidence, which included selective cleavage of the glucuronide linkage using photochemical reaction and lead tetraacetate decarboxylation reaction. Escins Ia, Ib, IIa, and IIb were found to exhibit an ethanol absorption-inhibitory effect and hypoglycemic activity in the oral glucose tolerance test in rats. Some structure–activity relationships are reported.

Key words escin Ia; escin IIa; horse chestnut; *Aesculus hippocastanum*; ethanol absorption inhibitor; hypoglycemic activity

Horse chestnut tree (*Aesculus hippocastanum* L., seiyou-tochinoki in Japanese) belongs to the small family Hippocastanaceae, and the fruit of this plant is a large globular capsule, which contains one to three seeds resembling those of the sweet chestnut, but having a bitter taste. The saponin mixture "escin" obtained from the seeds of the horse chestnut tree is widely employed in therapy of peripheral vascular disorders and also in cosmetics for prevention and treatment of cellulitis, because "escin" have been found to show antiinflammatory, antiedematous, and capillaro-protective activities.[2] The isolation and structure determination of saponin constituents in "escin" have been the target of many investigations and the structures of two major saponins were hitherto presumed on the basis of chemical and physicochemical evidence obtained by using the saponin mixture and sapogenol mixture.[3] Recently, the isolation of two major saponins from commercial β-escin was reported, but their MS data were merely analyzed on the basis of the previous presumed structures.[4]

In the course of our studies on new biologically active constituents of natural medicines,[5] we have isolated several saponins from *Aralia elata* (Japanese angelica tree, root cortex,[6] bark,[7] and young shoot[8]), *Camellia japonica* (camellia, seed),[9] *Polygala senega* var. *latifolia* (Japanese senega snakeroot, root),[1,10] *Beta vulgaris* (sugar beet, root),[11] and *Gymnema sylvestre* (leaf)[12] and reported their inhibitory effects on ethanol absorption and hypoglycemic activities. As a continuation of our studies to characterize saponin constituents with inhibitory activity on ethanol absorption and hypoglycemic activity, we have isolated five acylated polyhydroxyolean-12-ene 3-*O*-glucuronides named escins Ia (**1**), Ib (**2**), IIa (**10**), IIb (**11**), and IIIa (**13**) from the seed of horse chestnut tree.[13] In this paper, we present a full account of the structure elucidations of escins Ia (**1**), Ib (**2**), IIa (**10**), IIb (**11**), and IIIa (**13**), including the application of selective cleavage methods of the glucuronide linkage.[14] In addition, we describe the inhibitory effect on ethanol absorption and the hypoglycemic activity of escins Ia (**1**), Ib (**2**), IIa (**10**), and IIb (**11**) and the structure–activity relationships of acylated polyhydroxyolean-12-ene 3-*O*-glucuronide.[15]

The methanolic extract obtained from the seeds of horse chestnut tree was separated through the procedure shown in Chart 1. Thus, the methanolic extract was subjected to Diaion HP-20 column chromatography in order to remove the sugar and lipid components. The methanol-eluted fraction containing saponin constituents was separated by reversed-phase silica gel column chromatography to give the saponin fraction (the 90% aqueous methanol eluate). The saponin fraction was found to exhibit not only inhibitory activity on ethanol absorption but also hypoglycemic activity. The saponin fraction was further purified by repeated HPLC (YMC-Pack R&D ODS-5) separation to afford escins Ia (**1**), Ib (**2**), IIa (**10**), IIb (**11**), and IIIa (**13**).

Escins Ia (1) and Ib (2) Escin Ia (**1**) was isolated as colorless fine crystals of mp 224.9—226.7 °C. The IR spectrum of **1** showed absorption bands ascribable to carboxyl and α,β-unsaturated ester at 1731, 1719, 1653, and 1649 cm^{-1} and broad bands at 3453 and 1075 cm^{-1} suggestive of glycosidic structure. In the positive-mode FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 1153 $(M+Na)^+$ and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{55}H_{86}O_{24}$. Alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide–50% aqueous dioxane (1 : 1) liberated desacylescin I (**3**) together with acetic acid and tiglic acid. The organic acids were derived to the *p*-nitrobenzyl esters,[16] which were identified by HPLC analysis.

Acid hydrolysis of **3** with 10% aqueous sulfuric acid liberated protoaescigenin (**4**)[17] and escigenin (**5**),[17] while methanolysis of **3** with 9% hydrogen chloride in dry methanol furnished methyl D-glucoside and methyl D-glucuronide in a 2 : 1 ratio.[18] Since **3** was found to contain D-glucuronic acid in its oligoglycoside moiety, it was

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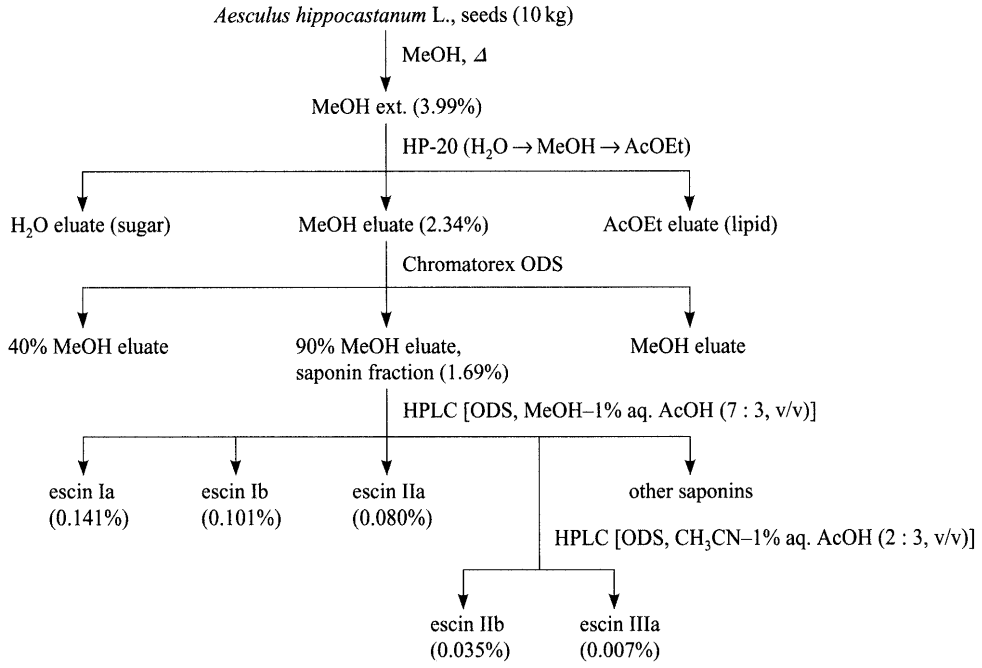


Chart 1

subjected to photochemical degradation, which is a selective cleavage method for the glucuronide linkage in oligoglycoside.[14,19] Irradiation of a methanolic solution of **3** with a 500 W high-pressure mercury lamp liberated **4**, so that **3** was shown to be a D-glucuronide of **4** having additional glucoside residues attached to the D-glucuronide moiety. Methylation of **3** with diazomethane in methanol yielded the methyl ester (**3a**), which was treated with sodium borohydride ($NaBH_4$) in methanol to give **3b**. Methanolysis of **3b** gave **4** and methyl D-glucoside, while complete methylation of **3b** by Hakomori's method furnished the pentadeca-*O*-methyl ether (**3c**). The ^{1}H-NMR (benzene-d_6) spectrum of **3c** showed signals assignable to three anomeric protons at δ 4.37, 4.40, and 4.96 (all d, J=7.0 Hz), suggestive of β-glycosidic linkages. Methanolysis of **3c** liberated 16,21,22,24,28-penta-*O*-methylprotoaescigenin (**4a**), methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, and methyl 3,6-di-*O*-methylglucopyranoside. Acetylation of **4a** with acetic anhydride in pyridine yielded the 3-*O*-acetate (**4b**) whose ^{1}H-NMR spectrum showed signals due to the 3-proton bearing an acetoxyl group [δ 4.46 (*t*-like, 3-H), 1.95 (s, 2''''-H)], together with five methoxyl groups. The MS and high-resolution MS of **4a** showed the molecular ion peak at m/z 576 of $C_{35}H_{60}O_6$ and fragment ion peaks, one at m/z 238 (**i**) of $C_{15}H_{26}O_2$ derived from the A/B ring and another at 338 (**iii**) of $C_{20}H_{34}O_4$ derived from the D/E ring, which were presumably formed through the characteristic retro-Diels-Alder type fragmentation at the C ring of the olean-12-en skeleton of **4a**, while **4b** showed the MS fragment ion peaks at m/z 280 (**ii**) and 338 (**iii**).

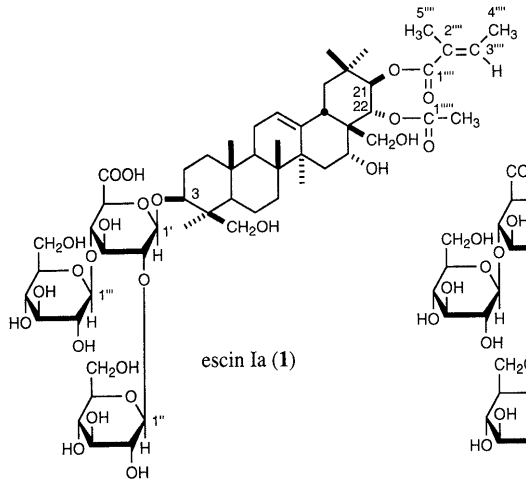
The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **3**, which were completely assigned by means of various NMR experiments,[20] showed signals due to three anomeric protons at δ 4.88 (1H, d, J=7.6 Hz, 3-*O*-Glc.A-1'), 5.54 (1H, d, J=7.6 Hz, 2'-*O*-Glc.-1''), and 5.16 (1H, d, J=7.6 Hz, 4'-*O*-Glc.-1'''). In the HMBC experiment on **3**, long-range correlations were observed between the anomeric proton (1'-H) of the glucuronic acid moiety and the 3-carbon of the protoaescigenin moiety and between the anomeric protons (1'', 1'''-H) of the D-glucose moieties and the 2' and 4'-carbons of the glucuronic acid moiety. The above-mentioned chemical and physicochemical evidence led us to clarify the structure of desacylescin I as **3**, which was found to be identical with desacylesculoside I obtained from the seed of Japanese horse chestnut tree (*Aesculus turbinata* BLUME).[21]

In order to obtain more chemical evidence regarding the oligosaccharide structure in **3**, the lead tetraacetate degradation method[14,22] was applied to **3**. Complete methylation of **3** furnished the pentadeca-*O*-methyl derivative (**3d**), which was treated with 5% aqueous potassium carbonate to give the tetradeca-*O*-methyl ester (**3e**). Decarboxylation of **3e** with lead tetraacetate [$Pb(OAc)_4$] in benzene afforded the 5'-acetoxyl mixture (**3f**). Alkaline treatment of **3f** with 0.2% sodium methoxide at room temperature furnished **4a** and the diacetal mixture (**6**),[23] which was subjected to sodium borohydride reduction to yield the glycitol oligoglycosides, **7**, **8**, and **9**. Methanolysis of **7**, **8**, and **9** liberated 3-*O*-methyl-L-arabitol (from **7**), 3-*O*-methyl-D-arabitol (from **8**) and 3-*O*-methyl-D-xylitol (from **9**), respectively, together with common methyl 2,3,4,6-tetra-*O*-methylglucopyranoside. Based on this evidence and examination of the spectral data, the structures of **7**, **8**, and **9** were characterized. On the other hand, treatment of **3f** with lithium aluminum hydride ($LiAlH_4$) was found to yield **4a** and **9**. These results indicated that **7** and **8** were produced secondarily during the alkaline treatment of **3f**, and $LiAlH_4$ reduction of the decarboxylation product was superior to the previous alkaline treatment[22] in lead tetraacetate degradation of glucuronide saponin.

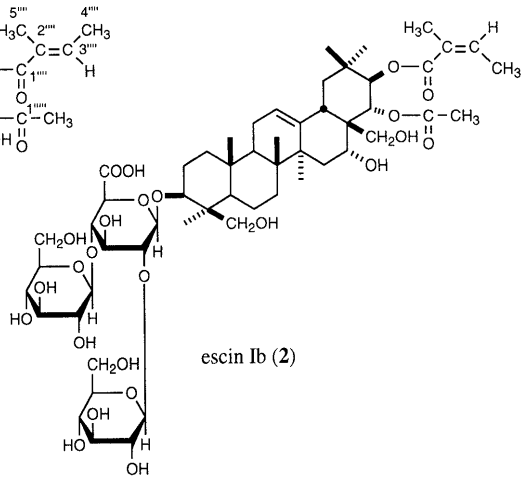
The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[20] of **1** showed signals assignable to tigloyl and

acetyl groups at δ 1.66 (3H, d, $J=6.9$ Hz, 4''''-H_3), 1.96 (3H, s, 5''''-H_3), 7.10 (1H, dq-like, 3''''-H), and 1.91 (3H, s, 2'''''-H_3), together with a desacylescin I moiety [δ 6.56 (1H, d, $J=10.2$ Hz, 21-H), 6.21 (1H, d, $J=10.2$ Hz, 22-H)]. The positions of two acyl groups in **1** were clarified by an HMBC experiment. Namely, long-range correlations were observed between the following protons and carbons: the 21-proton and the 1''''-carbonyl carbon of the tigloyl group, the 5''''-methyl protons and the 1''''-carbonyl carbon, the 22-proton and the 1'''''-carbonyl carbon of the acetyl group, and the acetyl methyl protons and the 1'''''-carbonyl carbon. Consequently, the structure of escin Ia was determined to be 21-tigloyl-22-acetylprotoaescigenin 3-*O*-[β-D-glucopyranosyl (1—2)][β-D-glucopyranosyl (1—4)]-β-D-glucopyranosiduronic acid (**1**).[24]

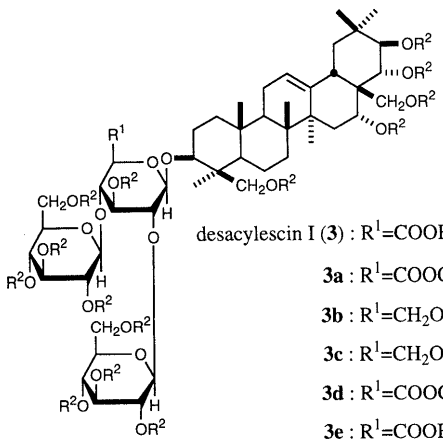
Escin Ib (**2**), also isolated as colorless fine crystals of mp 187.8—189.3 °C, liberated desacylescin I (**3**) and two organic acids, acetic acid and angelic acid, upon alkaline hydrolysis. The molecular formula $C_{55}H_{86}O_{24}$, which was the same as that of escin Ia (**1**), was determined from the positive-mode FAB-MS and by high-resolution MS measurement. Namely, in the positive-mode FAB-MS of **2**, a quasimolecular ion peak was observed at m/z 1153 $(M+Na)^+$. The carbon signals in the ^{13}C-NMR (Table 1) spectrum of **2** were shown to be superimposable on those of **1**, except for some signals assignable to an angeloyl group. The ^{1}H-NMR (pyridine-d_5) spectrum of **2** showed signals due to an angeloyl group [δ 2.02 (3H, s, 5''''-H_3), 2.11 (3H, d, $J=7.2$ Hz, 4''''-H_3), 5.95 (1H, dq-like, 3''''-H)] and an acetoxyl group [δ 1.91 (3H, s, 2'''''-H_3)]. Furthermore, correlation peaks were observed between the following protons and carbons: the 1'-proton [δ 4.91 (1H, d, $J=7.3$ Hz)] of the glucuronic acid moiety and the 3-carbon; the 1''-proton [δ 5.57 (1H, d, $J=7.3$ Hz)] of the 2'-*O*-D-glucosyl moiety and the 2'-carbon; the 1'''-proton [δ 5.18 (1H, d, $J=7.9$ Hz)] of the 4'-*O*-D-glucosyl moiety and the 4'-carbon; the 21-proton [δ 6.69 (1H, d, $J=10.2$ Hz)] of the protoaescigenin moiety and the 1''''-carbonyl carbon; the 5''''-methyl protons and the 2'''',1''''-carbons; the 22-proton [δ 6.17 (1H, d, $J=10.2$ Hz)] of the protoaescigenin moiety and the 1'''''-carbon (the acetyl carbonyl carbon); 2'''''-H_3 (the acetyl methyl protons) and



escin Ia (**1**)



escin Ib (**2**)



desacylescin I (**3**) : R^1=COOH, R^2=H

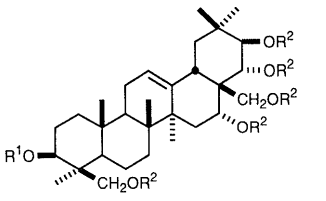
3a : R^1=$COOCH_3$, R^2=H

3b : R^1=CH_2OH, R^2=H

3c : R^1=CH_2OCH_3, R^2=CH_3

3d : R^1=$COOCH_3$, R^2=CH_3

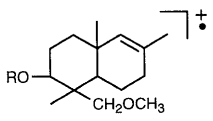
3e : R^1=COOH, R^2=CH_3



protoaescigenin (**4**) : R^1=R^2=H

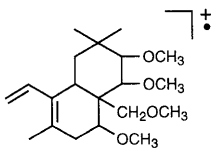
4a : R^1=H, R^2=CH_3

4b : R^1=Ac, R^2=CH_3

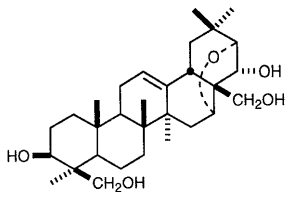


i : R=H

ii : R=Ac



iii



escigenin (**5**)

Chart 2

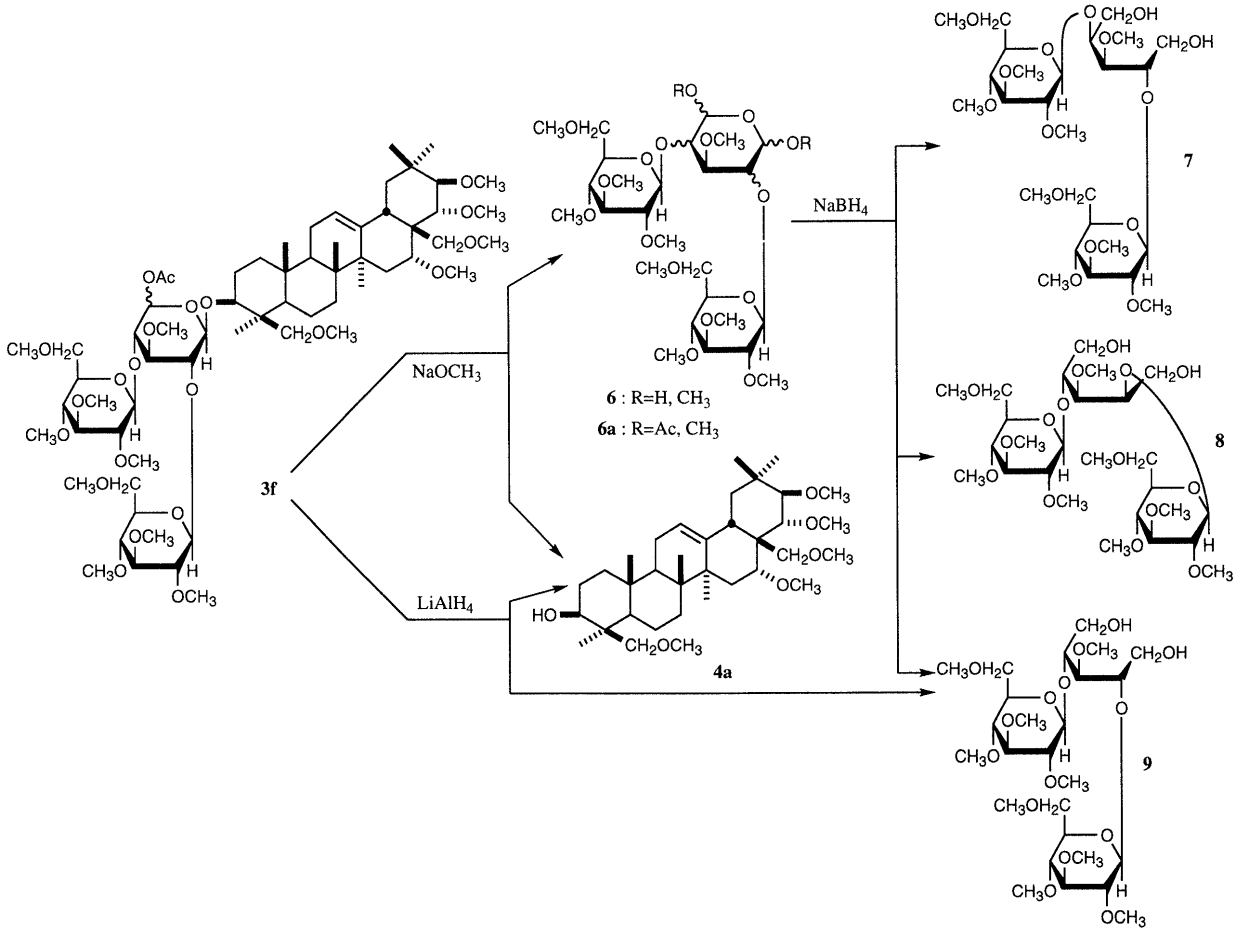


Chart 3

the 1''''-carbon. On the basis of the above-mentioned evidence, the structure of escin Ib was determined to be 21-angeloyl-22-acetylprotoaescigenin 3-*O*-[*β*-D-glucopyranosyl (1—2)][*β*-D-glucopyranosyl (1—4)]-*β*-D-glucopyranosiduronic acid (**2**).[24]

Escins IIa (10), IIb (11), and IIIa (13) Escins IIa (**10**) and IIb (**11**) were isolated as colorless fine crystals of mp 206.5—208.6 °C and 197.3—199.0 °C, respectively. Escins IIa (**10**) and IIb (**11**) were found to have the same molecular formula $C_{54}H_{84}O_{23}$, which was obtained from the quasimolecular ion peak in their positive-mode FAB-MS at *m/z* 1123 $(M+Na)^+$ and by high-resolution MS measurement. Alkaline hydrolysis of **10** and **11** furnished desacylescin II (**12**) and two organic acids (acetic acid and tiglic acid from **10**, acetic acid and angelic acid from **11**). Methanolysis of **12** liberated **4** together with methyl glycosides of D-glucose, D-glucuronic acid, and D-xylose in a 1 : 1 : 1 ratio.[18] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[20] of **12** showed signals due to the protoaescigenin moiety [*δ* 4.71 (1H, d, *J*=9.2 Hz, 21-H), 4.51 (1H, d-like, 22-H)], *β*-D-glucopyranosiduronic acid moiety [*δ* 4.97 (1H, d, *J*=6.6 Hz, 1′-H)], *β*-D-xylopyranosyl moiety [*δ* 5.21 (1H, d, *J*=7.1 Hz, 1″-H)], and *β*-D-glucopyranosyl moiety [*δ* 5.15 (1H, d, *J*=7.6 Hz, 1‴-H)]. The oligoglycoside structure of **12** was characterized by the HMBC experiment on **12**, which showed long-range correlations between the anomeric proton of the D-glucuronic acid moiety and the 3-carbon of the protoaescigenin moiety and between the anomeric protons of the D-xylopyranosyl moiety and the D-glucopyranosyl moiety and the 2′ and 4′-carbons of the D-glucuronic acid moiety, respectively.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[20] of **10** showed signals assignable to a tigloyl group [*δ* 1.66 (3H, d, *J*=7.3 Hz, 4''''-H_3), 1.96 (3H, s, 5''''-H_3), 7.08 (1H, dq-like, 3''''-H)] and an acetyl group [*δ* 1.91 (3H, s, 2''''-H_3)], together with two methine protons on carbons bearing an acyl group [*δ* 6.50 (1H, d, *J*=10.2 Hz, 21-H), 6.23 (1H, d, *J*=10.2 Hz, 22-H)]. In the HMBC experiment, long-range correlations were observed between the 21-proton and the carbonyl carbon (1''''-C) of the tigloyl group and between the 22-proton and the acetyl carbonyl carbon. Consequently, the structure of escin IIa was determined to be 21-tigloyl-22-acetylprotoaescigenin 3-*O*-[*β*-D-xylopyranosyl (1—2)][*β*-D-glucopyranosyl (1—4)]-*β*-D-glucopyranosiduronic acid (**10**).

The ^{1}H-NMR (pyridine-d_5) spectrum of **11** showed signals due to an angeloyl group [*δ* 2.02 (3H, s, 5''''-H_3), 2.10 (3H, d, *J*=7.2 Hz, 4''''-H_3), 5.98 (1H, dq-like, 3''''-H)], an acetoxyl group [*δ* 1.92 (3H, s, 2''''-H_3)], and two methine protons on carbons bearing an acyl group [*δ* 6.58 (1H, d, *J*=10.2 Hz, 21-H), 6.15 (1H, d, *J*=10.2 Hz, 22-H)]. The HMBC experiment on **11** showed long-range correlations between the 21-proton and the 1''''-carbon and between the 22-proton and the acetyl carbonyl carbon. Finally, comparison of the ^{13}C-NMR (Table 1) spectrum of **11** with those of **10** and **12** confirmed the structure of escin IIb as 21-angeloyl-22-acetylprotoaescigenin 3-*O*-[*β*-

Table 1. ^{13}C-NMR Data for **1**, **2**, **3**, **10**, **11**, **12**, **13**, and **14** (68 MHz, pyridine-d_5, δ_C)

	1	**2**	**3**	**10**	**11**	**12**	**13**	**14**
Sapogenol moiety								
C-1	38.6	38.6	38.5	38.9	38.9	38.9	38.9	38.9
C-2	26.6	26.6	26.5	26.6	26.6	26.6	26.6	26.6
C-3	91.2	91.2	91.1	90.7	90.7	90.7	89.3	89.3
C-4	43.8	43.8	43.6	44.3	44.3	44.3	39.6	39.6
C-5	56.2	56.2	56.1	56.4	56.4	56.5	55.8	55.8
C-6	18.6	18.6	18.5	18.8	18.8	18.8	18.5	18.5
C-7	33.3	33.3	33.2	33.4	33.4	33.4	33.2	33.3
C-8	40.0	40.0	39.9	40.1	40.1	40.0	40.1	40.1
C-9	46.8	46.8	46.8	46.8	46.8	46.9	47.0	47.1
C-10	36.5	36.5	36.3	36.6	36.6	36.6	36.8	36.9
C-11	24.1	24.1	23.9	24.1	24.1	24.1	23.9	23.9
C-12	122.7	122.6	122.8	122.7	122.7	122.7	122.7	122.7
C-13	142.9	142.9	143.8	142.9	142.9	143.9	143.0	144.0
C-14	41.8	41.7	41.9	41.8	41.8	42.1	41.8	42.1
C-15	34.7	34.7	34.2	34.7	34.6	34.3	34.7	34.4
C-16	68.1	68.1	67.8	68.1	68.1	67.9	68.2	69.6
C-17	48.0	48.1	47.2	48.0	48.1	47.3	48.1	47.3
C-18	40.2	40.2	41.1	40.2	40.2	41.3	40.2	41.3
C-19	47.5	47.3	48.2	47.3	47.3	48.2	47.3	48.3
C-20	36.5	36.3	36.2	36.5	36.3	36.4	36.5	36.4
C-21	79.4	79.0	78.6	79.4	78.9	78.8	79.4	78.8
C-22	74.5	74.5	77.3	74.4	74.5	77.4	74.6	77.6
C-23	22.5	22.5	22.4	22.7	22.7	22.7	28.1	28.1
C-24	63.4	63.4	63.2	62.9	62.9	62.9	16.8	16.8
C-25	15.6	15.6	15.5	15.5	15.8	15.5	15.7	15.7
C-26	16.8	16.8	16.7	16.8	16.8	16.9	17.0	17.0
C-27	27.4	27.4	27.3	27.4	27.4	27.4	27.5	27.4
C-28	64.0	64.0	68.3	63.9	64.0	68.4	64.0	68.6
C-29	29.6	29.5	30.4	29.5	29.5	30.5	29.6	30.5
C-30	20.1	20.3	19.3	20.1	20.2	19.4	20.2	19.4
Sugar moiety								
3-*O*-*β*-D-Glucuronopyranosyl moiety								
C-1′	104.6	104.6	104.5	104.8	104.9	104.8	105.1	105.1
C-2′	79.9	79.9	79.8	79.0	79.0	79.0	82.3	82.3
C-3′	76.5	76.5	76.3	76.3	76.3	76.3	76.0	75.9
C-4′	81.6	81.6	81.5	82.2	82.2	82.2	81.7	81.7
C-5′	75.8	75.8	75.6	75.6	75.6	75.6	75.4	75.4
C-6′	171.8	171.8	171.8	171.8	171.8	171.9	172.0	172.0
2′-*O*-*β*-D-Gluco- or galacto- or xylopyranosyl moiety								
C-1″	104.4	104.4	104.2	104.8	104.9	104.8	106.6	106.6
C-2″	75.8	75.8	75.6	75.7	75.7	75.7	74.6	74.6
C-3″	78.2	78.2	78.0	78.4	78.6	78.5	74.8	74.8
C-4″	69.9	69.9	69.7	70.8	70.8	70.8	69.6	67.9
C-5″	78.4	78.4	78.2	67.1	67.2	67.1	76.9	76.9
C-6″	61.6	61.6	61.5				61.6	61.5
4′-*O*-*β*-D-Glucopyranosyl moiety								
C-1‴	104.6	104.6	104.5	104.7	104.6	104.7	104.6	104.6
C-2‴	74.9	74.9	74.7	74.9	74.9	74.9	74.8	74.8
C-3‴	78.1	78.1	77.9	78.1	78.0	78.1	78.1	78.1
C-4‴	71.6	71.6	71.4	71.6	71.6	71.6	71.6	71.9
C-5‴	78.5	78.5	78.5	78.4	78.6	78.5	78.6	78.4
C-6‴	62.5	62.5	62.3	62.5	62.5	62.5	62.6	62.6
Acyl moiety								
Tigloyl or angeloyl moiety								
C-1⁗	168.0	167.9		168.0	167.8		168.0	
C-2⁗	129.6	129.1		129.5	129.0		129.6	
C-3⁗	136.8	137.0		136.8	137.0		136.8	
C-4⁗	14.2	15.9		14.2	15.5		14.2	
C-5⁗	12.4	21.0		12.4	21.0		12.4	
Acetyl moiety								
C-1⁗′	171.1	171.0		171.1	171.0		171.1	
C-2⁗′	20.9	20.9		20.9	20.8		20.9	

D-xylopyranosyl (1—2)][*β*-D-glucopyranosyl (1—4)]-*β*-D-glucopyranosiduronic acid (**11**).

Escin IIIa (**13**) was also isolated as colorless fine crystals of mp 194.1—196.5 °C and the IR spectrum of **13** showed absorption bands of hydroxyl, carboxyl, and α,β-unsaturated ester. Here again, the molecular formula $C_{55}H_{86}O_{23}$ was obtained from the quasimolecular ion peak [m/z 1137 $(M+Na)^+$] observed in the positive-mode

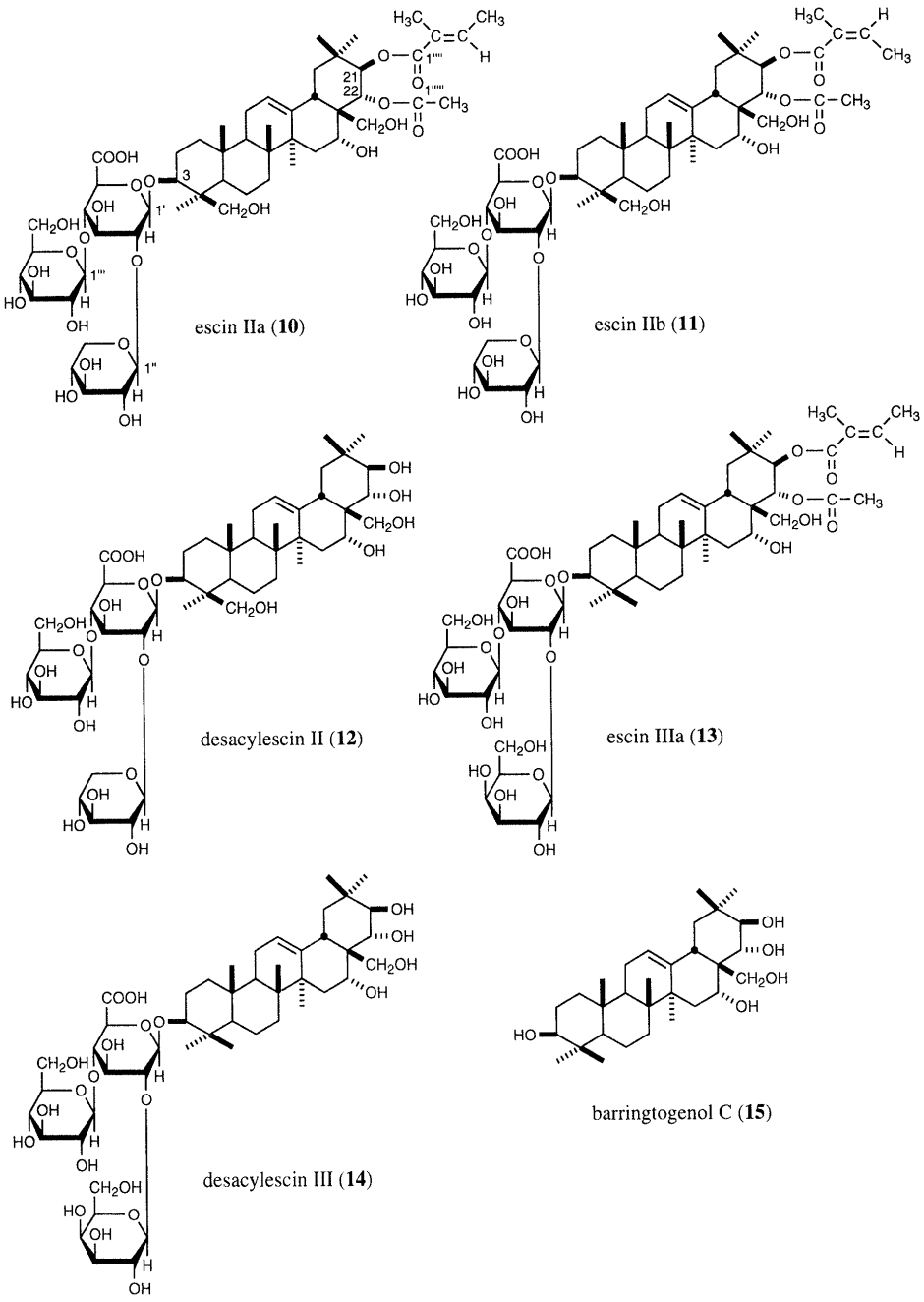


Chart 4

FAB-MS and by high-resolution MS measurement. The alkaline hydrolysis of **13** furnished desacylescin III (**14**) and two organic acids, tiglic acid and acetic acid. The methanolysis of **14** liberated barringtogenol C (**15**),[25] together with methyl glycosides of D-galactose, D-glucose, and D-glucuronic acid in a 1 : 1 : 1 ratio.[18] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectrum of **14** showed signals due to the β-D-glucuronopyranosyl moiety [δ 4.96 (1H, d, J=6.9 Hz, 1′-H)], β-D-galactopyranosyl moiety [δ 5.20 (1H, d, J=7.6 Hz, 1″-H)], β-D-glucopyranosyl moiety [δ 5.15 (1H, d, J=7.6 Hz, 1‴-H)], and the barringtogenol C moiety [δ 4.72 (1H, d, J=9.6 Hz, 21-H), 4.51 (1H, d-like, 22-H)]. In the HMBC experiment on **14**, long-range correlations were observed between the 1′-proton and the 3-carbon, between the 1″-proton and the 2′-carbon, and between the 1‴-proton and the 4′-carbon. On the basis of the above-mentioned evidence, the structure of **14** was characterized to be as shown.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectrum of **13** showed signals assignable to a tigloyl group and an acetyl group and the HMBC experiment on **13** showed long-range correlations between the following protons and carbons: 21-H and 1⁗-C; 22-H and 1⁗′-C; 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 4′-C; 5⁗-H_3 and 2⁗-C, 1⁗-C; 4⁗-H_3 and 3⁗-C; 2⁗′-H_3 and 1⁗′-C. Consequently, the structure of escin IIIa was determined to be 21-tigloyl-22-acetylbarringtogenol C 3-*O*-[β-D-galactopyranosyl (1—2)][β-D-glucopyranosyl (1—4)]-β-D-glucopyranosiduronic acid (**13**).

Inhibitory Activity of the Saponin Fraction of Horse Chestnut Seed, Escins Ia (1), Ib (2), IIa (10), and IIb (11), and Desacylescins I (3) and II (12) on Ethanol Absorption in Rats The tested samples (the saponin fraction, escins and desacylescins) were dissolved in water and then orally

administered to rats. At 1 h thereafter, 20% aqueous ethanol was orally administered and the ethanol concentration in blood collected at 1, 2, and 3 h later was assayed by the enzymatic method. Inhibitory effects of the saponin fraction, escins (**1**, **2**, **10**, **11**) and desacylescins (**3**, **12**) on ethanol absorption are summarized in Table 2. Single oral administration of 200 mg/kg of the saponin fraction of horse chestnut seed showed potent inhibitory activity on ethanol absorption. Escins IIa (**10**) and IIb (**11**) were also found to exhibit inhibitory activity at lower doses (50 mg/kg and 100 mg/kg), while escins Ia (**1**) and Ib (**2**) showed only weak activity. This implies that the 2′-*O*-xylopyranosyl moiety in **10** and **11** is required for potent inhibitory activity, and the 2′-*O*-glucopyranosyl moiety decreased the activity. Desacylescins I (**3**) and II (**12**) lacked the activity, so that the acyl groups in escins were also confirmed to be essential for the inhibitory activity.

Hypoglycemic Activity of the Saponin Fraction of Horse Chestnut Seed, Escins Ia (1), Ib (2), IIa (10), and IIb (11), and Desacylescins I (3) and II (12) Hypoglycemic activity of the saponin fraction of horse chestnut seed, escins (**1**, **2**, **10**, **11**), and desacylescins (**3**, **12**) was examined by using a bioassay to measure the inhibitory activity on the elevation of plasma glucose level in the oral glucose tolerance test in rats. As shown in Table 3, a single oral administration of 200 mg/kg of the saponin fraction of horse chestnut seed showed hypoglycemic activity. The major saponin constituents, escins Ia (**1**), Ib (**2**), IIa (**10**), and IIb (**11**) in the saponin fraction were found to exhibit hypoglycemic activity at a lower dose (100 mg/kg), and escin IIa (**10**) showed the most potent activity among them. In addition, escins IIa (**10**) and IIb (**11**) having the 2′-*O*-xylopyranosyl moiety in their oligosaccharide part were found to show much more potent activity than escins Ia (**1**) and Ib (**2**) having the 2′-*O*-glucopyranosyl moiety. On the other hand, desacylescins I (**3**) and II (**12**) lacked the activity. This evidence again indicated that the acyl groups in escins are essential for hypoglycemic activity.

Table 2. Inhibitory Effects of the Saponin Fraction, Escins Ia (**1**), Ib (**2**), IIa (**10**), and IIb (**11**) and Desacylescins I (**3**) and II (**12**) on Ethanol Absorption

	Dose (mg/kg, *p.o.*)	*n*	Ethanol concentration in blood (mg/ml) 1 h	2 h	3 h
Control		5	0.62±0.02	0.22±0.02	0.01±0.00
The saponin fraction	200	4	0.06±0.04**	0.10±0.04**	0.01±0.00
Control		10	0.54±0.01	0.19±0.01	0.03±0.01
Escin Ia (**1**)	100	5	0.50±0.02**	0.22±0.02	0.04±0.01
Escin Ib (**2**)	100	5	0.43±0.03**	0.02±0.01	0.04±0.02
Desacylescin I (**3**)	100	5	0.54±0.02	0.23±0.02	0.02±0.00
Escin IIa (**10**)	50	5	0.37±0.08	0.14±0.03	0.02±0.00
	100	5	0.08±0.04**	0.14±0.05	0.05±0.02
Escin IIb (**11**)	50	5	0.29±0.10	0.21±0.03	0.02±0.00
	100	5	0.14±0.04**	0.24±0.12	0.06±0.02
Desacylescin II (**12**)	100	5	0.54±0.02	0.23±0.02	0.02±0.00

* $p<0.05$. ** $p<0.01$.

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described in our previous paper.[1)]

Isolation of Escins Ia (1), Ib (2), IIa (10), IIb (11), and IIIa (13) The seeds of horse chestnut tree (10 kg, powder, a gift from Maruzen Pharmaceutical Co., Ltd., Hiroshima) were extracted with MeOH under reflux three times. After removal of the solvent from the MeOH solution under reduced pressure, the extract (399 g, 3.99%) was subjected to Diaion HP-20 column chromatography [(Nippon Rensui Co., 2.4 kg), $H_2O \rightarrow$MeOH$\rightarrow$AcOEt] to give the H_2O-eluted fraction, the MeOH-eluted fraction (234 g, 2.34%), and the AcOEt-eluted fraction. The MeOH-eluted fraction (234 g) was separated by reversed-phase silica gel column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical Ltd., 2.3 kg), 40% aqueous MeOH$\rightarrow$90% aqueous MeOH$\rightarrow$MeOH] to furnish the 90% MeOH-eluted fraction (the saponin fraction, 169 g, 1.69%). HPLC [YMC-Pack R&D ODS-5 (20×250 mm, i.d.), MeOH–1% aqueous AcOH (7 : 3, v/v)] separation of the saponin fraction (**6g**) yielded escins Ia (**1**, 501 mg, 0.141%), Ib (**2**, 360 mg, 0.101%), and IIa (**10**, 285 mg, 0.080%), a mixture of escins IIb and IIIa (160 mg, 0.045%), and another saponin fraction (4.5 g, 12.8%). A mixture of escins IIb and IIIa was separated by HPLC [YMC-Pack R&D ODS-5 (20×250 mm, i.d.), CH_3CN–1% aqueous AcOH (2 : 3, v/v) to give escins IIb (**11**, 126 mg, 0.035%) and IIIa (**13**, 33 mg, 0.007%).

Escin Ia (**1**): Colorless fine crystals from $CHCl_3$–MeOH, mp 224.9—226.7 °C, $[\alpha]_D^{25}$ −22.2° (c=0.6, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{55}H_{86}NaO_{24}$ $(M+Na)^+$: 1153.5406; Found: 1153.5405. IR (KBr): 3453, 1731, 1719, 1653, 1649, 1638, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.69, 0.82, 1.09, 1.32, 1.34, 1.81 (3H each, all

Table 3. Inhibitory Effects of the Saponin Fraction, Escins Ia (**1**), Ib (**2**), IIa (**10**), and IIb (**11**) and Desacylescins I (**3**) and II (**12**) on the Elevation of Plasma Glucose Level in the Oral Glucose Tolerance Test

	Dose (mg/kg, *p.o.*)	*n*	Plasma glucose concentration (mg/dl) 0.5 h		1 h		2 h	
Control (normal)		4	91.1±3.4**					
Control (glucose tolerance)		5	168.1±8.9	(77.0±5.5)				
The saponin fraction		5	139.1±9.4	(48.0±6.0)				
Control (normal)		5	81.9±6.0**		96.3±4.8**		87.9±4.4	
Control (glucose tolerance)		6	153.5±6.2	(71.6±6.2)	134.8±5.6	(38.5±5.6)	98.9±4.5	(11.0±4.5)
Escin Ia (**1**)	100	5	117.1±3.6**	(35.4±3.6**)	130.9±5.7	(34.6±5.7)	100.5±6.1	(12.6±6.1)
Escin Ib (**2**)	100	5	127.1±4.9*	(45.2±4.9*)	143.7±4.2	(47.4±4.2)	107.7±8.0	(19.8±8.0)
Escin IIa (**10**)	100	5	98.4±7.2**	(16.5±7.2**)	105.9±7.8*	(9.6±7.8*)	92.9±7.4	(5.0±7.4)
Control (normal)		5	78.5±4.2**		107.5±5.8**		97.0±1.9**	
Control (glucose tolerance)		5	143.0±3.9	(64.5±3.9)	140.6±4.6	(33.1±4.6)	120.3±4.6	(23.3±4.6)
Escin IIb (**11**)	100	2	107.9±7.3**	(29.4±7.3**)	123.6±6.9	(16.1±6.9)	113.5±5.7	(17.7±5.7)
Desacylesin I (**3**)	100	5	140.6±5.7	(62.1±5.7)	136.6±3.6	(29.1±3.6)	114.7±1.8	(17.7±1.8)
Desacylescin II (**12**)	100	5	146.2±4.5	(67.7±4.5)	140.4±7.5	(32.9±7.5)	118.9±3.8	(21.9±3.8)

* $p<0.05$. ** $p<0.01$. Values in parenthesis show the difference in plasma glucose concentration between the normal control and each sample treatment.

s, 25, 26, 29, 30, 23, 27-$H_3 \times 6$), 1.66 (3H, d, J=6.9 Hz, Tig-4''''-H_3), 1.91 (3H, s, Ac-2''''-H_3), 1.96 (3H, s, Tig-5''''-H_3), 3.06 (1H, m, 18-H), 3.42 (1H, dd-like, 3-H), 4.42 (1H, m, 16-H), 4.91 (1H, d, J=7.3 Hz, 3-*O*-Glc.A-1'-H), 5.18 (1H, d, J=7.9 Hz, 4'-*O*-Glc.-1'''-H), 5.57 (1H, d, J=7.3 Hz, 2'-*O*-Glc.-1''-H), 6.21 (1H, d, J=10.2 Hz, 22-H), 6.56 (1H, d, J=10.2 Hz, 21-H), 7.10 (1H, dq-like, Tig-3''''-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Positive-mode FAB-MS (*m/z*): 1153 $(M+Na)^+$.

Escin Ib (**2**): Colorless fine crystals from $CHCl_3$–MeOH, mp 187.8—189.3 °C, $[\alpha]_D^{25}$ −23.1° (c=0.6, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{55}H_{86}O_{24}Na$ $(M+Na)^+$: 1153.5407; Found: 1153.5459. IR (KBr): 3432, 1731, 1719, 1653, 1649, 1638, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.69, 0.82, 1.09, 1.31, 1.34, 1.81 (3H each, all s, 25, 26, 29, 30, 23, 27-$H_3 \times 6$), 1.91 (3H, s, Ac-2''''-H_3), 2.02 (3H, s, Ang-5''''-H_3), 2.11 (3H, d, J=7.2Hz, Ang-4''''-H_3), 3.07 (1H, m, 18-H), 3.40 (1H, dd-like, 3-H), 4.48 (1H, m, 16-H), 4.91 (1H, d, J=7.3 Hz, 3-*O*-Glc.A-1'-H), 5.18 (1H, d, J=7.9 Hz, 4'-*O*-Glc.-1'''-H), 5.57 (1H, d, J=7.3 Hz, 2'-*O*-Glc.-1''-H), 5.95 (1H, dq-like, Ang-3''''-H), 6.17 (1H, d, J=10.2 Hz, 22-H), 6.69 (1H, d, J=10.2 Hz, 21-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Positive-mode FAB-MS (*m/z*): 1153 $(M+Na)^+$.

Escin IIa (**10**): Colorless fine crystals from $CHCl_3$–MeOH, mp 206.5—208.6 °C, $[\alpha]_D^{25}$ −35.5° (c=0.6, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{54}H_{84}NaO_{23}$ $(M+Na)^+$: 1123.5301; Found: 1123.5315. IR (KBr): 3453, 1733, 1719, 1653, 1647, 1638, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.77, 0.84, 1.09, 1.32, 1.38, 1.83 (3H each, all s, 25, 26, 29, 30, 23, 27-$H_3 \times 6$), 1.66 (3H, d, J=7.3 Hz, Tig-4''''-H_3), 1.91 (3H, s, Ac-2''''-H_3), 1.96 (3H, s, Tig-5''''-H_3), 3.02 (1H, m, 18-H), 3.38 (1H, dd-like, 3-H), 4.43 (1H, m, 16-H), 4.94 (1H, d, J=6.9 Hz, 3-*O*-Glc.A-1'-H), 5.16 (1H, d, J=7.6 Hz, 4'-*O*-Glc.-1'''-H), 5.44 (1H, d, J=6.9 Hz, 2'-*O*-Xly.-1''-H), 6.23 (1H, d, J=10.2 Hz, 22-H), 6.50 (1H, d, J=10.2 Hz, 21-H), 7.08 (1H, dq-like, Tig-3''''-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Positive-mode FAB-MS (*m/z*): 1123 $(M+Na)^+$.

Escin IIb (**11**): Colorless fine crystals from $CHCl_3$–MeOH, mp 197.3—199.0 °C, $[\alpha]_D^{25}$ −19.4° (c=0.6, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{54}H_{84}NaO_{23}$ $(M+Na)^+$: 1123.5301; Found: 1123.5372. IR (KBr): 3453, 1736, 1719, 1658, 1649, 1630, 1076 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.77, 0.84, 1.08, 1.30, 1.38, 1.82 (3H each, all s, 25, 26, 29, 30, 23, 27-$H_3 \times 6$), 1.92 (3H, s, Ac-2''''-H_3), 2.02 (3H, s, Ang-5''''-H_3), 2.10 (3H, d, J=7.2 Hz, Ang-4''''-H_3), 3.02 (1H, m, 18-H), 3.42 (1H, dd-like, 3-H), 4.42 (1H, m, 16-H), 4.93 (1H, d, J=5.9 Hz, 3-*O*-Glc.A-1'-H), 5.16 (1H, d, J=7.6 Hz, 4'-*O*-Glc.-1'''-H), 5.44 (1H, d, J=6.9 Hz, 2'-*O*-Xly.-1''-H), 5.98 (1H, dq-like, Ang-3''''-H), 6.15 (1H, d, J=10.2 Hz, 22-H), 6.58 (1H, d, J=10.2 Hz, 21-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Positive-mode FAB-MS (*m/z*): 1123 $(M+Na)^+$.

Escin IIIa (**13**): Colorless fine crystals from $CHCl_3$–MeOH, mp 194.1—196.5 °C, $[\alpha]_D^{25}$ −17.2° (c=0.5, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{55}H_{86}NaO_{23}$ $(M+Na)^+$: 1137.5458; Found: 1137.5503. IR (KBr): 3432, 1734, 1719, 1655, 1649, 1630, 1076 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.86, 0.89, 1.29, 1.33, 1.84 (3H each, all s, 25, 26, 23, 30, 27-$H_3 \times 5$), 1.10 (6H, s, 24, 29-$H_3 \times 2$), 1.66 (3H, d, J=6.9 Hz, Tig-4''''-H_3), 1.91 (3H, s, Ac-2''''-H_3), 1.96 (3H, s, Tig-5''''-H_3), 3.03 (1H, m, 18-H), 3.30 (1H, dd-like, 3-H), 4.63 (1H, m, 16-H), 4.97 (1H, d, J=6.6 Hz, 3-*O*-Glc.A-1'-H), 5.15 (1H, d, J=7.6 Hz, 4'-*O*-Glc.-1'''-H), 5.21 (1H, d, J=7.6 Hz, 2'-*O*-Gal.-1''-H), 6.21 (1H, d, J=10.2 Hz, 22-H), 6.57 (1H, d, J=10.2 Hz, 21-H), 7.10 (1H, dq-like, Tig-3''''-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Positive-mode FAB-MS (*m/z*): 1137 $(M+Na)^+$.

Alkaline Hydrolysis of Escin Ia (1) Giving Desacylescin I (3) A solution of escin Ia (**1**, 100 mg) in 50% aqueous dioxane (5 ml) was treated with 10% aqueous KOH (5 ml) and the whole was stirred at 37 °C for 1 h. After removal of the solvent from a part (0.1 ml) of the reaction mixture under reduced pressure, the residue was dissolved in $(CH_2)_2Cl_2$ (2 ml) and the solution was treated with *p*-nitrobenzyl-*N*,*N*'-diisopropylisourea (10 ml), then the whole was stirred at 80 °C for 1 h. The rest of the reaction solution was subjected to HPLC analysis to identify the *p*-nitrobenzyl esters of acetic acid (**a**) and tiglic acid (**b**), HPLC conditions: column, TSK gel ODS-Prep (4.6 × 250 mm, i.d.); solvent, MeOH–H_2O (7 : 3, v/v); flow rate, 1.0 ml/min; t_R, **a**: 8.0 min; **b**: 17.0 min.

The rest of the reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a product (100 mg), which was subjected to silica gel column chromatography [3 g, $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to give desacylescin I (**3**, 80 mg).

Desacylescin I (**3**): Colorless fine crystals from $CHCl_3$–MeOH–H_2O, mp 234.0—236.3 °C, $[\alpha]_D^{24}$ −40.0° (c=2.04, pyridine). *Anal.* Calcd for $C_{48}H_{78}O_{22} \cdot 2H_2O$: C, 56.24; H, 7.87. Found: C, 56.49; H, 7.93. IR (KBr): 3388, 1724, 1630, 1060 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.75, 0.84, 1.29, 1.31, 1.33, 1.80 (3H each, all s, 25, 26, 29, 30, 23, 27-$H_3 \times 6$), 2.69 (1H, dd-like, 18-H), 3.43 (1H, dd-like, 3-H), 4.53 (1H, d-like, 22-H), 4.70 (1H, d, J=9.2 Hz, 21-H), 4.88 (1H, d, J=7.6 Hz, 3-*O*-Glc.A-1'-H), 4.96 (1H, br s, 16-H), 5.16 (1H, d, J=7.6 Hz, 4'-*O*-Glc.-1'''-H), 5.54 (1H, d, J=7.6 Hz, 2'-*O*-Glc.-1''-H).

Acid Hydrolysis of Desacylescin I (3) Giving Protoaescigenin (4) and Escigenin (5) A solution of **3** (20 mg) in MeOH (4 ml) was treated with 20% aqueous H_2SO_4 (4 ml) and the mixture was heated under reflux for 4 h. It was then poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated $NaHCO_3$ and H_2O, and dried over $MgSO_4$. After removal of the solvent from the extract under reduced pressure, the residue was separated by preparative TLC to give **4** (6 mg) and **5** (2 mg), both of which were shown to be identical with authentic samples[16] by co-TLC [$CHCl_3$–MeOH (6 : 1), benzene–MeOH (5 : 1), $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer)], mixed melting point determination and IR (KBr) comparison.

Methanolysis of Desacylescin I (3) A solution of **3** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the insoluble portion was removed by filtration. After evaporation of the solvent from the filtrate under reduced pressure, the residue was dissolved in pyridine (0.1 ml) and this solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.2 ml) for 1 h. The reaction solution was subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucoside (**c**) and methyl glucuronide (**d**). GLC conditions: 1) column, 5% butane-1,4-diol succinate on Uniport B (80—100 mesh), 3 mm × 2 m; column temperature, 150 °C; carrier gas, N_2; flow rate, 30 ml/min; t_R, **c**: 9 min 24 s (major), 10 min 28 s; **d**: 10 min 28 s, 19 min 42 s, 21 min 7 s (major). 2) column, 5% silicone SE-52 on Chromosorb WAW DMCS (80—100 mesh), 3 mm × 2 m; column temperature, 175 °C; carrier gas, N_2; flow rate, 35 ml/min; t_R, **c**: 12 min 14 s (major), 13 min 13 s; **d**: 6 min 52 s, 7 min 29 s (major), 8 min 29 s, 14 min 16 s. 3) column, CBR1-M25-025 (0.25 mm × 25 m, i.d.), column temperature, 140—240 °C (5 °C/min); carrier gas, He; flow rate 15 ml/min; t_R, **c**: 20 min 36 s, **d**: 18 min 0 s, 21 min 0 s, 21 min 18 min.

Photolysis of Desacylescin I (3) A solution of **3** (200 mg) in MeOH (550 ml) was irradiated internally for 1 h with a high-pressure mercury lamp (500W, Eikôsha P1H-500) equipped with a Vycor filter. It was neutralized with 10% aqueous K_2CO_3, then concentrated under reduced pressure to remove MeOH and diluted with water to give a precipitate (135 mg), which was collected by filtration. The precipitate was purified by preparative TLC [$CHCl_3$–MeOH (5 : 1)] to give protoaescigenin (**4**, 65 mg), which was shown to be identical with an authentic sample[16] by co-TLC (the same conditions as described above for the acid hydrolysis of **3**), mixed melting point determination and IR (KBr) comparison.

Diazomethane Methylation of Desacylescin I (3) Giving the Methyl Ester (3a) A solution of **3** (8.8 g) in MeOH (500 ml) was treated with ethereal diazomethane until the yellow color persisted. The solution was left standing for 1 h, then the solvent was removed under reduced pressure to furnish **3a** (8.9 g).

Desacylescin I methyl ester (**3a**): Colorless needles from MeOH, mp 223.5—225.0 °C, $[\alpha]_D^{27}$ −33.8° (c=1.41, pyridine). *Anal.* Calcd for $C_{49}H_{80}O_{22} \cdot 3H_2O$: C, 54.47; H, 8.06. Found: C, 54.75; H, 8.12. IR (KBr): 3375, 2942, 1734, 1071, 1035 cm^{-1}.

Reduction of Desacylescin I Methyl Ester (3a) with $NaBH_4$ Giving 3b A solution of the methyl ester (**3a**, 200 mg) in MeOH (20 ml) was treated with $NaBH_4$ (150 mg) and the mixture was stirred at room temperature (26 °C) for 2 h. Excess $NaBH_4$ was quenched with acetone, then the solution was neutralized with Dowex 50W × 8 (H^+ form) and filtered. Removal of the solvent from the filtrate yielded **3b** (192 mg).

3b: Colorless fine crystals from MeOH, mp 284.5—286.5 °C, $[\alpha]_D^{24}$ −30.2° (c=0.60, pyridine). *Anal.* Calcd for $C_{48}H_{80}O_{22}$: C, 58.04; H, 8.13. Found: C, 57.96; H, 8.26. IR (KBr): 3382, 2917, 1076, 1026 cm^{-1}.

Methanolysis of 3b A solution of **3b** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 2 h. After cooling, the reaction solution was neutralized with Ag_2CO_3 and filtered. Work-up of the filtrate gave a product, which was subjected to GLC analysis to identify methyl glucoside (**c**). GLC conditions: 4) column, 3% silicone SE-30 on Chromosorb WAW DMCS (80—100 mesh), 3 mm × 1 m; column tem-

perature, 150 °C; carrier gas, N_2; flow rate, 35 ml/min; t_R, **c**: 7 min 37 s (major), 8 min 36 s; 5) column, 15% diethylene glycol succinate on Chromosorb WAW (80—100 mesh), 3 mm × 2 m; column temperature, 150 °C; carrier gas, N_2; flow rate, 35 ml/min; t_R, **c**: 5 min 34 s (major), 6 min 4 s.

Complete Methylation of 3b Giving the Pentadeca-*O*-methyl Ether (3c) A solution of **3b** (200 mg) in dimethyl sulfoxide (DMSO) was treated with a dimsyl carbanion solution [20 ml, prepared with NaH (2 g) and DMSO (40 ml)]. The mixture was stirred in the dark at room temperature (15 °C) for 2 h, then treated with CH_3I (20 ml), and the whole was stirred for a further 12 h. The reaction mixture was poured into ice–water and the whole was extracted with AcOEt. The AcOEt extract was washed with 10% aqueous $Na_2S_2O_3$ and saturated saline, then dried over $MgSO_4$ and filtered. Evaporation of the solvent under reduced pressure afforded a residue, which was purified by preparative TLC [benzene–AcOEt (1 : 2)] to give **3c** (110 mg).

3c: Colorless needles from benzene–AcOEt, mp 111.0—113.0 °C, $[\alpha]_D^{27}$ −2.6° (c=0.44, CCl_4). *Anal.* Calcd for $C_{63}H_{110}O_{21}$: C, 62.87; H, 9.21. Found: C, 62.57; H, 9.12. IR (CCl_4): 2931, 1092 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (9H), 0.99, 1.16, 1.24 (3H each) (all s, *tert*-CH_3 × 6), 3.24 (9H), 3.36 (6H), 3.38 (3H), 3.51 (15H), 3.56, 3.59 (6H each) (all s, OCH_3 × 15), 4.26 (2H, d, J=7 Hz), 4.70 (1H, d, J=7 Hz) (1 min, 1 s, 1‴-H), 5.26 (1H, br s, $W_{h/2}$=9 Hz, 12-H). ^{1}H-NMR (benzene-d_6) δ: 0.89, 0.96, 1.05 (3H each), 1.12 (6H), 1.18 (3H) (all s, *tert*-CH_3 × 6), 3.14 (3H), 3.22, .3.26 (6H each), 3.29, 3.32, 3.38, 3.43, 3.52, 3.56 (3H each), 3.58 (9H), 3.81 (3H) (all s, OCH_3 × 15), 4.37, 4.40, 4.96 (1H each, all d, J=7 Hz, 1′, 1″, 1‴-H), 5.35 (1H, br s, $W_{h/2}$=7 Hz, 12-H).

Methanolysis of 3c Giving 16,21,22,24,28-Penta-*O*-methylprotoaescigenin (4a) A solution of **3c** (90 mg) in 9% HCl–dry MeOH (3 ml) was heated under reflux for 2 h, then neutralized with Ag_2CO_3 powder and filtered. Work-up of the filtrate yielded a residue, which was subjected to silica gel column chromatography [10 g, benzene–acetone (50 : 1→2 : 1)] to furnish **4a** (41 mg), methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**e**, 26 mg) and methyl 3,6-di-*O*-methylglucopyranoside (**f**, 12 mg).

Methyl glycosides (**e**, **f**) were shown to be identical with authentic samples by co-TLC [benzene–acetone (1 : 1), $CHCl_3$–MeOH (10 : 1), AcOEt] and GLC analysis. GLC conditions: 6) column, 5% butane-1,4-diol succinate on Uniport B (80—100 mesh), 3 mm × 2 m; column temperature, 150 °C; carrier gas, N_2; flow rate, 40 ml/min; t_R, **e**: 3 min 50 s, 4 min 38 s (major); 7) column, 15% diethylene glycol succinate on Uniport B (80—100 mesh), 3 mm × 2 m, column temperature, 170 °C; carrier gas, N_2; flow rate 50 ml/min; t_R, **e**: 4 min 11 s, 6 min 49 s (major); 8) column, 5% butane-1,4-diol succinate on Uniport B (80—100 mesh), 3 mm × 2 m; column temperature, 185 °C; carrier gas, N_2; flow rate, 40 ml/min, t_R, **f**: 9 min 42 s (major), 12 min 23 s; 9) column, 15% diethylene glycol succinate on Uniport B (80—100 mesh), 3 mm × 2 m; column temperature, 195 °C; carrier gas, N_2; flow rate, 50 ml/min; t_R, **f**: 16 min 12 s (major), 21 min 6 s.

16,21,22,24,28-Penta-*O*-methylprotoaescigenin (**4a**): A white powder, $[\alpha]_D^{27}$ +38.6° (c=1.63, CCl_4). High-resolution MS: Calcd for $C_{35}H_{60}O_6$ (M^+): 576.439; $C_{20}H_{34}O_4$ (**iii**): 338.245; $C_{15}H_{26}O_2$ (**i**): 238.193. Found: 576.440, 338.246, 238.190. IR (CCl_4): 3503, 2936 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H), 0.90 (9H), 1.21 (3H), 1.30 (3H) (all s, *tert-CH*$_3$ × 6), 3.24, 3.26, 3.29, 3.48, 3.52 (3H each, all s, OCH_3 × 5), 5.23 (1H, m, 12-H). MS (%) m/z: 576 (M^+, 5), 338 (**iii**, 6), 238 (**i**, 4).

Acetylation of 4a Giving the Monoacetate (4b) A solution of **4a** (72 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and the mixture was left standing for 24 h. It was then poured into ice-water and the resulting precipitate was collected by filtration. Crystallization of the precipitate from $CHCl_3$–MeOH–H_2O furnished **4b** (62 mg).

4b: Colorless needles from $CHCl_3$–MeOH–H_2O, mp 211.0—212.5 °C, $[\alpha]_D^{27}$ +28.9° (c=1.08, CCl_4). *Anal.* Calcd for $C_{37}H_{62}O_7$: C, 71.40; H, 10.26. Found: C, 71.81; H, 10.10. IR (CCl_4): 1738, 1243 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H), 0.88, 0.97 (6H each), 1.25 (3H) (all s, *tert*-CH_3 × 6), 1.95 (3H, s, OAc), 3.17 (3H), 3.22 (6H), 3.42, 3.46 (3H each) (all s, OCH_3 × 5), 4.46 (1H, t-like, 3-H), 5.16 (1H, br s, $W_{h/2}$=8 Hz, 12-H). MS (%) m/z: 618 (M^+, 4), 338 (**iii**, 9), 280 (**ii**, 5).

Complete Methylation of Desacylescin I (3) Giving the Pentadeca-*O*-methyl Derivative (3d) A solution of **3** (200 mg) in DMSO (25 ml) was treated with a dimsyl carbanion solution [20 ml, prepared with NaH (2 g) and DMSO (40 ml)]. The mixture was stirred in the dark at room temperature (15 °C) for 2 h, then treated with CH_3I (15 ml), and the whole was stirred for a further 12 h. The reaction solution was worked-up as described above for the complete methylation of **3b** to give a residue, which was purified by preparative TLC [benzene–acetone (7 : 2)] to afford **3d** (95 mg).

3d: A white powder, $[\alpha]_D^{13}$ −8.8° (c=1.50, CCl_4). *Anal.* Calcd for $C_{63}H_{108}O_{22}$: C, 62.15; H, 8.94. Found: C, 62.19; H, 8.92. IR (CCl_4): 1753, 1090 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (9H), 0.99, 1.15, 1.24 (3H each) (all s, *tert*-CH_3 × 6), 3.26 (9H), 3.38 (6H), 3.44 (3H), 3.49 (9H), 3.52 (3H), 3.57 (9H), 3.60 (3H) (all s, OCH_3 × 14), 3.76 (3H, s, $COOCH_3$), 4.24, 4.41, 4.67 (1H each, all d, J=7 Hz, 1′, 1″, 1‴-H), 5.24 (1H, br s, $W_{h/2}$=8 Hz, 12-H).

Alkaline Hydrolysis of 3d Giving the Tetradeca-*O*-methyl Ether (3e) A solution of **3d** (120 mg) in acetone (6 ml) was treated with 5% aqueous K_2CO_3 (3 ml) and the mixture was heated under reflux for 2 h. It was then treated with 5% aqueous HCl and the acidic solution was extracted with ether. The ether extract solution was washed with H_2O, dried over $MgSO_4$ and filtered. Removal of the solvent from the filtrate under reduced pressure gave the tetradeca-*O*-methyl ether (**3e**, 109 mg).

3e: A white powder, $[\alpha]_D^{27}$ −12.2° (c=1.51, CCl_4). *Anal.* Calcd for $C_{62}H_{106}O_{22}$: C, 61.88; H, 8.88. Found: C, 61.81; H, 8.82. IR (CCl_4): 1753, 1088 cm^{-1}. ^{1}H-NMR (benzene-d_6) δ: 1.04 (6H), 1.16, 1.18 (3H each), 1.29 (6H) (all s, *tert*-CH_3 × 6), 3.18 (6H), 3.24 (9H), 3.30 (3H), 3.43 (6H), 3.58 (12H), 3.66 (6H) (all s, OCH_3 × 14), 4.46, 4.59, 4.76 (1H each, all d, J=7 Hz, 1′, 1″, 1‴-H), 5.31 (1H, br s, $W_{h/2}$=10 Hz, 12-H), 6.80 (1H, br s, $W_{h/2}$=14 Hz, exchangeable with D_2O, COOH).

Lead Tetraacetate Oxidation of 3e Giving the Decarboxylation Product (3f) A solution of **3e** (109 mg) in benzene (6 ml) was treated with $Pb(OAc)_4$ (200 mg) and the mixture was heated under reflux for 5 h. After cooling, the reaction mixture was diluted with AcOEt and the solution was washed with H_2O. The organic layer was evaporated under reduced pressure to give the decarboxylation product (**3f**, 104 mg).

3f: A white powder, IR (CCl_4): 2931, 1765, 1221, 1087 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.13, 2.20 (totally 3H, both s, OAc), 5.74 (*ca.* 1/2H, d, J=7 Hz, 5′α-H), 6.23 (*ca.* 1/2H, d, J=3.5 Hz, 5′β-H).

Alkaline Degradation Followed by Acetylation of 3f Giving 4b and 6a A solution of **3f** (235 mg) in 0.2% NaOMe–MeOH (8 ml) was stirred at room temperature (24 °C) for **1** h. After neutralization with 10% HCl–dry MeOH, the mixture was evaporated to dryness under reduced pressure and the residue was acylated with Ac_2O–pyridine (1 : 1, 4 ml) at room temperature (33 °C) for 12 h. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. The usual work-up of the AcOEt extract followed by preparative TLC separation [benzene–acetone (7 : 2)] furnished **4b** (81 mg) and the acetal-type oligosaccharide acetate mixture (**6a**, 80 mg). 3-*O*-Acetyl-16,21,22,24,28-penta-*O*-methylprotoaescigenin (**4b**) was identical with an authentic sample on the basis of TLC, behavior, mixed melting point determination and IR (CCl_4) comparison. Preparative TLC [benzene–acetone (7 : 2)] of the acetate mixture (**6a**) gave a major constituent (16 mg).

Major component of **6a**: Colorless oil, $[\alpha]_D^{27}$ −19.8° (c=0.79, MeOH). IR (CCl_4): 1767, 1228 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.11 (3H, s, OAc), 3.36, 3.38, 3.44, 3.47, 3.52, 3.54, 3.57 (3H each), 3.62 (9H) (all s, OCH_3 × 10), 4.44, 4.47 (1H each, both d, J=7 Hz, anomeric H × 2), 4.95 (1H, d, J=2 Hz, 5′-H), 5.83 (1H, d, J=7 Hz, 1′-H). MS (%) m/z: 497 (14), 437 (6), 233 (12), 219 (33).

Alkaline Degradation Followed by $NaBH_4$ Reduction of 3f Giving 4a, 7, 8, and 9 A solution of **3f** (470 mg) in 0.2% NaOMe–MeOH (8 ml) was stirred at room temperature (24 °C) for 2 h. After neutralization with 10% HCl–dry MeOH, the solution was treated with $NaBH_4$ (150 mg) and the whole was stirred for 2 h. The reaction solution was neutralized with Dowex 50W × 8 (H^+ form) and filtered. Removal of the solvent from the filtrate under reduced pressure afford a residue, which was subjected to silica gel column chromatography [40 g, $CHCl_3$–MeOH (50 : 1→20 : 1)] to give **4a** (192 mg), **7** (43 mg), **8** (22 mg), and **9** (85 mg). 16,21,22,24,28-Penta-*O*-methylprotoaescigenin (**4a**) was identical with an authentic sample on the basis of TLC and IR (CCl_4) comparisons.

7: Colorless oil, $[\alpha]_D^{24}$ −1.97° (c=0.74, MeOH). High-resolution MS: Calcd for $C_{26}H_{51}O_{15}$ $(M+H)^+$: 603.323; Found : 603.323. IR (CCl_4): 3502, 1075 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.39 (6H), 3.41 (3H), 3.52 (6H), 3.55, 3.58 (3H each), 3.63 (6H) (all s, OCH_3 × 9), 4.47, 4.50 (1H each, both d, J=7 Hz, 1″, 1‴-H). MS (%) m/z: 603 [$(M+H)^+$, 1], 395 (10), 219 (24), 187 (100).

8: Colorless oil, $[\alpha]_D^{24}$ −14.5° (c=0.70, MeOH), High-resolution MS: Calcd for $C_{26}H_{51}O_{15}$ $(M+H)^+$: 603.323; Found: 603.324. IR (CCl_4): 3490, 1070 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.37 (3H), 3.39, 3.50, 3.61 (6H each), 3.62, 3.64 (3H each) (all s, OCH_3 × 9), 4.65, 4.73 (1H each, both d, J=7 Hz, 1″, 1‴-H). MS (%) m/z: 603 [$(M+H)^+$, 1], 395 (9), 299 (2),

219 (24), 187 (100).

9: Colorless oil, $[\alpha]_D^{30}$ −12.8° (c=1.91, MeOH). *Anal.* Calcd for $C_{26}H_{50}O_{15}$: C, 51.82; H, 8.36. Found: C, 51.71; H, 8.50. High-resolution MS: Calcd for $C_{26}H_{51}O_{15}$ $(M+H)^+$: 606.323; Found: 603.322. IR (CCl_4): 3494, 1075 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.37 (6H), 3.50 (9H), 3.58 (3H), 3.62 (9H) (all s, $OCH_3\times9$), 4.36, 4.46 (1H each, both d, J=7 Hz, 1″, 1‴-H). MS (%) m/z: 603 [$(M+H)^+$, 1], 395 (8), 299 (3), 219 (21), 187 (100)

Methanolysis of 7 Followed by Acetylation A solution of **7** (38 mg) in 10% HCl–dry MeOH (2 ml) was heated under reflux for 2 h. Work-up of the reaction solution as described for the methanolysis of **3b** gave a product, which was acetylated with Ac_2O–pyridine (1 : 1, 2 ml) at room temperature (34 °C) for 12 h. After work-up of the reaction mixture, the residue was separated by silica gel column chromatography [2 g, benzene–AcOEt (10 : 1)] to furnish methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (**e**, 12 mg) and 1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl-L-arabitol (10 mg). Methyl glucoside (**e**) was identified by GLC analysis (condition 6).

1,2,4,6-Tetra-*O*-acetyl-3-*O*-methyl-L-arabitol: Colorless oil, $[\alpha]_D^{27}$ −35.7° (c=0.57, MeOH), IR (CCl_4): 1750, 1246 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.03, 2.07 (3H each), 2.08 (6H) (all s, OAc×4), 3.50 (3H, s, OCH_3), 3.72 (1H, dd, J=3, 11 Hz, 3′-H). MS (%) m/z: 335 $(M+H)^+$, 189 (41), 129 (100), was identified by comparison of its physical data with the reported values {$[\alpha]_D$ −38° (MeOH)}.[26]

Methanolysis of 8 Followed by Acetylation A solution of **8** (30 mg) in 10% HCl–dry MeOH was heated under reflux for 2 h. Work-up of the reaction solution as described above furnished a residue, which was acetylated with Ac_2O–pyridine (1 : 1, 2 ml) to yield 1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl-D-arabitol (9 mg) and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (**e**, 10 mg), which were identified by GLC analysis (condition 6).

1,2,4,5-Tetra-*O*-acetyl-3-*O*-methyl-D-arabitol {$[\alpha]_D^{27}$ +41.9° (c=0.54, MeOH)} was identical with the L-isomer obtained from **7**, except for the sign of the specific rotation, on the basis of TLC, IR (CCl_4), and ^{1}H-NMR ($CDCl_3$) comparisons.

Methanolysis of 9 Followed by Acetylation A solution of **9** (5 mg) in 10% HCl–dry MeOH (1 ml) was heated under reflux for 2 h. After work-up of the reaction solution, the residue was dissolved in pyridine (0.1 ml) and the solution was treated with BSTFA (0.2 ml) for 1 h. The reaction solution was subjected to GLC analysis to identify methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**e**) and the TMS derivative of 3-*O*-methylxylitol (**g**). GLC conditions: 10) column, 5% butane-1,4-diol succinate on Uniport B (80—100 mesh), 3 mm×2 m; column temperature, 145 °C; carrier gas, N_2; flow rate, 25 ml/min; t_R, **e**: 15 min 8 s, 22 min 8 s (major); **g**: 14 min 50 s; 11) column, 5% silicone SE-52 on Chromosorb WAW DMCS (80—100 mesh), 3 mm×2 m; column temperature, 140 °C; carrier gas, N_2; flow rate, 30 ml/min; t_R, **e**: 5 min 16 s, 6 min 31 s (major); **g**: 18 min 22 s.

$LiAlH_4$ Degradation of 3f A solution of **3f** (68 mg) in dry ether (2 ml) was treated with $LiAlH_4$ (30 mg) and the mixture was stirred at room temperature (18 °C) for 2 h. After quenching of excess $LiAlH_4$ with wet ether, the reaction solution was neutralized with Dowex 50W×8 (H^+ form) and filtered. Removal of the solvent from the filtrate gave a residue, which was separated by preparative TLC [benzene–acetone (8 : 9)] to yield **4a** (24 mg) and **9** (10 mg). These were shown to be identical with the above-mentioned authentic samples by TLC and IR (CCl_4) comparisons.

Alkaline Hydrolysis of Escin Ib (2) Giving Desacylescin I (3) A solution of **2** (100 mg) in 50% aqueous dioxane (5 ml) was treated with 10% aqueous KOH (5 ml) and the mixture was stirred at 37 °C for 1 h. A part (0.1 ml) of it was evaporated to dryness under reduced pressure to give a residue, which was dissolved in $(CH_2)_2Cl_2$ (2 ml) and the solution was treated with *p*-nitrobenzyl-*N*,*N*′-diisopropylisourea (10 mg). The reaction solution was subjected to HPLC analysis to identify the *p*-nitrobenzyl esters of acetic acid (**a**) and angelic acid (**h**). HPLC conditions: the same as described above for alkaline hydrolysis of **1**, t_R, **a**: 8 min 0 s; **h**: 18 min 30 s. A major part of the alkaline hydrolysis solution was neutralized with Dowex HCR W×2 (H^+ form) and filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by silica gel column chromatography [3 g, $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to give **3** (78 mg), which was identical with an authentic sample on the basis of TLC and ^{13}C-NMR (pyridine-d_5) comparisons.

Alkaline Hydrolysis of Escins IIa (10) and IIb (11) Giving Desacylescin II (12) A solution of **10** (100 mg) in 50% aqueous dioxane (5 ml) was treated with 10% aqueous KOH (5 ml) and the mixture was stirred at 37 °C for 1 h. A part (0.1 ml) of it was worked-up as described above for alkaline hydrolysis of **1** and the *p*-nitrobenzyl esters of acetic acid and tiglic acid were identified by HPLC analysis (the same conditions as described above). The reaction solution was neutralized with Dowex HCR W×2 (H^+ form) and filtered. Removal of the solvent from the filtrate under reduced pressure yielded a residue, which was purified by silica gel column chromatography [3 g, $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to give **12** (82 mg).

A solution of **11** (100 mg) in 10% aqueous KOH–50% aqueous dioxane (1 : 1, 10 ml) was stirred at 37 °C for 1 h. By work-up of a part (0.1 ml) of the reaction mixture as described above, the *p*-nitrobenzyl esters of acetic acid and angelic acid were identified by HPLC analysis. From the remainder of the reaction mixture, **12** (83 mg) was obtained by the same procedure as described above.

Desacylescin II (**12**): Colorless fine crystals from $CHCl_3$–MeOH–H_2O, mp 234.0—236.2 °C, $[\alpha]_D^{25}$ +12.0° (c=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{47}H_{76}NaO_{20}$ $(M+Na)^+$: 999.4777; Found: 999.4804. IR (KBr): 3451, 1736, 1638, 1076 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.76, 0.87, 1.30, 1.34, 1.36, 1.83 (3H each, all s, 25, 26, 29, 30, 23, 27-$H_3\times6$), 2.75 (1H, dd-like, 18-H), 3.41 (1H, dd-like, 3-H), 4.51 (1H, d-like, 22-H), 4.71 (1H, d, J=9.2 Hz, 21-H), 4.92 (1H, d, J=6.6 Hz, 3-*O*-Glc.A-1′-H), 4.97 (1H, br s, 16-H), 5.15 (1H, d, J=7.6 Hz, 4′-*O*-Glc.-1‴-H), 5.21 (1H, d, J=7.1 Hz, 2′-*O*-Xly.-1″-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Positive-mode FAB-MS (m/z): 999 $(M+Na)^+$.

Methanolysis of Desacylescin II (12) A solution of **12** (55 mg) in 9% HCl–dry MeOH (1.5 ml) was heated under reflux for 1 h. After cooling, the reaction solution was neutralized with IRA-400 (OH^- form) and filtered. Evaporation of the solvent from the filtrate under reduced pressure gave a residue (50 mg) and 5 mg of the residue was dissolved in pyridine (0.1 ml) and BSTFA (0.2 ml). The reaction solution was subjected to GLC analysis to identify the TMS derivatives of methyl glucoside (**c**), methyl glucuronide (**d**), and methyl xyloside (**i**). GLC condition 3): t_R, **i**: 15 min 30 s, 16 min 0 s. The residue (50 mg) was purified by silica gel column chromatography [2 g, $CHCl_3$–MeOH (40 : 1)] to furnish **4** (13 mg), which was identical with an authentic sample on the basis of TLC and ^{13}C-NMR (pyridine-d_5) comparisons.

Alkaline Hydrolysis of Escin IIIa (13) Giving Desacylescin III (14) A solution of **13** (20 mg) in 10% aqueous KOH–50% aqueous dioxane (1 : 1, 10 ml) was stirred at 37 °C for 1 h. The reaction solution (0.1 ml) was worked-up as described above for alkaline hydrolysis of **1** and the *p*-nitrobenzyl esters of acetic acid and tiglic acid were identified by HPLC analysis (the same conditions as described above). The rest of the reaction solution was neutralized with Dowex HCR W×2 (H^+ form) and filtered. 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 (6 : 4 : 1)] to yield **14** (15 mg).

Desacylescin III (**14**): Colorless fine crystals from $CHCl_3$–MeOH–H_2O, mp 230.0—231.8 °C, $[\alpha]_D^{25}$ +14.4° (c=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{48}H_{78}NaO_{21}$ $(M+Na)^+$: 1013.4991; Found: 1013.4945. IR (KBr): 3410, 1736, 1638, 1076 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.86, 0.91, 1.08, 1.26, 1.31, 1.35, 1.84 (3H each, 25, 26, 24, 23, 29, 30, 27-$H_3\times7$), 2.74 (1H, dd-like, 18-H), 3.37 (1H, dd-like, 3-H), 4.51 (1H, d-like, 22-H), 4.72 (1H, d, J=9.6 Hz, 21-H), 4.96 (1H, d, J=6.9 Hz, 3-*O*-Glc.A-1′-H), 5.00 (1H, br s, 16-H), 5.15 (1H, d, J=7.6 Hz, 4′-*O*-Glc.-1‴-H), 5.20 (1H, d, J=7.6 Hz, 2′-*O*-Gal.-1″-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Positive-mode FAB-MS (m/z): 1013 $(M+Na)^+$.

Methanolysis of Desacylescin III (14) Giving Barringtogenol C (15) A solution of **14** (25 mg) in 9% HCl–dry MeOH (2.5 ml) was heated under reflux for 2 h. The reaction mixture (0.5 ml) was neutralized with Ag_2CO_3 and filtered. After evaporation of the solvent from the filtrate to dryness under reduced pressure, the residue was dissolved in pyridine (0.1 ml) and BSTFA (0.2 ml) and the mixture was left standing for 1 h. The reaction mixture was subjected to GLC analysis to identify the TMS derivatives of methyl glucoside (**c**), methyl glucuronide (**d**), and methyl galactoside (**j**). GLC condition 3): t_R, **j**: 19 min 0 s, 19 min 56 s.

The reaction mixture (2.0 ml) was neutralized with IRA-400 (OH^- form) and filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by silica gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to afford **15** (11 mg), which was identical with an authentic sample[25] on the basis of TLC, IR (KBr), and ^{1}H-NMR (pyridine-d_5) comparisons.

Bioassay Tests for Inhibitory Activity on Ethanol Absorption and Hypoglycemic Activity The methods of bioassay testing were the same as those used on senegasaponins and senegins from Senegae Radix, as described in our previous papers.[1,8,10]

Acknowledgement The authors are grateful to the Ministry of Education, Science, Sports and Culture of Japan for a Grant-in-Aid for Scientific Research (B) (Grant No. 07557292).

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