ESCINS-Ia, Ib, IIa, IIb, AND IIIa, BIOACTIVE TRITERPENE OLIGOGLYCOSIDES FROM THE SEEDS OF *AESCULUS HIPPOCASTANUM* L. : THEIR INHIBITORY EFFECTS ON ETHANOL ABSORPTION AND HYPOGLYCEMIC ACTIVITY ON GLUCOSE TOLERANCE TEST

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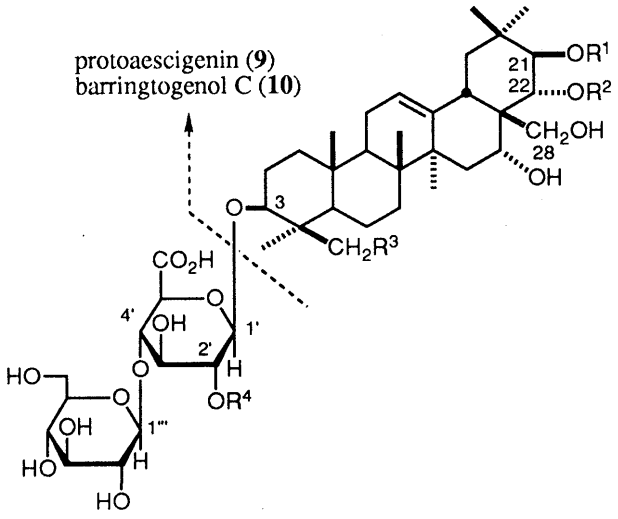
Five triterpene oligoglycosides named escins-Ia, Ib, IIa, IIb, and IIIa were isolated from the seeds of *Aesculus hippocastanum* L. and their chemical structures were determined on the basis of chemical and physicochemical evidence. Escins-Ia, Ib, IIa, and IIb were found to exhibit inhibitory effect on ethanol absorption and hypoglycemic activity on oral glucose tolerance test in rats. Among them, escins-IIa and IIb showed the higher activities for both bioassays, while desacylescins-I and II had no activity.

KEYWORDS escin IIa; escin IIb; *Aesculus hippocastanum*; ethanol absorption inhibitor; hypoglycemic activity; oral glucose tolerance test

Escin, one of the most important saponin constituents, is known as a mixture of saponins occurring in the seed of *Aesculus hippocastanum* L. (Seiyou-tochinoki in Japanese, Hippocastanaceae). Because of its antiinflamatory, anti-edamatous, and capillaro-protective proparties, escin is widely employed in therapy of peripheral vascular disorders and also in cosmetic field for prevention and treatment of cellulitis.[1] In regard to the chemical study of escin, it has been extensively investigated to shed light on its complex and unstable structure, so that the structures of two major saponins were presumed on the basis of chemical and physicochemical evidence obtained by using the saponin mixture or sapogenol mixture.[2] Recently, it was reported that the two major saponins were isolated from the commercial β-escin and then only their MS data were examined on the basis of the previous presumed structures.[3]



	R^1	R^2	R^3	R^4
escin-Ia (**1**):	tigloyl	Ac	OH	D-Glu
escin-Ib (**2**):	angeloyl	Ac	OH	D-Glu
escin-IIa (**3**):	tigloyl	Ac	OH	D-Xyl
escin-IIb (**4**):	angeloyl	Ac	OH	D-Xyl
escin-IIIa (**5**):	tigloyl	Ac	H	D-Gal
desacylescin-I (**6**):	H	H	OH	D-Glu
desacylescin-II (**7**):	H	H	OH	D-Xyl
desacylescin-III (**8**):	H	H	H	D-Gal
protoaescigenin (**9**):	H	H	OH	
barringtogenol C (**10**):	H	H	H	

D-Glu : β-D-glucopyranosyl
D-Xyl : β-D-xylopyranosyl
D-Gal : β-D-galactopyranosyl

During the course of our studied on the bioactive constituents of natural medicines, several saponins such as elatosides[4] from *Aralia elata* SEEM. and camelliasaponins[5] from *Camellia japonica* L. were found to show potent inhibitory effect on ethanol absorption. As a continuing part of our screening to find a new bioactive function of saponin, we have isolated five saponins named escins-Ia(**1**), Ib(**2**), IIa(**3**), IIb(**4**), and IIIa(**5**) from the seeds of *Aesculus hippocastanum* L. This communication deals with the structure elucidation of escins-Ia(**1**),[6] Ib(**2**),[6] IIa(**3**), IIb(**4**), and IIIa(**5**) and the inhibitory activities of **1**-**4** on ethanol absorption and on the elevation of plasma glucose level by oral glucose tolerance test in rats.

The alcoholic extract and saponin fraction from the seeds showed inhibitory effect on ethanol absorption and hypoglycemic activity. The saponin fraction with potent activities was purified by repeated HPLC separation to afford escins-Ia(**1**, 23.9 % from saponin mixture), Ib(**2**, 17.1 %), Ia(**3**, 13.6 %), IIb(**4**, 6.0 %), and IIIa(**5**, 1.5 %). Escin IIa(**3**), colorless fine crystals, mp 206.5-208.5°C, $[\alpha]_D$ -35.5°(MeOH), $C_{54}H_{84}O_{23}$, IR(KBr) : 3450, 1733, 1719, 1653, 1647, 1638, 1075 cm^{-1}, ^1H NMR (d_5-pyridine, δ) : 4.19(d, J = 7.3, 1'-H), 5.18(d, J = 7.9, 1'''-H), 5.57(d, J = 7.3, 1''-H), 6.21(d, J = 10.2, 22-H), 6.56(d, J = 10.2, 21-H), FAB-MS(m/z) : 1123(M+Na)$^+$, liberated acetic acid, tiglic acid, and desacylescin-II(**7**)[7] upon alkaline hydrolysis. Methano-

Table I. ^{13}C NMR Data for **1**, **2**, **3**, **4**, **5**, **6**, and **7** (68 MHz, d5-Pyridine, δc)

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

lysis of **7** furnished protoaescigenin(**9**)[8] together with methyl D-glucoside, methyl D-glucuronide, and methyl D-xyloside in a 1 : 1 : 1 ratio . The assignment of ^1H NMR(d5-pyridine, J in Hz) and ^{13}C NMR(Table I) data of **3**, **7**, and **9** was completely accomplished by COSY(^1H-^1H, ^1H-^{13}C), HMBC and HOHAHA(^1H-^1H, ^1H-^{13}C) experiment. HMBC correlations were observed between the following carbons and protons in the acyl and oligosaccharide moieties of **3** : [21-H&1''''-C (tigloyl carbonyl), 22-H&1''''-C(acetyl carbonyl), 1'-H&3-C, 1''-H&2'-C, 1'''-H&4'-C]. Based on these findings together with ROESY data for **3** and ^{13}C NMR comparisons for **3**, **7**, and **9**, the structure of escin-IIa has been determined as **3**.

Alkaline hydrolysis of escin-IIb(**4**)[9] furnished acetic acid, angelic acid, and **7**. Comparisons of ^1H NMR and ^{13}C NMR data for **4** with those for **3**, **7**, and **9** have corroborated the structure of escin IIb(**4**) as shown. The structures of escins-Ia(**1**),[10] Ib(**2**),[11] and IIIa(**5**)[12] were elucidated in the same way. By alkaline hydrolysis, **1** and **2** yielded desacylescin-I(**6**), which was identical with desacyl-esculoside I (aesculuside-B),[13] and organic acid(**1** : acetic acid and tiglic acid; **2** : acetic acid and angelic acid), while **5** furnished acetic acid, tiglic acid, and desacylescin-III(**8**)[14] which liberated methyl D-galactoside, methyl D-glucoside, and methyl D-glucuronide in a 1 : 1 : 1 ratio and barringtogenol(**10**) upon methanolysis. Finally detailed examinations of ^1H NMR and ^{13}C NMR experiments as described for **3** led us to furnish the structures of escin-Ia(**1**), Ib(**2**) and IIIa(**5**).

Inhibitory effects of escins-Ia(**1**), Ib(**2**), IIa(**3**) and IIb(**4**) and desacylescins-I(**6**) and II(**7**) on ethanol absorption are summarized in Table II. Escins(**1**-**4**) were found to exhibit inhibitory effect on ethanol absorption, while desacylescins(**6**, **7**) showed no activity. On the other hand, hypoglycemic effects of escins (**1**-**4**) and desacylescins(**6**, **7**) on oral glucose tolerance test are summarized in Table III. Escins(**1**-**4**) also showed hypoglycemic effect and desacylescins(**6**, **7**) did not have the activity, indicating that the acyl moiety in escins was essential to exerting the activities in these two bioassays. It is noteworthy that escins-IIa(**3**) and IIb(**4**) having 2'-*O*-xylopyranosyl group in the oligosaccharide moiety showed much more potent activities for both bioassays than escins-Ia(**1**) and Ib(**2**) having 2'-*O*-glucopyranosyl group. We are currently working on the further characterization of structure-activity relationships for acylated triterpene oligoglycosides.

Table II. Inhibitory Effects of Escins-Ia(**1**), Ib(**2**), IIa(**3**), and IIb(**4**) and Desacylescins-I(**6**) and II(**7**) on Ethanol Absorption

	Dose (mg /kg, p.o.)	n	Ethanol concentration in blood (mg / ml) 1 h	2 h	3 h
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**	0.20±0.01	0.04±0.02
Escin-IIa(**3**)	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(**4**)	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
Desacyl-escin-I(**6**)	100	5	0.54±0.02	0.23±0.02	0.02±0.00
Desacyl-escin-II(**7**)	100	5	0.54±0.01	0.20±0.01	0.02±0.00

* $p<0.05$, ** $p<0.01$

Table III. Inhibitory Effects of Escins-Ia(**1**), Ib(**2**), IIa(**3**), and IIb(**4**) and Desacylescins-I(**6**) and II(**7**) on the Elevation of Plasma Glucose Level by Oral Glucose Tolerance Test

	Dose (mg /kg, p.o.)	n	Plasma glucose concentration (mg / dl) 0.5 h	1 h	2 h
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(**3**)	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(**4**)	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)
Desacyl-escin-I (**6**)	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)
Desacyl-escin-II (**7**)	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)

* $p<0.05$, ** $p<0.01$

Each sample was orally administered to rats 30 min before oral administration of D-glucose(0.5g / kg). Values in parenthesis showed the difference in plasma glucose concentration between normal control and each sample treatment.

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