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Three New Triterpenoid Saponins from the Seeds of *Aesculus chinensis*

Xiu Wei YANG*, Jing ZHAO, Yu Xin CUI

National Laboratory of Natural and Biomimetic Drugs, Beijing Medical University, Beijing 100083

Abstract: Three new triterpenoid saponins, escins IVg (**1**), IVh (**2**) and VIb (**3**) were isolated from the seeds of *Aesculus chinensis* along with two known saponins, escin IIIa (**4**) and desacylescin I (**5**). Their structures were elucidated by spectroscopic analysis and chemical hydrolysis.

Keywords: *Aesculus chinensis*, Hippocastanaceae, escins IIIa, IVg, IVh and Vib, desacylescin I.

Escins, the anti-inflammatory principle from the seeds of *Aesculus chinensis* Bge. (Hippocastanaceae) consist of a series of analogous pentacyclic triterpenoid oligo-glycosides. During our work on the saponin constituents from the seeds, we have isolated and identified 8 saponins, *i. e.*, escins Ia, Ib, IVa, IVb, IVc, IVd, IVe, IVf isoescins Ia and Ib [1 - 4]. In continuation of our study, five more triterpenoid saponins were purified by preparative HPLC and identified on the basis of spectroscopic evidences. Among them, escin IIIa (**4**) and desacylescin I (=aesculuside B, **5**) were identified by comparison of spectral data with the reported literature data[5, 6]. In this paper, we report the structure elucidation of three new compounds.

Compound **1** was isolated as white amorphous powder. MALDI-TOF-MS showed quasimolecular ion peaks at *m/z* 1111 $[M+Na]^+$ and 1127 $[M+K]^+$, corresponding to the composition of $C_{53}H_{84}O_{23}$. Alkaline hydrolysis of **1** with 1% MeONa liberated the substance which had the same retention time with authentic desacylescin I in HPLC analysis and acid hydrolysis of **1** yielded glucose and glucuronic acid. The ^1H and ^{13}C NMR signals were assigned by a combination of HMQC, HMBC and NOESY spectra which included signals due to the protoaescigenin skeleton[1] [^1H NMR: δ0.62 (Me-25), 0.77 (Me-26), 1.26 (Me-23), 1.31 (Me-29), 1.40 (Me-30) and a broad singlet at δ5.42 (H-12)], a trisaccharide moiety [^1H NMR: β-anomeric protons at δ5.55 (d, *J*=8Hz, H-1''), 5.21 (d, *J*=8Hz, H-1''') and 4.77 (d, *J*=8Hz, H-1'); ^{13}C NMR: anomeric carbons at δ104.2 (C-1' and C-1''') and 104.1 (C-1'')] and a tigloyl group [^1H NMR: δ6.98 (H-3''''), 1.80 (Me-5'''') and 1.44 (Me-4''''); ^{13}C NMR: δ169.3 (C-1''''), 129.4 (C-2''''), 136.7 (C-3''''), 13.9 (C-4'''') and 12.2 (C-5'''')]. The attachment of the tigloyl group at C-22 was deduced from HMBC experiment, which showed long-range correlation

between H-22β (δ6.16) and the carbonyl carbon (δ169.3) of the tigloyl group. Furthermore, the trisaccharidie structure was characterized by HMBC correlations between the following pairs: C-3 (δ90.9) and H-1'; C-2' (δ79.5) and H-1" and C-4' (δ82.7) and H-1'". Comparison of the ^{13}C NMR spectral data ascribable to the sugar moiety revealed the identical trisaccharide unit with that of desacylescin I[5] and escin Ia[1] which was definitely identified by single crystal X-ray analysis. On the basis of the above evidences, the structure of **1** was determined as 22α-O-tigloylprotoaescigenin-3β-O-[β-D-glucopyra- nosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid, and named escin IVg.

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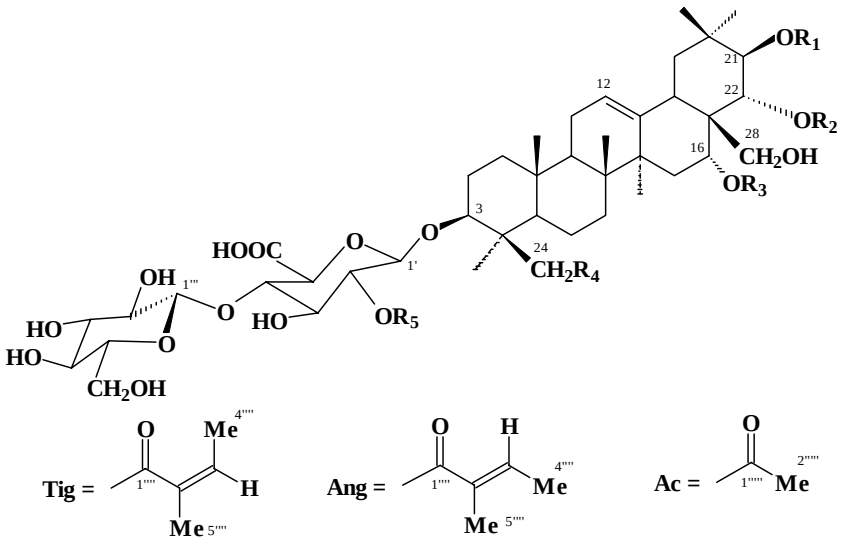
Figure Structures of Compounds **1**-**5**



Glu=β-D-glucopyranosyl
Gal=β-D-galactopyranosyl

	R_1	R_2	R_3	R_4	R_5
1	H	Tig	H	OH	Glu
2	H	Ang	H	OH	Glu
3	Ac	H	Ang	OH	Glu
4	Tig	Ac	H	H	Gal
5	H	H	H	OH	Glu

Compound **2** was isolated as white amorphous powder. The MALDI-TOF-MS showed the quasimolecular ion peaks at *m/z* 1111 $[M+Na]^+$ and 1133 $[M+2Na-H]^+$, corresponding to the molecular formula of $C_{53}H_{84}O_{23}$, identical with compound **1**. Comparison of the ^1H and ^{13}C NMR spectra with those of **1** led to the conclusion that an angeloyl group appeared in the structure instead of a tigloyl group. The HMBC experiment confirmed the same acylation position and the same structure of the trisaccharide moiety and alkaline hydrolysis of **2** also yielded desacylescin I. Therefore,

2 was established as 22α-O-angeloylprotoaescigenin-3β-O-[β-D-glucopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid, and named escin IVh. Compounds **1** and **2** are a pair of geometrical isomers.

Table ^{13}C NMR Spectral Data for Escins IVg (**1**), IVh (**2**), VIb (**3**), IIIa (**4**) and Desacylescin I (**5**) (δ values)[a]

C	1	2	3	4	5	C	1	2	3	4	5
1	38.3	38.3	38.4	38.8	38.3	1'	104.2	104.2	104.7	104.6	104.5
2	26.2	26.2	26.4	26.3	26.4	2'	79.5	79.6	79.4	82.5	79.5
3	90.9	90.9	91.2	89.9	90.9	3'	76.5	76.4	76.7	76.8	76.4
4	43.4	43.4	43.6	39.4	43.5	4'	82.7	82.7	82.4	81.2	81.9
5	55.9	55.9	56.0	55.7	55.9	5	75.4	75.5	75.8	74.2	75.5
6	18.4	18.3	18.4	18.4	18.4	6'	175.0	175.1	172.9	175.3	172.9
7	33.0	33.0	33.1	33.1	33.0	1"	104.1	103.9	104.1	105.2	104.0
8	39.7	39.7	39.9	40.0	39.8	2"	75.4	75.5	75.6	74.7	75.5
9	46.5	46.5	46.7	46.8	46.6	3"	78.1	78.2	78.2	74.7	78.1
10	36.2	36.2	36.3	36.4	36.3	4"	69.6	69.6	69.8	71.8	69.6
11	23.9	23.9	23.9	23.9	23.9	5"	77.4	77.4	78.0	76.6	77.9
12	123.2	123.0	123.2	123.3	122.7	6"	61.4	61.4	61.6	61.9	61.4
13	143.1	143.1	141.8	142.8	143.8	1'"	104.2	104.3	104.7	104.3	104.5
14	40.2	40.2	41.3	41.6	41.8	2'"	74.8	74.8	73.9	74.2	74.8
15	34.6	34.6	30.9	34.6	34.1	3'"	77.9	78.2	78.4	78.1	78.3
16	68.9	69.0	71.5	68.0	67.7	4'"	71.2	71.1	71.5	71.3	71.3
17	48.0	47.7	47.6	47.9	47.2	5'"	77.8	77.8	77.9	77.8	77.8
18	41.4	41.4	39.9	40.0	41.0	6'"	61.9	61.9	62.2	62.8	62.2
19	47.5	47.5	47.2	47.2	48.1	1""	169.3	169.4	168.3	167.9	
20	36.8	36.9	36.0	36.7	36.2	2""	129.4	129.3	129.2	129.4	
21	76.5	76.6	79.8	79.3	78.5	3""	136.7	136.5	136.0	136.8	
22	77.4	77.1	71.0	74.2	77.0	4""	13.9	15.8	15.9	14.2	
23	22.3	22.3	22.4	28.0	22.2	5""	12.2	20.8	21.0	12.4	
24	63.1	63.1	63.2	16.8	63.2	1""'			170.0	170.9	
25	15.4	15.4	15.5	15.7	15.4	2""'			22.2	20.8	
26	16.5	16.5	16.6	16.8	16.5						
27	27.3	27.4	27.0	27.4	27.2						
28	63.9	64.1	64.8	63.7	68.0						
29	30.1	30.1	30.0	29.5	30.4						
30	19.2	19.2	20.1	20.1	19.3						

[a] Measured in pyridine-d_5.

Compound **3** was obtained as white amorphous powder. The MALDI-TOF-MS exhibited molecular ion peak at *m/z* 1153 $[M+Na]^+$ corresponding to the molecular formula of $C_{55}H_{86}O_{24}$. It suggested that it may have the same molecular composition as escin Ia[1]. The ^{1}H and ^{13}C NMR spectra exhibited signals assignable to the proto-aescigenin skeleton[1], an angeloyl group [^{1}H NMR: δ5.87 (H-3""), 1.90 (Me-5"") and 1.98 (Me-4""); ^{13}C NMR: δ168.3 (C-1""), 129.2 (C-2""), 136.0 (C-3""), 15.9 (C-4"") and 21.0 (C-5"")], an acetyl group [^{1}H NMR: δ2.50 (Me-OAc); ^{13}C NMR: δ170.0 (C-1"""") and 22.2 (C-2"")] and a trisaccharide moiety [^{1}H NMR: δ4.79 (d, *J*=7.5Hz, H-1'), 5.33 (d, *J*=7.5Hz, H-1'") and 5.60 (d, *J*=7.5Hz, H-1"); ^{13}C NMR: δ104.7 (C-1' and C-1'") and 104.1 (C-1")]. Compared with the protoaescigenin skeleton of desacylescin I, however, C-16 shifted downfield for 3.8ppm while C-15 shifted upfield

for 3.2ppm. HMBC spectrum showed the correlation peak between H-16β (δ5.90) and the carbonyl carbon (δ168.3) of the angeloyl group. Thus the linkage site of the angeloyl group must be at C-16. The acetyl group was determined to linked to C-21 by deshielding of 1.3 ppm for C-21 and shielding of 6.0ppm for C-22 relative to desacylescin I and further suggested by NOE correlation between H-21α (δ5.95) and Me-OAc (δ2.50) in the NOESY spectrum. The sugar moiety was determined to be identical with desacylescin I for the chemical shifts of the sugar moiety in the ^{1}H and ^{13}C NMR spectra were coincident with those of desacylescin I, and the alkaline hydrolysate of **3** was desacylescin I. Consequently, the structure of **3** was confirmed as 16α-O-angeloyl-21β-O-acetylprotoaescigenin-3β-O-[β-D-glucopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)] -β-D-glucopyranosiduronic acid, and named escin VIb. It was the first C-16 acylated saponin ever isolated from escins.

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