A Pair of New Geometrically Isomeric Triterpenoid Saponins from the Seeds of *Aesculus chinensis*

Xiu Wei YANG*, Jing ZHAO, Yu Xin CUI, Li He ZHANG

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

Abstract: Two new triterpenoid saponins were isolated from the seeds of *Aesculus chinensis* by preparative HPLC. On the basis of chemical evidence and spectral data, their structures were established as 21β-tigloyl-28-acetylprotoaescigenin 3β-O-[β-D-xylopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid (isoescin IIa, **1**) and 21β-tigloyl-28-acetylprotoaescigenin 3β-O-[β-D-xylopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid (isoescin IIb, **2**), which are a pair of geometrical isomers.

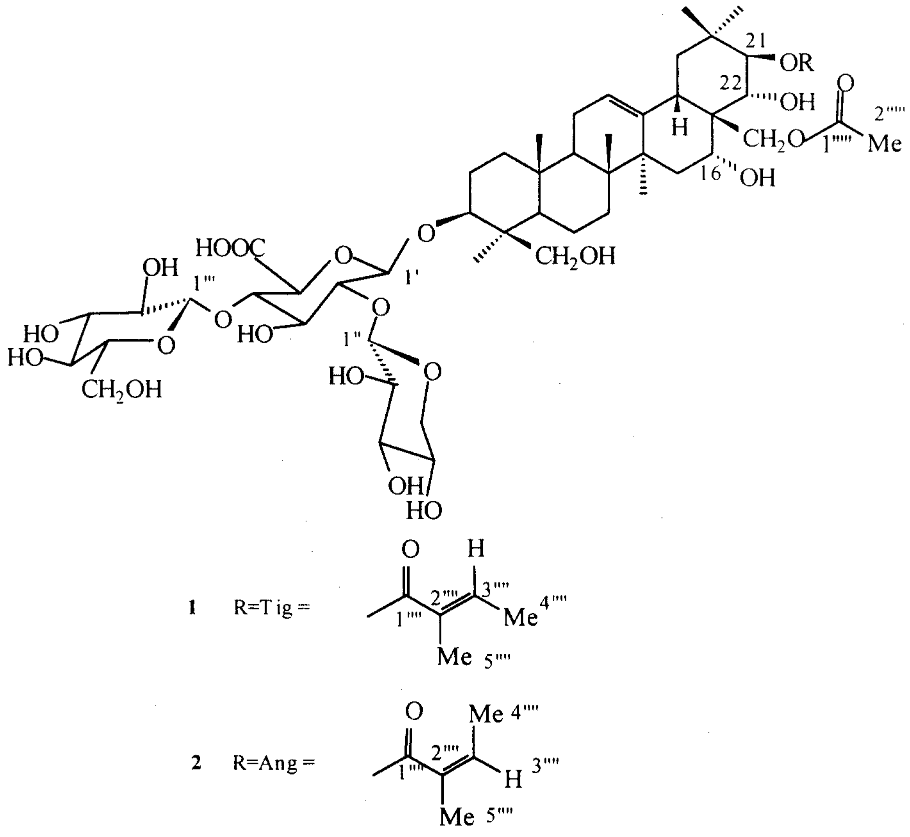
Keywords: *Aesculus chinensis*, isoescin **IIa** and **IIb**.

Aesculus chinensis Bge. (Hippocastanaceae) is one of the sources of traditional Chinese medicine "suo luo zi", which is specified in Chinese Pharmacopoeia (version 95)[1]. The bioactive saponin mixtures extracted from its seeds are known as escins, widely employed in the treatment of peripheral vascular diseases and in the cosmetic field in China. In previous papers, we have reported the isolation and identification of ten triterpenoid oligoglycosides, namely, escins Ia, Ib, IVa, IVb, IVc, IVd, IVe, IVf, IVg and IVh from escins[2, 3, 4]. In continuation of our work, another pair of new geometrically isomeric saponins, named isoescins IIa (**1**) and IIb (**2**) were isolated and identified by spectroscopic means and acid hydrolysis.

Compound **1** was isolated as an amorphous powder. Its IR spectrum showed absorption bands at 1713 and 1604cm^{-1} for α, β-unsaturated ester and the carboxyl group, and broad bands at 3416 and 1075cm^{-1} for hydroxyl groups, characteristic of the oligoglycosidic structure. The MALDI-TOF-MS exhibited a quasimolecular ion peak at *m/z* 1123 $[M+Na]^+$, corresponding to the molecular composition of $C_{54}H_{84}O_{23}$. Meanwhile, fragmental peaks at *m/z* 943 and 829 suggested the loss of a hexose and a pentose successively.

The ^{13}C NMR spectrum showed 54 carbon signals, among which olefinic resonances at δ123.0 and 142.6 were diagnostic of unsaturation at the 12-position in an oleanane skeleton, in conformity with the appearance of one broad singlet at δ5.44 in the ^{1}H NMR spectrum. The existence of oxy-substituent in the aglycone moiety was suggested by carbon signals at δ90.5, 67.5, 81.5, 71.2, 62.8 and 66.3. In the ^{1}H NMR spectrum, six quaternary methyl groups were observed at δ0.65 (Me-25), 0.88 (Me-26), 1.04 (Me-29), 1.27 (Me-23 and -30) and 1.78 (Me-27). All of the above NMR features accorded with the protoaescigenin[5] skeleton of the saponin. In addition, the presence of a tigloyl group was deduced by carbon resonances at δ168.5, 129.7, 136.3, 14.0 and 12.3 together with ^{1}H signals at δ1.55 (Me-4''''), 1.80 (Me-5'''') and 6.97 (H-3''''), while signals at δ170.7 and 20.7 in the ^{13}C NMR spectrum and δ1.97 (Me-2''''') in the ^{1}H NMR spectrum were assigned to an acetyl group. The attachments of the tigloyl group at C-21 and the acetyl group at C-28 were determined by the HMBC experiment and further confirmed by comparison of ^{13}C chemical shifts of **1** with those of escin IVa[2].

Scheme. Structures of Isoescins IIa and IIb



The presence of three β-anomeric monosaccharides was determined by anomeric carbons at δ104.5, 104.7 and 104.7 and corresponding protons at δ4.76 (d, *J*=7.5Hz, H-1'), 5.50 (d, *J*=7.0Hz, H-1'') and 5.19 (d, *J*=7.5Hz, H-1'''). On mild acid hydrolysis, **1** yielded glucuronic acid, glucose and xylose, revealing that it has a different trisaccharide moiety from previously isolated saponins. The chemical shifts of the sugar carbon atoms were found to be coincident with those of escin IIa[6], thus establishing the substitutions of a β-D-xylopyranosyl moiety and a β-D-glucopyranosyl moiety at C-2 and C-4 of a β-D-glucopyranosiduronic acid, which linked to C-3 of the aglycone.

Therefore the structure of **1** was elucidated as 21β-tigloyl-28-acetylprotoaescigenin 3β-O-[β-D-xylopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid, and named isoescin IIa.

Compound **2** was also obtained as an amorphous powder. In the IR spectrum, absorption bands due to hydroxyl groups were observed at 3414 and 1024cm^{-1} along with those due to the α, β-unsaturated ester and the carboxyl group at 1710 and 1604cm^{-1}. The MALDI-TOF-MS showed a quasimolecular ion peak at *m/z* 1123 [M+Na]$^+$, identical with **1**. Fragmental peaks at *m/z* 943 and 829 were also observed for **2**.

Table: ^{13}C NMR Spectral Data of Compounds **1** and **2** (δ Relative to TMS in pyridine-d_5, 125MHz)

C	1	2	C	1	2
1	38.7	38.7	1'	104.5	104.5
2	26.3	26.4	2'	79.5	78.9
3	90.5	90.5	3'	76.6	76.5
4	44.1	44.1	4'	83.2	83.3
5	56.2	56.2	5'	75.5	75.5
6	18.5	18.6	6'	175.1	175.5
7	33.2	33.2	1''	104.5	104.5
8	39.8	39.8	2''	75.5	75.5
9	46.6	46.6	3''	78.8	78.8
10	36.4	36.4	4''	70.7	70.7
11	24.0	24.0	5''	66.9	66.9
12	123.0	123.0	1'''	104.7	104.5
13	142.6	142.6	2'''	74.9	74.8
14	41.7	41.7	3'''	78.3	78.3
15	34.5	34.5	4'''	71.1	71.0
16	67.5	67.5	5'''	77.5	77.5
17	47.0	47.0	6'''	62.0	62.0
18	40.5	40.5	1''''	168.5	168.5
19	47.2	47.2	2''''	129.7	129.4
20	36.2	36.0	3''''	136.3	136.1
21	81.5	81.1	4''''	14.0	15.8
22	71.2	71.2	5''''	12.3	20.9
23	22.5	22.6	1'''''	170.7	170.6
24	62.8	62.8	2'''''	20.7	20.6
25	15.4	15.4			
26	16.8	16.8			
27	27.3	27.3			
28	66.3	66.3			
29	29.7	29.7			
30	20.0	20.1			

The ^{1}H and ^{13}C chemical shifts of **2** resembled those of **1**, which also exhibited signals belonging to the protoaescigenin skeleton, a trisaccharide moiety and an acetyl group. However, signals due to a tigloyl group disappeared. Instead, carbon signals at δ168.5, 129.4, 136.1, 15.8 and 20.9 and proton signals at δ1.94 (Me-5''''), 2.01 (Me-4'''') and 5.87 (H-3'''') were observed indicating the replacement by an angeloyl group. Furthermore, glucuronic acid, glucose and xylose were also detected by PC after acid hydrolysis of **2**.

Consequently, **2** was identified as 21β-angeloyl-28-acetylprotoaescigenin 3β-O-[β-D-xylopyranosyl (1→2)] [β-D-glucopyranosyl (1→4)]-β-D-glucopyranosiduronic acid, and named isoescin IIb. It is a geometrical isomer of **1**.

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