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A New Triterpenoid Saponin from *Anemone tomentosa*

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Abstract: A new triterpenoid saponin, tomentoside (**5**), together with prosapogenin CP 4 (**1**), huzhangoside B (**2**), C (**3**) and D (**4**) was isolated from the ethanolic extract of *Anemone tomentosa*. Its structure was established by spectroscopic and chemical evidence.

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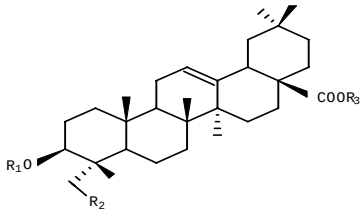
Keywords: *Anemone tomentosa*, triterpenoid saponin, tomentoside.

The roots of *Anemone tomentosa* (Maxim.) Péi are used as a folk medicine for the treatment of dysenteria in China[1]. Recently, two new coumarins and nine known compounds were isolated from the ethyl acetate extract which exhibited strong antifeedant activity against *Leucania Separata* Walker[2]. During the further investigation of the title plant, a new minor saponin, tomentoside, together with four known ones was isolated.

Tomentoside (**5**), amorphous powder, $[\alpha]_D^{25}$ -41.5(MeOH; *c* 0.26). Molecular formula was determined as $C_{70}H_{114}O_{34}$ by HRFABMS, *m/z*: 1497.7166 ($[M\text{-}H]^-$), calcd. 1497.7113. On acid hydrolysis, **5** gave oleanolic acid as aglycone and four kinds of monosaccharides, which were identified by TLC and PC as glucose, rhamnose, xylose and ribose respectively. Alkaline hydrolysis of **5** gave prosapogenin **5a**, whose ^{13}C NMR spectum disclosed the presence of 4 anomeric carbons (δ106.2, 104.3, 103.4 and 101.5).



	R_1	R_2	R_3
1	rib—(3)rha—(2)ara-	H	H
2	**rib—(3)rha—(2)**ara-	H	—glc(6)—glc(4)—rha
3	**rib—(3)rha—(2)**ara-	OH	—glc(6)—glc(4)—rha
4	**rib—(3)rha—(2)xyl-**	H	—glc(6)—glc(4)—rha
4a	rib—(3)rha—(2)xyl-	H	H
5	glc—(4)rib—(3)rha—(2)xyl-	H	—glc(6)—glc(4)—rha
5a	glc—(4)rib—(3)rha—(2)xyl-	H	H

Comparing the ^{13}C NMR signals with those of huzhangoside A (**4a**)[3], **5a** was found to contain one more glucose. The signals ascribable to aglycone and the inner two sugars (xylose and rhamnose) of the sugar chain were the same with those of **4a**, while C-4 of ribose was displaced downfield by 7.7 ppm, C-3 and C-5 upfield by 0.9 and 2.9 ppm

respectively. According to the rule of glycosylation shift, the additional glucose must be linked to C-4 of ribose. The ^{13}C NMR signals of compound **5** minus that of **5a** revealed that the ester-type sugar chain at C-28 hydrolysised by alkaline was –glc (6→1)-glc (4→1)-rha[3].

. Except for $[M]^-$ at *m/z* 1498, FAB^- mass spectrum showed a series of fragment ions at *m/z*: 1028 $(M\text{-}2glc\text{-}rha)^-$, 866 $(1028\text{-}glc)^-$, 734 $(866\text{-}rib)^-$, 587 $(734\text{-}rha)^-$ and 454 $(587\text{-}xyl)^-$, indicating the successive elimination of 2glc+rha, glc, rib, rha and xyl. This evidence supported the deduction of the sugar sequence by NMR data above.

Therefore, the structure of tomentoside can be established as 3-O-β-D-glucopyranosyl (1→4)-β-D-ribopyranosyl (1→3)-α-L-rhamnopyranosyl (1→2)-β-D-xylopyranosyl oleanolic acid 28-O–α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl ester.

By comparison with the published data[3], saponin **1**, **2**, **3** and **4** were identified to be prosapogenin CP4, huzhangoside B, huzhangoside C and huzhangoside D respectively.

Table-1 Chemical shifts of compounds **5** and **5a** (in pyridine-d_6)

C	**5**	**5a**	**C**	**5**	**5a**		**5**	**5a**		**5**
Aglycone moiety						Sugar moiety				
1	39.0	39.3	16	23.7	24.0	Xyl	106.0	106.2	Glc	95.5
2	26.8	27.0	17	46.9	46.9		79.6	79.4		73.7[e]
3	88.5	89.1	18	41.5	42.2		78.1[a]	77.7		78.5[a]
4	39.8	40.0	19	46.2	46.7		71.4[b]	71.3		70.6
5	56.1	56.5	20	30.7	31.2		66.9	66.8		77.0
6	18.4	18.8	21	33.9	34.4	Rha	101.3	101.5		69.0
7	32.4	33.3	22	33.0	33.4		71.7[b]	71.8	Glc	104.7
8	39.5	39.8	23	28.1	28.3		81.9	81.4		75.2
9	48.0	48.3	24	17.1	17.2		72.6[c]	72.8		76.3
10	37.0	37.3	25	15.6	15.7		69.6	69.9		78.5[a]
11	23.3	23.8	26	17.4	17.5		18.5	18.5		78.1[b]
12	122.8	122.8	27	26.0	26.3	Rib	104.5	104.3		61.1[d]
13	144.0	144.9	28	176.4	180.7		72.4[c]	72.0	Rha	102.6
14	42.0	42.3	29	33.1	33.4		69.6	68.9		72.6
15	28.5	28.5	30	23.6	23.8		78.5[a]	76.5		72.4
							62.4	62.3		73.8[e]
						Glc	103.4	103.4		70.2
							74.6	74.7		18.4
							78.5[a]	78.4		
							71.3[b]	71.5		
							77.8	77.9		
							61.7[d]	61.5		

[a~e] The values may be interchangeable in each column.

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