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Two New Oleanane Saponins from *Anemone anhuiensis*

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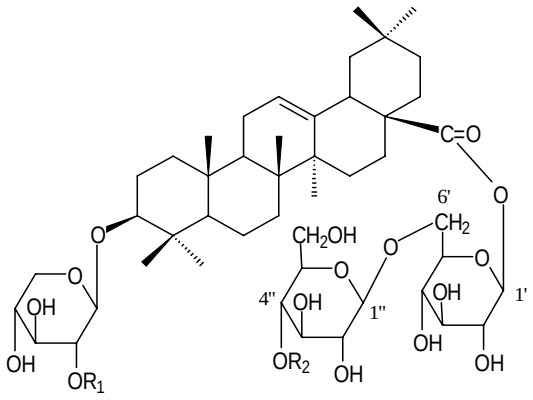
Abstract: Two new oleanane triterpene saponins, named as anhuienside C (**1**) and D (**2**), were isolated from the rhizomes of *Anemone anhuiensis* (Ranunculaceae). The structures of the new compounds were elucidated as 3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl ester (**1**) and 3-*O*-β-D-xylopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl ester (**2**), respectively.

Keywords: *Anemone anhuiensis*, Ranunculaceae, anhuiensides C and D, oleanane saponin.

Anemone anhuiensis Y. K. Yang, N. Wang *et* W. C. Ye is a new plant species found in the southeastern part of Anhui Province of China[1]. The rhizomes of this plant have been used in folk medicine for treatment of rheumatism and phlebitis. Previously, we have reported several known triterpene glycosides isolated from this plant[2]. Further investigation of the ethanol extract of this plant has now led to the isolation of two new oleanane saponins, anhuiensides C (**1**) and D (**2**), whose structures were determined by 1D and 2D NMR (HMQC, HMBC, ^{1}H-^{1}H DQF COSY) methods, FAB-MS, and hydrolysis.

Figure 1. Structures of anhuienside C (**1**) and D (**2**)



1 R_1 = α-L-rhamnopyranosyl R_2 = H

2 R_1 = H R_2 = α-L-rhamnopyranosyl

Table 1. ^{13}C NMR Chemical Shifts of **1** and **2** (ppm)[a]

Carbon	**1**	**2**	Carbon		**1**	**2**
1	39.4	39.1	C-3			
2	28.7	28.6	xyl	1	106.3	107.8
3	88.3	88.8		2	79.8	75.7
4	39.9	39.9		3	78.4	78.8
5	56.5	56.1		4	71.8	71.5
6	19.0	19.5		5	67.2	67.4
7	33.5	33.4	rha	1	102.2	
8	40.3	40.2		2	72.6	
9	48.4	48.4		3	72.8	
10	37.4	37.4		4	74.4	
11	23.8	23.7		5	70.1	
12	123.0	123.0		6	19.1	
13	144.3	144.2				
14	42.5	42.4	C-28			
15	27.2	27.1	glc	1'	95.8	95.8
16	24.2	24.2		2'	74.2	74.0
17	46.6	46.5		3'	78.9	78.9
18	42.1	41.9		4'	71.0	71.0
19	47.4	47.3		5'	78.2	78.2
20	31.1	31.1		6'	69.6	69.4
21	34.4	34.3	glc	1"	105.3	105.0
22	32.9	32.8		2"	75.4	75.6
23	28.4	28.5		3"	78.6	76.7
24	17.5	17.3		4"	71.7	78.4
25	16.1	16.0		5"	78.7	77.4
26	17.9	17.9		6"	62.8	61.5
27	26.5	26.4	rha	1		102.9
28	176.7	176.6		2		72.8
29	33.5	33.5		3		73.0
30	24.1	24.2		4		74.2
				5		70.6
				6		18.8

[a] Recorded in pyridine-d_5. Assignments were established by interpretation of the ^{13}C DEPT, HMQC, and HMBC spectra. Values given in boldface indicated the glycosidic positions.

The EtOH extract of the rhizomes was concentrated and filtered to give a solution. The solution was chromatographed on D101 macroporous resin column to give a fraction containing the mixture of saponins. The fraction was submitted to silica gel and C_{18} column successively and finally purified by HPLC to yield **1** (4.3 mg, 0.0019%) and **2** (12 mg, 0.0054%).

Anhuienside C (**1**) was obtained as an amorphous powder, $[\alpha]_D^{20}$ –8.4 (c=0.42, MeOH). The FAB-MS of **1** showed a quasi-molecular ion $[M+Na]^+$ at *m/z* 1081, consistent with a molecular formula of $C_{53}H_{86}O_{21}$. Acid hydrolysis of **1** yielded oleanolic

acid, glucose, rhamnose and xylose, which were identified by comparison with authentic samples by high performance thin layer chromatography (HPTLC). The ^{1}H and ^{13}C NMR data of **1** suggested the presence of a β-xylopyranosyl, a α-rhamnopyranosyl and two β-glucopyranosyl units, clearly indicated by four anomeric carbon signals at δ 106.3, 102.2, 105.3 and 95.8, and four anomeric proton signals at δ 4.80 (d, *J* = 7.2 Hz), 6.50 (brs), 5.01 (d, *J* = 7.6 Hz) and 6.24 (d, *J* = 8.0 Hz), and their absolute configurations were assumed to be D, L and D[3], respectively. The carbon signals at δ 88.3 (C-3) and 176.7 (C-28) revealed that both positions of oleanolic acid were glycosylated[4]. Furthermore, the ^{1}H and ^{13}C signals due to sugar moieties could be assigned by DQF-COSY and HMQC experiments (**Table 1** and **2**). Based on the above data, the interglycosidic linkages of the sugar chain were subsequently deduced from an HMBC experiment. Thus, in the HMBC spectrum of **1**, correlation peaks were observed between H-1 (δ 6.50) of rhamnose and C-2 (δ 79.8) of xylose, between H-1" (δ 5.01) of the terminal glucose and C-6' (δ 69.6) of the inner glucose, as well as between H-1' (δ 6.24) of the inner glucose and C-28 (δ176.7) of aglycon. Moreover, the HMBC spectrum revealed correlation peak between H-1 (δ 4.80) of xylose and C-3 (δ 88.3) of aglycon. Hence, the structure of anhuienside C (**1**) was elucidated as 3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl ester.

Table 2. ^{1}H NMR Chemical Shifts of the Sugar Moieties of **1** and **2** (ppm, *J* in Hz) [a]

Proton	**1**	**2**	Proton	**1**	**2**
C-3			C-28		
xyl 1	4.80 d (7.2)	4.81d (7.6)	glc 1'	6.24 d (8.0)	6.22 d (8.0)
2	4.20[b]	4.01 t (8.0)	2'	4.14[b]	4.10[b]
3	4.22[b]	4.17[b]	3'	4.24[b]	4.15[b]
4	4.16[b]	4.22[b]	4'	4.35[b]	4.25[b]
5	3.71 t (10.0)	3.77 t (10.8)	5'	4.11[b]	4.07[b]
	4.32[b]	4.39[b]	6'	4.35[b]	4.25[b]
rha 1	6.50 brs			4.73[b]	4.65[b]
2	4.87 brs		glc 1"	5.01 d (7.6)	4.97 d (7.6)
3	4.69 dd (3.2, 9.2)		2"	3.99 t (7.6)	3.93 t (7.6)
4	4.36[b]		3"	4.22[b]	4.16[b]
5	4.74[b]		4"	4.18[b]	4.39 t (9.2)
6	1.69 d (5.6)		5"	3.87[b]	3.63 t (9.2)
			6"	4.46 d (10.0)	4.09[b]
				4.32[b]	4.23[b]
			rha 1		5.83 brs
			2		4.76 brs
			3		4.54[b]
			4		4.31[b]
			5		4.95[b]
			6		1.68 d (6.4)

[a] Recorded in pyridin-d_5. Assignments were established by interpretation of the DQF-COSY, HMQC, and HMBC spectra. [b] Overlapped signals.

Anhuienside D (**2**) was obtained as an amorphous powder, $[\alpha]_D^{20}$ –36.3 (c=0.33, MeOH). The FAB-MS of **2** displayed a quasi-molecular ion at *m/z* 1081$[M+Na]^+$, consistent with a molecular formula of $C_{53}H_{86}O_{21}$. The ^{1}H and ^{13}C NMR spectra (**Table**

1 and **2**) suggested the presence of the same four sugar residues and aglycon as **1**. However, further comparison of ^{13}C NMR data for the saccharide portion indicated that **2** had different glycosidic chains at C-3 and C-28 positions of the aglycone from that of **1**. The oligosaccharide structure was confirmed by 2D NMR experiments. Thus, in the HMBC spectrum of **2**, correlation peaks were observed between H-1 (δ 5.83) of rhamnose and C-4" (δ 78.4) of the central glucose, between H-1" (δ 4.97) of the central glucose and C-6' (δ 69.4) of the inner glucose. Moreover, the HMBC spectrum revealed correlation peaks between H-1 (δ 4.81) of xylose and C-3 (δ 88.8) of aglycon, as well as between H-1' (δ 6.22) of the inner glucose and C-28 (δ 176.6) of aglycon. These findings led to the assignment of **2** as 3-*O*-β-D-xylopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl ester.

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