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TRITERPENOID SAPONINS FROM *ANEMONE HUPEHENSIS*

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Key Word Index—*Anemone hupehensis*; Ranunculaceae; triterpenoid saponins; hupehensis saponins F,G.

Abstract—Two new triterpenoid saponins, named hupehensis saponin F and G, were isolated from the water soluble part of *Anemone hupehensis* Lemoine. By chemical and spectroscopic evidence, their structures were elucidated as 3-*O*-β-D-ribopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl hederagennin-28-*O*-α-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→4)-β-glucopyranosyl(1→6)-β-D-glucopyranoside and 3-*O*-β-D-ribopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl hederagenin-28-*O*-β-glucopyranosyl(1→3)-α-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside, respectively. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

In Chinese folk medicine, *Anemone hupehensis* Lemoine, which is widely spread in China, is used for curing dysentery, infantile malnutrition, carbuncles, funculosis, sores and scrofula [1]. There was also a report about anemone having insecticidal properties. Previously we have reported the triterpenoid saponins with less than seven sugar units isolated from this plant [2, 3]. In this paper, we report two new saponins, hupehensis saponin F and G, which contain more than nine sugar units.

RESULTS AND DISCUSSION

The crude saponin fractions were subjected to repeated CC on silica gel, affording saponins **1** and **2**. The yields of the two saponins were 0.006 and 0.002% of the dry plant, respectively.

On acid hydrolysis, saponins **1** and **2** yielded hederagenin identified by comparison with an authentic sample. A comparison of the ^{13}C NMR signals due to the aglycone moieties with those of reported saponins revealed that they were all bisdesmosides of 3,28-di-*O*-glycosides [4].

Saponin **1** was hydrolysed with acid to yield D-glucose, L-rhamnose, L-arabinose and D-ribose as sugar components. The EI-mass spectrum of its acetate showed fragment ions at *m/z* 259 [terminal ribose $(Ac)_3]^+$, 489 [ribose $(Ac)_3$, rhamnose $(Ac)_2$], 273 [terminal rhamnose $(Ac)_3]^+$, 561 [rhamnose $(Ac)_3$ glucose $(Ac)_3]^+$. The ^{13}C NMR spectrum of sugar moieties (Table 1) indicated the presence of nine monosaccharide units. On 6 M NH_4OH hydrolysis, prosapogenin **1a** was obtained. By comparison with the authentic sample, prosapogenin **1a** was identified as prosapogenin CP_6, which was previously isolated from the same plant. L-Rhamnose and D-glucose were detected by complete hydrolysis of the solution from which prosapogenin CP6 was removed. The ^{13}C NMR signals due to a 28-*O*-saccharide were deciphered from the signals due to saponin **1** after removal of the signals due to prosapogenin CP_6. These signals indicated that there were six monosaccharide units. On comparison with the ^{13}C NMR spectrum of huzhangoside D, there were two groups of ^{13}C NMR signals the same as those due to the 28-*O*-saccharide of huzhangoside D [5]. That means that the 28-*O*-saccharide of saponin **1** contained two units of α-L-rhamnopyranosyl(1→4)-β-glucopyranosyl(1→6)-β-D-glucopyranoside. According to the glycosylation shift (δ 83.6) reported in the literature [6], this indicated that the α-L-rhamnopyranose was 3-*O*-glycosylated. Based on the above evidence, the structure of saponin **1** was established as 3-*O*-β-D-ribopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl hederagenin-28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→4)-β-glucopyranosyl(1→6)-β-D-glucopyranoside, which has been named as hupehensis saponin F.

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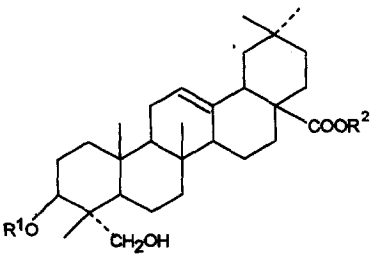

Saponin 1: R^1 = rib(1-->3)rha(1-->2)ara

R^2 = rha(1-->4)glc(1-->6)glc(1-->3)rha(1-->4)glc(1-->6)glc

Saponin 2: R^1 = rib(1-->3)rha(1-->2)ara

R^2 = glc(1-->6)rha(1-->4)glc(1-->6)glc(1-->3)rha(1-->4)glc(1-->6)glc

Saponin **2** was hydrolysed with acid to yield D-glucose, D-ribose, L-rhamnose and L-arabinose as sugar components. The EI-mass spectrum of its acetate showed fragment ions at *m/z* 331 [terminal glucose $(Ac)_4]^+$, 561 [glucose $(Ac)_4$ rhamnose $(Ac)_2]^+$, 259 [terminal ribose $(Ac)_3]^+$ and 489 [ribose $(Ac)_3$ rhamnose $(Ac)_2]^+$. The ^{13}C NMR spectrum due to the sugar moieties indicated the presence of 10 monosaccharide units. On 6 M NH_4OH hydrolysis, the obtained prosapogenin **2a** was identified as prosapogenin CP_6 by comparison with an authentic sample. It gave L-rhamnose and D-glucose by complete hydrolysis of the solution from which prosapogenin CP_6 was removed. The ^{13}C NMR signals due to a 28-*O*-saccharide were obtained from the signals due to saponin **1** minus the signals due to prosapogenin CP_6. The signals obtained indicated that there were seven monosaccharide units. On comparison with the ^{13}C NMR of saponin **1**, saponin **2** contained one more β-D-glucopyranose than saponin **1**. The glycosylation shift (δ 83.7) indicated that an α-L-rhamnopyranose was 3-*O*-glycosylated. Therefore, the structure of saponin **2** was elucidated as 3 - *O* - β - D - ribopyranosyl(1 $\rightarrow$ 3) - α - L - rhamno - pyranosyl(1 $\rightarrow$ 2)-α-L-arabinopyranosyl hederagenin-28-*O*-β-D-glucopyranosyl(1 $\rightarrow$ 3)-α-rhamnopyranosyl-(1 $\rightarrow$ 4) - β - D - glucopyranosyl(1 $\rightarrow$ 6) - β - D - gluco - pyranoside(1 $\rightarrow$ 3)-α-L-rhamnopyranosyl(1 $\rightarrow$ 4)-β-D-glucopyranosyl(1 $\rightarrow$ 6)-β-D-glucopyranoside, which has been named hupehensis saponin G.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded at 400 MHz in pyridine-d_5 using TMS as int. standard. EI-MS were measured at 40 eV accelerating voltage after acetylation. Optical rotations were measured with a J-20C automatic recording spectropolarimeter at 13° in MeOH.

Plant material. Roots and leaves of *Anemome hupehensis* Lemoine were collected in Kai-xan of Sichuan Province, China, and identified by Prof. S. C. Xiao. A specimen is deposited in the Herbarium of the Chengdu Institute of Biology.

Extractions and isolation of saponins. Dry roots and leaves (3.5 kg) were extracted with 95% EtOH. After removal of solvent by evapn, the combined extracts were suspended in H_2O, extracted with EtOAc several times and then with *n*-BuOH. The residue (210 g) was subjected to sepn on a D101 resin column. After diluting with large amounts of H_2O, and 15% EtOH–H_2O to remove most of the coloured material, EtOH was then used to elute crude saponins (3 g). The crude saponin fr. was chromatographed on a silica gel column, eluting with $CHCl_3$–MeOH–H_2O to yield frs **1–6**. Frs **1–4** had been isolated before. Frs 5 and 6 were purified by prep. TLC to give saponins **1** (218 mg) and **2** (90 mg) as powders. Compound **1**, $C_{82}H_{134}O_{44}$, $[\alpha]_D^{13}$ 18.7° (MeOH; *c* 2.53). EI-MS *m/z*: 259 [terminal ribose $(Ac)_3]^+$, 489 [ribose $(Ac)_3$ rhamnose $(Ac)_2]^+$, 273 [terminal rhamnose $(Ac)_3]^+$, 561 [rhamnose $(Ac)_3$ glucose $(Ac)_3]^+$. Compound **1a**, FAB-MS *m/z* 906 {$[M]^+$ ($C_{46}H_{74}O_{16}$) + Na + H}. Compound **1a** was identified as prosapogenin **CP_6** by comparison with an authentic sample. Compound **2**, $C_{88}H_{144}O_{49}$, $[\alpha]_D^{13}$ + 8.0° (pyridine, *c* 3.1). EI-MS *m/z*: 331 [terminal glucose $(Ac)_4]^+$. Compound **2a** was also identified as prosapogenin CP_6.

Acid hydrolysis of the saponins and identification of the resulting monosaccharide. Each saponin (10 mg) was heated with 5% H_2SO_4 under reflux for 7 hr. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. Sapogenin was detected in the $CHCl_3$ layer by TLC. The aq. layer was neutralized with $Ba(OH)_2$ and concentrated, then subjected to PC analysis with authentic samples, developing solvent BuOH–HOAc–H_2O (4:1:5) (upper layer), detection reagent: aniline-phthalate.

Alkaline saponification of saponins. A soln of saponin (20 mg) in 6-M NH_4OH was heated at 100° for 3 hr. The reaction mixt. was extracted with *n*-BuOH to give prosapogenin, which was identified by comparison with the authentic sample on TLC. The aq. layer was

Table 1. ^{13}C NMR spectral data of the saponin sugar moieties of compounds **1** and **2** (in pyridine-d_5, ppm)

	1	2		1	2		1	2
R1-ara-1	104.5	104.4	R2-glc-1	95.4	95.3	glc-1	104.3	104.7
2	75.1	74.9	2	73.6	73.5	2	75.8	75.7
3	73.6	73.5	3	77.7	77.5	3	76.1	76.0
4	68.6	68.5	4	70.5	70.4	4	78.1	78.0
5	65.8	65.6	5	76.8	76.7	5	76.8	76.7
rha-1	101.1	101.0	6	69.0	69.9	6	61.1	61.1
2	72.2	72.1	glc-1	104.3	104.4	rha-1	101.7	101.6
3	80.9	80.7	2	75.1	74.9	2	72.2	72.1
4	72.4	72.2	3	76.2	76.1	3	71.7	83.7
5	69.6	69.6	4	78.4	78.4	4	74.7	74.4
6	18.2	18.2	5	78.0	78.0	5	69.8	69.6
rib-1	104.3	104.2	6	61.1	61.0	6	18.0	18.0
2	72.4	72.2	rha-1	102.3	102.2	glc-1		104.7
3	70.0	69.9	2	71.8	71.7	2		76.0
4	68.2	68.1	3	84.0	83.6	3		78.0
5	64.9	64.8	4	72.5	72.4	4		70.9
			5	69.8	69.6	5		77.4
			6	18.2	18.2	6		61.0
			glc-1	105.7	105.5			
			2	75.1	74.9			
			3	77.4	77.4			
			4	71.0	70.9			
			5	76.8	76.7			
			6	69.3	69.2			

hydrolysed with 5% H_2SO_4, after being neutralized with $BaCO_3$ and concd, then subjected to PC analysis to detect the sugar units by comparison with authentic samples.

Acetylation of sapogenin. To each sapogenin (5 mg) was added Ac_2O–pyridine (1:1) (0.5 ml) in a micro-tube. After standing at room temp. for 48 hr the soln was evapd to dryness and subjected to EI-MS analysis.

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