

Article

## Cytotoxic Oleanane-Type Triterpenoid Saponins from the Rhizomes of *Anemone rivularis* var. *flore-minore*

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**Abstract:** Phytochemical investigation of the *n*-BuOH extract of the rhizomes of *Anemone rivularis* var. *flore-minore* led to the isolation of five new oleanane-type triterpenoid saponins **1–5**, together with five known saponins **6–10**. Their structures were determined by the extensive use of 1D and 2D NMR experiments, along with ESIMS analyses and acid hydrolysis. The aglycone of **4** and **5** was determined as 21 $\alpha$ -hydroxyoleanolic acid, which was reported in this genus for the first time. The cytotoxicity of these compounds was evaluated against four human cancer cell line, including HL-60 (promyelocytic leukemia), HepG2 (hepatocellular carcinoma), A549 (lung carcinoma) and HeLa (cervical carcinoma). The monodesmosidic saponins **6–8** exhibited cytotoxic activity toward all tested cancer cell lines, with IC<sub>50</sub> values in the 7.25–22.38  $\mu$ M range.

**Keywords:** *Anemone rivularis* var. *flore-minore*; triterpenoid saponin; cytotoxicity

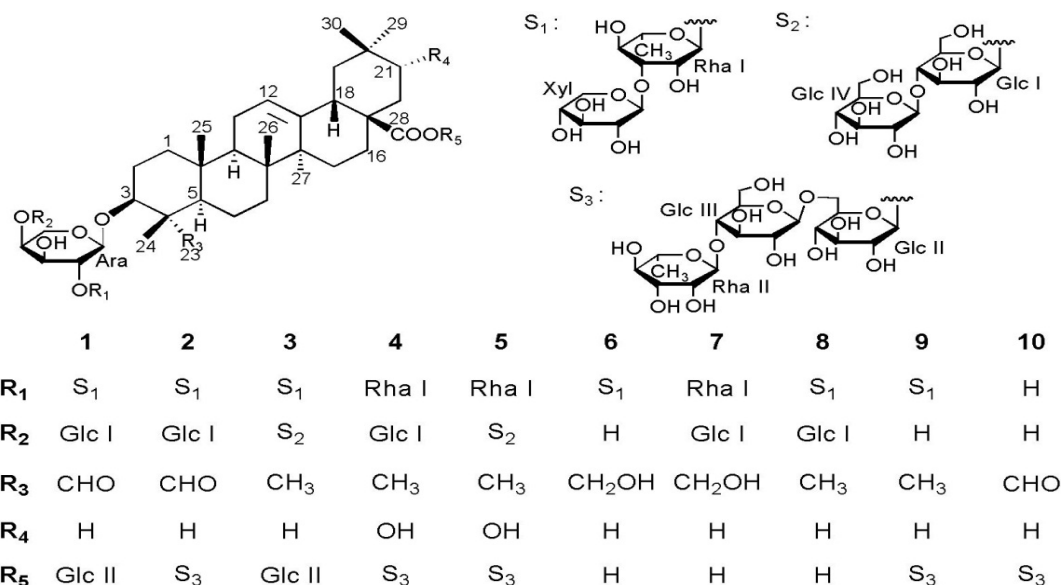
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## 1. Introduction

The genus *Anemone* (Ranunculaceae) consists of about 150 species with a nearly global distribution, of which about 50 species are found in China. More than 10 species of this genus have been used as Chinese folk medicines for a long time. For example, the rhizome of *A. raddeana*, named “Liangtoujian”, is recorded in the Chinese Pharmacopeia for the treatment of rheumatism and neuralgia [1]. Extensive phytochemical and pharmacological studies on this genus have proved the triterpenoid saponins to be the main bioactive substances, with potentially useful biological properties including antitumor, antibacterial, antiperoxidation, insect deterrence, etc. [2–9]. *Anemone rivularis* var. *flore-minore* is distributed mainly in the Tsinling Mountains in Shaanxi Province of China. The whole plants of this species are also used as a folk medicine named “Poniuqi” for the treatment of hepatitis, muscle and joint pain, emission, stranguria, edema, etc. [10].

Our previous phytochemical investigations of the whole plants of this species resulted in the isolation of a new gypsogenin saponin, a new diterpene glycoside, a new lignanoid glycoside, as well as a series of oleanane-type triterpenoid saponins [11,12]. As part of our ongoing search for new bioactive natural compounds from this genus [11–16], the present study of the rhizomes of *A. rivularis* var. *flore-minore* led to the isolation of five new oleanane-type saponins **1–5**, along with five known saponins **6–10** (Figure 1). Herein, we report the isolation and structural elucidation of these saponins, along with their cytotoxic activities against four human cancer cell lines, promyelocytic leukemia HL-60, hepatocellular liver carcinoma HepG2, lung carcinoma A549 and cervical carcinoma HeLa.

**Figure 1.** Structures of compounds **1–10**.



## 2. Results and Discussion

Compound **1** was obtained as a white amorphous powder and showed positive results to the Liebermann-Burchard and Molisch tests. The molecular formula C<sub>58</sub>H<sub>92</sub>O<sub>26</sub> was deduced from the [M+Na]<sup>+</sup> ion at *m/z* = 1,227.5779 (calcd. for C<sub>58</sub>H<sub>92</sub>O<sub>26</sub>Na<sup>+</sup>, 1,227.5775) in the positive ion mode HRESIMS.

**Table 1.**  $^1\text{H}$ - (500 MHz) and  $^{13}\text{C}$ -NMR (125 MHz) chemical shifts assignments for the aglycone moieties of compounds **1–5** in pyridine- $d_5$ .

C	1		2		3		4		5	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (m, J Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (m, J Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (m, J Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (m, J Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (m, J Hz)
1	38.2	0.98, 1.56 m	38.3	1.00, 1.60 m	38.8	1.44, 0.88 m	38.9	0.92, 1.46 m	39.0	0.95, 1.48 m
2	25.5	1.85, 2.09 m	25.5	1.89, 2.09 m	26.7	2.02, 1.81 m	26.7	1.85, 2.06 m	26.7	1.88, 2.10 m
3	81.6	4.05 m	81.7	4.08 m	88.6	3.23 dd (4.0, 11.6)	88.7	3.27 dd (3.9, 11.6)	88.7	3.29 dd (3.8, 11.6)
4	55.5	–	55.7	–	39.5	–	39.5	–	39.5	–
5	47.9	1.36 m	48.0	1.37 m	56.0	0.75 d (11.5)	56.0	0.78 d (11.5)	56.0	0.79 d (11.5)
6	20.6	0.96, 1.38 m	20.6	0.98, 1.42 m	18.5	1.42, 1.24 m	18.5	1.25, 1.43 m	18.5	1.26, 1.47 m
7	32.5	1.18, 1.42 m	32.5	1.19, 1.42 m	33.1	1.40, 1.23 m	33.1	1.24, 1.40 m	33.2	1.25, 1.41 m
8	40.2	–	40.3	–	39.8	–	39.8	–	39.8	–
9	48.2	1.68 m	48.2	1.71 m	48.0	1.60 m	48.1	1.66 m	48.1	1.67 m
10	36.3	–	36.3	–	37.0	–	37.0	–	37.1	–
11	23.4	1.91, 1.99 m	23.5	1.90, 2.02 m	23.7	1.93, 1.86 m	23.7	1.87, 1.93 m	23.7	1.86, 1.94 m
12	122.6	5.40 br s	122.6	5.41 br s	122.8	5.39 br s	122.8	5.43 br s	122.9	5.45 br s
13	144.1	–	144.2	–	144.1	–	144.2	–	144.3	–
14	42.2	–	42.2	–	42.1	–	42.3	–	42.3	–
15	28.3	1.14, 2.03 m	28.3	1.15, 2.06 m	28.3	2.27, 1.14 m	28.5	1.16, 2.30 m	28.6	1.18, 2.30 m
16	23.4	1.76, 2.01 m	23.4	1.77, 2.04 m	23.4	2.05, 1.91 m	27.0	2.36, 3.08 m	27.0	2.33, 3.10 m
17	47.1	–	47.2	–	46.9	–	47.3	–	47.3	–
18	41.7	3.13 dd (3.8, 13.5)	41.7	3.15 dd (3.9, 13.5)	41.6	3.16 dd (3.8, 13.4)	41.7	3.36 dd (3.2, 14.0)	41.8	3.35 dd (3.3, 13.9)
19	46.1	1.23, 1.74 m	46.1	1.23, 1.75 m	46.2	1.71, 1.21 m	41.3	1.71, 1.20 m	41.5	1.75, 1.22 m
20	30.6	–	30.7	–	30.7	–	35.7	–	35.7	–
21	33.9	1.11, 1.34 m	34.0	1.13, 1.36 m	33.9	1.30, 1.08 m	73.3	3.65 br s	73.3	3.66 br s
22	32.5	1.72, 1.89 m	32.5	1.72, 1.90 m	32.5	1.82, 1.74 m	39.6	2.25, 2.28 m	39.7	2.23, 2.29 m
23	206.3	9.61 s	206.4	9.66 s	28.1	1.30 s	28.1	1.29 s	28.2	1.31 s
24	10.5	1.32 s	10.6	1.34 s	17.2	1.15 s	17.2	1.16 s	17.2	1.17 s
25	15.6	0.89 s	15.7	0.89 s	15.5	0.83 s	15.6	0.87 s	15.7	0.88 s
26	17.5	1.05 s	17.5	1.06 s	17.4	1.06 s	17.4	1.09 s	17.4	1.10 s
27	26.2	1.21 s	26.2	1.21 s	26.1	1.23 s	25.6	1.33 s	25.7	1.35 s
28	176.6	–	176.5	–	176.5	–	176.5	–	176.5	–
29	33.1	0.86 s	33.1	0.86 s	33.1	0.86 s	28.3	1.14 s	28.4	1.15 s
30	23.7	0.88 s	23.7	0.87 s	23.7	0.87 s	24.9	1.00 s	25.0	1.01 s

The analysis of the NMR data (Tables 1 and 2) indicated that **1** was a saponin containing one triterpene aglycone and five monosaccharides. The 1D-NMR spectra showed signals for six tertiary methyl groups at  $\delta_{\text{H}}$  0.86 (H<sub>3</sub>-29), 0.88 (H<sub>3</sub>-30), 0.89 (H<sub>3</sub>-25), 1.05 (H<sub>3</sub>-25), 1.27 (H<sub>3</sub>-27) and 1.32 (H<sub>3</sub>-24), one olefinic proton signal at  $\delta_{\text{H}}$  5.40 (1H, br s) with two typical olefinic carbon signals at  $\delta_{\text{C}}$  122.6 and 144.1, one aldehyde proton signal at  $\delta_{\text{H}}$  9.61 (1H, s) with the corresponding aldehyde carbon signal at  $\delta_{\text{C}}$  206.3, and one carbonyl signal at  $\delta_{\text{C}}$  176.6, which revealed that the aglycone of **1** was an oleanolic acid derivative with one of the methyl groups substituted by an aldehyde function. The aldehyde function was located at C-23 on the basis of the downfield shift (+16.0 ppm) exhibited by

C-4 ( $\delta_C$  55.5) and the highfield shifts ( $-7.0$  ppm,  $-8.1$  ppm,  $-6.7$  ppm) exhibited, respectively, by C-3 ( $\delta_C$  84.6), C-5 ( $\delta_C$  47.9) and C-24 ( $\delta_C$  10.5) in comparison with the same carbon resonances in an oleanene skeleton bearing a Me-23 [16]. The correlations of H-23 ( $\delta_H$  9.61) with H-3 ( $\delta_H$  4.05) and H-5 ( $\delta_H$  1.36) observed in the NOESY spectrum indicated the  $\alpha$ -configuration for the 23-CHO function (Figure 2). The HMBC spectrum of 1 also allowed to determine the position of the aldehyde function by showing the correlations between H-23 ( $\delta_H$  9.61) and C-3 ( $\delta_C$  84.6), C-4 ( $\delta_C$  55.5) and C-24 ( $\delta_C$  10.5) (Figure 2). The assignments of the NMR signals associated with the aglycone moiety were derived from  $^1\text{H}$ - $^1\text{H}$  COSY, TOCSY, HSQC, HMBC and NOESY experiments. These data revealed the aglycone of 1 was gypsogenin [17,18]. The  $^{13}\text{C}$ -NMR shifts of C-3 at  $\delta_C$  81.6 and C-28 at  $\delta_C$  176.6 implied that sugar linkages were at both C-3 and C-28. The correlations of H-3 with H-23 and H-5 observed in the NOESY spectrum indicated the  $\beta$ -configuration for the 3-*O*-sugar moiety (Figure 2).

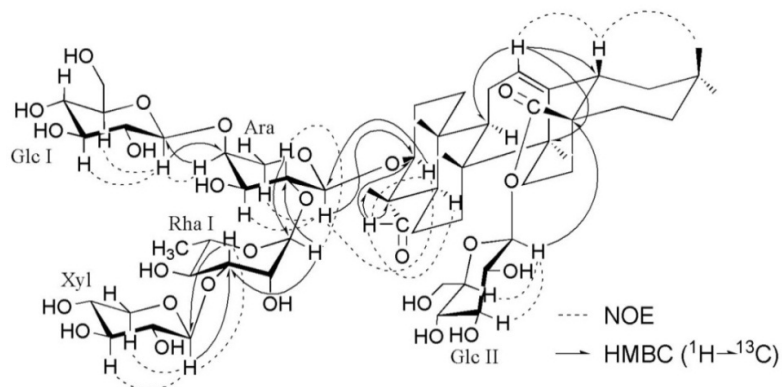
**Table 2.**  $^1\text{H}$ - (500MHz) and  $^{13}\text{C}$ -NMR (125 MHz) chemical shifts assignments for the sugar moieties of compounds 1–5 in pyridine- $d_5$  (m, J Hz).

C	1		2		3		4		5	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
3- <i>O</i> -sugar (Confirm here)										
Ara										
1	104.6	4.92 d (7.1)	104.6	4.91 d (7.2)	105.3	4.72 d (6.9)	105.0	4.77 d (6.2)	105.2	4.74 d (6.2)
2	75.6	4.48 m	75.5	4.50 m	75.5	4.50 m	76.4	4.50 m	76.1	4.49 m
3	75.5	3.91 d (10.3)	75.4	3.90 m	75.0	4.18 m	74.1	4.26 m	74.6	4.25 m
4	80.7	4.10 m	80.8	4.08 m	80.3	4.21 m	79.7	4.27 m	81.0	4.26 m
5	65.8	3.58, 4.35 m	65.9	3.56, 4.32 m	65.3	3.74, 4.38 m	64.5	4.40, 3.80 m	64.9	3.78, 4.41 m
Rha I										
1	101.3	6.33 s	101.2	6.34 s	101.4	6.30 s	101.8	6.15 s	101.7	6.26 s
2	72.0	4.88 br s	71.9	4.87 br s	71.9	4.89 m	72.3	4.71 m	72.2	4.67 m
3	82.9	4.73 m	82.9	4.75 m	82.9	4.72 m	72.6	4.59 m	72.4	4.60 m
4	72.9	4.45 m	73.0	4.47 m	73.0	4.48 m	74.1	4.28 m	74.0	4.25 m
5	69.5	4.71 m	69.6	4.72 m	69.7	4.66 m	69.8	4.62 m	69.7	4.72 m
6	18.5	1.56 d (6.1)	18.5	1.55 d (6.2)	18.5	1.55 d (6.2)	18.6	1.63 d (6.2)	18.6	1.64 d (6.2)
Xyl										
1	107.7	5.34 d (7.6)	107.6	5.35 d (7.7)	107.6	5.34 d (7.7)				
2	75.6	4.03 m	75.5	4.04 m	75.6	4.05 m				
3	78.5	4.13 m	78.5	4.15 m	78.5	4.14 m				
4	71.1	4.12 m	71.0	4.13 m	71.1	4.16 m				
5	67.4	3.68, 4.45 m	67.4	3.68, 4.23 m	67.5	3.73, 4.32 m				
Glc I										
1	106.9	5.08 d (7.9)	106.9	5.08 d (7.9)	106.2	5.03 d (8.0)	106.4	5.13 d (7.9)	106.3	5.04 d (8.0)
2	75.5	3.99 m	75.4	4.01 m	74.9	4.00 m	75.5	4.02 m	74.9	3.99 m
3	78.5	4.18 m	78.5	4.16 m	76.7	4.18 m	78.6	4.19 m	76.7	4.17 m
4	71.2	4.20 m	71.1	4.22 m	81.3	4.29 m	71.3	4.23 m	81.3	4.28 m
5	78.8	3.85 m	78.8	3.86 m	76.7	3.83 m	78.8	3.89 m	76.7	3.82 m
6	62.5	4.33, 4.45 m	62.4	4.36, 4.46 m	61.8	4.41, 4.51 m	62.6	4.37, 4.48 m	61.8	4.40, 4.51 m

Table 2. Cont.

C	1		2		3		4		5	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
Glc IV										
1					105.1	5.15 d (7.8)			105.1	5.15 d (7.8)
2					74.9	4.06 m			74.9	4.05 m
3					78.3	4.19 m			78.3	4.18 m
4					71.6	4.17 m			71.5	4.17 m
5					78.4	3.99 m			78.4	3.98 m
6					62.6	4.26, 4.52 m			62.5	4.27, 4.53 m
28-O-sugar										
Glc II										
1	95.6	6.31 d (8.2)	95.6	6.23 d (8.1)	95.7	6.32 d (8.2)	95.6	6.25 d (8.2)	95.7	6.23 d (8.1)
2	74.1	4.19 m	73.9	4.10 m	74.2	4.20 m	73.8	4.08 m	73.8	4.11 m
3	79.3	4.03 m	78.7	4.18 m	79.3	4.02 m	78.7	4.16 m	78.7	4.17 m
4	71.2	4.35 m	70.8	4.28 m	71.2	4.37 m	70.8	4.23 m	70.7	4.29 m
5	78.9	4.27 m	78.0	4.08 m	78.9	4.28 m	78.0	4.06 m	78.0	4.08 m
6	62.2	4.41, 4.44 m	69.2	4.31, 4.64 m	62.3	4.41, 4.46 m	69.1	4.28, 4.62 m	69.0	4.31, 4.63 m
Glc III										
1			104.9	4.97 d (7.7)			104.8	4.97 d (7.8)	104.9	4.98 d (7.8)
2			75.3	3.92 m			75.3	3.93 m	75.3	3.93 m
3			76.5	4.12 m			76.5	4.12 m	76.4	4.14 m
4			78.2	4.40 m			78.2	4.39 m	78.0	4.39 m
5			77.1	3.62 m			77.1	3.64 m	77.1	3.63 m
6			61.2	4.07, 4.18 m			61.2	4.07, 4.17 m	61.1	4.08, 4.18 m
Rha II										
1			102.7	5.84 s			102.7	5.84 s	102.7	5.85 s
2			72.6	4.66 m			72.5	4.65 m	72.6	4.65 m
3			72.7	4.53 m			72.7	4.52 m	72.7	4.53 m
4			74.0	4.31 m			74.0	4.30 m	74.0	4.32 m
5			70.3	4.95 m			70.3	4.94 m	70.2	4.95 m
6			18.5	1.68 d (6.2)			18.5	1.68 d (6.2)	18.5	1.62 d (6.2)

Figure 2. Key NOESY and HMBC correlations for compound 1.



The sugar moieties of **1** were determined to be L-arabinose (Ara), D-xylose (Xyl), L-rhamnose (Rha) and D-glucose (Glc) in a ratio of 1:1:1:2 by acidic hydrolysis followed by comparison of the GC retention times of the corresponding trimethylsilylated hydrolysates with those of the authentic samples prepared in the same manner in the literature [19]. Meanwhile, the  $^1\text{H-NMR}$  spectrum of compound **1** exhibited five anomeric protons at  $\delta_{\text{H}} = 6.33$  (s), 6.31 (d,  $J = 8.2$  Hz), 5.34 (d,  $J = 7.6$  Hz), 5.08 (d,  $J = 7.9$  Hz) and 4.92 (d,  $J = 7.1$  Hz), and one methyl group of a 6-deoxyhexopyranosyl moiety at  $\delta_{\text{H}} 1.56$  (d,  $J = 6.1$  Hz). The  $\beta$ -anomeric configurations for the xylose and glucose units were deduced from their  $^3J_{\text{H-1/H-2}}$  coupling constants (7.6–8.2 Hz). The arabinose unit was determined to have an  $\alpha$ -anomeric configuration on the basis of the  $^3J_{\text{H-1/H-2}}$  (7.1 Hz) value observed in the  $^4C_1$  form. Although the anomeric proton of the rhamnose moiety was observed as a singlet in the  $^1\text{H-NMR}$  spectrum, the  $^{13}\text{C-NMR}$  shift of Rha C-5 at  $\delta_{\text{C}} = 69.5$  indicated the  $\alpha$ -anomeric configuration [20,21]. All proton signals due to sugars were identified by careful analysis of the  $^1\text{H-}^1\text{H}$  COSY, TOCSY and NOESY spectra, and the carbon signals were assigned by HSQC and further confirmed by HMBC spectra (Table 1). All the monosaccharides were determined to be in their pyranose forms from their  $^{13}\text{C-NMR}$  data. The sequence and binding sites of the oligosaccharide chains were unambiguously defined by the HMBC experiment. A cross peak between C-3 of the aglycone and H-1 of Ara indicated that Ara was connected to C-3 of the aglycone and a cross peak between C-28 of the aglycone and H-1 of one of the glucose units (Glc II) indicated that Glc II was linked to C-28 of the aglycone.

Similarly, the linkage of one of the glucose units (Glc I) at C-4 of Ara was indicated by the cross peak Glc I H-1/Ara C-4, and the linkages of the terminal xyl at the C-3 of Rha in turn linked to C-2 of Ara were indicated by cross peaks Xyl H-1/Rha C-3 and Rha H-1/Ara C-2. The conclusion was confirmed by the NOESY correlations as shown in Figure 2. On the basis of the above analysis, the structure of compound **1** was thus elucidated as 3-*O*- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-arabinopyranosyl gypsogenin 28-*O*- $\beta$ -D-glucopyranosyl ester.

Compound **2** was also obtained as a white powder, and showed positive Liebermann-Burchard and Molisch reactions. HRESIMS of **2** showed the quasi-molecular ion at  $m/z$  1,535.6885 (calcd. for  $[\text{M}+\text{Na}]^+$  1,535.6882), establishing a molecular formula of  $\text{C}_{70}\text{H}_{112}\text{O}_{35}$ . The  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  data assignable to the aglycone moiety of **2** were identical to those of **1** (Table 1), suggesting the aglycone to also be gypsogenin. Seven anomeric protons [ $\delta_{\text{H}}$  6.34 (s), 6.23 (d,  $J = 8.1$  Hz), 5.84 (s), 5.35 (d,  $J = 7.7$  Hz), 5.08 (d,  $J = 7.9$  Hz), 4.97 (d,  $J = 7.7$  Hz) and 4.91 (d,  $J = 7.2$  Hz)] and seven anomeric carbons ( $\delta_{\text{C}}$  107.6, 106.9, 104.9, 104.6, 102.7, 101.2 and 95.6) were observed in the NMR spectra of **2**. Acid hydrolysis and GC analyses of **2** confirmed the NMR data of the sugar moieties, indicating the units as  $\alpha$ -L-arabinose,  $\alpha$ -L-rhamnose,  $\beta$ -D-glucose and  $\beta$ -D-xylose in a 1:2:3:1 ratio (Table 2).

The linkage points of the sugar units to each other and to C-3 and C-28 of the aglycone were established from the HMBC correlations of signals at  $\delta_{\text{H}}$  4.91 (H-1 of Ara) with  $\delta_{\text{C}}$  81.7 (C-3 of the aglycone),  $\delta_{\text{H}}$  6.34 (H-1 of Rha I) with  $\delta_{\text{C}}$  75.5 (C-2 of Ara),  $\delta_{\text{H}}$  5.35 (H-1 of Xyl) with  $\delta_{\text{C}}$  82.9 (C-3 of Rha I),  $\delta_{\text{H}}$  5.08 (H-1 of Glc I) with  $\delta_{\text{C}}$  80.8 (C-4 of Ara),  $\delta_{\text{H}}$  6.23 (H-1 of Glc II) with  $\delta_{\text{C}}$  176.5 (C-28 of the aglycone),  $\delta_{\text{H}}$  4.97 (H-1 of Glc III) with  $\delta_{\text{C}}$  69.2 (C-6 of Glc II) and  $\delta_{\text{H}}$  5.84 (H-1 of Rha II) with  $\delta_{\text{C}}$  78.2 (C-4 of Glc III) (Figure S1 in the Supplementary Data). Accordingly, compound **2** was identified as 3-*O*- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-arabino-pyranosyl gypsogenin 28-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl ester.

Compound **3** was positive to Liebermann-Burchard and Molisch tests. The molecular formula was established as  $C_{64}H_{104}O_{30}$  from the  $[M+Na]^+$  ion at  $m/z = 1,375.6507$  (calcd. for  $C_{70}H_{114}O_{34}Na^+$ , 1,375.6510) in the positive ion mode HRESIMS. The analysis of the NMR data (Tables 1 and 2) indicated that **3** was a saponin containing one triterpene aglycone and six monosaccharides. The aglycone of **3** was recognized to be oleanolic acid by  $^1H$ - and  $^{13}C$ -NMR analysis (Table 1), which was in full agreement with literature data [21]. The chemical shifts of C-3 ( $\delta_C = 88.6$ ) and C-28 ( $\delta_C = 176.5$ ) revealed that **3** was a bisdesmosidic glycoside. The presence of L-arabinose (Ara), D-xylose (Xyl), L-rhamnose (Rha) and D-glucose (Glc) in a 1:1:2:2 ratio was established by acid hydrolysis followed by GC analysis of the corresponding derivatives. Complete  $^1H$ - and  $^{13}C$ -NMR assignments of the sugar part were achieved by a combination of 2D-NMR experiments (Table 2). The NMR data of the sugar part of **3** were very similar to those obtained from **1**, except for a significant downfield shift of C-4 ( $\delta_C = 81.3$ ) of Glc I and the appearance of a set of additional signals, corresponding to a terminal  $\beta$ -D-glucopyranosyl group (Glc IV) in **3** which was attached at C-4 of Glc I. The sequence and binding sites of the sugar units connected to C-3 and C-28 of the aglycone were confirmed by the HMBC and NOESY correlations (Figure S2 in Supplementary Data). Hence, compound **3** was established as 3-*O*- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-arabinopyranosyl oleanolic acid 28-*O*- $\beta$ -D-glucopyranosyl ester.

Compound **4** was obtained as white amorphous powder and showed positive results to the Liebermann-Burchard and Molisch tests. In the positive-ion mode HRESIMS, a pseudomolecular ion peak at  $m/z$  1,405.6612  $[M+Na]^+$  (calcd. for  $C_{65}H_{106}O_{31}Na^+$ , 1,405.6616) was observed, which is consistent with a molecular formula  $C_{65}H_{106}O_{31}$ . The 1D-NMR data of **4** exhibited signals for seven tertiary methyl groups at  $\delta_H$  0.87 (H<sub>3</sub>-25), 1.00 (H<sub>3</sub>-30), 1.09 (H<sub>3</sub>-26), 1.14 (H<sub>3</sub>-29), 1.16 (H<sub>3</sub>-24), 1.29 (H<sub>3</sub>-23) and 1.33 (H<sub>3</sub>-27), one olefinic proton at  $\delta_H$  5.43 (1H, br s) with two typical olefinic carbon signals at  $\delta_C$  122.8 and 144.2, and one carbonyl signal at  $\delta_C$  176.5, which revealed that the aglycone of **4** was a derivative of oleanolic acid. Comparison of their chemical shifts between the aglycone and oleanolic acid indicated that they were the same except for the E-ring. Due to the change of chemical shift of C-21 from  $\delta_C$  31.4 in oleanolic acid [22] to  $\delta_C$  73.3 (+41.9) and the other of carbons such as C-18 [ $\delta_C$  41.7 (−1.2)], C-19 [ $\delta_C$  41.3 (−5.0)], C-20 [ $\delta_C$  35.7 (+4.8)], C-22 [ $\delta_C$  39.6 (+6.4)], C-29 [ $\delta_C$  28.3 (−6.9)] and C-30 [ $\delta_C$  24.9 (+0.2)], C-21 must be an oxygen-bearing methylene carbon in the aglycone of **4**, which was confirmed by the DEPT experiment. In the HMBC experiment, the correlation peaks H<sub>3</sub>-29/C-21 and H<sub>3</sub>-30/C-21 also allowed the location of a hydroxyl group at C-21 (Figure S3 in Supplementary data). The NOESY correlations between H-21 ( $\delta_H$  3.65) and H<sub>3</sub>-30 ( $\delta_H$  1.00) indicated the  $\alpha$ -orientation of 21-OH. The assignments of the NMR signals associated with the aglycone moiety were derived from  $^1H$ - $^1H$  COSY, TOCSY, HSQC, HMBC and NOESY experiments. These data revealed the aglycone of **4** was 21 $\alpha$ -hydroxy-oleanolic acid, which was in a good agreement with the literature data [23,24]. The  $^1H$ - and  $^{13}C$ -NMR spectra assignable to the sugar moieties of **4** were similar to those of **2** except for the absence of proton and carbon resonances for a terminal  $\beta$ -D-xylose moiety. The sequence and binding sites of the sugar units to each other and to the aglycone were confirmed by the HMBC and NOESY correlations (Figure S3 in the Supplementary Data). On the basis of the above analysis, the structure of compound **4** was elucidated as 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-arabinopyranosyl 21 $\alpha$ -hydroxyoleanolic acid 28-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl ester.

Compound **5** was also obtained as a white powder, showed positive Libermann-burchard and Molisch reactions. HRESIMS of **5** showed the quasi-molecular ion at  $m/z$  1,567.7150 (calcd. for  $[M+Na]^+$  1,567.7144), establishing the molecular formula of  $C_{71}H_{116}O_{36}$ . The 1D-NMR data assignable to the aglycone moiety of **5** were identical to those of **4** (Table 1), suggesting the aglycone also to be 21 $\alpha$ -hydroxy-oleanolic acid. The  $^1H$ - and  $^{13}C$ -NMR spectra assignable to the sugar moieties of **5** were similar to those of **4** except for the presence of proton and carbon resonances for an additional  $\beta$ -D-glucopyranose moiety (Glc IV). The downfield-shifted carbon signal of Glc I C-4 ( $\delta_C$  81.3) in the  $^{13}C$ -NMR spectrum and the presence of a correlation between Glc IV H-1 ( $\delta_H$  5.15) and Glc I C-4 ( $\delta_C$  5.15) in the HMBC spectrum indicated that Glc IV was attached to Glc I C-4. The conclusion was confirmed by the NOESY correlations (Figure S4 in the Supplementary Data). Hence, compound **5** was established as 3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-[ $\alpha$ -L-rhamno-pyranosyl-(1 $\rightarrow$ 2)]- $\alpha$ -L-arabinopyranosyl 21 $\alpha$ -hydroxyoleanolic acid 28-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl ester.

By comparing their physical and spectroscopic data with those reported in the literature, the known compounds were identified as sapindoside B (**6**) [25], pulsatilla saponin D (**7**) [26], 3-*O*- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-arabinopyranosyl oleanolic acid (**8**) [27], sieboldianoside B (**9**) [28], and 3-*O*- $\alpha$ -L-arabinopyranosyl gypsogenin 28-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl ester (**10**) [11], respectively.

The cytotoxic activity of saponins **1–10** against human leukemia HL-60 cells, human hepatocellular carcinoma HepG2 cells, human lung carcinoma A549 cells and human cervical carcinoma HeLa cells were evaluated by MTT colorimetric assay. Doxorubicin was used as positive control.

The  $IC_{50}$  value of each compound was measured on the basis of cell viability after 72 h treatment. As shown in Table 3, all monodesmosidic saponins **6–8**, which possess oligosaccharide chains at C-3 and a free carboxylic acid at C-28 of the aglycone, exhibited cytotoxic activity against all tested cancer cell lines with  $IC_{50}$  values ranging from 7.25 to 22.38  $\mu$ M. The bisdesmosidic saponins **2**, **4**, **5**, **9** and **10**, which possess the same  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl oligosaccharide chain at C-28, were all inactive. Earlier studies on the cytotoxicity of similar compounds have reached the same results [29,30].

**Table 3.** Cytotoxic activity of compounds **1–10** against four human cancer cell lines *in vitro*.

Compounds <sup>a</sup>	Cytotoxic activity ( $IC_{50}$ , $\mu$ M; mean $\pm$ SD, $n = 3$ )			
	HL-60	HepG2	A549	HeLa
<b>1</b>	82.16 $\pm$ 2.79	>100	>100	>100
<b>3</b>	45.32 $\pm$ 1.74	55.79 $\pm$ 2.57	75.68 $\pm$ 2.79	>100
<b>6</b>	12.32 $\pm$ 0.41	13.25 $\pm$ 0.38	19.42 $\pm$ 0.67	22.20 $\pm$ 0.42
<b>7</b>	9.57 $\pm$ 0.54	10.64 $\pm$ 0.47	22.38 $\pm$ 0.72	17.31 $\pm$ 0.81
<b>8</b>	7.25 $\pm$ 0.31	12.11 $\pm$ 0.87	9.89 $\pm$ 0.57	15.47 $\pm$ 0.73
Doxorubicin <sup>b</sup>	0.35 $\pm$ 0.05	0.50 $\pm$ 0.04	0.68 $\pm$ 0.10	0.66 $\pm$ 0.07

HL-60—human promyelocytic leukemia cell line; HepG2—human hepatocellular carcinoma cell line; A549—human lung carcinoma cell line; HeLa—human cervical carcinoma cell line; <sup>a</sup> Compounds **2**, **4**, **5**, **9** and **10** were inactive ( $IC_{50} > 100$   $\mu$ M) for all cell lines; <sup>b</sup> Doxorubicin was used as positive control.



Interestingly, the remaining two bisdesmosidic saponins **1** and **3** showed moderate cytotoxicity (**1** against HL-60; **3** against HL-60, HeLa and A549). This may be ascribed to the fact that these two saponins only possess one sugar moiety at C-28 of the aglycone. Therefore, we postulated that the shorter oligosaccharide chain at C-28 the better cytotoxic activity the saponins would exhibit.

### 3. Experimental

#### 3.1. General

Optical rotations were measured on a Perkin-Elmer 343 polarimeter. The ESIMS and HRESIMS were measured on a Micromass Quattro mass spectrometer. NMR experiments were performed on a Bruker AVANCE-500 spectrometer in pyridine-*d*<sub>5</sub> (99.95%, Sigma-Aldrich) with TMS as internal standard. GC was performed on a Finnigan Voyager apparatus using an L-Chirasil-Val column (0.32 mm × 25 m; injector temperature: 230 °C; column temperature: 100–180 °C, rate 5 °C/min; column head pressure: 12 Pa; carrier gas: He, 2 mL/min). HPLC was carried out on a Dionex P680 liquid chromatograph equipped with a UV 170 UV/Vis detector at 206 nm using a Diamonsil C18(2) column (21.2 × 250 mm i.d., 5 μm, Dikma Technologies Inc., Lake Forest, CA, USA). Materials for column chromatography (CC) were silica gel (10–40 μm, Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 (40–70 μm, GE-Healthcare, Uppsala, Sweden), and reversed phase silica gel ODS-A (50 μm, YMC Co., Ltd, Kyoto, Japan). TLC detection was achieved by spraying the silica gel plates (Qingdao Marine Chemical Inc) with 20% H<sub>2</sub>SO<sub>4</sub>-EtOH (v/v) solution followed by heating. The Liebermann–Burchardtest was made with acetic anhydride and sulfuric acid, and the Molisch test was made with α-naphthol and sulfuric acid. Chemical reagents for isolation were of analytical grade and purchased from Tianjin Fuyu Chemical Co. Ltd. (Tianjin, China).

#### 3.2. Plant Material

The rhizomes of *Anemone rivularis* var. *flore-minore* were collected on Taibai Mountain, Shaanxi Province, China, in September 2012, and identified by Prof. Ji-Tao Wang (Department of Pharmacognosy, School of Pharmacy, Shaanxi University of Chinese Medicine). A voucher specimen (NO.120922) has been deposited in the Herbarium of Shaanxi University of Chinese Medicine.

#### 3.3. Extraction and Isolation

The air-dried rhizomes of *A. rivularis* var. *flore-minore* (8 kg) were powdered and extracted with 70% EtOH (16 L) under reflux for three times (each for 2 h). The extract was evaporated *in vacuo* to yield a residue (1,300 g) which was suspended in water (10 L) and partitioned successively with petroleum ether (10 L × 2) and *n*-BuOH (10 L × 2). The *n*-BuOH extract (160 g) was separated by silica gel CC using a stepwise gradient of CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (10:1:0.05–6:4:0.8) to give nine fractions (Fr. 1–Fr. 9). Fr. 3 (4.5 g) was subject to silica gel CC with a CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O gradient (10:1:0.1–7:3:0.5) to give five sub-fractions (Fr. 3.1–Fr. 3.5). Fr. 3.2 (0.8 g) and Fr. 3.3 (1.2 g) were submitted to gel permeation chromatography on Sephadex LH-20 in MeOH to remove the pigments and carbohydrates, and further purified by semipreparative HPLC to give compound **6** [35 mg, MeOH-H<sub>2</sub>O (75:25), 8 mL/min, *t*<sub>R</sub> 22.0 min] and compound **7** [42 mg, MeOH-H<sub>2</sub>O (78:22), 8 mL/min,

$t_R$  19.5 min], respectively. Fr. 4 (3.5 g) was separated by silica gel CC with a stepwise gradient of  $\text{CHCl}_3$ –MeOH– $\text{H}_2\text{O}$  gradient (10:1:0.1–7:3:0.5) to yield three fractions (Fr. 4.1–Fr. 4.3). Compound **8** (26 mg) was obtained from Fr. 4.2 by semipreparative HPLC [MeOH– $\text{H}_2\text{O}$  (82:18), 8 mL/min,  $t_R$  16.5 min]. Fr. 6 (2.1 g) and Fr. 7 (15.5 g) were subjected to ODS CC using a stepwise gradient [MeOH– $\text{H}_2\text{O}$  (1:4–10:1)] to afford Fr. 6.1–Fr. 6.5 and Fr. 7.1–Fr. 7.6, respectively. Fr. 6.2 (0.9 g) were submitted to Sephadex LH-20 CC in MeOH and further purified by semipreparative HPLC [MeOH– $\text{H}_2\text{O}$  (75:25), 8 mL/min) to yield compound **9** (35 mg,  $t_R$  19.7 min) and compound **3** (26 mg,  $t_R$  25.2 min). Fr. 7.2 (1.1 g) and Fr. 7.3 (1.5 g) were subjected to semipreparative HPLC after CC over Sephadex LH-20 (MeOH), to give compound **10** [45 mg, MeOH– $\text{H}_2\text{O}$  (62:38), 6.5 mL/min,  $t_R$  18.5 min from Fr. 7.2], compound **1** [24 mg, MeOH– $\text{H}_2\text{O}$  (55:45), 6.0 mL/min,  $t_R$  29.3 min from Fr. 7.3] and compound **2** [30 mg, MeOH– $\text{H}_2\text{O}$  (55:45), 6.0 mL/min,  $t_R$  35.8 min from Fr. 7.3]. Fr. 7.4 (8.6 g) was separated by ODS CC again eluting with a gradient of MeOH– $\text{H}_2\text{O}$  (1:10–5:1) to afford Fr. 7.4.1–Fr. 7.4.6. Compound **4** [15 mg, MeOH– $\text{H}_2\text{O}$  (70:30), 8 mL/min,  $t_R$  18.5 min] and Compound **5** (19 mg, MeOH– $\text{H}_2\text{O}$  (65:35), 7 mL/min,  $t_R$  25.6 min) were obtained from Fr. 7.4.2 and 7.4.3, respectively. Purities of these compounds were determined >95% by HPLC.

Compound **1**: White amorphous powder;  $[\alpha]_D^{22} +12.3$  ( $c$  0.16, MeOH);  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data, see Tables 1 and 2; key HMBC and NOESY correlations, see Figure 2; ESIMS (pos. ion mode)  $m/z$  1227  $[\text{M}+\text{Na}]^+$ ; ESIMS (neg. ion mode)  $m/z$  1203  $[\text{M}-\text{H}]^-$ , 1071  $[\text{1203}-132]^-$ , 1041  $[\text{1203}-162]^-$ , 925  $[\text{1071}-146]^-$ , 909  $[\text{1071}-162]^-$ , 879  $[\text{1041}-162]^-$ ; HRESIMS (pos. ion mode)  $m/z$  1227.5779  $[\text{M}+\text{Na}]^+$  (calcd. for  $\text{C}_{58}\text{H}_{92}\text{NaO}_{26}$ , 1227.5775).

Compound **2**: White amorphous powder;  $[\alpha]_D^{22} -8.2$  ( $c$  0.18, MeOH);  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data, see Tables 1 and 2; key HMBC and NOESY correlations, see Figure S1 in the Supplementary Data; ESIMS (pos. ion mode)  $m/z$  1535  $[\text{M}+\text{Na}]^+$ , 1065  $[\text{1535}-146-162-162]^+$ ; ESIMS (neg. ion mode)  $m/z$  1511  $[\text{M}-\text{H}]^-$ , 1379  $[\text{1511}-132]^-$ , 1349  $[\text{1511}-162]^-$ , 1041  $[\text{1511}-146-162-162]^-$ ; HRESIMS (pos. ion mode)  $m/z$  1535.6885  $[\text{M}+\text{Na}]^+$  (calcd. for  $\text{C}_{70}\text{H}_{112}\text{NaO}_{35}$ , 1535.6882).

Compound **3**: White amorphous powder;  $[\alpha]_D^{22} -15.4$  ( $c$  0.20, MeOH);  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data, see Tables 1 and 2; key HMBC and NOESY correlations, see Figure S2 in the Supplementary Data; ESIMS (pos. ion mode)  $m/z$  1375  $[\text{M}+\text{Na}]^+$ , 1243  $[\text{1375}-132]^+$ , 1213  $[\text{1375}-162]^+$ , 1081  $[\text{1243}-162]^+$ , 1051  $[\text{1213}-162]^+$ ; ESIMS (neg. ion mode)  $m/z$  1351  $[\text{M}-\text{H}]^-$ , 1219  $[\text{1351}-132]^-$ , 1189  $[\text{1351}-162]^-$ , 1027  $[\text{1189}-162]^-$ ; HRESIMS (pos. ion mode)  $m/z$  1375.6507  $[\text{M}+\text{Na}]^+$  (calcd. for  $\text{C}_{70}\text{H}_{114}\text{NaO}_{34}$ , 1375.6510).

Compound **4**: White amorphous powder;  $[\alpha]_D^{22} +7.2$  ( $c$  0.11, MeOH);  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data, see Tables 1 and 2; key HMBC and NOESY correlations, see Figure S3 in the Supplementary Data; ESIMS (pos. ion mode)  $m/z$  1405  $[\text{M}+\text{Na}]^+$ , 1259  $[\text{1405}-146]^+$ , 1243  $[\text{1405}-162]^+$ , 935  $[\text{1405}-146-162-162]^+$ ; ESIMS (neg. ion mode)  $m/z$  1381  $[\text{M}-\text{H}]^-$ , 911  $[\text{1381}-146-162-162]^-$ ; HRESIMS (pos. ion mode)  $m/z$  1405.6612  $[\text{M}+\text{Na}]^+$  (calcd. for  $\text{C}_{65}\text{H}_{106}\text{NaO}_{31}$ , 1405.6616).

Compound **5**: White amorphous powder;  $[\alpha]_D^{22} -9.4$  ( $c$  0.18, MeOH);  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data, see Tables 1 and 2; key HMBC and NOESY correlations, see Figure S4 in the Supplementary Data; ESIMS (pos.

ion mode)  $m/z$  1567  $[M+Na]^+$ , 1421  $[1567-146]^+$ , 1405  $[1567-162]^+$ , 1097  $[1567-146-162-162]^+$ ; ESIMS (neg. ion mode)  $m/z$  1543  $[M-H]^-$ , 1073  $[1543-146-162-162]^-$ ; HRESIMS (pos. ion mode)  $m/z$  1567.7150  $[M+Na]^+$  (calcd. for  $C_{71}H_{116}NaO_{36}$ , 1567.7144).

### 3.4. Acid Hydrolysis and GC Analysis of the Sugar Moieties in 1–5

Compounds **1–5** (4 mg each) in 1 M HCl (dioxane–H<sub>2</sub>O 1:1, *v/v*, 5 mL) were heated at 95 °C for 6 h, respectively. The reaction mixture was evaporated *in vacuo* and the residue was extracted three times with CHCl<sub>3</sub>. The aqueous phase was concentrated and dissolved in pyridine (5 mL) and 1-(trimethylsilyl)imidazole (0.5 mL) at room temperature for 30 min. The reaction mixture was dried with a stream of N<sub>2</sub>. The residue was partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O. The organic layer was subjected to GC analysis using an L-Chirasil-Val column. The sugar units were identified by comparing the retention times of the corresponding trimethylsilylated derivatives with those of the authentic samples prepared in the same manner [19]. Retention times for authentic samples after being derivatized were 9.12 and 10.05 min (D-arabinose), 9.30 and 10.29 min (L-arabinose), 9.90 and 10.69 min (D-rhamnose), 9.70 and 10.58 min (L-rhamnose), 11.15 and 12.36 min (D-xylose), 11.27 and 12.30 min (L-xylose), 14.95 min (D-glucose), and 15.18 min (L-glucose), respectively. L-Arabinose, L-rhamnose, D-xylose and D-glucose were identified in a ratio of 1:1:1:2 for **1**, 1:2:1:3 for **2** and 1:2:1:4 for **3**, while the sugar moieties of **4** and **5** were identified as L-arabinose, L-rhamnose and D-glucose in the ratio of 1:2:3 and 1:2:4, respectively.

### 3.5. Assays for *in Vitro* Antitumor Activity

The cytotoxicity of compounds **1–5** against human promyelocytic leukemia HL-60 cells, human hepatocellular carcinoma HepG2 cells, human lung carcinoma A549 cells and human cervical carcinoma HeLa cells (all four cancer cell lines were obtained from ATCC, Manassas, VA, USA) was evaluated by MTT colorimetric assay described in previous papers [31], with doxorubicin (Sigma-Aldrich, St. Louis, MO, USA) as positive control. Briefly,  $4 \times 10^3$ /mL cells were added to 96-well plates (100  $\mu$ L/well), and incubated with various concentrations of drugs (80, 40, 20, 10, 5, 1 and 0.2  $\mu$ M) in triplex wells for 48 h at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere. After 48 h, 20  $\mu$ L 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solved in PBS was added to each well at a concentration of 5 mg/mL, and then incubated for another 4 h. The water-insoluble dark blue formazan crystals formed during MTT cleavage in actively metabolizing cells were dissolved in DMSO. The optical density of each well was measured with a Bio-Rad 680 microplate reader (Bio-Rad, Hercules, CA, USA) at 570 nm. Cytotoxicity was expressed as the concentration of drug inhibiting cell growth by 50% (IC<sub>50</sub>).

## 4. Conclusions

Phytochemical investigation of the rhizomes of *A. rivularis* var. *flore-minore*, led to the isolation of five new oleanane-type triterpenoid saponins **1–5** along with five known saponins **6–10**. Their structures were elucidated on the basis of spectroscopic studies and chemical evidence. These compounds were based on three types of aglycones, *i.e.*, gypsogenin, oleanolic acid and 21 $\alpha$ -hydroxyoleanolic

acid, and the third type of aglycone was found from the *Ranunculaceae* family for the first time. All of the compounds were tested for cytotoxicity against HL-60, HepG2, A549 and HeLa cell lines. The monodesmosidic saponins **6–8** exhibited cytotoxic activity toward all cancer cell lines, with IC<sub>50</sub> values in the 7.25–22.38 µM range.

### Supplementary Materials

Physical and spectroscopic data of compounds **6–10**, along with NOESY and HMBC correlations for compounds **2–5** are available as supporting data, which can be accessed at: <http://www.mdpi.com/1420-3049/19/2/2121/s1>.

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### Conflicts of Interest

The authors declare no conflict of interest.

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*Sample Availability:* Samples of the compounds **1–10** are available from the authors.

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