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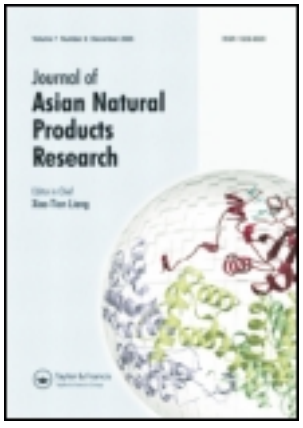

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Four new triterpenoid glycosides from the seed residue of Hippophae rhamnoides subsp. sinensis

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Four new triterpenoid glycosides from the seed residue of *Hippophae rhamnoides* subsp. *sinensis*

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Four new triterpenoid saponins (**1**–**4**) were isolated from the seed residue of *Hippophae rhamnoides* subsp. *sinensis*, named 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-13-ene-19-one-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**), 3-*O*-[β-D-glucopyranosyl (1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-13-ene-19-one-30-hydroxyolean-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**2**), 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl-13-ene-19-one-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**3**), and 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl-13-ene-19-one-30-hydroxyolean-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**4**), and their structures were elucidated on the basis of spectroscopic and chemical methods.

Keywords: *Hippophae rhamnoides* subsp. *sinensis*; hippophosides A–D; triterpenoid saponins

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1. Introduction

Hippophae rhamnoides L. (Sea buckthorn), a plant of family Elaeagnaceae, has been used for application as food and medicine in eastern countries, especially in traditional Mongolian and Tibetan medicine in China [1]. Previous investigations demonstrated that the seed extracts have sorts of biological effects, such as antioxidant [2], antilipemic [3], and antineoplastic [4]. As a part of our continuing search for bioactive components from the seed residue of *H. rhamnoides* subsp. *sinensis*, we present in this report the isolation and structure elucidation of four new oleanane-type saponins (**1**–**4**) (Figure 1).

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was elucidated as $C_{59}H_{94}O_{27}$ by the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data at *m/z* 1257.5886 $[M + Na]^+$. The acid hydrolysis, derivatization, and GC analysis gave D-glucose, L-rhamnose, and L-arabinose in **1**. The ^{1}H NMR spectrum (Table 1) revealed signals due to seven tertiary methyl groups (δ 0.60, 0.94, 0.96, 0.97, 1.09, 1.13, 1.13) and five anomeric protons (δ 4.68, 5.04, 5.14, 6.14, 6.16). The ^{13}C NMR spectrum (Table 2) showed signals of seven tertiary methyl carbons at δ 16.6, 17.0, 17.4, 20.9, 24.4, 25.3, and 28.2; a pair of olefinic carbons at δ 132.8 (C, C-18) and 152.0 (C, C-13); five anomeric carbons at δ 96.4, 101.0, 102.8, 104.8, and 106.2; six non-hydrogenated carbons at δ 37.3, 39.5, 43.2, 45.4, 45.5, and 53.0; and two carbonyl carbons at δ 174.8 and 208.1. The spectroscopic features suggested that

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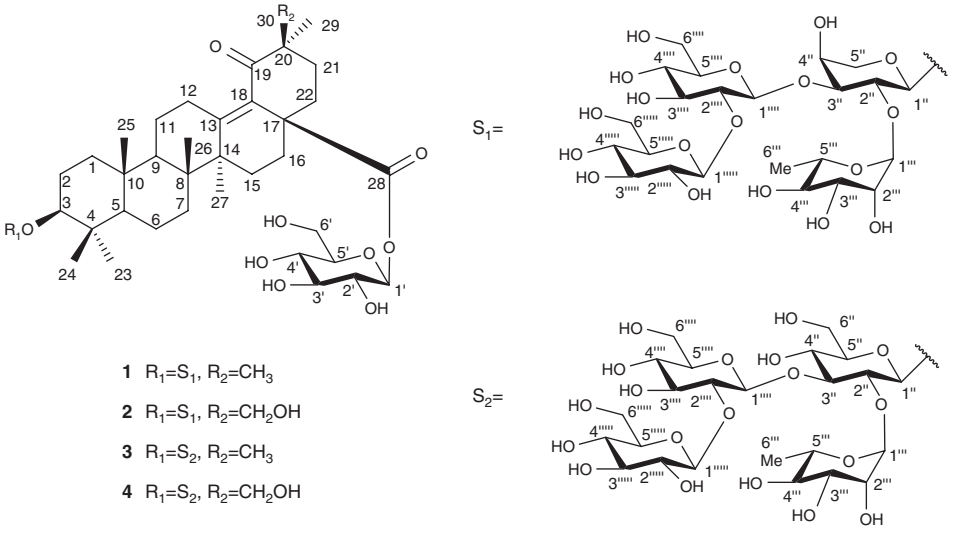

1 $R_1=S_1$, $R_2=CH_3$

2 $R_1=S_1$, $R_2=CH_2OH$

3 $R_1=S_2$, $R_2=CH_3$

4 $R_1=S_2$, $R_2=CH_2OH$

Figure 1. Structures of compounds **1**–**4**.

1 was an oleanane-type saponin with five sugars. The HMBC plot showed long-range correlations of the carbonyl carbon at δ 208.1 (C-19) with 3H-30 (δ 0.97) and 3H-29 (δ 1.13), and the olefinic carbons at δ 132.8 (C, C-18) and 152.0 (C, C-13) shifted to downfield, which established that a C═C bond existed between C-13 and C-18, and the carbonyl carbon was at C-19. The carbon chemical shifts, regarding C-29 and C-30 of **1**, were almost identical with the compounds **1** and **3** in the literature [5]. In addition, the long-range correlations of the C-3 (δ 88.2) with the anomeric proton H-1″ (δ 4.68), C-1″ (δ 104.8) with H-5″ (δ 3.60), and C-28 (δ 174.8) with the anomeric proton H-1′ (δ 6.14) (Figure 2) confirmed that the five sugars were present in two saccharide chains, one with arabinose attached to C-3 and the other to C-28. The unambiguous identification of the individual spin systems associated with the five monosaccharides and the assignments of their NMR resonances were established by the combined use of 1D and 2D NMR techniques (see Supplementary Material). For example, the spin–spin coupling of a secondary methyl group 3H-6‴ [δ 1.56 (d, $J = 6.0$ Hz)] and a carbinolic proton H-5‴ (δ 4.66) was clearly revealed by corresponding cross-peaks observed in the 2D ^1H–^1H COSY spectrum of **1** (see Supplementary Material), in agreement with the presence of a rhamnose moiety. All monosaccharides were in the pyranose form, and the anomeric configurations were α- for arabinose and rhamnose and β- for glucoses, determined by comparing the δ values with the compounds in the literature [6–8]. In the HMBC spectrum, the following correlations were observed: C-1‴ (δ 101.0) with H-2″ (δ 4.58), C-1⁗ (δ 102.8) with H-3″ (δ 4.14), and C-1⁗′ (δ 106.2) with H-2⁗ (δ 3.88) (Figure 2). Other additional heteronuclear long-range interactions involving proton and carbon atoms are described in the Supplementary Material. On the basis of this evidence, the structure of compound **1**, named hippophoside A, was elucidated to be 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-13-ene-19-one-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**).

Compound **2** was isolated as a white amorphous powder. Its molecular formula

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Table 1. ^{1}H NMR spectral data of **1**–**4** (**1** at 400 MHz, **2**–**4** at 600 MHz, in pyridine-d_5, δ_H, J in Hz).

Position	**1**	**2**	**3**	**4**
1	0.76 (t, $J = 9.6$)	0.77 (t, $J = 9.0$)	0.83 (t, $J = 9.6$)	0.77 (t, $J = 9.6$)
	1.46 (d, $J = 12.0$)	1.46 (d, $J = 12.0$)	1.40 (d, $J = 12.0$)	1.36 (d, $J = 12.0$)
2	1.96, 1.76 (m)	2.06, 1.85 (m)	2.20, 2.15 (m)	2.20, 2.15 (m)
3	3.16 (dd, $J = 11.6$, 4.4)	3.22 (dd, $J = 11.4$,4.8)	3.31 (dd, $J = 12.0$,4.8)	3.34 (dd, $J = 12.0$, 4.8)
5	0.64 (d, $J = 12.0$)	0.66 (d, $J = 12.0$)	0.67 (d, $J = 12.0$)	0.66 (d, $J = 12.0$)
6	1.21, 1.34 (m)	1.36, 1.55 (m)	1.38, 1.66 (m)	1.38, 1.65 (m)
7	1.14, 1.25 (m)	1.18, 1.31 (m)	1.18, 1.32 (m)	1.18, 1.31 (m)
9	1.39 (t, $J = 11.8$)	1.41 (t, $J = 12.0$)	1.40 (t, $J = 11.8$)	1.38 (t, $J = 12.0$)
11	1.39 (t, $J = 11.8$)	1.41 (t, $J = 12.0$)	1.42 (t, $J = 11.8$)	1.42 (t, $J = 12.0$)
12	1.43 (t, $J = 12.0$)	2.11 (t, $J = 6.0$)	1.47 (t, $J = 12.0$)	2.11 (t, $J = 6.0$)
15	2.83 (d, $J = 12.4$), 1.78	3.15 (d, $J = 12.6$), 1.77	2.94 (d, $J = 12.2$), 1.76	3.17 (d, $J = 12.6$), 1.76
16	1.38 (t, $J = 12.0$)	1.41 (t, $J = 12.0$)	1.40 (t, $J = 12.0$)	1.41 (t, $J = 12.0$)
21	2.17 (d, $J = 12.6$), 1.78	2.53 (d, $J = 12.6$), 1.63	2.25 (d, $J = 12.6$), 1.85	2.53 (d, $J = 12.6$), 1.63
22	2.41 (d, $J = 12.6$), 1.46	2.33 (d, $J = 12.6$), 2.08	2.53 (d, $J = 12.6$), 1.58	2.33 (d, $J = 12.6$), 2.08
23	1.09 (s)	1.17 (s)	1.18 (s)	1.18 (s)
24	0.96 (s)	1.06 (s)	1.08 (s)	1.07 (s)
25	0.60 (s)	0.64 (s)	0.63 (s)	0.63 (s)
26	0.94 (s)	1.04 (s)	1.04 (s)	1.04 (s)
27	1.13 (s)	1.18 (s)	1.18 (s)	1.18 (s)
29	1.13 (s)	1.47 (s)	1.21 (s)	1.47 (s)
30	0.97 (s)	3.96 (s)	1.04 (s)	3.97 (s)
28-*O*-Glc				
1′	6.14 (d, $J = 8.0$)	6.30 (d, $J = 9.6$)	6.27 (d, $J = 8.4$)	6.31 (d, $J = 9.6$)
2′	4.03 (m)	4.16 (m)	4.13 (m)	4.16 (m)
3′	4.12 (m)	4.21 (m)	4.20 (m)	4.20 (m)
4′	4.09 (m)	4.23 (m)	4.20 (m)	4.23 (m)
5′	3.87 (m)	3.98 (m)	3.99 (m)	3.99 (m)
6′	4.28, 4.39 (m)	4.38, 4.48 (m)	4.30, 4.42 (m)	4.34, 4.45 (m)
3-*O*	Ara	Ara	Glc	Glc
1″	4.68 (d, $J = 6.4$)	4.75 (d, $J = 6.6$)	4.78 (d, $J = 7.2$)	4.78 (d, $J = 7.8$)

(*Continued*)

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Table 1 – *continued*

Position	**1**	**2**	**3**	**4**
2″	4.58 (m)	4.68 (m)	4.27 (m)	4.28 (m)
3″	4.14 (m)	4.23 (m)	4.25 (m)	4.25 (m)
4″	4.45 (m)	4.57 (m)	4.15 (m)	4.14 (m)
5″	3.60 (m)	3.70 (m)	4.28 (m)	4.30 (m)
6″			4.30, 4.42 (m)	4.34, 4.45 (m)
2″-*O*-Rha				
1‴	6.16 (s)	6.31 (s)	6.47 (s)	6.47 (s)
2‴	4.51 (m)	4.63 (m)	4.74 (m)	4.74 (m)
3‴	4.37 (m)	4.50 (m)	4.51 (m)	4.52 (m)
4‴	4.12 (m)	4.25 (m)	4.26 (m)	4.26 (m)
5‴	4.54 (m)	4.66 (m)	4.80 (m)	4.79 (m)
6‴	1.56 (d, $J = 6.0$)	1.65 (d, $J = 6.0$)	1.75 (d, $J = 6.0$)	1.74 (d, $J = 6.0$)
3‴-*O*-Glc				
1⁗	5.04 (d, $J = 7.6$)	5.15 (d, $J = 7.2$)	5.15 (d, $J = 8.4$)	5.16 (d, $J = 8.4$)
2⁗	3.88 (m)	4.00 (m)	4.05 (m)	4.05 (m)
3⁗	3.72 (m)	3.87 (m)	3.83 (m)	3.82 (m)
4⁗	4.03 (m)	4.13 (m)	4.08 (m)	4.10 (m)
5⁗	4.07 (m)	4.18 (m)	4.15 (m)	4.15 (m)
6⁗	4.22, 4.36 (m)	4.31, 4.44 (m)	4.20, 4.35 (m)	4.29, 4.44 (m)
2⁗-*O*-Glc				
1⁗′	5.14 (d, $J = 7.6$)	5.27 (d, $J = 8.4$)	5.30 (d, $J = 7.8$)	5.30 (d, $J = 8.4$)
2⁗′	4.09 (m)	4.21 (m)	4.25 (m)	4.25 (m)
3⁗′	4.06 (m)	4.13 (m)	4.15 (m)	4.14 (m)
4⁗′	4.01 (m)	4.11 (m)	4.08 (m)	4.08 (m)
5⁗′	4.10 (m)	4.19 (m)	4.18 (m)	4.18 (m)
6⁗′	4.28, 4.39 (m)	4.38, 4.48 (m)	4.30, 4.42 (m)	4.34, 4.45 (m)

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Table 2. ^{13}C NMR spectral data of **1**–**4** (**1** at 100 MHz, **2**–**4** at 150 MHz, in pyridine-d_5, δ_C, J in Hz).

Position	1	2	3	4
1	39.4	39.3	39.4	39.4
2	26.7	26.7	26.8	26.8
3	88.2	88.2	88.4	88.4
4	39.5	39.5	39.5	39.5
5	56.2	56.1	56.0	56.1
6	18.4	18.3	18.2	18.3
7	34.8	34.8	34.8	34.8
8	45.4	45.5	45.4	45.5
9	51.2	51.2	51.0	51.1
10	37.3	37.2	37.2	37.2
11	28.2	28.2	28.2	28.2
12	36.0	31.1	35.9	31.1
13	152.0	152.1	152.1	152.1
14	43.2	43.2	43.1	43.2
15	27.3	27.3	27.2	27.3
16	22.9	22.9	22.9	22.9
17	53.0	53.0	53.0	53.0
18	132.8	133.6	132.8	133.6
19	208.1	207.9	208.1	207.9
20	45.5	52.1	45.5	52.1
21	34.2	31.9	34.2	31.9
22	31.8	34.0	31.8	34.0
23	28.2	28.1	28.0	28.1
24	17.0	17.0	17.0	17.0
25	16.6	16.6	16.6	16.6
26	17.4	17.5	17.4	17.5
27	20.9	21.0	21.0	21.0
28	174.8	175.1	174.8	175.1
29	25.3	21.1	25.3	21.0
30	24.4	66.6	24.3	66.6
28-*O*-Glc				
1′	96.4	96.4	96.4	96.4
2′	74.0	74.0	74.0	74.1
3′	78.7	78.8	78.8	78.8
4′	71.3	71.2	71.2	71.2
5′	79.3	79.4	79.4	79.4
6′	62.5	62.4	62.4	62.4
3-*O*	Ara	Ara	Glc	Glc
1″	104.8	104.9	104.9	104.9
2″	74.4	74.4	78.1	78.1
3″	82.2	82.4	87.8	87.8
4″	69.1	69.3	68.8	68.8
5″	65.4	65.6	77.1	77.1
6″			62.6	62.6
2″-*O*-Rha				
1‴	101.0	101.0	101.2	101.2
2‴	72.3	72.3	72.3	72.3
3‴	72.5	72.5	72.4	72.4
4‴	74.0	73.9	73.8	73.8
5‴	69.8	69.8	69.8	69.8
6‴	18.2	18.2	18.2	18.3
3‴-*O*-Glc				
1⁗	102.8	102.9	100.6	100.6
2⁗	84.5	84.4	84.8	84.8
3⁗	78.2	78.1	77.9	77.9
4⁗	71.0	70.9	70.6	70.6
5⁗	78.4	78.4	78.3	78.3
6⁗	62.0	61.9	61.8	61.8
2⁗-*O*-Glc				
1⁗′	106.2	106.2	106.7	106.8
2⁗′	76.1	76.0	76.0	76.1
3⁗′	78.3	78.3	78.0	78.0
4⁗′	70.6	70.6	70.4	70.4
5⁗′	78.7	78.7	78.7	78.7
6⁗′	62.5	62.4	62.0	62.0

was elucidated as $C_{59}H_{94}O_{28}$ by the HR-ESI-MS data at m/z 1273.5833 $[M + Na]^+$. The acid hydrolysis, derivatization, and GC analysis suggested the presence of D-glucose, L-rhamnose, and L-arabinose in **2** as **1**. Comparing the 1D and 2D NMR spectroscopic data of **2** with those of **1**, both were almost superimposable, except for some differences of the signals of aglycone. There were only six tertiary methyl carbons at δ 16.6, 17.0, 17.5, 21.0, 21.1, and 28.1 in **2**, but one more methylene carbon at δ 66.6 (C-30). Hence, it was deduced that one of the methyl carbons in **1** changed to methylene carbon with a hydroxyl, and this was confirmed by the methyl carbons at δ 24.4 (C-30) and δ 25.3 (C-29) in **1** substituted by the signals at δ 21.1 (C-29) and δ 66.6 (C-30) in **2**. The position of methylene is further confirmed by HMBC correlations from C-30 (δ 66.6) to 3H-29 (δ 1.47), C-20 (δ 52.1) with 3H-29 (δ 1.47), and C-19 (δ 207.9) with 3H-29 (δ 1.47). Moreover, the NOESY correlations of 3H-27 (δ 1.18) with 3H-29 (δ 1.47) suggested that the orientation of C_{29} was α. Thus, **2** was determined to be 3-*O*-[β-D-glucopyranosyl (1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-13-ene-19-one-30-hydroxyolean-

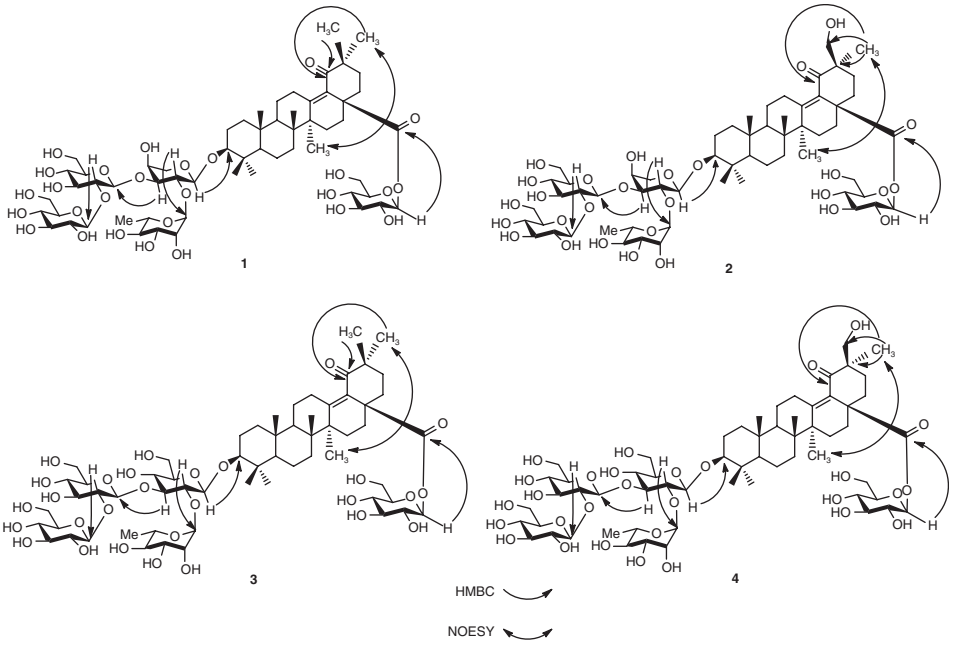

Figure 2. Selected HMBC and NOESY correlations for compounds **1**–**4**.

28-oic acid 28-*O*-β-D-glucopyranosyl ester (**2**) and was named hippophoside B.

Compound **3** was obtained as a white amorphous powder. Its molecular formula was elucidated as $C_{60}H_{96}O_{28}$ by the HR-ESI-MS data at *m/z* 1287.5979 $[M + Na]^+$. The ^1H and ^{13}C NMR signals of **3** were assigned after DEPT, HSQC, and HMBC experiments. A comparison of the ^1H and ^{13}C NMR signals of **3** with those of **1** clearly revealed that the aglycone part of **3** was identical to that of **1**, and **3** was suggested to be 13-ene-19-one-28-oic acid with five monosaccharide units, but one saccharide chain with four monosaccharides at C-3 was different from **1**. Acid hydrolysis suggested that the monosaccharides of this compound were L-rhamnose and D-glucose. The sequence of the glycan parts and glycoside sites were also proven by the following HMBC correlations: C-3 (δ 88.4) with H-1″ (δ 4.78) of the inner glucose, C-2″ (δ 78.1) of the inner glucose with H-1‴ (δ 6.47) of the rhamnose, C-3″ (δ 87.8) of the inner glucose with H-1⁗ (δ 5.15) of the inner glucose, and C-2⁗ (δ 84.8) of the inner glucose with H-1⁗′ (δ 5.30) of the glucose. Hence, the structure of 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl-13-ene-19-one-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**3**) was deduced for **3**, named hippophoside C.

Compound **4** was obtained as a white amorphous powder. Its molecular formula was elucidated as $C_{60}H_{96}O_{29}$ by the HR-ESI-MS data at *m/z* 1303.5944 $[M + Na]^+$. Acid hydrolysis of **4** gave the same results as those of **3**. Compound **4** had the same two sugar chains as **3** by comparison of the ^1H and ^{13}C NMR spectroscopic data of **4** with those of the moieties of **3**. Moreover, the aglycone part of **4** was identical to that of **2** by comparison of their ^1H and ^{13}C NMR spectroscopic data. The HMBC cross-peaks of C-28 (δ 175.1) with H-1′ (δ 6.31) and C-3 (δ 88.4) with H-1″ (δ 4.78) indicated that the sugar chains ether-linked was attached to C-3 and C-28, respectively. Besides, the NOESY

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correlations of 3H-27 (δ 1.18) with 3H-29 (δ 1.47) suggested that the orientation of C_{29} was α. Thus, **4** was determined to be 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl-13-ene-19-one-30-hydroxyolean-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**4**), named hippophoside D.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined with a Perkin-Elmer-341 polarimeter (San Jose, CA, USA). UV–vis spectra were recorded using a Techcomp 8500 spectrometer (Shanghai, China). IR spectra were recorded in KBr pellets on a Nicolet-NEXUS-670-FTIR spectrophotometer (Madison, WI, USA). NMR spectra were obtained with a Varian INOVA-400/600 instrument (Palo Alto, CA, USA). The ^{1}H and ^{13}C NMR chemical shifts were relative to solvent signals at $\delta_{H/C}$ 2.49/39.5 (DMSO-d_6) relative to tetramethylsilane. MS was measured on a Waters Q-Tof micro YA019 mass spectrometer (Leederville, WA, USA). Preparative HPLC was performed on a Shimadzu HPLC system consisting of two LC-8A pumps and an SPD-M10A detector. For preparative purposes, a Shimadzu PRC-ODS (15 μm, i.d. 20 mm × 250 mm; Kyoto, Japan) was used. Analytical GC was carried out on an Agilent 6890N system (H_2 flame ionization detector; Minneapolis, MN, USA) and a capillary column (30 m × 0.32 mm × 0.25 μm; Abel AB-5, Appleton, WI, USA). Column chromatography (CC) was performed on silica gel (100–200 or 200–300 mesh; Shanghai Sanpont Co., Ltd, Shanghai, China), D101 macroporous resin (Cangzhou Bon Adsorber Technology Co., Ltd, Cangzhou, China), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Silica gel HSGF254 (Yantai Jiangyou Guijiao Kaifa Co., Ltd, Yantai, China) was used for thin layer chromatography (TLC). Fractions were monitored by TLC on silica-gel plates sprayed with 10% H_2SO_4 in EtOH, followed by heating.

3.2 Plant materials

The seeds of *H. rhamnoides* subsp. *sinensis* were collected at Datong County of Qinghai Province, China, in July 2008 and identified by Dr Min-Sheng Cao of Qinghai General Health Bio-Science Co., Ltd. A voucher specimen (SIPITCM-080715) has been deposited at the Shanghai Institute of Pharmaceutical Industry.

3.3 Extraction and isolation

The seed residues of *H. rhamnoides* subsp. *sinensis*, which had been extracted through supercritical fluid extraction, were obtained from Qinghai General Health Bio-Science Co., Ltd (Xining, Qinghai, China). The air-dried seed residues of *H. rhamnoides* subsp. *sinensis* (12 kg) were extracted twice with 70% EtOH (120 l each) for 1 h. The extracts were combined and concentrated under reduced pressure to give a residue (900 g), which was suspended in 30% EtOH, and then passed through a D101 macroporous resin column eluted with EtOH–H_2O (30:70, 72 l and 70:30, 72 l (v/v)) to yield two fractions (Frs A and B). Fr. B (47 g) was chromatographed on a silica-gel column (10 × 60 cm) eluted with $CHCl_3$–MeOH–H_2O (9:1:0.1, 8:2:0.2, 7:3:0.5, and 6:4:1 (v/v)) and MeOH, each 15 l, to give Frs B1–B11. Fr. B9 (3.23 g) was loaded on silica-gel CC, eluted with $CHCl_3$–MeOH–H_2O (8:1:0.1, 6:1:0.1, and 4:1:0.1 (v/v)) and MeOH, each 0.5 l, to give Frs B9-1–B9-4. Fr. B9-3 (1.02 g) was subjected to Sephadex LH-20 column eluted with MeOH and pre-HPLC (MeOH–H_2O, 60:40 (v/v); 7 ml/min; UV detection 254 nm), and compounds **1** (35 mg, t_R: 25.461 min) and **3** (25 mg, t_R: 15.869 min) were obtained, respectively. Fr. B9-4 (1.36 g) was separated by a Sephadex

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LH-20 column eluted with MeOH and Pre-HPLC (MeOH–H_2O 52:48 (v/v); 7 ml/min; UV detection 254 nm) to yield compounds **2** (14 mg, t_R: 31.911 min) and **4** (25 mg, t_R: 23.881 min).

*3.3.1 3-O-[β-D-Glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-13-ene-19-one-28-oic acid 28-O-β-D-glucopyranosly ester (**1**)*

White amorphous powder; $[\alpha]_D^{25}$ −83.2 (*c* 0.45, MeOH). UV (MeOH) λ_{max} (log ε) nm: 254 (3.31); IR (KBr) ν_{max} cm^{-1}: 3423, 2927, 1736, 1638, 1458, 1384, 1075, 1028, 912, 589; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; ESI-MS *m/z*: 1257 $[M + Na]^+$ and 1071 $[M - 162 - H]^-$. HR-ESI-MS (positive) *m/z*: 1257.5886 $[M + Na]^+$ (calcd for $C_{59}H_{94}O_{27}Na$, 1257.5880).

*3.3.2 3-O-[β-D-Glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-13-ene-19-one-30-hydroxyolean-28-oic acid 28-O-β-D-glucopyranosly ester (**2**)*

White amorphous powder; $[\alpha]_D^{25}$ − 67.8 (*c* 0.46, MeOH). UV (MeOH) λ_{max} (log ε) nm: 216 (3.22), 256 (3.26); IR (KBr) ν_{max} cm^{-1}: 3422, 2925, 1735, 1655, 1458, 1384, 1261, 1074, 912, 592; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; ESI-MS *m/z*: 1273 $[M + Na]^+$ and 1249 $[M - H]^-$. HR-ESI-MS (positive) *m/z*: 1273.5833 $[M + Na]^+$ (calcd for $C_{59}H_{94}O_{28}Na$, 1273.5829).

*3.3.3 3-O-[β-D-Glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl-13-ene-19-one-28-oic acid 28-O-β-D-glucopyranosly ester (**3**)*

White amorphous powder; $[\alpha]_D^{25}$ −52.9 (*c* 0.49, MeOH). UV (MeOH) λ_{max} (log ε) nm: 217 (3.24), 253 (3.22); IR (KBr) ν_{max} cm^{-1}: 3423, 2924, 1736, 1654, 1458, 1384, 1075, 912, 569; ESI-MS *m/z*: 1287 $[M + Na]^+$ and 1101 $[M - 162 - H]^-$. HR-ESI-MS (positive) *m/z*: 1287.5979 $[M + Na]^+$ (calcd for $C_{60}H_{96}O_{28}Na$, 1287.5986).

*3.3.4 3-O-[β-D-Glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 3)]-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl-13-ene-19-one-30-hydroxyolean-28-oic acid 28-O-β-D-glucopyranosly ester (**4**)*

White amorphous powder; $[\alpha]_D^{25}$ −77.5 (*c* 0.45, MeOH). UV (MeOH) λ_{max} (log ε) nm: 256 (3.27); IR (KBr) ν_{max} cm^{-1}: 3421, 2926, 1735, 1655, 1458, 1384, 1262, 1075, 911, 570; ESI-MS *m/z*: 1303 $[M + Na]^+$ and 1279 $[M - H]^-$. HR-ESI-MS (positive) *m/z*: 1303.5944 $[M + Na]^+$ (calcd for $C_{60}H_{96}O_{29}Na$, 1303.5935).

3.4 Acid hydrolysis of 1–4

Compounds **1**–**4** (10 mg each) were refluxed in 1 mol/l HCl (1 ml) for 3 h. The mixture was neutralized with $NaHCO_3$ and then partitioned with dichloromethane. To the dried aqueous layer, pyridine and hydroxylamine hydrochloride (10 mg each) were added, and the solution was stirred at 95°C for 1 h. Then, after addition of acetic anhydride (1 ml each), the mixture was kept overnight. The acetylated derivatives were subjected to GC analysis to identify the sugars. Conditions for GC were as follows: AB-5 (30 m × 0.32 mm × 0.25 μm) column; column temp.: from 100 to 250°C, programmed increase 10°C/min and keeping 250°C for 5 min; injector and detector temp.: 250°C; injection volume: 2.0 ml; split ratio: 1:20; carrier gas: N_2 at 1 ml/min [9]. Under these conditions, the standard sugars gave peaks at t_R (min): 15.734 of D-glucose, 12.762 of L-rhamnose, and 12.951 of L-arabinose, respectively. Peaks

at t_R (min): 15.724, 12.759, and 12.962 of D-glucose, L-rhamnose, and L-arabinose for **1**–**2**, while 15.814 and 12.822 of D-glucose and L-rhamnose for **3**–**4**, were observed.

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