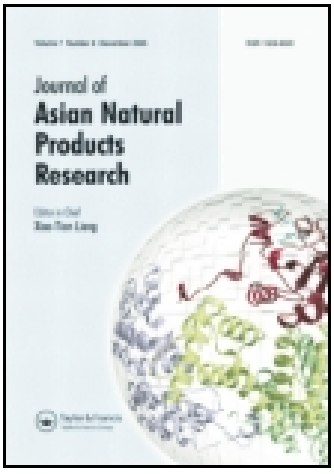
This article was downloaded by: [University of Winnipeg]
On: 28 August 2014, At: 02:38
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Hippophins C-F, four new flavonoids, acylated with one monoterpenic acid from the seed residue of Hippophae rhamnoides subsp. sinensis

Wen Gao [a], Chao Chen [a] & De-Yun Kong [a]
[a] State Key Laboratory of New Drug & Pharmaceutical Process, Shanghai Institute of Pharmaceutical Industry, Shanghai, 200040, China
Published online: 03 May 2013.

To cite this article: Wen Gao, Chao Chen & De-Yun Kong (2013) Hippophins C-F, four new flavonoids, acylated with one monoterpenic acid from the seed residue of Hippophae rhamnoides subsp. sinensis, Journal of Asian Natural Products Research, 15:5, 507-514, DOI: 10.1080/10286020.2013.787989

To link to this article: http://dx.doi.org/10.1080/10286020.2013.787989

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms &

Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Downloaded by [University of Winnipeg] at 02:38 28 August 2014

Journal of Asian Natural Products Research, 2013
Vol. 15, No. 5, 507–514, http://dx.doi.org/10.1080/10286020.2013.787989

Taylor & Francis
Taylor & Francis Group

Hippophins C–F, four new flavonoids, acylated with one monoterpenic acid from the seed residue of *Hippophae rhamnoides* subsp. *sinensis*

Wen Gao, Chao Chen and De-Yun Kong*

State Key Laboratory of New Drug & Pharmaceutical Process, Shanghai Institute of Pharmaceutical Industry, Shanghai 200040, China

(*Received 19 October 2012; final version received 18 March 2013*)

Four new flavonol glycosides (**1**–**4**), hippophins C–F, together with one known flavonoid (**5**), were isolated from the seed residue of *Hippophae rhamnoides* subsp. *sinensis*. The chemical structures of these compounds were characterized by 1D and 2D NMR, and HR-ESI-MS data. This report is a continuous research work on the systematic chemical investigation of plants of the genus *Hippophae* in our laboratory.

Keywords: *Hippophae rhamnoides* subsp. *sinensis*; seed residue; flavonol glycoside; hippophins

Downloaded by [University of Winnipeg] at 02:38 28 August 2014

1. Introduction

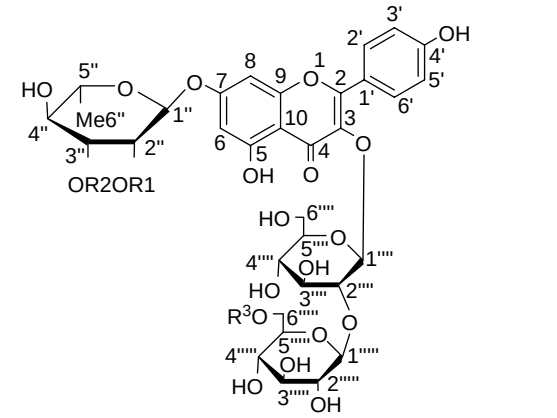
Hippophae rhamnoides subsp. *sinensis* is a plant of the genus *Hippophae* growing in west-north and north China [1]. The berry of this genus, used as a famous traditional medicine since ancient times by Tibetans and Mongolians, has several therapeutic uses due to its antioxidant, antibechic, antiviral, etc., activities [2]. Previous phytochemical studies of the genus *Hippophae* have led to the isolation of a number of flavonoids [3]. In addition, a new flavonoid acylated with one monoterpenic acid was obtained in our previous investigation on *H. rhamnoides* subsp. *sinensis* collected in Qinghai Province of China [4]. As a continuation of our systematic chemical investigation of plants of the genus *Hippophae*, the seed residues were studied. This article reported the isolation and structural elucidation of four new flavonoids acylated with one monoterpenic acid (**1**–**4**), together with one known flavonoid (**5**) (Figure 1).

2. Results and discussion

Hippophin C (**1**) was obtained as a yellow amorphous powder and its molecular formula was elucidated as $C_{54}H_{64}O_{26}$ by the HR-ESI-MS data at *m/z* 1151.3596 $[M + Na]^+$. The ^1H NMR spectrum (Table 1) of **1** presented a kaempferol skeleton, with typical signals at δ 6.86 and 6.40 (br s, each 1H) and AA′BB′ aromatic system signals at δ 8.03 (2H, d, $J = 8.8$ Hz) and 6.87 (2H, d, $J = 8.8$ Hz); a pair of aromatic protons at δ 6.70 (2H, s); one vinyl proton at δ 6.80 (1H, d, $J = 6.8$ Hz), a pair of *E*-vinyl protons at δ 7.34 (1H, d, $J = 16.0$ Hz) and 6.22 (1H, d, $J = 16.0$ Hz); a set of ABX-spin systems corresponded to the vinyl protons at δ 5.91 (1H, dd, $J = 17.2$, 11.8 Hz), 5.15 (1H, $J = 17.2$ Hz), 4.99 (1H, $J = 11.8$ Hz); a pair of methoxyl protons at δ 3.68 (6H, s), a methyl proton at δ 1.18 (3H, d, $J = 8.4$ Hz); and three anomeric protons at δ 5.56 (1H, br s), 5.46 (d, $J = 6.8$ Hz), and 4.62 (1H, d, $J = 6.0$ Hz). The ^{13}C NMR spectrum (Table 2) of **1** provided 54

*Corresponding author. Email: deyunk@yaohoo.com.cn

© 2013 Taylor & Francis



	R^1	R^2	R^3
1	H	S	E-sinapoyl
2	S	H	E-sinapoyl
3	H	S	E-feruloyl
4	S	H	E-feruloyl
5	H	H	E-sinapoyl

Figure 1. Structures of compounds **1–5**.

carbon signals, of which 15 were assigned to the aglycone and 18 to the sugar moiety. Analysis of the 18 carbon signals from the sugar moiety indicated the existence of one rhamnose at δ 98.3, 67.4, 73.8, 70.2, 69.0, and 17.9, glucose-I at δ 98.2, 83.2, 76.5, 69.7, 76.4, and 60.7, and glucose-II at δ 104.4, 74.6, 77.5, 70.0, 74.5, and 63.4, respectively. The anomeric proton at δ 5.56 (1H, br s) suggested α-configuration of rhamnose, and two glucoses were deduced to be β-configuration based on its ^{13}C NMR data [5]. Two methyl singlets (δ 12.3 and 27.8), three methylene groups (δ 23.3, 40.6, and 111.4), two methinyl groups (δ 145.2 and 143.8), two quaternary carbons (δ 127.2 and 71.4), and a carbonyl group (δ 166.4) were attributed a monoterpenoic acid moiety, and the signal of the β-vinyl proton of 2(*E*)-2-methyl-2-decanoic acid appeared downfield at δ 6.80 (1H, d, $J = 6.8$ Hz), different from that of the *cis*-isomer, appearing at δ 6.06 [6]. The remaining 11 carbons belonged to 4-hydroxy-3,5-dimethoxycinnamoyl (*E*-sinapoyl) at δ 167.1, 115.3, 145.9, 126.7, 106.1 × 2, 148.0 × 2, 138.4, and 56.1 × 2 [5]. The long-range correlations of H-1″ (δ 5.56) and C-7 (δ 161.3), H-1‴ (δ 5.46) and C-3 (δ 133.4), H-1⁗ (δ 4.62) and C-2‴ (δ 83.2), and H-6⁗ (δ 3.35) and C-9⁗′ (δ 167.1) were observed in the HMBC spectrum (Figure 2), suggesting that the rhamnose was attached to 7-OH and the glucose-I was attached to 3-OH of kaempferol, the glucose-II was attached to 2-OH of glucose-I and its 6-OH was substituted by 4-hydroxy-3,5-dimethoxycinnamoyl. A monoterpenoic acid moiety is located at 3″-OH of rhamnose compared with literature data at δ 98.2, 67.3, 73.7, 70.1, 68.7, and 17.9 [7].

Downloaded by [University of Winnipeg] at 02:38 28 August 2014

Downloaded by [University of Winnipeg] at 02:38 28 August 2014

Table 1. ^{1}H NMR spectral data of **1**–**4** (DMSO-d_6, δ_H, J in Hz).

Position	**1**	**2**	**3**	**4**
6	6.40 (br. s)	6.41(br. s)	6.39 (br. s)	6.38 (br. s)
8	6.86 (br. s)	6.85 (br. s)	6.81 (br. s)	6.79 (br. s)
2′	8.03 (d, $J = 8.8$)	8.04 (d, $J = 8.8$)	8.01 (d, $J = 9.0$)	8.03 (d, $J = 9.0$)
3′	6.87 (d, $J = 8.8$)	6.88 (d, $J = 8.8$)	6.83 (d, $J = 9.0$)	6.80 (d, $J = 9.0$)
5′	6.87 (d, $J = 8.8$)	6.88 (d, $J = 8.8$)	6.83 (d, $J = 9.0$)	6.80 (d, $J = 9.0$)
6′	8.03 (d, $J = 8.8$)	8.04 (d, $J = 8.8$)	8.01 (d, $J = 9.0$)	8.03 (d, $J = 9.0$)
Rha				
1″	5.56 (br s)	5.60 (br s)	5.60 (br s)	5.61 (br s)
2″	4.08 (m)	5.11 (m)	4.05 (m)	5.12 (m)
3″	4.98 (m)	4.08 (m)	4.96 (m)	4.06 (m)
4″	3.10 (m)	3.67 (m)	3.09 (m)	3.69 (m)
5″	3.58 (m)	3.58 (m)	3.59 (m)	3.59 (m)
6″	1.18 (d, $J = 8.4$)	1.18 (d, $J = 8.4$)	1.51 (d, $J = 8.5$)	1.51 (d, $J = 8.5$)
Monoterpenoic acyl				
3‴	6.80 (t, $J = 6.8$)	6.81 (t, $J = 6.8$)	6.80 (t, $J = 6.8$)	6.78 (t, $J = 7.0$)
4‴	2.19 (m)	2.19 (m)	2.19 (m)	2.19 (m)
5‴	1.54 (t, $J = 8.4$)	1.54 (t, $J = 8.4$)	1.54 (t, $J = 8.4$)	1.54 (t, $J = 8.4$)
6‴	5.91 (dd, $J = 17.2$, 11.8)	5.90 (dd, $J = 17.2$, 11.8)	5.91 (dd, $J = 17.5$, 10.5)	5.86 (dd, $J = 17.5$, 10.5)
7‴	5.15(dd, $J = 17.2$)	5.10 (dd, $J = 17.2$)	5.15 (dd, $J = 17.5$)	5.16 (dd, $J = 17.5$)
8‴	4.99 (dd, $J = 11.8$)	4.92 (dd, $J = 11.8$)	4.99 (dd, $J = 10.5$)	4.99 (dd, $J = 10.5$)
9‴	1.81 (s)	1.81 (s)	1.81 (s)	1.81 (s)
10‴	1.19 (s)	1.19 (s)	1.19 (s)	1.19 (s)
Glc I				
1⁗	5.46 (d, $J = 6.8$)	5.68 (d, $J = 6.8$)	5.64 (d, $J = 6.8$)	5.62 (d, $J = 6.8$)
2⁗	3.23 (m)	3.47 (m)	3.49 (m)	3.49 (m)
3⁗	3.34 (m)	3.45 (m)	3.46 (m)	3.45 (m)
4⁗	3.12 (m)	3.12 (m)	3.13 (m)	3.13 (m)
5⁗	3.10 (m)	3.46 (m)	3.41 (m)	3.40 (m)
6⁗	3.36, 3.56 (2 m)	3.27, 3.50 (2 m)	3.27, 3.46 (2 m)	3.27, 3.45 (2 m)
Glc II				
1⁗′	4.62 (d, $J = 6.0$)	4.61 (d, $J = 6.0$)	4.62 (d, $J = 8.0$)	4.62 (d, $J = 8.0$)
2⁗′	3.11 (m)	3.11 (m)	3.09 (m)	3.10 (m)

Downloaded by [University of Winnipeg] at 02:38 28 August 2014

Table 1 – *continued*

Position	1	2	3	4
3′′′′′	3.12 (m)	3.13 (m)	3.13 (m)	3.13 (m)
4′′′′′	3.15 (m)	3.16 (m)	3.18 (m)	3.17 (m)
5′′′′′	3.12 (m)	3.12 (m)	3.12 (m)	3.12 (m)
6′′′′′	3.35 (m)	3.34 (m)	3.35 (m)	3.34 (m)
Sinapoyl or feruloyl				
2′′′′′	6.70 (s)	6.72 (s)	7.35 (br.s)	7.34 (br s)
5′′′′′	–	–	6.84 (br s)	6.81 (br s)
6′′′′′	6.70 (s)	6.72 (s)	7.05 (br s)	7.07 (br s)
7′′′′′	7.34 (d, $J = 16.0$)	7.35 (d, $J = 16.0$)	7.33 ($J = 16.0$)	7.38 ($J = 16.0$)
8′′′′′	6.22 (d, $J = 16.0$)	6.23 (d, $J = 16.0$)	6.15 ($J = 16.0$)	6.12 ($J = 16.0$)
3′′′′′-OMe	3.68(s)	3.65 (s)	3.69(s)	3.69(s)
5′′′′′-OMe	3.68(s)	3.65 (s)	–	–

Thus, the structure of hippophin C was characterized as kaempferol-3-*O*-(6-*O*-*E*-sinapoyl)-β-D-glucosyl(1 → 2)-β-D-glucoside-7-*O*-[(2*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl(1 → 3)]-α-L-rhamnoside.

Hippophin D (**2**) was obtained as a yellow amorphous powder and its molecular formula was elucidated as $C_{54}H_{64}O_{26}$ by the HR-ESI-MS data at *m/z* 1151.3591 $[M + Na]^+$. Comparison of ^1H and ^{13}C NMR spectroscopic data of **2** with those of **1** indicated that compound **2** has the same aglycone, sugar moiety, *E*-sinapoyl, and monoterpenoic acid moiety as compound **1**. The HMBC correlations from H-1″ (δ 5.60) to C-7 (δ 161.2), H-1′′′′ (δ 5.68) to C-3 (δ 133.3), H-1′′′′′ (δ 4.61) to C-2′′′′ (δ 83.1), and H-6′′′′′ (δ 3.34) to C-9′′′′′′ (δ 167.0) indicated that the linkage sites among the above structure moieties in compound **2** were identical with those of compound **1**. The carbon signals of rhamnose at δ 95.5, 71.8, 68.5, 72.2, 70.2, and 18.0 revealed a monoterpenoic acid moiety located at 2″-OH of rhamnose compared with literature data at δ 95.3, 71.9, 68.4, 72.0, 70.1, and 17.8 [8]. Based on the above evidence, the structure of hippophin D was elucidated as kaempferol-3-*O*-(6-*O*-*E*-sinapoyl)-β-D-glucosyl(1 → 2)-β-D-glucoside-7-*O*-[(2*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl (1 → 2)]-α-L-rhamnoside.

Hippophin E (**3**) was obtained as a yellow amorphous powder and its molecular formula was elucidated as $C_{53}H_{62}O_{25}$ by the HR-ESI-MS data at *m/z* 1121.3486 $[M + Na]^+$. Comparison of ^1H and ^{13}C NMR spectroscopic data of **3** with those of **1** revealed that two compounds have the same flavonol core structure, sugar, and monoterpenoic acid moiety while one of the methoxyl groups was absent in **3**. The ^{13}C NMR signals suggested existence of 4-hydroxy-3-methoxycinnamoyl (feruloyl) at δ 126.6, 111.4, 147.9, 147.8, 115.4, 123.1, 145.8, 111.3, and 166.5 by comparison with literature data [7]. A pair of *E*-vinyl protons was verified by ^1H NMR signals at δ 7.33 (1H, d, $J = 16.0$ Hz) and 6.15 (1H, d,

Downloaded by [University of Winnipeg] at 02:38 28 August 2014

Table 2. ^{13}C NMR spectral data of **1**–**4** (DMSO-d_6, δ_C, J in Hz).

Position	1	2	3	4
2	156.3	156.4	156.1	156.1
3	133.4	133.3	133.1	133.1
4	177.7	177.7	177.5	174.3
5	160.2	160.2	160.2	160.1
6	99.3	99.3	99.3	99.2
7	161.3	161.2	161.1	161.0
8	94.4	94.4	94.2	94.2
9	155.9	155.9	155.8	155.8
10	106.0	106.0	105.7	105.7
1′	120.8	120.8	120.8	120.7
2′	131.1	131.1	131.1	131.1
3′	114.7	114.6	115.4	115.3
4′	161.0	161.1	160.8	160.7
5′	114.7	114.6	115.4	115.3
6′	131.1	131.1	131.1	131.1
Rha				
1″	98.3	95.5	98.1	95.4
2″	67.4	71.8	67.3	71.7
3″	73.8	68.5	73.7	68.3
4″	70.2	72.2	70.2	72.3
5″	69.0	70.2	69.0	70.2
6″	17.9	18.0	18.0	17.9
Monoterpenoic acyl				
1‴	166.4	166.5	166.5	166.5
2‴	127.2	126.8	127.0	129.7
3‴	143.8	143.8	143.7	142.9
4‴	23.3	23.3	23.2	23.2
5‴	40.6	40.7	40.6	40.6
6‴	71.4	71.4	71.3	71.3
7‴	145.2	145.9	145.0	144.9
8‴	111.4	111.4	111.3	110.6
9‴	12.3	12.3	12.2	12.1
10‴	27.8	27.8	27.8	27.7
Glc I				
1⁗	98.2	98.3	98.0	98.1
2⁗	83.2	83.1	84.1	84.1
3⁗	76.5	76.5	76.4	76.4
4⁗	69.7	69.8	69.8	69.7
5⁗	76.4	76.4	76.2	76.1
6⁗	60.7	61.0	60.4	60.3
Glc II				
1⁗′	104.4	104.4	104.7	104.4
2⁗′	74.6	74.2	74.6	74.6
3⁗′	77.5	77.4	77.5	77.4
4⁗′	70.0	70.0	70.0	70.1
5⁗′	74.5	74.4	74.5	74.6
6⁗′	63.4	63.3	63.3	63.1
Sinapoyl or feruloyl				
1⁗″	126.7	124.4	126.6	126.5
2⁗″	106.1	106.1	111.4	111.3
3⁗″	148.0	148.0	147.8	147.8
4⁗″	138.4	138.3	147.9	147.9
5⁗″	148.0	148.0	115.4	115.2
6⁗″	106.1	106.1	123.1	123.2
7⁗″	145.9	145.9	145.8	145.9
8⁗″	115.3	115.3	111.3	111.3
9⁗″	167.1	167.0	166.5	167.0
3⁗″-OMe	56.1	56.0	55.5	55.4
5⁗″-OMe	56.1	56.0	–	–

$J = 16.0$ Hz). Thus, the structure of hippophin E was characterized as kaempferol-3-*O*-(6-*O*-*E*-feruloyl)-β-D-glucosyl(1 → 2)-β-D-glucoside-7-*O*-[(2*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl(1 → 3)]-α-L-rhamnoside.

Hippophin F (**4**) was obtained as a yellow amorphous powder and its molecular formula was elucidated as $C_{53}H_{62}O_{25}$ by the HR-ESI-MS data at *m/z* 1121.3472 $[M + Na]^+$. Comparison of ^1H and ^{13}C NMR spectroscopic data of **4** with those of **3** revealed that the two compounds had a similar structure except for the substituent position in rhamnose, and comparison of carbon signals of **4** with those of **2** revealed a monoterpenoic acid moiety located at 2″-OH of rhamnose [8]. Thus, the structure of hippophin F was determined as kaempferol-3-*O*-(6-*O*-*E*-feruloyl)-β-D-glucosyl(1 → 2)-β-D-glucoside-7-*O*-[(2*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl (1 → 2)]-α-L-rhamnoside.

By comparing the NMR and MS data with those in the literature, one known flavonoid was identified as kaemferol-3-*O*-(6-*O*-*E*-sinapoyl)-β-D-glucosyl(1 → 2)-β-D-glucoside-7-*O*-α-L-rhamnoside (**5**) [5].

3. Experimental

3.1 Genenral experimental procedures

Optical rotations were determined with a Perkin-Elmer-341 polarimeter (Perkin-Elmer, San Jose, CA, USA). UV–vis spectra were recorded using a Techcomp 8500 spectrometer (Techcomp, Shanghai, China). IR spectra were recorded in KBr

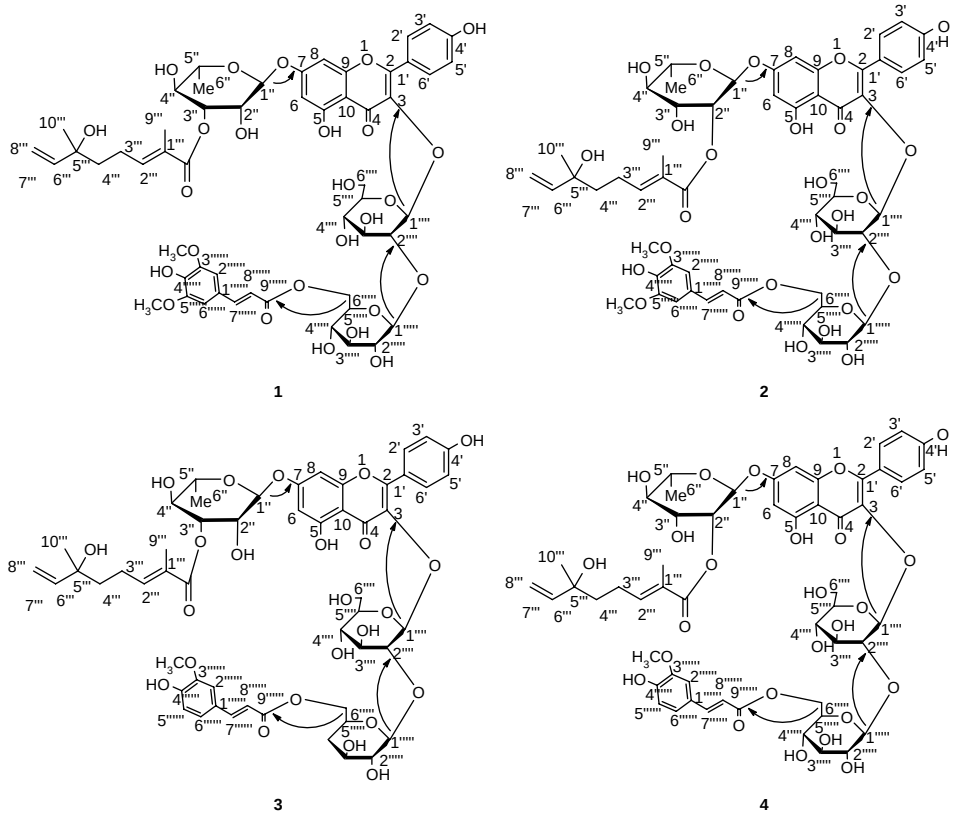


Figure 2. Selected HMBC correlations for compounds **1–4**.

pellets on a Nicolet-NEXUS-670-FTIR spectrophotometer (Nicolet, Madison, WI, USA). NMR spectra were obtained with a Varian INOVA-400/500 instrument (Varian, 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 (Waters, Leederville, WA, USA). Preparative HPLC was obtained 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, Shimadzu, Kyoto, Japan) was used. Analytical GC was carried out on Agilent 6890N system (H_2 flame ionization detector; Agilent, Minneapolis, MN, USA) and a capillary column (30 m × 0.32 mm × 0.25 μm; Abel AB-5; Appleton, WI, USA). Column chromatography was carried out 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), 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.

Downloaded by [University of Winnipeg] at 02:38 28 August 2014

Downloaded by [University of Winnipeg] at 02:38 28 August 2014

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* (2 kg) were extracted twice with 70% EtOH (20 liters each) for 1 h. The extracts were combined and concentrated under reduced pressure to a certain volume (2 liters), and then passed through a D101 macroporous resin column (5 kg) eluted with EtOH:H_2O (10:90, 45 liters; 70:30, 35 liters (v/v)) to yield two fractions (Fr. A and Fr. B). Fr. B (100 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) and MeOH, each 15 liters, to give Frs B1–B8. Fr B4 (5.03 g) was subjected to middle pressure liquid chromatography (Cheetah, Tianjin Agela Technology Co., Ltd, Tianjin, China) on silica gel (4.5 × 25.0 cm), eluted with $CHCl_3$:MeOH:H_2O (10:1:0.1, 8:1:0.1, 7:1:0.1, 6:1:0.1, 5:1:0.1, 4:1:0.1, and 3:1:0.1) and MeOH, each 1 liter, to give Frs B4-1–B4-10. Fr. B4-3 (116 mg) was subjected to Sephadex LH-20 column with MeOH and pre-HPLC (MeOH:H_2O 50:50, 6 ml/min, UV detection 254 nm), and compounds **1** (8 mg, t_R: 48 min), **2** (12 mg, t_R: 59 min), **3** (10 mg, t_R: 41 min), and **4** (11 mg, t_R: 52 min) were obtained, respectively. Fr. B6 (532 mg) was separated by a Sephadex LH-20 column with MeOH and pre-HPLC (MeOH:H_2O 32:68, 6 ml/min, UV detection 254 nm) to yield compound **5** (14 mg, t_R: 26 min).

3.3.1 *Kaempferol-3-O-(6-O-E-sinapoyl)-β-D-glucosyl(1 → 2)-β-D-glucoside-7-O-[(2E)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl (1 → 3)]-α-L-rhamnoside (**1**)*

A yellow amorphous powder; $[\alpha]_D^{20}$ −52.7 (c = 0.08, MeOH). UV (MeOH) λ_{max} (log ε) nm: 268 (3.35), 329 (3.38); IR (KBr) ν_{max} cm^{-1}: 3424, 2928, 1701, 1655, 1598, 1283, 1178, and 994; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; ESI-MS *m/z*: 1151 $[M + Na]^+$ and 1127 $[M - H]^-$. HR-ESI-MS (positive) *m/z*: 1151.3596 $[M + Na]^+$ (calcd for $C_{54}H_{64}O_{26}Na$, 1151.3584).

3.3.2 *Kaempferol-3-O-(6-O-E-sinapoyl)-β-D-glucosyl(1 → 2)-β-D-glucoside-7-O-[(2E)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl (1 → 2)]-α-L-rhamnoside (**2**)*

A yellow amorphous powder; $[\alpha]_D^{20}$ −50.1 (c = 0.08, MeOH). UV (MeOH) λ_{max} (log ε) nm: 268 (3.35), 329 (3.38); IR (KBr) ν_{max} cm^{-1}: 3427, 2930, 1705, 1651, 1597, 1285, 1177, and 989; for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; ESI-MS *m/z*: 1151 $[M + Na]^+$ and 1127 $[M - H]^-$. HR-ESI-MS (positive) *m/z*: 1151.3591 $[M + Na]^+$ (calcd for $C_{54}H_{64}O_{26}Na$, 1151.3584).

3.3.3 *Kaempferol-3-O-(6-O-E-feruloyl)-β-D-glucosyl(1 → 2)-β-D-glucoside-7-O-[(2E)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl (1 → 3)]-α-L-rhamnoside (**3**)*

A yellow amorphous powder; $[\alpha]_D^{20}$ −63.0 (c = 0.06, MeOH). UV (MeOH) λ_{max} (log ε) nm: 272 (4.34), 333 (4.41); IR (KBr) ν_{max} cm^{-1}: 3425, 2929, 1702, 1652, 1597, 1288, 1173, and 992; ESI-MS *m/z*: 1121 $[M + Na]^+$ and 1097 $[M - H]^-$. HR-ESI-MS (positive) *m/z*: 1121.3486

$[M + Na]^+$ (calcd for $C_{53}H_{62}O_{25}Na$, 1121.3478).

*3.3.4 Kaempferol-3-O-(6-O-E-feruloyl)-β-D-glucosyl(1 → 2)-β-D-glucoside-7-O-[(2E)-2,6-dimethyl-6-hydroxy-2,7-octadienoyl (1 → 2)]-α-L-rhamnoside (**4**)*

A yellow amorphous powder; $[\alpha]_D^{20}$ −61.4 ($c = 0.06$, MeOH). UV (MeOH) λ_{max} (log ε) nm: 272 (4.34), 332 (4.41); IR (KBr) ν_{max} cm^{-1}: 3427, 2932, 1704, 1651, 1598, 1287, 1170, and 985; ESI-MS *m/z*: 1151 $[M + Na]^+$ and 1127 $[M - H]^-$. HR-ESI-MS (positive) *m/z*: 1121.3472 $[M + Na]^+$ (calcd for $C_{54}H_{64}O_{26}Na$, 1121.3478).

3.4 Acid hydrolysis of 1–4

Compounds **1**, **2**, **3**, and **4** (5 mg each) were refluxed in 2 mol/l HCl (2 ml) for 3 h. The mixture was neutralized with $NaHCO_3$ and then partitioned with ethyl acetate. To the dried aqueous layer, pyridine and acetic anhydride (1 ml each) were added, and the mixture was kept overnight. The acetylated derivatives were subjected to GC analysis to identify the sugars. Conditions for GC: 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]. D-Glucose (t_R: 16.021 min)/L-rhamnose (t_R: 13.027 min) were detected from **1** to **4** (identical to authentic materials).

References

[1] Y.S. Liang and X.L. Chen, *Hippophae* **1**, 15 (1996).
[2] B.R. Yang, K.O. Kalimo, R.L. Tahvonen, L.M. Matilla, J.K. Katajisto, and H.P. Kallio, *J. Nutr. Biochem.* **11**, 338 (2000).
[3] M.Y. Xu, X.X. Sun, and W.X. Tong, *Hippophae* **7**, 32 (1994).
[4] J. Zhang, X.X. Weng, W. Gao, Z.J. Zhu, and D.Y. Kong, *Chin. J. Pharm.* **42**, 265 (2011).
[5] Z.P. Yong, C. Chen, H. Zhang, and J.H. Liu, *West China J. Pharm. Sci.* **25**, 633 (2010).
[6] Y. Okada, K. Koyama, K. Takahashi, T. Okuyama, and S. Sjibata, *Planta Med.* **40**, 185 (1980).
[7] Y. Takatoshi, S. Takashi, and K. Shizuo, *Chem. Pharm. Bull.* **35**, 97 (1987).
[8] T. Murakami, K. Kohno, A. Kishi, H. Matsuda, and M. Yoshikawa, *Chem. Pharm. Bull.* **48**, 1673 (2000).
[9] X.X. Weng, Y. Shao, Y.Y. Chen, W. Gao, L. Cheng, and D.Y. Kong, *J. Asian Nat. Prod. Res.* **13**, 749 (2011).

Downloaded by [University of Winnipeg] at 02:38 28 August 2014