New Flavonoids from *Oxytropis myriophylla*

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Eight compounds were isolated from *Oxytropis myriophylla*. On the basis of spectral analyses, their structures were elucidated to be (6*R*,9*R*)-roseoside (1), (6*R*,9*S*)-roseoside (2), adenosine (3), myriophylloside B (4), myriophylloside C (5), myriophylloside D (6), myriophylloside E (7), and myriophylloside F (8). Five flavonoids (4—8) were new compounds, and the three known compounds were isolated from this plant for the first time.

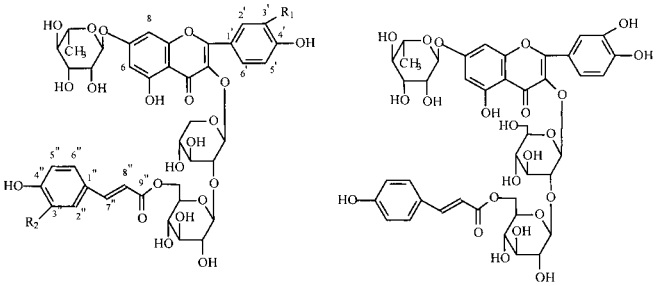
Key words *Oxytropis myriophylla*; flavonoid; myriophylloside B; myriophylloside C; myriophylloside D; myriophylloside E

Oxytropis myriophylla (Leguminosae) is a medicinal plant growing wild in southeast and northwest areas of China. It has been used as a folk medicine in China for the treatment of cold and inflammation of carbuncle swelling, pain and different types of bleeding. Studies on the chemical constituents and bioactivities have not been reported so far. In our recent research, a 95% EtOH extract of the plant was separated by repeated chromatography to give eight compounds. On the basis of spectral analyses, their structures were elucidated to be (6*R*,9*R*)-roseoside (**1**), (6*R*,9*S*)-roseoside (**2**), adenosine (**3**), quercetin-7-*O*-α-L-rhamnopyranosyl-3-*O*-(6″-*P*-coumaroyl)-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside (**4**), quercetin-7-*O*-α-L-rhamnopyranosyl-3-*O*-(6″-caffeoyl)-β-D-Glucopyranosyl-(1→2)-β-D-xylopyranoside (**5**), kaempferol-7-*O*-α-L-rhamnopyranosyl-3-*O*-(6″-*P*-coumaroyl)-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside (**6**), quercetin-7-*O*-α-L-rhamnopyranosyl-3-*O*-(6″-feruloyl)-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside (**7**), quercetin-7-*O*-α-L-rhamnopyranos-yl-1-3-*O*-(6″-*P*-coumaroyl)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**8**). Five flavonoids (**4**—**8**, Fig. 1) were new compounds called myriophylloside B (**4**), myriophylloside C (**5**), myriophylloside D (**6**), myriophylloside E (**7**), and myriophylloside F (**8**), and the known compounds were isolated from this plant for the first time.

Results and Discussion

The powders of *O. myriophylla* were extracted with 95% EtOH. The extract was suspended in water and extracted successively with EtOAc, *n*-BuOH. The *n*-BuOH soluble part was separated on D_{101} macroporous resin, silica gel, Rp-18 silica gel, Sephadex LH-20 column chromatography, and HPLC to obtain compounds **1**—**8**.

Compound **4**, yellow powder, has the molecular formula $C_{41}H_{44}O_{22}$ as revealed by high resolution (HR)-FAB-MS *m/z*: 887.2266 $[M-1]^-$. The UV spectrum of **4** showed absorption maxima at 348, 269 nm, which suggested a flavone structure. In the ^{1}H-NMR spectrum, the downfield signals at δ 12.67, 9.92, 9.80, and 9.23 suggested the existence of four hydroxy groups. A typical ABX system at δ 7.64 (1H, dd, J=8.5, 2.0 Hz), 7.54 (1H, d, J=2.0 Hz), 6.89 (1H, d, J=8.5 Hz) showed the presence of three aromatic protons in the B ring; two A-ring signals were at δ 6.54 (1H, d, J=2 Hz, H-8) and 6.34 (1H, d, J=2 Hz, H-6). An A_2B_2 pattern at δ 7.23 (2H, d, J=8.5 Hz), 6.67 (2H, d, J=8.5 Hz), the trans-olefinic protons at δ 7.33 (1H, d, J=15.5 Hz), 6.07 (1H, d, J=15.5 Hz), and ^{13}C signal at δ 166.9 (C-9″) implied a *P*-coumaroyl group. On acid hydrolysis, **4** gave glucose, rhamnose, and xylose, which were identified by co-TLC with authentic samples. Three anomeric signals at δ 5.51 (1H, d, J=6.0 Hz, xyl-H-1), 5.48 (1H, br s, rha-H-1), and 4.62 (1H, d, J=7.0 Hz, glu-H-1) were deduced from ^{1}H-NMR and 1D-TOCSY. In the ^{13}C-NMR spectrum of **4**, the signals for the aglycone were in good agreement with those of clovin,[1)] indicating that the aglycone (quercetin) of **4** is the same as that of clovin, and that both 3- and 7-hydroxy groups carried a sugar moiety. One- and two-dimensional NMR techniques (^{1}H-NMR, ^{13}C-NMR, COSY, HMQC, HMBC, and 1D-TOCSY) permitted assignments of the ^{1}H- and ^{13}C-NMR signals of **4** (see Tables 1, 2). In the HMBC spectrum, the correlation between H-6 at δ 4.28 of β-D-glucose and the ^{13}C signal at δ 166.9 of the *P*-coumaroyl group suggested linkage of the *P*-coumaroyl group to C-6 of β-D-glucose, the correlation between the anomeric signal of rhamnose at δ 5.48 and C-7 of aglycone at δ 161.9 indicated that rhamnose was attached to C-7 of aglycone, the correlation between the anomeric signal of β-D-glucose at δ 4.62 and C-2 of xylose at δ 83.2 implied that β-D-glucose was connected with C-2 of xylose, and the chemical shift of C-2 was judged from the correlations of ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY between two signals at δ 5.51 (δ_C 99.8) and 3.53 (δ_C 83.2). A correlation between anomeric signal δ 5.51 of β-D-xylose and C-3 (δ 133.8) of aglycone was found, and therefore β-D-xylose was determined to be linked to aglycone at the C-3 hydroxy group. The peaks of HR-FAB-MS at *m/z* 887.2266 $[M-1]^-$, 741.1681 $[M-rha]^-$, 595.1238 $[M-rha-P\text{-coumaroyl}]^-$, 447.0898 $[M-glc-P\text{-coumaroylxyl}]^-$, and 299.0189 $[M-glc-P\text{-coumaroylrha}-xyl]^-$ were also in good agreement with



4. R_1=OH R_2=H
5. R_1=OH R_2=OH
6. R_1=H R_2=H
7. R_1=OH R_2=OCH_3

8.

Fig. 1. Structures of Compounds **4**—**8**

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Table 1. ^{1}H-NMR Data of Compounds **4**—**8** (DMSO-d_6)

	4	**5**	**6**	**7**	**8**
A-ring					
6	6.34 (d, 2)	6.34 (br s)	6.39 (br s)	6.36 (d, 2)	6.34 (br s)
8	6.54 (d, 2)	6.60 (br s)	6.63 (br s)	6.56 (d, 2)	6.54 (br s)
B-ring					
2′	7.54 (d, 2)	7.54 (d, 2)	8.06 (d, 9)	7.52 (d, 2)	7.57 (d, 2)
3′	—	—	6.92 (d, 9)	—	—
5′	6.89 (d, 8.5)	6.87 (d, 8.5)	6.92 (d, 9)	6.89 (d, 8.5)	6.87 (d, 8.5)
6′	7.64 (dd, 8.5, 2)	7.61 (dd, 8.5, 2)	8.06 (d, 9)	7.63 (dd, 8.5, 2)	7.64 (dd, 8.5, 2)
Acyl group					
2″	7.23 (d, 8.5)	6.83 (d, 1.5)	7.24 (d, 9)	7.03 (d, 2)	7.23 (d, 8.5)
3″	6.67 (d, 8.5)	—	6.66 (d, 9)	—	6.70 (d, 8.5)
5″	6.67 (d, 8.5)	6.63 (d, 9)	6.66 (d, 9)	6.66 (d, 8.5)	7.24 (d, 8.5)
6″	7.23 (d, 8.5)	6.70 (dd, 9, 1.5)	7.24 (d, 9)	6.85 (dd, 8.5, 2)	6.70 (d, 8.5)
7″	7.33 (d, 15.5)	7.27 (d, 15.5)	7.36 (d, 15.5)	7.34 (d, 15.5)	7.33 (d, 16.0)
8″	6.07 (d, 15.5)	6.01 (d, 15.5)	6.10 (d, 15.5)	6.21 (d, 15.5)	6.03 (d, 16.0)
OCH_3	—	—	—	3.77 (s)	—
rha 1	5.48 (br s)	5.48 (br s)	5.50 (br s)	5.48 (br s)	5.48 (br s)
6	1.11 (d, 6)	1.11 (d, 6)	1.11 (d, 6)	1.12 (d, 6)	1.11 (d, 6)
Outer glc					
1	4.62 (d, 7)	4.61 (d, 7)	4.64 (d, 7)	4.63 (d, 7.5)	4.65 (d, 8)
2	3.14	—	—	—	—
3	3.25	—	—	—	—
4	3.18	—	—	—	—
5	3.50	—	—	—	—
6	4.28, 4.18	—	—	—	4.24, 4.16
Inner sugar	Xylose	Xylose	Xylose	Xylose	Glucose
1	5.51 (d, 6)	5.54 (d, 6)	5.56 (d, 6)	5.51 (d, 5.5)	5.67 (d, 6.5)
2	3.53	—	—	—	3.45
3	3.49	—	—	—	—
4	3.83	—	—	—	—
5	3.62, 3.01	—	—	—	—
6	—	—	—	—	—

the above deduced structure. Thus, compound **4** is quercetin-7-*O*-α-L-rhamnopyranosyl-3-*O*-(6″-*P*-coumaroyl)-β-D-glucopyranosyl-(1→2)-xylopyranoside, called myriophylloside B.

Compound **5**, yellow powder, was similar to **4** in the chemical shift of ^{1}H- and ^{13}C-NMR spectra of the aglycone and sugar moieties. The difference was that the ABX pattern of **5** at δ 6.83 (1H, d, J=1.5 Hz), 6.70 (1H, dd, J=9, 1.5 Hz), and 6.63 (1H, d, J=9 Hz) took the place of the A_2B_2 pattern of the *P*-coumaroyl group of **4**. A caffeoyl group was thus linked to C-6 of β-glucose. All protons and carbons were assigned by comparison of the ^{1}H- and ^{13}C-NMR spectra of **5** with those of **4**. Thus **5** is quercetin-7-*O*-α-L-rhamnopyranosyl-3-*O*-(6″-caffeoyl)-β-D-glucopyranosyl-(1→2)-xylopyranoside, called myriophylloside C.

Compound **6**. The proton and carbon signals in the spectra of **6** were simlar to those of **4**, except for the signals due to the A_2B_2 system at δ 8.06 (2H, d, J=9.0 Hz) and 6.92 (2H, d, J=9.0 Hz) in **6** replaced the ABX pattern of **4**. Thus **6** is kaempferol-7-*O*-α-L-rhamnopyranosyl-3-*O*-(6″-*P*-coumaroyl)-β-D-glucopyranosyl-(1→2)-xylopyranoside called myriophylloside D.

Compound **7**. In the ^{1}H- and ^{13}C-NMR spectra of **7**, the signals were similar to those of **5**, except that **7** has a methoxy group (δ_H 3.77). In a comparison of ^{13}C-NMR data with those of 6‴-feruloyl spinosin,[2] it was concluded that a feruloyl group was present and linked to C-6 of β-glucose. Thus **7** is quercetin-7-*O*-α-L-rhamnopyranosyl-3-*O*-(6″-feruloyl)-β-D-glucopyranosyl-(1→2)-xylopyranoside, called myriophylloside E.

Compound **8** was obtained as yellow powder. The signals of ^{1}H- and ^{13}C-NMR spectra were similar to those of **4**. However, the HR-ESI-MS gave a quasi-ion peak at *m*/*z* 941.2334 $[M+Na]^+$ and the determined molecular formula is $C_{42}H_{46}O_{23}$. 1D-TOCSY and the lack of a C-5 signal of β-xylose (δ_C 66.0) implied that a β-glucose in **8** took the place of a β-xylose in **4**, and HMBC showed that the linkage positions of three sugars (one rhamnose, two glucoses) were identical to **4** (Fig. 2). Thus **8** is quercetin-7-*O*-α-L-rhamnopyranosyl-3-*O*-(6″-*P*-coumaroyl)-β-D-glucopyranosyl-(1→2)-glucopyranoside, called myriophylloside F.

^{1}H- and ^{13}C-NMR data of compounds **1**—**3** were identical to those of known compound (6*R*,9*R*)-roseoside, (6*R*,9*S*)-roseoside[3] and adenosine.[4]

Experimental

General Experimental Procedures Melting points were determined on an X4 apparatus and are uncorrected. Optical rotations were recorded on an AA-10R Automatic Polarimeter (Optical Activity Ltd.). UV spectra were determined in MeOH on a TU-1901 UV-Vis spectrometer. The IR spectra were recorded on an Avatar 360 FT-IR spectrometer. ^{1}H- and ^{13}C-NMR, H–H COSY, HMQC, HMBC, and 1D-TOCSY were recorded with a Bruker AM-500 instrument. HR-FAB-MS and HR-ESI-MS were taken on a KYKY-ZHP-5# spectrometer. The compounds were isolated by preparative HPLC (Gilson 306 pump, 800C Dynamic Mixer, 506C system Interface,118 UV/Vis Detector, 201 Fraction Collector) and HPLC (Waters 600 pump, 600 Controller, 486 Tunable Absorbance Detector). Silica gel was purchased from Marine Chemical Factory, Qingdao. Sephadex LH-20 and Rp-18 (Chemical Reagent Factory, Tianjin) were used. TLC was performed on a Rp-18 precoated column (Merck).

Plant Material The plants were collected in Huherhaote, Inner Mongolia Autonomous Region, China and identified by Prof. Hubiao Chen. A specimen

Table 2. ^{13}C-NMR Data of Compounds **4**—**8** (DMSO-d_6)

	4	**5**	**6**	**7**	**8**
2	156.9	156.4	156.5	156.5	155.9
3	133.8	133.7	133.3	133.3	133.2
4	178.0	177.4	177.7	177.5	177.3
5	160.1	160.7	160.4	160.8	159.6
6	99.5	99.3	99.2	99.3	99.1
7	161.9	161.4	161.3	161.5	161.3
8	94.5	94.0	93.8	94.0	93.8
9	156.1	155.6	155.6	155.7	155.6
10	105.9	105.4	105.5	105.4	105.4
1′	121.1	120.4	120.2	120.5	120.7
2′	115.9	115.4	129.9	115.8	115.2
3′	144.9	144.9	115.6	144.8	144.2
4′	149.3	149.1	159.8	149.2	148.9
5′	116.4	115.8	115.6	115.5	115.9
6′	122.6	122.0	129.9	122.8	121.9
1″	125.8	125.2	124.8	125.5	124.8
2″	130.4	114.6	131.1	110.7	129.7
3″	116.0	145.5	115.4	147.7	111.5
4″	161.3	148.2	160.8	149.0	160.7
5″	130.4	115.4	131.1	115.3	129.7
6″	116.0	120.8	115.4	122.2	111.5
7″	145.5	145.0	144.5	145.1	144.8
8″	114.1	113.4	113.7	114.0	113.7
9″	166.9	166.3	166.4	166.4	166.2
OCH_3	—	—	—	55.5	—
rha 1	99.0	98.3	98.3	98.3	97.9
2	70.6	68.9	68.8	68.8	69.8
3	70.5	70.2	70.2	70.2	69.9
4	71.8	71.6	71.6	71.6	71.5
5	70.0	69.9	69.9	69.8	69.5
6	18.4	18.4	18.0	17.9	17.7
Outer glc	—	—	—	—	—
1	105.0	104.4	104.5	104.5	104.5
2	74.8	74.4	74.3	74.3	74.0
3	76.7	76.2	76.2	76.2	76.2
4	70.3	69.8	69.9	69.8	69.7
5	75.0	74.6	74.4	74.4	74.5
6	64.0	63.4	63.6	63.3	63.3
Inner sugar	Xylose	Xylose	Xylose	Xylose	Glucose
1	99.8	99.0	99.4	99.1	98.4
2	83.2	82.2	82.5	82.5	83.6
3	74.4	73.9	74.0	74.0	76.1
4	70.0	70,0	70.0	70.0	70.2
5	66.0	65.4	65.5	65.5	77.3
6	—	—	—	—	60.5

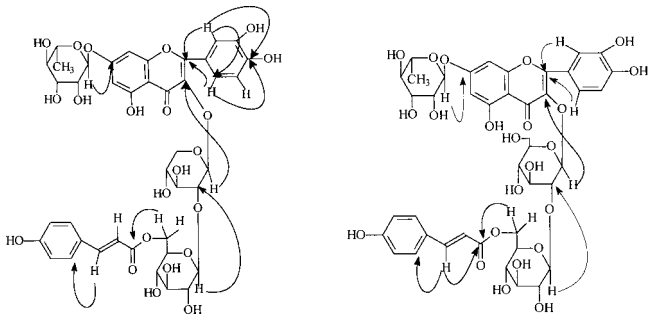

Fig. 2. Key Correlations of Compounds **4** and **8** in HMBC Spectra

was deposited in the Department of Natural Medicines, Peking University.

Extraction and Isolation The materials were extracted with 95% EtOH. The extracts were suspended in water and extracted with EtOAc and *n*-BuOH. The *n*-BuOH extract was subjected to D_{101} macroporous resin eluted with H_2O and 20%, 50%, and 90% EtOH.

The 20% EtOH fraction (21 g) was applied to a silica gel column and eluted with $CHCl_3$–MeOH–H_2O (65 : 35 : 10 lower layer) to afford 16 fractions. Fractions 5—7 were fractionated on an Rp-18 silica gel column (H_2O→40% MeOH) and then HPLC eluted with MeOH–H_2O (19 : 81) with a 1 ml/min of flow rate to yield compounds **1** (7.1 mg) and **2** (18.4 mg). Fraction 8—10 was chromatographed on an Rp-18 silica gel column (5→33% MeOH), then purified by preparative HPLC (reverse phase, MeOH–H_2O (15 : 85), flow rate=8 ml/min) to yield compound **3** (8.6 mg).

The 50% EtOH fraction (35 g) was subjected to a silica gel column eluted with $CHCl_3$–MeOH–H_2O (65 : 35 : 10 lower layer) to furnish six fractions. Fraction 2 was applied to an Rp-18 silica gel column eluted with increasing amounts of MeOH in H_2O (10 : 90→50 : 50) and then Sephadex LH-20 developed with MeOH–H_2O (10 : 90) to afford compounds **6** (80 mg) and **7** (70 mg). Fraction 3 was chromatographed on an Rp-18 silica gel column eluted with gradient MeOH–H_2O (20 : 80→50 : 50) and was purified by Preparative HPLC (reverse phase, MeOH–H_2O (25 : 75), flow rate= 8 ml/min) to give compounds **8** (27 mg) and **4** (197 mg). Fraction 5 was chromatographed on an Rp-18 silica gel column eluted with gradient MeOH–H_2O (20 : 80→50 : 50) to give compound **5** (60 mg).

Identification Compound **4**, yellow powder, mp 218—220 °C, $[\alpha]_D^{25}$ −167.5° (MeOH), UV λ_{max} (MeOH): 318, 256 nm, IR (cm^{-1}): 3358.4, 2923.7, 1654.7, 1602.5, 1168.9, 1060.4. HR-FAB-MS showed the molecular formula $C_{41}H_{44}O_{22}$, found 887.2266 $[M-1]^-$, calcd 887.2251, and fragment ions at *m/z* 741.1681 $[M-rha]^-$, 595.1238 $[M-rha-P\text{-coumaroyl}]^-$, 47.0898 $[M-xyl-glc-P\text{-coumaroyl}]^-$, and 299.0189 [aglycone]$^-$. ^{1}H- and ^{13}C- NMR data, see Tables 1 and 2.

Acidic hydrolysis of compound **4** on TLC plate: A sample (1 mg) was dissolved in 1 ml of MeOH and loaded on a TLC plate. The plate was suspended over a solution of 6 ml of 10 N HCl at a temperature of 60 °C for 30 min. After hydrolysis, HCl absorbed by the silica gel on the plate was evaporated. Then the plate was chromatographed using $CHCl_3$–MeOH–H_2O (6 : 4 : 1) as a development system and visualized by spraying with phenylamine–*ortho*-benzenedicarboxylic acid reagent, following by heating. This was used for identifying the sugars by comparison with authentic samples.

Compound **5**: Yellow powder, mp 205—207 °C, $[\alpha]_D^{25}$ −173.8° (MeOH). HR-FAB-MS showed the molecular formula $C_{41}H_{44}O_{23}$, found 903.2220 $[M-1]^-$, calcd 903.2200. ^{1}H- and ^{13}C- NMR data, see Tables 1 and 2.

Compound **6**: Yellow powder, mp 197—200 °C, $[\alpha]_D^{25}$ −120.3° (MeOH). HR-ESI-MS exhibited the molecular formula $C_{41}H_{44}O_{21}$, found 873.2468 $[M+1]^+$, calcd 873.2447. ^{1}H - and ^{13}C-NMR data, see Tables 1 and 2.

Compound **7**: Yellow powder, mp 212—214 °C, $[\alpha]_D^{25}$ −154.8° (MeOH), HR-ESI-MS showed the molecular formula $C_{42}H_{46}O_{23}$, found 941.2323 $[M+Na]^+$, calcd 941.2322. ^{1}H - and ^{13}C-NMR data, see Tables 1 and 2.

Compound **8** was obtained as yellow powder, mp 224—226 °C, $[\alpha]_D^{25}$ −168.6° (MeOH). HR-ESI-MS showed the molecular formula $C_{42}H_{46}O_{23}$, found 941.2334 $[M+Na]^+$, calcd 941.2321. ^{1}H- and ^{13}C- NMR data, see Tables 1 and 2.

Acknowledgement This project was supported by National Natural Science Foundation of China (No. 20172004).

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