MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. 2002; **40**: 603–608
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mrc.1060

Note

Four new ursane-type saponins from *Morina nepalensis* var. *alba*

Rong Wei Teng,* Hong Yan Xie, De Zu Wang and Chong Ren Yang**

Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China

Received 16 January 2002; Revised 7 May 2002; Accepted 13 May 2002

Four new ursane-type saponins, monepalosides C–F, together with a known saponin, mazusaponin II, were isolated from *Morina nepalensis* var. *alba* Hand.-Mazz. Their structures were determined to be 3-*O*-*α*-L-arabinopyranosyl-(1 → 3)-[*α*-L-rhamnopyranosyl-(1 → 2)]-*α*-L-arabinopyranosylpomolic acid 28-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside (monepaloside C, 1), 3-*O*-*α*-L-arabinopyranosyl-(1 → 3)-[*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-xylopyranosylpomolic acid 28-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside (monepaloside D, 2), 3-*O*-*α*-L-arabinopyranosyl-(1 → 3)-[*β*-D-glucopyranosy-(1 → 2)]-*α*-L-arabinopyranosylpomolic acid 28-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside (monepaloside E, 3) and 3-*O*-*β*-D-xylopyranosylpomolic acid 28-*O*-*β*-D-glucopyranoside (monepaloside F, 4) on the basis of chemical and spectroscopic evidence. 2D NMR techniques, including ^{1}H–^{1}H COSY, HMQC, 2D HMQC-TOCSY, HMBC and ROESY, and selective excitation experiments, including SELTOCSY and SELNOESY, were utilized in the structure elucidation and complete assignments of ^{1}H and ^{13}C NMR spectra.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; 2D NMR; *Morina nepalensis* var. *alba* Hand.-Mazz.; *Dipsacaceae*; monepalosides C–F; NMR complete assignments

INTRODUCTION

Morina nepalensis var. *alba* Hand.-Mazz. belonging to the family *Dipsacaceae* and the genus *Morina*, is a well known traditional Tibetan medicinal herb in China and has been used for the treatment of many diseases since ancient times.[1,2] We have reported two new ursane-type saponins, monepaloside A (**6**) and monepaloside B (**7**), caffeoylquinic acids, and two new flavonoid glycosides from the water-soluble fraction of the whole plant of *M. nepalensis* var. *alba*.[3–5] Further studies led to the isolation and identification of another four new ursane-type triterpenoid saponins. We report here their structure elucidation using chemical and spectroscopic methods. 2D NMR techniques, including ^{1}H–^{1}H COSY, HMQC, 2D HSQC-TOCSY, HMBC and ROESY, and selective excitation experiments, SELTOCSY and SELNOESY, were utilized in the structure elucidation and complete assignments of ^{1}H and ^{13}C NMR spectra.

RESULTS AND DISCUSSION

The n-butanol fractions of the ethanol extract of *M. nepalensis* var. *alba* Hand.-Mazz. were repeatedly subjected to silica gel and RP-8 and MCI gel CHP20 column chromatography to afford compounds **1–5** (Fig. 1).

The aglycone of saponins **1–4** was determined to be pomolic acid by comparing the ^{13}C NMR data with those for similar saponins whose aglycone was pomolic acid.[7–12] The assignments of the ^{13}C and ^{1}H signals of the aglycone were achieved by comparison with those reported in the literature[7–12] and further confirmed by 2D NMR data. The results are summarized in Tables 1 and 2.

Monepaloside C (**1**) was isolated as a white powder, m.p. 198–200 °C, $[\alpha]_D^{27}$ − 14.81° (*c* 0.41, pyridine). Its molecular formula was established as $C_{58}H_{94}O_{26}$ by the combination of negative ion high-resolution fast atom bombardment mass spectrometry (HR-FABMS), showing a quasi-molecular peak at *m/z* 1205.5979 $[M - H]^-$ (calcd for $C_{58}H_{93}O_{26}$: 1205.5955), and ^{13}C NMR (DEPT) spectra. The result was further confirmed by negative ion FABMS, showing a molecular peak at *m/z* 1206 (M^-).

The ^{1}H and ^{13}C NMR spectra showed five anomeric proton signals at δ 4.91 (d, J = 4.5 Hz), 5.06 (d, J = 6.8 Hz), 5.96 (brs), 6.20 (d, J = 7.9 Hz) and 5.04 (d, J = 8.3 Hz) and five anomeric carbon signals at δ 104.37, 103.95, 102.14, 95.86 and 105.34, respectively. Consequently, **1** was assumed to contain five sugar units. Sugar analysis by gas chromatography–mass spectrometry (GC–MS) revealed that **1** contained glucose, arabinose and rhamnose and

*Correspondence to: Rong Wei Teng , School of Botany, University of Melbourne, Victoria 3010, Australia.
E-mail: tengrongwei@hotmail.com
**Correspondence to: Chong Ren Yang , Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China. E-mail: cryang@public.km.yn.cn

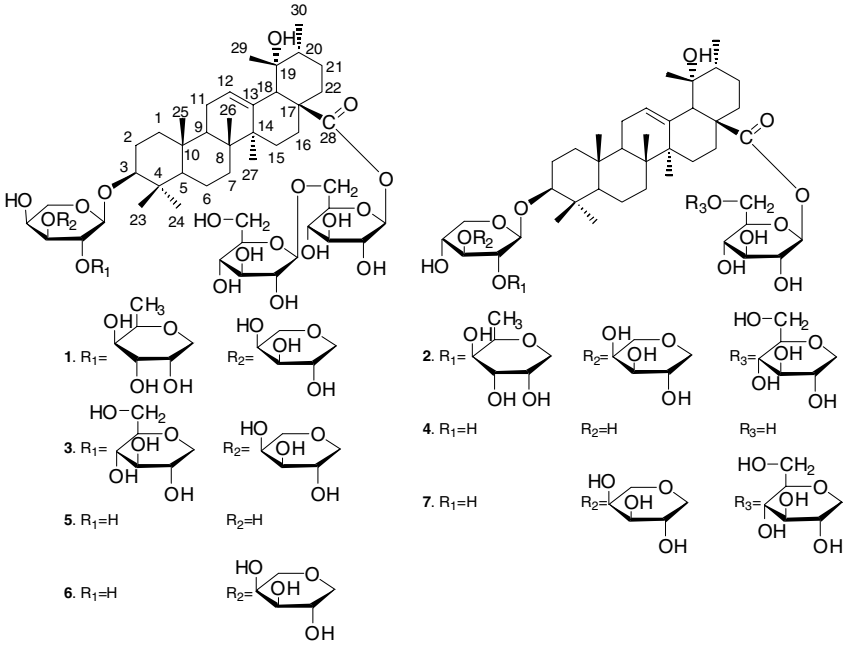

Figure 1. Structures of saponins **1–7**.

Table 1. ^{13}C NMR data for saponins **1–5** (125 MHz, pyridine-d_5)

C	1	2	3	4	5
1	39.19	39.23	39.06	38.93	38.94
2	26.77	26.78	26.75	26.74	26.74
3	88.46	88.54	89.27	88.79	88.85
4	39.64	39.61	39.80	39.62	39.61
5	56.11	55.29	56.07	55.97	56.00
6	18.72	18.72	18.84	18.73	18.76
7	33.59	33.60	33.64	33.53	33.54
8	40.62	40.60	40.65	40.59	40.58
9	47.87	47.86	47.87	47.79	47.80
10	37.85	37.82	37.86	37.77	37.81
11	24.13	24.11	24.18	24.09	24.10
12	128.50	128.47	128.56	128.47	128.46
13	139.36	139.34	139.37	139.30	139.33
14	42.22[a]	42.21[a]	42.23[a]	42.15[a]	42.14[a]
15	29.41	29.39	39.41	29.28	29.34
16	26.20	26.19	26.75	26.15	26.15
17	48.82	48.80	48.84	48.68	48.75
18	54.45	54.44	54.46	54.47	54.39
19	72.77	72.74	72.69	72.69	72.70
20	42.22[a]	42.21[a]	42.23[a]	42.15[a]	42.14[a]
21	26.77	26.78	26.75	26.74	26.74
22	37.12	37.10	37.12	37.04	37.07
23	28.25	28.11	28.22	28.25	28.31
24	17.08	17.01	16.87	16.97	16.96
25	15.88	15.90	15.80	15.69	15.76
26	17.53	17.52	17.57	17.45	17.49
27	24.67	24.66	24.69	24.62	24.62
28	177.23	177.19	177.24	177.01	177.15
29	27.13	27.10	27.18	27.08	27.08
30	16.79	16.77	16.80	16.73	16.72
3-*O*-Ara-1	104.37	Xyl: 105.34	Xyl: 105.31[a]	107.70	107.55
2	74.43	77.32	77.22	74.08[a]	72.95

Table 1. (*Continued*)

C	1	2	3	4	5
3	79.53	86.65	82.27	78.91	74.68
4	67.62	69.62	68.70	71.24[a]	69.63
5	64.19	66.06	65.77	67.13	66.79
ara-1	103.95	104.84	105.31[a]		
2	72.22	72.64	72.83		
3	74.03	74.39	74.50		
4	68.70	69.41[a]	69.46		
5	66.13	66.06	67.05		
6					
Rha-1	102.14	102.06	glc′: 104.44		
2	72.45	72.38	76.13		
3	72.68	72.54	78.72		
4	73.94	73.85[a]	72.40		
5	70.30	70.20	77.54		
6	18.68	18.72	63.22		
28-*O*-Glc-1	95.86	95.83	95.86	95.87	95.81
2	73.90	73.85[+]	73.91	74.08[a]	73.85
3	78.53	78.51	78.49	79.28	78.44
4	71.12	71.13	71.17	71.24[a]	71.14
5	78.03	78.02	78.02	78.56	77.99
6	69.62	69.41[a]	69.66	62.22	69.40
glc-1	105.34	105.49	105.31[a]		105.38
2	75.28	75.26	75.27		75.25
3	78.83	78.82	78.81		78.82
4	71.62	71.60	71.68		71.60
5	78.46	78.44	78.48		78.53
6	62.75	62.73	62.79		62.73

[a] Overlapped with other signals.

Table 2. ^{1}H NMR data for saponins **1–5** (500 MHz, pyridine-d_5, J in Hz)

C	1	2	3	4	5
1	1.49 (d, 12.7)	1.54	1.50	1.53	1.54
	0.89	0.94	0.86	0.95	0.92
2	1.97	2.04	2.01	2.13	2.14
	1.81	1.86	1.82	1.89	1.87
3	3.23 (dd, 3.8, 11.1)	3.28 (dd, 3.3, 10.6)	3.21 (dd, 3.8, 11.8)	3.33 (dd, 5.1, 10.4)	3.32 (dd, 4.0, 11.8)
5	0.77 (brd, 11.5)	0.80 (brd, 12.3)	0.75 (brd, 11.4)	0.83 (brd, 10.4)	0.81 (brd, 12.8)
6	1.43	1.49	1.47	1.48	1.46
	1.32	1.38	1.31	1.32	1.31
7	1.56 (d, 11.9)	1.60	1.56	1.58	1.59
	1.43	1.44	1.41	1.45	1.40
9	1.50	1.79	1.74	1.78	1.77
11	2.01	2.03	2.00	2.03	2.02
12	5.53 (t-like)	5.53 (t-like)	5.52 (t-like)	5.55 (t-like)	5.53 (t-like)
15	2.43 (dt, 3.4, 12.8)	2.43 (dt, 3.5, 13.6)	2.41 (dt, 4.1, 12.9)	2.47 (dt, 3.9, 14.0)	2.44 (dt, 3.6, 13.2)
	1.21	1.22	1.21	1.23	1.22
16	3.09 (dt, 3.4, 12.4)	3.09 (dt, 4.4, 13.4)	3.08 (dt, 3.8, 12.9)	3.11 (dt, 4.2, 14.0)	3.10 (dt, 4.3, 13.8)
	2.00	2.01	2.00	2.02	2.00
18	2.91 (s)	2.90 (s)	2.90 (s)	2.93 (s)	2.91 (s)
20	1.33	1.35	1.34	1.36	1.33
21	2.09	2.11	2.09	2.07	2.10
	1.95	1.97	1.94	1.96	1.95
22	1.25	1.25	1.25	1.25	1.24

(*continued overleaf*)

Table 2. (*Continued*)

C	1	2	3	4	5
23	1.13 (s)	1.20 (s)	1.20 (s)	1.29 (s)	1.25 (s)
24	1.07 (s)	1.13 (s)	1.07 (s)	1.00 (s)	0.96 (s)
25	0.89 (s)	0.91 (s)	0.88 (s)	0.91 (s)	0.93 (s)
26	1.16 (s)	1.16 (s)	1.15 (s)	1.19 (s)	1.17 (s)
27	1.68 (s)	1.68 (s)	1.66 (s)	1.70 (s)	1.68 (s)
29	1.35 (s)	1.35 (s)	1.35 (s)	1.39 (s)	1.35 (s)
30	1.02 (d, 6.2)	1.01 (d, 6.9)	1.02 (d, 6.6)	1.06 (d, 6.2)	1.02 (d, 6.9)
3-*O*-Ara-1	4.91 (d, 4.5)	Xyl: 4.80 (d, 7.6)	Xyl: 4.84 (d, 6.0)	4.82 (d, 7.3)	4.75 (d, 6.9)
2	4.60 (t, 5.9)	4.12	4.70	4.42	4.42
3	4.34	4.10	4.30	4.16	4.14
4	4.46	4.01	4.46	4.21	4.34
5	4.30	4.31	4.23	4.37	4.30
	3.78	3.63 (t, 10.6)	3.72 (t, 11.2)	3.77 (t, 10.4)	3.81 (t, 10.9)
ara-1	5.06 (d, 6.8)	4.93 (d, 6.8)	5.16 (d, 6.9)		
2	4.47	4.47	4.46		
3	4.17	4.09	4.13		
4	4.37	4.28	4.33		
5	4.34	4.30	4.28		
	3.76	3.78 (d, 12.3)	3.74		
Rha-1	5.96 (brs)	6.28 (brs)	glc′: 5.40 (d, 7.6)		
2	4.69 (brs)	4.81	3.99		
3	4.56 (dd, 3.4, 9.1)	4.58	4.11		
4	4.25	4.29	4.12		
5	4.49	4.65	3.58		
6	1.60 (d, 6.0)	1.64 (d, 5.4)	4.29		
			4.19		
28-*O*-Glc-1	6.20 (d, 7.9)	6.21 (d, 7.9)	6.18 (d, 7.9)	6.29 (d, 7.9)	6.21 (d, 7.9)
2	4.14	4.15	4.13	4.22	4.15
3	4.22	4.23	4.21	4.30	4.22
4	4.29	4.31	4.28	4.34	4.28
5	4.11	4.11	4.10	4.04	4.10
6	4.71 (brd, 11.1)	4.70	4.69	4.47 (brd, 11.5)	4.71 (brd, 11.2)
	4.33	4.34	4.29	4.40	4.34
glc-1	5.04 (d, 8.3)	5.04 (d, 7.7)	5.02 (d, 7.9)		5.04 (d, 8.2)
2	4.01	4.00	3.97		4.00
3	4.16	4.17	4.17		4.17
4	4.18	4.19	4.18		4.18
5	3.89	3.88	3.87		3.89
6	4.46	4.47	4.46		4.48 (brd, 11.8)
	4.32	4.33	4.31		4.32

indicated that **1** had an additional rhamnose compared with monepaloside A (**6**).[4]

The ^{13}C NMR data for the sugar units of **1** were very similar to those of monepaloside A (**6**)[4] except that **1** had one additional sugar unit. In the HMQC-TOCSY spectrum (t_m = 100 ms), only two carbon signals were correlated with the anomeric proton signal H-1 (δ 5.96, brs) of this unit; carbon signals of this sugar unit assigned by 2D NMR spectra were at δ 102.14, 73.94, 72.68, 72.45, 70.30 and 18.68, characteristic of an α-L-rhamnopyranosyl. Furthermore, the anomeric proton signals at δ 5.96 (brs) [^3J(H-1,H-2) < 5 Hz] indicated an α-configuration.[13–16]

It is interesting that the coupling constant ^3J(H-1,H-2) of the inner arabinopyranosyl was equal to 4.5 Hz, i.e. smaller than 5 Hz. It seemed that this was a β-L-arabinopyranosyl unit. In fact, the NMR data from 1D SELNOESY and 1D SELTOCSY proved that this unit was an α-L-arabinopyranosyl, because we observed NOE correlation between H-1 (δ 4.91, d, J = 4.5 Hz) of this arabinopyranosyl and its H-2 (δ 4.60, t, J = 5.9 Hz), H-3 (δ 4.34, m) and H-5b (δ 3.78, m) in the 1D SELNOESY spectrum and total correlation from H-1 (δ 4.91, d, J = 4.5 Hz) to H-4 (δ 4.46, m) in the 1D SELTOCSY spectrum. The 1D SELNOESY and 1D SELTOCSY spectra were obtained by irradiating the anomeric proton signal H-1 (δ 4.91, d, J = 4.5 Hz) to yield the sub-spectrum of the inner arabinopyranosyl with high digital resolution. It was deduced that the relatively small coupling constant ^3J(H-1,H-2) was due to the rapid conformational exchange of the α-L-arabinopyranoside between $^1C_4$ and $^4C_1$ conformations in solution.[17]

The C-2 of the inner α-L-arabinopyranosyl was shifted downfield from δ 71.83 to δ 74.43 and C-3 upfield from δ 83.85 to δ 79.53 on comparing ^{13}C NMR data with those for monepaloside A (**6**),[4] which suggested that the α-L-rhamnopyranosyl is linked at C-2 of the inner α-L-arabinopyranosyl. This deduction was further confirmed by an HMBC spectrum showing long-range correlation between Rha H-1 (δ 5.96, brs) and Ara C-2 (δ 74.43). Other linkage sites were also confirmed by the following long-range correlations from the HMBC spectrum: ara H-1 (δ 5.06, d, $J = 6.8$ Hz) and Ara C-3 (δ 79.53), Ara H-1 (δ 4.91, d, $J = 4.5$ Hz) and C-3 (δ 88.46) of the aglycone; glc H-1 (δ 5.04, d, $J = 8.3$ Hz) and Glc C-6 (δ 69.62), Glc H-1 (δ 6.20, d, $J = 7.9$ Hz) and C-28 (δ 177.23) of the aglycone. Hence the structure of saponin **1** was elucidated to be 3-*O*-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosylpomolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside and named monepaloside C.

Monepaloside D (**2**) was isolated as a white powder. Negative ion HR-FABMS established the molecular formula as $C_{58}H_{94}O_{26}$ (found *m/z* 1205.5918 $[M-H]^-$; calcd for $C_{58}H_{93}O_{26}$ 1205.5955). The ^{13}C NMR data for the sugar parts of **2** were very similar to those for **1** except for the NMR data due to the inner sugar unit linked at C-3 of the aglycone. Furthermore, **2** had one more terminal sugar unit in the oligosaccharide segment of C-3 of the aglycone than monepaloside B (**7**),[4] based on a comparison of their ^{13}C NMR data. By analyzing their structures, it could be concluded that **2** had one additional α-L-rhamnopyranosyl than monepaloside **B**, but an inner β-D-xylopyranosyl at C-3 of the aglycone in place of an inner α-L-arabinopyranosyl unit in **1**. Hence the structure of **2** was proposed as 3-*O*-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-xylopyranosylpomolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside and named moneparoside D.

Monepaloside E (**3**) was isolated as a white powder. Negative ion HR-FABMS established the molecular formula as $C_{58}H_{94}O_{27}$ (found *m/z* 1221.5967 $[M-H]^-$; calcd for $C_{58}H_{93}O_{27}$ 1221.5904). Based on a comparison of their ^{13}C NMR data, **3** was assumed to have one more sugar unit than monepaloside A (**6**).[4] This terminal sugar unit was determined to be a β-D-glucopyranosyl unit on the basis of GC–MS and NMR evidence. Compared with moneparoside A (**6**),[4] the C-2 and C-3 of the inner arabinopyranoyl of **3** shifted from δ 71.83 to δ 77.22 and from δ 83.15 to δ 82.27, respectively. These findings revealed the terminal glucopyranosyl could link at C-2 of the inner arabinopyranoyl group, which was confirmed by the HMBC spectrum, showing correlation between glc' H-1 (δ 5.40, d, $J = 7.6$ Hz) and Ara C-2 (δ 77.22). Hence the structure of **3** was determined to be 3-*O*-α-L-arabinopyranosyl-(1 → 3)-[β-D-glucopyranosy-(1 → 2)]-α-L-arabinopyranosylpomolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside and named moneparoside E.

Monepaloside F (**4**) was isolated as a white powder. Negative ion HR-FABMS established the molecular formula as $C_{41}H_{66}O_{13}$ (found *m/z* 765.4429 $[M-H]^-$; calcd for $C_{41}H_{65}O_{13}$ 765.4425). The ^1H and ^{13}C NMR spectra of **4** showed two anomeric proton signals at δ 4.82 (d, $J = 7.3$ Hz), 6.29 (d, $J = 7.9$ Hz) and two anomeric carbon signals at δ 107.70, 95.87 respectively. Hence **4** was assumed to contain two sugar units. The chemical shifts of C-3 (δ 88.79) and C-28 (δ 177.01) of the aglycone suggested that **4** was a bisdesmoside. Comparison of the ^{13}C NMR data with those for zigu-glucoside **I**[11,12] indicated that **4** was different from zigu-glucoside I in the sugar unit linked at C-3 of the aglycone. Furthermore, the ^{13}C NMR data for the sugar units of **4** closely resembled those of scabrioside A except that **4** had one allopyranosyl unit less than scabrioside A.[7] All the above findings suggested that **4** contained one β-D-xylopyranosyl unit linked at C-3 of the aglycone and one β-D-glucopyranosyl unit linked at C-28 of the aglycone. The sugar component was further confirmed by the HMQC-TOCSY spectrum[13–16] and the linkage sites were validated by an HMBC spectrum, showing long-range correlation between Xyl H-1 (δ 4.82, d, $J = 7.3$ Hz) and C-3 (δ 88.79) of the aglycone between Glc H-1 (δ 6.29, d, $J = 7.9$ Hz) and C-28 (δ 177.01) of the aglycone. Hence the structure of **4** was elucidated as 3-*O*-β-D-xylopyranosylpomolic acid 28-*O*-β-D-glucopyranoside and named monepaloside **F**.

Compound **5** was determined to be 3-*O*-α-L-arabinopyranosylpomolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside by comparing the ^{13}C NMR data with those for mazusaponin II first isolated from *Mazus miquelii* Makino.[6] The complete assignments of the ^1H and ^{13}C NMR spectra for saponins **1–5** were achieved on the basis of 2D NMR spectra, including ^1H–^1H COSY, HMQC, 2D HMQC-TOCSY, HMBC and ROESY.[13,15,16]

EXPERIMENTAL

General experimental procedures

Optical rotations were measured on a Horiba SEPA-300 polarimeter using a sodium lamp. Melting-points were measured on a Koffler melting-point apparatus produced at Sichuan University, China, and uncorrected. FAB Mass spectra were measured on a VG Autospect 3000 spectrometer. All NMR experiments were recorded on a Bruker DRX-500MHz spectrometer operating 500 and 100 MHz for ^1H and ^{13}C, respectively, equipped with an inverse detection 5 mm probe operating at room temperature. About 20–40 mg of sample were dissolved in pyridine-d_5 (0.4 ml) to record the NMR spectra using the lowest field signals of pyridine-d_5 (^1H, δ 8.71; ^{13}C, δ 149.9) as internal reference.

^1H and ^{13}C NMR spectra were acquired under standard conditions. The NMR conditions for all compounds were as follows: 1D spectra were acquired using 64K data points for ^1H and ^{13}C spectra. 32K data points were used for the processing with no window function for ^1H spectra and exponential function (LB = 4) for ^{13}C specta. 1D SELTOCSY and SELNOESY experiments used 100 and 300 ms as spin-lock time, respectively.

Standard pulse sequences were used for 2D spectra. Relaxation delays of 1.5 or 2 s were used for all 2D NMR experiments. 2D spectra used 1024 × 256 (H–H COSY, HMQC, ROESY and HMQC-TOCSY) and 2048 × 256 (HMBC) data point matrices, then zero filled to 1024 × 512

and 2048 × 512, respectively. A non-shifted sine window function was used along the F_1 and F_2 axes for H–H COSY, HMQC and HMBC and a 90° shifted sine window function was used along the F_1 and F_2 axes for ROESY and HMQC-TOCSY. The HMQC-TOCSY experiment utilized a 100 ms spin-lock as the mixing time to obtain the total correlations. The ROESY experiment used a 300 ms spin-lock as the mixing time. The HMBC experiment used a 62 ms as the delay time to obtain ^{1}H and ^{13}C long-range correlations. Z-PFG was used in HMQC, HMBC and DQF H–H COSY experiments. Data processing was carried out on an HP VL600 computer with Bruker XWINNMR programs (version 2.7).

Plant material

See previous papers.[3–5]

Extraction and isolation

Whole plant (3.4 kg) was extracted with hot ethanol three times to afford an ethanol extract. The extract was first partitioned between water and chloroform then between water and n-butanol. The n-butanol fraction (250 g) was subjected to silica gel column chromatography with EtOAc–acetone–H_2O (9 : 10 : 1) to give six fractions (Fr I–VI).

Fr III (22 g) was repeatedly subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O or EtOAc–acetone–H_2O, then to MCI gel CHP20 with aqueous methanol to afford **4** (2 mg). Fr IV (40 g) was subjected to dianion column chromatography then silica gel column chromatography with $CHCl_3$–MeOH–H_2O and RP-8 column chromatography with aqueous methanol repeatedly to afford **1** (160 mg), **2** (200 mg), **3** (100 mg) and **5** (150 mg).

Acid hydrolysis and GC–MS analysis

A solution of 2 mg of **1**, **2** or **3** in 2 M HCl–dioxane (1 : 1) (1 ml) was heated at 95 °C for about 6 h. The reaction mixture was blown to dryness with a stream of nitrogen. The residue was dissolved in pyridine (0.5 ml), then $(CH_3)_3SiNHSi(CH_3)_3$ (0.5 ml) was added. After 10 min at room temperature, the reaction mixture was blown to dryness with a stream of nitrogen. The residue was dissolved in diethyl ether then directly subjected to GC–MS analysis.

GC–MS experiments were carried out on an MD 800 instrument. Trimethylsilyl ether derivatives were separated using an HP Ac-5 capillary column (0.25 × 30 m). Nitrogen was used as the carrier gas. The initial column oven temperature was 180 °C, then increased at 5 °C min^{-1} to a final value of 240 °C. The sugars were determined by comparison of retention times (t_R) and MS behaviour with standard sugars: t_R (min) Glc 6.85 (*m/z* 482), Ara 4.19 (*m/z* 438), Xyl 5.06 (*m/z* 438), Rha 4.30 (*m/z* 452). The presence of arabinose, glucose and rhamnose in **1**, arabinose, xylose, rhamnose and glucose in **2** and arabinose and glucose in **3** was detected.

Spectral data

^{1}H and ^{13}C NMR data for compounds **1–5** are listed in Tables 1 and 2.

Monepaloside C (**1**): white powder, m.p. 198–200 °C, $[\alpha]_D^{27}$ − 14.81° (*c* 0.41, pyridine). Negative ion FABMS: *m/z* 1206 (M^-), 1074 [M − 132(arabinopyranosyl)]$^-$, 912 [M − H − 132 − 162(glucopyranosyl)]$^-$, 881 [M − H − 162 − 162]$^-$, 749 [M − H − 162 − 162 − 132]$^-$, 471 [M − H − 132 − 162 − 162 − 146(rhamnopyranosyl) − 132]$^-$. Negative ion HR-FABMS: *m/z* 1205.5979 [M − H]$^-$; calcd for $C_{58}H_{93}O_{26}$ 1205.5955.

Monepaloside D (**2**): white powder, m.p. 211–213.5 °C, $[\alpha]_D^{24}$ + 0.85° (*c* 0.30, MeOH). Negative ion FABMS: *m/z* 1205 [M − H]$^-$, 1074 [M − 132]$^-$, 881 [M − H − 162 − 162]$^-$, 749 [M − H − 162 − 162 − 132]$^-$, 471 [M − H − 132 − 162 − 162 − 146 − 132]$^-$. Negative ion HR-FABMS: *m/z* 1205.5918 [M − H]$^-$; calcd for $C_{58}H_{93}O_{26}$ 1205.5955.

Monepaloside E (**3**): white powder, m.p. 178–180.5 °C, $[\alpha]_D^{24}$ 0.00° (*c* 0.28, MeOH). Negative ion FABMS: *m/z* 1221 [M − H]$^-$, 1090 [M − 132]$^-$, 897 [M − H − 162 − 162]$^-$, 765 [M − H − 162 − 162 − 132]$^-$, 603 [M − H − 162 − 162 − 132 − 162]$^-$, 471 [M − H − 162 − 162 − 132 − 162 − 132]$^-$. Negative ion HR-FABMS: *m/z* 1221.5967 [M − H]$^-$; calcd for $C_{58}H_{93}O_{27}$ 1221.5904.

Monepaloside F (**4**): m.p. 170–172 °C, $[\alpha]_D^{19}$ 4.84 °C (*c* 0.31, MeOH). Negative ion FABMS: *m/z* 766 (M^-), 604 [M − 162]$^-$, 471 [M − H − 162 − 132]$^-$. Negative ion HR-FABMS: *m/z* 765.4429 [M − H]$^-$; calcd for $C_{41}H_{65}O_{13}$ 765.4425.

Mazusaponin II (**5**): white powder, $[\alpha]_D^{24}$ − 6.52° (*c* 0.58, MeOH), Negative ion FABMS: *m/z* 927 [M − H]$^-$, 765 [M − H − 162]$^-$, 603 [M − H − 162 − 162]$^-$, 471 [M − H − 162 − 162 − 132]$^-$.

REFERENCES

1. Delectis Florae Reipularis Sinicae Agendae Academiae Sinicae Edita. *Flora Reipublicae Popularis Sinicae*, vol. 73. Science Press: Beijing, 1986; No. 1, 44.
2. Yang JS, Chu CJC. *Dingqing Tibetan Medicine*, Version 1. Yunnan Nationality Press: Yunnan, 1989; 409.
3. Teng RW, Zhou ZH, Wang DZ, Yang CR. *Chin. J. Magn. Reson.* 2002; **19**: in press.
4. Teng RW, Xie HY, Wang DZ, Yang CR. *Chin. J. Org. Chem.* 2002; **22**: in press.
5. Teng RW, Xie HY, Li HZ, Liu XK, Wang DZ, Yang CR. *Magn. Reson. Chem.* 2002; **40**: 415.
6. Yaguchi E, Miyase T, Ueno A. *Phytochemistry* 1995; **39**: 185.
7. Baykai T, Panayir T, Tasdemir D, Sticher O, Calis I. *Phytochemistry* 1998; **48**: 867.
8. Ouyang MA, Wang HQ, Liu YQ, Yang CR. *Phytochemistry* 1997; **45**: 1501.
9. Baykal T, Panayir T, Tasdemir D, Sticher O, Calis I. *Phytochemistry* 1998; **48**: 867.
10. Zhao WM, Xu JP, Qin GW, Xu RS. *Phytochemistry* 1995; **39**: 191.
11. Qin WJ, Zhao JJ. Fukuyama Y, Yamada T. *Chin. Trad. Herbs Drugs* 1988; **19**(10): 2.
12. Qin GW, Chen MY, Xu RS. *Chin. Trad. Herbs Drugs* 1991; **22**(11): 483.
13. Teng RW, Wang DZ, Li CM, Ding ZT, Yang CR. *Chin. J. Magn. Reson.* 1999; **16**: 295.
14. King-Morris MJ, Serianni AS. *J. Am. Chem. Soc.* 1987; **109**: 3501.
15. Teng RW, Zhong HM, Chen CQ, Wang DZ. *Chin. J. Magn. Reson.* 1999; **16**: 389.
16. Teng RW, Wang DZ, Chen CQ. *Chin. Chem. Lett.* 2000; **11**: 337.
17. Tommasi ND, Autore G, Bellino A, Pinto A, Pizza C. Sorrentino R, Venturella P. *J. Nat. Prod.* 2000; **63**: 308.