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MAZUSAPONINS I–IV, TRITERPENE SAPONINS FROM *MAZUS MIQUELII*

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Key Word Index—*Mazus miquelii*; Scrophulariaceae; mazusaponin; pomolic acid; siaresinolic acid; bisdesmoside.

Abstract—From whole plants of *Mazus miquelii*, four new saponins designated as mazusaponins I–IV and a known saponin, ilexoside VIII, were isolated and the structures of the new compounds elucidated as 3-*O*-α-L-arabinopyranosyl siaresinolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, 3-*O*-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl siaresinolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester and 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, respectively, by spectroscopic data and chemical evidence.

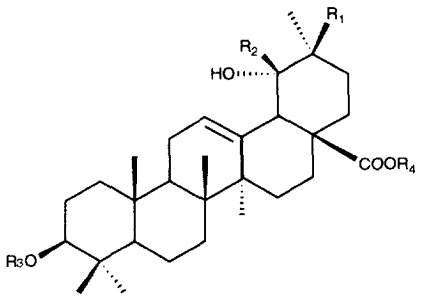
INTRODUCTION

In connection with a study on the saponins of some plants of the Scrophulariaceae [1–5], we have investigated *Mazus miquelii* Makino. We now report the isolation and the structure elucidation of four new saponins from the whole plants.

RESULTS AND DISCUSSION

The water extract of the whole plants was passed through a porous polymer gel Mitsubishi Diaion HP-20 column and the methanol eluate was chromatographed on a silica gel column to give 18 fractions. After purification of the polar fractions by semi preparative HPLC, four new saponins designated as mazusaponins I–IV (**1**–**4**) and a known saponin, ilexoside VIII (**5**) [6] were isolated.

Mazusaponin I (**1**), obtained as an amorphous powder, exhibited a $[M + Na]^+$ ion peak at *m/z* 951 in the FAB-mass spectrum. The ^1H NMR spectrum exhibited the presence of seven singlet methyl signals at δ0.94, 0.98, 1.02, 1.13, 1.14, 1.26 and 1.63, an olefinic proton signal at δ5.50 and three anomeric proton signals at δ4.76 (1H, *d*, $J = 7$ Hz), 5.01 (1H, *d*, $J = 8$ Hz) and 6.27 (1H, *d*, $J = 8$ Hz). Methanolysis of **1** with acetylchloride–methanol gave methyl siaresinate (**1a**) [7] as an aglycone moiety, while acid hydrolysis with 5% H_2SO_4 gave L-arabinose and D-glucose in the ratio 1:2 as a sugar moiety. The ^{13}C NMR spectrum of **1** revealed the presence of three anomeric carbon signals at δ95.8, 105.3 and 107.5, suggesting that **1** had one ester- and two ether-type glycosidic linkages. We employed the difference NOE experiment to decide the sugar sequences after assignment of most proton signals by detailed proton spin decoupling experiments starting from the irradiation of each anomeric proton signal. When the signal at δ4.76 owing to the H-1 of arabinosyl unit and at δ5.01 owing to the H-1 of glucosyl unit were irradiated, NOEs were



	R1	R2	R3	R4
1	CH_3	H	Ara	Glc^{6}—Glc
1a	CH_3	H	H	CH_3
2	H	CH_3	Ara	Glc^{6}—Glc
2a	H	CH_3	H	CH_3
2b	H	CH_3	Ara	Glc
3	CH_3	H	Ara^{2}—Rha	Glc^{6}—Glc
4	H	CH_3	Ara^{2}—Rha	Glc^{6}—Glc
5	H	CH_3	Ara^{3}—Glc	Glc^{6}—Glc

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observed at the signals at δ3.33 (1H, *dd*, J = 12, 4 Hz) owing to the H-3 of the aglycone unit and at δ4.33 (1H, *dd*, J = 12.5, 5 Hz); 4.67 (1H, *br d*, J = 12.5 Hz) owing to the H_2-6 of the ester linked glucosyl unit. The glycosylation shifts were observed at the C-3 (δ88.8) of the aglycone unit and the C-6 (δ69.5) of glucosyl unit. From the above evidence, the structure of **1** was concluded to be 3-*O*-α-L-arabinopyranosyl siaresinolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester.

Mazusaponin II (**2**), obtained as an amorphous powder, exhibited a $[M + Na]^+$ ion peak at m/z 951 in the FAB-mass spectrum. The ^{1}H NMR spectrum exhibited the presence of six singlet methyl signals at δ0.95, 0.97, 1.18, 1.25, 1.36 and 1.68, a doublet methyl signal at δ1.03 (3H, *d*, J = 6.5 Hz) and an olefinic proton signal at δ5.54 (1H, *t*-like, J = 3 Hz) and three anomeric proton signals at δ4.75 (1H, *d*, J = 8 Hz), 5.03 (1H, *d*, J = 8 Hz) and 6.20 (1H, *d*, J = 8 Hz). Acid hydrolysis with 5% H_2SO_4 gave L-arabinose and D-glucose in the ratio 1:2 as a sugar moiety, while an enzymatic hydrolysis with protease [8, 9] followed by methylation with diazomethane gave methyl pomolate (**2a**) as an aglycone moiety. In the ^{13}C NMR spectral data of **2**, the signals from the sugar moiety were in good agreement with those of **1**, although the signals from the carbons of the C, D and E-rings in the aglycone moiety were not identical. These data indicated that **2** was the 3,28-bisdesmoside of pomolic acid. The C–H long range couplings were observed in the HMBC spectrum to decide the sugar sequences. The correlation peaks between H-1 (δ4.75) of the arabinosyl unit and C-3 (δ88.9) of the aglycone unit; H-1 (δ5.03) of the glucosyl unit and C-6 (δ69.7) of glucosyl unit; H-1 (δ6.20) of the ester linked glucosyl unit and C-28 (δ177.1) of the aglycone unit showed that the sugar sequences were 3-arabinopyranosyl and glucosyl-(1 → 6)-glucosyl ester. A comparative study of the ^{13}C NMR signals from the sugar moiety of **2** with those of ziyu-glycoside (**2b**) [10] suggested the presence of one additional mole of glucosyl unit in **2**, which was linked to the C-6 of the ester linked glucosyl unit according to the glycosylation shift (+ 7.2 ppm at the C-6 of glucosyl unit). From these data, the structure of **2** was concluded to be 3-*O*-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester.

Mazusaponin III (**3**), obtained as an amorphous powder, exhibited a $[M + Na]^+$ ion peak at m/z 1098 in the FAB-mass spectrum. The ^{1}H NMR spectrum showed the presence of seven singlet methyl signals at δ0.93, 1.02, 1.08, 1.13, 1.14, 1.16 and 1.62, an olefinic proton signal at δ5.50 (1H, *t*-like, J = 3 Hz) and four anomeric proton signals at δ4.92 (1H, *d*, J = 5 Hz), 5.02 (1H, *d*, J = 8 Hz), 6.06 (1H, *br s*) and 6.28 (1H, *d*, J = 8 Hz). Methanolysis of **3** afforded methyl siaresinate (**1a**) as an aglycone moiety, while acid hydrolysis with 5% H_2SO_4 afforded L-arabinose, L-rhamnose and D-glucose in the ratio 1:1:2 as a sugar moiety. In the ^{13}C NMR spectral data of **3**, the signals from the aglycone moiety were in good agreement with those of **1**. Detailed proton spin decoupling experiments which started from the irradiation at each anomeric proton signal and difference NOE experiments involving irradiation at each anomeric proton signal enabled us to assign all proton signals of the sugar moiety (Table 1). When the signal from H-1 (δ6.06) of the rhamnosyl unit was irradiated, an NOE was observed at the signal due to H-2 (δ4.53) of the arabinosyl unit and when H-1 (δ4.92) of the arabinosyl unit was irradiated, an NOE was observed at H-3 (δ3.24) of the aglycone unit. When H-1 (δ5.02) of the glucosyl unit was irradiated, NOEs were observed at H_2-6 (δ4.34; 4.69) of the ester linked glucosyl unit. From the above evidence, the structure of **3** was decided to be 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl siaresinolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester. The glycosylation shifts in the ^{13}C NMR spectrum of **3** supported these sugar sequences.

Mazusaponin IV (**4**), obtained as an amorphous powder, exhibited an $[M + Na]^+$ ion peak at m/z 1098 in the FAB-mass spectrum. Acid hydrolysis afforded L-arabinose, L-rhamnose and D-glucose in the ratio 1:1:2 as a sugar moiety, while enzymatic hydrolysis with protease followed by methylation with diazomethane afforded methyl pomolate (**2a**) as an aglycone moiety. In the ^{13}C NMR spectral data of **4**, the signals from the sugar carbons were in good agreement with those of **3**, suggesting that the sugar sequences were the same as those of **3**. The difference NOE experiments involving irradiation at each anomeric proton signal supported the sugar sequences. The structure of **4** was characterized as 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester.

Compound **5** was assigned to ilexoside VIII by comparison of spectral data with the reported data [6]. This is the first report on the isolation of saponin from *Mazus* species.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR: Jeol GSX-500 FT NMR, with TMS as an int. standard FAB-MS: Jeol JMS-SX102 mass spectrometer.

Plant material. Mazus miquelii Makino was collected in Shizuoka, Japan in April 1993 and the voucher specimen is deposited in the herbarium, School of Pharmaceutical Sciences, University of Shizuoka.

Extraction and isolation. Dried whole (175 g) *Mazus miquelii* was extracted twice with hot H_2O. The extract was passed through a porous polymer gel Mitsubishi Diaion HP-20 column. After washing the column with H_2O, the adsorbed materials were eluted with 60% MeOH aq. (yield 15 g) and MeOH (yield 2 g), successively. The MeOH eluate was chromatographed on a silica gel column with $CHCl_3$–MeOH–EtOAc–H_2O (21:9:19:1) to afford 16 fractions (frs A–P). Fr. L (310 mg) was subjected to prep. HPLC (Develosil ODS-10/20, 50 mm × 50 cm × 2, 58% MeOH aq., recycle system) to afford **1** (47 mg) and **2** (73 mg). Fr. N (158 mg) was subjected to semi prep. HPLC (Develosil PhA-7, 20 mm × 25 cm, 27.5% MeCN aq., recycle system) to

Table 1. ^{1}H NMR spectral data of compounds **1**–**5** (in pyridine-d_5 at 35°)

	1	2	3	4	5
Aglycone moiety					
3	3.33 *dd* (12, 4)	3.33 *dd* (12, 4)	3.24 *dd* (12, 3.5)	3.23 *dd* (11, 4)	3.34 *dd* (12, 4)
5	0.84 *br d* (12)	0.84 *br d* (12,)	0.81 *br d* (11.5)	0.81 *br d* (12)	0.85 *br d* (12)
12	5.50 *t*-like (3)	5.54 *t*-like (3)	5.50 *t*-like (3)	5.54 *t*-like (3)	5.54 *t*-like (3)
18	3.53 *br s*	2.92 *s*	3.53 *br s*	2.92 *s*	2.93 *s*
19	3.57 *d* (3)		3.57 *d* (3)		
23	1.26 *s*	1.25 *s*	1.16 *s*	1.16 *s*	1.29 *s*
24	0.98 *s*	0.97 *s*	1.08 *s*	1.07 *s*	1.00 *s*
25	0.94 *s*	0.95 *s*	0.93 *s*	0.95 *s*	0.95 *s*
26	1.13 *s*	1.18 *s*	1.13 *s*	1.18 *s*	1.18 *s*
27	1.63 *s*	1.68 *s*	1.62 *s*	1.68 *s*	1.69 *s*
29	1.14 *s*	1.36 *s*	1.14 *s*	1.35 *s*	1.36 *s*
30	1.02 *s*	1.03 *d* (6)	1.02 *s*	1.03 *d* (6.5)	1.04 *d* (6.5)
Sugar moiety at C-3 (Ara)					
1	4.76 *d* (7)	4.75 *d* (7)	4.92 *d* (5)	4.91 *d* (4)	4.73 *d* (7)
2	4.40 *t* (7)	4.40 *dd* (8, 7)	4.53*	4.51*	4.56 *dd* (8, 7)
3	4.15*	4.14*	4.28*	4.26*	4.22*
4	4.31*	4.31*	4.27*	4.28*	4.42 *br s*
5	3.83 *dd* (12.5, 2.5)	3.81 *br d* (12)	3.82 *d* (10)	3.81 *br d* (10)	3.72 *br d* (12)
5	4.31*	4.29*	4.27*	4.27*	4.20*
(Rha)					
1			6.06 *s*	6.04 *s*	
2			4.70*	4.70*	
3			4.59 *dd* (9, 3)	4.58 *dd* (9.5, 3.5)	
4			4.27*	4.25*	
5			4.56*	4.54*	
6			1.61 *d* (6)	1.62 *d* (6)	
(Glc)					
1					5.35 *d* (8)
2					4.01 *t* (8)
3					4.22*
4					4.19*
5					3.97*
6					4.36 *dd* (12, 5)
6					4.52 *br d* (12)
Sugar moiety at C-28 (inner Glc)					
1	6.27 *d* (8)	6.20 *d* (8)	6.28 *d* (8)	6.21 *d* (8)	6.21 *d* (8)
2	4.12 *t* (8.5)	4.14*	4.13 *t* (9)	4.15 *t* (8.5)	4.15 *t* (8.5)
3	4.19*	4.21 *t* (9)	4.20*	4.22 *t* (8.5)	4.22*
4	4.28 *t* (9.5)	4.27 *t* (9)	4.27*	4.27*	4.28 *t* (9)
5	4.08 *m*	4.11*	4.10 *m*	4.12*	4.12*
6	4.33 *dd* (12, 5)	4.34 *dd* (11, 5)	4.34 *dd* (12, 5)	4.35 *dd* (12, 5)	4.36 *dd* (12, 5)
6	4.67 *br d* (12)	4.70 *br d* (11)	4.69 *br d* (12)	4.71 *dd* (12, 1.5)	4.71 *br d* (12)
(terminal glc)					
1	5.01 *d* (8)	5.03 *d* (8)	5.02 *d* (8)	5.04 *d* (8)	5.05 *d* (8)
2	3.97 *t* (8)	3.99 *t* (8)	3.92 *t* (8)	4.00 *t* (8)	4.00*
3	4.16*	4.17*	4.19*	4.19*	4.20*
4	4.18*	4.19 *t* (8.5)	4.18*	4.28*	4.20*
5	3.87 *m*	3.88 *m*	3.88 *m*	3.89 *m*	3.90 *m*
6	4.33 *dd* (12, 5)	4.34 *dd* (12, 5)	4.34 *dd* (12, 5)	4.35 *dd* (12, 5)	4.35 *dd* (12, 5)
6	4.45 *dd* (12, 2)	4.47 *dd* (12, 1)	4.47 *dd* (12, 2.5)	4.48 *dd* (12, 2.5)	4.48 *br d* (12)

*Overlapped with over signals.

afford **3** (28 mg) and **4** (24 mg). Fr. P (44 mg) was subjected to semi-prep. HPLC (Develosil ODS-10, 20 mm × 25 cm, 55% MeOH aq., recycle system) to afford **5** (4 mg).

Mazusaponin I (**1**). Amorphous powder, $[\alpha]_D^{22}$ −8.1° (MeOH; *c*0.81). FAB-MS *m/z*: 951 $[M + Na]^+$. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Mazusaponin II (**2**). Amorphous powder, $[\alpha]_D^{22}$ −8.5° (MeOH; *c*2.12). FAB-MS *m/z*: 951 $[M + Na]^+$. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Table 2. ^{13}C NMR spectral data of compounds **1**–**5** in (pyridine-d_5 at 35°)

	1	2	3	4	5
Aglycone moiety					
1	38.7	39.0	38.8	39.1	39.0
2	26.7	26.7	26.5	26.7	26.7
3	88.8	88.9	88.9	89.0	88.8
4	39.6	39.6	39.5	39.5	39.6
5	56.1	56.0	56.1	56.1	56.0
6	18.8	18.8	18.8	18.8	18.7
7	33.3	33.6	33.3	33.6	33.5
8	40.3	40.6	40.2	40.6	40.6
9	48.4	47.8	48.4	47.8	47.8
10	37.2	37.1	37.2	37.1	37.1
11	24.2	24.1	24.2	24.1	24.1
12	123.1	128.5	123.1	128.5	128.5
13	144.3	139.3	144.3	139.3	139.3
14	42.2	42.2	42.2	42.2	42.1
15	29.0	29.3	29.0	29.3	29.3
16	28.0	26.7	28.1	26.5	26.7
17	46.6	48.8	46.6	48.8	48.7
18	44.6	54.4	44.6	54.4	54.4
19	81.2	72.7	81.2	72.8	72.9
20	35.6	42.1	35.6	42.1	42.1
21	29.1	26.2	29.1	26.2	26.1
22	33.1	37.8	33.1	37.8	37.8
23	28.3	28.3	28.1	28.2	28.2
24	16.9	16.7	16.9	16.7	16.6
25	15.6	15.7	15.6	15.8	15.7
26	17.7	17.5	17.6	17.5	17.5
27	24.8	24.6	24.8	24.6	24.6
28	177.4	177.1	177.3	177.1	177.0
29	28.7	27.1	28.7	27.1	27.1
30	24.9	17.0	24.9	17.0	17.0
Sugar moiety at C-3 (Ara)					
1	107.5	107.4	104.7	104.6	107.3
2	72.9	72.9	76.1	76.1	71.9
3	74.6	74.6	73.6	73.5	84.2
4	69.5	69.5	68.5	68.4	69.3
5	66.7	66.6	64.4	64.3	66.9
(Rha)					
1			101.8	101.8	
2			72.4	72.4	
3			72.6	72.6	
4			74.1	74.1	
5			69.9	70.0	
6			18.6	18.6	
(Glc)					
1					106.4
2					75.8
3					78.7
4					71.7
5					78.4
6					62.8
Sugar moiety at C-28 (inner Glc)					
1	95.8	95.8	95.8	95.8	95.8
2	73.9	73.9	74.0	73.9	73.9
3	78.4	78.4	78.4	78.5	78.5
4	71.6	71.7	71.6	71.7	71.7
5	78.0	78.0	78.0	78.0	78.0
6	69.5	69.7	69.5	69.7	69.7
(terminal Glc)					
1	105.3	105.4	105.3	105.4	105.4
2	75.2	75.3	75.2	75.3	75.2
3	78.8	78.8	78.8	78.8	78.8
4	71.1	71.2	71.1	71.3	71.2
5	78.4	78.4	78.4	78.5	78.4
6	62.8	62.8	62.8	62.8	62.8

Mazusaponin III (**3**). Amorphous powder, $[\alpha]_D^{22}$ − 33.2° (MeOH; *c*1.58). FAB-MS *m/z*: 1098 [M + Na]$^+$. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Mazusaponin IV (**4**). Amorphous powder, $[\alpha]_D^{22}$ − 30.5° (MeOH; *c*1.36). FAB-MS *m/z*: 1098 [M + Na]$^+$. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Methanolysis of compound **1**. Compound **1** (20 mg) was refluxed with AcCl–MeOH (1:10) (5 ml) for 2 hr. The reagents were evapd off and the residue was subjected to prep. TLC [Kiesel gel PF_{254}, hexane–EtOAc (7:3)] to afford methyl siaresinate (**1a**) (5 mg). $[\alpha]_D$ and ^{1}H NMR data were identical to the reported data [7].

Acid hydrolysis of compounds **1**–**4**. Each compound (1 mg) was heated with 5% H_2SO_4–dioxane (1:1) (5 drops) at 100° for 1 hr. The reaction mixture was diluted with H_2O and partitioned between EtOAc and H_2O. The H_2O layer was passed through an Amberlite IRA-60E column. The eluate was concd to give a residue, which was treated with D-cysteine [11] (0.05 mg) in H_2O (0.03 ml) and pyridine (0.015 ml) at 60° for 1 hr with stirring. After the solvent was evapd and the reaction mixture dried, pyridine (0.015 ml), hexamethyl disilazane (0.015 ml) and trimethylsilyl chloride (0.015 ml) were added to the residue. The reaction mixture was heated at 60° for 30 min. The supernatant was applied to GC. From **1** and **2**, L-arabinose, D-glucose (1:2), from **3** and **4**, L-arabinose, L-rhamnose, D-glucose (1:1:2) were detected. GC conditions: column, Supelco, SPB™-1, 0.25 mm × 27 m; column temp., 230°; carrier gas, N_2; R_t, L-rhamnose 12.0 min, D-rhamnose* 11.7 min, L-arabinose 10.5 min, D-arabinose 9.9 min, D-glucose 17.7 min, L-glucose 17.0 min. The EtOAc layer was concd to dryness and the residue was methylated with CH_2N_2–Et_2O. From **1** and **3**, methyl siaresinate (**1a**) was detected by HPLC and TLC. HPLC conditions: column, Develosil ODS-5, 4.6 mm × 15 cm; solvent, MeCN–H_2O (17:3); flow 1.0 ml cm^{-1}; UV, 205 nm; R_t, 11.8 min. TLC conditions: Kiesel gel F_{254}; solvent C_6H_6–Me_2CO (93:7); R_f, 0.36.

Enzymatic hydrolysis of compounds **2** *and* **4**. Compound **2** (26 mg) was dissolved in EtOH (0.5 ml) and 0.1 M citric acid–0.2 M Na_2HPO_4 buffer (pH 4.0) (3 ml) and treated with protease (type XIII from *Aspergillus satoi*, Sigma) [9] (30 mg) at 37° for 5 days, then the reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was concd and the residue was methylated with CH_2N_2–Et_2O. The reaction product was purified

*The R_t for D-rhamnose was obtained from its enantiomer (L-rhamnose + L-cysteine).

by prep. TLC (Kiesel gel PF_{254}; hexane–EtOAc, 7:3) to give methyl pomolate (**2a**) (1 mg). $[\alpha]_D$ and ^1H NMR data were identical to the reported data [8]. Compound **4** (1 mg) was treated in the same manner and methyl pomolate (**2a**) was identified by HPLC and TLC. HPLC conditions: column, Develosil ODS-5, 4.6 mm × 15 cm; solvent, MeCN–H_2O (17:3); flow, 1.0 ml cm^{-1}; UV, 205 nm; R_t, 11.8 min. TLC conditions: Kiesel gel F_{254}; solvent, C_6H_6–Me_2CO (93:7); R_f, 0.33.

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