Two New Triterpenoidal Glycosides from *Medicago polymorpha* L.[1)]

Junei KINJO, Hadzuki UEMURA, Motoyuki NAKAMURA and Toshihiro NOHARA*

Faculty of Pharmaceutical Sciences, Kumamoto University, 5–1 Oe-honmachi, Kumamoto 862, Japan.
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Two new triterpenoid glycosides called medicago-saponins P_1 (1) and P_2 (2) were isolated together with five known glycosides from the aerial parts of *Medicago polymorpha* L. (Leguminosae). The structures of 1 and 2 were determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl caulophyllogenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside and the desglucoside of 1.

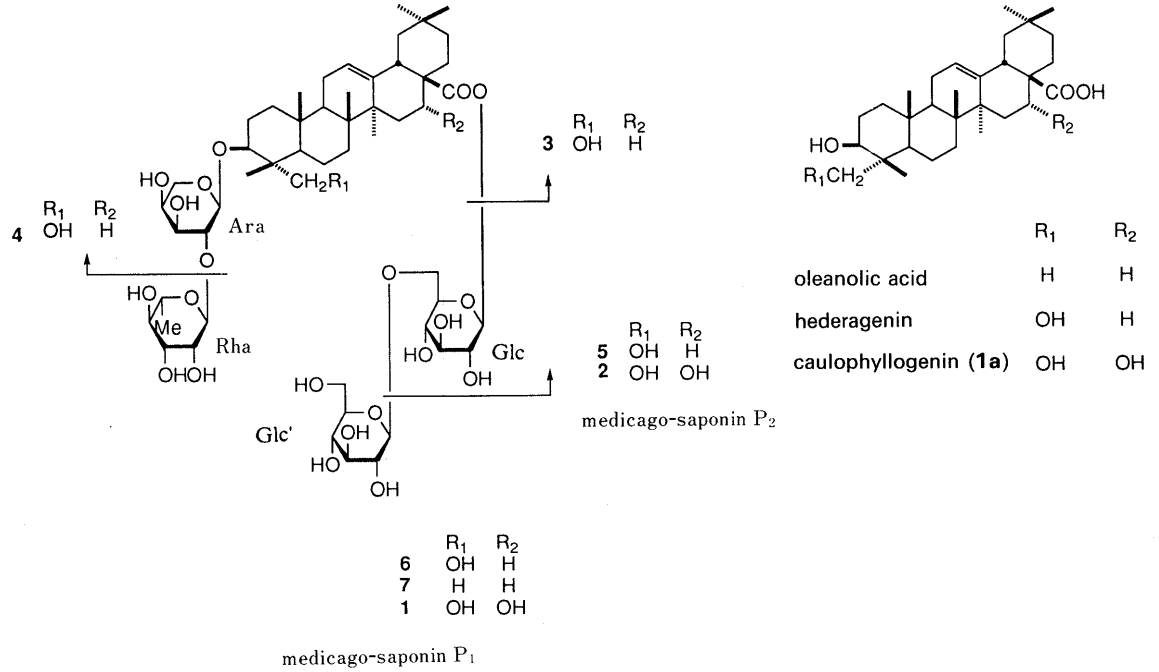
Keywords *Medicago polymorpha*; Leguminosae; triterpene saponin; oleanene glycoside; medicago-saponin; caulophyllogenin

Medicago polymorpha L. (syn *M. denticulata* WILLD. and *M. hispida* GAERTN.) is used as an important fodder together with alfalfa (*M. sativa* L.) worldwide. Although earlier researchers found that alfalfa included many saponins and reported their structures,[2)] there have been few reports concerning the saponins of *M. polymorpha*.[3)] During the course of our study on leguminous plants,[1)] we have investigated the saponins of Japanese *M. polymorpha*. This paper deals with the structural elucidation and identification of these saponins.

A methanolic extract of the aerial parts of *M. polymorpha* was partitioned with 40% MeOH and ethyl acetate. A combination of various chromatographies of the former phase resulted in the isolation of seven saponins (**1**—**7**). Saponins **3**—**7** were identified as sapindoside A (**3**),[4,5)] compound 6 (**4**),[6)] compound 9 (**5**),[5)] compound 10 (**6**)[5)] and compound 11 (**7**)[5)] comparing the negative FAB-MS, and the ^{1}H- and ^{13}C-NMR spectra with those of the published data.

Medicago-saponin P_1 (**1**) was obtained as a white amorphous powder, $[\alpha]_D$ −20.1° (pyridine). The IR spectrum of **1** featured absorptions of carbonyl (1735 cm^{-1}) and a hydroxy group. In the negative FAB-MS, **1** showed an $[M-H]^-$ ion at *m/z* 1089. Fragment ion peaks at *m/z* 765 [M−hexose−hexose]$^-$ and 619 [M−hexose−hexose−methylpentose]$^-$ were also observed. These MS data appeared to a greater extent than those of **6** by a hydroxy group. By acid hydrolysis, **1** gave **1a** as the sapogenol. The electron impact (EI)-MS of **1** showed a $[M]^+$ at *m/z* 488, indicating a hydroxy group located on **1a**. By the ^{1}H- and ^{13}C-NMR spectral data (as listed in Tables I—III), the location of the hydroxy group was determined to be at C-16α, which compares to those of hederagenin[7)] and acacic acid.[8)] Finally, **1a** was identified with caulophyllogenin[9a)] because of the agreement of various data. In addition, the signals for the sugar region (Table II) were superimposable on those of **6** in the ^{13}C-NMR. Since the absolute configurations of the sugar species were identified according to the method reported by Hara *et al.*,[10)] the structure of **1** was established to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl caulophyllogenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Medicago-saponin P_2 (**2**) was obtained as a white



amorphous powder, $[\alpha]_D$ −4.1° (MeOH). An acidic hydrolysis of **2** gave caulophyllogenin (**1a**) as the sapogenol. In the FAB-MS of **2**, an $[M+Na]^+$ ion at *m/z* 951 appeared to a lesser extent than that of **1** by a hexosyl moiety. Since the ^{13}C-NMR signals of the sugar region (Table II) showed a good coincidence with those of **5**, **2** was concluded to be the desglucosyl compound of **1**, that is, 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl caulophyllogenin 28-*O*-β-D-glucopyranoside.

Saponins with caulophyllogenin as their aglycone are very rare. Only two examples are known in nature. They were isolated from *Caulophyllum robustum*[9a] (Berberidaceae) and *Chrysanthellum procumbens*[9b] (Compositae). Medicago-saponins P_1 and P_2 are the third and fourth saponins known to have caulophyllogenin, respectively, and are the first examples from Leguminosae.

Table I. ^{13}C-NMR Data for Compounds **1**—**7** (Aglycone Moieties) and **1a** in Pyridine-d_5

Carbon No.	1a	1	2	3	4	5	6	7
1	38.9	38.9	38.9	38.9	38.8	38.9	38.8	38.8
2	27.7	26.0	26.1	26.2	26.1	26.0	26.0	26.4
3	73.6	80.9	81.2	81.0	81.9	80.9	80.9	88.7
4	42.2	43.3	43.3	43.5	43.5	43.3	43.3	39.4
5	47.2	47.1	47.1	47.7	47.6	47.5	47.5	55.8
6	18.7	18.0	18.1	18.2	18.2	18.2	18.0	18.4
7	33.3	32.9	32.9	33.2	32.8	32.6	32.6	33.0
8	40.0	39.9	40.0	39.7	40.0	39.8	39.7	39.8
9	48.9	47.6	47.6	48.1	48.2	48.0	48.0	47.9
10	37.3	36.8	36.8	36.9	36.9	36.7	36.7	36.9
11	23.9	23.7	23.7	23.8	23.9	23.7	23.7	23.7
12	122.5	122.5	122.6	122.6	122.9	122.8	122.7	122.7
13	145.1	144.2	144.5	144.8	144.2	143.9	143.9	144.0
14	42.9	41.8	42.0	42.1	42.9	42.0	42.0	42.0
15	36.2	35.9	35.7	28.3	28.3	28.1	28.1	28.2
16	74.7	74.1	73.4	23.6	23.4	23.2	23.2	23.3
17	48.6	48.9	49.2	46.6	47.0	46.8	46.9	46.9
18	41.5	41.0	41.2	42.0	41.7	41.6	41.5	41.6
19	47.3	46.9	47.1	46.4	46.2	46.0	46.0	46.1
20	31.1	30.5	30.6	30.9	30.8	30.6	30.5	30.6
21	36.2	35.7	35.7	34.2	34.0	33.8	33.8	33.9
22	32.9	31.9	31.8	32.8	32.5	32.4	32.3	32.4
23	68.2	63.7	63.8	64.0	64.5	63.8	63.8	15.5
24	13.1	13.7	13.7	14.0	13.6	13.8	13.7	28.0
25	16.1	16.1	16.2	16.1	16.2	16.0	16.0	16.8
26	17.6	17.4	17.5	17.5	17.6	17.4	17.4	17.4
27	27.2	27.0	27.1	26.2	26.0	25.9	25.8	25.9
28	178.0	175.8	176.4	180.2	176.6	176.3	176.4	176.4
29	33.3	33.0	33.0	33.2	33.1	33.0	32.9	33.0
30	24.8	24.5	24.6	23.8	23.7	23.5	23.5	23.5

Table II. ^{13}C-NMR Data for Compounds **1**—**7** (Sugar Moieties) in Pyridine-d_5

Carbon No.	1	2	3	4	5	6	7
Ara							
1	104.0	103.8	104.0	106.6	104.1	104.0	104.6
2	75.6	76.0	75.8	73.1	75.7	75.7	75.8
3	74.2	73.6	74.7	74.7	74.4	74.3	73.9
4	69.5	70.4	69.7	69.6	69.5	69.5	69.8
5	65.3	64.8	65.6	67.0	65.3	65.3	64.3
Rha							
1	101.4	101.4	101.6		101.5	101.5	101.6
2	72.1[a]	71.6[a]	72.3[a]		72.1[a]	72.1[a]	72.2[a]
3	72.3[a]	71.9[a]	72.5[a]		72.4[a]	72.3[a]	72.4[a]
4	73.7	73.4	74.1		73.9	73.7	73.4
5	69.0	68.6	69.3		69.1	69.0	68.4
6	18.3	18.2	18.5		18.3	18.3	18.4
Glc							
1	95.6	95.6		95.7	95.6	95.5	95.5
2	73.8	74.0		73.9	74.0	73.9	73.8
3	78.2[b]	78.8[b]		78.4[a]	79.1[b]	78.2[b]	78.3[b]
4	70.6	70.7		70.9	71.0	70.7	70.7
5	77.7	78.0[b]		78.0	78.7[b]	77.7	77.8
6	69.1	61.8		69.4	62.0	69.1	69.2
Glc							
1	105.0			105.3		105.0	105.1
2	74.9			75.2		74.9	75.0
3	78.1[b]			78.4[a]		78.2[b]	78.3[b]
4	71.2			71.5		71.3	71.4
5	78.4[b]			78.7[a]		78.5[b]	78.5[b]
6	62.3			62.6		62.4	62.5

a, b) In each vertical column may be interchanged.

Table III. ^{1}H-NMR Data for Compounds **1**—**7** and **1a** in Pyridine-d_5

	1a	1	2	3	4	5	6	7
tert-Me	1.02 (s)	0.96 (s)	0.95 (s)	0.94 (s)	0.87 (s)	0.88 (s)	0.86 (s)	0.89 (s)
	1.05 (s)	1.03 (s)	0.96 (s)	0.94 (s)	0.88 (s)	0.89 (s)	0.87 (s)	0.89 (s)
	1.08 (s)	1.03 (s)	0.99 (s)	1.00 (s)	0.94 (s)	0.96 (s)	0.99 (s)	0.89 (s)
	1.10 (s)	1.07 (s)	1.01 (s)	1.02 (s)	0.99 (s)	1.06 (s)	1.08 (s)	1.07 (s)
	1.19 (s)	1.17 (s)	1.12 (s)	1.06 (s)	1.14 (s)	1.12 (s)	1.14 (s)	1.11 (s)
	1.81 (s)	1.76 (s)	1.76 (s)	1.23 (s)	1.19 (s)	1.19 (s)	1.18 (s)	1.17 (s)
								1.26 (s)
H-18	3.64 (br d, J=13.9)	3.51 (br d, J=13.9)	3.47 (br d, J=13.0)	3.28 (br d, J=9.5)	3.20 (br d, J=13.2)	3.18 (br d, J=10.3)	3.19 (br d, J=13.9)	3.21 (br d, J=13.9)
H-3	4.22 (dd, J=11.0, 5.1)	—[a]	—[a]	4.12 (m)	—[a]	4.12 (m)	—[a]	3.24 (dd, J=12.1, 4.0)
H-16	5.26 (br s)	5.27 (br s)	5.29 (br s)					
H-12	5.68 (br s)	5.60 (br s)	5.61 (br s)	5.47 (br s)	5.45 (br s)	5.42 (br s)	5.42 (br s)	5.43 (br s)
Glc H-1		6.26 (d, J=8.1)	6.2 (d, J=8.8)		6.26 (d, J=8.1)	6.31 (d, J=8.1)	6.27 (d, J=8.1)	6.27 (d, J=8.1)
Glc′ H-1		5.02 (d, J=8.1)			4.98 (d, J=7.4)		5.05 (d, J=8.1)	5.04 (d, J=7.7)
Ara H-1		5.11 (d, J=5.9)	5.13 (d, J=5.9)	5.11 (d, J=6.6)	5.04 (d, J=7.3)	5.11 (d, J=5.9)	5.12 (d, J=5.9)	4.91 (d, J=5.1)
Rha H-1		6.23 (s)	6.05 (s)	6.24 (s)		6.21 (s)	6.25 (s)	6.12 (s)
Rha H-6		1.64 (d, J=6.6)	1.65 (d, J=6.6)	1.63 (d, J=5.9)		1.63 (d, J=5.9)	1.65 (d, J=5.9)	1.64 (d, J=6.2)

a) Overlapped with the other signals.

Experimental

The instruments and reagents used in this study were the same as those described in the previous papaer.[8]

Extraction and Isolation The fresh aerial parts(3.0 kg) of *Medicago polymorpha* collected in the medicinal garden of our Faculty were extracted with MeOH, and the extract (160 g) was partitioned with 40% MeOH and EtOAc. The aqueous extract (130 g) was subjected to Diaion HP-20 column chromatography using 0%→100% MeOH to give fractions 1 to 4. Each fraction was separated by Wako gel LP60 (0%→100% MeOH), Bondapak C_{18} (0%→100% MeOH) and silica gel ($CHCl_3$: MeOH : H_2O = 9 : 1 : 0.1→6 : 4 : 1) to provide compounds **1** (0.029%), **2** (0.0019%), **3** (0.0012%), **4** (0.0005%), **5** (0.011%), **6** (0.049%) and **7** (0.0006%).

Compound 1 (Medicago-Saponin P_1) A white amorphous powder, TLC *Rf*, 0.11 ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5). $[\alpha]_D^{28}$ −20.1° (*c* = 0.30, pyridine). IR (KBr): 3390 (ν_{O-H}), 1735 ($\nu_{C=O}$) cm^{-1}. Negative FAB-MS: *m/z* 1089 $[M-H]^-$, 765 $[M-H-Glc-Glc]^-$, 619 $[M-H-Glc-Glc-Rha]^-$. ^{1}H-NMR: Table III. ^{13}C-NMR: Tables I and II.

Characterization of Sapogenol and Sugars for 1 Compound **1** (12 mg) was dissolved in 2 N HCl/H_2O (2 ml) and heated at 90 °C for 2 h. After filtration of the mixture, the precipitate was recrystallized with MeOH to provide a sapogenol (**1a**), Colorless fine needles, mp > 300 °C, $[\alpha]_D^{28}$ +24.4° (*c* = 0.4, pyridine). IR (KBr): 3410 (ν_{O-H}), 1735 ($\nu_{C=O}$) cm^{-1}. EI-MS: *m/z* 488 $[M^+]$. ^{1}H-NMR: Table III. ^{13}C-NMR: Table I. The filtrate was neutralized by Amberlite MB-3 column chromatography. After evaporation, the residue (5 mg) was dissolved in pyridine (0.7 ml), then the mixture was added to a pyridine solution (2 ml) of L-cysteine methyl ester hydrochloride (0.06 mol/l) and warmed at 60 °C for 1 h. The mixture was evaporated under N_2 stream and dried *in vacuo*. The obtained syrup was trimethylsilylated with *N*-trimethylsilylimidazole (0.4 ml) at 60 °C for 1 h. After the addition of *n*-hexane (2 ml) and H_2O (2 ml), the *n*-hexane layer was taken off and checked by GC. The retention time (t_R) of the peaks was at 14.71 min (D-glucose, 14.75 min), 10.06 min (L-rhamnose, 10.11 min), and 8.34 min (L-arabinose, 8.36 min).

Compound 2 (Medicago-Saponin P_2) A white amorphous powder, TLC *Rf*, 0.30 ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5). $[\alpha]_D^{30}$ −4.1° (*c* = 0.54, MeOH). IR (KBr): 3410 (ν_{O-H}), 1735 ($\nu_{C=O}$) cm^{-1}. Positive FAB-MS: *m/z* 951 $[M+Na]^+$. ^{1}H-NMR: Table III. ^{13}C-NMR: Tables I and II.

Characterization of Sapogenol A sample of **2** was hydrolyzed in the same manner as above. The precipitate was identified with caulophyllogenin (**1a**) by TLC *Rf*, 0.23 ($CHCl_3$: MeOH = 20 : 1).

Compound 3 A white amorphous powder, TLC *Rf*, 0.61 ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5). $[\alpha]_D^{30}$ +19.9° (*c* = 0.48, MeOH). IR (KBr): 3410 (ν_{O-H}), 1700 ($\nu_{C=O}$) cm^{-1}. Positive FAB-MS: *m/z* 751 $[M+H]^+$. ^{1}H-NMR: Table III. ^{13}C-NMR: Tables I and II.

Compound 4 A white amorphous powder, TLC *Rf*, 0.30 ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5). $[\alpha]_D^{30}$ +17.7° (*c* = 0.40, MeOH). IR (KBr): 3415 (ν_{O-H}), 1725 ($\nu_{C=O}$) cm^{-1}. Positive FAB-MS: *m/z* 951 $[M+Na]^+$. ^{1}H-NMR: Table III. ^{13}C-NMR: Tables I and II.

Compound 5 A white amorphous powder, TLC *Rf*, 0.39 ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5). $[\alpha]_D^{30}$ +7.0° (*c* = 0.49, MeOH). IR (KBr): 3415 (ν_{O-H}), 1735 ($\nu_{C=O}$) cm^{-1}. Positive FAB-MS: *m/z* 935 $[M+Na]^+$. ^{1}H-NMR: Table III. ^{13}C-NMR: Tables I and II.

Compound 6 A white amorphous powder, TLC *Rf*, 0.18 ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5). $[\alpha]_D^{29}$ +30.9° (*c* = 0.33, MeOH). IR (KBr): 3395 (ν_{O-H}), 1715 ($\nu_{C=O}$) cm^{-1}. Negative FAB-MS: *m/z* 1073 $[M-H]^-$, 749 $[M-H-Glc-Glc]^-$. ^{1}H-NMR: Table III. ^{13}C-NMR: Tables I and II.

Compound 7 A white amorphous powder, TLC *Rf*, 0.26 ($CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5). $[\alpha]_D^{30}$ −11.1° (*c* = 0.41, MeOH). IR (KBr): 340 (ν_{O-H}), 1735 ($\nu_{C=O}$) cm^{-1}. Positive FAB-MS: *m/z* 1081 $[M+Na]^+$. ^{1}H-NMR: Table III. ^{13}C-NMR: Tables I and II.

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References and Notes

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