A New Oleanene Glucuronide Obtained from the Aerial Parts of *Melilotus officinalis*[1)]

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A new oleanene glucuronide called melilotus-saponin O_2 (1) was isolated together with three known ones (soyasaponin I, astragaloside VIII, wistariasaponin D) from the aerial parts of *Melilotus officinalis* (L.) PALLAS (Leguminosae). The structure of 1 was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl melilotigenin by spectroscopic and chemical methods.

Key words *Melilotus officinalis*; Leguminosae; triterpene saponin; oleanene glucuronide; melilotus-saponin; melilotigenin

Melilotus officinalis (L.) PALLAS is distributed worldwide and is known as common yellow melilot or medicinal sweet clover in English.[2)] This plant is used not only as food and forage but also as a medicine. The preventive effect of its extract of whole plant on experimental atherosclerosis in rabbits was reported.[3)] The effect of a medical preparation (Esberiven) using its extract was also evaluated on dermatological disease.[4)] Earlier researchers found that an extract of the aerial parts showed potent inhibitory activity on the migration of leucocytes, and one of the constituents responsible for the action was azukisaponin V.[5)] During our course of studies on leguminous plants,[1)] we also investigated the oleanene-type triterpene glucuronides (oleanene glucuronides) of the roots of *Melilotus officinalis* collected in Hokkaido, Japan.[6)] Herein, we describe the structural elucidation of oleanene glucuronides obtained from the aerial parts of the titled plant.

The aerial parts of *M. officinalis* were collected in the medicinal garden of Kumamoto University. A methanolic extract of the aerial parts of the titled plant afforded saponins (**1**—**4**) after various chromatographic purifications. Saponins **2**—**4** were identified as soyasaponin I (**2**),[7)] wistariasaponin D (**3**),[8)] and astragaloside VIII (**4**)[9)] by comparison with various data.

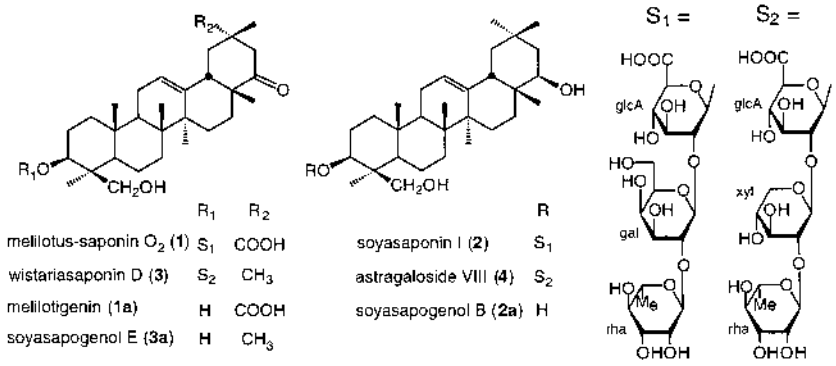
Melilotus-saponin O_2 (**1**) was obtained as a white amorphous powder, $[\alpha]_D$ −45.7° (pyridine). In the negative FAB-MS, **1** showed an $[M-H]^-$ ion at *m/z* 939. Fragment ion peaks at *m/z* 793 [M−methylpentose]$^-$ and 661 [M−methylpentose−pentose]$^-$ were also observed. The exact measurement under high resolution (HR) conditions showed that the composition is $C_{47}H_{72}NaO_{19}$ at *m/z* 963.4564 $[M+Na]^+$ in the HR/positive FAB-MS. The sapogenol obtained by enzymatic hydrolysis was identified with melilotigenin (**1a**).[5c,10)] The monosaccharide mixture obtained by acid hydrolysis of **1** revealed the presence of glucuronic acid, xylose and rhamnose by TLC. Their absolute configurations were determined to be the D-form (glucuronic acid, xylose) and the L-form (rhamnose), according to the procedure developed by Hara *et al.*[11)] In the sugar region of the ^{13}C-NMR spectrum for **1**, signals due to a sugar moiety were identical with those of **3** and **4**. Since the signal at C-3 of **1** was shifted to a lower field by glycosylation,[12)] the structure of **1** was deduced to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl melilotigenin. Although melilotigenin glycosides were already obtained from *Trifolium repens*,[13)] melilotus-saponin O_2 is the first example of a tri-glycosidic saponin having the same aglycone.

Experimental

The instruments and reagents used in this study were the same as those previously described.[6, 10)]

Extraction and Isolation The dried aerial parts (356 g) of *Melilotus officinalis* collected in the medicinal garden of Kumamoto University were extracted with MeOH. The extract (40 g) was partitioned with *n*-hexane and 80% MeOH. Removal of the solvent from the latter phase under reduced pressure gave an aqueous extract (35 g). The aqueous extract was subjected to Diaion HP-20 column (6×30 cm) chromatography using H_2O and MeOH. A part (5.2 g) of the MeOH eluate (10 g) was subjected to Sephadex LH-20 column (5×35 cm) chromatography using MeOH to give a total saponin fraction (0.73%). After silica gel column chromatography using $CHCl_3$:MeOH:H_2O=7:3:0.5—6:4:1, the saponin fractions were separated by silica gel (1-BuOH:AcOH:H_2O=8:1:0.1—4:1:2) to provide compounds **1** (0.032%), **2** (0.019%), **3** (0.020%) and **4** (0.19%), respectively. Soyasaponin I[7)] (**2**) and Astragaloside VIII[9)] (**4**) were identified by comparison of their t_R (HPLC)[7c,14)] and *Rf* (TLC) values with the authentic samples.

Compound 1 (Melilotus-Saponin O_2) A white amorphous powder, $[\alpha]_D^{14}$ −45.7° (*c*=0.50, pyridine). HR positive ion FAB-MS *m/z*: 963.4564 ($C_{47}H_{72}NaO_{19}$, Calcd for 963.4566). Negative ion FAB-MS *m/z*: 939



	R_1	R_2
melilotus-saponin O_2 (**1**)	S_1	COOH
wistariasaponin D (**3**)	S_2	CH_3
melilotigenin (**1a**)	H	COOH
soyasapogenol E (**3a**)	H	CH_3

	R
soyasaponin I (**2**)	S_1
astragaloside VIII (**4**)	S_2
soyasapogenol B (**2a**)	H

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$[M-H]^-$, 793 $[M-H-rha]^-$, 661 $[M-H-rha-xyl]^-$, 485 $[M-H-rha-xyl-glcA]^-$. ^{1}H-NMR (in pyridine-d_5): 0.69, 0.85, 1.19, 1.28, 1.45, 1.46 (each 3H, s, *tert*-Me×6), 1.82 (3H, d, J=6.1 Hz, rha H_3-6), 4.08 (1H, t, J=8.4 Hz, xyl H-3), 4.71 (1H, dd, J=3.1, 9.2 Hz, rha H-3), 4.93 (1H, d, J=7.9 Hz, glcA H-1), 5.27 (1H, s, H-12), 6.23 (1H, s, rha H-1). ^{13}C-NMR (in pyridine-d_5): 38.8, 26.3, 90.9, 44.0, 56.3, 18.5, 33.0, 39.7, 47.5, 36.4, 23.9, 123.2, 141.6, 42.0, 25.4, 27.4, 48.2, 47.4, 42.0, 45.2, 47.1, 216.8, 22.7, 62.8, 15.5, 16.7, 25.4, 20.9, 181.0, 21.9 (C-1—30), 104.9, 78.2, 76.4, 73.8, 77.8, 175.7 (glcA C-1—6), 102.4, 78.9, 78.0, 70.6, 66.5 (xyl C-1—5), 101.7, 72.0, 72.0, 73.9, 69.2, 18.6 (rha C-1—6).

Characterization of Sapogenol for 1 To a solution of **1** (4 mg) in acetate buffer (pH 5.0, 6 ml) was added glycyrrhizin hydrolase,[15] and the mixture was incubated at 37 °C for 40 h. When the hydrolysis was completed, the hydrolysate was evaporated and suspended in MeOH. After filtration of the suspension, the filtrate was methylated in ethereal CH_2N_2. The methylated sample was identified to be a methylester of melilotigenin[5c,10] by TLC. *Rf*s: 0.54 [$CHCl_3$: MeOH (19 : 1)], 0.83 [*n*-hexane : acetone (1 : 1)].

Characterization of Sugars for 1 A small amount of **1** (2 mg) was dissolved in 2 M HCl/H_2O (1 ml) and heated at 80 °C for 2 h. After neutralization of 2 M NaOH/H_2O, the sugar mixture was subjected to TLC analysis [TLC, Kieselgel 60 F_{254} (Merck Art. 5554), $CHCl_3$: MeOH : H_2O=6 : 4 : 1, *Rf*s: 0.09 (glucuronic acid), 0.45 (xylose), 0.61 (rhamnose).

D, L Determination of Sugars for 1 The absolute configuration of glucuronic acid was determined after $NaBH_4$ reduction, according to Tanaka *et al.*[16] A small amount of **1** (3 mg) in MeOH (0.5 ml) was methylated with ethereal CH_2N_2. To a solution of the methylated sample of **1** was added $NaBH_4$, and the mixture was kept at room temperature for 30 min. The reaction mixture was worked up with MCI gel CHP 20P. The MeOH eluate was evaporated and heated in 2 M HCl/H_2O at 90 °C for 3 h. The hydrolysate was subjected to MCI gel CHP 20P and Amberlite IRA-400 to give a sugar fraction. This fraction was dissolved in pyridine (0.1 ml), then the solution was added to a pyridine solution (0.2 ml) of L-cysteine methyl ester hydrochloride (0.1 mol/l) and heated at 80 °C for 1 h. The solvent was evaporated under a N_2 stream and dried *in vacuo* with heating. The remaining syrup was trimethylsilylated with trimethylsilylimidazole (0.1 ml) at 60 °C for 1 h. After the addition of *n*-hexane and H_2O, the *n*-hexane layer was taken out and checked by GC. The retention times of the peaks coincided with those of D-glucose (t_R 11.6 min), D-xylose (t_R 6.6 min) and L-rhamnose (t_R 8.0 min).

Wistariasaponin D (3)[8] A white amorphous powder, $[\alpha]_D^{14}$ −29.5° (*c*=0.46, pyridine). Negative ion FAB-MS *m/z*: 910 $[M-H]^-$, 764 $[M-H-rha]^-$, 632 $[M-H-rha-xyl]^-$. ^{1}H-NMR (in pyridine-d_5): 0.76, 0.85, 0.88, 0.96, 1.16, 1.31, 1.53 (each 3H, s, *tert*-Me×7), 1.81 (3H, d, J=6.1 Hz, rha H_3-6), 4.11 (1H, t, J=8.4 Hz, xyl H-3), 4.69 (1H, dd, J=3.1, 9.2 Hz, rha H-3), 5.02 (1H, d, J=7.9 Hz, glcA H-1), 5.25 (1H, s, H-12), 5.68 (1H, d, J=7.3 Hz, xyl H-1), 6.34 (1H, s, rha H-1). ^{13}C-NMR (in pyridine-d_5): 38.7, 26.6, 90.9, 44.2, 56.2, 18.5, 33.0, 39.7, 47.8, 36.4, 23.9, 123.9, 141.7, 42.0, 25.3, 27.2, 47.7, 47.5, 46.6, 34.0, 50.8, 215.6, 22.9, 62.8, 15.4, 16.7, 25.1, 20.9, 32.0, 25.4 (C-1—30), 105.4, 78.6, 77.4, 73.8, 77.7, 172.4 (glcA C-1—6), 102.5, 79.4, 78.3, 70.8, 66.7 (xyl C-1—5), 102.3, 72.3, 72.7, 74.3 69.4, 18.8 (rha C-1—6).

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References and Notes

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