A New Oleanene Glucuronide Having a Branched-Chain Sugar from *Melilotus officinalis*[1)]

Manabu UDAYAMA,[a] Junei KINJO,[a] Naotoshi YOSHIDA,[b] and Toshihiro NOHARA*,[a]

Faculty of Pharmaceutical Sciences, Kumamoto University,[a] 5-1 Oe-honmachi, Kumamoto 862–0973, Japan and Medicinal Plant Garden, Faculty of Pharmaceutical Sciences, Hokkaido University,[b] Nishi 6, Kita 12, Kita-ku, Sapporo 060–0812 Japan. Received September 19, 1997; accepted November 5, 1997

A new oleanene glucuronide called melilotus-saponin O_1 (1) was isolated together with three known ones from the roots of *Melilotus officinalis* (L.) PALLAS (Leguminosae). The structure of 1 was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)]-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B by spectroscopic and chemical methods.

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

Melilotus officinalis (L.) PALLAS is distributed worldwide and is known in English as common yellow melilot or medicinal sweet clover.[2)] This plant is used not only as a food and forage but also as a medicine. The preventive effect of its extract 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 the extract of aerial parts showed potent inhibitory activity on leucocyte migration and one of the constituents responsible for the action was azukisaponin V.[5)] During our course of studies on leguminous plants,[1)] we have investigated the oleanene-type triterpene glucuronides (oleanene glucuronides) of the roots of Japanese *Melilotus officinalis*. This paper deals with the structural elucidation and identification of these oleanene glucuronides.

A methanolic extract of the aerial parts of *M. officinalis* was first separated by Sephadex LH-20 column chromatography to get a crude saponin fraction. A combination of MCI gel and silica gel chromatographies resulted in the isolation of four saponins (**1**—**4**). Saponins **2**—**4** were identified as soyasaponin I (**2**),[6)] dehydrosoyasaponin I (**3**),[6b,7)] and acetyl-soyasaponin I (**4**)[8)] by direct comparison with the authentic samples.

Melilotus-saponin O_1 (**1**) was obtained as a white amorphous powder, $[\alpha]_D^{25}$ +5.7° (MeOH). In the negative FAB-MS, **1** showed an $[M-H]^-$ ion at *m/z* 1073. Fragment ion peaks at *m/z* 941 $[M-\text{pentose}]^-$ and 927 $[M-\text{methylpentose}]^-$ were also observed. The exact measurement under high resolution (HR) conditions showed that the composition is $C_{53}H_{86}NaO_{22}$ at *m/z* 1097.5519 $[M+Na]^+$ in the HR/positive FAB-MS. By acid hydrolysis, **1** gave soyasapogenol B as the sapogenol. The monosaccharide mixture obtained by acid hydrolysis revealed the presence of glucuronic acid, galactose, rhamnose and arabinose by TLC. Their absolute configurations were determined to be the D-form (glucuronic acid, galactose) and the L-form (arabinose, rhamnose), according to the procedure developed by Hara *et al.*[9)] In the sugar region of the ^{13}C-NMR spectrum for **1**, signals based upon the terminal rhamnopyranosyl and the terminal arabinopyranosyl residues were observed. Since the carbon signals due to the sapogenol moiety were superimposable on those of **2**,[6b)] these sugars were concluded to be composed of a branched-chain sugar which attached at C-3. The combination analyses of ^1H–^1H shift correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC) and heteronuclear multiple quantum coherence (HMQC) spectra of **1** gave the correlations shown in Fig. 1.

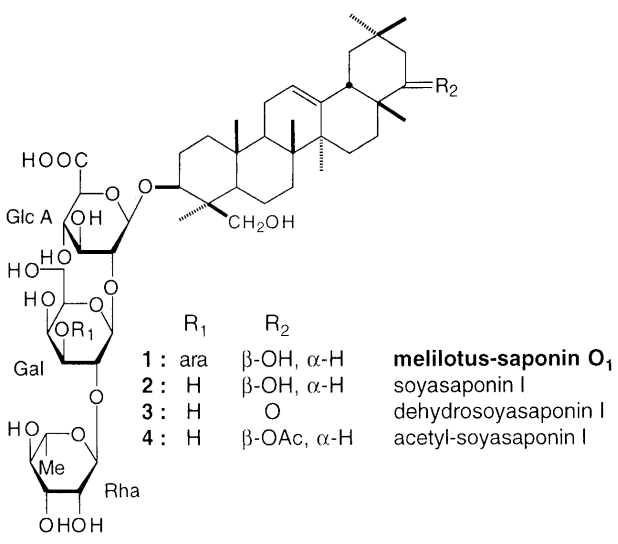
Consequently, the structure of **1** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)]-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B.

Meanwhile, we clarified that oleanene glucuronides are effective for experimental hepatitis.[10)] Since Kang *et al.* previously reported the potent inhibitory activity on leucocyte migration of oleanene glucuronides obtained from the titled plant,[5)] some of these could show anti-inflammatory actions on not only hepatitis but various other inflammations.

Experimental

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

Extraction and Isolation The dried roots (580 g) of *Melilotus officinalis* collected in the medicinal garden of Hokkaido University were extracted with MeOH, and the extract (18 g) was separated by Sephadex LH-20 column chromatography to give crude saponin fraction. After MCI gel CHP 20P column chromatography using 50%→100% MeOH to afford fractions 1 to 9, fractions 2, 3, 5 and 8 were separated by silica gel



* To whom correspondence should be addressed.

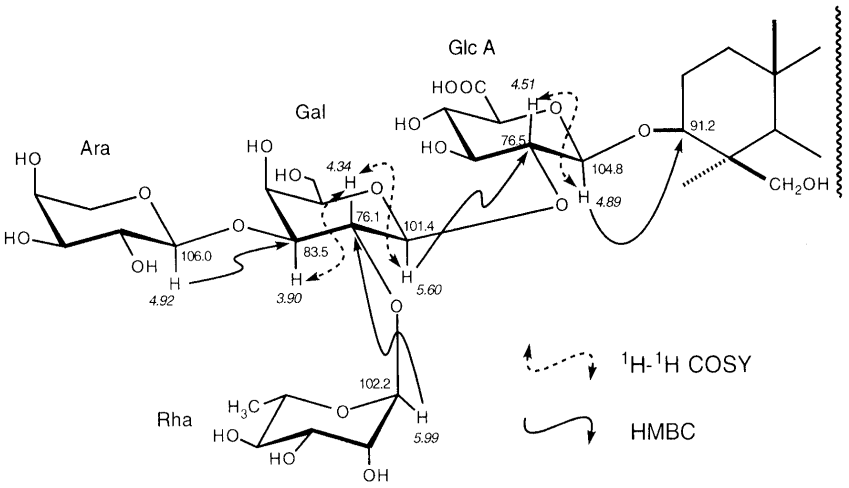


Fig. 1. ^1H–^1H COSY and HMBC Connectivities for Sugar Moiety of **1**

($CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to provide compounds **1** (0.005%), **2** (0.02%), **3** (0.0009%) and **4** (0.0009%), respectively.

Compound 1 (Melilotus-Saponin O_1) A white amorphous powder, $[\alpha]_D^{25}$ +5.7° (*c* = 0.50, MeOH). HR positive ion FAB-MS *m/z*: 1097.5519 ($C_{53}H_{86}NaO_{22}$, Calcd for 1097.5510). Negative ion FAB-MS *m/z*: 1073 $[M-H]^-$, 941 $[M-H-Ara]^-$, 927 $[M-H-Rha]^-$. ^{1}H-NMR (in pyridine-d_5): 0.72, 0.96, 1.01, 1.22, 1.26, 1.26, 1.44 (each 3H, s, *tert*-Me × 7), 1.72 (3H, d, *J* = 4.3 Hz, Rha H_3-6), 4.89 (1H, d, *J* = 5.9 Hz, Glc A H-1), 4.92 (1H, d, *J* = 6.7 Hz, Ara H-1), 5.30 (1H, s, H-12), 5.60 (1H, d, *J* = 7.7 Hz, Gal H-1), 5.99 (1H, s, Rha H-1). ^{13}C-NMR (in pyridine-d_5): 38.6, 26.3, 91.2, 43.7, 56.0, 18.4, 33.0, 39.7, 47.6, 36.3, 23.9, 122.4, 144.5, 42.2, 26.1, 28.7, 37.8, 45.4, 46.6, 30.6, 41.8, 76.1, 22.8, 63.3, 15.7, 16.9, 25.3, 20.7, 32.8, 28.6 (C-1—30), 104.8, 76.5, 73.5, 73.9, 77.9, 175.3 (Glc A C-1—6), 101.4, 76.1, 83.5, 68.7, 76.5, 61.5 (Gal C-1—6), 102.2, 71.8, 72.0, 73.7, 69.0, 18.6 (Rha C-1—6), 106.0, 71.9, 75.9, 70.6, 66.1 (Ara C-1—5).

Characterization of Sapogenol and Sugars for 1 A small amount of **1** was dissolved in 2 N HCl/H_2O (2 ml) and heated at 90 °C for 2 h. After addition of $CHCl_3$, the organic layer was identified to be soyasapogenol B[11] by TLC. *Rf*s: 0.34 [$CHCl_3$–MeOH (19 : 1)], 0.48 [*n*-hexane–acetone (2 : 1)]. The aqueous layer was neutralized with 2 N KOH/H_2O. The sugar mixture was subjected to TLC analysis [TLC, Kieselgel 60 F_{254} (Merck Art 5554), *n*-PrOH : acetone : H_2O = 5 : 3 : 1, *Rf*s: 0.06 (glucuronic acid), 0.44 (galactose), 0.58 (arabinose), 0.79 (rhamnose).

D, L Determination of Sugars of 1 A small amount of **1** 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 N 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 warmed at 60 °C for 2 h. The solvent was evaporated under N_2 stream and dried *in vacuo*. The remaining syrup was trimethylsilylated with trimethylsilylimidazole (0.1 ml) at 60 °C for 1 h. After addition of *n*-hexane and H_2O, the *n*-hexane layer was taken out and checked by GC. The retention times (t_R) of the peaks were 15.9 min (D-glucose), 9.0 min (L-arabinose), 10.8 min (L-rhamnose) and 16.9 min (D-galactose).

Soyasaponin I (2)[6] White amorphous powder, $[\alpha]_D^{25}$ −12.0° [*c* = 0.5, MeOH]. Positive ion FAB-MS *m/z* : 965 $[M+Na]^+$. HPLC, conditions see ref. 6*b*, (t_R: 31.4 min) and TLC, Kieselgel 60 F_{254} (Merck Art 5554), $CHCl_3$–MeOH–H_2O (6 : 4 : 1), *Rf*: 0.48; *n*-BuOH–AcOH–H_2O (4 : 1 : 5, upper), *Rf*: 0.31].

Dehydrosoyasaponin I (3)[7] White amorphous powder, $[\alpha]_D^{25}$ −11.7° [*c* = 0.5, MeOH]. Negative ion FAB-MS *m/z*: 939 $[M-H]^-$. HPLC, conditions see ref. 6*b*, (t_R: 33.5 min) and TLC, Kieselgel 60 F_{254} (Merck Art 5554), $CHCl_3$–MeOH–H_2O (6 : 4 : 1), *Rf*: 0.52; *n*-BuOH–AcOH–H_2O (4 : 1 : 5, upper), *Rf*: 0.30].

Acetyl-Soyasaponin I (4)[8] White amorphous powder, $[\alpha]_D^{25}$ −3.4° [*c* = 0.5, MeOH]. Negative ion FAB-MS *m/z*: 983 $[M-H]^-$. HPLC, conditions see ref. 6*b*, (t_R: 40.1 min) and TLC, Kieselgel 60 F_{254} (Merck Art 5554), $CHCl_3$–MeOH–H_2O (6 : 4 : 1), *Rf*: 0.54; *n*-BuOH–AcOH–H_2O (4 : 1 : 5, upper), *Rf*: 0.30].

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References and Notes

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