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TRITERPENE AND STEROIDAL GLYCOSIDES FROM THE GENUS *Melilotus* AND THEIR GENINS. V. MELILOTOSIDES A_1, B_1, AND C_1 FROM *Melilotus officinalis* ROOTS

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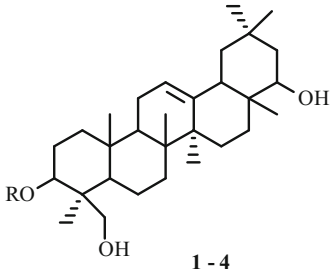
Three new triterpene oleanane glycosides, melilotosides A_1, B_1, and C_1, and non-glycosylated soyasapogenol B were isolated from Melilotus officinalis *(L.) Pall. (Fabaceae) roots. The structures of the glycosides were proved using chemical transformations and spectral data. Melilotoside A_1 is soyasapogenol B 3-O-β-D-xylopyranoside; melilotoside B_1, soyasapogenol B 3-O-[β-D-galactopyranosyl-(1-2)-β-D-xylopyranoside]; melilotoside C_1, soyasapogenol B 3-O-[α-L-rhamnopyranosyl-(1-2)-β-D-galactopyranosyl(1-2)-β-D-xylopyranoside].*

Keywords: *Melilotus officinalis*, Fabaceae, triterpene glycoside, melilotosides A_1, B_1, and C_1.

Our goal was to establish the structures of three new glycosides from *Melilotus officinalis* (L.) Pall. (Fabaceae) roots growing in Crimea (Zuya village). The new triterpene glycosides were called melilotosides A_1 (**1**), B_1 (**2**), and C_1 (**3**).



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1: R = β-D-Xyl*p*→; **2:** R = β-D-Gal*p*→^{2}β-D-Xyl*p*→
3: R = α-L-Rha*p*→^{2}β-D-Gal*p*→^{2}β-D-Xyl*p*→; **4:** R = H

Analysis of the total acid hydrolysis products of each of the studied compounds by PC and TLC (characterized by comparison of their chromatographic mobilities with known standards) showed that the algycon in each of them was soyasapogenol B (**4**) (3β,22β,24-trihydroxyolean-12-ene). The native aglycon was present in the $CHCl_3$ extract from the root extract of the plant. The carbohydrate part of melilotoside A_1 was xylose, the ^{13}C NMR spectrum of which corresponded to a single monosaccharide (one resonance for anomeric C atoms) (Table 1) [1]. A comparison of the chemical shifts of glycoside C atoms with the literature for the unsubstituted aglycon [2, 3] indicated that the sugar was bonded to C-3 of **4** (low-field shift from 80.2 to 91.2 ppm) (Table 1).

The carbohydrate part of melilotoside B1 contained xylose and galactose. The sequence of monosaccharides in the molecule was established by partial acid hydrolysis with chromatographic separation of the hydrolysis products and isolation of the single progenin, which was identified as melilotoside A_1 on the basis of TLC, comparison of physicochemical constants, and total acid hydrolysis. Thus, the carbohydrate component of melilotoside B_1 had a structure with sequentially alternating xylose and galactose.

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TABLE 1. Chemical Shifts of C Atoms of Melilotosides A_1 (**1**) and B_1 (**2**) (C_5D_5N, 0 = TMS, δ, ppm)

C atom	**1**	**2**	C atom	**1**	**2**
1	38.6	38.7	23	23.1	23.5
2	26.6	26.8	24	63.7	63.6
3	91.2	89.2	25	15.9	15.7
4	44.4	44.6	26	17.1	17.3
5	56.1	56.3	27	25.8	25.9
6	19.0	19.0	28	26.6	27.3
7	33.4	33.5	29	33.4	33.5
8	40.0	40.2	30	21.8	21.4
9	47.8	48.0	*β*-D-Xyl*p* unit		
10	36.6	36.8	1	107.4	105.5
11	24.1	24.3	2	75.4	84.1
12	122.5	122.7	3	78.2	78.0
13	144.9	145.1	4	71.1	70.7
14	42.4	42.6	5	66.7	66.7
15	26.6	27.3	*β*-D-Gal*p* unit		
16	28.8	28.7	1′		100.7
17	38.1	38.2	2′		75.7
18	45.4	45.5	3′		72.4
19	46.8	47.0	4′		69.9
20	31.0	31.1	5′		76.6
21	42.4	42.6	6′		62.3
22	75.7	75.8			

GC of the methanolysis products of melilotoside B_1 permethylate, which was prepared by methylation of the starting glycoside by the Hakomori method [4], identified methyl-3,4-di-*O*-methyl-D-galactopyranoside and methyl-2,3,4,6-tetra-*O*-methyl-D-galactopyranoside in a 1:1 ratio. Thus, the structure of the carbohydrate part of melilotoside B_1 was a xylopyranose glycosylated on the second C atom of galactopyranose. This was confirmed by comparing ^{13}C NMR spectra of melilotosides A_1 and B_1, which showed that C-2 of the xylopyranose underwent a paramagnetic shift from 75.4 to 84.1 ppm (Table 1).

Thus, melilotoside A_1 was soyasapogenol B 3-*O*-*β*-D-xylopyranoside; melilotoside B_1, soyasapogenol B 3-*O*-[*β*-D-galactopyranosyl-(1-2)-*β*-D-xylopyranoside].

Melilotoside C_1 contained galactose, rhamnose, and xylose. Its ^{13}C NMR spectrum was consistent with a 1:1:1 ratio of sugars (three resonances for anomeric C atoms) (Table 2) [1].

The bonding sequence of carbohydrate units in **3** was determined by partial acid hydrolysis and chromatographic separation of the two resulting progenins (**3a** and **3b**), which were analyzed by TLC, physicochemical constants, and total acid hydrolysis. As a result, **3a** was identified as melilotoside A_1; **3b**, melilotoside B_1. Thus, the carbohydrate component of melilotoside C_1 had successive placement of xylose, galactose, and rhamnose units. Resonances in the ^{13}C NMR spectrum for xylose, galactose, and rhamnose agreed with the literature for *β*-D-xylopyranose, *β*-D-galactose, and terminal *α*-L-rhamnose (Table 2), respectively [1, 5, 6].

GC of the permethylate products of melilotoside C_1 obtained by methylation of the starting glycoside by the Hakomori method [4] identified methyl-3,4-di-*O*-methyl-D-xylopyranoside, methyl-3,4,6-tri-*O*-methyl-D-galactopyranoside, and methyl-2,3,4-tri-*O*-methyl-L-rhamnopyranoside in a 1:1:1 ratio.

Comparison of chemical shifts of glycoside C atoms in its unsubstituted parts with the literature showed that the carbohydrate component was bonded to C-3 of the aglycon (low-field shift from 80.2 to 91.2 ppm [2]) and C-2 of *β*-D-xylopyranoside and *β*-D-galactopyranoside were substituted (low-field shifts from 75.8 to 84.5 ppm [5] and from 75.4 to 78.5 [1, 6], respectively) (Table 2).

Thus, melilotoside C_1 was soyasapogenol B 3-*O*-[*α*-L-rhamnopyranosyl-(1-2)-*β*-D-galactopyranosyl-(1-2)-*β*-D-xylopyranoside].

TABLE 2. Chemical Shifts of C Atoms of Melilotoside C_1 (**3**) and Its Progenins (**3a** and **3b**) (62.5 MHz, C_5D_5N, 0 = TMS, δ, ppm)

C atom	**3**	**3a**	**3б**	C atom	**3**	**3a**	**3б**
1	38.6	38.4	38.7	26	17.1	17.4	17.3
2	26.6	26.5	26.8	27	25.8	25.8	25.9
3	91.2	91.0	89.2	28	28.8	28.9	28.7
4	44.4	44.2	44.6	29	33.4	33.4	33.5
5	56.1	56.3	56.3	30	21.3	21.7	21.4
6	19.0	19.1	19.0	*β*-D-Xyl*p* unit			
7	33.4	33.6	33.5	1	105.4	107.4	106.4
8	40.0	40.3	40.2	2	84.5	75.8	84.1
9	47.8	47.9	48.0	3	78.1	78.4	77.8
10	36.6	36.5	36.8	4	71.1	71.1	70.0
11	24.3	24.1	24.2	5	66.7	66.6	66.6
12	122.5	122.6	122.7	*β*-D-Gal*p* unit			
13	144.9	144.8	145.1	1′	100.9		101.2
14	42.2	42.7	42.1	2′	78.5		75.4
15	26.6	26.5	26.3	3′	75.7		72.9
16	28.8	28.8	28.7	4′	71.2		70.1
17	38.1	38.4	38.2	5′	76.5		76.5
18	45.4	45.2	45.5	6′	61.7		62.5
19	46.8	46.7	47.0	*α*-L-Rha*p* unit			
20	31.0	31.0	31.1	1″	101.8		
21	42.4	42.7	42.6	2″	75.2		
22	75.7	75.4	75.8	3″	72.2		
23	23.1	23.8	23.5	4″	74.5		
24	63.7	63.9	63.6	5″	69.5		
25	15.9	15.9	15.7	6″	18.6		

EXPERIMENTAL

General Comments. We used solvent systems $CHCl_3$:MeOH (4:1, 1; 7:3, 2; 9:1, 4), $CHCl_3$:MeOH:H_2O (65:35:7, 3), benzene:acetone (10:1, 5), and BuOH:benzene:Py:H_2O (5:1:3:3, 6). TLC, CC, and PC conditions and spectral analyses have been published [7]; GC, [8].

Preparation of Glycosides. Ground air-dried *M. officinalis* roots collected during flowering were extracted with aqueous EtOH (70%). The extract was evaporated to one third the volume and extracted successively with $CHCl_3$ and BuOH. The BuOH extract was evaporated to afford total extracted compounds (100 g). Chromatography using systems 1-3 successively produced fractions enriched in **1**, **2**, and **3**.

Melilotosides A_1, B_1, and C_1 (1, 2, 3). Fractions enriched in glycosides were evaporated to dryness, dissolved in aqueous KOH (5%), refluxed on a water bath for 2 h, diluted with water to 100 mL, and extracted with BuOH. The BuOH was evaporated to dryness. The solid was chromatographed over SiO_2 with elution by systems 1–3 for **1**, **2**, and **3**, respectively. This afforded **1** (85 mg, 0.001% of air-dried raw material weight), $C_{36}H_{60}O_8$, mp (dec.) 215–219°C (system 1). IR spectrum (KBr, ν, cm^{-1}): 3200–3600 (OH), 1630 (C=C), **2** (200 mg, 0.004%), $C_{41}H_{68}O_{12}$, mp (dec.) 221–224°C (system 2). IR spectrum (KBr, ν, cm^{-1}): 3300–3600 (OH), 1630 (C=C); and **3** (0.54 g, 0.011%), $C_{47}H_{78}O_{16}$, mp (dec.) 239–240°C (system 3). IR spectrum (KBr, ν, cm^{-1}): 3580–3200 (OH), 1630 (C=C).

Total Acid Hydrolysis of 1–3. Glycosides **1–3** (40 mg, 50, 100 mg) were dissolved in aqueous H_2SO_4 (10 mL, 5%), refluxed on a sand bath for 24 h, diluted with water, and extracted multiple times with $CHCl_3$. The combined extracts were washed with water until neutral, evaporated to dryness, and chromatographed over silica gel using system 4 to afford the genin of **1** (5 mg); **2** (7 mg); and **3** (10 mg); which were identified as soyasapogenol B according to melting points and TLC behavior.

The hydrolysate was neutralized with $BaCO_3$ and evaporated to dryness with addition of a minimal amount of MeOH. PC of the solid using system 6 identified xylose in **1**; xylose and galactose in **2**; and xylose, galactose, and rhamnose in **3**.

Partial Acid Hydrolysis of 2 and 3. Glycoside (100 mg) was dissolved in aqueous H_2SO_4 (15 mL, 2.5%), refluxed on a sand bath for 4 h, diluted with water, and extracted multiple times with water-saturated BuOH. The combined BuOH extracts were washed until neutral, evaporated to dryness, and chromatographed successively using systems 4, 1, and 2 to isolate from **2** progenin **2a** (35 mg) and from **3**, **3a** (20 mg), mp (dec.) 214–220°C (system 1), and progenin **3b** (27 mg), mp (dec.) 218–223°C (system 2).

Total Acid Hydrolysis of Progenins 2a, 3a, and 3b. Progenins (5 mg each) were dissolved in aqueous H_2SO_4 (5 mL, 5%), refluxed on a sand bath for 24 h, neutralized with $BaCO_3$, filtered, and evaporated to dryness with addition of the minimal amount of MeOH. PC of the solid using system 6 identified xylose in **2a** and **3a** and xylose and galactose in **3b**.

Permethylates of 2 and 3. The melilotoside (50 mg) in anhydrous DMSO (10 mL) was stirred, treated in small portions with NaH (50 mg), treated dropwise with methyliodide (2 mL), stirred another 5 h, poured into aqueous sodium hyposulfite solution (50 mL, 2%), and extracted with $CHCl_3$. The usual work up of the $CHCl_3$ extract and evaporation of solvent gave a solid that was chromatographed over a column with elution by system 5. The isolated product underwent methanolysis.

GC of the methanolysis products detected in **2** methyl-3,4-di-*O*-methyl-D-xylopyranoside and methyl-2,3,4,6-tetra-*O*-methyl-D-galactopyranoside in a 0.96:1.04 ratio; in **3**, methyl-3,4-di-*O*-methyl-D-xylopyranoside, methyl-3,4,6-tri-*O*-methyl-D-galactopyranoside, and methyl-2,3,4-tri-*O*-methyl-L-rhamnopyranoside in a 0.97:1.05:1.02 ratio.

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