Chemistry of Natural Compounds, Vol. 30, No. 6, 1994

TRITERPENE AND STEROID GLYCOSIDES OF THE GENUS *Melilotus* AND THEIR GENINS

III. FUNKIOSIDE B AND PROTODIOSCIN FROM THE SEEDS OF *Melilotus tauricus*

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UDC 547.918:582.738

Two steroid glycosides belonging to the furostan series — funkioside B and protodioscin — have been isolated from the seeds of the plant Melilotus tauricus *(Bieb.).*

A qualitative analysis of glycosides of Crimean species of the *Melilotus* genus that we have performed [1, 2] has shown the presence in a methanolic extract of the seeds of Crimean sweet clover (*Melilotus tauricus* (Bieb.) Ser.) growing on the southern coast of the Crimea (in the region of the village of Partenit) of four substances assigned on the basis of qualitative reactions to the steroid glycosides of the spirostan and furostan series.

The present paper is devoted to a proof of the structures of two steroid glycosides of the furostan series.

As a result of the rechromatography of the total extractive substances under study, we isolated fractions enriched with compounds (**1**) and (**2**). To free them further from phenolic compounds, the latter were converted into phenolates and eliminated.

In TLC, the substances studied were revealed by the Ehrlich reagent [3] in the form of bright red spots. Their IR spectra had a weak broad band at 910 cm^{-1}. These results permitted their assignment to steroid glycosides of the furostan series.

On analyzing the products of complete acid hydrolysis of the glycosides with respect to their physicochemical constants, and also by TLC and PC, diosgenin and *D*-glucose were identified in the case of compound (**1**), and a comparison of physicochemical constants and chromatographic mobilities with those of specimens of known structure enabled it to be identified as funkioside B ((25R)-furost-5-ene-3β,22α,26-triol 26-O-α-*D*-glucopyranoside) [4].

In the case of compound (**2**), diosgenin, glucose, and rhamnose were identified. The ^{13}C NMR spectrum corresponded to a composition with a 2:2 ratio of the sugar residues (four signals in the region of anomeric carbon atoms [5]).

In the products of the methanolysis of the permethylate of (**2**), obtained by the Hakomori methylation of the glycoside, methyl 2,3,4,6-tetra-O-methyl-*D*-glucopyranoside, methyl 3,6-di-O-methyl-*D*-glucopyranoside, and methyl 2,3,4-tri-O-methyl-*L*-rhamnopyranoside were identified by GLC in a ratio of 0.95:1.07:1.89.

A comparison of the spectral results of the ^{13}C NMR APT (attached proton test) with those given in the literature [7] confirmed the structure of the aglycon component as (25R)-furostene-3β,22α,26-triol and showed substitution at C-3 (downfield shift from 75.3 to 78.2 ppm) and at C-26 (signal of a methylene carbon atom at 75.2 ppm).

The configurations of the glycosidic centers and the dimensions of the oxide rings were determiend and the position of attachment of the monosaccharides in the molecule was confirmed by the PMR method, using double homonuclear resonance, the observation of nuclear Overhauser effects (in the ordinary and the difference variants), and homonuclear (COSY) two-dimensional NMR spectroscopy, which permited an unambiguous interpretation of the signals in the PMR spectrum of the sugar components, and by the ^{13}C NMR spectrum of the glycoside and a comparison of the chemical shifts of the carbon atoms with literature figures for structural analogues.

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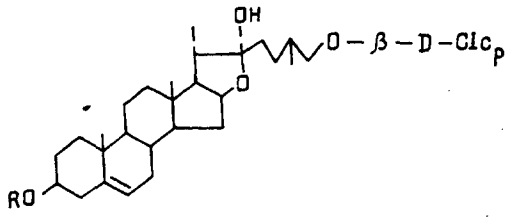
0009-3130/94/3006-0713$12.50

TABLE 1. Chemical Shifts of the Carbon Atoms (ppm, 0 — TMS, C_5D_5N) of Protodioscin (**2**)

C-Atom	δ, ppm	C-Atom	δ, ppm	C-Atom	δ, ppm
		Aglycon			
1	37.7	11	21.3	21	16.3
2	30.0	12	41.0	22	110.0
3	78.2	13	40.2	23	37.3
4	39.2	14	[illegible]7	24	28.5
5	143.5	15	32.5	25	34.4
6	121.9	16	81.3	26	75.2
7	31.9	17	64.0	27	17.5
8	35.9	18	16.5		
9	50.7	19	19.5		
10	37.2	20	40.6		
		Sugars			
	D-Glucose		*L*-Rhamnose		
1	100.5	1	103.0	1'	102.0
2	79.8	2	72.2	2'	72.4
3	78.0	3	72.9	3'	72.7
4	78.2	4	73.9	4'	73.6
5	76.8	5	69.5	5'	70.7
6	61.8	6	18.4	6'	18.6
1'	104.8				
2'	75.2				
3'	78.6				
4'	72.1				
5'	78.6				
6'	63.1				

The spin-spin coupling constants and characteristic splittings of the signals of the skeletal protons of the sugar residues showed two gluco- and two rhamno- configurations of monosaccharides in the pyranose forms, while the SSCCs corresponded to the β-configurations of the two glucose residues (7.5 Hz) (Table 2), and a comparative analysis of the chemical shifts in the ^{13}C NMR spectrum of both rhamnose residues with literature figures for terminal α-*L*-rhamnopyranoses unambiguously showed the α-configuration of their glycosidic centers (Table 1) [5].

Spatial propinquity was shown for H-1 of a β-*D*-glucopyranose and H-3 of the aglycon, for H-1 of one α-L-rhamnopyranose and H-2 of the same *D*-glucopyranose, and for H-1 of the other α-*L*-rhamnopyranose and H-4 of the same β-*D*-glucopyranose.



1. R = H 2. R = α-L-Rha p → (4) β-D-Glc p → (2) α-L-Rha p

TABLE 2. Chemical Shifts (ppm, 0 — TMS, C_5D_5N) and Spin-Spin Coupling Constants (J, Hz) of the Protons of Protodioscin (2)

Protons of aglycon and sugar	δ, ppm, J, Hz	Sugar Protons	δ, ppm, J, Hz
CH_3-18	0.88 s		D-galactose
CH_3-19	1.03 s	1	4.85 d $J_{1,2}$=7.5
H-3	3.70 s	2	4.05 t $J_{2,3}$=7.5
	L-Rhamnose	3	3.82 dd
1'''	6.14 s	4	4.17 dd $J_{4,5}$=9.0
2'''	4.65 d $J_{2,3}$=2.0	5	3.59 m
3'''	4.47 dd $J_{3,4}$=9.5	6a	4.01 dd $J_{6a,5}$=5.5
4'''	4.17 d $J_{4,5}$=9.5	6e	3.89 dd $J_{6e,5}$=2.5
5'''	4.63 dq $J_{5,6}$=6.5		$J_{6a,6e}$=11.5
6'''	1.52 d		
1''''	5.62 s	1''''	4.71 d $J_{1,2}$=7.5
2''''	4.54 d $J_{2,3}$=2.5	2''''	3.89 dd $J_{2,3}$=8.0
3''''	4.38 dd $J_{3,4}$=9.5	3''''	4.11 dd
4''''	4.20 dd $J_{4,5}$=9.0	4''''	4.08 dd
5''''	4.79 m	5''''	3.82 m $J_{5,6a}$=5.5
6''''	1.67 d $J_{5,6}$=6.5		$J_{5,6e}$=2.5
		6a''''	4.43 dd $J_{6a,6e}$=11.0
		6e''''	4.26 dd

Thus, glycoside (2) was identified as protodioscin ((25R)-furost-5-ene-3β,22β,26-triol 3-O-{[O-α-*L*-rhamnopyranosyl-(1→2)]-[O-α-*L*-pyranosyl-(1→4)]-β-*D*-glucopyranoside}.

EXPERIMENTAL

General Observations. The following solvent systems were used: 1) chloroform—methanol (8:2); 2) chloroform—methanol (7:3); 3) chloroform—methanol (20:1); 4) benzene—acetone (10:1); and 5) butanol—benzene—pyridine—water (5:1:3:3).

The conditions for TLC, CC, and PC and for spectral analysis are given in [1], and those for GLC in [9].

Isolation of the Glycosides. The total extractive substances obtained by standard methods [1] from 800 g of cleaned and comminuted *M. tauricus* seeds were chromatographed successively in systems 1 and 2, with the isolation of fractions enriched with substances (1) and (2). The fractions were evaporated to dry residues, solutions of which in 5% aqueous KOH were boiled for 2 h. After this the volume of each reaction mixture was made up to 100 ml and it was repeatedly extracted with butanol. The combined butanolic extract was evaporated to dryness and chromatographed with elution by the appropriate solvent system (1 for the fraction with substance (1), and 2 for the fraction with substance (2)). In this way we obtained 24 mg of funkioside B, $C_{33}H_{54}O_9$ (0.003% on the weight of the air-dry raw material), and 150 mg of protodioscin, $C_{51}H_{84}O_{22}$ (0.019% on the weight of the air-dry raw material). The IR spectra of each glycoside contained an absorption band at 910 cm^{-1}.

Complete Acid Hydrolysis of (1) and (2). Solutions of compounds (1) (20 mg) and (2) (80 mg) in 5 and 10 ml, respectively, of 2.5% sulfuric acid were boiled on a sand bath under reflux for 3 and 6 h, respectively. Then the reaction mixtures were diluted with water to 20 and 50 ml, after which they were extracted repeatedly with diethyl ether, and the extracts were washed with water to neutrality. The extract of the hydrolysate from compound (1) was evaporated to minimum

volume, and diosgenin was identified by TLC. The extract from the hydrolysate of compound (2) was evaporated to a dry residue, and this was chromatographed on silica gel in system 3 to give 23 mg of a product with mp 200-202°C, also identified as diosgenin.

The hydrolysates were neutralizd with barium carbonate and evaporated to dryness and the residues were treated with the minimum volume of methanol, after which PC in system 5 revealed glucose in the case of glycoside (**1**), and glucose and rhamnose in the case of glycoside (**2**).

Permethylate of (2). With constant stirring, 35 mg of sodium hydide was added to a solution of 30 mg of glycoside (**2**) in 5 ml of absolutely dry dimethyl sulfoxide, and the reaction mixture was left for 1 h. Then 2 ml of methyl iodide was added dropwise, and stirring was continued for 5 h. The reaction mixture was poured into 25 ml of 2% aqueous sodium thiosulfate and was extracted repeatedly with chloroform, after which the extract was washed, evaporated to dryness, and chromatographed on a column, with elution by system 4. The product isolated was subjected hydrolysis with 2.5% methanolic sulfuric acid.

Methyl 2,3,4,6-tetra-O-methyl-*D*-glucopyranoside, methyl 3,6-di-O-methyl-*D*-glucopyranoside, and methyl 2,3,4-tri-O-methyl-*L*-rhamnopyranoside were identified in the methanolysis products by GLC in a ratio of 0.95:1.07:1.89.

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