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

IV. TRILLIN AND DIOSCIN FROM THE SEEDS OF *Melilotus tauricus*

G. V. Khodakov,[a] Yu. A. Akimov,[a] and P. K. Kintya[b]

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Two steroid glycosides belonging to the spirostan series — trillin and dioscin — have been isolated from the seeds of the plant Melilotus tauricus *(Bieb.) Ser.*

In this paper we present the results of a study of steroid glycosides of the spirostan series detected by us previously in the seeds of Crimean sweet clover *Melilotus tauricus* (Bieb.) Ser. [1].

The glycosides were isolated by rechromatography of the total extract, with the production of fractions enriched with the desired compounds but contaminated with phenolic compounds, which it was possible subsequently to eliminate in the form of phenolates.

On TLC the compounds under consideration were revealed by vanillin/phosphoric acid in the form of yellow spots [2] and were not revealed by the Ehrlich reagent, which permitted them to be assigned to steroid glycosides of the spirostan series. We may consider as confirmation of this the formation of diosgenin ((25R)-spirost-5-en-3β-ol on the acid hydrolysis of the substances, and also absorption bands in the IR spectra at 900 and 920 cm^{-1} that are characteristic for steroid glycosides of the R-series.

Glucose was detected by PC in carbohydrate fractions of acid hydrolysates of compound (**1**), and TLC analysis of the progenin with an authentic marker permitted the identification of trillin (diosgenin 3-O-α-*D*-glucopyranoside) [3].

In a hydrolysate of compound (**2**) glucose and rhamnose were detected by PC and GLC in a ratio of 1:2. This result was in full agreement with ^{13}C NMR spectra, in which the signals of three anomeric carbon atoms were clearly traced at 100.8, 103.5, and 102.4 ppm (Table 1). TLC examination of the products of partial hydrolysis of the glycoside Mt-d [*sic*] permiited one of the progenins to identified as trillin.

Compound (**2**) was methylated by Hakomori's method [4]. The exhaustively methylated product, isolated by column chromatography, was subjected to methanolysis, with the subsequent GLC analysis of the components obtained, and methyl 3,6-di-O-methyl-*D*-glucopyranoside and methyl 2,3,4-tri-O-methyl-*L*-rhamnopyranoside were detected in a ratio of 0.95:2.07. Consequently, glycoside Mt-d was a monodesmoside with the structure shown in the scheme. This was confirmed by a comparison of the ^{13}C NMR spectrum with literature figures [5]; in particular, the C-3 atom of the aglycon component and C-2 and C-4 of the *D*-glucose residue had experienced a glycosylation effect.

The chemical shifts of the anomeric carbon atoms of glycoside (**2**), correlated with the literature [5], showed the α-configurations of the glycosidic centers of both rhamnoses and the β-configuration of the glucose.

Thus, glycoside (**2**) has been identified as dioscin (diosgenin (3-O-{[O-α-*L*-rhamnopyranosyl-(1→2)]-[O-α-*L*-rhamnopyranosyl-(1→4)]-β-*D*-glucopyranoside}.

a) State Nikitskii Botanical Garden, 334267, Yalta, Botanicheskoe Production Combine; b) Institute of Genetics, Academy of Sciences of the Republic of Moldava, 277018, Kishinev, ul. Lesnaya, 20. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 770-772, November-December, 1994. Original article submitted March 14, 1994.

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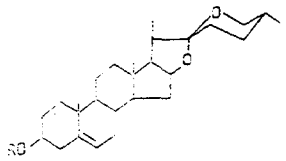
TABLE 1. Chemical Shifts of the Carbon Atoms (ppm, 0 — TMS, C_5D_5N) of Dioscin (**2**)

C-Atom	δ	C-Atom	δ	C-Atom	δ
		Aglycon			
1	37.3	11	20.8	21	16.5
2	30.4	12	40.4	22	110.9
3	78.4	13	40.6	23	37.4
4	39.0	14	56.8	24	28.8
5	141.0	15	32.0	25	34.6
6	121.7	16	81.5	26	75.8
7	31.8	17	64.3	27	17.9
8	31.5	18	16.7		
9	50.4	19	19.6		
10	37.0	20	40.9		
		Sugars			
D-Glucose		*L*-Rhamnose		*L*-Rhamnose	
1'	100.8	1'	103.5	1'	102.4
2'	79.0	2'	72.8	2'	72.4
3'	77.9	3'	72.1	3'	72.5
4'	78.0	4'	73.7	4'	73.8
5'	77.1	5'	69.2	5'	70.5
6'	61.5	6'	18.5	6'	18.7



$R = \alpha\text{-L-Rha}_p \rightarrow \beta\text{-D-Glc}_p \rightarrow 2\text{-L-Rha}_p$

EXPERIMENTAL

General observations are given in [1]. The following solvent sysyems were used: 1) chloroform—methanol (8:2); 2) chloroform—methanol (7:3); 3) chloroform—methanol (20:1); 4) benzene—acetone (10:1); and 5) benzene—pyridine—water (5:1:3:3).

Isolation of the Glycosides. Chromatography of the total extractive substances from the seeds of *M. tauricus*, with systems 1 and 2 successively, permitted fractions enriched with substances (**1**) and (**2**) to be obained. The fractions were evaporated to dry residues, and solutions of these in 5% aqueous KOH were boiled for 1.5 h. After this, the volume of each of the reaction mixtures was made up to 50 ml, and it was repeatedly extracted with butanol. The combined butanolic extract was concentrated to 1/4 volume and left to crystallize. This gave 21 mg of trillin $C_{33}H_{52}O_8$ (0.0029% on the weight of the air-dry raw material) and 58 mg of dioscin $C_{45}H_{72}O_{16}$ (0.0072% on the weight of the air-dry raw material).

The complete acid hydrolysis of (1) and (2) was carried out with 2.5% sulfuric acid for 5 h. TLC analysis in chloroform of the hydrolysate extract allowed the identification of diosgenin. In subsequent routine hydrolyzation by the BK method we detected: in compound 1 — glucose, in compound 2 — glucose and rhamnose.

Partial acid hydrolysis of (2) was conducted in a 0.5% solution of sulfuric acid in methanol for 2.5 h. The reaction mixture was diluted with water, and the methanol was evaporated off. The aqueous residue was extracted with butanol. The extract was washed to neutrality and evaporated to a dry residue, which was dissolved in the minimum amount of ethanol. TLC monitoring enabled one of the progenins to be identified as diosgenin 3-O-β-*D*-glucopyranoside.

The Permethylate of (2). With constant stirring, 35 mg of sodium hydride was added in small portions to a solution of 20 mg of glycoside (**2**) in 5 ml of absolutely dry dimethyl sulfoxide, and the 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 repeatedly extracted with chloroform, after which the chlororofm extract was washed, evaporated to dryness and subjected to column chromatography with elution by system 4. The product isolated was hydrolyzed with 2.5% methanolic sulfuric acid.

Among the hydrolysis products, methyl 3,6-di-O-methyl-*D*-glucopyranoside and methyl 2,3,4-tri-O-methyl-*L*-rhamnopyranoside were identified by TLC in a ratio of 0.95:2.07.

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