New triterpenoid glycosides from *Thalictrum minus* L.
11.* Structure of thalicoside H_1

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From the terrestrial part of *Thalictrum minus* L. (Ranunculaceae) a novel triterpenoid diglycoside was isolated. The genin of this glycoside is a new cycloartane triterpenoid. The structure of the glycoside was established on the basis of 1D and 2D NMR spectroscopy and FAB mass spectrometry as 22*S*,25-epoxy-3-*O*-β-D-galactopyranosyl-29-*O*-β-D-glucopyranosyl-9β,19-cyclo-20*S*-lanostane-3β,16β,24*S*,29-tetrol.

Key words: *Thalictrum*, Ranunculaceae, triterpenoid glycosides, cycloartane; 1D and 2D NMR spectroscopy, FAB mass spectrometry.

We have recently reported[1] the isolation and determination of the structure of two isomeric triterpenoid saponins from the Siberian chemorace of *Thalictrum minus* L. This paper is devoted to determination of the structure of a new minor saponin, thalicoside H_1 (**1**), by NMR spectroscopy and fast-atom bombardment (FAB) mass spectrometry.

Results and Discussion

A high-resolution FAB mass spectum of thalicoside H_1 exhibited a signal of the cluster molecular ion with *m/z* 837.4612 $[C_{42}H_{70}O_{15}Na]^+$. The ^1H and ^{13}C NMR spectra showed downfield signals at δH 5.16 and 5.29 ppm and δC 105.0 and 106.1 ppm. These signals were assigned to anomeric H and C atoms of the two monosacharide residues of **1**. The chemical shifts (CSs) of the carbohydrate units in the ^{13}C NMR spectrum of **1** (Table 1) are close to those of terminal β-D-galactopyranoside and β-D-glucopyranoside residues.[2]

The ^1H NMR spectrum of glycoside **1** contains an AB-system of two one-proton doublets at δ 0.32 and 0.58 ppm, J = 3.8 Hz, that proves the presence of a 9,19-cyclopropane ring in the molecule. Compound **1** also contains six methyl groups, as follows from the presence of five singlets at δ 0.91, 0.97, 1.30, 1.42, and 1.52 ppm and one doublet at δ 1.09 ppm in the ^1H NMR spectrum of **1** (Table 1). All these data give evidence that compound **1** is a 9,19-cyclolanostane triterpenoid derivative.

According to the molecular formula of glycoside **1** it contains 15 oxygen atoms including 5 oxygen atoms in the genin residue. Since the ^{13}C NMR spectrum of thalicoside **1** shows six signals characteristic of oxygen-bound carbon atoms at δ 71.1, 71.6, 76.6, 79.7, 81.7, and 82.8 ppm, an oxygen-containing (epoxide) cycle is present in compound **1**.

The comparison of the CS of the quaternary carbon atom at δ 44.9 ppm in the ^{13}C NMR spectrum of compound **1** with the literature data[3] (C(4), δ 41.4 ppm) points to the presence of an oxymethyl fragment at C(4). The CS of the carbon atom of the CH_2OR fragment at δ 71.1 ppm indicates its α-orientation and glycosylation at this position.[3] The assignment of two H(29) protons at δ 4.18 and 4.40 ppm in the ^1H NMR spectrum (Table 1) was made using data of the HETCOR spectrum.

The signals of the remaining carbon atoms in the ^{13}C NMR spectrum of compound **1** were interpreted on the basis of the results of an off-resonance decoupling experiment (Table 1) in comparison with literature data.[4]

After identifying fragments of the spin systems of the A, B, and C rings (DQF-COSY, TOCSY) we assigned signals of protons of the polycycle residue of the genin part of **1** (Table 1). The CSs of the H(3) (δ 4.46 ppm) and H(16) (δ 4.87 ppm) atoms indicate the presence of hydroxyl groups at these positions. We determined, with the help of the HETCOR spectrum, that the signals at δ 81.7 and 71.7 ppm in the ^{13}C NMR spectrum of compound **1** corresponded to oxygen-bound C(3) and C(16)

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Table 1. NMR data for compound **1** obtained from 1D and 2D experiments (C_5D_5N, δ Me_4Si = 0, ^1H (δ±0.01, ppm; 80 °C) and ^{13}C (δ±0.1, ppm; 26 °C))

Atom	^{13}C NMR, δ	^1H NMR, δ (*J*/Hz)	Atom	^{13}C NMR, δ	^1H NMR, δ (*J*/Hz)
1	32.1 t	1.14 m (α), 1.36 m (β)	24	77.6 t	4.31 m
2	29.4 t	2.02 m (α), 2.42 m (β)	25	82.8 s	—
3	81.7 d	4.46 m	26	26.3 q	1.30 s
4	44.9 s	—	27	23.4 q	1.52 s
5	40.7 d	2.06 m	28	19.4 q	0.97 s
6	20.7 t	0.77 m (α), 1.90 m (β)	29	71.1 t	4.18 m,* 4.40 m*
7	26.5 t	1.10 m (α), 1.67 m (β)	30	11.7 q	0.91 s*
8	48.3 d	1.94 m		3-*O*-β-D-Galp	
9	19.7 s	—	1	106.1 d	5.29 d (7.5), 5.56
10	25.9 s	—	2	73.3 d	4.51 m*
11	26.3 t	not identified	3	75.5 d	4.28 m*
12	33.6 t	not identified	4	70.4 d	4.60 m*
13	46.1 s	—	5	76.2 d	4.38 m*
14	47.0 s	—	6	63.0 t	4.46 m*
15	47.9 t	1.71 m (α), 2.07 m (β)			H(6′) not identified
16	71.7 d	4.87 m		29-*O*-β-D-Glcp	
17	52.5 d	2.18 m	1	105.0 d	5.16 d (7.5), 5.40
18	20.5 q	1.42 s	2	75.3 d	4.19 m*
19	30.4 t	0.32, 0.58 d (3.8)	3	78.6 d	4.31 m*
20	32.7 d	2.61 m	4	72.0 d	4.32 m*
21	15.4 q	1.09 d (6.9)	5	78.0 d	4.05 m*
22	79.7 d	4.26 m	6	62.4 t	4.43 m*
23	36.7 d	1.98 m, 2.36 m			H(6′) not identified

* Data of the HETCOR spectrum (23 °C).

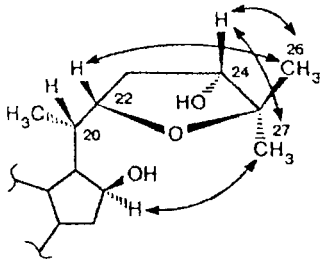
atoms respectively. According to the literature data[5] the signal of C(3) of 3β-OH-substituted triterpenoids appears at 75 ppm, and the effect of glycosylation at this position reaches 5—7 ppm. The downfield position of the C(3) signal is therefore a result of attachment of a carbohydrate chain at this atom of the genin. The relative configuration of the C(16)OH group was determined from the ^{13}C NMR spectrum, where the values of the β-, γ-, and σ-effects of the C(16)OH group indicated its β-orientation.[5]

The points of carbohydrate-genin joining followed from the interactions of C(3) (δC 81.7 ppm) and C(29) (δC 71.1 ppm) atoms with the anomeric protons of galactose and glucose, respectively, in the HMBC spectrum. Galactose is therefore at C(3), and glucose is at the C(29) position of the genin.

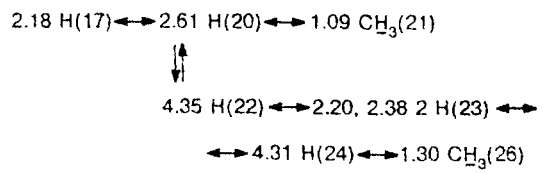
It has become evident after determining the structure of the polycyclic fragment of the molecule, as well as the quantitative and qualitative compositions of carbohydrates, that a side fragment (SF) of compound **1** contains two oxygen atoms connected with three carbon atoms with CSs of δ 77.6, 79.7, and 82.8 ppm in the ^{13}C NMR spectrum. The epoxide ring is therefore present in the SF.

The following proton-proton interactions were determined in the SF of glycoside **1** by 2D NMR spectroscopy (COSY, TOCSY, ROESY) using the CS of the H(17) atom (δ 2.18 ppm) as a reference point (see Scheme 1).

Scheme 1



The proton (δH 4.26 ppm) at the oxygen-bound carbon atom in the SF of compound **1** has a cross-peak with H(20) (δH 2.61 ppm) in the COSY spectrum. Its position could therefore be identified as H(22). The signal of H(22) also has cross-peaks with the signals of two protons at δ 2.20 and 2.38 ppm, which were identified as two H(23) protons. So, it is evident that a

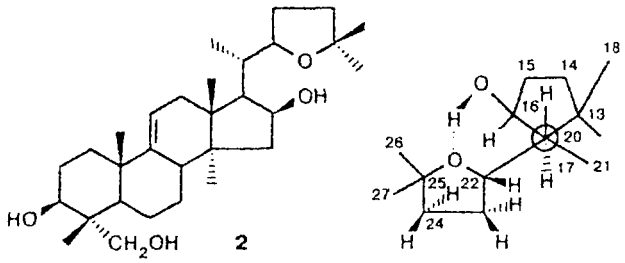


methylene group $C(23)H_2$ is present at the α-position relative to C(22). The protons of the $C(23)H_2$ group in turn interact with the proton of the CHOH-group (δ 4.31 ppm); hence the position of the secondary hydroxyl group in the SF was identified as C(24). The proton at C(24) interacts with two geminal methyl groups at C(25) (quaternary carbon atom, off-resonance).

Thus, the SF of thalicoside H_1 is a tetrahydrofuran 22,25-epoxide cycle, containing two *gem*-methyl groups at C(25) and a secondary hydroxyl group at C(24).

The relative configuration of substituents in the SF was determined as described below.

Earlier we have determined the structure of the artifact of thalicoside A (**2**), which was confirmed by X-ray crystallographic data.[6]

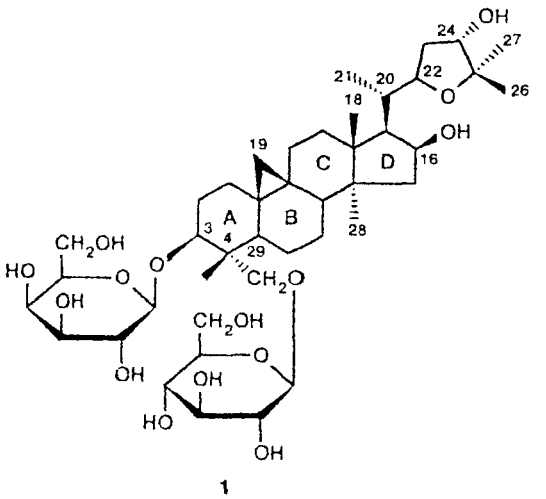


The structures of the D and SF rings in compounds **1** and **2** are similar and differ only by the presence of the C(24)OH group in compound **1**. The similar CSs of C(16) atoms in the ^{13}C NMR spectra of compounds **1** and **2** [7] could indicate the presence of an intramolecular hydrogen bond in compound **1** and, as a result, the stabilization of a conformer with sterically close C(16)OH group and the oxygen atom of the tetrahydrofurane ring. It was demonstrated with the help of Dreiding molecular models that H(16α) could give a cross-peak with one of the methyl groups (δ 1.52 ppm) observed in the ROESY spectrum in the case of *S*-configuration of C(22) only. Use of Dreiding molecular models as well as data of ROESY spectrum showed that the signal of H(22) has a cross-peak with the signal of the methyl group at 1.30 ppm, *i.e.*, with $C(26)H_3$ methyl group. Hence, the signal at 1.52 ppm should be assigned to $C(27)H_3$ methyl group. The signals of CH_3-groups in ^{13}C NMR spectrum (see Table 1) were identified by use of 2D NMR HMBC and HETCOR techniques.

The comparison of CSs of C(26) and C(27) methyl groups in the ^{13}C NMR spectrum of the compound **1** with those of the compound **2** indicates the presence of γ-effect of C(24)OH group on the methyl groups. It is known, that there are two types of γ-effects of OH groups: γ-*gauche*-effects and γ-*trans*-effects, the latter being predominantly shielding[5]. γ-*trans*-Effects are usually low (from 1.3 to −3.0 ppm)[5], whereas the values of γ-*gauche*-effects could reach−5.9 ppm.[8]

As follows from the observed value of γ-*trans*-effect of $CH_3(26)$ (27.9 − 26.3 = 1.6 ppm) and γ-*gauche*-effect of $CH_3(27)$ (28.9 − 23.4 = 5.5 ppm) of the compound **1**, the C(24)OH group exerts a stronger shielding effect on the C(27) group. The C(24)OH group should therefore have a *sin*-periplanar orientation relative to $C(27)H_3$ group. Such sterical orientation of C(24)OH group corresponds to *S*-configuration of C(24) chiral atom.

Thus, for thalicoside H_1 we propose the structure of 22*S*,25-epoxy-3-*O*-β-D-galactopyranosyl-29-*O*-β-D-glucopyranosyl-9β,19-cyclo-20*S*-lanostan-3β,16β,24*S*,29-tetrol (**1**).



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Experimental

Melting points were determined on a Kofler hot-plate apparatus. Optical rotations were measured with a POLAMAT A polarimeter. IR spectra were recorded with an IFS25 Fourier spectrometer. The isolation of the saponin fraction and parameters of registration of NMR and mass spectra were described in the reference[1].

Isolation of compound 1. A fraction of saponins (0.110 g) containing isomeric thalicosides of G and H groups was subjected to HPLC on Silasorb 12—C18 (10×250 mm) using a Yanako-2000L instrument with refractometeric detector. Elution with MeOH—H_2O = 55 : 45, 3 mL min^{-1} afforded compound **1** (0.0127 g).

The thalicoside H_1 (**1**), $C_{42}H_{70}O_{15}$, colorless crystals, m.p. 260—262 °C (pyridine), $[\alpha]^{17}_{546}$ +10.3° (*c* 0.34, Py). IR (KBr), ν/cm^{-1}: 3408 (OH), 3050 (9,19-cyclopropane ring), 1069 (C—O—). FAB MS, *m/z* (I_{rel} (%)): 837 $[M + Na]^+$ (5). The NMR data are given in Table 1.

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