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TRITERPENE SAPONINS FROM *Thalictrum minus*. VII. STRUCTURE OF THALICOSIDE E

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A new minor cycloartane glycoside — thalicoside E, 9,19-cyclo-20(S)-lanost-23-ene-3β,16β,22ξ,25,29-pentaol 3-O-β-D-galactoside 29-O-β-D-glucopyranoside — has been isolated from the epigeal part of Thalictrum minus *L. (Ranunculaceae).*

Continuing a study of the triterpene glycosides of *Thalictrum minus* [1-4], we have isolated a new glycoside. The present paper is devoted to the establishment of the structure of a triterpene glycoside containing a cycloartane genin that has not previously been described in the literature. The compound, which we have called thalicoside E (I) is a minor triterpene glycoside of low meadow rue.

It followed from the ^1H and ^{13}C NMR spectra of thalicoside E that the genin contains fragments showing the triterpene nature of the compound isolated: six methyl groups, one of which is secondary, a cyclopropane ring, a trisubstituted double bond, and five hydroxy groups (Table 1).

The glycosidic nature of the substance was shown by the results of mass spectrometry. Thus, the FAB mass spectrum of glycoside (I) contained a quasi-molecular peak with m/z 837 ($M + Na^+$) and cluster ions with m/z 615 ($M + Na^+ - Hex$-60) and 453 ($M + Na^+ - 2Hex$-60), showing the splitting out of one or two molecules of a hexose, respectively. It follows from the facts presented that a triterpene genin with a mass of 490 is glycosylated by two hexose molecules.

The CSs in the ^{13}C NMR spectrum for the carbohydrates (Table 2) corresponded to a terminal β-D-galactopyranoside and a terminal β-D-glucopyranoside rsidue [5]. A comparison of the ^{13}C NMR spectra of thalicoside E and of cycloartanol [6] showed that the new compound was a cycloartane derivative. Differences between them appeared in the structure of the side-chain and in substitution at the C-4 (ring A) and C-16 (ring D) atoms.

By using two-dimensional TOCSY and COSY experiments [7, 8], we identified the spin systems of rings A and D, The spin system of ring A includes the following protons: 1α (1.10) 1β (1.28)-2α (1.89) 2β (2.31)-3α (4.28 ppm). The signal of a hydroxymethylene proton (4.28 ppm) is in $^{13}C—^1H$ correlation with the signal of the C-3 carbon atom (81.91 ppm)

Formula

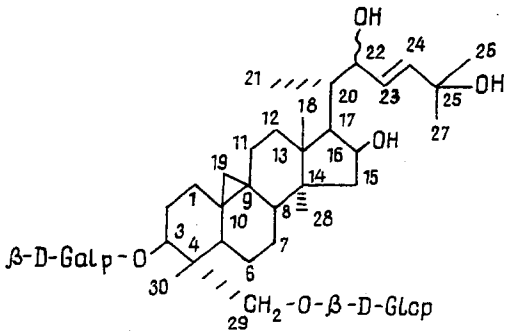

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TABLE 1. ^{13}C and ^{1}H Spectral Characteristics of Thalicoside E (genin moiety) from One- and Two-dimensional NMR Experiments (C_5D_5N; δ, ppm; J, Hz; TMS — 0)

C atom		DEPT	Chemical shift		SSCC	^1H/^1H
			^{13}C	^1H		
1	CH_2	32,17	(1α) 1,10 m			1β(1,28), 2α(1,89) 2β(2,31)
			(1β) 1,28 m			1α(1,10), 2β(2,31) 2α(1,89)
2	CH_2	29,46	(2α) 1,89 m			1α(1,10), 2β(2,31) 3α(4,28), 1β(1,28)
			(2β) 2,31 m			1β(1,28), 2α(1,89) 3α(4,28), 1α(1,10)
3	CH	81,91	(3α) 4,28 dd		^3J=3,0; 11,5	2α(1,89), 2β(2,31)
4	C	45,09				
5	CH	40,89	(5α) 1,95[a] dd		^3J=4,5; 12,4	6α(0,68), 6β(1,82)
6	CH_2	20,77	(6α) 0,68[b] m			5α(1,95), 6β(1,82) 7α(1,02), 7β(1,58)
			(6β) 1,82[c] m			5α(1,95), 6α(0,68) 7α(1,02), 7β(1,58)
7	CH_2	26,62[a]	(7α) 1,02[b] m			6α(0,68), 6β(1,82) 7β(1,58), 8α(1,18)
			(7β) 1,58[c] m			6α(0,68), 6β(1,82) 7α(1,02), 8α(1,18)
8	CH	48,41	(8α) 1,18[a] m			7α(1,02), 7β(1,82)
9	CH	20,03				
10	C	26,11				
11	CH_2	26,31[a]	Not revealed			
12	CH_2	37,76	Not revealed			
13	C	46,18				
14	C	47,46				
15	CH_2	48,52	(15α) 1,60 dd		^3J=8,2; ^2J=13,0	15β(2,00), 16α(4,74)
			(15β) 2,00 dd		^3J=4,8; ^2J=13,0	15α(1,60), 16α(4,74)
16	CH	72,00	(16α) 4,74 q d		^3J=4,8; 7,2; ^3J=8,2	15α(1,60), 15β(2,00) 17α(2,19)
17	CH	53,34	(17α) 2,19 dd		^3J=7,2; 11,2	16α(4,74), 20(2,59)
18	CH_3	20,52	1,36 c			
19	CH_2	30,44	(19′) 0,22 d		^2J=4,1	19′(0,49)
			(19) 0,49 d		^2J=4,1	19(0,22)
20	CH	36,79	(20S) 2,59 m		^3J=2,3; 7,0; ^3J=11,2	17α(2,19), 21(1,08) 22(4,70)
21	CH_3	15,46	1,08 d		^3J=7,0	20(2,59)
22	CH	76,31	4,70 q d		^4J=0,9; ^3J=2,3; 4,8	20(2,59), 23(6,21) 24(6,26)
23	CH	128,45	6,21 dd		^3J=4,8; 16,0	22(4,70), 24(6,26)
24	CH	139,84	6,26 dd		^4J=0,9; ^3J=16,0	22(4,70), 23(6,21)
25	C	69,98				
26	CH_3	30,85	1,44 s			
27	CH_3	30,92	1,46 s			
28	CH_3	19,58	0,83 s			
29	CH_2	71,21	(29′) 3,96		Not determined	29(4,21) 29′(3,96)
			(29) 4,21			
30	CH_3	11,78	0,87 s			

*a, b, c — alternative assignments within a column.

According to the literature, for 3β-OH-substituted triterpenes the signal of the C-3 carbon atom appears at 75 ppm and the effect of glycosylation at this position amounts to 5-7 ppm [6]. Consequently, the weak-field position of the C-3 signal (81.91 ppm) may be due to the attachment of a carbohydrate at this position.

The signal at 71.21 ppm belongs to a hydroxymethyl carbon atom (DEPT). It follows from a comparison of the ^{13}C NMR spectra of thalicoside E and of triterpenes with a 3β-OR or 4α-CH_2OR substituent that the hydroxymethyl fragment is present in the C-4 position, has the α-orientation, and is etherified by a carbohydrate [6].

The nature of the spin system of ring D — 15α (1.60) − 15β (2.00) − 16α (4.74) − 17α (2.19 ppm) — shows that there is a OH group in position 16 of ring D. In the ^{13}C NMR spectrum of thalicoside E the values of the β, γ, and δ effects on the 16-OH group correspond to its β-orientation [9].

Thus, we had established that the polycyclic part of the glycoside (I) molecule contains three methyl groups and three hydroxyls, two of which are glycosylated. Consequently, the remaining three methyl groups, two hydroxyls, and the double bond must be present in the side-chain.

TABLE 2. ^{13}C and ^{1}H Spectral Characteristics of Thalicoside E (carbohydrate moiety) from One- and Two-Dimensional NMR Experiments (C_5H_5N, δ, ppm, J, Hz, TMS — 0)

No. of the atom	DEPT	Galactose		Glucose	
		C	H	C	H
1	CH	106,20	5,28 $^{3}J=7,8$	104,98	5,04 $^{3}J=7,8$
2	CH	73,53	4,24	75,30	3,92
3	CH	75,58	4,04	78,75	4,05
4	CH	70,54	4,34	72,29	3,95
5	CH	76,26	4,0*	78,02	3,80
6	CH_2	63,25	4,26*	62,60	4,18*
			Not determined		4,21*

*The CSs were determined from 2D $^{13}C-^{1}H$ NMR spectra with an accuracy of ± 0.03 ppm.

The singlet nature of the signals of the protons of the 26- and 27-CH_3 groups in the ^{1}H NMR spectrum of thalicoside E showed the absence of a proton at C-25. The quaternary nature of this atom was also confirmed by the ^{13}C NMR spectrum (DEPT, Table 1). Furthermore, the CS of the signal of the C-25 carbon atom (69.98 ppm) showed the presence of an oxygen atom at C-25.

It became obvious from the facts given above that there is a tertiary alcohol group at C-25. In actual fact, in the FAB mass spectrum of thalicoside E the most intense ion is that of a fragment with m/z 777 [M + Na^+ — $(CH_3)_2CHOH$] formed as a result of the splitting out of isopropyl alcohol [10]. The splitting out of isopropanol is also characteristic for other fragmentary ions: m/z 615 [M + Na^+ — $(CH_3)_2CHOH$ — Hex], 453 [M + Na^+ — $(CH_3)_2CHOH$ — 2Hex].

The weak-field position of the signals of the C-26 and C-27 carbon atoms, together with their practically identical values (30.85 and 30.92 ppm) in the ^{13}C NMR spectrum, is characteristic for the $C_{23}H{=}C_{24}H-C_{25}(CH_3)_2OH$ fragment [11, 12]. The double bond in this fragment has trans-substitution ($J_{23,25}$ = 16.0 Hz).

The position of the fifth hydroxy group remained unelucidated. It could be in one of the two remaining positions — at C-20 or at C-22. The doublet nature of the signal of the protons of the 21-CH_3 group (1.08 ppm, ^{3}J = 7.0 Hz) in the ^{1}H NMR spectrum of the thalicoside excludes the presence of a hydroxyl at C-20. Consequently, the fifth hydroxy group is located at C-22.

The proposed structure of the side chain was confirmed by experiments with 2D NMR spectroscopy. Knowing the CS of H-17 (2.19 ppm) and using the TOCSY procedure we found the spin system of the side chain: 6.26, 6.21, 4.70, 2.59, 2.19, 1.08 ppm. Then, with the aid of a COSY experiment, we established the sequence of interaction of the protons responsible for the given signals.

H-17 (2.19) → H-20 (2.59) → H-22 (4.70) → H-23 (6.21) → H-24 (6.26)
H-21 (1.08)

Thus, we had established the structure of the genin, the qualitative and quantitative compositions of the carbohydrates, and the sites of glycosylation of the genin. It remained to determine the position of each carbohydrate.

We had previously established the structures of two new cycloartane glycosides from low meadow rue — thalicosides A and C [1, 4]. Both these compounds are bisdesmosides, each containing a β-D-galactopyranoside and a β-D-glucopyranoside residue in positions 3 and 29, respectively.

A comparison of the ^{13}C NMR spectra of thalicoside E and of thalicosides A and C [1, 4] showed close values of the CSs for the atoms of the polycyclic and carbohydrate moieties. This permitted the conclusion that thalicoside E is a bisdesmoside containing a β-D-galactopyranoside and a β-D-glucopyranoside residue in positions 3 and 29, respectively.

For thalicoside E we propose the structure 9,19-cyclo-20(S)-lanost-23-ene-3β,16β,22ξ,25,29-pentaol 3-O-β-D-galactoside 29-O-β-D-glucopyranoside.

EXPERIMENTAL

For general observations and the isolation of thalicoside E, see [4]. The glycoside was purified by droplet countercurrent chromatography in the chloroform–methanol–water (5:6:3.5) system [3].

Melting points were determined on a Boetius stage, and angles of rotation on a POLAMAT A polarimeter. The mass spectra of thalicoside E was recorded by A. L. Vereshchagin (IrIOKh) on an LKB-2091/PDP-11/34 instrument with a FAB ion source from Iontech. Ltd, Teddington. Ionization was brought about by a beam of accelerated xenon atoms with an energy of 6 kV at a discharge current of 1.2 mA. Glycerol with added NaCl was used as the matrix.

NMR spectra were recorded under the following conditions: Varian VXR 500S fitted with a SUN 3/50 computer with the standard VNMR equipment. The following procedures were used to obtain the two-dimensional $^{1}H-^{1}H$ and $^{13}C-^{1}H$ spectra:

COSY — the standard RELAYH program. Size of the matrix 2K × 0.5K, width of the spectrum 3000 Hz. In the pulse sequence we used two 90° pulses (COSY-90). The relaxation delay was 18 s. Before Fourier transformation the free induction decay signal was multiplied by a bell-shaped function with zero shift.

DQCOSY — the standard DQCOSY program in the phase-sensitive variant. Size of the matrix 2K × 1K, width of the spectrum 2000 Hz. Before Fourier transformation a Gaussian function with zero shift was used for weighting.

TOCSY — the standard TOCSY program in the phase-sensitive variant; for conditions, see [4].

2D $^{13}C-^{1}H$ — the standard HETCOR program; for conditions, see [4]. In a number of cases we used suppression of the signal of the residual protons of the solvent by presatuation for 1 s.

Thalicoside E (I) $C_{42}H_{70}O_{15}$, mp 249-251°C (chloroform–methanol (1:1)); $[\alpha]_{546}^{20}$ +4.7° (c 0.85; pyridine). FAB mass spectrum: m/z 837 $(M + Na)^+$, 777 $(M + Na^+ - 60)$, 615 $(M + Na^+ - 60 - Hex)$, 597 $(M + Na^+ - 60 - Hex - H_2O)$, 579 $(M + Na^+ - 60 - Hex - 2H_2O)$, 453 $(M + Na^+ - 60 - 2Hex)$, 435 $(M + Na^+ - 60 - 2Hex - H_2O)$, 417 $(M + Na^+ - 60 - 2Hex - 2H_2O)$. PMR spectra are shown in Tables 1 and 2.

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