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TRITERPENE SAPONINS FROM *Thalictrum minus*.

III. THE STRUCTURE OF THALICOGENIN

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The periodate oxidation with subsequent alkaline degradation of the products of the oxidation of the predominant saponin isolated from *Thalictrum minus* has yielded the native genin, which has been called thalicogenin. The structure of thalicogenin as 3β,16β,22(S),29-tetrahydroxy-9,19-cyclo-20(S)-lanost-24-ene has been established on the basis of chemical transformations and by spectral methods.

It has been reported previously that the acid hydrolysis of the predominating saponin isolated from *Thalictrum minus* (low meadow rue) gives a mixture of artefacts with a predominance of a compound for which the structure of 3β,16β,29-trihydroxy-22,25-epoxylanost-9(11)-ene (II) has been established [1-3]. In the present communication we describe the isolation of the native genin (I) and the determination of its structure.

The genin (I), which has been called thalicogenin, was obtained by the periodate oxidation of the main saponin of the plant under investigation followed by alkaline degradation of the oxidation products. According to the results of elementary analysis and its molecular mass (M^+ 474), thalicogenin corresponds to the formula $C_{30}H_{50}O_4$. The PMR spectrum has the signals of six methyl groups, and the mass spectrum (scheme 1) shows fragments with m/z (%): 329 (100) and 109 (17.3), which gives grounds for assuming that the compound isolated is a tetracyclic triterpenoid with an aliphatic side chain [4].

The structure of the side chain of thalicogenin was established in the following way. It followed from the PMR spectrum that compound (I) has a secondary methyl group (1.23 ppm,

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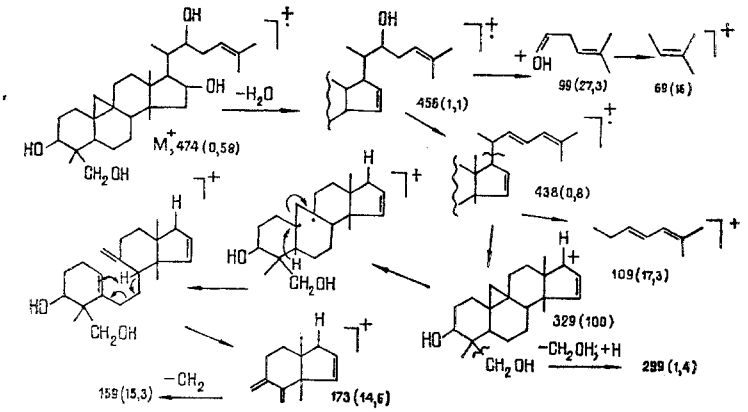


Scheme 1

3 H, d, J = 7.5 Hz) and two tertiary CH_3 groups at a double bond (1.68 ppm, 6 H, s) (Table 1).

The presence of a trisubstituted double bond in the side chain was confirmed by the ^1H NMR spectrum — 5.6 ppm, 1 H, m — and the ^{13}C NMR spectrum — 123.9 ppm, d, and 132.4 ppm (Tables 1 and 2).

The hydrogenation of (I) under mild conditions (Pd/C) led to compound (VI) with a saturated side chain (scheme 2). This was shown by the PMR spectrum of (VI) (Table 1) and the mass spectrum, in which an ion with m/z 111 (17.2%) corresponding to the hydrogenated side chain appeared.

A fragment with m/z 99 (27.3%) in the mass spectrum of (I) showed the presence of an OH group in the side chain (scheme 1). The location of the OH group at C-22 followed from a comparison of the positions of the H-22 signals in the PMR spectra of thalicogenin (I) and its acetate (III) (4.35 → 5.12 ppm) and in the artefact (II) and its acetate (V) (4.24 → 4.03 ppm). In the latter case, there was no paramagnetic shift because of the impossibility of the formation of an acetate at C-22.

The facts considered suggested the following arrangement of the structural elements of the side chain: A secondary methyl group is present at C-20 and a hydroxyl group at C-22, and the double bond is localized at C-24, at which there are two geminal CH_3 groups.

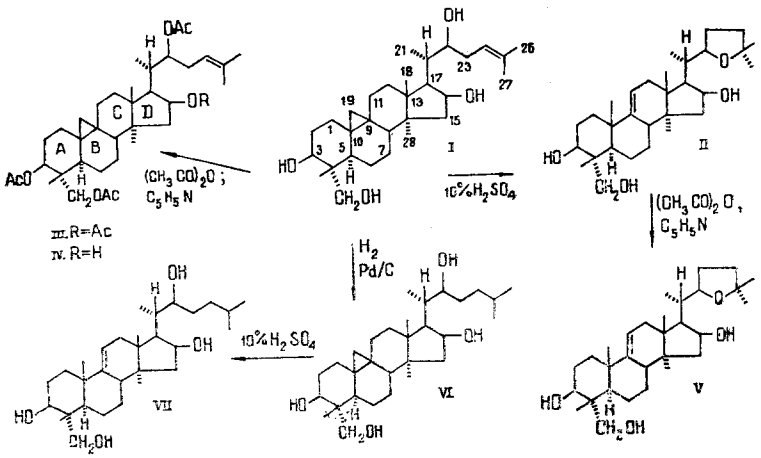
Chemical transformations confirmed this structure of the side chain: Heating (I) with an acid led to a compound with a closed side chain (II), while a compound not containing a double bond at C-24 (VI) lost its capacity for the cyclization of the side chain under the same conditions (scheme 2). Consequently, cyclization took place as a result of an attack of the OH group at C-22 on a carbocation at C-25.

The presence in the IR spectrum of an absorption band at 3030 cm^{-1} [5], and of a fragment with m/z 173 (14.6%) [6, 7] in the mass spectra of thalicogenin and its acetates suggested the presence of a cyclopropane ring in the molecule.

In the strong-field region of the PMR spectrum of thalicogenin there were two one-proton doublets (AB system) at 0.44 and 0.62 ppm (J = 4.5 Hz), which are characteristic for a methylene group of a cyclopropane ring. The presence of a three-membered ring was confirmed also by the chemical shifts of the carbon atoms of this fragment, C-9, C-10, and C-19, in the ^{13}C NMR spectra (Table 2) [8].

When thalicogenin was heated with dilute acid, simultaneously with the cyclization of the side chain there was the formation of a seventh CH_3 group and of a double bond in the 9(11) position (scheme 2), such transformations in rings A and B being possible if the cyclopropane fragment is located at C-9, 19 [9].

The thalicogenin molecule has four oxygen atoms. The acetylation of (I) gave two products (III) and (IV), the mass-spectral fragmentation of which showed that they were tetra- and triacetates of thalicogenin, respectively. The PMR spectrum of (I) contained four one-proton singlets at 5.08, 5.88, 5.91, and 6.49 which disappeared on the addition of deuteromethanol because of deuterium exchange. Thus, it was possible to assert that (I) contained four hydroxy groups one of which was, as mentioned above, present in the side chain.



Scheme 2

A negative periodate oxidation reaction of the genin showed the absence of vicinal hydroxy group in (I).

The multiplet with its center at 4.30 ppm in the PMR spectrum of thalicogenin related to a methine proton at C-3 (INDOR). The spin–spin coupling constants (SSCCs) ($J_{3,2}$ = 5.2 and $J_{3,2}$ = 11.0 Hz) showed the β orientation of the hydroxy group at C-3 in (I).

A fragment with m/z 173 (14.6%) in the mass spectrum of thalicogenin indicated that one of the hydroxy groups must be present in ring C or D. On comparing the PMR spectra of thalicogenin and its triacetate it was possible to see that the signal at 4.85 ppm remained in the same position, while on passing from the triacetate to the tetraacetate this signal underwent a paramagnetic shift by 0.88 ppm (Table 1). The apparently most hindered position in rings C and D is C-16, where the difficultly acetylatable hydroxy group must be present. In addition, it was established for (I) by INDOR experiments that the proton at C-16 interacted with two protons at C-15 (2.15 ppm, $J_{16,15}$ = 4.8 Hz, $J_{16,15}$ = 7.9 Hz) and with one proton at C-17 ($J_{16,17}$ = 8.4 Hz), giving rise to a sextet at 4.85 ppm. If the OH groups were present in any other position of the CD fragment, the signal of the geminal proton must have had a different form.

The mass spectrum of thalicogenin contained an ion with m/z 299 (1.4%), which is probably formed from an ion with m/z 329 by the loss of a $CH_2{=}\overset{+}{O}H$ group and the addition of hydrogen, which is characteristic for the fragmentation of primary alcohols (scheme 1). The presence in the PMR spectrum of thalicogenin of two doublets at 3.78 and 4.22 ppm (AB system, J_{AB} = 10.5 Hz) also confirmed the presence of a CH_2OH group in (I).

A comparison of the chemical shifts (CSs) in the ^{13}C NMR spectra of thalicogenin and its peracetate, of cycloartenol, and of hederagenin [10] showed the position of the OH groups in thalicogenin and its derivatives at C-3, C-16, and C-22 and permitted the position of the primary alcohol group to be determined as at C-29.

Calculations of the CSs in the ^{13}C NMR spectra of the CH_3 group at C-30 as a function of its cis-trans position with respect to the cyclopropane fragment [8] showed that in (I) these elements had the cis-1,3-diaxial arrangement (δ effect), and, consequently, the primary alcohol group at C-29 and the cyclopropane ring were located on different sides of the plane of the molecule.

In the PMR spectrum of (I) the SSCC of H-17 (2.40 ppm, $J_{17,20}$ = 10.8 Hz) permitted the conclusion that the rotation of the side chain about the 17-20 bond was hindered and the chain was orientated in such a way that H-17 and H-20 were present in the trans positions with respect to one another.

In the conversion of (I) into (II) (scheme 2), epimerization at the C-20 and C-22 asymmetric centers could not take place. Consequently, on the basis of the results of an x-ray structural analysis of (II) and a comparison of the ^{13}C CSs of the NMR spectra of thalicogenin and of the 22(R)- and 22(S)-hydroxycholestanols, we established that (I) has the 3β, 16β,20(S),22(S) configuration of the chiral centers.

Thus, thalicogenin has the structure of 3β,16β,22(S),29-tetrahydroxy-9,19-cyclo-20(S)-lanost-24-ene.

TABLE 1. Chemical Shifts and Spin–Spin Coupling Constants in the ^1H NMR Spectra of Compounds (I-VII) (δ, ppm, 0 — TMS, C_5D_5N, J, Hz)

Compound	2H-19	H-3	H-16	H-22	2H-29	>C=CH–		CH_3COO	CH_3
						H-21	H-11		
I	0,62; 0,44, d, J=4,5	4,30 q, J=5,2; 11,0	4,85, sx, J=4,8; 7,9; 8,4	4,35, m	4,22; 3,78 d, J=10 5	5 6, m	—	—	1,68 ($2CH_3$); 1,52; 1,23, d, (J=7,5); 1,16; 0,96
II	—	4,15, q, J=6,0; 12,0	4,59, sx, J=4,6; 7,6	4,.4, m	4,10; 3,63, d, J=10 5	—	5,24, d, J=6,0	—	1,18; 1,13; 1,09; 1,02 ($2CH_3$); 0,91, d, (J=7,0); 0,67
III	0,45; 0,25, d, J=4,4	5,07, m	5,56, sx, J=4.4; 8,0	5,12, m	4,20; 3,80, d, J=11,4	5,12, m	—	2,06; 2,04 2,02; 1,96	1,56 ($2CH_3$); 1,12; 1,06, d, (J=7,0); 0,85; 0,71
III ($CDCl_3$)	0.47; 0,21, d, J=4,5	5 56, m	6,03, m	5,64, m	4.45. 4 25, d, J=13,5	5,93, t J=9,0	—	2,18; 2,19 2,20; 2,21	1,76 ($2CH_3$); 1,69; 1,11; 0,92, d (J=7,5); 0,81
IV ($CDCl_3$)	0 44; 0,22, d, J=4,5	5,56, m	5,15. m	5,64, m	4,46. 4,23, d, J=13,5	5,79, t J=9,0	—	2,18; 2,20; 2,21	1,76 ($2CH_3$); 1,69; 1,11; 0,92, d (J=7,5); 0,81
V	—	4,97, q, J=4,6; 11,2	5,52 sx, J=4,4; 7,7	4,03, m	3,99; 3,89, d, J=11,8	—	5,11, d. J=6,25	2,05; 1,96; 1 87	1,18; 1,09; 1,06; 0,93, d (J=6,6); 0,82; 0,80; 0,54
VI	0,63; 0,30 d, J=4,5	4,21, q, J=5,2; 11.0	4 77, sx, J=4,4; 7,6	3,30, m	4,13; 3,67 d, J=10.0	—	—	—	1.43; 1,13, d (J=6,4); 1,06; 0,87; 0,84, d (J=6,4; $2CH_3$)
VII	—	4,14, m	4,42, sx, J=6,2; 7,7	3,30. m	4,10; 3 62, d, J=10.7	—	5,32, m	—	1,10; 1,01; 0,93, d (J=6,6); 0,81, d (J=6,2) 0.79, d (J=6,2); 0,86; 0,65;

*d — doublet; t — triplet; q — quartet; sx — sextet; m — multiplet.

TABLE 2. Chemical Shifts of the Carbon Atoms in the ^{13}C NMR Spectrum of Thalicogenin (C_5D_5N, ppm, TMS — 0)

C-atom	δ	C-atom	δ	C-atom	δ	C-atom	δ
1	32,6	9	20,3	17	53,3	25	132,4
2	31,0	10	26,0	18	20,7	26	26,0
3	74,8	11	26,5	19	30,5	27	18,2
4	44,9	12	33,7	20	36,2	28	19,7
5	42,3	13	45,3	21	14,8	29	68,5
6	21,4	14	47,6	22	75,8	30	11,4
7	26,9	15	49,0	23	34,0		
8	48,6	16	72,1	24	123,9		

EXPERIMENTAL

Melting points were determined on a Boëtius stage, and angles of rotation of Polamat A polarimeter. Mass spectra were recorded on a Varian MAT-212 chromato-mass spectrometer at an ionization energy of 79 eV. ^{13}C and ^{1}H NMR spectra were recorded on Bruker WP-200 and WM-250 instruments in the Fourier regime at room temperature. The solvents were C_5D_5N and $CDCl_3$, and the internal standard TMS. The accuracy of the measurements of the CSs for ^{1}H was ±0.12 Hz and for ^{13}C, ±0.03 ppm. IR spectra were recorded on a UR-20 instrument. The results of the elementary analyses of the compounds corresponded to the calculated figures.

Isolation of Thalicogenin. The main saponin (5 g) was dissolved in the minimum volume of methanol, the resulting solution was cooled to 15°C, and a 10% aqueous solution of sodium periodate (5 g) was added dropwise. After 24 h, the precipitate of inorganic salt that had formed was separated off and was carefully washed with a mixture of methanol and chloroform. The wash mixture was combined with the mother liquor and the whole was evaporated. The residue was subjected to hydrolysis with a 3% solution of KOH in 85% ethanol (100 ml) in a current of argon at 100°C for an hour. The reaction products were neutralized by KU-1 cation-exchange resin and extracted with ethyl acetate. The ethyl acetate extracts were washed with water and evaporated. This gave 1.3 g of a dry residue which was chromatographed on alumina in the ethyl acetate—methanol system with an increase in the proportion of methanol from 0 to 100%. This led to the isolation of 0.8 g of thalicogenin (I), $C_{30}H_{50}O_4$, mp 201-202°C (ethyl acetate), $[\alpha]_{546}^{22}$ + 34.5 (c 1.00, pyridine). ν_{max}^{KBr} cm^{-1}: 3400-3200 (OH), 3045 (>C=CH—). Mass spectrum, m/z (%): M^+ 474 (0.58), 459 (4.6), 456 (1.1), 441 (1.8), 438 (0.8), 369 (23.0), 329 (100), 311 (47.6), 299 (1.4), 293 (4.6), 173 (14.6), 159 (15.3), 145 (13.8), 109 (17.3), 99 (27.3), 69 (16.9).

3β,22(S),29-Triacetoxy-16β-hydroxy-9,19-cyclolanost-24-ene (IV) and 3β,16β,22(S),29-Tetraacetoxy-9,19-cyclolanost-24-ene (III). Compound (I) (500 mg) was dissolved in pyridine (10 ml), and the solution was treated with acetic anhydride (6 ml) and left for 24 h. After the usual working up, the reaction mixture (650 mg) was chromatographed on a column of silica gel with elution by hexane—acetone (10:1). This yielded 100 mg of (III), $C_{38}H_{58}O_8$, softening p. 73-76°C, $[\alpha]_{546}^{22}$ + 120° (c 1.00, pyridine). $\nu_{max}^{CCl_4}$, cm^{-1}: 1740 ester C=O). Mass spectrum, m/z (%): M^+ 642 (0.1), 582 (6.6), 522 (73.3), 462 (15.3), 402 (12.6), 293 (13.4), 173 (42.0), 109 (100), 99 (66.9), 69 (82.5).

When the column was eluted with the hexane—acetone (10:1.5) system, 30 mg of (IV), $C_{36}H_{56}O_7$, was obtained. $\nu_{max}^{CCl_4}$, cm^{-1}: 1730 (ester C=O), 3610 (OH). Mass spectrum, m/z (%): M^+ 600 (0.1), 540 (2.2), 480 (1.5), 420 (1.0), 293 (9.0), 173 (11.0), 149 (100), 109 (30.0).

3β,16β,29-Trihydroxy-22,25-epoxylanost-9(11)-ene (II) from (I). Compound (I) (71 mg) was dissolved in 6 ml of chloroform—methanol (1:2). Then 6 ml of 20% H_2SO_4 was added and the mixture was heated at 80°C for 5 h, cooled, and extracted with chloroform, and the extract was evaporated. The residue was chromatographed on a column of silica gel. Elution with the chloroform—benzene—ethyl acetate (9:6:4) system yielded 33 mg of compound (II), $C_{30}H_{50}O_4$, mp 148-152°C (hexane). Mass spectrum, m/z (%): M^+ 474 (0.2), 331 (0.9), 293 (0.5), 126 (15.2), 99 (100).

3β,16β,29-Triacetoxy-22,25-epoxylanost-9(11)-ene (V) from (II). A solution of 200 mg of compound (II) in 5 ml of pyridine was treated with 3 ml of acetic anhydride and the mixture was heated at 40°C for 4 h. After the usual working up, 230 mg of the peracetate (V) was obtained. $C_{36}H_{56}O_7$, mp 160-162°C (hexane), $[\alpha]_{546}^{22}$ + 73.2°(c 0.8, pyridine). $\nu_{max}^{CCl_4}$, cm^{-1}: 1740 (ester C=O). Mass spectrum, m/z (%): M^+ 600 (6.0), 540 (24.4), 480 (0.4), 293 (40.0). 126 (26.0), 99 (100).

3β,16β,22(S),29-Tetrahydroxy-9,10-cyclolanostane (VI) from (I). In a flask with a magnetic stirrer, 35 mg of thalicogenin dissolved in 10 ml of methanol was hydrogenated over Pd/C (5 mg) at room temperature. Hydrogenation was continued until the absorption of hydrogen ceased. This gave 25 mg of 24,25-dihydrothalicogenin, $C_{30}H_{52}O_4$, mp 109-112°C (chloroform–methanol–hexane). Mass spectrum, m/z (%): M^+ 476 (12.5), 458 (34.4), 443 (40.6), 425 (28.1), 329 (40.6), 187 (100), 173 (34.4), 159 (46.9), 111 (17.2), 109 (62.5), 107 (59.4), 95 (48.4).

3β,16β,22(S),29-Tetrahydroxylanost-9(11)-ene (VII). A mixture of 20 mg of 24,25-dihydrothalicogenin (VI) and 10 ml of 10% H_2SO_4 was heated on the boiling water bath for 8 h. After the usual working up, 15 mg of the crude product (VII), $C_{30}H_{52}O_4$, was obtained. Mass spectrum, m/z (%): 458 (M − 18) (66.5), 443 (100), 425 (58.2), 407 (18.3), 329 (21.9), 289 (19.5), 187 (25.9), 173 (37.6), 159 (40.1), 111 (55.8), 109 (47.4), 99 (38.1), 95 (63.7).

The Periodate Oxidation of (I). A solution of 5 mg of thalicogenin in ethanol (8 ml) was treated with 15 ml of H_2O, and 15 mg of sodium periodate was added. The mixture was left at 5°C for 3 days. The reaction product was extracted with butanol, and the butanolic extracts were washed with water and evaporated. The initial compound (I) was identified by TLC on silica gel in the methanol–ethyl acetate (5:95) system.

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SUMMARY

The periodate oxidation of the predominant saponin isolated from *Thalictrum minus* L. has given the native genin — thalicogenin — which has the structure of 3β,16β,22(S),29-tetrahydroxy-9,19-cyclo-20(S)-lanost-24-ene.

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