TRITERPENE GLYCOSIDES OF Thalictrum squarrosum.

III. STRUCTURES OF SQUARROGENINS 1 AND 2

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Two genins – squarrogenin 1 and squarrogenin 2 – have been isolated from nodding meadow rue by the hydrolysis of squarrosides A1 and A2. The compounds are epimeric at C-21 and have the following structures: 1 – (21R, 22S, 23R)-21-methoxy-21,23-epoxycycloart-24-ene-3β,22β,30-triol, $C_{31}H_{50}O_5$, mp 169-171°C (hexane–acetone), $[\alpha]_{546}^{20}$ −11.06° (c 4.52; pyridine); and 2 – (21S, 22S, 23R)-21-methoxy-21,23-epoxycycloart-24-ene-3β,22β,30-triol, $C_{31}H_{50}O_5$, mp 190-193°C (hexane–acetone), $[\alpha]_{546}^{20}$ +106.6° (c 0.3; pyridine). The results of ^1H and ^{13}C NMR spectroscopy and of mass spectrometry for the new compounds are given.

We have previously reported the isolation of squarrofuric acid – an artefact obtained as a result of the acid hydrolysis of a methanolic extract of Thalictrum squarrosum Stephan ex. Willd. (nodding meadow rue) [1, 2].

In the present paper we consider the determination of the structures of two new genins which have been called squarrogenins 1 and 2 (compounds (I) and (II), respectively). The two genins were obtained by the enzymatic hydrolysis of glycosides A1 and A2. The identity of the genins of glycosides A1 and A2 with compounds (I) and (II) was established on the basis of the results of a study of their ^1H and ^{13}C NMR spectra.

Squarrogenins 1 and 2 have the same mass (M^+ 502) and the same mass-spectral fragmentation in the region of high mass numbers. Genins (I) and (II) were assigned to the cycloartane triterpenoids on the basis of the characteristic signals of a cyclopropane ring in the ^1H and ^{13}C NMR spectra: two doublets in the 0.34-0.41 ppm region (AB system, ^2J = 4.0 Hz) and two signals of quaternary carbon atoms – C-9 (21.7; 21.8 ppm) and C-10 (26.0 ppm), respectively (Tables 1 and 2). The compounds each contained one trisubstituted double bond, five methyl groups at quaternary carbon atoms, and six carbon atoms linked to oxygen, one of which was a component of a methoxy group.

From the results of the mass spectrometry and the similarity of the PMR spectra of squarrogenins 1 and 2 it was possible to conclude that these compounds are isomers.

The acetylation of genins (I) and (II) yielded their acetates (compounds (III) and (IV), respectively). Consequently in each of them, the three oxygen atoms were present in hydroxy groups.

In the PMR spectra of (I) and (II) two doublets interacting in the manner of an Ab system (homonuclear double resonance) suggested the presence in each of these compounds of a primary alcohol group at a quaternary carbon atom. In actual fact, on passing from compounds (I) and (II) to their acetate derivatives (III) and (IV), the chemical shifts (CSs) of these signals changed correspondingly (see Table 1).

The values of the CSs of the C-4 atoms in the ^{13}C spectra of genins (I) and (II) showed the presence of a CH_2OH group at C-4 in each of them [3, 4]. The signals of the carbon atoms of these groups in squarrogenins (I) and (II) were present at 64.5 and 64.6 ppm, respectively. The CSs corresponded to compounds with the β-configuration of the hydroxymethyl group at C-4 [4, 5].

A quartet with a one-proton intensity having its center at 3.23 ppm, ^3J = 4.9, 10 Hz, shifting downfield in the acetates (III) and (IV), was assigned to Hα-3, geminal to a hydroxy group. The presence of a secondary alcoholic function at C-3 was also determined biogenetically.

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The mass spectra of genins (I) and (II) (m/z 330, M − side chain) confirmed that in the polycyclic moiety of the triterpenoids there were no double bonds and also no oxygen-containing functional groups other than those mentioned above.

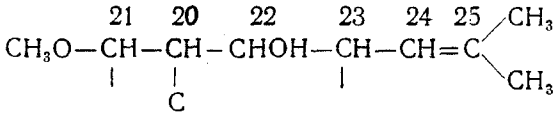
Thus, the polycyclic moieties of squarrogenins 1 and 2 were identical as was also shown by the practically coincident values of the CSs of carbon atoms from 1 to 15 in the ^{13}C NMR spectra of these compounds.

The structures of the side chains of the genins under investigation were established by ^{1}H NMR using homonuclear double resonance and by ^{13}C spectroscopy. In the ^{1}H NMR spectrum of each of compounds (I) and (II), the signals of the two geminal methyl groups in a relatively weak field (1.68 and 1.70 ppm, d) were broadened through allyl splitting from H-24.

The olefinic proton (H-24), apart from allyl splitting, also had a large SSCC with the vicinal proton at C-23. The CSs of H-23 (4.65 and 4.66 ppm) and C-23 (80.7 and 79.9 ppm for genins (I) and (II), respectively) showed a link of C-23 with an oxygen atom. In its turn, H-23 interacted with H-22, which is geminal to a hydroxy group. The presence of an alcoholic function in each genin at C-22 was shown by a 1-ppm downfield shift of the H-22 signal in the PMR spectra of acetates (III) and (IV).

Since on the periodate oxidation of the genins the C-22–C-23 bond was not cleaved, and on acetylation no acetate at C-23 was formed, the oxygen function at C-23 is not alcoholic.

Then considering the chain of interacting protons in the PMR spectra of (I) and (II), we detected a link of the H-22 signal with a multiplet in the strong field − H-20 (2.20 and 2.22 ppm, for genins (I) and (II), respectively). At the same time, H-20 interacted with H-21, and the latter appeared in the weak field in the form a doublet (^{3}J = 3.97 Hz). The CSs of H-21 (4.83 and 4.78 ppm) and of C-21 (104.9 and 108.7 ppm) in the ^{1}H and ^{13}C NMR spectra of compound (I) and (II) are characteristic for acetals and semiacetals, respectively. Since squarrogenins 1 and 2 did not form acetates at C-21, this atom is the acetal atom, one of the alkyl substituents of which is a methyl group (singlets with a three-proton intensity at 3.35 ppm and signals at 54.6 ppm in the spectra of (I) and (II)). Thus, the side chains of both compounds have the following structure:



Taking into account the nature of the C-21 and C-23 atoms it may be assumed that they are linked with one another through an oxygen bridge which is a component of a tetrasubstituted tetrahydrofuran (THF) ring with a $-OCH_3$ group at C-21 and a $(CH_3)_2C{=}CH$ fragment at C-23.

It must be mentioned that genins (I) and (II) are labile during chromatograpnhy on silica gel (particularly with proton-containing solvents, and squarrogenin 1 is partially converted into squarrogenin 2). Genin 2 is less labile.

As a result, we came to the conclusion that squarrogenins 1 and 2 are diastereomers with respect to the acetal carbon atom, i.e., 21α- and 21β-anomers.

The configurations of the chiral centers in the side chain were determined on the basis of ^{13}C NMR spectroscopy and the homonuclear Overhauser effect.

It is known that in the ^{13}C spectra of methyl furanosides with the 1,2-syn arrangement of the substituents the chemical shifts of carbon atoms 1 and 2 are present in a stronger field than in the case of 1,2-anti-substituted furanosides [6, 7]. In squarrogenin 1, the CSs of carbon atoms C-21 and C-20 (104.9 and 52.5 ppm, respectively) correspond to the syn-isomer (21α-OCH_3) and in squarrogenin 2 (C-21 and C-20, 108.7 and 55.6 ppm, respectively) to the anti-isomer (21β-OCH_3).

A proton interaction of the syn-1,3 type for Hα-21 and Hα-17 leads to a paramagnetic shift of the C-17 signal by 4.1 ppm [8] in squarrogenin 2 as compared with squarrogenin 1, which, on the one hand, well explains the difference in the C-17 CSs of compound (I) and (II) and, on the other hand, confirms the β-position of the methoxy group in genin (II).

When the signal of the H-23 proton was irradiated with an additional radio-frequency field, in each case a positive nuclear Overhauser effect was observed for the signal with a

TABLE 1. Chemical Shifts and Spin-Spin Coupling Constants of J, Hz; $CDCl_3$; 0 – TMS; s – singlet; br.s – broadened singlet;

Compound	H-3	2H-19	H-20	H-21	H-22	H-23
I	3.50 m 3,23** q ^3J=4,9; ^3J=10,0	0,37; 0.41 d ^2J=4,0	2,20 m	4.83 d ^3J=3,97	3,88 t ^3J=3,97	4,65 dd ^3J=9,16; 3.66
II	3,51 m 3,28** q ^3J=4,9; ^3J=10,0	0,34; 0,37 d ^2J=4,0	2,22 m	4,78 d ^3J=3,97	3,98 q ^3J=3,97, ^4J=2,14	4,66 dd ^3J=7,93, ^4J=1,53
III	4,63 m	0,44; 0,52 d ^2J=4,0		4,81 d ^3J=4,0	5,08 m	4,63 m
IV	4,75 ^3J=7,5; 2,0	0,41; 0,52 d ^2J=4,2		4,82 d ^3J=4,0	5,02 m	4,66 m

*The signals of the CH_3 groups have a singlet nature, with the
**In DMSO; 500 MHz.

TABLE 2. Chemical Shifts in the ^{13}C NMR Spectra of Compounds (I) and (II) (δ, ppm, C_5D_5N, 0 – TMS)

C atom*	I	II	C atom	I	II
1	32,4	32,4	17	40,7	44,8
2[a]	31,7	31,7	19[a]	31,3	30,7
3	80,1	80,2	20	52,5	55,6
4	43,8	43,8	21	104,9	108,7
5[b]	48,6	48,7	22	75,0	76,7
6	21,8	21,9	23	80,7	79,0
7[c]	27,0	26,9	24	121,4**	119,1**
8[b]	47,7	47,8	25	137,4**	139,4**
9	21,7	21,8	18	26,3	26,4
10	26,0	26,0	26	21,3	21,3
11[c]	26,7	26,5	27	19,8	19,8
12	36,1	35,9	28	19,8	18,9
13	45,3	45,6	29	18,4	18,6
14	48,4	48,5	30	64,5	64,6
15[a]	30,6	30,5	OCH_3	54,5	54,8
16[a]	30,0	27,8			

*a, b, c are alternative assignments.
**Solvent $CDCl_3$.

chemical shift of 2.2 ppm (H-20). This indicates the syn-arrangement of H-23 and H-20 relative to the plane of the five-membered ring in each of the genins.

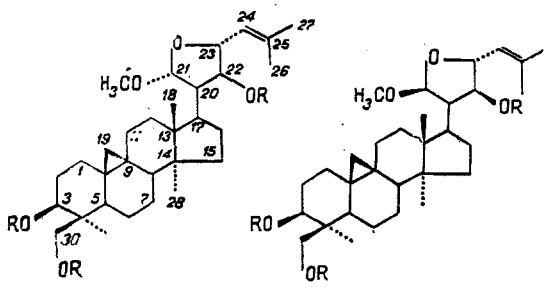
The presaturation of the proton at C-21 in (I) caused an enhancement of the signal at 4.65 ppm, relating to H-23, and the same action in (II) gave a NOE signal at 3.98 ppm (H-22). Consequently, H-23 occupies the β-position and H-22 the α-position both in (I) and in (II).

Finally, when the OCH_3 group in (I) was irradiated a small enhancement of the signal at 5.40 ppm (H-24) was observed. Similar presaturation in (II) caused a weak NOE on the signal at 4.66 ppm (H-23). This confirmed the syn-arrangement of the voluminous substituents at C-21 and C-23 in genin (I) and the syn-arrangement of OCH_3 at H-23 in genin (II). These facts show the relative configuration of the THF ring in squarrogenin 1: 21α-OCH_3, 22β-OH, and 23α-$CH{=}C(CH_3)_2$, while squarrogenin 2 is its anomer – with 21β-OCH_3.

the Protons in the PMR Spectra of Compounds (I-IV) (δ, ppm; d – doublet; t – triplet; q – quartet; m – multiplet)

H-24	2H-30	CH_3COO*	CH_3O*	CH_3*
5,40 dt ^3J=9,16, ^4J=1,22	3,40; 4,41 d ^2J=11,0		3,33	0,94; 1,01; 1,22; 1,78 d ^4J=1,22; 1,80 d, ^4J=1,22
5,35 dt ^3J=7,93, ^4J=1,53	3,47; 4,40 d ^2J=11,0		3,39	0,92; 1,06; 1,22; 1,78 d ^4J=1,53; 1,80 d ^4J=1,53
5,37 m	4,32; 4,36 d ^2J=11,0	2,03 2,04	3,30	0,89; 0,96; 1,05, 1,70 br.s, (CH_3×2)
5,39 m	4,32; 4,36 d ^2J=11,0	2,04; 2,04; 2,08	3,38	0,89; 0,96; 1,05; 1,68, br.s, 1,70, br.s.

exception of CH_3-26 and CH_3-27.



I. R=H–squarrogenin 1 II. R=H–squarrogenin 2
III. R = CH_3CO IV. R=CH_3CO

Thus, squarrogenin 1 is (21R, 22S, 23R)-21-methoxy-21,23-epoxycycloart-24-ene-3β,22β,30-triol, and squarrogenin 2 its (21S)-isomer.

EXPERIMENTAL

IR spectra were recorded on a Specord UV-VIS instrument in tablets with KBr. ^1H NMR spectra were recorded on a Bruker WP-200 (200.13 MHz) spectrometer, and ^{13}C NMR spectra on a JEOL FX-90 Q (22.49 MHz) spectrometer. TMS was used as internal standard. The NOE experiments were performed on a Bruker WM-250 (250 MHz for ^1H) spectrometer under the direction of V. V. Isakov and A. I. Kalinovskii (Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Division of the USSR Academy of Sciences, Vladivostok). The samples were previously degassed four times. Differential NOEs were recorded. The assignment of the signals of the carbon atoms was made by a comparative study of the spectra recorded under the conditions of complete decoupling from protons using the INEPT procedure and on the basis of literature information [4-7].

Mass spectra were taken on a LKB-2091 instrument at 310°C.

Melting points were determined in capillaries. Specific optical rotations were measured in pyridine on a Polarmat A polarimeter. Silica gel of type L40/100 was used for column chromatography, and L5/40 and Silufol plates for TLC. The following solvent systems were employed: hexane–acetone (with an increasing proportion of acetone from 0 to 15%); chloroform–methanol (with an increasing proportion of methanol from 0 to 5%); and chloroform–methanol–water (70:23:1).

The ratio of substance to sorbent was varied from 1:100 to 1:500.

Isolation of the Squarrogenins. A suspension of 400 mg of the sum of glycosides A1 and A2 in 100 ml of water was treated with 40 mg of the unpurified Helix pomatia enzyme and two drops of toluene. The reaction mixture was kept in a thermostat at 36°C with constant stirring for 14 days, with the periodic addition of 5-mg portions of enzyme and a drop of toluene. The course of the reaction was monitored by TLC in system 3. The reaction products were extracted with chloroform, and the chloroform extracts were washed with water. This

gave 80 mg of dry extract. The residue after extraction with chloroform was exhaustively extracted with butanol. The butanolic extracts after evaporation were again suspended in water, enzyme and toluene were added, and the mixture was thermostated under the conditions described above. The weight of chloroform extracts from the repeated enzymolysis amounted to 60 mg.

The reaction products were combined and chromatographed on a silica gel column in system 2. This gave 100 mg of the sum of the genins, which was rechromatographed on silica gel in system 1. When the proportion of acetone was 13%, 40 mg of compound (I) (squarrogenin 1) was eluted. Increasing the concentration of acetone to 14% gave 50 mg of compound (II) (squarrogenin 2).

Squarrogenin 1 (I). $C_{31}H_{50}O_5$, mp 169-171°C (hexane–acetone), $[\alpha]_{546}^{20}$ −11.06° (c 4.52; pyridine), ν_{max}^{KBr}, cm^{-1}: 3420-3442 (OH). Mass spectrum, m/z (%): M^+ 502(4.17); 484(100) - $M^+ - H_2O$; 469(50) - $M^+ - H_2O - CH_3$; 466(208) - $M^+ - 2H_2O$; 452(70.8) - $M^+ - CH_3OH - H_2O$; 437 (33.3) - $M^+ - CH_3OH - H_2O - CH_3$; 434(50) - $M^+ - CH_3OH - 2H_2O$; 419(16.7) - $M^+ - CH_3OH - 2H_2O - CH_3$; 400(41.7) - $M^+ - C_5H_{10}O_2$; 386(20.8) - $M^+ - C_6H_{12}O_2$; 368(66.7) - $M^+ - C_6H_{12}O_2 - H_2O$; 337(75.0) - $M^+ - C_6H_{12}O_2 - H_2O - OCH_3$; 330(8.3) - M^+ − side chain; 312(54.2) - M^+ − side chain $- H_2O$; 297(37.5) - M^+ − side chain $- H_2O - CH_3$. The ^1H and ^{13}C NMR spectra are given in Tables 1 and 2.

Squarrogenin 2 (II). $C_{31}H_{50}O_5$, mp 190-193°C (hexane–acetone), $[\alpha]_{546}^{20}$ +106.6° (c 0.3; pyridine), ν_{max}^{KBr}, cm^{-1}: 3040 (CH_2 of a cyclopropane ring), 3400-3420 (OH). Mass spectrum, m/z (%): 484(8.6) - $M^+ - H_2O$; 469(10); 466(2.9); 452(100); 437(11.4); 434(60); 419(55.7); 400 (12.9); 368(7.1); 349(92.9); 341(24.3); 297(51.4). The ^1H and ^{13}C NMR spectra are given in Tables 1 and 2.

Acetylation of Squarrogenin 1 (I) and of Squarrogenin 2 (II). A solution of 5 mg of genin (I) in 0.2 ml of pyridine was treated with 0.1 ml of acetic anhydride. The reaction mixture was left at room temperature for 5 h. The reaction products were worked up in accordance with the usual scheme. This gave 3 mg of 3,22,30-tri-O-acetylsquarrogenin 1 (III). Under similar conditions, 6 mg of 3,22,30-tri-O-acetylsquarrogenin 2 (IV) was obtained. Their PMR spectra are given in Table 1.

Attempted Periodate Oxidation of Squarrogenin 1 (I) and Squarrogenin 2 (II). A solution of 180 mg of the sum of squarrogenins 1 and 2 in 18 ml of methanol was treated with a solution of 400 mg of sodium periodate in 3 ml of water, and the mixture was stirred at room temperature for 18 h. After the oxidant had been destroyed with glycerol, TLC analysis in system 1 showed that the initial genins (I) and (II) had undergone no change.

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SUMMARY

The enzymatic hydrolysis of glycosides from the epigeal part of Thalictrum squarrosum (family Ranunculaceae) has given two new cycloartane genins which are isomers at an anomeric center. Squarrogenin 1 has the structure of (21R, 22S, 23R)-21-methoxy-21,23-epoxycycloart-24-ene-3β,22β,30-triol, while squarrogenin 2 is its (21S)-isomer.

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TRITERPENE GLYCOSIDES OF Thalictrum squarrosum.

IV. STRUCTURES OF SQUARROSIDES A1, A2, B1, AND B2

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Four new cycloartane glycosides have been isolated from a methanolic extract of Thalictrum squarrosum Stephan ex Willd.: squarroside A1 (I) – (21R, 22S, 23R)-3β-(β-D-glucopyranosyloxy)-21α-methoxy-21,23-epoxycycloart-24-ene-22β,30-diol, $C_{30}H_{60}O_{10}$; squarroside A2 (II) – the (21S)-epimer of compound (I); squarroside B1 (III) (21R, 22S, 23R)-3β-[O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyloxy]-21α-methoxy-21,23-epoxycycloart-24-ene-22β,30-diol, $C_{43}H_{70}O_{14}$; and squarroside B2 (IV) – the (21S)-epimer of compound (III). The proposed structures were determined on the basis of ^1H and ^{13}C NMR spectroscopy, FAB mass spectrometry, and chemical transformations.

In the present paper we consider the determination of the structures of four new triterpene glycosides from Thalictrum squarrosum Stephan ex Willd. which have been called squarrosides A1 (I), A2 (II), B1 (III), and B2 (IV).

The separation of the squarrosides that we had isolated into groups A and B was based on their belonging to monoside (group A) or bioside (group B) series. The compounds within each group are stereoisomers, and this has been responsible for the complexity and specificity of their individualization. After a number of attempts to separate the isomeric pairs, and the unfruitfulness of these attempts had become obvious only after the recording of NMR spectra, we came to the conclusion of the possibility of estimating the structures of the compounds without the separation of the isomeric pairs (of decisive importance in this question was an analysis of the NMR spectra obtained). It must be mentioned that chromatography was quite incapable of serving as an indicator in answering the question of the individuality of a particular compound, since the squarrosides of group A have close Rf values and the squarrosides of group B identical ones in, of course, the systems of solvents that we used (see Experimental part).

The proof of the structures of the triterpene glycosides of group A given below was carried out on fractions enriched to different degrees (about 90% of squarroside A1, and, correspondingly, 10% of squarroside A2, and also conversely) and with the use of derivatives. In a number of cases it was possible to obtain the latter in the individual state.

The monosidic nature of at least one of the squarrosides of group A was indicated by the presence, in the FAB mass spectra of enriched fractions, of a signal with m/z 687, corresponding to the cationized molecular ion $[M + Na]^+$ and of a peak of $[M + Na - 162]^+$ with m/z 525 showing that the carbohydrate moiety was a hexose.

The products of the enzymatic hydrolysis of squarrosides 1 and 2 were squarrogenins 1 and 2 (V and VI) – stereoisomers at C-21 [1]. The similarity of the ^{13}C NMR spectra of glycosides (I) and (II) (Table 1) showed the isomeric nature of this pair of compounds.

It can be seen that the spectra of glycosides A1 and A2 differ only by the values of the chemical shifts (CSs) of the signals of the carbon atoms sensitive to epimerization at C-21 (I: C-21, 104.8; C-20, 52.3; C-17, 43.3 ppm); (II: C-21, 108.5; C-20, 55.5; C-17,

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