APPLICATION OF ^{13}C NMR SPECTROSCOPY AND OF FAB MASS SPECTROMETRY IN THE INVESTIGATION OF THE MINOR TRITERPENOIDS OF *Thalictrum minus*

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Five minor triterpenoids have been isolated from the chloroform-soluble fraction of a methanolic extract of *Thalictrum minus* by chromatography on silica gel: (I) — oleanolic acid; (II) — oleanolic acid 3-O-α-L-arabinopyranoside; (III) — oleanolic acid 3-O-[O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside]; (IV) — oleanolic acid 3-O-[O-α-L-rhamnopyranosyl(1 → 2)-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside]; and (V) — oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside]. This is the first time that any of the compounds isolated have been detected in plants of the genus *Thalictrum*. The possibility has been shown of determining the structures of triterpene glycosides on the basis of ^{13}C NMR spectroscopy and FAB mass spectrometry without chemical transformations of the glycosides.

Traditional methods of investigating the chemical structures of natural glycosides include stages of methylation and chemical degradation followed by the identification of monosaccharides and the products of partial hydrolysis of the carbohydrate chains. In order to avoid these very laborious operations, recently a tendency has been observed to the use of a combination of physicochemical methods, including new methods of ionizing large nonvolatile molecules in the recording of mass spectra [1, 2]. We give the results of an investigation of the minor triterpenoids of the plant *Thalictrum minus* L. (low meadow rue); their chemical structures have been established without chemical degradation — by interpreting the results of ^{13}C NMR spectroscopy and FAB mass spectrometry.

We have previously reported on the isolation from the methanolic extract of low meadow rue of the predominating triterpene glycosides — thalicoside A [3] and thalicoside B [4]. The chloroform-soluble fraction of this extract contained a mixture of minor triterpenoids (I-V) (less than 0.01% on the weight of the raw material), which were separated by repeated chromatography on silica gel. It was found that compound (I) was a genin and (II)-(V) were glycosides.

The methylation of genin (I) with diazomethane led to its monomethyl derivative, the mass spectrum of which corresponded to that of methyl oleanolate. A chromatographic comparison of the triterpenoid (I) and its methyl derivative with authentic samples permitted genin (I) to be identified as oleanolic acid.

In the products of the acid hydrolysis of glycoside (III), L-arabinose and oleanolic acid were identified by the TLC method. A chromatographic comparison of glycoside (II) with oleanolic acid 3-O-α-L-arabinopyranoside showed their identity.

Oleanolic acid was also present as the genin of glycosides (III-V), as followed from the chemical shifts (CSs) of the genin parts of the ^{13}C NMR spectra of these glycosides (Table 1) [4] and the presence of a high-intensity fragment with m/z 439 $[(M_{genin} + H) - H_2O]^+$ in the mass spectra of each of these compounds.

The quasi-molecular ions in the mass spectra of (III-V) showed that glycoside (III) was a bioside of a pentose and a hexose, glycoside (IV) a trioside of a pentose, a hexose, and a deoxyhexose, and glycoside (V) a trioside of a pentose and two hexoses.

A study of the ^{13}C NMR spectra of glycosides (III-V) and a comparison of the CSs of the carbon atoms of the sugar residues with literature information [5] permitted the pentose to

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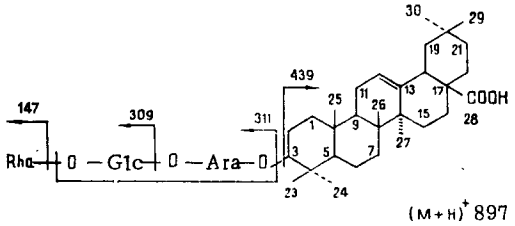

FAB mass-spectral fragmentation

be identified as α-L-arabinopyranose, the hexose as β-D-glucopyranose, and the deoxyhexose as α-L-rhamnopyranose.

In the FAB mass spectra of glycosides (III) and (IV), the peaks were observed of the quasi-molecular ions both of the individual monosaccharides (m/z 163, 147, and 133) and also of their blocks: for (III) — Glc-Ara (m/z 311); for (IV) — Glc-ARa (m/z 311) and Glc-Rha (m/z 309). Consequently, in compound (IV) the glucose was located between the Rha and the Ara (scheme).

The glycosylation of the arabinose residue by glucose at C-3 followed from a comparison of the CSs of the L arabinose residues in compounds (III) and (IV) and in methyl α-L-arabinopyranoside [5]. A paramagnetic shift of the signal of the C-3 atom of L-arabinose by 5.8 ppm unambiguously determined the position of attachment of the D-glucose residue.

It followed from the ^{13}C NMR spectra and molecular masses of compounds (III) and (IV) that glycoside (IV) contained one carbohydrate (L-rhamnose) residue more than (III), the L-rhamnose residue being attached at the C-2 position of the D-glucose residue ($\Delta\delta$ +2.7 ppm), since the CSs of the other atoms of the D-glucose and L-arabinose residues had scarcely changed.

Thus, glycoside (III) was oleanolic acid 3-O-[O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside], and glycoisde (IV) oleanolic acid 3-O-[O-α-L-rhamnopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside].

Like compound (IV), glycoside (V) contained three carbohydrate units [$(M + Na)^+$ 935], but one of the hexoses was attached to the genin by an ester bond (ν 1735 cm^{-1}). Glycosylation at C-28 of the genin was also confirmed by the CS of the anomeric carbon atom of this sugar residue —96.1 ppm. An analysis of the ^{13}C NMR spectrum of glycoside (V) showed that it was a bisdesmoside and contained two terminal β-D-glucopyranose residues.

The structures of the carbohydrate chains at C-3 of the genin in glycosides (III) and (V) was the same, as followed from the identity of the CSs of the atoms corresponding to it in the ^{13}C NMR spectra. Consequently, bisdesmoside (V) had the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside].

The native state of all the minor compounds was confirmed by an investigation of the plant fixed with methanol immediately after collection.

This is the first time that compounds (I-V) have been isolated from the genus *Thalictrum*. However, they have previously been obtained as progenins by the hydrolysis of thalicoside B [4].

In conclusion, we may mention that in the investigation of glycosides (III-V) by FAB mass spectrometry, a low intensity of the positive quasi-molecular ions and weak, uniformative, fragmentation of the whole molecule were observed. This fact is not unexpected, since a similar case has already been reported for related compounds [6]. Additions to the matrix of 0.1 N HCOOH, NaCl, and KCl did not lead to an increase of intensities in the high-mass region, while the addition of 0.1 N HCl permitted the observation for glycoside (V) of a signal with m/z 935, corresponding to $(M + Na)^+$.

EXPERIMENTAL

The ^{13}C NMR spectra were recorded on a Jeol FX-90Q spectrometer (22.49 MHz) at —25°C, δ scale, ppm, 0 — TMS, concentration of the samples 2%; (III) and (IV) in C_5D_5N and (V) in CD_3OD. The assignment of the signals was made by a comparative study of the spectra recorded under conditions of complete decoupling from protons using the INEPT procedure and on the basis of literature information [3, 5].

TABLE 1. Chemical Shifts in the ^{13}C NMR Spectra of Compounds (III-V) [(III and IV) — C_5D_5N; (V) — CD_3OD ppm, TMS — 0]*

C	Genin			C	Carbohydrates		
	III	IV	V		III	IV	V
1	38,9	38,9	40,1		L-Arabinose		
2	26,6	26,5	27,2				
3	88,9	89,1	91,2	1	106,9	106,9	107,5
4	39,6	39,5	40,5	2	74,3	74,6	74,6
5	56,1	56,0	57,3	3	79,2	78,9[a]	79,9
6	18,6[a]	18,6	19,7	4	71,6	71,9[c]	71,6
7	33,2[a]	33,3	33,5	5	65,7	65,1	66,5
8	40,0	39,9	41,1		D-Glucose at C-3		
9	48,2	48,2	b				
10	37,2	37,1	38,2	1	106,5	104,9	106,5
11	23,8	23,8	24,3	2	75,7	78,4[a]	75,7
12	122,5	122,5	124,1	3	78,5	78,4[a]	78,5[c]
13	144,9	144,8	145,1	4	73,5	72,9	73,5
14	42,3	42,3	43,2	5	78,4	78,2	78,4[c]
15	28,3	27,9	28,9	6	62,9	62,8	62,9
16	23,8	23,8	24,9				
17	46,8	46,7	b		L-Rhamnose		
18	42,1	42,1	42,9				
19	46,7	46,6	b	1		101,9	
20	30,9	31,0	31,8	2		72,0[c]	
21	34,4	34,4	35,2	3		72,4[d]	
22	33,3[a]	33,3	33,8	4		73,7	
23	28,4	28,3	29,2	5		70,0	
24	16,8	16,9	17,3	6		18,6	
25	15,5	15,5	16,3				
26	17,4	17,5	18,0		D-Glucose at C-28		
27	26,1	26,2	26,6				
28	180,5	180,1	178,4	1			96,1
29	33,3	33,3	33,9	2			74,3[a]
30	23,8	23,8	24,3	3			78,3[c]
				4			71,6
				5			78,3[c]
				6			62,8

*a, c, d — assignment ambiguous within one column; b) signals masked by the solvent. The spectra were taken by M. F. Larin.

The mass spectra of glycosides (III-V) were taken on a LKB-2091/PDP-11/34 instrument with an FAB-ion source from Iontech. Ltd, Teddington (United Kingdom). Ionization was carried out by a beam of accelerated xenon atoms with an energy of 6 kV at a discharge current of 1.2 mA. Glycerol was used as the matrix. The mass spectrum of methyl oleanolate was recorded on a MAT-212 instrument with direct introduction of the sample at an ionization energy of 70 eV and a temperature of 270°C. Melting points were measured on a Boëtius stage.

Type L 40/100 silica gel was used for column chromatography and L 5/40 for TLC with the solvent systems: 1) chloroform—methanol (10:1); 2) chloroform—methanol—water (70:12:1); 3) chloroform—methanol—water (70:23:4); and 4) ethyl acetate—methanol—water (3:2:1).

Compounds (I-V) were isolated by repeated column chromatography on silica gel in systems 2, 3, and 4 of the chloroform-soluble fraction of a methanolic extract of low meadow rue [7].

Oleanolic Acid (I). mp 300-301°C (from ethanol). Methyl oleanolate, $C_{31}H_{50}O_3$; mass spectrum, m/z (%): M^+ 470 (4), 452 (2), 262 (54.5), 203 (100), 190 (36.4).

Oleanolic Acid 3-O-α-L-Arabinopyranoside (II). By the TLC method in systems 2, 3, and 4, glycoside (II) was shown to be identical with oleanolic acid 3-O-α-L-arabinopyranoside. Compound (II) was hydrolyzed with 5% H_2SO_4, and the reaction mixture was neutralized and was treated with chloroform. In the chloroform extract, oleanolic acid was identified by TLC in system 1, and in the aqueous residue L-arabinose by TLC in system 3.

Oleanolic Acid 3-O-[O-β-D-Glucopyranosyl-(1 → 3)-α-L-arabinopyranoside] (III). $C_{41}H_{66}O_{12}$. mp 262-264°C (from methanol), $(M + H - CH_3)^+$ 736.

Oleanolic Acid 3-O-[O-α-L-Rhamnopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside (IV). $C_{47}H_{76}O_{16}$, mp 249-250°C (from methanol), $(M + H)^+$ 897.

Oleanolic Acid 28-O-β-D-Glucopyranoside 3-O-[O-β-D-Glucopyranosyl-(1 → 3)-α-L-arabinopyranoside. (V). $C_{47}H_{76}O_{17}$. mp 219-220°C (from methanol), $(M + Na)^+$ 935.

SUMMARY

Five minor triterpenoids (oleanolic acid and a monoside, a bioside, and two triosides of this acid) have been isolated from *Thalictrum minus* L., and have been identified by ^{13}C NMR spectroscopy and FAB mass spectrometry without chemical degradation of the glycosides.

This is the first time that any of these compounds have been detected in a plant of the genus *Thalictrum*.

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STEROID COMPOUNDS OF MARINE SPONGES.

VIII. 24-ISOPROPYL-5α-CHOLEST-22-ENE-2β,3α,6α-TRIOL TRISULFATE – A NEW STEROID IDENTIFIED IN THE SPONGE *Trachyopsis halichondrioides*

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A new sulfated steroid triol has been identified in extracts of the sponge *Trachyopsis halichondrioides* and its structure has been established as 24-isopropyl-5α-cholest-22-ene-2β,3α,6α-triol trisulfate. The possibility has been shown for the first time of the existence of identical side chains for free sterols and trisulfated steroids.

Continuing a study of the steroid compounds of sponges [1], we have established the structure of a new trisulfated steroid triol detected in the sponge of *Trachyopsis halichondrioides* (Halichondriidae).

Fractions of free sterols and of sulfated steroids giving a single spot on TLC in suitable systems were isolated from an ethanolic extract of the sponge by chromatography on Polychrome [2].

GLC and GLC-MS showed that the components of the free sterol fraction of this sponge were alcohols known previously [2, 3] – 24-isopropylcholesta-5,22-dien-3β-ol and its 22,23-dihydro analog.

Analysis of the mass and ^1H NMR spectra of the sulfated fraction revealed the presence in it not only of halistanol sulfate (1), but also of substances related to it (2, 3). However, we did not succeed in separating the isolated mixture of sulfated steroid triols with the aid of high-pressure liquid chromatography on a ODS column in ethanol–water systems.

Acid hydrolysis of the combined sulfates (1 + 2 + 3) and subsequent acetylation of the triols obtained led to a fraction of triacetates (1a, 2a, and 3a). According to GLC and GLC-

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