TRITERPENE GLYCOSIDES OF Hedera taurica

X. STRUCTURES OF COMPOUNDS F_4, I, AND J FROM THE LEAVES OF CRIMEAN IVY

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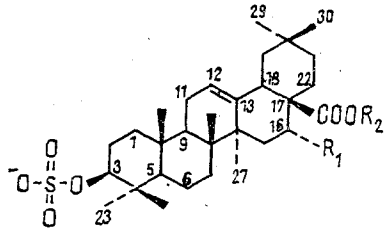
Echinocystic acid 3-sulfate and new 3-sulfates – of the 28-O-[O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl esters of oleanolic and echinocystic acids have been isolated from the leaves of Crimean ivy Hedera taurica Carr. (fam. Araliaceae).

We have previously described the establishment of the structures of glycosides F_0–F_3 from Crimean ivy leaves [1]. The preparation of this mixture in large amounts has permitted the detection of one more component, designated as F_4. Its preparative isolation was carried out by chromatography on silica gel, followed by conversion into the methyl ester for additional purification. Echinocystic acid and the sulfate anion were identified in an acid hydrolysate of F_4, but no sugars were detected. The PMR spectra of the methyl ester of F_4 showed a considerable downfield shift (1.3 ppm) of the H-3 signal in comparison with the H-3 signal in glycosylated echinocystic acid (3.24 ppm) [2]. The other signals in the PMR spectrum corresponded to the protons of an echinocystic acid residue. In the light of the results of acid hydrolysis, it was possible to assume that the C_3–OH group was acidified with sulfuric acid. This was confirmed by the presence of the bands of the ν_{as} and ν_s vibrations of a $O{=}\overset{|}{\underset{|}{S}}{=}O$ group in its IR spectrum (1390 and 1225 cm^{-1}). A comparison of the chemical shifts of the signals in the ^{13}C NMR spectrum of F_4 with literature information for synthetic echinocystic acid 3-sulfate [3] showed their agreement, which definitively confirmed the structure of compound F_4 as echinocystic acid 3-sulfate (I), isolated form the first time from a natural source



	R_1	R_2
I	OH	H
II	H	β-Glc$_p$-(6β)-Glc$_p$-(4α)-Rha$_p$
III	OH	β-Glc$_p$-(6β)-Glc$_p$-(4α)-Rha$_p$

We have described the isolation of taurosides I and J previously [2]. They were purified on silica gel, and glycoside I was additionally purified through its acetate. On acid hydrolysis, oleanolic and echinocystic acids, respectively, were found in taurosides I and J, and also rhamnose, glucose, and the sulfate anion. In the products of alkaline hydrolysis we identified the 3-sulfates of oleanolic and echinocystic acids as progenins. It was obvious that taurosides I and J were the sulfates F_0 and F_4 glycosylated in the carboxy groups.

In the region of anomeric C atoms in the ^{13}C NMR spectrum of tauroside I there were three signals. Consequently, the carbohydrate chain at the carboxy group consisted of a trisaccharide. Because of the low solubility of tauroside I in the standard solvent – pyridine – its PMR and ^{13}C NMR spectra were obtained in methanol-d_4. The assignments of the signals of the carbohydrate residues in the PMR spectrum were made with the aid of several

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TABLE 1. Chemical Shifts of the Signals of the ^{13}C Atoms of the Aglycon Moieties of Compounds F_4 (I), I (II) and J (III) (δ, ppm; O – TMS; C_5D_5N)

C atom	Compound				C atom	Compound			
	I	III	II	II*		I	III	II	II*
1	38,8	39 0	38 8	40,0	16	74,4	74,4	23,8	25,0
2	25,1	25,1	25,1	25,7	17	49,1	49,2	47,1	50,1
3	84,7	85,0	85,0	88,2	18	41,3	41,3	41,7	43,0
4	38,8	39,0	39,0	40,0	19	47,1	47.2	46,2	47,7
5	56,4	56 5	56,4	57,7	20	30,9	30,9	30,8	32,0
6	18,7	18,8	18,6	20,0	21	36,0	36,0	34,1	35,4
7	33,3	33,5	33,2	34,4	22	32,6	32,3	32,6	33,7
8	39,8	40,1	39,9	41,2	23	28,8	28,8	28,9	29,3
9	47,1	47,2	48,0	50,3	24	17,2	17,2	17,5	18,4
10	37,2	37,3	37,2	38,5	25	15,6	15,8	15,6	16,6
11	23,8	23,9	23,4	24,5	26	17,2	17,6	17,2	17,5
12	122,7	122,8	122,9	124,2	27	27,3	27,3	26,2	26,8
13	144,5	144,6	144,3	145,4	28	178,8	176,2	176,7	177,6
14	41,9	42,1	42,2	43,4	29	33,3	33,2	33,2	34,0
15	36,0	36,1	28,3	29,3	30	24,6	24,7	23,8	24,6
					–O–CH_3	51,8			

*Solvent – CD_3OD.

variants of two-dimensional homonuclear correlation spectroscopy (COSY, COSY + 2 stages of RCT, and ROESY), and complete assignments of the signals of the carbohydrate C atoms were made with the aid of two-dimensional heteronuclear correlation spectroscopy (HETCOSY). The nature of the splitting of the signals of the skeletal carbohydrate protons and the spin-spin coupling constants (SSCCs) corresponded to two β-glucose residues and one rhamnose residue.

Analysis of the positions of the signals of the anomeric protons showed that one of them was present in a substantially weaker field (5.30 ppm) and the C atom corresponding to it in a stronger field (96.2 ppm) than the others. Consequently, this glucose residue formed the acylglycosidic bond with the aglycone.

The same glucose residue was substituted in the OH group at C-6 (Δ_{C-6} + 7.7 ppm) while the other glucose residue was substituted in the C_4-OH group, since the C-4 signals experienced a downfield shift (Δ_{C-4} + 8.5 ppm) and there were negative β-effects (about 1 ppm) on the neighboring atoms. From the positions of its chemical shifts, the rhamnose residue was unsubstituted, i.e., terminal. Consequently, the trisaccharide consisted of O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl. The ^{13}C NMR spectrum of a saturated solution of tauroside I in pyridine showed complete agreement of the signals of the carbohydrate moiety with the signals of an analogous trisaccharide described previously [2], while those of the aglycon moiety coincided with those for a sulfated progenin from Bupleureum rotundifolium [3].

Thus, tauroside I was a new compound with the structure of 28-O-[O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]oleanolic acid 3-sulfate (II).

The ^{13}C NMR subspectra of the carbohydrate moieties of taurosides J and I were identical, while the chemical shifts of the signals of the aglycon moiety coincided with those for compound F_4. Consequently, the carbohydrate moiety of tauroside J was the trisaccharide O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-pyranosyl- and this was additionally confirmed by several variants of two-dimentional homonuclear correlation spectroscopy (COSY, ROESY, and HOHAHA). Thus, tauroside J was 28-O-[O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]echinocystic acid 3-sulfate (III) and it was also a new compound.

At the present time, only isolated reports have been published on the natural glycosides of sulfated triterpenoids from Bupleurum rotundifolium [3] and Patrinia scabiosaefolia [4].

EXPERIMENTAL

NMR spectra were taken on Bruker WM-250, AM-300, and AMX-400 instruments. Two-dimensional spectra were taken with the use of the standard procedures provided by the Bruker firm.

TABLE 2. Chemical Shifts of the Signals of the ^{13}C Atoms of the Carbohydrate Moieties of Taurosides I (II) and J (III) (δ, ppm; O – TMS; C_5D_5N)

C atom	Compound III	II	II*	C atom	Compound III	II	II*	C atom	Compound III	II	II*
—Glc—				—Glc—				—Rha			
1′	95,9	95,8	96,2	1″	105,0	105,0	104,7	1‴	102,8	102,8	103,4
2′	73,9	73,9	74,3	2″	75,4	75,5	75,8	2‴	72,6	72,7	72,9
3′	78,7	78,8	78,6	3″	76,5	76,6	77,2	3‴	72,8	72,8	72,7
4′	70,8	70,9	71,5	4″	78,3	78,3	80,0	4‴	74,0	74,1	74,2
5′	78,1	78,1	78,5	5″	77,2	77,2	77,3	5‴	70,4	70,4	71,1
6′	69,3	69,3	70,0	6″	61,3	61,3	62,3	6‴	18,6	18,8	18,4

*Solvent – CD_3OD.

TLC control was conducted on Silufol plates. Preparative separation was effected on silica gel L (40-100 μm). The following solvent systems were used: 1) chloroform–ethanol (20:1→6:1) saturated with water; 2) chloroform–ethanol–ammonia (30:10:1); and 3) chloroform–ethanol–water (20:6:1). We have described the conditions for acetylation, deacetylation, and acid and alkaline hydrolysis previously [5].

Triterpenoid F_4 (I). By the chromatographic separation of 60 g of an extract of Crimean ivy leaves containing weakly polar glycosides [1, 6] on silica gel in system 1 we obtained 0.4 g of crude F_4. Its additional purification in system 2 gave 0.13 g of F_4. After methylation with an ethereal solution of diazomethane followed by chromatography on silica gel in system 2, we obtained 50 mg of the pure ester of F_4 (Ia), $[\alpha]_D^{20}$ + 21° (c 2.5; methanol). PMR spectrum of Ia (δ, ppm, O – TMS, C_5D_5N): 4.54 (dd, $J_{2e,3}$ = 4.3 Hz, $J_{2a,3}$ = 11.5 Hz, H-3), 5.49 (t, $J_{11,12}$ = 3.5 Hz, H-12), 5.01 (Pt, $J_{15e,16e}$ = 3.3 Hz, H-16), 3.36 (dd, $J_{18,19e}$ = 4.0 Hz, $J_{18,19a}$ = 13.8 Hz, H-18), 0.62; 0.66; 0.98; 0.99; 1.06; 1.36; 1.72 (all s, 7 CH_3), 3.66 (s, O–CH_3), 0.7-2.8 (skeletal CH, CH_2). IR spectrum of Ia, ν_{max}^{KBr}, cm^{-1}: 1390 (ν_{as} O=S=O), 1225 (ν_S O=S=O).

Tauroside I (II) [2], after additional purification on silica gel with elution by system 2, was acetylated with acetic anhydride in pyridine. The acetate of tauroside I (IIa), after purification on silica gel in system 2 had $[\alpha]_D^{20}$ + 3° (c 1.5; chloroform). The deacetylation of IIa followed by chromatography in system 2 gave pure (II), $[\alpha]_D^{20}$ - 6° (c 6.4; methanol). PMR spectrum of II (δ, ppm, 0–TMS; CD_3OD) 5.30 (d, $J_{1,2}$ = 7.9 Hz, H-1'), 3.31 (t, $J_{2,3}$ = 8.5 Hz, H-2'), 3.35-3.40 (m, H-3', H-4'), 3.45-3.53 (m, H-5'), 4.04 (dd, $J_{6A,6B}$ = 12.0 Hz $J_{5,6A}$ = 1.5 Hz, H-6A'), 3.75 (dd, $J_{5,6B}$ = 5,0 Hz, H-6B'), 4.37 (d, $J_{1,2}$ = 8.4 Hz, H-1"), 3.19 (t, $J_{2,3}$ = 8.4 Hz, H-2"), 3.43 (t, $J_{3,4}$ = 8.7 Hz, H-3"), 3.50 (t, $J_{4,5}$ = 8.7 Hz, H-4"), 3.23 (m, H-5"), 3.76 (dd, $J_{6A,6B}$ = 12.0 Hz, $J_{5,6A}$ = 2.0 Hz, H-6A"), 3.61 (dd, $J_{5,6B}$ = 5.0 Hz, H-6B"), 4.81 (d, $J_{1,2}$ = 1.7 Hz, H-1"'), 3.81 (dd, $J_{2,3}$ = 3.4 Hz, H-2"'), 3.61 (dd, $J_{3,4}$ = 9.5 Hz H-3"'), 3.36 (t, $J_{4,5}$ = 9.5 Hz, H-4"'), 3.93 (dq, $J_{5,6}$ = 6.2 Hz H-5"'), 1.22 (d, H-6"'), 3.87 (dd, $J_{2e,3}$ = 4.5 Hz, $J_{2a,3}$ = 12.0 Hz, H-5"'), 1.22 (d, H-6"'), 3.87 (dd, $J_{2e,3}$ = 4.5 Hz, $J_{2a,3}$ = 12.0 Hz H-3), 5.18 (t, $J_{11,12}$ = 3.3 Hz, H-12), 0.73; 0.76; 0.84; 0.87; 0.89; 0.97; 1.08 (all s, 7 CH_3), 0.6-2.1 (skeletal CH, CH_2 of the aglycon). IR spectrum of (II) ν_{max}^{KBr} cm^{-1}: 1390 (ν_{as} O=S=O), 1230 (ν_S O=S=O).

Tauroside J (III) [2], after additional purification on silica gel in system 2 had $[\alpha]_D^{20}$ -15° (c 0.5 methanol). PMR spectrum of (III) (δ, ppm; O-TMS; pyridine-d_5): 6.20 (d, $J_{1,2}$ = 8.0 Hz, H-1'), 4.02 (t, $J_{2,3}$ = 8.3 Hz, H-2'), 4.16 (t, $J_{3,4}$ = 8.5 Hz, H-3'), 4.26 (t, $J_{4,5}$ = 8.6 Hz, H-4'), 4.04 (H-5'), 4.94 (d, $J_{1,2}$ = 7.6 Hz; H-1"), 3.90 (t, $J_{2,3}$ = 8.2 Hz, H-2"), 4.11 (t, $J_{3,4}$ = 9.0 Hz, H-3"), 4.38 (t, $J_{4,5}$ = 9.0 Hz, H-4"), 3.60 (ddd, H-5"), 4.0-4.2 (H-6"), 5.83 (d, $J_{1,2}$ = 1.3 Hz, H-1"'), 4.66 (dd, $J_{2,3}$ = 3.4 Hz, H-2"'), 4.54 (dd, $J_{3,4}$ = 9.2 Hz, H-3"'), 4.31 (t, $J_{4,5}$ = 9.2 Hz, H-4"'), 4.95 (dq, H-5"'), 1.67 (d, $J_{5,6}$ = 6.0 Hz, H-6"), 4.52 (dd, H-3), 5.53 (t, $J_{11,12}$ = 3.5 Hz, H-12), 5.27 (pt, $J_{15e,16e}$ = $J_{15a,16e}$ = 4.0 Hz, H-16), 3.45 (dd, $J_{18,19e}$ = 4.0 Hz, $J_{18,19a}$ = 13.8 Hz, H-18), 0.85; 0.94; 0.96; 0.99: 1.07; 1.32; 1.75 (all s, 7 CH_3), 0.7-2.8 (skeletal CH, CH_2). IR spectrum of (III), ν_{max}^{KBr}, cm^{-1}: 1390 (ν_{as} O=S=O), 1220 (ν_S O=S=O).

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GLYCOSIDES OF THE EPIGEAL PART OF Panax ginseng

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The qualitative and quantitative compositions of ginsenosides in the epigeal part of ginseng cultivated in the Maritime Territory have been determined. The concentration of ginsenosides in the epigeal part of this plant is fairly high and depends on the growth site and seasonal conditions. A method of alkaline hydrolysis for obtaining ginsenoside R_{g2} from R_e is described. Under these conditions for cleaving the glycosidic bond in the ginsenosides no epimerization at C_{20} is observed.

Ginseng is one of the best known medicinal plants. It is mainly the root of this plant that is used for medical purposes. The biological activity of ginseng roots is connected with the presence in them of ginsenosides – glycosides of the dammarane series. However, it has been found comparatively recently that some of the ginsengosides of ginseng roots are also present in the leaves, buds, and the flesh of the fruit in amounts of 6, 4, 1, and 6.4%, respectively, in several variations [1, 2]. As a rule, the total amount of ginsengosides in the epigeal parts of ginseng exceeds their amount in roots (1.45) [3]. A direct dependence of the biosynthesis of the ginsenosides in the leaves and buds of ginseng on the growth has also been found [4-6].

The increase in the plantations of cultivated ginseng in Maritime Territory is responsible for interest in the study of its epigeal part with the aim of establishing its qualitative and quantitative content of ginsenosides. The high yield of ginsenosides from the epigeal part of ginseng shows not only a potential possibility of isolating individual components but also the utilization of the whole epigeal part.

On investigating the glycoside composition of commercial ginseng roots, we isolated and identified 12 ginsenosides by the methods of ^{13}C and ^{1}H NMR, mass spectrometry, and high-performance liquid chromatography (HPLC) [7]. The procedure developed for the quantitative analysis of ginsenosides by the HPLC method [8] has permitted a comparative study to be made of extracts of the epigeal part of ginseng (leaves, flower buds, flesh of the fruit) of different populations, different growth sites, and different years of collection.

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