TRITERPENE GLYCOSIDES OF *Tetrapanax papyriferum*. IV. ACIDIC GLYCOSIDES FROM STEM BARK OF *T. papyriferum*

V. S. Strigunov,[1] V. I. Grishkovets,[1] A. S. Shashkov,[2] V. V. Kachala,[2] and N. V. Tolkacheva[1]

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Twelve acidic triterpene glycosides of oleanolic acid, two of which were new, were isolated from stem bark of Tetrapanax papyriferum *C. Koch (Araliaceae). The structures of these compounds were established using chemical methods and NMR spectroscopy.*

Key words: *Tetrapanax papyriferum*, Araliaceae, triterpene glycosides, oleanolic acid glycosides.

We isolated and established the structures of neutral triterpene glycosides from stem bark of *Tetrapanax papyriferum* C. Koch (Araliaceae) [1-3]. The present article provides structural data for 12 acidic triterpene glycosides from fractions St-E—St-K.

The isolation of glycosides St-E_1, St-I_1, St-J_1, and St-K_1 was reported earlier [1, 2]. Component St-F_1, which was obtained again by chromatography in acidic solvent systems of the St-F fraction that was not methylated by diazomethane [2], was further repeatedly chromatographed over silica gel. As a result, it separated into three glycosides of similar chromatographic mobilities that were denoted St-F_{1a}, St-F_{1b}, and St-F_{1c}. The glycoside fraction St-G, which had not been previously studied and appeared on TLC as an elongated diffuse spot or as several closely spaced partially overlapping spots (in solvent systems containing formic acid), was separated by chromatography over silica gel with elution by $CHCl_3$:propan-2-ol:water solvent systems with added formic acid. Three new glycoside fractions St-G_1, St-G_2, and St-G_3 were obtained after the separation. The first two fractions gave pure glycosides upon further separation. These were designated St-G_{1a}, St-G_{1b}, St-G_{1c}, and St-G_{2a}, St-G_{2b}, respectively.

Fraction St-G_3 is a mixture of triterpene glycosides. However, further separation was not performed because of the small amount. The chromatographic mobility of all glycosides increased upon adding formic acid to neutral solvent systems. This was consistent with the presence in these compounds of acidic monosaccharides that contain a free carboxylic acid. Total acid hydrolysis of the glycosides showed the presence in them of oleanolic acid as the aglycon. Alkaline hydrolysis of St-E_1, St-F_{1a}, St-F_{1b}, and St-G_{1a} did not change their chromatographic mobilities. This is consistent with localization of the carbohydrate on the C-3 hydroxyl of the aglycon. Furthermore, the ^{13}C chemical shifts in subspectra of the aglycon parts of these glycosides (primarily C-2—C-4, C-17, and C-18) are consistent with 3-O-glycosylated oleanolic acid [4]. Alkaline hydrolysis of the remaining glycosides showed the presence in them of a carbohydrate bonded to the aglycon carboxylic acid. The ^{13}C chemical shifts of the aglycon parts of these compounds are consistent with 3,28-disubstituted oleanolic acid. The chromatographic mobilities of the glycosides did not change after treatment with aqueous ammonia (10%). Therefore, they lack acyl groups.

St-E_1 (**1**), according to total acid hydrolysis, contains glucuronic acid. TLC results showed that **1** was identical in various solvent systems to an authentic specimen of oleanolic acid 3-O-β-D-glucuronopyranoside. This was confirmed by comparing ^{13}C chemical shifts in the NMR spectrum with those in the literature [4].

1) V. I. Vernadskii Tauria National University, 95007, Simferopol′, ul. Yaltinskaya, 4; 2) N. D. Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, B-334, Leninskii pr., 47. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 31-34, January-February, 2004. Original article submitted December 18, 2003.

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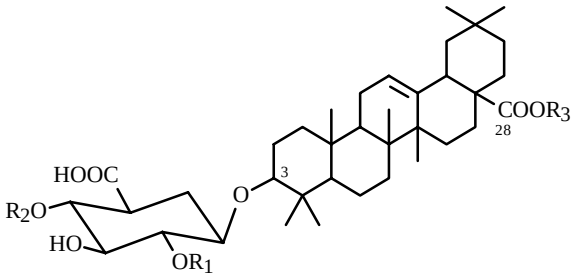

1 - 12

	R_1	R_2	R_3
1:	H-	H-	H-
2:	H-	α-L-Ara*f*	H-
3:	β-D-Gal-	H-	H-
4:	H-	H-	β-D-Glc-
5:	β-D-Gal-	α-*L*-Ara*f*	H-
6:	H-	α-L-Ara*f*	β-D-Glc-
7:	β-D-Gal-	H-	β-D-Glc-
8:	β-D-Gal-	α-L-Ara*f*	β-D-Glc-
9:	H-	H-	α-L-Rha-(1→4)-β-D-Glc-(1→6)-β-D-Glc-
10:	H-	α-*L*-Ara*f*	α-L-Rha-(1→4)-β-D-Glc-(1→6)-β-D-Glc-
11:	β-D-Gal-	H-	α-L-Rha-(1→4)-β-D-Glc-(1→6)-β-D-Glc-
12:	β-D-Gal-	α-L-Ara*f*	α-L-Rha-(1→4)-β-D-Glc-(1→6)-β-D-Glc-

Total acid hydrolysis of St-F_{1a} (**2**) is consistent with the presence in it of arabinose and glucuronic acid. The products of partial acid hydrolysis of **2** contain oleanolic acid and its 3-O-β-D-glucuronopyranoside. This defines the sequence of monosaccharides in the compound. The NMR spectrum of **2** showed the presence of two monosaccharides in the carbohydrate part. Two signals of anomeric C atoms of the monosaccharides were observed in the carbohydrate part of the ^{13}C NMR spectrum of **2** at low-field (106-109 ppm). The characteristic signal of the C-6 carboxyl of glucuronic acid was observed at 170 ppm. Signals of the remaining C atoms were assigned based on literature values for chemical shifts of substituted and unsubstituted sugars. Atom C-4 of the glucuronic acid had a positive α-effect (5.6 ppm), atoms C-3 and C-5, negative β-effects up to 0.9 ppm. This is consistent with localization of the arabinose on C-4 of glucuronic acid. The PMR spectrum of **2** exhibits signals of anomeric H atoms of the monosaccharides as a doublet for glucuronic acid with SSCC 8 Hz (β-configuration of the glycoside bond) and a doublet for arabinose with SSCC $J_{1,2}$ of about 1 Hz. The chemical shifts and the nature of the splitting of the signals for the remaining skeletal protons of the arabinose were found from double-resonance experiments. The magnitudes of the SSCC ($J_{2,3}$ = 3 Hz, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 10.5 Hz, and $J_{4,5'}$ = 0.5 Hz) unambiguously confirm the furanose form of arabinose and the α-configuration of its glycoside bond. Based on these results, it can be concluded that **2** is the already known glycoside R-3, which was isolated earlier from roots of tetrapanax [5]. This was confirmed by comparing the ^{13}C chemical shifts of the monosaccharides in the NMR spectrum of **2** with those for glycoside R-3.

Thus, **2** is the known oleanolic acid 3-O-α-L-arabinofuranosyl-(1→4)-O-β-D-glucuronopyranoside.

According to total acid hydrolysis, St-F_{1b} (**3**) contains monosaccharides galactose and glucuronic acid. The products of partial acid hydrolysis of **3**, like for **2**, contain oleanolic acid and oleanolic acid glucuronopyranoside. This defined the sequence of monosaccharides in the carbohydrate chain of **3**. A comparison of the ^{13}C NMR spectrum of **3** with that in the literature [5] identified the signals of the terminal galactopyranose atoms and the 2-substituted glucuronic acid. The assumption that **3** is the already known glycoside Rb-4, or oleanolic acid 3-O-β-D-galactopyranosyl-(1→2)-O-β-D-glucuronopyranoside, and was isolated previously from roots of tetrapanax was confirmed by comparing the ^{13}C NMR spectrum with that in the literature [5].

TABLE 1. ^{13}C Chemical Shifts of Aglycon and Carbohydrate Parts of **11** and **12** (δ, ppm, 0 = TMS, C_5D_5N)

C atom	Compound		C atom	Compound		C atom	Compound	
	11	**12**		**11**	**12**		**11**	**12**
				Aglycon part				
1	38.6	38.4	11	23.8	23.9	21	34.1	34.0
2	26.4	26.5	12	122.7	122.7	22	32.6	32.5
3	89.0	89.2	13	144.1	144.2	23	28.2	28.2
4	39.5	39.6	14	42.1	42.2	24	16.7	16.6
5	56.1	55.8	15	28.4	28.3	25	15.5	15.6
6	18.7	18.6	16	23.6	23.5	26	17.5	17.5
7	33.2	33.2	17	47.2	47.1	27	25.9	26.1
8	40.0	40.1	18	41.6	41.7	28	176.5	176.6
9	48.2	48.0	19	46.4	46.3	29	33.0	32.9
10	37.0	37.1	20	30.7	30.7	30	23.7	23.7
				Carbohydrate part				
GlcUA			Gal			Glc		
1	107.1	105.0	1	105.2	106.7	1	104.4	104.5
2	83.6	83.1	2	74.6	74.4	2	74.9	75.0
3	76.9	75.6	3	76.8	74.9	3	76.5	76.4
4	72.8	76.4	4	69.4	69.5	4	78.4	78.4
5	74.9	76.9	5	77.3	76.9	5	77.0	76.9
6	170.2	170.3	6	61.3	63.0	6	61.4	61.5
Ara			Glc*			Rha		
1		108.7	1	95.4	95.7	1	102.7	102.7
2		82.6	2	73.7	73.9	2	72.4	72.5
3		78.7	3	78.4	78.6	3	72.5	72.7
4		87.4	4	70.9	71.0	4	73.9	74.1
5		62.6	5	77.8	77.9	5	70.3	70.3
			6	69.2	69.4	6	18.3	18.2

*Glucose bonded to COOH of the aglycon.

The products of total acid hydrolysis of St-G_{1a} (**5**) contained galactose, arabinose, and glucuronic acid. The products of partial acid hydrolysis of **5** contained oleanolic acid and its glucuronopyranoside, like for **2**. The carbohydrate part of the ^{13}C NMR spectrum of **5** exhibits signals for anomeric atoms of three monosaccharides at 104-109 ppm. Furthermore, the signal for the carboxylic C of glucuronic acid appears at 172 ppm. The PMR of **5** has two doublets for H-1 of galactose and glucuronic acid with SSCC $J_{1,2}$ of about 8 Hz each and a singlet for yet another anomeric proton (SSCC $J_{1,2} < 1$ Hz), which is characteristic of α-arabinofuranose. The ^{13}C chemical shifts of arabinose and galactose are practically the same as those in glycosides **2** and **3**. Comparison of the ^{13}C shifts of glucuronic acid with the literature values for unsubstituted glucuronic acid [4] indicate that the additional carbohydrates are located on C-2 and C-4. This was confirmed by the α-effects of these atoms and the β-effects of the neighboring atoms. The bonding sequence of the monosaccharides and the type of glycoside bonds were established using two-dimensional ROESY spectroscopy. Assignments in the ROESY spectrum were made based on the COSY spectrum. Cross-peaks between H-1 of galactose and H-2 of glucuronic acid, H-1 of arabinose and H-4 of glucuronic acid, and H-1 of glucuronic acid and H-3 of the aglycon were identified in the ROESY spectrum.

Thus, **5** is oleanolic acid 3-O-[α-L-arabinofuranosyl-(1→4)-β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranoside, which was found earlier in roots of tetrapanax and was designated as Rb-2. Finally, glycosides Rb-2 and St-G_{1a} were confirmed to be identical by comparing the ^{13}C NMR spectra of these glycosides [5].

The structures of St-F_{1c} (**4**), St-G_{1b} (**6**), St-G_{1c} (**7**), and St-G_{2b} (**8**) were established by analyzing the products of hydrolytic decomposition and by comparing NMR chemical shifts with those obtained for **1**, **2**, **3**, and **5**. Alkaline hydrolysis

of **4**, **6**, **7**, and **8** produced progenins identical to **1**, **2**, **3**, and **5**, respectively. Total acid hydrolysis of **4**, **6**, **7**, and **8** showed that they contain the same monosaccharides as **1**, **2**, **3**, and **5** and an additional glucose. This indicated that **4**, **6**, **7**, and **8** are the 28-O-glucosides of compounds **1**, **2**, **3**, and **5**, respectively. Comparison of the ^{13}C NMR spectra of **4**, **6**, **7**, and **8** with those of **1**, **2**, **3**, and **5** and with literature data confirmed this assumption. Therefore, **4**, **6**, **7**, and **8** are the 28-O-β-D-glucopyranosyl esters of oleanolic acid 3-O-β-D-glucuronopyranoside, oleanolic acid 3-O-α-L-arabinofuranosyl-(1→4)-β-D-glucuronoyranoside, oleanolic acid 3-O-β-D-galactopyranosyl-(1→2)-β-D-glucoronopyranoside, and oleanolic acid 3-O-[α-L-arabinofuranosyl-(1→4)-β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranoside, respectively. Glycoside **4** was found in various species of aralia and ginseng [6] but has not been observed previously in *T. papyriferum*. Compound **6** was isolated from tetrapanax roots [5]; **7** and **8**, from its roots [7] as methyl esters and designated as R-1c and R-1a, respectively.

Acid hydrolysis of St-G_{2a} (**9**) produced glucose, rhamnose, and glucuronic acid. Alkaline hydrolysis of **9** produced the progenin, which was identical to **1**. The chromatographic mobility of **9** is similar to that of **8**. This indicated that it contains four monosaccharides. We think that **9** contains on the carboxyl of the aglycon the trisaccharide fragment α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl, which is known and often found in glycosides of Araliaceae plants.

The TLC and ^{13}C NMR spectrum of **9** were identical to those of an authentic specimen of the 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester of oleanolic acid 3-O-β-D-glucuronopyranoside, which was isolated previously from stems of various *Hedera* species [8, 9]. This glycoside has not been isolated before from *T. papyriferum*.

Alkaline hydrolysis of St-I_1 (**10**), St-J_1 (**11**), and St-K_1 (**12**) produced, like for **6**, **7**, and **8**, the progenins, which were identical to **2**, **3**, and **5**. Total acid hydrolysis of **10**, **11**, and **12** produced glucose, rhamnose, and glucuronic acid. Furthermore, the hydrolysate of **10** contained arabinose; of **11**, galactose; of **12**, arabinose and galactose. Considering that the chromatographic mobilities of these glycosides are rather slow, we assumed that they all contain the same trisaccharide on the COOH of the aglycon as **9** and that the structures of the carbohydrate chains on the C-3 hydroxyl of the aglycon in **10**, **11**, and **12** are the same as in **2**, **3**, and **5**. This was confirmed by the NMR spectra of **10**-**12**, which were compared with those of **6**-**8** and the ^{13}C NMR spectrum of the trisaccharide fragment α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl. Glycoside **10** was obtained previously from tetrapanax roots as the methyl ester and designated as R-1b [7]. It has not been found in other plants of the Araliaceae family. Glycoside **11** was found in *T. papyriferum* and plants of the Araliaceae family for the first time. Glycoside St-K_1 (**12**) is a new triterpene glycoside. It is possible that **1** and **4** are biosynthetic precursors of **9**; **2** and **6**, of **10**; **3** and **7**, of **11**; and **5** and **8**, of **12**.

EXPERIMENTAL

General comments, hydrolysis methods, and isolation of fractions St-E—St-K have been published [1]. The isolation and purification of St-E_1, St-I_1, St-J_1, and St-K_1 corresponding to **1**, **10**, **11**, and **12** from fractions St-E, St-I, St-J, and St-K were also described [1, 2]. Fractions St-F (750 mg) and St-G (620 mg) were obtained again from a larger quantity (118 g) of dried root bark of tetrapanax and were separated chromatographically into St-F_1 (420 mg), St-F_2 (300 mg), and St-G_1 (330 mg), St-G_2 (240 mg), St-G_3 (20 mg), respectively. Components F_1, G_1, and G_2 were further separated chromatographically over silica gel with gradient elution by $CHCl_3$: propan-2-ol solvent systems (5:1→1:1) saturated with water with added formic acid (20 cm^3 per 1 L). This produced St-F_{1a} (140 mg), St-F_{1b} (155 mg), St-F_{1c} (90 mg), St-G_{1a} (210 mg), St-G_{1b} (65 mg), St-G_{1c} (50 mg), St-G_{2a} (90 mg), and St-G_{2b} (135 mg).

The total acid hydrolysates of the glycosides contained the aglycon oleanolic acid and glucuronic acid. Arabinose was detected in the hydrolysates of **2**, **5**, **6**, **8**, **10**, and **12**; galactose, of **3**, **5**, **7**, **8**, **11**, and **12**; glucose, of **4** and **6**-**12**; rhamnose, of **9**-**12**. The products of partial acid hydrolysis of all glycosides contained oleanolic acid and oleanolic acid 3-O-β-D-glucuronopyranoside. Mild ammonolysis did not change the chromatographic mobilities of any glycosides.

Table 1 lists ^{13}C NMR data for **11** and **12**.

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