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TRITERPENE GLYCOSIDES OF *Tetrapanax papyriferum*. I. ISOLATION AND STRUCTURE OF GLYCOSIDES St-H_2 AND St-I_2 FROM STEM BARK

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Stem bark of Tetrapanax papyriferum *C. Koch., Araliaceae, yielded new triterpene glycosides 28-O-α-L-rhamnopyranosyl-(1→4)-O-(6-O-acetyl-β-D-glucopyranosyl)-(1→6)-O-β-D-glucopyranosyl esters of the 3-O-[β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-O-α-L-arabinopyranosides of oleanolic and echinocystic acids. The structures of these substances were established using chemical and physicochemical methods.*

Key words: *Tetrapanax papyriferum,* Araliaceae, triterpene glycosides, new glycosides of oleanolic and echinocystic acids.

Tetrapanax papyriferum C. Koch. (Araliaceae Juss.) is indigenous to Taiwan and is cultivated in South China. It was also successfully introduced to the Black-Sea coast of the Caucuses [1].

The glycoside composition of this plant has been studied previously by Japanese researchers. They described the isolation process and established the structures of triterpene glycosides from leaves [2-6] and roots [7, 8].

We describe the isolation of triterpene glycosides from stem bark of *Tetrapanax papyriferum* and establish the structures of two new glycosides. The triterpene glycosides were isolated by defatting and grinding stem bark and then extracting it with aqueous isopropanol. The solid left after evaporating the extract was separated by chromatography on silica gel with gradient elution by water-saturated $CHCl_3$—C_2H_5OH. Fractions St-A through St-K were obtained.

Fractions St-H and St-I were further purified of phenolic impurities by rechromatography on silica gel and were treated with diazomethane, which separated fraction St-H into two glycosides, St-H_1 with increasing chromatographic mobility owing to the presumed presence in it of glucuronic acid, the carboxyl of which is methylated by diazomethane, and St-H_2 with unchanged chromatographic mobility compared with the starting fraction. The analogous fraction St-I separated into two glycosides St-I_1 with increasing chromatographic mobility and St-I_2, with unchanged mobility.

According to acid hydrolysis, St-H_2 (**1**) contains arabinose, galactose, glucose, rhamnose, and oleanolic acid. Mild ammonolysis of **1** (treatment with aqueous-alcoholic ammonia) produces progenin **2**, total acid hydrolysis of which gives the same sugars and aglycone as in **1**. This suggests that **1** contains one or several acyl units. Strong base hydrolysis of **1** produces progenin **3**, the total acid hydrolysate of which contains arabinose, galactose, and glucose in addition to oleanolic acid. Partial acid hydrolysis of **3** gives oleanolic acid, the 3-O-α-L-arabinopyranoside of oleanolic acid, and two compounds with similar chromatographic mobilities, one of which was identical to an authentic sample of the 3-O-[β-D-glucopyranosyl-(1→3)]-O-α-L-arabinopyranoside of oleanolic acid, which was isolated previously from leaves of *Schefflеropsis angkae* (Araliaceae) [9]. This partially determines the structure of **3**.

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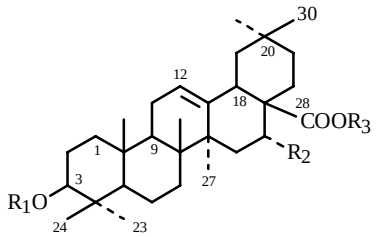

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	R_1	R_2	R_3
1:	βGlc*p*‴ $\xrightarrow{3}$ αAra*p*′→ (2↑ βGal*p*″)	H	←βGlc*p*‴‴←^{6}βGlc*p*‴‴←^{4}αRha*p*‴‴‴ (6↑ OAc)
2:	βGlc*p*‴ $\xrightarrow{3}$ αAra*p*′→ (2↑ βGal*p*″)	H	←βGlc*p*‴‴←^{6}βGlc*p*‴‴←^{4}αRha*p*‴‴‴
3:	βGlc*p*‴ $\xrightarrow{3}$ αAra*p*′→ (2↑ βGal*p*″)	H	H
4:	βGlc*p*‴ $\xrightarrow{3}$ αAra*p*′→ (2↑ βGal*p*″)	OH	←βGlc*p*‴‴←^{6}βGlc*p*‴‴ ←^{4} αRha*p*‴‴‴ (6↑ OAc)
5:	βGlc*p*‴ $\xrightarrow{3}$ αAra*p*′→ (2↑ βGal*p*″)	OH	←βGlc*p*‴‴←^{6}βGlc*p*‴‴←^{4}αRha*p*
6:	βGlc*p*‴ $\xrightarrow{3}$ αAra*p*′→ (2↑ βGal*p*″)	OH	H

PMR and ^{13}C NMR spectra of **1** have showed that it contains six monosaccharides (six signals of anomeric protons at 4.7-6.1 ppm in the PMR and six signals of anomeric C atoms at 95-105 ppm in the ^{13}C NMR spectrum). Signals of the remaining framework protons and C atoms of these monosaccharides were found using standard two-dimensional COSY and HETCOSY methods. The splitting pattern and spin—spin coupling constants (SSCC) found from the cross-peaks were used to determine unambiguously the mutual positions of framework protons of all monosaccharide units. This demonstrated that *α*-arabinose, *α*-rhamnose, *β*-galactose, and three *β*-glucose units were present.

The chemical shifts for the C atoms in the carbohydrate of **1** were compared with the literature values [10] and revealed that the rhamnose, galactose, and one of the glucoses are unsubstituted. Of the remaining monosaccharides, one of the glucoses is substituted at C-4; the other, at C-6; the arabinose, at C-2 and C-3. This follows from the observed positive α-effects (4.5-8 ppm) on these atoms and the negative (up to 3.6 ppm) *β*-effects on neighboring atoms compared with the literature values for the corresponding unsubstituted monosaccharides [10, 11]. Chemical shifts of the aglycone C atoms of **1** agree fully with the literature values for 3,28-di-O-glycosylated oleanolic acid [12].

The bonding sequence of the monosaccharides was established using two-dimenstional ROESY spectroscopy. Assignments in the ROESY spectrum were made based on the COSY spectrum. Cross-peaks H-1(galactose)—H-2(arabinose) and H-1(glucose)—H-3(arabinose) for the trisaccharide fragment on C-3 of the aglycone and H-1(rhamnose)—H-4(inner glucose) and H-1(inner glucose)—H-6(glucose), bound by an acylglycoside bond to the aglycone, and H-1(arabinose)—H-3(aglycone) were identified in the ROESY spectrum.

The high-field region of the PMR spectrum of **1** contains singlets of aglycone methyl protons and a 3H singlet with δ 1.9 ppm, the position of which is characteristic of acetate methyls. The low-field region of the ^{13}C NMR contains a signal for C-28 of the aglycone and a signal for the carbonyl of the acetyl with δ 170.6 ppm.

This indicates that **1** contains one acetyl on C-6 of the inner glucose in a trisaccharide chain on C-28 of the aglycone because a small (by 2.3 ppm) low-field shift of the C-6 signal of this unit and a high-field (by 3.5 ppm) shift of the signal of neighboring C-5 are observed in the ^{13}C NMR. This is usually observed for 6-O-acetylated glucopyranose [13].

Thus, glycoside St-H_2 is the 28-O-*α*-L-rhamnopyranosyl-(1→4)-O-(6-O-acetyl-*β*-D-glucopyranosyl)-(1→6)-*β*-D-glucopyranosyl ester of the 3-O-[*β*-D-glucopyranosyl-(1→3)]-[*β*-D-galactopyranosyl-(1→2)]-O-*α*-L-arabinopyranoside of oleanolic acid and is a new triterpene glycoside.

Glycoside St-I_2 (**4**), according to total acid hydrolysis, contains arabinose, galactose, glucose, rhamnose, and echinocystic acid. Mild ammonolysis of **4** produces progenin **5**, the total acid hydrolysate of which contains the same sugars and aglycone as in **4**. This indicated that **4** contains an acetyl, like in **1**. Total base hydrolysis of **4** produced progenin **6**, which, according to total acid hydrolysis, contains arabinose, galactose, glucose, and the aglycone echinocystic acid. Partial acid hydrolysis of **6** gives according to TLC echinocystic acid and the 3-O-α-L-arabinopyranoside of echinocystic acid, which partially determines the structure of **6**.

TABLE 1. Chemical Shifts of Protons in Aglycones of St-H_2 (**1**) and St-I_2 (**4**) (δ, ppm, 0 = TMS,C_5D_5N)

H-atom	Compound		H-atom	Compound	
	1	**4**		**1**	**4**
1	1.53, 0.93	1.55, 0.95	18	3.15	3.43
2	1.98, 1.80	2.00, 1.82	19	1.75, 1.22	2.66, 1.30
3	3.24	3.25	21	1.37, 1.15	-
5	0.76	0.77	22	1.93, 1.76	-
6	1.47, 1.30	1.45, 1.30	23	1.23	1.22
7	1.45, 1.30	-	24	1.06	1.07
9	1.59	1.72	25	0.89	0.90
11	-	-	26	1.02	1.05
12	5.39	5.55	27	1.22	1.71
15	2.18, 1.14	-	29	0.89	0.90
16	2.06, 1.88	-	30	0.93	0.95

TABLE 2. Chemical Shifts of Protons in Carbohydrates of St-H_2 (**1**) and St-I_2 (**4**) (δ, ppm, 0 = TMS, C_5D_5N)

H-atom	Compound		H-atom	Compound	
	1	**4**		**1**	**4**
Ara′			Glc′′′′		
1	4.70	4.70	1	6.06	6.06
2	4.58	4.60	2	4.01	3.97
3	4.27	4.28	3	4.07	4.08
4	4.44	4.45	4	4.07	4.08
5	3.65, 4.15	3.66, 4.14	5	4.01	4.03
Gal′′			6	4.22, 4.53	4.23, 4.55
1	5.20	5.20	Glc′′′′′		
2	4.32	4.32	1	4.87	4.87
3	3.99	3.99	2	3.81	3.83
4	4.42	4.42	3	4.00	4.02
5	3.80	3.80	4	3.90	3.91
6	4.20, 4.30	4.20, 4.30	5	3.76	3.76
Glc′′′			6	4.42, 4.57	4.44, 4.58
1	5.08	5.10	$C\underline{H}_3CO$-	1.90	1.90
2	3.89	3.90	Rha′′′′′′		
3	4.04	4.08	1	5.38	5.40
4	4.01	4.03	2	4.45	4.47
5	3.81	3.83	3	4.32	4.36
6	4.16, 4.33	4.18, 4.35	4	4.13	4.16
			5	4.56	4.60
			6	1.56	1.58

Signals were assigned and NMR spectra of **4** were analyzed analogously to those of **1**. The ^{13}C NMR spectrum of **4** differs from that of **1** only in the chemical shifts of the signals for the aglycone atoms (C-15, C-16, C-17, C-21, C-27, and C-30). Chemical shifts of the aglycone C atoms of **4** are consistent with 3,28-di-O-glucosylated echinocystic acid [12]. The nature of the monosaccharide units and their bonding sequence in addition to the location of the acetyl in **4** were determined as for **1**.

TABLE 3. Chemical Shifts of ^{13}C Atoms in Aglycones of St-H_2 (**1**) and St-I_2 (**4**) (δ, ppm, 0 = TMS, C_5D_5N)

C-atom	Compound		C-atom	Compound	
	1	**4**		**1**	**4**
1	39.0	39.0	16	23.5	74.2
2	26.4	26.5	17	47.1	49.3
3	89.0	89.0	18	41.7	41.3
4	39.6	39.6	19	46.4	47.1
5	56.1	56.1	20	30.7	30.6
6	18.6	18.5	21	34.1	35.8
7	33.2	33.4	22	32.6	31.8
8	40.0	40.1	23	28.2	28.1
9	48.1	47.2	24	16.7	16.7
10	37.1	37.0	25	15.5	15.6
11	23.8	23.8	26	17.5	17.5
12	122.9	122.6	27	25.9	27.1
13	144.1	144.3	28	176.4	175.9
14	42.2	42.0	29	33.0	33.0
15	28.2	36.0	30	23.7	24.8

TABLE 4. Chemical Shifts of ^{13}C Atoms in Carbohydrates of St-H_2 (**1**) and St-I_2 (**4**) (δ, ppm, 0 = TMS, C_5D_5N)

C-atom	Compound		C-atom	Compound	
	1	**4**		**1**	**4**
Ara′			Glc′′′′		
1	104.8	104.9	1	95.5	95.6
2	77.3	77.3	2	73.7	73.8
3	82.5	82.7	3	78.4	78.4
4	68.0	68.1	4	71.1	70.9
5	65.0	65.1	5	77.7	77.8
Gal′′			6	69.4	69.4
1	104.8	104.9	Glc′′′′′		
2	73.2	73.2	1	104.4	104.4
3	75.1	75.2	2	74.8	74.8
4	69.7	69.7	3	76.3	76.3
5	76.2	76.2	4	79.6	79.4
6	61.7	61.6	5	73.6	73.6
Glc′′′			6	63.7	63.6
1	104.5	104.5	$\underline{C}H_3CO$-	20.4	20.5
2	75.0	75.1	$CH_3\underline{C}O$-	170.6	170.6
3	77.9	78.0	Rha′′′′′′		
4	71.5	71.5	1	102.6	102.7
5	78.1	78.2	2	72.0	72.1
6	62.5	62.4	3	72.4	72.4
			4	73.6	73.6
			5	70.5	70.5
			6	18.1	18.2

Therefore, St-I_2 is the 28-O-α-L-rhmnopyranosyl-(1→4)-O-(6-O-acetyl-β-D-glucopyranosyl)-(1→6)-O-β-D-glucopyranosyl ester of the 3-O-[β-D-glucopyranosyl-(1→3)]-[β-D-galactopyranosyl-(1→2)]-O-α-L-arabinopyranoside of echinocystic acid. This glycoside is also a new triterpene glycoside.

EXPERIMENTAL

NMR spectra were recorded on a Bruker DRX-500 instrument (500 MHz for ^1H and 125 MHz for ^{13}C) in C_5D_5N at 30°C. Two-dimensional COSY, HETCOSY, and ROESY spectra were obtained by standard methods of Bruker. Spin-locking time in ROESY experiments was set at 100 ms. TLC monitoring was performed on Silufol UV-254 plates using $CHCl_3$—CH_3OH—NH_4OH (25%) (100:50:15, 100:40:10, 100:30:5), $CHCl_3$—CH_3OH—H_2O (100:40:7, 100:30:5), and C_6H_6—$(CH_3)_2CO$ (4:1). Glycosides and aglycones were detected using 10% alcoholic phosphotungstic acid; sugars, 10% alcoholic acid anilinium phthalate with subsequent heating to 100°C. Preparative separation and purification of the glycosides was carried out on silica gel L (40-100 μm).

Total acid hydrolysis was carried out by dissolving glycosides in dioxane and CF_3CO_2H (2 N) (1:1) and heating at 100°C for 2 h. The aglycone was extracted with benzene and analyzed by TLC on Silufol UV-254 plates using C_6H_6—$(CH_3)_2CO$ (4:1) and authentic samples. Sugar in the hydrolysate was identified by TLC using $CHCl_3$—CH_3OH—H_2O (100:40:7) or $CHCl_3$—CH_3OH—NH_4OH (25%) (100:40:10) and authentic samples.

Partial Acid Hydrolysis. Solutions of glycosides were heated in the same mixture for 15 min. Progenins were extracted by water-saturated *n*-butanol and analyzed by TLC using authentic samples and $CHCl_3$—CH_3OH—H_2O (100:30:5).

Total base hydrolysis was carried out by dissolving glycosides in KOH (10%) in H_2O—CH_3OH (1:1), heating at 100°C for 2 h with subsequent dilution with water, neutralization with aqueous H_2SO_4 (1 N) until weakly acidic, extraction of progenins with *n*-butanol, and analysis by TLC using $CHCl_3$—CH_3OH—H_2O (100:40:7).

Mild Ammonolysis. Solutions of glycosides were stored in aqueous ammonia (25%) and ethanol (1:1) at 20°C for 3 h, neutralized by cation exchanger KU-2-8 in the H^+-form, and analyzed by TLC using $CHCl_3$—CH_3OH—H_2O (100:40:7).

Methylation by Diazomethane. A solution of glycoside in aqueous CH_3OH (90%) was treated with diazomethane in ether until the yellow color was stable. The solution was evaporated after 15 min and analyzed by TLC.

Isolation and Purification of Glycosides. Dried and ground stem bark of *Tetrapanax papyriferum* (18 g) was defatted by C_6H_6—$CHCl_3$ (7:3, 3×150 mL) and extracted with aqueous 2-propanol (80%, 4×200 mL). The combined extracts were evaporated to give dry solid (2.3 g), which was separated by chromatography on silica gel (250 g) using gradient elution with water-saturated $CHCl_3$—C_2H_5OH (10:1 → 1:1) to give 11 fractions of triterpene glycosides St-A (20 mg), St-B (17 mg), St-C (38 mg), St-D (30 mg), St-E (150 mg), St-F (115 mg), St-G (95 mg), St-H (225 mg), St-I (250 mg), St-J (140 mg), and St-K (140 mg). Fractions St-H and St-I were further purified from phenolic compounds by rechromatography on silica gel with elution by water-saturated $CHCl_3$—C_2H_5OH (2:1). Both fractions were treated with diazomethane and chromatographed on silica gel with elution by the same solvent system to give pure glycosides St-H_1 (50 mg), St-H_2 (155 mg), St-I_1 (130 mg), and St-I_2 (47 mg).

Glycoside St-H_2 (1). The total acid hydrolysate of **1** contained arabinose, galactose, glucose, rhamnose, and oleanolic acid. Mild ammonolysis of **1** gives progenin **2**. The hydrolysate of **2** contains the same sugars and aglycone as that of **1**. Base hydrolysis of **1** gives progenin **3**. The acid hydrolysate of **3** contains arabinose, galactose, glucose, and oleanolic acid. Partial acid hydrolysis of **3** produces oleanolic acid, the 3-O-*α*-L-arabinopyranoside of oleanolic acid, and the 3-O-[*β*-D-glucopyranosyl-(1→3)]-O-*α*-L-arabinopyranoside of oleanolic acid. NMR spectra of **1** are given in Tables 1-4.

Glycoside St-I_2 (4). The total acid hydrolysate of **4** contains arabinose, galactose, glucose, rhamnose, and echinocystic acid. Mild ammonolysis of **4** gives progenin **5**. The hydrolysate of **5** contains the same sugars and aglycone as that of **4**. Base hydrolysis of **4** produces progenin **6**. The acid hydrolysate of **6** contains arabinose, galactose, glucose, and echinocystic acid. Partial acid hydrolysis of **6** produces echinocystic acid and the 3-O-α-L-arabinopyranoside of echinocystic acid. NMR data for **4** are given in Tables 1-4.

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