Triterpene Saponins from *Tetrapanax papyriferum* K. KOCH

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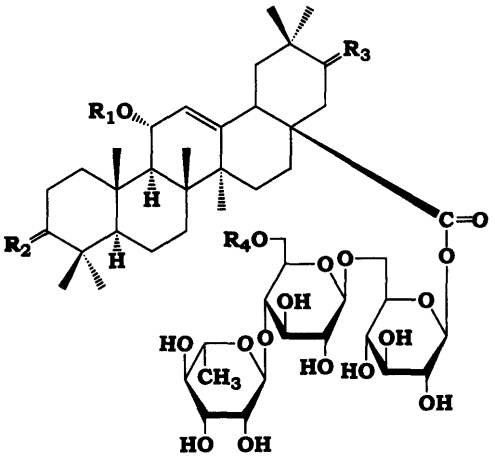
Four new oleanane-type saponins, papyrioside LA—LD, were isolated from the leaves of *Tetrapanax papyriferum*. The structures of these new compounds were elucidated as 11α-hydroxy-3,21-dioxo-olean-12-en-28-oyl-α-L-rhamnopyranosyl-(1→4)-(6-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-β-D-glucopyranoside, 11α-methoxy-3,21-dioxo-olean-12-en-28-oyl-α-L-rhamnopyranosyl-(1→4)-(6-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-β-D-glucopyranoside, 3α-hydroxy-11α-methoxy-21-oxo-olean-12-en-28-oyl-α-L-rhamnopyranosyl-(1→4)-(6-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-β-D-glucopyranoside and 21α-hydroxy-11α-methoxy-3-oxo-olean-12-en-28-oyl-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, respectively, on the basis of spectroscopic and chemical evidence.

Key words *Tetrapanax papyriferum*; Araliaceae; papyrioside; oleanane-type triterpene saponin

Tetrapanax papyriferum K. KOCH (Araliaceae) has been used as a material for paper production in Japan, where it is called "kamiyatsude." We previously reported the isolation and structural elucidation of saponins from the leaves of *Tetrapanax papyriferum*.[1-3] This paper deals with the isolation and structure elucidation of four new saponins obtained from the same source, papyrioside LA (**1**), LB (**2**), LC (**3**), LD (**4**). We also isolated papyrioside L-IIa—L-IId at the same time.

The *n*-butanol soluble part of the methanol extract was chromatographed on silica gel followed by Lobar RP-18 chromatography, and was subjected to repeated semi-preparative HPLC on an Asahipack ODP-50 reversed phase column. We isolated eight saponins (LA—LD, L-IIa—L-IId).

The ^{1}H- and ^{13}C-NMR spectra (pyridine-d_5) of **1**, **2**, **3** and **4** showed signals characteristic of an oleanane-type triterpene. Acid hydrolysis of pure aliquots allowed the characterization, by GC, of the sugar components of **1**—**4** as glucose and rhamnose.



	R_1	R_2	R_3	R_4
1	H	O	O	Ac
2	Me	O	O	Ac
3	Me	H, OH	O	Ac
4	Me	O	H, OH	H

Compound **1** exhibited an $[M+Na]^+$ ion peak at *m/z* 1020 in the FAB-MS, whose high-resolution MS (HR-MS) analysis established that the molecular formula of **1** is $C_{50}H_{76}O_{20}$. In connection with the aglycone moiety, the presence in the ^{1}H- and ^{13}C-NMR spectra of seven tertiary methyl signals at δ_H 1.05, 1.09, 1.16, 1.18, 1.19, 1.22 and 1.28, δ_C 16.2, 18.7, 21.5, 24.4, 25.0, 25.6 and 26.6 and eight methylene signals at δ_C 19.9, 25.7, 28.0, 33.2, 34.6, 41.5, 46.2 and 46.7 indicated that the aglycone was an oleanane-type triterpene (Tables 1 and 2). The presence of a tri-substituted olefinic linkage at 12-C/13-C was confirmed by the appropriate ^{1}H (δ 5.89) and ^{13}C (δ 129.4, 142.9) signals. One secondary hydroxyl group was assigned to the 11α position based on the presence of hydroxymethine signals at δ_H 4.57 (1H, dd, J=3.7, 9.2 Hz) and δ_C 66.8, and three carbonyl carbon signals assigned as 28-C, 21-C and 3-C were observed at δ_C 173.8, 212.2 and 216.6, respectively. From these data, the aglycone was assigned as 11α-hydroxy-3,21-dioxo-olean-12-en-28-oic acid. In connection with the sugar moiety, **1** exhibited three anomeric proton and carbon signals at δ_H 6.23 (1H, d, J=7.9 Hz, 1′-H), 5.53 (1H, brs, 1‴-H), 4.97 (1H, d, J=7.9 Hz, 1″-H); and δ_C 96.0 (1′-C), 102.8 (1‴-C), 104.8 (1″-C). Also, one acetyl group was observed (δ_H 1.93, δ_C 20.5, 170.5).

In the heteronuclear multiple bond connectivity (HMBC) experiment, long range correlations were observed between the following protons and carbons (1‴-H and 4″-C, 1″-H and 6′-C, 1′-H and 28-C, 6″-H and acetyl carbonyl carbon) (Fig. 1). A second attempt to determine the structure of the sugar-chain using the rotating frame Overhauser enhancement spectroscopy (ROESY) technique[4,5] allowed the observation of an NOE between 1‴-H and 4″-H, 1″-H and 6′-H (Fig. 1). Thus, **1** was determined to be 11α-hydroxy-3,21-dioxo-olean-12-en-28-oyl-α-L-rhamnopyranosyl-(1→4)-(6-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-β-D-glucopyranoside.

Compound **2** showed an $[M+Na]^+$ ion peak at *m/z*

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Table 1. ^{13}C-NMR Spectral Data of Compounds **1**—**4** in Pyridine-d_5

	1	2	3	4
Aglycone				
1	34.6	34.6	34.5	34.7
2	41.5	40.5	26.5	40.6
3	216.6	216.3	75.3	216.5
4	47.7	47.7	38.9	47.8
5	55.8	55.5	49.2	55.7
6	19.9	19.9	18.8	20.0
7	33.2	32.9	33.4	33.2
8	38.0	38.1	38.1	38.2
9	54.9	51.2	53.6	50.0
10	43.2	43.1	43.6	43.0
11	66.8	76.1	76.0	76.3
12	129.4	123.0	123.8	122.5
13	142.9	146.0	146.4	149.4
14	42.1	42.1	42.1	42.8
15	28.0	28.1	28.2	28.8
16	25.7	25.6	25.6	26.9
17	45.3	45.5	45.5	47.3
18	40.7	40.9	40.7	41.6
19	46.7	47.2	47.5	41.1
20	50.8	50.9	50.8	35.8
21	212.2	212.0	212.2	73.4
22	46.2	46.2	46.4	39.5
23	26.6	26.5	29.5	26.7
24	21.5	21.5	22.7	21.6
25	16.2	16.5	17.4	16.6
26	18.7	18.9	19.3	19.1
27	25.6	25.3	25.5	24.9
28	173.8	173.9	174.0	176.6
29	24.4	24.6	24.7	28.4
30	25.0	25.0	25.0	24.9
OMe		54.4	54.6	54.1
Glucose (in)				
1	96.0	96.1	96.1	95.8
2	76.2	76.3	76.3	76.5
3	78.5	78.6	78.7	78.8
4	70.7	70.8	70.9	70.9
5	78.0	78.0	78.1	78.1
6	69.4	69.6	69.7	69.3
Glucose (out)				
1	104.8	105.0	105.0	105.0
2	74.9	75.0	75.0	75.3
3	73.7	73.7	73.8	73.9
4	79.1	79.2	79.2	78.3
5	73.6	73.7	73.8	77.2
6	63.5	63.6	63.7	61.3
OCOCH$_3$	170.5	170.5	170.6	
OCOCH$_3$	20.5	20.6	20.6	
Rhamnose				
1	102.8	102.9	102.9	102.7
2	72.3	72.3	72.4	72.6
3	72.5	72.6	72.7	72.8
4	73.7	73.7	73.8	74.0
5	70.6	70.7	70.7	70.4
6	18.4	18.5	18.5	18.5

1034 in the FAB-MS. Combined with the result of HR-MS, its molecular formula was deduced to be $C_{51}H_{78}O_{20}$. The ^{1}H- and ^{13}C-NMR chemical shifts of **2** were similar to those of **1** except for the appearance of a methoxyl group (δ_H 3.27, δ_C 54.4) instead of a hydroxyl group, and methoxymethine signals were observed at δ_H 3.93 (11-H; −0.64 from **1**) and δ_C 76.1 (11-C; +9.3 from **1**). The HMBC experiment showed cross peaks between 11-H and the methoxyl carbon, and the methoxyl proton and 11-C. Therefore, the structure of **2** was characterized as 11α-methoxy-3,21-dioxo-olean-12-en-28-oyl-α-L-rhamnopyranosyl-(1→4)-(6-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-β-D-glucopyranoside.

The FAB-MS spectrum of **3** exhibited a peak at *m/z* 1036 $[M+Na]^+$, indicating a molecular formula of $C_{51}H_{80}O_{20}$, which was confirmed by HR-MS. Its ^{1}H- and ^{13}C-NMR chemical shifts resembled those of **2** except for the presence of 3α-hydroxymethine (δ_H 3.63, δ_C 75.3) and the absence of 3-C carbonyl carbon (δ_C 216.3). The chemical shifts, COSY and HMBC experiments indicated that the aglycone structure has to be 3α-hydroxy-21-oxo-olean-12-ene skeleton, and the sugar chain was same as **1** and **2**. From these data, the structure of **3** was determined to be 3α-hydroxy-11α-methoxy-21-oxo-olean-12-en-28-oyl-α-L-rhamnopyranosyl-(1→4)-(6-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-β-D-glucopyranoside.

A molecular formula of $C_{49}H_{78}O_{19}$ was deduced for **4** from its FAB-MS, which displayed a molecular ion peak at *m/z* 994 $[M+Na]^+$, and the HR-MS confirmed it. The structure of the aglycone was deduced to be a 21α-hydroxy-3-oxo-oleanane type from the presence of a 21α-hydroxymethine (δ_H 3.70, δ_C 73.4) and a 3-C carbonyl carbon (δ_C 216.5), by comparison of the NMR data of **2** and **3**. The sugar chain at 28-C was deduced to have no acetyl group from the absence of an acetyl signal in the ^{1}H- and ^{13}C-NMR, and by the observation of deacylation shifts at 6″-C (δ_C 61.3; −2.3) and 5″-C (δ_C 77.2; +3.5) of the glucose part compared to **2**. Taken together, these data indicated that **4** is 21α-hydroxy-11α-methoxy-3-oxo-olean-12-en-28-oyl-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Experimental

General Procedures Melting points were determined with a Yanagimoto microapparatus and are uncollected. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JMN A-500 FT-NMR spectrometer, and chemical shifts were given in ppm with tetramethylsilane as an internal standard. FAB-MS was recorded on a JEOL JMS-HX 110 mass spectrometer. Optical rotations were measured with a Jasco DIP-4 digital polarimeter. Gas chromatography (GC) was run on a Shimadzu GC-6A gas chromatograph. The Lobar column used was LiChroprep RP-18 (Merck). Semi-preparative HPLC was carried out on a column of Asahipak ODP-50 (10 mm × 250 mm). TLC was conducted on pre-coated silica gel plates (Merck Kieselgel 60F_{254} Art. 5715) with $CHCl_3$–MeOH–H_2O (61 : 32 : 7) as a developing solvent. Column chromatography was carried out on silica gel (Merck Kieselgel 60 Art. 7734).

Extraction and Isolation Dried powdered leaves (607.5 g) of *Tetrapanax papyriferum*, collected at Nagoya, Japan, were extracted with methanol (6 l × 2, 8 h in each) under reflux. The methanol extract was concentrated under reduced pressure and the residue (131.4 g) was suspended in water. The suspension was extracted with ether. The water layer was extracted with *n*-butanol and then the butanol soluble fraction was concentrated *in vacuo* to give a residue (34.3 g). The *n*-butanol extract was chromatographed on a silica gel with $CHCl_3$–MeOH–H_2O (80 : 20 : 2→70 : 30 : 3) to give four fractions (frs. 1—4). From frs. 1, 2, 3 and 4, **1** (35 mg), **2** (42 mg), **3** (24 mg), **4** (14 mg), L-IIa (406 mg), L-IIb (12 mg), L-IIc (124 mg) and L-IId (6 mg) were isolated by the Lobar RP-18 (33% aqueous CH_3CN) and semi-preparative HPLC (33% aqueous CH_3CN).

Papyrioside LA (1) Amorphous powder, $[\alpha]_D^{27}$ −21.5° (*c*=4.7, methanol). High-resolution FAB-MS: Calcd for $C_{50}H_{76}O_{20}Na$ $[M+Na]^+$, 1019.4828. Found, 1019.4776. FAB-MS 1020 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1, 2.

Papyrioside LB (2) Amorphous powder, $[\alpha]_D^{27}$ −23.9° (*c*=2.3, methanol). High-resolution FAB-MS: Calcd for $C_{51}H_{78}O_{20}Na$ $[M+Na]^+$, 1033.4984. Found, 1033.5029. FAB-MS 1034 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1, 2.

Table 2. ^{1}H-NMR Spectral Data of Compounds **1**—**4** in Pyridine-d_5

	1	2	3	4
Aglycone				
3			3.63 (1H, br s)	
11	4.57 (1H, dd, J=3.7, 9.2 Hz)	3.93 (1H, dd, J=3.7, 8.6 Hz)	3.87 (1H, dd, J=3.7, 8.5 Hz)	3.96 (1H, dd, J=3.7, 8.5 Hz)
12	5.89 (1H, d, J=3.7 Hz)	5.86 (1H, d, J=3.7 Hz)	5.85 (1H, d, J=4.3 Hz)	5.79 (1H, d, J=3.7 Hz)
18	3.64 (1H, br d)	3.71 (1H, dd, J=4.3, 14.0 Hz)	3.69 (1H, br d)	3.48 (1H, dd, J=4.3, 14.0 Hz)
21				3.70 (1H, d, J=3.7 Hz)
23	1.19 (3H, s)	1.17 (3H, s)	1.23 (3H, s)	1.16 (3H, s)
24	1.09 (3H, s)	1.07 (3H, s)	0.94 (3H, s)	1.08 (3H, s)
25	1.22 (3H, s)	1.12 (3H, s)	1.12 (3H, s)	1.14 (3H, s)
26	1.18 (3H, s)	1.12 (3H, s)	1.13 (3H, s)	1.19 (3H, s)
27	1.28 (3H, s)	1.25 (3H, s)	1.18 (3H, s)	1.40 (3H, s)
29	1.16 (3H, s)	1.23 (3H, s)	1.21 (3H, s)	1.17 (3H, s)
30	1.05 (3H, s)	1.10 (3H, s)	1.05 (3H, s)	1.03 (3H, s)
OMe		3.27 (3H, s)	3.29 (3H, s)	3.26 (3H, s)
Glucose (in)				
1	6.23 (1H, d, J=7.9 Hz)	6.25 (1H, d, J=7.9 Hz)	6.24 (1H, d, J=8.5 Hz)	6.31 (1H, d, J=7.9 Hz)
2	4.11[a]	4.10[a]	4.10[a]	4.14 (1H, dd, J=4.3, 9.2 Hz)
3	4.22 (1H, t, J=8.5 Hz)	4.22 (1H, t, J=9.2 Hz)	4.22 (1H, t, J=9.2 Hz)	4.22 (1H, t, J=8.5 Hz)
4	4.26 (1H, br d)	4.28 (1H, br d)	4.27 (1H, br d)	4.29 (1H, br d)
5	4.14 (1H, m)	4.14[a]	4.14 (1H, m)	4.13[a]
6	4.34[a]	4.34[a]	4.34[a]	4.41 (1H, m)
	4.73 (1H, br d)	4.71 (1H, br d)	4.71 (1H, br d)	4.70 (1H, br d)
Glucose (out)				
1	4.97 (1H, d, J=7.9 Hz)	4.95 (1H, d, J=7.9 Hz)	4.95 (1H, d, J=7.9 Hz)	4.98 (1H, d, J=7.9 Hz)
2	3.95 (1H, t, J=7.9 Hz)	3.95 (1H, t, J=7.9 Hz)	3.95 (1H, t, J=7.9 Hz)	3.95 (1H, t, J=7.9 Hz)
3	4.11[a]	4.10[a]	4.10[a]	4.13[a]
4	4.09[a]	4.10[a]	4.08[a]	4.41 (1H, t, J=9.2 Hz)
5	3.80 (1H, t, J=4.9 Hz)	3.81 (1H, t, J=4.9 Hz)	3.80 (1H, t, J=4.9 Hz)	3.67 (1H, br d)
6	4.54 (1H, br d)	4.54 (1H, br d)	4.54 (1H, br d)	4.11[a]
	4.62[a]	4.62[a]	4.62[a]	4.21 (1H, m)
$OCOCH_3$	1.93 (3H, s)	1.93 (3H, s)	1.93 (3H, s)	
Rhamnose				
1	5.53 (1H, br s)	5.54 (1H, br s)	5.53 (1H, br s)	5.84 (1H, br s)
2	4.63[a]	4.62[a]	4.62[a]	4.67 (1H, br d)
3	4.51 (1H, dd, J=3.1, 9.2 Hz)	4.50[a]	4.50 (1H, dd, J=3.7, 9.2 Hz)	4.55 (1H, br d)
4	4.33 (1H, t, 9.2 Hz)	4.32[a]	4.33 (1H, t, J=9.3 Hz)	4.32[a]
5	4.85 (1H, m)	4.85 (1H, m)	4.85 (1H, m)	4.96 (1H, m)
6	1.71 (3H, d, J=6.1 Hz)	1.71 (3H, d, J=6.7 Hz)	1.71 (3H, d, J=6.1 Hz)	1.71 (3H, d, J=6.1 Hz)

a) Overlapped with other signals.

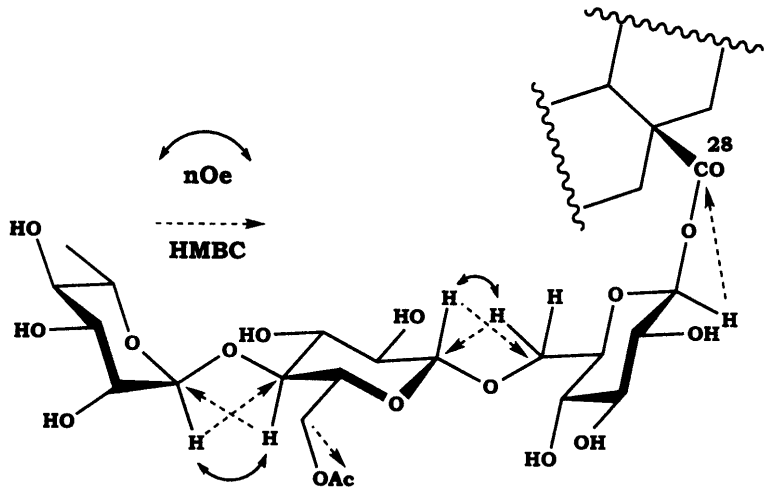

Fig. 1

Papyrioside LC (3) Amorphous powder, $[\alpha]_D^{27}$ −34.1° (c=1.6, methanol). High-resolution FAB-MS: Calcd for $C_{51}H_{80}O_{20}Na$ $[M+Na]^+$, 1035.5138. Found, 1035.5074. FAB-MS 1036 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1, 2.

Papyrioside LD (4) Amorphous powder, $[\alpha]_D^{27}$ −24.5° (c=0.7, methanol). High-resolution FAB-MS: Calcd for $C_{49}H_{78}O_{19}Na$ $[M+Na]^+$, 993.5035. Found, 993.5030. FAB-MS 994 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1, 2.

Acid Hydrolysis of Compounds 1—4 A sample of each compound (*ca.* 1 mg) was heated at 120 °C with 0.3 ml of 2 N trifluoroacetic acid for 5 h. The reaction mixture was concentrated to yield a residue, which was trimethylsilylated with 0.5 ml of trimethylsilylating reagent (TMS-HT, TCI) for 1 h. The trimethylsilyl derivative was subjected to GC, which identified the derivatives of glucose and rhamnose as 2 : 1. GC conditions:

column 3% SE-30 (3.2 mm × 2 m), column temperature 170 °C, injection temperature 190 °C, carrier gas N_2. t_R: glucose 32.8, 53.2 min, rhamnose 9.7, 13.2 min.

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