Minor Saponins from *Tetrapanax papyriferum*

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Four new minor saponins, papyrioside LE—LH, were isolated from the leaves of *Tetrapanax papyriferum*, and their structures were determined on the basis of spectroscopic evidence.

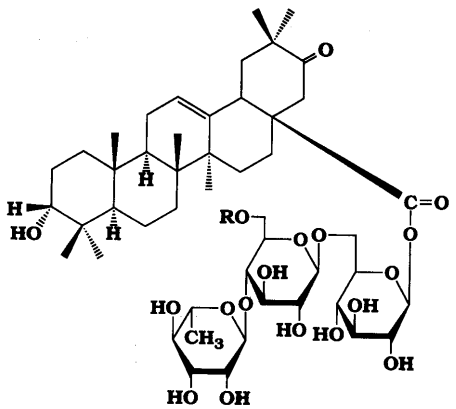
Key words *Tetrapanax papyriferum*; Araliaceae; papyrioside; triterpene saponin

Tetrapanax papyriferum K. KOCH (Araliaceae, Japanese name kamiyatsude) has been used as a material for paper production. We previously reported the isolation and structural elucidation of triterpenoid glycosides, called papyrioside L-IIa—L-IId,[1-3] LA—LD,[4] isolated from the leaves of *Tetrapanax papyriferum*. In a continuing investigation of oligoglycosidic constituents, we identified four new minor saponins designated as papyrioside LE (**1**), LF (**2**), LG (**3**) and LH (**4**). This paper deals with the isolation and structural elucidation of these saponins.

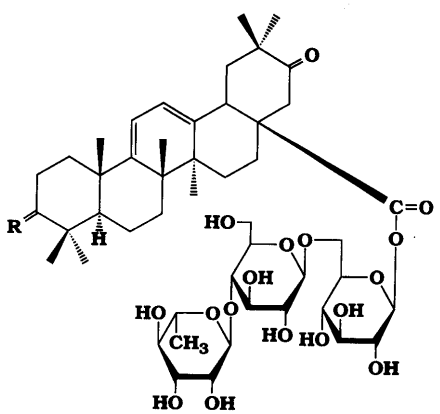
Compound **1** showed a $[M+Na]^+$ peak at *m/z* 963.4965 ($C_{48}H_{76}NaO_{18}$). The presence in the ^{1}H- and ^{13}C-NMR spectra of seven tertiary methyl signals at δ_H 0.90, 0.98, 1.06, 1.09, 1.11, 1.20, 1.21, δ_C 15.6, 17.5, 22.7, 24.6, 25.2, 26.0, 29.3, and nine methylene signals at δ_C 18.6, 23.8, 25.9, 26.3, 28.1, 33.1, 33.6, 46.5, 47.5, all originating from the aglycone moiety, indicated that the aglycone was an oleanane-type triterpenoid. The presence of a tri-substituted olefinic linkage at C-12/C-13 was confirmed by the appropriate ^{1}H (δ 5.63) and ^{13}C (δ 124.4, 141.9) signals. One secondary hydroxyl group was assigned to the 3α position based on the presence of hydroxymethine signals at δ_H 3.61 (br s), δ_C 75.3, two carbonyl carbon signals observed at δ_C 174.0 and 212.5 were assigned to C-28 and C-21, respectively, and the aglycone was assigned as 3α-hydroxy-21-oxooleane-12-en-28-oic acid. This aglycone was the same as papyriogenin F.[3]

On acidic hydrolysis, **1** provided glucose and rhamnose (GC) as a sugar moiety. In the ^{13}C-NMR spectrum, three anomeric carbons were observed at δ 96.1, 102.8, 105.1. In the ^{1}H-NMR spectrum, one doublet methyl signal belonging to rhamnose was observed. Thus, **1** should contain two glucose units and one rhamnose unit. In the ^{1}H-NMR spectrum, three anomeric protons were exhibited at δ 6.22 (1H, d, J=9 Hz), 5.84 (1H, br s), 4.96 (1H, d, J=8 Hz). All protons of the three sugar units were assigned unambiguously from the correlation spectroscopy (COSY) spectrum. A heteronuclear multiple quantum coherence (HMQC) experiment correlated all proton resonances with those of the corresponding carbons in each of the sugar units.

Information concerning the sequence of the oligosaccharide chains and the linkage sites to the aglycone were obtained by spatial correlation between two protons in a rotating frame Overhauser enhancement spectroscopy (ROESY) experiment,[5,6] as well as by the scalar coupling between a carbon and a proton of the neighboring residue in a heteronuclear multiple bond correlation (HMBC) experiment. In the ROESY spectrum, significant rotating frame nuclear Overhauser effect (rOe) correlation cross-peaks were observed between $H_{G'-1}$ (δ 4.96) and H_{G-6} (δ 4.31, 4.68), H_{R-1} (δ 5.84) and $H_{G'-4}$ (δ 4.39). From the HMBC spectrum, correlation peaks were observed from the following pairs: H_{G-1} (δ 6.22)/C-28 (δ 174.0), $H_{G'-1}$ (δ 4.96)/C_{G-6} (δ 69.5), H_{G-6} (δ 4.31)/$C_{G'-1}$ (δ 105.1), H_{R-1} (δ 5.84)/$C_{G'-4}$ (δ 78.4), $H_{G'-4}$ (δ 4.39)/C_{R-1} (δ 102.8).



	R
1	H
2	Ac



	R
3	
4	O

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

	1	**2**	**3**	**4**
Aglycone				
3	3.61[a)]	3.62 (1H, br s)	3.62 (1H, br s)	
11	2.00 (2H, br d, J=10 Hz)	1.97 (2H, br d, J=10 Hz)	5.83[a)]	5.75 (1H, d, J=7 Hz)
12	5.63 (1H, d, J=4 Hz)	5.62 (1H, d, J=14 Hz)	5.83[a)]	5.83 (1H, d, J=7 Hz)
18	3.61[a)]	3.61 (1H, br d, J=14 Hz)	3.76 (1H, dd, J=4, 14 Hz)	3.77 (1H, br d)
23	1.20 (3H, s)	1.21 (3H, s)	1.23 (3H, s)	1.15 (3H, s)
24	0.90 (3H, s)	0.90 (3H, s)	0.92 (3H, s)	1.08 (3H, s)
25	0.98 (3H, s)	0.98 (3H, s)	1.27 (3H, s)	1.21 (3H, s)
26	1.11 (3H, s)	1.10 (3H, s)	1.41 (3H, s)	1.38 (3H, s)
27	1.06 (3H, s)	1.06 (3H, s)	1.08 (3H, s)	1.08 (3H, s)
29	1.21 (3H, s)	1.22 (3H, s)	1.18 (3H, s)	1.20 (3H, s)
30	1.09 (3H, s)	1.09 (3H, s)	1.06 (3H, s)	1.10 (3H, s)
Glucose (inner)				
1	6.22 (1H, d, J=9 Hz)	6.21 (1H, d, J=8 Hz)	6.24 (1H, d, J=8 Hz)	6.24 (1H, d, J=8 Hz)
2	4.13[a)]	4.11 (1H, dd, J=7, 14 Hz)	4.13[a)]	4.13[a)]
3	4.21 (1H, t, J=9 Hz)	4.19 (1H, t, J=9 Hz)	4.20[a)]	4.21[a)]
4	4.32[a)]	4.27 (1H, t, J=9 Hz)	4.31[a)]	4.32[a)]
5	4.13[a)]	4.13[a)]	4.11[a)]	4.13[a)]
6	4.31[a)]	4.33[a)]	4.29[a)]	4.31[a)]
	4.68 (1H, br d, J=10 Hz)	4.69 (1H, br d)	4.67 (1H, br d)	4.68 (1H, br d)
Glucose				
1	4.96 (1H, d, J=8 Hz)	4.96 (1H, d, J=8 Hz)	4.96 (1H, d, J=8 Hz)	4.96 (1H, d, J=8 Hz)
2	3.94 (1H, t, J=8 Hz)	3.94[a)]	3.93 (1H, t, J=8 Hz)	3.94[a)]
3	4.11[a)]	4.10[a)]	4.11[a)]	4.10 (1H, dd, J=5, 8 Hz)
4	4.39 (1H, t, J=9 Hz)	4.07[a)]	4.39 (1H, t, J=9 Hz)	4.39 (1H, t, J=9 Hz)
5	3.66 (1H, br d)	3.81[a)]	3.66 (1H, br d)	3.67 (1H, br d)
6	4.10[a)]	4.54 (1H, dd, J=5, 12 Hz)	4.12[a)]	4.11[a)]
	4.19[a)]	4.63[a)]	4.19[a)]	4.19[a)]
$OCOCH_3$		1.93 (3H, s)		
Rhamnose				
1	5.84 (1H, br s)	5.53 (1H, s)	5.83 (1H, s)	5.84 (1H, s)
2	4.67[a)]	4.62[a)]	4.66[a)]	4.67[a)]
3	4.55 (1H, br d)	4.50[a)]	4.54 (1H, br d)	4.55[a)]
4	4.30 (1H, dd, J=6, 11 Hz)	4.32[a)]	4.30 (1H, br d)	4.31 (1H, br d)
5	4.96[a)]	4.85 (1H, dd, J=6, 9 Hz)	4.94[a)]	4.97[a)]
6	1.71 (3H, d, J=7 Hz)	1.71 (3H, d, J=6 Hz)	1.70 (3H, d, J=6 Hz)	1.70 (3H, d, J=6 Hz)

a) Overlapped with other signals.

Therefore, the structure of **1** was elucidated to be 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3α-hydroxy-21-oxoolean-12-en-28-oic acid.

Compound **2** showed a $[M+Na]^+$ peak at *m/z* 1005.5001 ($C_{50}H_{78}NaO_{19}$), and gave rise to ^{1}H- and ^{13}C-NMR spectra that were similar to those of **1**, except for the presence of signals due to an acetyl residue. A cross-peak between the $H_{G'\text{-}6}$ (δ 4.54) and acetyl carbonyl carbon (δ 170.6) in the HMBC spectrum suggested that the acetyl residue was linked to the 6-position of an outer glucose. When the ^{13}C-NMR data of **2** was compared with that of **1**, acylation shifts were observed at $C_{G'\text{-}5}$ (−3.5) and $C_{G'\text{-}6}$ (+2.3). Therefore, **2** was characterized to be 28-*O*-α-L-rhamnopyranosyl-(1→4)-(6-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-β-D-glucopyranosyl-3α-hydroxy-21-oxoolean-12-en-28-oic acid.

Compound **3** showed a $[M+Na]^+$ peak at *m/z* 961.4759 ($C_{48}H_{74}NaO_{18}$). The presence in the ^{1}H- and ^{13}C-NMR spectra of seven tertiary methyl signals, eight methylene signals, and one secondary hydroxyl group assigned to the 3α position, as well as two carbonyl groups assigned to C-28 and C-21, and two trisubstituted olefinic linkages as homoannular diene at C-9/C-11 and C-12/C-13, all originating from the aglycone moiety, indicated that the aglycone was assigned as 3α-hydroxy-21-oxoolean-9(11),12-dien-28-oic acid. The sugar chain of **3** was exactly the same as that of **1**, and **3** was elucidated to be 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3α-hydroxy-21-oxoolean-9(11),12-dien-28-oic acid.

Compound **4** showed a $[M+Na]^+$ peak at *m/z* 959.4592 ($C_{48}H_{72}NaO_{18}$), and gave rise to ^{1}H- and ^{13}C-NMR spectra that were similar to those of **3**, except for the presence of a carbonyl carbon signal (δ_C 216.1) assigned to 3-C and less of 3α-hydroxymethine signal. Therefore, **4** was 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3,21-dioxoolean-9(11),12-dien-28-oic acid. This aglycone was the same as papyriogenin B.[1)]

Compounds **3** and **4** might be artifacts at extraction, but this is the first time that a glycoside with papyriogenin B was obtained.

Experimental

General Procedures ^{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. Ultraviolet (UV) absorption spectra were determined on a Shimadzu UV-

Table 2. ^{13}C-NMR Spectral Data of **1**—**4** in Pyridine-d_5

	1	2	3	4
Aglycone				
1	33.6	33.6	32.4	34.7
2	26.3	26.3	27.0	37.8
3	75.3	75.1	74.6	216.1
4	40.1	37.9	38.3	47.3
5	49.3	49.3	45.3	51.9
6	18.6	18.6	18.5	19.7
7	33.1	33.1	32.4	31.6
8	37.9	37.5	39.5	38.6
9	47.9	47.9	156.8	154.1
10	37.5	41.9	43.1	43.2
11	23.8	23.8	115.3	117.5
12	124.4	124.4	122.2	122.0
13	141.9	141.8	142.5	143.3
14	41.9	40.1	41.1	40.9
15	28.1	28.1	27.4	27.4
16	25.9	25.9	26.4	26.3
17	45.5	45.5	50.6	50.6
18	41.3	41.2	39.6	39.6
19	47.5	47.5	47.5	47.5
20	51.1	51.6	45.5	45.5
21	212.5	212.5	212.1	212.0
22	46.5	46.5	46.2	46.1
23	29.3	29.3	29.3	27.0
24	22.7	22.7	22.8	21.5
25	15.6	15.6	25.3	25.2
26	17.5	17.5	21.1	20.5
27	26.0	26.0	20.5	20.2
28	174.0	174.0	174.1	174.1
29	24.6	24.7	24.6	24.6
30	25.2	25.2	25.0	25.1
Glucose (inner)				
1	96.1	96.0	96.1	96.5
2	76.5	76.3	76.5	76.5
3	78.6	78.7	78.6	78.6
4	70.9	70.9	70.9	70.9
5	78.1	78.1	78.1	78.1
6	69.5	69.6	69.5	69.5
Glucose				
1	105.1	105.0	105.0	105.1
2	75.2	75.0	75.3	75.3
3	73.7	73.8	73.8	73.8
4	78.4	79.3	78.4	78.4
5	77.2	73.7	77.2	77.2
6	61.4	63.7	61.4	61.4
OCOCH$_3$		170.6		
OCOCH$_3$		20.6		
Rhamnose				
1	102.8	103.0	102.8	102.0
2	72.6	72.4	72.6	72.6
3	72.8	72.7	72.8	72.8
4	74.0	73.8	74.0	74.0
5	70.4	70.7	70.3	70.4
6	18.5	18.5	18.5	18.5

160 spectrometer. Gas chromatography (GC) was run on a Shimadzu GC-6A. Semi-preparative HPLC was carried out on a column of Asahipak ODP-50 (10 mm × 250 mm). 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 (6l × 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). **1** (6 mg), **2** (10 mg), **3** (5 mg) and **4** (3 mg) were isolated by semi-preparative HPLC (CH_3CN : THF : H_2O = 33 : 1 : 66) from the chromatographed fractions.

Papyrioside LE (1) Amorphous powder, $[\alpha]_D^{25}$ −25.7° (c = 1.0, methanol). High-resolution FAB-MS Calcd for $C_{48}H_{76}NaO_{18}$ $[M+Na]^+$: 963.4929. Found: 963.4965. FAB-MS 964 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1, 2.

Papyrioside LF (2) Amorphous powder, $[\alpha]_D^{27}$ −14.4° (c = 1.9, methanol). High-resolution FAB-MS Calcd for $C_{50}H_{78}NaO_{19}$ $[M+Na]^+$: 1005.5035. Found: 1005.5001. FAB-MS 1006 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1, 2.

Papyrioside LG (3) Amorphous powder, $[\alpha]_D^{25}$ +12.5° (c = 1.0, methanol). UV λ_{max}^{MeOH} nm (log ε): 282 (2.57). High-resolution FAB-MS Calcd for $C_{48}H_{74}NaO_{18}$ $[M+Na]^+$: 961.4773. Found: 961.4759. FAB-MS 961 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1, 2.

Papyrioside LH (4) Amorphous powder, $[\alpha]_D^{25}$ +30.2° (c = 0.5, methanol). UV λ_{max}^{MeOH} nm (log ε): 282 (2.66). High-resolution FAB-MS Calcd for $C_{48}H_{72}NaO_{18}$ $[M+Na]^+$: 959.4617. Found: 959.4592. FAB-MS 959 $[M+Na]^+$. ^{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 (TFA) for 5 h. The reaction mixture was concentrated to yield a residue, which was trimethylsilylated with 0.5 ml of TMS-HT (trimethylsilylating reagent, TCI) for 1 h. The trimethylsilyl (TMS) derivative was subjected to GC, which identified the derivatives of glucose and rhamnose 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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References

1) Takai M., Amagaya S., Ogihara Y., *J. Chem. Soc., Perkin Trans. 1*, **1977**, 1801—1806.
2) Amagaya S., Takeda T., Ogihara Y., Yamasaki K., *J. Chem. Soc., Perkin Trans. 1*, **1979**, 2044—2047.
3) Asada M., Amagaya S., Takai M., Ogihara Y., *J. Chem. Soc., Perkin Trans. 1*, **1980**, 325—329.
4) Kojima K., Saracoglu I., Mutsuga M., Ogihara Y., *Chem. Pharm. Bull.*, **44**, 2107–2110 (1996).
5) Bothner-By A. A., Stephens R. L., Lee J. M., Warren C. D., Jeanloz R. W., *J. Am. Chem. Soc.*, **106**, 811—813 (1984).
6) Gosmann G., Guillaume D., Taketa A. T. C., Schenkel E. P., *J. Nat. Prod.*, **58**, 438—441 (1995).