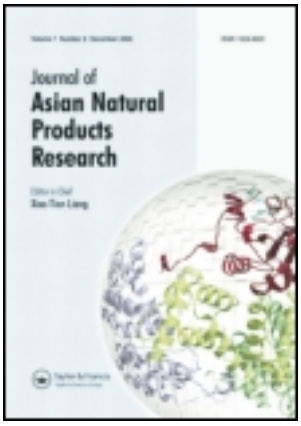

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Three new flavonoid glycosides from *Urena lobata*

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Three new flavonoid glycosides, kaempferol-3-*O*-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (**1**), kaempferol-4′-*O*-β-D-apiofuranosyl-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (**2**), and 5,6,7,4′-tetrahydroxy-flavone-6-*O*-β-D-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside (**3**), were isolated from the aerial parts of *Urena lobata* L., along with 10 known compounds (**4**–**13**). Their structures were determined based on spectroscopic methods including 1D and 2D NMR spectroscopy as well as HR-ESI-MS.

Keywords: Malvaceae; *Urena lobata* L; flavonoid glycoside

1. Introduction

As a traditional Chinese medicine, *Urena lobata* L. has been used to treat cold, fever, pain, or numbness caused by rheumatism, dysentery, edema, gonorrhea, leukorrhea, hematemesis, carbuncle, trauma bleeding, etc. In our earlier study on the chemical constituents of this plant, two new lignan compounds have been reported, i.e. ceplignan-4-*O*-β-D-glucopyranoside and 2,5-dihydroxy benzoic acid-7-(2,6-dimethyl-6-hydroxy-2,7-octadienoic acid)-anhydride-5-*O*-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside [1]. Further examination of this plant led to the isolation of three new flavonoid glycosides (**1**–**3**) and 10 known compounds (**4**–**13**). Herein, we report the isolation and the structural elucidation of the new compounds.

2. Results and discussion

Compound **1** was obtained as pale yellow amorphous powder with $[\alpha]_D^{26}$ −6.9. Its UV absorption maxima at 267, 349 nm, etc., and positive HCl–Mg and Molish reactions indicated that compound **1** was a flavonoid glycoside. The molecular formula $C_{32}H_{38}O_{19}$ was determined from its quasi-molecular ion peak at *m/z* 749.1902 $[M + Na]^+$ by its HR-ESI-MS, indicating 14 degrees of unsaturation. The ^{1}H NMR spectrum of **1** showed four aromatic protons of an AA′BB′ system at δ 8.04/6.74 (each 2H, d, $J = 8.8$ Hz), and an AB type signal at δ 6.70 (1H, d, $J = 2.0$ Hz, H-8) and 6.40 (1H, d, $J = 2.0$ Hz, H-6). In addition, it showed three anomeric protons at δ 5.50 (1H, d, $J = 7.6$ Hz), 5.55 (1H, s), and 5.48 (1H, d, $J = 1.3$ Hz). Acid hydrolysis with trifluoroacetic acid afforded aglycone, glucose, rhamnose, and apiofuranose, which were identified by comparing with authentic sample on CO-HP-Si-TLC. The apiofuranosyl moiety can make complexation reaction with boric acid, therefore we identified its 2-hydroxy and 3-hydroxy as cis [2]. The stereochemistry of sugar units

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was further confirmed by comparing their NMR spectral data with those in the literatures [3]. The ^{13}C NMR spectrum showed 32 carbon signals that were resolved as six methine aromatic carbons [C-2′/C-6′ (δ_C 132.4), C-3′/C-5′ (δ_C 118.6), C-6 (δ_C 100.3), C-8 (δ_C 95.2)], nine quaternary carbons (δ_C 107.5, 118.7, 134.1, 157.8, 157.8, 160.2, 163.0, 163.1, 179.2), six carbons for β-D-glucopyranosyl moiety (δ_C 62.6, 71.7, 78.2, 78.3, 79.1, 100.7), six carbons for α-L-rhamnopyranosyl moiety (δ_C 18.0, 71.6, 71.2, 72.1, 73.6, 99.8), and five carbons for β-D-apiofuranosyl moiety (δ_C 66.3, 75.6, 78.6, 80.9, 110.6) (Table 1). It was also confirmed by the HSQC and DEPT experiments. The ^1H and ^{13}C NMR spectra of **1** were almost identical to those of kaempferol-3-*O*-α-L-rhamnopyranoside-7-*O*-[α-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside], except for the different sugar linkages [4]. The HMBC correlations from H-1″ (δ 5.50) to C-3 (δ 134.1), from H-1 of apiofuranosyl to C-2″ at δ 79.1, and from H-1‴ (δ 5.55) to C-7 (δ 163.1) showed that β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranosyl moiety was attached to C-3, whereas the rhamnopyranosyl moiety was attached to C-7. Therefore, the structure of compound **1** was determined as kaempferol-3-*O*-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (Figure 1).

Compound **2** was isolated as yellow amorphous powder with $[\alpha]_D^{26}$ −2.3 and gave positive reactions with HCl–Mg and Molish reagents, indicating that it was also a flavonoid glycoside. The UV spectrum displayed absorption maxima at 270, 356 nm, etc. The molecular formula $C_{32}H_{38}O_{19}$, with 14 degrees of unsaturation, was deduced from its HR-ESI-MS, which showed a quasi-molecular ion peak at *m/z* 749.1901 $[M + Na]^+$, consistent with its NMR spectral data.

The ^1H and ^{13}C NMR spectra of **2** resembled those of **1**, except for the sugar linkage (Table 1). The locations of the sugar linkage were concluded to be C(3)–C(1″), C(7)–C(1‴), and C(4′)–C(1⁗) based on the HMBC experiment. The HMBC correlation of **2** from H-1″ (δ 5.52) to C-3 (δ 134.9) confirmed the attachment of β-D-glucosyl unit to the aglycone, and a down-field shift of C-2 (δ 157.8) was the other evidence for the linkage of β-D-glucose. The position of the rhamnose unit was confirmed by correlations from H-1‴ (δ 5.56) to C-7 (δ 163.2), and a down-field shift of H-6 (δ 6.45) and H-8 (δ 6.75) could be seen due to the glucosidation of C-7. Similarly, the position of β-D-apiofuranosyl unit was confirmed by correlations from H-1⁗ (δ 5.45) to C-4′ (163.1). A down-field shift of C-1′ (δ 122.7) and H-3′/5′ (δ 6.91) was the other evidence for the location of β-D-apiofuranosyl moiety. Therefore, compound **2** was elucidated as kaempferol-4′-*O*-β-D-apiofuranosyl-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (Figure 1).

Compound **3** was obtained as pale yellow amorphous powder. It showed positive reactions with HCl–Mg and Molish reagents, indicating that it was a flavonoid glycoside. The molecular formula was determined as $C_{26}H_{28}O_{14}$ based on the pseudo-molecular ion at *m/z* 587.1371 $[M + Na]^+$.

The ^1H NMR spectrum of **3** showed aromatic protons at δ 7.98/6.93 (each 2H, d, $J = 8.8$ Hz), 6.99 (1H, s, H-8), and 6.86 (1H, s, H-3). The anomeric protons of β-D-arabinopyranosyl and α-L-rhamnopyranosyl moieties appeared at δ 5.09 (1H, d, $J = 4.0$ Hz, H-1″) and 5.54 (1H, d, $J = 1.6$ Hz, H-1‴), and other characteristic proton signals for β-D-arabinopyranose and α-L-rhamnopyranose were also displayed in its ^1H NMR spectrum [5]. Acid hydrolysis with trifluoroacetic acid afforded arabinose and rhamnose, which were identified by comparing with authentic sample on CO-HP-Si-TLC. The ^{13}C NMR spectral data showed 26 carbon signals, including 15 carbons for the aglycone, five carbons for β-D-arabinopyranosyl moiety (δ_C 62.8, 69.9, 69.6, 70.4, 102.2), and six carbons for the

Table 1. ^{1}H, ^{13}C NMR, and HMBC spectroscopic data for compounds **1** and **2** (measured in DMSO-d_6).

	1				**2**			
No.	δ ^{13}C	δ ^{1}H (J in Hz)	DEPT	HMBC	δ ^{13}C	δ ^{1}H (J in Hz)	DEPT	HMBC
Flavonoids								
2	157.8	–	C	–	157.8	–	C	–
3	134.1	–	C	–	134.9	–	C	–
4	179.2	–	C	–	179.5	–	C	–
5	160.2	–	C	–	161.4	–	C	–
6	100.3	6.40 d (J = 2.0)	CH	C-7, C-8, C-10	100.5	6.45 s	CH	C-7, C-8, C-10
7	163.1	–	C	–	163.2	–	C	–
8	95.2	6.70 d (J = 2.0)	CH	C-6, C-10	95.4	6.75 s	CH	C-6, C-10
9	157.8	–	C	–	159.1	–	C	–
10	107.5	–	C	–	107.5	–	C	–
1′	118.7	–	C	–	122.7	–	C	–
2′	132.4	8.04 d (J = 8.8)	CH	C-2, C-1′, C-4′	132.2	8.08 d (J = 8.8)	CH	C-2, C-1′, C-4′
3′	118.6	6.74 d (J = 8.8)	CH	C-1′, C-2′	116.2	6.91 d (J = 8.8)	CH	C-1′, C-2′
4′	163.0	–	C	–	163.1	–	C	–
5′	118.6	6.74 d (J = 8.8)	CH	C-1′, C-6′	116.2	6.91 d (J = 8.8)	CH	C-1′, C-6′
6′	132.4	8.04 d (J = 8.8)	CH	C-2, C-1′, C-4′	132.2	8.08 d (J = 8.8)	CH	C-2, C-1′, C-4′
Glucosyl								
1″	100.7	5.50 d (J = 7.6)	CH	C-3	100.5	5.52 d (J = 7.2)	CH	C-3
2″	79.1	3.66 m	CH	–	78.0	3.64 m	CH	–
3″	78.2	4.04 m	CH	–	78.1	4.05 m	CH	–
4″	71.7	4.00 m	CH	–	71.5	4.01 m	CH	–
5″	78.3	3.59 m	CH	–	79.2	3.60 m	CH	–
6″	62.6	3.65, 3.44 m	CH_2	–	62.4	3.66, 3.46 m	CH_2	–
Rhamnosyl								
1‴	99.8	5.55 s	CH	C-7, C-5‴	99.6	5.56 s	CH	C-7
2‴	72.1	3.82 m	CH	–	71.9	3.85 m	CH	–
3‴	71.6	3.59 m	CH	–	71.1	3.82 m	CH	–
4‴	73.6	3.45 m	CH	–	73.5	3.44 m	CH	–
5‴	71.2	3.60 m	CH	–	70.9	3.61 m	CH	–
6‴	18.0	1.24 d (J = 6.1)	CH_3	C-4‴, C-5‴	18.0	1.24 d (J = 6.4)	CH_3	–

Table 1 – *continued*

	1				2			
No.	δ^{13}C	δ^{1}H (*J* in Hz)	DEPT	HMBC	δ^{13}C	δ^{1}H (*J* in Hz)	DEPT	HMBC
Apiofuranosyl								
1	110.6	5.48 d (J = 1.3)	CH	C-2″, C-3‴	110.6	5.45 s	CH	C-4′
2	78.6	4.06 m	CH	C-4‴, C-5‴	79.2	4.04 m	CH	
3	80.9	–	C		80.8	–	C	
4	75.6	3.71, 4.08 m	CH_2	C-1‴, C-2‴	75.3	3.71, 4.05 m	CH_2	
5	66.3	3.74 d (J = 11.2)	CH_2	C-2‴, C-3‴	66.0	3.73 d (J = 10.0)	CH_2	

α-L-rhamnopyranosyl moiety (δ_C 18.2, 69.7, 71.5, 71.9, 73.6, 99.5) (Table 2), which was also supported by the HSQC and DEPT experiments. The HMBC correlation of **3** from H-1″ (δ 5.09) to C-6 (δ 128.7) confirmed the attachment of an arabinose unit to the aglycone, and the position of the rhamnose unit was confirmed in a similar manner by the correlation from H-1‴ (δ 5.54) to C-7 (δ 155.3). Therefore, compound **3** was determined as 5,6,7,4′-tetra-hydroxyflavone-6-*O*-β-D-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside (Figure 1).

Compounds **4**–**13** were identified as 5,6,7,4′-tetrahydroxy-flavone-6-*O*-β-D-xylopyranosyl-7-*O*-α-L-rhamnopyranoside (**4**) [6], kaempferol-7-*O*-β-D-glucopyranosyl (1 → 3)-α-L-rhamnopyranoside (**5**) [7], kaempferol-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (**6**) [8], kaempferol-3-*O*-β-D-glucopyranoside (**7**) [9], 6,8-dihydroxykaempferol-3-*O*-β-D-glucopyranoside (**8**) [10], kaempferol-4′-*O*-β-D-glucopyranoside (**9**) [11], kaempferol-7-*O*-α-L-rhamnopyranoside (**10**) [12], kaempferol-7-*O*-α-L-rhamnopyranoside-4′-*O*-β-D-glucopyranoside (**11**) [13], kaempferol-3-*O*-glucopyranosyl(1 → 3)-β-D-glucopyranoside (**12**) [14], and kaempferol-3-*O*-β-robinobioside (**13**) [15] by comparing their spectroscopic data with those reported in the literatures.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a Perkin–Elmer 341 polarimeter (Perkin–Elmer Co., Norwalk, USA). UV spectral data were acquired on a JASCO P-1030 spectrophotometer (JASCO Corporation, Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were measured using a Bruker AVANCE DPX-400 spectrometer (Bruker Corporation, Fällanden, Switzerland). Mass spectra were measured using a Waters Q-TOF micro mass spectrometer (Waters Corporation, Milford, MA, USA). Column

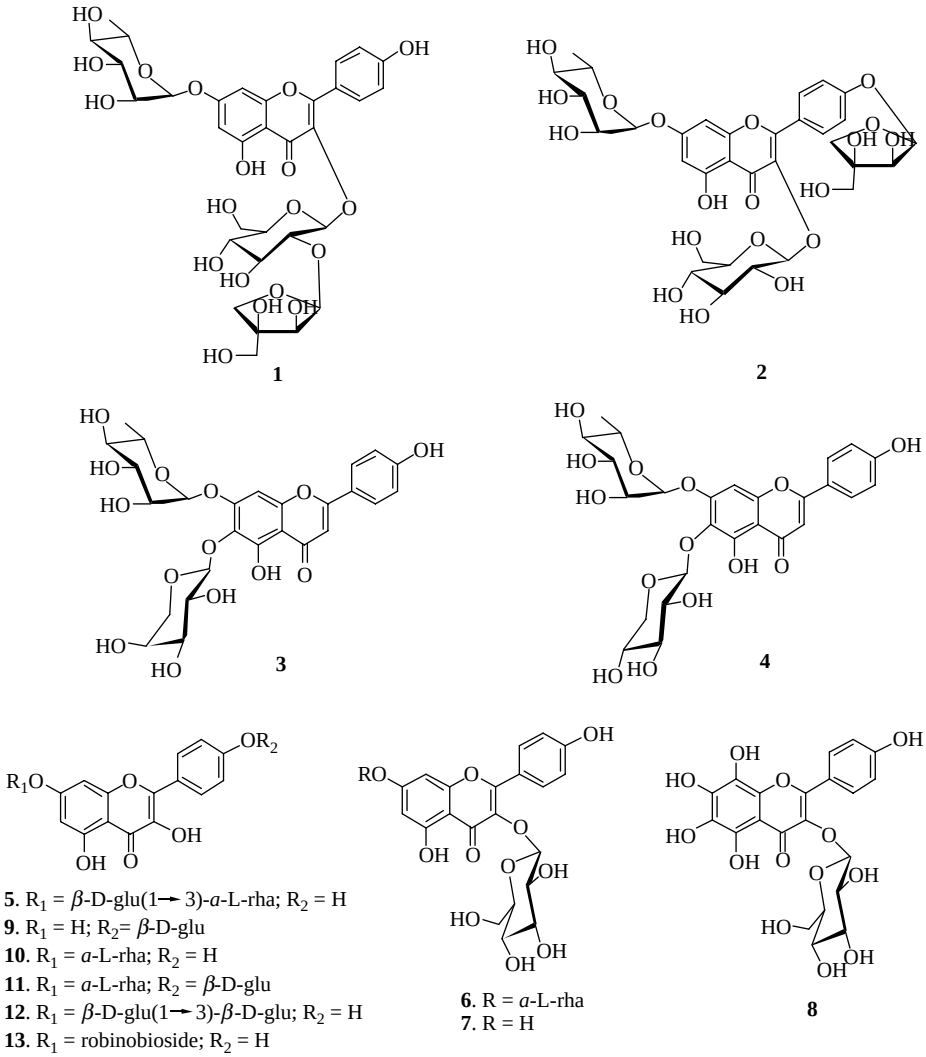

Figure 1. Structures of compounds **1**–**13**.

chromatography (CC) was carried out on Sephadex LH-20 (Pharmacia Corporation, New York, NY, USA), silica gel 100–200 mesh (Qingdao Haiyang Chemical Co., Ltd, Qingdao, China). Silica gel GF_{254} (Qingdao Haiyang Chemical Co.) was used for TLC.

3.2 Plant material

The aerial parts of *U. lobata* were collected from Quanzhou city of Fujian Province, China, and identified by Dr Tong Wu from Shanghai Institute of Pharmaceutical Industry. A voucher specimen (No. 20060823) is deposited in Shanghai Institute of Pharmaceutical Industry.

3.3 Extraction and isolation

The aerial parts of *U. lobata* (30 kg) were crushed and extracted with 95% EtOH (10 × 3 liters) under reflux. The extract was concentrated *in vacuum* to give a

Table 2. ^{1}H, ^{13}C NMR and HMBC spectroscopic data for compound **3** (measured in MeOH-d_4).

	3					**3**			
No.	$\delta\,^{13}C$	$\delta\,^{1}H$ (HSQC) (J in Hz)	DEPT	HMBC	No.	$\delta\,^{13}C$	$\delta\,^{1}H$ (HSQC) (J in Hz)	DEPT	HMBC
Flavonoids									
2	164.6	–	C	–	6′	128.9	7.98 d ($J = 8.8$)	CH	C-2,C-4′
3	102.2	6.86 s	CH	C-2, C-1′, C-10	Arabinosyl				
4	182.5	–	C	–	1″	102.2	5.09 d ($J = 4.0$)	CH	C-6
5	152.7	–	C	–	2″	69.9	3.91 m	CH	–
6	128.7	–	C	–	3″	69.9	3.91 m	CH	–
7	155.3	–	C	–	4″	70.4	3.48 m	CH	–
8	94.5	7.01 s	CH	C-6, C-7, C-9, C-10	5″	62.8	3.82, 3.31 m	CH_2	–
9	152.5	–	C	–	Rhamnosyl				
10	105.8	–	C	–	1‴	99.5	5.54 d ($J = 1.6$)	CH	C-7
1′	121.3	–	C	–	2‴	71.9	3.33 m	CH	–
2′	128.9	7.98 d ($J = 8.8$)	CH	C-2, C-4′	3‴	71.5	3.60 m	CH	–
3′	116.2	6.93 d ($J = 8.8$)	CH	C-1′, C-4′	4‴	73.6	3.32 m	CH	–
4′	161.6	–	C	–	5‴	69.7	3.43 m	CH	–
5′	116.2	6.93 d ($J = 8.8$)	CH	C-1′, C-4′	6‴	18.2	1.17 d ($J = 6.1$)	CH_3	–

residue (1.75 kg), which was dissolved in water (4 liters), and then partitioned with petroleum ether (3 × 2 liters), chloroform (3 × 2 liters), ethyl acetate (3 × 2 liters), and *n*-butanol (3 × 2 liters), respectively. The *n*-butanol fraction (345 g) was dissolved in water and passed through a D_{101} macroporous adsorption resin column (10 × 120 cm) eluted with H_2O:EtOH (100:0, 80:20, 60:40, 40:60, 20:80, 0:100, v/v) to yield seven fractions (Fr. 1–7). Fr. 2 (15.3 g) was chromatographed on a Sephadex LH-20 column with MeOH as eluent to give three subfractions. Subfraction 2 (5.2 g) was chromatographed on silica gel column (with a gradient of EtOAc–MeOH–H_2O from 30:2:1 to 5:2:1) to give **3** (10 mg), **4** (18 mg), **5** (6 mg), and **7** (5 mg). Fraction 4 (23.8 g) was applied to Sephadex LH-20 CC with MeOH as eluent to give six subfractions 4A–4F. Fraction 4B (3.6 g) was subjected to both Sephadex LH-20 CC (MeOH) and silica gel CC (with a gradient of EtOAc–MeOH–H_2O from 30:2:1 to 10:2:1), repeatedly, to give **1** (3 mg), **2** (6 mg), and **6** (12 mg). Fraction 4C (1.9 g) was repeatedly applied to Sephadex LH-20 CC (MeOH) and silica gel CC (with a gradient of EtOAc–MeOH from 20:1 to 3:1) to give **8** (3 mg), **9** (4 mg), **12** (1.2 mg), and **13** (1.7 mg). Fraction 4D (3.8 g) was repeatedly applied to Sephadex LH-20 CC (MeOH) and silica gel column (with a gradient of EtOAc–MeOH from 20:1 to 3:1) to give **10** (1.6 mg) and **11** (3 mg).

3.3.1 Kaempferol-3-O-β-D-apiofuranosyl (1 → 2)-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside

$C_{32}H_{38}O_{19}$; pale yellow amorphous powder; $[\alpha]_D^{26}$ −6.9 ($c = 0.61$, CH_3OH); IR ν_{max} (cm^{-1}): 3385, 1663, 1592, 1500; UV λ nm (CH_3OH, nm): 267, 349, etc. ^1H and ^{13}C NMR spectral data see Table 1; HR-ESI-MS: *m/z* 749.1902 $[M + Na]^+$ (calcd for $C_{32}H_{38}O_{19}Na$, 749.1905).

3.3.2 Kaempferol-4′-O-β-D-apiofuranosyl-3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside

$C_{32}H_{38}O_{19}$; pale yellow amorphous powder; $[\alpha]_D^{26}$ −2.3 ($c = 0.31$, DMSO); IR ν_{max} (cm^{-1}): 3384, 1665, 1593, 1500; UV λ_{max} (DMSO, nm): 270, 356, etc. ^1H and ^{13}C NMR spectral data see Table 1; HR-ESI-MS: *m/z* 749.1901 $[M + Na]^+$ (calcd for $C_{32}H_{38}O_{19}Na$, 749.1905).

3.3.3 5,6,7,4′-Tetrahydroxyflavone-6-O-β-D-arabinopyranosyl-7-O-α-L-rhamnopyranoside

$C_{26}H_{28}O_{14}$; pale yellow amorphous powder; $[\alpha]_{589}^{24.3}$ −54 ($c = 0.17$, CH_3OH); IR ν_{max} (cm^{-1}): 3400, 1660, 1607, 1499; UV λ_{max} (DMSO, nm): 275, 332, etc. ^1H and ^{13}C NMR spectral data see Table 2; HR-ESI-MS: *m/z* 587.1371 $[M + Na]^+$ (calcd for $C_{26}H_{28}O_{14}Na$, 587.1377).

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