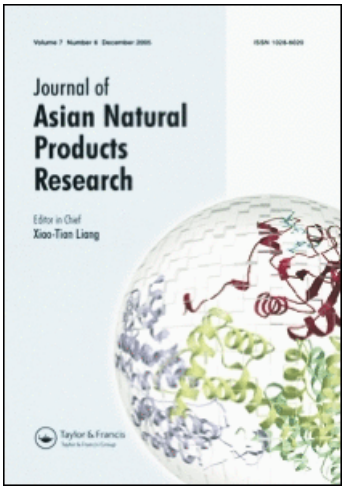

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Two new compounds from *Urena lobata* L.

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Two new compounds **1** and **2** have been isolated from the aerial parts of *Urena lobata* L. The structures of the two new compounds were established as ceplignan-4-*O*-β-D-glucoside (**1**) 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-glucoside (urenoside A) (**2**), on the basis of chemical and spectral evidence, including 1D and 2D NMR spectroscopic data as well as mass spectrometry (HR-ESI-MS).

Keywords: lignan; *Urena lobata* L.; ceplignan-4-*O*-β-D-glucoside; urenoside A

1. Introduction

Urena lobata L., a shrub with pink flowers, is cultivated in many tropical countries, including South America, Africa, Australia, and the USA (Florida). It has been used in traditional Chinese medicine for the treatment of cold, fever, pain, or numbness caused by rheumatism, dysentery, edema, gonorrhea, leucorrhea, hematemesis, carbuncle, trauma bleeding, etc. [1]. The methanolic extract of *U. lobata* L. roots showed a broad spectrum of antibacterial activity against G(+) and G(−) microorganisms [2], antioxidant activity (evaluated by measuring the DPPH free radical scavenging effect), and inhibitory action against nitric oxide release from macrophages [3]. Previously isolated compounds from *U. lobata* L. included flavonoids [4,5], C_{27}–C_{33} *n*-alkanes, β-sitosterol and stigmasterol [6], triglycerides [7], mangiferin [4], imperatorin [8], etc. However, the chemical constituents of *U. lobata* L. were not investigated systematically. In order to determine the bioactive principles and to explore this plant deeply, the 95% ethanol extract of *U. lobata* L. had been studied.

Here, we report the isolation and structural elucidation of two new compounds named ceplignan-4-*O*-β-D-glucoside (**1**) and 2,5-dihydroxy benzoic acid-7-(2,6-dimethyl-6-hydroxy-2,7-octadienoic acid) anhydride-5-*O*-β-D-apio furanosyl(1 → 2)-β-D-glucoside (urenoside A) (**2**) (Figure 1). Their structures were deduced on the basis of 1D and 2D NMR spectroscopic data as well as mass spectrometry (HR-ESI-MS).

2. Results and discussion

Compound **1** was obtained as a pale yellow amorphous powder with $[\alpha]_D^{26}$ −49.2 ($c = 0.40$, H_2O). The UV spectrum showed the absorption maximum at 264 nm. The IR spectrum showed the presence of an α,β-unsaturated carboxyl (3415 and 1680 cm^{-1}) and aromatic rings (1609 and 1500 cm^{-1}). The molecular formula was determined as $C_{24}H_{28}O_{12}$ by HR-ESI-MS at *m/z* 531.1475 $[M + Na]^+$.

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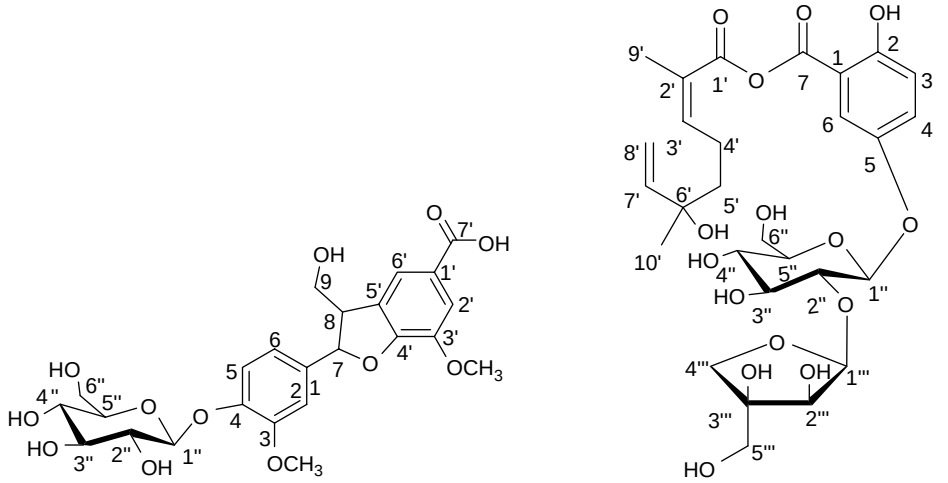

Figure 1. Structures of compounds **1** and **2**.

The ^{1}H NMR spectrum showed downfield signals of aromatic protons at δ 6.70 (1H, dd, $J = 8.4$, 1.6 Hz), 6.83 (1H, d, $J = 1.6$ Hz), 6.88 (1H, d, $J = 8.4$ Hz), forming an ABX system and two aromatic protons at δ 7.28 (1H, s), 7.31 (1H, s) forming an AB system. In the ^{1}H and ^{13}C NMR spectra of **1**, characteristic proton signals at δ_H 3.62 (3H, s), 3.70 (3H, s), and ^{13}C signals at δ_C 56.3, 56.4, indicated the presence of two methoxyl groups in **1**. The sugar moiety was determined as β-glucoside by acid hydrolysis and high-performance TLC comparison with an authentic β-glucoside sample. According to the ^{1}H and ^{13}C NMR spectral data shown in [9], the anomeric configuration was determined as β.

The ^{13}C NMR spectrum showed 24 carbon signals which were resolved as two methoxyl carbons at δ_C 56.3, 56.4, one methylene at δ_C 63.0, seven methine carbons at δ_C 110.7, 116.4, 119.0, 114.0, 119.1, 88.6, 52.7, eight quaternary carbons at δ_C 128.3, 131.1, 136.3, 143.5, 145.8, 149.3, 150.1, 175.1 for the aglycone moiety, and six carbons for the β-glucosyl moiety at δ_C 100.7, 73.2, 75.9, 69.7, 76.5, 60.8. This was also confirmed by the HSQC and DEPT experiments. The location of the sugar was concluded at C-4 based on the HMBC correlation from C-4 (δ 145.8) to H-1″ (δ 4.87).

The key HMBC correlations from H-7 to C-2, C-6, C-9, C-5′, from H-8 to C-1, C-4′, C-6′, from H-9 to C-7, indicated the presence of the benzofuran moiety (Figure 2). According to the literature [10], the chemical shifts of C-7 and C-8 at δ_C 86.5–87.5 and 55.0–56.5, respectively, indicated a 7*S*,8*R*-configuration; whereas 7*R*,8*S*-configuration corresponded to the chemical shifts of C-7 and C-8 at δ_C 88.5–89.5 and 52.5–53.5, respectively. Moreover, 7*S*,8*R*- and 7*R*,8*S*-configurations showed contrary optical rotation values, plus in the 7*S*,8*R*-configuration and minus in the 7*R*,8*S*-configuration. Thus, compound **1** was finally confirmed as a 7*R*,8*S*-configuration based on C-7 at δ_C 88.6, C-8 at δ_C 52.7, and $[\alpha]_D^{26}$ −49.2 ($c = 0.40$, H_2O).

Thus, the structure of compound **1** was elucidated as (7*R*,8*S*)-7,8-dihydro-7-(4-hydroxy-3-methoxyphenyl)-1′-formyl-3′-methoxyl-8-hydroxymethylbenzofuran-4-*O*-β-D-glucoside, named ceplignan-4-*O*-β-D-glucoside. The aglycone structure of compound **1** has been reported in [11].

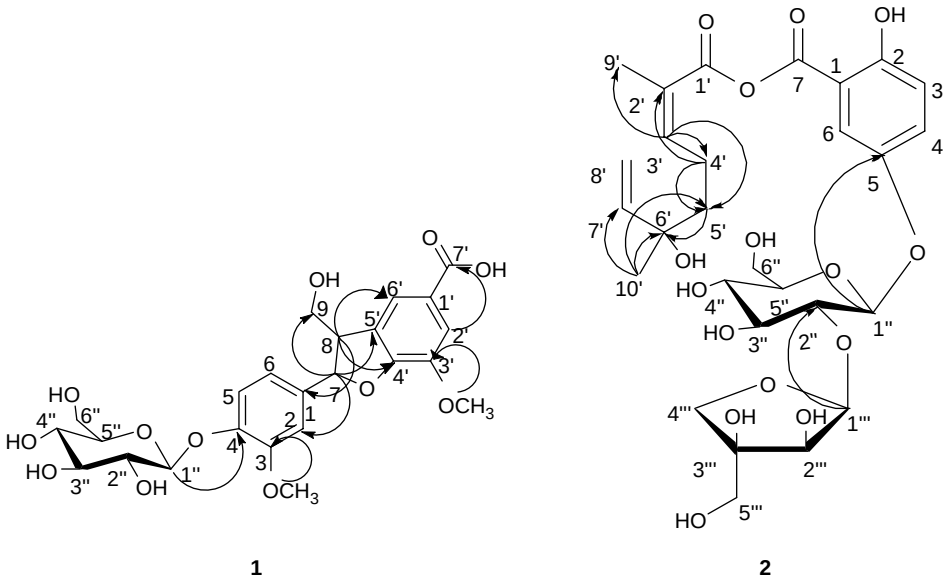

Figure 2. Important HMBC correlations of compounds **1** and **2**.

Compound **2** was obtained as a pale yellow amorphous powder with $[\alpha]_D^{25}$ −95.3 ($c = 0.39$, H_2O). The UV spectrum showed the absorption maximum at 313 nm. The IR spectrum showed the presence of two α,β-unsaturated carbonyls (1768 and 1723 cm^{-1}), conjugated olefinic bonds (1640 cm^{-1}), and an aromatic ring (1602 and 1497 cm^{-1}). The molecular formula was determined as $C_{28}H_{38}O_{15}$ by HR-ESI-MS at *m/z* 613.2137 $[M - H]^-$. The ^1H NMR spectrum showed aromatic protons at δ 7.27 (1H, d, $J = 3.0$ Hz), 6.92 (1H, dd, $J = 8.8$, 3.0 Hz), 6.66 (1H, d, $J = 8.8$ Hz) forming an ABX system, the proton signals at δ 5.77 (1H, dd, $J = 17.6$, 10.8 Hz), 5.04 (1H, dd, $J = 17.6$, 0.9 Hz), 4.98 (1H, dd, $J = 10.8$, 0.9 Hz) forming an ABX system. The olefinic proton at δ 6.38 (1H, dt, $J = 7.2$, 0.9 Hz) indicated the presence of three substituted C=C double bonds. There are two methyl signals at δ 1.42, 1.13, and two methylenes at δ 1.93, 1.40. In addition, the ^1H NMR spectrum displayed two anomeric protons at δ 5.27 (1H, s), 4.98 (1H, d, $J = 8.0$ Hz), suggesting the presence of two sugars. Acid hydrolysis of **2** with trifluoroacetic acid afforded glucose and apiose, which were identified by TLC comparison with an authentic sample. As the apiofuranose can complex with boric acid, its 2- and 3-hydroxyl groups are confirmed as *cis*-configurations.

The ^{13}C NMR spectral data showed 28 carbon signals, including three methine aromatic carbons at δ_C 117.5 (C-3), 121.8 (C-4), 116.8 (C-6), three quaternary aromatic carbons at δ_C 118.9 (C-1), 155.4 (C-2), 148.8 (C-5), and four olefinic carbons at δ_C 126.5, 145.1, 144.0, 112.6. In addition, two signals at δ_C 169.7, 175.0 indicated the presence of two carbonyls. The sp^3 carbon were distributed into two methyl, two methylene, and one quaternary carbon. Based on the literature [12] and the key HMBC correlations from H-3′ (δ 6.38) to C-4′, C-5′, C-9′, from H-4′ (δ 1.93) to C-2′, C-3′, C-5′, from H-5′ (δ 1.40) to C-3′, C-4′, C-6′, from H-10′ (δ 1.13) to C-5′, C-6′, C-7′, the presence of 2,6-dimethyl-6-hydroxy-2,7-octadienoic acid **a** in compound **2** was deduced. The rest of the signals were assigned to 2,5-dihydroxy

benzoic acid **b** [13], a β-apiofuranosyl and a β-glucosyl moiety [14]. The locations of the sugar–aglycone linkage were concluded to be C(5)—C(1″) and C(2″)—C(1‴) and based on the HMBC experiment. The HMBC correlations from H-1″ (δ 4.98) to C-5 (δ 148.8) confirmed the attachment of a β-glucosyl unit to the aglycone. The position of the β-apiofuranosyl unit was confirmed in a similar manner by the correlation from H-1‴ (δ 5.27) to C-2″ (77.1). There were three potential linkages between **a** and **b**, but in the HMBC spectrum, the correlations of H-3 (δ 6.66) to C-1′ (δ 169.7) and H-5′ (δ 1.40) or H-7′ (δ 5.77) to C-7 (δ 175.0) did not exist, suggesting that neither the ester of 6′-OH to **b** nor the ester of 2-OH to **a** were present. In addition, in the IR spectrum, the absorption peaks of two α,β-unsaturated carbonyls (1768 and 1723 cm^{-1}) were very strong, so we proposed carboxylic acids of **a** and **b** to be made of an anhydride.

Thus, the structure of compound **2** was elucidated as 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-glucoside, a novel compound, named urenoside A.

3. Experimental

3.1 General experiment procedures

UV–vis spectra were acquired on a JASCOP-1030 spectrophotometer. IR spectra were recorded as KBr pellets on a Perkin-Elmer 783 infrared spectrophotometer. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. 1D and 2D NMR spectra were measured with a Bruker AVANCE DPX-400 spectrometer. Mass spectra were taken with a Waters Q-TOF Micromass spectrometer. Column chromatography (CC) was performed on Sephadex LH-20 (Pharmacia, New York, USA), silica gel (100–200 mesh; Qingdao Haiyang Chemical Co. Ltd, Qingdao, China). Silica gel GF_{254} (Qingdao Haiyang Chemical Co. Ltd) was used for TLC.

3.2 Plant material

The aerial parts of *U. lobata* L. were collected from Quanzhou, Fujian Province of China, and identified by Dr Wu Tong from the Shanghai Institute of Pharmaceutical Industry. A voucher specimen (No. 20060823) is deposited at the 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 times × 3) under reflux. The extract was concentrated *in vacuo* to give a residue (1.75 kg), which was partitioned between petroleum ether (3 × 2 liters), chloroform (3 × 2 liters), ethyl acetate (3 × 2 liters), *n*-butanol (3 × 2 liters), and water (4 liters), respectively. The *n*-butanol fraction (345 g) was dissolved in water. The water-soluble fraction was passed through a D_{101} macroporous adsorption resin column (10 cm × 120 cm) and eluted with H_2O–EtOH (100:0, 80:20, 60:40, 40:60, 20:80, 0:100, v/v) to yield seven fractions (fractions 1–10). Fraction 1 (12.6 g) was chromatographed on a Sephadex LH-20 column with MeOH–H_2O as the eluent to give three subfractions. The resulting fraction 2 was chromatographed on a silica gel column (with a gradient of EtOAc–MeOH–H_2O from 30:2:1 to 5:2:1) to give **1** (2.1 mg). Fraction 4 (21.4 g) was applied to Sephadex LH-20 CC with MeOH as the eluent to give six subfractions 4A–4F. Fraction 4C (2.8 g) was subjected to both Sephadex LH-20 CC (MeOH) and a silica gel column (with a gradient of EtOAc–MeOH–H_2O from 30:2:1 to 10:2:1), repeatedly, to give **2** (3.4 mg).

3.3.1 Ceplignan-4-O-β-D-glucoside (1)

Pale yellow amorphous powder; $[\alpha]_D^{26}$ −49.2 (c = 0.40, H_2O); UV λ_{max} (MeOH) nm (log ε): 264 (3.141); IR ν_{max}: 3415, 1680, 1609, 1500 cm^{-1}. ^1H and ^{13}C NMR

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for compound **1** (measured in D_2O).

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
C-1	136.3		C-4′	150.1	
C-2	110.7	6.83 (1H, d, $J = 1.6$)	C-5′	128.3	
C-3	149.3		C-6′	119.1	7.28 (1H, s)
C-4	145.8		C-7′	175.1	
C-5	116.4	6.88 (1H, d, $J = 8.4$)	O*C*H$_3$	56.4	3.70 (3H, s)
C-6	119.0	6.70 (1H, dd, $J = 8.4, 1.6$)	O*C*H$_3$	56.3	3.62 (3H, s)
C-7	88.6	5.45 (1H, d, $J = 6.4$)	C-1‴	100.7	4.87 (1H, d, $J = 7.6$)
C-8	52.7	3.34 (1H, m)	C-2‴	73.2	3.66 overlapped
C-9	63.0	3.71 (2H, m)	C-3‴	75.9	4.04 overlapped
C-1′	131.1		C-4‴	69.7	4.00 overlapped
C-2′	114.0	7.31 (1H, s)	C-5‴	76.5	3.59 overlapped
C-3′	143.3		C-6‴	60.8	3.65, 3.44 overlapped

Table 2. ^{1}H and ^{13}C NMR spectroscopic data for compound **2** (measured in D_2O).

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
C-1	118.9		C-9′	11.5	1.42 (3H, s)
C-2	155.4		C-10′	26.3	1.13 (3H, s)
C-3	117.5	6.66 (1H, d, $J = 8.8$)	*Glucosyl*		
C-4	121.8	6.92 (1H, dd, $J = 8.8, 3.0$)	C-1″	98.8	4.98 (1H, d, $J = 8.0$)
C-5	148.8		C-2″	77.1	3.52 (1H, m)
C-6	116.8	7.27 (1H, d, $J = 3.0$)	C-3″	76.3	3.46 (1H, m)
C-7	175.0		C-4″	69.5	3.38 (1H, d, $J = 13.2$)
C-1′	169.7		C-5″	76.5	3.57 (1H, m)
C-2′	126.5		C-6″	60.8	3.77 (1H, dd, $J = 12.4$, 1.8), 3.58 (1H, dd, $J = 12.4$, 1.8) 3.58 (1H, dd, $J = 12.4$, 1.8)
C-3′	145.1	6.38 (1H, dt, $J = 7.2, 0.9$)	*Apiofuranosyl*		
C-4′	23.5	1.93 (2H, m)	C-1‴	108.6	5.27 (1H, s)
C-5′	40.0	1.40 (2H, m)	C-2‴	77.0	3.89 (1H, s)
C-6′	73.6		C-3‴	78.5	
C-7′	144.0	5.77 (1H, dd, $J = 17.6, 10.8$)	C-4‴	73.8	3.81 (1H, d, $J = 10.2$), 4.73 (1H, d, $J = 10.2$)
C-8′	112.6	5.04 (1H, dd, $J = 17.6, 0.9$), 4.98 (1H, dd, $J = 10.8, 0.9$)	C-5‴	66.7	4.01 (1H, d, $J = 11.6$), 4.06 (1H, d, $J = 11.6$)

spectral data: see Table 1. HR-ESI-MS *m/z*: 531.1475 $[M + Na]^+$ (calcd for $C_{24}H_{28}O_{12}Na$, 531.1478).

3.3.2 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-glucoside (2)

Pale yellow amorphous powder; $[\alpha]_D^{25}$ −95.3 ($c = 0.39$, H_2O); UV λ_{max} (MeOH) nm (log ε): 313 (3.940); IR ν_{max}: 1768, 1723, 1640, 1602, 1497, 1373 cm^{-1}. ^{1}H and ^{13}C NMR spectral data: see Table 2. HR-ESI-MS *m/z*: 613.2137 $[M - H]^-$ (calcd for $C_{28}H_{37}O_{15}$, 613.2132).

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