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A new isoflavanone from *Uraria crinita*

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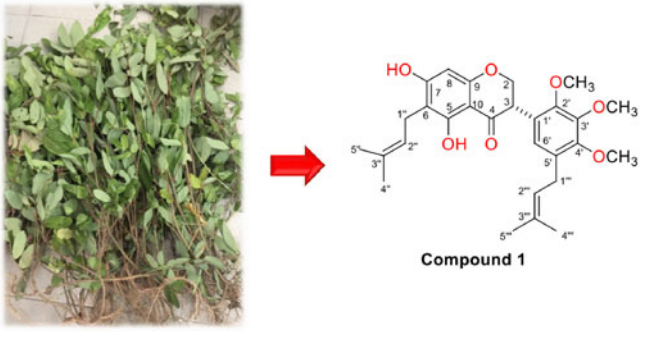
ABSTRACT

A new isoflavanone, (3*S*)-5,7-dihydroxy-2′,3′,4′-trimethoxy-6,5′-diprenylisoflavanone (**1**) and eight known compounds including five flavones (**2–6**), two triterpenes (**7–8**) and a steroid (**9**) were isolated from the whole plant of *Uraria crinita* (Leguminosae). The structure of **1** was elucidated by detailed spectroscopic means including IR, HR-ESI-MS, 1D and 2D NMR, and CD data. Compounds **1–9** were evaluated for their cytotoxicity against four human cancer cell lines KB (mouth epidermal carcinoma), HepG2 (hepatocellular carcinoma), Lu (lung carcinoma) and MCF7 (breast carcinoma). Compound **1** showed cytotoxic activity against the tested cell lines with IC_{50} values of 33.2, 29.4, 59.6 and 66.8 μM, respectively.



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1. Introduction

The genus *Uraria* Desv. (Leguminosae) comprises about 50 species mainly distributed in tropical and subtropical regions. There are 11 species occurring in Vietnam, some of

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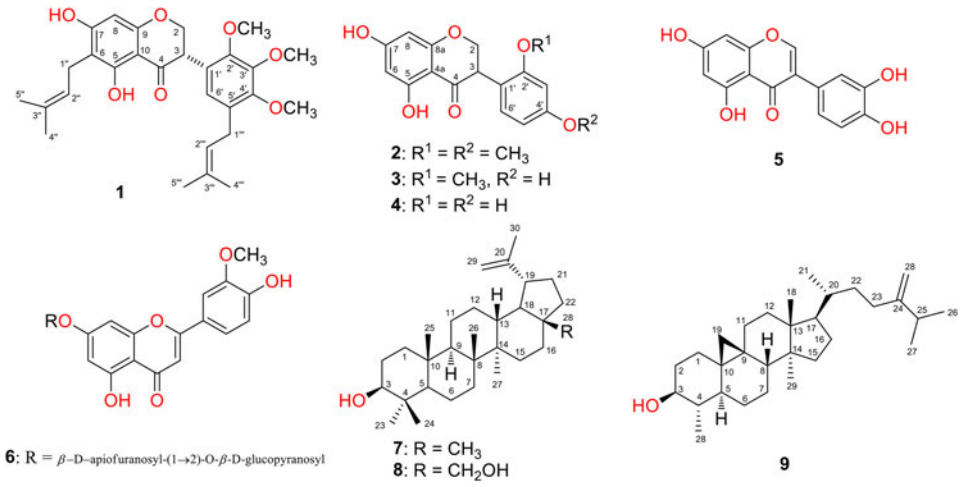

Figure 1. Compounds **1–9** isolated from the whole plant of *Uraria crinita*.

which are used as folk remedy (Pham 2000). *Uraria crinita* (L.) DC is an edible herb that is found in Vietnam, China, Taiwan, Thailand, Indonesia and Southeast Asia. *U. crinita* is used in Vietnamese traditional medicine for the treatment of rheumatism, diarrhoea, sprains, injuries and lung diseases (Pham 2000).

Uraria crinita has been reported to possess anti-oxidant, anti-inflammatory and osteogenic activities and to reduce the formation of stress-induced ulcers (Yen et al. 2001; Mao et al. 2014). Previous phytochemical studies of *U. crinita* revealed the presence of flavonoids, triterpenes, megastigmanes and nucleosides (Morita et al. 1977; Wang et al. 2009; Mao et al. 2014).

The chemical investigation of the whole plant of *U. crinita* collected in Vietnam led to the isolation of a new isoflavanone, (3*S*)-5,7-dihydroxy-2′,3′,4′-trimethoxy-6,5′-diprenylisoflavanone (**1**), in addition to eight known compounds, homoferreirin (**2**), isoferreirin (**3**), dalbergioidin (**4**), isoluteolin (**5**), 3′-methoxyapiin (**6**), lupeol (**7**), betuline (**8**) and cycloeucalenol (**9**) (Figure 1). The isolation and structural elucidation of compound **1**, and the cytotoxic activity of compounds **1–9** against four human cancer cell lines KB, HepG2, Lu and MCF7 are described herein.

2. Results and discussion

Compound **1** was obtained as a white solid. The IR spectrum of **1** indicated the presence of hydroxyl groups (3163 cm^{-1}), a chelated carbonyl group (1637 cm^{-1}), aromatic rings (1600 and 1459 cm^{-1}), and C–O–C bonds (1099 cm^{-1}). The molecular formula was determined to be $C_{28}H_{34}O_7$ from the $[M+H]^+$ ion peak at *m/z* 483.2344 in HR-ESI-MS spectrum.

The ^{1}H NMR spectrum of **1** exhibited signals characteristic of the protons of ring A [δ_H 5.95 (1H, s)], ring B [δ_H 6.63 (1H, s)] and ring C [δ_H 4.53 (1H, dd, J = 11.5, 11.0 Hz), 4.40 (1H, dd, J = 11.0, 6.0 Hz), 4.20 (1H, dd, J = 11.5, 6.0 Hz)] of an isoflavanone. The spectrum also showed the presence of three methoxyl groups [δ_H 3.88, 3.84, 3.83 (3H each, s)]; two phenolic hydroxyl groups [δ_H 12.59 (1H, s), 6.26 (1H, s)]; and two prenyl

units [δ_H 5.27 (1H, t, $J = 7.0$ Hz), 5.21 (1H, m), 3.36, 3.25 (2H each, $J = 7.0$ Hz), 1.82, 1.76, 1.71, 1.68 (3H each, s]. The downfield signal at δ_H 12.59 confirmed the presence of the chelated hydroxyl group at C-5 position. A one-proton singlet in the δ_H 5.90–6.10 region in the ^{1}H NMR spectrum suggested that ring A has a substituent at either C-6 or C-8 (Wandji et al. 1995).

The ^{13}C NMR and HSQC spectra displayed 28 carbon signals. Except for 15 carbons signals of the isoflavanone skeleton, three methoxyl carbon signals and ten carbon signals of two prenyl units were also observed.

The long-range correlations between the proton of the chelated hydroxyl group (δ_H 12.59) and C-5 (δ_C 161.7), C-6 (δ_C 106.9) and C-10 (δ_C 103.1) as well as the methylene protons H-1″ of the prenyl substituent (δ_H 3.36) and C-5 (δ_C 161.7), C-6 (δ_C 106.9) and C-7 (δ_C 163.5) in the HMBC experiment confirmed that the hydroxyl and the prenyl groups were located at C-5 and C-6, respectively. The other hydroxyl group located at C-7 was supported by the HMBC correlations of the hydroxyl proton (δ_H 6.26) with C-6 (δ_C 106.9), C-7 (δ_C 163.5) and C-8 (δ_C 95.2). Therefore, ring A had a 5,7-dihydroxy-6-prenyl substitution, whereas three methoxyl groups and the remaining prenyl unit were on ring B.

The deshielding of three methoxyl groups at δ_C 60.7, 60.7, 60.4 was in agreement with their *ortho*-disubstituted positions (Galeffi et al. 1997). Furthermore, the chemical shift of the methylene carbon C-1‴ at δ_C 28.2 suggested that one of the *ortho*-positions to the prenyl group was replaced by an oxygenated substituent and another by a hydrogen atom (Fukai and Nomura 1989). These data suggested that the position of three methoxyl groups and the prenyl unit was at C-2′, C-3′, C-4′ and C-5′, respectively. The exact location of the substitutes was also confirmed by HMBC experiment. The HMBC correlations between the proton H-3 (δ_H 4.20) and C-1′ (δ_C 123.1), C-2′ (δ_C 150.2) and C-6′ (δ_C 124.7); the aromatic proton H-6′ (δ_H 6.63) and C-3 (δ_C 47.9); the methoxyl protons (δ_H 3.83) and C-2′ (δ_C 150.2) confirmed that the proton and the methoxyl group were located at C-6′ and C-2′, respectively. Additional HMBC correlations between the proton H-6′ (δ_H 6.63) and C-1‴ (δ_C 28.2); the proton H-1‴ (δ_H 3.25) and C-4′ (δ_C 151.8), C-5′ (δ_C 130.8) and C-6′ (δ_C 124.7) determined the prenyl group to be at C-5′. Other HMBC correlations of the methoxyl protons at δ_H 3.88 and 3.84 with C-3′ (δ_C 146.4), and C-4′ (δ_C 151.8), respectively, and the protons H-6′ (δ_H 6.63), H-1‴ (δ_H 3.25) with C-4′ (δ_C 151.8) indicated that these two methoxyl groups were at both C-3′ and C-4′ positions.

The absolute configuration of C-3 was determined to be *S* from positive Cotton effect at 325 nm and negative Cotton effect at 347 nm in the CD spectrum (Galeffi et al. 1997; Vila et al. 1998). From the above spectral data, the structure of **1** was elucidated to be (3*S*)-5,7-dihydroxy-2′,3′,4′-trimethoxy-6,5′-diprenylisoflavanone.

The eight known compounds were determined as homoferreirin (**2**) (Huang et al. 2013), isoferreirin (**3**) (Rahman et al. 2007), dalbergioidin (**4**) (Durango et al. 2002), isoluteolin (**5**) (Dzoyem et al. 2018), 3′-methoxyapiin (**6**) (Momin and Nair 2002), lupeol (**7**) (Burns et al. 2000), betuline (**8**) (Siddiqui et al. 1988) and cycloeucalenol (**9**) (Hoa et al. 2014) by comparison of their spectral data with those of the literature.

Some isoflavanones are known to exhibit cytotoxic effects (Li et al. 2014; Zhou et al. 2018). Compounds **1–9** were evaluated for their cytotoxicity against KB, HepG2,

Table 1. Cytotoxic activities of compounds **1–9** against tested cell lines.

	IC_{50} (μM) ± SD			
Compound	KB	HepG2	Lu	MCF7
1	33.2 ±0.4	29.4 ±0.4	59.6 ±1.0	66.8 ±1.0
2	>200	>200	>200	>200
3	>200	>200	>200	>200
4	156.2 ±2.6	>200	>200	>200
5	>200	>200	>200	55.9 ±1.8
6	>200	>200	>200	>200
7	>200	>200	>200	150.0 ±3.5
8	144.6 ± 2.3	>200	>200	>200
9	>200	>200	>200	>200
Ellipticine[a]	1.3 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	2.2 ± 0.2

[a]Positive control.

Lu and MCF7 cell lines. Results were summarised in Table 1. Compound **1** was active against all the tested cancer cell lines with IC_{50} values of 33.2, 29.4, 59.6 and 66.8 μM, respectively. The other compounds exhibited slight or no activity.

3. Experimental

3.1. General experimental procedures

Optical rotation was measured with a Jasco P-2000 polarimeter (Jasco, Tokyo, Japan). IR spectra were recorded on Perkin Elmer Spectrum Two IR spectrometer (Perkin Elmer, Waltham, MA, USA). NMR spectra were taken on a Bruker Avance III 500 spectrometer (Bruker, Fällanden, Switzerland). HR-ESI-MS was obtained on a Sciex X500R QTOF mass spectrometer (Sciex, Massachusetts, USA). Circular dichroism (CD) spectra were recorded on a Chirascan Circular Dichroism Spectrometer (Applied Photophysics Ltd., Surrey, UK). Column chromatography was carried out on silica gel 60 (0.040–0.063 mm, Merck, Darmstadt, Germany) and Sephadex LH-20 (Amersham Pharmacia Biotech, Tokyo, Japan). Thin layer chromatography was performed on silica gel 60F254 (0.25 mm, Merck, Darmstadt, Germany). The spots were observed after spraying with solution of vanillin/sulfuric acid and heating for 5 min.

3.2. Plant material

The whole plant of *U. crinita* was collected in Phu Tho province, Vietnam in April 2018. The botanical identification was made by Assoc. Prof. Do Huu Thu, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (UC04/2018) was deposited at the Laboratory of Natural Products Research, Institute of Chemistry, VAST, Hanoi, Vietnam.

3.3. Extraction and isolation

The dried whole plant of *U. crinita* (1.3 kg) was ground and extracted three times with methanol-water (95:5, v/v) at room temperature. After the combined extracts were evaporated under reduced pressure at 45 °C, the residue was suspended in H_2O and then partitioned in turn between n-hexane, ethyl acetate and n-butanol, successively.

The organic solvents were evaporated to yield the corresponding extracts of 14.2 g, 8.0 g and 12.5 g, respectively.

The n-hexane extract (14.2 g) was subjected to silica gel column chromatography with a gradient mixture of n-hexane-EtOAc (from 50:1 to 1:1, v/v) to give 22 fractions. Fraction 7 was repeatedly chromatographed on silica gel column (n-hexane-EtOAc, 50:6, v/v) and then on Sephadex LH-20 column (CH_2Cl_2-MeOH, 1:9, v/v) to afford compound **1** (8 mg, 0.0006% w/w). Fraction 16 was chromatographed on silica gel column (n-hexane-acetone, 3:1, v/v) to give compound **2** (5 mg, 0.0004% w/w). Fraction 4 was purified on silica gel column chromatography (n-hexane-EtOAc, 50:4, v/v) to give compound **7** (10 mg, 0.0008% w/w). Fraction 10 was further chromatographed on silica gel column and eluted with n-hexane-EtOAc (5:1, v/v) to afford compound **8** (15 mg, 0.0012% w/w). Fraction 5 was chromatographed on silica gel column (n-hexane-acetone, 5:0.5, v/v) to afford compound **9** (4 mg, 0.0003% w/w).

The ethyl acetate extract (8.0 g) was chromatographed on silica gel column and eluted with CH_2Cl_2-MeOH (from 10:0 to 4:1, v/v) to give 15 fractions. Fraction 4 was purified on silica gel column (n-hexane-acetone, 3:2, v/v) to yield compound **3** (7 mg, 0.0005% w/w). Fraction 6 was purified on silica gel column and eluted with n-hexane-acetone (3:2.5, v/v) to yield compounds **4** (15 mg, 0.0012% w/w) and **5** (6 mg, 0.0005% w/w).

The n-butanol extract (12.5 g) was applied on a silica gel column eluded with CH_2Cl_2-MeOH-H_2O (from 9:1:0 to 0:9:1) to yield seven fractions. Fraction 5 was chromatographed on silica gel column (CH_2Cl_2-MeOH-H_2O, 4:1.5:0.15, v/v) and then on Sephadex LH-20 column (MeOH) to give compound **6** (4 mg, 0.0003% w/w).

(3S)-5,7-dihydroxy-2′,3′,4′-trimethoxy-6,5′-diprenylisoflavanone (1): White solid. Rf = 0.33 (n-hexane-EtOAc, 5:0.8). $[\alpha]_D^{25}$ −18° (*c* 0.12, $CHCl_3$). IR (KBr) ν_{max}: 3163, 2927, 1637, 1600, 1459, 1099 cm^{-1}. HR-ESI-MS *m/z* 483.2344 $[M+H]^+$, ($C_{28}H_{35}O_7^+$, calcd for 483.2383). ^1H NMR (500 MHz, $CDCl_3$) δ: 12.59 (1H, s, 5-OH), 6.63 (1H, s, H-6′), 6.26 (1H, s, 7-OH), 5.95 (1H, s, H-8), 5.27 (1H, t, *J* = 7.0 Hz, H-2″), 5.21 (1H, m, H-2‴), 4.53 (1H, dd, *J* = 11.5, 11.0 Hz, H-2a), 4.40 (1H, dd, *J* = 11.0, 6.0 Hz, H-2b), 4.20 (1H, dd, *J* = 11.5, 6.0 Hz, H-3), 3.88 (3H, s, 3′-OCH_3), 3.84 (3H, s, 4′-OCH_3), 3.83 (3H, s, 2′-OCH_3), 3.36 (1H, d, *J* = 7.0 Hz, H-1″), 3.25 (1H, d, *J* = 7.0 Hz, H-1‴), 1.82 (3H, s, H-4″), 1.76 (3H, s, H-5″), 1.71 (3H, s, H-4‴), 1.68 (3H, s, H-5‴). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 197.4 (C-4), 163.5 (C-7), 161.7 (C-5), 161.4 (C-9), 151.8 (C-4′), 150.2 (C-2′), 146.4 (C-3′), 135.4 (C-3″), 132.6 (C-3‴), 130.8 (C-5′), 124.7 (C-6′), 123.1 (C-1′), 122.6 (C-2‴), 121.6 (C-2″), 106.9 (C-6), 103.1 (C-10), 95.2 (C-8), 70.6 (C-2), 60.7, 60.7, 60.4 (3 × OCH_3), 47.9 (C-3), 28.2 (C-1‴), 25.8 (C-4″), 25.7 (C-4‴), 21.2 (C-1″), 17.9 (C-5″), 17.8 (C-5‴).

3.4. Cytotoxicity assay

Compounds **1–9** were evaluated for their cytotoxic activity against four human cancer cell lines, KB, HepG2, Lu and MCF7 by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay according to the method of Mosmann (1983) with some modifications. The tested cell lines were obtained from the American Type Culture Collection (ATCC, USA) and maintained in Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), and 1% antibiotic. The tested compounds

were initially dissolved in DMSO and then diluted into culture medium at concentrations of 256, 64, 16 and 4 μg/mL. The total concentration of DMSO added to the cells was <1%. Experimental cultures were plated in microtiter plates containing 10 μL of each test sample and 190 μL of the cell suspensions at a density of 3×10^4 cells/mL. Test plates were incubated in a humidified atmosphere of 5% CO_2, 37 °C for 72 h. Then, 10 μL (5 mg/mL) of the MTT solution in phosphate-buffered saline was added to each well, and incubated for an additional 4 h. After removal of the supernatant, 100 μL of DMSO was added to dissolve the formazan crystals and the absorbance was measured at 540 nm in a plate reader (BioTek, USA). All the experiments were performed three times with the mean absorbance values calculated. Ellipticine was used as positive control.

4. Conclusions

A new isoflavanone, (3*S*)-5,7-dihydroxy-2′,3′,4′-trimethoxy-6,5′-diprenylisoflavanone (**1**) in addition to eight known compounds, homoferreirin (**2**), isoferreirin (**3**), dalbergioidin (**4**), isoluteolin (**5**), 3′-methoxyapiin (**6**), lupeol (**7**), betuline (**8**) and cycloeucalenol (**9**) were obtained from the whole plant of *U. crinita*. Compound **1** was active against all the tested cancer cell lines, KB, HepG2, Lu and MCF7 with IC_{50} values of 33.2, 29.3, 59.6 and 66.8 μM, respectively.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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