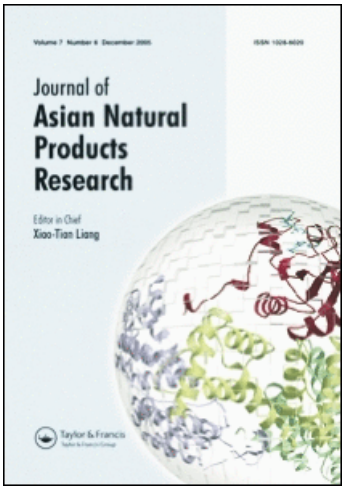

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ORIGINAL ARTICLE

New iridoids from the fruits of *Crescentia cujete*

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Four new 11-nor-iridoids, 6-*O*-*p*-hydroxybenzoyl-10-deoxyeucommiol (**1**), 6-*O*-benzoyl-10-deoxyeucommiol (**2**), 6-*O*-benzoyl-dihydrocatalpolgenin (a mixture of **3** and **4**), as well as two known iridoids, ningpogenin (**5**) and 6-*O*-*p*-hydroxybenzoyl-aucubin (**6**), were isolated from the fruits of *Crescentia cujete* Linn. The structures of these compounds were established on the basis of spectroscopic analysis.

Keywords: *Crescentia cujete*; calabash tree; Bignoniaceae; iridoids

1. Introduction

Crescentia cujete (Bignoniaceae), commonly known as the calabash tree, is a species of flowering plant that is native to Central and South America. It has been an important source of folk medicine [1], with both extracts and pulp of the seeds, fruits, leaves, and flowers being used to treat a variety of ailments. These include cold, other respiratory illnesses [2–4], hypertension [5], and the hemorrhagic effect of venomous snake bites [6]. The part of the tree that is used is the fruit. Its active ingredients have not yet been defined. Its uses are mainly in *phytopharmaceuticals*. The extract of the fruit is effective in the treatment of fever. In Vietnam, the dried fruit is used in folk medicine, the local name being 'Dao Tien'. A fruit decoction is taken orally to treat diarrhea, stomachache, cold, bronchitis, cough, asthma, and urethritis. Naphtoquinones [7], iridoid glycosides, aucubin, plumieride, and asperuloside [8] have already been reported as the constituents of the leaves of this plant. In this study, four new 11-nor-iridoids, 6-*O*-*p*-hydroxybenzoyl-10-deoxyeucommiol (**1**), 6-*O*-benzoyl-10-deoxyeucommiol (**2**), 6-*O*-benzoyl-dihydrocatalpolgenin (a mixture of **3** and **4**), as well as two known iridoids, ningpogenin and 6-*O*-*p*-hydroxybenzoylaucubin, were isolated from the fruits of *C. cujete* (Figure 1).

2. Results and discussion

Compound **1** was obtained as a colorless oil and was assigned a molecular formula of $C_{16}H_{20}O_5$ by negative HR-FAB-MS at *m/z* 291.1239 $[M - H]^-$, indicating seven degrees of unsaturation. The IR spectrum showed absorptions at 3398 and 1714 cm^{-1}, revealing the presence of hydroxyl and carbonyl groups. The ^{13}C

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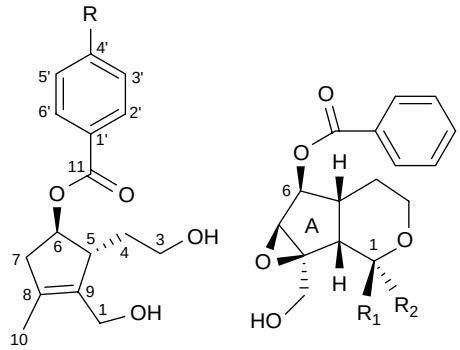

1 R = OH
2 R = H

3 R_1 = OH, R_2 = H
4 R_1 = H, R_2 = OH

Figure 1. Structures of compounds **1**–**4**.

NMR and DEPT spectra (Table 2) exhibited 16 carbons, including a carbonyl carbon (δ_C 168.3, s, C-11), three oxygen-bearing carbons (δ_C 57.1, CH_2, C-1; 61.5, CH_2, C-3; 80.0, CH, C-6), a methyl (δ_C 13.8, CH_3, C-10), a benzene ring, and a double bond (δ_C 134.5, C, C-8; 136.8, C, C-9). The NMR spectral data of **1** were similar to those of 10-deoxyeucommiol [9]. The key difference was that **1** had additional signals for a benzene ring and a carbonyl group. The signals at δ_H 7.85, 6.81 (each 2H, d, $J = 9.0$ Hz) indicated that the benzene ring was substituted by a *para*-hydroxyl group. The HMBC correlations (Figure 2) from the proton at δ_H 5.22 (H-6) and 7.85 (H-2′ and H-6′) to the carbonyl carbon (δ_C 168.3, C-11) revealed the presence of a *p*-hydroxybenzoyloxy group located at C-6. Therefore, compound **1** was proposed as 6-*O*-*p*-hydroxybenzoyl-10-deoxyeucommiol. This assignment was confirmed by 2D NMR analysis. The $^1H-^1H$ COSY spectrum of **1** proved the existence of a spin system from C-3 to C-7 unit. In the HMBC spectrum of **1**, the significant correlations between H-1 and C-5, C-8 and C-9, H-10 and C-7, C-8 and C-9 were observed. The β-orientation of H-5 and the hydroxyl group at C-6 were suggested by comparison of its NMR spectral data (chemical shifts and coupling constants) with those reported for 10-deoxyeucommiol [9].

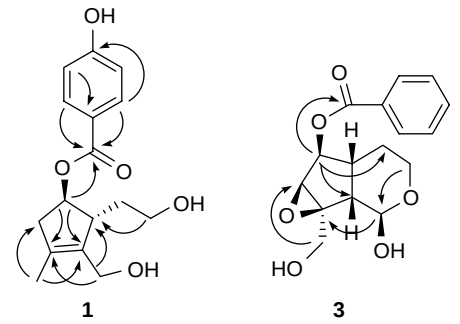

Figure 2. Key HMBC correlations of compounds **1** and **3**.

Compound **2** was obtained as a colorless oil and was assigned a molecular formula of $C_{16}H_{20}O_4$ by negative HR-ESI-MS at *m/z* 299.1262 $[M + Na]^+$. The ^1H and ^{13}C NMR spectra of **2** (Tables 1 and 2) were similar to those of **1**, which suggested that compound **2** possessed the same iridoid skeleton. The only difference between them was that the substituted hydroxyl on the benzene ring of **1** was absent in **2**, which was discovered by detailed MS and NMR analysis. Thus, compound **2** was assigned as 6-*O*-benzoyl-10-deoxyeucommiol.

Compounds **3** and **4** were obtained as inseparable pairs in the ratio of about 3:2, as shown by the integral intensity of corresponding signal multiplicities in their ^1H NMR spectrum. Their proton and carbon signals appeared as pairs. Both compounds had the same molecular formula of $C_{16}H_{18}O_6$ based on the positive HR-ESI-MS at *m/z* 329.1008 $[M + Na]^+$, which suggested an iridoid skeleton with eight degrees of unsaturation. The ^{13}C NMR and DEPT spectra of the mixture contained 14 carbon resonances for each compound and two totally overlapped resonances of C-11 and C-1′, seven of which belonged to a benzoyloxy group for **3** and **4**, respectively. Comparing

Table 1. ^{1}H NMR spectral data (500 MHz, J in Hz) of compounds **1**–**4**.

No.	**1**[a]	**2**[b]	**3**[c]	**4**[c]
1	4.25 (d, $J = 12.4$) 4.06 (d, $J = 12.4$)	4.26 (d, $J = 12.3$) 4.10 (d, $J = 12.3$)	4.64 (d, $J = 9.8$)	5.45 (d, $J = 4.9$)
3	3.70 (t, $J = 6.8$)	3.71 (t, $J = 6.8$)	3.96 (m) 3.60 (m)	4.07 (m) 3.54 (m)
4	1.97 (m) 1.54 (m)	1.98 (m) 1.57 (m)	1.86 (m) 1.58 (m)	1.92 (m) 1.68 (m)
5	3.01 (m)	3.08 (m)	2.52 (m)	2.35 (m)
6	5.22 (m)	5.29 (m)	5.39 (d, $J = 9.8$)	5.61 (d, $J = 9.8$)
7	2.92 (dd, $J = 17.5, 6.4$) 2.36 (d, $J = 17.5$)	2.95 (dd, $J = 17.8, 6.5$) 2.37 (d, $J = 17.8$)	3.77 (m)	3.82 (m)
9			2.39 (m)	2.60 (m)
10	1.76 (s)	1.72 (s)	4.14 (d, $J = 13.2$) 3.79 (d, $J = 13.2$)	4.10 (d, $J = 12.8$) 3.86 (d, $J = 12.8$)
2′, 6′	7.85 (d, $J = 9.0$)	7.99 (d, $J = 7.8$)	7.46 (m)[d]	7.46 (m)[d]
3′, 5′	6.81 (d, $J = 9.0$)	7.50 (dd, $J = 8.0, 7.8$)	8.07 (m)[d]	8.07 (m)[d]
4′		7.62 (d, $J = 8.0$)	7.58 (m)[d]	7.58 (m)[d]

Notes: [a] Measured in CD_3OD.
[b] Measured in CD_3COCD_3.
[c] Measured in $CDCl_3$.
[d] Signals in the same line were overlapped.

Table 2. ^{13}C NMR (125 MHz) spectral data of compounds **1**–**4**.

No.	**1**[a]	**2**[b]	**3**[c]	**4**[c]
1	57.1 CH_2	58.1 CH_2	95.2 CH	90.0 CH
3	61.5 CH_2	62.0 CH_2	62.1 CH_2	54.0 CH_2
4	34.8 CH_2	35.8 CH_2	22.6 CH_2	22.1 CH_2
5	51.6 CH	52.4 CH	34.4 CH	31.8 CH
6	80.0 CH	80.7 CH	74.7 CH	76.3 CH
7	44.9 CH_2	45.5 CH_2	58.3 CH	60.2 CH
8	134.5 C	133.2 C	65.0 C	65.8 C
9	136.8 C	138.6 C	43.4 CH	39.5 CH
10	13.8 CH_3	14.8 CH_3	61.4 CH_2	60.7 CH_2
11	168.3 C	167.9 C	167.0 C	167.0 C
1′	122.7 C	132.7 C	129.4 C	129.4 C
2′, 6′	132.8 CH	131.2 CH	128.3 CH[d]	128.4 CH[d]
3′, 5′	116.1 CH	130.3 CH	129.7 CH[d]	129.8 CH[d]
4′	163.5 C	134.8 CH	133.2 CH[d]	133.4 CH[d]

Notes: [a] Measured in CD_3OD.
[b] Measured in CD_3COCD_3.
[c] Measured in $CDCl_3$.
[d] Signals in the same line may be interchangeable.

the NMR spectral data of **3** and **4** with those of dihydrocatalpolgenin [10] revealed that **3** and **4** were esters formed by dihydrocatalpolgenin and benzoic acid at C-6, which was confirmed by HMBC correlations from the protons at δ_H 5.39 (H-6 in **3**) and δ_H 5.61 (H-6 in **4**) to an ester carbonyl carbon (δ_C 167.0, C-11 in **3** and **4**). Therefore, compounds **3** and **4** possessed the same planar structure and were identified as 6-*O*-benzoyl-dihydrocatalpolgenin. The stereochemistry of compounds **3** and **4** were suggested to be similar to that of dihydrocatalpolgenin by comparison of their NMR spectral data (chemical shifts and coupling constants) with those reported for dihydrocatalpolgenin [10].

The structures of the known iridoids isolated were identified as ningpogenin [11] and 6-*O*-*p*-hydroxybenzoylaucubin [12] by comparison of their spectroscopic data with literature values.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were measured on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained on a Tensor 27 with KBr pellets. NMR spectra were recorded on Bruker AV-400 and Bruker DRX-500 spectrometers. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. FAB-MS were recorded with a VG Autospec-3000 spectrometer. ESI-MS and HR-ESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer. Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 (5 μm, 9.4 × 150 mm) column. Preparative MPLC was performed on a Büchi apparatus equipped with Büchi fraction collector C-660, Büchi pump module C-605 and manager C-615. Silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China), RP-18 gel (40–75 μm; Fuji Silysia Chemical Ltd, Aichi, Japan), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography (CC). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

3.2 Plant material

The fruits of *C. cujete* were collected from Ho Chi Minh, Vietnam, in February, 2007. The plant was identified by Hsiehs Biotech Co. Ltd, Ho Chi Minh, Vietnam. The voucher specimen (HB 2007006) has been deposited in Hsiehs Biotech Co. Ltd.

3.3 Extraction and isolation

The air-dried pulp of the mature fruits of *C. cujete* (3.0 kg) was extracted with ethanol (8 liters × 3) at room temperature. The combined extract was concentrated *in vacuo* to give a crude residue (~ 183 g), which was suspended in H_2O and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract (~ 11.4 g) was subjected to silica gel CC (200–300 mesh, 5.0 × 60 cm), eluted with a $CHCl_3$–MeOH gradient (from 100:0 to 0:100, v/v) to afford fractions A–G. Fraction B (1.5 g) eluted with $CHCl_3$–MeOH (98:2, v/v) was separated by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1, v/v) CC to obtain fractions B1–B3. Fraction B1 (0.8 g) was subjected to preparative MPLC with a reversed-phased C_{18} column (MeCN–H_2O, 40–100%, v/v), followed by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1, v/v) CC to give pure compound **1** (9.6 mg). Fraction C (1.2 g) eluted with $CHCl_3$–MeOH (95:5, v/v) was purified by repeated silica gel CC eluted with $CHCl_3$–MeOH (from 80:1–5:1, v/v) and preparative HPLC to yield a mixture of **3** and **4** (2.6 mg) and subfraction C1, which was further separated by preparative HPLC to give **2** (10.0 mg). Fraction D (1.8 g) was separated by silica gel CC eluted with petroleum ether and acetone (from 10:1 to 1:1, v/v) to obtain subfractions D1–D4, of which D2 (0.5 g) was chromatographed on RP-C_{18} MPLC (MeOH–H_2O, 40–100%) and silica gel CC (petroleum ether–acetic ether, from 10:1 to 1:1, v/v) to afford ningpogenin (6.0 mg) and 6-*O*-*p*-hydroxybenzoylaucubin (8.6 mg).

3.3.1 6-O-p-Hydroxybenzoyl-10-deoxyeucommiol (*1*)

Colorless oil; $[\alpha]_D^{14}$ −70.1 ($c = 0.30$, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 203 (4.09), 258 (3.93) nm; IR (KBr) ν_{max}: 3419, 1686, 1609, 1282, 1165 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; FAB-MS (neg.) *m/z*: 291 $[M - H]^-$; HR-FAB-MS (neg.) *m/z*: 291.1239 $[M - H]^-$ (calcd for $C_{16}H_{19}O_5$, 291.1232).

3.3.2 6-O-Benzoyl-10-deoxyeucommiol (*2*)

Colorless oil; $[\alpha]_D^{25}$ − 3.4 ($c = 0.28$, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 241 (4.23) nm; IR (KBr) ν_{max}: 3398, 2925, 1713, 1283, 1117, 715 cm^{-1}, ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; ESI-MS (pos.) *m/z*: 299 $[M + Na]^+$; HR-ESI-MS (pos.) *m/z*: 299.1262 $[M + Na]^+$ (calcd for $C_{16}H_{20}O_4Na$, 299.1259).

3.3.3 6-O-Benzoyl-dihydrocatalpolgenin (*3* and *4*, mixture, 3:2)

Colorless oil; $[\alpha]_D^{25}$ − 122.6 ($c = 0.29$, CH_3Cl_3); UV ($CHCl_3$) λ_{max} (log ε): 241 (4.57) nm; IR (KBr) ν_{max}: 3422, 2924, 1717, 1280, 1114, 715 cm^{-1}, ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; ESI-MS (pos.) *m/z*: 329 $[M + Na]^+$; HR-ESI-MS (pos.) *m/z*: 329.1008 $[M + Na]^+$ (calcd for $C_{16}H_{18}O_6Na$, 329.1001).

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