A novel 10-hydroxycamptothecin-glucoside from the fruit of *Camptotheca acuminata*

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**Abstract**

Glycosides were isolated from the fruit of *Camptotheca acuminata* and identified using NMR, MS, UV and IR spectrometries. 10-O-(1-β-D-glycosyl) camptothecin (1) was identified for the first time in a natural material. In addition, compounds 2–4 were firstly reported from the fruits of *C. acuminata* and indentified as syringaresinol-4, 4′-O-bis-β-D-glucoside (2), hyperoside (3) and pumiloside (4), respectively. Two known compounds, vincoside-lactam (5) and strictosidinic acid (6), were also obtained.

**Keywords**

*Camptotheca acuminata*; glycoside; 10-O-(1-β-D-glycosyl) camptothecin; syringaresinol-4, 4′-O-bis-β-D-glucoside

**Article History**

Received 14 April 2015
Accepted 2 October 2015

**1. Introduction**

*Camptotheca acuminata* Decne, a plant found only in China, is a deciduous tree of the *Nyssaceae* family. This plant is the source of a natural quinoline alkaloid, 10-hydroxycamptothecin (HCPT). It has been reported that 10-hydroxycamptothecin has antitumour activity (inhibition of Topo I) with clinical applications in cancer therapeutics (Huang et al. 2003; Zhang 2005). 10-hydroxycamptothecin is also the precursor for the synthesis of several other anticancer drugs, such as topotecan (Kingsbury & Boehm 1991), 9-nitrocamptothecin (9-NC) and 9-aminocamptothecin (9-AC) (Cabri et al. 1995). Because only a trace amount of 10-hydroxycamptothecin is present in *C. acuminata* (Wang & Lin 2005), there is a need for additional sources of 10-hydroxycamptothecin.

In this study, six glycosides were obtained from the fruits of *C. acuminata* and were identified using NMR (including 1H NMR, 13C NMR, DEPT, HSQC, HMBC, COSY and NOESY), MS, UV and IR spectrometries. A glucose derivative of 10-hydroxycamptothecin, 10-O-(1-β-D-glycosyl)
camptothecin (compound 1), was isolated and identified for the first time from nature. This represents a potential new source of 10-hydroxycamptothecin. This finding is potential to increase the production of 10-hydroxycamptothecin in C. acuminata if a simple hydrolysis reaction is used. In addition, we identified syringaresinol-4,4′-O-bis-β-D-glucoside, hyperoside and pumiloside (compounds 2–4, respectively). Syringaresinol-4,4′-O-bis-β-D-glucoside has never been reported in extracts from C. acuminata. Hyperoside and pumiloside have not been previously found in its fruit. Two known compounds, vincoside-lactam (compound 5) and strictosidinic acid (compound 6), were also obtained from the extract (Figure 1).

2. Results and discussion

Compound 1 was obtained as a white flocculent crystal. The ESI-MS showed a quasimolecular ion peak at m/z 527.0 [M + H]^+ indicating a molecular weight of 526. The fragment ion at m/z 365.0 [M-162 + H]^+ suggested that compound 1 is a glycoside of hydroxycamptothecin (molecular weight of 364 and formula of C_{20}H_{16}N_{2}O_{5}) with a six-carbon glycosyl unit (C_{6}H_{10}O_{5}). The fragment ion at m/z 321.1 [M + H-162-44]^+ was generated by the loss of 44 (CO_{2}) atomic mass units, implying a lactone ring structure. The HR-ESI-MS of compound 1 exhibited a molecular ion peak at m/z 527.173[M + H]^+, supporting a molecular formula of C_{26}H_{26}N_{2}O_{10} {calculated for [M (C_{26}H_{26}N_{2}O_{10})+H^+]: 527.167}. IR spectroscopy showed absorption at 3392 cm^{-1} for hydroxyl groups, 1740 cm^{-1} for the carbonyl of a lactone ring, 1655 cm^{-1} for the carbonyl of lactam, 1590 and 1504 cm^{-1} for an aromatic ring. The 13C NMR spectrum showed 26 carbon signals, of which 6 were assigned to glucose and the remaining 20 were assigned to the aglycone. Except for the glucose signals, the 13C NMR spectrum of compound 1 is similar to that of 10-hydroxycamptothecin (Xu et al. 2005). Detailed analyses of the 1H NMR spectrum showed that compound 1 does not have a C10-OH signal, which is at δ 10.34 in 10-hydroxycamptothecin. Apart from the glucose residue signals, the other 1H NMR data for compound 1 were very close to those of 10-hydroxycamptothecin (Xu et al. 2005). The glucosyl unit was placed at C-10 based on an HMBC correlation from the anomeric proton (H-1′, δ 5.13) to the C-10 (δ 156.4). The magnitude of the coupling constant of the H-1′ signal (J = 7.8 Hz, in CD_{3}OD) defined the anomeric carbon configuration for glucose as β (Podlasek et al. 1995). HSQC, HMBC, 1H-COSY and NOESY correlations allowed for the complete assignments of the 1H and 13C-NMR signals of compound 1 (10-O-(1-β-D-glycosyl) camptothecin). The retention time of the peak in the HPLC of the derivative of sugar obtained after acid hydrolysis of compound 1 coincided with the derivative of D-glucose. The MP, UV and 13C NMR spectroscopic data for 10-O-(1-β-D-glycosyl) camptothecin are reported herein for the first time, and the 1H NMR spectrum signals were assigned according to HSQC and HMBC. Compound 1 is determined as 10-O-(1-β-D-glycosyl) camptothecin. It was obtained from nature for the first time.

Compound 2 was obtained as a white powder. The ESI-MS showed a quasimolecular ion peak at m/z 765 [M + Na]^+, indicating a molecular weight of 742. The +MS^2 (765) had a fragment ion at m/z 603 [M-162 + Na]^+ and + MS^3 (765→603) had a fragment ion at m/z 441 [M-2×162 + Na]^+, implying that compound 2 contained two hexose units. The aglucosyl weight of compound 2 is 418. The 13C NMR spectrum showed only 14 carbon signals, indicating that compound 2 has a highly symmetrical structure. According to the 1H and 13C NMR spectroscopic data, the molecular formula of 2 was determined to be C_{34}H_{46}O_{18}. The structure was identified as syringaresinol-4,4′-O-bis-β-D-glucoside by comparing the spectroscopic
data with the values in the literature (Gu et al. 2005; Zhang et al. 2008). Syringaresinol-4,4′-O-bis-β-D-glucoside has not been previously isolated from C. acuminata.

Compound 3 was obtained as a yellowish powder. The ESI-MS m/z 487 (M + Na)+ and 325 (M-162 + Na)+ indicated a molecular weight of 464 and a glycoside. The structure of 3 was elucidated by IR, MS, and NMR spectroscopies, and the spectroscopic data were compared with data from the literature (Dai et al. 2004; Wang et al. 2004). In addition, the HPLC retention time of 3 was consistent with that of an authentic hyperoside sample. Compound 3 was thus identified as hyperoside.

Compound 4 was obtained as a white flocculent crystal. It has a molecular weight of 512, as determined by the ESI-MS at m/z 511.7[M-H]+. The structure of 4 was elucidated by IR, MS and NMR spectroscopies, and the spectroscopic data were compared with data reported in the literature (Carte’ et al. 1990). Compound 4 was identified as pumiloside.

Compound 5 was elucidated by IR, MS, NMR spectrometries and comparison of its spectroscopic data with those reported in the literature (Yin & Hu 2005). Compound 5 was identified as vincoside-lactam.

Compound 6 was elucidated by IR, MS, NMR spectrometries. The assignments of C-2 and C-19 were corrected base on DEPT-experiment comparing with those reported in the literatures (Hamzah et al. 1994). Compound 6 was identified as strictosidinic acid.

3. Experimental

3.1. Plant material

The fruits of C. acuminata Decne were collected from the mountainous area of Enshi autonomous prefecture, located in the southwest of Hubei province in China. The research materials came from cultivated plants. The specimens were carefully identified by Professor Chen Keli (Hubei University of Chinese Medicine, China), and the voucher specimens (C200910) were deposited in College of Biological Engineering, Wuhan Polytechnic.

3.2. Extraction and isolation

The dried fruits of C. acuminata Decne (1.9 kg) were crushed into coarse powder and extracted with 50% ethanol at 80 °C. After removing the alcohol with a rotary evaporator, the concentrated extract were loaded onto X-5 styrene type non-polar macroporous resin column (surface area is 500–600 m² g⁻¹) and eluted with discontinuous gradients of 30, 50 and 70% ethanol. The 50% ethanol elution fraction was then loaded onto a CT-4S polystyrene-type macroporous adsorption resin column (particle size 0.3–1.2 mm, Nankai Hecheng, China), washed with 30% ethanol to remove water-insoluble impurities, and eluted with 50% ethanol in 6 fractions of 500 mL. After thin layer chromatography, the fractions containing similar spots were combined. After removing alcohol with a rotary evaporator, the concentrated eluates were purified using methanol–acetone precipitation. Compound 2 (approximately 50 mg) and 3 (approximately 60 mg) were directly obtained. The purities of compounds 2 and 3 were estimated to be > 94% by HPLC-UV analysis. Compounds 4–6 (approximately 45 mg) and 1 (approximately 38 mg) were obtained by further separation and purification by preparative HPLC. The purities of the four compounds were estimated to be > 97% based on analytical HPLC.
3.3. Chromatographic and spectroscopic methods

Preparative HPLC was carried out on a P270 preparative HPLC system (Chinese Dalian Elite) with ZORBAX Eclipse XOB-C18 Prep HT 21.2 x 150 mm 5 μm column (Agilent). HPLC was carried out on a LC-20AT HPLC (Shimadzu) with an Inertsil® ODS-SP 4.6 x 150 mm 5 μm column (Shimadzu).

Melting points were determined on a microscopic melting point determination apparatus (Reichert). UV spectra were recorded on a UV-1800 spectrophotometer (Shimadzu), and IR spectra were recorded on a VERTEX70 FT-IR spectrometer (Bruker, Germany). MS and HRMS were obtained with 1100 LC/MSD Trap XCT (Agilent, USA) and a 4800 Plus MALDI TOF/TOF™ Analyzer. 1H and 13C NMR spectra, HSQC and HMBC were recorded on a Unity-Inova 600 or Mercury VX-300 superconducting magnetic resonance spectrometer (Varian, USA). 1H-1H COSY and NOESY were recorded on Bruker Avance III 600 NMR spectrometer equipped with a TCI cryoprobe.

3.4. Characterisation

10-O-(1-β-D-glycosyl) camptothecin (1): white flocculent crystal, mp 228–232 °C, tR = 4.2 min (HPLC, CH3CN/H2O, 18:82, 1 mL min−1, 256 nm); ESI-MS m/z: +MS: 527.0 [M + H]⁺, 365.0 [M-162 + H]⁺, 321.1 [M-162-CO2 + H]⁺, 1052.8 [2 M + H]⁺; HR-ESI-MS: m/z = 527.173; UV (MeOH) ʎmax: 221 nm, 263 nm, 293 nm, 311 nm, 328 nm, 364 nm, 378 nm; IR (KBr) υmax (cm−1)=3392 (broad), 2926, 1740, 1655, 1622, 1590, 1504, 1240, 1164, 1075, 1047; 1H NMR (600 MHz, DMSO-d6): δ 5.26 (2H, s, H-5), 8.53 (1H, s, H-7), 7.64 (1H, s, H-9), 7.57 (1H, d, J = 9 Hz, H-11), 8.08 (1H, d, J = 9 Hz, H-12), 7.28 (1H, s, H-14), 5.42 (2H, s, H-17), 0.88 (3H, t, J = 7.2 Hz, H-18), 1.86 (2H, m, H-19), 5.13 (1H, d, J = 4.2 Hz, Glc-1′), 3.50–3.24 (4H, m, Glc-2′, 3′, 4′, 5′), 3.75(1H, m) and 3.52(1H, m) (Glc-6′), 6.54 (1H, s, 20-OH), 4.659 (1H, t, J = 6, 6′-OH), 5.484 (1H, s, 5.199 (1H, s) and 5.127 (1H, s) (2′,3′,4′-OH); 1H NMR (600 MHz, CD3OD): δ 5.11 (1H, d, J = 7.8 Hz, Glc-1″); 13C NMR (600 MHz, DMSO-d6): δ 151.12 (C-2), 146.03 (C-3), 50.67 (C-5), 130.59(C-6), 130.85 (C-7), 129.47 (C-8), 110.79 (C-9), 156.38 (C-10), 123.45 (C-11), 130.79 (C-12), 144.66 (C-13), 96.67 (C-14), 150.44 (C-15), 118.93 (C-16), 65.66 (C-17), 8.21 (C-18), 30.64(C-19), 72.82 (C-20), 172.99 (C-21), 157.24 (C-22), 100.57 (Glc-1′), 73.63 (Glc-2′), 77.62 (Glc-3′), 70.04 (Glc-4′), 77.07 (Glc-5′), 61.05 (Glc-6′).

Syringaresinol-4,4′-O-bis-β-D-glucoside (2): White powder (methanol-acetone), mp 255–257 °C. tR = 4.18 min (HPLC, CH3CN/H2O, 18:82, 1 mL min−1, 256 nm), ESI-MS m/z: +MS:765 [M + Na]⁺, + MS2 (765): 603 [M-162 + Na]⁺, + MS3 (765→603): 441 [M-2 × 162 + Na]⁺, -MS:777 [M + Cl]−, -MS2 (777): 579 [M-H-162], 417 [M-2 × 162-H]. UV (MeOH) ʎmax: 212 nm, 272 nm; IR (KBr) υmax (cm−1)=3393 (-OH), 2923, 2864, 1595, 1508, 1464, 1422, 1379, 1336, 1237, 1132, 1074, 1024, 993. 1H NMR (600 MHz, D2O): δ 6.69 (4H, s, H-2, 2′, 6, 6′), 4.78 (2H, s, H-7, 7′), 3.22 (2H, m, H-8, 8′), 3.88 (2H,d, J = 7.8 Hz, H-9a, 9′a), 4.22 (2H, dd, J = 7.2, 7.2 Hz, H-9e, 9′e), 3.76 (12H, s, OCH3), 4.90 (2H, d, J = 7.2 Hz, Glc-1″, 1‴), 3.18 (2H, s), 3.36 (2H, dd) and 3.43 (4H, m) (Glc-2′, 2‴, 3′, 3‴, 4′, 4‴, 5′, 5‴), 3.58 (2H, dd, J = 12.6, 5.4 Hz, Glc-6′, 6‴), 3.69 (2H,d, J = 12 Hz, Glc-6′, 6‴). 13C NMR (600 MHz, D2O): δ 138.5 (C-1, 1′), 104.5 (C-2, 2′, 6, 6′), 153.0 (C-3, 3′, 5, 5′), 133.5 (C-4, 4′), 86.1 (C-7, 7′), 53.6 (C-8, 8′), 72.1 (C-9, 9′), 56.8 (OCH3), 103.4 (C-1″, 1‴), 74.1 (C-2″, 2‴), 76.7 (C-3″, 3‴), 69.8 (C-4″, 4‴), 76.1 (C-5″, 5‴), 61.0 (C-6″, 6‴).
The characterisation of hyperoside (3), pumiloside (4), vincoside-lactam (5) and strictosidinic acid (6) are reported in the supplementary material.

### 3.5. Acid hydrolysis and derivatisation of sugar

Compound 1 (2 mg) was dissolved in 2 mL of CF₃COOH (4 mol L⁻¹) and was reacted for 3 h at 95 °C. The reaction solution was extracted with CH₂Cl₂ three times to remove impurities, and the aqueous layer was blown dry. The hydrolysate (from the aqueous layer), D-glucose and D-galactose (every 2 mg) were, respectively, put into three small flasks, and were added pyridine (0.5 mL) and L-cysteine methyl ester hydrochloride (1 mg), respectively. These three samples were heated at 60 °C for 60 min. Then, o-tolyl isothiocyanate (5 μl) was added to the reaction mixtures and further reacted at 60 °C for 60 min. Then, the reaction mixture was analysed by C₁₈ HPLC (acetonitrile–water 25–75, 0.8 mL min⁻¹, 35 °C) and detected by a UV detector (at 250 nm) (Takashi et al. 2007). The reaction mixture of compound 1 exhibited a peak at tR = 10.1 min and D-glucose and D-galactose derivatives at tR = 10.1 min and tR = 9.1 min.

### 4. Conclusion

In order to find new valuable compounds from the plant *C. acuminata*, we used a macroporous resin chromatography column and preparative HPLC, and the spectroscopy techniques of NMR, MS, IR and UV, to isolate and identify six glycosides from the fruit of *C. acuminata*. 10-O-(1-β-D-glycosyl) camptothecin was isolated from nature for the first time and represents a new potential source for 10-hydroxycamptothecin, which is an important compound with anticancer activity. Syringaresinol-4,4′-O-bis-β-D-glucoside was extracted from this plant for the first time. Hyperoside and pumiloside were also extracted from the fruit of this plant for the first time.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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