SUPPLEMENTARY MATERIAL

A novel 10-Hydroxycamptothecin-Glucoside from the fruit of

Camptotheca acuminata

Guo Qun* and Yuan Qiaoyu

Department of Pharmaceutical Technology, College of biological engineering, Wuhan polytechnic, Wuhan 430074, Hubei Province, China

*Corresponding author. Email: guoqun19@136.com

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 *Camptotheca 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

3. Experimental

3.1 *Plant material*

Figure S1. Fruits of *Camptotheca acuminata*

3.4 Characterization

Figure S2. ESI-MS of 10-O-(1-β-D-glycosyl) camptothecin(**1**)

Figure S3. HR-ESI-MS of 10-O-(1-β-D-glycosyl) camptothecin(**1**)

Figure S4. UV spectrum of 10-O-(1- β -D-glycosyl) camptothecin(**1**)

Figure S5. IR spectroscopy of 10-O-(1- β -D-glycosyl) camptothecin(**1**)

Figure S6. ^1H -NMR spectrum of 10-O-(1- β -D-glycosyl) camptothecin (**1**) in DMSO- d_6

Figure S7. ^1H -NMR spectrum of 10-O-(1- β -D-glycosyl) camptothecin (**1**) in CD_3OD

Figure S8. ^{13}C -NMR spectrum of 10-O-(1- β -D-glycosyl) camptothecin (**1**) in DMSO- d_6

Figure S9. HSQC spectrum of 10-O-(1- β -D-glycosyl) camptothecin (**1**) in DMSO-d₆

Figure S10. HMBC spectrum of 10-O-(1- β -D-glycosyl) camptothecin (**1**) in DMSO- d_6

Figure S11. ^1H - ^1H COSY spectrum of 10-O-(1- β -D-glycosyl) camptothecin (**1**) in DMSO- d_6

Figure S12. NOESY spectrum of 10-O-(1- β -D-glycosyl) camptothecin (**1**) in DMSO- d_6

Figure S13. Key HMBC, 1H-1H COSY and NOESY correlations of 10-O-(1-β-D-glycosyl) camptothecin (**1**)

Table S1. ¹³C and ¹H Spectral data and HMBC correlations for 10-O-(1-β-D-glycosyl) camptothecin (**1**) (600 MHz; in DMSO-d₆).

Position	¹³ C-NMR	¹ H-NMR	HMBC	Position	¹³ C-NMR	¹ H-NMR	HMBC
2	151.124			18	8.211	0.88 (3H,t,J=7.2)	H-18/C-19,20
3	146.033			19	30.635	1.86 (2H, m)	H-19/C-18,20,15,21
5	50.668	5.259(2H,s)	H-5/C-6,7,3,2	20	72.816		
6	130.591			21	172.989		
7	130.852	8.53(1H,s)	H-7/C-5,9,8,13,2	22	157.240		
8	129.473			Glc-1'	100.566	5.13 (1H,d,J=4.2)	H-1'/C-10
9	110.790	7.64(1H,s)	H-9/C-11,7,13,10	Glc-2'	73.629		
10	156.379			Glc-3'	77.616	3.496(1H,s),3.	
11	123.445	7.57(1H,d, J=9)	H-11/C-9,13,10	Glc-4'	70.037	4.81(1H,s),3.3 4.2(1H,s),3.23	
12	130.79	8.08(1H,d,9 Hz)	H-12/C-8,10	Glc-5'	77.065	8(1H,s)	
13	144.662			Glc-6'	61.051	3.751(1H,d,6. 6Hz) 3.524(1H,t,5. 7Hz)	
14	96.673	7.28(1H,s)	H-14/C-20,16,3,1 5	20-OH		6.54(1H, s)	
15	150.435			6'-OH		4.659 (1H, t, J=5.4)	
16	118.93			2',3',4'-OH		5.484(1H, s), 5.199(1H, s), 5.127(1H, s)	
17	65.658	5.42 (2H,s)	H-17/C-16,15,22, 21				

Figure S14. UV spectrum of syringaresinol-4,4'-O-bis- β -D-glucoside (**2**)

Figure S15. +MS of syringaresinol-4,4'-O-bis-β-D-glucoside (**2**)

Figure S16. $-MS$ of syringaresinol-4,4'-O-bis- β -D-glucoside (**2**)

Figure S17. IR spectrum of syringaresinol-4,4'-O-bis-β-D-glucoside (2)

Figure S18. ^1H NMR spectrum of syringaresinol-4,4'-O-bis- β -D-glucoside (**2**) in D_2O

Figure S19. ^{13}C -NMR spectrum of syringaresinol-4,4'-O-bis- β -D-glucoside (**2**) in D_2O

Hyperoside (**3**): yellowish powder (methanol-acetone), mp 245~246.5°C. tR=7.58 min (HPLC, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 20:80, 1 mL/min, 256 nm); ESI-MS m/z: +MS:487 ($\text{M}+\text{Na}^+$), +MS² (487): 325 ($\text{M}-162+\text{Na}^+$), 185 ($162+\text{Na}^+$), -MS: 463 ($\text{M}-\text{H}^-$), -MS² (463): 301 ($\text{M}-\text{H}-162$), -MS³ (463→301): 271, 179; UV (MeOH) λ_{max} : 257.0 nm, 300.5 nm, 359.0 nm; IR (KBr) $\nu_{\text{max}}(\text{cm}^{-1})$: 3300 (brs, OH), 2949 (CH_2), 2899 (CH), 1662 ($\text{C}=\text{O}$), 1608, 1562, 1514 (Ar), 1460, 1367, 1306, 1241, 1203, 1170, 1131, 1086, 1069, 1055, 1019, 999, 939, 919, 896, 877, 846, 821, 798, 763, 729, 709, 693, 656, 643. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 6.18 (1H,d, $J=2.1\text{Hz}$, H-6), 6.39 (1H, d, $J=2.4\text{Hz}$, H-8), 7.51 (1H,d, $J=1.8\text{Hz}$, H-2'), 6.80 (1H, d, $J=8.1\text{Hz}$, H-5'), 7.65 (1H, dd, $J=2.1\text{Hz}$, $J=7.8\text{Hz}$, H-6'), 5.37 (1H,d, $J=7.8\text{Hz}$, H-1"), 4.45 (2H, s, H-6"), 3.15-3.63 m (H-2", 3", 4", 5"), 12.62(1H,s, OH-5), 10.88 (1H, s, OH-7), 9.75(1H, s, OH-3'), 9.18 (1H, s, OH-4'), 5.14 (1H, brs, OH), 4.87 (1H, brs, OH); ^{13}C NMR(600 MHz, CD_3OD): δ 158.6 (C-2), 135.9 (C-3), 179.7 (C-4), 163.2 (C-5), 100.1 (C-6), 166.2 (C-7), 94.9 (C-8), 159.0 (C-9), 105.8 (C-10), 123.1 (C-1'), 116.3 (C-2'), 146.0 (C-3'), 150.1 (C-4'), 118.0 (C-5'), 123.2 (C-6'), 105.6 (C-1"), 73.4 (C-2"), 75.3 (C-3"), 70.2 (C-4"), 77.4 (C-5"), 62.2 C-6").

Figure S20. UV spectrum of hyperoside (**3**)

Figure S21. +MS of hyperoside (**3**)

Figure S22. - MS of hyperoside (**3**)

Figure S23. IR spectrum of hyperoside (**3**)

Figure S24. ¹³CNMR spectrum of hyperoside (**3**) in CD₃OD

Figure S25. ^1H NMR spectrum of hyperoside (**3**) in DMSO- d_6

Pumiloside (**4**): white flocculent crystal, mp>340°C, tR=9.24 min (HPLC, CH₃CN/H₂O, 18:82, 1 mL/min, 256 nm); ESI-MS m/z: 511.7 [M-H]⁺. UV (MeOH) λ_{max}: 213 nm, 244 nm, 314 nm, 328 nm; ¹H NMR (300 MHz, CD₃OD): δ 4.69 (1H, m, H-3), 5.40 (1H, d, J=9.9Hz, H-5), 4.73 (1H, overlapped, H-5), 8.25 (1H, d, J=7.5Hz, H-9), 7.41 (1H, t, J=6.9Hz, H-10), 7.62 (1H, t, J=8.7Hz, H-11), 7.68 (1H, d, J=6.9Hz, H-12), 2.55(1H, d, J=11.4Hz, H-14), 2.10(1H, q, J=12.2Hz, H-14), 3.19(1H, m, H-15), 7.16 (1H, s, H-17), 5.50 (2H, d, J=10.5Hz, H-18), 5.84 (1H, m, H-19), 2.68 (1H, m, H-20), 5.84 (1H, m, H-21), 4.71 (1H, overlapped, Glc-1'), 3.55 (1H, m, Glc-2'), 3.66 (1H, m, Glc-3'), 3.90 (1H, d, J=11.4Hz, Glc-4'), 3.41 (1H, m, Glc-5'), 4.66 (1H, brd, J=14.1Hz, H-6'a), 4.54 (1H, brd., J=14.4Hz, H-6'b). ¹³C NMR (300 MHz, CD₃OD): δ 152.5 (C-2), 61.9 (C-3), 49.3 (C-5, Overlapped by CD₃OD), 115.4 (C-6), 176.3 (C-7), 126.9 (C-8), 126.3 (C-9), 125.8 (C-10), 133.3 (C-11), 119.5 (C-12), 142.2 (C-13), 29.8 (C-14), 25.1 (C-15), 110.1 (C-16), 148.3 (C-17), 122.2 (C-18), 133.5 (C-19), 46.1 (C-20), 97.4 (C-21), 167.9 (C-22), 100.3 (C-1'), 75.2 (C-2'), 78.1 (C-3'), 71.6 (C-4'), 78.6 (C-5'), 64.9 (C-6').

Figure S26. UV spectrum of pumiloside (**4**)

Figure S27. MS spectrum of pumiloside (**4**)

Figure S28. ^1H NMR spectrum of pumiloside (4) in CD_3OD

Figure S29. ^{13}C NMR spectrum of pumiloside (**4**) in CD_3OD

Vincoside-lactam (**5**): white powder (methanol-acetone), mp 192~199°C; t_R=9.08 min (HPLC, CH₃CN/H₂O, 30:70, 1 mL/min, 256 nm); ESI-MS m/z: +MS: 499 [M+H]⁺, +MS² (499): 337 [M-162+H]⁺, +MS³ (499.4→337): 319 [337-H₂O]⁺, 267, 194, 171, 144; UV (MeOH) λ_{max}: 224nm, 269nm, 289nm; IR (KBr) ν_{max}(cm⁻¹): 3393 (OH, NH), 2923 (CH₂), 1704, 1654, 1579, 1438, 1353, 1307, 1280, 1193, 1153, 1075, 1016, 889, 742; ¹H NMR (300 MHz, CD₃OD): δ=4.7 (1H, Overlapped by CH₃OH, H-3), 5.06 (1H, d, J=11.4Hz, H-5a), 2.94 (1H, m, H-5b), 2.77 (2H, brs, H-6), 7.42 (1H, d, J=7.5Hz, H-9), 6.99 (1H, t, J=7.5Hz, H-10), 7.08 (1H, t, J=7.5Hz, H-11), 7.30 (1H, d, J=8.1Hz, H-12), 2.46 (1H, brd, J=12.9, H-14a), 1.44 (1H, d, J=12.9Hz, H-14b), 3.22 (1H, brd, J=8.1Hz, H-15), 7.45 (1H, s, H-17), 5.29 (1H, d, J=17.1Hz, H-18a), 5.19 (1H, d, J=10.5Hz, H-18b), 5.54 (1H, m, H-19), 2.72 (1H, m, H-20), 5.51 (1H, brs, H-21), 4.71 (1H, d, J=7.8Hz, Glc-1'), 3.31-3.61 (4H, overlapped, Glc-1', 2', 3'', 4', 5'), 3.91 (1H, brd, J=11.7Hz, H-6'a), 3.68 (1H, dd, J=11.1Hz, J=4.2Hz, H-6'b). ¹³C NMR (300MHz, CD₃OD): δ 134.8 (C-2), 55.1 (C-3), 41.5 (C-5), 22.3 (C-6), 109.6(C-7), 128.2 (C-8), 119.1 (C-9), 120.3 (C-10), 122.8(C-11), 112.3 (C-12), 138.5 (C-13), 27.6 (C-14), 32.9 (C-15), 109.3 (C-16), 149.3(C-17), 120.8 (C-18), 134.2 (C-19), 44.8 (C-20), 97.7 (C-21), 166.3(C=O), 99.8 (C-1'), 75.1 (C-2'), 78.2 (C-3''), 71.8 (C-4'), 78.6 (C-5'), 3.91 (1H, brd, J=11.7Hz, H-6'a), 3.68 (1H, dd, J=11.1Hz, J=4.2Hz, H-6'b).

Figure S30. UV spectrum of vincoside-lactam (**5**)

Figure S31. MS of vincoside-lactam (**5**)

Figure S32. IR spectrum of vincoside-lactam (5)

Figure S33. ^1H NMR spectrum of vincoside-lactam (**5**) in CD_3OD

Figure S34. ¹³CNMR spectrum of vincoside-lactam (**5**) in CD₃OD

strictosidinic acid (**6**): white precipitate, mp 204~207°C; tR=5.6 min (HPLC, CH₃CN/H₂O, 19:81, 1 mL/min, 256 nm); ESI-MS m/z: +MS: 517.1 [M+H]⁺, +MS² (517.4): 500 [M+H-OH]⁺, +MS³ (517.4→500.1): 337.8 [M+H-OH-162]⁺, 320, 251; UV (MeOH) λ_{max}: 218nm, 272nm, 278nm, 289nm, IR (KBr) ν_{max} (cm⁻¹): 3393(OH, NH), 2924 (CH₂), 2856, 1701, 1641, 1538, 1452, 1387, 1310, 1185, 1160, 1077, 1043, 993, 935, 805, 745; ¹H NMR (300 MHz, CD₃OD): δ 4.39 (1H, brd, J=11.7Hz, H-3), 3.09 (1H, m, H-5a), 2.97 (1H, overlapped, H-5b), 2.97 (2H, overlapped, H-6), 7.43 (1H, d, J=7.8Hz, H-9), 7.02 (1H, t, J=7.4Hz, H-10), 7.12 (1H, t, J=7.5Hz, H-11), 7.31 (1H, d, J=8.1Hz, H-12), 2.36 (1H, t, J=12.8, H-14a), 2.10 (1H, t, J=11.5Hz, H-14b), 2.97 (1H, overlapped, H-15), 7.56 (1H, s, H-17), 5.30 (1H, brd, J=17.1Hz, H-18a), 5.19 (1H, brd, J=10.5Hz, H-18b), 5.84 (1H, quint, H-19), 2.69 (1H, m, H-20), 5.81 (1H, s, overlapped, H-21), 4.82 (1H, d, J=7.5Hz, Glc-1'), 3.19-3.61 (4H, overlapped, Glc-1', 2', 3'', 4', 5'), 4.01 (1H, brd, J=11.4Hz, H-6'a), 3.67 (1H, dd, J=11.1Hz, J=6.4Hz, H-6'b); ¹³C NMR (300MHz, CD₃OD, DEPT-experiment): δ 129.33 (C-2), 51.1 (C-3), 41.8 (C-5), 18.4 (C-6), 106.1 (C-7), 126.4 (C-8), 117.9 (C-9), 119.4 (C-10), 122.2 (C-11), 111.1 (C-12), 137.0 (C-13), 33.9 (C-14), 32.8 (C-15), 112.4 (C-16), 152.2 (C-17), 117.7 (C-18), 135.1 (C-19), 44.5 (C-20), 95.4 (C-21), 174.8 (C=O), 99.2 (C-1'), 73.6 (C-2'), 76.8 (C-3'), 70.6 (C-4'), 77.5 (C-5'), 61.9 (C-6').

Figure S35. UV spectrum of strictosidinic acid (**6**)

Figure S36. IR spectrum of strictosidinic acid (**6**)

Figure S37. MS of strictosidinic acid (6)

Figure S38. ^1H NMR spectrum of strictosidinic acid (**6**) in CD_3OD

Figure S39. ¹³CNMR spectrum of strictosidinic acid (**6**) in CD₃OD

Figure S40. DEPT of strictosidinic acid (**6**) in CD₃OD

3.5 Acid hydrolysis and derivatisation of sugar

Figure S41. HPLC comparison of derivative of sugar obtained after acid hydrolysis of the compound **1** with the derivatives of D-glucose and D- galactose

Notes: A: derivative of D- galactose, B: derivatives of D-glucose,

C: derivative of sugar obtained after acid hydrolysis of the compound **1**