PERKIN 1 COMMUNICATION

Synthesis and absolute configuration of 9-β-D-glucosyloxycamptothecin, a new gluco camptothecin isolated from *Ophiorrhiza pumila* regenerated plants

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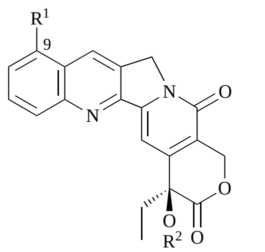
9-β-D-Glucosyloxycamptothecin, which has been obtained from the regenerated plantlets of *Ophiorrhiza pumila*, has been synthesized and its structure and the absolute configuration established.

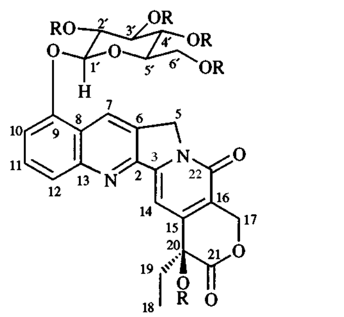
Introduction

Ophiorrhiza pumila Champ. (Rubiaceae) which grows on the Amami and Ryukyu Islands, Japan, produces a remarkable antitumor alkaloid, camptothecin **1**[1] and related alkaloids.[2a,b]



1 $R^1 = R^2$ = H : Camptothecin
2 R^1 = Glc, R^2 = H : 9-β-D-Glucosyloxycamptothecin
3 R^1 = Glc(OAc)$_4$, R^2 = Ac

During our chemical investigation of *O. pumila*,[2] we have recently succeeded in regenerating *O. pumila* from callus cultures. From the regenerated plantlets, a new hydroxycamptothecin glucoside **2** was obtained together with camptothecin **1** and related alkaloids.[2f] The new metabolite was characterized as the acetate and the structure was elucidated as 9-β-glucosyloxycamptothecin pentaacetate **3** based on spectroscopic studies.[2f] This compound was the second example of naturally occurring glycosidic camptothecins. We concluded that the position of the glucosyloxy group was at C-9 after an extensive NMR study, and the absolute stereochemistry at C-20 was most probably *S* as is the case with other natural camptothecins based on a CD spectral comparison (Fig. 1).[2f] The absolute configuration of the sugar, glucose, in **3** was assumed to be D as is encountered in almost all natural glucosides. The validity of the proposed structure including the absolute configuration at the chiral centers was unambiguously proved by the chiral total synthesis, which is described here.



3 R = Ac
2 R = H : 9-β-D-Glucosyloxycamptothecin

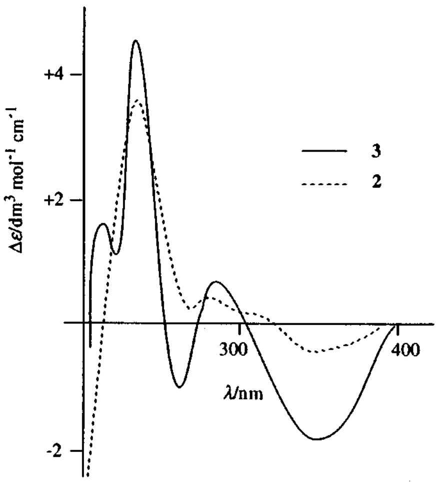
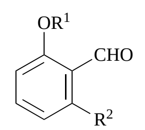
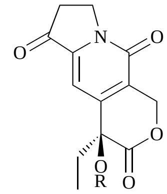


Fig. 1 Comparison of CD spectra for compounds **2** and **3**



4 R^1 = H, R^2 = NO_2
5 R^1 = Glc(OAc)$_4$, R^2 = NO_2
6 R^1 = Glc(OAc)$_4$, R^2 = NH_2



7 R = H
8 R = Ac

Results and discussion

The Friedländer condensation strategy[3] was adopted for the construction of the entire molecule. For this purpose, the A-ring moiety and CDE-ring counterpart were separately prepared. First, 6-glucosyloxy-2-aminobenzaldehyde **6**, corresponding to the A-ring part of **2**, was prepared from the known phenol **4**[4] as follows. The phenol **4** was condensed with tetra-*O*-acetyl bromoglucose in the presence of K_2CO_3 in acetone under reflux to give the 2-glucosyloxy-6-nitrobenzaldehyde **5** in 59% yield.[5] The nitro group in **5** was selectively reduced by hydrogenation (H_2, PtO_2) to afford segment **6**. In the ^{1}H NMR spectrum, the two protons of the amino group were observed as a broad singlet at δ 6.40. The CDE-ring moiety **8** was prepared from known (*S*)-**7**[3c] {$[a]_D^{20}$ +116.4 (*c* 0.51, $CHCl_3$)} through acetylation with AcCl (neat) in 85% yield. In the ^{1}H NMR spectrum, the acetyl methyl protons were observed at δ 2.16. Condensation of the two components **6** and **8** in 5% AcOH–MeOH under reflux gave the camptothecin skeleton **3** in 58% yield. The synthetic compound **3** was identical with the pentaacetate derived from the natural glucoside (TLC, UV, IR, ^{1}H and ^{13}C NMR, CD and mass spectra).[2f] Thus the structure of the new compound including the absolute configuration of the sugar moiety and the C-20 position was established. Deacetylation of **3** with K_2CO_3 (10 equiv.) in MeOH at room temperature gave 9-β-D-glucosyloxycamptothecin **2** in 80% yield.

In conclusion, 9-β-D-glucosyloxycamptothecin, the second example of naturally occurring camptothecinoid glucosides, was synthesized in a chiral manner and its absolute configuration was established.

Experimental

Selected data for the A-ring segment 6

Yellow needles, mp 135 °C (AcOEt) (Found: C, 53.63; H, 5.51; N, 3.06. $C_{21}H_{25}NO_{11}$ requires C, 53.96; H, 5.39; N, 3.00%); δ_H(400 MHz; $CDCl_3$) 10.26 (1 H, s, CHO), 7.20 (1 H, t, *J* 8.2†, aromatic-H), 6.40 (2 H, br s, NH_2), 6.33 (1 H, d, *J* 8.2, aromatic-H), 6.24 (1 H, d, *J* 8.2, aromatic-H), 5.15 (1 H, d, *J* 7.3, H-1′).

Selected data for the CDE-ring component 8

(Found: C, 58.85; H, 5.25; N, 4.79. $C_{15}H_{15}NO_6$ requires C, 59.01; H, 4.95; N, 4.59%); δ_H(500 MHz; $CDCl_3$) 6.76 (1 H, s, aromatic-H), 2.16 (3 H, s, $COCH_3$), 0.92 (3 H, dd, *J* 7.4 and 7.4, CH_3); CD (*c* 0.328 mmol dm^{-3}, MeOH, 25 °C) $\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1} (λ/nm) 0 (377), −0.42 (360), 0 (335), +0.25 (305), +0.14 (287), +2.78 (262), +2.05 (245), +7.90 (233).

Preparation of 9-β-D-glucosyloxycamptothecin tetraacetate 3

AcOH (0.2 cm^3) was added to a mixture of the A-ring segment **6** (219 mg, 0.441 mmol) and the CDE-ring component **8** (19 mg, 0.063 mmol) in dry MeOH (4 cm^3) and the mixture was refluxed under argon for 11.5 h. The reaction mixture was diluted with $CHCl_3$, washed with sat. aqueous $NaHCO_3$ and then with water, dried ($MgSO_4$) and evaporated. The residue was purified by MPLC (SiO_2, 20% AcOEt–$CHCl_3$) to afford the camptothecin derivative **3** (26.5 mg, 58%). [HRMS (FAB): found MH$^+$, 737.2167. Calc. for $C_{36}H_{37}N_2O_{15}$, 737.2194]; λ_{max}(MeOH)/nm 371 (sh), 356, 316, 299, 260; ν_{max}/cm^{-1} 1752, 1235, 1053; δ_H(400 MHz; $CDCl_3$) 8.68 (1 H, s, H-7), 7.94 (1 H, d, *J* 8.5, H-12), 7.73 (1 H, dd, *J* 8.5 and 7.9, H-11), 7.21 (1 H, s, H-14), 7.18 (1 H, d, *J* 7.9, H-10), 5.68 (1 H, d, *J* 17.3, H-17), 5.40 (1 H, d, *J* 17.3, H-17), 5.33 (1 H, d, *J* 19.8, H-5), 5.26 (1 H, d, *J* 19.8, H-5), 5.34 (1 H, d, *J* 7.8, H-1′), 2.28 (1 H, dd, *J* 13.9 and 7.5, H-19), 2.22 (3 H, s, COMe), 2.14 (1 H, dd, *J* 13.9 and 7.5, H-19), 2.09, 2.08, 2.07 and 2.06 (each 3 H, s, 4 × COMe) and 0.98 (3 H, dd, *J* 7.5 and 7.5, H_3-18); δ_C(125 MHz; $CDCl_3$) 153.0 (C-2), 146.0 (C-3), 50.2 (C-5), 128.5 (C-6), 126.0 (C-7), 121.1 (C-8), 152.2 (C-9), 110.2 (C-10), 130.2 (C-11), 124.3 (C-12), 149.4 (C-13), 96.2 (C-14), 145.8 (C-15), 120.6 (C-16), 67.1 (C-17), 7.5 (C-18), 31.8 (C-19), 75.9 (C-20), 167.5 (C-21), 157.3 (C-22), 99.1 (C-1′), 71.0 (C-2′), 72.3 (C-3′), 68.2 (C-4′), 72.3 (C-5′), 61.7 (C-6′); CD (*c* 0.054 mmol dm^{-3}, MeOH, 25 °C) $\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1} (λ/nm) 0 (396), −1.79 (345), 0 (306), +0.63 (283), 0 (269), −1.04 (263), 0 (253), +4.55 (232), +1.58 (217), 0 (210).

† *J* Values are given in Hz.

Preparation of 9-β-D-glucosyloxycamptothecin 2

A mixture of **3** (4.7 mg, 0.006 mmol) and K_2CO_3 (8.7 mg, 0.063 mmol) in dry MeOH (3 cm^3) was stirred at rt under argon for 2 h. The reaction mixture was subjected to Amberlite IR-120B eluted with H_2O. The water layer was extracted with Bu^{n}OH and the organic layer was washed with water, then evaporated. The residue was purified by SiO_2 column chromatography (25% MeOH–$CHCl_3$) to give **2** (2.7 mg, 80%). *m*/*z* (FAB, NBA) 527 (MH$^+$) [HRMS (FAB): found MH$^+$, 527.1660. Calc. for $C_{26}H_{27}N_2O_{10}$, 527.1666]; λ_{max}(MeOH)/nm 372 (sh), 359, 319, 304, 262; δ_H(400 MHz; [$^2H_6$]DMSO) 9.01 (1 H, s, H-7), 7.81 (1 H, d, *J* 7.9, H-12), 7.77 (1 H, dd, *J* 7.9 and 7.9, H-11), 7.36 (1 H, d, *J* 7.9, H-10), 7.34 (1 H, s, H-14), 6.53 (1 H, s, 20-OH), 5.42 (2 H, s, H-17), 5.34 (1 H, d, *J* 19.8, H-5), 5.26 (1 H, d, *J* 19.8, H-5), 5.11 (1 H, d, *J* 7.8, H-1′), 4.62 (1 H, dd, *J* 6.1 and 5.9, 6′-OH), 3.73 (1 H, m, H-6′), 3.44 (1 H, m, H-6′), 3.44 (3 H, m, H-3′–5′‡), 3.27 (1 H, m, H-2′‡), 1.86 (2 H, m, H-19) and 0.87 (3 H, m, H-18); δ_C(125 MHz; [$^2H_6$]DMSO) 152.8 (C-2), 145.5 (C-3), 50.4 (C-5), 129.2 (C-6), 126.5 (C-7), 120.4 (C-8), 153.0 (C-9), 96.8 (C-10), 130.5 (C-11), 122.4 (C-12), 148.6 (C-13), 110.2 (C-14), 150.0 (C-15), 119.1 (C-16), 65.3 (C-17), 7.8 (C-18), 30.3 (C-19), 72.4 (C-20), 172.5 (C-21), 156.9 (C-22), 101.1 (C-1′), 69.7 (C-2′)‡, 73.4 (C-3′)‡, 76.4 (C-4′)‡, 77.3 (C-5′)‡ and 60.7 (C-6′); CD (*c* 0.247 mmol dm^{-3}, MeOH, 25 °C); $\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1} (λ/nm) 0 (389), −0.42 (354), 0 (318), +0.31 (276), +0.26 (266), +3.44 (234), 0 (216).

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‡ Interchangeable.

References

1 (*a*) C. R. Hutchinson, *Tetrahedron*, 1981, **37**, 1047; (*b*) J.-C. Cai and C. R. Hutchinson, in *The Alkaloids*, ed. A. Brossi, Academic Press, New York, 1983, vol. 21, p. 101; (*c*) M. E. Wall and M. C. Wani, in *The Monoterpenoid Indole Alkaloids*, ed. J. E. Saxton, Wiley, London, 1994, ch. 13, p. 689.

2 (*a*) N. Aimi, M. Nishimura, A. Miwa, H. Hoshino, S. Sakai and J. Haginiwa, *Tetrahedron Lett.*, 1989, **30**, 4991; (*b*) N. Aimi, H. Hoshino, M. Nishimura, S. Sakai and J. Haginiwa, *Tetrahedron Lett.*, 1990, **31**, 5169; (*c*) N. Aimi, M. Ueno, H. Hoshino and S. Sakai, *Tetrahedron Lett.*, 1992, **33**, 5403; (*d*) M. Kitajima, S. Masumoto, H. Takayama and N. Aimi, *Tetrahedron Lett.*, 1997, **38**, 4255; (*e*) M. Kitajima, U. Fischer, M. Nakamura, M. Ohsawa, M. Ueno, H. Takayama, M. Unger, J. Stöckigt and N. Aimi, *Phytochemistry*, in press. (*f*) M. Kitajima, M. Nakamura, H. Takayama, K. Saito, J. Stöckigt and N. Aimi, *Tetrahedron Lett.*, 1997, **38**, 8997.

3 Shanghai No. 5 and No. 12 Pharmaceutical Plant, Shanghai Institute of Pharmaceutical Industrial Research and Shanghai Institute of Materia Medica, *Sci. Sin. Ser. B*, 1978, **21**, 87; (*b*) M. C. Wani, P. E. Ronman, J. T. Lindley and M. E. Wall, *J. Med. Chem.*, 1980, **23**, 554; (*c*) A. Ejima, H. Terasawa, M. Sugimori and H. Tagawa, *J. Chem. Soc., Perkin Trans. 1*, 1990, 27.

4 J. N. Ashley, W. H. Perkin and R. Robinson, *J. Chem. Soc.*, 1930, 382.

5 T. Yaegashi, S. Sawada, S. Okajima and T. Miyasaka, Jpn. Kokai Tokkyo Koho JapP 63 238 098.

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