Pergamon

Tetrahedron Letters, Vol. 38, No. 24, pp. 4255-4258, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00858-7

Isolation and Partial Synthesis of 3(*R*)- and 3(*S*)-Deoxypumiloside; Structural Revision of the Key Metabolite from the Camptothecin Producing Plant, *Ophiorrhiza pumila*

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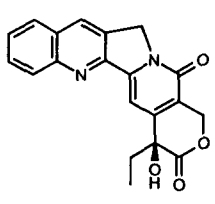
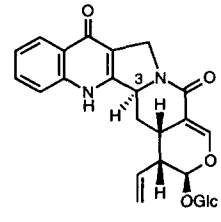
Abstract: Both the C-3 epimeric pair of 3(*R*)- and 3(*S*)-deoxypumiloside were found in *Ophiorrhiza pumila* (Rubiaceae), a source plant of camptothecinoid metabolites. These structures were confirmed by spectroscopic analysis and partial stereoselective syntheses. The configuration at C-3 of the previously reported "deoxypumiloside" is revised to 3(*R*) from 3(*S*). © 1997 Elsevier Science Ltd.

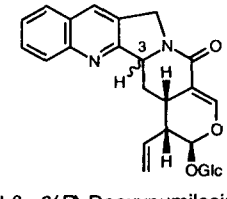
Camptothecin (**1**) is a natural molecule well-known for its potent biological properties such as inhibitory activities against tumor cells and DNA topoisomerase I[1] and activity against HIV-1.[2] From a biogenetic point of view, camptothecin (**1**) possessing a quinoline skeleton has been shown to be formed from the indole alkaloid, strictosidine (**2**).[3-5] Although strictosamide (**3**), a lactam derivative of strictosidine, was reported to be a biogenetic precursor of camptothecin,[3-5] "poststrictosamide biosynthetic events", so named by Hatchinson, have not yet been clarified. During our chemical investigation of camptothecin (**1**), we have found



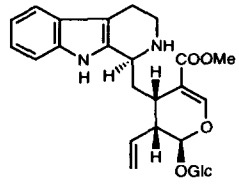
Camptothecin (**1**)



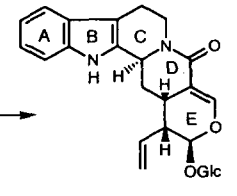
Pumiloside (**4**)



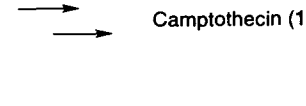
3H-β : 3(*R*)-Deoxypumiloside (**5**) ("Deoxypumiloside")
3H-α : 3(*S*)-Deoxypumiloside (**6**)



Strictosidine (**2**)



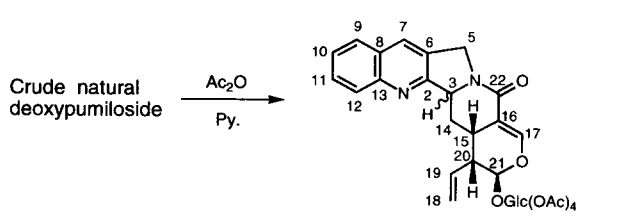
Strictosamide (**3**)



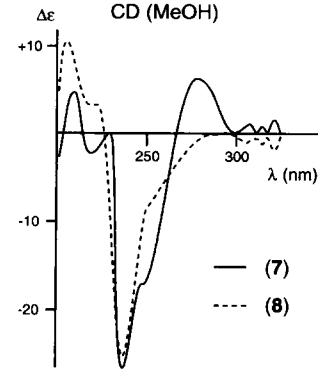
Camptothecin (**1**)

that *Ophiorrhiza pumila* (Rubiaceae) produces not only camptothecin but also a variety of camptothecin-related alkaloids.[6-8] Among them, pumiloside (**4**)[7] and deoxypumiloside[7] were considered to be biogenetic intermediates to camptothecin (**1**). Pumiloside (**4**) was also found in *Camptotheca acuminata* by Hecht and reported independently.[9] The presence of these hybrid-type molecules suggested that camptothecin formation from strictosamide (**3**) starts from the A/B ring conversion of the indole to a quinoline skeleton followed by D and E ring transformations. In order to find new secondary methabolites, which would produce further evidence for the camptothecin biosynthesis, an exhaustive investigation of the constituents in *O. pumila* was carried out. In this paper, we describe the isolation of both 3(*R*)- and 3(*S*)-deoxypumiloside as their tetraacetates and the unequivocal structural clarification by spectroscopic and synthetic methods.

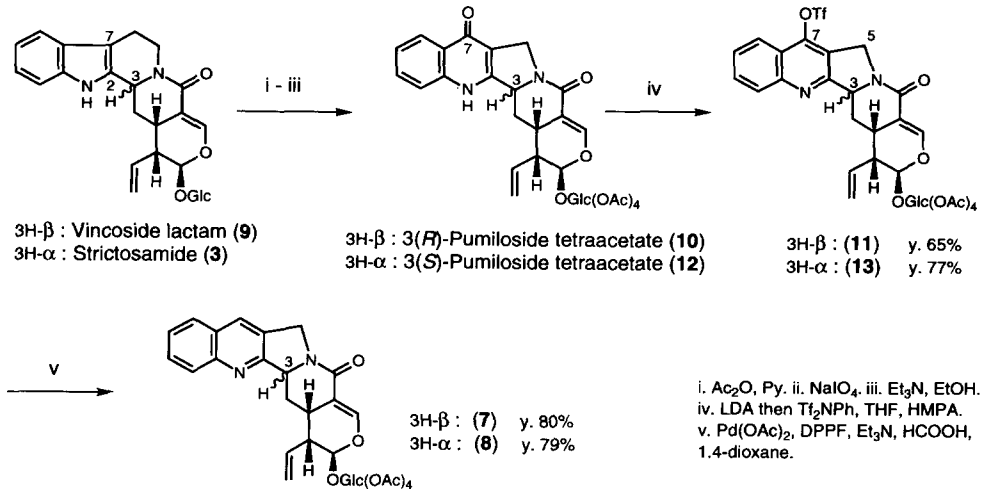
Acetylation of a crude natural deoxypumiloside fraction, which was isolated from *O. pumila*, led to the isolation of *two* acetates (**7** [10] and **8** [11]) in the ratio of 3:1. Both of the products exhibited the same UV absorptions (320, 313, 306, 300, 293, 236, 204 nm) and the molecular formula ($C_{34}H_{36}N_2O_{12}$) which agreed with the anticipated data of deoxypumiloside tetraacetate. The ^{13}C-NMR spectra of **7** and **8** were very similar except for the chemical shifts of the C-15 carbons (**7**: δ 28.3 ppm, **8**: δ 23.8 ppm). Furthermore, the CD spectra of **7** and **8** showed the opposite cotton effect in the region between 320-270 nm. These data clearly show **7** and **8** as epimeric isomers; most likely they are epimers at C-3. For elucidation of their configurations, careful NOE experiments were done. An irradiation of 15-H (δ 3.08) in **7** led to enhancement (10%) of the peak intensity of 3-H (δ 5.01), indicating that this compound has a 3(*R*)-configuration (3H-β). On the other hand, an irradiation of 3-H (δ 4.73) of the minor compound **8** led to enhancement (4%) of the peak intensity of 19-H (δ 5.80), indicating that it is the 3(*S*)-congener (3H-α).



3H-β : 3(*R*)-Deoxypumiloside tetraacetate (**7**)
3H-α : 3(*S*)-Deoxypumiloside tetraacetate (**8**)



To confirm these structures, we next undertook the partial syntheses of both deoxypumilosides using vincoside lactam (**9**) and strictosamide (**3**) as the starting materials, respectively. 3(*R*)-Pumiloside tetraacetate (**10**) was prepared from **9** by a three-step operation in 65% yield.[7] Thus, **10** was treated with LDA and then with *N*-phenyltrifluoromethanesulfonimide[12] in THF-HMPA at -78 ~ 0 °C to give the enol triflate **11** in 89% yield. In the ^{1}H-NMR spectrum, the peak of N_a-H disappeared and the methylene protons on C-5 shifted to a lower field (δ 5.50, 4.82) compared with those of **10** (δ 5.08, 4.55). UV absorptions of **11** (321, 308, 236, 205 nm) were similar to those of 3(*R*)-deoxypumiloside tetraacetate (**7**). These observations indicated that the trifluoromethanesulfonyl group was introduced to the oxygen at the C-7 position to form enol triflate. **11** thus



obtained was treated with palladium acetate, 1,1'-bis(diphenylphosphino)-ferrocene (DPPF), triethylamine and formic acid[13] in dioxane at 60 °C to afford the deoxygenated compound **7** in 80% yield. In the ^{1}H-NMR spectrum, a singlet peak due to 7-H was observed at δ 8.07. Spectroscopic data (UV, ^{1}H-, ^{13}C-NMR, MS, CD) of the synthetic compound were identical with those of the acetate of deoxypumiloside that we obtained from *O. pumila* and was reported in *Tetrahedron Letters* in 1990.[7] The present results clearly indicate that the formerly deduced stereochemistry is erroneous and the configuration of C-3 of "deoxypumiloside" should be revised from C-3(*S*) to C-3(*R*). This conclusion is further substantiated by a parallel stereoselective conversion. 3(*S*)-Deoxypumiloside tetraacetate (**8**) was prepared from strictosamide (**3**), which possesses the 3α-H configuration, *via* the reductive deoxygenation at the C-7 position in 3(*S*)-pumiloside tetraacetate (**12**). The synthetic compound **8** was identified as 3(*S*)-deoxypumiloside tetraacetate, the minor acetate that was obtained as the minor congener during acetylation of the crude "deoxypumiloside" fraction of *O. pumila*. From these synthetic studies, the absolute stereochemistry of both deoxypumilosides was unambiguously established.

In conclusion, we found that *O. pumila* produces both the 3(*R*)- and 3(*S*)-deoxypumiloside (**5** and **6**). We now abandon the name "deoxypumiloside", and this name appearing in previous literature[6,7] should be changed to read 3(*R*)-deoxypumiloside (**5**) hereafter. The structure of each tetraacetate compound including the absolute configuration was confirmed by spectroscopic data and partial syntheses. The findings that both the 3(*R*)- and 3(*S*)-deoxypumiloside are present in *O. pumila* and that 3(*R*)-deoxypumiloside is more richly abundant than the 3(*S*) congener are quite important in further clarification of the camptothecin biosynthesis.

Acknowledgment: Our thanks are due to the Ministry of Education, Science, Sports and Culture, Japan, for a Grant-in-Aid for Scientific Research (No. 08457578).

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10. 3(*R*)-Deoxypumiloside tetraacetate (**7**) : HR-MS (NBA) *m/z*: 665.2357 (Calcd for $C_{34}H_{37}N_2O_{12}$: 665.2347); FAB-MS (NBA) *m/z* (%): 665 (MH^+, 60), 154 (100); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.08 (d, 1H, *J* = 7.4 Hz, 12-H), 8.07 (1H, s, 7-H), 7.83 (dd, 1H, *J* = 8.0, 1.2 Hz, 9-H), 7.72 (ddd, 1H, *J* = 8.6, 7.1, 1.5 Hz, 11-H), 7.55 (ddd, 1H, *J* = 8.2, 7.1, 1.3 Hz, 10-H), 7.53 (d, 1H, *J* = 2.7 Hz, 17-H), 5.47 (ddd, 1H, *J* = 17.3, 9.9, 9.9 Hz, 19-H), 5.34 (d, 1H, *J* = 1.9 Hz, 21-H), 5.33-5.29 (br-d, 1H, *J* = 17.1 Hz, 5-H), 5.31 (dd, 1H, *J* = 17.1, 2.0 Hz, 18B-H), 5.27 (dd, 1H, *J* = 9.4, 9.4 Hz, 3'-H), 5.18 (dd, 1H, *J* = 10.0, 1.9 Hz, 18A-H), 5.12 (dd, 1H, *J* = 9.8, 9.8 Hz, 4'-H), 5.05 (dd, 1H, *J* = 9.5, 8.1 Hz, 2'-H), 5.01 (dd, 1H, *J* = 11.2, 3.0 Hz, 3-H), 4.97 (d, 1H, *J* = 8.0 Hz, 1'-H), 4.71 (dd, 1H, *J* = 16.6, 1.2 Hz, 5-H), 4.32 (dd, 1H, *J* = 12.5, 4.7 Hz, 6'-H), 4.16 (dd, 1H, *J* = 12.5, 2.2 Hz, 6'-H), 3.78 (ddd, 1H, *J* = 10.0, 4.7, 2.2 Hz, 5'-H), 3.08 (m, 1H, 15-H), 2.82 (ddd, 1H, *J* = 9.6, 5.6, 1.8 Hz, 20-H), 2.66 (ddd, 1H, *J* = 12.9, 3.6, 3.6 Hz, 14β-H), 2.11, 2.04, 2.02 and 2.01 (each s, 3H, 3 x OAc), 1.55 (ddd, 1H, *J* = 12.9, 12.3, 12.3 Hz, 14α-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 161.8 (C-2), 61.5 (C-3), 48.5 (C-5), 128.0 (C-6), 130.0 (C-7), 127.7 (C-8), 127.9 (C-9), 126.7 (C-10), 129.5 (C-11), 129.0 (C-12), 148.2 (C-13), 30.0 (C-14), 28.3 (C-15), 108.4 (C-16), 146.8 (C-17), 120.8 (C-18), 131.5 (C-19), 42.9 (C-20), 96.4 (C-21), 162.8 (C-22), 96.1 (C-1'), 70.6 (C-2'), 72.4 (C-3'), 68.2 (C-4'), 72.3 (C-5'), 61.8 (C-6'), 20.74 and 20.65 (each CO-*Me*), 20.57 (2 x CO-*Me*), 170.6 and 170.1 (each *C*O-Me), 169.4 (2 x *C*O-Me); CD (c = 0.21 mmol/l, MeOH, 21°C) Δε (λ nm): 0 (330), +1.46 (320), +0.29 (317), +0.88 (313), +0.29 (310), +1.17 (307), +0.58 (303), +6.56 (276), 0 (266), -16.62 (247), -27.12 (238), 0 (225), -2.33 (218), 0 (213), +5.83 (208).
11. 3(*S*)-Deoxypumiloside tetraacetate (**8**) : HR-MS (NBA) *m/z*: 665.2347 (Calcd for $C_{34}H_{37}N_2O_{12}$: 665.2347); FAB-MS (NBA) *m/z* (%): 665 (MH^+, 100), 154 (85); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.08 (d, 1H, *J* = 8.6 Hz, 12-H), 8.07 (1H, s, 7-H), 7.82 (d, 1H, *J* = 8.0 Hz, 9-H), 7.71 (ddd, 1H, *J* = 8.3, 6.9, 1.4 Hz, 11-H), 7.56 (ddd, 1H, *J* = 8.0, 6.9, 1.0 Hz, 10-H), 7.16 (d, 1H, *J* = 2.7 Hz, 17-H), 5.80 (ddd, 1H, *J* = 17.0, 9.9, 9.9 Hz, 19-H), 5.51 (dd, 1H, *J* = 17.2, 1.9 Hz, 18B-H), 5.05-4.98 (m, 1H, 5-H), 5.29 (d, 1H, *J* = 1.9 Hz, 21-H), 5.27 (dd, 1H, *J* = 9.5, 9.5 Hz, 3'-H), 5.40 (dd, 1H, *J* = 10.2, 1.6 Hz, 18A-H), 5.10 (dd, 1H, *J* = 9.8, 9.8 Hz, 4'-H), 5.03 (dd, 1H, *J* = 9.2, 8.1 Hz, 2'-H), 4.98 (d, 1H, *J* = 8.1 Hz, 1'-H), 4.78 (dd, 1H, *J* = 16.5, 1.1 Hz, 5-H), 4.73 (dd, 1H, *J* = 7.9, 4.1 Hz, 3-H), 4.30 (dd, 1H, *J* = 12.5, 2.2 Hz, 6'-H), 4.16 (dd, 1H, *J* = 12.5, 4.4 Hz, 6'-H), 3.77 (ddd, 1H, *J* = 10.0, 4.7, 2.3 Hz, 5'-H), 3.15 (m, 1H, 15-H), 2.72 (ddd, 1H, *J* = 9.5, 5.3, 1.4 Hz, 20-H), 2.62 (dd, 1H, *J* = 13.4, 5.0 Hz, 14α-H), 2.10 (m, 1H, 14β-H), 2.10 (s, 6H, 2 x OAc), 2.04 and 2.01 (each s, 3H, 2 x OAc); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 161.9 (C-2), 60.2 (C-3), 47.9 (C-5), 128.0 (C-6), 130.4 (C-7), 127.6 (C-8), 127.9 (C-9), 126.7 (C-10), 129.5 (C-11), 129.1 (C-12), 148.2 (C-13), 28.8 (C-14), 23.8 (C-15), 110.4 (C-16), 145.1 (C-17), 121.6 (C-18), 131.4 (C-19), 43.8 (C-20), 96.3 (C-21), 165.2 (C-22), 96.1 (C-1'), 70.4 (C-2'), 72.4 (C-3'), 68.1 (C-4'), 72.2 (C-5'), 61.7 (C-6'), 20.8 and 20.7 (each CO-*Me*), 20.6 (2 x CO-*Me*), 170.6, 170.1, 169.9 and 169.4 (each *C*O-Me); CD (c = 0.15 mmol/l, MeOH, 21°C) Δε (λ nm): 0 (325), -1.01 (320), -0.40 (317), -0.81 (313), -0.40 (310), -0.60 (307), -0.20 (300), -25.15 (238), 0 (227), +3.62 (218), +10.87 (206).
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(*Received in Japan* 10 *April* 1997; *revised* 28 *April* 1997; *accepted* 2 *May* 1997)