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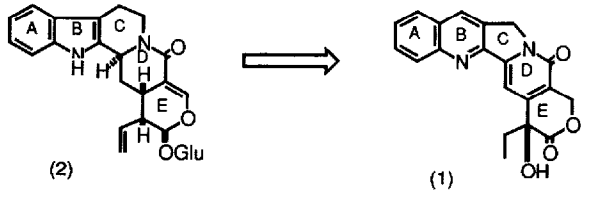
PUMILOSIDE AND DEOXYPUMILOSIDE; PLAUSIBLE INTERMEDIATES OF CAMPTOTHECIN BIOSYNTHESIS

Norio Aimi, Masashi Nishimura, Akiko Miwa, Hiroyuki Hoshino, Shin-ichiro Sakai, and Joju Haginiwa

Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Chiba 260, Japan

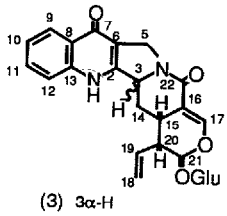
Summary -- Pumiloside and deoxypumiloside, long-sought plausible intermediates of camptothecin biosynthesis, were found in Ophiorrhiza pumila (Rubiaceae).

A great deal of chemical research towards camptothecin (**1**), a natural product which has strong antitumor activity and specific inhibitory activity to mammalian topoisomerase I, has been carried out by numerous research groups because of its remarkable biological activity and the novelty of the chemical structure.[1,2] In addition, strong attention has also been focused on its unique manner of biosynthetic construction of its molecule. Strictosamide (**2**) was proved to be the key precursor.[3] This finding indicated that the quinoline nucleus of **1** was formed from biological transformation of the indole moiety of **2**. Biogenetic oxidation of the D ring to pyridone and cleavage of the glucoside bond followed by several structural alterations on the E ring are also major structural changes. At some stage the vinyl group changes its form to the ethyl group. To clarify the exact features and sequences of these "poststrictosamide biosynthetic events" extensive study

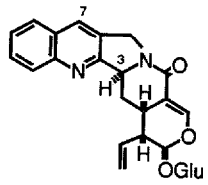


was carried out by Hutchinson et al.[1,2] Compound (**3**), the quinolone modification of strictosamide (**2**), was postulated as the possible intermediate. Some attempt in incorporation study was made by them but definite conclusion has not been obtained.[4] In this paper we report isolation of two new glucosidic alkaloids, pumiloside (**3**) and deoxypumiloside (**4**), from Ophiorrhiza pumila, the plant which also contain camptothecin (**1**). Pumiloside (**3**)

is the exactly same compound that was postulated by Hutchinson as the post-strictosamide intermediate of camptothecin biosynthesis, and deoxypumiloside (**4**) is the member which was expected to follow **3**. Laboratory syntheses of pumiloside (**3**) and its C-3 epimer (**5**) have also been made to obtain the unambiguous proof of the stereostructure.



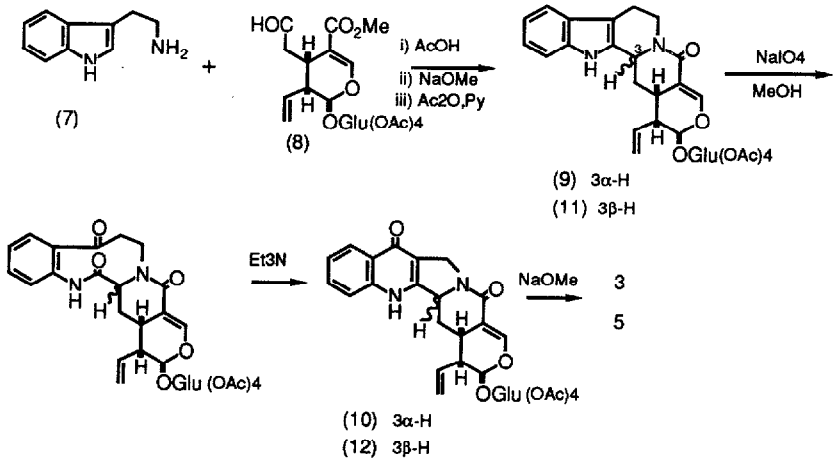
(3) 3α-H
(5) 3β-H
(6) 18,19-dihydro, 3α-H
(7) 18,19-dihydro, 3β-H



(4)

The whole plant of Ophiorrhiza pumila was extracted with methanol and the extract was partitioned between chloroform and water. From the chloroform layer camptothecin (**1**) [m/z 348.1142; calcd. for $C_{20}H_{16}N_2O_4$; m/z 348.1111] was obtained. Glycosides in the water layer were then transferred into butanol. Combination of open column and HPLC enabled us to obtain two new constituents. Compound A[5], now named pumiloside (**3**), showed the UV spectrum indicating the quinolone chromophore. The molecular formula, $C_{26}H_{28}N_2O_9$, was clarified with FAB-MS. The ^{1}H-NMR spectrum[5] demonstrated the structure depicted above.

With the purpose of clarifying the stereostructure laboratory synthesis of pumiloside was carried out. Closely related compounds (**6**) and (**7**) have been synthesized by Hutchinson et al.[6] and we followed the same sequence of reactions. Thus strictosamide acetate (**9**) prepared from tryptamine (**7**) and secologanin acetate (**8**) was oxidized with excess of sodium metaperio-



(9) 3α-H
(11) 3β-H
(10) 3α-H
(12) 3β-H

date. The resulting dicarbonyl derivative was treated with triethyl amine to give quinolone (**10**). Removal of the acetyl group gave the anticipated glucoside (**3**) as colorless prisms, mp > 300°C. Behaviors of this compound

on TLC and HPLC were identical with those of the natural pumiloside (**3**) and the identity was fully confirmed by comparison of their ^{1}H-NMR spectra. This forms the proof of α-H configuration at C-3 and respective configurations at C-15, 20, and 21 as well.

To obtain further decisive evidence of the stereochemistry at C-3 synthesis of 3-epipumiloside (**5**) was also carried out. Vincoside lactam acetate (**11**) was used as the starting material and the same sequence of reactions gave the objective compound (**5**) as colorless prisms, mp > 300°C. CD spectra were measured for pumiloside (**3**) and 3-epipumiloside (**5**). The opposite sign of the Cotton effect of the longest wave length region (350 - 300 nm), positive for (**3**) and negative for 3-epi isomer (**5**), clearly verified the assigned stereochemistry.

Compound B (**4**)[7] was obtained from the same plant as an amorphous powder. The FAB-MS indicated the molecular formula $C_{26}H_{28}N_2O_8$ by the M^+ +1 peak at m/z 497. This corresponds to a deoxy analogue of pumiloside and the structure was suggested as 7-deoxypumiloside (**4**) by the UV spectrum indicative of the quinoline chromophore. The ^{1}H-NMR spectrum[7] strongly supported this structure. The 3α-H configuration was indicated by the CD spectrum possessing the curve similar to pumiloside. It should be noted that this compound is another member of the long-sought poststrictosamide intermediates of camptothecin biosynthesis.

It is interesting to note that Ophiorrhiza pumila contains no trace of harman, lyaloside, and other member of β-carboline derivatives which were encountered in other species of Ophiorrhiza we already studied.[8]

ACKNOWLEDGMENTS; This work has been financially supported by a Grant-in-Aids for General Scientific Researches from the Ministry of Education, Science, and Culture of Japan.

References and Notes

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4) C. R. Hutchinson, M.-T. S. Hsia, A. H. Heckendorf, and G. J. O'loughlin, J. Org. Chem., **41**, 3493 (1976)
In the footnote part of this literature, as an unpublished result, they state failure to get positive incorporation of the administered synthetic compounds. There the authors put a quite appropriate remark that this kind of negative results should be treated very carefully. We also should like to point out that the administered synthetic compounds seem most likely to be 18,19-dihydro analogues of the plausible intermediate which we found in the nature this time.

5) Colorless prisms, mp > 300°C. FAB-MS m/z: 513 (M^++1, 8%) ($C_{26}H_{28}N_2O_9$). λ_{max} nm: 211, 241(sh), 244, 303(sh), 314, and 327. ^{1}H-NMR (500MHz, CD_3OD) δ :2.11(1H,ddd,J=12.8,12.8,10.3Hz,H-14β), 2.57(1H,dd,J=13.3, 3.7Hz,H-14α), 2.70(1H,dd,J=9.8,5.0Hz,H-20), 3.21(1H,dd,J=9.1,8.0Hz, H-2'), 3.36 - 3.27 (2H,m,H-4',H-5'), 3.39(1H,dd,J=8.9,8.9Hz,H-3'), 3.42 (1H,m,H-15), 3.68(1H,dd,J=12.0,5.9Hz,H-6'), 3.91(1H,dd,J=12.0,2.1Hz,H-6'), 4.54(1H,d,J=14.3Hz,H-5β), 4.72(1H,dd,J=14.3,2.2Hz,H-5α), 4.73(1H, d,J=8.0Hz,H-1'), 4.90(1H,m,H-3), 5.41(1H,dd,J=10.2,1.9Hz,H-18), 5.50 (1H,d,J=1.7Hz,H-21), 5.52(1H,dd,J=17.0,1.7Hz,H-18), 5.86(1H,ddd,J=17.4, 10.4,10.0Hz,H-19), 7.17(1H,d,J=2.5Hz,H-17), 7.42(1H,ddd,J=7.6,7.3,1.1 Hz,H-10), 7.71(1H,ddd,J=8.5,7.0,1.4 Hz,H-11), 7.64(1H,d,J=8.3Hz,H-12), and 8.27(1H,dd,J=8.3,1.1Hz,H-9).

6) C. R. Hutchinson, G. J. O'loughlin, R. T. Brown, and S. B. Fraser, J. C. S. Chem. Commun., **1974**, 928.

7) Amorphous powder. FAB-MS m/z: 497(M^++1, 41%). λ_{max} nm: 206, 235, 293, 300, 306, 312, and 320. ^{1}H-NMR (500MHz, CD_3OD) δ :1.54(1H,ddd,J=12.7,12.5, 12.4Hz,H-14α), 2.68(1H,ddd,J=12.6,3.6,3.6Hz,H-14β), 2.82(1H,ddd,J=8.1, 5.4Hz,H-20), 3.24(1H,dd,J=9.1,8.0Hz,H-2'), 3.29 - 3.35(2H,m,H-3',H-4'), 3.38(1H,m,H-15), 3.40(1H,m,H-5'), 3.69(1H,dd,J=12.0,5.64Hz,H-6'), 3.91 (1H,dd,J=12.0,2.1Hz,H-6'), 4.71(1H,d,J=16.8Hz,H-5α), 4.73(d,J=8.0Hz,H-1'), 5.09(1H,dd,J=11.6,3.3Hz,H-3), 5.20(1H,dd,J=10.7,1.9Hz,H-18), 5.24 (1H,d,J=17.3Hz,H-5β), 5.33(1H,dd,J=17.2,1.8Hz,H-18), 5.53(1H,ddd,J=17.1, 10.0,10.0Hz,H-19), 5.57(1H,d,J=1.9Hz,H-21), 7.52(1H,d,J=2.5Hz,H-17), 7.61(1H,ddd,J=7.6,7.6,1.1Hz,H-10), 7.76(1H,ddd,J=8.5,7.1,1.4Hz,H-11), 7.95(1H,dd,J=7.7Hz,H-9), 8.05(1H,d,J=8.5Hz,H-12), and 8.31(1H,s,H-7).

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