Pergamon

Tetrahedron 58 (2002) 9169–9178

TETRAHEDRON

Camptothecin-related alkaloids from hairy roots of *Ophiorrhiza pumila*

Mariko Kitajima,[a] Satoshi Yoshida,[a] Kyoko Yamagata,[a] Mio Nakamura,[a] Hiromitsu Takayama,[a] Kazuki Saito,[a] Hiroko Seki[b] and Norio Aimi[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*
[b]*Chemical Analysis Center, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*

Received 19 August 2002; accepted 20 September 2002

Abstract—Hairy roots of *Ophiorrhiza pumila*, a rubiaceous camptothecin-producing plant, were obtained. From an investigation of the secondary metabolites, it was found that the hairy roots produced camptothecin and its related alkaloids, i.e. (3*S*)-pumiloside, (3*S*)- and (3*R*)-deoxypumilosides and strictosamide. We also isolated two new camptothecinoids, OPHR-23 and OPHR-17, the structures of which including the absolute configurations, were determined by spectroscopic analyses and chemical conversion from tryptamine and secologanin.

1. Introduction

Camptothecin (**1**)[1] is a well-known anti-tumor alkaloid, which was first isolated from *Camptotheca acuminata* belonging to Nyssaceae. This compound possesses inhibitory activity against DNA topoisomerase I and also activity against HIV-1. Recently, semi-synthetic camptothecins such as irinotecan® and topotecan® have been used as clinical antitumor agents. In the course of our chemical studies on indole alkaloids, we found that *Ophiorrhiza pumila* (rubiaceae) produces camptothecin (**1**) and its related alkaloids such as (3*S*)-pumiloside (**2**), (3*S*)- and (3*R*)-deoxypumilosides (**3** and **4**) which might be plausible biogenetic precursors of camptothecin (**1**).[2] For the purpose of establishing an efficient production method of camptothecin (**1**) as well as finding novel secondary metabolites and a clue to clarification of the camptothecin biosynthetic pathway, we have also investigated the constituents of callus cultures[3] and regenerated plants[4] of *O. pumila*. Callus cultures produce anthraquinones that could not be detected in the wild plants, and the regenerated plants produce almost the same alkaloids as the wild plants. In continuation of our study, we obtained hairy roots[5] of *O. pumila* and investigated secondary metabolites of the hairy roots, resulting in the isolation and structure elucidation of alkaloids, including new camptothecinoids, which will be described here in detail.

2. Results and discussion

We succeeded in obtaining hairy roots of *O. pumila* by the direct inoculation of *Agrobacterium rhizogenes* (pRi15834; pGSGluc1) to the regenerated plant stems.[5] *Agrobacterium*-inoculated stems were maintained on the hormone-free half-strength Murashige and Skoog medium and after about 80 days hairy roots appeared. The hairy roots obtained were cultivated on the same medium under light at 25°C and frequently subcultivated on fresh medium at intervals of two weeks.

Hairy roots (190 g of wet weight) cultivated on the solid medium were extracted with hot MeOH to give the extract (2.3 g). The MeOH extract was then partitioned between H_2O and $CHCl_3$ and the aqueous layer was extracted with *n*-BuOH to give the $CHCl_3$ extract (263 mg), the *n*-BuOH extract (210 mg) and the water-soluble portion (1.83 g), respectively. From the extract, camptothecin-related alkaloids such as (3*S*)-pumiloside (**2**) (33.7 mg), (3*S*)- and (3*R*)-deoxypumilosides (**3**, 9.9 mg and **4**, 9.4 mg) and strictosamide (**5**, 9.4 mg) were isolated together with one new alkaloid, OPHR-23 (**6**, 0.4 mg). This result revealed that hairy roots of *O. pumila* maintained the alkaloid production ability. Although a small amount of (3*S*)-deoxypumiloside (**3**) was already isolated as a tetraacetate derivative from the wild plants,[2d] at this time, we obtained (3*S*)-deoxypumiloside (**3**) as a genuine form and in comparable amount with the 3-epimer (**4**).

The new alkaloid, OPHR-23 (**6**, 0.4 mg), having blue fluorescence under UV lamp at the wavelength of 365 nm, showed the characteristic UV spectrum of camptothecin at

Keywords: camptothecin; *Ophiorrhiza pumila*; alkaloids.
* Corresponding author. Tel./fax: +81-43-290-2901; e-mail: aimi@p.chiba-u.ac.jp

0040–4020/02/$ - see front matter
PII: S0040-4020(02)01196-1

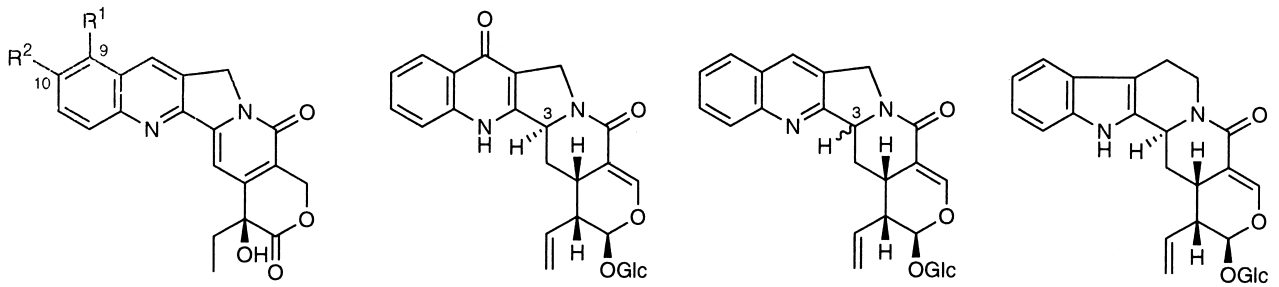


Figure 1.

362, 290, 254, 219 nm. From the HR-FAB-MS, the molecular formula was decided to be $C_{27}H_{28}N_2O_9$. The ^{1}H NMR spectrum revealed five aromatic protons due to the 2,3-disubstituted quinoline system [δ 8.67 (s, H-7), 8.16 (d, H-12), 8.11 (d, H-9), 7.85 (dd-like, H-11), 7.70 (dd-like, H-10)], one singlet aromatic proton due to H-14 (δ 7.02) and one methylene proton due to H_2-5 (δ 5.24), which are characteristic protons of camptothecin (**1**). A set of three protons on a vinyl group [δ 5.81 (ddd, J=17.1, 10.1, 9.1 Hz, H-19), 5.51 (br-d, J=17.1 Hz, H-18), and 5.44 (dd, J=10.1, 2.1 Hz, H-18)], an acetal proton at δ 5.46 (d, J=7.0 Hz, H-21), and one sugar unit containing an anomeric proton at δ 4.64 (d, J=7.9 Hz, H-1′) were also observed, which are very similar to those of strictosamide (**5**), (3*S*)-pumiloside (**2**), and deoxypumilosides (**3** and **4**), together with another acetal proton at δ 5.65 (s, H-17) and methoxy protons at δ 3.47 (s). The ^{13}C NMR showed 27 carbons including 16 sp^2 carbons, three acetal carbons (δ 97.9, 95.2 and 91.7), a methoxy carbon (δ 55.2) and carbons due to one glucose unit. In the HMBC spectrum, crosspeaks between the acetal proton at δ 5.65 and both carbons due to the methoxy group and the acetal carbon at δ 91.7 (C-21) and a crosspeak between the anomeric proton (δ 4.64, H-1′) and the same acetal carbon (C-21) were observed, suggesting that the methoxy group was located on C-17 (Figs. 1 and 2). From these data, the structure of this alkaloid was deduced to be **6**.

As the new compound (**6**) possesses a unique structure, we tried again to get this alkaloid from the hairy roots cultivated in the liquid medium. On the basis of the fundamental study on the culture condition, we cultivated the hairy roots in Gamborg's B5 medium containing sucrose (1%) under light (750 lx) at 25°C at 66 rpm. Hairy roots (about 375 g of wet weight) cultivated in the liquid medium were extracted with hot MeOH to give the extract (10.6 g). The MeOH extract was then partitioned between H_2O, $CHCl_3$ and *n*-BuOH to give the $CHCl_3$ extract (790 mg), the *n*-BuOH extract (1666 mg) and the water-soluble portion (6.03 g), respectively. Two new camptothecinoids (**6**, 0.3 mg and **7**, 0.4 mg), one of which was the same as the new camptothecinoid isolated from the hairy roots cultivated on the solid medium, were isolated together with camptothecin (**1**, 17.7 mg), (3*S*)-pumiloside (**2**, 9.9 mg), (3*R*)-deoxypumiloside (**4**, 1.3 mg) and strictosamide (**5**, 9.9 mg).

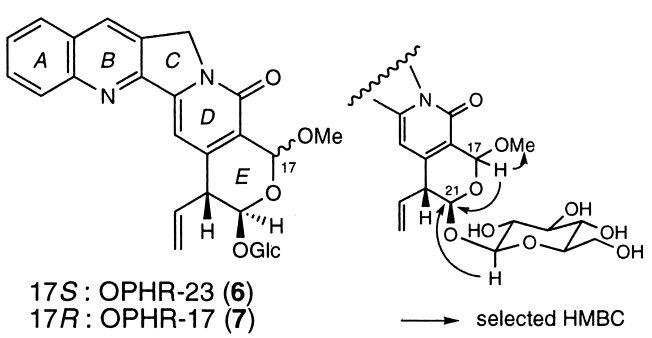
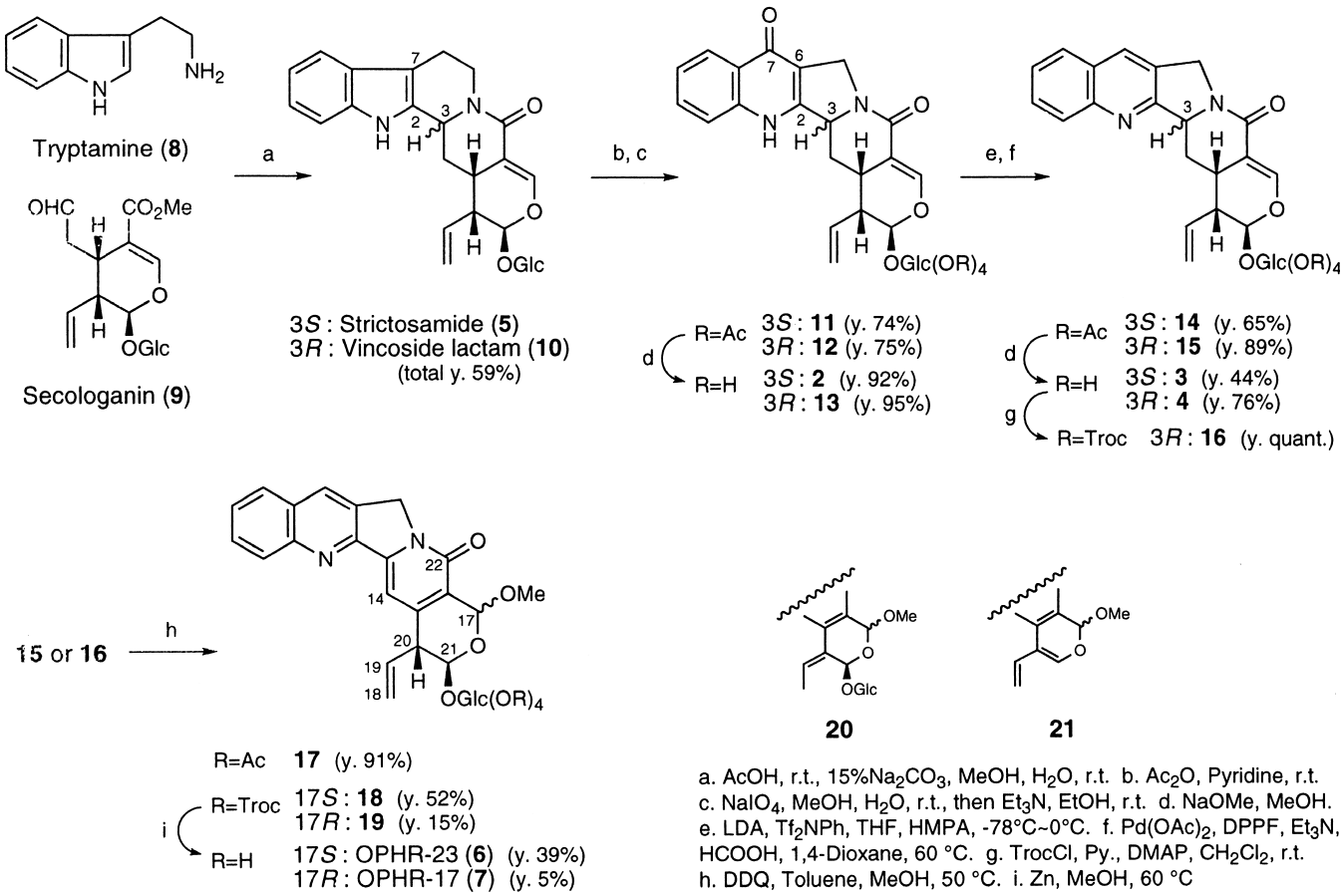


Figure 2.

The new alkaloid, OPHR-17 (**7**), having blue fluorescence under UV lamp (λ 365 nm), showed the UV spectrum (363, 280 sh, 254, 219 nm) and the molecular formula $C_{27}H_{28}N_2O_9$) which were the same as those of OPHR-23 (**6**). The ^{1}H NMR spectrum revealed five aromatic protons due to the 2,3-disubstituted quinoline system [δ 8.68 (s, H-7), 8.15 (d, H-12), 8.12 (d, H-9), 7.85 (dd-like, H-11), 7.70 (dd-like, H-10)], one singlet aromatic proton due to H-14 (δ 7.14) and one methylene proton due to H_2-5 (δ 5.25) as camptothecin, suggesting that the D-ring was oxidized to the pyridone ring. Furthermore, a set of three protons on a vinyl group at δ 5.82 (ddd, J=17.0, 10.2, 7.0 Hz, H-19), 5.37 (br-d, J=17.0 Hz, H-18), and 5.23 (br-d, J=10.2 Hz, H-18), two acetal protons at δ 5.53 (s, H-17), 5.45 (d, J=1.7 Hz, H-21), one anomeric proton at δ 4.65 (d, J=7.8 Hz, H-1′) and methoxy protons at δ 3.57 (s) were observed. The ^{13}C NMR showed 27 carbons including three acetal carbons (δ 97.6, 94.5, 94.3) and a methoxy carbon (δ 57.1) which are the same as those in **6**. From the above spectral data, the structure of OPHR-17 was presumed to be **7** and the new alkaloids **6** and **7** were considered to be a pair of diastereomers at the C-17 position.

We next planned the synthesis of two new camptothecinoids starting from tryptamine (**8**) and secologanin (**9**) to establish the structures, including the absolute configurations (Scheme 1). Condensation of **8** and **9** by the Pictet–Spenglar reaction under acidic condition followed by D-ring closure by treatment with alkaline gave strictosamide (**5**, y. 26%) having 3*S* and vincoside lactam (**10**, y. 33%) having 3*R* stereochemistry, respectively.[6] (3*S*)-Pumiloside tetraacetate (**11**) was prepared from **5** by a three-step procedure that included acetylation of the hydroxy groups in the glucose unit, oxidative bond cleavage at C2–C7 by using excess $NaIO_4$, and ring closure between C2 and C6 to give a

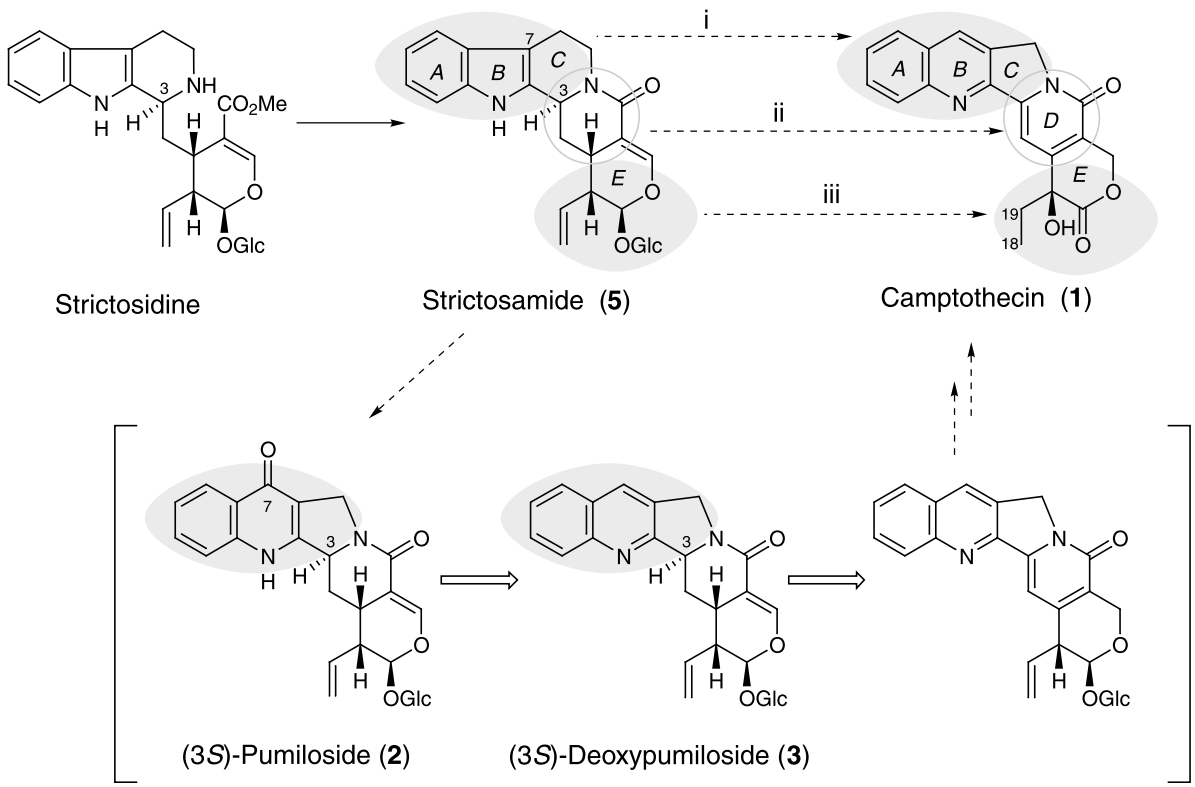


Scheme 1.

quinolone ring.[7] Removal of the protecting group of **11** gave (3*S*)-pumiloside (**2**). **11** was treated with lithium diisopropylamide (LDA) and then with *N*-phenyltrifluoromethanesulfonimide[8] in THF–HMPA. The enol triflate thus obtained was treated with $Pd(OAc)_2$, 1,1′-bis(diphenylphosphino)-ferrocene (DPPF), Et_3N and HCOOH in dioxane[9] to afford the deoxygenated compound (**14**). Deacetylation of **14** by NaOMe gave (3*S*)-deoxypumiloside (**3**), whose spectroscopic data, including the CD spectrum, were identical with those of natural **3** that was obtained from the hairy roots of *O. pumila*. The same procedure was applied to vincoside lactam (**10**) to give (3*R*)-deoxypumiloside (**4**).

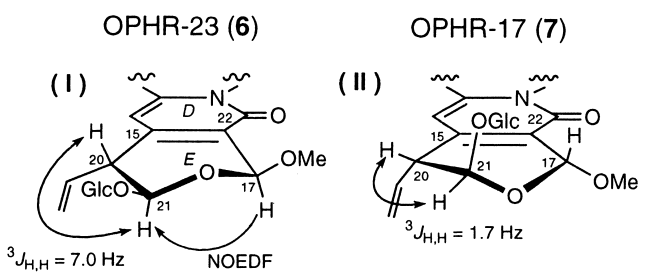
Next, (3*R*)-deoxypumiloside tetraacetate (**15**) was treated with DDQ in toluene–MeOH[10] at 50°C to give a mixture of 17-epimers of **17** in 91% yield in the ratio of 4:1 (major/minor). A mixture of these compounds showed the characteristic UV absorptions of camptothecin and a characteristic proton shifted to low field due to H-14 was observed in the ^1H NMR spectrum, suggesting the aromatization of the D-ring. As these compounds could not be separated, the mixture was treated with NaOMe in MeOH to give OPHR-23 (**6**) and OPHR-17 (**7**) along with some undesired compounds such as **20** and **21** (main product, y. 35%). To prevent elimination of the sugar unit and migration of the double bond at C18–C19 to C19–C20 during deprotection of the acetyl group in **17**, the protective group on the hydroxy functions in the sugar moiety was changed to a trichloroethoxycarbonyl (Troc) group which could be removed under acidic condition. Compound **16** having the Troc group on the hydroxy function was prepared from (3*R*)-deoxypumiloside (**4**) and then treated with DDQ in toluene–MeOH at 50°C to afford **18** and **19** in 52 and 15% yield, respectively. These compounds showed the characteristic UV absorptions of camptothecin (**18**: 358, 291, 253, 215 nm, **19**: 359, 288, 253, 218 nm), suggesting the aromatization of the D-ring. In the ^1H NMR spectrum, an aromatic proton at H-14 (**18**: δ 7.20, **19**: δ 7.16), an acetal proton at H-17 (**18**: δ 5.78, **19**: δ 5.64) along with two acetal protons at H-21 and H-1′, and 17-OMe were observed. Furthermore, three acetal carbons, one methoxy carbon, and aromatic carbons due to C-3, C-14–C-16 were observed in the ^{13}C NMR spectrum. Removal of the Troc group in each compound by treatment with Zn in AcOH gave OPHR-23 (**6**) and OPHR-17 (**7**), respectively. Each product was identified with the natural compound isolated from the hairy roots of *O. pumila* by comparison of the spectroscopic data, including CD spectra.

The configuration of the C-17 position in OPHR-23 (**6**) and OPHR-17 (**7**), a pair of diastereomers at C-17, was determined by using the NMR techniques (Fig. 3). The coupling constant between H-20 and H-21 in OPHR-23 (**6**) and OPHR-17 (**7**) showed different values, suggesting that each compound exists in a different conformation of the E-ring. Therefore, four possible stereostructures could be considered for OPHR-23 (**6**) and OPHR-17 (**7**). OPHR-23 (**6**) has a large coupling constant (7.0 Hz) of $^3J_{H20,H21}$ in the ^1H NMR spectrum. In the differential NOE experiments of



Scheme 2.

6, irradiation of H-17 led to enhancement of H-21. These observations revealed that **6** takes conformer I and has 17*S* configuration. Furthermore, the observed C–H long-range coupling constants between H-17 and C-15 (3.0 Hz), between H-17 and C-21 (4.9 Hz) and between H-17 and C-22 (1.5 Hz) obtained by PFG J-HMBC 2D spectroscopy[11] fitted well with the expected values from the torsion angles (Dreiding model), supporting the 17*S* configuration and conformation I in **6** (Fig. 3). On the other hand, OPHR-17 (**7**) has a small coupling constant of $^{3}J_{H20,H21}$ (1.7 Hz), suggesting that it takes conformation II. The coupling constants of $^{3}J_{H17,C15}$ (2.5 Hz), $^{3}J_{H17,C21}$ (6.2 Hz) and $^{3}J_{H17,C22}$ (1⩾ Hz) were in good agreement with those of **7** having 17*R*. From these results, the absolute configurations at the C-17 position of **6** and **7** were determined to be 17*S* and 17*R*, respectively.



	OPHR-23 (**6**)		OPHR-17 (**7**)	
	Torsion angle	$^{3}J_{H,C}$	Torsion angle	$^{3}J_{H,C}$
H17-C15	90°	3.0 Hz	90°	2.5 Hz
H17-C21	45°	4.9 Hz	45°	6.2 Hz
H17-C22	90°	1.5 Hz	90°	1>> Hz

Figure 3.

Camptothecin having a quinoline nucleus is biogenetically derived from strictosidine via strictosamide (**5**), that was actually proved by Hutchinson[1a,b] (Scheme 2). However, the biosynthetic pathway from strictosamide (**5**) to **1**, comprising (i) transformation of an indole 6–5–6 membered ring system of the ABC-ring to a quinoline 6–6–5 ring system, (ii) oxidation of the D-ring to a pyridone and (iii) removal of a glucose unit and structural conversions in the E-ring, is not clear yet. Existence of (3*S*)-pumiloside (**2**) and (3*S*)-deoxypumiloside (**3**) suggests a possibility that conversion of the ABC-ring of strictosamide (**5**) would be the next step after the formation of strictosamide. Although OPHR-23 (**6**) and OPHR-17 (**7**) seemed to be compounds similar to the presumed biosynthetic intermediate[12] from **3** to **1**, OPHR-23 (**6**) and OPHR-17 (**7**) might be artifacts during the extraction or isolation process. A similar observation was reported in the case of tetrahydroisoquinoline-type glucosides isolated from *Alangium lamarckii*.[10]

3. Conclusion

In conclusion, we have succeeded in obtaining the hairy roots of a camptothecin-producing plant, *O. pumila*, in which camptothecin and its related alkaloids, i.e. (3*S*)-pumiloside, (3*S*)- and (3*R*)-deoxypumilosides and strictosamide, are produced as in wild plants. The structures, including absolute configurations of the two new alkaloids, OPHR-23 and OPHR-17, isolated from the hairy roots were determined by spectroscopic analyses including J-HMBC

2D method and chemical synthesis from tryptamine and secologanin via pumilosides and deoxypumilosides.

4. Experimental

Melting points were determined on a Yamato MP-21 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM A-500, a JEOL JNM A-400 or a JEOL JNM FX270. ^{1}H NMR spectra were measured at 500 or 400 MHz. ^{13}C NMR spectra were measured at 125.65, 100 or 67.8 MHz. MS spectra were obtained by use of a JEOL JMS-AM20 (EI-MS), a Hitachi RMU-7M (HR-EI-MS) or a JEOL JMS-HX110 (FAB-MS and HR-FAB-MS). UV spectra were recorded on a JASCO V-560. CD spectra were measured by a JASCO J-720WI instrument. For TLC, precoated Silica gel 60 F_{254} plates (Merck, 0.25 mm thick) were used. For column chromatography, Silica gel 60 [Merck, 70–230 mesh (for open chromatography) and 230–400 mesh (for flash chromatography)], Aluminium oxide 90 [Merck] and DIAION HP20 [Mitsubishikasei] were used. For medium pressure liquid chromatography (MPLC), a C. I. G. prepacked column CPS-HS-221-05 (SiO_2) and CPO-HS-221-20 (ODS) [Kusano Kagakukikai] were used. For HPLC, Senshu Pak AQUASIL SS-752N (10ϕ×250 mm) [Senshu Scientific Co., Ltd] was used.

4.1. Extraction and isolation of alkaloids from hairy roots of *O. pumila*

From hairy roots cultivated on the solid medium. Hairy roots (190 g of wet weight), cultivated on the hormone-free half-length Murashige and Skoog (MS) solid medium containing sucrose (1%) under light at 25°C, were extracted with hot MeOH to give the extract (2.3 g). The MeOH extract was then partitioned between H_2O and $CHCl_3$. The organic layer was washed with H_2O, dried over $MgSO_4$ and then evaporated to give the $CHCl_3$ extract (263 mg). The aqueous layer was extracted with *n*-BuOH and the organic layer was evaporated to give the *n*-BuOH extract (210 mg). The aqueous layer was freeze-dried to give the water-soluble portion (1.83 g). The *n*-BuOH extract (210 mg) was separated by SiO_2 open column chromatography eluted with $CHCl_3$—MeOH–$CHCl_3$—$CHCl_3$–MeOH–H_2O—MeOH gradient to give the 12 fractions. The 20% MeOH–$CHCl_3$ eluate was subjected to MPLC (SiO_2) using 10% MeOH–$CHCl_3$ to give (3*S*)- and (3*R*)-deoxypumilosides (**3**, 9.9 mg and **4**, 9.4 mg), respectively. The 10% MeOH–$CHCl_3$ eluate of MPLC was purified by MPLC (SiO_2) using 20% MeOH–AcOEt to give the new compound, OPHR-23 (**6**, 0.4 mg). (3*S*)-Pumiloside (**2**, 24.1 mg) and strictosamide (**5**, 9.4 mg) were obtained from the 30–40% MeOH–$CHCl_3$ eluate of SiO_2 open column chromatography of the *n*-BuOH extract. (3*S*)-Pumiloside (**2**, 9.6 mg) was also obtained from the 50% MeOH–H_2O eluate of DIAION HP20 of the water-soluble portion.

From hairy roots cultivated in the liquid medium. Hairy roots (about 375 g of wet weight), cultivated in the Gamborg's B5 liquid medium containing sucrose (1%), were extracted with hot MeOH to give the extract (10.6 g). The MeOH extract was then partitioned between H_2O, $CHCl_3$ and *n*-BuOH to give the $CHCl_3$ extract (790 mg), the *n*-BuOH extract (1666 mg) and the water-soluble portion (6.03 g), respectively. Camptothecin (**1**, 17.7 mg) was obtained from the 50% AcOEt–MeOH—MeOH eluate of SiO_2 open column chromatography of the $CHCl_3$ extract. The *n*-BuOH extract (1666 mg) was separated by SiO_2 open column chromatography eluted with MeOH–$CHCl_3$ gradient to give 10 fractions. The 20 and 25% MeOH–$CHCl_3$ eluates were purified by using a combination of HPLC (SiO_2, 10% MeOH–$CHCl_3$) and MPLC (SiO_2, 10% MeOH–$CHCl_3$) to give OPHR-17 (**7**, 0.4 mg) and OPHR-23 (**6**, 0.3 mg), respectively. The 25% MeOH–$CHCl_3$ eluate gave strictosamide (**5**, 9.9 mg). (3*S*)-Pumiloside (**2**, 9.9 mg) and (3*R*)-deoxypumiloside (**4**, 1.3 mg) were obtained from the $CHCl_3$–MeOH–H_2O=10:5:1 eluate.

4.1.1. (3*S*)-Deoxypumiloside (3). HR-FAB-MS (positive, NBA) *m/z*: 497.1905 (MH^+) (Calcd for $C_{26}H_{29}N_2O_8$, 497.1924); FAB-MS (positive, NBA) *m/z*: 497 (MH^+); ^{1}H NMR (500 MHz, CD_3OD) δ: 8.24 (1H, s, H-7), 7.99 (1H, d, *J*=8.2 Hz, H-12), 7.89 (1H, d, *J*=8.2 Hz, H-9), 7.71 (1H, br-dd, *J*=8.2, 8.2 Hz, H-11), 7.55 (1H, dd, *J*=8.2, 8.2 Hz, H-10), 7.16 (1H, d, *J*=2.4 Hz, H-17), 5.83 (1H, ddd, *J*=17.1, 10.2, 10.2 Hz, H-19), 5.48 (1H, dd, *J*=17.1, 1.8 Hz, H-18), 5.47 (1H, d, *J*=1.5 Hz, H-21), 5.34 (1H, dd, *J*=10.2, 1.8 Hz, H-18), 4.94 (1H, d, *J*=16.5 Hz, H-5), 4.8 (overlapped, H-3), 4.74 (1H, d, *J*=16.5 Hz, H-5), 4.69 (1H, d, *J*=7.9 Hz, H-1′), 3.86 (1H, dd, *J*=12.0, 2.0 Hz, H-6′), 3.64 (1H, dd, *J*=12.0, 5.8 Hz, H-6′), 3.35 (1H, dd, *J*=8.8, 8.8 Hz, H-3′), 3.35 (overlapped, H-15), 3.30 (overlapped, H-5′), 3.25 (overlapped, H-4′), 3.16 (1H, dd, *J*=8.8, 7.9 Hz, H-2′), 2.68 (1H, m, H-20), 2.65 (1H, m, H-14), 2.02 (1H, br-ddd, *J*=12.0, 12.0, 12.0 Hz, H-14); ^{13}C NMR (125 MHz, CD_3OD) δ: 168.2 (C-22), 163.2 (C-2), 149.0 (C-13), 147.7 (C-17), 133.6 (C-19), 132.6 (C-7), 131.0 (C-11), 129.7 (C-6), 129.3 (C-8, 9), 129.0 (C-12), 128.0 (C-10), 121.3 (C-18), 110.6 (C-16), 99.8 (C-1′), 97.5 (C-21), 78.4 (C-5′), 78.0 (C-3′), 74.8 (C-2′), 71.6 (C-4′), 62.7 (C-6′), 61.6 (C-3), 49.1 (C-5), 46.0 (C-20), 30.2 (C-14), 25.1 (C-15); UV λ_{max} (MeOH) nm: 321, 313, 307, 300, 236, 206; CD (*c*=0.532 mmol/L, MeOH, 24°C) $\Delta\epsilon$ (λ nm): 0 (330), −1.58 (322), −5.03 (272), −3.79 (262), −23.61 (240), 0 (229), +5.86 (223), +4.12 (213), +12.93 (205).

4.1.2. (3*R*)-Deoxypumiloside (4). FAB-MS (positive, NBA) *m/z*: 497 (MH^+); ^{1}H NMR (500 MHz, CD_3OD) δ: 8.29 (1H, s, H-7), 8.04 (1H, d, *J*=8.4 Hz, H-12), 7.94 (1H, d, *J*=8.4 Hz, H-9), 7.75 (1H, dd-like, *J*=8.4, 8.4 Hz, H-11), 7.59 (1H, dd-like, *J*=8.4, 8.4 Hz, H-10), 7.51 (1H, d, *J*=2.5 Hz, H-17), 5.56 (1H, d, *J*=1.8 Hz, H-21), 5.52 (1H, ddd, *J*=17.1, 10.2, 10.2 Hz, H-19), 5.32 (1H, dd, *J*=17.1, 1.8 Hz, H-18), 5.23 (1H, d, *J*=17.1 Hz, H-5), 5.19 (1H, dd, *J*=10.2, 1.8 Hz, H-18), 5.07 (1H, dd, *J*=12.5, 3.8 Hz, H-3), 4.72 (1H, d, *J*=7.9 Hz, H-1′), 4.70 (1H, d, *J*=17.1 Hz, H-5), 3.91 (1H, dd, *J*=12.1, 1.9 Hz, H-6′), 3.69 (1H, dd, *J*=12.1, 5.6 Hz, H-6′), 3.40 (1H, dd, *J*=8.9, 8.9 Hz, H-3′), 3.38 (overlapped, H-15), 3.33 (overlapped, H-4′, 5′), 3.24 (1H, dd, *J*=8.9, 7.9 Hz, H-2′), 2.82 (1H, m, H-20), 2.67 (1H, ddd, *J*=12.5, 3.8, 3.8 Hz, H-14), 1.53 (1H, ddd, *J*=12.5, 12.5, 12.5 Hz, H-14); ^{13}C NMR (125 MHz, CD_3OD) δ: 165.7 (C-22), 163.2 (C-2), 149.2 (C-17), 148.9 (C-13), 133.7 (C-19), 132.3 (C-7), 131.0 (C-11), 129.7 (C-6), 129.4 (C-8,

9), 129.1 (C-12), 128.0 (C-10), 120.6 (C-18), 108.8 (C-16), 99.7 (C-1′), 97.5 (C-21), 78.4 (C-5′), 78.0 (C-3′), 74.8 (C-2′), 71.6 (C-4′), 62.7 (C-3, 6′), 49.6 (C-5), 44.8 (C-20), 31.1 (C-14), 29.2 (C-15); UV λ_{max} (MeOH) nm: 321, 313, 307, 300, 236, 206; CD (c=0.151 mmol/L, MeOH, 26°C) $\Delta\epsilon$ (λ nm): 0 (330), +1.82 (322), +6.76 (278), 0 (268), −25.28 (240), 0 (229), +1.50 (227), 0 (221), −0.38 (218), 0 (215), +3.70 (209).

4.1.3. OPHR-23 (6). Amorphous; HR-FAB-MS (positive, NBA) *m/z*: 547.1699 (MNa^+) (Calcd for $C_{27}H_{28}N_2O_9Na$, 547.1693); FAB-MS (positive, NBA) *m/z*: 525 (MH^+), 547 (MNa^+); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.67 (1H, s, H-7), 8.16 (1H, d, J=7.6 Hz, H-12), 8.11 (1H, d, J=7.6 Hz, H-9), 7.85 (1H, dd-like, J=7.6, 7.6 Hz, H-11), 7.70 (1H, dd-like, J=7.6, 7.6 Hz, H-10), 7.02 (1H, s, H-14), 5.81 (1H, ddd, J=17.1, 10.1, 9.1 Hz, H-19), 5.65 (1H, s, H-17), 5.51 (1H, br-d, J=17.1 Hz, H-18), 5.46 (1H, d, J=7.0 Hz, H-21), 5.44 (1H, dd, J=10.1, 2.1 Hz, H-18), 5.24 (2H, s, H_2-5), 5.01 (1H, d, J=5.5 Hz, OH), 4.96 (1H, d, J=4.9 Hz, OH), 4.92 (1H, d, J=5.2 Hz, OH), 4.64 (1H, d, J=7.9 Hz, H-1′), 4.51 (1H, dd, J=6.0, 6.0 Hz, OH), 3.68 (1H, m, H-6′), 3.53 (1H, m, H-20), 3.47 (3H, s, 17-OMe), 3.45 (overlapped, H-6′), 3.2–3.1 (2H, overlapped), 3.08 (1H, m), 2.99 (1H, m) (H-2′, 3′, 4′, 5′); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 157.8 (C-22), 152.5 (C-2), 147.9 (C-13), 147.4 (C-15), 145.5 (C-3), 134.6 (C-19), 131.5 (C-7), 130.3 (C-11), 129.9 (C-6), 129.0 (C-12), 128.5 (C-9), 128.0 (C-8), 127.6 (C-10), 122.3 (C-16), 121.0 (C-18), 99.2 (C-14), 97.9 (C-1′), 95.2 (C-17), 91.7 (C-21), 77.5, 77.0, 73.1 and 70.0 (C-2′, 3′, 4′, 5′), 61.0 (C-6′), 55.2 (OMe), 50.0 (C-5), 47.3 (C-20); UV λ_{max} (MeOH) nm: 362, 290, 254, 219; CD (c=0.382 mmol/L, MeOH, 24°C) $\Delta\epsilon$ (λ nm): 0 (390), −0.59 (364), 0 (334), −0.89 (307), 0 (280), +3.14 (260), 0 (254), −6.11 (239), 0 (230), +6.99 (223), 0 (216), −27.12 (205).

4.1.4. OPHR-17 (7). Amorphous; HR-FAB-MS (positive, NBA) *m/z*: 547.1715 (MNa^+) (Calcd for $C_{27}H_{28}N_2O_9Na$, 547.1693); FAB-MS (positive, NBA) *m/z*: 525 (MH^+), 547 (MNa^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.68 (1H, s, H-7), 8.15 (1H, d, J=7.8 Hz, H-12), 8.12 (1H, d, J=7.8 Hz, H-9), 7.85 (1H, dd-like, J=7.8, 7.8 Hz, H-11), 7.70 (1H, dd-like, J=7.8, 7.8 Hz, H-10), 7.14 (1H, s, H-14), 5.82 (1H, ddd, J=17.0, 10.2, 7.0 Hz, H-19), 5.53 (1H, s, H-17), 5.45 (1H, d, J=1.7 Hz, H-21), 5.37 (1H, br-d, J= 17.0 Hz, H-18), 5.25 (2H, s, H_2-5), 5.23 (1H, br-d, J= 10.2 Hz, H-18), 5.02 (1H, d, J=4.9 Hz, OH), 4.96 (1H, d, J=4.9 Hz, OH), 4.65 (1H, d, J=7.8 Hz, H-1′), 4.59 (1H, dd, J=6.0, 6.0 Hz, OH), 4.12 (1H, d, J=2.2 Hz, OH), 3.69 (1H, m, H-6′), 3.68 (1H, m, H-20), 3.57 (3H, s, 17-OMe), 3.45 (1H, m, H-6′), 3.2 (2H, overlapped), 3.03 (1H, m) (H-3′, 4′, 5′), 2.96 (1H, m, H-2′); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 157.7 (C-22), 152.6 (C-2), 147.9 (C-13), 145.7 (C-15), 145.5 (C-3), 135.8 (C-19), 131.5 (C-7), 130.3 (C-11), 129.9 (C-6), 128.9 (C-12), 128.5 (C-9), 128.0 (C-8), 127.6 (C-10), 121.2 (C-16), 118.9 (C-18), 99.9 (C-14), 97.6 (C-1′), 94.5 (C-17), 94.3 (C-21), 77.4, 76.7 and 69.9 (C-3′, 4′, 5′), 73.4 (C-2′), 61.0 (C-6′), 57.1 (OMe), 50.1 (C-5), 45.8 (C-20); UV λ_{max} (MeOH) nm: 363, 280 (sh), 254, 219; CD (c= 0.891 mmol/L, MeOH, 24°C) $\Delta\epsilon$ (λ nm): 0 (400), +0.36 (373), 0 (337), +0.51 (308), 0 (285), −0.41 (261), 0 (248), −1.59 (237), 0 (231), +4.17 (220), 0 (213), −10.30 (204).

4.2. Syntheses of OPHR-23 (6) and OPHR-17 (7)

4.2.1. Preparation of (3*S*)-pumiloside tetraacetate (11) from strictosamide tetraacetate. To a MeOH solution (60 mL) of strictosamide tetraacetate (806 mg, 1.210 mmol) was added an aqueous solution (30 mL) of $NaIO_4$ (6.0 g, 0.023 mol) at room temperature under Ar. The mixture was stirred for 22 h in dark. After concentration of MeOH, the residue was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. To a dry EtOH solution (50 mL) of the resulting dicarbonyl derivative was added dry Et_3N (2.0 mL, 0.014 mol) and the mixture was stirred for 16.5 h at room temperature under Ar. Water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by SiO_2 open column chromatography (MeOH–$CHCl_3$ gradient) to afford **11** (606 mg, y. 74%). *(3S)-Pumiloside tetraacetate* (**11**): Mp 218°C (dec., EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.60 (1H, br-s, *N*a-H), 8.36 (1H, d, J=7.6 Hz, H-9), 7.63 (1H, dd, J=7.6, 7.6 Hz, H-11), 7.55 (1H, d, J=7.6 Hz, H-12), 7.38 (1H, dd, J=7.6, 7.6 Hz, H-10), 7.10 (1H, d, J=1.9 Hz, H-17), 5.69 (1H, m, H-19), 5.25 (1H, dd, J=9.5, 9.5 Hz, H-3′), 5.21–5.15 (2H, m, H_2-18), 5.18 (1H, m, H-21), 5.06 (1H, dd, J=9.6, 9.6 Hz, H-4′), 5.01 (1H, dd, J=8.8, 8.8 Hz, H-2′), 4.95 (1H, d, J=8.3 Hz, H-1′), 4.75 (2H, m, H-3, H-5), 4.57 (1H, br-d, J=12.7 Hz, H-5), 4.28 (1H, dd, J=12.2, 4.3 Hz, H-6′), 4.13 (1H, d, J=12.2 Hz, H-6′), 3.75 (1H, br-d, J=8.5 Hz, H-5′), 3.12 (1H, m, H-15), 2.56 (2H, m, H-14, 20), 2.08 (s, 6H, OCOCH_3×2), 2.03 and 2.01 (each 3H, s, OCOCH_3×2), 2.1–2.0 (1H, m, H-14); UV λ_{max} (MeOH) nm: 328, 315, 303 (sh), 289 (sh), 245, 239 (sh), 213.

4.2.2. Preparation of (3*R*)-pumiloside tetraacetate (12) from vincoside lactam tetraacetate. To a MeOH solution (60 mL) of vincoside lactam tetraacetate (1161 mg, 1.743 mmol) was added an aqueous solution (30 mL) of $NaIO_4$ (6.0 g, 0.023 mol) at room temperature under Ar. The mixture was stirred for 41 h in dark. The residue obtained by the same work up procedure as above was dissolved in dry EtOH (50 mL) and then dry Et_3N (2.5 mL, 0.018 mol) was added to the solution. The mixture was stirred for 54 h at room temperature under Ar. (3*R*)-Pumiloside tetraacetate (**12**, 890 mg, y. 75%) was obtained via the same work up manner and purification as described above. *(3R)-Pumiloside tetraacetate* (**12**): Mp 213–217°C (EtOH); EI-MS *m/z* (%): 680 (M^+, 1), 43 (100); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.39 (1H, s, *N*a-H), 8.36 (1H, d, J=7.8 Hz, H-9), 7.65 (1H, dd-like, J=7.8, 7.8 Hz, H-11), 7.58 (1H, d, J=7.8 Hz, H-12), 7.49 (1H, d, J=2.2 Hz, H-17), 7.40 (1H, dd-like, J=7.8, 7.8 Hz, H-10), 5.46 (1H, ddd, J=17.1, 9.7, 9.7 Hz, H-19), 5.28 (1H, d, J=1.7 Hz, H-21), 5.24 (1H, d, J=17.1 Hz, H-18), 5.23 (1H, d, J= 9.7 Hz, H-18), 5.22 (1H, dd, J=9.5, 9.5 Hz, H-3′), 5.08 (1H, dd, J=9.5, 9.5 Hz, H-4′), 5.10–5.05 (2H, m, H-3, 5), 5.00 (1H, dd, J=9.5, 8.2 Hz, H-2′), 4.92 (1H, d, J=8.2 Hz, H-1′), 4.55 (1H, d, J=13.9 Hz, H-5), 4.30 (1H, dd, J=12.3, 4.7 Hz, H-6′), 4.14 (1H, dd, J=12.3, 2.2 Hz, H-6′), 3.76 (1H, m, H-5′), 2.90 (1H, m, H-15), 2.58 (1H, m, H-20), 2.47 (1H, m, H-14), 2.09, 2.04, 2.01 and 1.98 (each 3H, s, OCOCH_3×4), 1.61 (1H, ddd, J=12.4, 12.4, 12.4 Hz, H-14); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.5 (C-7), 170.6, 170.0, 169.47 and

169.46 (O*C*OCH$_3$×4), 162.4 (C-22), 150.6 (C-2), 147.0 (C-17), 140.9 (C-13), 132.3 (C-11), 131.5 (C-19), 125.8 (C-8), 125.3 (C-9), 124.4 (C-10), 120.9 (C-18), 118.8 (C-12), 114.3 (C-6), 107.7 (C-16), 96.2 (C-21), 96.0 (C-1′), 72.29 and 72.26 (C-3′, 5′), 70.5 (C-2′), 68.2 (C-4′), 61.8 (C-6′), 61.5 (C-3), 49.0 (C-5), 42.8 (C-20), 29.5 (C-14), 28.4 (C-15), 20.73, 20.65 and 20.56 (OCO*C*H$_3$×4); UV λ_{max} (MeOH) nm: 328, 315, 303 (sh), 289 (sh), 245, 239 (sh), 213; CD (c=0.171 mmol/L, MeOH, 23°C) $\Delta\epsilon$ (λ nm): 0 (340), −2.49 (327), −1.42 (315), 0 (295), −34.46 (245), 0 (230), +13.50 (215), 0 (205).

4.2.3. Preparation of (3*S*)-pumiloside (2). To a solution of (3*S*)-pumiloside tetraacetate (**11**, 10 mg, 0.015 mmol) in dry MeOH (2 mL) was added 1N NaOMe in MeOH (50 μL, 0.050 mmol) at room temperature under Ar. The mixture was stirred at the same temperature for 2 h. Water was added to the reaction mixture and the whole was extracted with *n*-BuOH. The organic layer was washed with water and evaporated. The residue was purified by SiO_2 open column chromatography (MeOH–$CHCl_3$=1:5) to give (3*S*)-pumiloside (**2**, 6.9 mg, y. 92%). The synthetic compound was identical with the natural (3*S*)-pumiloside[2a] (TLC, UV, ^{1}H NMR, CD, and MS). *(3S)-Pumiloside* (**2**): Mp >295°C (MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.08 (1H, s, *N*a–H), 8.12 (1H, dd, J=8.2, 1.2 Hz, H-9), 7.66 (1H, dd-like, J=8.2, 8.2 Hz, H-11), 7.60 (1H, d, J=8.2 Hz, H-12), 7.34 (1H, dd-like, J=8.2, 8.2 Hz, H-10), 7.04 (1H, d, J=2.8 Hz, H-17), 5.80 (1H, ddd, J=16.8, 10.1, 10.1 Hz, H-19), 5.47 (1H, dd, J=16.8, 1.8 Hz, H-18), 5.38 (1H, d, J=1.5 Hz, H-21), 5.34 (1H, dd, J=10.1, 1.8 Hz, H-18), 4.76 (1H, br-d, J=11.9 Hz, H-3), 4.54 (1H, d, J=7.9 Hz, H-1′), 4.47 (1H, br-d, J=13.7 Hz, H-5), 4.32 (1H, d, J=13.7 Hz, H-5), 3.69 and 3.43 (each 1H, m, H$_2$-6′), 3.3 (overlapped, H-15), 3.16 (2H, m, H-3′, 5′), 3.03 (1H, m, H-4′), 2.98 (1H, m, H-2′), 2.65 (1H, m, H-20), 2.5 (overlapped, H-14), 2.01 (1H, ddd, J=11.6, 11.6, 11.6 Hz, H-14); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 172.9 (C-7), 163.9 (C-22), 149.7 (C-2), 145.1 (C-17), 140.4 (C-13), 132.5 (C-19), 131.6 (C-11), 125.3 (C-8), 124.7 (C-9), 123.2 (C-10), 120.5 (C-18), 118.3 (C-12), 112.9 (C-6), 108.9 (C-16), 97.8 (C-1′), 94.8 (C-21), 77.3 (C-5′), 76.5 (C-3′), 73.1 (C-2′), 70.1 (C-4′), 61.0 (C-6′), 59.4 (C-3), 47.4 (C-5), 43.6 (C-20), 28.2 (C-14), 23.7 (C-15); CD (c=0.127 mmol/L, MeOH, 24°C) $\Delta\epsilon$ (λ nm): 0 (334), +1.27 (324), +0.51 (319), +0.99 (315), 0 (302), −19.52 (240), 0 (219), +11.48 (211).

4.2.4. Preparation of (3*R*)-pumiloside (13). To a solution of (3*R*)-pumiloside tetraacetate (**12**, 17 mg, 0.025 mmol) in dry MeOH (2.5 mL) was added 1N NaOMe in MeOH (50 μL, 0.050 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 1 h. The reaction mixture was directly subjected to SiO_2 open column chromatography (MeOH–$CHCl_3$ gradient) to give (3*R*)-pumiloside (**13**, 12.2 mg, y. 95%). *(3R)-Pumiloside* (**13**): Mp >300°C (MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.12 (1H, dd, J=8.0, 1.1 Hz, H-9), 7.65 (1H, dd-like, J=8.0, 8.0 Hz, H-11), 7.60 (1H, d, J=8.0 Hz, H-12), 7.34 (1H, overlapped, H-10), 7.34 (1H, d, J=2.4 Hz, H-17), 5.45 (1H, ddd, J=17.1, 10.0, 10.0 Hz, H-19), 5.42 (1H, d, J=1.5 Hz, H-21), 5.31 (1H, dd, J=17.0, 2.1 Hz, H-18), 5.19 (1H, dd, J=10.0, 2.1 Hz, H-18), 5.11 (1H, br-d, J=9.4 Hz, H-3), 4.74 (1H, dd, J=14.7, 2.1 Hz, H-5), 4.53 (1H, d, J=7.9 Hz, H-1′), 4.25 (1H, dd, J=14.7, 1.5 Hz, H-5), 3.69 (1H, m, H-6′), 3.39–3.16 (3H, m, H-15, 3′, 5′), 3.07–2.98 (2H, m, H-2′, 4′), 2.72 (1H, m, H-20), 2.53 (1H, m, H-14), 1.37 (1H, ddd, J=12.3, 12.3, 12.3 Hz, H-14) (H-6′ was buried under H_2O signal); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 172.7 (C-7), 161.6 (C-22), 149.8 (C-2), 146.4 (C-17), 140.2 (C-13), 132.6 (C-19), 131.5 (C-11), 125.3 (C-8), 124.7 (C-9), 123.2 (C-10), 120.0 (C-18), 118.3 (C-12), 112.9 (C-6), 107.1 (C-16), 97.9 (C-1′), 94.9 (C-21), 77.2 and 76.4 (C-3′, 5′), 73.1 (C-2′), 70.0 (C-4′), 61.0 (C-6′), 60.4 (C-3), 48.1 (C-5), 42.4 (C-20), 28.8 (C-14), 27.5 (C-15); CD (c=0.117 mmol/L, MeOH, 24°C) $\Delta\epsilon$ (λ nm): 0 (334), −1.88 (329), 0 (295), −34.52 (247), 0 (233), +14.98 (218).

4.2.5. Preparation of (3*S*)-deoxypumiloside tetraacetate (14). To a solution of (3*S*)-pumiloside tetraacetate (**11**, 606 mg, 0.891 mmol) in dry THF (30 mL) and dry HMPA (310 μL, 1.782 mmol), was added a THF solution (4 mL) of lithium diisopropylamide (LDA, 2 equiv.) at −78°C for 1 h under Ar. A THF solution (3 mL) of *N*-phenyltrifluoromethanesulfonimide (637 mg, 1.783 mmol) was added dropwise to the mixture and the reaction mixture was stirred at −78°C for 30 min. Then the temperature was gradually raised to room temperature over 1 h. Cold water was added to the reaction mixture. After the solvent was removed under reduced pressure, the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by SiO_2 open column chromatography ($CHCl_3$–MeOH gradient) to afford (3*S*)-7-triflate (590 mg, y. 82%). *(3S)-7-Triflate*: ^{1}H NMR (400 MHz, $CDCl_3$, selected data) δ: 8.17 (1H, br-d, J=8.3 Hz, H-9), 8.09 (1H, br-d, J=8.3 Hz, H-12), 7.84 (1H, dd-like, J=8.3, 8.3 Hz, H-10), 7.72 (1H, dd-like, J=8.3, 8.3 Hz, H-11), 7.18 (1H, d, J=2.6 Hz, H-17), 5.77 (1H, ddd, J=17.1, 10.2, 10.2 Hz, H-19), 5.52 (1H, dd, J=17.1, 1.6 Hz, H-18), 5.41 (1H, dd, J=10.2, 1.6 Hz, H-18), 2.11, 2.09, 2.04 and 2.01 (each 3H, s, OCO*C*H$_3$×4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.6, 170.1, 169.8, 169.4, 165.0, 163.8, 150.8, 146.9, 145.6, 131.2, 131.0, 129.4, 128.4, 123.6, 121.7, 121.3, 121.0, 120.4, 110.0, 96.2, 96.1, 72.34, 72.28, 70.4, 68.2, 61.7, 60.5, 46.6, 43.7, 29.7, 28.8, 23.7, 20.7, 20.63, 20.57; UV λ_{max} (MeOH) nm: 321, 308, 236, 205. To a solution of (3*S*)-7-triflate (5.9 mg, 0.007 mmol) in dry 1,4-dioxane (0.6 mL), were added a solution of DPPF (1.5 mg, 0.003 mmol), $Pd(OAc)_2$ (0.4 mg, 0.002 mmol) and dry Et_3N (5.6 μL, 0.040 mmol) in dry 1,4-dioxane (0.2 mL) and a solution of HCOOH (1.1 μL, 0.029 mmol) in dry 1,4-dioxane (0.2 mL) successively at room temperature under Ar. The reaction mixture was heated at 60°C for 30 min. A cold water was added to the mixture and the whole was extracted with $CHCl_3$. The organic layer was washed with 5% Na_2CO_3 aq. and then water, dried over $MgSO_4$ and evaporated. The residue was purified by preparative TLC (SiO_2, 5% MeOH–$CHCl_3$) to afford (3*S*)-deoxypumiloside tetraacetate (**14**, 3.8 mg, y. 79%). The synthetic compound was identical with the tetraacetate[2d] derived from natural (3*S*)-deoxypumiloside (TLC, UV, ^{1}H and ^{13}C NMR, CD, and MS).

4.2.6. Preparation of (3*R*)-deoxypumiloside tetraacetate (15). To a solution of (3*R*)-pumiloside tetraacetate (**12**, 10.5 mg, 0.015 mmol) in dry THF (0.5 mL) and dry HMPA (0.1 mL, 0.575 mmol), was added a THF solution (0.2 mL)

of LDA (2 equiv.) at −78°C for 1 h under Ar. A THF solution (0.2 mL) of *N*-phenyltrifluoromethanesulfonimide (29.1 mg, 0.082 mmol) was added dropwise to the mixture and the reaction mixture was stirred at −78°C for 30 min. Then the temperature was gradually raised to −15°C and the mixture was stirred for 30 min. (3*R*)-7-triflate (11.1 mg, y. 89%) was obtained via the same work up manner and purification as described above. *(3R)-7-Triflate*: Mp 185–187°C (dec., MeOH); FAB-MS (positive, NBA) *m/z*: 813 (MH^+); ^{1}H NMR (400 MHz, $CDCl_3$, selected data) δ: 8.16 (1H, br-d, *J*=8.0 Hz, H-9), 8.08 (1H, br-d, *J*=8.0 Hz, H-12), 7.84 (1H, dd-like, *J*=8.0, 8.0 Hz, H-10), 7.71 (1H, dd-like, *J*=8.0, 8.0 Hz, H-11), 5.50 (1H, d, *J*=17.2 Hz, H-5), 5.46 (1H, ddd, *J*=16.9, 9.8, 9.8 Hz, H-19), 2.11, 2.04, 2.02 and 2.01 (each 3H, s, OCOCH_3×4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.6, 170.0, 169.4, 163.9, 162.6, 150.7, 147.2, 146.8, 131.4, 131.0, 129.4, 128.4, 121.3, 120.95, 120.93, 120.4, 120.1, 116.9, 108.0, 96.3, 96.0, 72.3, 70.5, 68.2, 61.8, 61.7, 47.1, 42.9, 29.8, 28.0, 20.7, 20.64, 20.56; UV λ_{max} (MeOH) nm: 321, 308, 236, 205. To a solution of (3*R*)-7-triflate (224 mg, 0.275 mmol) in dry 1,4-dioxane (10 mL), were added a solution of DPPF (76.6 mg, 0.138 mmol), $Pd(OAc)_2$ (18.6 mg, 0.083 mmol) and dry Et_3N (250 μL, 1.764 mmol) in dry 1,4-dioxane (3 mL) and a solution of HCOOH (47 μL, 1.247 mmol) in dry 1,4-dioxane (2 mL) successively at room temperature under Ar. The reaction mixture was heated at 60°C for 1 h. The residue, which was obtained by the same work up procedure as described above, was purified by SiO_2 open column chromatography ($CHCl_3$–MeOH gradient) to afford (3*R*)-deoxypumiloside tetraacetate (**15**, 182 mg, y. quant.). The synthetic compound was identical with the tetraacetate[2d] derived from natural (3*R*)-deoxypumiloside (TLC, UV, ^{1}H and ^{13}C NMR, CD, and MS).

4.2.7. Preparation of (3*S*)-deoxypumiloside (3). To a suspension mixture of (3*S*)-deoxypumiloside tetraacetate (**14**, 20 mg, 0.030 mmol) in dry MeOH (1 mL) was added 1N NaOMe in MeOH (30 μL, 0.030 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 3 h. The reaction mixture was directly subjected to SiO_2 open column chromatography (20% MeOH–$CHCl_3$) and MPLC (10% MeOH–$CHCl_3$) to give (3*S*)-deoxypumiloside (**3**, 6.6 mg, y. 44%). The synthetic compound was identical with the natural (3*S*)-deoxypumiloside (TLC, UV, ^{1}H and ^{13}C NMR, CD, and MS).

4.2.8. Preparation of (3*R*)-deoxypumiloside (4). To a suspension mixture of (3*R*)-deoxypumiloside tetraacetate (**15**, 200 mg, 0.301 mmol) in dry MeOH (6 mL) was added 1N NaOMe in MeOH (301 μL, 0.301 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 5 h. The reaction mixture was directly subjected to SiO_2 open column chromatography (30% MeOH–$CHCl_3$) and MPLC (10% MeOH–$CHCl_3$) to give (3*R*)-deoxypumiloside (**4**, 113 mg, y. 76%). The synthetic compound was identical with the natural one (TLC, UV, ^{1}H and ^{13}C NMR, CD, and MS).

4.2.9. DDQ oxidation of (3*R*)-deoxypumiloside tetraacetate (15). A mixture of (3*R*)-deoxypumiloside tetraacetate (**15**, 27.7 mg, 0.417 mmol) and a solution of DDQ (60 mg, 0.264 mmol) in dry MeOH (0.5 mL) and dry toluene (0.5 mL) was stirred at 50°C for 3 h. The reaction mixture was directly subjected to Al_2O_3 column chromatography (5% MeOH–$CHCl_3$) and the residue was purified by MPLC (2% MeOH–$CHCl_3$) to afford **17** (26.2 mg, y. 91%, a mixture of 17-epimers=4:1). **17**: FAB-MS (positive, NBA) *m/z*: 693 (MH^+); UV λ_{max} (MeOH) nm: 361, 287, 253, 218 nm; Main product: ^{1}H NMR (500 MHz, $CDCl_3$, selected data) δ: 8.37 (s, 1H, H-7), 8.21 (1H, d, *J*=8.5 Hz, H-12), 7.92 (1H, d, *J*=8.5 Hz, H-9), 7.81 (1H, dd-like, *J*=8.5, 8.5 Hz, H-11), 7.65 (1H, dd-like, *J*=8.5, 8.5 Hz, H-10), 7.22 (1H, s, H-14), 5.79 (1H, s, H-17), 3.70 (3H, s, 17-OMe), 2.08, 2.05, 2.03 and 2.01 (each 3H, s, OCOCH_3×4); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.6, 170.3, 169.4, 158.8, 152.6, 148.9, 148.4, 145.7, 132.9, 131.0, 130.5, 129.7, 128.7, 128.1, 127.9, 122.6, 122.1, 100.9, 96.4, 96.1, 93.6, 72.9, 72.2, 71.0, 68.5, 62.1, 56.5, 49.8, 47.9, 20.7, 20.6.

4.2.10. Treatment of 17 with NaOMe. To a suspension mixture of **17** (a mixture of 17-epimers, 22.6 mg, 0.326 mmol) in dry MeOH (3 mL) was added 1N NaOMe in MeOH (16.3 μL, 0.016 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 5.5 h. The reaction mixture was directly subjected to SiO_2 open column chromatography (MeOH–$CHCl_3$ gradient). The residue was purified by MPLC (15% MeOH–$CHCl_3$) to give **21** (3.9 mg, y. 35%) together with OPHR-23 (**6**, 0.3 mg) and a mixture (3.0 mg) of OPHR-17 (**7**) and **20**. **21**: HR-FAB-MS (positive, NBA) *m/z*: 345.1224 (MH^+) (Calcd for $C_{21}H_{17}N_2O_3$, 345.1239); FAB-MS (positive, NBA) *m/z*: 345 (MH^+); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.37 (1H, s, H-7), 8.23 (1H, d, *J*=7.9 Hz, H-12), 7.93 (1H, d, *J*=7.9 Hz, H-9), 7.82 (1H, dd, *J*=7.9, 7.9 Hz, H-11), 7.66 (1H, dd, *J*=7.9, 7.9 Hz, H-10), 7.44 (1H, s, H-14), 7.08 (1H, s, H-21), 6.67 (1H, dd, *J*=17.1, 10.7 Hz, H-19), 6.35 (1H, s, H-17), 5.53 (1H, dd, *J*=17.1, 1.2 Hz, H-18), 5.33 (1H, d, *J*=19.2 Hz, H-5), 5.27 (1H, dd, *J*=10.7, 1.2 Hz, H-18), 5.27 (1H, overlapped, H-5), 3.66 (3H, s, 17-OMe); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 159.1, 152.9, 148.9, 145.9, 145.1, 140.8, 131.0, 130.5, 129.7, 129.1, 128.9, 128.1, 127.8, 115.5, 114.3, 113.1, 95.8, 95.7, 56.4, 49.9; UV λ_{max} (MeOH) nm: 355, 254, 219.

4.2.11. Preparation of 16 from (3*R*)-deoxypumiloside (4). To a suspension mixture of (3*R*)-deoxypumiloside (**4**, 133 mg, 0.269 mmol) and DMAP (14 mg, 0.115 mmol) in dry CH_2Cl_2 (10.5 mL) were added dry pyridine (3.5 mL, 43.27 mmol) and 2,2,2-trichloroethyl chloroformate (0.44 mL, 3.196 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 16.5 h. A cold water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by SiO_2 open column chromatography ($CHCl_3$) to afford **16** (323 mg, y. quant.). **16**: FAB-MS (NBA) *m/z*: 1192 (M^+), 1194, 1196, 1198, 1200, 1202, 1204; ^{1}H NMR (400 MHz, $CDCl_3$, selected data) δ: 8.08 (1H, d, *J*=8.1 Hz, H-12), 8.08 (1H, s, H-7), 7.83 (1H, d, *J*=8.1 Hz, H-9), 7.72 (1H, dd-like, *J*=8.1, 8.1 Hz, H-11), 7.56 (1H, dd-like, *J*=8.1, 8.1 Hz, H-10), 7.53 (1H, d, *J*=2.4 Hz, H-17), 5.47 (1H, ddd, *J*=17.1, 9.9, 9.9 Hz, H-19), 5.20 (1H, dd, *J*=9.9, 2.0 Hz, H-18), 4.86–4.65 (8H, m, COOCH_2CCl_3×4), 4.51 (1H, dd, *J*=12.2, 5.1 Hz, 6′-H), 4.43 (1H, dd, *J*=12.2, 2.7 Hz, 6′-H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.1, 162.0, 153.9, 153.5, 153.3, 153.2, 148.4, 147.2, 131.6, 130.5, 129.9, 129.3, 128.3, 128.1, 127.1, 121.3, 108.5, 97.0, 96.1, 94.6, 94.2, 76.7, 75.4, 73.1, 71.7, 66.2, 61.9, 48.9, 43.3, 30.3, 28.5.

4.2.12. DDQ oxidation of 16. A mixture of **16** (109 mg, 0.091 mmol) and a solution of DDQ (114 mg, 0.502 mmol) in dry MeOH (2.5 mL) and dry toluene (2.5 mL) was stirred at 50°C for 6 h. The reaction mixture was directly subjected to Al_2O_3 column chromatography (MeOH–$CHCl_3$ gradient). The residue was purified by MPLC (35% *n*-hexane–AcOEt) to afford **18** (58 mg, y. 52%) and **19** (17 mg, y. 15%). **18**: FAB-MS (positive, NBA) *m/z*: 1221 (MH^+), 1223, 1225, 1227, 1229, 1231; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.37 (s, 1H, H-7), 8.21 (1H, d, *J*=8.2 Hz, H-12), 7.92 (1H, d, *J*=8.2 Hz, H-9), 7.81 (1H, dd-like, *J*=8.2, 8.2 Hz, H-11), 7.65 (1H, dd-like, *J*=8.2, 8.2 Hz, H-10), 7.20 (1H, s, H-14), 5.80 (1H, m, H-19), 5.78 (1H, s, H-17), 5.52 (1H, br-d, *J*=10.1 Hz, H-18), 5.49 (1H, br-d, *J*=17.1 Hz, H-18), 5.43 (1H, d, *J*=7.3 Hz, H-21), 5.37 (1H, dd, *J*=9.2, 9.2 Hz, H-3′), 5.28 (1H, d, *J*=19.2 Hz, H-5), 5.23 (1H, d, *J*=19.2 Hz, H-5), 5.23 (1H, d, *J*=7.6 Hz, H-1′), 5.15 (1H, dd, *J*=9.2, 9.2 Hz, H-4′), 5.02 (1H, dd, *J*=9.2, 7.3 Hz, H-2′), 4.81–4.71 (8H, m, $COOCH_2CCl_3$×4), 4.44 (2H, m, H_2-6′), 3.99 (1H, m, H-5′), 3.70 (3H, s, 17-OMe), 3.45 (1H, br-dd, *J*=8.1, 8.1 Hz, H-20); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 158.8 (C-22), 153.7, 153.2, 153.0 and 152.8 ($COOCH_2CCl_3$×4), 152.6 (C-2), 148.9 (C-13), 148.1 (C-15), 145.8 (C-3), 132.7 (C-19), 131.0 (C-7), 130.5 (C-11), 129.7 (C-12), 128.8 (C-6), 128.1 (C-8, 9), 127.9 (C-10), 122.8 and 122.6 (C-16, 18), 100.7 (C-14), 96.3 (C-17), 95.7 (C-1′), 94.2, 94.1, 94.03 and 93.95 ($COOCH_2CCl_3$×4), 93.5 (C-21), 75.4 (C-2′), 73.1 (C-4′), 71.4 (C-5′), 66.1 (C-6′), 56.6 (17-OMe), 49.8 (C-5), 48.1 (C-20) (C-3′ and four Troc methylene carbons were buried under $CDCl_3$ signals.); UV λ_{max} (MeOH) nm: 358, 291, 253, 215; CD (*c*=0.253 mmol/L, MeOH, 23°C) $\Delta\epsilon$ (λ nm): 0 (395), −1.32 (355), −0.99 (308), 0 (284), +3.20 (260), 0 (254), −8.02 (238), 0 (230), +9.57 (221), 0 (214), −36.64 (205). **19**: FAB-MS (positive, NBA) *m/z*: 1221 (MH^+), 1223, 1225, 1227, 1229, 1231; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.37 (1H, s, H-7), 8.22 (1H, d, *J*=8.2 Hz, H-12), 7.93 (1H, d, *J*=8.2 Hz, H-9), 7.82 (1H, dd, *J*=8.2, 8.2 Hz, H-11), 7.65 (1H, dd, *J*=8.2, 8.2 Hz, H-10), 7.16 (1H, s, H-14), 5.81 (1H, m, H-19), 5.64 (1H, s, H-17), 5.44 (1H, d, *J*=0.9 Hz, H-21), 5.34–5.19 (5H, overlapped, H_2-5, H_2-18, H-3′), 5.15 (1H, d, *J*=7.6 Hz, H-1′), 5.10 (1H, dd, *J*=9.5, 9.5 Hz, H-4′), 4.92 (1H, dd, *J*=8.5, 7.6 Hz, H-2′), 4.82 (br-d, *J*=11.8 Hz), 4.81–4.75 (m), 4.71 (br-d, *J*=11.9 Hz), 4.58 (d, *J*=11.6 Hz), 4.47 (d, *J*=11.9 Hz, 8H, $COOCH_2CCl_3$×4), 4.51 (1H, dd, *J*=11.9, 4.9 Hz, H-6′), 4.43 (1H, dd, *J*=11.9, 2.4 Hz, H-6′), 4.02 (1H, m, H-5′), 3.70 (3H, s, 17-OMe), 3.59 (1H, br-d, *J*=8.2 Hz, H-20); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 158.6 (C-22), 153.7, 153.3 and 153.0 ($COOCH_2$-CCl_3×3), 152.8 (C-2), 152.4 ($COOCH_2CCl_3$), 148.9 (C-13), 145.8 (C-15), 145.4 (C-3), 134.8 (C-19), 131.0 (C-7), 130.5 (C-11), 129.7 (C-12), 128.7 (C-6), 128.1 (C-8, 9), 127.8 (C-10), 122.4 (C-16), 119.7 (C-18), 101.3 (C-14), 95.3 (C-1′), 94.9 (C-17), 94.8 (C-21), 94.2, 94.1, 94.01 and 93.97 ($COOCH_2CCl_3$×4), 75.0 (C-2′), 73.1 (C-4′), 71.0 (C-5′), 66.1 (C-6′), 57.3 (17-OMe), 49.8 (C-5), 46.7 (C-20) (C-3′ and four Troc methylene carbons were buried under $CDCl_3$ signals.); UV λ_{max} (MeOH) nm: 359, 288, 253, 218; CD (*c*=0.228 mmol/L, MeOH, 23°C) $\Delta\epsilon$ (λ nm): 0 (388), −0.79 (333), 0 (321), +1.76 (307), 0 (250), −7.19 (237), 0 (230), +22.88 (220), 0 (212), −36.00 (204).

4.3. Preparation of OPHR-23 (6) from 18

To a suspension mixture of **18** (18.0 mg, 0.147 mmol) in dry MeOH (1 mL) was added zinc dust (50 mg) at room temperature under Ar. The mixture was stirred at the same temperature for 19 h and under reflux for 2 h. The reaction mixture was filtrated and the filtrate was evaporated. The residue was purified by MPLC (10% MeOH–$CHCl_3$) to afford OPHR-23 (**6**, 3.0 mg, y. 39%). The synthetic compound was identical with the natural one (UV, ^{1}H and ^{13}C NMR, CD, and MS).

4.4. Preparation of OPHR-17 (7) from 19

To a suspension mixture of **19** (29.4 mg, 0.024 mmol) in dry MeOH (1.5 mL) was added zinc dust (60 mg) at room temperature under Ar. The mixture was stirred at 60°C for 7 h. The reaction mixture was filtrated and the filtrate was evaporated. The residue was purified by MPLC (10% MeOH–$CHCl_3$) to afford OPHR-17 (**7**, 0.6 mg, y. 5%). The synthetic compound was identical with the natural one (UV, ^{1}H and ^{13}C NMR, CD, and MS).

References

1. (a) Hutchinson, C. R. *Tetrahedron* **1981**, *37*, 1047. (b) Cai, J.-C.; Hutchinson, C. R. *The Alkaloids*; Brossi, A., Ed.; Academic: New York, 1983; Vol. 21, p 101. (c) Wall, M. E.; Wani, M. C. In *The Monoterpenoid Indole Alkaloids*; Saxton, J. E., Ed.; Wiley: London, 1994; p 689 Chapter 13. (d) Takayama, H.; Kitajima, M.; Aimi, N. *J. Synth. Org. Chem. Jpn* **1999**, *57*, 181.
2. (a) Aimi, N.; Nishimura, M.; Miwa, A.; Hoshino, H.; Sakai, S.; Haginiwa, J. *Tetrahedron Lett.* **1989**, *30*, 4991. (b) Aimi, N.; Hoshino, H.; Nishimura, M.; Sakai, S.; Haginiwa, J. *Tetrahedron Lett.* **1990**, *31*, 5169. (c) Aimi, N.; Ueno, M.; Hoshino, H.; Sakai, S. *Tetrahedron Lett.* **1992**, *33*, 5403. (d) Kitajima, M.; Masumoto, S.; Takayama, H.; Aimi, N. *Tetrahedron Lett.* **1997**, *38*, 4255.
3. Kitajima, M.; Fischer, U.; Nakamura, M.; Ohsawa, M.; Ueno, M.; Takayama, H.; Unger, M.; Stöckigt, J.; Aimi, N. *Phytochemistry* **1998**, *48*, 107.
4. (a) Kitajima, M.; Nakamura, M.; Takayama, H.; Saito, K.; Stöckigt, J.; Aimi, N. *Tetrahedron Lett.* **1997**, *38*, 8997. (b) Kitajima, M.; Nakamura, M.; Watanabe, A.; Takayama, H.; Aimi, N. *J. Chem. Soc., Perkin Trans. 1* **1998**, 389.
5. (a) Saito, K.; Sudo, H.; Yamazaki, M.; Koseki-Nakamura, M.; Kitajima, M.; Takayama, H.; Aimi, N. *Plant Cell Rep.* **2001**, *20*, 267. (b) Sudo, H.; Yamakawa, T.; Yamazaki, M.; Aimi, N.; Saito, K. *Biotechnol. Lett.* **2002**, *24*, 359. (c) Yamazaki, Y.; Urano, A.; Sudo, H.; Kitajima, M.; Takayama, H.; Yamazaki, M.; Aimi, N.; Saito, K., submitted.
6. Blackstock, W. P.; Brown, R. T.; Chapple, C. L.; Fraser, S. B. *J. Chem. Soc., Chem. Commun.* **1972**, 1006.
7. (a) Battersby, A. R.; Burnett, A. R.; Parson, P. G. *J. Chem. Soc. (C)* **1969**, 1193. (b) Hutchinson, C. R.; O'Loughlin, G. J.;

Brown, R. T.; Fraser, S. B. *J. Chem. Soc., Chem. Commun.* **1974**, 928.
8. Zheng, Q.; Yang, Y.; Martin, A. R. *Heterocycles* **1994**, *37*, 1761.
9. Cacchi, C.; Gattini, P. G.; Morea, E.; Orter, G. *Tetrahedron Lett.* **1986**, *27*, 5541.
10. Itoh, A.; Tanahashi, T.; Nagakura, N. *Heterocycles* **1998**, *48*, 499.
11. (a) Willker, W.; Leibfritz, D. *Magn. Reson. Chem.* **1995**, *33*, 632. (b) Takayama, H.; Ichikawa, T.; Kuwajima, T.; Kitajima, M.; Seki, H.; Aimi, N.; Nonato, M. G. *J. Am. Chem. Soc.* **2000**, *122*, 8635. (c) Hansen, P. E. *Prog. NMR Spectrosc.* **1981**, *14*, 175. (d) Sasaki, M.; Matsumori, N.; Maruyama, T.; Nonomura, T.; Murata, M.; Tachibana, K.; Yasumoto, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1672.
12. Hutchinson, C. R.; Heckendorf, A. H.; Daddona, P. E.; Hagaman, E.; Wenkert, E. *J. Am. Chem. Soc.* **1974**, *96*, 5609.