Facile Synthesis of a Novel Taxoid Closely Related to Bioactive Taxuspine D. Regio- and Stereo-Selective Hydration of Taxinine, a Naturally Occurring Taxane Diterpenoid

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Treatment of taxinine, which is a taxane diterpenoid readily available from needles of the Japanese yew *Taxus cuspidata*, with a large excess amount of sodium borohydride in slightly hydrous *N,N*-dimethylformamide at ambient temperature resulted in regio- and stereo-selective hydration at the C_{11}, C_{12}-double bond to give an isomeric taxuspine D derivative.

Key words taxinine; hydration; sodium borohydride; taxuspine D

Much attention has been paid to development of a new series of antitumor taxoids for the chemotherapeutic treatment of advanced solid cancers such as refractory ovarian cancer and metastatic breast cancer, because of the emergence of undesired side-effects and the rapid induction of P-glycoprotein (multi-drug resistance of cancer cells) in the course of the clinical use of Taxol® (paclitaxel, **1a**) and Taxotere® (docetaxel, **1b**), which are first-generation taxoids having a unique antimitotic mechanism of action.[1] Along this line, 56 taxane diterpenoids were isolated from stems and needles of the Japanese yew, *Taxus cuspidata* SIEB. *et* ZUCC. collected at Sapporo and their biological activities were evaluated.[2] Among them, taxuspine D (**2**), a new taxane diterpenoid, exhibits remarkable inhibitory activity towards Ca^{2+}-induced depolymerization of microtubules in a manner similar to that of the taxoids **1a**, **b**.[3] It should be noted that the taxoid **2** possesses neither a fused oxetane ring nor C_{13}-(*N*-acyl)phenylisoserinyl and C_2-*O*-benzoyl moieties, which had been regarded as very important for binding of taxoids to tubulin proteins to manifest the biological activities.[4]

In this paper, we describe a facile synthesis of a bioactive new taxoid (**4**), having a unique structure closely resembling that of the naturally occurring taxoid **2**, by regio- and stereo-selective hydration of taxinine (**3**), which is a taxane diterpenoid readily isolable (in 8.8×10^{-2}% yield) from needles of the Japanese yew.[5]

When a solution of the taxoid **3** in slightly hydrous *N,N*-dimethylformamide (DMF) was treated with a large excess amount (20 eq) of sodium borohydride ($NaBH_4$) at ambient temperature, the starting **3** was almost completely consumed after 7 h. Thin-layer chromatographic (TLC) analysis of the reaction mixture showed almost quantitative conversion of the taxoid **3** to a more polar compound **4**, which was easily isolated as an amorphous colorless powder by chromatographic purification with a short column. The product **4** showed a molecular ion peak at *m/z* 624 in the EI-MS. The molecular formula $C_{35}H_{44}O_{10}$ deduced from HR-FAB-MS (*m/z* 625.3035 $[M+H]^+$, $\Delta +2.3$ mmu) spectroscopic and elemental analyses, suggested the occurrence of hydration rather than reduction of **3** under the reaction conditions employed. Characteristic spectral data [IR absorption at 1707 cm^{-1}, a strong UV absorption at 278 nm, a prominent fragment ion peak at *m/z* 131 in EI-MS, proton signals at δ_H 6.63 (1H, d, $J=16$ Hz), 7.40 (3H, m), 7.63 (2H, m), and 7.68 (1H, d, $J=16$ Hz) ppm, and two carbon signals at δ_C 118.6 and 144.9 ppm] of the product **4** clearly indicated retention of the cinnamoyl moiety during the reaction. Two broad singlet signals at δ_H 5.47 and 5.91 ppm (δ_H 4.84 and 5.35 ppm for **3**) and a carbon signal at δ_C 122.1 ppm (δ_C 117.2 ppm for **3**) unambiguously indicated the involvement of the *exo*-methylene moiety in the product **4**. The marked upfield shifts (δ_C 59.7 and 75.7 ppm for **4**) of two olefinic carbons (δ_C 150.6 and 138.0 ppm) originating from the C_{11}- and C_{12}-positions in the starting **3**, a slight upfield shift (δ_H 2.28 ppm for **3**; δ_H 1.92 ppm for **4**) of a broad singlet signal for the C_{12}-methyl protons, and the appearance of a broad singlet signal at δ_H 2.30 ppm assignable to the C_{11}-proton in the product **4** indicated the occurrence of regio-selective hydration at the C_{11}, C_{12}-double bond of **3** under the conditions employed. The conversion of the cyclohexenone ring system in **3** to a cyclohexanone ring system for the formation of the product **4** was also supported by a pronounced downfield shift of the C_{13}-carbonyl carbon signal (δ_C 199.4 ppm for **3**, δ_C 209.8 ppm for **4**). The relative stereochemistry of **4** was elucidated from the Nuclear Overhauser and Exchange Spectroscopic (NOESY) spectrum, *i.e.*, correlation

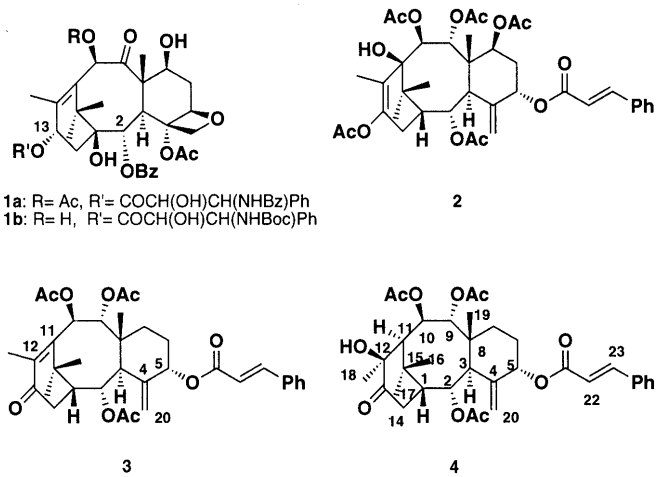

Fig. 1

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peaks of H-10 to H-11 and H_3-18 were observed in addition to four cross peaks indicating the correlations of H-2 to H-1, H-9, H_3-16, and H_3-19. Acetylation of the product **4** with acetic anhydride in pyridine at ambient temperature overnight resulted in complete recovery of the starting material, supporting the presence of the unreactive tertiary hydroxyl group in the molecule.

The formation of **4** in this reaction significantly depended upon the solvent employed and the concentration of $NaBH_4$. Analogous results were obtained by the use of slightly hydrous dimethyl sulfoxide or acetonitrile as a solvent, though a considerable delay of the reaction was observed in each case. The employment of anhydrous DMF in this reaction resulted in markedly decreased formation of the product **4**. The addition of a small amount of water to the anhydrous reaction media greatly enhanced the formation of **4** (*e.g.*, 81% yield in the case of the addition of 7 eq of water). Further addition of water to the reaction media, however, reduced the formation of **4**. In sharp contrast, the employment of an alcohol such as methanol or ethanol as a solvent caused decomposition of the starting **3** to give an undetermined complex mixture possessing high polarity as evidenced by TLC and ^{1}H-NMR spectroscopic analyses. When compound **3** was treated with 5 eq of $NaBH_4$ in slightly hydrous DMF for 7 h, the desired product **4** was obtained in 44% yield, together with the unreacted **3** (33%). The employment of 10 eq of $NaBH_4$ in this reaction caused a significant enhancement of the formation of **4**. These facts indicate that the combination of a large excess of $NaBH_4$ with a slightly hydrous aprotic solvent is a requisite for the preferential formation of **4** from **3**.

The formation of **4** in this reaction is formally explained by virtue of hydration at the C_{11}, C_{12}-double bond in **3** under the conditions employed. The regio- and stereoselectivity of this hydration seems to be due to the unique cage conformation of **3**[6] and the bulkiness of the β-acetoxy group at the *C*-10 position and of the *gem*-dimethyl groups at the *C*-15 position in **3**. Hydration of alkenes leading to the corresponding saturated alcohols can be accomplished under a variety of reaction conditions,[7] *e.g.*, in the presence of an acid catalyst such as sulfuric acid, *via* oxymercuration in Markownikov's manner followed by reductive demercuration with $NaBH_4$, and *via* hydroboration with reversed regioselectivity followed by oxidative carbon–boron bond-cleavage with hydrogen peroxide. Due to the inherent physicochemical properties of the double bond, most of the hydration initially proceeds in an electrophilic manner. To our knowledge, the present reaction system for the hydration of the double bond has no precedent. Alkaline hydrolysis of the taxoid **3** has been demonstrated to occur preferentially at the *C*-9 and *C*-10 ester moieties to give 9,10-di-*O*-deacetyltaxinine and subsequent ring-contraction leads to anhydrotaxininol.[8] In fact, the treatment of **3** with 10 eq of NaOH in slightly hydrous DMF gave polar complex mixtures with no detectable formation of compound **4**. Consequently, $NaBH_4$ appears to act as a base, not a reductant, in a slightly hydrous aprotic solvent, though we can not yet account in detail for the present result.

The C_{11}, C_{12}-double bond in **3** is regarded as quite unreactive to electrophilic and nucleophilic addition, as well as catalytic hydrogenation, for steric reasons arising from its situation in the bridge-head position, in spite of the presence of an adjacent carbonyl group.[5] As an exceptional case, the treatment of **3** with lithium aluminum hydride in boiling tetrahydrofuran reduces this double bond to give 2,5,9,10-tetra-*O*-deacylated 11,12-dihydrotaxinine (taxinol)[9] as the ultimate product. The present hydration provides another example of the chemical modification of the C_{11},C_{12}-double bond in the taxoid **3**.

The hydrated product **4** induced the differentiation of PC-12 tumor cells at concentrations of 10^{-7} mol order.[10] It is notable that the starting compound **3** lacks such biological activities and that the simple hydration of **3** generates biological activity.

Experimental

Melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotation was measured on a JASCO DIP-370 automatic polarimeter using chloroform as a solvent. Elemental analysis was carried out in the Microanalytical Center of our university. Mass spectra were recorded on a JEOL JMS-SX 102A instrument with a direct inlet system operating at 70 eV. IR spectra were recorded on a Perkin Elmer 1650 IR Fourier-transform spectrometer and UV spectra on a Shimadzu-260 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-EX 400 spectrometer using tetramethylsilane as the internal standard (peak multiplicity, s: singlet, d: doublet, m: multiplet, br s: broad singlet, dd: double doublet). TLC analyses were performed on Silica gel 60 F-254 plates (Merck Art. 5715, 0.25 mm thick) and TLC-scanning was carried out with a Shimadzu CS-9000 dual-wavelength flying-spot scanner. Peak detection on TLC was done at a wavelength of 278 nm, which is an absorption maximum band of the cinnamoyl moiety. Column chromatographic separation was performed with Merck Silica gel 60 (70—230 mesh).

Materials DMF and sodium borohydride ($NaBH_4$) purchased from chemical suppliers were used without further purification. Taxinine (**3**) was isolated as a crystalline compound (mp 265 °C) from needles of Japanese yew, *Taxus cuspidata*.[11]

Preparation of Isomeric Taxuspine D Derivative (4) A stirred solution of taxinine (**3**) (60.6 mg, 0.1 mmol) in DMF (6.0 ml) was treated with $NaBH_4$ (75.6 mg, 2.0 mmol) at ambient temperature and then the stirring was continued for 7 h. The reaction mixture was quenched with the addition of saturated ammonium chloride (1 ml), diluted with water (20 ml), and extracted with dichloromethane (2 × 30 ml). TLC analyses of the extract showed the presence of a major product, having a higher polarity than that of **3**, and trace amounts of more polar products, together with a trace amount of the starting **3**. The combined extract was washed well with brine, dried over anhydrous sodium sulfate, and evaporated to dryness. The resulting residue was purified by column chromatography with *n*-hexane–ethyl acetate (3/1) to isolate the product **4** (56.8 mg, 91%): mp 115 °C (from *n*-hexane–acetone); *Rf*: 0.34 (dichloromethane : ethyl acetate; 10/1); $[\alpha]_D^{20} = +10.7$ ($c = 0.43$); EI-MS *m/z* (rel. int. %) 624 (M^+, 1), 564 (1), 546 (1), 518 (1), 469 (33), 131 (100); IR (KBr) cm^{-1}: 1739 (C=O), 1707 (C=O); UV λ_{max} (EtOH) nm (ε): 278 (16800); ^{1}H-NMR ($CDCl_3$) δ: 7.68 (d, 1H, J=16 Hz, C23-H), 7.63 (m, 2H, benzene ring protons), 7.40 (m, 3H, benzene ring protons), 6.63 (d, 1H, J=16 Hz, C22-H), 6.00 (d, 1H, J=11 Hz, C9-H), 5.91 (s, 1H, C20-H), 5.81 (br s, 1H, C2-H), 5.66 (d, 1H, J=11 Hz, C10-H), 5.47 (s, 1H, C20-H), 5.35 (br s, 1H, C5-H), 2.89 (dd, 1H, J=7, 20 Hz, C14β-H), 2.60 (d, 1H, J=20 Hz, C14α-H), 2.30 (s, 1H, C11-H), 2.20 (s, 1H, OH), 2.06, 2.01, 2.00 (each s, each 3H, three *O*-acetyl protons), 1.92 (br s, 3H, C18-H_3), 1.89 (br d, 1H, J=7 Hz, C1-H), 1.79 (br s, 1H, C3-H), 1.63 (br s, 3H, C16-H_3), 1.6—1.8 (m, 4H, C6-H_2, C7-H_2), 1.33 (br s, 3H, C17-H_3), 0.91 (br s, 3H, C19-H_3); ^{13}C-NMR ($CDCl_3$) δ: 209.8, 169.6, 169.5, 169.0, 166.5, 144.9, 140. 2, 134.7, 130.2, 128.9 (2), 128.1 (2), 122.1, 118.6, 78.2, 78.1, 75.7, 73.4, 71.7, 59.7, 50.8, 44.0, 42.5, 35.6, 35.2, 34.6, 27.3, 26.4, 26.3, 26.2, 21.4, 21.1, 20.7, 18.4; HR-FAB-MS *m/z*: 625.3035 (Calcd for $C_{35}H_{45}O_{10}$: 625.3012); *Anal.* Calcd for $C_{35}H_{44}O_{10}1/2H_2O$: C, 66.33; H, 7.16. Found: C, 66.32; H, 7.23. The ^{1}H- and ^{13}C-NMR spectra were assigned on the basis of several types of two-dimensional

NMR data including ^1H–^1H Correlated Spectroscopy (COSY), ^{13}C–^1H COSY, and NOESY spectra. Detailed analyses of the ^1H–^1H and ^{13}C–^1H COSY spectra revealed connectivity of C-1 to C-14, C-2 to C-3, C-5 to C-6, and C-9 to C-10. The NOESY spectrum showed cross peaks for the correlation of H-2/H-1, H-9, H_3-16, and H_3-19, H-9/H-2, H_3-16, and H_3-19, and H-10/H-11 and H_3-18.

When the reaction (0.01 mmol scale) was carried out in slightly hydrous dimethyl sulfoxide or acetonitrile (1.0 ml) in place of DMF under analogous conditions, compound **4** was obtained in 45% yield (for the case of dimethyl sulfoxide) or 12% yield (for the case of acetonitrile) after 7 h, though TLC densitometric analyses showed the presence of a significant amount of unreacted **3** in each reaction mixture.

On the other hand, the employment of DMF carefully pretreated with molecular sieves 4A overnight decreased the formation of **4** (42% yield), in spite of the almost complete consumption of the starting **3**, suggesting concurrent occurrence of other reactions involving reductive *O*-deacylation of the ester moieties. The addition of 7 eq of water to the anhydrous reaction media in the 0.01 mmol scale experiment allowed the formation of **4** in 81% yield with the recovery of a trace amount of **3**. Further addition of water to the reaction media reduced the formation of **4** (70% yield in the case of the addition of 67 eq of water; 44% yield in the case of the addition of 200 eq of water) and increased the recovery of **3** (trace in the case of the 67 eq addition of water; 26% yield in the case of the 200 eq of water).

After treatment of **3** (0.01 mmol) with $NaBH_4$ (1.9 mg, 0.05 mmol) in DMF (1.0 ml) at ambient temperature for 7 h, TLC densitometric analysis of the reaction mixture showed 44% formation of **4** and 33% recovery of the starting **3**, together with the formation of small amounts of other undetermined products. The reaction of **3** (0.01 mmol) with $NaBH_4$ (0.10 mmol) under similar conditions afforded **4** in 70% yield with 12% recovery of **3**.

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