Convenient methods for regio- and/or chemo-selective O-deacylation of taxinine, a naturally occurring taxane diterpenoid

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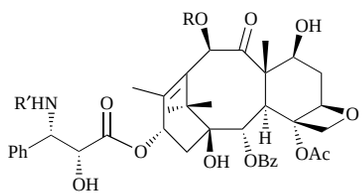
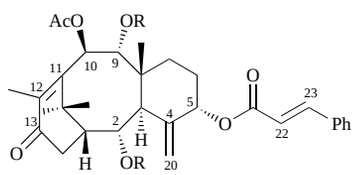
Selective O-deacylations of taxinine 1a, readily available from needles of *Taxus cuspidata*, at C-2, C-5 and C-9,10 have been accomplished by treatment with barium hydroxide octahydrate, sodium bis(2-methoxyethoxy)aluminium hydride (Red-Al®) or diisobutylaluminium hydride (DIBAL-H) under mild conditions to give 2,9,10-tri-*O*-deacetyltaxinine 4b, 2-*O*-deacetyltaxinine 4c, 5-*O*-decinnamoyltaxinine (taxinine A, 5a) and 2,5-di-*O*-deacyltaxinine (taxuspine G, 5b), respectively, which are expected to be useful synthetic intermediates for biologically active taxinine derivatives.

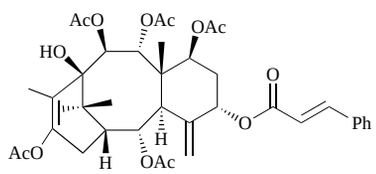
Paclitaxel (Taxol®), a taxane diterpenoid isolated from the bark of the Pacific yew (*Taxus brevifolia*) in 1971,[1] and its semisynthetic analogue, docetaxel (Taxotere®),[2] are the most promising antitumour agents available today, having a unique antimitotic mechanism of action, namely, as an inducer for the polymerization of tubulin α,β-heterodimers and a stabilizer of the resulting microtubule polymer.[3] Both taxoids exhibit significant antitumour activity against various types of advanced solid cancers, *e.g.*, refractory ovarian cancer,[4] metastatic breast cancer,[5] non-small-cell lung cancer[6] and head-and-neck cancer,[7] which have not effectively been treated by existing antitumour drugs. Chemotherapeutic treatment with these taxoids, however, has been shown to result in undesired clinical side-effects as well as the rapid emergence of drug resistance in the solid cancers by the induction of *P*-glycoprotein, which is a multi-drug transporter. Therefore, it has become essential to develop a new series of antitumour taxoids with fewer side-effects, higher solubility in water, and improved activity against various classes of tumours.[8]



Paclitaxel R = Ac, R′ = Bz
Docetaxel R = H, R′ = Boc



1 a R = Ac
b R = H



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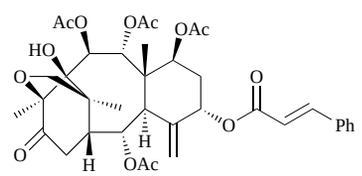

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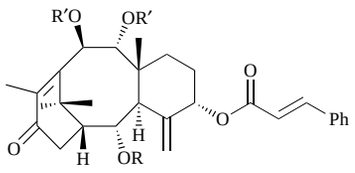
Results and discussion

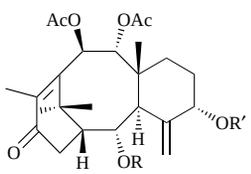
During the search for new bioactive and naturally occurring taxoids, it was found that non-paclitaxel-type compounds such as 2,9-di-*O*-deacetyltaxinine **1b**,[9] taxuspine D **2**[10] and taxagifine **3**[11] remarkably reduce the $CaCl_2$-induced depolymerization of microtubules in a manner similar to that of paclitaxel, and exhibit cytotoxicity against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells.[12] These observations prompted us to investigate the chemical modifications of taxinine **1a**, which possesses the unusually complex taxane ring-system and, furthermore, is readily obtainable (8.8×10^{-2}% isolated yield) from needles of the Japanese yew *Taxus cuspidata*. In this paper, we describe simple methods for regio- and/or chemo-selective O-deacylation of taxinine **1a**, leading to 2,9,10-tri-*O*-deacetyltaxinine **4b**, 2-*O*-deacetyltaxinine **4c**, 5-*O*-decinnamoyltaxinine **5a** and 2,5-di-*O*-deacyltaxinine **5b** which serve as synthetic intermediates for biologically active taxinine derivatives with proper modifications of the C-2, C-5, C-9 and C-10 substituents.

The taxoid **1a**, formed by thermal elimination of dimethylamine from the C-5 ester moiety of a taxane alkaloid (taxine II), was isolated as a beautiful crystalline compound in the alcohol extract of needles of the Japanese yew as early as 1925[13] and is known to be a component of needles,[9,14–17] fruits,[18] seeds,[19] twigs,[14a,20] bark[21] and heartwood[22] of various types of the Taxus species. A structural characteristic of taxinine **1a** is that it possesses an exocyclic methylene group at C-4, three O-acetyl groups at C-2, C-9 and C-10, an O-cinnamoyl group at C-5, and a carbonyl functional group at C-13, which could be chemically modified with ease. The structure of compound **1a** was determined on the basis of its ^{1}H NMR spectroscopic data and chemical reactions at almost the same time by both British and Japanese groups, the latter including one (Y. M.) of the authors, about thirty years ago.[13c] Its presumed absolute configuration was based on the X-ray crystallographic analytical data of 2,5,9,10-tetra-*O*-acetyl-14-bromotaxinol;[23] quite recently, the cage conformations in crystal and solution states were established by X-ray crystallographic analysis and rotating-frame nuclear Overhauser effect (ROE) NMR-

spectroscopic experiments of compound **1a** itself.[24] Compound **1a** is thus the first natural taxoid to be obtained in a pure state and structurally elucidated.



4 a R = Ac, R′ = H
b R = R′ = H
c R = H, R′ = Ac



5 a R = Ac, R′ = H
b R = R′ = H
c R = R′ = Ac

In early chemical studies on the structural elucidation of taxinine **1a**, it was demonstrated that its Zemplen methanolysis with 0.30–0.73 mol equiv. of sodium methoxide at 0 °C for 16–20 h results in the regioselective removal of both acetyl groups from the ester moieties on C-9 and C-10 to give 9,10-di-*O*-deacetyltaxinine **4a** in 56–93% yield.[15b,15c,25] In these studies, it was noted that there was no occurrence of (additional) O-deacetylation at the C-2 position leading to 2,9,10-tri-*O*-deacetyltaxinine **4b** or 2-*O*-deacetyltaxinine **4c** under the employed conditions, which was explained by virtue of the steric hindrance at this position in the molecule.

The methanolysis of taxinine **1a** was carried out with 0.5 mol equiv. of sodium methoxide at higher temperature (4 °C) in order to obtain the 2-*O*-deacetylated derivatives **4b**,**c**.† In this reaction, the expected compound **4b** was obtained as a crystalline product in 21% yield, together with 2-monoester compound **4a** (71% yield) and a trace amount of 9,10-diester **4c**. The structure of the major product **4a** was confirmed by ^{1}H NMR and UV spectral comparison with the authentic compound reported by Uyeo *et al.*[25] The structures of the minor products **4b**,**c** were well assigned on the basis of those microanalytical and spectra data, *e.g.*, pertinent disappearance of the singlet signals originating from the 2-, 9- and 10-*O*-acetyl groups (δ 2.06, 2.07 and 2.08 ppm for compound **1a**), upfield shifts of the doublet or broad doublet signals originating from methine protons at the C-2, C-9 and C-10 [δ 5.58 (br d, *J* 6.4, 2-H); 5.92 (d, *J* 10.3, 9-H) and 6.07 (d, *J* 10.3, 10-H) for **1a**; δ 4.07 (br d, *J* 9, 9-H), 4.20 (br d, *J* 6.4, 2-H) and 4.86 (d, *J* 9, 10-H) for **4b**; δ 4.32 (br t, *J* 6.4, 2-H), 5.80 (d, *J* 10, 9-H) and 6.02 (d, *J* 10, 10-H) ppm for **4c**] and retention of the two characteristic doublet signals for the cinnamoyl group [δ 6.46 (*J* 16) and 7.69 (*J* 16) ppm for taxinine **1a**] in ^{1}H NMR spectra. Treatment of the two minor products with acetic anhydride–4-(dimethylamino)pyridine (DMAP)/pyridine resulted in the reproduction of taxinine **1a** in almost quantitative yield, respectively, strongly supporting the structures of the deacetylated taxoids **4b**,**c**. Thus, under the reaction conditions described above, regio- and/or chemo-selective O-deacylation of taxinine **1a** proceeded with retention of the taxane ring system to form products **4a**–**c**. Prolongation of the reaction time or further addition of sodium methoxide to the reaction media to improve the chemical yields of the minor products **4b**,**c** in this reaction led instead to a decrease of the total yield of deacylation products **4a**–**c** due to side-reactions, presumably involving ring-contraction to anhydrotaxininol which is initiated by a retro-aldol cleavage of the 9,10-diol grouping under alkaline conditions, and demonstrated by Yamamoto *et al.*[27]

The use of barium hydroxide octahydrate in place of sodium methoxide in this reaction allowed for the formation of fully deacetylated compound **4b** in enhanced chemical yield (33%) with a decrease in the formation of compound **4a** (64%). On the other hand, no formation of compound **4b** was observed in the alkaline hydrolysis of taxinine **1a** with sodium carbonate monohydrate under analogous conditions (the yield of compound **4a** after 5 h: 18%). Thus, the chemoselective O-deacetylation of taxinine **1a** leading to 2,9,10-tri-*O*-deacetyltaxinine **4b** was accomplished by suitable choice of reagents and by carrying out the reaction at 4 °C, although there is a limitation to the formation of triol **4b** to avoid side-reactions.

Reduction of taxinine **1a** with a large (4.8 mol equiv.) amount of lithium aluminium hydride (LAH) in refluxing tetrahydrofuran (THF) has been reported to give taxininol[28] which is formed by reductive O-deacylation at C-2, C-5, C-9 and C-10 and the further reduction of the C-11–C-12 double bond. To control this reaction, treatment of compound **1a** with a lower (1.5 mol equiv.) amount of LAH at 60 °C was examined. Under these reaction conditions, the reductive O-deacylation proceeded at C-5 only (regio- and chemo-selectively) to give 5-*O*-decinnamoyltaxinine **5a** in 24% yield after 2 days, accompanied by the recovery of unconsumed starting material **1a**. This fact suggests that the regioselective O-deacylation of taxinine **1a** at the C-5 position can be accomplished by the choice of suitably active reducing agents. In order to substantiate this assumption, reductions of taxinine **1a** by using various types of reducing agents were carried out in order to improve the formation of compound **5a**.

$NaBH_4$ reduction in THF containing a small amount of methanol caused concurrent reduction and methanolysis of taxinine **1a** to give inseparable, highly polar, complex mixtures. On the other hand, employment of sodium bis-(2-methoxyethoxy)aluminium hydride (Red-Al®) as a reducing reagent resulted in the enhanced and smooth formation (50% yield) of the required hydroxy triacetate **5a**, even under mild conditions (room temp.; 0.5 h). This reaction, however, was accompanied by the reductive O-deacylation at both C-2 and C-5 of compound **1a** to give 2,5-di-*O*-deacyltaxinine **5b** in 22% yield. Product **5a** was identical with taxinine A,[29] which was isolated from *Taxus cuspidata*[14a,16a,16e,20] and *T. chinensis*[17] and which was also independently synthesized from taxinine **1a** by treatment with hydroxylamine hydrochloride.[30] The structure of compound **5b** was confirmed by ^{1}H NMR spectral comparison with taxuspine G[31] recently isolated from needles and stems of *T. cuspidata*, and was reconfirmed by its acetylation with acetic anhydride–DMAP in pyridine leading to taxinine H **5c**, which is a naturally occurring taxoid.[16a] When the reduction of taxinine **1a** was performed with 5.0 mol equiv. of Red-Al®, the yield of the 2,5-di-*O*-deacylated taxinine **5b** was markedly improved (32% yield), although the total yield of 5-*O*-deacylated products **5a** and **5b** was decreased because of the occurrence of over-reduction involving O-deacetylation at C-9 and/or C-10 to give inseparable, highly polar, complex mixtures.

In sharp contrast to the above results, treatment with two mol equiv. of diisobutylaluminium hydride (DIBAL-H) in dry THF at room temp. caused preferential O-deacetylation at C-2 of taxinine **1a** to give hydroxy diacetate **4c** which was obtained as a minor product in the methanolysis of taxinine **1a** as described above, together with a small amount of the 5-*O*-deacylated taxinine **5a**. Thus, the selective O-deacylation at C-2 and/or C-5 of taxinine **1a**, leading to products **5a**, **5b** and **4c** was accomplished by the use of Red-Al® or DIBAL-H as reducing agent. At present, clear reasons for the selectivity in the present reductive O-deacylations are not available even after consider-

† The synthesis of compound **4b** by methanolysis of a mixture of 2,9- and 2,10-di-*O*-deactylated taxinines, naturally occurring taxoids, under analogous conditions (0 °C; 16 h) has been recently reported without description of its physico-chemical data.[26]

ation of the chemical reactivity of the reducing agents and the steric crowdedness of the substrate molecule **1a**.

Distinct biological activities for taxinine **1a** itself have not been reported so far. In contrast to this fact, 9, 10-di-*O*-deacetyltaxinine **4a**, 2-*O*-deacetyltaxinine **4c** and 5-*O*-deacylated taxinine **5a** inhibited the function of *P*-glycoprotein in a high level (129–132%) compared with the case of verapamil, a typical functional inhibitor of *P*-glycoprotein, and increased cellular accumulation of antitumour vincristine in multidrug-resistant tumour cells. Furthermore, compound **4a** induced the differentiation of PC12 tumour cells in 10^{-6} m amounts. These results provide a promising clue to the development of a new generation of bioactive taxoids based on the chemical modifications of taxinine **1a**.

Experimental

Mps were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 polarimeter, using chloroform as solvent. $[\alpha]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analyses were 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 Infra Red Fourier-transform spectrometer, and UV spectra with 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. The chemical shifts are expressed in δ-values (parts per million). Peak multiplicities are denoted by s (singlet), d (doublet), t (triplet) and m (multiplet) or by a combination of these, *e.g.*, br s (broad singlet) and dd (double doublet), with coupling constants (J) given in Hz. All reactions were conducted under argon. Dry solvents were obtained using standard procedures. Anhydrous sodium sulfate was used for drying organic solvent extracts. 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 with a wavelength of 278 nm which is an absorption maximum band of the cinnamoyl moiety. Rotary evaporation was carried out under reduced pressure with the bath temperature below 35 °C unless otherwise specified. Column chromatography separation was performed with Merck silica gel 60 (70–230 mesh).

Methanolysis of taxinine 1a

To a stirred solution of taxinine **1a** (100 mg, 0.165 mmol) in a 1:1 mixture of dry CH_2Cl_2 and methanol (20 ml) was added sodium methoxide (Aldrich, 25 wt% solution in methanol) (0.38 ml, 0.083 mmol) and the mixture was kept in an ice-box (at 4 °C) overnight. After confirmation of complete consumption of the starting material by TLC analysis with developing solvent of CH_2Cl_2–AcOEt (10:1), the reaction mixture was diluted with CH_2Cl_2 (50 ml), washed with brine (20 ml), dried and evaporated to leave an oil, which was subjected to silica gel column chromatography, and elution with *n*-hexane–AcOEt (4:1), to separate 9,10-di-*O*-deacetyltaxinine **4a**, 2,9,10-tri-*O*-deacetyltaxinine **4b** and 2-*O*-deacetyltaxinine **4c**. TLC-densitometric analysis of the extract showed the formation of compounds **4a** and **4b** in 71% and 21% yield, respectively, together with a trace amount of compound **4c**.

9,10-Di-*O*-deacetyltaxinine 4a. Amorphous powder; mp 224–226 °C (from AcOEt–Et_2O)(lit.,[15b] 224–225 °C) (lit.,[15c] 216 °C) [lit.,[25] 222–223 °C (from hexane–benzene)]; $[\alpha]_D^{28}$ +169.6 (*c*, 1.00) [lit.,[15b] +181 (*c* 0.01–0.02, $CHCl_3$, at 21 °C)] [lit.,[25] +68.5 (*c* 0.97, $CHCl_3$)]; R_f 0.2 (CH_2Cl_2–AcOEt 10:1); *m*/*z* (EI) 522 (M^+, 10%), 462 (8), 420 (10), 131 (91) and 43 (100); ν_{max}(KBr)/cm^{-1} 3449, 1747, 1673 and 1235; λ_{max}(EtOH)/nm 276 (ε/dm^3 mol^{-1} cm^{-1} 30 400), 222 (14 800) and 217 (16 600); δ_H($CDCl_3$) 1.11 (3 H, br s, 8-Me), 1.23 (3 H, br s, 15-Me$^\beta$), 1.48, 1.71, 1.79 and 1.94 (each 1 H, each m, 6- and 7-H), 1.71 (3 H, br s, 15-Me$^\alpha$), 2.07 (3 H, s, OAc), 2.13 (3 H, br s, 12-Me), 2.15 (1 H, dd, *J* 2.0 and 6.8, 1-H), 2.40 (1 H, d, *J* 20.0, 14-H$^\alpha$), 2.67 (1 H, br s, 10-OH), 2.73 (1 H, br d, *J* 3.4, 9-OH), 2.83 (1 H, dd, *J* 6.8 and 20.0, 14-H$^\beta$), 3.36 (1 H, d, *J* 6.4, 3-H), 4.18 (1 H, dd, *J* 3.4 and 9.3, 9-H), 4.85 (1 H, s, 20-H), 4.90 (1 H, br d, *J* 9.3, 10-H), 5.32 (1 H, s, 20-H), 5.33 (1 H, br s, 5-H), 5.52 (1 H, dd, *J* 2.0 and 6.4, 2-H), 6.43 (1 H, d, *J* 16, 22-H), 7.43 (3 H, m, Ph), 7.63 (1 H, d, *J* 16, 23-H) and 7.76 (2 H, d, *J* 6.8, Ph); δ_C($CDCl_3$) 200.0, 169.8, 166.4, 155.4, 145.5, 142.5, 135.7, 134.6, 130.3, 128.9 (2), 128.4 (2), 118.0, 116.6, 78.7, 77.8, 73.3, 69.9, 48.8, 44.5, 43.2, 38.0, 37.6, 36.1, 28.5, 26.2, 25.3, 21.4, 17.7 and 14.1. The UV and ^{1}H NMR spectra of this product were almost identical with those of the authentic compound reported by Uyeo *et al.*,[25] except for the doublet signal at δ 4.18 in the ^{1}H NMR spectrum.

2,9,10-Tri-*O*-deacetyltaxinine 4b. Mp 117–119 °C (from AcOEt–Et_2O); $[\alpha]_D^{28}$ +151.8 (*c* 1.00); R_f 0.07 (CH_2Cl_2–AcOEt 10:1) (Found: C, 70.41; H, 7.99. $C_{29}H_{36}O_6$·8/9 H_2O requires C, 70.2; H, 7.7%) [HR FAB MS *m*/*z* 481.2599 (M^+ + 1). $C_{29}H_{37}O_6$ requires *M*, 481.2590]; *m*/*z* (EI) 480 (M^+, 8%), 462 (4) and 131 (100); ν_{max}(KBr)/cm^{-1} 3479, 1700, 1663, 1341, 1313 and 1181; λ_{max}(EtOH)/nm 279 (ε/dm^3 mol^{-1} cm^{-1} 25 700) and 222 (15 000); δ_H($CDCl_3$) 1.15 (3 H, br s, 8-Me), 1.25 (3 H, br s, 15-Me$^\beta$), 1.64 (3 H, br s, 15-Me$^\alpha$), 1.47, 1.72, 1.82 and 1.94 (each 1 H, each m, 6- and 7-H_2), 2.11 (3 H, br s, 12-Me), 2.23 (1 H, d, *J* 20.0, 14-H$^\alpha$), 2.34 (1 H, br d, *J* 6.8, 1-H), 2.77 (2 H, br, OH), 2.81 (1 H, dd, *J* 6.8 and 20.0, 14-H$^\beta$), 3.22 (1 H, d, *J* 6.4, 3-H), 4.07 (1 H, br d, *J* 9, 9-H), 4.20 (1 H, br d, *J* 6.4, 2-H), 4.86 (1 H, d, *J* 9, 10-H), 5.32 (1 H, br s, 5-H), 5.39 (1 H, s, 20-H), 5.43 (1 H, s, 20-H), 6.39 (1 H, d, *J* 16, 22-H), 7.41 (3 H, m, Ph), 7.62 (1 H, d, *J* 16, 23-H) and 7.74 (2 H, d, *J* 6.8, Ph); δ_C($CDCl_3$) 200.1, 166.5, 155.5, 145.7, 144.2, 135.2, 134.5, 130.3, 128.9 (2), 128.5 (2), 117.9, 117.4, 78.4, 77.7, 73.5, 68.2, 51.4, 45.2, 44.8, 38.0, 37.8, 35.8, 29.0, 26.5, 25.4, 17.8 and 14.0.

2-*O*-Deacetyltaxinine 4c. Mp 160–161 °C (from CH_2Cl_2–*n*-hexane); $[\alpha]_D^{28}$ +156.6 (*c* 1.00); R_f 0.3 (CH_2Cl_2–AcOEt 10:1) [Found: (EI) 564.2735 (M^+, 0.3%). $C_{33}H_{40}O_8$ requires *M*, 564.2732]; *m*/*z* 504 (0.3), 462 (0.3), 444 (2), 426 (5), 314 (10), 296 (8), 131 (91) and 43 (100); ν_{max}(KBr)/cm^{-1} 3527, 1746, 1714, 1671 and 1239; λ_{max}(EtOH)/nm 278 (ε/dm^3 mol^{-1} cm^{-1} 30 700) and 217 (18 000); δ_H($CDCl_3$) 0.97 (3 H, br s, 8-Me), 1.18 (3 H, br s, 15-Me$^\beta$), 1.71 (3 H, br s, 15-Me$^\alpha$), 1.73 and 2.00 (each 2 H, each m, 6- and 7-H_2), 2.05 (3 H, s, OAc), 2.08 (3 H, s, OAc), 2.23 (3 H, br s, 12-Me), 2.25 (1 H, d, *J* 20.0, 14-H$^\alpha$), 2.34 (1 H, br d, *J* 6.8, 1-H), 2.81 (1 H, dd, *J* 6.8 and 20.0, 14-H$^\beta$), 3.26 (1 H, d, *J* 6.4, 3-H), 4.32 (1 H, br t, *J* 6.4, 2-H), 5.34 (1 H, s, 20-H), 5.43 (1 H, br s, 5-H), 5.45 (1 H, s, 20-H), 5.80 (1 H, d, *J* 10, 9-H), 6.02 (1 H, d, *J* 10, 10-H), 6.38 (1 H, d, *J* 16, 22-H), 7.42 (3 H, m, Ph), 7.63 (1 H, d, *J* 16, 23-H) and 7.74 (2 H, d, *J* 7.3, Ph); δ_C($CDCl_3$) 199.7, 170.2, 169.7, 166.3, 150.7, 145.9, 143.6, 137.6, 134.5, 130.4, 129.0 (2), 128.5 (2), 118.1, 117.8, 78.1, 76.0, 73.5, 68.2, 51.5, 45.1, 44.7, 37.8, 37.6, 35.8, 28.7, 27.7, 25.5, 20.9, 20.7, 17.6 and 13.9.

Hydrolysis of taxinine 1a with $Ba(OH)_2$·8H_2O and Na_2CO_3·H_2O

To a stirred solution of taxinine **1a** (20 mg, 0.033 mmol) in a 1:1 mixture of dry CH_2Cl_2–methanol (2 ml) was added barium hydroxide octahydrate (Koso Chemical Co., Japan, 97% purity) (10.4 mg, 0.033 mmol) and the suspension was kept in an ice-box (at 4 °C) overnight. After confirmation of complete consumption of taxinine **1a** by TLC analysis as described above, the reaction mixture was diluted with AcOEt (30 ml) and then neutralized with dil. hydrochloric acid. The organic layer was washed with brine (10 ml) and dried. TLC analysis of the solution with developing solvent CH_2Cl_2–AcOEt (10:1) showed the formation of compound **4a** (64%) and **4b** (33%). After removal of the solvent under reduced pressure, the resulting residual oil was subjected to silica gel column chromatography and elution

with *n*-hexane–AcOEt (3:1), to separate products **4a** and **4b**. These products were identical with the authentic compounds (described above) in their ^{1}H NMR spectra.

When sodium carbonate monohydrate (4.1 mg, 0.033 mmol) was employed in place of barium hydroxide octahydrate in this reaction, no formation of compound **4b** was seen, and the formation of compound **4a** (18%) accompanied by the recovery of a large amount of the starting material **1a** was revealed by TLC analysis of the reaction mixture after it had been stirred for 5 h.

Reduction of taxinine 1a with LAH

To a clear, stirred solution of taxinine **1a** (10 mg, 0.0165 mmol) in dry THF (1 ml) was added LAH (Aldrich, 95% purity) (0.9 mg, 0.0225 mmol) at 0 °C and the mixture was stored in a freezer (at −18 °C) for 12 h and then stirred and warmed to 60 °C and kept at that temperature for 12 h. The resulting mixture was diluted with AcOEt (10 ml), washed with brine (5 ml), dried and evaporated to leave an oil, which was subjected to silica gel column chromatography and elution with *n*-hexane–AcOEt (3:1), to separate 5-*O*-decinnamoyltaxinine **5a** (1.9 mg, 24%) and the recovered substrate **1a** (6.5 mg, 65% recovery).

Compound **5a** had mp 254–255 °C (from AcOEt–Et_2O) (lit.,[16a] 254–255 °C) (lit.,[30] 252–254 °C); $[\alpha]_D^{28}$ +84.4 (*c* 1.00) [lit.,[16a] +106 ($CHCl_3$)] [lit.,[30] +43 (*c* 0.706, $CHCl_3$)]; R_f 0.1 (10:1 CH_2Cl_2–AcOEt) (Found: C, 65.40; H, 7.60. Calc. for $C_{26}H_{36}O_8$: C, 65.5; H, 7.6%) [Found: (EI) 476.2391 (M^+, 0.1%). Calc. for $C_{26}H_{36}O_8$: *M*, 476.2410]; *m*/*z* 458 (0.1), 83 (100) and 43 (88); λ_{max}(EtOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 6400). The IR and ^{1}H NMR spectra of compound **5a** were almost identical with those of taxinine A reported by Bathini *et al.*[30]

Reduction of taxinine 1a with sodium bis-(2-methoxyethoxy)-aluminium hydride (Red-Al®)

To a clear solution of taxinine **1a** (606 mg, 1.0 mmol) in dry THF (30 ml) was added Red-Al® (Aldrich, 65 wt% in toluene) (0.6 ml, 2.0 mmol) dropwise at 0 °C and the mixture was stirred at this temperature for 10 min and then at room temperature for 35 min. After confirmation of complete consumption of taxinine **1a** by TLC analysis, the reaction was quenched by the addition of saturated aq. dipotassium tartrate (20 ml). The resulting mixture was diluted with AcOEt (50 ml), washed with brine (20 ml), dried and evaporated to dryness to leave an oil, which was subjected to silica gel column chromatography, and elution with *n*-hexane–AcOEt (3:1), to isolate compound **5a** (238 mg, 50%), 2,5-*O*-deacyltaxinine **5b** (95 mg, 22%) and cinnamyl alcohol (69 mg, 43%). TLC analysis showed the presence of highly polar, complex mixtures, but no presence of compounds **5a** and **5b** in the AcOEt extract.

Compound **5b** had mp 285–286 °C (from AcOEt–Et_2O) [lit.,[31b] 295–298 °C (from $CHCl_3$–MeOH)]; $[\alpha]_D^{28}$ +123.6 (*c* 1.00) [lit.,[31a] +97 (*c* 0.21, $CHCl_3$) at 23 °C] [lit.,[31b] +147.8 (*c* 0.057, $CHCl_3$) at 28 °C] (Found: C, 66.09; H, 7.95. Calc. for $C_{24}H_{34}O_7$: C, 66.34; H, 7.89%); *m*/*z* (EI) 434 (M^+, 6%), 416 (3), 374 (8), 332 (15) and 43 (100). The IR, UV and ^{1}H NMR spectra of compound **5b** were almost identical with those of taxuspine G reported by Kobayashi *et al.*[31a] and Tong *et al.*[31b] TLC densitometric analyses of the reaction mixtures after treatment of compound **1a** (10 mg, 0.0165 mmol) with 2.5 or 5.0 mol equiv. of Red-Al® under analogous conditions (room temp.; 0.5 h) showed the formation of compounds **5a** and **5b** in 37 and 41% yield for the former case in 16 and 32% yield for the latter case, respectively.

Reduction of taxinine 1a with DIBAL-H

To a stirred solution of compound **1a** (606 mg, 1.0 mmol) in dry THF (10 ml) was added dropwise DIBAL-H (Aldrich, 1.0 M solution in toluene) (2.0 ml, 2.0 mmol) at 0 °C. After stirring of the mixture at room temp. for 0.5 h, the reaction was quenched by the addition of saturated aq. dipotassium tartrate (2 ml). The resulting mixture was diluted with AcOEt (20 ml), washed with brine (5 ml), dried and evaporated to leave an oil, which was subjected to silica gel column chromatography, and elution with $CHCl_3$–AcOEt (75:1–10:1), to separate compound **4c** (186 mg, 33%), the unchanged substrate **1** (170 mg, 28%) and compound **5a** (52 mg, 10%).

Acetylation of the O-deacylated taxinines 4a–c and 5a,b

To a stirred solution of the appropriate O-deacylated taxinine (0.02 mmol) in dry CH_2Cl_2 (1 ml) were added acetic anhydride (0.094 ml, 0.1 mmol) and DMAP (22 mg, 0.06 mmol) at 0 °C. The mixture was stirred at this temperature for 2 h and then at room temp. for 24 h. After confirmation of complete consumption of the starting material by TLC analysis with developing solvent of CH_2Cl_2–AcOEt (10:1), the reaction mixture was concentrated under reduced pressure and was then diluted with CH_2Cl_2 (20 ml). The solution was washed with brine (10 ml), dried and evaporated to dryness. The resulting residue was purified by column chromatography with *n*-hexane–AcOEt (3:1) as eluent. In this procedure, all of the O-deacylated taxinines **4a**–**c** were converted almost quantitatively to the same compound, which was identical in every respect with the taxinine **1a** employed as starting material for the selective O-deacylation described above, whereas the 5-*O*-decinnamoylated taxinines **5a**,**b** were converted to 5-*O*-acetyl-5-*O*-decinnamoyltaxinine (taxinine H, **5c**) in almost quantitative yield.

Compound **5c**; amorphous powder; $[\alpha]_D^{28}$ +60.9 (*c* 1.06) (lit.,[16a] +96 ($CHCl_3$)) [Found: *m*/*z* (EI), 518.2528 (M^+, 6%). $C_{28}H_{38}O_9$ requires *M*, 518.2516]; *m*/*z* 476 (5), 458 (8), 416 (13), 398 (18), 356 (23), 338 (21), 296 (45) and 43 (100); ν_{max}(KBr)/cm^{-1} 1740, 1675, 1373 and 1234; λ_{max}(EtOH)/cm^{-1} 268 (ε/dm^3 mol^{-1} cm^{-1} 11 200); δ_H($CDCl_3$) 0.91 (3 H, br s, 8-Me), 1.14 (3 H, br s, 15-Me$^{\beta}$), 1.70 and 1.83 (each 2 H, each m, 6- and 7-H), 1.76 (3 H, br s, 15-Me$^{\alpha}$), 1.99 (3 H, s, OAc), 2.05 (3 H, s, OAc), 2.06 (3 H, s, OAc), 2.08 (3 H, s, OAc), 2.21 (1 H, dd, *J* 2.0 and 6.8, 1-H), 2.25 (3 H, br s, 12-Me), 2.31 (1 H, d, *J* 20.0, 14-H$^{\alpha}$), 2.76 (1 H, dd, *J* 6.8 and 20.0, 14-H$^{\beta}$), 3.24 (1 H, d, *J* 6.4, 3-H), 4.86 (1 H, s, 20-H), 5.25 (1 H, br s, 5-H), 5.34 (1 H, s, 20-H), 5.53 (1 H, dd, *J* 2.0 and 6.4, 2-H), 5.89 (1 H, d, *J* 10.3, 9-H) and 6.02 (1 H, d, *J* 10.3, 10-H); δ_C($CDCl_3$) 198.9, 169.9, 169.7, 169.6, 169.2, 150.1, 141.6, 137.7, 117.4, 77.8, 75.6, 73.1, 69.5, 48.3, 44.3, 42.8, 37.5, 37.2, 35.8, 28.3, 27.3, 25.0, 21.2, 21.1, 20.7, 20.5, 17.3 and 13.7.

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