A New Taxane from the Hard Wood of *Taxus cuspidata*

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A new taxoid metabolite with an unusual double bond between C-13 and C-14 was isolated from the methanol extract of the hard wood of *Taxus cuspidata*. The structure was established as 2α,5α,7β,9α,10β,13β-hexaacetoxy-11β-hydroxyl-19β-benzoxytaxa-4(20),13-dien-12,16-epoxide (**1**), named 5,13-diacetyltaxinine M-13-enol, on the basis of spectral analysis including 1HNMR, 13CNMR, HMQC, HMBC, NOESY and confirmed by HR-FAB-MS.

**Key words:** *Taxus cuspidata*, Yew, Taxaceae, Hard Wood, Taxanes

**Introduction**

*Taxus* species have attracted a great deal of attention since paclitaxel (*Taxol®*), an anticancer drug used to treat ovarian, breast and lung cancers [1], was first isolated from the bark of *Taxus brevifolia* [2]. Since then, a large number of taxane diterpenoids have been isolated from various *Taxus* species [3 – 6]. *Taxus cuspidata*, an evergreen tree, is one of the most extensively studied yews, and more than 130 new taxanes have been reported [7 – 9]. As a continuation of our phytochemical study on *T. cuspidata* [10 – 14], we investigated the hard wood of *T. cuspidata* and isolated a new taxinine M-type taxane with a rare double bond between C-13 and C-14. We report herein the structure elucidation of this new compound (Fig. 1).

**Results and Discussion**

Compound **I** was isolated as a colorless amorphous powder from the methanol extract of *T. cuspidata* hard wood. The molecular composition of **I**, C_{39}H_{46}O_{16}, was derived by analysis of positive high-resolution FAB-MS at *m/z* = 809.2419 [M+K]^{+} and was further substantiated by the 13C NMR spectrum. The 1H and 13C NMR spectral data of **I** are summarized in Table 1. The 1H NMR spectrum disclosed well-dispersed characteristic signals of taxanoids [15, 16] including three-proton signals due to the two tertiary methyl groups at δ_{H} = 1.29, and 1.13. Six acetyl groups were observed between δ_{H} = 2.02 and 2.21, and were further confirmed by corresponding 13C NMR signals at δ_{C} = 168.0, 168.1, 169.8, 169.9, 170.2, and 172.5. A benzoyl group was also observed in both 1H and 13C NMR spectra: δ_{H} = 8.15 (2H, d, *J* = 7.6 Hz), 7.49 (2H, t, *J* = 7.6 Hz), 7.59 (1H, t, *J* = 7.6 Hz), and δ_{C} = 166.7 for the carbonyl of the benzoyl group. A pair of singlets at δ_{H} = 5.51 (1H, s) and 4.77 (1H, s) in the 1H NMR spectrum were the characteristic signals of taxane with an exo-double bond at C-4 [15, 16]. The 13C NMR spectrum of **I** revealed signals due to eight primary, four secondary, thirteen tertiary, and fourteen quaternary carbons. Of them, 17 sp^{2}-hybridized and 9 sp^{3}-hybridized carbon atoms are connected to oxygens judging from their chemical shifts. These carbons carried 45 hydrogens, indicating that the last hydrogen from the molecular formula was accommodated in a hydroxyl group. Indeed, a broad singlet signal was observed at δ_{H} = 4.00, which exhibited long range correlations with C-10, C-11, C-12, C-13, and C-15 in the HMBC experiment. This observation suggested that this hydroxyl group was attached to C-11. To clarify the intermolecular connectivities, the HMBC ex-
experiment and $^1$H-$^1$H-COSY spectra were performed (Fig. 2). Full assignments of the proton and carbon signals were secured by $^1$H-$^1$H-COSY, HSQC, and HMBC spectra. Detailed analysis of the HMBC correlations of H-18 to C-11, C-12, and C-13 confirmed that Me-18 was attached at C-12. In the HMBC spectrum, the cross-peaks of H-16 and H-17 to C-1, C-11, and C-15 indicated that C-16 and C-17 were connected at C-15. $^1$H-$^1$C long-range correlations between H-1 and C-1, C-13, C-14, and C-15, H-16, H-17 and C-1 and C-11, and between H-14 and C-1, C-12, C-13, and C-15 indicated the presence of a cyclohexane moiety (ring A). The cross-peaks of H-2 to C-3, C-8, H-3 to C-1, C-2, and C-8, H-10 to C-11, and C-15, H-1 to C-2, C-3, C-11, and C-15, H-9 to C-8 and C-11 in the HMBC spectrum suggested the presence of an eight-membered ring (ring B). Long-range coupling of H-19 to C-1, C-2, C-8, and C-9 implied that Me-19 was located at C-8. $^1$H-$^1$C long-range correlations between H-3 to C-4, C-5, and C-8, H-7 to C-6, C-8 were indicative of the presence of a cyclohexane moiety (ring C). Both H-16a and H-16b showed three-bond correlations with C-12 in the HMBC map indicating that a new ring was formed through an oxygen. This conclusion was further supported by the chemical shifts of C-12 ($\delta_C = 86.9$) and C-16 ($\delta_C = 82.0$) as well as the chemical shifts and coupling constants of H-16a and H-16b ($\delta_H = 3.76$, 1H, d, $J = 8.1$ Hz, H-16a; $\delta_H = 3.49$, 1H, d, $J = 8.1$ Hz, H-16b) [17–19]. From the $^1$H-$^1$H-COSY spectrum, it was possible to differentiate four discrete spin systems. A diagnostic signal at $\delta_H = 3.47$ typical for H-3$\alpha$ [15, 16] showed correlations with H-2 and H-20b in the $^1$H-$^1$H-COSY NMR map and exhibited HMBC correlations with C-1, C-2, C-4, C-5, C-7, C-8, C-9, C-19, and C-20. Using H-3 as a starting point, the spin system from H-3 to H-14 through H-2 and H-1 and the spin system from H-5 to H-7 through H-6a and H-6b were established. H-3 and H-5 also showed long-range correlations with H-20b and H-20a, respectively. Other three pairs of doublets were attributed to H-9 (1H, d, $J = 3.0$ Hz) and H-10 (1H, d, $J = 3.0$ Hz), and to the two geminal oxygenated methylenes (H-16 and H-19). These signals are the typical features of taxinine M-type taxane [17–19]. H-16 and H-19 resonated as a pair of doublets with relatively large coupling constants. The C-19-oxygenated methylene had a larger coupling constant than the C-16-oxygenated methylene because the latter is accommodated in a ring. H-2 resonated downfield as a broad doublet with a large coupling constant between H-3 and H-2 ($J = 10.3$ Hz).

According to the chemical shifts and HMBC correlations, five acetoxy groups were attached at C-2, C-5, C-7, C-9, and C-10, and the benzoyl group was positioned at C-13, as in all the other taxinine M-type taxanes. It is the first example of such taxane isolated from $Taxus$ sp. [3–5], although more than 15 taxinine M-type taxoids have been reported from $Taxus$ plants since the first one was reported in 1981 [19].

Compound I did not exhibit potential in vitro cytotoxicity screening against the human breast cancer MCF-7 and ovary cancer HAC-2 cell lines.

**Experimental Section**

**General**

Optical rotation values were recorded on a Jasco DIP-370 digital polarimeter. All NMR data were obtained at r.t.
Table 1. The $^1$H and $^{13}$C NMR data of 1 in CDCl₃ (300 MHz for $^1$H NMR and 75 MHz for $^{13}$C NMR).

<table>
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<tr>
<th>Position</th>
<th>$\delta^H$ (mult)</th>
<th>$J$ (Hz)</th>
<th>$^1$H-$^1$H-COSY</th>
<th>$\delta^C$</th>
<th>HMBC</th>
<th>NOESY</th>
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<td>3.9 (br.d)</td>
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<td>o</td>
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<td>166.7, o, i, m, p</td>
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<td></td>
<td>10, 11, 12, 13, 15</td>
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</table>

* These signals were exchangeable.
Plant material

The hard wood of *Taxus cuspidata* was collected in the fall of 2000 in the Toyama Prefecture in the north-west of Japan. The botanical identification was made by Professor T. Oritani of Toyama Prefectural University, Toyama, Japan. Several voucher specimens have been deposited in the Laboratory of Applied Bioorganic Chemistry, Graduate School of Agricultural Sciences, Tohoku University, Japan (no. NMC-2000-10-1).

Extraction and isolation

The air-dried hard wood (4500 g) of *T. cuspidata* was chipped and submerged in MeOH for one week at r. t. The methanolic extract was decanted and concentrated in vacuo; a dark-brown tar was obtained. The residue was diluted with brine and then extracted with CHCl₃. The CHCl₃-soluble portion was evaporated under reduced pressure to give a residue (40 g), which was loaded on to a silica gel column and eluted with increasing polarity of a mixed solvent (hexane-acetone). Fractions were pooled on the basis of their TLC to give 16 main fractions (Fr. 1 – 16). Fraction 5 was subjected to further chromatographic separations using stepwise elution (hexane-ethyl acetate) to give 6 sub-fractions (Fr. 5–1 to Fr. 5–6) according to TLC. Sub-fraction Fr. 5–3 was subjected to reverse-phase preparative HPLC, the material eluted at 44.17 min was collected, dried and further purified by preparative TLC using hexane-ethyl acetate as mobile phase. The preparative TLC plate was cut into small strips under UV. The compounds were carefully removed by scraping off the silica gel and then were exhaustively extracted with acetone. After solvent evaporation, the residue afforded compound I (4.5 mg).

2α,5α,7β,9α,10β,13β-Hexaacetoxy-11β-hydroxyl-19β-benzoytaxa-4(20),13-dien-12,16-epoxide (1)

Amorphous gum; [α]D⁰₂⁰ = −14° (c = 0.20, CHCl₃); ¹H and ¹³C NMR, HMBC, and NOESY spectral data, see Table 1. HRMS ((+)-FAB): m/z = 809.2419 (calcd. 809.2423 for C₃₉H₄₆O₁₆K, [M+K]⁺).

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