2-Deacetoxytaxinine B: A New Taxane from *Taxus wallichiana*

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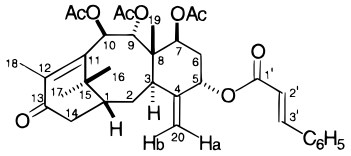
A new taxane derivative, 2-deacetoxytaxinine B (**1**), has been isolated from *Taxus wallichiana*, along with β-sitosterol, sciadopitysin, and methyl β-orcinolcarboxylate.

Taxus wallichiana (Zucc.) Pilger (syn. *T. baccata* L.) (Taxaceae) is widely distributed in Nepal at altitudes of 1800−4000 m. It is commonly known as the Himalayan yew and is locally recognized by various names such as "Burme Salla", "Dungre Salla", "Thuner", or "Kanhaswan", depending on location.[1] It is widely exploited as a traditional medicine by different ethnic groups of the Himalayan region. The leaves of *T. wallichiana* are reported to have emmenagogue, sedative, antispasmodic, and aphrodisiac properties. They are also used as a treatment for asthma, bronchitis, hiccough, indigestion, and epilepsy.[1] A water extract of leaves is reported to have good tranquillizing effects.[2] The isolation of the anticancer compound, paclitaxel (Taxol), and other taxane derivatives from *T. brevifolia* and other *Taxus* species, including *T. wallichiana*,[3−5] prompted the present investigation.

Repeated chromatography on Si gel of the hexane-soluble fraction of a MeOH extract of *T. wallichiana* leaves gave β-sitosterol and 2-deacetoxytaxinine B (**1**). Chromatography of the $CHCl_3$-soluble fraction of the extract yielded sciadopitysin and methyl β-orcinolcarboxylate.



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2-Deacetoxytaxinine B (**1**), mp 240 °C, had a molecular ion peak at *m/z* 606 in its EIMS (HRMS, $C_{35}H_{42}O_9$); the base peak was at *m/z* 131 due to the cinnamoyl ion (C_9H_7O), and a peak at *m/z* 458 arose from loss of cinnamic acid ($C_9H_8O_2$) from the M^+. The IR spectrum had bands at 3050 (aromatic), 1746 (ester), and 1625 (olefinic) cm^{-1}. The UV spectrum had λ_{max} at both 233 and 288 nm due to the presence of enone and cinnamate ester groups. The ^{1}H-NMR spectrum had well-dispersed signals suggestive of a taxane derivative containing three acetate groups and one cinnamate group.[3] Two sharp doublets (δ 5.92, 6.31, J = 10.8 Hz) could be assigned to H-9β and H-10α, respectively, and were deshielded by neighboring acetoxy groups. A third acetoxy group was located at C-7 (δ_{H-7} 5.53). The fact that Me-18 was a sharp singlet suggested that C-13 did not have a (β) hydrogen attached, but instead bore a ketone function. In accord with this, H_2-14 displayed a large J_{gem} = 19.6 Hz. By means of decoupling and COSY experiments the complete connection network was established for H_2-14−H-1−H_2-2−H-3−H_2-20−H-5−H_2-6−H-7. H-3 had allylic coupling with H-20a and H-20b. NOE enhancement of H-5 by H-20a and the lack of allylic coupling confirmed the β-equatorial disposition of H-5. The cinnamate group was located at C-5α, in accordance with what is observed in other taxinine derivatives. The detection of NOE enhancements from Me-18 to certain protons of the cinnamate residue (H-2′ and *o*-ArH) (in addition to H-3, H-7, and H-10) supports this assignment.[6] In $CDCl_3$, the signal from H-6α was obscured but became clear as a ddd signal on addition of C_6D_6. NOE difference spectroscopy revealed the H-6α and H-2β resonances and confirmed the location of other substituents, the relative configurations, and the conformation of the molecule.[6] The ^{13}C NMR spectrum was fully assigned by means of DEPT, HMQC, and HMBC spectra. The ^{1}H-^{13}C correlations observed in the HMBC spectrum were in accord with structure **1**.

Compound **1** is a new taxane derivative. It differs from taxinine B, isolated from *T. cuspidata*[6,7] and *T. mairei*,[8] through the lack of a 2-acetoxy group. Comptonine, isolated from *Austrotaxus spicata*, has the same oxygenation pattern as **1** with a 3-(dimethylamino)-2-hydroxy-3-phenylpropanoate ester instead of a cinnamate.[9]

Chromatography of the $CHCl_3$ extract gave sciadopitysin, previously reported from *T. wallichiana*[10,11] and *T. cuspidata*,[12] and methyl β-orcinolcarboxylate (atraric acid).[13] The latter substance is a rare plant product. It is possible that in the present instance it has come from adventitious lichen contaminants.

Experimental Section

General Experimental Procedures. Melting points were determined with an electrical melting point apparatus (Sunvic, Glasgow, UK), and are uncorrected. Optical rotations were determined on a Perkin-Elmer 243 polarimeter. UV spectra were recorded on a Shimadzu UV 160A spectrophotometer, and IR spectra, on a Nicolet 50X FTIR spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX-400 spectrometer at 400 MHz and 100 MHz, respectively, with TMS as internal reference, and HMQC and gradient-selected HMBC experiments were recorded on a Bruker AMX-600 spectrometer. EIMS mass spectra (low and high

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resolution) were recorded on an AEI MS902 spectrometer equipped with a DS30 data system. Qualigen (TA 27325) Si gel of grade 250−400 mesh was used for vacuum liquid chromatography (VLC) and flash chromatography, whereas E. Merck Si gel (60−120 mesh) was used for column chromatography. Precoated TLC plates (E. Merck, pr. no. 158807) were used.

Plant Material. The plant material was obtained in September 1995 from Herb Processing and Production Co. Ltd., Kathmandu, Nepal. A voucher sample has been deposited at the National Herbarium and Plant Laboratory, Godawari, Nepal (Voucher no. 90-725).

Extraction and Isolation. Air-dried, powdered needles and twigs (900 g) were extracted with MeOH (3 × 5 L) by percolation at room temperature. The combined extract was concentrated under reduced pressure to yield a viscous greenish black residue (326 g). The residue was suspended in H_2O (200 mL) and extracted (3 × 300 mL) with hexane, EtOAc, and $CHCl_3$ successively to afford hexane (22 g), EtOAc (4.2 g), and $CHCl_3$ (8.3 g) fractions. The hexane fraction (20 g) was further subjected to VLC, using gradients of hexane, EtOAc, and then MeOH. Material (3.51 g) in fractions 5−10, eluted with hexane−EtOAc mixtures, was rechromatographed by column chromatography with hexane−EtOAc gradients, collecting 20-mL fractions at the rate of 1 mL/min. Fractions 52−62, eluted with hexane−EtOAc (9:1), gave β-sitosterol (54 mg). Fractions 155−165, eluted with hexane−EtOAc (85:15), gave material that, on recrystallization (EtOAc−hexane), yielded **1** (15 mg, 1.7×10^{-3}%). The $CHCl_3$ fraction (8 g) was further fractionated by VLC. Fractions 4−5, eluted with hexane−EtOAc (95:5), were combined to give sciadopitysin[10−12] (30 mg). Fractions 10−12, eluted with hexane−EtOAc (1:1 to 1:3), were combined and the material rechromatographed by column chromatography to give methyl β-orcinolcarboxylate[13] (12 mg).

The known compounds, β-sitosterol, sciadopytsin, and methyl β-orcinolcarboxylate, were identified by comparison of their corresponding properties (mp, IR, MS, and NMR) with literature values.

2-Deacetoxytaxinine B (1): colorless prisms (EtOAc−hexane); mp 240 °C dec; $[\alpha]^{22}_D$ +71.7° (*c* 0.03, $CHCl_3$); TLC R_f 0.30 (EtOAc−hexane, 1:1); UV λ_{max} 282, 233 nm; IR (KBr) 3050 (CH), 1746 (C=O), 1625 (C=C), 1400, 1250 (OC) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.73 (2H, dd, J = 8.0, 1.4 Hz, *o*-ArH), 7.63 (1H, d, J = 16.0 Hz, H-3′), 7.41 (3H, m, ArH), 6.38 (1H, d, J = 16.0 Hz, H-2′), 6.31 (1H, d, J = 10.8 Hz, H-10α), 5.92 (1H, d, J = 10.8 Hz, H-9β), 5.53 (1H, dd, J = 5.2, 11.2 Hz, H-7α), 5.45 (1H, dd, J = 2.4, 3.6 Hz, H-5β), 5.31 (1H, d, J = 1.2 Hz, H-20a), 4.95 (1H, d, J = 1.7 Hz, H-20b), 3.06 (1H, dddd, J = 5.8, 1.8, 1.7, 1.2 Hz, H-3α), 2.94 (1H, dd, J = 19.6, 7.2 Hz, H-14β), 2.39 (3H, s, Me-18), 2.21 (1H, ddd, J = 7.2, 5.5, 1.8 Hz, H-1), 2.14, 2.13, 2.04 (3 × 3H, s, OAc-7, -9, -10), 2.06 (obscured, 1H, ddd, J = 14.6, 5.2, 2.4 Hz, H-6α), 2.03 (obscured, 1H, ddd, J = 16, 5.8, 1.8 Hz, H-2β), 1.98 (d, 1H, J = 19.6 Hz, C-14α), 1.90 (1H, ddd, J = 16.0, 5.5, 1.8 Hz, H-2α), 1.79 (1H, ddd, J = 14.6, 11.2, 3.6 Hz, H-6β), 1.63 (3H, s, Me-16), 1.13 (3H, s, Me-17), 0.88 (3H, s, Me-19); ^1H NMR (C_6D_6/$CDCl_3$, 1:1) selected peaks, 3.05 (1H, dddd, J = 5.8, 1.8, 1.7, 1.2 Hz, H-3α), 2.81(1H, dd, J = 19.6, 7.2 Hz, H-14β), 2.51 (3H, s, Me-18), 2.03 (1H, ddd, J = 14.6, 11.2, 3.6 Hz, H-6α), 1.89, 1.86, 1.81 (3 × 3H, s, OAc-7, -9, -10), 1.67 (2H, m, H-2β, H-6β), 1.56 (3H, s, Me-16), 1.03 (3H, s, Me-17), 0.84 (3H, s, Me-19); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 200.1 (s, C-1, 170.2 (s, O*C*OCH$_3$-9), 169.8 (s, O*C*OCH$_3$-7), 169.2 (s, O*C*OCH$_3$-10), 166.2 (s, C-1′), 152.3 (s, C-11), 147.0 (s, C-4), 146.0 (d, C-3′), 138.3 (s, C-12), 134.5 (s, C-1″), 130.4 (d, C-4″), 128.9 (d, C-3″, C-5″), 128.5 (d, C-2″, C-6″), 117.6 (d, C-2′), 114.6 (t, C-20), 75.8 (d, C-9), 74.4 (d, C-5), 73.0 (d, C-10), 69.9 (d, C-7), 46.6 (s, C-8), 40.9 (d, C-1), 39.6 (s, C-15), 37.1 (q, C-17), 36.9 (d, C-3), 34.0 (t, C-6), 25.8 (t, C-2), 25.6 (q, C-16), 21.4 (q, OCO*C*H$_3$), 20.9 (q, OCO*C*H$_3$), 20.8 (q, OCO*C*H$_3$), 14.1 (q, C-18), 12.9 (q, C-19); EIMS m/z 606 $[M]^+$ (0.5), 580 (1.7), 564 (0.5), 458 (2), 415 (1.5), 398, 356 (1.5), 338 (1.3), 314 (1.1), 131 (100), 43 (70); HREIMS m/z $[M]^+$ 606.2805 (calcd for $C_{35}H_{42}O_9$, 606.2828).

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