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A NEW TAXANE FROM *Taxus canadensis* NEEDLES

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A new 6/8/6-membered taxane was isolated from the needles of Taxus canadensis. *The structure was characterized as 10β,13α-diacetoxy-5α,9α-dihydroxytaxa-4(20),11-diene (**1**) on the basis of 1D and 2D spectrascopic data.*

Keywords: *Taxus canadensis,* Taxaceae, needles, taxane, diterpene, isolation.

Paclitaxel (Taxol®) [1] by virtue of its complex and densely functionalized, challenging and seductive structure, coupled with its novel mechanism of action, potent antitumor activity, and its clinical use as a powerful anticancer drug used for the treatment of ovarian and breast cancers as well as Kaposi′s sarcoma and non-small-cell lung cancers, has attracted the attention of many chemists and biological scientists. Indeed, taxol and its analogues continue to stimulate a great deal of interest in the isolation of further taxoids from plants in the genus *Taxus*. As a result, more than 550 taxane diterpenoids have been isolated and identified [2–4]. The isolation of new and minor taxanes is important in the context of the plant biosynthesis and metabolism of these compounds [5, 6].

The Canadian yew, *Taxus canadensis*, a low trailing bush very common in the Quebec region, is an interesting plant with unusual taxanes specific to this species [7, 8]. Although more than 70 new taxanes were isolated from the needles of the Canadian yew [7–9], the study of this yew's secondary metabolites continues to yield unique taxane derivatives [10, 11]. We have now isolated a new taxoid **1** from the needles of *T. canadensis*. In this communication, we report herein the structure elucidation of this taxane.

The molecular composition of **1** was established as $C_{24}H_{36}O_6$ from combined analysis of high-resolution FAB-MS, which provided a *quasi*-molecular ion at *m/z* 459.2143 $[M + K]^+$. This was in agreement with the ^{13}C NMR spectral data, in which 24 carbon resonances were observed. The ^1H NMR spectrum of **1** exhibited four three-proton signals due to the four tertiary methyl groups at δ 0.98, 1.44, 0.88, and 2.08, and two acetyl groups at 2.14 and 2.06, which was verified by the observation of ^{13}C NMR signals at δ 21.3, 170.4 and 21.0, 170.1. The ^1H NMR signals at δ 4.71 (1H, s) and 5.10 (1H, s) together with the signals at δ 146.5 and 110.7 in the ^{13}C NMR spectrum and the signal at δ_H 3.24 (1H, br.s) in the ^1H NMR spectrum are the characteristic of an *exo*-cyclic methylene and C-3 ring junction proton in a taxane with a 4(20)-double bond, respectively [12]. The connectivities of the protons on the taxane skeleton of **1** were determined by analysis of the ^1H–^1H COSY spectrum. Interpretation of HMBC permitted the assignment of the acetyl group. Using H-3 as a starting point, the connectivities from C-3 to C-2 to C-1 to C-14 were deduced from the ^1H–^1H COSY spectrum. The signal at δ 4.28 (1H, br.s) was assigned to H-5. Similarly, using H-5 as reference, the spin system derived from C-5 to C-6 to C-7 was readily interpreted from the analysis of the ^1H–^1H COSY spectrum. An isolated AB system signal resonating at δ_H 4.14 (1H, d, J = 9.8 Hz) and 5.89 (1H, d, J = 9.8 Hz) was attributed to H-9 and H-10, respectively. The chemical shift of H-10 indicated that an acetoxyl group was

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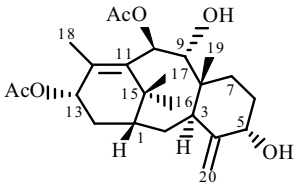
located at C-10, which was further confirmed by the HMBC experiment. H-10 exhibited a long-range correlation with the carbonyl carbon at δ_C 170.4. Another acetoxyl group was positioned at C-13, and another free hydroxyl group was located at C-5, judging from their chemical shifts and the splitting pattern of the corresponding protons. With all these spectral data taken into account, the structure of **1** was elucidated unequivocally as 10β,13α-diacetoxy-5α,9α-dihydroxytaxa-4(20),11-diene.



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EXPERIMENTAL

General Comments. Optical rotation was recorded on a JASCO DIP-370 digital polarimeter. Flash chromatography was performed on Silica gel 60 (230–400 mesh, EM Science). Thin-layer chromatography was conducted on Silica gel 60 F_{254} pre-coated TLC plates (0.25 mm or 0.5 mm, EM Science). The compounds were visualized on TLC plates with 10% sulfuric acid in ethanol and heating on a hot plate. Analytical HPLC was performed on a Waters 600 FHU delivery system coupled to a PDA 996 detector. Preparative chromatography was carried out on a Waters Delta Prep 3000 instrument coupled to a UV 486 tunable absorbance detector set at 227 nm and 210 nm (Waters, Montreal, Quebec, Canada) and a Partisil 10 ODS-2 MAG-20 preparative column (22 × 500 mm). The products were eluted with a 50 min linear gradient of acetonitrile (25 to 100 %) in water at a flow rate of 18 mL/min (preparative HPLC) and 3 mL/min (semi-preparative HPLC). Data were registered with Millenium^{TM} software (Waters).

All the NMR data were obtained at room temperature on a Bruker Avance-500 spectrometer at working frequencies 500.13 MHz for protons and at 125.77 MHz for carbon-13. The solvent was used as an internal reference for protons as well as for carbon-13; in the case of $CDCl_3$, the reference was set to 7.25 and 77.0 ppm. The various 2D spectra were acquired and processed using standard procedures. Positive ion MALDI mass spectrometry experiments performed on a Q-Tof Ultima Fast Atom Bombardment Mass Spectra (FAB-MS) were obtained with a Vacuum Generators ZAB-HS double-focusing instrument using a xenon beam having 8 kV energy at 1 mA equivalent neutral current. Low resolution mass spectra were obtained in glycerol. Samples were dissolved in 0.2 µL DMSO before addition of 0.5 µL glycerol.

Plant Materials. The needles of *Taxus canadensis* Marsh were collected in May of 2002 at St-Jean, Quebec, Canada. Several specimens have been deposited in our laboratory under accession voucher number 2002-5-1.

Extraction and Isolation. Air-dried needles of *Taxus canadensis* were ground (2.0 kg) and extracted with 8 L methanol four times at room temperature. The combined organic extracts were evaporated under reduced pressure. Water was added and lipids were removed by stirring the mixture with hexane. The aqueous phase was then salted and extracted with CH_2Cl_2. The combined CH_2Cl_2 extract was dried with anhydrous sodium sulfate, filtered and evaporated, yielding 45 g of a dark green extract. The CH_2Cl_2 extract was absorbed onto 50 g silica gel and subjected to column chromatography eluting with a CH_2Cl_2–MeOH gradient with increasing amounts of MeOH from 5% to 45%, yielding 25 fractions (Fr_{D-1} to Fr_{D-25}). The Fr_{D-15} (800 mg) was packed on a wet column chromatograph. Successive elution with hexane–acetone (65:40) yielded 12 fractions (Fr_{D-15-1} to $Fr_{D-15-12}$). Fraction Fr_{D-15-3} (15 mg) was separated by HPLC and finally yielded **1** (4 mg, t_R 36.34 min).

10β,13α-Diacetoxy-5α,9α-dihydroxytaxa-4(20),11-diene (**1**). Gum; $[\alpha]_D^{22}$ +34° (*c* 0.34, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.72 (1H, m, H-1), 1.68 (2H, m, H-2), 3.24 (1H, br.s, H-3), 4.28 (1H, br.s, H-5), 1.83 (1H, m, H-6a), 1.64 (1H, m, H-6b), 1.77 (2H, m, H-7), 4.14 (1H, d, J = 9.8, H-9), 5.89 (1H, d, J = 9.8, H-10), 5.69 (1H, ddq, J = 10.6, 4.6, 1.2, H-13), 2.77 (1H, ddd, J = 15.2, 10.6, 8.9, H-14a), 1.13 (1H, dd, J = 15.2, 4.6, H-14b), 0.98 (3H, s, Me-16), 1.44 (3H, s, Me-17), 2.08 (3H, d, J = 1.2, Me-18), 0.88 (3H, s, Me-19), 5.10 (1H, s, H-20a), 4.71 (1H, s, H-20b), 2.14 (3H, s, CH_3CO-10), 2.06 (3H, s, CH_3CO-13). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 21.3 (CH_3CO-10), 170.4 (CH_3*CO*-10), 21.0 (CH_3CO-13), 170.1 (CH_3CO-13), 39.6 (C-1), 27.4 (C-2), 36.1 (C-3), 146.5 (C-4), 74.5 (C-5), 29.1 (C-6), 25.3 (C-7), 43.8 (C-8), 76.8 (C-9), 76.3 (C-10), 138.8 (C-11), 138.8 (C-12), 70.2 (C-13), 32.4 (C-14), 38.9 (C-15), 32.2 (C-16), 26.3 (C-17), 15.7 (C-18), 17.4 (C-19), 110.7 (C-20), 21.3 (CH_3CO-10), 170.4 (CH_3*C*O-10), 21.0 (CH_3CO-13), 170.1 (CH_3*CO*-13); HR-FAB-MS: *m/z* 459.2143 $[M + K]^+$ (calcd for $C_{24}H_{36}O_6K$, 459.2149).

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