Taxanes from Rooted Cuttings of *Taxus canadensis*

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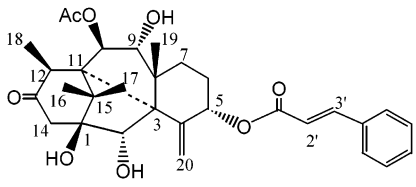
A 3,11-cyclotaxane (**1**), a new epoxytaxane (**2**), an *abeo*-taxane (3), and 26 known taxanes were isolated in rooted cuttings of the Canadian yew (*Taxus canadensis*) for the first time. Their chemical structures were characterized as 1*β*,2α,9α-trihydroxy-10*β*-acetoxy-5α-cinnamoyloxy-3,11-cyclotaxa-4(20)-en-13-one (**1**), 2α,9α,10*β*-triacetoxy-11,12-epoxy-20-hydroxytaxa-4-en-13-one (**2**), and 7*β*,9α,10*β*,13α-tetraacetoxy-11(15→1)*abeo*-taxa-4(20),11-diene-5α,15-diol (**3**). Metabolite **2** is a new taxane, metabolite **1** has been reported previously in the needles of *Taxus baccata*,[1,2] and metabolite **3** was previously discovered as a biotransformation product[3] but is now reported as a natural product for the first time.

Our group and others[4−7] have been investigating the taxane composition of Canadian yew needles since 1992, so only minor metabolites remain to be discovered. The composition of rooted cuttings that are used for the production of ornamental yews has never been investigated for *Taxus* species including the Canadian yew. We therefore contacted a nursery and obtained rooted cuttings of *Taxus canadensis* Marsh. (Taxaceae). Their taxane composition in comparison with that of the needles of the same species has not been reported.

9-Dihydro-13-acetylbaccatin III is specific to *Taxus canadensis* needles, where it is found as an abundant metabolite.[4−6] It has never been reported in other *Taxus* species. On the other hand, 10-deacetylbaccatin III, a major metabolite in the European *Taxus baccata*,[7] has also been found in the Canadian yew, albeit as a minor metabolite.[4] Climate probably has a big influence on *Taxus* spp. composition since we recently discovered a small *Taxus baccata* in Israel with only traces of 10-deacetylbaccatin III.[8]

In the present investigation, three new, **1**−**3**, and 26 known taxanes were isolated from rooted cuttings of the Canadian yew, *T. canadensis*. The new structures were elucidated by spectroscopic methods and characterized as 1*β*,2α,9α-trihydroxy-10*β*-acetoxy-5α-cinnamoyloxy-3,11-cyclotaxa-4(20)-en-13-one (**1**), 2α,9α,10*β*-triacetoxy-11,12-epoxy-20-hydroxytaxa-4-en-13-one (**2**), and 7*β*,9α,10*β*,13α-tetraacetoxy-11(15→1)*abeo*-taxa-4(20),11-diene-5α-diol (**3**). The epoxytaxane **2** (2α,9α,10*β*-triacetoxy-20-hydroxy-11,12-epoxytaxa-4-en-13-one) has never been reported, whereas the 3,11-cyclotaxane **1** was previously found in *T. baccata* needles,[1,2] and the *abeo*-taxane **3** was found for the first time as a natural product. We have previously obtained compound **3** as a biotransformation product.[3] The major taxanes in rooted cuttings differ from those in the needles of the mature plant.



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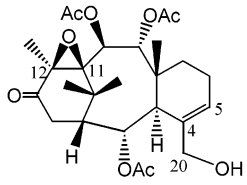

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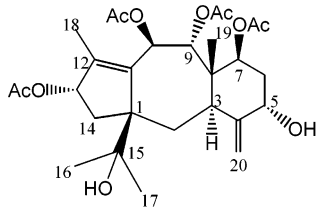

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Results and Discussion

Compound **1** was isolated as a colorless gummy substance with HRFABMS at *m/z* 577.2204 $[M + K]^+$. Analysis revealed that the molecular formula of **1** is $C_{31}H_{38}O_8$, indicative of 13 degrees of double-bond equivalence. Its ^1H NMR spectrum (Table 1) exhibited the characteristic signals of taxanes.[9,10] The chemical shift of the proton resonances due to an exomethylene moiety was observed at δ 5.94 and 5.80 (each 1H, s). The presence of a cinnamoyl moiety in **1** was revealed by the signals at δ 6.39 (1H, d, $J = 16.1$ Hz), 7.66 (1H, d, $J = 16.1$ Hz, *trans*-orientation), 7.55 (2H, m), and 7.38 (3H, m) in the ^1H NMR spectrum and by its UV absorption (λ_{max} 278 nm) in the HPLC analysis. In addition, the presence of one acetyl group and one ketone group was implied by the resonances at δ 2.16, 21.2, 172.4, and 213.9 in the ^1H NMR and/or ^{13}C NMR spectra. Since 12 out of 13 degrees of unsaturation deduced from the molecular formula were thus accounted for, the formula of **1** necessitated that this compound contains either an additional double bond or a saturated ring. Detailed examination of the ^1H NMR spectrum of **1** showed that it exhibited different spectral features compared to regular taxanes: the signal of H-3α, which usually appears at δ 3.2−3.6 with a coupling constant in a range ca. $J =$ 5.0−6.0 Hz, disappeared; one of the methyl groups gave rise to a doublet at δ 1.31 (3H, d, $J = 7.3$ Hz), which showed a coupling with a quartet signal at δ 3.49 (1H, q, $J = 7.3$ Hz) in the $^1H-^1H$ COSY spectrum. These characteristic signals were assigned as Me-18 and H-12, respectively. On analysis of these spectral features and available data, it became clear that compound **1** is a 3,11-cyclotaxane. This

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Table 1. ^{1}H and ^{13}C NMR Data for Taxane **1** in $CDCl_3$ (500 MHz for ^{1}H, 125 MHz for ^{13}C)

position	δ (H) mult[a]	*J* (Hz)	δ (C)[b]	HMBC	NOESY[c]
1			**78.3**		
2	4.78 (br s)	(COSY 14b)	78.6	1, 4, 8	$17^s/19^s$
3			**62.3**		
4			**142.5**		
5	5.59 (t)	8.8	75.8	6, 166.2	$6a^s$, $6b^m$
6a	2.20 (m)		26.0		5^s, $6b^s$, $7a^s$, 7b/17/19^w
6b	1.70 (o m)			7, 8	$6a^s$, 10^s
7a	1.93 (o m)		29.2		$7b^s$, 10^m
7b	1.34 (o m)				$7a^s$
8			**45.8**		
9	4.40 (d)	9.8	82.5	7, 8, 10, 19	2^w, $17^s/19^s$
10	5.40 (d)	9.8	84.0	9, 11, 12, 15, 172.5	$6b^s$, $7a^m$, 12^s, 18^s
11			**56.8**		
12	3.50 (q)	7.3	50.9	3, 13	10^s, 18^s, $20a^s$
13			**213.9**		
14a	3.05 (d)	19.8	46.4	1, 13, 15	$14b^s$, $20a^s$
14b	2.36 (d)	19.8			$14a^s$, 16^s
15			**45.0**		
16	1.12 (s)		23.0	1, 11, 15, Me-17	$14b^s$, $17^s/19^s$, 18^s
17	1.40 (o s)		23.3	1, 11, 15, Me-16	2^s, 5^w, 9^s, 16^s, (overlap 19)
18	1.31 (d)	7.3	15.8	11, 12, 13	12^s, 10^s, 16^s
19	1.40 (o s)		25.2	3, 7, 8, 9	see 17
20a	5.80 (s)		126.1	3	12^s, $14a^s$, $20b^s$
20b	5.60 (o s)			3, 4, 5	$20a^s$
−OAc	2.16 (s)		21.2	172.4	
1′			**166.2**		
2′	6.39 (d)	16.1	117.4	Ph-C1, 1′	
3′	7.66 (d)	16.1	145.3	1′, 2′, Ph-o	
Ph 3′			**134.4**		
o	7.55 (m)		128.2		
m, p	7.38 (m)		129.0		
			130.4		

[a] Mutiplicity: s, singlet; d, doublet; br, broad; t, triplet; m, mutiplet; o, overlapped. [b] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold represent quaternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm). [c] NOESY intensities are marked as strong (s), medium (m), or weak (w).

conclusion was further confirmed by the long-range C−H correlations. H-12 showed a three-bond correlation with C-3 in the HMBC spectrum. Combined analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra, together with the chemical shifts and coupling constants, enabled the assignment of all functional groups on the taxane skeleton. The acetyl group was located at C-10, since H-10 correlated with the carbonyl carbon of acetate (δ 172.5) in the HMBC spectrum; H-5 showed a three-bond correlation with the carbonyl of cinnamate (δ 166.2) in the HMBC experiment. The hydroxyl groups were connected to C-2 and C-9, as indicated by the chemical shifts of H-2 and H-9. The remaining hydroxyl group was attached to C-1, as indicated by the spin pattern of H-14a, H-14b, and H-2β. This assignment was verified by the chemical shift of C-1 (δ 78.3 ppm). To determine the relative stereochemistry of **1**, a NOESY experiment was performed. Both H-2 and H-9 showed NOE correlations with Me-17 and Me-19 and indicated that H-2 and H-9 adopted a β-orientation. The protons of Me-18 showed cross-peaks with the protons of Me-16 and suggested that Me-18 has a β-orientation while H-12 has an α-orientation. H-10 was α-oriented, as deduced from its NOE correlations with H-12 and H-6b. The structure of **1** was therefore characterized as 1β,2α,9α-trihydroxy-10β-acetoxy-5α-cinnamoyloxy-3,11-cyclotaxa-4(20)-en-13-one.

Compound **2** was also obtained as a colorless gum. Its molecular composition was $C_{26}H_{36}O_9$, as determined from combined analysis of its HRFABMS (*m/z* 531.1997) and ^{1}H and ^{13}C NMR spectral data. The ^{1}H NMR spectrum showed the characteristic signals due to the taxane skeleton,[9,10] including well-dispersed signals due to protons connected to oxygenated carbons and four tertiary methyl groups at δ 0.84, 1.90, 1.59, and 1.00. The connectivities of the protons on the taxane skeleton of **2** were determined by analysis of the ^{1}H−^{1}H COSY spectrum. Interpretation of the ^{1}H and ^{13}C NMR and HMBC spectra permitted the positional assignment of the functional groups. A pair of signals as an AB system, resonating at δ 6.12 (1H, d) and 5.20 (1H, d) with a large coupling constant (J = 11.2 Hz), were assigned as H-9 and H-10, and two acetoxy groups were attached to C-9 and C-10, respectively. This was indicated by the fact that H-9 correlated with C-7, C-8, C-10, and C-19 and a carbonyl signal at δ 169.3, while H-10 correlated with C-9, C-11, C-12, and C-15 and a carbonyl signal at δ 169.0 in the HMBC spectrum. The ^{13}C NMR signal at δ 208.1 suggested the presence of a C-13 ketone moiety. Accordingly, H_2-14 displayed a large coupling constant of J_{gem} = 20.1 Hz. Using H-14 as a starting point, the connectivities from C-14 to C-1 to C-2 to C-3 were deduced from the ^{1}H−^{1}H COSY spectrum. The absence of the characteristic signals due to an exocyclic methylene and the presence of two carbon signals in the olefinic region revealed that the C-4 double bond was endocyclic instead of exocyclic. The signals at δ 4.97 (1H, br d, J = 11.8 Hz) and 4.12 (1H, d, J = 11.8 Hz), which correlated with C-3, C-4, and C-5, were attributed to the methylenes of H-20a and H-20b, respectively. However, the downfield chemical shift of C-13 and upfield chemical shift of Me-18, especially H-10 and Me-16, as well as the lack of additional olefinic carbons from the ^{13}C NMR spectrum indicated that the endocyclic double bond at C-11,12 was instead a 11,12-epoxide (δ 65.1 and 59.6). This conclusion was supported by the HMBC spectrum and the molecular composition. H-10 resonated as δ 5.20 in **2**: this unusual upfield shift of H-10 was caused by the magnetic anisotropy effect of the 11,12-epoxy ring. H-10 in 11,12-epoxide taxoids is similar to H-5 in the taxanes with a C-4(20) oxirane ring,

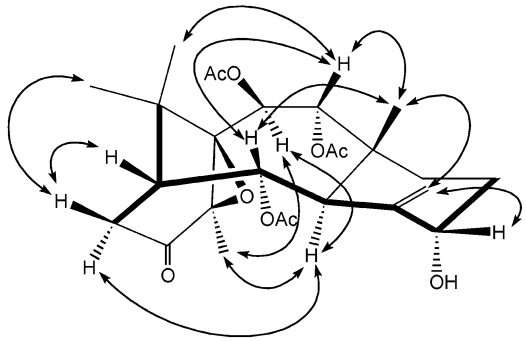

Figure 1. Relative stereochemistry of **2**. The arrows show selected NOE correlations.

both of which are dramatically shifted upfield when compared with corresponding protons in the taxanes with C-4(20),11-di-double bonds. The relative stereochemistry of **2** was established from analysis of the NOESY data, chemical shifts, and their coupling constants (Figure 1). The coupling constant between H-9 and H-10 ($J = 11.2$ Hz) and observed NOESY correlations of H-2/H-9 and H-9/H_3-17 established a chair-boat conformation for ring B, which is the typical conformation of natural taxanes. The β-orientations of H-2 and H-9 were assigned by NOESY correlations of H-2/H-1, H-2/H_3-17, H-2/H_3-19 and H-9/H-2, H-9/H_3-17, H-9/H_3-19. The α-orientation of H-10 was determined from the NOESY correlations of H-10/H-3 and H-10/H_3-18 (Figure 1). The β-orientation of the epoxide group at C-11 and C-12 was established by the NOESY correlations of H_3-18/H-3 and H_3-18/H-10. The upfield chemical shift of H_3-16 due to the presence of the C-11,-12-epoxide near H_3-16 and the γ-effect between the C-16 methyl group and 11,12-epoxide also suggested that the epoxide group has a β-orientation. From these data, the structure of **2** was established as 2α,9α,10β-triacetoxy-20-hydroxy-11,12-epoxytaxa-4-en-13-one.

Compound **3** was obtained as a colorless amorphous solid. The molecular composition of **3**, $C_{28}H_{40}O_{10}$, was established from the combined analysis of HRFABMS at *m/z* 575.2260 $[M + K]^+$ and the ^{13}C NMR spectrum. The ^1H NMR spectrum of **3** exhibited the three-proton signals due to the four tertiary methyl groups at δ 0.85, 1.10, 1.30, and 1.90, and four acetyl groups at a relatively lower field (δ 1.96, 1.98, 2.03, and 2.05), which was verified by the observation of ^{13}C NMR signals at δ 20.6/167.7, 20.6/169.7, 21.0/169.8, and 21.0/171.0. These signals suggested that **3** has a taxane-type skeleton.[6] The connectivities of the protons on the taxane skeleton of **3** were determined by analysis of the $^1H-^1H$ COSY spectrum. Interpretation of ^1H and ^{13}C NMR and HMBC spectra permitted the positional assignment of the functional groups. The ^1H NMR signals at δ 5.11 (1H, br s) and 4.76 (1H, br s) together with the signals at δ 111.4 in the ^{13}C NMR spectrum and δ 2.82 (1H, d, $J = 9.4$ Hz) in the ^1H NMR spectrum are characteristic of an exocyclic methylene and C-3 ring junction proton in a taxane with a 4(20)-double bond, respectively.[6] The one-proton doublet of doublets at δ 2.35 (1H, dd, $J = 14.8$, 9.6 Hz) and one-proton doublet at δ 1.43 (1H, br d, $J = 14.8$ Hz) were assigned to the C-2 methylene protons, H-2α and H-2β, respectively, on the basis of their geminal coupling ($J_{2\alpha,2\beta} = 14.8$ Hz) and coupling to H-3α. The signal at δ 4.33 (1H, br dd, $J = 2.6$, 1.9 Hz) was assigned to H-5β. Similarly, H-5β was correlated with the two one-proton multiplets at δ 1.97 and 1.79, which were assigned to H-6α and H-6β, respectively. Both H-6α and H-6β shared cross-peaks with the multiplets at δ 5.56 (1H, dd, $J = 11.1$, 5.2 Hz), which was assigned to H-7. A pair of isolated broad and broad doublet signals at δ 5.80 and 6.32 (1H, br d, $J = 8.9$ Hz) were attributed to H-9β and H-10α, respectively. The broad triplet signal of one proton resonating at δ 5.46 was assigned to H-13β; a pair of doublets of doublets at δ 2.48 ppm (1H, dd, $J = 13.9$, 7.5 Hz) and one-proton multiplet signal at δ 1.30 (1H, m) were assigned to H-14α and H-14β, respectively, according to their germinal coupling and coupling with H-13β in the $^1H-^1H$ COSY spectrum. All of the proton-bearing carbons were assigned by an analysis of the HMQC spectrum. The chemical shifts of H-7α, H-9β, H-10α, and H-13β indicated that four acetyl groups are attached to these positions, and these assignments were confirmed by observing the long-range correlations of these protons to corresponding carbonyl signals in the HMBC experiment. C-5 had a free hydroxyl group, as suggested by its chemical shift in the ^1H NMR spectrum. There are two signals relatively downfield (δ 75.3 and 62.8) which remained in the ^{13}C NMR spectrum, but only one bears a hydroxyl group, as required by the molecular composition. The signal at δ 75.3, which showed cross-peaks with H_3-16 and H_3-17 in the HMBC spectrum, was assigned to C-15 and one hydroxyl group attached to C-15. A remaining signal at δ 62.8 ppm was ascribed to C-1, an unusually downfield chemical shift as a quaternary carbon, which usually resonates around δ 43−46 in the normal 6/8/6-membered ring system skeleton,[9,10] and suggested that **3** possessed a 5/7/6 ring system skeleton.[9,10] Since no cross-peaks were observed between the H_3-16, H_3-17 and C-11 olefinic carbons in the HMBC spectrum, this further supported the 5/7/6 ring system skeleton, although three-bond correlations were not observed between H-2 and H-14 with C-11 in the HMBC spectrum. From the above results, the structure of **3** was established as 7β,9α,10β,13α-tetraacetoxy-11(15→1)*abeo*-taxa-4(20),11-diene-5α,15-diol. The relative stereochemistry of **3** was deduced from the NOESY experiment and coupling constants, as summarized in the Experimental Section.

The known compounds in the rooted cuttings of the Canadian yew were identified by NMR analysis and furthermore by comparison with reported data as 7β-acetoxytaxinine A (taxuspine F) (**4**),[11] baccatin III (**5**),[12,13] 5-decinnamoyltaxinine B-11,12-oxide (**6**),[14] 5α-decinnamoyltaxinine J (**7**),[15] taxayuntin (**8**),[16] taxuspine W (**9**),[17] baccatin IV (**10**),[13,14] taxinine M (**11**),[18] 1β-hydroxy-2α,7β,9α-trideacetylbaccatin I (**12**),[19] baccatin VI (**13**),[13] 13α-acetyl-13α-decinnamoyltaxchinin B (**14**),[20] taxuspine L (**15**),[21] 7β,13α-dideacetyl-9α,10β-didebenzoyltaxchinin C (**16**),[22] taxinine NN-3 (9α-deacetyltaxinine) (**17**),[23] paclitaxel (**18**),[24] 10β-deacetylcephalomanine (**19**),[25] 10β-deacetylbaccatin III (**20**),[26] 9-dihydro-13α-acetylbaccatin III (**21**),[4,5] taxinine (**22**),[27] 2α-deacetoxytaxinine J (**23**),[28] cephalomanine (**24**),[26] 5-decinnamoyltaxuspine D (**25**),[29] taxinine A (**26**),[30] 5-*epi*-canadensene (**27**),[31] 2α-deacetyl-5α-decinnamoyltaxinine J (**28**),[4] and 5α-decinnamoyltaxigifine (**29**).[32]

The relative amounts of these metabolites in the needles of the mature Canadian yew and in the rooted cuttings, which contains stems and roots, are surprising. In the mature Canadian yew, 9-dihydro-13-acetylbaccatin III is the most abundant metabolite, followed by taxinine and taxinine E.[4] The three major metabolites in rooted cuttings of the Canadian yew are 5-decinnamoyltaxagifine, 10-deacetylbaccatin III, and paclitaxel. The role of these secondary metabolites in the plant or in the rooted cuttings is presently unknown, but the differences between the metabolites of these various sources is intriguing.

Experimental Section

General Experimental Procedures. Flash chromatography was performed on silica gel 60 (230−400 mesh, EM Science). Thin-layer chromatography was performed on silica gel 60 F_{254} precoated TLC plates (0.25 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. Na_2SO_4 was the drying agent used in all workup procedures. Analytical HPLC was performed on a Waters 600 FHU delivery system coupled to a PDA 996 detector. Preparative and semipreparative HPLC were carried out on a Waters Delta Prep 3000 instrument coupled to a UV 486 tuneable absorbance detector set at 227 nm (Waters, Montreal, Quebec, Canada). Analytical HPLC was performed with two Whatman Partisil 10 ODS-2 analytical columns (4.6 × 250 mm) in series. Semipreparative HPLC was performed with two Whatman Partisil 10 ODS-2 Mag-9 semipreparative columns (9.4 × 250 mm) in series. Preparative HPLC was performed with a Partisil 10 ODS-2 MAG-20 preparative column (22 × 500 mm). The products were eluted with mixtures of acetonitrile in water with a flow rate of 18 mL/min (preparative HPLC) and 3 mL/min (semipreparative HPLC). All the reagents and solvents were of the best available commercial quality and were used without further purification. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter.

All the NMR data were obtained at room temperature on a Bruker Avance-500 spectrometer operating at 500.13 MHz for proton and at 125.77 MHz for carbon-13. The solvent was used as an internal reference (7.25 ppm for proton and 77.0 ppm for carbon-13). The various 2D spectra were acquired and processed using standard procedures. For phase-sensitive 2D experiments (NOESY, ROESY, and HSQC), the data were acquired using the TPPI phase mode. The NOESY experiment was obtained using a mixing time of 0.3 s and a relaxation delay of 1.5 s. The intensity of the cross-peaks in the NOESY experiment is designated as strong (s), medium (m), and weak (w). The ROESY (NOE in the rotating frame) experiment was used when NOESY proved to be unsuccessful or weak. Two mixing times were used in the ROESY: 0.3 and 0.5 s. Positive ion FABMS 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 of DMSO before addition of 0.5 μL of glycerol. HRFABMS was similarly obtained in glycerol-DMSO at a resolving power of 12 000.

Plant Material. Rooted cuttings of *Taxus canadensis* Marsh. (Taxaceae) were obtained by Mr. Mario Cramerstetter (accession voucher number 00357) in September 2001 from Cramer Nursery, 1002 Chemin St., Dominique, Les Cèdres, Québec, Canada.

Extraction and Isolation. Air-dried rooted cuttings of *T. canadensis* were ground into a fine powder (584 g), which was then extracted with 3 L of MeOH by shaking at room temperature for 24 h in the dark. The solution was removed and stored, and the plant material was extracted another six times, twice with MeOH and four times with a mixture of MeOH−CH_2Cl_2 (1:1). The combined organic extracts were evaporated to dryness and were redissolved in water. Lipids were removed from this mixture by extraction with hexane. The aqueous phase was then saturated with NaCl and extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness, yielding 8 g of a brown-colored extract. The hexane layer was also re-extracted using methanol and yielded another 15 g of a brown-colored extract.

The CH_2Cl_2 extract was redissolved in a minimum volume of CH_2Cl_2 and fractionated by column chromatography on silica gel (silica gel 60, 230−400 mesh, 200 g, bed size 5.5 × 22 cm). Forty-five fractions (Fr_{1-45}) were collected after elution with successive mixtures of ethyl acetate in CH_2Cl_2 (Fr_{1-5}: 33%, Fr_{6-15}: 50%, Fr_{16-19}: 66%, Fr_{20-45}: 100% ethyl acetate). Fractions were pooled on the basis of similar content as follows: Fr_{10-14} were combined, as were Fr_{15-23} and Fr_{27-33}. Fraction Fr_{10-14} was then further purified by preparative HPLC using a programmed isocratic run of 50 min (25% CH_3CN in H_2O), a linear gradient of 20 min (25% to 40% CH_3CN in H_2O), and a last isocratic step of 170 min (40% CH_3CN in H_2O). Both Fr_{15-23} and Fr_{27-33} were purified using a linear gradient of 120 min (25% to 100% CH_3CN in H_2O). Fr_8, Fr_9, and Fr_{26} were also additionally purified using a linear gradient of 50 min (25% to 100% CH_3CN in H_2O). Fractions collected between 27 and 29 min and 30.5 and 32 min of Fr_{10-14} were combined with the fraction collected between 45 and 45.6 min of Fr_{15-23} (named Fr_A) and was subsequently purified using a linear gradient of 70 min (25% to 100% CH_3CN in H_2O). The same program was used to further purify the fraction collected between 28.9 and 30.5 min of Fr_{10-14} (named Fr_B).

The fraction collected during time 44 and 44.8 min of Fr_{27-33} gave 2.5 mg of **1**. The fraction collected during time 31.2 and 32.3 min of Fr_9 gave 0.9 mg of **2** and 1.5 mg of **6**. Compound **3** (8.5 mg) was obtained from Fr_{10-14} during time 28.9 to 30.5 min. Compound **4** (4.5 mg) was obtained from three samples: from 26.9 to 27.5 min of Fr_A, from 40 to 41.5 min of Fr_{27-33}, and from 28.9 to 30.5 min of Fr_{10-14}. Compound **5** was collected during the same purification as compound **4** above and gave 2.5 mg: from 26.9 to 27.5 min of Fr_A and from 28.9 to 30.5 min of Fr_{10-14}. The fraction collected between 33.7 and 38 min of Fr_{10-14} was further purified and gave compound **7**, collected between 34.0 and 34.6 min. Additional compound **7** was also obtained from the 32.7 to 33.7 min of Fr_{10-14} for a total of 11.5 mg. Compound **8** (7.5 mg) was also obtained from these fractions. Compound **9** (3.5 mg) was obtained from 31.0 to 31.3 min of Fr_8. The fraction from 28.9 to 30.5 min of Fr_{10-14} was further purified, and the fraction collected between 29.9 and 30.5 min afforded compound **10**. Additional compound **10** was also obtained from 34.7 to 35.7 min of Fr_9 for a total of 5.5 mg. Compound **11** (6.5 mg) was obtained from 47.6 to 50 min of Fr_{15-23}. Compound **12** was obtained from 26.0 to 27.0 min of Fr_{27-33}, to give 3.5 mg. Compound **13** was identified in three fractions for a total of 8.5 mg: 39.1 to 39.3 min and 39.3 to 39.6 min of Fr_8 and 37.3 to 37.8 min of Fr_9. Compound **14** (2 mg) was identified from the fraction at 39.3 to 39.6 min of Fr_8. The fraction from 28.9 to 30.5 min of Fr_{10-14} was further purified to give 3.5 mg of compound **15**, collected between 30.9 and 31.2 min. Compound **16** (2.5 mg) was obtained from 31.0 to 31.9 min of Fr_{27-33}. Compound **17** (1 mg) was identified from the fraction from 45.1 to 45.8 min of Fr_{26}. The fraction from 33.7 to 38 min of Fr_{10-14} was further purified to give 31.5 mg compound **18**, identified in the fraction from 36.3 to 36.4 min. It was also identified from the purification of the methanol extract of the hexane layer between 34.0 and 35.9 min. Compound **19** was obtained from two fractions: 48.0 to 50 min of Fr_{27-33} and 28.6 to 29.5 min of Fr_{26} for a total of 11−12 mg. Compound **20** was obtained from the fraction from 27 to 29 min of Fr_{27-33}. Further purification of the fraction from 29 to 31 min also gave compound **20**, collected between 25.5 and 25.6 min, for a total of 38−39 mg. Compound **21** was identified in four fractions for a total of 26−27 mg: from 45.6 to 47.5 min of Fr_{15-23}, from 29.7 to 29.9 of Fr_B, from the further purification of the fraction from 32 to 32.7 min of Fr_{10-14}, and from 29.4 to 29.8 min of fraction Fr_A. Compound **22** (19−20 mg) and 19−20 mg of compound **23** were obtained from 50 to 53.4 min of Fr_{10-14}. The fraction from 33.7 to 38 min of Fr_{10-14} was further purified to afford 7−8 mg of compound **24** from the fraction from 35.2 to 35.3 min. Compound **25** was identified from four samples for a total of 12−13 mg: from 26.9 to 27.5 min of Fr_A, from 40.4 to 41.6 min of Fr_{15-23}, from 40.0 to 41.5 min of Fr_{27-33}, and from 26.9 to 27.4 min of Fr_B. Compound **26** was identified in three samples for a total of 15−16 mg: from 34.3 to 34.4 min of Fr_8, from 32.7 to 33.7 min of Fr_{10-14}, and from 32.3 to 32.5 min of Fr_A. Compound **27** (2−3 mg) was identified from the fraction from 44 to 45 min of Fr_{15-23}. Compound **28** was obtained from two samples for a total of 7−8 mg: from 38.8 to 40.4 min of Fr_{15-23} and from 38.6 to 40.0 min of Fr_{27-33}. Sample **29** was identified in four fractions for a total of 52−53 mg: from 33.9 to 37.0 min of Fr_{15-23}, from 24.1 to 24.7 min of Fr_{26}, from 34.0 and 36.0 min of Fr_{27-33},

Table 2. ^{1}H and ^{13}C NMR Data for Taxane **2** in $CDCl_3$ (500 MHz for ^{1}H, 125 MHz for ^{13}C)

position	δ (H) mult[a]	*J* (Hz)	δ (C)[b]	HMBC	NOESY[c]
1	1.94 (o m)		48.6		
2	5.83 (br m)	~2.4	71.4		1[s], 3[w], 9[s], 17[s], 19[s]
3	3.23 (br m)	5.7	40.9		2[w], 18[s]
4			**138.0**		
5	5.93 (m)		131.2		20b[s]
6a	2.10 (m)		21.9		
7a	1.96 (o m)		27.2		
7b	1.39 (td)	12.2, 12.2, 6.1			
8			**39.6**		
9	6.12 (d)	11.1	76.5	7, 8, 10, 19, 169.3	2[s], 17[s], 19[m]
10	5.20 (d)	11.2	71.2	9, 11, 12, 15, 160.0	3[w], 18[s]
11			**65.1**		
12			**59.6**		
13			**208.1**		
14a	3.04 (d)	20.1	37.6	1, 2, 13, 15	3[m], 14b[s], 20a[s]
14b	2.63 (o dd)	20.1, 8.4		1, 2, 13, 15	1[s], 14a[s], 16[w]
15			**39.1**		
16	0.84 (s)		28.7	1, 11, 15, Me-17	1[m], 17[s]
17	1.90 (s)		25.1	1, 11, 15, Me-16	2[s], 9[s], 16 [s]
18	1.59 (s)		15.0	11, 12, 13	3[s], 10[s]
19	1.00 (s)		18.0	3, 7, 8, 9	2[s], 6[m], 7a[w], 9[m], 20b[m]
20a	4.62 (br d)	~11.9	68.0		14a[m], 20b[s]
20b	4.12 (d)	11.7		3, 4, 5	5[s], 20a[s]
−OAc	n.a.[d]				

[a] Mutiplicity: s, singlet; d, doublet; br, broad; t, triplet; m, mutiplet; o, overlapped. [b] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold represent quaternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm). [c] NOESY intensities are marked as strong (s), medium (m), or weak (w). [d] Not assigned due to overlapping regions.

Table 3. ^{1}H and ^{13}C NMR Data for Taxane **3** in $CDCl_3$ (500 MHz for ^{1}H, 125 MHz for ^{13}C)

position	δ (H) mult[a]	*J* (Hz)	δ (C)[b]	HMBC	NOESY[c]
1			**62.8**		
2a	2.35 (dd)	14.8, 9.6	29.1	8, 15	2b[s], 9[s], 19
2b	1.43 (br d)	14			2a[s], 17[m], 20b[s]
3	2.82 (d)	9.4	37.4	2, 7	7[s], 14b[m]
4					
5	4.33 (br.dd)	~2.6, ~1.9	72.5		6a[m], 6b[m], 20a[s]
6a	1.97 (o.m)		35.7		5[m], 6b[s], 7[m]
6b	1.79 (m)				5[s], 6a[s], 19[s]
7	5.56 (dd)	11.1, 5.2	69.5	8, 19, 169.7	3[s], 6a[m], 10[s]
8			**44.9**		
9	5.80 (br)		77.1		2a[s], 19[s]
10	6.32 (br d)	~8.9	69.5		7[s],18[s]
11			**136.5**		
12			**146.2**		
13	5.46 (br t)	~7	79.5		13a[s], 14b[s], 16[w]
14a	2.48 (dd)	13.9, 7.5	43.9	15	1[s], 14b[s], 16[w]
14b	1.30 (o m)				see 17
15			**75.3**		
16	1.30 (s)		24.8	11, 15, Me-17	13[s], 17[s]
17	1.10 (s)		26.8	11, 15, Me-16	2b[s], 3[s], 14a[s] (overlap 14b)
18	1.90 (s)		11.7	11, 12, 13	10[s], 13[w]
19	0.85 (br d)		12.6	3, 7, 8, 9	2a[s], 6b[s], 9[m], 20b[w]
20a	5.11 (s)		111.4	5	5[s], 20b[s]
20b	4.76 (br s)			5	2b[s], 20a[s]
−OAc	2.05 (s)		21.0	169.8	
	2.03 (s)		21.0	171.0	
	1.98 (s)		20.6	169.7	
	1.96 (s)		20.6	167.7	
−OH	2.73 (br)				

[a] Mutiplicity: s, singlet; d, doublet; br, broad; t, triplet; m, mutiplet; o, overlapped. [b] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold represent quaternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm). [c] NOESY intensities are marked as strong (s), medium (m), or weak (w).

and from 22.3 to 22.4 min of the further purification of the fraction between 29.0 and 32 min from Fr_{27-33}.

1*β*,2α,9α-Trihydroxy-10*β*-acetoxy-5α-cinnamoyloxy-3,11-cycloaxa-4(20)-en-13-one (1): amorphous solid; $[\alpha]^{22}_D$ +7.3° (*c* 0.11, $CHCl_3$); ^{1}H and ^{13}C NMR spectral data, see Table 1; LRFABMS *m/z* 577 [M + K$^+$]; HRFABMS *m/z* 577.2201 [M + K]$^+$ (calcd for $C_{31}H_{38}O_8K$, 577.2204).

2α,9α,10*β*-Triacetoxy-20-hydroxy-11,12-epoxytaxa-4-en-13-one (2): amorphous solid; $[\alpha]^{22}_D$ −19.6° (*c* 0.07, $CHCl_3$); ^{1}H and ^{13}C NMR spectral data, see Table 2. LRFABMS *m/z* 531 (M + K$^+$); HRFABMS *m/z* 531.1997 [M + K]$^+$ (calcd for $C_{26}H_{36}O_9K$, 531.1996).

7*β*,9α,10*β*,13α-Tetraacetoxy-11(15→1)*abeo*-taxa-4(20),-11-diene-5α,15-diol (3): amorphous solid; $[\alpha]^{22}_D$ −10° (*c* 0.02, $CHCl_3$); ^{1}H and ^{13}C NMR spectral data, see Table 3. LRFABMS *m/z* 575 [M + K]$^+$; HRFABMS *m/z* 575.2260 [M + K]$^+$ (calcd for $C_{28}H_{40}O_{10}K$, 575.2259).

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Supporting Information Available: Structures of known compounds isolated from the rooted cuttings of the Canadian yew. This information is available free of charge via the Internet at http://pubs.acs.org.

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