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New Minor Taxanes Analogues from the Needles of *Taxus canadensis*

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Abstract—Seven new taxanes were isolated from the needles of the Canadian yew: unusual functional groups, positions and/or stereochemical features are described. Their chemical structures were rigorously characterized by detailed high resolution NMR analyses and confirmed by high resolution Fast Atom Bombardment Mass Spectrometry. Unlike paclitaxel and taxuspine D, these taxanes had no effect on tubulin assembly.

Introduction

We have been studying the composition of the Canadian yew since 1992.[1–3] The taxanes composition of this low trailing shrub has been shown to differ from other yews.[3,4] We have previously suggested[5–8] that a major conformational change of the core skeleton of a taxane can lead to unusual bioactivities. Indeed, taxuspine D (Fig. 1) which lacks the key elements essential for activity was found to promote the polymerization of tubulin with a potency corresponding to half of the activity of paclitaxel (Fig. 1). This bioactivity was explained by the major conformational change derived by the C12–C13 double bond enabling the C-5 cinnamoyl to mimic part of the C-13 side chain of paclitaxel.[7,8] Based on this hypothesis we have also designed and synthesized putative bioactive taxanes.[9]

In this publication, we have characterized seven new taxanes in the needles of the Canadian yew. In Figure 1, five taxanes with the same ring C but differing in rings A or B are shown, only one of these structures (taxane **4**) had been previously found in the seeds of *Taxus yunnanensis*.[10] Figure 2 shows the 3-D structure of the unusual 11,12-epoxy-taxane **5** with its NOESY correlations. In Figure 3, three new taxanes are shown: taxane **6** with a C4–C5 double bond, taxane **7** with a *cis*-cinnamoyloxy- side chain on C13, and taxane **8** with a C6/C10/C6 configuration. Unlike paclitaxel or taxuspine D, none of these taxanes showed any bioactivity in tubulin assembly.

Results and Discussion

Taxanes with 4(20)-ene,5α-*trans*-cinnamoyl-ring C (taxanes 1–5, Figs 1 and 2)

Taxanes **1**–**5** have ring C in common: a C4–C20 double bond and an α-*trans*-cinnamoyl group on C-5. The chemical shifts of the characteristic proton resonances due to the exomethylene C4–C20 group were observed at δ 5.89 (1H, s) and δ 5.68 ppm (1H, s) in the ^{1}H NMR spectra of taxane **2**. The presence of the *trans*-cinnamoyl moiety was revealed by the signals at δ 6.39 (1H, *d*, $J = 16.1$ Hz), 7.67 (1H, *d*, $J = 16.1$ Hz, *trans*-orientation), 7.55 (2H, *m*), and 7.37 (3H, *m*) in the ^{1}H NMR spectra. In addition, the fragment at *m*/*z* 131 (C_9H_7O) as well as $(M–cinn)^+$ deriving from fission of the cinnamoyl group are present in the FABMS data of these four taxanes. Taxanes **1** and **2** differ only in their C-9 substituent (a

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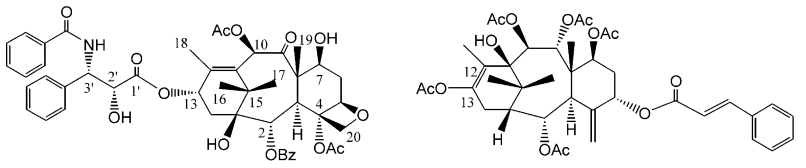

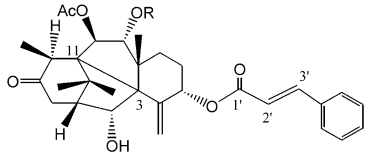

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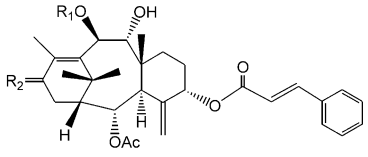

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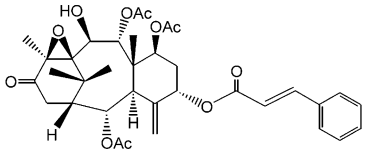

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Figure 1. Chemical structures of paclitaxel, taxuspine D and new taxanes analogues (**1**–**5**) isolated from the needles of *Taxus canadensis*.

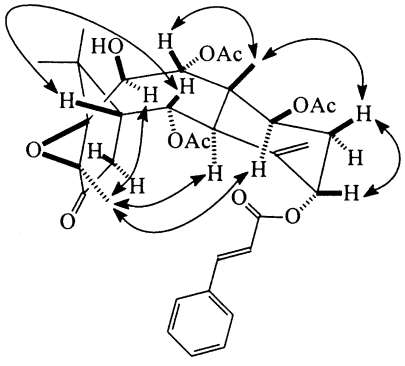
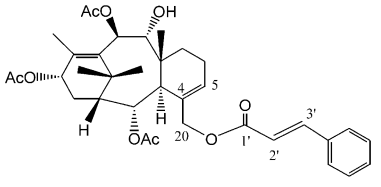


Figure 2. NOESY analysis of taxane **2**.

hydroxyl group for **1** and an acetoxy in **2**). The FAB-HR-MS analysis of taxane **2** revealed its molecular formula as $C_{33}H_{40}O_8$, indicating fourteen degrees of unsaturation. In taxane **2**, the presence of two acetyls and a keto-groups were implied by the resonances at δ 21.0, 171.0, 2.05 ppm; 21.0, 169.8, 2.04 ppm; and 215.3 ppm in the ^{13}C NMR and/or ^{1}H NMR spectra. Since thirteen out of fourteen unsaturation sites deduced from the molecular formula were thus accounted for, taxane **2** must contain an additional double bond or a saturated ring. Detailed examination of the ^{1}H NMR spectrum of **2** showed different spectral features from regular taxanes. The signal of H-3α (which usually appeared at δ 3.2–3.6 ppm with a coupling constant in the range of 5–6 Hz) had disappeared. One of the methyl groups gave rise to a doublet which showed a coupling with a quartet signal at δ 3.50 ppm (1H, *q*, J = 7.2 Hz) in



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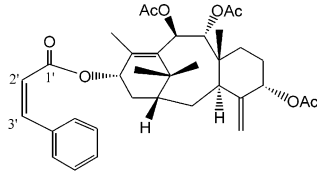

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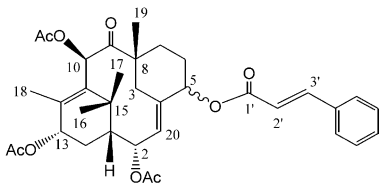

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Figure 3. Chemical structures of new taxanes analogues (**6**–**8**) isolated from the needles of *Taxus canadensis*.

the ^1H–^1H COSY spectrum. Further analysis of these features and available data suggest strongly that taxane **2** is a 3,11-cyclotaxane, a member of a minor group of taxanes. Additional confirmation of this structure was given by the long range correlations of H-12 to C-3 and H-10 to C-3 in the HMBC experiment. Combined analysis of the ^1H–^1H COSY, HSQC, HMBC spectra, together with chemical shifts and coupling constants allowed complete assignments of all the functional groups of taxane **2**. Two acetyl groups are located on C-9 and C-10; a free hydroxyl group is on C-2; a keto-group on C-13 and a *trans*-cinnamoyl group on C-5, as in most 3,11-cyclotaxanes.[12,13] The relative stereochemistry of taxane **2** shown in Fig. 1 was determined by the NOESY experiment. The NMR data of taxane **1** (experimental) and taxane **2** (Table 1) confirm their structures as 2α,9α-dihydroxy-10β-acetoxy-5α-cinnamoyloxy-3,11-cyclotaxa4(20)-ene-13-one for **1** and 2α-hydroxy-9α,10β-diacetoxy-5α-cinnamoyloxy-3,11-cyclootaxa4(20)-ene-13-one for **2**. High resolution mass spectrometry confirmed the elemental composition of their potassium quasimolecular ions.

The ^1H NMR spectra of taxane **3** showed well-dispersed signals. The HMBC experiment suggest a taxane derivative with a regular 6/8/6 ring system. The ^1H and ^{13}C NMR spectra (Table 2) indicate two acetate groups, two hydroxyls, a *trans*-cinnamoyloxy group as well as an exocyclic double bond. Taxane **3** is very similar to taxane **4** also isolated from the needles of the Canadian yew. They only differ in positions C-10 and C-13. In taxane **3**, C-10 is an acetyl versus a hydroxyl group in **4**, and on C-13 it is an α-hydroxyl group in taxane **3** and a ketone in **4.** The other difference between these two taxanes is that taxane **4** had already been isolated previously in the seeds of *Taxus yunnanensis*[10] whereas **3** is a novel taxane. Both of them however have been characterised for the first time in the needles of the Canadian yew. The stereochemistry of taxanes **3** and **4** established by the NOESY data is in accord with many taxanes. The structures of taxanes **3** and **4** are therefore 9α,13α-dihydroxy-2α,10β-diacetoxy-5α-cinnamoyloxy-taxa-4920),11-diene for **3** and 9α,10β-dihydroxy-2α-acetoxy-5α-cinnamoyloxy-taxa-4(20),11-dien-13-one for **4**.

Compound **5** was obtained as a colorless amorphous solid in a very low yield from the needles of *Taxus canadensis* (0.00002% w/w). The molecular composition of **5**, $C_{35}H_{42}O_{11}$, was established from combined analysis of high-resolution FAB-MS and ^{13}C NMR spectrum. The ^1H NMR spectrum of **5**, summarized in Table 3, exhibited four three-proton singlets due to the four tertiary methyl groups at δ 0.83, 1.83, 1.08, and 2.02 ppm, and three three proton singlets for acetyl groups at relatively lower field (δ 2.10–2.06 ppm). The ^{13}C NMR signals at δ 21.0, 170.0; 21.2, 170.1; and 21.2, 168.9 ppm confirmed these acetyl groups. The HMBC

Table 1. ^1H and ^{13}C NMR for taxane **2** in $CDCl_3$

Position	δ ^1H mult.[a] (*J* in Hz)	δ^{13}C[b]	HMBC	NOESY[c]
1	2.01 o.m	50.6	2, 3, 11, 14, 15. 17	2^s, $14a^w$, $14b^s$, 16^s, 17^s
2	5.15 br.d (*4.9*)	75.7	1, 3, 4, 8, 14	1^s, 17^s, 19^s
3	—	66.4		
4	—	142.9		
5	5.61 o.m	76.1	166.0	$6a^s$
6a	2.19 o.m	25.6		5^s, $6b^s$, $7b^s$
6b	1.77 o.m			
7a	1.78 o.m	31.0		
7b	1.28 o.m			See 18
8	—	44.4		
9	5.69 o.d (*9.7*)	82.1	7, 8, 10, 19, 171.0	17^s, 19^s
10	5.62 o.d (*9.7*)	79.4	3, 9, 11, 12, 15, 169.8	7a/$6b^s$, 12^s, 18^s
11	—	58.4		
12	3.50 q (*7.2*)	51.8	3, 11, 13, 15, 18	10^s, 18^s, $20a^s$
13	—	215.3		
14a	2.79 d (*20.6*)	38.2	1, 2, 13, 15	1^w, $14b^s$, $20a^s$
14b	2.50 dd (*20.6, 7.7*)		1, 2, 13	1^s, $14a^s$, 16^m
15	—	42.7		
16	1.20 s	26.6	1, 11, 17, (18-weak)	1^s, $14b^s$, 17^s
17	1.60 s	29.0	1, 11, 16, (9 weak)	1^s, 2^s, 9^s, 16^m, 19^s
18	1.27 d (*7.2*)	15.5	11, 12, 13	12^s, $10/5^m$, $6a^m$, $6b/7a^s$ (overlap 7b)
19	1.40 s	25.6	3, 7, 8, 9, (5-weak)	2^s, 9^s, $6b/7a^m$, $7b^m$, 17^s
20a	5.89 s	127.9	3, 5, (4, 6 weak)	12^s, $14a^s$, $20b^s$
20b	5.68 o.s		3, 4, 5	$20a^s$
OAc	2.05 s	21.0	171.0	
OAc	2.04 s	21.0	169.8	
1′	—	166.0		
2′	6.39 d (*16.1*)	117.7	C1-Ph, 3′, 1′	
3′	7.67 d (*16.1*)	145.3	1′, 2′,C-1-Ph, Ph-o	
Ph′		134.5		
O	7.55 m	128.1	3′, Ph-o, Ph-p	
m, p	7.37 m	128.7, 130.1		

[a]Mult. multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HSQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

Table 2. ^{1}H and ^{13}C NMR for Taxane **3** in $CDCl_3$

Position	δ ^{2}H mult.[a] (*J* in Hz)	$\delta^{13}C$[b]	HMBC	NOESY[c]
1	1.76 o.d (*9.3*)	48.0		
2	5.41 o.m	71.8		1^{s}, 3^{w}, 9^{s}, 17^{s}, 19^{m}
3	3.31 d (*5.5*)	44.0		$20a/2^{m}$, 10^{w}, $14b^{s}$, 18^{m}
4	—			
5	5.42 s	79.5		$20b^{s}$
6a	1.99 br.d (*14.6*)	28.3		
6b	1.77 o.m			
7a	1.90 m	25.7		
7b	1.33 m			
8	—	44.8		
9	4.26 d (*10.1*)	76.1	3, 7, 9, 19	2^{s}, 17^{s}, 19^{m}
10	5. 83 d (*10.1*)	76.4	9, 11,12, 15, 170.4	3^{w}, 7^{w}, $14b^{m}$, 18^{s}
11	—	133.3		
12	—	141.1		
13	4.53 br.dd (*9.5; 5.2*)	67.8		$14a^{s}$, 16^{s}, 18^{w}
14a	2.70 dt (*15.8, 9.5*)	32.52	12	1^{s}, 13^{s}, $14b^{s}$, 16^{w}
14b	1.59 o.m		2, 13	
15	—	37.3		
16	0.98 s	31.9	1, 11, 15, 17	1^{m}, 13^{s}, 17^{s}
17	1.57 s	26.3	1, 11, 15, 16	
18	2.31 s	16.0	11, 12, 13	3^{m}, 10^{s}, 13^{m}, $2'^{s}$
19	1.09 s	17.9	3, 7, 8, 9	2^{s}, $7a^{s}$, $6b^{m}$, 9^{s}, $20b^{w}$
20a	5.36 s	118.0		$20b^{s}$
20b	5.03 s		3, 5	5^{s}, $20a^{s}$
OAc	2.10 s	21.2	170.4	
OAc	2.05 s	21.4	169.7	
1′	—	166.5		18^{m}
2′	6.82 d (*15.9*)	117.8	C1 (Ph), 1′	
3′	7.72 d (*15.9*)	145.6	1′, 2′, Ph-o	
Ph′		134.7		
O	7.55 m			
m, p	7.38 m	128.1128.8; 130.2		

[a]Mult. multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HSQC and HMBC experiments (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

suggests a regular C6/C8/C6 structure. A cinnamoyloxy side chain on C-5 was confirmed both by the ^{1}H NMR: δ 7.64 (2H, dd, $J=8.0$, 2.1 Hz), 7.44 (1H, od), 7.38 (2H, om), 6.22 (1H, d, $J=16.0$ Hz, *trans*), and 7.68 (1H, *d*, $J=16.0$ Hz, *trans*) and the prominent peak at *m/z* 147 in the mass spectrum. The main difference between ring C of taxane **5** versus taxanes **1**–**4** is the presence of a C-7 acetoxy-group shown by its ^{1}H NMR shift: 1H at δ 5.54 ot, $J=\sim5.8$ Hz as well as its HMBC connectivity with a acetyl carbonyl group at 170.2 ppm. 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 ^{1}H-^{1}H COSY spectrum. The signal at δ 5.54 ppm (1H, t, $J=5.8$ Hz) was assigned to H-7, due to the correlation of C-7 with Me-19 in the HMBC experiment. Similarly, using H-7 as a starting point, the spin system derived from C-7 to C-6 to C-5 was readily interpreted from the analysis of ^{1}H–^{1}H COSY spectrum. The ^{13}C NMR signal at δ 209.2 ppm suggested the presence of C-13 non-conjugated carbonyl moiety. The downfield chemical shift of C-13 and the correlation of Me-18 with two relatively shielded carbons in the HMBC experiment (δ 66.3 and 59.4 ppm for C-11 and C-12 respectively) indicated that the endocyclic double bond is not present. In the HMBC, we also note that the two hydrogens at position 14 showed long-range C/H correlations to C-1, C-2, C-12, C-13 and C-15. The cross-peaks of H_3-16 and H_3-17 to C-1, C-11 and C-15 revealed that Me-16 and Me-17 are both attached to C-15. The cross-peaks of H_3-18 to C-11, C-12 and C-13 revealed that Me-18 is attached to two relativaly shielded quaternary carbons indicative of epoxide type of carbons. These correlations revealed the presence of a cyclohexane moiety (ring A). Cross-peaks of H-2 to C-8 and C-14, H-3 to C-1, C-7, C-8, C-19 and C-20, H-9 to C-7, C-8, C10 and C-19, together with H-10 to C-9, C-11, C-12, and C-15 showed the presence of an eight member ring (ring B). The cross-peaks of H-3 to C-7, C-20, H-20 to C-3, C-4 and C-5, H-7 to C-3, and C-19 and H-19 to C-3, C-7, C-8 and C-9 showed a presence of a cyclohexane moiety (ring C). Three acetoxy groups were attached at C-2, C-7 and C-9, respectively, as indicated from the observation of HMBC correlations of the protons to carbonyl esters and by observing the characteristic deshielding of those protons in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum of **5** showed two oxygenated tertiary carbon signals at δ 66.4 and 59.4 ppm assigned to C-11 and C-12 respectively. Judging from the ^{13}C NMR spectrum and molecular formular, the presence of an epoxide at C-11 and C-12 was strongly suggested. A free hydroxyl group is located at C-10 as indicated from its ^{1}H (δ 4.11 ppm, 1H, d, $J=10.1$ Hz) and ^{13}C NMR (δ 76.9 ppm). These 11,12-epoxy-taxanes are uncommon in nature.[11–13] The relative stereochemistry of **5** shown in Figure 2 was established from the NOESY spectral data as well as the NMR coupling constants. The coupling constant

Table 3. ^{1}H and ^{13}C NMR for Taxane **5** in $CDCl_3$

Position	δ ^{1}H mult.[a] (*J* in Hz)	δ^{13}C[b]	HMBC	NOESY[c]
1	1.96 o.m	51.6		2^{s}, $14a^{s}$, 16^{s}, 17^{s}
2	5.79 dd (*5.8, 1.4*)	68.4	8, 14, 169.2	1^{s}, 3^{w}, 9^{s}, 17^{s}, 19^{m}
3	3.05 d (*5.8*)	41.6	1, 7, 8, 19, 20	7^{s}, $14b^{s}$, 18^{s}
4	—	138.5		
5	5.50 dd (*~4.0, 1.5*)	76.4		$6a^{m}$, $6b^{m}$, $20b^{m}$
6a	2.10 o.m	34.9		5^{m}, $6b^{s}$, 7^{m}, $20b^{w}$
6b	1.86 o.m			$6a^{s}$, $5/7^{s}$, 19^{s}
7	5.54 o.t (*~5.8*)	69.0	3, 8, 19, 170.2	3^{s}, $6a/18^{s}$, 10^{s}
8	—	47.1		
9	5.98 d (*10.1*)	76.9	7, 8, 10, 19, 170.2	2^{s}, 17^{s}, 19^{m}
10	4.11 d (*10.1*)	71.1	9, 11, 12, 15	7^{s}, 18^{s}
11	—	66.3		
12	—	59.4		
13	—	209.2		
14a	2.69 dd (*20.8; 8.9*)	38.1	2, 12	1^{s}, $14b^{s}$, 16^{m}
14b	2.34 d (*20.8*)		1, 2, 13, 15	3^{s}, $14a^{s}$
15	—	38.0		
16	0.83 s	29.1	1, 11, 15, 17	1^{s}, $14a^{w}$, 17^{s}
17	1.83 s	25.4	1, 11, 15, 16	1^{s}, 2^{s}, 9^{s}, $14b^{w}$, 16^{s}
18	2.02 s	15.3	11, 12, 13	3^{s}, 7^{s}, 10^{s}, 2^{m}
19	1.08 s	13.5	3, 7, 8, 9	2^{m}, 9^{m}, 17^{m}
20a	5.57 s	120.6	3	$20b^{s}$
20b	5.11 s		3, 4, 5	5^{w}, $20a^{s}$
OAc	2.10 s	21.0	170.0	
OAc	2.06 s	21.2	170.1	
OAc	2.06 s	21.2	168.9	
1′	—	165.9		
2′	6.22 d (*16.0*)	116.3	1′, Ph-C1	
3′	7.68 d (*16.0*)	146.9	1′, 2′, Ph-o	
Ph′		134.1		
O	7.64 dd (*8.0, 2.1*)	128.4		
m, p	7.44 o.d, 7.38 o.m	129.0; 130.6		

[a]Mult. multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HMQC and HMBC experiments (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

between H-9 and H-10 (10.1 Hz) and the observed NOESY correlations of H-2/H-9, H-9/H_3-17 established a boat-chair conformation for ring-B, which is the typical taxane conformation. The β-orientation of H-2, H-5 and H-9 were assigned by the NOESY correlations of H-2/H_3-17, and H-9/H_3-17. The H-5 orientation can be deduced from the H-5/H-6b (and H-6b/H_3-19) and more importantly from the observation of a weak NOE between Me-18 and H-2′, an interaction due to the U-shape of the molecule in which Me-18 has NOE with protons on the α face of the molecule. The α-orientation of H-10 was obtained from the observation of the NOESY correlation of H-10/H_3-18 and H-10/H-7. The β-orientation of the epoxide group at C-11 and C-12 was established from the NOESY correlation of Me-18 with H-3, H-7 and H-10. The upfield chemical shift of Me-16 due to the presence of the C-11,C-12 epoxide near Me-16 suggested that the epoxide group had a β-orientation. From these data, the structure of **5** was established as 10β-hydroxy-2α,7β,9α-triacetoxy-5α-cinnamoy-11,12-epoxy-taxa-4(20)-en-13-one.

Unusual taxanes isolated from the needles of the Canadian yew (taxanes 6–8, Fig. 3, Tables 4–6). Two of these taxanes **6**, **7** have the usual C6/C8/C6 regular core skeleton whereas taxane **8** was shown to have C20 involved in a double bond with C4 in the B ring and C4 connected to C20, which in turn is connected to C8 leading to a C6/C10/C6 core skeleton. Taxane **6** was isolated as a colorless gum. Its ^{1}H NMR spectrum (Table 4) showed the characteristic signals of the taxane skeleton, including four tertiary methyl groups at δ1.02 (3H, s), 1.63 (3H, s), 1.97 (3H, s), and 1.12 ppm (3H, s) and three acetyl methyl groups at δ 2.10 (3H, *s*), 2.08 (3H, *s*), and δ 2.06 ppm (3H, s). A cinnamoyl group was suggested by the signals at δ 6.46 (1H, d, $J = 16.1$ Hz), 7.69 (1H, d, $J = 16.1$ Hz, *trans*-orientation), 7.53 (2H, m), and δ 7.39 (3H, m). Confirmation was derived from the presence of a prominent fragment peak at *m*/*z* 147, obtained from the loss of a cinnamoyl group in the mass spectra of **6**. The signals at δ 4.34 (1H) and 5.70 ppm (1H) with a large coupling constant ($J = 10.1$ Hz) were assigned to H-9 (a hydroxyl group) and H-10 (an acetoxy-group). The HMBC experiment confirmed this assignment. The two other acetyl groups were attached at C-2 and C-13 as deduced from the chemical shifts of corresponding protons and HMBC correlations. The presence of another AB system at δ 4.98 and 4.77 ppm with a large coupling constant of $J = 14.1$ Hz was attributed to the methylene of H-20. The signal at 125.3 ppm was correlated with the broad proton singlet at δ 5.79 ppm in the HSQC experiment, which revealed that C-4 double bond was endocyclic instead of exocyclic, as is usual in most natural taxoids. As this olefinic proton is coupled to H-6 protons in the COSY experiment, the signal at δ 5.79 was ascribed to H-5. The

Table 4. ^{1}H and ^{13}C NMR for Taxane **6** in $CDCl_3$

Position	δ ^{1}H mult.[a] (*J* in Hz)	δ^{13}C[b]	HMBC	ROESY[c]
1	1.71 br.d (*~9*)	46.3	3, 11, 15	2^{s}, $14a^{s}$, 16^{m}, 17^{m}
2	5.47 dd (*3.5, 1.0*)	71.9	1, 3, 8, 15, 169.3	1^{s}, 3^{w}, 9^{s}, 17^{s}, 19^{s}
3	3.31 br.s	44.4		2^{w}, 7^{m}, $14b^{m}$, 18^{m}, $20b^{w}$
4	—	133.3		
5	5.79 br.s	125.3		6^{s}, $20a^{m}$
6a,b	2.13 o.m	22.5		5^{s}, 7^{s}, 19^{s}
7a	2.13 o.m	26.8		3^{s}, 6^{s}, 10^{m}, 18^{m}
7b	1.27 o.m			
8	—	42.1		
9	4.34 d (*10.1*)	75.7	7, 8, 10, 19	2^{s}, 17^{s}, 19^{m}
10	5.70 d (*10.1*)	75.8	9, 11, 12, 15, 170.2	3^{m}, 18^{s}
11	—	136.1		
12	—	137.4		
13	5.59 br.d (*~10.5*)	69.1		$14a^{m}$, 16^{s}, 18^{w}
14a	2.76 ddd (*16.2, 10.0, 8.3*)	28.7		1^{s}, 13^{s}, $14b^{s}$, 16^{m}
14b	1.88 dd (*16.2, 3.2*)		1, 2, 13, 15	3^{s}, $14a^{s}$, $20b^{s}$
15	—	38.2		
16	1.01 s	32.7	1, 11, 15, 17	1^{s}, 13^{s}, $14a^{s}$, 17^{s}
17	1.63 s	25.8	1, 11, 15, 16	1^{s}, 2^{s}, 9^{s}, 16^{s}
18	1.97 s	15.6	11, 12, 13	3^{s}, 10^{s}
19	1.12 s	18.0	3, 7, 8, 9	
20a	4.98 d (*14.1*)	67.1		5^{s}, 6^{s}, $20b^{s}$
20b	4.77 d (*14.1*)		4, 166.6	3^{w},$14b^{m}$, $20a^{s}$
OAc	2.10 s	21.2	170.1	
OAc	2.08 s	20.9	170.3	
Oac	2.06 s	21.5	169.2	
1′	—	166.6		
2′	6.46 d (*16.1*)	117.9	1′, 3′, Ph-C1	
3′	7.69 d (*16.1*)	144.8	1′, 2′, Ph-o, Ph-C1	
Ph′		134.4		
o	7.53 m	128.0		
m, p	7.39 m	129.0; 130.4		

[a]Mult. multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HMQC and HMBC experiments (±0.2 ppm). The quaternary carbons are in bold characters.
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

cinnamoyl carbonyl carbon at δ 166.6 ppm is correlated in the HMBC experiment to proton H-20b and to H-2′ and H-3′, indicating that the cinnamoyl group is attached to C-20. Combined analysis of ^{1}H–^{1}H COSY, HSQC and HMBC spectral data permitted the assignment of the remaining proton and carbon signals of taxane **6**. In addition, two acetyls are connected to C-2 and C-13 hydroxyl groups. Therefore, the structure of **6** was unambiguously established to be 9α-hydroxy-2α,10β,13α-triacetoxy-20-cinnamoyloxy-taxa-4(5),11(12)-diene. The NOESY data (Table 4) proves the stereochemistry shown in Figure 3 for taxane **6**. High resolution mass spectrometry confirmed the elemental composition of the potassium quasimolecular ion of taxane **6**.

Taxane **7** was isolated as a colorless solid in a very low yield (0.00003%) from dried needles of the Canadian yew. The ^{1}H NMR spectrum of **7**, summarized in Table 5, exhibited the four-proton singlets due to the four methyl groups at δ 0.74, 1.10, 1.61, and 2.12 ppm. Three acetyl groups at relatively lower field (δ 2.00, 2.04 and 2.12 ppm) in the ^{1}H NMR were confirmed by the ^{13}C NMR signals at δ 20.4, 169.7; 20.2, 170.2; 20.8, 170.3 ppm of the corresponding methyl and carbonyl groups. The HMBC suggested that **7** had a taxane-type core skeleton. The HMBC correlations of H_3-18 to C-11, C-12, and C-13 and H_3-16 and H_3-17 to C-1, C-11 and C-15 revealed that Me-18 is connected to the olefinic carbon C-12 and Me-16 and Me-17 to C-15, implying that compound **7** has a regular 6/8/6-membered ring system. The ^{1}H NMR signals at δ 4.86 (1H, br.s), 5.23 ppm (1H, br.s), correlated to the ^{13}C signal at 113.4 ppm in the HSQC are characteristic of an exocyclic methylene group while the proton at δ 3.04 ppm (1H, d, $J=6.1$ Hz) is assigned to H-3 ring junction in a taxa-4(20),11-diene.[2,3] Using H-3 as a starting point, the connectivities from C-3 to C-2, C-1, C-14 and C-13 were deduced from the ^{1}H–^{1}H COSY spectrum. Similarly, the spin system from H-5 to H-6, and H-6 to H-7 was easily interpreted from ^{1}H–^{1}H COSY spectrum. The isolated AB system resonating at δ 5.86 and 6.06 ppm (each 1H, $J=10.6$ Hz) was attributed to H-9 and H-10, respectively. The presence of a cinnamoyloxy moiety in **7** was revealed by the signals at δ 6.14 (1H, *d*, $J=12.7$ Hz), 6.93 (1H, d, $J=12.7$), 7.65 (2H, m), and δ 7.3–7.4 (3H, m) in the ^{1}H NMR spectrum. Furthermore, the fragment at *m/z* 131 (C_9H_7O) and $(M–cinn)^+$ corresponding to the fission of a cinnamoyl group from the molecular ion was observed in the mass spectra. The unusual chemical shifts of H-2′ and H-3′, which are observed as an AX spin system with a coupling constant $J=12.7$ Hz instead of the normal ca. $J=16$–17 Hz, indicated that the double bond in the cinnamoyl group was of the *cis*-configuration.[14] Chemical shifts of H-5β, H-9β, and H-10α suggested that three acetyl groups were attached at C-5, C-9, and C-10. Indeed, we could

Table 5. ^{1}H and ^{13}C NMR for Taxane **7** in $CDCl_3$

Position	δ ^{1}H mult.[a] (*J* in Hz)	δ ^{13}C[b]	HMBC	ROESY[c]
1	1.83 o.m	39.6		
2	1.80 o.m	28.3		
3	3.04 br.d (*6.1*)	37.9		18^{s}, $14b^{w}$
4[d]	—			
5	5.41 br.t (∼*2.7*)	75.4		$20a^{s}$
6a	1.87 o.m	26.7		
6b	1.70 o.m			
7	1.80 o.m	27.1		
8	—	43.1		
9	5. 86 o.d (*10.6*)	76.7	7, 8, 10, 19, 170.3	17^{s}, 19^{w}
10	6.06 d (*10.6*)	71.8	9, 11, 12, 15, 169.7	18^{s}
11	—	134.7		
12	—	137.0		
13	5.84 o.m	69.9		$14a^{m}$, 16^{s}, 18^{w}
14a	2.72 dt (*14.6, 10.0*)	31.4	2, 12, 13	13^{s}, $14b^{s}$, $1/2^{s}$
14b	1.08 o.dd (*14.6, 7.0*)			See 16
15	—	40.1		
16	1.10 s	30.6	1, 11, 15, 17	1^{s}, 3^{m}, 13^{s}, $14a^{s}$, 17^{s}
17	1.61 s	26.5	1, 11, 15, 16	9^{s}, 16^{m}
18	2.12 s	14.4	11, 12, 13	3^{w}, 10^{s}, $2'^{s}$
19	0.74 s	17.1	3, 7, 8, 9	9^{m}, 2^{s}, 6^{s}, 7^{s}
20a	5.23 br.s	113.4		5^{s}, $20b^{s}$
20b	4.86 br.s		3	$20a^{s}$, 1^{w}, 2^{w}, 6^{w}, 7^{w}
OAc	2.12 s	20.8	170.3, 9	
OAc	2.04 s	20.2	170.2	
OAc	2.00 s	20.4	169.7, 10	
1′	—	164.8		
2′	6.14 d (*12.7*)	119.4		$3'^{s}$, 18^{w}
3′	6.93 d (*12.7*)	143.5		$2'^{s}$, 18^{w}
Ph-4′	—			
o	7.65 dd (*7.7, 1.7*)	129.3		
m, p	7.44–7.30 o.m	127.4, 128.6		

[a]Mult. multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HMQC and HMBC experiments (±0.2 ppm). The quaternary carbons are in bold characters.
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).
[d]The C-4 chemical shift could not be obtained from the HMBC experiment.

observe long-range H/C correlations of H-9 and H-10 protons with acetyl carbonyls in the HMBC. The H-5 and H-13 position are both deshielded either by an acetoxy group or a cinnamoyloxy group. As the HMBC do not reveal the position of the two remaining groups, we obtained confirmation of their position using ROESY. In the ROESY experiment we observed a strong ROE interaction between Me-18 and H-2′ and H-3′. This confirms that the remaining cinnamoyl group was connected at C-13. Comparison with other analogues also confirmed this positioning.[15,16] Based on the above analysis, the structure of **7** was assigned to be 5α, 9α,10β-triacetoxy-13α-*E*-cinnmaoyloxy-taxa-4(20),11-diene. The relative stereochemistry of **7** was obtained in a ROESY experiment and is shown in Figure 3.

Taxane **8** was another minor metabolite isolated as a white powder from the needles of the Canadian yew after purification. High resolution mass spectrometry showed that the elemental composition of the potassium quasimolecular ion of **8** was $C_{35}H_{42}O_9$ corresponding to *m/z*: 645.2764. The ^{1}H and ^{13}C NMR spectrum of **8** showed the characteristic signals of the taxane skeleton, including four tertiary methyl groups, three acetyl groups as well as a cinnamoyloxy-group. The UV absorption at 278 nm in HPLC analysis further supported the presence of the cinnamoyl group. In the ^{1}H NMR spectrum, **8** lacked the characteristic signal associated with H-3α, which usually appeared at ∼δ 2.3–3.6 ppm in most taxanes.[11,12] Moreover, neither a pair of signals corresponding to the AX system of an exocyclic methylene protons nor an AB quartet (at about δ 4.2 ppm with a coupling constant of ∼9 Hz) corresponding to an oxetane ring was observed in the ^{1}H NMR spectrum. Instead, an isolated AX spin system at δ 1.82 and 2.76 ppm with a coupling constant $J = 15.2$ Hz was observed. In the HSQC spectrum, one of the olefinic carbons at δ 122.1 ppm carried one proton (at δ 5.39 ppm), suggesting the presence of an endocyclic double bond. In the COSY experiment, this olefinic proton is correlated to H-2 (δ 5.80 ppm), which in turn is correlated to H-1 (δ 1.73), which is further correlated to H-14 (δ2.70 and δ 1.86 ppm) which finally is coupled to the acetylated H-13. These correlations prove that the olefinic H-3 proton belongs to the B ring. The skeleton of taxane **8** was characterized as a 6/10/6-ring system with a C-3/C-4 endocyclic double bond, that is, a taxane derivative as exemplified by taxine A derivatives.[17,18] The HMBC correlation of the Me-19 with the isolated methylene C-20 as well as with a ketone assigned as C-9 further confirm the taxine skeleton. The coupling constants and the NOESY spectra enabled us to establish the stereochemistry of the protons of taxane **8** as H-2β, H-5β, H-10α, and H-13β in accord with

Table 6. ^{1}H and ^{13}C NMR for Taxane **8** in $CDCl_3$

Position	δ ^{1}H mult.[a] (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY[c]
1	1.73 dd (*8.3, 1.9*)	47.1		2^{s}, $14a^{s}$, 16^{s}, 17^{s}
2	5.80 dd (*10.0, 1.8*)	70.8		1^{s}, 3^{w}, $17/19^{s}$, $3a^{s}$
3a	2.76 d (*15.2*)	35.7	4, 7, 8, 9	2^{s}, $3b^{s}$, $17/19^{s}$
3b	1.82 o.d (*15.2*)			
4	—	135.4		
5	5.57 br.s	72.3		$6b/3b^{s}$, $6a/7a^{m}$
6a	2.13 o.m	28.9		5^{s}, $6b^{s}$, $7b^{s}$, 10^{s}
6b	1.84 o.m			
7a	2.07 o.m	29.3		See 6a
7b	1.58 o.m			$6a/7a^{s}$, $14b/3b^{s}$
8	—	50.2		
9	—	206.6		
10	6.34 s	78.1	9, 11, 12, 15, 169.6	20^{w}, $6a/7a^{s}$, 18^{s}
11	—	130.5		
12	—	136.9		
13	5.39 o.m	69.2		$14a^{s}$, 16^{s}
14a	2.70 o.ddd (*16.1,8.9,10.9*)	27.5		1^{s}, 13^{s}, $14b^{s}$, 16^{s}
14b	1.86 o.dd (*16.1, 3.4*)			
15	—	37.8		
16	1.10 s	32.2	1, 11, 15, 17	1^{s}, 13^{s}, $14a^{s}$
17	1.27 s	25.4	1, 11, 15, 16	See 19
18	1.92 s	17.0	11, 12, 13	
19	1.25 s	27.0	7, 8, 9, 20	1^{s}, 2^{s}, $3a^{s}$
20	5.39 o.m	122.1		$14b^{s}$, 18^{s}
OAc	2.06 s	20.8	169.7	
OAc	2.00 s	21.3	170.3	
OAc	1.99 s	21.4	169.9	
1′	—	166.6		
2′	6.51 d (*16.0*)	118.1	1′, Ph-C1	18^{w}
3′	7.81 d (*16.0*)	145.7	1′, Ph-o	
Ph′		133.9		
O	7.50 m	128.1	3′	
m	7.40 o.m	129.1		
p	7.38 o.m	130.5		

[a]Mult. multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HMQC and HMBC experiments (±0.2 ppm). The quaternary carbons are in bold characters.
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

taxine A and most natural taxanes.[11,12,16] In the NOESY experiment, we observe correlation of H-2 with H-1, Me-17/Me-19 indicating their β relationship. In addition, the correlations of H-13 with H-14a and Me-16, and H-1 with H-14a, Me-16 and Me-17 suggest a β-configuration for H-13. NOESY correlations among H-10, H-7, H-3 and Me-18 in **8** implied that H-10 was α-oriented. These findings were consistent with an unusual cage conformation previously reported for taxuspine B and other taxin B derivatives.[19,20] Thus the 3-D-structure of **8** is shown in Figure 4. Taxane **8** was therefore characterized unambiguously as 5α-cinnamoyloxy-2α, 10β,13α-triacetoxy-2(3→20)*abeo*taxa-3(4),11-dien-9-one.

Results of bioactivity. These taxanes **1**–**8** showed no significant effect on tubulin assembly.[21,22] In 1979, Horwitz and co-workers[23] found that paclitaxel, unlike usual anti-cancer agents, promotes the assembly of tubulin to form super-stable microtubules by binding to tubulin. This activity short-circuits cell division and leads to apoptosis. A study of numerous paclitaxel analogues[24] established the key features essential for this bioactivity: a C-13 side chain with a C-2′-OH, a benzoyl group on C-2 and an oxetane on C-4–C-5. In 1999[5,25] we isolated and characterized from the needles of the Canadian yew two taxanes differing in the substituents and conformation of ring A, taxuspine D with a double bond on C12–C13 (instead of the usual C11–C12) or with an extra oxygenated ring taxagifine. They have no C-13 side chain but have a *trans*-cinnamoyl group on C-5. We were surprised to find that they promoted the polymerization of tubulin at the level of 1/3 to 1/2 the activity of paclitaxel. This result was confirmed by Kobayashi and co-workers[26,27] who found these taxanes in the stems of the Japanese yew. We found from molecular modeling studies[7] that the bioactivity of taxuspine D probably derives from the C-5 cinnamoyl which occupies a similar position in the binding of β-tubulin as the C-13 side chain of paclitaxel. We therefore concluded that by appreciably altering the conformation of the core structure of taxanes, the C-13 side chain requirement for bioactive taxanes could be replaced by an appropriate side chain on C-5. The lack

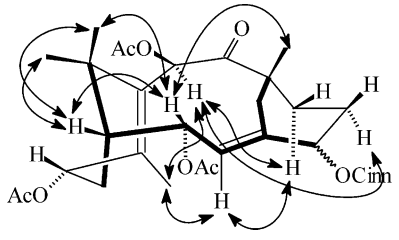

Figure 4. NOESY analysis of taxane **8**.

of bioactivity of taxanes **1**–**8** cannot be explained by the foregoing hypothesis. Work is in progress to elucidate this discrepancy.

Conclusion

In this publication, we have discovered in the needles of the Canadian yew novel taxanes with modified core skeletons (taxanes **1**, **2** and **8**) or unusual functional groups (oxirane at C11–C12, a C-13-cinnamoyloxy with a *cis*-configuration, double bonds at C3–C4 or C4–C5) and side chains at C-5, C-13 or C-20). The diversity of these taxane structures shows us the multitude of enzymatic reactions involved in their biosyntheses.

Experimental

Instrumentation

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 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 work up procedures. Analytical HPLC was performed on a Waters 600 FHU delivery system coupled to a PDA 996 detector. Preparative and semi-preparative HPLC were carried out on a Waters Delta Prep 3000 instrument coupled to a UV 486 Tunable Absorbance detector set at 227, 210 or 287 nm (Waters, Montreal, Quebec, Canada). Analytical HPLC was performed with two Whatman partisil 10 ODS-2 analytical columns (4.6×250 mm) in series. Semi-preparative HPLC was performed with two Whatman partisil 10 ODS-2 Mag-9 semi-preparative columns (9.4×250 mm) in series. Preparative HPLC was performed with one partisil 10 ODS-2 MAG-20 preparative column (22×500 mm). The products were eluted with a 50 min linear gradient of acetonitrile (25–100%) in water at a flow rate of 18 mL/min (preparative HPLC) and 3 mL/min (semi-preparative HPLC). All the reagents and solvents were of the best available commercial quality and were used without further purification.

NMR and mass spectrometry measurement

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 Fast Atom Bombardment Mass Spectra (FAB-MS) were obtained with a Vacuum Generators ZAB-HS double-focussing 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. FABHRMS was similarly obtained in glycerol–DMSO at a resolving power of 12,000.

Extraction, isolation and purification of taxanes. Air-dried needles of *Taxus canadesis* were ground (4.0 kg) and extracted with 24 L of methanol for one day at room temperature. The ground plants were filtered and extracted again with fresh solvent for another three times (each time with 8 L solvent, total 24 L) in three days. The combined organic extracts were evaporated under reduced pressure. Water (3 L) was added and lipids were removed by stirring the mixture with hexane (3×3 L). The hexane fraction was condensed into 1500 mL and extracted with 80% methanol four times (each 500 mL). The 80% methanol extract, after re-extracted with hexane two times (each 300 mL), was evaporated under reduced pressure and 1000 mL of water was added and extracted with ethyl acetate for three times (each 700 mL). The combined ethyl acetate extracts were dried with anhydrous sodium sulfate, filtered and evaporated yielding a dark brown extract 25 g. The aqueous phase was then salted (NaCl, 200 g) and extracted with CH_2Cl_2 (4×3 L). The combined CH_2Cl_2 extracts were dried with anhydrous sodium sulfate, filtered and evaporated yielding a dark green extract 115 g.

The ethyl acetate extract (25 g) was dissolved in 55 mL acetone and absorbed onto 40 g silica gel and subjected to a normal phase column chromatography (silica gel 230–400 mesh, 850 g, 25×9 cm) with elution with a mixture of CH_2Cl_2 and MeOH (1800:200, 1800:300, 1800:360, 1600:400, 700:300 v/v). Twenty seven fractions were obtained: $Fr_{E\text{-}1}$ to $Fr_{E\text{-}27}$.

$Fr_{E\text{-}1}$ (220 mg) was applied to 4 pre-coated TLC plates (20×20 cm, thickness 0.5 mm) and developed with a mixture of hexane/EtOAc (7:4). When the solvent reached the top of the TLC plate, it was separated into 9 bands detected at λ 254 nm with a UV lamp. The top band (band 9) eluted with acetone and gave 5 mg of residue after filtration and evaporation, and the residue was further purified with semi-preparative HPLC. The elution with a linear gradient of acetonitrile in water from 25 to 100% in 50 min at a flow rate of 3 mL/min yielded 2.5 mg of taxane **7** at a $t_R = 59.32$ min (analytical HPLC $t_R = 55.02$). $Fr_{E\text{-}2}$ (1.9 g) was dissolved in 5 mL acetone and absorbed onto 5 g silica gel and subjected to a normal phase column chromatography (silica gel 230–400 mesh, 100 g, 29×3 cm) and eluted with a mixture of hexane/EtOAc (600:400 mL). Twenty fractions were obtained ($Fr_{E\text{-}2\text{-}1}$–$Fr_{E\text{-}2\text{-}20}$). $Fr_{E\text{-}2\text{-}2}$ (400 mg) was subjected to preparative HPLC, eluted with a linear gradient of acetonitrile in water from 25 to 100% in

50 min at a flow rate of 18 mL/min. The material eluted at $t_R = 41.92$ min was collected, concentrated (33 mg) and applied to a preparative TLC (2×20×20 cm, thickness 0.25 mm). Development with a mixture of hexane/ethyl acetate (7:4) yielded taxane **8** (3.2 mg, $R_f = 0.44$). $Fr_{E\text{-}2\text{–}8}$ (145 mg) was subjected to preparative TLC (2.3×20×20, thickness 0.5 mm) and developed with CH_2Cl_2:$CNCH_3$ (100:7), the band at $R_f = 0.14$ was collected, eluted with acetone, filtered, dried and evaporated to yield a residue 49 mg. Further purification with preparative HPLC gave pure taxane **5** (1.2 mg, $t_R = 42.50$ min).

A portion of the methylene chloride extract (50 g) was absorbed onto 110 g silica gel and packed on a wet column chromatography (silica gel 230–400 mesh, 1320 g). Successive elution with CH_2Cl_2–MeOH gradient with increasing amounts of methanol from 5 to 45% (total 15 L) yielded 45 fractions ($Fr_{D\text{-}1}$ to $Fr_{D\text{-}45}$). The fractions 18–24 were pooled (3.5 g) after monitored by TLC and chromatographed over silica gel (195 g, 4.2×32 cm). Elution with hexane/ethyl acetate (600:500, 600:600, 500:600 and 400:700 mL) led to 23 fractions ($Fr_{D\text{-}18\text{–}1}$ to $Fr_{D\text{-}18\text{–}23}$). The $Fr_{D\text{-}18\text{–}4}$ (145 mg) was further separated by preparative HPLC and yielded pure taxane **6** (12 mg, $t_R = 49.02$ min). The $Fr_{D\text{-}18\text{–}6}$ and $Fr_{D\text{-}18\text{–}7}$ were combined (1.36 g) and packed on a wet column chromatography (silica gel 230–400 mesh, 60 g). Successive elution with CH_2Cl_2–$CNCH_3$ gradient with increasing amounts of $CNCH_3$ from 8.5 to 15% (total 2.5 L) yielded 24 fractions ($Fr_{D\text{-}18\text{–}6\text{-}1}$ to $Fr_{D\text{-}18\text{–}6\text{-}24}$). The $Fr_{D\text{-}18\text{–}6\text{-}20}$ and $Fr_{D\text{-}18\text{–}6\text{-}21}$ were combined (83 mg) and subjected to preparative HPLC. The material eluting at $t_R = 39.85$ min was collected and concentrated (5 mg) and further purified by preparative TLC (0.3×20×20 cm, thickness 0.25 mm). It was developed with a mixture of CH_2Cl_2/$CNCH_3$ (100:20, v/v) and yielded pure taxane **3** (1.8 mg, $R_f = 0.57$). The compound eluting at $t_R = 42.26$ min was identified as taxane **4** (15.5 m). The $Fr_{D\text{-}18\text{–}10}$ (78 mg) was applied to preparative TLC (CH_2Cl_2/$CNCH_3$, 100:8) and cut into 4 bands identifiable by a UV lamp. The band at $R_f = 0.14$ was further purified by preparative HPLC to give taxane **2** (2.8 mg, $t_R = 40.97$ min). The $Fr_{D\text{-}33}$ (700 mg) was separated with column chromatography (silica gel 57 g, 2.5×28 cm) and eluted with hexane/acetone (1300:800 mL) and yield 12 fractions ($Fr_{D\text{-}33\text{–}1}$ to $Fr_{D\text{-}33\text{–}12}$). The $Fr_{D\text{-}33\text{–}9}$ (37 mg) was further separated by preparative HPLC and obtained **1** (2.1 mg, $t_R = 43.84$ min).

2α, 9α-Dihydroxy-10β-acetoxy-5α-cinnamoyloxy-3(11)cyclotaxa-4(20)-ene-13-one, 1 (Scheme 1). $[\alpha]_D^{22}$ +57.0° (*c* 0.7, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, *J* = 16.0 Hz, 1H, H-3′), 7.55 (m, 2H, o-Ph′), 7.38 (m, 2H, m-Ph′), 7.38 (p, 1H, p-Ph′), 6.39 (d, *J* = 16.0 Hz, 1H, H-2′), 5.81 (s, 1H, H-20a), 5.65 (s, 1H, H-20b), 5.60 (t, *J* = 9.0 Hz, 1H, H-5), 5.32 (d, *J* = 9.5 Hz, 1H, H-10), 5.07 (d, *J* = 5.0 Hz, 1H, H-2), 4.37 (d, *J* = 9.5 Hz, 1H, H-9), 3.53 (q, 1H, *J* = 7.4 Hz, 1H, H-12), 2.80 (d, *J* = 20.8 Hz, 1H, H-14a), 2.50 (dd, *J* = 20.8, 7.7, 1H, H-14b), 2.20 (om, 1H, H-6a), 2.15 (s, 3H, OAc), 1.99 (dd, *J* = 7.5, 5.2 Hz, 1H, H-1), 1.92 (om, 1H, H-7a), 1.70 (om, 1H, H-6b), 1.46 (s, 3H, H-17), 1.41 (s, 3H, H-19), 1.32 (om, 1H, H-7b), 1.30 (od, 3H, H-18), 1.21 (s, 3H, H-16); ^{13}C NMR (125 MHz, $CDCl_3$) δ 215.3 (C-13), 172.6 (C-10, OAc), 165.9 (C-1′), 145.3 (C-3′), 143.5 (C-4), 134.3 (Ph′), 130.2 (Ph′-p), 128.8 (Ph′-m), 128.1 (Ph′-o), 126.5 (C-20), 117.7 (C-2′), 84.2 (C-10), 82.4 (C-9), 75.8 (C-5), 75.6 (C-2), 66.7 (C-3), 58.4 (C-11), 51.7 (C-12), 50.7 (C-1), 45.2 (C-8), 42.7 (C-15), 38.1 (C-14), 29.4 (C-7), 29.3 (C-17), 26.6 (C-16), 25.8 (C-6), 25.2 (C-19), 21.2 (OAc-CH_3), 15.7 (C-18); FAB-HR-MS: $C_{31}H_{38}O_7K$ $[M+K]^+$ required: 561.2255, found: 561.2255.

2α-Hydroxy-9α,10β-diacetoxy-5α-cinnamoyloxy-3,11-cyclotaxa-4(20)-ene-13-one, 2 (Scheme 1, Table 1). Gum; $[\alpha]_D^{22} = +122$ (*c* 0.03, $CHCl_3$); FAB-HR-MS for $C_{33}H_{42}O_8K$ $[M+K]^+$ requires 605.2517, found: 605.2517.

9α,13α-dihydroxy-2α,10β-diacetoxy-5α-cinnamoyloxy-taxa-4(20),11-diene, 3 (Scheme 1, Table 2). Gum; $[\alpha]_D^{22} = +26$ (*c* 0.05, $CHCl_3$); FAB-HR-MS for $C_{33}H_{40}O_8K$ $[M+K]^+$ requires 603.2360, found: 603.2359.

9α,10β-Dihydroxy-2α-acetoxy-5α-cinnamoyloxy-taxa-4(20),11-diene-13-one, 4 (Scheme 1). Gum; $[\alpha]_D^{22} = +110$ (*c* 0.05, $CHCl_3$), ^1H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H, o-Ph′), 7.64 (d, *J* = 16.0 Hz, 1H, H-3′), 7.44 (t, *J* = 7.7 Hz, 2H, m-Ph′), 7.39 (m, 1H, p-Ph′), 6.43 (d, *J* = 16.0 Hz, 1H, H-2′), 5.51 (dd, *J* = 6.2, 1.8 Hz, 1H, H-2), 5.32 (ot, *J* ~ 2.7 Hz, 1H, H-5), 5.31 (s, 1H, H-20a), 4.89 (d, *J* = 9.1 Hz, 1H, H-10), 4.84 (s, 1H, H-20b), 4.18 (d, *J* = 9.1 Hz, 1H, H-9), 3.36 (d, *J* = 6.1 Hz, 1H, H-3), 2.83 (dd, *J* = 20.0, 7.0 Hz, 1H, H-14a), 2.42 (d, *J* = 20.0, 1H, H-14b), 2.16 (s, 3H, H-18), 2.15 (om, 1H, H-1), 2.06 (s, 3H, OAc), 1.97 (br ddd, *J* = 14.3, 4.9, 2.4, 1H, H-6a), 1.79 (m, 1H, H-7a), 1.74 (om, 1H, H-6b), 1.70 (s, 3H, H-17), 1.50 (m, 1H, H-7b), 1.22 (s, 3H, H-16); 1.10 (s, 3H, H-19), ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.0 (C-13), 170.0 (C-2, OAc), 166.6 (C-1′), 155.5 (C-11), 145.4 (C-3′), 142.8 (C-4), 136.2 (C-12), 134.7 (Ph′), 130.3 (Ph′-p), 128.7 (Ph′-m), 128.2 (Ph′-o), 117.8 (C-2′), 116.4 (C-20), 78.5 (C-5), 77.6 (C-9), 73.2 (C-10), 69.6 (C-2), 48.8 (C-1), 44.7 (C-8), 43.0 (C-3), 38.7 (C-15), 37.4 (C-16), 36.1 (C-14), 28.3 (C-6), 26.2 (C-7), 25.1 (C-17), 21.4 (OAc-CH_3), 17.62 (C-19), 13.9 (C-18); FAB-HR-MS: $C_{31}H_{38}O_7K$ $[M+K]^+$ required: 561.2255, found: 561.2255. FAB-HR-MS: $C_{31}H_{38}O_7$ $[M+H]^+$ required: 523.2696, found: 523.2694.

10β-Hydroxy-2α,7β,9α-triacetoxy-5α-cinnamoyloxy-11,12-epoxy-taxa-4(20)-ene-13-one, 5 (Scheme 1, Table 3). Gum; $[\alpha]_D^{22} = +135$ (*c* 0.02, $CHCl_3$); FAB-HR-MS for $C_{35}H_{42}O_{11}K$ $[M+K]^+$ requires 677.2364, found: 677.2366.

9α-Hydroxy-2α,10β,13α-triacetoxy-20-cinnamoyloxy-taxa-4(5),11(12)-diene, 6 (Scheme 3, Table 4). Gum; $[\alpha]_D^{22} = +200$ (*c* 0.07, $CHCl_3$); HR-FAB-MS: $C_{35}H_{44}O_9K$ $[M+K]^+$ required: 647.2622, found: 647.2623.

5α,9α,10β-Triacetoxy-13α-*E*-cinnamoyloxy-taxa-4(20),11-diene, 7 (Scheme 3, Table 5). Gum;

$[\alpha]_D^{22} = +125$ (c 0.02, $CHCl_3$); FAB-HR-MS: $C_{35}H_{44}O_8K$ $[M+K]^+$ required: 631.2677, found: 631.2670.

5α-Cinnamoyloxy-2α,10β,13α-triacetoxy-2(3→2)*abeo*-taxa-4(20),11-dien-9-one, 8 (Scheme 3, Table 6). White power; $[\alpha]_D^{22} = -126$ (c 0.05, $CHCl_3$); FAB-HR-MS: $C_{35}H_{42}O_9K$ $[M+K]^+$ required: 645.2766, found: 645.2764.

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References and Notes

1. Zamir, L. O.; Nedea, M. E.; Belair, S.; Sauriol, F.; Mamer, O.; Jacqmain, E.; Jean, F. I.; Garneau, F.-X. *Tetrahedron Lett*. **1992**, *33*, 5173.
2. Zamir, L. O.; Nedea, M. E.; Zhou, Z.-H.; Belair, S.; Caron, G.; Jacqmain, E.; Jean, F. I.; Garneau, F.-X.; Mamer, O. *Can. J. Chem*. **1995**, *73*, 655.
3. Zhang, J.; Sauriol, F.; Mamer, O.; You, X.-L.; Alaoui-Jamali, M.; Batist, G.; Zamir, L. O. *J. Nat. Prod*. **2000**, *64*, 450 and references therein.
4. Gunawardana, G. P.; Premachandran, U.; Burres, N. S.; Whittern, D. N.; Henry, R.; Spanton, S.; McAlpine, J. B. *J. Nat. Prod*. **1992**, *55*, 1686.
5. Zamir, L. O.; Zhang, J.; Wu, J.-H.; Sauriol, F.; Mamer, O. *J. Nat. Prod*. **1999**, *62*, 1268.
6. Zamir, L. O.; Zhang, J.; Wu, J.-H.; Sauriol, F.; Mamer, O. *Tetrahedron* **1999**, *55*, 14323.
7. Wu, J.-H.; Zamir, L. O. *Anti-Cancer Drug Des*. **2000**, *15*, 73.
8. Wu, J.-H.; Zamir, L. O. *Anti-Cancer Drug Des*. **2000**, *15*, 441.
9. Nikolakakis, A.; Wu, J.-H.; Batist, G.; Sauriol, F.; Mamer, O.; Zamir, L. O. *Bioorg. Med. Chem*. **2002**, in press.
10. Shi, Q.-W.; Oritani, T.; Sugiyama, T.; Murakami, R.; Yamada, T. *J. Asia Nat. Prod. Res*. **1999**, *2*, 71.
11. Appendino, G. In *The Chemistry and Pharmacology of Taxol and Its Derivatives*, Farina, V., Ed.; Amsterdam, 1995; Vol. 22, pp 55–102.
12. Balologlu, E.; Kingston, D. G. I. *J. Nat. Prod*. **1999**, *62*, 1448; Kingston, D. G. I.; Molinero, A. A.; Rimoldi, J. M. In *Progress in the Chemistry of Organic Natural Products*, Herz, W.; Kirby, G.W.; Moore, R.E.; Steglich, W.; Tamm, C. H., Eds.; Springer, 1993, Vol. 61, p1.
13. Parmer, V. S.; Jha, A.; Bisht, K. S.; Taneja, P.; Sight, S. K.; Kumar, A.; Poonam, J. R.; Olsen, C. E. *Phytochemistry* **1999**, *50*, 1267.
14. Shen, Y.; Chen, C.; Hung, M. *Chem. Pharm. Bull*. **2000**, *48*, 1344.
15. Yeh, M. K.; Wang, J. S.; Liu, L. P.; Chen, F. Q. *Phytochemistry* **1988**, *27*, 1534.
16. Shinozaki, Y.; Fukamiya, N.; Fukushima, M.; Okano, M.; Mehira, T.; Tagahara, K.; Zhang, X.; Zhang, D.; Lee, K. *J. Nat. Prod*. **2001**, *64*, 1073.
17. Graf, E.; Kirfel, A.; Wolff, G. J.; Breitmaier, I. *Liebigs Ann. Chem*. **1982**, 376.
18. Appendino, G.; Cravotta, G.; Enriu, R.; Jakupovic, J.; Garibaldi, P.; Gabetta, B.; Bombardelli, E. *Phytochemistry* **1994**, *36*, 407.
19. Yue, Q.; Fang, Q. C.; Liang, X. T.; He, C. H.; Jing, X. L. *Planta Medica* **1995**, *61*, 375.
20. Kobayashi, J.; Ogiwara, A.; Hosoyama, H.; Shigemori, H.; Yoshida, N.; Sasaki, T.; Li, Y.; Iwasaki, S.; Naito, M.; Tsuruo, T. *Tetrahedron* **1994**, *50*, 7401.
21. We thank Dr. Haidera Khadidja in our group for the tubulin assay performed as described in ref 22.
22. Robley, C.; Williams, J.; James, C. L. *Methods in Enzymol*. **1982**, *85*, 376.
23. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665.
24. Kingston, D. G. I. *ACS Symposium Series* **1995**, *583*, 204.
25. Zamir, L. O.; Zhang, J.; Wu, J.-H.; Sauriol, F.; Mamer, O. *Tetrahedron* **1999**, *55*, 14323.
26. Kobayashi, J.; Hosoyama, H.; Shigemori, H.; Koiso, Y.; Iwasaki, S. *Experientia* **1995**, *51*, 592.
27. Kobayashi, J.; Hosoyama, H.; Katsui, T.; Yoshida, N.; Shigemori, H. *Tetrahedron* **1996**, *52*, 5391.