Five Novel Taxanes from *Taxus canadensis*

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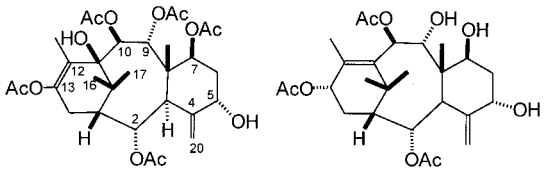
Five novel taxanes (**1**−**5**) have been isolated from the needles of *Taxus canadensis*, and their structures were elucidated on the basis of spectroscopic data. Two of these taxanes have structural features seldom found in yews, with a C-12, C-13 double bond and a hydroxymethylene at C-4. In addition, the 2D structures generated by molecular modeling of taxanes **1** and **2**, differing mostly in the location of the double bond in ring A, were compared. The different conformation of ring A derived from the C-12, C-13 double bond is particularly interesting because it implies that if a Taxol (paclitaxel) side chain were attached to C-13 it would have a different orientation from that in paclitaxel.

Since the discovery of the promising anticancer activity of Taxol® (paclitaxel), a unique diterpene alkaloid originally extracted from the bark of the pacific yew *Taxus brevifolia*, a large number of taxanes possessing various skeleton systems have been isolated from different yew species.[1,2] *Taxus canadensis* Marsh. (Taxaceae) has been shown to have specific taxanes not always found in other yews. 9-Dihydro-13-acetylbaccatin III,[3,4] five to seven times more abundant than paclitaxel, and the bicyclic[5−7] canadensene, epicanadensene, isolated from the needles of the Canadian yew, are the most striking examples. It is therefore important to try to elucidate the different taxanes produced by this species. In our continuing investigation on the metabolites of the needles of *T. canadensis*, we have previously reported 21 taxanes. [3−9] In this article, the isolation and structure elucidation of another five new taxanes (**1**−**5**) are reported.



1

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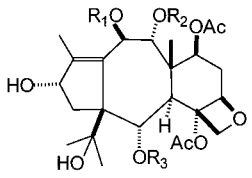
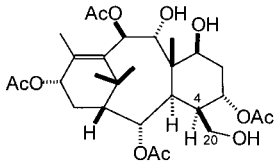


3 $R_1=R_2=H$, $R_3=Bz$
4 $R_1=R_2=R_3=Ac$



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Results and Discussion

Compound **1** was shown to have the composition $C_{30}H_{42}O_{12}$ from HRFABMS analysis. Its ^{1}H NMR, COSY, and HMQC spectra (^{1}H and ^{13}CNMR data, see Table 1) revealed the presence of five acetyl groups and four methyls (on quaternary carbons), two of which overlapped at the same chemical shift (δ 1.46 ppm). Two of the methyl singlets (δ 1.17 and 1.46 ppm) were COSY-correlated peaks, which means that they are geminal methyls (Me-16 and Me-17 located at C-15). The presence of two aliphatic methylene groups (δ 2.20 and 1.78 ppm, 6-2H and δ 2.57 and 2.38 ppm, 14-2H) and an olefinic type of methylene group (a pair of broad singlets at δ 5.27 and 5.04 ppm, 20-2H) were also observed. In the deshielded region, five protons (δ 5.72, 4.22, 4.72, 4.70, and 5.57 ppm) located on oxygenated carbons (C-2, -5, -7, -9, and -10) were identified through the analysis of their COSY correlations with the adjacent protons.

In the HMBC experiment on **1**, it was revealed that the two *gem*-dimethyl protons were both coupled to the same three carbons: the C-1 protonated carbon (δ 50.1 ppm) and two quaternary alkyl carbons (δ 78.4 and 40.9 ppm). These two quaternary carbons were assigned to C-11 and C-15, respectively. From the carbon chemical shifts of C-11, the presence of an oxygenated quaternary carbon was suspected. One methyl singlet (Me-18, δ 1.55 ppm) was correlated to the quaternary saturated carbon (δ 78.4 ppm) assigned to C-11 and was also coupled to two olefinic quaternary carbons (δ 124.0 and 144.1 ppm) assigned to C-12 and C-13, respectively. The very deshielded position observed for one of the olefinic carbons (C-13) indicated the presence of an acetoxy group. The other four acetoxy groups were revealed at the C-2, -7, -9, and -10 positions. One of the hydroxyl protons appearing as a relatively sharp singlet (δ 2.14 ppm) displayed HMBC correlations to the protonated C-10 and to three quaternary carbons, C-11, -12, and -13. These observations allowed us to confirm the presence of a hydroxyl group at C-11. Thus, the structure of compound **1** was established as shown. Comparing **1** with the only two taxanes (taxuspine D[10] and taxuspine P[11]) isolated from the stems of the Japanese yew, *Taxus cuspidata*, we found that their ^{1}H and ^{13}C NMR data are quite similar, except for the chemical shift of H-5 and the signals for the cinnamoyl group in taxuspine D and the 3-*N*,*N*-(dimethylamino)-3-phenylpropanoyl group in taxuspine P. They all possess the same unique skeleton with an enolacetate moiety in ring A. Because its relative stereochemistry was elucidated by NOESY data, **1** was named 5-decinnamoyltaxuspine D.

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

position	δ ^{1}H mult.[a] (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY
1	1.88 d (7.8)	50.1	2, 3, 11, 13, 16, 17	2, 14a, Me-16, Me-17
2	5.72 d (7.2)	68.8	3, 8, 14, 170.2	1, 3, Me-19
3	3.61 d (7.2)	40.5	1, 2, 4, 5, 8, 9, 19, 20	2, 7, 14b, 20b
4		147.4		
5	4.22 br t (8.3)	65.8	4, 6, 20	2, 6a, 20a, Me-19
6a	2.20 m	36.2		5, 6b, 7, Me-19
6b	1.78 m		5, 7, 8	5, 6a, 7
7	4.72 dd (10.0; 8.1)	70.4	3, 8, 9, 19, 170.2	3
8		43.1		
9	4.70 d (4.9)	74.5	3, 7, 8, 10, 11, 169.2	10, Me-17, Me-19
10	5.57 d (4.9)	76.4	3, 8, 9, 11, 170.2	9, Me-18, OH-11
11		78.4		
12		124.0		
13		144.1		
14a	2.57 ddd (18.5; 7.8; 1.2)	25.5		1, 14b, Me-16
14b	2.38 d (18.5)		1, 2, 12, 15	3, 14a, 20b, Me-17
15		40.9		
16	1.17 s	31.2	1, 11, 15, 17	1, 14a, Me-17
17	1.46 o s	23.8	1, 11, 15, 16	see Me-19
18	1.55 s	11.2	11, 12, 13	10
19	1.46 o s	14.5	3, 7, 8, 9	2, 5, 9, Me-16
20a	5.27 br s	109.6	4, 5	20b
20b	5.04 br s		3, 4, 5	3, 14b, 20a
OAc	2.20 s	20.5	168.5	
	2.17 s	20.8	170.2	
	1.97 s × 2	20.5	170.2	
	1.94 s	20.8	169.2	
OH-11	2.14 s		10, 11, 12, 15	10, Me-16

[a] Mult, multiplicity: br, broad; d, doublet; m, multiplet; o, overlapping; s, singlet; t, triplet. The precision of the coupling constants is ±0.5 Hz. [b] The ^{13}C chemical shifts were extracted from the HMQC and HMBC (for quaternary carbons) experiments (±0.2 ppm).

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

position	δ ^{1}H mult[a] (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY
1	1.77 dd (8.6; 1.6)	47.8	2, 3, 11, 13, 15	2, 14a, Me-16, Me-17
2	5.41 dd (5.5; 1.6)	70.7	1, 3, 14, 169.4	1, 3, 9, 20b, Me-17, Me-19
3	3.40 d (5.5)	39.7	1, 2, 4, 5, 7, 19, 20	2, 7, 14b, Me-18
4		145.7		
5	4.24 t (2.9)	75.8	3, 20	6a, 6b, 20a
6a	2.10 o m	39.2		
6b	1.59 o m			
7	4.44 dd (11.1; 5.1)	70.0	3, 9, 19	3, 6a, 6b, 10, Me-18
8		47.5		
9	4.37 d (10.3)	78.1	7, 8, 10, 19	2, 6a, Me-17, Me-19
10	6.08 d (10.2)	75.0	9, 11, 12, 15, 170.1	3, 7, Me-18
11		134.8		
12		137.8		
13	5.77 br ddq (10.5; 5.1; 1.2)	69.5	11, 12, 14, 170.1	14a, 14b, Me-16, Me-18
14a	2.63 ddd (15.6; 10.5; 8.8)	28.5	1, 2, 12, 13	1, 13, 14b, Me-16
14b	1.46 dd (15.6; 5.3)		1, 2, 13, 15	3, 14a, Me-18
15		37.3		
16	1.03 s	31.8	1, 11, 15, 17	1, 13, 14a, Me-17
17	1.60 o s	25.9	1, 11, 15, 16	1, 2, 9, Me-16, Me-19
18	2.13 d (1.4)	15.8	11, 12, 13	7, 10, 13
19	1.12 s	12.5	3, 7, 8, 9	2, 6b, 9, 20b, Me-17
20a	5.25 br s	116.0	3, 5	5, 20b
20b	4.96 t (1.5)		3, 4, 5	2, 6a, 20a, Me-19
OAc	2.13 s	21.1	170.1	
	2.09 s	21.1	170.1	
	2.06 s	20.9	169.4	

[a] Mult, multiplicity: br, broad; d, doublet; m, multiplet; o, overlapping; q, quadruplet; s, singlet; t, triplet. The precision of the coupling constants is ±0.5 Hz. [b] The ^{13}C chemical shifts were extracted from the HMQC and HMBC (for quaternary carbons) experiments (±0.2 ppm).

Compound **2** was determined to have the molecular formula of $C_{26}H_{38}O_9$ on the basis of HRFABMS data. The ^{1}H NMR spectrum (NMR data see Table 2) showed proton signals due to an exomethylene (δ 5.25 and 4.96 ppm), four methyls (δ 1.03, 1.12, 1.60, and 2.13 ppm), three acetyl methyls (δ 2.06, 2.09, and 2.13 ppm), and six oxymethines (δ 6.08, 5.77, 5.41, 4.44, 4.37, and 4.24 ppm). Detailed analysis of the COSY, HMQC, and HMBC spectra, especially the HMBC correlations of two *gem*-methyls (Me-16 and Me-17, δ 1.03 and 1.60 ppm) to C-1 (δ 47.8 ppm), -11 (δ 134.8 ppm), and -15 (δ 37.3 ppm) suggested that **2** possesses the normal 6/8/6 taxane skeleton. The three acetoxy groups were revealed at C-2, -10, and -13 from the HMBC cross-peak signals of H-2 (δ 5.41 ppm), -10 (δ 6.08 ppm), and -13 (δ 5.77 ppm) to the three acetyl carbonyl carbons (δ 169.4, 170.1 ppm). The C-5, -7, and -9 positions were found to be connected to hydroxyl groups deduced from the upfield shifts of the oxymethines. The structure of this compound was thus established as **2** and named 7,9-deacetyl-5-decinnamoyl taxinine J. The relative stereo-

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

position	δ ^{1}H mult[a] (J in Hz)	δ ^{13}C[b]	HMBC	NOESY
1		67.9		
2	6.10 d (7.5)	68.5	165.9	3, 9, Me-16/17, Me-19
3	3.12 d (7.5)	44.4	2, 8, 19, 20	2, 7, 10, 14b
4		79.9		
5	4.88 d (8.3)	84.7	3, 20	6a, 6b, 20b
6a	2.59 dt (15.4; 7.8)	34.5		5, 6b, 7
6b	1.87 o m			5, 6a
7	5.33 t (8.5)	71.8		3, 6a, 6b, 10
8		42.6		
9	4.30 d (9.8)	78.6	7, 10	2, 10, Me-19
10	4.60 d (10.0)	68.2	9, 12	3, 7, 9, Me-18
11		137.1		
12		147.5		
13	4.57 br s	77.5	1, 11, 12	14a, Me-16/17, Me-18
14a	2.29 dd (13.9; 6.8)	39.7	15	
14b	1.74 dd (14.1; 6.6)			3, 14a
15		75.8		
16/17	1.04 s	27.4	1, 15, 17	13, 14a
17/16	1.08 s	24.7	1, 15, 16	2, 14a
18	1.97 s	11.0	11, 12, 13	13
19	1.93 s	12.9	3, 7, 8, 9	2, 9, 20a
20a	4.50 d (7.3)	74.8		2, 20b, Me-19
20b	4.12 d (7.3)		4	20a
OAc	2.21 s	22.2	171.0	
	2.06 s	21.4	170.3	
OBz				
C-1		165.9		
o	8.01 d (7.9)	129.6		
m	7.47 t (7.6)	129.6		
p	7.60 t (7.1)	133.4		

[a] Mult, multiplicity: br, broad; d, doublet; m, multiplet; o, overlapping; s, singlet; t, triplet. The precision of the coupling constants is ±0.5 Hz. [b] The ^{13}C chemical shifts were extracted from the HMQC and HMBC (for quaternary carbons) experiments (±0.2 ppm).

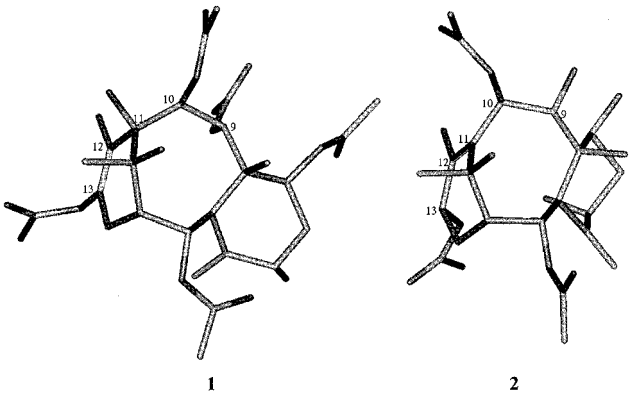

Figure 1. Tridimensional structures of taxanes **1** and **2** derived by molecular modeling.

chemistry of **2** was also demonstrated by NOESY correlations.

Compounds **1** and **2** both possess the 6/8/6 taxane ring system, with the only difference in the two skeletons being the position of one double bond ($\Delta^{12,13}$ in **1** and $\Delta^{11,12}$ in **2**). A large number of analogues of **2** have been found from nature, but **1** is now only the third example based on this unique taxane skeleton. Thus, molecular modeling was applied to study and compare the conformations of these two compounds. The final conformations of **1** and **2** are shown in Figure 1. The distances between H-2 and H-9 for **1** and **2** are 4.90 and 2.77 Å, respectively. The torsion angles H-9−C-9−C-10−H-10 for **1** and **2** were 81.0° and 155.2,° respectively. Inspection of NOE spectra of **1** showed that there was no NOE signal between H-2 and H-9, which supports the large distance between H-2 and H-9. The coupling constants between H-9 and H-10 for **1** and **2** were 4.9 and 10.2 Hz, respectively. These coupling constant data also support the large difference between the torsion angles H-9−C-9−C-10−H-10 in **1** and **2**. Ring B in **1** has more of a crown conformation. The large (10.2 Hz) coupling between H-9 and H-10 in **2** indicates a *trans*-diaxial orientation whereas the smaller coupling in **1** (4.9 Hz) can be interpreted as H-9 and H-10 being pseudoequatorial. This can also explain the very small NOE observed between H-2 and H-9, the strong NOE between H-9 and H-10, and the small NOE between H-7 and H-10. The unusual shielding observed for H-9 (4.7 ppm instead of 5.9 ppm) is due partly to the conformational change but most importantly from the anisotropy of the C-12,C-13 double bond: H-9 is located above the double bond in the shielding zone; H-7 is similarly influenced by the change in conformation of the B ring and also by the double bond at C-12,C-13.

In chloroform, compound **2** adopts a U-shape. However, the attachment of ring A to B in **1** is flatter than the one in **2**. The torsion angles C-12−C-11−C-10−C-9 for **1** and **2** were −66.0° and −102.9°, respectively. This can be explained by the different position of the double bond in the A rings between these two compounds. In compound **2**, the double bond is between C-11 and C-12. Because C-12 is shared by rings A and B, the double bond between C-11 and C-12 would constrain the conformation of ring A relative to ring B. This would contribute to the U-shape of compound **2**. For compound **1**, the double bond is between C-12 and C-13, and the bond between C-11 and C-12 is single. Therefore, ring A can adopt a flatter conformation relative to ring B. Consequently, the orientation of the ring A C-13 side chain will be changed if the double bond in ring A is at C-12,C-13 instead of at C-11,C-12. Hoemann et al.[12] showed that the orientation of the C-13 side chain is essential for the biological activity of paclitaxel. The skeleton from **1** might fit to optimize the orientation of the C-13 side chain. Indeed, Wicnienski et al. showed that 7-deoxy-$\Delta^{12,13}$-isotaxol is useful for the treatment of some cancers. [13]

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

position	δ ^{1}H mult[a] (*J* in Hx)	δ ^{13}C[b]	HMBC	NOESY
1		67.6		
2	6.02 d (7.7)	67.8		3, 9, 20a, Me-16, Me-17, Me-19
3	3.09 d (7.7)	43.7	1, 2, 5, 7, 8, 19, 20	2, 7, 10, 14b
4		79.9		
5	4.93 d (7.3)	85.0	4, 7	3, 6a, 6b, 20b
6a	2.52 dt (15.6; 8.0)	34.7		5, 6b, 7
6b	1.88 m			5, 6a
7	5.45 t (7.6)	70.2		3, 6a,10
8		43.6		
9	5.97 d (10.5)	76.6		2, 10, Me-19
10	6.25 d (10.5)	68.7		3, 7, Me-18
11		133.9		
12		150.6		
13	4.67 m	77.9		14a, 14b, Me-16, Me-17, Me-18
OH-13	1.78 o m			
14a	2.22 m	39.6		13, 14b, Me-16, Me-17
14b	1.55 o m		1, 2, 13, 15	3, 13, 14a, Me-16, Me-17
15		75.1		
16/17	1.05 br s	27.1	1, 15, 17	13, 14a, Me-17
17/16	1.12 s	25.0	1, 15, 16	2, 14a, Me-16
18	1.94 br s	11.4	11, 12, 13	10, Me-16/17
19	1.66 s	12.5	3, 7, 8, 9	2, 9, 20a
20a	4.51 br d (7.6)	74.9	3, 5	20b, Me-19
20b	4.38 d (7.6)		3, 4	5, 20a, Me-OAc
OAc	2.15 s	22.2	171.2	
	2.08 s	21.3	169.8	
	2.02 s	20.7	169.6	
	2.01 s	21.3	169.6	
	1.95 s	20.4	167.7	
OH-15	2.65 s		15, 16/17	2, 9

[a] Mult, multiplicity: br, broad; d, doublet; m, multiplet; o, overlapping; s, singlet; t, triplet. The precision of the coupling constants is ±0.5 Hz. [b] The ^{13}C chemical shifts were extracted from the HMQC and HMBC (for quaternary carbons) experiments (±0.2 ppm).

Compound **3** was shown to possess the molecular formula of $C_{31}H_{40}O_{11}$ by HRFABMS. Analysis of its ^{1}H NMR, COSY, and HMQC spectra (for NMR data see Table 3) revealed four methyls (δ 1.04, 1.08, 1.93, and 1.97 ppm), two acetyl methyls (δ 2.06 and 2.21 ppm), and one benzoyl (δ 8.01, 7.60, and 7.47 ppm). A pair of doublets at δ 4.50 and 4.12 ppm (J = 7.3 Hz) indicated the presence of an oxetane ring. Because the HMBC spectrum showed correlations of Me-16 and Me-17 to two identical quaternary carbons, which were assigned to C-1 (δ 67.9 ppm) and C-15 (δ 75.8 ppm), compound **3** was suspected of being an *abeo*-taxane.[14] Other HMBC correlations of H-10 (δ 4.60 ppm) to C-9 (δ 78.6 ppm) and C-12 (δ 147.5 ppm), and H-13 (δ 4.57 ppm) to C-1 (δ 67.9 ppm), C-11 (δ 137.1 ppm), and C-12 (δ 147.5 ppm) also supported this hypothesis. The benzoyloxy group was revealed to be located at C-2, inasmuch as the HMBC spectrum showed correlations between H-2 (δ 6.10 ppm) and the benzoyl carbonyl signal at δ 165.9 ppm. The upfield shift of the signals of H-9 (δ 4.30 ppm), -10 (4.60 ppm), and -13 (4.57 ppm) suggested a free hydroxyl group at C-9, -10, and -13, respectively. The two acetoxy groups must be at C-7 and C-4 as shown by the chemical shifts of H-7 (δ 5.33 ppm) and C-4 (δ 79.9 ppm). So, the structure of this compound was established as **3** and named 9,10,13-trideacetyl-*abeo*-baccatin VI. The relative stereochemistry of **3** was also determined by NOESY correlations.

HRFABMS showed the molecular formula of compound **4** to be $C_{30}H_{42}O_{13}$. Detailed analysis of the NMR spectra (^{1}H, COSY, HMQC, and HMBC, see Table 4) suggested that **4** possesses the same *abeo*-taxane skeleton as **3**. The differences are only in the oxygenation substitutuents: there are three acetoxy groups at C-2 (no benzoyloxy group as in **3**), C-9, and C-10 (no hydroxyl groups at C-9 and C-10 as in **3**), respectively. We also noted a COSY cross-peak from a singlet OH group located at δ 2.65 ppm with one on the *gem*-dimethyl group (δ 1.12 ppm), and HMBC correlations of this OH proton to C-15 and one of the *gem*-dimethyl carbons (δ 25.0 ppm). These observations confirmed an *abeo*-taxane structure in which an OH group is located at C-15 (δ 75.1 ppm). Thus, its structure was elucidated as **4** and named 2-acetyl-13-deacetyl-2-debenzoyl-*abeo*-baccatin VI. The NOESY correlations were used to establish the relative stereochemistry of this compound.

The molecular formula of compound **5** was established as $C_{28}H_{42}O_{12}$ by HRFABMS. Detailed analysis of the ^{1}H NMR, COSY, and HMQC spectra (Table 5) revealed that **5** was a normal taxane with a 6/8/6 ring system and with an opened oxetane ring (H-20 appeared as complex signals at δ 3.47 and 3.41 ppm, rather than a pair of doublets as seen in taxanes with an oxetane ring). Four methyls (δ 1.06, 1.13, 1.60, and 2.14 ppm) and four acetyl methyls (δ 2.10, 2.11, 2.14, and 2.20 ppm) were shown along with the connectivities of the protons from H-1 to H-7, H-9 to H-10, H-13 to H-14, and H-4 to H-20. The HMBC correlations of H-20 to C-4 and C-5, and Me-16 and Me-17 to C-1, -11, and -15 further confirmed the skeleton. The locations of the two acetoxy groups were revealed to be on C-2 and C-10 from the connection signals in the HMBC. The presence of the other two acetoxy groups at C-5 and C-13 was deduced from the chemical shifts of H-5 (δ 5.11 ppm) and H-13 (δ 5.89 ppm). The structure of this compound was thus assigned as **5** and named 7,9-deacetyltaxuspine L. Its relative stereochemistry was established on the basis of NOESY spectrum and by close comparison with the spectral data of that of taxuspine L [15] obtained from the stems of *T. cuspidata*.

Compounds **1**−**5** are novel taxanes isolated for the first time from the needles of *T. candensis*. There are only two other taxanes[10,11] (albeit with different substituents) reported with a double bond at C-12 and C-13 in ring A in the stems of the Japanese yew and relatively few taxanes with a CH−CH_2OH substituent at C-4.[15−21]

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

position	δ ^{1}H mult[a] (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY
1	1.91 br d (~8.1)	47.6		2, 14a, Me-16, Me-17
2	5.32 dd (5.9; 2.9)	71.3	8, 14, 170.1	1, 3, 9, Me-17, Me-19
3	2.62 o d	38.1		6a, 7, 14b, Me-18
4	2.00 o m	46.6		see 6a
5	5.11 m	71.3		6a, 6b, 20b
6a	1.98 o m	31.4		3, 5, 6b, 7, 14b, 20a
6b	1.77 ddd (14.7; 11.2; 2.7)			5, 6a, 19, 20b
7	4.14 dd (11.2; 4.6)	70.6	6, 9, 19	3, 6a,10, Me-18
8		45.2		
9	4.43 d (10.3)	78.0	7, 8, 10, 19	2, 10, Me-17, Me-19
10	5.97 d (10.2)	75.5	9, 11, 12, 15, 169.6	7, 9, Me-18
11		133.9		
12		137.6		
13	5.89 br t (8.6)	70.2		14a, Me-16, Me-18
14a	2.60 o m	27.5	1, 2, 12, 13	see 3
14b	1.47 dd (15.4; 8.1)			14a
15		37.7		
16	1.13 s	31.4	1, 11, 15, 17	13, Me-17
17	1.60 s	26.8	1, 11, 15, 16	1, 2, 9, Me-16
18	2.14 br s	14.8	11, 12, 13	7, 10
19	1.06 s	13.8	3, 7, 8, 9	2, 6b, 9, 20
20a	3.47 m	64.2	5	20b, Me-19
20b	3.41 m		4, 5	5, 6b, 20a, Me-19
OAc	2.20 s	21.5	170.0	
	2.14 o s	20.9	169.6	
	2.11 o s		170.5	
	2.10 s		170.1	

[a] Mult, multiplicity: br, broad; d, doublet; m, multiplet; o, overlapping; s, singlet; t, triplet. The precision of the coupling constants is ±0.5 Hz. [b] The ^{13}C chemical shifts were extracted from the HMQC and HMBC (for quaternary carbons) experiments (±0.2 ppm).

Experimental Section

General Experimental Procedures. ^{1}H, ^{13}C, HMQC, HMBC, and NOESY NMR spectral data were obtained on a Varian UNITY-500 spectrometer operating at 499.84 MHz for ^{1}H and at 125.69 MHz for ^{13}C. The spectra were obtained on 1−2 mg samples dissolved in $CDCl_3$, which was used as the internal reference. In the proton NMR spectra, the multiplicity shown in Tables 1−5 is the apparent one. Low-resolution xenon FABMS were obtained in glycerol with a VG ZAB-HS instrument. Samples were dissolved in 0.2 μL of DMSO before adding of 0.5 μL of glycerol. HRFABMS were similarly obtained in glycerol−DMSO at a resolving power of 12 000. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter.

Molecular modeling was performed on a Silicon Graphics R5000 workstation using the Discover program within Insight II package (version 97.0).[22] The structures were generated by modifying the crystal structure of 2-deacetoxydecinnamoyltaxinine J.[23] First, the initial structures were subjected to simulated annealing without constraints (the maximum temperature was 900 K). Cluster analysis revealed the existence of two two-conformer families for **1** and one for **2**. The lowest energy conformer for **2** was selected for further calculations. Because there were two conformer families for **1**, the lowest conformers in each family were compared with the NOE data, and the most consistent one was selected for further calculations. Second, the structures selected from simulated annealing were subjected to NOE-restraint molecular dynamics at 400 K for 100 ps, using the consistent valence force field (CVFF). The instant conformations were saved every 200 fs. The distance restraints from NOE spectra in $CDCl_3$ were classified as short, medium, and long, with distance ranges of 1.5−2.8, 2.8−3.5, and 3.5−5.0 Å, respectively. For compound **1**, 18 NOE distance restraints were employed in the calculation, whereas 21 NOE distance restraints were used for compound **2**. During the simulation, distance restraints were applied with a force constant of 100 kcal/Å/mol. The lowest energy conformations from the trajectories were selected for further minimizations. During minimization, the solute was solvated with a periodic 35 × 35 × 35 Å^3 $CDCl_3$ box containing 237 $CDCl_3$ molecules. The solvated systems were then energy minimized using 1000 steps of steepest descent and 1000 steps of conjugate algorithms with a root-mean-square gradient deviation of 0.001 Å. The NOE-restraints remained in place during these minimizations.

Unless otherwise specified, liquid column chromatography was performed on Si gel 60, 230−400 mesh (EM Science). Preparative HPLC was carried out on a Waters Delta Prep 3000 instrument coupled to a UV 486 tunable absorbance detector set at 227 nm (Waters) using a Partisil 10 ODS-2 MAG-20 preparative column (22 × 500 mm). Semipreparative HPLC was performed on the same system as the preparative one but using two Partisil 10 ODS-2 MAG-9 semipreparative columns (Whatman) connected in series (9.4 × 500 mm). Preparative TLC was carried out on Si gel 60 F_{254} precoated TLC plates, 0.25 mm (EM Science). The purified compounds were visualized by TLC on precoated TLC plates (Si gel 60 F_{254}, 0.25 mm, EM Science) with 10% H_2SO_4 in EtOH.

Plant Material. *T. canadensis* Marsh. is native to Northeast America including the Quebec region. The plant was collected in September 1997, in St.-Jean Quebec, and stored at 4 °C before drying when needed. In the herbarium of Montreal Botanical Garden several specimens are deposited.

Extraction and Isolation. Ground dried needles of *T. canadensis* (4.7 kg) were extracted and treated as described previously[7] to yield 119 g of a dark brown extract. A portion (50 g) of this extract was separated on a Si gel column (821 g, 10 × 21 cm) and eluted with hexane-CH_2Cl_2 (1:1, 2 L), CH_2Cl_2 (2 L), CH_2Cl_2-EtOAc [9:1 (2 L), 8:2 (2 L)], CH_2Cl_2−EtOAc−MeOH [700:295:5 (1 L), 700:290:10 (1 L), 700:280:20 (1 L), 700:250:50 (1 L), 600:350:50 (1 L), 500:400:100 (1 L), 400:400:200 (1 L), 300:500:200 (1 L)], and EtOAc−MeOH [7:3 (1 L), 1:1 (2 L)] to give fractions A (11.6−12.0 L), B (12.0−12.6 L), and C (12.6−13.4 L).

Fraction A (5.5 g) was applied to a Si gel column (105 g, 4 × 17.5 cm) with hexane−2-propanol [9:1 (100 mL), 8:2 (200 mL), 7:3 (300 mL), 6:4 (300 mL), 1:1 (300 mL)] and EtOAc−MeOH (1:1, 300 mL) to yield fraction D (400−540 mL). Fraction D (1.6 g) was then purified on a Si gel column (54 g, 2.5 × 36 cm) with CH_2Cl_2−EtOAc [8:2 (200 mL), 7:3 (200 mL), 6:4 (200 mL), 1:1 (200 mL), 4:6 (200 mL), 2:8 (200 mL)] and EtOAc (200 mL) to give fraction E (500−600 mL, 200 mg), which was then further purified on a preparative HPLC column eluting with a 70-min linear gradient of MeCN (25 to 100%) in H_2O at a flow rate of 18 mL/min followed by

preparative TLC (CH_2Cl_2−MeOH, 96:4) to afford compound **1** [15.1 mg, visualized on a TLC plate as a brown to black spot with R_f 0.80 (EtOAc)]; $[\alpha]^{24}_D$ +7.8° (*c* 0.10, $CHCl_3$). The structure of **1** was fully characterized by NMR (Table 1) and confirmed by high-resolution mass spectrometry data. FABMS *m/z* $[M + Na]^+$ 617.25731; $C_{30}H_{42}O_{12}Na$ requires 617.25739.

Fraction B (2.7 g) was subjected to a Si gel column (108 g, 4.5 × 18 cm) with hexane−EtOAc [8:2 (200 mL), 1:1 (300 mL), 4:6 (200 mL), 3:7 (200 mL), 2:8 (200 mL), 1:9 (200 mL)], EtOAc (600 mL), EtOAc−MeOH (8:2, 400 mL) to give fractions B1 (1380−1500 mL) and B2 (1500−1800 mL). Fraction B1 (205 mg) was separated on a preparative HPLC column eluting with a 70-min linear gradient of MeCN (25 to 100%) in H_2O at a flow rate of 18 mL/min to produce residue F (3.0 mg, t_R 27.8 min) and G (6.9 mg, t_R 24.8 min). Residue F was then purified by preparative TLC (CH_2Cl_2−MeOH, 98:2) to yield compound **3** (1.6 mg). Residue G was further purified on a semipreparative HPLC system eluting with a 50-min linear gradient of MeCN (25 to 100%) in H_2O at a flow rate of 3 mL/min to produce compound **4** (4.7 mg). Fraction B2 (1.05 g) was then applied to a Si gel column (55 g, 2.5 × 26 cm) with CH_2Cl_2 (200 mL) and CH_2Cl_2−MeOH [1000:5 (200 mL), 1000:10 (300 mL), 1000:15 (200 mL), 1000:20 (200 mL), 1000:30 (200 mL), 1000:40 (200 mL), 1000:50 (200 mL), 1000:60 (200 mL), 1000:70 (200 mL)] to yield fraction H (1220−1380 mL). Fraction H (489 mg) was again purified on a Si gel column (20 g, 2 × 17 cm) with CH_2Cl_2−EtOAc [8:2 (100 mL), 6:4 (100 mL), 4:6 (100 mL), 3:7 (100 mL), 2:8 (100 mL), 1:9 (100 mL)] and EtOAc (100 mL) to give fraction I (240−260 mL). Fraction I (25.3 mg) was further purified on a semipreparative HPLC system eluting with a 50 min linear gradient of MeCN (25 to 100%) in H_2O (flow rate: 3 mL/min) to afford compound **2** (4.6 mg). Compound **2** appeared as a brown spot (R_f 0.55, EtOAc); $[\alpha]^{24}_D$ +23.1° (*c* 0.23, $CHCl_3$), and **3** and **4** were both visualized as green spots with almost the same R_f value of 0.45 (EtOAc) on TLC plate; taxane **3** $[\alpha]^{24}_D$ +22.2° (*c* 0.01, $CHCl_3$); taxane **4** $[\alpha]^{24}_D$ −57.3° (*c* 0.16, $CHCl_3$). Their structures were fully established by NMR (Tables 2−4) and confirmed by HRMS. HRFABMS for **2**: *m/z* $[M + Na]^+$ 517.24121; $C_{26}H_{38}O_9Na$ requires 517.24135. HRFABMS for **3**: *m/z* $[M + Na]^+$ 611.24685; $C_{31}H_{40}O_{11}Na$ requires 611.24683. HRFABMS for **4**: *m/z* $[M + Na]^+$ 633.25234; $C_{30}H_{42}O_{13}Na$ requires 633.25231.

Fraction C (3.6 g) was applied to a Si gel column (108 g, 4.5 × 18 cm) with hexane−EtOAc [1:1 (400 mL), 3:7 (400 mL), 1:9 (400 mL)], EtOAc (600 mL), and EtOAc−MeOH (9:1, 400 mL) to yield fraction J (1240−1520 mL). Fraction J (1.25 g) was again purified on a Si gel column (28 g, 2 × 22.5 cm) with CH_2Cl_2 (100 mL) and CH_2Cl_2−MeOH [1000:5 (200 mL), 1000:10 (200 mL), 1000:15 (200 mL), 1000:20 (200 mL), 1000:25 (200 mL), 1000:30 (200 mL), 1000:35 (200 mL), 1000:40 (200 mL), 1000:50 (200 mL)] to afford fraction K (1640−1820 mL). Fraction K (137.4 mg) was further purified on the preparative HPLC system eluting with a 50-min linear gradient MeCN (25 to 100%) in H_2O at a flow rate of 18 mL/min followed by recrystallization to afford compound **5** (27.6 mg). It was shown to be a brown to black spot on TLC plate with R_f = 0.45 (EtOAc); $[\alpha]^{24}_D$ +42.9° (*c* 0.02, $CHCl_3$). The structure of **5** was fully elucidated by NMR (Table 5) and was supported by HRMS. HRFABMS for **5**: *m/z* $[M + Na]^+$ 577.26255; $C_{28}H_{42}O_{11}Na$ requires 577.26248.

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