New Minor Taxane Derivatives from the Needles of *Taxus canadensis*

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Seven minor taxane derivatives were isolated for the first time from the needles of *Taxus canadensis*. Four of these natural taxanes are *O*-glycosylated (**1**−**4**), with **4** being the only reported taxane with a glucose on ring C, and two have a dimethylamino-C-5 side chain (**5** and **6**). An unusual double bond at C-5(6) has been identified in taxane **7**.

The taxane composition of *Taxus canadensis* Marsh (Taxaceae) needles has been thoroughly investigated since 1992.[1−7] The most abundant taxane, 9-dihydro-13-acetylbaccatin III, is specific to this yew[1−3] and was converted to the anticancer drug paclitaxel (Taxol).[4] Hoping to get insights into the biosynthesis of taxanes, rooted cuttings of *Taxus canadensis* and *Taxus cuspidata* were investigated. The composition of taxanes from these sources was different from that of the mature species,[5−7] and there was no correlation with their biosynthesis.[8]

In the present work, the isolation and structural elucidation of six new taxanes (**1**, **3**−**7**) are reported, along with a known compound (**2**) isolated for the first time from a methanol extract of dried needles of the Canadian yew. Taxane **2** has been reported in rooted cuttings of *Taxus cuspidata*[7] and in the bark of the Chinese yew.[9]

Results and Discussion

A methanolic extract of the needles of *T. canadensis* was purified as described in the Experimental Section. Seven pure taxanes (**1**−**7**) were obtained.

The NMR data for compound **1** (Table 1) were very similar of that of **2** except for the absence of the proton signal at δ 3.88 ppm on the oxygenated carbon of the α-methylbutanoate side chain. Indeed, the molecular composition of **1**, $C_{35}H_{54}O_{12}$, established from the combined analysis of HRFABMS and 2D NMR spectral data, confirmed the structure. The HMBC correlations also confirmed the taxane skeleton with a 4(20) exocyclic double bond. The ^{1}H NMR signal at δ 4.97 (1H, dd, J = 9.4, 4.9 Hz) was assigned to H-14 and suggested that a side chain was attached to C-14. Detailed analysis of the ^{1}H−^{1}H COSY spectrum revealed an α-methylbutanoate group in taxane **1**. This moiety has been found in several natural taxane analogues.[10−12] The α-methylbutanoate group was connected to C-14, as deduced from the long-range correlation of H-14 to C-1′ (δ 174.4) in the HMBC experiment (Figure 1). The similarity of the NMR data of the α-methylbutanoate moiety with known taxanes revealed a similar stereochemistry.[10−12] The signal at δ 5.24 (1H, m) was assigned to H-5. The chemical shifts of H-5 and C-5 suggested that an acetyl group was attached to C-5, as confirmed by the HMBC correlation. Similarly, using H-5 as a starting point, the spin system derived from C-5 to C-7 through C-6 was readily interpreted from the analysis of the ^{1}H−^{1}H COSY spectrum. The signal at δ 5.32 (1H, dd, J = 11.9, 5.2 Hz), which showed long-range correlations with C-9, C-12, and C-15 in the HMBC spectrum, was attributed to H-10. Taxanes with a C-2, C-5, C-10, and C-14 oxygenated substitution pattern have a downfield chemical shift for C-1 (δ 59.5), a rather unusual chemical shift value for a nonoxygenated methine in the ^{13}C NMR spectrum.[10−12] Additionally, an anomeric carbon signal at δ 98.5 as well as five oxygenated carbons between δ 61.5 and 77.4 and six hydrogen signals between δ 3.22 and 4.32 indicated the existence of one sugar moiety in taxane **1**. The chemical shift, multiplicity, and coupling constant values indicated the presence of a glucopyranosyl unit. Analysis of the 2D NMR data (COSY, HMBC) (Figure 1, Table 1) as well as the MS fragment of $[M + H - 180]^+$ confirmed this suggestion. The coupling constant (J = 7.8 Hz) of the anomeric proton H-1″ indicated that this moiety was connected to the aglycon via a β-linkage (J = 6−8 Hz).[13,14] The anomeric proton H-1″ showed a long-range correlation with C-10. In addition, H-10 was correlated to C-1″ in the HMBC experiment, establishing that the glucose unit was attached to C-10. The orientations of the substituents on the taxane skeleton were confirmed by the coupling constants in ^{1}H NMR and HMBC experiments and the correlations in the NOESY experiment (Table 1). The strong NOE correlation of H-14 and H-1 and the small coupling constant between H-1 and H-14 suggested that their dihedral angle was about 90°: the C-14 side chain was therefore β-oriented with H-14α. The chemical structure of **1** was therefore determined as 2α,5α-diacetoxy-14β-2′α-methylbutanoate-10β-*O*-(β-D-glucopyranosyl)-taxa-4(20),11-diene. Taxane **1** is the first reported example in *T. canadensis* of a taxane with an *O*-glucosyl group on ring B.

Compound **2** [2α,5α-diacetoxy-14β-(2′*S*,3′*R*)-3′-hydroxy-2′α-methylbutanoate-10β-*O*-(β-D-glucopyranosyl)taxa-4(20),11-diene] has been detected also in the rooted cuttings of the Japanese yew, *Taxus cuspidata*,[7] and later in the bark of the Chinese yew, *T. yunnanensis*.[9]

Compound **3** was a colorless amorphous solid. Its molecular composition, $C_{32}H_{48}O_{12}$, was obtained from HRFABMS at *m/z* 663.2781 $[M + K]^+$. The ^{1}H and ^{13}C NMR spectra of **3** (Tables 2 and 3) showed the characteristic signals of C-14-substituted taxanes (H-2 and H-10 resonated as doublets of doublets, and C-1 had an unusual downfield shift at δ 59.3; carbon-14 appeared in the range of oxygenated carbons at δ 70.1 ppm).[10−12] The NMR data for the glucose unit were similar to those of **1** and **2**. The

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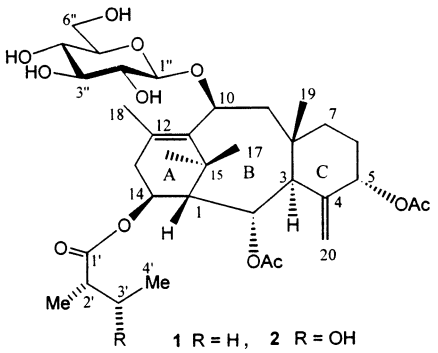

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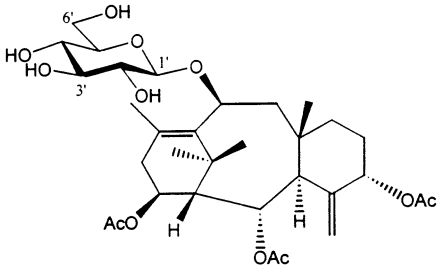

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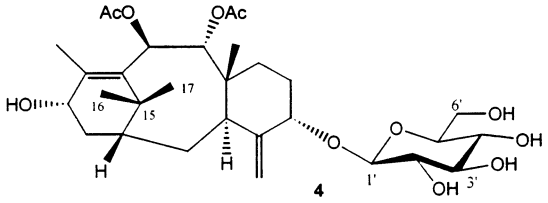

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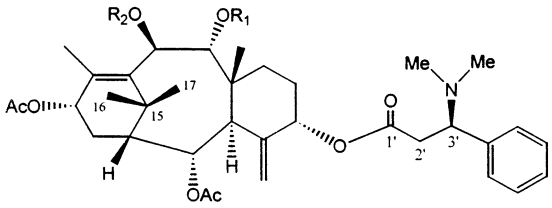
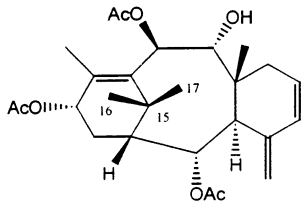


5 R_1 = Ac R_2 = H, **6** R_1 = H R_2 = Ac



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main differences were due to the presence of a C-14-acetyl group instead of the 2′-methylbutanoate found in **1** and **2**. The sugar residue on C-10 in **3** was confirmed by the HMBC correlations of H-10 to C-1′ and H-1′ to C-10. The β-configuration of the glucose unit was determined by the coupling constant (J = 7.8 Hz) of the anomeric proton H-1″.[13,14] The value for the ^{1}H and ^{13}C NMR data of C-14 is similar since in both cases C-14 is acylated. The NOESY correlations showed the same relative orientations as in taxanes **1** or **2**. Therefore, the structure of **3** was assigned as 2α,5α,14β-triacetoxy-10β-*O*-(β-D-glucopyranosyl)taxa-4(20),11-diene.

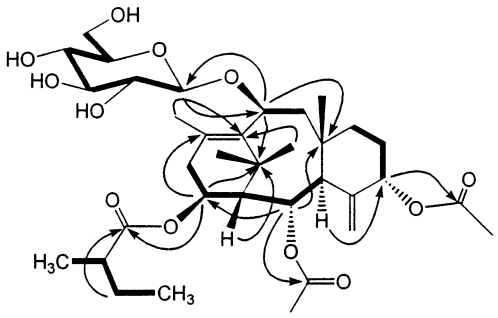

Figure 1. Arrows show the key HMBC correlations (H→C) of **1**. Bold lines indicate the ^{1}H−^{1}H COSY correlations. Most protons are omitted for clarity.

In the structures of compounds **4**−**7** we infer that the stereochemistry of the two methyls CH_3-16 and CH_3-17 is β-oriented for the following reasons: CH_3-16 is oriented β toward the A-six-membered ring because of its medium NOE with H-13 (also β). This NOE is a 1−4 interaction in a six-membered ring (involving four bonds!). The fact that we observed this NOE implies necessarily that the conformation of the A-six-membered ring must be in a boat form. Indeed, if we had a chair conformation, 1−4 interactions could not occur even when the CH_3-16 and H-13 are β. CH_3-17 is also oriented β but now toward the B-eight-membered ring because of its strong NOE with H-2 and H-9, which are both β. Although this CH_3-17 is α toward ring A, it is β toward ring B. Our notation emphasizes the fact that ring A is somewhat in a boat form with one methyl oriented toward ring A while the other is toward ring B.

Compound **4** was isolated as a colorless amorphous powder with a molecular formula of $C_{30}H_{46}O_{11}$, as established by HRFABMS. The ^{1}H and ^{13}C NMR data (Tables 2 and 3) suggested a taxane-type skeleton: four methyls, two acetates, and a pair of exoethylene protons.[10−12] Additionally, the signals of an *O*-glucosyl moiety were observed in both the ^{1}H and ^{13}C NMR spectra. The coupling constant (J = 7.8 Hz) for the anomeric proton H-1′ indicated that this moiety was connected to the aglycon via a β-linkage.[13,14] A set of isolated signals resonating at δ 5.79 (1H, d, J = 10.7 Hz) and 6.07 (1H, d, J = 10.7 Hz) was assigned to H-9 and H-10, respectively. The vicinal coupling between H-9 and H-10 with a large coupling constant of J = 10.7 Hz indicated their *trans*-orientation, as found in other 9,10-disubstituted taxanes.[10−12] Two acetyl groups were positioned at C-9 and C-10, as deduced from their chemical shifts and HMBC experiments. The signal at δ 4.69 (1H, t, J = 8.2 Hz) was attributed to H-13 on the basis of its long-range correlation with CH_3-18. The anomeric proton H-1′ (δ 4.24, 1H, d, J = 7.8 Hz) of the sugar exhibited a long-range correlation with C-5, clearly indicating that the glucose was attached to C-5. Thus, the structure of **4** was determined to be 9α,10β-diacetoxy-13α-hydroxy-5α-*O*-(β-D-glucopyranosyl)taxa-4(20),11-diene.

It is interesting to note that when the *O*-glucosyltaxanes (**1**−**4**) were dissolved in chloroform (1−2 mg in 0.3 mL), they solidified to gel-like substances, giving broad signals which were difficult to interpret. These compounds were therefore analyzed in acetone-d_6. It was still possible to measure the optical rotation since 1−2 mg was dissolved in 2 mL of chloroform.

Compound **5** was a colorless gummy substance. Its molecular composition was obtained by HRFABMS to be $C_{37}H_{51}NO_9$. The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) exhibited the usual signals for a taxane skeleton:[10−12] four tertiary methyl groups at δ 1.14, 1.74, 1.98, and 0.97, three

Table 1. ^{1}H and ^{13}C NMR Data of **1** in Acetone-d_6 (500 MHz for ^{1}H, 125 MHz for ^{13}C)

position	δ ^{1}H mult[a]	J (Hz)	δ ^{13}C[b,d]	HMBC[d]	NOESY[c]
1	1.82 (d)	2.2	59.5	2, 11, 15	
2	5.38 (dd)	6.6, 2.2	70.1	8, 14, **168.8**	1,^{s} 3,^{w} 9a,^{s} 17,^{s} 19,^{s} 20b^w
3	2.98 (d)	6.6	42.1	1, 2, 8, 19, 20	7a,^{s} 14,^{s} 18,^{s} 2^w
4			**142.7**		
5	5.24 (m)		77.7	6, **168.6**	6^s
6ab	1.77 (m)		28.6		
7a	1.96 (o m)		33.6		3,^{s} 5,^{w} 10,^{s} 7b,^{s} 20b^w
7b	1.19 (o m)				
8			**39.3**		
9a	2.36 (dd)	14.8, 12.4	44.7	8, 10, 19	2,^{s} 9b,^{s} 17,^{s} 19^s
9b	1.61 (m)			3, 7, 8, 10, 11	
10	5.32 (dd)	11.9, 5.2	70.9	9, 12, 15, **1″**	7a,^{s} 9a,^{w} 9b,^{s} 18,^{s} 1″ ^{m}
11			**135.5**		
12			**136.3**		
13a	2.87 (o dd)		39.7	1, 11, 12, 14, 18	13b,^{s} 14,^{s} 18,^{m} 13a,^{s} 16^s
13b	2.43 (dd)	18.9, 4.5			
14	4.97 (dd)	9.4, 4.9	69.7	1, 2, 13, **174.4**	1,^{m} 3,^{s} 13a^s
15			**36.8**		
16	1.19 (s)		30.9	1, 11, 15, 17-Me	
17	1.69 (s)		24.2	1, 11, 15, 16-Me	1,^{s} 2,^{s} 9a,^{s} 16^s
18	2.06 (s)		20.1	11, 12, 13	3,^{s} 10,^{s} 13a,^{s} 3′b^s
19	0.84 (s)		21.9	3, 7, 8, 9, 10 (weak)	2,^{s} 6,^{s} 7b,^{s} 9a,^{s} 9b,^{s} 20b,^{m} 20a/5^w
20a	5.24 (br s)		115.9	3, 4	20b^s
20b	4.85 (br s)			3, 4, 5	7a,^{m} 19,^{m} 20a^s
-OAc	2.14 (s)		21.0	**168.6**	
			168.6		
	1.96 (s)		20.4	**168.8**	
			168.8		
1′			**174.4**		
2′	2.32 (sextet)	7.0	40.7		
3′a	1.60 (o m)		26.6		
3′b	1.47 (m)				
Me-4′	0.86 (t)	7.3	10.8	2′, 3′	2′,^{w} 3′a,^{w} 3′b^w
Me-5′	1.08 (d)	6.9	16.0	1′, 2′, 3′	2′^{s}
1″	4.32 (d)	7.8	98.5	3″, 10	2″,^{w} 3″,^{s} 5″,^{s} 10,^{s} 17,^{w} 7b/16^m
2″	3.25 (t)	8.0	73.7	3″	
3″	3.33 (o m)		77.4	4″	
4″	3.33 (o m)		70.8	3″	
5″	3.22 (m)		76.5		
6″a	3.84 (dd)	11.3, 2.2	61.8		
6″b	3.65 (dd)	11.5, 5.5			

[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] ^{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). [d] Bold numbers represent quaternary carbons whose chemical shifts were obtained from HMBC experiments (±0.2 ppm).

acetyl groups at δ 2.12, 20.5, 170.0; 2.03, 19.9, 169.7; 1.96, and 21.3 168.7, and an exocyclic methylene group at δ 5.25 (1H, s), 4.85 (1H, s), 117.7, and 142.4. The signal at δ 3.18 (1H, d, J = 6.8 Hz) was characteristic of the C-3 ring junction proton in a taxa-4(20),11-diene analogue. In addition, five signals due to protons attached to oxygenated carbons were detected from both the ^{1}H and ^{13}C NMR data as well as the HMBC correlations. The signal resonating as a doublet at δ 5.77 is H-9 (which showed correlations with C-7, C-8, C-11, and C-19). Compound **5** is acetylated at C-9, as seen with the HMBC correlation of H-9 with a carbonyl signal at δ 169.7. The signal at δ 4.87, which showed cross-peaks with C-11, C-12, and C-15 in the HMBC spectrum, was attributed to H-10. The chemical shift of H-10 suggested that a hydroxyl group is attached to C-10. The large vicinal coupling constant between H-9 and H-10 (J = 10.2 Hz) demonstrated a *trans*-orientation. The spin system derived from H-3 to H-2 to H-1 to H-14 to H-13 was readily defined starting from H-3. The chemical shifts of H-2 (δ 5.47) and H-13 (δ 5.85) indicated acetylations on C-2 and C-13. Indeed, this was confirmed with HMBC correlations. The presence of a Winterstein's acid [3′-(*N*,*N*-dimethylamino)-3′-phenylpropanoyl] moiety in **5** was obtained from the signals at δ 2.17 (6H, s, N-CH_3), 2.88 (1H, broad, H-2′), 3.13 (1H, broad, H-2′), 3.78 (1H, broad, H-3′), 7.33 (4H, m, Ph-*o*, *m*), and 7.28 (H, m, Ph-*p*) in the ^{1}H NMR spectrum.[15,16] Further support was provided by the prominent fragment peaks at *m*/*z* 194 and 134 in the FABMS.[16] The location of the Winterstein's acid side chain was deduced to be at C-5 from the long-range correlations of H-5 (δ 5.17) to C-1′ (δ 170.0) in the HMBC spectrum. The relative stereochemistry of **5** was determined from the NOESY experiment. A coupling constant between H-9 and H-10 of 10.2 Hz indicated that the B-ring was in a chair-boat conformation. The β-orientations of H-2 and H-9 were deduced by the 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. Proton-10 was α, as deduced from the NOESY correlations of H-10/H-3 and H-10/H_3-18, while proton-13 was β-oriented because of the NOESY correlation between H-13 and H_3-16. In turn, proton 5 is in a pseudoequatorial position, and its ^{1}H NMR signal appeared as a broad triplet with a small coupling constant.[10,11,17] Proton-5 was β-oriented, because the NOESY correlations of H-5 to H-6a, H-6b, and H-20a, but not to H-7a, were consistent with the proposed relative stereochemistry (H-5β). The stereochemistry at C-3′ of the Winterstein's acid moiety is as shown from the similarity of its ^{1}H and ^{13}C NMR data with reported values.[9,17] The structure of **5** was therefore characterized as 10β-hydroxy-2α,9α,13α-triacetoxy-5α-(3′-(dimethylamino)-3′-phenyl)butanoate-taxa-4(20),11-diene.

The molecular formula of compound **6** was determined as $C_{37}H_{51}NO_9$ by HRFABMS, which is the same as for taxane **5**. The NMR data of **6** (Tables 2 and 3) were quite similar to those of **5**, with signals for four methyl groups,

Table 2. ^{1}H NMR Data of **3**−**6** (500 MHz, acetone-d_6)

position	**3**	**4**[a]	**5**	**6**
1	1.82 (d, 2.0)	1.76 (m)	1.86 (d, 9.7)	1.84 (br d, 9.5)
2	5.38 (dd, 6.4, 2.0)	1.74 (m)	5.47 (dd, 6.8, 2.2)	5.39 (dd, 6.5, 1.9)
3	2.97 (d, 6.4)	3.05 (m)	3.18 (d, 6.8)	3.13 (d, 6.5)
5	5.23 (m)	4.13 (br s)	5.17 (t, 2.5)	5.23 (t, 2.7)
6a	1.77 (o m)	1.97 (o m)	1.45 (o m)	1.46 (o m)
6b	1.77 (o m)	1.62 (o m)	1.11 (o m)	1.14 (m)
7a	1.97 (o m)	1.80 (o m)	1.58 (o m)	1.80 (o m)
7b	1.20 (o m)	1.64 (o m)	1.42 (o m)	1.29 (m)
9a	2.35 (dd,15.1,12.4)	5.79 (d, 10.7)	5.77 (d, 10.2)	4.23 (br d, 10.1)
9b	1.61 (dd, 15.1, 5.4)			
OH-9				4.45 (br)
10	5.31 (dd, 12.4, 5.4)	6.07 (d, 10.7)	4.87 (m)	5.80 (d, 10.1)
OH-10			4.14 (d, 3.9)	
13a	2.84 (dd, 19.2, 9.5)	4.69 (br t, 8.2)	5.85 (bt t, 8.4	5.82 (br t, 8.6)
13b	2.47 (dd, 19.2, 4.9)			
14a	4.97 (dd, 9.5, 4.9)	2.63 (m)	2.51 (m)	2.50 (dt, 15.1, 9.5)
14b		1.19 (m)	1.43 (o m)	1.46 (o m)
16	1.19 (s)	0.99 (s)	1.14 (s)	1.08 (s)
17	1.68 (s)	1.55 (s)	1.74 (s)	1.59 (s)
18	2.04 (s)	2.20 (s)	1.98 (s)	2.14 (s)
19	0.84 (s)	0.69 (s)	0.97 (s)	0.98 (s)
20a	5.23 (br s)	5.05 (s)	5.25 (s)	5.23 (s)
20b	4.88 (br s)	4.72 (s)	4.85 (s)	4.86 (s)
-OAc	2.13 (s)	2.00 (s)	2.12 (br)	2.10 (s)
	1.97 (s)	1.94 (s)	2.03 (s)	2.03 (s)
	1.96 (s)		1.96 (s)	1.96 (s)
1′	4.32 (d, 7.8)	4.24 (d, 7.8)		
2′a	3.25 (o.m)	3.18 (t, 7.8)	3.13 (br)	3.11 (o dd)
2′b			2.88 (br)	2.92 (dd, 14.1, 9.4)
OH-2′	4.22 (d, 3.9)			
3′	3.35 (o m)	3.34 (m)	3.78 (br)	3.80 (br t, 6.6)
OH-3′	4.18 (br s)			
4′	3.34 (o m)	3.34 (m)		
OH-4′	4.11 (br d, 2.7)			
5′	3.22 (o m)	3.23 (m)		
6′a	3.85 (o m)	3.80 (dd, 11.6, 2.7)		
6′b	3.66(o m)			
OH-6′	3.60 (t, 6.4)			
o, m		7.33 (m)	7.33 (m)	7.33 (m)
p		7.28 (m)	7.28 (m)	7.28 (m)
N-Me_2		2.17 (br)	2.17 (br)	2.17 (br)

[a] In $CDCl_3$.

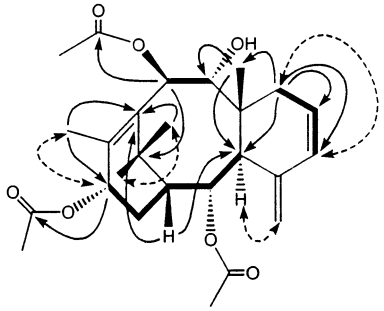

Figure 2. Arrows denote the key HMBC correlations (H→C) of **7**. Bold lines indicate the ^{1}H−^{1}H COSY correlations and dotted arrows show long-range correlations observed in the ^{1}H−^{1}H COSY spectrum.

three acetyl methyl groups, an exomethylene, and one Winterstein's acid moiety. The major differences were in the chemical shifts for H-9, H-10 and C-9, C-10. In taxane **5**, H-9/C-9 and H-10/C-10 resonated at δ 5.77/78.7 and 4.87/68.6, respectively, whereas in compound **6**, H-9/C-9 and H-10/C-10 resonated at δ 4.23/74.9 and 5.80/74.8, respectively. The HMBC correlations indicated that these differences were caused by a difference in acetylation pattern: **5** has an acetyl at C-9, whereas **6** is acetylated at C-10. The structure of **6** was, therefore, established as 9α-hydroxy-2α,10β,13α-triacetoxy-5α-(3′-(dimethylamino)-3′-phenyl)butanoate-taxa-4(20),11-diene.

Compound **7** was a colorless gum and minor metabolite. Its molecular composition was $C_{26}H_{36}O_7$, as deduced from the HRFABMS analysis. The ^{1}H NMR and ^{13}C NMR signals and HMBC correlations of **7** (Figure 2, Table 4) proved that it belongs to a regular C-6/C-8/C-6 taxane core skeleton with three acetyl groups on C-2, C-10, and C-13 and a hydroxyl group at C-9. The only missing signals were that of H-5/C-5. In addition, two olefinic protons/carbons resonated at δ 5.99/132.5 and 5.59/123.9, which from HMBC correlations implied that the additional double bond (aside from the C-4−C-20 exomethylene) was between C-5 and C-6. The HMBC spectrum supported the observed downfield chemical shifts of H-7a (δ 2.27, 1H, dd, J = 18.3, 6.1), H-7b (δ 2.11, 1H, m), and C-7 (δ 32.0) in **7**.[18] The structure of **7** was, therefore, established as 9α-hydroxy-2α,10β,13α-triacetoxytaxa-4(20),5(6),11(12)-triene. This is the first reported example of a taxane with a double bond between C-5 and C-6.

The Canadian yew seems to be different from other yews in its composition of taxanes. In this publication we have identified seven taxanes isolated for the first time in *T. canadensis*; six of these metabolites (**1**, **3**−**7**) are reported for the first time.

Experimental Section

General Experimental Procedures. See ref 6.

Plant Material. The needles of *Taxus canadensis* Marsh (Taxaceae) were collected in September 1997 at St-Jean, Quebec, Canada. Several specimens (under accession voucher number lz97-03) have been deposited in the herbarium of the Montreal Botanical Garden, Montreal, Canada.

Extraction and Isolation. Air-dried needles of *T. canadesis* were ground (4.0 kg) and extracted with 24 L of methanol for 1 day at room temperature. The ground needles were

Table 3. ^{13}C NMR Data of **3**−**6** (125 MHz, acetone-d_6)

position	**3**	**4**[a]	**5**	**6**
1	59.3	40.4	48.4	48.4
2	70.1	27.8	71.7	71.7
3	42.0	37.4	43.8	43.8
4	142.9	151.9	142.5	142.9
5	77.7	82.2	77.8	77.8
6	28.6	28.9	30.9	28.1
7	33.5	27.2	26.7	25.6
8	39.1	42.4	43.7	44.4
9	44.6	77.2	78.7	74.9
10	70.9	72.6	68.7	74.8
11	135.9	132.7	136.9	134.0
12	136.4	141.5	132.2	135.6
13	39.4	67.5	69.6	69.6
14	70.1	34.5	27.8	27.9
15	36.9	38.6	37.3	37.4
16	30.9	30.5	30.9	31.3
17	24.2	26.6	26.2	27.1
18	20.1	14.6	14.6	14.6
19	21.8	17.3	17.4	17.8
20	116.0	110.2	117.4	116.9
AcO	20.7	19.7	20.5	20.5
	168.8	169.5	170.0	169.9
	20.3	19.9	19.9	20.2
	169.2	169.0	168.7	169.3
	20.3			20.3
	169.2		168.7	168.9
1′	98.5	103.6	170.0	169.9
2′	73.8	73.4	39.7	39.6
3′	77.5	70.7		67.5
4′	70.9	76.7	67.6	
5′	76.5	76.2		
6′	61.8	62.0		
Ph-3′				138.9
o, m,			128.0	128.5
p			127.2	128.5
N-Me_2			41.8	42.0

[a] In $CDCl_3$.

filtered and extracted again with fresh solvent another three times (each time with 8 L of solvent, total 24 L) in 3 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 to 1.5 L and extracted with 80% methanol four times (each 500 mL). The 80% methanol extract, after being 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 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 of acetone and absorbed onto 40 g of silica gel and subjected to normal-phase column chromatography (silica gel 230−400 mesh, 850 g, 25 × 9 cm) with an elution 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-1} to Fr_{E-27}). Fr_{E-2} (1.9 g) was dissolved in 5 mL of acetone, absorbed onto 5 g of silica gel, and subjected to normal-phase column chromatography (silica gel 230−400 mesh, 100 g, 29 × 3 cm), eluted with a mixture of hexane−EtOAc (600−400 mL). Twenty fractions were obtained (Fr_{E-2-1} to Fr_{E-2-20}). Fr_{E-2-2} (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 per minute. The material eluted at t_R = 34.52 min was collected, concentrated, and applied to a preparative TLC column (1 × 20 × 20 cm, thickness 0.25 mm). Development with hexane−ethyl acetate (7:5) yielded taxane **7** (3.0 mg, R_f = 0.40). A portion of the CH_2Cl_2 extract (50 g) was absorbed onto 110 g of silica gel and subjected to column chromatography (silica gel 230−400 mesh, 1320 g). Successive elution with a CH_2Cl_2−MeOH gradient with increasing amounts of MeOH from 5% to 45% (total 15 L) yielded 45 fractions (Fr_{D-1} to Fr_{D-45}). Fractions Fr_{D-38} to Fr_{D-41} were combined (24 g) according to their TLC behavior, chromatographed over silica gel (770 g), eluted with hexane−acetone (3:2, 3000 mL; 1:1, 3000 mL; and 2:3, 3000 mL), and yielded 28 fractions (Fr_{D-38-1} to $Fr_{D-38-28}$). Fraction $Fr_{D-38-15}$ (1.8 g) was rechromatographed over silica gel (150 g, 4 × 30 cm), eluted with CH_2Cl_2−CH_3CN (8:7, 1000 mL; 8:5, 1000 mL; and

Table 4. ^1H and ^{13}C NMR Data of **7** in $CDCl_3$ (500 MHz for ^1H, 125 MHz for ^{13}C)

position	δ ^1H mult[a]	*J* (Hz)	δ ^{13}C[b,d]	HMBC[d]	NOESY[c]
1	1.66 (d)	8.9	47.3	1, 3, 11, 15	2,[s] 16[s]
2	5.48 (d)	2.8	72.1	1, 3, 4, 8, 14, 15	1,[s] 9,[s] 17,[s] 19[s]
3	2.99 (br s)	<1.8	44.0		7b,[m] 14b,[s] 18[m]
4			**141.9**		
5	5.99 (dd)	9.7, 1.5	132.5		6,[s] 20b[s]
6	5.59 (br t)	∼6−7	123.9		5,[s] 7a,[m] 7b,[w] 19[w]
7a	2.27 (dd)	18.3, 6.1	32.0	3, 5, 6, 8, 19	6,[s] 7b,[s] 19[s]
7b	2.11 (m)				3,[s] 5,[s] 6,[m] 7a,[s] 18[m]
8			**41.3**		
9	4.35 (d)	9.7	76.4	7, 8, 10, 19	2,[s] 17,[s] 19[m]
10	5.77 (d)	9.7	76.0	9, 11, 12, 15, **170.4**	3,[w] 7b,[s] 18[s]
11			**135.8**		
12			**137.5**		
13	5.53 (br dd)	∼10, ∼3	69.7	12, **170.5**	14a,[s] 16,[s] 18[m]
14a	2.74 (ddd)	8.9, 10, 15.7	29.3	1, 2, 12, 13	13,[s] 14b,[s] 16[m]
14b	1.69 (dd)	15.7, 4.2		1, 2, 13, 15, 17	3,[s] 14a[s]
15			**38.2**		
16	0.99 (s)		31.9	1, 11, 15, 17-Me	1,[s] 13,[s] 14a,[s] 17[s]
17	1.58 (s)		26.3	1, 11, 15, 16-Me	2,[s] 9,[s] 16[s]
18	1.91 (s)		15.8	11, 12, 13	3,[m] 10,[s] 7b,[s] 13[m]
19	1.02 (s)		18.8	3, 7, 8, 9	2,[s] 9,[s] 7a[s]
20a	5.55 (br s)		116.4		14b,[s] 20b[s]
20b	4.97 (br s)				5,[s] 20a[s]
−OAc	2.11 (s)		21.3	**170.4**	
			170.4		
	2.02 (s)		21.4	**169.5**	
			169.5		
	1.99 (s)		20.9	**170.5**	
			170.5		

[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 HSQC and HMBC experiments (±0.2 ppm). [c] NOESY intensities are marked as strong (s), medium (m), or weak (w). [d] Bold numbers represent quaternary carbons whose chemical shifts were obtained from HMBC experiments (±0.2 ppm).

2:1, 1000 mL) to afford 15 fractions ($Fr_{D-38-15-1}$ to $Fr_{D-38-15-15}$). Fraction $Fr_{D-38-15-12}$ (67 mg) was subjected to preparative TLC (2 × 20 × 20 cm, thickness 0.25 mm; hexane−acetone, 70:40) to give taxane **5** (2.0 mg, R_f = 0.35). Fractions $Fr_{D-38-16}$ to $Fr_{D-38-19}$ (4 g) were rechromatographed over silica gel (150 g, 4 × 30 cm), eluted with hexane−acetone (6:4, 5:4, 1:1) to afford 20 fractions ($Fr_{D-38-16-1}$ to $Fr_{D-38-16-20}$). Amorphous powder **6** (6 mg) was obtained from $Fr_{D-38-16-6}$. Fractions Fr_{D-42} to Fr_{D-45} were pooled (1.2 g), decolored with active carbon, and partitioned between water and ethyl acetate. The ethyl acetate-soluble fraction (200 mg) obtained was further separated by preparative HPLC (linear gradient of acetonitrile, same conditions as above) and afforded **3** (3.2 mg, t_R = 19.44 min), **2** (5.0 mg, t_R = 20.69 min), **4** (3.0 mg, t_R = 22.43 min), and **1** (5.5 mg, t_R = 26.52 min).

2α,5α-Diacetoxy-14*β*-2′α-methylbutanoate-10*β*-*O*-(*β*-D-glucopyranosyl)taxa-4(20),11-diene (1): amorphous solid; $[\alpha]^{22}_D$ +32° (*c* 0.15, $CHCl_3$); ^1H and ^{13}C NMR spectral data, see Table 1; LRFABMS *m/z* 705 [M + K^+]; HRFABMS *m/z* 705.3215 $[M + K]^+$ (calcd for $C_{35}H_{54}O_{12}K$, 705.3252), 689.3497 $[M + Na]^+$ (calcd for $C_{35}H_{54}O_{12}Na$, 689.3513).

2α,5α-Diacetoxy-14*β*-(2′*S*,3′*R*)-3′-hydroxy-2′α-methylbutanoate-10*β*-*O*-(*β*-D-glucopyranosyl)taxa-4(20),11-diene (2). This compound was first detected in rooted cuttings of *T. cuspidata*.[3]

2α,5α,14*β*-Triacetoxy-10*β*-*O*-(*β*-D-glucopyranosyl)taxa-4(20),11-diene (3): amorphous solid; $[\alpha]^{22}_D$ +39° (*c* 0.2, $CHCl_3$); ^1H and ^{13}C NMR spectral data, see Tables 2 and 3; HRFABMS *m/z* 663.27805 $[M + K]^+$ (calcd for $C_{32}H_{48}O_{12}K$, 663.27829).

9α,10*β*-Diacetoxy-13α-hydroxy-5α-*O*-(*β*-D-glucopyranosyl)taxa-4(20),11-diene (4): amorphous solid; $[\alpha]^{22}_D$ +33° (*c* 0.3, $CHCl_3$); ^1H and ^{13}C NMR spectral data, see Tables 2 and 3; HRFABMS *m/z* 621.2684 $[M + K]^+$ (calcd for $C_{30}H_{46}O_{11}K$, 621.2677), 605.2954 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_{11}Na$, 605.2938).

10*β*-Hydroxy-2α,9α,13α-triacetoxytaxa-4(20),5,11-triene (5): gum; $[\alpha]^{22}_D$ +37° (*c* 0.1, $CHCl_3$); ^1H and ^{13}C NMR spectral data, see Tables 2 and 3; HRFABMS *m/z* 692.3204 $[M + K]^+$ (calcd for $C_{37}H_{51}NO_9K$, 692.3201), 676.3441 $[M + Na]^+$ (calcd for $C_{37}H_{51}NO_9Na$, 676.3462), 194.1184 (calcd for $C_{11}H_{16}NO_2$, 194.1181), 134.0974 (calcd for $C_9H_{12}N$, 134.0970).

9α-Hydroxy-2α,10*β*,13α-triacetoxy-5α-(3′-(dimethylamino)-3′-phenyl)propionyloxytaxa-4(20),11-diene (6): amorphous powder; $[\alpha]^{22}_D$ +49° (*c* 0.1, $CHCl_3$); ^1H and ^{13}C NMR spectral data in acetone-d_6, see Tables 2 and 3; ^1H NMR (500 MHz, $CDCl_3$) δ 1.81 (1H, br d, *J* = 8.6 Hz, H-1), 5.33 (1H, dd, *J* = 6.4, 1.9 Hz, H-2), 3.05 (1H, d, *J* = 6.4 Hz, H-3), 5.20 (1H, t, *J* = 2.4 Hz, H-5), 1.43 (1H, m, H-6a), 1.01 (1H, br d, *J* = 15 Hz, H-6b), 1.66 (1H, m, H-7a), 1.16 (1H, td, *J* = 14.1, 4.0 Hz, H-7b), 4.25, (1H, dd, *J* = 9.8, 4.3 Hz, H-9), 2.18 (1H, d, *J* = 4.3 Hz, 9-OH), 5.76 (1H, d, *J* = 9.8 Hz, H-10), 5.81 (1H, br t, *J* = 8.4 Hz, H-13), 2.55 (1H, dt, *J* = 15.1, 9.6 Hz, H-14a), 1.43 (1H, m, H-14b), 1.09 (3H, s, Me-16), 1.58 (3H, s, Me-17), 2.12 (3H, s, Me-18), 0.98 (3H, s, Me-19), 5.24 (1H, br s, H-20a), 4.85 (1H, s, H-20b), 2.10 (3H, s, 10-CH_3CO−), 2.10 (3H, s, 13-CH_3CO−), 1.99 (3H, s, 2-CH_3CO−); ^{13}C NMR (125 MHz, $CDCl_3$) δ 48.4 (C-1), 72.2 (C-2), 43.9 (C-3), 142.3(C-4), 78.5 (C-5), 28.3 (C-6), 25.5 (C-7), 44.4 (C-8), 75.9 (C-9), 75.7 (C-10), 133.6 (C-11), 136.8 (C-12), 70.2 (C-13), 28.2 (C-14), 37.6 (C-15), 31.2 (C-16), 27.1 (C-17), 15.5 (C-18), 18.2 (C-19), 118.1(C-20), 21.5 (CH_3CO-10), 170.1 (CH_3CO-10), 21.5 (CH_3CO-2), 169.4 (CH_3CO-2), 21.3 (CH_3CO-13), 170.2 (CH_3CO-10); HRFABMS *m/z* 654.3659 [M + H]$^+$ (calcd for $C_{37}H_{52}O_9$, 654.3652).

9α-Hydroxy-2α,10*β*,13α-triacetoxy-5α-(3′-(dimethylamino)-3′-phenyl)propionyloxytaxa-4(20),11-diene (7): gum; $[\alpha]^{22}_D$ +47° (*c* 0.21, $CHCl_3$); ^1H and ^{13}C NMR, see Table 4; HRFABMS *m/z* 499.20968 $[M + K]^+$ (calcd for $C_{26}H_{36}O_7K$, 499.20981).

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Supporting Information Available: HMBC and NOESY data of **3**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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