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Taxanes with C-5-amino-side chains from the needles of *Taxus canadensis*

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Abstract

Five taxanes with an amino-side chain on C-5 were identified for the first time in the needles of the Canadian yew, *Taxus canadensis*. Their structures were characterized as 2α,7β,9α,10β,13-pentaacetoxy-11β-hydroxy-5α-(3′-*N,N*-dimethylamino-3′-phenyl)-propionyloxytaxa-4(20),12-diene (**1**), 2α,9α-dihydroxy-10β,13α-diacetoxy-5α-(3′-methylamino-3′-phenyl)-propionyloxytaxa-4(20), 11-diene (**2**), 2α17-dihydroxy-9α,10β,13α-triacetoxy-5α-(3′-*N,N*-dimethylamino-3′-phenyl)-propionyloxytaxa-4(20),11-diene (**3**), 2α-hydroxy-7β,9α,10β,13α-tetraacetoxy-5α-(2′-hydroxy-3′-*N,N*-dimethylamino-3′-phenyl)-propionyloxytaxa-4(20),11-diene (**4**), and 9α-hydroxy-2α,10β,13α-triacetoxy-5α-(3′-*N,N*-dimethylamino-3′-phenyl)-propionyloxytaxa-4(20),11-diene (**5**) on the basis of 1D-, 2D-NMR spectroscopic data and high-resolution fast atom bombardment MS analyses. Metabolite (**1**) was isolated from the needles of the Canadian yew for the first time but had previously been detected in the stems of the Japanese yew, whereas taxanes (**2**–**5**) are only now reported. Metabolite (**3**) is the first reported nitrogen-containing taxane with a 17-hydroxyl substitution.

Keywords: *Taxus canadensis*; Taxacae; Yew; Taxanes; C-5-amino-side chain; Metabolites

1. Introduction

Nature has been the primary source for most of the drugs presently on the market (Cragg et al., 1997; Newman et al., 2003). Pharmaceutical companies succeed in improving the biological activity of natural products by judicious choice of substituents. Paclitaxel (Taxol), the anticancer wonder drug of the 1990s was first isolated from the pacific yew *Taxus brevifolia* Nutt (Taxaceae) (Wani et al., 1971). Docetaxel, obtained by semi-synthesis, illustrate the power of synthetic modification of natural products (Kingston, 2001; Gueritte, 2001; Kingston et al., 2002). Extensive investigation of different *Taxus* species led to a large number of taxane structures (Baloglu and Kingston, 1999). Indeed, more than 400 taxanes are presently known (Banskota et al., 2002; Choudhary et al., 2002; Li et al., 2001, 2002, 2003; Nguyen et al., 2001; Shen et al., 2002a,b; Shinozaki et al., 2002), including many novel taxanes isolated from the needles of the Canadian yew, *Taxus canadensis* (Zhang et al., 2001; Shi et al., 2002, 2003a,b, 2004).

In the present publication, we are reporting characterization of five taxanes from the needles of the

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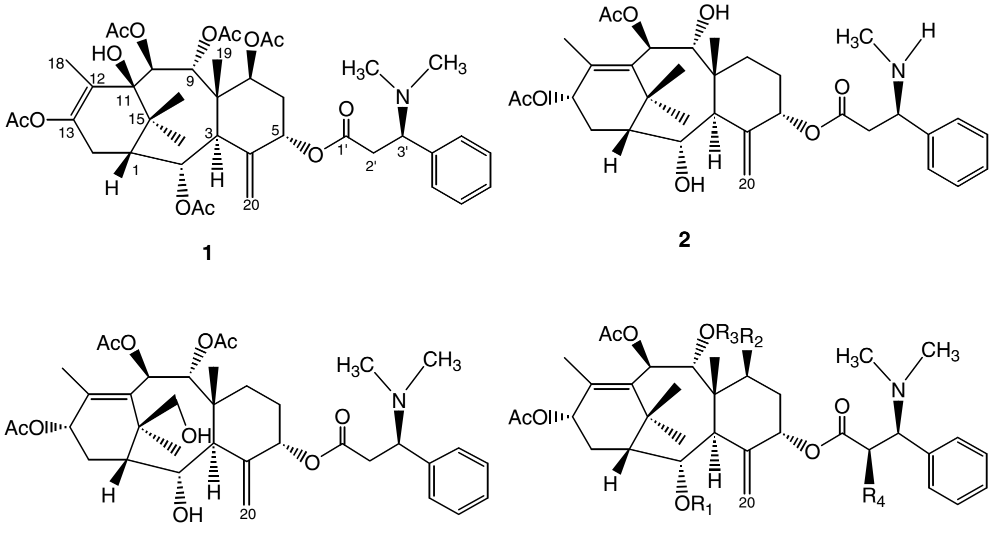
Canadian yew, four of which are new compounds. We have rigorously elucidated their chemical structures using 1D- (^{1}H NMR, ^{13}C NMR) and 2D-NMR (^{1}H–^{1}H COSY, HMQC or HSQC, HMBC and NOESY) methods and confirmed them by high resolution fast atom bombardment mass spectrometry (HRFABMS).

2. Results and discussion

A methanolic extract from the needles of *Taxus canadensis* was processed as described in the experimental section to give five taxanes containing nitrogen (**1**–**5**, Scheme 1).

Taxane **1** was obtained as a colorless amorphous solid. Its molecular composition, $C_{41}H_{55}NO_{13}$, was established from the combined analysis of HRFABMS at *m/z* 770.3752 $(M + H)^+$, 792.3571 $(M + Na)^+$ and 808.3310 $(M + K)^+$ and ^{13}C-NMR spectroscopic analyses. The ^{1}H-NMR spectrum of **1** (Table 1) exhibited the characteristic signals of a taxane with a Winterstein's acid moiety. Detailed interpretation of its 1D- and 2D-NMR including HSQC and HMBC spectra enabled us to establish the structure of taxane **1** as 2α,7β,9α,10β,13-pentaacetoxy-11β-hydroxy-5α-(3′-*N,N*-dimethylamino-3′-phenyl)-propionyloxytaxa-4(20),12-diene. A literature search showed that the structure of **1** is identical with taxuspine P isolated from the stem of the Japanese yew, *Taxus cuspidata* (Kobayashi et al., 1996). For comparison with taxuspine P, we recorded the NMR for **1** in benzene-d6 (Table 1). Differences between our NMR data for taxane **1** in benzene-d_6 and that in the literature was less than 0.04 ppm for ^{1}H-NMR and 0.2 ppm for ^{13}C-NMR spectroscopy.

Compound **2** was obtained as a colorless gummy substance. Its molecular composition ($C_{34}H_{47}NO_8$) was obtained by HRFABMS. The ^{1}H- and ^{13}C-NMR spectra (Table 2) showed signals due to four methyl groups at δ 1.06, 1.11, 1.51, and 1.98 ppm, two acetyl groups at δ 2.08, 21.3 and 170.2 and 1.66, 21.0 and 170.4 ppm, and an exocyclic methylene group at δ 5.39 (1H, s), 5.40 (1H, s), 118.8, and 143.5 ppm. These signals suggested that **2** has a taxane skeleton (Appendino, 1995; Kingston et al., 1993). The signal at δ 2.97 ppm (1H, d, $J = 6.8$ Hz) was characteristic of the C-3 ring junction proton in a taxa-4(20),11-diene analog (Appendino, 1995; Kingston et al., 1993). In addition, five proton signals attached to oxygenated carbons were observed from the chemical shift data and HMBC correlations. An AB system resonating at δ 4.19 ppm and at δ 5.76 ppm was assigned to H-9 and H-10 respectively based on the HMBC correlations (Table 2). The acetyl group was assigned to H-10 due to its correlation to a carbonyl carbon at δ 170.4 ppm. The *trans*-orientation of H-9 and H-10 was suggested by the large vicinal coupling constant observed (10 Hz). Using H-3 as a reference, the connectivities from C-3 to C-2 to C-1 to C-14 to C-13 were deduced from the ^{1}H–^{1}H COSY spectrum. The chemical shifts of H-2 (δ 4.06 ppm) and H-13 (δ 5.84 ppm) implied that a hydroxyl group and an acetyl group were attached to C-2 and C-13, respectively. The presence of a nor-Winterstein acid [3′-(*N*-methylamino)-3′-phenylpropanoyl] moiety in **2** was suggested from the signals at δ 2.32 (3H, s, N–CH_3), 2.83 (2H,



	R_1	R_2	R_3	R_4
4	H	OAc	Ac	OH
5	Ac	H	H	H

Scheme 1.

Table 1
^{1}H and ^{13}C NMR spectral data for **1** in acetone-d_6 (500 MHz for ^{1}H, 125 MHz for ^{13}C)

Position	δ (^{1}H), mult	J (Hz)	δ (^{13}C)	HMBC	NOESY
1	1.91 (o)		50.3		
2	5.77 (br.d)	6.7	67.8	8, 14, 15, 169.2	1, 17, 19
3	3.69 (d)	6.7	40.7	1, 4, 2/5, 8, 9, 19, 20	14b, 7, 5/20b
4	–		142.9		
5	5.15 (o.m)		67.8	6, 169.5	
6a	1.94 (o.m)		32.5		
6b	1.68 (o.m)				
7	4.67 (dd)	10.6, 8.4	69.8	3, 19, 169.3	3, 6a, 6b
8	–		42.7		
9	4.88 (d)	5.0	73.7	3, 7, 8, 10/11, 19, 168.2	10, 19
10	5.56 (d)	5.0	77.2	8, 9, 11, 12, 15, 168.1	9, 18,
11	–		77.1		
12	–		124.0		
13	–		143.5		
14a	2.54 (br.dd)	19.2, 7.4	25.6		1, 14b, 16
14b	2.36 (br.d)	19.2			3, 14a, 20b
15	–		41.2		
16	1.14 (s)		31.3	1, 11, 15, Me-17	1, 14a, 17
17	1.51 (s)		23.6	1, 11, 15, Me-16	2, 14a, 16
18	1.56 (br.s)		11.0	11, 12, 13	10
19	1.46 (s)		14.1	3, 7, 8, 9	2, 9, 5/20a
20a	5.14 (s)		109.7	3, 4, 5	19, 20b
20b	5.05 (s)			3, 4, 5	3, 14b, 20a
–OAc	2.20 (s)		19.5	168.0	
			168.0		
	2.11 (s)		20.1	168.1	
			168.1		
	2.03 (s)		19.9	169.0	
			169.0		
	1.91 (s)		19.7	168.0	
			168.0		
	1.85 (s)		19.9	168.2	
			168.2		
	1.82 (s)		20.2	169.2	
			169.2		
1′	–		169.5		
2′a	3.06 (dd)	14.7, 8.3	37.5	1′, 3′, Ph–C1	
2′b	2.69 (dd)	14.7, 7.2		1′, 3′, Ph–C1	
3′	3.97 (t)	7.6	65.7		
Ph-3′			137.4		
o, *m*,	7.32–7.31 (m)		127.6, 128.5	3′, Ph–C1	2′a, 3′, N–Me
p	7.26 (m)		127.0		
N–Me$_2$	2.11 (s)		40.9	1′, 3′, 4′	

m, H-2′), 4.00 (1H, br.dd, $J = 7.8$, 5.8 Hz, H-3′), 7.34 (2H, m, Ph-*o*), 7.33 (2H, m, Ph-*m*), and 7.28 ppm (H, m, Ph-*p*) in the ^{1}H NMR spectrum. Further support was provided by the fragment ions at *m/z* 120 and 180 in the FABMS spectrum (Fig. 1). Appendino et al. (1993) reported that removal of one methyl group from the dimethylamino of the Winterstein acid moiety could cause a marked upfield shift (ca. 8 ppm) of the remaining methyl group in the ^{13}C NMR spectrum. Indeed, the signal of N–Me of **2** resonated at δ 33.9 ppm. The location of the nor-Winterstein acid side chain was deduced to be at C-5 as observed from the long range correlation between H-5 (δ 5.26 ppm) and C-1′ (δ 171.0 ppm) in the HMBC spectrum. The relative stereochemistry of **2** was determined from chemical shifts, coupling constants and the NOESY experiment. A coupling constant between H-9 and H-10 of 10.0 Hz indicated that the B-ring was in a chair-boat conformation. The β-orientations of H-2 and H-9 were deduced by NOESY correlations of H-2/H-1, H-2/H$_3$-17, H-2/H$_3$-19, and H-9/H-2, H-9/H$_3$-17, H-9/H$_3$-19. The α-orientation of H-10 was assigned by the observation of NOESY correlations of H-10/H$_3$-18. The β-orientation of H-5 was inferred by comparison with analogs. H-13 was β as suggested by the observation of a correlation between H-13 and H$_3$-16. Thus, the structure of **2** was established as 2α,9α-dihydroxy-10β,13α-diacetoxy-5α-(3′-methylamino-3′-phenyl)-propionyloxytaxa-4(20),11-diene.

Table 2
^{1}H and ^{13}C NMR spectral data for **2** in CDCl$_3$ (500 MHz for ^{1}H, 125 MHz for ^{13}C)

Position	δ (^{1}H), mult	*J* (Hz)	δ (^{13}C)	HMBC	NOESY
1	2.05 (o.m)		51.3		2, 16, 17
2	4.06 (dd)	6.8, 2.1	69.8	8	1, *3*, 9, 17, 19
3	2.97 (d)	6.8	46.1	1, 2, 5, 8, 19, 20	7b, 18
4	–		143.5		
5	5.26 (t)	~2.6	79.0	3, 171.0	20, 1.70 (6)
6	1.65 (m)		28.7		
7a	1.80 (m)		25.7		
7b	1.30 (m)				
8	–		44.8		
9	4.19 (d)	10.0	75.8	7, 8, 10, 19	2, 17, 19
10	5.76 (d)	10.0	75.8	9, 11, 12, 15, 170.3	7b, 18
11	–		133.0		
12	–		135.8		
13	5.84 (br.t)	8.9	70.1		14a, 16
14a	2.48 (o.m)		27.9		13, 14b
14b	1.27 (o.m)				
15	–		37.2		
16	1.11 (s)		31.6	1, 11, 15, Me-17	1, 13, 17
17	1.51 (s)		27.4	1, 11, 15, Me-16	1, 2, 9, 16
18	1.98 (br.s)		15.0	11, 12, 13	3, 10, *2′*, *Ph3′*
19	1.06 (s)		18.2	3, 7, 8, 9	2, 9, 20, 1.70 (6)
20a	5.40 (s)		118.8	3, 4, 5	5
20b	5.39 (s)				
–OAc	2.08 (s)		21.3	170.2	
			170.2		
	1.66 (s)		21.0	170.4	
			170.4		
1′	–		171.0		
2′	2.83 (m)		42.6	3′, 140.5, 171.0	
3′	4.01 (br.dd)	7.8, 5.8	62.0	1′, 2′, 5′, Ph-126.9, 140.5	
Ph-3′	–		140.5		
	7.34 (m)		126.9	62.0, Ph–C1	2′, 3′, 5′
	7.33 (m)		128.7		
	7.28 (m)		128.0		
N–Me	2.321 (s)		33.9	3′	

The molecular formula of **3** was established as $C_{37}H_{51}NO_{10}$ [M + H]$^+$ by HRFABMS. The NMR data of **3** (Table 3) closely resembled those of taxuspine Z, which was isolated from the stems of *Taxus cuspidata* (Shigemori et al., 1997). Three acetyl methyl groups, an *exo*-methylene, and one Winterstein's acid moiety were observed. The only difference between **3** and taxuspine Z is the hydroxyl group on the C-17 position. The HMBC correlations of the hydroxymethylene protons to C-1 and C-16 indicated that this hydroxymethylene group was positioned at C-17 in compound **3**. The structure of **3** was therefore established as 2α17-dihydroxy-9α,10β,13α-triacetoxy-5α-(3′-*N,N*-dimethylamino-3′-phenyl)-propionyloxytaxa-4(20),11-diene, i.e. 17-hydroxy-taxuspine Z. In compound **3**, the signals due to the Winterstein's acid moiety were very broad, making assignments difficult. Mass spectrometry confirmed the presence of the Winterstein's acid moiety. Indeed, we found a molecular ion at 708.3150 (M + K$^+$), 692.3411 (M + Na$^+$), and 670.3591 (M + 1H), and fragment ion peaks at *m*/*z* 134.0976 ($C_9H_{12}N$) and 194.1185 ($C_{11}H_{16}NO_2$) in the HRFABMS (Fig. 1). In addition, the proton NMR data showed aromatic protons between δ 7.30 and 7.70 ppm (5H, m, Ph–H).

Compound **4** was isolated as a colorless gummy substance. Its molecular formula, $C_{39}H_{53}NO_{12}$, was deduced from HRFABMS analysis. The ^{1}H and ^{13}C NMR spectra (Table 4) exhibited signals of four methyl groups, four acetyl groups, and an exocyclic methylene group, suggesting that **4** has a taxane skeleton (Appendino, 1995; Kingston et al., 1993). The presence of the *N,N*-dimethyl-3-phenylisoseryl group was suggested by the signals at δ 3.93 (1H, broad, H-2′), 4.77 (1H, br., H-3′), 2.30 [6H, N–(CH$_3$)$_2$], and 7.31–7.45 (5H, Ph) in the ^{1}H NMR spectrum and the fragments at *m*/*z* 210 in the FABMS spectrum (Shi et al., 2000) (Fig. 1). The connectivities of the protons on the taxane skeleton of **4** were determined by analysis of the ^{1}H–^{1}H COSY spectrum. Interpretation of ^{1}H-, ^{13}C-NMR and HMBC spectra permitted the positional assignment of functional groups: a hydroxyl group attached to C-2, four acetoxy groups positioned at C-7, C-9, C-10 and C-13,

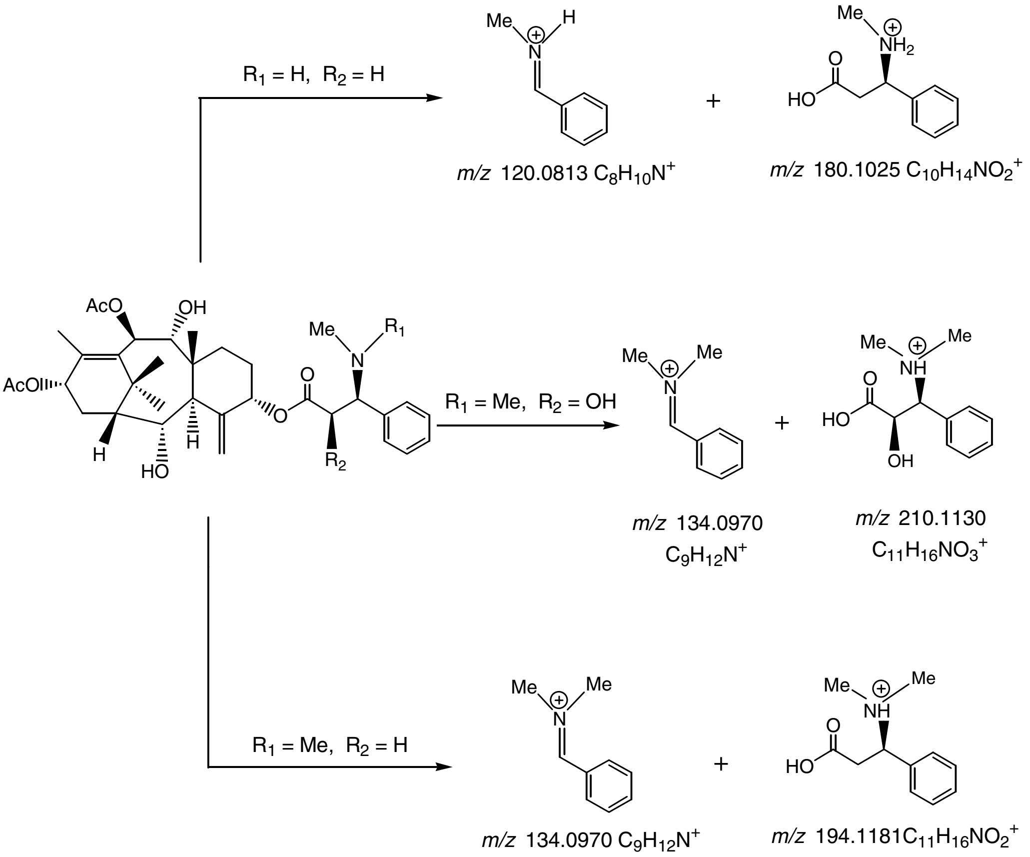


Fig. 1. Fragmentation pattern observed in positive ion FABMS/MS spectra of taxanes with different C-5-amino-side chains.

and an *N,N*-dimethyl-3-phenylisoseryl group was connected to C-5. The relative stereochemistry at all the positions of **4** was established from analysis of NOESY data and coupling constants. The structure of **4** was therefore characterized as 2α-hydroxy-7β,9α,10β,13α-tetraacetoxy-5α-[2′-hydroxy-3′-*N,N*-dimethylamino-3′-phenyl]-propionyloxy-taxa-4(20),11-diene.

Compound **5** was obtained as a colorless amorphous solid. Its molecular composition, $C_{37}H_{51}NO_9$, was established from combined analysis of HRFABMS at *m*/*z* 692.3201 $(M + K)^+$ and its ^{13}C NMR spectrum. The ^1H and ^{13}C NMR spectra of **5** (Table 5) exhibited the characteristic signals of a taxane including four tertiary methyls, three acetyl groups, and an exocyclic double bond. The presence of the Winterstein's acid moiety in **5** was indicated by the signals at δ 3.05 (1H, br., H-2′a), 2.87 (1H, br., H-2′b), 3.80 (1H, br., H-3′), 2.23 [6H, br.s, *N*–$(CH_3)_2$], and 7.25–7.33 ppm (5H, m, Ph–H), which was further verified by the fragment ion peaks at *m*/*z* 134.0974 ($C_9H_{12}N$) and 194.1184 ($C_{11}H_{16}NO_2$) in the FABMS (Fig. 1). Combined interpretation of ^1H–^1H COSY and HMBC spectra enabled us to assign the substitution groups on the taxane skeleton. Thus, the structure of **5** was unambiguously established as 9α-hydroxy-2α,10β,13α-triacetoxy-5α-(3′-*N,N*-dimethylamino-3′-phenyl)-propanoyloxytaxa-4(20),11-diene, **5** i.e. 2α-acetyl-9α-deacetyltaxuspine Z (Shigemori et al., 1997).

It is interesting to note the difference in the fragmentation pattern of the differently substituted nitrogen-containing taxanes (Fig. 1). These results rigorously confirm our structure assignments obtained from 1D and 2D NMR.

In summary, five nitrogen-containing taxanes were isolated from the needles of the Canadian yew. Four are new structures, while compound **1** was isolated previously from the stem of the Japanese yew, *Taxus cuspidata* (Kobayashi et al., 1996). Taxane **3** is the first example of a nitrogen-containing taxane with a hydroxyl group at C-17.

3. Experimental

3.1. Instrumentation

Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. Flash chromatography was performed on silica gel 60 (230–400 mesh EM Science). Thin layer chromatography was conducted on silica

Table 3
^{1}H and ^{13}C NMR spectral data for **3** in acetone-d_6 (500 MHz for ^{1}H, 125 MHz for ^{13}C)[a]

Position	δ (^{1}H), mult	J (Hz)	δ (^{13}C)	HMBC	NOESY
1	2.55 (m)		45.6		2, 16
2	4.21 (br.d)	5.5	70.4		1, 9, 17a, 19
3	2.92 (d)	5.5	45.4	1, 2, 8, 19, 20	2, 18, 7
4	–				
5	5.12 (br.t)	2.4	79.7		20b
6ab	1.27 (m)		32.5		
7	1.46 (m)		28.2		
8	–		45.0		
9	5.63 (d)	10.8	77.7	7, 8, 10, 19, 171.0	2, 17a, 19
10	5.95 (d)	10.8	72.5	9, 11, 12 15, 170.2	18
11	–		134.1		
12	–		139.2		
13	5.89 (o.m)		70.8		14a, 16
14a	2.46 (ddd)	14.7, 9.8, 9.6	28.3		13, 14b, *16*
14b	1.47 (dd)	14.7, 7.5			3, 14a, 20b
15	–		44.6		
16	1.23 (s)		25.6	1, 11, 15, 17	1, 13, 17b
17a	4.48 (br.d)	11.9	65.9		2, 9, 17b
17b	3.98 (br.d)	11.9		1, 16	*9*, 16, 17a
18	2.22 (v.br.s)		16.2		3, 10
19	0.82 (o.s)		18.2	3, 7, 8, 9	2, 9
20a	5.89 (br.s)		120.9		20b
20b	5.21 (br.s)				5, 20a
–OAc	2.02 (s)		21.7	171.0	
			171.0		
	1.96 (s)		21.7	170.2	
			170.2		
Ph	7.30–7.70 (br.m)				

[a] The signals of the side chain except aromatic ring were so broadened that their detection becomes difficult.

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. Analytical HPLC was performed on a Waters 600 FHU delivery system coupled to a PDA 996 detector. Preparative HPLC were carried out on a Waters Delta Prep 3000 instrument coupled to a UV 486 Tunable Absorbance detector set at 227 and 210 nm (Waters, Montreal, Que., Canada) and a Partisil 10 ODS-2 MAG-20 preparative column (22 × 500 mm). The products were eluted with a 50 min linear gradient of $CH_3CN \cdot H_2O$ (25–100%) in H_2O at a flow rate of 18 ml/min (preparative HPLC) and 3 ml/min (semi-preparative HPLC).

All NMR spectroscopic data were obtained at room temperature on a Bruker Avance-500 spectrometer at working frequencies 500.13 MHz for proton and at 125.77 MHz for carbon-13, respectively. The solvent was used as an internal reference for proton as well as for carbon-13; in the case of $CDCl_3$, the reference was set to 7.25 and 77.0 ppm while for acetone-d_6, the reference was set to 2.04 and 29.8 ppm for proton and carbon spectra, respectively. 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. Positive ion MALDI mass spectrometry experiments were performed on a Q-Tof UltimaFast 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.

3.2. Plant material

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

3.3. Extraction and isolation

Air-dried needles (4.0 kg) of *T. canadesis* were ground and extracted with MeOH at room temperature. The combined organic extracts were evaporated under reduced pressure. Water was added, and lipids were removed by extracting the mixture with hexane. The

Table 4
^{1}H and ^{13}C NMR spectral data for **4** in acetone-d_6 (500 MHz for ^{1}H, 125 MHz for ^{13}C)

Position	δ (^{1}H), mult	J (Hz)	δ (^{13}C)	HMBC	NOESY
1	2.06 (m)		52.1		
2	4.26 (td)	6.5, 1.9	68.7	3, 8, 14	1, 9, 17, 19
2-OH	3.52 (d)	6.5		2, 3	2, 14b, 20a
3	2.78 (o.d)		43.9	1, 2, 5, 7, 8, 19, 20	7, 10, 18, 14b
4	–				
5	5.15 (t)	~2.7	76.8		6a, 6b, 20b
6a	1.55 (m)		34.3		5, 6b, 19
6b	0.85 (br)				5, 6a, 7
7	5.29 (br.dd)	10.8, 4.3	69.6		6b, 10, 18
8	–		46.8		
9	5.80 (d)	10.8	75.5	7, 8, 10, 19, 169.5	2, 17, 19
10	6.16 (d)	10.8	71.4	8, 9, 11, 12, 15, 168.5	7, 18
11	–		132.7		
12	–		136.6		
13	5.91 (br.t)	~9	69.9		14a, 16, 18
14a	2.44 (dt)	15.2, 9.7	27.6	1, 2, 12, 13	1, 13, 14b, 16
14b	1.43 (o.m)			1, 2, 13, 15	14a, 16
15	–		37.5		
16	1.12 (s)		30.8	1, 11, 15, Me-17	1, 13, 14a, 17
17	1.66 (s)		27.0	1, 11, 15, Me-16	1, 2, 9, 16
18	2.24 (s)		14.7	11, 12, 13	7, 10, 13, 3′, Ph-*o*, Ph-*m*
19	0.95 (s)		13.0	3, 7, 8, 9	6a, 2, 9, 17
20a	5.82 (s)		121.3		3, 20b
20b	5.35 (s)				5, 20a
–OAc	2.07 (s)		19.8	170.2	
			170.2		
	2.03 (s)		20.4	168.5	
			168.5		
	2.02 (s)		19.7	169.4	
			169.4		
	1.94 (s)		19.8	168.1	
			168.1		
1′	–				
2′	3.93 (broad)				Ph-*o*
3′	4.77 (broad)		71.3		N–Me(4′), Ph-*o*
Ph3′					
o,	7.45 (br.)		130.1		2′, 3′, N–Me(4′)
m,	7.38 (t)	7.2	128.2		
p	7.31 (~t)		128.0		
N–Me$_2$	2.30 (br)		41.3		2′, 3′, Ph-*o*

aqueous phase was then salted and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried with anhydr. Na_2SO_4, filtered, and evaporated, to yield a dark green extract (115 g). A portion of the CH_2Cl_2 extract (50 g) was adsorbed onto 110 g silica gel and subjected to column chromatography eluting with a mixture of methylene chloride and methanol. A CH_2Cl_2–MeOH gradient was used with increasing amounts of MeOH from 5% to 45%. Forty-fractions were obtained: Fr$_{\text{D-1}}$ to Fr$_{\text{D-45}}$. Fr$_{\text{D-38}}$ to Fr$_{\text{D-41}}$ were combined (24 g) and subjected to column chromatography (silica gel) eluting with a mixture of hexane–acetone. Twenty-eight fractions were obtained (Fr$_{\text{D-38-1}}$ to Fr$_{\text{D-38-28}}$). Fr$_{\text{D-38-24}}$ (4 g) was re-chromatographed over silica gel eluted with hexane:acetone (3:2) to afford 20 fractions (Fr$_{\text{D-38-24-1}}$ to Fr$_{\text{D-38-24-20}}$).

Fraction Fr$_{\text{D-38-24-9}}$ (150 mg) was subjected to preparative HPLC. The material eluting at t_R = 38.24 min was further purified by preparative TLC (hexane-ethyl acetate 40:75) to yield taxane **1** (5.0 mg, $R_f = 0.14$). Part of Fr$_{\text{D-38-24-8}}$ (110 mg) was subjected preparative TLC (hexane–acetone 3:2) to yield **2** (7.0 mg, $R_f = 0.46$). Preparative TLC (20 cm × 20 cm) (hexane–acetone 5:6) of Fr$_{\text{D-38-24-17}}$ (25 mg) and isolating the region at $R_f = 0.40$ visible under UV light eluted with acetone and further purified by preparative TLC developed twice with CH_2Cl_2:CH_3CN (1:1) and (1:4) to give **3** (2.0 mg, $R_f = 0.16$).

Fractions Fr$_{\text{D-38-23}}$ to Fr$_{\text{D-38-28}}$ (2.0 g) were subjected to additional silica gel CC and eluted with CH_2Cl_2:CH_3CN = 7:3 and 6:4 affording 25 fractions (Fr$_{\text{D-38-23-1}}$ to Fr$_{\text{D-38-23-25}}$).

Table 5
^{1}H and ^{13}C NMR spectral data for **5** in $CDCl_3$ (500 MHz for ^{1}H, 125 MHz for ^{13}C)

Position	δ (^{1}H), mult	J (Hz)	δ (^{13}C)	HMBC	NOESY
1	1.81 (br.d)	8.6	48.4	2, 3, 11, 13, 15	2, 14a, 16, 17
2	5.33 (dd)	6.2, 1.9	72.2	1, 3, 8, 14, 15, 169.3	1, 3, 9, 17, 19
3	3.05 (d)	6.2	43.9	1, 2, 4, 5, 7, 8, 19, 20	2, 7b, 10, 18, 6a/14b
4	–		142.3		
5	5.20 (t)	~2.4	78.5 (broad)	3, 4, 20, 170.5	6a, 6b
6a	1.43 (o.m)		28.3		
6b	1.01 (br.d)	~15			3, 5, 6a, 7a
7a	1.66 (br.m)		25.5		7b, 6b
7b	1.16 (td)	14.1, 4.0			3, 5, 7a, 10, 18
8	–		44.4		
9	4.25 (dd)	9.8, 4.3	75.9	7, 8, 10, 19	2, 10, 17, 19, 9-OH
9-OH	2.18 (d)	4.3		8, 9/10	9, 10
10	5.76 (d)	10.0	75.7	8, 9, 11, 12, 15, 170.1	3, 7b, 9, 18, 9-OH
11	–		133.6		
12	–		136.8		
13	5.81 (br.t)	8.4	70.2	11, 12, 14, 170.2	14a, 16
14a	2.55 (dt)	15.1, 8.5	28.2	1, 2, 12, 13	1, 13, 14b, 16
14b	1.43 (o.m)				
15	–		37.6		
16	1.09 (s)		31.2	1, 11, 15, Me-17	
17	1.58 (s)		27.1	1, 11, 15, Me-16	1, 2, 9, 16
18	2.12 (s)		15.5	11, 12, 13	3, 7a, 10, 13
19	0.98 (s)		18.2	3, 7, 8, 9	2, 9, OH-9
20a	5.24 (s)		118.1	3, 4, 5	
20b	4.85 (s)			3, 4, 5	
–OAc	2.10 (br.)		21.5 (br)	170.1	
			170.1		
	2.10 (s)		21.5	170.4	
			170.4		
	1.99 (s)		21.3	169.6	
			169.6		
1′	–		170.6		
2′a	3.05 (br.o)		40.1 (broad)		
2′b	2.87 (br)				
3′	3.80 (br.)		68.1		
Ph	7.32–7.25 (m)		128.5		
N–Me$_2$	2.23 (br)		42.8		

Fraction Fr$_{D-38-23-24}$ (150 mg) was subjected to preparative HPLC and the material eluting at $t_R = 37.52$ min (40 mg) was further purified by preparative TLC (hexane–acetone 5:6) to give **4** (5.5 mg, $R_f = 0.26$).

Preparative HPLC of fraction Fr$_{D-38-22-5}$ (83 mg) and the material eluting at $t_R = 44.12$ min (25 mg) further purified by preparative TLC (hexane–acetone 70:45) gave **5** (4.0 mg, $R_f = 0.40$).

*3.4. 2α,7β,9α,10β,13-Pentaacetoxy-11β-hydroxy-5α-(3′-N,N-dimethylamino-3′-phenyl)-propanoyloxytaxa-4(20),12-diene (**1**)*

Amorphous solid; $[\alpha]_D^{22} + 39°$ (c 0.2, $CHCl_3$); for ^{1}H- and ^{13}C NMR spectral data, see Table 1; HRFABMS m/z 808.3310 $[M + K]^+$ (calcd for $C_{41}H_{55}NO_{13}K$, 808.3322), 792.3571 $[M + Na]^+$ (calcd for $C_{41}H_{55}NO_{13}Na$, 792.3606), 770.3752 $[M + H]^+$ (calcd for $C_{41}H_{55}NO_{13}H$, 770.3754), 134.0976 (calcd for $C_9H_{12}N$, 134.0970), 194.1178 (calcd for $C_{11}H_{16}NO_2$, 194.1181).

*3.5. 2α,9α-Dihydroxy-10β,13α-diacetoxy-5α-(3′-N-methylamino-3′-phenyl)-propanoyloxytaxa-4(20),11-diene (**2**)*

Gum; $[\alpha]_D^{22} + 47°$ (c 0.21, $CHCl_3$); for ^{1}H and ^{13}C NMR spectral data, see Table 2; HRFABMS m/z 620.3199 $[M + Na]^+$ (calcd for $C_{34}H_{47}NO_8Na$, 620.3220), 598.3380 $[M + H]^+$ (calcd for $C_{34}H_{47}NO_8H$, 598.3383), 120.0813 (calcd for $C_8H_{10}N$, 120.0813), 180.1022 (calcd for $C_{10}H_{14}NO_2$, 180.1025).

*3.6. 2α,17-Dihydroxy-9α,10β,13α-triacetoxy-5α-(3′-N,N-dimethylamino-3′-phenyl)-propanoyloxytaxa-4(20),11-diene (**3**)*

Amorphous powder; $[\alpha]_D^{22} + 39°$ (c 0.1, $CHCl_3$); for ^{1}H and ^{13}C NMR spectral data in acetone-D$_6$, see Table 3. HRFABMS m/z 708.3150 $[M + K]^+$ (calcd for $C_{37}H_{51}NO_{10}K$, 708.3164), 692.3411 $[M + Na]^+$ (calcd for $C_{37}H_{51}NO_{10}Na$, 692.3401), 670.3591 $[M + H]^+$ (calcd for $C_{37}H_{51}NO_{10}H$, 670.3580), 134.0976 (calcd for $C_9H_{12}N$, 134.0970), 194.1185 (calcd for $C_{11}H_{16}NO_2$, 194.1181).

3.7. 2α-Hydroxy-7β,9α,10β,13α-tetraacetoxy-5α-(2′-hydroxy-3′-N,N-dimethylamino-3′-phenyl)-propanoyloxy-taxa-4(20),11-diene (**4**)

Gum; $[\alpha]_D^{22} + 76°$ (*c* 0.21, $CHCl_3$); for ^1H and ^{13}C NMR spectral data, see Table 4; HRFABMS *m/z* 766.3205 $[M + K]^+$ (calcd for $C_{39}H_{53}NO_{12}K$, 766.3174), 750.3465 $[M + Na]^+$ (calcd for $C_{39}H_{53}NO_{12}Na$, 750.3447), 728.3646 $[M + H]^+$ (calcd for $C_{39}H_{53}NO_{12}H$, 728.3640), 134.0973 (calcd for $C_9H_{12}N$, 134.0970), 210.1129 (calcd for $C_{11}H_{16}NO_3$, 210.1130).

3.8. 9α-Hydroxy-2α,10β,13α-triacetoxy-5α-(3′-N,N-dimethylamino-3′-phenyl)-propanoyloxytaxa-4(20),11-diene (**5**)

Gum; $[\alpha]_D^{22} + 53°$ (*c* 0.21, $CHCl_3$); ^1H and ^{13}C NMR in $CDCl_3$ see Table 5; ^1H NMR in acetone-d_6 δ: 1.84 (1H, br.d, $J = 9.3$ Hz, H-1), 5.39 (1H, dd, $J = 6.4$, 1.9 Hz, H-2), 3.13 (1H, d, $J = 5.4$ Hz, H-3), 5.23 (1H, t, $J = 2.7$ Hz, H-5), 1.46 (1H, m, H-6a), 1.14 (1H, m, H-6b), 1.80 (1H, m, H-7a), 1.29 (1H, m, H-7b), 4.23 (1H, br.d, $J = 10.1$, H-9), 4.45 (1H, br. 9-OH), 5.80 (1H, d, $J = 10.1$, H-10), 5.82 (1H, br.t, $J = 8.6$ Hz, H-13), 2.50 (1H, dt, $J = 15.1$, 9.5 Hz, H-14a), 1.46 (1H, m, H-14b), 1.08 (3H, s, Me-16), 1.59 (3H, s, Me-17), 2.14 (3H, s, Me-18), 0.98 (3H, s, Me-19), 5.23 (1H, s, H-20a), 4.86 (1H, s, H-20b), 1.96 (3H, s, 2-CH_3CO-), 2.03 (3H, s, 10-CH_3CO-), 2.10 (3H, s, 13-CH_3CO-), 3.11 (1H, o.dd, H-2′a), 2.92 (1H, dd, $J = 14.1$, 9.4 Hz, H-2′b), 3.80 (1H, br.t, $J = 6.6$ Hz, H-3′), 7.27–7.33 (5H, m, Ph–H), 2.17 [6H, br., N–$(CH_3)_2$]; ^{13}C NMR in acetone-d_6 δ: 48.4 (C-1), 71.7 (C-2), 43.8 (C-3), 142.9 (C-4), 77.8 (C-5), 28.1 (C-6), 25.6 (C-7), 44.4 (C-8), 74.9 (C-9), 74.8 (C-10), 134.0 (C-11), 135.6 (C-12), 69.6 (C-13), 27.9 (C-14), 37.4 (C-15), 31.1 (C-16), 27.1 (C-17), 14.6 (C-18), 17.8 (C-19), 116.9 (C-20), 20.3 (2-CH_3CO-), 20.2 (10-CH_3CO-), 20.5 (13-CH_3CO-), 168.6 (2-$CH_3\mathit{CO}$-), 169.3 (10-$CH_3\mathit{CO}$-), 169.9 (13-$CH_3\mathit{CO}$-), 169.9 (C-1′), 39.6 (C-2′), 67.5 (C-3′), 138.9 (Ph–C), 128.5 (Ph–C), 42.0 (N–CH_3); HRFABMS *m/z* 692.3201 $[M + K]^+$ (calcd for $C_{37}H_{51}NO_9K$, 692.3204), 676.3461 $[M + Na]^+$ (calcd for $C_{37}H_{51}NO_9Na$, 676.3441), 654.3642 $[M + H]^+$ (calcd for $C_{37}H_{51}NO_9H$, 654.3659), 134.0974 (calcd for $C_9H_{12}N$, 134.0970), 194.1184 (calcd for $C_{11}H_{16}NO_2$, 194.1181).

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