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Taxoids from the needles of the Canadian yew

Junzeng Zhang[a], Francoise Sauriol[b], Orval Mamer[c], Lolita O. Zamir[a,d,*]

[a]*Univeristé du Québec, INRS-Institut Armand-Frappier-Santé Humaine, 531 Boul. des Prairies, Laval, Qué., Canada, H7N 4Z3*
[b]*Department of Chemistry, Queen's University, Kingston, Ont., Canada, K7L 3N6*
[c]*Biomedical Mass Spectrometry Unit, McGill University, 1130 Pine Avenue West, Montréal, Qué., Canada, H3A 1A3*
[d]*Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montréal, Qué., Canada, H3A 2K6*

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Abstract

Systematic characterization of the taxoids in the needles of *Taxus canadensis* led to the discovery of seven taxanes along with three known congeners. Their structures were rigorously established by spectroscopic methods as 15-benzoyl-10-deacetyl-2-debenzoyl-10-dehydro-*abeo*-baccatin III; 15-benzoyl-2-debenzoyl-7, 9-dideacetyl-*abeo*-baccatin VI; N-acetyl-N-debenzoyltaxol; 7,9,13-trideacetylbaccatin VI; 10-deacetyl-10-glycolylbaccatin IV; 1β-hydroxy-10-deacetyl-10-glycolylbaccatin I; and 7-deacetyltaxuspine L. These taxanes, specific to the Canadian yew, were co-isolated with taxacustin, taxagifine and 2-deacetyl-7,10-diacetyl-5-deaminoacyl taxine A previously found in *Taxus cuspidata, baccata*, and *yunnanensis*, respectively.

Keywords: *Taxus canadensis*; Taxaceae; Taxoids; Taxanes

1. Introduction

Since the discovery of the potent antitumor activity of paclitaxel (Taxol®), a naturally occurring diterpenoid from the bark of *Taxus brevifolia* (Wani et al., 1971), much attention has been paid to the investigation of various species of *Taxus* for new taxanes. To date, about 300 structural diverse taxane derivatives have been isolated and identified (Kingston et al., 1993; Appendino, 1995; Parmar et al., 1999; Baloglu and Kingston, 1999). *Taxus canadensis*, a low trailing bush very common in Quebec, has been shown to be an interesting plant with unusual taxanes specific to this yew. Its major taxane, 9-dihydro-13-acetylbaccatin III, was isolated from the needles in 1992 (Zamir et al., 1992a,b, 1995a; Gunawardana et al., 1992). It was at least five times more abundant than its co-metabolite paclitaxel. 9-Dihydro-13-acetylbaccatin III has only been found, in trace amounts, in the bark of one other yew, *Taxus chinensis* (Zhang et al., 1992). Canadensenes, bicyclic fully oxygenated structures, are also specific to the needles of the Canadian yew (Zamir et al., 1995b, 1998; Boulanger et al., 1996). Recently, further novel taxanes from *T. canadensis* needles were characterized (Zamir et al., 1996, 1999a,b).

We now report seven new taxanes **1**–**7** (Fig. 1) which have been isolated from the needles of the Canadian yew as co-metabolites of taxacustin (**8**), taxagifine (**9**), and 2-deacetyl-7,10-diacetyl-5-deaminoacyl taxine A (**10**), previously isolated from *Taxus cuspidata, baccata*, and *yunnanensis*, respectively. The structures of all the taxanes were rigorously established by spectroscopic methods.

* Corresponding author. Tel.: +1-450-687-5010 X4260; fax: +1-450-686-5501.
E-mail address: lolita.zamir@iaf.uquebec.ca (L.O. Zamir).

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2. Results and discussion

The NMR data of taxane **1** (Fig. 1, Table 1) showed the presence of an *abeo*-taxane skeleton with four C-methyls (1.52, 1.58, 1.76, and 2.25), one acetyl (2.21), and one benzoyl group (7.79, 7.58, and 7.47). The AB quartet of the two C-20 protons, typical of taxanes with an oxetane ring, was present (4.65, *d*, J = 8.4 Hz and 4.58 *d*, J = 8.4 Hz). The 11(15 → 1)-*abeo*-taxane structure was confirmed by HMBC correlations of the protons of Me-16/17 (1.58 and 1.52) with C-1(66.7), C-15(89.9) and Me-16/17 (22.7/22.2). The NMR data of taxane **1** (lacking H-9 and H-10; HMBC of the protons of Me-19 with C-9 at 204.5; downfield shift of C-12 to 164.1 due to enone conjugation) strongly suggested the presence of a 9, 10-diketo moiety. Additional confirmation was given by comparison of our natural product taxane **1** with the synthetic 15(16)-anhydro-11(15 → 1)-*abeo*-10-deacetyl-10-dehydrobaccatin III (**11**, Fig. 2), the major rearrangement product of 10-deacetylbaccatin III (Appendino et al., 1993). Their ^{1}H- and ^{13}C-NMR spectral data are very similar except for H-2, C-15 and C-16. The placement of the benzoyl group on C-15 was deduced by the unusual downfield shift of C-15 (89.9 ppm) reminiscent of wallifoliol **12** (C-15: 90.4 ppm, Fig. 2) which was also isolated from *T. canadensis* needles (Zamir et al., 1997). The relative stereochemistry of taxane **1** was established using the information contained in the NOESY spectrum. In addition, the NOESY cross-peaks between some phenyl protons of the benzoyl group and H-14a, OH-2 further confirmed the location of the benzoyl group. High resolution mass spectrometry confirmed the elemental composition of the sodiated quasimolecular ion of **1**. The structure of **1** is therefore 15-benzoyl-10-deacetyl-2-debenzoyl-10-dehydro-11(15 → 1)*abeo*-baccatin III, the first naturally occurring 9,10-diketo-*abeo*-taxane found to-date.

The NMR spectrum of taxane **2** also clearly, showed an 11(15 → 1)-*abeo*-taxane structure, confirmed by HMBC correlations of C-1(69.3), C-15 (91.0) and Me-16/17 (23.0) with protons of Me-16/17 (1.96/1.73). The presence of one pair of doublets for H-9/H-10 in the NMR spectrum is characteristic of oxygenated substituents. The location of the three acetates on C-4, C-10 and C-13 derives directly from analysis of the NMR data. As in taxane **1**, it seems that the benzoyl group is on C-15 (C-15: 91.0, Table 2). A comparison of ^{1}H- and ^{13}C-NMR values of taxane **2** with that of 7,9,10-trideacetyl-*abeo*-baccatin VI (Appendino et al., 1994), which differs only on C-2, C-10 and C-15, confirmed our analysis. The relative stereochemistry of taxane **2** was established using the information contained in the NOESY experiment (Table 2). High resolution mass spectrometry confirmed the elemental composition of the sodiated quasimolecular ion of **2**. The structure of taxane **2** was, therefore, 15-benzoyl-2-debenzoyl-7,9-dideacetyl-*abeo*-baccatin VI. Since we have noticed a benzoyl shift (from C-2 to C-20) during chemical rearrangement reactions (Zamir et al., 1997), we could therefore postulate a C-2 to C-15 benzoyl migration as a biosynthetic step. Compounds **1** and **2** showed no degradation products either during purification at room temperature or after lengthy storage while re-

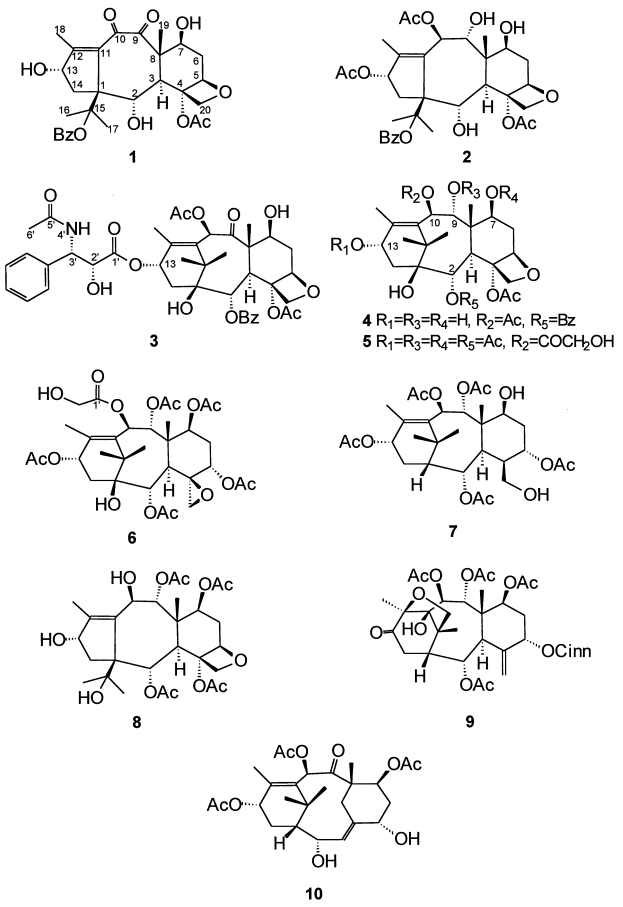

Fig. 1. Structures of isolated taxanes.

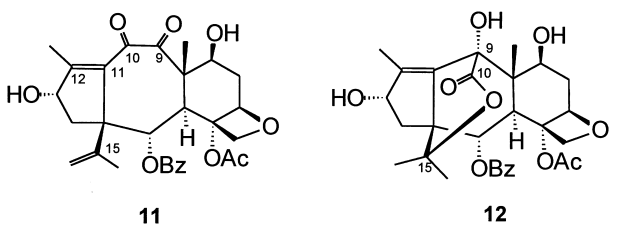

Fig. 2. Structures of some *abeo*-taxanes: **11** is a synthetic compound, whereas **12** is a natural taxane, wallifoliol isolated from *T. canadensis* needles.

frigerated. Tertiary benzoates are therefore apparently not labile in these taxanes.

The NMR spectrum of taxane **3** (Table 3) was very similar to that of paclitaxel (Chmurny et al., 1992) except for some differences in the C-13 side chain. The substituent on the secondary amine was not a benzoyl as for paclitaxel, but an acetyl. The placement of the acetyl on the 4′-NH was deduced from the HMBC correlations of C-5′ (169.9) with the proton on the secondary amine (6.23). The chemical shift of this proton overlaps with H-13. However, since HMBC experiments correlate protons to more distant carbons through their $^{2}J_{C-H}$ and $^{3}J_{C-H}$ scalar couplings, we are confident that the correlation with C-5′ is from 4′-NH and not H-13. The relative stereochemistry of taxane **3** was established to be identical to paclitaxel using the information contained in the NOESY experiment (Table 3). High resolution mass spectrometry confirmed the elemental composition of the sodiated quasimolecular ion of **3**. The structure of taxane **3**, is therefore, N-acetyl-N-debenzoylpaclitaxel (Fig. 1).

The structure of taxane **4** was easily determined since its NMR spectra (Table 4) were very similar to an abundant taxane in *Taxus canadensis* needles, 9-dihydro-13-acetylbaccatin III (Zamir et al., 1992a,b, 1995a; Gunawardana et al., 1992). The only difference is the placement of an acetyl on C-10 in taxane **4** instead of on C-13 in 9-dihydro-13-acetylbaccatin III, which derived from HMBC correlations (Table 4), as well as the comparison of H-10 and H-13 NMR shifts in both compounds. H-10 was deshielded by the presence of an acetyl on C-10 in taxane **4**, and H-2 by a benzoyl on C-2. All the other protons were not deshielded by the presence of an ester group and appeared at the usual chemical shifts for hydroxylated positions. The structure of taxane **5** is similar to **4** except with acetyl groups on C-2, C-7, C-9 and C-13. In addition, an unusual glycolic substituent–$^{1'}CO^{2'}$-CH_2OH on C-10 was derived from HMBC corre-

Table 1
^{1}H and- ^{13}C-NMR spectral data for taxane **1**

Position	δ ^{1}H Mult.[a] (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY
1		66.7		
2	4.52 *dd* (6.8; 4.9)	69.9	1, 3, 8, 14	3, Me-16, Me-17, Me-19, OH-2
2-OH	2.46 *d* (4.9)		2, 3	2, 14a, 14b, 20b, Bz-*o*
3	3.34 *d* (6.8)	43.6	1, 2, 4, 8, 19	2, 7, 14b, 20b
4		79.1		
5	5.04 *d* (8.8)	84.4	4, 7	6a, 20a
6a	2.67 *dt* (15.0; 7.6)	35.9		5, 6b, 7
6b	1.87 *ddd* (15.0; 9.5; 1.2)			6a, Me-19
7	4.15 *dd* (9.5; 7.6)	68.5		3, 5, 6b
8		56.3		
9		204.5		
10		192.0		
11		135.9		
12		164.1		
13	4.83 *br*/*t* (7.1)	76.7		
14a	2.76 *dd* (14.7; 7.1)	37.8	10, 11, 12, 15	13, 14b, Me-17
14b	1.91 *dd* (14.7; 7.8)		1, 2, 12, 15	3, 14a
15		89.9		
16	1.58 *o*/*s*	22.7	1, 15, 17	2, 13
17	1.52 *s*	22.2	1, 15, 16	2, 13, 14a
18	2.25 *d* (1.2)	14.0	11, 12, 13	
19	1.76 *s*	8.2	3, 7, 8, 9	2, 20b
20a	4.65 *d* (8.4)	75.6	4	20b
20b	4.58 *d* (8.4)		5	20a, Me-19
OAc	2.21 *s*	21.6	170.3	
OBz				
1′		165.4		
o	7.79 *dd* (8.3; 1.2)	128.8		Bz-*m*, Bz-*p*, OH-2, 14a
m	7.47 *t* (7.8)	128.8		
p	7.58 *t* (7.3)	132.9		

[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 quarternary carbons) experiments (±0.2 ppm).

lations between C-1′ (171.6) and H-10 protons (6.34) and the methylene protons of C-2′. The relative stereochemistry of taxanes **4** and **5** was established to be the same as in the corresponding baccatin VI and IV derivatives, respectively (Della Casa de Marcano and Halsall, 1975) using the information contained in the NOESY experiments (Tables 4 and 5). High resolution mass spectrometry confirmed the elemental compositions of the sodiated quasimolecular ions of **4** and **5**. The structures of taxanes **4** and **5** were, therefore, 7,9,13-trideacetylbaccatin VI and 10-deacetyl-10-glycolylbaccatin IV, respectively (Fig. 1).

Taxanes **6** and **7** are related to other taxanes found in *Taxus canadensis* needles, but with additional substituents. Taxane **6** differs from 1β-hydroxy-7,9-deacetylbaccatin I (Zamir et al., 1995a) by the presence of acetyl groups on C-7, C-9 instead of hydroxyl groups and a glycolic substituent $^{1'}COCH_2OH$ on C-10 instead of an acetyl. The acetyl groups on C-7 and C-9 could be easily located by the extra deshielding (~1.2–1.5 ppm) to the geminal protons on H-7 and H-9 (-CH-OAc) by comparison to an –OH(–CH–OH) as well as HMBC correlations (Table 6). The $^{1'}COCH_2OH$ group was observed by the corresponding NMR shifts and was placed on C-10 because of the HMBC correlation of C-1′ (171.5) with the H-10 proton (6.38). The relative stereochemistry of taxane **6** was established using the information contained in the NOESY experiment (Table 6). High resolution mass spectrometry confirmed the elemental composition of the sodiated quasimolecular ion of **6**. The structure of taxane **6** is, therefore, 1β-hydroxy-10-deacetyl-10-glycolylbaccatin I (Fig. 1). Taxane **6** is the first taxoid of the baccatin I type (4,20-epoxide) containing a glycolic substituent. Taxane **7** (Table 7) has an unusual hydroxymethyl group at an unoxygenated C-4 position (3.46 and 3.38) that we have previously observed in 7,9-dideacetyltaxuspine L (Zamir et al., 1999a). Indeed,

Table 2
^{1}H- and ^{13}C-NMR spectral data for taxane **2**

Position	δ ^{1}H Mult.[a] (J in Hz)	$\delta^{13}C$[b]	HMBC	NOESY
1		69.3		
2	4.74 *br/t* (4.3)	67.7	2, 3, 14, 15	3, 9, Me-16, Me-19, OH-2
2-OH	2.36 *br/d* (5.6)		3	2, 20, Bz-*o*
3	2.50 *d* (7.1)	44.7	1, 2, 7, 8, 19, 20	2, 7, 10
4		80.2		
5	4.98 *d* (8.8)	84.1		6a, 6b, 20
6a	2.58 *ddd* (15.4; 9.0; 8.3)	37.8		5, 6b, 7
6b	1.92 *o/m*			
7	4.45 *dd* (9.0; 7.8)	72.9	19	3, 6a, 10
8		42.2		
9	4.07 *d* (11.1)	77.8	7, 8, 10	2, Me-16, Me-19
10	6.04 *d* (11.1)	69.3	1, 9, 11, 12, Ac	3, 7, 9, Me-18
11		137.2		
12		146.9		
13	5.79 *t* (7.2)	78.4	11, Ac	14a, Me-16
14a	2.66 *dd* (14.4; 7.2)	35.9	11, 12, 15	13, 14b
14b	1.68 *o/m*		1, 2, 12, 13	14a
15		91.0		
16	1.96 *s*	23.0	1, 15, 17	2, 9, 13, 14a, Me-17
17	1.73 *s*	23.0	1, 15, 16	
18	1.75 *s*	11.8	11, 12, 13	
19	1.80 *s*	11.8	3, 7, 8, 9	
20a	4.60 *d* (8.5)	75.9	3, 4	5, Me-19
20b	4.58 *d* (8.5)			
OAc	2.15 *s*	21.6	169.3	
	2.09 *s*	20.8	170.5	
	2.00 *s*	20.8	170.3	
OBz				
1′		166.1		
o	7.85 *d* (8.3)	128.8	*o, p, 1′*	Bz-*m*, OH-2
m	7.46 *t* (7.8)	128.8		
p	7.56 *t* (7.3)	132.9		

[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 quarternary carbons) experiments (±0.2 ppm).

these compounds are very similar, the only difference being an extra acetyl on C-9 in taxane **7**. The NMR spectral data as well as the HMBC and NOESY correlations of **7** compared with 7,9-dideacetyltaxuspine L and taxuspine L (Wang et al., 1996a) proved that the structure of **7** was 7-deacetyltaxuspine L with the stereochemistry shown in Fig. 1. High resolution mass spectrometry confirmed the elemental composition of the sodiated quasimolecular ion of **7**. Taxane **7** and 7,9-dideacetyltaxuspine L are the only two examples found in *T. canadensis* of the relatively rare taxanes with a hydroxymethyl substituent at an unoxygenated C-4 position (Tanaka et al., 1994; Fuji et al., 1995; Wang et al., 1996a,b; Shen and Chen, 1997; Zhou et al., 1998; Yang et al., 1999).

The three known taxanes **8**, **9** and **10** were identified as taxacustin (Tong et al., 1993), taxagifine (Chauviere et al., 1982), and 2-deacetyl-7,10-diacetyl-5-deaminoacyl taxine A (previously mistakenly named 2-deacetyltaxin B) by comparison of their NMR spectral data to that in the literature (Yue et al., 1995). High resolution mass spectrometry confirmed the elemental compositions of the sodiated quasimolecular ions of taxanes **8**, **9**, and **10**. They are reported here for the first time from the needles of *T. canadensis*.

The list of taxanes specific to the needles of *T. canadensis* is increasing. Indeed, we can now add C-15-benzoylated taxanes and 9,10-diketo-*abeo*-taxanes to taxinine, taxinine E, the canadensenes, 7-oxygenated-3,11-cyclotaxanes and the abundant 9-dihydro-13-acet-

Table 3
^{1}H- and ^{13}C-NMR spectral data for taxane **3**

Position	δ ^{1}H Mult.[a] (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY
1		78.9		
2	5.68 *d* (7.1)	74.8	1, 3, 8, 14, Bz-1″	
3	3.79 *d* (7.1)	45.5	1, 2, 4, 7, 8, 19, 20	2, 7, 10, Me-18
4		81.1		
5	4.93 *d* (8.1)	84.4	4	
6a	2.54 *ddd* (15.4; 10.5; 6.8)	35.3	7, 8	5, 6b, 7
6b	1.86 *o*/*m*			5, 6a
7	4.40 *dd* (10.5; 6.8)	72.1	3, 6, 19	3, 6a, 10
8		58.3		
9		203.7		
10	6.28 *s*	75.3	9, 11, 12, 15, Ac	3, 7, Me-18
11		133.1		
12		141.9		
13	6.23 *o*/*m*	72.3	See NH	See NH
14	2.29 *o*/*m*	35.3		
15		43.0		
16	1.27 *s*	26.6	1, 11, 15, 17	13, 14, Me-17
17	1.15 *s*	21.6	1, 11, 15, 16	2, Me-17, Me-19
18	1.82 *s*	14.5	11, 12, 13	3, 7, 10
19	1.68 *s*	9.3	3, 7, 8, 9	2, 6b, 20b, Me-17
20a	4.30 *d* (8.5)	76.4	3, 4	5, 20b, Bz-*o*
20b	4.19 *d* (8.5)		5	2, 20a, Me-19
OAc	2.33 *s*	22.5	170.1	
	2.25 *s*	20.5	171.2	10
OBz				
1″		166.9		
o	8.11 *d* (8.0)	130.1	*o*, *p*, 1″	
m	7.51 *t* (7.8)	128.5		
p	7.62 *t* (8.0)	133.7		
C═O 1′		172.7		
CH 2′	4.67 *d* (2.3)	72.9	1′	13, 14, 3′, Ph
CH 3′	5.55 *dd* (8.8; 2.3)	54.5	Ph	
3′-Ph	7.43-7.32 *m*	128.5		3′, NH
		128.7		
		126.8		
NH 4′	6.23 *o*/*m*		11, 1′, 2′, 5′	14, 3′, Me-16, Ph, Me-6′
C═O 5′		169.9		
Me 6′	2.01 *o*/*s*	23.0	NH	NH

[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 quarternary carbons) experiments (±0.2 ppm).

ylbaccatin III. This latter compound (originally named 7, 9-deacetylbaccatin VI in Zamir et al., (1992a,b)) is abundant only in *Taxus canadensis* needles. This is not the first taxane which was found to be five to seven times more abundant than paclitaxel in yews, 10-deacetylbaccatin III was found to be abundant only in *Taxus baccata* in 1984 (Senilh et al., 1984). This key disclosure led to the semi-synthesis of paclitaxel and the discovery of docetaxel (Guéritte-Voegelein et al., 1986; Denis et al., 1988). However, 10-deacetylbaccatin III is found in all yews in about the same low yield as paclitaxel. This is not the case with 9-dihydro-13-acetylbaccatin III. The only other yew where it is found albeit as traces is in the bark of *Taxus chinensis* (Zhang et al., 1992). In addition to this abundant taxane, we find in the needles of the Canadian yew many taxanes which derive from this major compound (Zamir et al., 1995a, taxanes **4** and **5**, in this report). The biosynthesis of this unique abundant taxane is, therefore, intriguing, raising the possibility that *Taxus canadensis* may be the only yew having a dehydrogenase capable of reducing the C-9 keto-group of 10-deacetylbaccatin III.

3. Experimental

3.1. General

^{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. $CDCl_3$ was used as the internal reference (δ 7.25 ppm for ^{1}H and 77.0 ppm for ^{13}C). Low resolution FAB mass spectra were obtained in glycerol with a VG ZAB-HS instrument. Samples were dissolved in 0.2 μl DMSO before addition of 0.5 μl glycerol. HR-FAB MS was similarly obtained in glycerol-DMSO at a resolving power of 12,000.

Table 4
^{1}H- and ^{13}C-NMR spectral data for taxane **4**

Position	δ ^{1}H Mult.[a] (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY
1		78.5		
1-OH	1.67 *o*/*br*/*s*			2
2	5.73 *d* (6.0)	73.2	1, 3, 8, 14, 1′	3, 9, 10, Me-17, Me-19
3	3.11 *d* (6.0)	46.6	1, 2, 8, 19, 20	2, 7, 10, 14b
4		82.2		
5	4.93 *d* (8.9)	84.1	3, 4, 7	6a, 20a
6a	2.55 *ddd* (14.9; 8.9; 7.6)	37.8	7, 8	5, 6b, 7
6b	1.92 *ddd* (14.9; 9.8; 1.7)		4, 5, 7	6a, Me-19
7	4.44 *o*/*m*	73.7		
8		44.9		
9	4.45 *o*/*d* (10.1)	76.7		
10	6.14 *d* (10.1)	73.7	8, 9, 11, 12, Ac	2, 3, 7, 14a, Me-18
11		134.3		
12		142.9		
13	4.80 *br m*	68.2		14a, 14b, Me-16
14a	2.29 *o*/*dd* (14.7; 4.2)	38.4	1, 2, 12, 13	10, 13, 14b
14b	2.15 *o*/*m*			
15		42.6		
16	1.11 *s*	27.9	1, 11, 15, 17	13, 14a, Me-17
17	1.64 *s*	21.6	1, 11, 15, 16	2, 9, Me-16
18	2.12 *d* (1.2)	15.1	11, 12, 13	3, 10, 14a
19	1.81 *s*	12.3	3, 7, 8, 9	2, 6b, 9, 20b
20a	4.32 *d* (8.3)	76.4		20b, Bz-*o*
20b	4.17 *d* (8.3)			2, 20a, Me-19
OAc	2.25 *s*	22.7	171.6	
	2.15 *s*	21.1	170.3	
OBz				
1′		167.0		
o	8.11 *d* (7.5)	130.1	Bz-*m*, Bz-*p*, 1′	20a, Bz-*m*
m	7.48 *t* (7.9)	128.5	Bz-*o*	
p	7.61 *t* (7.6)	133.7	Bz-*o*	

[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 quarternary carbons) experiments (±0.2 ppm).

Liquid column chromatography was performed on silica 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). Semi-preparative HPLC was performed on the same system described above but using two Partisil 10 ODS-2 MAG-9 semi-preparative columns (Whatman) connected in series (9.4 × 500 mm). Preparative TLC was carried out on silica gel 60 F_{254} precoated TLC plates, 0.25 mm (EM Science).

3.2. Plant material

The needles of *T. canadensis* Marsh were collected in September 1997 at St-Jean, Quebec, Canada and air dried prior to extraction. Several specimens are kept in the herbarium of Montreal Botanical Garden.

3.3. Extraction and isolation

Dried, ground needles of *T. canadensis* (4.7 kg) were extracted as described previously (Zamir et al., 1998) to yield 119 g of a dark brown extract. A portion (50 g) of this extract was separated repeately on silica gel columns and eluted with n-hexane–CH_2Cl_2, CH_2Cl_2–EtOAc and EtOAc–MeOH, followed by preparative and semi-preparative HPLC (linear gradient of CH_3CN in water from 25% to 100% in 50 min or 70 min) and preparative TLC finally yielded **1** (1.2 mg), **2** (1.7 mg), **3** (1.5 mg), **4** (1.2 mg), **5** (4.1 mg), **6** (5.0 mg), **7** (1.2 mg), **8** (1.1 mg), **9** (17.8 mg), and **10** (8.4 mg).

15-Benzoyl-10-deacetyl-2-debenzoyl-10-dehydro-*abeo*-baccatin III (**1**): gum; HR-FAB MS for $C_{29}H_{34}O_{10}Na$ requires: 565.20497; found: 565.20470; ^{1}H- and ^{13}C-NMR, HMBC and NOESY spectral data (see Table 1).

15-Benzoyl-2-debenzoyl-7,9-dideacetyl-*abeo*-baccatin

Table 5
^{1}H- and ^{13}C-NMR spectral data for taxane **5**

Position	δ ^{1}H Mult.[a] (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY
1		78.6		
2	5.56 *d* (5.6)	72.7	1, 3, 8, 14, 15, Ac	3, 9, Me-17, Me-19
3	3.02 *d* (5.6)	47.3	1, 4, 5, 7, 8, 19, 20	2, 7, Me-18
4		81.2		
5	4.95 *d* (8.8)	83.9	3, 4, 7	6a, 6b, 7
6a	2.47 *dt* (14.7; 8.4)	34.5	4, 5, 7, 8	5, 6b, 7
6b	1.88 *dd* (14.7;10.0)		4, 5, 7	6a, Me-19
7	5.50 *br*/*t* (8.4)	71.7	3, 6, 8, 19, Ac	3, 6a, 6b, 10, Me-18
8		45.8		
9	5.92 *d* (11.4)	74.8	7, 8, 10, 11, 19, Ac	2, Me-17, Me-19
10	6.34 *d* (11.4)	72.1	8, 9, 11, 12, 15, 1′	7, Me-18
11		133.0		
12		142.4		
13	6.13 *t* (8.4)	69.6	11, 12, Ac	14a, Me-16
14a	2.13 *o*/*m*	34.9	1, 2, 13	
14b	2.05 *o*/*m*		1, 2, 13, 15	
15		42.8		
16	1.20 *s*	28.3	1, 11, 15, 17	13, Me-17
17	1.66 *s*	22.2	1, 11, 15, 16	2, 9, Me-16
18	2.02 *s*	15.0	11, 12, 13	
19	1.53 *s*	12.6	3, 7, 8, 9	6b, 20b
20a	4.51 *d* (8.1)	76.4	3, 4	20b
20b	4.17 *d* (8.1)		3, 4, 5	20a, Me-19
OAc	2.16 *s*	22.7	169.4	
	2.17 *s*	21.4	171.8	
	2.09 *s*	21.3	170.0	
	2.09 *s*	21.2	170.2	
	2.07 *s*	20.7	170.4	
$COCH_2$–OH				
1′		171.6		
–CH_2–	4.06 *d* (17.3)	60.6	1′	
	4.00 *d* (17.3)		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 quarternary carbons) experiments (±0.2 ppm).

Table 6
^{1}H- and ^{13}C-NMR spectral data for taxane **6**

Position	δ ^{1}H Mult.[a] (J in Hz)	δ ^{13}C[b]	HMBC	NOESY
1		75.6		
2	5.47 *o*/*d*	72.4	1, 8, Ac	9, 14b, Me-17
3	3.14 *d* (3.0)	41.7	1, 2, 4, 8, 19	7, 10, 13, 14b, 20a, Me-18
4		57.4		
5	4.21 *t* (2.8)	78.1	3, 4, 7, Ac	6a, 6b, 20b
6a	2.16 *o*/*m*	31.3		5, 6b
6b	1.74 *ddd* (13.6; 3.6; 1.9)			5, 6a, 7
7	5.48 *o*/*dd* (10.0; 4.3)	69.1	6, 8, 19	3, 6b, 10, Me-18
8		45.9		
9	6.08 *d* (11.1)	75.3	7, 8, 10, 11, Ac	2, Me-17
10	6.38 *d* (11.1)	72.4	9, 11, 12, 15, 1′	3, 7, Me-18
11		134.7		
12		141.4		
13	6.10 *t* (8.2)	71.4	11, 12, 14, Ac	3, Me-16
14a	2.53 *dd* (14.4;9.2)	39.0	1, 12, 13	13, 14b
14b	1.88 *dd* (14.4; 6.4)			2, 3, 14a, 20a
15		42.6		
16	1.23 *s*	28.9	1, 11, 15, 17	13, 14a, Me-17
17	1.61 *s*	22.3	1, 11, 15, 16	2, 9, Me-16
18	2.25 *s*	15.8	11, 12, 13	3, 7, 10
19	1.24 *s*	14.0	3, 7, 8, 9	2, 6a, 9
20a	3.53 *d* (5.5)	50.3	4	14b, 20b
20b	2.31 *d* (5.5)		4, 5	5, 20a
OAc	2.04 *s* × 2	21.1	169.5, 168.5	
	2.08 *s*	21.8	169.5	
	2.11 *s*	21.8	169.5	
	2.22 *s*	21.8	169.0	
$COCH_2$-OH				
1′		171.5		
$-CH_2-$	4.07 *dd* (17.5; 5.4)	60.9		
	4.02 *dd* (17.5; 4.3)			
OH	2.34 *t* (5.2)			

[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 quarternary carbons) experiments (±0.2 ppm).

VI (**2**): gum; HR-FAB MS for $C_{33}H_{42}O_{12}Na$ requires: 653.25740; found: 653.25720; ^{1}H- and ^{13}C-NMR, HMBC and NOESY spectral data (see Table 2).

N-Acetyl-N-debenzoyltaxol (**3**): gum; HR-FAB MS for $C_{42}H_{49}NO_{14}Na$ requires: 814.30510; found: 814.30474; ^{1}H- and ^{13}C-NMR, HMBC and NOESY spectral data (see Table 3).

7,9,13-Trideacetylbaccatin VI (**4**): gum; HR-FAB MS for $C_{31}H_{40}O_{11}Na$ requires: 611.24683; found: 611.24685; ^{1}H- and ^{13}C-NMR, HMBC and NOESY spectral data (see Table 4).

10-Deacetyl-10-glycolylbaccatin IV (**5**): gum; HR-FAB MS for $C_{32}H_{44}O_{15}Na$ requires: 691.25779; found: 691.25799; ^{1}H- and ^{13}C-NMR, HMBC and NOESY spectral data (see Table 5).

1β-hydroxy-10-deacetyl-10-glycolylbaccatin I (**6**): gum; HR-FAB MS for $C_{32}H_{44}O_{15}Na$ requires: 691.25779; found: 691.25799; ^{1}H- and ^{13}C-NMR, HMBC and NOESY spectral data (see Table 6).

7-Deacetyltaxuspine L (**7**): gum, HR-FAB MS for $C_{30}H_{44}O_{12}Na$ requires: 619.27305; found: 619.27279; ^{1}H- and ^{13}C-NMR, HMBC and NOESY spectral data (see Table 7).

Taxacustin (**8**): gum; HR-FAB MS for $C_{28}H_{40}O_{12}Na$ requires: 591.24175; found: 591.24170.

Taxagifine (**9**): colorless needles; HR-FAB MS for $C_{37}H_{44}O_{13}Na$ requires: 719.26796; found: 719.26789.

2-Deacetyl-7,10-diacetyl-5-deaminoacyl taxine A (**10**): gum; HR-FAB MS for $C_{26}H_{36}O_9Na$ requires: 515.22570; found: 515.22574.

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Table 7
^{1}H- and ^{13}C-NMR spectral data for taxane **7**

Position	δ ^{1}H Mult.[a] (J in Hz)	δ ^{13}C[b]	HMBC	NOESY
1	1.97 *o*/*m*	48.0		2, 14a
2	5.41 *d* (5.7)	71.4	1, 4, 14, Ac	1, 3, 9, Me-17, Me-19
3	2.63 *o*/*m*	38.9		4, 7, Me-18
4	2.00 *o*/*m*	46.9		3
5	5.11 *br*/*s*	71.5	3, 4, 7, Ac	6b, 20a, 20b
6a	1.99 *o*/*m*	31.1		
6b	1.71 *o*/*m*			
7	4.16 *dd* (12.1; 4.6)	70.4	19	3, 6a, 10, 7-OH
7-OH	3.47 *o*/*s*			
8		45.9		
9	5.99 *d* (10.8)	78.8	7, 8, 10, 19, Ac	2, Me-17, Me-19
10	6.14 *d* (10.8)	71.6	9, 11, 12, 15, Ac	7, Me-18
11		133.3		
12		137.7		
13	5.90 *t* (8.3)	70.7	11, 12, 14, Ac	14a, Me-16
14a	2.60 *o*/*m*	28.1		
14b	1.46 *o*/*m*		1, 13, 15	
15		37.6		
16	1.14 *s*	32.0	1, 11, 15, 17	1, 13
17	1.74 *s*	27.2	1, 11, 15, 16	
18	2.11 *o*/*s*	15.4	11, 12, 13	
19	0.78 *s*	13.8	3, 7, 8, 9	6b, 20a, 20b, 7-OH
20a	3.46 *o*/*m*	64.7		4, 5, 6b, 20b
20b	3.38 *br*/*m*			5, 6b, 20a
OAc	2.20 *s*	22.8	168.3	
	2.14 *s* ×2	21.9	169.8	
	2.10 *o*/*s*	21.3	169.8	
	2.00 *s*		168.9	

[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 quarternary carbons) experiments (±0.2 ppm).

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