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Novel Taxanes from the Needles of *Taxus canadensis*

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Abstract: Nineteen taxanes were characterized for the first time in the needles of the Canadian yew. Four of these metabolites are novel: taxinine-11, 12-oxide (**1**), 7-acetoxytaxuspine C (**2**), 5-cinnamoyltaxin B (**3**), and 7, 9-deacetyltaxinine B (**4**). Compound **1** is the second 11,12-epoxy-taxane known and 7-oxygenated-3, 11-cyclotaxane structures are unique to *Taxus canadensis*. In addition, taxinine and taxinine E were found to be major taxanes in the Canadian yew. The structures of all the taxanes were rigorously established by spectroscopic methods.

Keywords : Natural product; antitumour compounds; taxoids; epoxides.

Taxoids are promising novel agents against a wide spectrum of cancers including breast, ovary and lung carcinomas, with further potential activity in gastrointestinal tumours.[1] The two clinically used analogues are paclitaxel (taxol®) and docetaxel (taxotere®) [2], both of which are currently obtained by semi-synthesis [3,4]. The starting material is 10-deacetylbaccatin III, present in all yews but abundant only in the needles of *Taxus baccata*. [5] *Taxus canadensis*, a widespread [6] low trailing bush is a very interesting plant with taxanes specific to this yew. Its major taxane 9-dihydro-13-acetylbaccatin III (5-7 times more abundant

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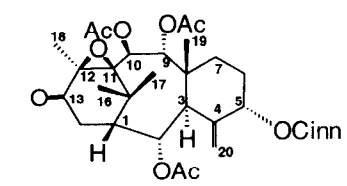

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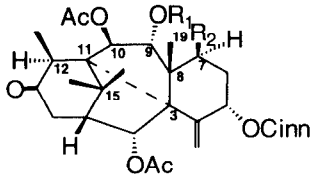

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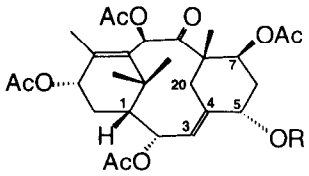
5 R_1=Ac, R_2=H
20 R_1=H, R_2=OH [13]



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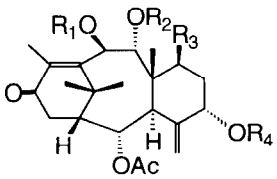
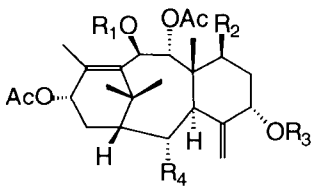
6 R=H



4 R_1=Ac, R_2=H, R_3=OH, R_4=Cinn
7 R_1=R_2=Ac, R_3=H, R_4=Cinn
8 R_1=R_2=Ac, R_3=R_4=H
9 R_1=R_2=Ac, R_3=OAc, R_4=Cinn
10 R_1=H, R_2=Ac, R_3=OAc, R_4=Cinn



11 R_1=Ac, R_2=H, R_3=Cinn, R_4=OAc
12 R_1=R_2=H, R_3=Cinn, R_4=OAc
13 R_1=Ac, R_2=R_3=H, R_4=OAc
14 R_1=Ac, R_2=R_4=OAc, R_3=Cinn
15 R_1=Ac, R_2=OAc, R_3=Cinn, R_4=H
16 R_1=Ac, R_2=OH, R_3=Cinn, R_4=H
17 R_1=Ac, R_2=R_4=H, R_3=Cinn
18 R_1=Ac, R_2=H, R_3=Cinn, R_4=OH

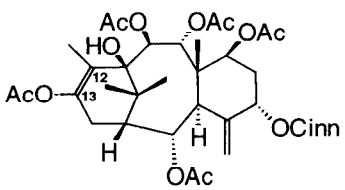


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Fig. 1. Structures of isolated taxanes

than paclitaxel in the Canadian yew depending on the collection site) was isolated in the needles in 1992 [7-9] and has only been found as traces in the bark of one other yew. [10] Its bicyclic fully oxygenated structures (canadensene and 5-epi-canadensene) suggest a possible different biosynthetic pathway. [11-13] These two and seventeen other taxanes have been identified in the needles of the Canadian yew. [7,9,13,14]

In this publication, we are reporting for the first time the detailed structures of nineteen additional taxanes isolated from the needles of *Taxus canadensis*, and four of them are novel taxanes. One of them is the second example of a natural taxane possessing an oxirane moiety at C-11-C-12 : taxinine-11,12-oxide (**1**, Fig.1); the first one, decinnamoyltaxinine B-11,12-oxide was obtained [15] from the needles and stems of *Taxus yunnanensis*. The 3,11-cyclotaxane (**2, 20**[13] , Fig. 1) bearing an oxygenated substituent on C-7 unique to the Canadian yew [16], exhibits interesting ring C conformations as shown by our data. Taxuspine D (**19**), previously found only in the stems of *Taxus cuspidata*, [17] with some reported bioactivity [18] has also been isolated from the needles of the Canadian yew. In addition, we have identified two taxanes (taxinine [19] and taxinine E) which could be isolated in a high yield (~0.03% yield from dry needles).

RESULTS AND DISCUSSION

Repetitive chromatographic steps enabled to separate 19 taxanes characterized for the first time in the needles of *T. canadensis*. The structural characterization of these compounds was performed using spectroscopic techniques (high resolution 2D NMR experiments, HMQC, [20] HMBC, [21] COSY, [22] NOESY, [23] and low and high resolution MS). The NMR data are shown in Tables 1-3.

Characterisation of taxanes ***1*** and ***2***

Taxane **1** (Fig. 1, Table 1) is characterized by the absence of a double bond between C-11 and C-12. In this new taxane, the usual double bond has been replaced by an epoxide. This was revealed from the HMBC experiments (which correlate protons to more distant carbons through their $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$ scalar couplings) where we observe that Me-18 is correlated to two relatively shielded quaternary carbons (64.3 and 59.4 ppm) and to a carbonyl. Me-16 and Me-17 are also coupled to one of these quaternary carbons (64.3 ppm). These relatively shielded quaternary carbons can be best explained by the presence of an epoxide on C-11 (64.3 ppm) and C-12 (59.4 ppm). The presence of a non-conjugated ketone at C-13 has also been detected by an HMBC experiment (Me-18 and H-14 are both correlated to the C-13 ketone). These data support the structure shown in Fig. 1 for taxane **1**. In addition, we compared the structures of taxane **1** and the known taxane **7** (same substituents but with a C-11-C-12 double bond instead of an epoxide). In the proton NMR, we note two major differences confirming our structure : the chemical shift of H-10 in **1** is greatly affected by the absence of a double bond (5.38 ppm instead of 6.06 ppm for taxane **7**); the C-13 ketone shift in **1** is very indicative of the absence of conjugation (208.1 ppm instead of 198.7 ppm for taxane **7**). The position of the acetyl and cinnamoyl groups have been confirmed by HMBC experiments.

The relative stereochemistry of taxane **1** was established using the information contained in the NOESY experiment. Ring B has the usual stereochemistry with H-2 correlating with protons on the β-side (upper)

Table 1. Proton and Carbon-13 NMR Data for Taxanes **1** and **2**

	Taxane 1		**Taxane 2**	
Position	H	C	H	C
1	1.94 (dd, *8.6, 1.5*)	51.1	2.17 (br.t)	48.1
2	5.74 (dd, *5.4, 1.5*)	69.9	6.09 (d, *5.1*)	76.0
3	3.11 (d, *5.4*)	43.8		65.5
4		140.8		141.6
5	5.49 (br.s)	78.5	5.73 (o.t, *10.4*)	73.9
6 (a)	2.02 (o.m)	27.7	2.70 (ddd, *14.6, 10.4, 4.4*)	30.6
(b)	1.81 (o.m)		2.05 (o.m)	
7	1.82 (o.m)	27.0	5.01 (d, *4.4*)	73.5
8		43.2		47.6
9	5.97 (d, *10.8*)	76.8	5.80 (d, *9.5*)	82.3
10	5.38 (d, *10.8*)	71.9	5.63 (d, *9.5*)	79.5
11		64.3		57.8
12		59.4	3.58 (q, *7.0*)	52.6
13		208.1		213.8
14 (a)	2.68 (dd, *20.3, 8.6*)	38.1	2.62 (d, *20.3*)	38.6
(b)	2.42 (d, *20.3*)		2.52 (dd, *20.3, 6.8*)	
15		38.4		42.7
16	0.82 (s)	28.9	1.23 (s)	26.9
17	1.83 (s)	25.3	1.69 (s)	28.3
18	1.99 (s)	15.8	1.29 (d, *7.0*)	15.7
19	0.98 (s)	18.0	1.42 (s)	24.4
20 (a)	5.50 (s)	119.9	5.84 (s)	128.6
(b)	5.21 (s)		5.72 (s)	
OAc	2.04 (s)	21.4, 169.7	2.07 (s)	21.4, 169.9
	2.05 (s)	20.8, 169.5	2.06 (s)	21.1, 169.1
	2.06 (s)	20.6, 169.2	2.04 (s)	20.9, 170.0
			1.95 (s)	20.9, 170.6
OCinn				
C=O		166.0		165.6
CH= 1'	6.26 (d, *16.0*)	117.1	6.35 (d, *16.1*)	117.5
=CH 2'	7.66 (d, *16.0*)	146.4	7.67 (d, *16.1*)	145.7
Ph-1''		134.1		134.2
Ph-*o*	7.60 (m)	128.3	7.53 (m)	128.2
Ph-*m*	7.42 (m)	129.0	7.37 (m)	128.9
Ph-*p*	7.42 (m)	130.6	7.37 (m)	130.5

a) δ in ppm b) J in Hz c) Mutiplicity: s, singlet; d, doublet; t, triplet; q, quadruplet; m, mutiplet; br., broad; o, overlapped

of the molecule (H-1, H-9, Me-17, Me-19) while H-10 correlates with protons on the α-side of the molecule (H-3, H-7, Me-18). The orientation of Me-18 in ring A is shown from the NOE correlations with protons on the α-side of the molecule (H-3, H-10, H-20a). This taxane in solution adopts a U shape as can be seen from

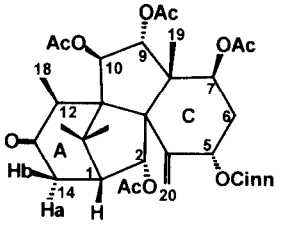
NOE's observed between some protons oriented on the α-side of ring A with C-ring protons. For example, Me-18 correlated with H-3, H-20a and H-1' of the cinnamoyl group. Despite the fact that H-5 overlapped with H-20a, its relative orientation could be deduced from the NOE observed between H-5 and Me-19 (on the β face of the molecule) and between H-1' of the cinnamoyl group and Me-18 (on the α side). High resolution mass spectrometry confirmed the elemental composition of the sodiated quasimolecular ion of **1**.

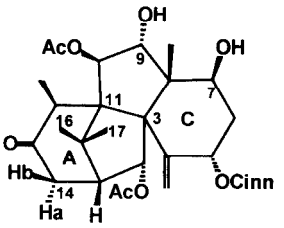
The second new taxane, **2** has a C-3-C-11 bond, resulting in the absence of a C-11-C-12 double bond and of a proton on C-3 and by the presence of a methyl doublet (Me-18) coupled to a neighboring methine proton at C-12. This tetracyclic structure was identified with HMBC experiments. The H-12 proton located on ring A exhibited correlation to C-11, C-13, C-15, Me-18 and more importantly to the C-3 of the last ring confirming the ring closure between C-3 and C-11. In addition, the Me-19 protons correlated to the protonated C-7 and C-9 carbons and to the quaternary C-3 and C-8 carbons. Taxane **2** contained four acetyls, one cinnamoyl group and a double bond at C-4-C-20. The methylene H-14 is characterized by relatively deshielded shifts (2.62 and 2.52 ppm) and a large geminal coupling constant (J=20.3 Hz) indicative of a neighboring carbonyl group. The presence of a carbonyl group at position 13 was confirmed by the HMBC experiment in which we observed the methyl doublet (Me-18), the methine H-12 and the H-14 protons correlated to a very deshielded carbonyl carbon (213.8 ppm).

Taxane **2** structure was similar to another taxane (**20**) isolated in our laboratory [13] from the needles of *Taxus canadensis*. The only difference between taxane **2** and **20** was that position 7 and 9 are hydroxylated in **20** instead of being acetylated. To further prove the similarity of the two structures, taxane **20** was converted to taxane **2** by acetylation. The NMR spectra of the product was identical to that of the new taxane **2** whose structure was therefore 7-acetoxytaxuspine C. High resolution mass spectrometry confirmed the elemental composition of the sodiated quasimolecular ion of **2**.

The NOESY spectra of taxane **2** (the acetylated version of taxane **20**) and **20** were examined and are summarized in table 2. For taxane **20**, the two bicyclic five-membered rings in the center of the taxane skeleton confered a lot of rigidity to the central core. We found the usual stereochemistry for H-2 correlating with protons on the β-side of the molecule (H-1, H-9, Me-17, Me-19) while H-10 correlated with protons on the α-side (H-7, H-6b, H-12, Me-18). From the NOE effects observed with H-10, it was not possible to decide on the relative stereochemistry at the C-12 center since H-10 had equally strong NOE with H-12 and Me-18 (H-10 is probably bisecting those two groups). The orientation of Me-18 could be deduced from it's NOE interaction with protons on the upper face of the molecule (Me-16), while the α-orientation of H-12 was confirmed from it's NOE with H-20b on ring C. This NOE could only be present if the molecule adopted a U-shape with the α-protons of the A-ring interacting with α-protons of ring C. This U-shape was also confirmed by observing an NOE interaction between H-14a and H-20a. The NOE observed between H-5 and Me-19

Table 2. NOE Observed for Taxanes **2** and **20** in $CDCl_3$ (NOE positive cross peaks)





Proton	Taxane 2	Taxane 20
H-1	**2, 14a, 14b, 16, 17**	**2**, 14b, **16, 17**
H-2	**1, 17, 19**	**1**, 9, **17, 19**
H-5	See H-20b	**6a,** 6b, 19
H-6a	**5, 6b**, 7	**5, 6b**, 19
H-6b	**6a**, 7, **10**, 12, **20b**	**6a, 10**
H-7	**6a, 6b, 19**	**6a, 6b, 10, 19**
H-9	**17, 19**	2, **17, 19**
H-10	**6b**, 7, **12, 18**	**6b, 7, 12, 18**
H-12	5/20b, **10, 18**, 20a, Hα, Hβ	**10, 18, 20b**
H-14a	1, **14b, 20a**	1, **14b, 20b**
H-14b	**1, 14a, 16**, 20a	**1, 14a, 16**
H-16	**1**, 14b, **17, 18**	**1, 14b, 17, 18**
H-17	**1, 2, 9, 16, 19**	1, **2, 9, 16**
H-18	**10, 12, 16,** Hα, Hβ	**10, 12, 16**
H-19	**2**, 7, **9**, 17	**2**, 5, 7, **9**
H-20a	14a, **20b**	**20b**
H-20b	**6a** , 12, **20a**	**12, 14a, 20a**

a) Protons in **bold** form indicate medium to strong NOE correlations

allowed us to postulate its β- orientation while H-7 was surely located on the α-face of the molecule as it has NOE with the H-10 proton. Contrary to other taxoid structures, H-7 (α) had also NOE with it's neighbor Me-19 on the β-face of the molecule. This NOE can be misleading but if we looked at molecular models with H-7 in the β-orientation, it was impossible to have a conformation of ring C in which this β-H-7 proton interacted with α-H-10. Also contrary to other taxoids, we observed a strong NOE between H-6b and H-10. These different NOEs indicated that ring C had a different conformation than in taxanes without a C-3-C-11 bond. Taxane **2** (the acetylated version of **20**), had similar NOE's as **20** except for H-10 that exhibit much weaker NOE with H-7 whereas H-6b had much stronger NOE with H-10. As the stereochemistry had to be the same at the C-7 center, we attributed these changes in NOE to a slight modification of the conformation of ring C.

In addition, the cinnamoyl group (protons α and β on the double bond), located at position 5 on the ring C had NOE interactions with the α-protons on ring A (CH-α and CH-β interact with H-12). In order to investigate the conformations of taxanes **2** and **20**, simulated annealing and NOE-restraint molecular dynamics were performed on both taxanes.

Molecular Modeling of Taxanes 2 and 20

The conformations generated from simulated annealing are the conformations in the gas-phase. We are however interested in the conformation in $CDCl_3$ solution. Therefore, the lowest energy conformers from each family were compared with the NMR NOE data and the most consistent ones were subject to NMR NOE-restraint molecular dynamic. The obtained conformations were solvated by periodic $CDCl_3$ box and minimized without NOE-restraint.

Cluster analysis from simulated annealing revealed the existence of two families of conformers for taxane **2** and **20**. For taxane **2**, only one family's lowest energy conformation was selected since the other one have a distance of 2.18 Å between H-7 and H-10 which violates the NOE data. In the final conformations of **2** (Fig. 2), ring A adapts a boat conformation and the 7-OAc is pseudo-axial. For taxane **20**, both of the lowest energy conformation of each family can not be ruled out by NOE data. Consequently, there are two conformations for taxane **20**. In Fig. 2 the two final conformations for **20** are superimposed together. Conformer A has 7-OH pseudo-equatorial (that is, H-7 is pseudo-axial) and conformer B has 7-OH pseudo-axial (that is, H-7 pseudo-equatorial). The conformations of ring A and the two bicyclic five-membered rings in the center of the two conformers are similar, the difference is in the last ring (called C in taxanes structures). In order to compare the energies of conformer A and conformer B in chloroform solution, semiempirical molecular orbital AM1-SM5.4A calculations, implemented in AMSOL 6.1 [24], were performed on conformer A and B. This method includes a modified Born method for the treatment of solvation. The calculated energies for conformer A and B are –256.4 kcal and -259.2 kcal, respectively. The difference is only 2.8 kcal. It is expected that these two conformers would co-exist in the chloroform solution. In the two tetracyclic taxanes **2** and **20**, ring A has a boat form, and the two bicyclic five-membered ring confers some rigidity to the central core. The last ring C seems to be restricted to one conformation when C-7 and C-9 hydroxyls are acetylated as in taxane **2** and adapt similar conformation as conformer B of taxane **20** (Fig.2). On the other hand, when C7 and C9 are hydroxyls (taxane **20**) ring C seems to be quite flexible and can exist as two twist-boat conformers A and B (Fig.2). Indeed, we observed that for taxane **20**, α H-7 had equally strong NOE interactions with α H-10 and β Me-19. These NOE correlations for **20** oringinated from two conformers, one in which H-7 is pseudo-axial (NOE with H-10) while the other one has H-7 pseudo-equatorial (NOE with Me-19). In taxane **2** the NOE observed suggest only one conformer H-7 has NOE with β Me-19 but not with α H-10.

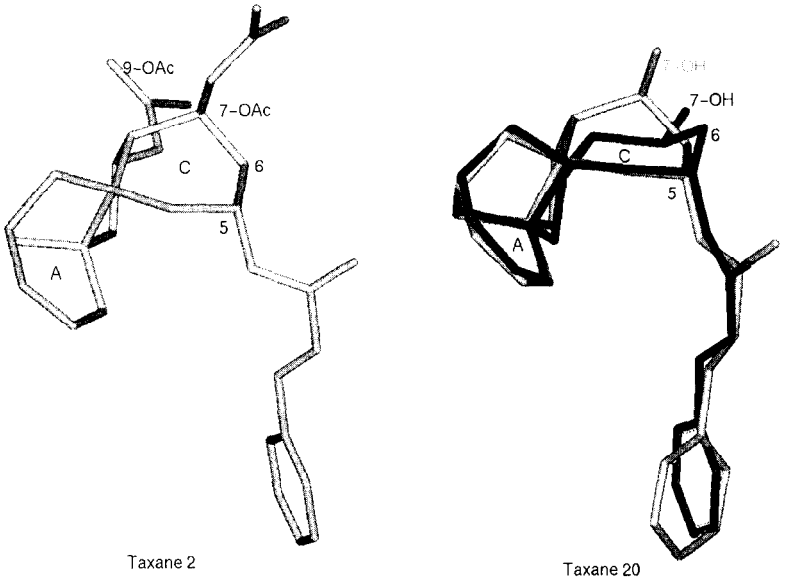


Fig. 2. Conformations of taxane **2** and conformer A (in black) and conformer B (in grey) of taxane **20** generated by NOE-restraint molecular dynamic. For clarity, hydrogen atoms are not shown. Side chains in taxane **2** except for 7-OAc, 9-OAc and 5-cinnamoyl are not shown. Side chains in taxane **20** except for 7-OH and 5-cinnamoyl are not shown.

*Characterisation of taxanes **3** and **4***

In the ^{1}H NMR spectrum of taxane **3** (Table 3), four tertiary methyls (δ 1.10, 1.26, 2.04 and 1.30), four acetyl methyls and one cinnamoyl group were observed. The HMQC experiment revealed the existence of an unusual olefinic proton (5.43 ppm/124.1 ppm) assigned to H-3. In the COSY experiment, this H-3 is correlated to a broad doublet (H-2 at 5.76 ppm) which in turn is also correlated to H-1 (1.69 ppm). The H-1 proton is correlated to H-14a (2.70 ppm) a proton of a methylene group, which is also correlated, to a deshielded proton assigned to H-13 (5.36 ppm). The correlation observed between two deshielded protons and a methylene group (2.24/2.04 ppm) allow us to assign the H-5 and H-7 protons (5.73 and 5.36 ppm respectively). The presence of an isolated methylene group assigned to position 20 has been noticed in the COSY spectra.

Table 3. Proton and Carbon-13 NMR Data for Taxanes **3** and **4**

	Taxane 3		**Taxane 4**	
Position	H	C	H	C
1	1.69 (d, *8.5*)	46.8	2.13 (o.d, *10.6*)	48.7
2	5.76 (d, *9.8*)	70.8	5.49 (d, *5.7*)	69.0
3	5.43 (d, *9.8*)	124.1	3.19 (d, *5.7*)	41.4
4		133.1		140.4
5	5.73 (d, *6.5*)	70.0	5.35 (o.br.s)	77.0
6 (a)	2.24 (td, *13.8, 6.5*)	32.7	2.20 (o.m)	37.5
(b)	2.04 (o.m)		1.72 (ddd, *14.5, 11.3, 3.5*)	
7	5.36 (o.m)	70.4	4.24 (dd, *10.3, 4.7*)	71.0
OH-7			3.66 (s)	
8		53.2		47.4
9		205.6	4.43 (dd, *9.9, 5.8*)	77.6
OH-9			3.91 (d, *5.8*)	
10	6.34 (s)	77.8	6.08 (d, *9.9*)	75.8
11		129.0		151.4
12		138.6		137.8
13	5.36 (o.d, *11.7*)	69.2		199.2
14 (a)	2.70 (ddd, *16.4, 16.4, 8.8*)	27.3	2.82 (dd, *20.0, 7.3*)	36.1
(b)	1.83 (dd, *16.4, 2.1*)		2.35 (d, *20.0*)	
15		37.8		37.9
16	1.10 (s)	32.0	1.14 (s)	37.2
17	1.26 (s)	25.0	1.65 (s)	25.6
18	2.04 (s)	17.1	2.26 (s)	14.2
19	1.30 (s)	20.4	1.19 (s)	12.9
20 (a)	2.77 (d, *15.4*)	35.4	5.37 (s)	118.2
(b)	1.98 (o.d)		4.94 (s)	
OAc	2.06 (s)	20.8, 169.8	2.15 (s)	21.4, 170.0
	2.13 (s)	20.8, 170.5	2.06 (s)	21.2, 169.6
	1.98 (s)	21.1, 170.3		
	1.92 (s)	21.1, 169.4		
OCinn				
C=O		166.5		166.2
CH= 1'	6.50 (d, *15.9*)	117.7	6.40 (d, *15.8*)	117.5
=CH 2'	7.80 (d, *15.9*)	146.2	7.66 (d, *15.8*)	146.1
Ph-1''		134.0		134.5
Ph-*o*	7.51 (m)	128.1	7.76 (d, *8.0*)	128.5
Ph-*m*	7.39 (m)	129.0	7.44 (t, *7.2*)	128.8
Ph-*p*	7.39 (m)	130.7	7.39 (d, *7.2*)	130.4

a) δ in ppm b) J in Hz c) Mutiplicity: s, singlet; d, doublet; t, triplet; m, mutiplet; br., broad; o, overlaped

The HMBC experiment is very useful to determine the core structure of this taxane. Methyl 18 is correlated as usual to the quaternary olefinic carbons C-11 and C-12 and to the oxygenated C-13 while methyl 16, 17 are correlated to C-1, C-11 and C-15. Methyl 19 has correlations to four carbons: the oxygenated C-7,

the ketone C-9, a quaternary carbon (C-8) and the isolated methylene group which has been assigned to C-20. The protons on that isolated methylene group show HMBC correlation to the olefinic carbons C-3 and C-4, allowing us to obtain the core structure of this modified taxoid. The H-5 proton is correlated in the HMBC to the cinnanoyl carbonyl group allowing us to propose 5-cinnamoyltaxin B for the structure of taxane **3**. Taxin B (**6**) [25] and taxuspine B [26] which are isolated from *T. yunnanensis* and *T. cuspidata* respectively, have the same core structure as **3** but with some different substituents. High resolution mass spectrometry confirmed the elemental composition of the sodiated quasimolecular ion of **3**.

The relative stereochemistry of **3** (Fig. 3) was determined using the 2D-NOESY experiment. On the β (upper) face of the molecule, we can observe NOE interactions between methyl-16, H-13 and H-14a. The H-1 proton interacts with H-14a, H-2 and methyl-17, which are therefore also oriented β. H-2 is correlated to H-20a, which in turn is correlated to methyl-19. Methyl-19 is correlated with H-6a which in turn is correlated to H-5 confirming it's β orientation. From the α face of the molecule, we observe NOE between H-7 and H-10. The NOE between H-3 and H-14b confirm α orientation. This NOE together with the long distance NOE observed between methyl-18, H-7 and CH-α of the cinnamoyl group allow us to postulate a U-shape molecule in which the α protons on ring A are close in space from the α protons on ring C.

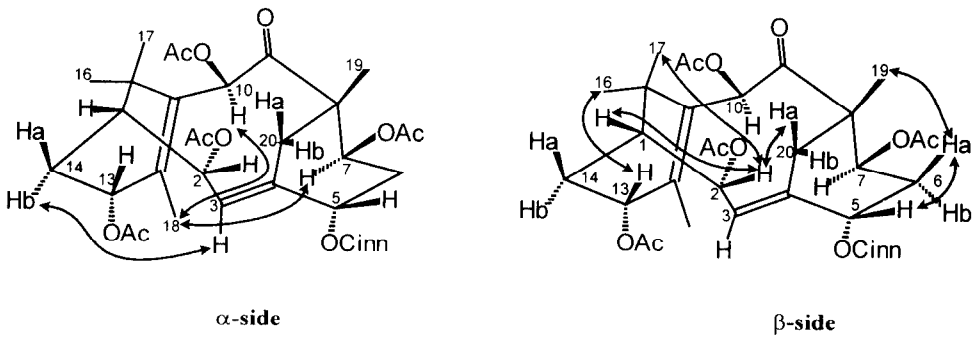


Fig. 3. Key NOESY correlations of taxane **3**

In the ^{1}H NMR spectrum of taxane **4** (Table 3), we can observe the presence of four tertiary methyls, two acetyls and one cinnamoyl group (a pair of doublets characteristic of trans-olefinic protons, and phenyl signal). We can also note the presence of an unsaturated exocyclic methylene group appearing as a pair of singlets at δ 5.37 and 4.94. The five protons attached to oxygenated carbons, (H-2, H-5, H-7, H-9 and H-10)

were assigned with the aid of ^{1}H-^{1}H COSY. The upfield shifts of H-7 and H-9 signals and their coupling with two hydroxyl protons allowed us to position the two hydroxyl groups. In the ^{13}C NMR spectrum (Table 3), one ketone carbonyl at C-13 and two double bonds at C-4, 20 and C-11, 12 were revealed. The core structure was confirmed to be the same 6/8/6 ring system as that of taxinine (**7**) by HMBC correlations. The HMBC experiment was also used to locate the acetoxy carbonyl carbons on C-2 and C-10, which thus left us with C-5 as the only possibility for the cinnamate substituent. The structure of compound **4** is then 7, 9-deacetyltaxinine B. High resolution mass spectrometry confirmed the elemental composition of the sodiated quasimolecular ion of **4**. The relative stereochemistry of **4** was determined by analysis of the ^{1}H-^{1}H couplings and NOESY correlations.

Characterization of taxanes ***5-19***

The fifteen known metabolites **5** to **19** have been isolated from other yews, with most of them from the needles. The exceptions are taxuspine C (**5**) [26], taxezopidine G (**18**) [27], and taxuspine D (**19**) [17] which were isolated only from the stems of the Japanese yew, 5-decinnamoyltaxinine E (**13**) [28] reported only in the seeds of *Taxus chinensis* and 2-deacetoxy-7-deacetyltaxinine J (**16**) [29] only from twigs of *Taxus maire*. They were identified by analysis of their NMR spectra (detailed NMR analysis and High Resolution Fast Atom Bombardment Mass Spectra) as taxuspine C (**5**) [26], taxin B (**6**) [25], taxinine (**7**) [30], taxinine A (**8**) [31], taxinine B (**9**) [32], 10-deacetyltaxinine B (**10**) [33], taxinine E (**11**) [32], 10-deacetyltaxinine E (**12**) [34], 5-decinnamoyltaxinine E (**13**) [28], taxinine J (**14**) [35], 2-deacetoxytaxinine J (**15**) [36], 2-deacetoxy-7-deacetyltaxinine J (**16**) [29], 2, 7-deacetoxytaxinine J (**17**) [37], taxezopidine G (**18**) [27] and taxuspine D (**19**).[17] Taxuspine D which was isolated from the needles of *Taxus canadensis* is a unique taxane with a double bond at C-12-C-13 and interesting biological activity [17,18] which was first isolated from the stems of *Taxus cuspidata*. Indeed, this taxane was found by Kobayashi and co-workers[18] to promote the polymerization of tubulin with a potency corresponding to half to one third that of taxol. We have confirmed this result in our laboratory with the taxane isolated from the Canadian yew. This activity is amazing considering that this compound has no side chain on C-13 a feature considered essential for activity. [38] In the experimental section, the analytical HPLC t_R are given as well as their isolation from the needles of *Taxus canadensis* and the purification procedures of these taxanes. It is interesting to note that the isolated yield of most of these taxanes is low (same as paclitaxel, ~0.003%) with few variations between yew species. The only exceptions were metabolites **7** and **11** which were found as major metabolites in the needles of the Canadian yew with isolation yields of 0.028% and 0.023% (based on dry needles) respectively. 9-Dihydro-13-acetylbaccatin III remains the most abundant taxane in *Taxus canadensis* (at least an isolated yield of 0.05% based on dry needles).

The unique composition of taxanes isolated from the Canadian yew (abundant 9-dihydro-13-acetylbaccatin III, taxinine, taxinine E, discovery of the canadensenes and 7-oxygenated-3, 11- cyclotaxanes

2, **20**) suggests an altered biosynthetic pathway of taxanes in this yew. The sequence of cyclizations and oxygenations must be different from other species. Biosynthetic experiments are in progress in our laboratory.

EXPERIMENTAL

Instrumentation

Unless otherwise specified, liquid column chromatography was performed on silica gel 60, 230-400 mesh (EM Science). Analytical HPLC was performed on a Waters 600 FHU pump system coupled with a 996 PDA detector (Waters) and two Whatman Partisil 10 ODS-2 analytical columns in series (4.6 × 250 mm). A 50 min gradient method (25% to 100% of CH_3CN in H_2O, run time: 60 min, flow rate: 1 mL/min) was used in verifying the purified compounds. 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 one Partisil 10 ODS-2 MAG-20 preparative column (22 × 500 mm), and either 50 min or 70 min linear gradient method (25% to 100% of CH_3CN in H_2O, flow rate: 18 mL/min) was used. Preparative TLC was performed with silica gel 60 F_{254} precoated TLC plates, 0.25 mm (EM Science). The purified compounds were also visualized by thin-layer chromatography on precoated TLC plates (silica gel 60 F_{254}, 0.25 mm, EM Science) with 10% sulfuric acid in ethanol.

NMR and Mass Spectrometry Measurement

All the NMR data were obtained at room temperature on a Bruker-AMX2-500 spectrometer operating at 500.13 MHz for proton and at 125.77 MHz for carbon-13. The solvent ($CDCl_3$) was used as an internal reference (7.25 ppm for ^1H and 77.0 ppm for ^{13}C). The various 2D spectra were acquired and processed using standard procedures. For phase sensitive 2D experiments (NOESY and HMQC), the data were acquired using the hypercomplex phase mode. The NOESY experiment was obtained using a mixing time of 0.4-0.5 s and a relaxation delay of 1 s. In the HMBC experiment the τ delay used to emphasize long-range coupling was set to 50 ms. Low-resolution FAB mass spectra were obtained in glycerol with a VG ZAB-HS instrument. High-resolution measurements were made similarly in glycerol-DMSO.

Molecular Modeling

Molecular modeling calculations for taxanes **2** and **20** were performed on Silicon Graphics R5000 workstation using the Discover program within InsightII package (InsightII version 97.0, Molecular Simulation, Inc.). First, the initial structures were subjected to simulated annealing without constraints (the maximum temperature 900K). Cluster analysis revealed the existence of two family conformers for taxane **2**

and **20**. The lowest energy conformers from each family were compared with the NMR NOE data and the most consistent ones were selected for further calculations. For taxane **2**, only one conformation was selected since the other one has the distance of H-7 and H-10 as 2.18 Å which violate the NOE data. For taxane **20**, two conformations were selected. Secondly, the strutures selected from simulated annealing were subjected to NMR NOE-restraint molecular dynamic at 300K using the consistent valence forcefield (CVFF). The system was equilibrated for 100 ps and the molecular dynamic was continued for another 500 ps for data collection. The instant conformations were saved every 250fs. The distance restraints from NOE spectra in CDCl3 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. When equivalent protons were present on a same heteroatom, a pseudoatom was created and a distance correction of 1 Å was applied. For taxane **2**, 25 NOE distance restraints were employed in the calculation whereas 28 NOE distance restraints were used for taxane **20**. In order to compare the two conformers of taxane **20**, the NOE data between H-6a and Me-19, H-7 and H-10, H-7 and Me-19 as well as H-6b and H-10 were not used in the NOE-restraint molecular dynamic. 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 periodic 35Åx35Åx35Å $CDCl_3$ box containing 232 $CDCl_3$ molecules. Without NMR NOE-restraint, the solvated systems were then energy minimized using 1000 steps of steepest descent and 3000 steps of conjugate gradient algorithms until root-mean-square gradient deviation was less than 0.001 Kcal/ Å.

Extraction of Taxus canadensis Needles and Isolation and Purification of Taxanes

Ground dried needles of *Taxus canadensis* (4.7 kg) were extracted with 18 L of CH_2Cl_2-MeOH (1:1, v/v) by shaking for 1 day at room temperature. The ground plants were filtered and extracted again with fresh solvent for another three days. The combined organic extracts were evaporated under reduced pressure. Water (3 L) was added and lipids were removed by stirring the mixture with hexane (4 x 3 L). The aqueous phase was then salted (NaCl, 300 g) and extracted with CH_2Cl_2 (4 x 3 L). The combined CH_2Cl_2 extracts were dried over anhydrous sodium sulfate, filtered and evaporated yielding a dark brown extract (119 g).

This extract was separated using dry-column chromatography on silica gel (Silica gel 60, 70-230 mesh, Selecto Science, 1.5 kg, 8 x 83 cm) eluted with CH_2Cl_2-*iso*-PrOH (9:1, 3.5 L). After elution, the silica gel was cut into 19 equal bands, and each band was individually eluted with EtOAc-MeOH (1:1, 600 mL). The eluent of the columns from bands 5 through 8 was combined and evaporated to yield 38 g of residue A, which was then subjected to silica gel column chromatography (840 g, 9.5 × 22 cm) with hexane (1 L), hexane-CH_2Cl_2 (3:1 and 1:1, each 2 L), CH_2Cl_2 (2 L), CH_2Cl_2-EtOAc (4:1, 3:2, 2:3 and 1:4, each 2 L), EtOAc (2 L) and EtOAc-MeOH [4:1 (2 L), 3:2 (4 L)] to yield fraction B (5.8 – 7.0 L), C (7.0 – 8.2 L), D (8.2 - 9.0 L) and E (9.0 – 10.0 L). Fraction B (4.3 g) gave colorless crystals (**7**, 726 mg) in Me_2CO, and the residue was applied

to a silica gel column (103 g, 3.5 × 29 cm) and eluted with hexane (200 mL), hexane-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 3:7 and 2:8, each 200 mL) and EtOAc (200 mL) to afford fraction B1 (960 – 1120 mL), B2 (1120 – 1220 mL) and B3 (1220 – 1320 mL). Compound **7** (569 mg) was again crystallized from fraction B1. The residue B1 (620 mg) was then purified on a preparative HPLC column and eluted with a 70 min gradient method and preparative TLC (hexane-*n*-BuOH, 9:1) to yield **1** (14.1 mg), **3** (55.7 mg), **5** (61.56 mg) and **11** (72.4 mg). Fraction B2 (231 mg) was also purified further by preparative HPLC (50 min gradient method) to yield **15** (63.9 mg) as colorless crystal. From fraction B3 (130 mg), compound **2** (4.4 mg), **9** (41.8 mg) and **14** (14.3 mg) were obtained by crystallization (for **9**, colorless crystals) and preparative HPLC (50 min gradient method) as well as preparative TLC (hexane-*n*-BuOH, 9:1 and CH_2Cl_2-MeOH, 97:3) respectively.

Fraction C (4.0 g) was subjected to a silica gel column (140 g, 3.5 × 39 cm) and eluted with hexane (200 mL), hexane-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 3:7, 2:8 and 1:9, each 200 mL), EtOAc (200 mL) and EtOAc-MeOH (9:1, 200 mL) to yield fraction C1 (1200 - 1240 mL, 481 mg), C2 (1440 – 1540 mL, 353 mg) and C3 (1540 - 1640 mL, 365 mg), which was then purified further on a preparative HPLC column with the above mentioned 50 min gradient method and preparative TLC (CH_2Cl_2-MeOH, 95:5 and hexane-EtOAc, 1:1) to afford **6** (22.0 mg), **8** (11.6 mg), **10** (3.3 mg), **12** (1.8 mg), **13** (22.2 mg), **16** (1.4 mg) and **19** (16.1 mg).

Fraction D (2.2 g) was applied to a silica gel column (54 g, 2.5 × 25 cm) and eluted with hexane-CH_2Cl_2 (1:1 and 8:2, each 200 mL), CH_2Cl_2 (200 mL), CH_2Cl_2-EtOAc (9:1, 8:2, 7:3, 6:4 and 1:1, each 200 mL), EtOAc (200 mL) and EtOAc-MeOH (8:2, 200 mL) to yield fraction D1 (1200 - 1620 mL). Further purification of D1 (97.3 mg) on a preparative HPLC column with the 50 min gradient method yielded **4** (10.8 mg).

Fraction E (5.7 g) was applied to a silica gel column (126 g, 4.5 × 21 cm) and eluted with hexane-CH_2Cl_2 [1:1 (300 mL), 4:6, 3:7, 2:8 and 1:9, each 200 mL], CH_2Cl_2 (200 ml), CH_2Cl_2-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 3:7 and 2:8, each 200 mL), EtOAc (200 mL) and EtOAc-MeOH (8:2, 400 mL) to afford fraction E1 (1440 - 1460 mL), E2 (1480 – 1640 mL), E3 (1660 – 1720 mL) and E4 (1820 - 1900 mL). Compounds **11** (504.7 mg) and **14** (11.6 mg) were obtained again as crystals from fractions E2 and E3 respectively. Further purification of fractions E1 (32.8 mg) and E4 (83.1 mg) on a preparative HPLC column with the 50 min gradient method as above combining with preparative TLC (hexane-*n*-BuOH, 8:2) afforded **17** (28.1 mg) and **18** (2.5 mg).

Taxinine-11, 12-oxide (**1**): t_R = 49.68 min (analalytical HPLC), was visualized as brown spot on TLC plate with R_f = 0.5 (hexane-EtOAc, 3:7). HRFABMS for $C_{35}H_{42}O_{10}Na$ $[M + Na]^+$ required: 645.26757, found: 645.26786. ^1H and ^{13}C NMR data see Table 1. ROESY correlations (strong one are shown in *italics* to differentiate with medium): H-2/H-*1, 9, 17, 19*; H3/H-7b or 17, *14b*, 18, 20a or 5; H-9/H-*2, 17, 19*; H-10/H-3, *7* or *17, 18*; H-14a/H-*1, 14b*, 16; H-14b/H-1, 3, *14a*; H-16/H-1, 14a; H-18/H-3, 5 or 20a, 10; H-19/H-2, 3, 9, 17, 20b; 20a/H-3, 17, 18, 19, 20b; H-20b/H-18, *19, 20a*. HMBC correlations: H-2/C-3, 14, acetyl carbonyl;

H-3/C-1, 2, 4, 8, 19, 20; H-5/C-7 or 6, 20, cinnamoyl carbonyl; H-9/C-7, 8, 10, 19, acetyl carbonyl; H-10/C-9, 11, 15, acetyl carbonyl; H-14a/C-2, 12,13; H-14b/C-1, 2, 13, 15; H-16/C-1, 11, 15, 17; H-17/C-1, 11, 15, 16; H-18/C-11, 12, 13; H-19/C-3, 7, 8, 9; H-20a/C-3, 5; H-20b/C-3, 4, 5.

7-Acetoxytaxuspine C (**2**): t_R = 45.16 min, visualized as brown spot on TLC plate with R_f = 0.60 (CH_2Cl_2-MeOH, 95:5). HRFABMS for $C_{37}H_{44}O_{11}Na$ $[M + Na]^+$ required: 687.27813, found: 687.27808. ^1H and ^{13}C NMR data see Table 1. HMBC correlations: H-1/C-2, 3, 11, 14, 15; H-2/C-3, 4, 8, 14, acetyl carbonyl; H-5/C-3, 7; H-6a/C-4, 5, 7, 8; H-7/C-3, 5, 6, 8, 19, acetyl carbonyl; H-9/C-7, 8, 10, 12, 19, acetyl carbonyl; H-10/C-9, 11, 12, 15, acetyl carbonyl; H-12/C-3, 11, 13, 15, 18; H-14a/C-1, 2, 13, 15; H-14b/C-1, 2, 13; H-16/C-1, 11, 15, 17; H-17/C-1, 11, 15, 16; H-18/C-11, 12, 13; H-19/C-3, 7, 8, 9; H-20a/C-3; H-20b/C-4.

5-Cinnamoyltaxin B (**3**): t_R = 48.42 min, visualized as brown spot on TLC plate with R_f = 0.80 (hexane-EtOAc, 4:6). HRFABMS for $C_{37}H_{44}O_{11}Na$ $[M + Na]^+$ required: 687.27813, found: 687.27808. ^1H and ^{13}C NMR data see Table 2. NOESY correlations: H-1/H-*2, 14a, 17*; H-2/H-*1, 17, 20a*; H-3/H-*14b*; H-5/H-6a, 6b, 18; H-6a/H-*5, 6b*; H-6b/H-*6a, 7*; H-7 or H-13/H-10, 14a, 16; H-10/H-7 or *13, 18*; H-14a/H-*1, 7* or *13, 14b, 16*; H-14b/H-*3, 14a*; H-16/H-*13, 14a*; H-17/H-*1, 2, 20a*; H-18/H-*7* or *13, 10*, olefinic protons in cinnamoyl group; H-19/H-*6a*; H-20a/H-*2, 19, 20b*; H-20b/H-*2* or *5, 20a*. HMBC correlations: H-1/C-2, 3, 11, 13, 14, 15; H-2/C-1, 3, 4, 14, acetyl carbonyl; H-3/C-4, 5, 20; H-5/C-3, 4, 7, cinnamoyl carbonyl; H-6a/C-5, 7, 8; H-6b/C-5, 7, 8; H-7 and H-13/C-9, 11, 12, acetyl carbonyl; H-10/C-9, 11, 12, 15, acetyl carbonyl; H-14a/C-1, 2, 12, 13; H-14b/C-1, 2, 13, 15; H-16/C-1, 11, 15, 17; H-17/C-1, 11, 15, 16; H-18/C-11, 12, 13; H-20a/C-3, 4, 5 or/and 7, 8, 9; H-20b/C-3, 4, 8, 9, 19.

7, 9-Deacetyltaxinine B (**4**): t_R = 42.15 min, visualized as yellowish brown spot on TLC plate with R_f = 0.45 (CH_2Cl_2-Me_2CO, 8:2). HRFABMS for $C_{33}H_{40}O_9Na$ $[M + Na]^+$ required: 603.25700, found: 603.25687. ^1H and ^{13}C NMR data see Table 2. NOESY correlations: H-1/H-*2, 3, 14a, 16, 17*; H-2/H-*1, 9, 17, 19*, OH-9; H-3/H-7, 14b; H-5/H-*6a, 6b*; H-6a/H-*5, 6b, 7*; H-6b/H-*5, 6a*; H-7/H-*3, 6b, 10, 18*; OH-7/H-9, OH-9; H-9/H-*2, 17, 19*; OH-9/H-2, 9, 17, 19, OH-7; H-10/H-*7, 18*; H-14a/H-*14b*, 16; H-14b/H-*3, 14a*; H-16/H-*14a*; H-17/H-*2, 9, 16*; H-18/H-*3, 7, 10*; H-19/H-*2, 9, 17, 20b*; H-20a/H-*20b*; H-20b/H-*20a*. HMBC correlations: H-1/C-2, 3, 11, 15; H-2/C-3, 8, 14, acetyl carbonyl; H-5/C-7; H-7/C-19; OH-7/C-6; H-9/C-7, 8, 10, 19; OH-9/C-8, 9; H-10/C-9, 11, 12, 15, acetyl carbonyl; H-14a/C-2, 12, 13; H-14b/C-1, 2, 13, 15; H-16/C-1, 11, 15, 17; H-17/C-1, 11, 15, 16; H-18/C-11, 12, 13; H-19/C-3, 7, 8, 9; H-20a/C-3, 5; H-20b/C-3, 5.

The known taxanes **5** to **19** were characterized by comparing their detailed NMR data with that of the known taxanes isolated from other yews, and HRFABMS confirmed the elemental compositions of the protonated (or sodiated) quasimolecular ions.

Taxuspine C (**5**): t_R = 48.14 min. $C_{35}H_{42}O_9Na$ $[M + Na]^+$ required: 629.27265, found: 629.27264.

Taxin B (**6**): t_R = 35.84 min. $C_{28}H_{38}O_{10}Na$ $[M + Na]^+$ required: 557.23627, found: 557.23630.

Taxinine (**7**): t_R = 52.16 min. $C_{35}H_{42}O_9Na$ $[M + Na]^+$ required: 629.27265, found: 629.27264.

Taxinine A (**8**): t_R = 34.68 min. $C_{26}H_{36}O_8Na$ $[M + Na]^+$ required: 499.23079, found: 499.23095.

Taxinine B (**9**): t_R = 48.84 min. $C_{37}H_{44}O_{11}Na$ $[M + Na]^+$ required: 687.27813, found: 687.27808.

10-Deacetyltaxinine B (**10**): t_R = 45.28 min. $C_{35}H_{42}O_{10}Na$ $[M + Na]^+$ required: 645.26757, found: 645.26786.

Taxinine E (**11**): t_R = 52.03 min. $C_{37}H_{46}O_{10}Na$ $[M + Na]^+$ required: 673.29887, found: 673.29904.

10-Deacetyltaxinine E (**12**): t_R = 47.15 min. $C_{35}H_{44}O_9Na$ $[M + Na]^+$ required: 631.28830, found: 631.28849.

5-Decinnamoyltaxinine E (**13**): t_R = 37.68 min. $C_{28}H_{40}O_9Na$ $[M + Na]^+$ required: 543.25700, found: 543.25684.

Taxinine J (**14**): t_R = 48.76 min. $C_{39}H_{48}O_{12}Na$ $[M + Na]^+$ required: 731.30435, found: 731.30432.

2-Deacetoxytaxinine J (**15**): t_R = 52.42 min. $C_{37}H_{46}O_{10}Na$ $[M + Na]^+$ required: 673.29887, found: 673.29904.

2-Deacetoxy-7-deacetyltaxinine J (**16**): t_R = 49.52 min. $C_{35}H_{44}O_9Na$ $[M + Na]^+$ required: 631.28830, found: 631.28849.

2, 7-Deacetoxytaxinine J (**17**): t_R = 55.66 min. $C_{35}H_{44}O_8Na$ $[M + Na]^+$ required: 615.29339, found: 615.29332.

Taxezopidine G (**18**): t_R = 47.79 min. $C_{35}H_{44}O_9Na$ $[M + Na]^+$ required: 631.28830, found: 631.28802.

Taxuspine D (**19**): t_R = 48.09 min. $C_{39}H_{48}O_{13}Na$ $[M + Na]^+$ required: 747.29926, found: 747.29926.

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