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5-Epi-Canadensene and other Novel Metabolites of *Taxus canadensis*

Lolita O. Zamir,*[a,b] Junzeng Zhang,[a] Kristina Kutterer,[a] Françoise Sauriol,[b]

[a] Université du Québec, Institut Armand-Frappier, 531 boul. des Prairies, Laval, Québec, Canada, H7N 4Z3

[b] Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Québec, Canada H3A 2K6

Orval Mamer,

Biomedical Mass Spectrometry Unit, McGill University, 1130 Pine Avenue West, Montreal, Québec, Canada H3A 1A3

Abdesslem Khiat and Yvan Boulanger

Département de Radiologie, Centre Hospitalier de l'Université de Montréal, Campus Saint-Luc, 1058 St-Denis, Montréal, Québec, Canada H2X 3J4

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Abstract: The Canadian yew distinguishes itself from other yews by the nature of its taxane metabolites. We are now reporting a new canadensene taxane whose stereochemistry is rigorously established. The three-dimensional structures of canadensene, 5-epi-canadensene and three other related bicyclic taxanes isolated from other yews were calculated using distance constraints derived from NMR data. The stereochemistry of the substituents, the polar acetate groups and the double bonds determine the 3D models. In addition, three new taxanes were also characterized and some biosynthetic speculations are presented.

The efficacy of paclitaxel **1** (Fig. 1) in improving the life of women affected with ovarian cancer at an advanced stage is practically uncontested.[1] This has triggered an intensive effort by medical researchers from the international community. Total syntheses of paclitaxel have been elegantly accomplished[2-6] but cannot yet compete with its semi-synthesis which depends on the isolation of 10-deacetylbaccatin III from *Taxus baccata.*[7] Indeed, 10-deacetylbaccatin III **2** (Fig. 1) is found in all yews but not in the same quantities as in the needles of the European yew (7 times more than paclitaxel).[8] *Taxus canadensis,* a small ramping bush abundant in Quebec, contains also a major taxane which is isolated from its needles: 9-dihydro-13-acetylbaccatin III **3** (Fig. 1).[9,10] It is interesting to note that this taxane, which is 5-7 times more abundant than paclitaxel in the Canadian yew depending on the collection site, has never been found in the needles of any other yew. It has only been detected in the bark of *Taxus chinensis* as a minor taxane.[11] A variety of derivatives of 9-dihydro-13-acetylbaccatin III **3** and a bioactive taxane have also been detected in *Taxus canadensis.*[12,13] In 1995, we have reported for the first time the isolation of a bicyclic oxygenated taxane that we named canadensene **4** (Fig. 2) to emphasize its origin

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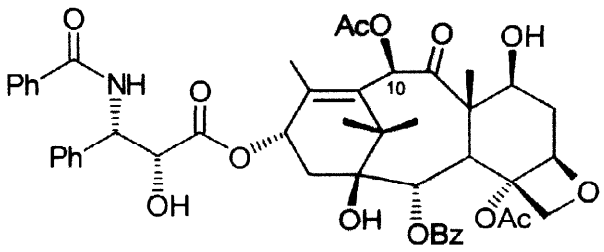
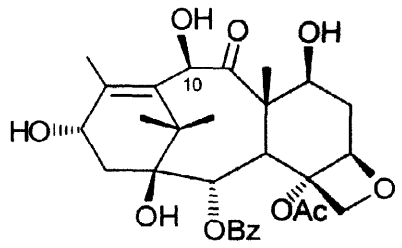
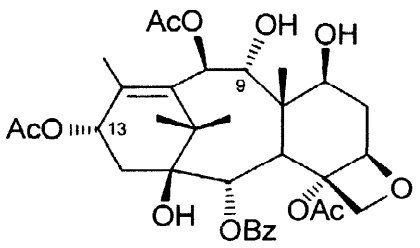


1 Paclitaxel



2 10-Deacetylbaccatin III

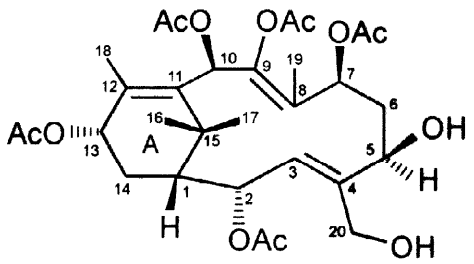


3 9-Dihydro-13-acetylbaccatin III

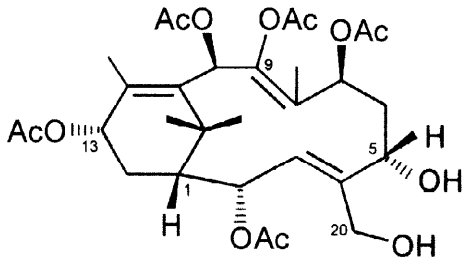
Fig. 1. Chemical structures for some taxanes isolated from the Canadian yew: paclitaxel found in all yews, 10-deacetylbaccatin III found in all yews but abundant only in *Taxus baccata* and 9-dihydro-13-acetylbaccatin III, a minor taxane found in bark of *Taxus chinensis*, abundant in needles of *Taxus canadensis* but not found in other yews.

from the Canadian yew.[14] The canadensene stereochemistry at C5 could not be established due to overlapping NMR signals. Molecular models calculated using NOE distance constraints showed a striking U-shape for the 3D model.[15] Other related bicyclic oxygenated taxanes were also found in the needles of *Taxus chinensis* and in the stems of *Taxus cuspidata*[16-20] (compounds **6-10**, Fig. 2).

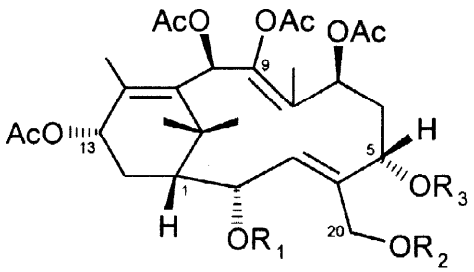
In this publication, we are reporting the isolation of 5-epi-canadensene **5** and the unambiguous characterization of its stereochemistry at C5 as 5α-OH on the basis of NMR studies. Overlapping NMR signals prevented this stereochemical identification for canadensene **4**. This allowed us also to determine that the published canadensene **4** is the stereoisomer with 5β-OH. We have used the NOE distance constraints derived from NMR to compare the three-dimensional structures of canadensene **4**, 5-epi-canadensene **5**, taxachitriene A **6**, taxuspine U **9** and taxuspine X **10** (Fig. 2). In addition, three new taxanes (**11-13**, Fig. 3) were isolated for the first time from the needles of *Taxus canadensis*. These taxanes have a bond between C3 and C11. Other taxanes differently substituted but with a C3-C11 bond were isolated from other yews,[20-26] however all of them have no substitution on C7. On the other hand, one of our novel taxane, compound **11** has a β-OH on C7 which is analogous to most taxanes, including paclitaxel **1**. The two other compounds **12** and **13** differ only in the position of the acetate substitutents (Fig. 3).



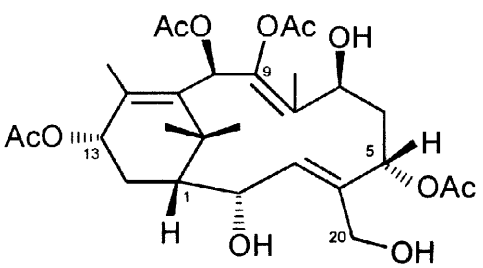
4 Canadensene



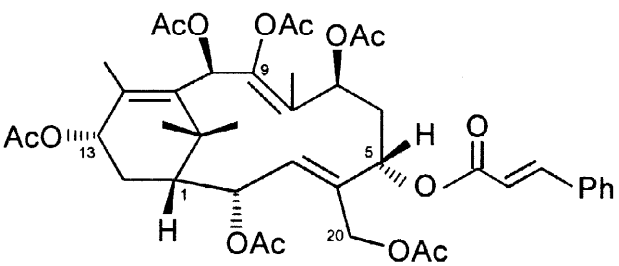
5 5-epi-Canadensene



6 Taxachitriene A: $R_1 = R_2 = Ac$; $R_3 = H$
7 Taxachitriene B: $R_1 = R_2 = H$; $R_3 = Ac$
8 5-Deacetyltaxachitriene B: $R_1 = R_2 = R_3 = H$

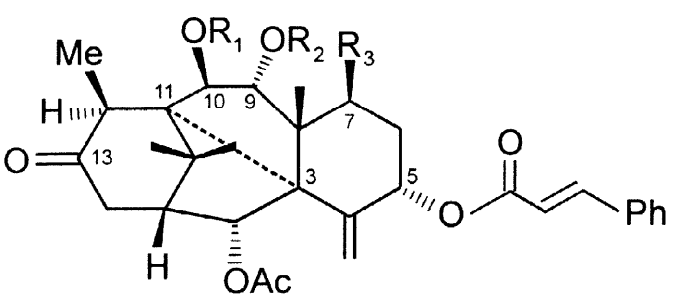


9 Taxuspine U



10 Taxuspine X

Fig. 2. Bicyclic oxygenated taxanes isolated from yews: canadensene found in *Taxus canadensis* needles,[14,15] 5-epi-canadensene found in *Taxus canadensis* needles (this work), taxachitrienes A and B and 5-deacetyltaxachitriene B found in *Taxus chinensis* needles,[16,17] taxuspines U and X isolated from *Taxus cuspidata* stems.[18-20]



11 $R_1 = Ac$; $R_2 = H$; $R_3 = OH$
12 $R_1 = Ac$; $R_2 = R_3 = H$
13 $R_1 = R_3 = H$; $R_2 = Ac$

Fig. 3. Chemical structures of new tetracyclic taxanes isolated from *Taxus canadensis* needles.

EXPERIMENTAL

Instrumentation

Unless otherwise specified, 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 one Partisil 10 ODS-2 MAG-20 preparative column (22 × 500 mm). Preparative TLC was performed on Silica gel 60 F_{254} precoated TLC plates, 0.25 mm (EM Science). The purified compounds were 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.

Extraction of Taxus canadensis Needles

Ground dried needles of *Taxus canadensis* (4.7 kg) were extracted with 18 l of dichloromethane/methanol (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 dichloromethane (4 x 3 l). The combined dichloromethane extracts were dried over anhydrous sodium sulfate, filtered and evaporated yielding a dark brown extract (119 g).

Isolation and Purification of 5-Epi-canadensene **5**

The above mentioned 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 dichloromethane/ isopropanol (9:1, 3.5 l). After elution, the silica gel was cut into 19 bands, and each band was individually eluted with ethyl acetate/methanol (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/dichloromethane [3:1 (2 l), 1:1 (2 l)], dichloromethane (2 l), dichloromethane/ethyl acetate [4:1 (2 l), 3:2 (2 l), 2:3 (2 l),1:4 (2 l)], ethyl acetate (2 l) and ethyl acetate/methanol [4:1 (2 l), 3:2 (4 l)] to yield fraction B (14.0 ~ 15.4 l). Fraction B (2.4 g) was applied to a silica gel column (62 g, 2.5 x 29 cm) and eluted with dichloromethane (200 ml), dichloromethane/acetone [95:5 (200 ml), 90:10 (200 ml), 85:15 (200 ml), 80:20 (200 ml), 75:25 (200 ml), 70:30 (200 ml), 60:40 (200 ml), 30:70 (200 ml)] and acetone (200 ml) to yield fraction C (1040 ~1100 ml, 232 mg), which was then purified on a reverse-phase column eluted with a 50 min linear gradient of acetonitrile (25 to 100%) in water at a flow rate of 18 ml/min to yield 5-epi-canadensene **5** (19.3 mg, t_R 26.5 min, pink spot on TLC plate with R_f = 0.45 (ethyl acetate)]. The product was fully characterized as 5-epi-

canadensene by NMR (see Results and Tables 1 and 2) and confirmed by high resolution mass spectrometry data. FAB MS: $(M + Na)^+$: 617.25711; $C_{30}H_{42}O_{12}Na$ requires 617.25740.

*Isolation and Purification of Taxanes **11**, **12** and **13***

Following the same extraction procedure as described above, another 50 g of dichloromethane extract from *Taxus canadensis* needles was obtained. This extract was separated on a silica gel column (821 g, 10 x 21 cm) eluted with hexane/dichloromethane (1:1, 2 l), dichloromethane (2 l), dichloromethane/ethyl acetate [9:1 (2 l), 8:2 (2 l)], dichloromethane/ethyl acetate/methanol [700:295:5 (1 l), 700:290:10 (1 l), 700:280:20 (1 l), 700:250:50 (1 l), 600:350:50 (1 l), 500:400:100 (1 l), 400:400:200 (1 l), 300:500:200 (1 l)] and ethyl acetate/methanol [7:3 (1 l), 1:1 (2 l)] to give fractions D1 (11.6 ~ 12.0 l), D2 (12.0 ~ 12.6 l) and D3 (12.6 ~ 13.4 l).

Fraction D1 (5.5 g) was applied to a silica gel column (105 g, 4 x 17.5 cm) with hexane/isopropanol [9:1 (100 ml), 8:2 (200 ml), 7:3 (300 ml), 6:4 (300 ml), 1:1 (300 ml) and ethyl acetate/methanol (1:1, 300 ml) to yield fraction E (400 ~ 540 ml). Fraction E (1.6 g) was then purified on a silica gel column (54 g, 2.5 x 36 cm) with dichloromethane/ethyl acetate [8:2 (200 ml), 7:3 (200 ml), 6:4 (200 ml), 1:1 (200 ml), 4:6 (200 ml), 2:8 (200 ml)] and ethyl acetate (200 ml) to yield fraction F (500 ~ 600 ml, 200 mg), which was further purified on a reverse-phase column eluting with 70 min linear gradient of acetonitrile (25 to 100%) in water at a flow rate of 18 ml/min followed by preparative TLC (dichloromethane/methanol, 95:5) to produce compound **11** (6.4 mg). The structure of **11** was fully characterized by NMR (Table 3) and confirmed by high resolution mass spectrometry data. FAB-MS: $(M + Na)^+$: 603.25687; $C_{33}H_{40}O_9Na$ requires 603.25700.

Fraction D2 (2.7 g) was subjected to a silica gel column (108 g, 4.5 x 18 cm) with hexane/ethyl acetate [8:2 (200 ml), 1:1 (300 ml), 4:6 (200 ml), 3:7 (200 ml), 2:8 (200 ml), 1:9 (200 ml)], ethyl acetate (600 ml), ethyl acetate/methanol (8:2, 400 ml) to yield fraction G (1500 ~ 1800 ml). Fraction G (1.05 g) was then applied to a silica gel column (55 g, 2.5 x 26 cm) and eluted with dichloromethane (200 ml), dichloromethane/methanol [1000:5 (200 ml), 1000:10 (300 ml), 1000:15 (200 ml), 1000:20 (200 ml), 1000:30 (200 ml), 1000:40 (200 ml), 1000:50 (200 ml), 1000:60 (200 ml), 1000:70 (200 ml)] to produce fraction H (1220 ~ 1380 ml). Fraction H (489 mg) was again purified on a silica gel column (20 g, 2 x 17 cm) with dichloromethane/ethyl acetate [8:2 (100 ml), 6:4 (100 ml), 4:6 (100 ml), 3:7 (100 ml), 2:8 (100 ml), 1:9 (100 ml)] and ethyl acetate (100 ml) yielding fraction I (1 ~ 400 ml).

Fraction D3 (3.6 g) was applied to a silica gel column (108 g, 4.5 x 18 cm) and eluted with hexane/ethyl acetate [1:1 (400 ml), 3:7 (400 ml), 1:9 (400 ml)], ethyl acetate (600 ml), ethyl acetate/methanol (9:1, 400 ml) to yield fraction J (1240 ~ 1520 ml). Fraction J (1.25 g) was again purified on a silica gel column (28 g, 2 x 22.5 cm) with dichloromethane (100 ml), dichloromethane/methanol [1000:5 (200 ml), 1000:10 (200 ml), 1000:15 (200 ml), 1000:20 (200 ml), 1000:25 (200 ml), 1000:30 (200 ml), 1000:35 (200 ml), 1000:40 (200 ml), 1000:50 (200

ml)] to produce fraction K (200 ~ 320 ml).

Fractions I and K were combined yielding 45.4 mg of residue which was further purified by preparative TLC (dichloromethane/methanol, 98:2) to finally produce compounds **12** (21.9 mg) and **13** (3.8 mg). Both products were visualized on TLC plate as brown spots with R_f =0.85 and R_f =0.70 (ethyl acetate). The structures of **12** and **13** were fully characterized by NMR and confirmed by high resolution mass spectrometry data. FAB-MS for **12**: $(M + H)^+$: 565.28006; $C_{33}H_{41}O_8$ requires 565.28014; FAB-MS for **13**: $(M + Na)^+$: 587.26196; $C_{33}H_{40}O_8Na$ requires 587.26209.

NMR and Mass Spectrometry Measurements

^1H, ^{13}C, HMQC, HMBC and NOESY NMR spectral data were obtained on a Varian UNITY 500 spectrometer operating at 499.84 MHz for proton and 125.69 MHz for carbon-13. The spectra were obtained on 1-2 mg samples dissolved in $CDCl_3$ which was used as the internal reference. In the proton NMR spectra, the multiplicity shown in the tables is the apparent one. Low resolution xenon fast atom bombardment (FAB) mass spectra were obtained in glycerol with a VG ZAB-MS instrument. Samples were dissolved in 0.2 μl DMSO before addition of 0.5 μl glycerol. High resolution FAB mass spectra were similarly obtained in glycerol-DMSO at a resolving power of 12000.

Molecular Modeling

Molecular modeling calculations for taxane compounds **4-10** were performed on the Silicon Graphics workstation with Molecular Simulations softwares (INSIGHT II, NMR REFINE, DGII, version 97). Chemical structures were constructed using the consistent valence forcefield and energy minimized for 1000 steps using the conjugate gradients algorithm. The energy minimized structures were then submitted to a distance geometry calculation using the NMR constraints from the literature for canadensene **4**[15] (23 constraints), taxachitriene A **6**[17] (16 constraints), taxuspine U **9**[18] (13 constraints) and taxuspine X **10**[19] (10 constraints), or from Table 2 for 5-epi-canadensene **5** (29 constraints). All NMR constraints were determined from NOE connectivities measured in NOESY spectra and recorded for samples dissolved in $CDCl_3$ except for **6** whose NOEs were determined by one-dimensional NOE difference spectra. Depending on the intensity of the NOESY connectivities, distance constraints were classified as short, medium and long and were assigned maximal values of 3.0, 4.0 and 5.0 Å, respectively. When the intensities of the NOESY connectivities were not reported (compounds **6, 9, 10**), all constraints were assigned a maximal value of 4.0 Å. When equivalent protons were present on a same heteroatom, a pseudoatom was created and a distance correction of 1 Å was applied. Twenty structures were produced for each compound by distance geometry, followed by 10,000 steps of simulated annealing (maximal temperature 1200 K) and conjugate gradients energy minimization with a root-mean-square gradient deviation of 0.001 Å (Fig. 4).

Final structures were analyzed by determining the number of constraint violations exceeding 0.5 Å and degree of convergence (root-mean-square distance deviations or RMSD) between structures.

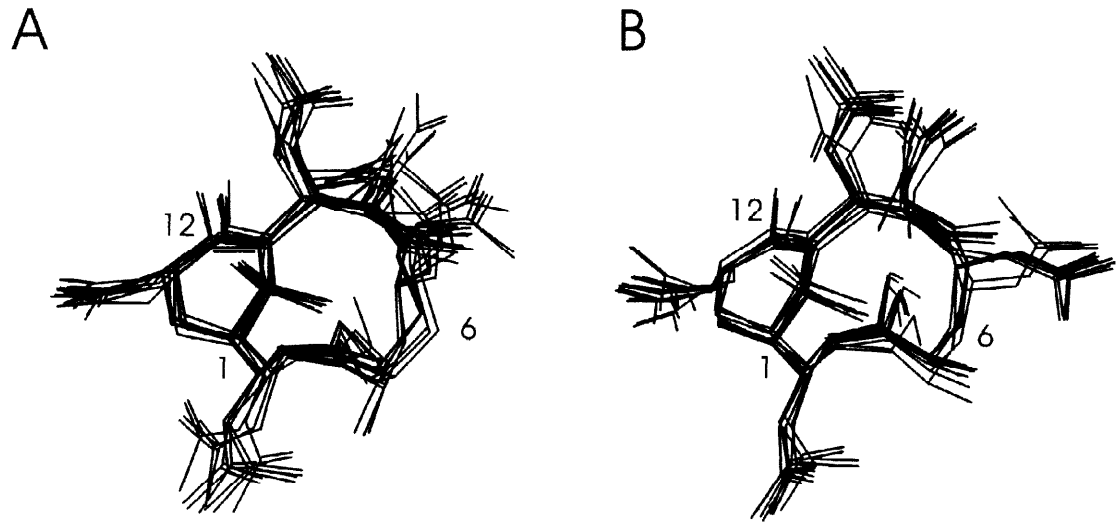


Fig. 4. Superposition of the lowest energy structures of (A) canadensene **4** and (B) 5-epi-canadensene **5** calculated by distance geometry using distance constraints derived from NOESY connectivities.

RESULTS AND DISCUSSION

Characterization of 5-Epi-canadensene

The structural characterization of epi-canadensene was performed using spectroscopic techniques (high resolution 2D NMR experiments, HMQC,[27] HMBC,[28] COSY,[29] NOESY,[30] and low and high resolution MS). The NMR data are shown in Table 1. The results of 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) were essential for the characterization of this compound. Analysis of the proton NMR, COSY and HMQC allowed us to write a bicyclic substructure. Only the most deshielded proton (6.89 ppm) remained unconnected. The only positions where this proton could be connected were C9, C10 or C11. The HMBC experiment showed indeed a correlation of this proton to both olefinic systems (C8-C9, C11-C12), to C15 and to an adjacent acetyl group, leading to an unambiguous assignment to H10. According to the proton spectra, there were five acetyl groups in the molecule: four attached to protonated carbons as seen in the HMBC experiment (2, 7, 10 and 13) and a fifth with no connection in the HMBC spectrum, and therefore located on a quaternary carbon. The olefinic C9 carbon is a quaternary olefinic carbon which according to its chemical shift can be substituted by an OAc group. The NMR data are compatible with only one structure:

canadensene.

Table 1. Proton and Carbon-13 NMR Data for 5-epi-Canadensene **5** in $CDCl_3$

Position	δ ^{1}H ppm	M[a]	J (Hz)	δ ^{13}C[b] ppm	Position	δ ^{1}H ppm	M[a]	J (Hz)	δ ^{13}C[b] ppm
1	1.76	br t	6.1, 6.9	46.6	OAc-9/10	2.21	s		20.0
2	5.75	dd	4.6, 11.5	70.7					**168.0**
OAc-2	2.01	s		21.4	12				**135.3**
				171.6	13	5.29	d	9.3	68.8
3	6.23	d	11.2	121.9	OAc-13	2.17	s		21.1
4				**142.3**					**170.7**
5	4.64	br s		67.9	14a	2.55	ddd	7.8, 9.3, 16.8	24.9
OH-5	3.72	br d	1.5		14b	2.25	d		16.8
6a	2.74	ddd	2.2, 8.3, 15.9	37.3	15				**35.7**
6b	2.02	o m			16	1.13	s		34.0
7	5.01	d	8.0	67.4	17	1.21	s		24.1
OAc-7	2.10	s		21.4	18	1.92	s		17.0
8				**123.2**	19	1.63	s		12.9
9				**144.3**	20a	4.56	dd	1.0, 12.7	57.5
10	6.89	br s		67.9	20b	3.46	br t	12.5	
OAc-9/10	1.96	s		20.6	OH-20	2.90	br dd	1.0, 9.5	
				168.0					

[a] M, multiplicity; br, broad, d, doublet, m, multiplet, o, overlapping, s, singlet, t, triplet.
[b] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold character represent quarternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm).

However, when we compare the chemical shifts observed for our compound with the reported values for canadensene[14,15], slight differences are apparent which must be attributable to a different stereochemistry. The stereochemistry around the two double bonds in this macrocycle was therefore determined on the basis of the following observations (Table 2):

1- H2 has a strong NOE connectivity with Me17 and H20a and a medium NOE connectivity with H1. H1 correlates with Me17, Me16, H2 and H14a. H13 interacts strongly with Me16 and H14a. These protons are therefore on the β-side (top) of the molecule as in paclitaxel **1**.

2- H3 correlates with protons on the α-side (bottom) of the molecule: H7 and H14b display weak NOE connectivities with Me18. H3 interacts also with OH-5, indicating that H3 and OH-5 are in *cis* positions on the α-side of the molecule. The fact that a strong NOE connectivity is observed between H5 and H20b (H5 being β) confirms the α-orientation of OH-5.

3- H10 displays a strong NOE connectivity with H7 and Me18. H7 interacts strongly with H10, H3, OH-5 and Me18. All these protons are therefore on the α-side of the molecule.

4- Me19 interacts with H6a and H20b on the other side of the macrocycle. This interaction necessitates that the two double bonds be spatially close and that Me19 be on the β-side of the molecule.

Table 2. NOESY observed for 5-epi-Canadensene **5** in $CDCl_3$ (NOE positive cross peaks)

Proton	NOE connectivities observed: s (strong), m (medium), w (weak)
H1	H2 (s), H14a (s), H14b (s), Me16 (s), Me17 (s)
H2	H1 (s), Me17 (s), H20a (s)
H3	OH-5 (m), H7 (m), H14b (m), Me18 (w)
H5	OH-5 (s), H6a (s), H6b (s), H20b (s), OH-20 (w)
OH-5	H3 (m), H5 (s), H6b (s), H7 (s)
H6a	H6b (s), Me19 (s), H20a (s), H20b (s)
H6b	H5 (s), OH-5 (w), H6a (s), H7 (w)
H7	H3 (m), OH-5 (s), H10 (s), Me18 (s)
H10	H7 (s), Me18 (s)
H13	H14a (s), H14b (m), Me16 (s), Me18 (s)
H14a	H1 (s), H13 (s), H14b (s), Me16 (s)
H14b	H3 (m), H13 (m), H14a (s)
H16	H1 (s), H14a (s)
H17	H1 (s), H2 (s), Me16 (s), H20a (s)
H18	H7 (s), H10 (s), H13 (s)
H19	H6a (s), H20b (s)
H20a	H2 (s), Me17 (s), H20b (s), OH-20 (m)
H20b	Me19 (m), H20a (s), OH-20 (s)
OH-20	OH-5 (s, negative), H20a (m)

The relative stereochemistries of the different groups in 5-epi-canadensene are very clearly established since there are no overlapping proton signals. On the previous canadensene isolated from the same yew,[14,15] overlapping signals for H5 and H20a prevented the determination of the H5 stereochemistry. The isolation of this stereoisomer allows us to re-examine the NOEs of canadensene and to conclude that the only difference between these two structures lies in the stereochemistry at C5. The substituent at C5 is a β-OH for canadensene and an α-OH for 5-epi-canadensene as illustrated in Fig. 2. These structures were confirmed by high resolution MS.

Molecular Modeling: 3D Structures of Canadensene and 5-Epi-Canadensene

The three-dimensional structures of canadensene **4** and 5-epi-canadensene **5** were calculated by distance geometry using the interproton distance constraints derived from NOESY data previously published[14,15] and from Table 2. The structures were refined by simulated annealing and energy minimization. No violation of the distance constraints of more than 0.5 Å was measured for any of the 20 final structures calculated for each molecule.

Sixteen structures out of 20 converged for **4** and 14 for **5**. The converging structures illustrated in Fig. 4 were of lower energy. The RMSD values calculated for all atoms in the 20 final structures are relatively low (1.5 Å for **4** and 1.6 Å for **5**), in agreement with the low structural dispersion illustrated in Fig. 4.

The molecular modeling results indicate that the dodecadiene cycle of both canadensenes is not very flexible. This is mostly due to the presence of two double bonds, of polar acetyl substituents and of gem-dimethyl groups on C15. The main difference in the 3D structures of these two stereoisomers lies in the C5, C6 and C7 region. The 5-epi-canadensene displays NOE correlations for H5 and OH-5 with H3, H6a, H6b, H7 and H20 (Table 2). These strong correlations were not observed for canadensene **4**.[14,15] Indeed, the minimal distances obtained in the lowest energy structures of this stereoisomer between the hydrogen of OH-5 and H3, H6, H7 and

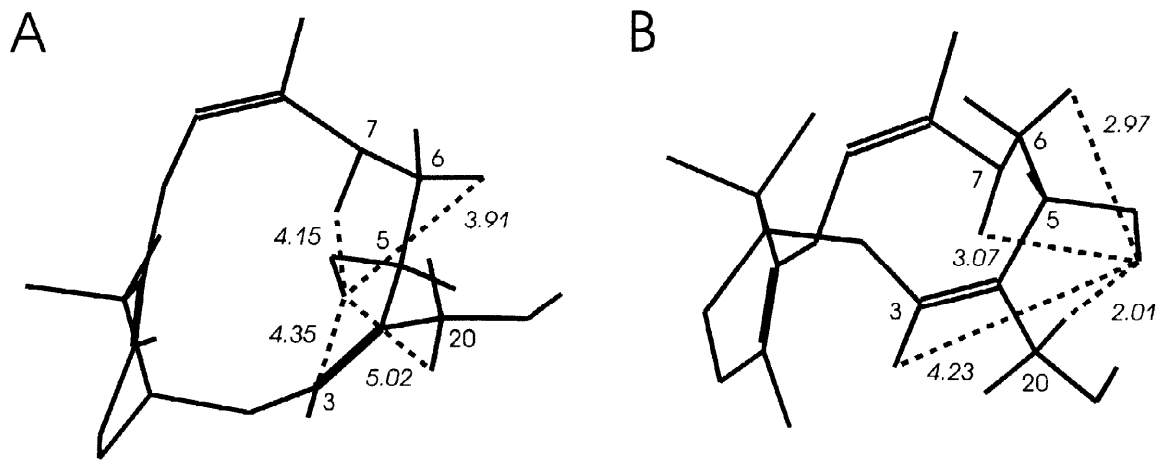


Fig. 5. Molecular models of the lowest energy structure of (A) canadensene **4** and (B) 5-epi-canadensene **5** illustrating the distances (in Å) between the OH-5 hydrogen and the neighbouring hydrogens.

H20 are 4.35 Å, 3.91 Å, 4.15 Å and 5.02 Å, respectively (Fig. 5A). In the case of 5-epi-canadensene **5**, the distances between the hydrogen of OH-5 and the same protons are much shorter: they are 4.23 Å, 2.97 Å, 3.07 Å and 2.01 Å with H3, H6, H7 and H20, respectively (Fig. 5B). The existence of OH-5 in the α-form as in **5** results in more unfavorable interactions with the neighbouring groups as a result of the shorter distances.

Molecular Modeling: 3D Structures of Related Bicyclic Taxanes from Different Yews

The chemical structures and NOE connectivities of several bicyclic taxanes related to canadensene have been reported.[16,18,19] Using the NOE connectivity data, three-dimensional structures for taxachitriene A **6**[16], taxuspine U **9**[18] and taxuspine X **10**[19] could be calculated and compared with those of canadensene **4** and 5-epi-canadensene **5**. It is interesting to note that canadensene is the only taxane of that group presenting a 5β-hydroxyl group, all the others existing in the 5α configuration. Fig. 6A presents a comparison of the bicyclic structures of

the two canadensenes **4** and **5** which differ mostly in the vicinity of C5 as a result of the stereochemical difference at this position. Taxuspine X was found to be the most different from 5-epi-canadensene **5** as a result of the interaction of Me18 in the six-membered ring and H23 of the cinnamate side chain on C5 (Fig. 6D). In contrast, the bicyclic core structure of taxuspine U is almost identical to that of **5** (Fig. 6C). This is because the OAc group at position 5 is located on the bottom side of the molecule and does not interact with the other β substituents. In contrast, when the acetyl group is on C20 as in taxachitriene A, the OAc-20 has a strong interaction with OH-5 (Fig. 6B) and the structure becomes distorted.

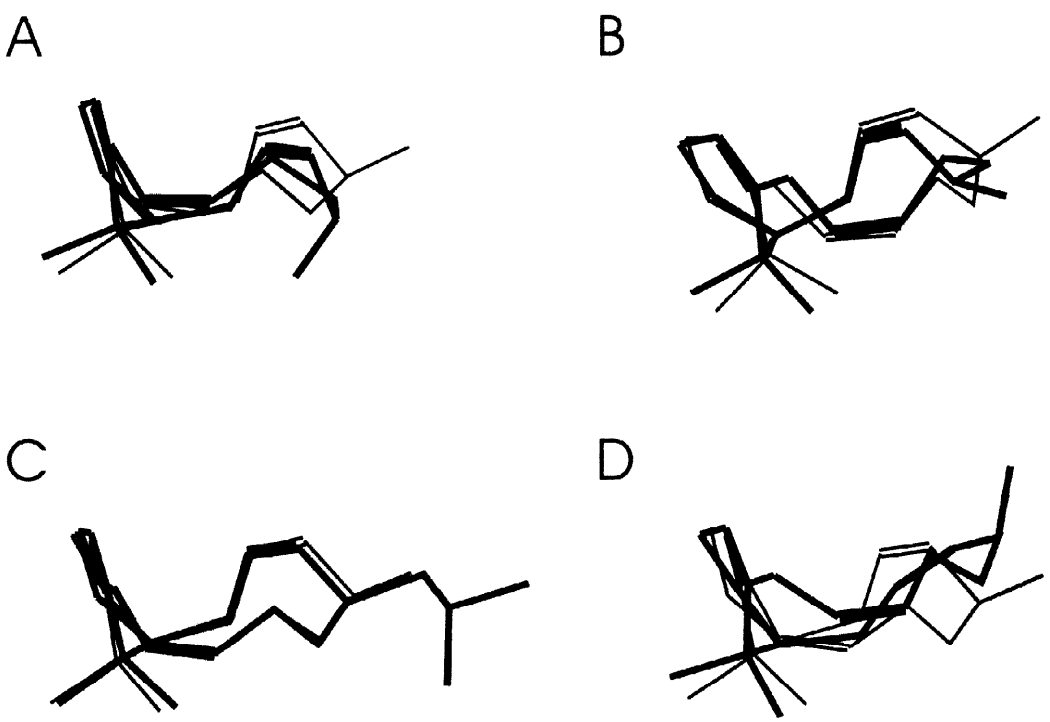


Fig. 6. Superposition of the bicyclic cores of 5-epi-canadensene **5** (thick line) and (A) canadensene **4**, (B) taxachitriene A **6** (C) taxuspine U **9** and (D) taxuspine X **10**. The structures correspond to the lowest energy conformations calculated by distance geometry with the NOE constraints.

*Novel Taxanes **11-13** Isolated from Taxus canadensis*

Three new and similar tetracyclic taxanes with a C3-C11 bond have been isolated from Canadian yew needles. Their structures have been characterized by NMR and MS and found to be those of compounds **11**, **12** and **13** shown in Fig. 3. Analogous tetracyclic taxanes have been reported before from other yews but with some different substituents.[20-26] It is the first time they have been isolated in *Taxus canadensis*. Compound **11** is also

the first compound of that series with an hydroxyl group on C7. The fact that *Taxus canadensis* is the only yew which produces in its needles different taxanes (accumulation of 9-dihydro-13-acetylbaccatin III **3**, and isolation of canadensenes) might suggest some biosynthetic differences from other yews.

Table 3. Proton and Carbon-13 NMR Data for Compound **11** in $CDCl_3$

Position	δ ^{1}H ppm	M[a]	J (Hz)	δ ^{13}C[b] ppm	Position	δ ^{1}H ppm	M[a]	J (Hz)	δ ^{13}C[b] ppm
1	2.16	o d	7.0	47.6	9	4.59	d	9.5	83.3
2	6.01	d	4.9	76.4	OH-9	3.57	br s		
OAc-2	2.06	s		21.0	10	5.44	d	9.6	83.3
				169.8	OAc-10	2.18	s		21.0
3				**64.7**					**172.9**
4				**141.7**	11				**57.8**
5	5.71	t	7.1	75.1	12	3.44	q	7.1	52.0
OR-5 CO				**165.4**	13				**214.0**
OR-5 CH	6.40	d	16.1	117.4	14a	2.65	d	20.5	38.8
	7.69	d	16.1	145.6	14b	2.50	dd	7.3, 20.5	
OR-5 Ph	7.39	m		128.7	15				**43.0**
				130.3	16	1.22	s		26.6
	7.54	m		128.1	17	1.58	s		29.4
6a	2.22	o m		33.5	18	1.29	d	7.3	15.7
6b	2.03	o m			19	1.47	s		19.1
7	4.26	br m		69.2	20a	5.75	s		125.9
OH-7	2.96	br s			20b	5.64	s		
8				**49.0**					

[a] M, multiplicity; br, broad, d, doublet, m, multiplet, o, overlapping, q, quartet s, singlet, t, triplet.
[b] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold character represent quarternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm).

NMR data consisting of ^{1}H and ^{13}C chemical shifts and ^{1}H-^{1}H coupling constants are presented in Table 3 for compound **11**. The assignment procedure was similar to that used for other taxanes and the major spectral characteristics for compound **11** are as follows. Me16 and Me17 are characterized by their shielded ^{1}H and ^{13}C chemical shifts and their absence of ^{1}H-^{1}H coupling. Their gem position is evident from the observation of a COSY connectivity with each other. Me18 is characterized by its ^{1}H-^{1}H coupling to a methine. The OAc methyl groups are characterized by their chemical shifts. The methylene groups (H6, H14 and H20) can be identified by the observation of a HMQC connectivity of two protons to a single carbon. H14 and H6 are distinguished by their different COSY connectivities, H6 connecting to two deshielded protons and H14 to a shielded and a deshielded neighbouring protons. Olefinic methylene protons (H20) display characteristic deshielded chemical shifts. Olefinic

Table 4. Proton and Carbon-13 NMR Data for Compound **12** in $CDCl_3$

Position	δ ^{1}H ppm	M[a]	J (Hz)	δ ^{13}C[b] ppm	Position	δ ^{1}H ppm	M[a]	J (Hz)	δ ^{13}C[b] ppm
1	2.15	o m		47.6	8				**45.0**
2	6.08	d	5.1	76.4	9	4.42	d	9.3	82.7
OAc-2	2.07	s		21.3	10	5.41	d	9.3	84.2
				169.6	OAc-10	2.16	s		21.0
3				**65.8**					**172.4**
4				**142.7**	11				**57.7**
5	5.63	t	9.0	76.4	12	3.57	q	7.3	52.0
OR-5 CO				**165.7**	13				**214.1**
OR-5 CH	6.38	d	15.9	117.7	14a	2.60	d	20.5	38.8
	7.66	d	16.1	145.3	14b	2.50	dd	7.1, 20.5	
OR-5 Ph	7.39	m		128.7	15				**42.6**
				130.3	16	1.22	s		26.6
	7.56	o m		128.1	17	1.56	s		29.8
6a	2.19	o m		26.0	18	1.32	d	7.2	15.7
6b	1.67	m			19	1.30	s		26.0
7a	2.01	m		29.4	20a	5.83	s		128.7
7b	1.12	td	2.0, 13.9, 13.9		20b	5.68	s		

[a] M, multiplicity; br, broad, d, doublet, m, multiplet, o, overlapping, q, quartet s, singlet, t, triplet.
[b] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold character represent quarternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm).

trans protons and aromatic protons in the cinnamate side chain display deshielded chemical shifts and characteristic coupling patterns. Identification of the tetracyclic structure could be accomplished with the HMBC experiments which connect protons and carbons through multiple bonds. The main HMBC connectivities observed were: Me16 and Me17 connecting with C1, C11 and C15; Me18 connecting to C11, C12 and C13; H14 connecting to C1, C2, C13 and C15; Me19 connecting to C3, C7, C8 and C9; H2 connecting to C3 and C8; H20 connecting to C3, C4 and C5; H10 connecting to the OAc-10 carbonyl. The existence of the C3-C11 bond was also demonstrated by HMBC: the C3-H12 connectivity is only possible if that bond exists. The cinnamate side chain structure was confirmed by HMBC connectivities between H5 and the ester carbonyl carbon as well as between the *trans* olefinic protons and the ester carbonyl carbon.

The stereochemistry of compound **11** was determined on the basis of the NOESY connectivities. The H2 proton displayed strong NOE connectivities with Me17 and Me19 and a medium NOE connectivity with H1. This pattern is similar to that observed in paclitaxel derivatives and corresponds to protons on the upper side of the molecule. H1 interacts with H2, H14b, Me16 and Me17 and is therefore also on the upper side of the molecule. Me 18 interacts only with Me16, suggesting that it is also on the upper side of the molecule. Me19 connects

strongly with H2 and moderately with H9 and H5, all protons on the upper part of the molecule. On the other

Table 5. Proton and Carbon-13 NMR Data for Compound **13** in $CDCl_3$

Position	δ ^{1}H ppm	M[a]	J (Hz)	δ ^{13}C[b] ppm	Position	δ ^{1}H ppm	M[a]	J (Hz)	δ ^{13}C[b] ppm
1	2.15	o m		47.7	8				**44.7**
2	6.11	d	5.1	76.7	9	5.49	d	9.2	87.1
OAc-2	2.07	s		21.1	OAc-9	2.15	s		20.8
				169.6					**172.6**
3				**66.2**	10	4.40	d	9.2	80.5
4				**142.5**	11				**59.3**
5	5.58	t	9.3	76.7	12	3.56	q	7.3	52.3
OR-5 CO				**165.7**	13				**215.2**
OR-5 CH	6.38	d	16.1	117.8	14a	2.58	d	20.3	38.6
	7.68	d	16.1	144.9	14b	2.51	dd	6.1, 20.8	
OR-5 Ph	7.39	m		128.9	15				**42.2**
				130.4	16	1.36	s		26.6
	7.56	o m		128.2	17	1.65	s		28.5
6a	2.23	m		25.8	18	1.55	d	7.3	16.4
6b	1.54	o m			19	1.26	s		26.8
7a	1.72	ddd	3.2, 4.6, 14.4	31.2	20a	5.84	s		129.3
7b	1.15	td	2.6, 13.6, 13.6		20b	5.66	s		

[a] M, multiplicity; br, broad, d, doublet, m, multiplet, o, overlapping, q, quartet s, singlet, t, triplet.
[b] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold character represent quarternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm).

hand, H10 shows strong NOE connectivities with H7, Me18 and H12; these protons must therefore be on the lower side of the molecule. OH-9 interacts with H7 and H10 which are located on the bottom part of the macrocycle. The stereochemistry of compound **11** determined by NMR data corresponds to that illustrated in Fig. 3. Similar analysis procedures were used to determine the structure and stereochemistry of taxanes **12** and **13** which are illustrated in Fig. 3. NMR data for **12** and **13** are given in Tables 4 and 5, respectively. The structures of **11-13** were confirmed by high resolution MS.

Biosynthetic Speculations

The elegant proof of the intermediacy of taxadiene[31,32] implies that in the biosynthesis of paclitaxel, a tricyclic hydrocarbon (taxadiene) is first formed, followed by oxidations of that structure. The discovery of taxanes in yews with rings B and C uncyclized (canadensene, 5-epi-canadensene, taxachitrienes and taxuspine) but with oxygenated substituents at carbons C2, C5, C7, C9, C10 and C13 (as in paclitaxel) might suggest that in these yews, the sequence of the reactions is different: first oxidations of a bicyclic moiety and then cyclization.

Support for this hypothesis is given by the same absolute stereochemistries at C1, C2, C7, C10 and C13 in these uncyclized metabolites as in paclitaxel.

On the basis of the molecular modeling work, distances between the oxygens of OH-5 and OH-20 were measured as 4.27 Å for canadensene and 3.08 Å for the 5-epi-canadensene. The 5-epi-canadensene seems to be more prone to cyclize to form an oxetane because of the proximity of the two hydroxyl groups. It is tempting to assume that one of them is used for the formation of a taxane whereas the other acts as a dead-end metabolite. Also, we have not isolated both compounds at the same time from the same plant. Preliminary studies in our laboratories suggest that the isolation of the different stereoisomers must be dependent on the season of the plant collection. The biosynthetic puzzle is still unsolved. Why only very few yew species (*Taxus canadensis* and *Taxus chinensis* needles, *Taxus cuspidata* stems) produce these compounds? Why both stereoisomers exist in the Canadian yew? It is doubtful that the bicyclic taxanes originate from the opening of a full oxygenated tricyclic taxane.[15] Indeed, nobody has yet reported such transformation by chemical reactions or *in vivo*. The diversity of compounds obtained from the Canadian yew might perhaps hint to two possible pathways to form taxanes.

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REFERENCES

1. McGuire, W. P.; Hoskins, W. J.; Brady, M. F.; Kucera, P. R.; Partridge, E. E.; Look, K. Y.; Clarke-Pearson, D. L.; Davidson, M. *N. Engl. J. Med.* **1996**, 334, 1.
2. Holton, R. A.; Somoza, C.; Kim, H. B.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C. L.; Vu, P.; Tang, S. H.; Zhang, P. S.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *A. C. S. Symposium Series*, **1995**, 583, 288.
3. Nicolaou, K. C.; Nantermet, P. G.; Ueno, H.; Guy, R. K.; Couladouros, E. A.; Sorensen, E. J. *J. Am. Chem. Soc.* **1995**, 117, 624-; Nicolaou, K. C.; Liu, J. J.; Yang, Z.; Ueno, H.; Sorensen, E. J.; Claiborne, C. F.; Guy, R. K.; Hwang, C. K.; Nakada, M.; Nantermet, P. G. *J. Am. Chem. Soc.* **1995**, 117, 634; Nicolaou, K. C.; Yang, Z.; Liu, J. J.; Nantermet, P. G.; Claiborne, C. F.; Renaud, J.; Guy, R. K.; Shibayama, K. *J. Am. Chem. Soc.* **1995**, 117, 645; Nicolaou, K. C.; Ueno, H.; Liu, J. J.; Nantermet, P. G.; Yang, Z.; Renaud, J.; Paulvannan, K.; Chadha, R. *J. Am. Chem. Soc.* **1995**, 117, 653.
4. Danishefsky, S. J.; Masters, J. J.; Young, W. B.; Link, J. T.; Snyder, L. B.; Magee, T. V.; Jung, D.K.; Isaacs, R. C. A.; Bormann, W. G.; Alaimo, C. A.; Coburn, C. A.; Digrandi, M. J. *J. Am. Chem. Soc.* **1996**, 118, 2843.
5. Mukaiyama, T.; Shiina, I.; Iwadare, H.; Sakoh, H.; Tani, Y.; Hasegawa, M.; Saitoh, K. *Proc. Japan Acad. Series B-Phys. Biol. Sci.* **1997**, 73, 95.
6. Wender, P.A.; Badham, N.F.; Conway, S.P.; Floreancig, P.E.; Glass, T.E.; Houze, J.B.; Krauss, N.E.; Lee, D.; Marquess, D.G.;

McGrane, P.L.; Meng, W.; **Natchus, M.G.; Shuker, A.J.;** Sutton, J.C.; Taylor, R.E. *J. Am. Chem. Soc.* **1997, 119,** 2757 **and** references therein.

7. Senilh, V.; Blechert, S.; Colin, M.; Guénard, D.; Picot, F.; Potier P.; Varenne, P. *J. Nat. Prod.* **1984,** 47, 131.
8. Denis, J. N.; Green, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988,** 110, 5917.
9. Zamir, L. O.; Nedea, M. E.; Bélair, S.; Sauriol, F.; Mamer, O.; Jacqmain, E.; Jean, F. I.; Garneau, F.-X. *Tetrahedron Lett.* **1992,** 33, 5173.
10. Gunawardana, G. P.; Premachandran, U.; Burres, N. S.; Whittern, D. N.; Henry, R.; Spanton, S.; McAlpine, J. B. *J. Nat. Prod.* **1992,** 55, 1686.
11. Zhang, S.; Chen, W. M.; Chen, Y. H. *Yaoxue Xuebao* **1992,** 27, 268.
12. Zamir, L. O.; Nedea, M. E.; Zhou, Z. H.; Bélair, S.; Caron, G.; Sauriol, F.; Jacqmain, E.; Jean, F. I.; Garneau, F.-X.; Mamer, O. *Can. J. Chem.* **1995,** 73, 655.
13. Zamir, L. O.; Nedea, M. E.; Zhou, Z. H.; Caron, G.; Sauriol, F.; Mamer, O. *Phytochemistry* **1996,** 41, 803.
14. Zamir, L. O.; Zhou, Z. H.; Caron, G.; Nedea, M. E.; Sauriol, F.; Mamer, O. *J. Chem. Soc. Chem. Commun.* **1995,** 529.
15. Boulanger, Y.; Khiat, A.; Zhou, Z.-H.; Caron, G.; Zamir, L. O. *Tetrahedron* **1996,** 52, 8957.
16. Fang, W. S.; Fang, Q. C.; Liang, X. T.; Yang, L.; Zheng, Q.T. *Tetrahedron* **1995,** 51, 8483.
17. Fang, W. S.; Fang, Q. C.; Liang, X. T. *Planta Medica* **1996,** 62, 567. 20-Deacetyltaxachitriene A was erroneously characterized as identical to canadensene. It is interesting to note that its 500 MHz proton NMR is identical to 5-epi-canadensene.
18. Hosoyama, H.; Inibishi, A.; Katsui, T.; Shigemori, H.; Kobayashi, J. *Tetrahedron* **1996,** 52, 13145.
19. Shigemori, H.; Wang, X.-X.; Yoshida, N.; Kobayashi, J. *Chem. Pharm. Bull.* **1997,** 45, 1205.
20. Kobayashi, J.; Shigemori, H. *Heterocycles* **1998,** 47, 1111.
21. Chiang, H. C.; Woods, M. C.; Nakadaira, Y.; Nakanishi, K. *Chem. Commun.* **1967,** 1201.
22. Ettouati, L.; Ahond, A.; Convert, O.; Poupat, C.; Potier, P. *Bull. Soc. Chim. France* **1989,** 687.
23. Kobayashi, J.; Ogiwara, A.; Hosoyama, H.; Shigemori, H.; Yoshida, N.; Sasaki, T.; Li, Y.; Iwasaki, S.; Naito, M.; Tsuruo, T. *Tetrahedron* **1994,** 50, 7401.
24. Kobayashi, J.; Irubushi, A.; Hosoyama, H.; Yoshida, N.; Sasaki, T.; Shigemori, H. *Tetrahedron* **1995,** 51, 5971.
25. Soto, J.; Castedo, L. *Phytochemistry* **1998,** 47, 817.
26. Appendino, G.; Lusso, P.; Gariboldi, P.; Bombardelli, E.; Gabetta, B. *Phytochemistry* **1992,** 31, 4259.
27. Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983,** 55, 301.
28. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986,** 108, 2093.
29. Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976,** 64, 2229.
30. Macura, S.; Ernst, R. R. *Mol. Phys.* **1980,** 41, 95.
31. Wildung, M. R.; Croteau, R. *J. Biol. Chem.* **1996,** 271, 9201.
32. Hezeri, M.; Croteau, R. *Planta Medica* **1997,** 63, 291.