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A novel minor metabolite (taxane?) from *Taxus canadensis* needles

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Abstract—A novel minor metabolite **1** with an unprecedented skeleton was isolated from the needles of *Taxus canadensis*. A biogenesis from taxinine an abundant taxane is proposed. This is the first example of a taxane with a 6/6/8/6-membered ring skeleton.

Taxus canadensis is a low trailing shrub ubiquitous to the Quebec region and its composition has been shown to be very different from other species.[1–13] Paclitaxel is found in its needles[1,2] in about the same yields as in other yew species. Its abundant taxane: 9-dihydro-13-acetylbaccatin III (5–7 times the amount of paclitaxel, depending on the collection site)[1,2] is specific to this variety and has only been found as *traces* in the bark of *T. chinensis*.[14] Taxinine and taxinine E are also isolated in high yield (~0.03% yield) from dry needles of *T. canadensis*.[6] We have now isolated from a methanolic extract of the needles of the Canadian yew a metabolite with an unprecedented skeleton which could derive from taxinine. In this communication, we determined its tetracyclic unusual structure and propose a putative biogenesis from taxinine.

The methanolic extract of the needles collected in Quebec was partitioned between hexane and water.[12,13] The aqueous layer was further extracted with dichloromethane. The dichloromethane soluble portion was purified by silica gel column chromatography followed by preparative thin layer chromatography and reversed-phase HPLC to afford a new metabolite (**1**, Fig. 1) with an unprecedented fused tetracyclic skeleton.[15]

The molecular composition of **1**, $C_{33}H_{40}O_9$, was established from combined analysis of high-resolution FABMS and ^{13}C NMR spectrum. The NMR spectral data of **1** is shown in Table 1. The ^1H NMR spectrum exhibited the three-proton signals due to the four methyl groups at δ 0.95, 1.43, 1.09, and 1.14. The extraction of this metabolite from *T. canadensis* and the four methyl groups might suggest it to be a taxane analog. Two acetyl groups were observed at relatively lower field (δ 2.17 and 2.04) as confirmed by ^{13}C NMR signals at δ 20.6, 172.2 and 21.0, 170.3. The proton signals due to the cinnamoyl group were observed at δ 7.59 (m, 2H), 7.40 (m, 3H), and 6.37 (d, $J=16.1$ Hz, 1H) and 7.68 (d, $J=16.1$ Hz, 1H, *trans*-oriented). This was further confirmed by UV absorption at 278 nm which we used in the HPLC analysis. A prominent peak at m/z 147 in the mass spectrum indicated the loss of a cinnamoyl group from **1**.

The connectivities of the protons of **1** were determined by analysis of the ^1H–^1H COSY spectrum. Interpretation of ^1H, ^{13}C NMR and HMBC spectra permitted the positional assignment of functional groups. In the ^1H NMR spectrum, **1** showed four protons attached to oxygenated carbons (two acetates, one cinnamate and one free hydroxyl group). A pair of signals resonating at δ 5.76 and 3.59 with a large coupling constant ($J=9.5$ Hz) was attributed to H-9 and H-10, with an

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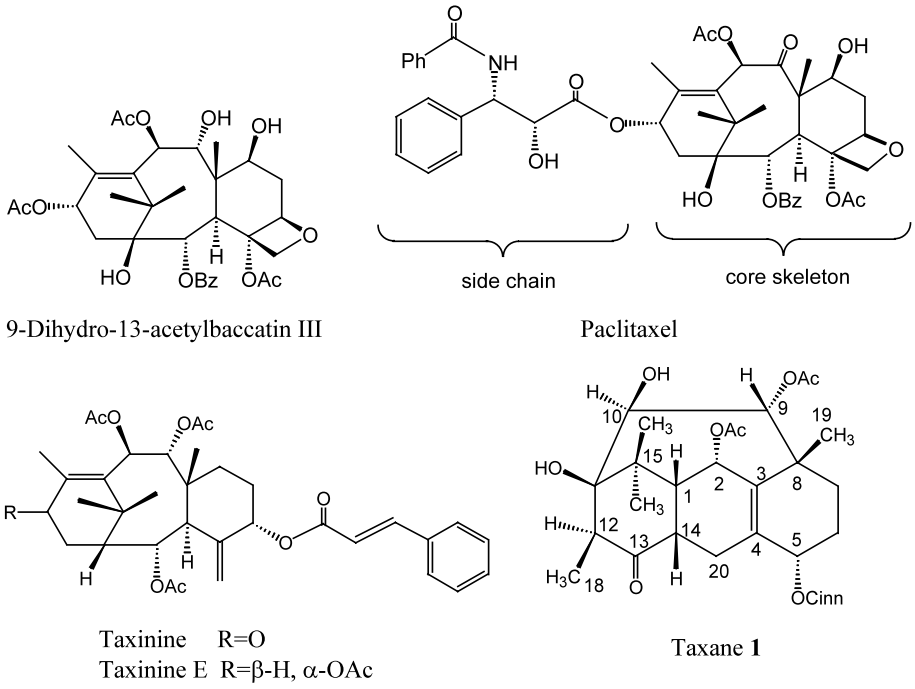

Figure 1. Some metabolites in the needles of *Taxus canadensis*.

acetyl and a hydroxyl group attached to C-9 and C-10, respectively. The 18-methyl resonating as a doublet at δ 1.09 indicated that the C-11,12 double bond was saturated. Carbon-11 resonated at δ 80.5 implying that a hydroxyl group was attached to C-11. An unconjugated ketone group, which resonated downfield at δ 215.1, was assigned to C-13 indicating that the C-11,12 double bond was saturated. The signal at δ 3.02, which correlated to C-13 and C-15 in the HMBC spectra, was assigned to H-14. Using H-14 as the starting point, the signals of H-1 and H-2 were confirmed by the ^{1}H–^{1}H COSY spectrum. The chemical shift of H-2 δ 6.00 (s) suggested an acetyl group attached to C-2. This was verified by the HMBC spectrum. The proton at C-3, which usually resonates around δ 2.5–3.8 as a doublet in the spectra of most taxanes was absent.[1–13] Instead, in the olefinic region **1** showed the presence of one tetra-substituted olefin (δ 139.0 and δ 139.9) as confirmed by the ^{13}C NMR and HMQC spectra. These observations indicated that a double bond was present between C-3 and C-4 as was confirmed by the HMBC correlations, as well as an unusual downfield chemical shift and split pattern of H-2 (δ 6.00, s).

The presence of another AB system at δ 2.89 (dd, J=15.8, 6.6 Hz, 1H) and 2.12 (dd, J=15.5, 1.3 Hz, 1H) was attributed to an isolated methylene of H-20. The unusually upfield chemical shift of H-20, together with their correlations to C-1, C-13 and C-14 in the HMBC spectrum, indicated that C-20 was connected to C-14 and formed a new 6-membered ring. The signal resonating at δ 5.30, which showed a weak cross-peak with H-20 in ^{1}H–^{1}H COSY spectrum and a cross-peak with the signal at δ 67.3 in the HMQC spectrum, was assigned to H-5. A cinnamoyloxy group was attached to C-5 as indicated by the HMBC spectrum. Accordingly, H-6 and H-7 were easily interpreted using H-5 as a starting point by ^{1}H–^{1}H COSY spectrum. Thus, the structure of **1** was rigorously characterized as 2α,9α-diacetoxy-5α-cinnamoyloxy-10β,11β-dihydroxy-14,20-cyclotaxa-3-ene-13-one.

The relative stereochemistry of **1** (Fig. 2) was elucidated from analysis of the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) data, chemical shifts and their coupling constants. The coupling constant between H-9 and H-10 (J=9.5 Hz) and observed NOESY correlations of H-2/H-9, H-2/H-17, H-9/H-17 established a chair–boat conformation for ring-**B**, which was the typical conformation of natural taxanes. The β-orientation of H-2 and H-9 were assigned by NOESY correlation of H-2/H-17, H-19/H-2, and H-9/H-17. The α-orientation of H-10 was applied by the observation of NOESY correlations of H-10/H-18 and H-10/H-12. We had no correlations for H-5, however, because we found this compound to be a taxane analog we postulate that H-5 has probably a β-orientation.

Compound **1** has a novel 6/6/8/6-membered ring system. The NOESY correlations show a cage-like backbone conformation similar to taxanes with 6/8/6 ring system even though it has an additional 6-membered ring formed between C-14 and C-20. A proposed mechanism for the formation of taxane **1** is shown in Fig. 3. We speculate that this metabolite might derive from taxinine which is the second most abundant taxane[6] after 9-dihydro-13-acetylbaccatin III[1,2] (Fig. 1) in *T. canadensis* needles. The enol-form of taxainine

Table 1. NMR data of **1** (500 MHz for ^{1}H, 125 MHz for ^{13}C, $CDCl_3$)

Position	δ (H) mult[a]	J (Hz)	δ (C)[b]	HMBC	NOESY[c]
1	2.41 (d)	8.9	51.9	2, 3, 13, 15, 20	2^{m}, 14^{s}, 16^{m}, 17^{m}
2	6.00 (s)		65.7	1, 3, 4, 8, 14, 15, 170.3	1^{m}, 9^{s}, 17^{s}, 19^{w}
3	–		**139.9**		
4	–		**139.0**		
5	5.30 (br.m)		67.3	4, 6/7, **167.1**	$6a^{w}$, $6b^{w}$, $20b^{w}$
6a	2.04 (o.m)		23.9		
6b	1.93 (m)				5^{s}, $6a^{s}$, 19^{m}
7	1.72 (m)		**24.0**	8, 19	$6a^{s}$, 10^{s}, 12^{s}, 19^{w}
8	–		**40.3**		
9	5.76 (d)	9.5	81.1	7, 8, 10, 19, **172.2**	2^{s}, 17^{s}, 10^{w}, 19^{s}, OH-10^{w}
10	3.59 (dd)	9.5, 7.0	78.5	9, 12, 15	7^{s}, 9^{w}, 12^{s}, 18^{s}, OH-10^{w}
OH-10	2.72 (br.d)	7.0			9^{w}, 10^{w}, 16^{w}, 17^{w}
11	–		**80.5**		
OH-11				12	16^{w}, 17^{w}, 18^{w}
12	2.77 (q)	6.8	51.0	10, 11, 13, 18	7^{m}, 9^{w}, 10^{s}, 18^{m}
13	–		**215.1**		
14	3.02 (m)		45.0	13, 15	1^{s}, 16^{m}, $20b^{s}$
15	–		**43.1**		
16	0.95 (s)		28.3	1, 11, 15, Me	1^{s}, 14^{s}, 17^{s}, OH-11^{w}
17	1.43 (s)		22.9	1, 11, 15, Me	1^{m}, 2^{s}, 9^{s}, 16^{s}, OH-10^{w}, OH-11^{w}
18	1.09 (d)	6.8	8.6	11, 12, 13	12^{s}, 9^{w}, 10^{w}, 16^{w}, OH-10^{s}, OH-11^{w}, $2'^{m}$
19	1.14 (s)		22.3	3, 7, 8, 9	2^{s}, $6a^{w}$, 7^{w}, 9^{s}, OH-10^{w}, OH-11^{w}, $2'^{w}$
20a	2.89 (dd)	15.8, 6.6	29.8	1, 14	14^{w}, $20b^{s}$
20b	2.12 (dd)	15.5, 1.3		1, 4, 13, 14	5^{m}, 14^{m}, $20a^{s}$
OAc	2.17 (s)		20.6	**172.2 (9)**	
	2.04 (s)		21.0	**170.3 (2)**	
1′	–		**167.1**		
2′	6.37 (d)	16.1	116.9	1′, Ph-C1	18^{w}, $3'^{s}$
3′	7.68 (d)	16.1	145.5	1′, 2′, Ph-o	
Ph′			**134.3**		
o	7.59 (m)		127.8		
m, p	7.40 (m)		128.6		
			130.2		

[a] Mutiplicity: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublets of doublets of doublets; m, mutiplet; o, overlapped.
[b] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold character represent quaternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm).
[c] NOESY intensities are marked as strong (s), medium (m), or weak (w).

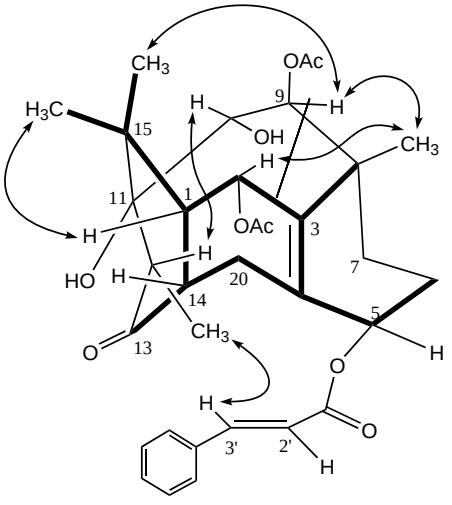

Figure 2. Relative stereochemistry of **1**, the arrows show selected key NOEs.

is shown as A in Fig. 3. Elimination of the allylic hydrogen at carbon-3 will lead to a carbanion which could easily attack the diene in ring A at C-14 (due to the U-cage conformation of taxanes), with the addition of the entity OH^{+} obtained by an oxygenase to give the enol form B in keto-enol equilibrium with C. The only step remaining is deacetylation of the acetyl at C-10 to give the tetracyclic metabolite **1**.

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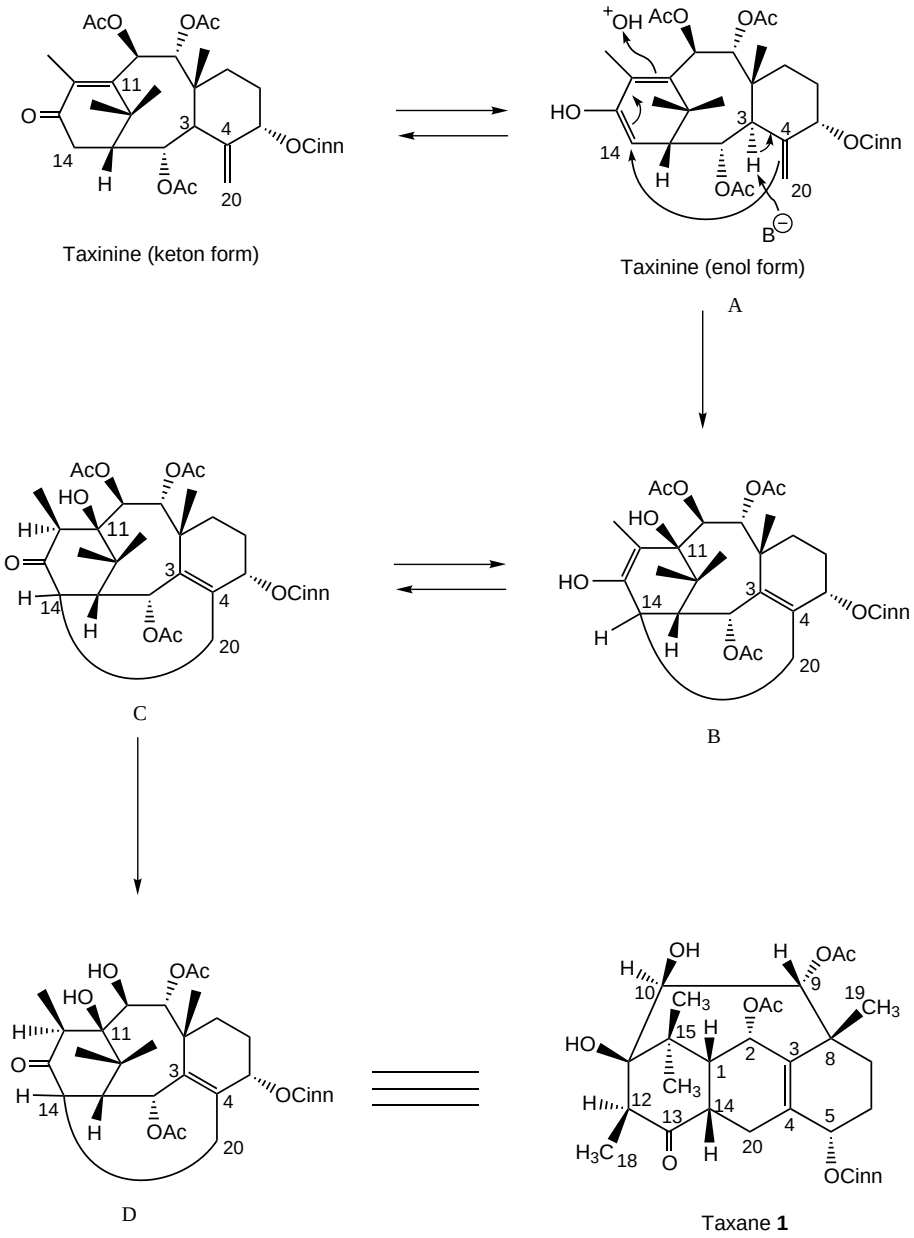

Figure 3. Proposed mechanism for the formation of **1**.

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15. General procedure for isolation and purification of metabolite **1**: air-dried needles of *T. canadensis* were ground (4.0 kg) and immersed in 24 L of methanol for 1 day at room temperature. The ground plants were filtered and extracted again with fresh solvent for another three times (each time with 8 L solvent, total 24 L) for 3 days. The combined organic extracts were evaporated under reduced pressure. Water (3 L) was added and lipids were removed by stirring the mixture with hexane (3×3 L). The aqueous phase was then saturated with NaCl (200 g) and extracted with CH_2Cl_2 (4×3 L). The combined CH_2Cl_2 extracts were dried with anhydrous sodium sulfate, filtered and concentrated below 45°C under reduced pressure to yield a viscous dark greenish extract (115 g). A portion of the methylene chloride extract (50 g) was absorbed onto 110 g silica gel and purified by flash chromatography (silica gel 230–400 mesh, 1320 g). Successive elution with a CH_2Cl_2–MeOH gradient with increasing amounts of MeOH from 5 to 45% (total 15 L) yielded 45 fractions ($Fr_{D\text{-}1}$ to $Fr_{D\text{-}45}$). The $Fr_{D\text{-}18}$ to $Fr_{D\text{-}24}$ were combined (3.5 g) in acetone and was absorbed on 7.5 g silica gel then applied to a column chromatography (silica gel 230–400 mesh, 195 g, 4.2×32 cm). Successive elution with hexane–EtOAc (6:5 and 1:1, each 1000 mL) yielded 22 fractions ($Fr_{D\text{-}18\text{-}1}$ to $Fr_{D\text{-}18\text{-}22}$). The $Fr_{D\text{-}18\text{-}12}$ (850 mg) was re-chromatographed on a small column (hexane:EtOAc = 3:2, 1800 ml) and obtained eight fractions. The first fraction was further purified by preparative TLC (CH_2Cl_2:CH_3CN = 8:2) followed by preparative HPLC and eluted with a linear gradient of acetonitrile in water from 25 to 100% for 50 min (flow rate of 18 ml per min) to yield taxane **1** (2 mg, $t_R = 39.03$ min). $[\alpha]_D^{22}$ −7 (*c* 0.06, $CHCl_3$). HRFABMS *m*/*z* 619.23093 (calcd for $C_{33}H_{40}O_9K$, 619.23094).