Isolation of a Putative Biogenetic Taxane Precursor from *Taxus Canadensis* Needles

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A novel *Taxus* metabolite having a pre-taxane structure in which rings *B*, *C* and *D* are not yet formed is isolated for the first time.

The unusual diterpene structure of taxol, its scarcity, its unique mode of action and its enormous therapeutic potential has triggered an intensive research effort from the international community. Very little is presently known[1,2] about the biosynthesis of the taxol core structure consisting of four rings (six-membered *A* and *C*, eight membered *B*, and four-membered *D*). There has been much speculation about the biosynthesis of the core ring structure of taxol[2] involving verticillol or verticillene type intermediates (Fig. 1). Verticillol is a natural product isolated from the Japanese umbrella pine[3] (*Sciadopitys verticillata*) which does not produce taxol or other taxanes. Verticillol which has never been reported in *Taxus* spp. has a core ring structure similar to that of taxol with rings *B*, *C* and *D* not yet formed. Verticillol has only one hydroxy group at C-12, and unlike taxol has no oxygenated substituents at C-2,5,7,9,10,13. In addition, in verticillol the hydrogen atom on C-1 is on the α face, whereas in taxol and all the known taxanes it is on the β face. Verticillene is a synthetic hydrocarbon[4] which was constructed with the taxane stereochemistry at C-1 (β) and the double bond at C-11, C-12. Similarly to verticillol, verticillene has no oxygenated substituents at C-2,5,7,9,10,13. The presumed cyclized form of verticillene, taxadiene (Fig. 1) has been generally assumed to be the hydrocarbon precursor to the diterpenoid backbone of taxol.[2] The implications were that oxygenations occur after cyclization. Numerous attempts of *in vitro* cyclization of verticillene, verticillol or their epoxides have failed.[5]

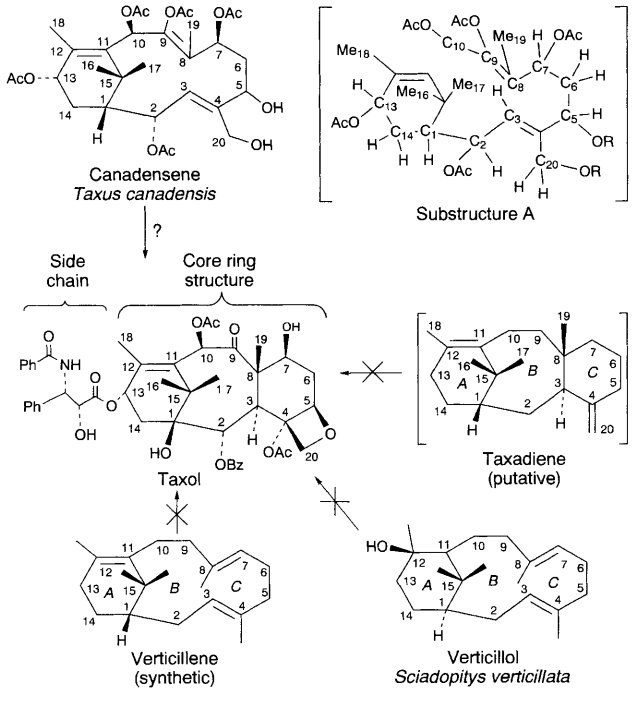

Fig. 1 Taxol and plausible precursors of its core ring structure. Verticillene is synthetic whereas verticillol and canadensene are natural products. Taxadiene is a hypothetical intermediate. Compounds in brackets have not been isolated. The dotted areas emphasize the possible derivations of the core ring structure of taxol.

Taxus canadensis seems to differ from other yews by its composition. Indeed, it is the only yew which accumulates 9-dihydro-13-acetyl-baccatin III[6,7] in its needles. In this publication, a novel natural product structurally related to verticillol was isolated from *Taxus canadensis*. This 4-hydroxy-methyl-8,12,15,15-tetramethyl-2,7,9,10,13-pentaacetoxy-bicyclo[9,3,1]pentadeca-5-hydroxy-3,8,11-triene is herein given the trivial name of canadensene (Fig. 1). The discovery of canadensene in *Taxus canadensis* and its structural similarity with the core ring of taxol (a co-metabolite) has considerable interest for biosynthetic studies.

The structure characterization of canadensene was performed using spectroscopic techniques (high resolution 2D NMR experiments, HMQC,[8] HMBC,[9] COSY,[10] NOESY[11] and low and high resolution MS). The NMR data are shown in Table 1. The ^{13}C NMR assignments are derived from HMQC (protonated carbons) and HMBC (quaternary carbons **4**, **8**, **9**, **11**, **12**, **15** in bold in Table 1). The results of the HMBC experiments (which correlate protons to more distant carbons through their $^{2}J_{C-H}$ and $^{3}J_{C-H}$ scalar couplings) were essential for the characterization of canadensene.

Initially in the analysis, substructure A was obtained (Fig. 1) and there was only one position where the carbon bearing the most deshielded proton (δ 7.31) might be connected: this is between C-11 and C-9. The HMBC experiment indeed shows correlation of that proton (H-10) to both olefinic systems (C-8, C-9, C-11 and C-12) and also correlation to an acetyl carbonyl carbon, allowing us to position the fourth acetyl group at that position. According to the proton spectra, there are five acetyl groups: four of them are attached to protonated carbons as seen in the HMBC experiment (C-2, C-7, C-10 and C-13). The fifth one does not show up in the HMBC experiment, allowing us to postulate that it is located on a quaternary carbon. The C-9 carbon is a quaternary olefinic carbon, which is acetylated as shown by its chemical shift.

The NMR information could not distinguish between the structure shown for canadensene in Fig. 1 and the elimination of water between C-20 and C-5 leading to an oxetane. The high resolution MS proved conclusively that the structure of canadensene is the one shown in Fig. 1. The following masses

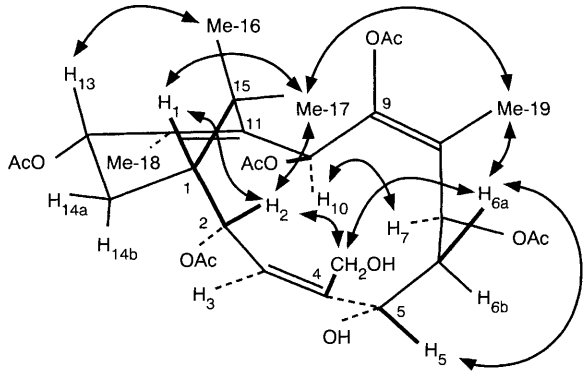

Fig. 2 NOESY analysis of canadensene

Table 1 NMR analysis of canadensene

Position	δ ^{1}H (mult)	*J*/Hz	δ ^{13}C	HMBC	NOESY
1	1.76 (br, dd)	6.4, 4.9	48.04	C2, C3, **C11**, C13, **C15**, Me16	H2(s), H14a(s), Me16(s), Me17(s)
2	5.822 (dd)	11.7, 4.9	70.96	C1, C3, **C4**, C14, **171.77**	H1(s), Me17(vs), H20a(vs) (or H5(vs))
3	6.143 (dd)	11.7, 2.0	122.98	C1, **C4**, C5, C20	H7(s), H5/20a(s), H14b(s)
4	—		**142.19**		
5	4.548 (o.m)	2.0	68.03	C3, **C4**, C5	
6a	2.791 (ddd)	12.7, 9.7, 2.9	38.34	C5/7, **C8**	H6b(vs), H5(s), Me19(s), H20b(s)
6b	1.99 (o.m)	—		**C4**, C5/7, **C8**	H6a(vs)
7	5.302 (d)	8.8	68.61	C5, C6, **C8**, **C9**, Me19, **170.35**	H10(s), H6b(s)
8	—		**125.53**		
9	—		**144.69**		
10	7.313 (br, s)		70.38	**C8**, **C9**, **C11**, **C12**, **C15**, **169.68**	Me18(vs), H7(s)
11	—		**137.19**		
12	—		**136.36**		
13	5.286 (d)	8.3	70.67	C1, **C11**, **C12**, C14, Me18, **172.60**	H10(s), 14a(s), 6b(s), Me16(w)
14a	2.546 (ddd)	17.1, 9.8, 7.3	26.29	C1, C2/13, **C12**	14b(vs), H1(s), H13(s), Me16(s),
14b	2.12 (o.m)	—			H14a(vs)
15	—		**36.80**		
16	1.130 (s)		33.94	C1, **C12**, **C15**, Me16	H1(s), H14a(s), Me17(s), H13(m)
17	1.257 (s)		25.41	C1, **C12**, **C15**, Me17	H2(vs), Me16(vs), Me19(s), H20b(m), H7/20a(m)
18	2.054 (s)		17.48	**C11**, **C12**, C13	H10(vs), H13/7(vs)
19	1.607 (d)	1.0	12.48	C7, **C8**, **C9**	H6a(s), H20b(m)
20a	4.543 (d)	12.7	58.62		
20b	3.666 (d)	12.7			
Ac	2.200 (s)		20.12	**170.10**	
Ac	2.131 (s)		21.00	**172.18**	
Ac	1.996 (s)		20.71	**171.35**	
Ac	1.971 (s)		21.00	**171.35**	
Ac	1.923 (s)		20.71	**169.68**	

were measured at R = 15 000 by peak matching against glycerol cluster ions with a Fisons ZAB-2F mass spectrometer: 595.27567 (MH^+, $C_{30}H_{43}O_{12}$ requires 595.27545), 577.26492 ($MH^+ - H_2O$, $C_{30}H_{41}O_{11}$ requires 577.26489), 535.25435 (MH^+ – AcOH, $C_{28}H_{39}O_{10}$ requires 535.25432), 475.23317 (MH^+ – 2AcOH, $C_{26}H_{35}O_8$ requires 475.23319).

The last step in establishing the structure is to determine the relative orientation of the various groups and determine the geometry around the C-3, C-4 and C-8, C-9 double bonds. There are many possible configurations for the large ring since it is not rigid. The most plausible stereochemical possibility for canadensene according to the NOESY experiments is shown in Fig. 2.

The discovery of canadensene implies that either the hypothetical hydrocarbon taxadiene is not a precursor or an alternative pathway exists for the biosynthesis of taxol.

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