Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 793–800

BIOORGANIC & MEDICINAL CHEMISTRY

Biotransformation of a 4(20),11(12)-Taxadiene Derivative

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Received 21 September 2000; accepted 3 November 2000

Abstract—A 4(20),11(12)-taxadiene derivative was converted to hydroxylated derivatives by *Cunninghamella elegans* AS3.2033 and *Cunninghamella elegans* var *chibaensis* ATCC 20230. Both microorganisms led to C-1 hydroxylations and conversion to a C-15-hydroxylated *abeo*-taxane. Additional products from the two fungi differed: a C-14 oxidation and a *trans–cis* isomerization of the cinnamoyl for one and an unprecedented hydroxylation at C-17 for the other.

Introduction

Filamentous fungi are known to carry out regio- and stereoselective hydroxylations of a wide range of organic compounds.[1–16] Unusual structures can be obtained which would not be accessible by chemical reactions. The main disadvantages of this route is that the products are not predictable and except for a few examples the yields are low. Despite our greater knowledge of chemical reactions, it seems that taxoids also do not follow expected routes.[17,18] One of the few high yield biotransformations reported involved *Absidia coerula* and 5α, 7β, 9α, 10β, 13α-pentaacetoxy-4(20),11-taxadiene, which was hydroxylated at C-1 and C-14 with yields of 39% and 26%, respectively.[4] These reactions were also obtained by *Cunninghamella echinulata, Cunninghamella elegans*, *Cunninghamella blakesleana and Rhizopus arrhizus* but less efficiently. In addition, these authors found that the taxoids were not metabolized by more than 25 collected fungal cultures if they contained a C-5 cinnamoyl group such as 2-deacetoxytaxinine J, taxinine J, taxinine, *O*-cinnamoyl-taxicin I triacetate.[5–7] Microbial hydroxylation at C-6 was shown to require a C-5-acetyl group.[5] We wanted to verify if this specificity of the microorganism would not change when other functional groups were altered. One possibility was to introduce multiple OH groups as plausible binding sites. The filamentous fungi, *C. elegans* AS 2033, *C. elegans* var chibaensis, (ATCC 20230) Kuwabara and Hoshino (IMI 199846), had been successful in other hydroxylations.

In this publication, we have found that with different varieties of the fungus *C. elegans,* we could obtain C-1 hydroxylation in high yield (44%) as well as a rearranged *abeo*-taxane (16%) on a taxoid with C-5-cinnamoyl present but no acetyls on C-7, C-9 and C-10. A C-14 hydroxylation as well as unprecedented isomerization of the cinnamoyl group and C-17 oxygenation were also observed.

Results and Discussion

Recently, C-1 and C-14 hydroxylations of taxoids were obtained from microbial transformations, particularly with *A. coerula*.[4] The structures required for biotransformation necessitated a C-5-acetate, and C-5-cinnamoylated taxanes were not metabolized. We wanted to investigate the role of C-5 cinnamoyl on these reactions and were surprised to find that the removal of the acetyls on C-7, C-9 and C-10 were essential for the catalyzed hydroxylations by the fungal species used in this study when the C-5 cinnamoyl was present.

Initially, the 4(20),11(12)-taxadiene derivatives possessing the 5-cinnamoyl group (taxinine J, 2-deacetoxytaxinine J; Fig. 1) were not metabolized by *C. elegans*

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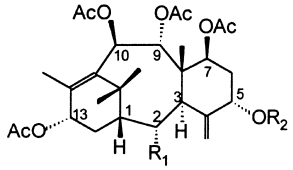
0968-0896/01/$ - see front matter
PII: S0968-0896(00)00299-6



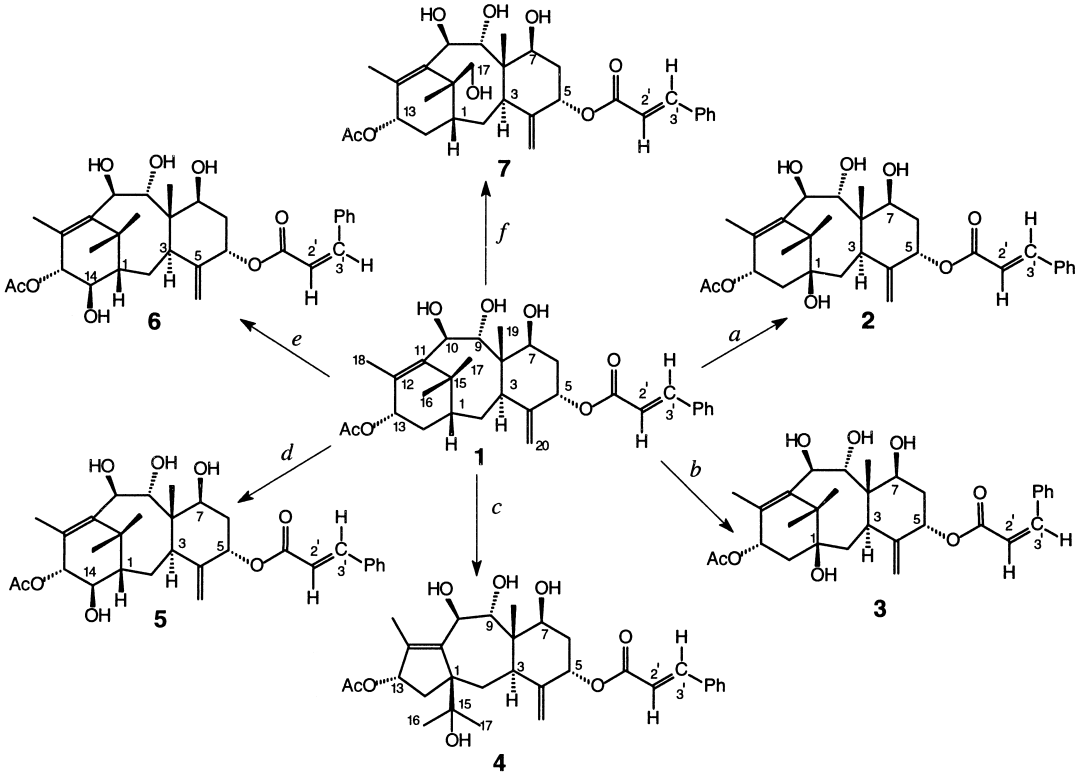
R_1=OAc	R_2=cinnamoyl (*trans*)	Taxinine J
R_1=H	R_2=cinnnamoyl (*trans*)	2-Deacetoxytaxine J
R_1=H	R_2=H	2-Deacetoxydecinnamoyltaxinine J
R_1=H	R_2=Ac	Taxa-4(20),11-diene-5α,7β,9α,10β,13α-pentaacetate

Figure 1. Some derivatives of taxinine J.

AS3.2033 and *C. elegans* ATCC 20230. However, when 2-deacetyldecinnamoyltaxinine J (Fig. 1) was also unchanged after incubation with the fungi, we decided to deacetylate C-7, C-9 and C-10 of 2-deacetoxytaxinine J which is readily available from *Taxus* species.[19] The derived 4(20),11(12)-taxadiene derivative (**1**; Scheme 1) gave hydroxylated products as well as a rearranged *abeo*-taxane upon incubation with *C. elegans* AS3. 2033 and *C. elegans* ATCC 20230. The characterization of these new taxoids is described in the following sections.

C-1β-Hydroxylation of the 4(20),11(12)-taxadiene derivative 1 (taxanes 2 and 3; Scheme 1, Table 1)

The same major taxoid was obtained from biotransformation with *C. elegans* AS3. 2033 and ATCC 20230, albeit in different yields (44% for the AS3. 2033 and 20% for ATCC 20230). HPLC and TLC data showed that this metabolite was more polar than the starting compound **1**, suggesting that perhaps **1** had been hydroxylated. Comparison of the ^{1}H NMR of **1** (see Experimental) and **2** (Table 1) shows some major differences in H-1 and H-14, as well as some detectable variations in H-2, H-3, H-13 and H-16, that is, all the protons around C-1. In taxane **2**, the H-1 signal (at δ 1.80 ppm) has disappeared, H-14a δ 2.55 ppm has a different pattern as it is now coupled to only two protons (dd, J=9.8; 15.0 Hz) instead of three protons (ddd, δ= 2.71 ppm, J=4.9; 9.8, 14.6 Hz) for taxane **1**. In addition, the chemical shift of H-14b is 1.53 ppm instead of 0.99 ppm for **1**. The most convincing evidence for the oxygenation at C-1 is the additional quaternary carbon at 75.9 ppm obtained from HMBC data, where H-2ab, H-14ab, H-16 and H-17 correlate to this carbon. This value is in accordance with C-1: 76.2 ppm for the C-1 β-OH for the biotransformation product of taxa-4(20),11-diene-5α, 7β,9α,10β,13α-pentaacetate (Fig. 1).[4] The structure of taxane **2** is therefore 1β-hydroxy-2-deacetoxytax-



Scheme 1. Microbial transformation products and yields of taxane **1** by fungi *C. elegans* AS3.2033 (A) and ATCC 20230 (B): (a) yields of taxane **2** by A: 44%, by B: 20%; (b) yield of taxane **3** by A: 2%, none by B; (c) yields of taxane **4** by A: 16%, by B: 6%; (d) yield of taxane **5** by A: 1%, none by B; (e) yield of taxane **6** by A: 1%, none by B; taxanes **5** and **6** were difficult to obtain in a pure state; (f) yield of taxane **7** by B: 6%, none by A.

inine J. High resolution mass spectrometry confirmed the elemental composition of the potassium quasimolecular ion of taxane **2**.

A minor metabolite with very similar spectral properties as taxane **2** was isolated only from the incubation of **1** with *C. elegans* AS3.2033. The only major differences in the ^{1}H NMR are in the positions H-2′ and H-3′ with their coupling constant ($J = 12.6$ Hz) typical of the *cis* isomer. In addition, H-3′ of taxane **3** is quite shielded compared to that of taxane **2**. The phenyl-*ortho* protons as well as H-7, H-5, H-20a, H-13, H-3 and Me-18 are also affected. The only explanation for these results is that in this metabolite the cinnamoyl is in the *cis* configuration. The influence on H-13 is not unusual since it is known that the core skeleton of taxanes is in U shape[20] and the C-5-cinnamoyl can mimic some of the C-13 side chain of paclitaxel.[21] High-resolution mass spectrometry confirmed the elemental composition of the potassium quasimolecular ion of taxane **3**. This is the first microbial *trans–cis* isomerization of a cinnamoyl group reported.

Rerrangement to an *abeo*-taxane (4; Scheme 1, Table 2)

The second highest yield compound obtained by *C. elegans* AS3 2033 (16%) and ATCC 20230 (6%) showed the presence of an *abeo*-taxane skeleton with four C-methyls (1.03, 1.15, 1.32, and 1.93) and one acetyl (1.49). The 11(15→1)-*abeo*-taxane structure was confirmed by the newly introduced carbon at 61.9 ppm and the carbon at 77.1 ppm which are attributed to C-1 and C-15, respectively. Additional HMBC correlations of the protons of Me-16/17 (1.15 and 1.32) with C-1 (61.9), C-15 (77.1), and Me-16/17 (27.5 and 25.0) support this structure. The positionning of the other groups are very similar to the starting material **1,** that is, an acetyl group on C-13, a *trans*-cinnamoyl group on C-5 and hydroxyls on C-7, C-9 and C-10. The relative stereochemistry of taxane **4** was established using the information contained in the NOESY experiment. High-resolution mass spectrometry confirmed the elemental composition of the potassium quasimolecular ion of taxane **4**.

C-14β-Hydroxylation of the 4(20),11(12)-taxadiene derivative 1 (taxanes 5 and 6; Scheme 1, Table 3)

Two minor metabolites were obtained on incubation of taxane **1** with *C. elegans* AS3.2033. The ^{1}H NMR of both of these compounds showed that the resonances corresponding to H-14a and H-14b had disappeared, while an oxygen-bearing methine signal appeared at 3.52 ppm. Confirmation was obtained by the resonance at 78.3 ppm in their ^{13}C NMR (Table 3). The stereochemistry of the 14-hydroxyl group was determined to be β- by the NOESY experiment. Indeed, H-14 correlates

Table 1. ^{1}H and ^{13}C NMR for taxane **2** in $CDCl_3$

Position	δ ^{1}H mult.[a] (*J* in Hz)	δ^{13}C[b]	HMBC	NOESY[c]
1		75.9		
2a	1.86 m	37.0	1, 3, 15	9^w
2b	1.80 m			
3	2.85 d (*4.9*)	39.9		7^s, 14b^m
4	—	146.5		
5	5.57 br s	75.2	165.9, 146.5	6a^s, 6b^s, 20a^s
6a/18	2.07 om	36.7		See 18
6b	1.76 om			
7	4.35 dd (*5.6, 11.4*)	71.2	Me-19	3^s, 6a^s, 6b^w, 10^m
8	—	45.6		
9	4.30 d (*10.2*)	79.8	Me-19, 8, 10/7	2a^m, 17^s, 19^m
10	5.02 od	71.3		7^m, 18^m
11	—	138.8		
12	—	135.8		
13	5.95 brt	71.1		14a^s, 16^s
14a	2.55 dd (*9.8, 15.0*)	41.7	1, 2, 12, 13	13^m, 14b^s
14b	1.53 odd (*6.4, 15.0*)			14a^w
15	—	43.7		
16	1.24 s	27.4	1, 11, 15, Me-17	13^w, 14a^w, 17^w
17	1.54 s	21.6	1, 11, 15, Me-16	2b^w, 9^w, 16^w
18/6a	2.08 s	15.6	11, 12, 13	3^w, 5^w, 6b^s, 7^w, 10^s
19	1.04 s	12.7	3, 7, 8, 9	2a^w, 6b^w, 9^w
20a	5.37 s	115.4	3, 5	5^s, 20b^s
20b	5.03 s			2b^s, 7^w, 9^w, 20a^s
OAc	1.74 s	20.7	170.8	
OCO	—	165.9		
2′	6.50 d (*16.0*)	118.1	165.9, 134.1	
3′	7.75 d (*16.0*)	145.6	165.9, Ph-*o*, 134.1	
Ph	—	134.1		
o	7.47 m	127.9		
m	7.40 m	130.4		
p	7.40 m	128.8		

[a]Mult, multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HMQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

Table 2. ^{1}H and ^{13}C NMR for taxane **4** in $CDCl_3$

Position	δ ^{1}H mult.[a] (*J* in Hz)	δ^{13}C[b]	HMBC	NOESY[c]
1	—	61.9		
2a/18	1.93 om	28.5	1, 3, 8	See 18
2b	1.36 d (*14.2*)		1, 3, 8	$2a^{s}$, 3^{w}, 17^{w}, $20b^{s}$
3	2.57 d (*8.2*)	38.4	8	7^{s}, $14b^{s}$, 10^{w}, $2a^{w}$, $2b^{w}$
4	—	145.6		
5	5.52 dd (*2.1, 3.0*)	74.6		$6a^{s}$, $6b^{s}$, $20a^{s}$
6a	2.13 ddd (*1.9, 4.6, 14.6*)	36.2		5^{s}, 7^{s}, $6b^{s}$
6b	1.82 ddd (*3.9, 11.4, 14.6*)			5^{s}, $6a^{s}$, 7^{w}, 19^{s}
7	4.29 dd (*4.9, 11.1*)	71.0	19	3^{w}, $6a^{s}$, $6b^{s}$, 10^{w}
8	—	44.0		
9	4.19 d (*9.8*)	81.7	7, 8, 10, 19	$2a^{s}$, 19^{w}
10	4.61 d (*9.8*)	69.2	1, 9, 11, 12	3^{w}, 7^{w}, 18^{w}
11	—	140.4		
12	—	142.2		
13	5.43 t (*7.0*)	79.7		16^{w}, $14a^{s}$, 18^{w}
14a	2.37 dd (*6.9, 13.8*)	44.2		13^{s}, $14b^{s}$, 17^{w}
14b	1.09 dd (*7.5, 13.8*)			$2b^{w}$, 3^{w}, 13^{s}, $14a^{s}$
15	—	77.1	11, 12, 15	
16	1.15 s	27.5	1, 15, Me-17	13^{s}, $14a^{s}$, 17^{w}
17	1.32 s	25.0	1, 15, Me-16	$14a^{w}$, 16^{w}
18/2a	1.93 s	11.2	11, 12, 13	$2b^{s}$, 3^{w}, 9^{s}, 10^{s}, 13^{s}, 19^{s}
19	1.03 s	12.4	3, 7, 8, 9	$2a^{m}$, $6b^{m}$, 9^{m}
20a	5.29 s	113.4	3, 5, 4	5^{m}, $20b^{s}$
20b	4.84 s		3, 5	$2b^{s}$, $20a^{s}$
OAc	1.49 s	20.4	171.0	
CO	—	165.5		
2	6.36 d (*16.0*)	118.4	C1(Ph), 165.5	
3	7.63 d (*16.0*)	144.6	C1 (Ph), CO	
Ph	—	134.4		
o	7.49 m	127.8		
m	7.38 om	128.9		
p	7.38 om	130.3		

[a]Mult, multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HMQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

with H-3, H-2a and H-1. It is interesting to note that the major difference in the NMR data of taxanes **5** (Table 3) and **6** (Experimental) shows isomerization of the double bond of the cinnamoyl group. In taxane **5** it is *trans,* whereas in taxane **6** it is *cis*. Indeed, H-3′ in **6** is quite shielded compared to taxane **5** (6.94 ppm instead of 7.76 ppm). The core skeleton in both taxanes have the same NOE correlations therefore the only difference is in the configuration of the cinnamoyl group. We had observed the same isomerization between taxanes **2** and **3**. High-resolution mass spectrometry confirmed the elemental composition of the potassium quasimolecular ion of taxanes **5** and **6**.

C-17-Hydroxylation of the 4(5),11(12)-taxadiene derivative 1 (taxane 7, Scheme 1, Table 4)

An unprecedented hydroxylation was observed in a reasonable yield (6%) on incubation of taxane **1** with *C. elegans* ATCC 20230. In taxane **7**, the original four methyl singlets in taxane **1** have been replaced by three methyl singlets and two doublet resonances at 4.60 ppm (11.8 Hz, 1 proton) and 3.26 ppm (11.8 Hz, 1 proton), suggesting the insertion of an oxygen on one of the methyls. This was confirmed by the appearance of a resonance at 70.5 ppm in the ^{13}C NMR of taxane **7**. The HMBC experiment showed a correlation between the proton at 3.26 ppm and carbons 16 and 11. This rules out methyls-18 and -19 since it would involve more than 3 bond scalar couplings. The hydroxylation could therefore be on Me-16 or Me-17. The NOESY experiment unambiguously determined that the hydroxylation occurred at Me-17. Indeed, H-13 has a correlation with Me-16 and not Me-17. Proton H-17a has a strong NOE with H-2 and H-9 and proton H-17b with H-1. High resolution mass spectrometry confirmed the elemental composition of the potassium quasimolecular ion of taxane **7**.

Conclusion

The good yields obtained in some of these reactions demonstrate the potential use of *C. elegans* in the biotransformation of taxane derivatives. Further work will be necessary to establish the rules underlying the fungus substrate selectivity. The metabolites which can be obtained are not accessible by conventional synthetic methods. It may be predicted from these findings that further exploration of this relatively new field may lead to useful insights into the fungal biotransformation pathways and, possibly, to a means of controlled biosynthetic formation of desired metabolites. The next step on this way, will involve isolating and purifying the enzymes responsible for these hydroxylations.

Table 3. ^{1}H and ^{13}C NMR for taxane **5** in $CDCl_3$

Position	δ ^{1}H mult.[a] (*J* in Hz)	δ^{13}C[b]	HMBC	NOESY[c]
1	1.76 om	50.1		
2a	1.70–1.81 m	26.2		
2b	1.96 om			
3	2.68 d (*5.6*)	37.4		7^{s}, 14^{s}, 18^{m}
4	—			
5	5.54 t (*2.7*)	75.3		$20a^{s}$
6a	2.07 om	36.9		
6b	1.80 om			
7	4.34 dd (*4.5, 11.5*)	71.4		3^{s}, $6a^{s}$, $10/20a^{s}$, 18^{s}
8	—	45.7		
9	4.31 d (*9.9*)	80.2		17^{s}, 19^{m}
10	5.03 om	71.3		$7/9^{m}$, 18^{s}
11	—	141.9		
12	—	131.9		
13	5.65 brm	81.4		
14	3.52 d (*4.8*)	78.3	1, 13, 15	3^{s}, $2a^{m}$ (1.96), 1
15	—	39.6		
16	1.26 s	31.6	1, 11, 15, Me-17	13^{s}, 17^{s}
17	1.58 s	27.0	1, 11, 15, Me-16	$2b/1$ $(1.8)^{s}$, 9^{s}, 16^{s}
18	2.11 s	15.5	11, 12, 13	3^{s}, 5^{w}, $6b^{s}$, 7^{s}, 10^{s}, 13^{w}
19	1.03 s	12.5	3, 7, 8, 9	$2a/1^{s}$, 9^{s}, 16^{w}
20a	5.35 s	115.5		5^{s}, $20b^{s}$
20b	5.00 s			2^{s}, $20a^{s}$
OAc	1.81 s	20.9	173.3	
C=O	—	165.9		
2′	6.48 d (*16.0*)	118.3	C1-Ph	Ph-o^{s}, 18^{w}
3′	7.76 d (*16.0*)	145.6	*o*-Ph	
Ph	—			
o	7.47 m	127.8		
m	7.40 m	130.5		
p	7.40 m	129.1		

[a]Mult, multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HMQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

Experimental

Instrumentation

Flash chromatography was performed on Silica gel 60 (230–400 mesh EM Science). Thin layer chromatography was conducted on Silica Gel 60 F254 pre-coated TLC plates (0.25 mm, EM Science). The compounds were visualized on TLC plates with 10% sulfuric acid in ethanol and heating on a hot plate. Na_2SO_4 was the drying agent used in all work up procedures. Analytical HPLC was performed on a Waters 600 FHU delivery system coupled to a PDA 996 detector. Preparative and semi-preparative HPLC were carried out on a Waters Delta Prep 3000 instrument coupled to a UV 486 Tunable Absorbance detector set at 227 nm (Waters, Montreal, Quebec, Canada). Analytical HPLC was performed with two Whatman partisil 10 ODS-2 analytical columns (4.6×250 mm) in series. Semi-preparative HPLC was performed with two Whatman partisil 10 ODS-2 Mag-9 semi-preparative columns (9.4×250 mm) in series. Preparative HPLC was performed with one partisil 10 ODS-2 MAG-20 preparative column (22×500 mm). The products were eluted with a 50 min linear gradient of acetonitrile (25–100%) in water at a flow rate of 18 mL/min (preparative HPLC) and 3 mL/min (semi-preparative HPLC). All the reagents and solvents were of the best available commercial quality and were used without further purification.

NMR and mass spectrometry measurement

All the NMR data were obtained at room temperature on a Bruker Avance-500 spectrometer operating at 500.13 MHz for proton and at 125.77 MHz for carbon-13. The solvent was used as an internal reference (7.25 ppm for proton and 77.0 ppm for carbon-13). The various 2-D spectra were acquired and processed using standard procedures. For phase sensitive 2-D experiments (NOESY and HMQC), the data were acquired using the TPPI phase mode. The NOESY experiment was obtained using a mixing time of 0.3 s and a relaxation delay of 1 s. The intensity of the cross-peaks in the NOESY experiment is designated as strong (s), medium (m) and weak (w). Positive ion fast atom bombardment Mass Spectra (FABMS) were obtained with a Vacuum Generators ZAB-HS double-focussing instrument using a xenon beam having 8 kV energy at 1 mA equivalent neutral current. Low resolution mass spectra were obtained in glycerol. Samples were dissolved in 0.2 μL DMSO before addition of 0.5 μL glycerol. FABHRMS was similarly obtained in glycerol-DMSO at a resolving power of 12,000.

Substrate: 2-deacetoxy-7,9,10-trideacetyltaxinine J, 1. 2-Deacetoxytaxinine J is abundant in various *Taxus* species.[19] It was deacetylated at positions 7, 9, and 10 to give the substrate 2-deacetoxy-7,9,10-trideacetyl-taxinine J, **1**, following the procedure of Sako et al.[22] To a solution of

Table 4. ^{1}H and ^{13}C NMR for taxane **7** in $CDCl_3$

Position	δ ^{1}H mult.[a] (*J* in Hz)	$\delta^{13}C$[b]	HMBC	NOESY[c]
1	1.86 br	37.0		
2a	1.70 dd (*4.0, 15.2*)	26.7	3, 8	3^{m}, Me-17^{m}, Me-19^{m}, $20b^{m}$
2b	1.63 dd (*4.9, 15.2*)			3^{m}, Me-17^{m}, Me-19^{m}, $20b^{m}$
3	2.91 br d (*4.2*)	36.2		7^{s}, $14b^{m}$, 18^{m}
4	—			
5	5.55 t (*2.4*)	75.3		$6a^{s}$, $6b^{s}$, $20a^{s}$
6a	2.10 ddd (2.2, 4.1, 14.1)	36.8		
6b	1.78 ddd (3.5, 11.7, 14.7)			
7	4.40 dd (*11.4, 4.8*)	71.1	Me-19	3^{s}, $6a^{m}$, $6b^{w}$, 18^{m}
8	—	45.6		
9	4.20 br d (*9.7*)	81.3		Me-17^{s}, Me-19^{w}
10	4.97 d (*10.3*)	71.3		7^{w}, $6b^{w}$, Me-18^{s}, $20a^{s}$
11	—	137.0		
12	—	136.5		
13	5.82 t (*8.4*)	70.5		$14a^{s}$, 18^{m}, 16^{s}
14a	2.74 dt (*14.8, 9.7*)	31.9	2, 12, 13	
14b	0.95 dd (*14.8, 7.2*)		1, 13, 15	3^{s}, $14a^{s}$
15	—	45.4		
16	1.34 s	26.6	1, 11, 15, Me-17	1^{m}, 13^{s}, $14a^{w}$, 17b(3.2)^{m}
17a	4.60 d (*11.8*)	70.5		2^{s}, 9^{s}, $17b^{s}$
17b	3.26 d (*11.8*)		16, 11	1^{s}, 16^{m}, $17a^{s}$
18	2.14 s	15.8	11, 12, 13	
19	1.04 s	12.8	3, 7, 8, 9	1w, 2w
20a	5.35 s	115.3		5^{s}, $20b^{s}$
20b	4.93 s		3, 5	2^{w}, $20a^{s}$
OAc	1.72 s	20.8	170.6	
C=O	—	166.1		
2	6.51 d (*16.0*)	118.2	C1-Ph, C=O	
3	7.75 d (*16.0*)	145.5	C1- Ph, *o*-P, C=O	
Ph	—	134.5		
o	7.48 m	128.0		
m	7.41 om	129.0		
p	7.41 om	130.5		

[a]Mult, multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.
[b]The ^{13}C chemical shifts were extracted from the HMQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).
[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

2-deacetoxytaxinine J (1000 mg, 1.54 mmol) in CH_2Cl_2/MeOH 1/1 (180 mL) was added a solution of $Ba(OH)_2 \cdot 8H_2O$ (486 mg, 1.54 mmol) in MeOH (20 mL). The reaction mixture was stirred at 4 °C for 14 h and diluted with 200 mL H_2O and extracted with CH_2Cl_2 (200 mL×3). The CH_2Cl_2 fraction was washed with brine, dried, filtered and evaporated in vacuo. The residue was purified by flash chromatography on a silica gel column (30 g, CH_2Cl_2/MeOH, 100/0 to 100/2) affording 190 mg 2-deacetoxy-7,9,10-trideacetyltaxinine J, **1**, and 660 mg recovered 2-deacetoxytaxinine J. Yield: 69% (based on recovered starting material). FABHRMS of **1**: $C_{31}H_{40}O_7K$ $[M+K]^+$ required 563.2411, found 563.2412; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, *J* = 16 Hz, 1H, H-3), 7.47 (m, 2H, H-Ph-o), 7.39 (m, 3H, H-Ph-m, p), 6.51 (d, *J* = 16 Hz, 1H, H-2′), 5.79 (br. t, *J* = 8.4 Hz, 1H, H-13), 5.55 (br.s, 1H, H-5), 5.33 (s, 1H, H-20a), 5.02 (d, *J* = 10.1 Hz, 1H, H-10), 4.96 (s, 1H, H-20b), 4.38 (dd, *J* = 11.1, 4.1 Hz, 1H, H-7), 4.30 (dd, *J* = 10.1, 4.5 Hz, 1H, H-9), 2.91 (br s, 1H, H-3), 2.70 (m, 1H, H-14b), 2.09 (s, 1H, Me-18), 1.72 (s, 13-Ac), 1.53 (s, Me-16), 1.16 (s, Me-17), 1.03 (s, Me-19), 0.99 (dd, *J* = 14.7, 6.9 Hz, 1H, H-14a); $^{13}CNMR$ (125 MHz, $CDCl_3$) δ 170.8 (OCOMe-13), 166.1(-OCO-), 146.9 (C-4), 145.4 (=C-3′), 139.2 (C-11), 134.2 (C-12), 134.2 (Ph-C_1), 130.6 (Ph-*p*), 129.0 (Ph-*m*), 128.0 (Ph-*o*), 118.5 (=C-2′), 115.2 (C-20), 75.5 (C-5), 71.6 (C-10), 71.5 (C-7), 70.8 (C-13), 45.4 (C-8), 40.2 (C-1), 39.6 (C-15), 36.9 (C-6), 36.5 (C-3), 32.1 (C-14), 31.7 (C-16), 27.4 (C-2), 27.0 (C-17), 21.0 (OCOMe-13), 15.6 (C-18), 12.9 (C-19).

Microorganisms and biotransformation procedure

C. elegans AS3.2033 and *C. elegans* var *chibaensis* ATCC 20230 were purchased from the Institute of Microbiology, Chinese Academy of Science, P. R. China and American Type Culture Collection (ATCC), respectively. Cultures were grown in Potato Dextrose (24 g/L, DIFCO laboratories) broth, and was incubated at 25 °C and 125 rpm, unless otherwise indicated. *C. elegans* AS3.2033 was first preserved on silica gel and kept at 4 °C to prevent mutation.[23] The seed culture was prepared by the addition of several grains of silica gel that absorbed the fungus to 30 mL of medium in a 125 mL Erlermeyer flask. The culture was incubated for 3 days. To 1 L of production medium in a 2 L flask was added 5 mL of the seed culture previously homogenized with a Polytron homogenizer. The culture was incubated for 3 days after which time it was inoculated with the substrate 2-deacetoxy-7,9,10-trideacetyltaxinine J **1** (50 mg in 1 mL DMSO). The culture was further incubated for 12 days, then homogenized and extracted with CH_2Cl_2 (500 mL×3) affording 276 mg extract. The exact same procedure and amounts were used in the incubation of this

substrate **1** with *C. elegans* var chibaensis ATCC 20230. The final CH_2Cl_2 extract obtained weighed 160 mg. Cultures with the same medium and substrate but without fungi were used as controls in the same experimental conditions.

Isolation and purification of the taxanes obtained from incubation of 2-deacetoxy-7,9,10-trideacetyl-taxinine J, 1, with *C. elegans* AS3.2033

The extract residue (276 mg) was applied on a silica gel flash chromatography column (19 g), eluted with CH_2Cl_2 (150 mL), CH_2Cl_2/MeOH (100/1, 100/2, 100/4, 100/10, 80/20 each 100 mL) and eleven 60 mL fractions were collected. Fractions 7 and 8 (40 mg) containing taxanes were combined, evaporated and further purified by preparative HPLC. Two pure compounds were isolated: taxane **2** ($t_R = 26.5$ min, 22 mg, 44%) and **4** ($t_R = 33.2$ min, 8 mg, 16%). A mixture at $t_R = 28.5$ min was collected and separated by preparative TLC (EtOAc/hexane, 7/1) to give pure taxane **3** ($R_f = 0.5$, 1 mg, 2%), and a mixture of taxanes **5** and **6** ($R_f = 0.4$, 1.0 mg, 2.0%) which was difficult to obtain in a pure state.

Isolation and purification of the taxanes obtained from incubation of 2-deacetoxy-7,9,10-trideacetyl-taxinine J, 1, with *C. elegans* var *chibaensis* ATCC 20230

The extract residue (160 mg) was applied on a silica gel flash chromatography column (10 g), eluted with CH_2Cl_2 (100 mL), CH_2Cl_2/MeOH (100/1, 100/2, 100/4, 80/20 each 100 mL) and seven 60 mL fractions were collected. Fractions 4–6 (35 mg) containing taxanes were combined, evaporated and further purified by preparative HPLC. Pure taxane **2** was isolated as a white powder ($t_R = 27.1$ min, 10 mg, 20%). A mixture at $t_R = 33.6$ min was collected and further separated by preparative TLC (EtOAc/hexane, 7:1) to afford taxanes **4** ($R_f = 0.35$, 3 mg, 6%), and **7** ($R_f = 0.45$, 3 mg, 6%).

1β-Hydroxy-2-deacetoxy-7,9,10-trideacetyl-taxinine J, 2. (White powder) FABHRMS: $C_{31}H_{40}O_8K$ $[M+K]^+$ required 579.2360, found 579.2361. For ^1H and ^{13}C NMR, HMBC, and NOESY spectral data see Table 1.

1β-Hydroxy-2-deacetoxy-7,9,10-trideacetyl-5-*cis*-cinnamoyl-taxinine J, 3. (Colorless gum) FABHRMS: $C_{31}H_{40}O_8K$ $[M+K]^+$ required 579.2360, found 579.2361. The NMR data is very similar to taxane **2**. We will therefore report only the differences (H-3′, H-2′, Ph-*o*, H-3, H-5, H-7, H-13, Me-18 and H-20a). ^1H NMR (500 MHz, $CDCl_3$) δ 7.65 (∼d, Ph-*o*), 7.47 (m, Ph-*o*, 2H), 6.94 (d, $J = 12.7$ Hz, 1H, H-3′), 6.08 (d, $J = 12.7$ Hz, 1H, H-2′), 6.05 (o.t, 1H, H-13), 5.46 (br. s, 1H, H-5), 5.30 (s, 1H, H-20a), 5.01 (o.s, 1H, H-20b), 4.97 (o.d, 1H, H-10), 4.27 (d, $J = 10.4$ Hz, 1H, H-9), 4.23 (dd, $J = 4.4$, 11.3 Hz, 1H, H-7), 2.78 (d, $J = 4.8$ Hz, 1H, H-3).

2-Deacetoxy-7,9,10-trideacetyl-11(15→1) *abeo*-taxinine J, 4. (Colorless gum) FABHRMS: $C_{31}H_{40}O_8K$ $[M+K]^+$ required 579.2360, found 579.2361. For ^1H and ^{13}C NMR, HMBC and NOESY spectral data, see Table 2.

14β-Hydroxy-2-deacetoxy-7,9,10-trideacetyl-taxinine J, 5. (Colorless gum) FABHRMS: $C_{31}H_{40}O_8K$ $[M+K]^+$ required 579.2360, found 579.2361. For ^1H and ^{13}C NMR, HMBC and NOESY spectral data, see Table 3.

14β-Hydroxy-2-deacetoxy-7,9,10-trideacetyl-5-*cis*-cinnamoyl-taxinine J, 6. (Colorless gum) FABHRMS: $C_{31}H_{40}O_8K$ $[M+K]^+$ required 579.2360, found 579.2361. The NMR data is very similar to taxane **5.** However, we were not able to separate taxanes **5** and **6** the best ratio we could obtain was 70:30 (**5**:**6**). In addition, the HMQC for **6** was too dilute to get all the shifts. We will therefore report only the differences (H-3′; H-2′; Ph-*o*; H-3; H-5; H-7; H-13; Me-18). ^1H NMR (500 MHz, $CDCl_3$) δ 6.94 (d, $J = 12.7$ Hz, 1H, H-3′), 6.08 (d, $J = 12.7$ Hz, 1H, H-2), 7.65 (∼d, Ph-*o*), 2.78 (d, $J = 4.8$ Hz, 1H, H-3), 5.46 (br s, 1H, H-5), 4.23 (dd, $J = 4.4$, 11.3 Hz, 1H, H-7), 6.05 (o.t. 1H, H-13), 1.97 (s, 3H, Me-18).

2-Deacetoxy-7,9,10-trideacetyl-17-hydroxy-taxinine J 7. (colorless gum) FABHRMS: $C_{31}H_{40}O_8K$ $[M+K]^+$ required 579.2360, found 579.2361. For ^1H and ^{13}C NMR, HMBC and NOESY spectral data, see Table 4.

Acknowledgements

We thank the Natural Science and Engineering Research Council of Canada and the Canadian Breast Cancer Research Initiative-Idea grant for support via an operating grants to L. O. Z. We thank the INRS-Institut Armand-Frappier for a predoctoral fellowship to D. A. Sun. Professor Lihe Zhang is gratefully acknowledged for a gift of *Cunninghamella elegans* AS3.2033 and Dr. Li Zhichao ((Zhongling (Huizhou) High Science and Technology Co. Ltd. P. R. China)) for a generous supply gift of a sample of 2-deacetoxy-taxinine J.

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