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Microbial and Reducing Agents Catalyze the Rearrangement of Taxanes

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Abstract—5α, 7β, 9α, 10β, 13α-Pentahydroxy-4(20),11(12)-taxadiene derivative **1** was converted to two unprecedented 1(15→11)*abeo*-taxanes and a taxane derivative with a C10–C11 double bond by *Absidia coerula* ATCC 10738a. A similar compound was obtained from treatment with zinc of a triacetoxy-4(20),11(12)-taxadiene derivative. © 2001 Elsevier Science Ltd. All rights reserved.

The use of enzymes in organic synthesis has been studied extensively since the 1980's.[1–5] Impressive advances have been reported using enzymes, which are functional in organic, aqueous or biphasic systems.[6–8] Recently, filamentous fungi have been used for taxanes[9–24] and stereospecific hydroxylations were discovered, some of them in relatively high yields.[12] These authors reported the C-1 (39% yield) and C-14 hydroxylations (26% yield) of 5α, 7β, 9α, 10β, 13α-pentaacetoxy-4(20),11(12)-taxadiene by *Absidia coerula*. We have also recently reported[25] the biotransformation of a 4(20),11(12)-taxadiene derivative by different microorganisms. The products included an efficient C-1-hydroxylation, and conversion to a C-15-hydroxylated 11(15→1)*abeo*-taxane. In addition, reported for the first time were isomerizations (*trans*- to *cis*-cinnamoyl) and a C-17-hydroxylation, which were detected but in low yields. The main disadvantages of the use of microorganisms is that the specificity of the substrate and the products obtained cannot be predicted. In addition, the yields are usually very low.

In this publication, we are reporting the biotransformation of the 4(20),11(12)-taxadiene derivatives **1**–**4** (Schemes 1 and 2) with *A. coerula* ATCC 10738a. Taxane **1** was the only one biotransformed. The taxane product **5** (Scheme 2) obtained in 32% yield has a C-13 ketone and a C10–C11 double bond, resulting from oxidation–reduction steps. In addition, two unprecedented 1(15→11)*abeo*-taxanes **6** and **7** (Scheme 2) were obtained in 42 and 4% yields, respectively. A chemical reduction (zinc/acetic acid) of a related 4(20),11(12)-taxadiene derivative **3** (Schemes 1 and 3) with a ketone on C-13, gave a major product **8** which has a similar structure to the taxane **5** obtained by biotransformation.

Results and Discussion

We intended to study the specificity of substrates with different fungi. *A. coerula* was known to catalyze C-1 and C-14 hydroxylations of 5α, 7β, 9α, 10β, 13α-pentaacetoxy-4(20),11(12)-taxadiene.[12] We have indeed reproduced these results when we used this same substrate and *A. coerula* ATCC 10738a. We found that the partially acetylated substrates, 5α,13α-dihydroxy, 7β, 9α, 10β-triacetoxy-4(20), 11(12)-taxadiene **2**[26] and 5α-hydroxy, 7β, 9α, 10β-triacetoxy-4(20), 11(12)-taxadien-13-one **3**,[27] gave no reaction when incubated with *A. coerula* ATCC 10738a. Similarly, 5α, 7β, 9α, 10β-tetrahydroxy-4(20), 11(12)-taxadien-13-one **4** (Scheme 1) was unreacted after incubation with this fungi. On the other

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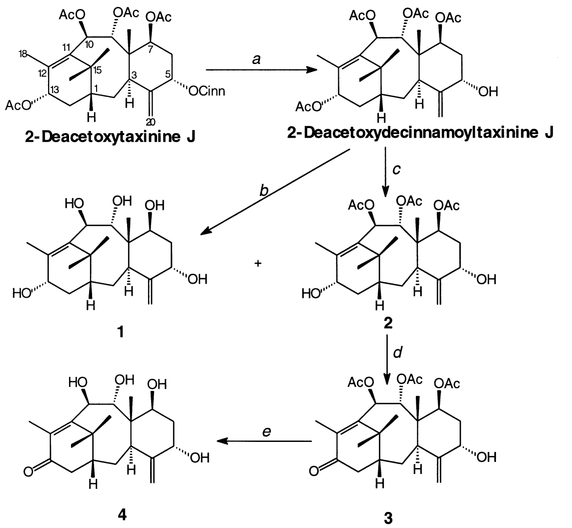
hand, the taxane derived from decinnamoylation and complete deacetylation of 2-deacetoxytaxinine J: 5α, 7β, 9α, 10β, 13α-pentahydroxy-4(20),11(12)-taxadiene **1**,[28] (Scheme 1) was a good substrate for *A. coerula* ATCC 10738a. Surprisingly, the expected C-1 and C-14 hydroxylations were not observed. Two major taxanes **5** (32% yield) and **6** (42% yield) were obtained as well as a minor product **7** (4%) (Scheme 2). The compound **5** was characterized as shown in Scheme 2 with a ketone at C-13 and a double bond at C10–C11. This is a new taxane, but taxoids with a C10–C11 double bond have previously been obtained by chemical reactions.[29] Taxanes **6** and **7** were found to be unprecedented 1(15→11)*abeo*-taxanes (Scheme 2).

An attempt to obtain a 12,13-isotaxane,[30] from the taxane derivative **1** by treatment with zinc/acetic acid failed and no taxanes similar to **5**–**7** were obtained. On the other hand, the triacetylated ketone **3** when treated with zinc/acetic acid gave a major compound **8** (23% yield) (Scheme 3). Its structure is similar to the biotransformation product **5**. The semi-syntheses of the substrates, characterization of the new taxoids (**5**–**8**) as well as plausible mechanisms for their formation are described in the following sections.

Semi-synthesis of 5α, 7β, 9α, 10β, 13α-pentahydroxy-4(20),11(12)-taxadiene, 1; 5α, 13α-dihydroxy-7β, 9α, 10β-triacetoxy-4(20),11(12)-taxadiene, 2; 5α-hydroxy,7β, 9α, 10β-triacetoxy-4(20),11(12)-taxadien-13-one, 3; and 5α, 7β, 9α, 10β-tetrahydroxy-4(20),11(12)-taxadien-13-one, 4 (Scheme 1)

The taxane derivatives (**1**–**4**) were semi-synthesized from 2-deacetoxytaxinine J.[31] The first step involved C-5-decinnamoylation according to a reported procedure.[32]

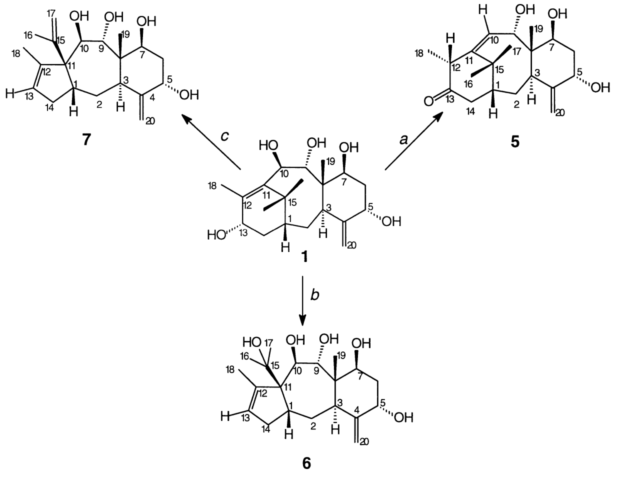


Scheme 1. Semi-syntheses of substrates 1–4. Reagents and conditions: (a) $NH_2OH.HCl/NaOAc$ in $EtOH/H_2O$, 80 °C, 24 h,[26,32] 90%; (b) LiOH, $MeOH/H_2O$, 1:1, 23 °C, 7 h, taxane **1** 78%, taxane **2** 7%; (c) LiOH, $MeOH/H_2O$, 1:1, 4 °C, 75 min taxane **2** not isolated but oxidized in situ according to (d); (d) CrO_3 in pyridine/CH_2Cl_2, 1:1, 23 °C, 2 h, taxane **3** 55%; (e) 1 N NaOH in MeOH, 23 °C, 10 h, taxane **4** 63%.

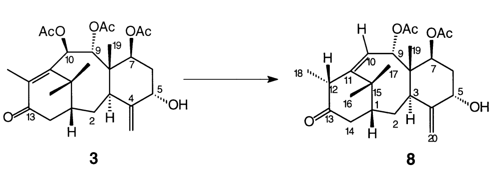
Upon treating the derived 2-deacetoxydecinnamoyltaxinine J[26] with excess $LiOH·H_2O$ for 7 h at room temperature, the major product involved complete deacetylation (**1**, 78% yield) and a minor compound with C-13-deacetylated (**2**, 7% yield). On the other hand, treatment of the same starting material with excess $LiOH·H_2O$ for 75 min at 4 °C gave a major compound **2** whose oxidation in situ yielded the substrate **3** with an overall yield of 55%. Compound **3** is not a new compound, it was previously isolated as a natural product.[27] The taxane derivative **4** was obtained by basic deacetylation of **3**.

5α, 7β, 9α-trihydroxy-4(20),10(11)-taxadien-13-one (5, Scheme 2, Table 1)

Comparison of the ^1H NMR of **5** (Table 1) to **1** (see Experimental) shows that ring C remains unchanged (Me-19, H-7, H-6a,b, H-5, H-20a,b). On the other hand, there are major differences in rings A and B, both in the ^1H and ^{13}C NMR. The first obvious finding in **5** is the ketone at C-13 (δ 211.3 ppm) and a characteristic CH_3–CH demonstrated by the H-12 quartet at δ 3.67 ppm ($J = 6.3$ Hz) coupled with the Me-18 doublet at δ 1.30 ppm ($J = 6.3$ Hz). This Me-18 is correlated in the HMBC to two quaternary carbons: the ketone C-13 and the olefinic carbon C-11. In the HMQC of **5**, we detected that the H-10 is attached to the olefinic carbon C-10. These observations proved that the original C11–C12 double bond has shifted to C10–C11 in **5**. As expected, H-10 is a doublet at δ 5.68 ppm ($J = 11.0$ Hz) coupled



Scheme 2. Microbial transformation products and yields of taxane **1** by fungi *A. coerula* ATCC 10738a: (a) yield of taxane **5**: 32%; (b) yield of taxane **6**: 42%; (c) yield of taxane **7**: 4%.



Scheme 3. Reduction of the αβ-unsaturated ketone **3**. Reagents and conditions: activated zinc and acetic acid 23 °C, 42 h, taxane **8** 23%.

with H-9 (δ 5.08 ppm, o.d. $J = 11.0$ Hz). The HMBC correlations in Table 1 for taxane **5** confirm these assignments. The NOESY data proves the stereochemistry shown in Scheme 2 for taxane **5**. In particular, the stereochemistry at the new stereocenter C-12 is demonstrated by the NOESY correlations: H-9 is β oriented because of its strong correlations with Me-19 and Me-17. In addition, a similarly strong correlation between H-12 and Me-16 proves that H-12 is also β oriented whereas Me-18 is α. High resolution mass spectrometry confirmed the elemental composition of the potassium quasimolecular ion of taxane **5**.

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

Position	δ ^{1}H mult.[a] J (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY[c]
1	2.12 o.m	44.1	11	
2a	1.88 ddd (*16.4, 5.9, 1.7*)	26.3	1, 3, 8, 15	$2b^{s}$, $9/20a^{w}$, 17^{w}, 19^{w}
2b	1.75 ddd(*16.4, 5.9, 2.0*)		1, 2, 8, 15	$2a^{s}$, $1/14b^{m}$, $20b^{s}$
3	2.22 br.d (*5.7*)	35.6	1, 2, 8	7^{s}, 10^{w}, $14b^{w}$
4	—	152.4		
5	4.29 t (*2.9*)	73.3		$6b^{w}$, $20a/2^{s}$
6a	2.03 ddd (*13.5, 5.0, 2.9*)	38.2		5^{w}, $6b^{s}$, 7^{w}
6b	1.54 ddd (*13.5, 11.4, 3.2*)			5^{s}, $6a^{s}$, 19^{s}
7	4.37 dd (11.4, 5.0)	68.1	6, 19	3^{s}, 10^{s}
8	—	51.3		
9	5.08 o.d (*11.0*)	77.6	7, 10, 11, 19	$2a^{m}$, 17^{s}, 19^{s}
10	5.68 d (*11.0*)	125.5	9, 11, 12, 15	3^{w}, 7^{s}, 18^{s}
11	—	144.2		
12	3.67 q (*6.3*)	52.1	10, 11, 13, 18	16^{s}, 18^{m}
13	—	211.3		
14a	2.77 dd (*18.5, 8.5*)	42.3	1, 2, 13	$14b^{s}$, 16^{w}
14b	2.09 o.d (*18.5*)		1, 2, 12, 13, 14, 15	$2b^{w}$, $14a^{s}$, 16^{s}
15	—	41.2		
16	1.40 s	30.5	1, 11, 15, 17	1^{w}, 12^{s}, $14a^{s}$, 17^{s}
17	1.60 s	26.5	1, 11, 15, 16	$1/14b^{w}$, $2a^{s}$, 9^{s}, 16^{s}, 19^{w}
18	1.30 d (*6.3*)	11.8	11, 12, 13	10^{s}, 12^{s}
19	0.98 s	11.3	3, 7, 8, 9	$2a^{w}$, $6b^{w}$, 9^{w}
20a	5.09 s	110.8	3, 8	5^{s}, $20b^{s}$
20b	4.74 d (*1.0*)		3, 4, 5, 8	$2b^{s}$, $20a^{s}$

[a] Mult. multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; 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 **6** in $CDCl_3$

Position	δ ^{1}H mult.[a] J (*J* in Hz)	COSY	δ ^{13}C[b]	HMBC	NOESY[c]
1	2.83 dt (*9.2, 4.9, 4.9*)	2a, 2b, 14a	39.3	11, 12, 15	$2a^{w}$, 9^{s}, $14a^{w}$, 16^{s}
2a	2.09 o.m	Overlap	32.1		—
2b	1.16 ddd (*14.2, 5.6, 1.8*)	1, 2a/3		3/14, 4, 8	$2a^{s}$, $14b^{s}$, $20b^{s}$
3	2.01 o.m	Overlap	37.9	2, 4, 8, 20	–
4	—		150.4		
5	4.27 t (*2.9*)	6a, 6b	73.3		$6b^{w}$, $20a^{w}$
6a	2.05 o.m	Overlap	37.4		—
6b	1.59 ddd (*14.0, 11.4, 3.0*)	5, 6a, 7		7	5^{m}, $6a^{s}$
7	4.32 dd (*11.4, 5.1*)	6a, 6b	68.3	6, 9, 19	10^{s}, $6a^{s}/3^{s}/18^{s}$
8	—		44.2		
9	4.04 d (*10.6*)	10	82.0	7, 10, 11, 19	1^{s}, 16^{m}, 19^{s}
10	4.81 d (*10.6*)	9	77.0	9, 11, 15	7^{s}, $6a^{s}/3^{s}/18^{s}$
11	—		60.4		
12	—		140.8		
13	5.49 d (*1.5*)	2a, 14b	126.7	2 (weak)	18^{m}
14a	2.28 dm	1, 13, 14b, 18	40.6	2, 11, 12	1^{s}, $14b^{s}$, 16^{w}
14b	1.73 br.dd (*15.4, 1.5*)	13, 14a, 18		12, 13	$2b^{s}$, 13^{m}, $14a^{s}$
15	—		89.6		
16	1.47		30.2	11, 15, 17	1^{s}, 9^{s}, $14a^{w}$
17	1.28		26.8	11, 15, 16	18^{w}
18	2.04		15.4	11, 12, 13	Overlap
19	0.94		11.8	3, 7, 8, 9	$2a^{w}$, $6b^{m}$, 9^{s}, $20b^{w}$
20a	5.00 s	3, 20b	110.7	4, 5, 6, 8	5^{s}, $20b^{s}$
20b	4.61 br.s	3, 20a		4, 5, 6, 8	$20a^{s}$, $2b^{m}$

[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 3. ^{1}H and ^{13}C NMR for taxane **7** in $CDCl_3$

Position	δ ^{1}H mult.[a] J (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY
1	2.81 o.m	44.1		
2a	1.67 o.m	23.6		Overlap
2b	1.56 dd (*15.0, 3.8*)		3, 4, 8, 11	1/3, 2a, 20b
3	2.83 o.m	34.1		
4	—	150.7		
5	4.33 o.m	73.3		20a
6a	2.11 o.m	38.4		
6b	1.71 o.m			
7	4.36 o.m	69.6		
8	—	43.7		
9	3.98 d (*7.5*)	76.9		17b, 19
10	4.18 d (*7.5*)	70.8		
11	—	64.8		
12	—	146.1		
13	5.54 br.s	126.2		18
14a	2.33 br.dd (*15.2, 10.2*)	34.9		14b
14b	2.14 br.dd (*15.2, 8.4*)			1/3, 14a, 18
15	—	144.0		
16	1.71 s	22.6	11, 15, 17	1/3, 17a
17a	5.15 s	114.9	11, 16	16, 17b
17b	5.05 s		15, 16	1, 9, 17a
18	1.75 br.s	16.8	11, 12, 13	13, 16
19	0.97 s	13.4	3, 7, 8, 9	2a, 9
20a	5.07 s	110.6	3, 5	5, 20b
20b	4.68 s		3, 4, 5	2b, 20a

[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).

Rearrangement to a major unprecedented 1(15→11)*abeo*-taxane (6, Scheme 2, Table 2)

The compound with the highest yield obtained from this biotransformation was taxane **6** (42%). Comparison of the ^{1}H NMR of **1** (see Experimental) and **6** (Table 2) shows that ring C in **6** is practically unchanged (Me-19, H-7, H-6a,b, H-5, H-20a,b). On the other hand, the ^{1}H and ^{13}C NMR of ring A and B of taxane **6** are different, in particular ring A. The signal for H-13 has disappeared from the HMQC spectra and a new olefinic proton appearing as a doublet at δ 5.49 ppm ($J = 1.5$ Hz) is connected to an olefinic carbon at δ 126.7 ppm. As this olefinic carbon is correlated with Me-18 in the HMBC experiment, and the olefinic proton shows COSY correlation to methylene proton H-14, the only position for this carbon is on C-13. At first, the structure of **6** seemed to be that of a usual 11(15→1)*abeo*-taxane with two newly introduced quaternary carbons at δ 60.4 ppm and δ 89.6 ppm. A close look at the HMBC experiment demonstrates that the *gem*-dimethyl group is attached to C-11 and not C-1. Indeed in the HMBC experiment, Me-18 shows correlations to the expected three carbons, C-11, C-12, and C-13. The assignment of the aliphatic type of quaternary carbon as C-11 is supported by several other correlated protons surrounding it within 2–3 bonds. The observation that the protons of Me-16/17 (δ 1.47 and 1.28 ppm) are correlated in the HMBC with C-11 (δ 60.4 ppm), C-15 (δ 89.6 ppm), and Me-16/17 (δ 30.2 and 26.8 ppm)

Table 4. ^{1}H and ^{13}C NMR for taxane **8** in $CDCl_3$

Position	δ ^{1}H mult.[a] J (*J* in Hz)	δ ^{13}C[b]	HMBC	NOESY[c]
1	2.12 o.m	43.8		
2a	1.97 o.m	25.8	1, 3, 8	
2b	1.76 o.m			
3	2.34 br.d (*5.4*)	35.8	1, 2, 8, 18	7^{s}, 10^{w}, $14b^{m}$
4	—	151.4		
5	4.27 t (*2.9*)	72.7	3, 20	$6a^{m}$, $6b^{m}$, $20a^{s}$
6a	1.89 o.m	36.2		
6b	1.59 o.m			
7	5.60 dd (*11.6, 5.2*)	67.6	6, 19, 169.6	3^{s}, $6a^{s}$, 10^{s}
8	—	51.1		
9	6.12 d (*11.3*)	77.2	7, 8, 10, 11, 19, 170.4	$2a^{m}$, 17^{s}, 19^{m}
10	5.72 d (*11.4*)	120.9	8, 9, 11	3^{w}, 7^{s}, 18^{s}
11	—	145.6		
12	3.64 q (*6.4*)	52.3	10, 11, 13, 18	16^{s}, 18^{m}
13	—	211.2		
14a	2.77 dd (*18.5, 8.8*)	42.2	1, 2, 13	$14b^{s}$, 16^{m}
14b	2.03 o.m		1, 2, 12, 15	
15	—	41.0		
16	1.37 s	30.5	1, 11, 15, 17	1^{m}, 12^{s}, $14a^{m}$, 17^{s}
17	1.71 s	26.0	1, 11, 15, 16	1^{s}, $2a^{s}$, 9^{s}, 16^{s}
18	1.32 d (*6.4*)	11.5	11, 12, 13	10^{s}, 12^{s}
19	0.87 s	11.8	3, 7, 8, 9	$2a^{m}$, $6b^{m}$, 9^{s}
20a	5.11 s	111.1	3, 5	5^{s}, $20b^{s}$
20b	4.77 s		3, 4, 5	$2b^{s}$, $20a^{s}$
OAc	2.03 s 2.01 s			

[a]Mult. multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; br, broad; m, multiplet; o, overlapping. The precision of the coupling constants is ±0.5 Hz.

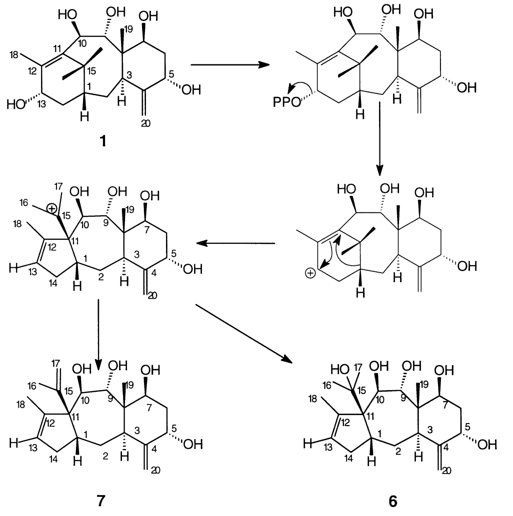
[b]The ^{13}C chemical shifts were extracted from the HMQC and HMBC experiments (for quaternary carbons) (±0.2 ppm).

[c]NOESY intensities are marked as strong (s), medium (m) or weak (w).

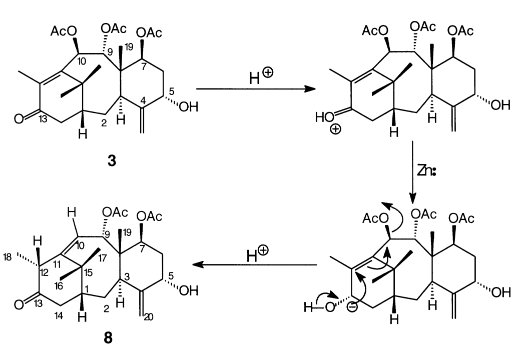
confirms that the structure **6** is an 1(15→11)*abeo*-taxane. Similarly, the proton H-1, which shows COSY correlations with H-2 and H-14, is correlated in HMBC with C-11 (δ 60.4 ppm), C-12 (δ 140.8 ppm) and C-15 (δ 89.6 ppm). These observations also confirm the structure assignment of **6**. The relative stereochemistry of taxane **6** was established using the information contained in the NOESY experiment shown in Table 2. High resolution mass spectrometry confirmed the elemental composition of the potassium quasimolecular ion of taxane **6**.

Rearrangement to a minor unprecedented 1(15→11)*abeo*-taxane (7, Scheme 2, Table 3)

A minor product (4% yield) was also isolated, purified and characterized. The significant differences in the ^{1}H NMR of **7** and **6** is the appearance of two extra olefinic singlets at δ 5.15 ppm and δ 5.05 ppm and only three methyl singlets (δ 1.71, 1.75, and 0.97 ppm) instead of four. The ^{13}C NMR also shows the appearance of two shielded olefinic carbons (δ 110.6 and 114.9 ppm), one for C-20 and the other one located at C-17. Similarly to **6**, we note the presence of the olefinic H-13/C-13 correlated to the Me-18 protons (δ 1.75 ppm) in the HMBC experiment. This methyl is further correlated to two other quaternary carbons, C-12 (δ 146.1 ppm) and C-11 (δ 64.8 ppm). The other deshielded methyl (δ 1.71 ppm) also has HMBC correlations with C-11 and two olefinic carbons C-15 and C-17 (δ 144.0 and 114.9 ppm, respectively). The H-17 protons, being correlated in the HMBC with C-11, C-15 and Me-16, further confirm that **7** has the structure shown in Scheme 2.



Scheme 4. Putative mechanism for the formation of the 1(15→11)*abeo*-taxanes **6** and **7**.



Scheme 5. Putative mechanism for the formation of taxane **8**.

5α-Hydroxy-7β, 9α-diacetoxy-4(20),10(11)-taxadien-13-one (8, Scheme 3, Table 4)

Comparison of the ^{1}H NMR of **8** and **5** (Table 1) shows the presence of two extra acetyl methyl groups (δ 2.03 and 2.01 ppm). All the protons in these two compounds are observed at similar shifts except for H-7 (δ 5.60 ppm) and H-9 (δ 6.12 ppm) that are significantly more deshielded than one can expect upon replacing the hydroxy group by acetoxy. The presence of a CH_3–CH group is deduced from the H-12 quartet at δ 3.64 ppm coupled to the Me-18 doublet at δ 1.32 ppm ($J = 6.4$ Hz). This methyl shows HMBC correlation to the ketone C-13 and to the olefinic quaternary carbon C-11. The H-10 olefinic doublet (δ 5.72 ppm), coupled to H-9 (δ 6.12 ppm), has an HMBC correlation to C-11 confirming the position of the shifted C10–C11 double bond. The position of each acetoxy group at C-7 and C-9 has been confirmed by HMBC, as the H-7 and H-9 protons show correlation to the carbonyl ester group. In the NOESY experiment, the β orientation of H-9 is confirmed by its interaction with Me-19 and Me-17. The α-stereochemistry of Me-18 in **8** is determined by the NOESY correlations of H-12 with Me-16 which are therefore both in the β-configuration. The correlation between H-3, H-7, H-10, and H-14b allow us to conclude that these protons have an α orientation.

Attempts to deacetylate **8** and convert it to **5** under different conditions [NaOH, MeOH; LiOH, MeOH; NaOMe, MeOH; $NH_2NH_2 \cdot H_2O$; $ZnBr_2$; $Ba(OH)_2 \cdot 8H_2O$] failed.

Putative mechanisms for the formation of taxanes 5–8 (Schemes 4 and 5)

One of the plausible mechanisms for the formation of these unusual 1(15→11)*abeo*-taxanes **6** and **7** is the following. The first step could be activation of the C13-OH by adding a good leaving group, such as pyrophosphate, etc. Upon leaving, it will generate a carbonium ion, which will cause the formation of the *abeo*-five-membered ring. The new carbonium ion is neutralized by addition of a hydroxyl group or formation of a double bond C15–C17 (Scheme 4). The reaction with zinc and acetic acid involves protonation of the carbonyl and donation of $2e^-$ from the zinc causing the shift of the double bond and elimination of the OAc anion (Scheme 5).

Conclusion

In this publication, we have shown that very similar taxanes with C10–C11 double bonds could be obtained from microbial reactions as well as from conditions with a reducing agent. This unexpected result is promising and indeed suggests that a reductase with a similar mechanism as the treatment with zinc might be one of the enzymes in *A. coerula* ATCC 10738a. Indeed, further research in these fields might lead to other similarities between chemical and microbial reactions, which could help us understand the mechanisms involved. This will eventually help us to understand the selectivity rules of these otherwise empirical reactions. The unprecedented 1(15→11)*abeo*-taxanes produced are presently under investigation in our laboratory to determine if addition of side chains might lead to bioactive taxanes.

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 unless otherwise noted. 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 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. Deuterated chloroform 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). Abbreviations regarding NMR data are: multiplicity (mult), singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublet (dd), broad (br), multiplet (m), overlapping (o). Positive ion Fast Atom Bombardment Mass Spectra (FAB-MS) 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. FAB-HR-MS was similarly obtained in glycerol-DMSO at a resolving power of 12,000.

Substrates: 5α, 7β, 9α, 10β, 13α-pentahydroxy-4(20),11(12)-taxadiene, 1; 5α, 13α-dihydroxy-7β, 9α, 10β-triacetoxy-4(20),11(12)-taxadiene, 2; 5α-hydroxy, 7β, 9α, 10β-triacetoxy-4(20),11(12)-taxadien-13-one, 3; 5α, 7β, 9α, 10β-tetrahydroxy-4(20),11(12)-taxadien-13-one, 4 (Scheme 1). 2-Deacetoxytaxinine *J* is abundant in various *Taxus* species.[31] Decinnamoylation of 2-deacetoxytaxinine J was accomplished on treatment with hydroxylamine as reported.[26,32] The derived 2-deacetoxydecinnamoyltaxinine J (244 mg, 0.47 mmol) in a solution of methanol/water (1:1, 5 mL) was treated with $LiOH.H_2O$ (160 mg; 3.80 mmol). After stirring at room temperature for 7 h, the reaction was worked up by dilution with ethyl acetate (40 mL) and washing with 10% $NaHCO_3$, water and brine. After drying, filtering and evaporating the solvent, the residue was purified by preparative HPLC. Two compounds were isolated: taxane 5α,7β,9α,10β,13α-pentahydroxy-4(20),11(12)-taxadiene **1** (128 mg, 78%) and 5α,13α-dihydroxy-7β,9α,10β-triacetoxy-4(20),11(12)-taxadiene **2** (16.5 mg, 7%).

1. $[\alpha]_D^{22}$ −199.2° (*c* 0.07, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.10 (s, 1H, H-20a), 4.99 (d, *J* = 9.5 Hz, 1H, H-10), 4.78 (s, 1H, H-20b), 4.40 (dd, *J* = 11.5, 5.0 Hz, 1H, H-7), 4.36 (br. d, *J* = 10.7 Hz, 1H, H-13), 4.32 (t, *J* = 2.5 Hz, 1H, H-5), 4.11 (d, *J* = 9.5 Hz, 1H, H-9), 3.09 (br.s, 1H, H-3), 2.81 (ddd, *J* = 15.5, 10.5, 8.7 Hz, 1H, H-14a), 2.07 (br.s, 3H, Me-18), 1.97 (br.ddd, *J* = 2.0, 4.0, 13.5 Hz, 1H, H-6a), 1.68-1.70 (o.m, 2H, H-2), 1.66 (o.m, 1H, H-1), 1.63 (o.m, 1H, H-6b), 1.44 (br.s, 3H, Me-17), 1.18 (dd, *J* = 15.5, 3.6 Hz, 1H, H-14b), 0.97 (br.s, 3H, Me-16), 0.96 (s, 3H, Me-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.5 (C-4), 138.9 (C-11), 138.8 (C-12), 111.7 (C-20), 80.5 (C-9), 73.7 (C-5), 72.6 (C-10), 70.7 (C-7), 68.4 (C-13), 45.8 (C-8), 39.6 (C-1), 39.6 (C-6), 38.7 (C-15), 34.9 (C-3), 32.9 (C-14), 32.9 (C-16), 26.7 (C-2), 25.9 (C-17), 17.0 (C-18), 12.1 (C-19); FAB-HR-MS for $C_{20}H_{32}O_5K$ $[M+K]^+$ requires: 391.1887; found: 391.1887.

2. $[\alpha]_D^{22}$ −16.4° (*c* 0.33, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.22 (d, *J* = 10.8 Hz, 1H, H-10), 5.81 (d, *J* = 10.8 Hz, 1H, H-9), 5.67 (dd, *J* = 11.4, 5.1 Hz, 1H, H-7), 5.16 (s, 1H, H-20a), 4.84 (d, *J* = 1.3 Hz, 1H, H-20b), 4.37 (o.m, 1H, H-5), 4.37 (o.m, 1H, H-13), 1.74 (o.m, 1H, H-1), 1.89 (o.m, 1H, H-2a), 1.79 (o.m, 1H, H-2b), 3.21 (d, *J* = 5.4 Hz, 1H, H-3), 2.84 (ddd, *J* = 15.3, 10.3, 8.4 Hz, 1H, H-14a), 2.32 (d, *J* = 1.1 Hz, 3H, Me-18), 2.07 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.95 (o.m, 1H, H-6a), 1.67 (o.m, 1H, H-6b), 1.53 (s, 1H, Me-17), 1.19 (o.m, 1H, H-14b), 0.92 (s, 3H, Me-16), 0.82 (s, 3H, Me-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.2 (C-4), 142.2 (C-12), 134.8 (C-11), 112.4 (C-20), 76.9 (C-9), 73.4 (C-5), 72.7 (C-10), 70.0 (C-7), 68.5 (C-13), 46.7 (C-8), 39.6 (C-1), 38.8 (C-15), 37.2 (C-6), 35.8 (C-14), 35.7 (C-3), 32.5 (C-16), 26.8 (C-2), 26.1 (C-17), 21.5 (Ac), 21.1(Ac), 20.9 (Ac), 16.7 (C-18), 12.5 (C-19); FAB-HR-MS for $C_{26}H_{38}O_8K$ $[M+K]^+$ requires: 517.2204; found: 517.2206.

The same starting material 2-deacetoxydecinnamoyltaxinine J (60 mg; 0.12 mmol) in a solution of methanol/water (1:1, 1.2 mL) was treated with $LiOH{\cdot}H_2O$ (36 mg; 0.86 mmol) at 4 °C for 75 min. The reaction mixture was quenched with brine and extracted with ethyl acetate. The organic phase was washed with brine, dried with $MgSO_4$, filtered and evaporated. To a solution of the residue in CH_2Cl_2 (1.8 mL) and pyridine (1.8 mL) was added CrO_3 (34 mg; 0.34 mmol). The reaction mixture was stirred at 23 °C for 2 h. The mixture was diluted with brine and extracted with CH_2Cl_2. The organic phase was washed with brine, dried with $MgSO_4$, filtered and evaporated. The residue was purified by preparative HPLC affording 5α-hydroxy,7β, 9α, 10β-triacetoxy-4(20),11(12)-taxadien-13-one: **3** (30 mg, 55% yield, $t_{R=}31.6$ min) and recovered starting material 2-deacetoxydecinnamoyltaxinine J (9 mg, 15% yield, $t_{R=}36.0$ min).

3. Specific rotation, ^1H and ^{13}C NMR (500 MHz, $CDCl_3$) as well as FABHRMS identical to literature.[27]

To a solution of 5α-hydroxy,7β, 9α, 10β-triacetoxy-4(20),11(12)-taxadien-13-one **3** (71 mg; 0.149 mmol) in methanol (14 mL) at 0 °C, was added 1 N NaOH (0.85 mL). The ice bath was removed and the solution was stirred at 23 °C for 10 h. The mixture was neutralized with 5% HCl and evaporated to remove the methanol. The aqueous residue was diluted in brine and extracted with ethyl acetate. The organic phase was dried ($MgSO_4$), filtered and evaporated to dryness. The residue was purified by preparative HPLC affording 5α, 7β, 9α, 10β-tetrahydroxy-4(20),11(12)-taxadien-13-one **4** (33 mg, 63%, $t_R=16.1$ min).

4. $[\alpha]_D^{22}$ +169.0° (*c 0*.10, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) 5.13 (o.s, 1H, H-20a), 5.11 (o.d, 1H, H-10), 4.83 (s, 1H, H-20b), 4.51 (dd, $J=11.1$, 4.8 Hz, 1H, H-7), 4.32 (t, $J=1.9$ Hz, 1H, H-5), 4.27 (d, $J=9.3$ Hz, 1H, H-9), 3.30 (br.d, $J=4.5$ Hz, 1H, H-3), 2.88 (dd, $J=19.4$, 7.4 Hz, 1H, H-14a), 2.12 (s, 3H, H-18), 2.04 (o.m, 1H, H-2a), 2.00 (o.d, $J=19.4$ Hz, 1H, H-14b), 1.71 (s, 3H, H-17), 1.25 (s, 3H, H-16), 1.11 (s, 3H, H-19); ^{13}C NMR ($CDCl_3$, HMQC data) 200.0 (C-13), 156.3 (C-4), 154.6 (C-11), 135.0 (C-12), 109.0 (C-20), 80.2 (C-9), 73.1 (C-5), 72.8 (C-10), 71.0 (C-7), 46.0 (C-8), 41.0 (C-1), 40.1 (C-15), 39.6 (C-14), 39.4 (C-16), 36.8 (C-6), 34.6 (C-3), 25.9 (C-2), 25.2 (C-17), 13.4 (C-18), 12.3 (C-19); FAB-HR-MS for $C_{20}H_{30}O_5Na$ $[M+Na]^+$ requires: 373.1991; found: 373.1992.

Microorganism and biotransformation procedure

A. coerulea ATCC 10738a was purchased from American Type Culture Collection. Cultures were grown in potato dextrose broth (24 g/L, DIFCO Laboratories) and incubated at 25 °C and 125 rpm. *A. coerulea* ATCC 10738a was first preserved on silica gel and kept at 4 °C to prevent mutation.[33] The seed culture was prepared by the addition of several grains of silica gel, which absorbed the fungus, to 30 mL of medium in a 125 mL Erlenmeyer flask. The culture was incubated for 3 days. To 1 L of potato dextrose broth in a 2 L flask was added 5 mL of the seed culture previously homogenized with a Polytron homogenizer. The culture was incubated for 1 day after which time it was inoculated with the substrate 5α, 7β, 9α, 10β, 13α-pentahydroxy-4(20),11(12)-taxadiene, **1** (40 mg in 1 mL DMSO). The culture was further incubated for 8 days, then homogenized and extracted with CH_2Cl_2 (400 mL×3) affording 91 mg extract. 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 5α, 7β, 9α, 10β, 13α-pentahydroxy-4(20),11(12)-taxadiene, 1 with *A. coerulea* ATCC 10738a. The above CH_2Cl_2 extract (91 mg) was first applied on a silica gel flash chromatography column (10 mL cylinder, 4 g), eluted with CH_2Cl_2/acetone (100:0, 20:1, 10:1: 5:1, 1:1, and 0:100 each 8 mL) and MeOH (10 mL) and fifteen 3 mL fractions were collected. Fractions 10–13 (37.9 mg) containing the taxanes were combined, evaporated and further purified by preparative HPLC to obtain the pure compounds: **5** (12 mg, 32%, $t_R=22.8$ min, colorless gum), **6** (17 mg, 42%, $t_R=29.1$ min, colorless gum), **7** (1.5 mg, 4%, $t_R=36.3$ min, colorless gum).

5α, 7β, 9α-Trihydroxy-4(20),10(11)-taxadien-13-one, 5 (Scheme 2, Table 1). $[\alpha]_D^{22}$ −148.8° (*c* 0.24, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1; FAB-HR-MS for $C_{20}H_{30}O_4K$ $[M+K]^+$ requires: 373.1781; found: 373.1783.

1(15→11)*abeo*-taxane 6 (Scheme 2, Table 2). $[\alpha]_D^{22}$ −58.5° (*c* 0.55, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; FAB-HR-MS for $C_{20}H_{30}O_4K$ $[M\text{-}H_2O+K]^+$ requires: 373.1781; found: 373.1783.

1(15→11)*abeo*-taxane 7 (Scheme 2, Table 3). $[\alpha]_D^{22}$ −24.0° (*c* 0.03, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) see Table 3; FAB-HR-MS: $C_{20}H_{30}O_4K$ $[M+K]^+$ required: 373.1781; found: 373.1783.

Reduction of 5α-hydroxy,7β, 9α, 10β-triacetoxy-4(20),11(12)-taxadien-13-one 3 with zinc/acetic acid. Taxane **3** (55 mg; 0.12 mmol) was dissolved in glacial acetic acid (0.65 mL) and treated with activated zinc (0.60 g; 9.18 mmol) at 23 °C for 42 h. The mixture was diluted with ethyl acetate and filtered through Celite. Heptane was added and the mixture was evaporated under reduced pressure. The residue was purified by semi-preparative HPLC affording a major product **8** (11 mg, 23% yield, $t_R=37.6$ min).

5α-Hydroxy-7β, 9α-diacetoxy-4(20),10(11)-taxadien-13-one, 8 (Scheme 3, Table 4). $[\alpha]_D^{22}$ −123.7° (*c* 0.30, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR

(125 MHz, $CDCl_3$) see Table 4; FAB-HR-MS: $C_{24}H_{34}O_6K$ $[M+K]^+$ required: 457.1993; found: 457.1993.

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