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TAXOIDS FROM CELL CULTURES OF *TAXUS CHINENSIS*

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Key Word Index—*Taxus chinensis*; Taxaceae; NMR-spectroscopy; Taxol®; taxoids.

Abstract—A cell culture of *Taxus chinensis* was found to produce taxoids in 4% yield (dry weight) after stimulation with methyl jasmonate. From the culture medium and cell mass, 16 taxoids were isolated. The structures were determined by two-dimensional gradient-enhanced DQF-COSY, HMQC, HMBC, and NOESY NMR experiments. The major taxoids of the culture were esterified at C-14 of the taxoid ring system. Five new taxoids were identified as minor metabolites and were assigned as 2α-benzoxy-4α,10β-diacetoxy-1β,7β,9α-trihydroxytax-11-ene, 2α-benzoxy-4α-acetoxy-1β,7β,10β-trihydroxy-9-dehydrotax-11-ene, 2α-benzoxy-4α,7β,9α,10β-tetraacetoxy-1β-hydroxytax-11-ene, 2α,4α,7β,9α,10β-pentaacetoxytax-11-ene and 2α,5α,7β,9α,10β-pentaacetoxy-4β,20-epoxytax-11-ene.

INTRODUCTION

Taxol® (**8**) [1] and the semi-synthetic related substance Taxotere® [2] have attracted considerable interest during the last ten years for their use in the chemotherapy of solid tumors. The supply crisis for Taxol® and its biogenetic precursor 10-deacetylbaccatin III (**1**), which can be chemically transformed into Taxotere® or Taxol®, has stirred the interest to produce these compounds by means of cell or even organ cultures. There are successful reports on the commercial scale fermentation of *Taxus* cells for the production of these natural products [3, 4]. In order to understand the complex metabolic pathways to taxoids and to unravel regulating and rate-limiting reactions in the biosynthetic process, the knowledge of the taxoid forming enzymes and their corresponding genes is a necessity. A full understanding of the taxoid biosynthetic pathway could provide the basis for biotechnological production of taxoids. The insertion of rate-limiting genes into whole plants or cell cultures might raise the yield of these medicinally desirable compounds.

Using a strain of *Taxus chinensis* (Pilger) Rehd. we previously investigated the biosynthesis of the major taxoid of this culture, 2α,5α,10β,14β-tetraacetoxy-4(20),11-taxadiene (taxuyunnanine C) [5]. We found that the terpenoid carbon skeleton of the taxoid is not of mevalonoid origin. Obviously, the deoxyxylulose pathway of isoprenoid biosynthesis [6–8] is operative in this plant cell culture. In order to gain further insight into the metabolite spectrum formed by this *Taxus* strain, we investigated the taxoid composition in cells and medium after cultivation. Moreover, we studied the kinetics of taxoid formation and the possibility to elicit the taxoid biosynthetic pathway.

As a result, 16 taxoids could be isolated from cells and medium of *T. chinensis*, and their structures could be elucidated by NMR techniques. Five new taxoids with unsubstituted C-13 and C-14 methylene ring atoms were found. The capacity to produce taxoids was considerably stimulated by the additon of methyl jasmonate [9].

RESULTS AND DISCUSSION

The cell culture used in this paper was described previously [5]. The strain was grown on larger scale in the presence or absence of methyl jasmonate in Erlenmeyer flasks. After reaching the stationary phase, taxoids were isolated from ether extracts of cells or from ether extracts of the culture medium by column chromatography and subsequent semi-preparative HPLC chromatography. The HPLC elution profiles of the combined taxoid-containing extracts from cells and medium are shown in Fig. 1. The 13 fractions indicated in Fig. 1 were collected and were subjected to homo- and heteronuclear NMR analysis (COSY, NOESY, HMQC, HMBC). The structures including the relative stereoconfigurations

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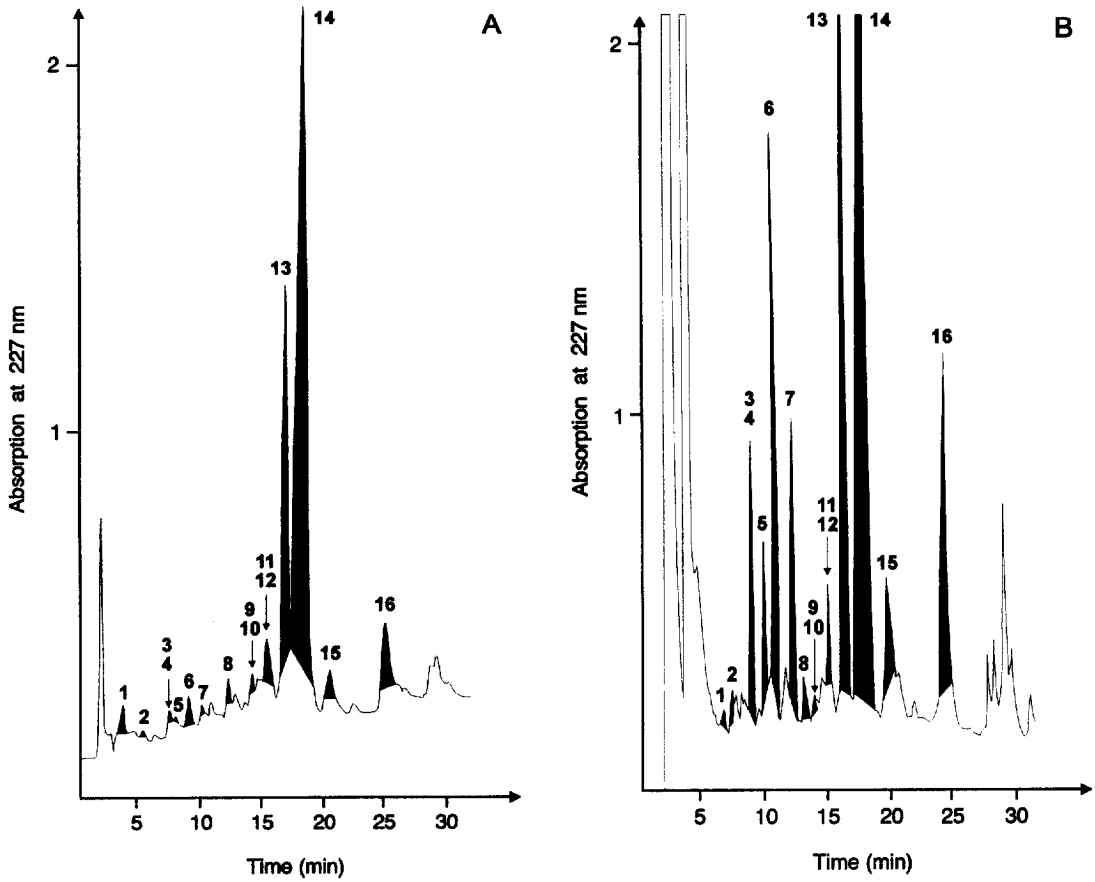
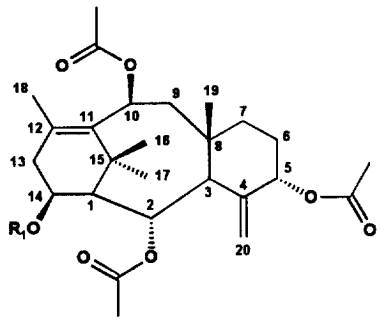


Fig. 1. HPLC profile of extracts from *T. chinensis* cell cultures. (A) untreated culture; (B) treated with 30 μM methyl jasmonate for 3 days. The extract injected corresponds in each case to 1.2 mg of dry cells. The numbers represent taxoids which were unequivocally identified by NMR.



14 R_1 = $COCH_3$

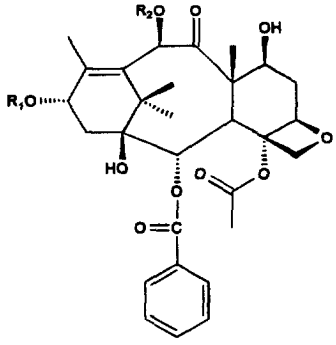
15 R_1 = $COCH_2CH_3$

16 R_1 = $COCHCH_2CH_3$ (CH_3)

13 R_1 = $COCH-CHCH_3$ (CH_3, OH)

of 16 taxoids could be established by rigorous interpretation of the correlation patterns in the NMR experiments. Table 1 summarizes the yield of the compounds after isolation with or without methyl jasmonate in the medium. As previously reported [5], by far the most abundant compound in this cultured strain was taxuyunnanine C (**14**) which is characterized by acetyl groups at C-14, C-2, C-5, and C-10, an unsubstituted methylene C-13, and a 4(20) exocyclic methylene function. This compound was originally described in roots of *T. yunnanensis* [10]. The related taxoids with a propionyl ester function (**15**), a (2′-methyl)butyryl ester function (**16**), and a (2′-methyl-3′-hydroxy)butyryl ester function (yunnanxane, **13**) at C-14 were also found in considerable amounts. These taxoids were already previously isolated and characterized from cell cultures of *T. chinensis* var. *mairei* [11] and *T. yunnanensis* [12].

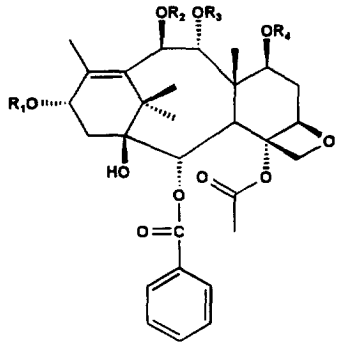
Six of the isolated taxoids (**1–3**, **5**, **8**, **10**) were hydroxylated or esterified at C-13, carried an oxetane ring, and a benzoic ester group at C-2. Taxol® (**8**), 10-deacetylbaccatin III (**1**), and baccatin III (**3**) [1, 13–16] were found in minor amounts under the conditions of cultivation of the cell culture. The taxoids **10** (baccatin VI) and **5** (7,9-deacetylbaccatin VI) could be identified as byproducts and were already described in *Taxus* species [16–19]. Compound **2** was recently obtained by chemical deacetylation of the cor-



8 R_1 = COCH(OH)—CHPh(NHBz); R_2 = $COCH_3$

3 R_1 = H; R_2 = $COCH_3$

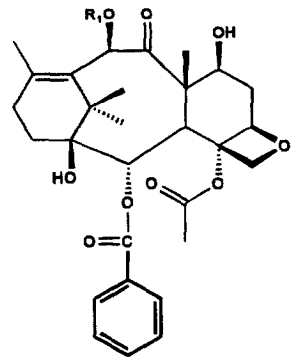
1 R_1, R_2 = H



2 R_1, R_3, R_4 = H; R_2 = $COCH_3$

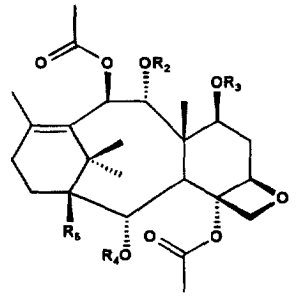
10 R_1, R_2, R_3, R_4 = $COCH_3$

5 R_1, R_2 = $COCH_3$; R_3, R_4 = H



7 R_1 = $COCH_3$

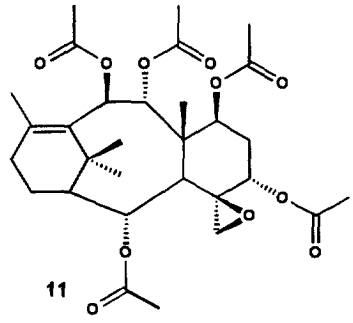
4 R_1 = H



9 R_2, R_3, R_4 = $COCH_3$; R_5 = H

12 R_2, R_3 = $COCH_3$; R_4 = COBz; R_5 = OH

6 R_2, R_3 = H; R_4 = COBz; R_5 = OH



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responding 13-acetyl compound but had not been observed as a natural product [20, 21].

Six of the analyzed taxoids (**4**, **6**, **7**, **9**, **11**, **12**) were found with unsubstituted methylene C-13 and C-14. The 9-dehydro taxoid **7** was previously described as a chemically synthesized product from the naturally occurring 10-deacetylbaccatin III [22].

The NMR signals of the taxoids **4**, **6**, **9**, **11**, **12** could not be assigned to any known taxoid structure and are therefore described in more detail (Tables 2–6). The ^{1}H NMR and ^{13}C NMR chemical shifts of **4** were similar to that of 13-dehydroxybaccatin III (**7**) with the exception of the singlet ^{1}H NMR signal at 5.26 ppm. An apparently corresponding singlet signal (H-10 of **7**) was found downfield shifted to 6.30 ppm in the ^{1}H NMR spectrum of **7**. Moreover, carbonyl and methyl signals which were assigned to the acetyl group at C-10 in **7** were absent in the NMR spectra of **4**. In a COSY experiment, the ^{1}H NMR signal at 5.26 ppm gave weak correlation peaks to the methyl ^{1}H NMR signals of C-16/C-17 and C-18. Long range ^{13}C-^{1}H correlation peaks were observed to C-7, C-8, C-9, C-11, C-12, and C-15 in a HMBC experiment. This connectivity pattern clearly indicates that the signal at 5.26 ppm is due to H-10 of **7**. A long range ^{13}C-^{1}H correlation peak to a carbonyl carbon of a hypothetical ester group at C-10 which was observed in the HMBC experiment with **4** was absent in the spectrum of **7**. Correlations through space were observed to methyl protons of C-18 and methine protons at C-3 and C-7 in a NOESY experiment aimed at the elucidation of the stereoconfiguration of H-10. All of these data gave firm evidence for the assignment of the 5.26 ppm signal to the methine proton at C-10 which carries a free hydroxy function in the β

Table 1. Taxoids from a cell culture of *T. chinensis*. The numbering of the compounds corresponds with the HPLC elution profile shown in Fig. 1

Compound	Systematic name	Trivial name	Yield [mg] control	Yield [mg l^{-1}] with 30 μM methyl jasmonate	Reference
1	2α-benzoxy-4α-acetoxy-1β,7β,10β,13α-tetrahydroxy-9α-dehydroxytax-11-ene	10-Deacetylbaccatin III	0.2	0.45	[13]
2	2α-benzoxy-4α,10β-diacetoxy-1β,7β,9α,13α- tetrahydroxytax-11-ene	9-Dihydrobaccatin III	0.02	0.2	[20, 21]
3	2α-benzoxy-4α,10β-diacetoxy-1β,7β,13α-trihydroxy-9-dehydrotax-11-ene	Baccatin III	0.04	0.6	[14–16]
4	2α-benzoxy-4α-acetoxy-1β,7β,10β-trihydroxy-9-dehydrotax-11-ene	13-Dehydroxy-10-deacetylbaccatin III	0.06	0.9	
5	2α-benzoxy-4α,10β,13α-triacetoxy-1β,7β,9α-trihydroxytax-11-ene	9-Dihydro-13-acetoxybaccatin III= 7,9-Deacetylbaccatin VI	0.22	0.9	[17]
6	2α-benzoxy-4α,10β-diacetoxy-1β,7β,9α-trihydroxytax-11-ene	9-Dihydro-13-dehydroxybaccatin III	0.4	3.2	
7	2α-benzoxy-4α,10β-diacetoxy-1β,7β-dihydroxy-9-dehydrotax-11-ene	13-Dehydroxybaccatin III	0.24	1.9	[22]
8	5β,20-epoxy-1,2,4,7,10,13-hexahydroxytax-11-ene-9-one-4,10-diacetate-2-benzoate-13-(2*R*,3*S*)-(benzoylamino)-hydroxyphenylpropionate (USAN)	Taxol®	0.1	0.4	[1]
9	2α,4α,7β,9α,10β-pentaacetoxytax-11-ene		0.1	0.5	
10	2α-benzoxy-4α,7β,9α,10β,13α-pentaacetoxy-1β-hydroxytax-11-ene	Baccatin VI	0.1	0.6	[16–19]
11	2α,5α,7β,9α,10β-pentaacetoxy-4β,20-epoxytax-11-ene	13-Deacetoxybaccatin I	0.05	0.75	
12	2α-benzoxy-4α,7β,9α,10β-tetraacetoxy-1β-hydroxytax-11-ene		0.06	0.85	
13	2α,5α,10β-triacetoxy-14β(2′-methyl-3′-hydroxy)butyryloxy-4(20),11-taxadiene	Yunnanxane	7.2	11.2	[11]
14	2α,5α,10β,14β-tetraacetoxy-4(20),11-taxadiene	Taxuyunnanine C	75	145	[10]
15	2α,5α,10β-triacetoxy-14β-propionyloxy-4(20),11-taxadiene		0.9	1.4	[11]
16	2α,5α,10β-triacetoxy-14β-(2′-methyl)butyryloxy-4(20),11-taxadiene		1.1	2.2	[11]

Table 2. ^{1}H NMR and ^{13}C NMR assignments of 2α-benzoxy-4α-acetoxy-1β,7β,10β-trihydroxy-9-dehydrotax-11-ene (**4**) in $CDCl_3$

Position	Chemical shift* ^{13}C ppm	Chemical shift* ^{1}H ppm	J_{HH}† Hz	Correlation experiments DQF-COSY	NOESY	DEPT	HMBC
1	80.62					C	3,14α,18, 13α(*w*)§,17,16,2
2	73.94	5.58 (*d*)	nd‡	3,14β	16,19,3,20β	CH	3,14α (*w*)
3	46.60	3.87 (*d*)	6.6 (2)	2,20α	14α,2,10,5,7	CH	19,5 (*w*),20α,20β,2
4	81.25					C	3,6α (*w*),5,20α
5	84.01	4.99 (*m*)	nd	6α,6β,20β	6α,3,20α	CH	6β,6α,20β
6	37.08	2.55(α) (*m*)	nd	6β,5,7	5,7,6β	CH_2	7
		1.83(β) (*m*)	nd	6α,5,7	6α		
7	72.03	4.23 (*dd*)	10.9(6α), 6.8(6β)	6α,6β	6α,10,3	CH	3,6β,6α, 19,5,10 (*w*)
8	57.82					C	3,6α,19,2,10 (*w*), 7 (*w*)
9	211.90					C	18 (*w*),19,10,7 (*w*), 3
10	75.09	5.26 (*s*)		16/17 (*w*),18 (*w*)	18,3,7	CH	
11	134.39					C	18,17,16,13β (*w*), 13α,10
12	139.66					C	13β (*w*),18,13α,10
13	29.79	2.66(β)(*ddd*)	19.0(13α), 11.7(14β), 5.5(14α)	13α,14β,14α,18	13α	CH_2	18,14α(*w*)
		1.84(α) (*m*)	nd	13β,14α,14β(*w*)	13β		
14	25.64	2.26(α) (*m*)	nd	14β,13β,13α	Bz2-2′/6′,3,14β	CH_2	2,13α (*w*),13β (*w*)
		1.64(β) (*m*)	nd	14α,13β,13α (*w*),2	14α		
15	42.09					C	14α,18 (*w*),17,16,1
16	20.87	1.08 (*s*)			2	CH_3	17
17	26.32	1.07 (*s*)				CH_3	16,18
18	20.53	1.94 (*s*)		13α	10	CH_3	
19	9.35	1.69 (*s*)			2	CH_3	3,7
20	76.49	4.29(α) (*d*)	8.5(20β)	20β,3	Bz2-2′/6′,5,20α	CH_2	3
		4.15(β) (*d*)	8.5(20α)	20α,5	2,20β		
Bz2-CO	166.84					C	Bz2-2′/6′,Bz2-3′/5′ (*w*),2
Bz2-1′	129.35					C	Bz2-3′/5′
Bz2-2′/6′	129.98	8.05 (*dd*)	8.4(Bz2-3′/5′),1.4 (Bz-4′)	Bz2-3′/5′Bz2-4′ (*w*)	Bz2-3′/5′,20α,14α	CH_2	Bz2-4′
Bz2-3′/5′	128.59	7.44 (*m*)	nd	Bz2-2′/6′,Bz2-4′	Bz2-2′/6′	CH_2	
Bz2-4′	133.59	7.57 (*m*)	nd	Bz2-3′/5′,Bz2-2′/6′ (*w*)		CH	Bz2-2′/6′
Ac4-CO	169.78					C	Ac4-Me
Ac4-Me	22.02	2.28 (*s*)				CH_3	

* Referenced to solvent signals at 7.24 ppm (^{1}H NMR) and 77.0 ppm (^{13}C NMR), respectively. ^{1}H NMR signal multiplicities are in parentheses (*s* = singlet, *d* = doublet, *t* = triplet, *m* = multiplet).

† Obtained from one-dimensional ^{1}H NMR spectra. Coupling partners are indicated in parentheses.

‡ nd, not determined due to signal overlapping.

§ *w*, weak cross peak intensity.

configuration, instead of a β acetyl group in the case of the taxoid **7**. It should be noted that the observed upfield shift of the ^{1}H NMR signal in **4** as compared to the corresponding signal in **7** is typical for methine protons with a free hydroxy function after deacetylation of the respective carbon atom. However, the presence or absence of an ester group of the taxoid skeleton was directly indicated by the presence or absence of a long-range ^{13}C-^{1}H correlation between the methine ^{1}H NMR signal of the taxoid ring atom and the carbonyl carbon of the ester group.

As described for the ^{1}H NMR signal at 5.26 ppm,

Table 3. ^{1}H NMR and ^{13}C NMR assignments of 2α,4α,7β,9α,10β-pentaacetoxytax-11-ene (**9**) in $CDCl_3$

Position	Chemical shift* ^{13}C ppm	^{1}H ppm	J_{HH}† Hz	Correlation experiments DQF-COSY	NOESY	DEPT	HMBC
1	49.61	1.48 (*d*)	nd‡	2,3 (*w*)§		CH	3,18 (*w*),17,16,2 (*w*)
2	70.57	5.53 (*d*)	nd	1,3	9,16,19	CH	3,1
3	43.52	2.85 (*d*)	5.4(2)	2,1 (*w*),20α	7,18	CH	19,5 (*w*),2 (*w*),1,20α,20β
4	81.25					C	3,6β (*w*),20α,5
5	83.98	4.94 (*dd*)	9.2 (6α),1.1 (6β)	6α,6β,20β	20α,6α	CH	6β (*w*),20β
6	34.54	2.49(α) (*m*)	nd	6β,5,7	7,5,6β	CH_2	7
		1.83(β) (*m*)	nd	6α,5,7	6α		
7	71.76	5.52 (*dd*)	nd	6α,6β	10,3,6α,18	CH	6α,19,9,5,3,6β
8	45.56					C	3,6β,19,9,2,7
9	75.52	5.82 (*d*)	11.3(10)	10	2,16,19	CH	19,10
10	71.22	6.09 (*d*)	11.1(9)	9	7,18	CH	9
11	131.51					C	18,17,16,13α (*w*),10,9 (*w*)
12	141.14					C	13β (*w*),18,13α (*w*),10
13	29.10	2.30(β) (*m*)	nd	13α,14β,18		CH_2	18
		1.76(α) (*m*)	nd	13β			
14	17.08	1.86(α) (*m*)	nd	14β		CH_2	2,13α (*w*)
		1.59(β) (*m*)	nd	14α,13β			
15	37.10					C	17,16,10,1
16	25.66	1.67 (*s*)		17	9,2,17	CH_3	17
17	31.44	1.02 (*s*)		16	16	CH_3	16
18	20.94	2.01 (*d*)	1.3(13β)	13β	10,7,3	CH_3	
19	12.43	1.47 (*s*)			2,9,20β	CH_3	3,9,7
20	76.57	4.49(α) (*d*)	8.0(20β)	20β,3	5,20β	CH_2	3
		4.21(β) (*dd*)	8.0(20α),0.7	20α,5	20α,19		
Ac2-CO	169.78¶						Ac2-Me,2
Ac2-Me	21.32¶	2.07¶ (*s*)					
Ac4-CO	169.37					C	Ac4-Me
Ac4-Me	21.57	1.99 (*s*)				CH_3	
Ac7-CO	169.81¶					C	7,Ac7-Me
Ac7-Me	22.02¶	2.14¶ (*s*)				CH_3	
Ac9-CO	170.16					C	9,Ac9-Me
Ac9-Me	20.79	2.04 (*s*)				CH_3	
Ac10-CO	169.18					C	10,Ac10-Me
Ac10-Me	21.07¶	1.94 (*s*)				CH_3	

* Referenced to solvent signals at 7.24 ppm (^{1}H NMR) and 77.0 ppm (^{13}C NMR), respectively. ^{1}H NMR signal multiplicities are in parentheses (*s* = singlet, *d* = doublet, *t* = triplet, *m* = multiplet).

† Obtained from one-dimensional ^{1}H NMR spectra. Coupling partners are indicated in parentheses.

‡ nd, not determined due to signal overlapping.

§ *w*, weak cross peak intensity.

¶ Assignments may be interchanged.

all of the ^{1}H NMR and ^{13}C NMR resonances were analyzed by the two-dimensional NMR experiments. The correlation pattern summarized in Table 2 gave redundant information for signal assignments and the structure of **7** was established as 2α-benzoxy-4α,10β-diacetoxy-1β,7β-dihydroxy-9-dehydrotax-11-ene (13-dehydroxybaccatin III).

Using the combined information from COSY, NOESY, DEPT, HMQC, and HMBC experiments (Tables 3–5), the ^{1}H NMR and ^{13}C NMR signals of **6**, **9** and **12** could also be assigned unequivocally. The carbon skeleton of the taxoid ring system was again clearly indicated by the network of observed correlations. The ester groups could be localized by HMBC information and chemical shift considerations as described for **7**. The taxoids **6**, **9** and **12** were characterized by hydroxy or ester functionalization at C-2, C-4, C-5, C-7, C-9 or C-10. C-1 was hydroxylated in **12** and **6**. The oxetane ring at C-4 and C-20 was obvious from the typical correlation pattern in COSY, NOESY, and HMBC experiments shown in Tables 3–5 with typical chemical shifts of the diastereotopic methylene C-20 protons in the range of 4.5–4.0 ppm. On the basis of the unambiguous NMR data, the structures of **6**, **9** and **12** were assigned as 2α-benzoxy-4α,10β - diacetoxy - 1β,7β,9α - tridroxytax - 11 - ene, 2α,4α,7β,9α,10β-pentaacetoxytax-11-ene and 2α-benzoxy-4α,7β,9α,10β-tetraacetoxy-1β-hydroxytax-

Table 4. ^{1}H NMR and ^{13}C NMR assignments of 2α-benzoxy-4α,7β,9α,10β-tetraacetoxy-1β-hydroxytax-11-ene (**12**) in $CDCl_3$

Position	Chemical shift* ^{13}C ppm	Chemical shift* ^{1}H ppm	J_{HH}† Hz	Correlation experiments DQF-COSY	NOESY	DEPT	HMBC
1	80.57					C	3,14α (*w*)§,18 (*w*), 13α (*w*),17,16,2
2	72.30	5.84 (*d*)	5.7 (3)	3,14β	9,16,19	CH	3
3	46.18	3.12 (*d*)	5.7 (2)	2,20α	18,10 (*w*),7	CH	19,5,20α,20β
4	81.74					C	3,6β (*w*),5,20α
5	83.81	4.93 (*d*)	8.7(6β)	6α,6β,20β	6α	CH	6α (*w*),6β,20β
6	34.41	2.51(α) (*m*)	nd‡	6β,5,7	6β,5,7	CH_2	7
		1.81(β) (*ddd*)	15.1(6β),9.7(7β), 1.5(7α)	6α,5,7	6α		
7	71.56	5.51 (*dd*)	9.5(6α),7.9(6β)	6α,6β	10,6α,18,3	CH	3,6β,6a,19,9,5
8	45.73					C	3,6α,19,9,2
9	75.14	5.90 (*d*)	11.2(10)	10	2,16	CH	18(*w*),17(*w*),19,10
10	70.75	6.14 (*d*)	11.2(9)	9	7,18,3 (*w*)	CH	9
11	132.36					C	18,17,16,13β (*w*), 13α,10 (*w*)
12	142.32					C	13β (*w*),18,13α,10
13	30.28	2.56(β) (*ddd*)	16.8(13α),12.0 (14β),4.5(14α)	13α,14β, 14α (*w*),18	17,13α	CH_2	18
		1.85(α) (*ddd*)	16.3(13β),10.5 (14α),2.7(14β)	13β,14α, 14β (*w*)	13β		
14	25.58	2.14(α) (*m*)	nd	14β,13α, 13β (*w*)	Bz2-2′/6′,14β	CH_2	17 (*w*),2
		1.67(β) (*m*)	nd	14α,13β, 13α (*w*),2	14α		
15	41.65					C	14α,18 (*w*),17,16,10
16	20.71	1.68 (*s*)		17	17,9,2	CH_3	17
17	28.00	1.08 (*s*)		16	13β,16	CH_3	16
18	20.70	2.04 (*d*)	1.1(13β)	13β	3,10,7	CH_3	
19	12.44	1.54 (*s*)			2,20β	CH_3	3,9,7
20	76.34	4.33 (α) (*d*)	8.4(20β)	20β,3	Bz2-2′/6′,20β	CH_2	3
		4.12(β) (*d*)	8.4(20α)20α	20α,5	20α,19		
Bz2-CO	166.82					C	Bz2-2′/6′,Bz2-3′/5′ (*w*),2
Bz2-1′	129.34					C	Bz2-3′/5′
Bz2-2′/6′	130.05	8.08 (*dd*)	8.3(Bz2-3′/5′),1.6(Bz-4′)	Bz2-3′/5′,Bz2-4′ (*w*)	20α,Ac4-Me, 14α	CH_2	Bz2-4′
Bz2-3′/5′	128.58	7.45 (*m*)	nd	Bz2-2′/6′,Bz2-4′		CH_2	
Bz2-4′	133.57	7.58 (*tt*)	7.3(Bz2-3′/5′),1.4(Bz-2′/6′)	Bz2-3′/5′,Bz2-2′/6′ (*w*)		CH	Bz2-2′/6′
Ac4-CO	169.77					C	Ac4-Me
Ac4-Me	22.12	2.26 (*s*)			Bz2-2′/6′	CH_3	
Ac7-CO	169.83					C	7,Ac7-Me
Ac7-Me	20.77	2.07 (*s*)				CH_3	
Ac9-CO	170.15					C	9,Ac9-Me
Ac9-Me	21.36	2.08 (*s*)				CH_3	
Ac10-CO	169.05					C	10,Ac10-Me
Ac10-Me	21.61	2.14 (*s*)				CH_3	

* Referenced to solvent signals at 7.24 ppm (^{1}H NMR) and 77.0 ppm (^{13}C NMR), respectively. ^{1}H NMR signal multiplicities are in parentheses (*s* = singlet, *d* = doublet, *t* = triplet, *m* = multiplet).

† Obtained from one-dimensional ^{1}H NMR spectra. Coupling partners are indicated in parentheses.

‡ nd, not determined due to signal overlapping.

§ *w*, weak cross peak intensity.

Table 5. ^{1}H NMR and ^{13}C NMR assignments of 2α-benzoxy-4α,10β-diacetoxy-1β,7β,9α-trihydroxytax-11-ene (**6**) in $CDCl_3$

Position	Chemical shift* ^{13}C ppm	^{1}H ppm	J_{HH}† Hz	Correlation experiments DQF-COSY	NOESY	DEPT	HMBC
1	80.43					C	3,14α (*w*)§,18 (*w*), 13α (*w*),17,16,2
2	72.50	5.69 (*dd*)	5.8(3), 1.0(14β)	3,14β	16,19,9	CH	3,14α
3	45.97	2.96 (*d*)	5.6(2)	2,20α	13α,18,14α,7	CH	19,5,20α,20β,2 (*w*), 7 (*w*)
4	82.32					C	3,6β (*w*),5,20αAc4-Me (*w*)
5	84.00	4.90 (*dd*)	9.5(6α), 1.5(6β)	6α,6β,20β	6α	CH	6α (*w*),6β,20β
6	37.70	2.49(α) (*ddd*)	14.8(6β), 9.2(7α), 7.5(7β)	6β,5,7	5,7,6β	CH_2	7
		1.88(β) (*ddd*)	15.0(6α), 9.9(7β), 1.6(7α)	6α,5,7	6α		
7	73.32	4.37 (*dd*)	9.8(6α), 7.5(6β)	6α,6β	18,6α,10,3	CH	3,6β,6α,19,9,5
8	44.83					C	3,6α,19,9,2
9	76.87	4.32 (*d*)	10.8(10)	10	16,19,2	CH	18 (*w*),17 (*w*),19,10
10	73.72	6.10 (*d*)	10.8(9)	9	18,7	CH	Ac10-Me (*w*)
11	133.57					C	18,17,16,13β (*w*), 13α,10 (*w*)
12	140.68					C	13β (*w*),18,13α,10
13	30.23	2.54(β) (*m*)	nd‡	13α,14β,14α (*w*),18	17,13α	CH_2	18
		1.79(α) (*m*)	nd	13β,14α,14β (*w*)	3,13β		
14	25.71	2.13(α) (*m*)	nd	14β,13α,13β (*w*)	14β,3	CH_2	2
		1.62(β) (*m*)	nd	14α,13β,13α (*w*)	14α		
15	41.79					C	14α (*w*),18 (*w*),17,16, 10,2 (*w*)
16	20.98	1.56 (*s*)		17	17,2,9	CH_3	17
17	27.96	1.07 (*s*)		16	16,13β	CH_3	16
18	20.47	1.93 (*d*)	1.1(13β)	13α	10,7,3	CH_3	
19	12.08	1.72 (*s*)			2,9,20β	CH_3	3,9,7
20	76.28	4.28(α) (*d*)	8.1(20β)	20β,3	20β,Bz2-2′/6′	CH_2	3
		4.14(β) (*dd*)	8.3(20α), 0.9(5)	20α,5	19,20α		
Bz2-CO	166.85					C	Bz2-2′/6′,Bz2-3′/5′ (*w*),2
Bz2-1′	129.32					C	Bz2-3′/5′
Bz2-2′/6′	129.98	8.05 (*dd*)	8.4(Bz2-3′/5′), 1.4(Bz-4′)	Bz2-3′/5′,Bz2-4′ (*w*)	20α	CH_2	Bz2-4′
Bz2-3′/5′	128.54	7.43 (*m*)	nd	Bz2-2′/6′,Bz2-4′		CH_2	
Bz2-4′	133.53	7.56 (*tt*)	7.5(Bz2-3′/5′), 1.4(Bz-2′/6′)	Bz2-3′/5′,Bz2-2′/6′ (*w*)		CH	Bz2-2′/6′
Ac4-CO	169.85					C	Ac4-Me
Ac4-Me	22.18	2.23 (*s*)				CH_3	
Ac10-CO	170.73					C	10,Ac10-Me
Ac10-Me	21.43	2.09 (*s*)				CH_3	

* Referenced to solvent signals at 7.24 ppm (^{1}H NMR) and 77.0 ppm (^{13}C NMR), respectively. ^{1}H NMR signal multiplicities are in parentheses (*s* = singlet, *d* = doublet, *t* = triplet, *m* = multiplet).

† Obtained from one-dimensional ^{1}H NMR spectra. Coupling partners are indicated in parentheses.

‡ nd, not determined due to signal overlapping.

§ *w*, weak cross peak intensity.

Table 6. ^{1}H NMR and ^{13}C NMR assignments of 2α,5α,7β,9α,10β-pentaacetoxy-4β,20-epoxytax-11-ene (**11**) in $CDCl_3$

Position	Chemical shift* ^{13}C ppm	Chemical shift* ^{1}H ppm	J_{HH}† Hz	Correlation experiments DQF-COSY	Correlation experiments NOESY	Correlation experiments DEPT	Correlation experiments HMBC
1	50.85	1.70 (*d*)	6.6 (14β)	2,14β,3 (*w*)§	2,16,17	CH	3,14β (*w*),18 (*w*), 13α (*w*),17,16,2
2	69.55	5.54 (*d*)	3.6(3)	1,3	9,16,19,1	CH	3,1
3	38.41	2.79 (*d*)	3.6(2)	2,1 (*w*)	18,14β,10 (*w*), 7,20α (*w*),5 (*w*)	CH	19,5 (*w*),2 (*w*),1
4	59.08					C	3,6α (*w*),20β
5	77.38	4.24 (*dd*)	3.4(6β),2.6(6α)	6α,6β	6β,6α,20β	CH	6α (*w*),20β
6	31.05	2.08(β) (*m*)	nd‡	6α,5,7	6α,5	CH_2	7,20α (*w*)
		1.75(α) (*ddd*)	14.6(6β),4.7(7), 2.6(5)	6β,5,7	6β,7,5		
7	69.03	5.47 (*dd*)	12.1(6β), 4.7(6α)	6α,6β	10,6α,18,3	CH	6α,19,9,5
8	46.64					C	3,6α,19,9,2,7
9	75.88	5.93 (*d*)	11.1(10)	10	2,16,19	CH	18 (*w*),19,10
10	71.70	6.14 (*d*)	11.0(9)	9	7,18,3 (*w*)	CH	9
11	132.65					C	18,17,16,13β (*w*), 13α,10 (*w*)
12	140.79					C	13β (*w*),18,13α,10
13	30.43	2.48(β) (*m*)	nd	13α,14α,18	17,13α	CH_2	18,1 (*w*)
		2.05(α) (*m*)	nd	13β	13β		
14	18.69	2.03(β) (*m*)	nd	14α	14α	CH_2	2
		1.62(α) (*m*)	nd	14β,13β	3,14β,20α		
15	37.59					C	18 (*w*),17,16,10,1
16	25.87	1.59 (*s*)		17	17,9,2,1	CH_3	17
17	31.34	1.04 (*s*)		16	13β,16,1	CH_3	16
18	21.49	2.19 (*d*)	0.9(13β)	13β	3,10,7	CH_3	
19	13.17	1.54 (*s*)			9,2	CH_3	3,9,7
20	49.90	3.58(α) (*d*)	5.4(20β)	20β	14α,20β,Ac2-Me,3 (*w*)	CH_2	
		2.26(β) (*d*)	5.6(20α)	20α	5,20α		
Ac2-CO	168.43						Ac2-Me,2
Ac2-Me	21.15	1.95 (*s*)			20α		
Ac5-CO	169.10					C	Ac5-Me,5 (*w*)
Ac5-Me	21.07	1.98 (*s*)				CH_3	
Ac7-CO	169.87					C	7,Ac7-Me
Ac7-Me	21.40	2.06 (*s*)				CH_3	
Ac9-CO	169.73					C	9,Ac9-Me
Ac9-Me	20.69	2.01 (*s*)				CH_3	
Ac10-CO	169.46					C	10,Ac10-Me
Ac10-Me	21.07¶	1.95 (*s*)				CH_3	

* Referenced to solvent signals at 7.24 ppm (^{1}H NMR) and 77.0 ppm (^{13}C NMR), respectively. ^{1}H NMR signal multiplicities are in parentheses (*s* = singlet, *d* = doublet, *t* = triplet, *m* = multiplet).

† Obtained from one-dimensional ^{1}H NMR spectra. Coupling partners are indicated in parentheses.

‡ nd, not determined due to signal overlapping.

§ *w*, weak cross peak intensity.

¶ Assignments may be interchanged.

11-ene, respectively. To the best of our knowledge these taxoids had not been described previously as natural products or as products of chemical synthesis.

No precedent of the NMR signal pattern of **11** was found in the literature. The spectra of **11** gave again typical chemical shifts and correlations for a taxoid ring system with acetyl ester functionalization at C-2, C-5, C-7, C-9, and C-10. However, the NMR data of the C-4/C-5/C-20 moiety differed drastically from those of metabolites containing an oxetane ring or an exocyclic methylene function. Specifically, the chemical shift of the diastereotopic protons at C-20 were found at 3.6 and 2.3 ppm (as compared with 4.5 and 4.2 ppm in oxetane ring-containing taxoids or 5.3 and 4.8 ppm in taxoids with an exocyclic 4(20) methylene function). However, recent ^{1}H and ^{13}C NMR data

of taxoids with a 4β,20-epoxy functionalization were similar to the ^{1}H NMR chemical shifts of the H-20 protons of **15** [23–26]. Moreover, the observed correlation patterns in COSY, NOESY, and HMBC experiments (Table 6) were in full accordance to the 4β,20-epoxy motif and **11** was assigned as 2α,5α,7β,9α,10β-pentaacetoxy-4β,20-epoxytax-11-ene (13-deacetoxybaccatin I). Taxoids with this epoxy ring system were proposed as possible direct precursors of taxoids containing the oxetane ring system [27, 28]. Interestingly, C-13, C-14, and C-1 were not functionalized in **11**. If **11** serves as a biosynthetic precursor of the highly functionalized taxoids, such as Taxol®, these positions would have to be oxidized *after* the oxetane ring formation in the late course of taxoid biosynthesis.

The time course of the formation of the major metabolite, taxuyunnanine C (**14**), in relation to dry weight of the culture is shown in Fig. 2. It is obvious that the production of taxoids is growth-linked and does not proceed in the stationary growth phase of the plant cell culture, as frequently reported for the production of secondary metabolites in microbial systems, e.g. [29]. The formation of taxuyunnanine C (**14**) stops when the culture enters the stationary phase (day 12). Subsequently, **14** decreases in concentration. It is not known whether **14** is degraded or whether it is transformed to the more complex taxoids found in this cell culture. The growth-linked production of **14** follows a pattern that was first established for an anthraquinone-producing *Morinda* cell culture [30] and has since then been confirmed in most cell culture systems.

The strain of *T. chinensis* under study appears to be a satisfactory system for studying various aspects of taxoid biosynthesis. Since this cell culture produces

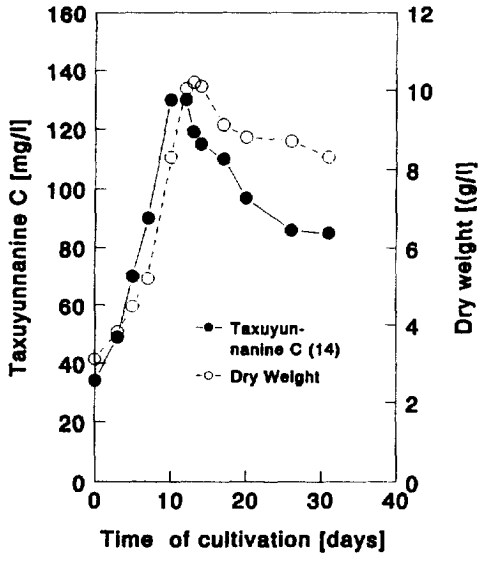

Fig. 2. Time course of taxuyunnanine C (**14**) formation and cell dry weight in the presence of 30 μM methyl jasmonate.

up to 4% (dry wt) of taxoids in a reasonably short time (10–12 days), we hope to be able to isolate enzymes involved in the formations of the later stages of the substituted taxane ring system formation that will supplement the excellent studies of Croteau and his colleagues [31] on taxadiene synthase and the C-5 hydroxylation of the taxa-4(5),11(12)-diene ring [28]. Futhermore, we hope to elucidate the structures of intermediates and metabolic sequences in the deoxyxylulose pathway that leads to the formation of taxuyunnanine C (**14**) in *Taxus* cell cultures [5]. Cell suspension cultures may once again prove to be the system of choice for clarifying complex secondary pathways [32].

EXPERIMENTAL

Plant material

A suspension culture of *T. chinensis* [5] was grown in modified B5 medium [33] in the absence or presence of 30 μM methyl jasmonate (Serva). The conditions have been described earlier [5].

Extraction and isolation of taxoids

Cells of *T. chinensis* grown in 17.5 l of medium for 28 days were harvested by centrifugation. The wet cell mass (211 g) was macerated in 2000 ml of MeOH. The supernatant was collected and evaporated to a small volume and the residue was extracted with diethylether. The supernatant was again collected and evaporated to a small volume. The concentrated extracts were combined and subjected to column and HPLC chromatography as described earlier [5]. The HPLC fractions were checked for purity by TLC (silica gel, $CHCl_3/CH_3CN$, 7:3). After developing, the plates were viewed at 254 nm. They were then sprayed lightly with a mixture of 0.5% vanillin and 3% H_2SO_4 in EtOH and were heated for 10 min at 60°C. Fractions containing taxoid mixtures were subjected again to HPLC separation.

In order to obtain sufficient amounts of minor metabolites, 30 liters of spent culture medium were passed through an XAD2 column (4 × 28 cm, 20–50 mesh) at 4°C. The column was washed with water, and the taxoids were eluted with 2 l of MeOH. The solvent was evaporated and the residue (580 mg) was used for HPLC separation [5].

Analytical HPLC. Separation of the crude extracts was performed with an Econosil C-18 column, 5 U, 4.6 × 250 mm, Alltech [5]. The column was developed using a gradient of 20 to 76% acetonitril for 20 min and subsequently by 100% acetonitril at a flow rate of 1 ml min^{-1}. The effluent was monitored photometrically (227 nm).

NMR measurements

^{1}H and ^{13}C NMR spectra were recorded at 20°C in $CDCl_3$ using a Bruker DRX 500 spectrometer equip-

ped with four frequency channels, pulsed field gradient accessory, lock switch unit, and an ASPECT station. Experimental setup and data processing was performed according to standard Bruker software (XWINNMR 1.1). ^{1}H NMR, DQF-COSY, NOESY, HMQC, HMQC-DEPT and HMBC experiments were measured using an inverse ^{1}H/^{13}C/^{15}N triple resonance probehead with Z gradient coil. ^{13}C NMR spectra were measured using a ^{13}C/^{1}H dual probehead.

*2α,5α,10β,14β-Tetraacetoxy-4(20)-11-taxadiene (taxuyunnanine C, **14**).* White amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.83 (3H, *s*, H-19), 1.11 (3H, *s*, H-17), 1.22 (1H, *dt*, *J* = 15.3, 3.5 Hz, H-7β), 1.62 (1H, *dd*, *J* = 14.5, 5.3 Hz, H-9α), 1.66 (3H, *s*, H-16), 1.79 (2H, *m*, H-6), 1.88 (1H, *d*, *J* = 2.2 Hz, H-1), 1.96 (1H, *dt*, *J* = 13.2, 7.0 Hz, H-7α), 2.00 (3H, *s*, H-2′), 2.04 (3H, *s*, Ac2-Me or Ac10-Me), 2.05 (3H, *s*, Ac10-Me or Ac2-Me), 2.08 (3H, *s*, H-18), 2.16 (3H, *s*, Ac5-Me), 2.37 (1H, *dd*, *J* = 14.8, 12.2 Hz, H-9β), 2.41 (1H, *m*, H-13β), 2.80 (1H, *dd*, *J* = 19.1, 9.3 Hz, H-13α), 2.91 (1H, *d*, *J* = 6.5 Hz, H-3), 4.85 (1H, *s*, H-20β), 4.98 (1H, *dd*, *J* = 9.2, 4.7 Hz, H-14), 5.26 (1H, *s*, H-20α), 5.28 (1H, *t*, *J* = 2.6 Hz, H-5), 5.34 (1H, *dd*, *J* = 6.5, 2.2 Hz, H-2), 6.05 (1H, *dd*, *J* = 12.1, 5.7 Hz, H-10); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.82 (*q*, C-18), 21.40 (*q*, Ac10-Me or Ac2-Me or 2′), 21.40 (*q*, Ac10-Me or Ac2-Me or 2′), 21.44 (*q*, Ac2-Me or Ac10-Me or 2′), 21.80 (*q*, Ac5-Me), 22.35 (*q*, C-19), 25.30 (*q*, C-16), 28.76 (*t*, C-6), 31.63 (*q*, C-17), 33.70 (*t*, C-7), 37.18 (*s*, C-15), 39.34 (*t*, C-13), 39.55 (*s*, C-8), 41.98 (*d*, C-3), 43.76 (*t*, C-9), 58.82 (*d*, C-1), 69.98 (*d*, C-10), 70.44 (*d*, C-14 or C-2), 70.44 (*d*, C-2 or C-14), 78.16 (*d*, C-5), 116.89 (*t*, C-20), 134.68 (*s*, C-12), 135.20 (*s*, C-11), 142.16 (*s*, C-4),169.75 (*s*, Ac5-CO), 169.95 (*s*, C-1′), 170.02 (*s*, Ac2-CO), 170.23 (*s*, Ac10-CO).

*2α,5α,10β-Triacetoxy-14β-propionyloxy-4(20),11-taxadiene (**15**).* White amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.80 (3H, *s*, H-19), 1.07 (3H, *t*, *J* = 7.7 Hz, H-3′), 1.08 (3H, *s*, H-17), 1.20 (1H, *dt*, *J* = 14.7, 3.4 Hz, H-7β), 1.60 (1H, *dd*, *J* = 15.3, 5.6 Hz, H-9α), 1.62 (3H, *s*, H-16), 1.76 (2H, *m*, H-6), 1.84 (1H, *d*, *J* = 2.2 Hz, H-1), 1.94 (1H, *m*, H-7α), 2.00 (3H, *s*, Ac2-Me or Ac10-Me), 2.02 (3H, *s*, Ac10-Me or Ac2-Me), 2.05 (3H, *s*, H-18), 2.14 (3H, *s*, Ac5-Me), 2.24 (2H, *q*, *J* = 7.6 Hz, H-2′), 2.34 (1H, *m*, H-9β), 2.37 (1H, *ddd*, *J* = 19.0, 4.6, 1.4 Hz, H-13β), 2.79 (1H, *dd*, *J* = 19.0, 9.2 Hz, H-13α), 2.89 (1H, *d*, *J* = 6.4 Hz, H-3), 4.81 (1H, *s*, H-20β), 4.98 (1H, *dd*, *J* = 9.2, 4.7 Hz, H-14), 5.23 (1H, *s*, H-20α), 5.25 (1H, *t*, *J* = 2.9 Hz, H-5), 5.31 (1H, *dd*, *J* = 6.5, 2.2 Hz, H-2), 6.02 (1H, *dd*, *J* = 12.1, 5.6 Hz, H-10); ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.12 (*q*, C-3′), 20.88 (*q*, C-18), 21.38 (*q*, Ac10-Me or Ac2-Me), 21.39 (*q*, Ac2-Me or Ac10-Me), 21.81 (*q*, Ac5-Me), 22.40 (*q*, C-19), 25.35 (*q*, C-16), 27.93 (*t*, C-2′), 28.82 (*t*, C-6), 31.67 (*q*, C-17), 33.75 (*t*, C-7), 37.25 (*s*, C-15), 39.53 (*t*, C-13), 39.61 (*s*, C-8), 42.07 (*d*, C-3), 43.83 (*t*, C-9), 59.03 (*d*, C-1), 70.13 (*d*, C-10), 70.35 (*d*, C-14), 70.59 (*d*, C-2), 78.26 (*d*, C-5), 116.90 (*t*, C-20), 134.79 (*s*, C-12), 135.28 (*s*, C-11), 142.21 (*s*, C-4), 169.91 (*s*, Ac5-CO), 170.14 (*s*, Ac2-CO), 170.37 (*s*, Ac10-CO), 173.50 (*s*, C-1′).

*2α,5α,10β-Triacetoxy-14β-(2′-methyl)butyryloxy-4(20),11-taxadiene (**16**).* White amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81 (3H, *s*, H-19), 0.85 (3H, *t*, *J* = 7.4 Hz, H-4′), 1.07 (3H, *d*, *J* = 7.4 Hz, H-5′), 1.09 (3H, *s*, H-17), 1.19 (1H, *dt*, *J* = 14.7, 3.4 Hz, H-7β), 1.42 (1H, *m*, H-3′α), 1.59 (1H, *m*, H-3′β), 1.59 (1H, *m*, H-9α), 1.63 (3H, *s*, H-16), 1.77 (2H, *m*, H-6), 1.84 (1H, *d*, *J* = 2.2 Hz, H-1), 1.91 (1H, *dt*, *J* = 13.2, 7.1 Hz, H-7α), 1.98 (3H, *s*, Ac2-Me or Ac10-Me), 2.02 (3H, *s*, Ac10-Me or Ac2-Me), 2.05 (3H, *s*, H-18), 2.14 (3H, *s*, Ac5-Me), 2.30 (1H, *hp*, H-2′), 2.34 (1H, *m*, H-9β), 2.36 (1H, *m*, H-13β), 2.82 (1H, *dd*, *J* = 19.2, 9.3 Hz, H-13α), 2.90 (1H, *d*, *J* = 6.5 Hz, H-3), 4.78 (1H, *s*, H-20β) 4.94 (1H, *dd*, *J* = 9.2, 4.7 Hz, H-14), 5.22 (1H, *s*, H-20α), 5.26 (1H, *t*, *J* = 2.6 Hz, H-5), 5.31 (1H, *dd*, *J* = 6.5, 2.2 Hz, H-2), 6.02 (1H, *dd*, *J* = 12.1, 5.7 Hz, H-10); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.56 (*q*, C-4′), 16.54 (*q*, C-5′), 20.90 (*q*, C-18), 21.32 (*q*, Ac10-Me or Ac2-Me), 21.36 (*q*, Ac2-Me or Ac10-Me), 21.86 (*q*, Ac5-Me), 22.44 (*q*, C-19), 25.36 (*q*, C-16), 26.71 (*t*, C-3′), 28.86 (*t*, C-6), 31.66 (*q*, C-17), 33.78 (*t*, C-7), 37.22 (*s*, C-15), 39.59 (*t*, C-13), 39.64 (*s*, C-8), 41.04 (*d*, C-2′), 42.11 (*d*, C-3), 43.85 (*t*, C-9), 59.22 (*d*, C-1), 70.07 (*d*, C-2 or C-10), 70.09 (*d*, C-10 or C-2), 70.52 (*d*, C-14), 78.11 (*d*, C-5), 116.77 (*t*, C-20), 134.80 (*s*, C-12), 135.30 (*s*, C-11), 142.24 (*s*, C-4), 169.75 (*s*, Ac5-CO), 169.90 (*s*, Ac2-CO), 170.18 (*s*, Ac10-CO), δ 175.59 (*s*, C-1′).

*2α,5α,10β-Triacetoxy-14β-(2′-methyl-3′-hydroxy)butyryl-oxy-4(20),11-taxadiene (Yunnanxane, **13**).* White amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.80 (3H, *s*, H-19), 1.08 (3H, *s*, H-17), 1.10 (3H, *d*, *J* = 7.1 Hz, H-5′), 1.15 (3H, *d*, *J* = 6.3, H-4′), 1.22 (1H, *dt*, *J* = 14.7, 3.4 Hz, H-7β), 1.58 (1H, *dd*, *J* = 14.5, 5.3 Hz, H-9α), 1.62 (3H, *s*, H-16), 1.79 (2H, *m*, H-6), 1.88 (1H, *d*, *J* = 2.2 Hz, H-1), 1.91 (1H, *dt*, *J* = 13.2, 7.1 Hz, H-7α), 1.97 (3H, *s*, Ac2-Me or Ac10-Me), 2.01 (3H, *s*, Ac10-Me or Ac2-Me), 2.05 (3H, *s*, H-18), 2.13 (3H, *s*, Ac5-Me), 2.30 (1H, *m*, H-2′), 2.33 (2H, *m*, H-9β), 2.36 (1H, *m*, H-13β), 2.80 (1H, *dd*, *J* = 19.2, 9.3 Hz, H-13α), 2.88 (1H, *d*, *J* = 6.5 Hz, H-3), 3.81 (1H, *m*, H-3′), 4.77 (1H, *s*, H-20β), 4.98 (1H, *dd*, *J* = 9.2, 4.7 Hz, H-14), 5.22 (1H, *s*, H-20α), 5.24 (1H, *t*, *J* = 2.6 Hz, H-5), 5.31 (1H, *dd*, *J* = 6.5, 2.2 Hz, H-2), 6.01 (1H, *dd*, *J* = 12.1, 5.7 Hz, H-10); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.83 (*q*, C-5′), 20.73 (*q*, C-4′), 20.85 (*q*, C-18), 21.32 (*q*, Ac10-Me or Ac2-Me), 21.34 (*q*, Ac2-Me or Ac10-Me), 21.81 (*q*, Ac5-Me), 22.39 (*q*, C-19), 25.30 (*q*, C-16), 28.81 (*t*, C-6), 31.60 (*q*, C-17), 33.73 (*t*, C-7), 37.18 (*s*, C-15), 39.41 (*t*, C-13), 39.58 (*s*, C-8), 42.06 (*d*, C-3), 43.79 (*t*, C-9), 46.91 (*d*, C-2′), 59.03 (*d*, C-1), 69.36 (*d*, C-3′), 69.97 (*d*, C-10), 70.39 (*d*, C-2), 70.62 (*d*, C-14), 78.12 (*d*, C-5), 116.82 (*t*, C-20), 134.57 (*s*, C-12), 135.33 (*s*, C-11), 142.16 (*s*, C-4), 169.70 (*s*, Ac5-CO), 169.86 (*s*, Ac2-CO), 170.14 (*s*, Ac10-CO), 174.67 (*s*, C-1′).

*2α-Benzoxy-4α,7β,9α,10β,13α-pentaacetoxy-1β-hydroxytax-11-ene (Baccatin VI, **10**).* White amorph-

ous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (3H, *s*, H-17), 1.58 (3H, *s*, H-19), 1.76 (3H, *s*, H-16), 1.83 (1H, *m*, H-6β), 1.97 (3H, *s*, Ac10-Me), 2.01 (3H, *d*, J = 1.3 Hz, H-18), 2.08 (3H, *s*, Ac7-Me), 2.08 (3H, *s*, Ac9-Me), 2.17 (3H, *s*, Ac13-Me), 2.18 (2H, *m*, H-14), 2.26 (3H, *s*, Ac4-Me), 2.49 (1H, *m*, H-6α), 3.16 (1H, *d*, J = 5.9 Hz, H-3), 4.10 (1H, *dd*, J = 8.4, 0.6 Hz, H-20β), 4.30 (1H, *d*, J = 8.2 Hz, H-20α), 4.94 (1H, *dd*, J = 8.9, 0.9 Hz, H-5), 5.50 (1H, *m*, H-7), 5.85 (1H, *d*, J = 6.0 Hz, H-2), 5.99 (1H, *d*, J = 11.3 Hz, H-9), 6.15 (1H, *ddd*, J = 9.7, 8.4, 1.4 Hz, H-13), 6.19 (1H, *d*, J = 11.3 Hz, H-10), 7.45 (2H, *m*, Bz2-3′/5′), 7.58 (1H, *tt*, J = 7.5, 1.4 Hz, Bz2-4′), 8.07 (2H, *dd*, J = 8.4, 1.4 Hz, Bz2-2′/6′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.74 (*q*, C-19), 14.96 (*q*, C-18), 20.75 (*q*, Ac7-Me or Ac9-Me), 20.91 (*q*, Ac10-Me), 21.07 (*q*, Ac13-Me), 21.32 (*q*, Ac9-Me or Ac7-Me), 22.25 (*q*, C-16), 22.70 (*q*, Ac4-Me), 28.25 (*q*, C-17), 34.47 (*t*, C-6), 35.09 (*t*, C-14), 42.76 (*s*, C-15), 45.75 (*s*, C-8), 47.27 (*d*, C-3), 69.63 (*d*, C-13), 70.36 (*d*, C-10), 71.74 (*d*, C-7), 73.23 (*d*, C-2), 75.00 (*d*, C-9), 76.37 (*t*, C-20), 78.86 (*s*, C-1), 81.47 (*s*, C-4), 83.83 (*d*, C-5), 128.61 (*t*, Bz2-3′/5′), 129.20 (*s*, Bz2-1′), 130.08 (*t*, Bz2-2′/6′), 133.59 (*s*, C-11), 133.68 (*d*, Bz2-4′), 141.24 (*s*, C-12), 166.91 (*s*, Bz2-CO), 168.88 (*s*, Ac10-CO), 169.11 (*s*, Ac4-CO), 169.84 (*s*, Ac7-CO), 170.16 (*s*, Ac9-CO), 170.44 (*s*, Ac13-CO).

2α-Benzoxy-4α,10β-diacetoxy-1β,7β,13α-trihydroxy-9-dehydrotax-11-ene (Baccatin III, 3). White amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.07 (3H, *s*, H-16), 1.07 (3H, *s*, H-17), 1.64 (3H, *s*, H-19), 1.80 (1H, *m*, H-6β), 2.03 (3H, *d*, J = 1.3 Hz, H-18), 2.21 (3H, *s*, Ac10-Me), 2.25 (3H, *s*, Ac4-Me), 2.26 (2H, *m*, H-14), 2.53 (1H, *m*, H-6α), 3.85 (1H, *d*, J = 7.0 Hz, H-3), 4.13 (1H, *d*, J = 8.4 Hz, H-20β), 4.27 (1H, *d*, J = 8.3 Hz, H-20α), 4.43 (1H, *dd*, J = 11.0, 6.8 Hz, H-7), 4.85 (1H, *dt*, J = 8.8, 1.4 Hz, H-13), 4.96 (1H, *m*, H-5), 5.59 (1H, *d*, H-2), 6.29 (1H, *s*, H-10), 7.45 (2H, *m*, Bz2-3′/5′), 7.57 (1H, *m*, Bz2-4′), 8.07 (2H, *dd*, J = 8.4, 1.4 Hz, Bz2-2′/6′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.41 (*q*, C-19), 15.53 (*q*, C-18), 18.56 (*q*, C-16), 20.87 (*q*, Ac10-Me), 22.53 (*q*, Ac4-Me), 26.91 (*q*, C-17), 35.60 (*t*, C-6), 38.61 (*t*, C-14), 42.66 (*s*, C-15), 46.14 (*d*, C-3), 58.63 (*s*, C-8), 67.81 (*d*, C-13), 72.19 (*d*, C-7), 74.91 (*d*, C-2), 76.22 (*d*, C-10), 76.39 (*t*, C-20), 79.03 (*s*, C-1), 80.75 (*s*, C-4), 84.42 (*d*, C-5), 128.59 (*t*, Bz2-3′/5′), 129.32 (*s*, Bz2-1′), 130.05 (*t*, Bz2-2′/6′), 131.70 (*s*, C-11), 133.62 (*d*, Bz2-4′), 146.44 (*s*, C-12), 167.00 (*s*, Bz2-CO), 170.63 (*s*, Ac4-CO), 171.28 (*s*, Ac10-CO), 204.14 (*s*, C-9).

2α-Benzoxy-4α,10β,13α-triacetoxy-1β,7β,9α-trihydroxytax-11-ene (5). White amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.22 (3H, *s*, H-17), 1.65 (3H, *s*, H-16), 1.78 (3H, *s*, H-19), 1.88 (1H, *m*, H-6β), 1.90 (3H, *d*, J = 1.5 Hz, H-18), 2.11 (3H, *s*, Ac10-Me), 2.16 (3H, *s*, Ac13-Me), 2.25 (3H, *s*, Ac4-Me), 2.25 (2H, *m*, H-14), 2.50 (1H, *ddd*, J = 14.8, 9.3, 7.4 Hz, H-6α), 3.02 (1H, *d*, J = 6.0 Hz, H-3), 4.14 (1H, *dd*, J = 8.1, 0.9 Hz, H-20β), 4.28 (1H, *d*, J = 8.1 Hz, H-20α), 4.40 (1H, *dd*, H-7), 4.41 (1H, *d*, H-9), 4.93 (1H, *dd*, J = 9.1, 1.1 Hz, H-5), 5.72 (1H, *d*, J = 6.0 Hz, H-2), 6.13 (1H, *qt*, J = 8.7, 1.6 Hz, H-13), 6.19 (1H, *d*, J = 10.9, H-10), 7.45 (2H, *m*, Bz2-3′/5′), 7.58 (1H, *tt*, J = 7.5, 1.4 Hz, Bz2-4′), 8.05 (2H, *dd*, J = 8.5, 1.4 Hz, Bz2-2′/6′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.47 (*q*, C-19), 14.83 (*q*, C-18), 21.22 (*q*, Ac13-Me), 21.32 (*q*, Ac10-Me), 22.55 (*q*, C-16), 22.85 (*q*, Ac4-Me), 28.25 (*q*, C-17), 35.30 (*t*, C-14), 37.89 (*t*, C-6), 43.00 (*s*, C-15), 44.82 (*s*, C-8), 47.07 (*d*, C-3), 69.75 (*d*, C-13), 73.18 (*d*, C-10), 73.50 (*d*, C-2), 73.91 (*d*, C-7), 76.55 (*t*, C-20), 76.75 (*d*, C-9), 78.72 (*s*, C-1), 82.06 (*s*, C-4), 84.04 (*d*, C-5), 128.61 (*t*, Bz2-3′/5′), 129.21 (*s*, Bz2-1′), 130.04 (*t*, Bz2-2′/6′), 133.67 (*d*, Bz2-4′), 134.92 (*s*, C-11), 139.49 (*s*, C-12), 166.98 (*s*, Bz2-CO), 169.38 (*s*, Ac4-CO), 170.48 (*s*, Ac13-CO), 170.58 (*s*, Ac10-CO).

2α-Benzoxy-4α,10β-diacetoxy-1β,7β,9α,13α-tetrahydroxytax-11-ene (9-Dihydrobaccatin III, 2). White amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08 (3H, *s*, H-17), 1.61 (3H, *s*, H-16), 1.78 (3H, *s*, H-19), 1.90 (1H, *ddd*, J = 14.9, 10.1, 1.6 Hz, H-6β), 2.07 (3H, *d*, J = 1.5 Hz, H-18), 2.11 (3H, *s*, Ac10-Me), 2.13 (1H, *dd*, H-14α), 2.22 (3H, *s*, Ac4-Me), 2.25 (1H, *ddd*, J = 15.7, 9.8, 1.0 Hz, H-14β), 2.49 (1H, *ddd*, J = 14.9, 9.3, 7.4 Hz, H-6α), 3.08 (1H, *d*, J = 5.9 Hz, H-3), 4.15 (1H, *dd*, J = 8.3, 0.8 Hz, H-20β), 4.29 (1H, *d*, J = 8.1 Hz, H-20α), 4.40 (1H, *d*, J = 11.0 Hz, H-9), 4.41 (1H, *dd*, J = 9.8, 7.5 Hz, H-7), 4.76 (1H, *m*, H-13), 4.90 (1H, *dd*, J = 9.4, 1.1 Hz, H-5), 5.70 (1H, *d*, J = 6.0, H-2), 6.13 (1H, *d*, J = 10.8 Hz, H-10), 7.45 (2H, *m*, Bz2-3′/5′), 7.58 (1H, *tt*, J = 7.5, 1.4 Hz, Bz2-4′), 8.08 (2H, *dd*, J = 8.5, 1.4 Hz, Bz2-2′/6′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.51 (*q*, C-19), 15.27 (*q*, C-18), 21.39 (*q*, Ac10-Me), 22.05 (*q*, C-16), 23.05 (*q*, Ac4-Me), 28.33 (*q*, C-17), 37.87 (*t*, C-6), 38.64 (*t*, C-14), 42.64 (*s*, C-15), 44.89 (*s*, C-8), 46.98 (*d*, C-3), 68.39 (*d*, C-13), 73.39 (*d*, C-2), 73.73 (*d*, C-10), 73.82 (*d*, C-7), 76.59 (*t*, C-20), 76.82 (*d*, C-9), 78.63 (*s*, C-1), 82.24 (*s*, C-4), 84.21 (*d*, C-5), 128.61 (*t*, Bz2-3′/5′), 129.30 (*s*, Bz2-1′), 130.08 (*t*, Bz2-2′/6′), 133.63 (*d*, Bz2-4′), 134.36 (*s*, C-11), 142.71 (*s*, C-12), 167.02 (*s*, Bz2-CO), 170.72 (*s*, Ac10-CO), 171.60 (*s*, Ac4-CO).

2α-Benzoxy-4α,10β-diacetoxy-1β,7β-dihydroxy-9-dehydrotax-11-ene (13-Dehydroxybaccatin III, 7). White amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.07 (3H, *s*, H-16), 1.09 (3H, *s*, H-17), 1.62 (3H, *s*, H-19), 1.67 (1H, *m*, H-14β), 1.83 (1H, *ddd*, J = 14.8, 10.8, 2.2 Hz, H-6β), 1.91 (1H, *ddd*, J = 19.3, 10.3, 2.5 Hz, H-13α), 1.93 (3H, *d*, J = 0.8 Hz, H-18), 2.20 (3H, *s*, Ac10-Me), 2.25 (1H, *ddd*, J = 15.6, 10.4, 5.6 Hz, H-14α), 2.27 (3H, *s*, Ac4-Me), 2.52 (1H, *ddd*, J = 16.4, 9.5, 6.8 Hz, H-6α), 2.69 (1H, *dddd*, J = 19.0, 11.9, 5.6, 1.3 Hz, H-13β), 3.75 (1H, *d*, J = 6.8 Hz, H-3), 4.13 (1H, *dd*, J = 8.3, 0.8 Hz, H-20β), 4.27 (1H, *d*, J = 8.3 Hz, H-20α), 4.41 (1H, *dd*, J = 10.8, 6.9 Hz, H-7), 4.96 (1H, *dd*, J = 9.7, 2.2 Hz, H-5), 5.56 (1H, *dd*, J = 6.8, 1.2 Hz, H-2), 6.30 (1H, *s*, H-10), 7.43 (2H, *m*, Bz2-3′/5′), 7.56 (1H, *tt*, J = 7.4, 1.4 Hz, Bz2-4′), 8.04 (2H, *dd*, J = 8.4, 1.7 Hz, Bz2-2′/6′); ^{13}C NMR (125 MHz, $CDCl_3$), δ 9.03 (*q*, C-19), 19.56 (*q*, C-16), 20.87 (*q*, C-18 or Ac10-Me), 20.88 (*q*, Ac10-Me or C-18), 21.99 (*q*, Ac4-Me), 25.35 (*t*, C-14), 26.52 (*q*, C-

17), 30.14 (*t*, C-13), 35.70 (*t*, C-6), 42.08 (*s*, C-15), 45.87 (*d*, C-3), 58.81 (*s*, C-8), 72.26 (*d*, C-7), 73.93 (*d*, C-2), 76.27 (*t*, C-20), 76.31 (*d*, C-10), 80.83 (*s*, C-1), 81.25 (*d*, C-4), 84.17 (*d*, C–5), 128.54 (*t*, Bz2-3′/5′), 129.27 (*s*, Bz2-1′), 129.93 (*t*, Bz2-2′/6′), 131.29 (*s*, C-11), 133.56 (*d*, Bz2-4′), 143.81 (*s*, C-12), 166.76 (*s*, Bz2-CO), 169.69 (*s*, Ac4-CO), 171.32 (*s*, Ac10-CO), 204.25 (*s*, C-9).

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