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Substrate specificity for the hydroxylation of polyoxygenated 4(20),11-taxadienes by *Ginkgo* cell suspension cultures

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Abstract

Three C-14 oxygenated taxanes isolated from callus cultures of *Taxus* spp., 2α,5α,10β,14β-tetra-acetoxy-4(20),11-taxadiene **3**, 2α,5α,10β-triacetoxy-14β-propionyloxy-4(20),11-taxadiene **4**, 2α,5α,10β-triacetoxy-14β-(2-methylbutyryl)-oxy-4(20),11-taxadiene **5**, and three deacetylated derivatives of **3**, 10β-hydroxy-2α,5α,14β-triacetoxy-4(20),11-taxadiene **6**, 14β-hydroxy-2α,5α,10β-triacetoxy-4(20),11-taxadiene **7**, 10β,14β-dihydroxy-2α,5α-diacetoxy-4(20),11-taxadiene **8**, could all be regio- and stereo-selectively hydroxylated at the 9α-position by *Ginkgo* cell suspension cultures to yield a series of new 9α,14β-dihydroxylated taxoids. The effects of functional groups, especially at C-14 of the substrates, on the biotransformation were also investigated. The results revealed that substrates with an acetoxyl group at C-14 could be more efficiently 9α-hydroxylated than those with a longer ester chain or a hydroxyl group at C-14. An acetoxyl or hydroxyl group at C-10 had no effect on the conversion rates of the substrates, but substrates with the hydroxyl group (compared with the acetoxyl analogues) could be converted into 9α-hydroxylated products more easily.

Keywords: Taxane; 4(20),11-Taxadiene; Hydroxylation; Cell suspension cultures; *Ginkgo biloba* L.

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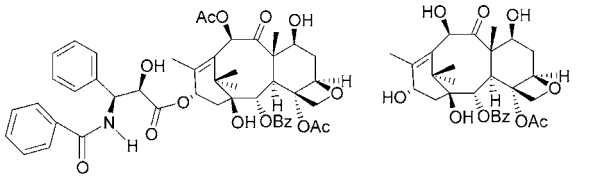
1. Introduction

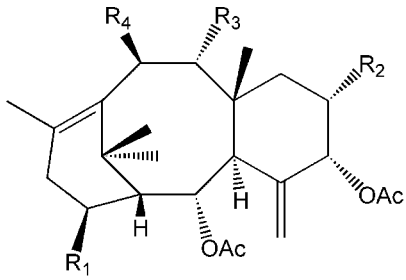
The diterpenoid paclitaxel **1** (Scheme 1), first isolated by Wani and Wall from the bark of the Pacific yew (*Taxus brevifolia* Nutt.) [1], is one of the most effective anticancer drugs used in clinical treatment for various cancers [2]. The structural complexity, significant biological activities of **1**, as well as its unique mechanism of action [3] have led to extensive research towards the synthesis of paclitaxel and other new analogs. The chemical synthesis of paclitaxel has been achieved both by semisynthesis [4], with 10-deacetyl baccatin III **2** as the starting material (Scheme 1), and by total synthesis [5]. However, it seems that the most promising approach for large-scale production of paclitaxel still relies on the combination of biological and semisynthetic methods [6]. As a good example, the Bristol–Myers Squibb Corporation reported recently the semisynthesis of paclitaxel in which biocatalysis was fully employed [6a]. Three novel enzymes were discovered that could convert a variety of taxanes in the plant extracts into a single molecule, 10-deacetyl baccatin III, an important synthetic precursor of paclitaxel. The concentration of 10-deacetyl baccatin III was increased by 5.5–24-folds in the extracts treated with the enzymes, and the difficult separation procedure could be avoided. Biocatalysis processes have also been reported for the preparation of C-13 paclitaxel side chain synthons [6a].

2α,5α,10β,14β-tetra-acetoxy-4(20),11-taxadiene **3** and its analogs, 2α,5α,10β-triacetoxy-14β-propionyloxy-4(20),11-taxadiene **4**, and 2α,5α,10β-triacetoxy-14β-(2-methylbutyryl)oxy-4(20),11-taxadiene **5** (Scheme 2) are major taxanes isolated in high yields (ca. 4% of dry weight) from callus cultures of *Taxus* spp. [7] The abundance of these 14β-hydroxylated taxanes makes them valuable for the semisynthesis of paclitaxel or other structurally related taxoids [8]. A number of studies on their structural modification by chemical and biocatalytic approaches were reported and encouraging progress has been achieved [9]. Recently, we also reported the specific hydroxylation of **3** at the 9α-position by *Ginkgo* cell suspension cultures [10]. That was the first report of 9α-hydroxylation of taxanes by plant cultures, and it might play an important role in the semisynthesis of new taxoids. Could the other closely related taxanes also be hydroxylated at 9α-position by *Ginkgo* cells? And how do the various substituents on substrates affect the biotransformation process? To confirm the capacity of the enzymes in *Ginkgo*



1 taxol **2** 10-deacetyl baccatin III

Scheme 1. Chemical structures of taxol and 10-deacetyl baccatin III.



Compounds	R_1	R_2	R_3	R_4
3	OAc	II	II	OAc
4	$OCOCH_2CH_3$	H	H	OAc
5	$OCOCH(CH_3)CH_2CH_3$	H	H	OAc
6	OAc	H	H	OH
7	OH	H	H	OAc
8	OII	II	II	OII
9	$OCOCH_2CH_3$	H	OH	OAc
10	$OCOCH(CH_3)CH_2CH_3$	H	OH	OAc
11	OAc	H	OH	OH
12	OAc	OH	H	OH
13	OAc	OH	OH	OH
14	OH	H	OH	OAc
15	OH	H	OH	OH

Scheme 2. The chemical structures of **3**–**15**.

cells responsible for the hydroxylation reactions, five taxadienes, **4**, **5**, **6**, **7**, and **8**, in addition to **3**, were tested as substrates for biotransformation by *Ginkgo* cells (Scheme 2). The effects of different substrates on the hydroxylation were investigated by determining the biotransformation rates of the substrates and the yields of the 9α-hydroxylated products.

2. Results

Six substrates with different substitution profiles were obtained, three of which, **3**, **4**, and **5**, were isolated from the callus cultures of *Taxus* spp. and characterized as described in [7a]. The only difference of these compounds is the length of the ester chain at C-14 position, wherein an acetoxyl group occurred in **3**, a propionyloxy group in **4**, and a (2-methylbutyryl)oxy group in **5**. Compound **3** was hydrolyzed under basic conditions as described [9a] to yield three major products identified as **6** (in 10% yield), **7** (in 30% yield), and **8** (in 15% yield). These six compounds were used as substrates in the biotransformation studies.

2.1. Biotransformation of *4* and *5* by Ginkgo cell suspension cultures

Ginkgo cell suspension cultures were verified to be able to convert **4** and **5**. The two substrates were, respectively, administered to the 15-day-old cell cultures, and one more polar product was, respectively, obtained after additional 6 days incubation. FAB mass spectrum of the product (**9**) derived from **4** showed a quasi molecular ion peak $[M + Na]^+$ at *m/z* 557, and HREIMS at 533.8720, consistent with the molecular formula $C_{29}H_{42}O_9$, suggesting the substitution of an additional hydroxyl group as compared with **4**. The ^{1}H-NMR spectrum of **9** was similar to that of **4** except that the signals corresponding to H-9α (δ1.64, dd, $J = 5.6$, 14.9 Hz) or H-9β(δ 2.39, m) in **4** disappeared, while an oxygen-bearing methine signal was observed at δ 4.21 (d, $J = 10.0$ Hz). As a result, the signal of H-10α (δ 6.06, dd, $J = 5.6$, 12.1 Hz) shifted to an upper field at δ 5.82 (d, $J = 10.0$ Hz), suggesting the existence of a hydroxyl group at C-9 position. This was confirmed by the signal of C-9 which significantly shifted downfield to δ 76.2 when compared with δ 43.9 in **4**. The ^{1}H-NMR spectrum of **9** also showed that the coupling constant of H-9 and H-10 was 10 Hz, which indicates α position of the 9-hydroxyl group. Therefore, **9** was determined to be the 9α-hydroxyl derivative of **4**. All the signals of **10** derived from **5** in ^{1}H-NMR, ^{13}C-NMR and FAB mass spectrum were all in good agreement with those of 9α-hydroxy-2α,5α,10β-triacetoxy-14β-(2-methylbutyryl)oxy-4(20),11-taxadiene [11]. The results showed that **4** and **5** could be specifically hydroxylated at 9α position by *Ginkgo* cell suspension cultures.

2.2. Biotransformation of *6, 7, and 8*

Three products were obtained from **6** added on day 15 and incubated with *Ginkgo* cells for additional six days. On the basis of the chemical and physical spectral data, their structures were identified as 9α,10β-dihydroxy-2α,5α, 14β-triacetoxy-4(20), 11-taxadiene (**11**, in 70% yield), [10] 6α,10β-dihydroxy-2α,5α, 14β-triacetoxy-4(20), 11-taxadiene (**12**, in 7.5% yield) [9d], and 6α,9α,10β-trihydroxy-2α,5α,14β-triacetoxy-4(20),11-taxadiene (**13**, in 10% yield), respectively. Also, three products were obtained from **7** under the same conditions, and characterized as 9α,14β-dihydroxy-2α,5α,14β-triacetoxy-4(20),11-taxadiene (**14**, in 20% yield), 6α,10-dihydroxy-2α,

5α,14β-triacetoxy-4(20),11-taxadiene(**12**, in 3% yield) [9d], and 6α,9α,10β-trihydroxy-2α, 5α,14β-triacetoxy-4(20),11-taxadiene (**13**, in 3% yield), respectively. Interestingly, 14-acetylated products (**12** and **13**) were obtained from 14-deacetylated substrate (**7**), which suggested that transfer of acetyl group from C-10 to C-14 or acetylation at C-14 accompanied with deacetylation at C-10 also occurred in the bioprocess.

From **8** was obtained one polar product and identified as 9α, 10β, 14β-trihydroxy-2α, 5α-diacetoxy-4(20),11-taxadiene (**15**, in 20% yield). The FAB mass spectrum showed a quasi molecular ion peak $[M + Na]^+$ at *m/z* 459, and HREIMS at 436.2460, consistent with the molecular formula $C_{24}H_{36}O_7$, suggesting the substitution of an additional hydroxyl group as compared with **8**. The ^{1}H-NMR spectrum of **15** was similar to that of **8**, except that the signals corresponding to H-9α (δ 1.66, dd, $J = 6.0$, 15.0 Hz) or H-9β (δ 2.34, m) in **8** disappeared, while an additional oxygen-bearing methine signal was observed at δ 4.10 (d, $J = 9.5$ Hz). The signal of H-10α (δ 5.10, dd, $J = 5.7$, 11.7 Hz) shifted to an upper field at δ 4.79 (d, $J = 9.5$ Hz). The above evidence suggested the existence of a hydroxyl group at C-9 position. This was confirmed by the signal of C-9 which significantly shifted downfield to δ 78.3 compared with δ 47.0 in **8**. The 9-hydroxyl group was also determined to be in the α position as described for **9** and **10**. Therefore, **15** was characterized as the 9α-hydroxyl derivative of **8**.

2.3. The effects of different substitution groups on the biotransformation reactions

According to the above results, all the six tested taxadienes could be hydroxylated at 9α-position by *Ginkgo* cells. Thus, in order to shed light on the effects of different substitution groups in substrates on the biotransformation process and the substrate-specificity of the responsible enzyme, the above six substrates were used for the biotransformation by the same *Ginkgo* cell suspension cultures, and the amounts of residual substrates and yielded 9α-hydroxyl products were used as indicators. The six substrates at the concentration of 35 mg/L were administered into the 15-day-old cell cultures, respectively. After six additional days of co-incubation, the reactions were stopped, and the extracts were analyzed by HPLC. The results (Table 1) showed that:

Table 1
The effects of different substitution groups of taxanes on the biotransformation by *Ginkgo* cell suspension cultures

Substrates	Biotransformation rates (%)	Yields of 9α-OH derivatives (%)
3	100	70 ± 6.5
4	80 ± 5.8	60 ± 3.8
5	50 ± 3.5	20 ± 1.2
6	100	75 ± 3.3
7	60 ± 6.0	40 ± 2.5
8	50 ± 4.6	30 ± 1.8

Note. Biotransformation rate, the percentage of transformed substrate; yields, the percentage of 9α-OH products, detected by HPLC. Each value represents mean ± SD ($n = 3$). For **3**–**8**, see Scheme 2.

(1) The length of ester chain at C-14 influenced the biotransformation significantly. The substrates with a shorter ester chain were more effectively converted than those with a longer one. For example, the biotransformation rate of **3** and the yield of its 9-hydroxylated product were higher than those of **4**, and **4** higher than those of **5**. In addition, the substrate with a hydroxyl group at C-14, such as **7** and **8**, was less effectively converted than those with an acetoxyl group, such as **3** and **6**. In other words, an acetyl group substituted at C-14 favored the bioprocess. (2) Either an acetoxyl group or a hydroxyl group substituted at C-10 position makes no difference on the biotransformation rate of the substrates, for example, both **3** and **6** were completely converted. However, substitution group at C-10 position influenced the yields of 9α-hydroxylated products, the yield of the 9α-hydroxylated product from the substrate with an hydroxyl group at C-10 (**6**) was higher than that with an acetoxyl group (**3**).

3. Discussion

CH oxidation is one of the most versatile reactions in organic chemistry since potentially useful oxyfunctionized synthons may be obtained from readily accessible hydrocarbons. Only a few conventional synthetic methods, which required extreme reaction conditions and yielded racemic products are, to date, available for this purpose. For this reason, a lot of efforts have been previously expended to develop efficient catalytic oxidations of hydrocarbons. For example, in the past several years, metal catalyzed asymmetric CH oxidations have been performed by employing chiral auxiliaries and optically active oxidants to afford enantiomerically enriched products [12]. Nevertheless, in most of the cases only a moderate enantio-selectivity has been achieved for unactivated alkanes. In this context, biomimetic studies have been carried out to gain insight into the mechanism of the catalytic CH oxidation and to enhance its stereo-selectivity. Despite all these efforts, the development of an effective catalytic asymmetric hydroxylation of unfunctionalized hydrocarbons with broad applicability remains a challenge [13]. Alternatively, microorganisms and plant cell suspension cultures have been successfully applied to the selective oxygenation of unactivated CH bonds in organic substrates [14]. However, it seems that the structures of substrates could greatly influence the biotransformation processes. From our results, the six tested taxanes could all be regio- and stereo-selectively hydroxylated by *Ginkgo* cells but in different yields. The substitution groups at C-14 could affect the reaction significantly, which suggested that the enzyme responsible for the hydroxylation is highly substrate-specific, and that longer C-14 substitution chain could inhibit the binding of substrates with the catalytic enzyme.

It is well known that 9α hydroxylation is probably one of the cytochrome P450-dependent oxidative steps in paclitaxel biosynthetic pathway [15]. So far, the enzyme and its encoding gene are still enigmatic, the biotransformation of taxanes by employing plant or microbial cells may biomimic some steps of taxoid biosynthesis, such as extensive oxidation of the taxane skeleton, therefore, which might give some hints to the paclitaxel biosynthesis.

In conclusion, six taxadienes with different substitution groups could be regio- and stereo-selectively hydroxylated at 9α-position by *Ginkgo* cell suspension cultures. This reaction not only provides a novel approach to prepare bioactive taxoids, but also might, to some extent, assist the biosynthetic studies of paclitaxel.

4. Materials and methods

4.1. General

Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. IR spectra were obtained on a Perkin–Elmer 983 spectrophotometer (KBr). NMR spectra (^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H-^{1}H-COSY, HMQC, HMBC, and NOESY) were recorded in $CDCl_3$ with Bruker DRX-500 or Varian INOVA-500 instrument (^{1}H-NMR, 500 MHz; ^{13}C-NMR, 125 MHz) and chemical shifts were recorded in δ using TMS as internal standard. FABMS spectra were measured on a KYKY-ZHP-5$^{\#}$ mass spectrometer in the positive mode, and HREIMS analyses were performed on a JEOL JMX HX-110 spectrometer (The Instrumental Analysis Center for Chemistry, Tohoku University, Japan). The *Ginkgo* suspension cells were cultured in 500-mL flask with 150 mL medium in the darkness at 25 °C, the inoculum size was 5 g/L of cell cultures (dry weight), shaking at the rate of 110 rpm. All media were autoclaved for 20 min at 121 °C, pH values were adjusted to 5.8 prior to autoclaving and the amount of sucrose addition in medium was 30 g/L [16]. All chemicals were obtained from Beijing Chemical Factory.

4.2. Substrate

2α,5α,10β,14β-tetra-acetoxy-4(20),11-taxadiene(**3**), 2α,5α,10β-triacetoxy-14β-\propionyloxy-4(20),11-taxadiene(**4**) and 2α,5α,10β-triacetoxy-14β-(2-methylbutyryl)oxy-4(20),11-taxadiene(**5**) were isolated from callus cultures of *Taxus* spp. [7a]. 10-Hydroxy-2α,5α,14β-triacetoxy-4(20),11-taxadiene(**6**) 14β-hydroxy-2α,5α,10β-triacetoxy-4(20),11-taxadiene(**7**), and 10β,14β-dihydroxy-2α,5α-diacetoxy-4(20),11-taxadiene(**8**) were prepared from the hydrolysis of **3** [9a]. All the substrates were characterized by chemical and spectral methods.

4.3. Biotransformation of *4*

One hundred milligrams of **4** was dissolved in 10 mL EtOH and distributed into 10 flasks of cell cultures on day 15. After additional six days of incubation, the cell cultures were filtered, the filtrate was collected, and extracted three times with EtOAc. All the extracted solutions were pooled and concentrated in vacuum at 40 °C to give 150 mg residue. The residue was separated on a silica gel column, (silica gel H, 5–40 mesh), eluted with acetone–petroleum ether (60–90 °C) (1:10–1:1) to yield 50 mg of **9**.

*9α-hydroxy-2α,5α,10-triacetoxy-14β-propionyloxy-4(20),11-taxadiene(**9**):* colorless needles. $[\alpha]_D^{25}$ + 45.45° (*c* 0.0067, MeOH); IR ν_{max} (KBr): 3432, 2940, 1726, 1636, 1373, 1236, 1023 cm^{-1}; ^{1}H-NMR δ 5.82(1H, d, J = 10.0 Hz, H-10), 5.34(1H, dd, J = 2.5, 6.5 Hz, H-2), 5.31(2H, brs, H-5, H-20a), 4.97(1H, dd, J = 5.0, 9.0 Hz, H-14), 4.91(1H, brs, H-20b), 4.21(1H, d, J = 10.0 Hz, H-9), 2.95(1H, d, J = 6.0 Hz, H-3), 2.86(1H, dd, J = 9.0, 19.0 Hz, H-13a), 2.42(1H, dd, J = 4.5, 19.5 Hz, H-13b), 2.28(2H, q, J = 7.5 Hz, 14-OCOC$\underline{H_2}$CH$_3$), 2.19(3H, s, 10-OCOCH$_3$), 2.14(3H, brs, 18-CH$_3$), 2.05(3H, s, 2-OCOCH$_3$), 2.01(3H, s, 5-OCOCH$_3$), 1.86(1H, d, J = 2.0 Hz, H-1), 1.83(2H, m, H-6), 1.71(1H, m, H-7a), 1.59(3H, s, 16-CH$_3$), 1.55(1H, m, H-7b), 1.11(3H, s, 17-CH$_3$), 1.11(3H, t, J = 7.5 Hz, 14-OCOCH$_2$C$\underline{H_3}$), 1.05(3H, s, 19-CH$_3$); ^{13}C-NMR δ 173.3(14-OCOCH$_2$CH$_3$), 170.5(10-OCOCH$_3$), 169.9(2-OCOCH$_3$), 169.7(5-OCOCH$_3$), 141.8(C-20), 137.0(C-11), 133.2(C-12), 117.6(C-20), 78.6(C-5), 76.2(C-9), 76.0(C-10), 70.1(C-2),70.0(C-14), 58.7(C-1), 44.7(C-8), 44.0(C-3), 39.6(C-15), 37.1(C-13), 31.5(C-17), 28.4(C-6), 27.9(14-OCO$\underline{C}$H$_2$CH$_3$), 26.1(C-16), 25.8(C-7), 21.9(C-18), 21.4(2-OCOCH$_3$), 21.3(5-OCOCH$_3$), 21.0(10-OCOCH$_3$), 17.5(C-19), 9.14(14-OCOCH$_2\underline{C}$H$_3$); FABMS (NBA) *m/z*: 557(M + Na, 30), 401(3), 341(9), 299(18), 281(30), 135(100); HREIMS for $C_{29}H_{42}O_9$ requires: 533.8714, found: 533.8720.

4.4. Biotransformation of **5**

To 15-day-old cell cultures, 100 mg of **5** was added, and the procedure was performed as for **4.** Ten milligrams of **10** was obtained.

*9α-hydroxy-2α,5α,10β-triacetoxy-14β-(2-methylbutyryl)oxy-4(20),11-taxadiene (**10**):* colorless needles. $[\alpha]_D^{25}$ + 65.40° (*c* 0.0054, MeOH); IR ν_{max} (KBr): 3456, 2940, 1726, 1620, 1373, 1236, 1023 cm^{-1}; ^{1}H-NMR δ 5.80(1H, d, J = 10.0 Hz, H-10), 5.32(1H, dd, J = 2.4, 6.3 Hz, H-2), 5.28(1H, brs, H-20a), 5.24(1H, brs, H-5), 4.93(1H, dd, J = 4.4, 8.8 Hz, H-14), 4.84(1H, brs, H-20b), 4.19(1H, d, J = 10.0 Hz, H-9), 2.93(1H, d, J = 6.3 Hz, H-3), 2.87(1H, dd, J = 9.2, 19.2, H-13a), 2.34(1H, m, 14-OCOC$\underline{H}$(CH$_3$)CH$_2$CH$_3$), 2.32(1H, dd, J = 4.5, 19.2 Hz, H-13b), 2.18(3H, s, 10-OCOCH$_3$), 2.12(3H, s, 18-CH$_3$), 2.05(3H, s, 2-OCOCH$_3$), 2.01(3H, s, 5-OCOCH$_3$), 1.83(2H, m, H-6), 1.82(1H, d, J = 2.4 Hz, H-1), 1.71(1H, m, H-7a), 1.58(3H, s, 16-CH$_3$), 1.55(1H, m, H-7b), 1.46(3H, m, 14-OCOCH(C$\underline{H_3}$)CH$_2$CH$_3$), 1.12(3H, s, 17-CH$_3$), 1.11(3H, d, J = 7.2 Hz, 14-OCOCH(CH$_3$)CH$_2$C$\underline{H_3}$), 1.06(3H, s, 19-CH$_3$), 0.85(2H, t, J = 7.2 Hz, 14-OCOCH (CH$_3$)C$\underline{H_2}$CH$_3$); ^{13}C-NMR δ 175.6(14-OCOCH(CH$_3$)CH$_2$CH$_3$), 170.1(2-OCOCH$_3$), 169.8(5-OCOCH$_3$), 169.6(10-OCOCH$_3$), 141.8(C-4), 137.0(C-11), 133.2(C-12), 117.5(C-20), 78.6(C-5), 76.2(C-9), 76.0(C-10), 70.0(C-2), 69.9(C-14), 59.0(C-1), 44.8(C-8), 44.1(C-3), 41.0(14-OCO$\underline{C}$H(CH$_3$)CH$_2$CH$_3$), 39.7(C-13), 36.8(C-15), 31.5(C-17), 28.5(C-6), 26.7(14-OCOCH($\underline{C}$H$_3$)CH$_2$CH$_3$), 26.2(C-16), 25.8(C-7), 22.4(C-19), 21.0(C-18), 21.3(10-OCOCH$_3$), 21.3(2-OCOCH$_3$), 20.9(10-OCOCH$_3$), 11.5(14-OCOCH (CH$_3$)$\underline{C}$H$_2$CH$_3$), 13.9(14-OCOCH(CH$_3$)CH$_2\underline{C}$H$_3$); FABMS (NBA) *m/z*: 585(M + Na, 15), 401(6), 341(16), 281(30), 136(100); HREIMS for $C_{31}H_{46}O_9$ requires: 562.2142, found: 562.3147.

4.5. Biotransformation of **6**

The procedure was similar to that described for **4**, except that 20 mg of **6** was used, and yielded 10 mg of **11**, 1.5 mg of **12**, and 2.0 mg of **13**.

*9α,10β-dihydroxyl-2α,5α,14β-tri-acetoxy-4(20),11-taxadiene(**11**):* needles; $[\alpha]_D^{25}$ + 40.60° (*c* 0.0061, MeOH); IR ν_{max} (KBr): 3432, 2940, 1726, 1636, 1373, 1236, 1023 cm^{-1}; ^{1}HNMR ($CDCl_3$, 500MHz) δ 5.37(1H, dd, $J = 6.5$, 2.3 Hz, H-2), 5.31(2H, brs, H-5, H-20), 4.97(1H, dd, $J = 4.5$, 8.5 Hz, H-14), 4.90(1H, brs, H-20), 4.80(1H, d, $J = 9.5$ Hz, H-10), 4.09(1H, d, $J = 9.6$ Hz, H-9), 2.93(1H, d, $J = 6.5$ Hz, H-3), 2.82(1H, dd, $J = 8.5$, 9.5 Hz, H-13), 2.46(1H, dd, $J = 5.0$, 19.0 Hz, H-13), 2.18(3H, s, 18-CH_3), 2.05(3H, s, $OCOCH_3$), 2.02(3H, s, $OCOCH_3$), 2.00(3H, s, $OCOCH_3$), 1.86(1H, d, $J = 2.1$ Hz, H-1), 1.81(1H, m, H-6), 1.77(1H, m, H- 7), 1.75(1H, m, H-6), 1.65(3H, s, 16-CH_3), 1.50(1H, m, H-7), 1.20(3H, s, 17-CH_3), 1.03(3H, s, 19-CH_3); $^{1\,3}$CNMR ($CDCl_3$, 500MHz) δ 169.9(2, 5, 14-$OCOCH_3$), 142.1(C-4), 136.7(C-11), 134.4(C-12), 117.6(C-20), 78.8(C-9), 78.5(C-5), 72.2(C-10), 70.6(C-14), 70.2(C-2), 59.0(C-1), 44.4(C-8), 44.2(C-3), 39.6(C-13), 37.4(C- 15), 31.8(C-17), 28.5(C-6), 26.5(C-16), 26.0(C-7), 21.8(C-18), 21.3(2, 10- $OCOCH_3$), 21.2(5-$OCOCH_3$), 17.6(C-19). FABMS (m/z): 501(M + Na, 6), 401(2), 341(1), 299(2), 154(100), 136(94); HREIMS for $C_{26}H_{38}O_8$ requires: 478.2567, found: 478.2575.

*6α,10β-dihydroxyl-2α,5α,14β-triacetoxy-4(20),11-taxadiene(**12**):* white powder. $[\alpha]_D^{25}$ + 47.1° (*c* 0.0072, MeOH); IR ν_{max} (KBr): 3432, 2940, 1726, 1636, 1373, 1236, 1023 cm^{-1}; ^{1}H-NMR δ 5.41(1H, s, H-20a), 5.36(1H, dd, $J = 2.0$, 6.0 Hz, H-2), 5.06 (1H, dd, $J = 6.0$, 12.0 Hz, H-10), 5.04(1H, brs, H-5), 5.01(1H, dd, $J = 9.5$, 5.5 Hz, H-14), 4.97(1H, s, H-20b), 3.89(1H, m, H-6), 2.95(1H, d, $J = 6.0$ Hz, H-3), 2.80(1H, dd, $J = 9.0$, 19.0 Hz, H-13a), 2.42(1H, dd, $J = 3.5$, 19.0 Hz, H-13b), 2.39(1H, m, H-9a), 2.20(3H, s, 18-CH_3), 2.06(3H, s, $OCOCH_3$), 2.02(3H, s, $OCOCH_3$), 1.98(1H, dd, $J = 4.5$, 14.5 Hz, H-7a), 1.94(3H, s, $OCOCH_3$), 1.91(1H, d, $J = 2.5$ Hz, H-1), 1.72(3H, s, 16-CH_3), 1.67(1H, dd, $J = 5.5$, 15.0 Hz, H-9b), 1.48(1H, d, $J = 14.5$ Hz, H-7), 1.19(3H, s, 17-CH_3), 1.04(3H, s, 19-CH_3); $^{1\,3}$C-NMR δ 169.9($OCOCH_3$), 169.9($OCOCH_3$), 169.5($OCOCH_3$), 138.6(C-4), 138.5(C-11), 132.3(C-12), 120.4(C-20), 81.6(C-5), 70.6(C-2), 70.2(C-6, C-14), 67.3(C-10), 58.8(C-1), 47.4(C-9), 41.5(C-3), 41.3(C-7), 39.6(C-15), 39.4(C-8), 37.5(C-13), 32.1(C-17), 25.4(C-16, C-19), 21.5(C-18), 21.5($OCOCH_3$), 21.4(OC-OCH_3), 21.0($OCOCH_3$); FABMS (NBA) *m/z*: 501(M + Na, 28), 401(4), 341(4), 299(8), 281 (12), 154(94), 136(100); HREIMS for $C_{26}H_{38}O_8$ requires: 478.2567, found: 478.2570.

*6α,9α,10β-trihydroxy-2α,5α,14β-triacetoxy-4(20),11-taxadiene(**13**):* colorless needles. IR ν_{max} (KBr): 3432, 2940, 1726, 1636, 1373, 1236, 1023 cm^{-1}; ^{1}H-NMR δ 5.46(1H, s, H-20a), 5.37(1H, dd, $J = 2.5$, 6.0 Hz, H-2), 5.08(1H, brs, H-5), 5.01(1H, s, H-20b), 4.98(1H, dd, $J = 5.1$, 9.3 Hz, H-14), 4.75(1H, d, $J = 9.5$ Hz, H-10), 4.01(1H, d, $J = 9.5$ Hz, H-9), 3.93(1H, m, H-6), 2.96(1H, d, $J = 6.0$ Hz, H-3), 2.82(1H, dd, $J = 8.6$, 18.6 Hz, H-13a), 2.44(1H, dd, $J = 5.1$, 18.6 Hz, H-13b), 2.20(3H, s, 18-CH_3), 2.06(3H, s, $OCOCH_3$), 2.02(3H, s, $OCOCH_3$), 2.00 (1H, m, H-7a), 1.96 (3H, s, $OCOCH_3$), 1.90 (1H, d, $J = 2.0$ Hz, H-1), 1.64(3H, s,

16-CH_3), 1.62(1H, d, $J = 4.0$ Hz, H-7b), 1.24 (3H, s, 17-CH_3), 1.20(3H, s, 19-CH_3); ^{13}C-NMR δ 170.0($OCOCH_3$), 169.8($OCOCH_3$), 169.4($OCOCH_3$), 138.2(C-4), 136.7(C-11), 134.8(C-12), 121.1(C-20), 82.0(C-5), 79.3(C-9), 72.1(C-10), 70.5(C-14), 69.8(C-2), 69.8(C-6), 58.7(C-1), 44.1(C-8), 43.6(C-3), 39.5(C-15), 37.4(C-13), 34.2(C-7), 31.8(C-16), 26.0(C-17), 21.6(18-CH_3, 19-CH_3), 21.3(OC-OCH_3), 21.3($OCOCH_3$), 20.8($OCOCH_3$); FABMS (NBA) *m/z*: 517 (M + Na, 7), 154(100), 136(82); HREIMS for $C_{26}H_{38}O_9$ requires: 494.2616, found: 494.2523.

4.6. Biotransformation of 7

The procedure was similar to that of described above as **4**, except that 300 mg of **7** was added, and gave 50 mg of **14**, 10 mg of **12**, and 10 mg of **13**.

*9α,14β-dihydroxy-2α,5α,10β-triacetoxy-4(20),11-taxadiene(**14**):* colorless needles. IR ν_{max} (KBr): 3432, 2940, 1726, 1636, 1373, 1236, 1023 cm^{-1}; ^{1}H-NMR δ 5.82(1H, d, $J = 10.0$ Hz, H-10), 5.41(1H, dd, $J = 6.5$, 2.3 Hz, H-2), 5.31(1H, brs, H-20a), 5.28(1H, brs, H-5), 4.95(1H, brs, H-20b),4.22(1H, d, $J = 10.0$ Hz, H-9) 4.09(1H, dd, $J = 5.0$, 9.0 Hz, H-14), 2.88(1H, d, $J = 6.0$ Hz, H-3), 2.67(1H, dd, $J = 9.0$, 19.0 Hz, H-13a), 2.53(1H, dd, $J = 5.0$, 19.0 Hz, H-13b), 2.15(3H, s, 10-$OCOCH_3$), 2.11(6H, s, 18-CH_3, 2-$OCOCH_3$), 2.08(3H, s, 5-$OCOCH_3$), 2.00(1H, brs, H-1), 1.81(2H, m, H-6), 1.77(1H, m, H-6a), 1.59(3H, s, 16-CH_3), 1.54(1H, m, H-7b), 1.17(3H, s, 17-CH_3), 1.04(3H, s, 19-CH_3). $^{1\,3}$C-NMR δ 170.5(2-$OCOCH_3$), 169.7(5-$OCOCH_3$), 169.6(10-$OCOCH_3$), 142.3(C-4), 137.5(C-11), 133.3(C-12), 117.4(C-20), 79.0(C-5), 76.2(C-10), 76.0(C-9), 70.6(C-2), 67.5(C-14), 63.2(C-1), 44.7(C-8),44.0(C-3), 42.3(C-13), 37.5(C-15), 31.2(C-17), 28.4(C-6), 26.4(C-7), 25.6(C-16), 21.9(C-18), 21.5(2-$OCOCH_3$), 21.3(10-$OCOCH_3$), 21.1(5-$OCOCH_3$), 17.4(C-19); FABMS (NBA) *m/z*: 501(M + Na, 15), 441(1), 419(2), 401(3), 341(4), 299(6), 154(77), 136(100); HREIMS for $C_{26}H_{38}O_8$ requires: 478.2567, found: 478.2566.

4.7. Biotransformation of 8

The procedure was similar to that of described above as **4**, also, 100 mg of **7** was added, and yielded 20 mg of **15**.

*9α,10β,14β-trihydroxy-2α,5α-diacetoxy-4(20),11-taxadiene(**15**):* colorless prism. $[\alpha]_D^{25}$ + 33.6° (*c* 0.0029, MeOH); IR ν_{max} (KBr): 3432, 2940, 1726, 1636, 1373, 1236, 1023 cm^{-1}; ^{1}H-NMR δ5.42(1H, dd, $J = 2.0$, 6.0 Hz, H-2), 5.30(1H, brs, H-20a), 5.26(1H, t, $J = 2.5$ Hz, H-5), 4.94(1H, brs, H-20b), 4.79(1H, d, $J = 9.5$ Hz, H-10), 4.10(1H, d, $J = 9.5$ Hz, H-9), 4.09(1H, dd, $J = 5.5$, 14.0 Hz, H-14), 2.87(1H, d, $J = 6.0$ Hz, H-3), 2.64(1H, dd, $J = 9.0$, 19.0 Hz, H-13a), 2.52(1H, dd, $J = 4.5$, 19.0 Hz, H-13b), 2.14(3H, s, $OCOCH_3$), 2.08(3H, s, 18-CH_3), 2.01(3H, s, $OCOCH_3$), 1.80(2H, m, H-6), 1.72(1H, d, $J = 2.0$ Hz, H-1), 1.70(1H, m, H-H-7a), 1.64(3H, s, 16-CH_3), 1.49(1H, m, H-7b), 1.20(3H, s, 17-CH_3), 1.02(3H, s, 19-CH_3); ^{13}C-NMRδ 169.7($OCOCH_3$), 169.6($OCOCH_3$), 142.4(C-4), 136.5(C-11), 135.1(C-12), 117.3(C-20), 79.1(C-5), 78.3(C-9), 72.0(C-2), 70.7(C-10), 67.7(C-14), 63.4(C-1), 44.4(C-8),

44.0(C-3), 42.3(C-13), 37.8(C-15), 31.5(C-7), 30.9(C-17), 28.5(C-6), 26.2(C-16), 25.9(C-19), 21.9(C-18), 21.5($OCOCH_3$), 21.3($OCOCH_3$); FABMS (NBA) *m/z*: 459(M + Na, 8), 299(4), 281(3), 154(100), 136(94); HREIMS for $C_{24}H_{36}O_7$ required: 436.2461, found: 436.2460.

4.8. The effects of different substitution groups on the biotransformation

Thirty-five mg/L of the six substrates were separately administered to the 15-day-old cell cultures and the enzymatic reactions were stopped and analyzed by HPLC after additional six days of incubation. The residues were dissolved in the HPLC mobile phase and diluted with the same solution to give 2.0 mL. The amounts of residual substrates and their 9-hydroxylated products were determined by HPLC. HPLC analyses were performed by using Zorbax C_{18} column (25 cm × 4.6 mm ID, 5 μm), eluted with methanol–acetonitrile–water (50:15:35, v/v/v) at a flow rate of 1.0 mL/min and detected at 227 nm.

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