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Combined biotransformations of 4(20),11-taxadienes

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Abstract—Taxuyunnanine C (**1**) and its analogs (**2** and **3**), the C-14 oxygenated 4(20), 11-taxadienes from callus cultures of *Taxus* sp., were regio- and stereo-selectively hydroxylated at the 7β position by a fungus, *Abisidia coerulea* IFO 4011, and it was interesting that the longer the alkyl chain of the acyloxyl group at C-14 became, the higher the yield of 7β-hydroxylated product was. Besides the three 7β-hydroxylated products (**5**, **9**, **17**), other nine new products (**7**, **11**, **12**, **14**, **15**, **16**, **18**, **20** and **21**) and six known products (**4**, **6**, **8**, **10**, **13** and **19**) were obtained. Subsequently, the acetylated derivatives (**24** and **27**) of 7β-and 9α-hydroxylated products of **1** were regio- and stereo-specifically hydroxylated at the 9α position by *Ginkgo* cells and 7β position by *A. coerulea*, respectively. Thus, the two specific oxidations have been combined. These bioconversions would provide not only valuable intermediates for the semi-synthesis of paclitaxel or other bioactive taxoids from **1** and its analogs, but also some useful hints for the biosynthetic pathway of taxoid in the natural *Taxus* plant.
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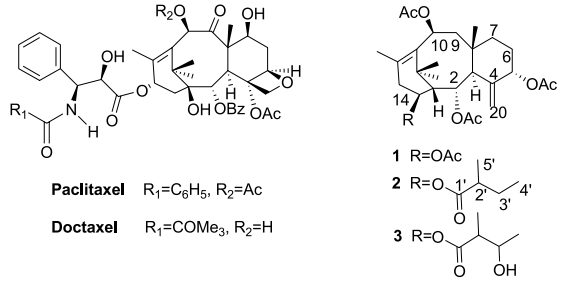
1. Introduction

The diterpenoid paclitaxel (Taxol®, Scheme 1), originally isolated from the Pacific yew (*Taxus brevifolia* Nutt.) in 1971,[1] exhibited remarkably high cytotoxicity and strong antitumor activity against different tumors resistantly treated by existing anticancer drugs.[2] It has been approved for the treatment of advanced ovarian and breast cancers,[3,4] and it is currently in clinical trials for treatment of lung, skin, head and neck cancers with encouraging results.[5] Since the discovery of paclitaxel in the late 1960s, its unique chemical structure, significant biological activity, as well as its novel mechanism of action[6,7] have led to research by scientists from different fields.[8,9]



Scheme 1. The structures of paclitaxel, docetaxel and taxanes from cell cultures of *T. chinensis*.

The source of paclitaxel has been a serious problem all the time since its only approved source was the bark of *T. brevifolia*, which yielded very low amount of paclitaxel (approximately 0.01% of the dry weight). Nowadays, the demand in clinics increases largely because its anticancer spectrum has been broadened and the wild *Taxus* trees have been forbidden to cut for the supply of paclitaxel by the governments of most countries in the world. This situation illustrates a serious resource crisis that has to be alleviated by using various approaches to produce alternate sources of paclitaxel, such as total synthesis, semisynthesis, nursery production of *Taxus* trees, fungal production, plant cell culture, etc.[10] Among them, plant tissue and cell culture of *Taxus* species is considered as one of the most promising approaches to produce paclitaxel and related taxanes. In the past decade, there have been a lot of successful reports and patents on the production of paclitaxel by callus or cell culture of various *Taxus* species, but the paclitaxel content was substantially different according to the particular *Taxus* species being investigated.[11–15] However, for most of these cases, only paclitaxel accumulation was reported, and its content was generally too low to scale-up industrially.

Keywords: *Ginkgo biloba* L.; *Abisidia coerulea* IFO 4011; Cell suspension cultures; 4(20), 11-Taxadiene; Biotransformation; Combination of 7β-and 9α-oxidations.

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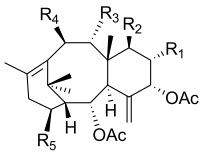
More than 10 years ago, a high and stable taxane-yielding callus strain of *T. chinensis*, (ca. 5–6% of the dry weight of cell cultures) was screened out of various callus strains of different *Taxus* species in our laboratory, which lost the ability to produce paclitaxel and other C-13 oxygenated taxoids, however, possessed the ability to produce C-14 oxygenated 4(20), 11-taxadienes only.[16,17] The three major taxanes are taxuyunnanine C [2α,5α,10β,14β-tetraacetoxytaxa-4(20),11-diene, **1**], 2α,5α,10β-triacetoxy- 14β-(2-methylbutyryl)oxytaxa-4(20),11-diene (**2**), and yunnanxane {2α,5α,10β-triacetoxy-14β-[3 (s)-hydroxy-2 (*R*)-methylbutyryl]oxytaxa- 4(20),11-diene, **3**} (Scheme 1). Their high contents in the cultures and their taxane-skeleton endow them with valuable potential for the semi-synthesis of paclitaxel or other bioactive taxoids. Unfortunately, these compounds have fewer functional groups with the skeleton in comparison with paclitaxel and other bioactive taxoids, such as at C-1, C-7, C-9 and C-13 positions. A number of studies on their structural modification by chemical and biocatalytic approaches were reported, and have achieved a lot of intriguing results.[18–25] However, the regio- and stereoselective introduction of oxygen functional groups at these positions seems to be very difficult through conventional chemical methods. In this context, the enzymatic conversion by employing microorganisms or plant cell suspension cultures is an alternative. In our previous investigation, 9α and 7β selectively hydroxylated products of these taxadeines were obtained successfully by cell suspension cultures of *G. biloba* and the fungus *A. coerulea*, respectively.[22–27] However, could these two selective oxidations be combined by the above two biocatalysts? So, as a part of our ongoing investigations, herein, we report the successful combination of the two reactions by subsequent biotransformation with the aid of chemical modification of the substrates. In addition, in our recent communication, only specific 7β-hydroxylations of **1**, **2** and **3** by *A. coerulea* were reported,[24] however, there are other fifteen products yielded from those bioprocesses, and nine of them are new compounds. These results will be presented in detail, and the effects of different substituents on the biotransformation will also be discussed.

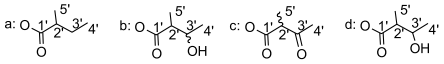
2. Results and discussion

After incubation with cell cultures of fungus *A. coerulea* for 7 days, five more polar metabolites were obtained from **1** by the combination of open silica gel chromatography and semi-prep. HPLC (Scheme 2). On the basis, of the physical and spectroscopic data, their structures were identified as 9α-hydroxy-2α,5α,10β,14β-tetraacetoxytaxa-4(20),11-diene (**4**, 1%),[22] 7β-hydroxy-2α,5α,10β,14β-tetraacetoxytaxa-4(20),11-diene (**5**, 5%),[24] 10β-hydroxy-2α,5α,14β-triacetoxytaxa-4(20),11-diene (**6**, 15%),[19–21,26] 6α-hydroxy-2α,5α,10β,14β-tetraacetoxytaxa-4(20),11-diene (**7**, 2%), 6α,10β-dihydroxy-2α,5α,14β-triacetoxytaxa-4(20),11-diene (**8**, 1%).[19–21,23] Among them, only **7** was a new compound.

The HREIMS, ^{1}H and ^{13}C NMR spectral data of **7** exhibited an elemental composition of $C_{28}H_{40}O_9$ (see Section 4.4.1), suggesting that a hydroxyl group may be introduced. The presence of an OH group in **7** was supported by the IR absorption at 3620 cm^{-1}. ^{1}H NMR spectrum of **7** was similar to that of **1** except that the signals of H-6α or H-6β (2H, δ 1.82, m) in **1** had disappeared, while a new oxymethine proton signal at δ 3.92 (1H, ddd, J=4.1, 5.1, 11.8 Hz) was observed, which was correlated with H-7 and H-5 in ^{1}H–^{1}H COSY spectrum, suggesting that the introduced OH group was at C-6 position. It was confirmed by the signal of C-6 which was substantially shifted downfield at δ 69.11 (d) compared with δ 28.88 (t) in **1**, and by the signal of H-6 which was correlated with C-5 and C-7 in HMBC spectrum. The stereochemistry of 6-OH was determined to be α-configuration by the NOE difference spectrum, in which the integration values of H-5, H-7β and H-19 were enhanced when H-6 was irradiated. Therefore, the structure of **7** was determined as 6α-hydroxy-2α,5α,10β,14β-tetraacetoxytaxa-4(20),11-diene. Although 6α hydroxylation of **1** occurred in the cases of fungus *Cunninghamella echinulata*, cell suspension cultures of *Catharanthus roseus* and *Ginkgo biloba* as the biocatalysts,[19–21,23] but in all cases, the C-10 acetyl group was simultaneously removed to afford **8**, thus this was the first time to get **7** from **1** by biotransformation.



Compounds	R_1	R_2	R_3	R_4	R_5
4	H	H	OH	OAc	OAc
5	H	OH	H	OAc	OAc
6	H	H	H	OH	OAc
7	OH	H	H	OAc	OAc
8	OH	H	H	H	OAc
9	H	OH	H	OAc	a
10	H	H	OH	OAc	a
11	OH	H	H	OAc	a
12	H	OH	H	OH	a
13 and 14 (isomer)	H	H	H	OAc	b
15 and 16 (isomer)	H	H	H	OAc	c
17	H	OH	H	OAc	d
18	H	H	OH	OAc	d
19	H	H	H	OH	d
20	OH	H	H	OAc	d
21	H	OH	H	OH	d



Scheme 2. The transformed products of **1**, **2** and **3** by *A. coerulea*.

Incubation of **2** with *A. coerulea* for 7 days yielded six metabolites (Scheme 2). By combination of ^{1}H NMR, ^{1}H–^{1}H COSY, ^{13}C NMR, DEPT, HMQC, HMBC, NOE, HREIMS and IR spectral analyses, their structures were determined to be 7β-hydroxy-2α,5α,10β-triacetoxy-14β-(2-methylbutyryl)oxytaxa-4(20),11-diene (**9**, 10%),[24] 9α-hydroxy-2α,5α,10β-triacetoxy-14β-(2-methylbutyryl)oxytaxa-4(20),11-diene (**10**, trace),[26] 6α-hydroxy-2α,5α,10β-triacetoxy-14β-(2-methylbutyryl)oxytaxa-4(20),11-diene (**11**, 2%), 7β,10β-dihydroxy-2α,5α-diacetoxy-14β-(2-methylbutyryl)oxytaxa-4(20),11-diene (**12**, trace), 2α,5α,10β-triacetoxy-14β-[3 (s)-hydroxy-2 (*R*)-methylbutyryl]oxytaxa-

4(20),11-diene (**13**, yunnanxane[28]) and 2α,5α,10β-triacetoxy-14β-[3(*R*)-hydroxy-2(*R*)-methylbutyryl]oxytaxa-4(20),11-diene (**14**, 3′-epimer of **13**, obtained as a mixture with **13**, totally in 5% yield). Among them, **11**, **12** and **14** were three new compounds.

The HREIMS spectrum of **11** showed a molecular ion peak $[M]^+$ at *m/z* 562.3154, consistent with the molecular formula of $C_{31}H_{46}O_9$, suggesting that an OH group may be introduced in comparison with **2**. The presence of an OH group in **11** was supported by the IR absorption at 3620 cm^{-1}. ^1H NMR spectrum of **11** was similar to that of **2** except that the signals of H-6α or H-6β (2H, δ 1.82, m) had disappeared, while an additional oxymethine signal at δ 3.96 (1H, ddd, $J=3.9$, 5.1, 11.8 Hz) was observed, suggesting that the OH group may be introduced at C-6 position. It was further supported by the signal of C-6 which was significantly shifted downfield to δ 68.16 (d) when compared with δ 28.88 (t) in **2**, and the correlations of this proton signal with C-5 and C-7 in HMBC spectrum. The stereochemistry of 6-OH was determined to be α-configuration by the NOE difference spectral experiment, in which the integration values of H-5, H-7β and H-19 were enhanced when H-6 was irradiated. Therefore, the structure of **11** was determined as 6α-hydroxy-2α,5α,10β-triacetoxy-14β-(2-methylbutyryl)oxytaxa-4(20),11-diene.

HRESIMS (negative) and HRESIMS (positive) of **12** displayed two quasi molecular ion peaks at *m/z* 519.3019 $[M-H]^+$ and 543.2963 $[M+Na]^+$, respectively, consistent with the molecular formula of $C_{29}H_{44}O_8$. The ^1H NMR spectrum of **12** was similar to that of **9**, but only two OAc signals were observed. The C-10 proton signal was shifted upfield to 0.9 ppm as compared with that of **9**, strongly suggesting a free OH group at C-10 in **12**. The ^1H NMR spectrum of **12** further showed that the resonances corresponding to H-7α or 7β [δ 1.24 (m); 1.98 (m)] in **2** had disappeared, and one new oxygen-bearing methine signal appeared at δ 3.83 (dd, $J=5.1$, 12.2 Hz), suggesting an insertion of an OH group at C-7 position in **12**. It was further supported by the HMBC experiment that this proton was correlated to C-5, C-6, C-8, C-9 and C-19. The stereochemistry of 7-OH was determined to be β-configuration by the NOE difference spectral analysis, in which the integration values of H-3, H-6α, H-10 and H-18 were enhanced when H-7 was irradiated. Thus, the structure of **12** was determined as 7β,10β-dihydroxy-2α,5α-diacetoxy-14β-(2-methylbutyryl)oxytaxa-4(20),11-diene, which could be biosynthesized from **9** via C-10 specific deacetylation.

13 and **14** were obtained as a mixture, clearly as a pair of isomers according to the ^1H and ^{13}C NMR spectra. The separation of these two isomers was tried by normal and reverse phase HPLC, however, it was troublesome and failed to give positive results. The ratio of this pair of isomers was 1:1 by the analysis of ^1H NMR. The NMR and IR spectral data of **13** were in good agreement with those of yunnanxane {2α,5α,10β-triacetoxy-14β-[3 (s)-hydroxy-2 (*R*)-methylbutyryl]oxytaxa- 4(20),11-diene}, and it was also supported by the HRMS experimental data.[28] The NMR data of **14** were very similar to those of **13** except that the signals of C-3′ and H-3′ were at δ 68.01 (δ 69.47 in yunnanxane) and δ 4.04 (δ 3.86 in yunnanxane), therefore, the structure of **14** was determined to be 2α,5α,10β-triacetoxy-14β-[3 (*R*)-hydroxy-2 (*R*)-methylbutyryl]oxytaxa- 4(20),11-diene, the 3′-epimer of yunnanxane. The result showed that **2** could not be specifically hydroxylated at C-3′ position by this fungus. To some extent, this result indicated that **3** was biosynthesized from **2** through C-3′ hydroxylation in the cell cultures of *T. chinensis*, however, in a specific manner.

3 was administered to 2-day-old cell cultures of *A. coerulea* and seven products were isolated by chromatographic methods after additional 7 days of incubation. Based upon the spectral and chemical data, their structures (Scheme 2) were identified as 2α,5α,10β-triacetoxy-14β-(2-methyl-3-keto)-butyryloxytaxa-4(20),11-diene (C-2′ diastereoisomers, **15** and **16**, totally in 10% yield), 7β-hydroxy-2α,5α,10β-triacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene (**17**, 15%),[24] 9α-hydroxy-2α,5α,10β-triacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene (**18**, 2%), 10β-hydroxy-2α,5α-diiacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene (**19**, 5%),[29] 6α-hydroxy-2α,5α,10β-triacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene (**20**, 2%), 7β,10β-dihydroxy-2α,5α-diacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene (**21**, trace). Among them, **15**, **16**, **18**, **20** and **21** were five new compounds.

15 and **16** were obtained as a mixture of C-2′ diastereoisomers. Normal and reverse phase HPLC had been used for their separation, unfortunately, both efforts failed. The ratio of this pair of isomers was 1:1 by the analysis of ^1H NMR. The HREIMS spectrum showed a molecular ion peak $[M]^+$ at *m/z* 560.2987, consistent with the molecular formula of $C_{31}H_{44}O_9$. However, the OH absorption at 3572 cm^{-1} in **3** was not observed in IR spectrum, suggesting that C-3′ OH group probably oxidized to keto group. The ^1H and ^{13}C NMR spectral data were very similar to those of **3** except that the signals of H-3′ at δ 3.86 (dq, $J=7.0$, 6.8 Hz) had disappeared, and the signal of C-3′ was shifted downfield to δ 203.47 (s) as compared with that of **3** at δ 69.47 (d), solidly indicating the presence of C=O group at C-3′ in **15** and **16** rather than an OH group. But how was **3** with a single stereochemistry at C-2′ converted to a pair of isomers with 2′ (*R*) and 2′ (s) configurations? There was no evidence for the mechanism of this conversion yet.

HREI mass spectrum of **18** exhibited a molecular ion peak $[M]^+$ at *m/z* 578.3102, consistent with the molecular formula of $C_{31}H_{46}O_{10}$, indicating the substitution of an additional OH group as compared with **3**. The ^1H NMR spectral data were very similar to those of **3** except that the signals corresponding to H-9α (δ 1.64, dd, $J=5.6$, 14.9 Hz) or H-9β (δ 2.38, m) in **3** had disappeared, while an oxygen-bearing methine signal was observed at δ 4.14 (d, $J=$ 9.8 Hz). And the signals of H-10α (δ 6.06, dd, $J=5.6$, 11.5 Hz) in **3** were shifted upfield to δ 5.75 (d, $J=9.8$ Hz). All of these suggested that an OH group might be introduced at C-9 position. It was confirmed by the signal of C-9 which was significantly shifted downfield to δ 76.23 (d) when compared with δ 43.88 (t) in **3**, and the correlations of this proton to C-3, C-7, C-8, C-10 and C-19 in HMBC spectrum.

The stereochemistry of 9-OH was determined to be α-configuration by the NOE difference spectrum, in which the integration values of H-16 and H-19 were enhanced when H-9 was irradiated. Accordingly, the structure of **18** was identified as 9α-hydroxy-2α,5α,10β-triacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene.

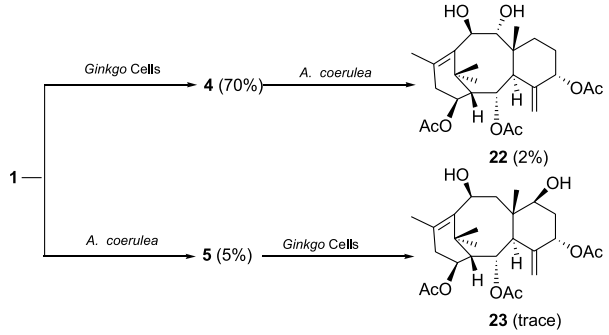
The HREIMS spectrum of **20** showed a molecular ion peak $[M]^+$ at *m/z* 578.3084, consistent with the molecular formula of $C_{31}H_{46}O_9$, suggesting that an OH group may be introduced in comparison with **3**. The ^{1}H NMR spectrum of **20** was similar to that of **3** except that the free methylene signals of H-6α or H-6β (2H, δ 1.80, m) had disappeared, while a new oxymethine signal at δ 3.96 (1H, ddd, J=3.9, 5.1, 11.8 Hz) was observed. In addition, in its ^{13}C NMR spectrum, the signal of C-6 was shifted downfield to δ 68.16 (d) as compared with that of **3** at δ 28.88 (t). These indicated that OH group was introduced at C-6 position. It was further confirmed by the observed correlations of this proton to C-5 and C-7 in HMBC spectrum. The stereochemistry of 6-OH was determined to be α-configuration by the NOE difference spectrum experiment, in which the integration values of H-5, H-7β and H-19 were enhanced when H-6 was irradiated. Therefore, the structure of **20** was determined as 6α-hydroxy-2α,5α,10β-triacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene.

The HRFABMS spectrum of **21** exhibited a quasi molecular ion $[M+Na]^+$ at *m/z* 559.2886, consistent with the molecular formula of $C_{29}H_{44}O_9$. The ^{1}H NMR spectrum of **21** was similar to that of **17** except that only two OAc groups were observed. The C-10 proton signal was shifted upfield to 0.9 ppm as compared with that of **17**, strongly suggesting the existence of a free OH group at C-10 in **21**. The ^{1}H NMR spectrum of **21** further showed that the resonances corresponding to H-7α or 7β [δ 1.24 (m); 1.96 (m)] in **3** had disappeared, and one additional oxygen-bearing methine signal appeared at δ 3.82 (dd, J=5.0, 11.4 Hz), indicating an insertion of an OH group at C-7 position in **21**. This was further supported by the HMBC experiment that this proton signal was correlated to C-5, C-6, C-8 and C-19. The stereochemistry of 7-OH was unambiguously determined to be β-configuration based on the NOE difference spectrum, in which the integration values of H-3, H-6α, H-10 and H-18 were enhanced when H-7 was irradiated. Therefore, the structure of **21** was determined to be 7β,10β-dihydroxy-2α,5α-diacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene (**21**), which might be biosynthesized from **17** by specific deacetylation at C-10.

From the above results, all of the substrates (**1**, **2** and **3**) could be regio- and stereo-selectively hydroxylated at 7β, 9α and 6α positions by the fungus *A. coerulea*, which suggested that the enzymes responsible for these reactions were highly substrate-specific. It was interesting that the longer the alkyl chain of acyloxyl group at C-14 became, the higher the yield of 7β-hydroxylated product was. Additionally, some other reactions also occurred, such as selective deacetylation at C-10 position for all of them, hydroxylation at C-3′ position for **2**, oxidation of OH group to C=O group for **3**. These results indicated that there were several types of enzymes involved in the bioprocess, in other words, biotransformation is an efficient approach to diversify natural products.

Thus, both 7β and 9α hydroxylations of this type of taxanes could be achieved by fungus *A. coerulea* and cell suspension cultures of *G. biloba*,[22,23] respectively. Clearly, 7β and 9α hydroxylations of **1** were constituted two key steps to the semi-synthesis of paclitaxel or other bioactive taxoids from **1** and /or its analogs, therefore, the combination of two oxidations would be of interest and importance, and the further efforts were carried out.

First, 9α and 7β hydroxylated products (**4**, **5**) were prepared as described before,[22–24] and bioconverted directly as the substrates by fungus *A. coerulea* and cell suspension cultures of *Ginkgo* following the method described as in Section 4, respectively. However, not as expected, the desired reactions-7β or 9α hydroxylation did not occur while 10-deacetylation occurred in the both cases and yielded products **22** and **23** (Scheme 3). Their structures were identified as 9α,10β-dihydroxy-2α,5α,14β-triacetoxy-taxa-4(20), 11-diene (**22**) and 7β,10β-dihydroxy-2α,5α,14β-triacetoxy-taxa-4(20),11-diene (**23**)[4] by IR, NMR, HRMS analyses. **22** was a known compound obtained firstly from the biotransformation of **1** by *Ginkgo* cells,[22] and **23** was a new compound.



Scheme 3. Subsequent biotransformation of **4** and **5** by *A. coerulea* and *Ginkgo* cells.

The HRFABMS spectrum of **23** exhibited a quasi molecular ion peak $[M+Na]^+$ at *m/z* 501.2472, consistent with the molecular formula of $C_{26}H_{38}O_8$, suggesting the removal of one acetyl group in comparison with the molecular of **5**. The presence of three acetoxyl groups in ^{1}H and ^{13}C NMR spectra of **23** confirmed the deduction. The ^{1}H NMR spectrum of **23** was similar to that of **5** except that the signal of H-10β was shifted to an upper field at δ 5.08 (dd, J=5.1, 12.0 Hz) as compared with that of **5** at δ 5.97 (dd, J=5.1, 12.0 Hz), suggesting the existence of an OH group rather than an OAc group at C-10. It was supported by its ^{13}C NMR spectrum in which the signal of C-10 was shifted to an upper field at δ 66.90 (d) compared with that of **5** at δ 69.58 (d). Therefore, **23** was elucidated to be 7β,10β-dihydroxy-2α,5α,14β-triacetoxy-taxa-4(20),11-diene, the 10-deacetyl derivative of **5**, might be formed through specific deacetylation.

The above results indicated somewhat that the enzymes responsible for 7β and 9α hydroxylations were strictly substrate-specific. Considering the effects of the structure of

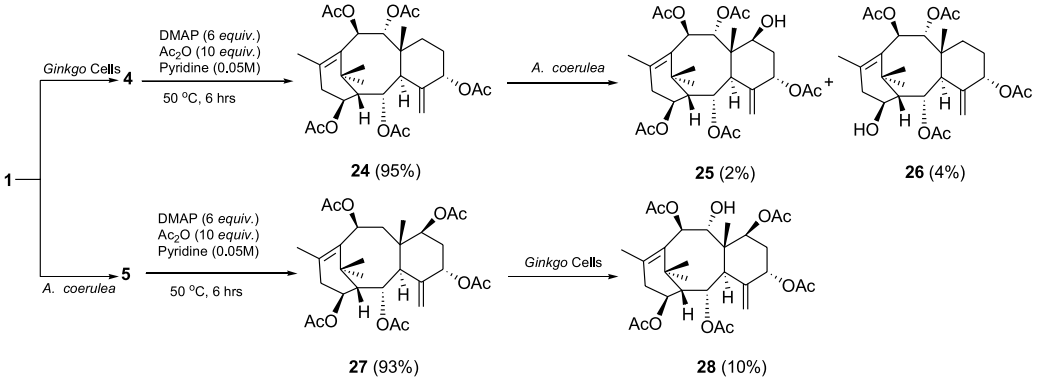
the substrate (substituent, polarity, conformation, etc.) on the biotransformation, we tried the following strategies. **4** and **5** were first acetylated by routinely chemical method, their acetylated products (**24**, **27**; Scheme 4) were subsequently biotransformed by fungus *A. coerulea* and cell suspension cultures of *Ginkgo*, respectively. Intriguingly, the desired hydroxylations took place in both cases, and their corresponding 7β and 9α hydroxylated products (**25** and **28**) were obtained in about 2 and 10% yields, respectively. Also one byproduct, compound **26** (4%) was formed in the former incubation. Their structures were determined on the basis of the ^{1}H NMR, ^{1}H–^{1}H COSY, ^{13}C NMR, DEPT, HMQC, HMBC, NOE, HRMS and IR spectral data.

The HRFABMS spectrum of **25** showed two quasi molecular ion peaks $[M+Na]^+$ and $[M+H]^+$ at *m/z* 601.2630 and 579.2736, respectively, consistent with the molecular formula of $C_{30}H_{42}O_{11}$, suggesting that an OH group may be introduced. The presence of an OH group in **25** was confirmed by the IR absorption at 3614 cm^{-1}. The ^{1}H NMR spectrum of **25** was similar to that of **24** except that the signals of H-7α or H-7β [2H, δ 1.68, m] in **24** had disappeared, while an additional oxymethine signal at δ 3.90 (dd, $J=5.4$, 11.5 Hz) was observed, indicating an OH introduction at C-7 position. It was supported by the signal of C-7 which was shifted to a downfield at δ 71.67 (d) as compared with δ 27.27 (t) in **24**, and by the correlations of this proton with C-5, C-6, C-8, C-9 and C-19 in HMBC. The stereochemistry of 7-OH was determined to be β-configuration by the NOE difference spectrum, in which the integration values of H-3, H-6α, H-10 and H-18 were enhanced when H-7 was irradiated. Thus, the structure of **25** was determined as 7β-hydroxy-2α,5α,9α,10β,14β-pentaacetoxy-taxa- 4(20),11-diene.

The HRFABMS spectrum of **26** exhibited two quasi molecular ion peaks $[M+Na]^+$ and $[M+H]^+$ at *m/z* 543.2571 and 521.2751, respectively, consistent with the molecular formula of $C_{28}H_{40}O_9$. The ^{1}H NMR spectrum of **26** was similar to that of **24** except that four OAc groups were observed, indicating that one acetyl group may be eliminated, and it was supported by the IR absorption at 3624 cm^{-1}. It was further confirmed by which the signals of H-14α and C-14 in ^{1}H NMR and ^{13}C NMR spectra of **26** were shifted upfield at δ 4.08 (dd, $J=5.0$, 9.0 Hz) and δ 67.56 (d) as compared with those of **24** at δ 4.97 (dd, $J=4.9$, 9.0 Hz) and δ 68.87 (d), respectively. So, compound **26** was identified to be 14β-hydroxy-2α,5α,9α,10β-tetraacetoxy-taxa-4(20),11-diene, the 14-deacetyl derivative of compound **24**.

The HRFABMS spectrum of **28** displayed two quasi molecular ion peaks $[M+Na]^+$ and $[M+H]^+$ at *m/z* 601.2626 and 579.2742, respectively, consistent with the molecular formula of $C_{30}H_{42}O_{11}$, implying that an OH group may be introduced. The presence of an additional OH group in **28** was confirmed by the IR absorption at 3620 cm^{-1}. The ^{1}H NMR of **28** was similar to that of **27** except that the signal of H-9α or H-9β (1H, δ 2.10, m; 1H, δ 1.98, m) in **27** had disappeared, however, a new oxymethine signal at δ 4.20 (1H, d, $J=11.5$ Hz) was observed, suggesting the introduction of an OH group at C-9 position. It was supported by the fact that the signal of C-9 was shifted downfield at δ 76.24 (d) in ^{13}C NMR spectrum of **28** as compared with that of **27** at δ 37.18 (t), and by the correlations of this activated carbon with H-3, H-7, H-10 and H-19 in HMBC spectrum. The stereochemistry of 9-OH was determined to be α-configuration by the NOE difference spectrum, in which the integration values of H-16 and H-19 were enhanced when H-9 was irradiated. Therefore, the structure of **28** was elucidated to be 9α-hydroxy-2α,5α,7β,10β,14β-pentaacetoxy-taxa-4(20),11-diene.

Thus, with the aid of simple chemical acetylation, the two selective 9α and 7β bio-oxidations of taxuyunanine C were combined by the cell cultures of *G. biloba* and fungus *A. coerulea*. The results suggested that the structure of the substrate (substituent, polarity, etc.) had influenced on the enzymatic process, and there have already been a great many of reports on it.[26,30–33] On the other hand, it was implied that subtle modification to the structure of substrate (i.e. substrate engineering) was one of the most efficient and simplest methods for obtaining helpful changes (e.g. biotransformation mode, yield, etc.) in biotransformation. Furthermore, the biotransformation of taxanes by employing plant and/or microbial cells may biomimic some steps of taxoid biosynthesis, not only extensive oxidations of the taxane skeleton, but also the order(s) of these functionalizations. It is well known that 9α and 7β oxidations are the two key steps in the taxoid biosynthesis, however, the order of the two steps of taxoids biosynthesis still remains unclear. Our results might somewhat provide a hypothesis that the 7β hydroxylation would occur before 9α functionalization or



Scheme 4. Combination of 9α and 7β oxidations of **1** by *A. coerulea* and *Ginkgo* cells.

after 9α acetoxylation, while, 9α hydroxylation would occur before 7β functionalization or after 7β acetoxylation. To some extent, the results were in good accordance with the results reported by Croteau and his colleagues recently.[34]

3. Conclusion

In conclusion, three C-14 oxygenated taxanes with different substitution groups at C-14 position could be regio- and stereo-selectively hydroxylated at 7β position by fungus *A. coerulea* in different yields. Moreover, nine new taxoids of the 18 products were obtained from these biotransformatios. Most importantly, the combination of 7β- and 9α-oxidations by two different biocatalytic systems, *A. coerulea* and *Ginkgo* cells, has been achieved with the aid of simple chemical modification of the substrates. The results would supply the very useful intermediates for the semi-synthesis of paclitaxel and other bioactive taxoids from readily available natural products—taxuyunanine C and/or its analogs, although in low yields. Additionally, these results might provide a useful tool to probe some important biosynthetic steps of taxoids and/or the order of these steps in *Taxus* plant.

4. Experimental

4.1. General

Optical rotations were obtained using a Horiba SEPA-200 polarimeter. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded with a Varian Unity-PS instrument using $CDCl_3$ as solvent and internal standard. ^{1}H NMR and ^{13}C NMR assignments were determined by ^{1}H–^{1}H COSY, DEPT, HMQC and HMBC experiments. HRFABMS were carried out on a JEOL-HX 110 FAB-mate instrument and HREIMS on a JEOL-HX 110 instrument. HRESIMS were performed on a Bruker Daltonics APEX II with a 7 T magnet, compounds formed sodiated ions under the ESI condition with a resolution range from 31,000 to 60,000. IR spectra were taken on a Hitachi 270-30 spectrometer in $CHCl_3$. Semi-preparative HPLC was performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil (GL Science, 25 cm×10 mm i.d.) stainless steel column and an YRU-883 RI/UV bi-detector, the flow rate was 5.0 mL/min unless otherwise mentioned. Silica gel (230–300 mesh) was employed for flash column chromatography, analytical TLC plates (silica gel 60 F_{254}, Merck) were visualized at UV_{254} and by spraying 5% H_2SO_4 (in EtOH) followed by heating. Pyridine for acetylation was distilled from CaH_2, and the reactions were run under an atmosphere of N_2.

4.2. Substrates

Compounds **1**–**3** (purities: >95% by HPLC analyses) were isolated from callus cultures (Ts-19 strain) of *T. chinensis* and identified by chemical and spectral methods.[16,17] the substrates were dissolved in EtOH (50 mg/mL) before use.

4.3. Organisms, media and cultivation conditions

The cell suspension cultures of *G. biloba* were cultivated in 500 mL Erlenmeyer flask with 150 mL of liquid MS medium supplemented with 0.5 mg/L of naphthalene acetic acid, 0.5 mg/L of 6-benzylaminopurine and 0.2 mg/L of 2,4-dichlorophenoxy acetic acid on the rotary shaker at 110 rpm at (25±2) °C in the dark.[35] The inoculum size was 5 g/L of cell cultures (dry weight) and subcultured every 21 days. The cell cultures were maintained in the above conditions before use for the biotransformation. The fungus, *A. coerulea* IFO4011was purchased from Institute for Fermentation, Osaka, Japan (IFO), and kept on solid PDA medium containing potato (200 g/L), sucrose (20 g/L) and agar (2%) at 4 °C. The seed cultures were prepared in 500 mL flask with 150 mL of liquid medium (PDA medium without agar) and incubated for 2 days. Seed cultures (5 mL) was added to 500 mL flask and shaken at 110 rpm at (25±2) °C in the dark for the use of biotransformation.

4.4. Biotransformation of 1 with *A. coerulea*

1 (400 mg) was dissolved in EtOH (8.0 mL), distributed among forty flasks of 2-day-old cultures and incubated for additional 7 days, after which the cultures were filtered under vacuum, and the filtrate was saturated with NaCl and extracted 5 times with ethyl acetate. All the extracts were pooled, dried with anhydrous Na_2SO_4, and concentrated under vacuum at 40 °C to give 700 mg of residue. The dried cell cultures were extracted thrice by sonication with ethyl acetate, the resulting extracts were pooled and concentrated under vacuum at 40 °C to afford 136.9 mg of residue. The above two parts of extracts were combined and separated by combination of open silica gel chromatography and normal phase semi-prep. HPLC to afford **1** (260 mg, 65%; analyzed by TLC and ^{1}H NMR), **4** (4.5 mg, ca. 1%; t_R=7.6 min; mobile phase: hexane/ethyl acetate=50/50, v/v), **5** (20 mg, ca. 5%; t_R=11.3 min; mobile phase: hexane/ethyl acetate=50/50, v/v), **6** (60 mg, ca. 15%; t_R=8.1 min; mobile phase: hexane/ethyl acetate=50/50, v/v), **7** (9.0 mg, ca. 2%; t_R=19.6 min; mobile phase: hexane/ethyl acetate=50/50, v/v), and **8** (4.0 mg, ca. 1%; t_R=11.7 min; mobile phase: hexane/ethyl acetate=30/60, v/v).

4.4.1. 6α-Hydroxy-2α,5α,10β,14β-tetraacetoxy-taxa-4(20),11-diene (7). White powder; $[\alpha]_D^{20}$ +44.9° (*c* 0.5, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3620, 2936, 1732, 1436, 1240, 1102, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.90 (1H, d, *J*=2.0 Hz, H-1), 5.35 (1H, dd, *J*=2.1, 6.4 Hz, H-2), 2.89 (1H, d, *J*=6.3 Hz, H-3), 5.43 (1H, d, *J*=3.7 Hz, H-5), 3.92 (1H, ddd, *J*=4.1, 5.1, 11.8 Hz, H-6), 1.82 (1H, dd, *J*=12.4, 12.4 Hz, H-7β), 1.56 (1H, dd, *J*=5.1, 12.7 Hz, H-7α), 2.35 (1H, dd, *J*=12.2, 14.6 Hz, H-9β), 1.68 (1H, dd, *J*=5.4, 14.9 Hz, H-9α), 6.03 (1H, dd, *J*=5.6, 12.2 Hz, H-10), 2.81 (1H, dd, *J*=9.0, 19.0 Hz, H-13β), 2.44 (1H, dd, *J*=4.9, 19.0 Hz, H-13α), 4.99 (1H, dd, *J*=4.9, 9.3 Hz, H-14), 1.65 (3H, s, H-16), 1.12 (3H, s, H-17), 2.11 (3H, br s, H-18), 0.86 (3H, s, H-19), 5.38 (1H, s, H-20a), 4.94 (1H, s, H-20b), 2.24, 2×2.05, 2.02 [3H each, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 58.8 (d, C-1), 70.4 (d, C-2), 41.2 (d, C-3), 140.0 (s, C-4), 80.4 (d, C-5), 69.1 (d, C-6), 42.2 (t, C-7), 38.0 (s, C-8), 43.5 (t, C-9), 69.9 (d, C-10), 135.4 (s, C-11), 134.9 (s, C-12), 39.4 (t, C-13), 70.2 (d, C-14), 37.3 (s, C-15), 25.4 (q,

C-16), 31.8 (q, C-17), 21.0 (q, C-18), 23.4 (q, C-19), 119.7 (t, C-20), 21.7, 2×21.42, 21.4 [q, OAc (CH_3)], 171.0, 170.1, 170.0, 169.9 [s, OAc (CO)]; HREIMS *m/z* 520.2666 $[M]^+$ (calcd 520.2672 for $C_{28}H_{40}O_9$).

4.5. Biotransformation of 2 with *A. coerulea*

The procedures were performed as described in Section 4.4, except that 540 mg of **2** was used, finally 1162 mg of extract (472 mg for filtrate, 690 mg for cell cultures) was afforded. The extract was fractionated and separated by combination of open silica gel chromatography and normal phase semi-prep. HPLC (in this experiment the flow rate was 4 mL/min) to give **2** (360 mg, 66.7%; analyzed by TLC and ^{1}H NMR), **9** (60 mg, ca. 10%; t_R=34.6 min; mobile phase: hexane/ethyl acetate=70/30, v/v), **10** (4 mg; t_R=23.8 min; mobile phase: hexane/ethyl acetate=75/25, v/v), **11** (12 mg, ca. 2%; t_R=28.1 min; mobile phase: hexane/ethyl acetate=60/40, v/v), **12** (3 mg; t_R=15.9 min; mobile phase: hexane/ethyl acetate=60/30,v/v), **13** and **14** (isomers, 28 mg, ca.5%; t_R=31.7 min; mobile phase: hexane/ethyl acetate=70/30, v/v).

4.5.1. 6α-Hydroxy-2α,5α,10β-triacetoxy-14β-(2-methyl)-butyryloxytaxa-4(20),11-diene (11). White powder; $[\alpha]_D^{20}$ +47.9° (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3620, 2936, 1732, 1436, 1374, 1240, 1102, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.92 (1H, d, *J*=2.0 Hz, H-1), 5.39 (1H, dd, *J*=2.2, 6.6 Hz, H-2), 2.94 (1H, d, *J*=6.4 Hz, H-3), 5.47 (1H, d, *J*=3.9 Hz, H-5), 3.96 (1H, ddd, *J*=3.9, 9.3, 16.1 Hz, H-6), 1.86 (1H, dd, *J*=12.5, 12.5 Hz, H-7β), 1.59 (1H, dd, *J*=5.6, 12.5 Hz, H-7α), 2.35–2.45 (1H, m, H-9β), 1.72 (1H, dd, *J*=5.9, 14.9 Hz, H-9α), 6.07 (1H, dd, *J*=5.6, 12.2 Hz, H-10), 2.88 (1H, dd, *J*=9.2, 19.0 Hz, H-13β), 2.42 (1H, dd, *J*=4.4, 19.0 Hz, H-13α), 5.02 (1H, dd, *J*=4.6, 9.0 Hz, H-14), 1.70 (3H, s, H-16), 1.16 (3H, s, H-17), 2.15 (3H, br s, H-18), 0.90 (3H, s, H-19), 5.41 (1H, s, H-20a), 4.94 (1H, s, H-20b), 2.37 (1H, dq, *J*=6.8, 7.3 Hz, H-2′), 1.68 (1H, dq, *J*=6.8, 7.3 Hz, H-3′a), 1.49 (1H, dq, *J*=7.1, 7.3 Hz, H-3′b), 0.92 (3H, t, *J*=7.3 Hz, H-4′), 1.15 (3H, d, *J*=7.3 Hz, H-5′), 2.29, 2.09, 2.05 [3H each, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 59.1 (d, C-1), 70.2 (d, C-2), 41.3 (d, C-3), 140.0 (s, C-4), 80.3 (d, C-5), 69.2 (d, C-6), 42.2 (t, C-7), 38.0 (s, C-8), 43.6 (t, C-9), 69.9 (d, C-10), 135.4 (s, C-11), 135.0 (s, C-12), 39.7 (t, C-13), 70.0 (d, C-14), 37.3 (s, C-15), 25.4 (q, C-16), 31.7 (q, C-17), 21.0 (q, C-18), 23.4 (q, C-19), 119.6 (t, C-20), 175.7 (s, C-1′), 41.1 (d, C-2′), 26.8 (t, C-3′), 11.6 (q, C-4′), 16.6 (q, C-5′), 21.8, 21.3, 21.4 [q, OAc (CH_3)], 171.0, 170.1, 169.9 [s, OAc (CO)]; HREIMS *m/z* 562.3154 $[M]^+$ (calcd 562.3142 for $C_{31}H_{46}O_9$).

4.5.2. 7β,10β-Dihydroxy-2α,5α-diacetoxy-14β-(2-methyl)-butyryloxytaxa-4(20),11-diene (12). White powder; $[\alpha]_D^{20}$ +59.5° (*c* 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3624, 2972, 2940, 1732, 1460, 1376, 1244, 1214, 1154, 1084, 1058, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.87 (1H, d, *J*=2.0 Hz, H-1), 5.42 (1H, dd, *J*=2.2, 6.6 Hz, H-2), 2.83 (1H, d, *J*=6.6 Hz, H-3), 5.33 (1H, t, *J*=3.4 Hz, H-5), 2.05–2.15 (1H, m, H-6α), 1.58–1.62 (1H, m, H-6β), 3.83 (1H, dd, *J*=5.1, 12.2 Hz, H-7), 2.25–2.35 (1H, m, H-9β), 2.10–2.16 (1H, m, H-9α), 5.08 (1H, dd, *J*=5.4, 11.7 Hz, H-10), 2.83 (1H, dd, *J*=9.3, 19.0 Hz, H-13β), 2.37 (1H, dd, *J*=5.1, 19.0 Hz, H-13α), 4.95 (1H, dd, *J*=4.9, 9.3 Hz, H-14), 1.76 (3H, s, H-16), 1.19 (3H, s, H-17), 1.95 (3H, br s, H-18), 0.76 (3H, s, H-19), 5.28 (1H, s, H-20a), 4.88 (1H, s, H-20b), 2.26–2.38 (1H, m, H-2′), 1.64–1.72 (1H, m, H-3′a), 1.45–1.52 (1H, m, H-3′b), 0.89 (3H, t, *J*=7.3 Hz, H-4′), 1.12 (3H, d, *J*=6.8 Hz, H-5′), 2.18, 2.07 [3H each, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 59.4 (d, C-1), 70.1 (d, C-2), 40.4 (d, C-3), 140.4 (s, C-4), 77.5 (d, C-5), 37.5 (t, C-6), 69.3 (d, C-7), 44.4 (s, C-8), 40.5 (t, C-9), 66.9 (d, C-10), 139.0 (s, C-11), 132.2 (s, C-12), 37.0 (t, C-13), 70.2 (d, C-14), 37.4 (s, C-15), 25.4 (q, C-16), 32.0 (q, C-17), 21.2 (q, C-18), 16.7 (q, C-19), 117.9 (t, C-20), 175.7 (s, C-1′), 41.1 (d, C-2′), 26.8 (t, C-3′), 11.6 (q, C-4′), 16.6 (q, C-5′), 21.8, 21.4 [q, OAc (CH_3)], 169.8, 169.9 [s, OAc (CO)]; HRESIMS (negative) *m/z* 519.3019 $[M-H]^+$ (calcd 519.2958 for $C_{29}H_{43}O_8$) and HRESIMS (positive) *m/z* 543.2963 $[M+Na]^+$ (calcd 543.2934 for $C_{29}H_{44}O_8Na$).

4.5.3. 2α,5α,10β-Triacetoxy-14β-[3(*R*)-hydroxy-2(*R*)-methyl]-butyryloxytaxa-4(20),11-diene (14). White powder (mixed with **13**); IR ν_{max} ($CHCl_3$): 3620, 2994, 2936, 1728, 1646, 1240, 1102, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.88 (1H, d, *J*=2.0 Hz, H-1), 5.34 (1H, dd, *J*=2.2, 6.6 Hz, H-2), 2.93 (1H, d, *J*=6.4 Hz, H-3), 5.29 (1H, br s, H-5), 1.76–1.86 (2H, m, H-6), 1.97 (1H, ddd, *J*=6.3, 13.0, 13.0 Hz, H-7α), 1.20–1.26 (1H, m, H-7β), 2.35–2.45 (1H, m, H-9β), 1.64 (1H, dd, *J*=5.8, 13.9 Hz, H-9α), 6.06 (1H, dd, *J*=5.6, 11.9 Hz, H-10), 2.85 (1H, dd, *J*=9.3, 19.0 Hz, H-13β), 2.38–2.46 (1H, m, H-13α), 5.03 (1H, dd, *J*=4.6, 9.0 Hz, H-14), 1.67 (3H, s, H-16), 1.13 (3H, s, H-17), 2.10 (3H, s, H-18), 0.85 (3H, s, H-19), 5.28 (1H, s, H-20a), 4.83 (1H, s, H-20b), 2.40–2.45 (1H, m, H-2′), 4.04 (1H, dq, *J*=6.3, 5.6 Hz, H-3′), 1.16 (3H, d, *J*=7.3 Hz, H-4′), 1.17 (3H, d, *J*=7.0 Hz, H-5′), 2.18, 2.06, 2.04 [s, 3H each, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 59.2 (d, C-1), 70.5 (d, C-2), 42.1 (d, C-3), 142.2 (s, C-4), 78.2 (d, C-5), 28.9 (t, C-6), 33.8 (t, C-7), 39.7 (s, C-8), 43.9 (t, C-9), 70.0 (d, C-10), 135.4 (s, C-11), 134.6 (s, C-12), 39.5 (t, C-13), 70.8 (d, C-14), 37.3 (s, C-15), 25.4 (q, C-16), 31.7 (q, C-17), 20.9 (q, C-18), 22.5 (q, C-19), 116.9 (t, C-20), 174.7 (s, C-1′), 46.6 (d, C-2′), 68.0 (d, C-3′), 19.9 (q, C-4′), 11.0 (q, C-5′), 21.9, 21.4, 20.9 [q, OAc (CH_3)], 170.2, 170.0, 169.8 [s, OAc (CO)]; HREIMS *m/z* 562.3143 $[M]^+$ (calcd 562.3142 for $C_{31}H_{46}O_9$).

4.6. Biotransformation of 3 with *A. coerulea*

The procedures were carried out as described in Section 4.4, except that 500 mg of **3** was used, finally 973 mg of extract (500 mg for filtrate, 473 mg for cell cultures) was afforded. The extract was fractionated and separated by combination of open silica gel chromatography and normal phase semi-prep. HPLC to yield 300 mg of **3** (ca. 60%; analyzed by TLC and ^{1}H NMR), 50 mg of the mixture of **15** and **16** (ca. 10%; isomers, t_R=13.1 min; mobile phase: hexane/ethyl acetate=7/3, v/v), 76 mg of **17** (ca. 15%; t_R=16.8 min; mobile phase: hexane/ethyl acetate=50/50, v/v), 2 mg of **18** (t_R=12.9 min, mobile phase: hexane/ethyl acetate=50/50, v/v), 25.0 mg of **19** (ca. 5%; t_R=14.7 min; mobile phase: hexane/ethyl acetate=50/50,v/v), 10 mg of **20** (ca. 2%; t_R=18.0 min; mobile phase: hexane/ethyl acetate=1/2, v/v), and 2 mg of **21** (t_R=20.5 min; mobile phase: hexane/ethyl acetate=1/4, v/v).

4.6.1. 2α,5α,10β-Triacetoxy-14β-(2-methyl-3-keto)-butyryloxytaxa-4(20),11-diene (isomers, 15 and 16, the

ratio was 1:1 by ^{1}H NMR analysis). White powder; IR ν_{max} ($CHCl_3$): 3000, 2940, 1730, 1646, 1456, 1376, 1320, 1246, 1230, 1156, 1106, 1072, 1018 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.86 (1H, br s, H-1), 5.35 (1H, dd, J=2.4, 6.6 Hz, H-2), 2.910 and 2.904 (1H, d, J=6.1 Hz, H-3, **15** and **16**), 5.28 (1H, br s, H-5), 1.75–1.85 (2H, m, H-6), 1.90–2.02 (1H, m, H-7β), 1.20–1.28 (1H, m, H-7α), 2.32–2.44 (1H, m, H-9β), 1.60–1.70 (1H, m, H-9α), 6.04 (1H, dd, J=5.5, 11.9 Hz, H-10), 2.84 (1H, dd, J=9.3, 19.3 Hz, H-13β), 2.36–2.44 (1H, m, H-13α), 5.033 and 5.024 (1H, dd, J=5.1, 9.8 Hz, H-14, **15** and **16**), 1.65 (3H, s, H-16), 1.08 and 1.07 (3H, s, H-17, **15** and **16**), 2.09 (3H, br s, H-18), 0.84 (3H, s, H-19), 3.471 and 3.465 (1H, q, J=7.2 Hz, H-2′, **15** and **16**), 2.22 and 2.21 (3H, s, H-4′, **15** and **16**), 1.32 (3H, d, J=7.3 Hz, H-5′), 2.086 (3H, s, 2-OAc), 2.176 and 2.168 (3H, 5-OAc, **15** and **16**), 2.043 and 2.035 (3H, s, 10-OAc, **15** and **16**); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 59.02 and 59.00 (d, C-1, **15** and **16**), 70.39 and 70.33 (d, C-2, **15** and **16**), 42.13 and 42.11 (d, C-3, **15** and **16**), 142.19 and 142.14 (s, C-4, **15** and **16**), 78.2 (d, C-5), 29.0 (t, C-6), 33.80 and 33.77 (t, C-7, **15** and **16**), 39.7 (s, C-8), 43.8 (t, C-9), 70.00 and 69.98 (d, C-10, **15** and **16**), 135.44 and 135.34 (s, C-11, **15** and **16**), 134.53 and 134.44 (s, C-12, **15** and **16**), 39.27 and 39.16 (t, C-13, **15** and **16**), 71.7 (d, C-14), 37.20 and 37.18 (s, C-15, **15** and **16**), 25.3 (q, C-16), 31.6 (q, C-17), 21.4 (q, C-18), 22.5 (q, C-19), 116.94 and 116.91 (t, C-20, **15** and **16**), 169.43 and 169.35 (s, C-1′, **15** and **16**), 53.59 and 53.57 (d, C-2′, **15** and **16**), 203.47 and 203.33 (s, C-3′, **15** and **16**), 28.64 and 28.57 (q, C-4′, **15** and **16**), 12.7 (q, C-5′), 21.4 (q, 2-OAc), 21.9 (q, 5-OAc), 20.92 and 20.90 (q, 10-OAc, **15** and **16**), 169.98 and 169.91 (s, 2-OAc, **15** and **16**), 169.76 and 169.73 (s, 5-OAc, **15** and **16**), 170.23 and 170.21 (s, 10-OAc, **15** and **16**); HREIMS m/z 560.2987 $[M]^+$ (calcd 560.2985 for $C_{31}H_{44}O_9$).

4.6.2. 9α-Hydroxy-2α,5α,10β-triacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene (18). White powder; $[\alpha]_D^{20}$ +45.8° (c 0.4, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3624, 3540, 3036, 2988, 1726, 1644, 1456, 1374, 1248, 1178, 1108, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.81 (1H, d, J=2.0 Hz, H-1), 5.28 (1H, dd, J=2.2, 6.6 Hz, H-2), 2.87 (1H, d, J=6.6 Hz, H-3), 5.25 (1H, br s, H-5), 1.73–1.79 (2H, m, H-6), 1.60–1.70 (1H, m, H-7β), 1.49 (1H, dd, J=5.5, 13.6 Hz, H-7α), 4.14 (1H, d, J=9.8 Hz, H-9), 5.75 (1H, d, J=9.8 Hz, H-10), 2.81 (1H, dd, J=9.0, 19.0 Hz, H-13β), 2.28–2.35 (1H, m, H-13α), 4.94 (1H, dd, J=4.6, 9.0 Hz, H-14), 1.53 (3H, s, H-16), 1.05 (3H, s, H-17), 2.07 (3H, br s, H-18), 0.99 (3H, s, H-19), 5.25 (1H, s, H-20a), 4.81 (1H, s, H-20b), 2.32 (1H, dq, J=7.0, 7.4 Hz, H-2′), 3.79 (1H, dq, J=7.0, 6.4 Hz, H-3′), 1.13 (3H, d, J=6.4 Hz, H-4′), 1.08 (3H, d, J=7.4 Hz, H-5′), 2.12, 2.05, 1.96 [3H each, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 58.9 (d, C-1), 70.0 (d, C-2), 44.1 (d, C-3), 141.8 (s, C-4), 78.6 (d, C-5), 28.5 (t, C-6), 25.9 (t, C-7), 44.1 (s, C-8), 76.2 (d, C-9), 76.0 (d, C-10), 136.8 (s, C-11), 133.3 (s, C-12), 39.6 (t, C-13), 70.5 (d, C-14), 37.1 (s, C-15), 26.2 (q, C-16), 31.5 (q, C-17), 21.0 (q, C-18), 17.5 (q, C-19), 117.7 (t, C-20), 174.8 (s, C-1′), 47.0 (d, C-2′), 69.5 (d, C-3′), 20.9 (q, C-4′), 14.0 (q, C-5′), 21.9, 21.4, 21.3 [q, OAc (CH_3)], 170.5, 169.9, 170.0 [s, OAc (CO)]; HREIMS m/z 578.3102 $[M]^+$ (calcd 578.3091 for $C_{31}H_{46}O_{10}$).

4.6.3. 6α-Hydroxy-2α,5α,10β-triacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene (20). White powder; $[\alpha]_D^{20}$ +44.4° (c 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3612, 2940, 1730, 1606, 1458, 1376, 1320, 1240, 1166, 1112, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.91 (1H, d, J=2.2 Hz, H-1), 5.35 (1H, dd, J=2.2, 6.6 Hz, H-2), 2.89 (1H, d, J=6.6 Hz, H-3), 5.43 (1H, d, J=3.9 Hz, H-5), 3.92 (1H, ddd, J=3.9, 5.3, 12.0 Hz, H-6), 1.82 (1H, dd, J=12.4, 12.4 Hz, H-7β), 1.56 (1H, dd, J=5.1, 12.5 Hz, H-7α), 2.35 (1H, dd, J=12.2, 14.9 Hz, H-9β), 1.68 (1H, dd, J=5.6, 14.9 Hz, H-9α), 6.03 (1H, dd, J=5.6, 12.0 Hz, H-10), 2.84 (1H, dd, J=9.2, 19.0 Hz, H-13β), 2.39 (1H, dd, J=4.6, 19.0 Hz, H-13α), 5.02 (1H, dd, J=4.6, 9.3 Hz, H-14), 1.63 (3H, s, H-16), 1.12 (3H, s, H-17), 2.11 (3H, br s, H-18), 0.86 (3H, s, H-19), 5.38 (1H, s, H-20a), 4.91 (1H, s, H-20b), 2.39 (dq, J=7.0, 7.3 Hz, H-2′), 3.86 (1H, dq, J=6.3, 7.0 Hz, H-3′), 1.20 (3H, d, J=6.3 Hz, H-4′), 1.15 (3H, d, J=7.3 Hz, H-5′), 2.25, 2.05, 2.02 [3H each, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 58.9 (d, C-1), 70.2 (d, C-2), 41.3 (d, C-3), 134.0 (s, C-4), 80.3 (d, C-5), 69.1 (d, C-6), 42.2 (t, C-7), 38.0 (s, C-8), 43.5 (t, C-9), 69.8 (d, C-10), 135.4 (s, C-11), 134.8 (s, C-12), 39.5 (t, C-13), 70.6 (d, C-14), 37.2 (s, C-15), 25.3 (q, C-16), 31.7 (q, C-17), 22.0 (q, C-18), 23.4 (q, C-19), 119.7 (t, C-20), 174.8 (s, C-1′), 47.0 (d, C-2′), 69.5 (d, C-3′), 20.9 (q, C-4′), 14.0 (q, C-5′), 21.8, 21.4, 21.4 [q, OAc (CH_3)], 171.0, 170.1, 169.9 [s, OAc (CO)]; HREIMS m/z 578.3084 $[M]^+$ (calcd 578.3091 for $C_{31}H_{46}O_{10}$).

4.6.4. 7β,10β-Dihydroxy-2α,5α-diacetoxy-14β-(3-hydroxy-2-methyl)-butyryloxytaxa-4(20),11-diene (21). White powder; $[\alpha]_D^{20}$ +45.0° (c 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3624, 2992, 2944, 1732, 1644, 1456, 1376, 1320, 1242, 1220, 1168, 1114, 1048, 1004 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.90 (1H, d, J=2.0 Hz, H-1), 5.42 (1H, dd, J=2.2, 6.6 Hz, H-2), 2.89 (1H, d, J=6.4 Hz, H-3), 5.23 (1H, t, J=3.9 Hz, H-5), 2.08–2.16 (1H, m, H-6α), 1.60–1.70 (1H, m, H-6β), 3.82 (1H, dd, J=5.0, 11.4 Hz, H-7), 2.29 (1H, dd, J=5.4, 14.9 Hz, H-9β), 1.68 (1H, dd, J=10.7, 14.9 Hz, H-9α), 5.08 (1H, dd, J=5.4, 10.7 Hz, H-10), 2.80 (1H, dd, J=9.3, 18.8 Hz, H-13β), 2.39 (1H, dd, J=4.9, 18.8 Hz, H-13α), 4.99 (1H, dd, J=4.9, 9.3 Hz, H-14), 1.75 (3H, s, H-16), 1.19 (3H, s, H-17), 1.95 (3H, br s, H-18), 0.76 (3H, s, H-19), 5.29 (1H, s, H-20a), 4.89 (1H, s, H-20b), 2.39 (1H, dq, J=7.0, 7.3 Hz, H-2′), 3.86 (1H, dq, J=7.0, 6.3 Hz, H-3′), 1.21 (3H, d, J=7.3 Hz, H-4′), 1.16 (3H, d, J=7.3 Hz, H-5′), 2.19, 2.03 [3H each, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 59.2 (d, C-1), 70.1 (d, C-2), 40.4 (d, C-3), 140.9 (s, C-4), 77.6 (d, C-5), 37.5 (t, C-6), 69.2 (d, C-7), 44.4 (s, C-8), 40.5 (t, C-9), 66.9 (d, C-10), 139.1 (s, C-11), 132.0 (s, C-12), 39.5 (t, C-13), 70.8 (d, C-14), 37.4 (s, C-15), 25.4 (q, C-16), 31.9 (q, C-17), 21.1 (q, C-18), 16.7 (q, C-19), 118.0 (t, C-20), 174.8 (s, C-1′), 47.0 (d, C-2′), 69.5 (d, C-3′), 14.1 (q, C-4′), 20.9 (q, C-5′), 21.8, 21.4 [q, OAc (CH_3)], 169.8, 169.6 [s, OAc (CO)]; HRFABMS m/z 559.2886 $[M+Na]^+$ (calcd 559.2883 for $C_{29}H_{44}O_9Na$).

4.7. Biotransformation of 4 by *A. coerulea*

The procedures were followed as described in Section 4.4, except that 200 mg of **4** (prepared from biotransformation of **1** by *Ginkgo* cells[23]) was used, finally 373 mg of extract (200 mg for filtrate, 173 mg for cell cultures) was resulted. The extract was fractionated and separated by combination of open silica gel chromatography and normal phase semi-prep. HPLC. **4** (160 mg) (80%; t_R=11.9 min) and 4.0 mg of

22 (ca. 2%; t_R=15.3 min) were obtained, the HPLC mobile phase was the mixture of hexane and ethyl acetate (70/30, v/v).

4.8. Biotransformation of 5 by cell suspension cultures of *G. biloba*

Compound **5** (20 mg) was dissolved in EtOH (0.4 mL), distributed between 2 flasks of 15-day-old cell cultures and incubated for 6 days, after which the cultures were filtered under vacuum and the filtrate was saturated with NaCl and extracted 5 times with ethyl acetate. All the extracts were pooled, dried with anhydrous Na_2SO_4, and concentrated under vacuum at 40 °C to give 30 mg of residue. The dried cell cultures were extracted 3 times by sonication with ethyl acetate, the resulting extracts were pooled and concentrated under vacuum at 40 °C to afford 40 mg of residue. 12 mg of **5** (60%; t_R=12.3 min) and 2.0 mg of **23** (ca. 10%; t_R=14.2 min) were obtained by combination of open silica gel column chromatography and semi-prep. HPLC (mobile phase: hexane/ethyl acetate=70/30, v/v).

4.8.1. 7β,10β-Dihydroxy-2α,5α,14β-triacetoxy-taxa-4(20),11-diene (23). White powder; $[\alpha]_D^{20}$ +42.5° (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3624, 2992, 2944, 1732, 1644, 1456, 1376, 1242, 1114, 1048 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.87 (1H, d, *J*=2.0 Hz, H-1), 5.42 (1H, dd, *J*=2.2, 6.6 Hz, H-2), 2.83 (1H, d, *J*=6.1 Hz, H-3), 5.33 (1H, t, *J*=3.4 Hz, H-5), 2.06–2.14 (1H, m, H-6α), 1.58–1.64 (1H, m, H-6β), 3.83 (1H, dd, *J*=5.1, 11.7 Hz, H-7), 2.30 (1H, dd, *J*=5.1, 15.1 Hz, H-9β), 2.09–2.24 (1H, m, H-9α), 5.08 (1H, dd, *J*=5.1, 12.0 Hz, H-10), 2.83 (1H, dd, *J*=9.3, 19.3 Hz, H-13β), 2.37 (1H, dd, *J*=4.9, 19.0 Hz, H-13α), 4.95 (1H, dd, *J*=4.9, 9.3 Hz, H-14), 1.76 (3H, s, H-16), 1.19 (3H, s, H-17), 1.95 (3H, br s, H-18), 0.75 (3H, s, H-19), 5.28 (1H, s, H-20a), 4.88 (1H, s, H-20b), 2.18, 2.07, 2.03 [3H each, s, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 59.3 (d, C-1), 70.1 (d, C-2), 40.4 (d, C-3), 140.9 (s, C-4), 77.5 (d, C-5), 37.6 (t, C-6), 69.2 (d, C-7), 44.4 (s, C-8), 40.5 (t, C-9), 66.9 (d, C-10), 139.2 (s, C-11), 132.3 (s, C-12), 39.6 (t, C-13), 70.2 (d, C-14), 37.4 (s, C-15), 25.5 (q, C-16), 31.9 (q, C-17), 21.2 (q, C-18), 16.7 (q, C-19), 118.0 (t, C-20), 21.8, 21.5, 21.4 [q, OAc (CH_3)], 170.2, 169.8, 169.6 [s, OAc (CO)]; HRFABMS *m/z* 501.2472 $[M+Na]^+$ (calcd 501.2464 for $C_{26}H_{38}O_8Na$).

4.9. Acetylation of 4 and 5

Into 50 mL of egg-plant flask, 195 mg of **4** (0.375 mmol) and 274.5 mg (2.25 mmol) of 4-(dimethylamino)-pyridine (DMAP) were added and dissolved with 7.5 mL of dry pyridine at ambient temperature by stirring, then 0.352 mL of Ac_2O (3.75 mmol) was added. After 7 h incubation at 50 °C, the reaction was quenched by adding 20 mL of sat. NaCl aq and extracted with ethyl acetate (4×20 mL). The extract was washed with 2 M HCl (3×20 mL) until the pH<5, followed with sat. $NaHCO_3$ aq (2×15 mL) until the pH 7–8, then with sat. NaCl aq (2×10 mL), dried over anhydrous $NaSO_4$ and concentrated to afford 257 mg of residue. The resulting residue was applied to an open silica gel column and eluted with the mixture of hexane and ethyl acetate (7/3, v/v) to afford 200 mg of **24**. The same procedure was performed in the acetylation of **5** (50 mg, 0.096 mmol), finally 50 mg of **27** was obtained.

4.9.1. 2α,5α,9α,10β,14β-Pentaacetoxy-taxa-4(20),11-diene (24). White powder; $[\alpha]_D^{20}$ +50.8° (*c* 1.2, $CHCl_3$); IR ν_{max} ($CHCl_3$): 2964, 1734, 1436, 1374, 1236, 1224, 1114, 1022 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.93 (1H, d, *J*=2.2 Hz, H-1), 5.40 (1H, dd, *J*=2.4, 6.6 Hz, H-2), 2.96 (1H, d, *J*=6.6 Hz, H-3), 5.30 (1H, t, *J*=3.6 Hz, H-5), 1.78–1.85 (1H, m, H-6a), 1.65–1.75 (1H, m, H-6b), 1.64–1.72 (2H, m, H-7), 5.79 (1H, d, *J*=10.3 Hz, H-9), 6.01 (1H, d, *J*=10.5 Hz, H-10), 2.84 (1H, dd, *J*=9.0, 19.0 Hz, H-13β), 2.43 (1H, dd, *J*=4.9, 19.0 Hz, H-13α), 4.97 (1H, dd, *J*=4.9, 9.0 Hz, H-14), 1.71 (3H, s, H-16), 1.12 (3H, s, H-17), 2.14 (3H, br s, H-18), 0.84 (3H, s, H-19), 5.32 (1H, s, H-20a), 4.87 (1H, s, H-20b), 2.17, 2.04, 2.03, 2.01, 2.00 [3H each, s, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 58.5 (d, C-1), 70.2 (d, C-2), 44.1 (d, C-3), 141.4 (s, C-4), 78.3 (d, C-5), 28.3 (t, C-6), 27.3 (t, C-7), 44.4 (s, C-8), 72.5 (d, C-9), 76.8 (d, C-10), 137.3 (s, C-11), 132.8 (s, C-12), 39.4 (t, C-13), 68.9 (d, C-14), 37.0 (s, C-15), 25.8 (q, C-16), 31.6 (q, C-17), 21.8 (q, C-18), 17.3 (q, C-19), 118.1 (t, C-20), 21.4, 21.3, 21.1, 21.0, 20.8 [q, OAc (CH_3)], 170.0, 169.9, 169.8, 169.7, 169.6 [s, OAc(CO)]; HRFABMS *m/z* 585.2676 $[M+Na]^+$ (calcd 585.2676 for $C_{30}H_{42}O_{10}Na$); 563.2857 $[M+H]^+$ (calcd 563.2856 for $C_{30}H_{43}O_{10}$).

4.9.2. 2α,5α,7β,10β,14β-Pentaacetoxy-taxa-4(20),11-diene (27). White powder; $[\alpha]_D^{20}$ +35.5° (*c* 1.4, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3004, 2940, 1730, 1650, 1432, 1374, 1250, 1222, 1216, 1182, 1104, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.90 (1H, d, *J*=2.2 Hz, H-1), 5.39 (1H, dd, *J*=2.2, 6.6 Hz, H-2), 2.85 (1H, d, *J*=6.6 Hz, H-3), 5.34 (1H, t, *J*=2.4 Hz, H-5), 2.04–2.12 (1H, m, H-6α), 1.65–1.74 (1H, m, H-6β), 2.05–2.15 (1H, m, H-9β), 1.94–2.12 (1H, m, H-9α), 5.85 (1H, dd, *J*=6.0, 11.5 Hz, H-10), 2.82 (1H, dd, *J*=9.3, 19.0 Hz, H-13β), 2.42 (1H, dd, *J*=4.9, 19.0 Hz, H-13α), 4.95 (1H, dd, *J*=4.9, 9.0 Hz, H-14), 1.66 (3H, s, H-16), 1.14 (3H, s, H-17), 2.08 (3H, br s, H-18), 0.83 (3H, s, H-19), 5.32 (1H, s, H-20a), 4.94 (1H, s, H-20b), 2.19, 2.10, 2.06, 2.02, 2.01 [3H each, s, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 58.8 (d, C-1), 69.7 (d, C-2), 41.0 (d, C-3), 140.5 (s, C-4), 76.8 (d, C-5), 33.8 (t, C-6), 70.6 (d, C-7), 43.1 (s, C-8), 37.2 (t, C-9), 69.8 (d, C-10), 135.2 (s, C-11), 134.9 (s, C-12), 39.4 (t, C-13), 70.4 (d, C-14), 37.4 (s, C-15), 25.4 (q, C-16), 31.6 (q, C-17), 21.3 (q, C-18), 17.8 (q, C-19), 118.4 (t, C-20), 21.7, 21.5, 2×21.4, 21.0 [q, OAc (CH_3)], 170.4, 170.0, 169.9, 169.9, 169.5 [s, OAc(CO)]; HRFABMS *m/z* 585.2676 $[M+Na]^+$ (calcd 585.2676 for $C_{30}H_{42}O_{10}Na$); 563.2857 $[M+H]^+$ (calcd 563.2856 for $C_{30}H_{43}O_{10}$).

4.10. Biotransformation of 24 by *A. coerulea*

Compound **24** (200 mg) was used as substrate in this experiment, and the procedures were performed as described in Section 4.4, finally 321 mg of extract was obtained (210 mg for filtrate, 101 mg for cells). 162 mg of **24** (81%; t_R=9.6 min), 4.2 mg of **25** (ca. 2%; t_R=11.1 min) and 8.5 mg of **26** (ca. 4%; t_R=15.16 min) were obtained by combination of open silica gel chromatography and normal phase semi-prep. HPLC (mobile phase: hexane/ethyl acetate=70/30, v/v).

4.10.1. 7β-Hydroxy-2α,5α,9α,10β,14β-pentaacetoxy-taxa-4(20),11-diene (25). White powder; $[\alpha]_D^{20}$ +38.4° (*c* 0.2, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3614, 2972, 2884, 1732,

1646, 1436, 1374, 1240, 1102, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.88 (1H, d, J=2.2 Hz, H-1), 5.41 (1H, dd, J=2.2, 6.0 Hz, H-2), 2.81 (1H, d, J=6.2 Hz, H-3), 5.31 (1H, t, J=2.6 Hz, H-5), 2.09–2.15 (1H, m, H-6α), 1.67–1.73 (1H, m, H-6β), 3.90 (1H, dd, J=5.4, 11.5 Hz, H-7), 5.80 (1H, d, J=11.0 Hz, H-9), 6.05 (1H, d, J=11.5 Hz, H-10), 2.86 (1H, dd, J=9.0, 19.0 Hz, H-13β), 2.40 (1H, dd, J=4.9, 19.0 Hz, H-13α), 4.95 (1H, dd, J=4.9, 9.0 Hz, H-14), 1.71 (3H, s, H-16), 1.21 (3H, s, H-17), 2.15 (3H, br s, H-18), 1.31 (3H, s, H-19), 5.30 (1H, s, H-20a), 4.88 (1H, s, H-20b), 2.17, 2.04, 2.03, 2.01, 2.00 [3H each, s, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 58.9 (d, C-1), 70.4 (d, C-2), 45.2 (d, C-3), 140.2 (s, C-4), 83.2 (d, C-5), 31.3 (t, C-6), 71.7 (d, C-7), 45.6 (s, C-8), 73.6 (d, C-9), 70.3 (d, C-10), 135.5 (s, C-11), 133.2 (s, C-12), 39.4 (t, C-13), 68.9 (d, C-14), 37.4 (s, C-15), 26.1 (q, C-16), 31.7 (q, C-17), 21.9 (q, C-18), 12.7 (q, C-19), 117.7 (t, C-20), 21.4, 21.3, 21.1, 21.0, 20.8 [q, OAc (CH_3)], 170.4, 170.1, 169.9, 169.3, 168.8 [s, OAc (CO)]; HRFABMS m/z 601.2630 $[M+Na]^+$ (calcd 601.2625 for $C_{30}H_{42}O_{11}Na$); 579.2796 $[M+H]^+$ (calcd 579.2805 for $C_{30}H_{43}O_{11}$).

4.10.2. 14β-Hydroxy-2α,5α, 9α,10β-tetraacetoxy-taxa-4(20),11-diene (26). White powder; $[\alpha]_D^{20}$ +35.8° (c 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3624, 2940, 1736, 1376, 1226, 1022 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.80 (1H, d, J=2.0 Hz, H-1), 5.48 (1H, dd, J=2.2, 6.3 Hz, H-2), 2.92 (1H, d, J=6.3 Hz, H-3), 5.27 (1H, t, J=2.9 Hz, H-5), 1.80–1.88 (1H, m, H-6a), 1.65–1.71 (1H, m, H-6b), 1.68–1.75 (2H, m, H-7), 5.81 (1H, d, J=10.3 Hz, H-9), 6.02 (1H, d, J=10.5 Hz, H-10), 2.76 (1H, dd, J=9.0, 18.5 Hz, H-13a), 2.54 (1H, dd, J=4.4, 18.0 Hz, H-13b), 4.08 (1H, dd, J=5.0, 9.0 Hz, H-14), 1.72 (3H, s, H-16), 1.18 (3H, s, H-17), 2.16 (3H, s, H-18), 0.85 (3H, s, H-19), 5.33 (1H, s, H-20a), 4.91 (1H, s, H-20b), 2.16, 2.08, 2.04, 2.02 [3H each, s, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 63.1 (d, C-1), 70.5 (d, C-2), 44.1 (d, C-3), 141.9 (s, C-4), 78.7 (d, C-5), 28.4 (t, C-6), 27.2 (t, C-7), 44.4 (s, C-8), 76.8 (d, C-9), 72.5 (d, C-10), 137.9 (s, C-11), 132.8 (s, C-12), 42.2 (t, C-13), 67.6 (d, C-14), 37.5 (s, C-15), 26.1 (q, C-16), 31.3 (q, C-17), 20.8 (q, C-18), 17.3 (q, C-19), 118.0 (t, C-20), 22.0, 21.6, 21.5, 21.1 [q, OAc (CH_3)], 170.1, 170.0, 169.7, 169.4 [s, OAc (CO)]; HRFABMS m/z 543.2571 $[M+Na]^+$ (calcd 543.2570 for $C_{28}H_{40}O_9Na$); 521.2751 $[M+H]^+$ (calcd 521.2751 for $C_{28}H_{41}O_9$).

4.11. Biotransformation of 27 by cell suspension cultures of *G. biloba*

Compound **27** (50 mg) was used as the substrate in this experiment, and the procedures were performed as described in Section 4.8, finally 95 mg of extract was afforded (63 mg for filtrate, 32 mg for cells). 31 mg of **27** (ca. 60%; t_R=10.1 min), 6.2 mg of **28** (ca. 10%; t_R=12.9 min) were obtained by combination of open silica gel chromatography and normal phase semi-prep. HPLC (mobile phase: hexane/ethyl acetate=70/30, v/v).

4.11.1. 9α-Hydroxy-2α,5α,7β,10β,14β-pentaacetoxy-taxa-4(20),11-diene (28). White powder; $[\alpha]_D^{20}$ +40.6° (c 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3620, 3026, 2974, 1734, 1644, 1436, 1240, 1102 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.87 (1H, d, J=2.3 Hz, H-1), 5.34 (1H, dd, J=2.3, 6.5 Hz, H-2), 2.93 (1H, d, J=6.5 Hz, H-3), 5.33 (1H, t, J=2.6 Hz, H-5), 2.05–2.15 (1H, m, H-6α), 1.60–1.70(1H, m, H-6β), 5.40 (1H, dd, J=5.4, 11.5 Hz, H-7), 4.20 (1H, d, J=11.0 Hz, H-9), 5.85 (1H, d, J=11.5 Hz, H-10), 2.80 (1H, dd, J=9.0, 19.0 Hz, H-13β), 2.38 (1H, dd, J=4.9, 19.0 Hz, H-13α), 4.94 (1H, dd, J=4.9, 9.0 Hz, H-14), 1.65 (3H, s, H-16), 1.16 (3H, s, H-17), 2.13 (3H, s, H-18), 1.25 (3H, s, H-19), 5.25 (1H, s, H-20a), 4.88 (1H, s, H-20b), 2.19, 2.15, 2.06, 2.01, 2.00 [3H each, s, OAc (CH_3)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 59.0 (d, C-1), 70.2 (d, C-2), 43.6 (d, C-3), 142.1 (s, C-4), 77.2 (d, C-5), 31.3 (t, C-6), 70.8 (d, C-7), 44.3 (s, C-8), 76.2 (d, C-9), 76.0 (d, C-10), 136.5 (s, C-11), 133.3 (s, C-12), 39.4 (t, C-13), 68.7 (d, C-14), 37.2 (s, C-15), 26.2 (q, C-16), 31.7 (q, C-17), 21.9 (q, C-18), 12.0 (q, C-19), 118.6 (t, C-20), 21.4, 2×21.3, 21.1, 20.6 [q, OAc (CH_3)], 170.5, 2×170.0, 169.9, 168.6 [s, OAc (CO)]; HRFABMS m/z 601.2626 $[M+Na]^+$ (calcd 601.2625 for $C_{30}H_{42}O_{11}Na$); 579.2806 $[M+H]^+$ (calcd 579.2805 for $C_{30}H_{43}O_{11}$).

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