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Selective Microbial Hydroxylation and Biological Rearrangement of Taxoids

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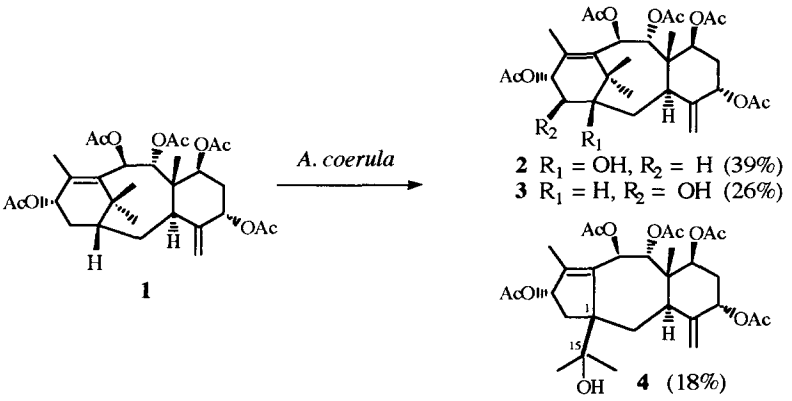
Abstract: 5α,7β,9α,10β,13α-Pentaacetoxy-4(20),11-taxadiene **1** was selectively transformed into its 1β,14β-hydroxylated derivatives **2**, **3**, and 11(15→1)abeotaxane **4** by different filamentous fungi. Microbial transformation of a series of taxoids with a 4(20) exocyclic double bond was described. The microbial enzymatic systems may be served as a useful tool to mimic some steps of taxoid biosynthesis in *Taxus* spp.. © 1997 Elsevier Science Ltd.

The novel diterpenoid paclitaxel (Taxol®), a potent antitumor agent, was originally isolated[1] from *Taxus brevifolia*. Its unusual biological activity, intriguing structure and scarcity have prompted extensive chemical and biological investigations into its production. The most promising options for large scale production of taxol are likely cell culture and semisynthesis.[2] Phytochemical investigations for different *Taxus* spp. have grown exponentially in the past few years. As a result, more than 200 taxoids were found in nature and hundreds of structurally related products were synthesized before 1994.[3] The most common group of taxoids consisted of those compounds with a C-4(20) exocyclic double bond. Taxine B and some related taxoids occur in higher contents in *Taxus baccata* and other *Taxus* spp., and this makes them an interesting starting material for semisynthetic studies toward taxol analogs.[4]

Microorganisms such as filamentous fungi are known to carry out regio- and stereoselective hydroxylations of a wide range of natural or synthetic hydrophobic organic compounds. The potential and limitations of such reactions, as a tool for performing preparative hydroxylation and functionalization, may be well determined by biotransformation of the taxane diterpenoids. *Taxomyces andreanae*, an endophytic fungus, originally isolated from the bark of *Taxus brevifolia*, is able to produce paclitaxel in culture media.[5] This exciting finding provides a potential alternative for the production of paclitaxel, and also provides the basis for the transformation of taxoids by microbial systems. In our previous studies on the biotransformation of taxoids with an oxygen substituent at C-14, we reported the selective hydroxylation at C-6α by the fungi *Cunninghamella* species.[6] As part of our continuing study on the biotransformation and biosynthesis of taxoids, we have had a long-standing interest in hydroxylations of the taxane skeletons at different positions. We report herein our studies on selective microbial hydroxylation of taxoids at sites of C-1 and C-14, and biological rearrangement reactions of taxoids.

Preliminary screening studies with 25 collected fungal cultures[6] showed that 2α-deacetoxytaxinine J, taxinine J, taxinine, O-cinnamoyl-taxicin I triacetate[3,7] were not metabolized by these fungi. In our previous

studies[6] on the microbial transformation of taxoids with an oxygen substituent at C-14, we were aware of the hydroxylation at C-6 having high substrate specificity, and the acetyl groups present at C-5 and C-14 are essential for this type hydroxylation. Based on this consideration, we used hydroxylamine[8] to selectively cleave the 5-O-cinnamoyl groups (in yields 50-78%), and also to prepare the corresponding acetylated derivatives at C-5. We used these taxoids as substrates to examine the abilities of biotransformation of the fungal cultures.



Surprisingly, compound **1** could be easily transformed into three more polar metaboiltes by *Absidia coerula*, *Cunninghamella echinulata*, *Cunninghamella elegans*, *Cunnighamella blakesleana*, *Rhizopus arrhizus*. Among these cultures, *A. coerula* seems to be the most efficient strain. In a preparative experiment without the optimization of culture and incubation conditions, a mixture of **2** and **3** (3:2, 75% yield, determined by HPLC eluted by MeCN/H_2O) and pure **4** (20% yield) were isolated by flash chromatography, using CH_2Cl_2-MeOH as eluent (200:1 and 100:1, v/v) after 5 days incubation of compound **1** (50 mg/L of culture media) with *A. coerula*. The mixtures of these two products were exceedingly difficult to separate; while after acetylation with Ac_2O, a small part of the mixture was transformed into a less polar compound **5**, and compound **2** was unchanged. Compounds **2** and **5** were easily separated by flash chromatography.

FABMS of **2** showed a $[MH]^+$ fragment peak at m/z 579, consistent with the molecular formula of $C_{30}H_{42}O_{11}$. A detailed analysis of the NMR spectra (^{1}H-, ^{1}H-^{1}H COSY, ^{13}C- and DEPT)[9] showed that **2** was the 1β-hydroxylated derivative of **1**.[10] FABMS of **5** established the molecular formula of $C_{32}H_{44}O_{12}$. In the ^{1}H-NMR spectrum of **5**, the signals for six acetyl groups and four methyl groups were observed, and a new doublet at δ 4.59 was observed, suggested that a novel acetoxy group was introduced at a methylene carbon; the broad triplet of H-13 at δ 5.96 in **1** was replaced by a well-resolved doublet doublets at δ 6.30. A detailed analysis of the NMR spectra (^{1}H-, ^{1}H-^{1}H COSY, NOE, ^{13}C- and DEPT)[11] of **5** and those of the mixture of **2** and **3** exhibited that **3** and **5** were 14β-hydroxy- and 14β-acetoxy derivatives of **1**, respectively. FABMS showed that **4** had the same molecular ion as that of **2**. This product showed unusual chemical shifts for C-1 (62.8 ppm) and C-15 (75.3 ppm) when compared with those of **1**. In the ^{1}H-NMR spectra, the signal of CH_3-16 showed a marked upfield shift (Δδ -0.26 ppm) compared with those of **1**,[12] and the signal of H-9 showed

unusual broad doublet. These data and a detailed analysis of ^{1}H-^{1}H COSY, ^{13}C-NMR and DEPT spectral data[13] confirmed that **4** was a 11(15→1)abeotaxane derivative. The structure of **4** was very similar to those of brevifoliol and its analogs, previously isolated from different *Taxus* spp.. Chemical transformation of baccatin III derivatives into this type abeotaxane has been reported. [14]

Compound **1** can easily be obtained from *T. baccata*, *T. mairei* and other *Taxus* spp.. 2α-Deacetoxytaxinine J has been isolated from *T. yunnanensis*, *T. mairei*, *T. cuspidata* and other *Taxus* species in relatively high amounts, which was easily transformed into 5α-hydroxy-7β,9α,10β,13α-tetraacetoxy-4(20),11-taxadiene with hydroxylamine in yield of 78%. 2'β-Deacetoxyaustrospicatine and its analogs can easily be transformed into C-5 cinnamate derivatives through deamination with MeI/K_2CO_3,[4,7] and can be also used as the potential sources for the selective hydroxylation at C-1 and C-14 by microorganisms. The novel C-1 and C-14 hydroxylated derivatives may be used as useful intermediates for the semisynthesis of taxol analogs and other new taxoids.

It has been reported very recently that cyclization of geranylgeranyl diphosphate to taxa-4(5),11(12)-diene is the committed step of taxol biosynthesis in pacific yew. The responsible enzyme, taxadiene synthase from *T. brevifolia* has been isolated and partially purified. A cDNA encoding taxadiene synthase has also been obtained.[15] Alternatively, a series of taxoids with a 14-oxygen substituent and a 4(20)-exocyclic methylic double bond are obtained in very high amounts from cell cultures of *Taxus yunnanensis*, *Taxus chinensis* and other *Taxus* spp..[6] These exciting findings may offer an alternative for the preparation of taxane skeleton molecules on a large scale. The introduction of critical functional groups such as the hydroxyl groups at sites of C-1 and/or C-14 may be effected through selective microbial hydroxylation reactions, and this type of biohydroxylation reaction may be served as a powerful method for the preparation of C-1 and C-14 hydroxylated derivatives on a large scale. Microbial rearrangement of taxoids with a 6/8/6 taxane ring into abeotaxane provided useful experimental proofs for the biogenesis of abeotaxane in *Taxus* spp.

It is clear that microbial enzymatic systems may be a useful tool to mimic some important steps of taxoid biosynthesis, and are also useful for the better understanding of the biosynthetic pathways of paclitaxel and other taxoids in *Taxus* spp..

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(d, 1H, J = 1.2, H-20), 5.02 (d, 1H, J = 1.5, H-20), 2.85 (d, 1H, J = 5.8, H-3), 2.49 (dd, 1H, J = 14.8, 9.7, H-14), 2.19 (d, 3H, J = 1.2, CH_3-18), 2.18 (s, 3H, OAc-5), 2.07, 2.06, 2.03, 2.00 (4s, 12H, OAc-7, 9, 10, 13), 1,98 (m, 1H, H-2), 1.92 (ddd, 1H, J = 14.7, 5.2, 2.5, H-6), 1.82 (d, 1H, J = 15.0, H-6), 1.78 (m, 1H, H-2), 1.62 (s, 3H, CH_3-16), 1.59 (dd, 1H, J = 14.8, 7.5, H-14), 1.21 (s, 3H, CH_3-17), 0.85 (s, 3H, CH_3-19). ^{13}C-NMR ($CDCl_3$, 125 MHz) 170.2, 170.2, 169.9, 169.7, 169.2 (s, $OCOCH_3$-5, 7, 9, 10, 13), 145.8 (s, C-4), 139.4 (s, C-12), 134.2 (s, C-11), 115.9 (t, C-20), 76.2 (s, C-1), 76.2 (d, C-9), 74.6 (d, C-5), 71.2 (d, C-13), 70.9, 69.7 (d, C-7,10), 46.4 (s, C-8), 43.8 (s, C-15), 41.1 (d, C-3), 41.0 (t, C-14), 37.1 (t, C-2), 34.0 (t, C-6), 27.3 (q, C-16), 22.1, 21.7, 21.4, 21.4, 20.9, 20.8 (q, $OCOCH_3$-5, 7, 9, 10, 13 and C-17), 14.9 (q, C-18), 13.2 (q, C-19).

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