Published in final edited form as:

Nat Prod Rep. 2012 June 1; 29(6): 683–696. doi:10.1039/c2np20021j.

The Early Stages of Taxol Biosynthesis: An Interim Report on the Synthesis and Identification of Early Pathway Metabolites

Jennifer Guerra-Bubb[†], Rodney Croteau[‡], and Robert M. Williams^{†,§}

[†] Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, USA

[‡] Institute of Biological Chemistry, Washington State University, Pullman, WA, 99164-6340, USA

[§] University of Colorado Cancer Center, Aurora, Colorado

Abstract

The biosynthesis of the anti-cancer drug taxol (paclitaxel) has required the collaborative efforts of several research groups to tackle the synthesis and labeling of putative biosynthetic intermediates, in concert with the identification, cloning and functional expression of the biosynthetic genes responsible for the construction of this complex natural product. Based on a combination of precursor labeling and incorporation experiments, and metabolite isolation from *Taxus* spp., a picture of the complex matrix of pathway oxygenation reactions following formation of the first committed intermediate, taxa-4(5),11(12)-diene, is beginning to emerge. An overview of the current state of knowledge on the early-stages of taxol biosynthesis is presented.

1 Introduction

Clinical oncologists, biologists and chemists have been irresistibly drawn to taxol¹ (**1**, paclitaxel) and its semi-synthetic congener taxotere (**2**) due to their promising spectrum of antineoplastic activity, unique mechanism of action, and the synthetic challenge that the complex, and densely functionalized ring system poses.^{2–9} Taxol was first isolated from the bark of the Pacific yew, *Taxus brevifolia* Nutt.¹⁰ Unfortunately, the yew is slow growing and is primarily found in environmentally sensitive areas of the Pacific Northwest, and stripping the tree of its bark kills the yew. It takes three trees to obtain ~10 Kg of bark from which 1 gm of taxol can be isolated.⁷ FDA approval of taxol for the treatment of advanced ovarian cancer² has created a severe supply and demand problem. Collection of renewable *Taxus* sp. needles and clippings has attenuated this problem somewhat, but as the drug becomes more widely adopted, particularly for use earlier in the course of cancer intervention and for new therapeutic applications, pressure on the yew population is likely to increase worldwide. Alternative means of taxol production are being vigorously pursued since cost and availability will continue to be significant issues.

To date, totally synthetic methods have fallen short as an alternative source for taxol production,^{11–25} due to the complex structure of taxol that mandates lengthy and expensive synthetic routes. A method that has proven viable is the semi-synthesis of taxol from 10-deacetylbaaccatin III, a renewable, which is isolated from the needles of yew species.^{3,7} The commercial supply of taxol and semi-synthetic precursors will have to increasingly rely on biological methods of production. This can be reasonably achieved by: (1) extraction from intact *Taxus* plants, (2) *Taxus* cell culture,²⁶ or (3) microbial systems.^{27–35} In order to produce large quantities of taxol or a pharmacophoric equivalent by semi-synthetic approaches, or perhaps by genetically engineered biosynthetic methods, it is essential to gain a better understanding of the detailed biosynthetic pathways^{26–37} in *Taxus* sp.

In 1966, Lythgoe and coworkers proposed a biosynthetic pathway in which the tricyclic carbon framework of the taxoids was envisioned to arise by the sequential intramolecular cyclizations of the double bonds of geranylgeranyl pyrophosphate (ggPP) to 1(*S*)-verticillene (**3**) and then to taxa-4(20),11(12)-diene (**4**, Scheme 1).^{38,39}

Our highly collaborative program has been aimed at manipulating taxoid biosynthesis at the genetic level in cell culture; as such, the slow steps in the biosynthetic pathway need to be elucidated. Since ggPP serves as a starting material for many other biosynthetic pathways,⁴⁰⁻⁴⁴ any number of the steps may be rate-limiting in the secondary metabolic flux to taxol. As a first step toward targeting the slow steps in the biosynthesis of taxol, we deployed several parallel strategies involving: (1) the biosynthetic production of lightly oxygenated taxoids using synthetic, tritium-labeled substrates; (2) total synthesis and isotopic labeling of pathway metabolites; (3) isolation and structure determination of pathway intermediates; (4) identification, cloning and functional expression of the biosynthetic genes; (6) incorporation of candidate pathway metabolites into taxol *in vivo* and; (7) mechanistic studies on the individual pathway enzymes.

We discovered that the “Lythgoe taxadiene” (**4**) is not the first committed intermediate on the taxoid biosynthetic pathway, but rather that taxa-4(5),11(12)-diene (**5**, Scheme 2) is the major metabolite.⁴⁵⁻⁵² This was demonstrated by a total synthesis^{51,52} of both **4** and **5** and administration of synthetic ¹³C-labeled taxa-4(20),11(12)-diene (**4**) to microsomes of *Taxus Canadensis*.⁴⁷ Cross-over experiments and the lack of detectable biotransformation of the Lythgoe diene (**4**) in *Taxus* microsomes had initially cast considerable doubt on the intermediacy of this substance in taxoid biosynthesis.⁵⁰ On the other hand, using ggPP as a substrate, taxa-4(5),11(12)-diene (**5**) has been shown to be the primary product of taxadiene synthase, an enzyme recently characterized, and the corresponding gene cloned and functionally expressed.⁴⁵⁻⁴⁸ However, recent results from our laboratories have revealed that recombinant taxadiene synthase indeed produces ~5% of the Lythgoe diene (**4**) plus <1% percent of the 3,4-diene isomer (**6**).⁵⁰

2 Taxadiene Synthase

Taxadiene synthase from *Taxus* species catalyzes the first committed step in the biosynthesis of taxol and related taxoids by the cyclization of the universal diterpenoid precursor (*E,E,E*)-geranylgeranyl diphosphate (ggPP) to the parent hydrocarbon taxa-4(5), 11(12)-diene (**5**). Following the remarkable one-step construction of taxadiene, an extended and complex series of oxygenation and acylation reactions ensue, culminating in the production of taxol.^{45,53} Taxadiene synthase, which has been isolated from both yew saplings⁴⁶ and cell cultures,⁵⁴ catalyzes a slow, but apparently not rate limiting step in the taxol biosynthetic pathway.⁵⁴ A cDNA encoding the taxadiene synthase from *T. brevifolia* has been obtained by a homology-based PCR cloning method.⁴⁸ A truncated version of the enzyme, in which the plastidial targeting peptide of the preprotein has been deleted, has been functionally expressed in *Escherichia coli* and shown to resemble the native enzyme in kinetic properties.⁵⁵ Native taxadiene synthase is constituted of 862 amino acids with a molecular weight of 98.3 kD. The recombinant enzyme (after deletion of the plastid-targeting sequence) is constituted of 783 amino acids and has a molecular weight of ~80 kD. Overexpression of the truncated, recombinant synthase has been successfully accomplished in *E. coli*. This significant milestone has made available sufficient amounts of the enzyme, and the enzyme products, to permit a more detailed study of the electrophilic cyclization reaction cascade. Recently, the X-ray crystal structure of a truncated variant of taxadiene synthase was reported by Koksai and coworkers.⁵⁶

2.1 Mechanism of Taxadiene Synthase

In collaboration with the Croteau laboratory, the Coates laboratory (University of Illinois) and the Floss laboratory (University of Washington), we have determined the fascinating mechanism of the “one-step” cyclization of ggPP to taxa-4(5),11(12)-diene (**5**) as shown in Scheme 3.⁵⁰

The Coates lab focused on the stereochemistry of the A-ring formation⁵⁷ and demonstrated that, as expected, the pyrophosphate-bearing carbon atom undergoes a clean Walden inversion, and that the attack of the C11/C12 π -system on the incipient cation to form the A-ring quaternary center is an antarafacial reaction. Our group has focused on the stereochemistry and mechanistic implications of the unprecedented migration of the C11 hydrogen atom to C7.⁵⁰ The Floss lab synthesized the requisite deuterated ggPP substrate (shown in Scheme 3) and this substrate was converted into taxadiene *via* soluble recombinant synthase from *Taxus cuspidata*. We observed >90% retention of deuterium at C7 in taxadiene (**5**). Our laboratory elucidated the stereochemistry of the deuterium migration by 2D NMR and 1D TOCSY and has unambiguously established that the deuterium atom migrates to the α -face of C-7. The observation that the deuterium transfer from C11 to C7 occurs with such a high degree of isotopic retention, coupled with the exclusive transfer to the α -face of C7, suggests that the mechanism for the transfer of the deuterium from C11 to C7 occurs *intramolecularly*. Such an intramolecular proton transfer during an olefin-cation cyclization reaction is highly unusual.⁵⁰

Of further interest, is the facial bias of the final C8/C3 olefin-cation cyclization. Considering the known relative stereochemistry of the taxoid B/C ring juncture, the C3/C4 π -system *must* attack the putative C8 cation (**8**) formed immediately following the C11/C7 proton migration, from the *same face* to which the newly installed proton at C7 migrated. Molecular modeling of this process, which formally involves a criss-cross of bond formation across the C7/C8 π -system, proved quite insightful. The molecular model of the C8 cation suggests that, upon pyramidalization at C7 and fashioning of the C11/C12 olefin, the conformation of the 12-membered ring twists, relative to that of the C12 cation, rocking the C3/C4 π -system into the correct facial orientation for capture of the C8 cation establishing the *trans*-fused B/C ring juncture.

Taxadiene synthase is therefore a remarkable terpene cyclase that appears to function by non-covalent binding and ionization of the substrate ggPP and mediates an enantio- and face-selective polyolefin-cation cascade, involving the formation of three carbon-carbon bonds, three stereogenic centers, and the loss of a hydrogen atom in “a single step”. The apparently unassisted C-11/C-7 intramolecular proton transfer mechanism of taxadiene synthase is seemingly rare in this regard, and suggests that this enzyme type is capable of mediating complex olefin-cation cyclizations, with absolute stereochemical fidelity, by conformational control alone. These provocative experimental results have provided important insight into the molecular architecture of the first dedicated step of taxol biosynthesis that creates the taxane carbon skeleton. We further note that these observations have broad relevance and implications for the general mechanistic capability of the large family of terpenoid cyclization enzymes that catalyze related electrophilic reaction cascades.

2.2 Overproduction of Taxadiene

With the production of large scale advanced intermediates enroute to taxol being quite limited, work towards developing routes aimed at overproduction of metabolites have been explored. Developments in metabolic engineering, as well as synthetic biology, have enabled new avenues for the overproduction of complex natural products through the optimization of microbial hosts.^{58,59} Using a partitioned metabolic pathway utilizing the

upstream methylerythritol-phosphate (MEP) forming IPP and the heterologous downstream terpenoid forming pathway, Ajikumar, *et al* reported that they were successfully able to develop a multivariate-modular approach towards metabolic-pathway engineering and were able to increase titers of taxadiene to a ~ 1g/L, essentially a 15,000-fold increase in an *E. coli* strain.⁶⁰ Additionally, Boghigian, *et al*, have compared the heterologous production of taxadiene in two lineages of *E. coli*, K and B.⁶¹ Boghigian was able to show that the K-derivative outperformed the B-derivative and probed this finding by applying global transcript profiling to the two strains, thus revealing notable differences in pyruvate metabolism and central metabolism. Further studies demonstrated that temperature had a significant effect on taxadiene production and that indole inhibited the growth of both strains, which was consistent with their earlier observations.⁶¹

Metabolic engineering has also been applied in the realm of overexpressing natural product metabolites to help foster a more amenable route toward taxadiene production.⁶² Boghigian identified targets both within and outside of the isoprenoid precursor pathway and then tested them for over-expression in a heterologous *E. coli* host.⁶² The use of an algorithm for identifying over-expression targets is unique, since most metabolic engineering efforts have used computational methods to model cellular metabolism.

Collectively, the use of both heterologous hosts and metabolic engineering are paving the way for the over-expression of taxadiene synthase and future endeavors of developing routes toward the overexpression of downstream taxol pathway genes.

3 Efforts Toward Biosynthetic Intermediates to Probe the 5 α -Hydroxylase Pathway

3.1 Synthesis of Taxa-4(5),11(12)-diene (5) and Taxa-4(20),11(12)-diene (4)

The total syntheses of taxa-4(20),11(12)-diene (**4**) and taxa-4(5),11(12)-diene (**5**) are outlined in Scheme 4. Synthetic access to these substrates (albeit in racemic form) has proven to be critical in penetrating the early stages of taxol biosynthesis. After evaluating several alternatives, we decided to adapt a route originally devised by Shea and Davis,^{63,64} that relied on the use of an intramolecular Diels-Alder (IMDA) cyclization reaction forming the A-B-ring system with an aromatic C-ring. This strategy was also explored by Jenkins and coworkers⁶⁵⁻⁶⁷ to access a model A-B/(saturated) C-ring system containing the C-19 methyl group with the correct relative stereochemistry, but devoid of functionality in the C-ring. This IMDA approach for the synthesis of the taxadienes (**4** and **5**) was easily employed by installing a functional group at C-4 in the C-ring for the ultimate installation of the C-20 carbon atom and the 4(20)- or 4(5)-double bonds. The synthesis illustrated in Scheme 4 closely parallels the Jenkins synthesis with the important difference being the early introduction of a hydroxyl group in the C-ring precursor, which ultimately became the functionalized C-4 carbon of the taxadienes.

The key intermediate in this synthesis proved to be ketone **25**, which could be elaborated into the “Lythgoe diene” (**4**) by a simple Wittig reaction or, alternatively, to taxa-4(5), 11(12)-diene (**5**) by a Grignard-dehydration sequence (this procedure also gives **4**). The late-stage installation of the C-20 carbon atom by either approach turned out to be very significant, in that we were readily able to introduce tritium, deuterium and/or ¹³C at C-20 by the appropriate choice of relatively inexpensive, commercially available, isotopically labeled versions of methyl iodide.

3.2 Synthesis of Taxa-4(20),11(12)-dien-5 α -ol (**26**)

We have further established that the first hydroxylation product in the taxol pathway is taxa-4(20),11(12)-dien-5 α -ol (**26**, Scheme 5) through total synthesis and bioconversion of this substance into taxol, 10-deacetylbaccatin III and cephalomannine *in vivo* in *Taxus brevifolia*.⁶⁸ Furthermore, a synthetic sample of taxa-4(20),11(12)-dien-5 α -acetate (**27**) has been used to establish that, following hydroxylation of **5** to **26**, the next step in the pathway appears to be acetylation of **26** to acetate **27**.⁶⁹ The Croteau lab has cloned and expressed taxa-4(20),11(12)-dien-5 α -ol-*O*-acetyl transferase from *Taxus canadensis* in *E. coli*.⁶⁹

Due to the very low yield of natural taxadiene obtainable from biological sources (an extract from 750 kg of *Taxus brevifolia* bark powder yielded⁴⁵ ~1 mg of 85% pure **5**) and the remaining uncertainty as to the sequence of hydroxylation reactions to taxoids,^{45-48,70-73} synthetic access to these isotopically labeled tricyclic diterpenes has become essential for several reasons: (1) synthesis provides radio-labeled substrates from which pathway metabolites can be made by enzymatic biotransformation and; (2) the synthetic metabolites facilitate the identification, isolation and structure elucidation of new pathway metabolites that are produced biosynthetically in very tiny amounts. The second point was amply demonstrated by our synthesis of taxa-4(20),11(12)-dien-5 α -ol (**26**) which preceded the identification and isolation of this substance from *Taxus brevifolia*.⁷¹⁻⁷³ Indeed, the identification of this substance as a biosynthetic intermediate would have proven far more difficult if the synthetic reference sample were not available. The first point has also been amply demonstrated, since synthetic, tritium-labeled **26** and the corresponding acetate **27** have served as key substrates from which numerous intermediate hydroxylation products (diol, triol, tetraol and pentaol) have been produced biosynthetically. It should also be noted that taxol has now been identified in several strains of microorganisms,²⁷⁻³⁵ and our laboratory has provided samples of stable- and radio-isotopically labeled taxadienes to numerous other laboratories interested in identifying pathway intermediates to taxoids produced in other biological systems.

The synthesis of taxa-4(20),11(12)-dien-5 α -ol (**26**) is illustrated in Scheme 6 and proceeds *via* selenium dioxide oxidation of the synthetic Lythgoe diene (**4**). We observed exclusive formation of the naturally configured 5 α -alcohol (**26**) from this reaction whose stereochemistry was confirmed by extensive 2D NMR analysis (¹H, nOe, DQF-COSY, HMQC, HMBC). To interrogate the possible intermediacy of the corresponding β -alcohol diastereomer **28**, synthetic **26** was oxidized to the corresponding C-5 ketone with Dess-Martin periodinane and stereoselectively reduced with lithium aluminum hydride to deliver **28**. This authentic, synthetic sample made it possible to interrogate taxadiene hydroxylase for the possible production of trace amounts of this diastereomer, which was not observed.

Preparative incubations of synthetic C20-[³H]-taxadiene (**5**) with *Taxus* microsomes were next carried out to accumulate sufficient biosynthetic product (~3 nmol) for full spectrum GCMS analysis. Comparison of GC retention time and mass spectral fragmentation pattern of the TLC-purified biosynthetic product to those of authentic taxa-4(20),11(12)-dien-5 α -ol (**26**) indicated that the two compounds were identical. Final experimental corroboration was secured through the conversion of the purified, tritium-labeled biosynthetic product (42.8 nCi) to the corresponding 3,5-dinitrobenzoate ester (90% yield), which was diluted with authentic ($\pm$)-taxa-5(20),11(12)-dien-5 α -yl dinitrobenzoate, and recrystallized to constant specific activity and melting point (4.67 ± 0.09 nCi/ μ mol, m.p. 171 °C, dec.). These analyses, including quantitative evaluation of the radiochemical crystallization studies, revealed that a minimum of 87% of the oxygenation product of taxa-4(5),11(12)-diene (**5**) is taxa-4(20),11(12)-dien-5 α -ol (**26**).

We were also able to detect low levels of **26** as a natural metabolite in *Taxus* sp. bark extract. Thus, radiochemically-guided fractionation of a *T. brevifolia* bark extract (0.25 μ Ci of ($\pm$)-[20- 3 H]taxa-4(20),11(12)-dien-5 α -ol diluted into an extract of 250 kg dry bark) utilizing argentation TLC, and reverse-phase chromatography, before and after alkaline hydrolysis of half of the extract, followed by GC-MS analysis of the partially purified product, demonstrated the alcohol to be present in the 5-10 μ g/kg range and ester(s) of **26** to be present in the 25-50 μ g/kg range.

3.3 Elucidation of the Mechanism of Taxadiene Hydroxylase

We have examined the interesting mechanistic question regarding the cytochrome P450 mediated conversion of taxa-4(5),11(12)-diene (**5**) into taxa-4(20),11(12)-dien-5 α -ol (**26**) with a contrathermodynamic allylic transposition.⁶⁸ We designed an experiment to discern if the cytochrome P450 hydroxylation of taxa-4(5),11(12)-diene (**5**) to taxa-4(20),11(12)-dien-5 α -ol (**26**) proceeds through an epoxide intermediate or an allylic radical species. No isomerization of taxa-4(5),11(12)-diene (**5**) to the 4(20),11(12)-diene isomer (**4**) (or *vice versa*) was observed in *Taxus* sp. microsomes (or *Spodoptera* microsomes enriched in the recombinant 5 α -Hydroxylase)⁶⁸ under standard assay conditions but in the absence of NADPH or in the absence of O₂, or in the presence of CO, 100 μ M miconazole, or 100 μ M clotrimazole (all conditions under which hydroxylation activity is negligible), nor was isomerization observed in boiled controls containing all cofactors and reactants. Similarly, no interconversion of either positional isomer was observed in the presence of Mg⁺², NAD⁺, NADH, NADP⁺, or flavin cofactors, at pH values from 4~10. From these studies, we concluded that taxa-4(5),11(12)-diene (**5**) is not appreciably isomerized to taxa-4(20),11(12)-diene (**4**) under physiological conditions, and that the migration of the double bond from the 4(5)- to the 4(20)-position in the process of taxadienol formation is an inherent feature of the cytochrome P450 oxygenase reaction with taxa-4(5),11(12)-diene (**5**) as substrate. It is of further interest to note that taxa-4(20),11(12)-diene (**4**) is but a minor natural product of *Taxus* sp. formed by taxadiene synthase that is an adventitious, yet efficient, substrate for the 5 α -hydroxylase.

Previous efforts to evaluate the 5 α -hydroxylation reaction by the native enzyme, by search for an epoxide intermediate and through the use of [20- 2 H₃]taxa-4(5),11(2)-diene to examine a kinetic isotope effect (KIE) on the deprotonation step, failed to distinguish between two reasonable mechanistic possibilities.⁷¹

The two mechanistic manifolds that were considered involve: (1) preliminary conversion of the 4(5)-double bond of taxa-4(5),11(12)-diene (**5**) to the corresponding 4(5)-epoxide (**29**), followed by ring-opening to carbenium ion species **30** and β -proton elimination from the C20 methyl group to yield the allylic alcohol product (**26**) or, (2) an alternate route involving cytochrome P450-mediated abstraction of hydrogen radical from the C20 methyl of the substrate (**5**) to yield the allylic radical (**31**) to which oxygen is added at C5 yielding **26** (Scheme 7).

The utilization of the isomeric taxa-4(20),11(12)-diene (**4**) by the hydroxylase, with efficiency comparable to that of the natural substrate, would appear to rule out an intermediate epoxide (**29**) in the reaction cycle. These results instead suggest a mechanism involving abstraction of a hydrogen radical from C20 for **5** (or from C5 in the case of the 4(20)-isomer **4**), leading to the common intermediate, allylic radical **31**, followed by oxygen insertion selectively from the 5 α -face of this radical intermediate to accomplish the net oxidative rearrangement. It also appears that the somewhat tighter binding of the 4(20)-isomer substrate (**4**) may be a reflection that this isomer more closely mimics the allylic radical intermediate.⁶⁸

3.4 Over-Production of Taxa-4(20),11(12)-dien-5 α -ol

As with the studies on the overproduction of taxadiene, Ajikumar and coworkers have also engineered a P450-based oxidation to convert taxadiene to taxa-4(20),11(12)-dien-5 α -ol.⁶⁰ The engineered strain of *E. coli* improved the production of taxa-4(20),11(12)-dien-5 α -ol some 2400-fold over Dejong's yeast alternative.⁷⁴ Interestingly, work towards the metabolic engineering of taxa-4(20),11(12)-dien-5 α -ol formation in tobacco demonstrated that a novel taxane was also being produced as a by product (Scheme 8, **32**).⁷⁵

Rontein and coworkers reported that the rearrangement of taxadiene to OCT is mediated by CYP725A4 and does not rely on additional enzymes or factors.⁷⁵ Furthermore, it was also demonstrated by Ajikumar and coworkers that the production of OCT (**32**) is likely due to the functional plasticity of the 5- α -hydroxylase with its chimeric CYP450 enzymes.⁶⁰

4 Syntheses of Lightly Functionalized Taxoids

To approach subsequent biosynthetic steps considered to involve hydroxylation and acylation of the taxane core,⁷⁶ lightly oxygenated taxoids bearing the C5-hydroxyl group were prepared to evaluate them as bioconversion intermediates in *Taxus* cell feeding studies and as substrates for both native and recombinant enzymes from *Taxus*.

4.1 Synthesis of Taxa-4(20),11(12)-dien-2 α ,5 α -diol

We have completed a synthesis of the **2 α ,5 α -diol (39)** as shown in Scheme 9 utilizing the Shea-Jenkins type II IMDA construction⁷⁷ that was developed and refined in the course of our first total synthesis of taxa-4(5),11(12)-diene (**5**).^{51,52,63-67} The key issue came down to devising a means to reduce the ketone moiety of **21** to the **2 α -alcohol**.

During the course of synthetic studies on taxol, Wender¹² reported the dissolving metal reduction of a substrate structurally related to **21** affording a mixture of α - and β -epimeric alcohols. Indeed, reduction of ketone **21** (Na⁰/EtOH/Et₂O) resulted in a 1.3:1 separable mixture (flash column silica gel chromatography) of **22** and **33** respectively in 58% yield, along with unreacted starting material (13%). We found that when the reduction of **21** is conducted at -40°C the ratio **22:33** can be reversed to 1:1.3 and the yield increased to 79% (Scheme 9). This synthesis provided the first authentic specimen of this material and proved invaluable in identifying this material as a component of *Taxus* tissue extracts,⁷⁷ but its role as a biosynthetic pathway intermediate is presently uncertain and may be verified by radio labeling and incorporation experiments.

4.2 Synthesis of Taxa-4(20),11(12)-dien-2 α ,10 β -diol-5 α -acetate from a Japanese Yew-derived Taxadien-tetraol

Extensive effort was expended on devising totally synthetic approaches to lightly oxygenated taxoids bearing oxygen atoms at C5 and also at either C9 and/or C10 but without success.⁷⁸ We then turned to semi-synthetic approaches using naturally occurring taxoid shunt metabolites that accumulate in *Taxus* sp. heartwood. We acquired over 100 kg of Japanese yew heartwood from a wood sculptor in Japan (Hida Ichii Ittoubori Kyoudou Kumiai of The Engraving Craftsman Association, Japan; see Figure 2) and have extracted more than 3.5 grams of 2 α ,5 α ,10 β -triacetoxy-14 β -(2-methyl)-butyryloxytaxa-4(20),11-diene (**40**) as a raw material for the preparation of a host of lightly oxygenated taxoids. We have completed the semisynthesis of taxa-4(20),11(12)-dien-5 α -yl-acetate-10 β -ol (**47**)⁷⁹ and taxa-4(20),11(12)-dien-5 α -yl-acetate-2 α ,10 β -diol (**49**)⁸⁰ as shown in Schemes 10 and 11. Thus, selective manipulation of the hydroxyl groups of this substrate has led to the synthesis taxa-4(20)-11(12)-diene-5 α -yl-acetate-10 β -ol (**47**, Scheme 10) in tritium-labeled form in preparatively useful amounts. This has provided a key substrate that the Croteau laboratory

has utilized for characterization of downstream hydroxylases. In a first pass, we prepared 32 mgs of tritium-labeled **46** with a specific activity of 11.5 Ci/mol. The Barton deoxygenation protocol permitted the facile tritium labeling of these materials through the purchase of commercially available $\text{Bu}_3\text{Sn}^3\text{H}$ of high specific activity.

Using the same approach, we prepared taxa-4(20),11(12)-dien-5 α -yl-acetate-2 α ,10 β -diol (**49**).⁸⁰ Substrates bearing the 5 α -acetate residue have proven particularly important and, in some cases, difficult to selectively prepare.

For example, diol **49**, prepared by the radical deoxygenation of **42b** (Scheme 11), was subjected to a variety of acetylation conditions. After extensive work, it was found that treatment of **48** with LHMDs at -18°C and careful addition of acetyl bromide, cleanly afforded the C-5 mono-acetate, although only in 12% isolated yield. Fortunately, unreacted **48** could be readily recovered in 74% yield. Removal of the silyl residue with $\text{HF}\beta\text{pyridine}$ gave taxa-4(20),11(12)-dien-5 α -yl-acetate-2 α ,10 β -diol- (**49**, Scheme 11).

Substrate **42b** turned out to be a very useful species for the preparation of several triols as well as constituting an alternate source of the 5,10-diol-mono-acetate (**47**) as shown in Scheme 12. The acylation of **42b** with phenylthionochloroformate, was non-regioselective giving a separable mixture of **50**, **51** and **52**. Tin hydride-based deoxygenation of each species provided the first authentic specimens of taxa-4(20),11(12)-dien-5 α , 10 β ,14 β -triol (**53**) and taxa-4(20),11(12)-dien-2 α , 5 α ,10 β -triol (**54**). Substitution of $\text{Bu}_3\text{Sn}^2\text{H}$ or $\text{Bu}_3\text{Sn}^3\text{H}$ provided the corresponding isotopomers.

Compound **47b** has also constituted an alternative and expedient source for the synthesis of tritiated C5-mono-acetate **27b** (Scheme 13). This has proven significant since the totally synthetic route initially devised to prepare **47** described above, requires extensive time and effort to repeat.⁷⁹

5 Deoxygenation of Taxusin

Taxusin is an abundantly available taxoid that makes up a significant weight% of *Taxus* sp. heart wood. We obtained 5 kg of *Taxus brevifolia* heartwood chips from Prof. Croteau from which we extracted and purified 4.5 grams of taxusin and developed a tetraol protection strategy.

5.1 Attempt to Deoxygenate Taxusin at C-13

Despite considerable effort, we were unable to effect the deoxygenation of taxusin at C13 without concomitant migration of the 11(12)-bridgehead alkene. This would have provided convenient access to the 5,9-diol, 5,10-diol and 5,9,10-triol that are potential candidates as pathway metabolites. This approach has been abandoned in favor of the successful deoxygenation of a taxadien-tetraol obtained from Japanese yew heartwood described above.

Despite the failure to utilize taxusin to prepare C13-deoxytaxoids, we have developed deoxygenation strategies for the other hydroxyl residues at C9 and C10.⁸¹ Thus, treatment of taxusin with potassium carbonate in methanol, selectively removes the C5 and C13 acetate residues (**56**, Scheme 14). Protection of the C5 and C13 hydroxyl groups as the triethylsilyl (TES) ethers and LAH removal of the acetates furnished **57**. Xanthate formation gave a mixture of **58a** and **58b** which could be subjected to radical deoxygenation and fluoride-based desilylation to produce taxa-4(20),11(12)-dien-5 α ,9 α ,13 α -triol (**60**) and taxa-4(20),11(12)-dien-5 α ,10 β ,13 α -triol (**61**).⁸¹

The mixture of **59a/b**, also provided an opportunity for the synthesis of the corresponding taxa-4(20),11(12)-diene-5 α , 13 α -diol (**64**). As shown in Scheme 15, **59a/b** was treated with LHMDS, carbon disulfide and methyl iodide to give a mixture of the xanthates **62a/b**, which could be separated on column but was used as a mixture in the following step. Barton deoxygenation of **62a/b** furnished the protected diol **63** in 79% yield. Final treatment of **63** with HF β pyridine complex afforded taxa-4(20),11(12)-diene-5 α ,13 α -diol (**64**).⁸¹

While this route provided the first authentic specimens of **60** and **64**, it proved to be capricious due to the lability of the *O*-TES ethers. As such, a more robust, second-generation synthesis was devised as shown in Scheme 16 where *O*-diethyl-*iso*-propylsilyl ethers (ODEIPS) were employed. In addition, we found that conversion of taxusin into the corresponding tetraol, followed by protection of the 9,10-diol unit as its cyclic carbonate proved advantageous and reproducible.⁸²

6 Subsequent Biosynthetic Transformations of the 5-Hydroxytaxadiene Core

6.1 10 β and 13 α -hydroxylase

The predicted second and third hydroxylation steps in the pathway are the 13 α - and 10 β -hydroxylations. The responsible microsomal enzymes have been fully characterized, and the respective cytochrome P450 genes have been cloned.^{83,84} The enzymes, originally obtained from *T. cuspidata*,⁸³ show conversion of taxa-4(20),11(12)-diene-5 α -ol (**26**) (as well as its C20 tritium labeled isotope), to the subsequent diols, but 10 β -hydroxylase prefers taxa-4(20), 11(12)-diene-5 α -acetate (**27**) (as well as its C20 tritium labeled isotope) as substrate and 13 α -hydroxylase prefers taxa-4(20),11(12)-diene-5 α -ol (**26**) (Scheme 17). The 13 α -Hydroxylase was characterized as a member of the CYP725A gene family, whereas the 10 β -hydroxylase was determined to be of the CYP725A1 gene line. Subsequent attempts at hydroxylation of either the 10 β /13 α -diols to deliver the corresponding triol were unsuccessful with either of the 10 β /13 α -hydroxylases.⁸³

6.2 2 α - and 7 β -hydroxylase

Work towards the identification and characterization of the 2 α - and 7 β -hydroxylases utilized what is a presumed dead-end metabolite from yew heartwood, (+)-taxusin (**55**). This metabolite actually proved to be a useful surrogate to examine the oxygenations on route to taxol (**1**). Studies using (+)-taxusin (**55**) showed that it could be hydroxylated to give either 2 α -hydroxytaxusin (**73**) or 7 β -hydroxytaxusin (**72**) via their corresponding hydroxylases and that these two intermediates could be further elaborated with the opposing enzyme to give 2 α , 7 β -dihydroxytaxusin (**74**) (Scheme 18). Unfortunately, no structurally simpler taxoids could be utilized by these hydroxylases suggesting that they function later in the biosynthesis of taxol.^{84,85}

Furthermore, the 2 α - and 7 β -hydroxylases genes have been cloned from *Taxus* species and are very similar to those of the CYP725A gene family. Studies show that both of these enzymes operate sequentially with 7 β hydroxylation occurring first, and 2 α -hydroxylation occurring second.⁸⁵ Through catalytic as well as binding studies⁸⁶, it was found that 7 β -hydroxylase was not able to convert less functionalized taxadienes or highly substituted taxoids, which further supported the concept that 7 β -hydroxylation is an intermediate step in the biosynthetic pathway.

6.3 Uncharacterized Hydroxylases

Oxygenation at C-9 is assumed to happen early in the biosynthetic pathway.⁴ A cDNA clone for 9 α -hydroxylase was tentatively identified using taxa-4(20),11(12)-diene-5 α -ol (**26**) as a substrate by in vivo feeding of yeast that were transformed with P450 sequences, but subsequent identification of the product has not been performed due to a lack of material, and the enzyme has not been characterized⁸⁷. Two additional oxidation steps, at C1 and in the side-chain, remain uncharacterized.⁸⁴

6.4 Biosynthetic Conversion to the 5,14-diol: A Case of Aberrant Metabolism

Additional work has been done surrounding the biosynthetic conversion to 14-hydroxylated taxoids. We synthesized a large (150 mg) quantity of tritium-labeled taxa-4(20),11(12)-dien-5 α -ol^{52,71-73} (**26b**) and have used this as a substrate (~1 mg aliquots, the current maximum scale based on microsomal preps) for biosynthetic conversion to more polar products in *Taxus* microsomes. This substrate actually gives three products with a molecular mass of 304 (diol) and three products with a molecular mass of 320 (triol). We obtained 200 μ g of the major diol (a heroic, maxi-prep experiment) and have purified this substance by HPLC. Detailed ¹H NMR analysis (principally 1D TOCSY) revealed that the structure was that of a 5 α ,14 β -diol (**75**, Scheme 19).^{69,88}

Since it is quite unlikely that such a metabolite is on the pathway to taxol, this substance presumably arose *via* a C-14-specific P450 hydroxylase that converts a more highly oxygenated taxoid into the C-14 β -alcohol and accepted **26b** as an adventitious substrate. Due to the low yield and difficulty in separating the other two minor diols, we are focusing on the conversion of taxa-4(20),11(12)-dien-5 α -acetate (**27**) into the diol mono-acetates *via* the relevant recombinant P450 hydroxylases.

A clone for the 14 β -hydroxylase was discovered by in vivo screening of transformed yeast cells bearing candidate P450's genes,⁷⁶ and the corresponding recombinant enzyme was characterized.^{76,88-90} Further studies determined that the 14 β -hydroxylase is able to convert taxa-4(20),11(12)-diene-5 α -yl-acetate, 10 β -diol (**47b**) to taxa-4(20),11(12)-diene-5 α -yl-acetate, 10 β ,14 β -triol, **76** (Scheme 20).

In the case of taxol, hydroxylation at the C-14 position does not occur, but the accumulation of metabolites that are hydroxylated at C-14 is quite significant. The availability of the cDNA encoding for the 14 β -hydroxylase suggests a possible strategy to suppress this gene thereby permitting redirection of the pathway flux toward taxol. Further examination of the 14 β -hydroxylase revealed that it contained several of the typical characteristics of cytochrome P450 enzymes, namely, the oxygen binding motif, a heme-binding motif with the PFG element, and a conserved cysteine at position 444. Alternately, the enzyme contained an additional number of residues at the N-terminus.⁸⁸

7 Studies on the Formation of the Oxetane Ring

The biosynthetic pathway to taxol also includes five acyltransferase-catalyzed steps. Each transacylase delivers an acyl group from acyl CoA to a pathway intermediate.⁹¹⁻⁹³ Previous theories indicate that the ene-acetoxy functional group of taxa-4(20),11(12)-diene-5 α -yl-acetate (**26**) transforms into the 5-acetoxy-4(20)-epoxy moiety with subsequent rearrangement to the oxetane (**77**) (Scheme 21).⁹⁴

Alternatively, recent studies by Ondari and Walker now suggest another possibility. Ondari showed that when using advanced taxanes deacetylated at C4, a regioselective acetylation with a recombinantly expressed *Taxus* 10-deacetylbaicatin 10 β -O-acetyltransferase (**DBAT**) catalyzes transfer to the tertiary C4 position on the oxetane ring, in this case, on 4-

DAB (79).⁹⁵ This new evidence adds another possibility to the sequence of events in the biosynthesis of taxol.

8 C13 Acylation: Final Step in the Biosynthesis of Taxol

Extensive work has been done decoding the final steps towards the production of taxol, namely, the acylation at C13. Early *in vivo* studies showed that the intact side chain⁴ was not incorporated into taxol, whereas β -phenylalanine and α -phenylalanine and phenylisoserine were incorporated. These findings suggested that β -phenylalanine is attached to baccatin III, and that this side chain is subsequently hydroxylated and then N-benzoylated to complete the biosynthesis of taxol.⁹⁶ Walker and coworkers discovered a cDNA clone encoding a taxoid C13 *O*-phenylpropanoyltransferase which yielded a recombinant enzyme that selectively catalyzed the selective 13-*O*-acylation of baccatin III with β -phenylalanoyl CoA (83) as the acyl donor, forming N-debenzoyl-2'-deoxytaxol (84).^{91,92} Interestingly, 2*S*- α -phenylalanine (81) is converted to 3*R*- β -phenylalanine (82) by phenylalanine aminomutase (PAM).⁹⁷ The stereochemical mechanism of PAM actually involves the removal and interchange of the pro-3*S* hydrogen and the amino group, which subsequently rebonds at C3 with retention of configuration (Scheme 23)⁹⁷

Furthermore, the crystal structure as well as additional mechanistic insight into the phenylalanine aminomutase has been recently reported.⁹⁸

9 Conclusion

Overall, a combination of total synthesis and semi-synthesis has been used to prepare taxoids as putative substrates for interrogating the substrate specificity of the cytochrome P450 enzymes involved in hydroxylations of the tricyclic hydrocarbon core of taxol. Both stable-isotope and radio-isotope labeling have been employed (feeding study results summarized in Table 1), with the former being utilized more dominantly at the present time. Furthermore a greater understanding of the biosynthetic pathway is being gained and is helping to piece together the puzzle of the biosynthesis of taxol. Although much work has yet to be complete, especially with late stage intermediates, advances in bioengineering and overexpression of recombinant enzymes are leading the way in pushing the biosynthetic pathway towards a functional synthesis of taxol.

Acknowledgments

The studies performed by the Williams and Croteau groups reported in this review were supported by the National Institutes of Health (Grant CA70375 to RMW and CA55254 to RBC).

11 References

1. Paclitaxel the generic name for Taxol®, is a registered trademark for Bristol-Myers Squibb. Due to greater familiarity with the word taxol, this will be used throughout this application and the symbol ® has been deleted for the sake of clarity and brevity.
2. Georg G, Ali S, Zygmunt J, Jayasinghe L. Exp. Op. Ther. Pat. 1994; 4(2):109–120.
3. Boa AN, Jenkins PR, Lawrence MJ. Contemp. Org. Synth. 1994:47–75.
4. Floss, HG.; Moeck, U. Taxol: Science and Applications. CRC Press; Boca Raton: 1995. Biosynthesis of Taxol.
5. Guenard D, Guerittevoegelein F, Potier P. Acc. Chem. Res. 1993; 26(4):160–167.
6. Kingston DGI. Pharmacol. Ther. 1991; 52(1):1–34. [PubMed: 1687170]
7. Nicolaou KC, Dai WM, Guy RK. Angew. Chem. Int. Ed. Eng. 1994; 33:15–44.
8. Rohr J. Angew. Chem. Int. Ed. Eng. 1997; 36:2190–2195.

9. Swindell CS. *Org. Prep. Proced. Int.* 1991; 23(4):465–543.
10. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. *J. Am. Chem. Soc.* 1971; 93(9):2325–2327. [PubMed: 5553076]
11. Holton RA, Juo RR, Kim HB, Williams AD, Harusawa S, Lowenthal RE, Yogai S. *J. Am. Chem. Soc.* 1988; 110(19):6558–6560.
12. Wender PA, Mucciario TP. *J. Am. Chem. Soc.* 1992; 114(14):5878–5879.
13. Nicolaou KC, Yang Z, Liu JJ, Ueno H, Nantermet PG, Guy RK, Claiborne CF, Renaud J, Couladouros EA, Paulvannan K, et al. *Nature.* 1994; 367(6464):630–634. [PubMed: 7906395]
14. Nicolaou KC, Nantermet PG, Ueno H, Guy RK, Couladouros EA, Sorensen EJ. *J. Am. Chem. Soc.* 1995; 117(2):624–633.
15. Nicolaou KC, Liu JJ, Yang Z, Ueno H, Sorensen EJ, Claiborne CF, Guy RK, Hwang CK, Nakada M, Nantermet PG. *J. Am. Chem. Soc.* 1995; 117(2):634–644.
16. Nicolaou KC, Yang Z, Liu JJ, Nantermet PG, Claiborne CF, Renaud J, Guy RK, Shibayama K. *J. Am. Chem. Soc.* 1995; 117(2):645–652.
17. Nicolaou KC, Ueno H, Liu JJ, Nantermet PG, Yang Z, Renaud J, Paulvannan K, Chadha R. *J. Am. Chem. Soc.* 1995; 117(2):653–659.
18. Holton RA, Somoza C, Kim HB, Liang F, Biediger RJ, Boatman PD, Shindo M, Smith CC, Kim SC, Nadizadeh H, Suzuki Y, Tao CL, Vu P, Tang SH, Zhang PS, Murthi KK, Gentile LN, Liu JH. *J. Am. Chem. Soc.* 1994; 116(4):1597–1598.
19. Holton RA, Kim HB, Somoza C, Liang F, Biediger RJ, Boatman PD, Shindo M, Smith CC, Kim SC, Nadizadeh H, Suzuki Y, Tao CL, Vu P, Tang SH, Zhang PS, Murthi KK, Gentile LN, Liu JH. *J. Am. Chem. Soc.* 1994; 116(4):1599–1600.
20. Masters JJ, Link JT, Snyder LB, Young WB, Danishefsky SJ. *Angew. Chem., Int. Ed.* 1995; 34(16):1723–1726.
21. Wender PA, Badham NF, Conway SP, Floreancig PE, Glass TE, Granicher C, Houze JB, Janichen J, Lee DS, Marquess DG, McGrane PL, Meng W, Mucciario TP, Muhlebach M, Natchus MG, Paulsen H, Rawlins DB, Satkofsky J, Shuker AJ, Sutton JC, Taylor RE, Tomooka K. *J. Am. Chem. Soc.* 1997; 119(11):2755–2756.
22. Wender PA, Badham NF, Conway SP, Floreancig PE, Glass TE, Houze JB, Krauss NE, Lee DS, Marquess DG, McGrane PL, Meng W, Natchus MG, Shuker AJ, Sutton JC, Taylor RE. *J. Am. Chem. Soc.* 1997; 119(11):2757–2758.
23. Morihira K, Hara R, Kawahara S, Nishimori T, Nakamura N, Kusama H, Kuwajima I. *J. Am. Chem. Soc.* 1998; 120(49):12980–12981.
24. Paquette LA, Wang HL, Su Z, Zhao MZ. *J. Am. Chem. Soc.* 1998; 120(21):5213–5225.
25. Mukaiyama T, Shiina I, Iwadare H, Saitoh M, Nishimura T, Ohkawa N, Sakoh H, Nishimura K, Tani Y, Hasegawa M, Yamada K, Saitoh K. *Chem.-Eur. J.* 1999; 5(1):121–161.
26. Han KH, Fleming P, Walker K, Loper M, Chilton WS, Mocek U, Gordon MP, Floss HG. *Plant Science.* 1994; 95(2):187–196.
27. Stierle A, Strobel G, Stierle D. *Science.* 1993; 260(5105):214–216. [PubMed: 8097061]
28. Strobel G, Yang XS, Sears J, Kramer R, Sidhu RS, Hess WM. *Microbiology-Uk.* 1996; 142:435–440.
29. Strobel GA, Hess WM, Ford E, Sidhu R, Yang X. *J. Ind. Microbiol.* 1996; 17:417–423.
30. Strobel GA, Hess WM, Li JY, Ford E, Sears J, Sidhu RS, Summerell B. *Australian Journal of Botany.* 1997; 45(6):1073–1082.
31. Hoffman AK, W. Waroapng J, Strobel G, Griffin D, Arbogast B, Barofsky D, Boone RB, Ning L, Zheng P, Daley L. *Spectroscopy.* 1999; 13:22–32.
32. Stierle A, Stierle D, Strobel G, Bignami G, Grothaus P. *Bioactive Metabolites of the Endophytic Fungi of Pacific Yew, Taxus brevifolia. Taxane Anticancer Agents, American Chemical Society.* 1995:82–97.
33. Shrestha K, Strobel GA, Shrivastava SP, Gewali MB. *Planta Med.* 2001; 67(4):374–6. [PubMed: 11458463]
34. Li JY, Sidhu RS, Ford EJ, Long DM, Strobel GA. *J. Ind. Microbiol. Biotech.* 1998; 20:259–264.

35. Metz AM, Haddad A, Worapong J, Long DM, Ford EJ, Hess WM, Strobel GA. *Microbiology*. 2000; 146:2079–2089. [PubMed: 10931912]
36. Fleming PE, Knaggs AR, He XG, Mocek U, Floss HG. *J. Am. Chem. Soc.* 1994; 116(9):4137–4138.
37. Fleming PE, Mocek U, Floss HG. *J. Am. Chem. Soc.* 1993; 115(2):805–807.
38. Harrison JWS, R.M. Lythgoe B. *J. Chem. Soc. (C)*. 1966:1933–1945.
39. Gueritte-Voegelein F, Guenard D, Potier P. *J. Nat. Prod.* 1987; 50(1):9–18. [PubMed: 2885400]
40. Herbert, BA. *The Biosynthesis of Secondary Metabolites*. Chapman and Hall; London: 1981.
41. Nakanishi, K.; Goto, T.; Ito, S.; Nozoe, S. *Natural Products Chemistry*. Vol. Vol. 1. Kodansha, Ltd; Tokyo: 1974.
42. Towers, GH. *Biochemistry of the Mevalonic Acid Pathway to Terpenoids*. In: Stafford, HA., editor. *Recent Advances in Phytochemistry*. Vol. Vol. 24. Plenum Press; New York: 1990.
43. Luckner, M. *Secondary Metabolism in Microorganisms, Plants and Animals*. Springer-Verlag; Berlin: 1990.
44. Eisenrich WM, B. Hylands PJ, Zenk MH, Bacher A. *Proc. Natl. Acad. Sci. U. S. A.* 1996; 93:6431–6436. [PubMed: 8692832]
45. Koepp AE, Hezari M, Zajicek J, Vogel BS, LaFever RE, Lewis NG, Croteau R. *J. Biol. Chem.* 1995; 270(15):8686–8690. [PubMed: 7721772]
46. Hezari M, Lewis NG, Croteau R. *Arch. Biochem. Biophys.* 1995; 322(2):437–444. [PubMed: 7574719]
47. Lin X, Hezari M, Koepp AE, Floss HG, Croteau R. *Biochemistry*. 1996; 35(9):2968–77. [PubMed: 8608134]
48. Wildung MR, Croteau R. *J. Biol. Chem.* 1996; 271(16):9201–9204. [PubMed: 8621577]
49. Hezari M, Croteau R. *Planta Med.* 1997; 63(4):291–295. [PubMed: 9270370]
50. Williams DC, Carroll BJ, Jin Q, Rithner CD, Lenger SR, Floss HG, Coates RM, Williams RM, Croteau R. *Chem. Biol.* 2000; 7(12):969–977. [PubMed: 11137819]
51. Rubenstein SM, Williams RM. *J. Org. Chem.* 1995; 60(22):7215–7223.
52. Rubenstein, SM. *Dissertation: Elucidating the Biosynthetic Pathway to Taxol*. Colorado State University; 1996.
53. Walker KC, R. *Rec. Adv. Phytochem.* 1999; 33:31–50.
54. Hezari M, Ketchum REB, Gibson DM, Croteau R. *Arch. Biochem. Biophys.* 1997; 337(2):185–190. [PubMed: 9016812]
55. Williams DC, Wildung MR, Jin AQW, Dalal D, Oliver JS, Coates RM, Croteau R. *Arch. Biochem. Biophys.* 2000; 379(1):137–146. [PubMed: 10864451]
56. Koksai M, Jin Y, Coates RM, Croteau R, Christianson DW. *Nature*. 2011; 469:116–122. [PubMed: 21160477]
57. Jin Q, Williams DC, Hezari M, Croteau R, Coates RM. *J. Org. Chem.* 2005; 70(12):4667–75. [PubMed: 15932303]
58. Tyo KE, Alper HS, Stephanopoulos GN. *Trends Biotechnol.* 2007; 25(3):132–137. [PubMed: 17254656]
59. Ajikumar PK, Tyo K, Carlsen S, Mucha O, Phon TH, Stephanopoulos G. *Mol. Pharm.* 2008; 5(2): 167–190. [PubMed: 18355030]
60. Ajikumar PK, Xiao WH, Tyo KEJ, Wang Y, Simeon F, Leonard E, Mucha O, Phon TH, Pfeifer B, Stephanopoulos G. *Science*. 2010; 330(6000):70–74. [PubMed: 20929806]
61. Boghigian BA, Salas D, Ajikumar PK, Stephanopoulos G, Pfeifer BA. *Appl. Microbiol. Biotechnol.* 2012; 93:1651–1661. [PubMed: 21850432]
62. Boghigian BA, Armando J, Salas D, Pfeifer BA. *Appl. Microbiol. Biotechnol.* 2012; 93:2063–2073. [PubMed: 22124721]
63. Shea KJ, Davis PD. *Angew. Chem. Int. Ed. Eng.* 1983; 22:419.
64. Shea KJ, Davis PD. *Angew. Chem. Int. Ed. Eng.* 1983; 22:546.
65. Bonnert RV, Jenkins PR. *J. Chem. Soc. Perkin Trans. 1*. 1989:413.
66. Brown PA, Jenkins PR. *J. Chem. Soc. Perkin Trans. 1*. 1986:1303.

67. Brown PA, Jenkins PR, Fawcett J, Russell DR. *J. Chem. Soc. Perkin Trans. 1*. 1984;253.
68. Jennewein S, Long RM, Williams RM, Croteau R. *Chem. Biol.* 2004; 11:379–387. [PubMed: 15123267]
69. Wheeler AL, Long RM, Ketchum RE, Rithner CD, Williams RM, Croteau R. *Arch. Biochem. Biophys.* 2001; 390:265–278. [PubMed: 11396929]
70. Hezari M, Croteau R. *Planta Med.* 1997; 63(4):291–295. [PubMed: 9270370]
71. Hefner J, Rubenstein SM, Ketchum RE, Gibson DM, Williams RM, Croteau R. *Chem. Biol.* 1996; 3(6):479–489. [PubMed: 8807878]
72. Borman S. *Chem. Eng. News.* 1996; 74(27):27–29.
73. Rubenstein SM, Vazquez A, Sanz-Cervera JF, Williams RM. *J. Labelled Compd. Radiopharm.* 2000; 43(5):481–491.
74. DeJong JM, Liu YL, Bollon AP, Long RM, Jennewein S, Williams D, Croteau RB. *Biotechnol. Bioeng.* 2006; 93(2):212–224. [PubMed: 16161138]
75. Rontein D, Onillon S, Herbette G, Lesot A, Werck-Reichhart D, Sallaud C, Tissier A. *J. Biol. Chem.* 2008; 283(10):6067–75. [PubMed: 18167342]
76. Croteau R, Ketchum RE, Long RM, Kaspera R, Wildung MR. *Phytochem. Rev.* 2006; 5(1):75–97. [PubMed: 20622989]
77. Vazquez A, Williams RM. *J. Org. Chem.* 2000; 65(23):7865–7869. [PubMed: 11073592]
78. Lenger SR. Dissertation: Studies on the Total Synthesis of Putative Intermediates in the Biosynthesis of Taxol. 2003
79. Horiguchi T, Rithner CD, Croteau R, Williams RM. *J. Labelled Compd. Radiopharm.* 2008; 51(9-10):325–328.
80. Horiguchi TR, C.D. Croteau R, Williams RM. *J. Org. Chem.* 2002; 67:4901–4903. [PubMed: 12098303]
81. Li H, Horiguchi T, Croteau R, Williams RM. *Tetrahedron.* 2008; 64(27):6561–6567. [PubMed: 19122848]
82. Horiguchi TR, C.D. Croteau R, Williams RM. *Tetrahedron.* 2003; 59:267–273.
83. Jennewein S, Rithner CD, Williams RM, Croteau RB. *Proc. Natl. Acad. Sci. U. S. A.* 2001; 98(24): 13595–13600. [PubMed: 11707604]
84. Kaspera R, Croteau R. *Phytochem. Rev.* 2006; 5(2):433–444. [PubMed: 20622990]
85. Chau M, Croteau R. *Arch. Biochem. Biophys.* 2004; 427(1):48–57. [PubMed: 15178487]
86. Chau M, Jennewein S, Walker K, Croteau R. *Chem. Biol.* 2004; 11(5):663–672. [PubMed: 15157877]
87. Walker K, Fujisaki S, Long R, Croteau R. *Proc. Natl. Acad. Sci. U. S. A.* 2002; 99(20):12715–20. [PubMed: 12232048]
88. Jennewein S, Rithner CD, Williams RM, Croteau R. *Arch. Biochem. Biophys.* 2003; 413(2):262–70. [PubMed: 12729625]
89. Ketchum RE, Rithner CD, Qiu D, Kim YS, Williams RM, Croteau RB. *Phytochemistry.* 2003; 62(6):901–909. [PubMed: 12590117]
90. Ketchum REG, D.M. Croteau R, Shuler ML. *Biotechnol. Bioeng.* 1999; 62:97–105. [PubMed: 10099517]
91. Walker K, Long R, Croteau R. *Proc. Natl. Acad. Sci. U. S. A.* 2002; 99(14):9166–9171. [PubMed: 12089320]
92. Walker K, Fujisaki S, Long R, Croteau R. *Proc. Natl. Acad. Sci. U. S. A.* 2002; 99(20):12715–12720. [PubMed: 12232048]
93. Walker K, Croteau R. *Phytochemistry.* 2001; 58(1):1–7. [PubMed: 11524108]
94. Gueritte-Voegelein F, Guenard D, Potier P. *J. Nat. Prod.* 1987; 50(1):9–18. [PubMed: 2885400]
95. Ondari ME, Walker KD. *J. Am. Chem. Soc.* 2008; 130(50):17187–17194. [PubMed: 19007225]
96. Long RM, Croteau R. *Biochem. Biophys. Res. Commun.* 2005; 338(1):410–417. [PubMed: 16137660]
97. Walker KD, Klettke K, Akiyama T, Croteau R. *J. Biol. Chem.* 2004; 279(52):53947–53954. [PubMed: 15494399]

98. Feng L, Wanninayake U, Strom S, Geiger J, Walker KD. *Biochemistry*. 2011; 50(14):2919–2930. [PubMed: 21361343]

Figure 1.
The structure of Taxol (**1**) and Taxotere (**2**).

Figure 2.
Small sculpture made from Japanese Yew by Hida Ichii Ittoubori Kyoudou Kumiai of The Engraving Craftsman Association, Japan.

Scheme 1.

Early biosynthetic proposal from ggPP to taxol.

Scheme 2.
Products of recombinant taxadiene synthase.

Scheme 3.
Mechanism of taxadiene synthase.⁵⁰

Scheme 4.

Scheme 4.
Total synthesis of taxa-4(5),11(12)-diene (**5**) and taxa-4(20),11(12)-diene (**4**).

Scheme 6.
Synthesis of taxadiene 5α- and 5β-ols (**26** and **28**).

Scheme 7.
Mechanism of taxadiene hydroxylase.⁶⁸

Scheme 8.
Production of Novel Taxane, OCT.

Scheme 9.

Synthesis of taxa-4(20),11(12)-dien-2 α ,5 α -diol (**39**).

Scheme 10.

Synthesis of tritiated taxa-4(20),11(12)-dien-5 α -yl-acetate-10 β -ol (**47**).⁷⁹

Scheme 11. Synthesis of taxa-4(20),11(12)-dien-5 α -yl-acetate-2 α ,10 β -diol (**49**).⁸⁰

Scheme 12.

Synthesis of the 5,10,14-triol; the 2,5,10-triol and the 5,10-diol.

Scheme 13. Alternative route to taxa-4(20),11(12)-diene-5 α -acetate

Scheme 14.

Synthesis of the 5,9,13-triol and the 5,10,13-triol by deoxygenation of taxusin.

Scheme 15. Synthesis of taxa-4(20),11(12)-dien-5 α ,13 α -diol.

Scheme 16.

Alternative syntheses of the 5,9,13-triol and the 5,10,13-triol by deoxygenation of taxusin.

Scheme 17.
13 α - and 10 β -hydroxylase

Scheme 18.
2α- and 7β-Hydroxylase Activity.

Scheme 19.
Biosynthetic formation of the 5 α , 14 β -diol (75).

Scheme 20.
14 β -hydroxylase Activity.

Scheme 21.
Original Theory of Oxetane Ring Formation.

Scheme 22.
Acylation of C4 in Advanced Taxanes.

Scheme 23.
C13 Side-chain Construction and Implementation.

Table 1

Summary of Feeding Studies.

Taxoid	Incorporation into Taxol in Cell Culture	Detected as a Natural Metabolite
Yes	Yes
?	?
Yes	Yes
Yes	Yes
Yes	Yes
Yes	?
Tentative	?
Yes	?
?	?
No	?

Taxoid	Incorporation into Taxol in Cell Culture	Detected as a Natural Metabolite
Tentative	?