JOC Article

Studies on Taxol Biosynthesis. Preparation of Taxa-4(20),11(12)-dien-5α-acetoxy-10β-ol by Deoxygenation of a Taxadiene Tetraacetate Obtained from Japanese Yew

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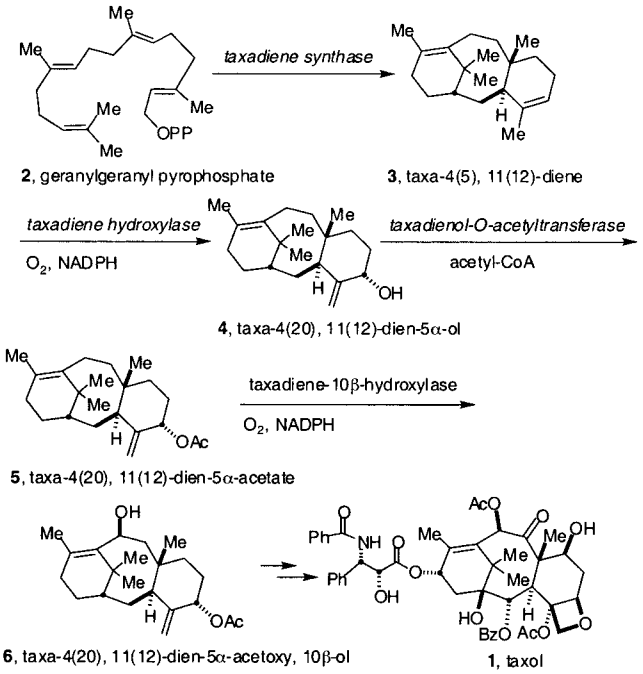
Taxa-4(20),11(12)-dien-5α-acetoxy-10β-ol **6** has been identified as an early stage intermediate involved in the biosynthesis of taxol (Paclitaxel). This compound has been efficiently prepared by Barton deoxygenation of the C-2- and C-14-hydroxyl groups on a derivative semisynthetically prepared from taxa-4(20),11(12)-dien-2α,5α,10β-triacetoxy-14β-(2-methyl)butyrate (**7**), a major taxoid metabolite isolated from Japanese yew heart wood. The synthetic methodology is amenable for the preparation of isotopically labeled congeners that will be useful to probe further intermediate steps in the biosynthesis of taxol.

In connection with ongoing studies on the biosynthesis of taxol (Paclitaxel, **1**),[1,2] our laboratory needed to prepare several lightly oxygenated taxoids with the aim of elucidating intermediate hydroxylation steps in the biosynthetic pathway. To fully gain command of taxol biosynthesis in genetically engineered *Taxus* sp. cell culture systems, a detailed understanding of the steps of taxol biosynthesis and the identification of the associated genes is essential.

A combination of in vivo feeding studies and investigations with cell-free enzyme systems, using Yew stem tissue or suspension cultured *Taxus* sp. cells as bioconversion vectors, have recently revealed that the early steps of the taxol biosynthetic pathway proceed in sequence from the initial conversion of geranylgeranyl pyrophosphate (**2**) to the parent diene **3** catalyzed by taxadiene synthase (Scheme 1).[3,4] Following this first committed step in taxol biosynthesis, taxadiene hydroxylase, a cytochrome P-450-dependent enzyme, regioselectively hydroxylates **3** with allylic rearrangement, to taxa-4(20),11(12)-dien-5α-ol (**4**).[5] Taxadienol-*O*-acetyltransferase subsequently acylates **4** to provide the acetate **5**, which has proven to be a superior substrate for downstream hydroxylation reactions.[6]

SCHEME 1. Early Steps in Taxol Biosynthesis



Initial observations from our laboratories reveal that after the formation of **5**, downstream hydroxylations

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(2) Paclitaxel is the generic name for taxol, a registered trademark of Bristol-Myers Squibb; because of its greater familiarity, the term "taxol" is used throughout.

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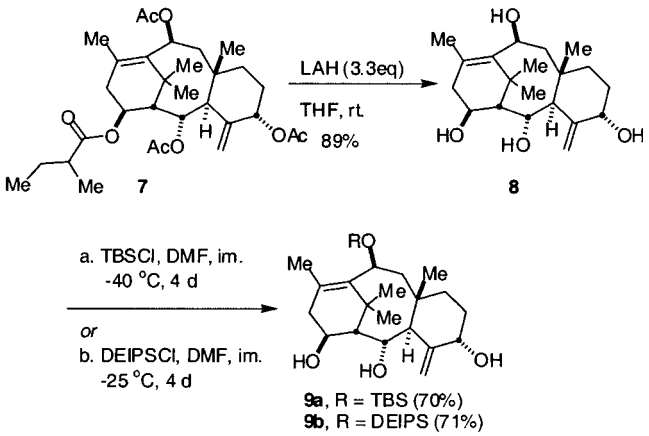
SCHEME 2



enter a very complex matrix and the elucidation of a single linear path to taxol has proven extremely challenging. This appears to be due to the softening of substrate specificity by several of the remaining cytochrome P-450 enzymes that can accept **5** as a suitable substrate in vitro. Two major approaches are being concurrently investigated to identify the genes and associated intermediates from **5** to taxol. In the first approach, we have obtained a set of related full-length cytochrome P-450 clones by the method of differential display of mRNA-reverse transcription-PCR, followed by traditional library screening. Clones were selected based on homology to other plant cytochrome P-450s and used to individually transform *Saccharomyces cerevisiae* and the transformed yeast clones were screened for oxygenation activity with several taxoids as substrates. One such clone produced taxa-4(20),11(12)-dien-5α-acetoxy-10*β*-ol (**6**) using taxa-4(20),11(12)-dien-5α-acetate (**5**) as a substrate.[7] This material has been characterized by ^{1}H NMR and mass spectroscopy and has been shown to incorporate into taxol in vivo.

In a second approach, microsomal bioconversion of **5** with *Taxus* sp. microsomes to more polar products, however, yields several diol monoacetates including taxa-4(20),11(12)-dien-5α-acetoxy-2α-ol[8] and taxa-4(20),-11(12)-dien-5α-acetoxy-13α-ol along with other, as yet unidentified oxygenation products.[9]

Due to the very low yield of the intermediate metabolites that may be obtained from natural sources, we have relied heavily on synthetic, tritium-labeled taxadienes **3**,[10] **4**,[5] and **5**[5] as substrates from which in vivo and in vitro bioconversion strategies have been utilized to identify lightly oxenated taxoids downstream of these substances. Using tritium-labeled **5** as a substrate, we have identified several diol, triol, and higher polyols from *Taxus* sp. suspension cell cultures by GC-MS, and, in a few instances where more than 20 *μ*g was produced and purified, ^{1}H NMR techniques were applied to identify the structures of three diol monoacteates (vida supra). Since diol monoactetate **5** appears to enter an oxygenation "matrix" and is converted into a host of polyols, we have focused on the downstream oxygenation reactions of **6**, a verified taxol pathway metabolite. However, production of **6** by bioconversion of **5** gives at best, sub-milligram quantities of this substance, which is insufficient for the substrate requirements of our planned bioconversion experiments. We have thus devoted considerable effort to devising totally synthetic and semisynthetic methods to prepare this compound. A further restriction we have placed on our synthetic planning requires that the convenient installation of radioisotopic tags be easily attainable.

Herein, we report a method to semisynthetically prepare **6** from taxa-4(20),11(12)-dien-2α,5α,10*β*-triacetoxy-14*β*-(2-methyl)butyrate (**7**), a component of Japanese Yew heart wood.[11] This method appears easily applicable for the preparation of multi-milligram quantities of tritium-labeled **6**.

Taxa-4(20),11(12)-dien-2α,5α,10*β*-triacetoxy-14*β*-(2-methyl)butyrate **7** was prepared by extraction from Japanese yew heart wood. Although the yield of **7** is less than that of taxusin,[12] the main taxoid component in Yew heart wood, compound **7** does not possess the C-13 allylic hydroxyl group. We have devoted considerable efforts to deoxygenate taxusin at C-13, but all attempts to reductively remove this hydroxyl function were attended by allylic transposition of the bridgehead olefin to the 12-(13) position.[13] Compound **7**, therefore, appeared to be an excellent precursor for the synthesis of lightly oxygenated C-13-deoxy taxoids.

As shown in Scheme 2, all of the acyl groups of **7** were removed by treatment with LAH to give taxa-4(20),11-(12)-dien-2α,5α,10*β*,14*β*-tetraol **8**[14] in good yield. Next, the C-10-hydroxyl group was selectively protected as the corresponding *tert*-butyldimethylsilyl ether (TBS) **9a** or as the corresponding diethylisopropylsilyl ether (DEIPS) **9b** by treatment with the corresponding silyl chlorides at low temperature. Deoxygenation via hydride attack on C-2- and/or C-14- mesyl or tosyl groups was considered, but appeared to be difficult due to steric hindrance on the congested and convex α-face at C-14 as well as to steric hindrance imposed by the C-17 methyl group on the *β*-face at C2.

Thus, Barton deoxygenation strategies were examined.[15] Xanthate esters were formed from the sterically more accessible C-2 hydroxyl group of triols **9a** and **9b** by treatment with base followed by *O*-alkylation with carbon disulfide and methyl iodide. This protocol furnished the requisite substrates **10a** and **10b** in 55% and 44% yields, respectively (Scheme 3). Resubjecting **10a** and **10b** to the xanthate ester acylation conditions yielded

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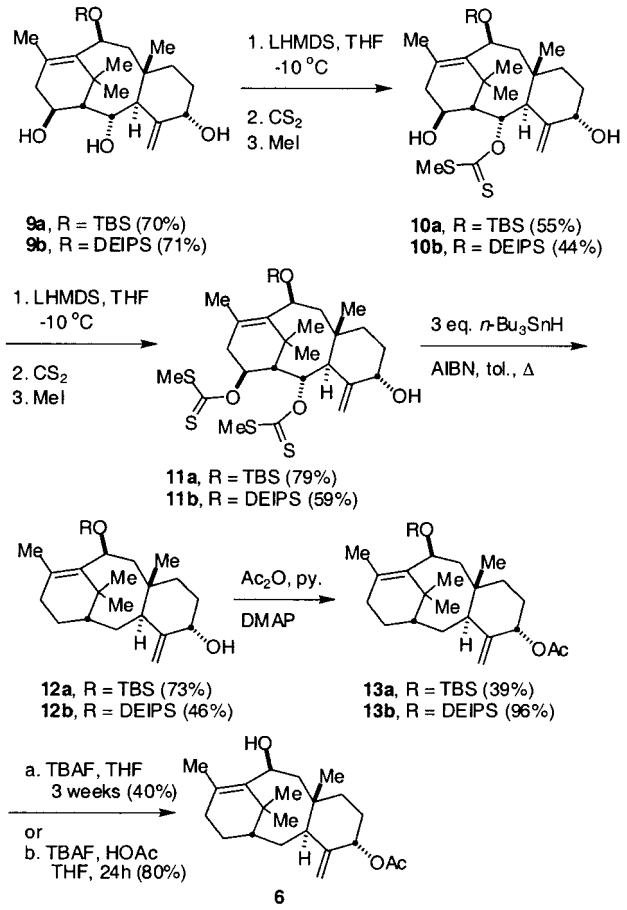
SCHEME 3



the bis-xanthates **11a** and **11b** in 79% and 59% yields, respectively.

Treatment of **11a** and **11b** with tri-*n*-butyltin hydride in the presence of AIBN in toluene at 100−120 °C gave the desired deoxygenation products **12a** and **12b** in 73% and 46% yields, respectively. Acetylation of these substances with acetic anhydride in pyridine containing DMAP provided the corresponding acetates **13a** and **13b** in 39% and 96% isolated yields, respectively. Removal of the *O*-TBS group from **13a** required treatment with TBAF in THF for 20 days, affording **6** in 40% yield. The DEIPS group was more easily removed requiring treatment with TBAF for 1 day to give **6** in 80% isolated yield. The semisynthetic **6** prepared by either route proved to be identical by ^{1}H NMR and ^{13}C NMR to that of natural taxa-4(20),11(12)-dien-5α-acetoxy-10*β*-ol (**6**).[7] The substitution of *n*-Bu_3Sn^3H for *n*-Bu_3SnH in the Barton deoxygenation step (**11** → **12**) is reasonably expected to yield the requisite ^{3}H-labeled **6**.

In summary, taxa-4(20),11(12)-dien-5α-acetoxy-10*β*-ol (**6**) has been efficiently prepared from the readily available taxa-4(20),11-dien-2α,5α,10*β*-triacetoxy-14*β*-(2-methyl)butyrate **7**, one of the main taxoid components in Japanese Yew heart wood. The synthetic methodology described here is readily amenable for the preparation of isotopically labeled congeners that are required for biosynthetic studies. In addition, the synthesis recorded here provides further corroboration of the structure assigned to **6** that was based on extensive 2D NMR methods. Utilization of this procedure is presently being explored to probe further steps in the biosynthesis of taxol and will be reported on in due course.

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Supporting Information Available: Full experimental procedures and ^{1}H NMR spectra of all new compounds reported in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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