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An efficient synthesis of 4(5),11(12)-taxadiene derivatives and microbial mediated 20-hydroxylation of taxoids

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Abstract

4(20),11(12)-Taxadiene **1** is specifically converted into 4(5),11(12)-taxadiene **5** via Claisen-like rearrangement and Barton radical desulfurization (cleavage of C–S bond) in high yield. Taxoid **5** and its deoxygenated derivatives are potential biosynthetic precursors of paclitaxel and other taxoids. 20-Hydroxylated metabolite **8** is one major product from microbially mediated hydroxylation of **5**. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: rearrangement; radicals and radical reactions; desulfurization; taxoids; microorganisms; hydroxylation.

Since the discovery of paclitaxel (Taxol®),[1] a potent antitumor drug, great progress has been made in understanding its biological activity and chemistry. The most promising approaches for a large scale production of taxol rely on biological and semisynthetic methods. Advances in genetic engineering of *Taxus* would provide new approaches for improvement of the production of paclitaxel by biological methods. The cyclization of geranylgeranyl diphosphate to 4(5),11(12)-taxadiene is the first dedicated step in the biosynthesis of paclitaxel and other related taxoids.[2a] The responsible enzyme, taxadiene synthase and its cDNA has been isolated from *Taxus brevifolia* and overexpression of the enzyme has been recently achieved in *E. coli*.[2e,f] The next step of the pathway is known to be the transformation of 4(5),11(12)-taxadiene to 5α-hydroxy-4(20),11(12)-taxadiene via cytochrome P-450 mediated hydroxylation.[2d]

In order to gain a better understanding of the biosynthetic pathway of different taxoids, in particular, the role of 4(5),11(12)-taxadienes, it is important to obtain a large quantity of 4(5),11(12)-taxadiene derivatives with different oxygen substituents. The total synthesis of taxa-4(5),11(12)-diene and taxa-4(20),11(12)-diene has been achieved, but is lengthy and the overall yield is low.[4] Chemical or biological isomerization of these two types of taxoid has not yet been described. Microorganisms such as filamentous fungi are known to carry out biotransformation of taxoids, and can perform regio- and stereoselective hydroxylation.[5] We report herein a versatile and efficient method for the synthesis of 4(5),11(12)-

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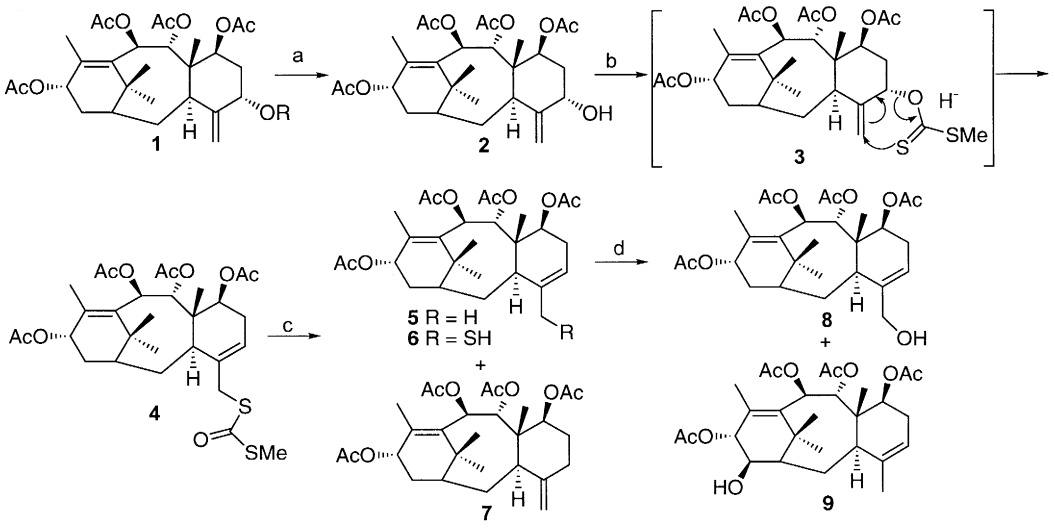
taxadiene derivatives from the 4(20),11(12)-taxadiene **1** (readily available from *Taxus* species[5b]), and microbially mediated 20-hydroxylation of 4(5),11(12)-taxadiene derivatives.

Our approach is illustrated in Scheme 1. Treatment of **1** with hydroxylamine at 80°C afforded 5-hydroxy derivative **2** in high yield.[5b] Treatment of **2** with carbon disulfide and sodium hydride in THF gave the 20-dithiocarbonate derivative **4** in excellent yield (rather than the 5-xanthate derivative). The structure of **2** was fully elucidated by extensive spectral analysis (^{1}H, ^{1}H–^{1}H COSY, ^{13}C, DEPT, MS). The proposed mechanism is considered to proceed via an intermediate **3**, which undergoes Claisen-like rearrangement to give the desired product **4**.



Scheme 1. *Reagents and conditions*: (a) $NH_2OH \cdot HCl$ (10 equiv.), NaOAc, EtOH/H_2O, 80°C, 60 h (85%); (b) NaH (5 equiv.), THF/CS_2 (2:1), rt, 2 h, then MeI (10 equiv.), rt, 16 h (92%); (c) BuSnH (5 equiv.), *p*-cymene, 150°C, 24 h (65% of **5**, 5% of **7**); (d) *Absidia coerula*, 100 mg of **5** per litre of culture, 27°C, 200 rpm, 9 days, 10% of **8**, 3% of **9**

Dithiocarbonate **4** was reduced with tributyltin hydride upon heating at 150°C in *p*-cymene or xylene to produce 4(5),11(12)-taxadiene **5** in good yield. A detailed analysis of ^{1}H, ^{1}H–^{1}H COSY, ^{13}C, DEPT-NMR and FABMS confirmed the structure of **5**.[7] While Barton radical deoxygenation of primary alcohols is well documented,[6] in our case it is most likely that this reaction proceeds by tributyltin mediated radical fission of the allylic C–S bond, rather than the usual C–O bond cleavage. The rearranged product, 4(20),11(12)-taxadiene **7** is also produced in low yield. The structure of another minor product **6** corresponds with thioester bond cleavage, further supporting the Claisen-like rearrangement mechanism. Compound **5** and its subsequent deoxygenated derivatives could serve as potential intermediates for the biosynthesis of paclitaxel or other taxoids.

Microbial enzymatic systems may be useful tools to mimic some steps of taxoid biosynthesis, such as extensive oxidation of the taxane skeleton. In the case of compound **5** hydroxylation was expected to proceed either at the 5-position followed by allylic shift of the double bond from 4(5) to 4(20), to produce compound **2**, or at the 20-position to produce compound **8**. Biotransformation of **5** by *Absidia coerula*, a filamentous fungus, under standard incubation conditions[5] gave one major product **8** in moderate yield. Combined analysis of FABMS and the ^{13}C NMR spectra suggested the molecular formula of **8** was $C_{28}H_{40}O_9$. A detailed analysis of the NMR spectra (^{1}H, ^{1}H–^{1}H COSY, ^{13}C, DEPT) showed that compound **8** is the 20-hydroxylated metabolite of **5**. Oxidation of **5** with SeO_2 and *t*-BuOOH also produced **8** selectively in 70% yield, which further supported the structural elucidation of **8**. Very recently, a 20-hydroxylated taxoid has been isolated from *Taxus mairei*.[3] This type of taxoid may be a potential

intermediate of taxol biosynthesis. In addition, a minor metabolite, 14β-hydroxylated derivative **9** was also produced. Previously, we described another example of 14-hydroxylation of taxoids.[5b]

Here we report a versatile and powerful method for preparation of 4(5),11(12)-taxadiene derivatives from a 4(20),11(12)-taxadiene, readily available from *Taxus spp*. This method, combined with Barton radical deoxygenation, should be a useful approach to the synthesis of a variety of 4(5),11(12)-taxadiene derivatives with different oxygen substitutions which can be used to probe the biosynthesis of taxoids in *Taxus spp*. In addition, we have described the first example of microbial 20-hydroxylation of taxoids, thus providing experimental evidence for the biosynthesis of 20-hydroxylated taxoids.

Acknowledgements

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7. Spectral data for **5**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.08 (1H, d, *J*=11.0), 5.89 (1H, d, *J*=11.0), 5.54 (1H, d, *J*=8.1), 5.33 (1H, t, *J*=8.7), 5.25 (1H, br.s), 2.90 (1H, br.s), 2.84 (1H, m), 2.33 (1H, m), 2.06, 2.03, 2.03, 2.01, 1.97 (15H, 5s), 2.03 (1H, m), 1.85–1.76 (2H, m), 1.66 (3H, s), 1.61 (1H, m), 1.59 (3H, s), 1.36 (1H, dd, *J*=15.4, 3.6), 0.97 (3H, s), 0.94 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.88, 169.88, 169.75, 168.82, 136.69, 136.18, 135.91, 118.52, 75.94, 71.87, 71.24, 69.30, 44.47, 39.49, 38.95, 38.26, 32.53, 31.97, 30.45, 25.47, 25.17, 22.86, 20.91, 20.51, 20.47, 20.32, 15.71, 12.35. HRFABMS: calcd for $C_{28}H_{40}O_8Na$ $[M+Na]^+$ 527.26464, found 527.26209.