Published in final edited form as:

Tetrahedron. 2008 ; 64(27): 6561–6567. doi:10.1016/j.tet.2008.04.008.

Studies on Taxol Biosynthesis: Preparation of Taxadiene-diol- and triol-Derivatives by Deoxygenation of Taxusin

Hui Li^a, Tohru Horiguchi^a, Rodney Croteau^c, and Robert M. Williams^{a,b}

^aDepartment of Chemistry, Colorado State University, Fort Collins, Colorado 80523, USA

^bUniversity of Colorado Cancer Center, Aurora, Colorado

^cInstitute of Biological Chemistry, Washington State University, Pullman, WA, 99164-6340, USA

Abstract

The putative taxol biosynthesis metabolites, taxa-4(20),11(12)-diene-5 α , 13 α -diol (**7**), taxa-4(20), 11(12)-diene-5 α , 9 α , 13 α -triol (**9**), and taxa-4(20),11(12)-diene-5 α , 10 β , 13 α -triol (**10**), have been prepared by Barton deoxygenation of the C-9 and C10-hydroxyl groups of protected derivatives of taxusin, a major taxoid metabolite isolated from Yew heart wood. The synthetic protocol devised, is amenable for the preparation of isotopically labeled congeners that will be useful to probe further intermediate steps in the biosynthesis of taxol.

Introduction

The approval of taxol (Paclitaxel, **1**) as a clinically useful therapeutic agent against ovarian and other types of cancer continues to stimulate interest in both the chemical synthesis and biogenesis of this agent.^{1,2} Numerous approaches for the synthesis of the taxane framework³ and for taxol itself⁴ have been reported but the architectural complexity of the taxol core mandates lengthy syntheses, with low overall yields rendering chemical synthesis approaches to this drug impractical for large-scale manufacturing of clinical-grade material. The bark of *Taxus brevifolia* Nutt., the Pacific yew tree, served as the initial source for commercial-scale production of taxol.¹ However, this species grows in environmentally sensitive areas of the Pacific Northwest and has become an untenable source for large-scale taxol production. Alternative approaches for taxol production such as semi-synthesis from 10-deacetylbaccatin III, which can be isolated from the needles of the European yew *Taxus baccata*, a renewable resource, were subsequently adopted as the current commercial method for taxol production.⁵ Additionally, it is reasonable to anticipate that taxol will become utilized for other types of cancer and as a consequence, pressure on the population of yew species worldwide will increase significantly. Alternative sources for taxol production based on biological systems have become an important goal and our laboratories have sought to employ emerging technologies based on the genetic manipulation of *Taxus* sp. cell cultures to address this problem. In order to meet these objectives, we have sought to elucidate a detailed understanding of the steps of taxol biosynthesis and the identification of the associated genes.

© 2008 Elsevier Ltd. All rights reserved.

Correspondence to: Robert M. Williams.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Previously, we have reported a combination of *in vivo* feeding studies and investigations with cell-free enzyme systems, using *Taxus* sp. stem tissue or suspension cultured *Taxus* sp. cells as bioconversion vectors. The experimental results of these investigations have led us to surmise that the early steps of taxol biosynthesis proceed in sequence from the initial conversion of geranylgeranyl pyrophosphate (**2**) to the parent diene **3** catalyzed by taxadiene synthase (Scheme 1).^{6,7} Following this first committed step in taxol biosynthesis, taxadiene hydroxylase, a cytochrome P-450-dependent enzyme, regioselectively hydroxylates **3** with allylic transposition of the 4,5-double bond, to the first oxygenated metabolite, taxa-4(20),11(12)-diene-5 α -ol (**4**).⁸ The resulting intermediate taxadienol **4** can be subsequently acetylated through the action of taxadienol-*O*-acetyltransferase to provide the corresponding acetate **5**.^{9a} Studies have shown that taxa-4(20),11(12)-diene-5 α -acetate (**5**) was converted to taxa-4(20),11(12)-diene-5 α ,10 β -diol-5-acetate (**6**) by taxoid 10 β -hydroxylase.^{10,12a} Experiments also demonstrated that taxa-4(20),11(12)-diene-5 α -ol (**4**) was converted by a 13 α -hydroxylase to taxa-4(20),11(12)-diene-5 α ,13 α -diol (**7**; Scheme 1),^{12b} thereby introducing uncertainties as to the precise order of the early hydroxylation and C5-acetylation diverging from the confirmed intermediate taxadienol (**4**). The complementary but opposite substrate selectivities of the 13 α -hydroxylase and the 10 β -hydroxylase, which favors taxadien-5 α -ol and taxadien-5 α -acetate as substrates respectively, as well as feeding studies of *Taxus* sp. cells with the relevant intermediates,¹³ suggest a bifurcation in the early stages of Taxol biosynthesis.¹⁴ Based on the relative abundance of the several hundreds defined taxoids bearing oxy-functional groups at various positions, the subsequent order of hydroxylation reactions downstream appears likely to occur at C10, followed by C13 and C9, and later C7 and C2, the precise timing of acetylation at C5 is, however, uncertain.¹⁴ Considering numerous possible parallel sequences of oxygenation, we have decided to investigate a series of plausible intermediates downstream from **4** by way of the C13-oxygenated species⁷ that can be converted into taxa-4(20),11(12)-diene-5 α ,9 α ,13 α -triol (**9**) or taxa-4(20),11(12)-diene-5 α ,10 β ,13 α -triol (**10**). In order to evaluate the possible intermediacy of such species, we required access to authentic specimens of **7**, **9** and **10** since we have found that sufficiently good substrates for subsequent hydroxylation reactions are often produced in trace amounts and whose structures are very difficult to confirm. We report herein, the semi-synthesis of **7**, **9** and **10** by radical deoxygenation of taxusin.

Results and Discussion

Taxusin was selected as a starting substrate due to its structural relatedness to a variety of putative pathway metabolites and its high natural abundance in European and Japanese yew heart woods. Previous studies from our laboratory have revealed that taxusin can be deacetylated efficiently to the corresponding tetraol and re-protected with modest efficiency to form a series of differentially protected polyols that can be selectively deoxygenated by classical radical deoxygenation methods with the exception of the C13 alcohol.¹⁵ All attempts to remove the C13 alcohol results in rearrangement of the C11(12)-bridgehead alkene to the C12(13) alkene. Despite our failure to deoxygenate taxusin at C-13, this substrate proved to be useful for making lightly oxygenated taxoids with oxygenation at C13.

Taxusin was partially deacylated by treatment with K₂CO₃ in THF/MeOH at 0 °C for 3 days, which gave the 5,13-diol **12** predominantly with some 9,10-diol. A small amount of the more polar taxusin tetraol was produced but easily separated (Scheme 2). The diol **12** was treated with TESCl in the presence of imidazole in DMF, followed by deacetylation of the 9,10-diacetyl groups using lithium aluminum hydride (LAH) in THF, which provided diol **13** in high yield. Exposure of **13** to LHMDs followed by addition of carbon disulfide and methyl iodide, gave an inseparable mixture of the xanthates **14a** and **14b** in approximately 1:1 ratio. The C-9 and C-10-hydroxyl residues were successfully removed by Barton deoxygenation respectively to give an inseparable mixture of **15a** and **15b**. Finally, removal of the *O*-TES

ethers by treatment with TBAF in the presence of acetic acid in THF, gave the desired triols taxa-4(20), 11(12)-diene-5 α , 10 β , 13 α -triol (**10**) and taxa-4(20), 11(12)-diene-5 α , 9 α , 13 α -triol (**9**), which were separable by silica gel chromatography (Scheme 2).

The mixture of **15a/15b**, also proved useful for the synthesis of the corresponding taxa-4(20), 11(12)-diene-5 α , 13 α -diol. As shown in Scheme 3, **15a/15b** was subjected to treatment with LHMDs in THF followed by the addition of carbon disulfide and methyl iodide to give a mixture of the xanthates **16a/16b**, which could be separated on column but was used as a mixture in the following step. Barton deoxygenation of **16a/16b** yielded the protected diol **17** in 78% yield. Final treatment of **17** with HF-pyridine complex afforded taxa-4(20), 11(12)-diene-5 α , 13 α -diol (**7**) (Scheme 3).

While this route provided the first authentic specimens of **9** and **10**, 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 4 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.

Taxusin was converted into tetraol **18** by global reductive removal of the acetates with LAH. Cyclic carbonate formation followed by protection of the C5 and C13 hydroxyl groups as *O*-DEIPS ethers proceeded in 62% overall yield. Reductive removal of the cyclic carbonate followed by xanthate formation, gave the inseparable xanthates **22a/22b**, which were directly subjected to Barton deoxygenation conditions. Final removal of the silyl ethers with *tetra-n*-butylammonium fluoride (TBAF) produced **9** and **10** (73% and 10% yields, respectively). Similarly, **23a** and **23b** were converted to **7** in good overall yield as shown in Scheme 5.

The synthesis of these substrates with deuterium labeling is enabled through the use of tri-*n*-butyltin deuteride in the radical deoxygenation step.

Experimental

5,13-Dideacetyltaxusin (12)

To a solution of taxusin (120 mg, 0.24 mmol) in THF/MeOH (4 mL, 1:1) was added K₂CO₃ (49 mg, 0.36 mmol) at 0 °C. The mixture was stirred for 3 days at 0 °C, after which time, EtOAc (20 mL) and sat. NH₄Cl (aq) (5 mL) were added to the mixture. The organic layer was separated and washed with brine. The organic layer was concentrated and purified by chromatography (silica gel, hexanes/EtOAc 1:1), yielding **12** (80 mg, 80%). ¹H NMR (300 MHz, CDCl₃) δ 6.05 (d, *J* = 10.2 Hz, 1 H), 5.69 (d, *J* = 10.2 Hz, 1 H), 5.04 (s, 1 H), 4.69 (s, 1 H) 4.40-4.24 (br s, 1 H), 4.28 (s, 1 H), 3.32 (d, *J* = 8.4 Hz, 1 H), 3.24 (d, *J* = 4.5 Hz, 1 H), 2.90-2.70 (comp, 2 H), 2.20 (d, *J* = 1.2 Hz, 3 H), 2.02 (s, 3 H), 1.98 (s, 3 H), 1.94-1.54 (comp, 7 H), 1.49 (s, 3 H), 1.24-1.16 (m, 1 H), 0.88 (s, 3 H), 0.68 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 170.5, 170.1, 152.3, 141.7, 134.8, 110.7, 77.7, 74.3, 73.6, 68.5, 43.4, 39.5, 38.6, 36.3, 35.8, 32.8, 30.5, 27.2, 26.6, 25.9, 21.2, 20.9, 17.1, 16.7; IR (NaCl) 3450 (br), 2944, 1724, 1242 cm⁻¹; HR-MS calcd for C₂₄H₃₅O₆ (M⁺-H) 419.2434, found 419.2422.

5 α ,13 α -Bis(triethylsilyloxy)-taxa-4(20),11(12)-diene-9 α ,10 β -diol (13)

To the solution of **12** (1.32 g, 3.13 mmol) and imidazole (534 mg, 7.85 mmol) in DMF (5 mL), TESCO (991 mg, 6.57 mmol) was added at 0 °C. The mixture was stirred for 24 h at rt. The mixture was diluted by diethyl ether (40 mL) and washed with water, NH₄Cl (aq), and brine and dried over Na₂SO₄, yielding a crude mixture, which was used in the next reaction without further purification. Thus, a solution of the crude diacetate prepared above and LAH (238 mg,

6.26 mmol) in THF (10 ml) was stirred at rt for 15 h. The mixture was diluted by THF-H₂O (3:1, 10 ml) at 0 °C. The mixture was filtered and the filtrate was diluted with EtOAc. The mixture was washed by NH₄Cl (aq) and brine, dried over Na₂SO₄, and condensed *in vacuo*, yielding diol **13** (1.60 g, 90 %). ¹H NMR (400 MHz, CDCl₃) δ 4.91 (s, 1 H), 4.80 (d, J = 9.8 Hz, 1 H), 4.82-4.75 (m, 1 H), 4.58 (s, 1 H), 4.15 (t, J = 2.6 Hz, 1 H), 4.12 (d, J = 9.8 Hz, 1 H), 2.98 (d, J = 5.6 Hz, 1 H), 2.36 (td, J = 9.2, 14.0 Hz, 1 H), 2.48-2.22 (br s, 2 H), 1.99 (s, 3 H), 1.76-1.50 (comp, 7 H), 1.50 (s, 3 H), 1.17 (s, 3 H), 1.18-1.10 (m, 1 H), 1.2-0.88 (comp, 21 H), 0.70-0.55 (comp, 12 H); ¹³C NMR (100 MHz, CDCl₃) δ 155.4, 140.0, 135.5, 109.4, 79.6, 74.7, 73.0, 69.4, 43.1, 42.0, 39.7, 36.7, 35.9, 32.0, 31.6, 29.0, 28.5, 25.6, 18.4, 14.9, 7.2, 5.1, 4.7.

Taxa 4(20), 11(12)-diene-5α, 10β, 13α-triol (10)

Procedure A—To the solution of **13** (137 mg, 0.24 mmol) in THF (3 mL), LHMDS (0.25 mmol, 1 M THF solution) was added at 0 °C. After stirred for 5 min, CS₂ (0.1 mL) was added to the mixture. The mixture was stirred for 30 min at rt, then MeI (16 μL, 36 mg, 0.25 mmol) was added. The mixture was stirred for another 14 h at rt. The mixture was diluted with CH₂Cl₂ (20 mL) and washed with water. The organic layer was dried over Na₂SO₄, and concentrated *in vacuo*. The residue was purified by preparative TLC (hexanes/EtOAc 30:1), yielding an inseparable mixture of **14a** and **14b** (75 mg, 47 %).

To a solution of **14a** and **14b** (75 mg, 0.11 mmol) obtained above in toluene (2 mL), AIBN (cat.) and *n*Bu₃SnH (63 μL, 0.23 mmol) were added. The mixture was stirred at reflux for 3 h. The mixture was concentrated and purified by flash chromatography (silica gel, hexanes/EtOAc 11:1), yielding an inseparable mixture of **15a** and **15b** (54 mg, 86 %).

To a solution of **15a** and **15b** (67 mg, 0.12 mmol) in THF were added TBAF (0.26 mmol, 1 M THF solution) and AcOH (1 drop). After 9 h additional TBAF (0.52 mmol) was added and the mixture was stirred for 72 h. The mixture was diluted with EtOAc and washed with NH₄Cl (aq) and brine. The organic layer was concentrated and purified by preparative TLC (silica gel, hexanes/EtOAc 1:1, then CHCl₃/MeOH 20:1), yielding triol **10** (20 mg, 51 %) and triol **9** (15 mg, 38 %).

Procedure B—A solution of **23a** and **23b** (280 mg, 0.485 mmol) and TBAF (651 mg, 2.50 mmol) in DMF (30 mL) was stirred at 55 °C for 12 h. The mixture was diluted with EtOAc (75 mL) and washed with H₂O (3 × 50 mL) and brine (50 mL). The organic layer was concentrated and purified by flash chromatography (silica gel, eluted with hexanes/EtOAc 3:2 then 1:1), yielding triol **9** (120 mg, 77%) and triol **10** (27 mg, 17%). ¹H NMR (300 MHz, CDCl₃) δ 5.15 (dd, J = 5.7, 11.7 Hz, 1 H), 4.97 (s, 1 H), 4.64 (s, 1 H), 4.38-4.22 (comp, 2 H), 3.22 (s, 1 H), 2.88-2.60 (comp, 3 H), 2.18 (dd, J = 11.7, 14.4 Hz, 1 H), 2.12-2.00 (m, 1 H), 2.01 (d, J = 1.2 Hz, 3 H), 1.72-1.52 (comp, 7 H), 1.48 (s, 3 H), 1.38-1.10 (comp, 2 H), 0.93 (s, 3 H), 0.66 (s, 3 H); ¹³C NMR (100 MHz, CD₃CN) 156.2, 142.5, 136.9, 109.6, 75.2, 69.1, 68.7, 48.4, 41.5, 39.8, 39.7, 37.6, 35.9, 34.1, 34.0, 32.3, 25.9, 22.5, 21.5, 17.2; IR (KBr) 3349 (br), 2927, 1652, 1456, 1004, 894 cm⁻¹; HR-MS (M⁺+Na) calcd for C₂₀H₃₂O₃Na 343.2244, found 343.2240.

Taxa 4(20), 11(12)-diene-5α, 9α, 13α-triol (9)

¹H NMR (300 MHz, CDCl₃) δ 5.03 (s, 1 H), 4.69 (s, 1 H), 4.40-4.30 (br s, 1 H), 4.32 (s, 1 H), 4.14-4.02 (m, 1 H), 3.55 (s, 1 H), 2.96-2.70 (comp, 3 H), 2.54 (dt, J = 3.8, 13.2 Hz, 1 H), 2.30-2.15 (br s, 1 H), 2.00 (s, 3 H), 1.93 (dt, J = 5.1, 13.2 Hz, 1 H), 1.80-1.60 (comp, 5 H), 1.55-1.45 (m, 1 H), 1.38-1.32 (m, 1 H), 1.33 (s, 3 H), 1.20 (dd, J = 3.9, 15.3 Hz, 1 H), 0.92 (s, 3 H), 0.83 (s, 3 H); ¹³C NMR (75 MHz, acetone-d₆) δ 156.4, 138.2, 136.3, 109.6, 76.8, 75.9, 69.6, 46.7, 40.9, 40.6, 38.1, 37.6, 37.1, 32.8, 32.5, 28.4, 26.6, 26.3, 19.0, 17.6; IR (NaCl) 3350 (br), 2941, 1448, 1101 cm⁻¹; HR-MS calcd for C₂₀H₃₂O₃Na (M⁺+Na) 343.2244, found 343.2240.

5 α , 13 α -Bis(triethylsilyloxy)-10 β -S-methyl dithiocarboxytaxa-4(20), 11(12)-diene (16a) and 5 α , 13 α -di-(triethylsilyloxy)-9 α -S-methyl dithiocarboxytaxa-4 (20), 11(12)-diene-10 β -ol (16b)

To the solution of the mixture of **15a** and **15b** (62 mg, 0.11 mmol) in THF (2 mL), LHMDs (0.12 mL, 1 M THF solution) was added at 0 °C. After stirring for 10 min, CS₂ (0.1 mL) was added to the mixture. The mixture was stirred for 2 h at rt, then MeI (7 μ L, 0.12 mmol) was added. The mixture was stirred for 3 h at rt. The mixture was diluted with CH₂Cl₂ (20 mL) and washed with water. The organic layer was dried over Na₂SO₄, and concentrated *in vacuo*. The residue was purified by PTLC (hexanes/EtOAc 100:1), yielding **16a** (41 mg, 57%) and **16b** (20 mg, 28 %). These were used for the next step without further purification.

5 α , 13 α -Bis(triethylsilyloxy)-taxa-4(20), 11(12)-diene (17)

To a solution of the mixture of **16a** and **16b** (61 mg, 0.095 mmol) obtained above in toluene (2 mL), AIBN (cat.) and *n*Bu₃SnH (52 μ L, 0.19 mmol) were added. The mixture was stirred at reflux for 3 h. The mixture was concentrated and purified by chromatography (silica gel, heptane), yielding **17** (40 mg, 79 %). ¹H NMR (400 MHz, benzene-*d*₆) δ 4.96-4.78 (m, 1 H), 4.64 (s, 1 H), 4.184 (t, *J* = 3.0 Hz, 1 H), 3.33 (m, 1 H), 2.90 (dt, *J* = 3.6, 12.8 Hz, 1 H), 2.44 (td, *J* = 9.2, 13.6 Hz, 1 H), 2.41 (dt, *J* = 4.8, 13.2 Hz, 1 H), 2.14 (s, 3 H), 2.15-2.00 (comp, 2 H), 1.80-1.60 (comp, 5 H), 1.38 (s, 3 H), 1.44-1.34 (m, 1 H), 1.30-1.20 (m, 1 H), 1.16 (s, 3 H), 1.14-1.00 (comp, 18 H), 1.00-0.90 (comp, 2 H), 0.80-0.62 (comp, 15 H); ¹³C NMR (100 MHz, benzene-*d*₆) 156.8, 137.0, 133.8, 108.7, 75.0, 70.1, 42.6, 41.7, 40.8, 40.5, 37.0, 36.5, 35.0, 32.9, 31.1, 30.1, 28.2, 26.3, 23.3, 15.3, 7.8, 7.7, 5.8, 5.4; IR (NaCl): 2951, 2361, 1457, 1239, 1118, 1062, 1007, 886, 838, 739 cm⁻¹;

Taxa 4(20), 11(12)-diene-5 α , 13 α -diol 7

Procedure A—To a solution of **17** (25 mg, 0.047 mmol) in pyridine (2 mL) was added HF-pyridine (0.2 mL, 70 % solution in pyridine) at 0 °C. The mixture was stirred for 3 h at room temperature and was diluted with EtOAc (30 mL) and washed with saturated CuSO₄, water, NH₄Cl (aq) and brine. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by chromatography (silica gel, CHCl₃/MeOH 9:1), yielding **7** (12 mg, 84 %).

Procedure B—To a solution of the mixture of **26** (60 mg, 0.107 mmol) in THF (10 mL) were added TBAF (140 mg, 0.535 mmol). The mixture was stirred at 55 °C overnight and concentrated and purified by flash chromatography (silica gel, hexanes/EtOAc 3:2), yielding diol **7** (27 mg, 83%). ¹H NMR (300 MHz, CDCl₃) δ 4.96 (s, 1 H), 4.63 (s, 1 H), 4.38-4.24 (br s, 1 H), 4.27 (t, *J* = 2.6 Hz, 1 H), 3.51 (br s, 1 H), 2.85 (dd, *J* = 5.4, 13.2 Hz, 1 H), 2.83-2.70 (comp, 2 H), 2.23 (dt, *J* = 5.7, 12.9 Hz, 1 H), 2.18-2.09 (br s, 2 H), 1.95 (s, 3 H), 2.00-1.90 (m, 1 H), 1.82-1.60 (comp, 5 H), 1.32 (s, 3 H), 1.28-1.16 (comp, 2 H), 1.00 (ddd, *J* = 2.4, 4.5, 13.2 Hz, 1 H), 0.88 (s, 3 H), 0.60 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 153.7, 139.5, 132.5, 109.5, 74.7, 68.7, 40.4, 39.6, 39.24, 39.18, 36.2, 35.8, 32.4, 32.1, 31.4, 27.4, 25.4, 24.9, 22.2, 16.3; IR (NaCl): 3359 (br), 2921, 1439, 1008 cm⁻¹. HR-MS calcd for C₂₀H₃₂O₂Na (M⁺+Na) 327.2294, found 327.2292.

Cyclic carbonate (19)

A solution of tetraol **18** (6.41 g, 19.1 mmol) and CDI (5.0 g, 30.8 mmol) in toluene (300 mL) was heated at 70 °C overnight. After cooled to room temperature, the mixture was concentrated and the residue was purified by flash chromatography (silica, 1:1 EtOAc/hexanes) to give **19** as a white powder (4.80 g, 70 %). ¹H NMR (300 MHz, acetone-*d*₆) δ 5.79 (d, *J* = 12.0 Hz, 1 H), 5.09 (s, 1 H), 4.95 (d, *J* = 12.0 Hz, 1 H), 4.72 (s, 1 H), 4.45 (app dt, *J* = 4.5, 9.8 Hz, 1 H), 4.31 (d, *J* = 2.4 Hz, 1 H), 4.17 (d, *J* = 2.1 Hz, 1 H), 3.76 (d, *J* = 9.6 Hz, 1 H), 3.16-3.10 (br s 1 H), 2.84 (ddd, *J* = 9.0, 10.2, 15.3 Hz, 1 H), 2.18 (d, *J* = 1.2 Hz, 3 H), 1.99 (dd, *J* = 4.5, 13.5 Hz,

1 H), 1.86-1.80 (comp, 5 H), 1.46 (s, 3 H), 1.48-1.40 (m, 1 H), 1.18 (d, $J = 4.8, 15.3$ Hz, 1 H), 1.03 (s, 3 H), 0.92 (s, 3 H); ^{13}C NMR (75 MHz, acetone- d_6) δ 155.7, 154.0, 150.5, 133.7, 110.8, 88.2, 81.1, 75.1, 69.3, 41.5, 41.4, 40.2, 37.6, 36.8, 33.9, 31.7, 28.8, 27.1, 26.3, 18.3, 17.8; IR (NaCl): 3407 (br), 2944, 2877, 1801, 1190, 1016 cm^{-1} . HR-MS calcd for $\text{C}_{21}\text{H}_{30}\text{O}_5\text{Na}$ ($\text{M}^+ + \text{Na}$) 385.1991, found 385.1982.

Cyclic carbonate (20)

A solution of diol **19** (475 mg, 1.31 mmol), imidazole (700 mg, 10.2 mmol) and DEIPSCl (0.63 ml, 0.34 mmol) in DMF (7 ml) was stirred at rt for 40 h. The mixture was diluted with EtOAc (25 mL), washed with H_2O (3×25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4 . After filtration, the filtrate was concentrated and the residue was purified by flash chromatography (silica, 1:20 then 1:10 EtOAc/hexanes) to give **20** as a clear liquid (726 mg, 89 %). ^1H NMR (300 MHz, CDCl_3) δ 5.58 (d, $J = 12.0$ Hz, 1 H), 5.06 (d, $J = 12.0$ Hz, 1 H), 4.96 (s, 1 H), 4.84 (app t, $J = 9.0$ Hz, 1 H), 4.60 (s, 1 H), 4.18 (t, $J = 2.3$ Hz, 1 H), 2.70-2.63 (s, 1 H), 2.41 (td, $J = 9.3, 14.1$ Hz, 1 H), 2.03 (d, $J = 0.6$ Hz, 3 H), 1.88-1.73 (comp, 2 H), 1.67-1.55 (comp, 4 H), 1.50 (s, 3 H), 1.53-1.44 (m, 1 H), 1.18 (s, 3 H), 1.10-0.83 (comp, 30 H), 0.70-0.54 (comp, 8 H); ^{13}C NMR (75 MHz, CDCl_3) δ 155.1, 152.2, 149.2, 129.2, 110.1, 87.1, 79.6, 77.0, 74.1, 69.4, 42.0, 39.6, 39.4, 35.7, 35.4, 32.4, 30.9, 28.2, 28.0, 25.3, 17.6, 17.5, 17.4, 15.4, 13.0, 12.8, 7.20, 7.18, 7.15, 7.10, 3.7, 3.5, 3.4, 3.1; IR (NaCl): 2944, 2877, 1809, 1463, 1184 cm^{-1} . HR-MS calcd for $\text{C}_{35}\text{H}_{62}\text{O}_5\text{Si}_2\text{Na}$ ($\text{M}^+ + \text{Na}$) 641.4034, found 641.4050.

5 α , 13 α -Bis(diethylisopropylsilyloxy)-taxa-4(20),11(12)-diene-9 α , 10 α -diol (21)

A mixture of the cyclic carbonate **20** (1.5 g, 2.42 mmol) and LiAlH_4 (184 mg, 4.85 mmol) in THF (150 mL) was stirred at rt for 6 h whereupon EtOAc (10 mL) was added slowly to quench excess LiAlH_4 . The mixture was partitioned between EtOAc (80 mL), H_2O (40 mL) and brine (40 mL). The aqueous layer was extracted with EtOAc (2×80 mL) and the combined organic layers were washed with brine (150 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by flash chromatography (silica gel, 1:7 then 1:3 EtOAc/hexanes) to give **21** as a white solid (1.44 g, 100%). ^1H NMR (300 MHz, CDCl_3) δ 4.89 (s, 1 H), 4.80 (t, $J = 1.2$ Hz, 1 H), 4.76 (d, $J = 9.9$ Hz, 1 H), 4.57 (s, 1 H), 4.15 (s, 1 H), 4.10 (d, $J = 9.9$ Hz, 1 H), 2.90 (d, $J = 5.4$ Hz, 1 H), 2.50-2.20 (br s, 2 H), 2.33 (td, $J = 9.3, 13.2$ Hz, 1 H), 1.97 (d, $J = 1.2$ Hz, 3 H), 1.72-1.52 (comp, 7 H), 1.47 (s, 3 H), 1.15 (s, 3 H), 1.04-0.84 (comp, 27 H), 0.84 (s, 3 H), 0.70-0.54 (comp, 8 H); ^{13}C NMR (75 MHz, CDCl_3) δ 155.1, 139.7, 135.3, 109.6, 79.4, 74.7, 72.7, 69.3, 42.9, 42.0, 39.4, 36.6, 35.5, 31.9, 31.4, 29.0, 28.5, 25.5, 18.3, 17.6, 17.54, 17.52, 17.49, 14.5, 13.1, 12.9, 7.24, 7.21, 7.17, 3.8, 3.6, 3.5, 3.2; IR (NaCl): 3402 (br), 2944, 1463, 1072, 1014 cm^{-1} . HR-MS calcd for $\text{C}_{34}\text{H}_{64}\text{O}_4\text{Si}_2\text{Na}$ ($\text{M}^+ + \text{Na}$) 615.4241, found 615.4238.

5 α , 13 α -Bis(diethylisopropylsilyloxy)-9 α -S-methyl dithiocarboxytaxa-4(20), 11(12)-diene (24b)

A solution of KHMDS (1.18 mL, 0.5 M in toluene, 0.59 mmol) was added to **23b** (200 mg, 0.347 mmol) in THF (20 mL) at -10°C . The mixture was stirred at 0°C for 10 min and then at room temperature for 20 min, whereupon carbon disulfide (36 μL , 0.58 mmol) was added, and the solution was stirred for another 30 min. MeI (36 μL , 0.54 mmol) was then added and the mixture was allowed to stir overnight. The mixture was then partitioned between EtOAc (25 mL) and 1% AcOH in water (25 mL) and the organic layer separated. The organic layer was washed with brine (25 mL) and dried over Na_2SO_4 . After filtration, the filtrate was concentrated and the residue was purified by flash chromatography (silica gel, hexanes then 1:100 EtOAc/hexanes) to give **24b** as a clear oil (210 mg, 91 %). ^1H NMR (300 MHz, CDCl_3) δ 6.20 (dd, $J = 5.7, 11.7$ Hz, 1 H), 4.92 (s, 1 H), 4.80 (app t, $J = 9.0$ Hz, 1 H), 4.61 (s, 1 H), 4.18 (s, 1 H), 3.19 (d, $J = 5.1$ Hz, 1 H), 2.89 (t, $J = 12.9$ Hz, 1 H), 2.56 (s, 3 H), 2.55-2.48 (br s, 1 H), 2.32

(td, $J = 9.0, 14.1$ Hz, 1 H), 2.20–2.05 (m, 1 H), 1.98 (s, 3 H), 1.80–1.55 (comp, 6 H), 1.63 (s, 3 H), 1.17–1.08 (m, 1 H), 1.11 (s, 3 H), 1.05–0.90 (comp, 26 H), 0.71 (s, 3 H), 0.70–0.60 (comp, 8 H) ^{13}C NMR (75 MHz, CDCl_3) δ 215.6, 154.2, 136.3, 132.1, 109.9, 90.7, 74.4, 60.4, 45.2, 41.3, 39.6, 36.8, 35.2, 21.2, 30.7, 30.5, 28.9, 28.5, 26.7, 19.0, 18.3, 17.7, 17.6, 17.55, 17.53, 14.7, 13.1, 12.9, 7.29, 7.25, 7.2, 3.8, 3.6, 3.5, 3.2; IR (NaCl): 2945, 2872, 1645, 1466, 1240, 1078 cm^{-1} ; HR-MS calcd for $\text{C}_{36}\text{H}_{65}\text{O}_3\text{S}_2\text{Si}_2$ ($\text{M}^+ - \text{H}$) 665.3914, found 665.3905.

5 α , 13 α -Bis(diethylisopropylsilyloxy)-4(20), 11(12)-diene (25)

A solution of **24b** (160 mg, 0.24 mmol), AIBN (20 mg, 0.12 mmol), Bu_3SnH (140 mg, 127 μL , 0.48 mmol) in toluene (15 mL) was heated at 115°C for 3 h. The mixture was concentrated and the residue purified by flash chromatography (silica gel, hexanes then 1:100 EtOAc / hexanes) to give **25** as clear oil (100 mg, 74%). ^1H NMR (300 MHz, CDCl_3) δ 4.81 (s, 1 H), 4.75 (t, $J = 8.9$ Hz, 1 H), 4.55 (s, 1 H), 4.14 (t, $J = 3.5$ Hz, 1 H), 3.06 (d, $J = 6.3$ Hz, 1 H), 2.76 (ddd, $J = 5.4, 12.6, 14.1$ Hz, 1 H), 2.33 (td, $J = 9.3, 13.8$ Hz, 1 H), 2.16 (dt, $J = 4.1, 13.2$ Hz, 1 H), 2.10–1.90 (comp, 2 H), 1.89 (s, 3 H), 1.80–1.40 (comp, 6 H), 1.37 (s, 3 H), 1.33–1.12 (comp, 3 H), 1.05 (s, 3 H), 1.02–0.84 (comp, 25 H), 0.65 (s, 3 H), 0.74–0.62 (comp, 8 H); ^{13}C NMR (75 MHz, CDCl_3) δ 156.4, 136.4, 133.3, 107.8, 73.9, 69.3, 42.0, 41.2, 39.9, 39.7, 36.8, 35.7, 34.8, 32.1, 30.7, 30.1, 27.8, 25.4, 22.7, 17.7, 17.6, 17.5, 14.6, 13.1, 12.9, 7.33, 7.30, 7.27, 7.2, 3.9, 3.7, 3.5, 3.3; IR (NaCl): 2941, 1458, 1016 cm^{-1} ; IR (NaCl): 2941, 2877, 1463, 1059, 1013 cm^{-1} ; HR-MS calcd for $\text{C}_{34}\text{H}_{63}\text{O}_2\text{Si}_2$ ($\text{M}^+ - \text{H}$) 559.4365, found 559.4367.

Acknowledgments

We thank the National Institutes of Health (Grant CA 70375 to R.M.W., and Grant CA 55254 to R.C.) for financial support. We thank the Yamada Science Foundation for fellowship support (to T.H.).

References

- (a) Suffness, M. Taxane anticancer agents: Basic science and current status. Georg, G.I.; Chen, T.T.; Ojima, I.; Vyas, D.M., editors. Washington, DC: American Chemical Society; 1995. p. 1–17. (b) Suffness, M.; Wall, M.E. Taxol: Science and applications. Suffness, M., editor. Boca Raton, FL: CRC Press; 1995. p. 3–25. (c) Holmes, F.A.; Kudelka, A.P.; Kavanagh, J.J.; Huber, M.H.; Ajani, J.A.; Valero, V. Taxane anticancer agents: Basic science and current status. Georg, G.I.; Chen, T.T.; Ojima, I.; Vyas, D.M., editors. Washington, DC: American Chemical Society; 1995. p. 31–57. (d) Golspiel B.R. Pharmacotherapy 1997;17:110S–125S. [PubMed: 9322878]
- 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.
- For a review, see: Swindell C.S. Org. Prep. Proced. Int 1991;23:465–543.
- (a) Holton R.A., Somoza C., Kim H.B., Liang F., Biediger R.J., Boatman D., Shindo M., Smith C.C., Kim S., Nadizadeh H., Suzuki Y., Tao C., Vu P., Tang S., Zhang P., Murthi K.K., Gentile L.S., Liu J.H. J. Am. Chem. Soc 1994;116:1597–1598. (b) Holton R.A., Somoza C., Kim H.B., Liang F., Biediger R.J., Boatman D., Shindo M., Smith C.C., Kim S., Nadizadeh H., Suzuki Y., Tao C., Vu P., Tang S., Zhang P., Murthi K.K., Gentile L.S., Liu J.H. J. Am. Chem. Soc 1994;116:1599–1600. (c) Nicolaou K.C., Nantermet P.G., Ueno H., Guy R.K., Coulandouros E.A., Sorensen E.J. J. Am. Chem. Soc 1995;117:624–633. (d) Nicolaou K.C., Liu J.J., Yang H., Claiborne C.F., Hwang C.K., Nakada M., Nantermet P.G., Ueno H., Guy R.K., Sorensen E.J. J. Am. Chem. Soc 1995;117:634–644. (e) Nicolaou K.C., Liu J.J., Yang H., Claiborne C.F., Renaud J., Nantermet P.G., Guy R.K., Shibayama K. J. Am. Chem. Soc 1995;117:645–652. (f) Nicolaou K.C., Ueno H., Liu J.J., Yang H., Renaud J., Nantermet P.G., Paulvannan K., Chadha R. J. Am. Chem. Soc 1995;117:653–659. (g) Danishefsky S.J., Masters J.J., Young W.B., Link J.T., Snyder L.B., Magee T.B., Jung D.K., Isaacs R.A., Bornman W.G., Alaimo C.A., Coburn C.A., DiGrandi M.J. J. Am. Chem. Soc 1996;118:2843–2859. (h) Wender P.A., Badham N.F., Conway S.P., Floreancig P.E., Glass T.E., Granicher C., Houze J.B., Janichen J., Lee D., Marquess D.G., McGrane P.L., Meng W., Mucciato T.P., Muhlebach M., Natchus M.G., Paulsen H., Rawlins D.B., Satkofsky J., Shuker A.J., Sutton J.C., Taylor R.E., Tomooka K.I. J. Am. Chem. Soc 1997;119:2755–2756. (i) Wender P.A., Badham N.F., Conway S.P., Floreancig P.E.,

- Glass TE, Houze JB, Krauss NE, Lee D, Marquess DG, McGrane PL, Meng W, Natchus MG, Shuker AJ, Sutton JC, Taylor RE. *J. Am. Chem. Soc* 1997;119:2757–2758. (j) 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;5:121–161. (k) Kusama H, Hara R, Kawahara S, Nishimori T, Kashima H, Nakamura N, Morihira K, Kuwajima I. *J. Am. Chem. Soc* 2000;122:3811–3820.
5. Nicolaou KC, Dai W-M, Guy RK. *Angew. Chem., Int. Ed. Engl* 1994;33:15–44.
6. (a) Hezari M, Croteau R. *Planta Medica* 1997;63:291–295. [PubMed: 9270370] (b) Walker K, Croteau R. *Phytochem* 2001;58:1–7.
7. (a) Koepp AE, Hezari M, Zajicek J, Vogel BS, LaFever RE, Lewis NG, Croteau R. *J. Biol. Chem* 1995;270:8686–8690. [PubMed: 7721772] (b) Hezari M, Lewis NG, Croteau R. *Arch. Biochem. Biophys* 1995;322:437–444. [PubMed: 7574719] (c) Lin X, Hezari M, Koepp AE, Floss HG, Croteau R. *Biochem* 1996;35:2968–2977. [PubMed: 8608134]
8. Hefner J, Rubenstein SM, Ketchum REB, Gibson DM, Williams RM, Croteau R. *Chem. Biol* 1996;3:479–489. [PubMed: 8807878]
9. (a) Walker K, Ketchum REB, Hezari M, Gat J, wfnierfield D, Goleniowski M, Barthol A, Croteau R. *Arch. Biochem. Biophys* 1999;364:273–279. [PubMed: 10190984] (b) Walker K, Schoendorf A, Croteau R. *Arch. Biochem. Biophys* 2000;374:371–380. [PubMed: 10666320]
10. Schoendorf A, Rithner CD, Williams RM, Croteau R. *Proc. Nat. Acad. Sci. USA* 2001;98:1501–1506. [PubMed: 11171980]
11. Vazquez A, Williams RM. *J. Org. Chem* 2000;65:7865–7869. [PubMed: 11073592]
12. (a) Wheeler AL, Long RM, Ketchum REB, Rithner CD, Williams RM, Croteau R. *Arch. Biochem. Biophys* 2001;390:265–278. [PubMed: 11396929] (b) Jennewein S, Rithner CD, Williams RM, Croteau R. *Proc. Nat. Acad. Sci. USA* 2001;98:13595–13600. [PubMed: 11707604]
13. Ketchum REB, Croteau R. *Plant metabolomics (biotechnology in agriculture and forestry)* 2006;57:291–309.
14. Kaspera R, Croteau R. *Phytochem. Rev* 2006;5:433–444.
15. (a) Horiguchi T, Rithner CD, Croteau R, Williams RM. *J. Org. Chem* 2002;67:4901–4903. [PubMed: 12098303] (b) Horiguchi T, Rithner CD, Croteau R, Williams RM. *Tetrahedron* 2003;59:267–273. (c) Ketchum REB, Horiguchi T, Qiu D, Williams RM, Croteau RB. *Phytochem* 2007;68:335–341.

Scheme 1.

Possible divergence of taxadiene to lightly oxygenated taxoids and convergence to a putative pentaol.

Scheme 2.

Synthesis of taxa-4(20), 11(12)-diene-5 α , 10 β , 13 α -triol (**10**) and taxa-4(20), 11(12)-diene-5 α , 9 α , 13 α -triol (**9**).

Scheme 3.
Synthesis of taxa-4(20), 11(12)-diene-5α, 13α-diol.

Scheme 4.
Cyclic carbonate route to **9** and **10**.

Scheme 5.
Conversion of **23b** to **7**.