DISSERTATION

ELUCIDATING THE BIOSYNTHETIC PATHWAY OF TAXOL

Submitted by

Steven Marc Rubenstein

Department of Chemistry

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 1996

COLORADO STATE UNIVERSITY

March 28, 1996

WE HEREBY RECOMMEND THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY STEVEN MARC RUBENSTEIN ENTITLED "ELUCIDATING THE BIOSYNTHETIC PATHWAY OF TAXOL" BE ACCEPTED AS FULFILLING IN PART THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Advisor

Department Head

ABSTRACT OF DISSERTATION

ELUCIDATING THE BIOSYNTHETIC PATHWAY OF TAXOL

The total synthesis of (±)-taxa-4(5),11(12)-diene, (±)-taxa-4(20),11(12)-diene, (±)-taxa-4(20),11(12)-diene-5(β)-ol, and (±)-taxa-4(20),11(12)-diene-5(α)-ol is described. The syntheses rely upon selenium-based methodology developed by Krief for the introduction of the tetrasubstituted double bond in diene **201** and an intramolecular Diels-Alder reaction, methodology developed by Shea and Jenkins, to form the AB ring system of taxol in compound **208**. The synthetic route was used to introduce stable and radiolabeled atoms into the target compounds.

The incubation of ^{13}C labeled (±)-taxa-4(20),11(12)-diene with taxadiene synthase has helped confirm the first committed biosynthetic step to taxol, in *Taxus brevifolia,* as being the direct cyclization of geranylgeranyl diphosphate to taxa-4(5),11(12)-diene. The incubation of tritium labeled (±)-taxa-4(5),11(12)-diene with a cytochrome P-450 microsomal cell-free extract produced a new mono-oxygenated product that had the same g.l.c. retention time and mass spectral fragmentation pattern as taxa-4(20),11(12)-diene-5(α)-ol. Taxa-4(20),11(12)-diene-5(β)-ol could not be found in this assay. Tritium labeled taxa-4(20),11(12)-diene-5(α)-ol was subsequently incubated with *Taxus brevifolia* stem discs and was incorporated into taxol.

In addition, taxa-4(20),11(12)-diene-5(α)-acetate acted as a better substrate than taxa-4(20),11(12)-diene-5(α)-ol in the conversion to the more polar product(s) when incubated with the cytochrome P-450 microsomal cell-free extract. The more polar product(s) is/are presumed to be more highly hydroxylated biosynthetic intermediates enroute to taxol.

Steven M. Rubenstein
Department of Chemistry
Colorado State University
Fort Collins, Co 80523
Spring 1996

Acknowledgments

I have realized that it takes more than one person to earn a Ph.D. degree while only one person actually receives it. With that thought in mind, I would like to thank all of the past and present members from the Williams', Hegedus's, Meyers', Greenberg's, and Stermitz's research groups that I have had a chance to interact with over the years. I would specifically like to thank Greg Miknis for helping me make the transition from undergraduate research to graduate research. In addition, I would like to thank Dr. Hegedus, Dr. Meyers, Dr. Greenberg, and Dr. Stermitz for always having their doors open for me.

Due to the Boehringer-Ingelheim fellowship that was graciously awarded to me by the organic faculty at Colorado State University, I had the opportunity to visit Dr. Croteau's lab at Washington State University. Not only did Dr. Croteau, Mehri, Jeff, Jerry, and Tom teach me many practical Biosynthetic laboratory techniques they gladly welcomed me as a member of their group. I am indebted to them for their time and patience while I was there.

Finally, I would like to thank Dr. Robert M. Williams, better known as Bob, for providing me an environment in which research could be performed expeditiously and allowing me the freedom to grow as a chemist while subtly guiding me throughout this project.

DEDICATION

This dissertation is dedicated to my parents who have always been behind me one hundred percent in everything I've either done or attempted to do. Without their help and support, the work that went into writing this dissertation would have been much harder if not impossible. I would also like to dedicate this dissertation to my wife (Pam) who made just as many sacrifices, if not more, as I have had to make in obtaining a Ph.D. The excellent job that she has done in raising our children (Marc, Sean, and Kristen) will always be, in my mind, a far more important and rewarding endeavor than any written dissertation. All of you have made this accomplishment much more meaningful and I thank you from the bottom of my heart.

TABLE OF CONTENTS

CHAPTER 1

THE BIOSYNTHESIS OF TAXOL

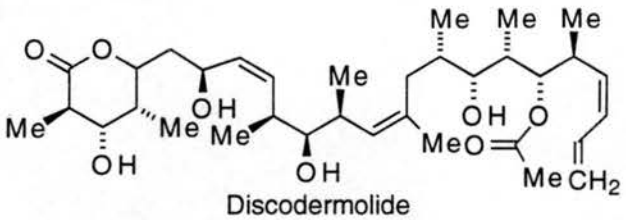
1.1 Introduction

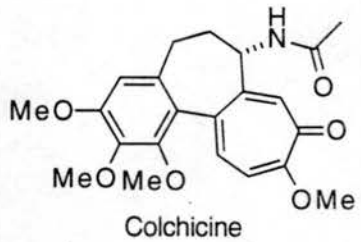
The scientific community has recently put great emphasis on the elucidation of the mechanism of action, biosynthesis, semi-synthesis and total synthesis of the potent, and commercially significant anti-cancer drug taxol along with several biologically active analogues.[1] Chemists and biologists alike have been drawn to taxol due to its promising spectrum of antineoplastic activity, its unique mechanism of action, and the synthetic challenge that the complex, and densely functionalized ring system poses.

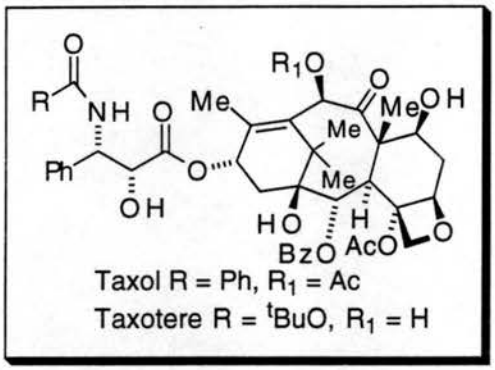
Taxol belongs to a group of anticancer agents known as the spindle poisons, Scheme 1.1. The word "spindle" refers to the microtubule proteins found in eucaryotic cells. The relative concentration of these proteins at any given time during the life cycle of the cell is important. By changing the relative concentration of the microtubule protein one can induce cell death. Most of the compounds that belong to this class such as colchicine, vincristine, and vinblastine are known to inhibit the polymerization of tubulin into microtubules, Scheme 1.2.[1c]

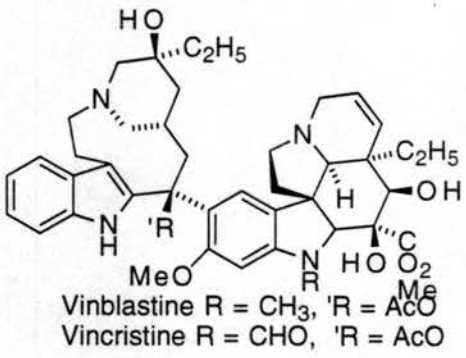
Taxol, however, has been observed to accelerate the polymerization rate of tubulin to microtubules. The resulting proteins are resistant to depolymerization conditions such as low temperature and/or calcium ions.

Scheme 1.1 Spindle Poisons



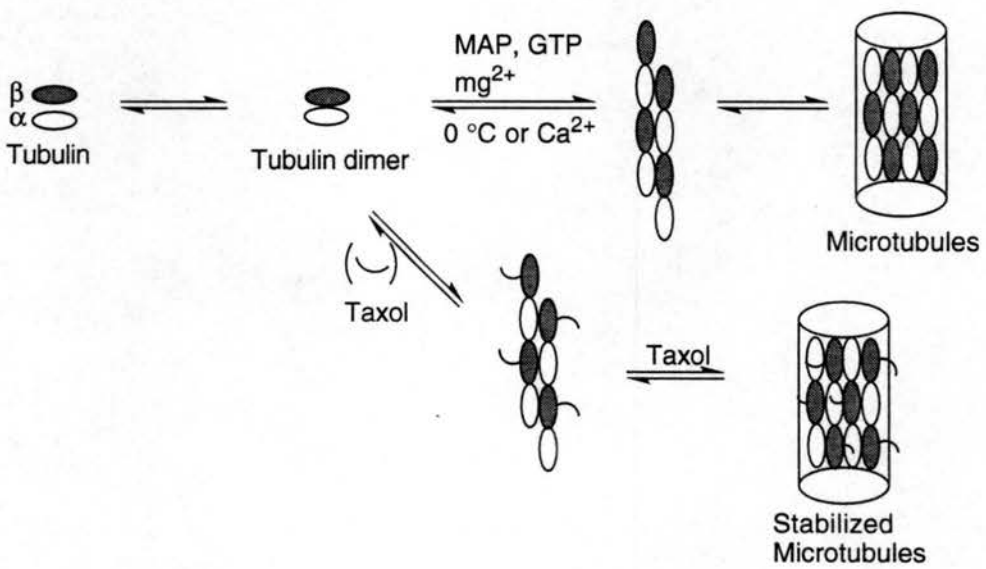






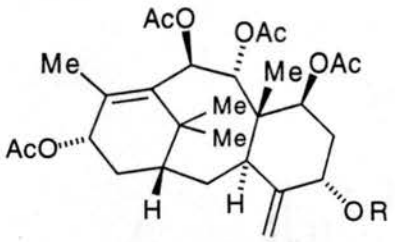
Recently, discodermolide has been added to this class of compounds because it can polymerize tubulin to microtubules approximately ten times better than taxol in *in vitro* tubulin polymerization assays.[1i]

Scheme 1.2 Microtubule Assembly.



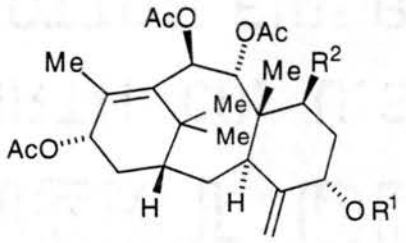
Isolation of taxol from the bark of *Taxus brevifolia* [2] was the primary method for taxol production. Ten kilograms of bark are needed to isolate one gram of taxol.[1c] Unfortunately, stripping the tree of its bark destroys the yew. The current protocol in treating cancer patients with taxol requires the use of two grams per patient. In addition, the recent FDA approval of TAXOL® [3] for the treatment of refractory ovarian and breast cancers makes taxol isolation from *T. brevifolia* infeasible over the long term.[1c] Taxol has also been isolated from the fungus, *Taxomyces andreanae*, by Stierle and Strobel. However, only 50 ng of taxol can be isolated from a one liter of fungus culture.[4] Due to the limited availability of taxol, this research group became interested in developing an alternative method for taxol production.

To date, totally synthetic methods have fallen far short as an alternative source for taxol production, because of the highly complex nature of the taxol structure which mandates lengthy and expensive synthetic routes. A method which shows promise and has recently been approved by the FDA, is the semi-synthesis of taxol from 10-deacetylbaccatin III which can be isolated from the needles, a renewable source, of the European yew, *Taxus baccata*.[5]. Recently, Hanson and co-workers described how an enzyme from *Nocardiodes albus* SC13911 can specifically hydrolyze the C-13 esters found on a variety of naturally occurring taxanes while an enzyme from *N. luteus* SC13912 can specifically hydrolyze the C-10 acetate from a variety of naturally occurring taxanes.[6] These two enzymes have been shown to increase the amount of 10-deacetyl baccatin III isolated from various crude materials of *Taxus* species by a factor of 4 to 24. Based on these results, semisynthesis has become a viable alternative for taxol production in large quantities. In order to ensure a steady supply of taxol, alternative methods should also be developed. A biosynthetic method could be one such alternative approach. In order to develop a biosynthetic method it would be essential to gain a better understanding of the detailed biosynthetic pathway(s) for the production of taxol in *Taxus brevifolia* and other



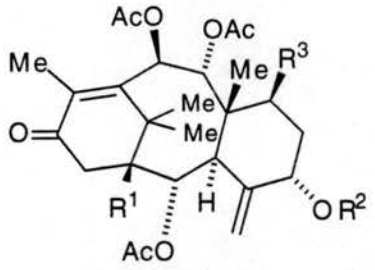
R = Ac (Taxusin)
R = H
R = cinnamoyl

Chattopadhyay, S.K..; et. al. 1995; ref. 9a



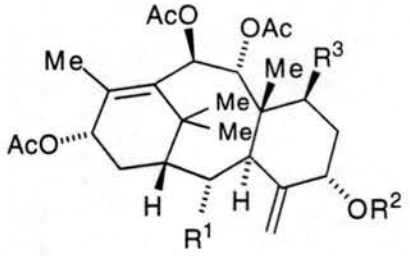
R^1	R^2
$COCH_2CH(NMe)_2Ph$	OAc
cinnamoyl	H
cinnamoyl	OAc

Kobayashi, J.; et. al. 1994; ref. 9b



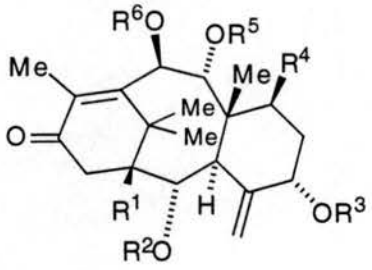
R^1	R^2	R^3
H	cinnamoyl	H
H	cinnamoyl	H
H	cinnamoyl	OAc
OH	cinnamoyl	H
H	$COCH_2CH(NMe)_2Ph$	H

Kobayashi, J.; et. al. 1994; ref. 9b



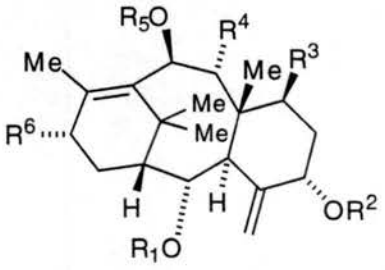
R^1	R^2	R^3
H	Ac	H
H	Ac	OAc
OAc	cinnamoyl	H
OAc	cinnamoyl	OAc
OAc	Ac	H
OAc	Ac	OAc
OAc	H	OAc
OAc	$COCH(OH)CH(NMe_2)Ph$	H
OAc	$COCH(OAc)CH(NMe_2)Ph$	H
OAc	$COCH_2CH(NMe_2)Ph$	H

Potier, P.; et. al. 1987, ref. 8



R^1	R^2	R^3	R^4	R^5	R^6
H	Ac	cinnamoyl	H	Ac	Ac
H	Ac	H	H	Ac	Ac
H	Ac	cinnamoyl	OAc	Ac	Ac
H	Ac	Ac	H	Ac	Ac
OH	H	H	H	H	H
OH	Ac	cinnamoyl	H	Ac	Ac
H	Ac	$COCH_2CH(NMe_2)Ph$	H	Ac	Ac

Potier, P.; et. al. 1987, ref. 8



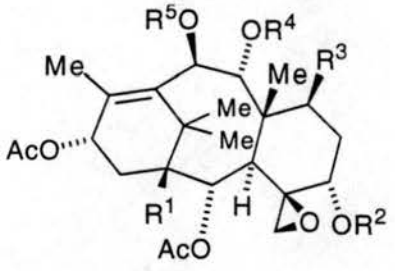
R^1	R^2	R^3	R^4	R^5	R^6
$OCOC(CH_3)=CH(CH_3)$	Ac	OAc	H	OAc	H
$OCOC(CH_3)=CH(CH_3)$	H	OAc	OAc	OAc	H
$OCOC(CH_3)=CH(CH_3)$	Ac	OAc	OAc	OAc	H
H	H	H	OH	OH	OH
H	H	H	OAc	OAc	OH

Potier, P.; et. al. 1987, ref. 8

Figure 1.1 Class A Naturally Occurring Taxanes

related taxol-producing species. Specifically, deciphering and then controlling the slow steps of taxol production through gene manipulation would be required.

There are more than 100 naturally occurring taxanes.[7] The majority of these compounds can be grouped into three classes and are shown figures 1.1-1.2.[7-11] The miscellaneous naturally occurring taxanes are shown in figures 1.3 and 1.4.[7-9] The class **A** type of compounds are similar in that they all posses a C-4 exomethylene group and a C-5 alpha alcohol. Class **B** is similar to class **A** except the C-4 exomethylene is oxidized to an oxirane and the class **C** set of compounds all contain the intact oxetane moiety found in taxol. Based on the amount of natural taxoids isolated from each class, Potier proposed that the class **A** type of compounds are formed first, followed by conversion to Class **B** and then to class **C** type compounds. The class C type of compounds are then converted to taxol.[8]



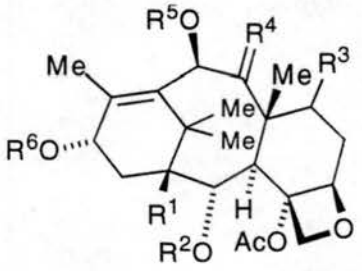
R^1	R^2	R^3	R^4	R^5
H	Ac	OAc	Ac	Ac
H	H	OAc	Ac	Ac
OH	Ac	OAc	Ac	Ac
OH	Ac	OH	Ac	Ac
H	cinnamoyl	H	H	Ac
H	$COCH_2CH(NMe_2)Ph$	H	H	Ac
H	$COCH_2CH(NMe_2)Ph$	H	Ac	H

Potier, P.; et. al. 1987, ref. 8

Zhang, S.; et. al. 1994; ref. 9c

Fuji, K.; et. al. 1992; ref. 11

Class **B** Type Compounds



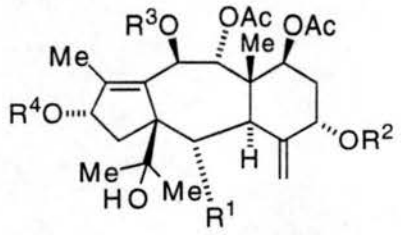
R^1	R^2	R^3	R^4	R^5	R^6
OH	Bz	OAc(β)	OH(α)	H	H
OH	Bz	OH(α)	O	Ac	H
OH	Bz	OH(β)	O	Ac	H
OH	Bz	OH(β)	O	H	H
OH	Ac	OAc(β)	OAc(α)	Ac	Ac
H	Ac	OAc(β)	OAc(α)	Ac	Ac
OH	Bz	OAc(β)	OAc(α)	Ac	Ac
OH	COC_5H_{11}	OAc(β)	OAc(α)	Ac	Ac

Potier, P.; et. al. 1987, ref. 8

Kobayashi, J.; et. al. 1995; ref. 10

Class **C** Type Compounds

Figure 1.2 Class B and C Naturally Occurring Taxanes



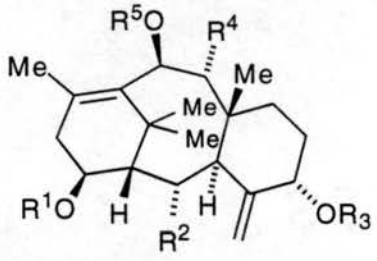
R^1	R^2	R^3	R^4
OAc	H	Bz	H
OAc	H	Bz	Ac
OAc	H	H	Ac
H	cinnamoyl	Bz	H
OAc	H	Bz	cinnamoyl
H	cinnamoyl	Bz	Ac
H	cinnamoyl	Bz	cinnamoyl

Chattopadhyay, S.K.; et. al. 1995; ref. 9a

Fuji, K.; et. al 1995; ref. 9d

Kobayashi, J.; et. al. 1995; ref. 9i

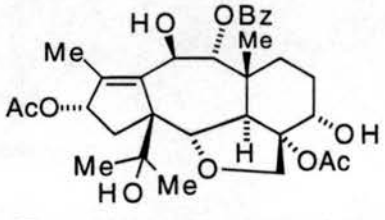
Tanaka, K.; et.al. 1994; ref. 9j



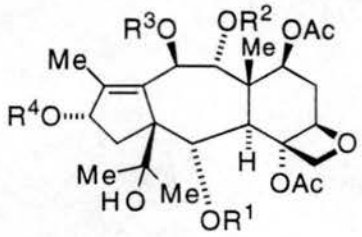
R^1	R^2	R^3	R^4	R^5
$COCH(Me)CH_2CH_3$	OAc	Ac	OAc	Ac
Ac	OAc	Ac	H	Ac
$COCH(Me)CH_2CH_3$	OAc	H	OAc	Ac
$COCH(Me)CH_2CH_3$	OAc	Ac	H	Ac
OH	OAc	Ac	H	H
$COCH_2CH_3$	OAc	H	H	H
COCH(Me) OH	H	Ac	H	H

Zhang, H.; et. al. 1994; ref. 7

Zhang, H.; et. al. 1995; ref. 9e



Zhang, H.; et. al. 1995; ref. 9g



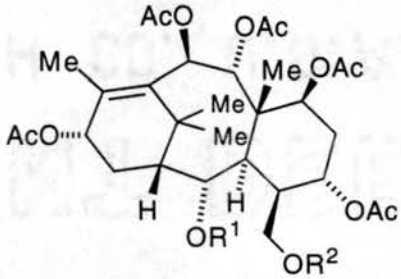
R^1	R^2	R^3	R^4
Ac	Ac	Bz	cinnamoyl
Bz	Bz	Bz	Ac
Ac	Bz	Bz	Ac
Bz	Bz	H	Ac
Bz	Bz	Ac	Ac
Ac	Bz	H	cinnamoyl
Ac	Ac	Bz	H
Ac	Ac	H	Ac
Ac	Ac	H	H
Bz	Ac	H	H
Ac	Bz	H	H
Ac	Bz	H	cinnamoyl

Fuji, K.; et. al 1995; ref. 9d

Zhang, H.; et. al. 1995; ref. 9g

Fang, Q-Y.; et. al. 1995; ref. 9h

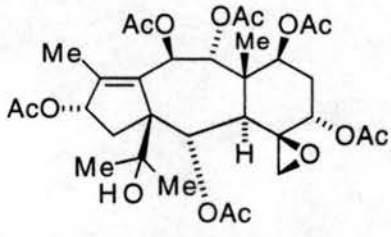
Tanaka, K.; et.al. 1994; ref. 9j



R^1	R^2
Ac	cinnamoyl
H	Ac

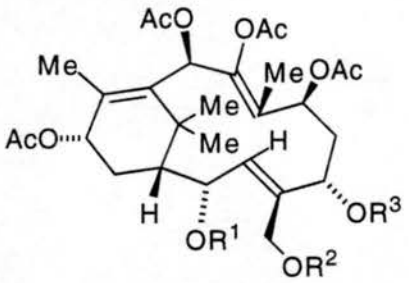
Fuji, K.; et. al 1995; ref. 9d

Tanaka, K.; et.al. 1994; ref. 9j



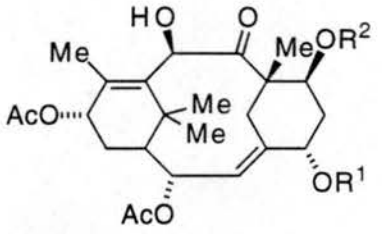
Zhang, S.; et. al. 1994; ref. 9c

Figure 1.3 Miscellaneous Naturally Occurring Taxanes



R^1	R^2	R^3
Ac	Ac	H
H	H	Ac

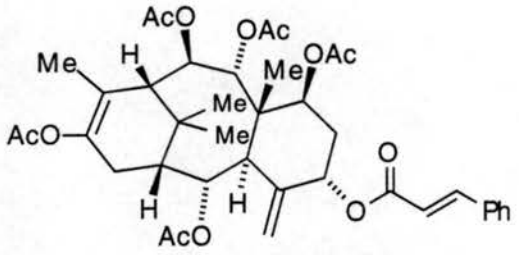
Fang, W-S.; et. al. 1995; ref. 9f



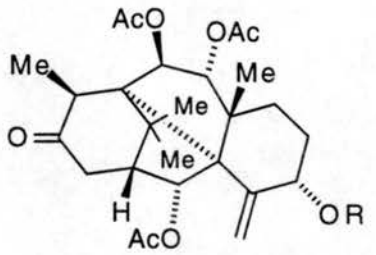
R^1	R^2
cinnamoyl	Ac
$COCH(OH)CH(NMe_2)Ph$	H

Potier, P.; et. al. 1987, ref. 8

Kobayashi, J.; et. al. 1995; ref. 9i



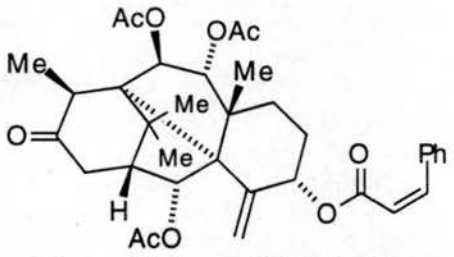
Kobayashi, J.; et. al. 1995; ref. 9i



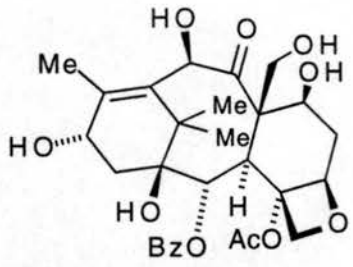
R = H, Ac, cinnamoyl

Potier, P.; et. al. 1987, ref. 8

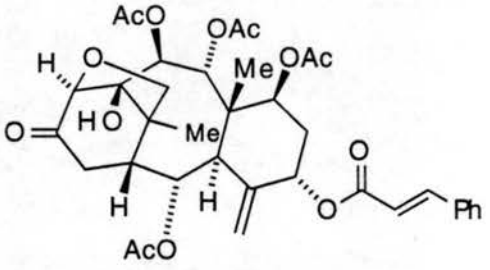
Kobayashi, J.; et. al. 1995; ref. 9i



Kobayashi, J.; et. al. 1994; ref. 9b



Potier, P.; et. al. 1987, ref. 8

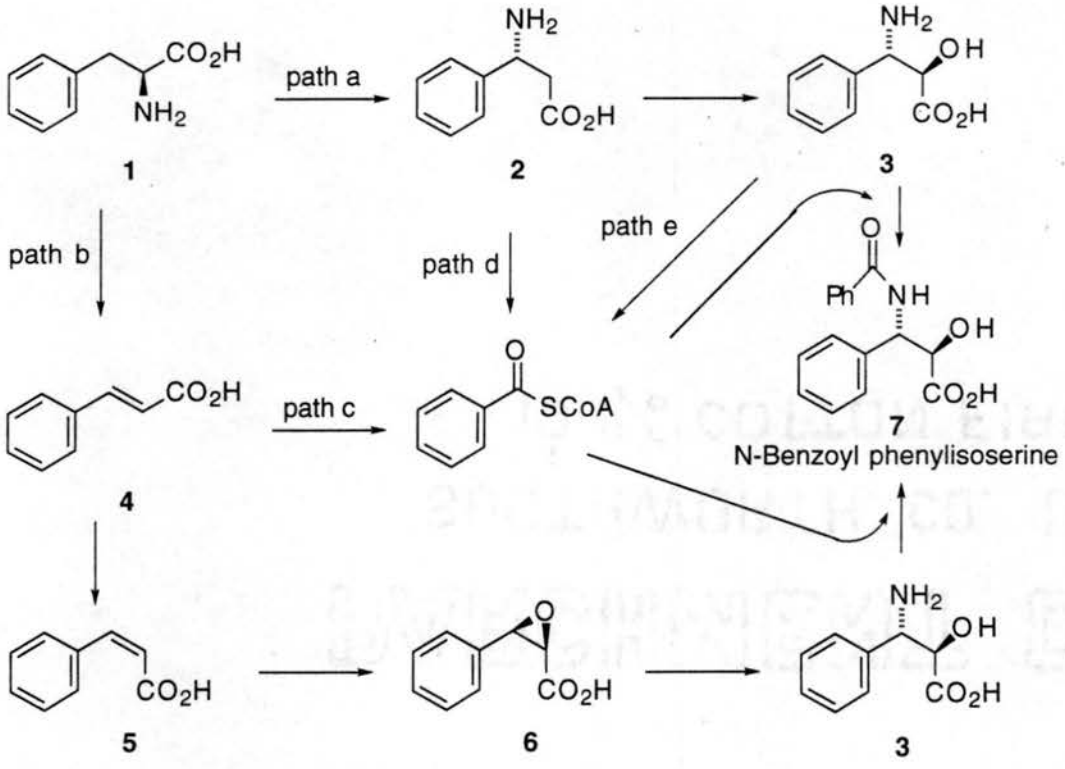


Potier, P.; et. al. 1987, ref. 8

Figure 1.4 Miscellaneous Naturally Occurring Taxanes Continued

1.2 The Biosynthetic Origin of the Phenylisoserine Side Chain of Taxol

Scheme 1.3 Proposed Biosynthetic Pathway to Taxol's Sidechain.



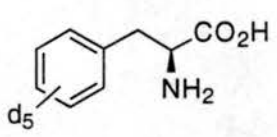
Floss and co-workers were the first to make significant progress towards elucidating the biosynthetic pathway to taxol in *Taxus brevifolia* .[1h, 12a] They have specifically focused on the *de novo* synthesis of the N-benzoyl phenylisoserine **7** side chain as well as the stage of the biosynthesis at which the side chain gets attached to the diterpene core structure. Scheme 1.1 outlines their proposed biosynthetic routes to taxol's side chain. In order to determine which pathway (**a** or **b**) is operative they synthesized compounds **2**-**4**, and **6** in deuterated form. Compound **1** is commercially available in deuterated form. Deuterated compounds **1a**-**4a**, and **6a** were then incubated separately with pieces of bark and cambial tissue from *Taxus brevifolia* for 96h. The taxoids produced were separated from the bark and cambial tissue and then purified by HPLC (High Performance Liquid Chromatography) and subjected to mass spectral analysis.

Figure 1.2 shows deuterated compounds **1a**-**4a**, and **6a** and their percent incorporation into taxol and cephalomannine.

From figure 1.2 it can be seen that phenylalanine **1a**, β-phenylalanine **2a**, and phenylisoserine **3a** were all incorporated into taxol and cephalomannine as their M+5 isotopomers. Mass spectral fragmentation patterns showed the deuterium label to be located exclusively in the side chain for all three labeled precursors. β-phenylalanine **2a** and phenylisoserine **3a** were also observed to incorporate exclusively into the side chain of taxol as their M+10 isotopomers. However, *trans*-cinnamic acid **4a** and epoxide **6a** did not incorporate into taxol or cephalomannine. Based on these results they concluded that: (1) a phenylalanine ammonia-lyase reaction was not operative in converting phenylalanine **1** into phenylisoserine **3** and benzoyl-CoA through *trans*-cinammic acid **4**; (2) phenylalanine **1** is converted to phenylisoserine **3** by way of an aminomutase reaction followed by a C-2 hydroxylation on β-phenylalanine **2**; and (3) β-phenylalanine **2** and/or phenylisoserine **3** are/is converted into benzoyl-CoA which is then used to acylate phenylisoserine **3** to give the fully functionalized side chain of taxol. These results seem to favor paths a/d and/or a/e while suggesting that path b/c is a highly unlikely route to taxol's side chain. It is worth noting that path b/c is a known pathway to benzoyl-CoA in other plant systems.[12]

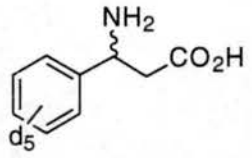
There are several naturally occurring taxanes that have Winterstein's acid, N-dimethyl-β-phenylalanine (a potential side chain precursor), attached at C-5.[8] The diterpene core structure of taxol is cup-shaped thus bringing C-5 and C-13 in to close proximity. Based on these facts and Floss's initial results, there are still questions concerning the biosynthesis of taxol's side chain. For instance, is the fully functionalized phenylisoserine side chain intermolecularly attached to baccatin III or is it possible for phenylisoserine, or an analogue thereof, to first become attached to the C-5 position followed by an intramolecular transesterifiction at C-13? This hypothesis was initially proposed by Potier.

Subsequent work by Floss and co-workers addressed these questions. Their results are shown in figure 1.3.[12b]



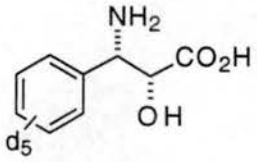
1a

0.9% (M+5) incorp. into taxol/cephalomannine



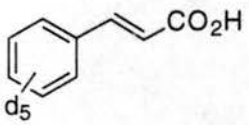
2a

2.8% (M+5) incorp. into taxol/cephalomannine
2.6% (M+10) incorp. into taxol



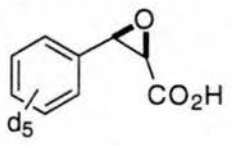
3a

1% (M+5) incorp. into taxol/cephalomanine
0.9% (M+10) incorp. into taxol



4a

0% incorp. into taxol/cephalomannine



6a

0% incorp. into taxol/cephalomannine

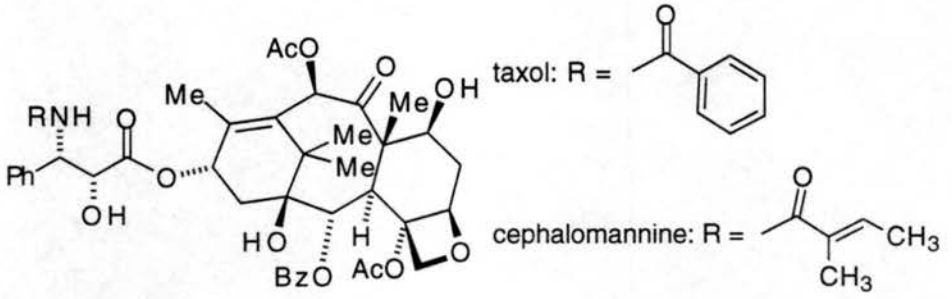
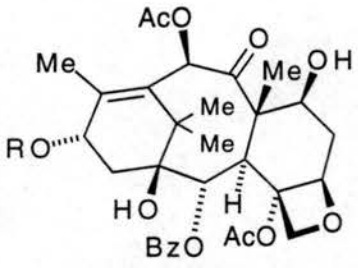


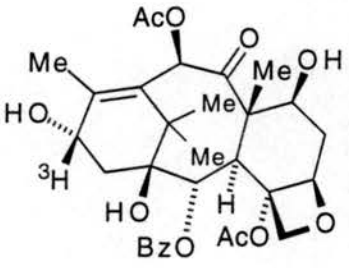
Figure 1.5 Deuterated compounds **1a-4a**, and **6a** and their Percent Incorporation into Taxol and Cephalomannine.

In order to address the question of whether the side chain is coupled to the C-13 alcohol via an intramolecular transesterification or an intermolecular coupling, compounds **8a** and **9a** were synthesized. [13-^{3}H]-Baccatin III **8a** and [10-acetyl-$^{2}H_3$, 13-$^{2}H_1$]-baccatin III **9a** were incubated separately as previously described and both showed incorporation into taxol. Degradation studies were performed on **8a** showing that the tritium label was still at the C-13 position. Mass spectrometry showed that **9a** was incorporated into taxol and cephalomannine as their M+4 isotopomers indicating that the C-10 acetyl group does

not hydrolyze during the reaction conditions. Based on these results, it's unlikely that an intramolecular transesterifiction is taking place.

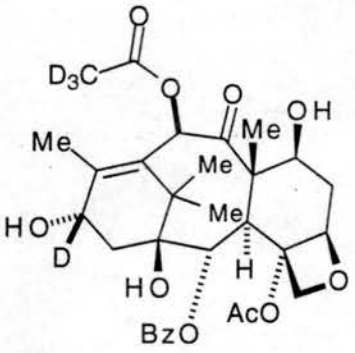


Baccatin III: R = H



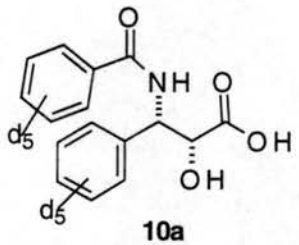
8a

0.1% incorporation into taxol



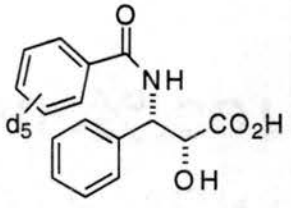
9a

1% (M+4) incorp. into taxol
material was not hydrolyzed



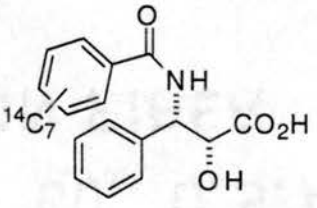
10a

1.7% (M+5) incorp. into taxol
small amount of M+10 detected



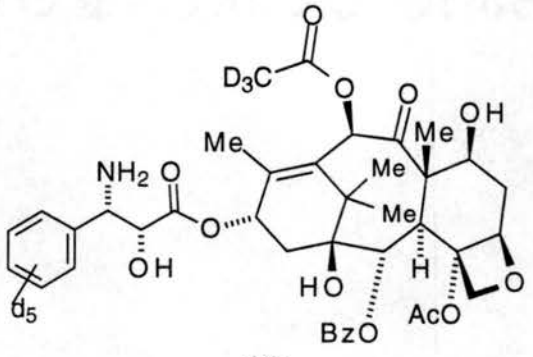
11a

1.7% (M+5) incorp. into taxol



12a

92% of the radioactivity was found in the intact side chain



13a

5% (M+8) incorp. into taxol and cephalomannine
<1% of M+5 and M+3 isotopomers

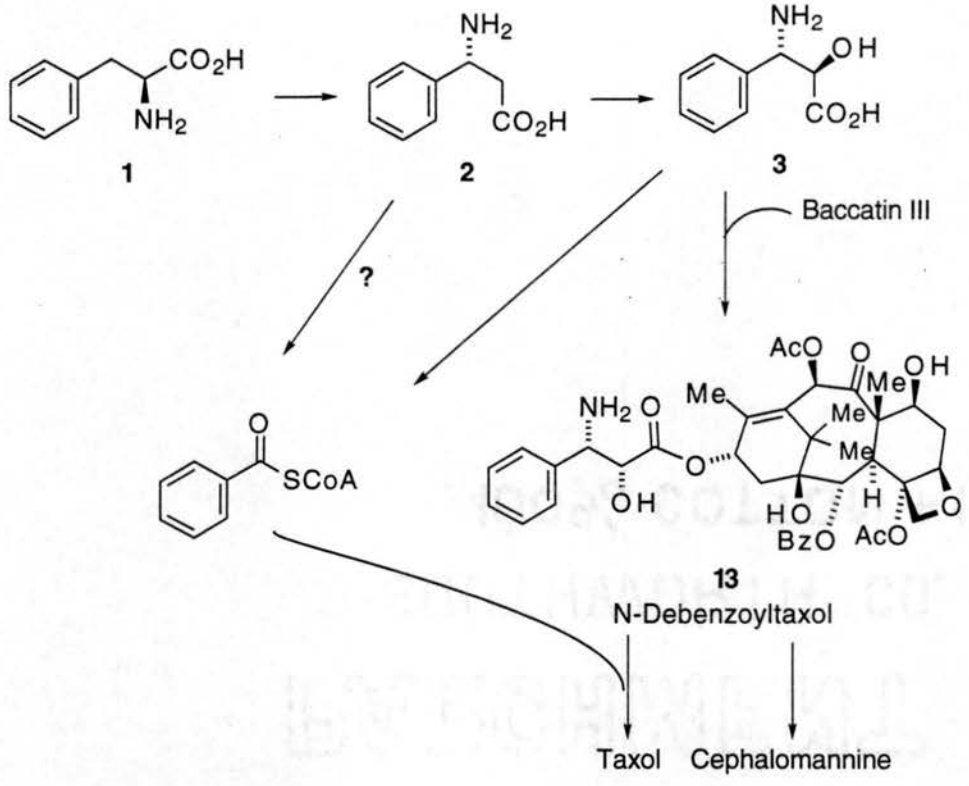
Figure 1.6 Deuterated/Tritiated compounds **8a-13a** and their Percent Incorporation into Taxol and Cephalomannine.

Deuterated compound **11a** was also found to incorporate into taxol but it was not known whether the deuterated N-benzoyl phenylisoserine side chain was attached as a

intact unit or if the N-benzoyl group was first hydrolyzed followed by phenylisoserine coupling to the diterpene core and then reacylation of the deuterated N-benzoyl moiety. In order to test this idea, **10a** was synthesized and incubated with bark and cambial tissue from *Taxus brevifolia*. As outlined in figure 1.3, **10a** incorporated into taxol as its M+5 isotopomer thus showing that the N-benzoyl group was hydrolyzed either before or after attachment to the diterpene core structure. There was still concern as to the origin of this debenzoylation; was it an indiscriminate process? In order to test this hypothesis, ^{14}C labeled N-benzoyl phenyl isoserine **12a** was synthesized and incubated as previously described. The ^{14}C labeled N-benzoyl phenylisoserine side chain **12a** isolated contained 92% of the original amount of the radiolabel. The results suggest that the side chain is not attached as an intact unit to the diterpine core structure of taxol but rather phenylisoserine **3** is attached first followed by N-benzoylation. In order to test this proposal, Floss and co-workers synthesized and then incubated deuterated compound **13a**. N-Debenzoyltaxol **13a** did incorporate into taxol as its M+8 isotopomer thus confirming the idea that N-benzoylation takes place after phenylisoserine ring attachment. Scheme 1.2 shows a revised biosynthetic pathway in which higher order taxanes such as taxol and cephalomannine are produced from phenylalanine **1** and baccatin III by *Taxus brevifolia*.

Significant progress has been made towards a better understanding of how the phenylisoserine side chain of taxol is produced and when it is coupled to baccatin III. However, there still remain many questions. For example, does β-phenylalanine **2** first couple to baccatin III followed by C-2 oxidation? Is 10-deacetylbaccatin III, an abundant naturally occurring compound, a precursor to baccatin III? These and many other questions are currently being addressed by Floss's group.

Scheme1.4 Biosynthetic Pathway for Taxol Production.



1.3 The Biosynthetic Origin of the Diterpene Core Structure of Taxol

In 1966, Lythgoe and co-workers proposed a biosynthetic pathway (Scheme 1.3) in which the tricyclic carbon framework of the taxoids arose by the sequential intramolecular cyclizations of the double bonds of geranylgeranyl diphosphate **14**, a known a diterpene precursor, [13] via cation **15** to 1-(S)-verticilline **16** and then to taxa-4(20),11(12)-diene **18** via cation **17**.[14] The elaboration of taxol from this simple diterpene skeleton would then involve a series of nine two-electron enzymatic oxidations, acylations and attachment of the side chain to produce taxol.

It should be noted that 1-(S)-verticilline **16** and taxa-4(20),11(12)-diene **18** have not been isolated to date, while three structurally related compounds, verticillol **19** [15], cembrene **20** [16], and casbene **21** [17] (Figure 1.4) have been isolated. Based on the preceding observations, It's possible that 1-(S)-verticilline **16** and taxa-4(20),11(12)-diene

18 are not biosynthetic precursors to taxol while cembrene **20** and casbene **21** are possible precursors. It is highly unlikely that verticillol **19** is a biosynthetic precursor to taxol since it has the incorrect stereochemistry at the C-1 position.

Scheme1.5 Lythgoe's Proposed Biosynthetic Origin of the Diterpene Core Structure of Taxol.

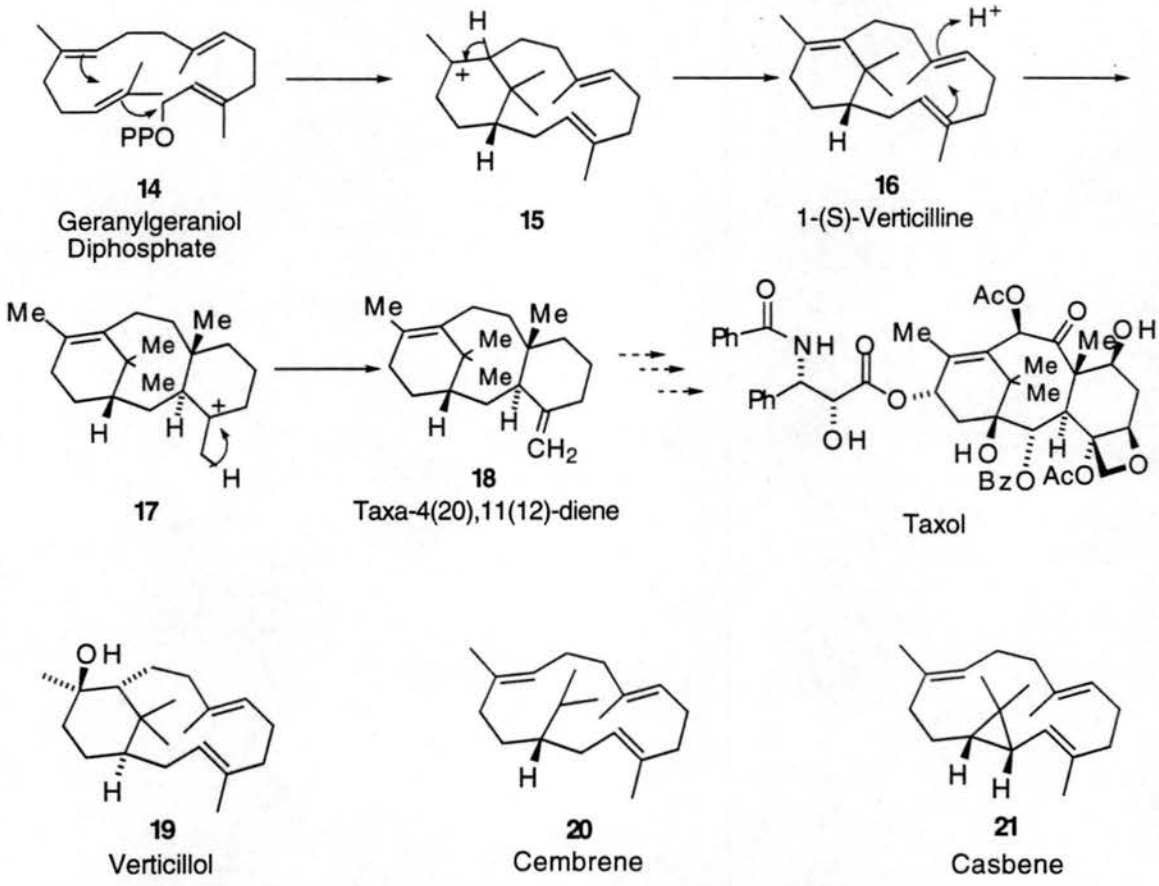
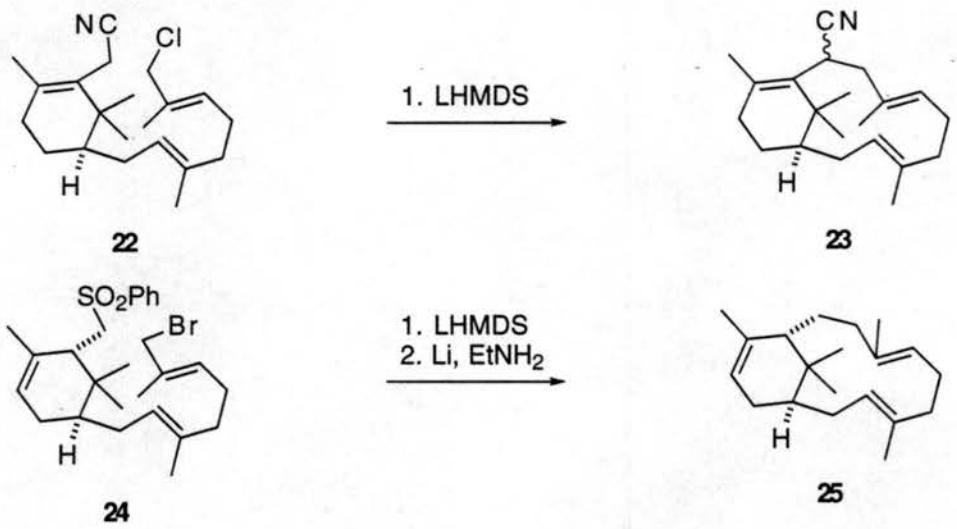


Figure 1.7 Putative Biosynthetic Precursors to Taxol.

Nine years after Lythgoe's initial proposal, the first synthetic approaches to putative biosynthetic precursors verticillol **19** and 1-(S)-verticilline **16** were attempted by Kato and co-workers, Scheme 1.4. [18] Starting from geranyl precursors, compounds **22** and **24** were synthesized and subjected to intramolecular ring closure to yield diterpenes **23** and **25** respectively. Both of these compounds have the incorrect relative stereochemistry at the C-1 position and, in addition compound **25** has the incorrect C-7/C-8 double bond

geometry thus making these routes impractical for the synthesis of naturally occurring biosynthetic precursors of taxol.

Scheme 1.6 Synthesis of Diterpenes **23** and **25**.

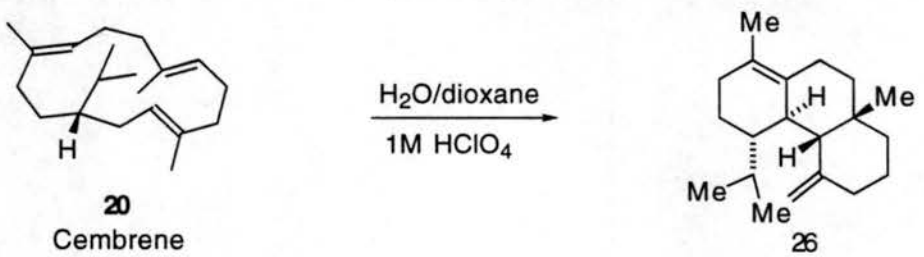


22 **23**

24 **25**

Also, during this time, the first *in-vitro* cyclization of a putative biosynthetic precursor was carried out, Scheme 1.5.[16] The acid catalyzed cyclization of cembrene **20** yielded compound **26** instead of a taxoid type of skeleton. In 1985 1-(S)-verticilline **16** was synthesized for the first time by Pattenden and co-workers, Scheme 1.6.[19] Starting with 3-isobutylcyclohexenone **27** and E-3,7-dimethylocta-2,6-dienyl bromide **28**, compound **29** was synthesized in eight steps in 5% overall yield. Dialdehyde **29** was coupled under McMurry conditions followed by a 1,5-H sigmatropic rearrangement to yield 1-(S)-verticilline **16** in 20% yield for the two steps.

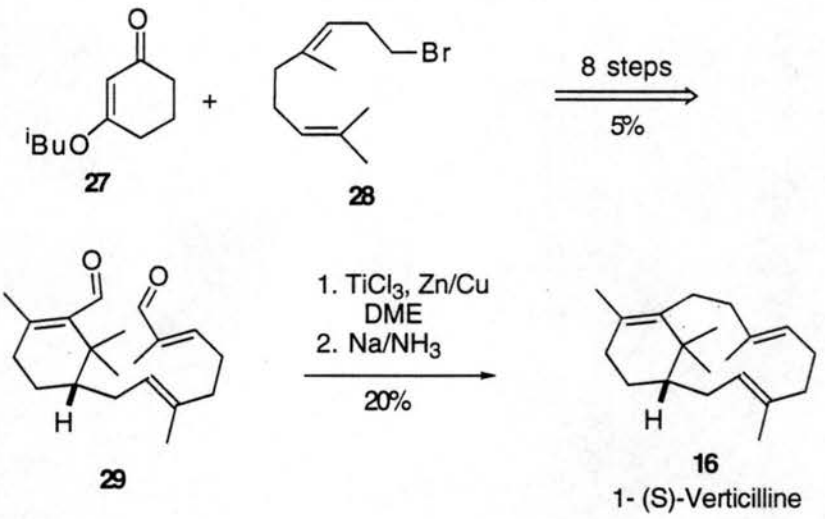
Scheme 1.7 Acid Catalyzed Cycylization of Cembrene **20**.



20
Cembrene

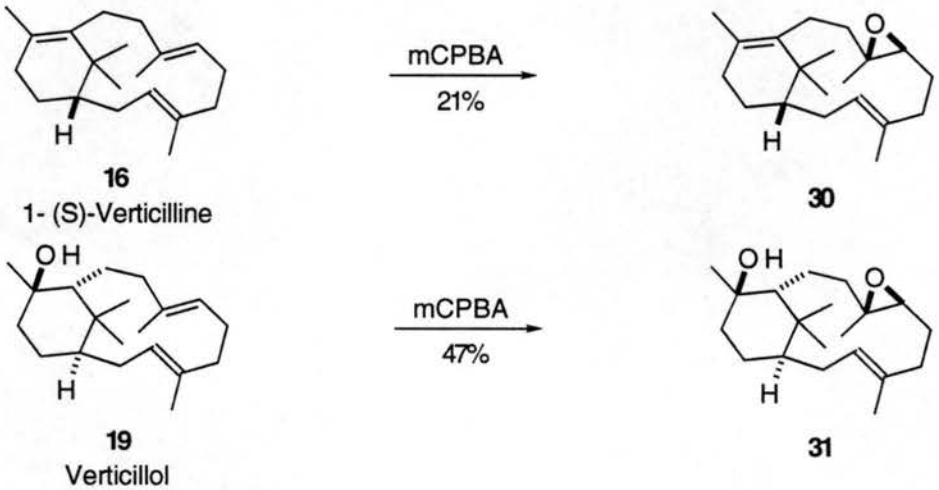
26

Scheme 1.8 The Synthesis of 1-(S)-Verticilline **16**.



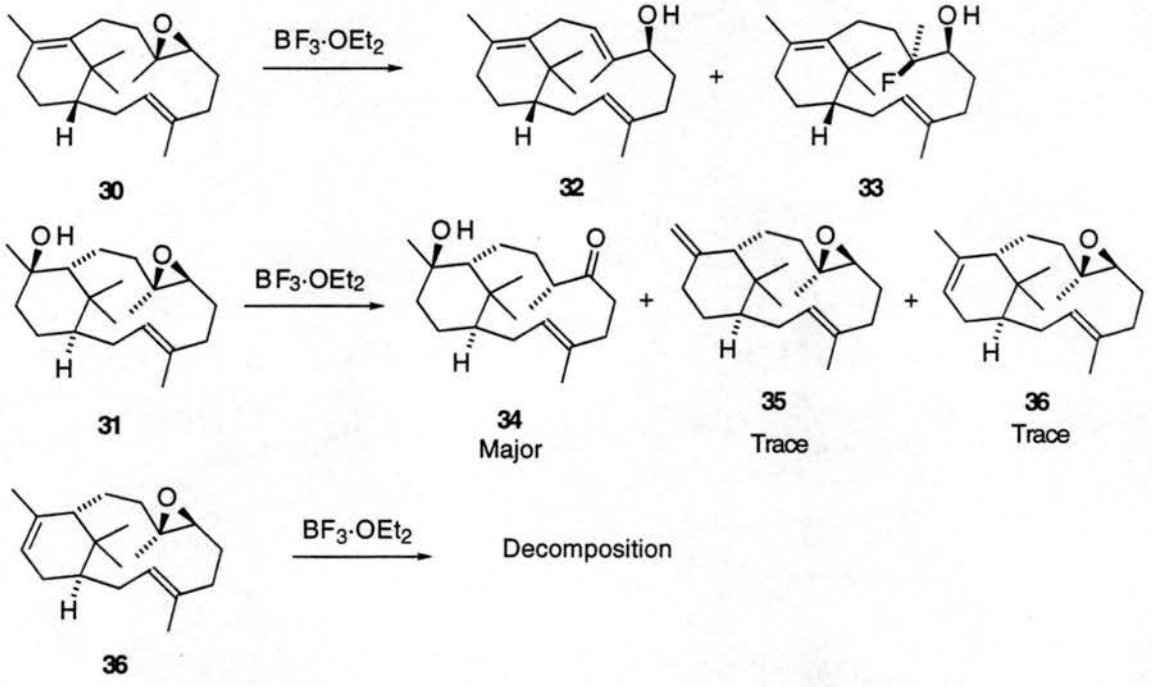
1-(S)-Verticilline **16** and verticillol **19**, isolated from *Sciadopitys verticillata*, were then epoxidized (Scheme 1.7) in order to activate the C-8 carbon for an *in-vitro* transannular cyclization, Scheme 1.8. [20] Subjecting epoxide **30** to Lewis acidic conditions yielded allylic alcohol **32** (25%) and fluorinated alcohol **33** (18%). There was no sign of 1-(S)-verticilline-7β-ol formation. Similarly, epoxide **31**, under Lewis acidic conditions, yielded ketone **34** as the major product plus epoxides **35** and **36** in trace amounts.

Scheme 1.9 Epoxidation of 1-(S)-Verticilline **16** and Verticillol **19**.



Epoxide **36** was further treated with $BF_3 \cdot OEt_2$ only to yield decomposition products. It is hard to draw any concrete conclusions from this set of experiments except to say that under chemical Lewis acidic conditions, functionalized putative biosynthetic precursors **30** and **31** do not form taxane skeletal systems.

Scheme 1.10 Attempted Transannular Cyclizations of Epoxides **30**, **31**, and **36**.

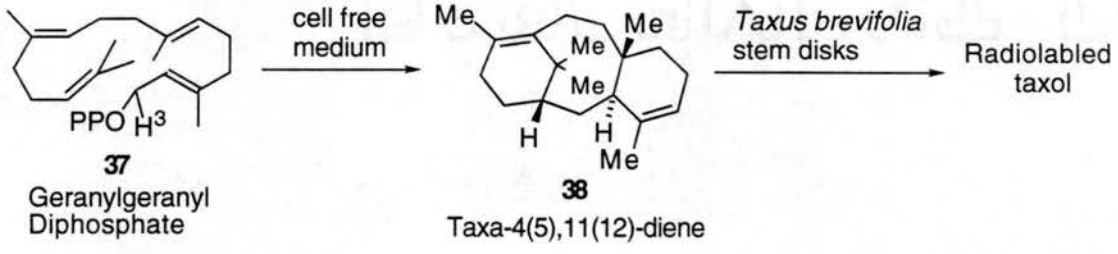


It wasn't until 1995 that significant progress was made towards better understanding the origin of the diterpene core structure of taxol; almost 30 years after Lythgoe's original proposal. Croteau and co-workers incubated [1-^{3}H] geranylgeranyl diphosphate **37** (Scheme 1.9) in a cell-free medium prepared from sapling yew stems. After one hour of incubation, a new radioactive pentane soluble hydrocarbon was formed.[21] The isolated hydrocarbon was then incubated with *Taxus brevifolia* stem disks for eight days. The taxoids were then separated from the reaction mixture and purified. Taxol, cephalomannine, and baccatin III were just a few of the taxoids found that were radiolabeled. Because the amount of radioactive hydrocarbon formed from [1-^{3}H]

geranylgeranyl diphosphate **37** is so small as to preclude structural determination via spectroscopic methods, radio-label guided fractionation was performed.

The radio-labeled hydrocarbon was mixed with a nonpolar extract from 750 Kg of dried *Taxus brevifolia* powder obtained from Hauser Chemical Research. Radio-label guided fractionation was performed and 850 μg of radioactive hydrocarbon was isolated. Mass spectral analysis, one- and two-dimensional NMR analyses were performed to structurally assign the radioactive hydrocarbon as taxa-4(5),11(12)-diene **38** (Scheme 1.9). Additional searching of the nonpolar extract for 1-(S)-verticilline **16** and taxa-4(20),11(12)-diene **18** proved unsuccessful.

Scheme 1.11 Taxa-4(5),11(12)-diene **38** is a Biosynthetic Precursor to Taxol.



37
Geranylgeranyl
Diphosphate

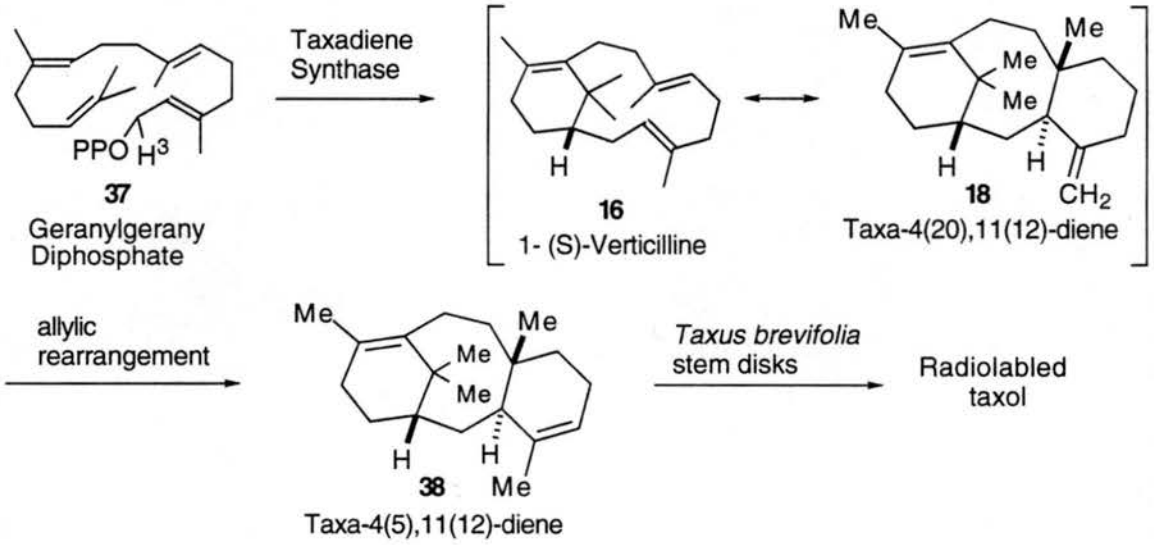
38
Taxa-4(5),11(12)-diene

The diterpene cyclase responsible for converting tritiated geranylgeranyl diphosphate **37** to taxa-4(5),11(12)-diene **38** was subsequently isolated and partially purified.[22] The taxadiene cyclase is primarily located in the peeled bark and adhering cambial cells and is a monomeric protein with a molecular weight of 79 Kd. The cyclase has a lower activity as compared to other similar cyclases and requires the following conditions for optimum output of product: (1) the pH of the medium should be at 8.5 and (2) the synthase requires a divalent ion, preferably Mg^{2+}, for activity.

1.4 Conclusion

As previously mentioned, the diterpene cyclase has a lower activity as compared to other similar cyclases and taxa-4(5),11(12)-diene **38** is present in extremely small amounts in *Taxus brevifolia*. This suggests that the first slow step in the biosynthetic pathway to taxol is the cyclyization of geranylgeranyl diphosphate **14** to taxa-4(5),11(12)-diene **38**. As stated earlier, isolation of 1-(S)-verticilline **16** and taxa-4(20),11(12)-diene **18** from the *Taxus brevifolia* extract was unsuccessful. Does this mean that they are not biosynthetic precursors to taxol or could they just be transient intermediates (Scheme 1.10)? What are the subsequent oxidative steps leading to taxol? In order to answer these questions and the many more that will arise during the course of studying the biosynthetic pathway of taxol, it would be desirable to develop a synthetic route(s) that would allow for the synthesis of taxa-4(5),11(12)-diene **38**, taxa-4(20),11(12)-diene **18**, and other putative biosynthetic precursors. The synthetic route(s) should allow for: (1) quick access to the desired compounds; (2) incorporation of stable and radiolabeled isotopes; and (3) the synthesis of the desired compounds in large quantities. Chapter 3 outlines our progress in this area.

Scheme 1.12 **16** and **18** are Putative Biosynthetic Precursors to Taxol.



CHAPTER 2

THE CHEMICAL SYNTHESIS OF TAXOL AND RELATED COMPOUNDS

2.1 Introduction

The chemical synthesis of complex biologically active molecules is an important endeavor for the organic chemist.[23] Synthesizing complex molecules usually requires the development of new and improved methodologies. Developing new and improved methodologies is important because it should allow for quick and convenient access to large quantities of structurally complex molecules that are biologically active. Another important aspect of total synthesis is the ability to modify the synthetic routes to make analogues more biologically active than their parent compounds.

Since the structural elucidation of taxol in 1971[2], many research groups across the world have either developed new methodologies or used existing ones in an attempt to synthesize the diterpene core structure of taxol.[1] Intramolecular, metal-mediated ring closure, ring expansions and contractions, and cycloadditions are the three general classes of reactions that have been used to synthesize the diterpene moiety. In 1988 Greene and co-workers described the first semi-synthesis of taxol starting from 10-deacetylbaccatin III, a natural product isolated from *Taxus baccata*.[24] Since that time, and more than 20 years after taxol's structural elucidation, only Holton's, Nicolaou's, and Danishefsky's research groups have synthesized taxol from commercially available starting materials. [25-27] The

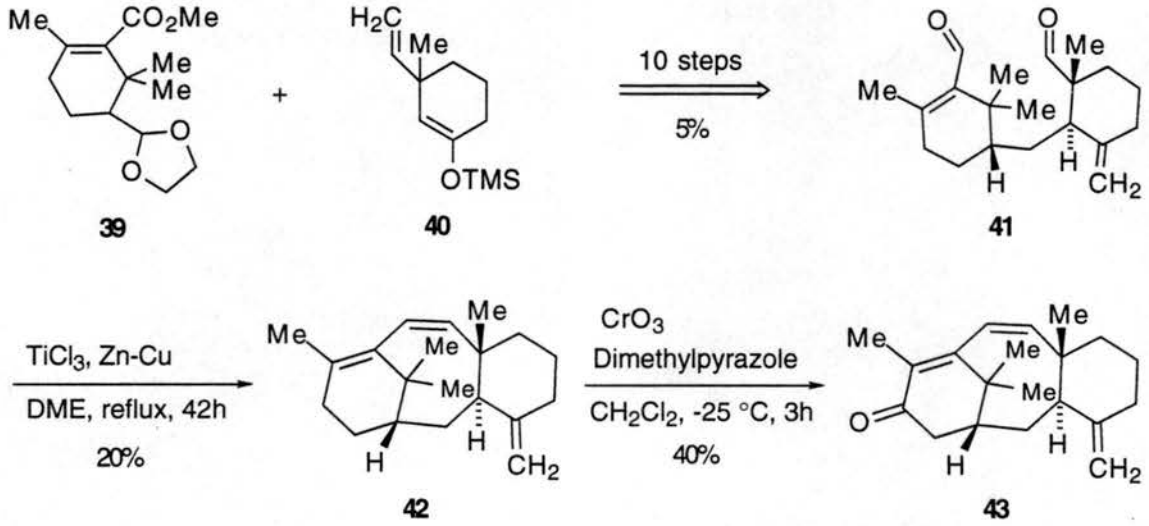
next section outlines some of the main strategies used in attempts to synthesize the diterpene core of taxol.

2.2 Synthetic Strategies towards the Diterpene Core Structure of Taxol

2.2a Intramolecular Metal-Mediated Ring Closures

In 1986 Kende and co-workers were the first group to synthesize a completely intact racemic taxane core structure.[28] Using standard chemical transformations, compound **41** was synthesized in 5% overall yield in 10 steps starting from precursors **39** and **40** (Scheme 2.1). Dialdehyde **41**, which has the same relative stereochemistry at C-1, C-3, and C-8 as taxol, was then subjected to the McMurry coupling protocol which afforded diterpene **42** in 20% yield. Oxidation of **42** with chromium trioxide/dimethylpyrazole complex produced **43** in 44% yield. The key to this successful synthesis relied upon the intramolecular McMurry coupling.

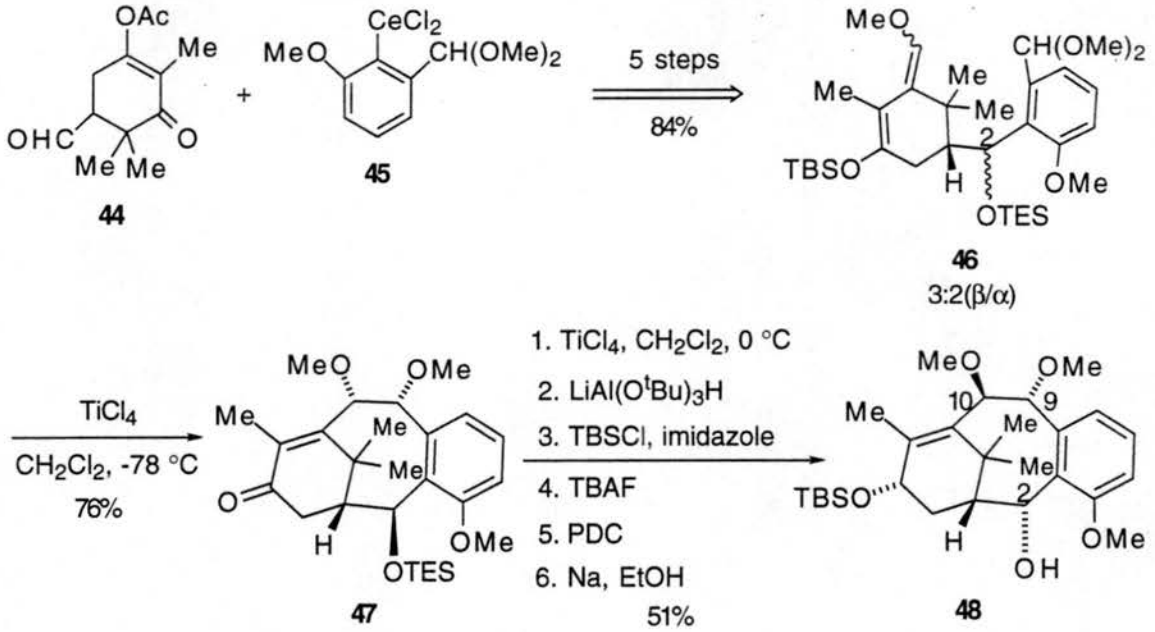
Scheme 2.1 Kende's Approach to Taxol's Diterpene Core Structure.



A few years later, Kuwajima and co-workers published their own route towards the ABC ring system of taxol,[29a] which relied upon forming an eight-membered ring via a

Lewis acid mediated intramolecular cyclization. Scheme 2.2 outlines their most recent progress towards synthesizing a functionalized taxane type molecule using the methodology just described.[29b]

Scheme 2.2 Kuwajima's Approach to Taxol's Diterpene Core Structure.

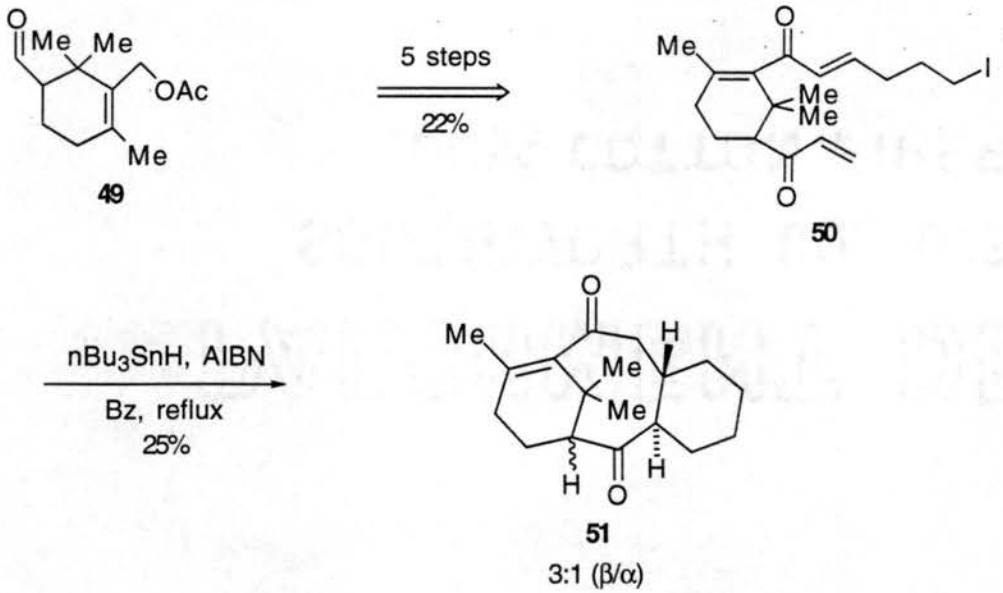


Compound **46** was formed as a 3:2 mixture of β and α diastereomers at the C-2 position (taxane numbering) respectively, in 5 steps and in 84% overall yield from compounds **44** and **45**. The β–diastereomer was allowed to reacted with tin tetrachloride at -78 °C to form **47** as the kinetic product in 76% yield. Compound **47** was then converted to **48** in 51% yield via the six step reaction sequence outlined in scheme 2.2. Compound **48** has the same relative stereochemistry at C-1, C-2, C-10, and C-13 in addition to having the appropriate functionality at C-2, C-9, C-10, and C-13 as in the taxane class of compounds.

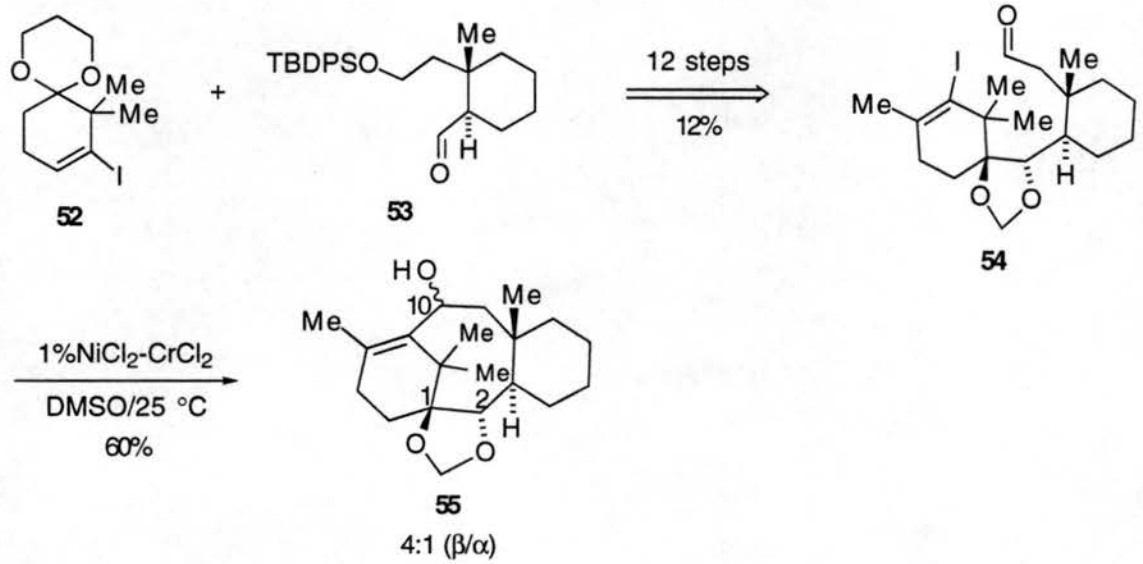
In 1992 Pattenden and co-workers were the first and only group to use a tandem radical macrocyclization approach towards synthesizing the ABC ring system of taxol.[30] Cyclohexenealdehyde **49** was converted in 5 steps to **50** in 22% overall yield, Scheme

2.3. Compound **50** then underwent a tandem 12-endo, 8-endo radical cyclization to produce compound **51** as a 3:1 mixture of epimers at C-1. The major diastereomer has the same relative stereochemistry at C-1, C-3, and C-8 as taxol.

Scheme 2.3 Pattendon's Approach to Taxol's Diterpene Core Structure.



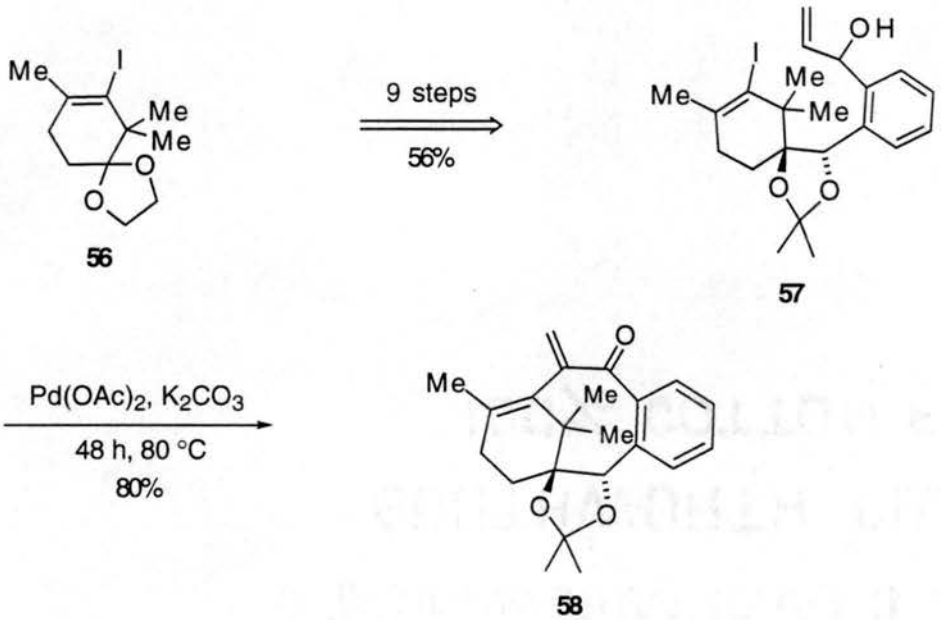
Scheme 2.4 Kishi's Approach to Taxol's Diterpene Core Structure.



In 1993 a series of synthetic papers were published by various authors in which they attempted to synthesize the taxane core structure. Scheme 2.4 outlines the work Kishi and co-workers performed in this endeavor.[31] Compound **54** was synthesized in 12% yield over 12 steps from precursors **52** and **53**. Nickel/chromium metal-mediated ring closure gave **55** as a 4:1 mixture of epimers at C-10. The major diastereomer has the correct relative stereochemistry and functionality at C-1, C-2, C-3, C-8, and C-10.

Danishefsky and co-workers also published a paper during this time related to the synthesis of the taxane framework.[32] His approach relied on using an intramolecular Heck reaction to close the eight-membered ring, Scheme 2.5. Iodoalkene **56** was converted to **57** in 9 steps in 56% yield. Compound **57** then underwent the Heck reaction to give the taxane analogue **58** in 80% yield. Compound **58** has the same relative stereochemistry and appropriate functionality at C-1, C-2, and C-9 as taxol.

Scheme 2.5 Danishefsky's Approach to Taxol's Diterpene Core Structure.

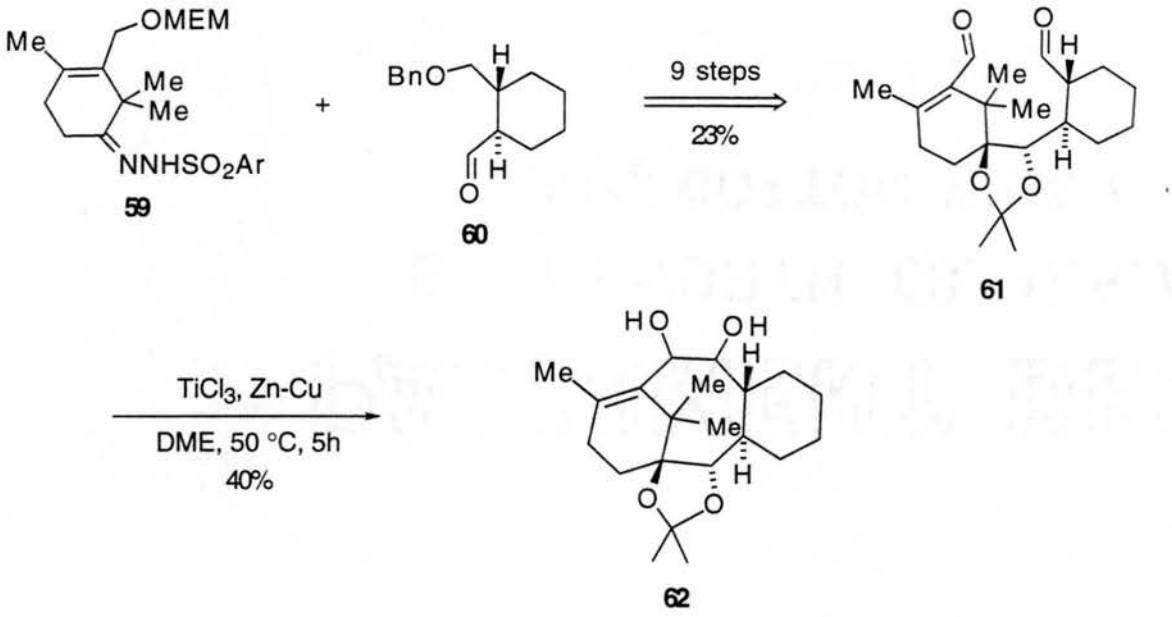


56 **57** **58**

Scheme 2.6 outlines Nicolaou's approach to the taxane ring system.[33] The Nicolaou route relied, as did the Kende route in 1986, on closing the eight membered ring

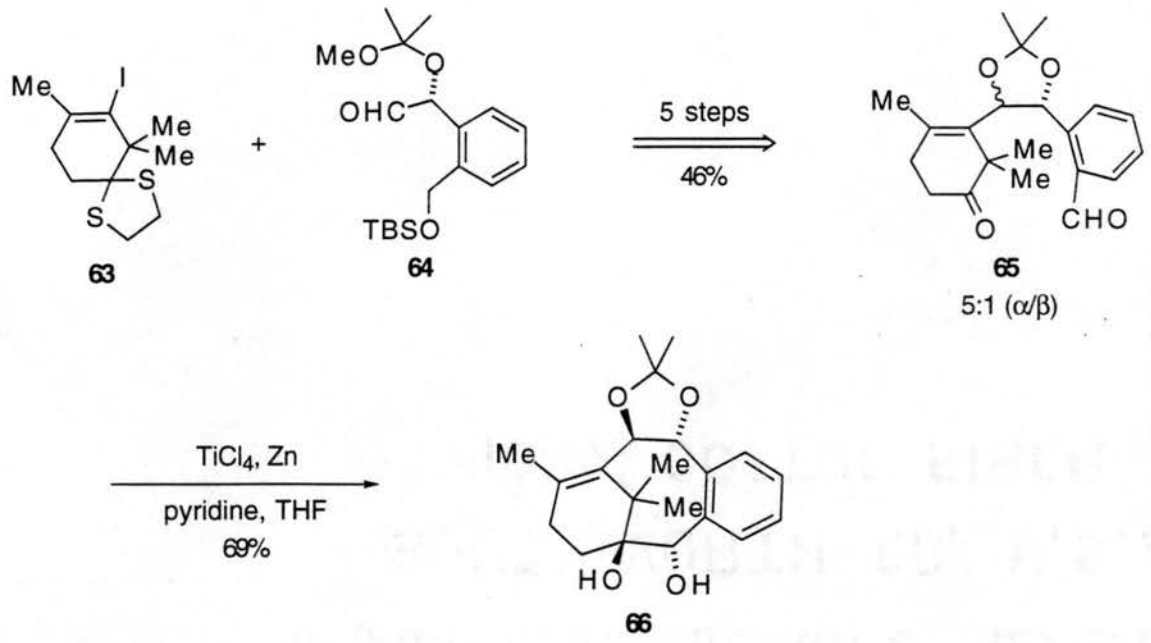
using the McMurry coupling protocol. Starting with precursors **59** and **60**, Nicolaou and co-workers were able to synthesize compound **61** in 23 % over 9 steps. The McMurry coupling reaction was then used to synthesize compound **62** in 40% yield from **61**. Nicolaou's product has the same relative stereochemistry at C-1, C-2, C-3, and C-8 as taxol in addition to having the appropriate functionality at C-1, C-2, C-3, C-9, and C-10.

Scheme 2.6 Nicolaou's Approach to Taxol's Diterpene Core Structure.



Swindell used a similar strategy in constructing the taxane core framework.[34] Iodoalkene **63** and aldehyde **64** were coupled followed by conversion to **65** in 46% yield over 5 steps as a 5:1 (α/β) mixture of diastereomers with respect to C-10. The minor β–diastereomer was subjected to Mukaiyama pinacol coupling conditions to yield compound **66** in 69% yield. The taxoid framework synthesized by Swindell and co-workers has the same relative stereochemistry at C-1, C-2, and C-10; in addition to having the appropriate functionality at C-1, C-2, C-9, and C-10 as taxol.

Scheme 2.7 Swindell's Approach to Taxol's Diterpene Core Structure.

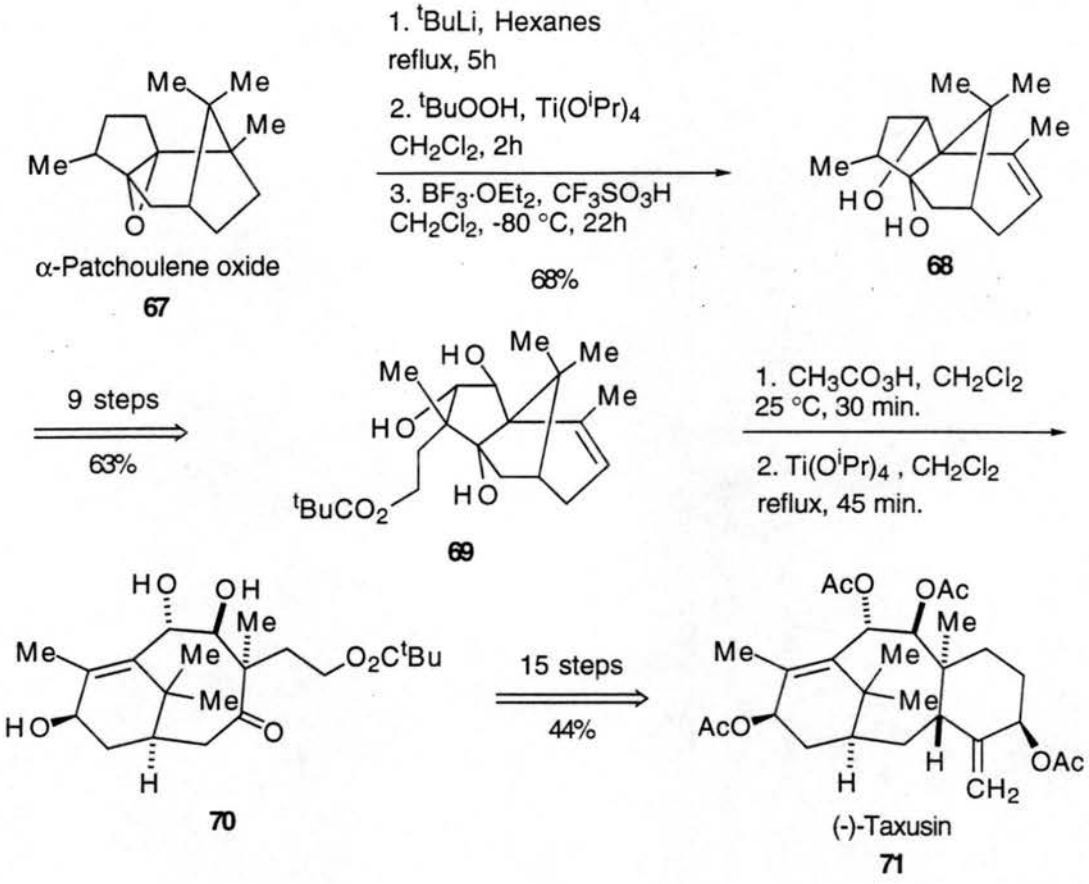


2.2b Ring Expansions and Contractions

In 1984 Holton described his strategy for synthesizing molecules that have the taxane diterpene core structure.[35] His methodology employs the Lewis acid catalyzed rearrangement of enantiomerically pure patchoulene oxide, available in bulk from International Flavors and Fragrances, followed by epoxidation and ring expansion. Scheme 2.8 outlines the first total synthesis of a naturally occurring taxane from commercially available starting materials using the methodology just described.[36]

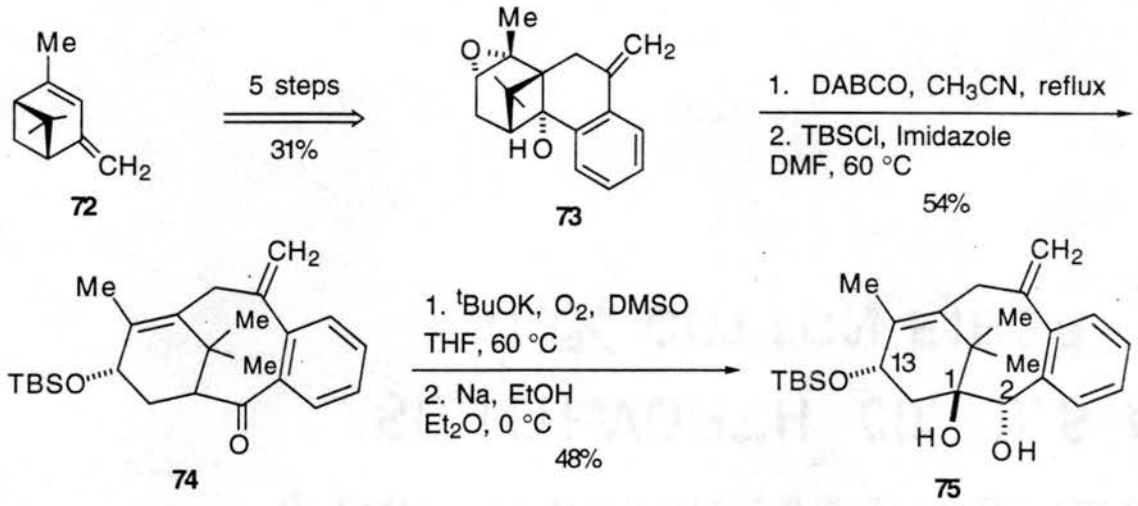
Epoxide ring opening followed by epoxidation and ring rearrangement on enantiomerically pure α-patchoulene oxide **67** furnished **68** in 68% yield. Compound **68** was converted to **69** in 63% yield over 9 steps. Compound **69** was then epoxidized followed by a ring expansion/rearrangement under Lewis acidic conditions to produce the AB ring system of taxusin **70**. Compound **70** was then converted to (-)-taxusin **71** in 44% over 15 steps.

Scheme 2.8 The Total Synthesis of (-)-Taxusin.



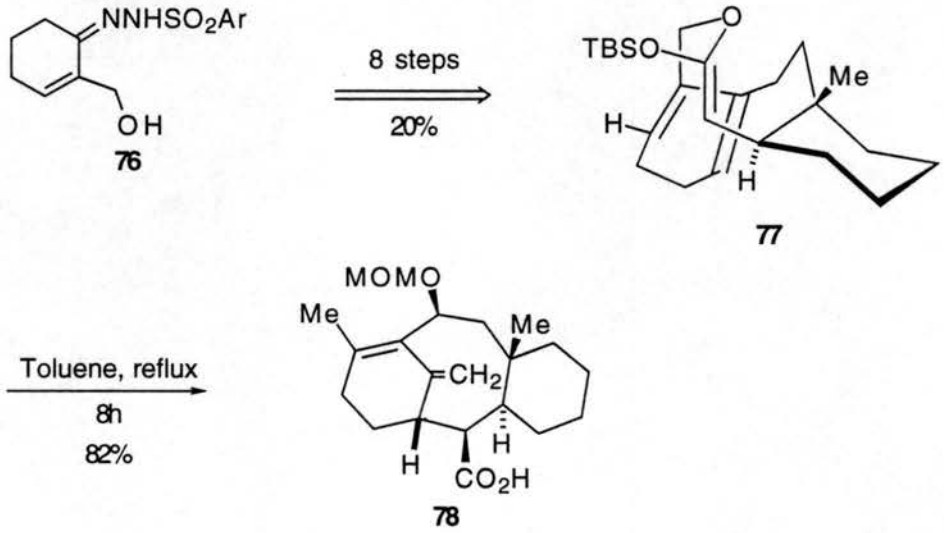
In 1992 Wender and Mucciaro published a similar ring expansion/rearrangement strategy.[37] Scheme 2.9 outlines their strategy towards synthesizing taxoid type molecules using enantiomerically pure pinene as their starting substrate. Pinene **72** was converted to the tricyclic system **73** in 31% yield over 5 steps. Compound **73** then underwent a ring expansion immediately when treated with DABCO under refluxing conditions followed by alcohol protection to give taxane skeletal system **74** in 54% overall yield from **73**. Compound **74** was then stereoselectively oxidized at C-1 followed by a stereoselective reduction at C-2 to yield **75** in 48% yield for the two steps. Taxoid **75** has the same relative stereochemistry and appropriate functionality at C-1, C-2, and C-13 as taxol.

Scheme 2.9 Wender's Approach to Taxol's Diterpene Core Structure.



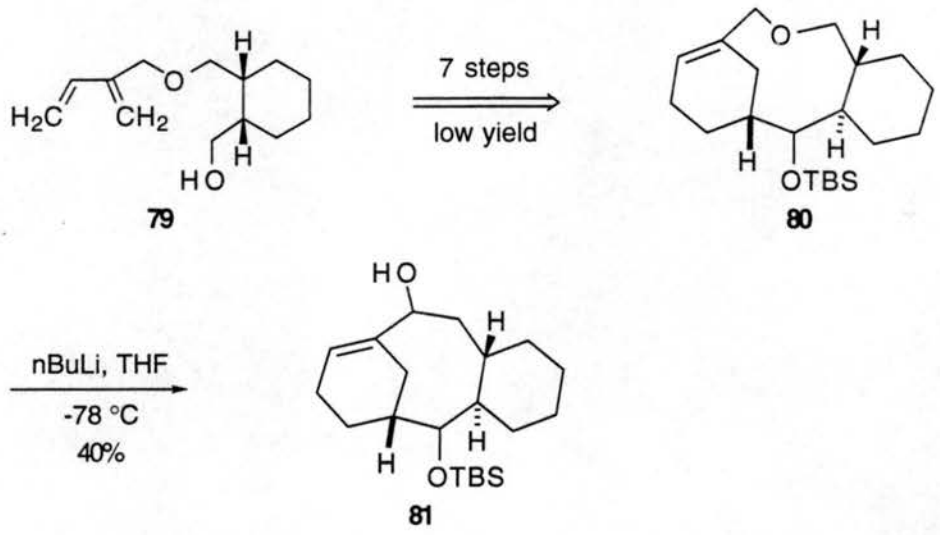
Scheme 2.10 outlines the Claisen-rearrangement based methodology developed by Funk and co-workers to access the taxane skeleton.[38] Ketene acetal **77** was synthesized in 20% yield over 8 steps starting from hydrazone **76**. Compound **77** then underwent the Claisen rearrangement when heated at reflux in toluene for 8h to form the taxoid type compound **78** in 82% yield.

Scheme 2.10 Funk's Approach to Taxol's Diterpene Core Structure.



Another strategy which relies on a rearrangement for the synthesis of a taxoid type compound is outlined in scheme 2.11. Yadov and co-workers synthesized ether **80** in low yield over 7 steps from compound **79**.[39] Ether **80** was then subjected to Wittig rearrangement conditions, which produced compound **81** in 40% yield.

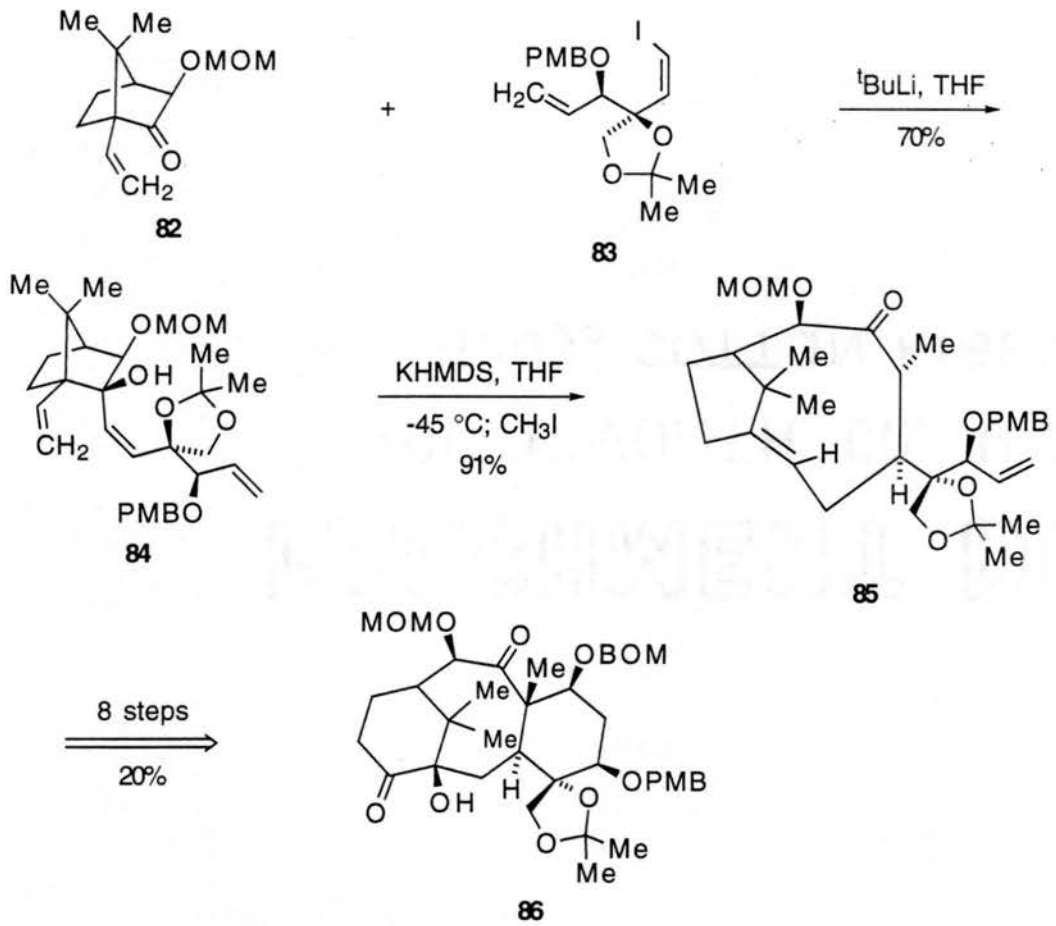
Scheme 2.11 Yadov's Approach to Taxol's Diterpene Core Structure.



An anionic oxy-Cope rearrangement-based strategy towards the taxane skeletal system has been employed by several research groups. This methodology was originally developed by Martin and co-workers[40] and expanded upon by Paquette's research group.[41] Scheme 2.12 outlines the recent advances that Paquette and co-workers have made in attempting to synthesize taxol using the anionic oxy-Cope rearrangement as their key reaction. Camphor derivative **82** was coupled with the lithium anion of iodoalkene **83** to afford **84** in 70% yield. Compound **84** then underwent an anionic oxy-Cope rearrangement to yield **85** in 91% yield. The bicyclic system was converted in 8 steps to taxane **86** in 20 % yield. The taxane molecule **86** has the same relative stereochemistry at

C-1, C-3, C-4, C-7, C-8, and C-10 as taxol. In addition this compound has the appropriate functionality at C-1, C-4, C-5, C-7, C-9, and C-10 for conversion into taxol.

Scheme 2.12 Paquette's Approach to Taxol's Diterpene Core Structure.

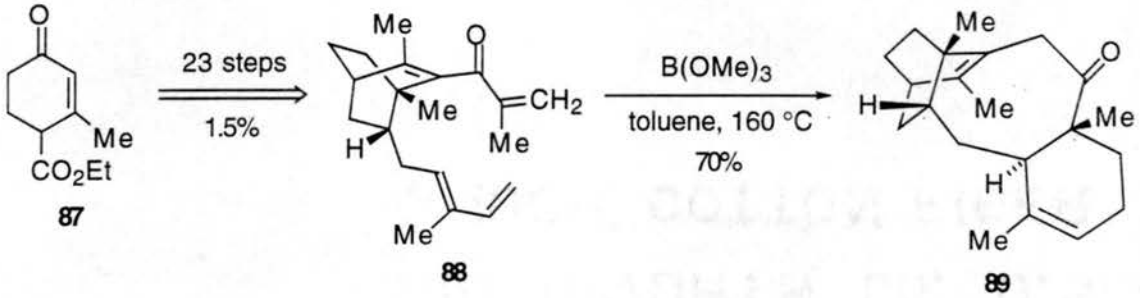


2.2c Cycloadditions

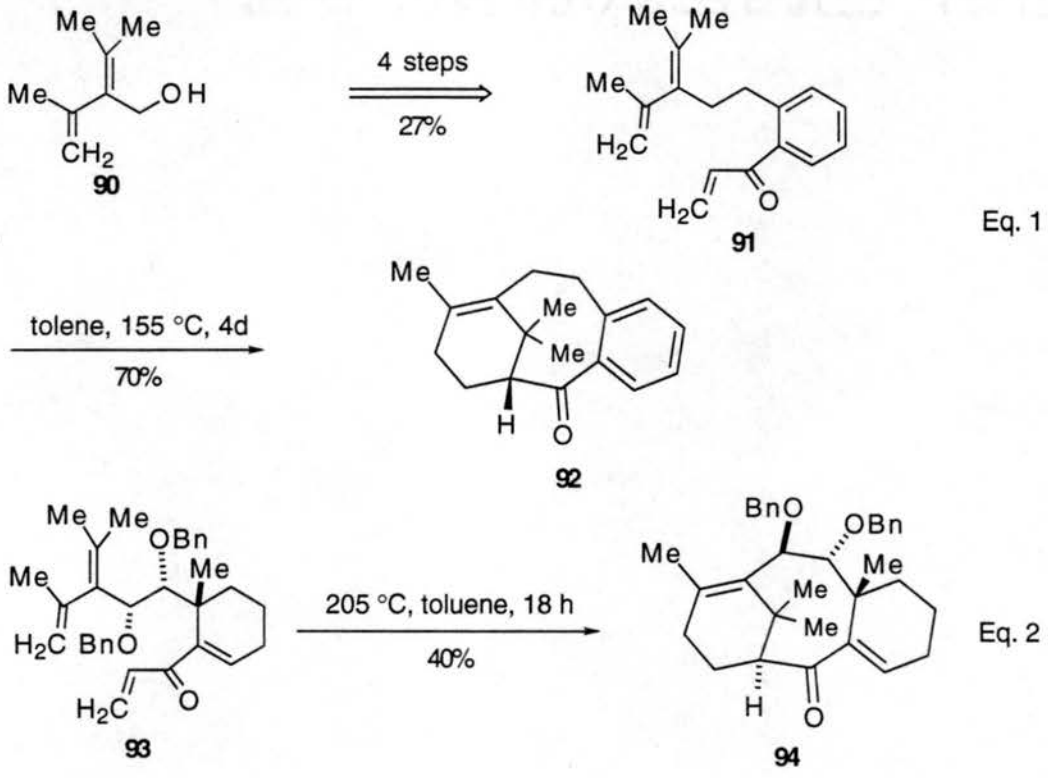
In 1983 two research groups published papers showing how an intramolecular Diels-Alder reaction based methodology could be used to access the taxane class of compounds. Scheme 2.13 outlines Sakan and Craven's results[42] while scheme 2.14 outlines Shea's results.[43] As outlined in scheme 2.13, enone **87** was converted to Diels-Alder precursor **88** in 1.5% yield over 23 steps. Compound **88** then under went a thermal intramolecular Diels-Alder reaction to produce adduct **89** in 70% yield. Compound **89**

has the same relative stereochemistry at C-1, C-3, and C-8 in addition to having the same functionality at C-9 as taxol.

Scheme 2.13 Sakan's Approach to Taxol's Diterpene Core Structure.



Scheme 2.14 Shea's Approach to Taxol's Diterpene Core Structure.

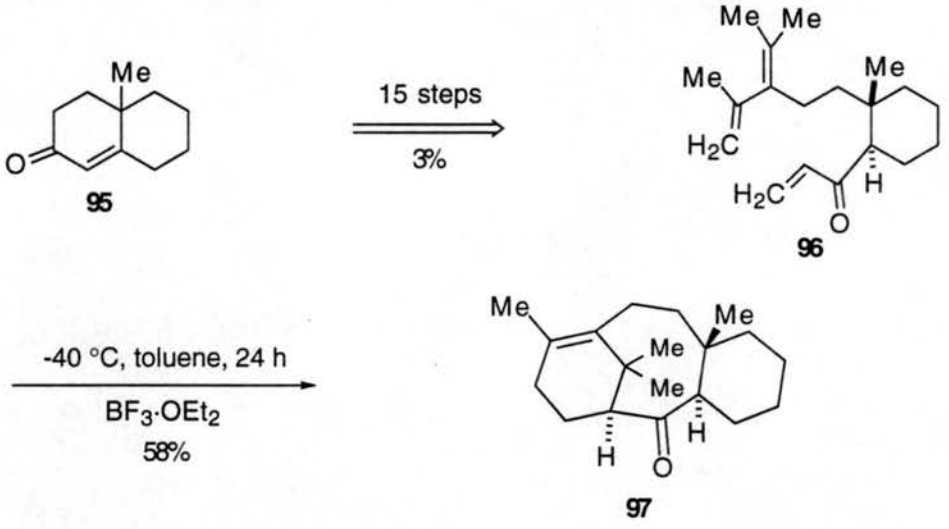


Shea and Davis were also able to access the taxane skeleton using an intramolecular Diels-Alder approach, as shown in equation 1, Scheme 2.14.[43a] Diels-Alder precursor **91** was synthesized in 27% yield over 4 steps from dienol **90**. Compound **91** then underwent

a thermal Diels-Alder reaction to produce taxane skeleton **92** in 70% yield. Equation 2, Scheme 2.14, outlines Shea's most recent attempt to synthesize a more functionalized taxane skeleton. Diels-Alder adduct **94** was synthesized under thermal conditions in 40% yield from **93**. Compound **94** has the appropriate functionality at C-2, C-9, and C-10. This product also has the same relative stereochemistry at C-8 and C-10 as taxol while having the incorrect relative stereochemistry at C-1.

A similar Diels-Alder strategy has also been used by Jenkins and co-workers to synthesize the taxane skeleton with the same relative stereochemistry at C-1, C-3, and C-8 as taxol (scheme 2.15).[44] Diels-Alder precursor **96** was synthesized in 3 % yield over 15 steps from enone **95**. Compound **96** then under went a Lewis acid catalyzed intramolecular Diels-Alder reaction to yield taxoid **97** in 58%.

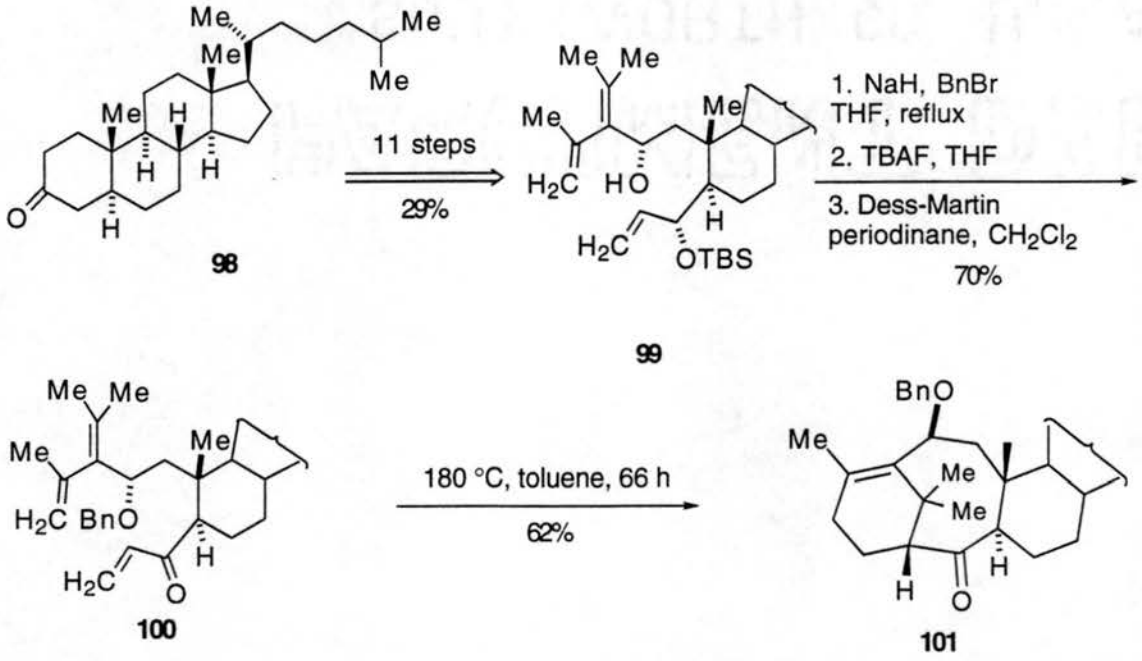
Scheme 2.15 Jenkin's Approach to Taxol's Diterpene Core Structure.



Recently, Danishefsky and co-workers described the degradation of steroidal compound **98** in 11 steps to yield **99** as a 5:2 (α/β) mixtures of diastereomers with respect to C-10 (taxane numbering) in 29% yield, Scheme 2.16.[45] The minor β diastereomer was converted to Diels-Alder precursor **100** in 70% yield by the 3 step sequence outlined in scheme 2.16. This compound then underwent a thermal intramolecular Diels-Alder

reaction at 180 °C for 66h to give taxoid hybrid **101** in 62% yield. The C-1 stereochemistry of **101** differs from Shea's taxoid **94** in that it has the same relative stereochemistry as taxol as well as at C-3, C-8, and C-10. Compound **101** was synthesized in an attempt to probe the structure activity Relationship of taxol. However, the experimental details concerning the biological activity have not been published.

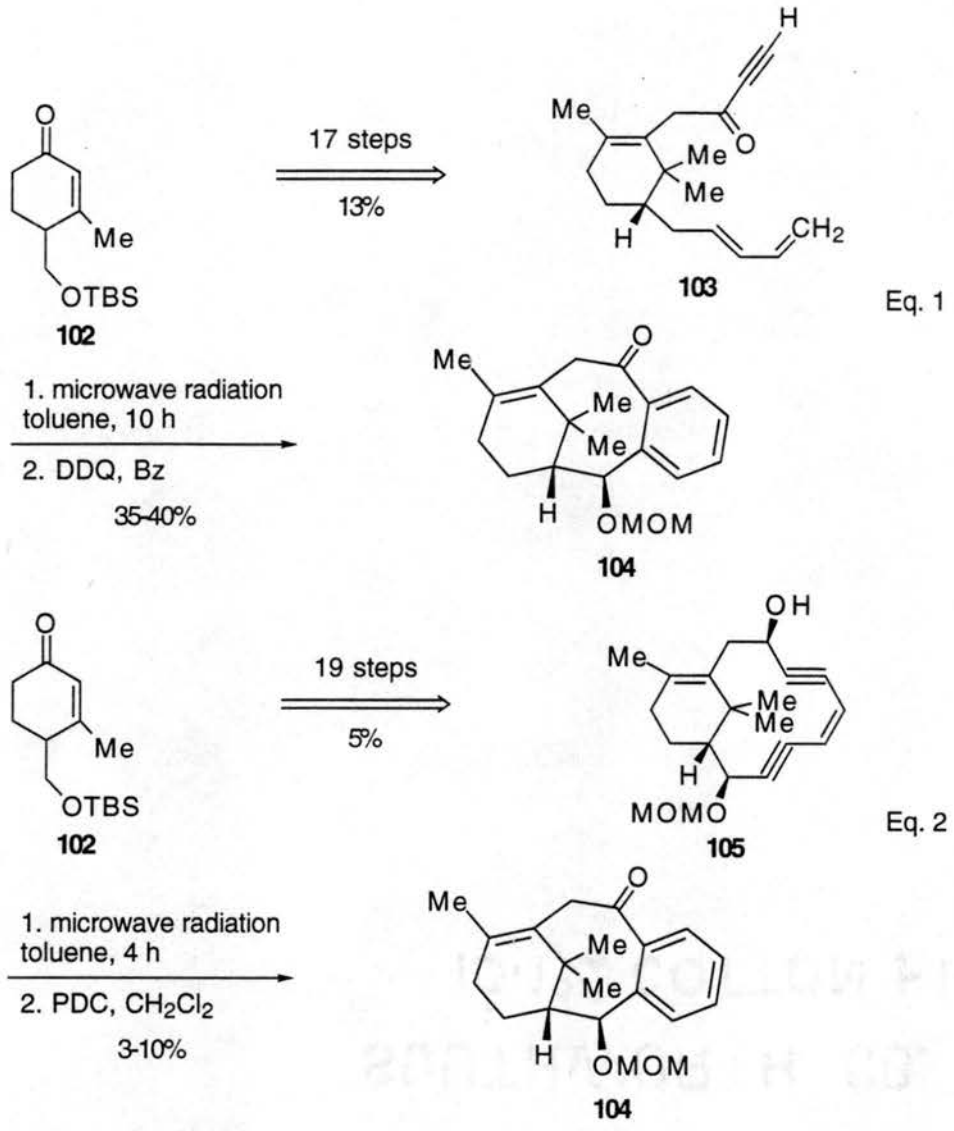
Scheme 2.16 Danishefsky's Approach to Taxol's Diterpene Core Structure.



Scheme 2.17 outlines the two strategies Fallis and co-workers used to access the taxane ring system;[46] equation 1 outlines their intramolecular Diels-Alder methodology. [46a] Starting with enone **102**, they were able to synthesize diene **103** in 13% yield over 17 steps. A thermally induced (via microwave radiation) Diels-Alder reaction followed by DDQ oxidation gave taxane **104** in 30-40% yield. Compound **104** has the same relative stereochemistry at C-1 in addition to having the appropriate functionality at C-2 and C-9 as taxol. Fallis's other strategy also relied on starting substrate **102**, Equation 2.[46b] This time **102** was converted to **105** in 5% yield over 19 steps. Enediyne **105** was subjected to

microwave radiation and presumably underwent a Bergman cyclization followed by PDC oxidation to give what appeared to be **104** in 3-10% yield.

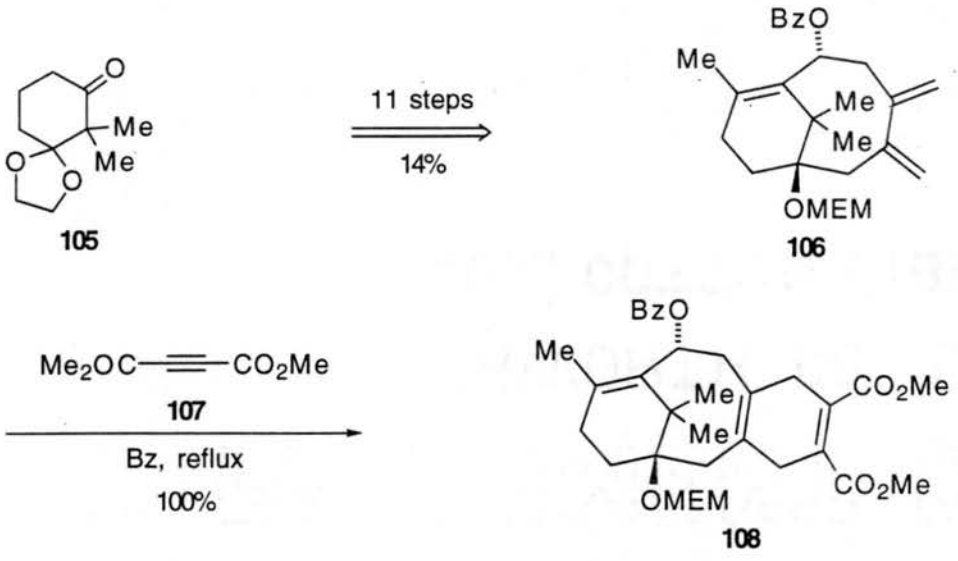
Scheme 2.17 Fallis's Approach to Taxol's Diterpene Core Structure.



Wang's methodology for accessing the ABC ring of the taxane class of compounds is outlined in scheme 2.18.[47] Diene **106** was synthesized in 14 % yield over 11 steps from ketone **105**. Dimethyl acetylenedicarboxylate **107**, when heated with **106**, afforded

tricyclic compound **108** in quantitative yield. Compound **108** has the same relative stereochemistry and appropriate functionality at C-1 and C-10 as taxol.

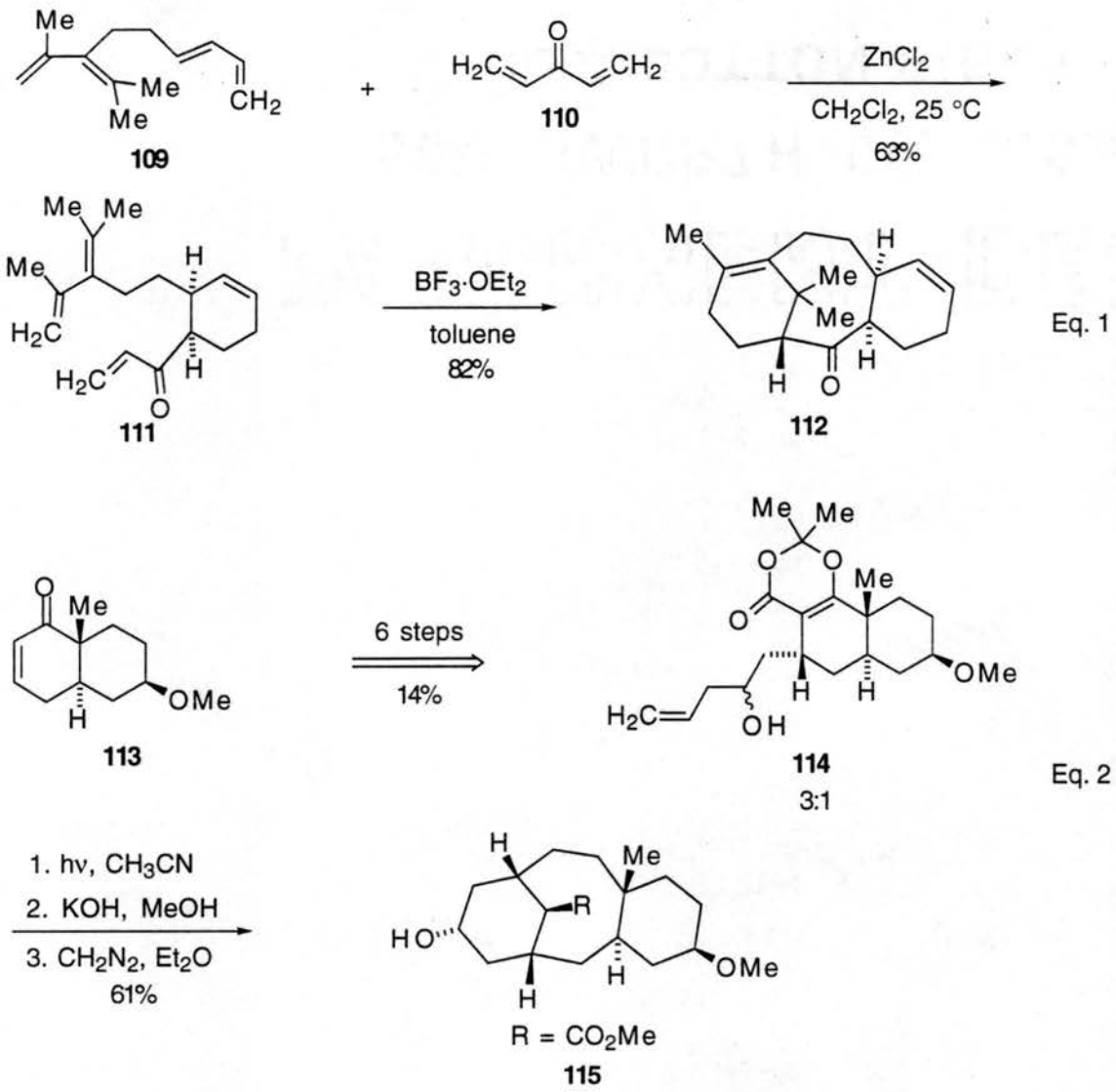
Scheme 2.18 Wang's Approach to Taxol's Diterpene Core Structure.



Winkler and co-workers published two strategies for accessing the taxane skeletal system, Scheme 2.19.[48] Equation 1 outlines a tandem intramolecular Diels-Alder based strategy for accessing the ABC ring system.[48a] Tetraene **109**, obtained in 4 steps from readily available starting materials, under went a chemoselective Lewis acid catalyzed intermolecular Diels-Alder reaction with ketone **110** to yield **111** in 63 %. Compound **111** then underwent a second Lewis acid catalyzed intramolecular Diels-Alder reaction to produce **112** in 82% as a single diastereomer. Taxane **112** has the same relative stereochemistry at C-1 and C-3 in addition to having the appropriate functionality at C-2 as taxol. It's worth mentioning that neither Lewis acid used could effect the tandem Diels-Alder reactions in sequence. Winkler's second approach relied on an intramolecular [2+2] photocycloaddition reaction.[48b] His most recent work in this area is outlined in equation 2. Enone **113** was converted to **114** as a 3:1 mixture of diastereomers at C-13 (taxane numbering) in 14 % yield over 6 steps. The major diastereomer then under went a photochemical [2+2] cycloaddition followed by ring opening and esterification to produce

taxoid **115** in 82% over the 3 steps. Compound **115** has the appropriate functionality at C-13 and C-5 in addition to having the same relative stereochemistry at C-1, C-3, C-8, and C-13.

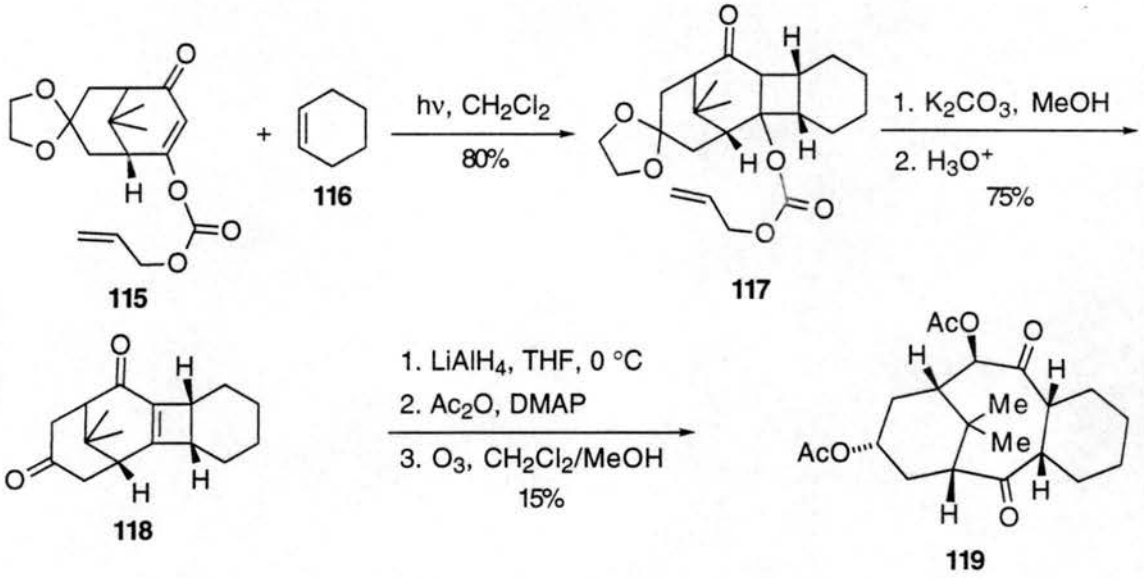
Scheme 2.19 Winkler's Approach to Taxol's Diterpene Core Structure.



Scheme 2.20 outlines Blechert's methodology towards accessing the diterpine core of taxol.[48] Compound **117** was synthesized in 80% yield via a [2+2] photocycloaddition from enone **115** and cyclohexene **116**. Elimination followed by deprotection afforded

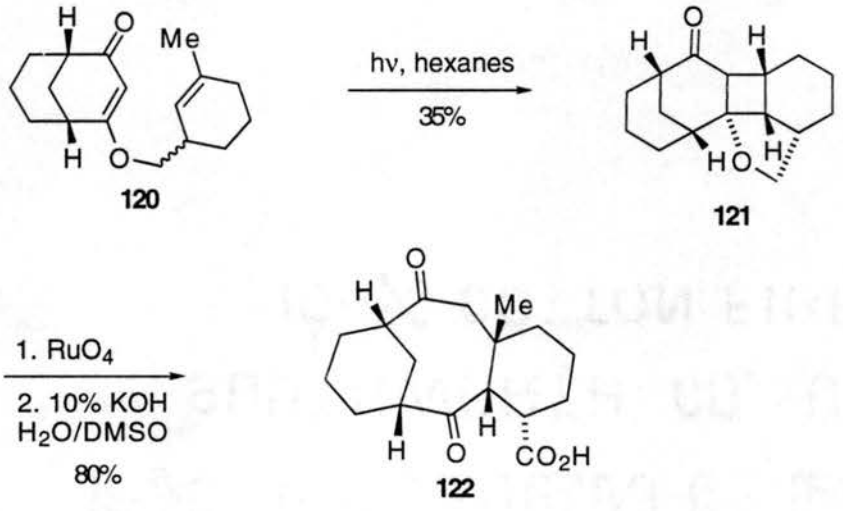
compound **118** in 75% yield. Taxoid **119** was synthesized in 15% yield over the 3 steps outlined in scheme 2.20. Compound **119** has the same relative stereochemistry at C-1, C-8, C-10, and C-13 in addition to having the appropriate functionality at C-2, C-9, C-10, and C-13 as taxol but has the incorrect stereochemistry at C-3.

Scheme 2.20 Blechert's Approach to Taxol's Diterpene Core Structure.



115 **116** **117** **118** **119**

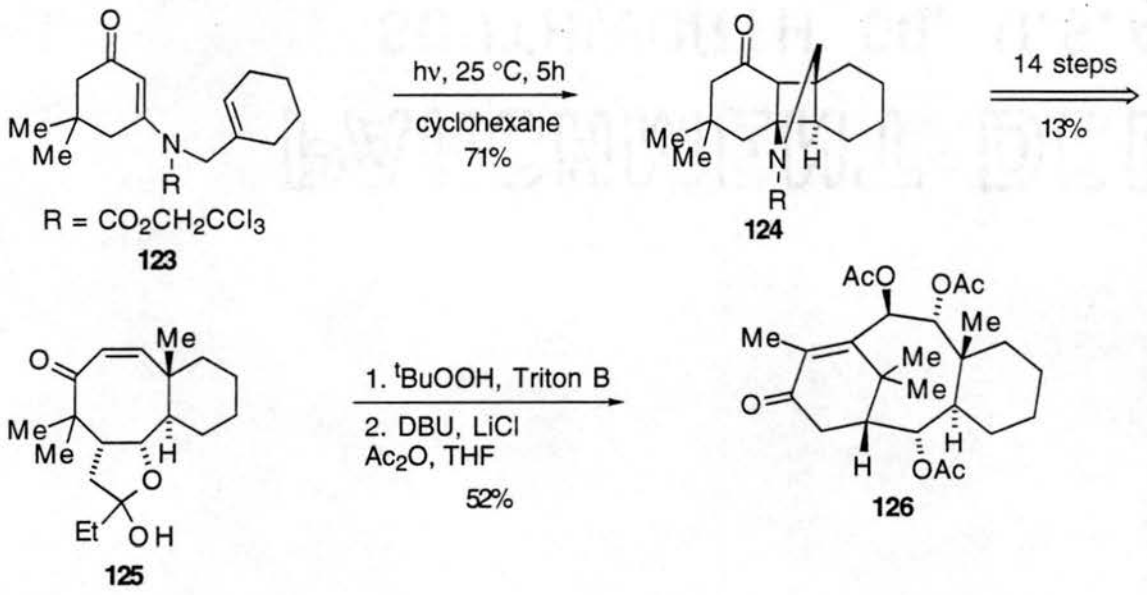
Scheme 2.21 Inouye's Approach to Taxol's Diterpene Core Structure.



120 **121** **122**

Inouye reported a similar approach to access the ABC taxane ring system, Scheme 2.21.[50] Enone **120** underwent an intramolecular [2+2] photocycloaddition to afford **121** in 35% yield. Oxidation followed by lactone ring opening produced **122** in 80% yield over 2 steps from **121**. Taxane **122** has the appropriate functionality at C-2 and C-10.

Scheme 2.22 Swindell's Approach to Taxol's Diterpene Core Structure.



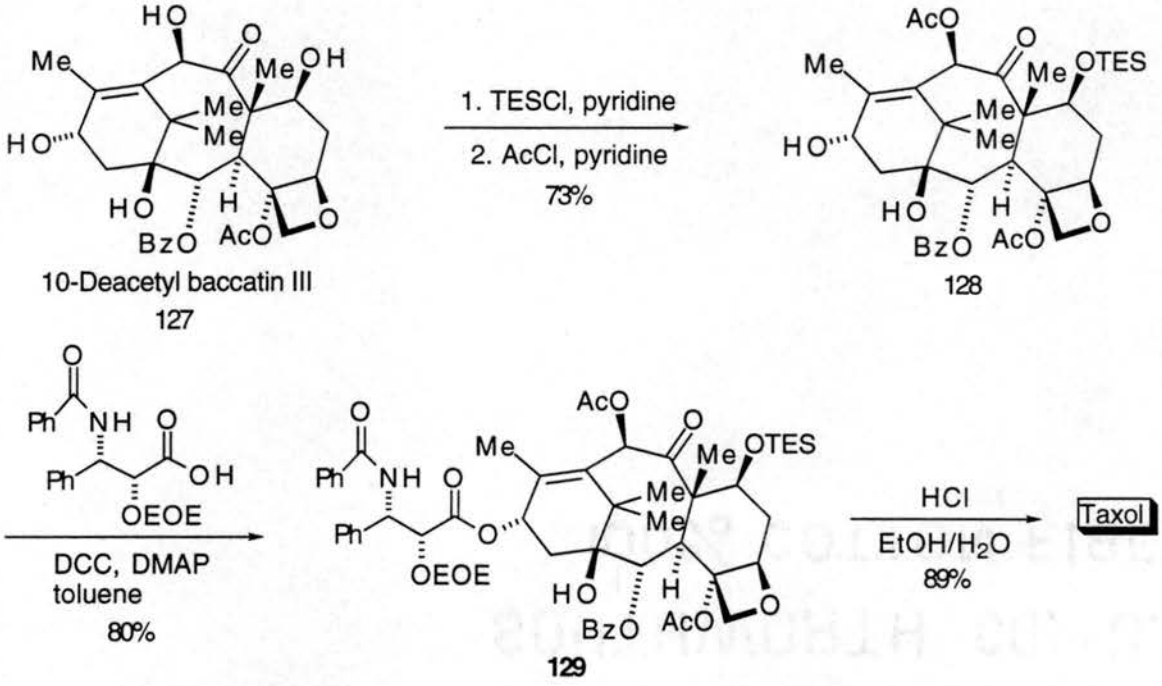
Another intramolecular [2+2] photocycloaddition based methodology was reported by Swindell and co-workers, Scheme 2.22.[51] The [2+2] photocycloaddition of **123** yielded **124** in 71%. Enone **125** was synthesized in 13% yield over 14 steps from **124**. Compound **125** was then epoxidized followed by an Aldol cyclization and Payne rearrangement to afford **126** in 52% yield. Taxane **126** has the same relative stereochemistry at C-1, C-2, C-3, C-8, and C-10 in addition to having the appropriate functionality at C-2, C-3, C-8, C-9, C-10, and C-13 as taxol.

2.3 Total Syntheses of Taxol

2.3a The Semi-synthesis of Taxol

To date, only four successful approaches to taxol have been published.[24-27] The first approach to taxol was published by Greene and co-workers in 1988.[24] The success of their synthesis relied upon obtaining 10-deacetyl baccatin III from natural sources, thus making their approach a semi-synthetic one. Scheme 2.23 outlines their strategy.

Scheme 2.23 Greene's Approach to Taxol.



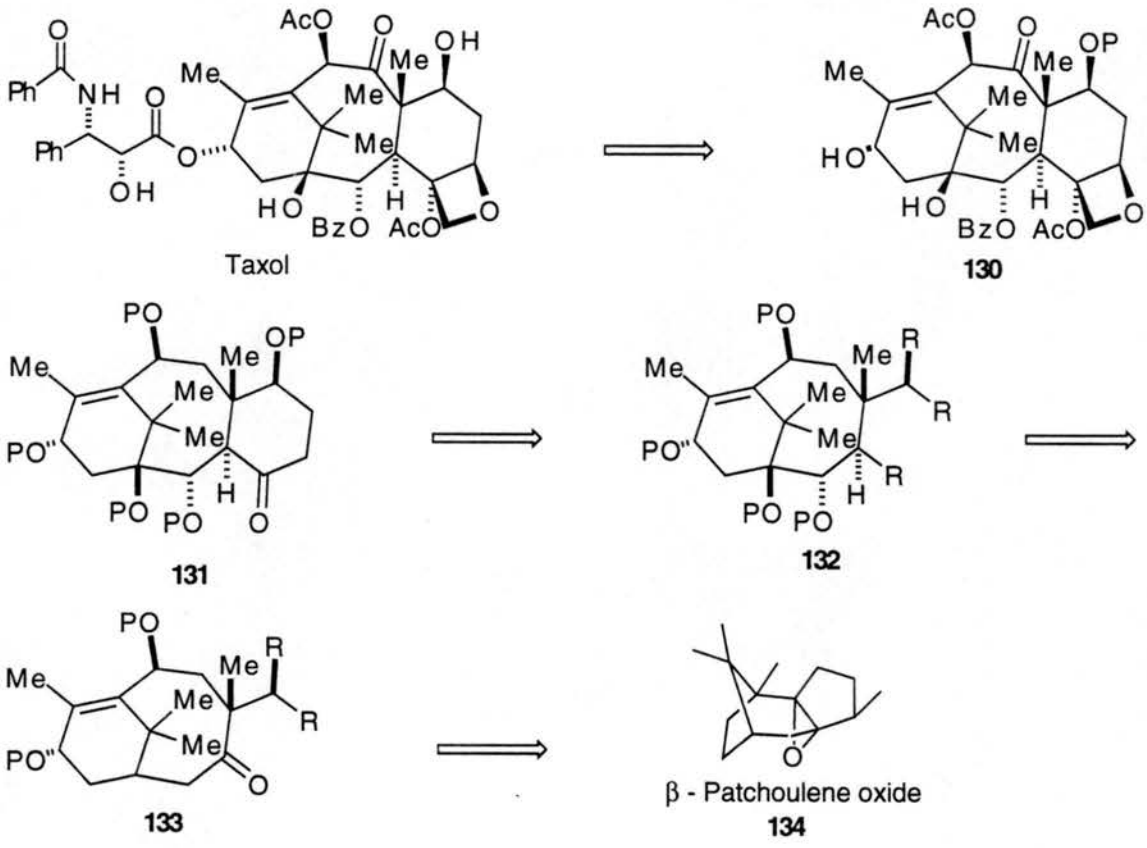
10-Deacteyl baccatin III **127** was selectively silylated at the C-7 position followed by selective acetylation at C-10 to afford the suitably protected baccatin III species **128**. (2R,3S)-N-Benzoyl-O-(1-ethoxyethyl)-3-phenylisoserine was coupled to **128** to yield **129** in 80% yield. Deprotection of **129** under acidic conditions afforded taxol in 89% yield. Due to the limited availability of taxol from natural sources and the fact that 10-deacetyl

baccatin III can be isolated from renewable sources, producing taxol in large quantities by this approach seems to be quite an attractive prospect.

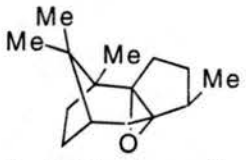
2.3b Holton's Approach to Taxol

Holton and Nicolaou were the first to independently publish the total synthesis of taxol from relatively simple, commercially available starting materials. Scheme 2.24 outlines Holton's retrosynthetic analysis of taxol.[25] Taxol was envisioned to arise from suitably protected baccatin III **130** which in turn could arise from the tricyclic system **131**. Taxoid **131** could potentially come from the AB ring system **132** which in turn could arise from β-patchoulene oxide via **133**. Scheme 2.25 outlines their results in this endeavor.

Scheme 2.24 Holton's Retrosynthetic Analysis of Taxol.



Scheme 2.25 Holton's Total Synthesis of Taxol.

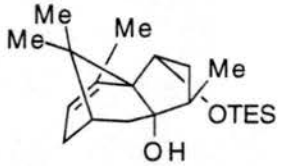


β - Patchouline oxide

134

1. ^{t}BuLi, hexanes, reflux
2. ^{t}BuOOH, Ti(OiPr)$_4$, CH_2Cl_2
3. $BF_3{\cdot}OEt_2$, CF_3SO_3H, CH_2Cl_2, -80 °C
4. TESCl, pyridine

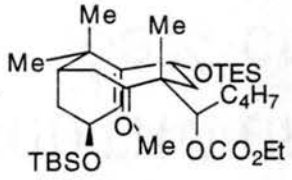
69%



135

1. ^{t}BuOOH, Ti(O^{i}Pr)$_4$, CH_2Cl_2
2. TBSOTf, CH_2Cl_2
3. $MgBrN(iPr)_2$, THF
4. 4-pentenal, THF, -23 °C
5. phosgene, EtOH, CH_2Cl_2

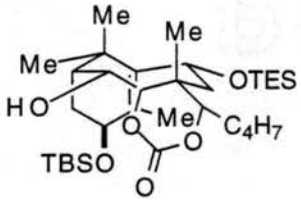
70%



136

1. LDA, THF
(+)-Camphorsulfonyl oxaziridine
2. REDAL, toluene
3. Phosgene, pyridine, CH_2Cl_2

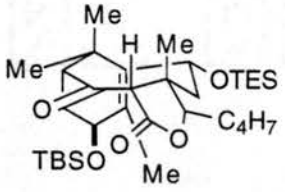
97%



137

1. Swern, CH_2Cl_2
2. LTMP, THF, -25 °C
3. SmI_2, THF; SiO_2

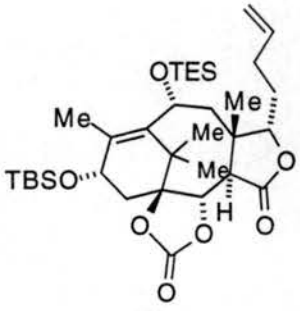
77%



138

1. LTMP, THF
(±)-Camphorsulfonyl oxaziridine
2. REDAL, THF
3. Phosgene, pyridine, CH_2Cl_2

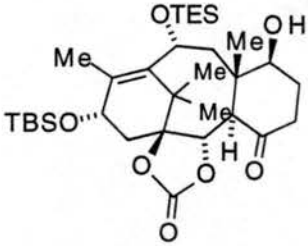
77%



139

1. O_3, CH_2Cl_2
2. $KMnO_4$, KH_2PO_4
Acetone, H_2O
3. CH_2N_2, Et_2O
4. LDA, THF, -78 °C
5. 2-methoxy propene, THF
6. PhSK, DMF, 80 °C

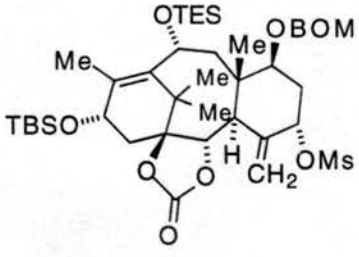
55%



140

1. BOMCl, $EtN(iPr)_2$, CH_2Cl_2
2. LDA, TMSCl, THF, -78 °C
3. MCPBA, hexanes
4. MeMgBr, CH_2Cl_2, -45 °C
5. $MeOCON^{-}SO_2N^{+}Et_3$
toluene, reflux
6. MsCl, pyridine

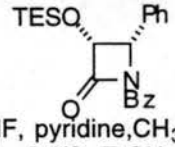
41%



141

1. ^{t}BuOK, THF;
$(PhSe(O))_2O$; ^{t}BuOK, 0 °C
2. Ac_2O, pyridine, DMAP
3. TASF, THF
4. LiHMDS, THF, 0 °C

Taxol

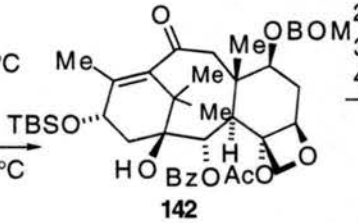


5. HF, pyridine,CH_3CN, 0 °C
6. H_2, Pd/C, EtOH, reflux

57%

1. OsO_4, pyridine/Et_2O, 0 °C
2. DBU, toluene, reflux
3. Ac_2O, pyridine, DMAP
4. HF, pyridine, CH_3CN, 0 °C
5. PhLi, THF, -78 °C
6. TPAP, NMO, CH_2Cl_2

17%



142

β-Patchoulene oxide was converted to **135** using the 4 step sequence shown in Scheme 2.25. Epoxy alcohol fragmentation followed by enolate formation, pentenal addition, and protection of the secondary alcohol as its ethyl carbonate, yielded bicyclic system **136** in 70% yield. Compound **136** was oxidized at its C-2 position (taxane numbering) followed by reduction and carbonate formation to afford **137** in 97%. The tricyclic compound was then oxidized followed by undergoing the Chan rearrangement under basic conditions. The resulting tertiary alcohol was reduced with samarium diiodide followed by treatment with silica gel to yield lactone **138** as a 6:1 mixture of *cis*- and *trans*-fused ring systems. The *cis*-fused ring system is shown in scheme 2.25.

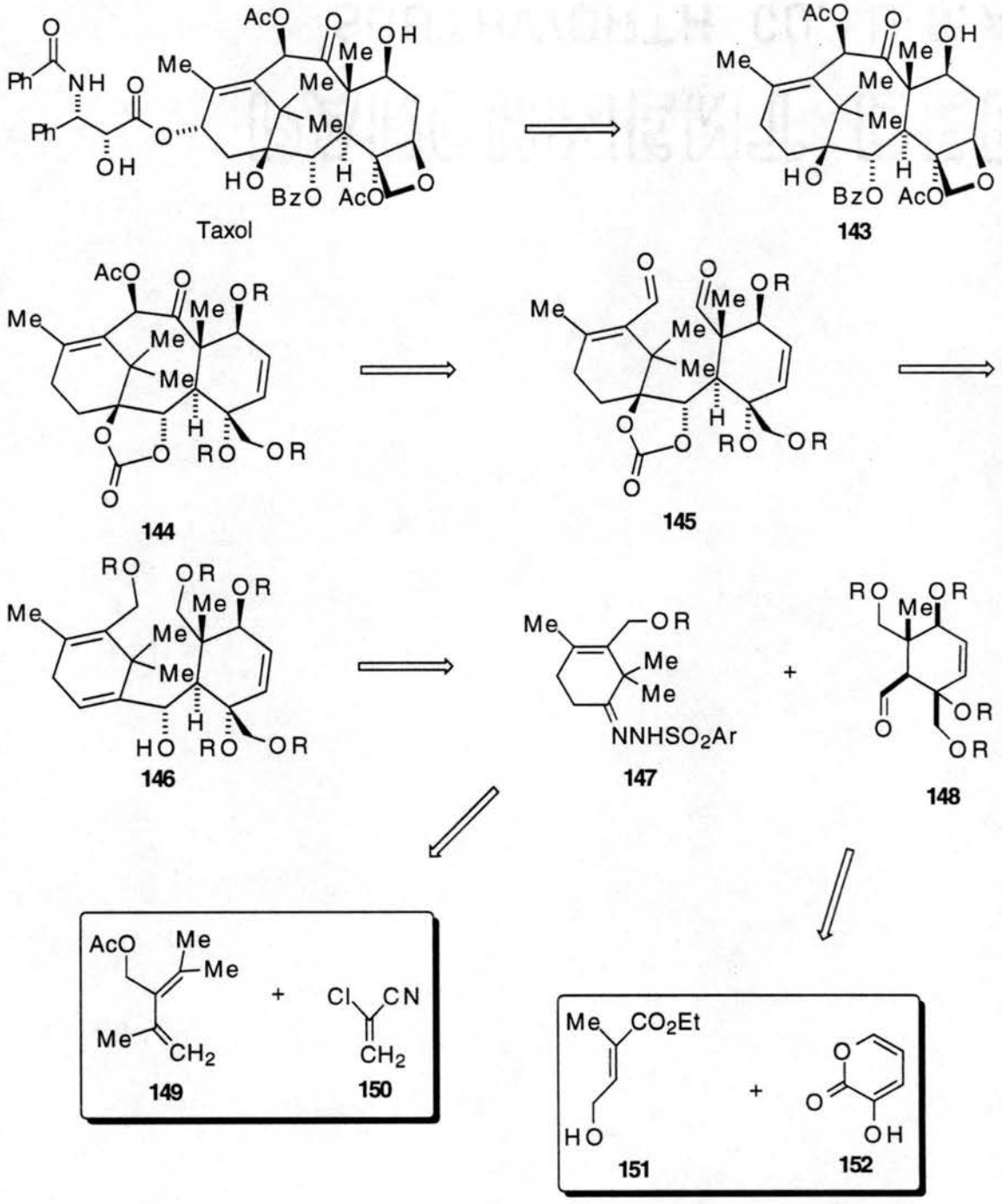
The minor trans ring fused ring system was converted to the cis fused system using potassium butoxide followed by silica gel to produce **138** as a single diastereomer in 77% yield from **137**. Lactone **138** apparently underwent a selective C-1 deprotonation followed by oxidation and reduction to afford the diol which was then protected as its cyclic carbonate **139**. The terminal alkene of lactone **139** was then ozonolyzed, oxidized and converted to its methylester. The methylester then underwent the Dieckmann condensation followed by C-7 alcohol protection and decarboxylation to afford taxoid **140** in 55% yield after acidic work-up.

The absolute stereochemistry and structure of **140** was confirmed by chemical correlation to baccatin III. Compound **140** was then C-7 protected followed by oxidation at C-5 and installation of the C-20 carbon. The allylic alcohol was mesylated to yield **141** in 41% over the 6 steps. The C-4 acetate and oxetane moiety were then installed by dihydroxylating the terminal alkene followed by heating at reflux under basic conditions and treating the tertiary alcohol with acetic anhydride. The triethylsilyl group was selectively cleaved followed by regioselective carbonate ring opening with phenyllithium and oxidation at C-10 to afford **142** in 17% yield from **141**. Compound **142** was then oxidized at C-9 followed by acetylation at C-10 to yield a suitably protected baccatin III

molecule. The C-13 alcohol was deprotected and then coupled to Ojima's lactam[52] followed by deprotection to yield taxol in 5% yield over the last 6 steps.

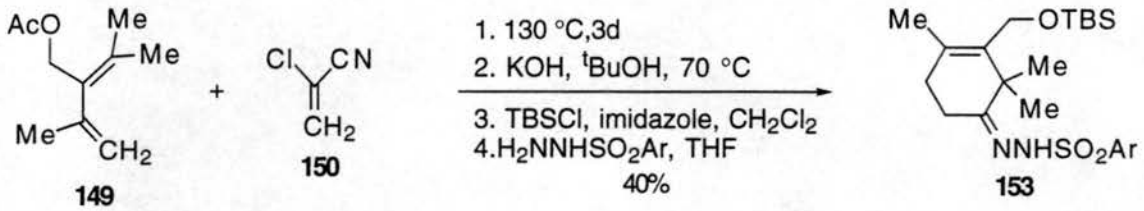
2.3c Nicolaou's Approach to Taxol

Scheme 2.26 Nicolaou's Retrosynthetic Analysis of Taxol.

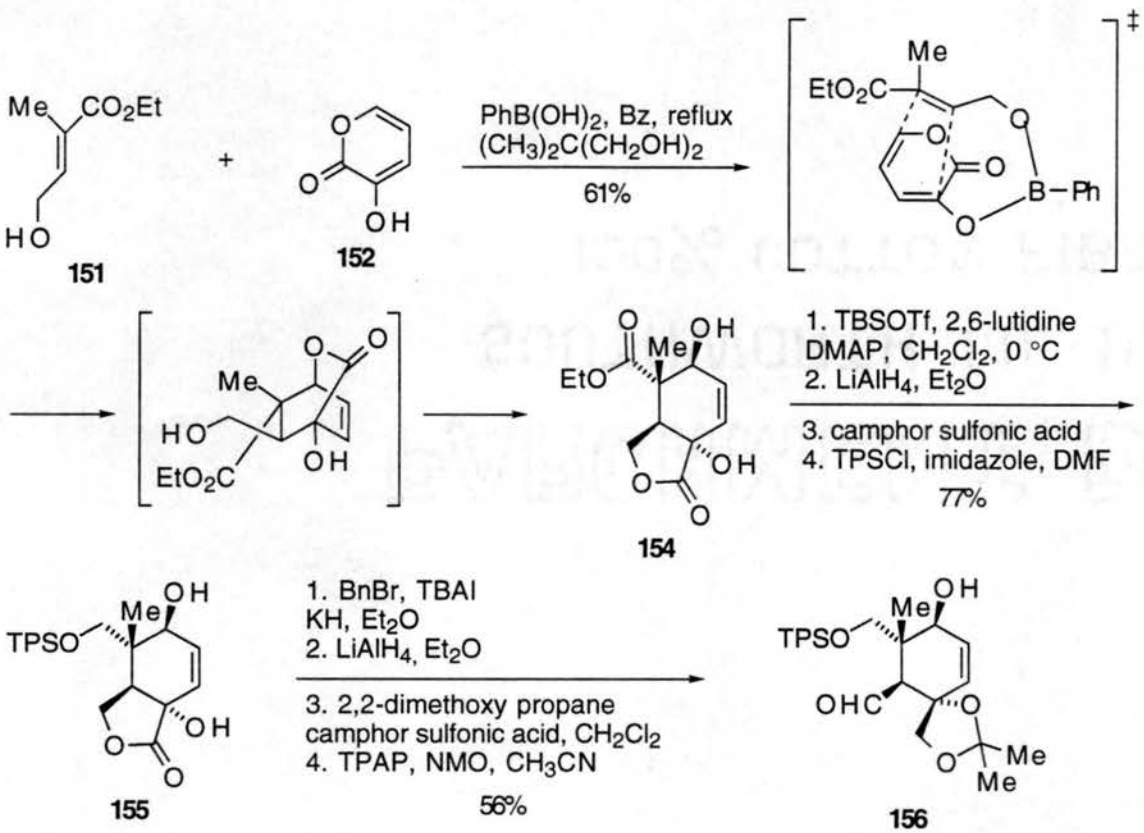


Nicolaou's retrosynthetic analysis of taxol is outlined in Scheme 2.26. As with Holton's retrosynthetic analysis, Nicolaou envisioned baccatin type compound **143** as a penultimate precursor to taxol.[26] Selective oxidation at C-5 of **144** would allow for oxetane installation. Taxane **144** was envisioned to come from dialdehyde **145** via a McMurry coupling. Compound **145** would then come from chemoselectively epoxidizing **146** followed by regioselective epoxide ring-opening and diol protection. Nicolaou then

2.27 Nicolaou's Synthesis of the A Ring Precursor.



2.28 Nicolaou's Synthesis of the C Ring Precursor.



envisioned using A and C ring precursors **147** and **148** respectively in a Shapiro reaction to afford **146**. The A ring precursor was envisioned to arise from diene **149** and dienophile **150**, while the C ring was envisioned to come from diene **151** and dieneophile **152** via the Diels-Alder reaction.

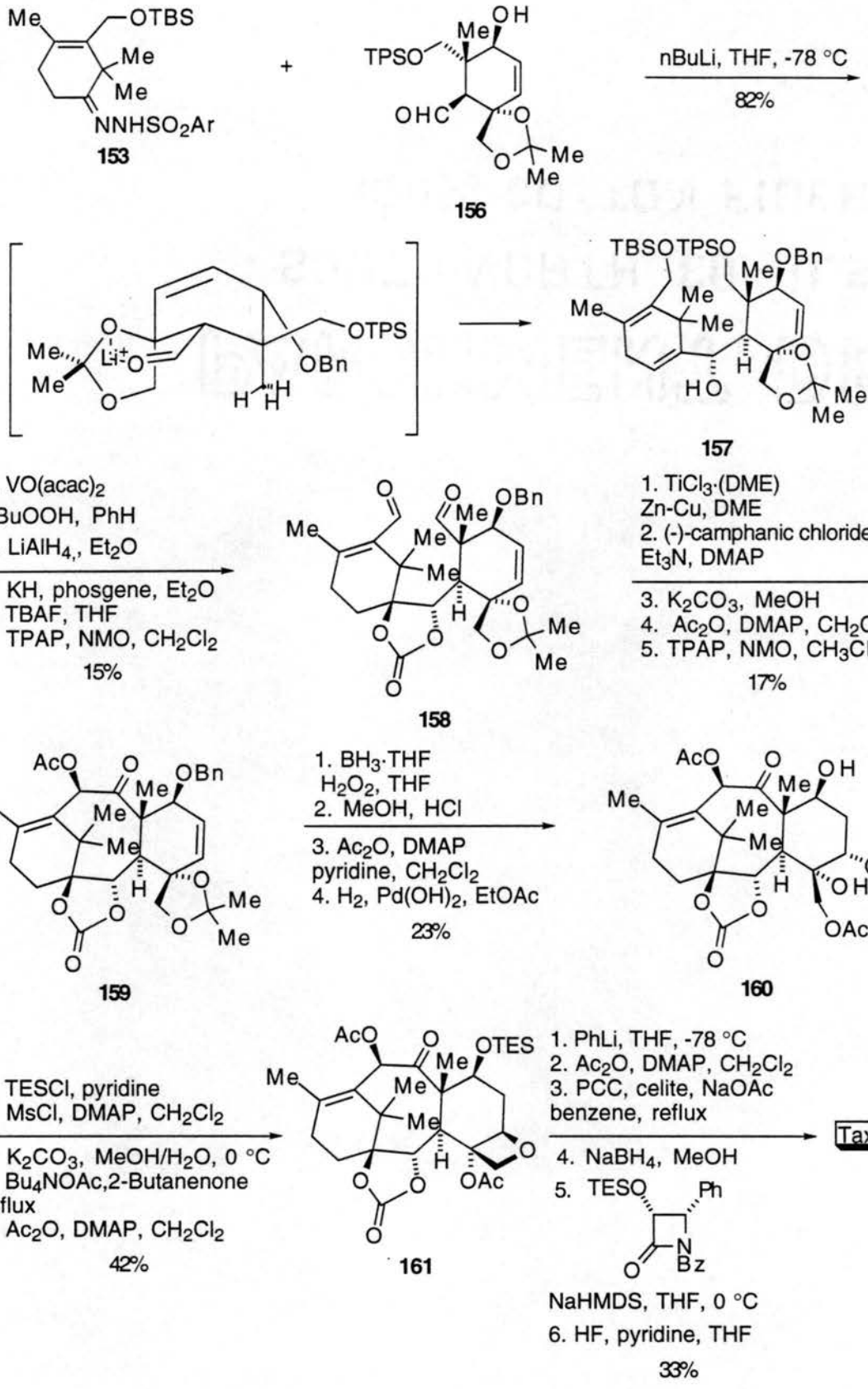
Scheme 2.27 outlines the Nicolaou approach to the A ring precursor. Diene **149** and dienophile **150** under went a thermal [4+2] cycloaddition to give **153** with the correct regiochemistry. The ketone functionality was unmasked under basic conditions followed by alcohol protection and hydrazone formation to yield **153** in 40% over 4 steps.

The B ring precursor was synthesized as outlined in Scheme 2.28. Initial intermolecular Diels-Alder adduct formation between dienophile **151** and diene **152** gave the incorrect regiochemistry. Thus a boron tethered intramolecular Diels-Alder reaction was performed followed by translactonization to yield **154** in 64%. The secondary and tertiary alcohols of compound **154** were protected as their TBS ethers followed by ester reduction and selective deprotection of the secondary alcohol. The primary and secondary alcohols were protected followed by lactone reduction and acetonide formation. The unprotected primary alcohol was then oxidized to afford aldehyde **156** in 56% yield over the 4 steps.

Hydrazone **153** and aldehyde **156** were then coupled using the Shapiro protocol to give **157** as a single diastereomer in 82% yield. The diastereoselectivity is thought to arise from the lithium chelated complex shown in scheme 2.29. This lithium complex exposes the β-face of the aldehyde carbon while the α-face is blocked by the angular C-8 (taxane numbering) methyl group.

Dialdehyde **158** was then synthesized by the 5 step sequence outlined in Scheme 2.29. The Shapiro coupled product **157** was chemoselectively epoxidized followed by a regioselective epoxide opening to yield the C-1 alcohol which was subsequently protected with the C-2 alcohol as its cyclic carbonate. The C-10 and C-9 primary silylated alcohols

2.29 Nicolaou's Total Synthesis of Taxol.

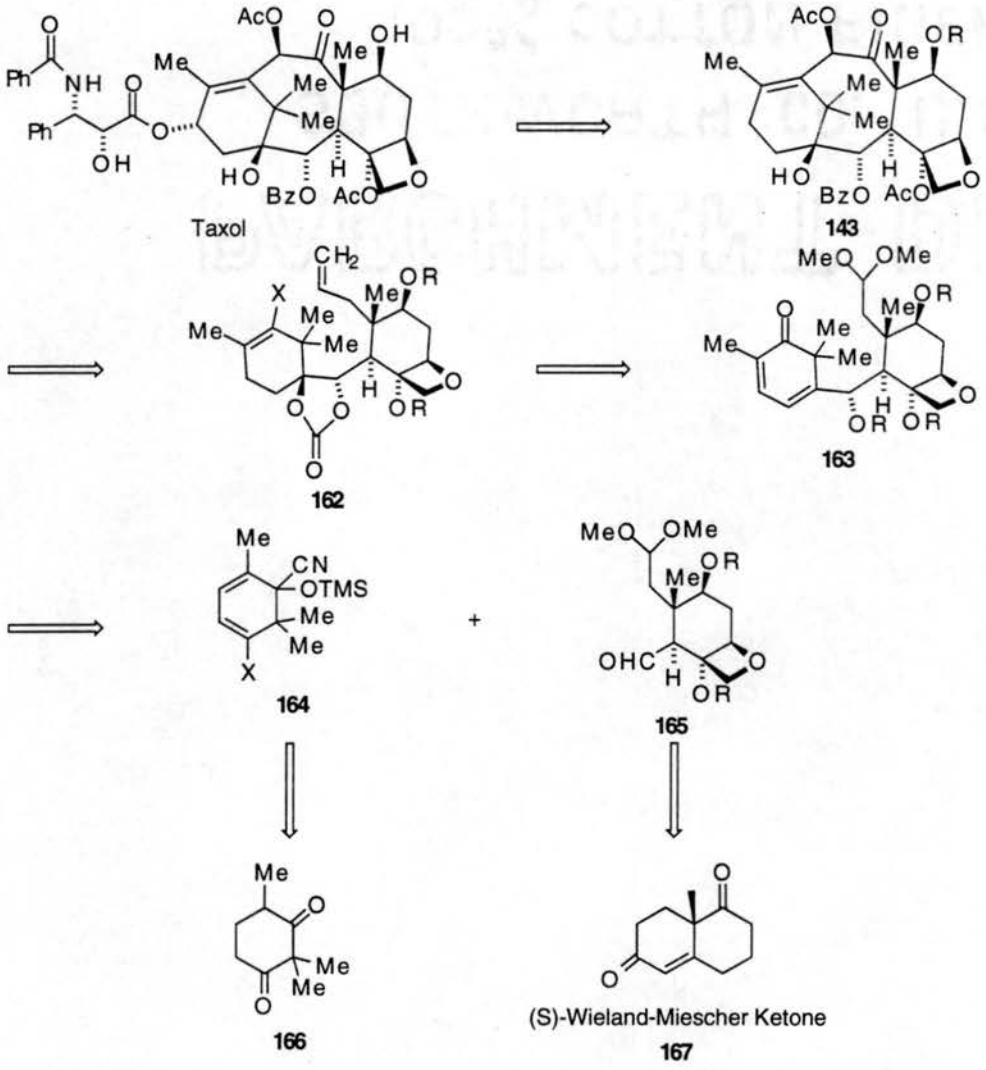


were deprotected followed by oxidation to afford dialdehyde **158** in 15% yield which was subsequently coupled using the McMurry protocol to afford the C-10, C-9 diol as a single diastereomer in 25% yield in addition to three undesired products totaling 65% of the recovered mass. The racemic diol formed from the McMurry coupling was esterified with (-)-camphanic chloride and the 1:1 mixture of diastereomers was chromatographically separated. The unnatural camphanic taxane ester was subjected to crystallographic analysis thus securing the relative and absolute stereochemistry of both diastereomers. The camphanic ester corresponding to the natural taxanes was hydrolyzed followed by selective C-10 acetylation and C-9 oxidation which afforded taxoid **159** enantiomerically pure in 17% yield over 5 steps. The C-5, C-6 alkene of **159** was regioselectively oxidized in a 2:1 ratio with respect to C-5 .

Acetonide deprotection followed by C-20 acetylation and C-7 deprotection yielded **160** (23%). Taxane **160** was reprotected at C-7 followed by mesylation at C-5. The C-20 acetate was hydrolyzed followed by oxetane ring formation and C-4 acetylation to afford the baccatin III species **161** in 42% yield over the 5 steps. The carbonate on **161** was regioselectively opened using phenyl lithium followed by reacetylation at C-10. Oxidation at the C-13 carbon followed by a stereoselective reduction yielded a suitably protected form of baccatin III which was subsequently coupled with Ojima's[52] lactam and deprotected to afford taxol in 33% yield over the last 6 steps.

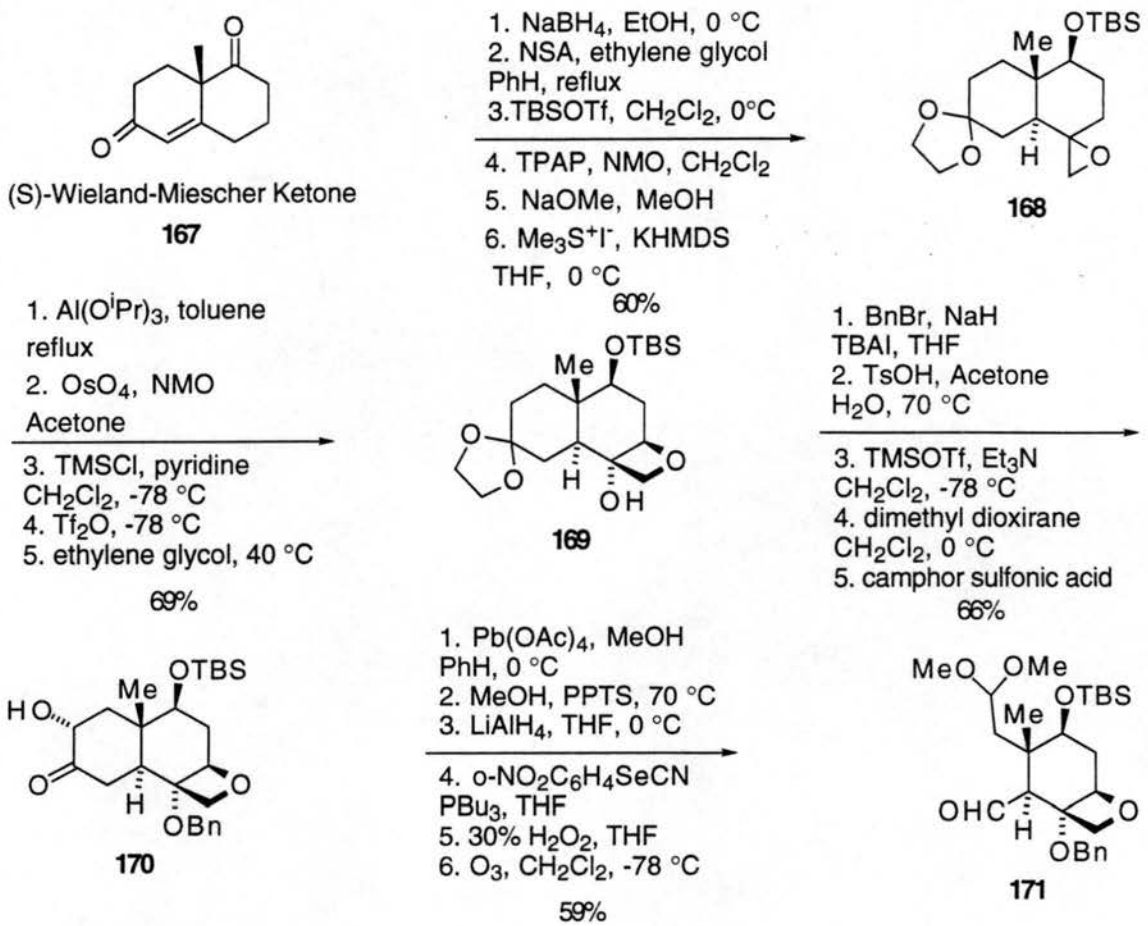
2.3d Danishefsky's Approach to Taxol

Scheme 2.30 Danishefsky's Retrosynthetic Analysis of Taxol.



Scheme 2.30 outlines Danishefsky's retrosynthetic analysis of taxol. Like Holton and Nicolaou, Danishefsky envisioned synthesizing taxol from suitably protected baccatin III species **143**. Compound **143** would then be synthesized from **162** via an intramolecular Heck reaction. Compound **162** would then derive from **163** via A and CD ring precursors **164** and **165** respectively. The A ring precursor would ultimately emanate from diketone **166** while the CD ring precursor would arise from (S)-Wieland-Miescher Ketone **167**.

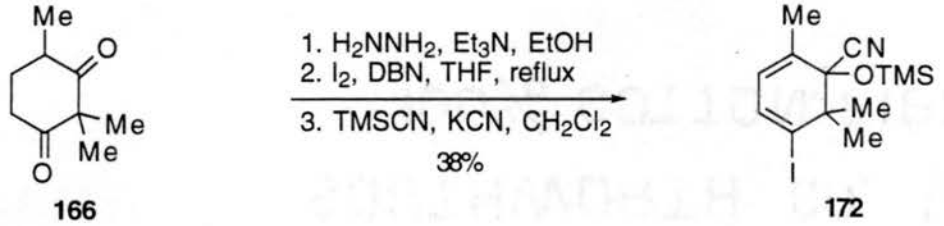
Scheme 2.31 Danishefsky's Synthesis of the CD Ring Precursor.



Epoxide **168** was synthesized from the Wieland-Miescher ketone **167** in 6 steps as outlined in Scheme 2.31.[27] Compound **168** was then subjected to epoxide ring opening conditions followed by dihydroxylation, C-20 alcohol protection and C-5 activation. The C-20 alcohol was then deprotected followed by oxetane formation which gave **169** in 69% yield. Compound **169** was C-4 protected followed by ketal hydrolysis and enol ether formation. The enol ether was then oxidized followed by silyl deprotection which afforded **170** in 66% yield over the 5 steps. The CD ring precursor was then synthesized by lead tetraacetate ring opening followed by acetal formation and ester reduction. The resulting

primary alcohol was converted to CD ring precursor **171** using the Grieco protocol[53] followed by ozonolysis in 59% yield over the 6 steps just described.

Scheme 2.32 Danishefsky's Synthesis of the A Ring Precursor.

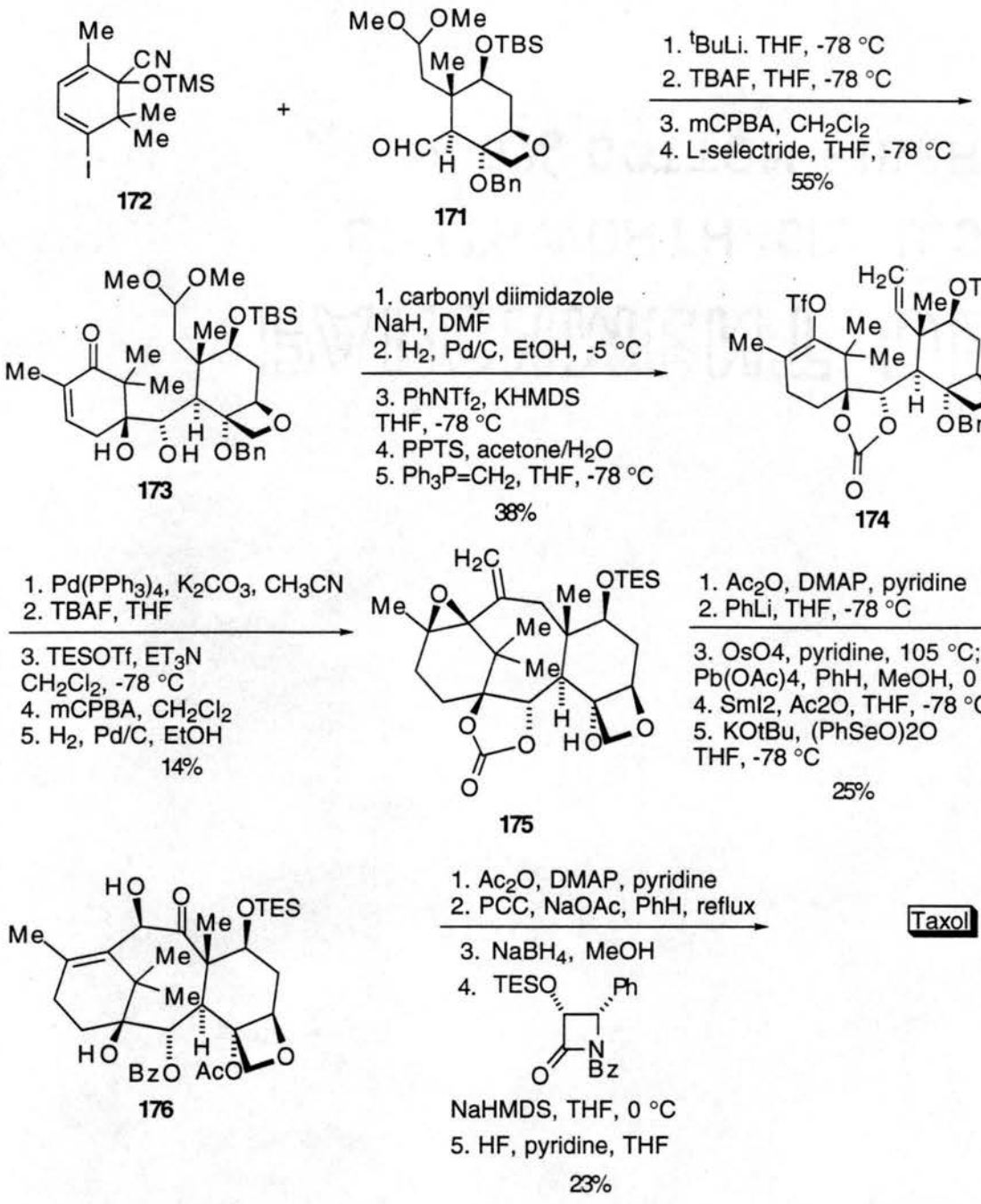


166 **172**

Scheme 2.32 outlines the synthesis of the A ring precursor. Iodo diene **172** was synthesized in 38% yield over 3 steps. Hydrazone formation of **166** at the least hindered ketone followed by iodo diene formation and ketone masking led to **172**.

Scheme 2.33 outlines how the A and CD ring precursors were used to construct baccatin III. Compound **172** was subjected to a lithium-halogen exchange followed by a presumed stereoselective addition to CD ring precursor **171** (the authors did not mention whether this was a stereoselective addition). The ketone was then unmasked followed by chemoselective epoxidation and regioselective epoxide ring-opening to yield **173** in 56% over 4 steps. Diol **173** was then protected followed by enone reduction and triflate formation. The dimethyl acetal was then hydrolyzed and the resulting aldehyde underwent a Wittig reaction to afford **174** in 38% yield over the 5 steps. Compound **174** was then subjected to the intramolecular Heck reaction protocol which formed the ABC ring system in 49% yield. The resulting taxoid was deprotected at C-7 followed by reprotection, chemoselective epoxidation, and C-4 deprotection which afforded epoxide **175** in 14% yield over 5 steps.

2.33 Danishefsky's Total Synthesis of Taxol.



Taxane **175** was acetylated at C-4 followed by regioselective carbonate opening using phenyl lithium. The C-10 alkene was oxidized followed by C-11, C-12 alkene reintroduction using samarium diiodide. The C-9 and C-10 carbons were then converted to their appropriate functionalities and stereochemistries using the seleninic protocol described

earlier by Nicolaou to yield **176** in 25% over the 5 steps.[26] Baccatin III analogue **176** was then acetylated at C-10 followed by C-13 oxidation and stereoselective reduction. The C-13 alcohol was then coupled to Ojimas' lactam[52] followed by deprotection to afford taxol in 23% yield over the last 5 steps.

2.4 Conclusion

To date, only four successful approaches to taxol have been published.[24-27] The first successful synthesis of taxol, as described in section 2.3a, relied on obtaining 10-deacetyl baccatin III from natural sources, thus making this approach a semi-synthetic one. Because 10-deacetyl baccatin III can be isolated from the needles (a renewable source) of *Taxus baccata*, this approach is the most practical of the four total syntheses for producing taxol on large scale.

Of the three total syntheses of taxol, starting from relatively simple starting materials, Holton's approach is linear while, Nicolaou's and Danishefsky's are convergent. Holton's research group used enantiomerically pure β-patchoulene oxide to control the relative and absolute stereochemistry to achieve the total synthesis of taxol. They were able to synthesize taxol in 42 steps from β-patchoulene oxide in 0.6% overall yield. The key to Holton's success relied on the epoxy alcohol fragmentation methodology used to generate a suitable AB ring precursor of taxol.

Nicolaou's research group was able to synthesize taxol in a convergent manner in 0.06% yield over 25 steps starting from suitably functionalized A and C ring precursors. However, the longest linear sequence in this synthesis was 39 steps. The key to Nicolaou's success relied on the C-9, C-10 carbon-carbon bond-forming intramolecular McMurry reaction. Unlike Holton's synthesis, the Nicolaou synthesis produced racemic material and required a resolution step late in the synthesis.

Danishefsky's research group was able to synthesize taxol in a convergent manner in 0.02% yield over 24 steps starting from suitably functionalized A and C ring precursors.

However, the longest linear sequence in this synthesis was 46 steps. Unlike Nicolaou's strategy which closed the B ring of taxol via the C-9, C-10 carbon bond, Danishefsky relied on closing the B ring of taxol using an intramolecular Heck reaction which formed the C-10, C11 carbon-carbon bond. Also, Holton's and Nicolaou's approaches relied on installing the oxetane functionality late in their syntheses while Danishefsky installed the oxetane functionality early in the synthesis. Danishefsky's strategy is similar to Holton's in that the approach also relied on an enantiomerically pure substrate, the (S)-Wieland-Miescher ketone, to control the relative and absolute stereochemistry throughout the synthesis. Although all three syntheses combined probably increased the world's supply of taxol by only a few milligrams, it is hoped the strategies developed will be used to synthesize more biologically active analogues of taxol.

CHAPTER 3

THE CHEMICAL SYNTHESIS OF (±)-TAXA-4(5),11(12)-DIENE AND (±)-TAXA-4(20),11(12)-DIENE

3.1 Introduction

As discussed in chapter 2, totally synthetic methods are unlikely to provide an economically viable alternative source for the production of taxol or a pharmacophoric equivalent. However, taxol production relying on plant cell cultures or genetically altered microorganisms holds potential as an alternative method. In order for plant cell cultures to provide an economically feasible alternative for taxol production, they would have to produce one to two milligrams of taxol per liter per day.[54b] To date, plant cell cultures can only produce 1.5-15 mg of taxol per liter per 30 days.[54] Plant cell cultures, and thus taxol production, are extremely sensitive to environmental perturbations and subculturing.[54a] Oxygen, carbon dioxide, ethylene, and methyl jasmonate are just a few chemicals that have been shown to perturb taxol production in a plant cell line.[54b,c]

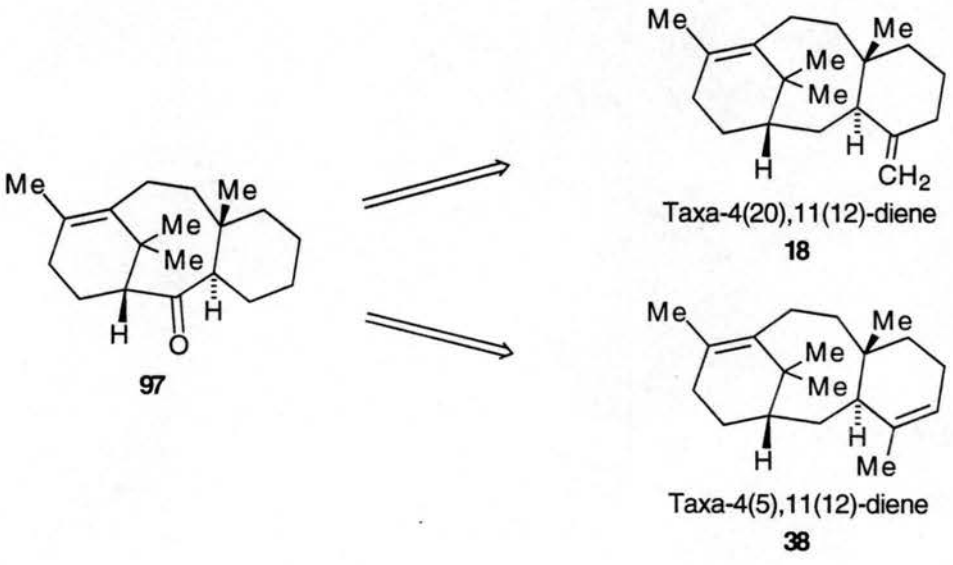
Another way to increase taxol production in a given plant cell line would be to genetically alter that cell line. Alternatively, genetically altering a microorganism to produce taxol would also be desirable. Microorganisms such as *Escherichia coli* and *streptomyces* species are relatively hardy and robust.[55] This feature, and the fact that plant cell cultures are sensitive to external perturbations, make it desirable to genetically engineer-in the

enzymes responsible for taxol production into an *E. coli* strain. This would hopefully produce an *E. coli* strain that would produce taxol in greater amounts and would also be more reliable in producing taxol with respect to the parent plant cell line.

Understanding the biosynthetic pathway of taxol production in *Taxus* species should greatly aid in the development of taxol production via the biosynthetic approaches just mentioned. Performing feeding experiments using synthesized putative biosynthetic precursors to taxol with sections from *Taxus sp.* trees or *Taxus sp.* cell cultures may lead to incorporation into taxol and other higher order taxanes which is imperative for understanding the biosynthetic pathway for taxol production. Since our research group is interested in producing taxol in a biosynthetic manner, we found it important to develop a synthesis that would allow for quick access to the desired compound(s) in large quantities and with incorporation of stable and radio labeled isotopes late in the synthesis.

3.2 Results and Discussions

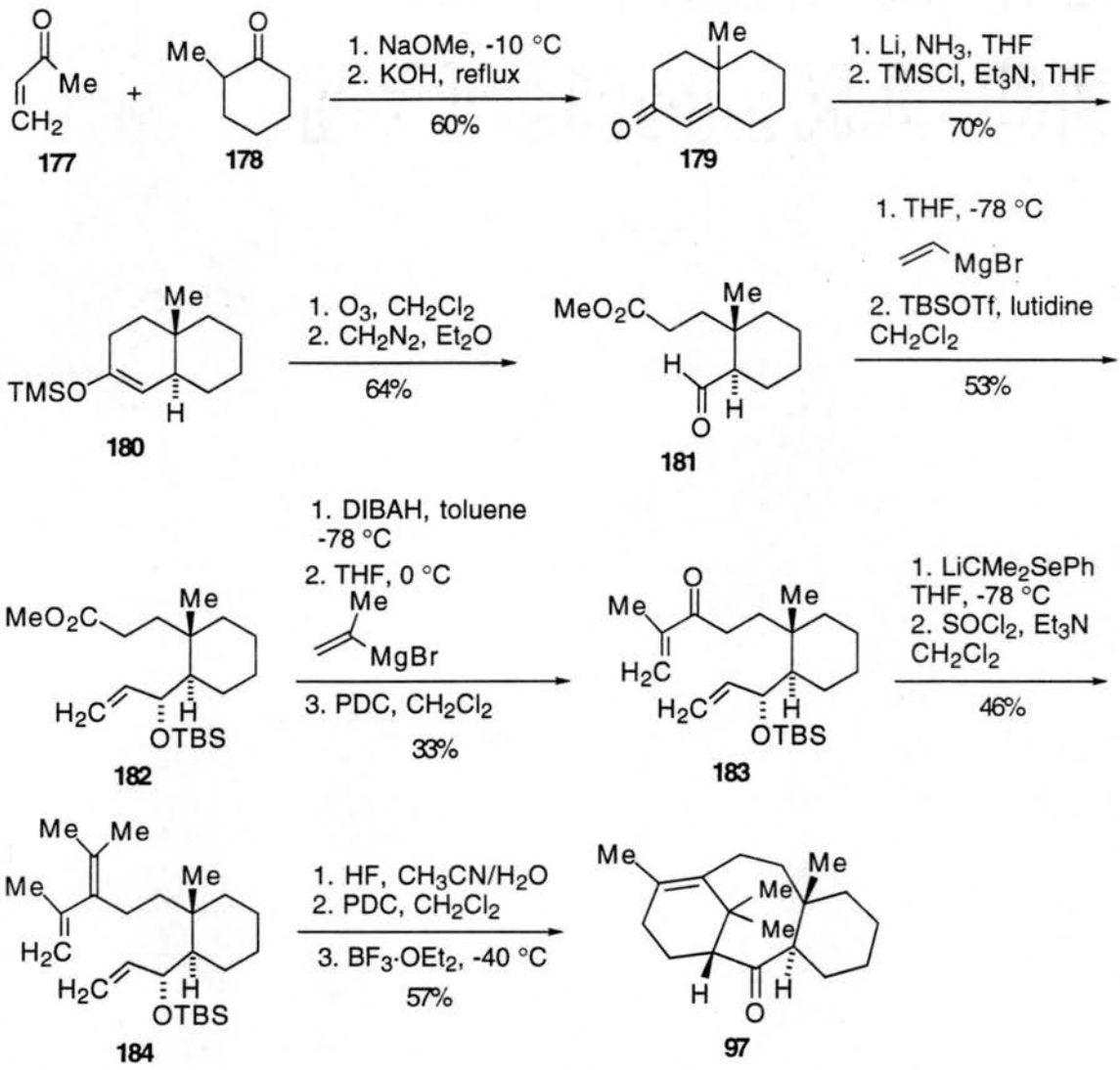
Scheme 3.1 Proposed Synthetic Origin of Compounds **18** and **38**.



Based on Croteau's initial results and Lythgoe's proposal, as discussed in chapter 1, synthesizing taxa-4(5),11(12)-diene (**38**) and taxa-4(20),11(12)-diene (**18**) in radiolabeled and nonradiolabeled form should aid in deciphering the biosynthetic pathway of taxol production when incubated with *Taxus brevifolis* stem discs. It's surprising that even after 30 years, there were no reports concerning the synthesis of Lythgoe's putative biosynthetic precursor **18**. Thus, the development of a synthetic route that would allow access to both taxa-4(5),11(12)-diene (**38**) and taxa-4(20),11(12)-diene (**18**) was required. A review of the literature, revealed that Jenkins' group had synthesized taxane **97**. This taxane is structurally similar to taxa-4(5),11(12)-diene (**38**) and taxa-4(20),11(12)-diene (**18**).[44] Conscripting Jenkins' synthetic route should allow access to compounds **18** and **38** (Scheme 3.1).

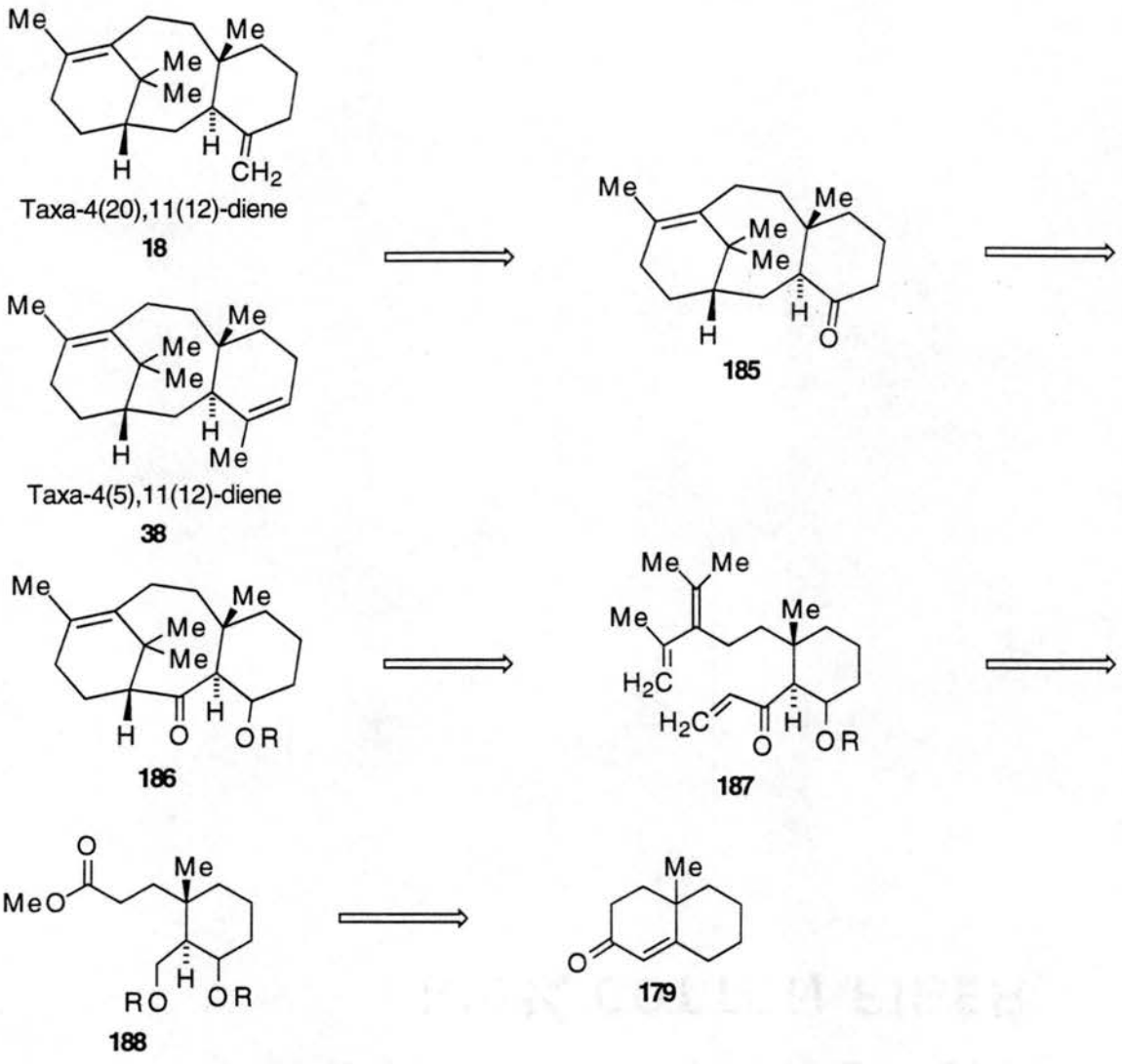
Scheme 3.2 outlines Jenkins' synthesis of taxane **97**. Methyl vinyl ketone **177** and 2-methylcyclohexanone **178** underwent a Robinson annulation reaction under basic conditions followed by steam distillation to yield **179** (60%). Enol ether **180** was synthesized using lithium and ammonia followed by trapping of the enolate as it trimethyl silyl ether in 70% yield from enone **179**. Ozonolysis followed by esterification afforded **181** as a single diastereomer in 64% yield from enol ether **180**. Aldehyde **181** then underwent a stereoselective Grignard addition followed by allylic alcohol protection to yield **182** (53%). Attempts to crystallize derivatives of **182** failed. The relative stereochemistry at C-2 (taxane numbering) was assigned based on the open chain model of Cram's rule. Ester **182** was then reduced to its aldehyde followed by 2-propenyl magnesium bromide addition and PDC oxidation which afforded **183** in 33% yield. A 1,2 chemoselective addition of 2-lithio-2-phenylselenopropane, generated from n-butyllithium and 2,2-bis(phenylseleno)propane, to enone **183** followed by thionyl chloride elimination afforded **184** in 46% yield for the two steps. Taxane **97** was then synthesized in three steps from **184**. Silyl deprotection followed by allylic alcohol oxidation and intramolecular [4+2] cycloaddition afforded **97** in 57% yield from **184**.

Scheme 3.2 Jenkin's Synthesis of Taxane **97**.



Scheme 3.3 outlines a retrosynthetic analysis of taxanes **18** and **38** using the strategy developed by Jenkins. Taxadienes **18** and **38** could arise from the common ketone **185** which in turn would come from taxane **186**. The only difference between ketone **186** and Jenkins' taxane **97** is the protected hydroxyl group at C-4 (taxane numbering). Therefore, the anticipated target molecules could arise from intermediate **187** via an intramolecular Diels-Alder reaction. Compound **187** would then arise from ester **188** by way of Jenkin's starting substrate enone **179**.

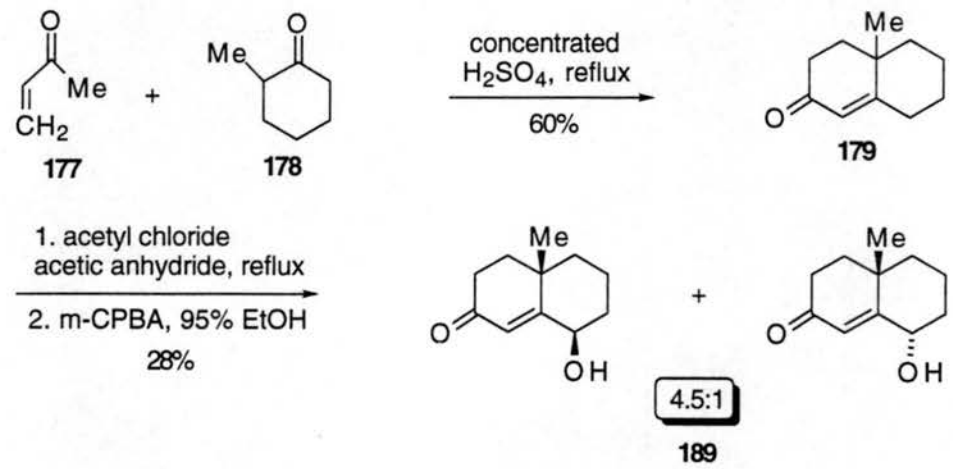
Scheme 3.3 The Retrosynthetic Analysis of Taxadienes **18** and **38**.



With a synthetic strategy in hand, bicyclic enone **179** was synthesized as described by Jenkins.[44] However, The Robinson Annulation reaction followed by steam distillation yielded enone **179** in 20% yield rather than the 60% yield described by Jenkins. Review of the literature led to an alternative procedure for synthesizing bicyclic enone **179**.[56] Rather than performing the Robinson Annulation reaction under basic conditions at low temperature, Heathcock and co-workers described the same reaction under acidic conditions at reflux. Under refluxing, acidic conditions enone **179** was synthesized in 60% yield (Scheme 3.4); this reaction proved efficient on a 2 Kg scale.

With significant amounts of **179** in hand, introduction of the C-4 (taxane numbering) hydroxyl group was then examined. Scheme 3.4 outlines the results. Enone **179** was heated at reflux with acetyl chloride and acetic anhydride[57] to yield the dienol acetate followed by m-CPBA oxidation[58a] which afforded alcohol **189** as a 4.5:1 mixture of separable epimers at C-4. Both reaction steps can be carried out on a 300 gm scale. The relative stereochemistries were assigned by comparing the ^{1}H NMR data with the ^{1}H NMR data of an already published paper on microbial oxidations.[58b] Installation of the hydroxyl group in the decalone system at this point provided a C-4 functional group for the ultimate introduction of the C-20 carbon atom of the taxadiene systems **18** and **38**.

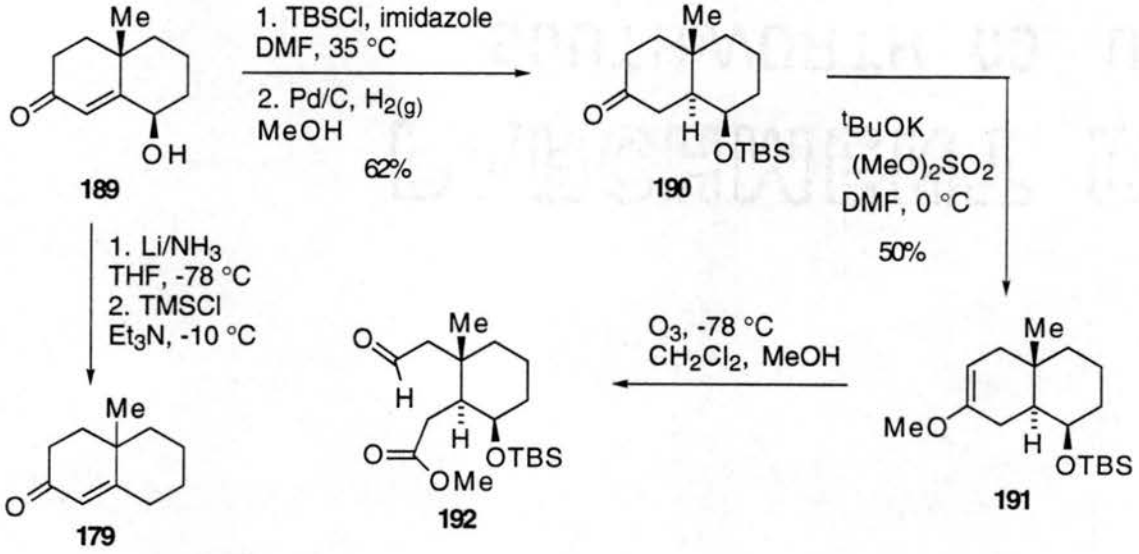
Scheme 3.4 The Synthesis of Diastereomers **189**.



The major C-4 β-diastereomer was then subjected to the reductive conditions followed by enolate trapping as outlined by Jenkins.[44] Decalone **179** was isolated instead of the desired 3,4 enol ether (steroid numbering), Scheme 3.5. It is well known[59] that enolization, under thermodynamic conditions, of trans decalone systems such as **190** yield 2,3 enolates rather than 3,4 enolates (steroid numbering). A selective enolization, under kinetic conditions, followed by trapping might afford the desired 3,4 enol ether (steroid numbering) which could then be ozonolyzed and esterified to yield our version of **181**.

Scheme 3.5 outlines the results. Alcohol **189** was protected as a tert-butyldimethyl silyl ether[60] with the expectation that the angular methyl group in conjunction with the silyl ether would effectively block the β-face of **189**. After alcohol protection, the resulting enone under went a selective hydrogenation[61] to afford **190** in 62% yield for the two steps; both steps can be run on a 100 gm scale. The ring system was assigned as being *trans*-fused based on NOE (Nuclear Overhauser Effect) spectral analysis and was later confirmed by X-ray crystallographic analysis. The kinetic enolate of ketone **190** was formed followed by dimethyl sulfate trapping to yield **191** (50%). The structure of enol ether **191** was confirmed by ozonolysis which afforded the undesired ester **192**.[44]

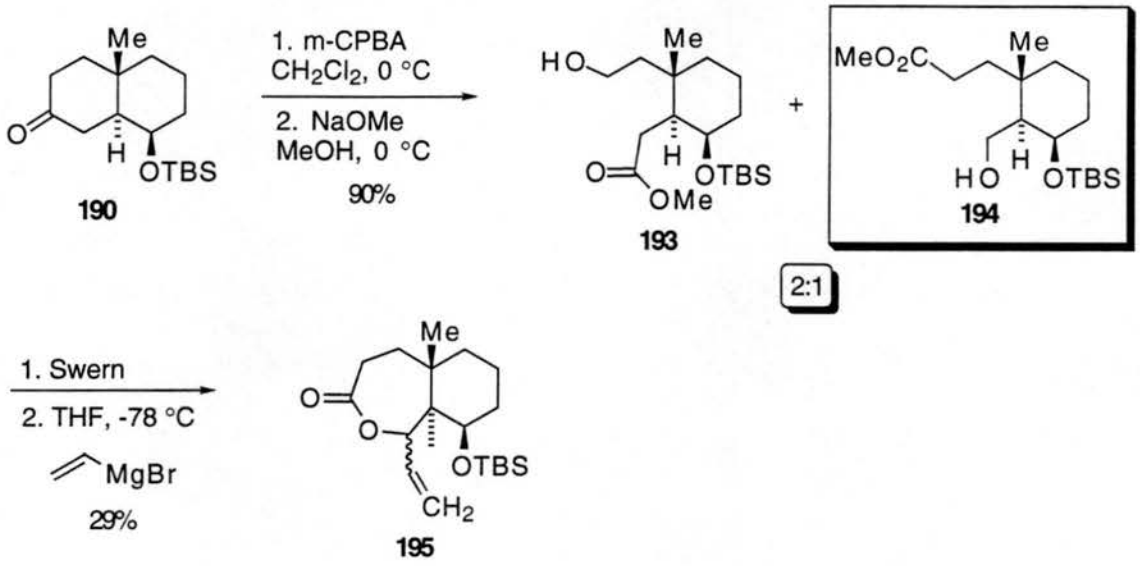
Scheme 3.5 The Syntheses of Enone **179** and Ester **192**.



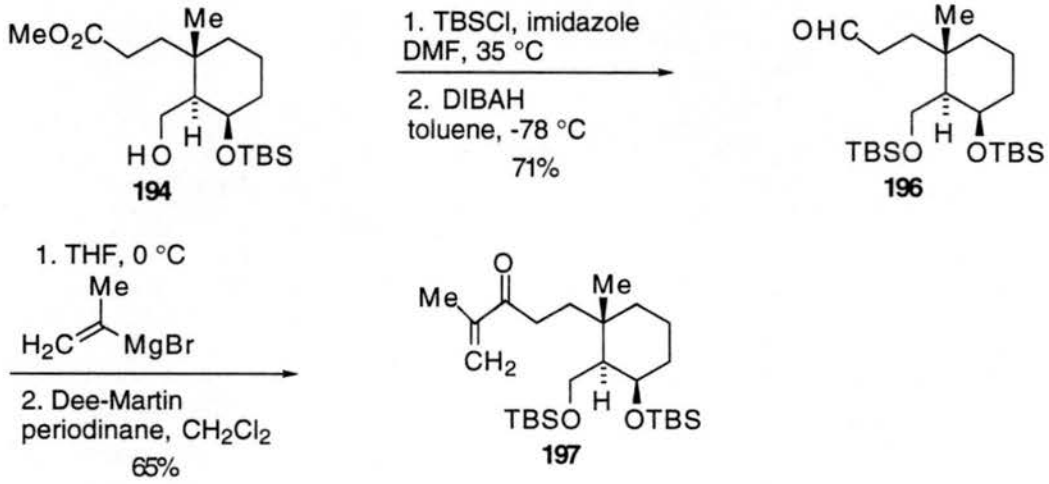
An alternative synthesis of the desired ester was attempted as outlined in Scheme 3.6. Ketone **190** was subjected to Baeyer-Villiger conditions[62] which yielded a 2:1 mixture of regioisomers that were extremely difficult to separate by column chromatography. The mixture of lactones was opened using sodium methoxide which furnished an easily separable 2:1 (undesired:desired) mixture of esters **193** and **194** respectively. Despite the poor regioselectivity, it was possible to isolate a preparatively

useful amount of the desired isomer **194**. Ester **194** was then oxidized[63] followed by vinyl magnesium bromide addition which afforded lactone **195** (based on the ^{1}H NMR spectra) in 29% yield for the 2 steps. Because of the low yield in this last step, and the poor regioselectivity observed for the Baeyer-Villiger reaction, the installation of the diene portion of the molecule was attempted first with the expectation of obtaining a higher overall yield. Scheme 3.7 outlines the results.

Scheme 3.6 The Synthesis of Lactone **195**.



Scheme 3.7 The Synthesis of Enone **197**.



Alcohol **194** was protected as a silyl ether[60] followed by diisobutyl aluminum hydride reduction[44] which furnished aldehyde **196** in 71% yield for the 2 steps. Enone **197** was synthesized by reacting **196** with 2-propenyl magnesium bromide[44] which furnished the allylic alcohol as a single diastereomer. Des-Martin periodinane oxidation[64] then afforded the desired product in 66% yield for the 2 steps. With enone **197** in hand, the installation of the tetrasubstituted double bond, which would produce the desired diene, was followed as described by Jenkins.[50]

However, following the protocol described by Jenkins[44] gave disappointing results. The 2,2-bis(phenylseleno)propane **199** was synthesized[65] from benzene selenol **198** and acetone in 15% yield. Compound **199** was then converted to its α-selenoalkyllithium using tert-butyllithium in ether[66] rather than n-butyllithium in tetrahydrofuran as described by Jenkins[44]. Initial attempts using n-butyllithium in tetrahydrofuran failed. The α-selenoalkyllithium then added chemoselectively to enone **197** which furnished **200** in 67% yield (Scheme 3.8).

Scheme 3.8 The Synthesis of Allylic alcohol **200**.

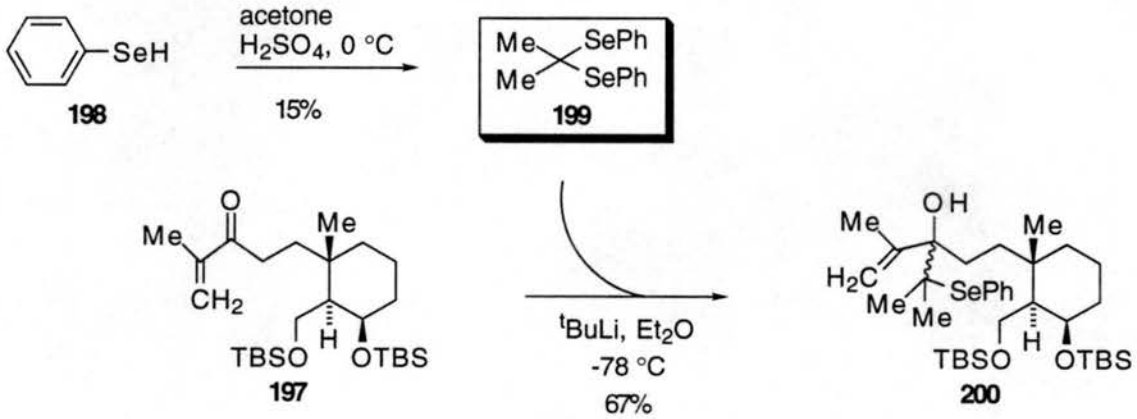
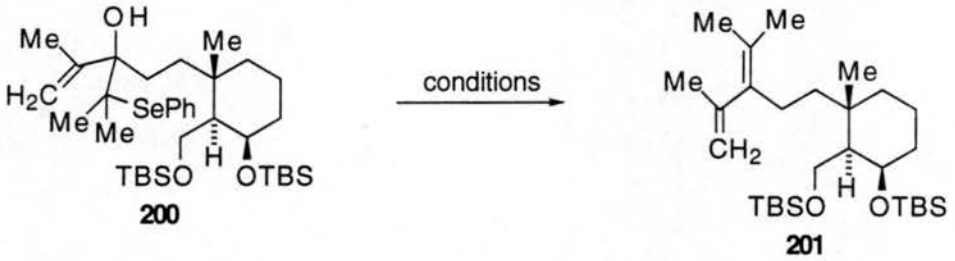


Table 3.1 outlines the various conditions[66] used in an attempt to synthesize **201**. From the table it can be seen that efforts to synthesize diene **201** from **200** were

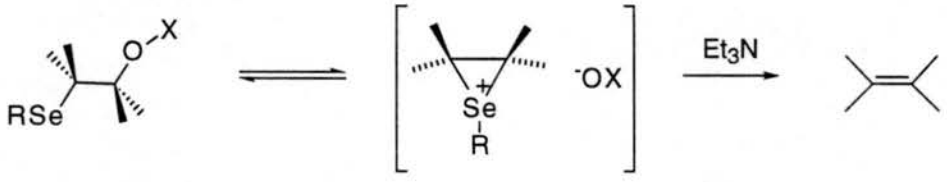
unsuccessful. A mechanistic rational, developed by Krief[66], for the elimination is outlined in Scheme 3.9. It is believed that in order for the elimination to occur, the selenyl moiety must be *trans*,anti-periplanar to the activated hydroxyl moiety. Once this is achieved, a seleniranium salt is formed followed by elimination of the selenyl moiety in the presence of triethyl amine to furnish the desired alkene.

Table 3.1 The Various Conditions Used in an Attempt to Synthesize **201**.



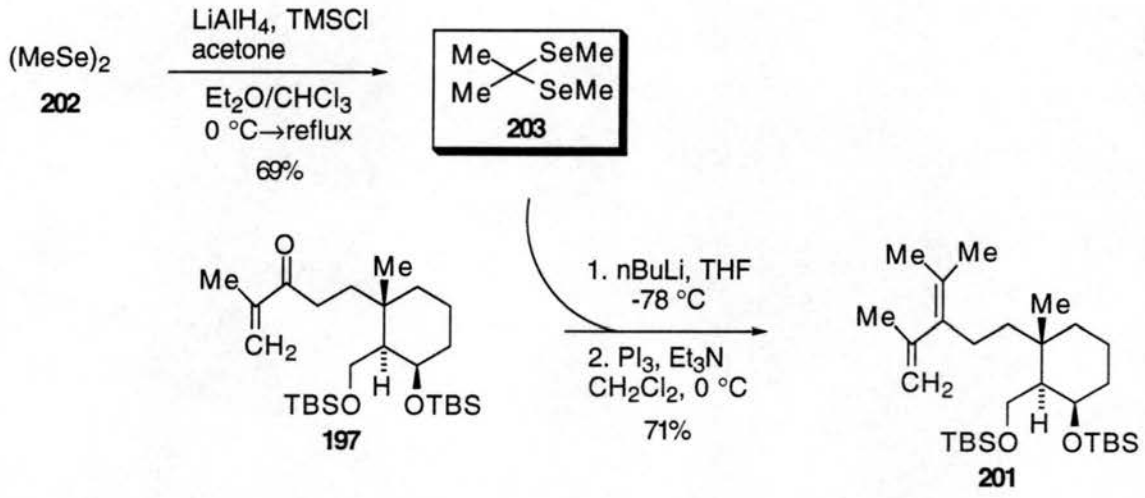
ENTRY	CONDITIONS	RESULTS
1)	$SOCl_2$, Et_3N, CH_2Cl_2 0 °C→25 °C, 3.5 hr.	decomposition
2)	H_3CSO_2Cl, Et_3N, CH_2Cl_2 0 °C→25 °C, 2.5 days.	no reaction
3)	PI_3, Et_3N, CH_2Cl_2 0 °C→25 °C, 22 hr.	decomposition
4)	$POCl_3$,NaH, CH_2Cl_2 0 °C→25 °C, 2.5 days.	decomposition
5)	P-TsOH·H2O, pentane reflux, 2.5 days	decomposition
6)	$(F_3CCO)_2O$, Et_3N, CH_2Cl_2 0 °C→25 °C, 2.5 days.	no reaction
7)	$(ClOC)_2$, DMF, CH_2Cl_2 0 °C→25 °C, 22 hr.	decomposition

Scheme 3.9 Krief's Mechanistic proposal for Alkene Formation.



Based on Krief's mechanistic rational, it seemed reasonable to assume that diene **201** was not being produced because compound **200** was unable to adopt the *trans*, anti-periplanar configuration needed, with respect to the selenyl and hydroxyl moieties, to effect the elimination. This prompted us to synthesize a sterically less demanding selenium analogue of **200** in the hope that the *trans*, anti-periplanar configuration could be adopted and thus afford the elimination product **201**. Scheme 3.10 outlines the results in this endeavor.

Scheme 3.10 The Synthesis of Diene **201**.

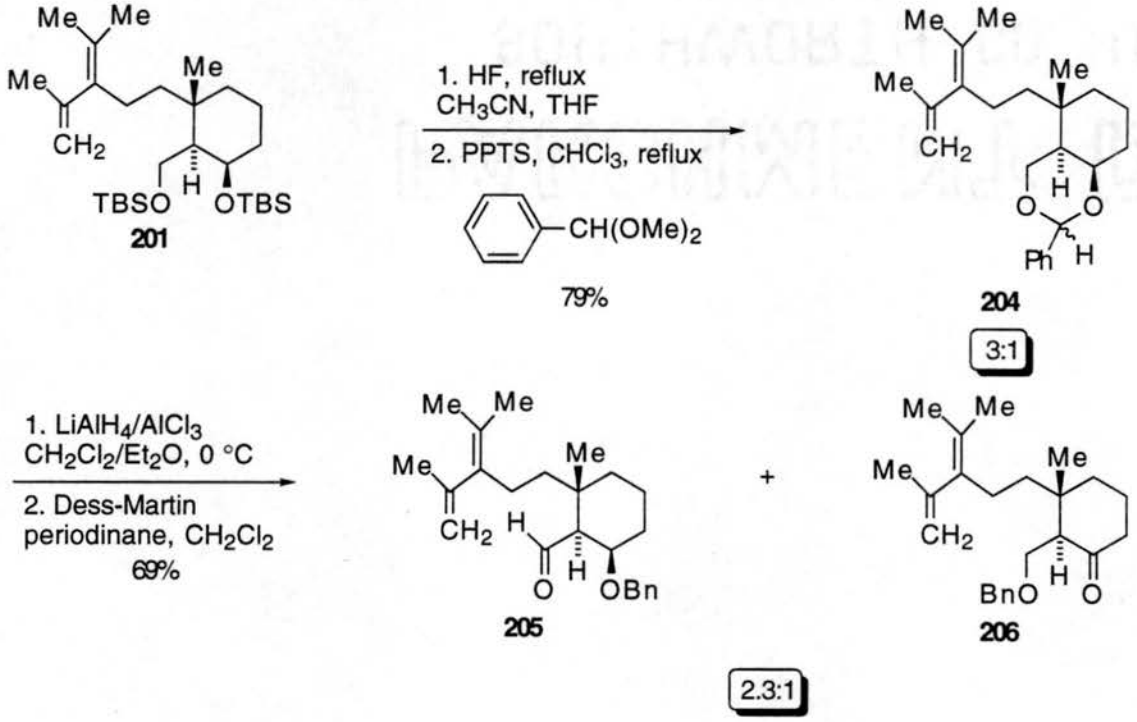


2,2-Bis(methylseleno)propane **203** was synthesized[67] in 69% yield from dimethyldiselenide **202**. Compound **203** was converted to its α-selenoalkyllithium using n-butyllithium in tetrahydrofuran as described by Jenkins[44] (the α-selenoalkyllithium could not be generated using tert-butyllithium in ether). The α-selenoalkyllithium then added chemoselectively to enone **197** which yielded the allylic alcohol as a 4:1 mixture of diastereomers that was taken on directly. Elimination using phosphorous triiodide and triethyl amine[68] furnished diene **201** in 71% yield for the 2 steps (Scheme 3.10).

With diene **201** in hand, installation of the dienophile portion of the molecule was next addressed. It was anticipated that selective deprotection of the primary alcohol over

the secondary alcohol could be achieved. However, after considerable experimentation, this could not be achieved.[69] An analogue of **197**, in which the secondary alcohol was protected as its tert-butyldiphenylsilyl ether, was also synthesized. It was assumed that having a hardier silyl protecting group on the secondary alcohol would allow for the selective deprotection of the primary alcohol. After considerable experimentation, selective deprotection could not be achieved.[69] Based on these results, a slightly different strategy in which to access the dienophile portion of the molecule was developed. Scheme 3.11 outlines the results.

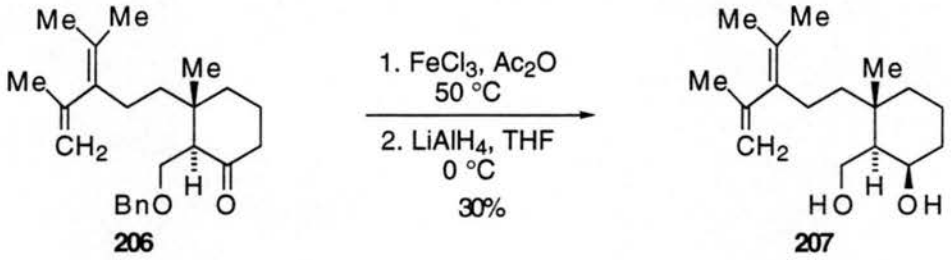
Scheme 3.11 The Synthesis of Aldehyde **205** and Ketone **206**.



Benzylidine acetal **204** was synthesized as a 3:1 mixture of diastereomers in 79% yield from diene **201** using hydrofluoric acid[43] and benzaldehyde dimethyl acetal.[70] Interestingly, attempts to protect the diol system with benzaldehyde in the presence of a Lewis acid led to a regioselective hetero Diels-Alder reaction between benzaldehyde and the diene functionality. Lithium aluminum hydride and aluminum trichloride were used to

selectively open the benzylidine acetal to afford an inseparable 4:1 mixture of unprotected primary and secondary diols respectively.[71] The inseparable mixture was oxidized with Dess-Martin reagent to furnish Aldehyde **205** and ketone **206** as a 2.3:1 mixture of separable compounds in 69% yield for the 2 steps.[64] Ketone **206** was recycled back into the synthetic route (Scheme 3.12) while aldehyde **205** was taken on as shown in Scheme 3.13.

Scheme 3.12 The Synthesis of Diol **207**.



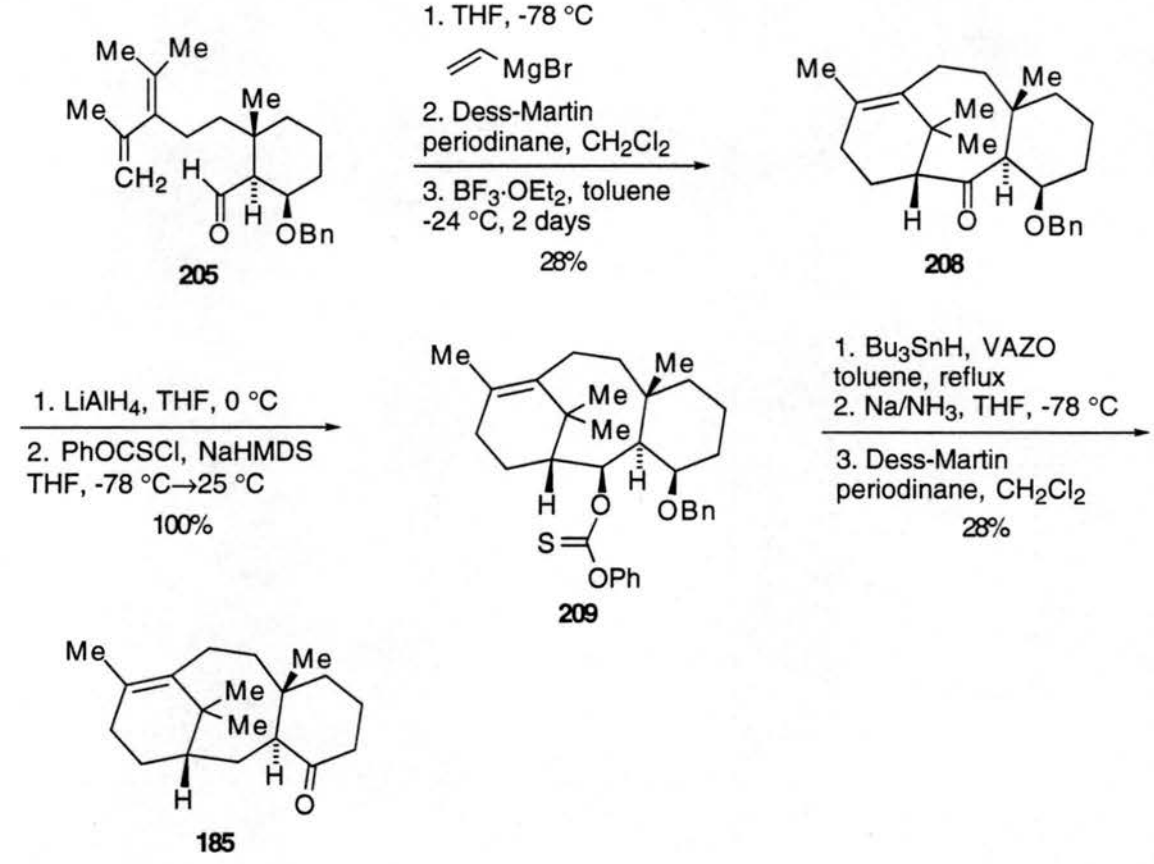
Cleavage of the benzyl ether followed by acetylation of the resulting primary alcohol gave the desired product in 30% yield.[72] Concurrent deacetylation and stereoselective reduction with lithium aluminum hydride afforded diol **207** in quantitative yield. Diol **207** can then be converted into benzylidine acetal **204** as outlined in Scheme 3.11.

Alternatively, aldehyde **205** was allowed to react with vinyl magnesium bromide to furnish a 5.4:1 diastereomeric mixture of allylic alcohols.[43] Originally, these diastereomers were separated and subjected to pyridinium dichromate oxidation. The major diastereomer furnished the desired enone in 40% yield after 5 days while the minor diastereomer afforded the desired enone in 60% yield after 18 hr. Alternatively, the mixture was oxidized with Dess-Martin reagent[64] which produced the intact Diels-Alder precursor in 77% yield after 1hr. An intramolecular [4+2] cyclization under Lewis acidic conditions afforded taxane **208** in 28% yield for the 3 steps.[44] The relative stereochemistry at C-1, C-3, and C-8 was evident from ^{1}H NMR NOE experiments and was finally corroborated by

X-ray crystallographic analysis of the subsequent transformation product **185**. A quantity of 7.4 gm of taxane **208** was synthesized using this protocol. However, the Diels-Alder reaction does not go to completion when run on a >500 mg scale. This presents a problem because the Diels-Alder product and precursor are inseparable. In order to get around this problem, the [4+2] cyclization was run many times in parallel on <500 mg scale.

Taxane **208** was stereoselectively reduced to the C-2 alcohol followed by xanthate ester formation[73] using phenyl chlorothionoformate which afforded **209** in quantitative yield for the 2 steps. The C-2 ester is believed to have the stereochemistry shown, since the top face is blocked by both C-16 and C-19 (taxane numbering) methyl groups thus forcing

Scheme 3.13 The Synthesis of Taxoid **185**.



lithium aluminum hydride to attack from the bottom face. Deoxygenation of the C-2 xanthate[73] ester **209** followed by benzyl ether deprotection and Dess-Martin periodinane

oxidation[64] furnished ketone **185** as a crystalline solid in 28% yield for the 3 steps. The structure and relative stereochemistry was firmly established by X-ray crystallographic analysis, as shown in Figure 3.1.

Ketone **185** served as the key intermediate for the final elaboration to both taxa-4(20),11(12)-diene (**18**) and taxa-4(5),11(12)-diene (**38**). The exomethylene group of **18** was conveniently installed utilizing methylene triphenylphosphorane[74] to furnish (±)-**18** in 80% yield, as shown in Scheme 3.14. The natural product **38** was prepared by condensing ketone **185** with methylmagnesium bromide in the presence of $CeCl_3$[75] to give a single stereoisomeric tertiary alcohol **210** in 80% yield which is presumed to have the

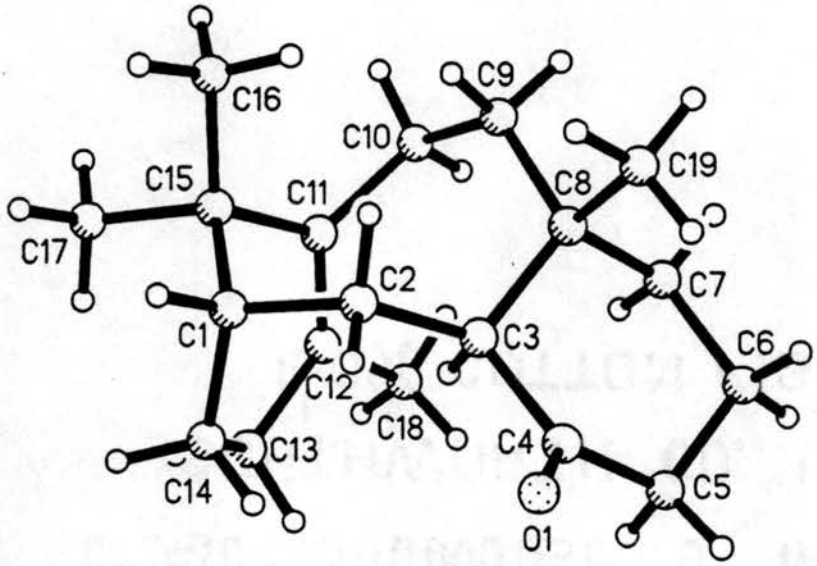
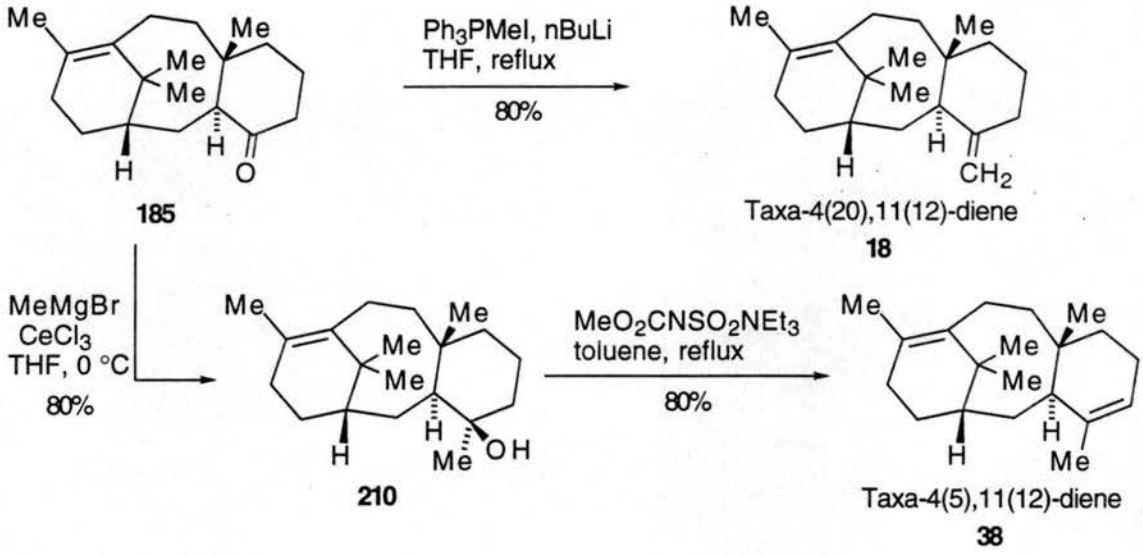


Figure 3.1 X-Ray Stereostructure of Compound **185**.

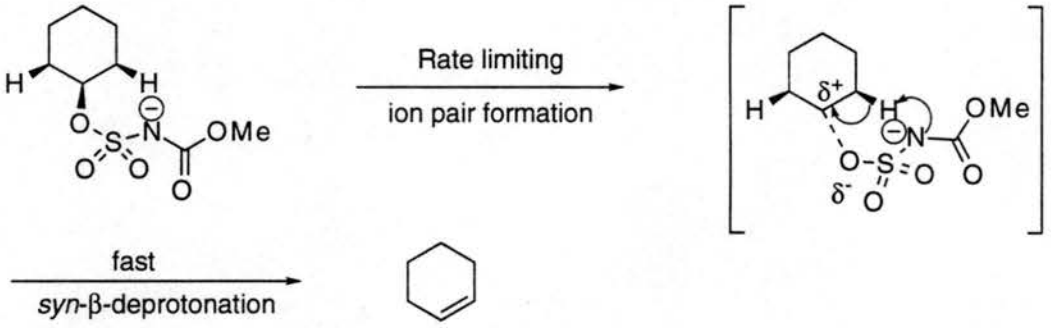
indicated α-stereochemistry.[25b] Dehydration of **210** with the Burgess reagent[25b] gave an approximately 1 : 1 mixture of taxa-4(20),11(12)-diene (**18**) and taxa-4(5),11(12)-diene (**38**) in 80% combined yield (Scheme 3.14). Comparison of synthetic **38** with an authentic, natural sample by capillary gc, ei-mass spec, ^1H NMR and ^{13}C NMR firmly established the identity of this substance.[21,76] The authentication of the structure of synthetic

38 also serves to corroborate and secure the structural assignment very recently made for the natural product isolated from Pacific Yew bark.[21]

Scheme 3.14 The Synthesis of Taxadienes **18** and **38**.



Scheme 3.15 Proposed Mechanism Based on Experimental Data.

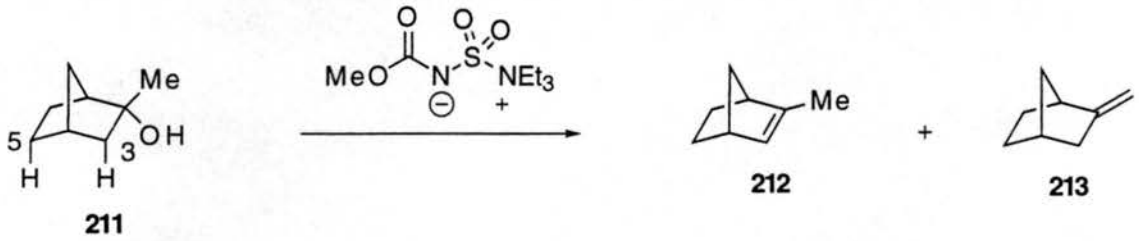


The equal ratio of products formed is assumed to be of kinetic origin based on experimental data (Table 3.2) that reveals that **18** could not be isomerized to **38** in any detectable amount under a variety of reaction conditions. The Burgess reagent is known to form alkyl-N-carbomethoxy sulfamates with a wide variety of alcohols which subsequently undergo an intramolecular *syn*-deprotonation followed by elimination.[77a] Burgess and co-workers observed the first order kinetics, activation parameters, and β-isotope effect data

collected were consistent with a mechanism that has formation of an ion pair as the rate limiting step followed by a fast *syn*-β-deprotonation by the departing anion (Scheme 3.15).

When acyclic alcohols having more than one kind of β-proton were dehydrated using the Burgess reagent, the more substituted (Saytzeff) alkene was produced as the major product, assuming no rearrangements can take place. Interestingly, when 2-endo-methylbicyclo[2.21]heptan-2-ol (**211**) was allowed to react with the Burgess reagent the Saytzeff product (**212**) and Hofmann product (**213**) were produced in a 1:1 ratio (Scheme 3.16). The unexpected faster rate of formation of **213** relative to **212** was attributed to greater strain energy in the transition state for the formation of **212**. Also, it was hypothesized that the C-5 endo hydrogen sterically interfered with the ion pair transition state geometry necessary for the C-3 endo deprotonation thus leading to **212**.[77a]

Scheme 3.16 Dehydration of **211**.

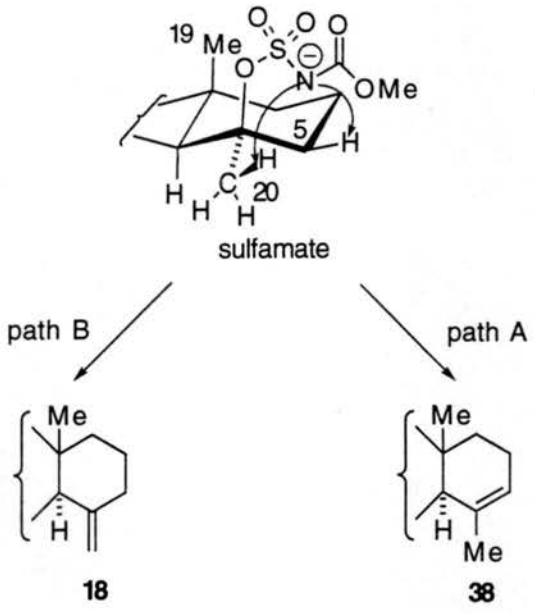


211 **212** **213**

The same kind of rationale could also be used to explain the observed ratio of regioisomers **18** and **38** when reacting **210** with the Burgess reagent as outlined in Scheme 3.14. Scheme 3.17 outlines the two possible fates for the resulting sulfamate. As stated earlier, taxadienes **18** and **38** were formed in a 1:1 ratio. It is proposed, based on the previous work of Burgess and co-workers[77a] with **211**, that the Saytzeff product (**38**) was not formed in greater amounts because of the possible increase of strain energy in the transition state upon deprotonating the C-5 exo hydrogen (path A) relative to the C-20 hydrogen (path B). In addition, models suggest that in order for the C-5 deprotonation to occur the C-19 Methyl group will sterically interfere with one of the lone pair orbitals on

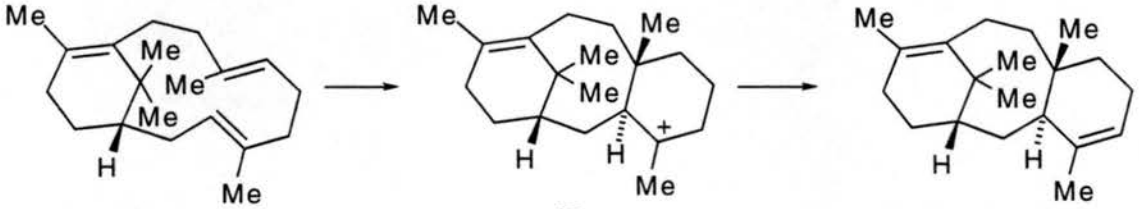
the C-4 oxygen while, no such steric interaction exists for the intramolecular C-20 hydrogen abstraction.

Scheme 3.17 Dehydration of **210** via a Sulfamate.



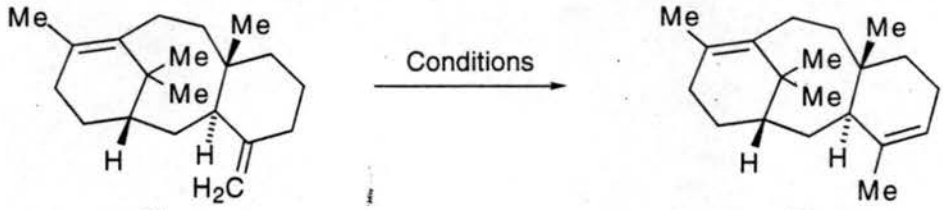
The proposed cyclization of 1S-verticilline **16** via cation **17** (Scheme 3.18), constitutes a reasonable mechanistic pathway for the formation of taxa-4(5),11(12)-diene **38**.[14] We attempted to generate cation **17**, from taxa-4(20),11(12)-diene **18** in an effort to

3.18 Proposed Biosynthetic Origin of Taxa-4(5),11(12)-diene **38**.



examine the intrinsic tendency of this species to effect the isomerization of **18**→**38**. Thus, treatment of taxa-4(20),11(12)-diene (**18**) under the reaction conditions outlined in Table 3.2[77b,c] provided, in all cases, no evidence for the production of compound **38**.

Table 3.2 The Attempted Isomerizations of **18** to **38**.



18
Taxa-4(20),11(12)-diene

38
Taxa-4(5),11(12)-diene

ENTRY	CONDITIONS	RESULTS
1)	$HCl_{(g)}$, Et_2O, 0 °C	decomposition
2)	Conc. HCl, Et_2O, 0 °C	decomposition
3)	Conc. H_2SO_4, EtOH, 0 °C	decomposition
4)	$BF_3{\cdot}OEt_2$, Et_2O, 0 °C	decomposition
5)	$PdCl_2$, toluene, reflux	no reaction
6)	$RhH(CO)(P(Ph_3))_3$, reflux	no reaction

3.3 Conclusion

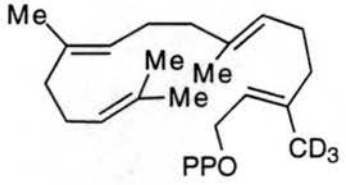
The intermediacy of the Lythgoe structure **18**[14] in the biosynthesis of the taxoids presently remains, at best, uncertain and, based on the very recent work of Croteau and associates,[21] seems unlikely. The present syntheses of taxadienes **18** and **38** are fairly lengthy; however, these routes provided significant amounts of material for biosynthetic feeding experiments. In addition, they also provide convenient access to [20-$^3H_3$]-, [20-$^2H_3$]-, and [20-^{13}C]-labeled taxadienes **18** and **38**, as described in Chapter 4. The synthesis of these radio-labeled biosynthetic intermediates will be useful probes to determine the sequence of hydroxylation reactions from **38** into the taxoid manifold.

CHAPTER 4

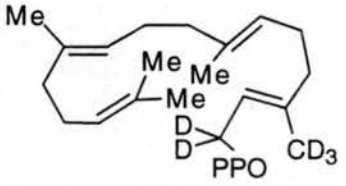
BIOSYNTHETIC EXPERIMENTAL RESULTS

4.1 Introduction

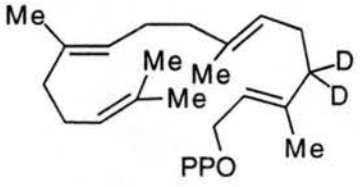
The fact that taxa-4(5),11(12)-diene (**38**) is a biosynthetic precursor to taxol raises several important questions. For example, what is the mechanism of taxa-4(5),11(12)-diene (**38**) production from geranylgeranyl diphosphate (**14**) ? Also, is it possible for putative biosynthetic precursors such as taxa-4(20),11(12)-diene (**18**), 1-(S)-verticilline (**16**), and/or casbene (**21**) to act as transient intermediates along the way to taxa-4(5),11(12)-diene (**38**) and thus to taxol? These questions were recently addressed by Croteau and Floss.[78]



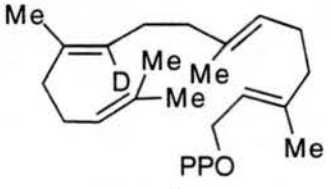
214



215



216

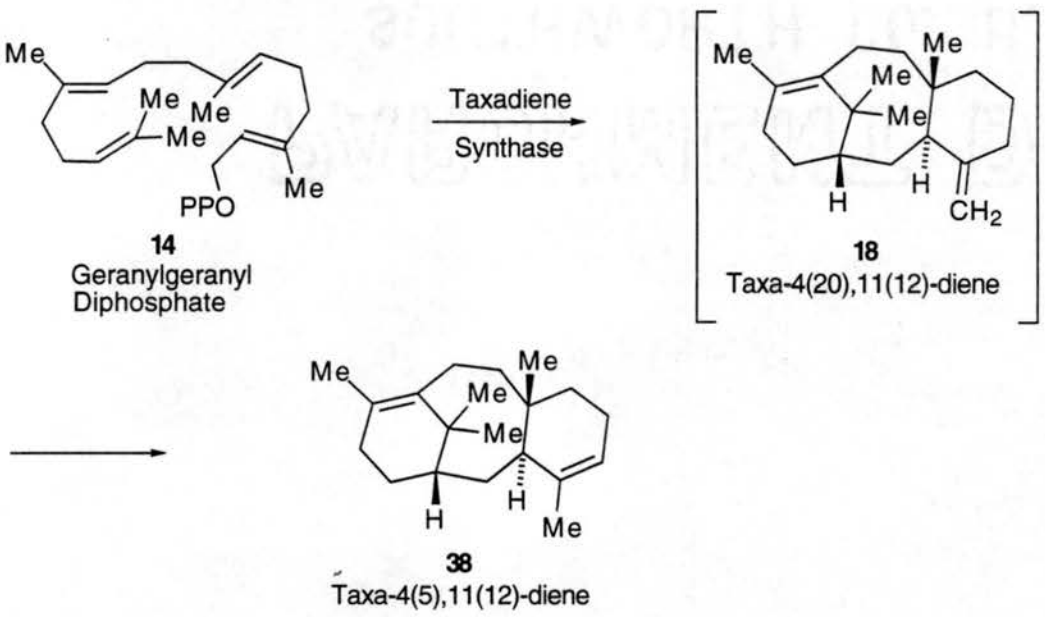


217

Figure 4.1 The Deuterated Compounds Used as Mechanistic Probes.

Figure 4.1 lists the deuterated compounds synthesized by Floss and associates which were used as mechanistic probes.[78] A partially purified preparation of taxadiene synthase from *Taxus brevifolia* stems was used in these mechanistic studies.[22] Under optimal conditions, geranylgeranyl diphosphate (**14**) was converted to taxa-4(5),11(12)-diene (**38**) in 4-5% yield without detectable formation of taxa-4(20),11(12)-diene (**18**), 1-(S)-verticilline (**16**), cembrene (**20**), and/or casbene (**21**). This observation argues against the synthesis of these olefins as free intermediates, but does not address the possibility that such intermediates are enzyme-bound.[78]

Scheme 4.1 Proposed Conversion of **14** to **38** Via **18**.



14
Geranylgeranyl Diphosphate

18
Taxa-4(20),11(12)-diene

38
Taxa-4(5),11(12)-diene

In order to test the mechanistic possibility that geranylgeranyl diphosphate (**14**) is cyclized to taxa-4(20),11(12)-diene (**18**) followed by isomerization to taxa-4(5),11(12)-diene (**38**) (Scheme 4.1), geranylgeranyl diphosphate isotopomers **214** and **215** were synthesized and incubated separately with taxadiene synthase (Scheme 4.2). Conversion efficiencies of **214** and **215** were calculated to be 4.0% and 3.6% respectively compared to 4.2% for the control assay in which tritiated geranylgeranyl diphosphate (**37**) was

used.[78] The observed rate suppression for **215** probably reflects a secondary kinetic isotope effect which suggests that ionization of geranylgeranyl diphosphate may be rate-limiting. Similar kinetic isotope effects have been observed with other terpenoid cyclizations.[79]

Scheme 4.2 The Cyclization of Isotopomers **214** and **215** Using Taxadiene Synthase.

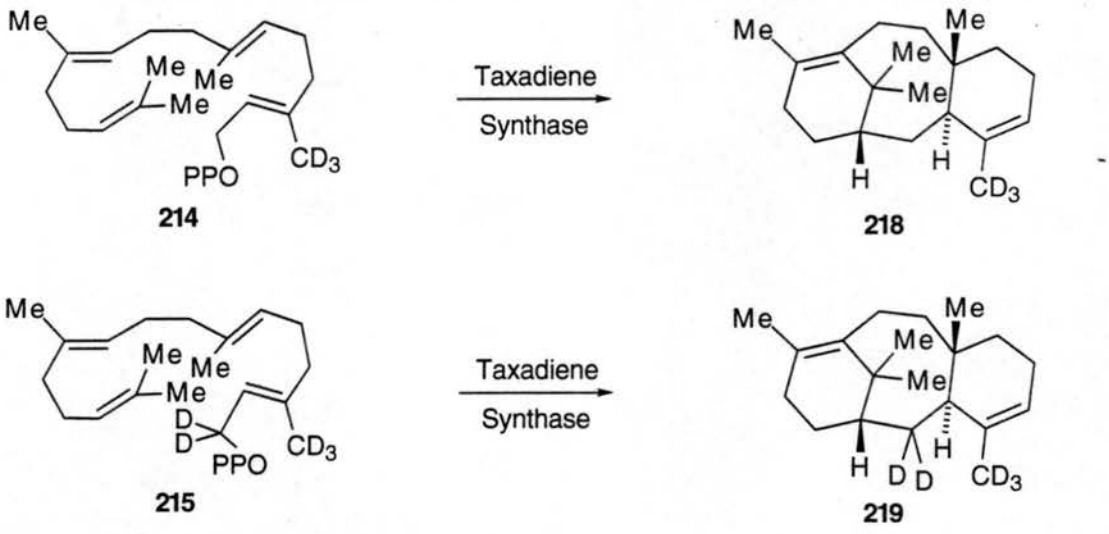


Table 4.1 Mass Spectral Analysis of Taxa-4(5),11(12)-diene Derived from Isotopomers of Geranylgeranyl Diphosphate.

		Fragment ions (m/z)	
Substrate (Atom% ^2H)[a]	P (Atom% ^2H)[a]	P-15 (CH_3)	C-ring (C_9H_{14})
37	272	257	122
215 (>95)	277 (>95)	262	127
214 (>95)	275 (>95)	260	125
216 (91)	273 (90)	258	123
217 (>95)	273 (85±5)	258	123
37/^2H_2O (100)	273 (8±2)	—	—

[a] Atom % deuterium corrected for background contribution. No entry indicates not reliably determined.

Table 4.1 outlines the mass spectral results for the incubations of the isotopomers of geranylgeranyl diphosphate with taxadiene synthase.[78] The major ion peaks considered were the parent (P), P-15 (CH3), and the C-ring fragment ions. Figure 4.2 outlines the

proposed mechanism for C-ring fragment ion formation. From the table it can be seen that both **214** and **215** retain all of their deuterium labeling (Scheme 4.2).

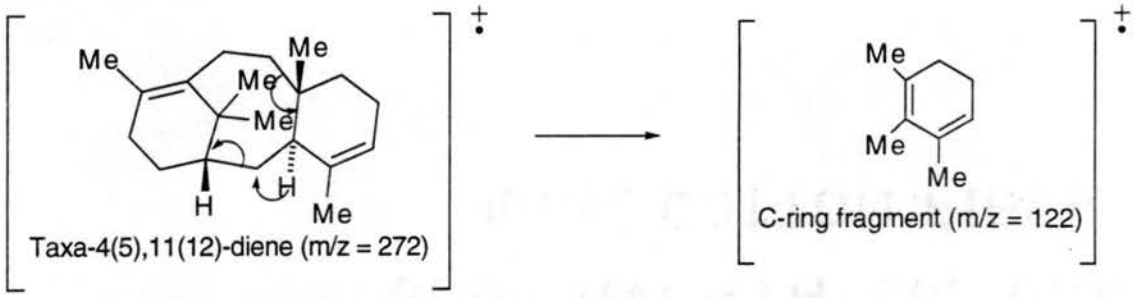
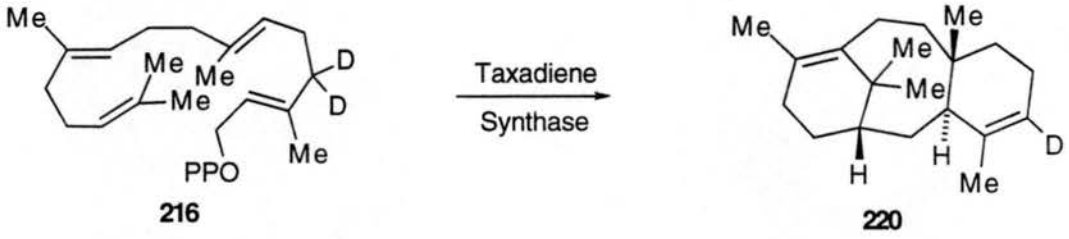


Figure 4.2 Proposed Mechanism of Formation for the C-ring Fragment in the mass spectrometer.

Scheme 4.3 The Cyclization of Isotopomers **216** Using Taxadiene Synthase.

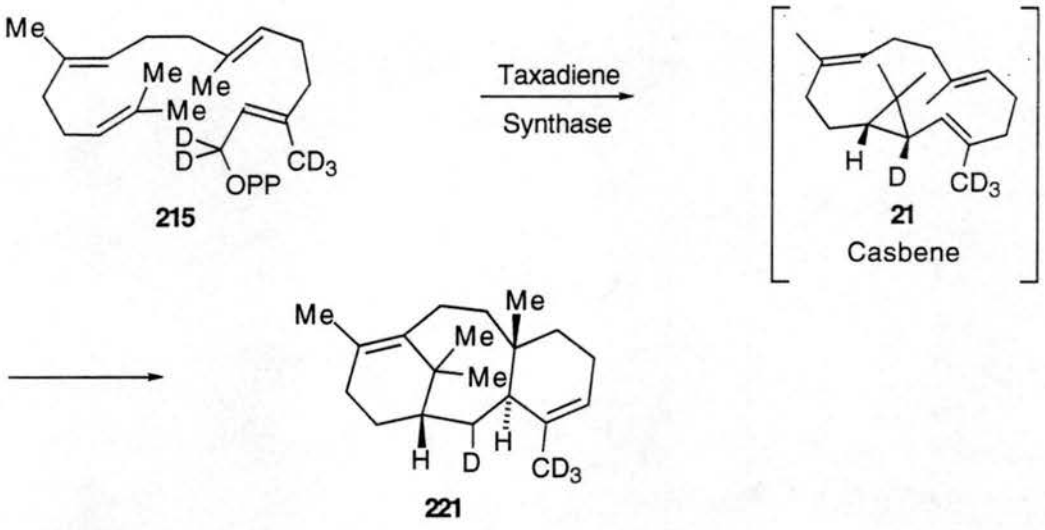


216 **220**

Scheme 4.3 outlines an additional experiment that was performed to examine the presumed C-5 (taxane numbering) deprotonation step in the cyclization to taxa-4(5),11(12)-diene. From Table 4.1 it can be seen that isotopomer **216** underwent cyclization to C-5(^{2}H)-taxa-4(5),11(12)-diene **220** with concurrent loss of a deuterium atom at the C-5 position (taxane numbering) from **216**. Based on these results, it was concluded that geranylgeranyl diphosphate is directly cyclized to taxa-4(5),11(12)-diene rather than proceeding through intermediate **18** (Scheme 4.1). In addition, these results appear to disfavor casbene **21** as an intermediate on route to C-20-($^2H_3$)-taxa-4(5),11(12)-diene (**221**). As outlined in Scheme 4.4, if casbene (**21**) were an intermediate, then one deuterium from C-2 (taxane numbering) of isotopomer **215** would be missing thus yielding **221** instead of taxadiene isotopomer **219**.[78]

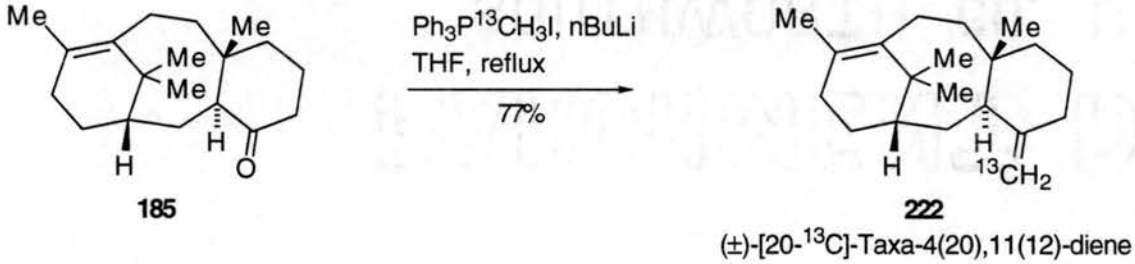
Several additional experiments were performed in an attempt to evaluate the role of (±)-[10-^{2}H]-verticilline (**223**), (±)-[20-^{13}C]-taxa-4(20),11(12)-diene (**222**), and (±)-casbene (**21**) as potential transient intermediates in the reaction cascade leading to taxa-4(5),11(12)-diene.[78]

Scheme 4.4 Proposed Conversion of **215** to **221** Via **21**.

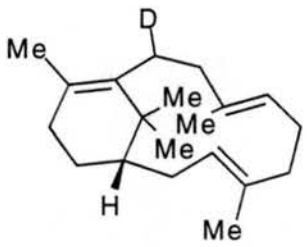


Scheme 4.5 outlines the synthetic route used to synthesize **222** which was subsequently used in Croteau's and Floss' study. Ketone **185**, prepared as previously described in Chapter 3, was subjected to a Wittig reaction with methyl-^{13}C-triphenyphosphonium iodide (99 atom% ^{13}C, purchased from Aldrich) to furnish **222** in 77% yield.

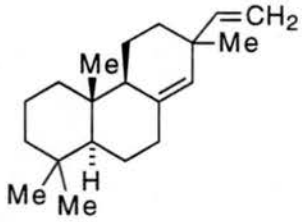
Scheme 4.5 The Synthesis of (±)-[20-^{13}C]-taxa-4(20),11(12)-diene (**222**).



The first set of experiments performed by Croteau and associates was designed to intercept a putative intermediate on route to taxa-4(5),11(12)-diene and thus dilute the product generated from tritium-labeled geranylgeranyl diphosphate (**37**). Tritium-labeled geranylgeranyl diphosphate was incubated with (±)-[10-^{2}H]-verticilline (**223**), sandaracopimaradiene (**224**), a natural product isolated from *Taxus brevifolia* which could not possibly serve as an intermediate in the taxadiene biosynthesis (Figure 4.2), (±)-[20-^{13}C]-taxa-4(20),11(12)-diene (**222**), and (±)-casbene (**21**) separately in a 1:1 ratio in the presence of taxadiene synthase. All compounds showed a 9-14% nonspecific inhibition of [^{3}H]-taxa-4(5),11(12)-diene formation indicating that little, if any, intervention of the olefins during cyclization had occurred. Also, mass spectral analysis of the [^{3}H]-taxa-4(5),11(12)-diene formed showed no stable isotope dilution.[78]



223
(±)-Verticilline



224
Sandaracopimaradiene

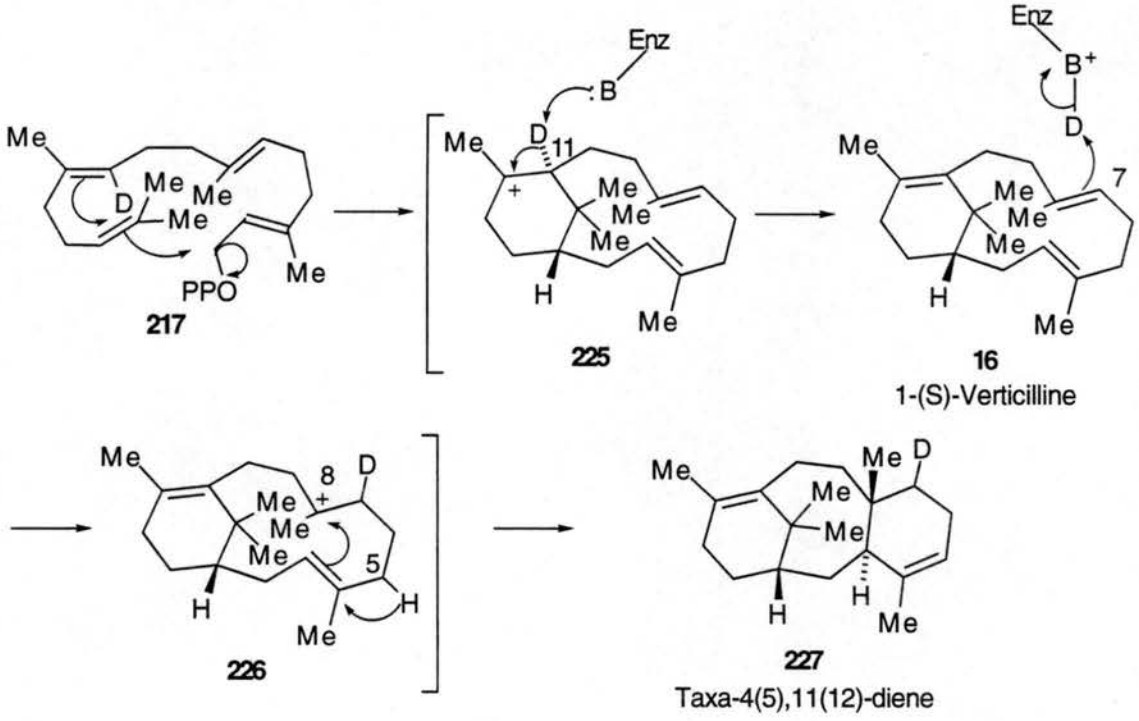
Figure 4.2 Additional Compounds Used in Croteau's studies.

The second set of experiments performed by Croteau and associates involved incubating (±)-[10-^{2}H]-verticilline (**223**), sandaracopimaradiene (**224**), (±)-[20-^{13}C]-taxa-4(20),11(12)-diene (**222**), and (±)-casbene (**21**) separately in a 1:1 ratio with tritium-labeled geranylgeranyl diphosphate (**37**) in the presence of taxadiene synthase and looking for trapped radioactivity in the putative biosynthetic precursors verticilline, taxa-4(20),11(12)-diene, and casbene. Each putative precursor was purified and examined for its radioactive content using a LSC (Liquid Scintillation Counter). Casbene, taxa-4(20),11(12)-diene, and sandaracopimaradiene contained negligible radioactivity, while

verticilline contained 2.8% of the tritium label as compared to [^{3}H]-taxa-4(5),11(12)-diene.[78]

The last set of experiments performed involved the direct examination of the conversion of the putative biosynthetic precursors to taxa-4(5),11(12)-diene using taxadiene synthase. Thus, incubation of taxadiene synthase separately with (±)-casbene, (±)-verticilline, and (±)-taxa-4(20),11(12)-diene showed no conversion to taxa-4(5),11(12)-diene. Based on the preceding results, it is likely that casbene and taxa-4(20),11(12)-diene are not transient intermediates along the way to taxa-4(5),11(12)-diene starting from geranylgeranyl diphosphate while verticlline cannot be ruled out as a transient intermediate.

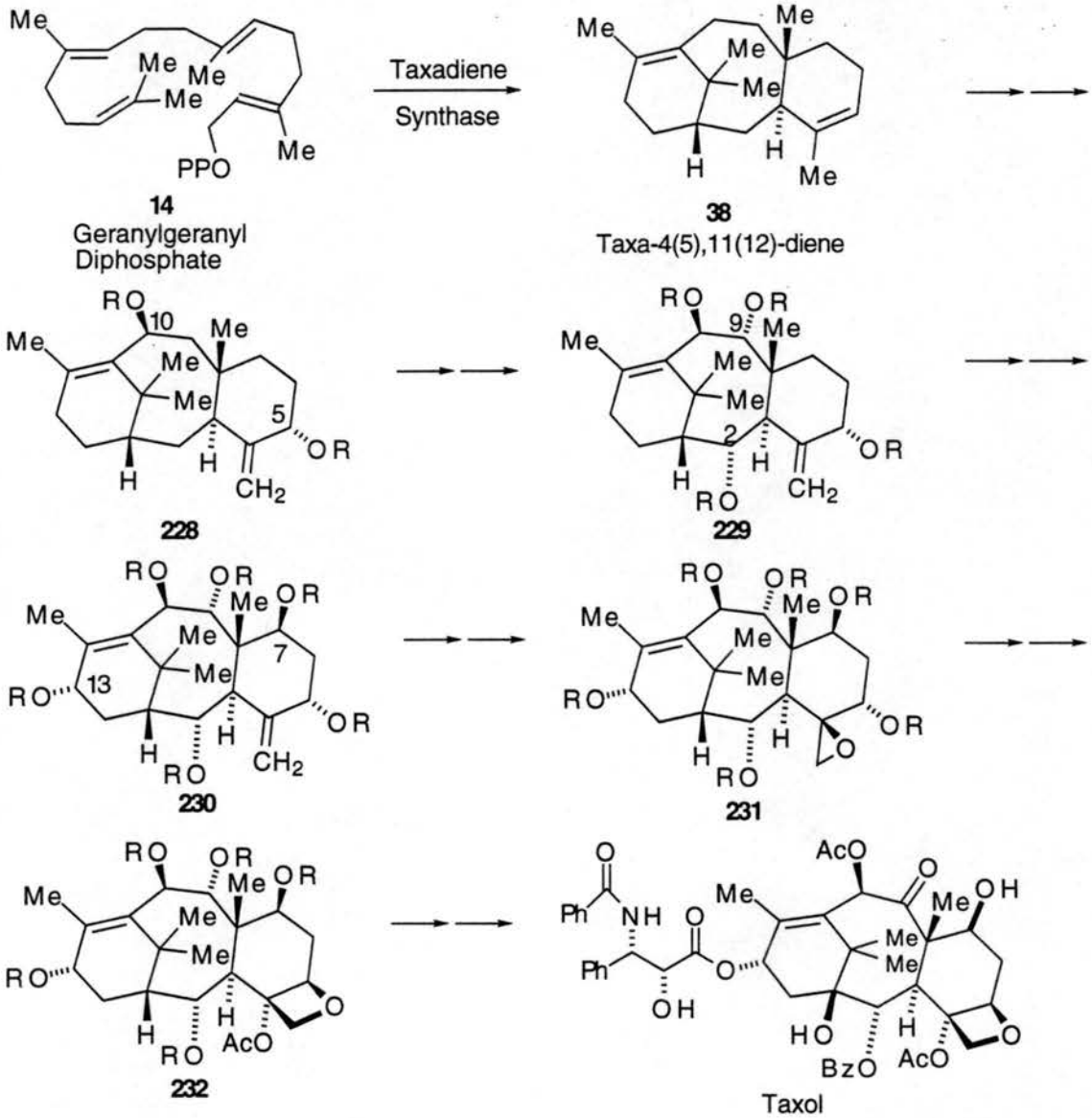
Scheme 4.6 The Taxadiene Synthase Catalyzed Intramolecular Proton Transfer.



Inspection of models by Floss[1h] shows that it is possible for geranylgeranyl diphosphate to undergo A-ring closure followed by an enzyme mediated 11-α

deprotonation to yield 1-(S)-verticilline as a transient intermediate. The protonated enzyme could then transfer the proton to C-7 which would then induce B/C ring closure followed by C-5 deprotonation to furnish taxa-4(5),11(12)-diene.

Scheme 4.7 Proposed Hydroxylation Steps Leading to Taxol

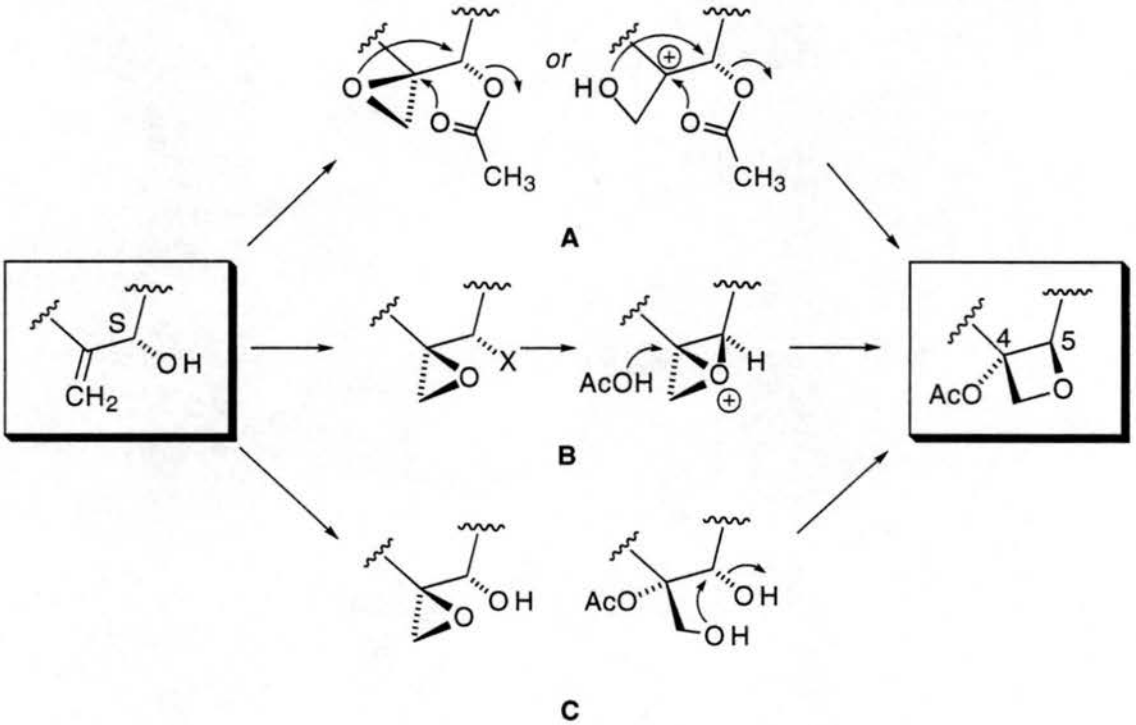


In order to test this hypothesis, the enzymatic conversion of [^{3}H]-geranylgeranyl diphosphate to taxa-4(5),11(12)-diene in ^2H_2O was examined. Table 4.1 shows that the taxadiene generated is largely devoid of deuterium, a result consistent with (non-solvent

donated) intramolecular proton transfer. An additional experiment was performed to directly test Floss's hypothesis. Deuterated geranylgeranyl diphosphate (**217**) was incubated with taxadiene synthase. Table 4.1 outlines the mass spectral results of this experiment while Scheme 4.6 illustrates these results. From the table, one can conclude the C-11 deuterium (taxane numbering) gets abstracted by the taxadiene synthase followed by intramolecular deuterium transfer to C-7. The C-7 protonation presumably generates a C-8 cation which promotes the B/C ring closure followed by C-5 deprotonation to furnish taxa-4(5),11(12)-diene.[78]

The first committed step in the biosynthetic pathway of taxol production has been established based on the structural identification of taxa-4(5),11(12)-diene, its mechanistic origin from geranylgeranyl diphosphate, and its subsequent incorporation into taxol. Scheme 4.7 outlines an updated biosynthetic route, originally proposed by Gueritte-Voegelein and co-workers[8] and later by Floss[1h], to taxol from geranylgeranyl diphosphate.

Scheme 4.8 Possible Mechanisms for Oxetane Formation from the 5-(S)-Alchol.

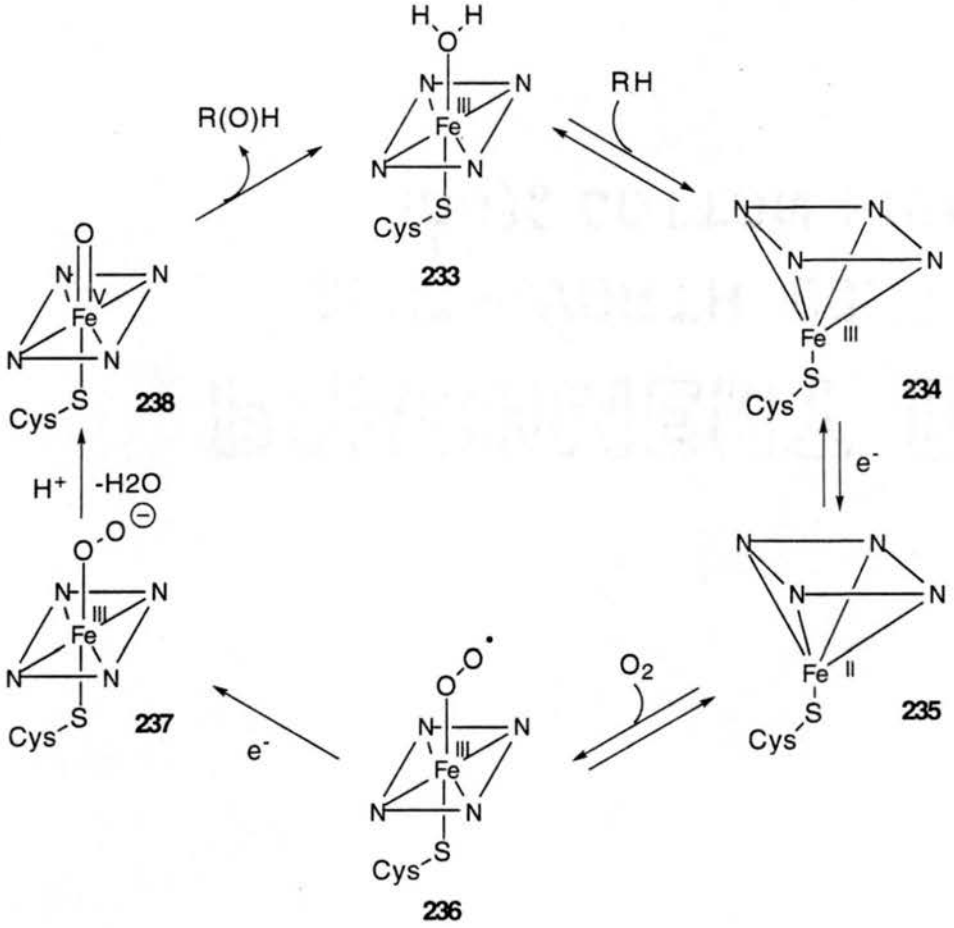


Based on the preceding observations by Floss, Croteau and associates,[21,22,78] geranylgeranyl diphosphate cyclizes to taxa-4(5),11(12)-diene. It is proposed that the first hydroxylation steps occur at C-5 and C-10, based on naturally occurring taxanes, thus furnishing **228**. The subsequent hydroxylations at C-2, C-9 are then thought to occur to afford **229**. It is believed that **229** is further oxidized at C-13 and C-7 to produce taxoid **230** followed by epoxidation at the C-4,C-20 double bond to yield **231**. Epoxide **231** is then thought to undergo ring expansion to produce the intact oxetane ring of **232**. The three most likely mechanisms for this transformation are outlined in Scheme 4.8. Of the three possible routes shown, only route C has been modeled successfully in a simpler system while route B has not been modeled successfully. Athough untested, path A has been proposed by Gueritte-Voegelein and co-workers[8] as the most plausible route for oxetane ring formation.[1h] Oxidation at C-1 and C-9 followed by C-13 side chain installation and acylation(s) of **232** would then furnish taxol. In addition, it is likely that a series of putative acylations/deacylations are required throughout the biosynthesis of taxol.

The plant cytochrome P-450 enzymes are probably responsible for the oxidations on taxa-4(5),11(12)-diene that lead to taxol production. This class of enzymes oxidize a wide variety of terpene type compounds via hydroxylations and epoxidations.[80] Scheme 4.9 outlines a plausible mechanism for plant cyctochrome P-450 oxidations based on work performed by Dawson and Sligar dealing with the bacterial cyctochrome P-450$_{CAM}$ oxidative system isolated from *Pseudomonas putida*.[81a] The terpene hydroxylase systems contain a cytochrome protein and a NAD(P)H-cytochrome P-450 reductase (flavoprotein) which oxidizes terpenes in the presence of molecular oxygen. The low spin Fe^{3+} porphyrin complex (**233**) becomes high spin upon substrate binding (**234**). An electron is then transferred from NAD(P)H via a CP-450 reductase to furnish a high spin Fe^{2+} -substrate bound complex (**235**). Molecular oxygen then binds to **235** to afford low spin Fe^{3+} radical **236**. A second electron is then transferred to furnish what is believed to be the Fe^{3+} -peroxo species (**237**). This complex is protonated followed by loss of water to afford the

oxo-ferryl species (**238**). The resulting reactive species (**238**) then furnishes the oxidized terpene, water, and the regenerated Fe^{3+} cytochrome protein (**233**).[80, 81a]

Scheme 4.9 Proposed Cytochrome P-450 oxidation of Terpenes.



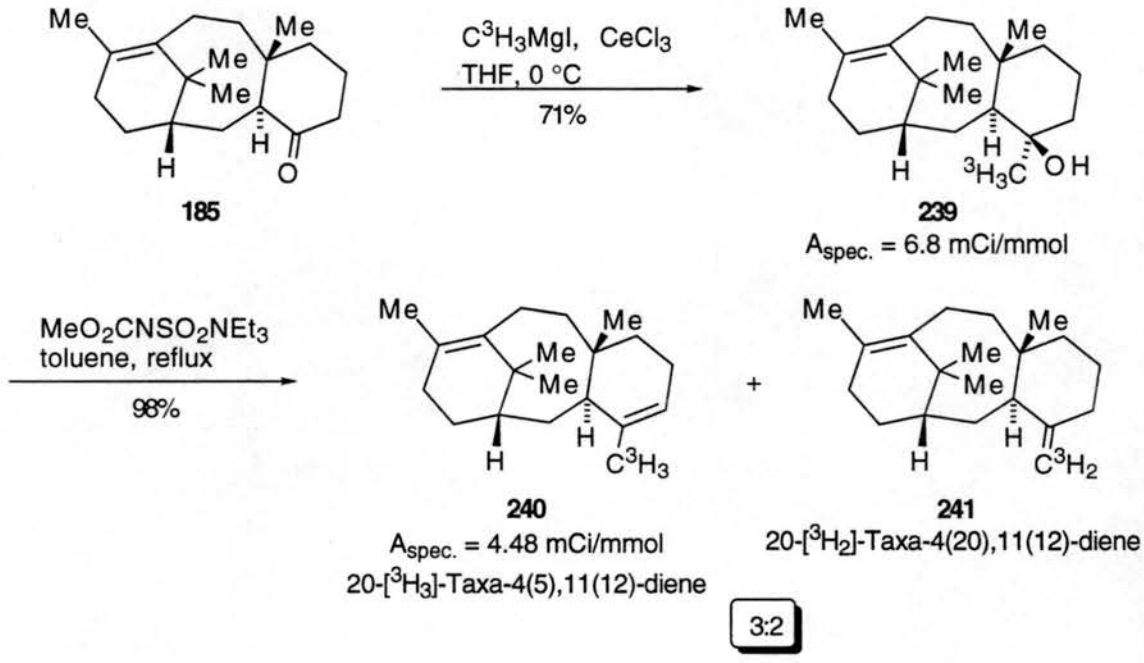
In order to demonstrate the involvement of a cytochrome P-450 enzyme, one needs to isolate the cyctochrome and the NAD(P)H-cytochrome P450 reductase activity located in the light membrane (microsomal) fraction of the plant cell. The requirement of NAD(P)H and molecular oxygen are indicators that a cytochrome P-450 enzyme is at work. Additional experiments such as carbon monoxide inhibition followed by regeneration of activity using blue light (450 nm) are also indicators of cytochrome P-450 activity.[80]

4.2 Results and Discussion

4.2a The First Hydroxylation Step

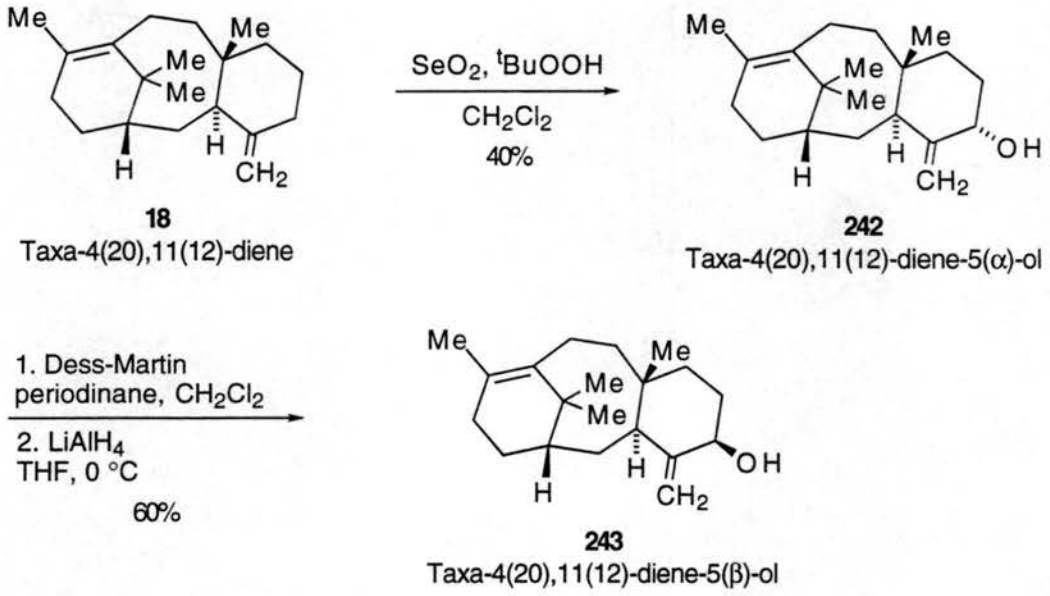
In order to elucidate the subsequent oxidations on taxa-4(5),11(12)-diene to taxol, a convenient synthesis of [^{3}H]-taxa-4(5),11(12)-diene was needed. Scheme 4.10 outlines the approach to [^{3}H]-taxa-4(5),11(12)-diene. Tritiated methylmagnesium iodide was generated by heating at reflux, 10 mCi of tritiated methyl iodide (purchased from Amersham), diluted with unlabeled methyl iodide, with magnesium in ether. The tritium-labeled Grignard was slowly added to a solution of cerium trichloride and ketone **185** at 0 °C in THF to afford alcohol **239** in 77% yield with a calculated specific activity of 6.8 mCi/mmol. Taxadienes **240** and **241** were synthesized as an approximate 3:2 mixture respectively in 98% yield using the Burgess reagent. The calculated specific activity for 20-[$^3H_3$]-taxa-4(5),11(12)-diene (**240**) is 4.48 mCi/mmol.

Scheme 4.10 The Synthesis of Tritium Labeled Taxadienes **240** and **241**.



Cell cultures of *Taxus canadensis* were used as the source for the microsomal cytochrome P-450 systems. The microsomal cytochrome systems were isolated via centrifugation. The pellet was resuspended in an assay buffer and a portion of the resulting solution was transferred, diluted with more assay buffer, and incubated with 20-[$^3H_3$]-Taxa-4(5),11(12)-diene (**240**) in the dark at 31 °C for 70 min. The reaction was subsequently extracted with ether and was observed to contain a new unidentified radioactive mono-oxygenated product, presumed to be taxa-4(20),11(12)-5(α)-ol (**242**), based on g.l.c.-M.S. analysis.

Scheme 4.11 The Synthesis of Taxa-4(20),11(12)-diene-5-ols **242** and **243**.



18
Taxa-4(20),11(12)-diene

242
Taxa-4(20),11(12)-diene-5(α)-ol

243
Taxa-4(20),11(12)-diene-5(β)-ol

The C-5 α allylic alcohol was synthesized as outlined in Scheme 4.11. Taxa-4(20),11(12)-diene (**18**) was oxidized using tert-butyl hydroperoxide with a catalytic amount of selenium dioxide to afford taxa-4(20),11(12)-diene-5(α)-ol (**242**) in 40% yield. Because it is mechanistically possible, though highly unlikely, that taxa-4(20),11(12)-diene-5(β)-ol (**243**) could also act as a precursor to the oxetane functionality of taxol, this compound was also synthesized as outlined in Scheme 4.11. Taxa-4(20),11(12)-diene-

5(α)-ol (**242**) was oxidized using the Dess-Martin periodinane reagent followed by a stereoselective reduction to furnish taxa-4(20),11(12)-diene-5(β)-ol (**243**) in 60% yield for the two steps. The relative stereochemistry at C-5 for alcohols **233** and **234** were assigned based on ^{1}H spin-spin coupling constants and nOe studies (see Appendix).

Taxa-4(20),11(12)-diene-5(α)-ol (**242**) had the same g.l.c. retention time and mass fragment pattern as the radioactive mono-oxygenated product produced by the incubation of taxadiene **240** with the cell-free cytochrome P-450 microsomal assay. On the other hand, exhaustive searching for taxa-4(20),11(12)-diene-5(β)-ol (**243**) in the microsomal extract proved unsuccessful. Tritium labeled taxa-4(20),11(12)-diene-5(α)-ol was subsequently synthesized in 52% yield as outlined in Scheme 4.11 using **241**. The taxadienol was calculated to have a specific activity of 3.54 mCi/mmol. The [C-20-$^3H_2$]-taxa-4(20),11(12)-diene-5(α)-ol was then incubated with *Taxus brevifolia* stem discs for a two, four, and six day time-course period. The specific incorporation of taxa-4(20),11(12)-diene-5(α)-ol into taxol, 10-deacetylbaccatin III, and cephalomannine is outlined in Table 4.2. The rate of taxa-4(20),11(12)-diene-5(α)-ol incorporation is similar to the rate of incorporation of taxa-4(5),11(12)-diene into these advanced taxanes and thus, confirms that taxa-4(20),11(12)-diene-5(α)-ol is a biosynthetic intermediate of taxol.

Table 4.2 Specific Incorporation of Taxa-4(20),11(12)-diene-5α-ol into Advanced Taxoids.

	Time-Course (days)		
Advanced Taxoids	2	4	6
Taxol	0.28%	0.58%	0.77%
10-Deacetylbaccatin III	0.96%	1.65%	3.07%
Cephalomannine	0.16%	0.28%	0.35%

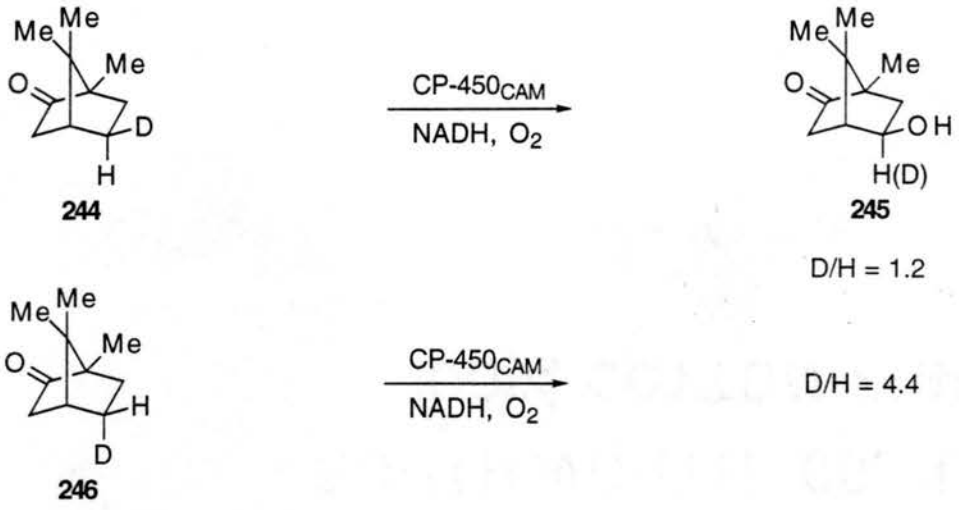
Additional experiments were performed on the cell-free cytochrome P-450 microsomal assay in Croteau's lab, they include carbon monoxide inhibition studies followed by blue light regeneration and comparing the rate of oxidation of the complete CP-450 system with the CP-450 system that was missing either flavins, oxygen, NADPH, or a NADPH regenerating system. The results from these experiments are consistant for the

operation of a cytochrome P-450 enzyme.[80b] Based on these results, it appears the first hydroxylation step in the biosynthetic pathway to taxol is the cytochrome P-450-mediated stereoselective C-5 oxidation of taxa-4(5),11(12)-diene (**38**) to taxa-4(20),11(12)-diene-5(α)-ol (**242**).

4.2b Mechanism of the Cytochrome P-450 Enzyme

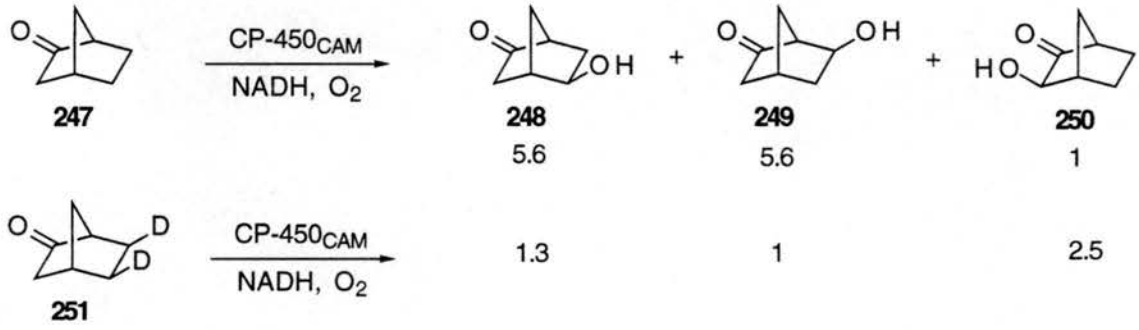
Initial mechanistic studies on the CP-450_{CAM} enzyme, isolated from *Psuedomonas putida*, by Sligar and Dawson have shown that deuterium kinetic isotope effects as it relates to the overall velocity of the reaction is either very small or non-existent.[81a-c] In most cases, the second electron transfer usually controls the overall rate of the oxidation. However, significant kinetic isotope effects have been observed in these systems when product regioselectivity and intramolecular hydrogen/deuterium competition abstraction experiments were examined. For example, Sligar has shown that when camphor (**244**) (which has 90% of the deuterium label at the 5-exo position) was incubated with the CP-450_{CAM} system, a reaction known to furnish 5-exo-alcohol (**245**) exclusively, the ratio of deuterium to hydrogen at the endo position was 1.2:1 (Scheme 4.12). When camphor (**246**) (which had 95% of the deuterium label at the 5-endo position) was incubated with the CP-450_{CAM} system, **245** was formed exclusively. The ratio of deuterium to hydrogen at the endo position was 4.4:1. These results show that hydrogen abstraction preferentially takes place at the C-5-exo position however, when there is a deuterium located at this position a significantly greater amount of C-5-endo abstraction takes place. It was assumed that the hydrogen abstraction proceeded via a radical pathway rather than a hydride pathway since no iron-oxo species are known to formally abstract hydride anion. The cytochrome P-450_{CAM} and other microsomal P-450 systems are expected to proceed through the same mechanism.[81b]

Scheme 4.12 CP-450$_{CAM}$-Mediated Oxidations on **244** and **246**.



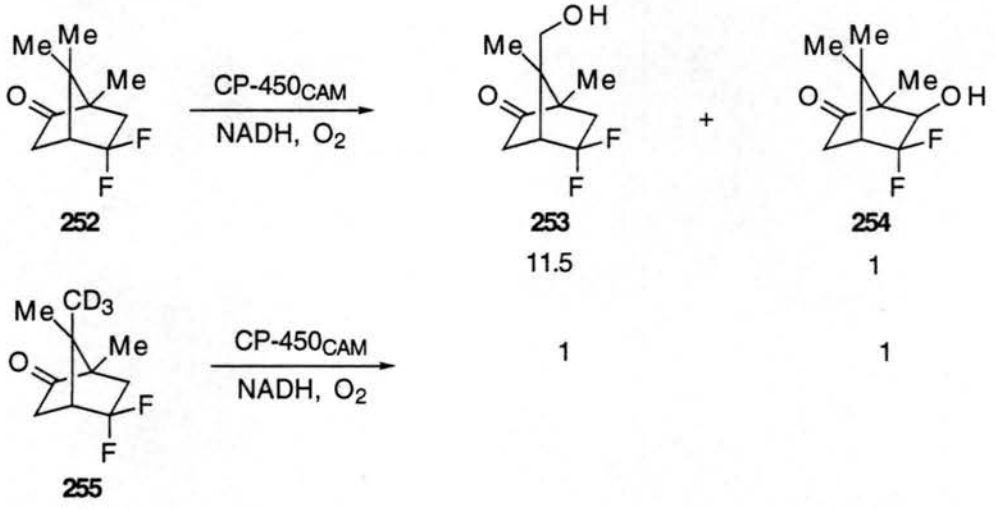
Sligar has also shown how kinetic isotope effects can control the regiochemistry observed for the CP-450$_{CAm}$ oxidations (Scheme 4.13). Unlabeled norcamphor (**247**) was oxidized by the CP-450$_{CAM}$ system at the C-5 (**248**), C-6 (**249**), and C-3 (**250**) positions in a 5.6:5.8:1 ratio respectively. When the 5,6-exo-dideuteronorcamphor (**251**) was subjected to the same conditions, a significant kinetic isotope effect was observed causing a decrease in the production of **248** and **249** and an increase in the production of **250**. An intrinsic isotope effect of 3.78 was calculated. This value is small and was proposed to be masked by some other slow step, ie. the second electron transfer step.[81c]

Scheme 4.13 Regiochemical Control via a Deuterium Kinetic Isotope Effect.

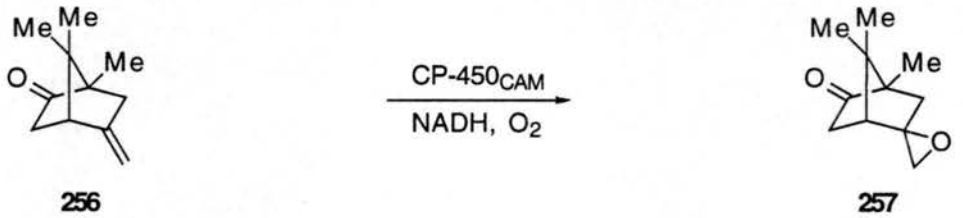


Dawson has also observed a significant kinetic isotope effect during a CP-450_{CAM} oxidation on camphor derivative **255**. Scheme 4.14 outlines the results. When camphor derivative **252** was incubated with the CP-450_{CAM} system, alcohols **253** and **254** were formed in a 11.5:1 ratio respectively. When camphor derivative **255** was subjected to the same conditions a kinetic isotope effect of 11.8 was observed which caused a decrease in the production of **253** and an increase in the production of **254**. Because of the high kinetic isotope effect, it was proposed that the hydrogen abstraction was the sole rate-limiting step and that the overall velocity was not effected by the second electron transfer step. In addition , Dawson has shown that the CP-450_{CAM} system can also epoxidize camphor derivative **256** which furnished **257** as outlined in Scheme 4.15.[81a]

Scheme 4.14 CP-450_{CAM}-Mediated Oxidations on **252** and **255**.



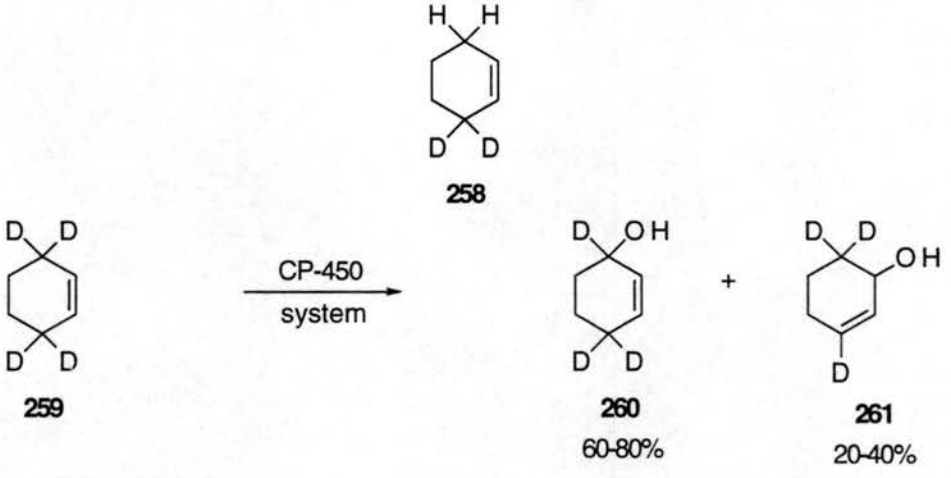
Scheme 4.15 CP-450_{CAM}-Mediated Epoxidation.



Groves has shown that cycloalkene **258** underwent a hydrogen/deuterium competitive abstraction experiment using a cytochrome P-450 system in which a kinetic isotope effect of ~5 was calculated (Scheme 4.16). Groves also examined the position of oxygenation using alkene **259** as outlined in Scheme 4.16. The major product formed was the unrearranged allylic alcohol **260** in 60-80% yield along with rearranged allylic alcohol **261** in 20-40% yield. A radical mechanism can explain both the kinetic isotope effect observed for alkene **258** and partial allylic rearrangement observed for alkene **259** when incubated with a CP-450 system.[81d]

Based on the cytochrome P-450 systems previously discussed, it appears that the rate limiting step can either be transfer of the second electron to form the iron-peroxo species **237** or homolytic hydrogen abstraction. In some cases both steps appear to be partially rate limiting. In addition, it appears that the CP-450_{CAM} enzyme is promiscuous and thus is capable of oxidizing a wide variety of camphor derivatives.

Scheme 4.16 CP-450-Mediated Alyllic Oxidation.

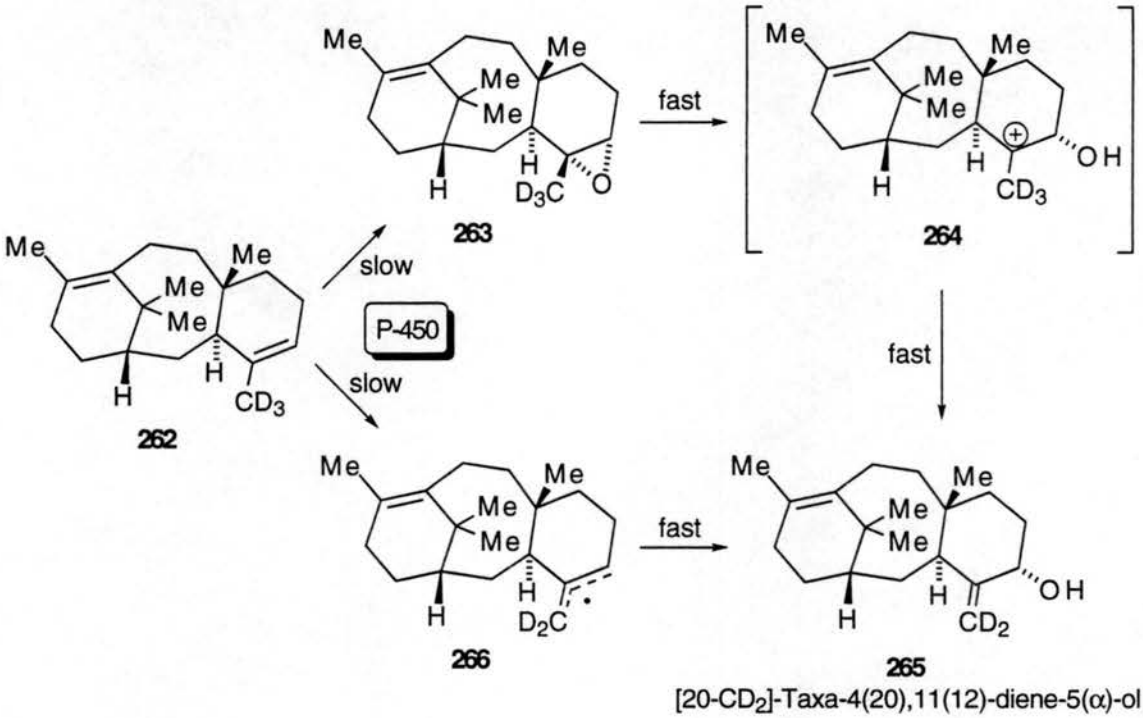


If a cytochrome P-450 enzyme is responsible for the C-5 oxidation, based on literature precedent, there are two plausible mechanisms for this observed oxidation as outlined in Scheme 4.17.[81, 82] The first mechanism involves a presumed rate-limiting

epoxidation step followed by a fast sequential ring opening to furnish cation **264** and deuteride elimination to produce the desired allylic alcohol **265**. The second mechanism involves a presumed rate-limiting carbon deuterium homolytic bond cleavage to furnish allylic radical species **266** followed by a fast oxygen transfer to provide allylic alcohol **265**.

It was anticipated that [C-20-CD_3]-taxa-4(5),11(12)-diene **262** could be utilized to distinguish between the two proposed mechanisms outlined in Scheme 4.17. Taxadiene **262** was expected to produce a kinetic isotope effect of 1-12 depending on the importance of the homolytic hydrogen abstraction as it relates to the rate-limiting transition state while a negligible kinetic isotope effect was expected if the epoxide mechanism was operable assuming the deprotonation during the E1-type elimination was not rate-limiting.[82]

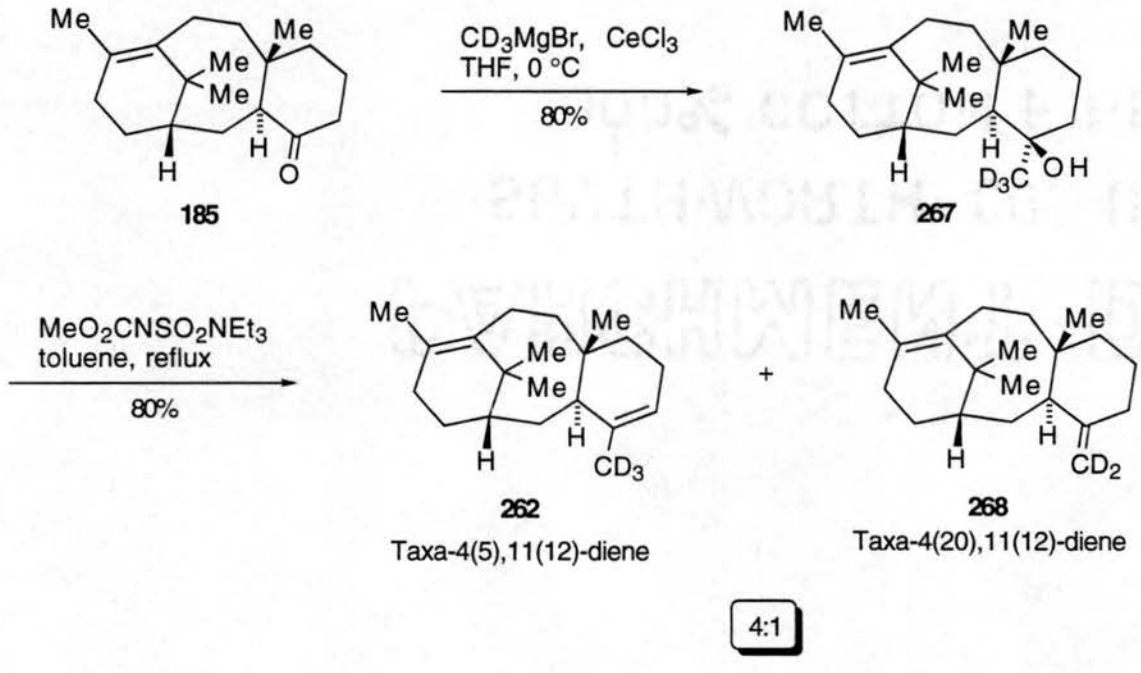
Scheme 4.17 Proposed Mechanisms for Taxa-4(20),11(12)-diene-5(α)-ol Formation.



Scheme 4.18 outlines the synthesis of taxadienes **262** and **268**. Ketone **185** was allowed to react with deuterated methylmagnesium bromide (99 atom D% purchased from Aldrich) in the presence of cerium trichloride which afforded alcohol **267** in 80% yield. As discussed in Chapter 3, unlabeled taxadienes **18** and **38** were synthesized as a 1:1 mixture from alcohol **210**. However, a total kinetic isotope effect of ~4 was seen when the kinetically formed taxadienes **262** and **268** were synthesized as a 4:1 mixture respectively from alcohol **267** in 80% yield using the Burgess reagent.

This result was somewhat unexpected since Burgess and co-workers have suggested that in other systems the rate-limiting step of an elimination reaction using the Burgess reagent involves formation of an ion pair followed by a fast β-*cis*-deprotonation (Scheme 3.15).[77a] If this mechanism were operable in the taxadiene system **267** no kinetic isotope effect should have been observed. The mechanism for dehydrating **267** to afford **262** and **268** must therefore be more concerted in which β-*cis*-deprotonation is involved in the transition state.

Scheme 4.18 The Synthesis of Deuterium-Labeled Taxadienes **262** and **268**.

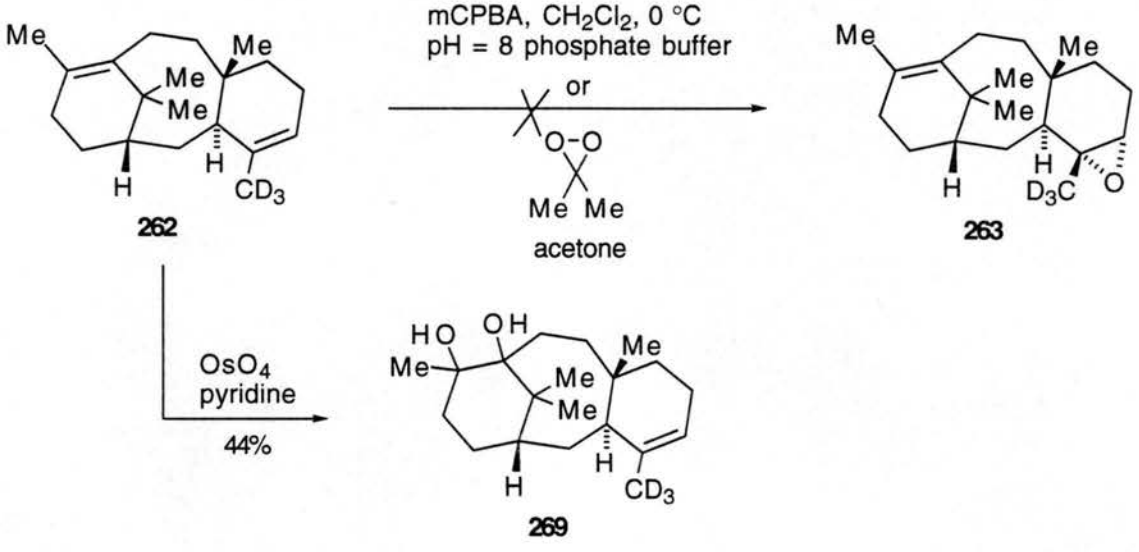


$$\text{Total Kinetic Isotope Effect} = (\text{primary})(\text{secondary})^2(\beta)^3 \qquad \text{Eq. 4.1}$$

$$\text{Primary } K_H/K_D = \frac{0.1865\ \text{K·cm}}{398\ \text{K}}\ (3000\ \text{cm}^{-1}) \qquad \text{Eq. 4.2}$$

The observed total kinetic isotope effect of 4 is the product of the primary, secondary, and β-isotope effects as outline in Equation 4.1. If one assumes that the transition state is linear and symmetrical and the ground state carbon-hydrogen bond stretching frequency is 3000 cm^{-1} then the upper limit for the observed primary kinetic isotope effect at 398 K is 4.4, based on Equation 4.2.[81e] The observed total kinetic isotope effect is very close to the calculated upper limit. Based on Equation 4.1, it would appear that the secondary and β-isotope effects are negligible.

Scheme 4.19 Attempted synthesis of epoxide **263**.



The synthesis of epoxide **263** was also attempted with the desire of directly testing the proposed epoxide mechanism. Even though the C-11(12) double bond is more

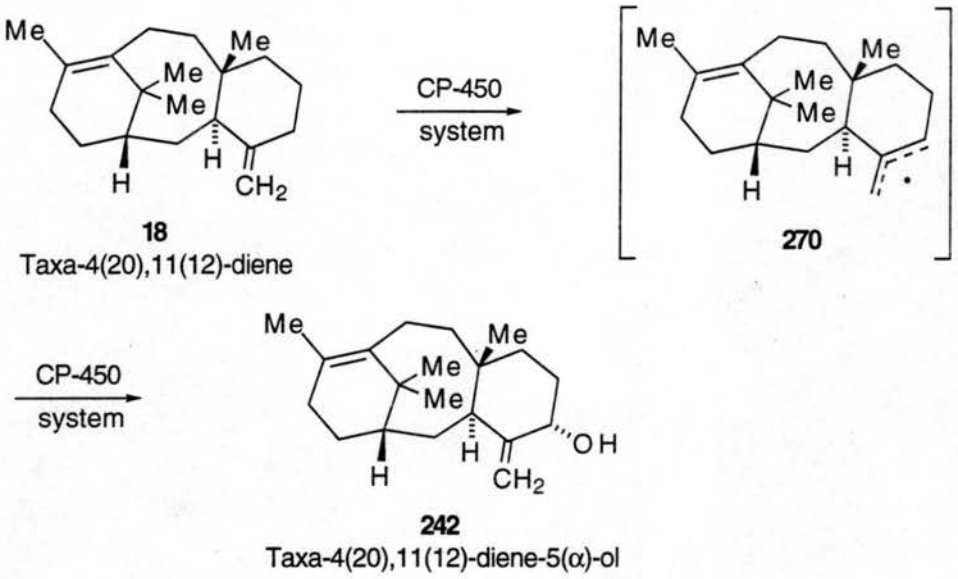
electron-rich than the C-4(5) double bond, it was anticipated that steric effects would allow for the selective epoxidation at the C-4(5) double bond. Unfortunately, all attempts to synthesize epoxide **236** either directly or indirectly were unsuccessful, as shown in Scheme 4.19.

Direct epoxidation using mCPBA under basic conditions or dimethyl dioxirane under neutral conditions afforded an unidentifiable mixture of products. An indirect dihydroxylation reaction was also employed. However, instead of isolating the desired C-4, C-5 diol, the C-11, C-12 diol was observed as the major product.

Deuterated taxadiene **262** and tritiated taxadiene **240** were incubated separately with the cytochrome P-450 microsomal extract in the dark at 31 °C for 70 min. It should be noted that the amount of tritium in the tritium-labeled taxadiene **240** is negligible compared to the amount of deuterium in deuterated taxadiene **262** and is thus considered unlabeled as it pertains to this experiment. The amount of taxa-4(20),11(12)-diene-5(α)-ol produced by each incubation was quantified relative to an internal standard (abietadienol). Unexpectedly, the deuterated diene produced 1.7 times more taxa-4(20),11(12)-diene-5(α)-ol than the tritium-labeled diene. This result indicates that, within the limits of experimental error, there was no significant deuterium isotope effect in this transformation. While this result might argue in favor of the epoxide mechanism, it is possible that the rate limiting step for both postulated mechanisms is the release of the product from the active site of the hydroxylase enzyme or the transfer of the second electron to the CP-450 active site as previously described . Continuing efforts are underway to synthesize epoxide **263** in an effort to determine the relevance of this hypothetical intermediate.

If one assumes that the cytochrome P-450 enzyme system responsible for converting **18** to **242** is as promiscuous as the CP-450CAM enzyme, then a series of indirect experiments could be performed that might help elucidate the mechanism of this transformation. Scheme 4.20 outlines one such experiment.

Scheme 4.20 Allylic Radical Generation via a Cytochrome P-450 Enzyme.

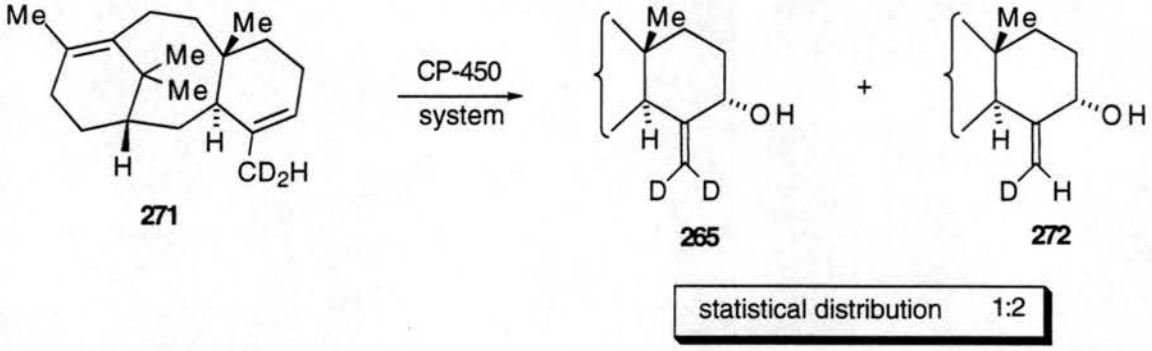


18
Taxa-4(20),11(12)-diene

270

242
Taxa-4(20),11(12)-diene-5(α)-ol

Scheme 4.21 Intramolecular Hydrogen/Deuterium Competition Experiment.



271

265

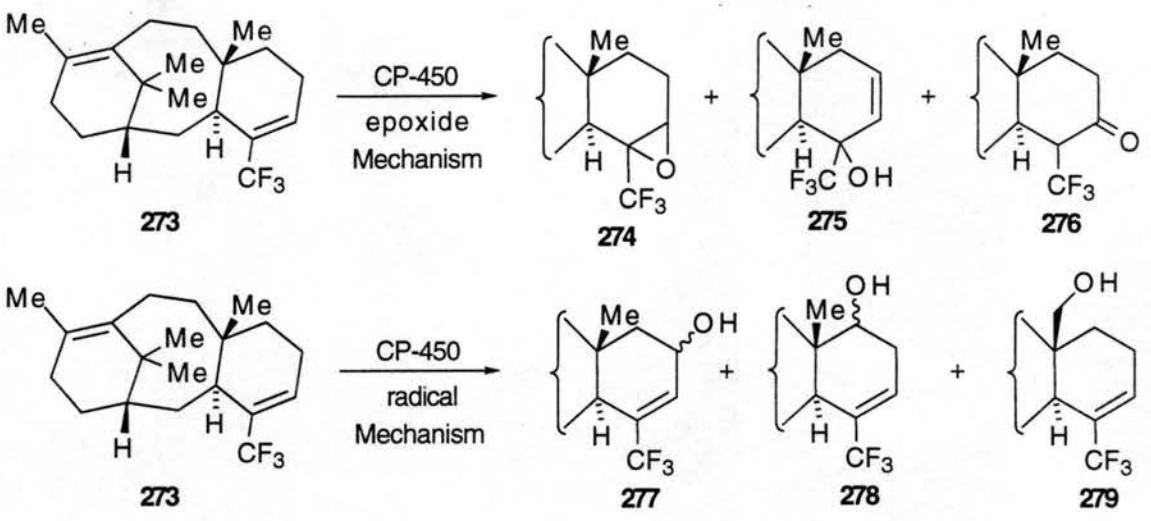
272

If the homolytic bond cleavage mechanism is operable, then **18** could lose a hydrogen at C-5 and thus generate allylic radical **270** which could then undergo oxidation at C-5 to yield **242**. Scheme 4.21 outlines another experiment that could aid in determining the mechanism of this cytochrome P-450 oxidation.

If taxadiene **271** was oxidized by the CP-450 system via the epoxide mechanism and if the E1-type elimination was not rate-limiting or if the radical abstraction was not rate-limiting then one would expect to see a statistical 1:2 ratio of allylic alcohols **265** and **272**

respectively. However, if hydrogen abstraction was at least partially rate-limiting, as expected in the radical mechanism, then one would expect to see a **265** to **272** ratio approaching 1:1. Scheme 4.22 outlines a third experiment that might aid in distinguishing between the two proposed mechanisms.

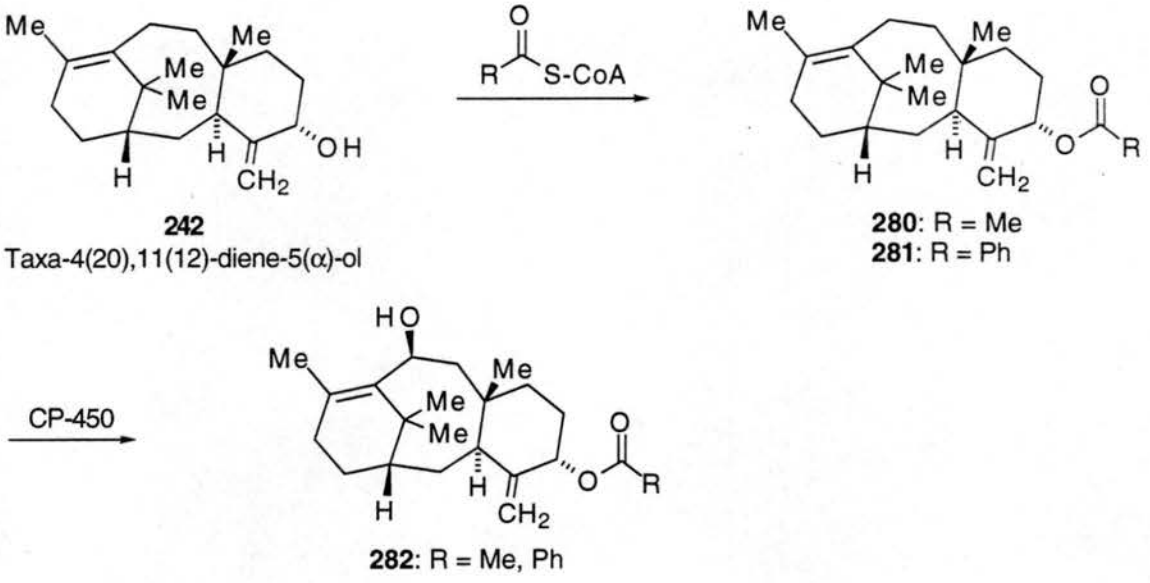
Scheme 4.22 Proposed Products from the CP-450-mediated Oxidation of 273.



If the epoxide mechanism were operable then **273** would be expected to furnish **274**. Taxadiene **274** could rearrange to produce **275** and/or **276** depending on the stability of **274** and the work-up conditions. If the radical mechanism were operable, then **273** would be expected to furnish monooxygenated compounds such as **277**, **278**, and **279**. If this assay could be run on a large enough scale, one could then directly test for the production of these proposed intermediates via ^{1}H NMR analysis. Alternatively, If compounds **274-279** could be synthesized then one could run HPLC traces of the synthesized standards against the CP-450 assay to detect if any of the proposed products were produced.

4.2c The Third Biosynthetic Step: Acylation at the C-5 Alcohol

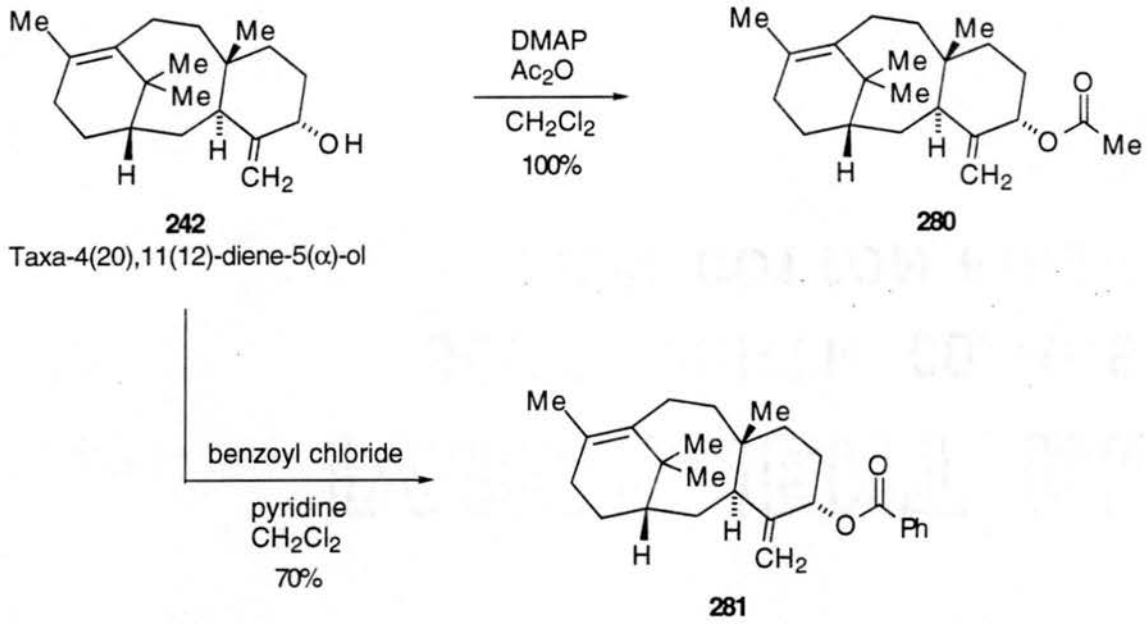
Scheme 4.23 Putative Acylation Required for Subsequent CP-450 Hydroxylation.



As mentioned earlier, a series of putative acylation/deacylation reactions are probably required to convert taxa-4(5),11(12)-diene to taxol. It is possible that in order for taxadienol **242** to get converted to the next hydroxylated product, an acylation at the C-5 alcohol is required, as shown in Scheme 4.23. This rationale can also hold for the subsequent hydroxylation reactions. In order to test this theory, compounds **280** and **281** were synthesized in tritium-labeled form from [C-20-$^3H_2$]-taxa-4(20),11(12)-diene-5(α)-ol using standard chemical procedures, as shown in Scheme 4.24.[83, 26b] Compound **242** was converted to acetate **280** in quantitative yield using acetic anhydride and to benzoate **281** in 70% yield using benzoyl chloride and pyridine.

[C-20-$^3H_2$]-taxa-4(20),11(12)-diene-5(α)-ol and [C-20-$^3H_2$]-taxa-4(20),11(12)-diene-5(α)-acetate were incubated separately with the cytochrome P-450 microsomal extract in the dark for 2h at 31 °C. The reactions were worked up and purified to yield the following observed results based on LSC (Liquid Scintillation Counting). The tritiated

Scheme 4.24 The Synthesis of **280** and **281**.



242
Taxa-4(20),11(12)-diene-5(α)-ol

280

281

5(α)-acetate was hydrolyzed in about 25% yield to afford the 5(α)-alcohol while 5-10% of the 5(α)-acetate was converted to unidentifiable more polar product(s). The tritiated 5(α)-acetate was converted to the more polar product(s) about twice as fast as the tritiated 5(α)-alcohol, even though a substantial amount was hydrolyzed, based on LSC. Although this number is qualitative, this experiment does support the hypothesis that acylation reactions are required to convert taxa-4(5),11(12)-diene to taxol. Also, it appears the cell-free microsomal extract used has a significant amount of esterase activity. In order to quantitatively compare the conversion rates of the tritiated 5(α)-acetate with the tritiated 5(α)-alcohol, a microsomal extract without esterase activity will be needed. The development of this assay is currently in progress in Croteau's lab.

Because the 5(α)-acetate (**280**) acts as a better substrate than 5(α)-alcohol in the P-450 microsomal assay, it is desirable to find out if there is a transacylase enzyme that can convert taxa-4(20),11(12)-diene-5(α)-ol (**242**) to taxa-4(20),11(12)-diene-5(α)-acetate (**280**). The transacylase assay was prepared from the green tips of a Pacific Yew sapling via a series of filtration and centrifugation steps. Incubation of tritiated taxa-4(20),11(12)-

diene-5(α)-ol with the transacylase system (exclusion of light not necessary) at 31 °C for 1.5 h showed conversion to tritiated taxa-4(20),11(12)-diene-5(α)-acetate. Continuing efforts in this area, including the viability of taxa-4(20),11(12)-diene-5(α)-benzoate as a precursor to the next hydroxylation step, is in progress in Croteau's lab.

4.2d *Taxus cuspidata* Cell Cultures Used in Biosynthetic Feeding Experiments

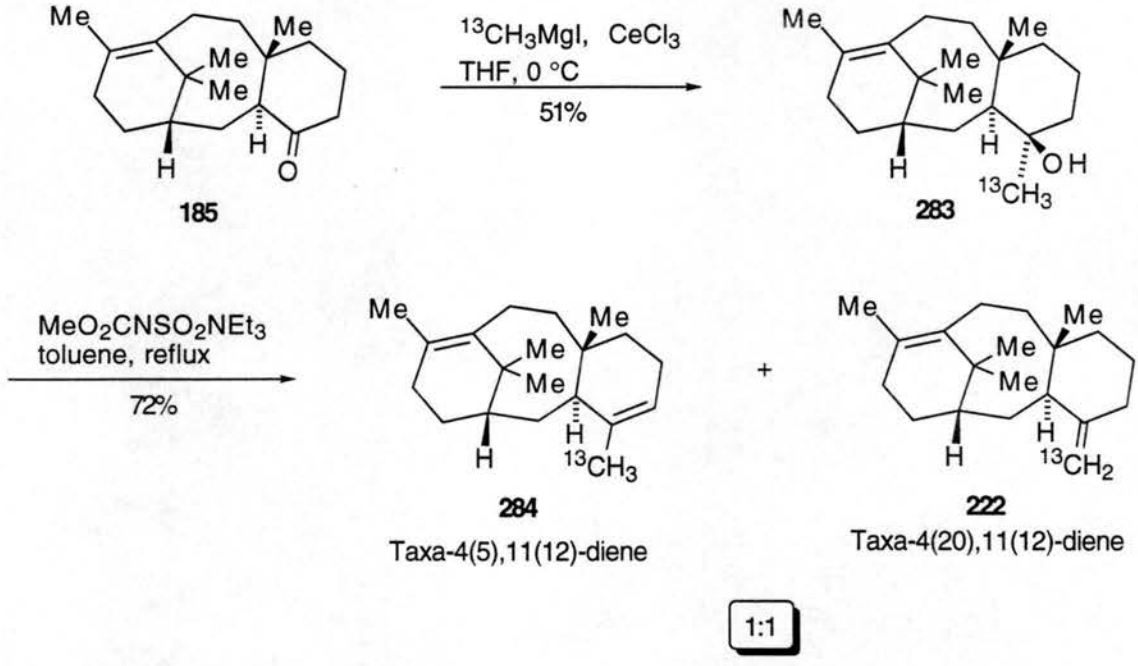
Cell cultures of *Taxus cuspidata* were considered as an alternative medium for performing biosynthetic feeding experiments.[84] *Taxus* cell cultures grow much faster than the mature *Taxus* tree and thus produce up to three times as much taxol per gram of dry weight of tissue. They also to excrete up to 80% of the taxol produced into the surrounding medium thus, making isolation quicker and easier.[85] Based on these advantages that *Taxus* cell cultures have over mature *Taxus* trees, it is anticipated that performing feeding experiments using cell cultures would allow for higher incorporation rates with a quicker, easier workup and purification protocol. In addition, higher incorporation rates should lead to the isolation of lightly hydroxylated intermediates of taxol in greater amounts which in turn should speed up the process of elucidating the biosynthetic pathway of taxol.

Recently, Floss and Linden have performed feeding experiments using deuterated β and α phenylalanine with *Taxus cuspidata* cell cultures. The deuterated phenylalanine was dissolved in ethanol, added to the suspension of cell cultures, and allowed to incubate for seven days. There was no observed incorporation. One potential problem with the experiment could have been cellular uptake of the deuterated substrate.[86]

Dimethyl sulphoxide is known to transverse cell membranes quite easily. When the biosynthetic feeding experiments were performed in collaboration with Linden's research group, DMSO (dimethyl sulphoxide) was separately incubated with one batch of the cell cultures while four other batches were incubated with 1.25 mg of [C-20-$^{13}CH_3$]-taxa-4(5),11(12)-diene **284** (Scheme 4.25) in the presence of ethanol for 7 days. The DMSO

blank run, in addition to the four batches treated with taxadiene **284**, produced the same amount of taxol as compared to the control run. However, after workup and purification of the four feeding experiment batches, taxol could not be detected by FAB mass spectral analysis and thus ^{13}C incorporation could not be ascertained. Because the cell culture is stable to DMSO, subsequent feeding experiments will be performed in the presence of DMSO in the hope of obtaining detectable incorporations. Additional modifications in the feeding experiment and workup procedures are currently in progress.

Scheme 4.25 The Synthesis of ^{13}C Labeled Taxadienes **284** and **222**.



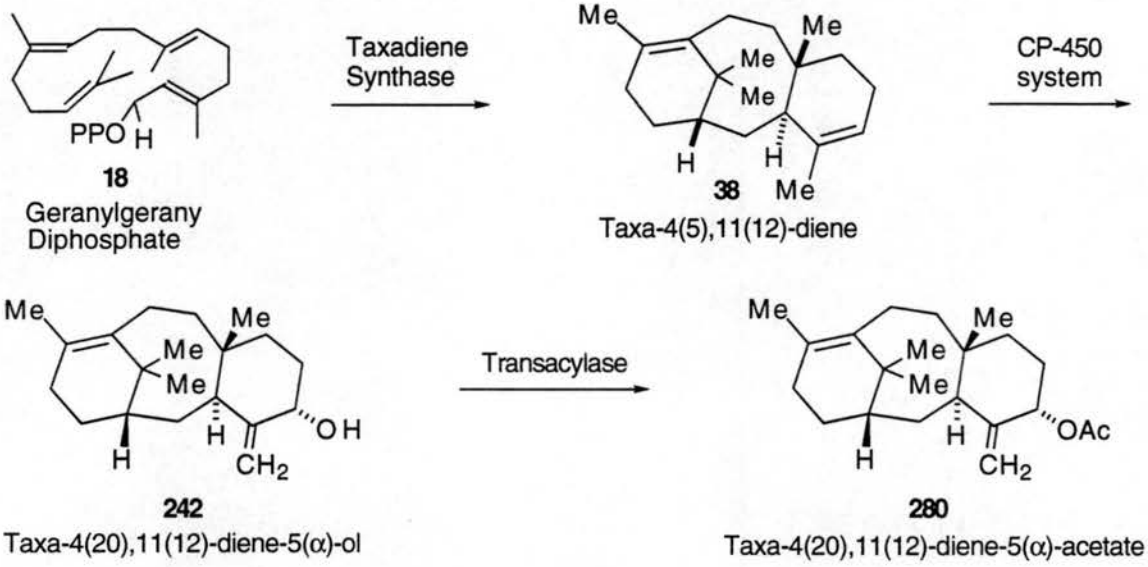
4.3 Conclusions and Future proposals

Based on the preceding experiments it appears that the conversion of taxa-4(5),11(12)-diene (**38**) to taxa-4(20),11(12)-diene-5(α)-ol (**242**) is the first hydroxylation step in the biosynthesis of taxol. In addition, the first hydroxylation step is mediated by a cytochrome P-450 enzyme. It appears that the rate limiting step for the first hydroxylation reaction is not the homolytic C-20 carbon, hydrogen bond cleavage. Further chemical

studies our under way to synthesize epoxide **263** in order to directly test its viability as a precursor to taxa-4(20),11(12)-diene-5(α)-ol (**242**).

It was also observed that taxa-4(20),11(12)-diene-5(α)-acetate (**280**) acted as a better substrate than taxa-4(20),11(12)-diene-5(α)-ol (**242**) in the cytochrome P-450 microsomal assay conversion to the unidentifiable higher hydroxylated product(s). In addition, the transacylase assay produced taxa-4(20),11(12)-diene-5(α)-acetate (**280**) from taxa-4(20),11(12)-diene-5(α)-ol (**242**). Scheme 4.26 summarizes the steps elucidated, to date, in the taxol biosynthetic pathway.

Scheme 4.26 The Early Steps in Taxol Biosynthesis.

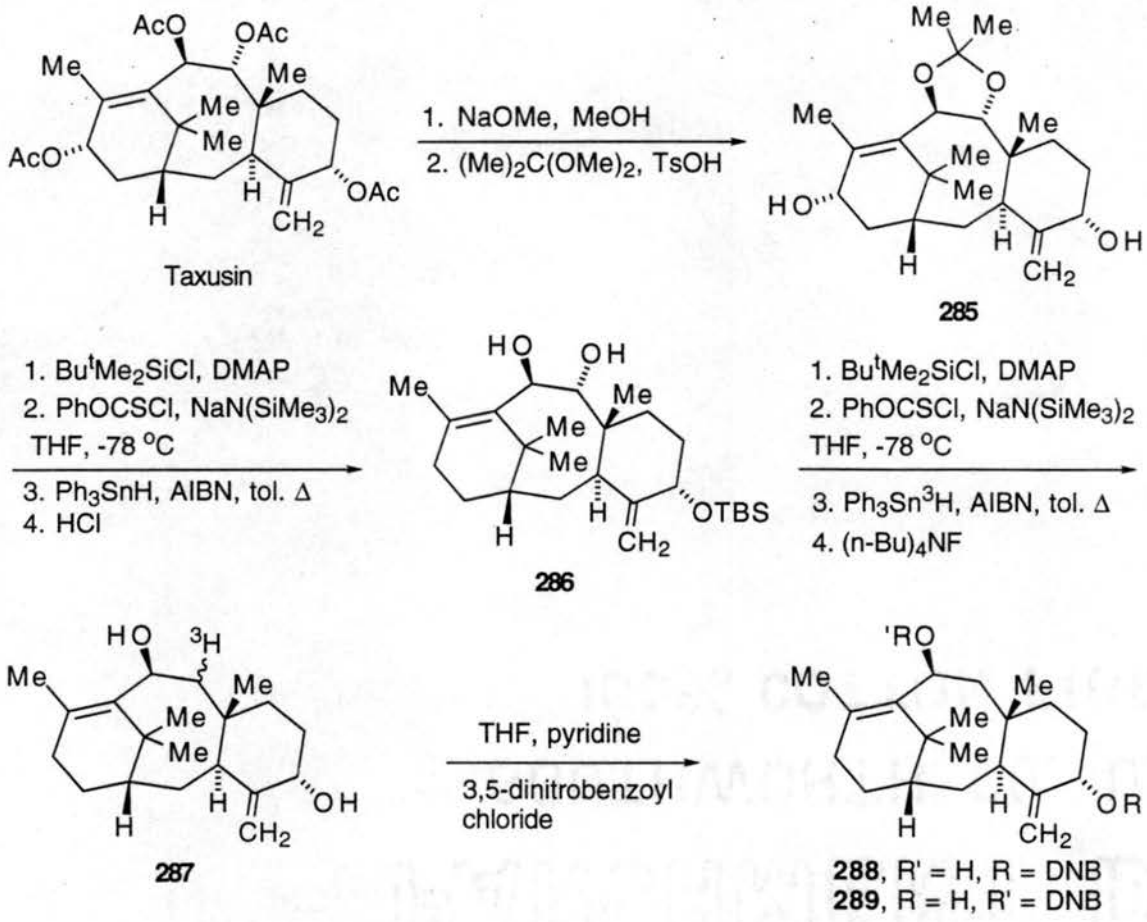


The cell culture incubation method, though initially unsuccessful, still shows promise especially in light of the fact that DMSO does not effect taxol production in the *Taxus cuspidata* cell line.

The immediate future direction of this project involves synthesizing the next putative hydroxylated products **286**, **287**, and **292** in radiolabeled form. Since the functionality present in taxa-4(20),11(12)-diene-5(a)-ol (**242**), will limit what can be reasonably synthesized from this substrate, it was felt that deoxygenation of taxusin

(Scheme 4.27) will provide an alternative means for accessing the putative taxoids, taxa-4(20),11(12)-diene-5,10-diol **287** and taxa-4(20),11(12)-diene-5,9,10-triol (see structure **286**).

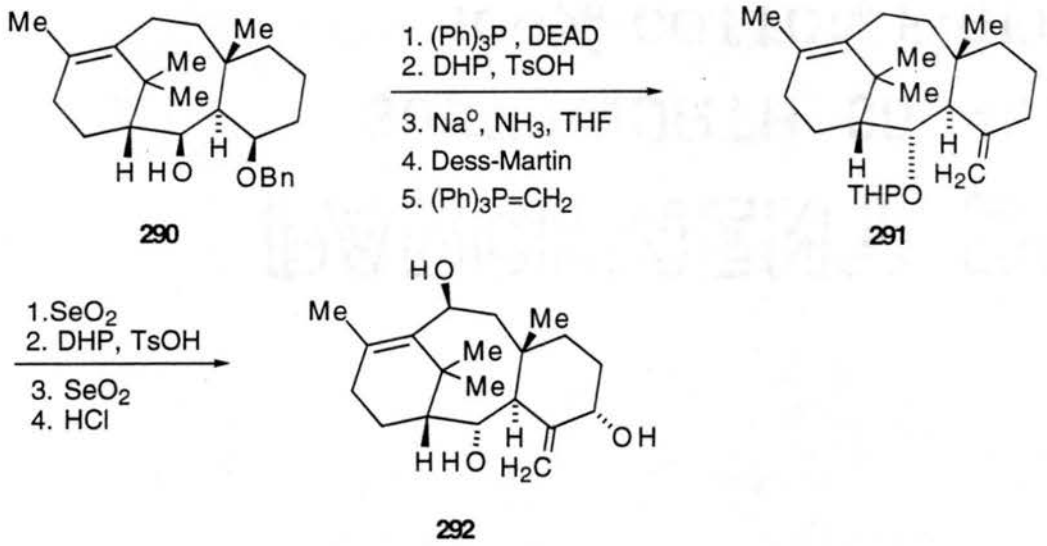
4.27 Proposed Degradation of Taxusin to Yield the Putative Second Hydroxylation Product.



As shown in Scheme 4.27, deacetylation of taxusin followed by protection of the 9,10-diol and monosilylation is expected to give a mixture of the desired C-5 silyl ether and undesired C-13 silyl ether. Thionoformate acetylation of the desired C-13 alcohol, tin hydride reduction and removal of the acetonide is expected to furnish **286**. Protection of the less hindered C-10 alcohol, followed by a second round of Barton deoxygenation at C-

9 provides the 5,10-diol **287**. This sequence permits the convenient introduction of the tritium radiolabel (via the tin tritiide).

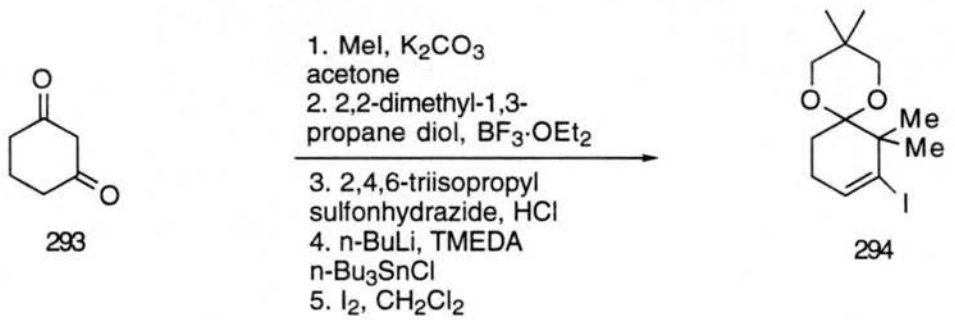
Scheme 4.28 The Proposed Synthesis of Putative Precursor **292**.



290 **291** **292**

Unfortunately, taxusin cannot provide the C-2-hydroxylated material **292**. Compound **290** (Scheme 4.28) can easily be synthesized as outlined in Scheme 3.13. Mitsunobo inversion followed by alcohol protection should furnish the C-2 protected alcohol with the correct relative stereochemistry as compared to taxol. C-4 deprotection followed by oxidation and Wittig olefination, which permits the convenient installation of either a ^{13}C or ^{3}H label at C-20, should afford taxoid **291**. Putative precursor **292** could then be synthesized from **291** via the 4 step sequence shown in Scheme 4.28.

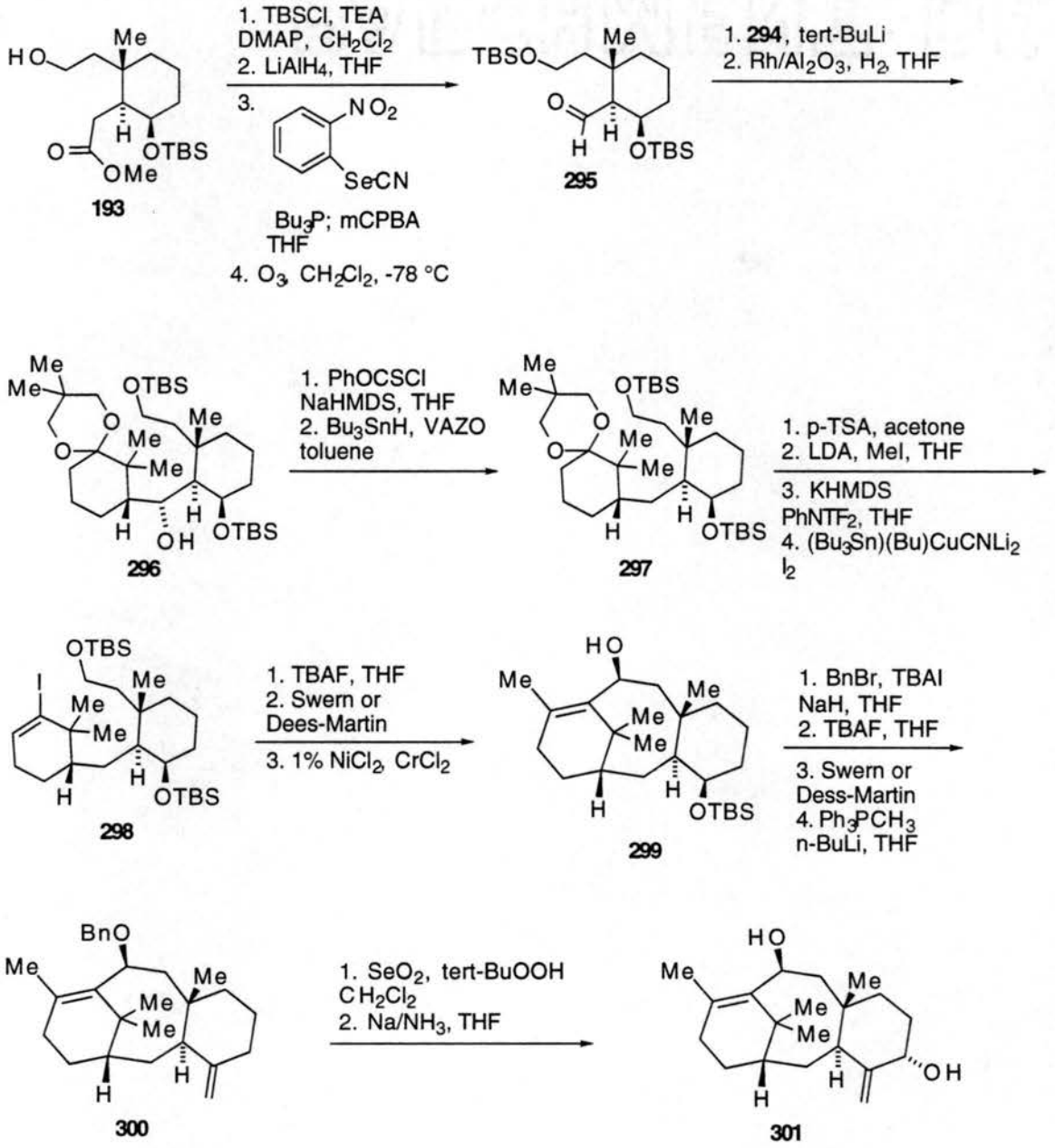
Scheme 4.29 The Synthesis of Alkene **294**.



293 **294**

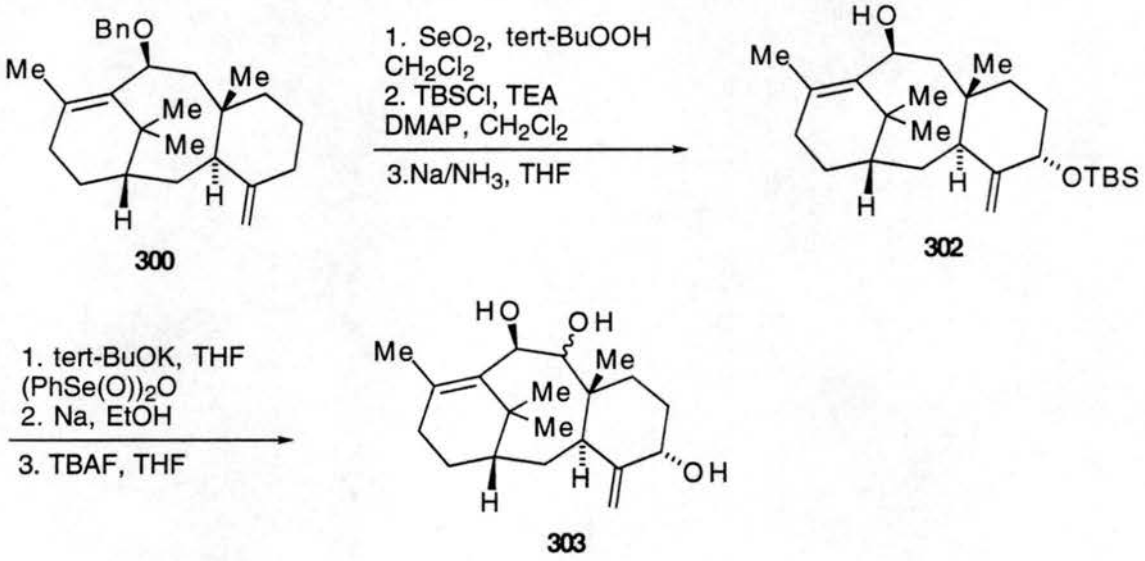
An alternative approach to putative biosynthetic precursors **280**, **286**, and **292** is outlined in Schemes 4.29-4.32. The alternative approach would rely on using the nickel/chromium metal-mediated methodology developed by Kishi and co-workers.[31] Protection of **193**, synthesized as outlined in Scheme 3.6, followed by ester reduction, alkene formation, and ozonolysis should afford aldehyde **295**, as outlined in Scheme 4.30.

Scheme 4.30 The Proposed synthesis of Putative Biosynthetic Precursor **301**.



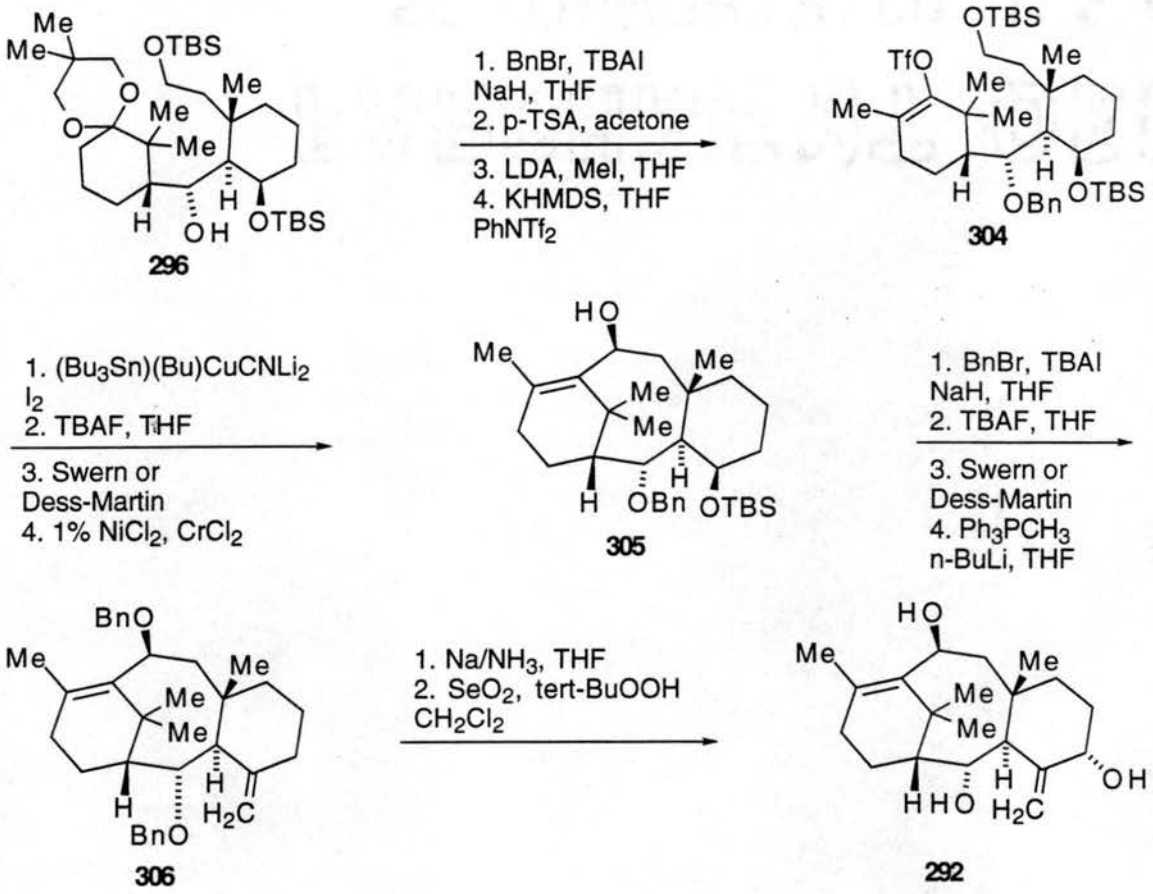
The aldehyde should then couple to the lithium anion of **294** followed by hydrogenation to furnish **296** based on literature precedent.[31] Compound **294** was synthesized by Kishi and co-workers as outlined in Scheme 4.29.[31] Deoxygenation of **296** should produce **297**. Deprotection, alkylation, triflate formation, and iodonation should furnish **298** from **297**. Selective silyl deprotection followed by oxidation and metal-mediated ring closure should afford taxane **299**. Protection the C-10 alcohol as it benzyl ether followed by silyl deprotection, oxidation, and Wittig olefination, which permits the convenient installation of either a ^{13}C or ^{3}H label at C-20, should produce taxoid **300**. This compound could then be oxidized followed by benzyl ether deprotection which should afford putative biosynthetic intermediate **301**. Scheme 4.31 outlines a plausible approach to putative precursor **303**.

Scheme 4.31 The Synthesis of Putative Biosynthetic Precursor 303.



Selenium dioxide oxidation followed by C-5 protection and C-10 deprotection should furnish **302** starting from taxane **300**. Oxidation and reduction at C-9 followed by deprotection should afford **303** as a mixture of diastereomers with the a-C-9 diastereomeric triol as the desired compound. Scheme 4.32 outlines a plausible approach to putative biosynthetic precursor **292** conscripting the approach developed by Kishi and co-workers.[31]

Scheme 4.32 The Synthesis of Putative Biosynthetic Precursor 292.



Alcohol protection, deketalization, alkylation, and reaction with N-phenyltrifluoromethanesulfonimide should produce triflate **304**. Iodination, primary alcohol deprotection, oxidation followed by metal-mediated ring closure should furnish taxane **305**. Protection of the C-10 alcohol as it benzyl ether followed by silyl deprotection, oxidation, and Wittig olefination, which permits the convenient installation of either a ^{13}C or ^{3}H label at C-20, should produce taxoid **306**. Compound **306** could then be converted to **292** via deprotection and allylic oxidation.

As this work progresses, a better understanding of the exact target to synthesize will become apparent for identifying the early hydroxylation steps in taxol biosynthesis. The targets described above need only be made in 10-20 mg amounts and it is felt that the synthetic routes shown above should prove entirely satisfactory in this capacity.

CHAPTER 5
EXPERIMENTAL SECTION

5.1 General Synthetic Procedures

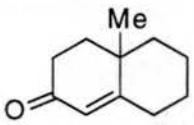
^{1}H NMR and ^{13}C NMR spectra were obtained on the Bruker AC 300 MHz spectrometer. Chemical shifts are reported in parts per million from $CHCl_3$ as the internal standard. Infrared spectra were recorded on Perkin-Elmer 1600 Series FTIR and are reported as λ_{max} in cm^{-1}. Melting points were determined in open-ended capillary tubes on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by M-H-W laboratories, Phoenix, Az, and are accurate to within the calculated values by ±0.4%. High-resolution mass spectra were carried out by UCR Mass Spectrometry Facility, Department of Chemistry, University of California at Irvine, Irvine, Ca. and CSU Mass Spectrometry Facility, Department of Chemistry at Colorado State University, Fort Collins, Co. Thin layer chromatography (TLC) was performed on 0.25-mm E. Merck precoated silica gel glass plates. Visualization on TLC was achieved with ultraviolet light, an I_2 developing chamber, and/or heating of TLC plates submerged in a 7% solution of phosphomolybdic acid in 95% ethanol. Reagents and solvents were commercial grades and were used as supplied unless otherwise stated and with the following exceptions. Tetrahydrofuran, toluene, and diethyl ether were freshly distilled from sodium

benzophenone ketyl. Dichloromethane was freshly distilled from CaH_2. DMF was dried over 3-Å molecular sieves. All moisture sensitive reactions were carried out in glassware that was flame dried under high vacuum (0.5-2.0 mm Hg) and then purged with Argon. The term "concentrated" refers to the removal of volatile solvents using an aspirated rotary evaporator.

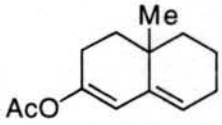
2D-NMR spectra was recorded at 500.1 MHz (^{1}H, DQF-COSY, HMQC, HMBC) and at 125.8 MHz on a Bruker AM-500 instrument, and at 300.1 and 75.5 MHz ($^{13}C\{^1H\}$, DEPT) using a Bruker AC-300P instrument. All experiments were run at 28 °C on a 18 mM solution in $CDCl_3$. Chemical shifts are reported in δ (ppm) using $CHCl_3$ as an internal standard set at δ 7.24 ppm for ^{1}H NMR and δ 77.0 ppm for ^{13}C NMR. The DFQ-COSY[87] was run in the phase sensitive mode. The $512t_1$ increments of 16 scans each were sampled in 2K data points for each of the $512t_1$ increments. Zero-filling the F_1 domain to 1K and a π/2 shifted sine-bell apodization function were applied in both F_1 and F_2 domains prior to double Fourier transformation. The HMQC spectrum was measured employing the pulse sequence of Bax et al.[88] No X-decoupling during acquisition was used. Delay Δ was set to 3.40 ms corresponding to the average one bond carbon-proton coupling constant, 147 Hz. In this experiment, 128 t_1 increments were sampled in 2K data points using 16 scans for each of the t_1 increments. Data in the F_1 domain were zero-filled to 256K and the sine-bell squared weighting function phase shifted by π/2 was applied in both F_1 and F_2 domains prior to double Fourier transformation. Analogous parameters were adopted for the HMBC spectrum that was obtained using the pulse sequence of Bax and Summers[89] and involved low-pass J-filtering to suppress correlations due to one-bond couplings. Delay D_2 was set to 91 ms, 83 ms corresponding to the average long-range (through two or three bonds) carbon-proton coupling constant, 5.5Hz, 6.0 Hz for taxa-4(20),11(12)-diene and taxa-4(20),11(12)-diene-5α-ol respectively.

The proton connectivities for taxa-4(20),11(12)-diene, taxa-4(20),11(12)-diene-5α-ol and, taxa-4(20),11(12)-diene-5β-ol were determined by DFQ-COSY. The HMQC spectra was used to assign the signals of all carbons directly attached to their protons. The HMBC spectrum was used to assign the quaternary carbons and to check the correctness of the connectivities established by the interpretation of the other spectra.

5.2 Chemical Synthesis Experimentals

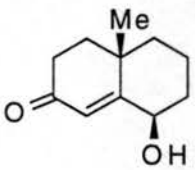


4,4a,5,6,7,8-hexahydro-4a-methylnapthlen-2(3H)-one. To 2-methylcyclohexanone (1.48 L, 12.19 mol) at 0 °C under argon was added methyl vinyl ketone (1.27 L, 15.24 mol) and concentrated H_2SO_4 (9 mL). The solution was allowed to stir for 1 h in an ice bath followed by warming up to 25 °C, and then refluxed for 17 h. The mixture was then diluted with hexanes (3 L) and washed 1x with 5% KOH solution (3 L). The resulting solution was dried over Na_2SO_4, concentrated, and purified by distillation (boiling point 115 °C at 2 mm) to yield 1.2 Kg (60%) of product as a pale yellow oil. IR (Neat) 2930, 2860, 1677, 1635 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.65 (s,1H), 2.43 (ddd, J = 17, 13.3, 6.1 Hz, 1H), 2.35-2.1 (m, 3H), 1.9-1.75 (m, 2H), 1.73 (dd, J = 6, 3.4 Hz, 1H), 1.7-1.55 (m, 3H), 1.45-1.25 (m, 2H), 1.19 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 199.6, 170.5, 124.1, 41.5, 38.0, 35.9, 33.9, 32.7, 27.1, 22.0, 21.7. Anal. Calcd for $C_{11}H_{16}O$: C, 80.43; H, 9.83. Found: C, 80.64; H, 9.61.

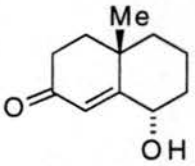


2-methoxy-4a-methyl-3,4,4a,5,6,7-hexahydronapthalene. To the starting enone (302 g, 1.84 mol) under argon was added acetic anhydride (1.9 L, 20.2 mol), and acetyl chloride (0.92 L, 12.9 mol). This solution was refluxed for 2 days, cooled to 25 °C, and poured over ice followed by stirring for 2 h. Et_2O (4 L) was added and the layers separated. The Et_2O layer was

washed 2x with saturated aqueous $NaHCO_3$ (1 L) and 1x with H_2O (1 L). The aqueous layers were combined and extracted 2x with Et_2O (2 L). The resulting Et_2O layers were washed 2x with saturated aqueous $NaHCO_3$ (1 L), 1x with H_2O (1 L), dried over Na_2SO_4, and concentrated. The crude oil was purified by distillation (boiling point 120°C at 5 mm) to give 250 g (66%) of product as a yellow oil. IR (Neat) 2927, 2859, 1755, 1681, 1633 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.65 (d, J = 2 Hz, 1H), 5.38 (dd, J = 3.7, 3.7 Hz, 1H), 2.55-2.4 (m, 1H), 2.2-1.95 (m, 3H), 2.1 (s, 3H), 1.85-1.55 (m, 2H), 1.55-1.4 (m, 3H), 1.28 (ddd, J = 13.4, 13.4, 3.6 Hz, 1H), 1.0 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.2, 147.3, 138.4, 124.2, 116.9, 36.8, 36.6, 31.8, 25.4, 24.8, 23.1, 21.0, 18.3.



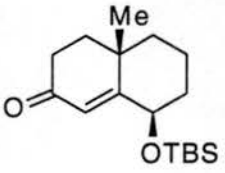
A



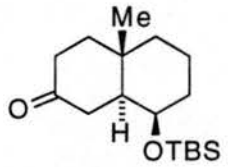
B

8β-hydroxy-4a-methyl-4a,4,5,6,7,8-hexahydro-2(3H)-napthalenone (A) and 8α-hydroxy-4a-methyl-4a,4,5,6,7,8-hexahydro-2(3H)-napthalenone (B). To a 0.6M solution of m-CPBA (855 g, 2.48 mol) in 95% EtOH at 25 °C was added a 0.8M solution of the dienol acetate (327 g, 1.59 mol) in 95% EtOH and allowed to stir for 2 h. $Na_2S_2O_3$ (427 g, 2.7 mol) is added to a 1M solution of $NaHCO_3$ (307 g, 3.66 mol). This solution was then slowly added to the reaction mixture and stirred for 1 h. The resulting solution was then partially concentrated followed by the addition of H_2O (1 L) and Et_2O (2 L). The layers were separated and the aqueous layer was extracted 6x with Et_2O (1.5 L). The organic layer was dried over Na_2SO_4, concentrated and the crude solid was purified by column chromatography (4:1, 2:1, 1:1 hexanes/EtOAc) to give 100 g (35%) of **A** as a dark orange oil and 22 g (8%) of **B** as a yellow solid (m.p. 121.9-122.8 °C). **A**: IR (Neat) 3408, 2930, 2862, 1656 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 6.1 (s, 1H), 4.25 (ddd, J = 12, 5.6, 2 Hz, 1H), 3 (s, 1H), 2.43 (ddd, J = 17, 13, 6.2 Hz, 1H) 2.3 (ddd, 17, 4.4, 4.4 Hz, 1H), 2.2-2.1 (m, 1H), 1.9-1.5 (m, 5H), 1.45-1.22 (m, 2H), 1.18 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.2,

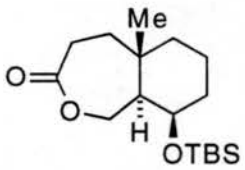
171.6, 120.1, 68.8, 41.1, 38.3, 36.7, 36.6, 33.8, 22.9, 20.1. Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.28; H, 8.95. Found: C, 73.24; H, 9.11. **B**: IR (Neat) 3412, 2934, 1700, 1674 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.73 (s, 1H), 4.28 (dd, J = 2.7, 2.7 Hz, 1H), 2.53 (ddd, J = 17.4, 14.6, 5.3 Hz, 1H), 2.45-2.25 (m, 3H), 2.13-1.9 (m, 2H), 1.81 (ddd, J = 13.7, 13.7, 4.1 Hz, 1H), 1.72-1.45 (m, 4H), 1.4 (s, 3H), 1.28 (ddd, J = 13.7, 13.7, 3.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.8, 168.0, 126.4, 72.4, 56.3, 55.8, 41.1, 39.3, 34.3, 32.2, 24.0, 16.1. HRMS Calcd for $C_{11}H_{16}O_2$ 180.1146, found 180.1145.



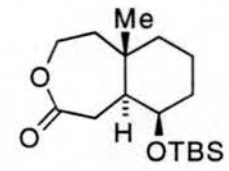
To a 3M solution of allylic alcohol (38 g, 0.21 mol) in DMF under argon was added imidazole (46 g, 0.66 mol), tert-butyldimethylsilyl chloride (100 g, 0.66 mol), and allowed to stir for 18 h at 35 °C. The crude reaction mixture was poured into a separatory funnel followed by H_2O (500 mL) and Et_2O (500 mL). The layers were separated and the aqueous layer was extracted 3x with Et_2O (500 mL). The organic layer was washed 1x with H_2O (250 mL), saturated aqueous $NH_4^+Cl^-$ solution (250 mL), brine (250 mL), dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (32:1 hexanes/EtOAc) to give 46.5 g (75%) of product as a yellow oil. IR (Neat) 2928, 2856, 1680 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.7 (s, 1H), 4.22 (dd, J = 2.6, 2.6 Hz, 1 Hz), 2.55 (ddd, J = 17, 14.7, 5.3 Hz, 1H), 2.33 (ddd, J = 17.1, 3.5, 3.5 Hz, 1H), 2.05 (apparent ddq, J = 14, 4.8, 2.7 Hz, 1H), 1.9-1.72 (m, 2H), 1.72-1.55 (m, 2H), 1.55-1.39 (m, 2H), 1.35 (s, 3H), 1.25 (ddd, J = 13.5, 13.5, 3.4 Hz, 1H), 0.82 (s, 9H), 0.0 (s, 3H), -0.5 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.9, 167.7, 125.6, 73.1, 41.5, 39.7, 35.6, 34.9, 34.3, 25.6, 24.1, 17.9, 16.3, -4.7, -5.1. Anal. Calcd for $C_{17}H_{30}O_2Si$: C, 69.34; H, 10.27. Found: C, 69.23; H, 10.31.



To a 0.3M solution of protected allylic alcohol (103 g, 0.35 mol) in anhydrous MeOH under argon was added Pd/C (43 g, 0.02 mol) followed by bubbling H_2(g) through the solution for 3 minutes. The solution was allowed to stir for 2 days under a balloon head of pressure. The mixture was concentrated, taken up in EtOAc (500 mL) and filtered through a plug of silica gel. The silica gel was washed 3x with EtOAc (200 mL). The organic layer was concentrated, and purified by column chromatography (32:1, 16:1 hexanes/EtOAc) to give 85 g (83%) of product as a colorless oil. IR (Neat) 2946, 1715 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 3.7 (ddd, J = 2.6, 2.6, 2.6 Hz, 1H), 2.67 (dd, J = 14.6, 14.6 Hz, 1H), 2.46 (ddd, J = 15.7, 13.8, 6.7 Hz, 1H), 2.26 (dddd, J = 15.6, 4.6, 2.1, 2.1 Hz, 1H), 2.0 (ddd, J = 15, 3.4, 2.3 Hz, 1H), 1.87 (apparent ddq, J = 14.2, 4.5, 3.3 Hz, 1H), 1.78-1.68 (m, 1H), 1.6-1.3 (m, 6H), 1.2 (s, 3H), 1.07 (ddd, J = 13.4, 13.4, 3.4 Hz, 1H), 0.85 (s, 9H), 0.0 (s, 3H), -0.05 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 212.9, 70.9, 47.4, 42.9, 42.3, 40.3, 38.2, 33.8, 33.1, 25.7, 18.5, 17.9, 16.7, -4.7, -5.2. Anal. Calcd for $C_{17}H_{32}O_2Si$: C, 68.86; H, 10.87. Found: C, 68.76; H, 10.67.



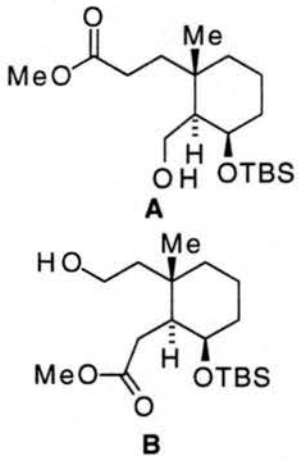
A



B

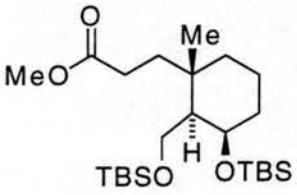
Lactone (A) and Lactone (B). To a 0.3M solution of m-CPBA (87.5 g, 0.25 mol) in CH_2Cl_2 at 25 °C was added a 0.2M solution of ketone (50 g, 0.17 mol) in CH_2Cl_2 and allowed to stir for 18 h. $Na_2S_2O_3$ (45 g, 0.29 mol) was added to a 1M solution of $NaHCO_3$ (32.7 g, 0.39 mol). This solution was slowly added to the reaction mixture and allowed to stir for 1 hr. The layers were separated and the aqueous layer was extracted 1x with Et_2O (1 L). The organic layers were combined, washed 2x with saturated aqueous $NaHCO_3$ (1 L), dried over Na_2SO_4, and concentrated to give 45.3 g (86%) of **A** (Rf 0.37 4:1 hexanes/EtOAc) and **B**

(0.32 4:1 hexanes/EtOAc) as an oil. The crude mixture was taken on. **A**: IR (Neat) 2930, 2856, 1736 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.35 (dd, J = 13.2, 8.2 Hz, 1H), 4.0 (d, J = 2.7 Hz, 1H), 3.87 (d, J = 13.2 Hz, 1H), 2.76 (ddd, J = 14.8, 11.6, 3.8 Hz, 1H), 2.45 (ddd, J = 14.6, 5.2, 3.5 Hz, 1H), 1.9-1.6 (m, 2H), 1.5-1.3 (m, 6H), 1.15 (s, 3H), 1.2-1.0 (m, 1H), 0.85 (s, 9H), 0.01 (s, 3H), -0.01 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.9, 71.9, 70.2, 50.1, 40.8, 39.9, 34.9, 33.9, 30.0, 25.7, 25.6, 19.4, 17.8, 16.4, -4.4, -5.4. Anal. Calcd for $C_{17}H_{32}O_3Si$: C, 65.33; H, 10.32. Found: C, 65.72; H, 10.50. **B**: IR (Neat) 2930, 2857, 1733 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.35 (dd, J = 13.2, 11.2 Hz, 1H), 4.05 (ddd, J = 13.3, 5.4, 1.9 Hz, 1H) 3.86 (d, J = 2.8 Hz, 1H), 2.89 (dd, J = 14.6, 11.3 Hz, 1H), 2.2 (d, J = 14.6 Hz, 1H), 1.8-1.3 (m, 7H), 1.15 (s, 3H), 1.3-1.05 (m, 2H), 0.85 (s, 9H), 0.0 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.6, 73.2, 64.7, 46.1, 44.8, 41.2, 36.7, 35.2, 34.0, 25.7, 19.3, 17.9, 16.1, -4.4, -5.2.

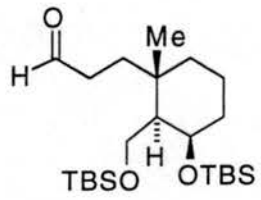


Ester (A) and Ester (B). To a 0.45M solution of the lactone mixture (110 g, 0.35 mol) in MeOH at 0 °C Was added sodium methoxide (47.6 g, 0.88 mol) and allowed to stir for 1 h. Concentrated HCl was then added dropwise until a pH of 3 was reached. H_2O (500 mL) and EtOAc (500 mL) were added and the aqueous layer was extracted 3x with EtOAc (250 mL). The organic layer was washed 1x with saturated aqueous $NaHCO_3$ solution (250 mL), 1x with brine (250 mL), dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (4:1, 2:1 hexanes/EtOAc) to give 33.8 g (28%) of **A** (R_f 0.5 2:1 hexanes/EtOAc) and 54.2 g (62%) of **B** (R_f 0.33 2:1 hexanes/EtOAc) as pale yellow oils. **A**: IR (Neat) 3466, 2931, 2857, 1740 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.2 (ddd, J = 7.3, 3.6, 3.6 Hz, 1H), 3.9 (dd, J = 10.5, 10.5 Hz, 1H), 3.7 (dd, J = 10.5, 3.5 Hz, 1H), 3.62 (s, 3H), 2.24 (dd, J = 9.8,

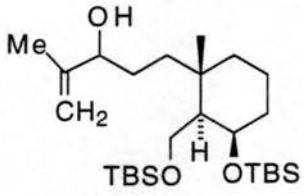
8 Hz, 1H), 1.8-1.45 (m, 7 H), 1.25-1.1 (m, 2H), 0.9 (s, 3H), 0.85 (s, 9H), 0.02 (s, 3H), -0.02 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.6, 69.9, 60.3, 51.6, 48.3, 35.5, 35.4, 35.2, 31.4, 28.5, 25.7, 23.8, 18.4, 17.9, -4.6, -5.2. Anal. Calcd for $C_{18}H_{36}O_4Si$: C, 62.74; H, 10.53. Found: C, 62.88; H, 10.69. **B**: IR (Neat) 3358, 2933, 2857, 1738 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 3.95 (ddd, J = 6, 3, 3 Hz, 1H), 3.69 (dd, J = 7.5, 7.5 Hz, 2H), 3.62 (s, 3H), 2.55 (dd, J = 16.5, 8.4 Hz, 1H), 2.3 (dd, 16.5, 4.2 Hz, 1H), 1.85 (ddd, J = 7.6, 3.6, 3.6 Hz, 1H), 1.75-1.6 (m, 1H), 1.55 (dd, J = 7.5, 7.5 Hz, 2H), 1.48-1.2 (m, 5H), 0.98 (s, 3H), 0.85 (s, 9H), 0.0 (s, 3H), -0.02 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.8, 69.0, 58.9, 51.4, 44.4, 44.1, 36.3, 35.5, 32.8, 30.4, 25.8, 22.8, 18.0, 17.4, -4.5, -5.4.



To a 3M solution of ester (13 g, 37.8 mmol) in DMF was added imidazole (7.8 g, 113 mmol) and ter-butyldimethylsilyl chloride (17 g, 113 mmol) and allowed to stir for 14 h at 35 °C. The crude reaction mixture was poured over H_2O (100 mL) and extracted 3x with Et_2O (100 mL). The organic layer was washed 1x with H_2O (100 mL), 1x with saturated $NH_4^+Cl^-$ solution (100 mL), 1x with brine (100 ml), dried over Na_2SO_4, and concentrated. The oil was purified by column chromatography (32:1 hexanes/EtOAc) to give 12.3 g (71%) of product as a pale yellow oil. IR (Neat) 2916, 1745 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.17-4.1 (m, 1H), 3.7-3.65 (m, 2H), 3.6 (s, 3H), 2.3-2.15 (m, 2H), 1.8-1.55 (m, 4H), 1.4-1.1 (m, 5H), 0.95 (s, 3H), 0.86 (s, 9H), 0.84 (s, 9H), 0.01(s, 3H), -0.01 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.8, 67.5, 60.1, 51.4, 49.9, 37.6, 37.0, 34.8, 33.6, 28.6, 25.9, 25.8, 22.1, 18.1, 17.0, -4.6, -5.1, -5.3, -5.4. Anal. Calcd for $C_{24}H_{52}O_4Si_2$: C, 62.83; H, 10.98. Found: C, 63.02; H, 10.89.

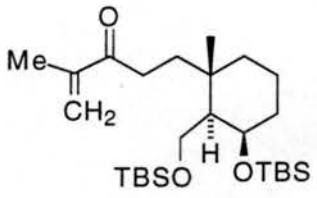


To a 0.07M solution of ester (12.5 g, 27.3 mmol) in toluene at -78 °C was added a 1.5M solution of DIBAH (18.2 mL, 27.3 mmol) and allowed to stir for 1 h. The resulting mixture was quenched with 1M HCl (200 mL) and extracted 3x with Et_2O (150 mL). The organic layer was washed 2x with brine (100 mL), dried over Na_2SO_4, and concentrated to give 11.7 g (100%) of product as a colorless oil and was taken on without further purification. IR (Neat) 2930, 2856, 1729 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 9.75 (t, J = 1.9 Hz, 1H), 4.09-4.18 (m, 1H), 3.7-3.6 (m, 2H), 2.4-2.3 (m, 2H), 1.8-1.5 (m, 4H), 1.4-1.1 (m, 5H), 1.0 (s, 3H), 0.88 (s, 9H), 0.84 (s, 9H), 0.03 (s, 3H), 0.01 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 202.9, 67.6, 60.3, 50.0, 38.6, 37.3, 34.7, 34.5, 33.6, 25.9, 25.8, 22.1, 18.2, 18.0, 16.9, -4.6, -5.1, -5.3, -5.4. Anal. Calcd for $C_{23}H_{48}O_3Si_2$: C, 64.44; H, 11.29. Found: C, 64.59; H, 11.16.

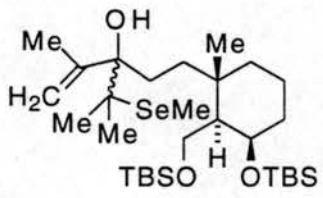


To a 0.4M solution of magnesium (960 mg, 40 mmol) in THF was added a catalytic amount of iodine and freshly distilled 2-bromopropene (3.3 mL, 36.4 mmol) slowly with concurrent heating. The resulting solution was refluxed for 0.5 h and cooled to 0 °C. To this solution was added a 0.36M solution of aldehyde (13 g, 30.3 mmol) in THF dropwise. The reaction mixture was allowed to stir for 2 h at 0 °C, quenched with saturated $NH_4^+Cl^-$ solution (100 mL), and extracted 3x with Et_2O (100 mL). The organic layer was dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (32:1 hexanes/EtOAc) to give 9.95 g (71%) of product as a single diastereomer (pale yellow oil). IR (Neat) 3349, 2930, 2856 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.93-4.88 (m, 1H), 4.83-4.78 (m, 1H), 4.18-4.1 (m, 1H), 3.95 (dd, J = 6.3, 6.3 Hz, 1H), 3.69-3.62 (m, 2H), 1.8-1.6 (m, 1H), 1.7 (s, 3H), 1.55-1.4 (m, 3H), 1.4-1.1 (m, 8H), 0.95 (s, 3H), 0.87 (s, 9H), 0.86 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H), 0.02 (s,

3H), 0.01 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.8, 115.5, 111.3, 77.7, 77.3, 67.5, 60.1, 50.3, 38.8, 37.6, 34.9, 33.9, 28.8, 26.3, 26.1, 22.8, 18.5, 18.3, 17.7, 17.6, -4.3, -4.8, -4.9, -5.0. Anal. Calcd for $C_{26}H_{54}O_3Si_2$: C, 66.32; H, 11.56. Found: C, 66.50; H, 11.64.

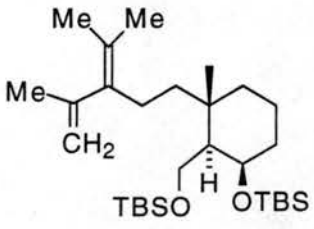


To a 0.1M solution of allylic alcohol (19.9 g, 42.34 mmol) in CH_2Cl_2 at 25 °C was added Dess-Martin reagent (35.9 g, 84.7 mmol) and was allowed to stir for 16 h. $Na_2S_2O_3 \cdot 5H_2O$ (31.5 g, 1127 mmol) was added to a 1M solution of $NaHCO_3$ (8.2 g, 97.4 mmol). This solution and Et_2O (100 mL) were added to the reaction mixture and allowed to stir for 30 min. Saturated aqueous $NaHCO_3$ solution (200 mL) was added and the mixture was extracted 3x with Et_2O (100 mL). The organic layer was washed 2x with saturated aqueous $NaHCO_3$ solution (100 mL), dried over Na_2SO_4, and concentrated. The crude oil was passed through a plug of silica gel (8:1 hexanes/EtOAc) to give 18 g (91%) of product as a clear colorless oil. IR (Neat) 2930, 2857, 1682 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.95-5.9 (m, 1H), 5.73-5.7 (m, 1H), 4.2-4.1 (m, 1H), 3.7-3.6 (m, 2H), 2.6 (dd, J = 8.6, 8.6 Hz, 1H), 1.85 (dd, J = 1.2, 0.74 Hz, 3H), 1.8-1.5 (m, 4H), 1.4-1.15 (m, 5H), 0.98 (s, 3H), 0.88 (s, 9H), 0.85 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H), 0.01 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 202.7, 144.6, 124.1, 67.4, 59.9, 50.2, 37.2, 34.9, 33.6, 31.8, 29.7, 25.9, 25.85, 25.81, 22.2, 18.2, 18.1, 17.8, 17.1, -4.6, -5.1, -5.2, -5.3. Anal. Calcd for $C_{26}H_{52}O_3Si_2$: C, 66.60; H, 11.18. Found: C, 66.63; H, 11.05.

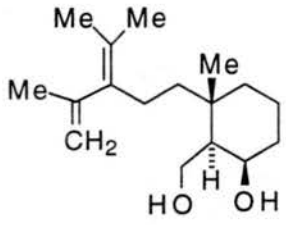


To a 0.23M solution of 2,2-bis(methylseleno)propane[67] (3.5 g, 15.3 mmol) in THF at -78 °C was added a 1.6M solution of nBuLi (9.5 mL, 15.3 mmol) and allowed to stir for 1 h. To this

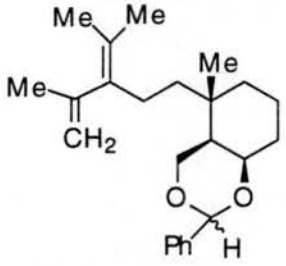
solution was added a 0.35M solution of enone (7.14 g, 15.3 mmol) in THF dropwise and the resulting solution was allowed to stir 18 h as the temperature rose to 25 °C. To the reaction was added H_2O (100 mL), extracted 3x with Et_2O (100 mL), the organic layer washed 2x with brine (100 mL), dried over Na_2SO_4, and concentrated to give 9.2 g of product (100%) as an ≈ 4:1 mixture of diastereomers (pale yellow oil). The crude oil was taken on without further purification. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.05 (s, 1H), 4.89 (m, 1H), 4.15 (s, 1H), 3.72 (m, 1H), 3.61 (m, 2H), 2.0 (s, 3H), 1.78 (s, 3H), 1.48 (s, 3H), 1.40 (s, 3H), 0.9 (s, 3H), 0.85 (s, 18H), 0.0 (s, 12H).



To a 0.14M solution of phosphorous triiodide (13.1 g, 37.7 mmol) in CH_2Cl_2 at 0 °C was added a solution of triethylamine (15.6 mL, 111 mmol) and selenium analogue (9.2 g, 15.3 mmol) in CH_2Cl_2 (64 mL) dropwise. The resulting solution was allowed to stir for 1 h and then filtered through a plug of silica gel. The silica gel was then washed 2x with Et_2O (100 mL), the organic layer was washed 1x with 1M HCl (100 mL), 1x with brine (100 mL), dried over Na_2SO_4, and concentrated. The crude oil was passed through a plug of silica gel (32:1 hexanes/EtOAc) to give 5.4 g (71%) of product as a pale yellow oil. An analytical sample was prepared by column chromatography (hexanes). IR (Neat) 2929, 2856, 1463 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.9-4.83 (m, 1H), 4.51 (d, J = 2.6 Hz, 1H), 4.15 (br s, 1H), 3.7-3.6 (m, 2H), 1.98 (dd, J = 17.4, 8.1 Hz, 2H), 1.72 (s, 3H), 1.55 (s, 6H), 1.4-1.2 (m, 9H), 0.95 (s, 3H), 0.89 (s, 9H), 0.88 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H), 0.04 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.9, 137.1, 124.3, 112.7, 67.2, 59.5, 49.9, 41.3, 37.2, 34.9, 33.7, 29.7, 26.0, 25.9, 24.9, 22.9, 21.7, 19.5, 18.2, 18.1, -4.6, -5.1, -5.2, -5.3. Anal. Calcd for $C_{29}H_{58}O_2Si_2$: C, 70.38; H, 11.81. Found: C, 70.57; H, 11.63.

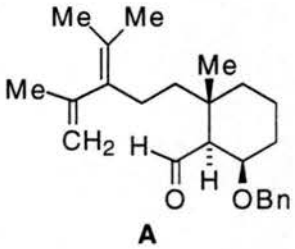


To a 0.05M solution of diene (5.8 g, 11.7 mmol) in THF/CH_3CN (1:1) was added 48% HF (2.3 mL, 117 mmol) and refluxed for 40 minutes. The mixture was cooled to 25 °C, H_2O (100 mL) followed by solid $NaHCO_3$ were added until a PH of 3 was reached. The resulting solution was extracted 3x with $CHCl_3$ (150 mL), the organic layer dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (2:1 hexanes/EtOAc) to give 2.8 g (90%) of product as a white solid (m.p. 94.6-95.0 °C). IR (Neat) 3300, 2926, 2870, 1631, 1445 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.88 (sextet, J = 1.4 Hz, 1H), 4.5 (dd, J = 2.8, 0.8 Hz, 1H), 4.18 (ddd, J = 7.7, 3.9, 3.9 Hz, 1H), 3.95 (dd, J = 11, 9 Hz, 1H), 3.77 (dd, J = 11, 3.9 Hz, 1H), 3.6 (s, 2H, D_2O exch.), 2.05-1.88 (m, 2H), 1.72 (dd, J = 1 Hz, 3H), 1.72-1.59 (m, 4H), 1.62 (s, 6H), 1.45-1.31 (m, 2H), 1.2-1.1 (m, 3H), 0.95 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.5, 136.6, 124.6, 112.9, 69.6, 60.7, 47.2, 38.8, 35.6, 35.1, 31.2, 24.7, 24.4, 22.7, 21.7, 19.4, 18.7. Anal. Calcd for $C_{17}H_{30}O_2$: C, 76.63; H, 11.36. Found: C, 76.47; H, 11.42.

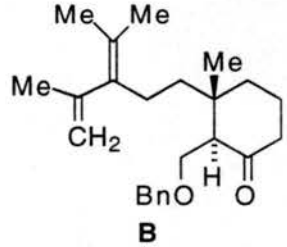


To a 0.2M solution of diol (9.8 g, 36.8 mmol) in $CHCl_3$ was added PPTS (1.9 g, 7.4 mmol) and benzaldehyde dimethyl acetal (7.7 mL, 51.6 mmol). The mixture was refluxed with concurrent azeotropic removal of MeOH using a dean-stark trap for 1 h (note: replenished lost $CHCl_3$ during azeotropic removal of MeOH). The mixture was cooled saturated aqueous $NaHCO_3$ solution (100 mL) was added and the aqueous layer was extracted 3x with Et_2O (50 mL). The organic layers combined, dried over Na_2SO_4, and concentrated. The excess benzaldehyde dimethyl acetal was removed by kugelrohring the oil at 100 °C under 18 mm of pressure to give 11.5 g (88%) of product as a 3:1 mixture of diastereomers (pale yellow oil). An analytical sample was separated. R_f

0.66 (8:1 hexanes/EtOAc): IR (Neat) 3070, 3021, 2931, 2861, 1630, 1455 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.55-7.45 (m, 2H), 7.4-7.25 (m, 3H), 5.5 (s, 1H), 4.92-4.88 (m, 1H), 4.55 (d, J = 2.7 Hz, 1H), 4.4 (d, J = 12 Hz, 1H), 4.15-4.05 (m, 1H), 3.93 (dd, J = 12, 3.8 Hz, 1H), 2.1-1.79 (m, 4H), 1.75 (s, 3H), 1.67 (s, 3H), 1.66 (s, 3H), 1.58-1.38 (m, 4H), 1.3 (s, 3H), 1.3-1.12 (m, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.6, 139.1, 136.8, 128.8, 128.3, 126.5, 124.7, 112.9, 102.1, 76.3, 67.7, 41.7, 40.8, 37.2, 34.8, 32.0, 24.7, 23.7, 22.7, 21.7, 19.4, 17.3. Anal. Calcd for $C_{24}H_{34}O_2$: C, 81.31; H, 9.67. Found: C, 81.54; H, 9.73. R_f 0.61 (8:1 hexanes/EtOAc): IR (Neat) 3070, 3033, 2922, 2868, 1452, 1387 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.5-7.4 (m, 2H), 7.4-7.3 (m, 3H), 5.8 (s, 1H), 4.92-4.88 (m, 1H), 4.53 (d, J = 2.7 Hz, 1H), 4.35 (ddd, J = 12.6, 5, 5 Hz, 1H), 4.06 (d, 2.8 Hz, 1H), 4.03 (s, 1H), 2.35-2.15 (m, 2H), 2.1-1.88 (complex, 2H), 1.75-1.65 (m, 2H), 1.72 (s, 3H), 1.65 (s, 6H), 1.55-1.35 (m, 2H), 1.35-1.1 (m, 3H), 0.85 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.5, 138.9, 136.5, 128.7, 128.3, 126.1, 124.9, 113.1, 93.8, 72.4, 64.5, 40.7, 36.4, 35.4, 33.2, 24.7, 24.3, 23.8, 22.8, 21.7, 19.9, 19.5. Anal. Calcd for $C_{24}H_{34}O_2$: C, 81.31; H, 9.67. Found: C, 81.49; H, 9.44.



A

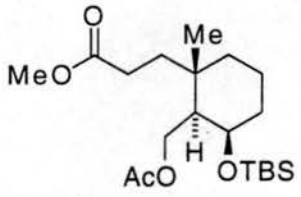


B

Aldehyde (A) and Ketone (B). To a 0.12M solution of acetals (11.5 g, 32.5 mmol) in CH_2Cl_2/Et_2O (1:1) at 0 °C was added $LiAlH_4$ (2.6 g, 64.9 mol) and allowed to stir for 5 min. To this solution was slowly added a 4M solution of $AlCl_3$ (17.3 g, 129.8 mmol) in Et_2O. The ice bath was taken away and the solution was allowed to stir for 30 min. as the temperature rose to 25 °C. EtOAc (50 mL) was added dropwise followed by H_2O (200 mL). The mixture was extracted 3x with Et_2O (150 mL), the

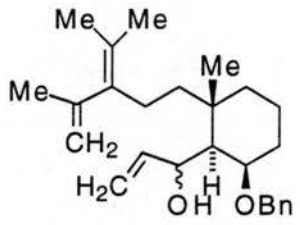
organic layer washed 2x with H_2O (100 mL), dried over Na_2SO_4, and concentrated to yield 11.5 g (100%) of an inseparable 4:1 mixture of alcohol as a pale yellow crude oil which was taken up in CH_2Cl_2 (320 mL). To this solution was added Dess-Martin reagent (27.4 g, 64.7 mmol) and allowed to stir for 4.5 h. $Na_2S_2O_3 \cdot 5H_2O$ (24.1 g, 97 mmol) was added to a 1M solution of $NaHCO_3$ (6.3 g, 74.4 mol) and this solution with Et_2O (100 mL) was added to the reaction mixture and allowed to stir for 30 min. Saturated aqueous $NaHCO_3$ solution (150 mL) was added and the mixture was extracted 3x with Et_2O (200 mL), the organic layer was washed 2x with saturated aqueous $NaHCO_3$ solution (150 mL), dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (64:1, 32:1 hexanes/EtOAc) to give 5.52 g (48%) of **A** and 2.4 g (21%) of **B** both as pale yellow oils. **A**: IR (Neat) 3069, 3027, 2932, 2858, 1720 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 9.98 (d, J = 4.7 Hz, 1H), 7.4-7.2 (m, 5H), 4.89 (sextet, J = 1.4 Hz, 1H), 4.51 (dd, J = 2.7, 0.8 Hz, 1H), 4.49 (s, 2H), 3.88 (ddd, J = 8.7, 4.3, 4.3 Hz, 1H), 2.45 (dd, J = 4.7, 4.7 Hz, 1H), 2.05-1.75 (m, 5H), 1.73 (dd, J = 0.9, 0.9 Hz, 3H), 1.65 (s, 3H), 1.55 (s, 3H), 1.55-1.5 (m, 2H), 1.42 (dd, J = 11.8, 5.5 Hz, 1H), 1.37 (dd, 10.3, 6.8 Hz, 1H), 1.24 (ddd, J = 13.8, 10.3, 7.3 Hz, 1H), 1.05 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.7, 146.3, 138.4, 136.1, 128.3, 127.4, 127.3, 125.0, 113.2, 75.5, 70.3, 58.4, 38.4, 36.2, 35.3, 28.5, 24.5, 24.3, 22.7, 21.7, 19.4, 18.9. Anal. Calcd for $C_{24}H_{34}O_2$: C, 81.30; H, 9.67. Found: C, 81.24; H, 9.67. **B**: IR (Neat) 3070, 3033, 2932, 2868, 1710 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.4-7.2 (m, 5H), 4.9-4.86 (m, 1H), 4.52 (1/2 ABq, J = 11.9 Hz, 1H), 4.51 (m, 1H), 4.45 (1/2 ABq, J = 11.9 Hz, 1H), 3.85 (dd, J = 9.2, 8.2 Hz, 1H), 3.45 (dd, J = 9.3, 3.5 Hz, 1H), 2.61 (dd, J = 8, 3.4 Hz, 1H), 2.4-2.25 (m, 2H), 2.2-2.05 (m, 2H), 1.95-1.7 (m, 2H), 1.7 (s, 3H), 1.65 (s, 3H), 1.63 (s, 3H), 1.6-1.2 (m, 4H), 0.75 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 211.8, 146.4, 138.5, 136.1, 128.3, 127.7, 127.5, 125.1, 113.2, 73.4, 65.6, 58.7,

58.6, 41.2, 41.1, 39.5, 35.3, 24.9, 22.7, 21.7, 21.4, 19.5. Anal. Calcd for $C_{24}H_{34}O_2$: C, 81.29; H, 9.67. Found: C, 81.15; H, 9.65.

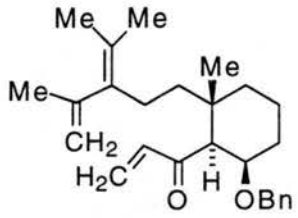


To a 0.2 M solution of benzyl ether (332 mg. 0.94 mmol) in acetic anhydride was added $FeCl_3$ (76 mg, 0.47 mmol). The mixture was heated to 50 °C for 24 h followed by cooling to 25 °C, the slow addition of H_2O (5 mL) and stirring for 30 min. The reaction was extracted 3x with hexanes (10 mL) and the organic phase dried over Na_2SO_4 and concentrated. The crude oil was purified by column chromatography using a 32:1, 16:1 (hexanes/ethyl acetate) gradient elution to provide the product (85 mg, 30%) as a pale yellow oil. IR (Neat) 2937, 2870, 1736, 1710, 1628, 1440, 1365, 1236, 1032, 891 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.89 (dq, J = 2.7, 1.5 Hz, 1H), 4.52 (dd, J = 2.8, 0.9 Hz, 1H), 4.36 (dd, J = 11, 9.3 Hz, 1H), 4.15 (dd, J = 11, 3.2 Hz, 1H), 2.6 (dd, J = 9.2, 3.4 Hz, 1H), 2.43-2.22 (m, 2H), 2.07 (apparent dt, J = 10.1, 2.9 Hz, 2H), 1.99-1.87 (m, 1H), 1.98 (s, 3H), 1.86-1.74 (m, 1H), 1.79 (dd, J = 8.1, 3.2 Hz, 1H), 1.73 (s, 3H), 1.66-1.49 (m, 1H), 1.63 (s, 6H), 1.44-1.34 (m, 2H), 0.77 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 210.7, 171.1, 146.3, 135.9, 125.4, 113.5, 59.8, 56.9, 41.4, 41.3, 39.7, 35.7, 24.9, 22.7, 21.8, 21.0, 20.9, 19.5.

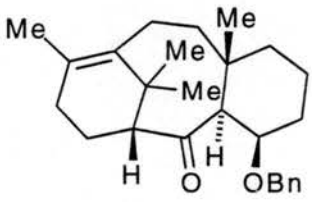
To a 0.03 M solution of the acetylated alcohol (85 mg, 0.28 mmol) in THF at 0 °C was added $LiAlH_4$ (11 mg, 0.28 mmol) and the resulting mixture was allowed to stir for 30 min. The reaction was quenched with 1 M HCl (10 mL) and extracted 3x with Et_2O (15 mL). The organic phase was washed 2x with brine (15 mL), dried over Na_2SO_4, and concentrated to furnish the desired diol in quantitative yield.



Allylic alcohol. To a 0.1M solution of aldehyde (5.5 g, 15.6 mmol) in THF at -78 °C was added a 0.45M solution of vinylmagnesium bromide (69 mL, 31.2 mmol) in THF dropwise and allowed to stir for 1h. The mixture was quenched with 1M HCl (100 mL) and extracted 3x with Et_2O (100 mL). The organic layer was washed 2x with brine (75 mL), dried over Na_2SO_4, and concentrated to give 5.9 g (100%) of product as a 5.4:1 mixture of diastereomers (pale brown oil). The crude oil was taken on directly. The diastereomers were isolated by column chromatography (128:1, 64:1 hexanes/EtOAc) to give clear colorless oils. R_f 0.54 (8:1 hexanes/EtOAc): IR (Neat) 3461, 3069, 2910, 1630 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.4-7.25 (m, 5H), 5.85 (ddd, J = 17, 10.4, 3.9 Hz, 1H), 5.35 (ddd, J = 17, 2, 2 Hz, 1H), 5.13 (ddd, J = 10.3, 2, 2 Hz, 1H), 4.91-4.88 (m, 1H), 4.6-4.52 (m, 3H), 4.45 (d, J = 8.2 Hz, 1H), 4.3 (1/2 ABq, J = 11.3 Hz, 1H), 4.18-4.11 (m, 1H), 1.74 (dd, J = 1.3, 1.3 Hz, 3H), 1.65 (s, 3H), 1.5-1.38 (m, 7H), 1.3 (s, 3H), 1.15 (dddd, J = 14.2, 12.7, 3.9, 3.9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.4, 142.6, 137.9, 136.7, 128.5, 127.7, 127.5, 124.7, 113.5, 113.1, 76.6, 71.7, 70.4, 49.3, 41.4, 38.3, 36.1, 28.6, 24.8, 23.6, 22.8, 21.7, 19.5, 16.9. Anal. Calcd for $C_{26}H_{38}O_2$: C, 81.61; H, 10.02. Found: C, 81.8; H, 10.18. R_f 0.37 (8:1 hexanes/EtOAc): IR (Neat) 3429, 3069, 2921, 2868, 1630 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 6.1 (ddd, J = 17.1, 10.3, 6.7 Hz, 1H), 5.18 (ddd, J = 17.2, 1.6, 1.6 Hz, 1H), 5.02 (ddd, J = 10.3, 1.4, 1.4 Hz, 1H), 4.9-4.85 (m, 1H), 4.58 (1/2 ABq, J = 11.7 Hz, H), 4.53-4.47 (m, 2H), 4.28 (1/2 ABq, J = 11.7 Hz, 1H), 3.85 (ddd, J = 6.3, 3.3, 3.3 Hz, 1H), 2.58 (s, 1H, D_2O exch.), 2.1-1.9 (m, 3H), 1.72 (s, 3H), 1.73-1.65 (m, 1H), 1.63 (s, 6H), 1.5-1.25 (m, 7H), 1.15 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.9, 142.1, 138.9, 137.1, 128.3, 127.3, 127.0, 124.4, 114.0, 112.8, 78.2, 74.9, 70.6, 50.9, 41.7, 37.6, 36.5, 28.3, 25.1, 23.7, 22.8, 21.7, 19.5, 17.7. Anal. Calcd for $C_{26}H_{38}O_2$: C, 81.61; H, 10.02. Found: C, 81.38; H, 9.86.

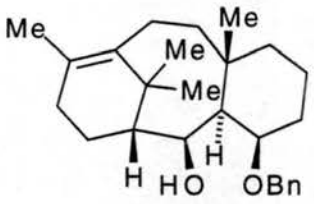


To a 0.1M solution of allylic alcohols (5.9 g, 15.6 mmol) in CH_2Cl_2 was added Dess-Martin reagent (11.1 g, 26.1 mmol) and allowed to stir for 12 h. $Na_2S_2O_3 \cdot 5H_2O$ (11.6 g, 46.8 mmol) was added to a 1M solution of $NaHCO_3$ (3 g, 35.9 mol) and this solution with Et_2O (50 mL) was added to the reaction mixture and allowed to stir for 30 min. Saturated aqueous $NaHCO_3$ solution (75 mL) was added and the mixture was extracted 3x with Et_2O (50 mL). The organic layer was washed 2x with saturated aqueous $NaHCO_3$ (50 mL), dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (32:1 hexanes/EtOAc) to give 4.2 g (91%) of product as a clear colorless oil. IR (Neat) 3048, 3027, 2932, 2868, 1694, 1673 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.2 (m, 5H), 6.41 (dd, J = 17.4, 10.4 Hz, 1H), 6.12 (dd, J = 17.4, 1.2 Hz, 1H), 5.64 (dd, J = 10.4, 1.2 Hz, 1H), 4.9-4.87 (m, 1H), 4.54 (1/2 ABq, J = 12.4 Hz, 1H), 4.51 (dd, J = 2.7, 0.73 Hz, 1H), 4.44 (1/2 ABq, J = 12.4 Hz, 1H), 3.77 (ddd, J = 10.8, 5.1, 5.1 Hz, 1H), 3.34 (d, J = 5.2 Hz, 1H), 2.1-1.75 (m, 5H), 1.75-1.6 (m, 1H), 1.7 (s, 3H), 1.62 (s, 3H), 1.6 (s, 3H), 1.45-1.15 (m, 4H), 0.85 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 202.9, 146.5, 139.9, 138.9, 136.4, 128.2, 127.3, 127.1, 126.4, 124.9, 113.2, 76.6, 70.3, 54.0, 37.3, 37.0, 32.0, 26.5, 25.1, 24.9, 22.8, 21.7, 19.7, 19.5. Anal. Calcd for $C_{26}H_{36}O_2$: C, 82.05; H, 9.54. Found: C, 82.08; H, 9.63.



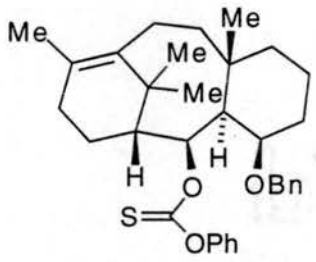
To a 0.15M solution of diene (321 mg, 0.84 mmol) in toluene at -78 °C was added $BF_3 \cdot OEt_2$ (0.21 mL, 1.68 mmol) and the resulting solution was allowed to sit at -23 °C for 48 h. Saturated aqueous $NaHCO_3$ solution (10 mL) was added and the mixture was extracted 3x with Et_2O (15 mL). The organic layer dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (64:1

hexanes/EtOAc) to give 99 mg (31%) of product as an oil which solidifies under vacuum (mp 75.7-77.0 °C). [It was found that scaling the reaction above 500 mg of the ketodiene, resulted in moderately lower yields as a result of the reaction not going to completion; the product and the starting material are not separable.] IR (Neat) 3016, 2911, 2868, 1683, 1456 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.45-7.34 (m, 2H), 7.34-7.15 (m, 3H), 4.6 (1/2 ABq, J = 12.1 Hz, 1H), 4.4 (1/2 ABq, J = 12.1 Hz, 1H), 3.55 (d, J = 2.5 Hz, 1H), 3.25 (d, J = 2.1 Hz, 1H), 2.81 (dt, J = 13.2, 5.6 Hz, 1H), 2.6-2.45 (m, 1H), 2.42 (d, J = 8.7 Hz, 1H), 2.19-2.02 (m, 1H), 2.02-1.6 (m, 7H), 1.8 (s, 3H), 1.5-1.38 (m, 1H), 1.28-1.1 (m, 2H), 1.25 (s, 3H), 1.2 (s, 3H), 1.1 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 214.9, 139.4, 137.6, 130.4, 128.0, 127.1, 126.9, 76.5, 71.8, 62.9, 51.2, 40.8, 38.9, 38.4, 38.3, 29.9, 29.8, 28.9, 25.7, 25.3, 25.2, 22.1, 18.6, 17.7. Anal. Calcd for $C_{26}H_{36}O_2$: C, 82.05; H, 9.54. Found: C, 82.02; H, 9.43.

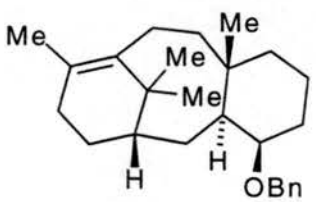


To a 0.07M solution of ketone (1.3 g, 3.4 mmol) in THF at 0 °C was added $LiAlH_4$ (136 mg, 3.4 mmol) and allowed to stir for 45 min. The mixture was quenched with 1M HCl (25 mL) and extracted 3x with Et_2O (25 mL). The organic layer was washed 1x with brine (25 mL), dried over Na_2SO_4, and concentrated to give 1.3 g (100%) of product as a single diastereomer (clear colorless oil). The crude was taken on directly. An analytical sample was prepared by column chromatography (64:1 hexanes/EtOAc). IR (Neat) 3586, 3006, 2928, 1454 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.2 (m, 5H), 4.63 (1/2 ABq, J = 11.7 Hz, 1H), 4.23 (1/2 ABq, J = 11.7 Hz, 1H), 3.85 (d, J = 3.1 Hz, 1H), 3.4 (d, J = 2.6 Hz, 1H), 2.73 (apparent dt, J = 13.5, 5 Hz, 1H), 2.57 (apparent dt, J = 14.5, 4.7 Hz, 1H), 2.38-2.21 (m, 1H), 2.28 (d, J = 3.2 Hz, 1H), 2.2-1.98 (m, 4H), 1.93 (dd, J = 8.9, 3 Hz, 1H), 1.85-1.6 (m, 3H), 1.65 (s, 3H), 1.55 (s, 3H), 1.45-1.35 (m, 1H), 1.28 (s, 3H), 1.28-0.98 (m, 4H), 1.0 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ

139.1, 138.5, 128.3, 127.8, 127.3, 127.2, 88.4, 86.6, 71.8, 49.5, 43.3, 41.9, 41.3, 38.6, 38.0, 32.7, 29.0, 28.4, 28.3, 26.1, 25.2, 23.9, 21.4, 18.2. Anal. Calcd for $C_{26}H_{38}O_2$: C, 81.63; H, 10.01. Found: C, 81.82; H, 10.12.

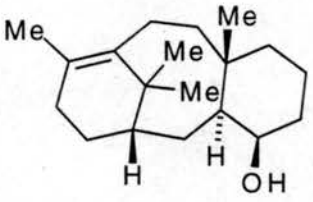


To a 0.06M solution of alcohol (1.3 g, 3.4 mmol) in THF at -78 °C was added phenyl chlorothionoformate (2.3 mL, 16.9 mmol) followed by a 1M solution of sodium bis(trimethylsilyl)amide (3.7 mL, 3.7 mmol) and allowed to stir for 10 min. The dry ice bath was removed and the mixture was allowed to sir for 18 h. EtOAc (50 mL) was added and the solution was washed 1x with H_2O (50 mL), 1x with brine (50 mL), dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (128:1, 64:1, 32:1 hexanes/EtOAc) to give 1.7 g, (100%) of product as a yellow solid (mp 153.1-153.8 °C). IR (Neat) 3004, 2925, 1485, 1450, 1283, 1190 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.4-7.2 (m, 8H), 6.8-6.7 (m, 2H), 5.52 (d, J = 3.7 Hz, 1H), 4.53 (1/2 ABq, J = 11.1 Hz, 1H), 4.33 (1/2 ABq, J = 11.1 Hz, 1H), 3.49 (d, J = 2.7 Hz, 1H), 2.84 (apparent dt, J = 13.5, 5.2 Hz, 1H), 2.68 (d, J = 2.7 Hz, 1H), 2.6 (dd, J = 13.7, 5.2 Hz, 1H), 2.5-2.38 (m, 1H), 2.35 (dd, J = 9.1, 3.7 Hz, 1H), 2.3-2.12 (m, 2H), 2.08 (br d, J = 15.9 Hz, 1H), 1.9-1.7 (m, 3H), 1.71 (s, 3H), 1.51 (s, 3H), 1.5-1.1 (m, 5H), 1.3 (s, 3H), 1.05 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.5, 153.5, 139.5, 138.2, 129.2, 128.8, 128.0, 127.4, 126.8, 126.1, 122.3, 96.8, 85.1, 72.1, 46.1, 42.1, 41.1, 38.5, 38.4, 32.2, 28.9, 28.8, 27.3, 25.9, 25.2, 22.2, 21.6,18.0.

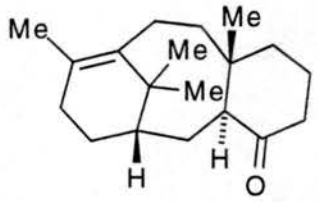


To a 0.03M solution of xanthate ester (1.76 g, 3.4 mmol) in toluene was added VAZO (348 mg, 1.0 mmol) followed by tributyltin hydride (4.6 mL, 16.9 mmol) and the resulting mixture was refluxed for 2 h. The mixture was then concentrated and the oil chromatographed (128:1, 64:1, 32:1 hexanes/EtOAc) to give 660

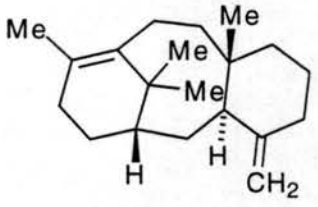
mg (53%) of product as a clear colorless oil. IR (Neat) 3008, 2925, 1455 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.2 (m, 5H), 4.58 (1/2 ABq, J = 12.2 Hz, 1H), 4.24 (1/2AB q, J = 12.2 Hz, 1H), 3.2 (ddd, J = 2.8, 2.8, 2.8 Hz, 1H), 2.1-1.6 (m, 6H), 1.65 (s, 3H), 1.4-1.1 (m, 5H), 1.3 (s, 3H), 1.05-0.9 (m, 4H), 1.0 (s, 3H), 0.95 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 140.0, 138.5, 129.5, 128.1, 126.8, 126.7, 83.9, 71.7, 43.5, 41.1, 39.5, 39.3, 38.6, 37.9, 32.3, 31.0, 30.2, 29.2, 25.5, 25.3, 24.9, 23.2, 21.8, 17.9. Anal. Calcd for $C_{26}H_{38}O$: C, 85.19; H, 10.45. Found: C, 85.03; H, 10.48.



To a 0.07M solution of benzyl ether (660 mg, 1.8 mmol) in THF at -78 °C was added NH_3 (25 mL). Na was added until the blue color of the solution persisted and allowed to stir for 1 h. The mixture was quenched with saturated aqueous $NH_4^+Cl^-$ solution (10 mL) and warmed up to 25 °C. H_2O (20 mL) and Et_2O (20 mL) were added and the reaction mixture was extracted 3x with Et_2O (20 mL). The organic layer was washed 1x with brine (20 mL), dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (32:1, 16:1, hexanes/EtOAc) to give 419 mg (84%) of product as a clear colorless oil which solidifies under vacuum (mp 110.5-111 °C). IR (Neat) 3368, 2926, 1456, cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 3.7 (br s, 1H), 2.7 (apparent dt, J = 13.3, 3.8 Hz, 1H), 2.42-2.28 (m, 1H), 2.17 (ddd, J = 5.4, 2.7, 2.7 Hz, 1H), 2.13-1.55 (m, 8H), 1.65 (s, 3H), 1.48-1.35 (m, 3H), 1.32 (s, 3H), 1.28 (dd, J = 4.7, 2.5 Hz, 1H), 1.25-1.15 (m, 3H), 1.02-0.92 (m, 1H), 1.0 (s, 3H), 0.95 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.2, 129.6, 76.3, 43.5, 41.2, 39.4, 38.9, 38.4, 37.8, 34.2, 32.0, 30.9, 30.1, 25.5, 24.9, 23.1, 21.8, 17.5. Anal. Calcd for $C_{19}H_{32}O$: C, 82.55; H, 11.67. Found: C, 82.47; H, 11.72.



To a 0.1M solution of alcohol (274 mg, 0.99 mmol) in CH_2Cl_2 was added Dess-Martin reagent (842 mg, 1.98 mmol) and allowed to stir for 1 h. $Na_2S_2O_3 \cdot 5H_2O$ (0.7 g, 2.97 mmol) was added to a 1M solution of $NaHCO_3$ (0.19 g, 2.28 mmol) and this solution with Et_2O (15 mL) was added to the reaction mixture and allowed to stir for 30 min. Saturated aqueous $NaHCO_3$ solution (20 mL) was added and the mixture was extracted 3x with Et_2O (20 mL). The organic layer was washed 2x with saturated aqueous $NaHCO_3$ solution (20 mL), dried over Na_2SO_4, and concentrated. The crude oil was purified by column chromatography (32:1 hexanes EtOAc) to give 181 mg (67%) of product as a white solid (mp 97.7-100 °C). IR (Neat) 2927, 1711, 1457 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 3.06 (d, J = 6H, 1H), 2.85 (apparent dt, J = 13.7, 5.3 Hz, 1H), 2.4-1.8 (m, 10H), 1.8-1.7 (m, 2H), 1.78 (s, 3H), 1.45-1.2 (m, 3H), 1.3 (s, 3H), 1.12 (ddd, J = 15, 10, 4.9 Hz, 1H), 1.0 (s, 3H), 0.95-0.79 (m, 1H), 0.62 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 213.5, 137.5, 130.1, 77.2, 51.4, 42.7, 42.4, 40.8, 39.5, 39.3, 36.8, 30.6, 29.9, 25.3, 24.7, 24.1, 23.4, 22.7, 22.2, 21.9. Anal. Calcd for $C_{19}H_{30}O$: C, 83.14; H, 11.03. Found: C, 82.89; H, 11.17.



Taxa-4(20,11(12)-diene. To a 0.04M solution of methyltriphenyl- phosphonium iodide (59 mg, 0.15 mmol), flame dried, in THF at 25 °C was added a 1.6M solution of nBuLi (0.09 mL, 0.15 mmol) in hexanes and allowed to reflux for 1 h. To this solution was added a 0.02M solution of ketone (20 mg, 0.073 mmol) in THF and allowed to reflux for 18.5 h. The reaction was cooled to 25 °C and saturated aqueous $NH_4^+Cl^-$ solution (10 mL) followed by hexanes (10 mL) were added. The phases were separated and the aqueous layer was extracted 2x with hexanes (10 mL). The organic layer was washed 1x with brine (10 mL), dried over Na_2SO_4, and concentrated. The crude

oil was purified by preparative TLC (100 % hexanes) to give 16 mg (80%) of taxa-4(20),11(12)-diene as a clear colorless oil. IR (Neat) 2929, 1644, 1458, 1376, 880 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.7 (dd, J = 2.9, 1.4 Hz, 1H), 4.48 (d, J = 1.1 Hz, 1H), 2.83-2.7 (m, 1H), 2.59 (br s, 1H), 2.4-2.2 (m, 2H), 2.15-1.75 (m, 6H), 1.75-1.69 (m, 1H), 1.73 (s, 3H), 1.3-1.1 (m, 3H), 1.3 (s, 3H), 1.0 (s, 3H), 0.6 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.8, 137.9, 129.7, 105.2, 43.7, 42.7, 40.3, 40.2, 39.4, 38.4, 38.0, 30.8, 30.2, 28.9, 25.5, 24.8, 24.1, 22.9, 22.8, 22.0. Anal. Calcd for $C_{20}H_{32}$: C, 88.16; H, 11.84. Found: C, 88.40; H, 11.88.

The ^{13}C NMR experiment revealed a total of twenty carbon resonances (see table 1) of which nine corresponded to methylene carbons, four to methyl carbons, five to quaternary carbons, and two to methine carbons via ^{13}C DEPT and HMQC experiments. One of the methylene carbon resonances and three of the quaternary carbon resonances appears downfield between 100 to 160 ppm. All remaining carbon resonances appear upfield between 20 to 50 ppm.

The ^1H NMR spectrum (see table 1) showed two proton resonances downfield between 4.4 and 4.8 ppm. All other signals were found upfield between 0.5 and 2.9 ppm. In the DFQ-COSY spectrum, both vinyl CH_2-20 (δ 4.7 and 4.5 ppm) protons exhibited cross peaks to two different sets of protons corresponding to methine CH-3 (δ 2.61 ppm) and to one of the methylene CH_2-5 (δ 1.88 and 2.24 ppm) protons located at 1.88 ppm. The HMQC spectrum was then used to assign the CH-3 (δ 42.7) and the CH_2-5 (δ 38.4 ppm) carbon signals. The CH-3 resonance showed only one crosspeak in the HMBC spectrum; belonging to the CH_2-20 methylene carbon. However, CH_2-5 protons showed cross peaks to the CH_2-20 carbon and to a quaternary carbon (δ 153.8 ppm) which was subsequently assigned as C-4.

The methine CH-3 proton was used to assign the methylene CH_2-2 (δ 1.59 and 1.62 ppm) protons and its carbon signal (δ 28.9 ppm) was assigned via the HMQC

spectrum. The CH_2-2 signals, in the DQF-COSY, exhibited a connectivity to methine CH-1 (δ 1.73) and the carbon resonance (δ 43.7 ppm) was assigned by analysis of the HMQC spectrum.

The methyl groups CH_3-16 (δ 1.02 ppm) and CH_3-17 (δ 1.31 ppm) were assigned based on previous analysis on a similar molecule. The CH_3-16 (δ 30.8 ppm) and the CH_3-17 (δ 25.5 ppm) carbon signals were assigned using the HMQC spectra. Further analysis of the HMBC spectrum showed the gem-dimethyl groups (CH_3-16, CH_3-17) and the CH-1 proton exhibiting a crosspeak to quaternary carbon C-15 (δ 39.4 ppm). The remaining upfield quaternary carbon (δ 40.2 ppm) was then assigned as C-8. The methyl group (δ 0.61 ppm) displayed a connectivity to the C-8 carbon in the HMBC spectrum and was thus assigned as CH_3-19. The last unlabeled methyl group (δ 1.75 ppm) was then assigned as CH_3-18 and its carbon signal (δ 22.0 ppm) established via the HMQC experiment.

Additional Interpretation of the HMBC spectra showed connectivities between the gem dimethyl groups and C-15 and with quaternary carbon C-11 (δ 137.9 ppm). The CH_3-18 protons displayed crosspeaks to C-11, C-12 (δ 129.7 ppm), and to methylene carbon CH_2-13 (δ 30.2 ppm).

The methylene CH_2-13 proton resonances (δ 1.79, 2.1 ppm) were assigned using the HMQC spectra. The DFQ-COSY spectrum displayed connectivities between CH_2-13 and CH_2-14 (δ 1.22, 2.01 ppm) while the HMBC spectra showed CH_2-13 proton connectivities to C-11 and CH_2-14 (δ 22.8 ppm). The methylene CH_2-14 displayed DFQ-COSY crosspeaks with CH-1 and HMBC connectivities with C-15, CH_2-13, and CH-1.

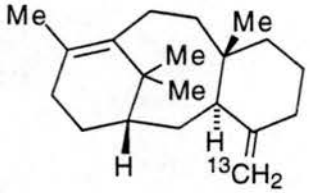
Further analysis of the HMBC spectra shows CH_2-5 proton cross peaks with carbons C-4, CH_2-20, and CH_2-6 (δ 24.1 ppm). The CH_2-6 proton resonances were assigned as 1.58 and 1.6 ppm using the HMQC spectra. The methylene CH_2-6 protons exhibited cross peaks with CH_2-7 (δ 1.15, 1.85 ppm) protons in the DFQ-COSY spectra. the CH_2-7 carbon (δ 38.0 ppm) resonance was assigned using the HMQC spectra. The

remaining two carbons at 24.8 ppm and 40.3 ppm were assigned as CH_2-10 and CH_2-9 respectively. The CH_2-10 protons (δ 2.06, 2.78 ppm) and the CH_2-9 protons (δ 1.2, 2.0 ppm) were assigned using the HMQC spectra. The CH_2-10 proton at 2.78 ppm displayed connectivities to quaternary carbons C-11 and C-12 while the CH_2-9 proton at 1.2 ppm exhibited a cross peak to methine CH-3 in the HMBC spectra.

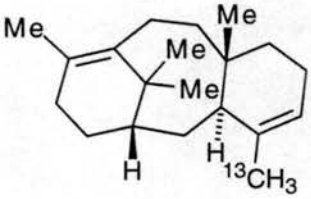
Table 5.1
Taxa-4(20),11(12)-diene[a]

Group	^{1}H shift	^{13}C shift
CH-1	1.73	43.7
CH_2-2	1.59, 1.62	28.9
CH-3	2.61	42.7
C-4	—	153.8
CH_2-5	1.88, 2.24	38.4
CH_2-6	1.57, 1.58	24.1
CH_2-7	1.15, 1.85	38.0
C-8	—	40.2
CH_2-9	1.2, 2.0	40.3
CH_2-10	2.06, 2.79	24.8
C-11	—	137.9
C-12	—	129.7
CH_2-13	1.79, 2.3	30.2
CH_2-14	1.22, 2.01	22.8
C-15	—	39.4
CH_3-16	1.02	30.8
CH_3-17	1.31	25.5
CH_3-18	1.75	22.0
CH_3-19	0.61	22.9
CH_2-20	4.5, 4.71	105.2

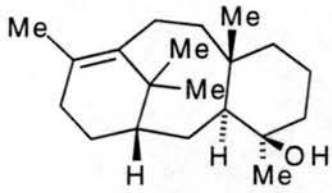
a. The experiment was run at 28 °C on a 18 mM solution of $CDCl_3$. The chemical shifts are reported in δ (ppm) using $CHCl_3$ as an internal standard set at δ 7.24 ppm for ^{1}H NMR and at δ 77.0 ppm for ^{13}C NMR.



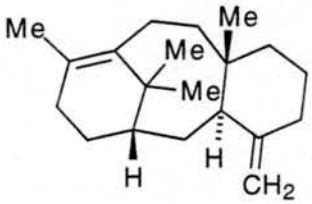
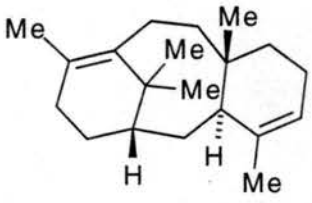
(C-20-$^{13}CH_2$)-Taxa-4(20),11(12)-diene To a 0.07M solution of [^{13}C]-methyltriphenyl phosphonium iodide (99 Atom % purchased from Aldrich) (115 mg, 0.29 mmol) in THF at 25 °C was added a 1.4M solution of nBuLi (0.2 mL, 0.29 mmol) in hexanes and allowed to reflux for 30 min. To this solution was added a 0.04 M solution of ketone (39 mg, 0.14 mmol) in THF and allowed to reflux for 18.5 h. The reaction was cooled to 25 °C and saturated aqueous $NH_4^+Cl^-$ solution (15 mL) followed by hexanes (15 mL) were added. The phases were separated and the aqueous layer was extracted 2x with hexanes (15 mL). The organic layer was washed 1x with brine (15 mL), dried over Na_2SO_4, and concentrated. The crude oil was purified by preparative TLC (100 % hexanes) to give 27 mg (80% based on recovered starting material) of [^{13}C]-taxa-4(20),11(12)-diene as a clear colorless oil. IR (Neat) 2928, 1620, 1457, 1376, 872 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.7 (dd, J = 154, 1.3 Hz, 1H), 4.5 (d, J = 154 Hz, 1H), 2.76 (m, 1H), 2.6 (br s, 1H), 2.4-2.2 (m, 2H), 2.1-1.7 (m, 6H), 1.75-1.69 (m, 1H), 1.75 (s, 3H), 1.65-1.5 (m, 4H), 1.3 (s, 3H), 1.3-1.1 (m, 3H), 1.1 (s, 3H), 0.6 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.8 (d, J = 73.5 Hz), 137.9, 129.7, 105.2, 43.7, 42.6, 40.24, 40.21, 39.4, 38.4 (d, J = 2.4 Hz), 38.0, 30.8, 30.2, 28.9 (d, J = 3.5 Hz), 25.5, 24.8, 24.1 (d, J = 1.9 Hz), 22.9, 22.7, 22.0.



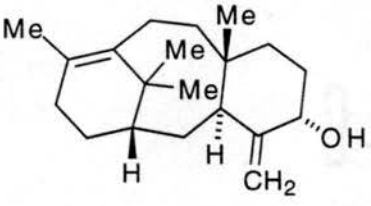
(C-20-$^{13}CH_3$)-Taxa-4(5),11(12)-diene. The same procedures were followed as described for the tritium labeled taxadienes except ^{13}C methyl iodide (99 atom% purchased from Aldrich) was used instead of ^{3}H methyl iodide. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.5 (d, J = 45.5 Hz), 137.7, 129.6, 121.1, 44.3, 41.4, 39.8, 39.0, 38.5, 37.3, 30.7, 29.8, 28.4, 26.3, 24.5, 24.0, 23.3, 22.6, 21.7, 21.5.



To a 0.02M solution of ketone (28 mg, 0.102 mmol) in THF was added anhydrous $CeCl_3$ (252 mg, 1.02 mmol); the resulting mixture was allowed to stir for 2h. This solution was cooled to 0 °C and a 3.1 M solution of MeMgBr (0.33 mL, 1.02 mmol) was added dropwise. The reaction mixture was allowed to stir for 30 min. at 0 °C. The reaction was quenched with saturated ammonium chloride solution (10 mL). The layers were separated and the aqueous layer was extracted twice with Et_2O (10 mL). The organic layer was washed once with brine solution (20 mL), dried over anhydrous Na_2SO_4, and concentrated to give 28 mg (93%) of product as a pale yellow oil. The crude, oily product was directly used for the next step without further purification. An analytical sample was prepared by column chromatography (silica gel, 32:1, hexanes/EtOAc). IR (neat) 3477, 2931, 1461, 1371 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 2.68 (apparent dt, J = 14.76, 6.74 Hz, 1H), 2.35-2.19 (m, 1H), 2.12-1.95 (m, 3H), 1.89 (dd, J = 5.38, 2.56 Hz, 1H), 1.8 (ddd, J = 13.23, 3.75, 3.75 Hz, 1H), 1.75-1.55 (m, 7H), 1.62 (s, 3H), 1.55-1.21 (m, 4H), 1.32 (s, 3H), 1.2-1.1 (m, 1H), 1.18 (s, 3H), 1.0 (s, 3H), 0.95 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.2, 128.8, 74.1, 43.5, 42.9, 41.4, 41.3, 39.4, 39.0, 38.9, 32.1, 31.7, 29.7, 26.8, 25.5, 24.8, 24.4, 22.2, 21.4, 19.0. HRMS Calcd for $C_{20}H_{34}O$: 290.2609, found: 290.2610.

Taxa-4(20),11(12)-diene and Taxa-4(5),11(12)-diene. To a 0.02M solution of alcohol (28 mg, 0.097 mmol) in toluene at reflux temperature was added Burgess reagent ($MeO_2CNSO_2NEt_3$) (46 mg, 0.193 mmol) and the resulting mixture was allowed to stir at reflux temperature for 10 min. The mixture was cooled to 25 °C and diluted with EtOAc (15 mL). The organic layer was washed once with brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude oil was purified by preparative TLC (silica gel, hexanes) to give 8 mg (Rf = 0.59, 30.5 %) of Taxa-4(20),11(12)-diene and 8 mg (Rf = 0.51, 30.5 %) of Taxa-4(5),11(12)-diene. Data for Taxa-4(5),11(12)-diene: IR (Neat) 2941, 1449, 1374 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.27 (m, 1H), 2.6 (ddd, J = 14.8, 10.0, 5.2 Hz, 1H), 2.5 (brs, 1H), 2.38-2.21 (m, 1H), 2.2-1.95 (m, 3H), 1.95-1.55 (m, 8H), 1.69 (s, 3H), 1.68 (s, 3H), 1.4 (ddd, J = 14.8, 5.5, 5.5 Hz, 1H), 1.31 (s, 3H), 1.18 (dd, J = 12.7, 6.0 Hz, 1H), 1.0 (s, 3H), 0.8 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.5, 137.7, 129.5, 121.1, 44.3, 41.4, 39.8, 39.0, 38.5, 37.3, 30.7, 29.8, 28.4, 26.3, 24.5, 23.9, 23.2, 22.6, 21.6, 21.5. This substance was found to be identical to an authentic, natural sample by ^1H NMR, ^{13}C NMR, ei-mass spec. and capillary gc.[20]



Taxa-4(20),11(12)-diene-5α-ol. To a 0.04M solution of SeO_2 (7 mg, 0.07 mmol) in CH_2Cl_2 was added ^tBuOOH (26 μL, 0.26 mmol) and the resulting mixture was allowed to stir for 0.5 h. To this solution was added a 0.07M solution of taxa-4(20),11(12)-diene (36 mg, 0.13 mmol) in CH_2Cl_2 and allowed to stir for 7h. The reaction mixture was condensed and the crude oil was purified by preparative TLC (silica gel, 4:1 hexanes/EtOAc) to give 14 mg (Rf = 0.75 2:1 hexanes/EtOAc , 39 %) of

product as a colorless oil. IR (neat) 3397, 2925, 1448 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 4.91 (dd, J = 1.4, 1.4 Hz, 1H), 4.62 (ddd, J = 1.7, 1.1, 0.5 Hz, 1H), 4.23 (dd, J = 2.7, 2.7 Hz, 1H), 3.31-3.28 (m, 1H), 2.82 (dt, J = 13.5, 5.3 Hz, 1H), 2.36-2.28 (m, 1H), 2.24 (ddd, J = 13.1, 13.1, 6.8 Hz, 1H), 2.1-1.93 (m, 3H), 1.87 (ddd, J = 18.6, 10.6, 3.1 Hz, 1H), 1.81 (dd, J = 1.1, 1.1 Hz, 3H), 1.78-1.68 (m, 3H), 1.62 (ddd, J = 15.5, 5.9, 2.4 Hz, 1H), 1.54 (ddd, J = 15.3, 5.3, 2.2 Hz, 1H), 1.32 (s, 3H), 1.28-1.18 (m, 2H), 1.17 (s, OH, 1H), 1.02 (s, 3H), 0.97 (ddd, J = 13.3, 4.3, 2.9 Hz, 1H), 0.59 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.9, 136.8, 130.5, 108.7, 74.6, 43.5, 40.0, 39.9, 39.2, 35.4, 32.7, 30.8, 30.16, 30.13, 28.0, 25.4, 24.7, 22.9, 22.1, 21.3. HRMS Calcd for $C_{20}H_{32}O$: 288.2453, found: 288.2448.

The ^{13}C NMR experiment revealed a total of 20 carbon resonances (see table 5.2) for taxa-4(20),11(12)-diene-5α-ol of which eight correspond to methylene carbons, four to methyl carbons, five to quaternary carbons, and three to methine carbons via ^{13}C DEPT and HMQC experiments. One of the methine resonances, one of the methylene resonances, and three of the quaternary resonances appears downfield between 74 and 160 ppm. All remaining carbon resonances appear upfield between 20 and 45 ppm.

The ^1H NMR spectrum (see table 5.2) shows five distinct signals between 2.7 and 5.0 ppm. All other proton signals can be found between 0.5 and 2.4 ppm. In the DQF-COSY experiment both vinyl CH_2-20 protons (δ 4.61, 4.91 ppm) exhibited a cross peak with methine proton CH-3 (δ 3.28 ppm). The CH_2-20 (δ 108.7 ppm) and CH-3 (δ 35.4 ppm) carbons were assigned using the HMQC spectra. Analysis of the HMBC experiment showed connectivities between the CH_2-20 protons with the CH-3 and the quaternary C-4 (δ 155.9 ppm) carbons.

The HMQC spectra displayed a proton resonance at 4.22 ppm connected to methine carbon CH-5 (δ 74.6 ppm). The CH-5 signal showed DQF-COSY connectivities to CH_2-6 (δ 1.75-1.6 ppm) and to the hydroxyl proton (δ 1.116 ppm). The CH-5 methine proton

also exhibited HMBC connectivities to methine CH-3, methylene CH_2-7 (δ 32.7 ppm), methylene CH_2-6 (δ 30.16 ppm), and methyl CH_3-19 (δ 22.1 ppm) carbons. The CH_2-6 and the CH_2-7 (δ 0.98, 2.33 ppm) signals exhibited DQF-COSY connectivities to each other. The HMBC spectra displayed cross peaks between CH_2-7 protons with quaternary C-8 (δ 40 ppm), CH-3, CH_2-6, and CH_3-19 carbons.

The methine CH-3 proton exhibited cross peaks to CH_2-2 (δ 1.54, 1.62 ppm) in the DQF-COSY experiment. The HMQC experiment was used to assign methylene CH_2-2 (δ 28 ppm) carbon. Further analysis of the HMBC spectra showed CH_2-2 proton connectivities to CH-1 (δ 43.5 ppm), C-8, C-15 (δ 39.2 ppm), CH-3, and CH_2-14 (δ 22.9 ppm). In the DQF-COSY spectrum the CH_2-2 resonances displayed connectivities to CH-1 (δ 1.72 ppm) while the CH_2-14 protons (δ 1.25, 2.1 ppm) exhibited connectivities to each other, CH-1, and CH_2-13 (δ 1.87, 2.31 ppm). The methylene CH_2-13 protons displayed cross peaks to carbons CH-1, CH_2-14, and CH_3-18 (δ 21.3 ppm) while the CH_3-18 resonance (δ 1.81 ppm) exhibited connectivities to carbons CH-1, and CH_2-13 (δ 30.13 ppm) in the HMBC experiment.

The carbon resonances at 30.8 and 25.4 ppm and the proton resonances at 1.02 and 1.31 ppm were assigned to methyl CH_3-16 and methyl CH_3-17 respectively based on literature precedent. The methyl groups CH_3-16 and CH_3-17 showed carbon connectivities to quaternary carbons C-15, and C-11 (δ 136.8 ppm) in the HMBC spectrum. The remaining quaternary carbon at 130.5 ppm was assigned as C-12.

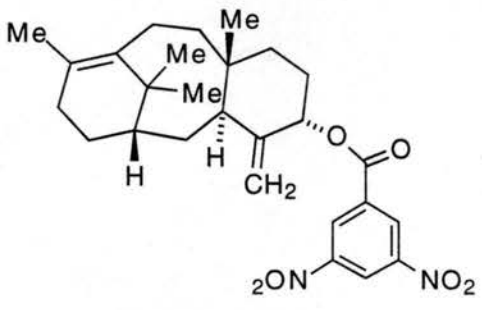
The methylene carbon signal at (δ 24.7 ppm) displayed connectivities to two protons at 2.08 and 2.82 ppm. The proton signal at 2.82 ppm showed cross peaks in the HMBC spectra to carbons C-11, CH-1, and C-15 and was thus labeled as a methylene CH_2-10 proton. The remaining methylene carbon (δ 39.9 ppm) was labeled as CH_2-9. The HMQC experiment displayed proton connectivities at 1.21 and 1.99 ppm to methylene

carbon CH_2-9. These proton signals exhibited crosspeaks to themselves and to CH_2-10 signals in the DFQ-COSY spectrum.

Table 5.2
Taxa-4(20),11(12)-diene-5α-ol[a]

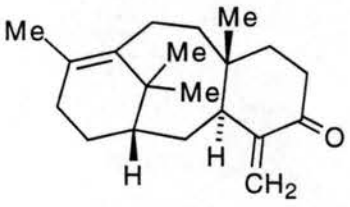
Group	^{1}H shift	^{13}C shift
CH-1	1.72	43.5
CH_2-2	1.58	28.0
CH-3	3.28	35.4
C-4	—	155.9
CH_2-5	4.22	74.6
CH_2-6	1.75	30.16
CH_2-7	0.98, 2.23	32.7
C-8	—	40.0
CH_2-9	1.21, 1.99	39.9
CH_2-10	2.08, 2.82	24.7
C-11	—	136.8
C-12	—	130.5
CH_2-13	1.87, 2.31	30.13
CH_2-14	1.25, 2.1	22.9
C-15	—	39.2
CH_3-16	1.02	30.8
CH_3-17	1.31	25.4
CH_3-18	1.81	21.3
CH_3-19	0.59	22.1
CH_2-20	4.61, 4.91	108.7

a. The experiment was run at 28 °C on a 18 mM solution of $CDCl_3$. The chemical shifts are reported in δ (ppm) using $CHCl_3$ as an internal standard set at δ 7.24 ppm for ^{1}H NMR and at δ 77.0 ppm for ^{13}C NMR.



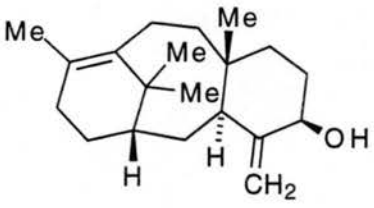
Taxa-4(20),11(12)-diene-5α-3,5-dinitrobenzoate. To a 0.03M solution of Taxa-4(20),11(12)-diene-5α-ol (20 mg, 0.07mmol) in CH_2Cl_2 was added pyridine (0.1 mL, 1.2 mmol) and the resulting mixture was cooled to 0 °C. To

this solution was added 3,5-Dinitrobenzoyl chloride (74 mg, 0.32 mmol) and allowed to stir for 4h as the mixture warmed to 25 °C. The reaction mixture was quenched with saturated aqueous NaHCO3 (5 mL), extracted 3x with Et2O (10 mL). The organic layer washed 1x with brine (20 mL), dried over Na2SO4, condensed to give an oil which was purified by preparative TLC (silica gel, 4:1 hexanes/EtOAc) to give 26 mg (Rf = 0.28 4:1 hexanes/EtOAc , 79 %) of product as a yellow solid. An analytical sample was prepared by recrystallization (decomposes at 171.5 °C). IR(neat) 3098, 2923, 1723, 1626, 1544, 1451, 1344, 1272, 1159 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 9.22 (t, J = 2.1 Hz, 1H), 9.15 (d, J = 2.1 Hz, 2H), 5.58 (t, J = 3 Hz, 1H), 5.28 (s, 1H), 4.98 (s, 1H), 3.0 (d, J = 4.2 Hz, 1H), 2.77 (apparent dt, J = 7.2, 14.7 Hz, 1H), 2.3-1.9 (m, 7H), 1.85-1.45 (m, 4H), 1.7 (s, 3H), 1.4-1.1 (m, 3H), 1.37 (s, 3H), 1.0 (s, 3H), 0.7 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.5, 149.3, 148.6, 137.7, 134.7, 129.4, 129.1, 122.3, 114.5, 80.8, 43.5, 40.1, 39.7, 39.1, 37.9, 33.6, 30.6, 30.2, 28.3, 27.9, 25.4, 24.7, 22.8, 22.4, 21.5. HRMS Calcd for $C_{27}H_{34}N_2O_6$: 482.2417, found: 482.2471.



5-Keto-taxa-4(20),11(12)-diene. To a 0.01M solution of Taxa-4(20),11(12)-diene-5α-ol (6 mg, 0.02 mmol) in CH_2Cl_2 was added Dess-Martin periodinane reagent (29 mg, 0.07 mmol); the resulting mixture was allowed to stir for 2 h. $Na_2S_2O_3 \cdot 5H_2O$ (15 mg, 0.06 mmol) was added to a 1M solution of $NaHCO_3$ (4 mg, 0.046 mmol); this solution with Et_2O (10 mL) was added to the reaction mixture and the resulting mixture was allowed to stir for 30 min. Saturated aqueous $NaHCO_3$ solution (10 mL) was added and the mixture was extracted with Et_2O (3 x 10 mL). The organic layer was washed twice with saturated aqueous $NaHCO_3$ solution (10 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude oil was purified by preparative TLC (silica gel, 4:1 hexanes/EtOAc) to give 4 mg (67%) of product as a colorless oil. IR (neat)

2928, 1697, 1458 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.81 (d, J = 2.5 Hz, 1H), 5.11 (d, J = 2.5 Hz, 1H), 2.87 (sextet, J = 2.5 Hz, 1H), 2.75 (apparent dq, J = 13.3, 3.8 Hz, 1H), 2.51-2.44 (m, 2H), 2.35-1.95 (m, 5H), 1.85-1.6 (m, 3H), 1.6 (s, 3H), 1.48-1.37 (m, 2H), 1.35 (s, 3H), 1.28-1.15 (m, 2H), 1.04 (s, 3H), 0.7 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.9, 151.9, 137.1, 129.8, 116.6, 43.2, 40.4, 39.6, 39.3, 37.9, 36.5, 33.9, 30.4, 29.8, 27.9, 25.5, 24.5, 23.5, 22.4, 21.5. HRMS Calcd for $C_{20}H_{30}O$: 286.2375, found: 286.2378.



Taxa-4(20),11(12)-diene-5β-ol. To a 0.01M solution of 5-keto-taxa-4(20),11(12)-diene (4 mg, 0.014 mmol) in THF at 0 °C was added $LiAlH_4$ (0.6 mg, 0.014 mmol) and the resulting mixture was allowed to stir for 15 min. The reaction mixture was quenched with H_2O (10 mL) and the aqueous layer was extracted 2x with Et_2O (10 mL). The organic layer was dried over Na_2SO_4 and condensed to give 4 mg (100%) of product as a clear colorless oil. An analytical sample was prepared by preparative TLC (silica gel, 4:1 hexanes/EtOAc) (Rf = 0.25, 4:1 hexanes/EtOAc). IR (neat) 3334, 2927, 2858, 1646, 1452 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.03 (s, 1H), 4.68 (s, 1H), 3.92-3.8 (m, 1H), 2.83-2.69 (m, 1H), 2.52 (br s, 1H), 2.4-2.2 (m, 1H), 2.15-1.82 (m, 5H), 1.82-1.5 (m, 5H), 1.7 (s, 3H), 1.5-1.1 (m, 4H), 1.3 (s, 3H), 1.0 (s, 3H), 0.6 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.7, 137.7, 129.6, 102.2, 74.2, 43.5, 40.3, 39.8, 39.5, 39.3 35.5, 33.3, 30.8, 30.1, 29.3, 25.4, 24.7, 22.9, 22.7, 21.9. HRMS Calcd for $C_{20}H_{32}O$: 288.2453, found: 288.2449.

The ^{13}C NMR experiment revealed a total of 20 carbon resonances (see table 5.3) for taxa-4(20),11(12)-diene-5β-ol of which eight correspond to methylene carbons, four to methyl carbons, five to quaternary carbons, and three to methine carbons via ^{13}C DEPT

and HMQC experiments. One of the methine resonances, one of the methylene resonances, and three of the quaternary resonances appears downfield between 74 and 160 ppm. All remaining carbon resonances appear upfield between 20 and 45 ppm.

The ^{1}H NMR spectrum (see table 5.3) shows six distinct signals between 2.2 and 5.2 ppm. All other proton signals can be found between 0.5 and 2.1 ppm. In the DQF-COSY experiment both vinyl CH_2-20 protons (δ 4.68, 5.05 ppm) exhibited a cross peak with methine proton CH-3 (δ 2.58 ppm). The CH_2-20 (δ 102.2 ppm) and CH-3 (δ 40.3 ppm) carbons were assigned using the HMQC spectra. Analysis of the HMBC experiment showed connectivities between the CH_2-20 protons with the CH-3, quaternary C-4 (δ 155.9 ppm), and CH-5 (δ 74.2 ppm) carbons.

The HMQC spectra displayed a proton resonance at 3.9 ppm connected to methine carbon CH-5. The CH-5 signal showed DQF-COSY connectivities to CH_2-6 (δ 1.42, 1.99 ppm). The CH_2-6 carbon (δ 33.3 ppm) was assigned using the HMQC spectra. The CH_2-6 and the CH_2-7 (δ 1.18, 1.71 ppm) signals exhibited DQF-COSY connectivities to each other. The HMBC spectra displayed cross peaks between CH_2-6 proton (δ 1.42 ppm) with CH_2-7 and CH-5 carbons, while CH_2-7 proton (δ 1.18 ppm) displayed connectivities to CH_3-19 (δ 22.9 ppm), CH-3, and quaternary C-8 (δ 39.8 ppm) carbons.

The methine CH-3 proton exhibited cross peaks to CH_2-2 (δ 1.6-1.7 ppm) in the DQF-COSY experiment. The HMQC experiment was used to assign methylene CH_2-2 (δ 29.3 ppm) carbon. Further analysis of the HMBC spectra showed CH_2-2 proton connectivities to C-4, CH-1 (δ 43.5 ppm), CH-3, and CH_2-14 (δ 22.7 ppm). In the DQF-COSY spectrum the CH_2-2 resonances displayed connectivities to CH-1 (δ 1.75 ppm) and CH-3, while the CH_2-14 protons (δ 1.22, 2.0 ppm) exhibited connectivities to each other, CH-1, and CH_2-13 (δ 1.78, 2.32 ppm). The CH_2-13 carbon (δ 30.1 ppm) was assigned using the HMQC spectra. The methylene CH_2-13 protons displayed cross peaks to carbons CH-1, CH_2-14, and CH_3-18 (δ 21.9 ppm) while the CH_3-18 resonance (δ 1.72

ppm) exhibited connectivities to carbons C-11 (δ 137.7 ppm), C-12 (δ 129.6 ppm), and CH_2-13 in the HMBC experiment.

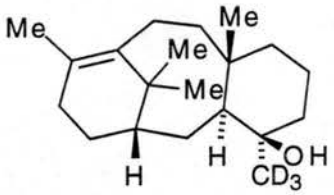
The carbon resonances at 30.8 and 25.4 ppm and the proton resonances at 1.01 and 1.32 ppm were assigned to methyl CH_3-16 and methyl CH_3-17 respectively based on literature precedent. The methyl groups CH_3-16 and CH_3-17 showed carbon connectivities to C-15, C-11, and CH-1 in the HMBC spectrum.

The methylene CH_2-10 carbon signal at (δ 24.7 ppm) displayed connectivities to two protons at 2.08 and 2.72 ppm. The proton signal at 2.72 ppm displayed proton connectivities to its geminal and vicinal CH_2-9 (δ 1.27, 2.02 ppm) neighbors. The HMQC experiment was used to assign carbon CH_2-9 (δ 39.5 ppm).

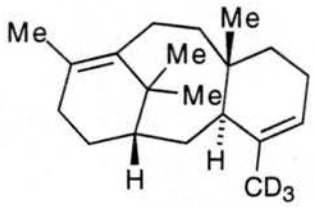
Table 5.3
Taxa-4(20),11(12)-diene-5β-0l[a]

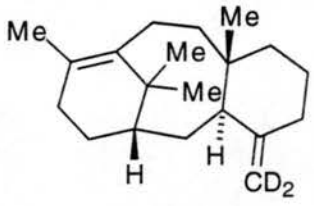
Group	^{1}H shift	^{13}C shift
CH-1	1.75	43.5
CH_2-2	1.6-1.7	29.3
CH-3	2.58	40.3
C-4	—	155.7
CH_2-5	3.9	74.2
CH_2-6	1.42, 1.99	33.3
CH_2-7	1.18, 1.71	35.5
C-8	—	39.8
CH_2-9	1.27, 2.02	39.5
CH_2-10	2.08, 2.77	24.7
C-11	—	137.7
C-12	—	129.6
CH_2-13	1.78, 2.32	30.1
CH_2-14	1.22, 2.0	22.7
C-15	—	39.3
CH_3-16	1.01	30.8
CH_3-17	1.32	25.4
CH_3-18	1.72	21.9
CH_3-19	0.6	22.9
CH_2-20	4.68, 5.05	102.2

a. The experiment was run at 28 °C on a 18 mM solution of $CDCl_3$. The chemical shifts are reported in δ (ppm) using $CHCl_3$ as an internal standard set at δ 7.24 ppm for ^{1}H NMR and at δ 77.0 ppm for ^{13}C NMR.

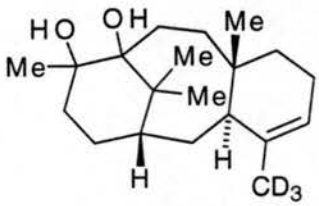


To a 0.02M solution of ketone (76 mg, 0.28 mmol) in THF was added anhydrous $CeCl_3$ (205 mg, 0.83 mmol); the resulting mixture was allowed to stir for 2h. This solution was cooled to 0 °C and a 3.1 M solution of (99+ atom% purchased from Aldrich) CD_3MgBr (0.83 mL, 0.83 mmol) was added dropwise. The reaction mixture was allowed to stir for 30 min. at 0 °C. The reaction was quenched with saturated ammonium chloride solution (15 mL). The layers were separated and the aqueous layer was extracted twice with Et_2O (15 mL). The organic layer was washed once with brine solution (20 mL), dried over anhydrous Na_2SO_4, and concentrated to give 65 mg (80%) of product as a pale yellow oil. The crude, oily product was directly used for the next step without further purification. An analytical sample was prepared by column chromatography (silica gel, 32:1, hexanes/EtOAc). IR (neat) 3468, 2927, 1455, 1381 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 2.68 (apparent dt, J = 14.8, 6.8 Hz, 1H), 2.35-2.19 (m, 1H), 2.12-1.95 (m, 3H), 1.89 (dd, J = 5.4, 2.5 Hz, 1H), 1.8 (ddd, J = 13.4, 3.8, 3.8 Hz, 1H), 1.75-1.55 (m, 7H), 1.62 (s, 3H), 1.55-1.21 (m, 4H), 1.32 (s, 3H), 1.2-1.1 (ddd, J = 12.3, 4.9, 2.79 Hz, 1H), 1.0 (s, 3H), 0.95 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.3, 128.9, 43.5, 42.9, 41.4, 41.2, 39.4, 39.0, 38.9, 31.7, 29.7, 26.8, 25.5, 24.8, 24.4, 22.2, 21.5, 19.0. HRMS Calcd for $C_{20}H_{31}D_3O$: 293.2844, found: 293.2784.



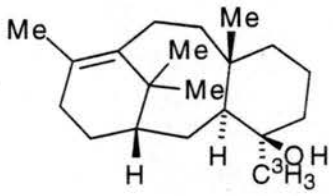


(C-20-CD_3)-Taxa-4(5),11(12)-diene and (C-20-CD_2)-Taxa-4(5),11(12)-diene . To a 0.02M solution of alcohol (65 mg, 0.22 mmol) in toluene at reflux temperature was added Burgess reagent ($MeO_2CNSO_2NEt_3$) (106 mg, 0.44 mmol) and the resulting mixture was allowed to stir at reflux temperature for 10 min. The mixture was cooled to 25 °C and diluted with EtOAc (45 mL). The organic layer was washed once with brine (45 mL), dried over anhydrous Na2SO4, and concentrated. The crude oil was purified by using $AgNO_3$ impregnated silica gel and a 80:1, 40:1 (hexanes/ Et_2O) gradient elution to give 36 mg (60 %) of (C-20-CD_3)-Taxa-4(5),11(12)-diene and 12 mg (20 %) of (C-20-D_2)-Taxa-4(20),11(12)-diene. Data for (C-20-CD_3)-Taxa-4(5),11(12)-diene : IR (Neat) 2922, 1458, 1375 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.27 (m, 1H), 2.6 (ddd, J = 14.8, 10.2, 5.4 Hz, 1H), 2.5 (m, 1H), 2.38-2.21 (m, 1H), 2.2-1.95 (m, 3H), 1.95-1.48 (m, 8H), 1.64 (s, 3H), 1.4 (ddd, J = 11.8, 6.4, 6.4 Hz, 1H), 1.31 (s, 3H), 1.18 (dd, J = 12.6, 6.0 Hz, 1H), 1.0 (s, 3H), 0.8 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.5, 137.7, 129.6, 121.1, 44.3, 41.4, 39.7, 39.0, 38.5, 37.3, 30.7, 29.8, 28.4, 26.3, 24.5, 23.2, 22.6, 21.6, 21.5. HRMS Calcd for $C_{20}H_{27}D_3$: 275.2689, found: 275.2686.

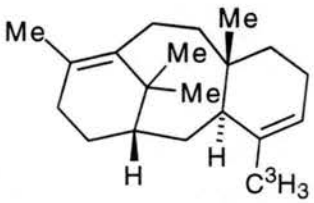


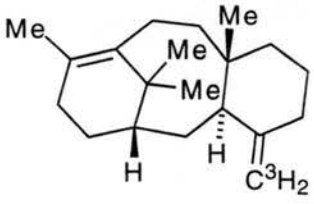
To a 0.02 M solution of the deuterated taxadiene (16 mg, 0.06 mmol) in pyridine was added OsO_4 (22 mg, .9 mmol) and allowed to stir for 12 h followed by the addition of 20% $Na_2S_2O_4$ (4 mL) which was stirred for 6 h. The mixture was diluted with H_2O (10 mL), extracted three times with EtOAc (10 mL), and dried over Na_2SO_4. The crude oil was purified by preparative TLC (silica gel) using EtOAc as the developing solvent to yield 8 mg (44%) of the undesired diol. IR (Neat) 3450, 2908, 1459 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.5-5.4 (m, 1H), 2.3-1.33 (m, 18H), 1.3 (s, 3H),

1.22 (s, 3H), 1.12 (s, 3H), 0.9 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.8, 123.2, 77.9, 74.9, 44.3, 43.3, 41.1, 36.1, 35.8, 33.3, 30.4, 29.7, 26.7, 25.4, 22.7, 20.7, 17.3.



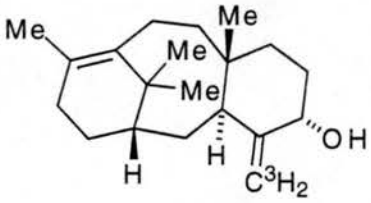
To a 0.2 M solution of magnesium (29 mg, 1.2 mmol) in Et_2O was added a catalytic amount of iodine followed by methyl iodide (65 mL, 1 mmol) diluted with 10 mCi of tritiated methyl iodide (obtained from Amersham). The resulting solution was refluxed for 1h. To a 0.1M solution of ketone (146 mg, 0.53 mmol) in THF was added anhydrous $CeCl_3$ (197 mg, 0.79 mmol); the resulting mixture was allowed to stir for 2.5 hr. This solution was cooled to 0 °C and the freshly made C^3H_3MgI was added dropwise. The reaction mixture was allowed to stir for 1 hr at 0 °C. The reaction was quenched with saturated ammonium chloride solution (30 mL). The layers were separated and the aqueous layer was extracted twice with Et_2O (30 mL). The organic layer was washed once with brine solution (30 mL), dried over anhydrous Na_2SO_4, and concentrated to give 110 mg (71%) of the alcohol, with a calculated specific activity of 6.8 mCi/mmol. as a pale yellow oil. The crude, oily product was directly used for the next step without further purification.



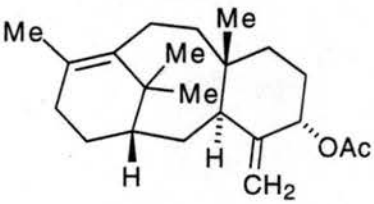


(C-20-$^3H_3$)-Taxa-4(5),11(12)-diene and (C-20-$^3H_2$)-Taxa-4(20),11(12)-diene. To a 0.02M solution of alcohol (110 mg, 0.38 mmol) in toluene at reflux temperature was added Burgess reagent ($MeO_2CNSO_2NEt_3$) (181 mg, 0.76 mmol) and the resulting mixture was allowed to stir at reflux temperature for 10 min. The mixture was cooled to 25 °C and diluted with EtOAc (45 mL). The organic layer was washed once with brine (45 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude oil

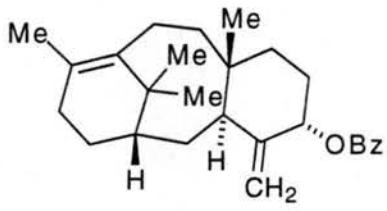
was purified by using $AgNO_3$ impregnated silica gel and a 80:1, 40:1 (hexanes/ Et_2O) gradient elution to give 57 mg (Rf = 0.21 (50:1(hexanes/ Et_2O), 55 %) of (C-20-$^3H_3$)-Taxa-4(5),11(12)-diene, with a calculated specific activity of 4.48 mCi/mmol, and 40 mg (Rf = 0.13 (50:1(hexanes/ Et_2O), 39 %) of (C-20-$^3H_2$)-Taxa-4(20),11(12)-diene.



(C-20-$^3H_2$)-Taxa-4(20),11(12)-diene-5α-ol. To a 0.02M solution of SeO_2 (40 mg, 0.15 mmol) in CH_2Cl_2 was added ^tBuOOH (13 mL, 0.3 mmol) and the resulting mixture was allowed to stir for 0.5 h. To this solution was added a 0.02M solution of (C-20-$^3H_2$)-Taxa-4(20),11(12)-diene (40 mg, 0.15 mmol) in CH_2Cl_2 and allowed to stir for 7 hr. The reaction mixture was condensed and the crude oil was purified by preparative TLC (silica gel, 4:1 hexanes/EtOAc) to give 21 mg (Rf = 0.75 2:1 hexanes/EtOAc , 52 %) of (C-20-$^3H_2$)-Taxa-4(20),11(12)-diene-5α-ol, with a calculated specific activity of 3.54 mCi/mmol, as a colorless oil.



Taxa-4(20),11(12)-diene-5α-acetate. To a 0.02M solution of Taxa-4(20),11(12)-diene-5α-ol (2 mg, 0.007mmol) in CH_2Cl_2 was added DMAP (4 mg, 0.03 mmol) and acetic anhydride (14 mL, 0.14 mmol). The resulting mixture was allowed to stir for 24 hr at 25 °C. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (5 mL) and extracted 3x with Et_2O (10 mL). The organic layer washed 1x with 1 M NaOH (10 mL), 1x with brine (20 mL), dried over Na_2SO_4, and condensed to give product (100%)



Taxa-4(20),11(12)-diene-5α-benzoate. To a 0.02M solution of Taxa-4(20),11(12)-diene-5α-ol (2 mg, 0.007mmol) in CH_2Cl_2 was added pyridine (10 mL, 0.13 mmol) and benzoyl chloride (7 mL, 0.06 mmol). The resulting mixture was allowed to stir for 24 hr at 25 °C. The reaction mixture was concentrated taken up in THF (5 mL) and saturated Na_2CO_3 (2 mL) solution and stirred for 30 min. The resulting solution was extracted 3x with Et_2O (10 mL), dried over Na_2SO_4, and concentrated to give product which was purified by preparative TLC (silica gel, 8:1 hexanes/EtOAc) 2mg (70%).

5.3 General Biosynthetic Procedures

Plants and standards - Two year old *Taxus brevifolia* saplings in active growth were obtained from the Weyerhaeuser Research Center, Centralia WA. Taxoid standards (taxol, cephalomannine) were obtained from Hauser Chemical Research Inc., Boulder, CO. *Taxus Canadensis* cells were obtained from Plant, Soil and Nutrition Laboratory of the USDA Agricultural Research Service, Ithica, New York.

Microsomal Cell-Free Extract Preparation - *Taxus Canadensis* cells (40 g) were frozen in liquid N_2 and pulverized in a mortar and pestal to break open the cells. The ground up cells were transferred to a 200 mL extraction buffer solution and allowed to stir at low speed for 30 min. The mixture was then allowed to gravity filter through eight layers of cheeses clothe for 20 min. The cheeses clothe was gently squeezed to collect some of the remaining solution, over squeezing will ruin the preparation (note: all of the preceding steps should be carried out in a 4 °C cold room). The resulting extraction buffer was transferred to chilled (0 °C) centrifuge tubes and centrifuged for 30 min. at 3000g. The resulting supernatant was transferred to another set of chilled centrifuge tubes and centrifuged for 20 min. at 10 Kg. The supernatant was then transferred to a pair of 100 mL polycarb centrifuge tubes and ultra-centrifuged for 90 min. at 190 Kg and 4 °C to afford

two small pellets (one in each tube). The supernatant (this should have trans-acylase activity) was decanted and discarded. The pellets were resuspended in 1 mL of chilled (0 °C) resuspension buffer and transferred to a homogenizer and homogenized. The resulting solution was then assayed (in triplicate) for its protein concentration using a Microscale Bradford-Biorad Protein Concentration Assay kit. The protein mixture can be frozen in liquid N_2 and stored in the -80 °C freezer for prolonged period of time.

Extraction Buffer Base Preparation for the Microsomal Assay - To 200 mL of distilled H_2O was added EGTA (ethylenebis[oxyethylenenitrilo]tetraacetic acid) (190 mg), HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid) (5.95 g), $MgCl_2 \cdot 6H_2O$ (51 mg), and glycerol (37.5 mL). The resulting solution was adjusted to a pH of 7.6 using NaOH or HCl followed by the addition of PMSF (α-toluenesulfonyl fluoride) (40 mg) and 4'-bromoacetophenone (50 mg). The solution was then allowed to stir for 24 h (note: the buffer base solution can be stored in a freezer for extended periods of time).

Extraction Buffer Preparation for the Microsomal Assay - The following chemicals should be added to the 200 mL buffer base solution immediately prior to use. To the 200 mL buffer base solution was added procain·HCl (682 mg), lidocain·HCl (678 mg), glycerol-1-phosphate (810 mg), ascorbate (220 mg), $NaHCO_3$ (238 mg), and DDT (dithiothreitol) (193 mg). The resulting solution was adjusted to a pH of 7.2-7.6 using NaOH or HCl followed by the addition of PVP·40 (polyvinyl pyrrolidine) (2.5 g), PVPP (polyvinylpoly pyrrolidine) (2.5 g), and XAD-resin (25 g).

Resuspension Buffer Preparation for the Microsomal Assay - To 250 mL of distilled H_2O was added of EGTA (95 mg), HEPES (1.49 g), $MgCl_2 \cdot 6H_2O$ (508 mg), KCl (466 mg), and glycerol (50 mL). The resulting solution was adjusted to a pH of 7.2 using NaOH or HCl. This solution can be stored for prolonged periods of time at 4 °C. Before the resuspension buffer can be used to resuspend the microsomal pellet, DDT (20 mg) and a 1 mg/mL stock solution of leupeptin (25 μL) must be added per 25 mL of resuspension buffer.

Transacylase Cell-Free Extract Preparation - The very light green tips of *Taxus brevifolia* (4 g) were frozen in liquid N_2 and pulverized in a mortar and pestal to break open the tissue. The ground up tissue were transferred to a 40 mL extraction buffer solution followed by the addition of XAD resin (2.4 g) and PVPP (2.4 g). The resulting solution was shaken vigorously followed by stirring at low speed for 30 min. The mixture was then allowed to gravity filter through four layers of cheeses clothe for 20 min. The cheese cloth was squeezed vigorously to collect the remaining solution (note: all of the preceding steps should be carried out in a 4 °C cold room). The resulting extraction buffer was transferred to chilled (0 °C) centrifuge tubes and centrifuged for 30 min. at 4650g. One mL of the resulting supernatant was set aside for the crude assay while the rest of the supernatant was slowly passed dropwise through an AMICON filter fitted with a YM membrane (note: if the supernatant is passed through the filter too quickly the acylase enzymes will pass through the membrane) leaving 4 mL of supernatant above the YM membrane. The supernatant solution was pipetted from the AMICON filter and loaded onto a Biorad (cat# 732-2010) disposable 10 mL desalting column. The 4 mL of supernatant was allowed to gravity filter through the column using 12 mL of assay buffer as the eluent while collecting 4 at 3 mL fractions. Each fraction including the crude supernatant fraction were then assayed for transacylase activity.

Transacylase Extraction Buffer Preparation - To 500 mL of distilled H_2O was added TRIS (tris[hydroxymethyl]aminomethane) (50 mmol) and borate (150 mmol). The solution was then adjusted to a pH of 7.6 at 0 °C followed by the addition of mercaptoethanol (0.5 mmol). This solution can be stored for prolonged periods of time.

Transacylase Assay Buffer Preparation - To 200 mL of distilled H_20 was added imidazole (1.36 g) and DTT (30 mg). The resulting solution was adjusted to pH 8.

***Taxus brevifolia* Buffer Preparation** - To 100 mL of distilled H_2O was added EDTA (7.6 mg), phenylalanine (33 mg), sodium benzoate (14.4 mg), KH_2PO_4 (68 mg), DTT (14.4 mg), sucrose (342.3 mg), $MgCl_2$ (20.3 mg), KCl (14.9 mg), tween 20 (50

mL), sodium acetate (13.6 mg), and sodium diphosphate (4.5 mg). The resulting solution was adjusted to pH 5.5 using NaOH or HCl. This solution can be stored for prolonged periods of time in a freezer.

AG (Ascorbic acid/Glutamine) preparation - To 10 mL of distilled H_2O was added ascorbic acid (25 mg) and glutamine (146 mg). This solution should be freshly prepared.

***Taxus canadensis* Buffer Preparation** - To 250 ml of distilled H_2O was added Gibco's Gamborg B-5 medium (5.8 g), a 0.1 mM stock solution of 6-benzylamonpurine (25 mL), and a 1mM stock solution of naphthalene acetic acid (671 mL). the resulting solution was adjusted to a pH of 5.5 using KOH followed by sterilization for 15 min. at 121 °C.

5.4 Biosynthetic Feeding Experimentals

The CP-450 oxidizing rate of taxa-4(20),11(12)-diene-5(α)-acetate as compared to taxa-4(20),11(12)-diene-5(α)-ol - To 500 µg of the microsomal cell-free proteins in resuspension buffer in a test tube with a secure lid was added of a 1:1 0.5 mM solution of FMN/FAD (flavin mononucleotide/flavin adenosine nucleotide) (10 µL) followed by the addition of a freshly prepared 100 mM solution of NADPH (10 µL) and enough resuspension buffer to bring the total assay volume to 1 mL. Tritiated taxa-4(20),11(12)-diene-5(α)-acetate (dpm = 200,000) was diluted in a minimum volume of pentane and added to the microsomal assay. The test tube was wrapped in tin foil and placed in a shaker bath at 31 °C for 2 h. The incubation was terminated by the addition of NaCl (250 mg) and Et_2O (2 mL). The resulting solution was vortexed and centrifuged for 2 min. The Et_2O layer was pipetted from the crude assay and passed over a plug of $MgSO_4$. The assay solution was extracted two more times with Et_2O (1 mL) and passed over the plug of $MgSO_4$ as described above. The $MgSO_4$ was washed with Et_2O (5 mL) and the combined Et_2O layer was then concentrated using a speed vacuum. Taxa-

4(20),11(12)-diene-5(α)-acetate, taxa-4(20),11(12)-diene-5(α)-ol, and an unidentified new polar product(s) were purified by silica gel chromatography (using a disposable pipette as the column) using a gradient elution of pentane, 1:1 (pentane/Et_2O), Et_2O. Tritiated taxa-4(20),11(12)-diene-5(α)-ol (dpm = 200,000) was also incubated with the microsomal proteins, workedup and purified as described above to yield taxa-4(20),11(12)-diene-5(α)-ol and the unidentified new more polar product(s). The tritiated taxa-4(20),11(12)-diene-5(α)-acetate was observed to undergo hydrolysis to taxa-4(20),11(12)-diene-5(α)-ol in addition to being oxidized to the unidentified more polar product(s) two times as fast as the tritiated taxa-4(20),11(12)-diene-5(α)-ol. The analysis was based on liquid scintillation counting using 10 mL of a cocktail solution consisting of 0.4% (w/v) omnifluor (Dupont/New England Nuclear) dissolved in 30% ethanol in toluene (^3H efficiency ~42%).

The Kinetic Isotope Effect of (C-20-CD_3)-taxa-4(5),11(12)-diene - To 500 μg of the microsomal cell-free proteins in resuspension buffer in a test tube with a secure lid was added of a 1:1 0.5 mM solution of FMN/FAD (flavin mononucleotide/flavin adenosine nucleotide) (10 μL), a freshly prepared 100mM solution of NADPH (10 μL), a 500 mM solution of glucose-6-phosphate (10 μL), a 25 μg/100 μL stock solution of glucose-6-phosphate dehydrogenase (10 μL), and enough resuspension buffer to bring the total assay volume to 1 mL. (C-20-CD_3)-Taxa-4(20),11(12)-diene (23.35 nmol) was diluted in a minimum volume of pentane and added to the microsomal assay. The test tube was wrapped in tin foil and placed in a shaker bath at 31 °C for 70 min. The incubation was terminated by the addition of NaCl (250 mg) and a 9:1 solution of pentane/Et_2O (2 mL). The resulting solution was vortexed and centrifuged for 2 min. The 9:1 pentane/Et_2O layer was pipetted from the crude assay and passed over a plug of $MgSO_4$. The assay solution was extracted two more times with the 9:1 solution of pentane/Et_2O (1 mL) and passed over the plug of $MgSO_4$ as described above. The $MgSO_4$ was washed with the 9:1 solution of pentane/Et_2O (5 mL) and the combined pentane/Et_2O layer was then concentrated using a speed vacuum. Taxa-4(20),11(12)-diene, taxa-4(20),11(12)-diene-

5(α)-ol were purified by silica gel chromatography (using a disposable pipette as the column) using a gradient elution of pentane, 9:1 (pentane/Et_2O) followed by preparative TLC (silica gel impregnated with 8% $AgNO_3$) using a 3:2 pentane/Et_2O solution as the eluent. The control, tritiated taxa-4(20),11(12)-diene (23.35 nmol, dpm = 950,000), was also incubated with the microsomal proteins, worked up and purified as described above to yield taxa-4(20),11(12)-diene and taxa-4(20),11(12)-diene-5(α)-ol. The taxa-4(20),11(12)-diene-5(α)-ol isolated from each assay was separately taken up in hexane and 1.94 ng of abietadienol (an internal standard) was added to each taxadienol solution. The relative areas of abietadienol and taxa-4(5),11(12)-ol for each assay were analyzed by a HP-5890 series II g.l.c. using an RTX-50 column. It was observed that (C-20-CD_3)-taxa-4(5),11(12)-diene was converted to taxa-4(20),11(12)-diene-5(α)-ol 1.7 times faster than the control (tritiated taxa-4(5),11(12)-diene).

Transacylase Activity Assayed for Taxa-4(20),11(12)-diene-5(a)-ol - To 100 μL of each transacylase cell-free extract fraction, including the crude fraction, in a test tube with a secure lid was added a 10 mM solution of acetyl coenzyme A (15 μL) in a 50 mM solution of MES-buffer (4-morpholineethanesulfonic acid) followed by taxa-4(20),11(12)-diene-5(α)-ol (dpm = 200,000) in a minimum amount of pentane. The resulting five assays were incubated, exclusion of light not necessary, in a shaker bath at 31 °C for 1.5 h. The work-up procedure is the same as the taxa-4(20),11(12)-diene-5(α)-acetate microsomal assay. After the work-up, the organic layer of each assay was spiked with unlabeled taxa-4(20),11(12)-diene-5(α)-acetate. Each assay was purified by preparative TLC (silica gel impregnated with 8% $AgNO_3$) using a 8:1 hexanes/EtOAc solution as the eluent. The taxa-4(20),11(12)-diene-5(α)-acetate was subsequently isolated and observed to contain radioactivity. The analysis was based on liquid scintillation counting using 10 mL of a cocktail solution consisting of 0.4% (w/v) omnifluor (Dupont/New England Nuclear) dissolved in 30% ethanol in toluene (^3H efficiency ~42%).

The *invivo* Incorporation of (C-20-C^3H_2)-taxa-4(20),11(12)-diene-5(α)-ol using *Taxus brevifolia* Stem Discs - To a sterilized glass vial was added tritiated taxa-4(20),11(12)-diene-5(α)-ol (dpm = 200,000) in a minimum amount of pentane that was evaporated using air. 1.5 ml of the *Taxus brevifolia* buffer solution was filtered through a Nalgene 0.2 μm syringe filter into the glass vial. *Taxus brevifolia* stem discs (1.75 g) prepared from sapling stem sections that had been surfaced sterilized in 70% EtOH (1 sec.) followed by 3% sodium hypochlorite, 0.5% Tween 20 (1 min.) and washed two times in distilled H_2O (1 min. followed by 5 min.) were cut 1-2 mm thick using sterile technique. The preceding steps were performed in a sterilized laminar flow hood. The stem discs and tritiated taxadienol were vacuum infiltrated six times and incubated for two days at 25 °C in the air and light with slow shaking on a bi-directional rotator. The buffer solution was pipetted out of the vial and placed in a test tube followed by the addition of a 6:3:1 solution of CH_3Cl/CH_3CN/MeOH (1 mL) to the remaining stem discs in the glass vial. The organic solution was mixed well with the stem discs followed by transferring via pipette to the test tube containing the buffer solution. The buffer/organic biphasic mixture was vortexed well followed by centrifugation for two minutes. The organic layer was transferred via pipette to another test tube. This extractive process was repeated two more times. The stem discs were frozen in liquid N_2 and pulverized with a mortar and pestal to break open the tissue. The tissue was extracted three times as described above for the buffer solution except 3 mL of organic solution was used instead of 1 mL. The organic phases from both the buffer and stem discs extraction were combined and concentrated using a speed vacuum. The crude mixture was diluted with taxol (2 mg) and run up a preparative TLC plate (silica gel) using 20:1 (CH_3Cl/MeOH). The taxol zone was scraped off and observed to contain radioactivity. The analysis was based on liquid scintillation counting using 10 mL of a cocktail solution consisting of 0.4% (w/v) omnifluor (Dupont/New England Nuclear) dissolved in 30% ethanol in toluene (^3H efficiency ~42%).

***Taxus cuspidata* Cell Culture Feeding Experiment** - To 40 mL of sterilized buffer solution was added AG solution (1 mL), *Taxus cuspidata* cells (~1 g), and (C-20-$^{13}CH_3$)-taxa-4(5),11(12)-diene (1.25 mg) dissolved in 250 μL of EtOH. The preceding steps are performed in a sterilized laminar flow hood. The resulting mixture was shaken for seven days under an atmosphere of O_2 (10%), CO_2 (0.5%), and C_2H_2 (5 ppm). The cells were then filtered off through a buchner funnel and washed with H_2O (10 mL). The *Taxus* cells were transferred to a 125 Erlenmeyer flask followed by the addition of CH_2Cl_2 (75 mL) and sonicated for 30 min. at 25 °C. The cells were then filtered off through a buchner funnel and the organic layer set aside. The aqueous supernatant was extracted three times with CH_2Cl_2 (75 mL). The organic phases from the aqueous and cell extractions were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude material was run up a preparative plate (slica gel) using 1:1 (hexane/EtOAc) as the eluent. The zone where taxol should be was scraped off the TLC plate and was analyzed for its taxol content. FAB mass spectral analysis showed no detectable taxol formation.

REFERENCES AND FOOTNOTES

(1) For reviews see: (a) Georg, G.I.; Ali, S.M.; Zygmunt, J.; Jayasinghe, L.R. *Exp. Opin. Ther. Patents.* **1994**, *4*, 109. (b) Boa, A.N.; Jenkins, P.R.; Lawerence, N.J. *Contemp. Org. Synth.* **1994**, 47. (c) Nicolaou, K.C.; Dai,W-M.; Guy, R.K. *Angew. Chem. Int. Ed. Eng.* **1994**, *33*, 15. (d) Guenard, D.; Gueritte-Voegelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160. (e) Kingston, D.G. *Pharmac. Ther.* **1991**, *52*, 1. (f) Swindell, C.S. *Org. Prep. Proc. Inter.* **1991**, *23*, 465. (g) Rowinsky, E.K.; Cazenave, L.A.; Donehower, R.C. *J. Nat. Canc. Inst.* **1990**, *82*, 1247. (h) Floss, H.G.; Mocek, U., in *Taxol: Science and Applications* (Suffness, M., Ed.), CRC Press, Boca Raton, Florida, **1995**. (i) Haar, E.; Kowalski, R.J.; Hamel, E.; Lin, C.M.; Longley, R.E.; Gunasekera, S.P.; Rosenkranz, H.S.; Day, B.W. *Biochemistry* .**1996**, *35*, 243.

(2) Wani, M.C.; Tayler, H.L.; Wall, M.E.; Coggon, P.; McPhail, A.T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.

(3) Stull, D.P.; Scales, T.A.; Daughenbaugh, R.; Jans, N.A.; Bailey, D.T. *Appl. Biochem. and Biotechnol.* **1995**, *54*, 133.

(4) Stierle, A.; Stierle, D.; Strobel, G. *Science*. **1993**, *260*, 214.

(5) McNeil, C. *J. Nat. Canc. Instit.* **1995**, *87*, 1106.

(6) Hanson, R.L.; Wasylyk, J.M.; Nanduri, V.B.; Czulino, N.D.; Patel, R.N.; Szarka, L. *J. Biol. Chem.* **1994**, *269*, 22145.

(7) Zhang, H.; Takeda, Y.; Minami, Y.; Yoshida, K.; Matsumoto, T.; Xiang, W.; Mu, O.; Sun, H. *Chemistry Lett.* **1994**, 957.

(8) Voegelein-Gueritte, F.; guenard, D.; Potier, P. *J. Nat. Prod.* **1987**, *50*, 9.

(9) (a) Chattopadhyay, S.K.; Sharma, R. *Phytochemistry*. **1995**, *39*, 935. (b) Kobayashi, J.; Ogiwara, A.; Hosoyama, H.; Shigemori, H.; Yoshida, N.; Sasaki, T.; Li, Y.; Iwasaki, S.; Naito, M.; Tsuruo, T. *Tetrahedron*, **1994**, *50*, 7401. (c) Zhang, H.; Lee, C.T-L.; Chen, K.; Kashiwada, Y.; Zhang, D-C.; McPhail, A.T.; Lee, K-H. *J. Chem. Soc. Chem. Commun.* **1994**, 1561. (d) Fuji, K.; Tanaka, K.; Li, B.; Shinga, T.; Yokoi, T.; Sun, H.; Taga, T. *Tetrahedron*. **1995**, *51*, 10175. (e) Zhang, H.; Sun, H. *J. Nat.*

Prod. **1995**, *58*, 1153. (f) Fang, W-S.; Fang, Q-C.; Liang, X-T.; Lu, Y.; Zheng, Q-T. *Tetrahedron.* **1995**, *51*, 8483. (g) Zhang, H.; Tadeda, Y.; Sun, H. *Phytochemistry.* **1995**, *39*, 1147. (h) Fang, Q-C.; Fang, Q-Y.; Liang, X-T.; He, C-H. *Phytochemistry.* **1995**, *39*, 871. (i) Kobayashi, J.; Hosoyama, H.; Koiso, Y.; Iwasaki, S. *Experimentia.* **1995**, *51*, 592. (j) Tanaki, K.; Fuji, K.; Yokoi, T.; Shinga, T.; Li, B.; Sun, H. *Chem. Pharm. Bull.* **1994**, *42*, 1539. (k) Zamir, L.O.; Nedea, M.E.; Zhou, Z-H.; Belair, S.; Caron, G.; Sauriol, F.; Jacqmain, E.; Jean, F.I.; Garneau, F-X.; Mamer, O. *Can. J. Chem.* **1995**, *73*, 655.

10) Kobayashi, J.; Inubushi, A.; Hosoyama, H.; Yoshida, N.; Sasaki, T.; Shigemori, H. *Tetrahedron.* **1995**, *51*, 5971.

(11) Fuji, K.; Tanaka, B.; Li, T.; Shingu, H.; Taga, S.; Taga, T. *Tetrahedron Lett.* **1992**, *33*, 7915.

(12) (a) Fleming, P.E.; Knaggs, A.R.; He, X-G.; Mocek, U.; Floss, H.G. *J. Am. Chem. Soc.* **1994**, *116*, 4137. (b) Fleming, P.E.; Mocek, U.; Floss, H.G. *J. Am. Chem. Soc.* **1993**, *115*, 805.

(13) Alonso, W.R.; Croteau, R. *Methods Plant Biochem.* **1993**, *9*, 239.

(14) (a) Harrison, J.W.; Scrowston, R.M.; Lythgoe, B. *J. Chem. Soc. (C).* **1966**, 1933; see also: (7) Gueritte-Voegelein, F.; Guenard, D.; Potier, P., *J. Nat. Prods.* **1987**, *50*, 9.

(15) (a) Erdtman, H.; Norin, T.; Sumimoto, M.; Morrison, A. *Tetrahedron Lett..* **1964**, *5*, 3879. (b) Karlsson, B.; Pilloti, A-M.; Soderholm, A-C.; Norin, T.; Sundin, S.; Sumimoto, M. *Tetrahedron..* **1978**, *34*, 2349.

(16) Dauben, W.G.; Hubbell, J.P.; Oberhansli, P.; Thiessen, W.E. *J. Org. Chem* . **1979**, *20*, 44, 669.

(17) Robinson, D.R.; West, C.A. *Biochemistry*. **1970**, *9*, 70.

(18) (a) Kato, T.; Takayanagi, H.; Suzuki, H.; Uyehara, T. *Tetrahedron Lett*. **1978**, *19*, 1201. (b) Kumagai, T.; Ise, F.; Uyehara, T.; Kato, T. *Chemistry Lett*. **1981**, 25.

(19) Jackson, C.B.; Pattenden, G. *Tetrahedron Lett*. **1985**, *26*, 3393.

(20) (a) Begley, M.J.; Jackson, C.B.; Pattenden, G. *Tetrahedron Lett*. **1985**, *26*, 3397. (b) Begley, M.J.; Jackson, C.B.; Pattenden, G. *Tetrahedron*. **1990**, *46*, 4907.

(21) Koepp, A.E.; Hezari, M.; Zajicek, J.; Vogel, B.S.; LaFever, R.E.; Lewis, N.G.; Croteau, R. *J. Biol. Chem*. **1995**, *270*, 8686.

(22) Hezari, M.; Lewis, N.G.; Croteau, R. *Arch. Biochem. Biophys*. **1995**, *322*, 437.

(23) Corey, E.J.; Cheng, X-M., in *The Logic of Chemical Synthesis*, John Wiley & Sons, New York, **1989**.

(24) Denis, J-N.; Greene, A.E.; Guenard, D.; Gueritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc*. **1988**, *110*, 5917.

(25) (a) Holton, R.A.; Somoza, C.; Kim, H.B.; Liang, F.F.; Biediger, R.J.; Boatman, P.D.; Shindo, M.; Smith, C.C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.;

Tang, S.; Zhang, P.; Murchi, K.K.; Gentile, L.N.; Liu, J.H. . *J. Am. Chem. Soc.* **1994**, *116*, 1597. (b) Holton, R.A.; Somoza, C.; Kim, H.B.; Liang, F.F.; Biediger, R.J.; Boatman, P.D.; Shindo, M.; Smith, C.C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murchi, K.K.; Gentile, L.N.; Liu, J.H. . *J. Am. Chem. Soc.* **1994**, *116*, 1599.

(26) (a) Nicolaou, K.C.; Yang, Z.; Liu, J.J.; Ueno, H.; Nantermet, K.; Guy, K.; Claiborne, C.F.; Renaud, J.; Couladouros, E.A.; Paulvannan, K.; Sorensen, E.J. *Nature* **1994**, *367*, 630. (b) Nicolaou, K.C.; Yang, Z.; Liu, J.J.; Ueno, H.; Guy, K.; Couladouros, E.A.; Sorensen, E.J. *J. Am. Chem. Soc.* **1995**, *117*, 624. (c) Nicolaou, K.C.; Yang, Z.; Liu, J.J.; Ueno, H.; Guy, K.; Claiborne, C.F.; Sorensen, E.; Hwang, C-K.; nakeda, M.; Nantermet, K. *J. Am. Chem. Soc.* **1995**, *117*, 634. (d) Nicolaou, K.C.; Yang, Z.; Liu, J.J.; Nantermet, K.; Claiborne, C.F.; Renaud, J.; Guy, K.; Shibayama, K. *J. Am. Chem. Soc.* **1995**, *117*, 645. (e) Nicolaou, K.C.; Ueno, H.; Liu, J.J.; Nantermet, K.; Yang, Z.; Renaud, J.; Paulvannan, K.; Chadha, R. . *J. Am. Chem. Soc.* **1995**, *117*, 653. (f) Nicolaou, K.C.; Guy, K. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2079.

(27) (a) Masters, J.J.; Link, J.T.; Snyder, L.B.; Young, W.B.; Danishefsky, S.J. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1723. (b)) Masters, J.J.; Jung, D.K., Danishefsky, S.J.; Snyder, L.B.; Park, T.K.; Isaacs, R.C.A.; Alaimo, C.A.; Young, W.B. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 452. (c) Grandi, M. J.D.; Coburn, C.A.; Isaacs, R.C.A.; Danishefsky, S.J. *J. Org. Chem.* **1993**, *58*, 7728. (d) Magee, T.V.; Bornmann, W.G.; Isaacs, R.C.A.; Danishefsky, S.J. *J. Org. Chem.* **1992**, *57*, 3274.

(28) Kende, A.S.; Johnson, S.; Sanfilippo, P.; Hodges, J.C.; Jungheim, L.N. *J. Am. Chem. Soc.* **1986**,*108* , 3513.

(29) (a) Horiguchi, Y.; Furakawa, T.; Kuwajima, I. *J. Am. Chem. Soc.* **1989**,*111*,, 8277. (b) Seto, M.; Morihira, K.; Horiguchi, Y.; Kuwajima, I. *J. Org. Chem.* **1994**,*59*,, 3165.

(30) Hitchcock, S.A.; Pattendon, G. *Tetrahedron Lett.* **1992**, *33*, 4843.

(31) (a) Kress, M.H.; Ruel, R.; Miller, W.H.; Kishi, Y. *Tetrahedron Lett.* **1993**, *34*, 5999. (a) Kress, M.H.; Ruel, R.; Miller, W.H.; Kishi, Y. Tetrahedron Lett. **1993**, *34*, 6003.

(32) Masters, J.J.; Jung, D.K.; Bornmann, W.G.; Danishefsky, S.J. *Tetrahedron Lett.* **1993**, *34*, 7253.

(33) Nicolaou, K.C.; Yang, Z.; Sorensen, E.J.; Nakada, M. *J. Chem. Soc. Chem. Commun.* **1993**, 1024.

(34) Swindell, C.S.; Fan, W.; Klimko, P.G. *Tetrahedron Lett.* **1994**, *35*, 4959.

(35) Holton, R.A. *J. Am. Chem. Soc.* **1984**, *106*, 5731.

(35) Holton, R.A.; Juo, R.R.; Kim, H.B.; Williams, A,D.; Harusawa, S.; Lowenthal, R.E.; Yogai, S. *J. Am. Chem. Soc.* **1988**, *110*, 6558

.

(37) (a) Wender, P.A.; Mucciaro, T.P. . *J. Am. Chem. Soc.* **1992**, *114*, 5878. (b) Wnder, P.A.; Floreancig, P.E.; Glass, T.E.; Natchus, M.G.; Shuker, A.J.; Sutton, J. *Tetrahedron Lett.* **1995**, *36*, 4939.

(38) Funk, R.L.; Daily, W.J.; Parvez, M. *J. Org. Chem.* **1988**, *53*, 4143.

(39) (a) Yadav, J.S.; Ravishankar, R. *Tetrahedron Lett.* **1991**, *32*, 2629. (b) Yadav, J.S.; Ravishankar, R.; Samala, L. *Tetrahedron Lett.* **1994**, *35*, 3617.

(40) Martin, S.F.; White, J.B.; Wagner, R. . *J. Org. Chem.* **1982**, *47*, 3190.

(41) (a) Paquette, L.A.; Bailey, S. *J. Org. Chem.* **1995**, *60*, 7849. (b)) Paquette, L.A.; Montgomery, F.J.; Wang, T-Z. *J. Org. Chem.* **1995**, *60*, 7857.

(42) Sakan, K.; Craven, B.M. . *J. Am. Chem. Soc.* **1983**, *103*, 3732.

(43) (a) Shea, K.J.; Davis, P.D. *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 419. (b) Jackson, R.W.; Shea, K.J. *Tetrahedron Lett.* **1994**, *35*, 1317.

(44) Jenkin, P.R.; Bonnert, R.V. *J. Chem. Soc. Perkin Trans I.* **1989**, 413.

(45) (a) Park, T.K.; Kim, I.J.; Danishefsky, S.J. *Tetrahedron Lett.* **1995**, *36*, 1015. (b) Park, T.K.; Kim, I.J.; Danishefsky, S.J.; Gala, S. *Tetrahedron Lett.* **1995**, *36*, 1019. (c) Masters, J.J.; Jung, D.K.; Danishefsky, S.J.; Snyder, L.B.; Park, T.K.; Isaacs, R.C.A.; Alaimo, C.A.; Young, W.B. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 452.

(46) (a) Lu, Y.F.; Fallis, A.G. *Tetrahedron Lett.* **1993**, *34*, 3367. (b)) Lu, Y.F.; Harwig, C.W.; Fallis, A.G . *J. Org. Chem.* **1993**, *58*, 4202.

(47) Wang, Z.; Warder, S.E.; Perrier, H.; Grimm, E.L.; Bernstein, M.A. *J. Org. Chem.* **1993**, *58*, 2931.

(48) (a) Winkler, J.D.; Kim, H.S.; Kim, S. *Tetrahedron Lett.* **1995**, *36*, 687. (b) Winkler, J.D.; Subrahmanyam, D. *Tetrahedron.* **1992**, *48*, 7049.

(49) Blechert, S.; Muller, R.; Beitzel, M. *Tetrahedron.* **1992**, *48*, 6953.

(50) Kujima, T.; Inouye, Y.; Kakisawa, H. *Chem. Lett.* **1985**, 323.

(51) Swindell, C.S.; Patel, B.P. *J. Org. Chem.* **1990**, *55*, 3.

(52) Ojima, I.; Habus, I.; Zho, M.; Georg, G.I.; Jayasinghe, L.R. *J. Org. Chem.* **1991**, *56*, 1681.

(53) Grieco, P.A.; Gilman, S.; Nishizawa, M. *J. Org. Chem.* **1976**, *41*, 1485.

(54) (a) Srinivasan, V.; Pestchanker, L.; Moser, S.; Hirasuna, T.J.; Taticek, R.A.; Shuler, M.L. *Biotech. bioengin.* **1995**, *47*, 666. (b) Mirjalili, N.; Linden, J.C. *Biotech. Bioengin.* **1995**, *48*, 123. (c) Mirjalili, N.; Linden, J.C. *Biotech. Progress.* **1996**, *12*, 110.

(55) Abramowicz, D.A. *Biocatalysis*; Van Nostrand Reinhold: New York, **1990**.

(56) Heathcock, C.H.; Ellis, J.E.; McMurray, J.E.; Coppolino, A. *Tetrahedron Lett.* **1971**, *52*, 4995.

(57) Julia, S.; Rasseur, M.J. *Bull. Soc. Chim. Fr.* **1962**, 374.

(58) (a) Wege, P.M.; Clark, R.D.; Heathcock, C.H. *J. Org. Chem.* **1976**, *41*, 3144. (b) Holland, H.L.; Auret, B.J. *Can. J. Chem.* **1975**, *53*, 2041.

(59) Huffman, J.W.; Balke, W.H. *J. Org. Chem.* **1988**, *53*, 3828.

(60) Corey, E.J.; Vankateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

(61) Beeson, C.; Dix, T.A. *J. Org. Chem.* **1992**, *57*, 4386.

(62) DeBoer, A.; Ellwanger, R.E. *J. Org. Chem.* **1974**, *39*, 77.

(63) Mancuso, A.J.; Swern, D. *Synthesis*. **1981**, 165.

(64) (a) Dess, D.B.; Martin, J.C. *J. Org. Chem.* **1983**, *48*, 4155. (b) Ireland, R.E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.

(65) Dumont, W.; Krief, A. *Angew. Chem. Int. Ed. Eng.* **1977**, *16*, 540.

(66) Krief, A. *Tetrahedron*. **1980**, *36*, 2531.

(67) Krief, A.; Cravador, A. *C.R. Acad. Sc. Paris serie C.* **1979**, 267.

(68) Krief, A.; Halazy, S. *J. Chem. Soc. Chem. Commun.* **1979**, 1136.

(69) Greene, T.H.; Wuts, P.G.M., in *Protective Groups in Organic Synthesis.*, John Wiley & Sons, INC, New York, **1991**.

(70) (a) Bartlett, P.A.; Akerfeldt, K. *Carbohydr. Res.* **1986**, *158*, 137. (b) Ferro, V.; Molerino, M.; Stick, R.V.; Tilbrook, G.; Mattew, D. *Aust. J. Chem.* **1988**, *41*, 813.

(71) Mori, Y.; Morishima, N. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2061.

(72) Brown, M.J.; Overman, L.E. *J. Org. Chem.* **1991**, *56*, 1933.

(73) Williams, R.M.; Im, M-N.; Cao, J. *J. Am. Chem. Soc.* **1991**, *113*, 6976.

(74) Leopold, E.J. *Organic Synthesis*, **1985**, *64*, 164.

(75) Imamoto, T.; Takiyama, N.; Nakamura, K.; Hatajima, T.; Kamiya, Y., *J. Am. Chem.Soc.*, **1989**, *111*, 4392.

(76) Authentication of the synthetic material by comparison to natural **38** by capillary gc and ei-mass spec analysis was kindly carried out by Professor Rodney Croteau of the Institute of Biological Chemistry, Washington State University.

(77) (a) Burgess, E.; Penton, H.R.; Taylor, E.A. *J.Org. Chem.* **1973**, *38*, 26. (b) Hubert, A.J.; Reimlinger, H. *Synthesis*. **1969**, 405. (c) Davies, N.R. *Aust. J. Chem.* **1964**, *17*, 212.

(78) Lin, X.; Hezari, M.; Koepp, A.E.; Floss, H.G.; Croteau, R. *J. Biol. Chem.* **1996**, in press.

(79) (a) Croteau, R.; Wheeler, C.J.; Cane, D.E.; Ebert, R.; Ha, H-J. *Biochemistry*. **1987**, *26*, 5382. (b) Wagschal, K.C.; Pyun, H-J.; Coates, R.M.; Croteau, R. *Arch. Biochem. Biophys*. **1994**, *308*, 477.

(80) (a) Mihaliak, L.A.; Karp. F.; Croteau, R. *Meth. Plant Biochem*. **1993**, *9*, 261. (b) Hefner, J.; Rubenstein, S.M.; Ketchum, R.E.B.; Gibson, D.M.; Williams, R.M.; Croteau, R. *Chem. and Biol*. **1996**, submitted.

(81) (a) Kadkhodayan, S.; Coulter, E.D.; Maryniak, D.M.; Bryson, T.A.; Dawson, J.H. *J. Biol. Chem*. **1995**, *270*, 28042. (b) Gelb, M.H.; Heimbrook, D.C.; Malkonen, P.; Sligar, S.G. *Biochemistry*. **1982**, *21*, 310. (c) Atkins, W.M.; Sligar, S.G. *J. Am. Chem. Soc*. **1987**, *109*, 3754. (d) Groves, J.T. *J. Chem. Ed*. **1988**, *27*, 1610. (e) Lowry, T.H.; Richardson, K.S., in *Mechanism and Theory in Organic Chemistry*. Harper and Row Publishers, New York, **1987**, 234.

(82) Oritz de Montellano, P.R., in *Cytochrome P-450: Structure, Mechanism, and Biochemistry*. (Oritz de Montellano, P.R., Ed.), Plenum Press, New York, **1986**, 217-271.

(83) Haines, A.H. *Adv. Carbohydr. Chem. Biochem*. **1976**, *337*, 11.

(84) Generously supplied by Dr. James Linden, Colorado State University.

(85) Seki, M.; Takeda, M.; Furusaki, S. *J. Chem. End. Jpn*. **1995**, *28*, 488.

(86) unpublished results.

(87) Piatini, U.; Soerenson, O.W.; Ernst, R.R. *J. Am. Chem. Soc*. **1982**, *104*, 6800.

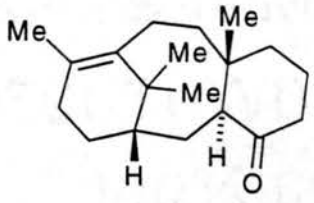
(88) Bax, A.; Griffey, R.H.; Hawkins, B.L. *J. Magn. Reson*. **1983**, *55*, 301.

(89) Bax, A.; Summers, M.F. *J. Am. Chem. Soc*. **1986**, *108*, 2093.

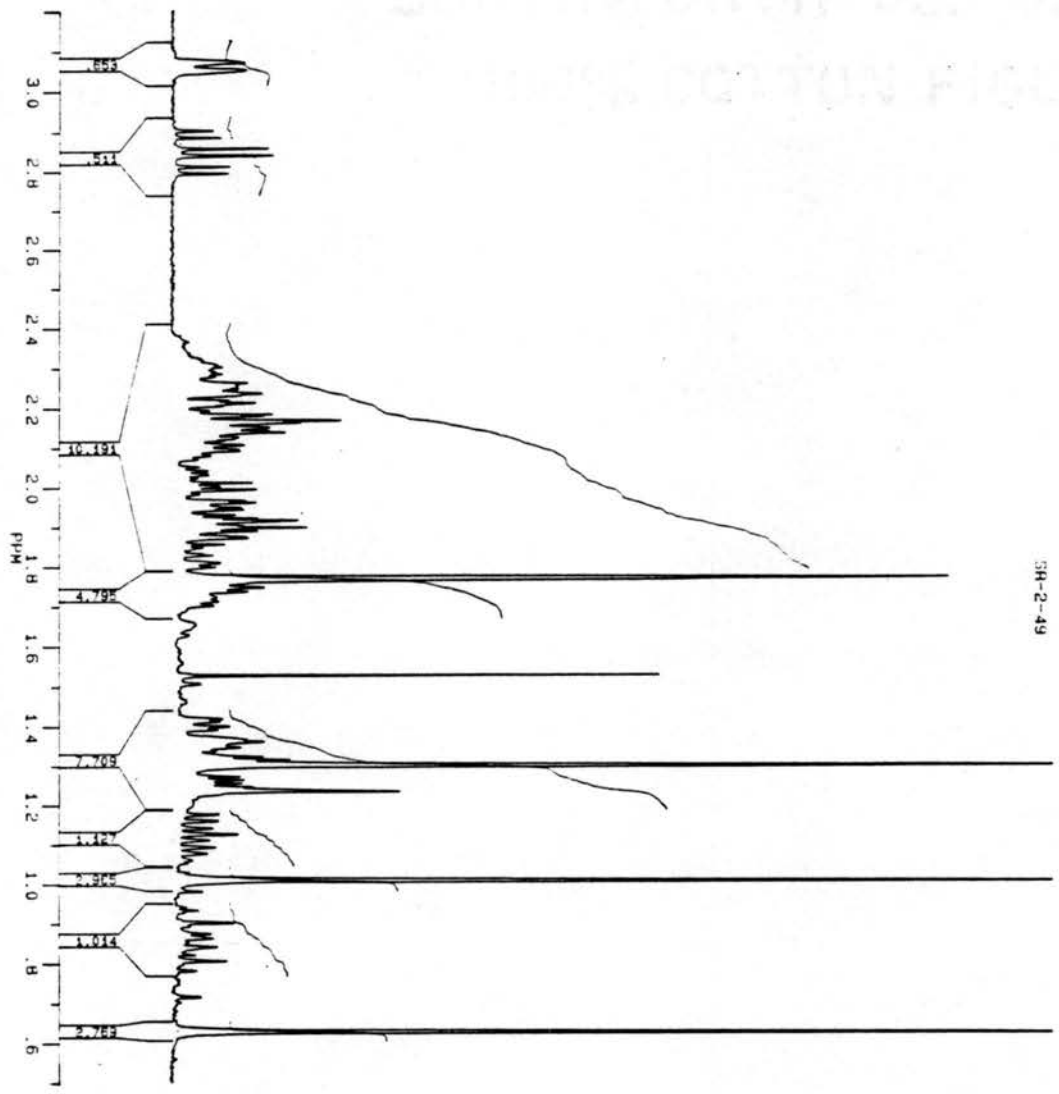
APPENDIX 1

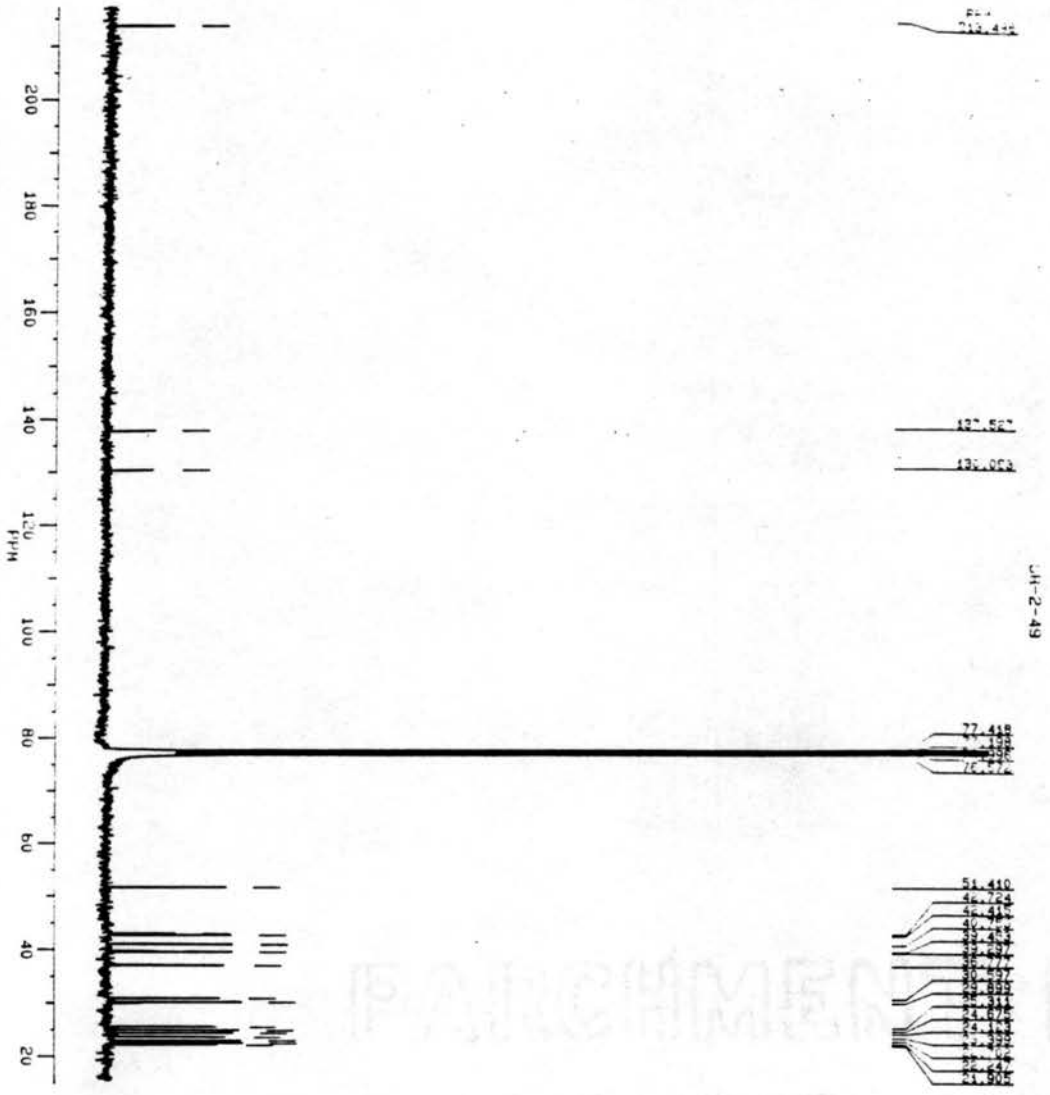
SPECTRAL DATA

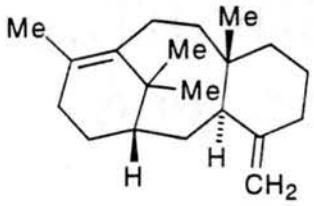
Aprendix 1 contains spectral data on ketone **185**, taxa-4(20),11(12)-diene, taxa-4(5),11(12)-diene, taxa-4(20),11(12)-diene-5(α)-ol, taxa-4(20),11(12)-diene-5(β)-ol, taxa-4(20),11(12)-diene-5(α)-acetate, and taxa-4(20),11(12)-diene-5(α)-benzoate.



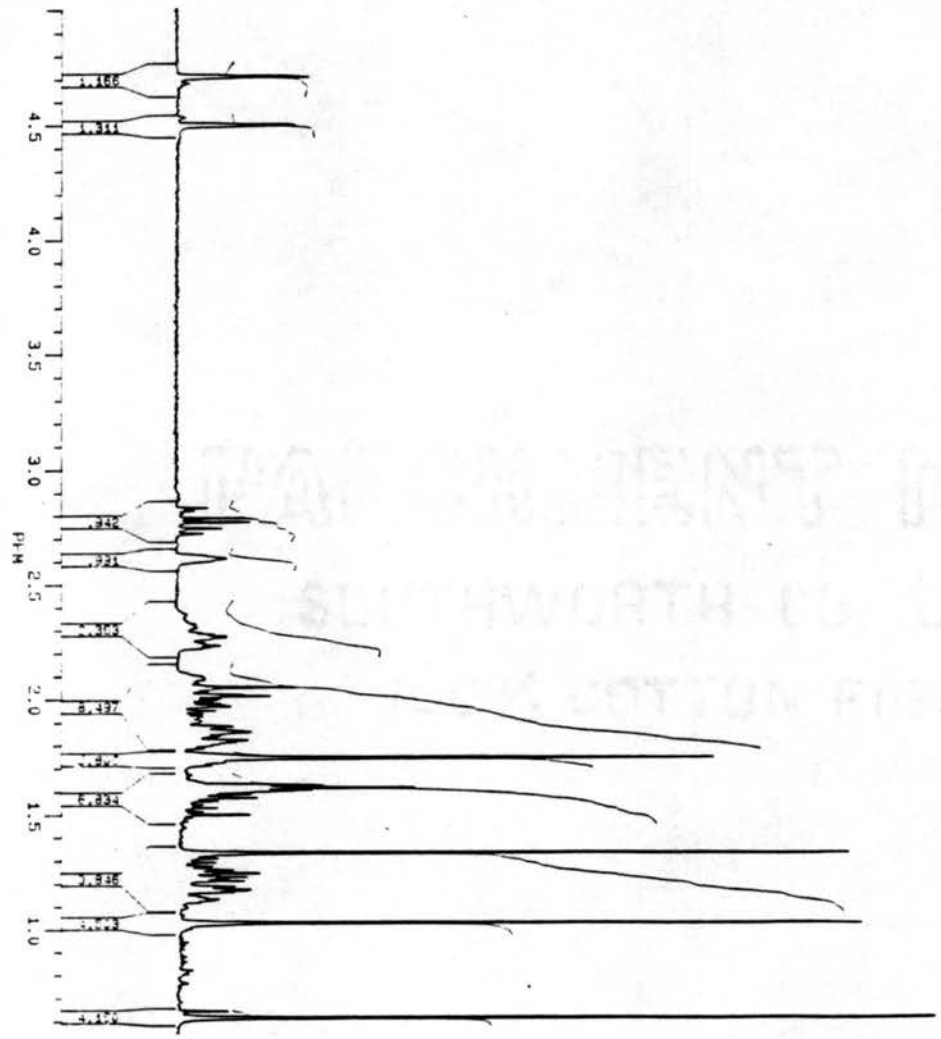
185


.859
.911
10.191
4.795
7.709
1.127
2.905
1.014
2.769
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
PPM
SH-2-49

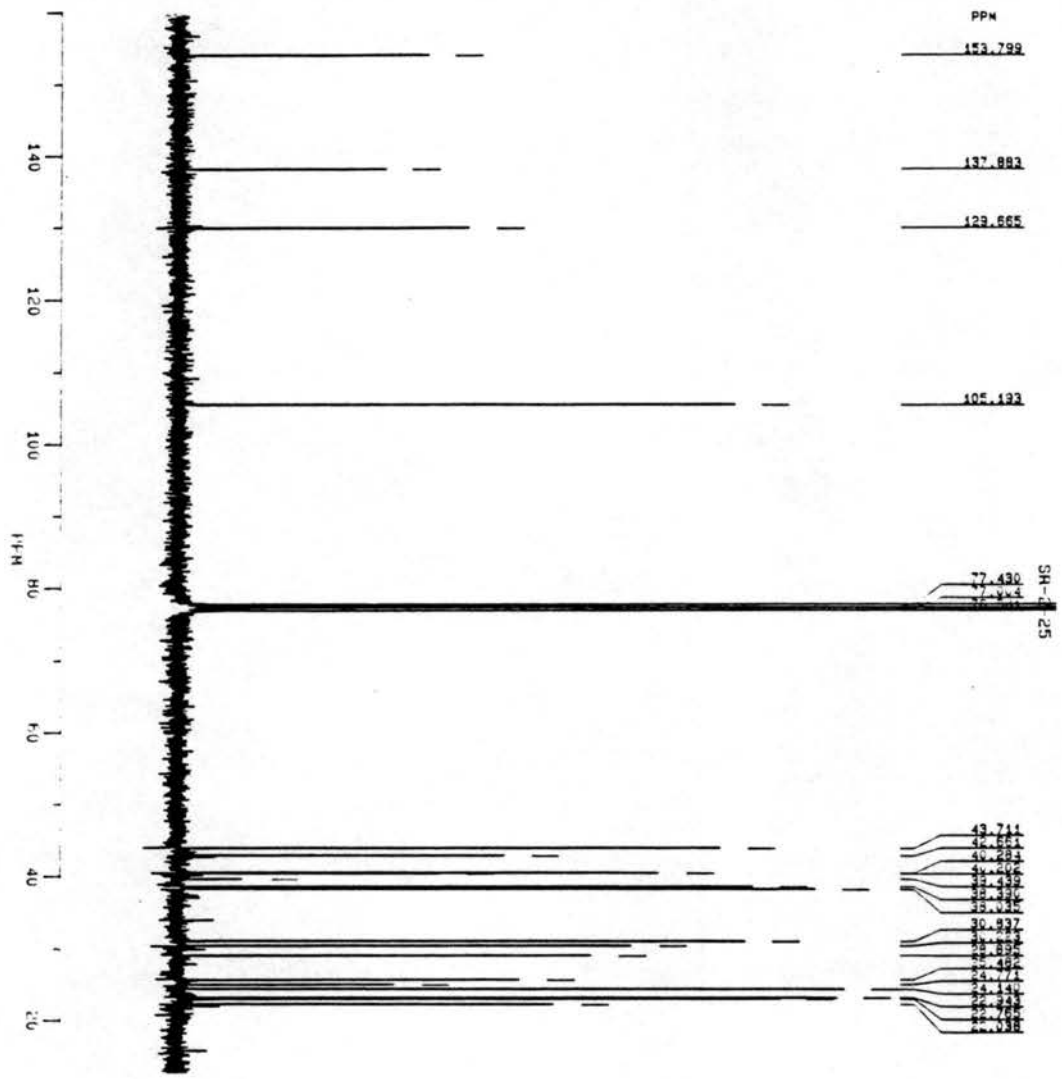


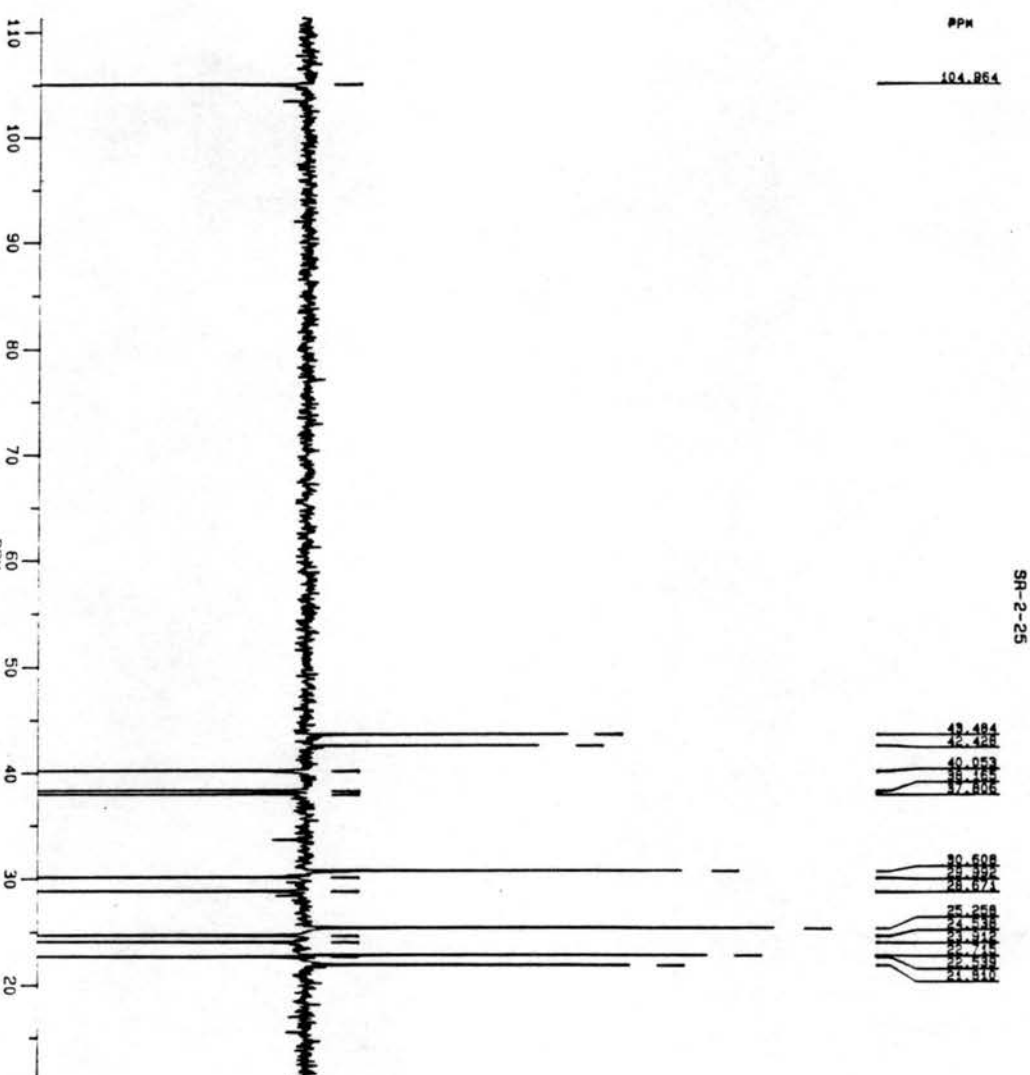


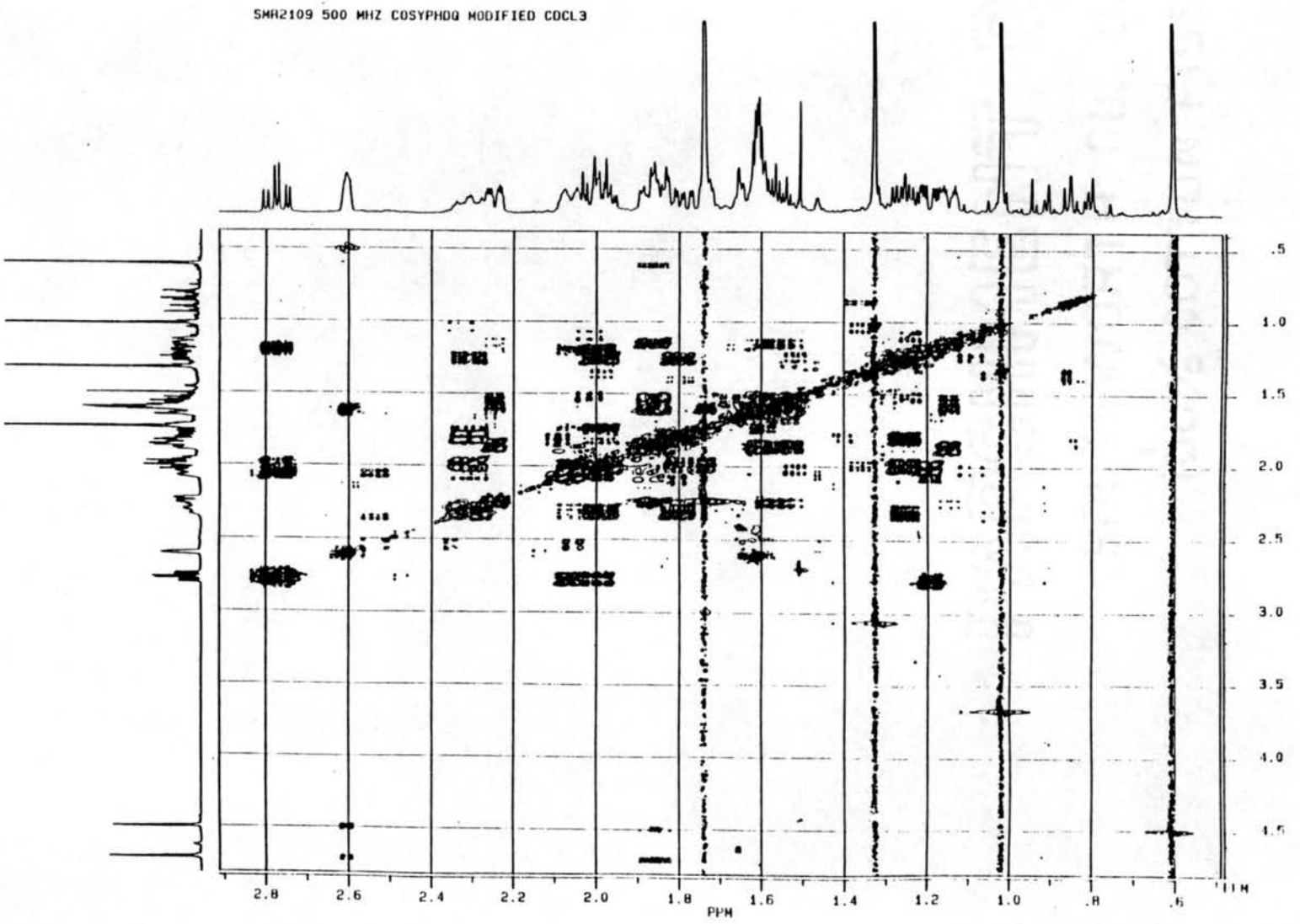
Taxa-4(20),11(12)-diene

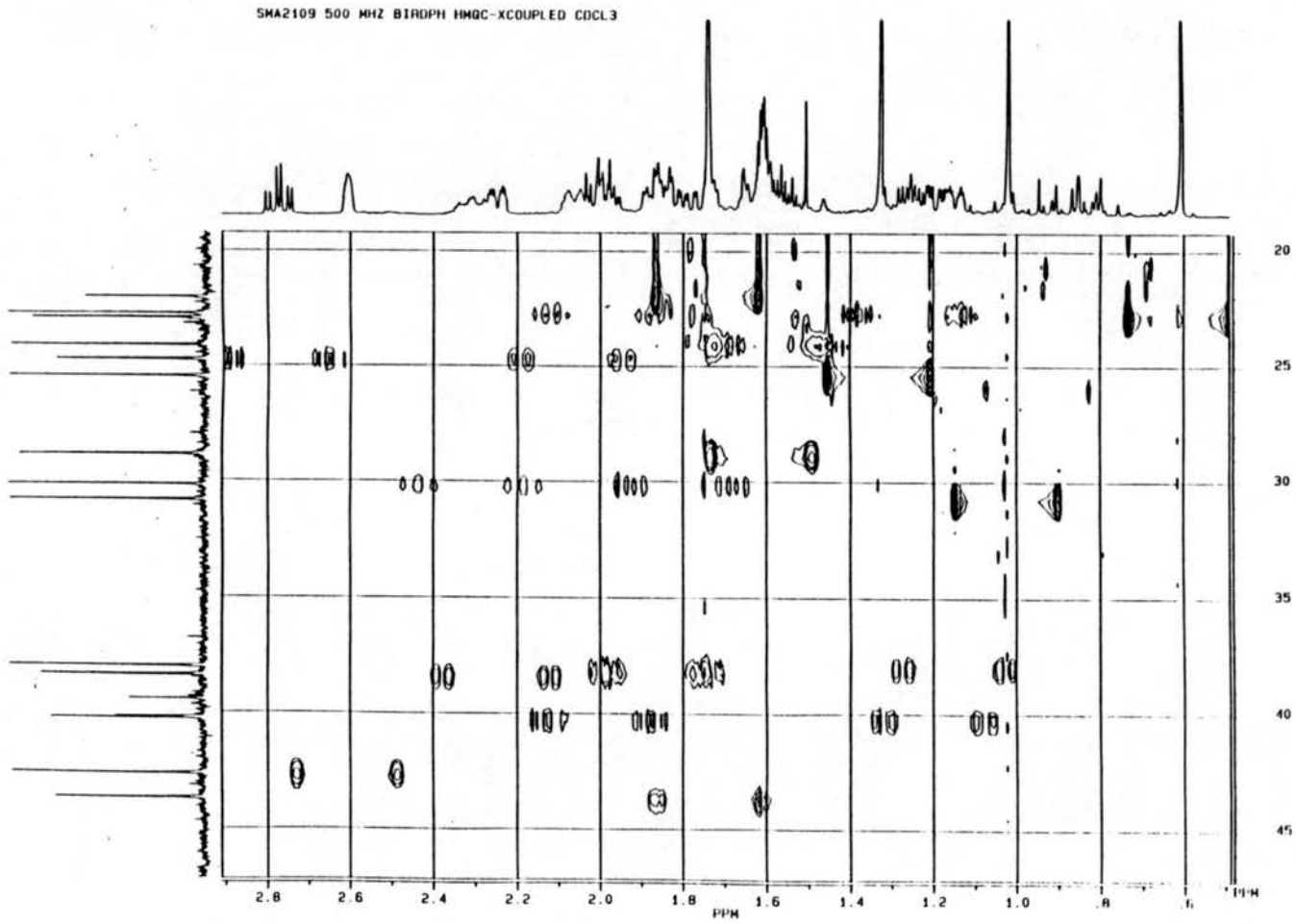


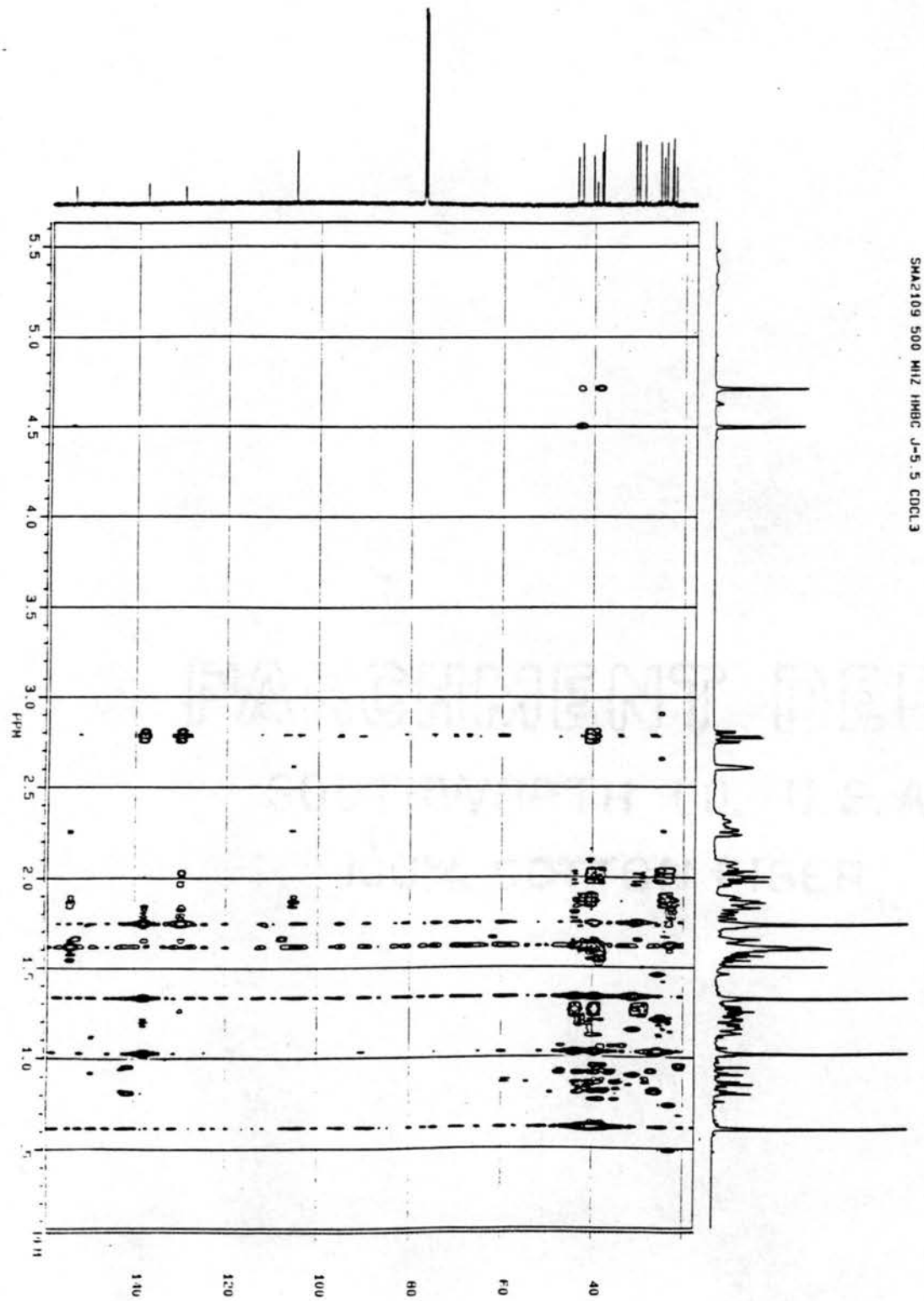
PROTON NMR IN CDCL3

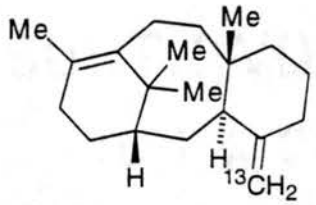

PPM
153.799
137.883
129.665
105.193
77.430
SH-4-25
43.711
30.937
24.771
24.149
140
120
100
80
60
40
20
H+1


SA-2-25
PPM
104.864
43.484
42.428
40.053
37.806
30.608
28.671
25.258
21.810
110
100
90
80
70
60
PPM
50
40
30
20

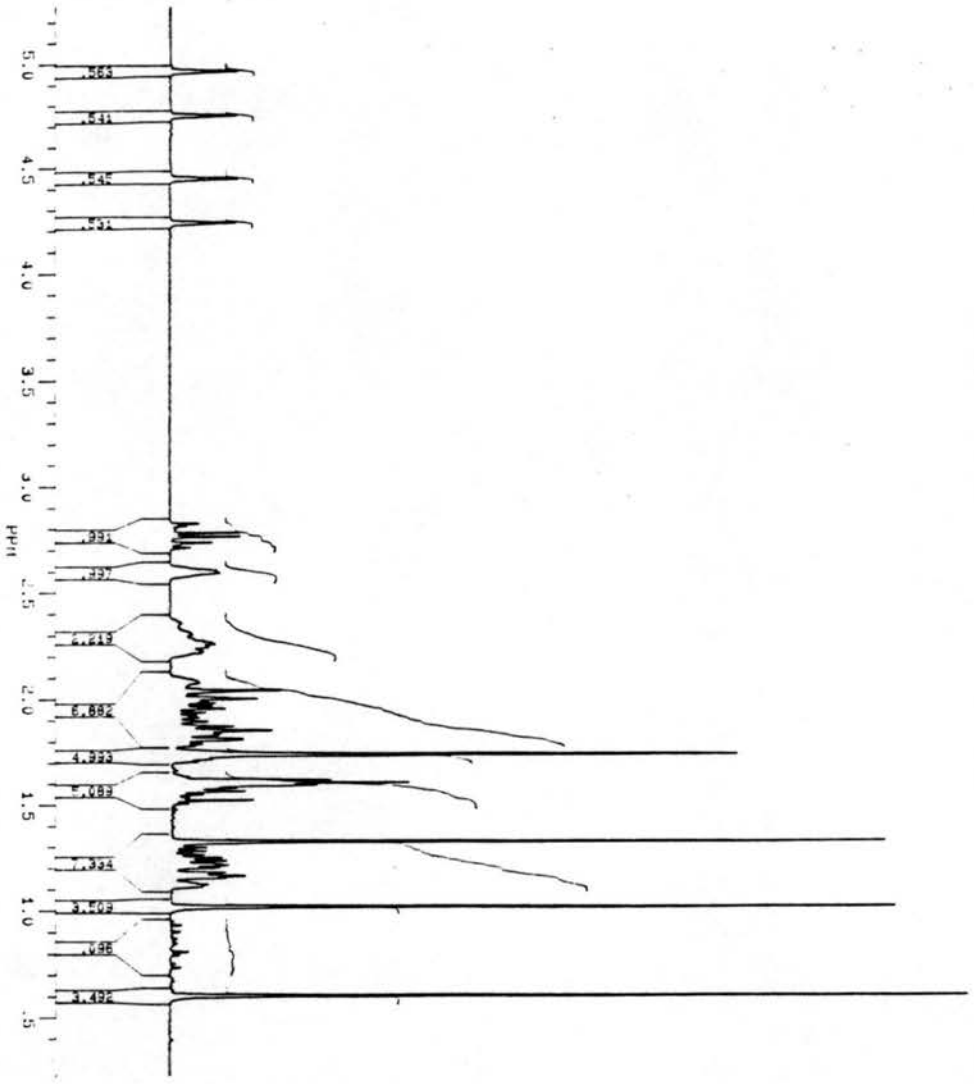

SMR2109 500 MHZ COSYPHDQ MODIFIED CDCL3
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
PPM
.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5


SMA2109 500 MHZ BIRDPH HMQC-XCOUPLED CDCL3
20
25
30
35
40
45
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
PPM

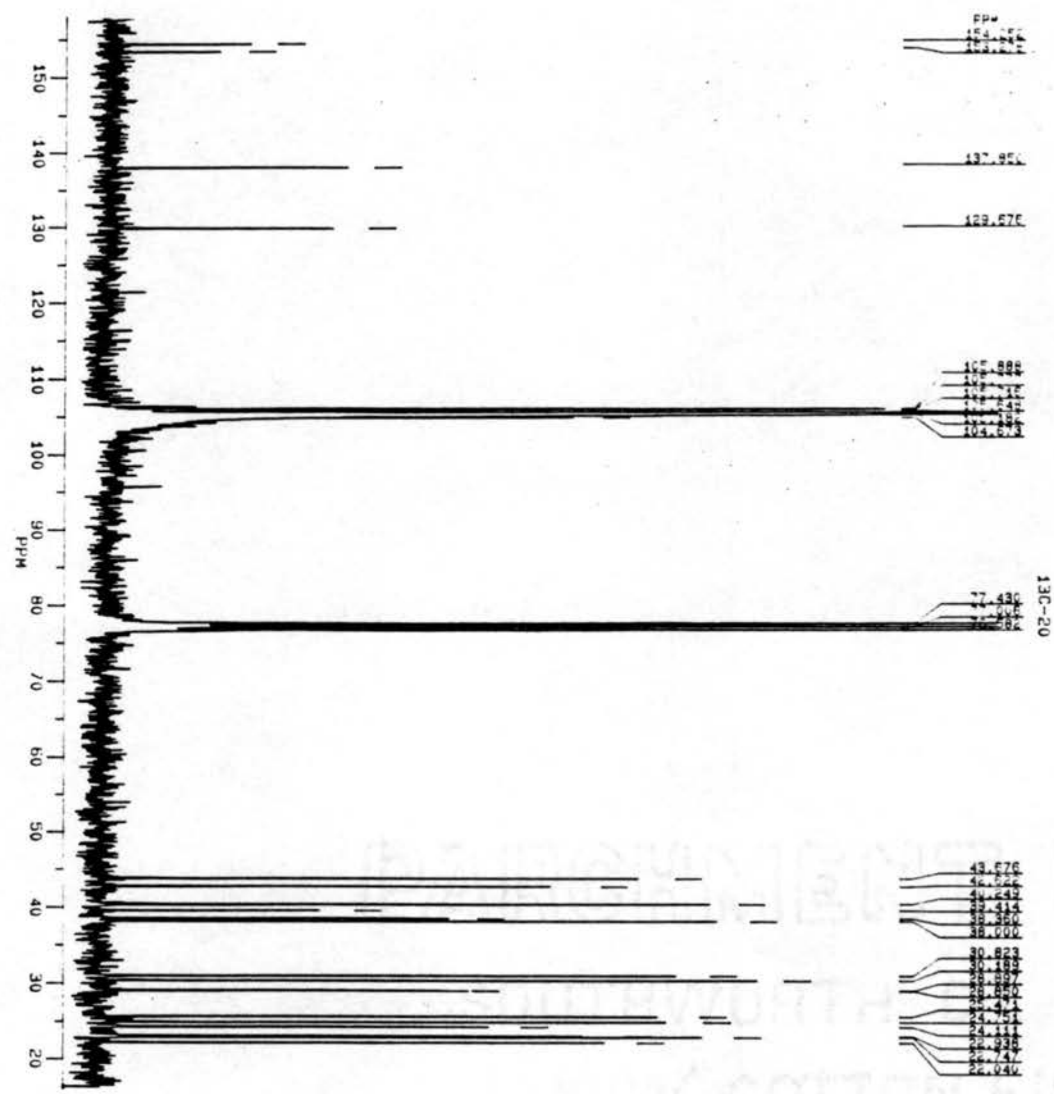

SHA2109 500 MHZ HMBC J=5.5 CDCL3
PPM
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
140
120
100
80
60
40

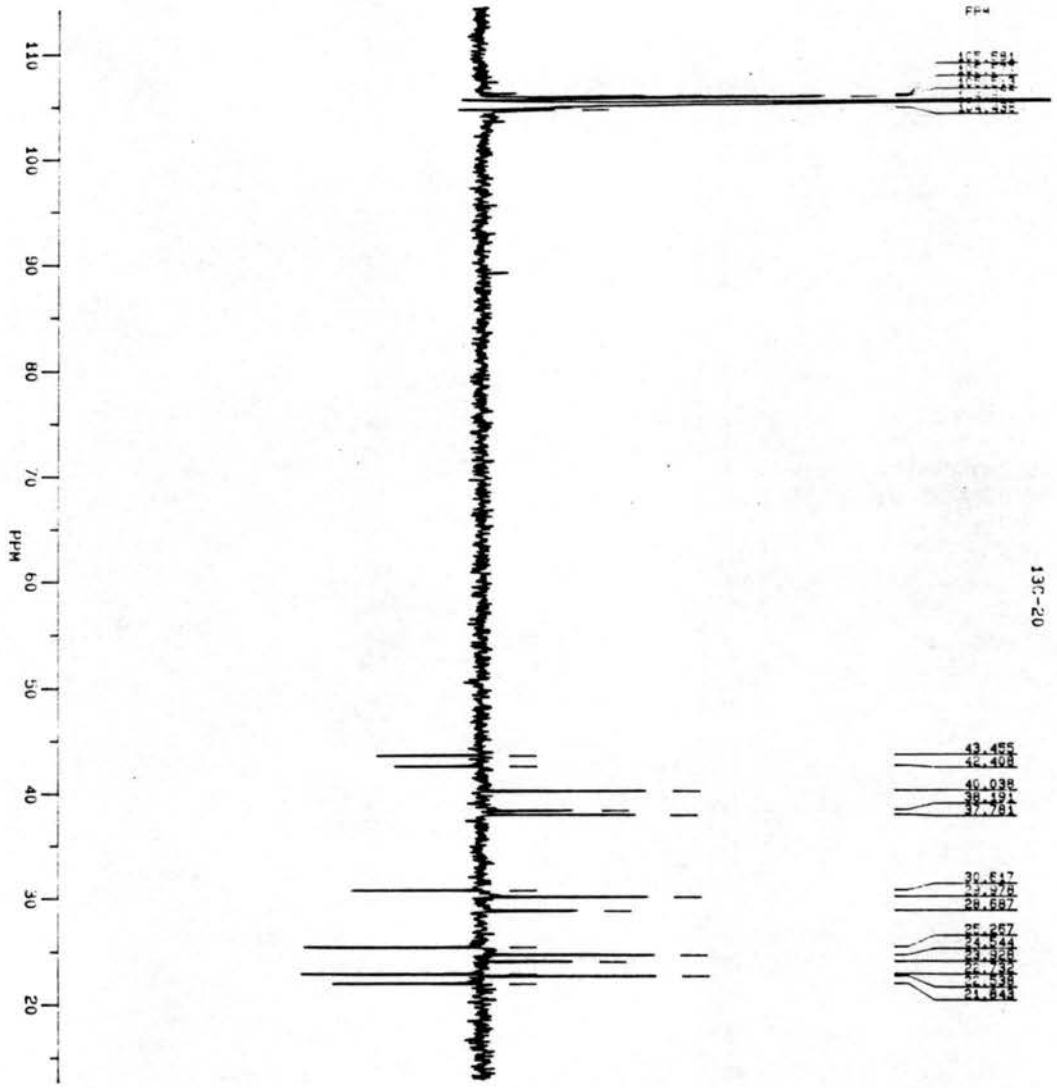


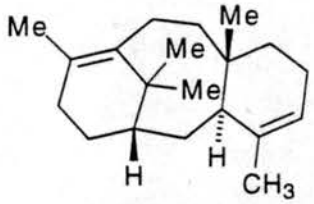
[20-^{13}C]-Taxa-4(20),11(12)-diene



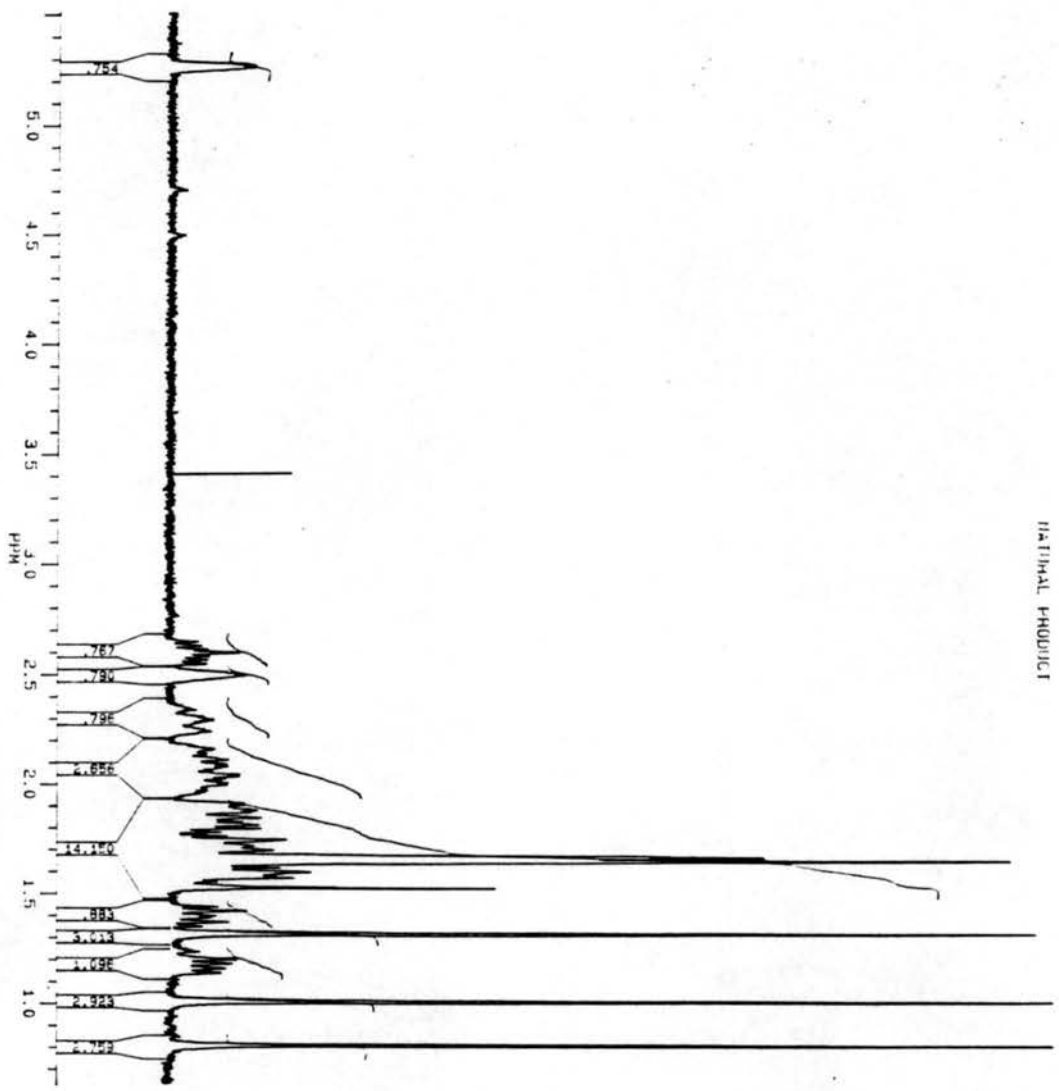
TAXOL-13C AT C-20

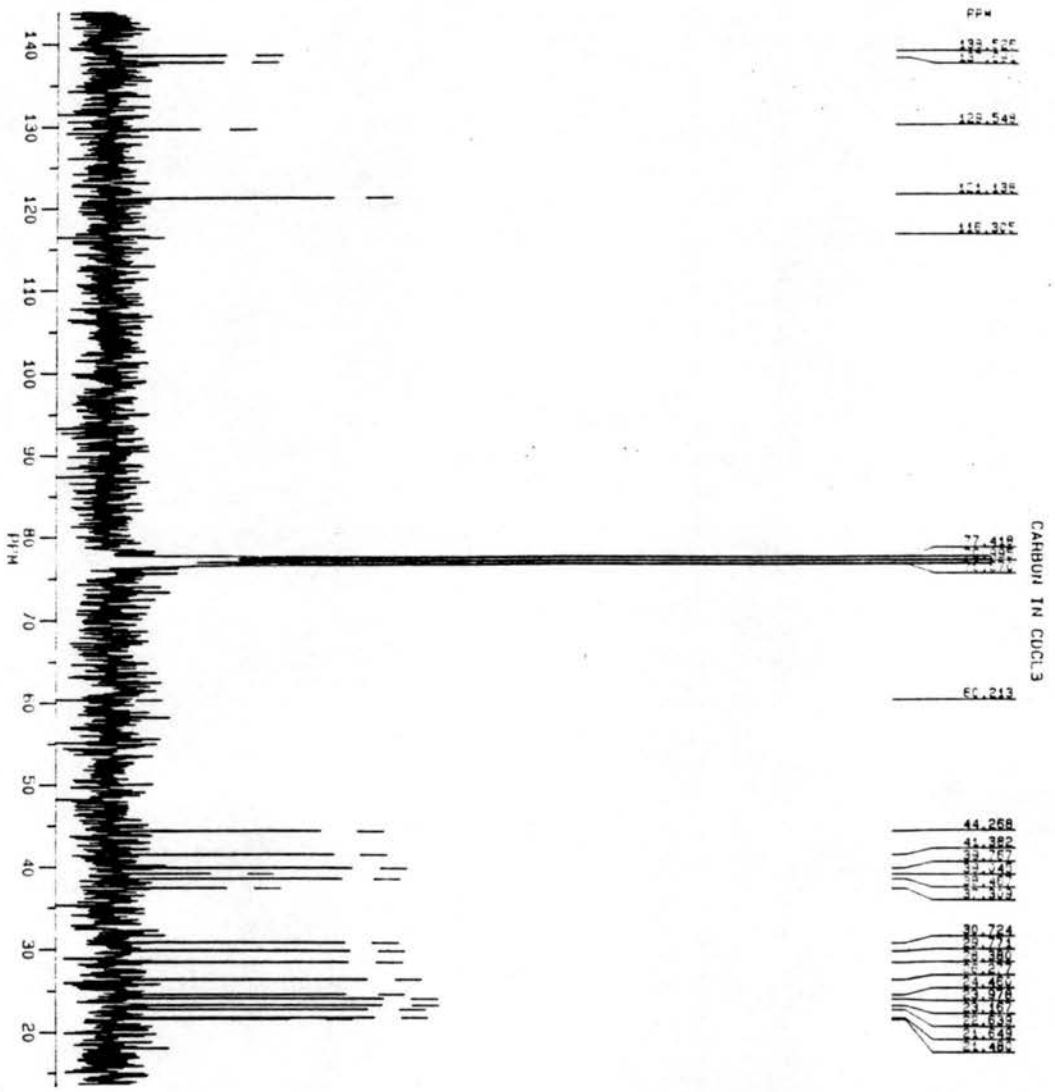

13C-20
PPM
150
140
130
120
110
100
90
80
70
60
50
40
30
20

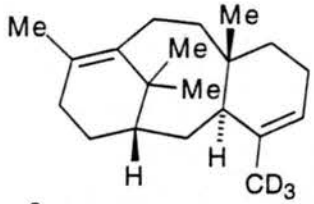

PPM
110
100
90
80
70
60
50
40
30
20
PPM
13C-20
43.455
42.498
40.038
38.491
37.781
30.617
29.927
28.687
25.267



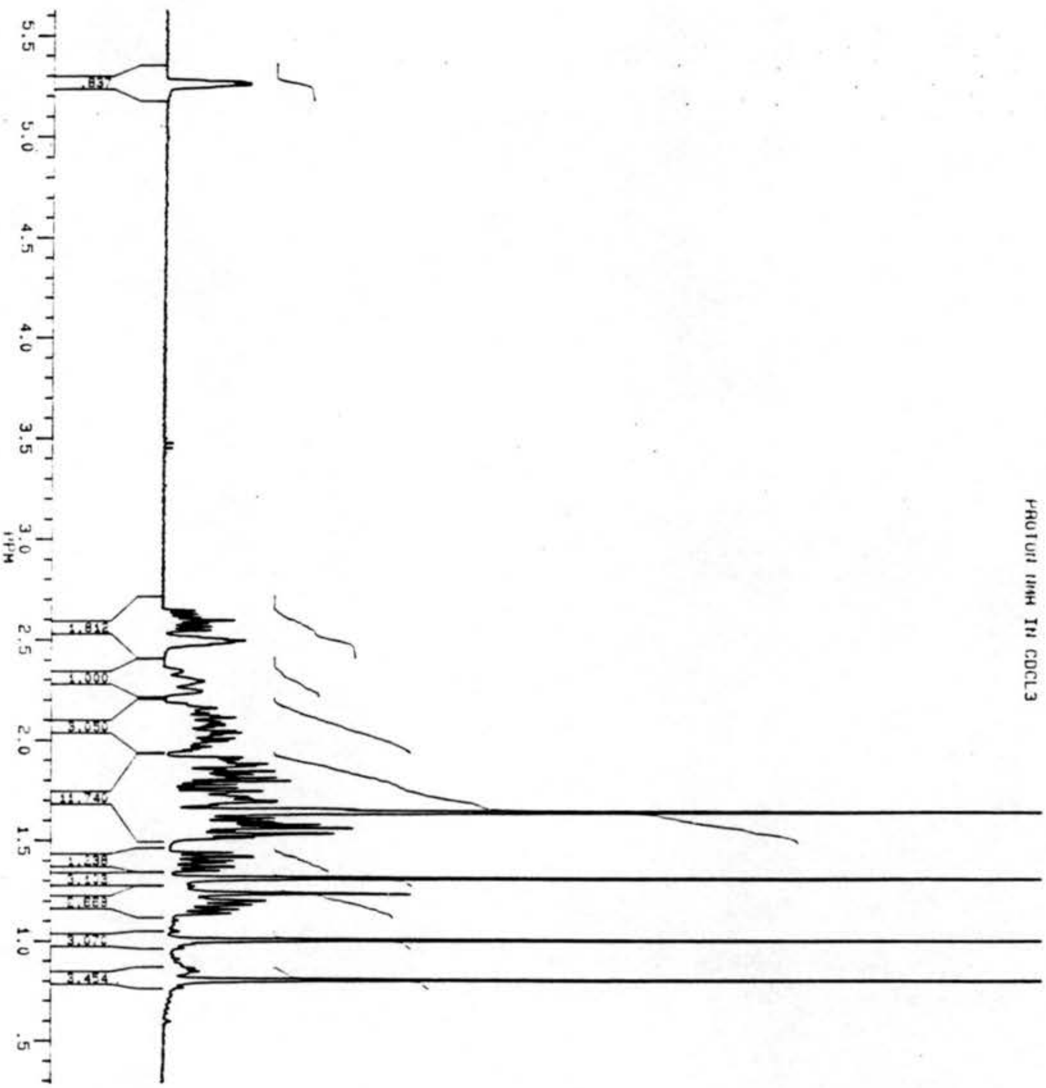
Taxa-4(5),11(12)-diene

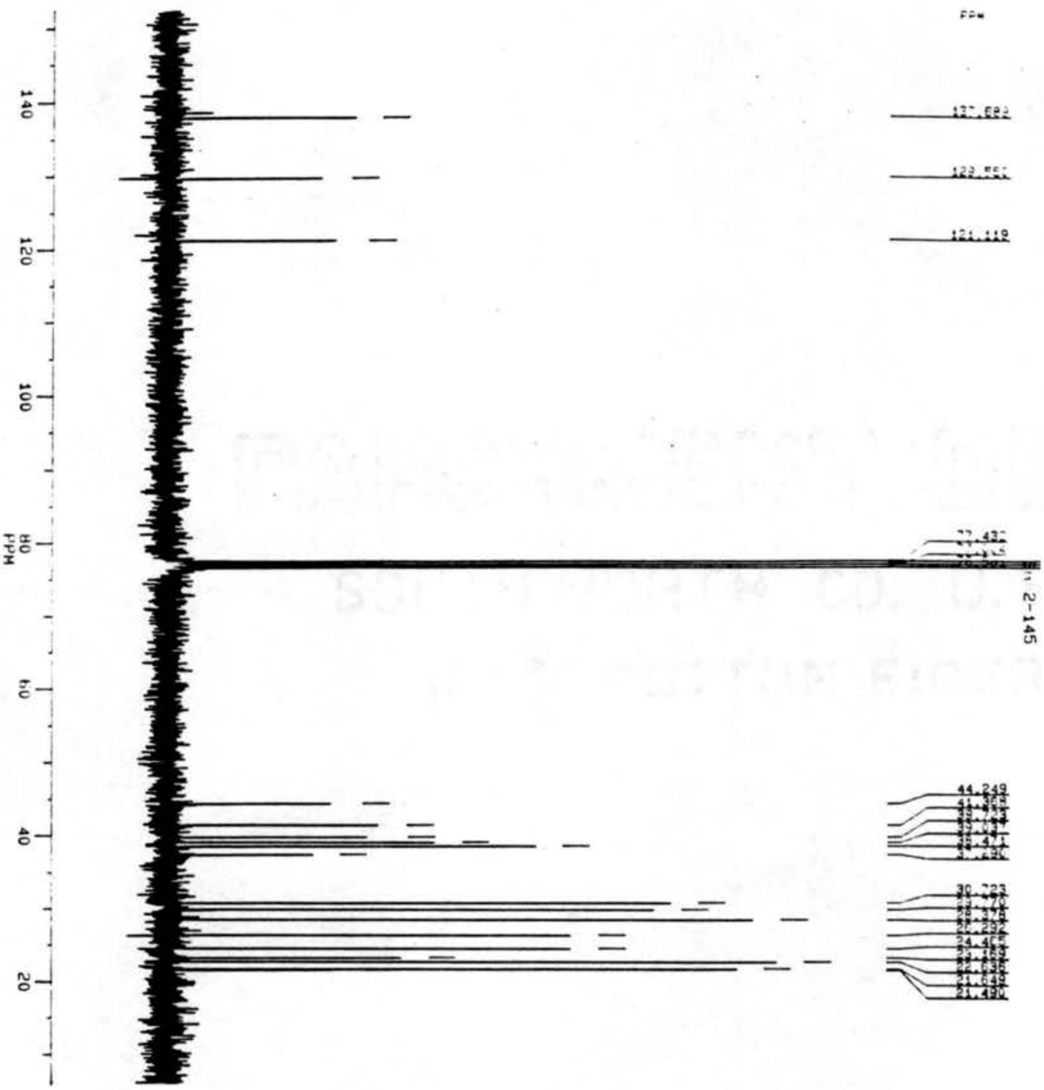

.754
.767
.780
.795
2.656
14.150
.883
3.433
1.095
2.925
2.749
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
PPM
NATIONAL PRODUCT

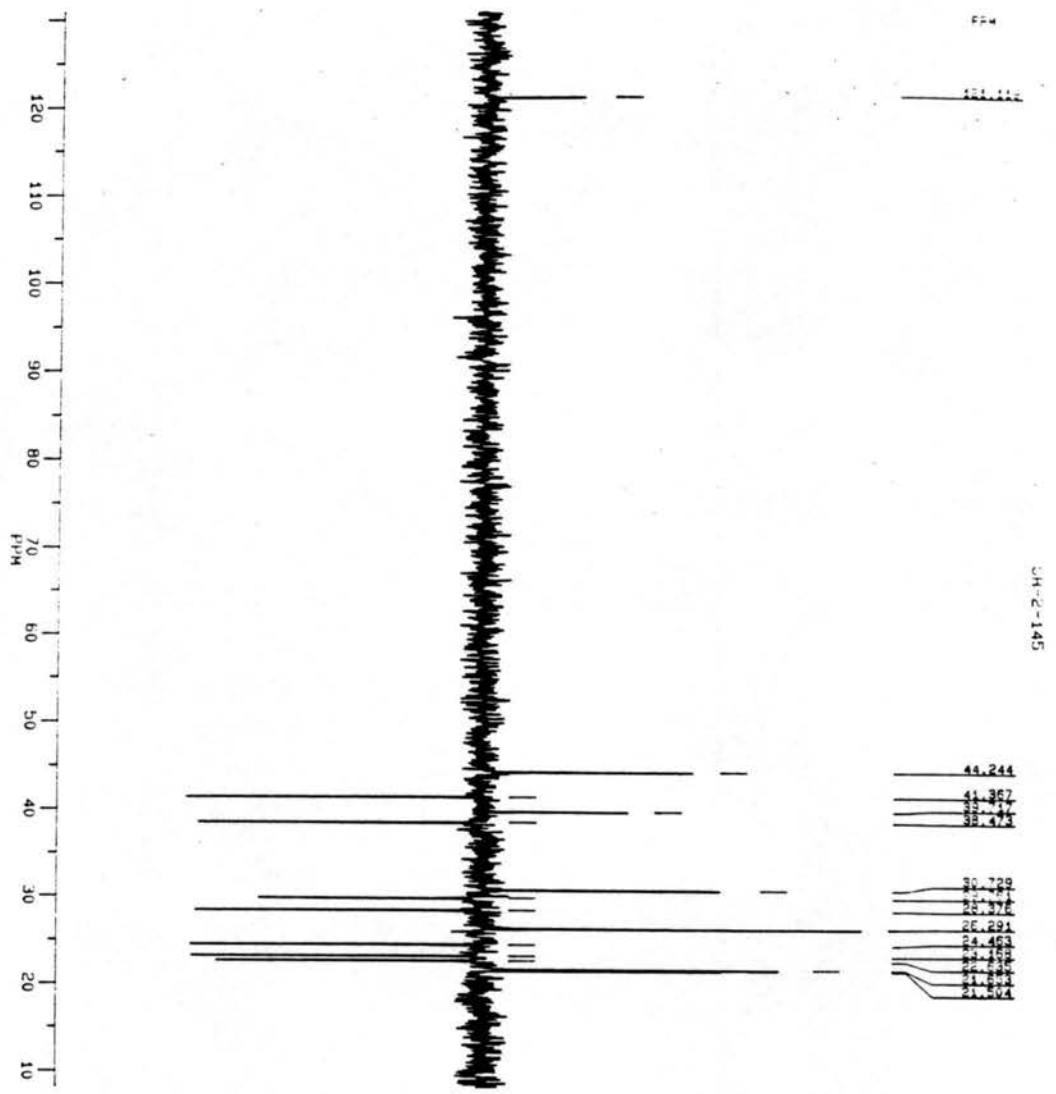

PPM
128.549
60.213
44.268
41.382
30.724
CARBON IN CDCL3
PPM

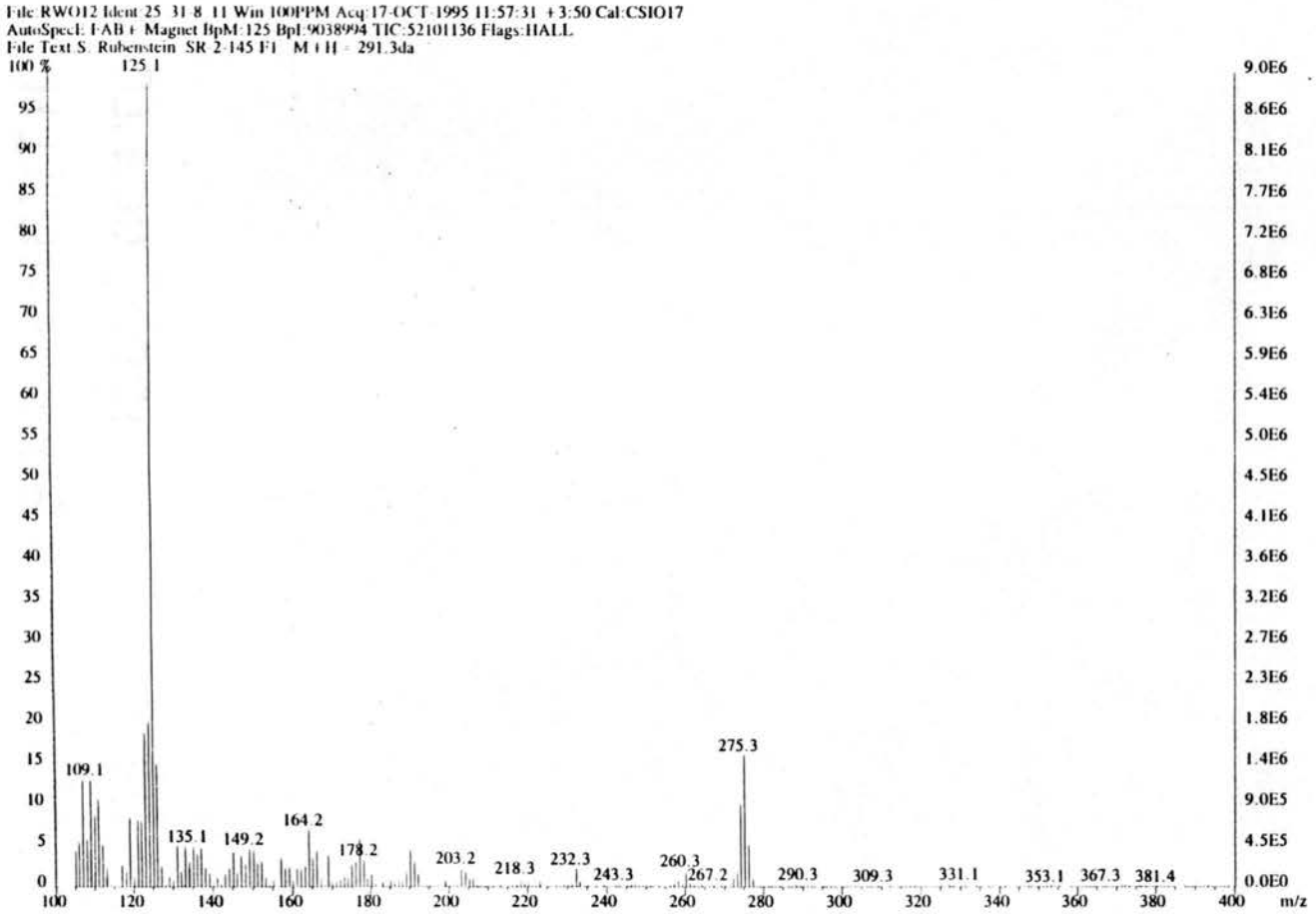


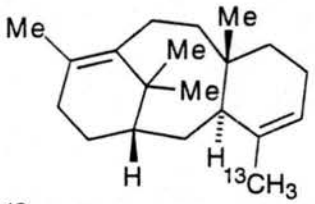
[20-C^2H_3]-Taxa-4(5),11(12)-diene


PROTON NMR IN CDCL3
PPM
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5

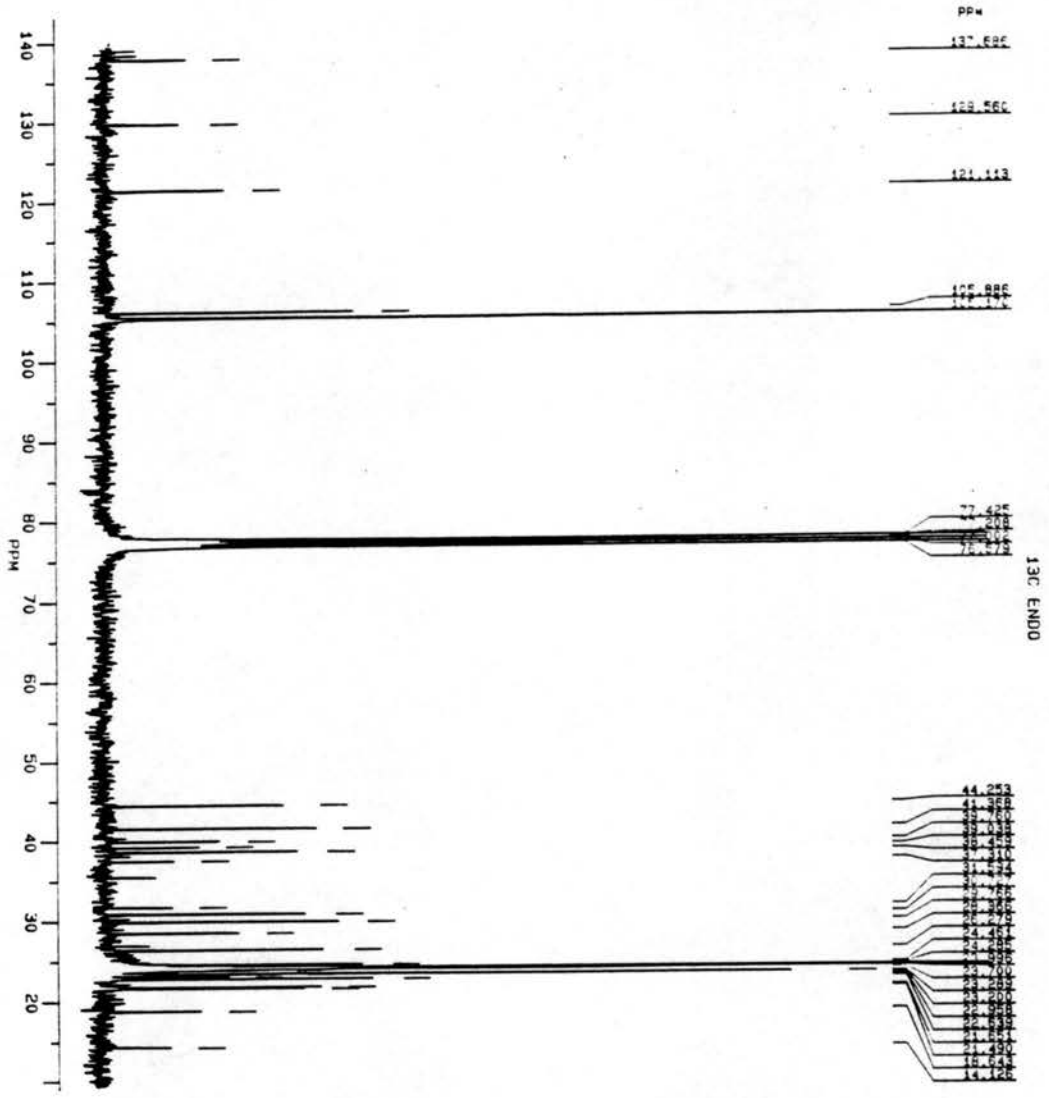

140
120
100
80
PPM
60
40
20
121.119
44.249
21.490
2-145

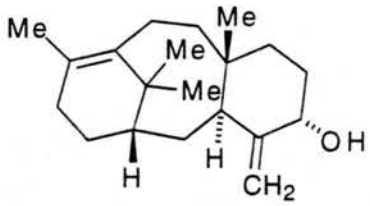

PPM
120
110
100
90
80
70
60
50
40
30
20
10
44.244
41.367
26.291


File:RWO12 Ident:25_31-8_11 Win:100PPM Acq:17-OCT-1995 11:57:31 +3:50 Cal:CSIO17
AutoSpecE:FAB+ Magnet BpM:125 BpI:9038994 TIC:52101136 Flags:HALL
File Text:S. Rubenstein SR 2-145 F1 M+H = 291.3da
100 %
125.1
109.1
135.1
149.2
164.2
178.2
203.2
218.3
232.3
243.3
260.3
267.2
275.3
290.3
309.3
331.1
353.1
367.3
381.4
9.0E6
8.6E6
8.1E6
7.7E6
7.2E6
6.8E6
6.3E6
5.9E6
5.4E6
5.0E6
4.5E6
4.1E6
3.6E6
3.2E6
2.7E6
2.3E6
1.8E6
1.4E6
9.0E5
4.5E5
0.0E0
m/z

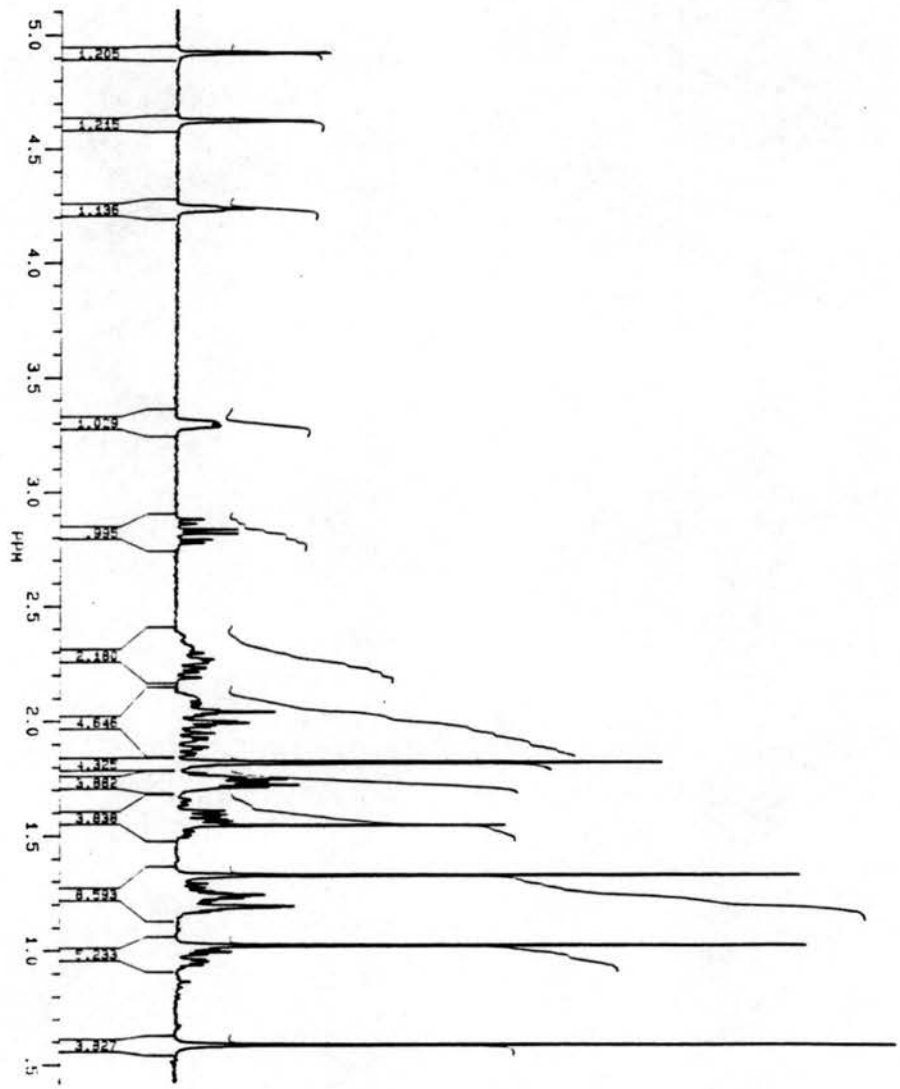


[20-$^{13}CH_3$]-Taxa-4(5),11(12)-diene

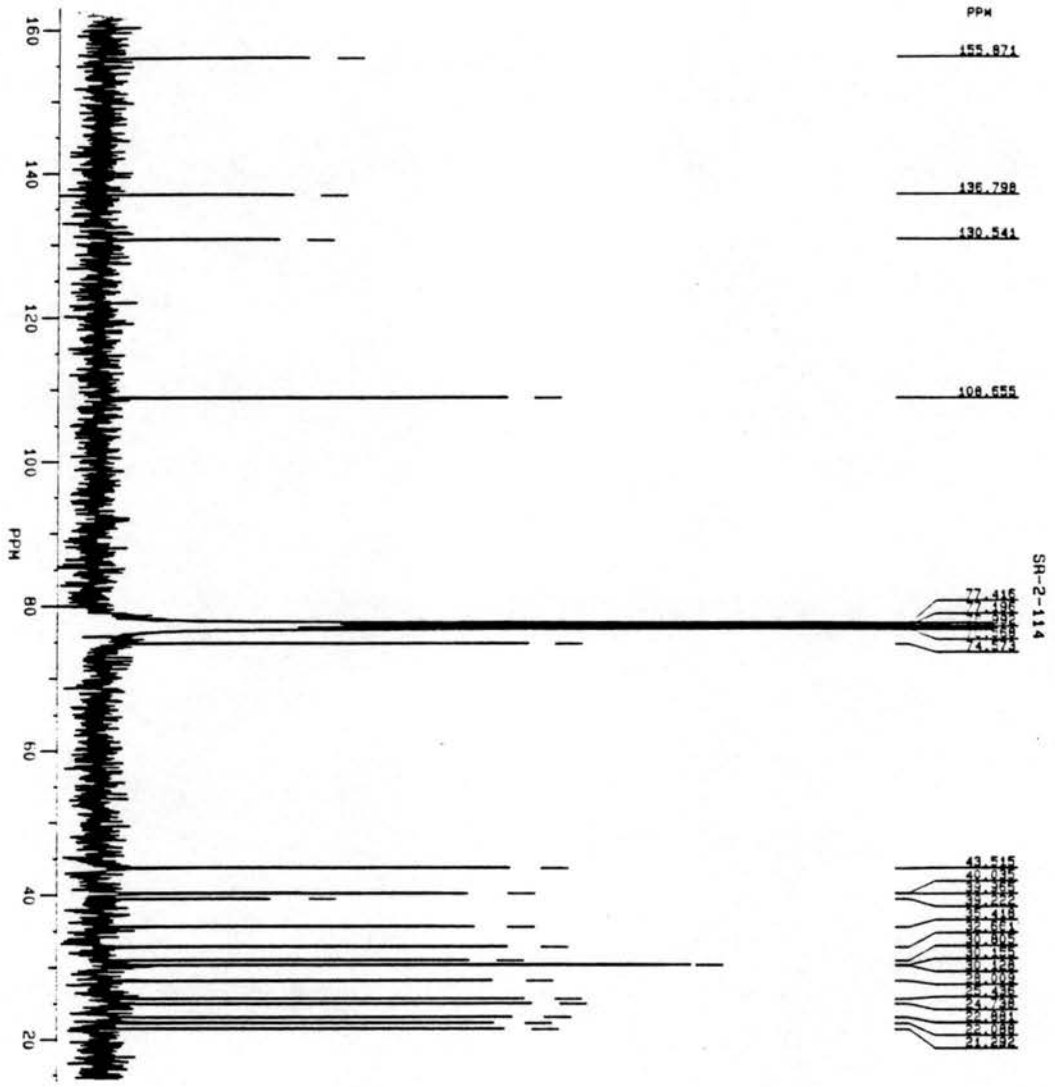

PPM
137.686
128.566
121.113
105.886
77.425
44.253
41.368
PPM
13C ENDO

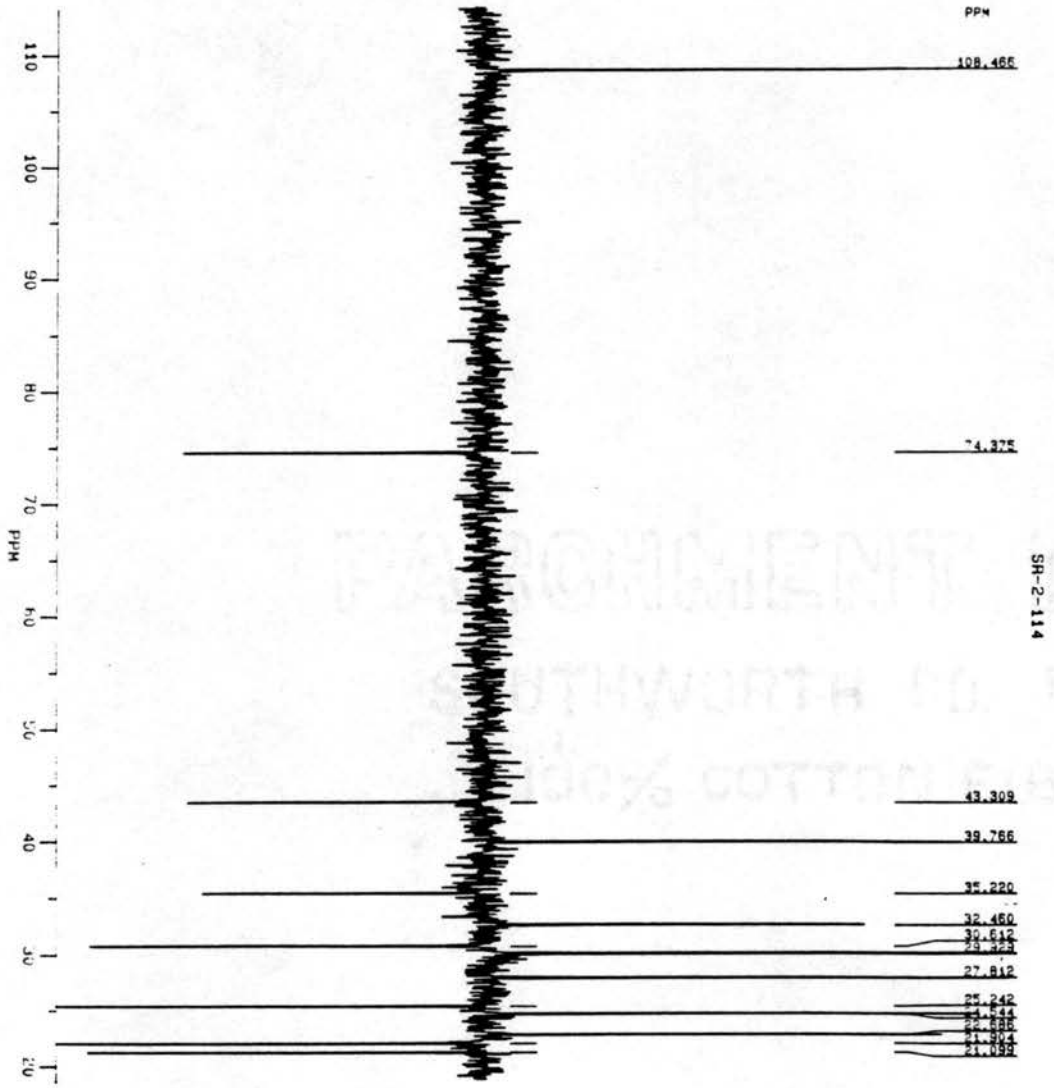


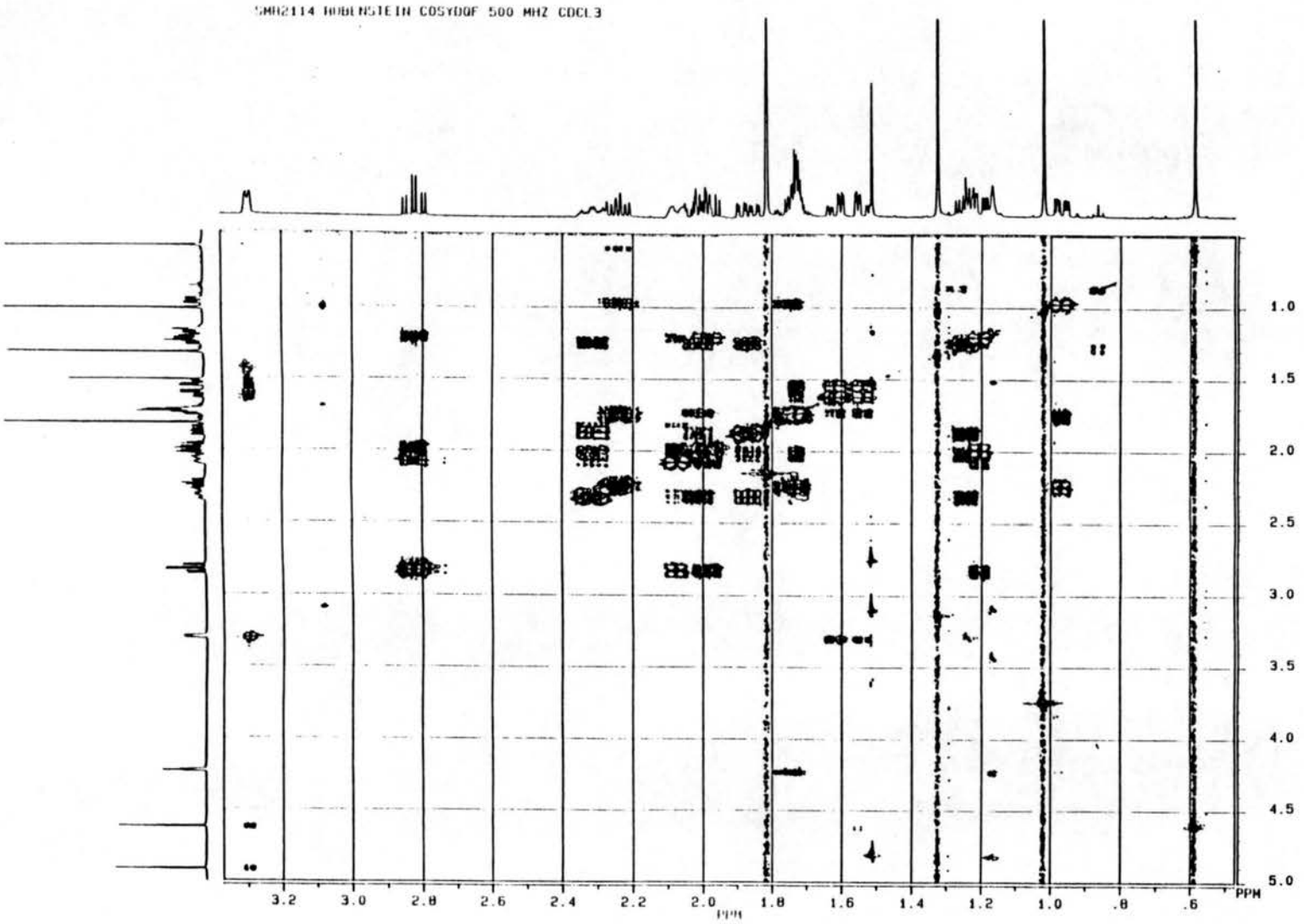
Taxa-4(20),11(12)-diene-5(α)-ol


5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
PPM
1.205
1.215
1.136
1.049
.935
2.186
4.646
1.324
1.882
1.838
8.593
1.233
3.327

SR-2-114


PPM
155.871
136.798
130.541
108.655
77.415
43.515
SR-2-114
160
140
120
100
80
60
40
20
PPM


PPM
108.466
74.375
43.309
39.766
35.220
32.460
30.612
27.812
SR-2-114
PPM


SMR2114 ROBENSTEIN COSYDQF 500 MHZ CDCL3
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
PPM
PPM

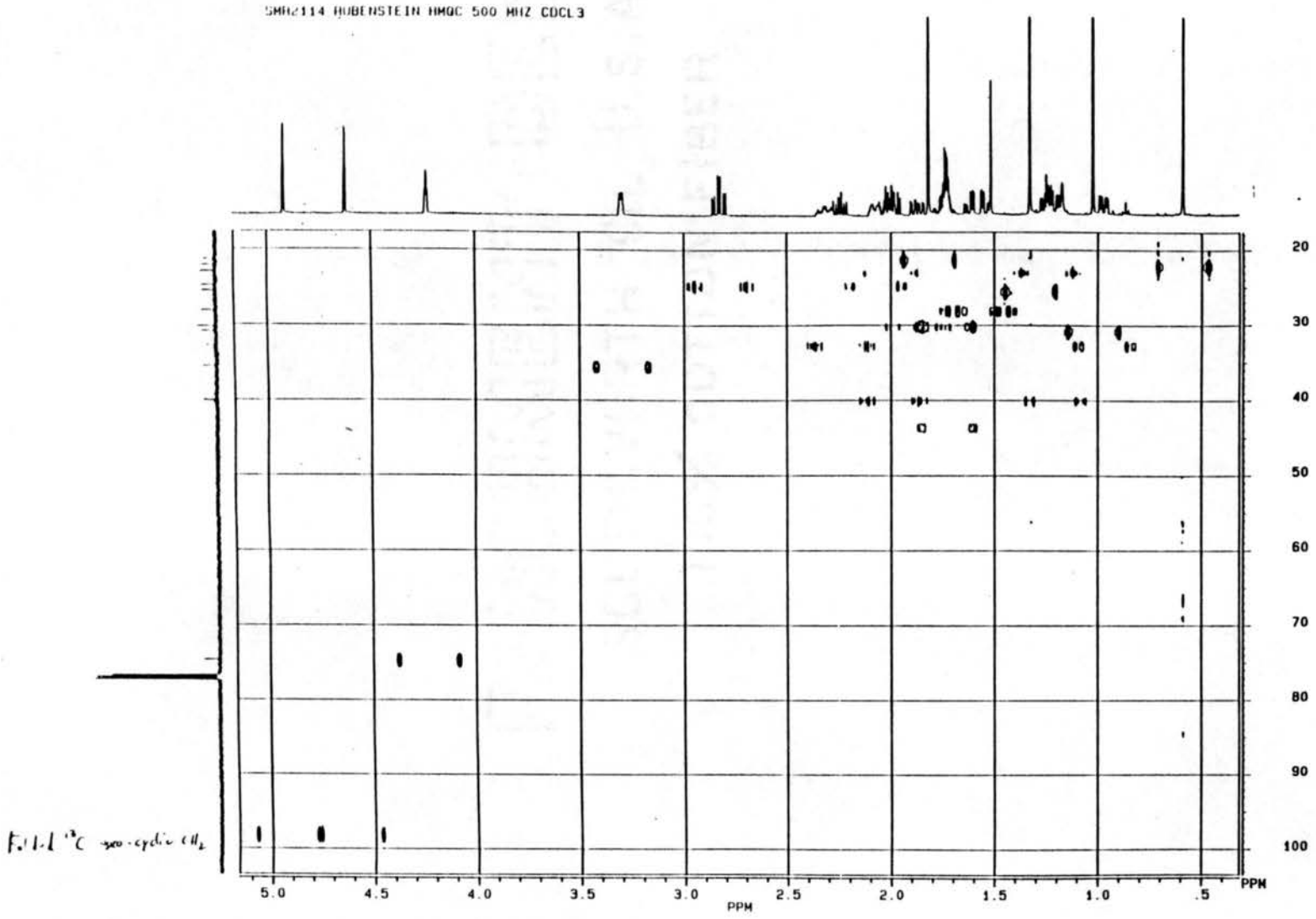
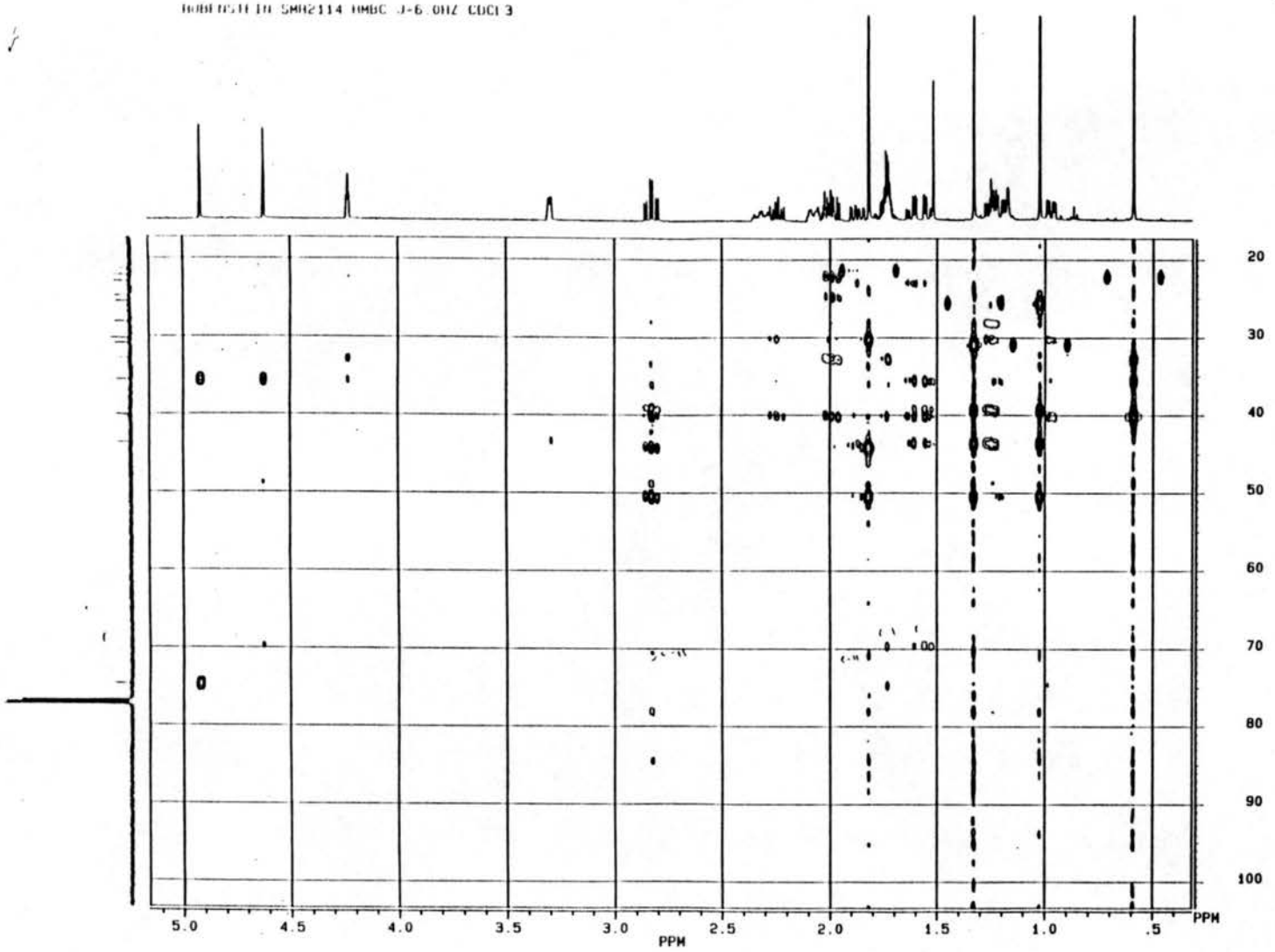
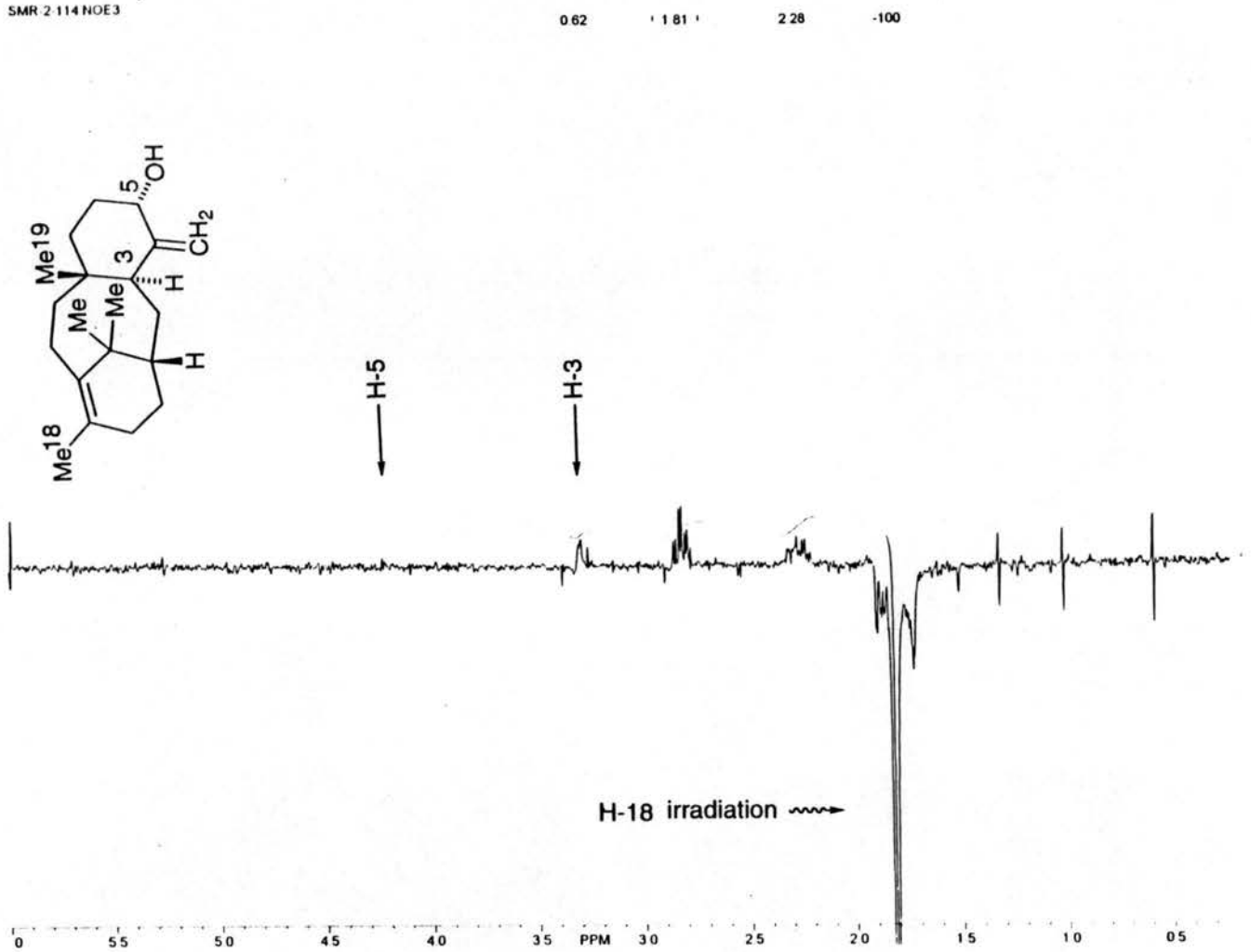
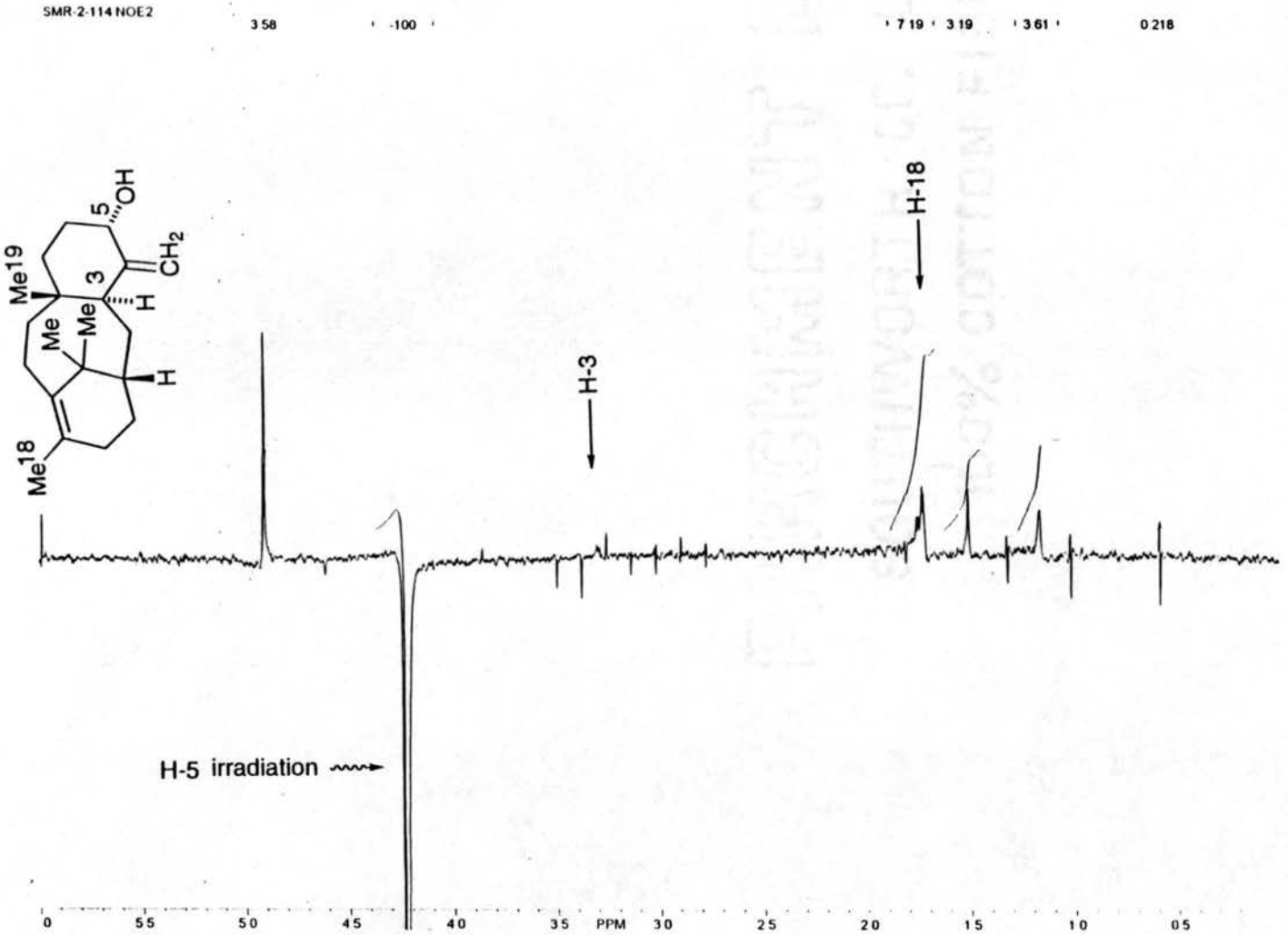
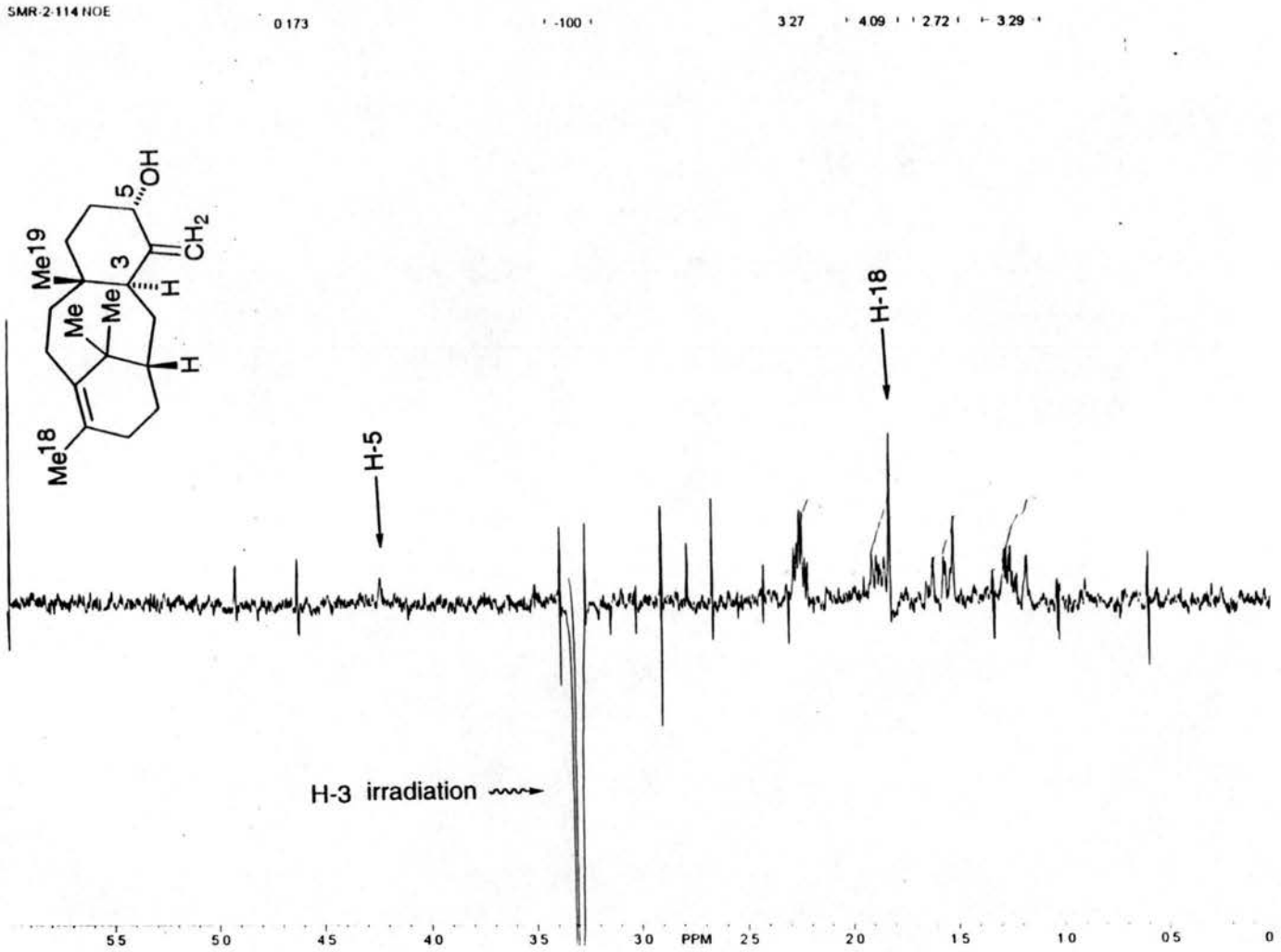


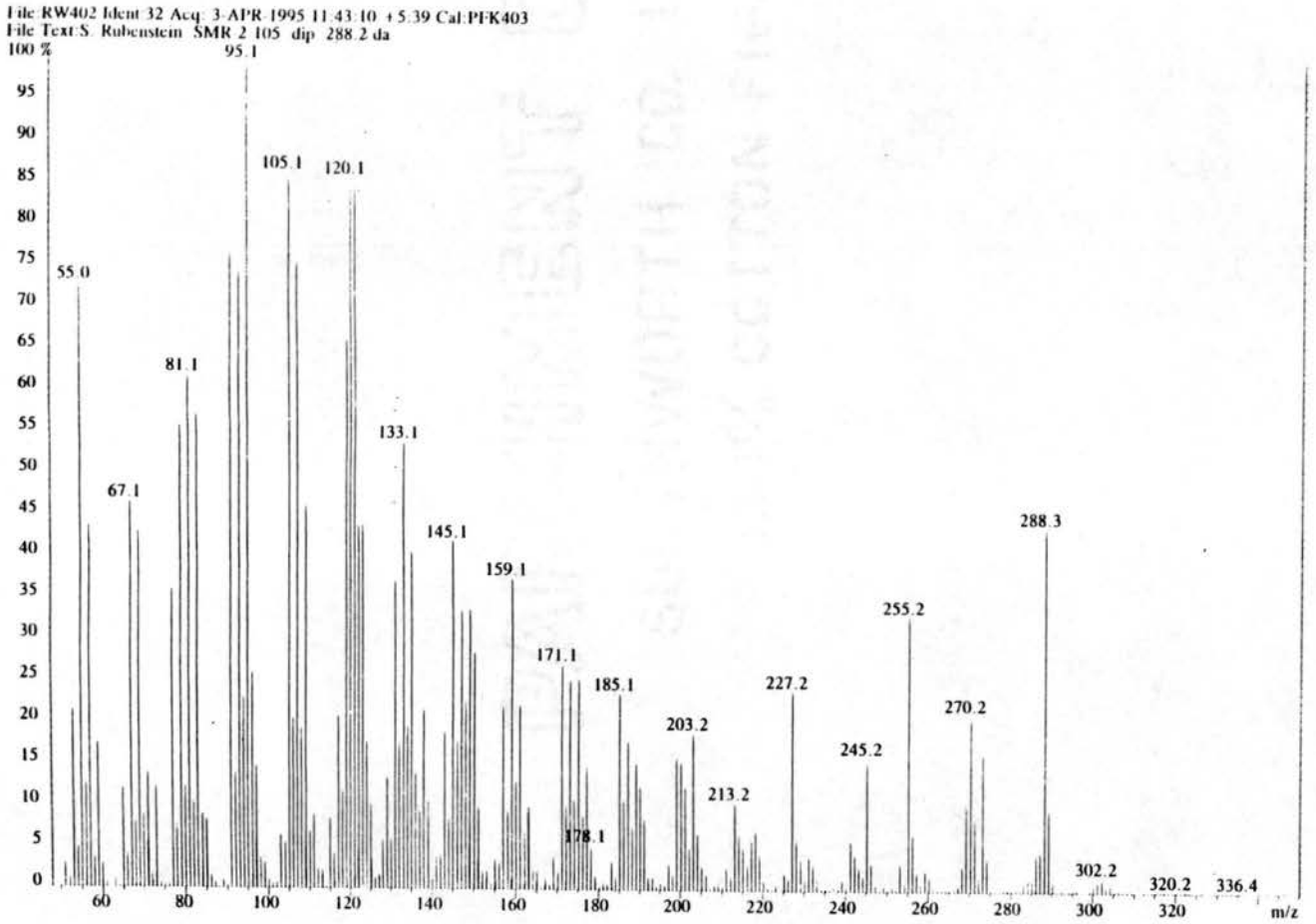
Fig 1.1 ^{13}C 300 cyclic CH_2

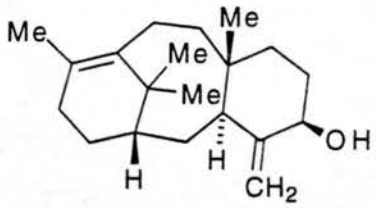

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
PPM
PPM
20
30
40
50
60
70
80
90
100


SMR 2-114 NOE3
0.62
1.81
2.28
-100
5
OH
CH2
Me19
3
H
Me
Me
H
Me18
H-5
H-3
H-18 irradiation
0
55
50
45
40
35
PPM
30
25
20
15
10
05

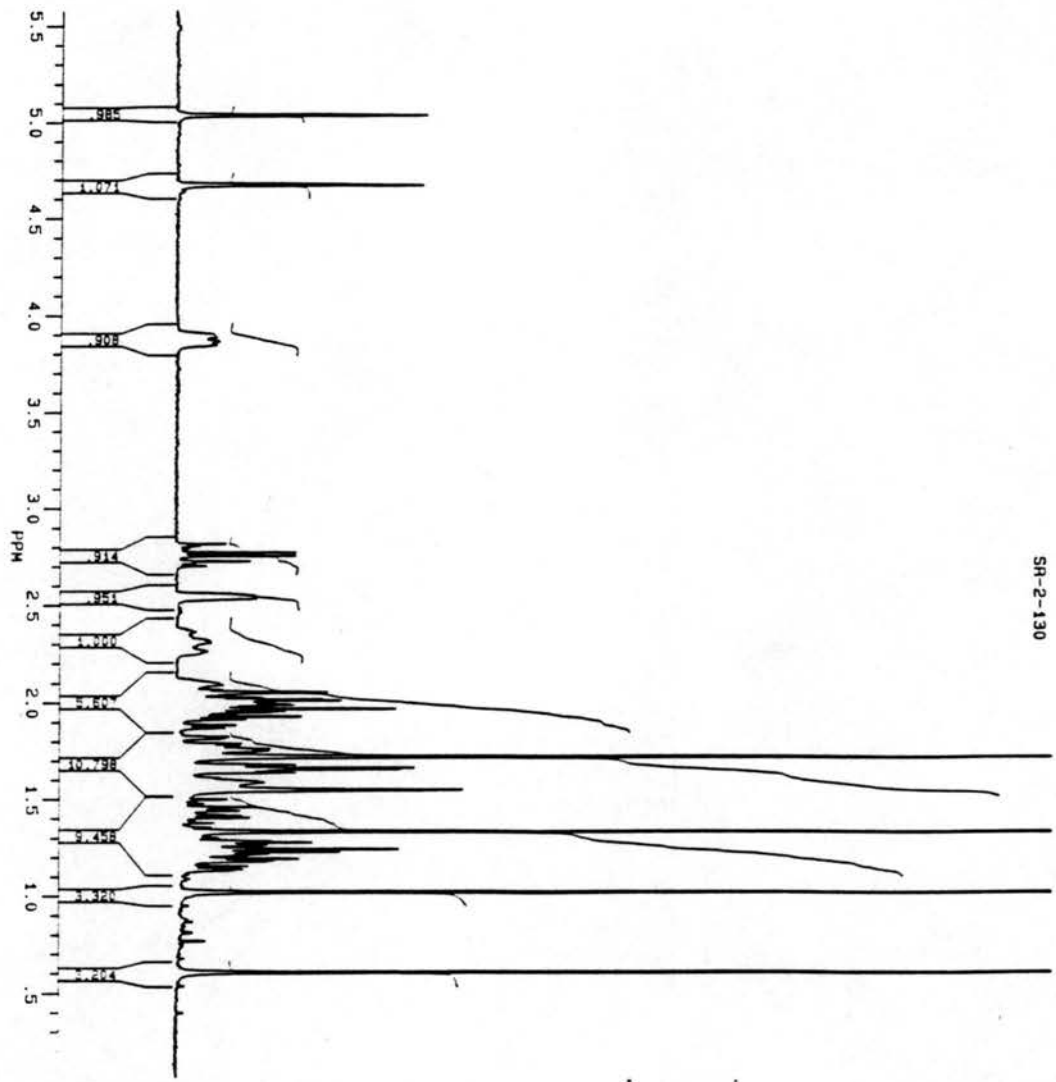

SMR-2-114 NOE2
3 58
-100
7 19
3 19
3 61
0 218
OH
5
CH2
Me19
3
H
Me
Me
H
Me18
H-18
H-3
H-5 irradiation
0
55
50
45
40
35
PPM
30
25
20
15
10
05

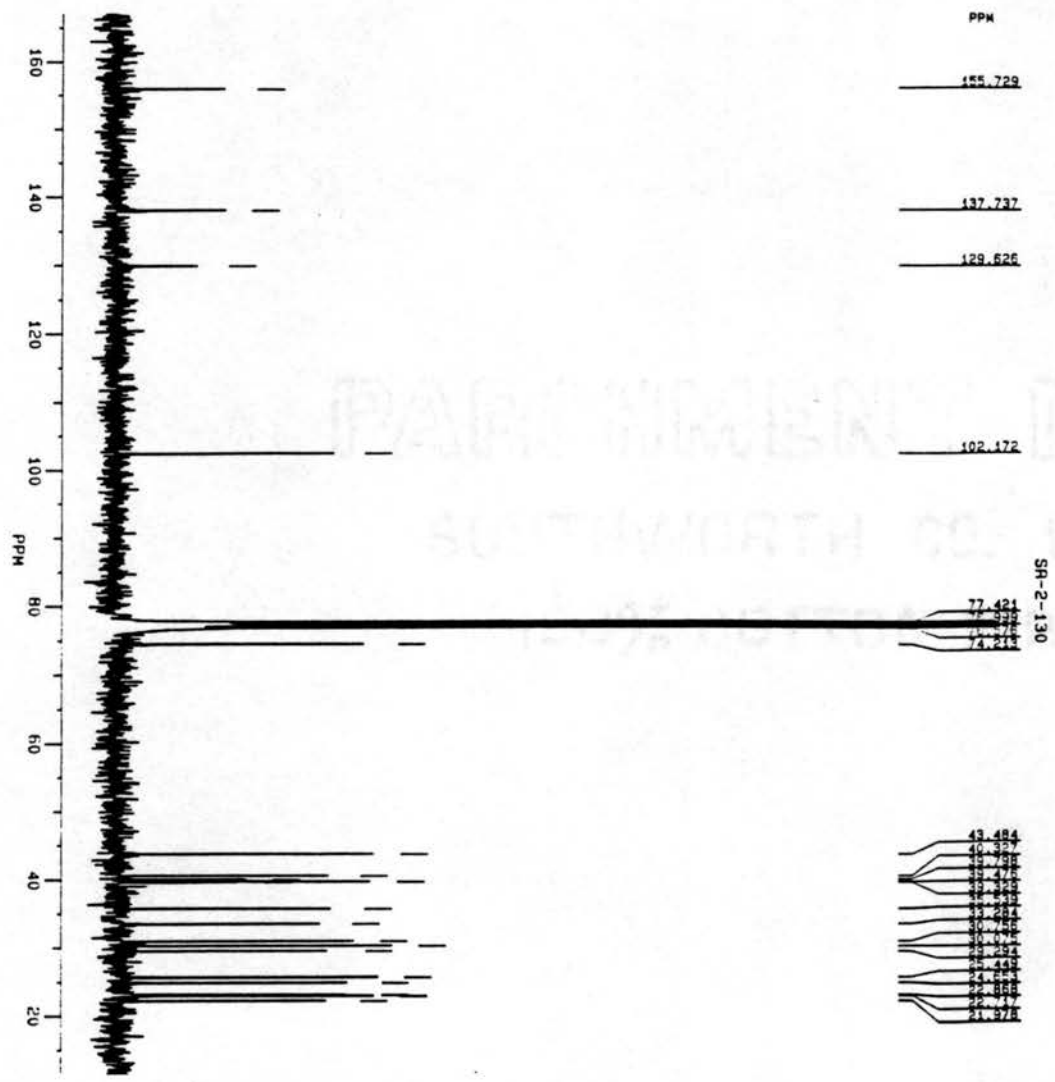

SMR-2-114 NOE
0.173
-100
3.27
4.09
2.72
3.29
OH
5
Me19
3
CH2
H
Me
Me
H
Me18
H-5
H-18
H-3 irradiation
55
50
45
40
35
30
PPM
25
20
15
10
05
0

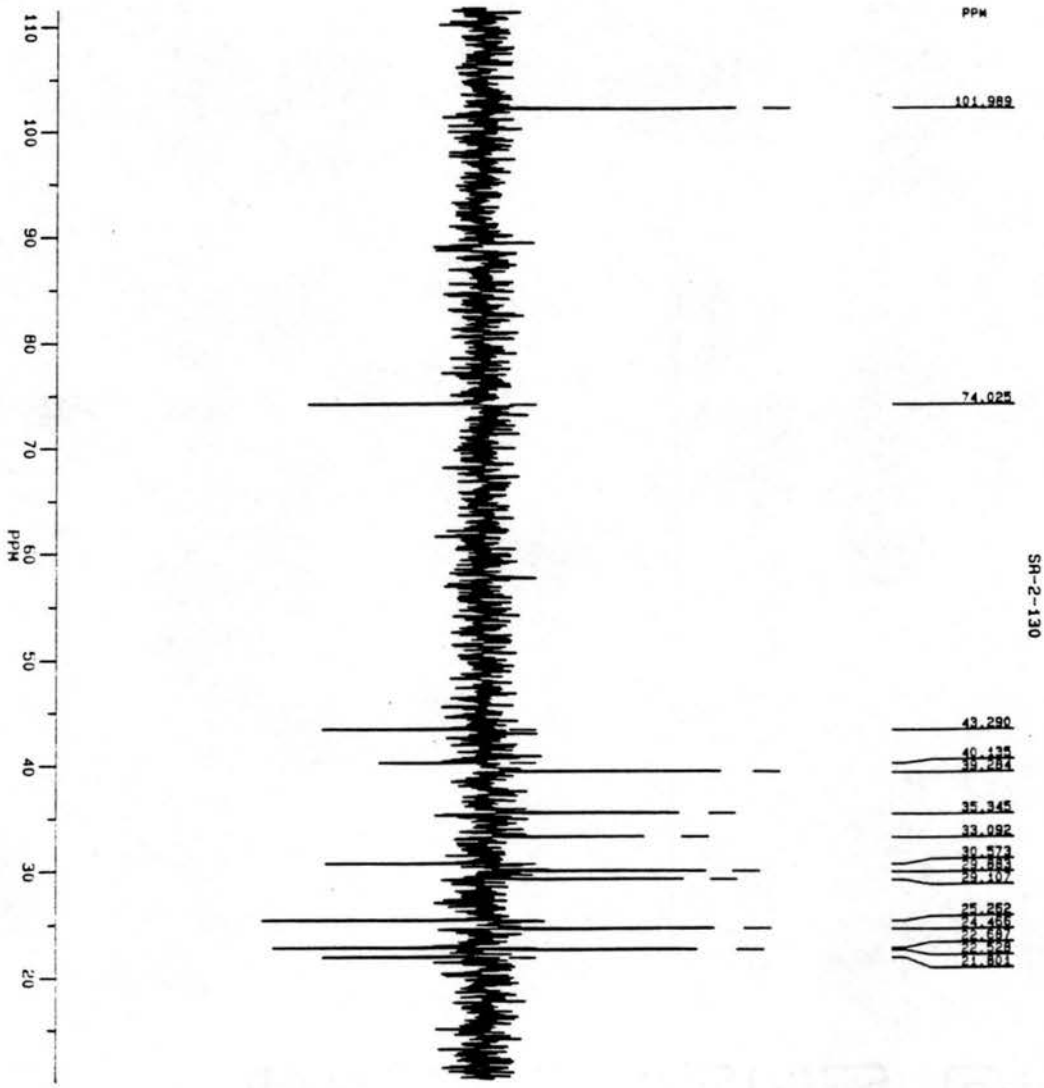

File:RW402 Ident 32 Acq: 3-APR-1995 11:43:10 +5:39 Cal:PFK403
File Text:S. Rubenstein SMR 2 105 dip 288.2 da
100 %
95.1
105.1
120.1
55.0
81.1
133.1
67.1
145.1
288.3
159.1
255.2
171.1
227.2
185.1
270.2
203.2
245.2
213.2
178.1
302.2
320.2
336.4
m/z

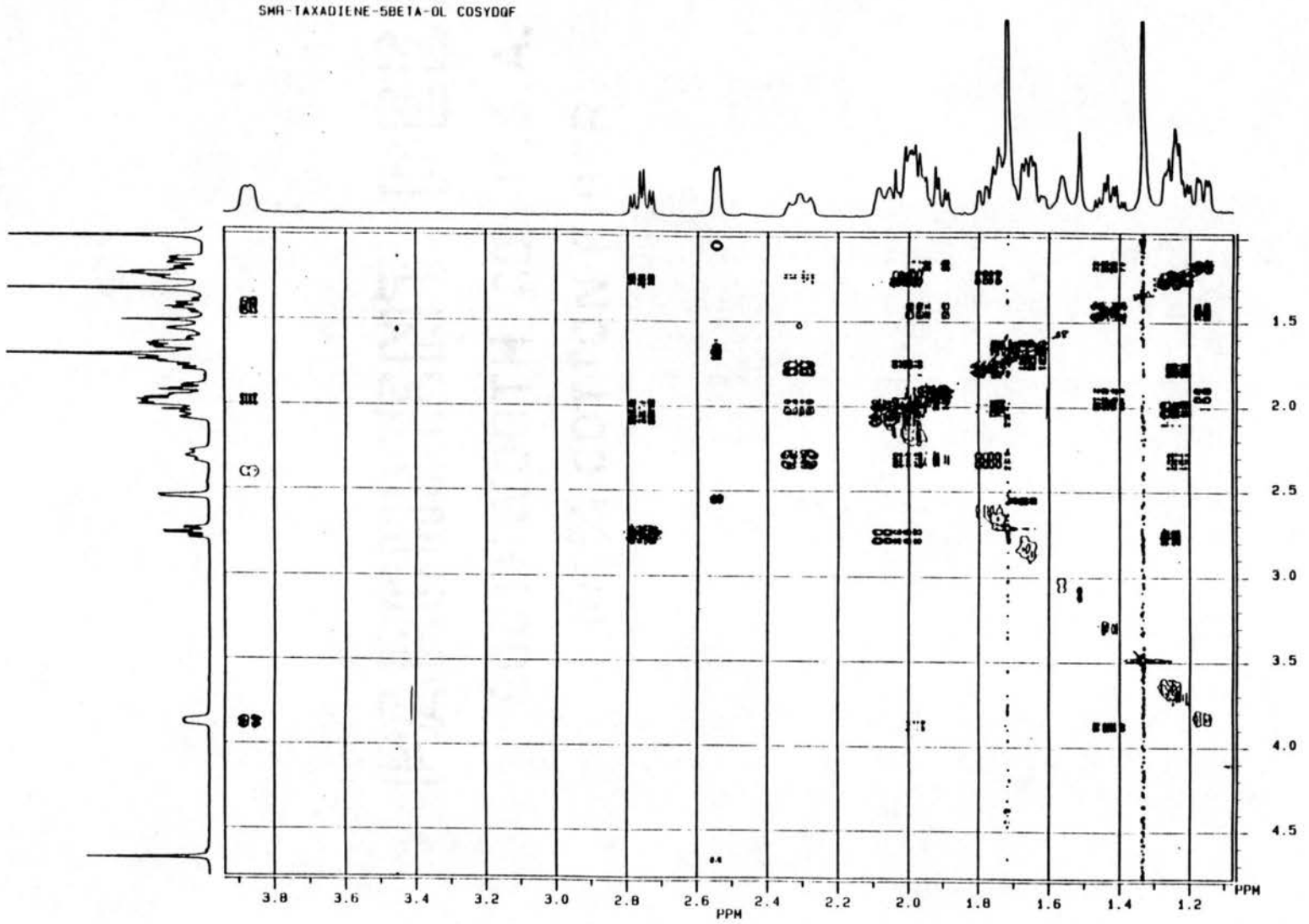


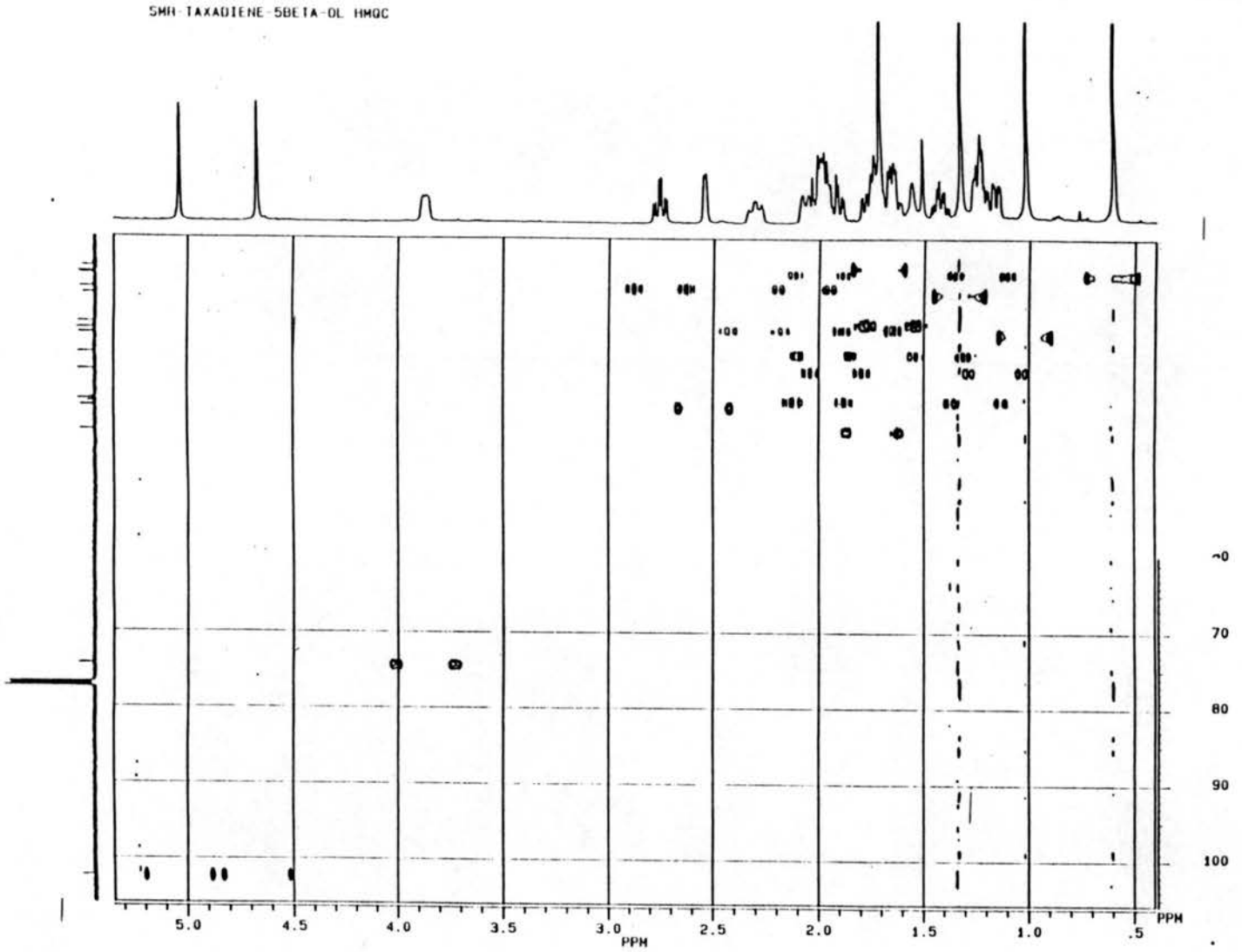
Taxa-4(20),11(12)-diene-5(β)-ol

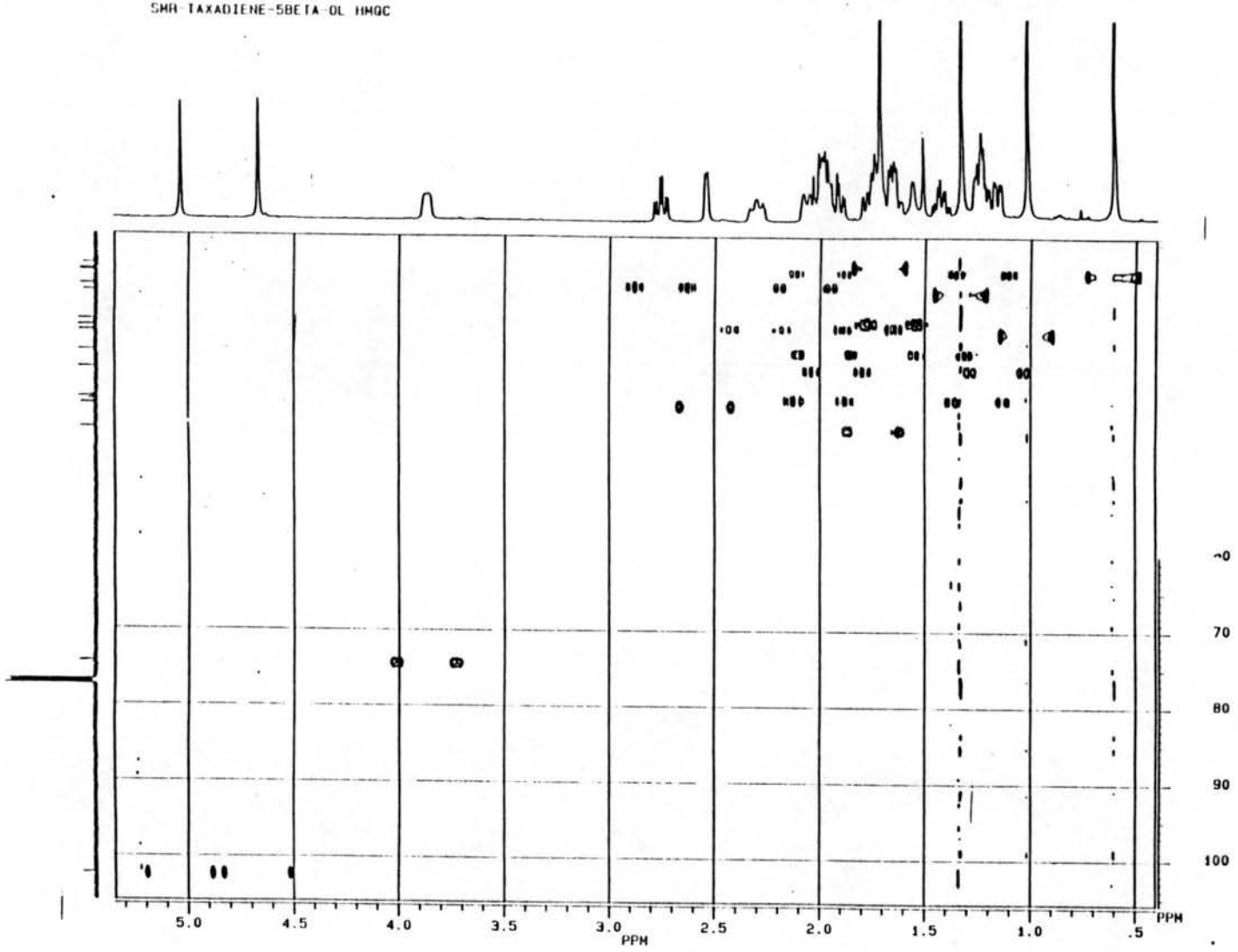

SR-2-130
PPM
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5

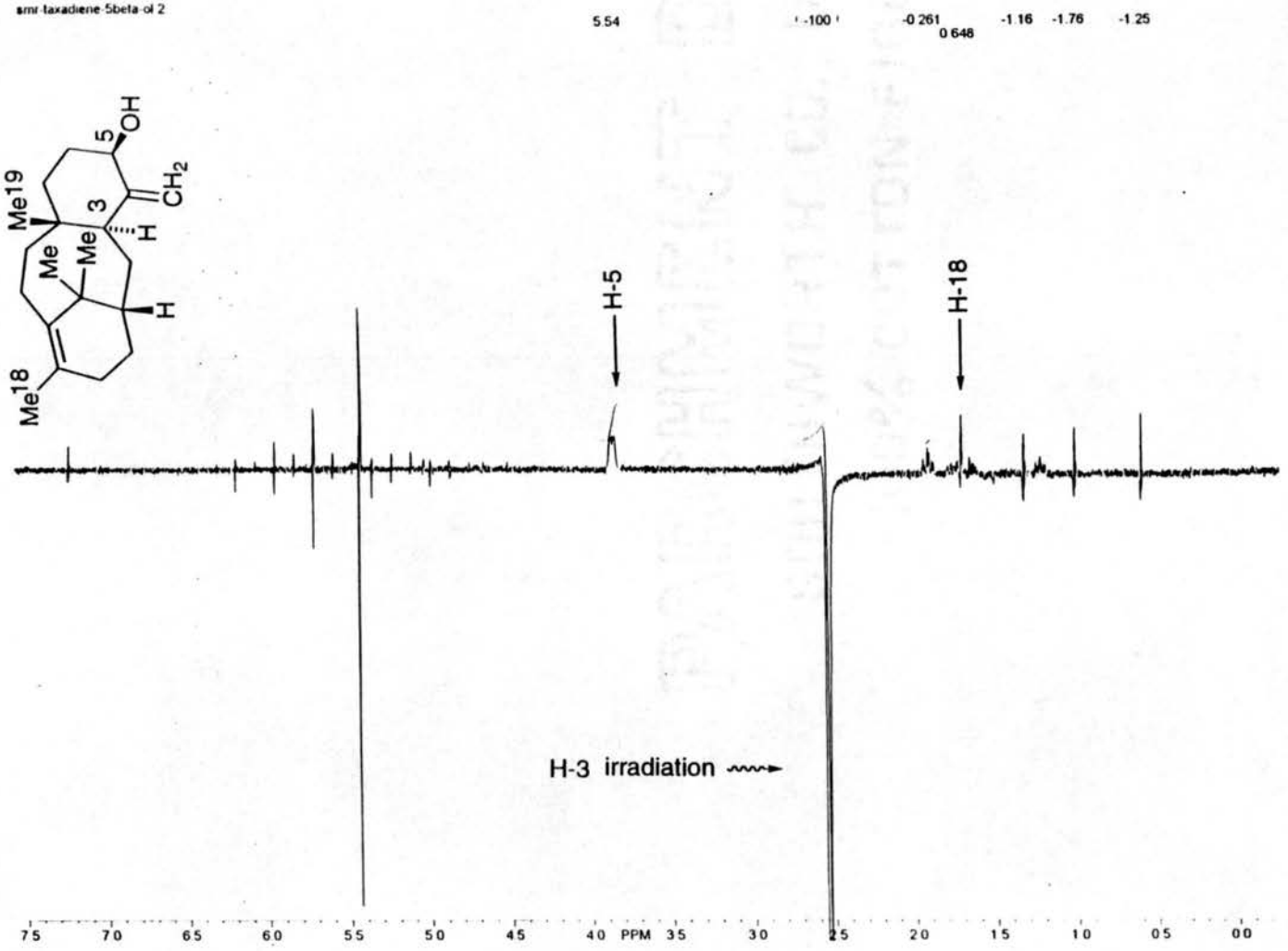

PPM
155.729
137.737
129.626
102.172
77.421
74.211
43.484
SA-2-130
160
140
120
100
80
60
40
20
PPM

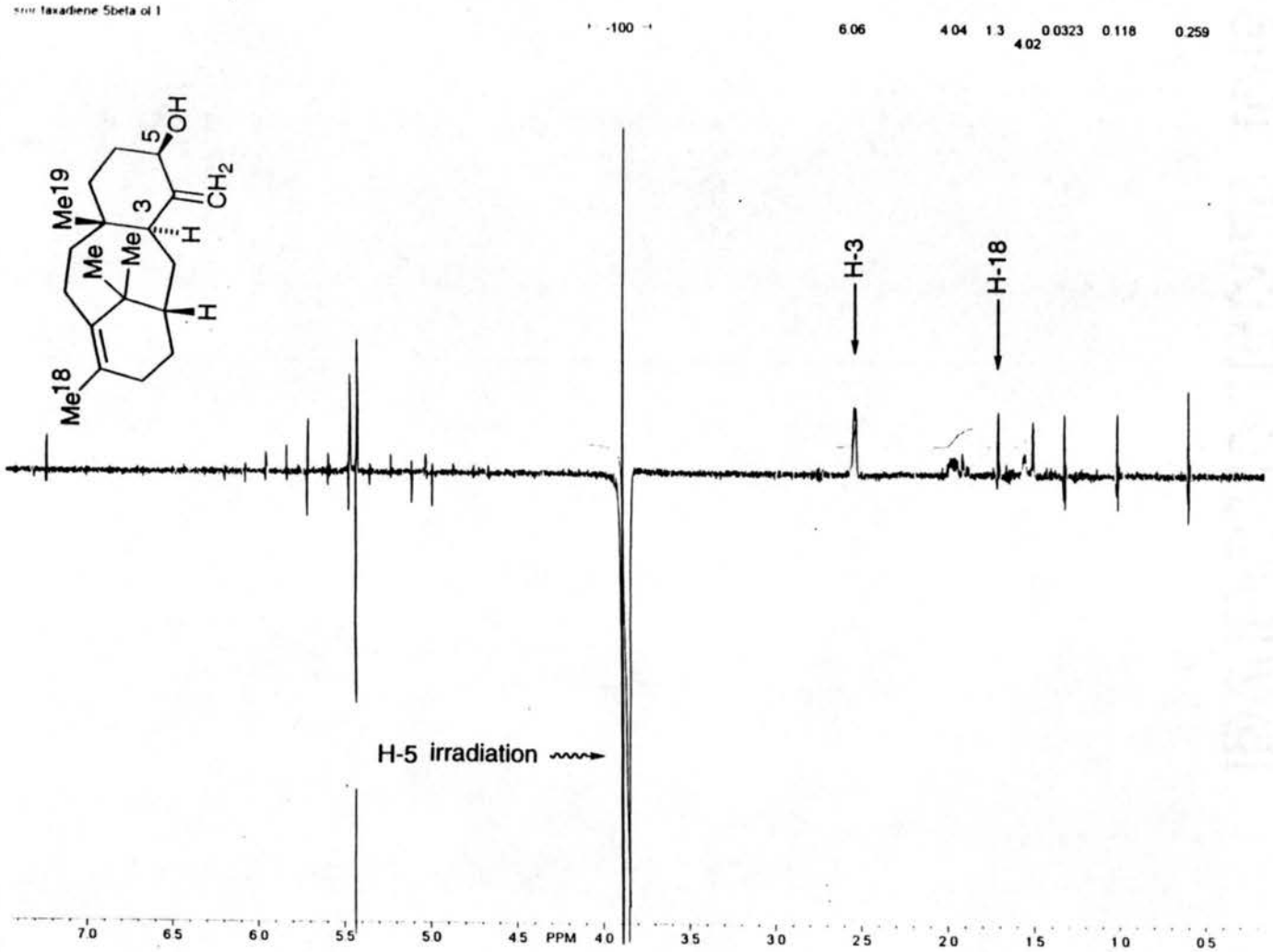

PPM
101.989
74.025
43.290
40.435
39.281
35.345
33.092
30.573
29.884
29.107
25.262
24.406
22.697
22.369
21.801
SR-2-130
PPM
110
100
90
80
70
60
50
40
30
20

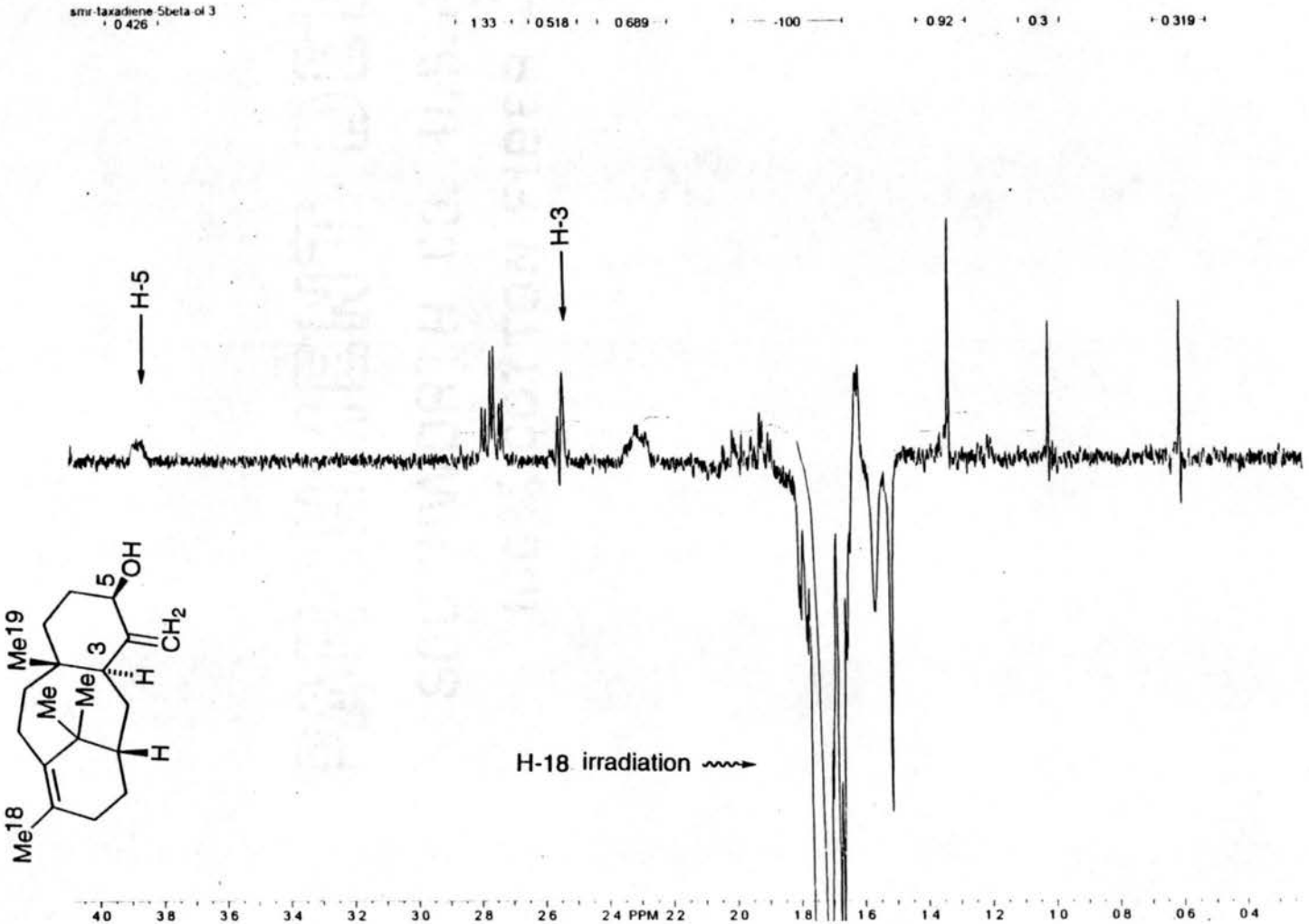

SMR-TAXADIENE-5BETA-OL COSYDQF
1.5
2.0
2.5
3.0
3.5
4.0
4.5
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
PPM
PPM

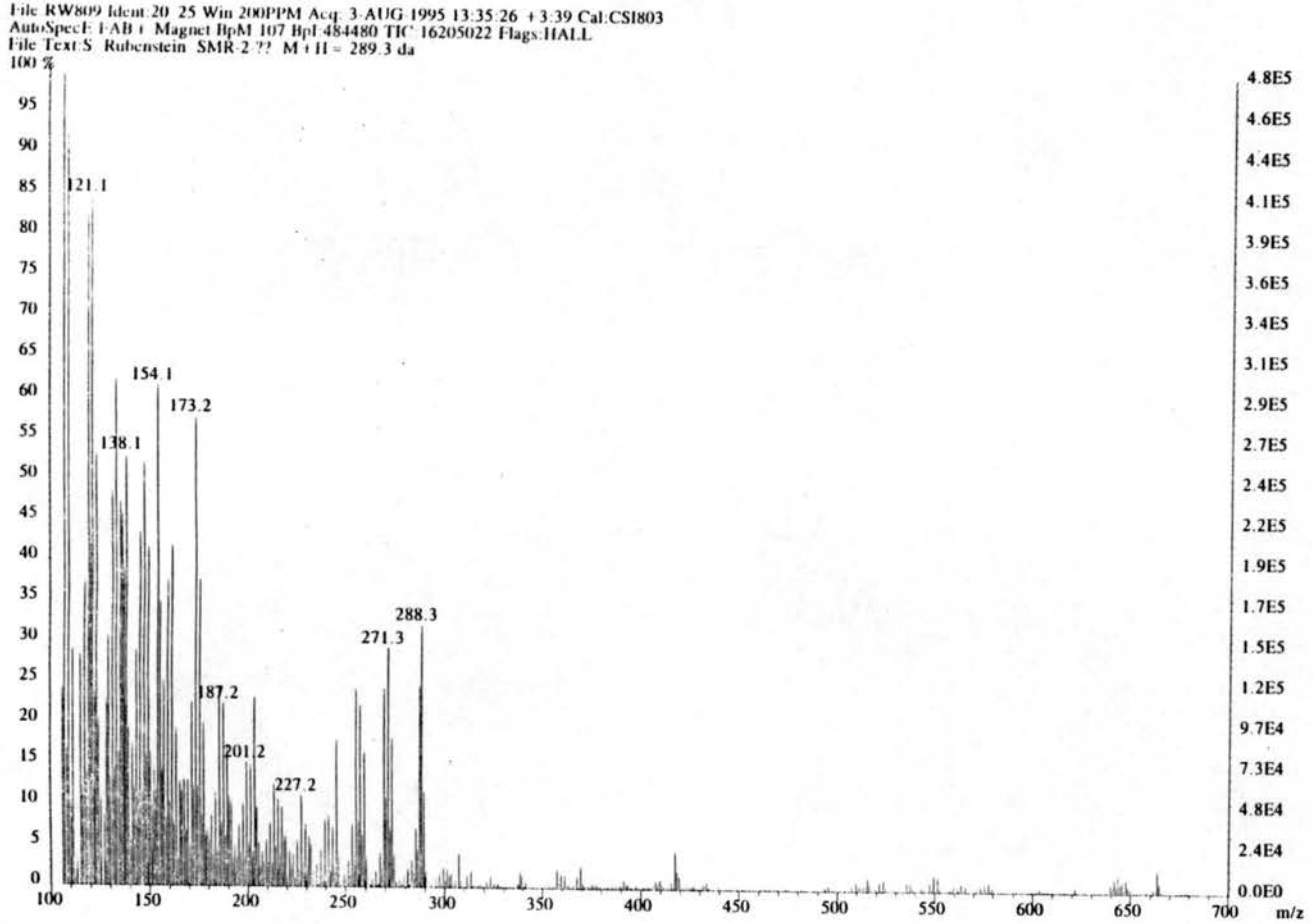

5MH-TAXADIENE-5BETA-OL HMQC
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
PPM
70
80
90
100

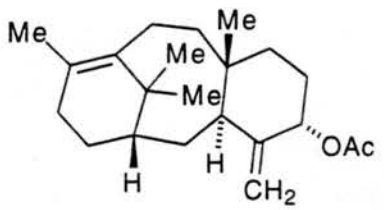

5MR-TAXADIENE-5BETA-OL HMQC
5.0
4.5
4.0
3.5
3.0
PPM
2.5
2.0
1.5
1.0
.5
PPM
70
80
90
100


smr-taxadiene-5beta-ol 2
5.54
-100
-0.261
0.648
-1.16
-1.76
-1.25
OH
5
CH2
Me19
3
H
Me
Me
H
Me18
H-5
H-18
H-3 irradiation
75
70
65
60
55
50
45
40
PPM
35
30
25
20
15
10
05
00

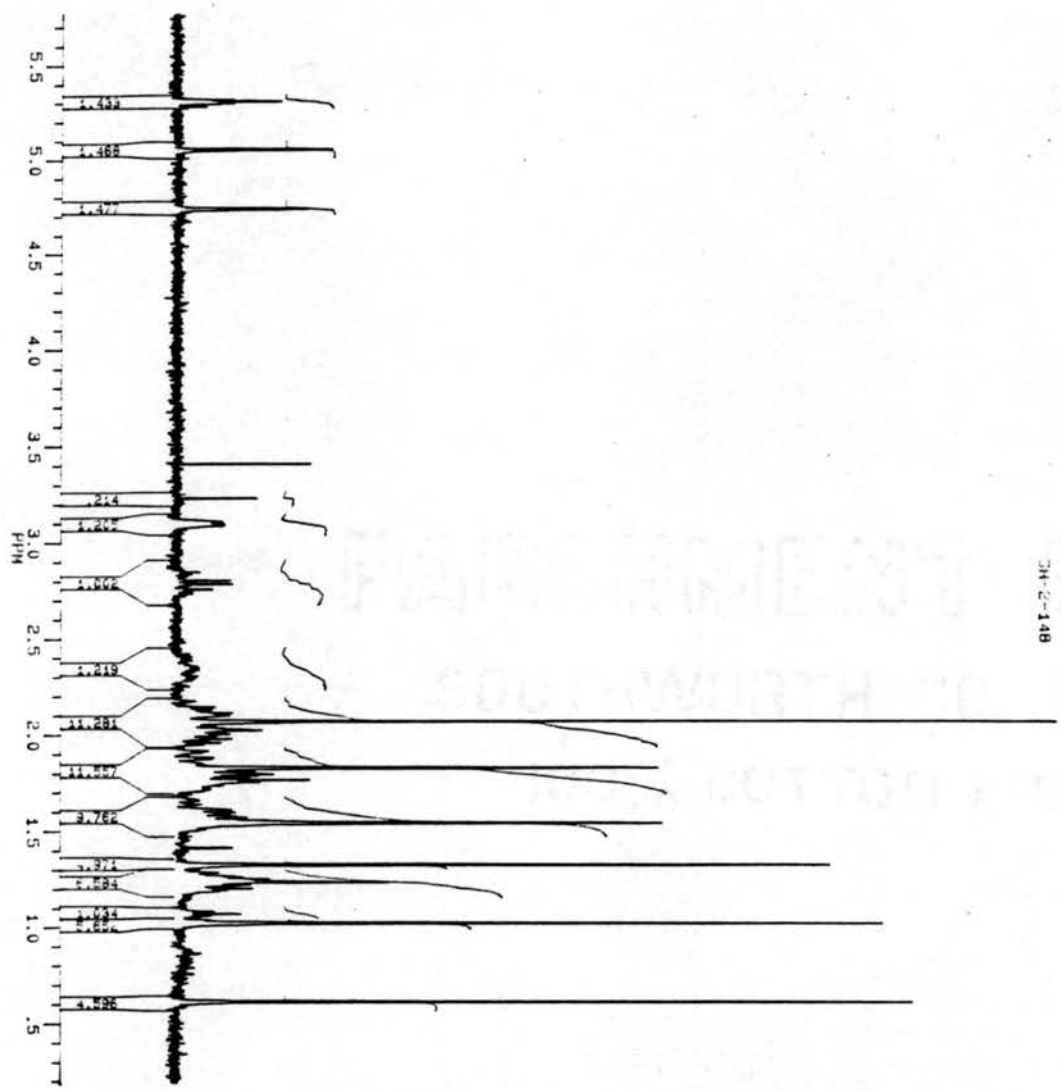

taxadiene 5beta ol 1
-100
6.06
4.04
1.3
4.02
0.0323
0.118
0.259
OH
5
Me19
3
CH2
H
Me
Me
H
Me18
H-3
H-18
H-5 irradiation
7.0
6.5
6.0
5.5
5.0
4.5
PPM
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

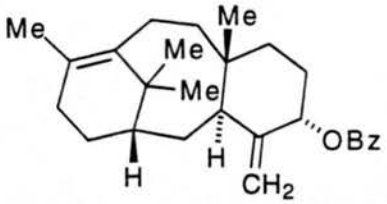

smr-taxadiene-5beta-ol 3
0.426
1.33
0.518
0.689
-100
0.92
0.3
0.319
H-5
H-3
H-18 irradiation
OH
5
CH_2
Me19
3
H
Me
Me
H
Me^{18}
40 38 36 34 32 30 28 26 24 PPM 22 20 18 16 14 12 10 08 06 04


File RW809 Ident 20 25 Win 200PPM Acq 3-AUG-1995 13:35:26 +3:39 Cal:CSI803
AutoSpecE EAB+ Magnet BpM 107 BpI 484480 TIC 16205022 Flags:HALL
File Text:S Rubenstein SMR-2 ?? M+H = 289.3 da
100 %
121.1
154.1
173.2
138.1
288.3
271.3
187.2
201.2
227.2
m/z

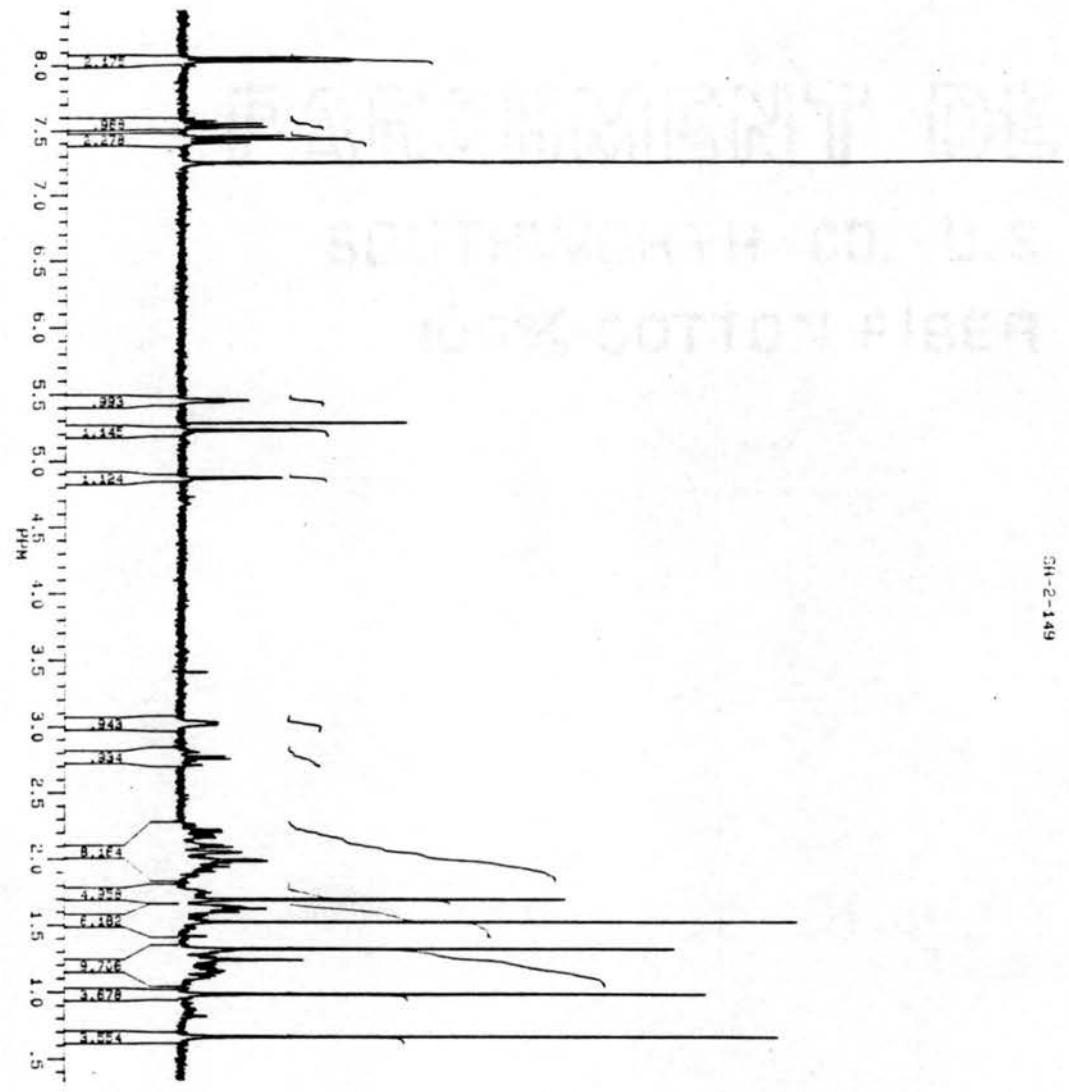


Taxa-4(20),11(12)-diene-5(α)-acetate


5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
PPM
1.439
1.468
1.477
.214
1.267
1.302
1.319
11.281
11.357
3.762
1.971
1.994
1.994
4.896
SH-2-148



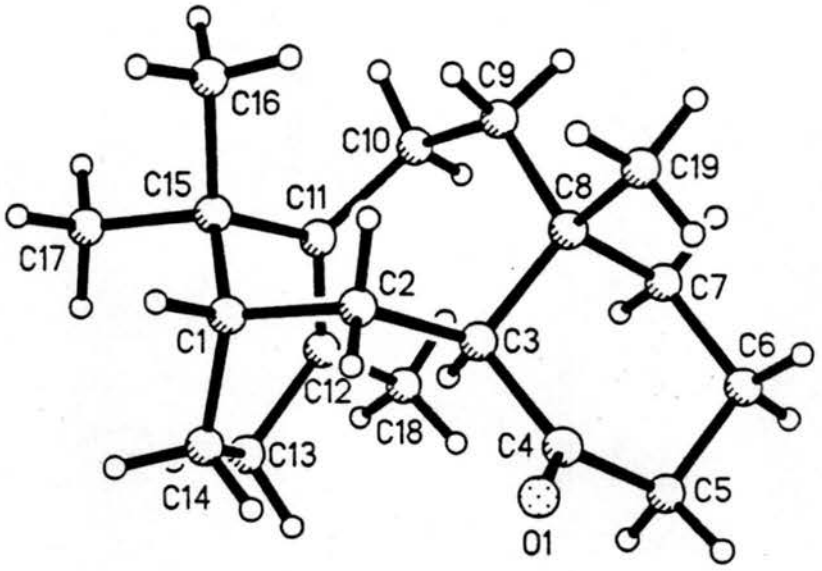
Taxa-4(20),11(12)-diene-5(α)-benzoate


8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
PPM
.993
1.145
1.124
.943
.934
8.164
4.286
2.102
9.746
3.878
9.444
SH-2-149

APPENDIX 2

X-RAY CRYSTALLOGRAPHIC DATA

Appendix 2 contains X-ray crstallographic coordinates for ketone **185**.



STRUCTURE DETERMINATION SUMMARY for rw33

Crystal Data

Empirical Formula	$C_{19}\ H_{30}\ O$
Color; Habit	clear colorless thin rods
Crystal Size (mm)	0.03 x 0.06 x 0.42
Crystal System	Monoclinic
Space Group	C2/c
Unit Cell Dimensions	$\underline{a}$ = 33.937(7) Å
	$\underline{b}$ = 7.586(2) Å
	$\underline{c}$ = 29.153(6) Å
	β = 121.21(3)°
Volume	6419(3) $Å^3$
Z	16
Formula Weight	274.4
Density(calc.)	1.136 Mg/m^3
Absorption Coefficient	0.067 mm^{-1}
F(000)	2432

Solution and Refinement

System Used	Siemens SHELXTL PLUS (UNIX)
Solution	Direct Methods
Refinement Method	Full-Matrix Least-Squares
Quantity Minimized	$\sum w(F_o - F_c)^2$
Absolute Structure	N/A
Extinction Correction	N/A
Hydrogen Atoms	Riding model, fixed isotropic U
Weighting Scheme	$w^{-1} = \sigma^2(F) + 0.0014F^2$
Number of Parameters Refined	361
Final R Indices (obs. data)	R = 6.70 %, wR = 7.55 %
R Indices (all data)	R = 13.16 %, wR = 9.35 %
Goodness-of-Fit	1.20
Largest and Mean Δ/σ	0.001, 0.000
Data-to-Parameter Ratio	6.1:1
Largest Difference Peak	0.29 $e\text{Å}^{-3}$
Largest Difference Hole	-0.25 $e\text{Å}^{-3}$

Data Collection

Diffractometer Used	Siemens P4/Unix
Radiation	MoKα (λ = 0.71073 Å)
Temperature (K)	173
Monochromator	Highly oriented graphite crystal
2θ Range	2.0 to 45.0°
Scan Type	2θ–θ
Scan Speed	Variable; 1.00 to 60.00°/min. in ω
Scan Range (ω)	1.00° plus Kα–separation
Background Measurement	Stationary crystal and stationary counter at beginning and end of scan, each for 50.0% of total scan time
Standard Reflections	3 measured every 97 reflections
Index Ranges	$0 \leq h \leq 36$, $0 \leq k \leq 8$ $-31 \leq \ell \leq 26$
Reflections Collected	4280
Independent Reflections	4195 (R_{int} = 4.44%)
Observed Reflections	2191 ($F > 4.0\sigma(F)$)
Absorption Correction	N/A

Table 1. Atomic coordinates ($x10^5$) and equivalent isotropic displacement coefficients ($Å^2 x10^4$)

	x	y	z	U(eq)
O(1)	1165(15)	61683(64)	14751(19)	477(26)
C(1)	14352(20)	61622(79)	17834(23)	257(31)
C(2)	9164(20)	66715(77)	14218(24)	280(31)
C(3)	7302(19)	81413(77)	16241(23)	229(29)
C(4)	3040(20)	76023(94)	16437(24)	316(32)
C(5)	1541(21)	88847(89)	19144(24)	335(33)
C(6)	1092(22)	107586(87)	17046(27)	374(35)
C(7)	5192(20)	113106(85)	16566(23)	279(30)
C(8)	6332(20)	99932(86)	13409(22)	265(30)
C(9)	10320(20)	107113(79)	12820(24)	267(31)
C(10)	15255(19)	108628(74)	17829(23)	243(30)
C(11)	17060(18)	91751(72)	20925(23)	184(28)
C(12)	17459(18)	89052(77)	25694(22)	200(28)
C(13)	17748(21)	70762(79)	27875(23)	311(31)
C(14)	15554(21)	56051(80)	23503(24)	325(33)
C(15)	17790(19)	75677(81)	18223(22)	250(30)
C(16)	17257(22)	78853(88)	12728(25)	378(35)
C(17)	22814(19)	68882(88)	21736(25)	366(34)
C(18)	17133(21)	103140(84)	29141(24)	328(33)
C(19)	2169(20)	98316(97)	7588(24)	374(33)
O(2)	-9965(15)	174877(63)	17567(17)	415(24)
C(21)	-15932(20)	176616(80)	1333(24)	278(32)
C(22)	-15949(21)	170966(79)	6445(23)	280(31)
C(23)	-12791(19)	155819(73)	9865(22)	210(28)
C(24)	-9712(21)	160677(88)	15761(24)	259(32)
C(25)	-6173(20)	146966(93)	19263(24)	360(33)
C(26)	-8291(20)	128588(89)	18569(24)	348(32)
C(27)	-11469(20)	123818(81)	12675(23)	294(31)
C(28)	-15215(19)	137742(76)	9503(22)	197(27)
C(29)	-18525(19)	130992(82)	3750(22)	270(29)
C(30)	-16952(20)	129394(80)	-363(22)	239(29)
C(31)	-15061(20)	146478(74)	-1160(22)	190(29)
C(32)	-10568(21)	148659(78)	674(22)	225(31)
C(33)	-8404(21)	166707(84)	1582(25)	324(32)
C(34)	-11108(21)	181544(83)	2480(27)	358(36)
C(35)	-18134(20)	163031(81)	-3288(23)	266(31)
C(36)	-23286(20)	160284(88)	-5273(25)	360(34)
C(37)	-18187(21)	170285(87)	-8336(24)	349(33)
C(38)	-6983(21)	134149(83)	2661(26)	347(35)
C(39)	-18262(21)	139734(85)	11977(24)	325(33)

* Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor

Table 2. Bond lengths (Å)

O(1)-C(4)	1.227 (8)	C(1)-C(2)	1.561 (8)
C(1)-C(14)	1.542 (10)	C(1)-C(15)	1.541 (9)
C(2)-C(3)	1.542 (10)	C(3)-C(4)	1.533 (11)
C(3)-C(8)	1.575 (9)	C(4)-C(5)	1.498 (11)
C(5)-C(6)	1.524 (10)	C(6)-C(7)	1.527 (11)
C(7)-C(8)	1.538 (11)	C(8)-C(9)	1.549 (11)
C(8)-C(19)	1.551 (7)	C(9)-C(10)	1.554 (7)
C(10)-C(11)	1.502 (8)	C(11)-C(12)	1.341 (10)
C(11)-C(15)	1.540 (9)	C(12)-C(13)	1.508 (9)
C(12)-C(18)	1.510 (10)	C(13)-C(14)	1.562 (8)
C(15)-C(16)	1.535 (10)	C(15)-C(17)	1.553 (8)
O(2)-C(24)	1.221 (9)	C(21)-C(22)	1.554 (11)
C(21)-C(34)	1.538 (10)	C(21)-C(35)	1.547 (8)
C(22)-C(23)	1.535 (8)	C(23)-C(24)	1.523 (8)
C(23)-C(28)	1.574 (8)	C(24)-C(25)	1.515 (8)
C(25)-C(26)	1.533 (10)	C(26)-C(27)	1.525 (8)
C(27)-C(28)	1.539 (8)	C(28)-C(29)	1.544 (7)
C(28)-C(39)	1.544 (12)	C(29)-C(30)	1.549 (11)
C(30)-C(31)	1.517 (9)	C(31)-C(32)	1.339 (9)
C(31)-C(35)	1.542 (8)	C(32)-C(33)	1.511 (9)
C(32)-C(38)	1.516 (9)	C(33)-C(34)	1.558 (11)
C(35)-C(36)	1.545 (9)	C(35)-C(37)	1.563 (11)

Table 3. Bond angles (deg)

C(2)-C(1)-C(14)	111.8(6)	C(2)-C(1)-C(15)	115.1(5)
C(14)-C(1)-C(15)	109.9(4)	C(1)-C(2)-C(3)	117.6(5)
C(2)-C(3)-C(4)	113.8(5)	C(2)-C(3)-C(8)	117.4(6)
C(4)-C(3)-C(8)	108.2(5)	O(1)-C(4)-C(3)	121.7(7)
O(1)-C(4)-C(5)	122.7(7)	C(3)-C(4)-C(5)	115.5(6)
C(4)-C(5)-C(6)	112.6(7)	C(5)-C(6)-C(7)	112.7(6)
C(6)-C(7)-C(8)	113.6(5)	C(3)-C(8)-C(7)	108.4(6)
C(3)-C(8)-C(9)	114.5(5)	C(7)-C(8)-C(9)	110.1(5)
C(3)-C(8)-C(19)	109.1(5)	C(7)-C(8)-C(19)	109.7(5)
C(9)-C(8)-C(19)	105.0(6)	C(8)-C(9)-C(10)	120.0(6)
C(9)-C(10)-C(11)	114.0(5)	C(10)-C(11)-C(12)	122.9(6)
C(10)-C(11)-C(15)	119.4(6)	C(12)-C(11)-C(15)	116.9(5)
C(11)-C(12)-C(13)	121.8(6)	C(11)-C(12)-C(18)	125.4(5)
C(13)-C(12)-C(18)	112.5(6)	C(12)-C(13)-C(14)	114.4(5)
C(1)-C(14)-C(13)	115.4(5)	C(1)-C(15)-C(11)	105.6(6)
C(1)-C(15)-C(16)	110.9(5)	C(11)-C(15)-C(16)	116.5(5)
C(1)-C(15)-C(17)	110.5(5)	C(11)-C(15)-C(17)	109.8(4)
C(16)-C(15)-C(17)	103.6(6)	C(22)-C(21)-C(34)	113.0(5)
C(22)-C(21)-C(35)	114.3(5)	C(34)-C(21)-C(35)	109.3(6)
C(21)-C(22)-C(23)	118.8(6)	C(22)-C(23)-C(24)	113.1(5)
C(22)-C(23)-C(28)	116.4(4)	C(24)-C(23)-C(28)	108.4(5)
O(2)-C(24)-C(23)	122.6(5)	O(2)-C(24)-C(25)	121.5(5)
C(23)-C(24)-C(25)	115.8(6)	C(24)-C(25)-C(26)	112.4(5)
C(25)-C(26)-C(27)	112.0(6)	C(26)-C(27)-C(28)	113.3(5)
C(23)-C(28)-C(27)	108.5(5)	C(23)-C(28)-C(29)	115.0(5)
C(27)-C(28)-C(29)	109.5(5)	C(23)-C(28)-C(39)	109.7(5)
C(27)-C(28)-C(39)	109.3(5)	C(29)-C(28)-C(39)	104.7(5)
C(28)-C(29)-C(30)	121.2(5)	C(29)-C(30)-C(31)	113.2(5)
C(30)-C(31)-C(32)	122.0(5)	C(30)-C(31)-C(35)	120.4(6)
C(32)-C(31)-C(35)	116.9(5)	C(31)-C(32)-C(33)	122.1(6)
C(31)-C(32)-C(38)	125.8(6)	C(33)-C(32)-C(38)	111.7(5)
C(32)-C(33)-C(34)	114.1(6)	C(21)-C(34)-C(33)	116.2(5)
C(21)-C(35)-C(31)	105.7(4)	C(21)-C(35)-C(36)	111.0(6)
C(31)-C(35)-C(36)	116.0(5)	C(21)-C(35)-C(37)	110.5(5)
C(31)-C(35)-C(37)	109.9(6)	C(36)-C(35)-C(37)	103.7(4)

Table 4. Anisotropic displacement coefficients ($Å^2 x 10^4$)

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	343(28)	418(33)	609(34)	-134(27)	203(26)	0(28)
C(1)	333(38)	193(36)	281(36)	-7(32)	184(32)	-56(31)
C(2)	336(38)	180(37)	327(38)	-51(31)	172(32)	-80(31)
C(3)	236(34)	208(37)	210(34)	17(30)	92(29)	23(28)
C(4)	227(37)	395(46)	228(37)	-49(36)	49(31)	49(34)
C(5)	246(37)	521(49)	308(37)	-40(35)	194(33)	32(36)
C(6)	346(40)	385(46)	407(41)	41(36)	205(35)	21(36)
C(7)	246(34)	350(41)	262(35)	30(32)	147(30)	50(31)
C(8)	256(37)	305(39)	183(35)	20(31)	78(30)	51(30)
C(9)	296(37)	207(37)	298(37)	26(31)	155(32)	84(30)
C(10)	247(36)	190(37)	251(35)	-17(30)	102(31)	32(30)
C(11)	180(33)	154(35)	251(35)	20(27)	135(29)	-1(28)
C(12)	154(32)	220(35)	221(35)	32(28)	94(29)	26(29)
C(13)	355(38)	259(40)	236(36)	75(34)	94(32)	57(32)
C(14)	333(39)	213(38)	404(42)	18(32)	173(35)	25(32)
C(15)	274(36)	302(40)	212(36)	30(31)	152(30)	-8(31)
C(16)	473(43)	319(41)	412(42)	-25(36)	280(37)	-32(34)
C(17)	282(37)	359(42)	485(44)	59(33)	218(35)	17(35)
C(18)	358(41)	306(41)	300(38)	6(33)	154(34)	-27(32)
C(19)	268(38)	489(47)	276(38)	-9(35)	77(33)	79(35)
O(2)	457(29)	402(32)	363(28)	-122(25)	197(24)	-209(25)
C(21)	365(39)	154(36)	340(39)	10(31)	199(33)	10(31)
C(22)	339(38)	228(38)	304(37)	18(32)	189(32)	45(31)
C(23)	255(35)	204(35)	188(33)	-15(29)	128(30)	-58(28)
C(24)	283(38)	282(42)	273(37)	-174(34)	187(33)	-113(33)
C(25)	212(37)	613(51)	203(36)	-104(37)	71(32)	24(36)
C(26)	256(37)	450(47)	288(38)	44(35)	105(32)	132(35)
C(27)	360(38)	285(39)	268(36)	76(33)	184(33)	132(32)
C(28)	216(33)	187(34)	181(31)	-35(28)	98(28)	8(27)
C(29)	276(35)	257(37)	253(35)	-84(32)	121(30)	8(30)
C(30)	304(35)	292(39)	144(32)	-45(31)	134(29)	-60(28)
C(31)	256(38)	173(35)	178(33)	38(30)	139(30)	-8(27)
C(32)	295(40)	191(36)	216(35)	16(31)	152(32)	8(29)
C(33)	264(36)	378(45)	327(40)	23(33)	151(32)	27(33)
C(34)	475(45)	235(39)	454(43)	-97(35)	304(38)	-53(34)
C(35)	328(38)	271(39)	254(36)	63(33)	189(31)	36(31)
C(36)	381(42)	344(44)	310(38)	84(35)	147(35)	100(33)
C(37)	403(40)	319(42)	304(38)	34(35)	169(33)	42(33)
C(38)	347(39)	269(41)	472(44)	41(33)	245(36)	0(34)
C(39)	423(40)	341(42)	278(37)	-61(35)	229(34)	-44(32)

The anisotropic displacement factor exponent takes the form:

$-2\pi^2(h^2a^{*2}U_{11} + \ldots + 2hka^*b^*U_{12})$

Table 5. H-Atom coordinates ($x10^4$) and isotropic displacement coefficients ($Å^2 x10^3$)

	x	y	z	U
H(1A)	1482	5142	1622	80
H(2A)	864	7024	1078	80
H(2B)	736	5631	1366	80
H(3A)	968	8343	1990	80
H(5A)	374	8873	2293	80
H(5B)	-137	8516	1862	80
H(6A)	82	11557	1943	80
H(6B)	-168	10848	1359	80
H(7A)	785	11442	2010	80
H(7B)	455	12439	1483	80
H(9A)	943	11864	1125	80
H(9B)	1051	9976	1026	80
H(10A)	1731	11245	1669	80
H(10B)	1525	11751	2017	80
H(13A)	1623	7077	2989	80
H(13B)	2093	6792	3031	80
H(14A)	1765	4629	2463	80
H(14B)	1279	5197	2331	80
H(16A)	1421	8313	1027	80
H(16B)	1948	8742	1308	80
H(16C)	1775	6800	1140	80
H(17A)	2491	7778	2194	80
H(17B)	2344	6626	2527	80
H(17C)	2319	5840	2016	80
H(18A)	1696	11452	2761	80
H(18B)	1442	10122	2932	80
H(18C)	1981	10263	3269	80
H(19A)	156	10956	584	80
H(19B)	286	8985	566	80
H(19C)	-49	9454	767	80
H(21A)	-1777	18710	2	80
H(22A)	-1516	18113	872	80
H(22B)	-1905	16781	537	80
H(23A)	-1076	15359	856	80
H(25A)	-474	15047	2295	80
H(25B)	-382	14646	1837	80
H(26A)	-1000	12823	2035	80
H(26B)	-587	12001	2023	80
H(27A)	-1293	11274	1245	80
H(27B)	-965	12235	1104	80
H(29A)	-2118	13854	219	80
H(29B)	-1955	11951	407	80
H(30A)	-1463	12043	82	80
H(30B)	-1953	12571	-375	80
H(33A)	-817	16974	-147	80
H(33B)	-533	16619	465	80
H(34A)	-929	18540	614	80
H(34B)	-1141	19138	25	80
H(36A)	-2359	15577	-239	80
H(36B)	-2488	17133	-647	80
H(36C)	-2459	15203	-819	80
H(37A)	-1956	16173	-1117	80
H(37B)	-1994	18101	-950	80
H(37C)	-1509	17259	-744	80

H(38A)	-847	12289	202	80
H(38B)	-535	13475	79	80
H(38C)	-485	13563	644	80
H(39A)	-1974	12870	1175	80
H(39B)	-1638	14316	1567	80
H(39C)	-2056	14859	1005	80