MODIFIED TAXOLS AS ANTICANCER AGENTS

by

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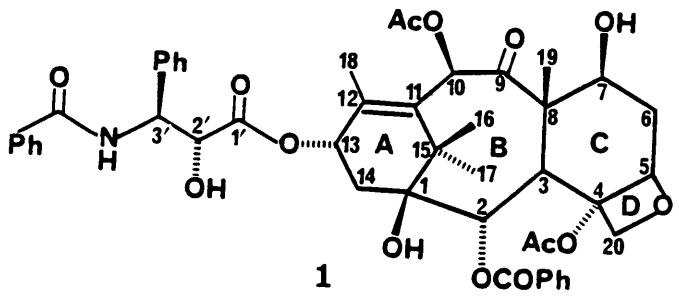
1.0 INTRODUCTION

1.1 PURPOSE

It was the purpose of this research, through the discipline of synthetic organic chemistry, to improve cancer therapy. The focal point of this work was the potent antitumor natural product taxol **1**. In general terms the goals of this investigation were to gain an understanding of the chemical reactivity of taxol and to determine, to as great an extent as possible, the factors which contribute to the activity of the drug.



1

1.2 IMPORTANCE OF NATURAL PRODUCTS IN CANCER THERAPY

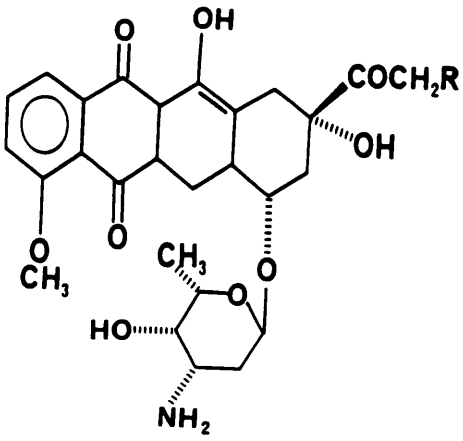
Within the field of cancer research natural products have played an important role. The first modern classes of anticancer drugs to become available were the naturally occurring

androgens and estrogens in 1940.[1] Since that time a wealth of chemotherapeutic agents have been isolated from natural sources, including the antibiotic compounds daunomycin **2** and adriamycin **3** which were isolated from Streptomyces peucetius.[2] Other antibiotic compounds used in cancer therapy are: bleomycin, mithramycin, mitomycin C and dactinomycin.

Natural products obtained from plants have also played an important part in cancer therapy. The antineoplastic compounds vincristine **4** and vinblastine **5** were isolated from the periwinkle plant which had been used in folk medicine for centuries.[3] Modified forms of natural products have also found their way into clinical use. Epipodophyllotoxin from the May apple, Podophyllum peltatum, led to the clinically used semisynthetic analogues VM26 **6** and VP16-213 **7**.

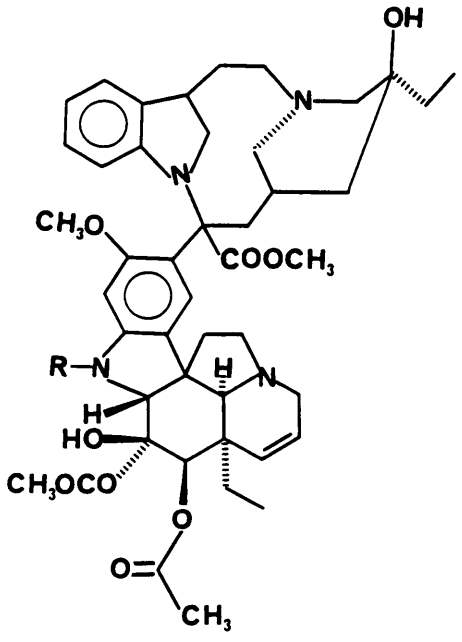
Many of these chemotherapy agents have not only been successful in marginally prolonging the life span of patients but have been able to effect cures. Daunomycin and vincristine are able to cure up to 72% of patients with non-Hodgkins lymphoma. Methotrexate, dactinomycin and vinblastine will cure 70% of those with advanced trophoblastic tumors.[1] Clearly natural products in cancer therapy are an indispensable tool in the fight against the second leading cause of death in the United States.

While the final administration of cancer chemotherapy lies with the physician it must begin with the chemist. Isolation



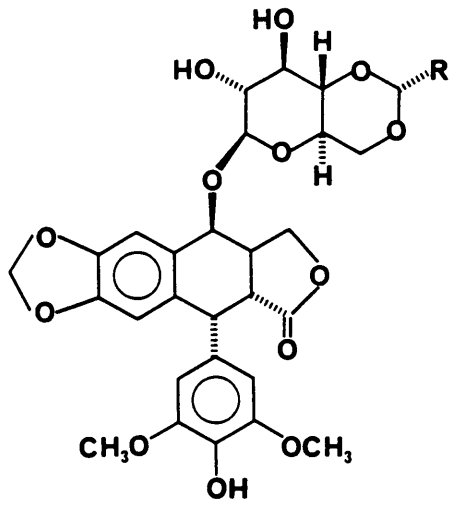
2 R=H

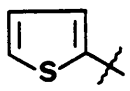
3 R=OH



4 R=CHO

5 R=CH_3



6 R =  VM 26

7 R = CH_3— VP 16-213

and characterization of new chemotherapentic agents is the first step that is taken by the chemist in producing a new cancer drug. Once a drug has been isolated there are several ways in which the chemist can contribute further. One of these is to structurally modify the drug to produce a more active or less toxic drug. Another way is to synthesize a series of derivatives from the original compound in order to determine the structure-activity relationship of the drug and possibly determine its chemical mechanism of action.

It is important for the progress of cancer therapy that new, not necessarily more active, chemotherapy agents are found. Most gains in clinical administration of drugs in the last ten years have been achieved by using protocols with several drugs. In this way the problems of toxicity and of loss of response to the drug upon repeated administration have been reduced. New drugs are needed therefore, to be used in combination with other anticancer agents.

1.3 REVIEW OF THE LITERATURE

1.3.1 TAXANES

The yew plant, in which taxol is found, has been known since antiquity as an extremely toxic plant. The first modern attempts of chemistry to isolate the constituents of the yew were made in 1856 by Lucas who extracted a crude material

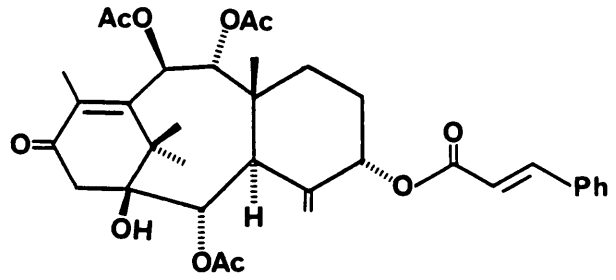
from Taxus baccata which he called taxine. Two of the earliest compounds characterized which possess the taxane skeleton were o-cinnamoyltaxicin I triacetate **8** and o-cinnamoyltaxicin II triacetate **9**.[4]

Numerous other compounds have been isolated from several species of yew plant. Characterization of these compounds was carried out using chemical and instrumental studies, including x-ray crystallography, the Cotton effect to determine absolute configuration and the nuclear Overhauser effect to determine relative stereochemistry.[5,6] Several reviews have been published covering most of the compounds containing the taxane skeleton isolated from yew species.[7,8,9]

The first compound isolated which has the same functionality around the taxane skeleton as taxol does was Baccatin III **10**.[10] After the discovery of taxol a third compound having the ring functionality of taxol was reported - cephalomannine **11** isolated from Taxus cephalomanni.[11]

Once the ability of taxol to exert an antimitotic effect via polymerization of microtubule protein was established, an assay measuring the ability of compounds to stabilize microtubule polymers was used to follow the fractionation of material from the yew plant.[12] The end purpose of this was to isolate compounds having the same type of biological activity as taxol.

Using this procedure a number of compounds were isolated from Taxus baccata which possessed the ability to stabilize



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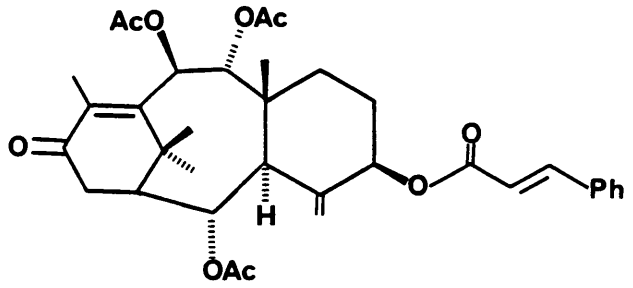

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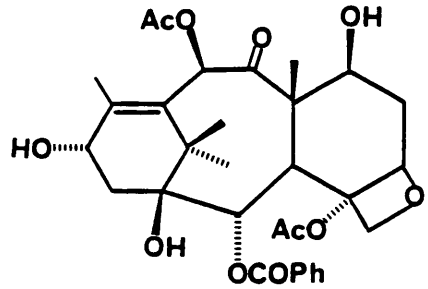

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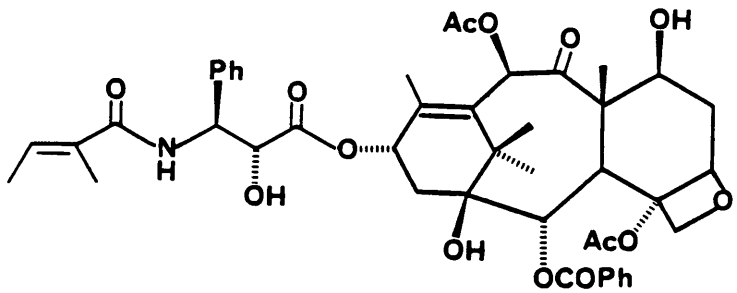

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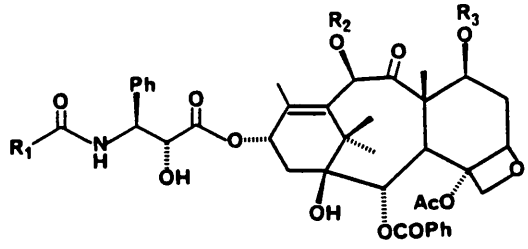
microtubule polymers (Table 1).[13] The biological activities of these compounds, along with two modified taxols also produced in this research - 2'-acetyltaxol and 7-acetyltaxol - were published recently.[14]

In the past several years other new compounds have been isolated which possess the taxane skeleton.[15] Some of these contain the ester side chain of taxol or analogues of it[16,17] (Table 2).

1.3.2 TAXOL

Taxol **1** was first isolated by M.E. Wall and associates at Research Triangle Institute and the results were published in 1971.[18] Isolation of taxol was guided by the 9KB test, an assay that tests the cytotoxicity of a compound, and the P-388 test, an *in vivo* assay against leukemia. Taxol was isolated from the bark of the western yew - *Taxus brevifolia*. Subsequent large scale isolation (8000 lbs of yew bark) for clinical testing of the drug yielded taxol in only 0.008% yield. The procedure used by Polyscience Inc. for the large scale isolation of taxol is shown in Scheme 1.[19] The structure determination of taxol was carried out using ir, nmr, uv and mass spectrometries. X-ray diffraction studies were not possible with taxol itself, as suitable crystals could not be grown, but were performed on crystals

Table 1. Microtubule Polymer Stabilizing Compounds Isolated from *Taxus baccata*



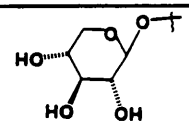
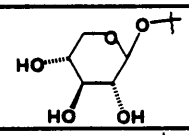
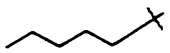
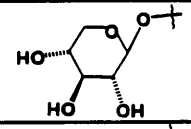
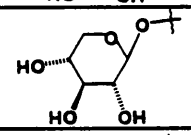
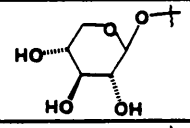
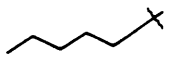
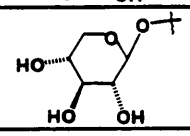
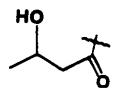
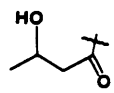
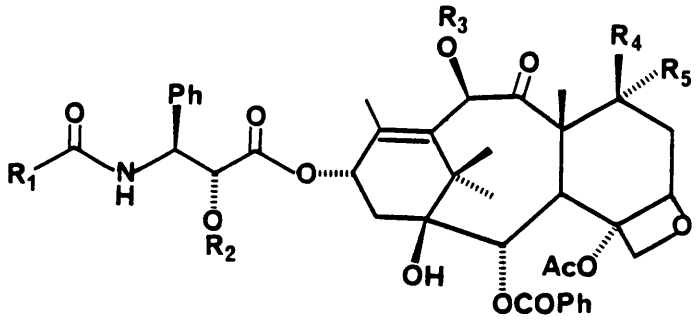
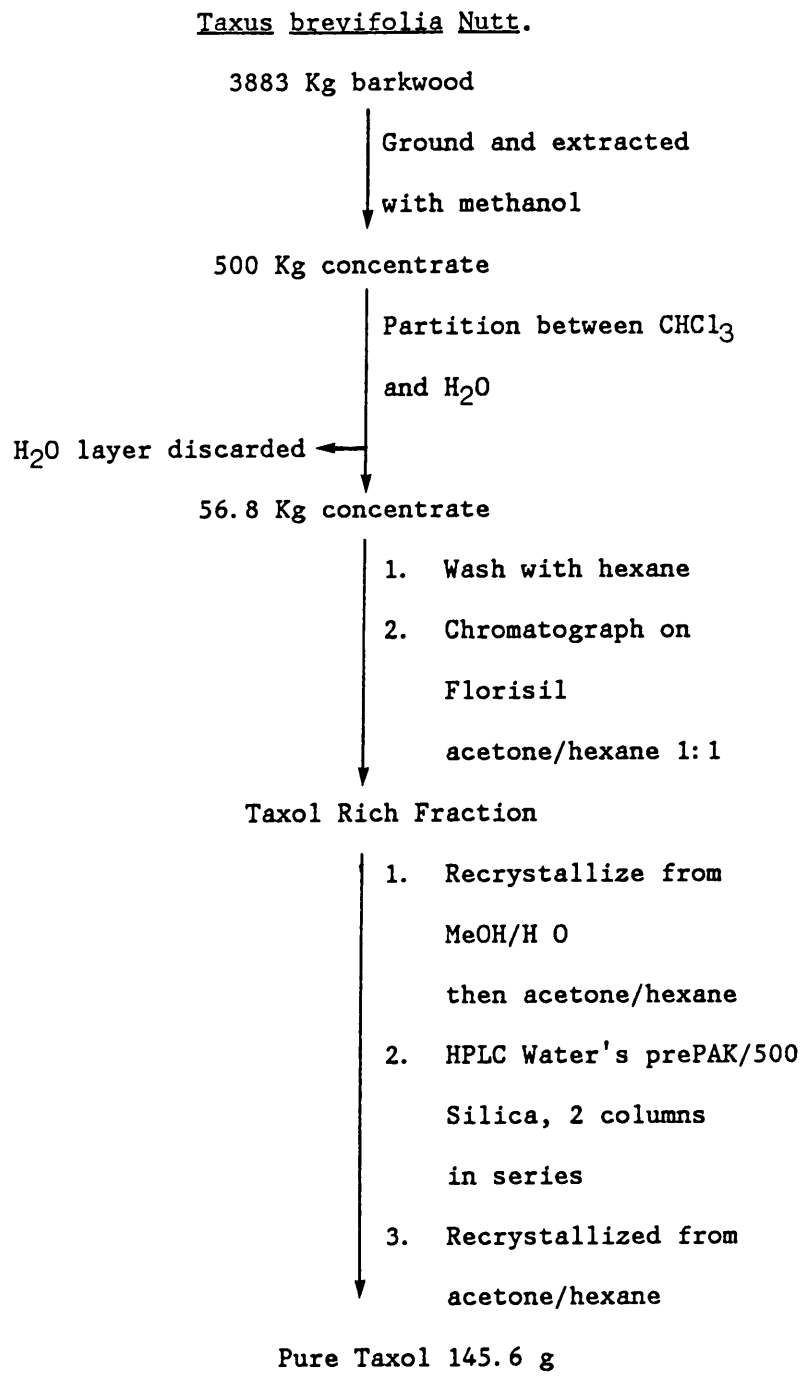
Compound	R_1	R_2	R_3
12	Ph	H	
13		H	
14		H	
15	Ph	Ac	
16		Ac	
17		Ac	
18			H
19	Ph		H

Table 2. Recently Isolated Taxol-like Compounds

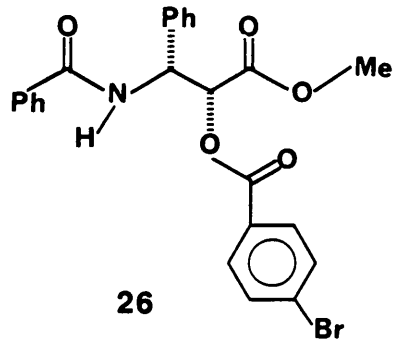


COMPOUND	R_1	R_2	R_3	R_4	R_5
20	Ph	Ac	Ac	OH	H
21	Ph	Ac	H	OH	H
22		H	H	OH	H
23	Ph	H	H	OH	H
24	Ph	H	H	H	OH
25		H	H	H	OH



Scheme 1. Large Scale Isolation of Taxol from Taxus brevifolia

of an iodoacetyl derivative of baccatin III and on the methyl ester p-bromobenzoate of the side chain of taxol **26**.



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The structure of taxol along with the numbering of the skeleton and rings is shown in Figure 1 on page 13. It is impossible to give a precise description of taxol's three dimensional shape as it is quite complex but the gross structure is that of a cup with the beta face being convex and the alpha face concave. On a smaller scale the A ring is in a boat form with positions 13 and 15 pointing up. The B ring is a boat, chair form with positions 11, 15, 1, 2, and 3 forming the chair with position 2 pointing down; the rest of the B ring is in a boat form with position 9 pointing up. The C ring is in a boat form with positions 6 and 3 pointing down.

Once isolated, taxol was found to be a potent antitumor agent possessing activity in a wide range of cancer assays, as shown in Table 3 .[20]

Having been shown to be very active in anticancer screening tests and not to cause an unmanageable level of side effects taxol was approved for Phase I clinical trials. In

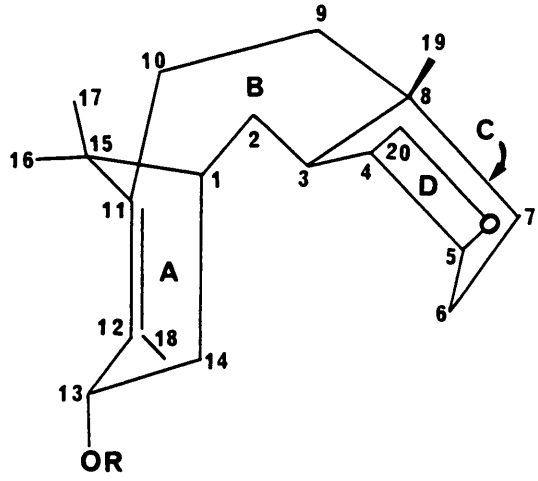

Figure 1. Three Dimensional Structure of the Taxol Skeleton

Phase I clinical trials the maximum nontoxic dosage for a drug is determined. Taxol passed the Phase I trials and currently is in Phase II clinical trials in which the protocol for a drug is optimized.

1.4 TAXOL'S MECHANISM OF ACTION

The importance of taxol as an anticancer drug lies not only in its activity in the tumor assays but also in its unique mechanism of action. In order to understand taxol's mechanism of action knowledge of what takes place during cell division is necessary as this is where taxol exerts its effect.

Cell division involves the synthesis of nucleic acid and other proteins, replication of the DNA, division of the nuclear material and formation of two new cells. In the discussion of taxol a structure called the mitotic spindle will be of primary interest. The mitotic spindle is composed of threads of protein which are polymers of the protein tubulin (alpha and beta tubulin). The mitotic spindle is responsible for pulling the pairs of chromosomes apart during mitosis (Figure 2). After the chromosomes have been separated and before the cell has finished dividing the microtubule polymers must depolymerize so that the mitotic spindle is destroyed.

Investigation of the mechanism of the biological activity of taxol showed that it acted as a mitotic spindle poison.[21] Further investigation showed that taxol acted by increasing the extent to which microtubule polymers form and by preventing the microtubule polymer from disassembling once formed.[22,23] In *in vitro* tests it was found that tubulin will polymerize in the presence of taxol alone, without the presence of exogenous guanine 5'-triphosphate or microtubule associated proteins, materials which had previously been necessary for the polymerization of tubulin *in vitro*.[24,25] Taxol was found not only to promote microtubule polymer formation but to bind to the polymers formed in a ratio of one mole of taxol to one mole of tubulin dimer (one mole each of alpha and beta tubulin).[26,27]

Table 3.

In Vivo Activity of Taxol

Assay	Test/Control
3PS31 P-388 Lymphocytic leukemia	190 + (a)
3B131 B-16 Melanocarcinoma	226 ++ (a)
3C361 Colon 26	161 + (a)
3LE21 L-Lymphoid leukemia	139 + (a)
3C2G5 CX-1 Colon xenograft	-23 + (b)
3C9G5 CX-5 Colon xenograft	-31 + (b)
3LKG5 LX-1 Lung xenograft	+ (b)
3MBG5 MX-1 Breast xenograft	++ (B)

(a) - These tests measure the ability of a drug to prolong life in mice: numbers >125 are considered to correspond to active drugs.

(b) - These tests measure the ability of a drug to limit the growth of human tumors in mice: numbers <42 are considered to correspond to active drugs.

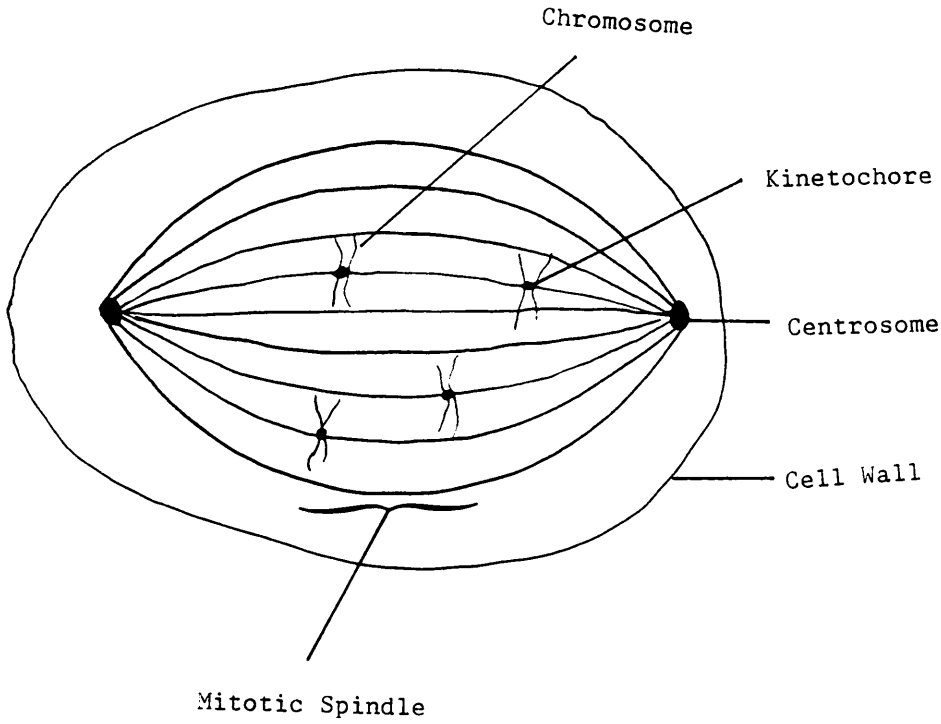

Figure 2. Mitotic Spindle and Mitosis

It is not yet completely understood how taxol's promotion of the formation of a structure necessary for cell division causes cell division to stop. Research has been conducted which suggests that the structures in a cell normally responsible for production and organization of the microtubule polymers - the centrosomes and kinetochores - no longer have the power to effect the synthesis of the organized spindle required for cell division.[28, 29, 30] It has also been shown that the growth of spindles in the presence of taxol during anaphase when the chromosomes are separating, and when spindles normally have stopped forming, can push the nuclear material back together thereby temporarily reversing cell division.[31]

Taxol's mechanism of action is unique. While there are other anticancer drugs such as vincristine, vinblastine, podophyllotoxin and colchicine which exert their activity by interacting with microtubule polymer formation, these other drugs work by destroying the microtubule polymers. Taxol is the only drug that works by promoting the formation of these polymers. It is this uniqueness of taxol's mechanism of action that makes it such a promising candidate for clinical use.

1.5 STATEMENT OF THE PROBLEM

The three broad objectives areas that were addressed in this research were:

1. Determination of the factors which contribute to the activity of taxol;
2. Preparation of active water soluble taxol analogues;
3. Finding an active taxol derivative that could be obtained from more abundant taxanes.

The bulk of this investigation was comprised of determining the factors which contribute to the activity of taxol. Portions of taxol that were modified include:

1. 2'-Hydroxyl and 7-hydroxyl groups
   a. Substitution
   b. Oxidation
   c. Stereochemistry changes
2. Oxetane ring
3. Ester side chain

Determination of the structure activity relationships of taxol will, in the process of modification, yield information about taxol's chemistry. It may also indicate what reaction or reactions taxol undergoes *in vivo* and make possible the

preparation of a less complex anticancer drug with taxol's mechanism of action.

The preparation of a water soluble taxol analogue is important for clinical administration of the drug. Taxol is not water soluble and so must be given in conjuction with emulsifying agents. In its Phase I clinical trials taxol itself did not show excessive toxic effects but severe allergic reactions were caused by the emulsifiers with which taxol is given. In one case the shock caused by allergic reaction caused the death of a patient.[32] If taxol did not possess a unique mechanism of action it might not have been approved for Phase II clinical trials because of this problem of formulating the drug.

As has previously been stated, on a large scale taxol is available from the bark of Taxus brevifolia in only 0.008% yield and the cost of the isolation is at least one thousand dollars per gram. There are compounds obtained from the yew plant in much higher yields than taxol, such as O-cinnamoyl taxicin-I triacetate. From the information gained in this research it may eventually be possible to modify these natural products so that they are active.

1.6 GENERAL EXPERIMENTAL TECHNIQUES

Common experimental techniques carried out in this project will be addressed here and are valid for the experimental procedure sections of all chapters.

1.6.1 SYNTHESIS

Reactions requiring anhydrous conditions were carried out by:

1. Drying reagents, including taxol, in vacuo at room temperature for several hours;
2. Carrying out reactions under an argon or nitrogen atmosphere;
3. Transferring solutions and liquids via syringe;
4. Using dried solvents.[35] A typical drying procedure involved stirring with CaH_2 overnight, fractional distillation, and storage over molecular sieves in a septum sealed brown glass bottle.

The term 'usual work up' means: 1. Dilution of the reaction mixture with several mL of an organic solvent (usually CH_2Cl_2), 2. Washing with 1.0 N HCl, 5% $NaHCO_3$ and water, 3. Drying with $MgSO_4$, 4. Filtering through a cotton plug in a

Pasteur pipette and, 5. Evaporation of the solvent on a rotary evaporator.

The taxol used in this project was isolated by Polysciences Inc. for the National Cancer Institute. The final step in the purification of taxol by Polysciences (Scheme 1) involved recrystallization; the mother liquor from this procedure was an impure mixture of taxol and cephalomannine. This sample was occassionally used in this project to investigate reactions where a product was not to be isolated and is referred to as a 6/4 mixture of taxol and cephalomannine.

1.6.2 ISOLATION AND CHARACTERIZATION

Analytical chromatography was carried out using E. M. Reagents, Silica Gel 60 F_{254}, aluminum backed, 0.2 mm thickness tlc plates. Preparative thin layer chromatography was carried out using ANALTECH silica gel GF 20 x 20 cm, 1000 µm thickness plates. All compounds were visualized using a 254 nm UV light. High pressure liquid chromatography (hplc) analysis and isolation work used a Waters Associates Model M-6000A pump, a Glenco injection valve and a Waters Associated Model 441 Absorbance Detector at 254 nm. For preparative hplc an E. Merck column, 250 mm x 10 mm, LiChrosorb RP-8, 10 µm was used. Crude samples, purified by preparative hplc, were first dissolved in a minimal amount of CH_2Cl_2; injection volumes did not exceed 30 µL. Analytical hplc was carried

out using an Alltech 25 cm x 4.6 mm RP-8 10 µm column or a Waters Associates Radial Pak RLM-100 RP-8, 10 µm cartridge system.

The nmr spectra for the modified taxols were obtained on a Bruker WP 270 spectrometer, operating at 270 MHz for proton spectra and 67.93 MHz for carbon spectra. The spectra were obtained at room temperature in $CDCl_3$ solution and $CDCl_3$ was used for calibrating the spectra 7.24 ppm for proton spectra and 77.0 ppm for carbon spectra.

The mass spectra were all obtained by the use of the fast atom bombardment (FAB) method, and most were obtained by the Midwest Center for Mass Spectrometry at the University of Nebraska; the remainder were obtained by the Middle Atlantic Mass Spectrometry Laboratory at Johns Hopkins University School of Medicine. The notation RCOOH will be used in tables of mass spectral data and corresponds to the C-13 ester side chain as an acid.

Infrared spectra were obtained using a Perkin-Elmer 710B infrared spectrometer. All samples for infrared analysis were prepared as KBr pellets. Hydroxyl and carbon hydrogen stretchings were not recorded as complete elimination of water from KBr was impossible and the C-H stretching in taxol and its derivatives is too weak to be of any value for structure determination. The notations s, m, w and sh will be used in the infrared spectral data tables and correspond to strong, moderate, weak and shoulder.

2.0 C-2' AND C-7 SUBSTITUTIONS AND EPIMERIZATIONS OF TAXOL

2.1 INTRODUCTION

The purpose of the work presented in this chapter is the determination of some of the factors which control the bioactivity of taxol. The fundamental rationale for attempting a modification of a bioactive compound for determining its structure-activity relationships is to choose a modification that changes the compound in such a way that the chemical reactivity or shape of the molecule is changed.

Before this project was begun almost no research had been published on preparing modified taxols. Initial inspection of taxol showed two groups which were candidates for modification: the C-2' and C-7 hydroxyl groups (Figure 3).

The C-2' hydroxyl group is of interest for two reasons. The first of these is that the side chain of taxol is necessary for activity; the natural product baccatin III (taxol without the side chain) possesses only a fraction of the activity of taxol.[33] Modifying the side chain therefore may modify activity. Secondly, the ester linkage joining the side chain to taxol is unusually susceptible to basic hydrolysis and this is presumably due to the C-2' hydroxyl group,[18] so that modifying this group may well change the chemical reactivity of taxol.

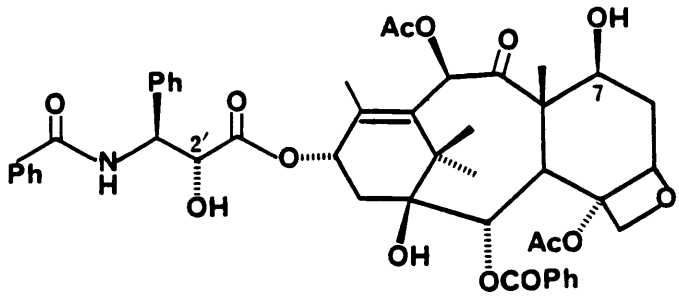

Figure 3. C-2' and C-7 Hydroxyl Groups of Taxol

The C-7 hydroxyl group is of interest as it has been shown that under basic conditions one transformation taxol undergoes is C-7 epimerization. The C-7 position therefore is a chemically reactive one and modifying it may change taxol's biological activity.

Modifications of the C-2' and C-7 positions were divided into two classes. The first of these was substitution reactions in which acetyl or silyl groups were added. These transformations, as expected, were straightforward and were among the first reactions carried out in this project. The second type of modification to be carried out was epimerizations of the C-2' and C-7 positions. These transformations required more sophistication and these reactions were carried out towards the end of the taxol project.

2.2 RESULTS AND DISCUSSION

2.2.1 SUBSTITUTION OF C-2' AND/OR C-7

Acetylation of taxol in pyridine with one equivalent of acetic anhydride for two hours at room temperature yielded 2'-acetyltaxol in nearly quantitative yield* (Scheme 2). Acetylation of both the C-2' and C-7 hydroxyl groups required an excess of acetic anhydride and a reaction time of 24 hours at room temperature. The product 2',7-diacetyltaxol had previously been obtained by Kingston and Ovington[17] via acetylation of a crude extract from the yew plant but this was the first time the reaction had been carried out on pure taxol.

The relatively slow acetylation of the C-7 position with respect to the C-2' position was the first evidence of what became a general rule with taxol - groups directly attached to the taxane skeleton are sterically hindered. The taxol skeleton is complex and contains a great variety of functional groups. There are no positions where a substituent of the skeleton would not have steric interaction with other substituents or with the skeleton itself. In the case of the

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* The term quantitative yield is used when analysis of the crude reaction by tlc or hplc showed only one product; isolated yield were always less than quantitative due to loss from handling milligram amounts of sample during work up.

C-7 hydroxyl group the major steric interactions come from the fact that C-7 is a 'neopentyl' center, and the presence of the adjacent oxetane ring (Figure 4 on page 26).

The structure assignment of 2'-acetyltaxol was made employing ^{1}H-nmr, mass and infrared spectrometries. Of these, ^{1}H-nmr was the most powerful, and this was true for all taxol derivatives in this project. Correct interpretation of the spectrum of 2'-acetyltaxol required a detailed understanding of the ^{1}H-nmr spectrum of taxol. The ^{1}H-nmr spectrum of taxol is shown in Figure 5.

The spectrum of taxol is divided into three major areas: from 1.0 to 2.5 ppm the three proton singlets for the methyl group and acetates are seen, from 2.5 to 7.0 ppm the signals for the protons directly attached to the skeleton and the side chain are seen, and downfield of 7.0 ppm the signals for the aromatic protons are seen with the <u>ortho</u> protons of the C-2 benzoate and the C-3' phenyl amide seen downfield of 7.5

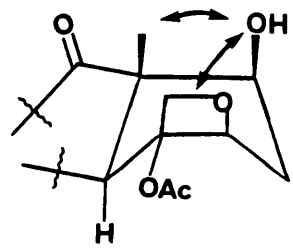


Figure 4. Steric Interactions of the C-7 Hydroxyl Group of Taxol

Scheme 2. Acetylations of Taxol

ppm. This type of pattern is seen for all taxol derivatives in this project and enables one at a glance to look at a proton spectrum and tell if the compound is a taxol-like compound.

Peak shapes in addition to chemical shifts and multiplicities were important for the assignment of the spectra of taxol analogues. From 1.0 ppm to 2.5 ppm in the spectrum of taxol the sharpest singlets are due to the acetate signals. Signals for the methyl groups at C-18 and C-19 are downfield of the signals for the geminal dimethyl groups of C-16 and C-17. Long range coupling causes the signal for the protons at C-18 to be broader than that for C-19 and the signal for C-17 to be broader than that of C-16. These differences in sharpness of peaks are seen consistently in the spectra of taxol derivatives throughout this research.

Peak shape is also important for peak assignment in the middle portion of the taxol spectrum. The 2.5 - 7.0 ppm section of the taxol spectrum is composed primarily of doublets. Of these doublets there are only three which have similar peaks shape: the signals for the protons at C-3', C-2 and N-H. These signals are widely separated by chemical shift in this and all other taxol derivatives in this project. The other signals in the taxol spectrum possess unique peak shapes. The signal for the proton at C-10 is the only sharp one proton singlet in the spectrum of taxol and the signal for the C-13 proton is the only triplet. The signals

for the C-3' and C-7 protons are both doublets of doublets but the C-3' signal is characterized by a relatively large and a small coupling constant while the coupling constants for C-7 do not differ greatly for any taxol derivative. The signal for C-5 is the broadest doublet in the taxol spectrum and even though for some taxol derivatives it is seen as a doublet of doublets its gross shape is constant. The signal for C-2' for taxol is the most intense of the doublet signals, possessing only a 2 Hz coupling constant. The signals for the C-20 oxetane protons form the only AB quartet seen in the taxol spectrum. In the aromatic region of the taxol spectrum the ortho protons of the C-2 benzoate are seen downfield of the ortho protons for the C-3' phenyl amide.

The characterization data for 2'-acetyltaxol are shown in Table 4 and the proton spectrum is shown in Figure 6 on page 31. The only gross change in the proton spectrum of 2'-acetyltaxol, as expected, is that the signal for the C-2' proton is shifted downfield from 4.78 ppm (d, J=3) in taxol to 5.51 ppm (d, J=3). The mass spectrum of 2'-acetyltaxol (fast atom bombardment, methanol/thioglycerol matrix) shows peaks at m/z 896 $(MH)^+$ and 836 $(MH-H_2O)^+$ indicating a molecular weight of 895 corresponding to the addition of one acetate to taxol. Peaks occurring at m/z 569 $(MH-RCOOH)^+$ and m/z 509 $(MH-RCOOH-HOAc)^+$, where RCOOH is the ester side chain lost as an acid, indicate the addition of the acetate occurred on the side chain. The infrared spectrum of

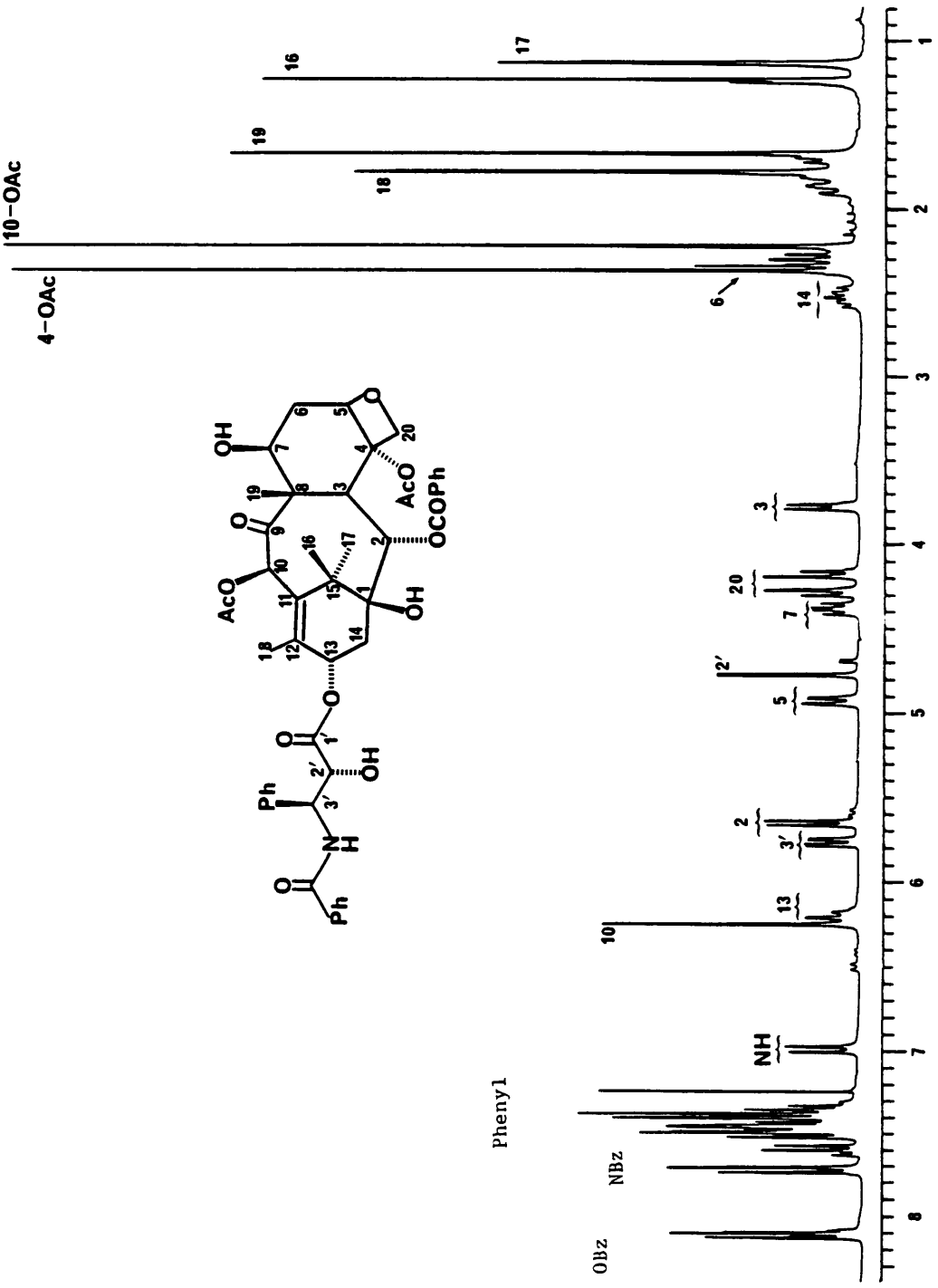


Figure 5. 270 MHz ^{1}H-NMR Spectrum of Taxol.

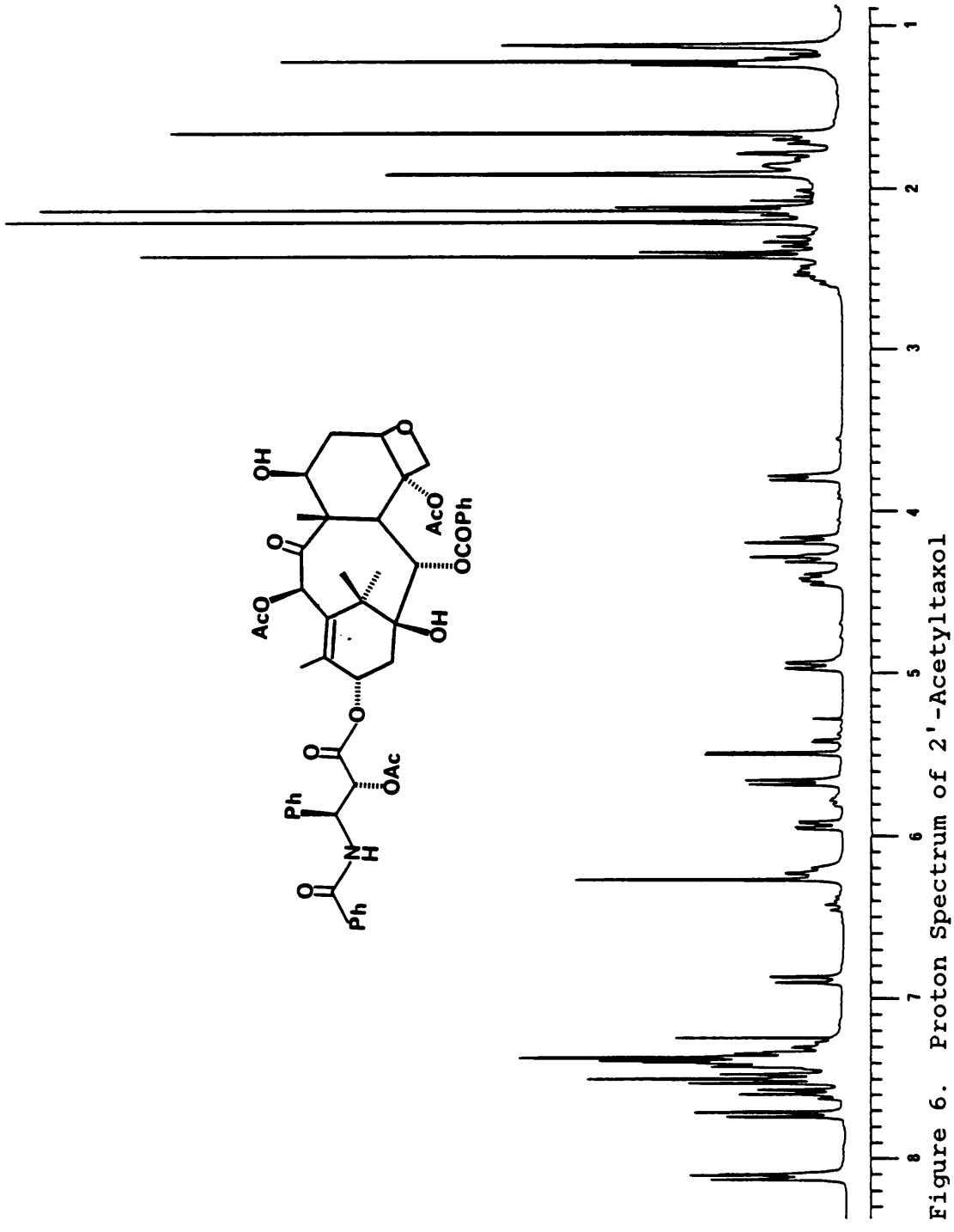
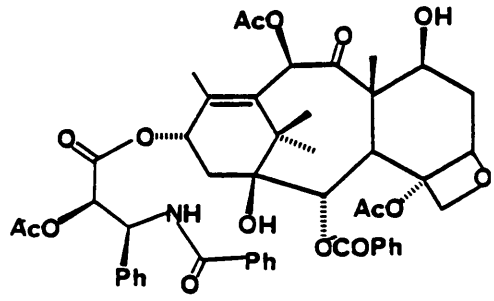


Figure 6. Proton Spectrum of 2'-Acetyltaxol

Table 4 Characterization Data for 2'-Acetyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.69 (d, 7)
3	3.81 (d, 7)
5	4.97 (dd, 2,7)
6	2.3-2.5 (m)
7	4.43 (dd, 6.5,10)
10	6.29 (s)
13	6.25 (br t, 8)
14	2.3-2.5 (m)
16	1.14 (s)
17	1.27 (s)
18	1.68 (s)
19	1.93 (br s)
20	4.15 (d, 8) 4.24 (d, 8)
2'	5.51 (d, 3)
3'	5.95 (dd, 3,9)
N-H	6.88 (d, 9)
OAcs	2.16 (s), 2.23 (s), 2.38 (s)
3' NBz	7.74 (d, 7), 7.4 (m)
2 OBz	8.10 (d, 7), 7.4 (m)
3' Ph	7.4 (m)



Mass Spectral Data

896 $(MH)^+$, 836 $(MH-H_2O)^+$,

569 $(MH-RCOOH)^+$,

509 $(MH-RCOOH-HOAc)^+$,

105 $(C_7H_5O)^+$.

RCOOH - ester side chain

Infrared Spectral Data

1740 s, 1675 m, 1535 w,

1465 w, 1385 m, 1245 s,

1080 m, 990 m.

s-strong, m-moderate,

w-weak

2'-acetyltaxol does not differ significantly from that of taxol.

Although the only major change in the spectrum of 2'-acetyltaxol when contrasted with the spectrum of taxol was seen for the C-2' proton signal the proton spectrum of a mixture of taxol and 2'-acetyltaxol in Figure 7 shows that small differences in the spectra are seen throughout. Separate sets of peaks are seen not only for those protons on the ester side chain but for almost all the protons including: C-10, C-2, C-5, C-3, one proton at C-20, C-19 and both acetates.

Analysis of a model of taxol shows that some of these positions are spatially close to C-2' but others are not even remotely connected. The protons at C-19 and C-10 along with the acetate at C-10 are separated from the side chain by the bulk of the taxol skeleton.

An explanation for the minor changes in the nmr spectra seen in Figure 7 is that taxol is a compact molecule and changing any one part of it is going to cause conformation changes throughout. There are two major practical implications of this behavior. A high resolution nmr spectrum of a taxol derivative is analogous to a fingerprint for that compound; no other taxol derivative will have a spectrum that is identical or even differs by only one or two peaks from the original compound. The second implication is that a high resolution nmr spectrum of a taxol derivative is a good mea-

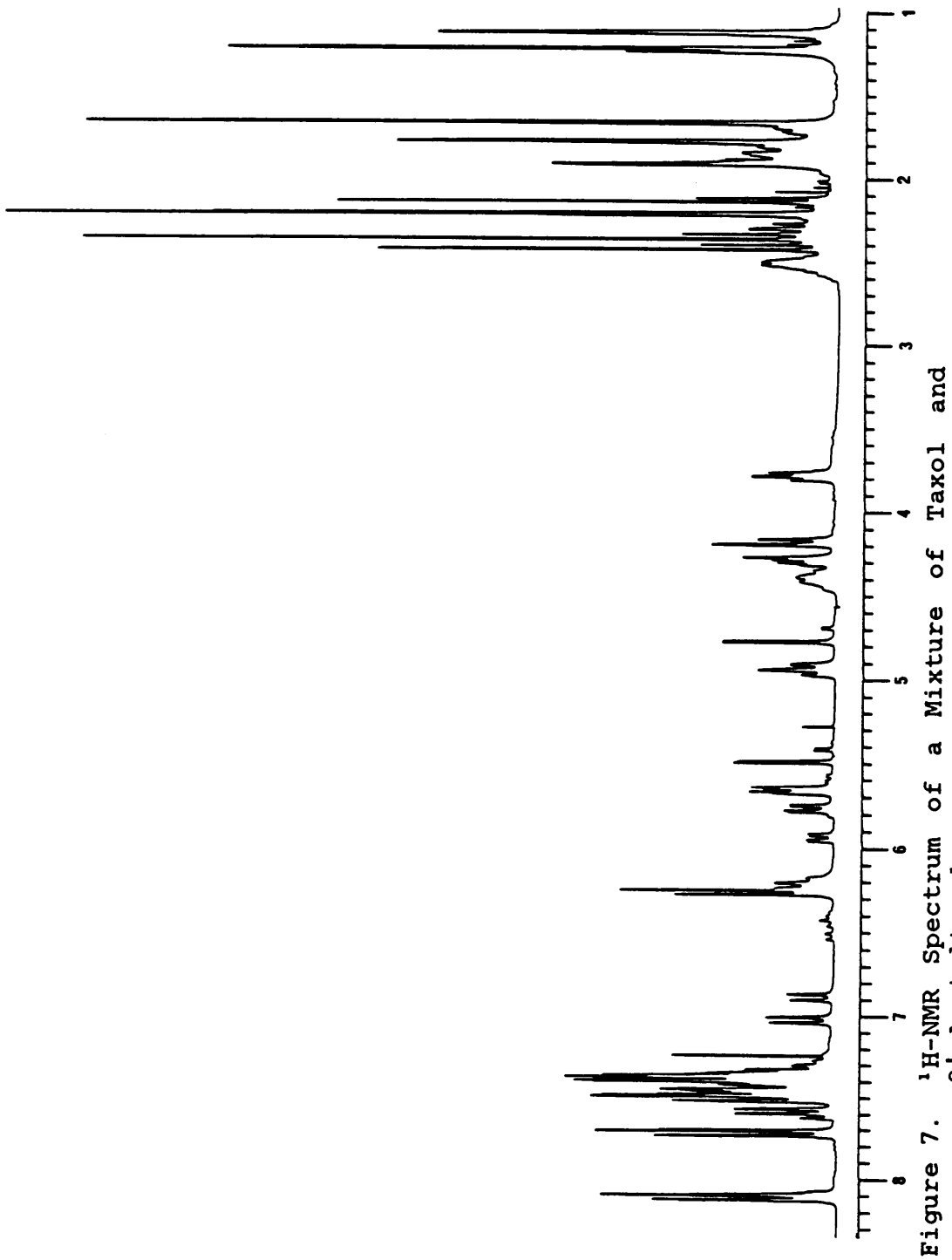


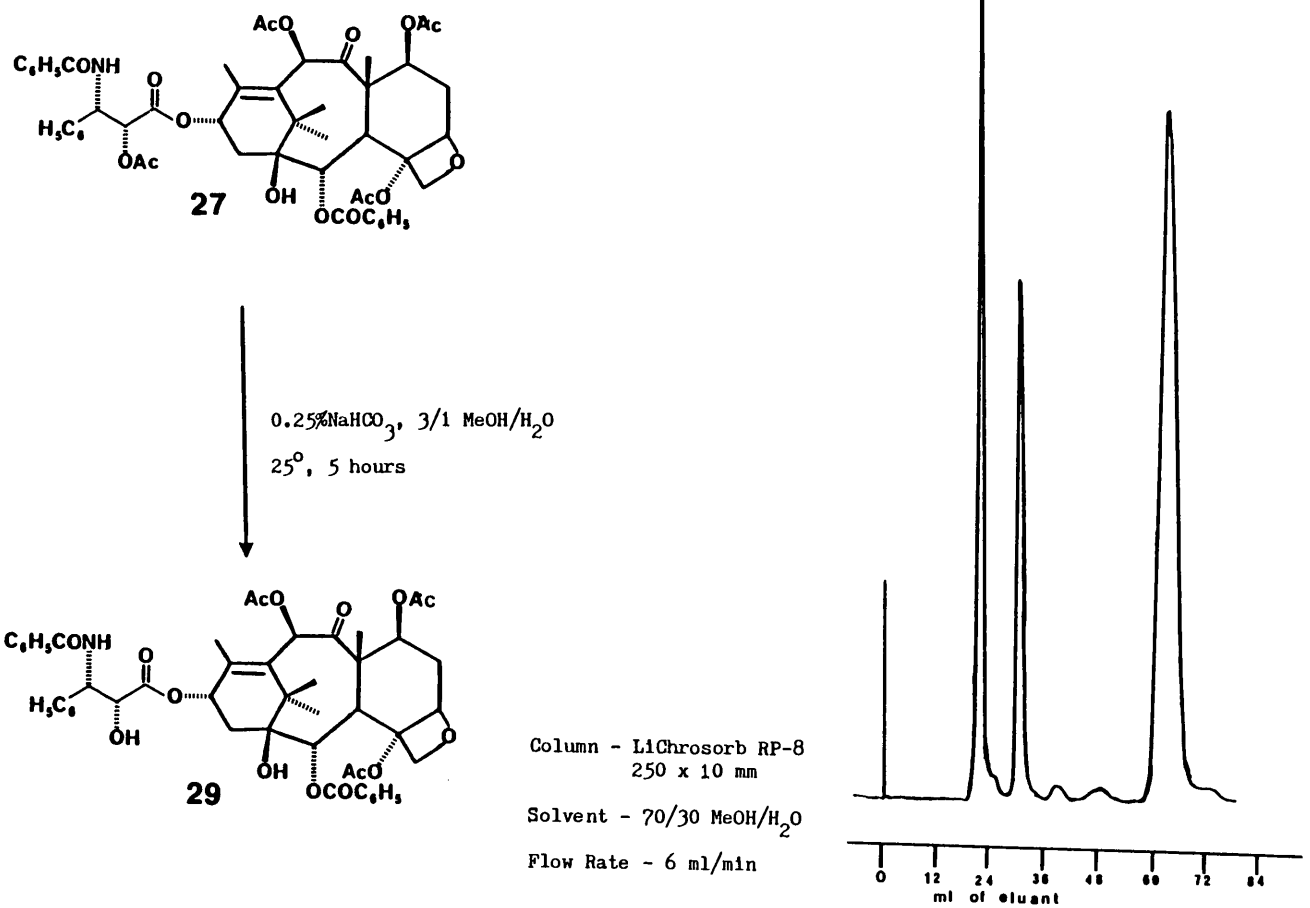
Figure 7. ^{1}H-NMR Spectrum of a Mixture of Taxol and 2'-Acetyltaxol

sure of purity. The spectrum of taxol shown in Figure 5 shows some impurity peaks, yet this sample was characterized as greater than 99% pure by Polysciences Inc.[19] Proton spectra therefore are valid measures of purity for taxol analogues in this project. Elemental analysis, usually used to measure purity, is of little value in determining the purity of taxol derivatives.

After the preparation of 2'-acetyltaxol and 2',7-diacetyltaxol the one remaining taxol acetate to be synthesized was 7-acetyltaxol. Acetylation of the C-1 hydroxyl group was not considered to be a reasonable goal as that group is extremely sterically hindered; it is tertiary, a 'neopentyl' center and has steric interactions with the taxane skeleton.

Synthesis of 7-acetyltaxol was achieved through basic solvolysis of 2',7-diacetyltaxol. A sample of 2',7-diacetyltaxol was subjected to mildly basic conditions (0.25% $NaHCO_3$ 3:1, $MeOH/H_2O$) at room temperature for several hours (Scheme 3). The preparative hplc chromatogram for the reaction is shown in Figure 8. The major products from the reaction were 7-acetyltaxol, 7-acetyl-baccatin III and the methyl ester of the side chain.[18] The two side products resulted from methanolysis of the ester linkage joining the side chain to taxol.

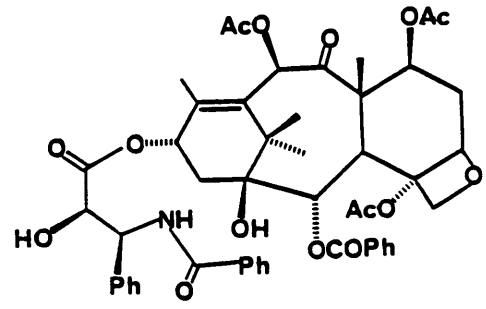
The characterization data for 7-acetyltaxol are shown in Table 5. Characterization of 7-acetylbaccatin III is dis-



Scheme 3. Preparation of 7-Acetyltaxol

Table 5 Characterization Data for 7-Acetyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.66 (d, 7)
3	3.87 (d, 7)
5	4.91 (dd, 8,1)
6	2.2 (m)
7	5.52 (dd, 8,11)
10	6.19 (s)
13	6.15 (br t, 8)
14	2.5 (m)
16	1.16 (s)
17	1.12 (s)
18	1.61 (s)
19	1.81 (br s)
20	4.15 (d, 8), 4.29 (d, 8)
2'	4.78 (d, 3)
3'	5.78 (dd, 3,9)
N-H	7.11 (d, 9)
OAcs	2.31 (s), 2.11 (s), 1.97 (s)
3' NBz	7.74 (d, 8), 7.4 (m)
3' Ph	7.4 (m)
2' OBz	8.09 (d, 8), 7.52 (t, 8), 7.4 (m)



Mass Spectral Data

896 $(MH)^+$, 836 $(MH-HOAc)^+$,

818 $(MH-HOAc-H_2O)^+$,

611 $(MH-RCOOH)^+$,

551 $(MH-RCOOH-HOAc)^+$,

105 $(C_7H_5O)^+$,

286 $(RCOOHH)^+$,

268 $(RCOOHH-H_2O)^+$.

Infrared Spectral Data

1765 s, 1750 s, 1680 m,

1650 w, 1530 w, 1510 w,

1470 w, 1450 w, 1265 s,

1010-1145 mw, 1130 mw,

1080 mw, 1065 mw.

cussed in Chapter 3. The signal for the C-7 proton at 5.52 ppm (dd, J=8,11) is shifted downfield from the C-7 signal of taxol at 4.33 ppm (dd, J=7,10). The peak for the C-2 proton at 4.78 ppm (d, J=3) is similar to that of taxol at 4.71 ppm (d, J=3). The mass spectral data for **29** indicates the same molecular weight as for 2'-acetyltaxol - 895, but the peaks at m/z 611 $(MH-RCOOH)^+$, 551 $(MH-RCOOH-H_2O)^+$ and 551 $(MH-RCOOH-HOAc)^+$ all involving loss of the side chain as an acid (RCOOH) are each 42 mass units higher than the analogous peaks for 2'-acetyltaxol. In the mass spectra for 2'-acetyltaxol and other C-2' acylated derivatives in this project, peaks are seen resulting from loss of the C-2' acyl substituent by matrix solvolysis before analysis; this loss of a C-2' acyl substituent was not seen for 7-acetyltaxol.

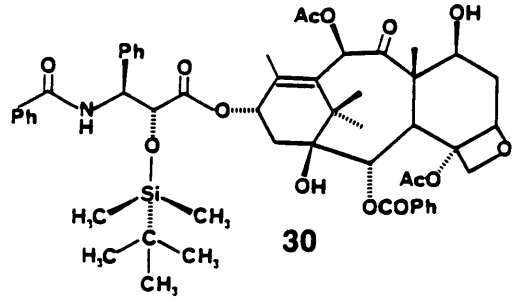
The occurrence of the side reaction did shed light on the reactivity of the C-1' ester linkage. Monitoring of the solvolysis of 2',7-diacetyltaxol showed that in the initial stages of the reaction cleavage of the C-1' ester was not taking place. During the later stages of the reaction C-1' ester cleavage could be seen and the rate of reaction increased as the concentration of 7-acetyltaxol increased. The significance of this reactivity data is that cleavage of the C-1' ester linkage was not taking place on diacetyltaxol but rather on 7-acetyltaxol. This supports the hypothesis that the sensitivity of the C-1' ester linkage to nucleophiles is due to the presence of the alpha hydroxyl group. This be-

havior is of importance to this structure activity relationship study as the alpha hydroxy ester group of taxol may react with a nucleophile *in vivo* as part of taxol's mechanism of action.

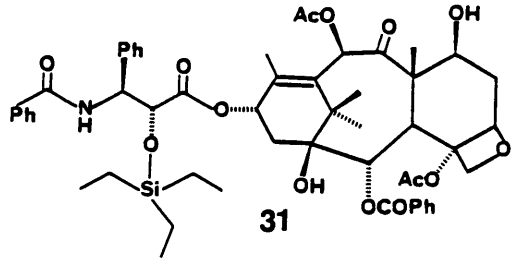
In addition to C-2' acetylated taxol the C-2' silyl ethers, 2'-triethylsilyltaxol **31** and 2'-tertiarybutyldimethylsilyltaxol **30**, were prepared from taxol using imidazole as catalyst and the respective silyl chlorides. The products were purified by preparative tlc or reverse phase preparative hplc. These taxol derivatives were designed to be final products as opposed to C-2' protected taxol intermediates. The biological activity data for 2'-acetyltaxol will raise a question about the possibility of the C-2' acetyl group being removed when the compound is tested *in vivo*. The silyl ether taxol derivatives will give additional information about the importance of the C-2' position to activity as it would be expected that these groups would not be easily removed even under *in vivo* conditions.

Both of the taxol silyl ethers were chromatographically homogenous and ^{1}H-nmr showed each to be a pure taxol-like compound. Even before purification 2'-triethylsilyltaxol was the only ultraviolet absorbing compound seen on tlc while 2'-tertiarybutyldimethylsilyltaxol contained a small amount of a less polar ultraviolet absorbing compound which probably was the disilylated derivative. The only major changes seen in either ^{1}H-nmr spectrum was the presence of the peaks for

the silyl moiety protons at 0.80 ppm (s), -0.04 ppm (s) and -0.29 ppm (s) for 30 and 0.57 ppm (m) and 0.90 ppm (m) for 31. The signal for the C-2' proton in each derivative was also shifted upfield slightly from 4.71 ppm (d, J=3) in taxol to 4.62 ppm (d, J=3) for **30** and 3.91 ppm (d, J=3) for **31**. The mass spectrum of **31** showed peaks at m/z 990 $(MNa)^+$ and 968 $(MH)^+$ indicating the addition of one triethylsilyl group. The mass spectrum of **30** indicated the molecular weight of the compound as 967 by peaks at m/z 968 $(MH)^+$ and 908 $(MH-HOAc)^+$. A peak at m/z 400 $(RCOOHH)^+$, where RCOOH is the side chain lost as an acid, showed that the silylation occurred on the side chain.



30



31

2.2.2 EPIMERIZATIONS

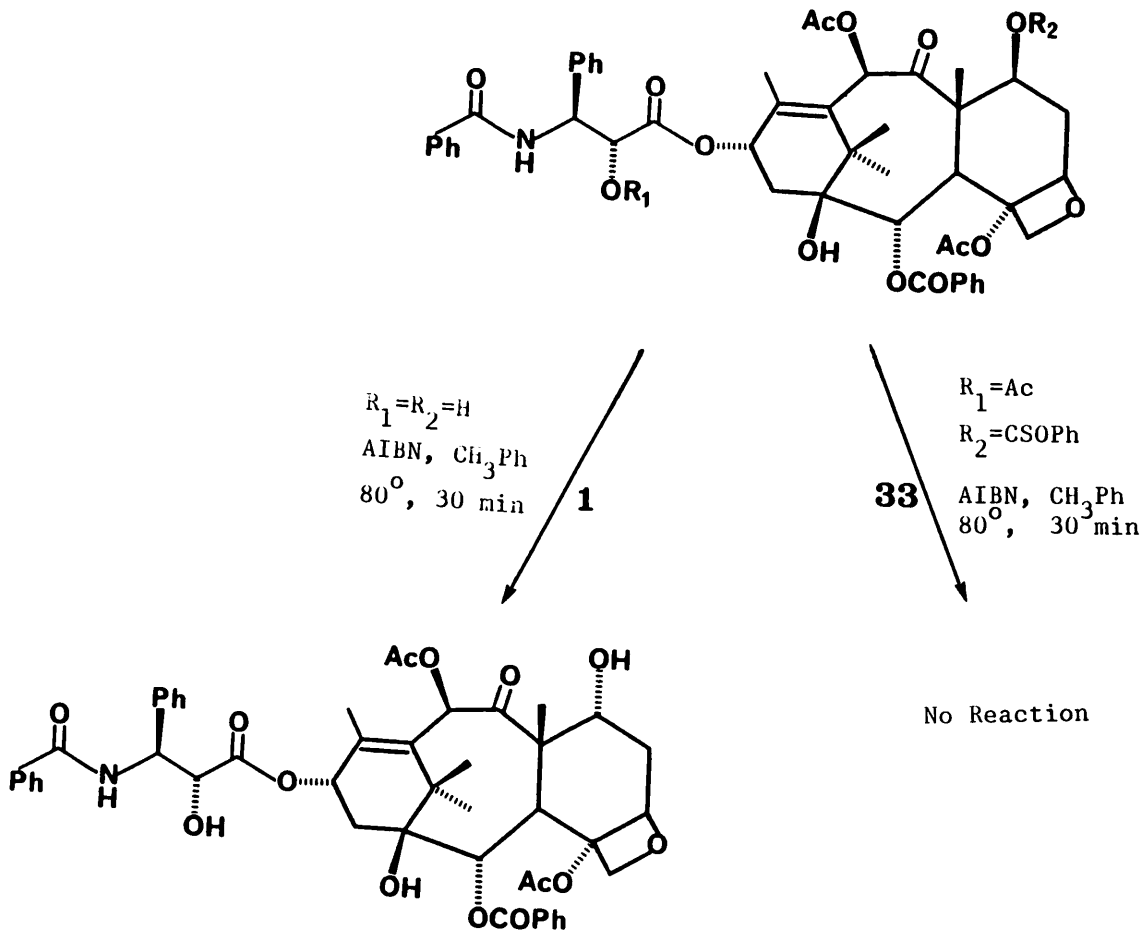
2.2.2.1 C-7 EPIMERIZATION

Epimerization of the 7 position of taxol has been reported to occur under basic conditions ($NaHCO_3$, MeOH).[33] Under these conditions however epimerization of this center was only one of several processes occurring, so that prior to this research 7-epitaxol was an unknown compound. To achieve

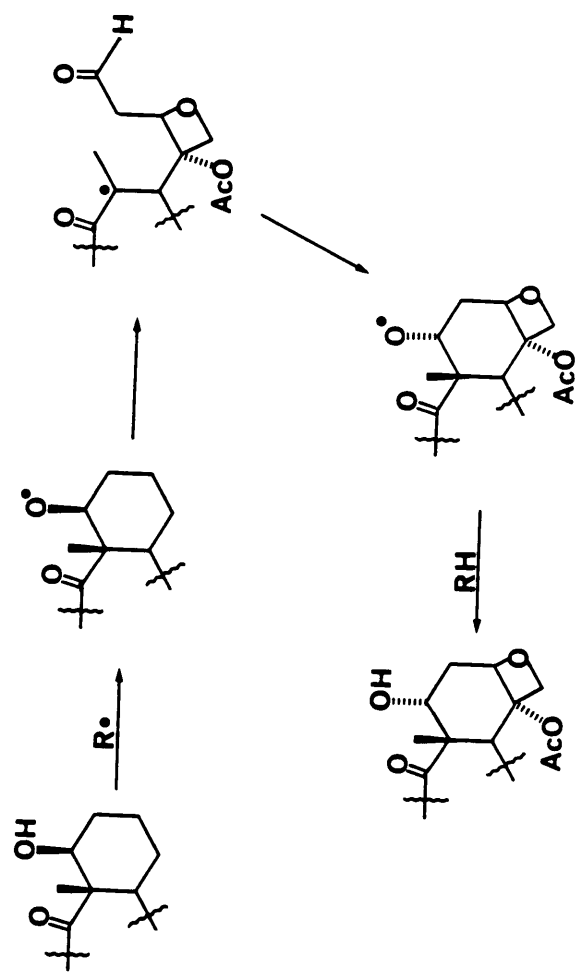
the goal of selective C-7 epimerization of taxol it was necessary to suppress the unwanted side reactions which were all nucleophilic attacks at the ester linkages of taxol. In order to do this a reaction was needed which would epimerize a beta hydroxy ketone but would not affect esters.

The epimerization of C-7 of taxol was effected by heating taxol at 80° in toluene with a catalytic amount of azobis-(isobutyronitrile) for 30 minutes. Under identical conditions a taxol substituted at C-7 with a phenyloxythionyl [33] group did not react at all (Scheme 4). This reactivity data showed that the epimerization of the 7 position did not take place via abstraction of a hydrogen radical from C-7 but rather by a radical analogue of the retro aldol reaction (Scheme 5). Analysis of the crude reaction solution showed 7-epitaxol as the only ultraviolet absorbing compound present. Removal of non-taxane impurities was achieved by preparative tlc. The ^{1}H-nmr of 7-epitaxol showed that it was a taxol-like compound.

The structure assignment of 7-epitaxol was made through comparison of the ^{1}H-nmr spectra of taxol, 10-deacetyl-7-epitaxol, and 10-deacetyltaxol. Contrasting the spectra of 10-deacetyltaxol and 10-deacetyl-7-epitaxol showed three major differences in the spectra. The signal for the C-7 proton is shifted upfield from 4.18 ppm (m) for 10-deacetyltaxol to 3.66 ppm (br t, J=3,12) for 10-deacetyl-7-epitaxol, the signals for the C-20 protons change from an AB quartet 4.25



Scheme 4. 7-Epimerization of Taxol Under Radical Conditions



Scheme 5. Possible Mechanism of the 7-Epimerization of Taxol Under Radical Conditions

ppm, to a broad singlet 4.39 ppm and the signal for the C-10 proton is shifted downfield from 5.20 ppm (s) to 5.42 ppm (s). The signal for C-5 in 10-deacetyltaxol is a broad doublet (J=6) and in 10-deacetyl-7-epitaxol the signal changes to a triplet.

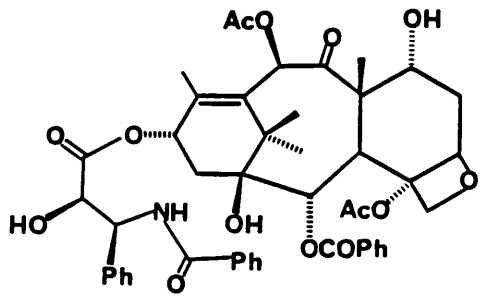
The ^1H spectrum of 7-epitaxol, shown in Table 6, when contrasted with the spectrum of taxol shows the same changes that were seen between the spectra of 10-deacetyltaxol and 10-deacetyl-7-epitaxol. The signal for the C-7 proton in 7-epitaxol 3.67 ppm (br d, J=12) is upfield of the analogous signal in taxol, 4.33 ppm (dd, J=4,11). The C-20 proton signal is seen as a broad singlet at 4.36 contrasted with the AB quartet of taxol at 4.17 ppm (d, J=8), 4.27 ppm (dd, J=8). The signal for C-10, 6.76 ppm (s) is shifted downfield 0.50 ppm from the analogous taxol signal and the coupling constants for the C-5 proton signal have changed from 4.92 ppm (dd, J=2,8) in taxol to 4.89 ppm (dd, J=5,8) in 7-epitaxol.

The mass spectrum of 7-epitaxol shows peaks at m/z 892 $(MK)^+$ 876 $(MNa)^+$ and 854 $(MH)^+$ indicating a molecular weight of 853, the same as that of taxol. Peaks below m/z 650 were not recorded in the spectrum of 7-epitaxol. The infrared spectrum of 7-epitaxol does not differ significantly from that of taxol.

The complete epimerization of C-7 taxol and the conditions necessary to achieve it were in contrast to the reported behavior of 10-deacetyltaxol.[33] The epimerization of 10-de-

Table 6 Characterization Data for 7-Epitaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	6.75 (d, 8)
3	3.90 (d, 8)
5	4.89 (dd, 5,8)
6	2.3 (m)
7	3.67 (br d, 12)
10	6.76 (s)
13	6.22 (br t, 9)
14	2.2 (m)
16	1.17 (s)
17	1.13 (br s)
18	1.77 (d, 1)
19	1.64 (s)
20	4.36 (br s)
2'	4.78 (d, 2)
3'	5.88 (dd, 2,9)
N-H	6.98 (d, 9)
OAcs	2.46 (s), 2.17 (s)
2 OBz	8.17 (d m, 7), 7.4 (m)
3' NBz	7.70 (d m, 7), 7.4 (m)
3' Ph	7.4 (m)



Mass Spectral Data

892 $(MK)^+$,

876 $(MNa)^+$,

854 $(MH)^+$.

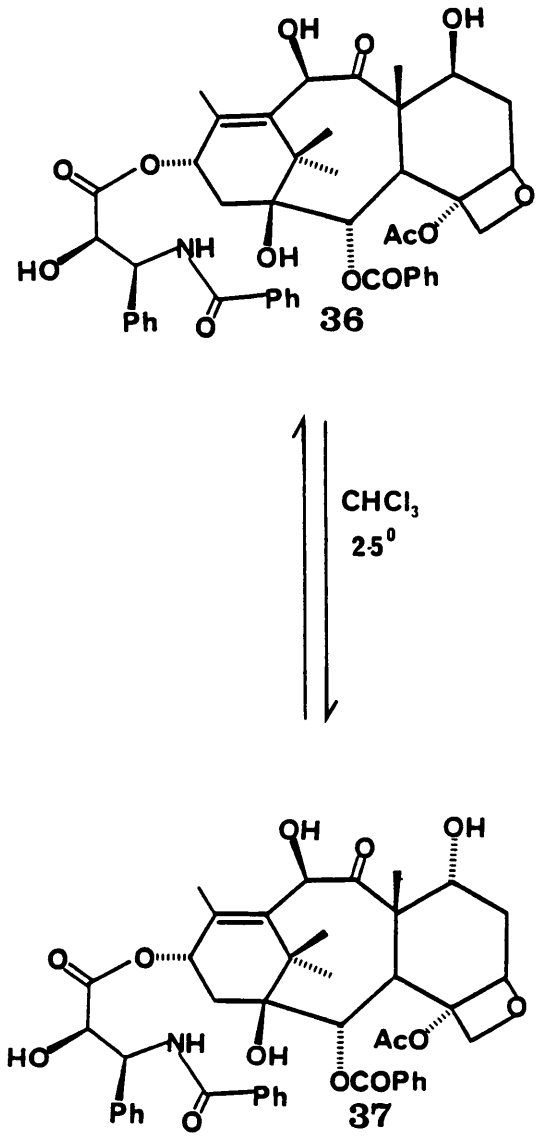
Infrared Spectral Data

1760 s-sh, 1720 s,

1660 m, 1535 w, 1500 w,

1470 w, 1390 m, 1260 s,

1110 m, 1060 m, 720 m.



Scheme 6. 7-Epimerization of 10-Deacetyltaxol

acetyltaxol took place at room temperature upon standing overnight in chloroform solution and the two C-7 epimers present formed an equilibrium mixture (Scheme 6). C-7 epimerization of taxol or of any taxol analogue has not been seen during the five years of this research project.

An explanation for the reactivity of taxol and 10-deacetyltaxol lies in hydrogen bonding between groups at C-9, C-10 and C-7. In taxol hydrogen bonding between the C-7 hydroxyl and the C-9 ketone is not possible because the groups are not close enough and the C-19 methyl group blocks any interaction between C-9 and any beta group at C-7. When 7-epitaxol is formed hydrogen bonding is possible between the C-7 hydroxyl and C-10 ketone and provides the driving force for the reaction. Evidence of the interaction between C-7 and C-9 is seen in the ^{1}H-nmr spectrum of 7-epitaxol in which the signal for the C-10 proton is shifted downfield by 0.5 ppm when contrasted with taxol; the hydrogen bonding also causes 7-epitaxol to be less polar that taxol when seen by tlc.

In 10-deacetyltaxol hydrogen bonding can exist between the C-9 hydroxyl and the C-10 ketone. There are two consequences of this bonding. The hydrogen bonding withdraws electron density from C-10 allowing the epimerization, via a retro aldol reaction, to occur more readily. The hydrogen bonding already present in 10-deacetyltaxol makes the bonding between C-7 and C-10 in 7-epi-10-deacetyltaxol less important so that

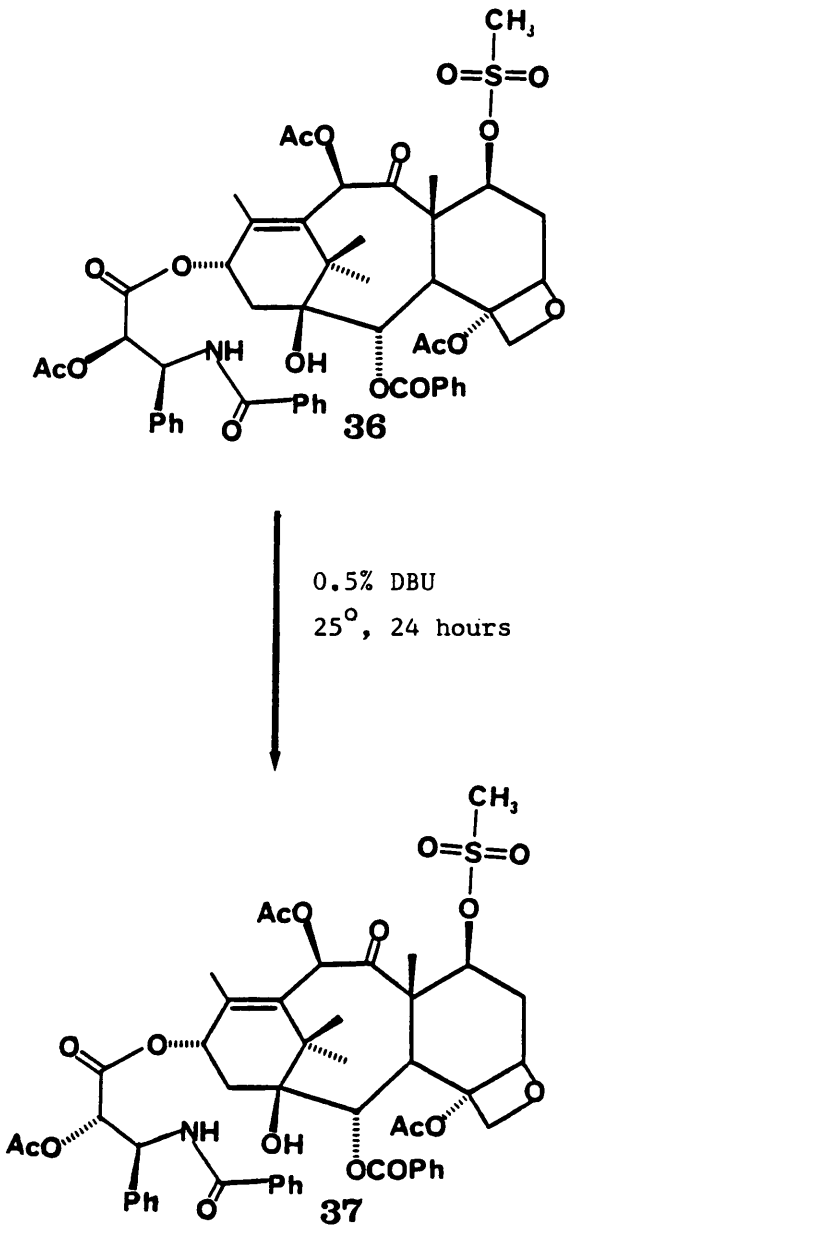
there is less of a driving force for the reaction and the epimers exist as an equilibrium mixture.

2.2.2.2 C-2' EPIMERIZATION OF TAXOL

Epimerization of the C-2' position of taxol was achieved through the reaction of 2'-acetyl-7-methanesulfonyltaxol with 1,8-diazabicycloundecene (DBU) in CH_2Cl_2 at room temperature for 24 hours (Scheme 7). The product 2'-epiacetyl-7-methanesulfonyltaxol was obtained in 16% yield after isolation via tlc (silica gel, 9/2 CH_2Cl_2 /2-butanone) and the starting material was recovered in 4% yield. Information on other products obtained is included in Chapter 7.

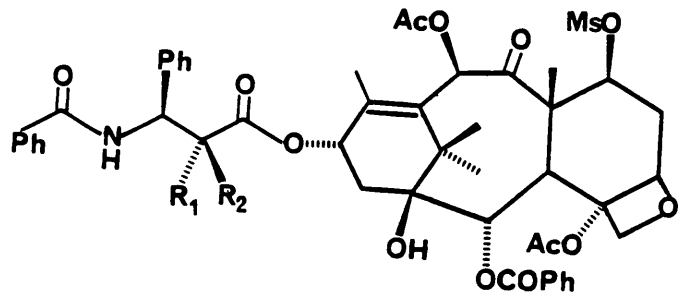
The ^{1}H-nmr spectra of the starting material **36** and the C-2' epimer **37** are shown in Table 7. The two spectra are similar except for the C-2', C-3' coupling constant. In **36** this constant is 3 Hz and in **37** 6 Hz. In all taxol derivatives with aliphatic side chains, with the exception of **37**, the coupling constant was never seen as greater than 3 Hz. A 3 Hz change in this coupling constant therefore is extraordinary and can not be explained without invoking a change in stereochemistry at C-2'.

The mass spectrum of 2'-epiacetyl-7-methanesulfonyltaxol gave peaks at m/z 996 $(MNa)^+$, 974 $(MH)^+$, 914 $(MH-HOAc)^+$ and 587 $(MH-RCOOH-SOCH_2)^+$, where RCOOH is the side chain lost as an acid. The molecular weight, 973, is the same as that of the



Scheme 7. 2'-Epimerization of 2'-Acetyl-7-methanesulfonyltaxol

Table 7 (Part 1 of 2). ^{1}H-NMR Spectra for 2'-Acetyl-7-methanesulfonyltaxol and 2'-Epiacetyl-7-methanesulfonyltaxol



36 R_1=OAc, R_2=H

37 R_1=H, R_2=OAc

Position	Shift (ppm from TMS) Coupling (hertz) 37	Shift (ppm from TMS) Coupling (hertz) 36
2	5.63 (d, 7)	5.68 (d, 7)
3	3.87 (d, 7)	3.92 (d, 7)
5	4.91 (d, 8)	4.92 (d, 9)
6	2.9-3.1 (m)	2.9-3.1 (m)
7	5.30 (dd, 7,10)	5.34 (dd, 7,10)
10	6.39 (s)	6.50 (s)
13	6.11 (br t, 8)	6.19 (br t, 9)
14	2.2 (m)	2.1-2.2 (m)
16	1.19 (s)	1.14 (s)

Table 7 (Part 2 of 2). ^{1}H-NMR Spectra for 2'-Acetyl-7-methanesulfonyltaxol and 2'-Epiacetyl-7-methanesulfonyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)	Shift (ppm from TMS) Coupling (hertz)
17	1.20 (s)	1.23 (s)
18	2.01 (br s)	1.56 (s)
19	1.80 (s)	1.78 (s)
20	4.12 (d, 8), 4.29 (d, 8)	4.16 (d, 8), 4.31 (d, 8)
2'	5.62 (d, 6)	5.52 (d, 3)
3'	5.86 (dd, 6,8)	5.93 (dd, 3,9)
N-H	6.88 (d, 8)	6.91 (d, 9.5)
OAcs	2.40 (s), 2.20 (s), 2.14 (s)	2.41 (s), 2.16 (s), 2.14 (s)
2 OBz	8.09 (d m, 7), 7.61 (t, 7), 7.4 (m)	8.09 (d m, 7), 7.60 (t, 7), 7.4 (m)
3' NBz	7.78 (d m, 7), 7.4 (m)	7.73 (d m, 7), 7.4 (m)
3' Ph	7.4 (m)	7.4 (m)
Mesyl	3.07 (s)	3.09 (s)

starting material. The m/z 587 peak indicated that both the mass of the taxane skeleton moiety and the side chain were not from that of the starting material.

While the C-2' epimer was obtained in low yield there was less 2'-acetyl-7-methanesulfonyltaxol recovered. The C-2' epimer therefore is more stable than the normal stereochemistry, at least when C-2' is acetylated. Newman projections of the C-2', C-3' bond of **36** and **37** are shown in Figure 9.

In order to synthesize 2'-epitaxol a taxol derivative was prepared which was substituted at C-2' and C-7 with a 2,2,2-trichloroethyloxycarbonyl (troc) group. This protecting group is one which can be removed with zinc in acetic acid.[34] It had been used by Senilh et. al. to protect C-2' of taxol, and the literature procedure indicated that at room temperature in pyridine C-2' monosubstitution of taxol took place in 2 hours. In this project however reaction of taxol at 0° in acetonitrile with several equivalents of pyridine and 2,2,2-trichloroethylchloroformate caused disubstitution to take place in 5 minutes (Scheme 8).

After work up 2',7-di(2,2,2-trichloroethyloxycarbonyl)-taxol was homogenous on analytical tlc and ^{1}H-nmr showed the compound to be greater than 95% pure. Because the compound was to be used as an intermediate and not a final product it was not purified further. The characterization data for 2',7-di(2,2,2-trichloroethyloxycarbonyl)taxol are shown in Table 8.

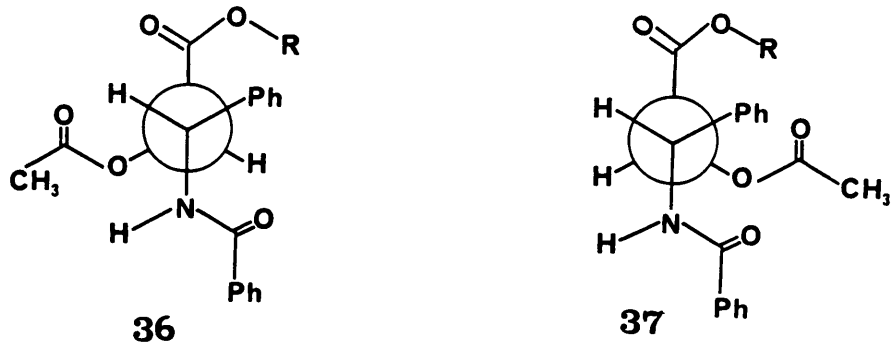


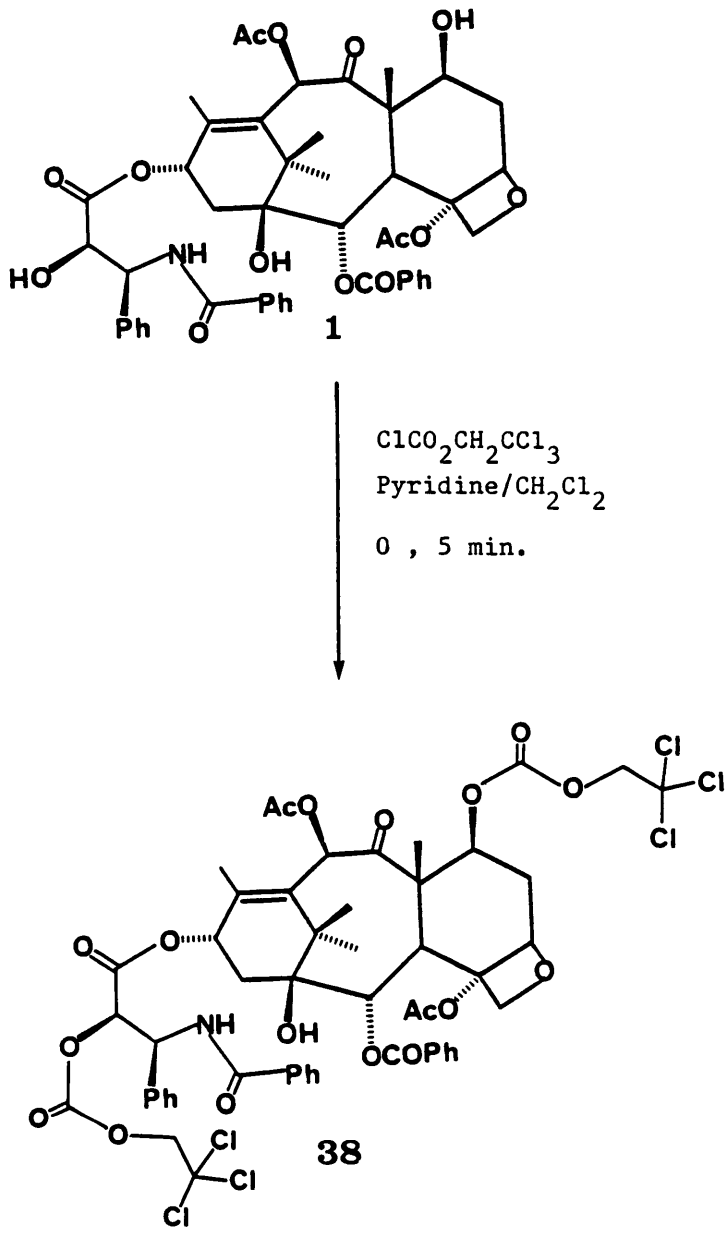
Figure 9. Newman Projections of
2'-Acetyl-7-methanesulfonyltaxol and
2'-Epiacetyl-7-methanesulfonyltaxol

The mass spectrum of 2',7-di(2,2,2-trichloroethyloxycarbonyl)taxol gave peaks at m/z 1202 $(MH)^+$, 1142 $(MH-HOAc)^+$, 1124 $(MH-HOAc)^+$, and 684 $(MH-RCOOH-HOAc)^+$ where RCOOH is the side chain lost as an acid. The molecular weight, 1201, indicated by the mass spectrum corresponds to the addition of two 2,2,2-trichloroethyloxycarbonyl (troc) protecting groups. The m/z 684 peak shows that one protecting group had been added to the taxane skeleton and one to the side chain.

The ^{1}H-nmr spectrum of 2',7-di(troc)taxol shows that the signals for protons at C-2', 5.52 ppm (d, J=3) and C-7, 5.56 ppm (dd, J=7,11) are downfield of the analogous taxol signals at 4.71 ppm (d, J=3) and 4.33 ppm (dd, J=7,11). The assignment of the protons on the C-2' protecting group, 4.80 ppm (d, J=12) and 4.73 ppm (d, J=12) and the C-7 protecting group 5.01 ppm (d, J=12) and 4.62 ppm (d, J=12) was based on comparison of the spectrum of 2',7-di(troc)taxol with those for 7-(troc)baccatin III (Chapter 3) and 2'-(troc)taxol (Chapters 4 and 7). The proton signals for the C-7 protecting group are always seen as two well separated doublets and the signals for the C-2' protecting group are seen as a distorted AB quartet.

The infrared spectrum of 2',7-di(troc)taxol shows the same peaks as taxol with the addition of intense absorbance at 1775 cm^{-1} due to the carbonate carbonyl stretching.

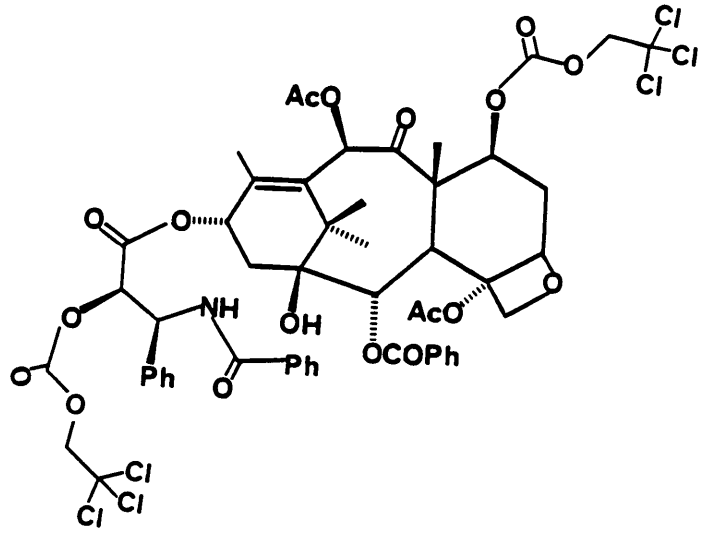
Reaction of 2',7-di(2,2,2-trichloroethyloxycarbonyl)taxol with DBU in methylene chloride at 25°, which was expected to



Scheme 8. Preparation of 2',7-Di(2,2,2-trichloroethyloxycarbonyl)taxol

Table 8 (Part 1 of 2). Characterization Data for

2',7-Di(2,2,2-trichloroethyloxycarbonyl)taxol



Position	Shift (ppm from TMS) Coupling (hertz)
2	5.69 (d, 7)
3	3.95 (d, 7)
5	4.96 (br d, 10)
6	2.4 (m)
7	5.56 (dd, 7,11)
10	6.35 (s)
13	6.24 (br t, 9)
14	2.6 (m)
16	1.20 (s)
17	1.16 (s)

Mass Spectral Data

1202 $(MH)^+$, 1142 $(MH-HOAc)^+$,

1124 $(MH-HOAc-H_2O)^+$,

684 $(MH-HOAc-RCOOH)^+$,

460 $(RCOOHH)^+$,

442 $(RCOOHH-H_2O)$,

105 $(C_7H_5O)^+$.

High Resolution

$(MH)^+$-$C_{63}H_{53}NO_{18}Cl_6$ -

1202.1406

Calculated - 1202.1475

Table 8 (Part 2 of 2). Characterization Data for 2',7-Di(2,2,2-trichloroethyloxycarbonyl)taxol

Position	Shift (ppm from TMS) Coupling (hertz)
18	1.92 (br s)
19	1.82 (s)
20	4.33 (d), 4.18 (d)
2'	5.52 (d, 3)
3'	6.02 (dd, 3,9)
N-H	6.90 (d, 9)
OAcs	2.13 (s), 2.47 (s)
2 OBz	8.12 (d m, 7), 7.60 (t, 7), 7.4 (m)
3' NBz	7.74 (d m, 7), 7.4 (m)
3' Ph	7.4 (m)
2'-troc	4.80 (d, 12), 4.73 (d, 12)
7-troc	5.01 (d, 12), 4.62 (d, 12)

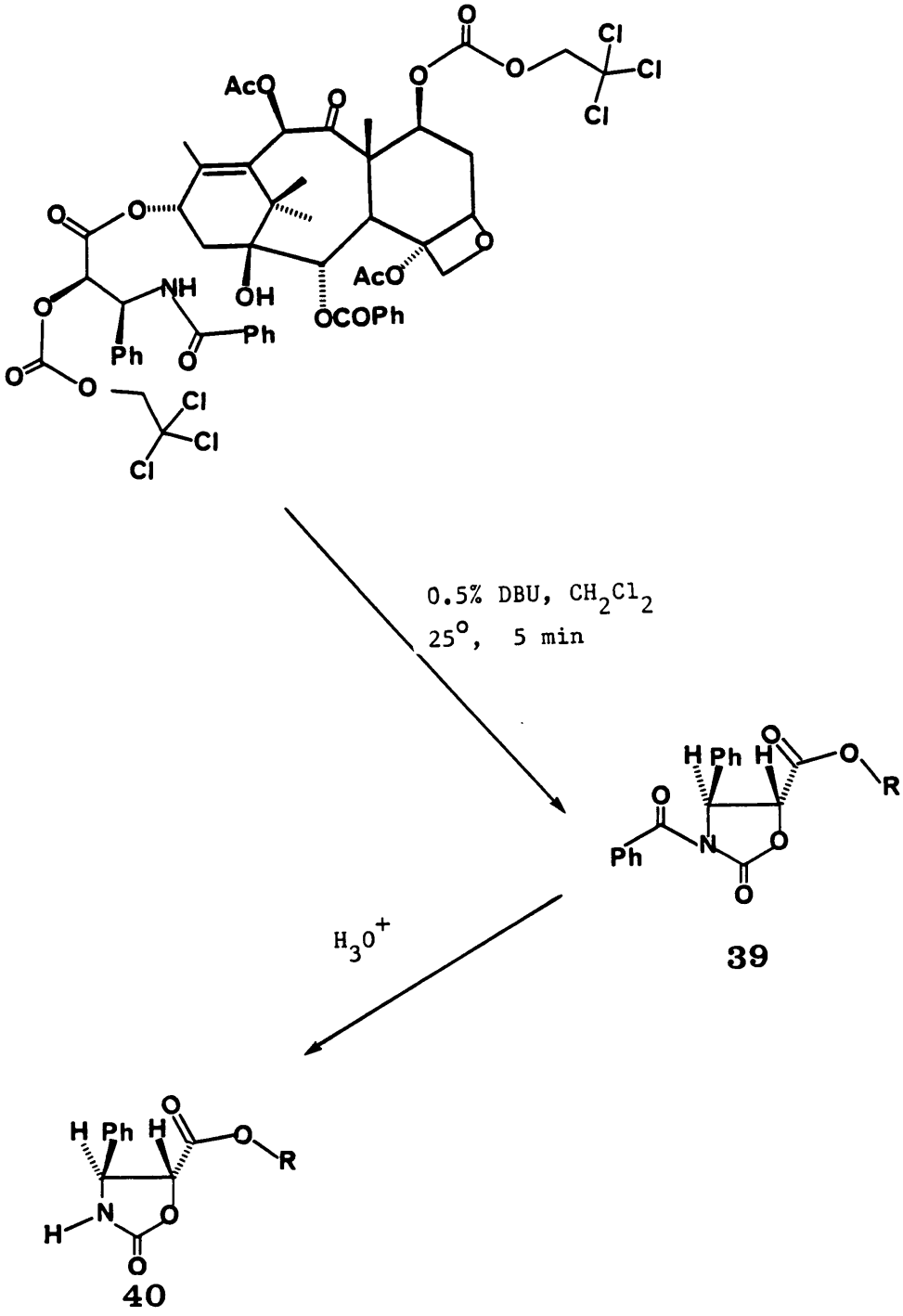
Infrared Spectral Data

1775 s, 1750 ms-sh, 1690 mw, 1675 mw, 1555 w, 1535 w, 1475 mw, 1397 m, 1290 s-sh, 1250 s, 1130-980 m, 830 m, 790 mw, 720 m.

take in excess of 12 hours to consume the starting material, was complete as soon as the reaction was analyzed by tlc (1 minute). At that point one major product was seen by tlc; after work up including washing with 1.0 N HCl an additional minor product was seen. After isolation (by preparative tlc) and characterization of the products the reactions having taken place were found to be those shown in Scheme 9. Reaction of taxol with DBU under identical reaction conditions required several hours before anything other than taxol could be seen on tlc.

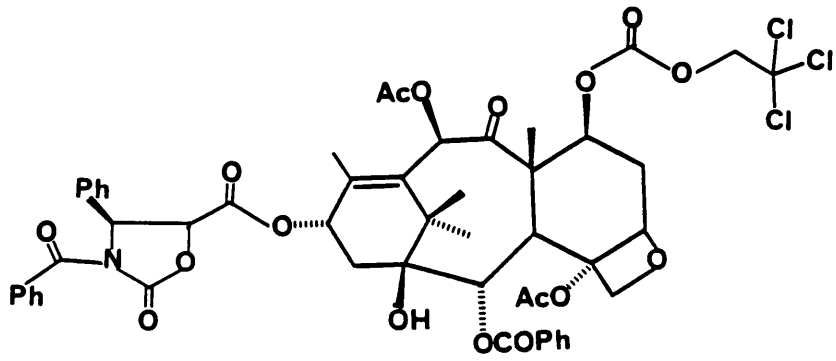
Structural assignment of the taxol analogues with the cyclic urethane side chains was achieved by analysis of their ^{1}H-nmr, ir and mass spectra. Both derivatives were chromatographically homogenous and ^{1}H-nmr showed each to be a pure compound. The characterization data for **39** are shown in Table 9 and the characterization data for **40** are shown in Table 10.

Inspection of the ^{1}H-nmr spectrum of **39** immediately showed that some sort of transformation had taken place on the side chain of 2',7-di(2,2,2-trichloroethyloxycarbonyl)taxol but not on the taxane skeleton. All of the signals for the protons on the main skeleton of 2',7-di(2,2,2-trichloroethyloxycarbonyl)taxol are present with no major change in the spectrum of **39**. The peaks associated with the side chain of 2',7-di(2,2,2-trichloroethyloxycarbonyl)taxol: peaks at 5.52 ppm (d, J=3), 6.90 ppm (d, J=9), 6.02 ppm (dd, J=3,9)



Scheme 9. Reaction of 2',7-Di(2,2,2-trichloroethyloxycarbonyl)taxol with DBU

Table 9 (Part 1 of 2). Characterization Data for **39**



Position	Shift (ppm from TMS) Coupling (hertz)
2	5.65 (d, 7)
3	3.93 (d, 7)
5	4.90 (br d, 9)
6	2.38 (m)
7	5.56 (m)
10	6.34 (s)
13	6.33 (br t, 9)
14	2.27 (d, 9)
16	1.24 (s)
17	1.17 (s)

Mass Spectral Data

1054 $(MH)^+$, 994 $(MH-HOAc)^+$,

976 $(MH-HOAc-H_2O)^+$,

683 $(MH-RCOOH-HOAc)^+$,

743 $(MH-RCOOH)^+$,

105 $(C_7H_5O)^+$.

High Resolution

$(MH)^+C_{51}H_{50}NCl_3O_{17}$ -

1054.2277

Calculated - 1054.2224

Table 9 (Part 2 of 2). Characterization Data for **39**

Position	Shift (ppm from TMS) Coupling (hertz)
18	2.00 (br,s)
19	1.79 (s)
20	4.11 (d, 8.5), 4.28 (d, 8.5)
2'	4.94 (d, 6)
3'	5.71 (d, 6)
OAcs	2.14 (s), 1.97 (s)
2 OBz	8.04 (d, 8), 7.4 (m)
troc	5.01 (d, 12), 4.62 (d, 12)
3' NBz	7.71 (d, 8), 7.4 (m)

Infrared Spectral Data

1820 ms, 1775 s, 1750 s,

1720 m-sh, 1665 mw, 1475 w,

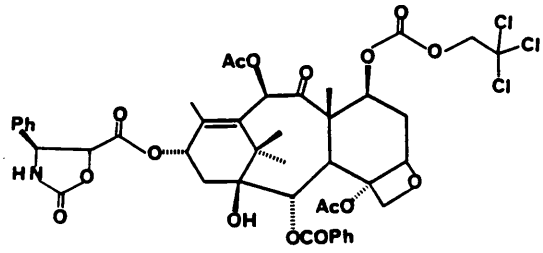
1390 m, 1260 s, 1195 m-sh,

1110 m, 1080 m,

1040-1060 mw.

Table 10 Characterization Data for **40**

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.65 (d, 7)
3	3.94 (d, 7)
5	4.91 (dd, 9,1)
6	2.5 (m)
7	5.57 (dd, 7,11)
10	6.33 (s)
13	6.31 (br t, 9)
14	2.24 (d, 9)
16	1.22 (s)
17	1.13 (s)
18	1.79 (s)
19	2.01 (s)
20	4.11 (d, 8), 4.28 (d, 8)
2'	4.82 (d, 6)
3'	5.07, (d, 6)
N-H	5.70 (br s) , 5.07
OAcs	2.10 (s), 2.13 (s)
2 OBz	8.06 (d, 7), 7.62 (t, 7) 7.4 (m)
troc	5.00 (d), 4.62 (d)
3' Ph	7.4 (m)



Mass Spectral Data

988 $(MK)^+$, 972 $(MNa)^+$,

950 $(MH)^+$, 890 $(MH-HOAC)^+$,

683 $(MH-RCOOH)^+$,

623 $(MH-RCOOH-HOAc)^+$,

105 $(C_7H_5O)^+$.

High Resolution

$(MH)^+$ - 950.1867

Calculated - 950.1962

$C_{44}H_{46}NO_{16}Cl_3$

Infrared Spectral Data

1810 s-sh, 1775 m,

1740 s, 1460 w, 1390 m,

1280 s-sh, 1260 s,

1240 s-sh, 1100 m,

1080 m, 995 m, 720 w.

were not seen at all in the spectrum of **39**. In place of the side chain signals seen for **38** two coupled sharp doublets were seen for **39** at 5.71 ppm (d, J=6) and 4.94 ppm (d, J=6). Sharp doublets have almost never been seen for taxol analogues and whenever they are present they must be explained by protons being in positions where the possibility of long range coupling is eliminated. The presence of the sharp doublets along with the absence of a signal for the C-3' amide proton and the shifting of the meta protons of the phenyl amide so they could not be clearly seen indicated that the reaction taking place involved the C-3' amide.

The mass spectrum of **39** indicated the molecular weight as 1053 by the presence of peaks at m/z 1054 $(MH)^+$, 994 $(MH-HOAc)^+$, and 976 $(MH-HOAc-H_2O)^+$. The molecular weight of 1053 was a loss of 148 mass units ($C_2H_3Cl_3O$) when compared to **38**. Among other peaks in the mass spectrum a peak at m/z 312 $(RCOOHH)^+$ where RCOOH is the side chain lost as an acid, showed that the loss of mass occurred on the side chain.

The infrared spectrum of **40** does not show the amide peak seen at 1780 cm^{-1} for **38**. The spectrum does show a major new absorbance at 1820 cm^{-1} due to the carbonyl absorbance of the cyclic urethane.

The ^1H-nmr data for **40** are similar to those for **39** but the changes that are present clearly show that the compound is the N-debenzoylated derivative of **39**. In the ^1H-nmr spectrum of **40** the sharp doublets seen for **39** are seen as a doublet

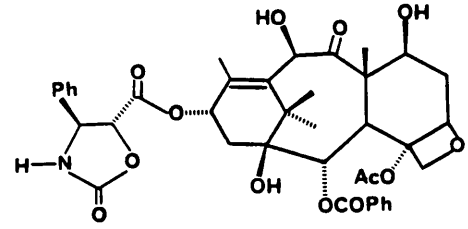
at 4.82 ppm (d, J=6) for the C-2' proton coupled to a broad doublet at 5.07 ppm (J=6) coupled to a broad singlet at 5.70 ppm. The change in the ^{1}H-nmr spectrum was due to the presence of an amide proton in **40**. The mass spectrum of **40** shows a loss of 106 mass units (C_7H_6O) when contrasted with **39**. The infrared spectrum shows the urethane carbonyl peak shifting from 1820 cm^{-1} to 1800 cm^{-1}.

In order to prepare a derivative suitable for biological activity testing **40** had its 7 position deprotected via reaction with zinc dust in methanol and acetic acid at room temperature for 1 hour. Characterization data for the product **41** are shown in Table 11. The changes seen in the spectra of the deprotected product **41** can all be explained by removal of the trichloroethyloxycarbonyl group. The peaks in the ^{1}H-nmr spectrum caused by the trichloroethyloxycarbonyl protons are absent and the signal for the C-7 proton is shifted upfield to 4.39 ppm (dd, J=4,11). The carbonate carbonyl stretching in the infrared spectrum, which had been seen at 1775 cm^{-1}, is absent.

This reaction occurred because it was entropy favored as the nucleophile and electrophile were held close to each other and it was also favored by the good leaving group 2,2,2-trichloroethoxide. As with many reactions that have taken place during this research project unexpected results are not necessarily bad results. The reaction of 2',7-di(2,2,2-trichloroethyloxycarbonyl)taxol with DBU did not

Table 11. Characterization Data for **41**

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.64 (d, 7)
3	3.77 (d, 7)
5	4.88 (br d, 8)
6	2.1 (m)
7	4.39 (dd, 4,11)
10	6.25 (s)
13	6.33 (br t, 9)
14	2.5 (m)
16	1.24 (s)
17	1.13 (br s)
18	1.91 (br s)
19	1.18 (s)
20	4.31 (d, 8), 4.26 (d, 8)
2'	4.94 (d, 6)
3'	5.73 (d, 6)
OAcs	1.94 (s), 2.22 (s)
2 OBz	8.04 (d m, 7), 7.45 (m)
3' NBz	7.69 (d m, 7.51), 7.60 (m), 7.45 (m)
3' Ph	7.45 (m)



Mass Spectral Data

880 $(MNa)^+$

858 $(MH)^+$

569 $(MH-RCOOH)^+$

509 $(MH-RCOOH-HOAc)^+$

105 $(C_7H_5O)^+$

Infrared Spectral Data

1815m, 1740s, 1720s-sh, 1670m, 1465w, 1390m, 1255s, 1200m, 1095m.

give the desired C-2' epimer but that result can be achieved through the work on preparing taxols with totally synthetic side chains; and the 2'-epiacetyltaxol derivative that was prepared will allow for unequival assignment of the isomers of the taxol side chain (see Chapter 3). The ring closure reaction that did take place achieved something that was even more important than isomerizing the C-2' position - it substituted the phenyl amide of taxol and then showed that the original nitrogen substituent can be easily removed. Substitution of the nitrogen of taxol is, as will be fully explained in the Chapter 3, the best chance that currently exists for synthesizing a more active form of taxol. Substitution of the amide and hydrolysis of the benzoyl group would be a simple high yielding method to achieve this.

2.3 EXPERIMENTAL PROCEDURE

2'-Acetyltaxol (**27**) (100 mg scale) - Taxol (119 mg.) was dissolved in 1.0 mL of dry pyridine. To this solution was added 70 µL of acetic anhydride. The reaction was allowed to proceed at room temperature under a nitrogen atmosphere for a period of 1 hour 45 minutes. The reaction was stopped by the addition of 1 mL of water. The products were extracted with several mL of CH_2Cl_2, washed with 0.1 N HCl and then water, followed by drying of the organic solution with $MgSO_4$ and evaporation of the solvent. The product was purified

using preparative high pressure liquid chromatography (RP-8, 65/35 MeOH/H_2O). Analysis by hplc of the crude product showed that the reaction yielded 92% C-2' monoacetyltaxol and 8% 2',7-diacetyltaxol.

2'-Acetyltaxol (**27**) (1.00 g scale) - Taxol (1.00g) was dissolved in 10.0 mL of dry CH_2Cl_2 and to this solution 200µL of dry pyridine was added along with acetic anhydride (116 µL, 1.02 eq.). The reaction was stirred and left to stand overnight. The reaction was worked up and the product obtained as needle shaped crystals by evaporation of an 8/2 CH_2Cl_2/hexane solution with a nitrogen stream. Analysis by hplc showed the product to be >99% 2'-acetyltaxol with a trace of diacetyltaxol present. Yield-1.02g, 98%.

2',7-Diacetyltaxol (**28**) (Limited Reaction Time) - Taxol (104 mg) was dissolved in 1 mL of dry pyridine to which acetic anhydride (185 µL, 15 eq) had been added. After stirring at room temperature for 10 hours the reaction was worked up. Analysis by hplc showed that the products were 15% 2'-acetyltaxol and 85% 2',7-diacetyltaxol. The 2',7-diacetyltaxol was isolated by preparative hplc, 7/3 methanol/water. Yield-70 mg, 62%. The ^{1}H-nmr spectrum is identical to that published[17] and the product cochromatographed on hplc with authentic 2',7-diacetyltaxol.

2',7-Diacetyltaxol (Excess Reaction Time) - Taxol (100 mg) was dissolved in 1 mL of dry pyridine and acetic anhydride (0.358 mL, 30 eq) was added. The reaction was allowed to proceed for 72 hours at room temperature. At the end of the reaction 10 mL of water was added which precipitated all of the diacetyltaxol. The mixture was filtered and washed with several mL of water. Any remaining traces of pyridine were removed in vacuo. This method produced 100% pure diacetyltaxol (analysis by hplc) and the only losses in yield were from sample handling. Isolated yield-0.105 g, 96%.

7-Acetyltaxol (29) - 2',7-Diacetyltaxol (220 mg) was partially dissolved in 100 mL of a 0.25% $NaHCO_3$ solution in 3/1 methanol/water at room temperature. The reaction was stirred vigorously with a magnetic stirring bar for 5 hours at room temperature. Because an unwanted side reaction began to occur towards the end of this reaction it was monitored by hplc so that it could be stopped as soon as all of the diacetyltaxol had reacted. The reaction was stopped by the addition of several milliliters of acetic acid. The methanol was evaporated in vacuo, the products extracted with methylene chloride, and final purification was achieved with preparative hplc, (RP-8, 70/30, methanol/water). Yield-90 mg, 47% of 7-acetyltaxol. Characterization data for 7-acetlytaxol is shown in Table 5. Yield - 7-acetylbaccatin III-25 mg, 36%. Yield - methyl ester of the side chain - 15 mg, 23%.

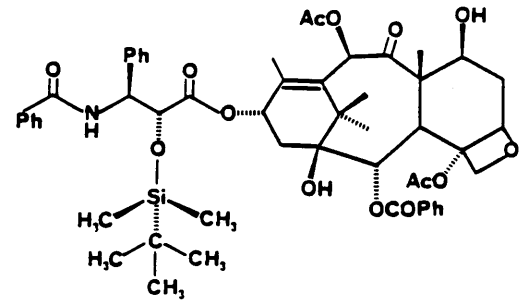
Characterization data for 7-acetylbaccatin III is shown in Table 18 in Chapter 3. The ^{1}H-nmr and mass spectra for the side chain agreed with published data.[33]

2'-Tertbutyldimethylsilyltaxol - Taxol (5 mg) was reacted with 25 µL (5 eq) of a silylating solution consisting of 18 mL dry dimethylformamide, 10.2 g tertiarybutyldimethylsilyl-chloride imidazole (9.2 mg). The reaction was allowed to proceed at room temperature for 12 hours at which time it was worked up and the product isolated by preparative hplc (85/15, MeOH/H_2O). Characterization data are shown in Table 12.

2'-Triethylsilyltaxol - Taxol (25 mg) was dissolved in 1.0 mL of dimethylformamide and this solution had triethylsilyl-chloride (103µL, 25 eq) and imidazole (84 mg, 50 eq) added to it. The reaction was allowed to proceed at room temperature for 3 hours and was then worked up. Analysis by tlc showed no taxol remaining at the end of the reaction. Rf 2': triethylsilyltaxol, 0.88; taxol, 0.45, (silica gel, 6/4, ethylacetate/hexane). The products were obtained using preparative tlc (1/1 ethyl acetate/hexane) 2'-Triethylsilyl-taxol Rf 0.54. Yield - 18 mg, 75%. Characterization data are shown in Table 13.

Table 12 Characterization Data for 2'-t-Butyldimethylsilyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.68 (d, 6.5)
3	3.79 (d, 6.5)
5	4.97 (dd, 1.5,10)
6	2.2-2.5 (m)
7	4.41 (dd, 6,11)
10	6.24 (s)
13	6.24 (br t, 8)
14	2.2-2.5 (m)
16	1.29 (s)
17	1.13 (s)
18	1.69 (s)
19	1.90 (br s)
20	4.17 (d, 8.5) 4.30 (d, 8.5)
2'	4.62 (d, 2)
3'	5.71 (dd, 2,9)
N-H	7.06 (d, 9)
OAcs	4.17 s, 4.30 (s)
3' NBz	7.71 (d, 8), 7.4 (m)
2 OBz	8.11 (d, 8)
3' Ph	7.4 (m)
Silyl	-0.80(9H), -0.04(3H), -0.29(3H)



Mass Spectral Data

968 $(MH)^+$, 908 $(MH-HOAc)^+$,

569 $(MH-RCOOH)^+$,

551 $(MH-RCOOH-H_2O)^+$,

509 $(MH-RCOOH-HOAc-H_2O)^+$,

400 $(RCOOHH)^+$,

105 $(C_7H_5O)^+$.

Infrared Spectral Data

1745 s, 1670 m, 1535 w,

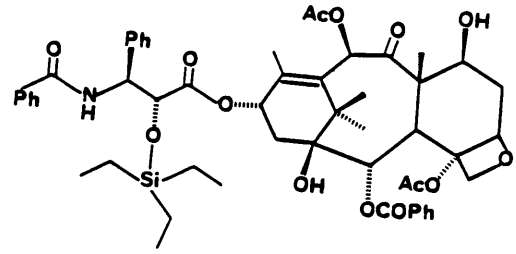
1510 w, 1490 w, 1385 m,

1260 s, 1120 m, 1085 m,

850 m, 790 m, 720 w.

Table 13 Characterization Data for 2'-Triethylsilyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.67 (d, 7)
3	3.77 (d, 7)
5	4.90 (d, 9)
6	5.3 (m)
7	4.40 (dd, 6.5)
10	6.41 (s)
13	6.17 (br t, 8)
14	2.5 (m)
16	1.25 (s)
17	1.14 (s)
18	1.68 (s)
19	1.89 (s)
20	4.17 (d, 8), 4.28 (d, 8)
2'	3.91 (d, 3)
3'	5.78 (dd, 3,9)
N-H	7.00 (d, 9)
OAcs	2.36 (s), 2.17 (s)
2 OBz	8.10 (d m, 8), 7.60 (t, 7)
3' NBz	7.74 (d m, 8),
3' Ph	7.4 (m)
Silyl	0.57 (m), 0.90 (m)



Mass Spectral Data

990 $(MNa)^+$, 968 $(MH)^+$.

Infrared Spectral Data

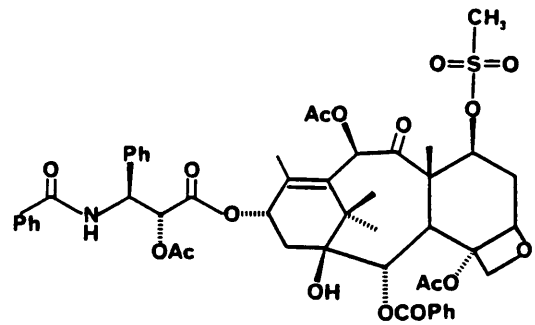
1740 s, 1680 m, 1665 m,
1535 w, 1505 w, 1475 w,
1385 w, 1245 s, 1110 m,
1090 m, 950 w, 830 w,
720 m.

C-7 Epimerization of Taxol - A sample of taxol (19.7 mg) and freshly prepared azobis(isobutyronitrile), (0.9 mg were dried in vacuo at room temperature for 1 hour and then dissolved in 2.0 mL of dry toluene. The solution was heated on an oil bath to 80° for 30 minutes after which it was diluted with 6 mL of EtOAc and worked up as usual. Isolation of the product was by preparative tlc (silica gel, 1000 μm, 3/2, EtOAc/Hexane). Preparative tlc showed only the product band, Rf 0.46, and a very faint taxol band, Rf 0.20. Yield-16.5 mg, 84%. Characterization data for 7-epitaxol is shown in Table 6. This reaction was repeated successfully three additional times but when it was attempted six months later using the same AIBN sample it could not be duplicated. The use of freshly prepared AIBN therefore was critical.

2'-Epiacetyl-7-methanesulfonyltaxol - 2'-Acetyl-7-methanesulfonyltaxol (50 mg) was dissolved in 2.0 mL of dry CH_2Cl_2 and DBU (50μL) was added. The reaction was allowed to proceed at room temperature for 26 hours and 40 minutes, at which time it was worked up. The products were isolated using preparative tlc (silica gel, 9/2 CH_2Cl_2/methylethylketone). Yield 2'-epiacetyl-7-methanesulfonyltaxol - 8 mg, 16 %, 2'-acetyl-7-methanesulfonyltaxol - 2 mg, 4%. A more detailed description of this experiment is included in Chapter 6. Characterization for data 2-acetyl-7-methanesulfonyltaxol

Table 14 Data for 2'-Acetyl-7-methanesulfonyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.68 (d, 7)
3	3.92 (d, 7)
5	4.92 (d, 9)
6	2.9-3.1 (m)
7	5.34 (dd, 7,10)
10	6.50 (s)
13	6.19 (br t, 9)
14	2.1-2.2 (m)
16	1.19 (s)
17	1.18 (s)
18	1.80 (s)
19	2.01 (s)
20	4.16 (d, 8), 4.31 (d, 8)
2'	5.52 (d, 3)
3'	5.93 (dd, 3,9)
N-H	6.91 (d, 9.5)
OAcs	2.41 (s), 2.16 (s), 2.14 (s)
3' NBz	7.73 (d, 7), 7.4 (m)
2' OBz	8.09 (d, 7), 7.60 (t, 7), 7.4 (m)
3' Ph	7.4 (m)
Mesyl	3.09 (s)



Mass Spectral Data

996 $(MNa)^+$, 974 $(MH)^+$.

Infrared Spectral Data

1740-1775 s, 1745 max,

1680 m, 1575-1440 w,

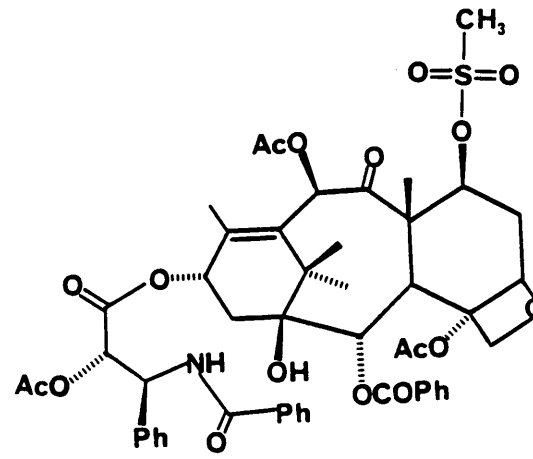
1395 m, 1360 m,1285 m,

1260 s, 1200 m,

1150-1090 w.

Table 15. Data for 2'-Epi-acetyl-7-methanesulfonyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.63 (d, 7)
3	3.87 (d, 7)
5	4.91 (d, 8)
6	2.90-3.05 (m)
7	5.30 (dd, 7,10)
10	6.39 (s)
13	6.11 (br t, 8)
14	2.2 (m)
16	1.14 (s)
17	1.23 (s)
18	1.56 (s)
19	1.78 (s)
20	4.12 (d, 8), 4.29 (d, 8)
2'	5.62 (d, 6)
3'	5.86 (dd, 6,8)
N-H	6.88 (d, 8)
OAcs	2.40 (s), 2.20 (s), 2.14 (s)
2 OBz	8.09 (d, 7), 7.61 (t, 7), 7.4 (m)
3' NBz	7.78 (d, 7), 7.4 (m)
3' Ph	7.4 (m)
Mesyl	3.07 (s)



MassSpectral Data

996 $(MNa)^+$, 974 $(MH)^+$,

914 $(MH\text{-}HOAc)^+$,

587 $(MH\text{-}RCOOH\text{-}SOCH_2)^+$.

Infrared Spectral Data

1740 s, 1670 m, 1535 w,

1505 w, 1470 w,

1390-1350 m, 1290 m-sh,

1250 s, 1195 m, 1115 m.

are on Table 5 and for 2'-epiacetyl-7-methanesulfonyltaxol are on Table 15.

2',7-Di(2,2,2-trichloroethyloxycarbonyl)taxol (**38**) - Taxol (50 mg) was dissolved in 1.0 mL of dry CH_2Cl_2 and the solution was cooled to 0° in an ice bath. To the taxol solution was then added 25 µL of dry pyridine and 25 µL of 2,2,2-trichloroethylchloroformate. After 5 minutes tlc showed only one compound present. The reaction was then worked up to yield pure 2',7-di(2,2,2-trichloroethylformyl)-taxol, Rf 0.67, (silica gel, 1/1 ethyl acetate/hexane). Yield - 65 mg, 93%. Characterization data are shown in Table 8.

Reaction of 2',7-Di(troc)taxol **38** with DBU - A sample of 2',7-di(troc)taxol (112 mg) was dissolved in 1.5 mL of dry CH_2Cl_2 and 10 µL of DBU was added. When the reaction was checked by tlc (2/3, EtOAc/Hexane) after 4 minutes all of the starting material had reacted and two products, Rf 0.43, 0.55 were seen. The reaction was worked up by washing with 1.0 N HCl, water and then drying and filtering. After work up a third product with Rf 0.20 was seen. The products were isolated by preparative tlc (2/3, EtOAc/Hexane). Three products were obtained - **39**, 38 mg, 39%; **40**, 13.5 mg, 15% and 17.7 mg of an impure product (Rf 0.60) which ^{1}H-nmr indicated probably resulted from transfer of the chlorocarbonate to the C-3'

amide and transfer of the benzoyl group to C-2'. The third product was not of interest and so further purification and characterization was not carried out. The reaction of 2',7-di(troc)taxol with DBU was carried out on a preparative scale only once and the procedure was not optimized. The characterization data for **39** are shown in Table 9 and for **40** in Table 10.

C-7 Deprotection of **39** - The procedure described above was repeated with the difference that instead of isolating the products the crude reaction mixture was subjected to deprotection by reaction with 200 mg Zn dust in 2.0 mL 9/1, MeOH/HOAc at room temperature for 30 minutes. The products were isolated via preparative tlc (1/1, EtOAc/Hexane).* The only product that could be obtained in pure form was **41**, 20 mg, 28%, Rf 0.24. The characterization data for **41** is shown in Table 11.

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* For any researchers wishing to repeat this reaction it is suggested that purification be carried out before the deprotection step. The deprotection reaction is essentially a quantitative transformation and no chromatography would be required in the work up.

3.0 BACCATIN III

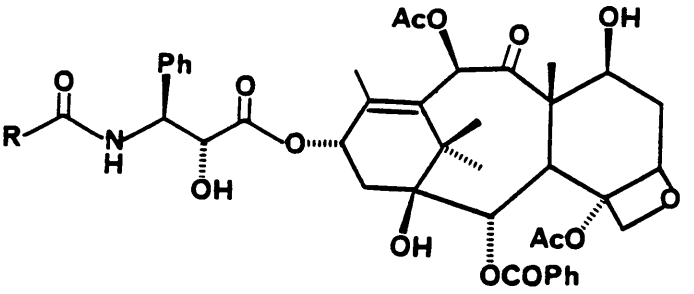
3.1 INTRODUCTION

Research conducted in this portion of the taxol project had the overall goal of laying the foundation necessary for producing a more active form of taxol, which it is hoped will ultimately be accomplished by preparing taxols with totally synthetic modified side chains. The specific aims of this particular research were to selectively remove the side chain of taxol so that modified side chains can be attached, and to determine how the C-13 hydroxyl group produced can be selectively esterified.

The feasibility of preparing a more active form of taxol via substitution of a totally synthetic side chain is supported by two major pieces of information.

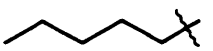
The first of these is that natural products, **11** and **17**, have been isolated which have the structure of taxol **1**, except for differing amide functionality. Each of these compounds possesses biological activity comparable to that of taxol.

It has also been shown that a significant change in the activity of a bioactive compound can be made by varying the polarity of that compound, even though the modifications are not at a location involved in the reactivity of the compound



11 R =

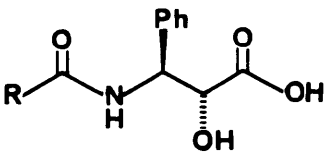
17R =



1 R =

or in the binding of the compound to its receptor site. The polarity of a bioactive compound can be expressed by the biophysical constant P.[39] P is a partition coefficient for a compound between 1-octanol and water. In somewhat simplistic terms the partitioning of a compound between octanol and water is analogous to what the compound will do *in vivo*. In order to be transported through the blood a compound must be water soluble and in order to be transported through the cell membrane a compound must be fat soluble to some extent. Therefore there is an optimum polarity for a compound, independent of the actual binding or reactivity of the drug, at which the drug can be easily transported to the cell and through the cell wall.

One of the ultimate aims of the overall taxol research project is to prepare a taxol derivative with this optimum polarity, and this can in principle be done by the preparation of a suitably modified sidechain **42** and its attachment to the taxol nucleus, which is known as baccatin III. The achievement of this objective requires a source of baccatin III and it is this aspect which will be addressed first.



R = groups of varying lipophilicity

42

3.2 RESULTS AND DISCUSSION

3.2.1 PREPARATION OF BACCATIN III FROM TAXOL VIA BASIC SOLVOLYSIS

The major piece of work, before this research project, in which a taxol without the side chain (baccatin III) was produced was conducted by Powell et. al.[35] In this reaction cephalomannine was subjected to solvolysis in a water and methanol solution with 1% $NaHCO_3$ as base (Scheme 10). Powell also subjected taxol to basic solvolysis. Reaction of taxol with sodium methoxide in methanol produced the methyl ester of the side chain and the taxane products were "complex and uncharacterized."

In Powell's solvolysis of cephalomannine there were three major processes taking place: removal of the ester side chain, removal of the C-10 acetyl group and epimerization of the 7 position. The yield of baccatin III was 19%. In addition to these processes additional reactions accounted for 26% of the starting material being uncharacterized.

Most of the products from the solvolysis of cephalomannine did not possess the ester side chain. This result does not preclude the possibility that removal of the side chain was

the fastest process occurring and the other products arose from decomposition of the initially produced baccatin III. In order to test the hypothesis that baccatin III is the initial major product a mixture of taxol and cephalomannine* was subjected to solvolysis in methanol with triethylamine. Reaction temperatures were either 25° or 0° and the concentration of triethylamine ranged from 0.01% to 1%. The reactions were monitored by hplc. All reaction conditions produced a mixture of products from the start. Since, in this portion of the taxol project there was only one specific desired product, products other than baccatin III were not isolated and characterized.

The methanolysis of taxol showed the necessity of a modification of technique to stop or slow unwanted processes during the reaction. An indication of how this might be accomplished was gained from the acetylation of an extract from Taxus brevifolia by Kingston and Ovington.[17] From this reaction 10-deacetyl-2',7-diacetyltaxol was isolated (Scheme 11).

The acetylation of the Taxus brevifolia extract showed that the 10 position is more sterically hindered than either the 7 or the 2' position. Therefore use of a more hindered nucleophile might stop the deacetylation of the 10 position,

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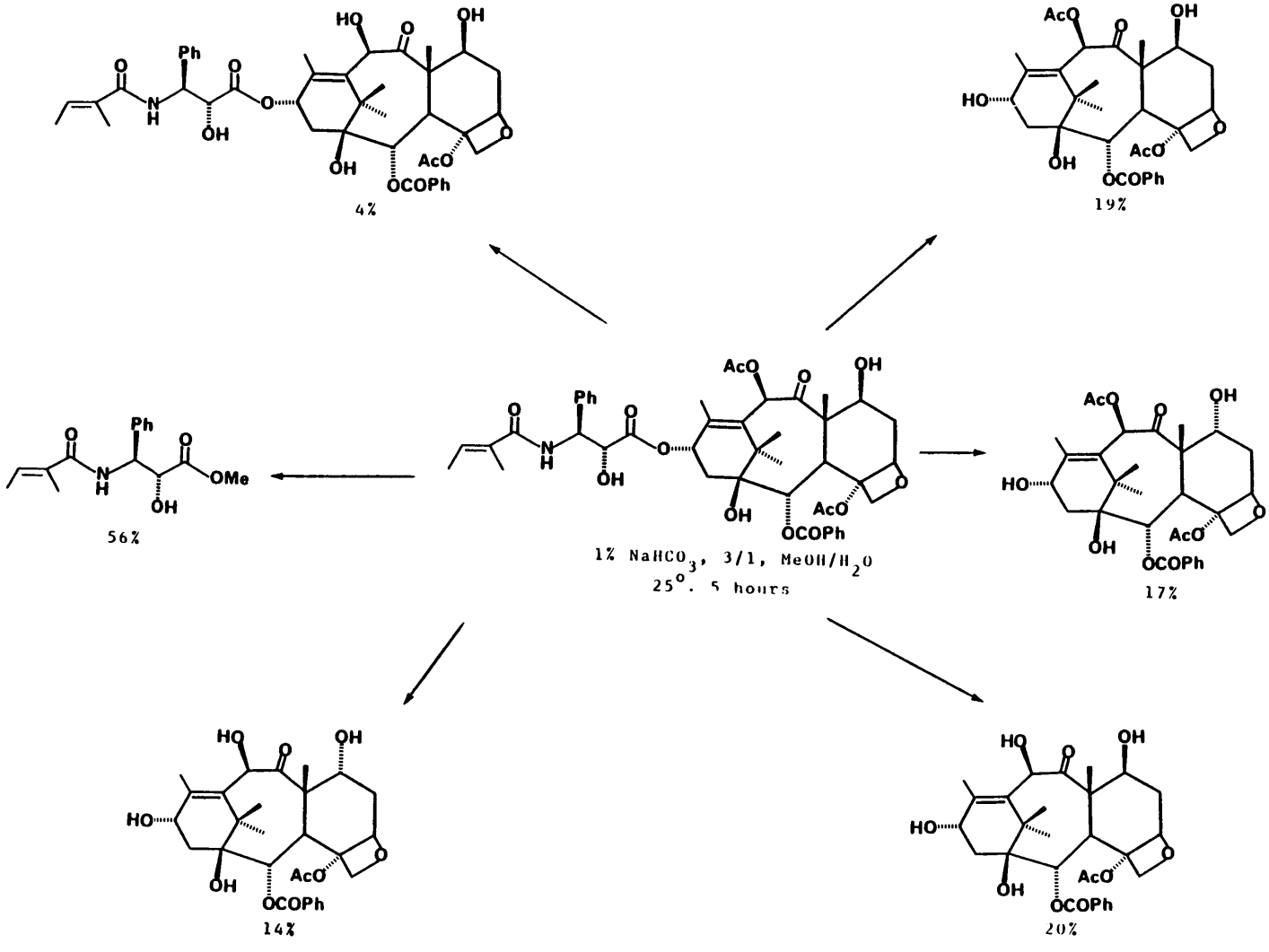
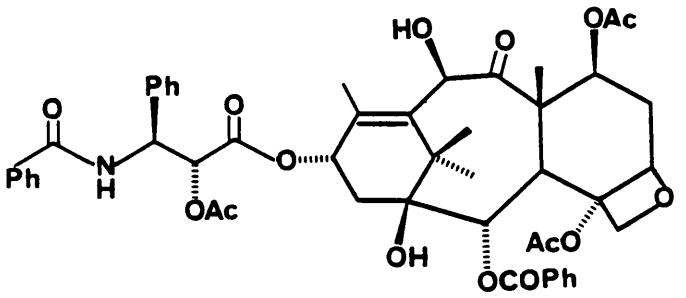
* A mixture of taxol and cephalomannine was used because removal of the side chain for either compound lead to the same product.



Scheme 10. Basic Solvolysis of Cephalomannine

Extract from Taxus brevifolia

Ac_2O, Pyridine
25°, 24 hours

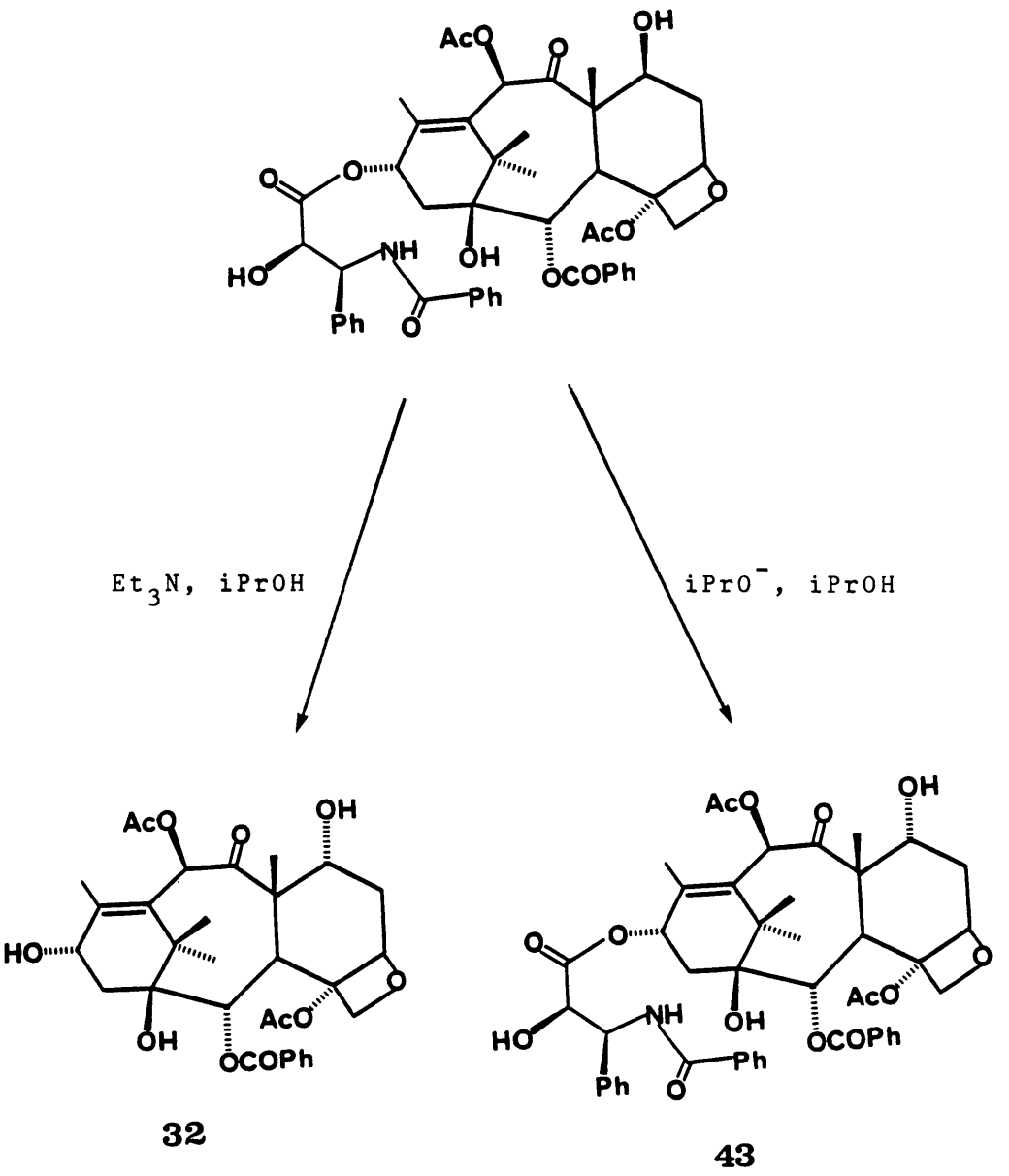


Scheme 11. Acetylation of a Taxus brevifolia Extract

leaving only epimerization of the 7 position as a major unwanted reaction. Since Powell[33] had indicated that the epimerization of the 7 position was expected to be an equilibrium process it was considered to be a problem that could be overcome by recycling the undesired 7 epimer - baccatin V.

Solvolysis of taxol in isopropanol was conducted using either triethylamine or sodium isopropoxide as base. The solvolysis with triethylamine as base produced only a less polar compound (as indicated by hplc) which was not isolated but on the basis of its polarity was assigned the structure 7-epitaxol 13 (Scheme 12). Solvolysis with isopropoxide as base produced, from the start, the same less polar compound along with baccatin III **11** and baccatin V **43**. These were not isolated but cochromatographed with authentic samples of baccatin III and baccatin V. Eventually all of the taxane products were converted into baccatin V.

Solvolysis of taxol with the more hindered solvent isopropanol with triethylamine or isopropoxide as base did stop the deacetylation at position 10 and in general prevented decomposition of the taxane skeleton. The reactions failed to give a high yield of the desired product baccatin III however as the assumption made before the reactions that epimerization of the 7 position was an equilibrium process was incorrect. The mixture of C-7 epimers seen in the methanolysis of cephalomannine by Powell was not caused by the ep-



Scheme 12. Basic Isopropanolysis of Taxol

imerization being an equilibrium process but rather by the reaction not having reached equilibrium when it was stopped.

3.2.2 PREPARATION OF BACCATIN III FROM TAXOL VIA HYDRIDE REDUCTION

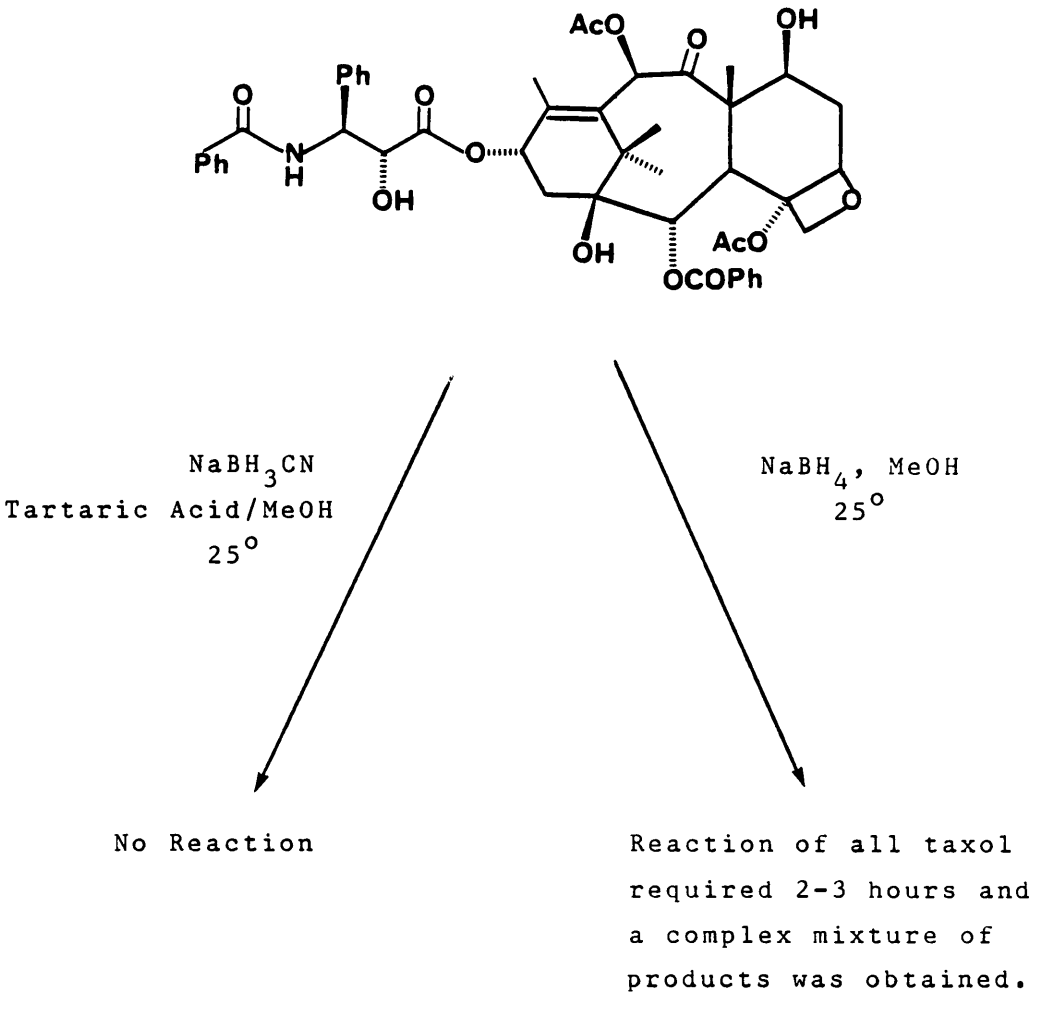
The selective removal of the ester linked side chain of taxol was effected in high yield via reduction of the ester group by hydride reducing agents. The first attempt to prepare baccatin III in this manner was the reaction of taxol with sodium cyanoborohydride in methanol in the presence of citric acid. Sodium cyanoborohydride is a mild reducing agent that is stable under mildly acidic conditions and requires a pH of 4 to reduce ketones.[37] The reducing agent was too mild to produce any reaction with taxol, either at the C-1' ester or the C-10 ketone (Scheme 13). An explanation for this lack of reactivity will be given later.

Further experimentation involved the stronger reducing agent sodium borohydride, which is frequently used to reduce ketones in the presence of esters.[38] Reaction of taxol with sodium borohydride in methanol at room temperature required several hours to consume the starting material and hplc monitoring showed that the reaction produced only a mixture of polar products from which no major product could be isolated (Scheme 13).

A straightforward explanation for the mixture of products produced in the reaction can be given because, as has already been shown, taxol and baccatin III are not stable in basic methanol. The mixture of products therefore is due to sodium borohydride in methanol being too basic for taxol or baccatin III to be stable in.

In order to compensate for the instability of taxol in base, isopropanol, a solvent in which sodium borohydride has been reported to be stable for days at room temperature, was used for the reaction.[39] Reaction of taxol with sodium borohydride in isopropanol at room temperature for 1 hour gave a mixture of the reduced side chain and comparable amounts of baccatin III and baccatin V, all of which were isolated and characterized (Scheme 14). The epimerization of the 7 position showed that even though borohydride in isopropanol is much less basic than when methanol is used as solvent, it is still basic enough that baccatin III is not stable in it. This problem was overcome by running the reaction at 0°, following the reaction carefully by hplc, and stopping the reaction by addition of acetic acid as soon as it was complete.

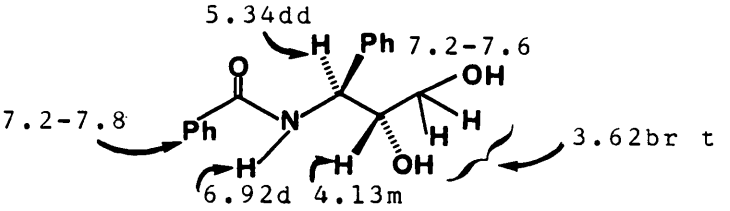
A final improvement in the procedure involved the use of tetrabutylammonium borohydride as reducing agent, which is soluble in organic solvents.[40] Baccatin III was obtained from taxol using tetrabutylammonium borohydride at room tem-



Scheme 13. Reaction of Taxol with $NaBH_3CN$ or $NaBH_4$ in Methanol

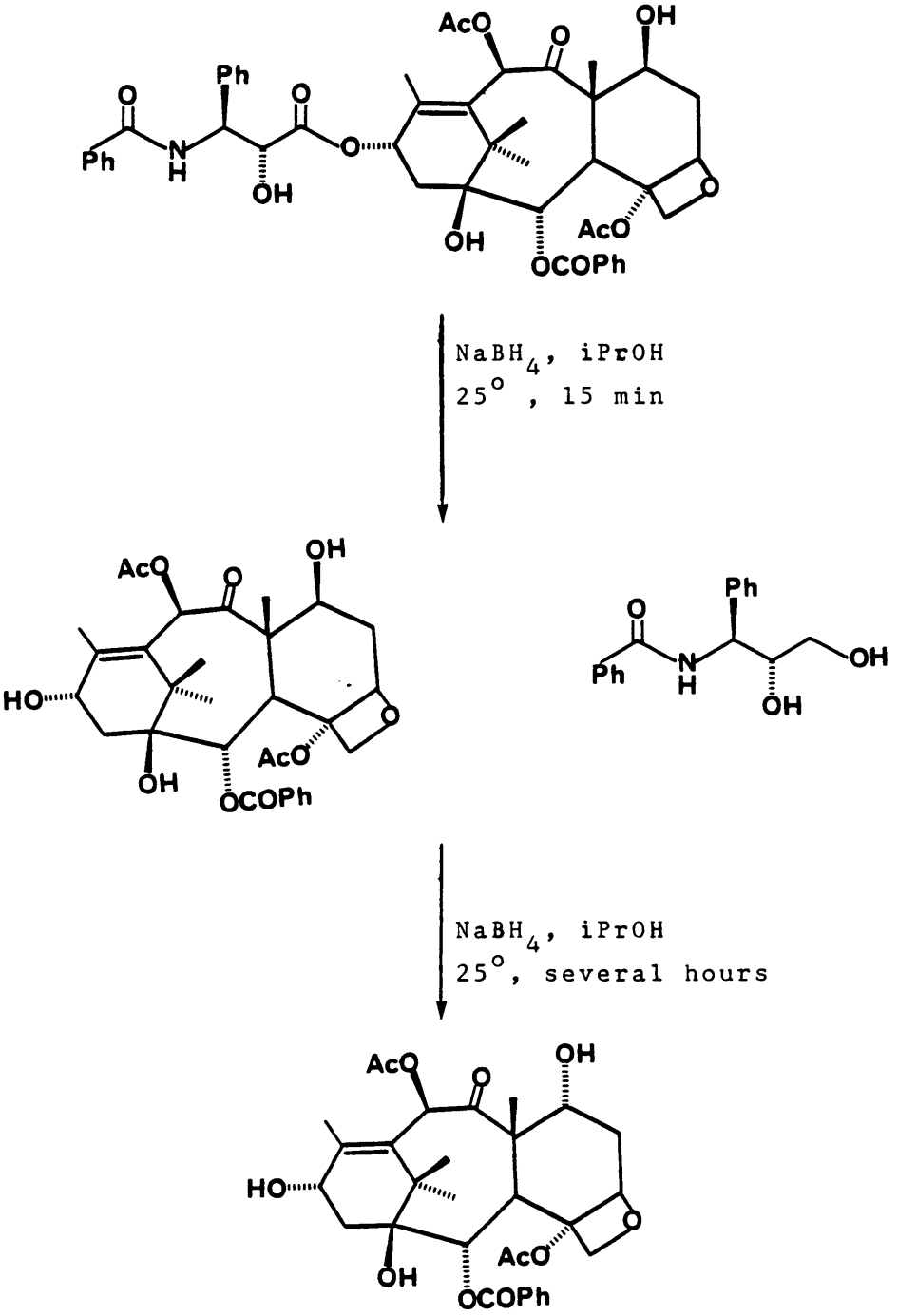
perature and, once produced, the baccatin III did not react further to yield baccatin V or any other product (Scheme 15).

The ^{1}H-nmr spectrum for the baccatin III, obtained via hydride reduction of taxol, matched the values published for the known natural product.[33] Characterization of the reduced side chain of taxol was accomplished using ^{1}H-nmr, ir, and mass spectrometries. The mass spectrum showed the molecular weight of the compound to be 271, m/z 272 $(MH)^+$. The infrared spectrum showed the absence of ester carbonyl absorption and the presence of the amide carbonyl with absorbance at 1655 cm^{-1}. This rules out the possibility that the side chain product possesses either ester or carboxylic acid functionality. The proton nmr assignments for the side-chain alcohol **44** are shown in Figure 10. The downfield shift of the amide proton is caused by the solvent d_6-DMSO. The same effect is seen when the proton spectrum of taxol is obtained using d_6-DMSO as a solvent.

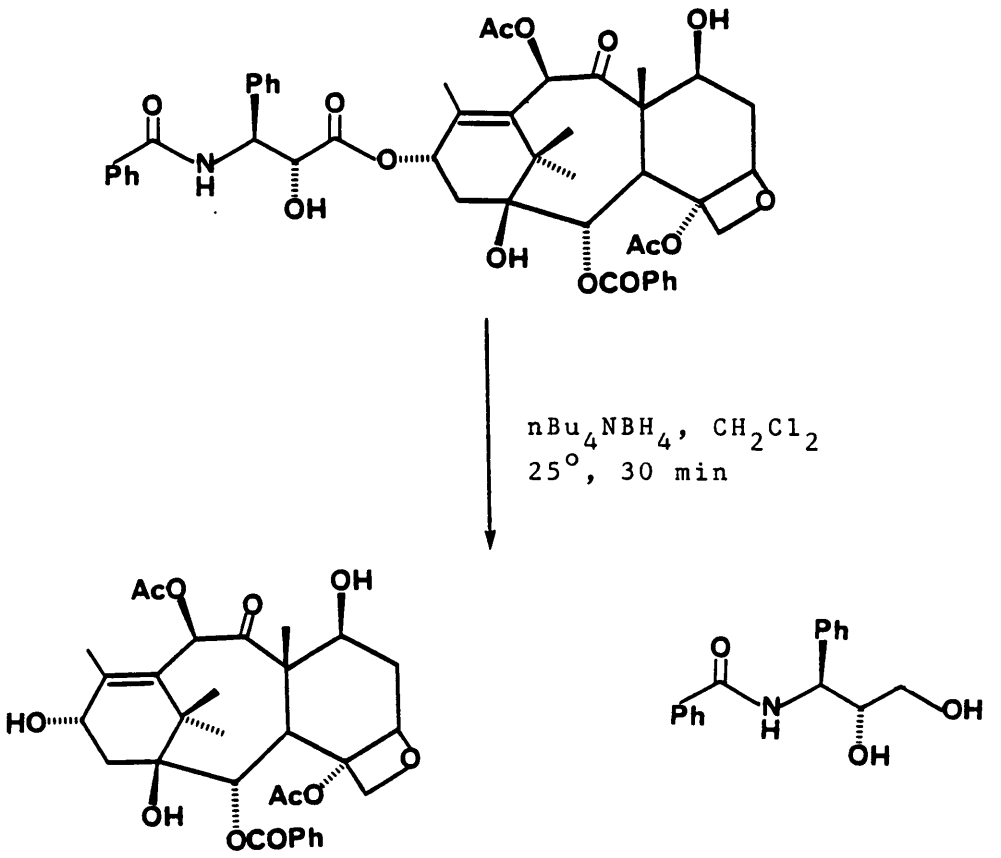


44

Figure 10. Proton NMR Assignments for the Side-chain Alcohol



Scheme 14. Reaction of Taxol with $NaBH_4$



Stable Indefinitely

Scheme 15. Reaction of Taxol with $(nBu)_4NBH_4$

The mixture of taxol and cephalomannine which had been used to produce baccatin III was obtained, by Polysciences Inc., from the recrystallization of impure taxol. Yields of baccatin III using this crude material were 40-50%. When a sample of pure taxol was used the yield of isolated baccatin III was 97%.

3.2.2.1 REACTION MECHANISM

The reduction of taxol by borohydride is an unusual reaction in that an ester is being reduced in the presence of a ketone, and borohydride is commonly used to reduce ketones in the presence of esters.

The reactivity of the ester linkage at the 13 position of taxol is due to the hydroxyl group alpha to the ester. The importance of the alpha hydroxy functionality was shown in two experiments in which it was not present: the reaction of 2'-acetyltaxol with sodium borohydride in isopropanol or with tetrabutylammonium borohydride in methylene chloride. In the reaction with sodium borohydride several hours at room temperature were required to consume the 2'-acetyltaxol and there were several processes occurring, as shown by the hplc chromatogram (Figure 11). While these products were not isolated, from what is known about the reactivity of taxol it is possible to assign tentative structures to the peaks seen by hplc. The slightly basic solution of isopropanol

epimerized the 7 position and also removed the C-2' acetyl group. The products from the removal of the C-2' acetyl group then reacted with the borohydride, with the side chain being reduced, so that only a small amount of these products were seen by hplc. Reaction of 2'-acetyltaxol with tetrabutylammonium borohydride in CH_2Cl_2 produced no reaction at all as there were no nucleophiles present to remove the C-2' acetyl group. These reactions show that the presence of a C-2' acetyl group on taxol prevents the side chain from being reduced by borohydride.

The reactivity of the ester linkage at position 13 therefore can be explained as shown in Scheme 16. In the first step of the reaction the borohydride condenses with the 2' hydroxyl group producing an intermediate in which the reducing agent has been brought close to the ester center it is to react with. In the reduction step an intramolecular five membered ring is formed, which in terms of entropy of activation, is much more favored than the intermolecular reaction necessary for an isolated ester. Activation of an ester by an alpha hydroxyl group was also seen by Plaumann et al.[41] (Scheme 17). Aromatic esters are normally reduced only slowly by BH_3. The reactivity of the ester in Scheme 17 was ascribed to the influence of the adjacent hydroxyl group.

Further evidence for the role of the 2' hydroxyl group in the reduction of the ester linkage and an explanation for the low reactivity of borohydride in methanol is contained in

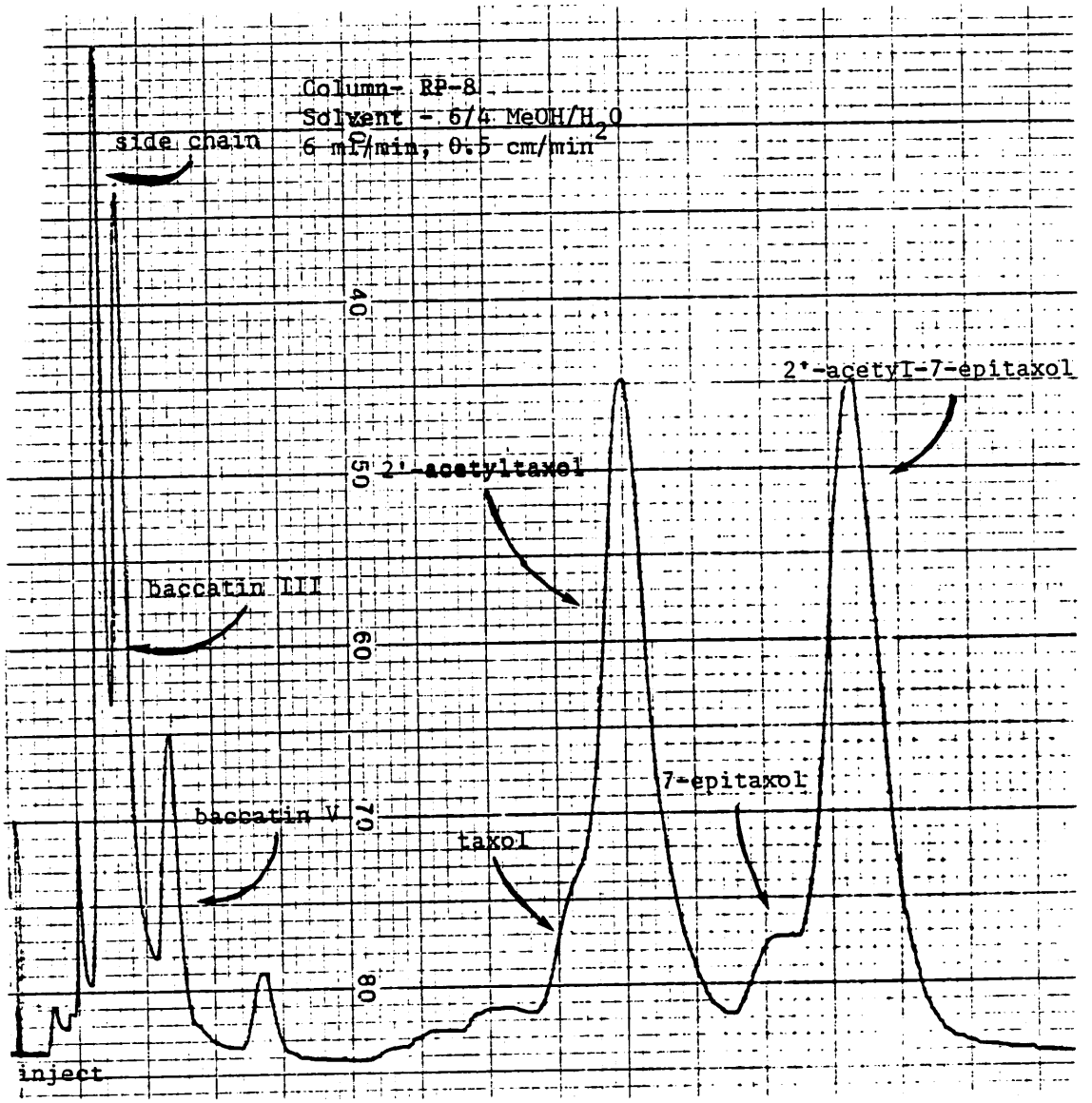
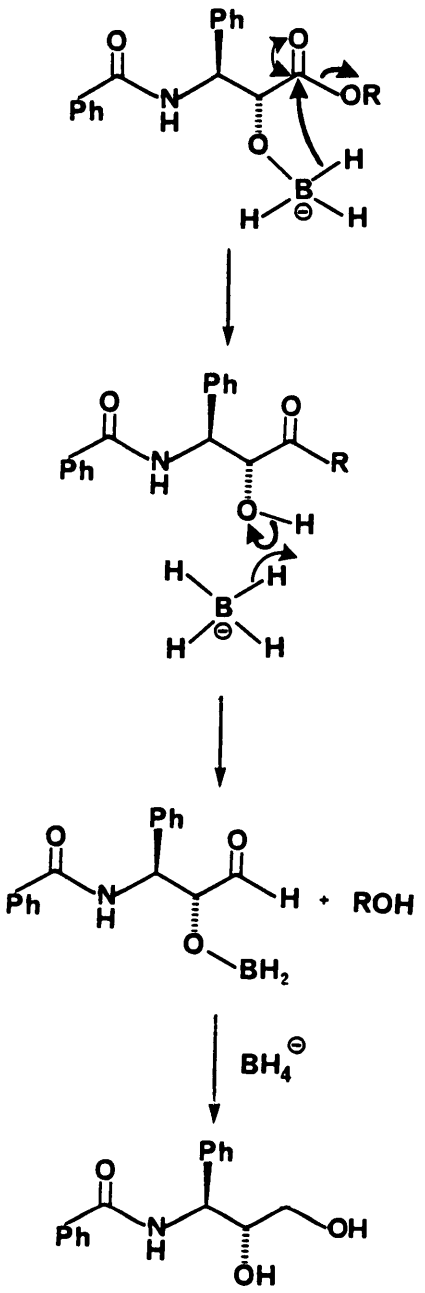
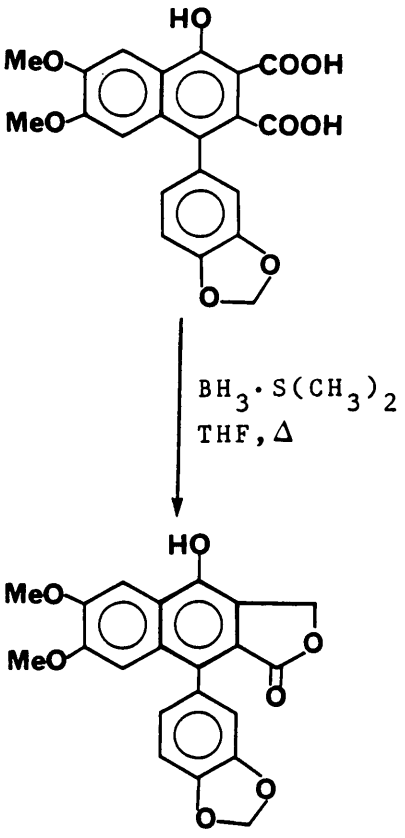


Figure 11. HPLC Chromatogram of the Reaction of 2'-Acetyltaxol with $NaBH_4$ in 2-Propanol



Scheme 16. Mechanism of the Reduction of Taxol by Borohydride

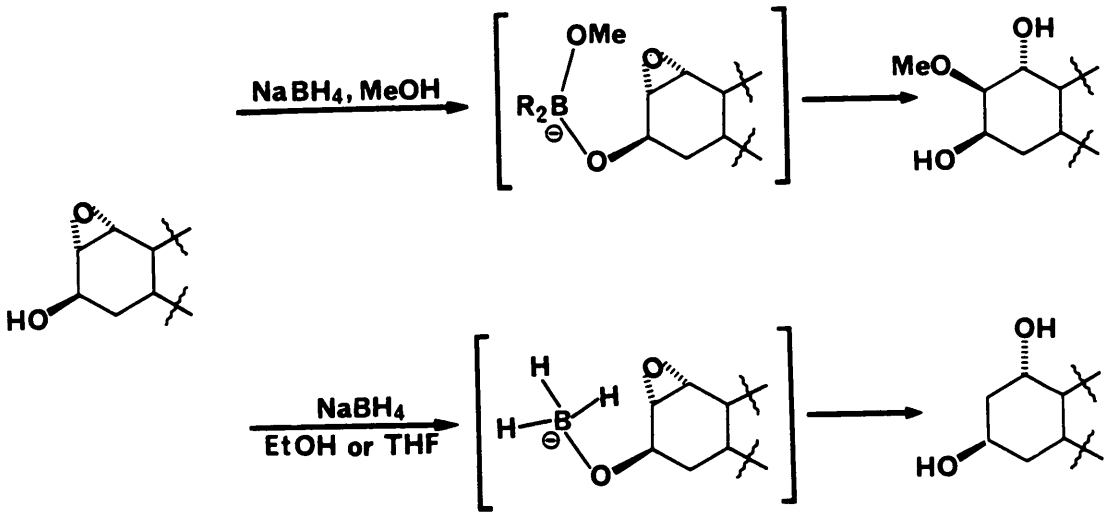


Scheme 17. Activation of an Ester by an Alpha Hydroxyl Group

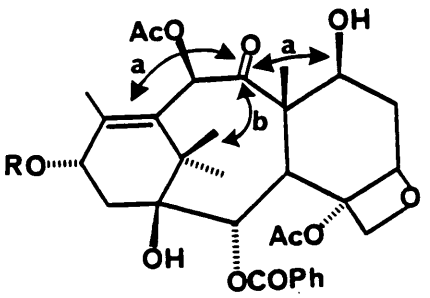
work conducted by Weissenberg et al. (Scheme 18).[42] In this work steroid epoxides, normally not reduced by sodium borohydride, were reduced when a trans diaxial alpha hydroxyl group was present. When methanol was used as solvent a methoxide group attacked the epoxide and when ethanol or THF was used hydride attack occurred. This reaction not only shows the activation of borohydride by an alcohol but also that borohydride behaves differently in methanol than in more hindered alcoholic solvents.

Cleavage of the taxol side chain by borohydride in methanol might have taken place by addition of methoxide to the ester. The transition state in such a reaction would be more crowded than the analogous hydride addition. As has been shown by the forcing conditions necessary to acetylate baccatin III, an ester linkage at the 13 position is sterically hindered. It is because of these steric factors that addition of methoxide by borohydride to the ester linkage at C-13 would have been a slower process than hydride addition.

The failure of the ketone at position 10 to react with the hydride reagents can be explained by the steric hindrance of the position. Approach to the ketone from the beta face is blocked by the gem dimethyl groups at 16 and 17. Approach to the ketone from the alpha face is blocked by the concave face of the taxol skeleton (Figure 12).



Scheme 18. Reaction of an Epoxy Alcohol with Borohydride



a - Folded ring structure blocks the alpha face.

b - Geminal dimethyl groups block the beta face.

Figure 12. Steric Hindrance of Taxol's C-10 Keto Group

3.2.3 ACETYLATION OF BACCATIN III

The second portion of the baccatin III project to be addressed was selective acylation of the C-13 hydroxyl group of baccatin III. Review of the literature with regard to this problem gave conflicting information. Powell et al.[33] reported that mild acetylation of baccatin III yielded 13-acetylbaccatin III. Senilh et al.[14] reported that acetylation of baccatin III yielded 7-acetylbaccatin III. Neither article reported characterization data for the compound(s).

Elimination of the uncertainty evident in the literature was accomplished by acetylation of baccatin III and characterization of the product. Acetylation of baccatin III with acetic anhydride in pyridine at room temperature for 4 hours yielded a single product in addition to unreacted baccatin III. Characterization of this product showed it to be 7-acetylbaccatin III **45**.

Characterization of the product by ^{1}H-nmr showed that the major change from the spectrum of baccatin III was the presence of a peak at 5.58 ppm (dd, J=2,11). The signal corresponding to the protons at C-7 in baccatin III at 4.4 ppm (m) was absent and the signal corresponding to the proton at C-13 in baccatin III at 4.82 ppm (br t, J=8) was present at 4.83 ppm (br t, J=9). In all taxol-like compounds characterized by ^{1}H-nmr in this entire research project (except for those not possessing an oxetane) the protons at C-13 are seen as

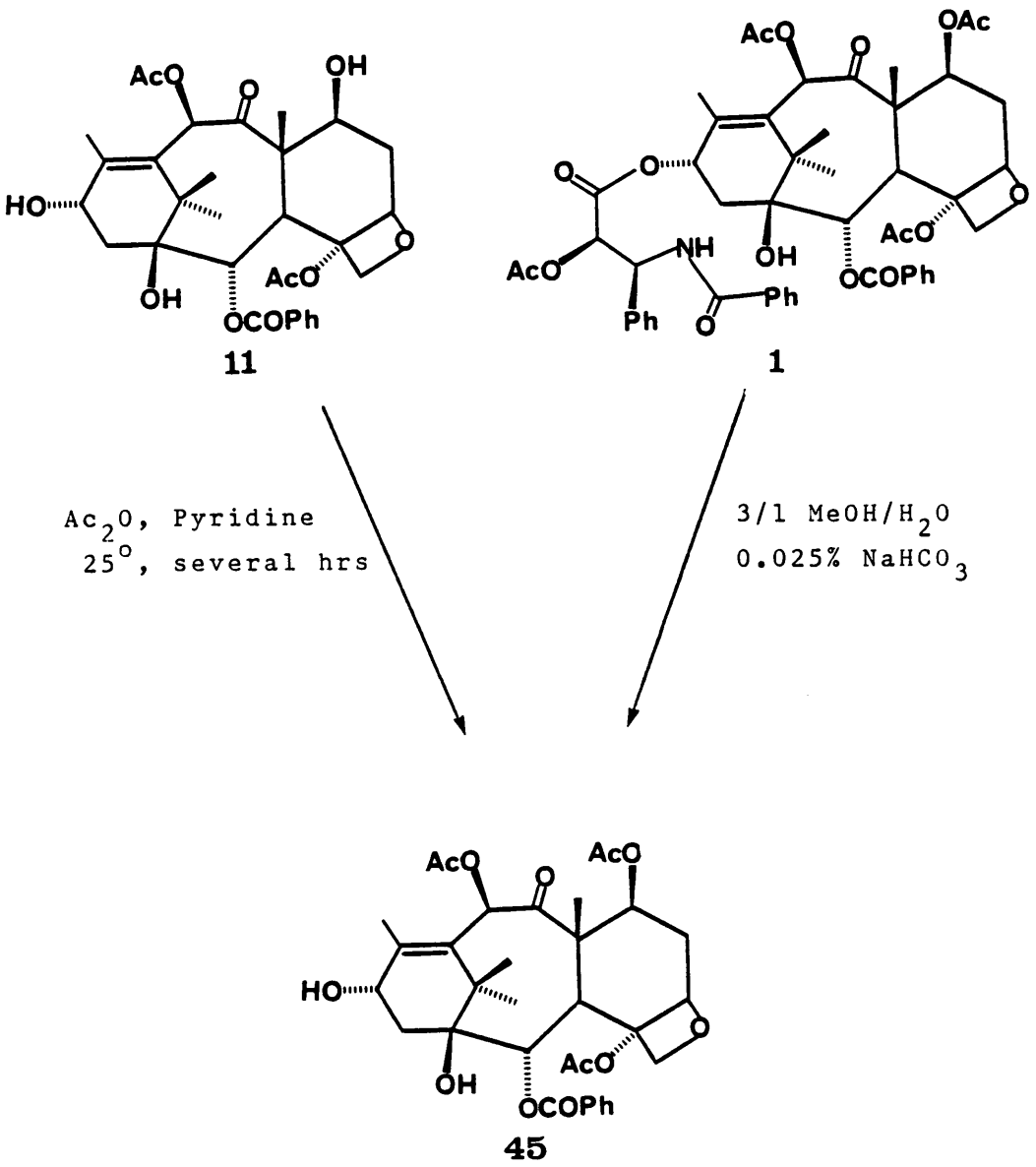
broad triplets and those at C-7 are seen either as multiplets or doublet of doublets.

Characterization of the product by mass spectrometry showed the molecular weight to be 628 which corresponds to the addition of one acetyl group to baccatin III. Losses of water and acetic acid(s) were also seen in the mass spectrum. In addition to these losses prominent $M-2^+$ peaks were seen. These peaks were caused by loss of hydrogen, which from C-13 would have formation of an enone as its driving force.

The ^{1}H-nmr, ir, and mass spectra of the 7-acetylbaccatin III obtained form acetylation of baccatin III matched the spectra of the 7-acetylbaccatin III obtained as a side product in the methanolysis of 2',7-diacetyltaxol (Scheme 19).

The acetylation of baccatin III showed that the C-7 hydroxyl group of baccatin III is more reactive, with respect to esterification, than the C-13 hydroxyl group. Preparation of C-13 esterified baccatin III therefore must entail protection of the C-7 hydroxyl, esterification of the C-13 hydroxyl and deprotection of the C-7 hydroxyl.

The C-7 hydroxyl of baccatin III was protected by reacting baccatin III with 2,2,2-trichloroethyloxycarbonylchloride for 5 minutes at 0° (Scheme 20). This protecting group had been used by Senilh[14] to protect the C-7 hydroxyl group of taxol and can be cleaved with zinc in methanol or acetic acid.

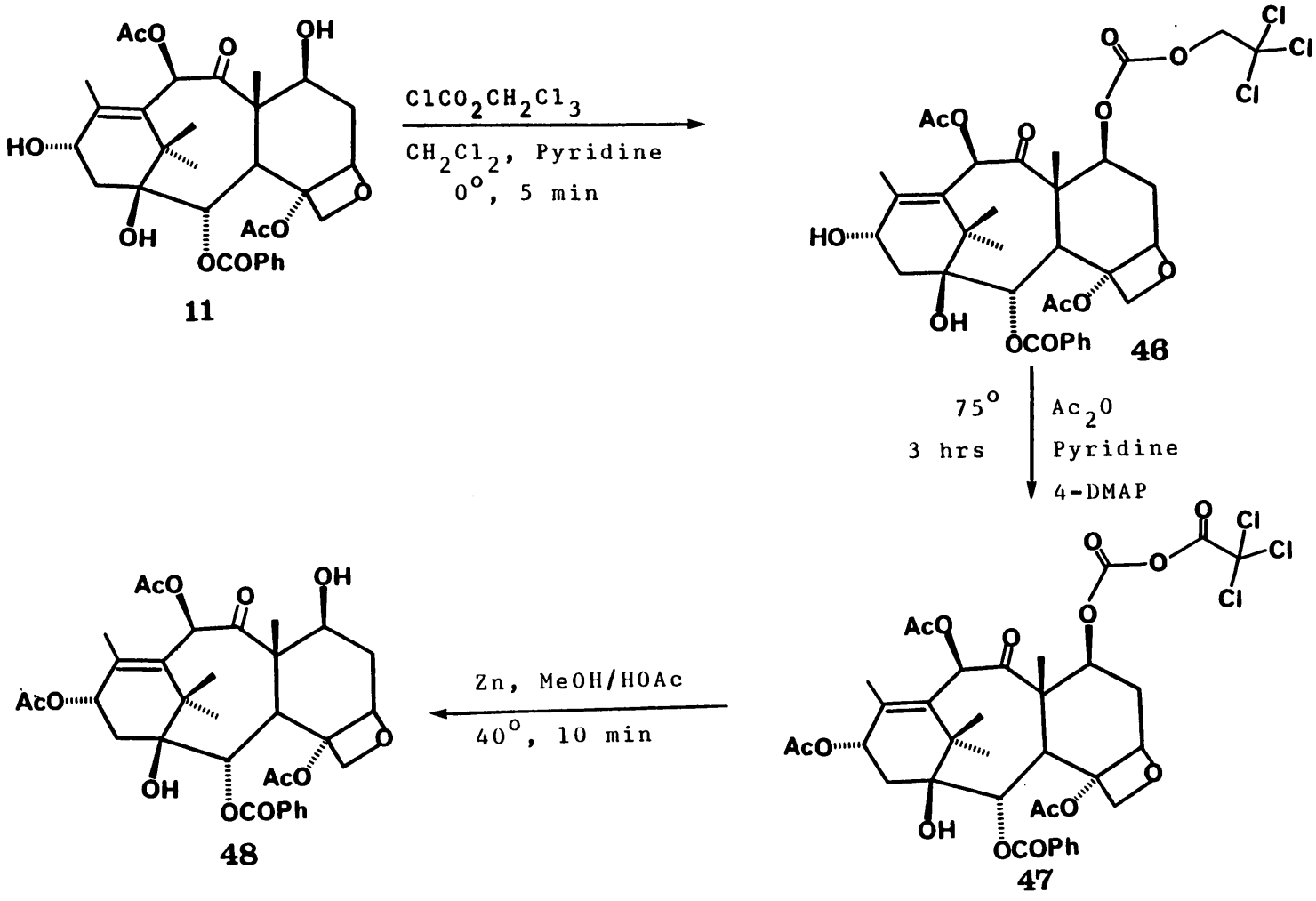


Scheme 19. Preparation of 7-Acetylbaccatin III from Baccatin III or 2',7-Diacetyltaxol

The major change in the ^{1}H-nmr spectrum of 7-(2,2,2-trichloroethyloxycarbonyl)baccatin III 46 when contrasted with baccatin III is the shift of the C-7 proton signal from 4.4 ppm (m) to 5.60 ppm (dd, J=7,10). The methylene protons of the protecting group were seen in the ^{1}H-nmr spectrum at 4.62 ppm (d, J=12) and 5.02 ppm (d, J=12). The mass spectrum gave the molecular weight as 760 and also showed peaks for loss of acetic acid and water. Absorption for the carbonate carbonyl stretching was seen in the infrared spectrum at 1775 cm^{-1}.

Acetylation of the C-13 hydroxyl group was achieved with excess acetic anhydride, pyridine, 4-dimethylaminopyridine and heating at 75° for several hours. Deprotection of C-7 was then effected by stirring 13-acetyl-7-(2,2,2-trichloroethyloxycarbonyl)baccatin 47 with zinc in methanol and acetic acid at room temperature (Scheme 20).

The ^{1}H-nmr signal for the C-13 proton of 13-acetylbaccatin III occurs at 6.16 ppm (td, J=1,8) and its chemical shift is almost identical to that seen for the C-13 proton of taxol 6.18 ppm (br t, J=8). The signal for the C-7 proton occurs at 4.42 ppm (br t, J=8). The mass spectrum gave the molecular weight as 628 and peaks resulting from loss of acetic acid and water were also present. The infrared spectrum does not differ significantly from that of baccatin III and does not show a peak at 1775 cm^{-1} which had been seen when the carbonate protecting group was present.



Scheme 20. Selective Acylation of the 13 Position of Baccatin III

3.3 EXPERIMENTAL PROCEDURE

Methanolysis of Taxol/Cephalomannine - Samples of a 6/4 taxol/cephalomannine mixture (approximately 1 mg each) were reacted with a series of solutions of triethylamine in methanol. The concentration of triethylamine ranged from 0.01% to 1.0% and the reaction temperatures were either 0° or 25°. The reactions were monitored by hplc solely for the purpose of determining if a reasonable yield of baccatin III could be obtained under optimized conditions. Authentic samples of baccatin III and baccatin V were used as standards.*

All of the reactions produced similar results, differing only in the rate of reaction - a complex mixture of products at all stages of the reactions. For example, hplc analysis (RP-8, analytical column, 6/4, CH_3CN/H_2O, 2 mL/min), of the taxol cephalomannine sample in 0.5% triethylamine/methanol at 25° after reacting 2 hours showed:

---

* Authentic samples of Baccatin III and Baccatin V were obtained from R. G. Powell.

Compound (a)	% of Mixture(b)	Retention Time (min)
7-Epi(taxol/cephalomannine)	22	19.3
Taxol/cephalomannine	26	14.3
10-Deacetyl-7-epi(taxol/cephalomannine)	5	10.4
10-Deacetyl(taxol/cephalomannine)	5	8.8
Polar mixture of products	42	<5

(a) - No compounds were isolated; the products are what would be expected from their polarities and literature reports.[33]

(b) - By peak area.

Reaction of Taxol/Cephalomannine with Triethylamine in 2-Propanol - A sample of 6/4 taxol/cephalomannine was dissolved in 1.0% triethylamine in 2-propanol at 25° for 24 hours and at 40° for 3 hours. At that point hplc analysis, (6/4, CH_3CN/H_2O, 2 mL/min) showed: 45% taxol/cephalomannine and 55% 7-epitaxol/7-epicephalomannine by peak area.

Solvolysis of Taxol/Cephalomannine with Sodium Isopropoxide in 2-Propanol - A 0.01M sodium isopropoxide in isopropanol solution was prepared by adding a 60% NaH dispersion in oil (35 mg) to dry isopropanol (90 mL) and the solution was cooled to 0° after the evolution of hydrogen had ceased. A

6/4 mixture of taxol/cephalomannine (8.4 mg) was then dissolved in 0.300 mL of the isopropoxide/isopropanol solution at 0°. After 1.67 hours hplc analysis (analytical, RP-8, 6/4 CH_3CN/H_2O, 2 mL/min) showed: 22% 7-epitaxol/cephalomannine, 13% taxol/cephalomannine, 37% baccatin V, and 12% baccatin III by peak area. From this point on the amount of baccatin V present increased with decreasing amounts of the other specified products.

Stability of Taxol in Tartaric Acid - Taxol (1 mg) was added to a 0.1M solution of tartaric acid in methanol whose pH had been adjusted to 3.0 with NaOH. The solution was analyzed by reverse phase analytic hplc over a period of 72 hours (6/4 $MeOH/H_2O$, 2 mL/min). During this time no decomposition of the taxol was seen.

Reaction of Taxol with Sodium Cyanoborohydride - Taxol (1 mg) and $NaBH_3CN$ (10 mg) were added to a 0.1M solution of tartaric acid in methanol (1 mL). The reaction was stirred at room temperature and analyzed by hplc. After 24 hours at room temperature no reaction could be seen by hplc (RP-8 analytical column, 65/35 $MeOH/H_2O$, 2 mL/min) Rf of taxol - 5.8 min, Rf of tartaric acid - 0.8 min. At that point the reaction was heated to 40° for an additional 24 hours at the end of which hplc analysis again showed no reaction at all having taken place.

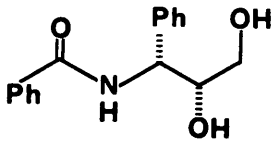
Rate of Reaction of Taxol with Sodium Borohydride in Methanol or 2-Propanol - A sample of taxol (1 mg) was dissolved in 1.0 mL of dry MeOH along with 7.6 mg of $NaBH_4$ at room temperature. The reaction was monitored by hplc (7/3 MeOH/H_2O) for the purpose of determining the rate at which taxol reacted. After 1.33 hours hplc showed that 18% of the starting taxol (by peak area) remained. After standing overnight (15 hours) hplc showed no taxol and a mixture of polar products present. In contrast to the slowness of the reaction in methanol, reaction of a sample of taxol (1mg) in 1 mL of 2-propanol with 7.6 mg $NaBH_4$ (which only partially dissolved) was completely reacted after 30 minutes at room temperature.

Reaction of Taxol with $NaBH_4$ in 2-Propanol - Taxol (20 mg) was reacted with $NaBH_4$ (10 mg) in 1 mL of dry 1-propanol. The reaction was stirred at room temperature for 1 hour. The reaction was stopped by the addition of a drop of acetic acid and the products were extracted with CH_2Cl_2 after the addition of several mL of water. The products were purified using preparative hplc RP-8, 55/45, MeOH/H_2O, 6 mL/min, 0.5 cm/min. Three products were isolated: a reduced side chain of taxol 44 (2.4 mg), baccatin III 11 (4.2 mg) and baccatin V 43 (5.4 mg). Characterization data for the products are shown in Table 16 and Table 17.

Reaction of a Taxol/Cephalomannine Mixture with $NaBH_4$ in 2-Propanol at 0° - Sample consisting of approximately 6/4 taxol/cephalomannine (190 mg) was dissolved in 10 mL of dry 2-propanol at 0° along with $NaBH_4$ (120 mg). The reaction was stirred and monitored by hplc RP-8 analytical column. The reaction was allowed to proceed for 3 hours, 20 minutes at which time it was stopped by the addition of acetic acid. As previously described the products were extracted after the addition of water. The sample was purified using a Chromatotron® (Silica Gel, 1000um, 95/6, $CHCl_3$, MeOH). The only taxane product present was baccatin III as shown by hplc analysis of the crude reaction mixture.

Reaction of Taxol/Cephalomannine with Tetrabutylammonium Borohydride - A mixture of 6/4 taxol/cephalomannine (12.5 mg) was reacted with tetrabutylammonium in 1.0 mL of dry CH_2Cl_2 at room temperature. The reaction was complete after 45 minutes but was allowed to stand at room temperature for 14 hours to see if the baccatin III produced was stable. After 14 hours hplc showed less than 1% baccatin V present (with respect to baccatin III). The reaction was monitored by hplc (analytical, RP-8, 2mL/min, 55/45 MeOH/H_2O). The products had the following retention times: reduced side chains of taxol and cephalomannine, 3.2 min; baccatin III, 4.9 min; baccatin, 7.7 min.

Table 16 Characterization Data for the Reduced Side Chain of Taxol



Position	Shift (ppm from TMS) Coupling (hertz)
1'	3.62 (br t, 2.5)
2'	4.13 (m)
3'	5.34 (dd, 3.5,8)
N-H	6.92 (d, 8), 8.37 in DMSO
3' NBz	7.80 (d, 7), 7.2-7.6 m
3' Ph	7.2-7.6 (m)

m.p. 147-148

Mass Spectral Data

272 $(MH)^+$, 155, 135,

119, 105, 103.

High Resolution

$(MH)^+$ - $C_{16}H_{17}NO_3$ - 272.1287

Calculated - 272.1313

Infrared Spectral Data

3570 s, 3375 s, 3100 w,

3000 w, 2980 w, 1655 s,

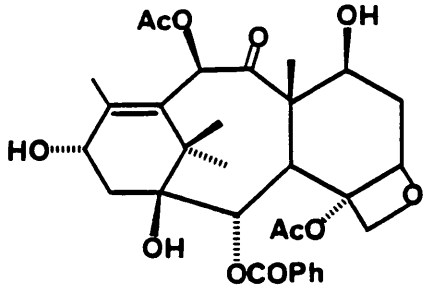
1550 s, 1515 w, 1475 w,

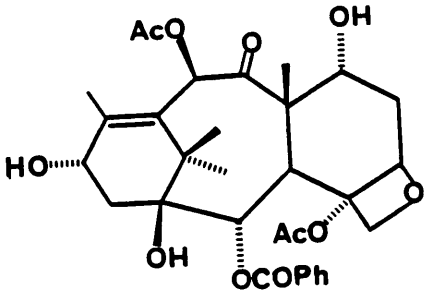
1410 w, 1355 w, 1130 mw,

1010 mw, 1045 mw,

770 mw, 715 m.

Table 17 The ^1H NMR Spectra of Baccatin III and Baccatin V





Position	Shift (ppm from TMS) Coupling (hertz)	Shift (ppm from TMS) Coupling (hertz)
2	5.58 (d, 7)	5.71 (d, 7.5)
3	3.84 (d, 7)	4.04 (d, 7.5)
5	4.94 (dd, 2,8)	4.93 (dd, 3,5)
6	2.4 (m)	2.1 (m)
7	4.4 (m)	3.67 (d t)
10	6.28 (s)	6.82 (s)
13	4.82 (br t, 9)	4.8 (m)
14	2.4 (m)	2.3 (m)
16	1.22 (s)	1.11 (s)
17	1.12 (s)	1.05 (s)
18	1.78 (br s)	1.99 (d, 1.5)
19	1.66 (s)	1.63 (s)
20	4.4 (d), 4.26 (d, 8)	4.35 (d), 4.36 (d, 9)
OAcs	2.20 (s), 2.24 (s)	2.20 (s), 2.35 (s)
2 OBz	7.46 (m), 8.05 (dd, 2,8)	7.52 (m), 8.12 (dd, 2,8)

High Yield Preparation of Baccatin III **11** from Taxol - A sample of taxol (100 mg) was dissolved in 2.0 mL of dry CH_2Cl_2 and reacted with nBu_4N (50 mg) for the purpose of determining the isolated yield of baccatin III from the reaction. After a reaction time of 1 hour at room temperature the reaction was stopped by the addition of 0.5 mL acetic acid. After stirring for an additional 10 minutes the solvents were removed *in vacuo* and baccatin III isolated via preparative tlc (silica gel, 1000µm, 6/4, EtOAc/Hexane). The product was shown to be homogenous by tlc and ^{1}H-nmr. Yield-65 mg, 97%.

Reaction of 2'-Acetyltaxol with Sodium Borohydride - A sample of 2'-acetyltaxol (4.0 mg) was dissolved in 0.300 mL of a saturated $NaBH_4$/2-propanol solution at 0°. After 30 min hplc showed no reaction taking place and the reaction was warmed to room temperature. After 2.5 hours at room temperature hplc (Figure 11) showed that most of the mixture consisted of starting material and 2'-acetyl-7-epitaxol.

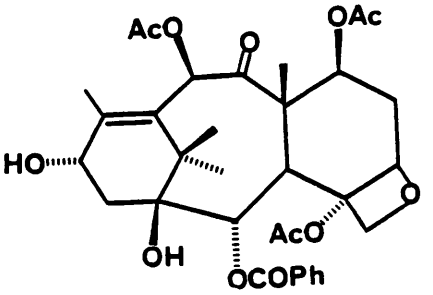
Reaction of 2'-Acetyltaxol with Tetrabutylammonium Borohydride - A sample of 2'-acetyltaxol (1 mg) was dissolved in 1 mL of dry CH_2Cl_2 along with tetrabutylammonium borohydride (5 mg) at room temperature. Over a period of 48 hours tlc analysis (silica gel, 6/4, EtOAc, Hexane) showed no reaction taking place.

Acetylation of Baccatin III - Baccatin III (35 mg) was dissolved in 1.0 mL of dry pyridine along with acetic anhydride (70 µL) at room temperature. The reaction was allowed to proceed for 4 hours at which time it was worked up as usual. Isolation of the product was carried out by prep. tlc (silica gel, 6/4, EtOAc, Hexane) to yield 7-acetylbaccatin III 45, Rf 0.47, yield 13 mg, 35%, and baccatin III, Rf 0.34, yield 18 mg, 51%. Characterization data for 7-acetylbaccatin III are shown in Table 18.

Preparation of 7-(2,2,2-Trichloroethyloxycarbonyl)baccatin III **46** - Baccatin III (104 mg) and 4-dimethylaminopyridine (2.8 mg) were dissolved in 1.0 mL of dry CH_2Cl_2 and pyridine (50 µL). To this solution 2,2,2-trichloroethylchloroformate eq) was added at room temperature. After 40 minutes an additional 30 µL of the 2,2,2-trichloroethylchloroformate was added as not all of the baccatin III had reacted. The reaction was worked up as usual 3 minutes after this second addition of chloroformate to yield 7-(2,2,2-trichloroethyloxycarbonyl)baccatin of the crude product by tlc (silica gel, 7/3, EtOAc/Hexane) showed the product (Rf 0.62) to be at least 95% pure; only a very faint spot with Rf 0.88 was seen as an impurity. This impurity was most likely the disubstituted product. Characterization data for 7-(troc)baccatin III are shown in Table 19.

Table 18. Characterization Data for 7-Acetylbaccatin III

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.60 (d, 7)
3	3.98 (d, 7)
5	4.95 (br d, 9)
6	2.5-2.7 (m)
7	5.58 (dd, 2,11)
10	6.24 (s)
13	4.83 (br t, 8)
14	2.2 (m)
16	1.11 (s)
17	1.05 (s)
18	1.77 (s)
19	2.08 (s)
20	4.02 (d, 8.5), 4.30 (d, 8.5)
OAcs	2.27 (s), 2.13 (s), 2.00 (s)
2 OBz	8.09 (t, 7), 7.59 (t, 7), 7.46 (t, 7)



Mass Spectral Data

651 $(MNa)^+$, 649 $(MNa-H_2)^+$,

629 $(MH)^+$, 627 $(MH-H_2)^+$,

569 $(MH-HOAc)^+$,

567 $(MH-HOAc-H_2)^+$,

551 $(MH-HOAc-H_2O)^+$,

509 $(MH-2HOAc)^+$.

Infrared Spectral Data

1770 s, 1745 s, 1700 w,

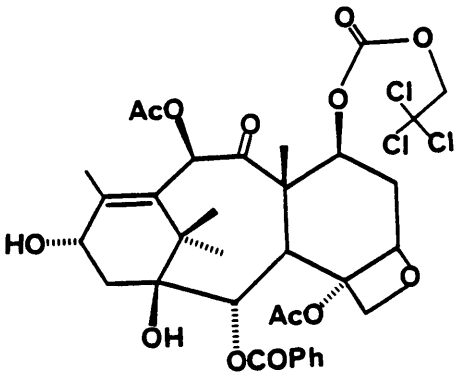
1395 mw, 1260 s, 1200 w,

1145-980 broad band-w,

730 mw.

Table 19 Characterization Data for 7-(2,2,2-Trichloroethyloxycarbonyl) baccatin III

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.62 (d, 7)
3	4.00 (d, 7)
5	4.97 (d, 9)
6	2.6 (m), 2.3 (m)
7	5.60 (dd, 7,10)
10	6.37 (s)
13	4.82 (br t, 7)
14	2.0-2.3 (m)
16	1.11 (s)
17	1.07 (s)
18	1.80 (s)
19	2.10 (s)
20	4.00 (d, 8), 4.31 (d, 8)
OAcs	4.28 (s), 4.14 (s)
2 OBz	8.08 (t, 8), 7.60 (t, 8), 7.47 (t, 8)
troc	4.62 (d, 12), 5.02 (d, 12)



Mass Spectral Data

761 $(MH)^+$, 701 $(MH-HOAc)^+$,

683 $(MH-H_2O-HOAc)^+$.

Infrared Spectral Data

1770 s, 1730 s, 1660 w,

1550 w, 1385 m, 1290 s-sh,

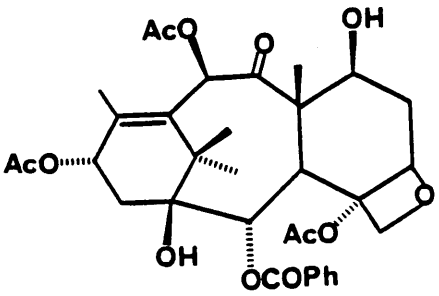
1260 s, 1115 w, 1160w,

980 w, 720 w.

Preparation of 13-Acetylbaccatin III - 7-(2,2,2-Trichloroethyloxycarbonyl)baccatin (70 mg) was dissolved in 1.0 mL of dry CH_3CN along with 4-dimethylaminopyridine (2.4 mg) and acetic anhydride (100 µL). The reaction was conducted for 2.5 hrs at 75° after which it was worked up in the usual manner to yield crude 7-(2,2,2-trichloroethyloxycarbonyl)-13-acetylbaccatin Analytical tlc (silica gel, 7/3, EtOAc/Hexane) showed in addition to the product with Rf 0.58 a good deal of streaking but no single major impurity. The product was purified by prep. tlc (1/1, EtOAc/Hexane Rf 0.65). The purified protected 13-acetylbaccatin III was reacted with Zn dust (100 mg) in 2 mL of 1/1 MeOH/HOAc at 40° for 10 min. The reaction was worked up by filtering, evaporating the solvent in vacuo, redissolving in CH_2Cl_2 and refiltering to remove Zn salts. The product 13-acetylbaccatin III was tlc pure (silica gel, 1/1 EtOAc/Hexane, Rf 0.14) and also was shown to be pure by proton nmr. Characterization data for 13-acetylbaccatin III are shown in Table 20.

Table 20 Characterization Data for 13-Acetylbaccatin III

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.64 (d, 7)
3	3.81 (d, 7)
5	4.95 (dd, 1.5,9)
6	2.45-2.60 (m)
7	4.42 (br t, 8)
10	4.28 (s)
13	6.16 (t d, 1,8)
14	2.2 (m)
16	1.21 (s)
17	1.10 (s)
18	1.65 (s)
19	1.89 (s)
20	4.14 (d, 8), 4.28 (d, 8)
OAcs	2.30 (s), 2.22 (s), 2.18 (s)
2 OBz	8.05 (d, 7.5), 7.40 (t, 7.5), 7.47 (t, 7.5)



Mass Spectral Data

651 $(MNa)^+$, 629 $(MH)^+$,

611 $(MH-H_2O)^+$,

551 $(MH-HOAc-H_2O)^+$,

509 $(MH-2HOAc)^+$.

High Resolution

$(MH)^+$ - $C_{33}H_{40}O_{12}$ - 629.2541

Calculated - 629.2599

Infrared Spectral Data

1760 s-sh, 1740 s,

1725 s-sh, 1670 w,

1570 w, 1390 w,

1295 m-sh, 1255 s,

1120 w, 1090 m, 1060 m,

1000 w, 720 w.

4.0 OXIDIZED TAXOLS AND RELATED PRODUCTS

4.1 INTRODUCTION

This section of the taxol project addresses the relationship of the oxidation levels of C-2' and C-7 of taxol to biological activity. The structure-activity relationships of the D ring will also be investigated. This will be possible because oxidation of the C-7 hydroxyl group activates the protons of C-6 allowing a beta elimination involving the D ring to occur, Figure 13.

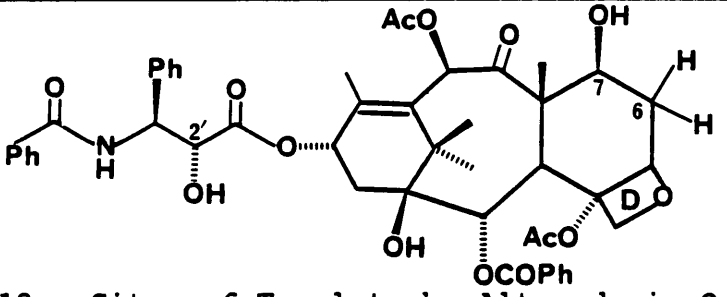


Figure 13. Sites of Taxol to be Altered via Oxidations

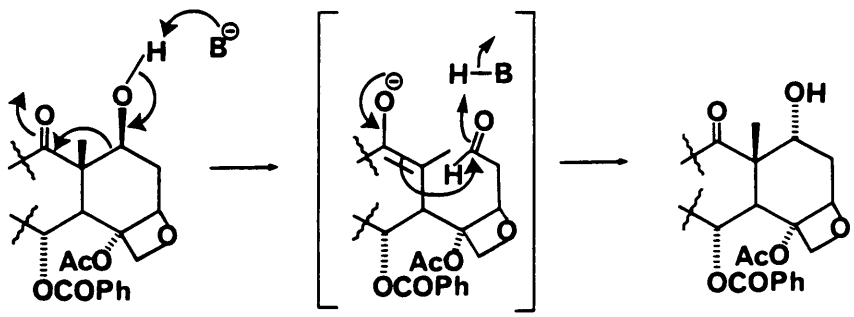
The a priori rationale for hypothesizing that oxidation of the C-2' and/or the C-7 hydroxyl group will cause a change in the biological activity of taxol is that either group may be involved in a chemical reaction in vivo.

The 7 position of taxol is known to undergo epimerization, presumably via a retro aldol reaction (Scheme 21). Oxidation of this hydroxyl group would prohibit a retro aldol reaction

from taking place. While active taxol derivatives possessing an acetyl[43] or xylosyl[14] group at C-7 are known, it is conceivable that these are hydrolyzed in vivo. This possibility would not exist in the case of C-7 being oxidized to a ketone.

Two hypotheses for the mechanism of action of taxol are that the C-13 ester linkage, activated by the alpha hydroxyl group, acts as an acylating agent or that the C-2' hydroxyl group assists taxol in binding to its receptor site. In either of these cases oxidation of the C-2' hydroxyl group to a ketone could change the activity of taxol.

Determination of the relationship of the oxetane ring of taxol to biological activity is of interest for two reasons. The first of these is that several compounds, containing the taxane ring structure but not the oxetane ring, have been obtained from the yew plant in yields higher than taxol.[9] The necessity of an oxetane ring for biological activity may be the key factor in determining if these compounds can be transformed into active anticancer compounds in high yield. The second reason is that the oxetane ring imposes a great degree of rigidity on the taxane system. Opening of the oxetane may change the shape of the skeleton and so affect the binding of the modified drug to its receptor site.



Scheme 21. Base Catalysed C-7 Epimerization of Taxol

4.2 RESULTS AND DISCUSSION

4.2.1 OXIDATION OF TAXOL

Jones oxidation of 2'-N-carbobenzoxy-β-alanyltaxol **49** yielded 2'-N-carbobenzoxy-β-alanyl-7-oxotaxol **50** in a reaction in which no trace of other products could be seen by tlc analysis.* Deprotection by hydrogenation in methanol with palladium on carbon as catalyst yielded 7-oxotaxol **51**. The product 7-oxotaxol was also obtained via Jones oxidation of 2'-(2,2,2-trichloroethyloxycarbonyl)taxol **52** followed by deprotection using zinc in methanol and acetic acid. The synthesis of 7-oxotaxol, in addition to yielding the desired product, showed that the C-2' hydroxyl group can be protected and deprotected under neutral or mildly acidic conditions.

Preparation of the protected taxol 2'-(2,2,2-trichloroethyloxycarbonyl)taxol had been reported by Senilh[14] but no characterization data had been given. The published procedure for preparation of 2'-(2,2,2-trichloroethyloxycarbonyl)taxol involved reaction of taxol with 2,2,2-trichloroethylchloroformate in pyridine at room temperature for several hours. In this research it was found that reaction of taxol with

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* The synthesis and characterization of this C-2' protected taxol is included in Chapter 7.

2,2,2,-trichloroethylchloroformate at -23° with addition of 1 equivalent of the chloroformate over 45 minutes yielded 85% of the monosubstituted product along with small amounts of taxol and the disubstituted product.

Addition of the 2,2,2-trichloroethyloxycarbonyl protecting group caused the signal for the C-2' proton to be shifted downfield to 5.52 ppm (d, J=2.5) and the signals for the protecting group protons are seen as a distorted AB quartet at 4.73 ppm (d, J=11) and 4.79 ppm (d, J=11). Peaks in the mass spectrum at m/z 1028 $(MH)^+$ and 509 $(MH-RCOOH-HOAc-H_2O)^+$ showed the addition of one protecting group to the side chain of taxol. The carbonate carbonyl absorbance was seen in the infrared spectrum at 1780 cm^{-1}.

The 7-oxotaxol produced by both methods was homogenous on tlc and ^{1}H-nmr showed that only one taxol-like compound was present in each case. The major change in the ^{1}H-nmr spectrum was seen for the C-6 protons. In taxol the signal for these protons is seen as a multiplet at 2.0 ppm, while in 7-oxotaxol the signals occur at 3.10 ppm (d, J=19) and 2.59 ppm (dd, J=6,19). Irradiation of the doublet at 2.59 ppm caused the doublet at 3.10 ppm to collapse to a singlet, and the doublet for the C-5 proton at 5.06 ppm (J=6) also collapsed to a singlet.

The mass spectrum of 7-oxotaxol gave the molecular weight as 851, with m/z 852 $(MH)^+$, a loss of 2 mass units when contrasted with taxol. Major peaks in the mass spectrum at m/z

589 $(MNa-RCOOH)^+$, 567 $(MH-RCOOH)^+$, 549 $(MH-RCOOH-H_2O)^+$ and 507 $(MH-RCOOH-HOAc)^+$, where RCOOH is the side chain as an acid, show that the change in molecular weight occurs on the taxane skeleton. The spectral changes seen when 7-oxotaxol was contrasted with taxol were also seen when the ^{1}H-nmr and mass spectra of 2'-N-carbobenzoxy-β-alanyltaxol and 2'-(2,2,2-trichloroethyloxycarbonyl)-7-oxotaxol **53** were contrasted with their respective unoxidized forms.

As with the other C-2' protected taxols, oxidation of 2'-acetyltaxol proceeded to yield a single product as shown by tlc and ^{1}H-nmr analysis. The ^{1}H-nmr spectrum of the 2'-acetyl-7-oxotaxol product **54** included the C-6 protons at 2.84 ppm (d, J=19) and 3.09 ppm (dd, J=6,19). The mass spectrum gave the molecular weight as 893, with m/z 894 $(MH)^+$, two less than 2'-acetyltaxol, and peaks involving loss of the side chain showed that the loss of two mass units occurred on the taxane skeleton.

Attempted deprotection of 2'-acetyl-7-oxotaxol was carried out using 0.025% $NaHCO_3$ in a water-methanol solution with the reaction being followed by hplc. Reaction of the starting material to form a less polar product required 30 minutes. This initially formed product completely decomposed to a mixture of polar products in 1.5 hours. This decomposition occurred well within the time which had been required for removal of the C-2' acetyl group of 2',7-diacetyltaxol. Using KCN in ethanol at 0°, conditions reported to be suit-

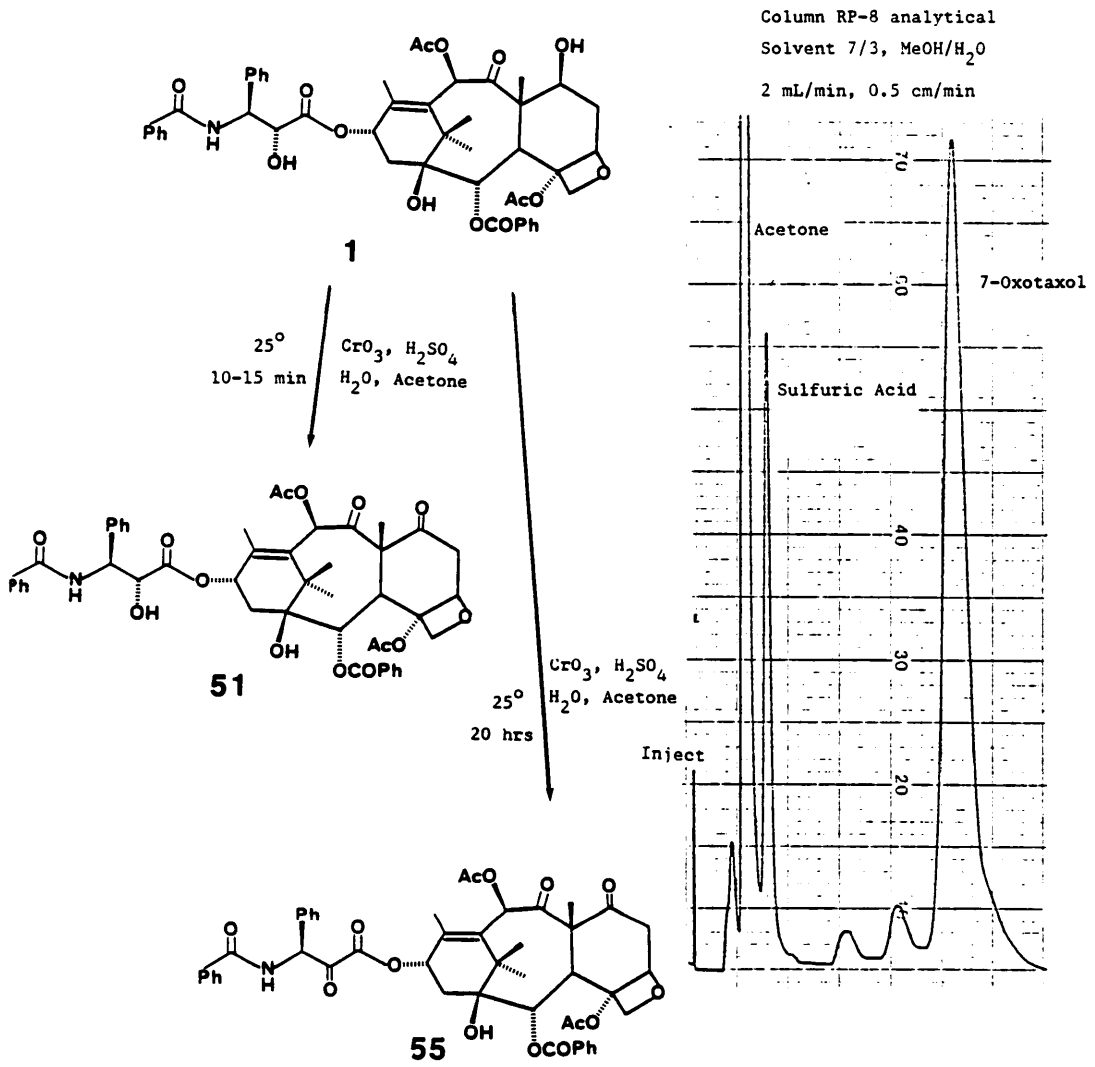
able for base sensitive compounds,[44] also produced decomposition of the starting material to a complex mixture of polar products. Decomposition of 2'-acetyl-7-oxotaxol indicated the severe base instability of taxol derivatives possessing a C-7 keto group.

It was later found that 7-oxotaxol could be obtained directly from taxol without protection of the C-2' hydroxyl group. Jones oxidation of taxol at 25° for 20 minutes and isolation of the product by preparative reverse phase hplc yielded 7-oxotaxol (Scheme 22).

Preparation of 2',7-dioxotaxol from taxol via Jones oxidation required a reaction time of 20 hours at 25° (Scheme 22). Isolation and purity of the compound will be discussed later.

The ^{1}H-nmr spectrum of 2',7-dioxotaxol, as with the 7-oxotaxols, showed the C-6 protons shifted downfield at 2.82 ppm (dd, J=1,19) and 3.07 ppm (dd, J=7,19). The signal for the proton at C-2', 4.73 ppm (d, J=3) in taxol, was absent. The signal for the proton at C-3' was seen as a sharp doublet at 6.41 ppm (J=5.5) contrasted with the same proton seen at 5.73 ppm (dd, J=3,9) in taxol.

The mass spectrum of 2',7-dioxotaxol gave the molecular ion peak at m/z 850 $(MH)^+$, a loss of four mass units when contrasted with taxol. A peak at m/z 507 $(MH-RCOOH-HOAc)^+$, contrasted with the analogous peak at m/z 509 in taxol,



Scheme 22. Jones Oxidations of Taxol

showed a loss of two mass units for the taxane skeleton and two mass units for the side chain.

After work-up the crude 2',7-dioxotaxol was seen on tlc as a major product spot with some streaking. Analysis of the crude product by ^{1}H-nmr also showed one major product with numerous small peaks from impurities but no one major taxol-like impurity. The crude product was purified by reverse phase hplc using 7:3 methanol/water as eluant. After isolation, which included rotary evaporation of the methanol and extraction of the product with ethyl acetate, ^{1}H-nmr showed that 2',7-dioxotaxol was contaminated with approximately 40% of a second taxol-like compound. Analysis of the purified 2',7-dioxotaxol by hplc showed the presence of a compound less polar than 2',7-dioxotaxol which had not been present during the initial isolation.

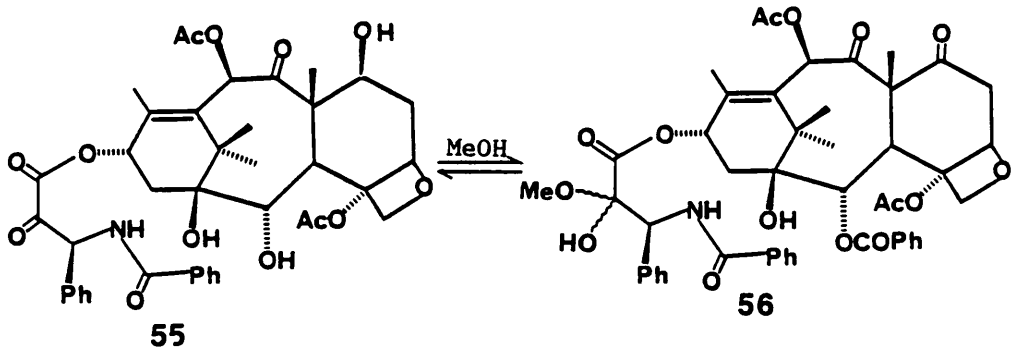
The ^{1}H-nmr spectrum of the mixture of 2',7-dioxotaxol and the second taxol-like compound showed two sets of signals which did not differ in multiplicity and differed in chemical shift by no more than 0.15 ppm. The spectrum did show the presence of a sharp singlet at 4.42 ppm and a small singlet at 4.47 ppm due to methanol as an impurity.

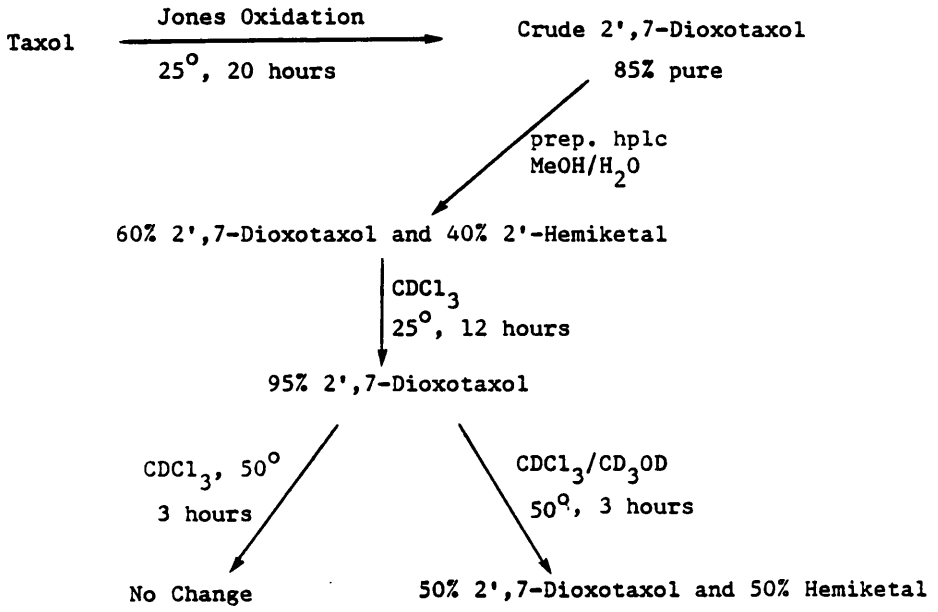
Upon standing overnight in $CDCl_3$ solution ^{1}H-nmr showed that the signals due to the second taxol-like compound had diminished to approximately five percent of those of 2',7-dioxotaxol. The sharp singlet at 4.42 ppm had diminished greatly and the methanol impurity peak had increased

so that it was the size that the 4.42 ppm singlet had originally been. Characterization and biological testing were performed on this 95% pure sample of 2',7-dioxotaxol. Heating the $CDCl_3$ solution at 50° for several hours produced no change in the ^{1}H-nmr. Addition of CD_3OD and heating at 50° for several hours however caused the ^{1}H-nmr spectrum to again exhibit peaks due to 2',7-dioxotaxol and those of the second taxol-like compound.

This spectral and chemical reactivity evidence indicate that in methanolic solution 2',7-dioxotaxol **55** was in equilibrium with its C-2' hemiketal **56** (Scheme 23). The singlet seen at 4.42 ppm in the ^{1}H-nmr spectrum was due to the methoxy group at C-2'. When the hemiketal was converted back into 2',7-dioxotaxol upon standing in $CDCl_3$ the loss of the methoxy group was seen along with a proportional increase in the MeOH peak. As with the ^{1}H-nmr spectra of all taxol analogues seen in this project a transformation at one center of the compound will cause small changes in the chemical shifts of almost all of the protons.

The importance of the formation of the hemiketal of 2',7-dioxotaxol under unusually mild conditions is that it showed the electrophilic character of the C-2' keto group. If taxol's mechanism of action is that the C-2' hydroxyl binds to a hydroxyl or amino group when taxol is bound to tubulin then C-2' oxidized taxols may also show activity. The C-2' oxidized taxols would be able to do this by having





Scheme 23. Reaction of 2',7-Dioxotaxol with Methanol

the group which would normally hydrogen bond to taxol's C-2' hydroxyl group attack the C-2' keto group. This would cause the C-2' oxidized taxol to be bonded to the receptor site in a reversible fashion.

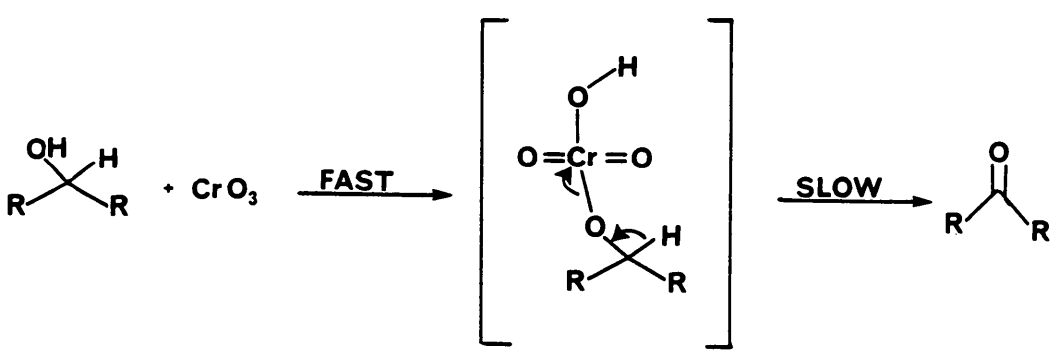
Jones oxidation of 7-acetyltaxol at room temperature for 24 hours yielded 2'-oxo-7-acetyltaxol **57**. This product was purified by preparative tlc with EtOAc and hexane as eluants so that the formation of a C-2' hemiketal was avoided. The ^{1}H-nmr spectrum showed the same changes for the side chain protons that had been seen for 2',7-dioxotaxol. The signal for C-3' 6.47 (d, J=9) was shifted downfield from that of 7-acetyltaxol 5.81 ppm (dd, J=3,9). The two sets of signals for the C-6 protons seen in the 7-oxotaxols were absent; the C-6 protons of 2'-oxo-7-acetyltaxol being seen as a complex multiplet at 2.2-2.5 ppm. Upon standing several days in $CDCl_3$ solution the product became discolored and decomposed so an infrared spectrum was not obtained.

The mass spectrum showed ions at m/z 894 $(MH)^+$ and 834 $(MH-HOAc)^+$ indicating the molecular weight as 893, two less than 7-acetyltaxol. Peaks at m/z 611 $(MH-RCOOH)^+$ and 551 $(MH-RCOOH-HOAc)^+$ where RCOOH is the side chain as an acid showed that the loss of two mass units from 7-acetyltaxol occurred on the side chain.

The Jones oxidations of taxol, 2'-acetyltaxol and 7-acetyltaxol showed the stability of taxol under acidic conditions. In a separate experiment taxol was dissolved in a 23%

H_2SO_4 solution which was identical to that used for the Jones oxidations except for the absence of CrO_3. In this solution tlc showed that most of the taxol remained unchanged even after 60 hours at room temperature; the taxol which had reacted appeared as a streak.

It is not suprising that the two secondary hydroxyl groups of taxol exhibit greatly differing rates of oxidation. The Jones oxidation of alcohols occurs by a two step mechanism (Scheme 24). The second step of the reaction is rate limiting so that factors which increase the rate of the second step increase the rate of the entire reaction.[45,46,47] Since the 7-hydroxyl group of taxol is sterically hindered then the even more crowded intermediate chromate ester will be relatively unstable and decompose to the product quickly. The 2'-hydroxyl group on the other hand is not only relatively uncrowded but, more importantly, decomposition of the intermediate chromate ester yields an alpha ketoester. Due to two electrophilic carbons being alpha to each other an alpha ketoester is less stable than an isolated ketone.

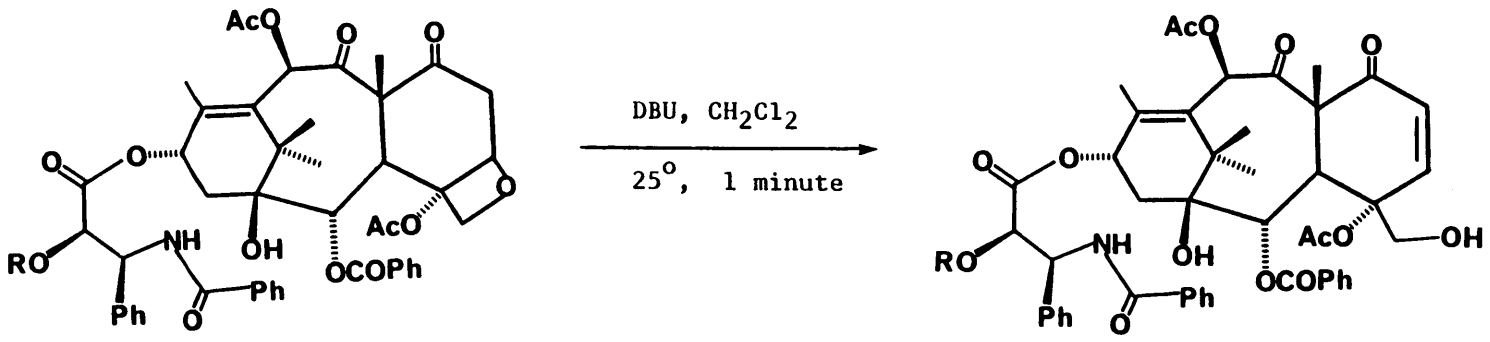


Scheme 24. Jones Oxidation Mechanism

4.2.2 BETA ELIMINATION REACTIONS OF 7-OXOTAXOLS

Reaction of 2'-N-CBZ-β-alanyl-7-oxotaxol and 2'-acetyl-7-oxotaxol with 1,8-diazabicycloundecene in methylene chloride at 25° quantitatively gave the D-seco products **58** and **59**. The reactions were complete as soon as checked by tlc (less than one minute), (Scheme 25).

The ^{1}H-nmr spectra of the D ring secotaxols are strikingly different from their parent compounds. In Table 21 the major differences in the ^{1}H spectra of 2'-acetyl-7-oxotaxol **54** and 2'-acetyl-7-oxo-6-dehydro-5,0-secotaxol **58** are listed. Two new peaks which appeared in the spectrum of **58** are those for the vinyl protons at C-6 and C-5, 6.01 ppm (d, J=10) and 7.00 ppm (d, J=10). The large difference in chemical shift and 10 Hz coupling constant are expected for the α and β protons of an enone.[48] Irradiation of either of these signals causes the other to collapse to a sharp singlet. The doublets seen for these protons are very sharp and indicated isolated protons. In contrast to this 2'-acetyl-7-oxotaxol shows several doublets but due to the complexity of the molecule producing long range coupling none of these are sharp. The only other taxol derivatives in this project which contain an oxetane ring and show a sharp doublet are the C-2' oxidized taxols



2 R = OAc

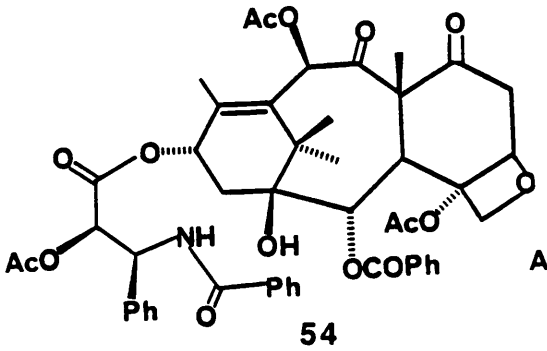
41 R = N-CBZ-βalanyl

58 R = OAc

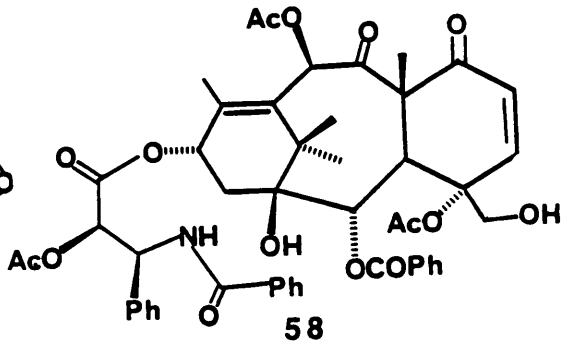
59 R = N-CBZ-βalanyl

Scheme 25. Preparation of D-Secotaxols via Beta Elimination

Table 21 Major Differences in the Proton Spectra of **54** and **58**



54



58

Position	Shift (ppm from TMS) Coupling (hertz)	Shift (ppm from TMS) Coupling (hertz)
5	5.08 (d, 5)	7.00 (d, 10)
6	2.84 (d, 19), 3.09 (dd, 7,19)	6.01 (d, 10)
13	6.19 (br t, 7)	5.87 (br d, 11)
14	2.3-2.4 (m)	2.96 (dd, 4,16) 2.44 (dd, 11,16)
20	4.28 (d, 8), 4.47 (d, 8)	4.12 (d , 12), 4.37 (d , 12)
2 OBz	Meta 7.4 (m)	7.20 (t, 8)
20 Hydroxy	---	4.60 (s)
4 Acetate	2.43 (s)	1.70 (s)

which show a sharp doublet for the C-3' proton. The signals for the C-7 and C-6 protons in 2'-acetyl-7-taxol seen at 5.08 ppm (d, J=5) and 2.84 ppm (d, J=19), 3.09 ppm (dd, J=7,19) are absent in the spectrum of 2'-acetyl-7-oxo-6-dehydro-5,O-secotaxol.

The signals for the C-20 geminal protons of **58** at 4.12 ppm (d, J=12) and 4.37 ppm (d, J=12) show only a small chemical shift change from the analogous signals of **27**. 4.28 ppm (d, J=8) and 4.47 ppm (d, J=8). The significant difference in the two sets of signals is the coupling constants.

The major factor in determining the coupling constant for geminal protons is the angle formed by the protons and the connecting carbon. The relationship between this angle and the coupling constant is given by the Karplus equation in Figure 14.[49]

In taxol the geminal coupling constant for the C-20 protons is 8 Hz which corresponds to an angle greater than the 109° expected for a tetrahedron. An oxetane however is not a tetrahedron; the angles within the ring are 90°, and therefore the angles between the protons on the ring will be greater than 109°. The largest coupling constant seen for the C-20 protons of a compound possessing the taxane skeleton and an oxetane ring is 9.5 hertz for 10-deacetylbaccatin V **60**.[33]

In 10-deacetylbaccatin V hydrogen bonding between the C-7 hydroxyl and the C-4 acetoxy group, which would not be pos-

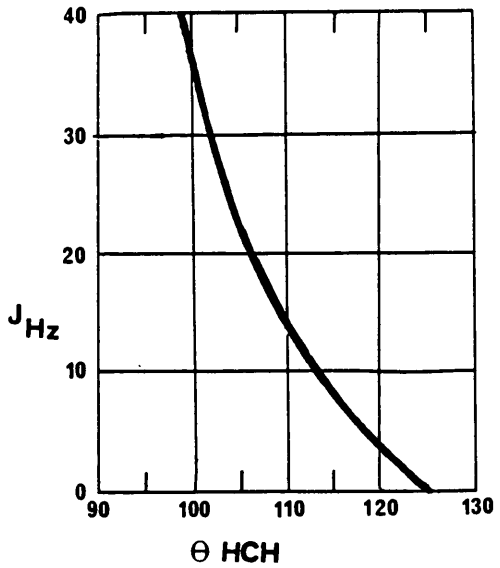
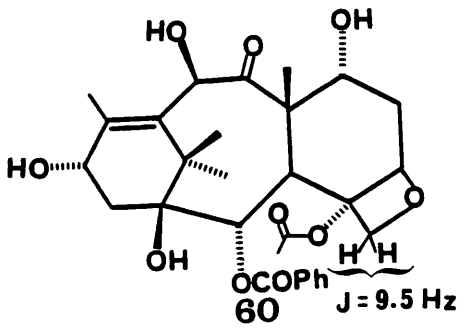


Figure 14. Bond Angle Coupling Constant Relationship for Geminal Protons



60

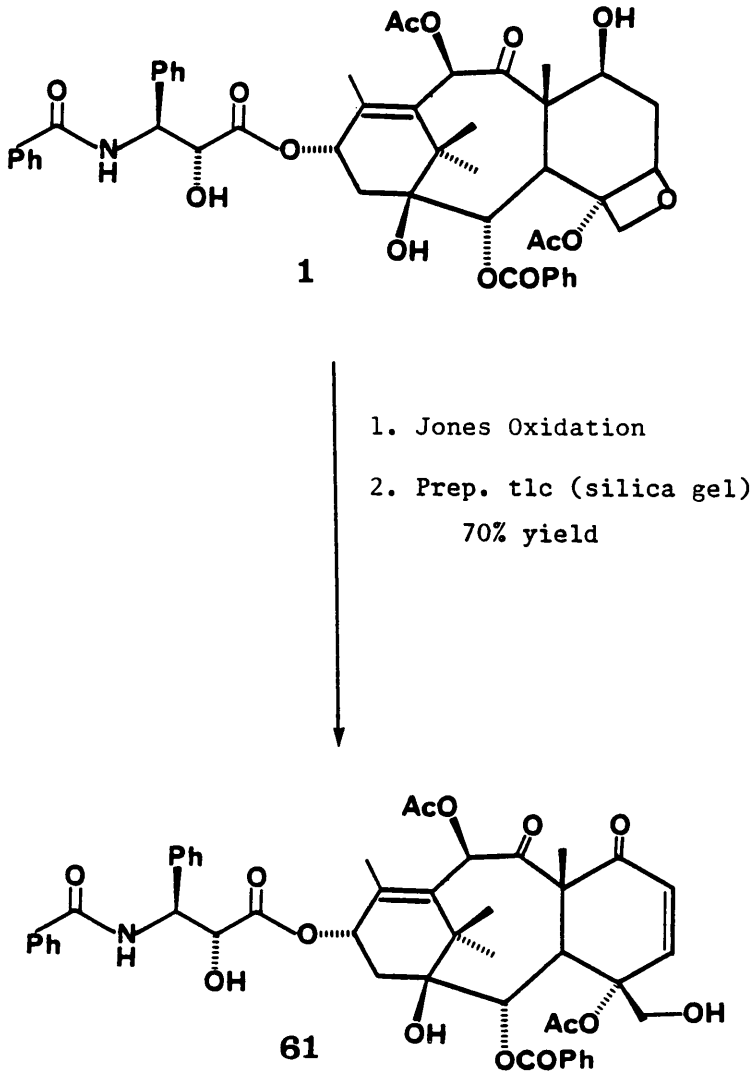
sible for taxol, was reported. This hydrogen bonding could have changed the shape of the oxetane and so a slight increase in the coupling constant is seen. In **68** the 12 hertz coupling constant corresponds to an angle close to 109° This 109° angle would be the result of the strained oxetane opening and the formation of an unstrained tetrahedral C-20.

The extreme change in chemical shift of the C-4 acetoxy group of **58** 1.70 ppm (s) when contrasted with the C-4 acetoxy group of **27** 2.43 ppm (s), indicates a major change in the electronic environment of that group. The groups directly attached to the C-4 acetoxy are not changing but opening of the D ring could spatially move the methyl portion of the acetoxy in relation to the rest of the molecule. This movement could be caused by a change in conformation of the taxane skeleton or by the C-4 acetoxy group hydrogen bonding to the hydroxyl at C-20 in **58**. The C-20 hydroxyl group of **58** is seen as a singlet at 4.60 ppm. The changes in the signals

for C-13 and C-14 are due to conformational changes of the taxane skeleton and will be discussed later.

As expected the mass spectra of the D ring seco taxols are essentially the same as those of the starting materials. The beta elimination does not involve a change in the molecular weight for the entire compound and distribution of mass between the skeleton and side chain is not changed. The beta elimination also does not produce any new moiety that can be lost as a neutral nonradical fragment.

The opening of the oxetane rings of the 7-oxotaxol was found also to occur when the compounds were purified by preparative tlc on silica gel. A pure sample of 2'-N-CBZ-β-alanyl-7-oxotaxol was streaked onto a preparative tlc plate (silica gel, 1000 μm) and the plate was developed with ethyl acetate and hexane to yield one narrow less polar band as the only product. Characterization of this product showed it to be 2'-N-CBZ-β-alanyl-7-oxo-6-dehydro-5,O-secotaxol. The ring opening did not take place on analytical tlc plates as **48** and **59** could be seen as separate spots on silica gel analytical tlc plates. The ring opening on silica gel allowed the compound 7-oxo-6-dehydro-5,O-secotaxol **61** to be prepared in one step from taxol. Jones oxidation of taxol and purification of the product by preparative tlc yielded the D seco product **61** in 70% yield (Scheme 26). A further indication of the ease with which the beta elimination can occur was seen with samples of 7-oxotaxol stored as solids at room



Scheme 26. Preparation of 7-Oxo-6-dehydro-5,0-secotaxol from Taxol

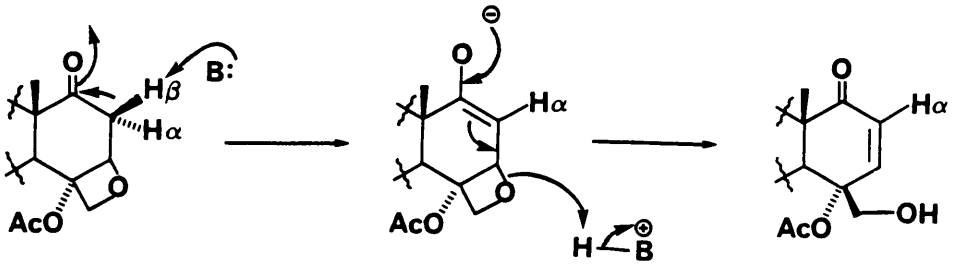
temperature for several months, which nmr showed had all to some extent decomposed to 7-oxo-6-dehydro-5,O-secotaxol.

The oxetane ring opening of the 7-oxotaxols, under exceptionally mild conditions, proceeded by the two step mechanism shown in Scheme 27. If the reaction were concerted the alpha proton at C-6 would have to have been abstracted. This is highly unlikely as the rings of taxol form a cup shape with the alpha face being concave and therefore quite sterically hindered.

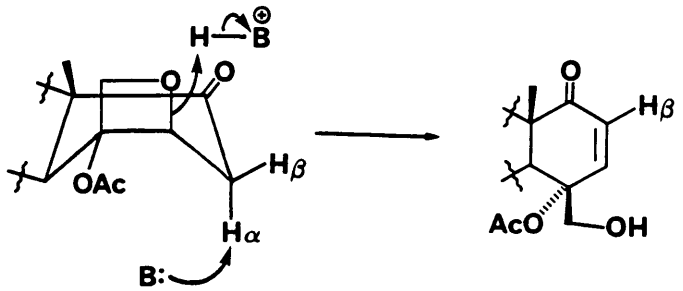
There are two major driving forces for the D ring opening of the 7-oxotaxols. The first of these is that the strain inherent in four membered rings is released with opening of the D ring. The second driving force is that the skeleton of taxol gains a measure of flexibility with the oxetane ring removed. With the ring present the taxane skeleton is rigid, as the oxetane imposes the requirement that all of its bonds exist in the same plane.

An indication of the change in the conformation of the taxane skeleton that occurred upon opening of the oxetane ring is shown in the ^{1}H-nmr spectrum of 7-oxo-6-dehydro-5,O-secotaxol with the spectrum of 7-oxotaxol (Figure 15). In all taxol derivatives seen in this project in which the oxetane ring is intact the signal for the proton at C-13 is a broad triplet, 6.19 ppm (br t, J=7) in **1**, and the signals for the C-14 protons are seen as a complex multiplet at or near 2.4 ppm, for example 2.3-2.4 ppm in taxol. In

Two Step Mechanism



One Step Mechanism



Scheme 27. Possible Mechanisms of the Beta Elimination Ring Opening Reaction

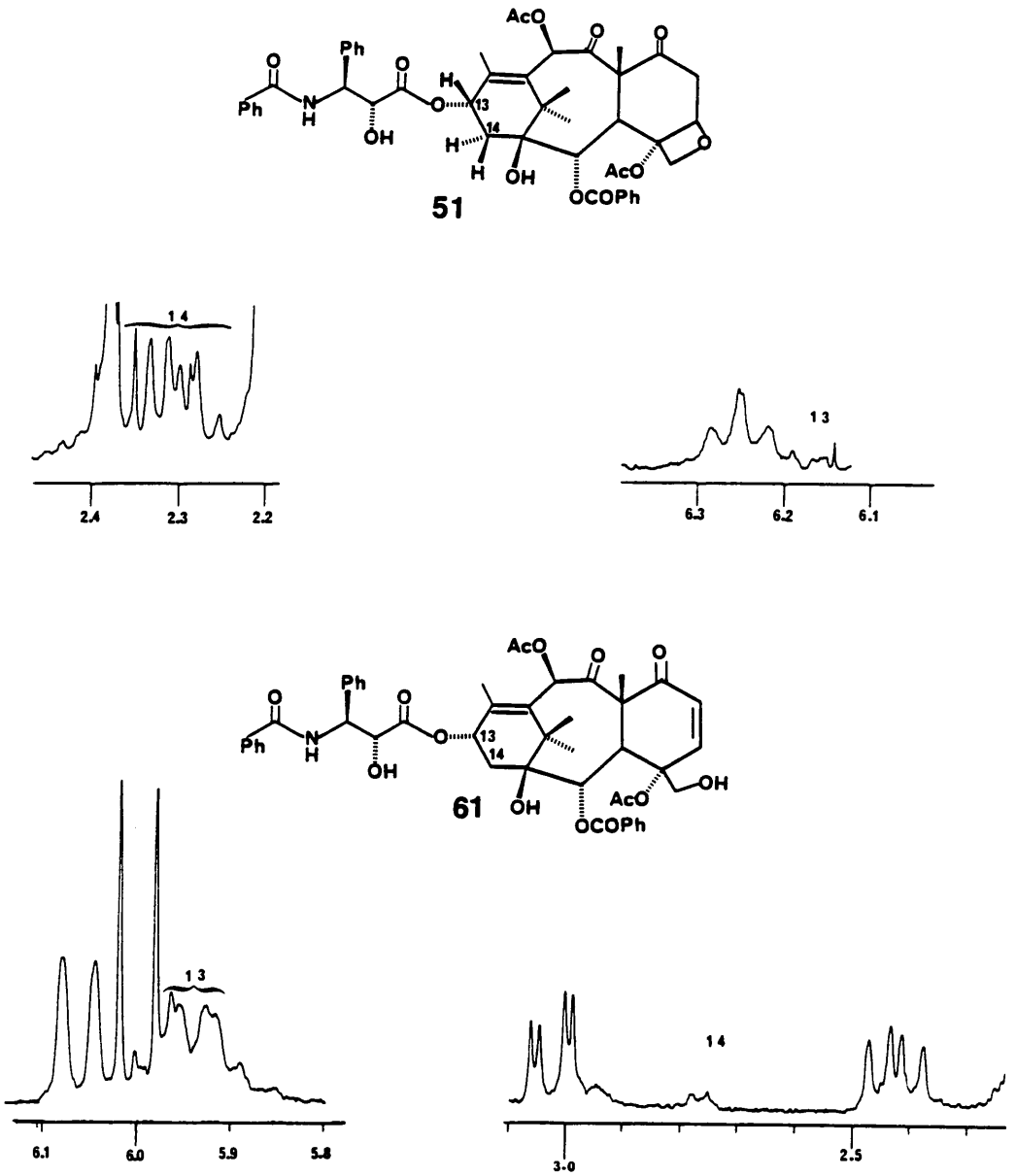


Figure 15. C-13 and C-14 Proton Signals for **51** and **61**

7-oxo-6-dehydro-5,O-secotaxol the signal for the C-13 proton is seen as a doublet of doublets at 5.95 ppm (J=4,11) and the signals for the C-14 protons are well differentiated at 3.03 ppm (dd, J=4,16) and 2.43 ppm (dd, J=11,16). This ^{1}H-nmr data must be explained by a conformational change as the 13 and 14 positions are not close, either through bonds or spatially, to the position undergoing transformation in the D ring opening reactions.

The ^{1}H-nmr data for the D ring seco taxols clearly show that the taxane skeleton of taxol exists in a conformation which, due to steric interactions and/or bond angle strain, is less stable than the conformation the skeleton can attain when the oxetane ring is not present. Because a change in conformation can affect a drug's ability to bind to its receptor site this change in conformation is very much a positive result. Activity results for the D ring seco taxols will yield information on how rigid the steric requirements are for a drug to bind to taxol's receptor site.

4.2.2.1 ATTEMPTED ACETYLATION OF 7-OXO – 6 – DEHYDRO-5,O-SECOTAXOL

Acetylation of 7-oxo – 6 – dehydro-5,O-secotaxol was attempted for the purpose of obtaining additional characterization data. Even though the C-20 hydroxyl group is

primary, acetylation with acetic anhydride in pyridine at room temperature resulted in no reaction at all. When the acetylation was conducted using acetic anhydride, pyridine and 4-dimethylaminopyridine at room temperature the analysis showed that the starting material decomposed into a complex mixture of products seen as a streak.

While the acetylation reactions were not successful in their primary goal they did give information about the structure and reactivity of the D ring seco taxol compound. The failure of the acetylation with acetic anhydride and pyridine shows that the C-20 hydroxyl group is sterically hindered, being a 'neopentyl' center and also spatially close to the C-19 methyl group (Figure 16). Decomposition in the presence of 4-dimethylaminopyridine may well be due to the enone functionality being susceptible to nucleophilic attack: this initial attack could lead to a variety of products (Scheme 27).

4.2.3 REDUCTION OF D RING SECO TAXOLS

The successful opening of the D ring of taxol via beta elimination contributed much toward determining the relationship of the D ring to the activity of taxol as it is the first known reaction in which the oxetane has been modified. The compound 7-oxo-6-dehydro-5,O-secotaxol will not

however give a definitive answer about the structure activity relationship of the oxetane ring because taxol has been modified in more ways than just opening of the oxetane ring. A superior taxol analogue therefore would be one in which the enone moiety of the D ring seco taxols had been modified so that the C ring functionality more closely resembled taxol's.

Attempted reduction of 7-oxo-6-dehydro-5,O-secotaxol with tetrabutylammonium borohydride or with diborane in THF produced a complex mixture of products from the start of each reaction as shown by tlc. Attempted reductions via hydrogenation using palladium on alumina as catalyst with a hydrogen atmosphere or formic acid as a source of hydrogen were shown by ^{1}H-nmr to have produced no change in the enone moiety.

Clean reduction of 7-oxo-6-dehydro-5,O-secotaxol was accomplished under a hydrogen atmosphere with platinum on carbon as catalyst. Product isolation consisted of filtering

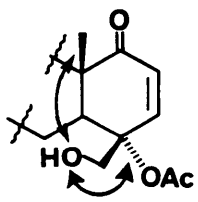
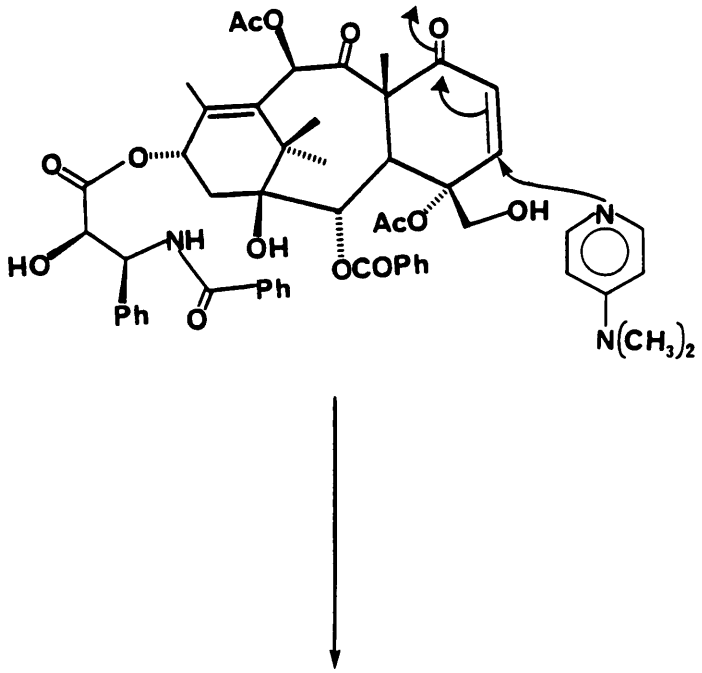


Figure 16. Major Steric Interactions of the C-20 Hydroxyl of 7-Oxo-5,6-dehydro-5,O-secotaxol



Complex Mixture of Products

Scheme 28. Decomposition of **61** by 4-Dimethylaminopyridine

the catalyst, evaporating off the methanol solvent on a rotary evaporator and preparative hplc isolation of the product, 77% isolated. The hplc chromatograms of the starting material and crude product are shown in Figure 17.

The assigned structure for the product from the hydrogenation of 7-oxo-6-dehydro-5,0-secotaxol and two reasonable reaction pathways are shown in Scheme 28. Chromatograms of the starting material and crude product are shown in Figure 17. Assignment of the structure **62** as the final form of the hydrogenated **61** was made only after careful analysis of the ^{1}H-nmr and mass spectra. The major differences in the ^{1}H-nmr spectra of **61** and **62** are shown in Table 22.

The taxane skeleton, which taxol and all* known naturally occurring taxol-like compounds possess, contains four nonacetoxy methyl groups, Figure 18. These methyl groups cause one of the identifying features of the ^{1}H-nmr spectrum of a taxol-like compound to be that only singlets integrating for three protons are seen in the 1.0 ppm to 2.0 ppm portion of the spectrum.

Inspection of the ^{1}H-nmr spectrum of **62** immediately showed that this was not true with that taxol analogue: a doublet, integrating as three protons was seen at 1.39 ppm (J=7). Irradiation of this signal caused the quartet of doublets seen at 3.10 ppm (J=2,7) to collapse to a doublet (J=2). The

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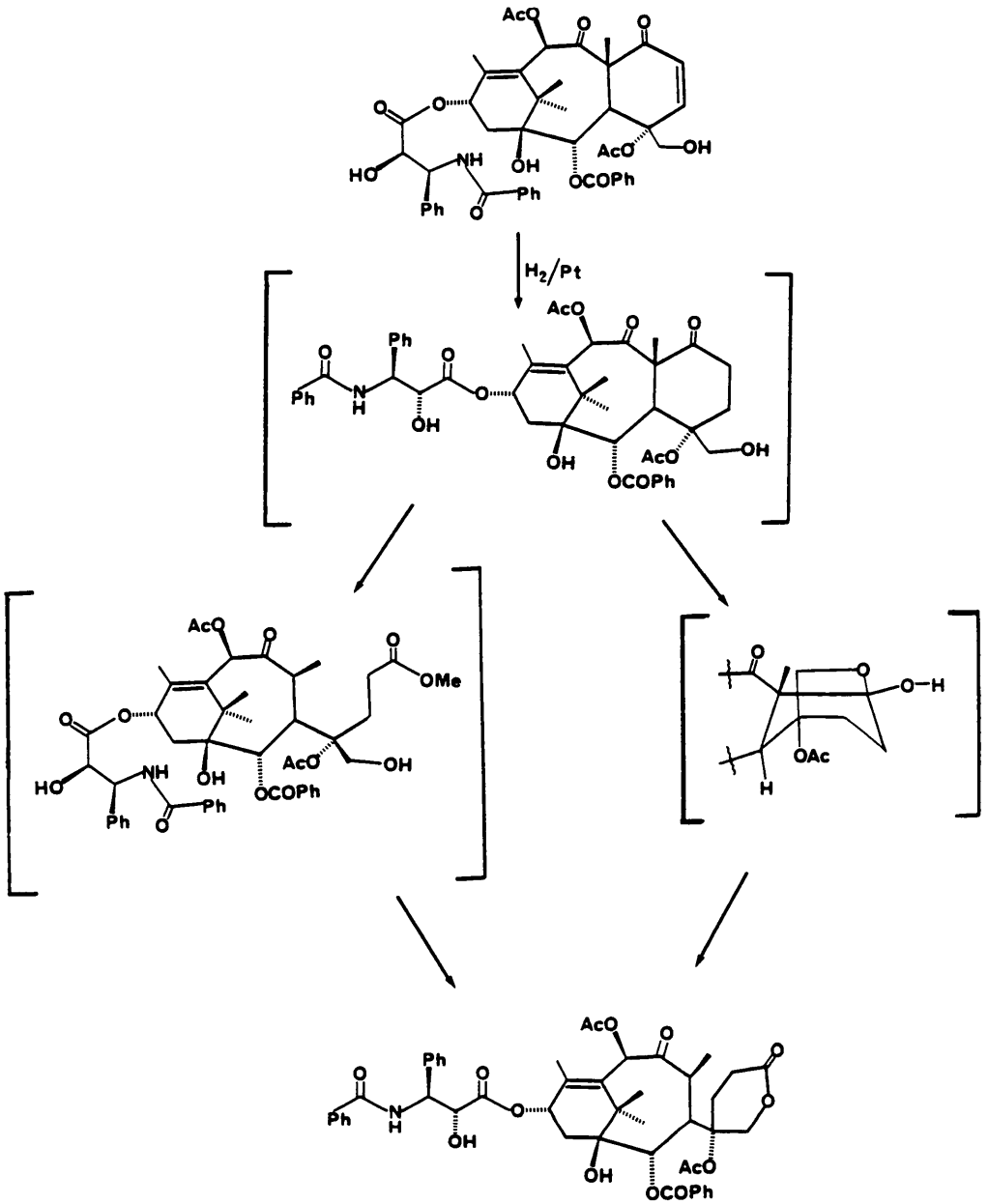
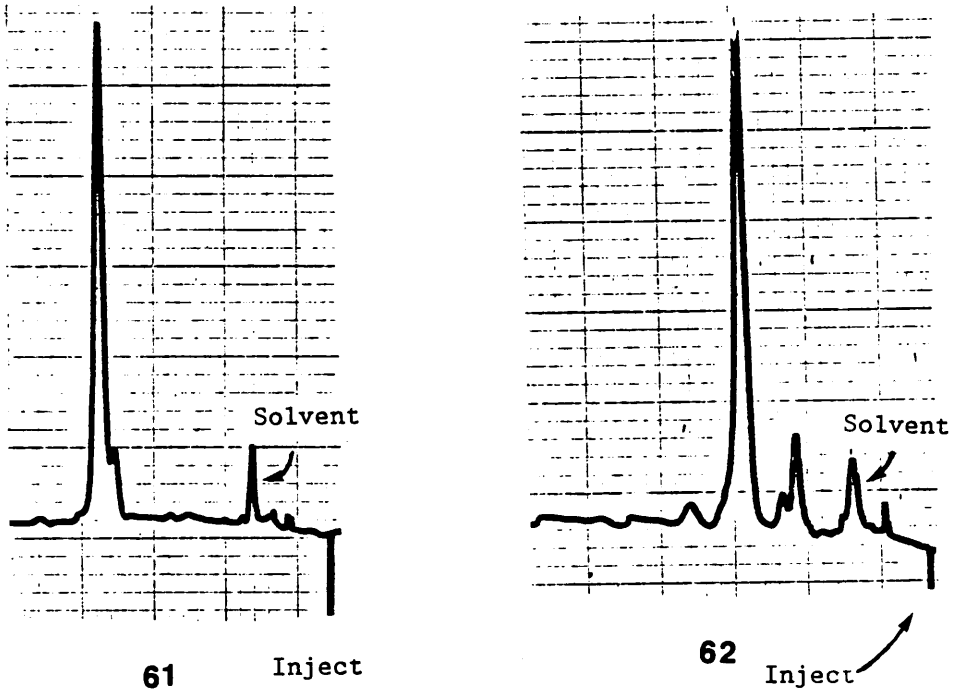
* The only exception is 19-hydroxybaccatin III.



Scheme 29. Possible Mechanisms for the Formation of Lactone **62**



Column RP-8, Radial Compression

7/3, MeOH/H_2O, 2 mL/min

0.5 cm/min

Figure 17. HPLC Chromatograms of 61 and 62 Before Isolation

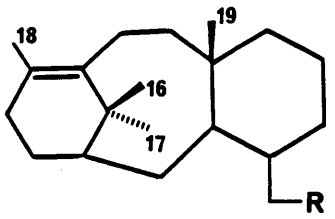
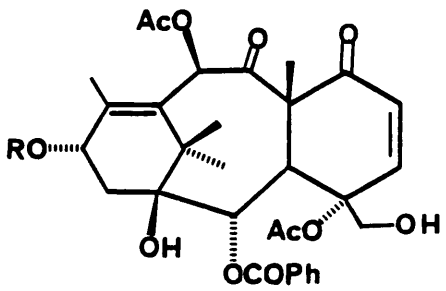
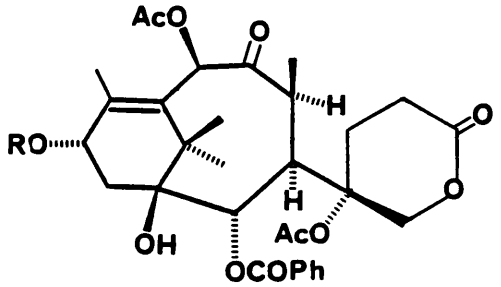


Figure 18. Methyl groups of the Taxane Skeleton

possibility that the methyl doublet resulted from hydrogenation of the C-11, C-12 double bond was discounted as the mass spectrum shows that only one equivalent of hydrogen had been added during the reaction and ^{1}H-nmr, by the absence of the two sharp doublets for C-5 and C-6 seen for **62**, showed that the C-5, C-6 double bond had been hydrogenated. Also, the conditions used to hydrogenate produced no reaction when used with taxol. Further proof that the methyl doublet was due to C-19 was obtained by irradiation of the quartet of doublets coupled to it. This caused the methyl doublet to collapse to a sharp singlet and the signal for the proton at C-3, 3.77 ppm (dd, J=2.7) to collapse to a doublet (J=7). Irradiation of the C-3 proton caused the quartet of doublets to collapse to a quartet (J=7) and the signal for the C-2 proton at 5.60 ppm (d, J=6) to collapse to a singlet. Compound **62** is the first compound seen in this research in which the ^{1}H-nmr signal for the proton at C-3 has appeared as anything other than a doublet.

Table 22 Major Differences in the Proton Spectra of **61** and **62**





Position	Shift (ppm from TMS) Coupling (hertz) **61**	Shift (ppm from TMS) Coupling (hertz) **62**
2	5.60 (d, 6)	5.73 (d, 7)
3	4.18 (d, 6)	3.77 (dd, 2,7)
5	7.00 (d, 10)	2.3-2.5 (m)
6	6.00 (d, 10)	2.3-2.5 (m)
8	---	3.10 (q,d, 2,7)
19	1.83 (br s)	1.39 (d, 7)
20	4.35 (d, 12), 4.18 (d, 12)	4.68 (br d, 11), 4.43 (d, 11)
C-20 OH	4.60 (s)	---

Integration of the ^{1}H-nmr of **62** showed the presence of six nonmethyl protons from 2.0 ppm to 2.6 ppm; these signals would be due to the protons at C-5, C-6 and C-14. The C-5, C-6 protons are coupled to each other and their chemical shifts could not be precisely determined. The signals for the C-14 protons could be more precisely determined by decoupling experiments. Decoupling experiments were performed by irradiating at 2.0 ppm through 2.6 ppm at intervals of 0.1 ppm with the decoupling power corresponding to 30 Hz (0.11 ppm). After each decoupling experiment the splitting pattern for the C-13 proton was observed, 6.05 ppm (br dd, J=4,10). Irradiation at 2.3 ppm caused the C-13 proton signal to collapse to a broad doublet (J=10) as did irradiation at 2.6 ppm (J=4) indicating that the signals for the C-14 protons occurred in these areas.

The ^{1}H-nmr signals for the C-20 protons of **62** are shifted downfield from 4.18 ppm (d, J=12) and 4.35 ppm (d, J=12) **61** to 4.43 (d, J=11) and 4.68 (br d, J=11). The 11 Hz coupling constant shows that the hydrogenation did not cause the reformation of the oxetane ring. The change in chemical shift is somewhat less than what might be expected to occur upon acylation of the C-20, hydroxyl but the change in the electronic environment of the C-20 protons is more than simple acylation of the hydroxyl group.

The assignment of the stereochemistry at position 8 was made from the 2 Hz coupling constant between the protons at

C-8 and C-3. With the assigned stereochemistry the dihedral angle between these two protons would be approximately 60° and a coupling constant of 2 Hz would be predicted; epimerization at position 8 would give a dihedral angle of approximately 180° and the coupling constant would be predicted to be 9 Hz.

In epimerization reactions of C-7 of the taxane skeleton via a retro aldol reaction in this research and in research by Powell[33] epimerization of C-8 was not seen. Epimerization of C-8 would cause gross changes in the taxane conformation and steric interaction associated with the C-19 methyl group pointing into the concave face of the skeleton. Senilh[14] reported that reaction of baccatin III with HCl in methanol produced 8-epibaccatin III but no characterization data was given for the compound and so the structure assignment could not be verified.

The hydrogenation of 7-oxo-5,0-secotaxol was repeated using deuterated methanol as solvent. The purpose of this reaction was to show that the proton at C-8 in **62** originated from the solvent. Work up for this reaction consisted only of filtering off the catalyst, evaporation of the solvent on a rotary evaporator and analysis of the compound by ^{1}H-nmr. This was done because it had already been shown that the product **62** could be isolated in high yield from **61** and the presence of a small amount of impurities would not cause

difficulties in seeing the absence of the C-8 proton signal and collapse of the C-19 methyl group signal to a singlet.

The ^{1}H-nmr of the product hydrogenated in deuterated methanol clearly showed that the enone had been reduced as no trace of the sharp doublets at 7.0 ppm and 6.0 ppm for the protons of the enone was seen. The ^{1}H-nmr did not show one major taxol-like product but rather a complex mixture of products. Sharp singlets at 6.35 ppm, 6.49 ppm and 6.73 ppm characteristic of the signal for C-10 of taxol-like compounds showed that at least three taxol-like compounds were present. Upon standing in $CDCl_3$ solution for several days the changing ^{1}H nmr spectrum showed that the complex mixture of products reached an equilibrium state composed mostly of two taxol-like compounds. Some of the chemical shift and multiplicities that could be seen in the ^{1}H nmr spectrum of this equilibriated mixture are shown in Table 23 along with tentative peaks assignments.It can be seen from the ^{1}H-nmr data that neither of the major compounds present in the mixture were **62**.

The results of this reaction yielded important information about the reactivity of the hydrogenated D ring seco taxol. The results show that the formation of the lactone seen in **62** did not occur immediately upon hydrogenation of the enone but rather the intermediate compounds seen in Scheme 28 were in equilibrium. If the conditions were such that formation of the lactone could occur, perhaps by prolonged heating on

Table 23 Partial Proton Peak Assignments for the Mixture of Products from the Hydrogenation of **61**

Position	Shift (ppm from TMS) Coupling (hertz)
C-2	5.54 (d), 5.45 (d)
C-3'	6.05 (m), 5.80 (dd)
C-10	6.81 (s), 6.48 (s)
C-13	6.05 (m), 5.87 (br t)
C-14	2.14 (dd)
C-16, C-17	1.08 (s), 1.09 (s), 1.17 (s), 1.18 (s)
C-20	4.5 (d), 4.08 (d)
OAcs, C-18, C-19	2.20 (s), 2.18 (s), 2.15 (s), 1.94 (s), 1.89 (s), 1.87 (s), 1.60 (s)
2 OBz	8.12 (d), 8.03 (d), 7.19 (t)
3' NBz	7.83 (d), 7.88 (d)

a rotary evaporator, then a stable product is formed which is not in equilibrium with any other product.

The information gained concerning the reactivity of the hydrogenated D ring seco taxol made possible modification of the experimental technique for the hydrogenation so that a nonrearranged hydrogenated D ring seco taxol could be prepared. Hydrogenation of 2'-acetyl-7-oxo-6-dehydro-5,O-seco-taxol by platinum on carbon under a hydrogen atmosphere was carried out. Ethyl acetate was used as solvent instead of methanol to avoid nucleophilic attack on the C-7 keto group. Work up consisted of filtering off the catalyst, rotary evaporation of the solvent with a 30° water bath as heat source, stopping the rotary evaporator as soon as almost all of the ethyl acetate had been removed and removing traces of ethyl acetate by drying the product in a vacuum desiccator for several hours at 25°. The sample was then dissolved in $CDCl_3$ and subjected to ^{1}H-nmr analysis.

Initial analysis of the sample showed that it was a 4:1 mixture of two taxol-like compounds. Upon standing overnight in $CDCl_3$ solution the minor component was converted into the major component and only one taxol-like compound **63** was seen in the ^{1}H-nmr spectrum. In addition to the product **63** the proton spectrum showed numerous minor impurity peaks amounting to no more than 10% of the integration of the product peaks. Additional purification at this point was not attempted as it might have caused decomposition of the product.

If the 90% pure hydrogenated product is biologically active then further purification would be warranted.

The structure of the hydrogenated product 2'-acetyl-7-oxo-5,0-secotaxol along with characterization data for the compound are shown in Table 24. The mass spectral data did not include a molecular ion but peaks at m/z 551 $(MH-RCOOH-H_2O)^+$ and m/z 328 $(RCOOHH)^+$ show that one equivalent of hydrogen had been added to the taxane skeleton and that the mass of the side chain was unchanged.

Careful analysis of the ^{1}H-nmr spectrum of **63**, comparing and contrasting it with other known taxol analogues left no doubt that the compound is a hydrogenated D ring seco taxol that had not undergone rearrangement to form a lactone. The absence of the sharp doublets at 7.00 ppm and 6.01 ppm seen for **58** showed that the enone double bond had been hydrogenated. There is a doublet at 7.03 ppm (J=9) but irradiation of this signal caused the signal for the C-3' proton 6.30 ppm (dd, J=2,9) to collapse to a 2 Hz doublet. Irradiation of the C-3' signal caused the 7.03 ppm doublet and the C-2' proton doublet at 5.30 (J=2) to collapse to singlets. The doublet at 7.03 ppm therefore is due to the amide proton and not due to any vinyl proton.

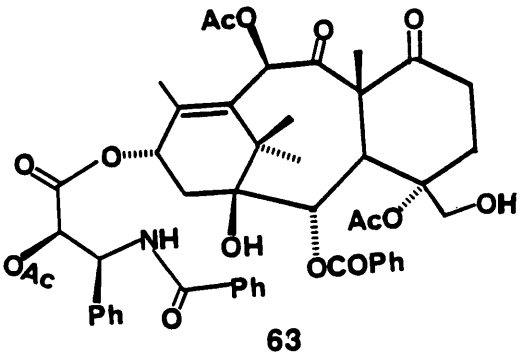
There are numerous indications from the ^{1}H-nmr spectrum that the oxetane ring is not present in **63** but that the rest of the taxane skeleton is intact. In taxanes possessing an oxetane the *meta* protons of the C-2 benzoate can not be

differentiated from the other aromatic signals seen from 7.25 ppm - 7.6 ppm portion of the spectrum. Opening of the oxetane ring to form **61** caused these meta protons to be shifted upfield to 7.20 ppm (t, J=8) which is separate from the rest of the aromatic signals. In **62** the changing structure of the C ring caused these protons to again be lost in the 7.25 ppm - 7.6 ppm portion of the spectrum. In **63** the benzoate ortho protons can again be clearly seen at 7.20 ppm (t, J=8), suggesting that the functionality near the benzoate in **63** is similar to that in **61**.

One of the significant sets of proton signals seen for **58** is due to the C-14 protons, occurring at 2.44 ppm (dd, J=11,16) and 2.96 ppm (J=4,16) where in 2'-acetyl-7-oxotaxol the signals were seen as a multiplet at 2.2 ppm. The change in position of the C-14 proton signals was due to changing conformation of the taxane skeleton. In **62** the C-14 proton signals were shifted to 2.5 ppm and 2.3 ppm showing that a change in conformation from 7-oxo-6-dehydro-5,O-secotaxol had occurred due to opening of the fused C ring. In the proton spectrum of **63** the signals for the C-14 protons occur at 3.10 ppm (dd, J=4,15.5) and 2.45 ppm (multiplicity unknown). These assignments were made by selective decoupling of the C-14 protons from each other and from the C-13 proton. The reappearance of the C-14 proton signals near 2.4 ppm and 3.0 ppm for **63** indicates that the A ring is in a conformation

Table 24 Characterization Data for 2'-Acetyl-7-oxo-5,0-secotaxol **63**

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.57 (d, 5)
3	4.10 (d, 5)
5	2.3-2.5 (m)
6	2.3-2.5 (m)
10	6.51 (s)
13	5.87 (br d, 10)
14	3.10 (dd, 4,15.5), 2.45 (br d, 15.5)
16	1.14 (s)
17	1.05 (s)
18	2.02 (br s)
19	1.60 (s)
20	4.05 (d, 11.5) 4.56 (d, 11.5)
2'	5.30 (d, 2)
3'	6.30 (dd, 2,9)
N-H	7.03 (d, 9)
OAcs	2.21 (s), 2.16 (s), 1.80 (s)
2 OBz	8.18 (d, 8) , 7.20 (t, 8) , 7.4 (m)
3' NBz	7.79 (d, 8), 7.4 (m)
3' Ph	7.4 (m)
C-20 OH	4.34 (br s)



63

Mass Spectral Data

551 $(MH\text{-}RCOOH\text{-}H_2O)^+$,

509 $(MH\text{-}RCOOH\text{-}HOAc)^+$,

328 $(RCOOHH)^+$,

268 $(RCOOH\text{-}HOAc)^+$,

105 $(C_7H_5O)^+$.

Infrared Spectral Data

1745 s, 1720 m-sh, 1675 m,

1530 w, 1500 w, 1470 w,

1385 w, 1280 m-sh, 1240 s,

1105 m, 1085 m, 1050 m.

seen when the oxetane ring is not present and the A,B,C fused ring system is.

The 12 Hz coupling constant for the C-20 protons of **63** shows that the oxetane ring is not present. The chemical shifts for the C-20 protons of **63**, 4.05 ppm (d, J=11.5) and 4.56 ppm (d, J=11.5) are upfield of the C-20 proton signals for **58** at 4.43 ppm (d, J=11) and 4.67 ppm (br d, J=11) indicating that the C-20 hydroxyl group is not esterified. A hydroxyl peak assigned to C-20 at 4.34 ppm (br s) was also seen for **63**.

4.3 EXPERIMENTAL PROCEDURES

General Experimental Procedure - The Jones oxidizing reagent consisted of 0.67 g of CrO_3, 0.58 mL H_2SO_4 diluted to 2.3 mL with H_2O.[50] Work up for the oxidation reactions consisted of stopping the reactions by addition of several drops of 2-propanol, filtering through a 0.45 μm pore size centrifugal filter to remove precipitated chromium salts, dilution with several mL of CH_2Cl_2, washing with 5% $NaHCO_3$ and water, followed by drying with $MgSO_4$, filtration of the solution and evaporation of the solvent.

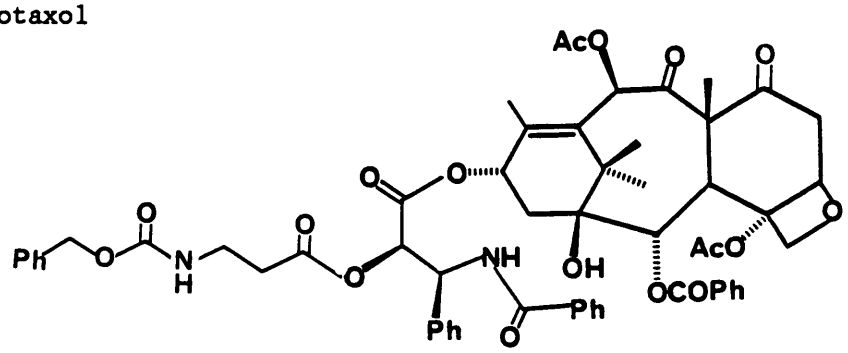
Oxidation of 2'-N-Carbobenzoxy-β-alanyltaxol (49) - 2'-N-CBZ-β-alanyltaxol (51 mg) was dissolved in distilled acetone (1.5 mL) at room temperature. To this solution was added

Jones oxidizing reagent (50 µL). After 10 minutes tlc analysis showed the reaction to be complete and quantitative (silica gel, 1/1, EtOAc/Hexane, Rf **49** - 0.25, Rf **50** - 0.36). The reaction was then worked up as usual. Jones oxidations of C-2' protected taxols were the cleanest reactions carried out in this research project. After simple work up no other products could be seen either by tlc or ^{1}H-nmr. Characterization data for **50** is shown in Table 25.

C-2' Deprotection of 2'-N-Carbobenzoxy-β-alanyl-7-oxotaxol - 2'-N-Carbobenzoxy-β-alanyl-7-oxotaxol (41 mg) was dissolved in 6.3 mL of 40% formic acid in methanol and 5% Pd/C (24 mg) was added as catalyst. After 1 hour the catalyst was centrifuged off. The solvents were then removed via rotary evaporation and then in a vacuum desiccator overnight and the residue was then dissolved in methanol. After 30 minutes the methanol was removed *in vacuo*. The reaction was worked up as usual to yield ^{1}H-nmr and tlc pure 7-oxotaxol **51**. Yield - 31 mg, 89%. Characterization data for 7-oxotaxol are shown in Table 26.

Preparation of 2'-(2,2,2-trichloroethyloxycarbonyl)taxol (52) - Taxol (50 mg) was dissolved in 1.0 mL of CH_2Cl_2 and 100µL of pyridine. The solution was cooled to -23° in a CCl_4/dry ice bath. Over 45 minutes 2,2,2-trichloroethylchloroformate (8.0 µL, 1 eq) was added. The reaction was then

Table 25(Part 1 of 2). Characterization Data for 2'-N-CBZ-β-Alanyl-7-oxotaxol



Position	Shift (ppm from TMS) Coupling (hertz)
2	5.78 (d, 7)
3	4.29 (d, 7)
5	5.07 (d, 6)
6	3.07 (dd, 6,19), 2.84 (d, 19)
10	6.42 (s)
13	6.18 (br t, 8)
14	2.2 (m)
16	1.15 (s)
17	1.17 (s)
18	1.88 (s)
19	2.04 (s)
20	4.30 (d, 8), 4.45 (d, 8)
2'	5.51 (d, 3)
3'	6.00 (dd, 3,9)

Mass Spectral Data

1079 $(MNa)^+$, 1057 $(MH)^+$,

997 $(MH-HOAc)^+$.

Infrared Spectral Data

1770 m-sh, 1740 s, 1675 m,

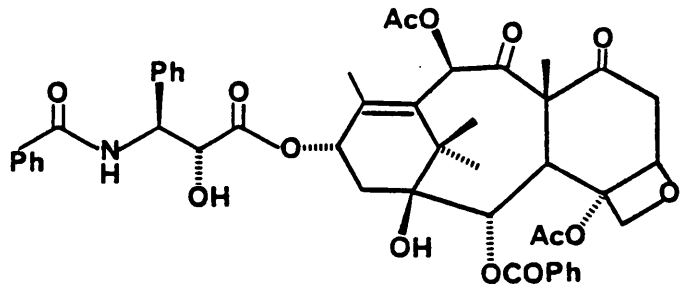
1540 m, 1475 w, 1385 mw,

1255 s, 1050-1110 m.

Table 25(Part 2 of 2). Characterization Data for 2'-N-CBZ-β-Alanyl-7-oxotaxol

Position	Shift (ppm from TMS) Coupling (hertz)
N-H	7.2-7.5
OAcs	2.18 (s), 2.46 (s)
3' NBz	7.77 (d, 8), 7.4 (m)
2 OBz	8.14 (d, 7), 7.4 (m)
Beta Alanine	
2	3.5 (m)
3	2.6 (m)
Bz	4.87 (br s, 6)
NH	5.16 (br t, 8)
3' Ph	7.4 (m)

Table 26 (Part 1 of 2). Characterization Data for 7-Oxotaxol



Position	Shift (ppm from TMS) Coupling (hertz)
2	5.74 (d, 7)
3	3.78 (d, 7)
5	5.06 (br d, 6)
6	3.10 (d , 19), 2.59 (dd, 6,19)
10	6.18 (s)
13	6.06 (br t , 9)
14	2.45 (m)
16	1.10 (s)
17	1.07 (s)
18	1.41 (s)
19	1.78 (br s)
20	4.43 (d, 8), 4.67 (d, 8)
2'	4.91 (d, 2)

Mass Spectral Data

890 $(MK)^+$, 874 $(MNa)^+$,

852 $(MH)^+$, 788 $(MH-HOAc)^+$,

774 $(MH-HOAc-H_2O)^+$,

589 $(MNa-RCOOH)^+$,

567 $(MH-RCOOH)^+$,

549 $(MH-RCOOH-H_2O)^+$,

507 $(MH-RCOOH-HOAc)^+$.

Infrared Spectral Data

1750 s, 1730 m-sh, 1685 m,

1665 mw-sh, 1535 w,

1510 w, 1395 m, 1290 s-sh,

1260 s, 1060-1120 m.

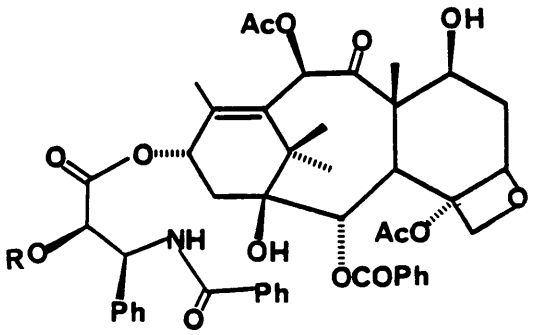
Table 26 (Part 2 of 2). Characterization Data for 7-Oxotaxol

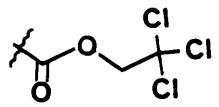
Position	Shift (ppm from TMS) Coupling (hertz)
3'	5.87 (dd, 2,9)
N-H	7.16 (d, 9)
OAcs	1.90 (s), 2.10 (s)
2 OBz	8.16 (d, 8), 7.4 (m)
3' NBz	7.79 (d, 8), 7.4 (m)
3' PH	7.4 (m)

worked up. After work tlc showed the monosubstituted product **52** to be the major product, Rf 0.39, with small amounts of taxol, Rf 0.11, and disubstituted taxol **38** Rf 0.74, 1/1 ethyl acetate/hexane. The product 2'-(2,2,2-trichloroethyloxycarbonyl)taxol was isolated by preparative tlc with 1/1 ethyl acetate/hexane as solvent: (Rf 0.56) Yield-51 mg, 85%. Characterization data for **52** are shown in Table 27.

Oxidation and Deprotection of 2'-(2,2,2-Trichloroethyloxycarbonyl) - 2'-(2,2,2-Trichloroethyloxycarbonyl)taxol (51 mg) was dissolved in 3.0 mL of distilled acetone and Jones oxidizing reagent (50 µL) was added at room temperature. After 11 minutes tlc showed the reaction to be complete and it was stopped and worked up to yield tlc pure 2'-(2,2,2-trichloroethyloxycarbonyl)-7-oxotaxol **53**, Rf (1/1 ethyl-acetate/hexane) 0.69. All of the 2'-(2,2,2-trichloroethyloxycarbonyl) -7- oxotaxol was dissolved in 9/1 methanol/acetic acid (2 mL) and Zn dust (40 mg) was added and the reaction was stirred for 10 minutes at room temperature to yield 7-oxotaxol. The reaction was worked up by filtering off the excess zinc, evaporating most of the solvents, redissolving in CH_2Cl_2 and washing with 5% $NaHCO_3$ and water, drying with $MgSO_4$, filtering and evaporating. The product was homogenous on tlc without chromatography purification.

Table 27 Characterization Data for 2'-(2,2,2-Trichloroethyloxy-carbonyl) taxol





Position	Shift (ppm from TMS) Coupling (hertz)
2	5.56 (d, 7)
3	3.79 (d, 7)
5	4.95 (d, 9)
6	2.4 (m)
7	4.41 (m)
10	6.27 (s)
13	6.27 (br t, 8.5)
14	2.4 (m)
16	1.11 (s)
17	1.21 (s)
18	1.65 (s)
19	1.87 (s)
20	4.19 (d, 8), 4.21 (d, 8)
2'	5.51 (d, 2.5)
3'	6.03 (dd, 2.5,9)
N-H	6.91 (d, 9)
OAcs	2.20 (s), 2.44 (s)
2 OBz	8.13 (d, 8), 7.60 (t, 8), 7.4 (m)
3' NBz	7.74 (d, 7), 7.4 (m)
3' Ph	7.4 (m)
troc	4.73 (d, 11) 4.79 (d, 11)

Mass Spectral Data

1028 $(MH)^+$,

509 $(MH-RCOOH-HOAc)^+$.

Infrared Spectral Data

1780 s-sh, 1740 s,

1690 m-sh, 1675 m, 1530 w,

1505 w, 1485 w, 1390 m,

1290 s-sh, 1255 s.

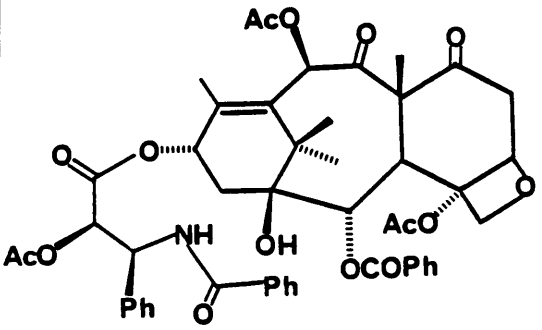
2'-Acetyl-7-oxotaxol (54) - 2'-Acetyltaxol (19 mg) was dissolved in 0.30 mL of acetone. To this solution Jones oxidizing reagent (11 µL) was added at room temperature. After 30 minutes the reaction was stopped and worked up to yield pure 2'acetyl-7-oxotaxol. 2'-Acetyltaxol Rf 0.35, 2'-Acetyl-7-oxotaxol Rf 0.54, (6/4 ethyl acetate/hexane). Characterization data are shown in Table 28. Yield–17 mg, 90%.

Attempted Deacylations of 2'-Acetyl-7-oxotaxol With $NaHCO_3$ - A small amount of 2'-acetyl-7-oxotaxol (0.5mg) was treated with 3.0 mL of a solution of 3/1/0.01 methanol/water/$NaHCO_3$. This is a reaction that had been shown to remove the 2' acetyl group of 2',7-diacetyltaxol. The reaction was followed by hplc (RP-8, 63/35 MeOH/H_2O, 2mL/min). After 30 minutes at room temperature hplc showed an initial major product which was less polar than 2'-acetyl-7-oxotaxol; after 1.5 hours at room temperature all of this product had decomposed into a variety of polar compounds.

Attempted Deacylations of 2'-Acetyl-7-oxotaxol with KCN/EtOH - 2'-Acetyl-7-oxotaxol (1 mg) was dissolved in 0.3 mL of 0.75% KCN in 95% ethanol at 0°. Hplc monitoring showed that a great variety of polar compounds were produced from the start of the reaction. Seventy percent of the starting material had decomposed after 45 minutes at 0°.

Table 28. Characterization Data for 2'-Acetyl-7-oxotaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.77 (d, 6)
3	4.29 (d, 6)
5	5.08 (d, 5)
6	2.84 (d, 19), 3.09 (dd, 19,6)
10	6.42 (s)
14	2.3-2.4 (m)
16	1.16 (s)
17	1.18 (s)
18	1.88 (s)
19	2.05 (s)
20	4.28 (d, 8), 4.47 (d, 8)
2'	5.53 (d, 3)
3'	5.94 (dd, 9,3)
N-H	6.88 (d, 9)
OAcs	2.43 (s), 2.20 (s) 2.14 (s)
2 OBz	8.14 (d, 8), 7.64 (t, 7), 7.4 (m)
3' NBz	7.73 (d, 7), 7.4 (m)
3' Ph	7.4 (m)



Mass Spectral Data

916 $(MNa)^+$, 894 $(MH)^+$,

567 $(MH-RCOOH)^+$,

549 $(MH-RCOOH-H_2O)^+$,

507 $(MH-RCOOH-HOAc)^+$.

Infrared Spectral Data

1765 (s), 1755 (s), 1675 (m),

1535 (w), 1505 (w), 1475 (w),

1390 (m), 1280 (ms-sh),

1250 (s), 1195 (mw-sh),

1125-1040 (m).

7-Oxotaxol via Jones Oxidation of Taxol - Taxol (20 mg) was dissolved in 0.100 mL of distilled acetone and treated with 20 µL of Jones reagent. The reaction was allowed to proceed at room temperature for 20 minutes at which time it was worked up. The product was isolated by preparative hplc (RP-8, 7/3, MeOH/H_2O, 6 mL/min). The product was homogenous on tlc. Yield - 10 mg, 50%.

Preparation of 2',7-Dioxotaxol (55) - Taxol (24 mg) was dissolved in distilled acetone (0.40 mL) at room temperature. To this solution was added Jones oxidizing reagent (50 µL). After 8 minutes tlc analysis (6/4 ethyl acetate/hexane) showed a small amount of starting material, Rf 0.11, a major product with Rf 0.22 (7-oxotaxol), and two very faint product spots Rf 0.32 and 0.46. After 4 hours tlc showed no taxol, 50% 7-oxotaxol, 35% dioxotaxol (Rf 0.32) and 15% of the product with Rf 0.46. After a total reaction time of 24 hours dioxotaxol was the major product in addition to the minor product spot at 0.46. The reaction was worked up as usual to yield a crude product. ^{1}H-nmr showed this product to be approximately 85% dioxotaxol and the remainder several taxol-like impurities. The crude product was purified by preparative hplc (70/30 methanol/H_2O), retention time 7.0 minutes. The product was isolated by evaporating the methanol *in vacuo* at 40° and extracting the product with ethyl acetate; ^{1}H-nmr analysis of this 'purified' product

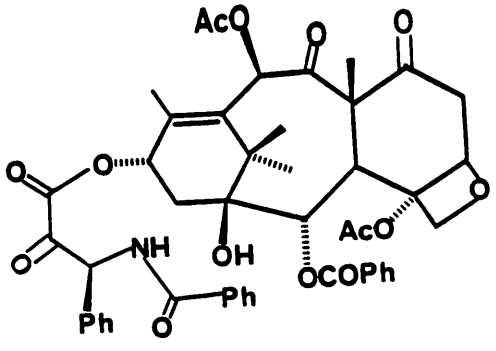
showed a second taxol-like compound present. Reinjection onto the hplc showed a previously absent impurity **56** (retention time - 8 minutes 20 seconds). After standing overnight in $CDCl_3$ solution a second spectrum was obtained and the only product present was 2',7-dioxotaxol. The sample then had 1 mL of CD_3OD added to it and was heated to 50° for 3.5 hours. A ^1H nmr spectrum obtained after this showed the two taxol-like compounds that were first seen after isolation by hplc. The characterization data for 2',7-dioxotaxol are shown in Table 29 and some of the proton nmr signals for the hemiketal are shown in Table 30.

Jones Oxidation of 7-Acetyltaxol - 7-Acetyltaxol (10 mg) was dissolved in distilled acetone (0.3 mL) and had Jones oxidizing reagent (50 µL, excess) added to it at room temperature. The reaction was ended after 24 hours and worked up as usual to yield crude 2'-oxo-7-acetyltaxol **57**. The product was purified via preparative tlc (6:4, EtOAc/Hexane, silica gel). Yield - 6.5 mg, 65%. Characterization data are shown in Table 31.

Sulfuric Acid Stability of Taxol - A mixture of taxol and cephalomannine (4.5 mg) was dissolved in 120 µL and 10 µL of 23% aqueous H_2SO_4 was added. The reaction was monitored by tlc, 7/3 ethyl acetate/hexane, taxol Rf 0.41. No spots other than taxol/cephalomannine could be seen in the first several

Table 29 Characterization Data for 2',7-Dioxotaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.72 (d, 6)
3	4.72 (d, 6)
5	5.04 (br d, 7)
6	2.82 (dd, 1,19), 3.07 (dd, 7,19)
10	6.42 (s)
13	6.12 (dd, 8,10)
14	2.15 (m)
16	1.15 (s)
17	1.14 (s)
18	1.84 (s)
19	2.00 (s)
20	4.22 (d, 8), 4.41 (d, 8)
3'	6.41 (d, 5.6)
N-H	7.12 (d, 5.6)
OAcs	2.14 (s), 2.19 (s)
2 OBz	8.04 (d, 8), 7.62 (t, 8), 7.4 (m)
3' NBz	7.80 (d, 8), 7.4 (m)
3' Ph	7.4 (m)



Mass Spectral Data

850 $(MH)^+$, 790 $(MH-HOAc)^+$,

772 $(MH-HOAc-H_2O)^+$,

507 $(MH-RCOOH-HOAc)^+$.

High Resolution

$(MH)^+$ - $C_{47}H_{47}NO_4$ - 850.2861

Calculated - 850.3075

Infrared Spectral Data

1745 (s), 1730 (m-sh),

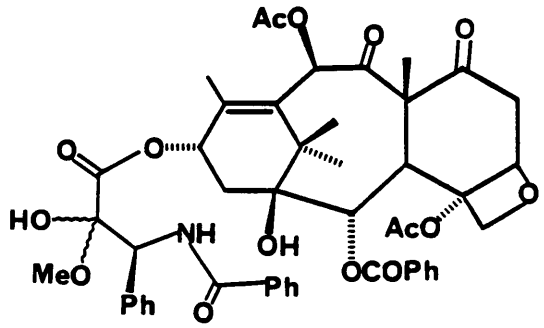
1670 (m), 1500 (w),

1475 (w), 1280 (s),

1250 (s), 1120 (m),

1085 (w), 1060 (w), 720 (m).

Table 30 Partial Proton NMR Data for the 2'-Methylhemiketal of 2',7-Dioxotaxol

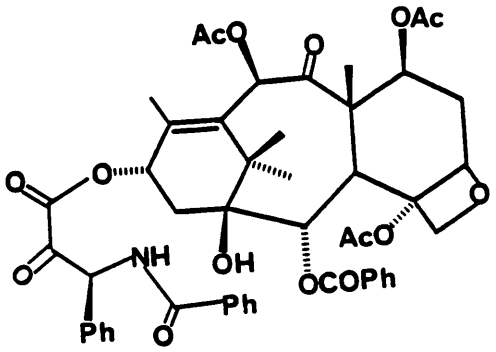


Position	Shift (ppm from TMS) Coupling (hertz)
2	5.73 (d, 7)
10	6.30 (s)
13	6.02 (br t, 7)
20	4.45 (d, 6)
2 OBz	8.14 (d, 7),
3' NBz	7.73 (d, 8),
2' MeO	3.42 (s)
MeOH	3.47 (s)

Peaks which are clearly differentiated from 2',7-dioxotaxol.

Table 31 Characterization Data for 7-Acetyl-2'-oxotaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.65 (d, 7)
3	3.96 (d, 7)
5	4.97 (d, 9)
6	2.2-2.5 (m)
7	5.50 (dd, 6,10)
10	6.72 (s)
13	6.18 (br t, 9)
16	1.18 (s)
17	1.14 (s)
18	1.77 (s)
19	1.96 (s)
20	4.12 (d, 8), 4.28 (d, 8)
3'	6.47 (d)
N-H	7.22 (d)
OAcs	2.16 (s), 2.13 (s), 2.02 (s)
2 OBz	7.73 (d,), 7.4 (m)
3' NBz	7.53 (d) 7.4 (m)
3' Ph	7.4 (m)



Mass Spectral Data

894 $(MH)^+$, 834 $(MH-HOAc)^+$,

611 $(MH-RCOOH)^+$,

551 $(MH-RCOOH-HOAc)^+$.

High Resolution

$(MH)^+$ - $C_{49}H_{51}NO_{15}$ -

894.3322

Calculated - 894.3338

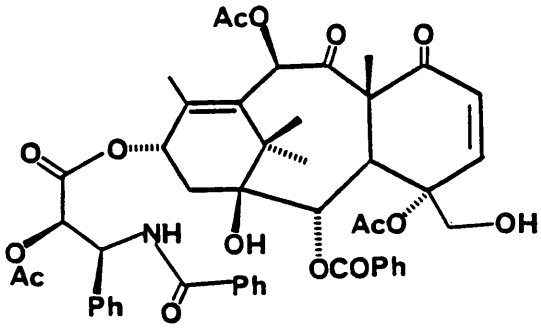
hours. After 19 hours two faint nontaxol spots could be seen, Rfs 0.31, 0.12. After a total time of 60 hours tlc showed a complex mixture of products all of which were more polar than taxol/cephalomannine. Even at that point taxol/cephalomannine were the major compounds present. Taxol and cephalomannine appeared as one spot during tlc analysis.

2'-Acetyl-7-oxo-5,6-dehydro-5,O-secotaxol (**58**) - 2'-Acetyl-7-oxotaxol (150 mg) was dissolved on 0.5 mL of 0.5% DBU in CH_2Cl_2 at room temperature. As soon as the reaction was checked by tlc (1 minute) it was complete. The reaction was then worked up to yield 2'-acetyl-7-oxo-5,6-dehydro-5,O-secotaxol. Yield-19 mg, 95%. Characterization data for **58** are shown in Table 32.

2'-N-carbobenzoxy-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol (**59**) - 2'-N-carbobenzoxy-β-alanyl-7-oxotaxol (10 mg) was dissolved in 1.0 mL of dry CH_2Cl_2 and had 3.0 µL of DBU added. When the reaction was first checked by tlc after 10 minutes the reaction was complete. The reaction was worked up to yield 2'-N-carbobenzoxy-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol. Rf 2'-N-carbobenzoxy-β-alanyl-7-oxotaxol 0.53, 2'-N-carbobenzoxy-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol 0.63, 6/4 ethyl acetate/hexane. Yield-8.2 mg, 82%. Characterization data for **59** are shown in Table 33.

Table 32 Characterization Data for 2'-Acetyl-7-oxo-6-dehydro-5,0-secotaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.62 (d, 6)
3	4.17 (d, 6)
5	7.00 (d, 10)
6	6.01 (d, 10)
10	6.38 (s)
13	5.87 (br d, 11)
14	2.96 (dd, 16,4), 2.44 (dd, 16,11)
16	1.06 (s)
17	1.20 (s)
18	1.55 (s)
19	1.90 (br s)
20	4.12 (d, 12), 4.37 (d, 12)
2'	5.31 (d, 2)
3'	6.31 (dd, 2,9)
N-H	7.00 (d, 9)
OAcs	1.70 (s), 2.17 (s), 2.21 (s)
2 OBz	8.22 (d, 8), 7.4 (m), meta 7.20 (t, 8)
3' NBz	7.80 (d, 8), 7.4 (m)
3' Ph	7.4 (m)
Hydroxyl	4.60 (br s)



Mass Spectral Data

916 $(MNa)^+$, 894 $(MH)^+$,

876 $(MH-H_2O)^+$,

834 $(MH-HOAc)^+$,

549 $(MH-RCOOH-H_2O)^+$,

507 $(MH-RCOOH-HOAc)^+$.

High Resolution

$(MH)^+$ - $C_{49}H_{51}NO_{15}$ - 894.3236

Calculated - 894.3338

Infrared Spectral Data

1760 s, 1745 s, 1675 m,

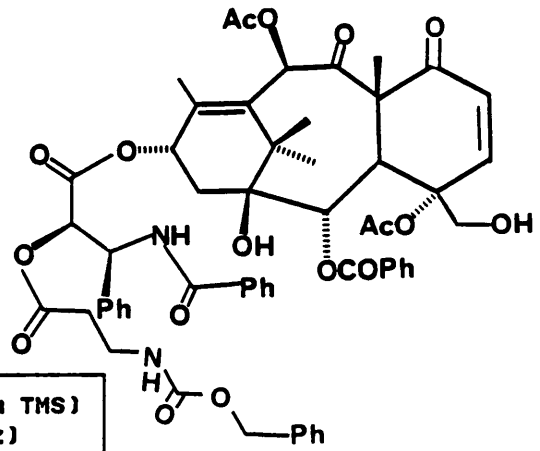
1530 w, 1505 w, 1470 w,

1390 m, 1280 s-sh,

1240 s, 1105 m,

1085 m, 1075 m, 720 m.

Table 33 (Part 1 of 2). Characterization Data for 2'-(N-Carbobenzoxy-β-alanyl)-7-oxo-6-dehydro-5,0-secotaxol



Position	Shift (ppm from TMS) Coupling (hertz)
2	5.63 (d, 7)
3	3.18 (d, 7)
5	7.01 (d, 10)
6	6.01 (d, 10)
10	6.37 (s)
13	5.88 (br d, 9)
14	2.98 (dd, 4,12), 2.40 (dd, 12,16)
16	1.05 (s)
17	1.20 (s)
18	1.54 (s)
19	1.90 (s)
20	4.11 (d, 12), 4.38 (d, 12)
2'	5.32 (d, 2)
3'	6.35 (dd, 2,5)

Mass Spectral Data

567 $(MH-RCOOH)^+$,

549 $(MH-RCOOH-H_2O)^+$,

507 $(MH-RCOOH-HOAc)^+$,

491 $(RCOOHH)^+$.

Infrared Spectral Data

1770-40 s, 1675 m, 1545 m,

1475 w, 1395 m, 1290 s-sh,

1245 s, 1190 m, 1110 m,

1090 m, 1060 m, 720 w.

Table 33 (Part 2 of 2). Characterization Data for 2'-(N-Carbobenzoxy-β-alanyl)-7-Oxo-6-dehydro-5,0-secotaxol

Position	Shift (ppm from TMS) Coupling (hertz)
N-H	7.2-7.6 (d)
OAcs	2.22 (s), 1.68 (s)
2 OBz	8.28 (d), 7.4 (m)
3' NBz	7.83 (d), 7.4 (m)
3' Ph	7.4 (m)
N-CBZ-β-alanine	
2	3.45 (m), 3.65 (m)
3	2.63 (m)
NH	5.18 (br t, 7)
Benzylic	4.89 (br s)
3' Ph	7.4 (m)

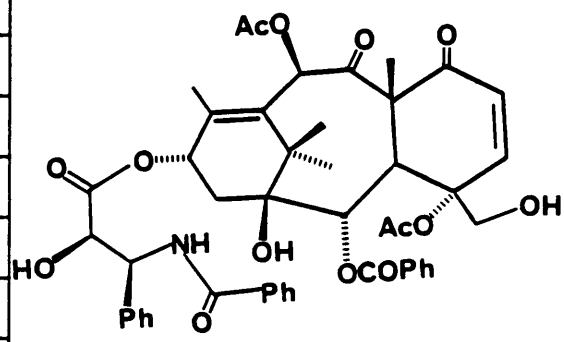
2'-N-carbobenzoxy-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol **59** - A sample of 2'-N-carbobenzoxy-β-alanyl-7-oxotaxol (30 mg) which was pure to begin with was streaked onto a silica gel preparative tlc plate and the plate was developed with 55/45 ethyl acetate/hexane. The developed plate showed only one narrow band Rf 0.85. This product was 2'-N-carbobenzoxy-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol. Yield 28 mg, 93%.

7-Oxo-5,6-dehydro-5,O-secotaxol (**61**) - Taxol (50 mg) was dissolved in 0.5 mL of acetone and to this mixture Jones oxidizing reagent (20 µL) was added at room temperature. After 20 minutes the reaction was stopped, worked up and the product isolated by preparative tlc, 1/1 ethyl acetate/hexane, Rf 0.53. Yield-35 mg, 70%. Characterization data for **61** are shown in Table 34.

Reaction of 2'-N-Carbobenzoxy-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol with Acetic Anhydride/Pyridine - 2'-N-Carbobenzoxy-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol (100 mg) was dissolved in 2.0 mL of dry pyridine and acetic anhydride (200 µL) was added at room temperature. The reaction was allowed to proceed for 3 hours and then worked up. Analysis by tlc and nmr showed that the only compound present at the end of the reaction was starting material.

Table 34 Characterization Data for 7-Oxo-6-dehydro-5,0-secotaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.60 (d, 6)
3	4.18 (d, 6)
5	7.00 (d, 10)
6	6.00 (d, 10)
10	6.37 (s)
13	5.95 (br dd, 4,10)
14	3.04 (dd, 4,16)
16	1.20 (s)
17	1.08 (s)
18	1.83 (br s)
19	1.56 (s)
20	4.35 (d, 12), 4.18 (d, 12)
2'	4.82 (br s)
3'	6.08 (d, 9)
N-H	7.12 (d, 9)
OAcs	C-10 2.20 (s), C-20 1.77 (s)
2 OBz	(2H) 8.20 (d, 8), (2H) 7.11 (t, 8) 7.4 (m)
3' NBz	7.79 (d, 7.5), 7.4 (m)
3' Ph	7.4 (m)



Mass Spectral Data

890 $(MK)^+$, 874 $(MNa)^+$,

852 $(MH)^+$, 834 $(MH-H_2O)^+$,

549 $(MH-RCOOH-H_2O)^+$,

507 $(MH-RCOOH-HOAc)^+$.

RCOOH = C-13 side chain

Infrared Spectral Data

1765 s, 1745 s, 1685 m,

1695 m-sh, 1530 mw,

1505 mw, 1470 mw,,

1380 mw, 1265 s, 1230 s,

1100 m, 1075 m, 1045 m.

		C-20 OH	4.60 (s)

Reaction of 2'-N-CBZ-β-alanyl-7-oxo5,6-dehydro-5,O-secotaxol with Acetic Anhydride/4-DMAP - 2'-N-CBZ-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol (15 mg) and 4-dimethylamino pyridine were dissolved in dry pyridine (200 µL). Acetic anhydride (100 µL) was then added. The reaction was allowed to proceed for 19 hours. At the end of the reaction the solution had become colored orange/red. The reaction was worked up as usual. Analysis of the crude products by tlc showed a mixture of products.

Reaction of 2'-Acetyl-7-oxotaxol with Borohydride - 2'-Acetyl-7-oxotaxol (14 mg) and $(Bu)_4NBH_4$ (6 mg) were dissolved in 0.30 mL of dry CH_2Cl_2 at room temperature. The reaction was followed by tlc and all of the starting material had reacted after 8 minutes. The reaction was stopped by adding several drops of acetone and was then worked up. Analysis by nmr and tlc showed the major product to be 2'-acetyl-7-oxo-5,6-dehydro-5,O-secotaxol. This product is not stable in BH_4^- so this resulted in some decomposition.

Reaction of 2'-N-Carbobenzoxy-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol with $BH_3 \cdot THF$ - 2'-N-Carbobenzoxy-β-alanyl-7-oxo-5,6-dehydro-5,O-secotaxol (4.3 mg) was dissolved in 0.5 mL of dry CH_2Cl_2 at room temperature and 1.0 M $BH_3 \cdot THF$ (10 µL) was added. After 30 minutes no reaction was seen taking place and so additional $BH_3 \cdot THF$ (250 µL) was added. After

an additional 2 hours reaction time the reaction was stopped by the addition of 1 mL of methanol. All solvent were then evaporated in vacuo and the reaction was analysed by tlc (50/50 ethyl acetate/hexane, silica gel). Tlc showed starting material present Rf 0.45, and a more polar streak in which product spots too numerous to count accurately were present.

Hydrogenation of 2'-acetyl-7-oxo-5,6-dehydro-5,O-secotaxol with Palladium - A sample of 2'-acetyl-7-oxo-5,6-dehydro-5,O-secotaxol (10 mg) was dissolved in 2.0 mL of ethyl acetate. After adding 4.0 mg of 5% Pd/C the mixture was placed under one atmosphere of hydrogen. Over a period of twelve hours the reaction was followed by thin layer chromatography which showed no reaction at all taking place.

Hydrogenation of 7-Oxo-6-dehydro-5,O-secotaxol with Platinum as Catalyst - A sample of 7-oxo-6-dehydro-5,O-secotaxol (35 mg) was dissolved in 10 mL of methanol and 5% Pt/C (17 mg, Engelhard) was added. The reaction mixture was stirred under a hydrogen atmosphere at room temperature for 3 hours, at which point hplc analysis (see Figure 17) showed no starting material present. The catalyst was filtered off, the products redissolved in a minimal amount of CH_2Cl_2 and purified by preparative hplc (RP-8, 7/3, MeOH/H_2O, 6 mL/min). Yield

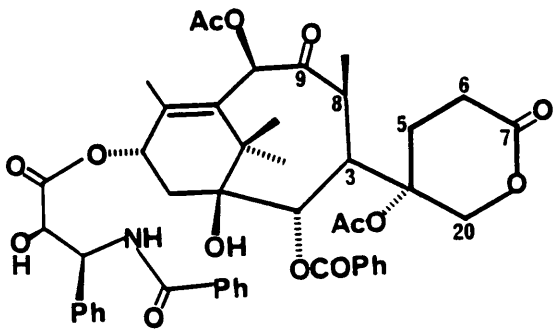
of **62** - 27 mg, 77%. Characterization data are shown in Table 35.

In a separate experiment the procedure described above was followed with the exception that CD_3OD was used as solvent and the crude reaction mixture was analyzed by ^1H-nmr with no purification by hplc. Analysis by ^1H-nmr immediately after hydrogenation showed a complex mixture of products present. Over the course of several days nmr showed the mixture changing and reaching an equilibrium state (see Table 23).

Preparation of 2'-Acetyl-7-oxo-5,O-secotaxol via Hydrogenation of 2'-Acetyl-7-oxo-6-dehydro-5,O-secotaxol - A sample of 2'-acetyl-7-oxo-6-dehydro-5,O-secotaxol (39 mg) was dissolved in 5 mL of EtOAc and 23 mg of 5% Pt/C was used as catalyst. The mixture was stirred under a hydrogen atmosphere for 3 hours at which time the catalyst was filtered off and the solvent removed on a rotary evaporator heating with a 30° water bath. Traces of solvent were removed by drying the sample in a vacuum desiccator at room temperature for several hours. The sample was dissolved in $CDCl_3$ and analyzed by ^1H nmr which showed a 4/1 mixture of two taxol-like compounds. After standing overnight only the major taxol-like compound **63** was seen by nmr analysis, some impurity peaks were present but integrated for no more than 10% of the peak area. After standing in $CDCl_3$ solution for 2 weeks at room temperature

Table 35 Characterization Data for **62**

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.73 (d, 7)
3	3.77 (dd, 2,7)
5	2.3-2.5 (m)
6	2.3-2.5 (m)
8	3.10 (qd, 2,7)
10	6.18 (s)
13	6.05 (br dd, 4,10)
14	2.2-2.5 (m)
16	1.08 (s)
17	1.06 (s)
18	1.77 (br s)
19	1.39 (d, 7)
20	4.68 (br d, 11), 4.43 (d, 11)
2'	4.91 (dd, 2,5)
2'-OH	3.44 (d, 5)
3'	5.87 (dd, 2,9)
N-H	7.14 (d, 9)
OAcs	2.18 (s), 1.89 (s)
2 OBz	8.14 (dd, 1,8), 7.58 (t, 8), 7.4 (m)
3' Ph	7.4 (m)
3' NBz	7.78 (dd, 1,8), 7.4 (m)



Mass Spectral Data

876 $(MNa)^+$, 854 $(MH)^+$,

509 $(MH\text{-}RCOOH\text{-}HOAc)^+$,

286 $(RCOOHH)^+$.

High Resolution

$(MH)^+$ - $C_{47}H_{49}NO_{14}$ - 854.3246

Calculated - 854.3389

Infrared Spectral Data

1800 m-sh, 1765 s, 1753 s,

1740 s, 1690 m-sh, 1667 s,

1643 m.

nmr showed the product to be unchanged. Characterization data for **63** are shown in Table 24.

5.0 D RING SECO TAXOLS VIA ELECTROPHILIC PROCESSES

This section of the taxol project addresses the preparation of more taxol derivatives which are differentiated from taxol by the absence of the D ring - the oxetane. In Chapter 4 the preparation of several D ring seco taxols was reported which were to be tested for biological activity. Preparation of additional D ring seco taxols was necessitated because the D ring seco taxols previously prepared all had been modified in more ways than simple opening of the oxetane. Biological activity results from a series of D seco taxols would give a more accurate picture of the necessity of the presence of the oxetane for biological activity.

In contrast to the oxetane cleaving reactions presented in Chapter 4, all of which originated from a beta elimination of an oxidized taxol, the reactions presented in this chapter involved electrophilic oxetane cleavage on unmodified taxol. There are many electron rich sites in taxol and reading of the literature did not reveal any reagent that would be selective for cleavage of oxetane rings in the presence of the wide variety of functional groups present in taxol; no reactions opening the oxetane of a taxane have been reported prior to this work. The absence of precedent for the selective opening of an oxetane did not prevent attempting the transformation; taxol is an extraordinarily complex molecule

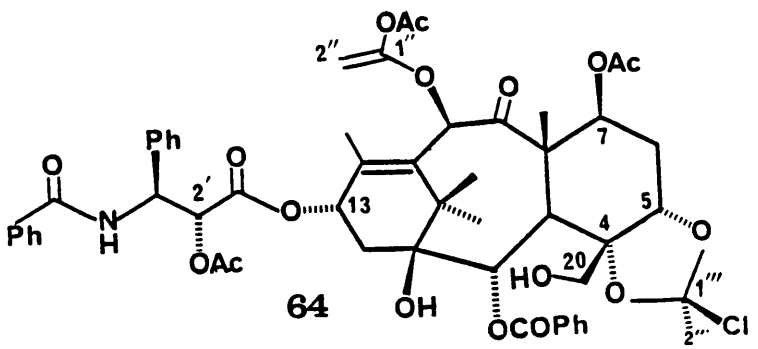
and there is no reasonable model compound for it. Absence of literature precedent could not be substituted for experimental results.

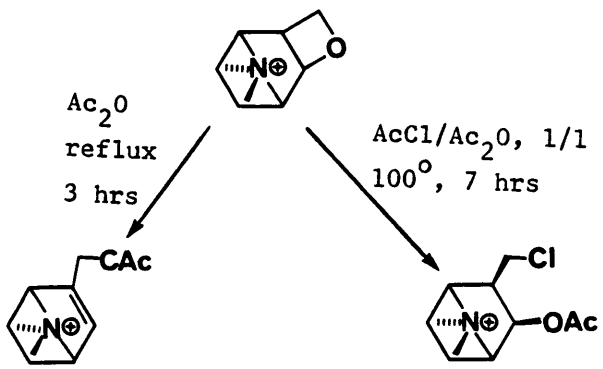
Three electrophilic reagents were chosen to cleave taxol's oxetane: acetyl chloride, acetic anhydride and triethyloxonium tetrafluoroborate. Acetyl chloride and acetic anhydride were chosen as Kovacs et. al.[51] had used these reagents to cleave an oxetane ring (Scheme 29). Triethyloxonium tetrafluoroborate had been shown by Raber and Guida to cleave an ether linkage with assistance by an ester (Scheme 29).[52]

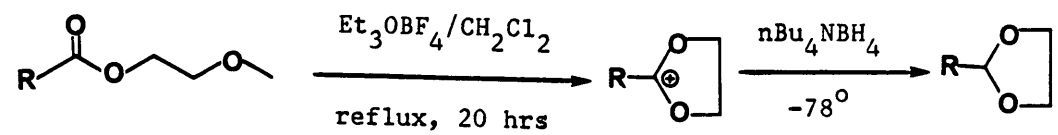
5.1 RESULTS AND DISCUSSION

5.1.1 REACTION OF TAXOL WITH ACETYL CHLORIDE/HCL

Reaction of taxol with a refluxing solution of 1/1 acetyl chloride/acetic anhydride for three hours gave in 85% isolated yield the diacetyl, enol acetate, D ring seco, chloroketaltaxol **64**. Analysis of the isolated sample by ^{1}H-nmr and tlc showed it be at least 95% pure.







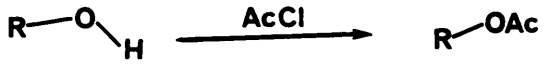
R = alkyl or aryl

Scheme 30. Ester Assisted Ether Cleavages

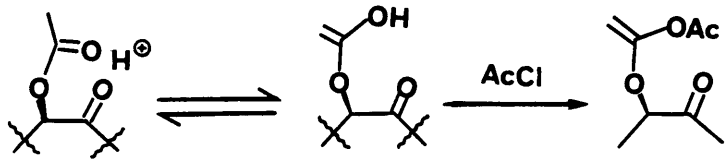
The reaction was also carried out using acetyl chloride alone with no change in results. When acetyl chloride and acetic anhydride were used part of the work-up consisted of washing with 0.1% $NaHCO_3$ or addition of methanol. When acetyl chloride alone was used work up consisted only of evaporation of the acetyl chloride; the product was the same in all cases. Isolation of the product in all reactions was by preparative tlc (silica gel, 1/1 EtOAc/Hexane). Analysis by hplc (RP-8, analytical, 3/1, MeOH/H_2O, 2 mL/min) showed the product to be approximately 95% pure with a major part of the impurities being 2',7-diacetyltaxol; ^{1}H-nmr analysis showed one major taxol-like compound and small impurity peaks. The sample was not purified further as the impurities did not interfere with characterization or biological test results. Reaction of taxol with refluxing acetic anhydride for 7 hours was also carried out; after work up hplc analysis showed only a complex mixture of products present.

Three processes occurred in the synthesis of **64**: acetylation of the C-2' and C-7 hydroxyl groups, acetylation of the C-10 acetate and addition of HCl to the C-5 - oxygen bond with assistance by the C-4 acetate. (Scheme 30) Addition of acetyl chloride to open the oxetane did not occur as the reaction conditions were not as vigorous as those used by Kovacs. The acetyl chloride used in the synthesis of **64** was obtained from a 500 mL bottle that was nearly empty, was not distilled before use and so contained a significant, though

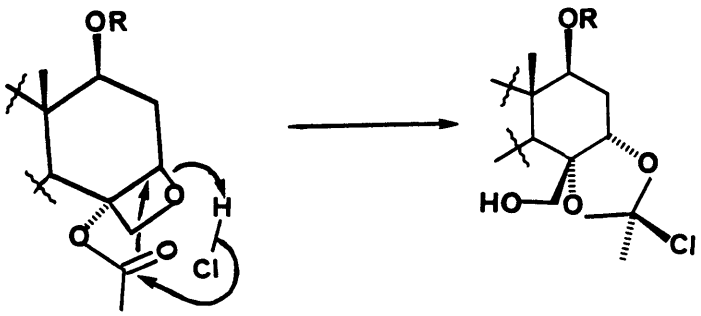
1. Acetate Formation



2. Formation of an Enol Acetate



3. Concerted Addition of HCl to Form a Ketal



Scheme 31. Processes Occuring During the Synthesis of **64**

undetermined amount of HCl. Use of fresh acetyl chloride caused the reaction to proceed poorly, yielding mainly 2',7-diacetyltaxol.

The reaction at C-10 was a separate process from that taking place at the D ring; in order to keep an explanation of the structural assignment as simple as possible the structural assignment at C-10 and the D ring will be presented separately. Assignment of the C-2' and C-7 acetates along with the C-10 functionality will be addressed first.

Obtaining a 270 ^{1}H MHz-nmr spectrum of **64** was the first step in the characterization of the compound.* The ^{1}H-nmr spectrum of **64** is shown in Figure 19. A comparison of the ^{1}H-nmr spectrum of **64** and 2',7-diacetyltaxol is shown in Table 36. The spectra clearly show that, as expected, the C-2' and C-7 hydroxyls were acetylated; the signals from the C-2' and C-7 protons of **64** are almost identical to those of 2',7-diacetyltaxol.

The spectra also show major differences in the signals of protons located on or close to the A ring. The signal for the C-13 proton of 2',7-diacetyltaxol occurs at 6.15 ppm (t, J=8), in taxol the signal also occurs at 6.15 ppm (t, J=8) and in 13-acetylbaccatin III the signal occurs at 6.16 ppm (t, J=8). In **64** however the signal for the C-13 proton signal

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* A reliable molecular weight was desired as the first characterization datum for **64** but, as will be shown, this was not possible.

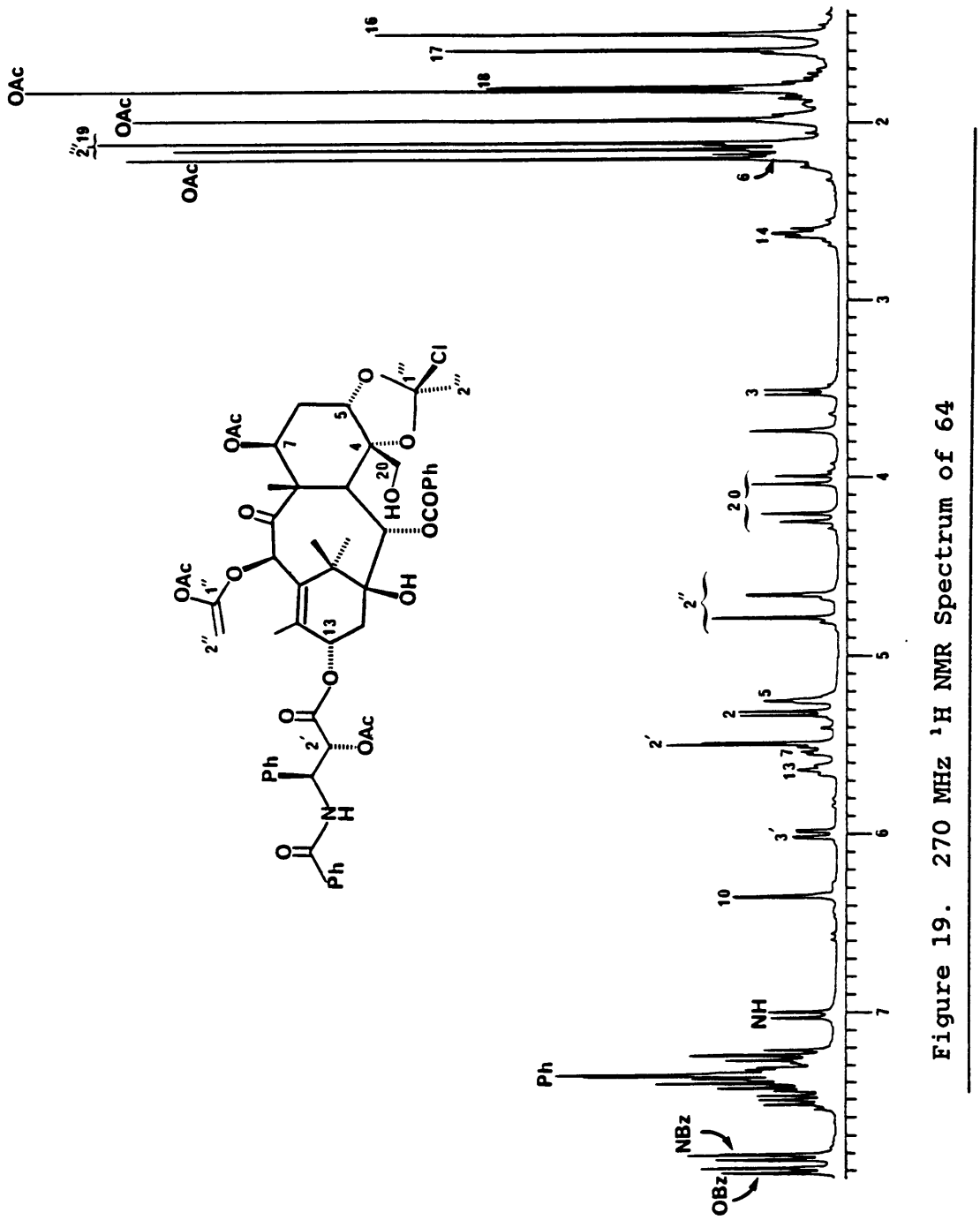
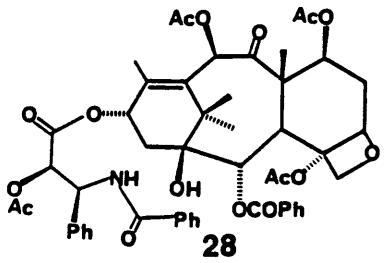
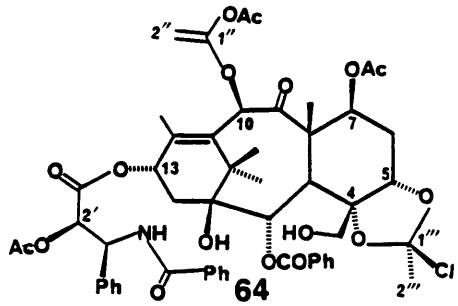


Figure 19. 270 MHz ^{1}H NMR Spectrum of 64

Table 36 (Part 1 of 2). Comparison of the ^1H nmr Spectra of **28** and **64**



28



64

Position	Shift (ppm from TMS) Coupling (hertz)	Shift (ppm from TMS) Coupling (hertz)
2	5.60 (d, 7)	5.34 (d, 7)
3	3.87 (d, 7)	3.52 (d, 7)
5	4.90 (d, 9)	5.25 (t, 2.5)
6	2.2 (m)	2.2 (m)
7	5.53 (m)	5.52 (dd, 4,11)
10	6.18 (s)	6.35 (d, 0.7)
13	6.15 (t, 8)	5.54 (br t, 7)
14	2.55 (m)	2.60 (dd, 7,13), 2.67 (dd, 7,13)
16	1.14 (s)	1.58 (br s)
17	1.09 (s)	1.49 (s)
18	1.74 (br s)	1.78 (br s)
19	1.91 (s)	2.09 (s) or 2.14 (s)
20	4.11 (d, 8), 4.25 (d, 8)	4.01 (d, 12), 4.21 (d, 12)

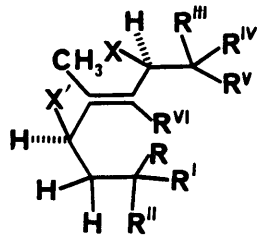
Table 36 (Part 2 of 2). Comparison of the ^1H nmr Spectra of **28** and **64**

Position	Shift (ppm from TMS) Coupling (hertz)	Shift (ppm from TMS) Coupling (hertz)
2'	5.50 (d, 3)	5.48 (d, 2)
3'	5.89 (dd, 3,9)	6.00 (dd, 2,9)
N-H	---	6.99 (d, 9)
OAcs	1.96 (s), 2.08 (s), 6H 2.36 (s)	1.82 (s), 1.92 (s), 2.18 (s)
2 OBz	7.45 (t, 7), 7.52 (t, 7), 8.06 (dd, 1,7)	7.90 (d m, 7), 7.29 (t, 7), 7.4 (m)
3' NBz	7.33 (m), 7.45 (t, 7), 7.67 (dd, 1,7)	7.82 (d m, 7), 7.4 (m)
3' Ph	7.3 (m)	7.4 (m)
C-2'''	---	2.09 or 2.14
Vinyl	---	4.78 (br s), 4.67 (br s)
C-20 OH		3.70 (s)
		Peaks at 1.82 ppm, 1.92 ppm, and 2.18 ppm did not appear when the reaction was run with d_3-AcCl.

occurs at 5.54 ppm (t, J=8) which is upfield of that seen for the C-13 esterified baccatin IIIs (such as taxol) but downfield of the C-13 proton signal for baccatin III, 4.82 ppm (br t, J=9). Irradiation of the C-13 proton signal of **64** caused the two doublets of doublets produced by the C-14 protons, 2.60 ppm (J=7,13), 2.67 ppm (J=7,13), to collapse to two doublets (J-13) and the signal for the C-10 proton, 6.35 ppm (d, J=0.7) to collapse to a singlet.

The signal for the C-13 proton shows that the C-13 position is esterified, the presence of the side chain also supports this, but that some change is taking place affecting the A ring. The small homoallylic coupling between the C-13 and C-10 protons, which is unique to **64**, shows that the C-10 to C-13 functionality is intact. This conclusion considered along with the coupling of the C-14 protons to the C-13 proton indicates that the structure shown in Figure 20 must be present in **64**.

The ^{1}H-nmr spectrum of **64** also showed the methyl singlets for C-17 at 1.58 (br s) and C-16 at 1.49 ppm (s) in contrast to the analogous signals of 2',7-diacetyltaxol for C-17 at 1.09 ppm (br s) and C-16 1.14 (s). This change in chemical shift of the C-16 and C-17 geminal methyl had never been seen before in any taxol-like compound. For all taxol derivatives in this project except for **64**, the methyl signals for C-16 and C-17 occur upfield of 1.25 ppm. As with the C-13 and C-10

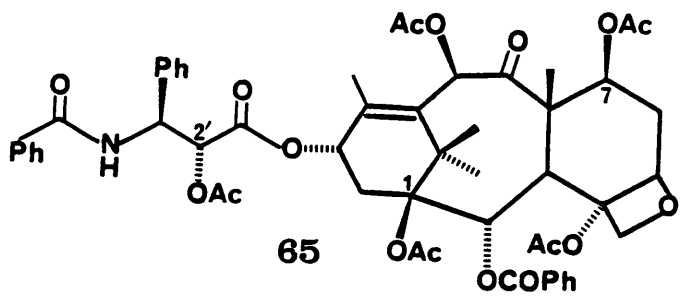


X,X'= Electronegative Groups

Figure 20. C-9 to C-1 Skeleton of **64**

signals the C-16 and C-17 methyl signals of **64** indicate that some transformation had taken place on or near the A ring.

The first structure that was considered as a possibility for **64**, which would explain the change in the A ring peaks, was 1,2',7-triacetyltaxol **65**. In order to test this hypothesis a sample of **64** was reacted with 0.025% $NaHCO_3$ in 3/1 MeOH:H_2O at room temperature. These conditions had previously been used to remove the C-2' acetyl group from 2',7-diacetyltaxol. In contrast to the reaction with 2',7-diacetyltaxol basic sovolysis of **64** produced a complex mixture of products from the start of the reaction. One of the products isolated from the solvolysis was the methyl ester of the side chain of taxol, which is a known compound. This showed that in the reaction of AcCl with taxol no reaction, other than acetylation, had taken place on the side chain. This reactivity data indicated that **64** was not



65

1,2',7-triacetyltaxol. Careful integration of the ^{1}H-nmr spectrum also showed that only eight three proton singlets were present.

A second hypothesized structural change that would explain the proton nmr data was epimerization at C-13. The ^{1}H-nmr data showing changes in the signals from the A ring also suggested epimerization at C-13. However, the presence of the C-13 proton signal as a broad triplet with a 7 Hz coupling constant showed that C-13 had not epimerized. The C-13 proton signal requires dihedral angles of 20° and 140° between the C-13 and C-14 protons[48]. Manipulation of a model of taxol showed that this conformation can be attained without any noticeable bond strain. Epimerization at C-13 would produce dihedral angles of 20° and 80° with corresponding coupling constants of 7 hertz and 0 Hz so that the C-13 proton signal would be seen as a doublet.

The presence of an enol acetate at C-10 of **64** is consistent with changing nmr spectral data for the proton at C-10

and those of the A ring. The enol acetate also allowed for an explanation of other unusual proton nmr spectral data such as the ^{1}H-nmr spectrum of **64** showing the presence of broad singlets at 4.78 ppm and at 4.67 ppm. One proton singlets had previously not been seen in this area of the proton spectrum for any taxol derivative. Irradiation of either of the singlets caused the other singlet to sharpen and increase in intensity. Addition of D_2O to the $CDCl_3$ did not cause the signals to disappear. Irradiation of the singlet at 4.67 ppm not only caused the singlet at 4.78 ppm to increase in intensity but the C-17 methyl signal at 1.58 ppm also increased in intensity so that it was slightly more intense than the C-16 signal. Irradiation of the C-16 methyl signal also caused the singlet at 4.67 ppm to increase in intensity so that it was more intense than the 4.78 ppm singlet. The decoupling power used corresponded to 2 Hz and the changes in intensity did not require repeated scans but could be seen after only 2 scans.

Prior to the assignment of the structure of **64** the possibility of this behavior being due to long range coupling was considered. Inspection of the skeleton in Figure 20 however shows that for the addition of a proton which is long range coupled to the C-17 methyl and which had not been present in taxol to take place, cleavage of the A ring would have to have occurred. No reasonable mechanism or driving force exists for the A ring to open under the conditions of the reaction.

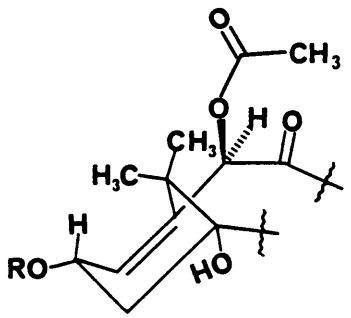


Figure 21. C-10 Portion of Taxol

The enol acetate at C-10 allowed for an explanation of the behavior of the singlets at 1.58 ppm and 4.67 ppm - the changes in signal intensity were due to a nuclear Overhauser effect. Nuclear Overhauser effects are seen when protons are spacially close but are not directly coupled. For example in taxol the proton at C-13 and the C-16 methyl groups have been shown to exhibit at nOe as the stereochemistry of the A ring forces these groups together. In this case of **64** however there is no ring system forcing the vinyl proton and the methyl group together. Analysis of the taxane skeleton indicated the reasons that an nOe is seen between the two groups.

A three dimensional structure of the C-10 portion of taxol is shown in Figure 21. The C-10 acetoxy group of taxol is spatially close to the geminal dimethyl groups at C-15; manipulation of a model of taxol showed that the methyl group of the acetoxy residue could easily touch the carbons of C-16

and C-17 without producing any bond strain in the model. Because the acetoxy group can rotate, the methyl group will not be close to the geminal dimethyl groups and no nOe is seen in taxol.

In **64**, as in taxol, the functional group at C-10 is spatially close to the geminal dimethyl groups and there is free rotation present. The factors which govern the conformation of the group at C-10 relative to the taxane skeleton however have changed. A three dimensional drawing of the C-10 area of **64** is shown in Figure 22.

In **64** free rotation of the bonds at C-10 is going to be such that the total amount of steric strain is minimized. Analysis of a model of **64** showed that it is not possible for all groups at C-10 to be in an uncrowded environment, because of the constraint that the atoms attached to C-1" are 180° apart and coplanar. Movement of one group therefore produces an opposite movement of the other. In **64** the acetoxy group at C-1" is larger than the terminal methylene group. Rotation of the bonds at C-10 is such that the acetoxy group is pointed away from the bulk of the taxane skeleton. This will cause the terminal methylene group to be pointed in toward the geminal dimethyl groups and for this reason an nOe was seen.

The nOe coupling between the broad singlet at 4.67 ppm and the C-17 methyl singlet in the proton spectrum of **64** can be explained by the structure of **64** but is not conclusive proof

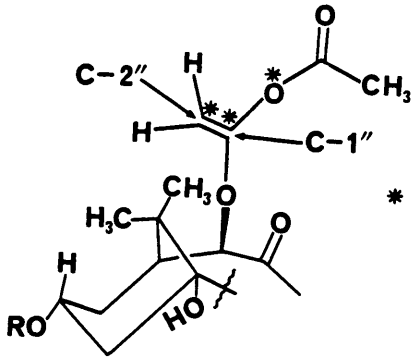


Figure 22. C-10 Area of **64**

of an enol acetate at C-10. Consideration of both broad one proton singlets in the proton spectrum of **64** coupled with data from the mass spectrum and ^{13}C spectrum of **64** did show conclusively that an enol acetate of an acetate is present in the structure of **64**.

Compound **64** presented great difficulties in obtaining a mass spectrum. A sample sent to VG Instruments for fast atom bombardment (FAB) mass spectral analysis resulted in reporting that no data could be obtained. Samples sent to the Midwest Center for Mass Spectrometry at the University of Nebraska and to Johns Hopkins University, also for FAB analysis, resulted in some data but no parent ion. The data that was obtained, however, considered in light of mass spectral data for other taxol analogues did give important information about the structure of **64**.

The peaks with highest m/z seen in the mass spectrum obtained from the Midwest Center for mass spectrometry were at

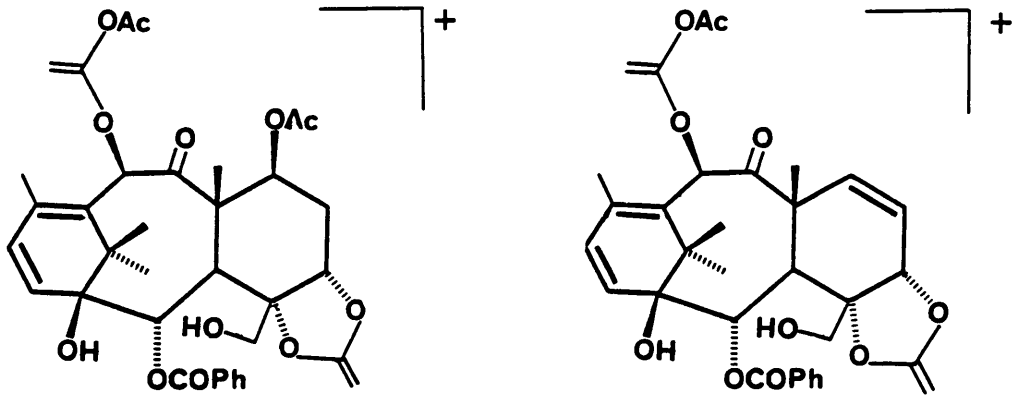


Figure 23. m/z 653 and 593 ions for **64**

m/z 653 and 593. The ions associated with these peaks are shown in Figure 23. Even before structural assignment had been made these peaks clearly showed the addition of 2 acetates, or their equivalents, to the taxane skeleton. This conflicted with the ^{1}H-nmr data in which all of the methyl singlet peaks could be accounted for by acetylation of the C-2' and C-7 hydroxyl groups. Resolution of this conflict was gained by analysis of the ^{13}C spectrum.

The ^{13}C spectra of **64** provided information which led to the structural assignment of **64** and, once the structure was assigned, gave a large amount of data supporting the structural assignment.* The broad band decoupled ^{13}C spectrum and of **64** is shown in Figure 24. An INEPT spectrum was also obtained and is shown in Figure 25. The spectra were compared and contrasted with the published ^{13}C spectrum of baccatin

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* An in depth discussion of ^{13}C data will be presented later.

III[16] and with broad band decoupled, INEPT and selective proton decoupled spectra of taxol obtained in this research.

A striking characteristic of the ^{13}C spectra of **64** is that there are four methylene groups present. Taxol and baccatin III possess only three methylene groups; the methylene groups of baccatin III occur at 35.74 ppm for C-6, 38.80 ppm for C-14 and 72.33 ppm for C-20, the analogous groups for taxol occur as overlapping peaks at 35.87 ppm and the C-20 signal at 72.03 ppm. These peaks were approximately matched in the spectrum of **64** by peaks at 35.74 ppm, 37.94 ppm and 63.69 ppm. The additional peak occuring in the spectrum of **64** was at 113.08 ppm which is indicative of a terminal methylene group. Selective decoupling of either of the vinyl protons caused the triplets for the terminal methylene carbon to collapse to a doublet.

The presence of geminal vinyl proton signals in the nmr spectrum of **64** along with the mass spectral data showing the addition of three acetates to the taxane skeleton and ^{13}C data showing the addition of a terminal methylene group all agree with the enol acetate present in **64**.

The position of the enol acetate of an acetate in **64** was conclusively shown by a labelling experiment in which taxol was refluxed with deuterated acetyl chloride for three hours and worked up as in previous reactions. The sample of **64** obtained from this reaction was contaminated with approximately 60% 2',7-diacetyltaxol. The large amount of

2',7-diacetyltaxol present was due to pure acetyl chloride being employed for the reaction; the only HCl available for the reaction came from reaction of the acid chloride with the C-2' and C-7 hydroxyl groups. The mixture of products was not purified further as the ^{1}H nmr signals for 2',7-diacetyltaxol are known and did not interfere with analysis of the ^{1}H-nmr spectrum of **64**. The ^{1}H-nmr spectrum of the deuterated **64** showed all of the peaks of nondeuterated **64** except for three three proton singlets at 1.82 ppm, 1.92 ppm and 2.18 ppm; these missing signals correspond to the acetates at C-2', C-10 and C-7. The broad vinyl protons could be clearly seen. The presence of the vinyl proton signals showed that these protons originated from an acetate on taxol and not from acetyl chloride. The only possibilities for the reactive acetate were the acetates at C-10 and C-4, and as will be shown later the acetate at C-4 had undergone transformation to form a ketal. The only possibility therefore for the position of the enol acetate was C-10.

5.1.2 STRUCTURAL ASSIGNMENT OF THE C RING

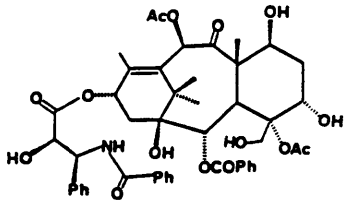
The second part of the structure assignment of **64** to be addressed is the opening of the D ring and formation of the suprisingly stable chloro ketal. Structural assignment of this moiety was based on a variety of techniques: comparison of the ^{1}H-nmr spectrum of **64** with other D seco taxols, ^{13}C

spectral data, unique mass spectral data and the reaction mechanism. Analysis of the spectral data for **64** showed that the assigned structure is the only possibility that would account for all the data.

The ^{1}H-nmr spectrum of **64** shows that the oxetane is not present and that C-5 had been epimerized. Signals in the proton spectrum of **64** characteristic of D ring seco taxols include:

1. A 12 hertz coupling constant for the C-20 protons signals;
2. The signal for the meta benzoate protons shifted upfield to 7.24 ppm (7, J=8);
3. Presence of the C-20 hydroxyl group signal at 3.70 ppm (s).

In addition to these data the signal for the C-5 proton, 5.25 ppm (t, J=2.5) was characteristic of a reversal of stereochemistry at C-5. In 5-epihydroxy-5,O-secotaxol **66** synthesized in this project and in the known natural products **67** and **68** the signal for C-5 is seen as a triplet with a 3 hertz coupling constant. The chemical shift of the C-5 proton indicates that the position is not substituted by a hydroxyl group as the peak for the proton at C-5 of 5-epihydroxy-5,O-secotaxol is seen at 3.88 ppm (t, J=3).



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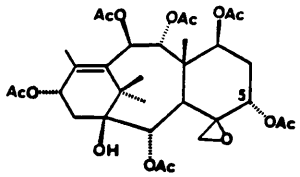

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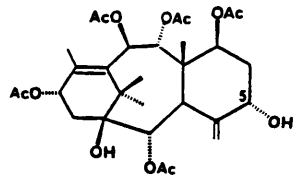


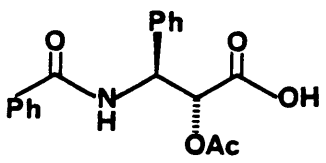
68

The signals for the C-14 protons of **64** at 2.60 ppm (dd, J=7,13) and 2.67 ppm (dd, J=7,13) indicate a different conformation of the taxane skeleton for **64** than would be present if only the A, B and C rings were present. In 7-oxo-6-dehydro-5,O-secotaxol, 7-oxo-5,O-secotaxol and 5-epihydroxy-5,O-secotaxol the signals for the C-14 protons occur as doublets of doublets at 2.95-3.05 ppm and 2.45 ppm and the positions of these signals are due to the conformation the taxane skeleton exists in when only the A, B and C rings are present.

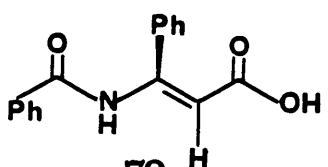
The appearance of the C-13 proton signal as a broad triplet (J=7) in the proton spectrum of **64** yielded a tremendous amount of information about the conformation of the skeleton of **64**. In all the D ring seco taxol analogues prepared in this project, except for **64**, the signal for the C-13 proton is seen as a doublet of multiplets or as a doublet of doublets with a large difference between coupling constants. In addition to this there are no known taxane compounds not possessing an oxetane in which the C-13 proton signal is seen

as a triplet. Whatever functional group had been added during the opening of the oxetane ring of taxol must impose the same constraint on the conformation of the taxane ring system as the oxetane; the ketal in **64** is capable of doing this. A five membered ring, while not as planar as an oxetane, is a fairly flat structure. The ketal in **64** imposed the constraint that the alpha face bonds at C-4 and C-5 be parallel. This would also cause the beta face bonds at C-4 and C-5 to be parallel and this is the conformational constraint that the oxetane imposed on the taxane skeleton. The splitting of the C-13 proton in **64** is sufficient to show that the taxane conformation of **64** is the same as that of taxol.

The FAB mass spectrum (methanol, thioglycerol matrix) of **64** obtained from the Midwest Center for Mass Spectrometry, contained unique data which strongly supported formation of an enol acetate of an acetate at C-10 and opening of the oxetane and formation of a chloroketal. Major peaks were seen in the mass spectrum at m/z 653, 593, 429, 411, 369, 351, 328, and 268. The peaks at m/z 328 and 268 are due to the side chain ions **69** and **70**.



69



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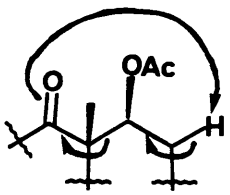
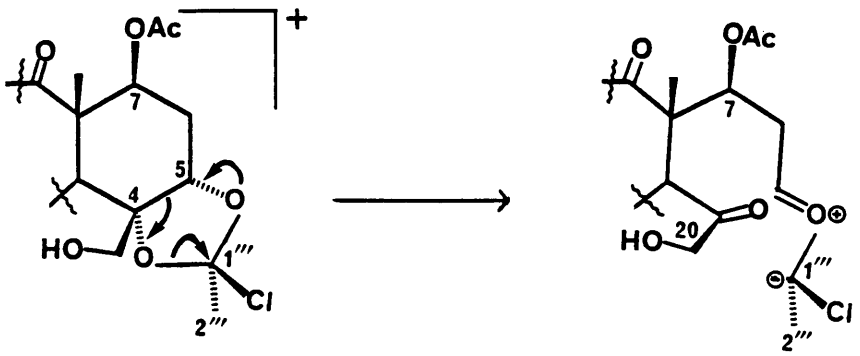


Scheme 32. Cleavage of the C-6, C-7 Bond of **64**

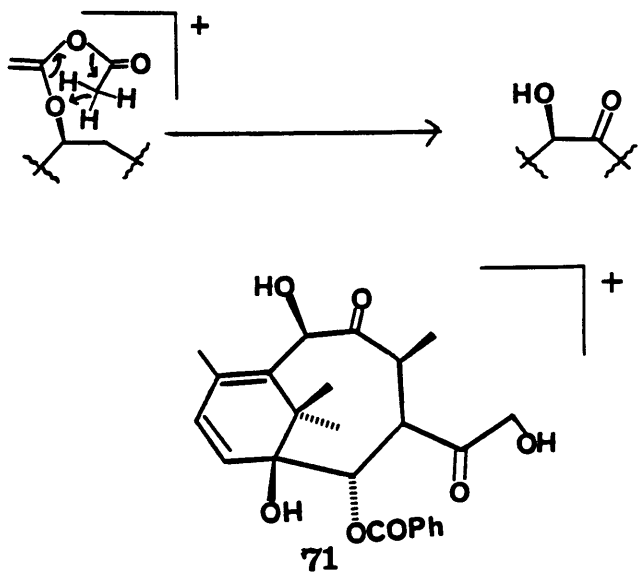
The unusual peaks were the ones at m/z 429, 411, 369, and 351. The lower mass peaks of this set are due to loss of water and/or an acetic acid equivalent. The key peak which had to be explained was at m/z 429. This peak is unique among the FAB spectra of taxol derivatives because it must involve loss of the side chain (this has always been seen) and loss of some portion of the taxane skeleton. Loss of portions of the taxane skeleton has never been identified in the mass spectra because relatively high (such as 429) m/z peaks in the mass spectra of taxol derivatives in this research have always involved the loss of neutral components. To lose a portion of the skeleton, such as the C ring, cleavage of two bonds was necessary. In the C ring it has always been possible to have cleavage of the C-7, C-6 bond as shown in Scheme 31 but no reasonable mechanism has existed for cleavage of another bond in the ring.

In **64** however the chloroketal functionality allowed for a reasonable pathway for cleavage of the C-4, C-5 bond (Scheme

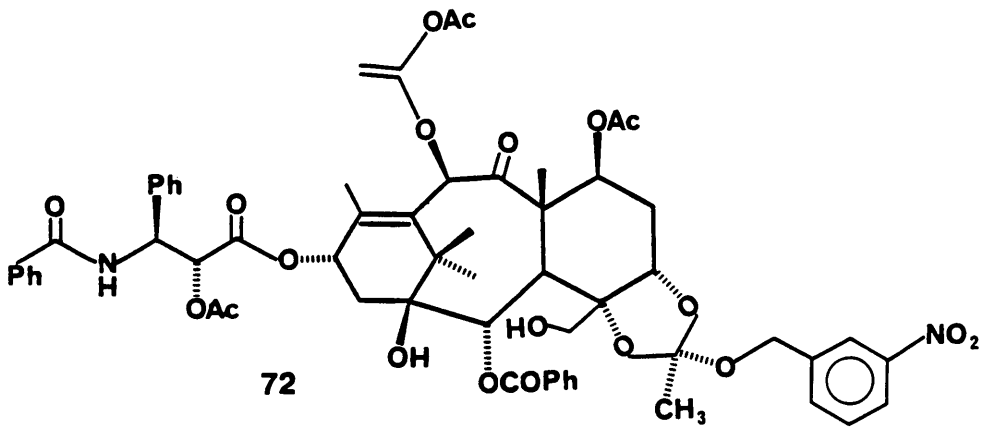
Cleavage of the C-4, C-5 Bond



Loss of Ketenes from C-10



Scheme 33. Mass Spectral Losses Seen for **64**



72

32). The loss of the C ring coupled with coss of the side chain and loss of two ketenes from C-10 (Scheme 33) gives rise to the ion **71** with m/z 429 for MH^+.

A second mass spectrum of **64** was obtained from Johns Hopkins University employing fast atom bombardment spectrometry with 2-nitro-benzylalconol as the matrix. Only peaks above m/z 910 were recorded. Peaks are present in this mass spectrum at m/z 1133, 1113, 1091, 1071, 1049, 1029, 980, and 918. None of these correspond to $(MH)^+$ or $(MNa)^+$ for **64**. The mass spectral data can be explained by the peaks in the spectrum originating from condensation of **64** with the matrix. The compound resulting from this condensation is **72**. The peak at m/z 1133 is **72**. The other peaks in the spectrum resulted from loss of acetate at C-2' by nucleophilic attack of the matrix before analysis and/or loss of 2 ketenes from C-10 and/or loss of nitrobenzylalcohol from the ketal and addition of H^+ or Na^+.

Compound **64** possesses such an unusual structure that ^{13}C nmr spectral data was necessary for proof of the structure. Obtaining the ^{13}C spectra necessary for peak assignment of a taxol derivative required a relatively large amount of sample (about 100 mg) and approximately 50 hours for data acquisition. For these reasons, and because ^{13}C spectra have not been required for structure assignments, ^{13}C spectra of other taxol derivatives in this project have not been obtained.

Accurate interpretation of the ^{13}C data for **64** required an understanding of the ^{13}C data for other taxanes. The only published ^{13}C spectra of taxanes are those of baccatin III, 19-hydroxy baccatin III and 13-oxobaccatin III.[16] The spectral data for baccatin III is shown in Table 37. Interpretation of the spectrum of **64** could not be achieved only by comparison with baccatin III's spectrum as baccatin III does not possess the side chain of taxol and of **64**; ^{13}C spectral data for taxol was obtained and peak assignments made.

The ^{13}C spectral assignments for taxol are shown in Table 38. Assignment of the spectrum of taxol involved acquisition of 18 separate spectra: a broad band decoupled spectrum, a coupled spectrum, an INEPT spectrum and 15 selective proton decoupled spectra. The peaks which could not be assigned by INEPT and/or selective proton decoupling were assigned by comparison with the spectrum of baccatin III. It was impossible to unambiguously assign the ester carbonyl carbon peaks as the six signals are grouped closely and are not coupled

to any protons. Many of the aromatic carbons of taxol appear as overlapping signals between 128 ppm and 130 ppm and could not clearly be differentiated.

Carbon spectra of **64** obtained included broad band decoupled, coupled, INEPT and selective proton decoupled. The broad band decoupled spectrum of **64** is shown in Figure 24., the INEPT spectrum is shown in Figure 25. The peak assignments for **64** are listed in Table 39.

The complex structure differences between taxol and **64** make many of the peak assignments in Table 39 tentative. The purpose of obtaining ^{13}C spectra of **64** was not to make unambiguous peak assignments for all carbons but to show that the functional groups added to taxol in the synthesis of **64** could be accounted for in the ^{13}C spectrum. This goal was accomplished by selective proton decoupling for C-2", C-10, C-20 and C-5. The INEPT spectrum showed the presence of five quarternary peaks immediately downfield of the overlapping aromatic peaks between 128 ppm and 130 ppm. Also shown by the INEPT spectrum was the presence of six ester (or amide) carbonyls, which agreed with the assigned structure of **64**. Tentative peak assignments for the other peaks based on comparison with taxol's spectrum gave no data which contradicted the structure assignment of **64**.

In the ^{13}C spectrum of baccatin III there are two quaternary peaks between 132 and 150 ppm corresponding to C-11 at 146.36, and C-12 at 132.06 ppm. In the spectrum of taxol

there are three quaternary peaks in this area: C-11 at 133.97, C-12 at 141.89 and C-1 of the C-3' phenyl at 138.35. In **64** the broad band decoupled and INEPT spectra show that five quarternary peaks are present between 132 and 150 ppm at 133.85, 136.92, 137.45, 144.27, and 145.29. Assignments for these peaks can not involve the C-1 carbons of the C-2 benzoate and C-1 of the C-3' phenyl amide. In baccatin III, C-1 of the benzoate is seen at 128.68 and in taxol it occurs between 128 and 130 ppm as does C-1 of the phenyl amide. No transformations in taxol had taken place in the reaction with acetyl chloride in which the shift of the benzoate or phenyl amide would be affected significantly. Tentative peaks assignments were made for these quaternary peaks but the presence of these five quaternary peaks alone is strong supportive evidence for the presence of the enol acetate and the chloro ketal in **64**.

The C-1" carbon of the enol acetate, being substituted with two electron drawing substituents, would be expected to occur relatively far downfield and is seen as the peak at either 144.27 ppm or 145.29 ppm, with the other peak corresponding to C-11. Peaks due to ketals occur from 90 ppm to 112 ppm and the shift caused by the presence of the chlorine would approximate the +23.5 ppm change seen when the shift of methylene chloride at 54.0 ppm is contrasted with the shift of chloroform at 77.5 ppm.

The upper limit for the position of C-2''' therefore would be approximately 135.5 ppm. The peak due to C-2''' would be expected to be the most upfield of the quaternary signals and was assigned to the peak at 133.85 ppm. The C-1 carbon of the C-3' phenyl group had been subjected to little or no change in chemical environment during the transformation of taxol and the peak at 137.45 ppm which is closest to the 138.5 ppm signal seen in taxol was assigned to this carbon. The remaining signal at 136.92 ppm was assigned to C-12.

The INEPT spectrum of **64** indicated that the C-20 carbon signal is at 63.79 ppm contrasted with 72.33 ppm for baccatin III and 72.03 ppm for taxol. This change in peak position shows that C-20 in **64** is hydroxylated; substitution by an ether or an acetate would cause the signal to be downfield of 63.79 ppm and substitution by a chlorine would cause it to be upfield.[48]

Selective proton decoupling allowed for assignment of the C-5 carbon signal of **64** to be 71.57 ppm and the analagous signal for taxol is seen at 73.43 ppm. The similarity of the chemical shifts for C-5 in **64** and in taxol agreed with substitution of C-5 by an ether or an acetate but not a hydroxyl or a chlorine.

The signal for C-4 of **64** is probably present between 72 ppm and 78 ppm but since it is a quarternary peak overlapping a methine peak it can not be seen by INEPT or selective proton decoupling. The change in substitution that C-4 is undergo-

ing is that of being substituted by an external acetate to being substituted by an internal ether; little change in chemical shift would be expected by this transformation.

Based on mechanistic and stability considerations there were several structures other than the chloro ketal that were hypothesized. The characterization data for **64** showed that any alternative structure could not agree with more than one or two of the data points presented, and it is thus concluded that structure **64** is the correct one for this compound.

Table 37 ^{13}C NMR Chemical Shifts of Baccatin III[16]

Position	Shift (ppm from TMS) Multiplicity	Position	Shift (ppm from TMS) Multiplicity
1	79.16 (s)	15	42.83 (s)
2	76.30 (d)	16	15.47 (q?(a))
3	46.27 (d)	17	22.55 (q?(a))
4	80.98 (s)	18	27.04 (q)
5	68.04 (d)	19	9.49 (q)
6	35.74 (t)	20	72.33 (t)
7	76.49 (d)	CO on Ac	171.25, 170.66 (bs)
8	58.88 (s)	CH on Ac	20.93 (q)
9	204.13 (s)	CO on Bz	167.15 (s)
10	84.55 (d)	p-Benzoyl	133.68 (d)
11	146.36 (s)	o-Benzoyl	130.18 (d)
12	132.06 (s)	1-Benzoyl	129.59 (s)
13	75.13 (d)	m-Benzoyl	128.68
14	38.80 (t)		(a)-Interchangeable assignments

Table 38 (Part 1 of 2). ^{13}C Assignment for Taxol

Position	Shift (ppm)	Carbon type by Inept	Assignment method
1	78.79	q	B
2	75.47	t	A
3	45.73	t	A
4	81.04	q	B
5	73.20	t	A
6	35.64	s	B
7	76.33	t	A
8	58.39	q	B
9	203.39	q	B
10	75.15	t	A
11	133.74	q	B
12	141.66	q	B
13	75.47	t	A
14	35.64	s	B
15	43.05	q	B

Table 38 (Part 2 of 2). ^{13}C Assignment for Taxol

Position	Shift (ppm)	Carbon type by Inept	Assignment method
16	26.55	p	A
17	21.33	p	A
18	14.6	p	A
19	9.2	p	A
20	71.8	s	A
1'	172.48*	q	B
2'	84.25	t	A
3'	54.81	t	A
C-4 OAc	21.92	p	A
C-10 OAc	20.54	p	A
C-3' N Bz CO	166.68*	q	B
C-2 OBz CO	167.22*	q	B
Acetates CO	170.19* 170.79*	q q	B B
C-1 of C-3' Ph	138.12	q	B
p-N Bz	133.13	t	B
p-OBz	133.39	t	B
aromatics	128-130	--	--

A - Selective proton decoupling

B - Chemical Shift Arguments,

INEPT and comparison with Baccatin III

* - May be interchangeable

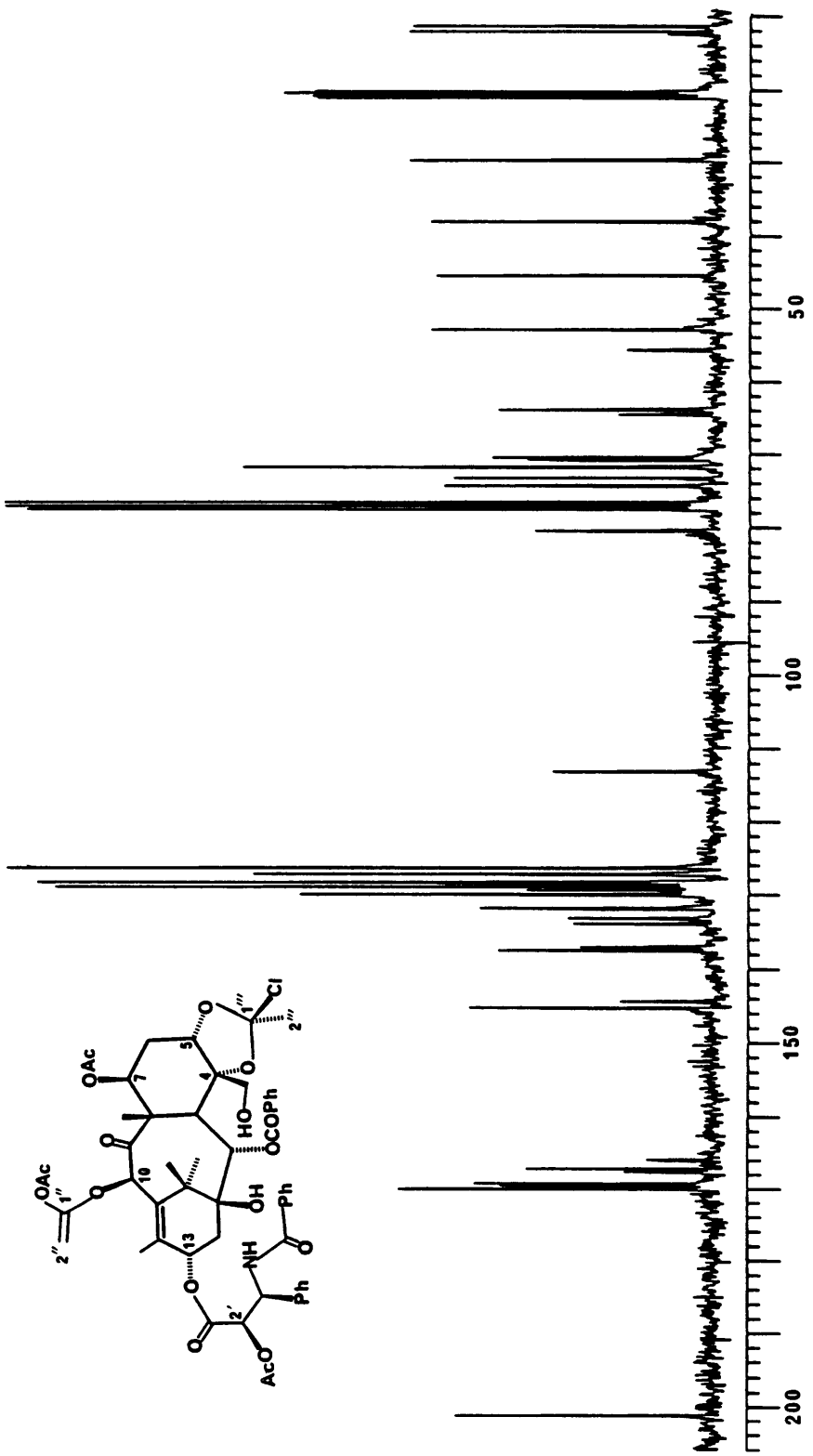


Figure 24. ^{13}C Broad Band Decoupled Spectrum of **64**

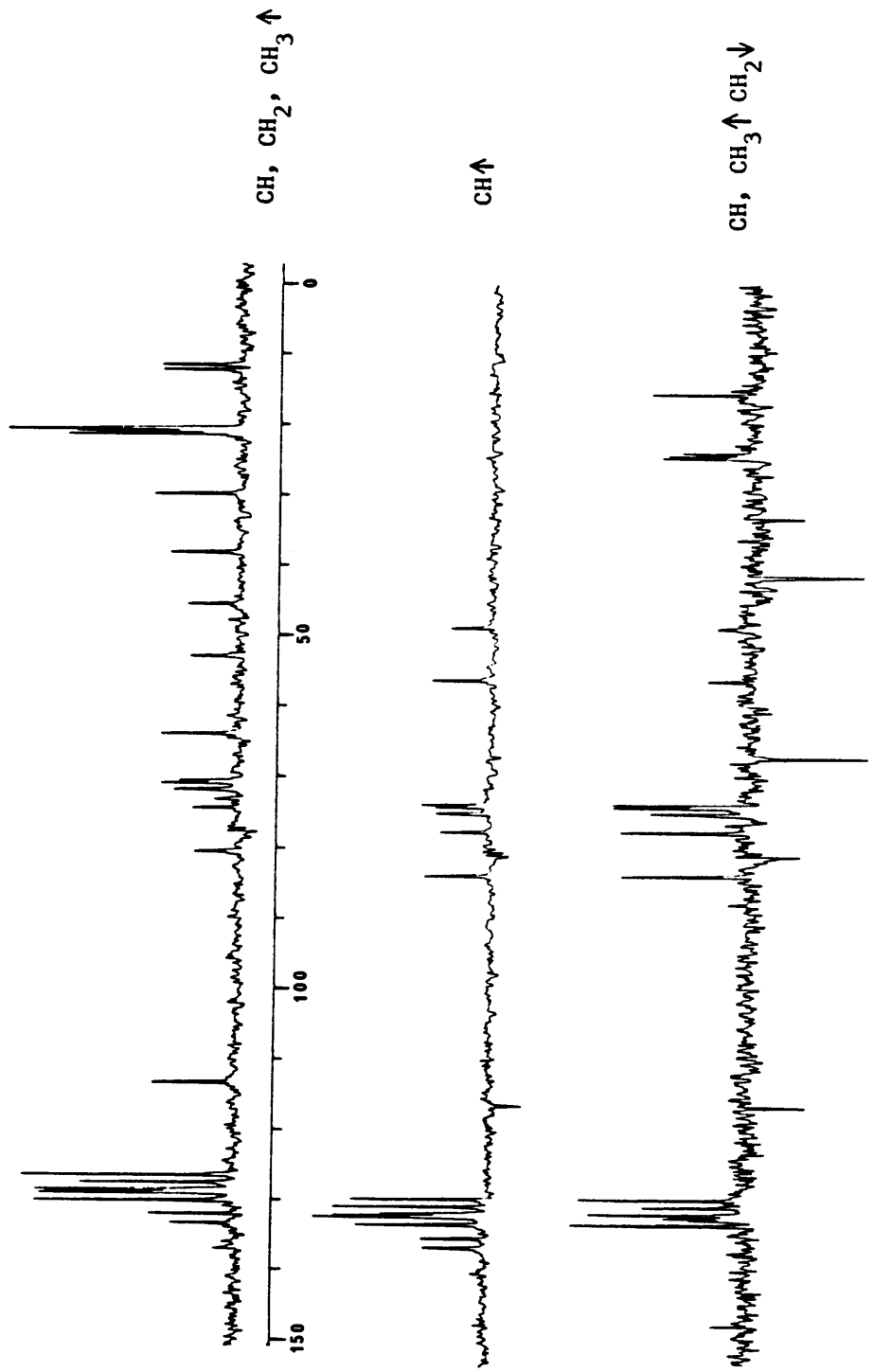
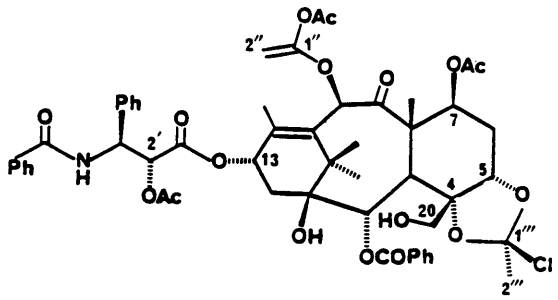


Figure 25. INEPT Spectrum for 64

Table 39 (Part 1 of 3). ^{13}C Assignment for **64**



Position	Shift (ppm)	Carbon type by INEPT	Assignment method
1	*	--	--
2	74.26	t	C
3	45.37	t	D
4	*	--	--
5	71.69	t	A
6	29.65	s	C
7	73.16	t	C
8	55.59	q	C
9	201.16	q	C
10	70.37	t	A
11	145.29	q	B
12	136.92	q	B
13	80.36	t	A
14	38.01	s	C
15	*	--	--

Table 39 (Part 2 of 3). ^{13}C Assignment for **64**

Position	Shift (ppm)	Carbon type by INEPT	Assignment method
16	11.99	p	A
17	11.28	p	A
18	20.19-21.05	p	C
19	20.19-21.05	p	C
20	63.79	s	D
2'	70.68	t	C
3'	52.85	t	D
1"	20.19-21.05	q	C
2"	144.27	s	A
1'''	133.95	q	C
2'''	20.19-21.05	p	B
CO_2R CO	165.99, 167.16, 167.63, 169.29, 169.60, 170.07	q	D
CH of Acetates	20.19-21.05	p	C
Aromatic C-1 of C-3' Ph	137.45	q	B
p-Benzoate	133.85	t	C
p-Amide	131.93	t	C
aromatics	126-130	--	--

Table 39 (Part 3 of 3). ^{13}C Assignment for 64

p - primary, s - secondary,

t - tertiary, q - quarternary

* - quarternary overlapping peaks and an

unassigned quarternary peak at 64.42 ppm.

Assignment Methods

A - Selective Proton Decoupling

B - Chemical Shift Arguments (see text)

C - Chemical Shift Arguments (see reference 16) and comparison to the spectra of taxol and baccatin III

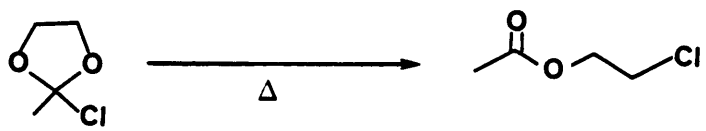
D - INEPT showed no other carbons of this type having similar shifts

5.1.3 STABILITY OF THE CHLORO KETAL OF 64

Proof of the structural assignment for 64 must not only include an explanation for all the characterization data but also for the stability of **64**. Compound **64** was formed in refluxing acetyl chloride and so is stable at 80°. Preparation of **64**, where acetic anhydride in addition to acetyl chloride was used, included methanol in the work up; where acetyl chloride alone was used **64** was only exposed to acetyl chloride, hexane and ethyl acetate. In either case the product was the same. A $CDCl_3$ solution of **64** saturated with D_2O produced no decomposition after standing 4 days and a 1/1 $CDCl_3/CD_3OD$ solution of **64** produced no reaction after several hours at room temperature.

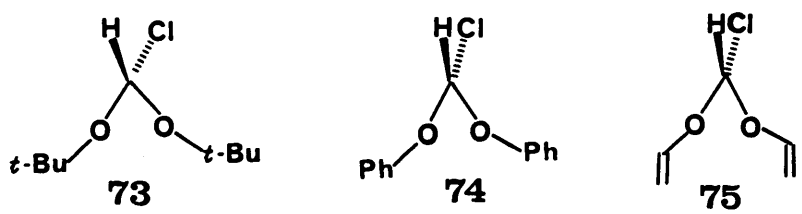
On first consideration this data showing the stability of **64** produced would preclude the presence in **64** of the chloro ketal group, since chloride is a good leaving group and the positive charge produced by chloride removal would be stabilized by two alpha oxygens. The basic principles of organic chemistry would suggest that the chloro ketal of 64 could not survive the conditions it had been exposed to. However, literature precedent for the stability of chloro ketals coupled with analysis of the three dimensional structure of the taxane ring structure show that the chloro ketal of **64** would be expected to be a stable structure.

Several researchers have attempted the preparation of chloro ketals but only obtained decomposition products. An example of a decomposition of an intermediate chloro ketal which occurred quantitatively on distillation is shown in Scheme 34.[53] Decomposition of the chloro ketal involved SN2. attack by Cl^- on a carbon beta to the chlorine substituted carbon. Decomposition of other halogenated ketals were all ascribed to nucleophiles atacking the beta carbon in an SN_2 manner.



Scheme 34. Thermal Rearrangement of a Chloroketal

Modifying the structure of the chloro ketal so that SN2 attack could not take place resulted in preparation of the stable chloro ketals, **73**[54] , **74**[55] , **75**[56] .



The decomposition of the halogenated ketals by an SN2 process is supported by the stability of **73**; if decomposition oc-

curred by an SN1 reaction **73** would be expected to be extremely unstable.

Analysis of the structure of the C ring and chloro ketal of **64** shown in Figure 26 shows that decomposition would not occur readily. Decomposition of **64** when heated would involve SN2 attack at either C-4 or C-5. Approach to C-5 is not just hindered but effectively blocked by the C ring, the C-18 methyl group and the acetate at C-7. Approach to C-4 is blocked by the same groups and C-4 is also a tertiary carbon. Even when **64** was heated in refluxing acetyl chloride no pathway existed for transformation of the chloro ketal.

The second question to be addressed is the stability of chloro ketals to water and methanol. Formation of the methoxy ortho ester shown in Scheme 35 required reaction with methanol and triethylamine in methylene chloride and petroleum ether at room temperature for 1 hour.[59] Transformation of the chloro neopentyl ketal **73** to an ortho ester required NaOMe in Et_2O as a suspension; decomposition of the ketal required NaOH as shown in Scheme 35.[54]

The reactivity data for chloro ketals indicates that a methoxide or hydroxide is necessary to displace chloride, so that the reactions possess enough SN2 character to take place via back side attack. The conditions **64** was stable in involved neutral water or methanol and so would not cause the chloro ketal to be changed. Even if neutral conditions had caused the chloro ketals in Scheme 35 to react the chloro

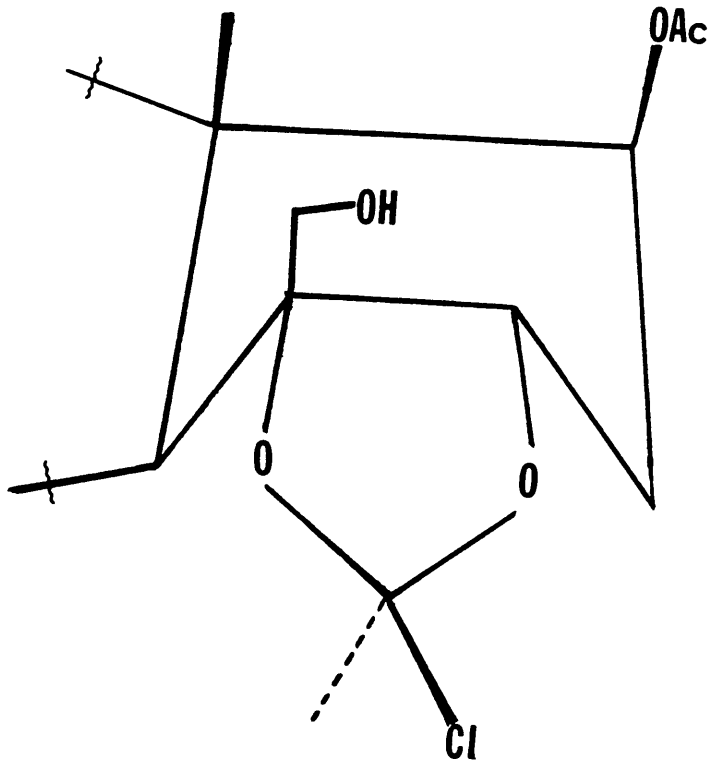
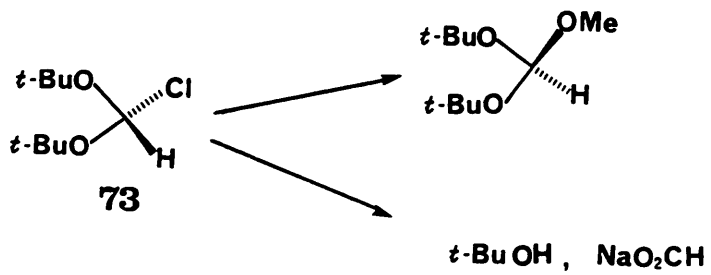


Figure 26. C and D Rings of **64**

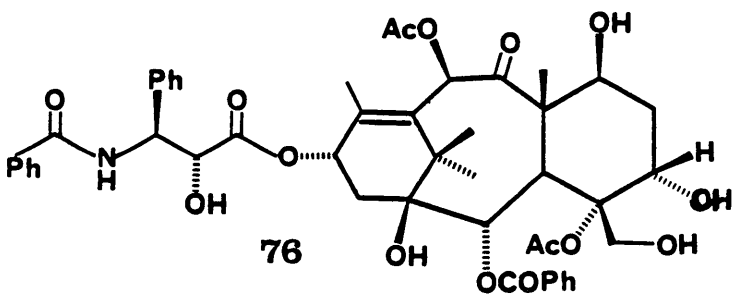


Scheme 35. Nucleophilic Substitution Reactions of Chloroketals

ketal of **64** would probably be stable to neutral water or methanol. Approach to the chloro ketal for an SN2 reaction is completely blocked by the concave shape formed by the A and B rings of the taxane skeleton.

5.1.4 REACTION OF TAXOL WITH TRIETHYLOXONIUM TETRAFLUOROBORATE

Reaction of taxol with excess (12 eq.) triethyloxonium tetrafluoroborate CH_2Cl_2 at 0° for 45 minutes yielded the D ring seco taxol analogue **76**. Work up consisted of adding 6/1 1.0 N HCl/THF to hydrolyze reactive intermediates and isolation of the product via preparative tlc (silica gel, 1/1 EtOAc/Hexane). The isolated yield was 35%; numerous other compounds were present but **76** was the only compound that could be isolated in a pure form. The compound was homogenous on tlc and ^{1}H-nmr showed the **76** to be a pure taxol-like compound.



76

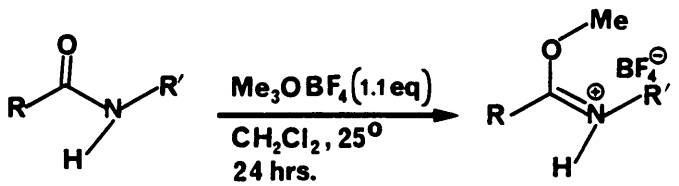
The FAB mass spectrum of **76** gave peaks at m/z 911 $(MK)^+$, 894 $(MNa)^+$, 872 $(MH)^+$, 854 $(MH-H_2O)^+$ indicating a molecular weight of 871 which corresponds to the addition of water to taxol. Peaks in the mass spectrum at m/z 609 $(MNa-RCOOH)^+$, 569 $(MH-RCOOH-H_2O)^+$ and 527 $(MH-RCOOH-HOAc)^+$ where RCOOH is the side chain as an acid, indicated that the addition of water occurred on the taxane skeleton.

The ^{1}H-nmr spectrum of **76** is shown in Table 40. Conspicuous by their absence from the ^{1}H-nmr spectrum, because Et_3OBF_4 is an ethylating agent, are any signals that could be interpreted as coming from an ethyl group. In addition to this the signals for the C-5 proton, 3.88 (br s), and the C-20 protons, 3.85 (d, J=11.5), 4.03 (d, J=11.5) were shifted upfield when contrasted with the analogous proton signals of taxol, C-5 at 4.92 ppm (dd, J=2,8), and C-20 at 4.17 ppm (d, J=8). The upfield change in chemical shift showed that C-20 and C-5 in **76** were substituted with hydroxyl rather than ether groups. The change in coupling for C-5 indicated a change of stereochemistry and the change in coupling constant for the C-20 protons from 8 to 12 Hz was indicative of the change in H-C-H bond angle associated with opening of the oxetane.

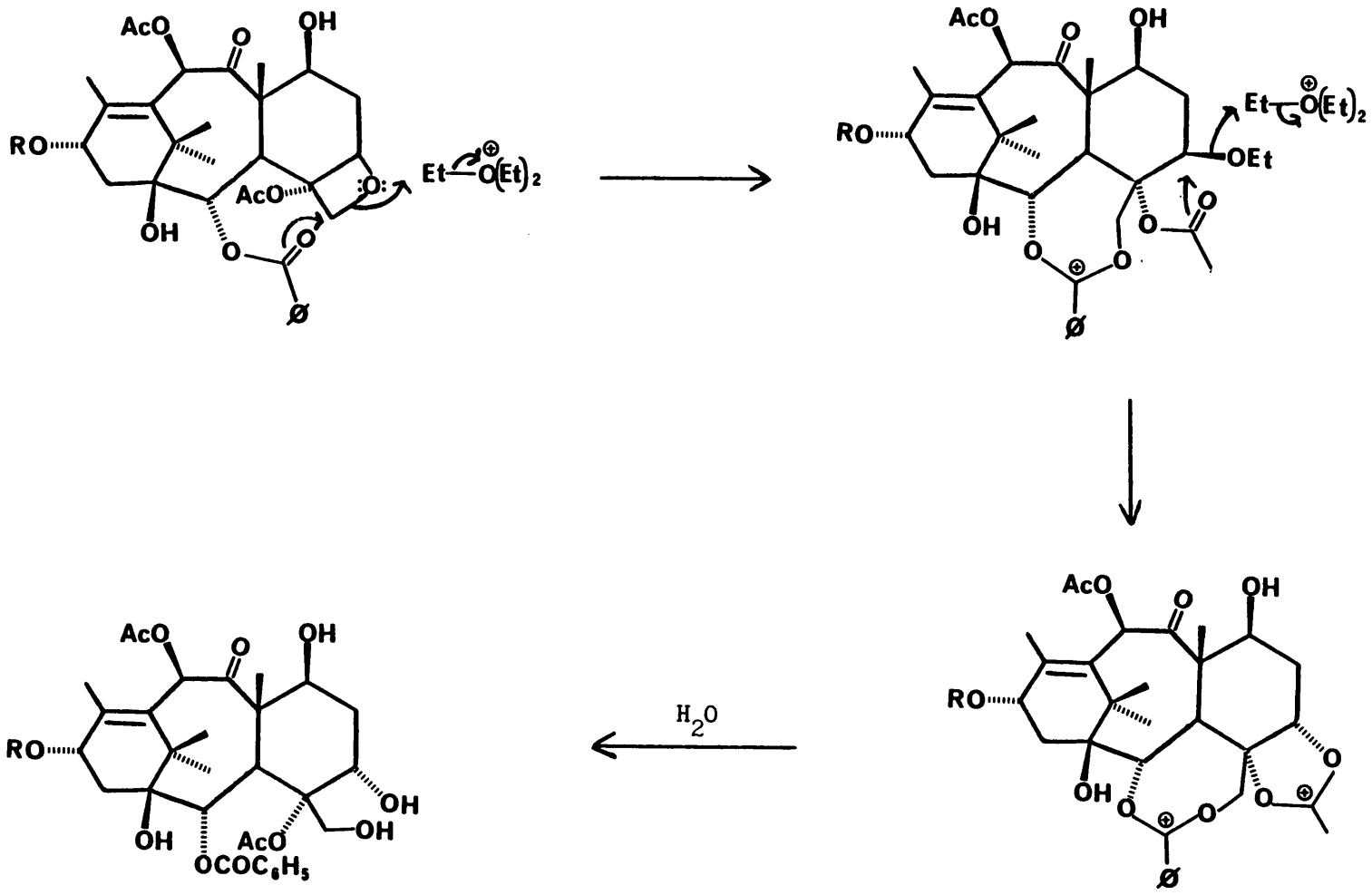
The splitting patterns for C-13, 6.05 (br dd, J=4,10), and C-14, 2.43 (dd, J=10,16) and 3.05 (dd, J=4,16) were caused by conformational changes associated with a taxane skeleton possessing only A, B, and C rings. The upfield shift of the

C-4 acetoxy group at 1.89 ppm (s), 2.38 ppm (s) in taxol, was caused by opening of the oxetane. The upfield shift of the benzoate meta protons, 7.17 (t, J=8), 7.4 (m) in taxol, is caused by the proximity of the C-20 hydroxyl group. Explanations for these changes in the nmr spectrum of **76** is based on comparison with the spectra of the D ring seco taxols described in Chapter 4.

The formation of **76** from taxol can be explained by a reaction mechanism in which ether linkages are cleaved with assistance from ester groups (Scheme 37). This type of reactivity had been seen by Raber and Guida[52] (Scheme 29), but a higher temperature and considerably longer reaction time was required for cleavage of their ether than for that of taxol. More vigorous reaction conditions would also have produced reaction at the C-3' N benzoyl group as it had been shown by Chen and Benoiton[86] that amides, especially phenyl amides, react readily with Meerwein's reagent to form imino ether fluoroborates (Scheme 36). The selective reaction of the oxetane with Meerwein's reagent was due to the ring strain of the oxetane and the reaction pathway.



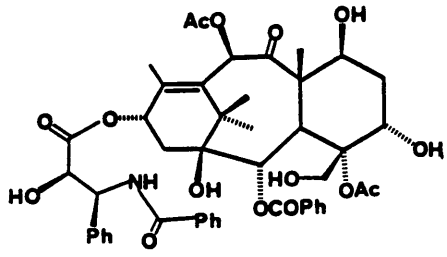
Scheme 36. Reaction of an Amide with Meerwein's Reagent



Scheme 37. Reaction of Taxol with Meerwein's Reagent

Table 40 Characterization Data for 5-Epihydroxy-5,0-secotaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.56 (d, 6)
3	4.03 (d, 6)
5	3.88 (br s)
6	2.2 (m)
7	4.49 (dd, 4,11) (dm without added D_2O)
10	6.57 (s)
13	6.01 (br dd, 4,11)
14	2.43 (dd, 10,16), 3.05 (dd, 4.5,16)
16	1.12 (s)
17	1.10 (s)
18	2.08 (br s)
19	1.22 (s)
20	3.85 (d, 11.5)
2'	4.70 (d, 2), (br s without added D O)
3'	5.92 (d, 2,9)
N-H	7.19 (d, 9)
OAcs	2.21 (s), 1.62 (s)
2 OBz	8.03 (d,m, 7.5), 7.4 (m), 7.17 (t, 8)
3' NBz	7.80 (d,m, 8), 7.4 (m)
3' Ph	7.4 (m)
OH	3.52 (br s), 3.71 (s), 4.14 (s)



Mass Specral Data

911 $(MK)^+$, 894 $(MNa)^+$,

872 $(MH)^+$, 854 $(MH-H_2O)^+$,

812 $(MH-HOAc)^+$,

609 $(MNa-RCOOH)^+$,

569 $(MH-RCOOH-H_2O)^+$,

527 $(MH-RCOOH-HOAc)^+$.

Infrared Spectral Data

1745 s, 1670 m, 1535 w,

1505 w, 1475 w, 1395 m,

1120 m, 1080 m, 1060 (m).

A full understanding of the reaction of taxol with Meerwein's reagent requires several aspects of the reaction to be addressed:

1. Spatial relationship of the C-2 benzoate to C-20 - Analysis of a model of taxol showed that the carbonyl of the ester can reach C-20 without any steric or bond angle strain being produced;
2. Order of steps - The initial step of the reaction may occur with assistance by either the C-2 benzoate or the C-4 acetate. When the reaction was stopped after 15 minutes, instead of 45, ^{1}H-nmr analysis showed that no one intermediate was quickly formed;
3. Transesterification - Decomposition of the dication product in Scheme 37 can lead to four products via transesterification. The three products, other than 76, may have been present in the crude reaction mixture but could not be isolated in pure form.

5.1.5 SUMMARY AND PROPOSED WORK

Reaction of taxol with acetyl chloride/HCl or triethyloxonium tetrafluoroborate achieved the objective of producing D seco taxols. The biological activity results from these taxol analogues together with activity results from the C ring seco taxols discussed in Chapter 4 will give accurate

information concerning the importance of the oxetane to the biological activity of taxol.

The ring opening reactions showed that taxol's oxetane is unusually susceptible to electrophilic attack. The keys to this sensitivity to nucleophiles are the close ester groups - the C-4 acetate and the C-20 benzoate. Openings of the oxetane with assistance by the C-4 acetate and/or C-20 benzoate produced resonance stabilized carbonium ions in addition to relieving the strain inherent in a four membered ring.

Reaction of taxol with acetyl chloride/HCl yielded the taxol analogue **64** and also suggested other taxol derivatives that should be synthesized - a taxol analogue modified only by formation of the enol acetate at C-10 and a taxol derivative modified only by opening of the D ring with formation of the chloro ketal. A taxol derivative modified only by formation of the enol acetate should be able to bind to taxol's receptor site because C-10 modified taxols have been shown to be biologically active. The enol acetate at C-10 is a potential acetylating agent. A C-10 taxol enol acetate might be able to bind to taxol's receptor site and acetylate a free hydroxyl or amino group which could produce a cytotoxic effect.

Synthesis of a taxol modified only by formation of the chloro ketal will allow for differentiation of the importance of the oxetane and the conformation of the taxane system for

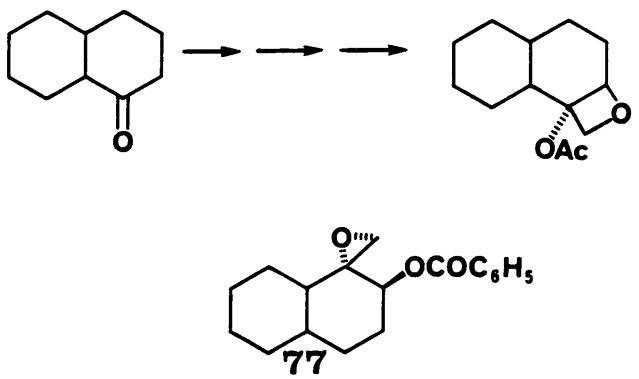
binding of a drug to taxol's receptor site. It has been shown that the chloro ketal of **64** causes the taxane skeleton to have the same conformation as that of taxol but not possess an oxetane.

Preparation of these taxol analogues should be straight forward. Synthesis of a taxol modified only by an enol acetate would involve protection of the 2' and 7 hydroxyl groups, reaction of the protected taxol with acetyl chloride and pyridine followed by deprotection of the hydroxyl groups. Synthesis of the chloro ketal of taxol would involve reaction of taxol with HCl in an inert nonnucleophilic solvent.

One of the initial objectives of this research project was a model study designed to transform an allylic alcohol to an acetoxy oxetane (Scheme 38). This study, which was partially completed with the preparation of the epoxy benzoate **77** before the structure assignment for the chloroketal was made, should not be continued because: 1.) the rearrangement proposed for the allylic alcohol is not likely to occur on a taxane and 2.) the chloro ketal of **77** was envisioned as an intermediate in the allylic alcohol rearrangement and so is a superior "model" than a nontaxane compound.

Conversion of **64** back to an oxetane containing compound may be possible by isolation of the chloroketal followed by reaction with a electrophile other than H^+. One possibility for this would be reaction of the chloroketal with a lithium salt in a polar nonprotic solvent at high temperatures. As

will be discussed in Chapter 6 a 2',7-disubstituted taxol was stable indefinitely in the presence of $LiSO_4$ or LiCl in dimethylformamide at temperatures of 90° to 130°. Therefore if conditions can cause the chloroketal to reform the oxetane then the reaction will not be an equilibrium process.



Scheme 38. Oxetane Model Study

5.2 EXPERIMENTAL PROCEDURE

Reaction of Taxol with Acetyl Chloride/Acetic Anhydride - Taxol (100 mg) was dissolved in 8.0 mL of 1/1 acetyl chloride/acetic anhydride.* The reaction was stirred and refluxed for 3 hours with a 70° water bath as the heat source. At the end of the reaction period the acetyl chloride was removed on a rotary evaporator, the residue was dissolved in 50 mL of CH_2Cl_2 and washed with 40 mL of 10% $NaHCO_3$, additional $NaHCO_3$ was added until evolution of CO_2 had stopped. The organic solution was then dried with $MgSO_4$, several mL of CCl_4 were added and all the solvents removed on a rotary evaporator. The product was isolated by preparative tlc (silica gel, 1000 µm, 1/1, hexane/EtOAC, Rf 0.34), yield 95 mg, 80%. The reaction was repeated changing the work up procedure to evaporation of the acetyl chloride, addition of several mL of methanol and evaporation of all solvents followed by preparative tlc. This work up procedure did not change the yield or purity of the product and was much more convenient to follow. Characterization data other than the infrared spectrum for **64** are included in the Results and Discussion section. Ir - 1765 s, 1670 m, 1535 w, 1510 w, 1470 w, 1385 m, 1290 m-sh, 1240 s, 1100 m, 1055 m.

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* All acetyl chloride used in these reactions was obtained from a nearly empty 500 mL bottle.

Reaction of Taxol with Acetyl Chloride - Taxol (23 mg) was dissolved in 2.0 mL of acetyl chloride. The reaction flask was equipped with a stirring bar and reflux condenser and the reaction was heated to reflux on a water bath for 3 hours. At the end of the reaction the acetyl chloride and HCl were removed on a rotary evaporator. The residue was dissolved in $CDCl_3$ and analysed by ^{1}H-nmr which showed the mixture to be **64** with numerous minor impurity peaks present.

Reaction of Taxol with d_3-Acetyl Chloride - A sample of taxol (50 mg) was refluxed with 3 mL of deuterated acetyl chloride, from a newly opened vial, as previously described. After work up preparative tlc showed two major bands present, one of which corresponded to **64**. After isolation the **64** band was shown by proton nmr to consist of a 6/4 mixture of 2',7-diacetyltaxol and **64**. All of the peaks present in the nondeuterated spectrum of **64** could be seen by nmr with the exception of the singlets 1.82 ppm, 1.92 ppm, and 2.18 ppm.

Basic Solvolysis of **64** - A sample of **64** (90 mg) was stirred with 30 mL of a 0.25% $NaHCO_3$, 3/1, $MeOH/H_2O$ solution. The reaction was monitored by hplc (analytical column, RP-8, 3/1, $MeOH/H_2O$) which showed a complex mixture of more polar products developing from the start of the reaction. After 2.5

hours the reaction was stopped by the addition of 2 mL of HOAc. The methanol was then evaporated, the residue redissolved in CH_2Cl_2 and worked up. Preparative tlc (silica gel, 1000 μm, 1/1, EtOAc/Hexane, 2 developments) was carried out but ^{1}H-nmr showed that none of the bands isolated was a pure product. The least polar band isolated (Rf 0.60) was shown by proton nmr to consist mostly of the methyl ester side chain of taxol.[33]

Partial Reaction of Taxol with Triethyloxonium Tetrafluoroborate - Taxol (103 mg) was dissolved in 2.8 mL of dry methylene chloride and coooled to 0° in an ice bath. To the taxol solution Et_3OBF_4 was added (140 μL of 1.0 M in CH_2Cl_2, 12 eq.). The reaction was allowed to proceed for 20 minutes at 0° after which time 5 mL of 6/1 1,4-dioxane/1.0 N HCl was added. Most of the solvents were evaporated *in vacuo* and then the products extracted with ethyl acetate which was washed and dried. A ^{1}H-nmr spectrum of the crude reaction was obtained and showed that the major compound present was taxol.

Reaction of Taxol with Triethyloxonium Tetrafluoroborate - Taxol (100 mg) was dissolved in 3.0 mL of dry CH_2Cl_2, cooled to 0° in an ice bath and stirred. To this stirred solution was added 140 μL of a 1.0 M solution of triethyloxonium tetrafluoroborate in CH_2Cl_2 (12 eq). After 65 minutes at 0° tlc

analysis (silica gel, 4/6, EtOAc/CH_2Cl_2) showed that most of the taxol had reacted. At that point 5 mL of 6/1 1,4-dioxane/1.0 N HCl was added and the mixture stirred for several minutes at room temperature. Most of the solvents were removed via rotary evaporation. The residue was dissolved in EtOAc, washed with water, dried with $MgSO_4$, dried, filtered and evaporated. The product 5-epihydroxy-5,O-seco-taxol **76** was isolated via preparative tlc (silica gel, 1000 μm, 8/2, EtOAc/Hexane) Rf of **76** - 0.68. Yield - 40 mg (38%). Analysis of the sample by proton nmr showed one product present with only a few very small impurity peaks so that was greater than 95% pure.

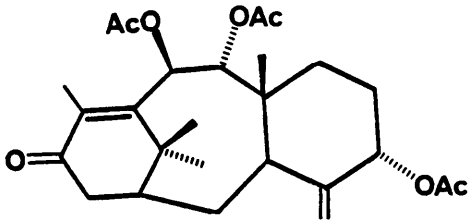
Any researchers wishing to repeat this reaction should keep in mind that the key for successful synthesis of **76** is to follow the reaction by frequently checking the progress of the reaction by tlc. On tlc the products will be seen as a streak and no one product easily identified. It is not important however to watch the development of products but to watch the depletion of taxol; stop the reaction when approximately 10% to 20% of the starting material remains. If the reaction is allowed to continue further the product will decompose.

6.0 ATTEMPTED C-7 DEOXYGENATION OF TAXOL

6.1 INTRODUCTION

The goal of reducing the C-7 hydroxyl group of taxol to a saturated hydrocarbon is the determination of the need for oxygen functionality at C-7 for *in vitro* and/or *in vivo* activity. Previously in this project taxol had been modified at C-7 via oxidation, subsitution of an acetate and addition of a sugar via a carbonate linkage. All of these modifications left the C-7 oxygen of taxol intact. The biological activities of these taxol derivatives therefore will not give a definitive answer concerning the necessity of there being an oxygen-containing functionality of some sort at C-7 for biological activity.

Determination of the necessity of oxygen functionality at C-7 will further the goal of obtaining a more abundant supply of a semisynthetic active taxol-like compound. There are several compounds containing the taxane skeleton containing compounds available from the yew plant which do not possess oxygen functionality at C-7. An example of one of these compounds is taxusin **78** which is available from *Taxus cuspidata* in 0.4% yield.[58] Oxygenation of taxusin at C-7 would be extremely difficult if not impossible.



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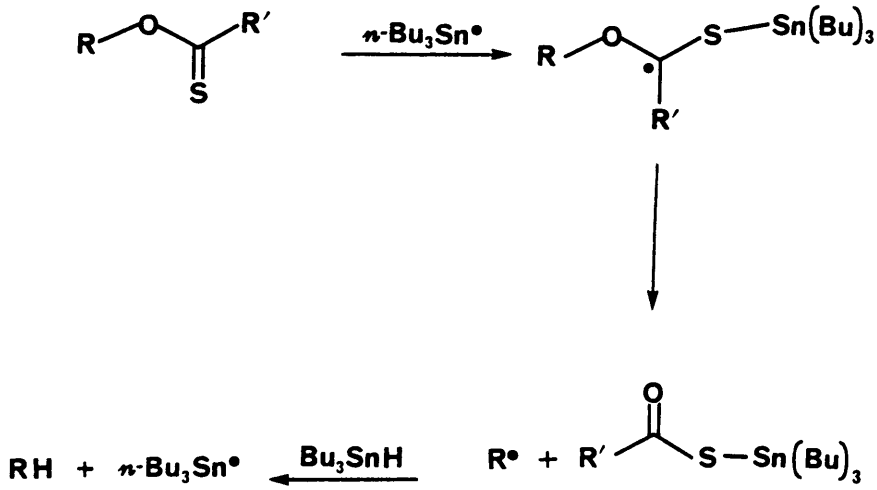
Two strategies directed at deoxygenating C-7 of taxol were chosen. The first of these involved addition of a thionyl containing group to the C-7 hydroxyl group and deoxygenation with tributyltin hydride. The second strategy consisted of transformation of the C-7 hydroxyl group to a methanesulfonate followed by elimination and hydrogenation of the double bond.

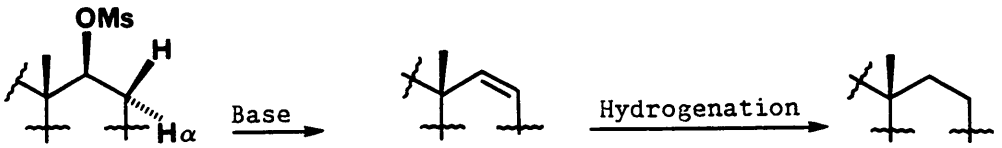
The radical deoxygenation strategy employed a reaction developed by Barton and McCombie.[59,60] The reaction mechanism is shown in Scheme 40. The first step of the reaction is attack of a tin radical at the sulfur of the thionyl. The intermediate carbon radical resulting from this attack decomposes to deoxygenate the position originally hydroxylated. A hydrocarbon is produced when the carbon radical abstracts a hydrogen radical from tributytin hydride. The strategy offered the advantage that only two reactions are necessary for the entire deoxygenation process.

The second strategy chosen for the deoxygenation of taxol was the elimination reaction shown in Scheme 39. The elimination reaction would involve abstraction of the trans co-



Scheme 39. Barton Deoxygenation Reaction

planar hydrogen Hα. Hydrogenation of the double bond could be achieved without competitive reduction of the hindered C-11, C-12 double bond.[61]



Scheme 40. Proposed C-7 Deoxygenation of Taxol

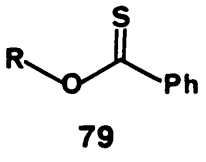
Experimental results from radical reactions designed to deoxygenate C-7 will be presented first, followed by elimination reaction results and finally proposals for future research will be given.

6.2 RESULTS AND DISCUSSION

6.2.1 ATTEMPTED RADICAL DEOXYGENATION

Deoxygenation of C-7 of taxol by tributyltin hydride required that the hydroxyl be converted to a thionyl containing group. The first thionyl containing group chosen to substitute the C-7 position of taxol for deoxygenation was the thionobenzoate **79**. The thionobenzoate group is among the most reactive of the thionyl containing functional groups as the intermediate **80** produced from the initial attack of tri-

butyl tin hydride is resonance stabilized by the adjacent phenyl group.



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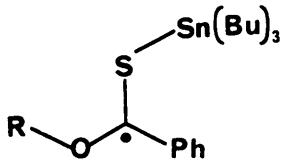

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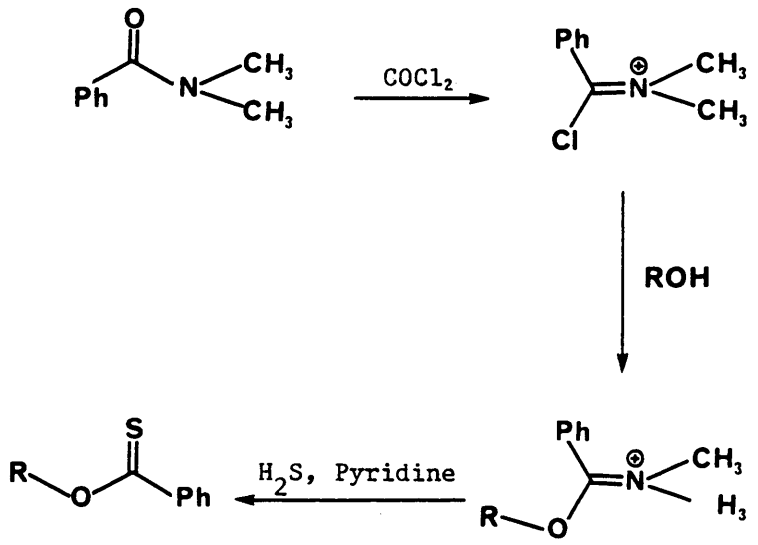
A relatively mild method for the conversion of an alcohol to a thionobenzoate was developed by Barton[49] and is shown in Scheme 41. Barton indicated that the reactions proceeded at room temperature even with sterically hindered alcohols. Previously, preparation of thionobenzoates required NaOH, and this is one step taxol could not survive. Reaction of the C-2' protected taxol 2'-N-CBZ-β-alanyltaxol with two equivalents of the colorless imidoyl chloride in CH_2Cl_2 at room temperature produced a yellow solution after several minutes. The reaction was allowed to proceed for 2.5 hours at which time tlc analysis showed no starting material remaining and one product spot present. Pyridine was was syringed in and H_2S was then bubbled through the solution for ten minutes. After addition of the H_2S the light yellow color of the solution deepened and then the solution started to become cloudy. After one hour the reaction mixture had become dark and cloudy and a large amount of black precipitate was present. After work up and preparative tlc no pure

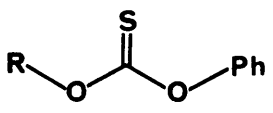


Scheme 41. Formation of a Thionocarbonyl Compound

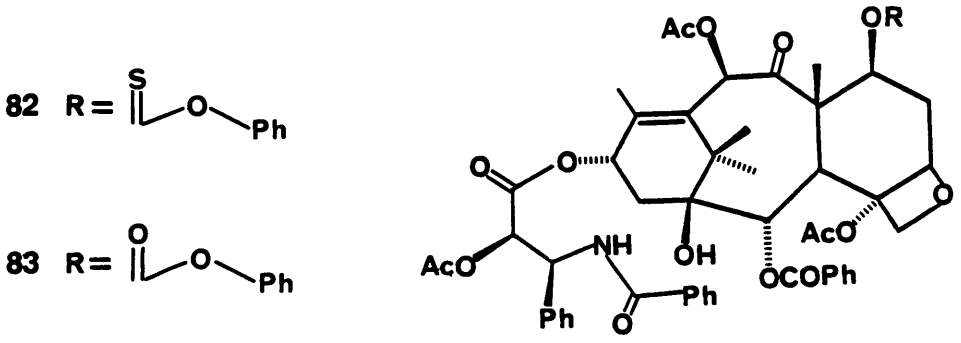
product could be isolated as a complex mixture of products was present.

The failure of 2'-N-CBZ-β-alanyltaxol to be converted into its 7-phenylthionylbenzoate derivative can be explained in light of the susceptibility of taxol to nucleophilic attack. In this project and other work[18,33] taxol was found to decompose quickly in the presence of basic methanol or isopropanol. Taxol has been exposed to pyridine and methanol and/or water during work up in this research project and no decomposition had been seen. Hydrogen sulfide however is more acidic than water and more nucleophilic. Therefore it is thought that the synthesis of the C-7 thionobenzoate was not successful as the hydrogen sulfide caused the taxol derivative to decompose.

The result of the attempted preparation of the C-7 thionobenzoate derivative of taxol emphasized the need to keep taxol away from nucleophiles. A thionyl functional group which can be added to a hydroxyl under non-nucleophilic conditions is the phenyloxythionyl group[62] **81**. This derivative can be synthesized via reaction of the alcohol with phenylchlorothionocarbonate using pyridine and 4-dimethylaminopyridine as catalysts.



81



The preparation of a C-7 phenyloxythionylcarbonyltaxol was achieved by the reaction of 2'-acetyltaxol with phenylchlorothionocarbonate. The phenylchlorothionocarbonate was obtained from Aldrich Chemical Company and was labelled as 99% pure. The reaction of 2'-acetyltaxol with the phenylchlorothionocarbonate was carried out at room temperature in CH_2Cl_2 with pyridine and 4-dimethylaminopyridine[63] as catalysts. The reaction required 96 hours to consume all of the starting material. At the end of the reaction the solution was worked up as usual and isolation of the products was achieved via preparative hplc (RP-8, 7/3 MeOH:H_2O). Two products were obtained via preparative hplc: 2'-acetyl-7-phenyloxythiocarbonyltaxol **82** (49% isolated yield) and 2'-acetyl-7-phenyloxycarbonyltaxol **83** (19% yield).

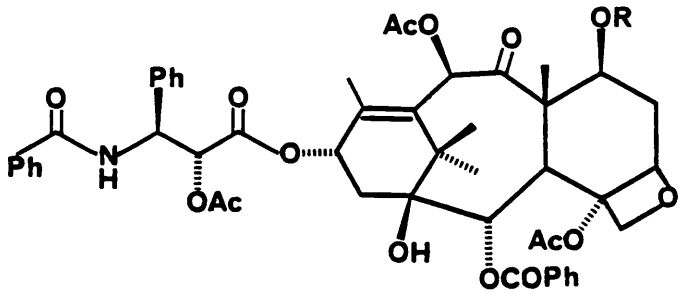
The product 2'-acetyl-7-phenyloxycarbonyltaxol can not be explained by the reaction of 2'-acetyltaxol with phenylchlorothionocarbonate. The appearance of the product can be

explained by the commercially obtained phenylchlorothionocarbonate having been contaminated with phenylchloroformate and it is thought that this was the case.

The ^{1}H-nmr spectra of 2'-acetyl-7-phenyloxythiocarbonyltaxol and 2'-acetyl-7-phenyloxycarbonyltaxol are shown in Table 41. The major change seen when the spectrum of 82 is contrasted with that of the starting material 2'-acetyltaxol is the signal for the C-7 proton, 4.43 ppm (dd, J=7,10) in 2'-acetyltaxol, is shifted downfield to 6.11 ppm (dd, J=7,10) and the signal for the C-6 protons, 2.3-2.5 ppm (m) in 2'-acetyltaxol is also shifted downfield to 3.0 ppm (m). Integration of the ^{1}H-nmr spectrum of **82** also showed the presence of an additional five aromatic protons. The only major difference between the spectra of 2'-acetyl-7-phenyloxythiocarbonyltaxol and 2'-acetyl-7-phenyloxycarbonyltaxol is that the signal for the C-7 protons is shifted upfield from 6.11 ppm (dd, J=7,10) to 5.52 ppm (dd, J=7,11).

The FAB mass spectrum for 2'-acetyl-7-phenyloxythiocarbonyltaxol agreed with the structural assignment. Peaks were seen at m/z 1054 $(MNa)^+$, 1032 $(MH)^+$, 972 $(MH-HOAc)^+$, and 954 $(MH-HOAc-H_2O)^+$. Signals below m/z 900 were not recorded. Peaks in the mass spectrum of 2'-acetyl-7-phenyloxycarbonyltaxol at m/z 1016 $(MH)^+$ and 951 $(MH-HOAc)^+$ indicated a molecular weight of 1015, sixteen less than **83**. A peak at m/z 818 $(MH-HOAc-HOCO_2C_6H_5)^+$ showed that the difference in molecular weight between **82** and **83** was due

Table 41 (Part 1 of 2). Characterization Data for **82** and **83**



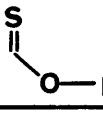
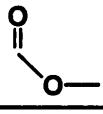
Position	82 R= 	83 R= 
2	5.75 (d, 7)	5.69 (d, 7)
3	4.03 (d, 7)	3.96 (d, 7)
5	5.02 (br d, 8)	4.98 (dd, 1,8)
6	3.0 (m)	2.65 (m)
7	6.11 (dd, 7,10)	5.52 (dd, 7,11)
10	6.41 (s)	6.40 (s)
13	6.26 (br t, 9)	6.20 (br t, 8.5)
14	2.3-2.5 (m)	2.3-2.5 (m)
16	1.24 (s)	1.21 (s)
17	1.20 (s)	1.17 (br s)
18	2.00 (br s)	1.96 (br s)
19	1.92 (s)	1.85 (s)
20	4.37 (d, 8), 4.24 (d, 8)	4.35 (d, 8), 4.18 (d, 8)
2'	5.55 (d, 3)	5.51 (d, 3)
3'	5.97 (dd, 3,9)	5.92 (dd, 3,9)

Table 41 (Part 2 of 2). Characterization Data for **82** and **83**

Position	82	83
N-H	6.89 (d, 9)	6.90 (d, 9)
OAcs	2.49 (s), 2.25 (s), 2.16 (s)	2.41 (s), 2.19 (s), 2.13 (s)
2 OBz	8.13 (d m, 7), 7.4 (m)	8.10 (d m, 8), 7.4 (m)
3' NBz	7.76 (d m, 7), 7.4 (m)	7.72 (d m, 7), 7.4 (m)
3' Ph	7.4 (m)	7.4 (m)
R	7.4 (s)	7.4 (s)

Mass Spectral Data **82**

1054 $(MNa)^+$,

1032 $(MH)^+$,

972 $(MH\text{-}HOAc)^+$,

954 $(MH\text{-}HOAc\text{-}H_2O)^+$.

Mass Spectral Data **83**

1016 $(MH)^+$, 956 $(MH\text{-}HOAc)^+$,

938 $(MH\text{-}H_2O\text{-}HOAc)^+$,

818 $(MH\text{-}HOAc\text{-}C_6H_5CO_3H)^+$,

689 $(MH\text{-}RCOOH)^+$,

629 $(MH\text{-}RCOOH\text{-}HOAc)^+$

328 $(RCOOHH)^+$

Infrared Spectral Data **82**

1760 s, 1745 s, 1675 m

1500 w, 1395 w, 1260 s

1075 m.

Infrared Spectral Data **83**

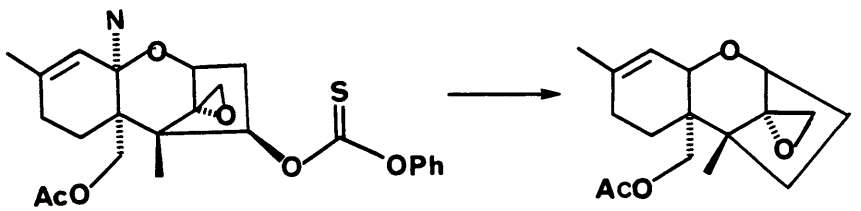
1760 s, 1745 s, 1675 m,

1500 w, 1395 w, 1280 s,

1240 s.

to the functionality at C-7. High resolution mass spectrometry analysis of **83** agreed with the elemental composition $C_{56}H_{57}NO_{17}$; experimental $(MH)^+$ 1016.3627; calculated 1016.3553.

The phenyloxythionyl group is less reactive than the thionobenzoate group due to loss of resonance stabilization of the intermediate radical involved in the reaction. Deoxygenation reactions employing a phenyloxythionyl group and tributyl tin hydride require use of the free radical initiator azobis(isobutyronitrile), (AIBN), and heating to 80°. Employing these conditions Schuda[64] was able to synthesize 15-acetoxy-4-deoxyverrucarol in 88% yield with a reaction time of 46 hours at 75° (Scheme 42). The hydroxyl group removal in this reaction was 'neopentyl' and may be comparable to the C-7 hydroxyl of taxol with respect to steric hindrance.



Scheme 42. Deoxygenation of 15-Acetoxyverrucarol

The stability of taxol under the reaction conditions necessary for deoxygenation was determined. This was done because taxol possesses sites capable of stabilizing radicals; C-3 and C-5 are tertiary, C-13 and C-18 are allylic, C-10 and

C-2' are alpha to a carbonyl and C-3' is benzylic. It was not expected therefore that taxol would be stable indefinitely under the reaction conditions necessary for deoxygenation.

Taxol was dissolved in dry toluene along with a catalytic amount of AIBN and the solution was heated to 80° for 15 minutes followed by addition of several equivalents of tributyltin hydride. Analysis by tlc showed a product forming even before the addition of the tin hydride. After a total reaction time of 30 minutes the reaction was worked up and the product isolated. The product was 7-epitaxol; characterization data and the experimental procedure for 7-epitaxol are included in Chapter 2.

A sample of taxol and cephalomannine was treated in the same manner as described for the taxol sample. The reaction was monitored by tlc (silica gel, 6/4 EtOAc/Hexane). After heating to 80° for 40 minutes tlc showed only a mixture of 7-epitaxol and 7-epicephalomannine Rfs 0.42 and 0.49; taxol/cephalomannine have Rf 0.29. After heating at 80° for 20 hours tlc analysis indicated that approximately half of the 7-epitaxol and 7-epicephalomannine remained with the rest of the material being seen as a complex mixture, all of which was more polar than 7-epitaxol and 7-epicephalomannine. The stability of taxol under the conditions necessary for deoxygenation, coupled with the reaction time necessary to synthesize 15-acetoxy-4-deoxyverrucarol, did not give a clear

indication as to whether decomposition or deoxygenation of 2'-acetyl-7-phenyloxythionyltaxol would be the faster process.

2'-Acetyl-7-phenyloxythionyltaxol was reacted with nBu_3SnH and a catalytic amount of AIBN for 14 hours at 70° and 7 hours at 85°. The reaction was followed by tlc (silica gel, 1/1, EtOAc/Hexane) which showed a mixture of products developing from the start of the reaction. The reaction was continued until the starting material was seen as a faint spot by tlc. After work up hplc analysis of the reaction mixture showed that the product mixture was extremely complex and no product could be isolated.

The reaction of 2'-acetyl-7-phenyloxythiocarbonyltaxol and tributyltin hydride showed that decomposition occurred faster than the deoxygenation reaction. Because taxol possesses no functionality which would have prevented formation of a radical at C-7 the slowness of the deoxygenation reaction was ascribed to steric hindrance at C-7.

The problems encountered at C-7 could not have been overcome by the radical methodology developed by Barton and further experimentation this area was not carried out.

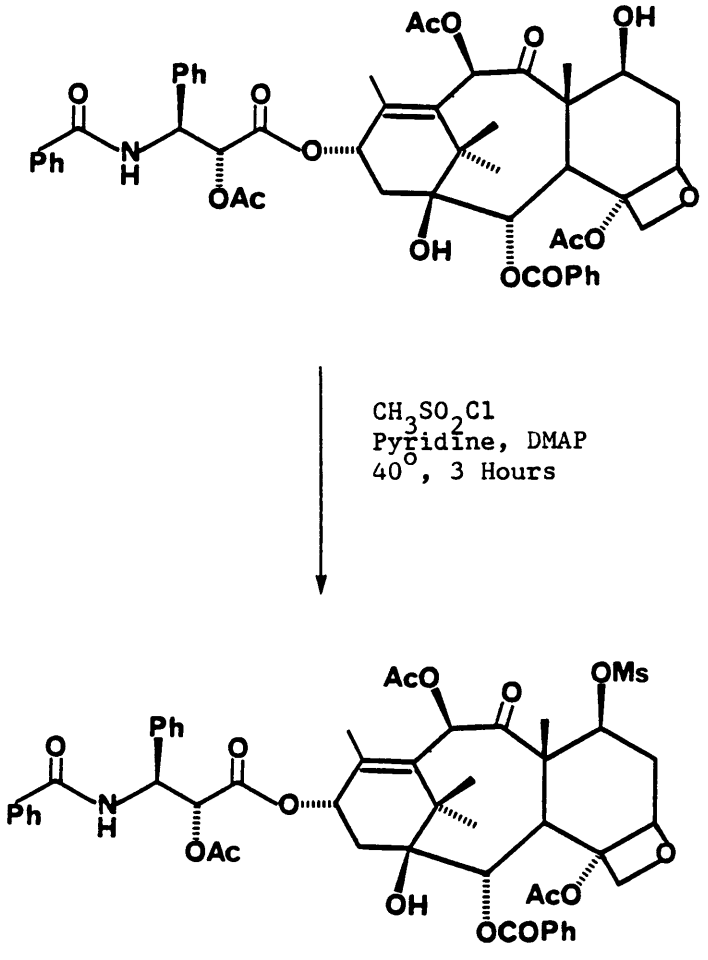
6.2.2 ATTEMPTED C-7 DEOXYGENATION OF TAXOL VIA BETA ELIMINATION

The second strategy investigated which was aimed at deoxygenaton of the C-7 position of taxol was to convert the C-7 hydroxyl group to a good leaving group, eliminate it and then hydrogenate the resulting double bond.

A methanesulfonate was chosen as the initial target functional group for C-7. This group was chosen rather than a toluenesulfonate as the smaller size of methanesulfonyl chloride would cause less crowding at the 7-position as opposed to the larger toluenesulfonyl chloride.

Reaction of taxol with methanesulfonyl chloride in deuterated pyridine at room temperature was followed by proton nmr for three hours. No reaction at all could be detected by nmr during this period. Preparation of 2'-acetyl-7-methanesulfonyltaxol **36** was achieved by reaction of 2'-acetyltaxol with methanesulfonyl chloride in pyridine at 40° for three hours using 4-dimethylaminopyridine as catalyst (Scheme 43). The reaction proceeded in nearly quantitative yield and work up with no chromatography was all that was required for isolation of the product which was homogenous on tlc and by ^{1}H-nmr analysis.

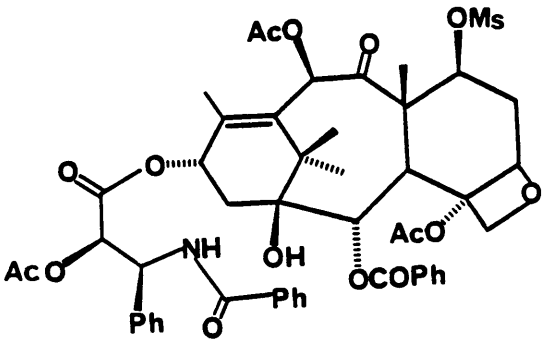
The characterization data for 2'-acetyl-7-methanesulfonyltaxol is shown in Table 42 . The proton nmr spectrum for the compound showed several changes when contrasted with



Scheme 43. Mesylation of 2'-Acetyltaxol

Table 42. Characterization Data for 2'-Acetyl-7-methanesulfonyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.68 (d, 7)
3	3.92 (d, 7)
5	4.92 (d, 9)
6	2.9-3.1 (m)
7	5.34 (dd, 7,10)
10	6.50 (s)
13	6.19 (br t, 9)
14	2.1-2.2 (m)
16	1.14 (s)
17	1.23 (s)
18	1.56 (br s)
19	1.78 (s)
20	4.16 (d, 8), 4.31 (d, 8)
2'	5.52 (d, 3)
3'	5.93 (dd, 3,9)
N-H	6.91 (d, 9.5)
OAcs	2.41 (s), 2.16 (s), 2.14 (s)
C-2' OBz	8.09 (d,m, 7), 7.10 (t, 7), 7.4 (m)
C-3' Ph	7.4 (m)
C-3' NBz	7.73 (d m, 7), 7.4 (m)
Mesyl	3.09 (s)



Mass Spectral Data

996 $(MH)^+$, 874 $(MNa)^+$.

Peaks below m/z 900 not recorded.

Infrared Spectral Data

1745 s, 1680 m, 1575-1440 w, 1285 s, 1150-1020 w, 1090 m.

the spectrum of the starting material. The peak for the C-7 proton of **36** is at 5.34 ppm (dd, J=7,10), downfield of the analogous peak in 27 at 4.43 (dd, J=6.5,10). The methanesulfonyl groups also causes the peaks for the C-6 protons of **36** at 2.9-3.1 ppm (m) to be downfield of the C-6 protons for **27** at 2.3-2.5 ppm (m). The methyl peak for the methanesulfonyl group occurs at 3.09 ppm as a sharp three proton singlet. The mass spectrum for 2'-acetyl-7-methanesulfonyltaxol shows peaks at m/z 996 $(MH)^+$ and 974 $(MNa)^+$ indicating a molecular weight of 973. The molecular weight of **36** is 78 greater than that of **27** which is correct for the substitution of a methanesulfonyl group.

Reaction of 2'-acetyl-7-methanesulfonyltaxol with a 1/1 solution of Et_3N/CH_2Cl_2 at room temperature for 5 hours produced no change in the starting material. The failure of triethylamine to cause any transformation of 2'-acetyl-7-methanesulfonyltaxol showed that a stronger base would be needed if elimination were to be achieved. The hindered base potassium tertiary-butoxide was chosen as it was not expected to undergo nucleophilicly attack on the ester sites of **36**. Reaction of 2'-acetyl-7-methanesulfonyltaxol with KOtBu under a variety of conditions to be described failed to selectively effect elimination; under some conditions no reaction took place but when reactions did start they produced complex mixtures of products from the beginning. Reaction of 2'-acetyl-7-methanesulfonyltaxol with a slight excess of

KOtBu as a suspension in dry CH_2Cl_2 with a catalytic amount of 18-crown-6 present produced no reaction at room temperature after 2 hours. Addition of t-butanol did not cause any reaction to take place, as shown by tlc monitoring after 45 minutes. The addition of several equivalents of KOtBu at that point caused the starting material to decompose so that it was seen as a streak on tlc after 25 minutes. Reaction of **36** with several equivalents of KOtBu in t-butanol at room temperature for 20 minutes also produced a complex mixture of products that was seen as a streak on a silica gel analytical tlc plate.

Reaction of 2'-acetyl-7-methanesulfonyltaxol with a slight excess of KOtBu and a slight excess of 18-crown-6 in dry acetonitrile for 1 hour at 0° left most of the starting material unchanged. In addition to the starting material several product spots were seen on tlc along with a significant amount of streaking. This reactivity datum shows that from the beginning of the reaction with KOtBu a complex mixture of products was produced from the start of the reaction. An alkoxide therefore was too strong a base to use to effect elimination without decomposition also taking place. To try and overcome this problem a weaker base was next chosen to be tested.

Reaction of 2'-acetyl-7-methanesulfonyltaxol (50 mg) with 0.025% DBU in CH_2Cl_2 at room temperature was carried out for 26 hours 40 minutes. The reaction was monitored by tlc and

stopped when the starting material spot had grown faint. After work up preparative tlc (silica gel, 9/2, CH_2Cl_2/2-butanone) yielded three product bands, Rfs 0.80 (8 mg), 0.55 (5 mg), 0.38 (20 mg), in addition to the starting material, Rf 0.70 (2 mg), (Scheme 44). Analysis of each of the products obtained from prepararative tlc showed that in every case the methanesulfonyl group at C-7 was intact. The structures of the products from the reaction will be discussed further but for the purpose of deoxygenating the C-7 position of taxol the presence of the methanesulfonyl peak at 3.09 ppm in the proton spectrum of each product was sufficient to show that the elimination had not taken place.

The failure of base to eliminate the C-7 methanesulfonyl group was due to steric hindrance. In order for the elimination to occur the proton on the alpha face would have to be abstracted (Figure 27) because it is <u>trans</u> coplanar to the methanesulfonyl group. The alpha hydrogen is pointed into the concave face of the taxane skeleton and a large base such as DBU would be able to abstract the proton only with great difficulty. This steric problem could be minimized by using smaller bases for the elimination reaction but smaller bases would also be nucleophilic, which would cause taxol to decompose.

The product isolated by preparative tlc with Rf 0.80 was assigned the structure 2'-epi-acetyl-7-methanesulfonyltaxol **37** and is discussed in more detail in Chapter 2. The product

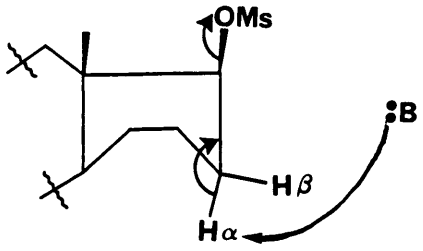


Figure 27. Reaction Mechanism for Elimination of Methanesulfonic Acid from **36**

with Rf 0.55 was found to be one pure taxol-like compound by ^{1}H-nmr which was assigned the structure 7-methanesulfonyl-taxol **84**; its characterization data is shown in Table 43 . In the spectrum of **84** the peak for the C-2' proton is at 4.78 ppm (d, J=2) and for **36** it is seen at 5.52 ppm (d, J=3). The C-2' peak in the spectrum of taxol is seen at 4.73 ppm (d, J=3). The mass spectrum of **84** agreed with the assigned structure; peaks at m/z 954 $(MH)^+$ and 932 $(MH)^+$ indicating a molecular weight of 931, 42 less than the starting material.

The product band with Rf 0.38 was analyzed by ^{1}H-nmr and found to be a mixture. The presence of three sharp singlets of comparable intensity between 6.4 ppm and 6.55 ppm corresponding to C-10 protons showed that the mixture contained three taxol-like compounds. Even though a mixture of products was present it was possible to determine that each of the products possessed a methanesulfonyl group by the following rationale. Between 4.3 ppm and 5.2 ppm the only peak

present was a very broad doublet corresponding to the C-5 protons of the products. The absence of any other peaks in this area showed that C-13 was esterified in each of the products and so each of the products contained the three aromatic rings found in the starting material. Integration between 7.3 ppm and 8.2 ppm therefore corresponded to fifteen protons for each of the products. Integration of the broad singlet at 3.10 ppm due to the methanesulfonyl groups showed that the peak area was exactly one third of the aromatic protons. No major compound was present which did not possess a methanesulfonyl group.

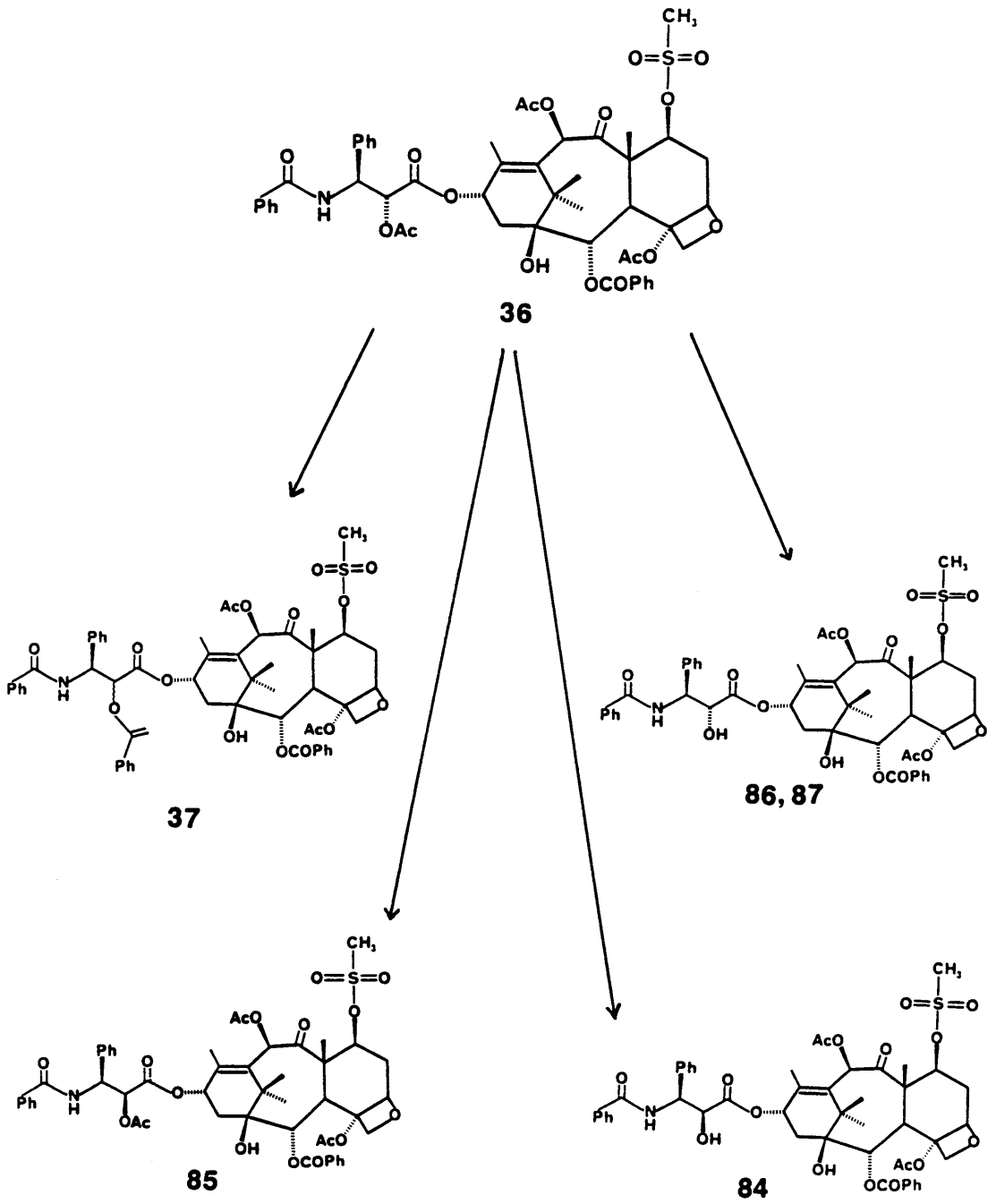
For purposes of C-7 deoxygenation of taxol, characterization of the mixture of products was not required beyond the point that the presence of the methanesulfonyl groups was ascertained. The mixture was purified further however for the general goal of determining the reactivity of taxol under basic conditions. Preparative hplc enabled us to isolate a pure compound from the mixture along with the remaining two compounds as a mixture.

The proton spectrum of the pure compound **85** is shown in Table 47. The spectrum for the compound is nearly identical to that for 7-methanesulfonyltaxol; differences in chemical shift between the two spectra differ by no more than 0.1 ppm for any peak. There is a difference in coupling constants when the two spectra are contrasted; the C-2' proton signal for **84** is a 2 Hz doublet while that for **84** is a 4 Hz doublet.

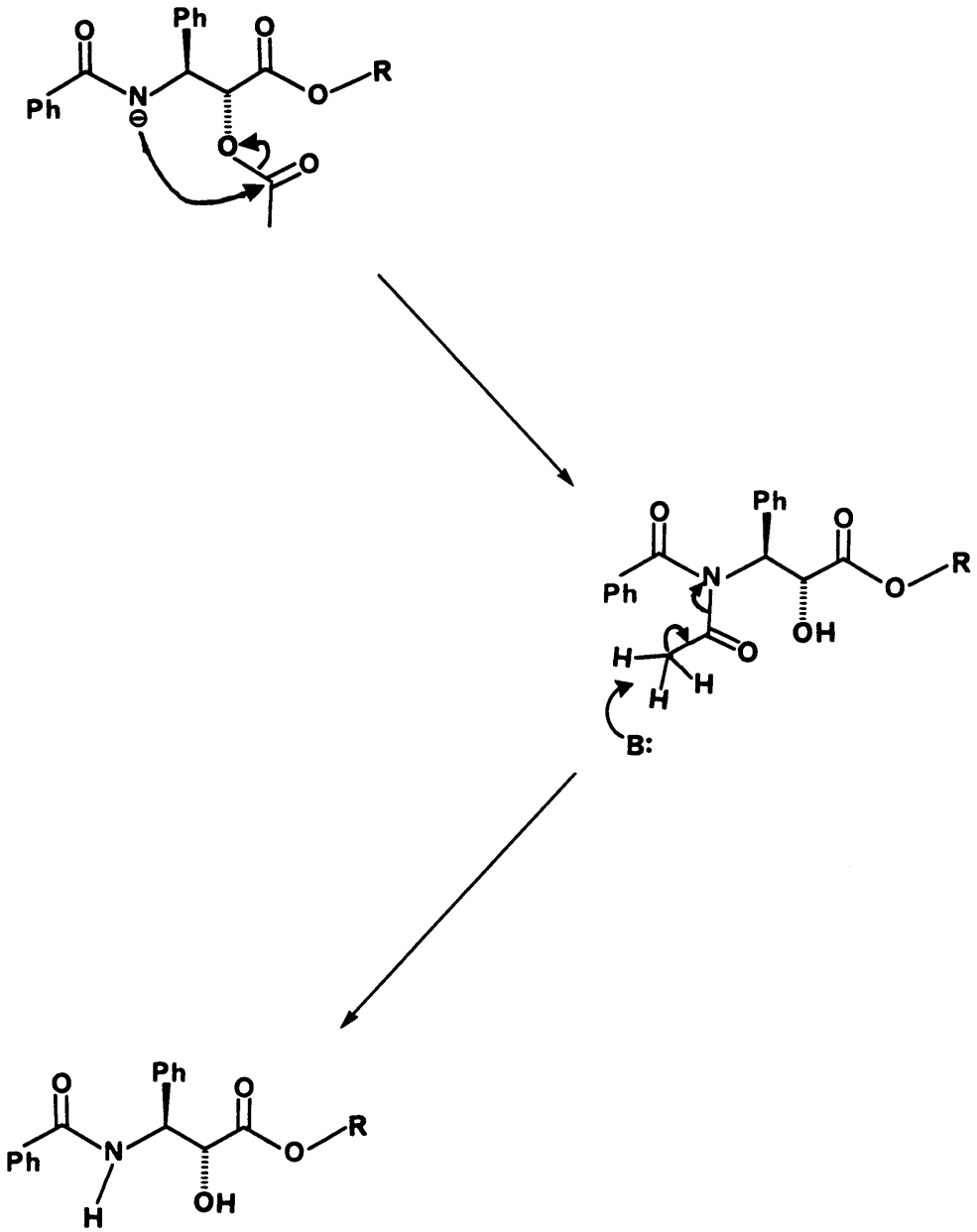
A coupling constant for C-2' larger than 3.0 hertz is unusual for a taxol-like compound and was seen for 2'-epiacetyl-7-methanesulfonyltaxol (J=6). Compound **85** can be tentatively assigned the structure epi-2',7-methanesulfonyltaxol.

Overlapping of the peaks in the proton nmr spectrum made only a tentative assignment of structures possible for the mixture of the taxol-like compounds. Some information about the structure of the compounds could be gained however. The spectrum showed only a peak for C-5 between 4.3 ppm and 5.2 ppm. and two broad triplets near 6.3 ppm were also present. These data indicated that the ester side chain was attached in both compounds. The spectrum also showed that the methanesulfonyl group was present in both compounds by the presence of two sharp singlets near 3.10 ppm. Absent from the spectrum was an amide proton near 7.0 ppm for either compound. It is possible that transacylation of the acetyl from C-2' to C-3' N and of the benzoyl from C-3' N to C-2 had taken place.

The reaction of 2'-acetyl-7-methanesulfonyltaxol with DBU produced unusual results in that most of the products obtained had been deacetylated at the 2' position but none of the products had lost the C-13 ester side chain. When the C-2' acetate of 2',7-diacetyltaxol was removed by basic solvolysis (Chapter 2) 7-acetyltaxol was obtained in 47% yield and cleaved 7-acetylbaccatin III, with the side chain cleaved was obtained in 36% yield. The solvolysis of



Scheme 44. Reaction of 2'-Acetyl-7-methanesulfonyltaxol with DBU



Scheme 45. Removal of Acetate from 2'-Acetyl-7-methanesulfonyltaxol

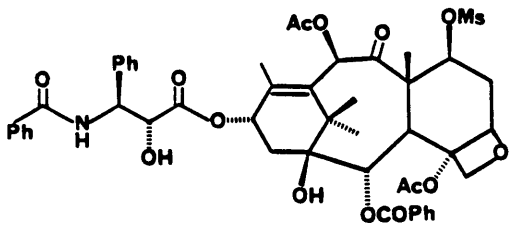
2',7-diacetyltaxol occurred via nucleophilic attack at ester linkages; if the removal of acetate from **36** had occurred by nucleophilic attack then it would have been expected that cleavage of the side chain would also have taken place. A possible explanation for the removal of acetate from the side chain while the side chain was left intact is beta elimination of ketene from C-2' by DBU (Scheme 45).

The steric problems associated with the alpha hydrogen at C-6 would prevent an elimination reaction from taking place as long as the reaction mechanism was primarily E2, i.e., as long as abstraction of a proton was required as the driving force for the reaction. In order to overcome this difficulty a different strategy was tested.[65] In these reactions 2'-acetyl-7-methanesulfonyltaxol was heated in DMF with LiCl or $LiSO_4$. The mechanism for the reaction involves lithium complexation of the C-7 oxygen and at least partial C-7 - oxygen bond breaking followed by abstraction of a proton at C-6.

Reaction of 2'-acetyl-7-methanesulfonyltaxol with $LiSO_4$ in dimethylformamide at 60° for 30 minutes and then, after the addition of pyridine, at 100° for 1 hour and 130° for three hours produced no change in the starting material, as indicated by hplc. At the end of the reaction period the solution had become cloudy and dark brown. After work up, ^{1}H-nmr of the crude reaction mixture showed a complex pattern of intense peaks upfield of 2.5 ppm; below 2.5 ppm the only

Table 43 Characterization Data for 7-Methanesulfonyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.66 (d, 7)
3	3.89 (d, 7)
5	4.90 (br d, 9)
6	2.9-3.1 (m)
7	5.30 (dd, 7,10)
10	6.46 (s)
13	6.16 (br t, 9)
14	2.2-2.4 (m)
16	1.17 (s)
17	1.21 (s)
18	1.83 (br s)
19	1.78 (s)
20	4.29 (d, 8), 4.14 (d, 8)
2'	4.78 (d, 2)
3'	5.89 (dd, 2,9)
N-H	7.04 (d, 9)
OAcs	2.33 (s), 2.15 (s)
C-2' OBz	8.09 (d,m, 8), 7.4 (m)
C-3' Ph	7.4 (m)
C-3' NBz	7.76 (d,m, 8), 7.4 (m)
Mesyl	3.08 (s)



Mass Spectral Data

954 $(MNa)^+$, 932 $(MH)^+$,

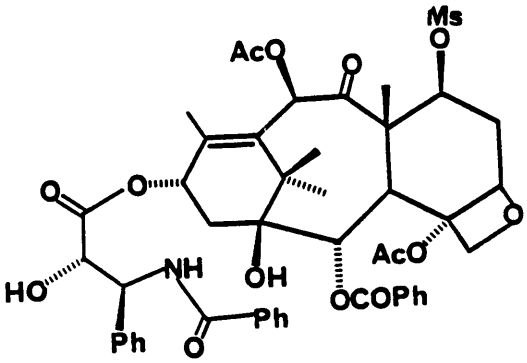
872 $(MH\text{-}HOAc)^+$,

854 $(MH\text{-}CH_2SO_2)^+$,

587 $(MH\text{-}RCOOH\text{-}HOAc)^+$

Table 44 Proton Spectrum of 2'-Epi-7-methanesulfonyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.63 (d, 7)
3	3.89 (d, 7)
5	4.93 (dd, 1,9)
6	2.9-3.1 (m)
7	5.31 (dd, 7,10)
10	6.42 (s)
13	6.07 (br t, 9)
14	2.2-2.4 (m)
16	1.13 (s)
17	1.23 (s)
18	1.64 (br s)
19	1.78 (s)
20	4.30 (d, 8), 4. 0 (d, 8)
2'	4.89 (d, 4)
3'	5.76 (dd, 4,8.5)
N-H	7.06 (d, 8.5)
OAcs	2.42 (s), 2.15 (s)



C-2 OBz	8.05 (d m, 8), 7.4 (m)	C-3' NBz	7.72 (d,m, 8), 7.4 (m)
C-3' Ph	7.4 (m)	Mesyl	3.09 (s)

peaks seen were those of the starting material. The decomposition seen upon heating was the solvent decomposing and not the taxol derivative.

Reaction of taxol was also carried out with LiCl in dimethylformamide at 90° for 60 hours. Monitoring of the reaction throughout this period by hplc (analytical RP-8, 7/3, MeOH/H_2O) showed that no reaction occurred to any significant extent. The failure of 2'-acetyl-7-methanesulfonyltaxol to react under the conditions described was a disappointment as far as C-7 deoxygenation was concerned but was not without a positive aspect. The reaction conditions that the 2'-7-disubstituted taxol survived were extremely vigorous. Since taxol has been shown to react or decompose in the presence of nucleophiles, electrophiles, acids, bases, reducing agents and oxidizing agents the identification of harsh conditions under which it is stable may be important information for carrying out selective transformations in future research.

Another strategy envisioned for eliminating the C-7 hydroxy was to derivatise the position as a trifluoromethanesulfonyl group. Reaction of a mixture of 2'-acetyltaxol and 2'-acetylcephalomannine was reacted with a slight excess of trifluoromethanesulfonicanhydride at 0° with pyridine as catalyst. After several minutes tlc analysis showed no reaction taking place. The reaction was warmed to room temperature and several additional equivalents of the anhydride

were added. After ten minutes at room temperature tlc analysis showed approximately 25% of the starting material remaining. Also present were too many products to count and the plate had a streaky quality.

The initial reaction of taxol with trifluoromethanesulfonic anhydride was carried out before the sensitivity of taxol to electrophiles was fully understood (see Chapter 5). Further reactions, with modification of technique, were not carried out because it was expected that trifluoromethanesulfonyic anhydride was too strong an electrophile to react selectively at the C-7 hydroxyl group.

Two additional reactions designed to deoxygenate the C-7 positon of taxol were carried out: reaction of 2'-acetyl-7-oxotaxol with triphenylphosphinemethylide[66] and reaction of 2'-acetyl-7-methanesulfonyltaxol with tetrabutylammonium borohydride.[67]

The Wittig reaction with the 7-oxotaxol was carried out before the extreme base instability of the compound was established (see Chapter 4). The reaction was followed by hplc which showed a mixture of products but no triphenylphosphine oxide. Isolation of 5 product bands by preparative tlc and analysis by ^{1}H-nmr showed that no pure product could be obtained. In all of the spectra of mixtures of products no sharp doublets at 6.0 ppm and 7.0 ppm were seen. In most of the spectra a broad doublet or broad doublets near 6 ppm could be seen which are indicative of the C-13 proton signals

for D-seco taxols. The reactivity data indicate that 2'-acetyl-7-oxotaxol underwent a beta-elimination to produce a D-seco taxol; excess Wittig reagent then attacked the enone leading to a complex mixture of products none of which involved the transformation of a carbonyl group to an alkene.

Reaction of 2'-acetyl-7-methanesulfonyltaxol with tetrabutylammonium borohydride produced no reaction after 66 hours at 25°. Reduction of the mesylate would have required SN2 attack by hydride at C-7. The reduction was prevented by the concave face of the taxol skeleton prohibiting approach of the hydride.

6.2.3 SUMMARY AND PROPOSED RESEARCH

Attempted C-7 deoxygenation of taxol was unsuccessful due to reaction limitations imposed by the complexity of the taxol structure. Radical deoxygenation via reduction of a 7-phenyloxythionyltaxol with tributyltin hydride failed due to the instability of taxol under radical conditions. Deoxygenation of a 7-methanesulfonyltaxol by elimination could not be achieved due to steric crowding of the C-6 alpha hydrogen. A 7-trifluoromethanesulfonyltaxol could not be selectively prepared because of the sensitivity of several of taxol's functional groups to electrophiles.

The attempted taxol deoxygenation reactions presented in this chapter did not exhaust all the reasonable strategies

available. A deoxygenation method which had not been attempted was dissolving metal reductions such as those developed by Ireland[68] and Liu[69] for phosphoroamidates. Dissolving metal reduction was not attempted because derivatization of C-7 would require conversion of the alcohol to an alkoxide; taxol would not be expected to survive such conditions.

Radical deoxygenation of C-7 might be achieved through the reduction of a selenocarbonate by tributyltin hydride.[59] The reaction proceeds initially by attack of a tin radial on selenium. Even with hindered alcohols the reaction proceeds within one hour at 80° but higher temperatures are usually required to obtain the hydrocarbon product as opposed to an alcohol or a formyl derivative. Reduction could be achieved with a reaction time small enough that decomposition of taxol would be minimal.

Deoxygenation via elimination might be achieved through converting the C-7 hydroxyl group to a derivative more reactive than methanesulfonyl but less reactive than trifluoromethanesulfonyl. One such derivative is a 2,2,2-trifluoroethanesulfonyl (tresylate).[70] Solvolysis rates for tresylate esters are on the order of 200 times greater than those for methanesulfonyl and 400 times smaller than those of trifluoromethanesulfonyl esters. A C-7 tresylate ester of taxol could be prepared without electrophilic attack taking place at other sites and still be reactive

enough to allow an elimination reaction to take place via an E1 mechanism.

6.3 EXPERIMENTAL PROCEDURE.

Attempted Preparation of 2'-N-CBZ-β-alanyl-7-Phenylthionyl-taxol - N,N Dimethylbenzamide (310 mg) was reacted with 2.50 mL of a 12.5% solution of $COCl_2$ in benzene for a period of 12 hours at room temperature to yield a white crystalline salt. The solvent and excess phosgene were removed by evaporation on a rotary evaporator with a drying tube placed between the reaction flask and the rest of the apparatus. The residual salt was then dissolved in 4.20 mL of CH_2Cl_2.

2'-N-CBZ-β-alanyltaxol (25 mg) had 0.10 mL (2 eq) of the clear, colorless imidoylchloride solution added to it. After several minutes a yellow color had developed.

After a reaction time of 150 minutes tlc showed one product and no starting material remaining and 10 µL of dry pyridine were added to the taxol reaction solution. After addition of the pyridine H_2S was bubbled through the solution for 5 minutes. Upon addition of the H_2S the color of the solution darkened beyond yellow and the reaction became cloudy within 2-3 minutes. Upon standing for one hour a dark precipitate developed in addition to the solution being cloudy. The reaction was worked up by washing with water, 0.1 N HCl, and 5% $NaHCO_3$. An attempt to purify the sample

was made using preparative tlc with 60/40 hexane/ethyl acetate as the solvent. Numerous (>15) UV absorbing bands were seen and at least six of these were colored yellow. Scraping off the yellow bands and washing off the products yielded no product that could be characterized. The products were either liquids or in very small quantity (<0.1 mg) or decomposed (most of the yellow bands were starting to darken as soon as the tlc plate was withdrawn from the developing tank). This reaction was repeated several times with slightly changed conditions each time and in no case could characterizable product be isolated.

2'-Acetyl-7-phenylthionocarbonyltaxol - 2'-Acetyltaxol (110 mg) was dissolved in 1.0 mL of a solution consisting of 10.0 mL CH_2Cl_2, 270 µL pyridine and 82 mg 4-dimethylaminopyridine. To this solution was added 70 µL of phenylchlorothionocarbonate. The reaction was allowed to stand at room temperature under a nitrogen atmosphere for 96 hours. The reaction was worked up by diluting with CH_2Cl_2, washng with 0.1 N HCl, 10% $NaHCO_3$ and water. The products were purified by preparative hplc (70/30 $MeOH/H_2O$). Two products were obtained **83** 13 mg (19% yield) and **82** 61 mg (49% yield). The characterization data for **82** and **83** are shown in Table 41 .

Stability of Taxol under Radical Deoxygenation Conditions - Taxol (19.7 mg) and azobisisobutyronitrile (0.9 mg) were

dried in vacuo in a 5 mL round bottomed flask. After drying for an hour the taxol and AIBN were dissolved in 2.0 mL of dry toluene. The solution was heated to 80° for 15 minutes at the end of which 40 μL of nBu_3SnH was added. The reaction solution was kept at 80° for an additional 15 minutes. The reaction was worked up by diluting with 6 mL ethyl acetate, washing with several mL of 0.1 N HCl and then with water. The solution was then dried with $MgSO_4$ and then filtered. After evaporation the product, 7-epitaxol, was purified by preparative tlc (60/40 EtOAc/Hexane; Rf of product 0.46, Rf of taxol 0.26). There was a faint band of taxol present. Characterization of 7-epitaxol is discussed in Chapter 2.

The reaction was also carried out with a taxol/cephalomannine mixture and the reaction was conducted for an extended period to determine the stability of the C-7 epitaxol under the reaction conditions to be used for deoxygenation. A mixture of taxol and cephalomannine (20 mg) was dissolved in 2.0 mL of dry toluene along with 1.4 mg of AIBN. The solution was heated to 80° and maintained at that temperature for 15 minutes at which time 38 μL of nBu_3SnH was added. The reaction was monitored by tlc over the course of 24 hours at 80°. Analysis by tlc showed that approximately half of the 7-epitaxol/7-epicephalomannine had decomposed into a complex mixture of products seen as a streak.

Reaction of 2'-Acetyl-7-phenylthiocarbonyltaxols with AIBN/nBu_3SnH - 2'-Acetyl-C-7-phenylthionocarbonyltaxol (11 mg) was dissolved in 2.0 mL of dry benzene which had been dried over sodium. To this solution was added 1.5 mg of AIBN and the solution was heated to 85° for a period of 15 minutes. At the end of the 15 minute period 25 µL of nBu_3SnH was added. The reaction was continued at 85° for 7 hours and then at 70° for 14 hours. The reaction was analyzed using tlc (50/50 EtOAc/Hexane) and the reaction was stopped when the spot corresponding to the starting material had grown faint. Throughout the reaction tlc showed numerous products and had a streaky quality. At the end of the reaction the products were dissolved in CH_3CN and the tin compounds were removed with hexane. Analysis of the reaction products by hplc after work up showed there to be a high number of products present along with some starting material.

Reaction of 2'-Acetyltaxol with Methanesulfonylchloride in Pyridine - 2'-Acetyltaxol (34 mg) was dissolved in 1.0 mL of deuterated pyridine. To this solution was added 15.3 µL (1.1 eq) of a solution of 90µL of methanesulfonylchloride in 0.5 mL of deuterated pyridine. The solution was left to stand at 0° for 2 hours at and 3 hours at 25°. In transferring the sample to an nmr tube for analysis water was accidentally introduced which made nmr analysis impossible. The reaction

was worked up and tlc analysis showed that no product other than the starting material was present.

Reaction of 2'-Acetyltaxol with Methanesulfonylchloride/4-DMAP in Pyridine - 2'-Acetyltaxol (30 mg) was added to a dry nmr tube (5mm) along with 3.6 mg of 4-dimethylaminopyridine (1.0 eq). To the acetyltaxol and DMAP was added 0.50 mL of deuterated pyridine. The reaction was put into a sonicator so that the acetyltaxol and DMAP would dissolve quickly. The reaction was conducted at 40° for 3 hours at which time an nmr spectrum was obtained. The spectrum showed only one compound present (one taxol-like compound) but it was not clear if the compound was the desired product. The reaction was continued for an additional 3 hours at which time another spectrum was obtained and a spectrum of C-2'-acetyltaxol in deuterated pyridine was also obtained. The second spectrum of the product was identical with the first one obtained and did not match that of 2'-acetyltaxol.

The reaction was diluted with CH_2Cl_2 and washed with 1 N HCl and then 10% $NaHCO_3$ and water, dried and evaporated. At that point tlc analysis showed only one product present, identified as 2'-acetyl-7-mesyltaxol, Rf 0.50 (EtOAc/hexane, 60/40). 2'-Acetyltaxol has Rf 0.32 in the same system. Isolated yield 31 mg, 95%. Characterization data are shown in Table 42 .

Reaction of 2'-Acetyl-7-methanesulfonyltaxol with Triethylamine - A small amount (<0.5 mg) of 2'-Acetyl-7-methanesulfonyltaxol had 1 ml of dry triethylamine added to it. Within a few minutes it became apparent that the taxol derivative was not going to dissolve, and 1 mL of CH_2Cl_2 was then added. The reaction was followed by tlc. After a period of 5 hours no reaction at all could be seen by tlc.

Reaction of 2'-Acetyl-7-methanesulfonyltaxol with Potassium tert-butoxide and 18-Crown-6 - 2'-Acetyl-7-methanesulfonyltaxol (4.7 mg) was dissolved in 200µL of a mixture consisting of potassium t-butoxide (116 mg), 18-crown-6 (14 mg) and 100mL of hplc grade CH_2Cl_2 (0.002% H_2O). The reaction was stirred at room temperature. The reaction was followed by thin layer chromatography (6/4 ethyl acetate/hexane, silica gel plates). After two hours tlc showed virtually no reaction taking place. At that point an additional 400 µL of the CH_2Cl_2 mixture was added along with 40 µL of dry tert-butanol. After 45 minutes tlc again showed no reaction taking place. At that point 7 mg of potassium tertiary butoxide was added.

Twenty five minutes after the addition of the KOtBu, tlc showed no starting material present but rather a streak. The reaction was stopped 50 minutes after the addition of the KOtBu and was worked up by diluting with CH_2Cl_2, washing with water then 0.1 N HCl, drying with $MgSO_4$ filtering and evapo-

rating. Analysis by tlc showed nine distinct spots and much streaking ; Rfs 0.94, 0.83, 0.68, 0.50, 0.36, 0.24, 0.14, 0.08, 0.0; Rf of starting material was 0.46. The product mixture was too complex to isolate any one product.

Reaction of 2'-Acetyl-7-methanesulfonyltaxol with Potassium tert-butoxide in tert-Butanol - 2'-Acetyl-7-methanesulfonyltaxol (4 mg) and KOtBu (2 mg) were dissolved in 1.0 mL of t-butanol freshly distilled from calcium hydroxide. The reaction was conducted at room temperature and followed by tlc. (6/4 ethyl acetate/hexane, silica gel tlc plates). After twenty minutes no starting material could be seen but only a spot at the origin. The reaction was worked up as previously described and the products were analyzed by tlc. Methylene chloride/methanol solvents with tlc showed only streaks and very minor product spots. With 100% ethyl acetate as solvent the products just began to move from the origin. All of the products therefore were much more polar that the starting material.

Reaction of 2'-Acetyl-7-methanesulfonyltaxol with Potassium tertiary butoxide with One Equivalent of 18-Crown-6 - 2'-Acetyl-7-methanesulfonyltaxol (4.5 mg) was dissolved in 0.400 mL of a solution consisting of KOtBu (34.3 mg), 18-crown-6 (77.4 mg) and 20 mL of dry acetonitrile. The reaction solution was stirred at 0° for one hour. The reaction

was worked up as previously described and analyzed by tlc. Analysis by tlc showed mostly starting material present with several products. Rfs = 0.55, 0.49 (starting material), 0.38, 0.17 and 0.0. There was also a good deal of streaking on the tlc plate; (6/4 ethyl acetate/hexane, silica gel plates).

Reaction of 2'-Acetyl-7-methanesulfonyltaxol with 1,8-Diazabicyclo [5.4.0.] undec-7-ene (DBU) - 2'-Acetyl-7-methanesulfonyltaxol (50 mg) was dissolved in 2.0 mL of dry CH_2Cl_2 and DBU (50 µL) was added. The reaction was allowed to proceed at room temperature for 26 hours, 40 minutes at which time it was stopped by washing with several mL of 1.0 N HCl, then water, drying with $MgSO_4$, filtering and evaporating. The products were isolated using preparative tlc (silica gel, 90/20 CH_2Cl_2/Methyl Ethyl Ketone). Four bands were isolated — Rf 0.80, 8 mg **37**; Rf 0.71, 2 mg **36**; Rf 0.55, 5 mg **84**, and a mixture with Rf 0.38, 20 mg. Characterization data and structure assignments are included in the Results and Discussion section.

Reaction of 2'-Acetyl-7-methanesulfonyltaxol with Lithium Sulfate in Dimethylformamide with Heating - 2'-Acetyl-7-methanesulfonyltaxol (18 mg) was dried *in vacuo* along with $LiSO_4$ (6 mg). To these reagents was added 1.0 mL of dry dimethylformamide, which dissolved all of the taxol derivative

but only partially dissolved the $LiSO_4$. The reaction was well stirred with a magnetic stirring bar and was followed by hplc (RP-8, 6/4 CH_3CN/H_2O, 2 mL/min, Rf-6 min). The reaction was heated in an oil bath to 65° for 30 minutes and no reaction could be seen taking place. The reaction then was heated to 100° and had dry pyridine (5 µL) added to it. After 1 hour at 100° still no reaction could be seen taking place and so the temperature was raised to 130° and kept there for 3 hours. At the end of this period the solvent was becoming brown and cloudy. The reaction was worked up in the usual manner and the crude reaction mixture analysed by ^{1}H-nmr. The spectrum obtained showed numerous impurity peaks above 2.5 ppm but below 2.5 ppm only peaks resulting from the starting material were present.

Reaction of 2'-Acetyl-7-methanesulfonyltaxol with Lithium Chloride in Dimethylformamide - 2'-Acetyl-7-methanesulfonyltaxol (20 mg) was dissolved in dry dimethylformamide along with lithium chloride (40 mg). The solution was stirred and heated to 90°. The reaction was checked periodically by hplc (analytical RP-8, 7/3 $MeOH/H_2O$). Over the course of 60 hours hplc showed no UV absorbing compounds present other than the starting material.

Reaction of 2'-Acetyltaxol/2'-Cephalomannine with Trifluoromethanesulfonic Anhydride - A mixture of 2'-acetyltaxol and

2'-acetylcephalomannine (20 mg) was dissolved in dry CH_2Cl_2 (0.30 mL) at 0° and pyridine (5 µL) was then added. A solution of trifluoromethanesulfonic anhydride (100 µL) in CH_2Cl_2 (300 µL) was then added (16 µL) at 0°. Immediately upon addition of the anhydride the solution became yellow. After several minutes the reaction was checked by tlc (6/4, EtOAc/Hexane) which showed no reaction had taken place. At that point the reaction was removed from the ice bath and allowed to warm to room temperature and additional anhydride solution was added (50 µL). The yellow color deepened upon addition of more anhydride and after five minutes the solution was yellow/green. Analysis by tlc ten minutes after the addition of the anhydride showed aproximately 25% of the starting material remaining along with numerous product peaks and a streak. The reaction was worked up at that point and tlc analysis of the crude reaction mixture showed that no one isolatable product was present.

Reaction of 2'-Acetyl-7-oxotaxol with Triphenylphosphinemethylide - The Wittig reagent triphenylphosphinemethylide was prepared in the following manner. Sodium hydride (0.0772 g 60% dispersion in mineral oil) was washed with two 1 ml portions of hexane. Dry DMSO (1.0 mL) was then added to the sodium hydride and the mixture was stirred at 70° for 15 minutes by which time the evolution of hydrogen had ceased and the mixture was heated with methyltriphenylphosphonium-

bromide (0.520 g) in 2.0 mL of dry DMSO. Immediately upon addition of the phosphonium salt a dark yellow/light orange color developed.

The Wittig reagent solution (0.6 mL) was added to 2'-acetyl-7-oxotaxol (145 mg) which had been dissolved in 1.5 mL of DMSO. After 20 minutes at room temperature and under an argon atmosphere the reaction was checked by hplc, which showed a mixture of products, no starting material and no triphenylphosphine oxide. The reaction was checked again by hplc several times over the course of 2 hours. During this time no change was seen in the hplc chromatogram. After 2 1/2 hours of reaction time an additional 250 µL of the Wittig reagent was added. The reaction was checked by hplc 30 minutes after this addition and again no change was seen; at this point the reaction was stopped. The reaction was worked up by: 1. Dilution of the reaction to 12 mL with CH_2Cl_2; 2. Washing the organic layer with an equal volume of 1 N HCl. At this the dark red color of the organic solution faded to light yellow; 3. Washing with water; 4. Drying with $MgSO_4$, filtering and evaporating; 5. Purifying the products by preparative tlc. All products bands isolated by preparative tlc were mixtures.

Reaction of 2'-Acetyl-7-methanesulfonyltaxol with Tetrabutylammonium Borohydride - Tetrabutylammonium borohydride (2.5 mg) and 2'-acetyl-7-methanesulfonyltaxol were added to a 5

mL round bottomed flask along with a stirring bar. The reagents were then dissolved by the addition of 1.0 mL of CH_2Cl_2. At room temperature no reaction was seen using tlc within 7 hours. The reaction was left to stand for several days (66 hours). At the end of this time the reaction was again checked by tlc, which showed only starting material present.

7.0 WATER SOLUBLE TAXOLS

7.1 INTRODUCTION

The work to be presented in this chapter addressed the synthesis of biologically active water-soluble taxols. One of the major obstacles that taxol faces in becoming a routinely clinically used drug is its lack of water solubility. The production of an active water soluble taxol derivative would generate two major benefits. The first of these is that taxol could be given in a water based solution. Taxol itself must be given in conjuction with emulsifying agents and this is not the vehicle of choice for clinical administration of a drug. The second benefit is that once the drug was present *in vivo* it could be more easily transported throughout the body.

The methods which were used to prepare water soluble taxols involved the addition of polar groups to the C-2' or C-7 positions. These polar groups included: an amino acid, β-alanine; a sugar, α-D-glucose; and a dicarboxylic acid, succinic acid.

7.2 RESULTS AND DISCUSSION

7.2.1 2'-β-ALANYLTAXOL

7.2.1.1 SYNTHESIS

Preparation of 2'-β-alanyltaxol was achieved via coupling of taxol with the N- protected N-carbobenzoxy(CBZ)-β-alanine followed by deprotection of the amine. Reaction of taxol with N-CBZ-β-alanine using dicyclohexylcarbodiimide (DCC) as the coupling agent[71] produced 2'-N-CBZ-β-alanyltaxol **49** in 88% yield after isolation via preparative tlc (silica gel, 1/1, EtOAc/Hexane) or flash chromatography[72] (silica gel, 3/7, EtOAc/CH_2Cl_2), (Scheme 46). An hplc chromatogram of the crude reaction mixture is shown in Figure 28. The reaction, monitored by tlc, was 90% complete in 15 hours but extended reaction times were also employed to allow the reaction to proceed close to completion. Extended reaction times did not cause any C-2', C-7 disubstituted product to form.

The side product N(N'-CBZ-β-alanyl)dicyclohexylurea **88** has been commonly seen in ester-producing reactions using DCC and β-alanine because the activated acid intermediate **89** produced in the reaction can be attacked by two species.[73] Attack by the alcohol yields the desired ester but attack by another molecule of acid yields an anhydride which can be attacked by dicyclohexylurea to yield the side product.

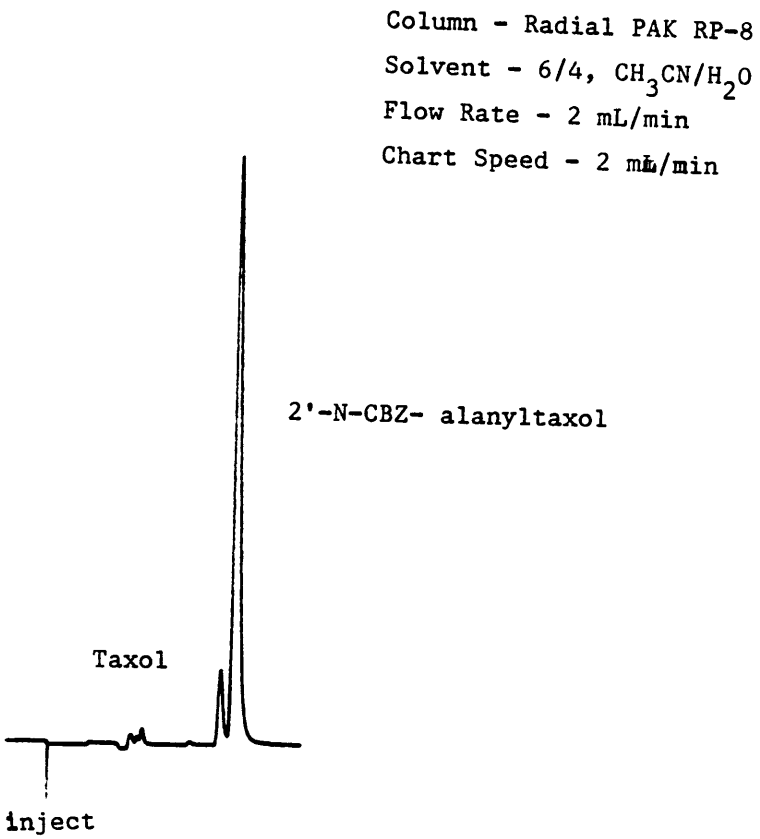
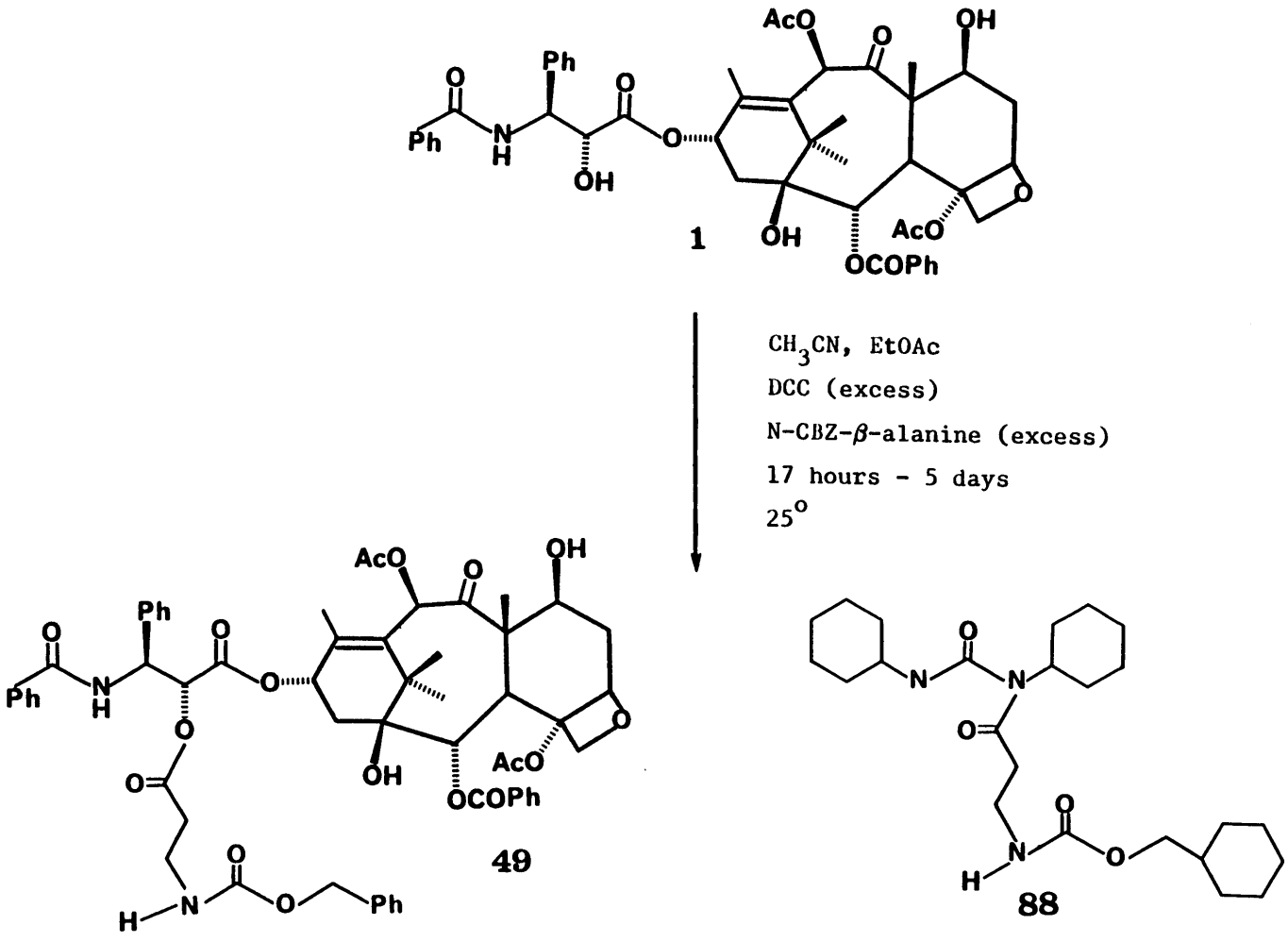
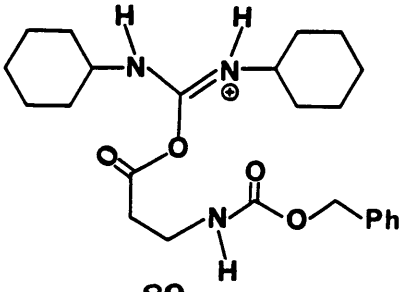


Figure 28. HPLC Chromatogram of Crude 2'-N-CBZ-β-alanyltaxol



Scheme 46. Preparation of 2'-N-CBZ-β-alanyltaxol

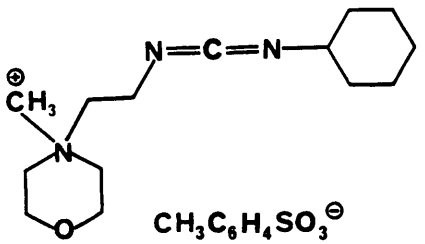
Minimization of the side reaction was achieved by using CH_3CN or EtOAc as solvents, in which N-CBZ-β-alanine is only sparingly soluble.[74,75]



89

It was best to use an excess of DCC and the protected β-alanine for two reasons. The first reason was that the side reaction consumed some of the reagents. The second reason was that reverse phase hplc would separate taxol from 2'-N-CBZ-β-alanyltaxol but would not separate 2'-N-CBZ-β-alanyltaxol and N(N-CBZ-β-alanyl)dicyclohexylurea. Silica gel chromatography would separate 2'-N-CBZ-β-alanyltaxol from the side product but would not separate taxol well from 2'-N-CBZ-β-alanyltaxol. Excess reagents therefore were used to drive the reaction to completion so that silica gel chromatography could be used as almost no taxol was present. The excess reagents present at the end of the reaction did not present a problem. The excess DCC was decomposed to dicyclohexylurea by the addition of water, and most of the dicyclohexylurea and N-CBZ-β-alanine were removed by filtration.

The product 2'-N-CBZ-β-alanyltaxol could be purified much more readily if the side product could be removed without chromatography. In an attempt to accomplish this, taxol was reacted with N-CBZ-β-alanine using the coupling reagent 1-cyclohexyl-3-(2-morphilinoethyl)-carbodiimide-metho-p-toluenesulfonate as the coupling agent.[76] Any side products from this reaction would have been water soluble and could have been removed by washing. The coupling reagent used is less active than DCC and had only been reported in the preparation of amides. In the case of taxol no trace of 2'-N-CBZ-β-alanyltaxol could be seen by hplc even after seven days at room temperature.



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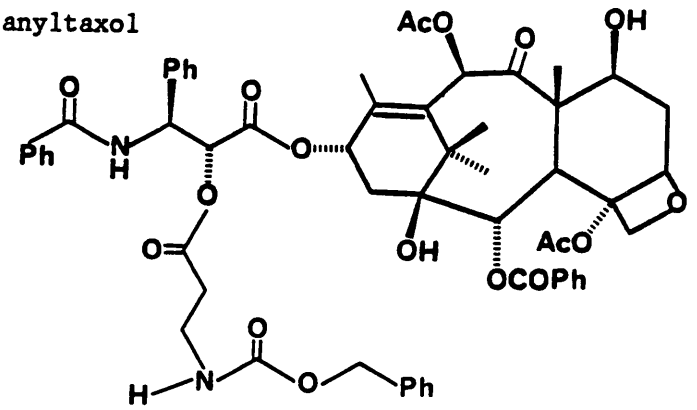
Characterization data for 2'-N-CBZ-β-alanyltaxol are shown in Table 45. The only major change for the taxol derived protons in the ^{1}H-nmr spectrum when contrasted with the ^{1}H-nmr spectrum of taxol was that the signal for C-2', 4.73 ppm (d, J=3) in taxol, was shifted downfield to 5.46 ppm (d, J=3). This downfield shift is consistent with acylation of the C-2' hydroxyl group. The signal for the C-7 proton of **49**, 4.43 ppm (d, J=6,10) was essentially unchanged when com-

pared with the analogous taxol signals 4.38 ppm (dd, J=4,10). The C-7 signal for **49** showed that no reaction had taken place at that position. Integration of the aromatic region of the ^{1}H-nmr spectrum clearly showed the presence of 20 aromatic protons. The other signals for the N-CBZ-β-alanyl protons did not overlap with the signals from the taxol derived protons and could be assigned without difficulty. The mass spectrum for **49** indicated a molecular weight of 1058 by the presence of peaks at m/z 1081 $(MNa)^+$, and 1059 $(MH)^+$. Peaks in the mass spectrum resulting from loss of the side chain at m/z 569 $(MH-RCOOH)^+$, and 509 $(MH-RCOOH-HOAc)^+$ showed that addition of the protected amino acid took place on the side chain.

Preparation of a second protected β-alanyl taxol derivative 2'-N-tertiarybutyloxycarbonyl-β-alanyltaxol (2'-N-tBOC-β-alanyltaxol) **91** was achieved in the same manner as the CBZ protected derivative. The reaction proceeded in only 50% isolated yield with the remainder of material being unreacted taxol; this occurred even with excess DCC and N-tBOC-β-alanine. The characterization data for 2'-N-tBOC-β-alanyltaxol are shown in Table 46. The proton spectrum of **91** is similar to 2'-N-CBZ-β-alanyltaxol with the exception that instead of benzyl proton signals the signals for the tertiary butyl proton are seen at 1.36 ppm (6H) and 1.40 ppm s (3H). Two unusual pieces of data in the proton spectrum are the downfield shift of the C-3' amide proton so

Table 45 (Part 1 of 2). Characterization Data for 2'-N-CBZ-β-Alanyltaxol



Position	Shift (ppm from TMS) Coupling (hertz)
2	5.47 (d, 7)
3	3.79 (d, 7)
5	5.66 (dd, 1,8)
6	2.2 (m)
7	4.43 (dd, 6,10)
10	6.28 (s)
13	6.24 (br t, 9)
14	2.4 (m)
16	1.19 (s)
17	1.10 (s)
18	1.55 (s)
19	1.91 (s)
20	4.18 (d, 8), 4.29 (d, 8)

Mass Spectral Data

1081 $(MNa)^+$, 1059 $(MH)^+$,

999 $(MH-HOAc)^+$,

569 $(MH-RCOOH)^+$,

509 $(MH-RCOOH-HOAc)^+$

Infrared Spectral Data

1760 s, 1725 s,

1745 s, 1680 m,

1545 m, 1485 w,

1395 w, 1265 s,

1285 m, 1200 w,

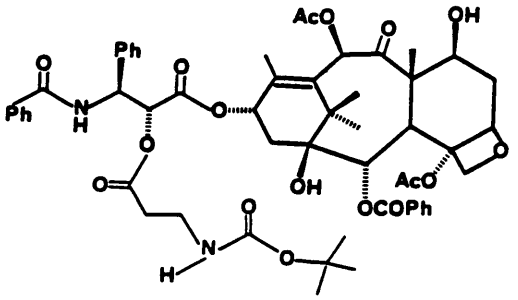
1090 m, 1070 w.

Table 45 (Part 2 of 2). Characterization Data for 2'-N-CBZ-β-Alanyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2'	5.46 (d, 3)
3'	6.00 (dd, 3,9)
N-H	7.4 (d, 9)
OAcs	2.18 (s), 2.44 (s)
2 OBz	8.17 (d, 7), 7.4 (m)
3' NBz	7.79 (d, 7), 7.4 (m)
N-CBZ-β-Alanine	
2	3.45 (m), 3.55 (m)
3	2.6 (m)
NH	5.17 (br t, 6)
Bz	4.87 (s)
Ph	7.4 (m)

Table 46. Characterization Data for 2'-N-tBOC-β-Alanyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.65 (d, 7)
3	3.77 (d, 7)
5	4.95 (br d, 9)
6	2.4 (m)
7	4.11 (dd, 7,11)
10	6.26 (s)
13	6.20 (br t, 9)
14	2.2 (m)
16	1.19 (s)
17	1.10 (s)
18	1.65 (s)
19	1.90 (s)
20	4.17 (d, 8), 4 29 (d, 8)
2'	5.42 (d, 4)
3'	5.93 (dd, 4,10)
N-H	7.4 (m)
OAcs	2.18 (s), 2.40 (br s)
3' NBz	7.68 (d m, 7.5), 7.4 (m)
2 OBz	8.12 (d,m, 7.5) 7.4 (m)
3' Ph	7.4 (m)



Mass Spectral Data

1063 $(MK)^+$, 1047 $(MNa)^+$,

1025 $(MNa)^+$,

925 $(MH-CO_2-CH_2C(CH_3)_2)^+$,

907 $(MH-CO_2-CH_2C(CH_3)_2-H_2O)^+$,

969 $(MH-CH_2C(CH_3)_2)^+$.

Infrared Spectral Data

1260 s-sh, 1245 s,

1675 m, 1500 w,

1460 w, 1390 m,

1260 s.

that it is hidden under the aromatic peaks and the large 4 Hz coupling constant between the C-2' and C-3' protons. Both of these changes are due to hydrogen bonding; this will be discussed in more detail for the 2'-β-alanyltaxol.

Deprotection of 2'-N-CBZ-β-alanyltaxol to yield 2'-β-alanyltaxolformate **92** was effected using 5% Pd/C or 5% Pd/Al_2O_3 as catalyst and formic acid as hydrogen source.[78] The catalysts were obtained from Engelhard; when 5% Pd/C from Aldrich was used the reaction often did not work. Formic acid was was used as hydrogen source for two reasons; it is a much more active form of hydrogen for removal of CBZ protecting groups, and the reaction yields the β-alanine derivative as a salt, which is more water soluble than the neutral form. Reaction of **49** with the hydrogen donor solvent cyclohexene with palladium as catalyst produced no reaction. Hydrogenation of **49** under a hydrogen atmosphere with methanol as solvent and palladium as catalyst converted **49** into taxol. The stability of 2'-β-alanyltaxol in methanol will be discussed later.

The characterization data for 2'-β-alanyltaxol formate is shown in Table 47. The difference in the ^{1}H-nmr spectrum of 2'-β-alanyltaxol when contrasted with the N-protected derivative are due to the absence of the protecting group. The carbobenzoxy signals are not seen in the spectrum of **92** and a broad peak between 5.0 and 5.5 ppm was present corresponding to the amine peaks and also any excess formic acid and/or

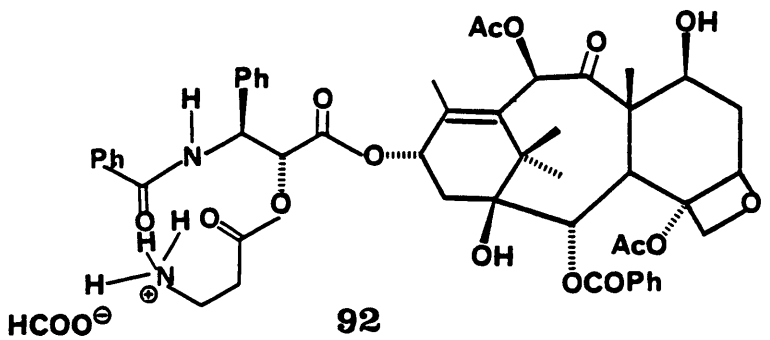


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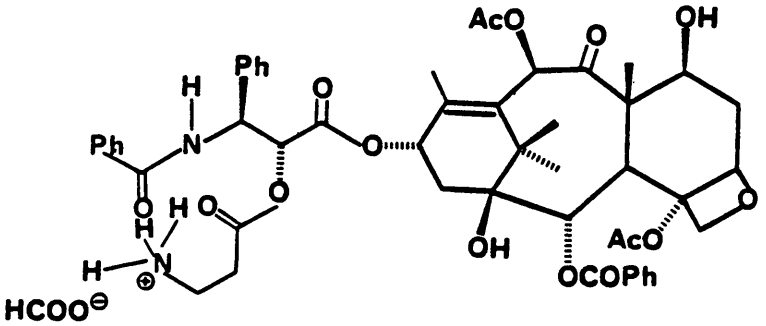
Table 47 (Part 1 of 2). Characterization Data for 2'-β-Alanyltaxol-formate



Position	Shift (ppm from TMS) Coupling (hertz)
2	5.62 (d, 7)
3	3.71 (d, 7)
5	4.91 (br d, 9)
6	2.4 (m) , 1.8 (m)
7	4.36 (dd , 7.10)
10	6.27 (s)
13	6.06 (br t , 9)
14	1.8 (m), 2.1 (m)
16	1.16 (s)
17	1.09 (br s)
18	1.84 (br s)
19	1.62 (s)
20	4.14 (d, 8), 4.27 (d, 8)
2'	5.61 (d, 5)

Mass Spectral Data

925 $(MH-HCO_2H)^+$,

509 $(MH-RCOOH-HOAc-H_2O)^+$

Addition of D_2O caused N-H to collapse to a singlet, C-3' to a doublet. All HCOOH, NH and H_2O peaks disappear (including 8.08 s), i.e., adding D_2O results in the free amine being present in $CDCl_3$.

Table 47 (Part 2 of 2). Characterization Data for 2'-β-Alanyltaxol-formate

Position	Shift (ppm from TMS) Coupling (hertz)
3'	5.91 (dd , 5,8)
N-H	8.28 (d , 8)
OAcs	C-4 2.39 (s), C-10 2.16 (s)
2 OBz	8.09 (d m, 7.5), 9.4 (m)
3' NBz	7.76 (d m, 8), 7.4 (m)
3' Ph	7.4 (m)
β-Alanine	
2	4.15-4.35 (m)
3	2.70-2.95 (m), 5.0-5.5
HCOOH	8.08 (s)

water. The proton spectrum shows that the amine functionality is strongly hydrogen bonded to the C-3' amide. The coupling constant of 5 Hz between C-2' and C-3' is unusual and can be explained by the amine and the C-3' amine forming a ring which would change the dihedral angle between C-2' and C-3'. The chemical shift of the C-3' amide proton signal at 8.28 ppm (d, J=8) contrasted with that of taxol at 7.01 (d, J=9) also shows that the amide is hydrogen bonded. A similar change in chemical shift of the C-3' amide proton signal was seen when a spectrum of 2'-acetyltaxol was obtained with C_5D_5N as solvent or when a proton spectrum of the reduced side chain of taxol (Chapter 3) was obtained with d_6-DMSO as solvent. Except for some excess formic acid and water no impurity peaks were seen in the proton spectrum of **92**.

Deprotection of 2'-N-tBOC-β-alanyltaxol was achieved via reaction of **91** with a solution of 5/6/1, EtOH/EtOAc/conc. HCl at room temperature for 4 hours. During the course of the reaction removal of the tBOC groups was not the only process taking place; when all of starting material had been consumed most of the 2'-β-alanyltaxol produced had decomposed into a complex mixture of products and the remaining product could not be isolated. The tBOC protecting group therefore was not the protecting group of choice for preparing 2'-β-alanyltaxol.

7.2.1.2 WATER SOLUBILITY OF 2'-β-ALANYLTAXOL

In order to determine if 2'-β-alanyltaxol is a suitable drug for clinical use its aqueous solubility was measured. An excess of the taxol derivative was stirred with water for 45 minutes and then filtered through a 0.45 μm pore size centrifugal filter. The saturated solution filtered with great difficulty; only approximately 50 μL of saturated solution was obtained before the filter was completely plugged. A sample of taxol was treated in the same manner; the saturated taxol sample filtered with no difficulty. The amounts of derivatives in the saturated solutions were determined by obtaining hplc chromatograms of the samples and measuring the peak areas, and comparing these with the areas of standard samples of taxol.

HPLC chromatograms of the saturated taxol and 2'-β-alanyltaxol solutions are shown in Figure 29. The chromatogram for 2'-β-alanyltaxol shows that the derivative has a longer retention time than taxol on a reverse phase column. The reason for this was that the amine group bonded to the small amount of unsilated silica sites present on most reverse phase hplc columns. This also caused the 2'-β-alanyltaxol peak to be broad and its retention time to change with differing amounts of sample injected. Quantitation of the peak areas from the two chromatograms showed that

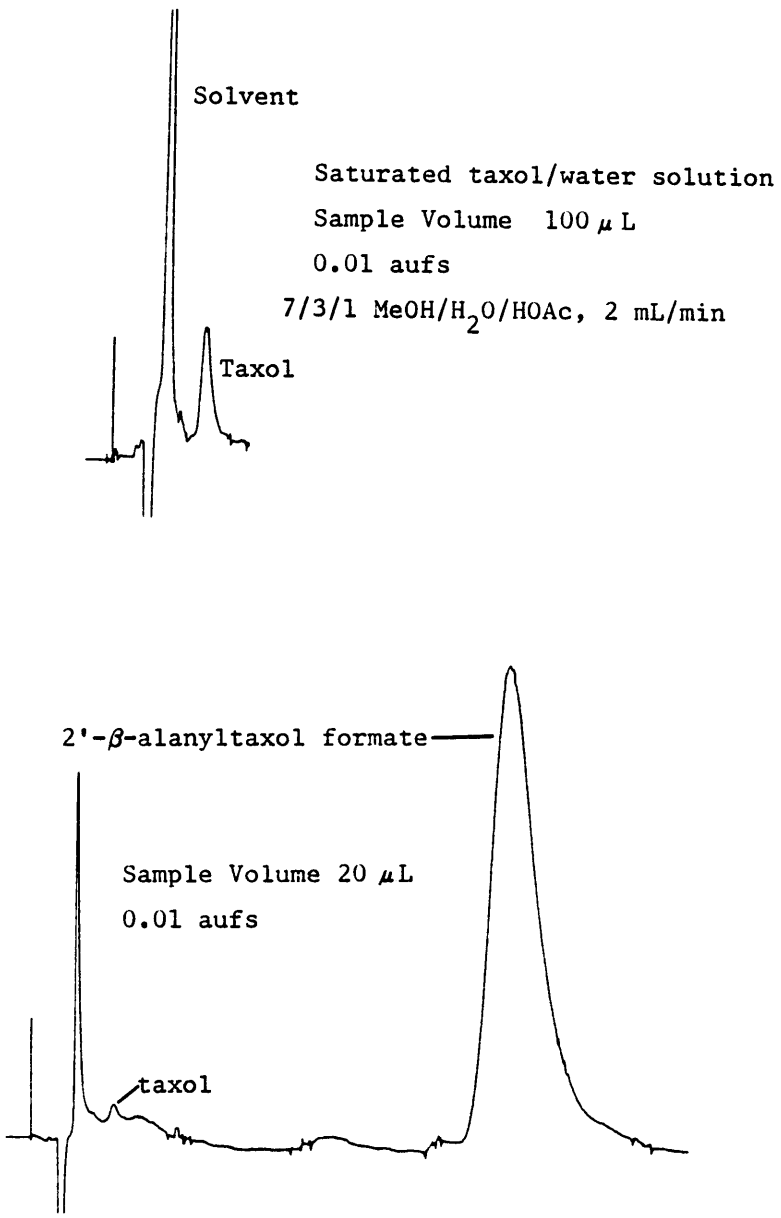


Figure 29. Comparison of the Water Solubility of Taxol and 2'-β-alanyltaxol formate

2'-β-alanyltaxol is 88 times more water soluble than taxol when filtered through a 0.45 μm pore size filter.

Quantitation of the amount of 2'-β-alanyltaxol present in the saturated aqueous solution was achieved by preparing a standard sample of 1.72 mg 2'-β-alanyltaxol formate in methanol and injecting the sample onto an hplc column. The combined peak areas for taxol and 2'-β-alanyltaxol (MeOH caused taxol to form) were used to determine the concentration of β-alanyltaxol in the aqueous solution. The solubility of 2'-β-alanyltaxol in the aqueous solution was 2.2 mg/mL.

In order to obtain a more water soluble drug which would cause less irritation than a formate salt, the National Cancer Institute requested that 2'-β-alanyltaxol hydrochloride (**93**) be prepared. This derivative could not be prepared by hydrogenation of the protected derivative in the presence of HCl because deprotection of the N-t-BOC derivative had shown that 2'-β-alanyltaxol is not stable in the presence of HCl. In order to overcome this difficulty the reaction conditions to produce the hydrochloride had to be such that the β-alanyl derivative was exposed to HCl for as brief a period as possible.

The hydrochloride of 2'-β-alanyltaxol was obtained by first synthesizing 2'-β-alanyltaxol formate as previously described. The 2'-β-alanyltaxol was actually a mixture of taxol and the β-alanyl derivative because an insufficient amount of formic acid was present in the hydrogenation step.

The β-alanyltaxol was purified and converted to the hydrochloride by filtering through a flash chromatography column. The taxol and 2'-β-alanyltaxol sample were dissolved in EtOAc* and filtered through a silica gel column with ethyl acetate as solvent. The Rf of taxol with ethyl acetate is close to 1.0 while the Rf of 2'-β-alanyltaxol is 0.0. After the taxol had been removed several equivalents of HCl in EtOAc were added to the column and the β-alanyltaxol HCl was removed by using 1/1 CH_2Cl_2/MeOH as solvent. The Rf of 2'-β-alanyltaxol with this solvent is nearly 1.0 while the excess HCl would not be removed from the column. The 2'-β-alanyltaxol HCl obtained by this method was homogenous on tlc (1/7, MeOH/CH_2Cl_2); the analytical tlc plate had to be greatly overloaded before even a trace of any peak other than the product could be seen. This method of preparing 2'-β-alanyltaxol HCl ensured that the taxol derivative was in the presence of excess HCl for no more than 15 to 30 seconds.

The solubility of 2'-β-alanyltaxol HCl was determined in the same manner as for the formate salt with a few differences. A saturated aqueous sample filtered through a 0.45 μm pore size filter was prepared along with a saturated aqueous sample filtered through Whatman No. 1 filter paper;

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* Several hundred mL of EtOAc were required to dissolve 1 g of the mixture.

a saturated solution in 9/1, H_2O/EtOH* filtered through a 0.45 μm pore size filter was also prepared. A standard solution of 2'-β-alanyl HCl in methanol was also prepared; unlike the formate salt the hydrochloride did not decompose to taxol in methanol solution. Quantitation, by peak area on hplc, of the concentrations of **93** in each solution gave the following results:

1. Aqueous solution, 0.45 μm pore size filter - 2.9 mg/mL

2. Aqueous solution, Whatman No. 1 filter paper - 11.2 mg/mL

3. 9/1, EtOH/H_2O solution, 0.45 μm filter - 3.1 mg/mL

The difference in the amount of 2'-β-alanyltaxol present in solution when the sample was filtered through a 0.45 μm pore size filter and Whatman No. 1 filter paper is indicative of the manner in which 2'-β-alanyltaxol behaves in water - the compound forms a fine suspension rather than dissolving as individual molecules. When the aqueous solutions were filtered micelles smaller than the pore size of the filter passed through. This type of behavior resulted in the large

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* This solvent solution can be used for clinical administration of a drug.

difference in solubility being seen with changing filter pore size.

The difference in solubility seen with changing filter size was not the only data that indicated that 2'-β-alanyltaxol formed a suspension in an aqueous medium. When excess 2'-β-alanyltaxol was added to a stirred aqueous solution at no time could discrete particles be seen. As 'excess' 2'-β-alanyltaxol was added the suspension became slightly cloudy and viscous. Continuing addition eventually caused the aqueous suspension to become extremely viscous so that it was similar to gelatin in consistency. The second piece of evidence showing a driving force for formation of a suspension was obtained during proton nmr analysis of 2'-β-alanyltaxol formate. The salt was dissolved in $CDCl_3$, readily filtered through a 0.45 μm pore size filter and a proton spectrum was obtained. Deuterated water was then added, the nmr tube shaken and a second spectrum obtained a few minutes later. The second spectrum showed the absence of the amine protons and also showed the absence of the formyl protons; the formic acid had been extracted into the aqueous layer and the free amine was left in organic solution. Upon standing overnight the 2'-β-alanyltaxol was extracted out of the organic solution and formed a viscous white suspension in the aqueous layer.

The behavior of 2'-β-alanyltaxol formate in water can, in simple terms, be readily explained. Addition of β-alanine

to taxol increases the water solubility of the compound by adding a polar group, but the amine is only one polar site in a large structure. The taxane skeleton is no more hydrophilic in 2'-β-alanyltaxol than it is in taxol. In aqueous solution therefore the 2'-βalanyltaxol molecules cluster together with the taxane skeletons pointing inward forming a hydrophobic environment and the β-alanyl portion pointing outward towards the aqueous environment. This type of behavior would be expected of any water-soluble taxol derivative if that derivative was functionalized by a polar group at only one position.

The formation of a suspension of 2'-β-alanyltaxol in water is not a hindrance to clinical administration of the drug, since drugs that are not water soluble can be given as suspensions with emulsifying agents. It is expected that 2'-β-alanyltaxol will be superior to taxol in that it could be administered with less emulsifying agents than taxol.

7.2.1.3 STABILITY OF 2'-β-ALANYLTAXOL

In order to accurately evaluate the biological tests results for 2'-β-alanyltaxol formate and to assess the possibility of using the taxol derivative as a 'prodrug' the stability of the compound in diffent solvent systems was determined.

The taxol derivative was found to quickly decompose to taxol in neutral methanol solution. A sample of 2'-N-CBZ-β-alanyltaxol hydrogenated in methanol solution with a hydrogen atmosphere produced only taxol after three hours. A sample of 2'-N-CBZ-β-alanyltaxol formate dissolved in methanol decomposed to taxol within two hours at room temperature. Hydrogenation of 2'-N-CBZ-β-alanyltaxol in methanol with less than one equivalent of formic acid caused a mixture of unreacted starting material and taxol to be produced.

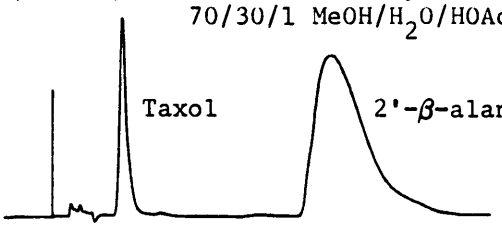
In aqueous solution 2'-β-alanyltaxol was relatively stable. An excess of 2'-β-alanyltaxol was stirred with water (pH 5.4) and filtered through a cotton plug. The saturated solution was analyzed by hplc which showed that only approximately 10% decomposition to taxol had taken place after 24 hours.

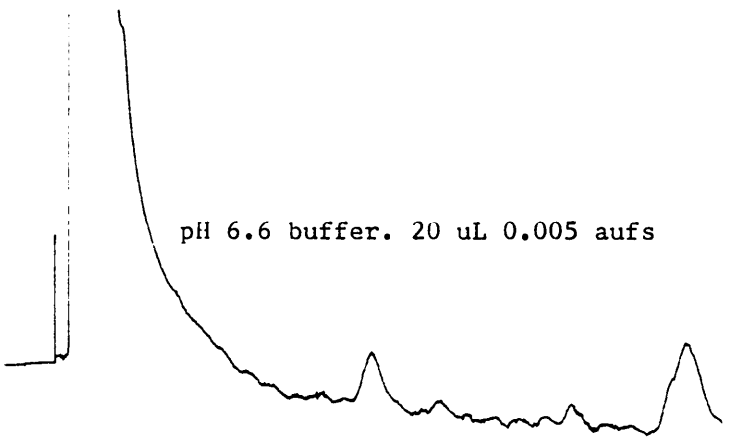
The stability of 2'-β-alanyltaxol at 37° in a pH 6.6 a buffer used in one of the <u>in vitro</u> tests for taxol derivatives was also determined. A mixture of taxol and 2'-β-alanyltaxol was incubated in the biological buffer and the stability of the 2'-β-alanyltaxol was monitored by hplc (Figure 30). Over the course of 20 hours all of the 2'-β-alanyltaxol decomposed into taxol.

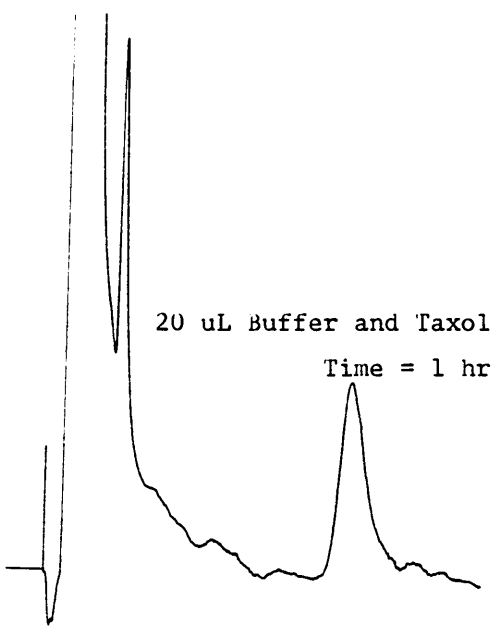
The facile decompositon of 2'-β-alanyltaxol to taxol via nucleophilic attack on the ester linkage is unusual reactivity, ester linkages are not normally cleaved in neutral

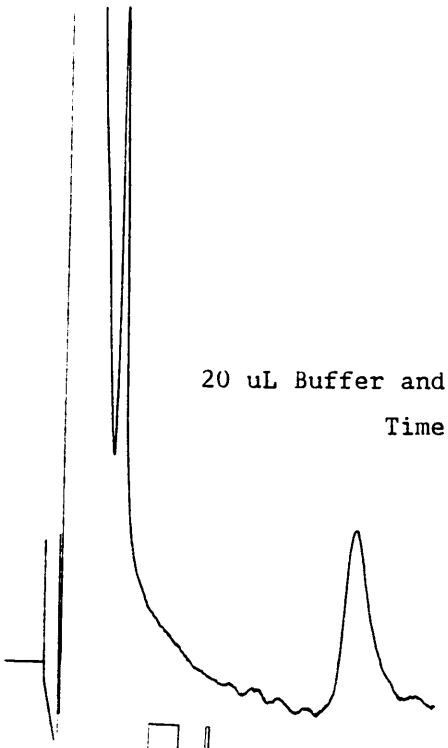
methanol solution or in pH 6.6 aqueous solution at 37°. The key to the reactivity of 2'-β-alanyltaxol is the cyclic hydrogen bonded form that the compound exists in the conformation on page 297. The amino group of the β-alanine is bent around to hydrogen bond to the C-3' amide and to the ester linkage bonding the β-alanine to the side chain. This hydrogen bonding activates the ester linkage with respect to nucleophilic attack.

Starting Mixture for the Study of the Stability of
2'-β-alanyltaxol formate at pH 6.6 in Aqeous Solution
70/30/1 MeOH/H_2O/HOAc, 2 ml/min 0.5 cm/min

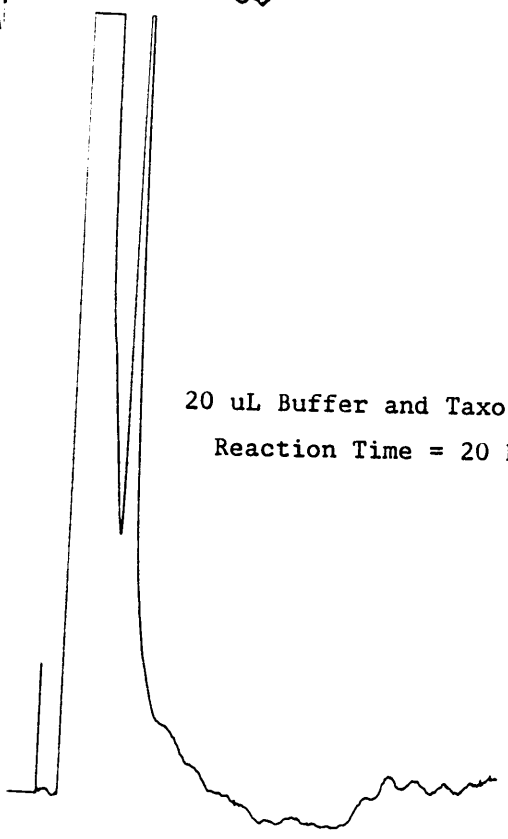








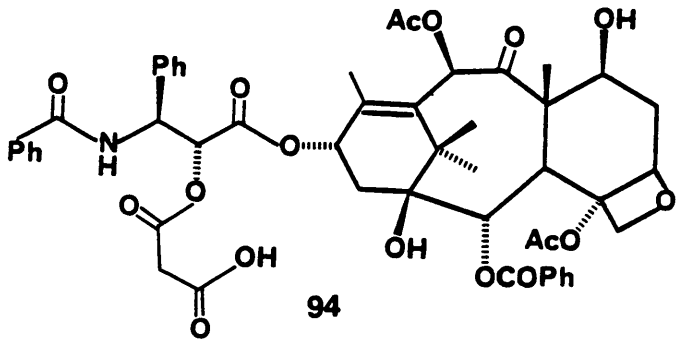
20 uL Buffer and Taxols, 0.005 aufs
Time = 2 hrs 45 min



20 uL Buffer and Taxols
Reaction Time = 20 hrs

7.2.2 2'-SUCCINYLTAXOL

Reaction of taxol with succinic anhydride yielded 2'-succinyltaxol in 96% yield after a reaction time of 2 hours at room temperature. After simple work up the product was shown to be homogenous on tlc and ^{1}H nmr showed no other products present. No trace of taxol or a disubstituted taxol was present; extended reaction times or elevated temperatures did not cause any C-7 substituted product to form.



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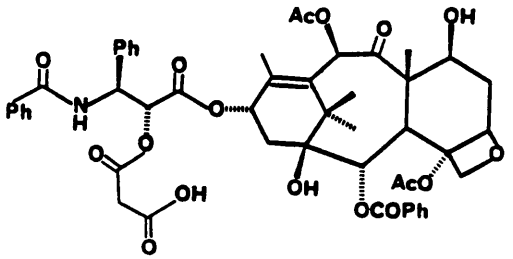
The characterization data for 2'-succinyltaxol is shown in Table 48. Contrasted with taxol the major change seen in the ^{1}H-nmr spectrum of 2'-succinyltaxol is the downfield shift of the C-2' proton signal to 5.51 ppm (d, J=3). The succinyl protons are seen as multiplets centered at 2.6 ppm. The 2 Hz coupling constant between the C-2' and C-3' protons and the chemical shift of the C-3' amide proton at 7.07 (d, J=9) show that the succinyl substituent is not hydrogen bonded to the C-3' amide as was the case with 2'-β-alanyltaxol.

Table 4.8 Characterization Data for 2'-Succinyltaxol

Position	Shift (ppm from TMS) Coupling (hertz)
2	5.67 (d, 7)
3	3.78 (d, 7)
5	4.96 (br d, 9)
6	2.2 (m)
7	4.48 (dd, 6,11)
10	6.27 (s)
13	6.21 (br t, 8)
14	2.2 (m)
16	1.20 (s)
17	1.11 (s)
18	1.90 (s)
19	1.69 (s)
20	4.17 (d, 8), 4.30 (d, 8)
2'	5.51 (d, 3)
3'	5.97 (dd, 3,9)
N-H	7.07 (d, 9)
OAcs	2.20 (s), 2.43 (s)
2 OBz	8.10 (d,m, 8), 7.4 (m)



Mass Spectral Data

976 $(MNa)^+$, 954 $(MH)^+$,

569 $(MH-RCOOH)^+$,

551 $((MH-RCOOH-H_2O)^+$,

509 $(MH-HOAc)^+$,

386 $(RCOOHH)^+$,

368 $(RCOOHH-H_2O)^+$

Infrared Spectral Data

1240 s, 1675 m,

1535 w, 1390 m,

1265 s, 1185 m,

1095 w, 1065 w.

Position	Shift (ppm from TMS) Coupling (hertz)
3' NBz	7.73 (d,m, 8), 7.4 (m)
Succinyl	2.6 (m)

The water solubility of 2'-succinyltaxol was determined in the same manner as that of 2'-β-alanyltaxol. Saturation of water with 2'-succinyltaxol did not cause a suspension to form; the excess 2'-succinyltaxol precipitated as discrete particles. The saturated solution was filtered through a 0.45 μm pore size filter with no difficulty. The solubility of 2'-succinyltaxol in water was determined to be 1.3 mg/mL.

7.2.3 GLUCOSYL TAXOL

7.2.3.1 PREPARATION OF PROTECTED GLUCOSYLTAXOLS

It was thought that the preparation of an active water soluble taxol derivative could be achieved via addition of a sugar to the hydroxyls of taxol. This was supported by the isolation of active 7-xylosyltaxols by Senilh et. al.[13] The condensation of sugar halides with alcohols , the Koenigs-Knorr reaction, is a widely used reaction in sugar chemistry, so that new methodology did not have to be developed before preparation of a taxol-sugar derivative could be attempted.[78, 79, 80]

In order to test the feasibility of preparing an ether linked taxol-sugar derivative the commercially available 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide **95** and siver salicylate were chosen as reagents. The galactosyl bromide had been shown to form ether linkages with tertiar-

y-butanol at room temperature with silver salicylate as catalyst.[81]

2'-Acetyltaxol was reacted with silver salicylate and 95 in CH_2Cl_2 at room temperature for 48 hours; several additional equivalents of the sugar and silver compounds were added over the course of the reaction. Monitoring of the reaction by tlc (3/2, EtOAc/Hexane) showed a spot developing which was bright blue when seen with a 254 nm light, in addition to the dark starting material spot. After work up the products were isolated by preparative tlc (3/2, EtOAc/Hexane). Two products were obtained with Rf 0.73 and 0.46. The product with Rf 0.46 was the starting material. Proton nmr analysis of the compound with Rf 0.73 clearly showed that it was not a taxol-like compound. No aromatic protons were present in the proton spectrum and the major peaks that were present were numerous singlets near 2.0 ppm corresponding to the sugar acetates. This product resulted from an intramolecular or intermolecular reaction of the galactyl bromide and did not involve 2'-acetyltaxol. Further experimentation to produce an ether linked taxol sugar derivative was not carried out as the galactosyl bromide could not withstand harsher reaction conditions without decomposing.

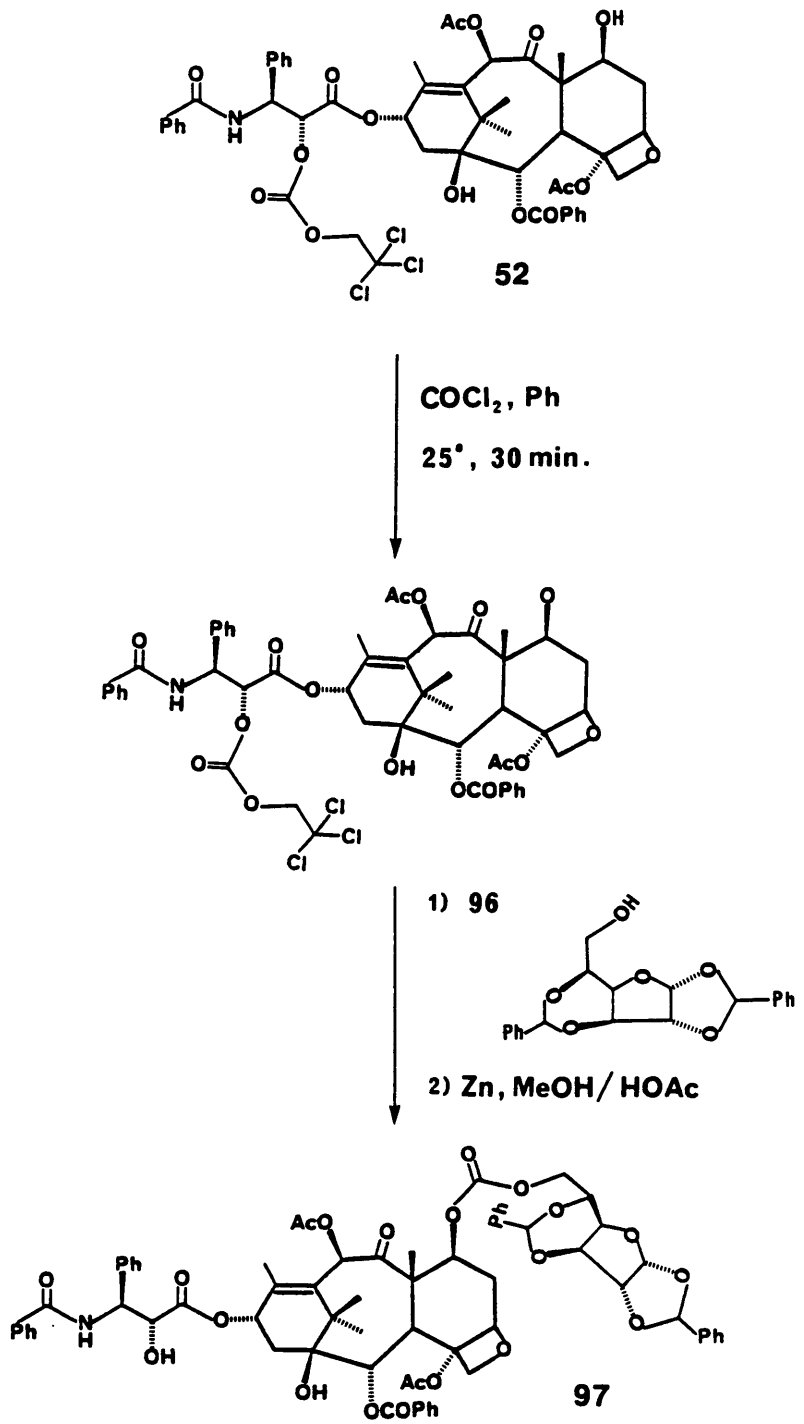
The difficulty with the steric crowding at the C-7 position was overcome by first reacting the C-7 hydroxyl group of taxol with phosgene to form a chloroformyl taxol deriva-

tive. This 7-chloroformyltaxol was then reacted with a protected sugar to form a carbonate linked sugar derivative. This strategy had the advantage that in the step in which large groups are reacting the site of reaction was two bonds removed from the crowded C-7 position rather than one bond.

Following this methodology 7-(6-O-carbonyl-(1,2,3,5-di-O-benzylidene)-α-D-glucosyl)taxol (**97**) was prepared as shown in Scheme 47. The product **97** was obtained in only 40% yield. The low yield was due to incomplete formation of the chloroformate in the first step of the reaction and modification of experimental technique could greatly increase the yield. After work up the product was isolated using flash chromatography.

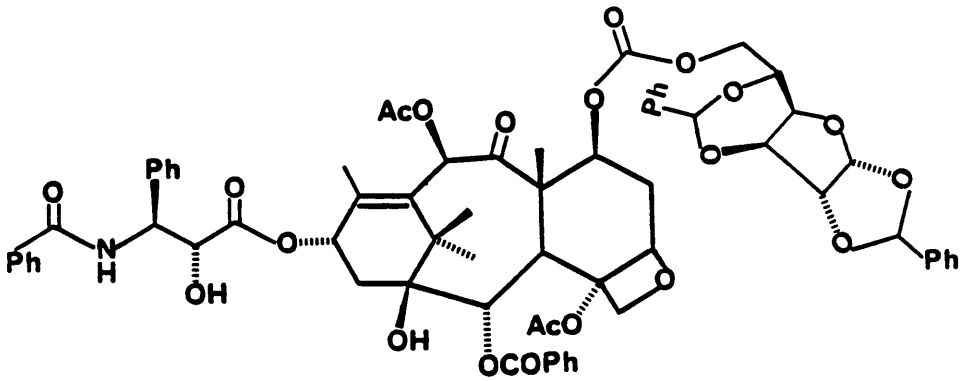
The characterization data for **97** are shown in Table 47. The proton spectra for the carbonate linked protected sugar taxols are the most complex spectra in this project, but peak assignment was straightforward. The peaks for the taxol derived protons have essentially the same chemical shifts and peaks shape as they do in the spectrum of taxol. The one differing peak is that for the carbonate-linked C-7 position; the signal for the C-7 proton of **97** is seen at 5.50 ppm (dd, J=7,10). This shift is nearly identical to that of the C-7 proton signal for 2',7-diacetyltaxol, 5.53 ppm (dd, J=7,10).

Most of the peaks for the sugar derived protons appear as closely grouped multiplets and could not be individually assigned, either by chemical shift arguments or selective pro-



Scheme 47. Preparation of a 7-Glucosyltaxol

Table 49 (Part 1 of 2). Characterization Data for **97**



Position	Shift (ppm from TMS) Coupling (hertz)
2	5.68 (d, 7)
3	3.91 (d, 7)
5	4.91 (br d, 9)
6	1.8 (m)
7	5.50 (dd, 7,10)
10	6.27 (s)
13	6.20 (br t, 9)
14	2.6 (m)
16	1.20 (s)
17	1.16 (br s)
18	1.84 (br s)
19	1.82 (s)
20	4.17 (d, 8), 4.21 (d, 8)
2'	4.74 (d, 2)

Mass Spectral Data

1236 $(MH)^+$,

1130 $(MH-PhCHO)^+$,

1070 $(MH-HOAc-PhCHO)^+$,

951 $(MH-RCOOH)^+$,

891 $(MH-RCOOH-HOAc)^+$,

836 $(MH-ROCO_2H)^+$,

785 $(MH-RCOOH-PhCHO)^+$,

RCOOH-side chain as as acid

$R'OCO_2H$-glucosyl moiety as a carbonic acid

High Resolution

(MH) $C_{54}H_{61}NO_{21}$ - 1237.4268

Calculated - 1237.4475

Table 49 (Part 2 of 2). Characterization Data for **97**

Position	Shift (ppm from TMS) Coupling (hertz)
3'	5.79 (dd, 2,9)
N-H	7.05 (d, 9)
OAcs	1.99 (s), 2.38 (s)
2 OBz	8.10 (d,m, 8), 7.4 (m)
3' NBz	7.74 (d,m, 8), 7.4 (m)
3' Ph	7.4 (m)
Glucosyl	6.22 (d, 4), (C-1), 6.14 (s), (Bz), 5.82 (s), (Bz), 4.80 (m), (2H), 4.5-4.7 (m), (3H), 4.15 (d, 2)

Infrared Spectral Data

1245 s, 1240 s-sh,

1675 m, 1535 w,

1505 w, 1475 w,

1420 m, 1395 m,

1280 s, 1240 m-sh,

1100 m s, 1000 m,

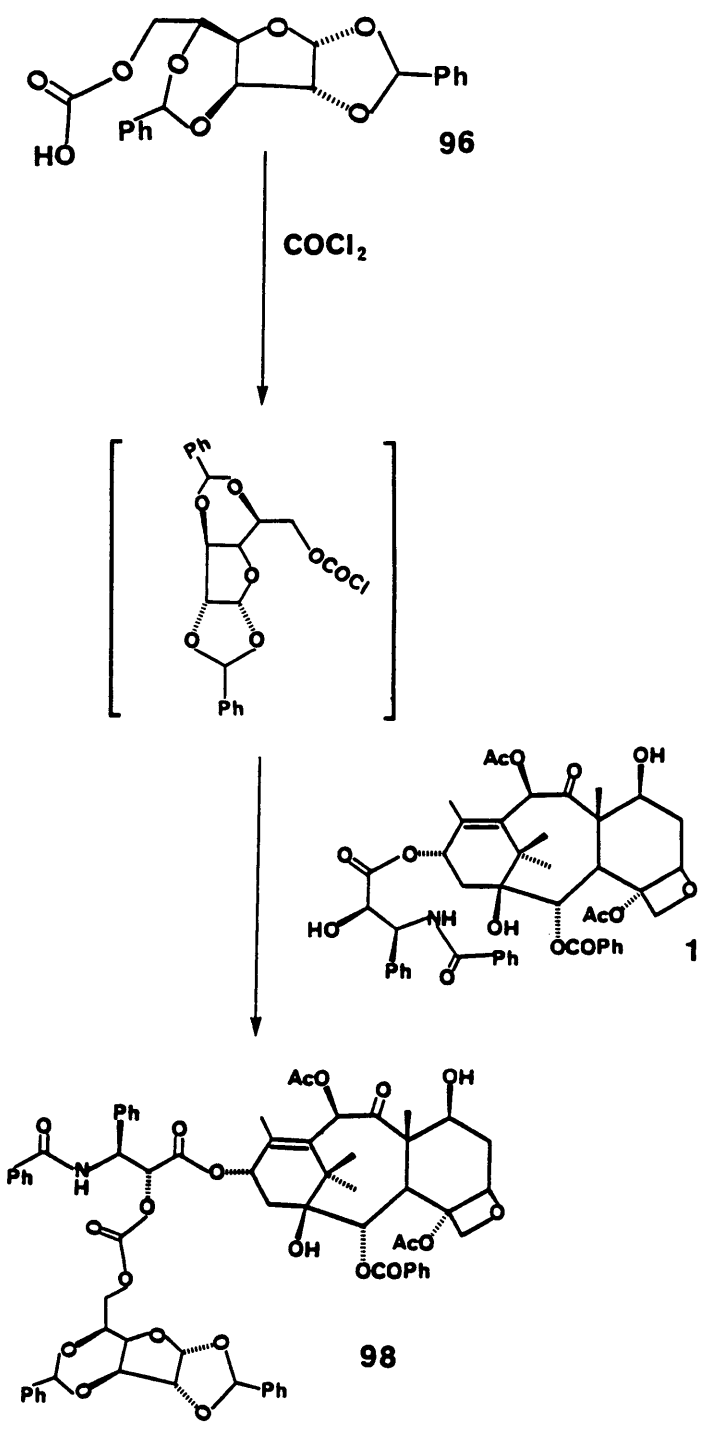
720 w.

ton decoupling. The benzylidene peaks at 6.14 ppm (s) and 5.82 ppm (s) and the C-1 proton peak at 6.22 ppm (d, J=4) could be assigned.

The mass spectral data for **97** indicates a molecular weight of 1235, m/z 1236 $(MH)^+$, which is correct for the addition of the protected sugar. Other peaks in the mass spectrum resulted from loss of benzaldehyde from the sugar m/z 1130, loss of the sugar as a carbonic acid m/z 836, and loss of the side chain as a carboxylic acid m/z 951. The infrared spectrum shows the addition of two absorption frequencies at 1240 cm^{-1} and 1100 cm^{-1} from the glucosyl moiety.

The preparation of a glucosyltaxol by first synthesizing a chloroformyltaxol was not the method of choice. The method suffered from the disadvantages that incomplete formation of the chloroformate and/or introduction of any water with the protected sugar would both detract from the yield. The preferred method would be to form a chloroformyl sugar derivative[82] and react that with taxol. Due to the steric crowding at C-7 a glucosylchloroformate would not yield the desired product; the 2' position however is not sterically crowded and a glucosyl chloroformate was used to introduce a protected glucose at C-2' of taxol.

The C-2' glucosylated taxol derivative was prepared as shown in Scheme 48. The protected sugar was reacted with phosgene to form the chloroformate at room temperature and then taxol was introduced. The reaction of taxol with the



Scheme 48. Preparation of a 2'-Glucosyltaxol

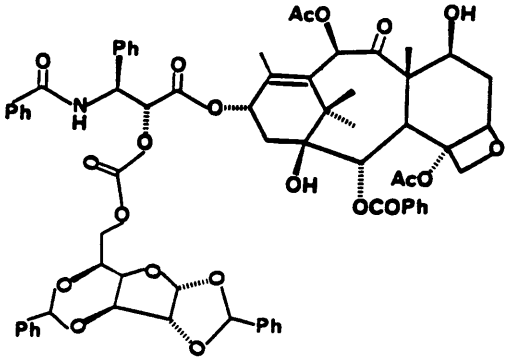
chloroformyl glucose was catalyzed by pyridine and was complete within 1 hour at room temperature. The product 2'-(6,O-carbonyl-1,2,3,5-di-O-benzylidene)-α-D-glucosyltaxol **98** was isolated by flash chromatography in 67% yield. A sample of the glucosyl chloroformate was also worked up by the addition of MeOH to form a methyl carbonate. A proton spectrum of this compound was obtained to help verify the peak assignments for **97** and **98**.

The characterization data for **98** are shown in Table 48. The proton spectrum is similar to that of **97** with the exception that the C-2' proton signal is shifted downfield to 5.43 ppm (d, J=2.5) and the C-7 proton signal is at 4.69 ppm (dd, J=6,11). The mass spectrum of **98** indicates a molecular weight of 1235 by the presence of peaks at m/z 1258 $(MNa)^+$ and 1236 $(MH)^+$. The losses of benzaldehyde, acetic acid, the side chain as an acid and the glucosyl moiety as a carbonic acid which had been seen for **97** were also seen for **98**. The loss of the side chain as an acid for **98** unlike in the mass spectrum for **97** also caused loss of the glucosyl moiety producing a peak at m/z 509.

7.2.3.2 DEPROTECTION OF (DIBENZYLIDENEGLUCOSYL)TAXOLS

A sample of 6/4 2'-acetyltaxol/2'-cephalomannine was treated in the manner described for 2'-(troc)taxol and a mixture of 2'-acetyl-7-(6-O-carbonyl-1,2,3,5-di-O-

Table 50 (Part 1 of 2). Characterization Data for **98**



Position	Shift (ppm from TMS) Coupling (hertz)
2	5.67 (d, 7)
3	3.89 (d, 7)
5	4.96 (br d, 9)
6	1.8 (m)
7	4.69 (dd, 6,11)
10	6.24 (s)
13	6.24 (br t, 9)
14	2.4 (m)
16	1.18 (s)
17	1.11 (br s)
18	1.84 (br s)
19	1.66 (s)
20	4.18 (d, 8), 4.30 (d, 8)
2'	5.43 (d, 2.5)

Mass Spectral Data

1258 $(MNa)^+$, 1236 $(MH)^+$,

1070 $(MH\text{-}HOAc\text{-}PhCHO)^+$,

836 $(MH\text{-}R'OCO_2H)^+$,

776 $(MH\text{-}HOAc\text{-}R'OCO_2H)^+$,

509 $(MH\text{-}RCOOH)^+$.

$R'OCO_2H$ - Glucose moiety as a carbonic acid

RCOOH - entire ester side chain

Table 50 (Part 2 of 2). Characterization Data for **98**

Position	Shift (ppm from TMS) Coupling (hertz)
3'	6.01 (dd, 2.5,9)
N-H	6.92 (d, 9)
OAcs	2.20 (s), 2.44 (s)
2 OBz	8.12 (d,m, 8), 7.4 (m)
3' NBz	7.72 (d,m, 8), 7.4 (m)
3' Ph	7.4 (m)
Glucosyl	7.4 (m), 6.20 (d, 3.5), 6.10 (s), 5.86 (s), 4.80 (d, 3.5), 4.63 (br s), 4.28-4.35 (m), (3H), 4.18 (br s)

Infrared Spectral Data

1235 s, 1675 m,

1535 w, 1505 w,

1485 w, 1390 m,

1290 s-sh, 1250 s,

1085 m, 1035 m,

1000 m, 720 w.

benzylidene-α-D-glucosyl)(taxol/cephalomannine) **99** was obtained. This sample was used to investigate deprotection reactions and was not purified or characterized.

Removal of benzylidene groups from carbohydrates has been reported to take place via hydrogenation with palladium as catalyst.[83] Reaction of **99** with hydrogen in methanol solution with Pd/Al_2O_3 as catalyst was monitored by tlc over a period of 24 hours. During the reaction no product other than the starting material could be seen.

Reaction of **99** with hydrogen in 99/1, MeOH/conc. HCl with 5% Pt/C as catalyst caused all of the starting material to disappear after 3 hours and a major nonpolar spot (1/1, EtOAc/Hexane, Rf 0.90) to appear on tlc. Isolation of this nonpolar product by filtration of the sample in CH_2Cl_2 through silica gel yielded a small amount of volatile liquid* which cochromatographed with benzaldehyde and had the odor of benzaldehyde. This data showed that solvolysis and not hydrogenolysis had taken place.

Reaction of **99** with 5/4/3 EtOAc/MeOH/conc. HCl at room temperature for 1 hour caused all of the starting material to react and two product spots, in addition to benzaldehyde, were seen by tlc analysis (100% EtOAc, Rfs - 0.95 minor, 0.64 major). It was assumed that these two spots corresponded to

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* No taxol derivative is a liquid.

the monodeprotected and dideprotected compounds, but this may not have been true.

Deprotection of the protected glucosyl taxol **97** was carried out via acid hydrolysis as previously described. A reaction time of 1 hour was required for reaction of 90% of the starting material and a total reaction time of 6 hours was required before one product spot was seen by tlc analysis (3/2, EtOAc/Hexane, Rf 0.15). A mass spectrum and proton spectrum for the product was obtained. The mass spectal data in Table 51 shows peaks corresponding to the desired product 7-(6-O-carbonyl-α-D-glucosyl)taxol **100** but numerous peaks were present at relatively high m/z which could not be accounted for. The proton spectrum of the product showed that a complex mixture of products was present.

Acid hydrolysis of the C-2' glucosyl taxol derivative **98** yielded the same results as for **97**. Acid hydrolysis was carried out for five hours at room temperature to yield a product which appeared pure by tlc analysis. Mass spectral data for the compound included peaks which were probably due to the desired 2'-(6-O-carbonyl-α-D-glucosyl)taxol product **101** but other peaks were also present. The ^{1}H-nmr spectrum for the compound showed it to be a complex mixture.

At the point in the taxol project that deprotection of the protected glucosyl taxol derivatives was carried out the only indication of the stabity of taxol in acidic media had been gained from the Jones oxidation reactions (Chapter 4). The

Table 51. Mass Spectral Data for Impure Glucosyl Taxols

100	101
1082 $(MNa)^+$	1060 $(MH)^+$
1060 $(MH)^+$	551 $(MH\text{-}RCOOH\text{-}H_2O)^+$
1042 $(MH\text{-}H_2O)^+$	509 $(MH\text{-}RCOOH\text{-}HOAc)^+$
1000 $(MH\text{-}HOAc)^+$	
775 $(MH\text{-}RCOOH)^+$	
757 $(MH\text{-}RCOOH\text{-}H_2O)^+$	
715 $(MH\text{-}RCOOH\text{-}HOAc)^+$	
697 $(MH\text{-}RCOOH\text{-}HOAc\text{-}H_2O)^+$	

oxidation reactions had shown that taxol was stable for extended periods at room temperature in the presence of 4.5 M H_2SO_4; it was expected that in the presence of 1.2 M HCl taxol would also be stable. The reaction of taxol with acetyl chloride/HCl described in Chapter 5 however showed that the oxetane ring of taxol is not stable in the presence of HCl. The sensitivity of the oxetane to HCl was, in all probability, the cause of a mixture of products being obtained in the hydrolysis of the protected glucosyl taxols.

Further investigation of methods to deprotect the glucosyl groups of **97** and **98** was not carried out. This was because results which were being received concerning the stability and activity of 2'-β-alanyltaxol showed that this derivative would be the taxol derivative of choice for a clinically useful soluble taxol.

7.3 EXPERIMENTAL PROCEDURE

Preparation of 2'-N-CBZ-β-alanyltaxol **49** - Taxol (209 mg) was added to a 25 mL round-bottomed flask along with dicyclohexylcarbodiimide (400 mg) and N-CBZ-β-alanine (200 mg). The reactants were dissolved in 10.0 mL of dry acetonitrile. The reaction was stirred at room temperature for 15 hours. At the end of the reaction the dicyclohexylurea which had precipitated was removed by filtration, the solvent removed *in vacuo* and the product purified by preparative tlc (1000 μm

thickness, silica gel, 55/45 ethyl acetate/hexane, 2 developments). Yield - 234 mg (88%), 99% pure as shown by hplc analysis. A small amount of the product from the side reaction - N(N-CBZ-β-alanyl)dicyclohexylurea was also isolated. The characterization data for **49** are shown in Table 45.

Reaction of Taxol with N-CBZ-β-alanine Using 1-Cyclohexyl-3-(2-morpholinoethyl)-carbodiimidemetho-p-toluenesulfonate as Catalyst - Taxol (5 mg) and N-CBZ-β-alanine (5 mg) were dissolved in dry CH_3CN along with 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimidimetho-p-toluenesulfonate (10 mg) at room temperature. The reaction was monitored by hplc. Over the course of 5 days nothing other than starting material could be seen by hplc.

Preparation of 2'-N-tBOC-β-alanyltaxol - Taxol (95 mg), N-tBOC-β-alanine (100 mg) and dicyclohexylcarbodiimide (58 mg) were dissolved in a round bottomed flask equipped with a magnetic stirrer with 4.0 mL of dry acetonitrile. The reaction was stirred at room temperature for 21 hours at which point there was still taxol present but tlc analysis indicated that no further reaction was taking place. The reaction was worked up in the same manner as that with the carbobenzoxy-β-alanyl derivatives. Characterization data for **91** are shown in Table 46.

Preparation of 2'-β-alanyltaxol Formate - 2'-N-CBZ-β-alanyltaxol (159 mg) was dissolved in 25 mL of a 40% formic acid in methanol solution. To this solution was added 5% Pd/C (98 mg) and the reaction mixture was stirred. The reaction was followed by hplc; after 35 minutes at room temperature it was found to be 80% complete. After a total reaction time of 75 minutes the reaction was stopped by filtering off the catalyst. The reaction solution was evaporated using a rotary evaporator with a 40-50° water bath as heat source and a water aspirator as the vacuum source to yield a glassy film. The product was then redissolved in CH_2Cl_2 and hexane was added. The solvents were evaporated *in vacuo* again to yield a white powdery product. Yield - 89%, 131 mg, hplc analysis showed a small taxol peak (<1%) in addition to the β-alanyltaxol salt. Characterization data for **92** are shown in Table 47.

Preparation of β-Alanyltaxol Hydrochloride (**93**) from β-Alanyltaxol Formate - A 1 g sample of taxol and 2'-β-alanyltaxol formate* was dissolved in 250 mL of EtOAc and was filtered through a 3 cm x 6 cm silica gel column, as

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* A mixture was present because not enough formic acid had been used in the initial step of hydrogenating the protected derivative.

in flash chromatography, using 2 column lengths of EtOAc as eluent. After the taxol had been removed by EtOAc, 0.60 mL (6 eq) of conc. HCl in 50 mL of EtOAc was added to the column and the resulting 2'-β-alanyltaxol HCl was eluted with 2 column lengths of 1/1, CH_2Cl_2/MeOH and the solvents were removed *in vacuo*. The resulting 2'-β-alanyltaxol HCl was homogenous on tlc (7/1, CH_2Cl_2/MeOH, Rf - 0.56); the tlc plate had to be overloaded before even a small trace of any spot other than the product could be seen. Yield - 0.35 g taxol and 0.60 g 2'-β-alanyltaxol HCl, 92% based on unrecovered taxol.

Reaction of 2'-CBZ-β-alanyltaxol with Cyclohexene and Palladium - A mixture of 2'-CBZ-β-alanyltaxol (10 mg) and 5% Pd/C in 1.0 mL of a 2/10 cyclohexene/CH_2Cl_2 solution was stirred at room temperature. The reaction was monitored by tlc over a period of 7 days. At no point during this time could anything other than starting material be seen.

Deprotection of 2'-N-tBOC-β-alanyltaxol - 2'-N-tBOC-β-alanyltaxol (1 mg) was reacted with a solution of ethyl acetate (0.2 mL), ethanol (0.25 mL) and concentrated hydrochloric acid (25 μL). The reaction was followed by hplc, (RP-8, 70/30/1 MeOH/H_2O/HOAc, 2 mL/min). After 30 minutes at room temperature all of the starting material had dissappeared to yield the β-alanyl derivative but numerous

other peaks were also seen on the chromatogram showing that the β-alanyltaxol was not stable under the reaction conditions.

Water Solubility of 2'-β-alanyltaxol Formate Salt - An excess of 2'-β-alanyltaxol was shaken with water for a period of 45 minutes at the end of which the mixture was partially filtered through a 0.45 μm pore size centrifugal filter. In the same manner taxol was shaken with water for 45 minutes and then filtered. The two filtered samples were then quantitatively analyzed by hplc. Analysis by hplc showed the β-alanyl taxol derivative to be present at 88 times the amount of taxol.

When an excess amount of C-2'-β-alanyltaxol formate salt was stirred vigorously with water in a 0.3 mL reaction vial with a Wheaton spin vane a suspension of the taxol derivative was formed. Adding even more taxol derivative never resulted in a precipitate but rather in the formation of a gel. Once the suspension of the β-alanyltaxol formed, the undissolved compound could not be precipitated by centrifugation and the suspension would pass through a centrifugal filter only with great difficulty. Several hours would be required to filter one mL of the suspension.

The chromatograms for the saturated solutions of taxol and 2'-β-alanyltaxol formate has a retention time much greater than that of taxol on a reverse phase column. The reason for

this is that the amine group binds to the small amount of unsilated silica groups present on most reverse phase hplc columns. This also causes the β-alanyltaxol formate peak to be broad and its retention time changed with changing amounts of compound injected on the column. The chromatogram for the β-alanyl derivative also shows a taxol peak, as approximately 1% of taxol was present in the product; the chromatogram therefore was actually saturated with respect to taxol and 2'-β-alanyltaxol formate. Quantitation of the amount of 2'-β-alanyltaxol formate in aqueous solution was accomplished by dissolving 1.72 mg of 2'-β-alanyltaxol formate in methanol and injecting 5 µL of this solution onto the hplc using the same conditions employed for the aqueous solution. Comparison of the peak areas showed that the solubility of the 2'-β-alanyltaxol in water is 2.2 mg/mL.

Water Solubility of 2'-β-Alanyltaxol HCl - The water solubility of 2'-β-alanyltaxol HCl was determined in the same manner as that of 2'-β-alanyltaxol formate with several modifications. A saturated solution of 2'-β-alanyltaxol HCl was prepared and filtered, with difficulty, through a 0.45 µm pore size centrifugal filter as was done for the formate salt. A second sample was filtered through a centrifugal filter using Whatman No. 1 filter paper; this filtration also proceeded with great difficulty. A third sample was prepared with 9/1, H_2O/EtOH as solvent and filtered through a 0.45 µm

pore size centrifugal filter. Each of the saturated solutions was quantitated via hplc by comparison of peak area with a 9.5 mg 2'-β-alanyltaxol HCl/MeOH standard solution. The standard solution was stable throughout the analysis; no decomposition to taxol was seen. The solubilities of 2'-β-alanyltaxol HCl are shown on page 302.

Stability of 2'-β-Alanyltaxolformate in Methanol - A standard solution of 2'-β-alanyltaxol formate in methanol was prepared for quantitation of the solubility of 2'-β-alanyltaxol formate in water. When this sample was injected onto the hplc, approximately 15 minutes after being prepared, a large amount (25%) of taxol was seen. Upon standing the 2'-β-alanyltaxol formate decomposed completely to taxol.

Stability of 2'-βAlanyltaxol Formate Salt in Aqueous Solution - A saturated solution of the formate salt of β-alanyltaxol was prepared by stirring excess β-alanyltaxol with water for 45 minutes and then filtering off the undissolved taxol derivative through a cotton plug. The solution was analyzed by hplc over a period of several days. Chromatographic analysis showed that the β-alanyltaxol formate decomposed slowly to taxol. After 24 hours this decomposition had occurred to approximately 10 percent of completion.

Stability of 2'-β-alanyltaxol Formate Salt in Biological pH Buffer - The buffer solution which was used in the microtubule polymer assembly tests was prepared, consisting of 0.1 M 2(N-Morpholino)ethanesulfonic acid, 0.5 mM $MgCl_2$ and 1.0 M ethyleneglycol-bis-(β-amino-ethyl ether) N,N' tetra-acetic acid (EGTA). The pH of the buffer was adjusted to 6.6 by adding 0.6 N NaOH.[61]

A solution of 2'-β-alanyltaxol and taxol (75 μL) consisting of 9.5 mg taxol and 2'-β-alanyltaxol in 3.0 mL of 20% formic acid/methanol was added to 3.0 mL of the buffer previously described. This concentration was ten times that used in the microtubule polymer assembly promotion tests but was necessary so that peaks could clearly be seen above the noise on the hplc chromatogram. Testing of the solution with Hydrion pH paper showed that no significant change in pH had occurred by the addition of the formic acid. The solution was analyzed periodically by hplc and was incubated at 37° in a water bath. Analysis by hplc showed that after 20 hours essentially none of the starting 2'-β-alanyltaxol remained but had cleanly been converted into taxol. Chromatograms showing the starting mixture of taxol and 2'-β-alanyltaxol formate to taxol are shown in Figure 30.

2'-Succinyltaxol - Taxol (206 mg), 4-dimethylaminopyridine (2.9 mg) and succinic anhydride (49 mg) were added to a 5 mL vial equipped with a magnetic stirrer and were dried in vacuo

for 2 hours. After drying, 2.0 mL of dry pyridine was added and the solution was stirred at room temperature for 2.5 hours. At the end of the reaction several mL of water was added which produced a white precipitate and opaque suspension. Several mL of CH_2Cl_2 were then added to extract the product. The white aqueous suspension did not disappear until 1 mL of conc. HCl was added. The methylene chloride layer was dried with $MgSO_4$, filtered and evaporated. Analysis of the sample at this point by tlc (7/1, CH_2Cl_2/MeOH, Rf 0.50) showed no trace of any other product present, and proton nmr spectroscopy also showed the product to be pure. Yield - 221 mg, 96%. Characterization data for **94** are shown in Table 48.

Preparation of Silver Salicylate - Silver salicylate was prepared following the procedure of Wulff.[92] Salicylic acid (2.6 g) was dissolved in 20 mL of ethanol and then neutralized with NaOH (1.51 g in 5 mL of H_2O). This solution was then dripped into a solution of silver nitrate (3.4 g) in 10 mL of 1/1 ethanol/water over 15 minutes. The product which precipitated was filtered off and washed with ethanol (10 mL) and diethylether (10 mL).

Reaction of 2'-Acetyltaxol with 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl - Silver salicylate (27.3 mg) and 2'-acetyltaxol (21 mg) and acetobromo-α-D-galactose were dissolved in dry CH_2Cl_2 (0.40 mL) at room temperature. The

reaction was followed by tlc. After 28 hours an additional 39.6 mg of silver salicylate and 43.6 mg of acetobromo-α-D-galactose added. After a total reaction time of 48 hours the reaction was stopped by filtering off the silver catalyst. The products were isolated by preparative tlc on silica gel using 6/4 ethyl acetate/hexane as solvent. Two products were obtained with Rf 0.73 and 0.46. The more polar product was shown by nmr to be starting material and the less polar compound was not a taxol-like compound.

Preparation of 1,2:3,5-Di-O-benzylidene-α-D-glucose (**96**) - The method of Wood was used for the preparation of **96**. Freshly distilled benzaldehyde (150 mL), acetic acid (19 mL), α-D-glucose (25 g) and $ZnCl_2$ (50 g) which had been fused for 45 minutes, were placed in a round bottomed flask and shaked at 25° for 17 hours. At the end of the reaction the solution was poured onto 200 mL of water and ice, extracted with 3 100 mL portions of Et_2O, the organic solution was washed with water and then 5% $NaHCO_3$, dried with $MgSO_4$ followed by the addition of 1 g of charcoal and the solution was filtered. The solvents were then removed *in vacuo*. The resulting syrupy gel was recrystallized from 50 mL of EtOH, requiring 3 days at 0° for the crystals to fully develop. The crude product was recrystallized again from EtOH and then from EtOAc to yield **96**. Yield 3.3 g, 7%, mp 160-162°, lit. 160-

161. The ^{1}H-nmr of the product matched the range of values for the protected sugar given in the literature.

7-(6-O-Carbonyl-1,2,3,5-di-O-benzylidene)-α-D-glucosyltaxol - A sample of 2'-(2,2,2-trichloroethyloxycarbonyl)taxol (0.700 g), 1,2,3,5-di-O-benzylidene-α-D-glucose (0.291 g) and 4-dimethylaminopyridine (8 mg) were dried separately in an Abderholden drying apparatus for several hours using refluxing EtOAc as heat source. The C-2' protected taxol derivative was dissolved in 6 mL of dry CH_3CN, 100 µL of dry pyridine was added and the solution was cooled to 0° in an ice bath. To this solution was added 0.619 mL (1.1 eq) of a 12.5% solution of $COCl_2$ in benzene. After 30 minutes 6 mL of CH_2Cl_2 and 4 mL of CH_3CN were added to dissolve a white precipitate which had developed. After a total reaction time of 1 hour tlc showed all of the starting material had reacted (1/1, EtOAc/hexane, product Rf 0.50). After 1 hour 20 minutes the 7-chloroformyltaxol derivative was added to the protected sugar and 4-DMAP at room temperature along with an additional 100 µL of pyridine. Analysis of the reaction by tlc showed that after 35 minutes no further reaction was taking place. After a total reaction time of 1 hour 10 minutes the reaction was worked up to yield a mixture of products including the taxol sugar derivative (Rf 0.75), 2'-(troc)-7-chloroformyltaxol (Rf 0.46) and 2'-(troc)taxol (Rf 0.31). The crude reaction mixture was reacted with Zn

dust (1 g) in 20 mL of 95/5 MeOH/HOAc at room temperature. Over the course of 3 hours an additional gram of Zn dust was added as the starting material spot with Rf 0.75 did not react completely. After a total reaction time of 4 hours rhe reaction was worked up and products purified by flash chromatography (45/55, EtOAc/Hexane). Three products were isolated; 60 mg of a nontaxol product which had the same Rf as the starting material and was probably a sugar dimer, 300 mg of **97** and 200 mg of taxol. Characterization data for **97** are shown in Table 49.

The reaction to produce **97** was conducted on a large scale only once and reaction conditions were not optimized for yield. The low yield of desired product and the significant amount of taxol recovered at the end of the reaction sequence was probably due to incomplete formation of the chloroformyltaxol in the initial reaction. Incomplete formation of the chloroformyl taxol will decrease the yield in two ways: taxol was left unreacted and the excess phosgene would have reacted when the sugar derivative was added to form a carbonate linked sugar dimer, one equivalent of phosgene would have reacted with two equivalents of protected sugar. Any researchers wishing to repeat this reaction should allow the reaction of taxol with phosgene to proceed for a longer time.

2'-(6-O-Carbonyl-1,2,3,5-di-O-benzylidene)-α-D-glucosyltaxol - The protected sugar 1,2;3,5-di-O-benzylidene-α-D-glucose (210 mg) was partially dissolved in 5 mL of dry Et_2O and 100 µL of pyridine were added. To this mixture 0.48 mL of 12.5% $COCl_2$ in benzene was added (1.75 eq). The addition of the phosgene caused a white precipitate to form. The reaction was stirred for 2 hours at room temperature at which time a solution of taxol (250 mg, 1 eq) in 4 mL of CH_2Cl_2 was added. Over the course of an hour the white precipitate which had been present dissolved. At that point monitoring of the reaction by tlc (6/4, EtOAc/Hexane) showed the product **98** present, Rf 0.39, and taxol, Rf 0.15, and no further reaction taking place. The reaction was worked up and the product **98** isolated by flash chromatography (6/4 EtOAc/Hexane). Yield - 244 mg, 67%. Characterization data for **98** are shown in Table 50.

Deprotection of Di-O-benzylidene Glucosyl Taxol - A sample of 2'-acetyltaxol/2'-acetylcephalomannine was treated in the manner described for 2'-(troc)taxol to obtain 2'-acetyl-7-(6-O-carbonyl-1,2,3,5-di-O-benzylidene-D-glucosyl) (taxol/cephalomannine) which was used as a crude product to investigate sugar deprotection reactions.

Hydrogenolysis

A sample of the glucosyl (taxol/cephalomannine) mixture (50 mg) was dissolved in 5 mL of MeOH and 25 mg of 5% Pd on alumina was added to the solution. The reaction was stirred and placed under one atmosphere of hydrogen at room temperature. The reaction was monitored by tlc (3/2, EtOAc/Hexane) which showed no reaction even beginning to occur over the course of 24 hours.

A sample of the glucosyl (taxol/cephalomannine) mixture (47 mg) was dissolved in 10 mL of EtOH and 5% Pt/C (9.8 mg) was added. The reaction was stirred under one atmosphere for 24 hours at which time tlc analysis showed no significant reaction had taken place.

A sample of the glucosyl (taxol/cephalomannine) (50 mg) was dissolved in 1.0 mL of 99/1 MeOHY/conc. HCl and 5% Pt/C (25 mg) was added. After three hours of stirring under a hydrogen atmosphere tlc analysis (1/1 EtOAc/Hexane) showed an intense nonpolar spot, Rf 0.90, and a small spot at the origin, but no starting material. The reaction was stopped by filtering off the catalyst and was worked up. The crude product was redissolved in several mL of CH_2Cl_2 and filtered through a Pasteur pipette filled with silica gel to isolate the nonpolar compound. After evaporation of the solvent a small amount of liquid was obtained which had the characteristic odor of benzaldehyde and which cochromatographed with benzaldehyde on tlc.

Hydrolysis

A sample of the glucosyl (taxol/cephalomannine) mixture was dissolved in 2 mL of 5/4/1, EtOAc/MeOH/conc. HCl, at room temperature. After reacting for 1 hour the reaction was worked up and tlc analysis (EtOAc) showed two major spots, Rfs 0.95 and 0.64.

The sample of 7-(6-O-carbonyl-1,2,3,5-di-O-benzylidene-α-D-glucosyl)taxol was dissolved in 10 mL of 5/4/1, EtOAc/MeOH/conc. HCl. The reaction was monitored by tlc analysis and required three hours before a single spot was seen (3/2, EtOAc/Hexane, Rf 0.15). The reaction was worked up and a proton nmr spectrum and a mass spectrum were obtained. The mass spectrum showed peaks corresponding to 7-(6-O-carbonyl-α-D-glucosyl)taxol but the nmr spectrum showed a complex mixture of products present. Further tlc analysis using CH_2Cl_2/MeOH solvent systems showed that a mixture of products was present.

A sample of α-(6-O-carbonyl-1,2,3,5-di-O-benzylidene-α-D-glucosyl)taxol (130 mg) was treated in the same manner as the C-7 derivative, with the same results. The sample was subjected to hydrolysis for 3 hours at room temperature and the reaction was worked up. Mass spectrometry analysis indicated that some of the desired product was present but proton nmr analysis and tlc analysis with CH_2Cl_2/MeOH solvent systems showed that a complex mixture of products was present.

8.0 BIOLOGICAL ACTIVITY OF MODIFIED TAXOLS AND SUMMARY OF TAXOL'S REACTIVITY

In this chapter the knowledge gained about taxol in this research project will be presented. This presentation will be divided into biological activity and chemical reactivity, with biological activity being addressed first.

8.1 BIOLOGICAL ACTIVITY OF MODIFIED TAXOLS

8.1.1 ASSAY TESTS

Three different assays were employed for evaluation of the modified taxols as anticancer agents: a microtubule polymer assembly assay, the KB cell culture assay and P-388 *in vivo* leukemia assay.

The microtubule polymer assembly assay was created and carried out on the modified taxols by Susan Horwitz at the Albert Einstein Institute.[22] The microtubule polymer promotion assay involves incubating a drug with microtubule protein. The ability of the drug to polymerize the microtubule protein was then measured by the increase in optical density of the solution which was measured by UV absorption; an increase in optical density indicated assembly activity.

With respect to the environment that the drug being tested encounters the microtubule polymer assembly test was the simplest. The drugs encountered no enzymes that could modify them; the test was carried out under mild conditions, pH 6.6, 37°, 30 minutes, and there were no cell membranes for the drugs to cross. The microtubule polymer assembly assay showed if a drug could function by taxol's mechanism of action. The test was not an absolute indicator of a drug's clinical ability due to the simple nature of the assay. Several milligrams of a compound were required for the microtubule assay.

The KB cell culture measured the ability of a drug to inhibit the growth of cancer cells. In the KB assay a drug at varying concentrations was incubated with cancer cells. The growth of the treated samples was then compared with that of untreated cells and the assay result was reported as the concentration of drug necessary to have one half the growth (as measured by protein production) in the treated sample as in the control sample. The KB assay was more complex than the assembly assay and its results were a better indicator of a drug's clinical usefulness. The drug must cross a cell membrane and the chemical environment of a cell could modify the drug being tested. One disadvantage of the KB cell assay is that it did not give a measure of toxicity of a drug. Several milligrams of each taxol derivative were required for

the KB assays which were carried out at the University of Miami School of Medicine.

The P-388 *in vivo* assay is the most complex of the tests performed on modified taxols. The P-388 assay involves giving a single dose, or repeated doses, of a drug being tested to a mouse which has P-388 lymphoma; a series of test animals and dosages are used. The life spans of the treated animals are then compared to those of genetically identical but untreated mice. The assays results are given as the lifetime of treated animals (t) divided by that of control animals (c) multiplied by 100, together with the dosage of drug necessary to achieve the result. Unlike the KB assay, high dosages in the P-388 assay will yield poor results due to the drugs becoming toxic at high concentrations. The P-388 assay is the best indicator of the three assays of the clinical anticancer abilities of a drug. The P-388 test required at least 100 mg of a drug and so was carried out on only a select number of modified taxols. The assays were carried out by the National Cancer Institute.

8.1.2 C-2' AND C-7 MODIFIED TAXOLS

The *in vitro* microtubule polymer assembly test results for C-2' and C-7 modified taxols are shown in Table 52.[43] The *in vitro* tests clearly showed that substitution of the C-2'

Table 52. Activity of C-2' and C-7 Modified Taxols in Microtubule Polymer Assembly Assay System

Compound	Activity
Taxol	+
2'-Acetyltaxol	-
7-Acetyltaxol	+
7-Epitaxol	+
2',7-Diacetyltaxol	-

hydroxyl by an acetate removes all biological activity; in the presence of 2'-acetyltaxol no polymerization of tubulin could be detected. As expected, 2',7-diacetyltaxol also showed no biological activity. Contrasted with the 2'-acetate, 7-acetyltaxol and 7-epitaxol polymerized microtubule protein nearly as well as taxol. The presence of a hydroxyl group on the beta face of taxol therefore is not necessary for biological activity.

The in vivo tests, P-388 and KB cytotoxicity, for the C-2' and C-7 modified taxols are shown in Tables 52 and 53. The in vivo tests results, on first consideration, seemed to contradict the in vitro results. The C-2' modified taxols - 2'-acetyltaxol and 2'-N-CBZ-β-alanyltaxol showed nearly as much activity as taxol while 7-acetyltaxol had only 0.25% as much activity as taxol in the cytotoxicity test.

The cytotoxicity and P-388 activity of the C-2' esterified taxols can be explained from knowledge of chemical reactivity. In Chapters 2 and 7 it was shown that C-2' ester substituents are readily hydrolysed and it is thought that in vivo this happened also. The activity of the C-2'-esterified taxols resulted from the drugs being converted back into taxol. This hypothesis is supported by the inactivity of the 2'-triethylsilylstaxol and in the cytotoxicity test. It would be expected that a hindered silyl group would not be cleaved as readily as an ester group.

Table 53 Activity of C-2' and C-7 Modified Taxols in KB Cell Culture Assay System

Compound	ED_{50} (μg/mL)
Taxol	0.00001
2'-Acetyltaxol	0.00002
7-Acetyltaxol	0.004
2'-Triethylsilyltaxol	0.3
7-Epitaxol	0.00003
2',7-Diacetyltaxol	0.03
2'-N-CBZ-β-alanyltaxol	0.0001
2'-N-β-Alanyltaxol Formate	0.01
Cyclic urethane **41**	0.2, 0.35

Table 54 Activity of C-2' and C-7 Modified Taxols in P-388 *in vivo* Assay System

Compound	Test/Control x 100 (dose, mg/kg)
Taxol	156 (5)
2'-Acetyltaxol	140 (20)
7-Acetyltaxol	130 (26)
2',7-Diacetyltaxol	100 (all doses)

The relative inactivity of 2'-β-alanyltaxol formate in the KB cell test suggests that the cleavage of C-2' acyl substituents takes place with assistance by an enzyme. If the cleavage took place via attack of water on a compound not bound to an enzyme then 2'-β-alanyltaxol formate which is less stable in water than 2'-acetyltaxol (Chapter 7) would have been more active in the KB assay.

The *in vitro* and *in vivo* results for 7-acetyltaxol can not be readily explained with the current knowledge of taxol's mechanism of activity. The *in vivo* test results can not invalidate the *in vitro* results. The *in vitro* tests showed that esterification of the 7 position did not affect interaction of the drug with microtubule protein, which is taxol's mechanism of action. The *in vivo* test systems are more complex than the *in vitro* and it may be that a 7-hydroxyl group is necessary for some function other than polymerization of microtubule protein, such as transport across the cell membrane.

The *in vivo* and *in vitro* test results showed that with respect to taxol's mechanism of action the C-2' position is critical and the C-7 is not. The tests also showed that for purposes of preparing clinically useful drugs substitution of C-2' with a hydrolyzable group may be carried out but substitution of C-7 will probaby not lead to a useful drug.

Table 55 Activity of 7-Acetyl-2'-oxotaxol in KB Cell Culture Assay

Compound	ED (μ g/mL)
Taxol	0.00001
7-Acetyltaxol	0.004
7-Acetyl-2'-oxotaxol	0.02

8.1.3 OXIDIZED TAXOLS

The KB cytotoxicity assay results for the oxidized taxol 7-acetyl-2'-oxotaxol are shown in Table 55. The other oxidized taxols were not included in this table as they all possessed 7-oxo groups and it was expected that these derivatives were transformed into the D-seco derivatives.

The KB assay results for 7-acetyl-2'-oxotaxol showed that the compound possesses considerably less activity than taxol but that most of this loss of activity was due to the presence of the 7-acetyl group. The 2'-oxidized derivative was 20% as active as 7-acetyltaxol. This was a definite diminishing of activity but is a small change when compared with the difference in activity between taxol and 2'-triethylsilyltaxol. Transformation of the C-2' hydroxyl to a ketone therefore did not significantly alter the activity of the drug. Two possible explanations for the activity

of the 2'-oxotaxol are: 1) The keto group is attacked by water and exists as a hemiketal which would have a 2'-hydroxyl group, or, 2) The 2'-keto group was no larger than the 2'-hydroxyl it was replacing and so the derivative could fit into taxol's receptor site. In either case the activity result showed that there is not an absolute requirement for a taxol derivative to possess a C-2' hydroxyl group in order to be biologically active.

The KB cell culture assay results for the D-seco taxols, and the 7-oxotaxols which were precursors to the D-seco taxols, are shown in Table 56. The activity results are consistent and unequivocal - removal of the oxetane functionality from taxol destroys the activity of the taxol derivative. This loss of activity could be due to loss of the oxetane or the changing conformation of the taxane ring system (see Chapters 4 and 5).

The P-388 assay results for the water soluble taxols, 2'-β-alanyltaxol and 2'-succinyltaxol are shown in Table 57. The P-388 assay system results were the most important results for these derivatives as the water soluble taxols were designed to be clinically useful drugs.

The P-388 assay showed 2'-succinyltaxol to be active but not as active as taxol. This result coupled with 2'-succinyltaxol being less water soluble than 2'-β-alanyltyaxol (Chapter 7) indicates that 2'-succinyl

Table 56. Activity of D-Secotaxols in the KB Cell Culture Assay System

Compound	ED_{50} (g/mL)
Taxol	0.00001
Taxol AcCl Product **64**	2.5
7-Oxotaxol	0.5
2'-Acetyl-7-oxotaxol	1.5
5-epi-hydroxy-5,0-secotaxol	0.2
2',7-Dioxotaxol	12.0
C Ring Lactone **62**	2.0
2'-Acetyl-7-oxo-5,0-secotaxol	1.0

Table 57. P-388 Assay Activity of the Water Soluble Taxols

Compound	T/C, Dose (mg/kg)
Taxol	156 (5)
2'-β-Alanyltaxol	153 (40)
2'-Succinyltaxol	129 (44)

taxol is not the taxol derivative of choice for clinical administration.

The activity of 2'-β-alanyltaxol in the *in vivo* assay was nearly identical to that of taxol. The water soluble taxol required a higher dosage than taxol but that is due to the water solubility of the drug. The P-388 assay involves a lymphoma localized in the peritoneum of mice and the drug to be tested is injected intraperitoneally.[32] A sparingly water soluble drug such as taxol remains in the vicinity of where it was injected while a water soluble drug would be transported throughout the mouse. The higher dosage of 2'-β-alanyltaxol required for optimum results showed that the drug can be transported *in vivo* before decomposing into taxol. The water soluble 2'-β-alanyltaxol was also found to be active in the *in vitro* assay. This activity was due to decomposition of the drug to taxol even under *in vitro* conditions as was shown in stability tests described in Chapter

7. Barring unforseen difficulties in clinical administration of the drug 2'-β-alanyltaxol is the drug of choice for a water soluble taxol.

8.2 SUMMARY OF TAXOL'S CHEMICAL REACTIVITY

Prior to this project knowledge of the chemical reactivity of taxol was limited to the ester groups of the compound being susceptible to nucleophilic attack. In this project, taxol, with respect to chemical activity, has been transformed from a 'black box' into a compound for which some definite rules concerning its chemical behavior are known. The chemical behavior of several areas of taxol is summarized below:

A. Taxol's Side Chain

1. The C-2' hydroxyl activates the C-1' ester linkage with respect to nucleophilic attack (Chapters 2 and 3).

2. The C-2' position can be epimerized by a hindered base (Chapters 2 and 3).

3. The C-2' hydroxyl is readily substituted by silyl or acyl groups (Chapters 2 and 7).

4. The C-2' hydroxyl is slowly oxidized by Jones reagent (Chapter 4).

5. The side chain is not sensitive to electrophiles (Chapter 5).

6. The C-3' amide becomes nucleophilic in the presence of base and can attack an electrophile attached to C-2' (Chapters 2 and 6).

B. C-10 Acetate

1. The C-10 acetate is sensitive to the electrophile acetyl chloride and can, in high yield, be converted to an enol acetate (Chapter 5).

C. C-9 Ketone

1. The C-9 ketone is sterically hindered; it is stable indefinitely in the presence of borohydride (Chapter 3).

D. C-7 Hydroxyl

1. The C-7 position can be epimerized in nearly quantitative yield (Chapter 2).

2. The C-7 hydroxyl can be quickly oxidized by Jones reagent but the resulting derivatives are susceptible to beta elimination under mildly basic conditions (Chapter 4).

3. The C ring of a 7-oxo-D-secotaxol can be opened via nucleophilic attack at C-7 (Chapter 4).

4. The C-7 hydroxyl is sterically hindered (Chapters 2, 6 and 7).

E. Oxetane

1. Taxol's oxetane ring is extraordinarily sensitive to electrophilic attack and beta elimination. Ring opening can take place with assistance by the C-4 acetate or the C-2 benzoate. Ring opening relieves bond angle strain and changes the conformation of the taxane ring system (Chapters 4 and 5).

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MODIFIED TAXOLS AS ANTICANCER AGENTS

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(ABSTRACT)

Modifications of the potent anticancer agent taxol were carried out in order to gain an understanding of the chemical reactivity of the drug and the factors which contribute to its biological activity. The C-2' and/or the C-7 hydroxyl groups of taxol were substituted with acetyl, βalanyl, silyl, succinyl, trichloroethyloxycarbonyl or carbonate linked dibenzylidene protected glucosyl groups. The C-7 position was selectively epimerized under free radical conditions and a 2'-epiacetyltaxol was produced via base catalysed epimerization. The C-2' amide became nucleophilic in the presence of base and could attack a C-2; acyl substituent. The C-13 ester side chain was selectively reduced by borohydride. The 7 position of taxol was selectively oxidized by Jones reagent and longer reaction times also oxidized the 2' position. The D rings of the 7 oxotaxols were readily opened via beta elimination; hydrogenation of the double bond in the enone of the D seco products produced a product in which the C ring was opened. The D ring was also susceptible to electrophilic attack. Reaction of taxol with triethyloxonium tetrafluoro-

borate or acetyl chloride/HCl produced D seco taxols. C-7 deoxygenation was not achieved due to steric hindrance at C-7 and the instability of taxol under free radical conditions.

Biological testing of modified taxols showed that substitution of the C-2' hydroxyl removed biological activity but that C-2' acyl groups were readily removed in vivo. The water soluble 2'-βalanyltaxol possessed in vivo activity equal to that of taxol. Substitution of the C-7 hydroxyl did not inhibit the ability of a taxol derivative to polymerize tubulin but did decrease in vivo activity; epimerization of C-7 decreased in vivo activity slightly. A 2'-oxotaxol was found to be less active than, but still comparable to, its nonoxidized analogue. All taxol derivatives having a 7-oxo group and/or not possessing a D ring lost almost all biological activity.