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Synthesis of Podophyllotoxin and Analogs

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A thesis submitted in partial fulfilment

of the requirements for the degree of

Doctor of Philosophy

in the

Department of Chemistry

University of Ottawa

Ottawa, Canada

April, 1987

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ISBN 0-315-40718-2

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

Acknowledgements

The financial support of the Natural Sciences and Engineering Reseasch Council of Canada and of Bristol Laboratories, Syracuse, NY, is gratefully acknowledged.

I would like to thank my supervisor Professor Tony Durst for his excellent suggestions, encouragement, direction and zeal in this project.

The nmr specta were recorded by Mr. Raj Capoor, to whom I am very grateful. Dr. Clem Kasakof is to be credited with his excellent mass spectral service.

I would like to thank my parents for their support, encouragement, patience, kindness and great helpfulness over the years.

I am greatly indebted to Joy Tunnoch for typing the entire experimental section as well as doing an excellent job in drawing the majority of the structures.

The text of this thesis was processed using the document preparation system LaTeX.

Above all I must thank my Savior and Lord for the marvelous design and creation of the very atoms with which we work! He is also to be credited with the design and truly the first synthesis of podophyllotoxin, which is also enantiospecific, via Podophyllum Peltatum!

"For of Him, and through Him, and to Him,
are all things: to whom be glory for ever. Amen."

Romans 11:36

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Abbreviations

n-BuLi	*n*-butyllithium
DMF	dimethylformamide
h	hours
d	days
IR	infrared
LDA	lithium diisopropyl amide
mp	melting point
MS	mass spectrum
nmr	nuclear magnetic resonance
PTLC	preparative thin layer chromatography
THF	tetrahydrofuran
tlc	thin layer chromatography
TsOH	*p*-toluenesulfonic acid
PCC	pyridinium chlorochromate
TMS	trimethylsilyl
Me	methyl
Et	ethyl
Bn	benzyl
conc	concentrated

Abstract

A highly stereoselective synthesis of (±)-podophyllotoxin is described. The correct relative stereochemsitry of the four cheral centers in podophyllotoxin is produced via an intramolecular Diels-Alder reaction involving an *o*-quinodimethane, generated by the thermal ring opening of a *trans*-2-arylbenzocyclobutenol derivative.

The syntheses of two analogs of podophyllotoxin are described in which similar methods, to that used for podophyllotoxin, are utilized. Several changes were practicable in the analog syntheses which resulted in shorter and more efficient preparations.

The stereoselectivity as well as the rate of the intramolecular Diels-Alder reactions was found to be solvent dependent. Polar solvents such as nitromethane and DMF were found to favor the required products as well as giving the highest reaction rates.

Part I

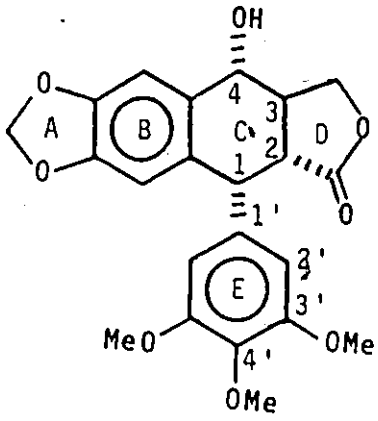
Introduction

Chapter 1

Origin and Use of Podophyllotoxin and its Derivatives

1.1 Isolation, Structure and Background

Podophyllum peltatum, commonly named the May Apple, has long been known for its medicinal properties. It was known to the North American Indians several hundred years ago as a cathartic, anthelmintic and a poison. It was used by American settlers as a herbal medicine and was included in the US Pharmacopoeia from 1863 to 1942. In 1835 a resin was isolated, from the roots of the may apple, which contained the components responsible for the biological activity.[1] In 1880 the active compound was isolated and named podophyllotoxin.[2] The structure and configuration of podophyllotoxin was reported in 1958 by Hartwell and Schrecker as 1.[3] X-ray crystal analysis of 2′-bromopodophyllotoxin confirmed Hartwell's original assignment of absolute configuration as (1R, 2R, 3R, 4R).[4] In the late 1960's the use of O-glycosides of podophyllotoxin in treatment of a variety of cancers, rekindled an interest in podophyllotoxin.

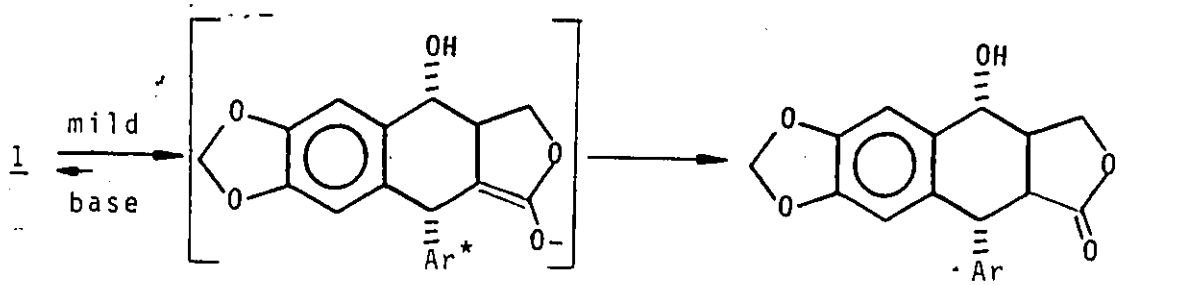


Podophyllotoxin 1

Natural products containing a 2,3-dibenzyl butane system have been termed "lignans".[5] Podophyllotoxin is thus one such compound, as can be noted by breaking the C1-aryl(B-ring) bond. Podophyllotoxin has as its key structural feature a 5-membered lactone *trans* fused to carbons 2 and 3 of a tetrahydronaphthalene containing a C1 aryl substituent with *cis*-1,2 stereochemistry. This *cis*-1,2 stereochemistry places the aryl ring E perpendicular to the average plane of the other rings. Finally, the hydroxyl group at C4 gives podophyllotoxin 4 contiguous chiral centres.

1.2 Characteristic Chemistry

The strained *trans* C/D ring junction and axially locked C1 aryl substituent make podophyllotoxin very susceptable to epimerization at C2 via it's ester enolate 2. Treatment with weak bases, such as sodium acetate in ethanol,[6] produces almost complete conversion to the more stable *cis* fused picropodophyllotoxin 3. Picropodophyllotoxin has little or no useful biological activity.

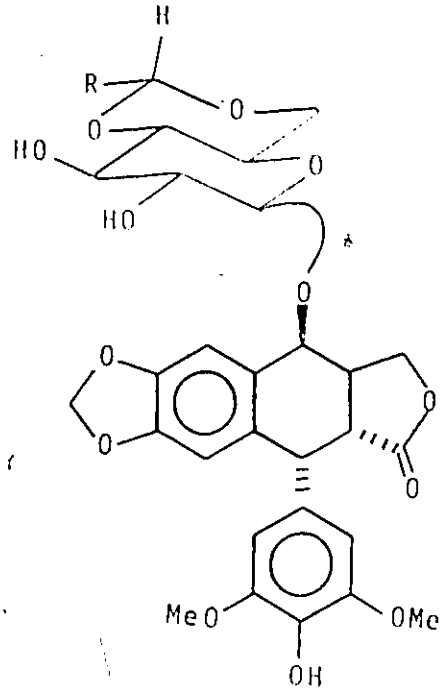


1 2 Picropodophyllotoxin 3

* Unless otherwise indicated, Ar equals 3,4,5-trimethoxyphenyl in all formulae throughout this thesis.

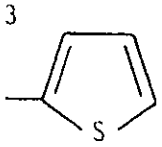
1.3 Biologically Active Podophyllotoxin Derivatives

Podophyllotoxin itself has significant cytotoxic activity but is also quite toxic, with the LD_{50} in mice being approximately 30 mg/kg of body weight. Many derivatives have been prepared (by modification of the natural compound) with the goal of obtaining compounds which retain the cytotoxicity of podophyllotoxin but have low enough toxicity to be used clinically. Work by Kuhn and von Wartburg of Sandoz (Basel, Switzerland) yielded two successful examples, Etoposide 4 and Teniposide 5, which are O-glycosides of the C4 epimer, C4' O demethylated.[7] Etoposide is currently used clinically against a variety of cancers including bladder cancer. It is considered one of the most effective chemical agents against small cell lung cancer.[8] Its use against this type of cancer generally gives 6-9 months of remission, but no long term cures.

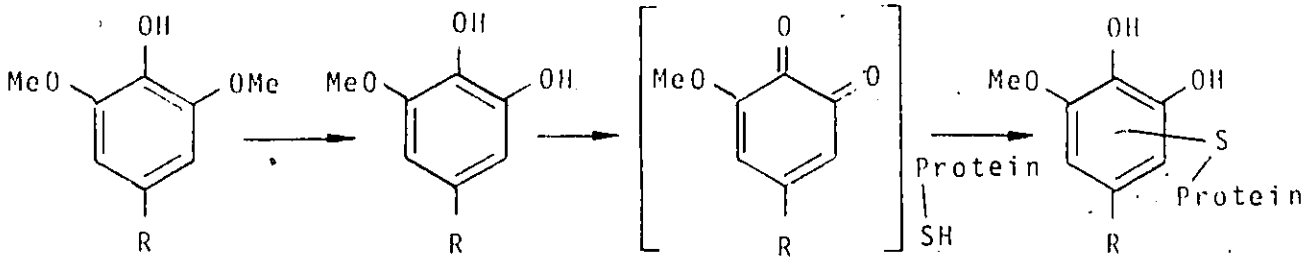


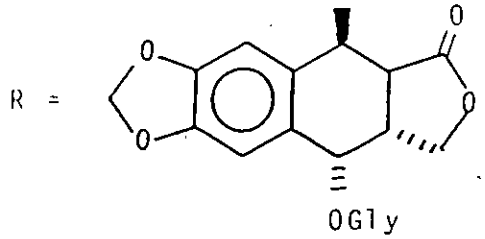
Etoposide 4 R= CH_3

Teniposide 5 R=



The exact biochemical basis for the biological activity of podophyllotoxin and its derivatives is yet uncertain but has been under investigation for several years. Biochemical studies have indicated that podophyllotoxin has a different mode of action than does Etoposide.[9] Because Etoposide is of greater interest than podophyllotoxin due to its increased biological activity, the majority of biochemical studies in the past few years have been focused on it. The most recent studies have indicated that the mechanism of action of Etoposide involves O-demethylation of the C3′ methoxy group to give 6 which may be oxidized to the quinone 7. Quinone 7 could then act as a Michael receptor capable of covalent binding to nucleophilic cell components, resulting in cytotoxicity.[10]





In order to further this study it is of interest to make derivatives with various types of substitution on rings B and E. To prepare such derivatives, with the goal of producing better anticancer agents and understanding their mechanism of action, a synthesis with sufficient generality is required.

It should be noted that a synthesis yielding epipodophyllotoxin 8 is equivalent to one yielding podophyllotoxin because the O glycosides (e.g. Etoposide), which are of interest for biochemical studies, are C4 epimers of podophyllotoxin. Glycosylation of podophyllotoxin takes place with inversion at C4.

Chapter 2

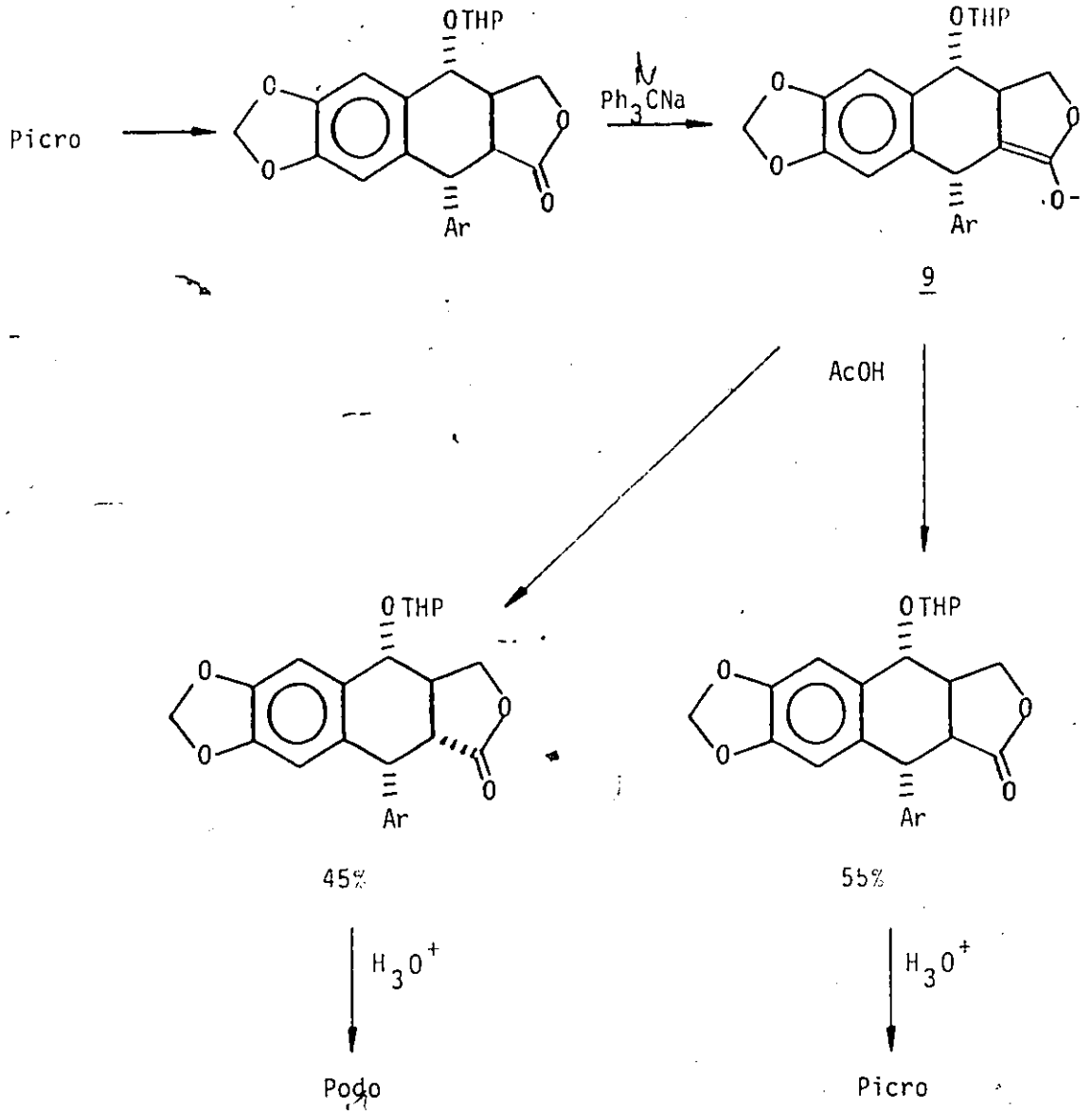
Previous Syntheses of Podophyllotoxin

Several syntheses of podophyllotoxin have been reported, the early ones having the disadvantage that the conversion of the more stable picropodophyllotoxin to podo[1] was required. Two later syntheses were very efficient although modification of stereochemistry was required in the course of the synthesis.

2.1 Early Podophyllotoxin Synthesis

The first synthesis of podophyllotoxin was reported by Gensler in 1966.[11] Two more were reported by Kende in 1977 and Murphy in 1980.[12] All three syntheses produced the more stable picropodophyllotoxin which had been previously converted to podophyllotoxin by kinetic protonation of the enolate 9.[11] The kinetic protonation step is an unfortunate limitation because it produces 55% picropodophyllotoxin which must be recycled (Scheme 2.1). Separation of podo from picropodophyllotoxin requires fractional crystallization, a method which may fail in an analog preparation. Since these syntheses have been carefully reviewed [19] they will not be disussed in detail.

[1] the shorter versions podo and picro will be used in place of podophyllotoxin and picropodophyllotoxin when convenient.



Scheme 2.1: Conversion of Picro to Podophyllotoxin by Kinetic Protonation

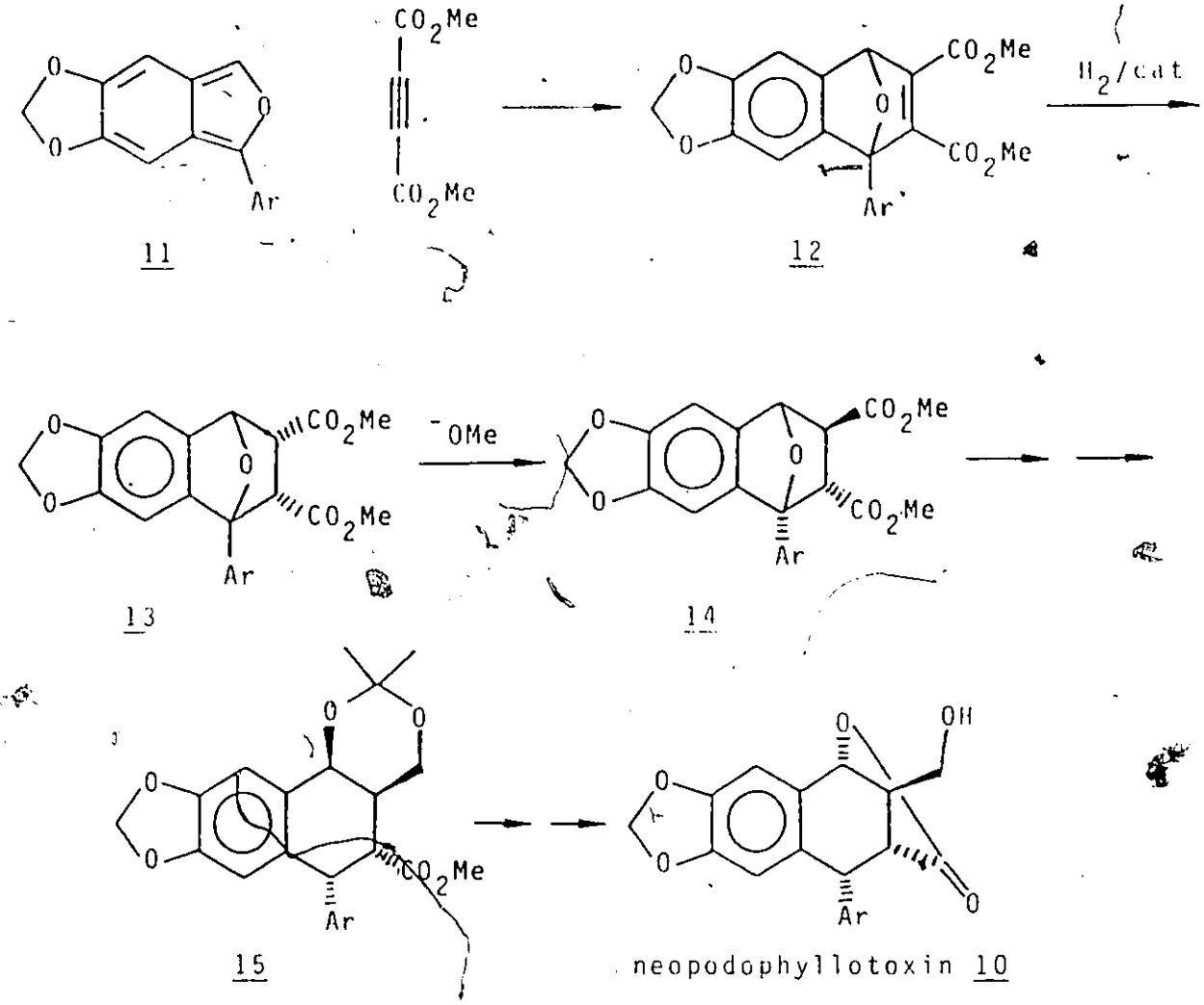
2.2 Synthesis of 1 Not Proceeding Via Picropodophyllotoxin

Recently three syntheses of podophyllotoxin have been reported which do not require the unfavorable conversion of picro to podophyllotoxin in the final stages of the synthesis. Each of these routes lead initially to other isomers of podophyllotoxin, namely neopodophyllotoxin 10 and epipodophyllotoxin 8, which have the same relative stereochemistry at C1 C2 and C3 as does podo, and which had previously been converted to podo.[13] In addition, one of the three suffers greatly from a highly non-stereoselective step involving the formation of the C2-C3 bond.

2.2.1 The Rodrigo Synthesis

In 1981 Rodrigo reported a very efficient synthesis of neopodophyllotoxin 10.[14] Inversion of stereochemistry was required at two chiral centres in the synthesis; the inversions were thermodynamically favorable.

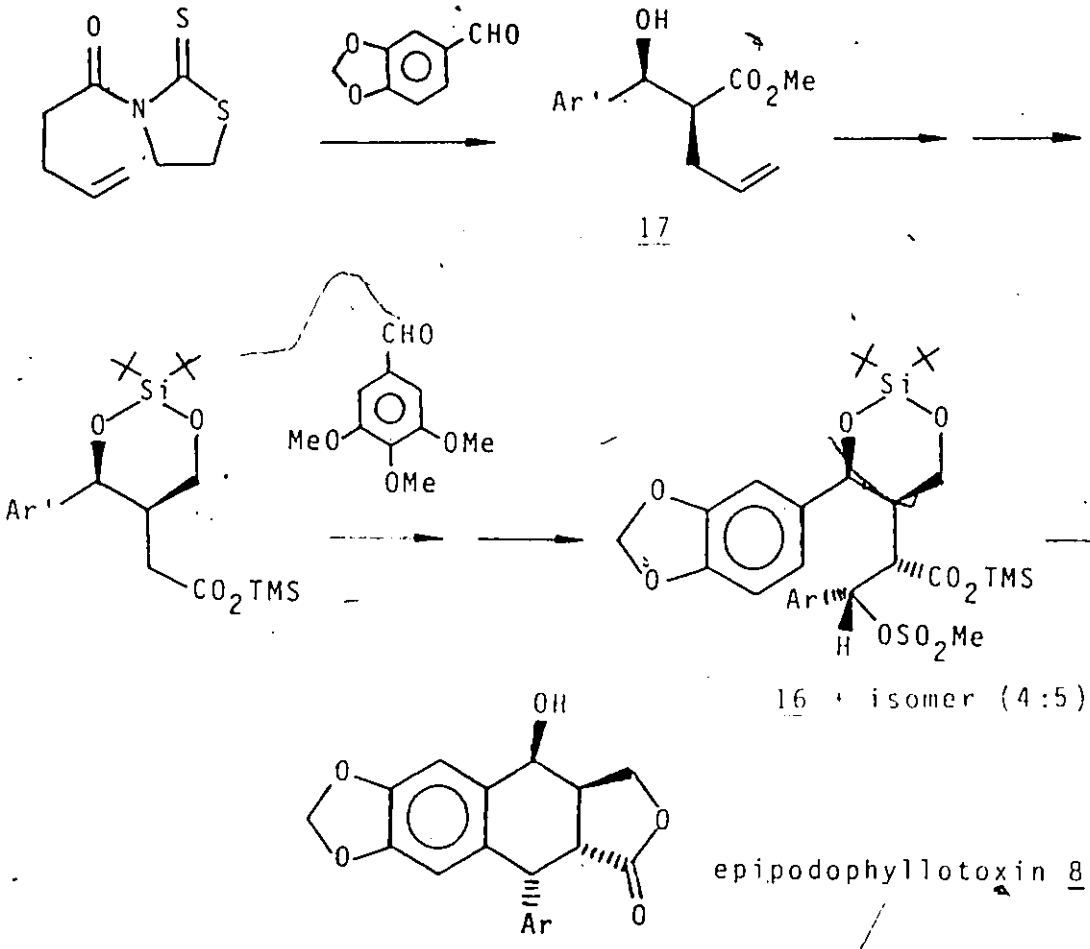
A Diels-Alder reaction of isobenzofuran 11 and dimethylacetylenedicarboxylate produced the basic carbon skeleton. Stereospecific hydrogenation of 12 and base catalyzed epimerization of 13 at C3 using sodium methoxide in methanol established the correct C2-C3 relative stereochemistry. Selective reduction of the C3 ester in 14 and C1-O stereospecific hydrogenolysis was followed by acetonide formation of the diol to give 15. The acetonide



forced the ester moiety into the equatorial conformation which allowed basic hydrolysis of the ester without epimerization. Treatment of the resulting carboxylic acid with dilute aqueous acid to remove the acetonide, was accompanied by epimerization at C4 and subsequent lactonization which produced neopodophyllotoxin.

2.2.2 Vandewalle's Synthesis

In 1985 Vandewalle reported a synthesis of epipodophyllotoxin 8.[16] The synthesis suffered the disadvantage of having a non stereospecific step which produced greater than a 50% proportion of an intermediate with incorrect stereochemistry.

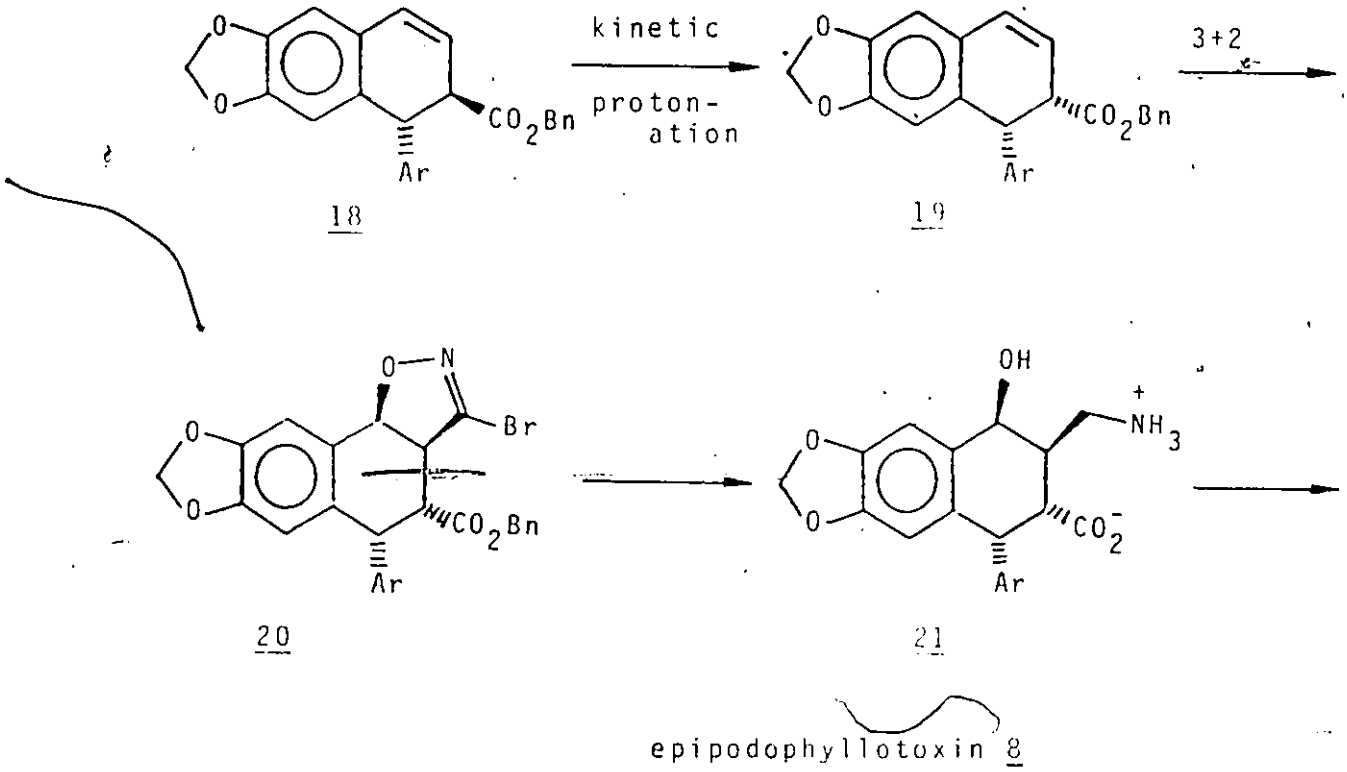


The key step was the stereoselective ring closure, via electrophilic substitution, of mesylate 16. The preparation of 16 was, however, less rewarding. Although the first aldol condensation yielding 17 was stereoselective, the

second to give 16 was non-stereoselective yielding a 4:5 ratio of correct to incorrect isomers.

2.2.3 The Vyas and Doyle Synthesis

In 1986 Vyas and Doyle reported a synthesis that produced epipodophyllotoxin 8.[16] One step involving inversion of stereochemistry was required, but this step proceeded with complete conversion.



Kinetic protonation of trans ester 18 (prepared in 8 steps from piperonal) gave only cis ester 19. Regio and stereospecific (3+2)-cycloaddition of bromonitrile oxide yielded only adduct 20. Reduction to give amino acid 21 followed by diazotization with nitrous acid yielded epipodophyllotoxin.

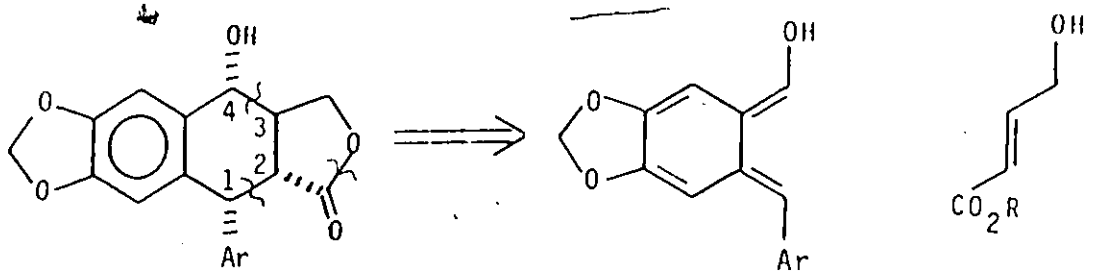
Chapter 3

Direct Generation of all Four Chiral Centres in Podophyllotoxin

Although some of the syntheses mentioned in chapter 2 are very efficient and may have great potential for preparation of derivatives required for biological evaluation, it was noted that all required stereochemical modification during the course of the synthesis. These stereochemical modification steps are not likely to cause a loss in generality of these syntheses, however a direct synthesis would certainly be advantageous.

3.1 Retrosynthetic Analysis

If the lactone is formed last then the C1-C2 and the C3-C4 bonds could be formed by a Diels-Alder reaction which could, in principle, produce the correct stereochemistry at all four chiral centres. In order for this to occur a correctly substituted *o*-quinodimethane 22 and crotonate 23 would be required, and these must combine with the desired regio and stereochemistry. These possibilities were investigated earlier in simpler model systems by Khan and others.

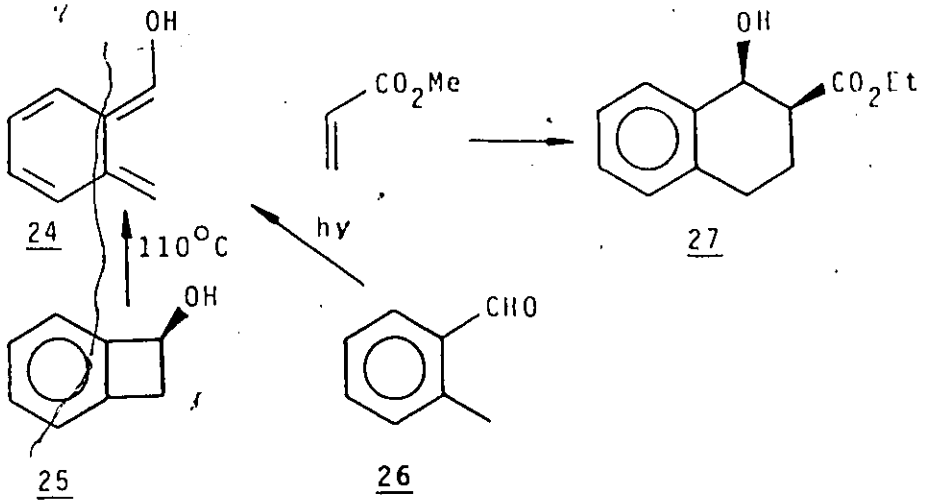


1 22 23

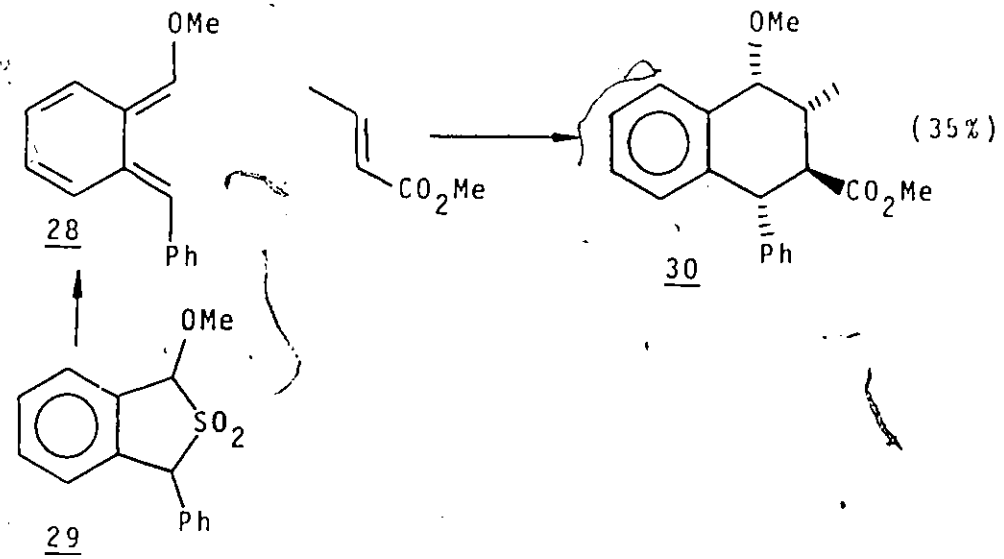
3.2 Model Experiments

The required *o*-quinodimethanes may be generated by methods such as thermal ring opening of the appropriately substituted benzocyclobutenols, thermal extrusion of sulfur dioxide from a dihydrobenzothiophene sulfone, or by photoenolization of an ortho substituted benzaldehyde.

Experiments in which a 1-hydroxy substituted *o*-quinodimethane 24 was generated both by thermolysis of benzocyclobutenol 25 and photolysis of o-methyl benzaldehyde 26, and trapped by Diels-Alder reaction with acrylate, yielded a product with both incorrect regio and stereochemistry, i.e., 27.[17]



An experiment in which methoxy and phenyl substituted *o*-quinodimethane 28 was generated by thermal extrusion of sulfur dioxide from sulfone 29, and trapped by Diels-Alder reaction with crotonate, yielded a product with the correct regiochemistry but the incorrect stereochemistry 30.[18]



The regio- and stereochemical problems encountered in the model experiments indicate the need for a system in which these variables are controlled.

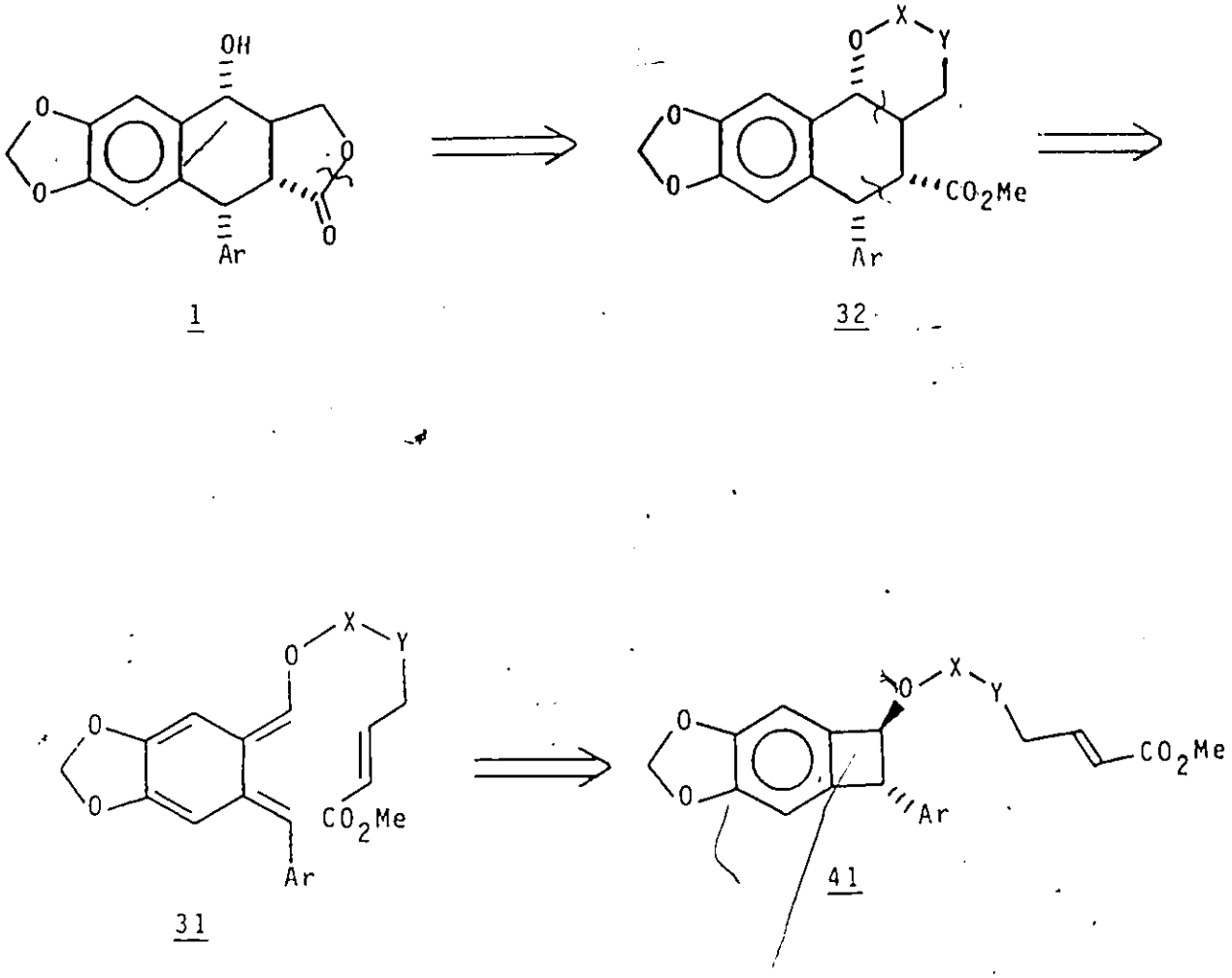
3.3 Intramolecular Approach

Due to the regio and stereochemical problems encountered in the model systems which made use of an intermolecular Diels-Alder reaction, an intramolecular approach was examined.

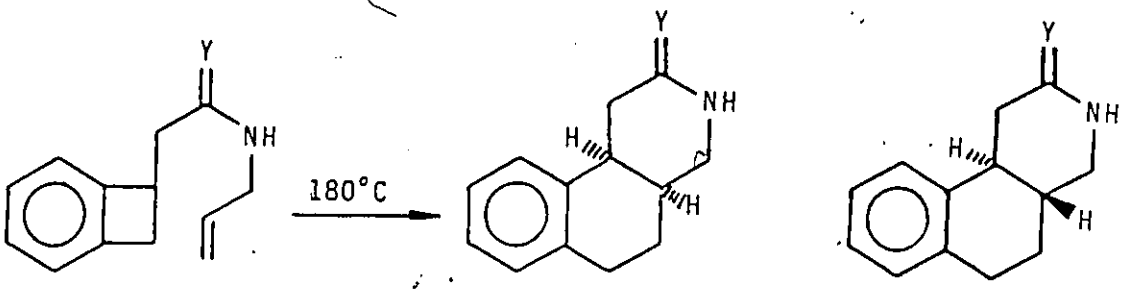
3.3.1 Retrosynthetic Analysis

Using the same retrosynthetic approach as before except that the diene and dienophile components are connected by a bridge between the hydroxyl groups 31,[19] it was rationalized that the Diels-Alder reaction should produce the correct regio and stereochemistry, i.e., 32 (Scheme 3.1). Incorrect regiochemistry is impossible due to structural limitations. The stereochemistry should be correct since trans fused bicyclic 4.4.0 systems are less strained than cis.[20]

The effect of various bridging systems on the stereochemistry of ring fusion is somewhat uncertain. Previous examples such as 33 and 34 have shown the bridging moiety can have a large effect on the stereochemistry of ring fusion [21].

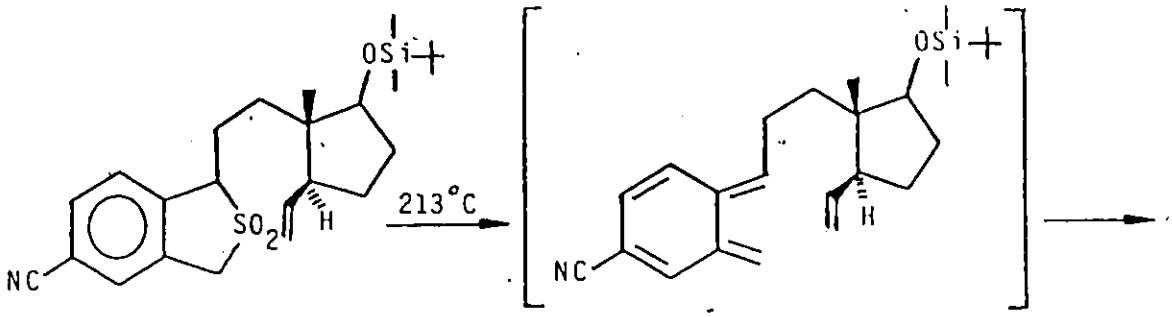


Scheme 3.1: Proposed Intramolecular Approach

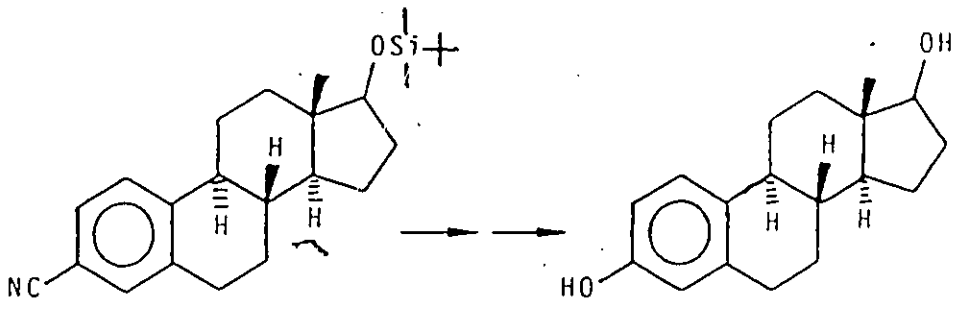


33	Y = H_2	12 %	87 %
34	Y = O	79 %	20 %

The intramolecular Diels-Alder reaction of *o*-quinodimethanes has been used in the synthesis of many natural products. The enantioselective synthesis of (+)-estradiol 35 using sulfone 36 to produce the required *o*-quinodimethane 37 and subsequent cycloaddition, to give intermediate 38 containing the correct stereochemistry at the ring fusion, is a representative example of the reaction's preparative utility.[22]



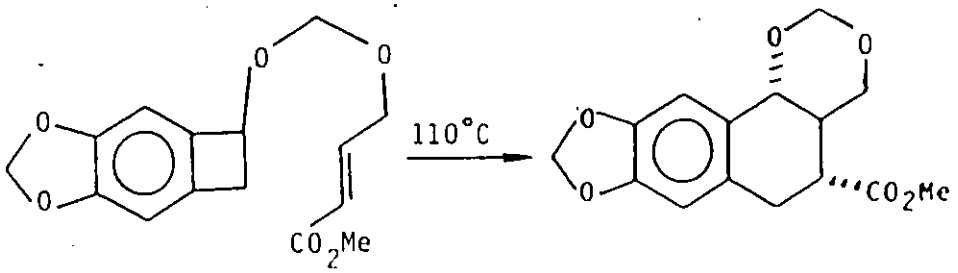
36 37



38 35 (+)-estradiol

3.3.2 Model System

The model system 39, in which a monosubstituted *o*-quinodimethane was used, produced the correct regio and stereochemistry 40.[19]



39 40

The fact that the intramolecular model system produced the correct regio and stereochemistry at C4, C3 and C2 was encouraging, but the question of what difference the 2-aryl substituent (required for podophyllotoxin) would make remained to be investigated. The Woodward-Hoffman Rules predict that ring opening of the *trans*-1,2 substituted benzocyclobutene 41 should produce the required *E,E*-*o*-quinodimethane 31 (Scheme 3.1). If the dienophile in 31 added to diene in the endo mode, as in the case of model system 39, then the required relative stereochemistry for 1 should be produced. If however the dienophile were to add in the exo mode, to give a cis fused isomer of 32, then the relative stereochemistry of epiisopodophyllotoxin 42 [19] would be produced.

In this thesis, a successful synthesis of (±)-podophyllotoxin 1, using the synthetic methodology proposed in this section, is described.

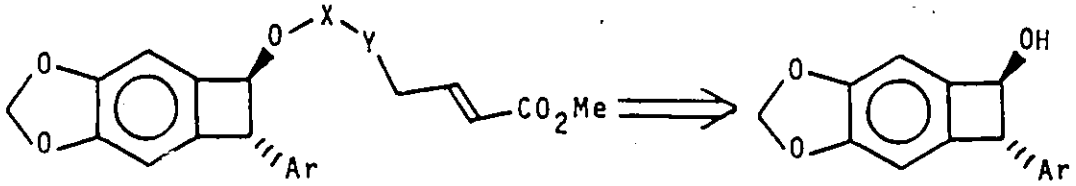
Part II

Results and Discussion

Chapter 4

Preparation of 2-Arylbenzocyclobutenols

In 3.3.1 a synthesis of podophyllotoxin was proposed in which an intramolecular Diels-Alder reaction would produce the correct relative stereochemistry at all four chiral centres. In order to accomplish this goal it was noted that a precursor for *o*-quinodimethane 31 was required. One such precursor is the *trans*-1,2 substituted benzocyclobutene 41, which should produce 31 upon thermal conrotatory ring opening. In order to prepare 41, the *trans*-2-arylbenzocyclobutenol 44 was required.



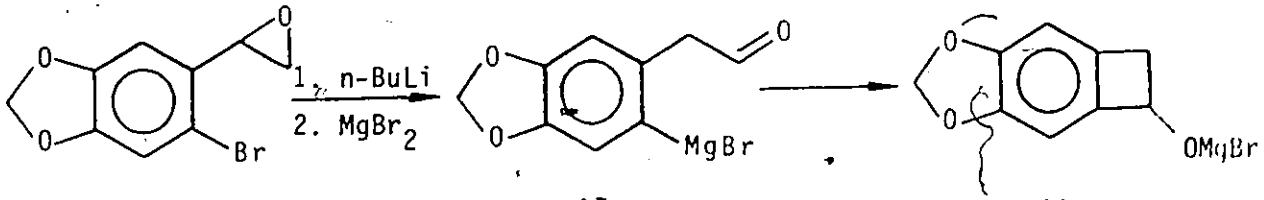
41 44

4.1 Previous Attempts at Synthesis of *trans*-2-Aryl-benzocyclobutenols

Examination of the literature revealed that no synthesis of benzocyclobutenols bearing a 2-aryl substituent had been described. In 1985 Jung reported a lengthy description of how all known methods had failed to produce 44 or analogs thereof.[20] Many of the methods attempted by Jung had also been tried by previous workers in our laboratory, without success.[19] Several of the previously attempted synthesis of 2-arylbenzocyclobutenols are shown in Figure 4.1.

4.2 Further Unsuccessful Attempts

The method involving butyllithium and magnesium bromide treatment of an *ortho*-bromoaryl epoxide as shown in example 2 of Figure 4.1, was applied to the methylenedioxy substituted compound 45. This method is sucessful with compounds not containing the phenyl group attached to the epoxide ring and is known to involve lithiation of the aryl bromide followed by Lewis acid catalyzed rearangement of the epoxide moiety to aldehyde 47 (accompanied by lithium magnesium exchange) and subsequent ring closure to give alkoxide 48.[23]



47 48

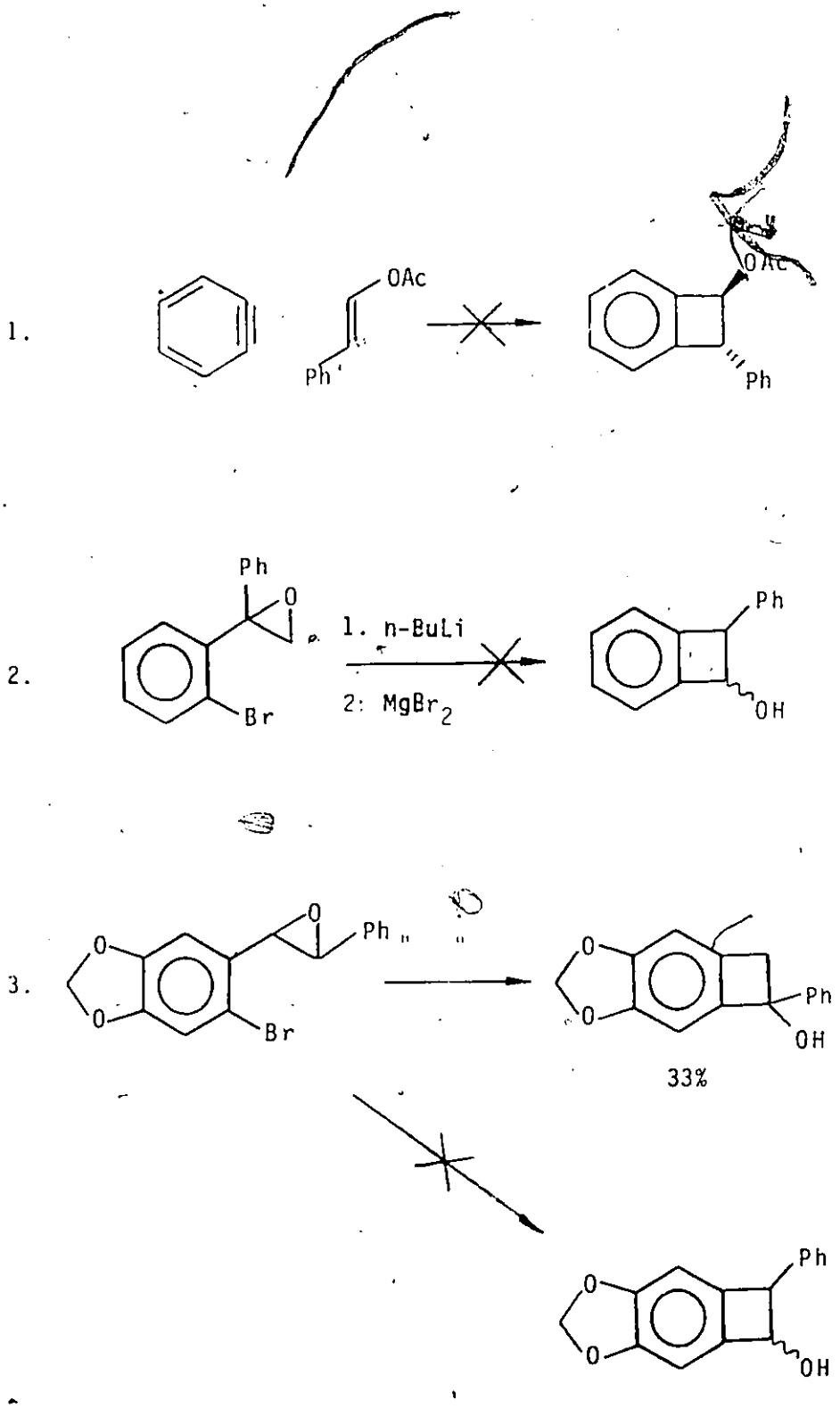
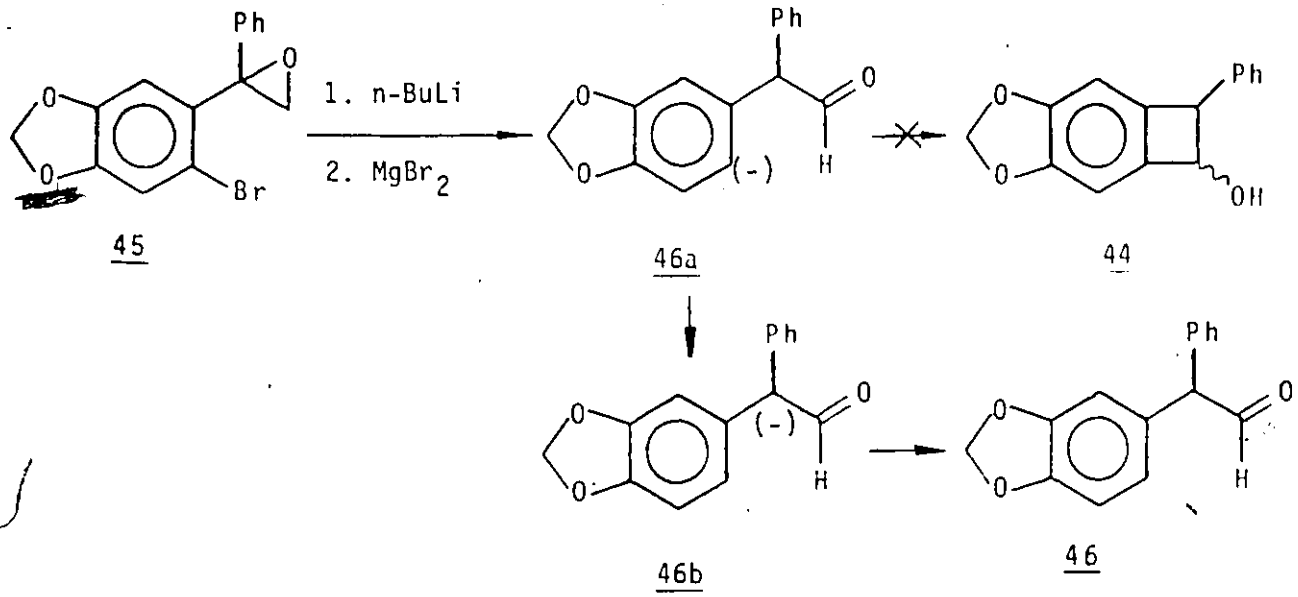


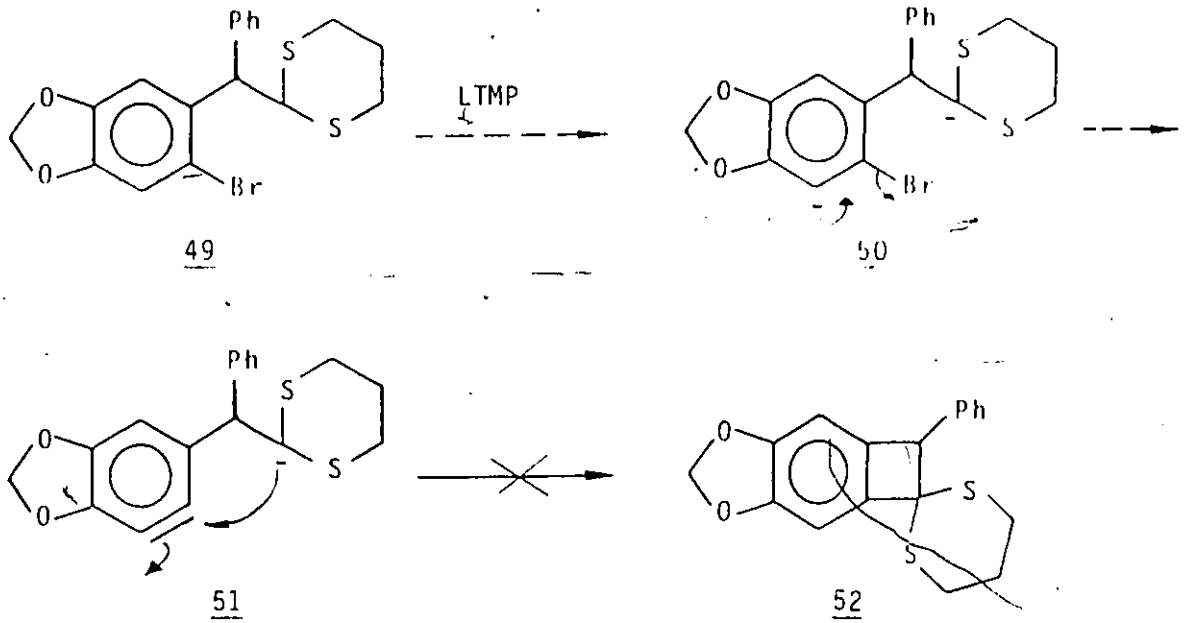
Figure 4.1: Previous Attempts at Synthesis of 2-Arylbenzocyclobutenols

Exposure of 45 to the typical conditions: halogen-metal exchange with n-butyllithium at -78°C followed by addition of magnesium bromide etherate and warming the reaction mixture to 0°C prior to quenching with aqueous ammonium chloride, resulted in the formation of the aldehyde 46 (pmr δ = 9.80 due to the aldehyde H) as the only characterizable product in 10% yield. No evidence for the formation of 44 was obtained. The formation of 46 may



result from proton transfer (intra or intermolecular) in the intermediate 46a to the more stable 46b.

A method which had not been previously reported, in which dithiane 49 was treated with 3 equivalents of lithium tetramethylpiperidide in THF at -78 to 25°, was attempted. It was proposed that the base would produce di-anion 50 which should eliminate bromide ion to give benzyne 51. Sub-

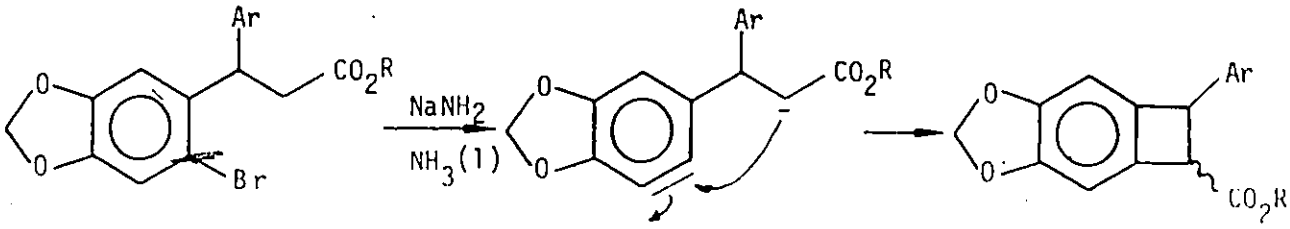


sequent nucleophilic attack of the carbanion on the benzyne should yield benzocyclobutene 52 upon protonation of the resulting anion. The product dithiane could then be hydrolysed to a ketone and, in principle, reduced to yield the desired 2-arylbenzocyclobutenol. The attempt was unsuccessful, yielding only an uncharacterizable mixture of products. One possible reason for the failure of this approach may be that benzyne formation from 49 preceeded formation of the dithiane anion, thereby eliminating the potential for the desired intramolecular benzyne trapping.

4.3 First Successful Synthesis of a 2-Arylbenzocyclobutenol

In view of the difficulties encountered in the previous attempts to synthesize 2-arylbenzocyclobutenols, we decided to try a longer but safer route to these compounds. It has been known for some time that the intramolecular attack

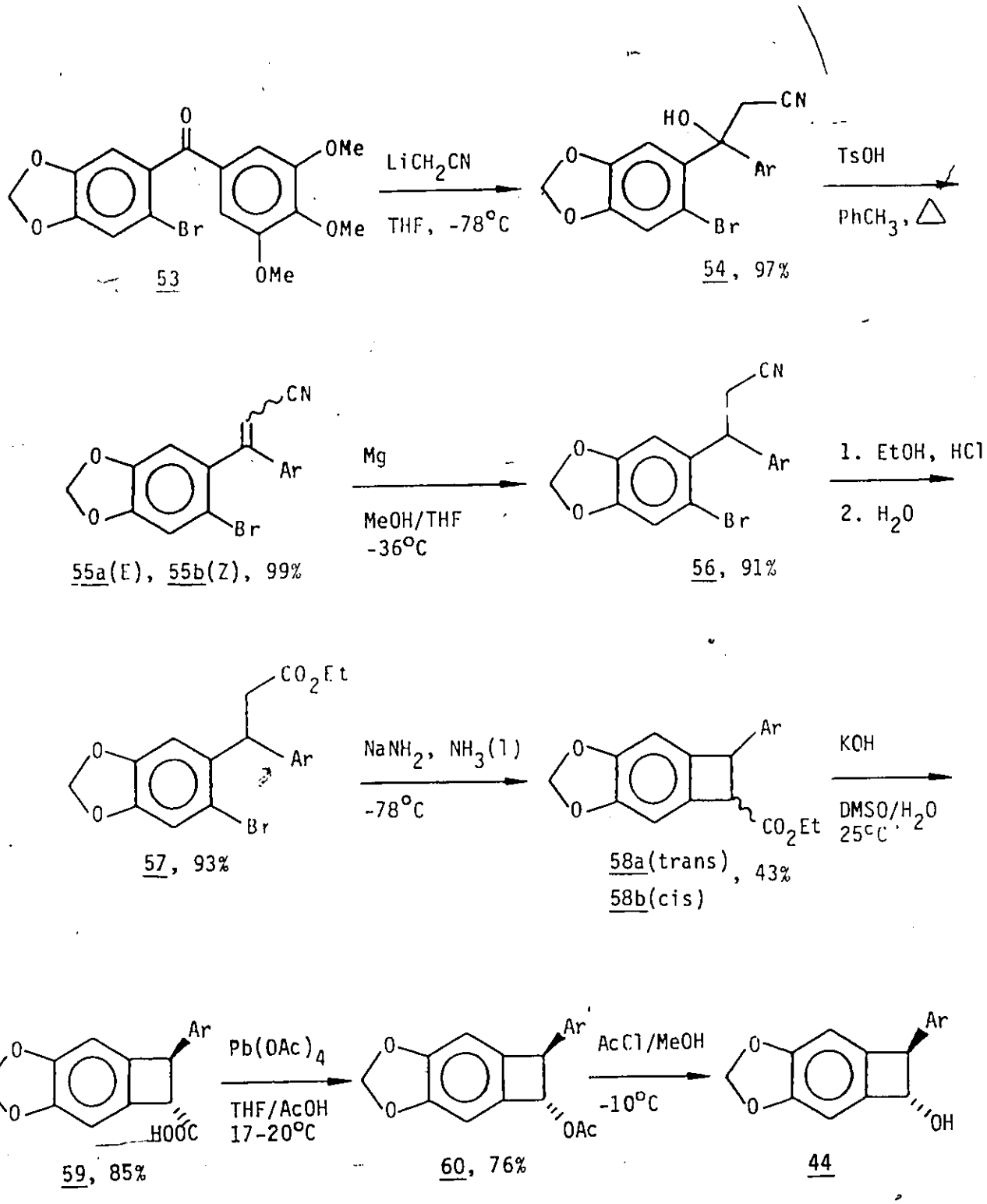
of a carbanion, such as an enolate, on a benzyne is a synthetically useful method for the construction of the benzocyclobutane ring.[24] The benzyne-enolate intermediates have been generated most commonly by deprotonation and dehydrohalogenation of a bromoarylester, using a strong base such as sodium amide in liquid ammonia. The method had been used before but



only when Ar=H. There was however no reason to expect that a simple Ar group would have an adverse effect on the cyclization. It was anticipated that the carboalkoxy group could be converted to the required hydroxyl via oxidative decarboxylation.

The successful synthesis of 2-arylbenzocyclobutenol 44 using the above methodology is illustrated in Scheme 4.1.[25] The route has been published in preliminary form [25] using a slightly different method (than that of Scheme 4.1) to prepare the ester 57. The reason for presenting a different method for the preparation of 57 in Scheme 4.1 is due to reproducibility problems with our previously published method.

Shortly after our publication, Jung and coworkers published a variation of our approach with the major differences being the method of constructing an equivalent of ester 57 (they used the respective nitrile) and the use of LDA



Scheme 4.1: Synthesis of 2-Arylbenzocyclobutenol 44.[25]

in THF to generate the key benzyne intermediate.[26] The overall yield of 44 via the two procedures was not substantially different.

Ketone 53, previously prepared in three steps from piperonal in 60% overall yield [19], was reacted with lithioacetonitrile in THF at -78°C to give alcohol 54, mp 171-172°C in 97% yield. The alcohol was characterized by infrared bands at 3300-3600 and 2257 cm^{-1} due to the alcohol and nitrile groups. The proton nmr contains two doublets(J=16Hz) at 3.33 and 3.40 ppm due to the two hydrogens α to the nitrile, in addition to the expected aromatic and methylenedioxy peaks. The two α protons are diastereotopic due to the adjacent chiral centre.

Acid catalyzed dehydration of 54 yielded a 1:1 mixture of E and Z alkenes 55a and 55b in 99% yield. The alkenes were characterized by an infrared band at 2248 cm^{-1}, due to the nitrile group. The proton nmr spectra of the two isomers are as follows: 55a δ(ppm) 3.79(s,6H,), 3.86(s, 3H), 5.89(s, 1H), 6.06(s, 2H), 6.47(s, 2H), 6.76(s, 1H), 7.10(s, 1H). 55b 3.81(s, 6H), 3.88(s, 3H), 5.44(s, 1H), 6.04(s, 2H), 6.70(s, 1H), 6.71(s, 2H), 7.04(s, 1H). The stereochemistry of the isomers was assigned on the basis of the proton chemical shift differences in the bromoaryl rings. The E isomer should have greater deshielding in the bromoaryl ring than the Z isomer due to the fact that translation of electron withdrawing effects are greater through E substituents than Z substituents on alkenes. The chemical shift differences of the trimethoxyaryl ring protons obey the same reasoning.

Dissolving metal reduction of the alkene mixture 55a and 55b, using magnesium in methanol/THF, yielded the saturated nitrile 56, mp 157°C in 91% yield. Nitrile 56 was characterized by an infrared band at 2248 cm^{-1},

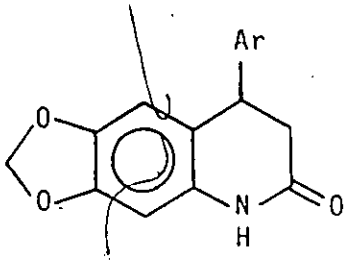
due to the nitrile group, and its proton nmr spectrum which contained (in addition to the expected aromatic and methylenedioxy peaks) a doublet at 2.95 ppm (J=7.4Hz, 2H) and a triplet at 4.75 ppm (J=7.4 Hz, 1H) due to the nitrile α hydrogens and the benzylic hydrogen.

The conditions for the magnesium in methanol reduction were critical. Reduction of the aryl bromide resulted when the reaction temperature was above -36°C. The cosolvent THF was required to increase the solubility of the compounds in the reaction mixture.

Acid catalyzed ethanolysis of the nitrile moiety followed by hydrolysis of the resulting imino ester yielded ester 57 mp 113°, in 93% yield. Ester 57 was characterized by an infrared band at 1734 cm^{-1} and its proton nmr which is similar to that of nitrile 56 except for the addition of a triplet at 1.13 ppm (J=7.2 Hz, 3H) and a quartet at 4.05 ppm (J=7.2 Hz, 2H) due to the ethoxy group.

Reaction of bromoarylester 57 with sodium amide (4 equivalents) in liquid ammonia, at -78°C for 10 minutes, yielded a 1:1 mixture of *trans* and *cis* benzocyclobutene esters 58a and 58b in 42% yield. The benzocyclobutene esters were characterized by infrared bands at 1730 cm^{-1} for 58a and 1725 cm^{-1} for 58b. The two isomers were distinguished on the basis of their proton nmr spectra. In addition to the expected aryl, methylenedioxy and ester signals, isomer 58a has two doublets at 3.88 and 4.65 ppm (J=2.1 Hz, 1H each), due to the ester α and dibenzylic protons. The 2.1 Hz coupling indicated 58a has *trans* stereochemistry. Isomer 58b has a similar proton nmr spectrum except that the two protons on the benzocyclobutene ring at 4.46 and 4.83 ppm have a coupling of 5.7 Hz, which indicates *cis* stereochemistry.

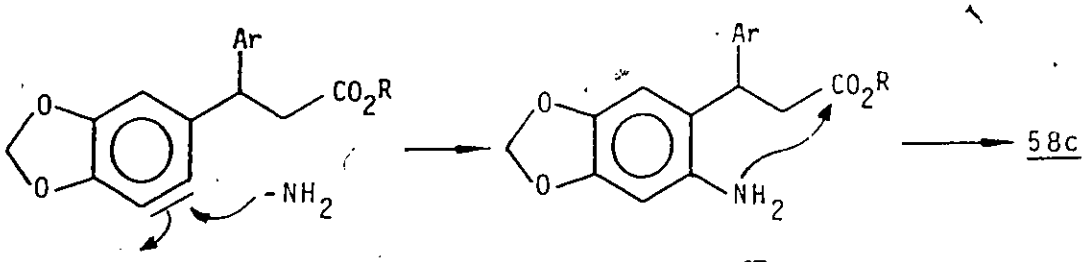
The reaction also gave a crystalline byproduct which was determined to have structure 58c, mp 212-213°C, in 11% yield. The byproduct 58c was



58c

characterized by infrared bands at 3420 and 1680 cm^{-1} due to the amide group, as well as proton nmr signals at δ 2.80(dd, J=16.0, 8.8 Hz, 1H), 2.87(dd, J=16.0, 6.6 Hz, 1H) and 4.09(dd, J=8.8, 6.6 Hz, 1H) due to the secondary and tertiary hydrogens respectively.

Formation of 58c was presumably via nucleophillic addition of amide ion to the benzyne followed by ring closure to form an amide.



The cyclization reaction was very fast, even at -78°C, and it was important to quench the reaction very shortly after completion in order to

maximize the yield. Optimum yields were obtained with four equivalents of sodium amide. The use of three equivalents or less, two are required stoichiometrically, resulted in recovery of some starting material. The benzocyclobutene esters were found to be very labile; extensive decomposition occurred by leaving them open on the bench for a few days.

Saponification of the mixture of esters 58a and 58b at 25°C using KOH in DMSO-water was accompanied by isomerization and afforded the *trans* acid 59 mp 145°, in 85% yield. The infrared spectrum of 59 contains bands at 2500-3500, 1700 and 1730 cm^{-1} characteristic of a carboxylic acid. The proton nmr spectrum of 59 was similar to the trans ester 58a without the ethoxy signals. The two benzocyclobutene ring protons produce two doublets at 3.93 and 4.67 ppm having a coupling of 1.9 Hz, characteristic of the *trans* stereochemistry.

Oxidative decarboxylation of 59 using lead tetraacetate in THF-acetic acid at 17-20°C afforded the benzocyclobutyl acetate 60 mp 134-135°C, in 76% yield. This compound was characterized by an infrared band at 1735 cm^{-1} and a 3H singlet at 2.12 ppm (3H) due to the acetate group as well as two doublets at 4.38 and 5.45 ppm (J=1.8 Hz, 1H each) due to the dibenzylic hydrogen and the hydrogen α to the acetoxy group in its proton nmr spectrum. The 1.8 Hz coupling between the two hydrogens indicates a *trans* relationship about the cyclobutane ring. The proton nmr spectrum of 60 is shown in Figure 4.2.

Careful control of the temperature was required and it was important to use only one equivalent of lead tetraacetate. Large amounts of a compound containing an acetate group on the methylenedioxy bridge 61 were

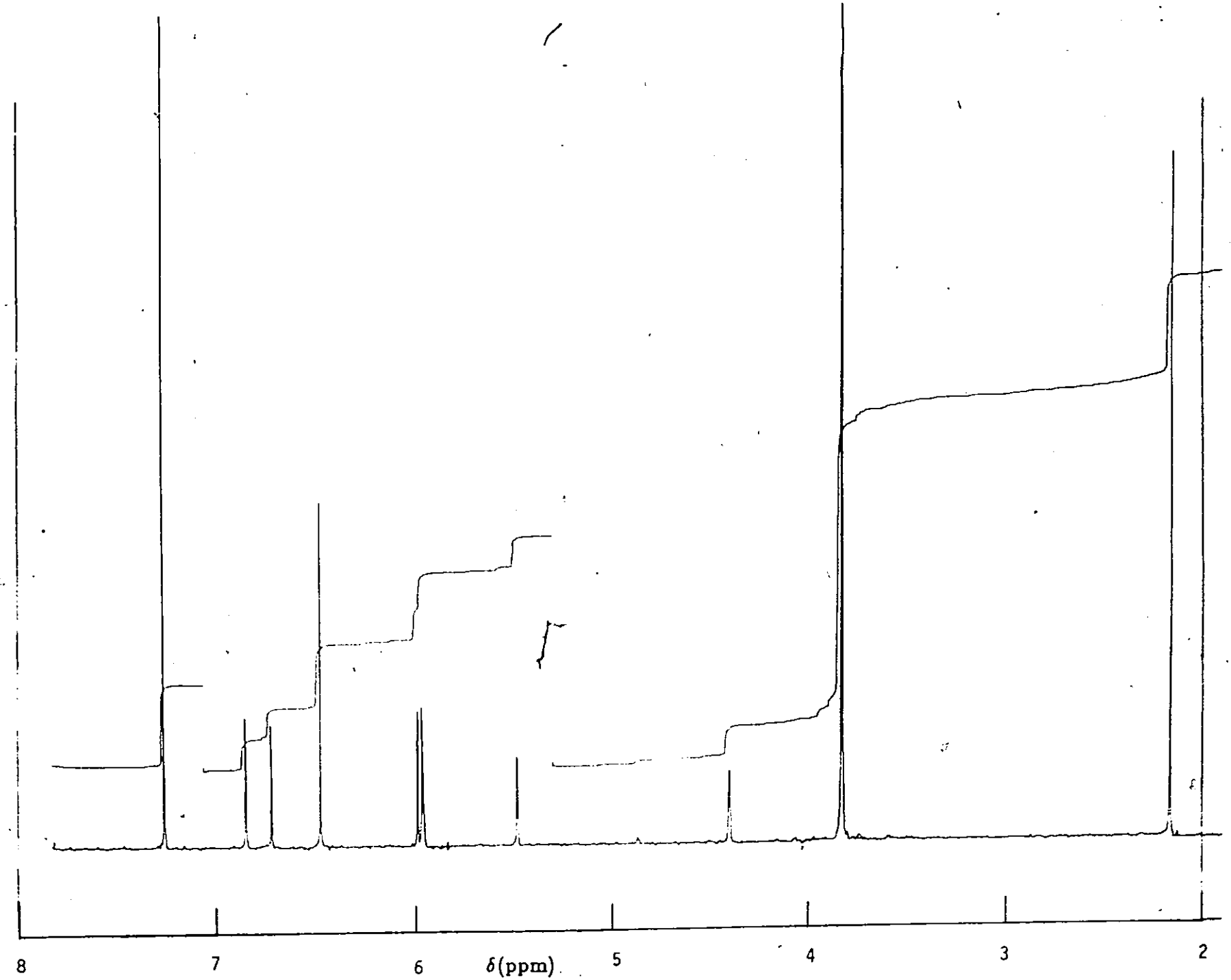
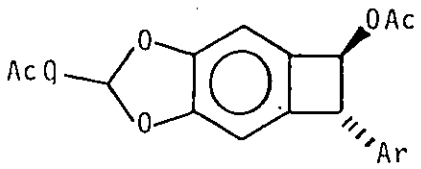


Figure 4.2: Proton NMR Spectrum of 2-Arylbenzocyclobutyl Acetate 60

produced, if the temperature was too high or excess lead tetraacetate was used. Compound 61 was characterized by its proton nmr which showed a

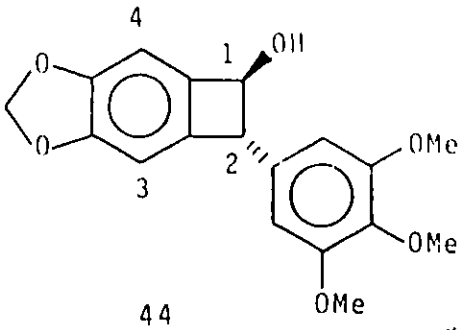


61

second acetate peak at 2.1 ppm as well as a singlet at 7.7 ppm (1 H), due to the remaining methylenedioxy proton, in place of the usual methylenedioxy signals at 5.93 and 5.96 ppm.

Deprotection of the acetate 60 to give the alcohol 44 was accomplished by acid catalyzed methanolysis, using HCl generated by the reaction of acetyl chloride with methanol as catalyst. Conversion of 60 to 44 required 5h at -10°C and was performed in a mixture of methanol-methylene chloride, in which 60 was sufficiently soluble.

The alcohol 44 was characterized by an infrared band at 3200-3510 cm^{-1} due to the hydroxyl group. The proton nmr showed, singlets at 4.77(1H), 4.17(1H), 6.71(1H), 6.84(1H), 6.40(2H), 3.80(6H), 3.82(3H) and two doublets at 5.93(J=2.0, 1H) and 5.96(J=2.0, 1H) due to H1, H2, H3, H4, the aryl ring, the methoxy groups on the aryl ring and the two methylenedioxy protons, respectively (see structure for numbering). The fact that the

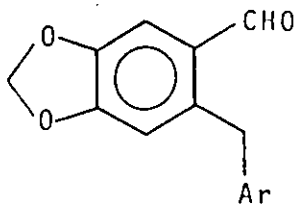


44

two benzocyclobutene ring protons H1 and H2 are singlets, or doublets with couplings too small to be detected, indicates that the *trans* stereochemistry was retained in the acetate methanolysis. A shift of the α acetoxy hydrogen signal from 5.45 ppm in acetate 60 to 4.77 ppm in alcohol 44, as well as the absence of the acetate peak at 2.12 ppm, verified the removal of the acetate group. A broad proton nmr signal with variable chemical shift (1H), which overlapped the 4.77 ppm signal in the spectrum reported, was due to the hydroxyl group in 44. Other proton nmr spectra of 44 have the OH signal at a different location.

The alcohol 44 was found to be extremely labile. If the methanolysis reaction was performed at temperatures above 0° or if basic conditions were used for the methanolysis (e.g. potassium carbonate in methanol), partial or complete conversion to aldehyde 62[1] resulted. Even short periods of standing in a neutral solution at 25°C or concentration of solutions of 44 resulted in

[1]Aldehyde 62 has been previously reported.[19]



62

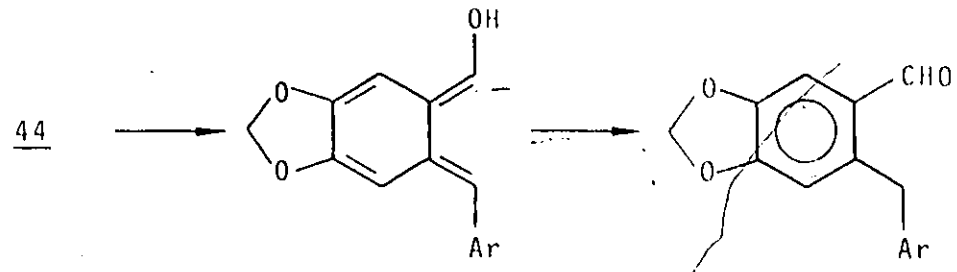
the formation of considerable amounts of aldehyde 62.

The ready conversion of 44 to 62 was easily monitored by nmr (probe temperature = 30°C). Over a 5 minute period the amount of alcohol to aldehyde conversion was noted to be about 10%.

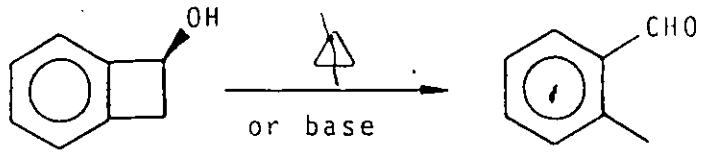
Due to the extremely labile character of benzocyclobutenol 44, the workup of the methanolysis reaction was carried out at 0°C and the solvent was not completely removed. Residual methanol was removed by several partial evaporations of a carbon tetrachloride solution, via rotary evaporator, at temperatures between 0 and 25°C. Due to its instability, the alcohol 44 was stored in the form of the more stable acetate 60. This compound can be stored for long periods of time in the freezer.

The extreme lability of the 2-arylbenzocyclobutenol 44 relative to benzocyclobutenol itself was unexpected and may explain why other workers had such difficulty in their attempts to prepare it.[19,20]

The mechanism of conversion of alcohol 44 to aldehyde 62 was assumed to be via thermal electrocyclic ring opening of the benzocyclobutene ring to yield *o*-quinodimethane 63, followed by 1,5-sigmatropic rearangement to



give aldehyde 62. The reason for the alcohol's instability is somewhat uncertain but can be explained as being due to a number of factors. Benzocyclobutenol 64 produces the respective aldehyde 65 when heated or subjected



to strong base.[17] Thus compound 64 undergoes the same process but under much more severe conditions. The 2-aryl group is therefore responsible for lowering the activation energy of the process described above. If the rate determining step is assumed to be the ring opening, then the effect of the 2-aryl group might be explained as follows:

1. An increased strain in the benzocyclobutene ring, due to the position

of the 2-aryl group, thus raising the energy of starting material.

2. A gain in conjugation of the π system and the 2-aryl group upon ring opening of the benzocyclobutene ring to form *o*-quinodimethane 63, thus lowering the energy of 63.

The two factors would be expected to lower the activation energy of the ring opening and therefore make the conversion of alcohol 44 to aldehyde 62 more facile. The mechanism by which basic conditions increase the rate of aldehyde formation is assumed to be via formation of the alkoxide, which undergoes the ring opening reaction to give the *o*-quinodimethane more easily than the alcohol. This rate enhancement upon formation of an alkoxide is known with other electrocyclic processes as well, the anionic oxy-Cope rearrangement being a well known example.[27] The alkoxide effect has been explained as being due to a stabilization of the alkoxide in the transition state, by delocalization which is not present in the starting alkoxide, thus lowering the activation energy due to a greater increase in the energy of the starting material than the transition state.[28]

Chapter 5

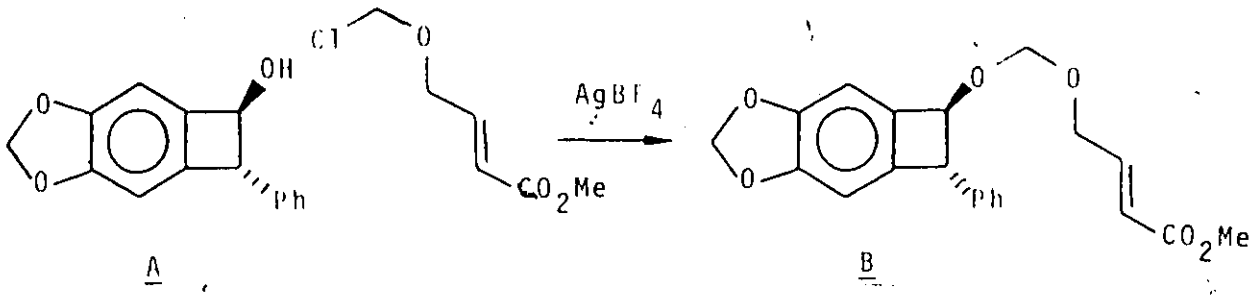
Synthesis of (±)-Podophyllotoxin Via 2-Arylbenzocyclobutenol 44

It was noted in Chapter 4 that the 2-arylbenzocyclobutenol 44 was needed in order to prepare the *o*-quinodimethane precursor 41, required for the proposed Diels-Alder based podophyllotoxin synthesis. In Chapter 4 the first synthesis of 44 was presented and its extreme lability was noted.

5.1 Coupling of Benzocyclobutenol 44 With a Crotyl Moiety

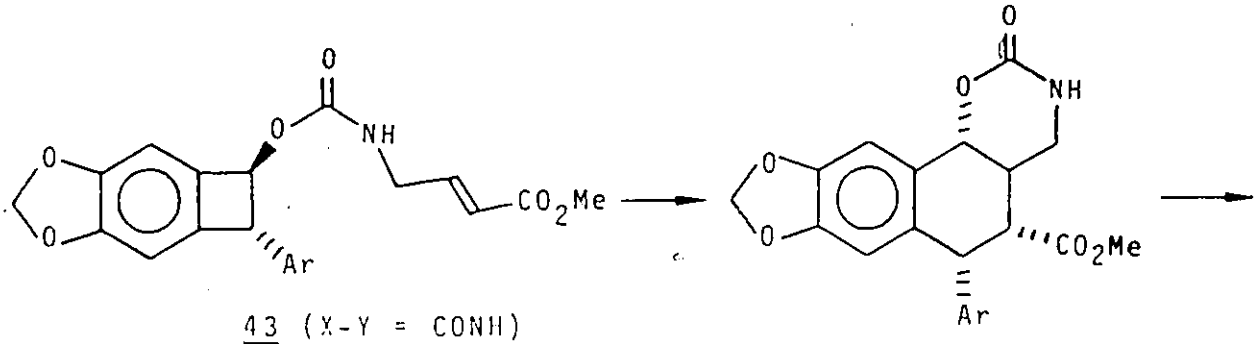
The very labile character of 44, especially toward mildly basic conditions, dictated that the connection between 44 and a crotonic ester to prepare 41 be made under essentially neutral conditions. It removed the possibility of using a carbonate (X-Y = COO, as proposed by Jung [20]) or a mixed acetal (X-Y = CH_2O, used by Glinski and Durst [19]) since the preparation of mixed acetals or mixed carbonates generally requires basic catalysis.

An attempt was made to couple the benzocyclobutenol A to a chloromethyl ether, using silver ion as catalyst, in order to prepare the mixed acetal B, but the yield was low and the results uncertain.

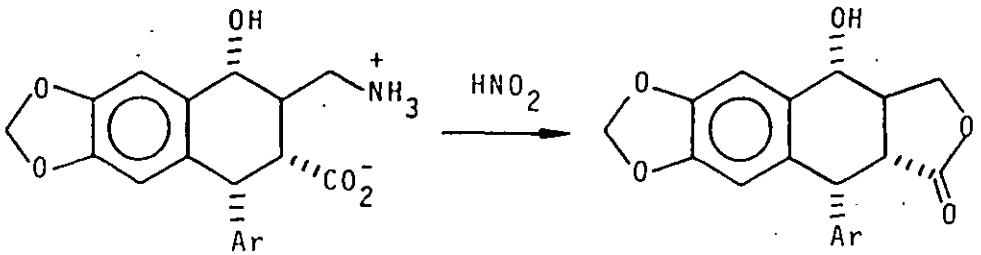


A B

Isocyanates are known to couple with alcohols under neutral conditions and the reaction may be catalyzed by use of catalysts such as triphenyltin acetate.[29] Thus it was decided to connect 44 to the required *trans*-crotyl ester moiety using a urethane function (X-Y = CONH). A second advantage of the urethane connecting group was the expected cyclic urethane 32 (the



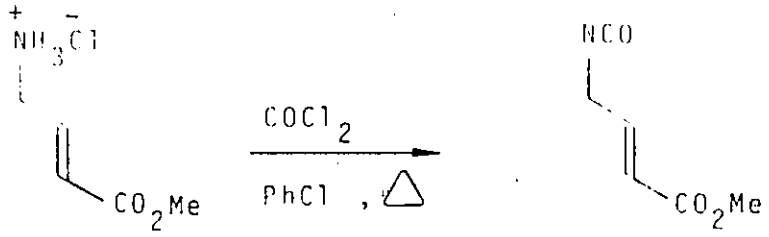
43 (X-Y = CONH) 32 (X-Y = CONH)



66 1

product of the anticipated Diels-Alder reaction) should be hydrolyzable to amino acid 66, which upon diazotization would be expected produce the required lactone. The diazotization of a similar amino acid had been previously used to generate the lactone ring in epipodophyllotoxin 17 by Vyas and Doyle of Bristol-Myers [16].

Thus methyl *trans*-aminocrotonate hydrochloride 67[1] was reacted with phosgene in refluxing chlorobenzene to yield isocyanate 68 bp 65°C (0.075 mm) in 57% yield. Isocyanate 68 showed the characteristic isocyanate and

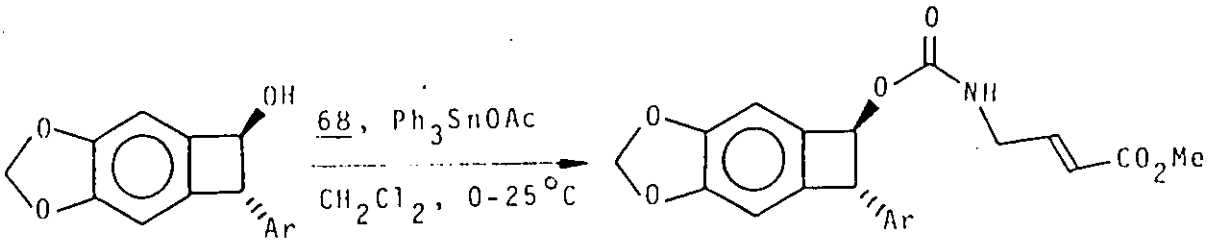


67 68, 57%

ester bands at 2257 and 1725 cm^{-1}; its proton nmr displayed absorptions at δ(ppm) 3.74 (s, 3H), 4.13 (dd, J=4.3, 2.1 Hz, 2H), 6.10(dt, J=15.4, 2.1 Hz, 1H), 6.88(dt, J=15.4, 4.3 Hz, 1H).

Coupling of 68 and benzocyclobutenol 44, using triphenyltin acetate as catalyst in methylene chloride at 0-25°C, yielded urethane 69 as white needles, mp 141-142°C in 47% yield.

[1] 67 was prepared in 54% yield by reaction of 4-bromocrotonic acid with excess ammonia followed by esterification with HCl in methanol, according to the methods of Pinza and Brehm.[30]



44 69, 47%

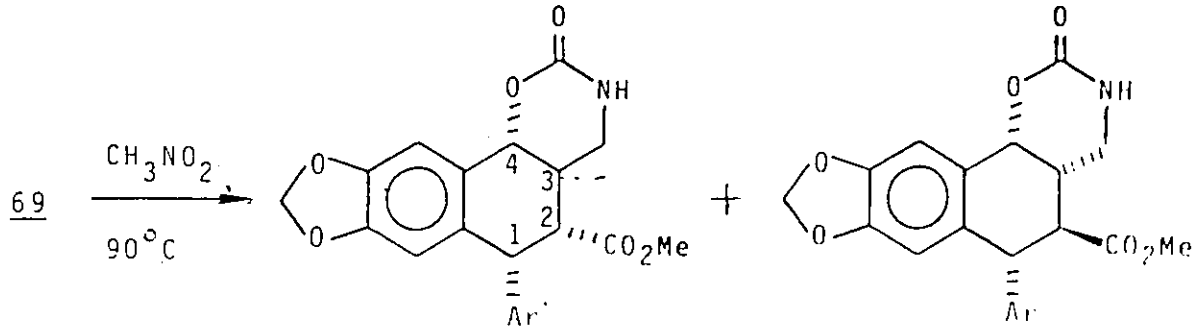
Due to the labile character of benzocyclobutenol 44, it was necessary to ensure the coupling reaction be very fast and at a temperature below 25°C. To fulfill this requirement it was necessary to evaporate the reaction mixture to a viscous oil in order to increase the concentration of the reactants and thus obtain a reasonable reaction rate. Under these conditions of high concentration the reaction was complete almost instantaneously. At lower concentrations the reaction was very slow, thus allowing extensive conversion of 44 to the aldehyde 62.

Urethane 69 was characterized by infrared bands at 3340 and 1720 cm^{-1} due to the NH, urethane and ester carbonyls respectively. The proton nmr spectrum of 69 showed, in addition to the usual aromatic and methylenedioxy signals, two doublets at 4.36 and 5.47 ppm (J=0.6 Hz, 1H each) due to the two benzocyclobutene ring protons, a singlet at 3.72 ppm (3H, OCH_3), a multiplet at 4.00 ppm (2H, $NHC\underline{H}_2$), a broad signal at 4.90 ppm (1H, NH), a double triplet at 5.96 ppm (J=15.9, 1.9 Hz, 1H, $=C\underline{H}CO_2CH_3$), and a second double triplet at 6.92 ppm (J=15.9, 4.6Hz, 1H, $-C\underline{H}=CHCO_2CH_3$). The observed signals characteristic of the crotyl moiety verify that the coupling

reaction took place, while the 15.9 Hz coupling between the vinyl protons indicated the double bond had retained the required E stereochemistry. The very small 0.6 Hz coupling between the benzocyclobutene ring protons defines their *trans* stereochemical relationship.

5.2 Thermolysis of Benzocyclobutene 69

Thermolysis of benzocyclobutene 69 in nitromethane at 90°C for 5h, yielded a 3:1 ratio of *trans* and *cis* fused intramolecular Diels-Alder products 70 and 71 as determined by proton nmr of the crude mixture. Separation of the two isomers was achieved via fractional recrystallization from methanol followed by PTLC of the mother liquor to yield 70 as colorless cubes mp 272°C and 71 as white needles mp 234°C in 82% total yield. The *trans*



Diels-Alder product 70 was characterized by infrared bands at 3385, 1735 and 1712 cm^{-1} due to the NH, ester and urethane groups. The *cis* Diels-Alder product 71 was characterized by infrared bands at 3300, 3400 and 1700-1740 cm^{-1} due to the NH, ester and urethane groups. The proton nmr of 70 and 71 are shown, in Figures 5.1 and 5.2, and the signals assigned,

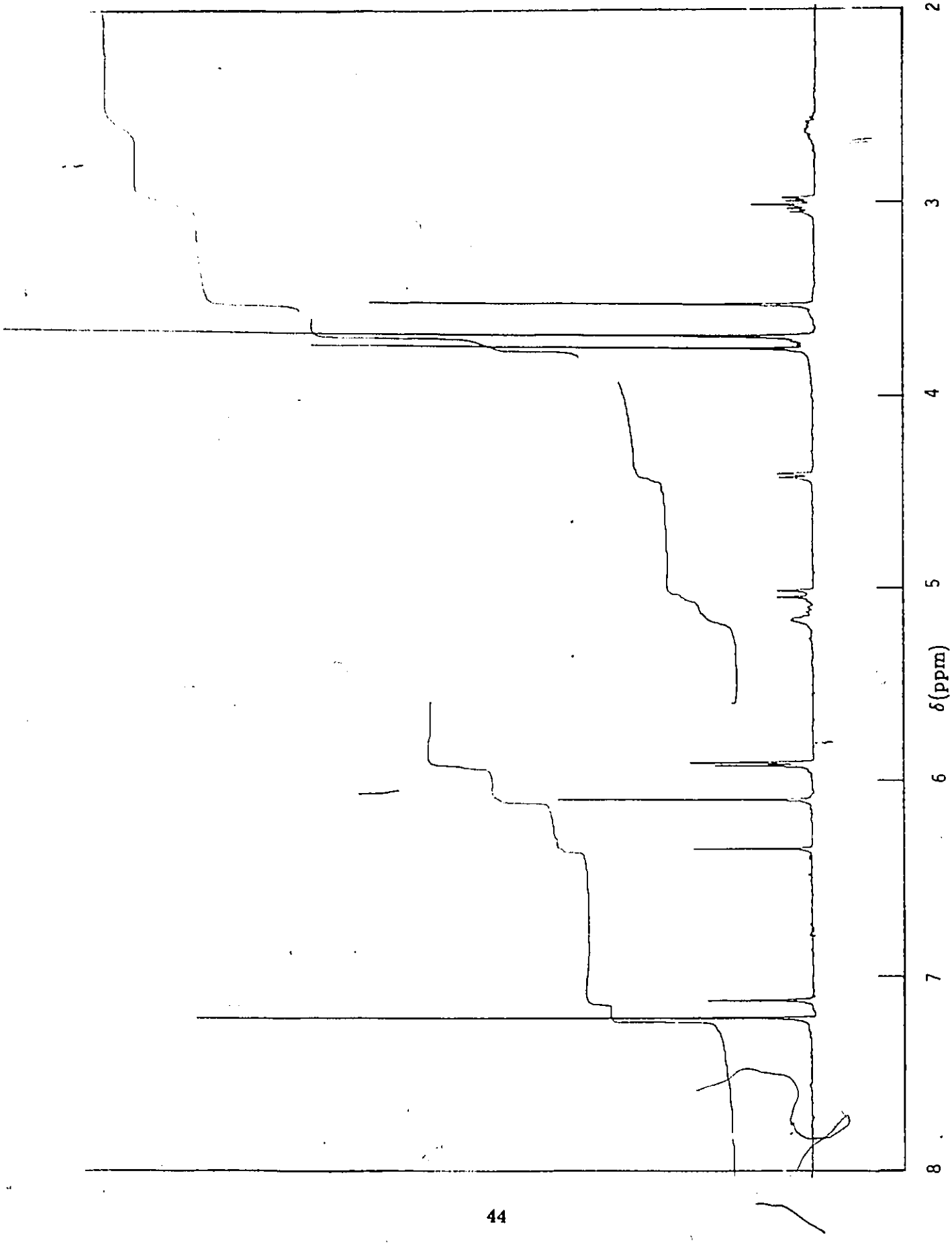


Figure 5.1: Proton NMR Spectrum of 70

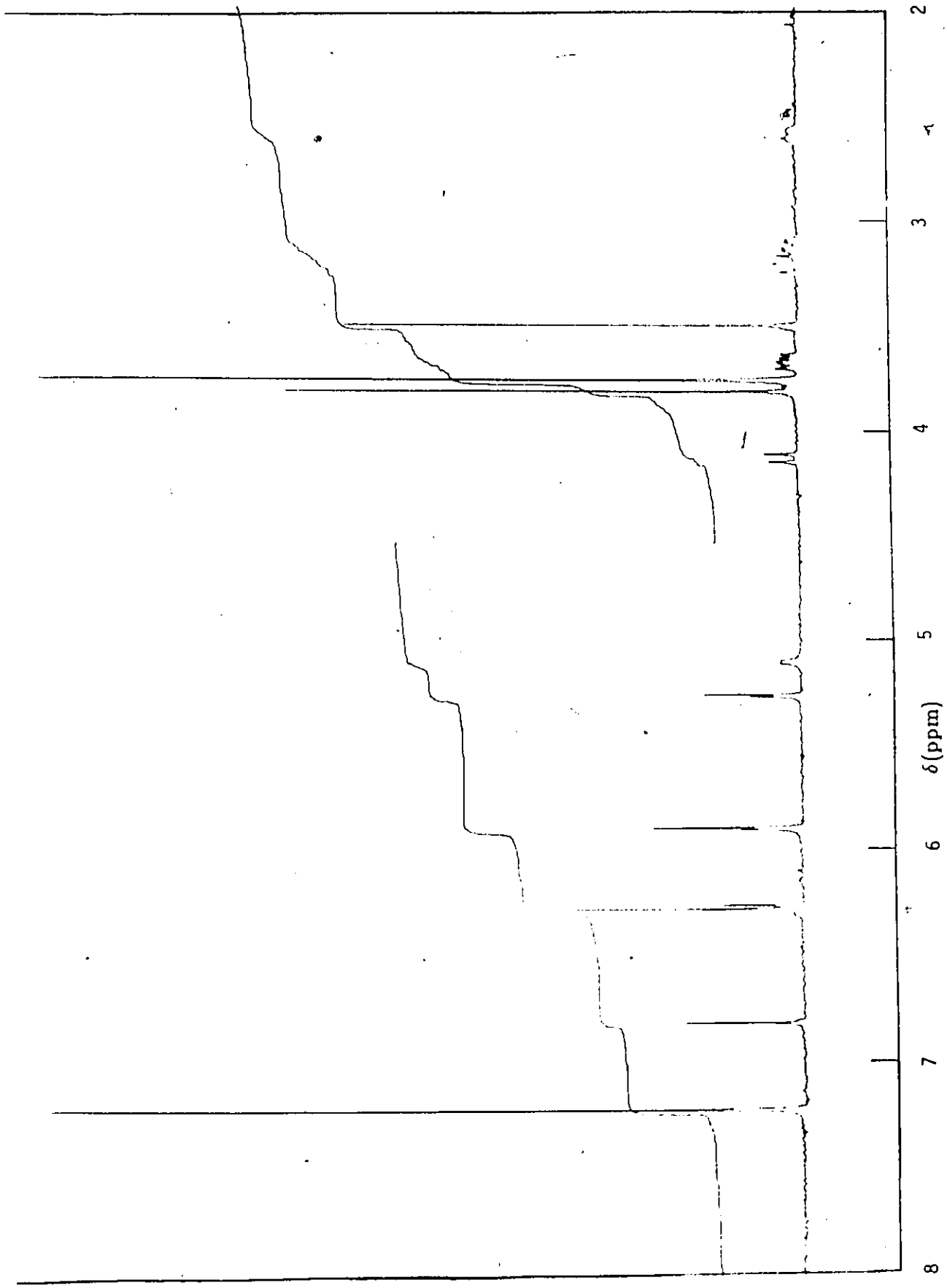
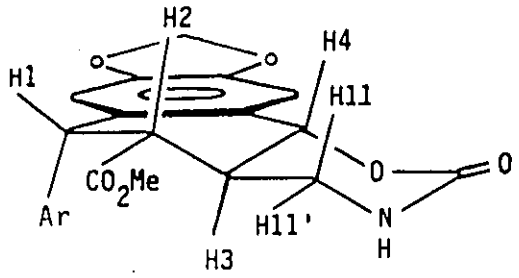
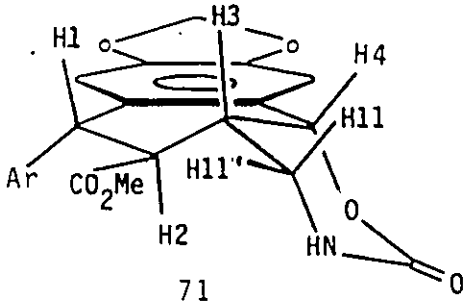


Figure 5.2: Proton NMR Spectrum of 71



70



71

Chemical Shifts (ppm)

Compound	H1	H2	H3	H4	H11	H11'
70	4.44	3.03	2.64	5.05	3.03	3.74
71	4.14	3.21	2.58	5.28	3.12	3.64

Coupling Constants (Hz)

Compound	J_{1-2}	J_{2-3}	J_{3-4}	J_{3-11}	$J_{3-11'}$	$J_{11-11'}$	J_{11-NH}	$J_{11'-NH}$
70	6.0	12(?)	9.9	12(?)	-	12(?)	0	-
71	11.5	11.8	2.7	4.0	5.0	12.5	0	0

Figure 5.3: Assignment of Signals in Proton NMR of 70 and 71

in Figure 5.3. The stereochemical assignment of 70 and 71 was made on the basis of the vicinal coupling constants between H1 and H2, and between H3 and H4. The spectrum of 70 showed couplings of 6.0 and 9.9 Hz for H1-H2 and H3-H4 respectively, consistent with *cis*-1,2 and *trans*-3,4 stereochemical relationships. In contrast, 71 showed couplings of 11.5 and 2.7 Hz for H1-H2 and H3-H4 respectively, indicating *trans*-1,2 and *cis*-3,4 stereochemical relationships.

Thus the benzocyclobutene derivative 69 underwent the expected ring opening followed by an intramolecular Diels-Alder reaction to give the two possible products 70 and 71. The *trans* fused product 70 contained the same relative stereochemistry as podophyllotoxin 1, namely *cis*-1,2, *trans*-2,3 and *trans*-3,4. The *cis* fused product 71 contained the relative stereochemistry of epiisopodophyllotoxin 42 [19], namely *trans*-1,2 and *trans*-2,3, and *cis*-3,4. It was encouraging to find that 70 (which was the desired isomer) was also the major product, however greater stereoselectivity would definitely have been an asset. Further studies (to be discussed later) indicated that the stereoselectivity could be influenced by external factors such as the solvent. In particular, polar solvents increased the amount of the desired *trans* product and also the reaction rate, hence the use of nitromethane in this case. Thus the moderate selectivity in this initial trial was not a great disadvantage.

5.3 Conversion of Diels-Alder Product 70 to Podophyllotoxin

With Diels-Alder product 70 in hand, which has the correct relative stereochemistry of the four chiral centres in podophyllotoxin, all that was required

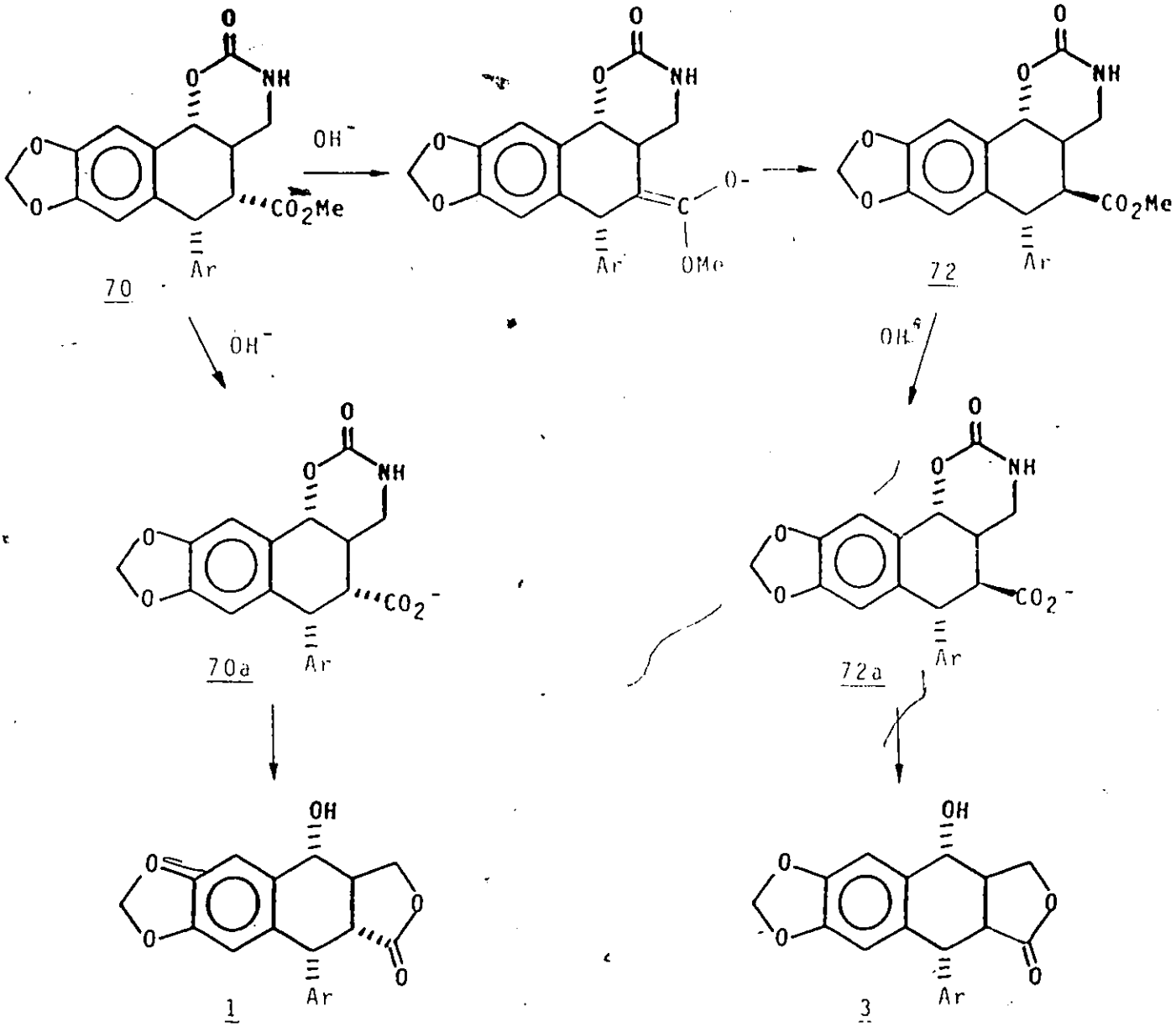
to complete the synthesis was hydrolysis of the ester and urethane functions followed by diazotization of the resultant γ-amino acid to produce the γ-lactone.

5.3.1 Basic Hydrolysis of Ester and Urethane in One Pot

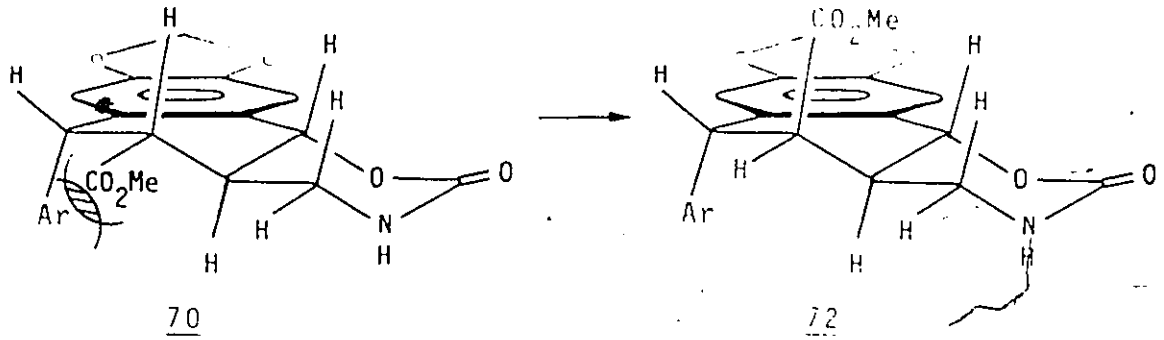
Hydrolysis of both the ester and urethane functions was accomplished by refluxing 70 in aqueous lithium hydroxide. The resulting solution was treated with acetic acid and sodium nitrite. Extraction of the reaction mixture with ethyl acetate and chromatography of the crude product yielded a white solid with the same TLC R_f (0.5, hexane-ethyl acetate = 1:3) as podophyllotoxin. Proton nmr revealed however that the white solid consisted of a 1:1 mixture of picro and podophyllotoxin. Both the proton nmr and infrared spectra of the mixture were identical to those of the authentic compounds. More careful examination by TLC using a different solvent system also revealed that two compounds with the same R_fs as picro and podophyllotoxin were present (methylene chloride-acetone = 7:1, podo R_f = 0.40, picro R_f = 0.45).

It was therefore concluded that epimerization of the stereochemistry at C2, which had been so carefully established by the Diels Alder, competed with the hydrolysis of the methyl ester. This could only have occurred by deprotonation at C2 to give the ester enolate, followed by protonation to yield the C2 epimer 72. Subsequent hydrolysis then converted 70 and 72 to their respective carboxylates 70a and 70b. Further hydrolysis of the urethane functions in 70a and 70b to yield their respective amino acids followed by diazotization, yielded a mixture of podo and picropodophyllotoxin. The

1:1 mixture appeared to be kinetic rather than thermodynamic due to the fact that milder conditions, such as aqueous lithium hydroxide in THF at 25°C prior to refluxing, produced almost exclusively picropodophyllotoxin. Thus the previous 1:1 mixture represented a kinetic competition between



the ester epimerization and hydrolysis, not simply an equilibrium between 70 and 72. This unwanted behavior was totally unexpected, particularly because the carboethoxy group in 70 is in an equatorial position which would normally be more stable than the axial position. Thus it seemed unlikely that the observed epimerization of 70 to 72 should occur. The most likely explanation for the observed result was that the axially locked aryl group in 70 has significant steric interaction with the *cis*-carbomethoxy group, epimerization at C2 to give 72 relieves this interaction.



70 72

5.3.2 Avoiding the C2 Epimerization Problem

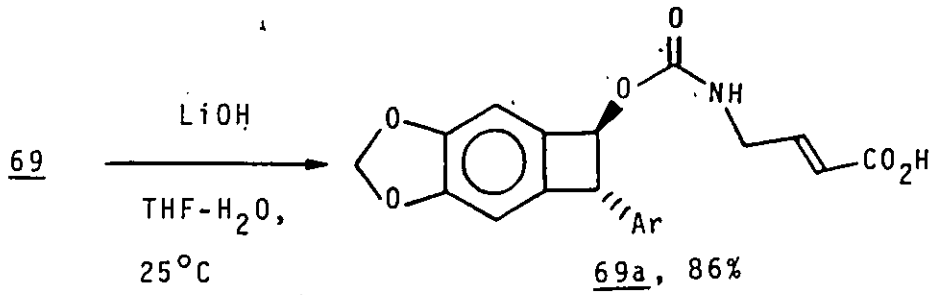
The epimerization problem encountered with basic hydrolysis of the ester function in 70 could in principle be avoided by one of several methods. Three such methods are as follows:

1. Hydrolysis of the ester prior to the Diels-Alder reaction.
2. "Hydrolysis" of the ester in 70 by a non basic method.
3. Use of an ester other than methyl which could be easily removed by a non basic method.

The use of an ester other than methyl would require either preparation of isocyanate 68 with another ester group or conversion of the methyl ester in 69 to another ester. The former method would not be practical because the method used to prepare the isocyanate would likely be incompatible with any special ester (particularly those which are acid sensitive). The conversion of the methyl ester in 69 to another ester would likely require two more steps and was therefore undesirable.

The most attractive method found in the literature for non-basic removal of methyl esters was halogenolysis using lithium iodide in refluxing DMF,[31] the mechanism being nucleophilic attack by iodide ion on the methyl group with the assistance of lithium ion acting as an electrophile to activate the ester. Although the yields were reported to be good, the conditions seemed rather severe (DMF, LiI, 153°C), and thus this method was not carefully considered as our first method of choice. The method was however used later with great success, in the preparation of an analog of podophyllotoxin, and will be discussed in detail in Chapter 7.

It was found that the methyl ester in 69 could be hydrolyzed selectively without hydrolysis of the urethane using aqueous lithium hydroxide in THF at 25°C. Thus acid 69a mp 106-108°C was obtained in 86% yield from 69. Acid 69a was characterized by infrared bands at 2900-3400 and 1690-1720

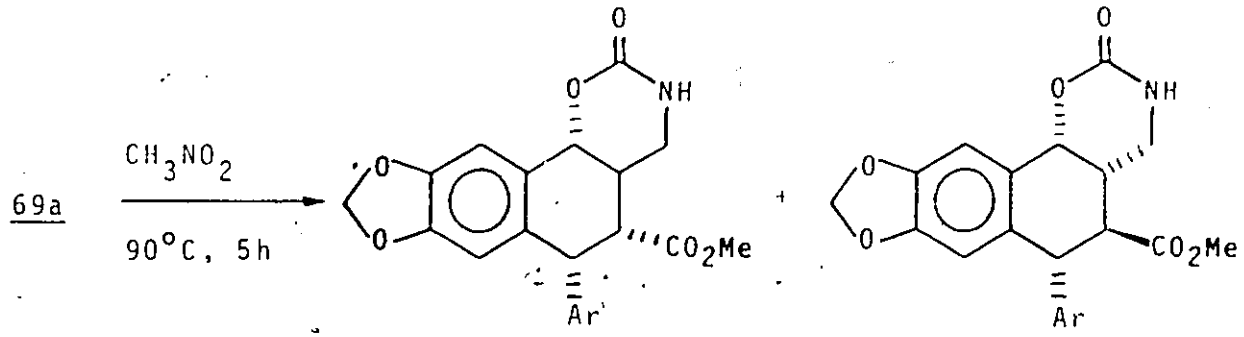


69a, 86%

cm^{-1} due to the carboxylic acid and urethane groups. The proton nmr of 69a was almost identical to the parent ester 69 except for very small chemical shift variations and the absence of a methyl ester signal.

Thus it was found that the methyl ester in 69 could be easily hydrolyzed prior to the Diels-Alder reaction. Assuming that the presence of the carboxylic acid would have no adverse effect on the thermolysis reaction, the expected "tricyclic" acid 70a should undergo basic hydrolysis of the urethane function to yield 66 without epimerization at C2.

Thermolysis of 69a in nitromethane at 90°C for 5h yielded the *trans* and *cis* fused acids 70a and 71a in a 5:1 ratio, as determined by 300 MHz proton nmr examination of the crude reaction mixture after treatment with diazomethane. The ratio was based on the integration of the carbomethoxy signals. The acids were characterized by conversion to their methyl esters using diazomethane. The methyl esters thus obtained were identical to those obtained upon thermolysis of 69 (ie. 70 and 71). The total yield of methyl esters 70 and 71 from 69a was 60%.

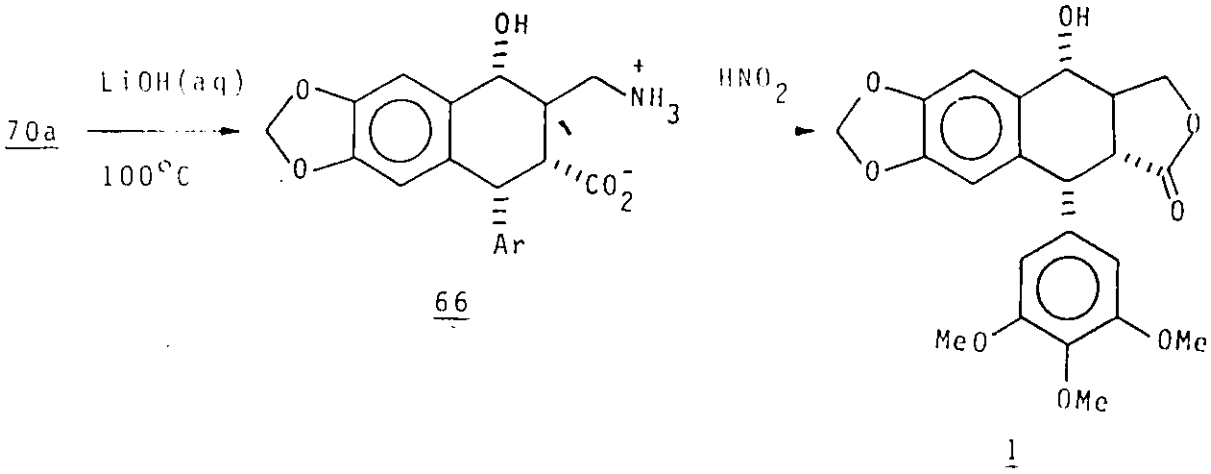


70a 5:1 (60% total) 71a

Thus the thermolysis reaction of acid 69a, produced a total yield of "tricyclic" product slightly less than thermolysis of the respective methyl ester

69 but with higher stereoselectivity!

Hydrolysis of the crude mixture of acids 70a and 71a in refluxing aqueous lithium hydroxide followed by treatment with sodium nitrite, while buffering the solution to pH4 using KH_2PO_4, yielded (±)-podophyllotoxin 1 mp 236-237°C in 23% yield from the methyl ester 69. The proton nmr and infrared spectra of the product obtained are identical to those of authentic podophyllotoxin. The mp of (±)-podophyllotoxin has not been reported.

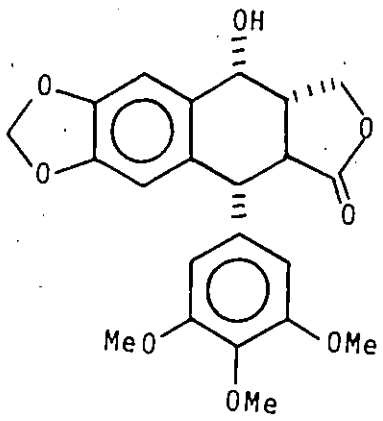


66

1

The proton nmr of the crude product showed no signals due to picropodophyllotoxin.

The mixture of acids 70a and 71a was used without purification or separation due to the low solubility of the acids in common organic solvents. The product expected from the *cis* fused acid 71a, epiisopodophyllotoxin 42 previously prepared by Glinski and Durst [19], was not observed in the proton nmr of the crude product. This observation was somewhat difficult



42 epiisopodophyllotoxin

to explain, other than some unexpected side reaction had occurred, such as one resulting from trapping the diazonium salt by the *cis*-4-hydroxyl group to give an oxetane carboxylic acid.

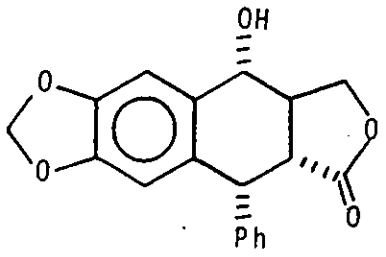
Thus (±)-podophyllotoxin has been synthesized in six steps from *trans*-2-(3,4,5-trimethoxyphenyl)-4,5-(methylenedioxy)benzocyclobutyl acetate 60 in 11%, nonoptimized, yield. The route has been published in preliminary form.[32]

Chapter 6

Preparation of Podophyllotoxin Analogs

In Chapter 1 attention was drawn to the need for a method capable of producing podophyllotoxin analogs with various types of substitution on rings B and E. In Chapters 4 and 5, a synthesis of (±)-podophyllotoxin was described in which the four chiral centres were established directly by use of a highly stereospecific Diels-Alder reaction.

In order to demonstrate the generality of the synthesis, with respect to the preparation of podophyllotoxin analogs with various substitution on the aryl rings, two analogs were prepared. The first analog 73 contained the same substitution as podophyllotoxin on the B ring, but the E ring was replaced



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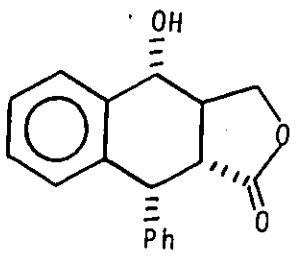
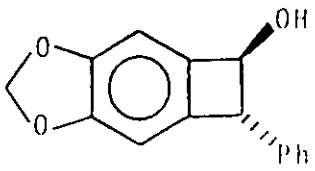


74

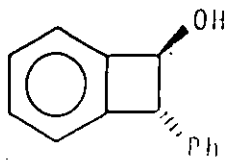
by a phenyl group. The second derivative 74 contained no functionality on either of rings B and E.

6.1 Preparation of Benzocyclobutenols

In order to prepare the two podophyllotoxin derivatives 73 and 74, we required access to the benzocyclobutenols 75 and 76.



75

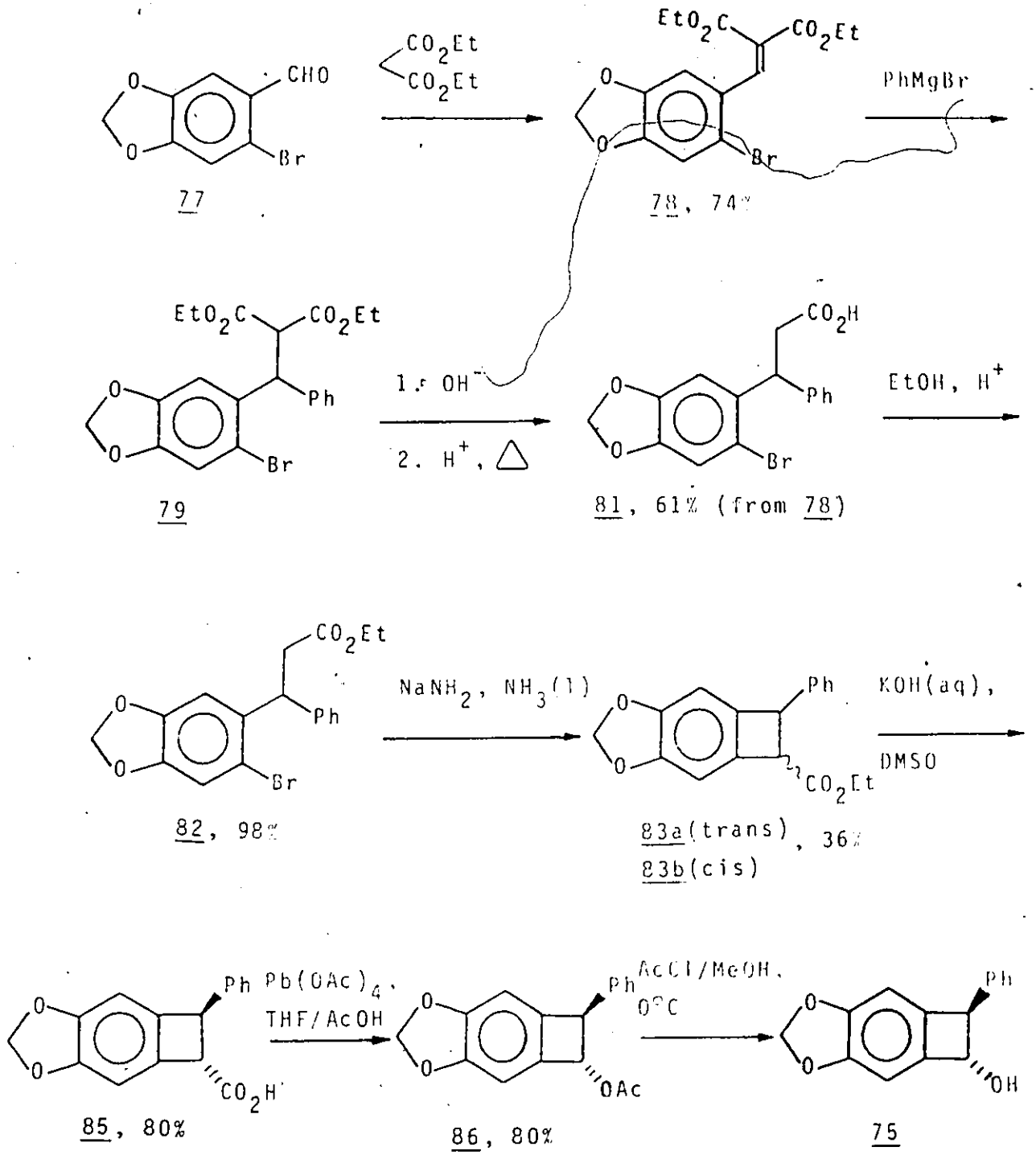


76

6.1.1 Synthesis of *trans*-2-Phenyl Benzocyclobutenol 75

The benzocyclobutenol 75 was prepared following the methodology used for the preparation of 44. The synthesis has been published in preliminary form [25] and is illustrated in Scheme 6.1.

The actual method used to prepare the *o*-bromoarylester 82, shown in Scheme 6.1, was slightly shorter than the route presented in Scheme 4.1 for the preparation of the analogous compound 57. A similar route to that shown in Scheme 6.1 was used for the preparation of 57 and has also been reported in the same preliminary publication.[25] The 1,4-conjugate addition step introducing the 3,4,5-trimethoxyaryl group was however, not found to be consistently reproducible, thus the method is not reported in this thesis.



Scheme 6.1: Synthesis of 2-Phenylbenzocyclobutenol 75.[25]

Condensation of 6-bromopiperonal 77 and diethyl malonate in refluxing toluene using piperidinium benzoate as catalyst, yielded the α,β-unsaturated diester 78 mp 50-52°C in 74% yield. The infrared spectrum contains bands at 1724, 1629, 1617 and 1599 cm^{-1}, characteristic of the unsaturated diester. The proton nmr contains the usual aromatic and methylenedioxy signals as well as a singlet at 7.90 ppm due to the vinyl proton and signals characteristic of the two carboethoxy groups (two 3H triplets at 1.26 and 1.35 ppm (J=7.4 Hz) and two quartets at 4.28 and 4.29 ppm (J=7.4 Hz)).

Addition of phenylmagnesium bromide to the unsaturated diester 78 in ether-benzene at -78 to 0°C, afforded the Michael adduct 79 as a yellow oil in quantitative yield. The crude product was used without purification in the preparation of 81. The Michael adduct 79 was characterized by infrared bands at 1753 and 1734 cm^{-1} due to the ester groups and its proton nmr, which contained a 5H multiplet found at δ = 7.1 to 7.3 ppm due to the phenyl group and two 1H doublets at 4.21 and 5.26 ppm (J=13.2 Hz) assigned to the two adjacent methine hydrogens.

The crude diester was saponified to the diacid 80, which was not purified or characterized. Thermal decarboxylation of 80 in refluxing toluene for 1 day, gave the monoacid 81 mp 190-192°C in 61% yield from 79. The acid 81 was characterized by infrared bands at 2500-3600 and 1709 cm^{-1} due to the carboxylic acid group. The proton nmr spectrum included (in addition to the usual aromatic and methylenedioxy signals) two doublets at 3.00 and 3.01 ppm (J=8.6 Hz, 1H each) and a triplet at 4.98 ppm (J=8.6 Hz, 1H), due to the two hydrogens α to the carboxylic acid and the benzylic hydrogen, respectively.

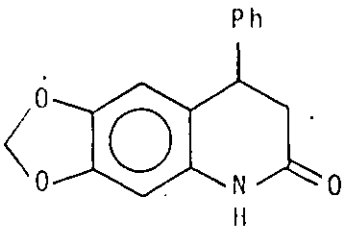
Acid catalyzed esterification of the acid 81 with ethanol provided the ethyl ester 82 in 98% yield as a pale yellow oil. The ester showed the characteristic ester band at 1732 cm^{-1} in the infrared and its proton nmr was similar to the acid 81 except for the addition of a triplet at 1.12 ppm (J=7 Hz, 3H) and a quartet at 4.04 ppm (J=7 Hz, 2H), due to the ethoxy group.

Thus the required *o*-bromoester 82 was prepared in 4 isolation steps (5 chemical steps), without chromatography, in 44% overall yield from bromopiperonal. This is in contrast to the method used in Scheme 4.1 for the preparation of 57 (6 steps, 73% overall yield). The method used for the preparation of 82 had a lower yield but possesses the advantage of two fewer isolation steps and is better suited for large scale preparations.

The conversion of 82 to the required benzocyclobutenol 75 was performed using the same method, with almost identical conditions, as that used in the preparation of 44 in Scheme 4.1. The yields obtained in the conversion of 82 to 75 were also similar to those obtained in the preparation of 44.

Thus treatment of the bromoarylester 82 with sodium amide (four equivalents) in liquid ammonia gave 36% of a 1:1 mixture of *trans* and *cis* benzocyclobutene esters 83a and 83b. IR: 1729 cm^{-1}. Proton NMR: *trans* 3.89(d, J=2.3 Hz, 1H), 4.73(d, J=2.3 Hz, 1H). *cis* 4.49(d, J=5.8 Hz, 1H), 4.91(d, J=5.8 Hz, 1H).

A similar byproduct to that obtained in the cyclization of 57 was also obtained. The δ lactam 84 mp 204-205°C was formed in 20% yield. IR: 3340, 1673 cm^{-1}. Proton NMR: δ 2.63(dd, J=16.0, 5.7 Hz, 1H), 2.80(dd, J=16.0, 6.5 Hz, 1H), 4.19(t, J=6.1 Hz, 1H).



84

Saponification of the mixture of esters 83a and 83b (KOH, DMSO-water) was accompanied, as before, by isomerization to produce the *trans* acid 85 mp 140°C (dec) as white crystals in 80% yield. IR: 2500-3600, 1709 cm^{-1}. Proton NMR: δ 3.93(d, J=2.6 Hz, 1H), 4.75(d, J=2.6 Hz, 1H).

Oxidative decarboxylation of 85 with lead tetraacetate in THF-acetic acid yielded 54% of the acetate 86 mp 83-84°C as colorless cubes. Due to incomplete crystallization from the mother liquor, the actual yield was likely about 80%. IR: 1740 cm^{-1}. Proton NMR: δ 2.12(s, 3H), 4.46(s, 1H), 5.45(s, 1H). The proton nmr spectrum of 86 is shown in figure 6.1.

Deprotection of the acetate 86 using acetyl chloride in methanol at 0°C for 3h, using a similar workup to that of 44 produced a carbon tetrachloride solution of 75. IR: 3100-3600 cm^{-1}. Proton NMR: δ 2.30(d, J=7.7 Hz, OH), 4.25(s, 1H), 4.80(d, J=7.7 Hz, 1H), 5.93(s, 2H), 6.70(s, 1H), 6.83(s, 1H), 7.1-7.3(aromatics, 5H).

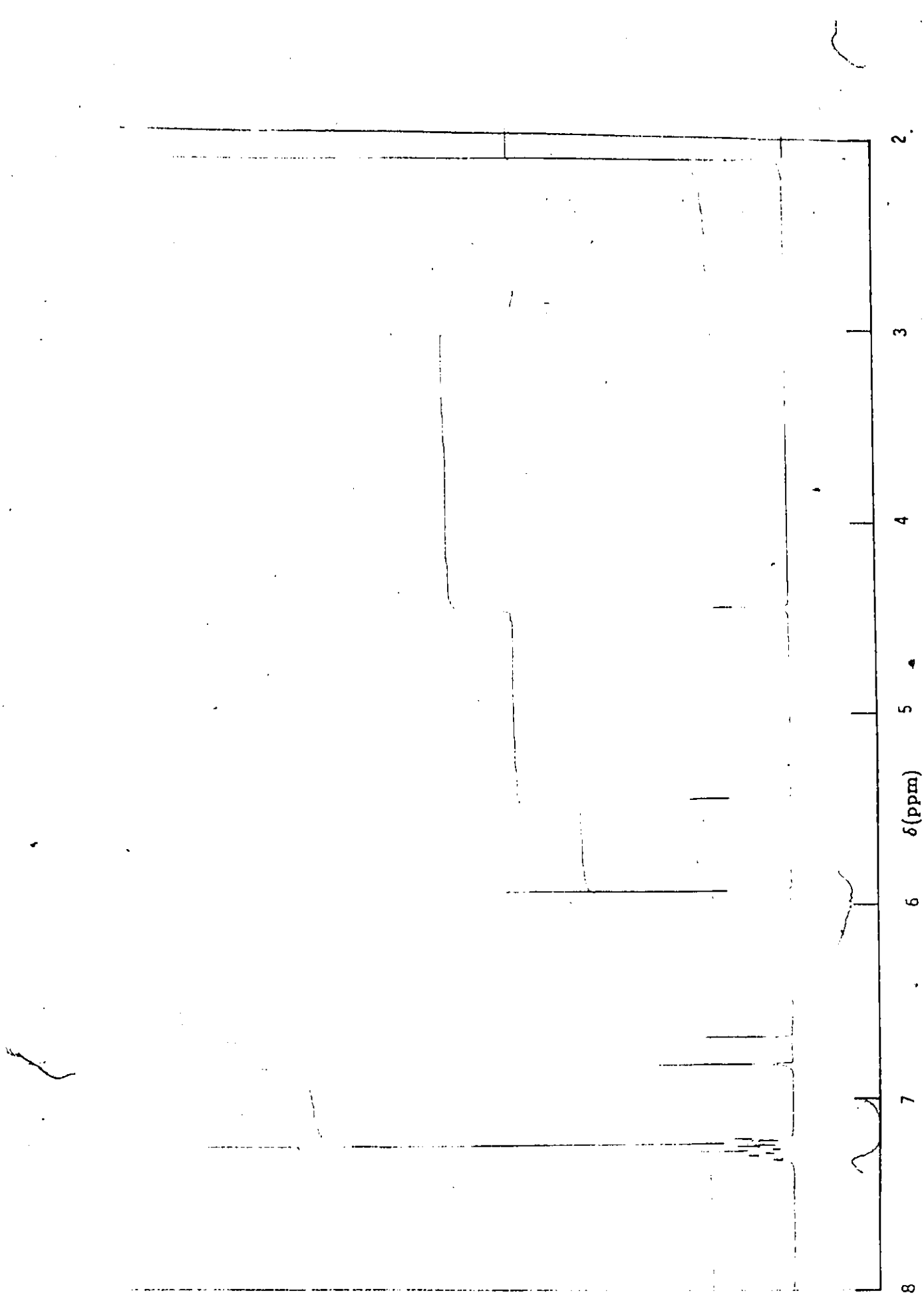
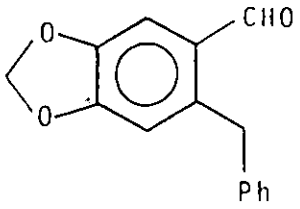


Figure 6.1: Proton NMR Spectrum of 2-Phenylbenzocyclobutyl Acetate 86.

The benzocyclobutenol 75 was found to be similar to 44 in that it was also very labile, yielding the aldehyde 87[1] upon heating or treatment with



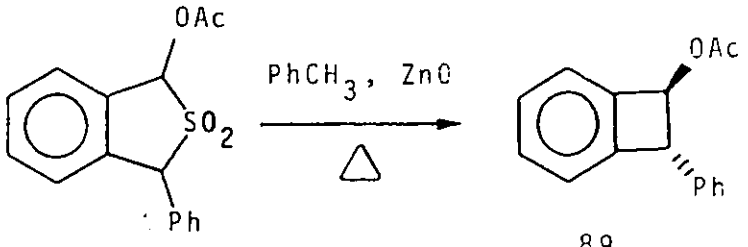
87

base. Thus, as in the case of 44, the alcohol 75 was stored in the form of its more stable acetate.

6.1.2 Preparation of Benzocyclobutenol 76

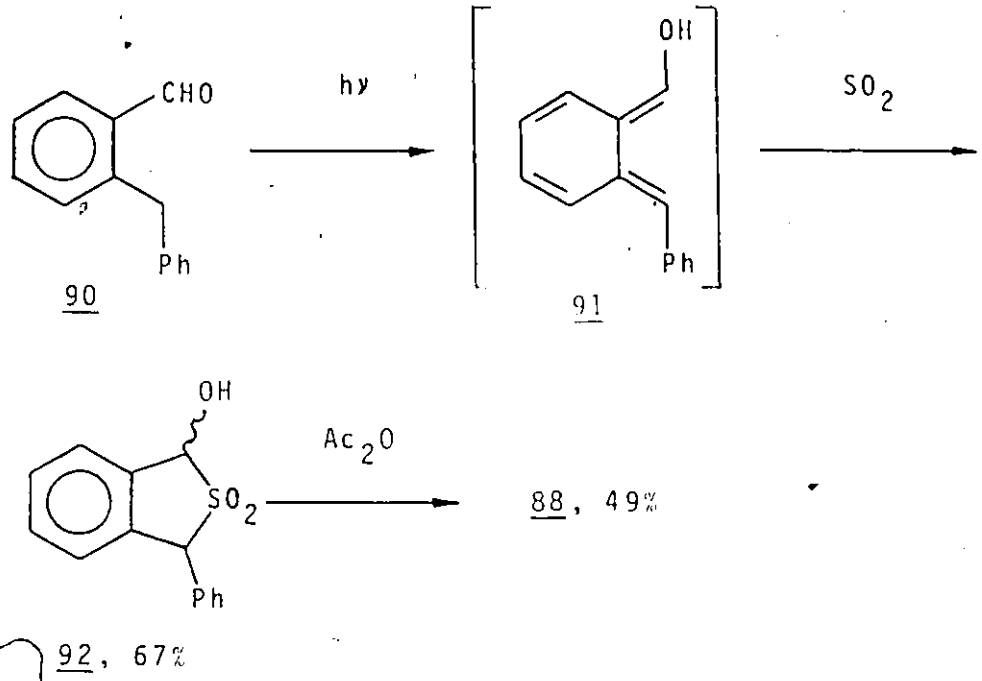
Charlton and Alauddin [33] recently reported that thermolysis of the sulfone 88 in refluxing toluene, in the presence of zinc oxide, produced *trans*-2-phenylbenzocyclobutyl acetate 89 in 76% yield. Upon repeating the reaction, we found the zinc oxide was not necessary.

[1]Proton NMR of 87: δ 4.34(s, 2H), 6.02(s, 2H), 6.64(s, 1H), 7.1-7.3(aromatics, 5H), 7.33(s, 1H), 10.13(s, 1H).



88 89

The sulfone 88 had been previously prepared by Charlton[2] in our laboratory.[34] Photoenolization of *o*-benzylbenzaldehyde 90 produced the intermediate *o*-quinodimethane 91, which was trapped by sulfur dioxide to yield the hydroxy sulfone 92 as a 1:1 mixture of *cis* and *trans* isomers in 67% yield.



90 91

88, 49%

92, 67%

Acetylation of the alcohol mixture 92 with acetic anhydride produced a 3:2 mixture of *cis* and *trans* acetates, from which the *cis* isomer 88 was isolated by fractional crystallization in 49% yield. It was found that the *trans* isomer could be isomerized to reestablish the 3:2 mixture of *cis* to *trans* by treatment with a base such as triethylamine. Recrystallization in the presence of triethyl amine produced complete conversion of the *trans* to *cis* isomers.

Removal of the acetate group in 89 to yield *trans*-2-phenylbenzocyclobutenol

[2]Visiting professor from University of Manitoba.

76 was performed as before, using acetyl chloride in methanol at -5°C for 5h. IR: 3300-3500 cm^{-1}. Proton NMR: δ 4.10(s, 1H), 4.96(s, br,2H), 7.0-7.6(aromatics, 9H).

This benzocyclobutenol too was found to be very labile, producing the aldehyde 90 upon heating or treatment with base. It was therefore stored in the form of its more stable acetate.

6.2 Preparation of Podophyllotoxin Analogs from Benzocyclobutenols 75 and 76

The podophyllotoxin analogs 73 and 74 were prepared from the respective benzocyclobutenols 75 and 76 following the methods described for the conversion of 44 to (±)-podophyllotoxin in Chapter 5. The synthesis of 73 and 74 are outlined in Scheme 6.2.

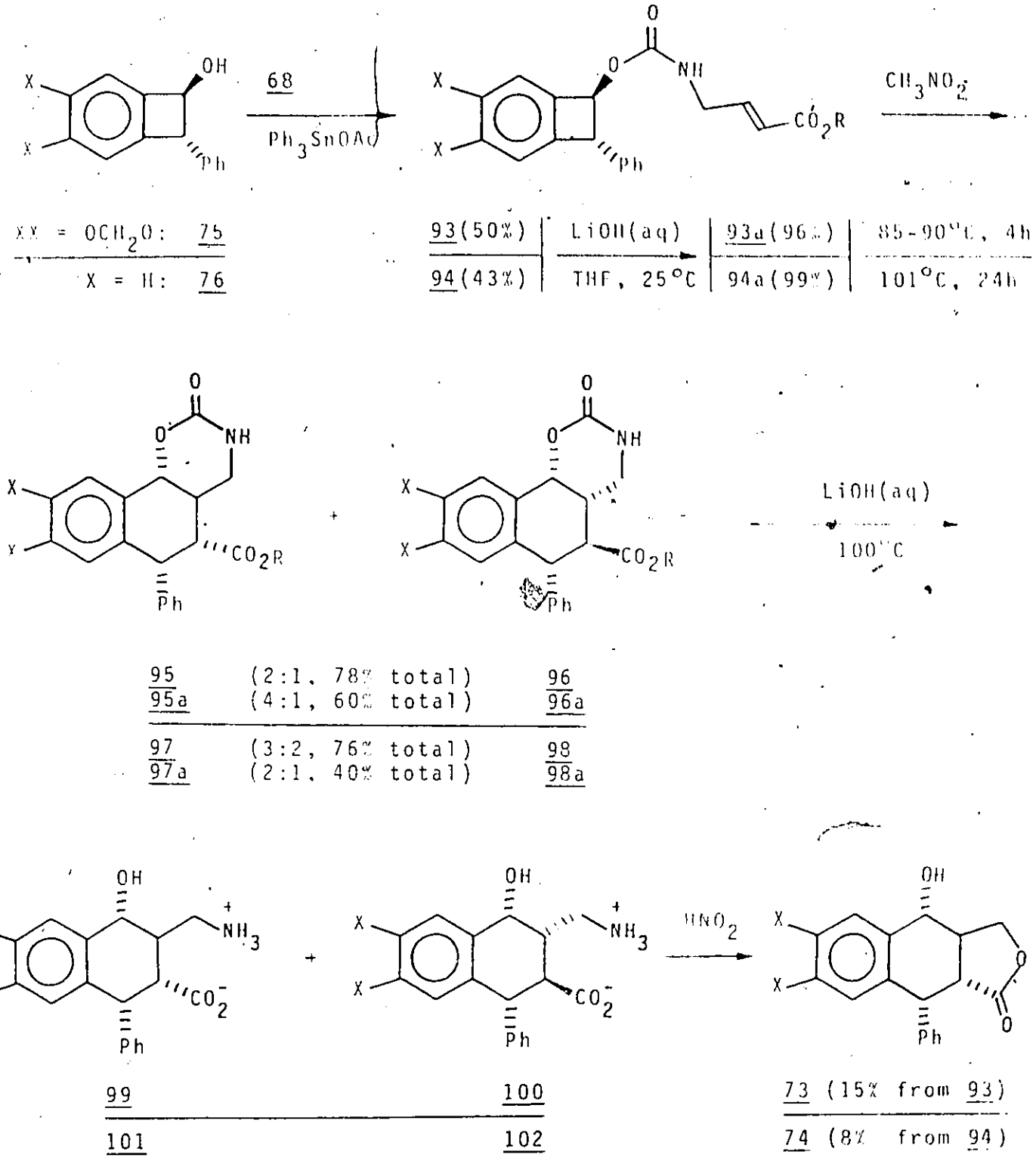
The alcohol 75 and isocyanate 68 were coupled using triphenyltin acetate as catalyst, under similar conditions to those described in Chapter 5 (0-25°C, slow evaporation), yielding 50% of urethane 93 mp 110-111°C as white needles from the acetate 86. IR: 3300-3460, 1723 cm^{-1}. Proton NMR: δ 3.72(s, 3H), 4.01(m, 2H), 4.44(s, 1H), 4.9(br, NH), 5.46(s, 1H), 5.96(dt, J=16, 1.9 Hz, 1H), 6.92(dt, J=16, 5 Hz, 1H).

Coupling of the alcohol 76 to isocyanate 68 using the same conditions, afforded the urethane 94 mp 93-94°C, as white needles in 43% yield. IR: 3340, 1700-1730 cm^{-1}. Proton NMR: δ 3.73(s, 3H), 4.03(m, 2H), 4.64(s, 1H), 4.93(m, NH), 5.67(d, 1.4 Hz, 1H), 5.97(dt, J=16.0, 2.0 Hz, 1H), 6.93(dt, J=16.0, 4.9 Hz, 1H).

The proton nmr spectra of 93 and 94 are shown in Figures 6.2 and 6.3.

R = Me: 93, 94, 95, 96, 97, 98.

R = H: 93a, 94a, 95a, 96a, 97a, 98a.



Scheme 6.2: Preparation of Podophyllotoxin Analogs 73 and 74.

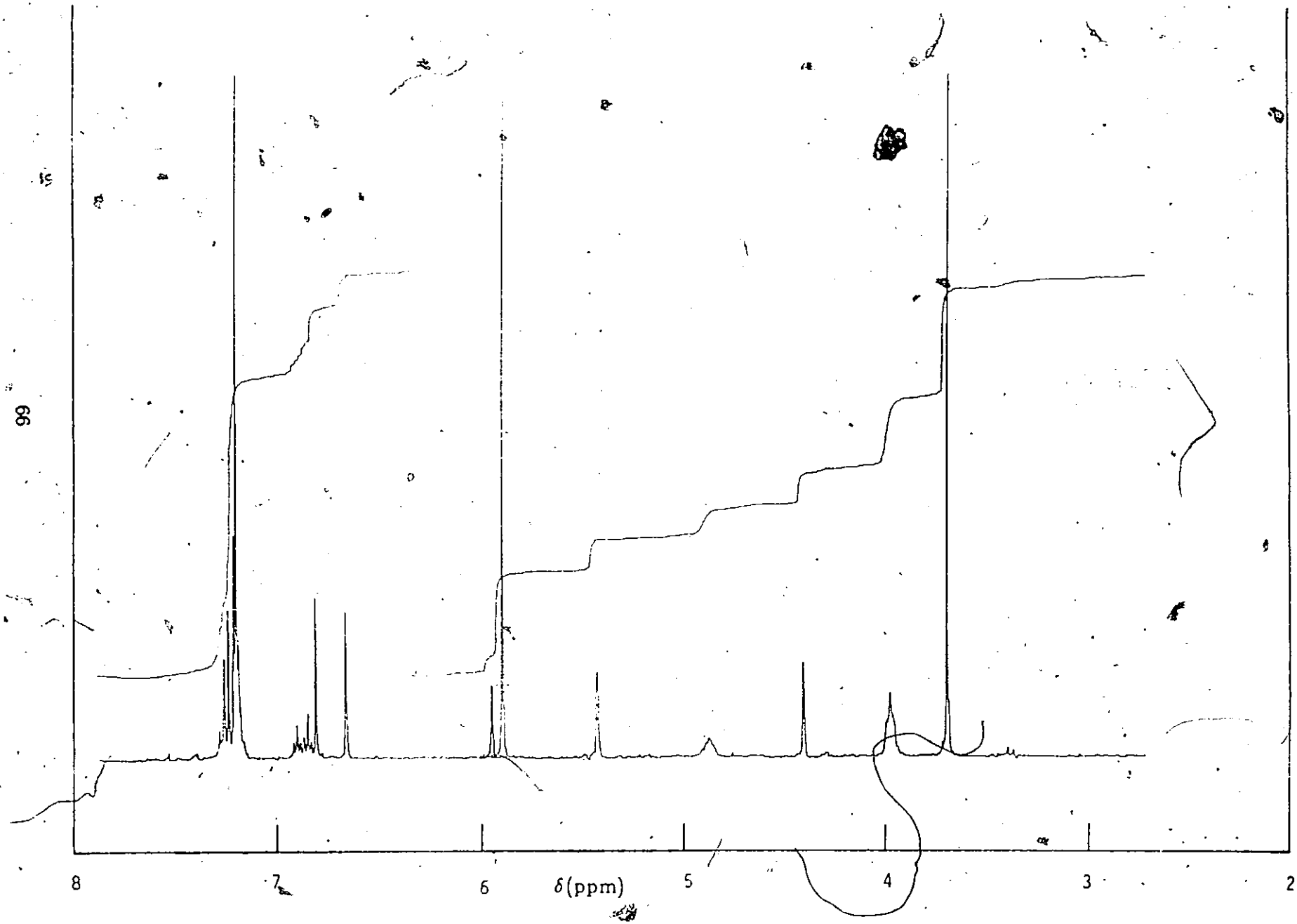


Figure 6.2: Proton NMR Spectrum of Urethane 93.

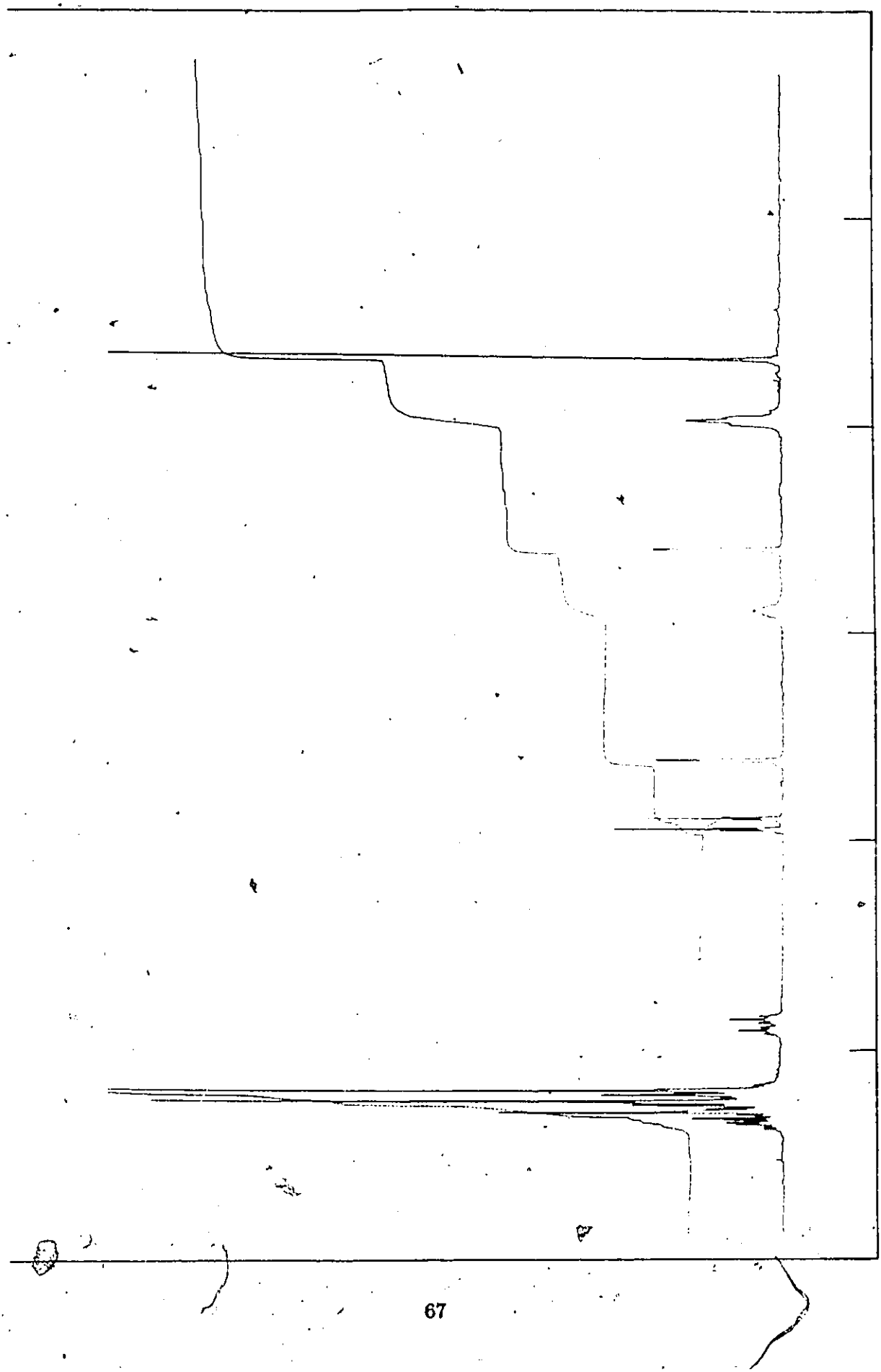


Figure 6.3: Proton NMR Spectrum of Urethane 94.

Selective hydrolysis of the methyl esters in 93 and 94 using aqueous lithium hydroxide in THF at 25°C, afforded the acids 93a and 94a in 96% and >99% crude yield. Recrystallization from methylene chloride-hexane produced white needles, mp 156-157°C and 130-131°C. Both acids showed a broad absorption in the 2400-3600 region due to the carboxylic acid groups. Their nmr spectra were very similar to those of the corresponding methyl esters with the ester peak near $\delta = 3.7$ being absent.

Thermolysis of the acid 93a in nitromethane for 4h at 85-90°C yielded a 4:1 mixture of the *trans* and *cis* fused acids 95a and 96a, as characterized by proton nmr analysis of the crude methyl esters obtained from diazomethane treatment of the crude acid mixture. The *trans* and *cis* methyl esters; 95 white needles mp 300-302°C and 96 white needles mp 230-231°C were obtained in 50 and 10% yields respectively. IR: 95 3300-3500, 1715 cm^{-1}. 96 3300-3600, 1700-1750 cm^{-1}. Proton NMR: 95 δ 4.51(d, J=5.9 Hz, 1H), 5.07(d, J=10.2 Hz, 1H). 96 4.22(d, J=11.5 Hz, 1H), 5.29(d, J=2.2 Hz, 1H).

The acid 94a was heated in refluxing nitromethane (101°C) for 1 day. Treatment with diazomethane afforded a 2:1 mixture of the *trans* and *cis* fused tricyclic methyl esters. The *trans* ester 97 mp 260°C was isolated in 27% yield as white needles. IR: 3420, 1728, 1706 cm^{-1}. Proton NMR: δ 4.65(d, J=5.8 H, 1H), 5.18(d, J=10.2 Hz, 1H). The *cis* ester 98 mp 169-170°C was produced in 13% yield as white needles. IR: 3260, 1714 cm^{-1}. Proton NMR: δ 4.34(d, J=11.2 Hz, 1H), 5.40(d, J=2.5 Hz, 1H).

The proton nmr of 95, 96, 97 and 98 are reproduced in Figures 6.4, 6.5, 6.6 and 6.7. The signals are assigned in Figure 6.8.

The crude mixture of acids 95a and 96a, prepared by thermolysis of crude

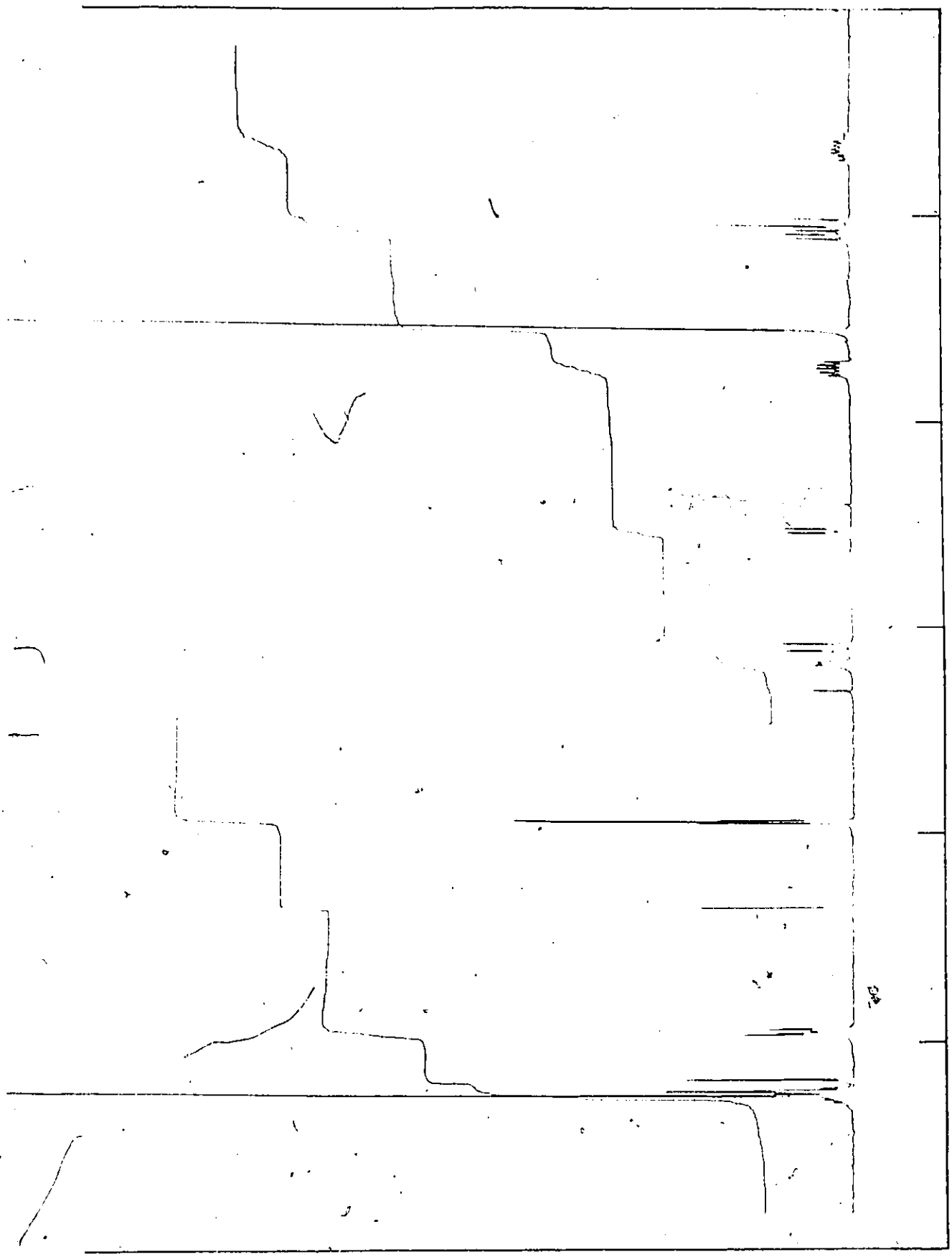


Figure 6.4: Proton NMR Spectrum of 95.

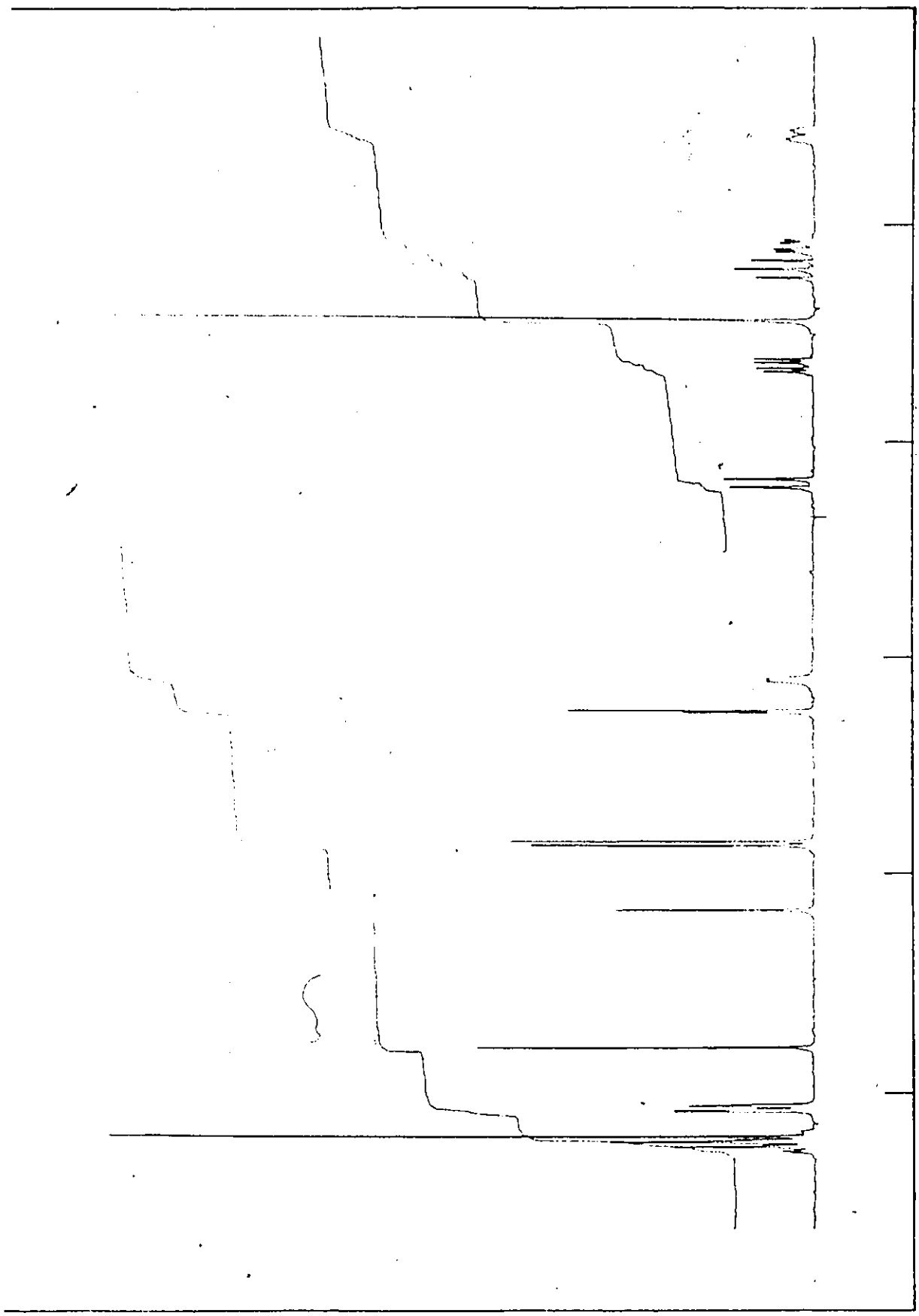


Figure 6.5: Proton NMR Spectrum of 96.

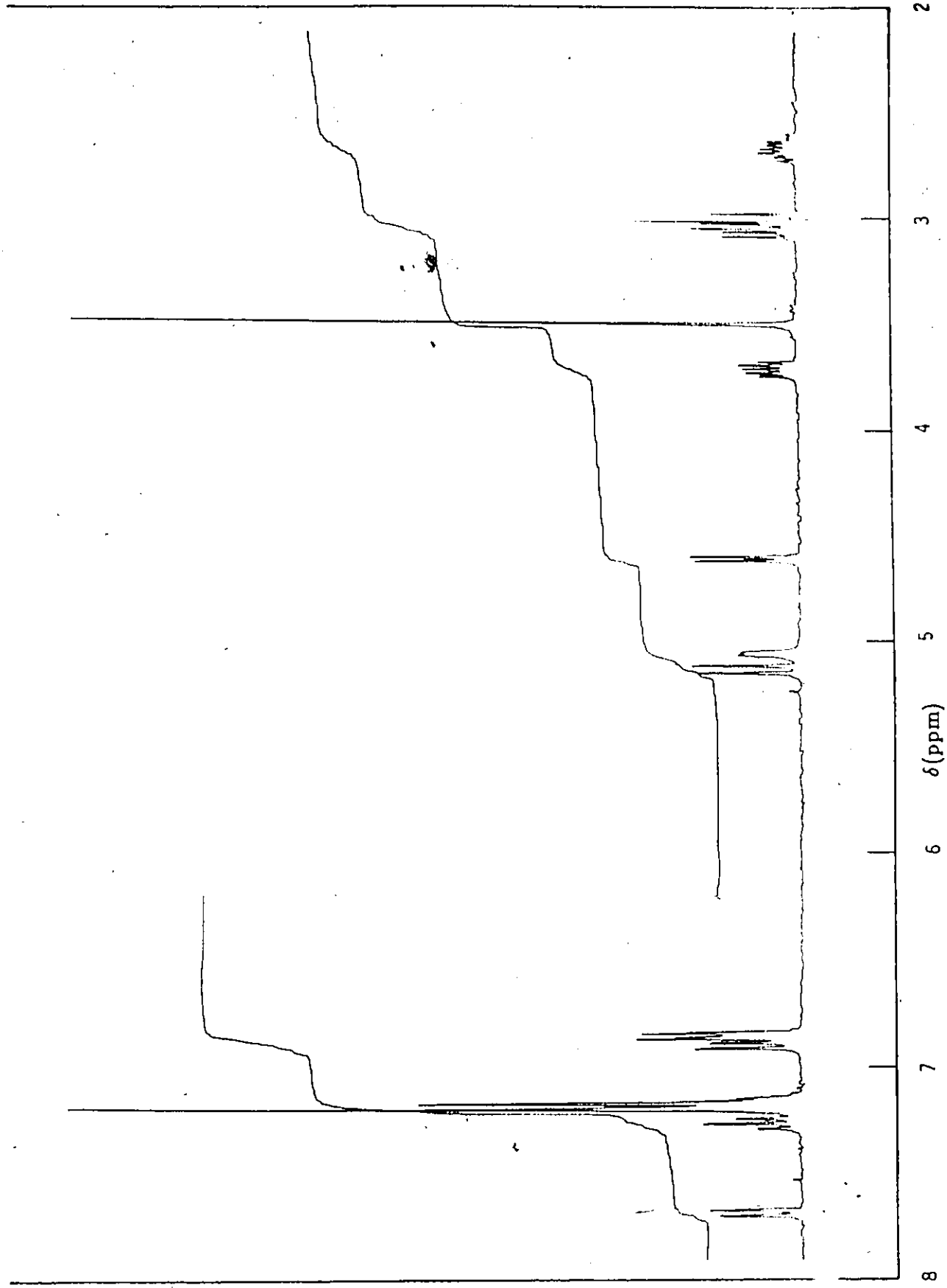


Figure 6.6: Proton NMR Spectrum of 97.

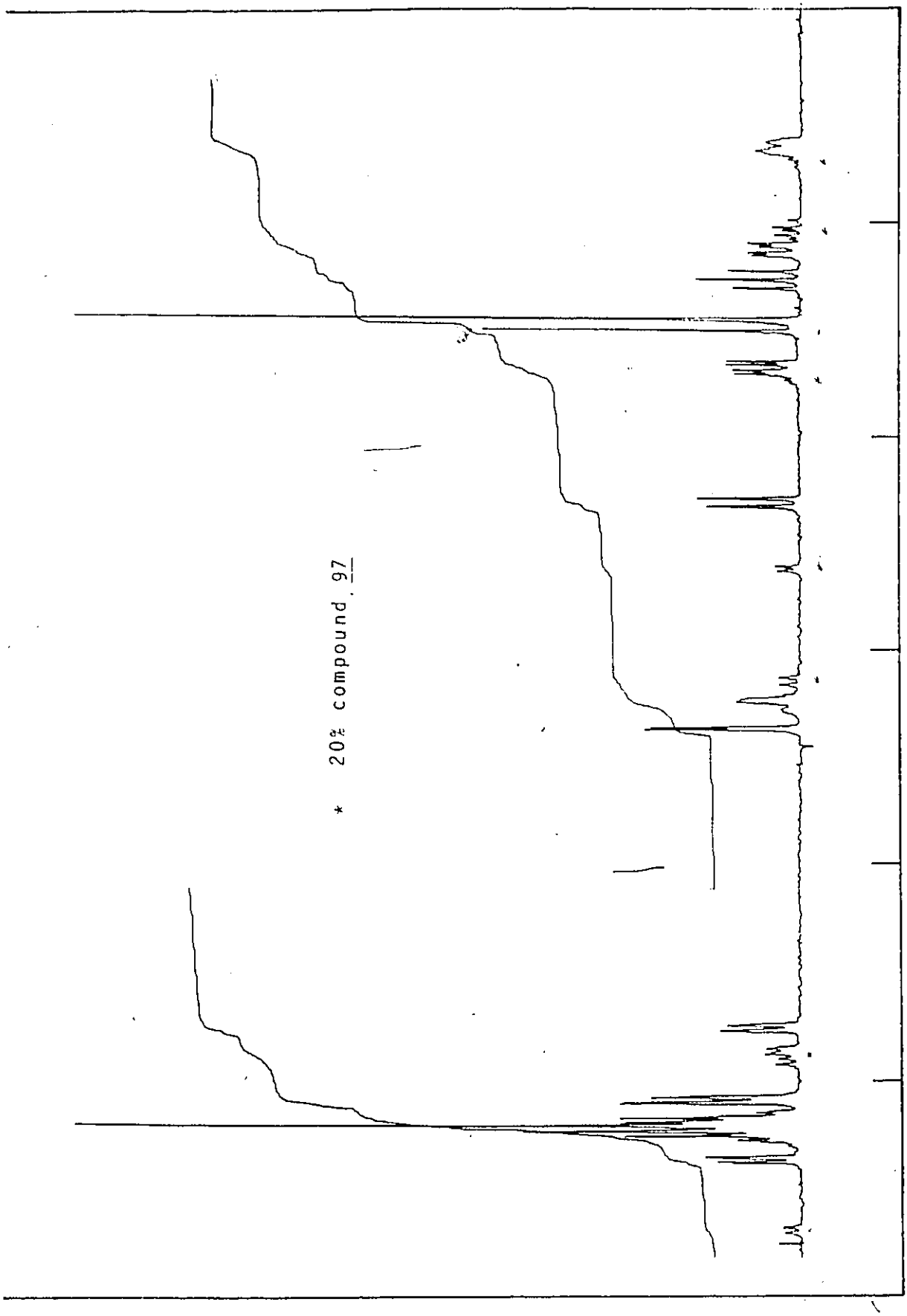
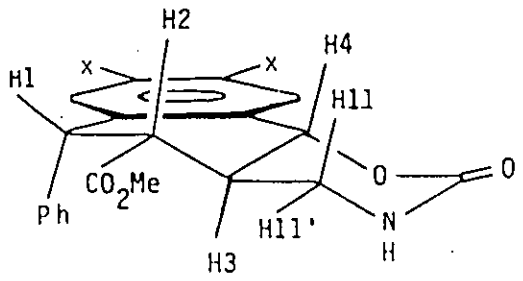
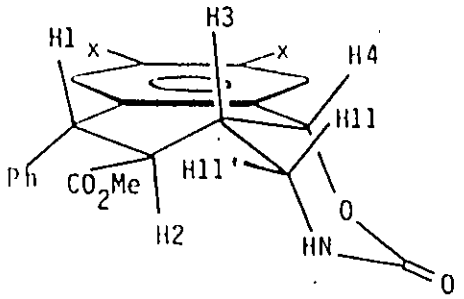


Figure 6.7: Proton NMR Spectrum of 98.





XX = OCH_2O: 95 96

X = H: 97 98

Chemical Shifts (ppm)

Compound	H1	H2	H3	H4	H11	H11'
95	4.51	3.05	2.63	5.07	3.02	3.72
96	4.22	3.23	2.61	5.29	3.12	3.68
97	4.65	3.10	2.70	5.18	3.06	3.75
98	4.34	3.29	2.66	5.40	3.15	3.70

Coupling Constants (Hz)

Compound	J_{1-2}	J_{2-3}	J_{3-4}	J_{3-11}	$J_{3-11'}$	$J_{11-11'}$	J_{11-NH}	$J_{11'-NH}$
95	5.9	12.1	10.2	11.0	5.4	11.0	0	4.4
96	11.5	11.5	2.8	4.3	4.8	12.6	1.4	0
97	5.9	10.9	10.2	10.9	5.2	10.9	0	5.2
98	11.7	11.7	2.5	4	4.9	12.7	2	0

Figure 6.8: Assignment of Signals in Proton NMR of 95, 96, 97 and 98.

93a, was refluxed in aqueous lithium hydroxide for 0.5h to hydrolyze the urethane function. The amino acids 99 and 100 thus produced were diazotized using sodium nitrite at pH4. The yield podophyllotoxin analog 73, obtained as white plates mp 247-248°C, from 93 was 15% after chromatography and recrystallization from methylene chloride-hexane. The hydroxy-lactone 73 was characterized by infrared bands at 3400 and 1760 cm^{-1} due to the alcohol and γ-lactone respectively. Podophyllotoxin has similar alcohol and lactone bands in the infrared. The proton nmr spectrum of 73 is similar to that of podophyllotoxin except for small chemical shift differences as well as the absence of the signals due to the trimethoxy aryl ring in exchange for the phenyl group signals.

Similar hydrolysis conditions converted the crude mixture of acids 97a and 98a, prepared by refluxing crude 93a in nitromethane, to the amino acids 101 and 102. Diazotization, as before, produced 74 mp 226-227°C as white plates, in 8% yield from 94. IR: 3420, 1756 cm^{-1}. The proton nmr was once again similar to podophyllotoxin except for the expected changes due to the absence of the functionality in the aryl rings.

The proton nmr spectra of 73 and 74 and podophyllotoxin are reproduced in Figures 6.9, 6.10 and 6.11, the signals are assigned in Figure 6.12.

The most noticable trend in the chemical shift differences between 73 and podophyllotoxin was a small downfield trend of the signals due to the protons nearest the pendant aryl ring. Specifically H1(4.63 ppm) and H2(2.86) in 73 were downshifted by 0.05 and 0.04 ppm relative to the respective podophyllotoxin protons. This downfield trend was expected because the electron donating effect of the trimethoxyaryl ring (in podophyllotoxin) would not

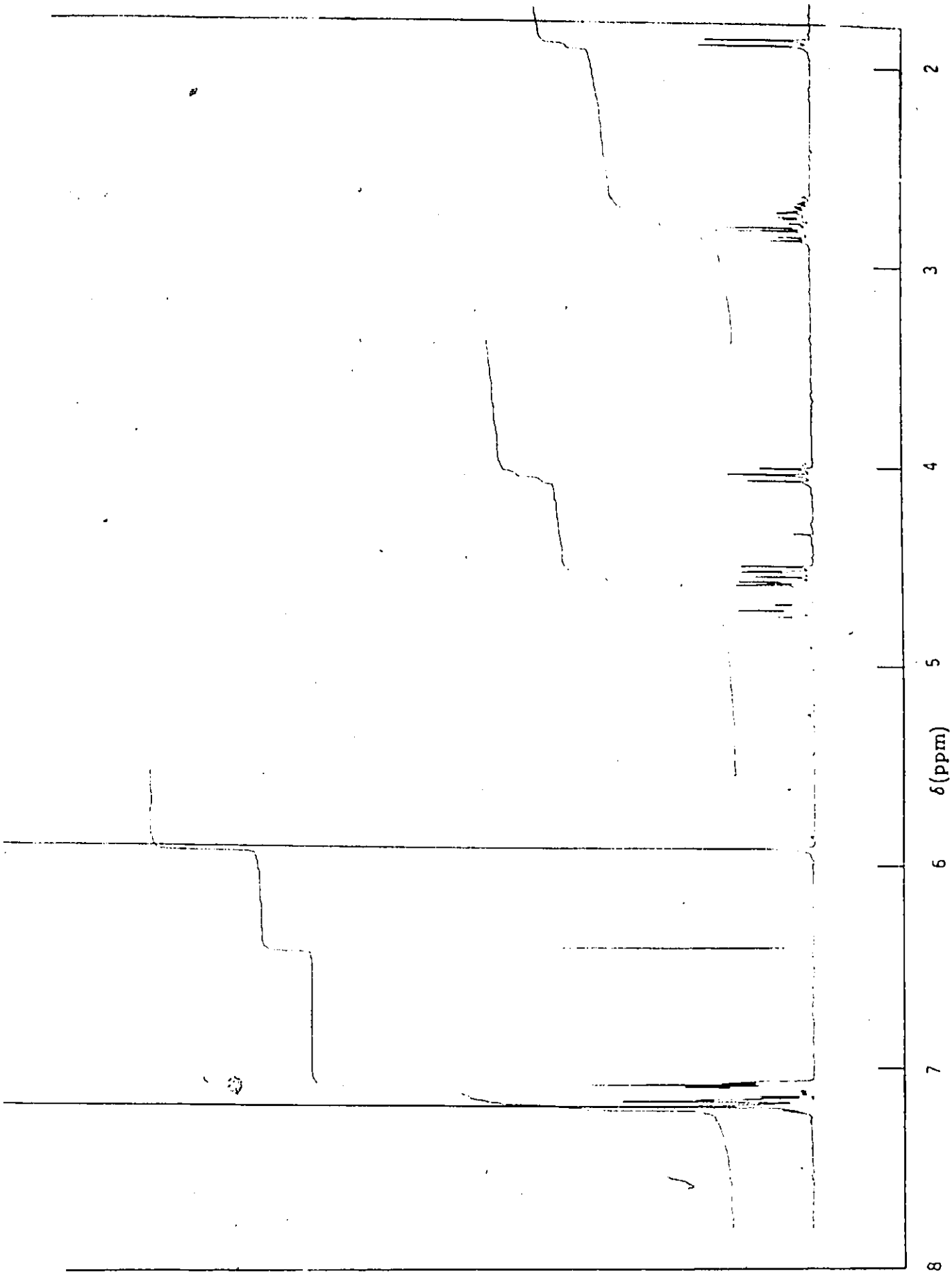


Figure 6.9: Proton NMR Spectrum of 73.

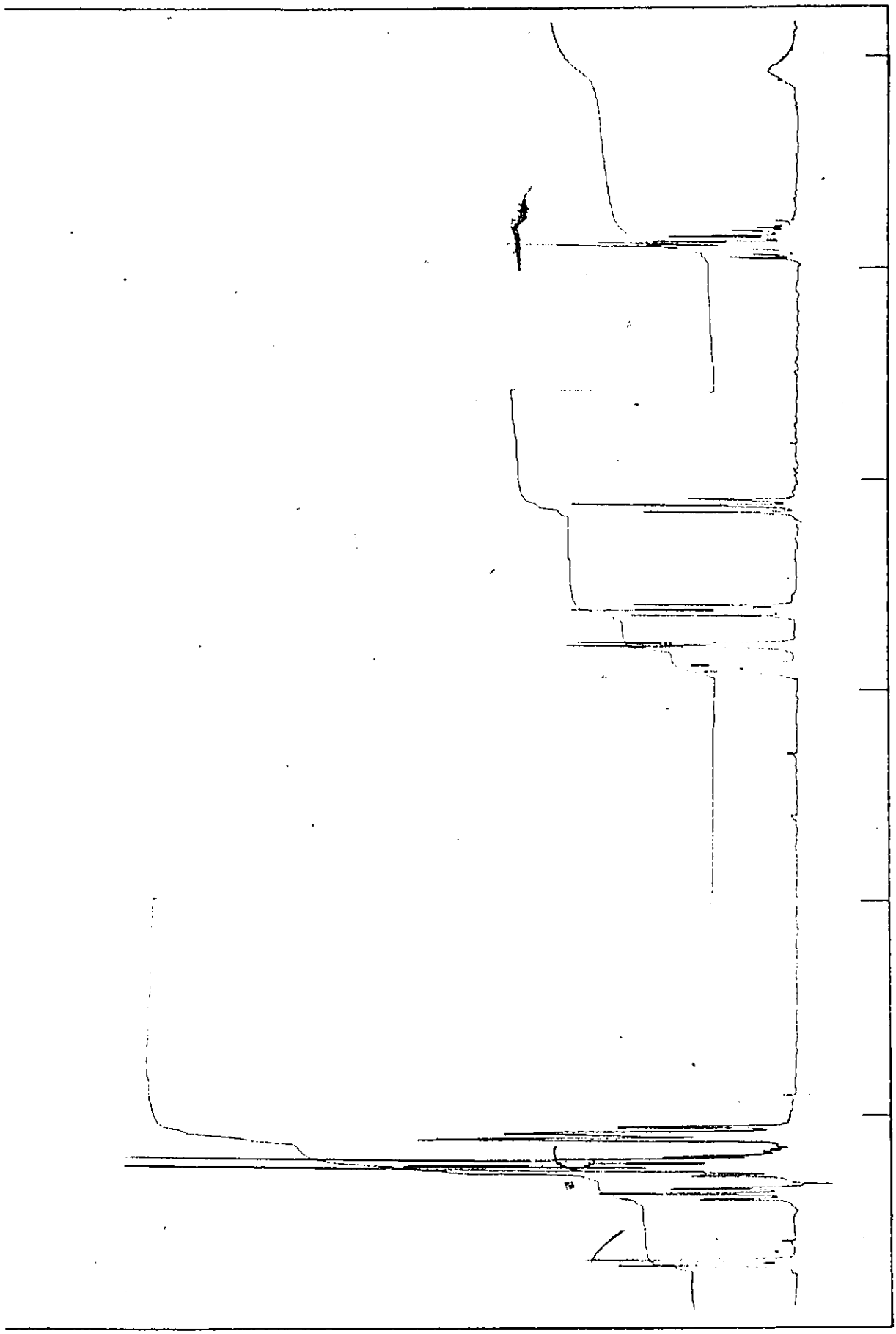


Figure 6.10: Proton NMR Spectrum of 74.

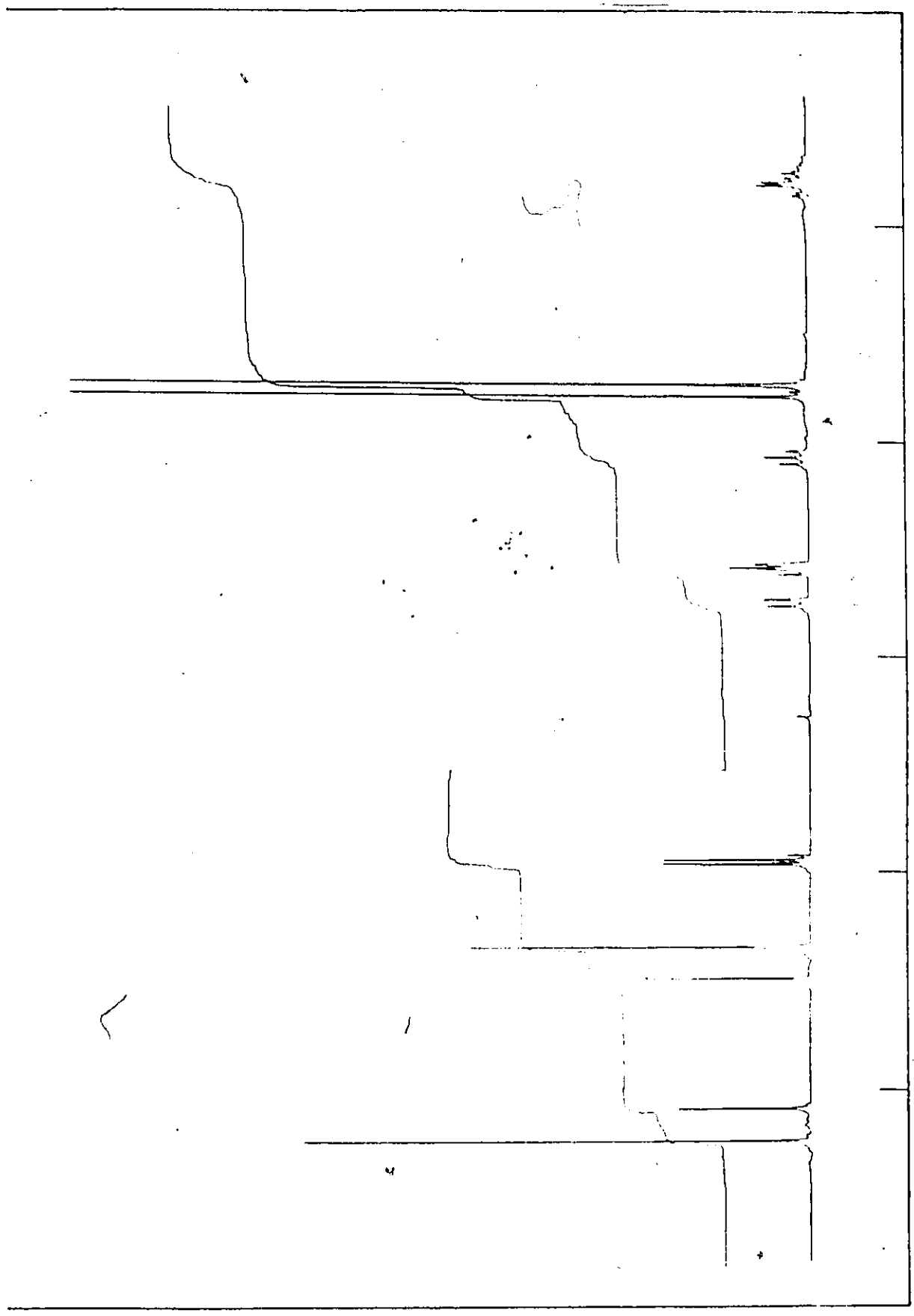
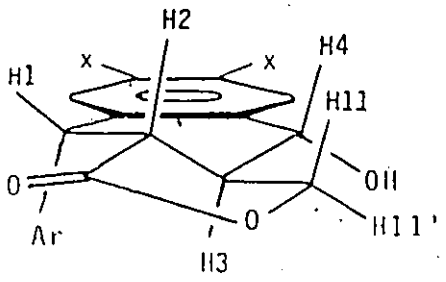


Figure 6.11: Proton NMR Spectrum of Podophyllotoxin.



XX=OCH_2O, Ar=3,4,5-trimethoxyphenyl: 1 podophyllotoxin

XX=OCH_2O, Ar=Ph: 73

X=H, Ar=Ph: 74

Chemical Shifts (ppm)

Compound	H1	H2	H3	H4	H11	H11'
1	4.58	2.82	2.77	4.76	4.08	4.60
73	4.63	2.86	2.76	4.76	4.08	4.57
74	4.77	2.89	2.85	4.88	4.11	4.60

Coupling Constants (Hz)

Compound	J_{1-2}	J_{2-3}	J_{3-4}	J_{3-11}	$J_{3-11'}$	$J_{11-11'}$
1	4.9	14	9.0	9.5	6.8	9.4
73	4.8	14.3	9.2	9.4	7.1	9.2
74	4.3	14.2	8.8	10.0	6.5	9.7

Figure 6.12: Assignment of Signals in the Proton NMR of 73, 74 and Podophyllotoxin.

be present in the compound where the E ring is a phenyl group. A similar trend was noted by comparison of the synthetic intermediates involved in the preparation of the two compounds.

The proton NMR of analog 74 obeys similar reasoning to that of 73, the absence of the methylenedioxy group producing the expected downfield shifts of the protons nearest the aromatic rings. In particular, H1(4.77 ppm), H2(2.89), H3(2.85) and H4(4.88) were downfield relative to their respective podo protons by 0.19, 0.07, 0.08, and 0.19 ppm.

In the synthesis of podophyllotoxin analog 73 the overall yield from 93 (15%) was not significantly different from that obtained in the (±)-podophyllotoxin synthesis of Chapter 5 (23%). The yield reported in Chapter 5 was based on a very small scale synthesis, yielding only 2 mg, and may have considerable uncertainty. A similar small scale synthesis of 73 had a yield of 26%.

The yield of 8% in the synthesis of 74 was, however, significantly lower than that obtained with 1 and 73. The factor responsible for this low yield was the thermolysis reaction of 94a in which the yield of the required tricyclic acid, isolated as its methyl ester, was only 27%. The yields of the analogous reactions, producing 70 and 95, were 62 and 50% respectively.

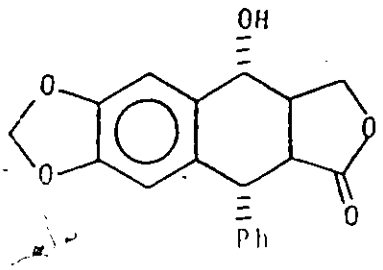
Thermolysis of 94a required a higher temperature (101°C) and a much longer reaction time (1 day) than the same reaction for 69a and 93a (90°C, 5h). The more severe conditions, in the presence of the carboxylic acid group, were thought to be responsible for unwanted side reactions such hydrolysis of the urethane function. Similar conditions using the methyl ester 94 produced 97 in a more acceptable 56% yield.

An alternative higher yield method for the preparation of 74, involving thermolysis of the methyl ester 94, is described in Chapter 7.

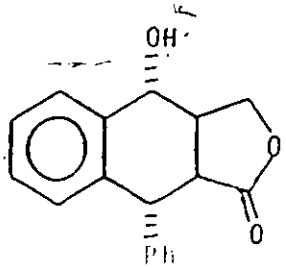
6.3 Conversion of Podophyllotoxin Analogs to Picropodophyllotoxin Analogs

It was noted in Chapter 1 that mild base treatment of podophyllotoxin converts the strained *trans* fused lactone ring to the more stable *cis* fused picropodophyllotoxin. In order to establish that the podophyllotoxin analogs described here have similar characteristics, they were converted to their respective picro forms under the same conditions.

Thus the podo analogs 73 and 74 were treated with aqueous sodium acetate in refluxing ethanol, to yield their respective picro analogs 103 mp 185-186°C and 104 mp 148-149°C both as white cotton-like crystals. IR:



103



104

103 3430, 1763 cm^{-1}. 104 3430, 1760 cm^{-1}. The proton nmr spectra were similar to picropodophyllotoxin apart from the expected changes due to the variation in aryl ring substitution. Similar reasoning to that used in the case of the podo analogs also applies in the comparison of the respective picro analog's proton nmr spectra to picro.

The spectra of 103, 104 and picropodophyllotoxin are shown in Figures 6.13, 6.14, and 6.15, their proton assignments are given Figure 6.16.

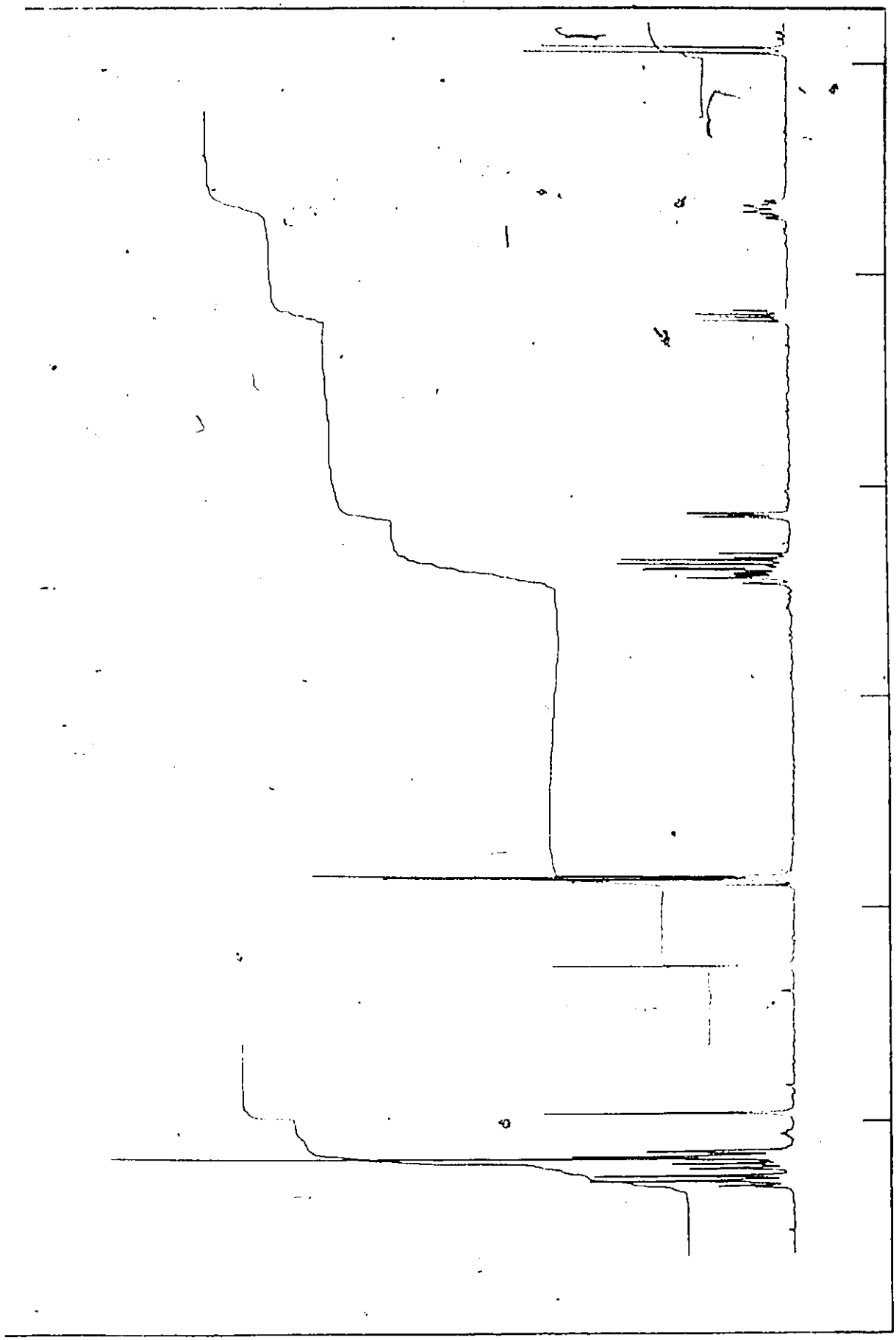


Figure 6.13: Proton NMR Spectrum of 103.

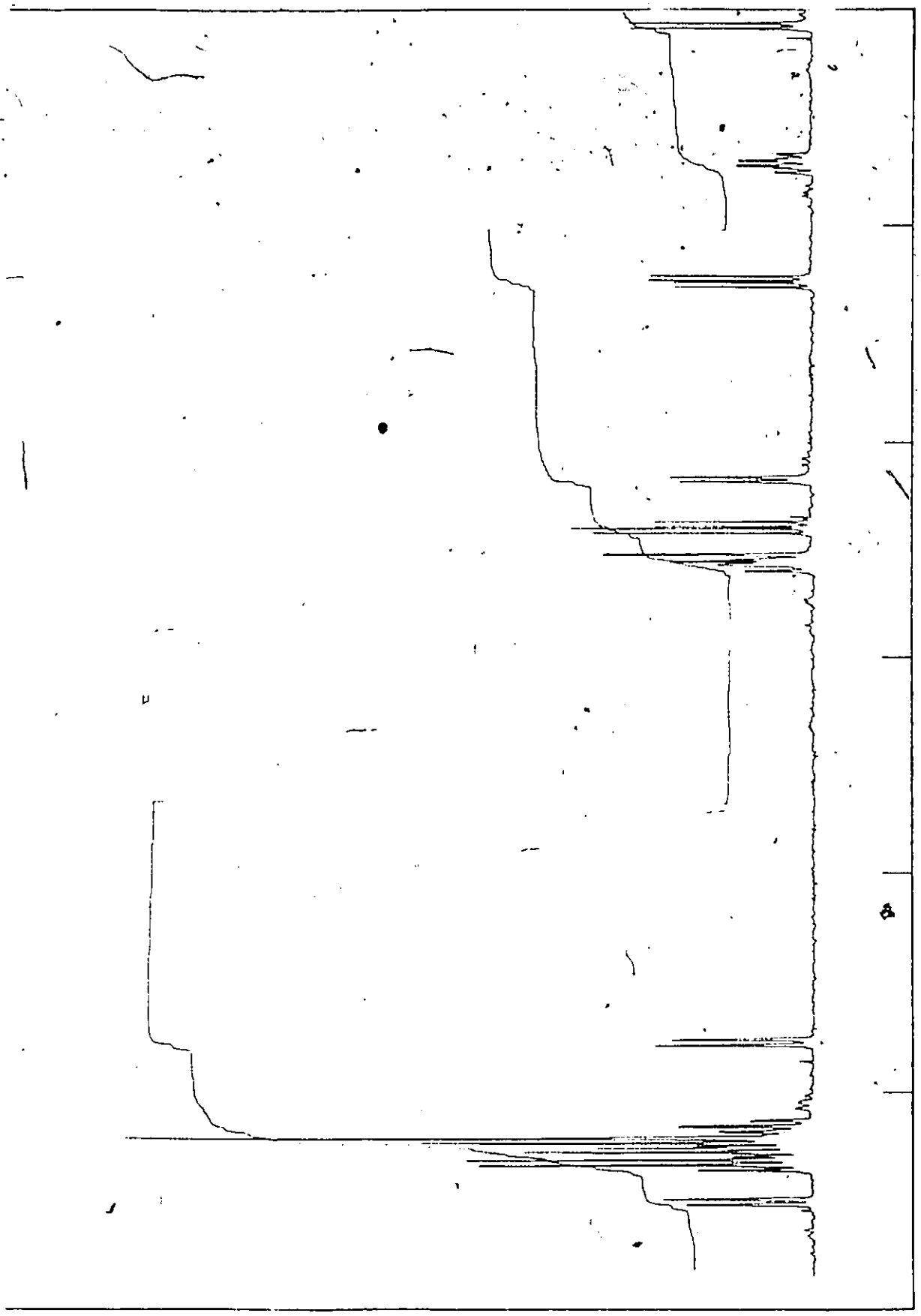


Figure 6.14: Proton NMR Spectrum of 104.

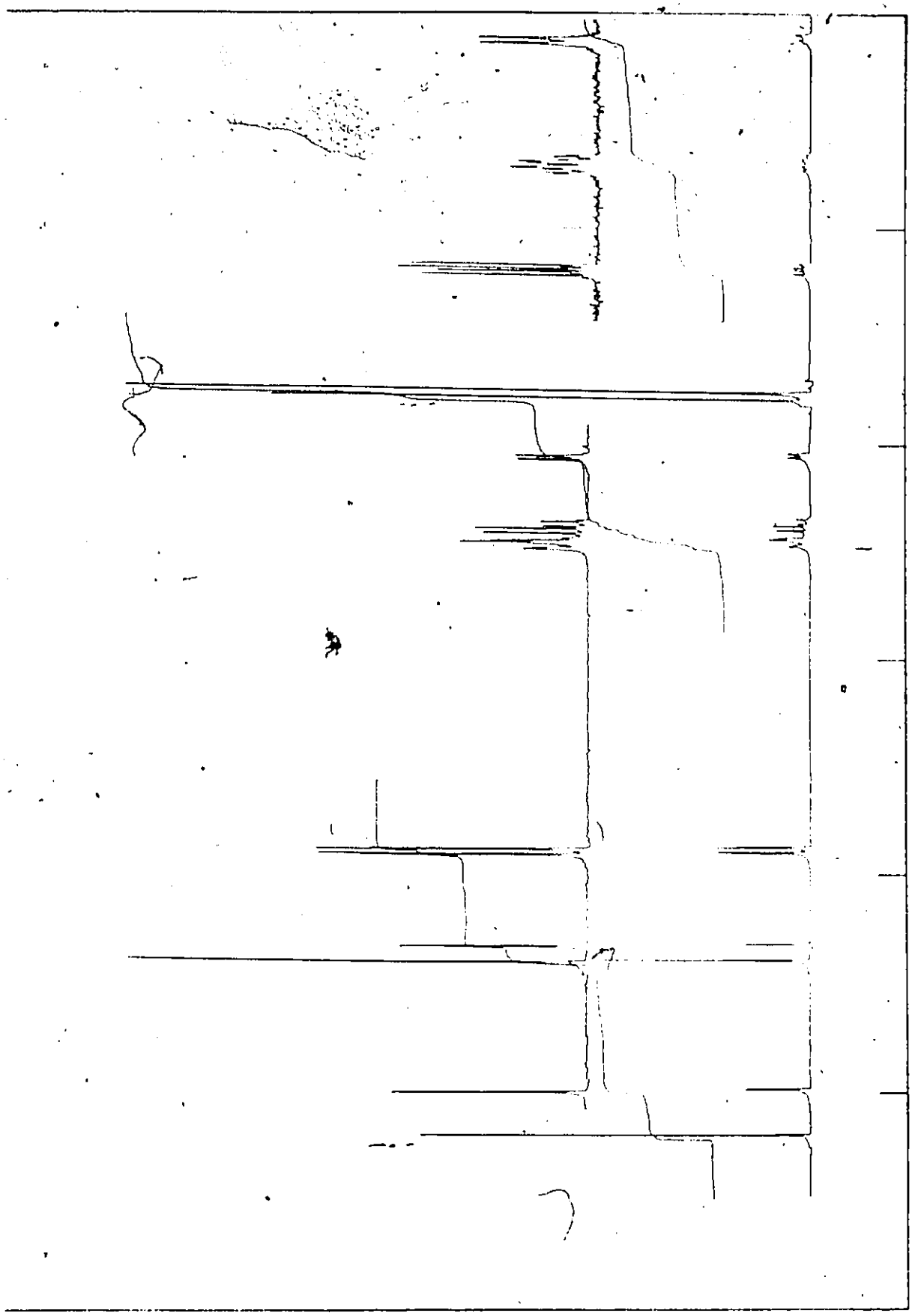
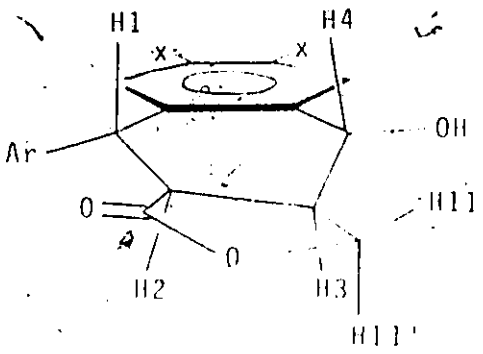


Figure 6.15: Proton NMR Spectrum of Picropodophyllotoxin.



XX=)CH_2O, Ar=2,3,4-trimethoxyphenyl: 1 picropodophyllotoxin

XX=OCH_2O, Ar=Ph: 103

X=H, Ar=Ph: 104

Chemical Shifts (ppm)

Compound	H1	H2	H3	H4	H11	H11'
3	4.10	3.22	2.73	4.48	4.50	4.41
103	4.20	3.25	2.74	4.50	4.47	4.40
104	4.21	3.28	2.73	4.60	4.57	4.43

Coupling Constants (Hz)

Compound	J_{1-2}	J_{2-3}	J_{3-4}	J_{3-11}	$J_{3-11'}$	$J_{11-11'}$
3	5.1	9.2	7	2.1	6.1	9.6
103	5.0	9.3	7.8	2.4	6.1	9.6
104	6.2	9.5	7.4	2.0	6.4	9.6

Figure 6.16: Proton NMR Assignments for 103, 104 and Picropodophyllotoxin.

Chapter 7

Variations in Methodology of Analog Synthesis

A viable synthesis of (±)-podophyllotoxin was presented in Chapters 4 and 5. In Chapter 6, similar methodology was employed for preparation of the two podophyllotoxin analogs 73 and 74. The synthesis produced analog 73 in acceptable yield, but 74 was obtained with only half the yield of the others. The yield of the thermolysis reaction of the acid 94a was reduced, possibly because the reaction conditions were more severe than those necessary for thermolysis of the analogous compounds containing substituted aryl rings. Thermolysis of the methyl ester 94 was found to produce the required tricyclic methyl ester 97 with a much more acceptable yield. Thus if conversion of the methyl ester to its acid could be carried out under nonbasic conditions, which would not cause epimerization at C2 (Chapter 5), thermolysis of the methyl ester 94 could be used in the place of the acid 94a.

7.1 Use of Lithium Iodide in DMF for Non-basic Cleavage of the Methyl Ester

In Chapter 5 a method was discussed briefly in which methyl esters could be cleaved by the use of lithium iodide in refluxing DMF. The high temperature (153°C) required was thought to be undesirable and the method was not

attempted. In view of the possible yield advantage to be gained by using such a method in the synthesis of 74, this route was carefully examined for conversion of the methyl ester 97 to its acid 97a. Refluxing the solution, using similar conditions to those described in the literature, was found to require long reaction times and resulted in extensive thermal decomposition.

Because the conditions given in the literature for this reaction were considered to be rather severe (refluxing DMF, 153°C), an attempt was made to carry out the demethylation at a lower temperature. It was noted in Chapter 5 that the mechanism of the halogenolysis reaction involves nucleophilic displacement of the methyl group by iodide to produce methyl iodide and the lithium carboxylate.[31] The reverse reaction can also occur and thus the reactants and products are in equilibrium. In order to shift the equilibrium to the product carboxylate, methods have been reported in which the methyl iodide is removed by addition of a nucleophile such as cyanide, or by simply refluxing the solution to evaporate it. Cyanide ion being somewhat basic however, was found to produce partial epimerization at C2.

A variation, which had not been previously reported, was found to be successful:

1. The methyl iodide was evaporated by passing nitrogen over the reaction mixture to shift the equilibrium toward the products.

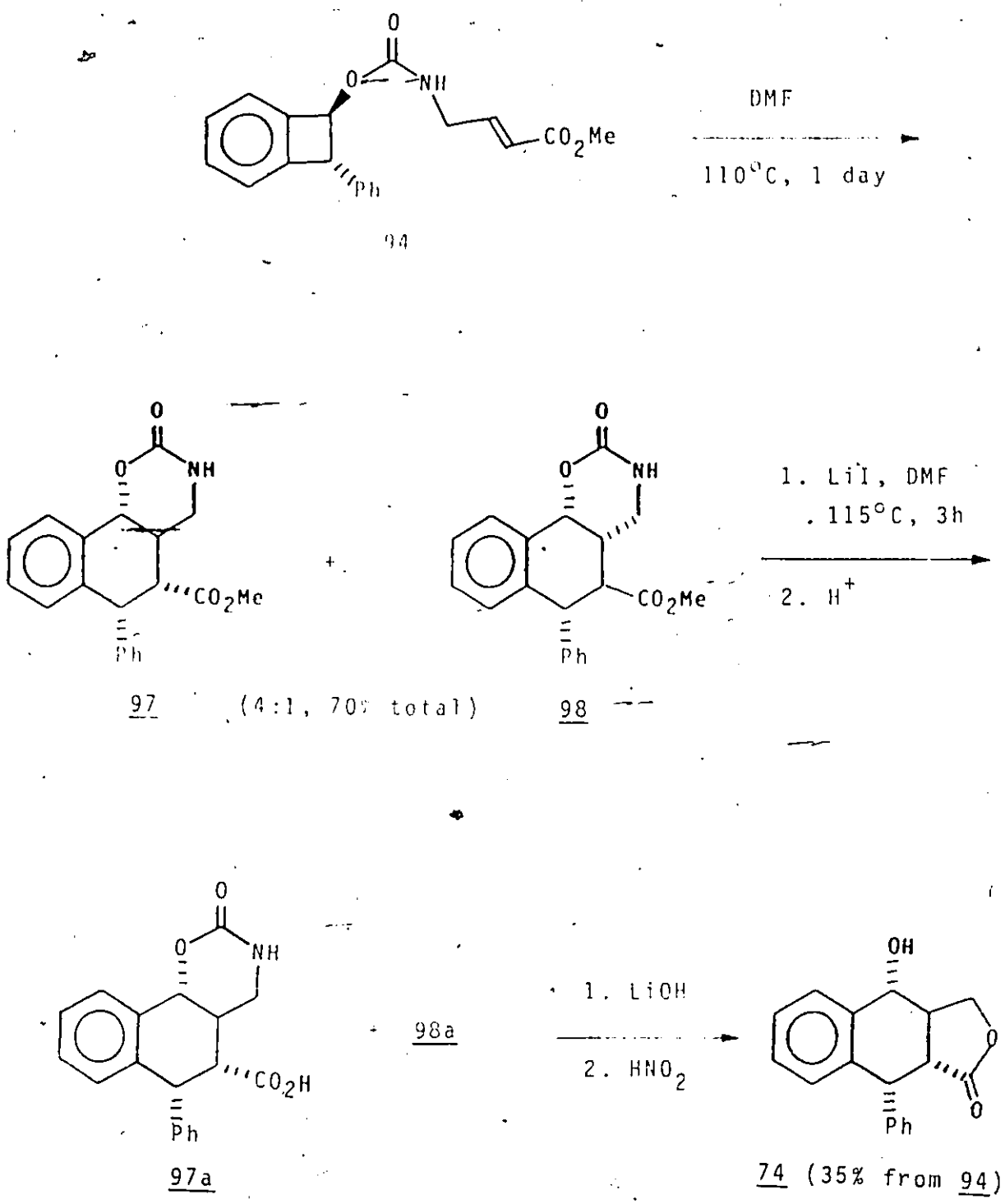
2. Large amounts of lithim iodide were used in high concentrations to enhance both the equilibrium positon and the reaction rate.

Thus the tricyclic methyl ester 97 (80mg, 0.25mmol) and LiI (1.2g, 9.0mmol) in 2 mL DMF was heated to 115°C for 3h while the solution was

stirred vigorously with nitrogen passing over the suface. Aqueous workup afforded crude acid 97a which was converted to the podophyllotoxin derivative 74, by the usual methods of urethane hydrolysis and diazotization, in 55% yield. The crude product was examined by proton nmr and found to contain none of the picro analog 104.

In order to simplify the overall conversion of the benzocyclobutene 94 to the podophyllotoxin analog 74, it was postulated that the thermolysis reaction could be performed in DMF instead of nitromethane, thus eliminating the need for isolation of the tricyclic ester. Thermolysis of 94 in DMF at 110°C for 1 day produced a 4:1 mixture of the *trans* and *cis* fused tricyclic esters 97 and 98, from which 97 could be isolated in 56% yield. Conversion of the resulting mixture of esters to the lactone 74, using the method described above, afforded 74 in 35% overall yield. The method described above is outlined in Scheme 7.1.

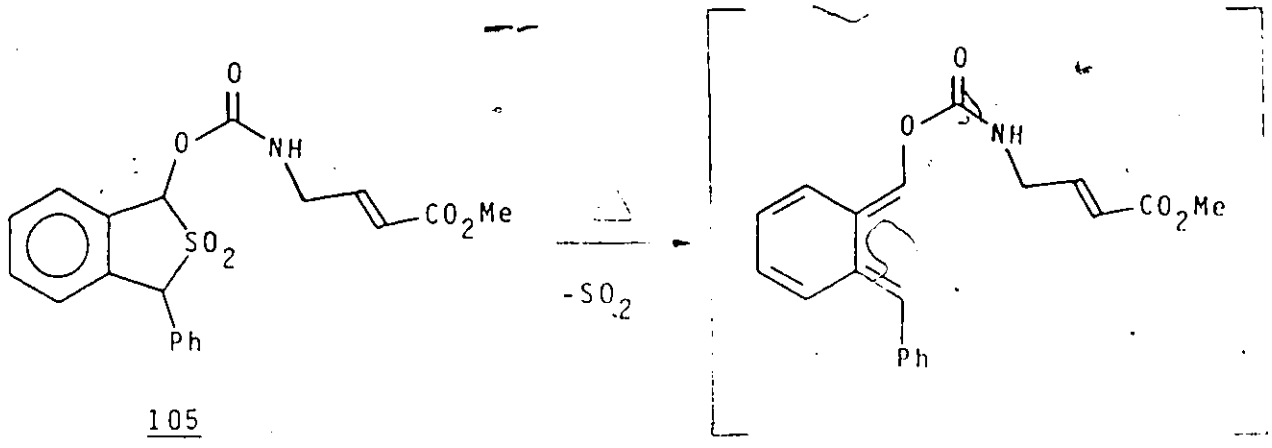
Comparison of the overall yield in the method just described, for the conversion of 94 to 74 (35%), to that of the method described Chapter 6 (8%) shows that a remarkable improvement was made. It was also noteworthy that the thermolysis reaction in DMF produced a 4:1 ratio of the *trans* to *cis* fused isomers, whereas the same reaction of both the acid 94a and the ester 94 in nitromethane afforded a ratio of only about 2:1.



Scheme 7.1: Conversion of Benzocyclobutene 94 to Lactone 74 Via Cleavage of the Methyl Ester with LiI in DMF.

7.2 Use of Sulfones as *o*-quinodimethane precursors

In Chapter 3 it was noted that the required E,E-*o*-quinodimethanes may also be generated by thermal extrusion of sulfur dioxide from a *cis* substituted sulfone. Thus the sulfone 105 should, in principle, produce the same *o*-quinodimethane as the benzocyclobutene 94.

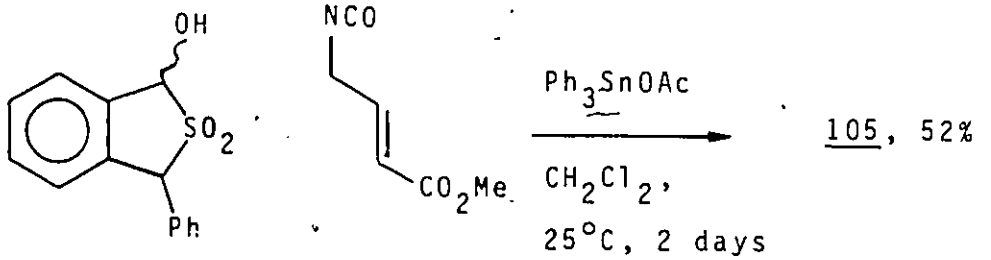


105

The sulfone 105 could, in principle, be prepared from the known hydroxy-sulfone 92 [34] by coupling it to the isocyanate 68 under the same conditions as the benzocyclobutenols. If both the coupling and the thermolysis reactions were sucessful, the lactone 74 could be prepared in 2 fewer steps than the method previously described! That is, the need for acetylation of 92 and the thermolysis of 88 would be eliminated.

Thus a solution of the hydroxy-sulfone 92, isocyanate 68 and triphenyltin acetate in methylene chloride was evaporated to a viscous oil at 25°C. Examination of the reaction mixture with IR and proton nmr revealed only starting materials. The solution was diluted with methylene chloride and reevaporated several times over a 2 day period, after which IR indicated the

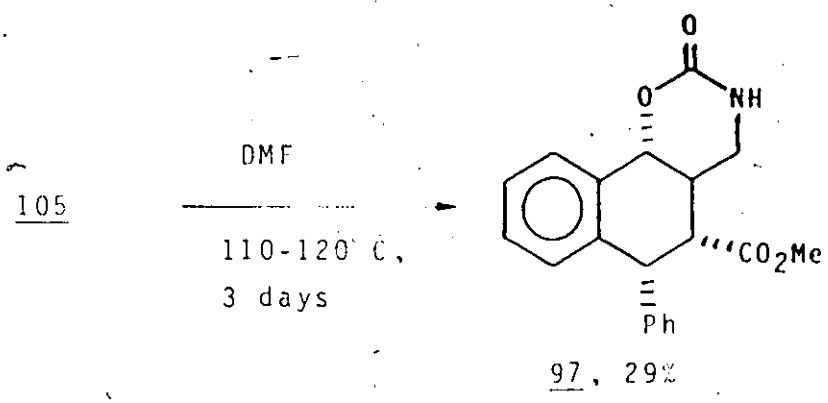
absence of an isocyanate band at 2257 cm^{-1}. Purification on silica (hexane-ethyl acetate = 2:1) afforded 105 in 52% yield as a pale yellow foam. The IR of 105 shows bands at 3300-3550, 1745 and 1723 cm^{-1} due to the urethane and ester groups. Proton nmr spectroscopy produced signals at δ 3.72(s, 3H), 4.07(m, 2H), 5.17(m, NH), 6.00(dt, J=15.8, 1.7 Hz, 1H) and 6.92(dt, J=15.8, 4.9 Hz, 1H) due to the methylcrotyl moiety, and two 1H singlets at 5.55 and 6.67 ppm due to the two benzylic hydrogens. The fact that only two benzylic proton signals were produced indicated that the product was likely one isomer. The relative stereochemistry of the two substituents on the cyclic sulfone ring could not be assigned on the basis of the spectroscopic data.



The coupling reaction was much slower with the hydroxy-sulfone (2 days) than in the case of the respective benzocyclobutenol (minutes). This marked difference was attributed to a reduced nucleophility of the hydroxyl group due to the electron withdrawing effect of the sulfone.

The assignment of relative stereochemistry about the cyclic sulfone was made on the basis of the geometry of the product obtained upon thermoly-

sis. The thermolysis was carried out in DMF at 110 to 120°C for 3d. Nitrogen was continuously passed through the reaction mixture to purge sulfur dioxide and prevent it from retrapping the intermediate *o*-quinodimethane. Evaporation of the DMF followed by chromatography and recrystallization afforded in 29% yield a solid which was identical in every respect to the tricyclic urethane 97.



97, 29%

The fact that only the required tricyclic product was produced indicated that the E,E-*o*-quinodimethane intermediate had resulted upon thermal sulfur dioxide extrusion from the sulfone 105. Since the cheleotropic elimination of sulfur dioxide is suprafacial,[35] the two substituents on the sulfone ring in 105 must be *cis*.

The above two step conversion of the sulfone 92 to the tricyclic intermediate used in the synthesis of the podophyllotoxin analog 74 thus represents a shorter approach than via the benzocyclobutenes. The entire synthesis from the commercially available *o*-benzylbenzaldehyde only involves 3 isolation or 6 chemical steps with 6% overall yield. If the respective aldehydes required for synthesis of other podophyllotoxin derivatives were easily obtained, this

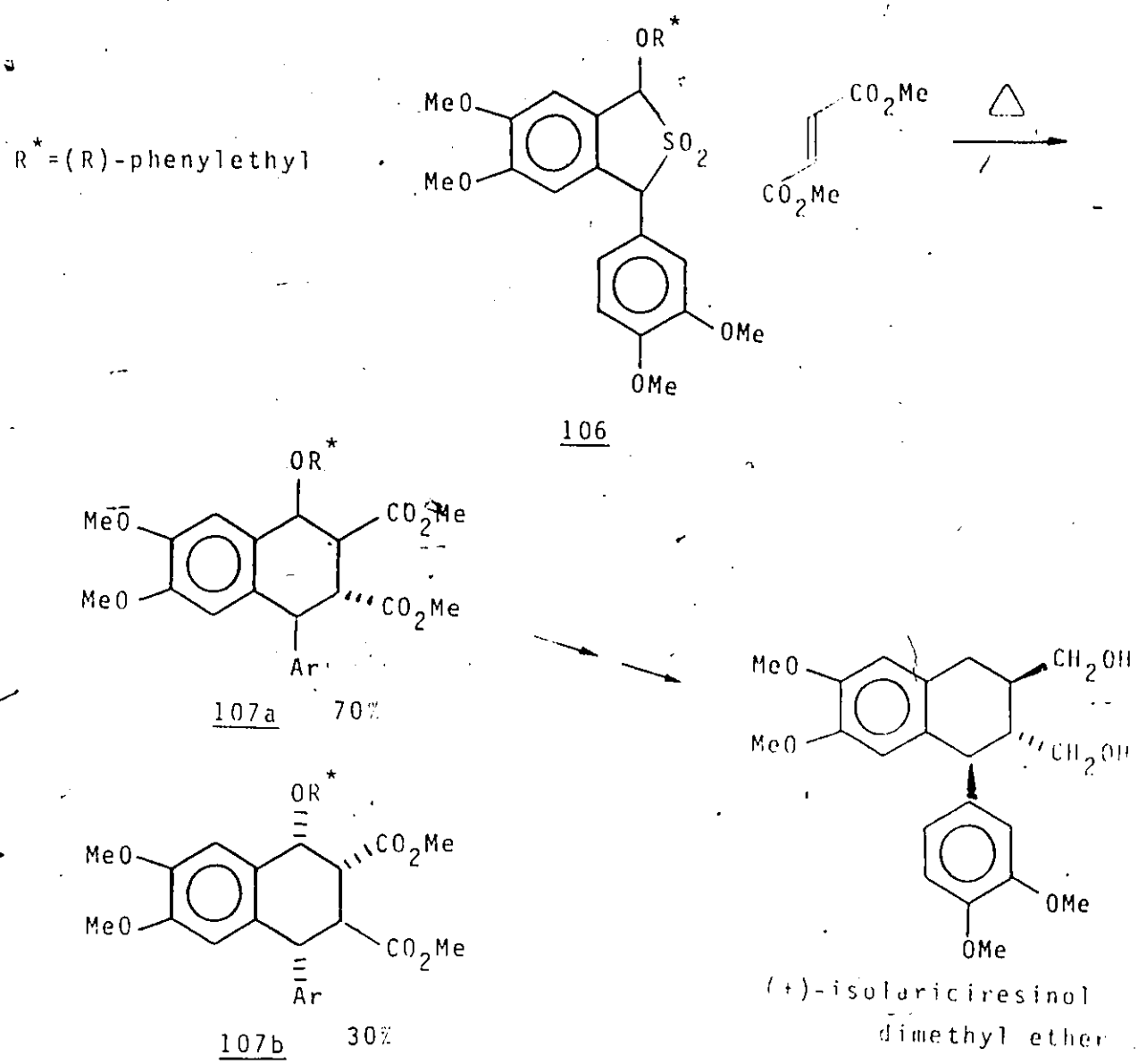
method would be much shorter than the previously discussed syntheses via the benzocyclobutenols.

Attempts to perform the thermolysis of sulfone 105 larger scale were less rewarding. Yields dropped to less than 10% when gram ammounts of 105 were thermolysed. The cause of this difficulty was most likely the inability, with the available apparatus, to effectively remove the sulfur dioxide. Retrapping of the intermediate *o*-quinodimethane with sulfur dioxide thus competed with the Diels-Alder reaction, thereby effectivly lowering the reaction rate and producing extensive thermal decomposion of the reactants due to the longer reaction time required.

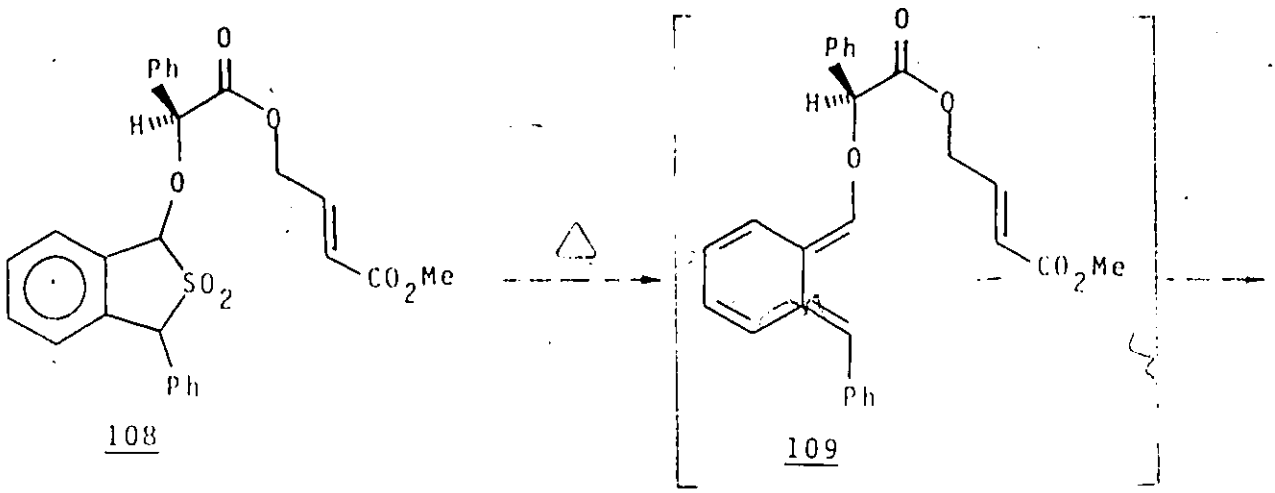
The simple appartaus used for passing nitrogen through the solution, a small tube below the surface, could in principle be replaced by a more effective system, thus possibly eliminating the problem associated with use of a larger scale.

7.3 An Attempt at Asymmetric Induction using a Chiral Bridging Moiety

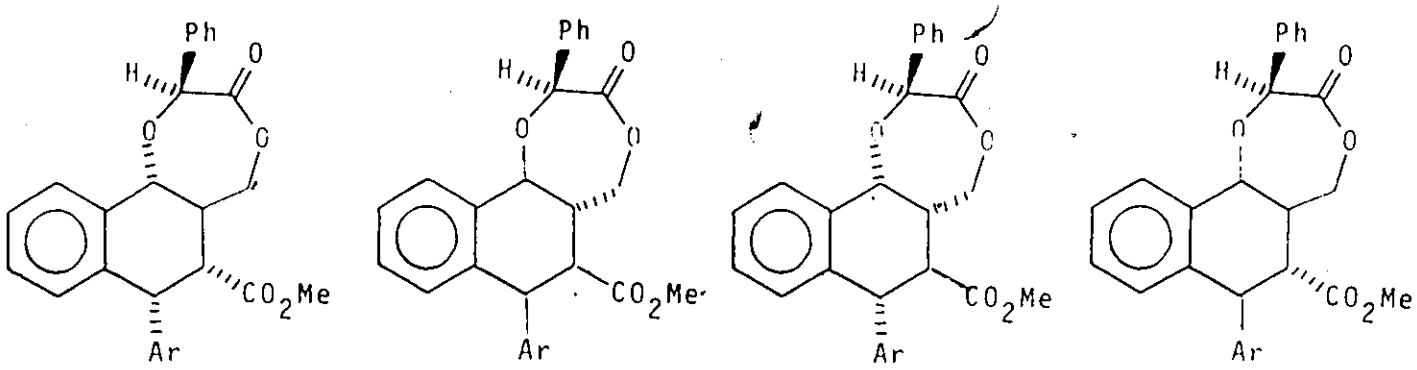
Charlton and co-workers have reported that *o*-quinodimethanes, containing a chiral alkoxy group at C1, showed significant diastereoselecivity upon intermolecular Diels-Alder reaction with dienophiles.[36] For example, the sulfone 106, when heated in the presence of dimethylfumarate, afforded the two diastereomers 107a and 107b in a 83:17 ratio. Removal of the chiral auxialiary and reduction produced the lignan (+)-isolariciresinol dimethyl ether in 83% optical purity.[37]



This methodology could, in principle, be extended to our intramolecular system by use of a chiral bridging group. One such system was considered, the sulfone 108, in which a mandeloyl moiety was used as the bridging group. Thermolysis of 108 should produce the *o*-quinodimethane 109, which is expected to undergo an intramolecular Diels-Alder reaction, to yield the four possible products due to exo and endo addition of the dienophile to either face of the diene: 110a, 110b, 110c and 110d. If both the stereoselectivity of endo over exo addition (the case of the system previously used for the



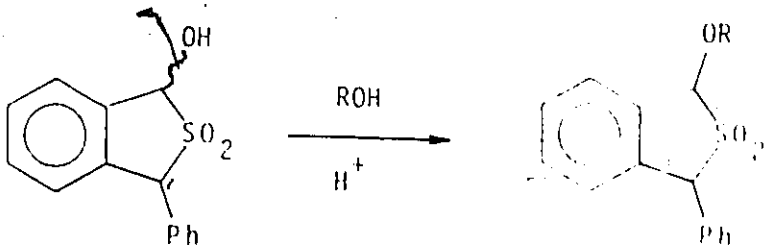
108 109



110a 110b 110c 110d

podophyllotoxin synthesis) as well as the π face selectivity (observed in the systems of Charlton) were to occur, the isomer with both correct relative and absolute stereochemistry could be produced stereoselectively.

Reaction of an alcohol with the hydroxy-sulfone 92 in the presence of an acid catalyst has been found, by Charlton and Durst [34], to yield the ethers 112 predominantly as the *cis* isomers. Thus the same sulfone should,



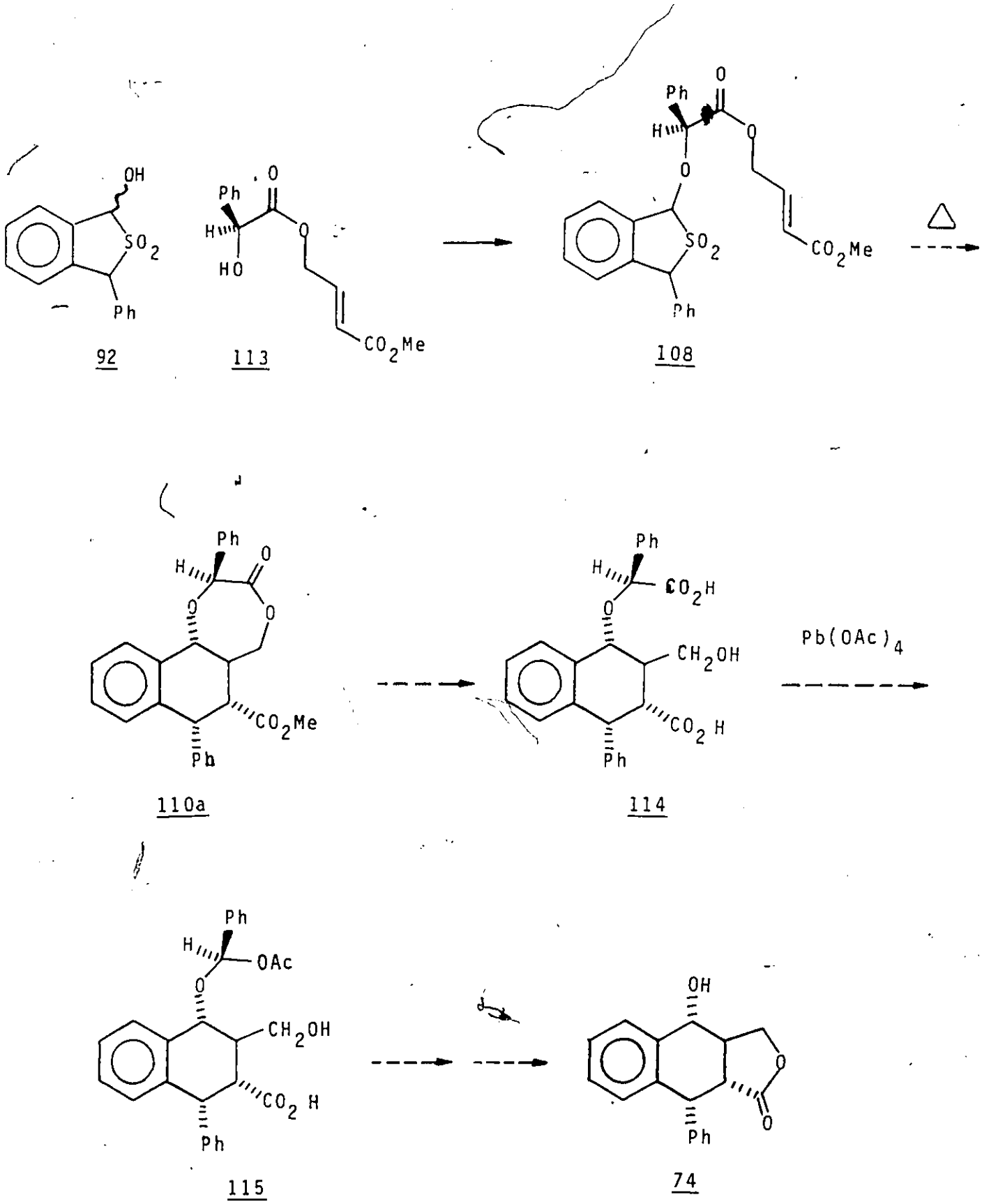
92 112

in principle, react with the alcohol 113 to produce the required ether 108 (Scheme 7.2).

Hydrolysis of the isomer 110a should yield the diacid 114. Oxidative decarboxylation of acids with lead tetraacetate is known to be accelerated by cation stabilizing groups.[1] Treatment of the diacid 114 with lead tetraacetate should selectively decarboxylate the mandelic acid group and afford the acetate 115. Finally, hydrosysis of the acetate and lactonization would yield the podophyllotoxin derivative 74 as a single enantiomer.

Methyl *trans*-4-bromocrotonate 116 and (±)-mandelic acid 117 were coupled under phase transfer conditions to yield the hydroxy ester 113 mp 64-65°C, as fine white needles in 32% yield. The infrared spectrum showed the expected alcohol band at 3450 cm^{-1}, and two ester bands at 1743 and 1716 cm^{-1}. Proton NMR: δ 3.38(br, OH), 3.71(s, 3H, CH_3), 4.80(m, 2H, CH_2), 5.23(s, 1H, CH), 5.77(dt, J=15.8, 2.0 Hz, 1H, =CHCO-), 6.84(dt, J=15.8, 4.6 Hz, 1H, $CH_2C\underline{H}$=), 7.3-7.5(aromatics, 5H).

[1]The mechanism involves formation of a carbocation, on the carbon from which the carboxyl was removed, as the rate determining step.



Scheme 7.2: Proposed Asymmetric Induction.

The hydroxy-sulfone 92 and alcohol 113 were coupled using borontrifluoride etherate as catalyst to yield the ether 108 as a mixture of diastereomers in 43% yield. The infrared spectrum showed two ester bands as 1753 and 1725 cm^{-1} as well as bands at 1318 and 1176 cm^{-1} due to the sulfone. The proton nmr indicated the presence of at least 3 diastereomers in about equal amounts: 3 sets of signals similar to the alcohol 113, but with slightly different chemical shifts; a 3H multiplet at 5.66-5.98 ppm due to the two benzylic and one vinyl proton, a 14H multiplet at 7.1-7.6 ppm characteristic of the 3 aryl groups.

The reaction could in principle produce four diastereomers of 108, two *cis* and two *trans*. The fact that 3 isomers of 108 were produced indicates that at least one *cis* isomer was produced. If two *cis* isomers were present then both would yield the same *o*-quinodimethane:

The mixture of diastereomers 108 was heated in refluxing toluene for 5h while nitrogen was bubbled through the solution. Chromatography produced no characterizable products.

The failure of the reaction to produce the expected products was attributed to the fact that formation of a seven membered ring, as in the expected product 110a, is not as entropically favorable as is the formation of a six membered ring, which was successful in the other systems such as 105. The intramolecular Diels-Alder reaction desired may have encountered competion from other reactions, such as dimerization of the *o*-quinodimethane and other intermolecular Diels-Alder reactions.

In the previous chapters is was noted that polar solvents such as DMF produced more efficient thermolysis reactions than less polar solvents such

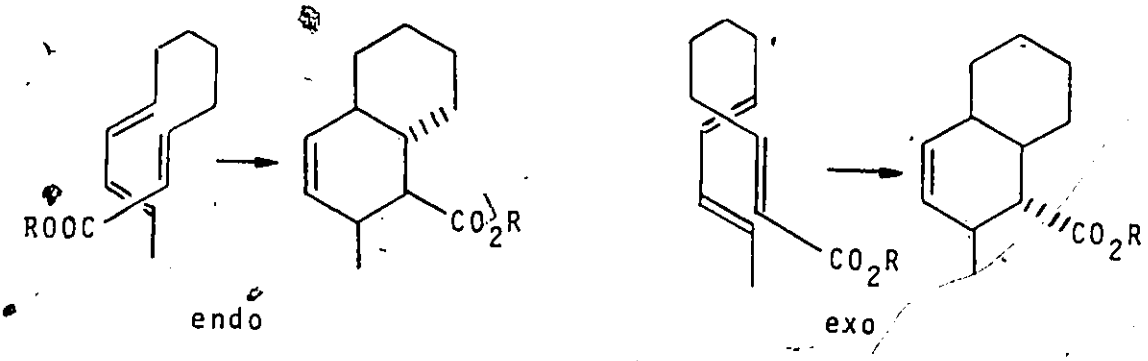
as toluene. Further efforts to thermolyse 108 in more polar solvents may prove fruitful.

Chapter 8

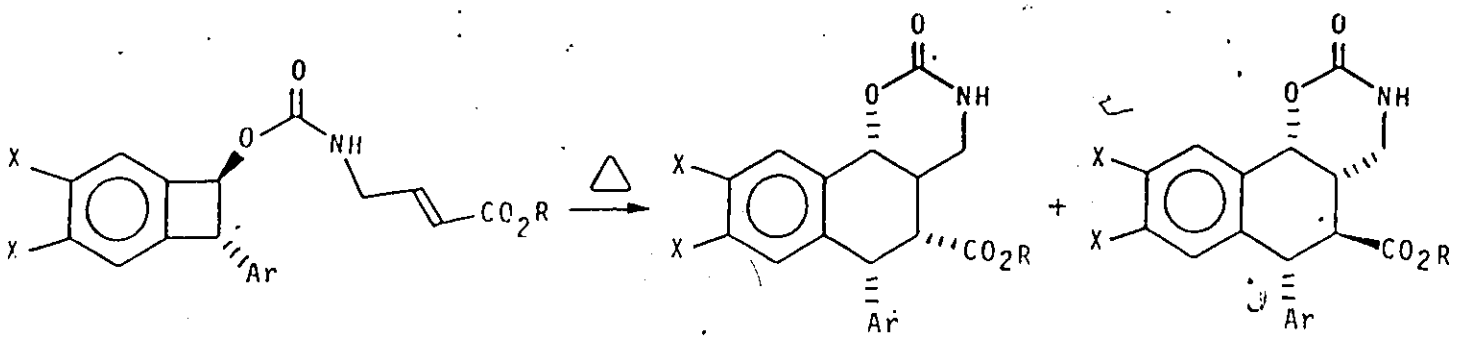
Factors Effecting the Ratio of Exo to Endo Products and Rate in Intramolecular Diels-Alder Reactions

8.1 Influences on Product Ratio

It was noted in the previous chapters that the ratio of endo to exo addition of the crotyl moiety to the *o*-quinodimthane in the intramolecular Diels-Alder reactions was influenced by several factors. In Table 8.1, results of the various systems examined are compiled. Endo addition, in which the ester moiety is over the diene, is usually prefered in intermolecular systems. Exo addition is usually not prefered in the absence of steric effects.[39].



From Table 8.1 it is seen that the ratio of endo to exo products was directly related to both the solvent polarity and the polarity of the crotyl



Compound	R	XX	Ar	Solvent	Temp(C)	Time	Endo:Exo	Yield (endo,%)
69	Me	OCH_2O	3,4,5-tri-methoxyphenyl	CH_3NO_2	101	1.5h	3:1	62
69a	H	"	"	"	90	5h	5:1	50
69a	H	"	"	H_2O/ dioxane = 95:1	100	1h	>10:1	36
-	(=)	"	"	H_2O	90	8h	>10:1	35
93	Me	OCH_2O	phenyl	cyclo-hexane	81	2d	1:4	-
"	"	"	"	$PhCH_3$	110	10h	1:1	-
"	"	"	"	CH_3NO_2	86	4h	2:1	47
"	"	"	"	MeOH	65	1d	2.5:1	-
93a	H	"	"	CH_3NO_2	85-90	4h	4:1	50
94	Me	H	phenyl	$PhCH_3$	90-100	5d	1:1	-
"	"	"	"	CH_3NO_2	101	1d	1.5:1	51
"	"	"	"	DMF	110	1d	4:1	56
94a	H	"	"	CH_3NO_2	101	1d	2:1	27

Table 8.1: Endo - Exo Ratios with Various Systems

derivative. Thus solvents such as nitromethane, DMF, methanol and water produced the highest ratios of endo to exo products. Use of the carboxylic acid in place of the respective methyl ester also produced a higher endo to exo ratio. The effect of using the respective carboxylate was even more pronounced, affording complete stereospecificity. The yield of the required product was often reduced, however, by use of solvents such as water, possibly because of the very limited solubility of the compounds in water, and thus solvents such as nitromethane and DMF were found most useful.

In general, solvent effects are not found to be important in Diels-Alder reactions, with the exception of a few isolated examples. A similar solvent effect, to that observed in our systems, has been reported by Berson in intermolecular Diels-Alder systems.[39] For example, methyl crotonate in decalin at 30°C reacted with cyclopentadiene to afford a 52:48 ratio of endo to exo products, while the same reaction in nitromethane produced a 59:41 ratio. Other solvents such as acetic acid afforded a 70:30 ratio. DMF was intermediate between decalin and nitromethane (56:44).

Berson suggested that the transition state for endo addition has a greater dipole moment than that of the exo. Thus polar sovents would stabilize the endo transition state more than the exo.

The solvent effects observed in our systems were somewhat larger than those observed by Berson but may arise for similar reasons.

The carboxylic acids displayed a greater preference for the endo products than their esters. The reason for this difference is not easily explained.

8.2 Factors Effecting the Thermolysis Reaction Rate

It is obvious from the data in Table 8.1 that the rate of formation of the "tricyclic" products was considerably faster in the more polar solvents. The rate determining step is presumably thermal ring opening of the benzocyclobutene ring. Thus the polar solvents must be lowering the activation energy of ring opening by raising the energy of the starting material or stabilizing the transition state. The reason for this solvent effect on the stability of the benzocyclobutene or the *o*-quinodimethane is not well understood.

Attention should be drawn to the fact that the methylenedioxy group in ring B also produced a large rate enhancement in the reaction, 69 and 93 required 5h at 90°C in nitromethane while 94 required 1 day at 101°C. The reason for the large effect of the methylenedioxy group on the reaction rate can be attributed to its electron donating ability. This electron donating effect decreases the energy of the product (*o*-quinodimethane) more than that of the starting material. Delocalization of the oxygen lone pairs into the *o*-quinodimethane would result in a net stabilization, whereas delocalization into the aryl ring of the benzocyclobutene would result in little stabilization as it would destroy the aromaticity. Because the *o*-quinodimethane is expected to be similar in energy to the transition state, the activation energy of the reaction would thus be reduced by the presence of the methylenedioxy group.

Part III

Summary

In Chapters 1 and 2 the need for a general synthesis of podophyllotoxin was established, by which analogs could be prepared. A direct synthesis of podophyllotoxin was proposed, in Chapter 3, where the four chiral centres would be generated by an intramolecular Diels-Alder reaction.

A highly stereoselective synthesis of (±)-podophyllotoxin was developed and is described in Chapters 4 and 5. The correct relative stereochemistry of the four chiral centres in podophyllotoxin was produced via an intramolecular Diels-Alder reaction involving an *o*-quinodimethane, generated by the ring opening of a *trans*-2-arylbenzocyclobutenol derivative. The synthesis of the previously unknown *trans*-2-arylbenzocyclobutenol intermediate was also described.

Using similar methodology, to that used for the preparation of (±)-podophyllotoxin, two analogs were prepared. It was found that several changes were possible, in the analog synthesis, which resulted in fewer steps as well as increased yields. The changes involved in the analog synthesis include use of a sulfone in place of the benzocyclobutenol for generation of the *o*-quinodimethane and cleavage of a methyl ester using lithium iodide in DMF, in place of saponification prior to the Diels-Alder reaction.

The stereoselectivity, of the Diels-Alder reaction, was found to be dependent on factors such as the solvent and dienophile carboxylic acid derivative. Use of polar solvents such as DMF and nitromethane was found to afford the highest yields of the required products. The polar solvents also gave higher reaction rates, in the thermolysis reactions, than less polar solvents such as toluene.

Approximately 300 mg of each podophyllotoxin analog was eventually

produced. These will be forwarded to Bristol-Myers for conversion into their Etoposide analogs and biological screening!

Part IV

Experimental

General

The usual workup involved extraction with the specified solvent, washing the organic extracts with water then saturated sodium chloride, drying the organic solution with magnesium sulfate and evaporating the solvents on a rotary evaporator.

Tetrahydrofuran was distilled over sodium-benzophenone under a nitrogen atmosphere immediately prior to use. Diisopropyl amine and DMF were distilled from calcium hydride under nitrogen. Acetonitrile was distilled over phosphorous pentoxide under a nitrogen atmosphere. All other solvents or reagents were distilled or of reagent grade quality.

Column chromatography was accomplished using Baker 60-200 mesh silica as the adsorbant. Thin layer chromatography was performed on Merck 60F 254 and Kieselgel 60 F-254 precoated silica plates of 0.25 mm thickness.

Melting points were determined using a Gallenkamp digital melting point apparatus and are uncorrected. Infrared spectra were obtained using a Perkin Elmer 783 spectrophotometer. Absorptions are noted as strong (s), medium (m), weak (w) or broadened (br). Mass spectra were recorded on an AEIMS 9025 instrument. The peak intensities are given as a % of the base peak intensity. Proton NMR spectra were taken in deuterochloroform, unless otherwise noted, using a Varian XL-300 300 MHz spectrometer. The chemical shifts are relative to the internal standard tetramethylsilane. The coupling patterns are noted as singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of triplets (dt), broadened (br) or multiplet (m).

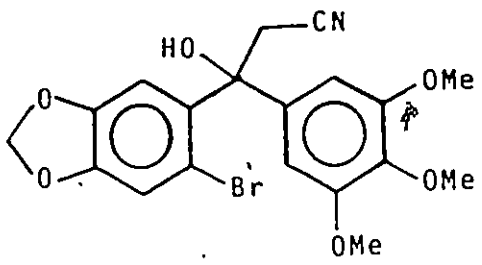
Chapter 9

(±)-Podophyllotoxin

9.1 Benzocyclobutenol 44

9.1.1 Preparation of 54

n-BuLi (14.5 mL, 41.0 mmol) was added to diisopropyl amine (6.8 mL, 48.2 mmol) in 170 mL THF at -78°C. After 20 minutes acetonitrile (2.20 mL, 42.1 mmol) was added. After an additional 10 minutes, ketone 40 [19] (13.5g, 34.2 mmol) in 125 mL THF was added over a 10 minute period. The reaction mixture was stirred for a further 10 minutes then quenched with saturated ammonium chloride. The usual extractive workup followed by recrystallization from ether-methylene chloride yielded 54 (14.5g, 97%) as white crystals.



$C_{19}H_{18}BrNO_6$ 436.26

MP 171-172°C.

IR(neat) $\nu_{max}(cm^{-1})$ 3300-3600(m), 2257(w).

MS m/e(%) 437(15), 435(14), 229(96), 227(100).

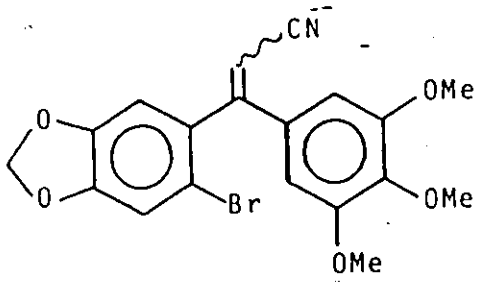
^{1}H NMR δ(ppm) 3.33(d, J=16.0 Hz, 1H), 3.40(d, J=16.0 Hz, 1H), 3.44(s, 1H, br, OH), 3.77(s, 6H), 3.83(s, 3H), 6.05(d, J=1.5 Hz, 1H), 6.06(d, J=1.5 Hz, 1H), 6.48(s, 2H), 7.01(s, 1H), 7.33(s, 1H).

Analysis Calcd. for $C_{19}H_{18}BrNO_6$: C, 52.31; H, 4.16. Found: C, 52.35; H, 4.31.

9.1.2 Preparation of 55a and 55b

A solution of 54 (14.0g, 33.5 mmol) and toluenesulfonic acid monohydrate (1.0g) in 250 mL benzene was refluxed for 40 minutes. The solution was cooled, washed with saturated $NaHCO_3$ solution, H_20, saturated NaCl solution, dried over $MgSO_4$ and evaporated to yield a mixture of 55a and 55b (13.3g, 99%) as a cream colored solid.

Recrystallization from ether yielded 55a (9.2g, 68%) as colourless needles.



$C_{19}H_{16}BrNO_5$ 418.24

<u>55a</u>

MP 159-161°C.

IR(neat) $\nu_{max}(cm^{-1})$ 2219 (w).

MS m/e(%) 419(18), 417(18), 307(100).

^{1}H NMR δ(ppm) 3.79(s, 6H), 3.86(s, 3H), 5.89(s, 1H), 6.06(s, 2H), 6.47(s, 2H), 6.76(s, 1H), 7.10(s, 1H).

Analysis Calcd. for $C_{19}H_{16}BrNO_5$: C, 54.56; H, 3.86. Found: C, 54.94; H, 4.13.

<u>55b</u>

IR(neat) $\nu_{max}(cm^{-1})$ 2219 (w).

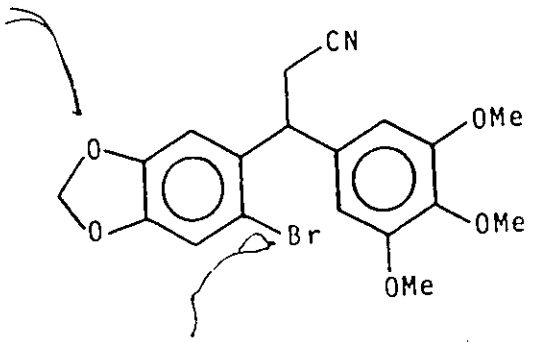
MS m/e(%) 419(18), 417(18), 307(100).

^{1}H NMR δ(ppm) 3.81(s, 6H), 3.88(s, 3H), 5.44(s, 1H), 6.04(s, 2H), 6.70(s, 1H), 6.71(s, 2H), 7.04(s, 1H).

9.1.3 Preparation of 56

Magnesium turnings (8.0g, 329 mmol) in 100 mL of methanol were activated with a few crystals of iodine at 25°C until H_2 was rapidly evolved. The mixture was then cooled to -36°C and a solution of 55a and 55b (13.2g, 31.4 mmol) in 150 mL THF was added.

The reaction mixture was stirred for 2 hours at -36°C, then poured into 200 mL 10% HCl. Extractive workup (ether) followed by recrystallization from ether-methylene chloride afforded 56 (12.0g, 91%) as colourless prisms.



$C_{19}H_{18}BrNO_5$ 420.26

MP 157°C.

IR(neat) ν_{max}(cm^{-1}) 2248(w).

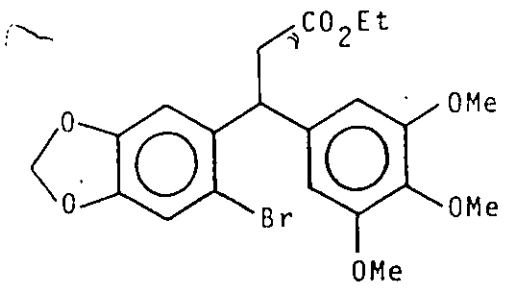
MS m/e(%) 421(47), 419(47), 381(19), 379(19), 300(100).

^{1}H NMR δ (ppm) 2.95(d, J=7.4 Hz, 2H), 3.82(s, 3H), 3.83(s, 6H), 4.75(t, J=7.4 Hz, 1H), 5.96(s, 2H), 6.45(s, 2H), 6.61(s, 1H), 7.03(s, 1H).

Analysis Calcd. for $C_{19}H_{18}BrNO_3$: C, 54.30; H, 4.32. Found: C, 54.08; H, 4.32.

9.1.4 Preparation of 57

A suspension of 56 (11.6g, 24.8 mmol) in 150 mL of ethanol was saturated with $HCl_{(g)}$ at 25°C. Methylene chloride (50 mL) was added, the solution refluxed 1 hour and allowed to stand 15hrs at 25°C. The solution was evaporated to 100 mL and diluted with 100 mL ethanol. Water (20 mL) was added and the solution refluxed for 4hrs to hydrolyze the imino ester. The solution was cooled, poured into water (500 mL) and the solid isolated by filtration. Recrystallization from ethanol yielded 57 (12.0g, 93%) as colourless needles.



$C_{21}H_{23}BrO_7$ 467.31

MP 113°C.

IR(neat) $\nu_{max}(cm^{-1})$ 1734 (s).

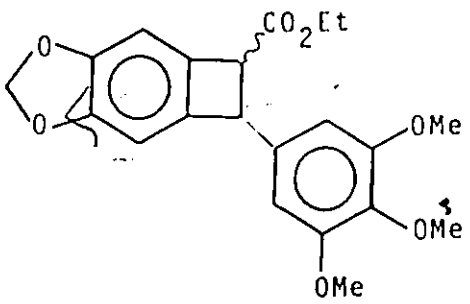
MS m/e(%) 468(42), 466(42), 300(62), 299(100).

^{1}H NMR δ(ppm) 1.13(t, J= 7.2 Hz, 3H), 2.92(d, J= 8.3 Hz, 2H), 3.79(s, 3H), 3.80(s, 6H), 4.05(q, J=7.2 Hz, 2H), 4.92(t, J= 8.3 Hz, 1H), 5.92(d, J= 1.35 Hz, 1H), 5.93(d, J=1.35 Hz, 1H), 6.45(s, 2H), 6.64(s, 1H), 6.99(s, 1H).

Analysis Calcd. for $C_{21}H_{23}BrO_7$: C, 53.98; H, 4.96. Found: C, 53.59; H, 4.93.

9.1.5 Preparation of 58a and 58b

Sodium (1.19g, 51.8mmol), cut in small pieces was added to 200 ml of liquid ammonia at -78°C (distilled from sodium under nitrogen). Stirring was continued for five minutes and anhydrous ferric chloride (0.2g, 1.2mmol) was added. The temperature was slowly raised to almost reflux and in 10 minutes the deep blue color had disappeared, leaving a grey suspension. The solution was cooled to -78°C and 57 (6.00g, 12.8mmol) in 40 mL THF was added, upon which a deep wine coloured solution was produced. After a further 10 minutes, ammonium chloride (20g) was added portionwise and the ammonia allowed to boil off. Water (200 mL) was added and the mixture acidified with concentrated hydrochloric acid. Extractive workup with methylene chloride followed by chromatographic purificaton on silica (50g, hexane-ethyl acetate = 3:1) yielded a 1:1 mixture of benzocyclobutene esters 58a and 58b (2.09g, 42%) as a pale yellow foam.



$C_{21}H_{22}O_7$ 386.40

<u>58a</u>

IR(neat) $\nu_{max}(cm^{-1})$ 1730(s).

MS m/e(%) 386(22), 313(100).

^{1}H NMR δ(ppm) 1.30(t, 7.0 Hz, 3H), 3.80(s, 6H), 3.81(s, 3H), 3.88(d, 2.1 Hz, 1H), 4.22(q, 7.0 Hz, 2H), 4.65(d, 2.1 Hz, 1H), 5.90(d, 1.3 Hz, 1H), 5.94(d, 1.3 Hz, 1H), 6.49(s, 2H), 6.72(s, 1H), 6.78(s, 1H).

<u>58b</u>

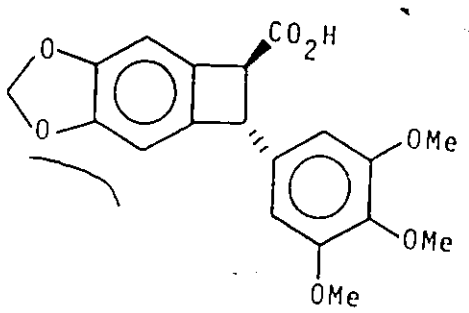
IR(neat) $\nu_{max}(cm^{-1})$ 1725(s).

MS m/e(%) 386(22), 313(100)

^{1}H NMR δ(ppm). 0.79(t, 7.2 Hz, 3H), 3.73(q, 7.2H, 2H), 3.77(s, 6H), 3.78(s, 3H), 4.46(d, 5.7 Hz, 1H), 4.83(d, 5.7 Hz, 1H), 5.92(d, 1.3 Hz, 1H), 5.96(d, 1.3 Hz, 1H), 6.39(s, 2H), 6.68(s, 1H), 6.77(s, 1H).

9.1.6 Preparation of 59

To a solution of 58a and 58b (1.96g, 5.47 mmol) in 3.0 mL DMSO (cooled in a bath at 17°C), was added 3.0 mL 50% KOH. The mixture was stirred for 1 hour, then mixed with 30 mL water and acidified at 0°C with conc HCL. The usual extractive workup followed by recrystallization from ether/methylene chloride yielded 59 (1.54g, 85%) as white crystals.



$C_{19}H_{18}O_7$ 358.34

MP 145°C.

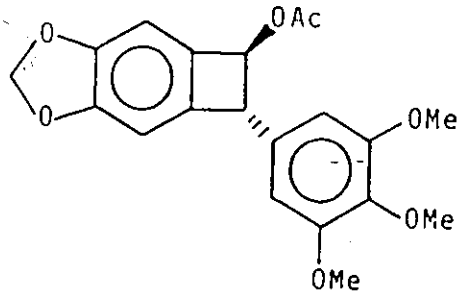
IR(neat) $\nu_{max}(cm^{-1})$ 1700(s), 1730(m), 2500-3500(m).

MS m/e(%) 358(93), 283(100).

^{1}H NMR δ(ppm) 3.81(s, 6H), 3.82(s, 3H), 3.93(d, J= 1.9 Hz, 1H), 4.67(d, J= 1.9 Hz, 1H), 5.91(d, J= 1.5 Hz, 1H), 5.96(d, J= 1.5 Hz, 1H), 6.48(s, 2H), 6.72(s, 1H), 6.79(s, 1H).

9.1.7 Preparation of 60

To a solution of 59 (1.53g, 4.30 mmol) in 45 mL THF and 10 mL acetic acid (cooled in a bath at 18°C), was added $Pb(OAc)_4$ (90%) (2.10g, 4.26 mmol). The solution was stirred 1 hr, during which time a heavy white precipitate formed. The usual extractive workup (ether, including sat. $NaHCO_3$ wash), followed by purification on a silica column (20g, hexane-ethyl acetate = 3:1) and recrystallization from ether yielded 60 (1.15g, 76%) as white crystals.



$C_{20}H_{20}O_7$ 372.37

MP 134-135°C.

IR(neat) $\nu_{max}(cm^{-1})$ 1735(s).

MS (C.I. ether) m/e(%) 373(1), 313(100).

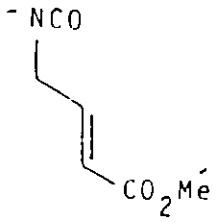
^{1}H NMR δ(ppm) 2.12(s, 3H), 3.81(s, 6H), 3.82(s, 3H), 4.38(d, J=1.8 Hz, 1H), 5.45(d, J= 1.8 Hz, 1H), 5.93(d, J= 2.0 Hz, 1H), 5.96(d, J= 2.0 Hz, 1H), 6.45(s, 2H), 6.70(s, 1H), 6.83(s, 1H).

Analysis Calcd. for $C_{20}H_{20}O_7$: C, 64.51; H 5.41. Found: C, 64.81; H, 5.55.

9.2 Conversion of 60 to (±)-Podophyllotoxin

9.2.1 Preparation of 68

A suspension of methyl trans-4-aminocrotonate hydrochloride (2.90g, 19.2 mmol) in 100 mL chlorobenzene was refluxed for 5h with phosgene bubbling through. Evaporation of the chlorobenzene and distillation yielded 68 (1.59g, 57%) as a colourless liquid.



$C_6H_7NO_3$ 141.13

BP 65°C at 0.075 torr.

IR(neat) $\nu_{max}(cm^{-1})$ 2257(vs), 1725(s).

MS m/e(%) 141(15), 100(81), 98(38), 86(100), 82(75).

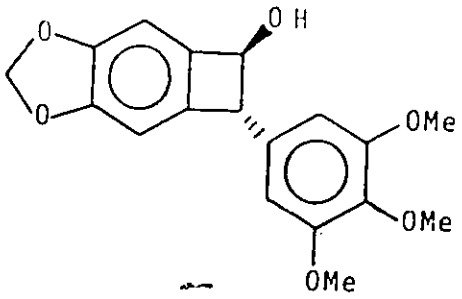
^{1}H NMR δ(ppm) 3.74(s, 3H), 4.13(dd, J=4.3, 2.1 Hz, 2H), 6.10(dt, J=15.4, 2.1 Hz, 1H), 6.88(dt, J=15.4, 4.3 Hz, 1H).

9.2.2 Preparation of 44 and 69

A solution of 60 (168.1mg , 0.451 mmol) in 2 mL methylene chloride was added to 4.0 mL methanol, to which had been added 1.0 ml of acetyl chlo-

ride, at -10°C. The reaction mixture was stirred 5 hrs at -10°C, then diluted with 10 mL methylene chloride, washed with water until the pH was neutral, washed with saturated NaCl and dried over magnesium sulfate. The organic extract was then diluted with carbon tetrachloride (10 mL) and evaporated to 5 mL. The evaporation procedure was then repeated. The resultant solution of 44 was used for the next reaction without further manipulation. The entire workup procedure was performed at 0°C.

To the above solution of 44 (diluted with 5 mL methylene chloride), was added isocyanate 68 (72.1 mg, 0.511 mmol) and triphenyltin acetate (25.2 mg, 0.062 mmol) at 0°C. The solvent was evaporated at 0°C to yield a viscous oil which was allowed to warm to 25°C. Purification on a silica column (5g, methylene chloride then methylene chloride-ethyl acetate = 10:1), followed by recrystallization from methylene chloride-hexane yielded 69 (103.7 mg, 47%) as white needles.

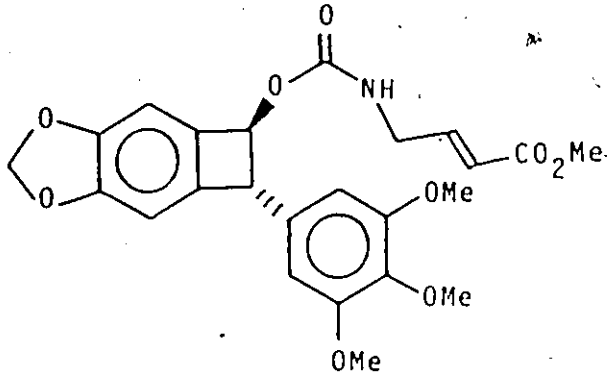


44

$C_{18}H_{18}O_6$ 330.34

IR(neat) $\nu_{max}(cm^{-1})$ 3200-3510 (m).

^{1}H NMR δ(ppm) 3.80(s, 6H), 3.82(s, 3H), 4.17(s, 1H), 4.77(bs, 2H), 5.93(d, J=2.0 Hz, 1H), 5.96(d, J=2.0 Hz, 1H), 6.40(s, 2H), 6.71(s, 1H), 6.84(s, 1H).



69

$C_{24}H_{25}NO_9$ 471.46

MP 141-142°C.

IR(neat) ν_{max}(cm^{-1}) 1720(s), 3340(m, br)

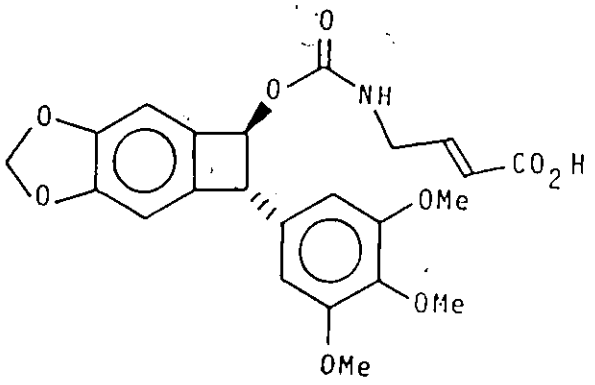
MS m/e(%) 471(58), 312(100)

^{1}H NMR δ(ppm) 3.72(s, 3H), 3.80(s, 6H), 3.81(s, 3H), 4.00(m, 2H), 4.36(d, J= 0.6 Hz, 1H), 4.90(m, NH), 5.47(d, J= 0.6 Hz, 1H), 5.93(d, J= 1.3 Hz, 1H), 5.95(d, J= 1.3 Hz, 1H), 5.96(dt, J= 15.9, 1.9 Hz, 1H), 6.45(s, 2H), 6.70(s, 1H), 6.85(s, 1H), 6.92(dt, J= 15.9, 4.6 Hz, 1H).

9.2.3 Preparation of 69a

Aqueous LiOH (2M, 0.2 ml) was added to a solution of 69 (62.2 mg, 0.132 mmol) in 10 ml THF and 2 ml water at 25°C. A small second phase was

produced. Stirring was continued for 3.5 h. The THF was evaporated at reduced pressure and the solution acidified with 10% HCl. The usual extractive workup with methylene chloride followed by recrystallization from methylene chloride-hexane yielded 69a (52.1 mg, 86%) as a white crystalline solid.



$C_{23}H_{23}NO_9$ 457.44

MP 106-108°C.

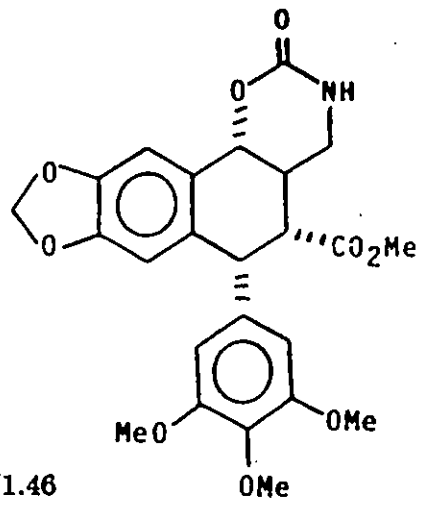
IR(neat) $\nu_{max}(cm^{-1})$ 1690-1720(s), 3310(m), 2900-3400(m).

MS m/e(%) 457(40), 312(100).

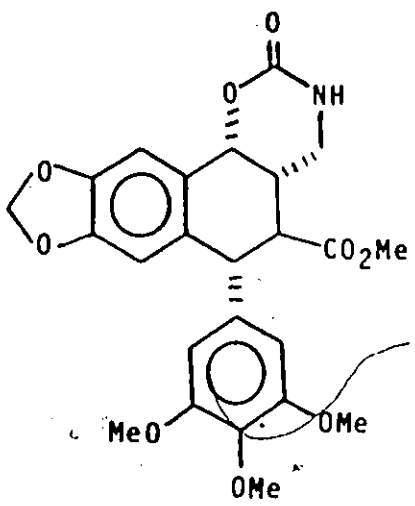
^{1}H NMR δ(ppm) 3.80(s, 6H), 3.81(s, 3H), 4.04(m, 2H), 4.37(s, 1H), 4.96(m, NH), 5.48(s, 1H), 5.94(d, J= 1.3 Hz, 1H), 5.96(d, J=1.3 Hz, 1H), 5.97(dt, J= 15.6, 1.7 Hz, 1H), 6.46(s, 2H), 6.71(s, 1H), 6.86(s, 1H), 7.02(dt, J= 15.6, 4.8 Hz, 1H).

9.2.4 Preparation of 70 and 71 from 69

A solution of 69 (51.2 mg, 0.109 mmol) in 5 mL nitromethane was refluxed for 1.5h. Evaporation of the solvent and recrystallization from methanol followed by PTLC (ethyl acetate) of the mother liquor yielded 70 (31.9 mg, 62%) as colourless cubes and 71 (10.1 mg, 20%) as white needles. The PMR of the crude reaction mixture indicated a 3:1 ratio of 70 to 71.



70



71

$C_{24}H_{25}NO_4$ 471.46

70

MP 272°C.

IR(KBr pellet) $\nu_{max}(cm^{-1})$ 1712(vs), 1735(s), 3385(m), 3200-3600(m).

MS m/e(%) 471(100%). exact mass: 471.1527, calc. for $C_{24}H_{25}NO_9$ 471.1527

^1H NMR δ(ppm) 2.64(m, 1H), 3.03(m, 2H), 3.56(s, 3H), 3.72(s, 6H), 3.78(s, 3H), 3.74(m, 1H), 4.44(d, J= 6.0 Hz, 1H), 5.05(d, J=9.9 Hz, 1H), 5.14(m, 1H), 5.92(d, J= 1.3 Hz, 1H), 5.94(d, J=1.3 Hz, 1H), 6.12(s, 2H), 6.37(s, 1H), 7.15(s, 1H).

Analysis Calcd. for $C_{24}H_{25}NO_4$: C, 61.14; H, 5.34. Found: C, 61.18; H, 5.58.

71

MP 234°C.

IR (KBr pellet) $\nu_{max}(cm^{-1})$ 1700-1740(vs), 3300(m), 3400(m).

MS m/e(%) 471(48), 398(49), 339(100).

^{1}H NMR δ(ppm) 2.58(m, 1H), 3.12(dd, J= 12.5, 4.0 Hz, 1H), 3.21(t, 12.0 Hz, 1H), 3.51(s, 3H), 3.64(dd, J= 12.5, 5.0 Hz, 1H), 3.77(s, 6H), 3.83(s, 3H), 4.14(d, J= 11.5 Hz, 1H), 5.28(d, J= 2.7 Hz, 1H), 5.91(d, J= 1.3 Hz, 1H), 5.92(d, J= 1.3 Hz, 1H), 6.28(s, 1H), 6.30(s, 2H), 6.82(s, 1H).

9.2.5 Thermolysis of 69a

A solution of 69a (10.0 mg, 21.9 mmol) in 10 mL nitromethane was heated to 90°C for 5h. The nitromethane was evaporated and the residue suspended in 10 mL methylene chloride. Excess diazomethane in ether was added, the solvents were evaporated and the residue recrystallized from methanol to yield 70 (5.0 mg, 50%). PMR of the crude esters indicated a 5:1 mixture of 70 to 71.

9.2.6 Preparation of (±)-Podophyllotoxin from 69

The crude 69a prepared as before from 69 (14.1 mg, 0.030 mmol) was dissolved in 15 mL nitromethane and heated to 90°C for 5h. The nitromethane was then evaporated and the residue was refluxed in aqueous LiOH (0.2M, 10 mL) for 0.5h. After cooling, the solution was acidified with 10% HCl,

buffered to pH 4 with 0.2 KH_2PO_4 and cooled to 0°C. Sodium nitrite (0.3g, 3.5 mmol) was added followed by 10% HCl (0.5 mL)to give pH 4. The solution was stirred 18h during which nitrogen evolved and a tan coloured precipitate was produced. The usual extractive workup with ethyl acetate solvent followed by PTLC (methylene chlorid–acetone = 10:1) yielded (±)-podophyllotoxin (2.8 mg = 23%) as white crystals.

MP 336-337°C.

IR, MS and ^{1}H NMR identical to authentic podophyllotoxin.

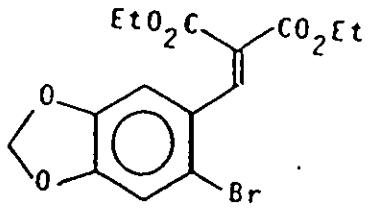
Chapter 10

Podophyllotoxin Analog 73

10.1 Benzocyclobutyl Acetate 86

10.1.1 Preparation of 78

A solution of bromopiperonal (60.0g, 0.262 mol), diethyl malonate (42.0g, 0.262 mol) and piperidine (2.6g, 0.031 mol) in 100 mL toluene was refluxed in a flask equiped with a Dean-Stark apparatus. Refluxing was continued 48h followed by the usual extractive workup with ether as a solvent (including a wash with 10 % HCl). A silica filtration (200g, hexane-ethyl acetate = 7:1) followed by recrystallization from ethanol-water yielded 78 (71.5g, 74%) as pale yellow prisms.



$C_{15}H_{15}BrO_6$ 371.18

MP 50-52°C.

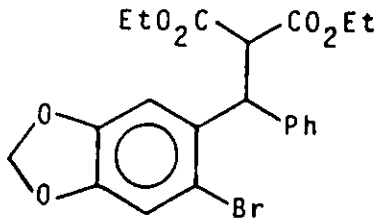
IR(neat) $\nu_{max}(cm^{-1})$ 1724(s), 1629(m), 1617(m), 1599(w).

MS m/e(%) 372(9), 370(9), 327(97), 325(100).

^{1}H NMR δ(ppm) 1.26(t, J=7.4 Hz, 3H), 1.35(t, J=7.4 Hz, 3H), 4.28(q, J=7.4 Hz, 2H), 4.29(q, J=7.4 Hz, 2H), 5.99(s, 2H), 6.93(s, 1H), 7.04(s, 1H), 7.9(s, 1H).

10.1.2 Preparation of 79

A solution of bromobenzene (33.2 mL, 0.315 mol), in 160 mL diethyl ether was prepared. A few crystals of iodine and 10 mL of the bromobenzene solution was added to magnesium turnings (8.84g, 0.364 mol). After the reaction had started, the remainder of the bromobenzene solution was added dropwise at a rate that produced a vigorous reflux. After the addition was complete, the solution was refluxed for an additional 30 minutes. The above solution was cooled to 25°C and added over a 15 minute period to a solution of 78 (90.0g, 0.242 mol) in 450 mL ether and 180 mL benzene at -78°C. After the addition was complete, the solution was warmed to 0°C. A heavy yellow precipitate which formed over the addition dissolved upon warming the solution to 0°C, resulting in a deep yellow solution. The reaction mixture was stirred for an additional 25 minutes, then quenched with saturated ammonium chloride. The usual extractive workup with ether solvent, yielded 79 (108.9g, 100%, crude) as a pale yellow oil. Crude 79 was used in the preparation of 80 without purificaton.



$C_{21}H_{21}BrO_6$ 449.30

IR(neat) $\nu_{max}(cm^{-1})$ 1735(m), 1734(s).

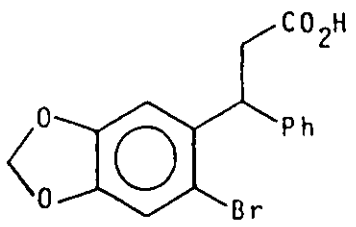
MS m/e(%) 450(25), 448(25), 369(100).

^{1}H NMR δ(ppm) 1.00(t, J=7.5 Hz, 3H), 1.09(t, J=7.5 Hz, 3H), 3.98(dq, J=7.5, 1.1 Hz, 2H), 4.05(q, J=7.5 Hz, 2H), 4.21(d, J=13.2 Hz, 1H), 5.26(d, J=13.2 Hz, 1H), 5.89(d, J=2 Hz, 1H), 5.93(d, J=2 Hz, 1H), 6.86(s, 1H), 6.96(s, 1H), 7.1-7.3(aromatics, 5H).

10.1.3 Preparation of 80 and 81

A solution of 79 (108.9g, 0.242 mol, crude) and 100 mL 50% aqueous KOH in 400 mL ethanol was refluxed for 2h. After cooling, the mixture was acidified with concentrated HCl and diluted with 200 mL water. The usual extractive workup with ethyl acetate solvent yielded crude di-acid 80 as a yellow solid. Crude di-acid 80 was decarboxylated without purification or characterization by refluxing in 500 mL toluene for one day. Acid 81 (51.7g, 61%) was

obtained as colourless cubes by recrystallization from toluene followed by a second recrystallization from 1,2-dichloroethane.



$C_{16}H_{13}BrO_4$ 349.18

MP 190-192°C.

IR(neat) $\nu_{max}(cm^{-1})$ 3300-3600(m), 2500-3600(w), 1709(s).

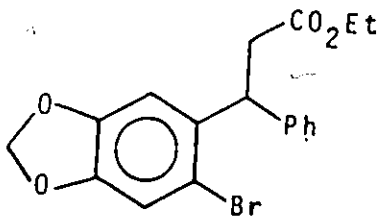
MS m/e(%) 350(46), 348(56), 269(85), 210(100).

^{1}H NMR δ(ppm) 3.00(d, J=8.6 Hz, 1H), 3.01(d, J=8.6 Hz, 1H), 4.98(t, J=8.6 Hz, 1H), 5.90(d, J=2 Hz, 1H), 5.93(d, J=2 Hz, 1H), 6.65(s, 1H), 6.99(s, 1H), 7.1-7.3(aromatics, 5H).

Analysis calcd. for $C_{16}H_{13}BrO_4$: C, 55.04; H, 3.75. Found: C, 54.62; H, 4.03.

10.1.4 Preparation of 82

A solution of 81 (44.2g, 0.127 mol) and 1.0g p-toluenesulfonic acid in 800 mL ethanol was refluxed for one day. The ethanol was evaporated under vacuum, and the usual extractive workup with ether solvent yielded 82 (46.7g, 98%) as a pale yellow oil.



$C_{18}H_{17}BrO_4$ 377.23

IR(neat) $\nu_{max}(cm^{-1})$ 1732(s).

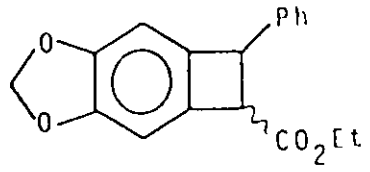
MS m/e(%) 378(18), 376(17), 297(100).

^{1}H NMR δ(ppm) 1.12(t, J=7 Hz, 3H), 2.94(d, J=8.5 Hz, 2H), 4.04(q, J=7 Hz, 2H), 4.98(t, J=8.5 Hz, 1H), 5.90(d, J=2 Hz, 1H), 5.93(d, J=2 Hz, 1H), 6.68(s, 1H), 6.98(s, 1H), 7.1-7.3(aromatics, 5H).

10.1.5 Preparation of 83a and 83b

Sodium (6.10g, 0.265 mol) cut in small pieces was added portionwise to 700 mL of liquid ammonia (distilled directly from the cylinder) while stirring at -78°C. Anhydrous ferric chloride (0.6g, 3.7 mmol) was added in two portions, one after half of the sodium was added and the second after all the

sodium was added. The temperature was slowly raised to near reflux and in 20 minutes the deep blue colour had disappeared leaving a dark grey suspension. The solution was cooled to -78°C and 82 (25.0g, 66.3 mmol) in 90 mL THF was added over one minute. The resulting deep wine solution was stirred for an additional 8 minutes and quenched by portionwise addition of ammonium chloride (50g). A similar workup to that of 58a and 58b followed by chromatographic purification on silica (10g, hexane-ethyl acetate = 3:1) yielded a 1:1 mixture of benzocyclobutene esters 83a and 83b (7.13g, 36%) as a yellow oil.



$C_{18}H_{16}O_4$ 296.32

83a

IR(neat) $\nu_{max}(cm^{-1})$ 1729(s).

MS m/e(%) 296(11), 223(100).

^{1}H NMR δ(ppm) 1.29(t, J=7.1 Hz, 3H), 3.89(d, J=2.3 Hz, 1H), 4.22(q, J=7.1 Hz, 2H), 4.73(d, J=2.3 Hz, 1H), 5.90(d, J=1.4 Hz, 1H), 5.92(d, J=1.4 Hz, 1H), 6.72(s, 1H), 6.77(s, 1H), 7.2-7.3(aromatics, 5H).

83b

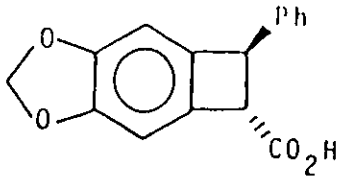
IR(neat) $\nu_{max}(cm^{-1})$ 1729(s).

MS m/e(%) 296(9), 223(100).

^{1}H NMR δ(ppm) 0.75(t, J=7.1 Hz, 3H), 3.61(dq, J=10.7, 7.1 Hz, 1H), 3.66(dq, J=10.7, 7.1 Hz, 1H), 4.49(d, J=5.8 Hz, 1H), 4.91(d, J=5.8 Hz, 1H), 5.92(d, J=1.4 Hz, 1H), 5.94(d, J=1.4 Hz, 1H), 6.68(s, 1H), 6.78(s, 1H), 7.1-7.3(aromatics, 5H).

10.1.6 Preparation of 85

To a solution of 83a and 83b (11.2g, 37.8 mmol) in 30 mL DMSO (cooled in a bath at 17°C) was added 30 mL 50% KOH. The mixture was stirred for 4h, mixed with 300 mL water, washed with methylene chloride and acidified at 0°C with conc HCl. The usual extractive workup with methylene chloride, followed by recrystallization from methylene chloride- hexane yielded 85 (8.14g, 80%) as white crystals.



$C_{16}H_{12}O_4$ 268.27

MP 140°C (dec).

MS m/e(%) 268(41), 233(100).

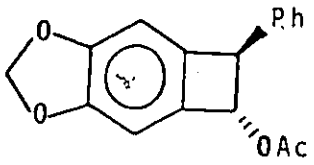
^{1}H NMR δ(ppm) 3.93(d, J=2.6 Hz, 1H), 4.75(d, J=2.6 Hz, 1H), 5.90(d, J=1.6 Hz, 1H), 5.93(d, J=1.6 Hz, 1H), 6.73(s, 1H), 6.78(s, 1H), 7.1-7.4(aromatics, 5H).

Analysis Calcd. for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.69; H, 4.95.

10.1.7 Preparation of 86

To a solution of 85 (7.20g, 26.8 mmol) in 250 ml THF and 10 mLacetic acid (cooled in a bath at 18°C), was added $Pb(OAc)_4$ (90%) (12.9g, 26.6 mmol). The solution was stirred for 1h and a heavy white precipitate formed. The usual extractive workup with ether, followed by purification on a silica column (50g, hexane-ethyl acetate = 5:1), and recrystallization from methylene chloride-hexane yielded 86 (4.09g, 54%). The mother liquor contained 80%

of 86, thus giving a corrected yield of about 90%. Preparation of 75 may be performed on crude 86.



$C_{17}H_{14}O_4$ 282.30

MP 83-84°C.

IR(neat) $\nu_{max}(cm^{-1})$ 1740(s).

MS (C.I. ether) m/e(%) 283(2), 282(9), 281(40), 239(22), 223(100).

^{1}H NMR δ(ppm) 2.12(s, 3H), 4.46(s, 1H), 5.45(s, 1H), 5.94(s, 2H), 6.69(s, 1H), 6.83(s, 1H), 7.2-7.3(aromatics, 5H).

Analysis Calcd. for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.06; H, 4.91.

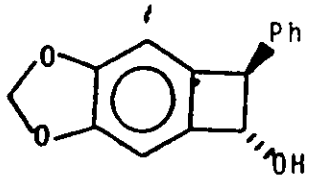
10.2 Podo Analog 73

10.2.1 Preparation of 75 and 93

A solution of 86 (3.03g, 10.7 mmol) in 30 mL methylene chloride was added to 60 mL of methanol at 0°C to which had been added 15 mL acetyl chloride. The reaction mixture was stirred 3h at 0°C, then diluted with 60 mL

methylene chloride. Workup was similar to 44 to yield a solution of 75 in 20 mL carbon tetrachloride.

To the above solution of 75 (diluted with 100 mL methylene chloride), was added isocyanate 68 (1.52g, 10.8 mmol) and triphenyltin acetate (0.40g, 0.98 mmol) at 0°C. The solvent was evaporated at 0°C to yield a viscous oil which was allowed to warm to 25°C. Purification on a silica column (100g, methylene chloride then methylene chloride-ethyl acetate = 10:1), followed by recrystallization from methylene chloride-hexane yielded 93 (2.06g, 50%) as white needles.

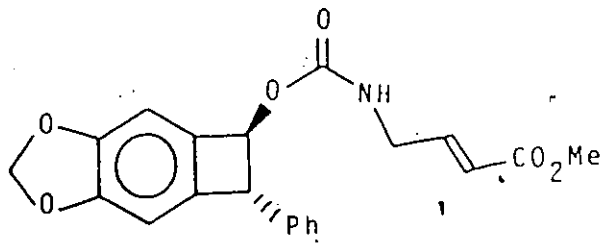


75

$C_{15}H_{12}O_3$ 240.26

IR(neat) $\nu_{max}(cm^{-1})$ 3100-3600(m).

^{1}H NMR δ(ppm) 2.30(d, J=7.7 Hz, OH), 4.25(s, 1H), 4.80(d, J=7.7 Hz, 1H), 5.93(s, 2H), 6.70(s, 1H), 6.83(s, 1H), 7.1-7.3(aromatics, 5H).



93

$C_{21}H_{19}NO_6$ 381.38

MP 110-111°C.

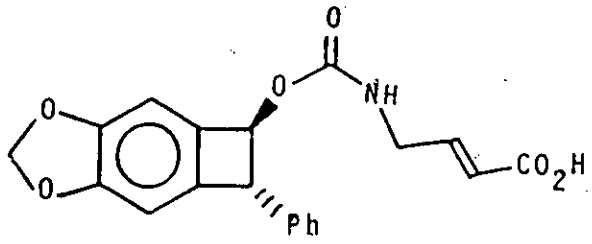
IR(neat) $\nu_{max}(cm^{-1})$ 3300-3460(m), 1723(s).

MS m/e(%) 381(15), 240(86), 222(100).

^{1}H NMR δ(ppm) 3.72(s, 3H), 4.01(m, 2H), 4.44(s, 1H), 4.9(br, NH), 5.46(s, 1H), 5.93(s, 2H), 5.96(dt, J=16, 1.9 Hz, 1H), 6.70(s, 1H), 6.85(s, 1H), 6.92(dt, J=16, 5 Hz, 1H), 7.1-7.3(aromatics, 5H).

10.2.2 Preparation of 93a

Aqueous LiOH (2M, 5 mL) was added portionwise to a solution of 93 (1.65g, 4.33 mmol) in 100 mL THF and 20 mL water at 23°C. Two phases were produced. Stirring was continued for 7h, after which the THF was evaporated at reduced pressure and the solution acidified with 10% HCl. The usual extractive workup with methylene chloride solvent yielded crude 93a (1.52g, 96%) as a light yellow-brown solid. A small sample was recrystallized from methylene chloride-hexane for characterization.



$C_{20}H_{17}NO_6$ 367.36

MP 156-157°C.

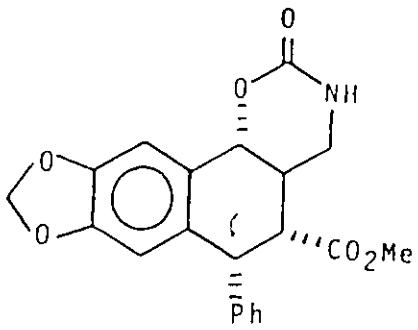
IR(neat) $\nu_{max}(cm^{-1})$ 2400-3600(w), 3300(m), 3410(m), 1690(s).

MS m/e(%) 367(7), 240(51), 239(38), 223(31), 222(62), 44(100).

^{1}H NMR δ(ppm) 4.04(m, 2H), 4.44(s, 1H), 4.94(br, NH), 5.47(s, 1H), 5.93(s, 2H), 5.97(dt, J=15.9, 1.8 Hz, 1H), 6.70(s, 1H), 6.85(s, 1H), 7.01(dt, J=15.9, 5 Hz, 1H), 7.2-7.3(aromatics, 5H).

10.2.3 Preparation of 95 and 96 from 93

A solution of 93 (64 mg, 0.17 mmol) in 5 mL nitromethane was heated to 86°C for 4h. Evaporation of the solvent and recrystallization from methylene chloride-hexane followed by PTLC (ethyl acetate) of the mother liquor yielded 95 (30 mg, 47%) as white needles and 96 (20 mg, 31%) as white needles. The PMR of the crude reaction mixture indicated approximately a 2:1 ratio of 95 to 96.



$C_{21}H_{19}NO_6$ 381.38

95

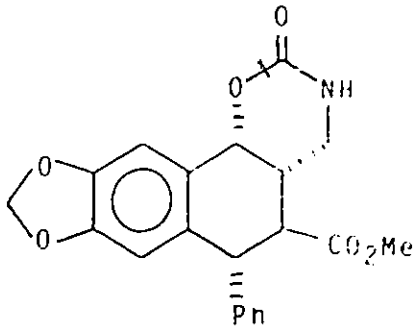
MP 300-302°C.

IR (KBr pellet) $\nu_{max}(cm^{-1})$ 3300-3500(m), 1715(s).

MS m/e(%) 381(86), 308(63), 249(100).

^{1}H NMR δ(ppm) 2.63(m, 1H), 3.02(t, J=11.0 Hz, 1H), 3.05(dd, J=12.1, 5.9 Hz, 1H), 3.53(s, 3H), 3.72(ddd, J=11.0, 5.4, 4.4 Hz, 1H), 4.51(d, J=5.9 Hz, 1H), 5.07(d, J=10.2 Hz, 1H), 5.15(m, NH), 5.90(d, J=1.3 Hz, 1H), 5.91(d, J=1.3 Hz, 1H), 6.33(s, 1H), 6.9-7.0(aromatics, 2H), 7.16(s, 1H), 7.2-7.3(aromatics, 3H).

Analysis Calcd. for $C_{21}H_{19}NO_6$: C, 66.14; H, 5.02. Found: C, 66.00; H, 5.37.



96

MP 230-231°C

IR(KBr pellet) $\nu_{max}(cm^{-1})$ 3300-3600(s), 1700-1750(s).

MS m/e(%) 381(17), 308(39), 249(87), 28(100).

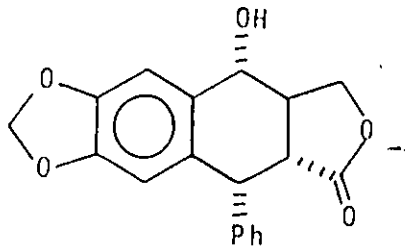
^{1}H NMR δ(ppm) 2.61(m, 1H), 3.12(ddd, J=12.6, 4.3, 1.4 Hz, 1H), 3.23(t, J=11.8 Hz, 1H), 3.47(s, 3H), 3.68(dd, J=12.5, 4.8 Hz, 1H), 4.22(d, J=11.2 Hz, 1H), 5.29(d, J=2.8 Hz, 1H), 5.14(m, NH), 5.88(d, J=1.3 Hz, 1H), 5.90(d, J=1.3 Hz, 1H), 6.20(s, 1H), 6.82(s, 1H), 7.1-7.3(aromatics, 5H).

10.2.4 Thermolysis of 93a

A solution of 93a (3.8 mg, 0.010 mmol) in 2 mL nitromethane was heated to 85-90°C for 4h. The nitromethane was evaporated and the residue suspended in 5 mL methylene chloride and treated with excess diazomethane in ether. Evaporation of the solvents yielded a 5:1 mixture of 95 and 96 as indicated by PMR. Compound 96 was isolated in 51% yield.

10.2.5 Preparation of 73 from 93

Crude 93a (1.65 mg, 4.33 mmol) prepared as before was heated in 120 mL nitromethane at 86°C for 5.5 h. The nitromethane was then evaporated and the residue was refluxed in aqueous LiOH (0.2M, 200 mL) for 0.5h. After cooling to 23°C, KH_2PO_4 (8g) and $NaNO_2$ (.4g) were added followed by 10% HCl (about 60 mL) to give pH 4. The solution was stirred 20h followed by the usual extractive workup with ethyl acetate. Purification on silica (30 g, methylene chloride then methylene chloride - ethyl acetate = 10:1) followed by recrystallization from methylene chloride-hexane yielded 73 (0.207 g, 15%) as white plates.



73

$C_{19}H_{16}O_5$ 324.33

MP 247-248°C

IR(KBr pellet) $\nu_{max}(cm^{-1})$ 3400(s), 1760(s).

MS m/e(%) 324(100).

^{1}H NMR δ(ppm) 2.76(m, 1H), 2.86(dd, J=14.3, 4.8 Hz, 1H), 4.08(t, J=9.4 Hz, 1H), 4.57(dd, J=8.9, 7.1 Hz, 1H), 4.63(d, J=4.8 Hz, 1H), 4.76(d, J=9.2 Hz, 1H), 5.94(s, 2H), 6.44(s, 1H), 7.1-7.3 (aromatics, 6H).

Analysis Calcd. for $C_{19}H_{16}O_5$: C, 70.36; H, 4.97. Found: C, 70.65; H, 5.36.

Chapter 11

Podophyllotoxin Analog 74

11.1 Benzocyclobutyl Acetate 89

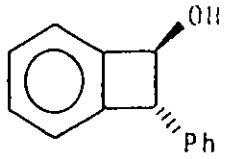
trans-2-Phenyl Benzocyclobutyl Acetate 89 was prepared according to the method previously reported by Charlton and Alauddin.[33]

11.2 Podo Analog 74

11.2.1 Preparation of 76 and 94

A solution of 89 (0,405g, 1.70 mmol) in 10 mL methylene chloride was added to 8 mL methanol at -5°C, to which 2 mL acetyl chloride had been added. The reaction mixture was stirred 5h at -5°C. Workup was as before (44 and 86) to yield a carbon tetrachloride solution of 76.

To the above solution of 76 at 0°C (diluted with 10 mL methylene chloride), was added isocyanate 68 (0.237g, 1.68 mmol) and triphenyltin acetate (84.0 mg, 0.205 mmol). The solvent was evaporated at 0°C to produce a viscous oil which was allowed to warm to 25°C. Purificaton on a silica column (15g, methylene chloride, followed by methylene chloride-ethyl acetate = 20:1), followed by recrystallization from ether-hexane yielded 94 (0.245g, 43%) as white needles.

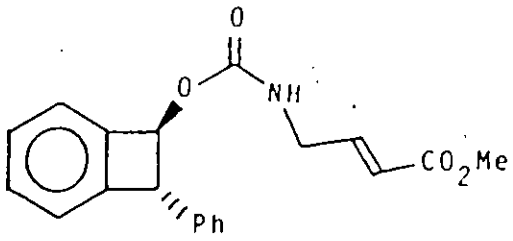


76

$C_{14}H_{14}O$ 198.26

IR(neat) $\nu_{max}(cm^{-1})$ 3300-3500(m).

^1H NMR δ(ppm) 4.10(s, 1H), 4.96(s, br, 2H), 7.0-7.6(aromatics, 9H).



94

$C_{20}H_{19}NO_4$ 337.37

MP 93-94°C.

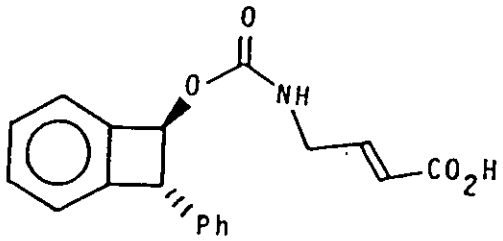
IR(neat) $\nu_{max}(cm^{-1})$ 3340(m), 3400(w), 1722(s), 1700-1730(s).

MS m/e(%) 337(0.8), 196(81), 198(100).

^{1}H NMR δ(ppm) 3.73(s, 3H), 4.03(m, 2H), 4.64(s, 1H), 4.93(m, NH), 5.67(d, 1.4 Hz, 1H), 5.97(dt, J=16.0, 2.0 Hz, 1H), 6.93(dt, J=16.0, 4.9 Hz, 1H), 7.2-7.6(aromatics, 9H).

11.2.2 Preparation of 94a

Aqueous LiOH (2M, 1 mL) was added portionwise to a solution of 94 (130 mg, 0.341 mmol) in 25 mL THF and 5 mL water at 23°C. Stirring was continued for 3h, after which the THF was evaporated at reduced pressure and the solution acidified with 10% HCl. The usual extractive workup with methylene chloride solvent yielded crude 94a (125 mg, 100%) as a light yellow-brown solid. A small sample was recrystallized from methylene chloride-hexane for characterization.



$C_{19}H_{17}NO_4$ 323.34

MP 130-131°C.

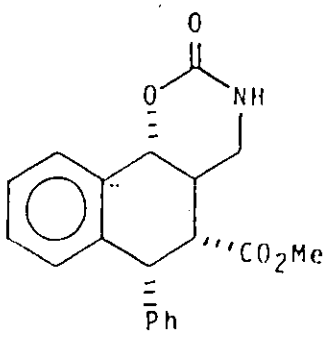
IR(neat) $\nu_{max}(cm^{-1})$ 3320(m), 2800-3460(m), 1700(br).

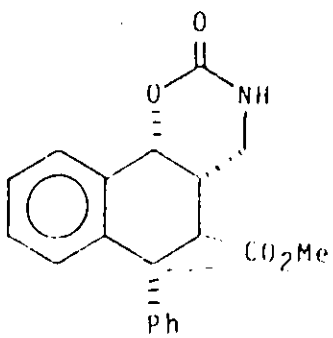
MS m/e(%) 323(2), 196(100).

^{1}H NMR δ(ppm) 4.06(m, 2H), 4.65(s, 1H), 4.96(m, NH), 5.67(s, 1H), 5.98(dt, J=15.8, 1.8 Hz, 1H), 7.03(dt, J=15.8, 4.8 Hz, 1H), 7.2-7.4(aromatics, 9H).

11.2.3 Preparation of 97 and 98

A solution of 94 (26.8 mg, 0.079 mmol) in 4 mL DMF was heated to 110°C for 1 day. Evaporation of the DMF and recrystallization from ethyl acetate followed by PTLC (ethyl acetate) of the mother liquor yielded 97 (15 mg, 56%) as white needles and 98 (3 mg, 11%) as white needles. PMR of the crude reaction mixture indicated approximately a 4:1 ratio of 97 to 98.





$C_{20}H_{19}NO_4$ 337.37 97 98

97

MP 260°C.

IR(KBr pellet) $\nu_{max}(cm^{-1})$ 3420(s), 1728(m), 1706(s).

MS m/e(%) 337(53), 196(84).

^{1}H NMR δ(ppm) 2.70(m, 1H), 3.06(t, J=10.9 Hz, 1H), 3.10(dd, J=10.9, 6.0 Hz, 1H), 3.54(s, 3H), 3.75(quintet, J=5.2 Hz, 1H), 4.65(d, J=5.8 Hz, 1H), 5.11(d, J=3.9 Hz, NH), 5.18(d, J=10.2 Hz, 1H), 6.9-7.8(aromatics, 9H).

Analysis Calcd. for $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68. Found: C, 71.25; H, 5.65.

98

MP 169-170°C.

IR(neat) ν_{max}(cm^{-1}) 3260(m), 1714(vs).

MS m/e(%) 337(5), 263(65), 205(69), 30(100).

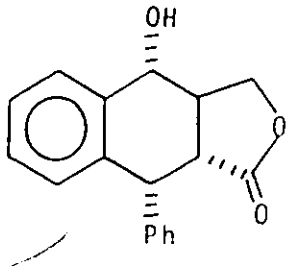
^{1}H NMR δ(ppm) 2.66(m, 1H), 3.15(ddd, J=2, 4, 12 Hz, 1H), 3.29(t, 12.2 Hz, 1H), 3.48(s, 3H), 3.70(dd, J=12.7, 4.9 Hz, 1H), 4.34(d, J=11.2 Hz, 1H), 5.27(m, NH), 5.40(d, J=2.5, 1H), 6.7-7.4(aromatics, 9H).

11.2.4 Thermolysis of 94a

A solution of 94a (14.5 mg, 0.039 mmol) in 10 mL nitromethane was refluxed 1 day. The nitromethane was evaporated and the residue mixed with 5 mL methylene chloride and treated with excess diazomethane in ether. Evaporation of the solvents yielded a 2:1 mixture of 97 and 98 as indicated by PMR. PTLC yielded 27% of 97.

11.2.5 Preparation of 74 via 94a

Crude 94a was prepared as before from 94 (70 mg, 0.21 mmol). The crude 94a in 120 mL nitromethane was heated to reflux for 1 day and the nitromethane was then evaporated. The residue was refluxed in aqueous LiOH (0.2M, 20 mL) for 0.5h. After cooling to 23°C, KH_2PO_4 (.5g) and $NaNO_2$ (.4g) were added followed by 10% HCl (about 4 mL) to give pH 4. The solution was stirred 20h followed by the usual extractive workup with methylene chloride. Purification by PTLC (hexane-ethyl acetate = 1:1) followed by recrystallization from methylene chloride-hexane yielded 74 (4.7 mg, 8%) as white plates.



$C_{18}H_{16}O_3$ 280.32

MP 226-227°C.

IR(KBr pellet) $\nu_{max}(cm^{-1})$ 3420(s), 1756(s).

MS m/e(%) 280(100).

^{1}H NMR δ(ppm) 2.06(br, OH), 2.85(m, 1H), 2.89(dd, J=14.2, 5 Hz, 1H), 4.11(t, J=10.0 Hz, 1H), 4.60(dd, J=9.3, 6.5 Hz, 1H), 4.77(d, J=4.3 Hz,

1H), 4.88(d, J=8.8 Hz, 1H), 7.0-7.7(aromatics, 9H).

Analysis Calcd. for $C_{18}H_{16}O_3$: C, 77.12; H, 5.75. Found: C, 76.95; H, 5.85.

Chapter 12

Variations in Analog synthesis - Podo to Picro Analog Conversion

12.1 Modifications in the Synthesis of 74

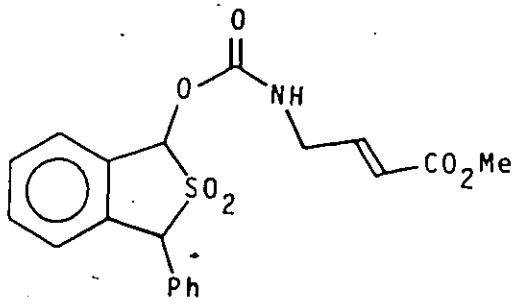
12.1.1 Preparation of 74 via LiI Demethylation

A solution of 94 (125 mg, 0.371 mmol) in 15 mL DMF was heated to 110°C for 1 day. The DMF was evaporated to 2 mL, LiI (1.2g, 9.0 mmol) was added and the solution was heated to 115°C for 3h with nitrogen passing over. The solution was cooled and mixed with 1% HCl (15 mL). The usual workup with ethyl acetate (except not filtered or dried) yielded a brown white solid. The residue was refluxed in aqueous LiOH (0.2M, 75 mL) for 0.5h. After cooling to 23°C, KH_2PO_4 (6g) and $NaNO_2$ (3g) were added followed by 10% HCl (15 mL) to give pH 4. The solution was stirred 20h followed by the usual workup with methylene chloride solvent. Purification on silica (1.5g, methylene chloride-ethyl acetate = 10:1) followed by recrystallization from methylene chloride-hexane yielded 74 (36 mg, 35%) as white plates.

12.1.2 Preparation of 105

A solution of 92 (2.60g, 10.0 mmol), isocyanate 68 (1.41g, 10.0 mmol) and triphenyltin acetate (0.50g, 1.2 mmol) in 50 mL methylene chloride was

evaporated to a viscous oil. The solution was diluted and re-evaporated several times over a 2 day period. Purification on silica (50g, hexane-ethyl acetate = 2:1) yielded 105 (2.08g, 52%) as a pale yellow foam.



$C_{20}H_{19}NO_6S$ 401.43

IR(neat) $\nu_{max}(cm^{-1})$ 3460(m), 3300-3550(w), 1723(s), 1745(m).

^{1}H NMR δ(ppm) 3.73(s, 3H), 4.07(m, 2H), 5.17(m, NH), 5.55(s,1H), 6.00(dt, J=15.8, 1.7 Hz, 1H), 6.67(s, 1H), 6.92(dt, J=15.8, 4.9 Hz, 1H), 7.0-7.6(aromatics, 9H).

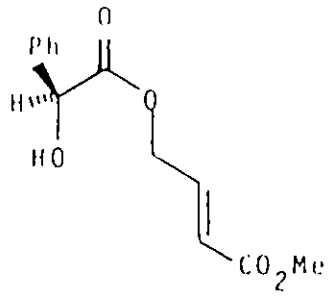
12.1.3 Thermolysis of 105

Nitrogen was continuously passed through a solution of 105 (126.2 mg, 0.559 mmol) in 25 mL DMF (dried/calcium hydride) while heating at 110-120°C for 3 days. Evaporation of the DMF, recrystallization from methylene chloride-hexane, purification of the mother liquor by PTLC (hexane-ethyl acetate = 1:3) and recrystallization yielded 97 (30.3 mg, 29%).

12.2 Attempted Assymetric Induction

12.2.1 Preparation of 113

A solution of methyl trans-4-bromocrotonate 116 (0.484g, 2.70 mmol) in 1 mL methylene chloride was added to a solution of (±)-mandelic acid 109 (0.565g, 3.71 mmol) and 0.5g benzyltrimethylammonium chloride in 1 mL 10% sodium bicarbonate at 25°C. The mixture was stirred vigorously for 4 days. The usual extractive workup with methylene chloride solvent, followed by recrystallization from ether-hexane yielded 113 (0.256g, 32%) as fine white needles.



$C_{13}H_{14}O_5$ 250.25

MP 64-65°C

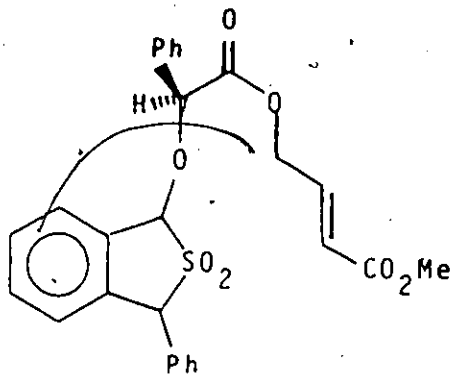
IR(neat) $\nu_{max}(cm^{-1})$ 3450(m), 1743(s), 1716(s).

MS m/e(%) 107(100), 100(63).

^{1}H NMR δ(ppm) 3.38(br, 0H), 3.71(s, 3H), 4.80(m, 2H), 5.23(s, 1H), 5.77(dt, J=15.8, 2.0 Hz, 1H), 6.84(dt, J=15.8, 4.6 Hz, 1H), 7.3-7.5(aromatics, 5H).

12.2.2 Preparation of 108

Boron trifluoride etherate (0.6 mL, 5 mmol), was added to a solution of 92 (0.379g, 1.46 mmol) and 113 (0.247g, 0.988 mmol) in 5 mL methylene chloride at 0°C. After stirring for 10 minutes the temperature was raised to 25°C and stirring was continued for 4h. The solution was washed with 10% sodium bicarbonate followed by the usual extractive workup and purification on silica (hexane-ethyl acetate = 3:1) to yield 108 (0.208g, 43%) as a pale yellow foam.



$C_{27}H_{24}SO_7$ 492.54

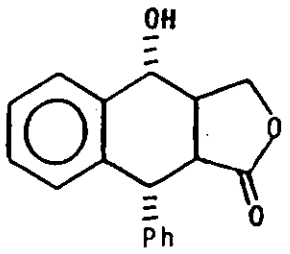
IR(neat) $\nu_{max}(cm^{-1})$ 1753(m), 1725(S), 1318(s), 1176(s).

^{1}H NMR δ(ppm) 3.66, 3.80, 4.00(3s, 3H), 4.68, 4.76, 4.86(3m, 2H), 5.27, 5.32(2m, 1H), 5.66-5.98(m, 3H), 6.72-6.92(3dt, 1H), 7.1-7.6(aromatics, 14H).

12.3 Picro Analogs

12.3.1 Preparation of 104

A solution of 74 (3.0 mg, 0.011 mmol) in 1 mL ethanol containing 0.5 mL 10% aqueous sodium acetate was refluxed for 2h. The solution was evaporated to 1 mL upon which fine white cotton-like crystals formed. The crystals were washed with water and recrystallized from methylene chloride-hexane to yield 104 (2.0 mg, 67%) as white cotton-like crystals.



$C_{18}H_{16}O_3$ 280.32

MP 148-149°C.

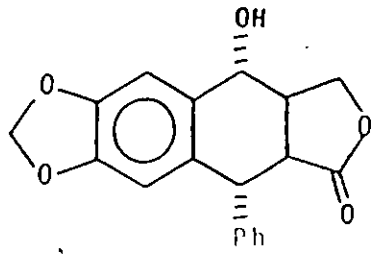
IR(KBr pellet) $\nu_{max}(cm^{-1})$ 3430(m), 1760(s).

MS m/e(%) 280(100).

^{1}H NMR δ(ppm) 2.10(d, J=7.4 Hz, OH), 2.73(m, 1H), 3.28(dd, J=9.5, 6.1 Hz, 1H), 4.21(d, J=6.2 Hz, 1H), 4.43(dd, J=9.6, 6.4 Hz, 1H), 4.57(dd, J=9.6, 2.0 Hz, 1H), 4.60(t, J=7.4 Hz, 1H), 6.7-7.6(aromatics, 9H).

12.3.2 Preparation of 103

Compound 73 (5.0 mg, 0.015 mmol) was treated under identical conditions to 74 in the preparaion of 104, to yield 103 (3.0 mg, 60%) as white cotton-like crystals.



$C_{19}H_{16}O_6$ 324.33

MP 185-186°C.

IR(KBr pellet $\nu_{max}(cm^{-1})$ 3430(m), 1763(s).

MS m/e(%) 324(100).

^{1}H NMR δ(ppm) 1.98(d, J=7.8 Hz, OH), 2.74(m, 1H), 3.25(dd, J=9.3, 5.1 Hz, 1H), 4.20(d, J=4.9 Hz, 1H), 4.40(dd, J=9.6, 6.1 Hz, 1H), 4.47(dd, J=9.6, 2.4 Hz, 1H), 4.50(t, J=7.8 Hz, 1H), 5.90(d, J=1.4 Hz, 1H), 5.91(d, J=1.4 Hz, 1H), 6.32(s, 1H), 7.02(s, 1H), 7.2-7.4(aromatics, 5H).

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Claims to Original Research

1. The two *trans*-2-arylbenzocylobutenols 44 and 75 were prepared via intramolecular attack of an enolate on a benzyne to form the benzocyclobutene ring. Oxidative decarboxylation of the benzocyclobutene carboxylic acids 59 and 85 resulted in formation of the *trans*-2-arylbenzocyclobutyl acetates 60 and 86. Deprotection of the acetates required careful acid catalized methanolysis at -10 to 0°C to prevent conversion of the sensitive alcohols 44 and 75 to the aldehydes 62 and 87. The previously prepared *trans*-2-phenylbenzocyclobutyl acetate 89 was deprotected by the same method and the resulting alcohol 76 found to be also very labile.

2. Isocyanate 68 was prepared and coupled with each of the three benzocyclobutenols 44, 75 and 76 using triphenyltin acetate as catalyst to afford the urethanes 69, 93 and 94.

3. The benzocyclobutene urethane derivatives 69, 93 and 94 were thermolysed to each yield the *trans* and *cis* fused Diels-Alder products 70, 71, 95, 96, 97 and 98. The ratio of *trans* to *cis* products was solvent dependent as was the reaction rate. Polar solvents such as nitromethane and DMF were found to increase the reaction rate as well as the ratio of *trans* to *cis* fused products. The *trans* fused products, which had the same relative stereochemistry as podophyllotoxin, were formed in ratios of about 5:1 when the polar solvents were used.

4. The *trans* fused Diels-Alder product 70 was converted to (±)-podophyllotoxin by ester and urethane hydrolysis and diazotization of the

resulting amino acid. Epimerization at C2 was circumvented by selective hydrolysis of the methyl ester in 69 prior to the Diels-Alder reaction. Similar treatment of 93 produced the podophyllotoxin analog 73.

5. The podophyllotoxin analog 74 was prepared by a similar route from 93 but a more efficient route was found to be via the LiI halogenolysis of the methyl ester after the Diels-Alder reaction.

6. The sulfone 105 was prepared and thermolysed to afford the tricyclic urethane 97 by a significantly shorter route than via the benzocyclobutene derivative 94.

7. Conversion of the podophyllotoxin analogs 73 and 74 to the picropodophyllotoxin analogs 103 and 104 occurred under similar conditions to those reported for the conversion of podophyllotoxin to picropodophyllotoxin.