ISOLATION, STRUCTURE ELUCIDATION AND APPROACHES TO THE PARTIAL SYNTHESIS OF NEW TAXOL ANALOGS

by

Ru Chen

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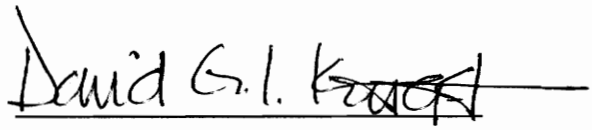
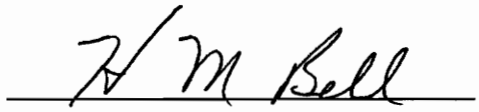


D. G. I. Kingston, Chairman

N. Castagnoli



H. M. Bell

July 14, 1994

Blacksburg, Virginia

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ABSTRACT

The western yew, *Taxus brevifolia*, was for many years the source of the important anticancer drug taxol, but it also contains many other natural taxoids. These compounds are of interest both from a structural viewpoint and also as a starting point for the synthesis of paclitaxel analogs. As a complement to our ongoing studies on the chemistry of paclitaxel and on its structure-reactivity relationships, we have investigated *T. brevifolia* extracts and have isolated two new taxoids. These are the A-nortaxol analog 7,13-deacetyl-9,10-debenzoyltaxchinin C **2.1** and 9-deacetyl-9-benzoyl-10-debenzoylbrevifoliol **2.4**. The structures were elucidated by spectral methods, including extensive 2D nmr techniques. An approach to the synthesis of a novel A-nortaxol analog from one of the new compounds has been developed.

ACKNOWLEDGMENTS

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Great gratitude is extended to the author's parents, Zhihua Chen and Xianghua Mei, for their love and inspiration. Finally, the author would like to express her appreciation to her husband, Wenjun, for his understanding and tolerance.

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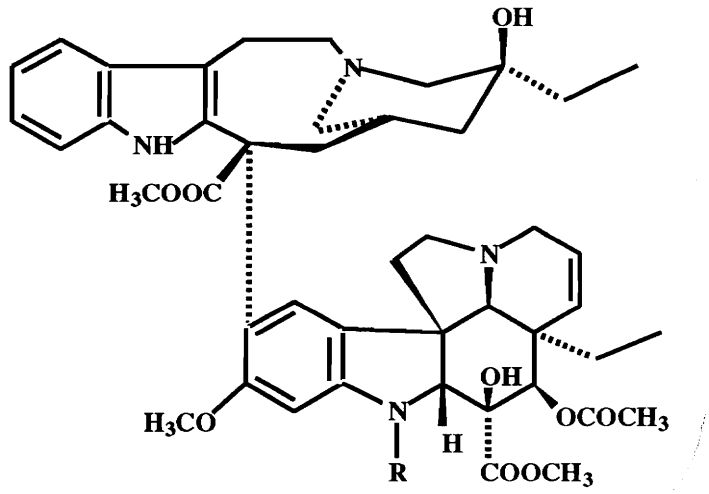
1.1 Natural Products in Cancer Chemotherapy

Natural products have played an important role in the treatment of various diseases for thousands of years. In western medicine, about one-fourth of currently used drugs are derived from plants (1). Thousands of natural products have been tested as potential antitumor compounds (2). The use of naturally occurring plant-derived anticancer agents dates back to as early as 1550 BC (3), but it is only in the recent decades that natural products as anticancer agents have been studied from a scientific point of view. According to the National Cancer Institute program for discovery of new and clinically useful anticancer drugs from plants, there are 3 - 4 % of plant species which produce a wide variety of anticancer agents with very diverse structural types (4 - 7).

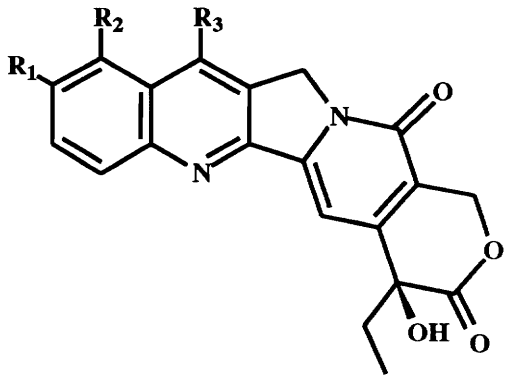
The vinca alkaloids vincristine **1.1** and vinblastine **1.2** were isolated from *Catharanthus roseus*, whose common name is periwinkle. Although their mechanism of action is not completely understood, they are used extensively in combination with other agents for the treatment of a wide variety of cancers, including leukemia, bladder cancer, testicular cancer (8) and lymphomas such as Hodgkin's disease (9).

The alkaloid camptothecin **1.3** was isolated from the tree *Camptotheca acuminata* (10). Clinical studies from the Peoples Republic of China gave encouraging results in a number of cancers, including gastrointestinal cancers, head and neck tumors and bladder carcinoma (11). To search for analogs with greater water solubility and lower toxicity, two promising compounds were obtained. One of them, CPT-11 **1.4** has been shown to be effective against refractory leukemia and lymphoma in a phase II trial in Japan (12); the other one, topotecan **1.5** (13) has shown promising results in phase I clinical trials in the USA and Europe.

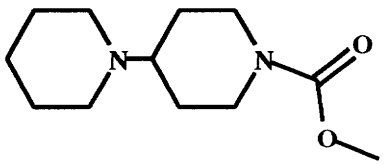


1.1 Vincristine: R = CHO

1.2 Vinblastine: $R = CH_3$



1.3 Camptothecin: $R_1 = R_2 = R_3 = H$

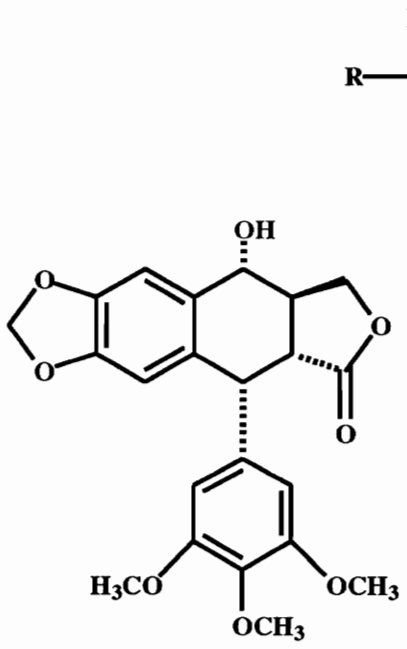
1.4 CPT-11: $R_1 =$ 

$R_2 = H$; $R_3 = CH_3CH_2$

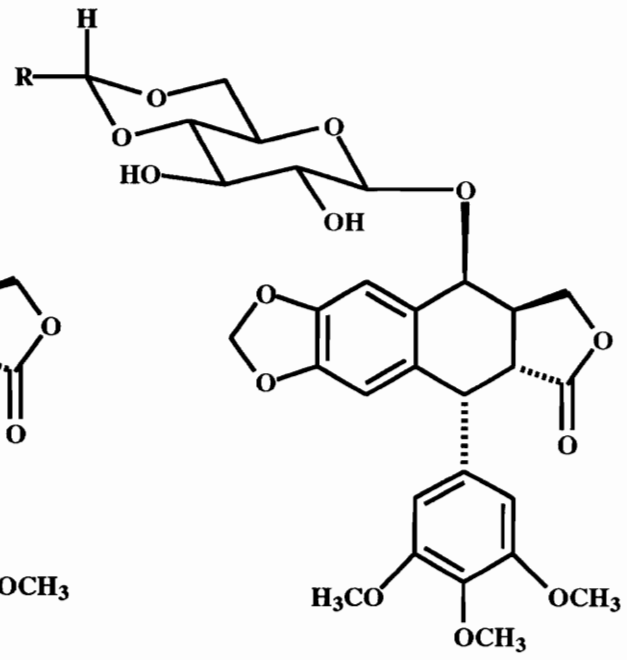
1.5 Topotecan: $R_1 = OH$; $R_3 = H$

$R_2 = CH_2N(CH_3)_2$

The lignan podophyllotoxin **1.6** isolated from the May apple (*Podophyllum peltatum)* has been converted to the derivatives etoposide **1.7** and teniposide **1.8** (14, 15) which represent the most useful non-alkaloid natural products in cancer chemotherapy. As one of the most active single active agents in small cell lung cancer, etoposide (VP16-213) is also active in testicular cancer (16). Teniposide (VM26) shows activity against Hodgkin's disease and other malignant lymphomas, pediatric refractory neuroblastoma, and brain tumors in adults (14). These two compounds and the two vinca alkaloids (vinblastine and vincristine) are currently clinically used drugs.

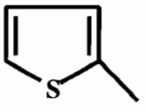


1.6 Podophyllotoxin



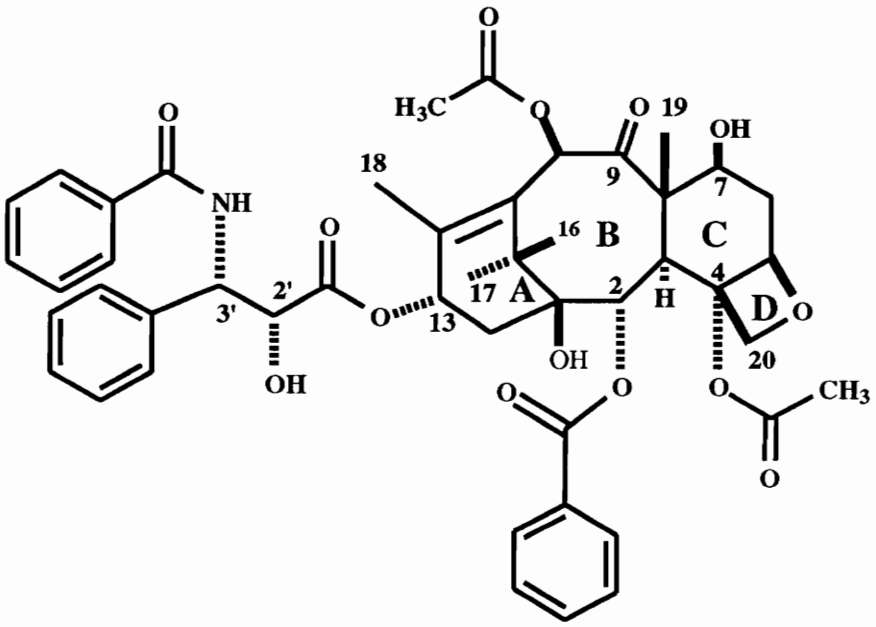
1.7 Etoposide: R = CH_3
(VP16-213)

1.8 Teniposide: R =
(VM26)



A number of microorganism-derived compounds, mostly antibiotics, also play a significant role in the anticancer field. There are many other promising natural product candidates which have a bright future in the clinic.

The most recent member of the family of clinically useful natural anticancer agents is Taxol® **1.9**, the taxane diterpenoid from *Taxus brevifolia*.. [1]It was approved in 1992 as a chemotherapeutic agent for the treatment of drug refractory advanced ovarian cancer by the U.S. Food and Drug Administration (FDA).



1.9 Paclitaxel

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[1]. The name Taxol is a registered trademark of the Bristol-Myers Squibb Company, and the generic name paclitaxel will thus be used hereafter.

1.2 Paclitaxel and Taxane Diterpenoids (Taxoids)

The compounds of plants in the Taxaceae have been studied for over a hundred years. The yew plant, from which paclitaxel was isolated, has been known as an extremely toxic plant since antiquity.

The first modern attempt to isolate the constituents of the yew was made by Lucas. In 1856, Lucas (17) extracted a crude material which he called taxine from the needles and other parts of the English yew, *Taxus baccata*. It was later found to be a mixture of many alkaloids (18 -20). In 1963, Taylor reported the isolation of a compound called baccatin (21) which was later named baccatin I by Halsall (22) from the heartwood of *T. baccata*. Halsall himself isolated baccatin II, III and IV.

In the early 1960's, a continuing effort by the National Cancer Institute (NCI) to discover novel cancer chemotherapeutics resulted in a wide screening of various plants. In 1962, the bark of the western yew, *Taxus brevifolia*, was collected from an Oregon forest (23). In 1964, a crude extract of the bark showed cytotoxic activity against leukemia cells and inhibitory action against a variety of tumors. In 1966, the Wall group at the Research Triangle Institute in North Carolina isolated the bioactive component of *T. brevifolia* from the dried stem bark in 0.02 % yield and named it paclitaxel (24). Paclitaxel was also found in other *Taxus* species, such as *T. baccata* and *T. cuspidata* (25). Its molecular structure including absolute stereochemistry was revealed by X-ray crystallographic analysis undertaken at Duke University. The molecular model (Figure 1) shows the beautiful molecular architecture and the highly compact nature of paclitaxel.

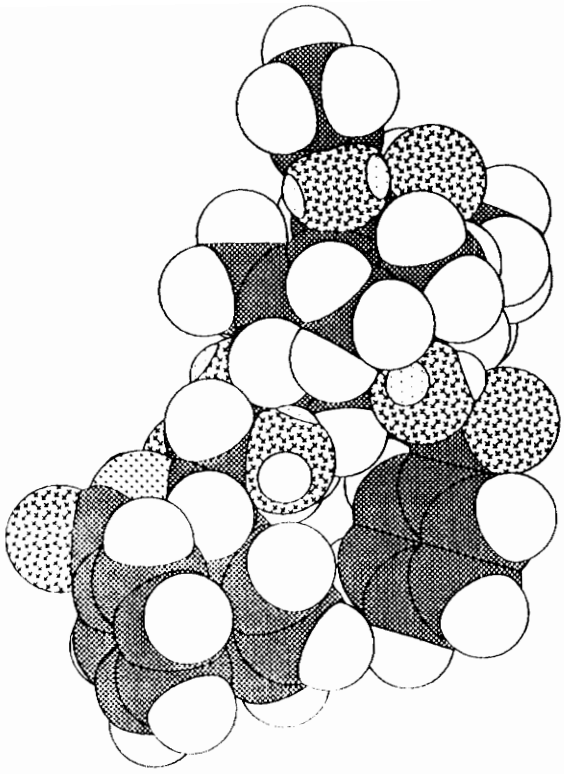


Figure 1. Molecular models of paclitaxel

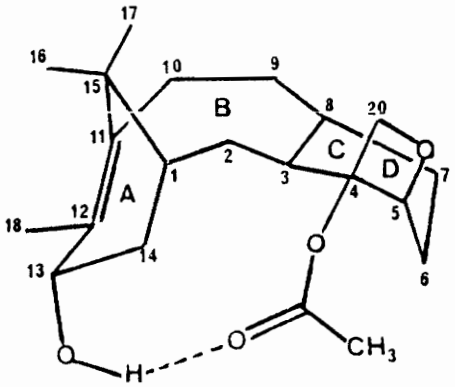
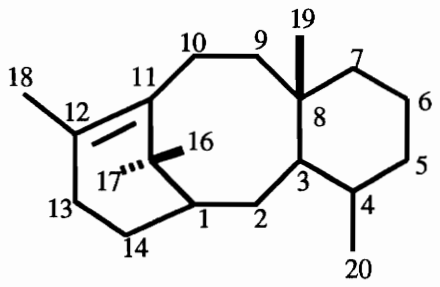


Figure 2. 3D Structure of the taxane skeleton

1.3 Structure of the Taxane Skeleton



1.10 Taxane skeleton

All the taxane diterpenoids have a relatively complex structure with an unusual carbocyclic framework (or a closely related skeleton) as depicted in **1.10**. It was established by a combination of chemical, and spectroscopic studies, and was confirmed by x-ray crystallographic analysis. The numbering system used for the taxane skeleton **1.10** was adopted by IUPAC (26).

The tricyclic ring system of the taxoids possesses a flexible and inverted cup-shaped conformation, which is obviously from its three-dimensional (Figure 2) representation. The six membered ring A exists as a distorted boat form, cis-fused to the eight membered ring B which has a boat-chair conformation. The conformation of the six membered ring C which is trans-fused to ring B is highly dependent on the functionality located at C-4,(5). Almost all known taxoids contain an angular methyl group at C-8 and are also characterized by the existence of a fully substituted bridgehead olefin at C-11,(12).

1.4 The Families of Taxoids

There are several different ways to sort the taxoids, based on the nature of the oxygenation pattern, on the presence or absence of basic side chains, and on other structural features. Taxoids can be divided into several classes according to the nature of the oxygenation at C-20 and the presence or absence of basic side chains, and this is the classification that will be used here.

1.4.1 Taxoids with a C-4,(20) Exocyclic Double Bond

There are over 50 examples of this class **1.11** (27). This largest single group of natural taxoids has a C-4,(20) double bond, and an α-oxygen function at C-5; which are defined as structural characteristics of this class of compound. Almost all of them have a β-oxygen substituent at C-10. Most of them are also oxygenated at C-9 and are not oxygenated at C-1.

They are further divided into three subclasses based on the nature of the oxygenation of C-5. These are:

a) Taxoids with a hydroxyl or acetate group at C-5 **1.12**.

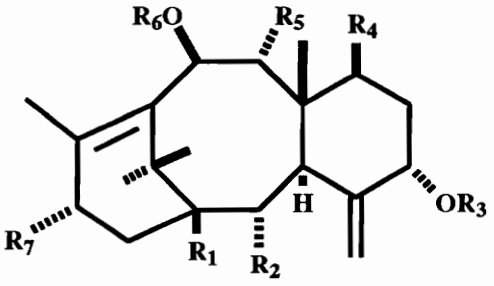
b) Basic taxoids: taxoids with a basic side chain at C-5 **1.13**.

c) Taxoids with a C-5 cinnamoyl group **1.14**.

1.4.2 Taxoids with a C-4,(20) Epoxide

This is a small class which consists of less than 20 examples **1.15**. All of them have an acetoxyl group at C-2, and the C-5 oxygen is substituted with hydrogen, acetyl,

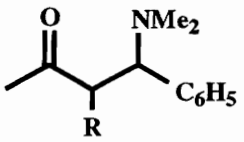
cinnamoyl or a basic side chain. All compounds of this class isolated to date are also oxygenated at C-9, C-10 and C-13. Several compounds esterified at C-9 with nicotinic acid (from *Austrotaxus spicata*) **1.16** are an interesting sub-group of this class.



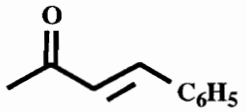
1.11

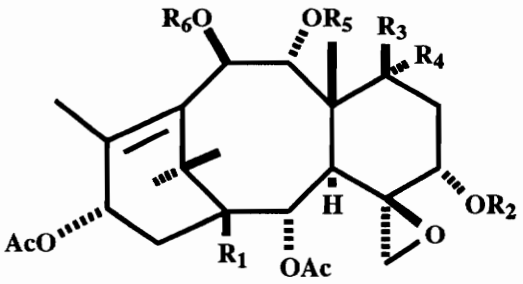
1.12 R_3 = H or Ac

1.13 R_3 =

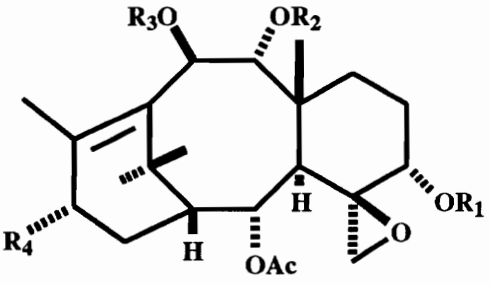


1.14 R_3 =

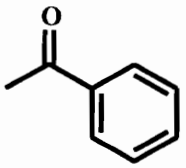




1.15



1.16 R_1 =



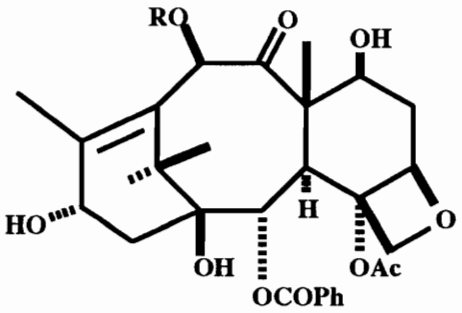
1.4.3 Taxoids with an Oxetane Ring

The second largest class of the taxane diterpenoids, with more than thirty current members, is the most important class, since paclitaxel is one of the members. All of the

members of this class are highly oxygenated, with oxygen substituents at C-2, C-4, C-7, C-9, C-10, C-13, and even the C-1 position, which is usually not oxygenated in the other series of compounds. These compounds can be easily divided into two subclasses without or with a complex ester side chain at C-13.

a) simple taxoids with an oxetane ring

This subclass consists of about twenty members, including paclitaxel's potential semisynthetic precursors baccatin III **1.17** and 10-deacetyl baccatin III **1.18**.

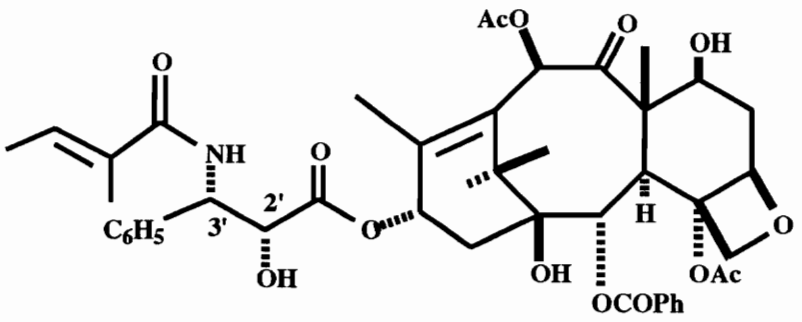


1.17 R = OAc

1.18 R = H

b) Taxoids with a complex C-13 side chain and an oxetane ring

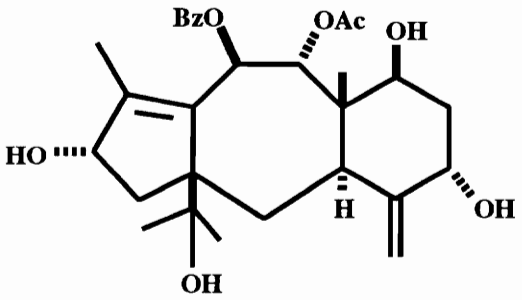
The most important anticancer agent paclitaxel is one of this small subclass, which also includes the important related compound cephalomannine **1.19**.



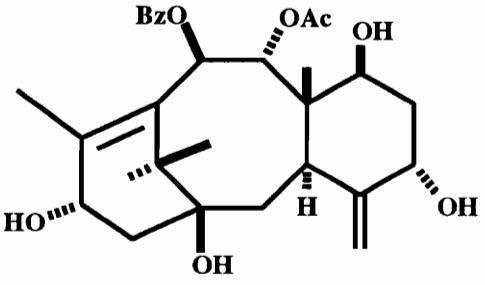
1.19

1.4.4 A-nortaxoids

A-nortaxoids (or 11 (15 → 1) - *abeo* taxoids, as they are more correctly described (28)) are a class of taxoids with a 5-membered A-ring instead of the normal 6-membered ring. These AB ring contracted taxanes have a novel 5/7/6 ring system rather than the 6/8/6 ring system of the normal taxoid skeleton. First discovered as synthetic compounds which were reported by Kingston's group (28) and later by Wahl and his coworkers (29), a small group of naturally occurring A-nortaxoids were isolated only very recently. Brevifoliol **1.20** isolated from *T. brevifolia* (30), was the first natural taxoid with an A-nortaxane skeleton; although it was initially assigned a normal taxane skeleton **1.21** which was later corrected to the A-nortaxane skeleton (31,32,33).

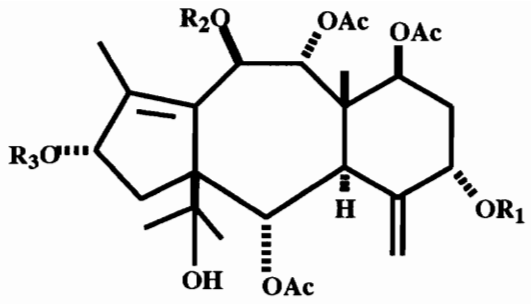


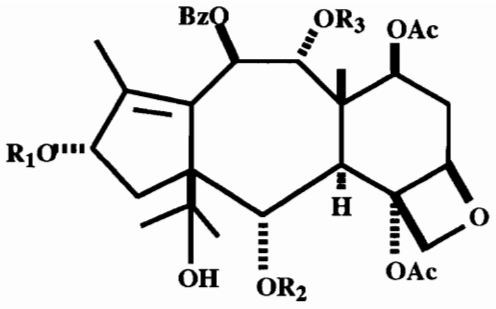
1.20



1.21

Taxchinin A **1.22** isolated from *Taxus chinensis* (30) was the first naturally occurring taxoid to be correctly assigned the A-nortaxane skeleton (34). Taxchinin B **1.23**, C **1.24**, D **1.25**, and G **1.26** were isolated from the same plant by the same research group (35,36).





1.22 $R_1 = R_3 = H$, $R_2 = Bz$

1.25 $R_1 = H$, $R_2 = Bz$, $R_3 = Ac$

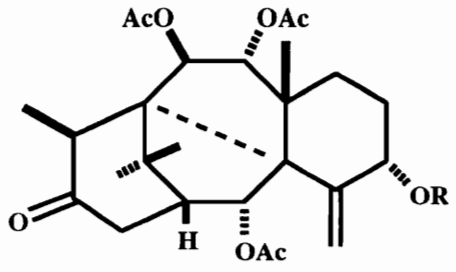
1.26 $R_1 = R_2 = H$, $R_3 = Ac$

1.23 R_1 = Cinnamoyl,

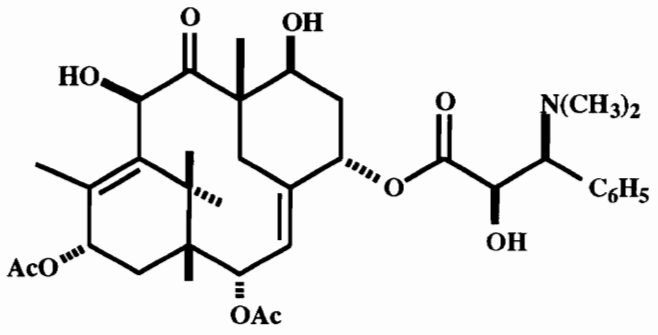
$R_2 = R_3 = Ac$

1.24 $R_1 = Ac$, $R_2 = R_3 = Bz$

The two new taxoids which will be presented in this thesis are new additions to this rapidly-growing family. They are proposed to be named as a brevifoliol derivative and a taxchinin C derivative. In addition to brevifoliol from *T. brevifolia* and the taxchinins from *T. chinensis*, compounds with this ring system have also been isolated from *T. baccata* (33,37) and *T. wallichiana* (38).



1.27



1.28

1.4.5 Miscellaneous Taxoids

This group includes six diverse taxoids. Three of them with a C-3,(11) bond **1.27** are particularly interesting compounds. This type compounds can be obtained both by isolation from plants and by preparation from the irradiation of taxinine (39, 40). Another interesting compound is the alkaloid taxine A **1.28**, which has a rearranged taxane diterpenoid skeleton (41).

1.5 Importance of Paclitaxel

1.5.1 Structure and Activity Relationship

It has been shown that both the C-13 phenylisoserine side chain and the diterpene skeleton of paclitaxel are essential for biological activity (24). One of the interesting structural features of the paclitaxel diterpene skeleton is the oxetane ring, which plays a critical role in cytotoxicity (28). The C-2 benzoate can also significantly affect its activity. Some articles gave a very plausible review of the structure-activity relationships. Figure 3 is reproduced from a very recently published review by Dr. Kingston (42), which showed detailed structure-activity relationships.

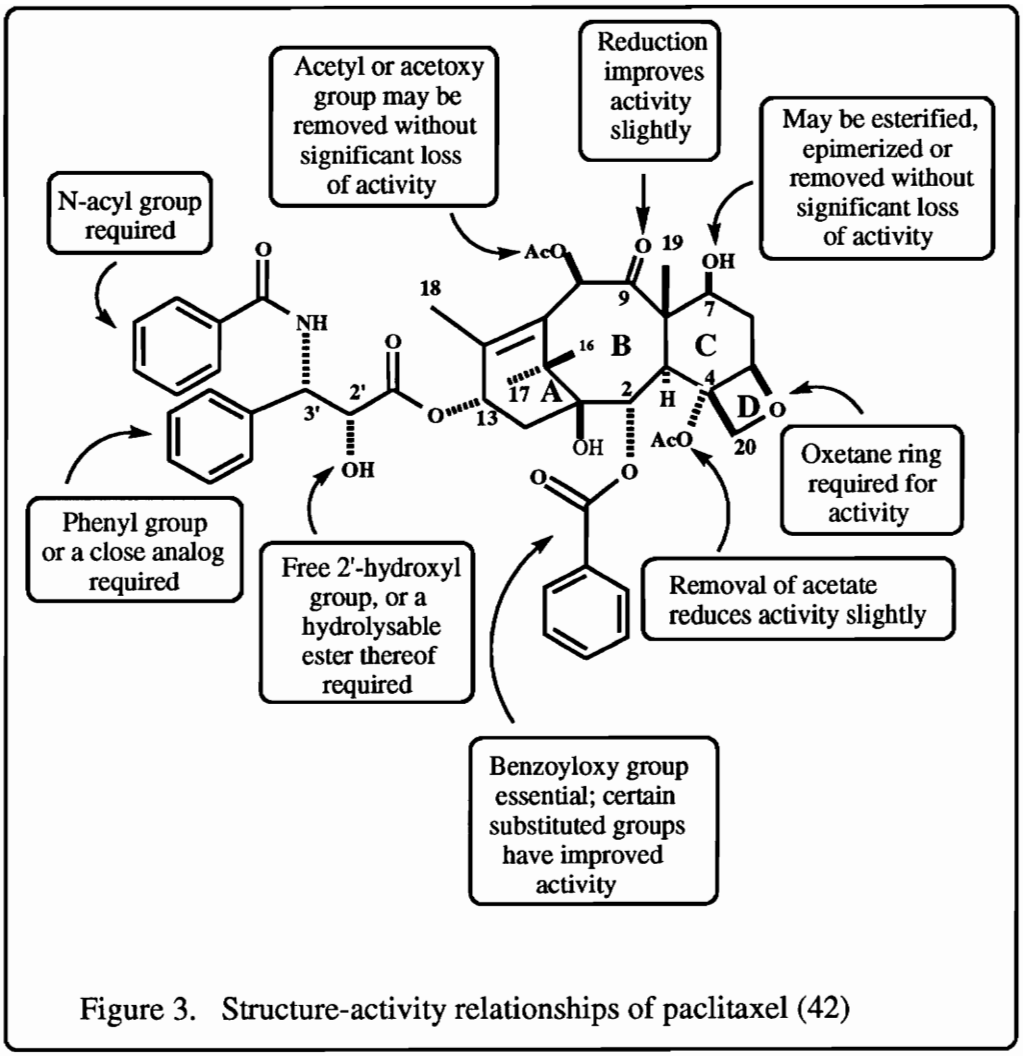


Figure 3. Structure-activity relationships of paclitaxel (42)

1.5.2 Novel Mechanism of Action

Microtubules play a key role in mitosis, maintenance of cell shape, cell motility, and intracellular transport. They are self-assembling and self-disassembling structures that are in dynamic equilibrium with tubulin dimers, the protein subunits of which they are composed. A substance that interferes with microtubules can disrupt cell growth and function.

As a mitotic spindle poison, unlike other known tubulin binders which prevent the formation of microtubules such as vinca alkaloids, paclitaxel inhibits the depolymerization of microtubules (44). Studies have also shown that paclitaxel enhances both the rate and yield of microtubule assembly (Figure 4). Paclitaxel's binding to the microtubule is highly specific, reversible and temperature independent. Because tumor cells usually divide much more rapidly than normal cells, paclitaxel inhibits tumor activity by disrupting cancer cells.

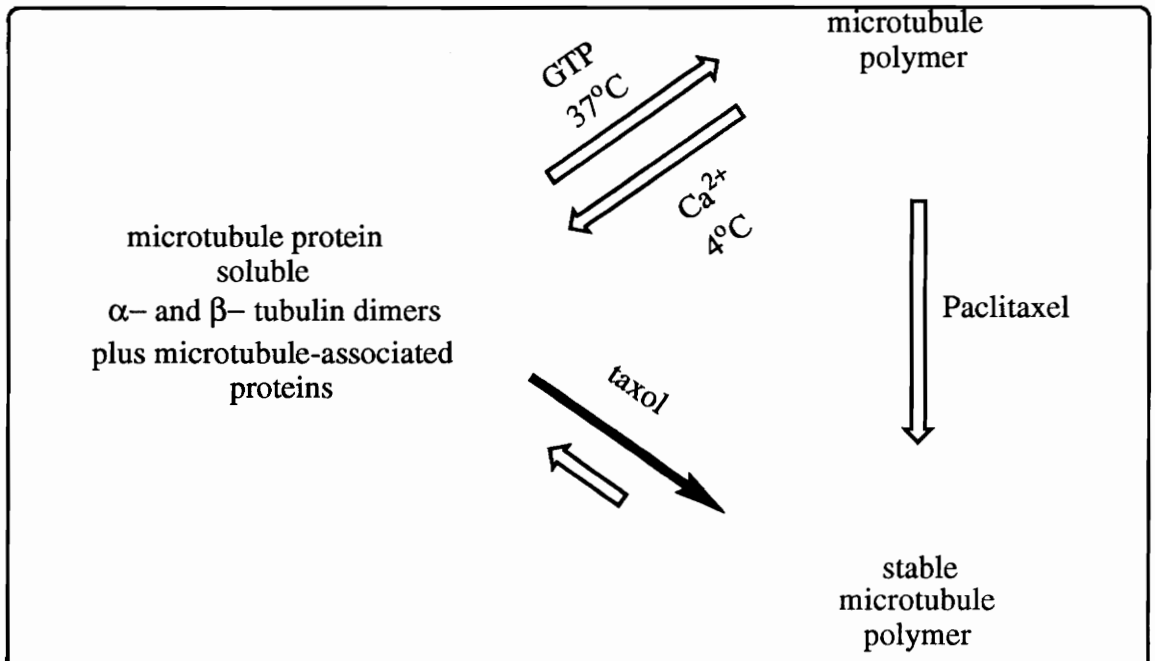
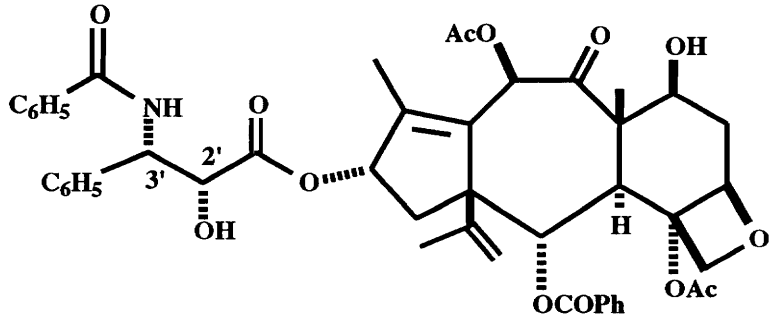


Figure 4. Paclitaxel alters the normal equilibrium between soluble tubulin dimers and polymerized microtubules. Taxol enhances the polymerization of tubulin into stable microtubules in the absence of GTP, a cofactor normally required for microtubule polymerization in vitro, and stabilizes microtubules to cold and Ca^{2+} (43).

1.5.3 Bioactivity of Paclitaxel and A-nortaxol

The activities of paclitaxel and related analogs are determined by their ability to promote the polymerization of microtubules or inhibit their depolymerization (45), *in vitro* cytotoxicity assays, and *in vivo* tumor models (46). There are three important assay systems, the P-388 *in vivo* lymphocytic leukemia system, the 9 KB cell culture system, and the microtubule assembly and binding assays, which are usually performed to investigate the activity of paclitaxel at the cellular level because they give the information about the intrinsic activity of paclitaxel and related compound.



1.29

Biological activities of paclitaxel and A-nortaxol **1.29**, which were determined in both the KB cell culture assay and a tubulin depolymerization assay are shown in Table 1 (28). The assay results on the ring-contracted paclitaxel derivative were almost as active as paclitaxel in the tubulin depolymerization assay. This result are not surprising since although A-nortaxol has a highly modified A ring, molecular models indicate that its shape is not significantly different from that of paclitaxel itself. This interesting biological activity result makes the A-nortaxoids important precursors of new paclitaxel analogs. The reasons for lack of KB activity of A-nortaxol may be due to its instability or lack of transportation to get into the cell or other factors.

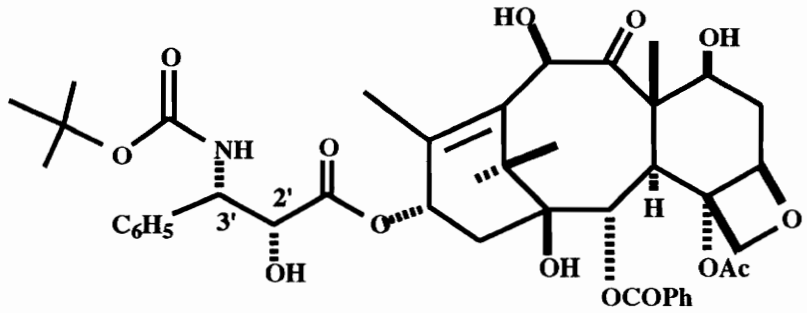
Table 1. Bioactivity of Paclitaxel and A-nortaxol (28)

compounds	ED_{50}(g/mL) in KB cell culture	ID_{50}(M) in tubulin depolymerization assay
paclitaxel	0.00001	0.3
A-nortaxol	2.0	0.9

1.5.4 Clinical Trials

Based upon its biological data, especially its significant activity against B16 melanoma, paclitaxel (46) was chosen as a clinical candidate in 1977. Phase I clinical trials of paclitaxel began in the early 1980's, and various protocols were tried to observe its effects on solid tumors and adult leukemia (47 - 49). Phase II trials of paclitaxel began in 1985, and yielded exciting results of paclitaxel's remarkable activity against advanced refractory ovarian cancer (50). The results obtained from phase II clinical trials also showed paclitaxel's activity against metastatic breast cancer (51), non-small cell lung cancer (52,53), and head and neck cancer (54). Paclitaxel's activity against ovarian and breast cancer is currently in Phase III clinical trials.

Taxotére® **1.30**, a semi-synthetic paclitaxel analog developed in France (55), was in its initial clinical trials. Taxotére showed better activity than paclitaxel *in vitro* (56,57).



1.30

1.6 Problems of Paclitaxel as An Anticancer Agent

1.6.1 Limited Availability from Natural Sources

Paclitaxel is obtained only in low yields from the very slowly growing western yew tree. To obtain one kilogram of paclitaxel, 10,000 kilograms of yew bark are required. A relatively large amount of paclitaxel is needed to treat a patient (almost two grams under current protocols) (58).

Since removing the bark from tree destroys the tree, it endangers the survival of this species. Currently cell culture and semisynthesis are two promising options for large scale production of paclitaxel. As mentioned before, Taxotere, an important semi-synthetic paclitaxel analog, was prepared from a precursor of paclitaxel, 10-deacetyl baccatin III. 10-Deacetyl baccatin III can be isolated from the needles of *T. baccata* (59). In contrast to the bark, the needles can regenerate and thus provide a continuous source of precursor.

1.6.2 Poor Aqueous Solubility

One of the major obstacles for routine clinical use of paclitaxel is its lack of water solubility; as a result, it must be given in conjunction with emulsifying agents. During the Phase I clinical trials, several allergic reactions were caused by the emulsifiers given in conjunction with paclitaxel, which even caused the death of a patient (60). It is only because of its unique mechanism of action that paclitaxel was approved for further clinical trials.

1.6.3 Cancers Show Resistance to Paclitaxel

Some cancers showed multi-drug resistance to paclitaxel (61,62); paclitaxel was pumped out from the cells.

1.7 Objective of This Work

As a conclusion to the above statement, our objective is to develop new paclitaxel analogs with improved bioactivity and hopefully good activity against MDR-resistant cell lines through the approach of isolating new taxane diterpenoids and modifying them chemically to be paclitaxel derivatives.

II. ISOLATION AND STRUCTURE ELUCIDATION OF TWO NEW TAXOIDS FROM *TAXUS BREVIFOLIA*

2.1 Introduction

The clinical activity of paclitaxel against breast and ovarian cancers (63) has spurred a worldwide search for better sources and improved analogs of this drug. Although over 100 taxoids have been isolated to date (27), new taxoids continue to be isolated, and many of these could serve as precursors to new analogs of paclitaxel.

The western yew, *Taxus brevifolia*, was the original source of paclitaxel (24), and it continues to be a source of other natural taxoids (64, 65 ,30). In continuation of our work on this plant, we have examined a pre-paclitaxel fraction from a large-scale isolation of paclitaxel and have obtained two new taxoids with the uncommon A-ring contracted taxane skeleton.

Structures were elucidated by spectral methods, including high and low resolution fast atom bombardment mass spectra (hrfabms), uv spectra, ir spectra and especially nmr spectra. Extensive nmr techniques were used. Those were ^{1}H, ^{13}C, APT, DEPT, proton decoupling, DQCOSY, TOCSY (66), HETCOR (67), HMQC and HMBC. HMBC was the most useful technique in confirming the presence of the ring contracted A-nortaxoids. The definitions of some nmr techniques were given below:

APT - *A*ttached *P*roton *T*est

DEPT - *D*istortionless *E*nhancement by *P*olarization *T*ransfer

DQCOSY - *D*ouble *Q*uantum Filtered *C*orrelation *S*pectroscop*y*

TOCSY - *To*tal *C*orrelation *S*pectroscop*y*

HETCOR - *Het*eronuclear *Cor*relation Spectroscopy

HMQC - *H*eteronuclear *M*ultiple-*Q*uantum *C*orrelation

HMBC - *H*eteronuclear *M*ultiple-*B*ond *C*orrelation

HOMO2DJ - *H*omonuclear 2D J-Resolved Spectroscopy

In particular the comparison of nmr data of these two new compounds with those of taxchinin C **1.24** and brevifoliol **1.20** confirmed the structures of new taxoids.

2.2 Results and Discussion

2.2.1 Isolation

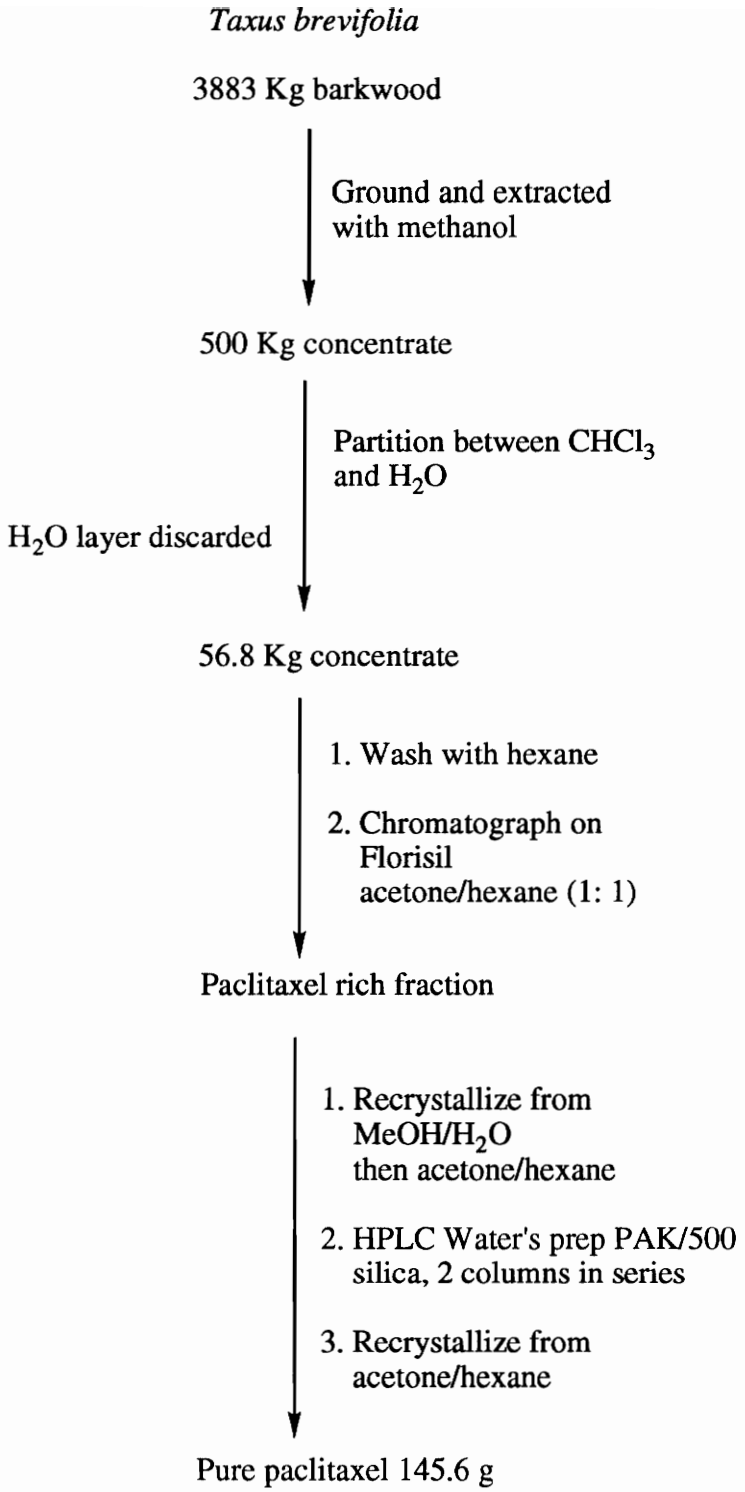
The starting material was a 'pre-paclitaxel' fraction from a previous large-scale isolation of paclitaxel from *T. brevifolia* . Scheme 1 was the flowsheet of this large-scale isolation of paclitaxel.

Partitioning of this fraction between CH_2Cl_2 and H_2O, followed by cc on silica gel and finally repeated preparative tlc, yielded the two new taxoids **2.1** and **2.4**. Scheme 2 showed the flowsheet of the isolation of these two compounds.

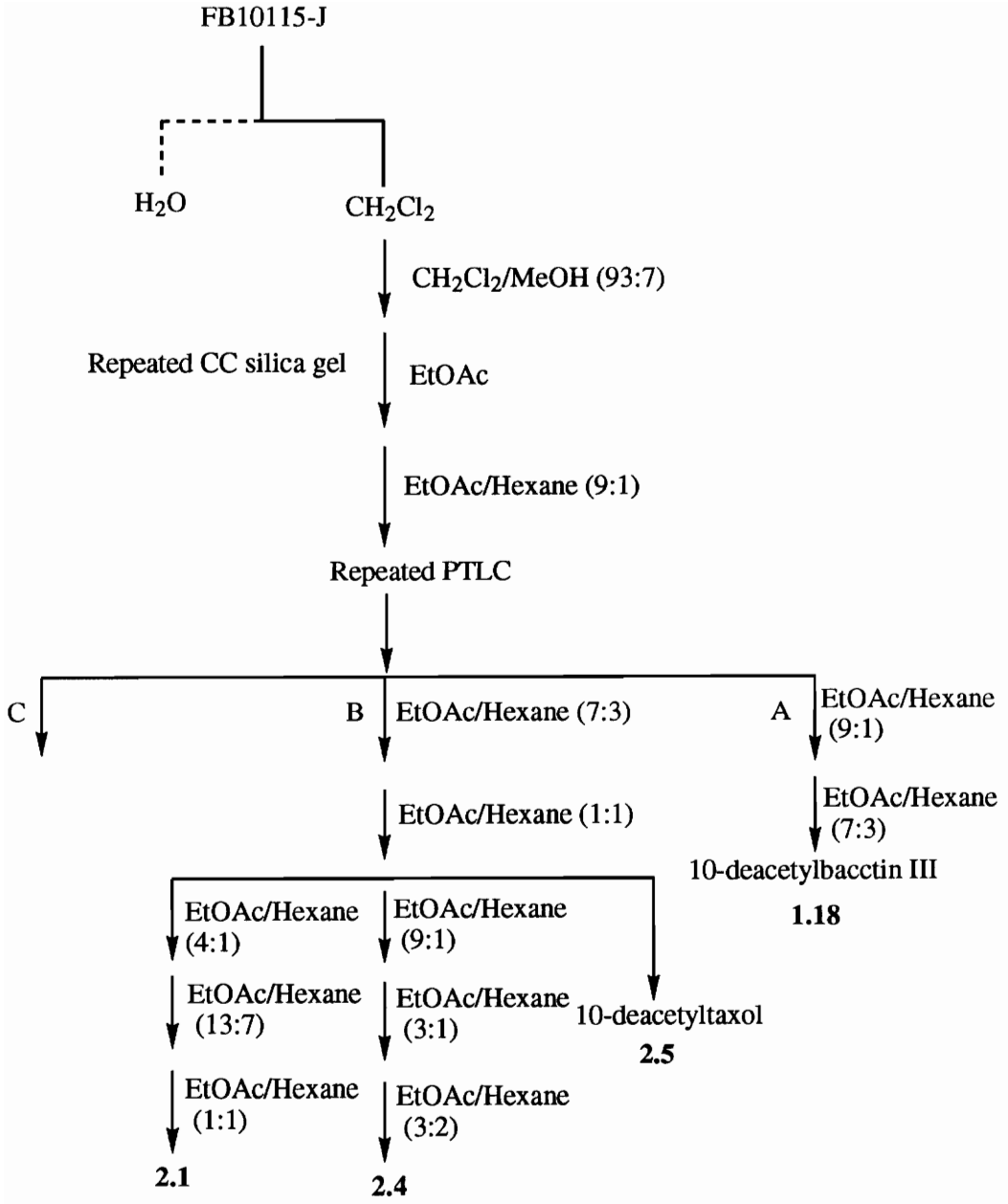
2.2.2 Structure Elucidation

2.2.2.1 7,13-Deacetyl-9,10-debenzoyltaxchinin C 2.1

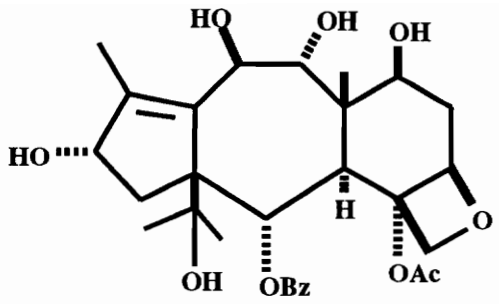
Compound **2.1** had a composition of $C_{29}H_{38}O_{10}$ as determined by hrfabms and ^{13}C-nmr spectroscopy (Figure 6). Its ^{1}H-nmr spectrum (Figure 5) showed characteristic taxoid signals, including singlets for four methyl groups at 1.05, 1.08, 1.84, and 1.93 ppm, and a pair of doublets for oxetane protons at 4.08 and 4.43 ppm. The presence of an oxetane ring was further supported by a ^{13}C-nmr signal for the methylene carbon at 74.7 ppm and by ^{1}H decoupling and TOCSY experiments (Figure 7) to verify the coupling of



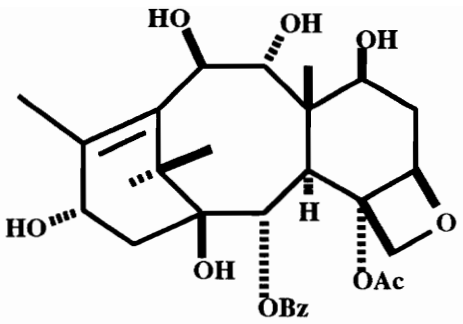
Scheme 1. Large scale isolation of paclitaxel from *Taxus brevifolia*



Scheme 2. Modified isolation scheme for **2.1, 2.4**



2.1



2.2

the H-20α and H-20β protons. Characteristic signals for a benzoate group were observed in both the ^{1}H nmr (7.46, 7.60, and 7.99 ppm) and the ^{13}C nmr spectra.

The basic ^{1}H- and ^{13}C-nmr assignments for **2.1** were made by a combination of DEPT (Figure 8), HETCOR (Figure 9), TOCSY, and HMBC (Figure 10) techniques, and are shown in Table 2. The doublet at 6.04 ppm was assigned to the C-2 proton, deshielded by the benzoate group, and this was coupled to the C-3 proton at 3.00 ppm. HMBC correlations of the *ortho* -benzoyl proton at 7.99 ppm with the carbonyl signal at 166.2 ppm assigned the latter as being due to the benzoate carbonyl group, and this signal in turn was correlated with the proton signal at 6.04 ppm, confirming the location of the benzoate group at C-2. The appearance of the C-2 and C-3 protons as an isolated spin system confirmed the fully substituted nature of C-1 and C-4.

The remaining key question was that of the assignment of the ring system as that of paclitaxel and related compounds **2.2** or of the newer brevifoliol type **2.1**. Two key results support the brevifoliol skeleton for **2.1**. The first was the assignment of the ^{13}C-nmr signal at 76.4 ppm to C-15, an assignment supported by HMBC correlations with the protons on C-2 and C-14. The downfield nature of this signal indicated that C-15 must be oxygenated, which is consistent with structure **2.1** but not with structure **2.2.** Secondly, the lack of a diagnostic three-bond correlation from H-16 and H-17 to C-11 (32), which is

a characteristic HMBC correlation of paclitaxel (Figure 11), eliminated structure **2.2** from consideration.

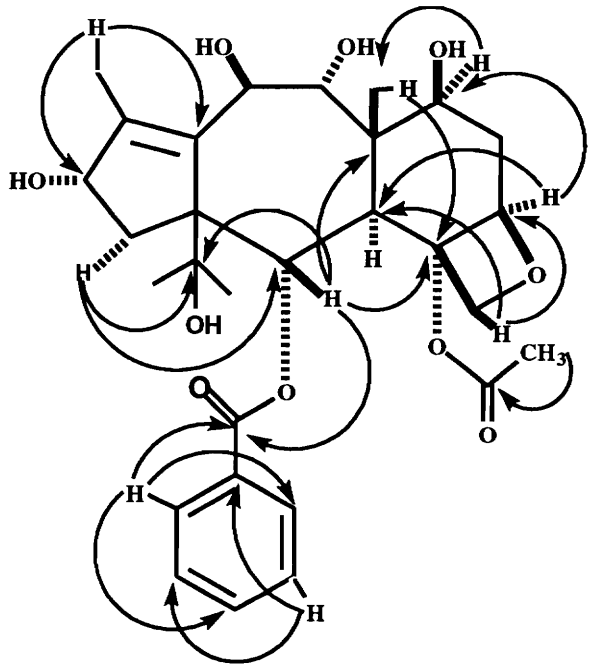


Figure 10. HMBC correlations of compound **2.1**

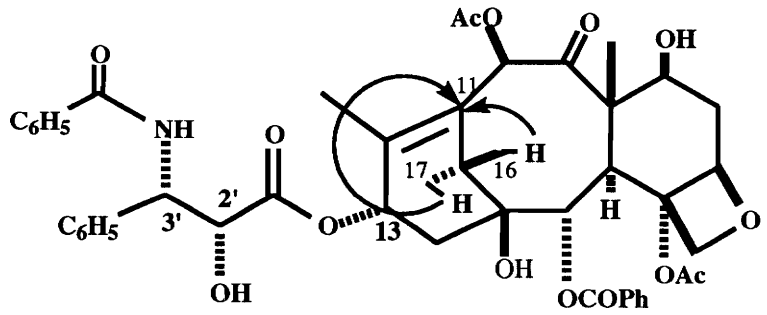


Figure 11. Diagnostic three-bond HMBC correlation of paclitaxel

Table 2. Nmr data of 7,13-deacetyl-9,10-debenzoyltaxchinin C **2.1**

Position	^{1}H[a]	^{13}C[b]	TOCSY	HMBC[c]
1		67.7		
2	6.04 (1H, d, 7.4)	68.8	H-3	C-3, C-4, C-8, C-15, O$\underline{C}$OC$_6$H$_5$
3	3.00 (1H, d, 7.4)	44.5	H-2	C-1, C-2, C-4, C-8, C-19, C-20
4		80.4		
5	4.89 (1H, brd, 8.5)	85.1	H-6α	C-3, C-4, C-7
6	α 1.80 (1H, m)	37.2	H-5, H-6β, H-7	C-7, C-8
	β 2.55 (1H, ddd, 16.0, 8.2, 1.5)		H-6α, H-7	
7	4.20 (1H, brt, 8.2)	72.4	H-6α, H-6β	C-19
8		42.6		
9	4.30 (1H, d, 10.4)	80.7	H-10	
10	4.55 (1H, d, 10.4)	68.7	H-9	
11		137.2		
12		146.8		
13	4.58 (1H, brt)	77.6	H-14α , H-14β	
14	α 1.75 (1H, dd, 15, 7.4)	39.4	H-14α , H-13	C-1, C-2, C-13, C-15,
	β 2.25 (1H, dd, 15, 7.4)		H-14β , H-13	C-18
15		76.4		
16	1.08 (3H, s)	24.7		
17	1.05 (3H, s)	27.7		C-16
18	1.93 (3H, s)	11.4		C-11, C-12, C-13
19	1.84 (3H, s)	12.2		C-9
20	α 4.43 (1H, d, 7.9)[d]	74.7	H-20β	C-3, C-4, C--5
	β 4.08 (1H, d, 7.9)		H-20α	
OCOC$\underline{H}_3$	2.20 (3H,s)	22.4		O$\underline{C}$OCH$_3$
O$\underline{C}$OCH$_3$		171.1		
O$\underline{C}$OC$_6$H$_5$		166.2		
Ar i		129.9		
o	7.99 (2H, d, 7.2)	129.6	m-Ar	O$\underline{C}$OC$_6$H$_5$
m	7.46 (2H, m)	128.6	o-Ar, p-Ar	
p	7.60 (1H, m)	133.9	m-Ar	

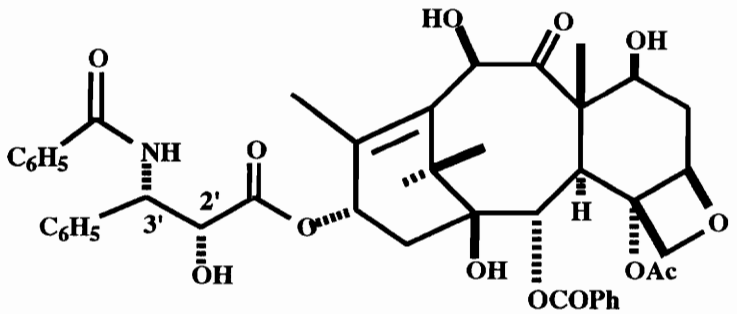
a. Multiplicity and apparent coupling constant(s) in Hz in parentheses.

b. ^{13}C assignments of protonated carbons were confirmed by a HETCOR experiment, and carbon types were assigned by a DEPT experiment.

c. The HMBC experiment was performed with the second delay in the J filter segment set for J = 8 Hz and J = 10 Hz. Data from both experiments are combined in this column.

d. The assignments of 20a and 20b were made by comparison with data for taxol (70); the assignments for taxchinin B (36) are reversed.

The comparison of the ^{1}H, ^{13}C-nmr data of **2.1** and those of taxchinin C **1.24** (Table 3) strongly supported the structure assignment. These data matched very well except the proton chemical shifts of C-7, C-9, C-10, and C-13. In the case of taxchinin C, substituted by acetoxyl or benzoxyl groups rather than hydroxyl groups, the chemical shifts were more downfield. The structure of the new compound is thus established as a derivative of taxchinin C **1.24** (36) and specifically as 7,13-deacetyl-9,10-debenzoyltaxchinin C.



2.5

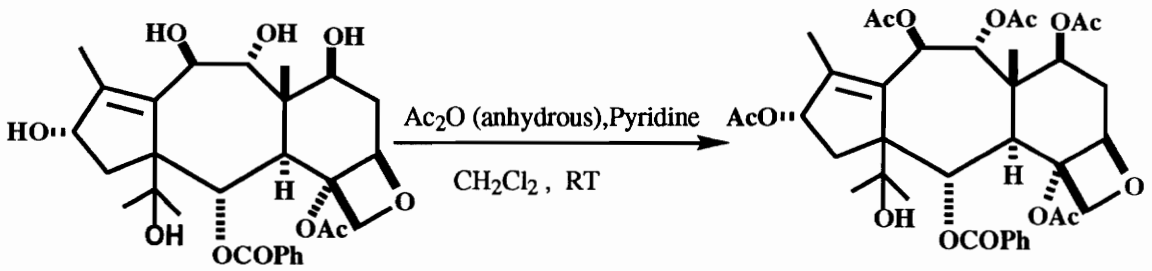
Table 3. ^{1}H and ^{13}C nmr assignments for taxchinin C **1.24** (36)

C	^{1}H	^{13}C
1		68.1
2	6.55 (d, 7.3)	68.4
3	3.18 (d, 7.7)	44.4
4	–	79.1
5	4.97 (d, 7.3)	84.8
6	2.70 (m)	34.8
7	5.67 (t, 8.1)	70.6
8	–	43.8
9	6.56(d,11.3)	77.5
10	6.77 (d, 11.0)	68.5
11	–	136.4
12	–	148.0
13	5.71 (t, 7.7)	78.8
14	2.45, H (dd, 7.3, 14.3)	36.7
15	–	75.8
16	1.21 (s)	27.7
17	1.21 (s)	25.4
18	1.97 (s)	11.8
19	1.87(s)	13.1
20	4.19, H (d, 7.7) 5.19, H (d, 7.7)	74.6
OCOMe (C-4)	2.17 (s)	21.6
OCOMe (C-4)	–	170.5
OCOMe (C-7)	2.17 (s)	21.0
OCOMe (C-7)	–	169.4
OCOMe (C-9)	1.83 (s)	21.9
OCOMe (C-9)	–	171.0
OCOPh		134.1
		129.1
	8.04 (d, 7.3)	133.4
	7.75 (d, 7.3)	130.6
	7.63 (m)	129.5
	7.49 (m)	129.0
	7.35 (m)	128.7
	7.22 (m)	128.2
OCOPh		164.7 166.8 166.1

It is noteworthy that an unusual 4-bond HMBC correlation was detected between H-14β and C-18; this is probably due to the W-effect. Correlations are not observed

between H-14β and C-11 or C-12, but a similar failure to observe these correlations is seen in the HMBC spectrum of paclitaxel (68). To confirm the above arguments, an HMBC experiment was carried out on paclitaxel as a control. The result was consistent with the literature.

Acetylation of **2.1** yielded a tetraacetate derivative **2.3** in which the proton signals for C-7, C-9, C-10, and C-13 had shifted downfield, indicating that these position in **2.1** carried secondary hydroxyl groups (Scheme 3).

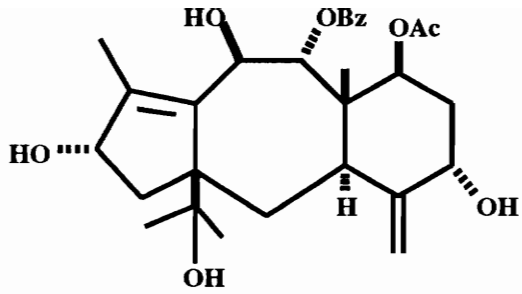


2.1 **2.3**

Scheme 3. Acetylation of **2.1**

2.2.2.2 9-Deacetyl-9-benzoyl-10-debenzoylbrevifoliol 2.4

The second new compound **2.4** had a composition of $C_{29}H_{38}O_8$ as deduced by hrfabms and ^{13}C nmr spectroscopy. Its ^1H and ^{13}C nmr spectra (Figure 16, 17) were similar in many respects to those of compound **2.1**, with the significant difference that signals for an exocyclic methylene group (δ_H 4.82 and 5.10 ppm, δ_C 150.0, 111.8 ppm) replaced those for the oxetane ring. Signals for one acetate group and one benzoate group could also be identified.



2.4

One interesting feature of both ^{1}H and ^{13}C nmr spectra that was observed in the spectra of **2.4** but not in those of **2.1** was that the signals were very broad when the spectra were obtained at room temperature. Increasing the temperature to 57°C, however, yielded normal narrow linewidths, indicating that line broadening was due to a slow equilibrium between two or more conformational isomers. This behavior has been observed previously in taxoids that were subsequently shown to have the brevifoliol skeleton (33, 38), and appears to be characteristic of this structural type.

A comparison of the ^{1}H- and ^{13}C-nmr spectra of **2.4** with those of brevifoliol **1.20** (Table 5) (31) indicated that **2.4** differed from brevifoliol primarily in lacking one acetate group. The locations of the acetate and benzoate groups in **2.4** were confirmed by acetylation and by HMBC experiments (Figure 21). Acetylation of **2.4** (Scheme 4) yielded a triacetate derivative **2.8** in which the signal for C-5, C-10, and C-13 had shifted downfield by approximately 1 ppm, indicating that these positions in **2.4** carried secondary hydroxyl groups. HMBC experiments then related the benzoate carbonyl group both to the *ortho* protons at 8.00 ppm and to the C-9 proton at 5.70 ppm, and the acetate carbonyl group to the acetate methyl group at 1.87 ppm and the C-7 proton at 5.51 ppm. The assignment of the signal at 5.70 ppm to C-9 (rather than C-10) was made on the basis of HMBC correlations of the C-10 proton at 4.85 ppm with C-1 and C-12.

Table 4. Nmr data of 9-deacetyl-9-benzoyl-10-debenzoylbrevifoliol **2.4**

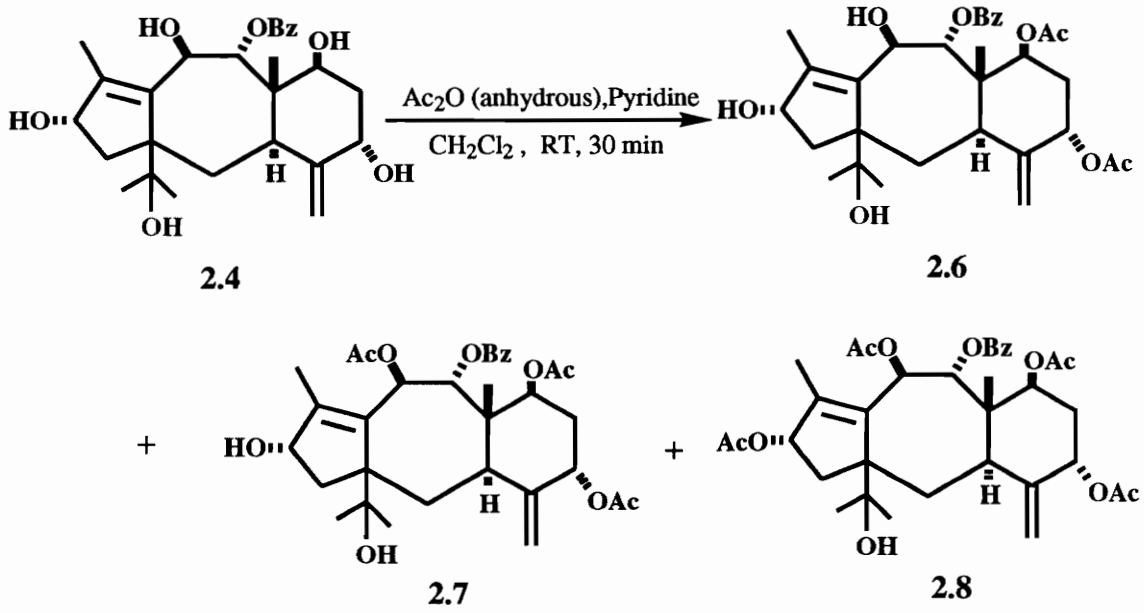
Position	^{1}H[a]	^{13}C[b]	TOCSY	HMBC[c]
1		61.2		
2	a 1.58 (1H, d, 14.3)	28.9	H-2b	C-1, C-3, C-8, C-14, C- 15
	β 2.30 (1H, dd, 14.3, 9.0)		H-2a, H-3	
3	2.84 (1H, d, 9.0)	37.1	H-2b	C-1, C-4, C-7, C-8, C-19, C-20
4		150.0		
5	4.36 (1H, brs)	72.9	H-6α	
6	α 1.70 (1H, m)	35.6	H-6β, H-7	
	β 1.99 (1H, brd, 11.5)		H-6α, H-7	
7	5.51 (1H, dd, 11.5, 5)	71.0	H-6α, H-6β	O$\underline{C}$OCH$_3$, C-19
8		45.5		
9	5.70 (1H, d, 9.2)	80.2	H-10	O$\underline{C}$OC$_6$H$_5$
10	4.85 (1H, d, 9.2)	68.0	H-9	C-1, C-12
11		137.4		
12		145.9		
13	4.43 (1H, brt, 7.0)	78.0	H-14α , H-14β	C-11, C-12
14	α 1.35 (1H, dd, 14.0, 7.0)	46.4	H-14α , H-13	C-11, C-12, C-13, C-15,
	β 2.39 (1H, dd, 14.0, 7.0)		H-14β , H-13	
15		76.5		
16	1.07 (3H, s)	27.1		C-1
17	1.39 (3H, s)	25.9		C-1, C-15, C-16
18	1.75 (3H, s)	11.9		C-12
19	1.03 (3H, s)	12.8		
20	α 5.10 (1H, s)[d]	111.8		C-3, C--5
	β 4.80 (1H, s)			
OCOC$\underline{H}_3$	1.87 (3H, s)	21.6		O$\underline{C}$OCH$_3$
O$\underline{C}$OCH$_3$		170.8		
O$\underline{C}$OC$_6$H$_5$		167.0		
Ar i		130.6		
o	8.00 (2H, d, 7.9)	129.6	m-Ar	O$\underline{C}$OC$_6$H$_5$
m	7.40 (2H, m)	128.2	o-Ar, p-Ar	
p	7.53 (1H, m)	132.7	m-Ar	

a. Multiplicity and apparent coupling constant(s) in Hz in parentheses.

b. ^{13}C assignments of protonated carbons were confirmed by a HETCOR experiment, and carbon types were assigned by a DEPT experiment.

c. The HMBC experiment was performed with the second delay in the J filter segment set for J = 8 Hz and J = 10 Hz. Data from both experiments are combined in this column.

d. The assignments of 20a and 20b were made by comparison with data for taxol (68); the assignments for brevifoliol (31) are reversed.



2.4 2.6 2.7 2.8

Scheme 4. Acetylation of **2.4**

Table 5. ^{1}H and ^{13}C nmr assignments for brevifoliol **1.20** (31)

C	^{1}H	^{13}C
1		75.9
2	1.49, H (m) 2.36, H (dd, 8.8, 13.0)	29.1
3	2.77 (d, 8.8)	37.9
4	–	149.0
5	4.45 (br s)	72.4
6	1.84, H (dd, 5.0, 14.0) 2.02, H (dd,11.4, 14.0)	36.0
7	5.57 (dd, 5.0, 11.4)	70.1
8	–	45.0
9	6.06(bd)	77.1
10	6.53 (d,10.6)	70.2
11	–	133.9
12	–	151.5
13	4.38 (t,7.4)	76.7
14	2.46, H (dd, 7.4 Hz, 14.0) 1.29, H (dd, 7.4 Hz, 14.0)	47.3
15	–	62.4
16	1.05 (s)	26.9
17	1.35 (s)	24.8
18	2.01 (s)	12.0
19	0.90 (s)	12.9
20	4.83, Hα (s) 5.19, Hβ (s)	
OCOMe (C-7)	1.75 (s)	20.7
O<u>C</u>OMe (C-7)	–	169.9
OCO<u>Me</u> (C-9)	2.08 (s)	21.4
O<u>C</u>OMe (C-9)	–	170.5
O<u>C</u>OPh (C-10)	–	164.3
ipso-OCOPh	–	129.3
o-OCOPh	7.87 (d, 7.8)	129.4
m-OCOPh	7.43 (t, 7.8)	128.7
p-OCOPh	7.56 (t, 7.8)	133.2

These results, taken in conjunction with the published spectra of brevifoliol and the additional correlations summarized in Table 4, establish the structure of **2.4** as the brevifoliol analog 9-deacetyl-9-benzoyl-10-debenzoylbrevifoliol.

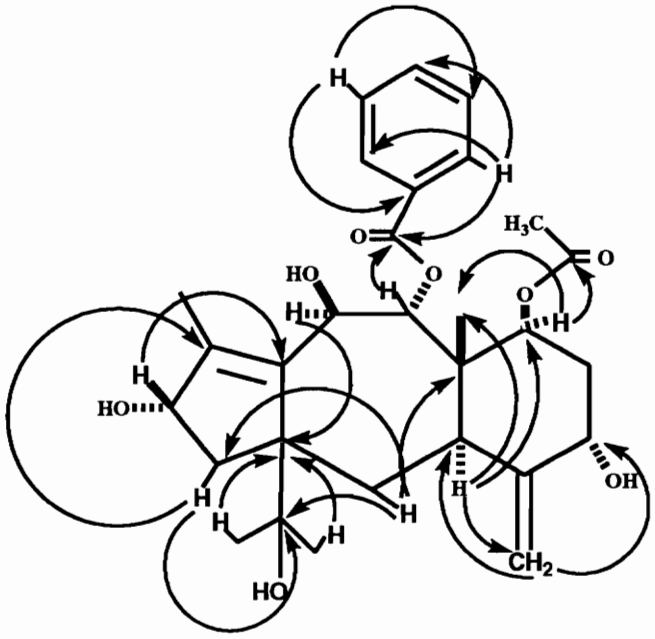


Figure 21. HMBC correlation of compound **2.4**

2.3 Experimental

GENERAL EXPERIMENTAL PROCEDURES — Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Ftir spectra were recorded as KBr pellets on a Nicolet 710 FT-IR spectrometer. Uv spectra were obtained on a Perkin-Elmer 330 spectrophotometer. Mass spectra were obtained on a Kratos MS50 mass spectrometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Nmr spectra were recorded on a Varian Unity 400 spectrometer in $CDCl_3$. ^1H chemical shifts were recorded in ppm from internal TMS and ^{13}C chemical shifts were based on the $CHCl_3$ signal at 77.0 ppm.

FRACTION FB10115-J — *T. brevifolia bark* was processed as previously described (65) to yield a paclitaxel-rich fraction which was crystallized to give crude paclitaxel. The mother liquors from this crystallization were rechromatographed on a Florisil' column with elution of acetone-hexane, 50-50, to yield additional crude paclitaxel. Fraction FB10115-J eluted just before paclitaxel on this column.

ISOLATION OF COMPOUNDS **2.1** AND **2.4** — A 35 g portion of dark brown syrupy residue of FB10115-J was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 extract (32 g dark brown syrup) was subjected to chromatography on silica gel (3 times) with elution by CH_2Cl_2 / MeOH (93:7), EtOAc, and EtOAc / Hexane (9:1) respectively. Fractions which eluted after 10-deacetylbaccatin III were combined and purified by repeated preparative tlc (silica gel F_{254}, 20×20 cm) with development by EtOAc/hexane. A total of 62.5 mg of compound **2.1** and 105.2 mg of compound **2.4** was obtained.

COMPOUND **2.1** — Mp 162°; $[\alpha]^{25}_D$ - 15° (c=10, $CHCl_3$), uv (EtOH) λ_{max} (log ε) 240 nm (4.52); ir (KBr) ν_{max} 3405, 2995, 2976, 2930, 2891, 1736, 1715, 1663, 1449, 1369, 1269, 1240, 1175, 1111, 1067, 1043, 1024, 731,712 cm^{-1}; fabms *m/z* $[M+Na]^+$· 569 (51), 499 (35), 476 (43), 413 (32), 349 (10), 326 (18), 242 (6), 199 (26), 176 (100), 173 (60), 136 (8); hrfabms *m/z* $[M+Na]^+$· 569.23260, calc for $C_{29}H_{38}O_{10}Na$ 569.23624; ^1H- and ^{13}C-nmr, see Table 2.

COMPOUND **2.4** — Mp 152°; $[\alpha]^{25}_D$ +18° (c=10, $CHCl_3$); uv (EtOH) λ_{max} (log ε) 228 nm (5.93); ir (KBr) ν_{max} 3380, 2974, 2939, 2896, 1715, 1663, 1373, 1281, 1176, 1111, 1096, 1069, 1026, 730, 712 cm^{-1}; fabms *m/z* $[M+Na]^+$ 537 (58), $[M-H_2O- OH]^+$ 479 (20), $[M-COCH_3-2\ OH]^+$ 421 (43), 357 (14), 329 (68), 219 (5), 154 (100); hrfabms

m/z [M + Na]$^{+\cdot}$ 537.24610, calc for $C_{29}H_{38}O_8Na$ 537.24644; ^1H and ^{13}C-nmr, see Table 4.

ACETYLATION OF COMPOUND **2.1** — Compound **2.1** (5.1 mg) in dry CH_2Cl_2 (50 µl) and dry pyridine (56.6 µl) was mixed with Ac_2O (51 µl). The mixture was stirred at room temperature overnight, diluted with EtOAc and extracted with H_2O. The organic layer was washed with dilute HCl and H_2O, dried and evaporated. The crude products were isolated by preparative tlc with EtOAc/hexane (3:2). The 7,9,10,13-tetraacetate **2.3** (3.7 mg) was obtained. Mp 130 -134°C; fabms *m/z* $[M+Na]^+$ 737 (10), $[M+H]^+$ 715 (3), 105 (100); ^1H nmr 8.01 (d, 7.2, 2H), 7.62 (m, 1H), 7.44 (m, 2H), 6.36 (d, 7.5, 1H), 6.31 (d, 10.2, 1H), 5.69 (t, 7.2, 1H), 5.02 (brt, 1H), 4.94 (d, 8.1, 1H,), 4.46 (d, 7.9, 1H), 4.14 (d, 7.9, 1H), 3.04 (d, 7.2, 1H), 2.54 (m, 1H), 2.39 (m, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.76 (s, 3H), 1.12 (s, 3H), 1.07 (s, 3H), 1.06 (s, 3H).

ACETYLATION OF COMPOUND **2.4** — Compound **2.4** (20 mg) in dry CH_2Cl_2 (100 µl) and dry pyridine (20 µl) was mixed with Ac_2O (20 µl). The mixture was stirred at room temperature for 35 minutes, diluted with EtOAc and extracted with H_2O. The organic layer was washed with dilute HCl and H_2O, dried and evaporated. The crude products were isolated by preparative tlc with EtOAc/hexane (3:2). The 5,10,13-triacetate **2.8** (2.7 mg) and the 5-monoacetate **2.6** (1.3 mg) were obtained directly, and the 5,10-diacetate **2.7** (4.4 mg) was obtained by further ptlc with EtOAc/hexane (7:3). Compound **2.8** Mp 120 - 123°C; fabms *m/z* [$M+Na]^+$ 663 (5), 105 (100); ^1H nmr 7.96 (d, 7.2, 2H), 7.55 (m, 1H), 7.42 (m, 2H), 6.55 (d, 9.2, 1H), 6.15 (brs, 1H), 5.56 (m, 1H), 5.55 (brs, 1H),

5.38 (brt, 1H), 5.27 (brs, 1H), 4.91 (brs, 1H), 2.74 (d, 8.4, 1H, 2.50 (m, 1H), 2.08 (s, 6H), 2.04 (s, 6H), 1.80 (s, 3H), 1.35 (s, 3H), 1.12 (s, 3H), 0.98 (s, 3H).

III. SYNTHESIS OF THE PACLITAXEL SIDE CHAIN

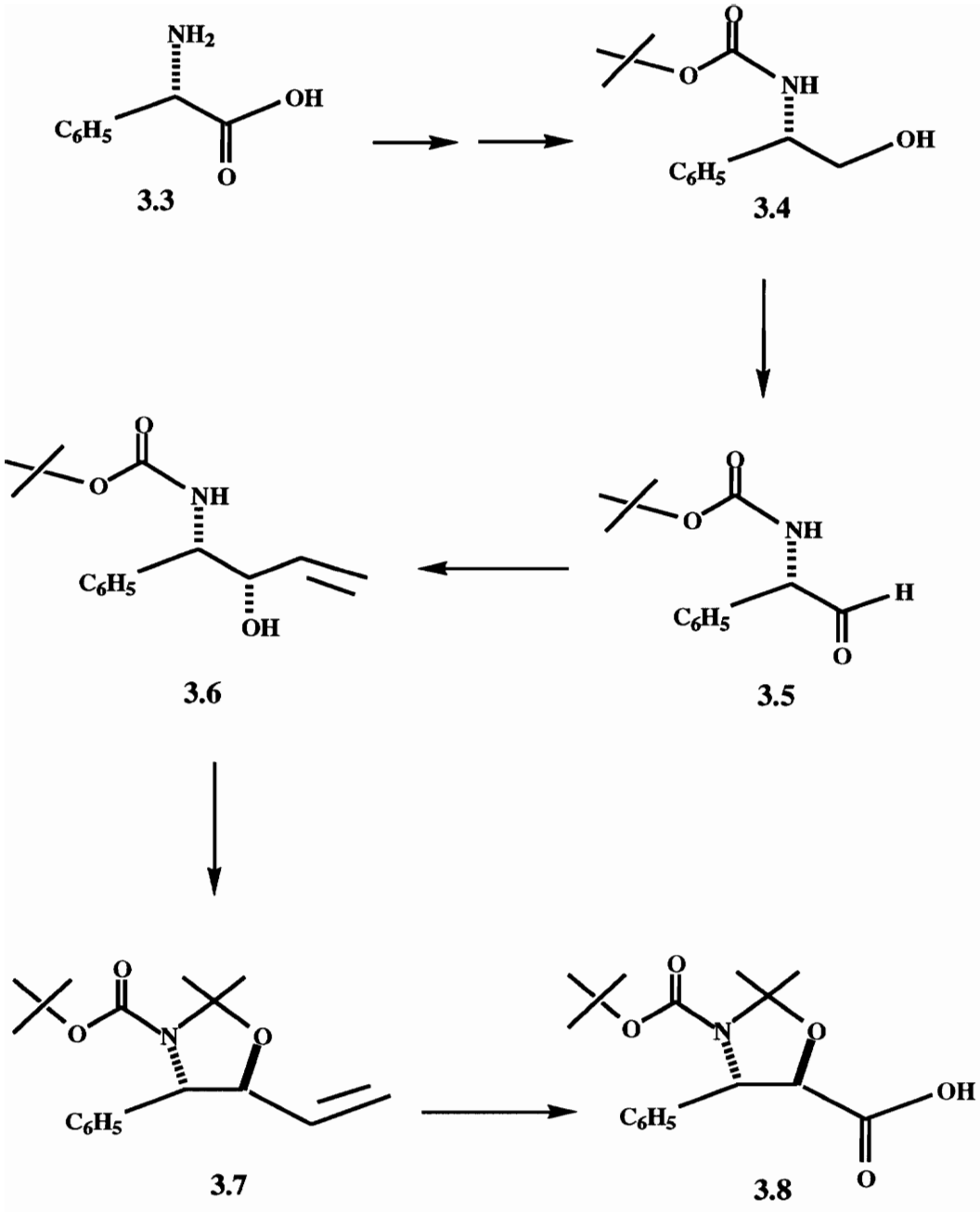
3.1 Introduction

Paclitaxel and its most important derivative taxotere possess a 2'R, 3'S phenylisoserine side chain which plays a very important role in their biological activity (69, 70). These substances can be secured in good yield from the appropriate hydroxyl protected side chain (71, 72) or β-lactam (73) and naturally abundant 10-deacetyl baccatin III in its protected form. The need for a highly efficient enantioselective synthesis of the required side chain has been created by the anticancer potential of these compounds. A number of methods for the synthesis of side chain derivatives has been published (71, 74-77).

3.2 Results and Discussion

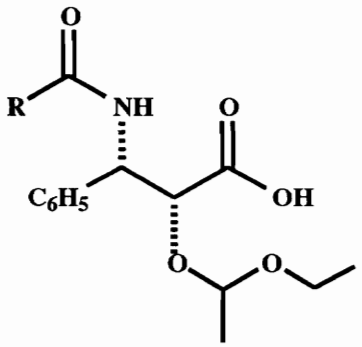
3.2.1 Synthetic Strategy

Several research groups have developed different synthetic methods for the phenylisoserine side chain through very diverse pathways. The synthetic method used in this work was a combination of two different pathways which were developed by two research groups. Since the side chain analogs **3.1, 3.2** synthesized by Greene's method (78) were very unstable and decomposed rapidly upon isolation, we took the advantage of using the acetonide protection system to protect the 1,2 amino alcohol moiety **3.6** by Commercon's method (79). The use of this protecting group circumvented the problem of the labile ethyl vinyl ether protecting group at C'-2. These methods had previously been



Scheme 5. Overall synthetic strategy of paclitaxel side chain

developed by other workers in our group, but they are recorded in detail here for the first time. The overall synthetic strategy was based upon the hypothesis that the aldehyde derived from alcohol would, under the appropriate conditions, undergo chelation-controlled carbonyl addition and produce preferentially the desired threo 1,2 amino alcohol derivative (Scheme 5).



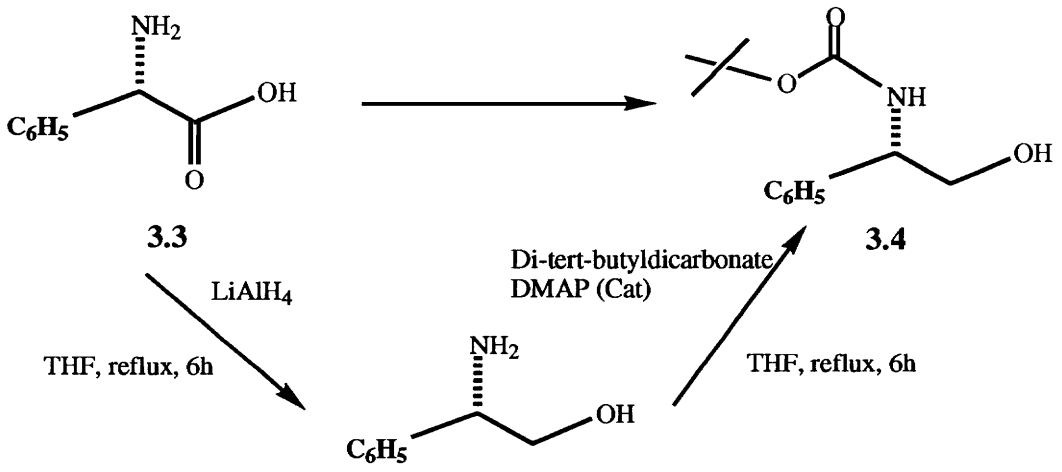
3.1 $R = C_6H_5$

3.2 $R = (CH_3)_3CO$

3.2.2 Synthesis

3.2.2.1 Amino Alcohol Derivative 3.4

Preparation of **3.4** was achieved by a one-pot operation which involved lithium aluminum hydride reduction followed by in situ derivatization (Scheme 6). Inexpensive, enantiomerically pure amino acid (S)-(+)-phenylglycine **3.3** was readily reduced to amino alcohol by $LiAlH_4$ after refluxing for 6 hours. This reaction mixture was immediately treated with di-tert-butyl dicarbonate and the resulting mixture was then refluxed for another 6 hours. Starting material was converted to the amino alcohol derivative **3.4** in 70% yield. The in situ treatment eliminated the necessity of isolating the polar amino alcohol, thus the yield was substantially improved. The ^1H-nmr spectrum (Figure 24) was consistent with the published data (78).

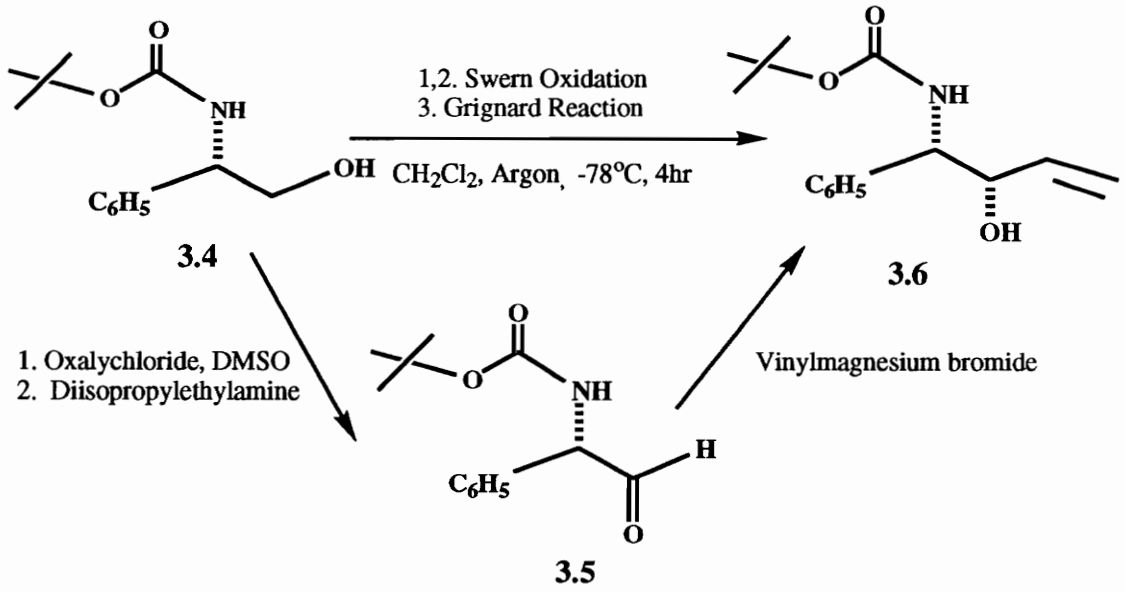


Scheme 6. Reduction and in situ derivatization of **3.3**

3.2.2.2 Olefin 3.6

Preparation of **3.6** was approached through the Swern oxidation (80, 81) and then the Grignard reaction (Scheme 7). This one-pot operation was the most crucial one in this multi-step synthesis of side chain , and required extremely dry and temperature controlled conditions. At -60°C, the amino alcohol derivative **3.4** was introduced to a stirred solution in which DMSO reacted with oxalyl chloride for 5 min at -78°C. The reaction mixture was treated with base (diisopropylethylamine) at -35°C to produce the required the amino aldehyde derivative as a Swern oxidation product. The carbonyl addition was carried out by adding the Swern oxidation mixture to the Grignard reagent (vinylmagnesium bromide) at -78°C, and then stirring at room temperature for 3 hours. Extended reaction time was employed to allow the reaction to proceed to completion. The pure amino alcohol derivative was obtained in 51% yield after chromatographic purification. Representative changes in the ^{1}H-nmr spectrum (Figure 25) of **3.6** relative to **3.4** were the appearance of three

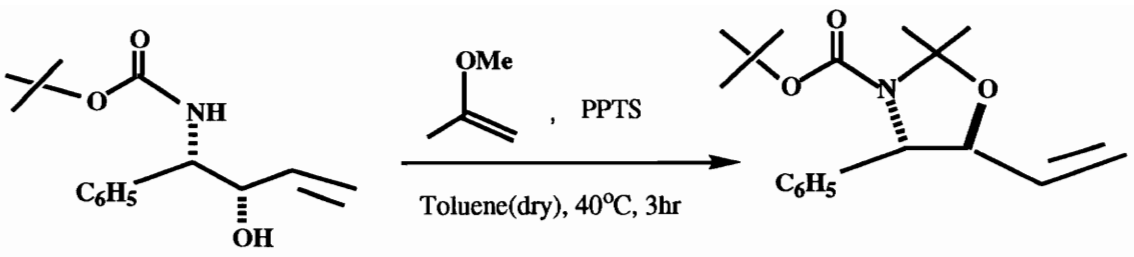
olefinic protons between 5.2 to 5.9 ppm, and the downfield shift of C-2' proton from 3.85 (2H) to 4.38 ppm (1H).



Scheme 7. Swern oxidation and Grignard reaction of **3.4**

3.2.2.3 Protected Olefin 3.7

In order to obtain acid from olefin, the oxidation reaction has to be performed. To carry out this oxidation reaction, the 1,2 amino alcohol moiety must be protected. The dimethyl ketal protection was found to be suitable protection (Scheme 8). Stirring compound **3.6** with 2-methoxypropene and a catalytic amount of pyridinium tosylate at 40°C, the reaction was completed in 2.5 hours. After simple work-up, the desired compound **3.7** was obtained in 87% yield. Characteristic data in ^1H nmr (Figure 26) were the disappearance of OH, NH protons and the appearance of the two methyl signals at 1.77, 1.75 ppm.

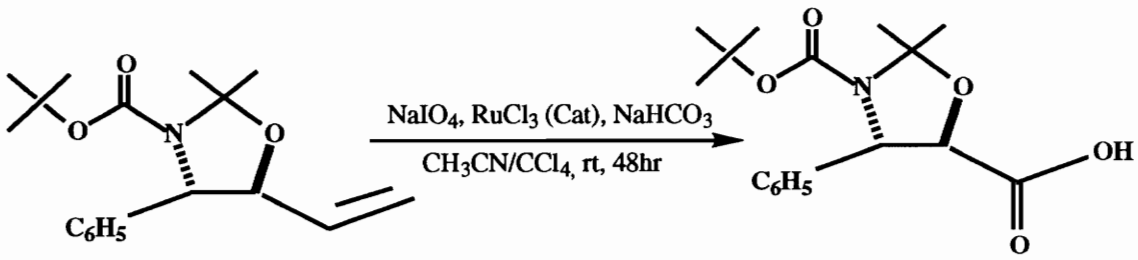


3.6 3.7

Scheme 8. Protection of **3.6**

3.2.2.4 Protected side chain acid 3.8

The side chain acid can be prepared via the double bond oxidation. Once the suitable olefin was obtained, the next step was to oxidize olefin to acid. The method available in the literature (78) used sodium periodate and a catalytic amount of ruthenium chloride in the presence of sodium bicarbonate (Scheme 9). Monitored by TLC, the oxidation reaction was completed in 24 hours in 83% yield. Illustrative changes in the ^1H nmr spectrum (Figure 27) of **3.8** were the appearance of the acidic OH proton at 9.2 ppm, and the disappearance of the three olefinic protons.



3.7 3.8

Scheme 9. Double bond oxidation of **3.7**

3.3 Experimental

AMINO ALCOHOL DERIVATIVE **3.4** — (S)-(+)-phenylglycine (5 g) was carefully added portionwise from the top of the condenser to a stirred mixture of 2.5 g $LiAlH_4$ in 100 ml of THF at reflux under argon. After the addition, the condenser was rinsed with 7 ml of THF, and the mixture was refluxed for an additional 6 h. Then the mixture was allowed to cool to room temperature and was slowly treated with 3 ml of 10% aqueous NaOH followed by 5 ml of H_2O and was stirred for 5 min. A solution of 7 g of di-tert-butyl dicarbonate and 100 mg of 4-(dimethylamino) pyridine in 40 ml of CH_2Cl_2 was introduced and the resulting mixture was refluxed for another 6 h. The mixture was allowed to cool to room temperature and was filtered through a pad of anhydrous Na_2SO_4, which then was washed with three portions of CH_2Cl_2. The solvents were dried by the rotavapor to give the crude product. This white yellow powder was crystallized from CH_2Cl_2-cyclohexane to give 5.5 g **3.4**. ^1H nmr: δ7.38-7.26 (m, 5H), 5.20 (brs, 1H), 4.78 (brs,1H), 3.85 (brs, 2H), 2.31 (brs, 1H), 1.45 (brs, 9H). The ^1H nmr spectrum of **3.4** is consistent with the literature data (78). Other data, see literature (78).

OLEFIN **3.6** — To a stirred solution of 4.05 ml (5.09 g) of oxalyl chloride in 50 ml dry CH_2Cl_2 at -78°C under argon was added 5 ml (5.5 g) of dimethyl sulfoxide (DMSO). The reaction mixture was stirred for 5 min at -78°C and was allowed to warm to -60°C over 20 min, then 5.5 g of alcohol **3.4** dissolved in 55 ml of CH_2Cl_2-DMSO (9: 1) was added over 15 min. The mixture was allowed to warm to -35°C over 20 min, at -35°C, stirred for 5 min and then treated over 4 min with 36 ml diisopropylethylamine. Removing the cooling bath for 5 min, then at -78°C (in cooling bath again), the mixture was added with a double-tipped needle to a room temperature solution 250 ml of vinylmagnesium bromide in 1: 1 THF-CH_2Cl_2. After stirring for 3 h, the mixture was treated with 20 ml

ethyl alcohol and 100 ml saturated aqueous NH_4Cl. The reaction solution was diluted with CH_2Cl_2, and acidified with dilute HCl, then washed with H_2O, brine, and dried with Na_2SO_4 anhydrous. Purification of the reaction product by silica gel chromatography with EtOAc / CH_2Cl_2 (5:95) gave 3.1 g **3.6**. ^1H nmr: δ7.38-7.25 (m, 5H), 5.94 (m, 1H), 5.38 (brs, 1H), 5.37 (m, 1H), 5.10 (m, 1H), 4.70 (brs, 1H), 4.38 (brs, 1H), 2.32 (brs, 1H), 1.48 (brs, 9H). The ^1H nmr spectrum of **3.6** is consistent with the literature data (78). Other data, see literature (78).

PROTECTED OLEFIN **3.7** — In a 100 ml round-bottom flask, 3.1 g **3.6** was dissolved in 10 ml dry toluene. 218 mg pyridinium p-toluene-sulfonate (pyridinium tosylate) and 8 ml 2-methoxypropene were added into the solution(exothermic). The reaction mixture was stirred at 40°C for 2 h 10 min. Diluted with EtOAc, the organic layer was separated, washed with H_2O and 5% $NaHCO_3$ aqueous solution, brine, dried over anhydrous Na_2SO_4 and evaporated. The crude reaction product was thus purified by column chromatography on silica gel and 3.1 g **3.7** was obtained as thick oil. Cims, eims and GC-MS did not yield an identifiable molecular ion. ^1H nmr: δ7.38-7.22 (m, 5H), 5.94 (m, 1H), 5.20 (m, 1H), 5.10 (m, 1H), 4.38 (brs, 1H), 4.22 (t, 1H), 1.77 (s, 3H), 1.75 (s, 3H), 1.01 (brs, 9H).

PROTECTED SIDE CHAIN ACID **3.8** — To a stirred mixture of 1.9 g **3.7** in 15 ml CH_3CN and 15 ml CCl_4, $NaHCO_3$ solution (4.25 g $NaHCO_3$ in 25 ml H_2O) was added, then 7.05 g $NaIO_4$ was added in small portions. After finishing the addition, the mixture was stirred for 5 min, and then was treated with 318 mg $RuCl_3$. Reaction was completed after 24 h stirring. Reaction solution was diluted by EtOAc. After discarding organic impurity, another 30 ml EtOAc was added into the aqueous layer, which was then acidified by dilute HCl. The organic phase was washed with brine, dried with anhydrous Na_2SO_4

and evaporated to give 1.75 g **3.8** (83%). Mp 135-138°C (lit. 137°C (79)), $[\alpha]_D$ + 5.2° (C=1.1, $CHCl_3$), Cims *m/z* $[MH]^+$ 322 (4), 309 (9), 278 (7), 266 (29), 250 (17), 222(100), 206 (16), 146 (9), 121 (8), 91 (12). ^1H nmr: δ9.20 (brs, 1H), 7.38-7.24 (m, 5H), 5.10 (brs, 1H), 4.60 (d, 5.7, 1H), 4.38 (brs, 1H), 1.77 (s, 3H), 1.75 (s, 3H), 1.05 (brs, 9H). These data are consistent with the literature data (79).

IV. THE DEVELOPMENT OF A SEMISYNTHETIC APPROACH TO NEW PACLITAXEL ANALOGS

4.1 Introduction

Because of paclitaxel's importance as an anticancer agent and its problems associated with solubility and availability, the preparation of new paclitaxel analogs is an important endeavor, especially in view of the fact that the semisynthetic paclitaxel analog Taxotere was reported to be approximately twice as active as paclitaxel in some assays (56). Over one hundred naturally occurring taxoids which possess an unusual tricyclic ring system have been isolated from various *Taxus* species, and many of these could serve as precursors to the semisynthesis of new paclitaxel analogs. Since synthetic A-nortaxol analogs showed interesting biological activity (28), these two new A-nortaxoids isolated by the author become important precursors for new paclitaxel derivatives. The presence of the oxetane ring at the C-4,(5) position which is essential for biological activity and the presence of numbers of hydroxyl groups which enable to improve water solubility make taxchinin C derivative **2.1** a very promising candidate for the synthesis paclitaxel like analogs.

4.2 Results and Discussion

4.2.1 Semisynthetic Strategy

Several groups have developed methods of coupling suitably protected baccatin III or 10-deacetylbaccatin III with 2'-O, 3'-N protected phenylisoserine side chain, thus provided various ways to prepare paclitaxel and its analogs. These approaches involved

different acylating agents, including β-lactam (82), (2R, 3S)-N-benzoyl-)-(1-ethoxyethyl)-3-phenylisoserine (83), oxazinone (84) and protected side chain acid **3.7** (79).

4.2.2 Protecting Strategy

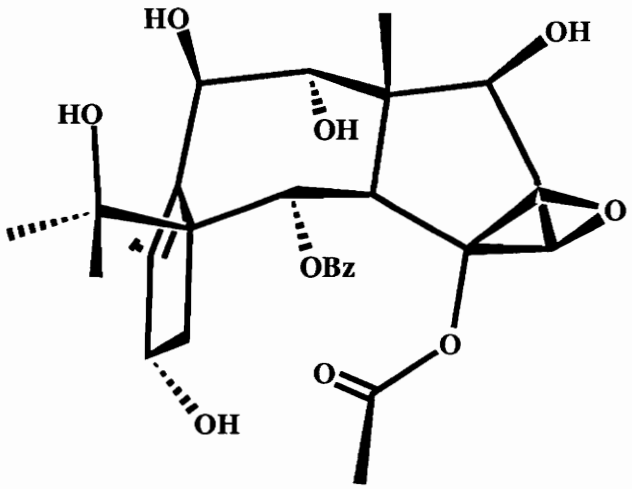


Figure 28. 3D-view of compound **2.1**

The C-13-hydroxyl group in baccatin III or 10-deacetyl baccatin III is highly hindered and it forms an intramolecular hydrogen bond with the C-4 acetate, thus the C-7 OH group is more reactive than the C-13 OH group (85). The acylation of baccatin III at the C-13 OH group can only be achieved if the C-7 OH group is suitably protected. In analogy with baccatin III, the newly isolated compound **2.1** possesses four secondary OH groups at C-7, C-9, C-10, C-13 positions, which make the selective protection of OH groups a very challenging task. The molecular modeling studies of this compound (Figure 28) revealed that it has a similar conformation to that of baccatin III. Therefore there is a need to protect the C-7, C-9, and C-10 hydroxyl groups before attempting acylation at the C-13 position. The C-7 and C-9 OH groups are a 1,3-diol and the C-9, C-10 OH groups

are a 1,2-diol, thus one may be able to selectively protect these groups as a cyclic acetal or ketal or cyclic may be able to selectively protect these groups as a cyclic acetal or ketal or a cyclic carbonate in one reaction. The remaining OH group could then be protected by suitable silyl protection, most probably with a triethylsilyl group.

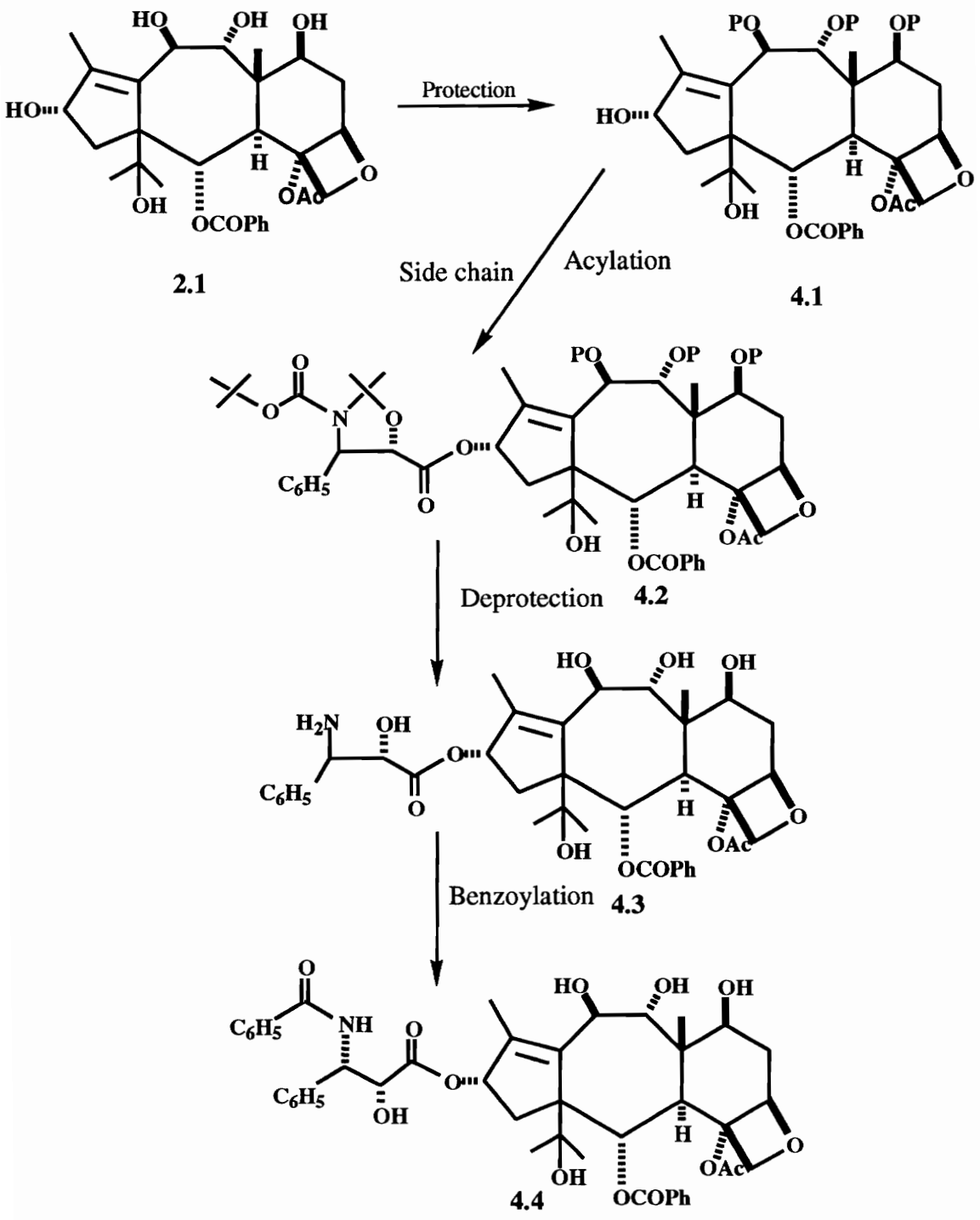
4.2.3 Semisynthetic Scheme

Once the suitably protected derivative **4.1** of compound **2.1** is available, acylation at the C-13 position to synthesize a new A-nortaxol analog could achieved by the proposed Scheme 10. With the side chain **3.7** in hand DCC / PP acylation of **4.1** would likely yield the desired coupled product **4.2**. Then, deprotection of **4.2** could be achieved by formic acid to yield **4.3**. Acylation of **4.3** at the C-3'-Nitrogen would then afford the desired new A-nortaxol analogs **4.4** (Scheme 10).

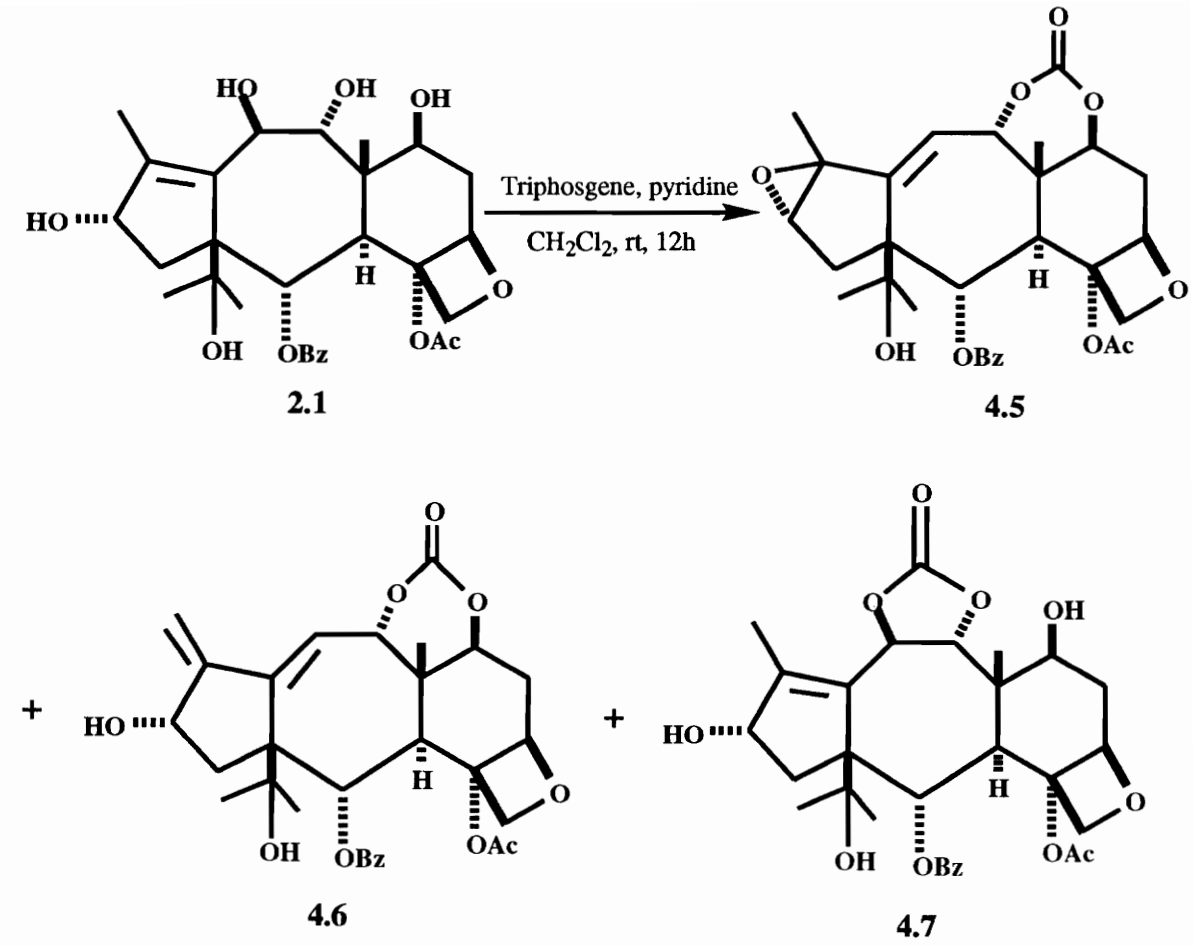
4.2.4 Protection of 2.1

With the "tied together" protection in mind, several protecting group were tried under various conditions. In a microgram-scale reaction, TLC analysis of reactions with 2-methoxypropene, 2,2-dimethoxypropane, and trichloroethylchloroformate showed several spots, thus, the selective protections were not successful.

In another attempt compound **2.1** was treated with triphosgene under basic conditions (pyridine) at room temperature over a period of 12 hours; three major spots were observed on the TLC plate. Isolation of these three compounds by PTLC afforded compounds **4.5, 4.6,** and **4.7** (Scheme 11).



Scheme 10. Proposed semisynthesis approach of new A-nortaxol analog



Scheme 11 Reaction with triphosgene

4.2.5 Proposed Structure Assignments of compound 4.5, 4.6, and 4.7

4.2.5.1 Compound 4.5

The distinct differences in the ^{1}H, ^{13}C nmr spectra (Figure 29, 30) of compound **4.5** relative to compound **2.1** were the evident downfield chemical shifts of the C-10 proton and carbon (δH 4.55 → 6.25 ppm, δC 68.7 → 125.0 ppm), and the upfield shift of

the olefinic C-12 carbon (δC 146.8 ppm → 72.0 ppm), which were consistent with the proposed structure for compound **4.5**. These assignments were further supported by the upfield shift of the C-13 proton (δH 4.58 → 4.09 ppm) due to the loss of the α-substituted olefin and the lower coupling constant between H-9 and H-10 (J = 10.4 → 3.97 ppm) caused by the reduction of the dihedral angle. The downfield shift of the C-7, C-9 protons resulted from the cyclic carbonate protection. Additional nmr data including TOCSY, DEPT, HETCOR, assignments are given in Table 6. Thus, compound **4.5** is proposed to have above structure.

4.2.5.2 Compound 4.6

The ^{1}H, ^{13}C-nmr spectra of compound **4.6** (Figure 34, 35) were very similar to those of **4.5**. The significant differences were the disappearance of one methyl signal and the appearance of two olefinic signals, which was confirmed by a DEPT (Figure 36) experiment. In addition to the same arguments as with **4.5** for the C-7, C-9 position and the coupling constants between H-9 and H-10, the structure assignment was supported by

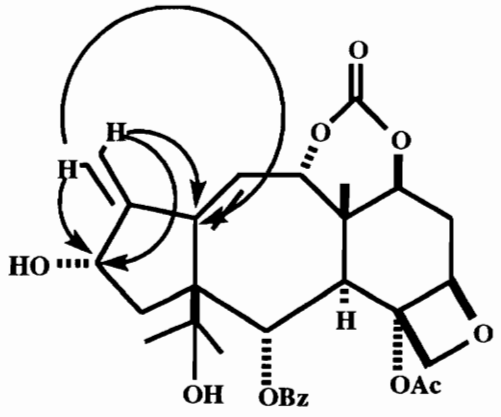


Figure 44. Key HMBC correlation of **4.6**

an HMBC correlation from olefin protons H-18 to C-11, C-13 (Figure 44). Additional nmr data are given in Table 7. Thus, compound **4.6** may be proposed to have the assigned structure.

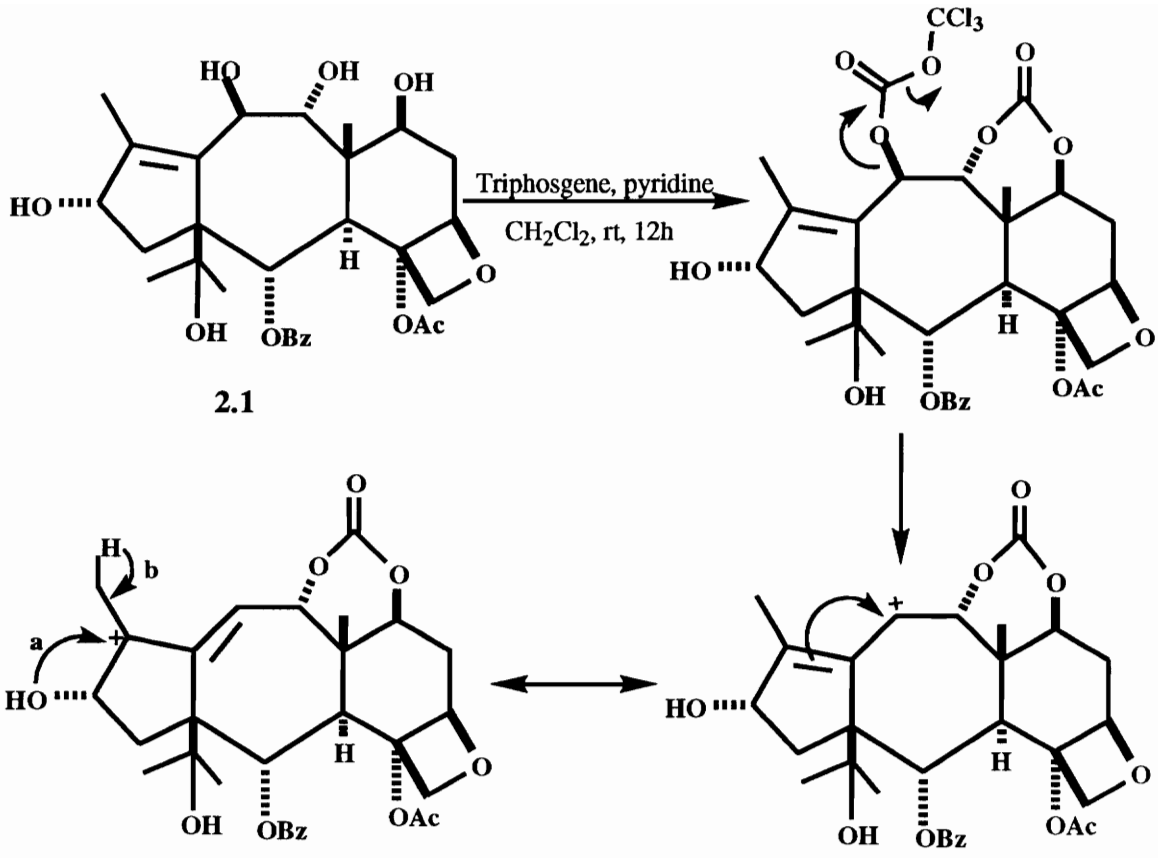
4.5.2.3 Compound 4.7

From the available nmr data of compound **4.7** (Figure 39 - 43) which is the most polar one of these three compounds, the structure of compound **4.7** may be proposed tentatively as a 9,10 cyclic carbonate which was supported by the downfield chemical shift of C-9 (δH 4.30 → 4.50 ppm), and C−10 (δH 4.55 → 5.58 ppm). Other nmr data (TOCSY, HETCOR, DEPT, and HMBC) of compound **4.7** seem agree with the proposed structure except a unexplainable strong methylene signal (δH 1.25, δC 29.6 ppm). Because of the shortage of this compound we can not make sure that whether this signal is due to impurity or not. Therefore, the structure of compound **4.7** is tentative only.

4.2.6 Possible Mechanism of Reaction

One possible mechanism for this reaction could be proposed as follows. It is assumed that cyclic carbonate protection first took place between C-7, C-9 diol. The protecting group formed at C-10 is a good leaving group, which can form a secondary allylic carbocation at C-10. This secondary carbocation has a more stable resonance form which has a tertiary carbocation. This tertiary cation generated at C-12 has two options open. It can undergo either nucleophilic attack by 13-OH to form epoxide **4.2** or proton loss to form olefin to get dienone **4.3** (Scheme 12).

A reaction in which epoxide formulation or proton loss and double bond migration is concerted with loss of the leaving group at C-10 is also possible.



Scheme 12 One possible mechanisms for the reaction of **2.1** with triphosgene

4.3 Experimental

PREPARATION OF COMPOUNDS **4.5**, **4.6**, AND **4.7** — Starting compound **2.1** (30 mg)was dissolved in dry CH_2Cl_2 (500 µl). After adding pyridine (250 µl), triphosgene was added during the stirring. The reaction mixture was stirred at room temperature for 12 h, then diluted with EtOAc, organic layer was separated, washed with H_2O, dilute HCl and brine, dried over Na_2SO_4 and evaporated. TLC showed three major spots with Rf values of 0.25, 0.51 and 0.80 (EtOAc: Hexane = 1: 1). The crude product

was purified on PTLC (EtOAc: Hexane = 1: 1). The fastest moving band (Rf 0.80) gave **4.5** (7.0 mg), the middle band (Rf 0.51) gave **4.6** (5.0 mg) and lower band (Rf 0.25) gave **4.7** (3.5 mg).

COMPOUND **4.5** — Mp 128 - 131°C; $[\alpha]^{25}_{D}$ +21° (c=10, $CHCl_3$); lrfabms did not yield an identifiable molecular ion. ^{1}H and ^{13}C nmr, see Table 6.

COMPOUND **4.6** — Mp 145 - 148°C; $[\alpha]^{25}_{D}$ -12° (c=10, $CHCl_3$); lrfabms did not yield an identifiable molecular ion. ^{1}H and ^{13}C nmr, see Table 7.

COMPOUND **4.7** — Mp 160 - 164°C, $[\alpha]^{25}_{D}$ +65° (c=10, $CHCl_3$); lrfabms did not yield an identifiable molecular ion. ^{1}H nmr 8.00 (d, 8.4, 2H), 7.62 (m, 1H), 7.48 (m, 2H), 6.01 (d, 7.7, 1H), 5.69 (d, 12.3, 1H), 4.92 (d, 8.2, 1H), 4.49 (d, 12.3, 1H), 4.38 (m, 3H), 4.16 (d, 7.9, 1H), 2.70 (m, 1H), 2.55 (d, 7.7, 1H), 2.25 (d, 9, 1H), 2.17 (s, 3H), 2.09 (d, 9.4, 1H), 1.98 (s, 3H), 1.89 (s, 3H), 1.84 (m, 1H), 1.45 (d, 8.6, 1H), 1.25 (s, 2H), 1.12 (s, 3H), 1.08 (s, 3H); ^{13}C nmr 169.8, 165.6, 152.3, 151.0, 134.0, 132.4, 129.6, 129.1, 128.8, 128.6, 86.9, 84.6, 81.3, 78.8, 78.4, 75.0, 74.0, 70.8, 69.2, 64.1, 47.8, 45.7, 41.4, 35.8, 29.6, 26.6, 25.9, 21.7, 13.8, 12.6.

Table 6. Nmr data of compound **4.5**

Position	1_Ha	13_Cb	TOCSY	HMBC
1		66.1		
2	5.91 (1H, d, 7.6)	63.5	H-3	C-3, C-8, C-14, C-15, O<u>C</u>OC_6H_5
3	3.10 (1H, d, 7.6	38.9	H-2	C-1, C-4, C-5, C-7, C-8, C-19, C-20
4		79.1		
5	5.02 (1H, brd, 8.4)	84.1	H-6α	C-3
6	α 2.02 (1H, m) β 2.74 (1H, m)	33.4	H-5, H-6β, H-7 H-6α, H-7	C-8
7	4.55 (1H, brt, 8.7)	76.4	H-6α, H-6β	C-19
8		44.8		
9	4.96 (1H, d, 3.97)	84.4	H-10	
10	6.25 (1H, d, 3.97)	125.0	H-9	
11		150.5		
12		73.4		
13	4.09 (1H, brt)	81.1	H-14α , H-14β	C-11
14	α 2.28 (1H, dd, 15, 7.4) β 2.48 (1H, dd, 15, 7.4)	35.5	H-14α , H-13 H-14β , H-13	C-1, C-2, C-13, C-15, C-18
15		81.6		
16	1.04 (3H, s)	23.6		C-1, C-17
17	0.96 (3H, s)	24.9		C-1, C-16
18	1.78 (3H, s)	25.4		C-11, C-13
19	1.88 (3H, s)	12.6		C-3, C-7, C-8, C-9
20	α 4.22 (1H, d, 8.0)[c] β 4.32 (1H, d, 8.0)	72.0	H-20β H-20α	C-3
OCOC<u>H</u>3	2.21(3H,s,)	21.5		O<u>C</u>OCH3
O<u>C</u>OCH3		169.5		
O<u>C</u>OC_6H_5		165.9		
Ar i		128.0		
o	8.02 (2H, d, 7.2)	128.9	m-Ar	O<u>C</u>OC_6H_5
m	7.49 (2H, m)	129.7	o-Ar, p-Ar	
p	7.66 (1H, m)	134.2	m-Ar	
C=O		146.9		

a. Multiplicity and apparent coupling constant(s) in Hz in parentheses.

b. ^{13}C assignments of protonated carbons were confirmed by a HETCOR experiment, and carbon types were assigned by a DEPT experiment.

c. The assignments of 20α and 20β were made by comparison with data for taxol (68); the assignments for taxchinin B (36) are reversed.

Table 7. Nmr data of compound **4.6**

Position	^{1}H[a]	^{13}C[b]	TOCSY	HMBC
1		65.6		
2	5.99 (1H, d, 7.8)	63.9	H-3	C-3, C-4, C-8, C-15, O<u>C</u>OC$_6$H$_5$
3	3.01 (1H, d, 7.8)	44.3	H-2	C-1, C-2, C-4, C-7, C-8, C-19, C-20
4		84.0		
5	5.00 (1H, brd, 8.4)	85.1	H-6α	C-4, C-7
6	α 2.04 (1H, m) β 2.73 (1H, m)	33.4	H-5, H-6β, H-7 H-6α, H-7	C-7, C-8
7	4.75 (1H, brt, 8.8)	76.3	H-6α, H-6β	C-19
8		38.9		
9	5.10 (1H, d, 3.7)	85.0	H-10	
10	6.10 (1H, d, 3.7)	116.3	H-9	
11		142.5		
12		146.4		
13	4.46 (1H, brt)	77.5	H-14α , H-14β	
14	α 1.40 (1H, dd, 10.1, 2.0) β 2.44 (1H, dd, 10.1, 2.0)	37.8	H-14α , H-13 H-14β , H-13	C-11, C-12,C-13, C-15
15		80.4		
16	1.26 (3H, s)	24.9		C-17
17	0.99 (3H, s)	25.8		C-16
18	5.09 (1H, s) 5.26 (1H, s)	104.4		C-11, C-13
19	1.87 (3H, s)	12.6		
20	α 4.20 (1H, d, 8.1)[c] β 4.38 (H, d, 8.1)	73.9	H-20β H-20α	C-4
OCOC<u>H</u>$_3$	2.20 (3H, s)	21.6		O<u>C</u>OCH$_3$
O<u>C</u>OCH$_3$		169.7		
O<u>C</u>OC$_6$H$_5$		165.9		
Ar i		128.9		
o	8.02 (2H, d, 7.2)	128.8	m-Ar	O<u>C</u>OC$_6$H$_5$
m	7.49 (2H, m)	129.7	o-Ar, p-Ar	
p	7.64 (1H, m)	134.0	m-Ar	
C=O		147.0		

a. Multiplicity and apparent coupling constant(s) in Hz in parentheses.

b. ^{13}C assignments of protonated carbons were confirmed by a HETCOR experiment, and carbon types were assigned by a DEPT experiment.

c. The assignments of 20α and 20β were made by comparison with data for taxol (68); the assignments for taxchinin B (36) are reversed.

V. CONCLUSIONS AND OUTLOOK

An investigation of *Taxus brevifolia* extracts afforded two new taxoids with the uncommon brevifoliol (or taxchinin A) skeleton. The structures were elucidated by spectral methods and in particular by comparison of nmr data with those of taxchinin C and brevifoliol. Extensive 2D nmr techniques were used. HMBC was the most useful technique in confirming the presence of the ring contracted A-nortaxol compounds. The synthesis of a protected paclitaxel side chain was completed by a modification of literature methods, and an approach to the synthesis of a novel A-nortaxol analog from one of the newly isolated compounds has been developed. The reaction of 7, 13-deacetyl-9,10-debenzoyl taxchinin C **2.1** with triphosgene yielded three compounds. The structures of two of them were proposed on the basis of spectral data (1D and 2D nmr), and the structure of the third compound is tentative only.

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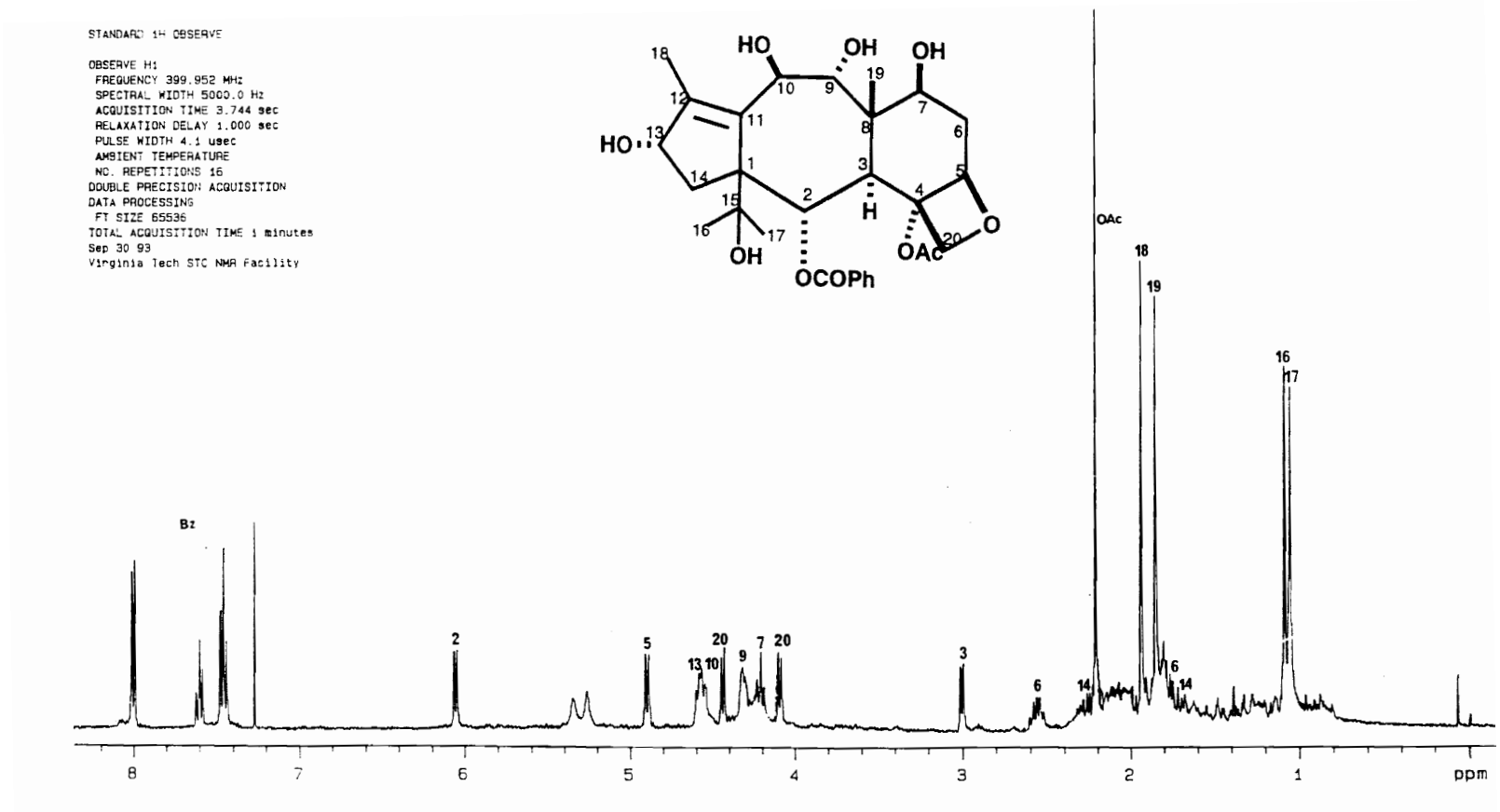


Figure 5. ^{1}H-NMR spectrum of compound **2.1**

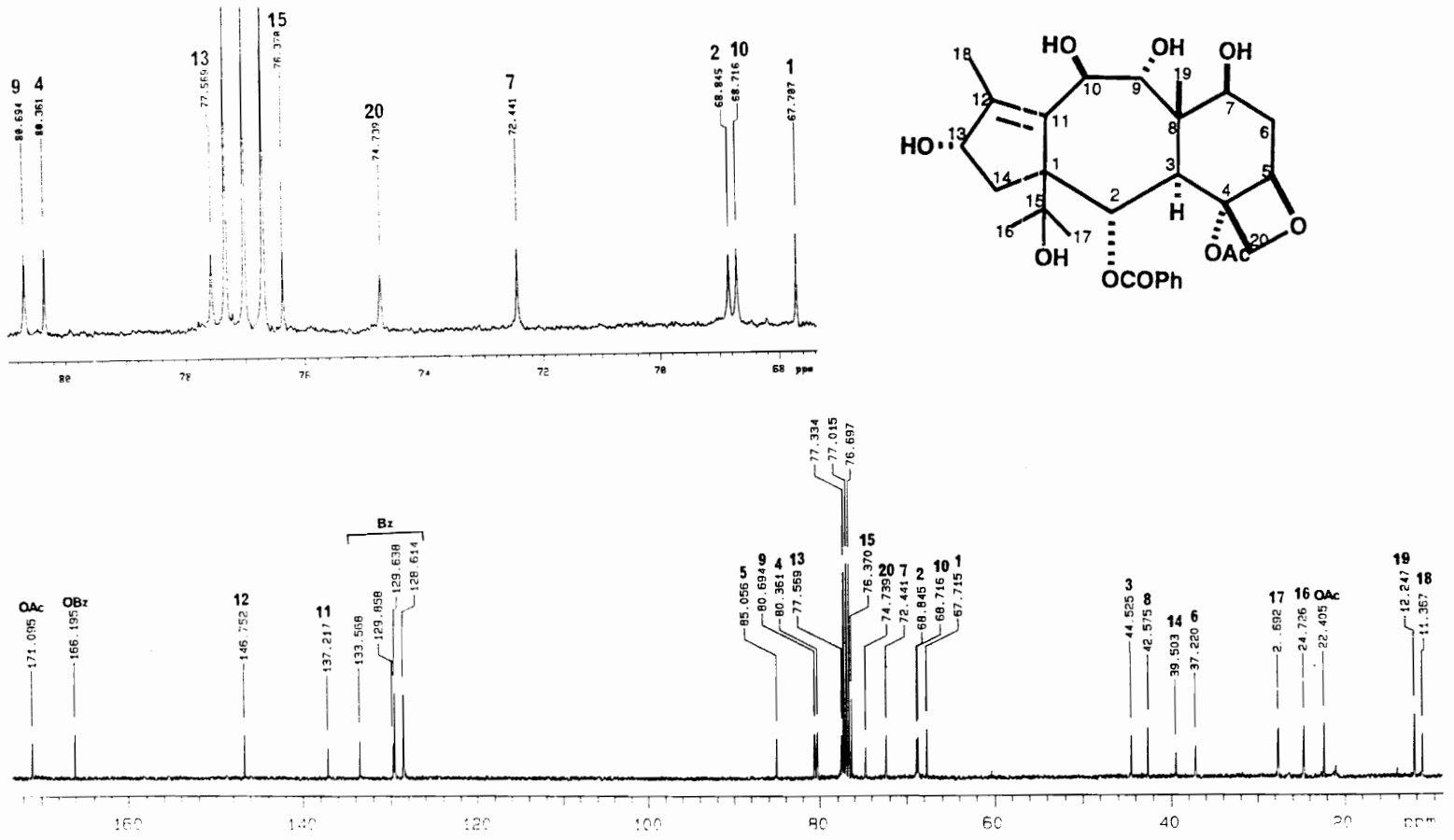
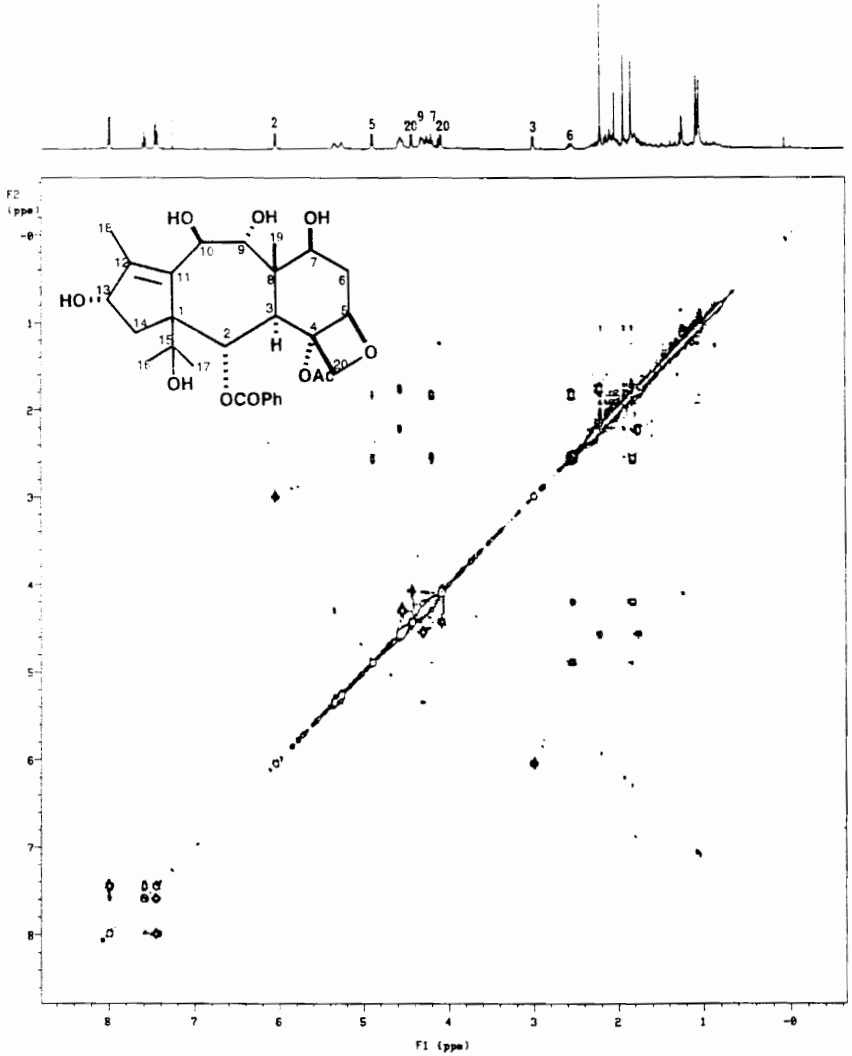


Figure 6. ^{13}C-NMR spectrum of compound **2.1**



STANDARD 1H OBSERVE

exp2 pulse sequence: tocsy

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date	Oct 5 93	dn	H1	array	phase
solvent	CDCl3	dof	0	arraydim	500
file	exp	da	nnn		
ACQUISITION		dmm	c	1	phase
sfrq	399.952	def	200	1	1
tn	H1	dpwr	30	2	2
at	0.136	homo	y		
np	1024	PROCESSING			
sw	3766.5	sb	0.102		
fb	2100	sbs	-0.068		
bs	16	wtfile			
ss	64	proc	ft		
tpwr	50	fn	1024		
pw	36.0	math	f		
pllvl	56				
pl	18.0	werr			
p2	undefined	wexp			
d1	1.000	wba			
presat	0	wnt			
mix	0.015	2D PROCESSING			
trim	0.002e	sb1	0.050		
tof	-800.3	sbs1	-0.033		
nt	1	wtfile1			
ct	1	proc1	ft		
alock	n	fn1	1024		
gain	14	DISPLAY			
FLAGS		sp	-258.1		
il	n	wp	3766.5		
in	n	vs	100		
dp	y	sc	80		
hs	nn	wc	140		
aspul	y	hzmm	26.90		
2D ACQUISITION		is	500.00		
sw1	3766.5	rf1	258.1		
ni	250	rfp	0		
phase	arrayed	th	4		
2D DISPLAY		ins	1.000		
sp1	-258.1	ai cdc ph			
wp1	3766.5				
sc2	0				
wc2	140				
rf11	258.1				
rfp1	0				

Figure 7. TOCSY spectrum of compound **2.1**

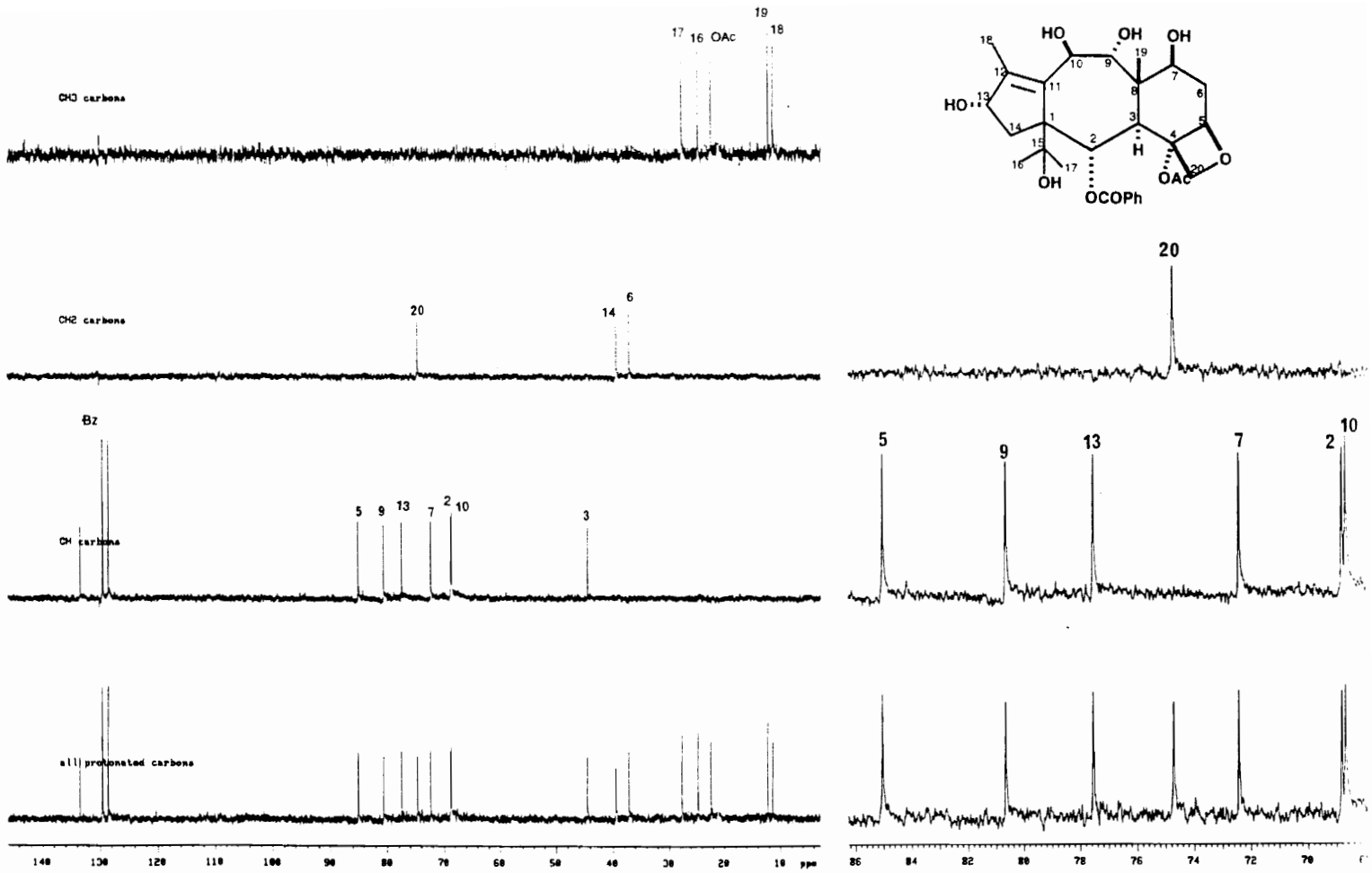


Figure 8. DEPT spectrum of compound **2.1**

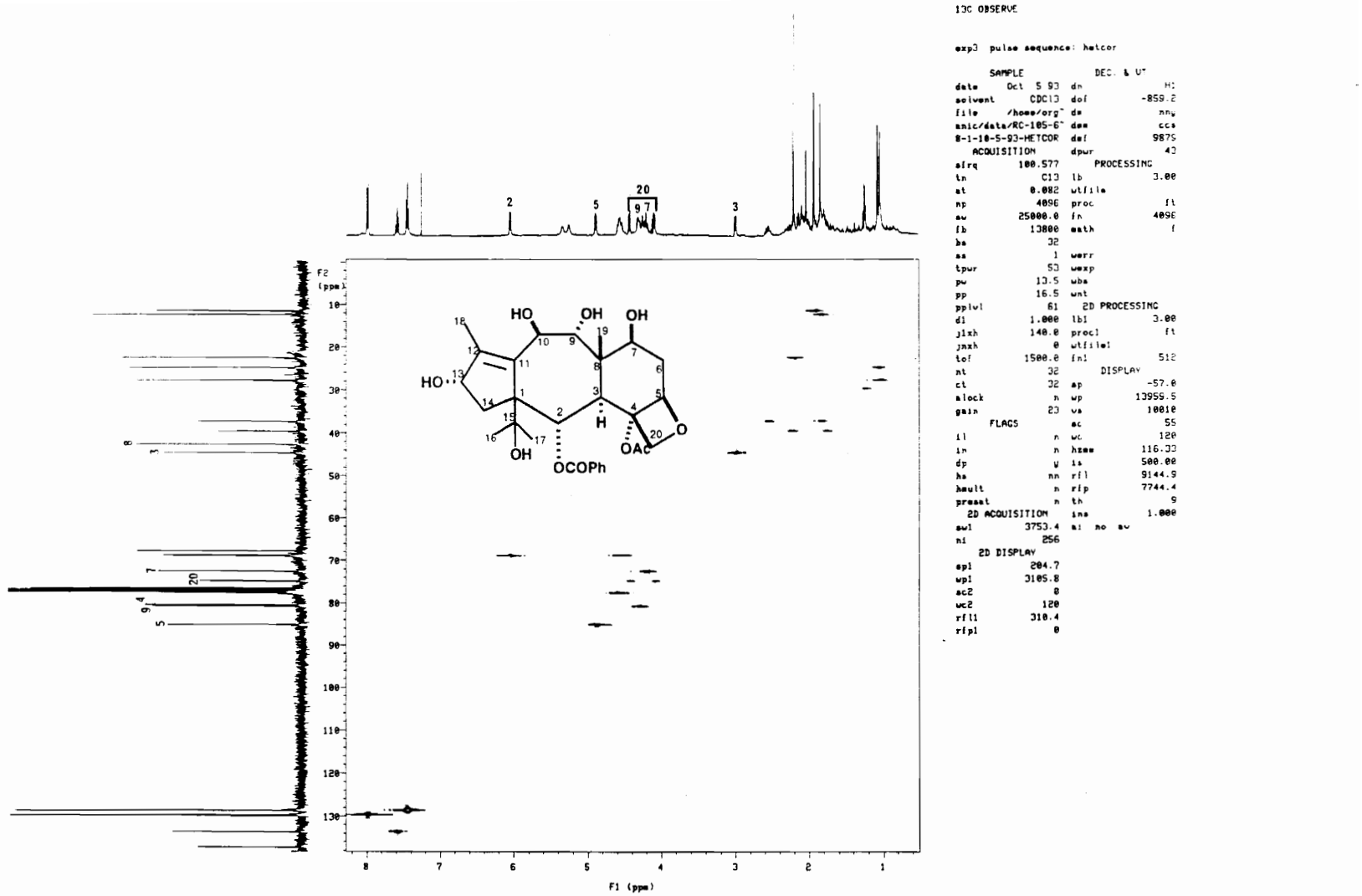


Figure 9. HETCOR spectrum of compound **2.1**

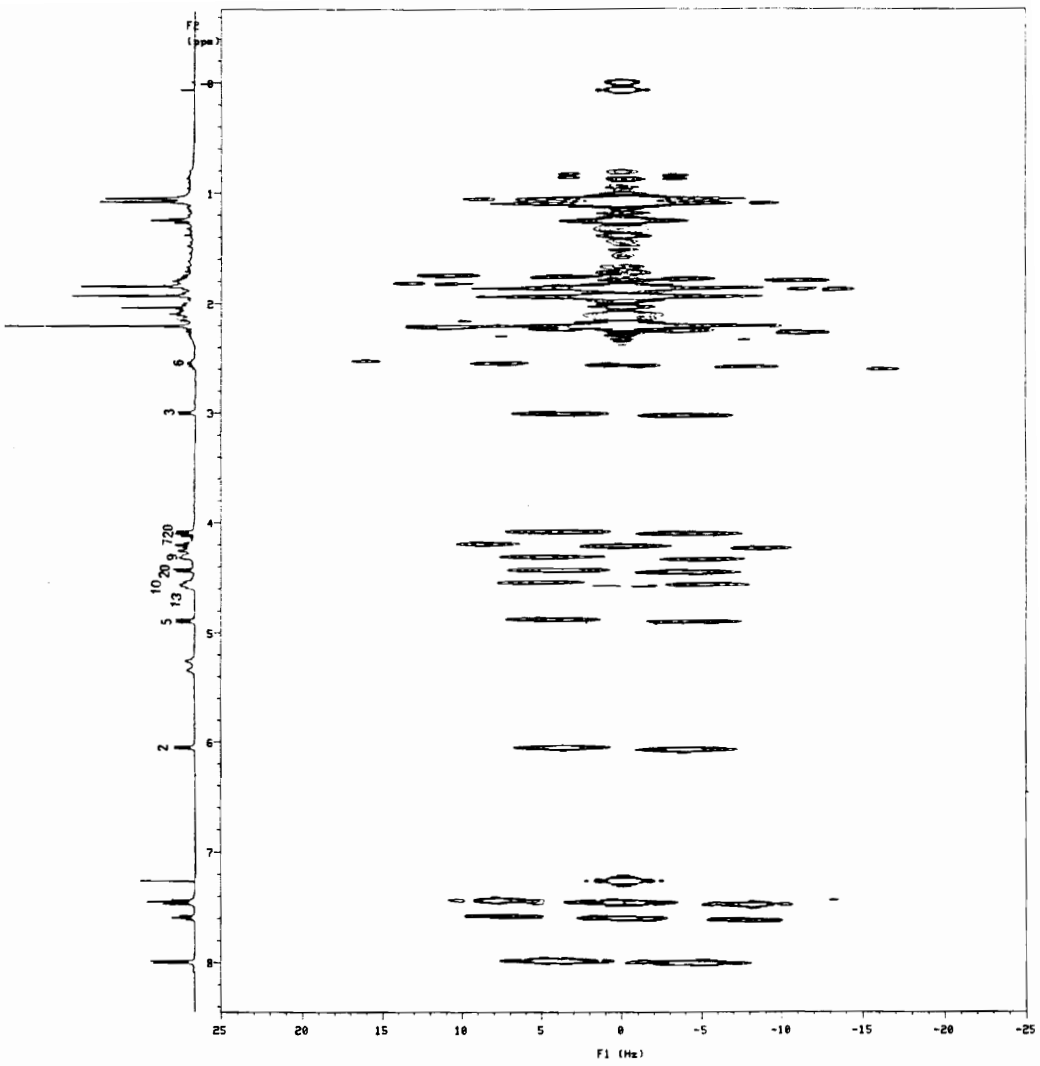


Figure 12. HOMO2DJ spectrum of compound **2.1**

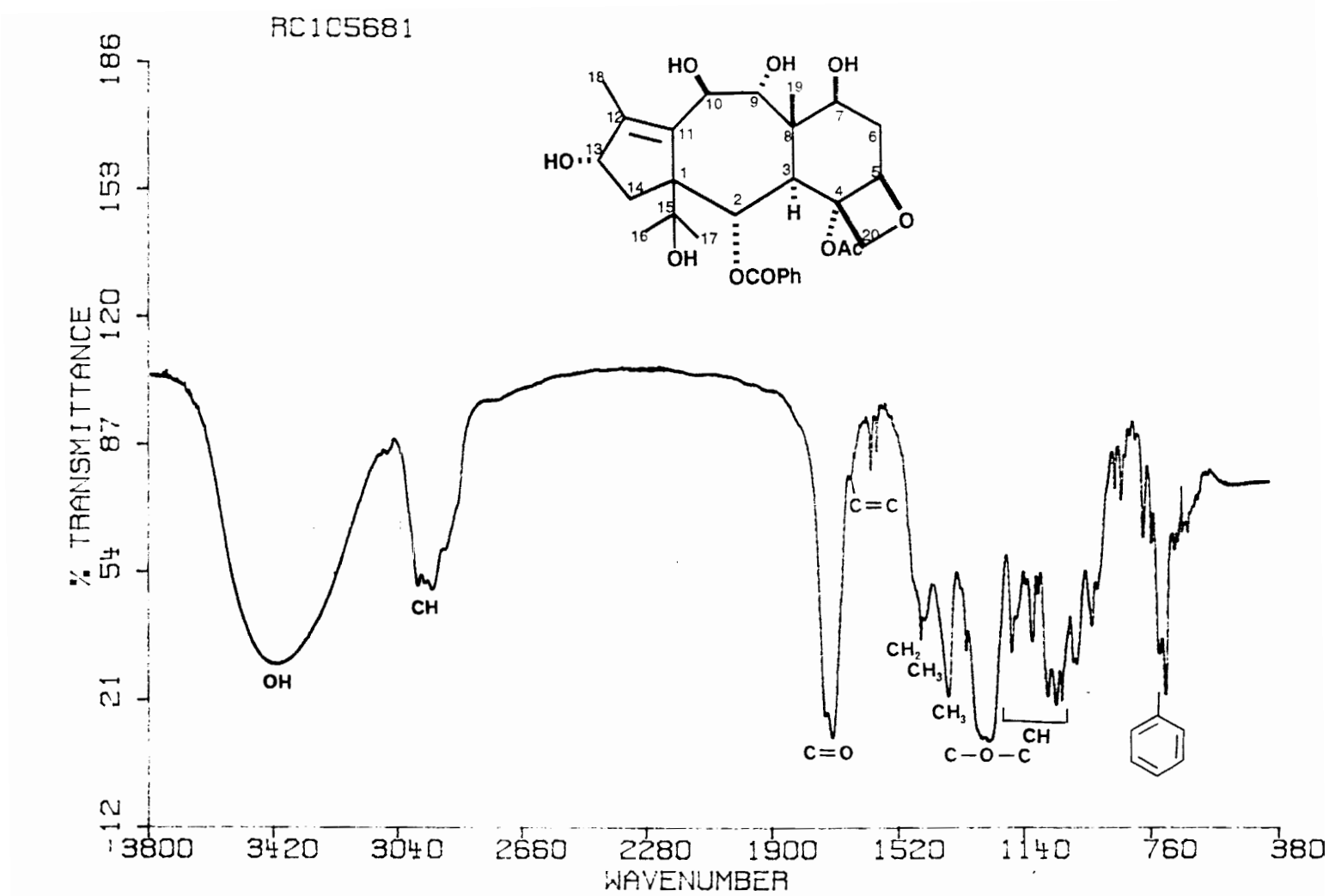


Figure 13. FT-IR spectrum of compound **2.1**

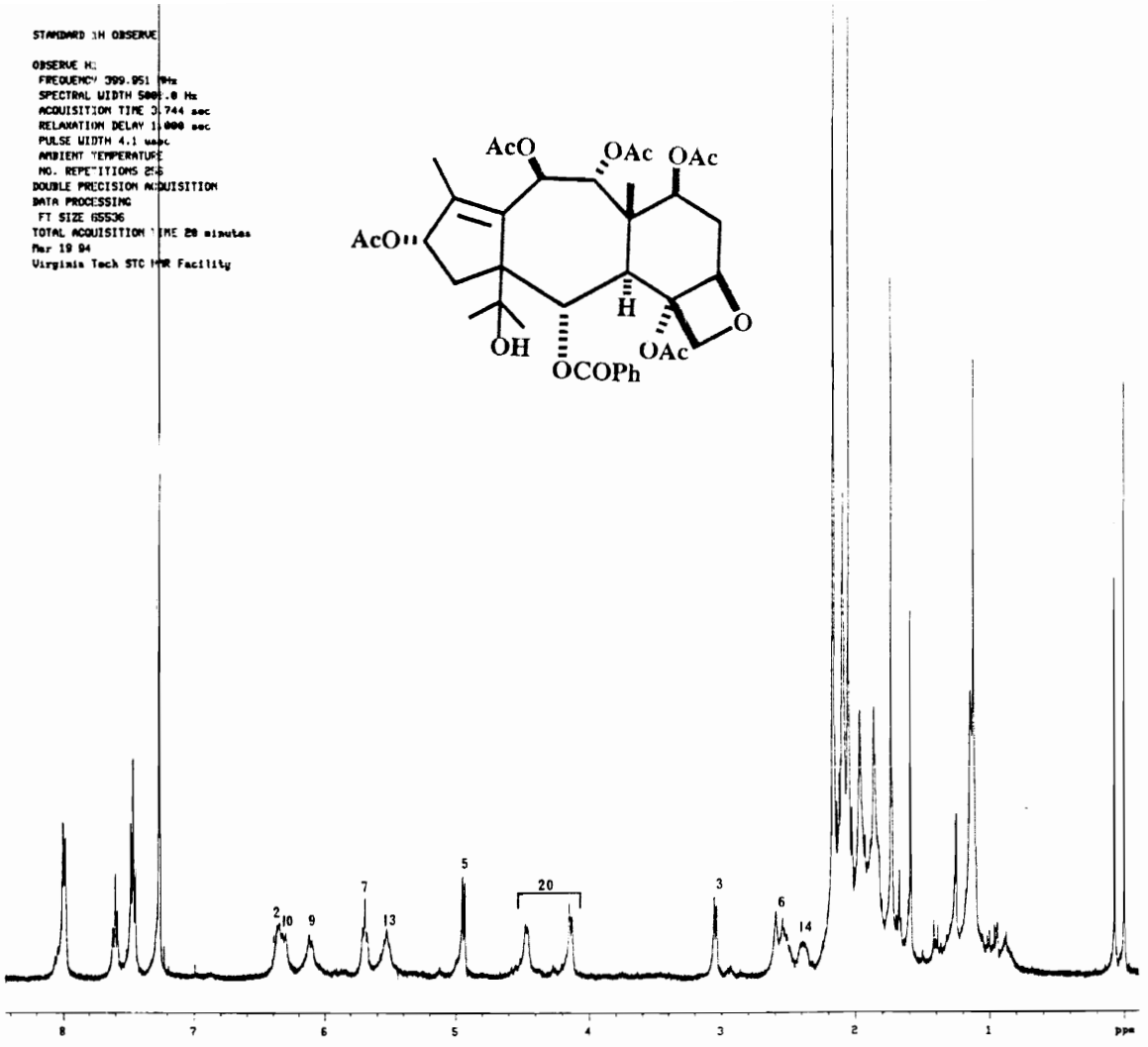


Figure 14. ^{1}H-NMR spectrum of compound **2.3**

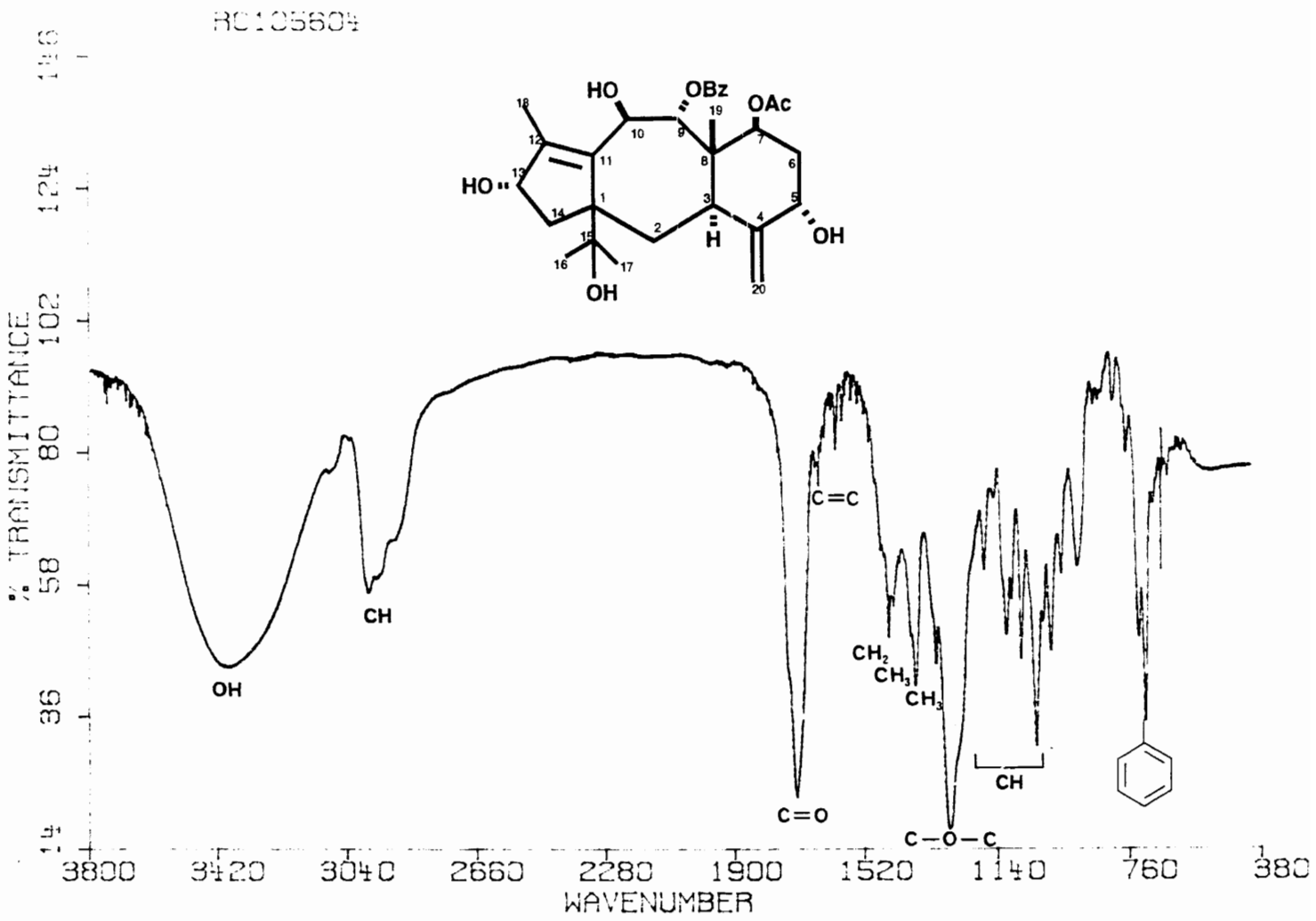


Figure 15. FT-IR spectrum of compound **2.4**

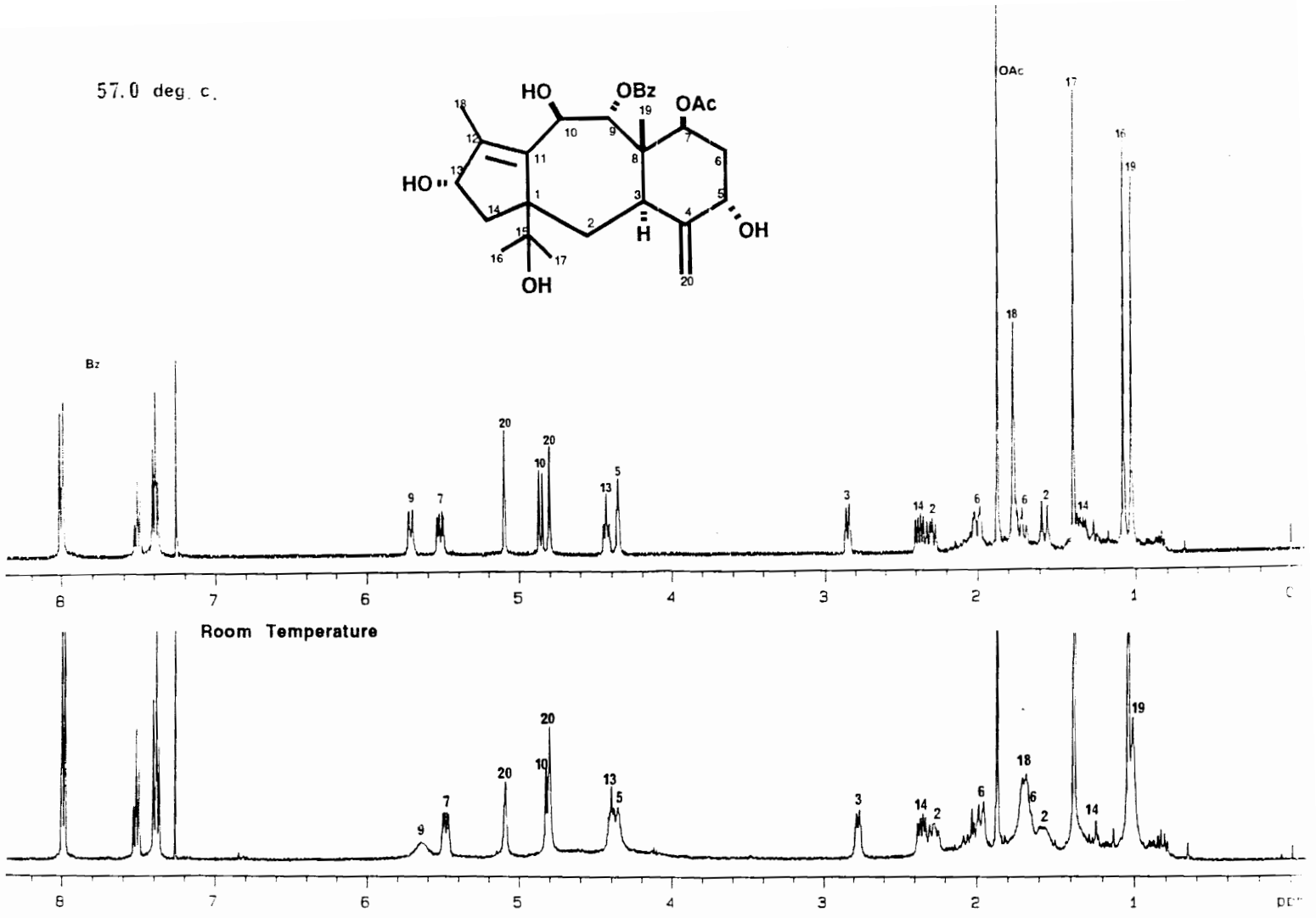


Figure 16. ^{1}H-NMR spectrum of compound **2.4**

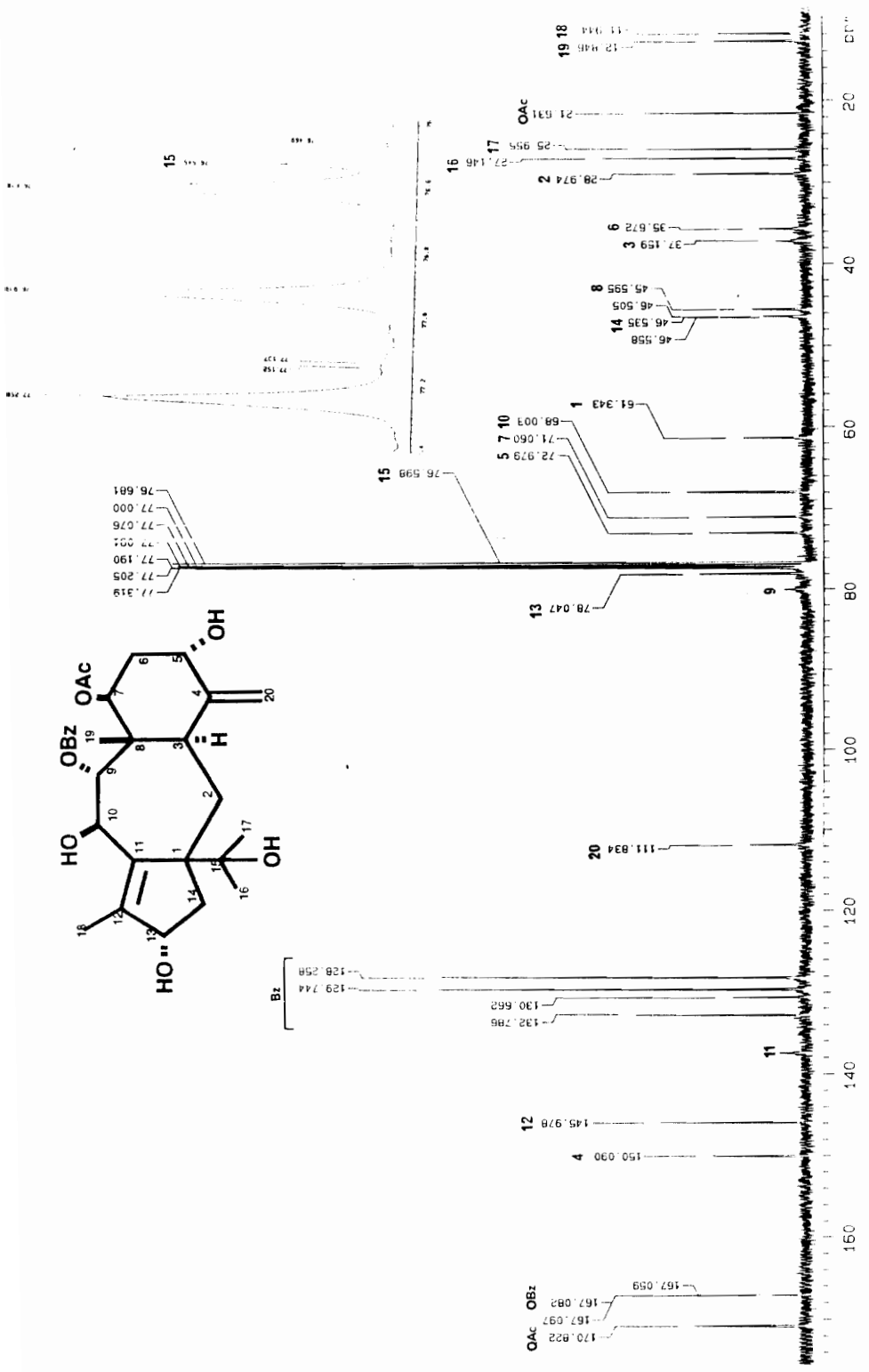
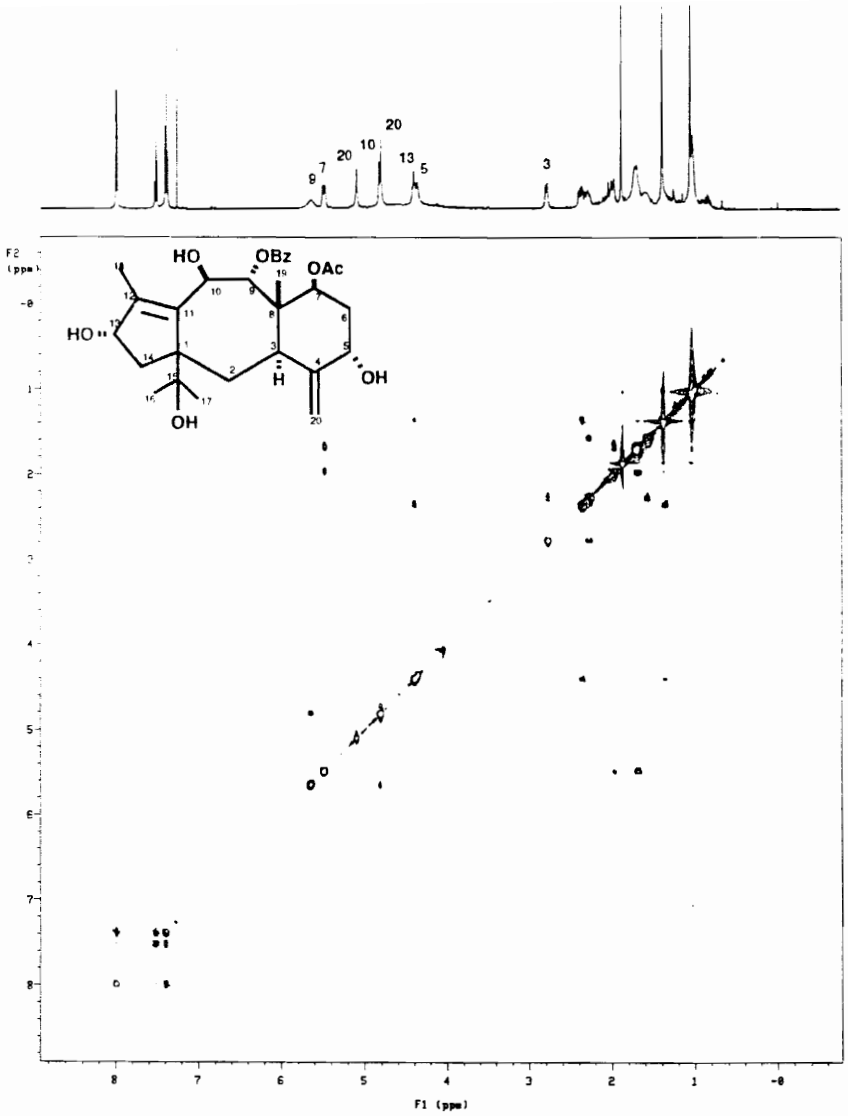


Figure 17. ^{13}C-NMR spectrum of compound **2.4**



RC-105-60-4

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solvent	CDCl3	dof	0	arraydim	512
file	exp	dm	nnn		
ACQUISITION		dmm	c	1	phase
sfrq	399.952	dmf	200	1	1
tn	H1	dpwr	30	2	2
at	0.132	homo	y		
np	1024	PROCESSING			
sw	3867.0	lb	0.095		
fb	2200	lbs	-0.066		
bs	16	wtfile			
ss	32	proc	ft		
tpwr	50	fn	1024		
pw	36.0	math	f		
pl1w1	56				
pl	18.0	werr			
p2	undefined	wexp			
d1	0.600	wbs			
presat	0	wnt			
mix	0.012	2D PROCESSING			
trim	0.0020	lb1	0.049		
tof	-802.5	lbs1	-0.033		
nt	1	wtfile1			
ct	1	proc1	ft		
alock	n	fn1	1024		
gain	8	DISPLAY			
FLAGS		sp	-310.5		
il	n	wp	3867.0		
in	n	vs	125		
dp	y	sc	80		
hs	nn	wc	140		
aspul	y	hzmm	27.62		
2D ACQUISITION		is	393.06		
sw1	3867.0	rf1	310.5		
ni	256	rfp	0		
phase	arrayed	th	4		
2D DISPLAY		ins	1.000		
sp1	-310.5	ai cdc ph			
wp1	3867.0				
sc2	0				
wc2	140				
rf11	310.5				
rfp1	0				

Figure 18. TOCSY spectrum of compound **2.4**

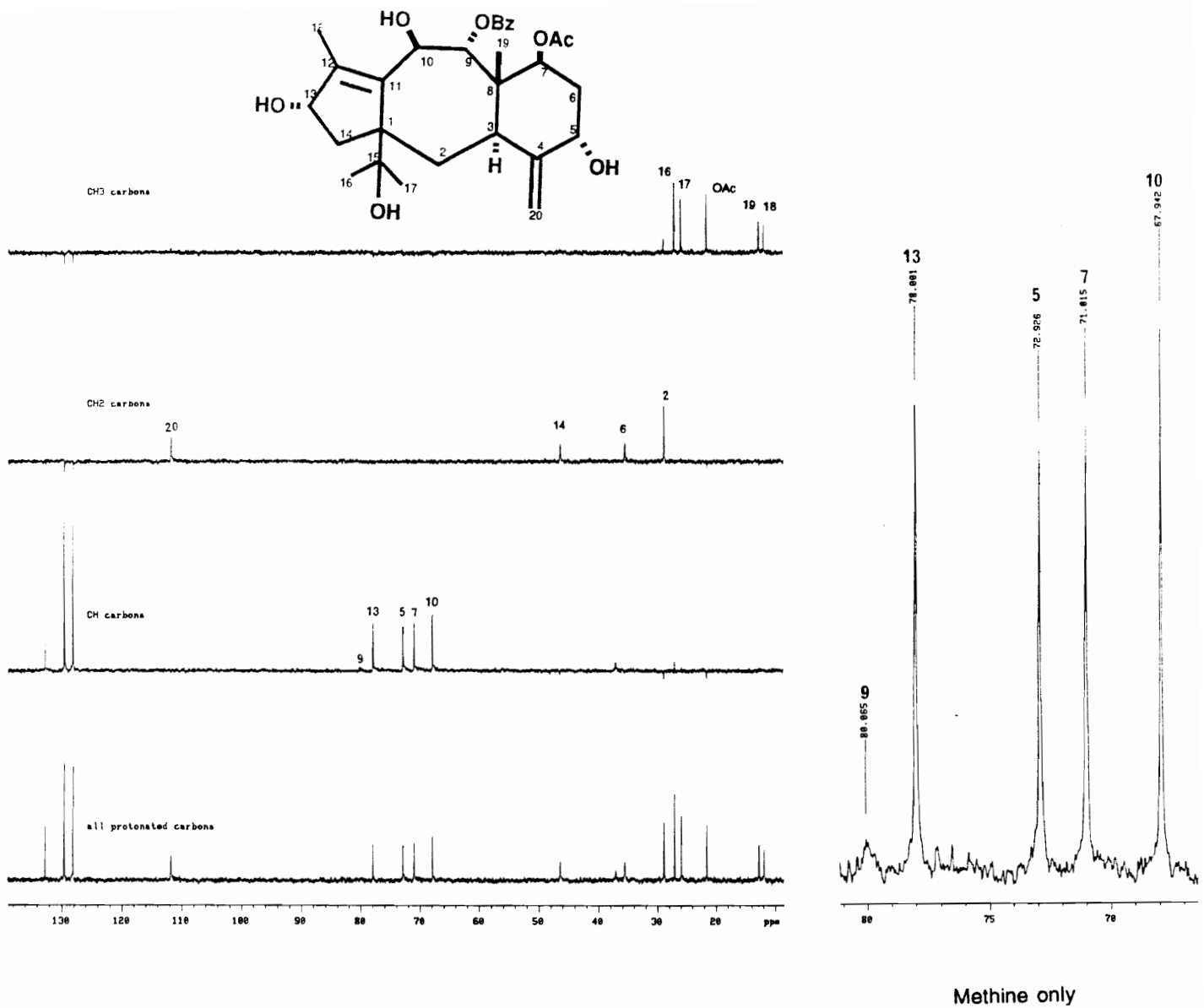

Figure 19. DEPT spectrum of compound **2.4**

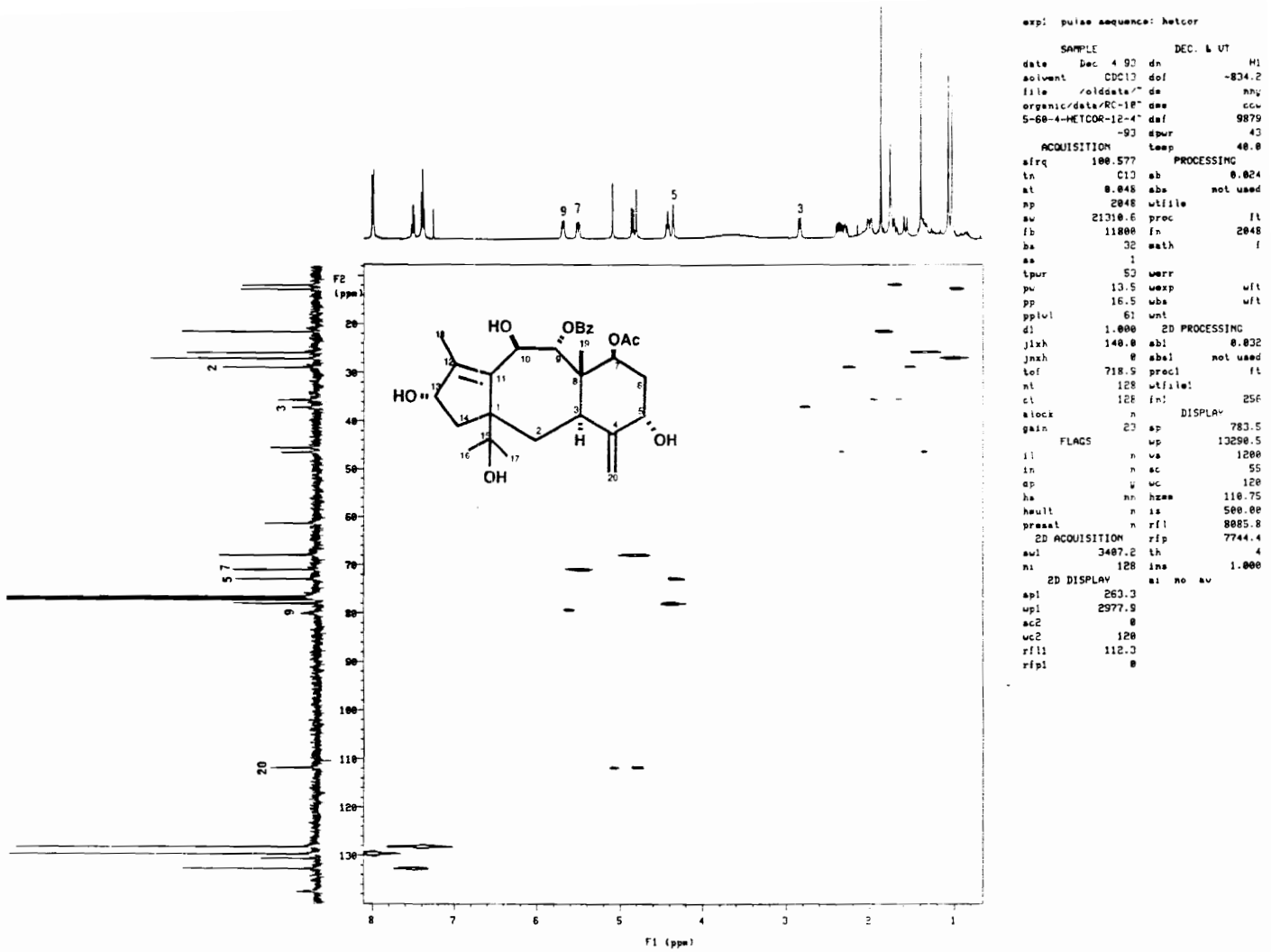

Figure 20. HETCOR spectrum of compound **2.4**

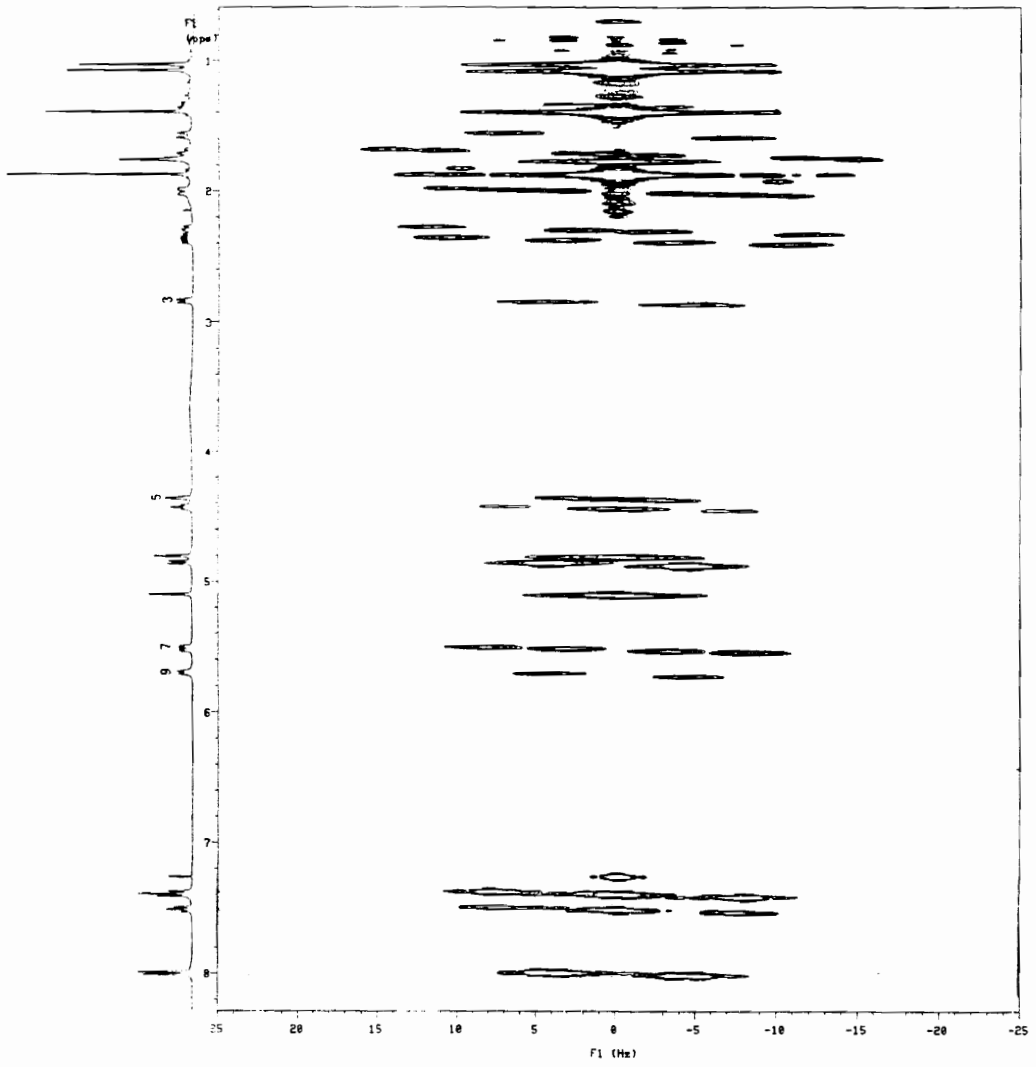

Figure 22. HOMO2DJ spectrum of compound **2.4**

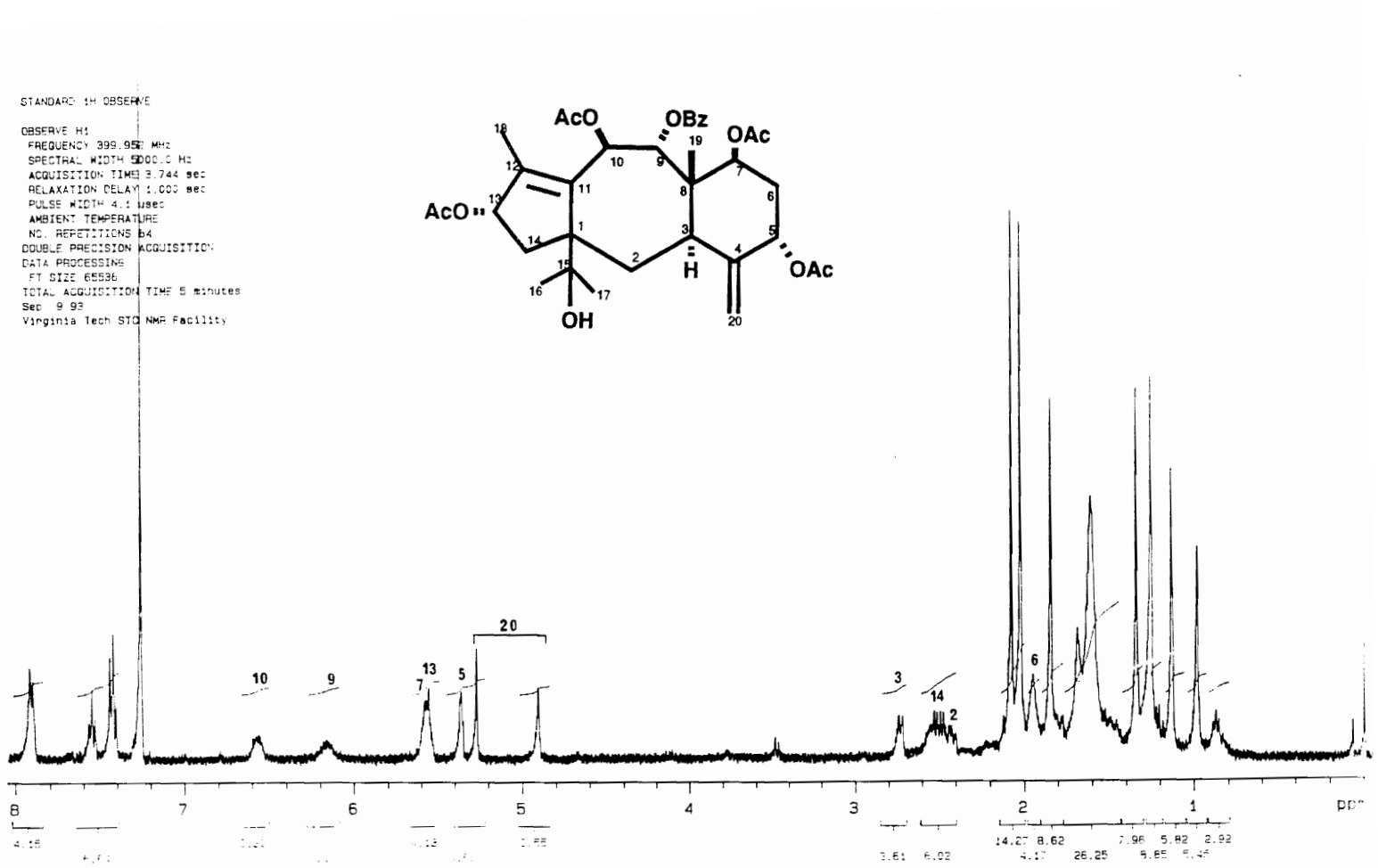

Figure 23. ^{1}H-NMR spectrum of compound **2.8**

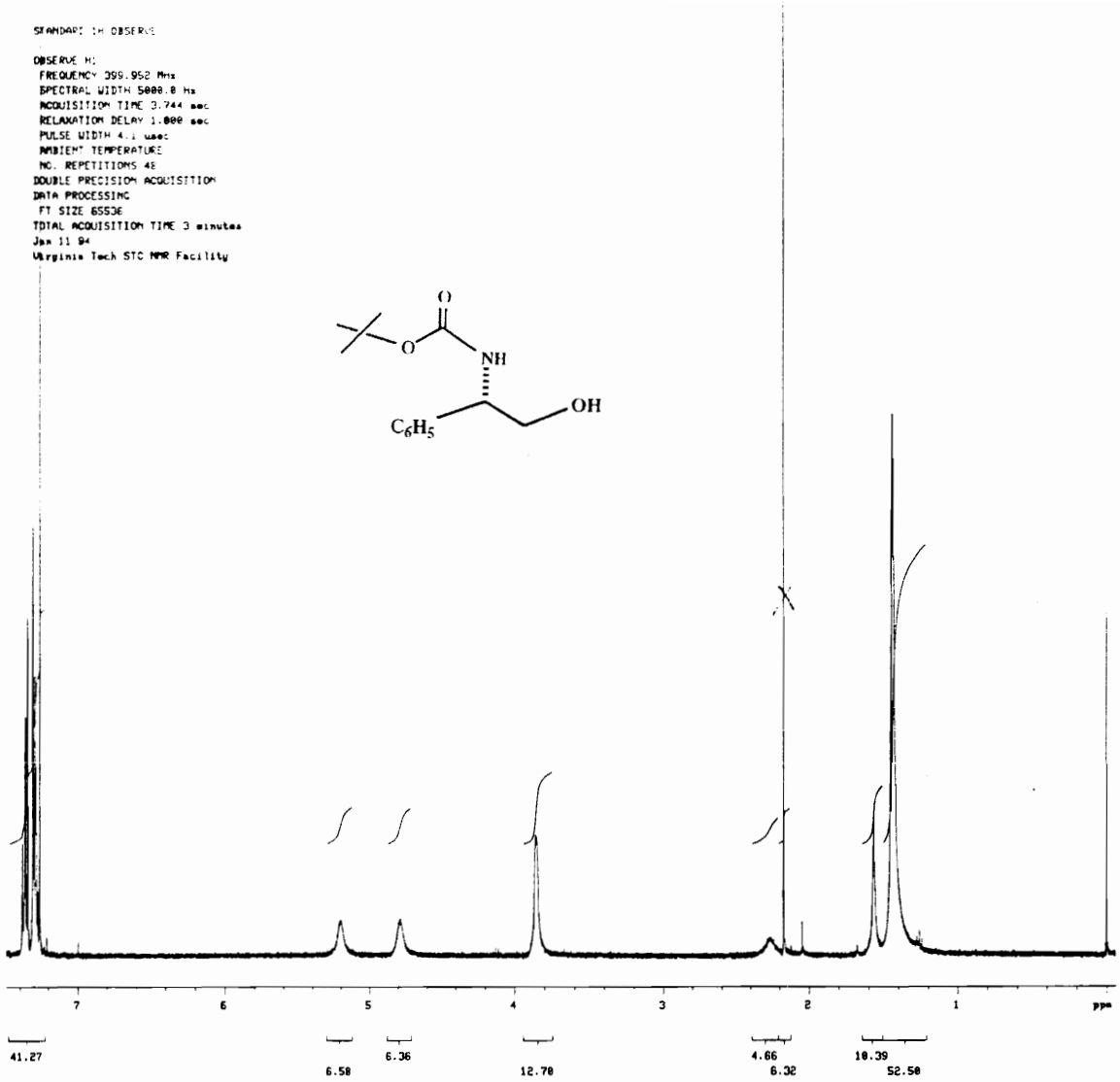

Figure 24 ^{1}H-NMR spectrum of compound **3.4**

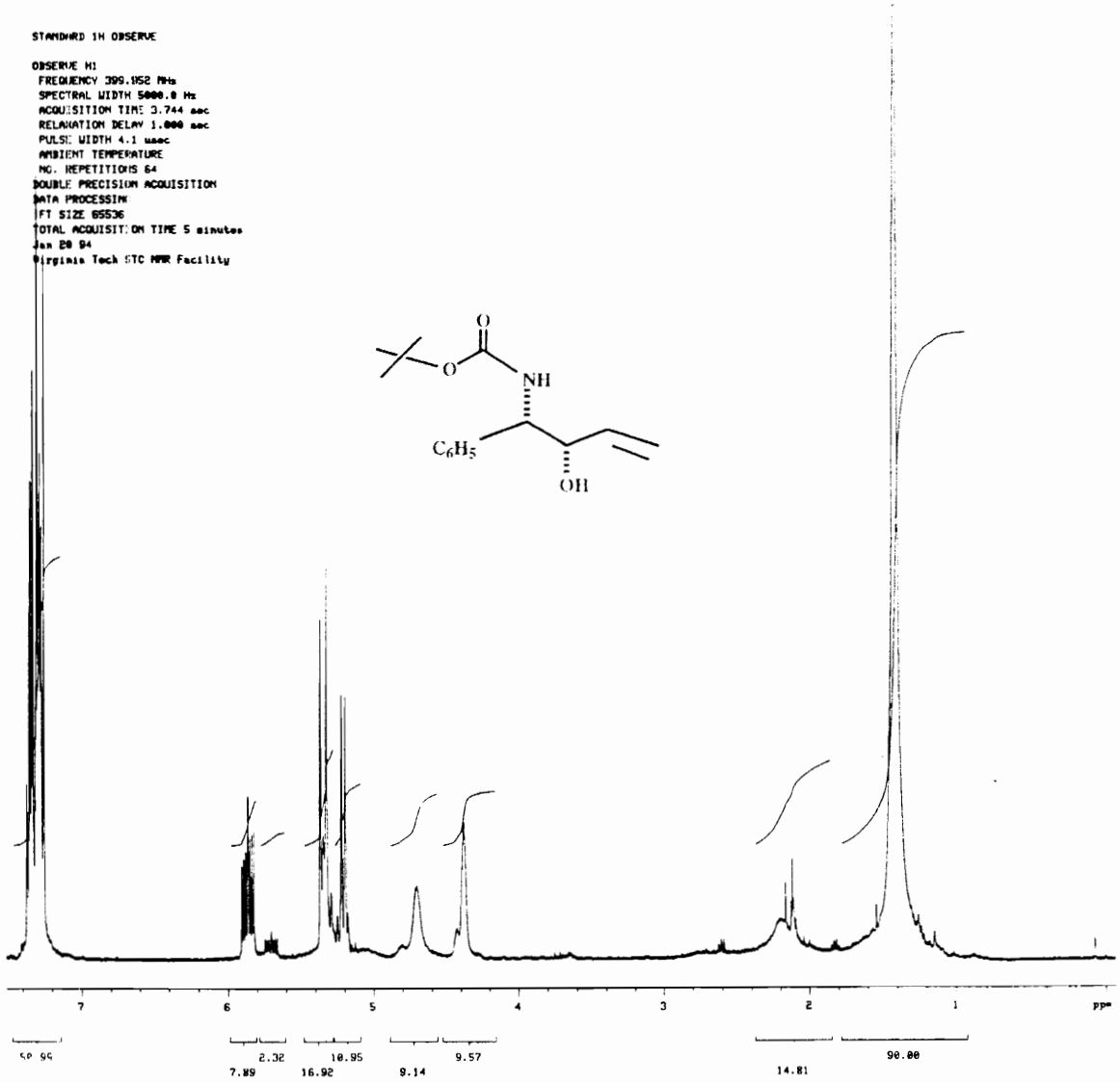

Figure 25. ^{1}H-NMR spectrum of compound **3.6**

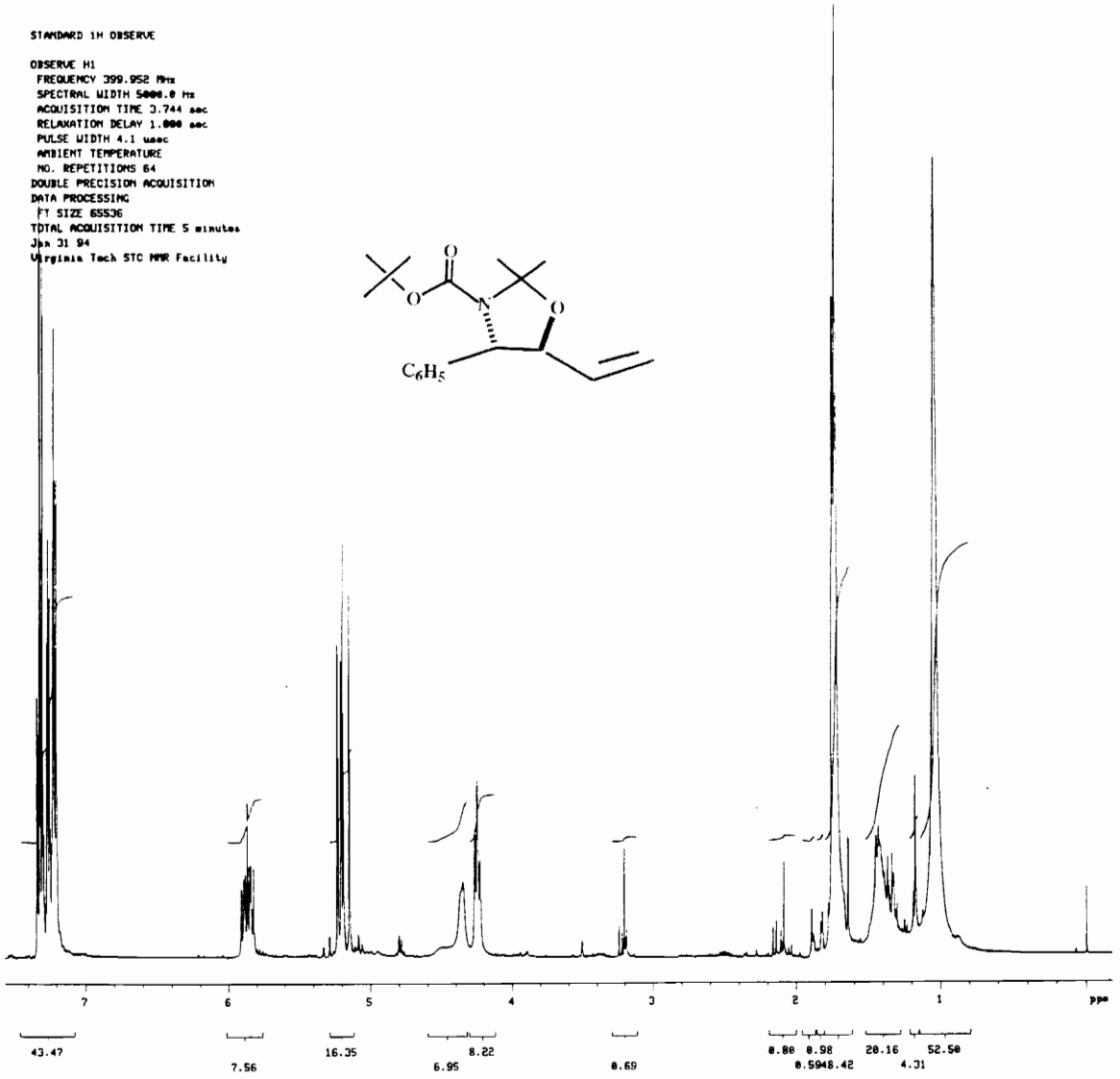

Figure 26. ^{1}H-NMR spectrum of compound **3.7**

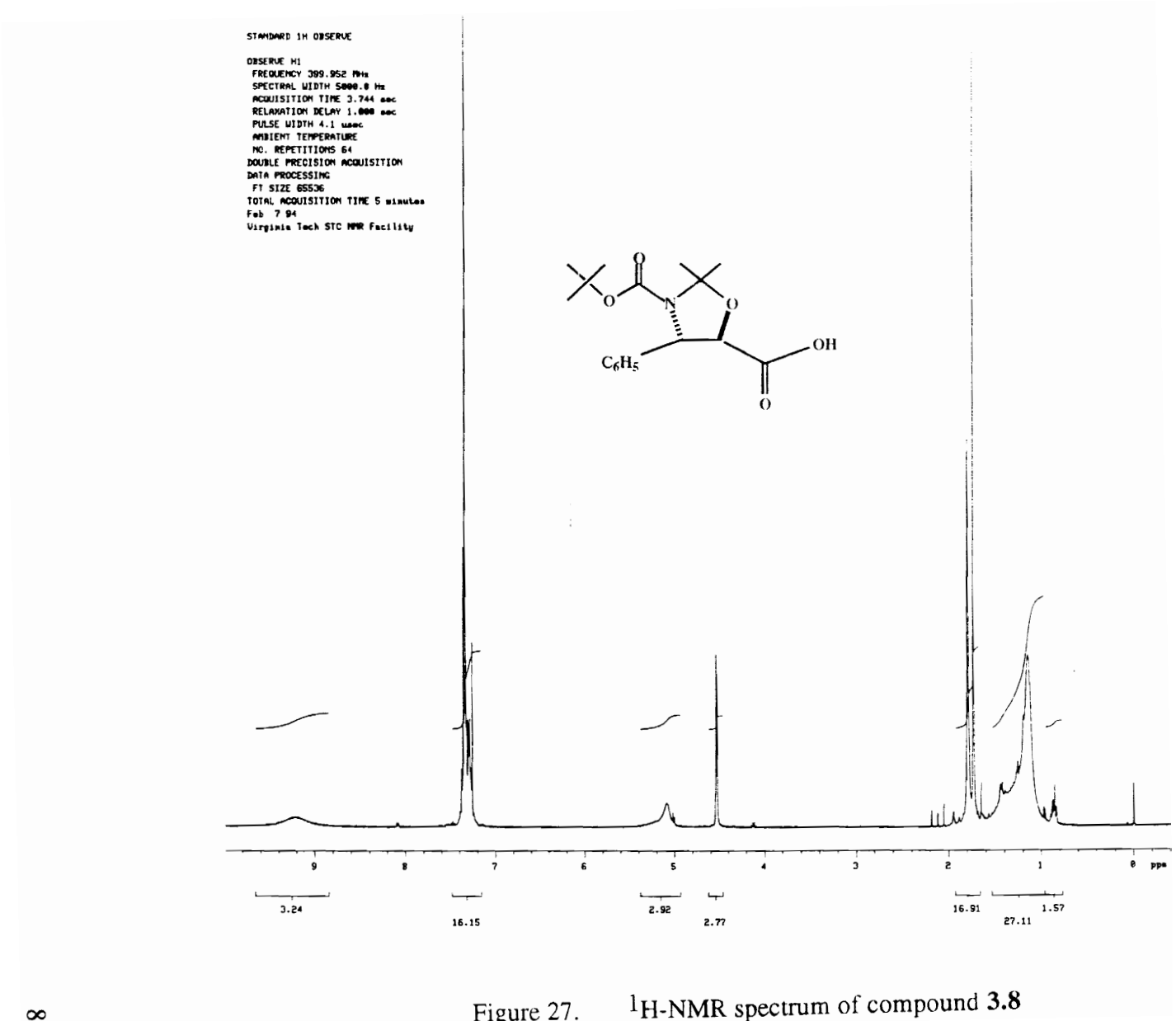

Figure 27. ^{1}H-NMR spectrum of compound **3.8**

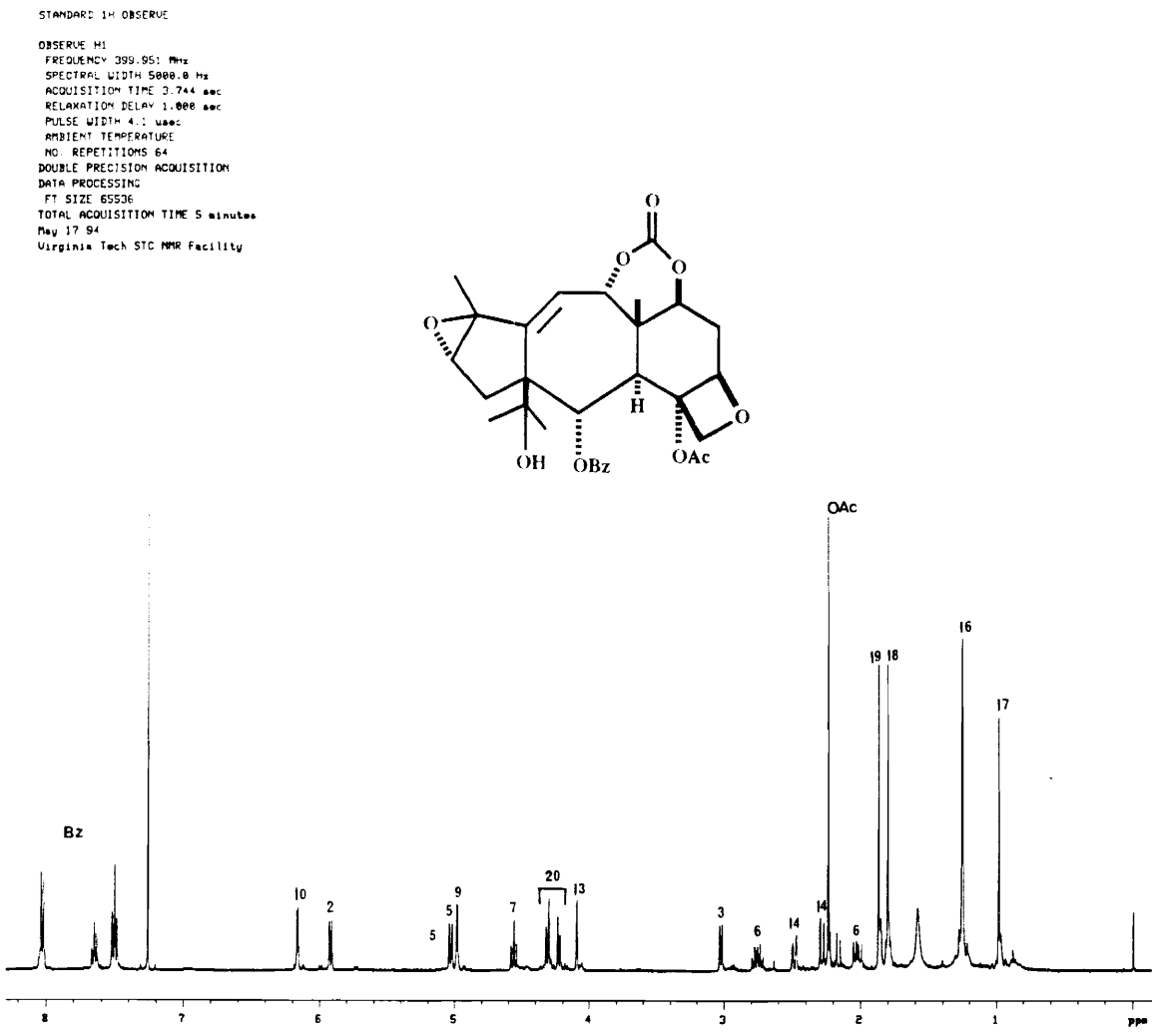

Figure 29. ^{1}H-NMR spectrum of compound **4.5**

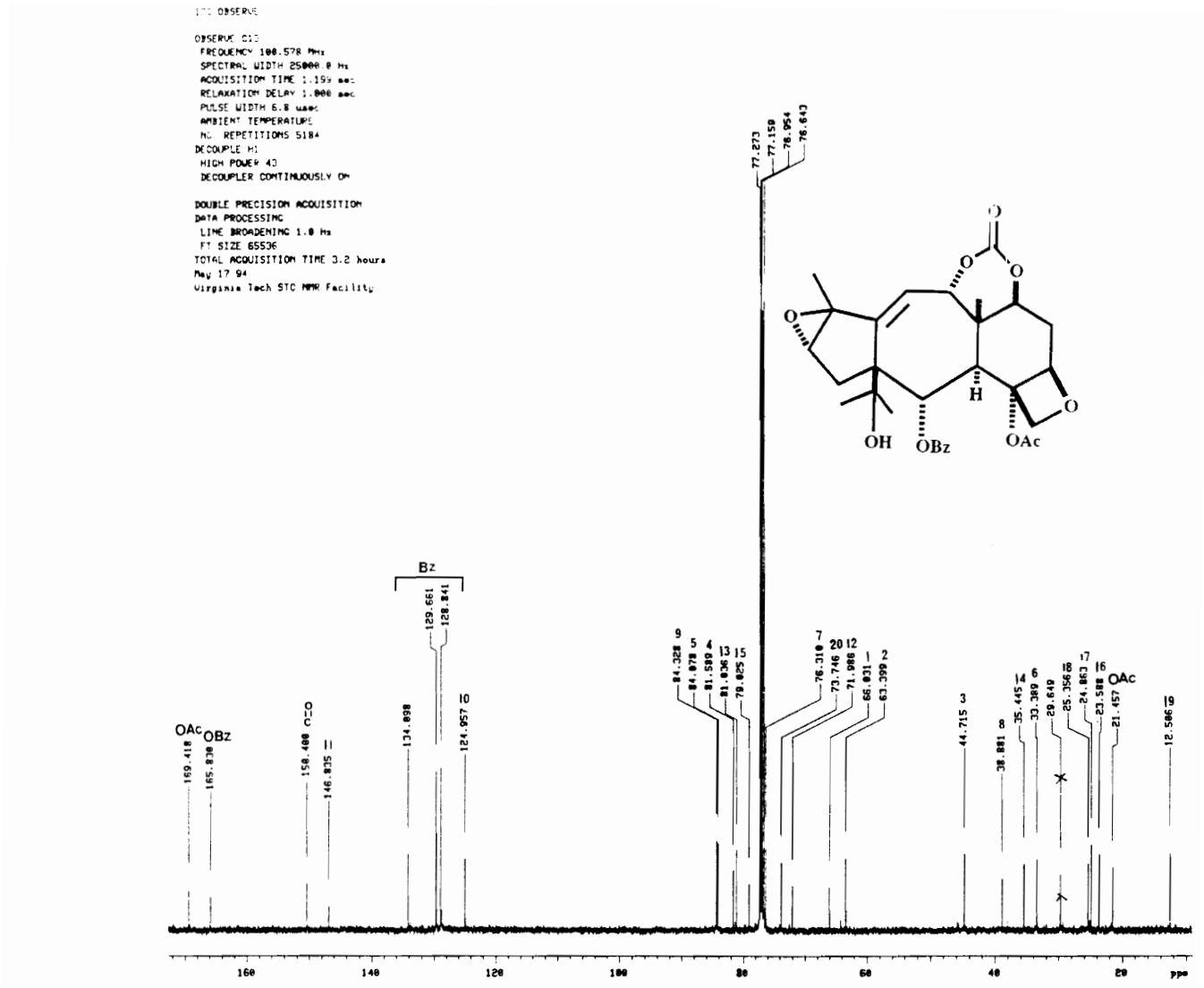

Figure 30. ^{13}C-NMR spectrum of compound **4.5**

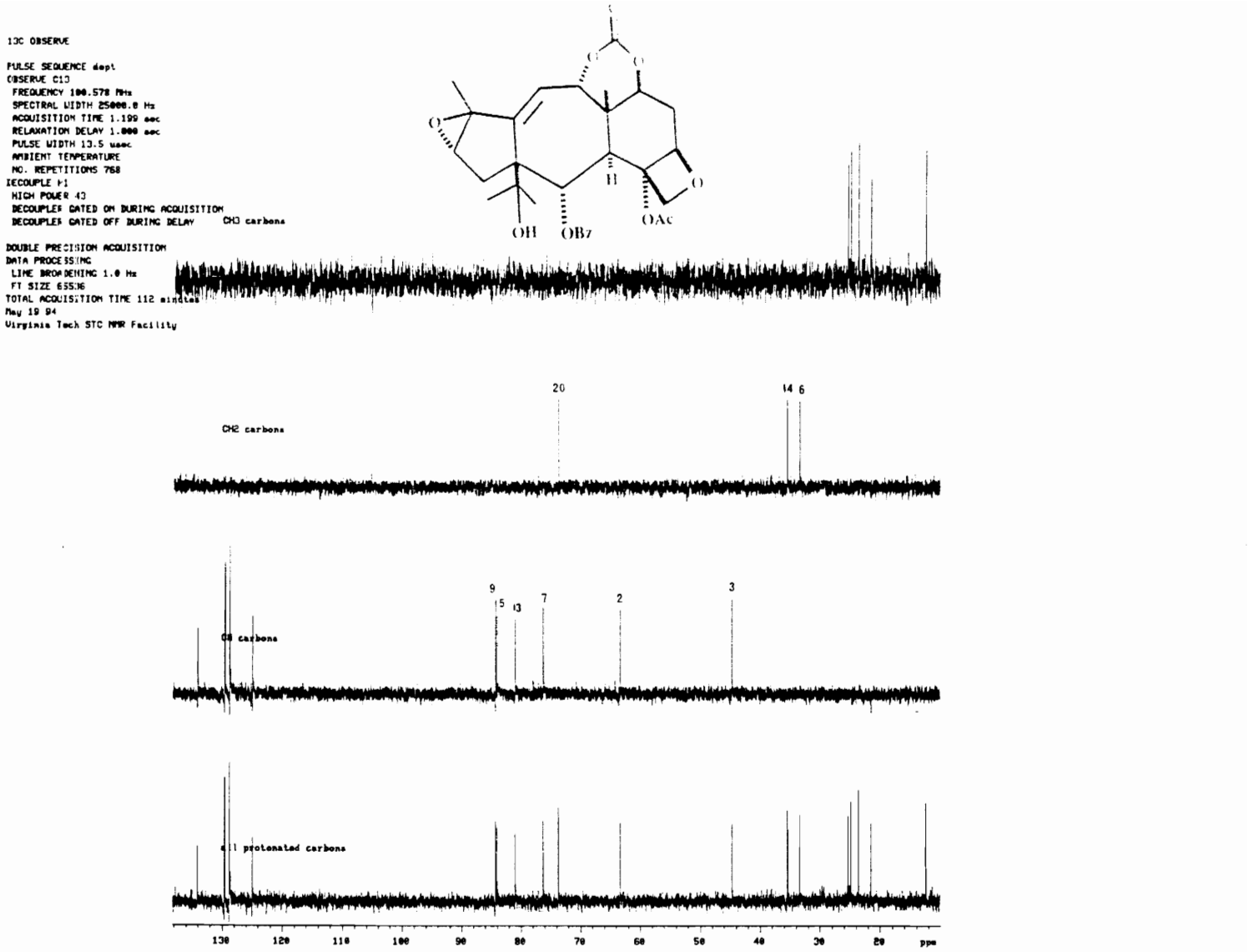

Figure 31. DEPT spectrum of compound **4.5**

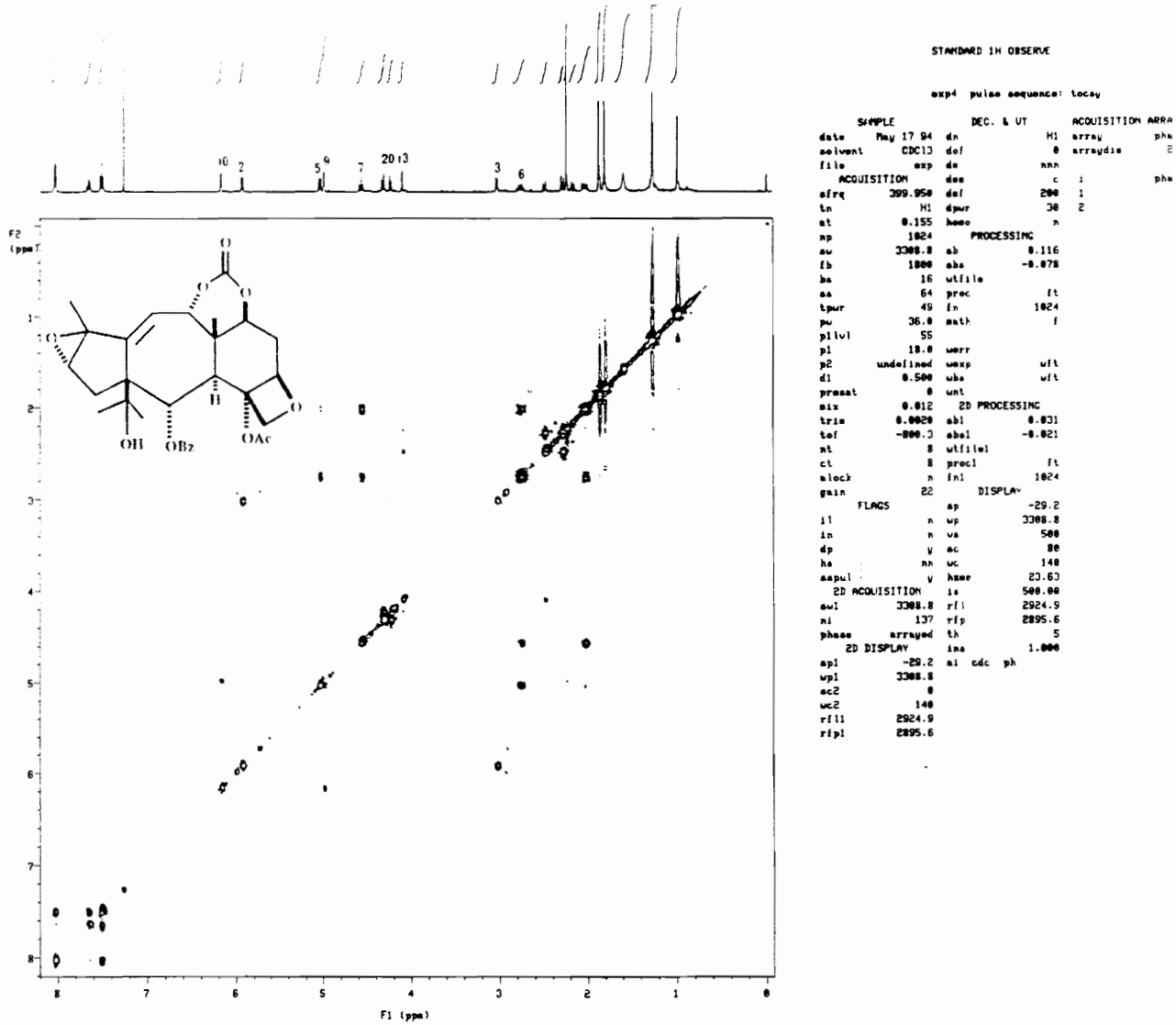

Figure 32. TOCSY spectrum of compound **4.5**

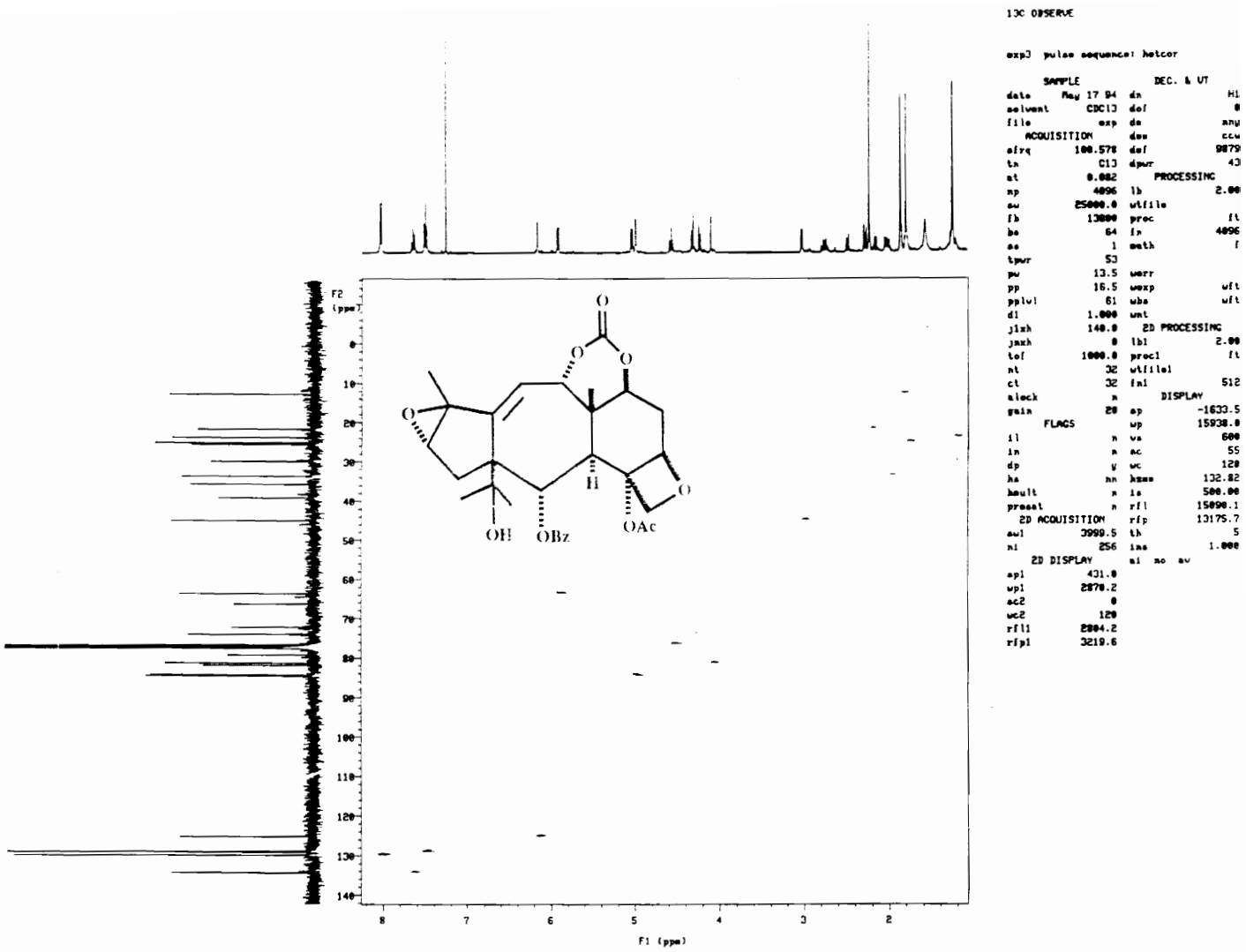

Figure 33. HETCOR spectrum of compound **4.5**

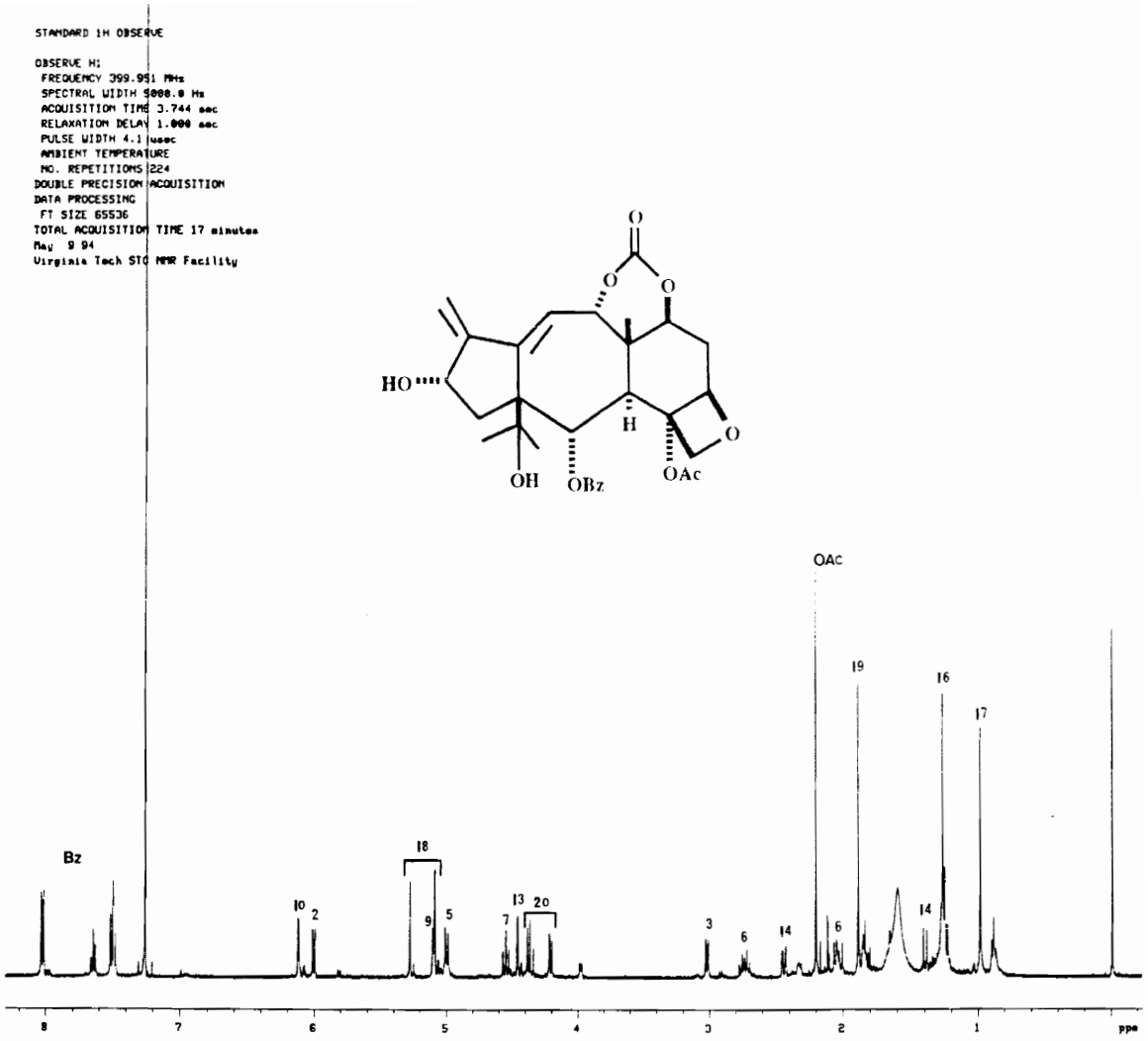

Figure 34. ^{1}H-NMR spectrum of compound **4.6**

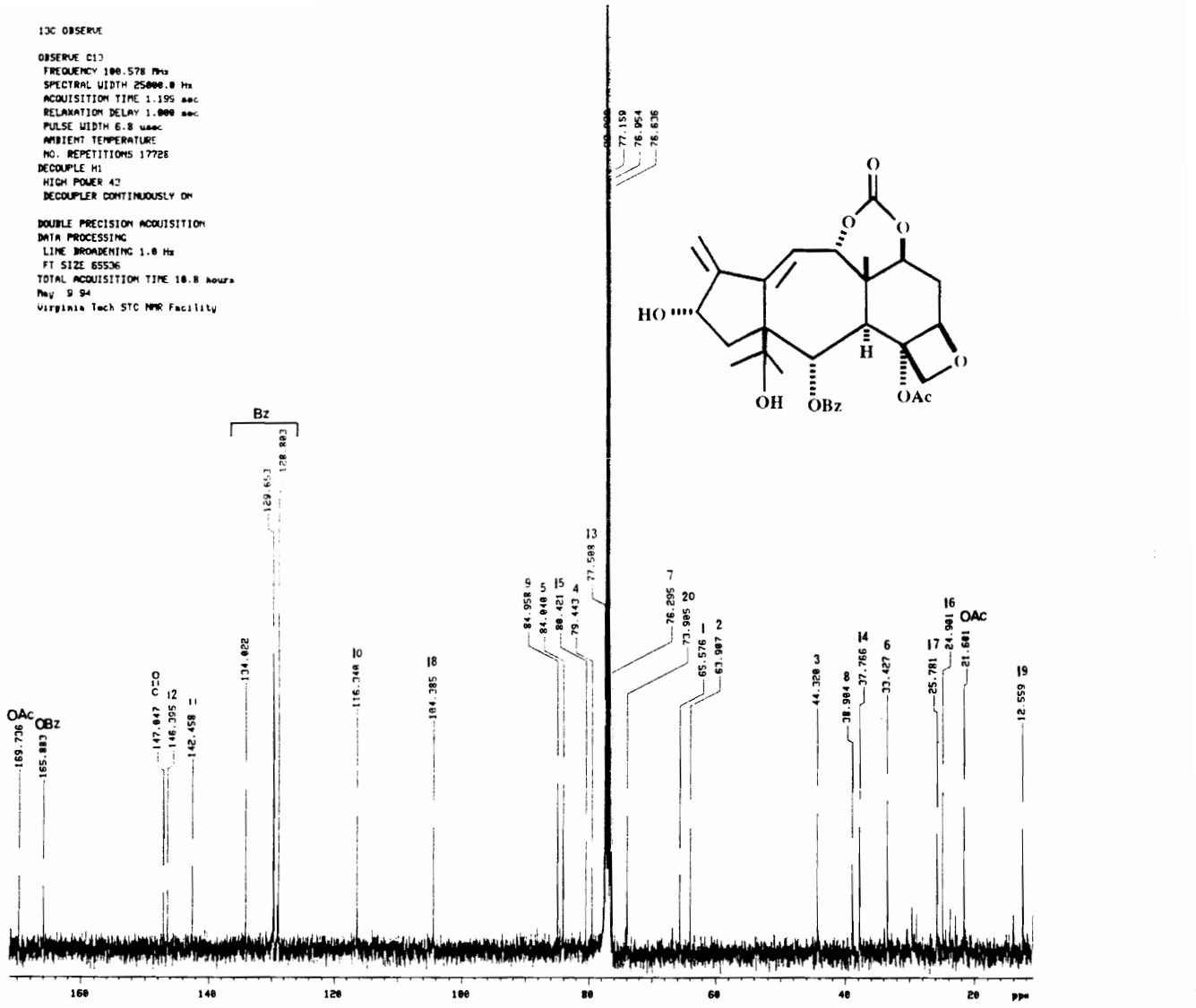

Figure 35. ^{13}C-NMR spectrum of compound **4.6**

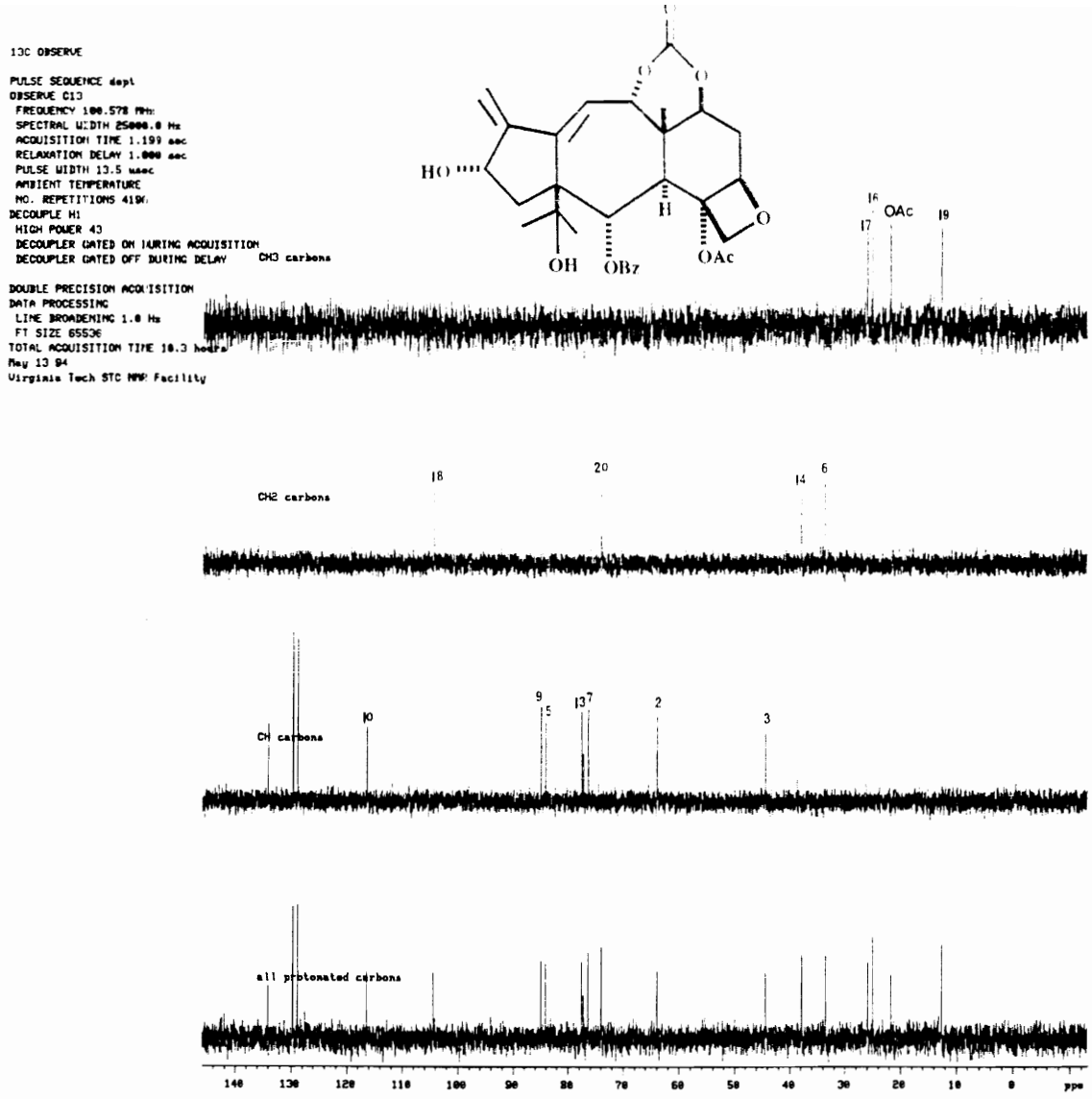

Figure 36. DEPT spectrum of compound **4.6**

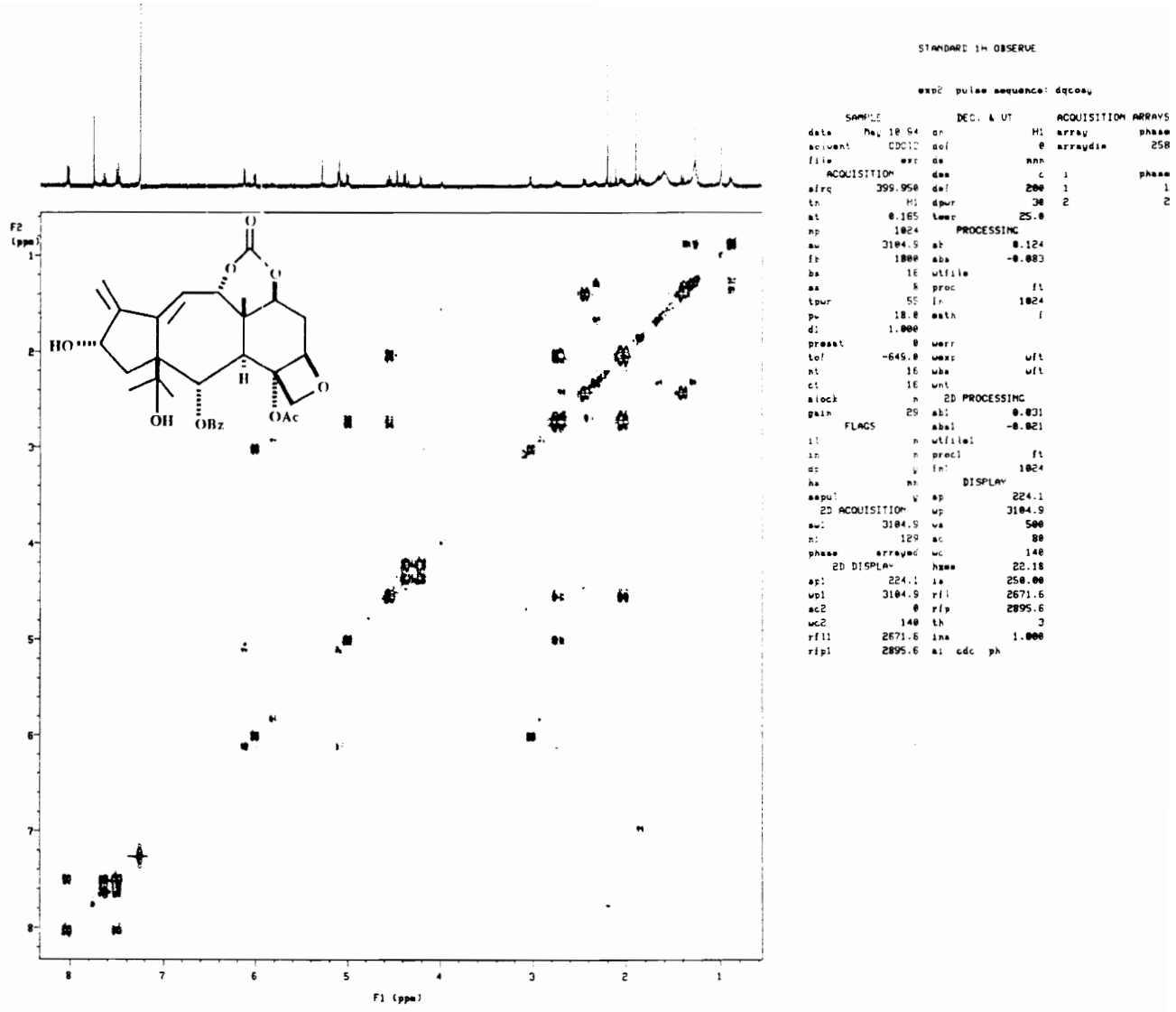

Figure 37. TOCSY spectrum of compound **4.6**

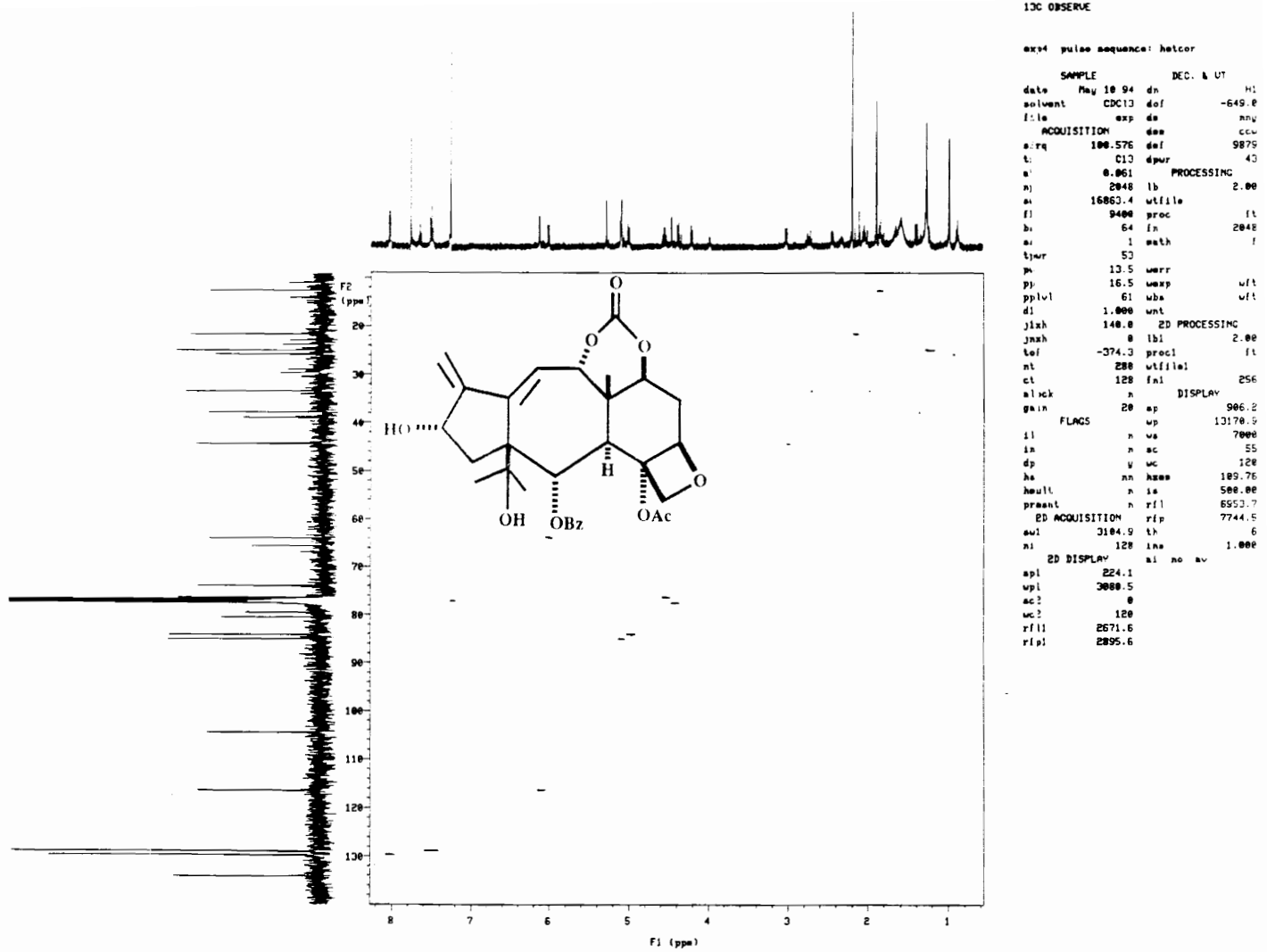

Figure 38. HETCOR spectrum of compound **4.6**

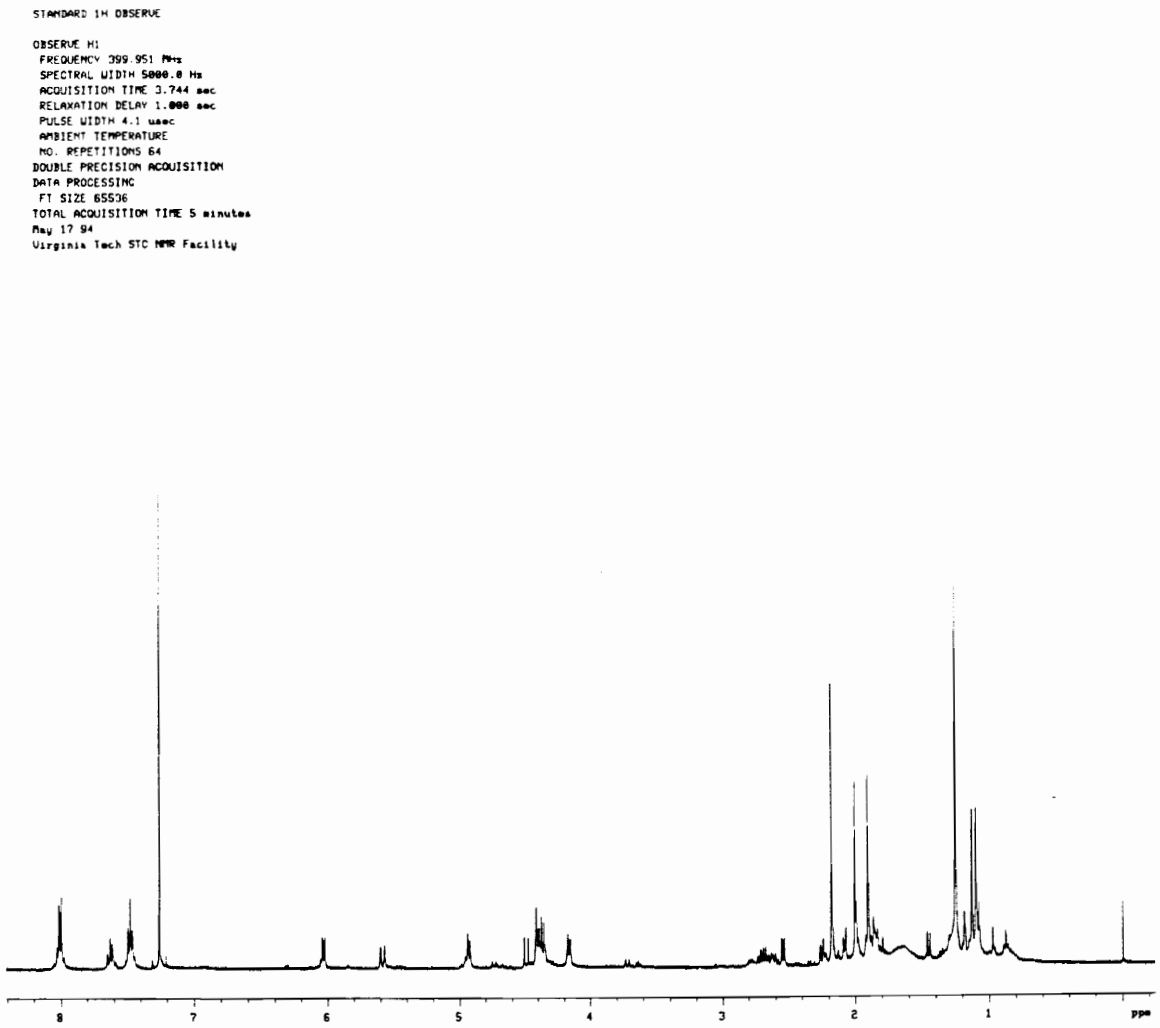

Figure 39. ^{1}H-NMR spectrum of compound **4.7**

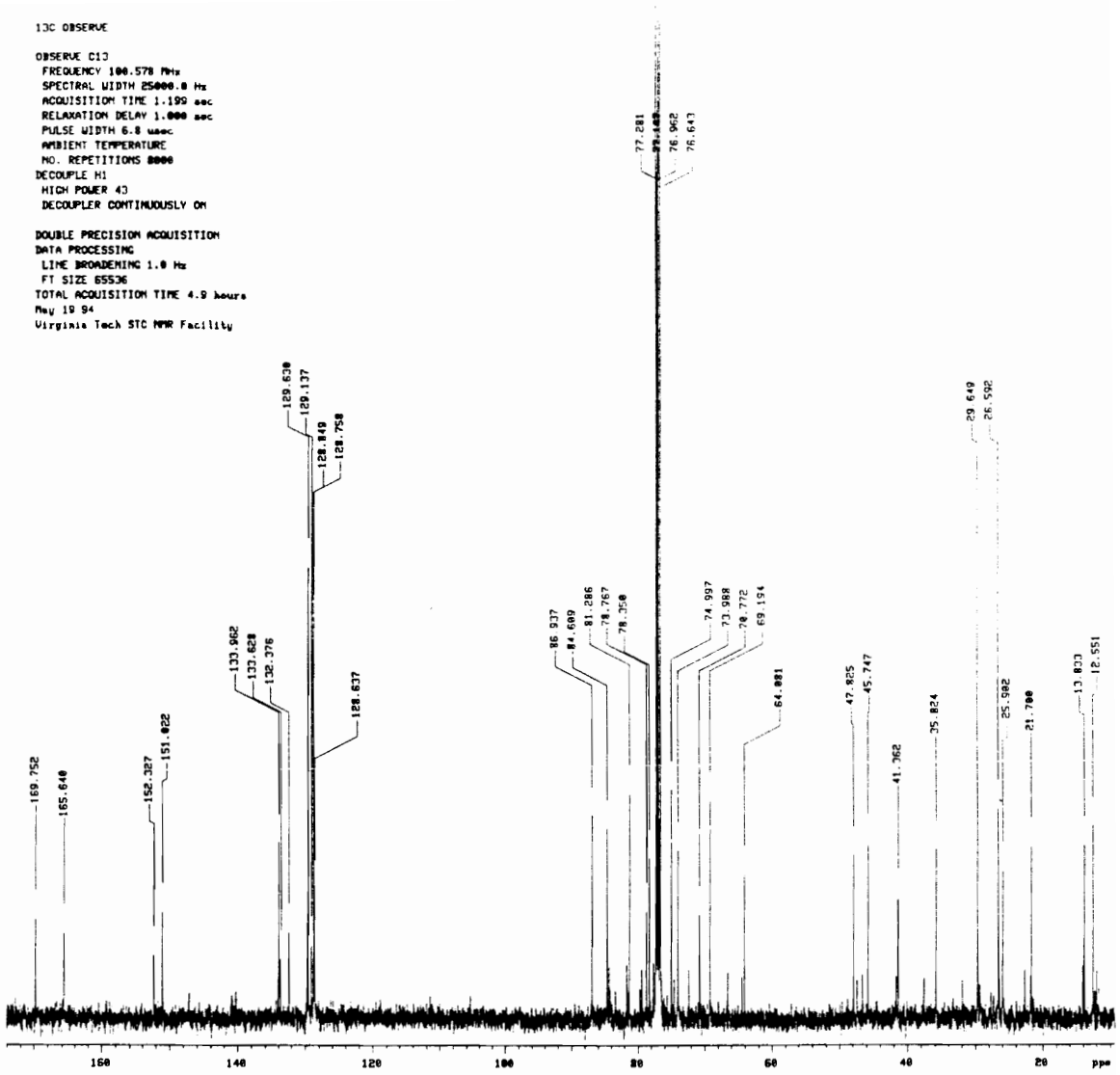

Figure 40. ^{13}C-NMR spectrum of compound **4.7**

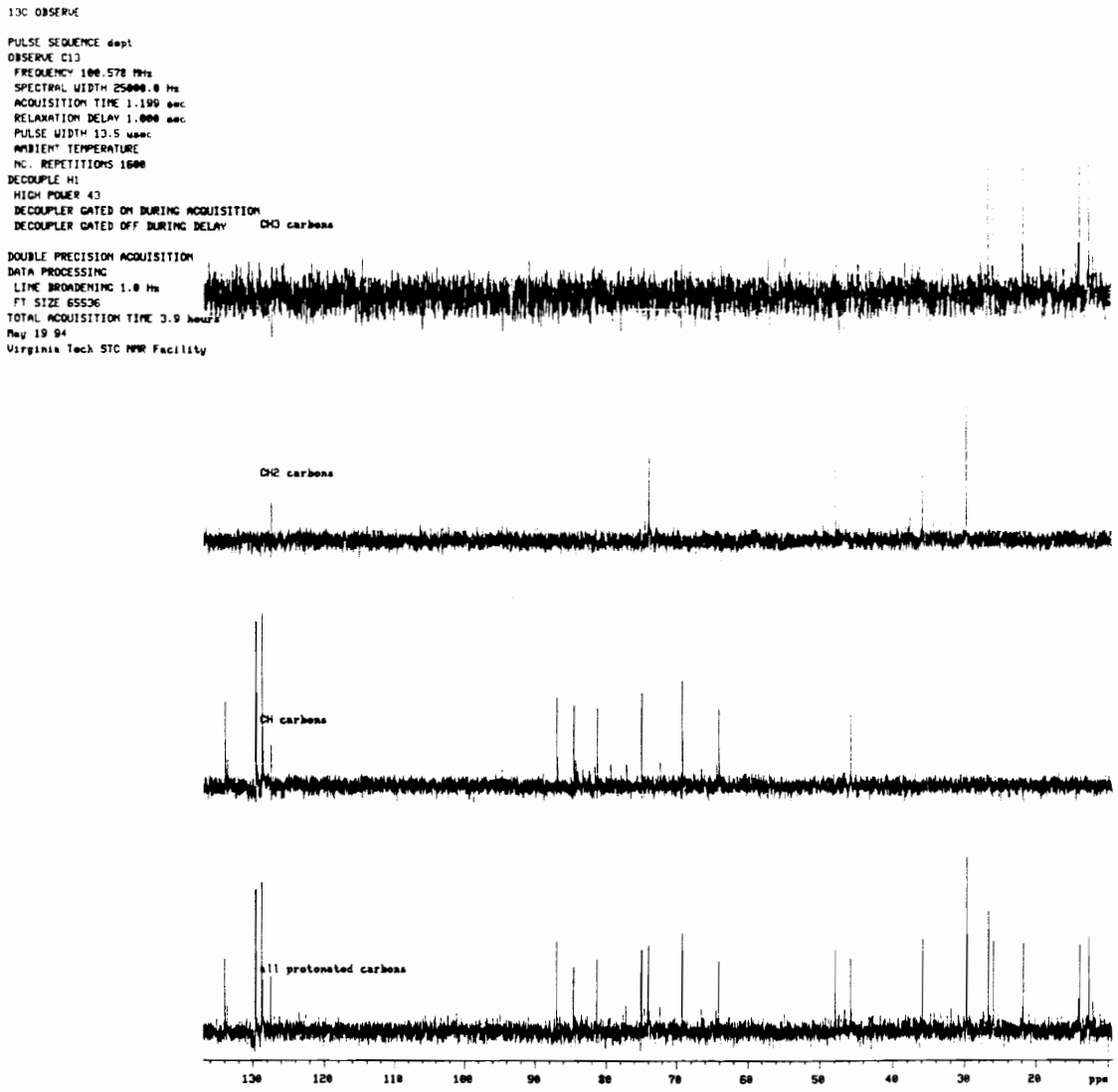

Figure 41. DEPT spectrum of compound **4.7**

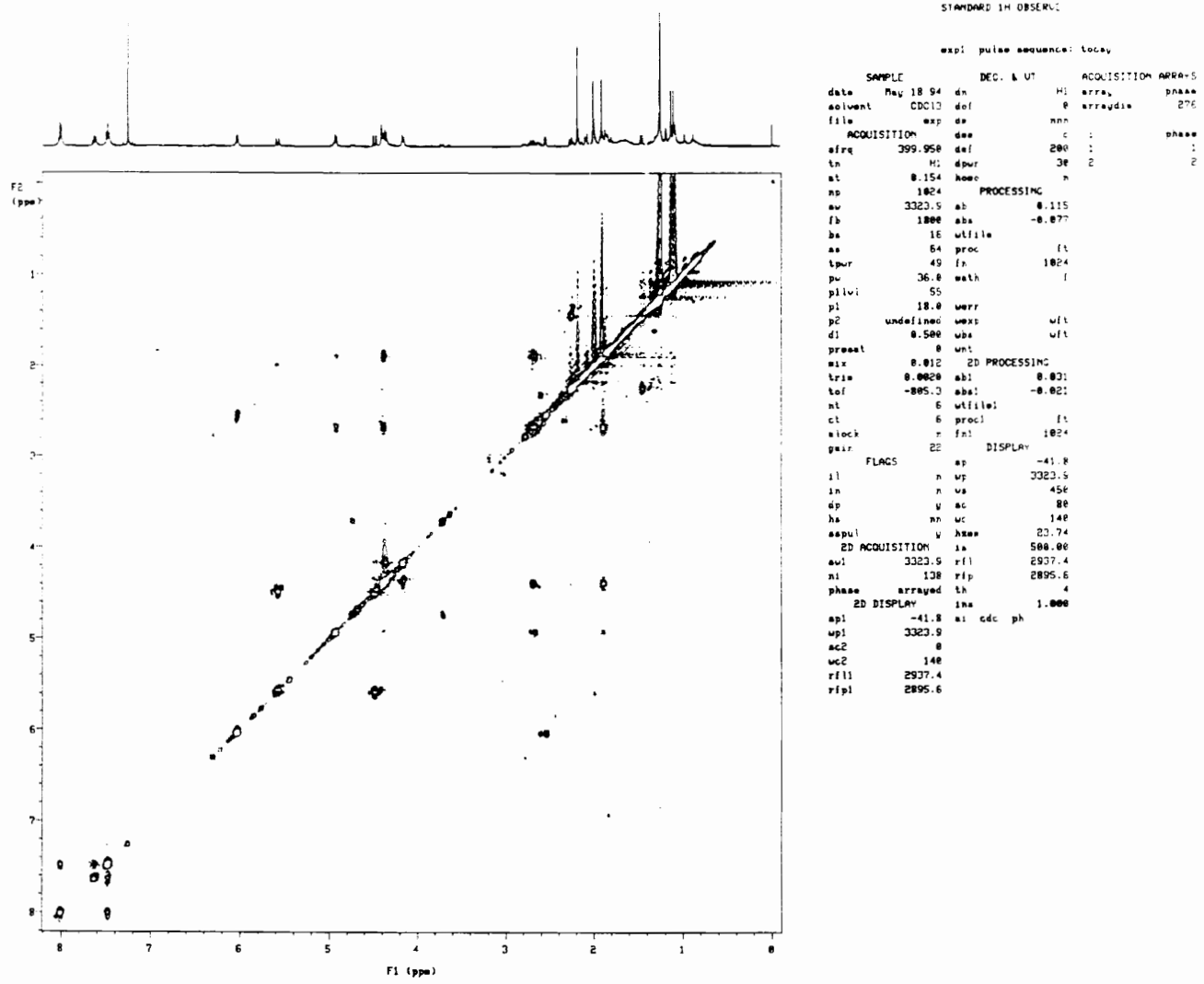

Figure 42. TOCSY spectrum of compound **4.7**

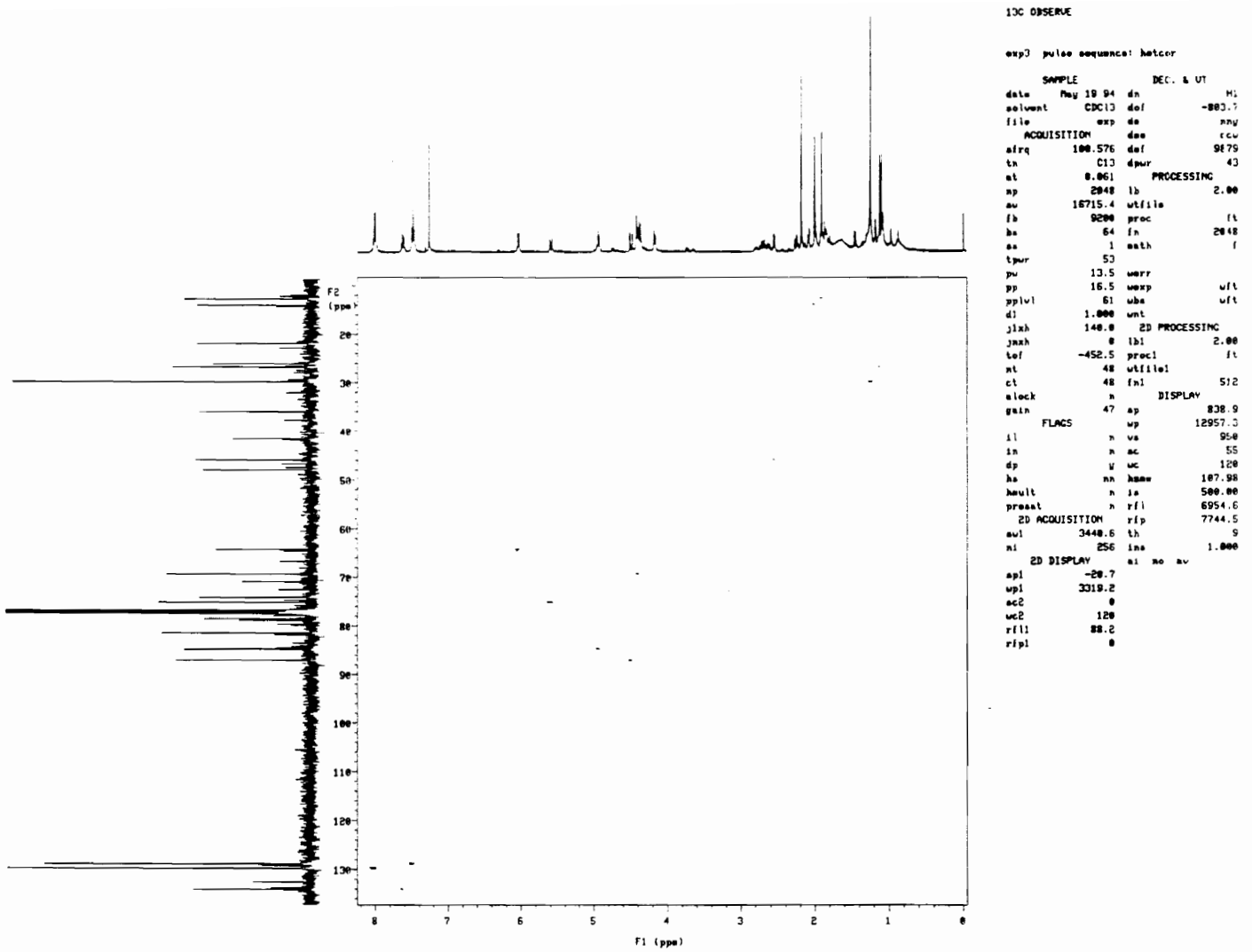

Figure 43. HETCOR spectrum of compound **4.7**

VITA

Ru Chen was born on January 30, 1963 in Nanchang, China. She received a Bachelor of Science in Applied Chemistry from Central-South University of Technology, China in 1984. She then worked as a chemist in Changsha Research Institute of Metallurgy, China. In Dec., 1990, she came to Blacksburg, Virginia to visit her husband. She entered Virginia Polytechnic Institute and State University in 1992. Since then, she served as a teaching assistant and a research assistant in the Department of Chemistry until August, 1994. She worked under the direction of Dr. David G. I. Kingston, and she was awarded a Master of Science in Chemistry in July of 1994.