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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

Experience is the name everyone
gives to their mistakes.

Oscar Wilde

But I shall let the little I have learnt go forth into the day in order that someone better than I may guess the truth, and in his work may prove and rebuke my error. At this I shall rejoice that I was yet a means whereby this truth has come to light.

Albrecht Dürer

Acknowledgements

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I would like to express my deep gratitude to all my friends for their support whenever it was needed. I would also like to thank my parents and family for their tolerance of me during my stay at university. Their support in the past few years is greatly appreciated.

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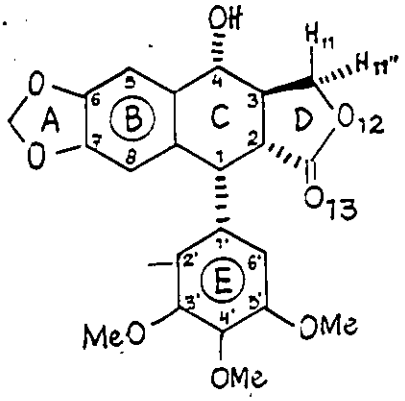
LIST OF SCHEMES

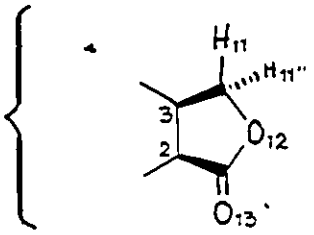
ABBREVIATIONS

AcOH	acetic acid
n-BuLi	n-butyllithium
DCC	dicyclohexylcarbodiimide
DMF	dimethylformamide
h	hours
HMPT	hexamethylphosphoramide
IR	infrared
LDA	lithium diisopropyl amide
mp	melting point
MS	mass spectra
NMR	nuclear magnetic resonance
PTLC	preparative thin layer chromatography
rt	room temperature
TBAF	tetrabutyl ammonium fluoride
TBDMS	tert-butyldimethylsilyl
TBDMSCl	tert-butyldimethylsilyl chloride
THF	tetrahydrofuran
THP	tetrahydropyranyl
TLC	thin layer chromatography
TsOH	p-toluenesulfonic acid
°	refers to degrees Celcius (°C)

NUMBERING SYSTEMS

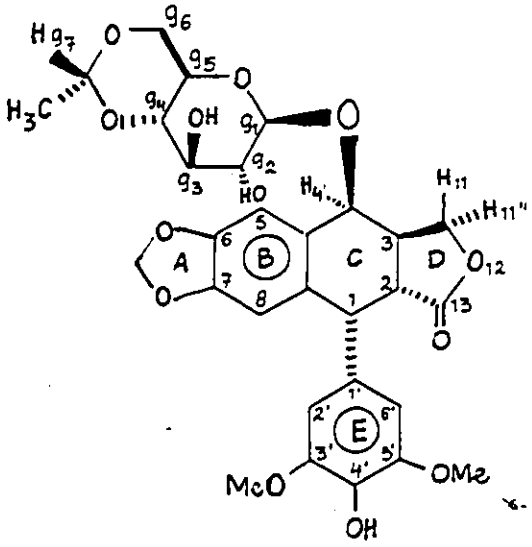
I. Podophyllotoxin Derivatives

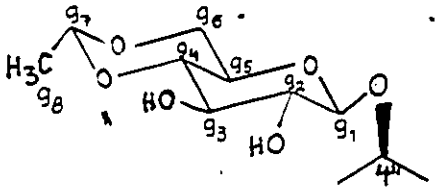


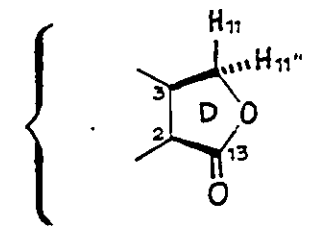


Picro

II. Etoposide Derivatives







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INTRODUCTION

Plant products have been used to treat cancer patients for many years; and, could be considered historically to be the first anticancer drugs. Today, the vinca alkaloids are part of every medical oncologist's resources and play an important role in treating diverse diseases such as the leukemias, lymphomas, breast and testicular cancers, pediatric solid tumors and malignant melanomas. They have been found to be a vital part of most of the successful combination regimens that have led to major therapeutic breakthroughs in chemotherapy.

Podophyllum peltatum linnaeus (family: Berberidaceae), also known as May Apple, American Mandrake, Indian Apple, Wild Lemon or Duck's Foot, can be found in moist, shady woodlands and marshy meadows throughout the region east of the Mississippi and from southeastern Canada to the Gulf of Mexico[1,2]. Two other species of this plant can be found elsewhere in the world. The Indian species, Podophyllum emodi, was discovered by Wallich in 1824 and grows in the interior ranges of the Himalaya Mountains from Sikkim to Hazara. The third species, Podophyllum sikkimensis, also growing in the Himalayan region, was discovered in 1950 by Chatteejee and Mukerjee.

Most of the biological activity of these plants resides in the alcohol soluble portion of the roots. This was known to the North American Indian several hundred years ago who used its properties as a cathartic, as an anthelmintic and as a mortal poison. The alcohol-soluble resin, which is a complex mixture of lignans and flavonol pigments, is known as podo-

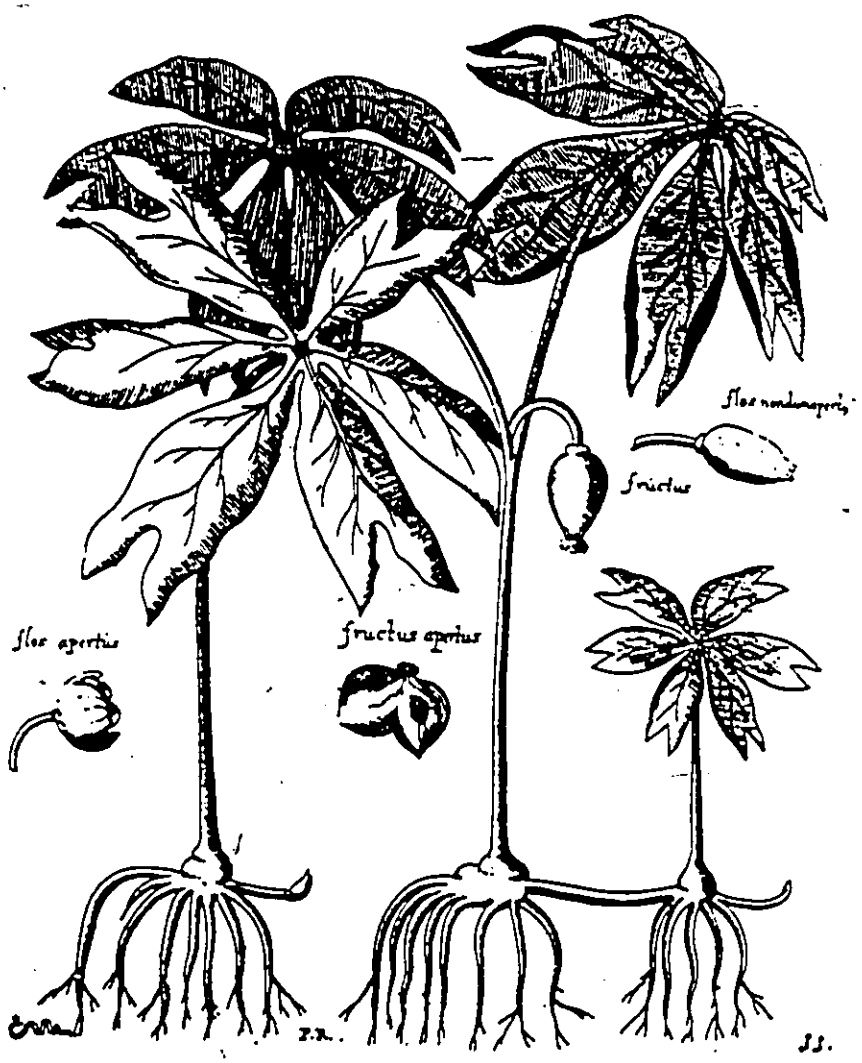


FIGURE 1: PODOPHYLLUM PELTATUM (MAY APPLE)

phillin or podophyllum. Podophyllum became very popular in the United States as a cathartic and chologogue and was included in the first edition of the United States Pharmacopoeia in 1820[3]. In 1835 Dr. John King was one of the first researchers to prepare and administer podophyllum clinically. However, serious side effects were observed as described in the following excerpts of a letter to a colleague of Dr. King[4].

> "...She was in severe pain and distress, cramps in the stomach and extremities, pulse small and feeble, extremities cold, excessive vomiting, and hypercatharsis, and apparently sinking rapidly. Her condition greatly resembled that of a person suffering from a fatal attack of Asiatic cholera.
>
> ...By perseverance in this course (of treatment), the patient recovered in six or seven days, but unfortunately, with some chronic gastroenteritic abnormal condition, that remained for years."

The fifteenth revision of the U.S. Pharmacopoeia gives the following excerpt as a method for preparing podophyllum from P. peltatum[5]:

> "Extract the drug (Podophyllum in fine powder, 1000 gm) by slow percolation until it is exhausted of its resin, using alcohol as the menstruum. Concentrate the percolate by evaporation until the

> residue has the consistency of a thin syrup, and pour this, with constant stirring, into 1000 ml of water containing the hydrochloric acid (10 ml) and previously cooled to a temperature below 10°. Allow the precipitate to settle, decant the clear liquid, and wash the precipitate with two 1000 ml portions of cold water. Dry the resin, and powder it."

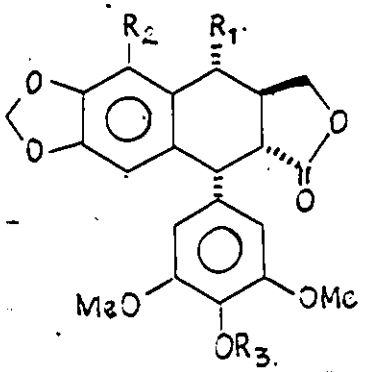
Since its early discovery, podophyllum has been used in the treatment of various ailments including cancer and other growths. In 1845, Good wrote[6]:

> "Some Physicians and Practitioners recommend the powdered root as an escharotic to cleanse foul and ill-conditioned ulcers and dispose them to heal and to promote the exfoliation or removal of carious or rotten bones.... It is also said to destroy proud flesh without any injury to the sound parts."

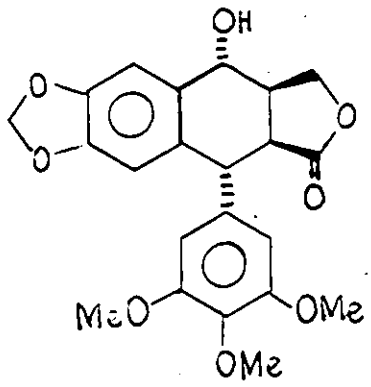
After being dropped from the twelfth edition of the U.S. Pharmacopoeia (1942), Kaplan published a report showing that podophyllum was successful in the clinical treatment of a venereal wart (Condyloma acuminatum)[7]. Between 1942 and 1960, podophyllum was studied in many clinical conditions including diseases of the skin due to infectious agents, nonspecific dermatoses, metabolic diseases, gout, rheumatoid arthritis, and, benign and malignant growths[8].

A lot of effort has gone into the isolation and identification of the components of podophyllum. In 1880 Podwyssotzki had isolated a white crystalline substance which he named podophyllotoxin and which he believed to be the active component[9]. By 1947, only two components of podophyllum, podophyllotoxin and quercetin, had been isolated due to the lack of techniques of separation. However, in 1947, Hartwell and his co-workers were able to isolate three new components of podophyllum, two from P. peltatum and one from P. emodi[10,11,12]. The first two components, α-peltatin and β-peltatin, were initially believed to be isomeric to podophyllotoxin but it was shown later that their correct empirical formulas were $C_{21}H_{20}O_8$ and $C_{22}H_{22}O_8$ respectively[11,12,13]. The third compound was identified as 4'-demethylpodophyllotoxin. By the late 1950's, sixteen components of podophyllum had been isolated and characterized. Their structures are shown in Table 1, It is interesting to note that the podophyllum resin varies in chemical composition from the different species as can be seen in Table 2. For example, podophyllotoxin can be found in P. peltatum and P. emodi but not in P. sikkimenis while the peltatins are characteristic of only P. peltatum. As expected, the percentage of the sixteen components varies with species. This can be seen with podophyllotoxin which varies in yields from 35-50% to 10% for P. emodi and P. peltatum respectively[12,13,14,15].

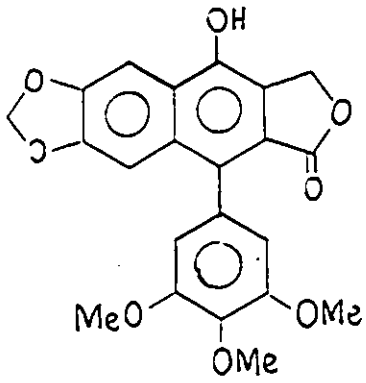
TABLE 1: COMPOUNDS ISOLATED FROM PODOPHYLLIN



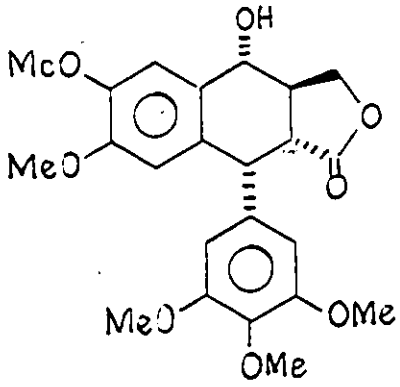
NAME	#	R_1	R_2	R_3
podophyllotoxin	1	OH	H	CH_3
α-peltatin	2	H	OH	H
β-peltatin	3	H	OH	CH_3
4'-demethylpodophyllotoxin	4	OH	H	H
deoxypodophyllotoxin	5	H	H	CH_3
podophyllotoxin glucoside	6	O-glucosyl	H	CH_3
α-peltatin glucoside	7	H	O-glucosyl	H
β-peltatin glucoside	8	H	O-glucosyl	CH_3
4'-demethylpodophyllotoxin glucoside	9	O-glucosyl	H	H



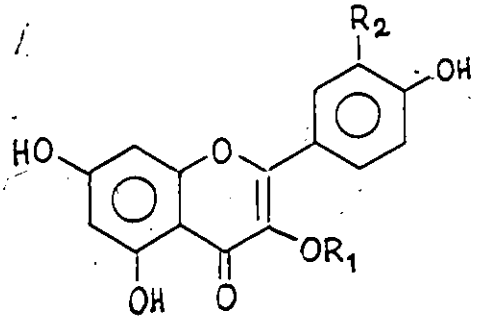
picropodophyllotoxin 10



tetradehydropodophyllotoxin 11



sikkimotoxin 12

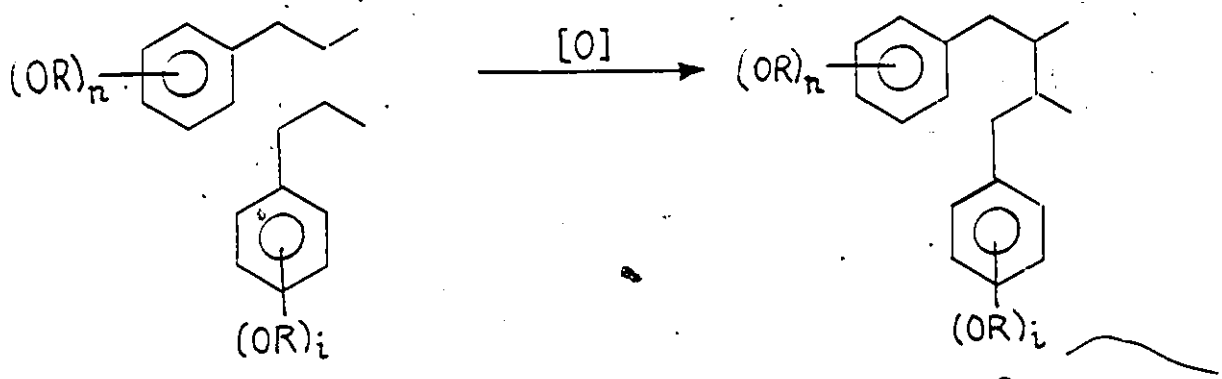


NAME	#	R_1	R_2
quercetin	13	H	OH
isorhamnetin	14	H	OCH_3
quercetin 3-galactoside	15	galactosyl	OH
kaempferol	16	H	H

TABLE 2: VARIOUS RESINS OF PODOPHILLIN

NAME	Present in		
	P. peltatum	P. emodi	P. sikkimensis
Podophyllotoxin	+	+	
α-Peltatin	+		
β-Peltatin	+		
4'-Demethylpodophyllotoxin		+	
Sikkimotoxin			+
Picropodophyllin Glucoside	+	+	
Quercetin	+	+	+
Isorhamnetin			+
3-Galactosidylquercetin			+

Table 1 shows that podophyllum contains two categories of components; four are flavonol pigments and twelve are members of the lignan family. Haworth used the term "lignan" to describe the class of optically active plant products which contain the 2,3-dibenzylbutane skeleton and are probably derived by dimerization of two C_6-C_3 units at the β-carbon atoms of the two side chains as follows[16]:



18 19

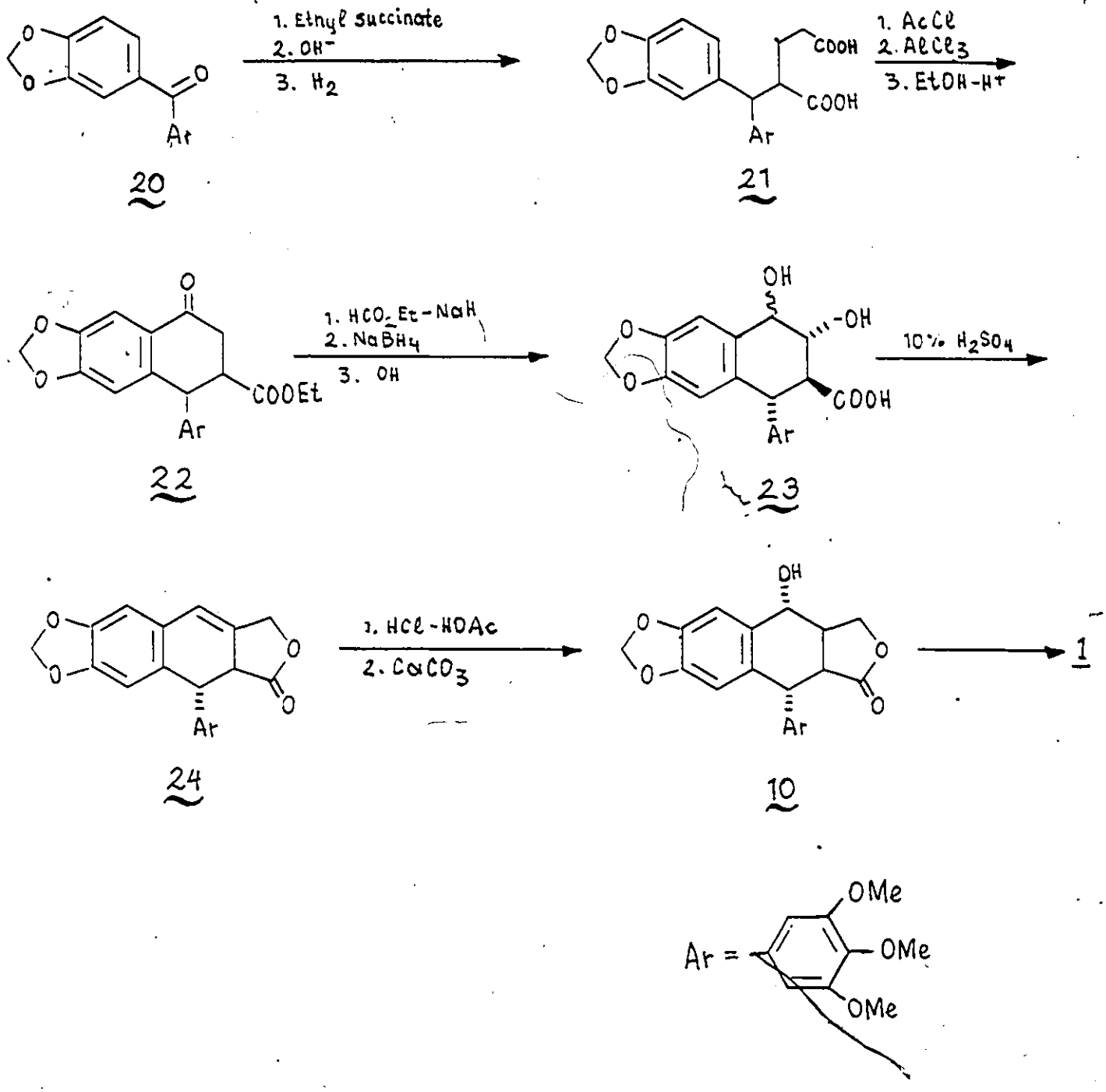
In 1953, Hartwell and Schrecker published a report showing the absolute configuration and structure of podophyllotoxin[17]. Petcher's x-ray crystal strueture analysis of 2'-bromopodophyllotoxin in 1973 confirmed the absolute configuration as (1R, 2R, 3R, 4R)[18]. Hartwell and Schrecker postulated that the antimitotic and antitumor activity of the podophyllum lignans was closely related to their unique configuration at C-1, C-2, C-3 and C-4 [cis-(1:2)-trans (2:3)-trans-(3:4)] and the highly strained, trans-fused γ-lactone moiety[17].

Since podophyllotoxin had shown promise as an anticancer drug, much research has gone into its synthesis. The first synthesis of podophyllotoxin was reported by Gensler and co-workers in 1954 and 1966 (Scheme 1)[19,20,21,22]. Under mild basic conditions, podophyllotoxin will epimerize at the C-2 position to picropodophyllotoxin. This thermodynamic equilibrium gives a mixture of podophyllotoxin and picropodophyllotoxin in the ratio of 3:97[21]. Interestingly, this epimerization results in almost total loss of the cytotoxic activity[21,22].

The first synthesis of podophyllotoxin by Gensler in 1954 and 1966 was directed toward the synthesis of the more stable picropodophyllotoxin isomer 10 followed by isomerization to the active podophyllotoxin. Gensler tried to overcome the thermodynamic problem by kinetic proton trapping of the rigid enolate of 4-O-tetrahydropyranylpicropodophyllotoxin with acetic acid[22]. This still yielded a mixture of podophyllotoxin and picropodophyllotoxin but in a ratio of 45:55 (Scheme 2).

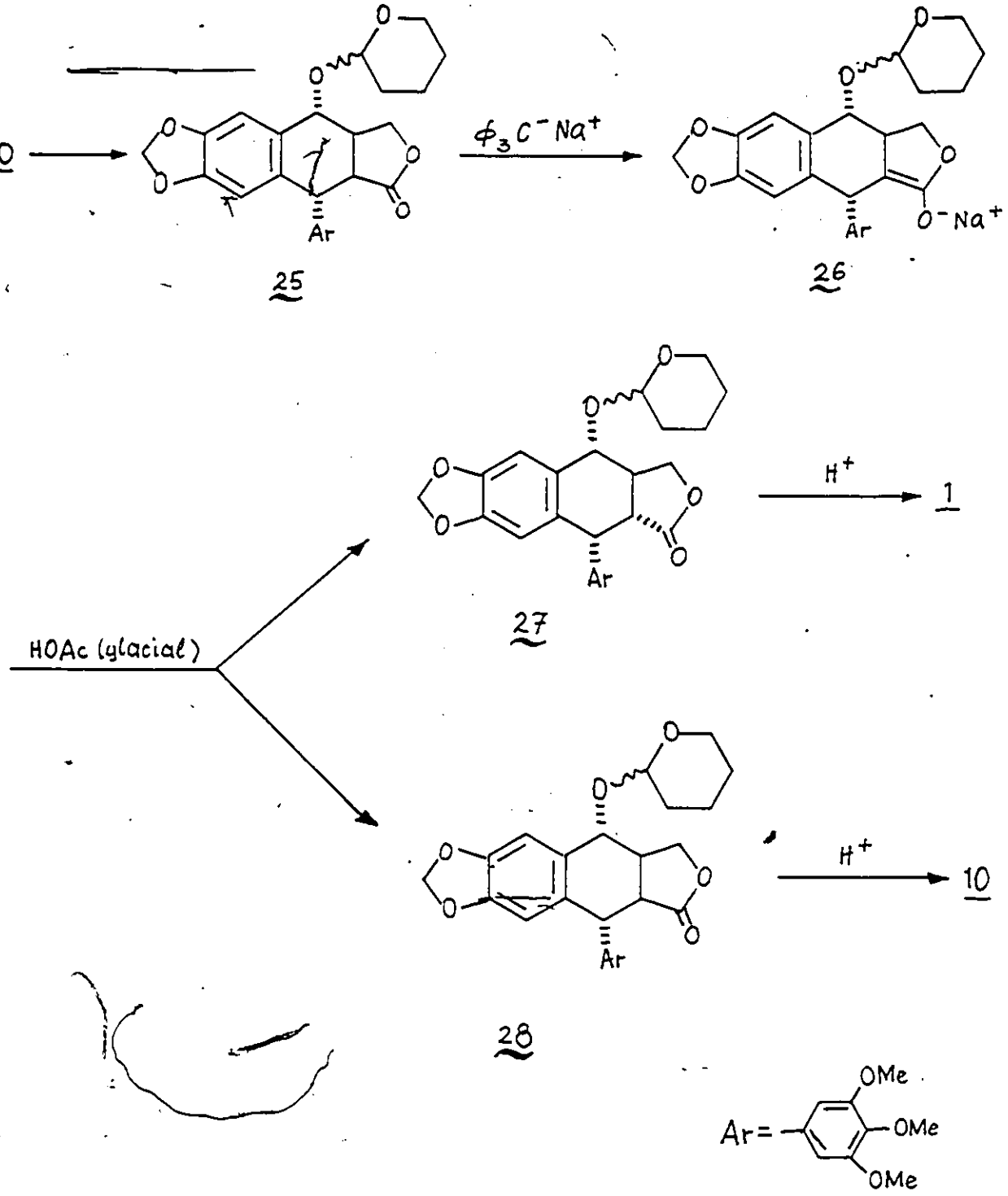
Kende synthesized (±)-podophyllotoxin in 12 steps from piperonal (Scheme 3) but he also required as a final step the unfavourable (difficult) isomerization of picropodophyllotoxin to podophyllotoxin[23,24]. Kende had used the TBDMS as the oxygen protecting group.

Rodrigo's synthesis of (±)-podophyllotoxin avoided this problem by synthesizing (±)-neopodophyllotoxin 36 (Scheme 4)[25,26].

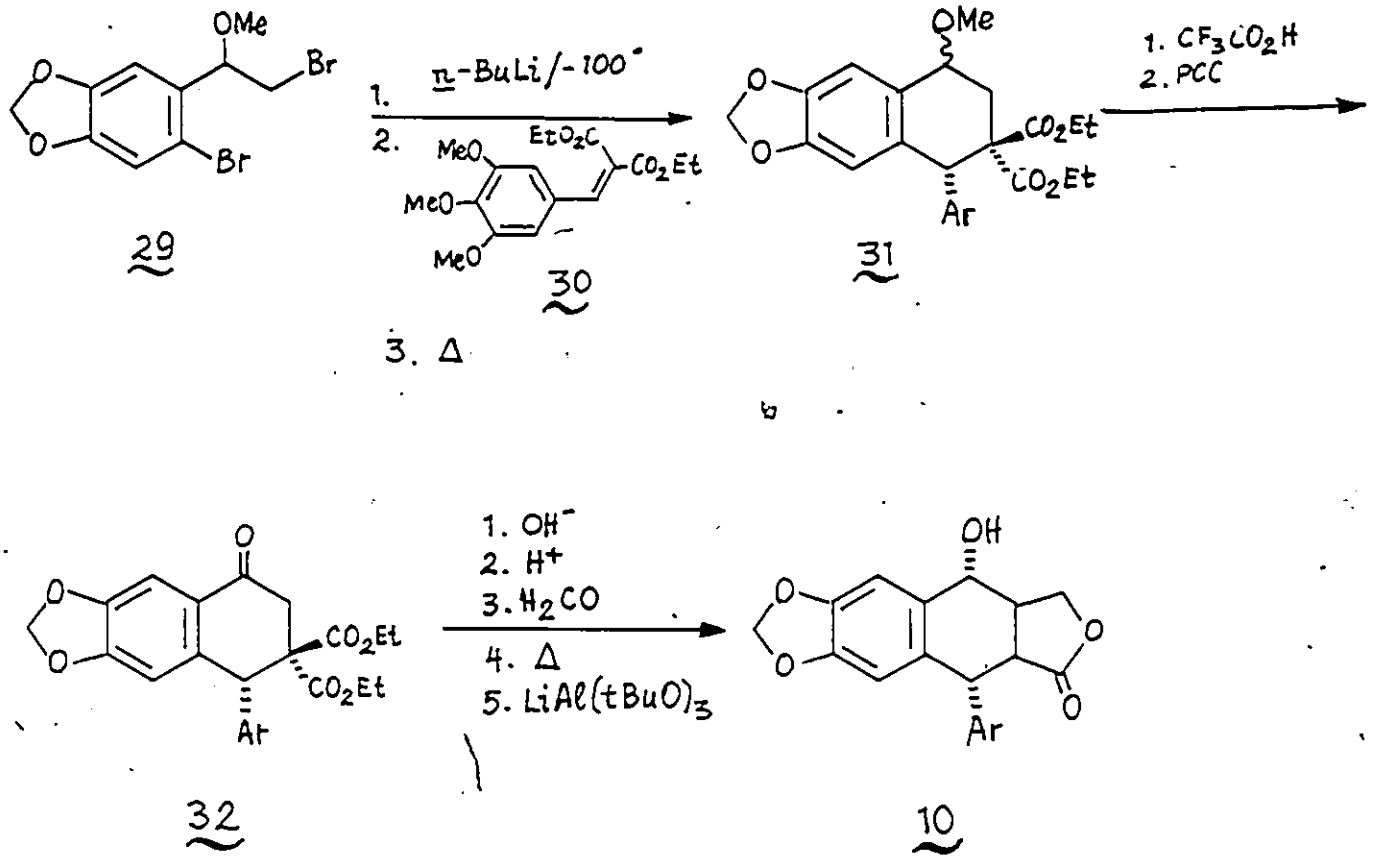


20 21 22 23 24 10 1

SCHEME 1: GENSLER'S SYNTHESIS OF PODOPHYLLOTOXIN (1954, 1966)



SCHEME 2: CONVERSION OF PICROPODOPHYLLOTOXIN TO PODOPHYLLOTOXIN

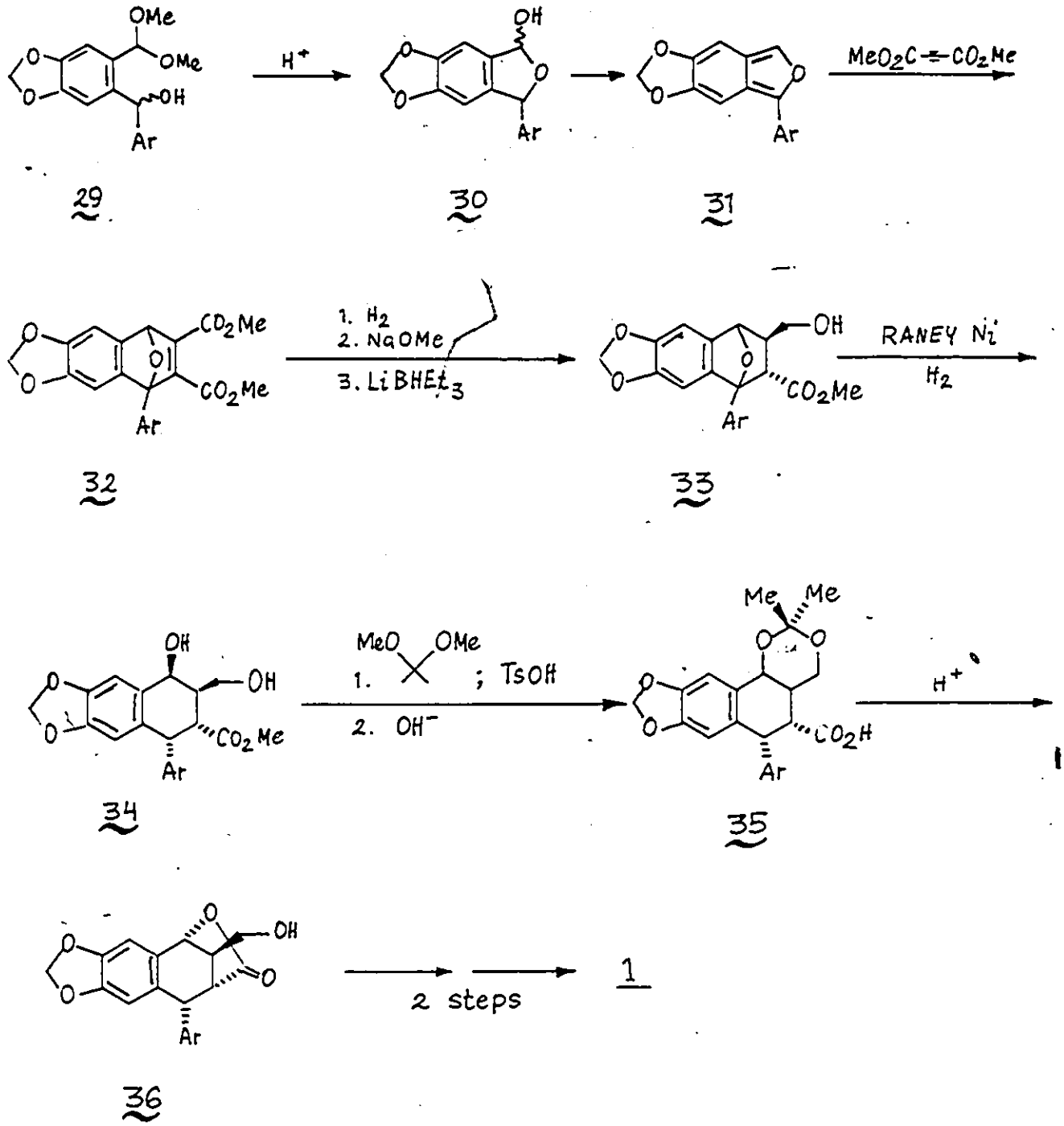


29 30 31 32 10

1. tBuMeSiCl/IMIDAZOLE
2. LDA
3. PY · HCl
4. NEt_3 · HF

→ 1 + 10 (1:1)

SCHEME 3: KENDE'S SYNTHESIS OF PODOPHYLLOTOXIN (1981)



SCHEME 4: RODRIGO'S SYNTHESIS OF PODOPHYLLOTOXIN

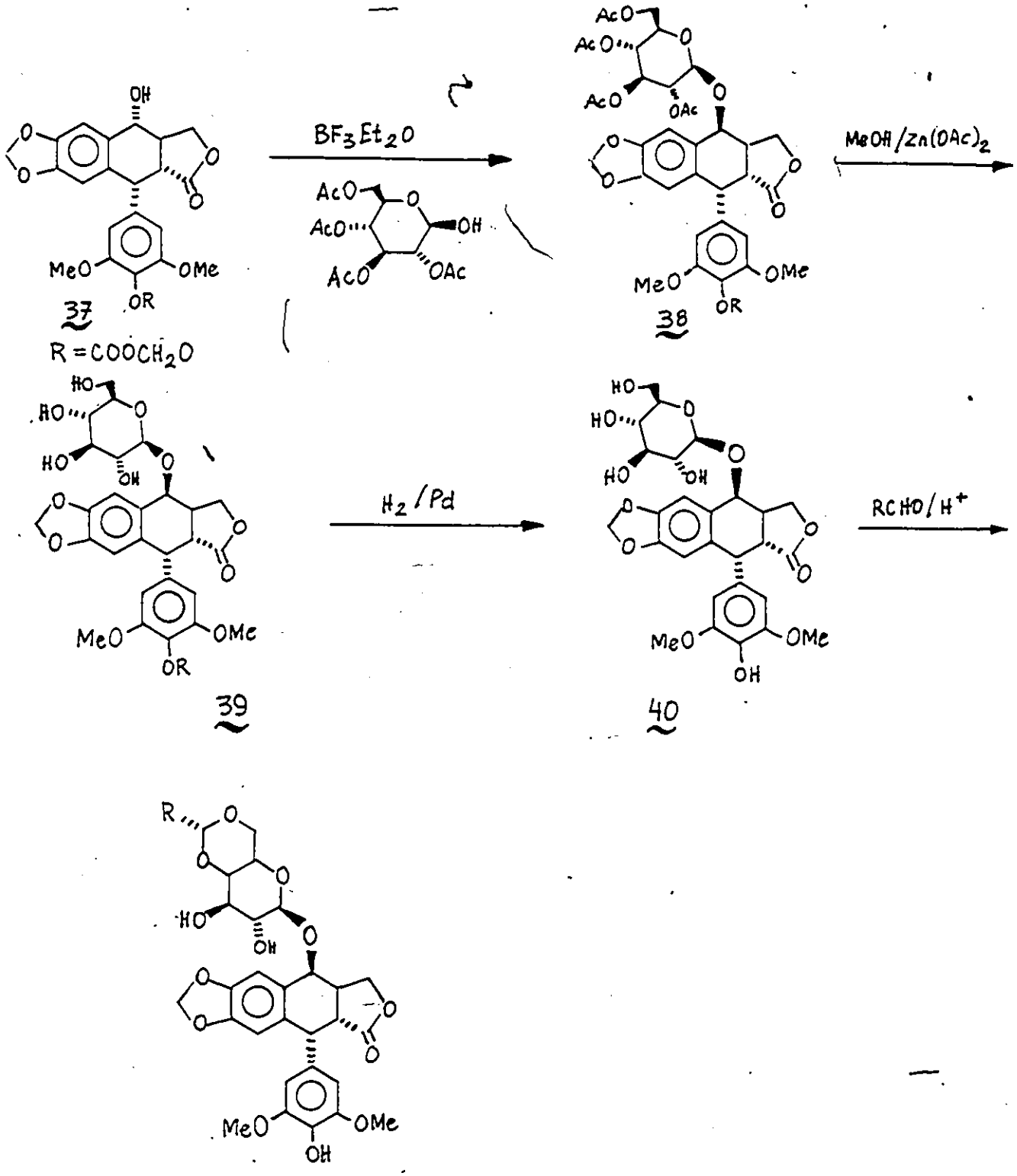
Neopodophyllotoxin 36 can be readily converted to podophyllotoxin by first opening of the lactone ring with base to give podophillic acid followed by lactonization with DCC[27].

The effect of podophyllotoxin and the peltatins on lymphomas and other implanted tumors in mice resulted in acute damage in all cases within twenty-four hours after a single subcutaneous injection. 4'-Demethyl analogues of podophyllotoxin have also shown similar results[28]. The antimitotic activity of podophyllotoxin apparently disrupts the assembly of microtubules, thus arresting the dividing cells in metaphase[29,30]. The toxicity of podophyllotoxin and its analogues, however, made them unsuitable as anticancer agents. Gensler suggested that epimerization at the C-2 position may constitute the primary method of detoxification by the cell and thus leading to loss in chemotherapeutic effectiveness[22].

Gensler attempted to synthesize C-2 analogues of podophyllotoxin to overcome this eperimization problem but was not successful in synthesizing derivatives which retained the desired trans-fused γ-lactone ring[31]. Gensler then decided to replace the lactone group by first reducing the carbonyl group to a methylene group and then replacing the lactone oxygen with a variety of substituents[32]. However, these compounds were found to be less effective[33].

Kuhn and von Wartburg developed a method for preparing glycosides of podophyllotoxin and its analogues[34,35] (Scheme 5). The cyclic acetals of 4'-demethylepipodophyllotoxin β-glycoside, which are epimeric at the C-4 position, were found to exhibit a high activity in in vitro testing but as important, were much less toxic in in vivo testing against mouse lymphocytic leukemia (L-1210)[36]. Two of these derivatives which showed promising results were: 4'-demethyl-1-O-(4,6-O-ethylidene-β-D-glucopyranosyl)-epipodophyllotoxin (VP-16 or Etoposide), 42; and, 4'-demethyl-1-O-(4,6-O-(2-thenylidene)-β-D-glucopyranosyl)-epipodophyllotoxin (VM-26 or Teniposide) 44 (Table 3). Both of these compounds have been cleared for use as chemotherapeutic agents in Western Europe, U.S.A. and Canada. Etoposide, the more commonly used derivative, is active against small cell lung cancer (oat cell lung cancer), bladder cancer and several types of solid brain tumors[37,38,39,40]. Etoposide is prescribed either by itself or in combination with other anticancer agents.

Surprisingly, Stähelin has shown that both compounds, 42 and 44, possess a different mechanism of action compared to podophyllotoxin[41]. These glycosides prevent the cells from entering mitosis in the late S or G_2 phase of the cell cycle unlike podophyllotoxin which arrests the cells in metaphase (see Figure 2). In fact, they have been shown not to bind to tubulin[42]. Much research has gone into determining this mechanism of action but to date there is no clear answer[43].



SCHEME 5: PREPARATION OF EPIPODOPHYLLOTOXIN GLYCOSIDES

TABLE 3: EFFECT OF CYCLIC ACETAL DERIVATIVES ON L-1210 LEUKEMIA

	R	MOUSE LEUKEMIA L-1210 % SURVIVAL TIME INCREASE
41	$CH_2CH=CH$	121
42	CH_3	167
43	CH_2CH_3	97
44	 X=S	121
45	X=O	136
46	C_6H_5	97
47	$C_6H_5CH_2$	46
48	1-naphthyl	95
49	$(CH_3)_3C$	57
50	$p\text{-}FC_6H_4$	64
51	$p\text{-}CH_3C_6H_4$	64

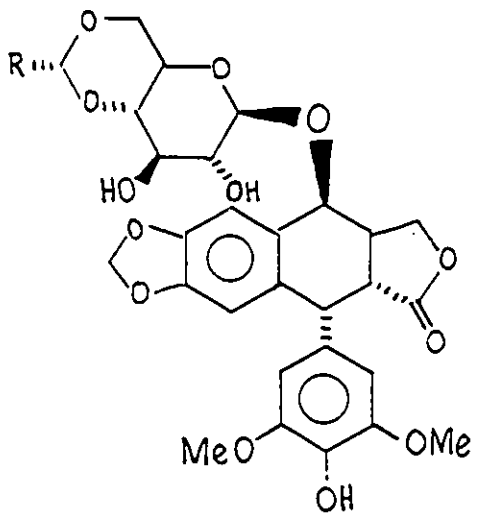
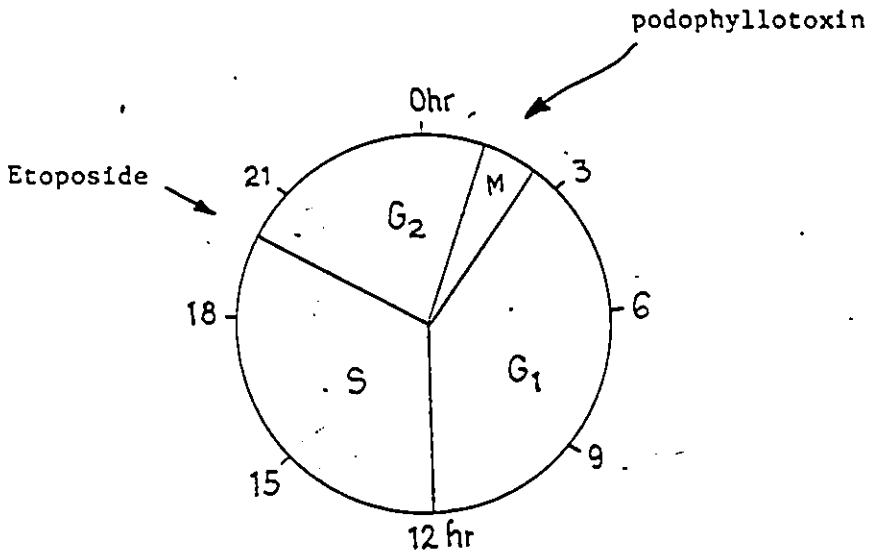


FIGURE 2: POINT OF INTERFERENCE OF PODOPHYLLOTOXIN AND ETOPOSIDE IN THE CELL CYCLE



RESULTS AND DISCUSSION

Part I

Synthesis of Nonenolisable Podophyllotoxin Derivatives

i) Reaction of the dianion of podophyllotoxin with hexachloroethane

This project commenced shortly after Glinski reported that 2-chloropodophyllotoxin had promising anticancer activity in addition to lower toxicity when compared to podophyllotoxin[44]. It was therefore decided to prepare several more 2-substituted derivatives for biological screening and to investigate further the effect of the 4-substituent on the podo to picro ratio*.

To this end the dianion, 52, of podophyllotoxin was prepared by treatment of podophyllotoxin 1 with two equivalents of LDA at -78° in dry THF. The enolate was treated with excess hexachloroethane at -78° for 30 minutes, warmed to 0° and stirred for an additional 1 h. Normal workup furnished a 2:3 mixture of 2-chloropodophyllotoxin and 2-chloropicropodophyllotoxin, respectively, in an overall yield of 80%[45].

* Nomenclature: The following nomenclature has been adopted for the substituted derivatives of podophyllotoxin. Derivatives having the same stereochemistry at carbons 2 and 3 as podophyllotoxin are named as being derived from the same. Similarly, derivatives having the same stereochemistry at carbons 2 and 3 as picropodophyllotoxin are named as being derived from picropodophyllotoxin.

To facilitate separation and identification of the isomers the mixture was converted into the 4-OSiMe$_3$ ethers using Me$_3$SiCl and pyridine in CH_2Cl_2. The 4-OTMS isomers were then separated by PTLC (ethyl acetate/hexanes 1:3, 3 developments) to afford 4-O-trimethylsilyl-2-chloropodophyllotoxin 53 (Figure 3) and 4-O-trimethylsilyl-2-chloropicropodophyllotoxin 54 (Figure 4). The yields of 53 and 54 were 24% and 32%, respectively. The structures of the C-2 substituted lignan lactone derivatives described in this thesis were assigned mainly on the basis of their high field ^{1}H NMR spectra. Substitution at C-2 was always clearly evident by the presence of a sharp singlet due to H_1 in the 4.1-4.8 ppm range (Table 4).

The stereochemistry at C-2 and C-3 was assigned on the basis of the value of the coupling constants $J_{H_3H_{11}}$ and $J_{H_3H_{11'}}$ and on variations in the chemical shifts of the aromatic protons. It had been observed earlier by Gensler et al that $J_{H_3H_{11}}$ and $J_{H_3H_{11'}}$ were higher in podophyllotoxin and derivatives ($J_{H_3H_{11}}$ = 9.0 Hz and $J_{H_3H_{11'}}$ = 8.0 Hz for 1) than in picropodophyllotoxin ($J_{H_3H_{11}}$ = 6.0 Hz and $J_{H_3H_{11'}}$ = 1.5 Hz). Furthermore in the series of podo compounds the chemical shift of the aromatic singlet due to $H_{2'}H_{6'}$ was consistently at higher field than either H_5 or H_8. In contrast, in picropodophyllotoxin $H_{2'}H_{6'}$ is at lower field than H_8. Glinski has used these criteria in the assignment of the configuration of the 2-methyl and 2-chloro derivatives[44].

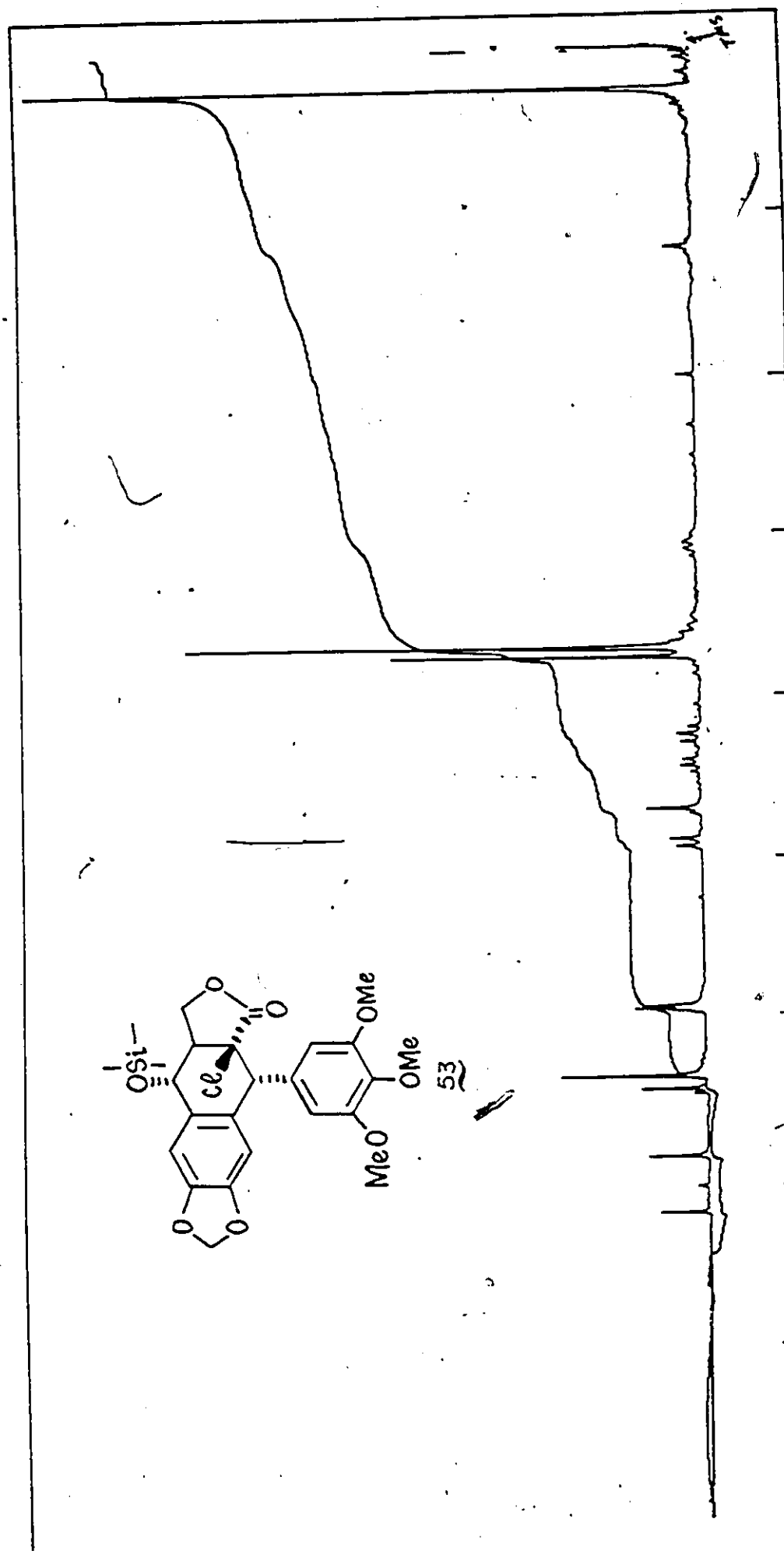


FIGURE 3: ^1H NMR SPECTRUM OF 4-O-TRIMETHYLSILYL-2-CHLOROPODOPHYLLOTOXIN

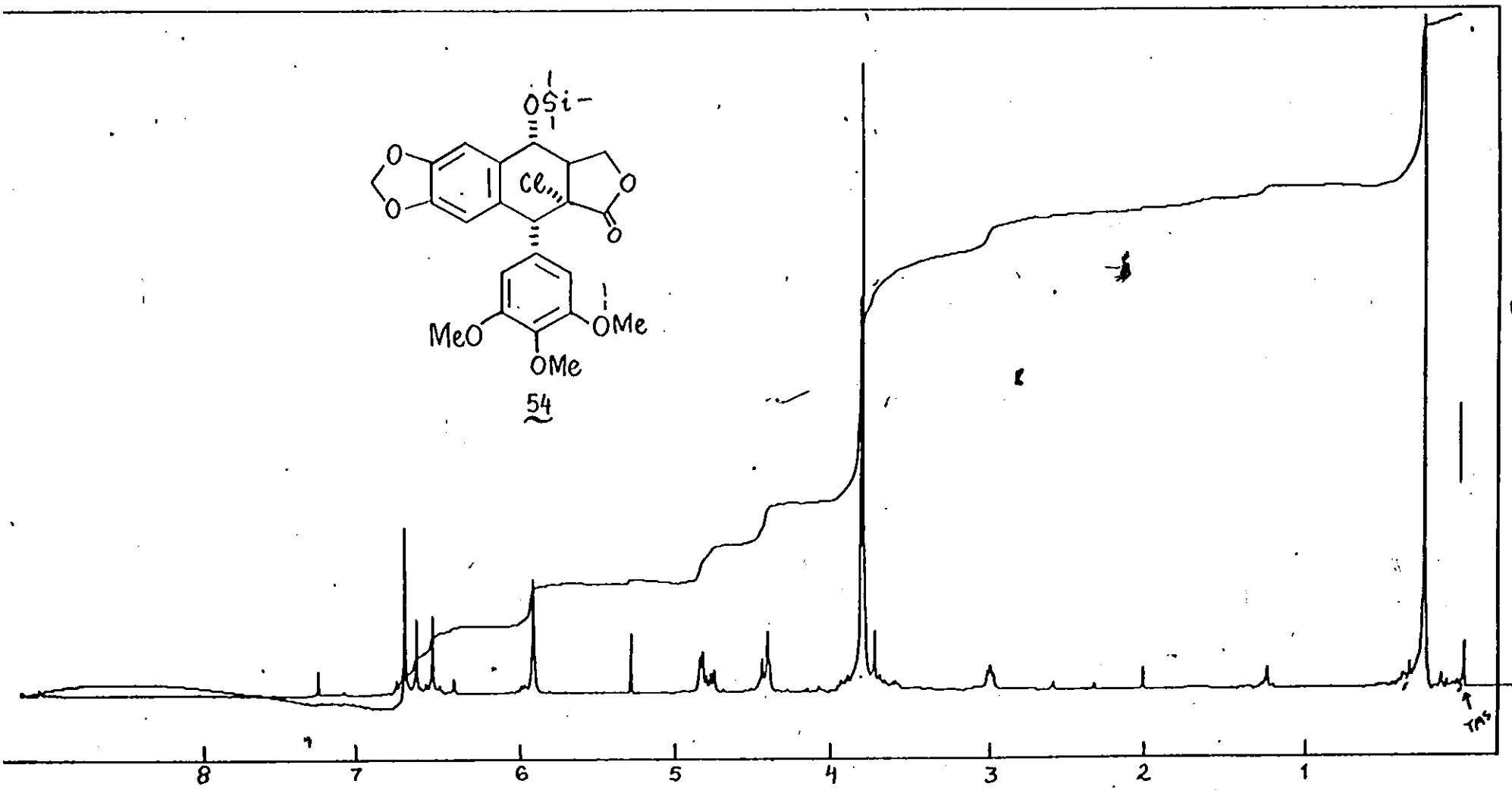


FIGURE 4: ^1H NMR SPECTRUM OF 4-O-TRIMETHYLSILYL-2-CHLOROPICROPODOPHYLLOTOXIN

The less polar isomer 53 showed a singlet at δ = 4.75 due to H_1 and aromatic singlets at 6.41 (2H), 6.49 (1H) and 6.93 (1H) indicative of the podo stereochemistry. This assignment was corroborated by the 9.9 and 7.1 Hz coupling constants for $J_{H_3H_{11}}$ and $J_{H_3H_{11}}$". Hydrolysis of the 4-OTMS group afforded 2-chloropodophyllotoxin 55 (mp 105-108°, lit. mp 106-110°[44]) having a similar NMR with that prepared earlier by Glinski. The relevant chemical shifts and coupling constants show the same pattern for both 53 and 55.

The other NMR peaks for 53 were as expected. The presence of the TMS group was clearly indicated by a singlet at 0.26 ppm. The methylene-dioxy protons are magnetically non-equivalent and appeared as two doublets centered at 5.97 and 5.98 ppm (J = 0.5 Hz). The presence of the methoxy groups occurred as two peaks (1:2 ratio) at 3.80 and 3.75 ppm due to $H_{4'}$ and $H_{3',5'}$ respectively. The MS of 53 showed a M^+ at 520 with $M^+ + 2$ = 522 in a 3:1 ratio, thereby confirming the presence of one chlorine atom in this molecule.

In contrast 54 was assigned the picro configuration. This was based both on the aromatic pattern [δ = 6.55 (H_8), 6.65 (H_5), 6.72 ($H_{2'}H_{6'}$] and the observation of a 4.6 Hz coupling constant for $J_{H_3H_{11}}$". $J_{H_3H_{11}}$ was negligible. In addition 54 also showed singlets at δ = 0.27 ($SiMe_3$), 3.82 ($H_{3'}H_{5'}$), 3.83 ($H_{4'}$) and 4.43 (H_1). Again as in 53 the MS showed clearly the presence of only one chlorine atom.

Hydrolysis of the 4-OTMS group in 54 was carried out in 73% yield in a 4:1 acetic acid/H_2O mixture at room temperature for 1 h. 2-Chloropicropodophyllotoxin 56 thus obtained had a mp of 99-104°. It showed aromatic singlets at 6.61 (H_8), 6.75 (H_5) and 6.77 ($H_{2'}H_{6'}$) suggesting the picro stereochemistry (Figure 5). Again $J_{H_3H_{11'}}$ and $J_{H_3H_{11}}$ were found to be significantly smaller than the coupling in podophyllotoxin or in 53 or 55.

Attempted desilylation of 54 with tetrabutylammonium fluoride in THF gave the naphthalene lactone 57, mp 283-285°, lit. mp 270° in 51% yield[47,48]. Compound 57 had NMR peaks at 3.83 (s,6H) 3.93 (s,3H) 5.35 (s,2H), 6.05 (s,2H), 6.53 (s,2H) 7.12 (s,1H) 7.18 (s,1H) and 7.68 (s,1H). The ease with which 54 was aromatized to 57 via initial loss of HCl followed by H_2O is consistent with a trans arrangement at C-1 and C-2 (Scheme 6). This trans arrangement at C-1 and C-2 was clearly evident in 53 as desilylation of 53 with tetrabutylammonium fluoride in THF gave 2-chloropodophyllotoxin 55 in 60% yield and not the aromatized derivative 57.

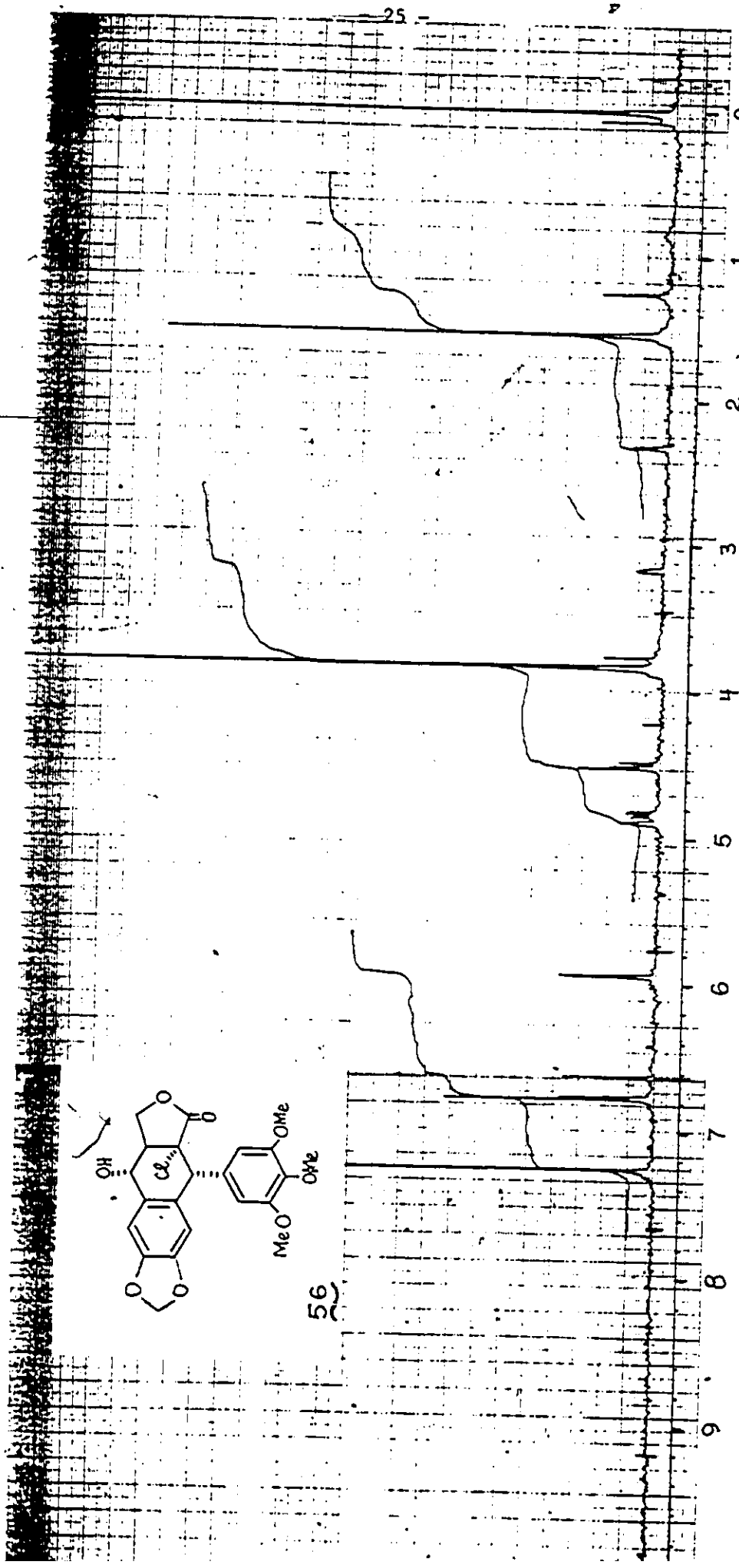
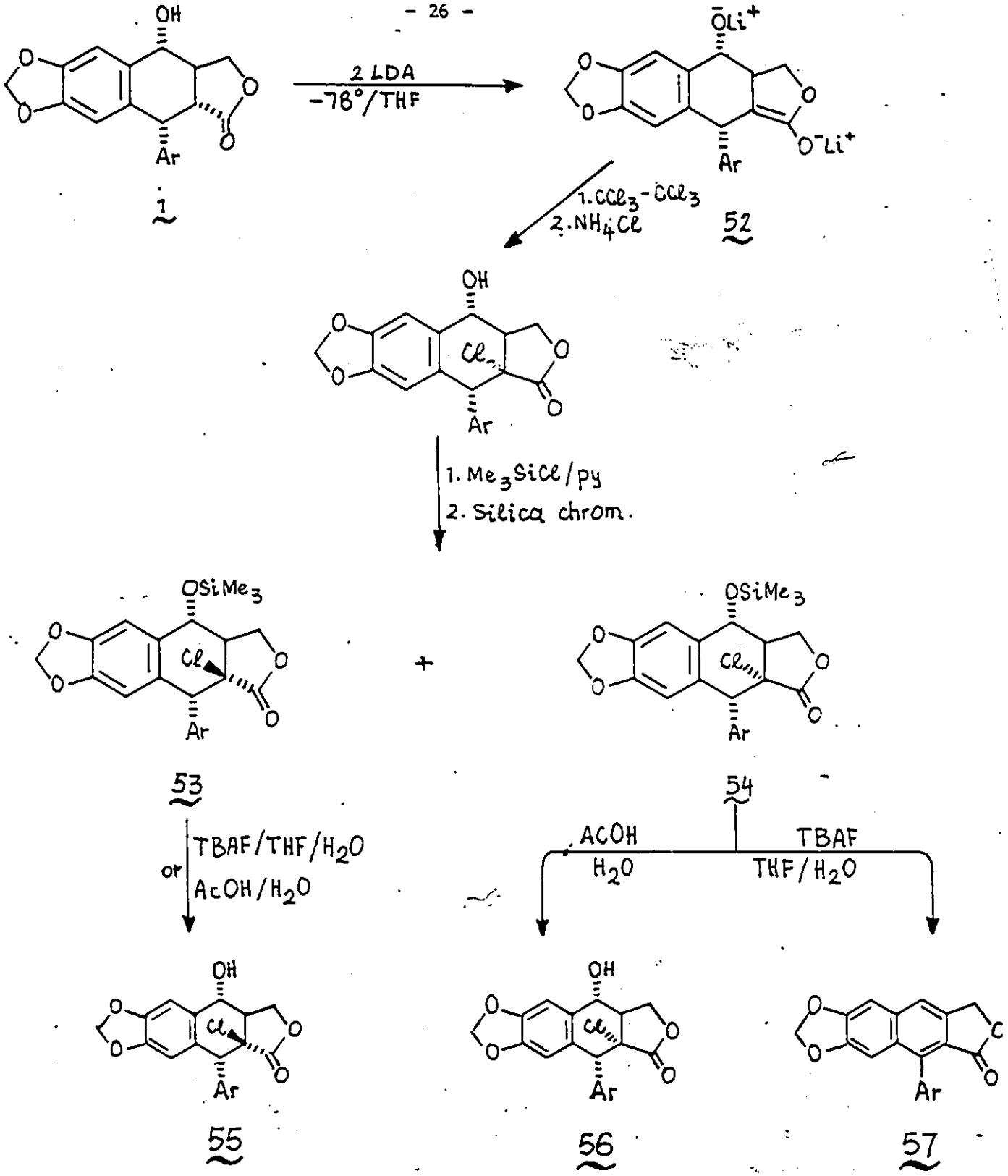


FIGURE 5: ^1H NMR SPECTRUM OF 2-CHLOROPICROPODOPHYLLOTOXIN



SCHEME 6: DIANION ROUTE TO 2-CHLORO-PODO/PICROPODOPHYLLOTOXIN

The ease of loss of HCl from the silylated derivatives 53 and 54 under thermal conditions was qualitatively observed by varying the insertion probe temperature in the mass spectra of these compounds. 4-OSiMe$_3$-2-chloropodophyllotoxin 53 showed a strong molecular ion cluster at 520/522 with a probe temperature of 200° (Figure 6). In contrast the 2-chloro picro isomer 54 showed no evidence of the 520/522 peaks at 200°; however, it gave a very strong peak at m/e = 394 indicative of $M^{+\cdot}$-HCl-HOSiMe$_3$ (Figure 7). When the probe temperature was lowered to 170° the molecular ion cluster at 520/522 was observed. The ease of loss of HCl in 54 is in agreement with a transoid arrangement between H and Cl at C-1 and C-2 respectively.

ii) Reaction of the dianion of podophyllotoxin with bromine

The bromination of the dianion of podophyllotoxin was briefly investigated. Treatment of the dianion 52 with Br_2 for 2.5 h at -78° followed by warming to 0° for 1.5 h gave after workup a complex mixture from which only one nonpolar component was isolated in pure form via preparative thin layer chromatography. This compound had: mp 228-230°; ^{1}H NMR: 3.80 (s,3H), 3.95 (s,3H) 4.09 (s,3H) 5.40 (s,1H), 5.42 (s,1H), 6.09 (s,2H) 6.60 (s,1H) 6.86 (s,1H) 7.22 (s,1H) and 7.72 (s,1H); and, MS: $M^{+\cdot}$ = 472, $M^{+\cdot}$ + 2 = 474 (1:1 ratio). The TLC behaviour of this compound was quite similar to the naphthalene lactone 57 thus suggesting that an aromatization reaction had taken place. This was corroborated by both the ^{1}H NMR and the mass spectrum.

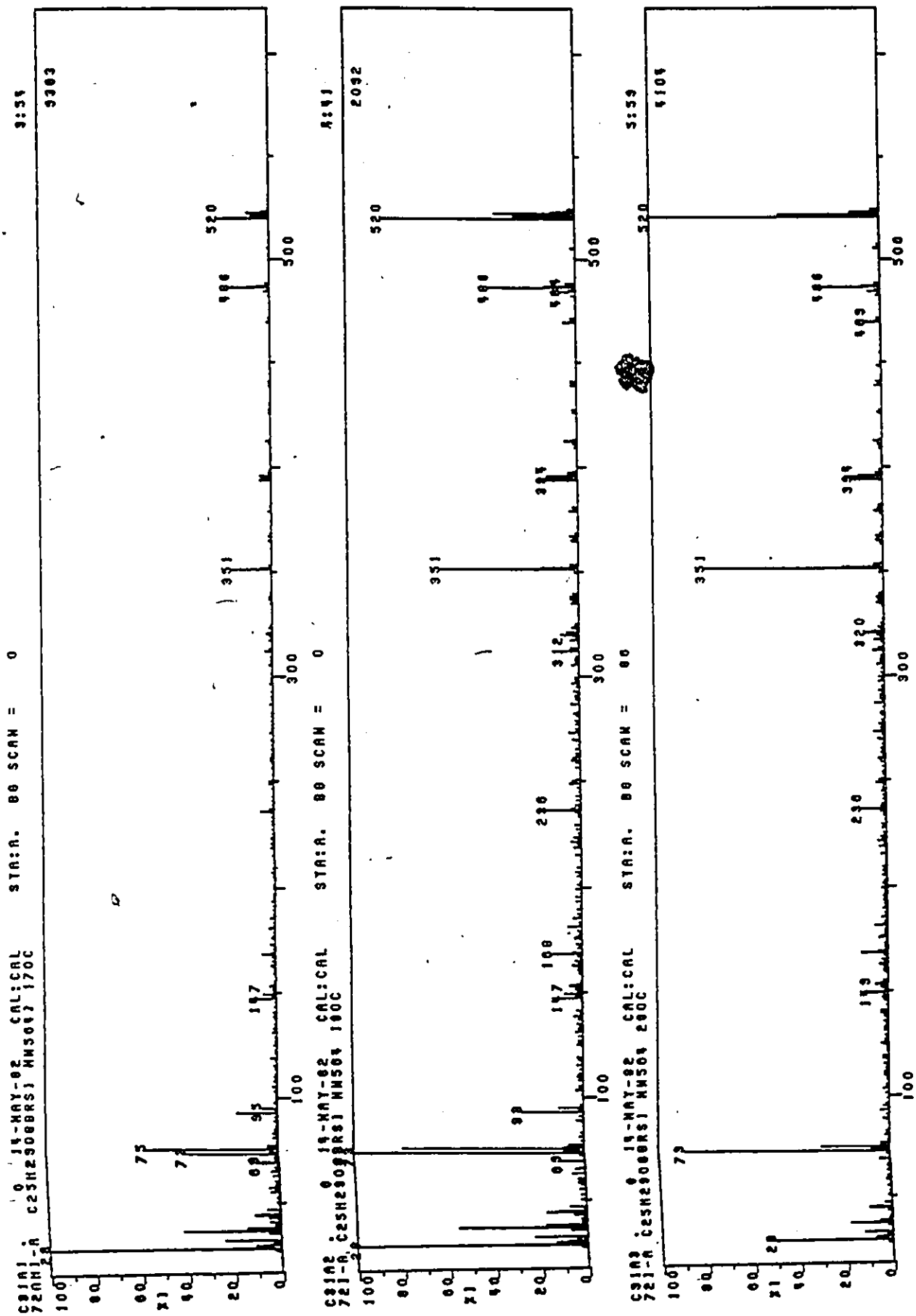


FIGURE 6: MASS SPECTRA OF 53 WITH VARIOUS PROBE TEMPERATURES

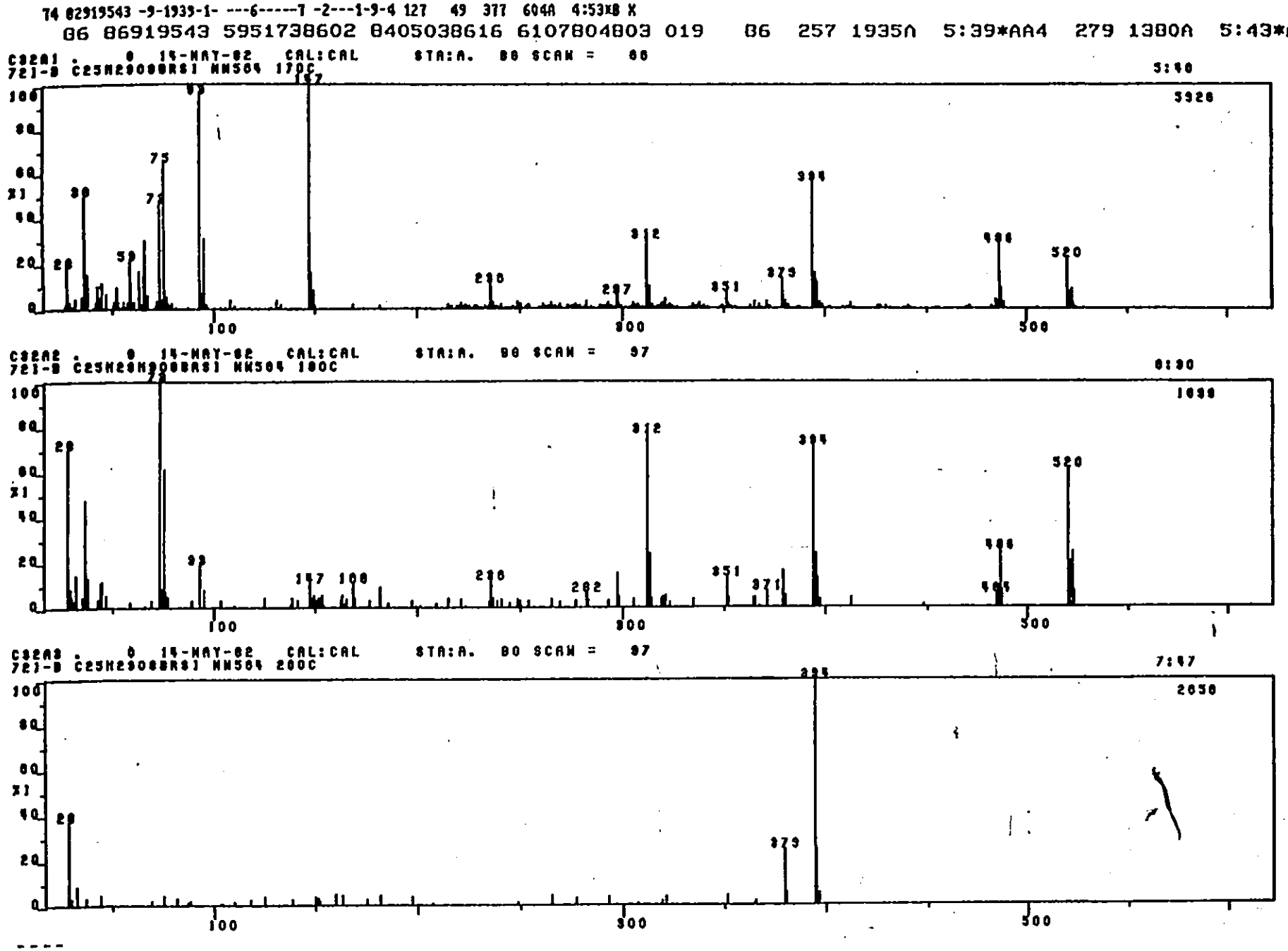
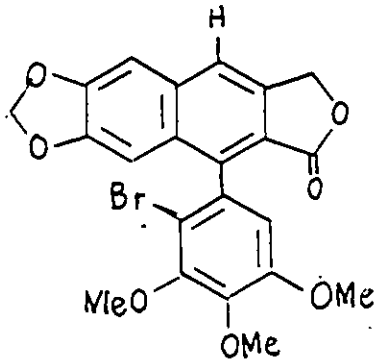


FIGURE 7: MASS SPECTRA OF 54 WITH VARIOUS PROBE TEMPERATURES

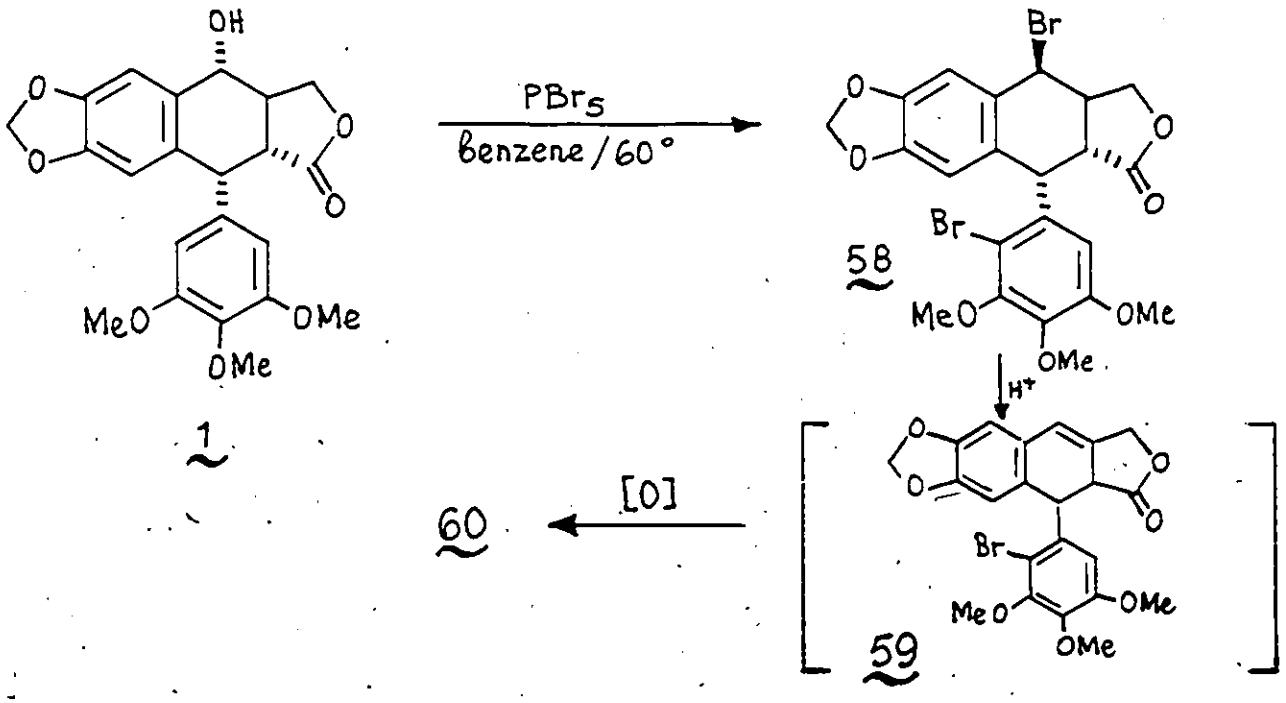
In addition the MS indicated the presence of one Br atom. Its position was suggested by the proton NMR which at δ = 3.80, 3.95 and 4.09 ppm showed three nonequivalent methoxy groups thereby placing the bromine in ring E.



60

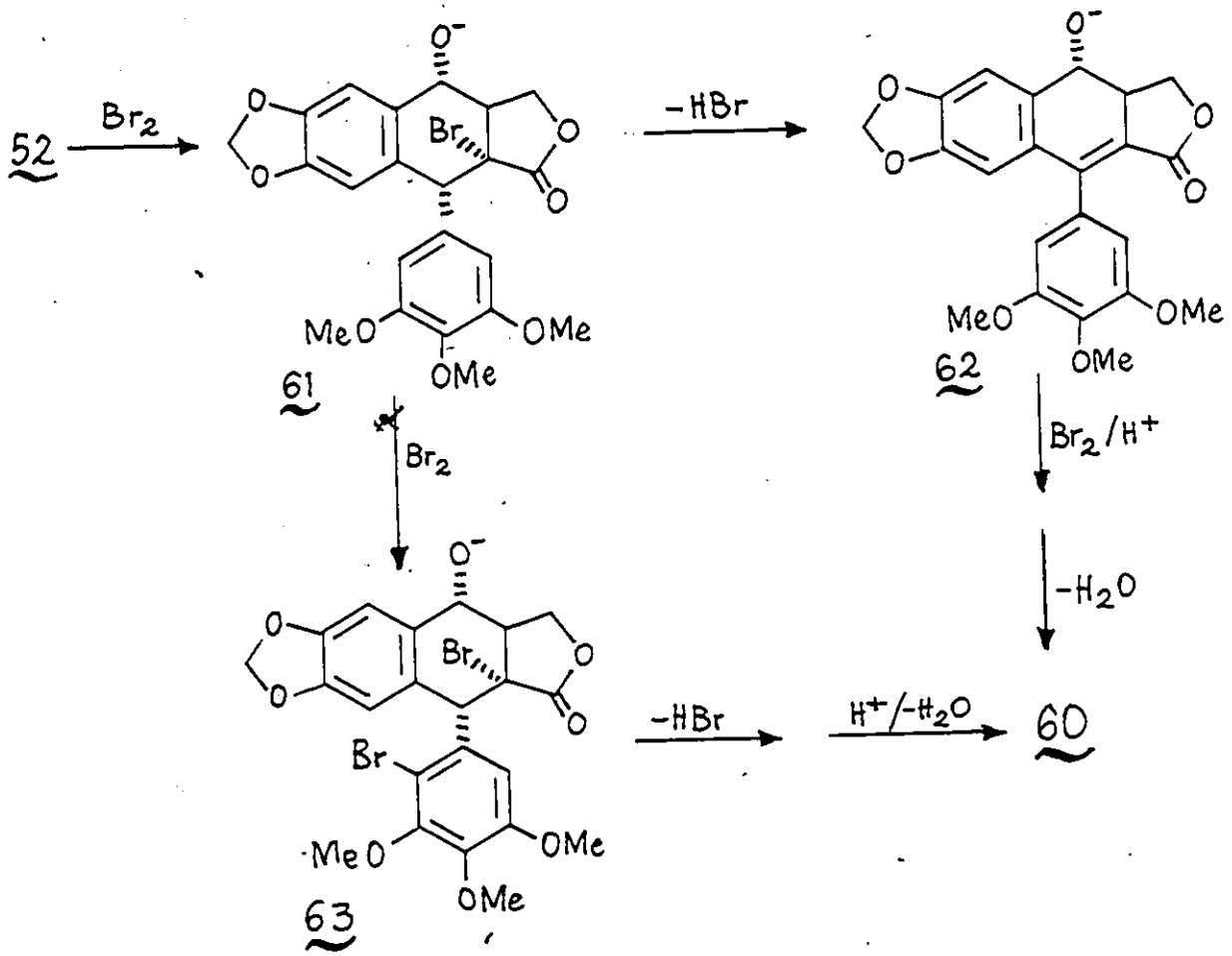
The proposed structure 60 is identical to that reported by Ayres and Lim[49]. These authors had prepared 60 by boiling a solution of 2'-bromoepipodophyllotoxin[58] in acetone containing 2N-hydrochloric acid (Scheme 7). Ayres had postulated that the formulation of 60 proceeded via the intermediate 59. This intermediate, an α-apopodophyllotoxin, could not be purified as either the chloro or bromo derivative owing to the ease of the subsequent dehydrogenation step.

SCHEME 7: PREPARATION OF 2'-BROMONAPHTHALENE LACTONE



1 58 60

Several mechanisms can be suggested for the formation of 60 during the bromination of the dianion 52. The most plausible are shown below.



Bromination of 52 would be expected to yield the picro isomer as the major product. Since the hydrogen at C-1 and the bromine at C-2 are trans to each other, a base catalysed elimination of HBr is highly probable either before or after further bromination of ring E. Ayres and Lim have shown that the ring E in podophyllotoxin is reactive towards a brominating agent. Further loss of H_2O upon workup would yield 60.

iii) Reaction of the anion of 4-O-tetrahydropyranylpodophyllotoxin with various bromine reagents

The methylation, chlorination and bromination of the dianion 52 do not yield the 2-substituted derivatives having the podophyllotoxin configuration in major amounts. Glinski had earlier shown that methylation and chlorination of the 4-OTHP derivative 27 gave the 2-substituted podophyllotoxin derivatives as the major or exclusive product[44]. In order to prepare other possible 2-substituted podophyllotoxin derivatives we continued this work using this protected derivative of podophyllotoxin.

Treatment of 4-O-tetrahydrapyranylpodophyllotoxin, obtained in 85% yield from podophyllotoxin 1 and dihydropyran in the presence of p-toluenesulfonic acid as catalyst, with one equivalent of LDA at -78° in dry THF generated the enolate 64. This enolate was trapped with bromine (-78° to rt, 15 minutes) and then quenched with a saturated solution of Na_2SO_3. Preparative thin layer chromatography afforded, in order of elution, 4-O-tetrahydropyranyl-2-bromopodophyllotoxin 65 and 4-O-tetrahydropyranyl-2-bromopicropodophyllotoxin 66 in yields of 35% and 21%, respectively (Figures 8 and 9).

Similarly, treatment of the enolate 64 with carbon tetrabromide afforded 65 and 66 in yields of 11 and 33%, respectively. Trapping of the enolate 64 with benzene sulfinyl bromide gave a mixture of 3 components. They were identified, in order of elution, as 65, 66 and 27 in yields of

16, 32 and 40%, respectively. The MS of both 65 and 66 showed M^+ at 576 and $M^+ + 2$ at 578 in a 1:1 ratio, thereby confirming the presence of only one bromine atom in the molecule.

The stereochemistry at C-2 and C-3 for compounds 65 and 66 could not be made on the basis of the coupling constants $J_{H_3H_{11}}$ and $J_{H_3H_{11}"}$ because of the complexity of the NMR spectra due to the presence of diastereomeric mixtures caused by the 4-OTHP group. However, the stereochemistry assignments were based on the variations in the chemical shift patterns of the aromatic protons. The chemical shift pattern for H_5,H_8 and $H_{2'}H_{6'}$ in 65 (Figure 7) was consistent to that of the podo series since $H_{2'}H_{6'}$ appeared to be at higher field than protons H_5 and H_8. In contrast, the pattern for these protons in 66 (Figure 8) showed a downfield shift for $H_{2'}H_{6'}$ characteristic of the picro configuration.

Hydrolysis of the THP derivative 65 using Glinski's procedure afforded 2-bromopodophyllotoxin 67, mp 89-93°, in 51% yield (Figure 10). This compound showed a singlet at δ = 4.91 due to H_1 and aromatic singlets at δ = 6.42 (2H), 6.52 (1H) and 7.10 (1H) strongly indicative of the podo stereochemistry. This assignment was corroborated by the large coupling constants of 9.6 and 6.8 Hz for $J_{H_3H_{11}}$ and $J_{H_3H_{11}"}$ respectively. The remaining chemical shifts and coupling constants of 67 showed the same pattern as that found in 2-chloropodophyllotoxin (Tables 4 and 5).

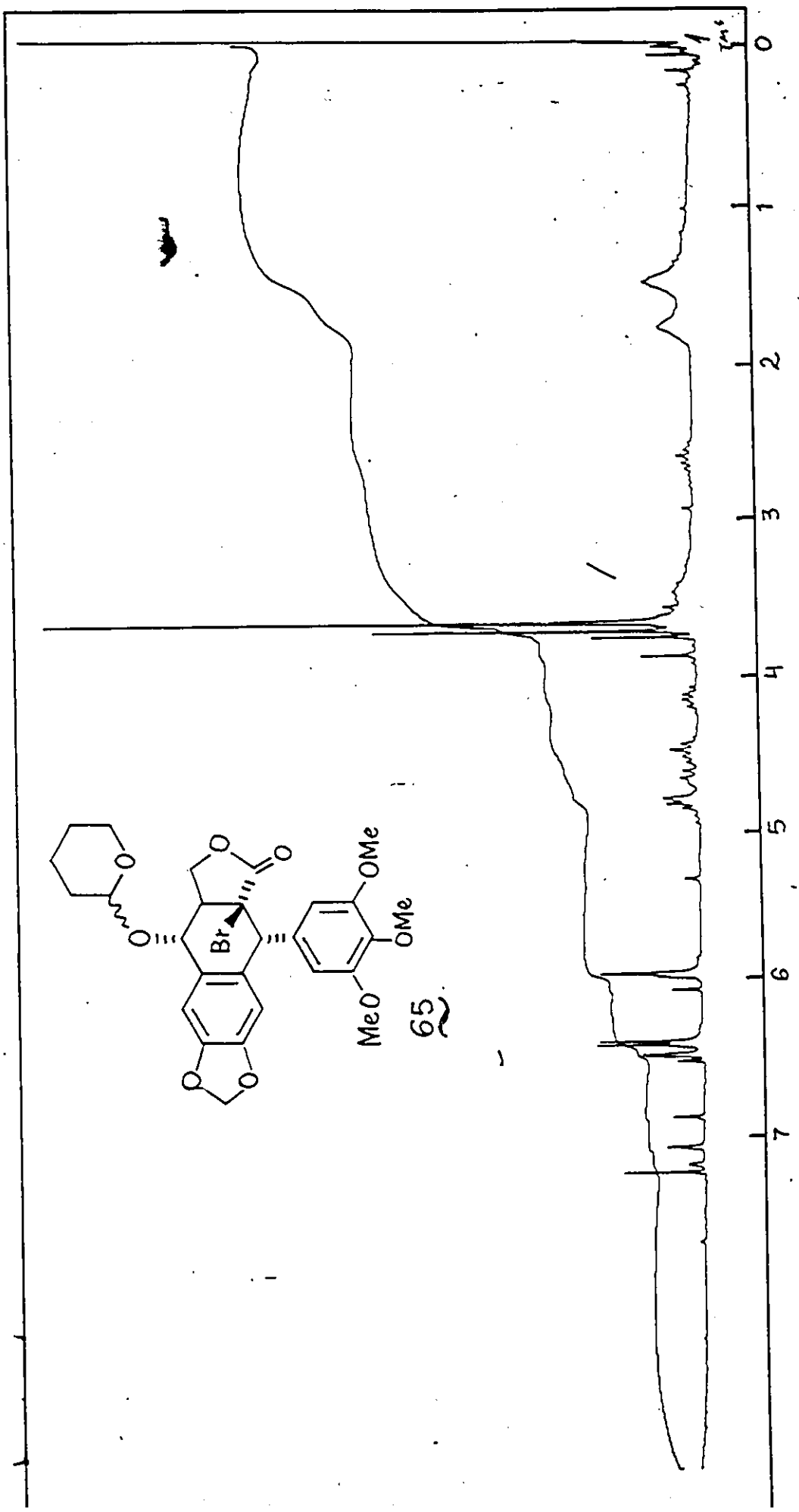


FIGURE 8: ^{1}H NMR SPECTRUM OF 4-OTHP-2-BROMOPODOPHYLLOTOXIN

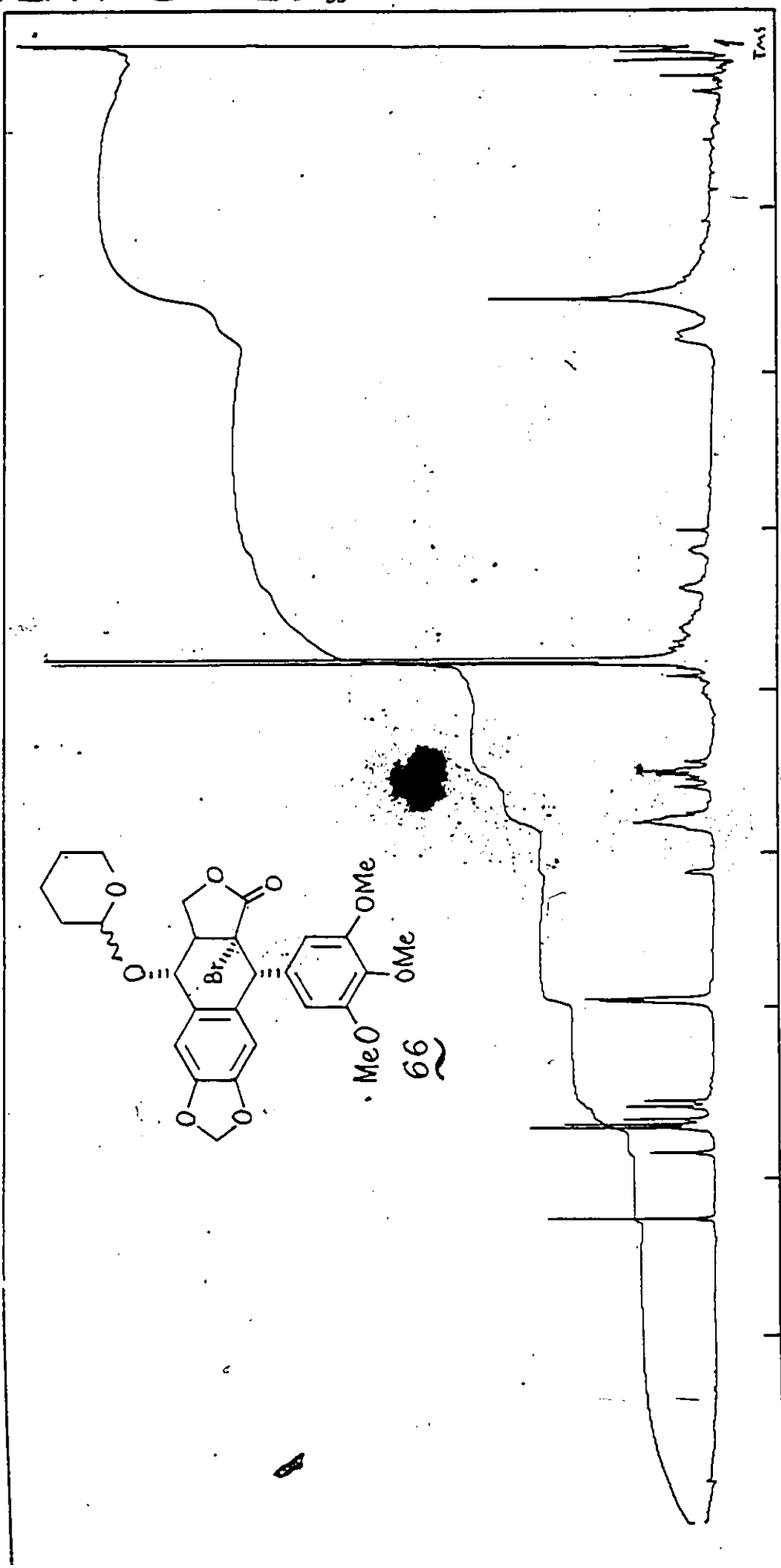


FIGURE 9: ^1H NMR SPECTRUM OF 4-OTHP-2-BROMOPICROPODOPHYLLOTOXIN

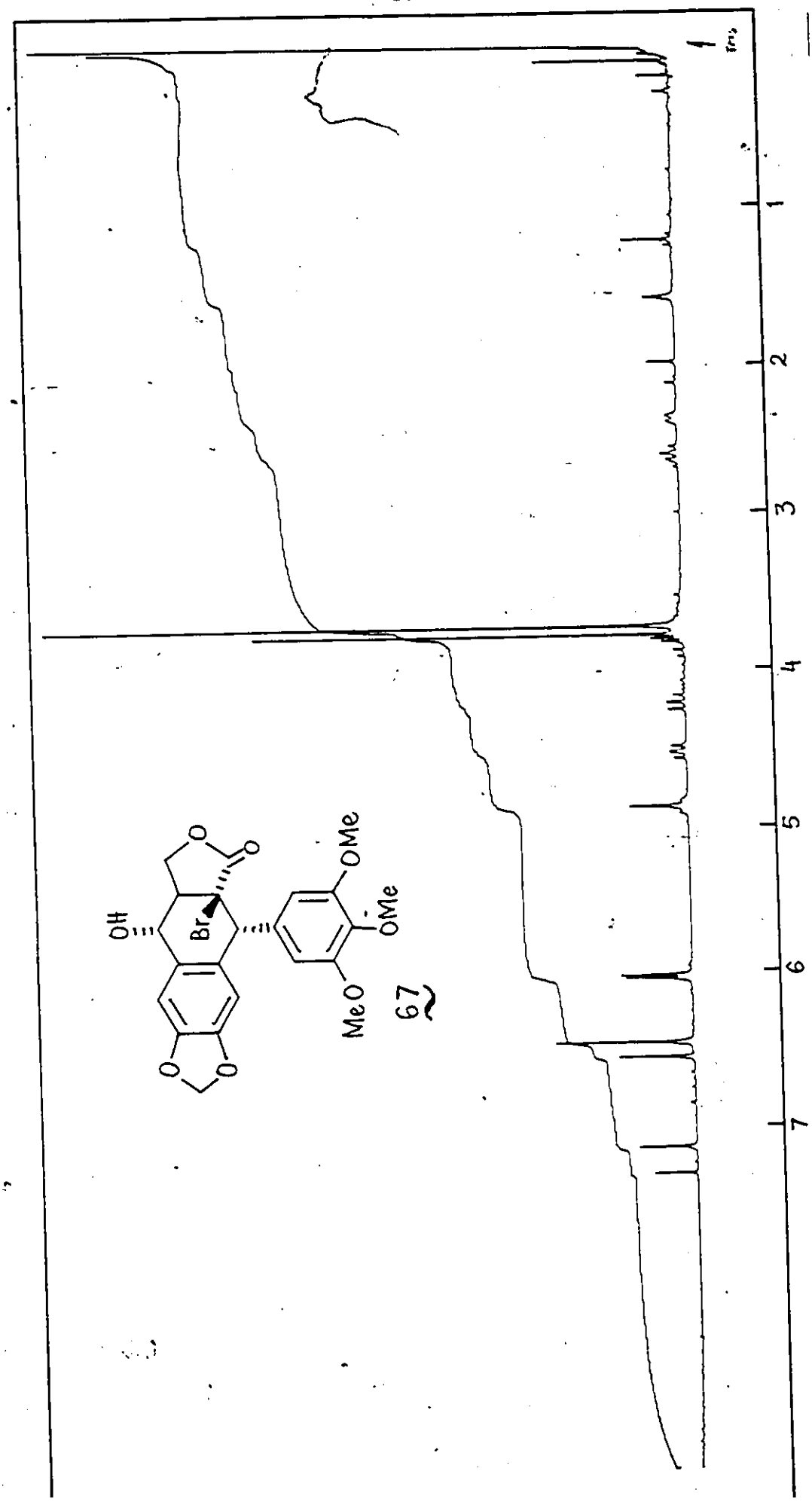


FIGURE 10: ^1H NMR SPECTRUM OF 2-BROMOPODOPHYLLOTOXIN

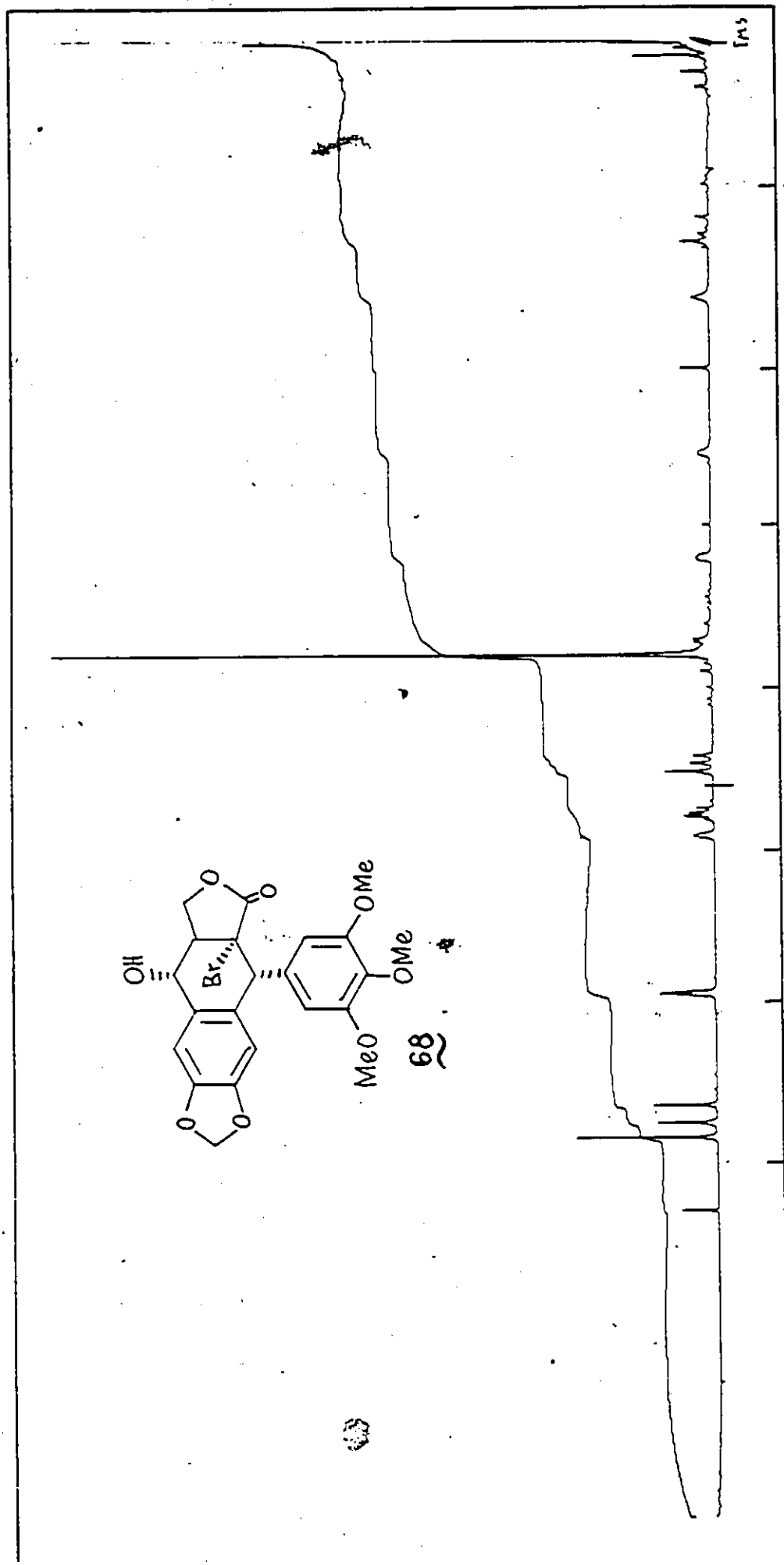


FIGURE 11: ^1H NMR SPECTRUM OF 2-BROMOPICROPODOPHYLLOTOXIN 68

Hydrolysis of the more polar isomer 66 gave 2-bromopicropodophyllotoxin 68, mp 93-98°, in 60% yield. Singlets at δ = 6.61 (1H), 6.72 (1H), and 6.81 (2H) for the aromatic protons indicated a picro structure (Figure 11). As in 2-chloropicropodophyllotoxin, only a 4.8 Hz coupling constant for $J_{H_3H_{11}}$ was observed and $J_{H_3H_{11}}$ was found to be negligible. The remaining chemical shifts and coupling constants for 68 were similar to those found in 2-chloropicropodophyllotoxin.

iv) Reaction of the anion of 4-O-tetrahydropyranylpodophyllotoxin with dimethyldisulfide

In order to complete the synthesis of a series of non-halogen 2-substituents of podophyllotoxin we attempted to prepare the 2-thiomethyl derivatives. Such groups are typically introduced to a carbonyl group via reaction of the enolate with sulfur electrophiles e.g. disulfides (RSSR) or thiolsulfonates (RSO_2SR).

Treatment of 4-O-tetrahydropyranylpodophyllotoxin 27 with one equivalent of LDA at -78° in dry THF generated the enolate 64 which was subsequently trapped with dimethyl disulfide (-78° to rt). Normal workup and PTLC gave only one isomer 69 in 54% yield which could not be placed into either the 2-podo- or 2-picro- series.

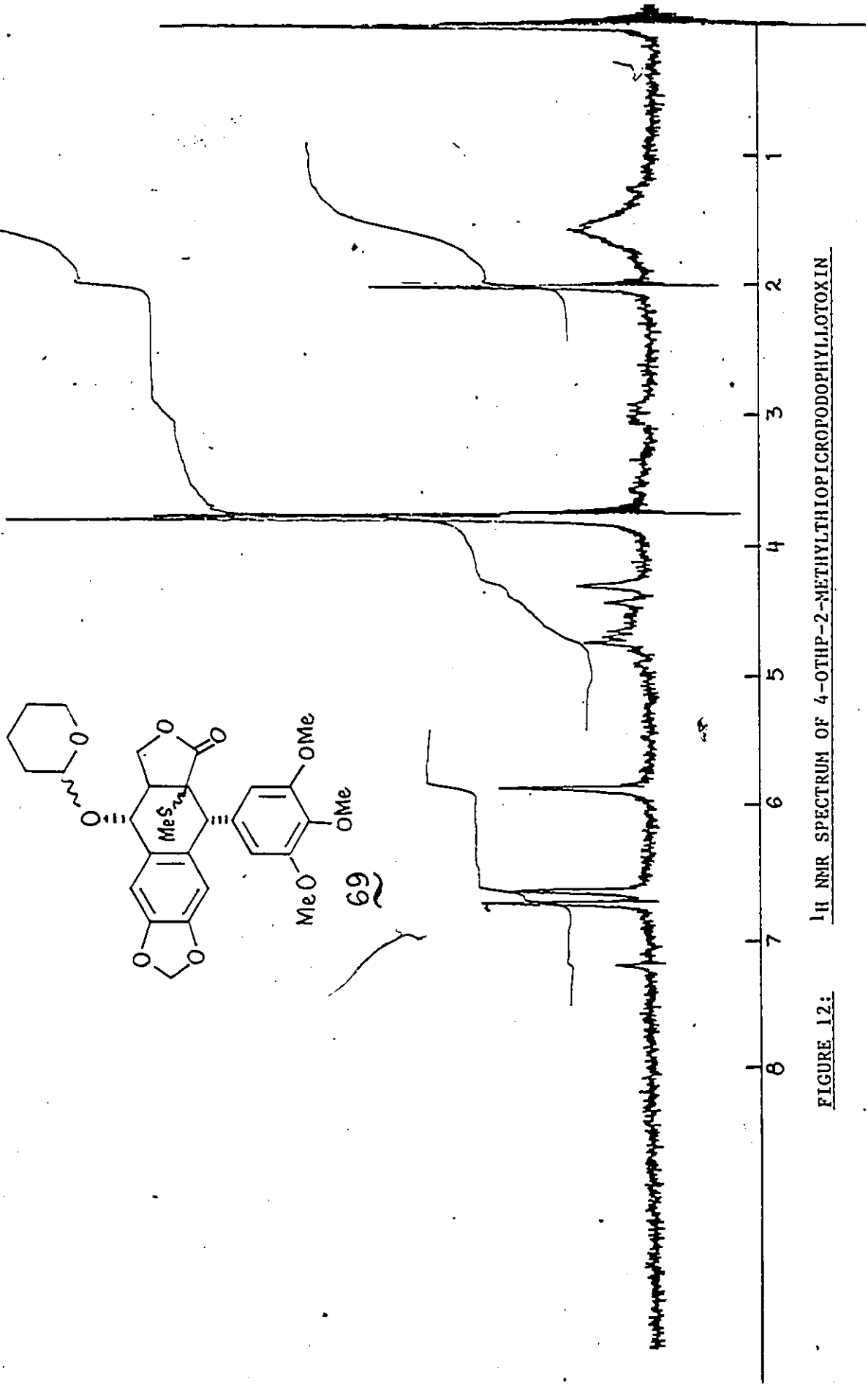


FIGURE 12: ^1H NMR SPECTRUM OF 4-OTHP-2-METHYLTHIOPICROPODOPHYLLOTOXIN

The MS of 69 showed a M^+ at 544 confirming the introduction of a methylthio group. The NMR showed a singlet at 2.01 ppm (3H) thereby corroborating the above conclusion. As in the 2-bromo derivatives 65 and 66 the stereochemistry between C-2 and C-3 could not be made on the basis of the coupling constants $J_{H_3H_{11}}$ and $J_{H_3H_{11}"}$ due to the presence of diastereoisomers. In addition, the stereochemistry assignment of 69 could not be assumed on the chemical shift pattern of the aromatic protons as was in the case for 65 and 66. The chemical shift pattern for H_5, H_8 and $H_{2'}H_{6'}$ showed only two singlets at δ = 6.70 (2H) and 6.80 (2H) which was uncharacteristic for either a podo or picro isomer (Figure 12).

Hydrolysis of 69 was carried out using a 5% HCl/THF solution (1:9) which afforded, in 70% yield, a single compound eventually identified as 2-(methylthio)picropodophyllotoxin 70, mp 97-101°. The NMR spectrum of 70 showed aromatic singlets at δ =6.72 (H_8), 6.75 (H_5) and 6.91 ($H_{2'}H_{6'}$) suggesting the picro stereochemistry (Figure 13). This assignment was confirmed by the small coupling constants of 4.7 and 2.6 Hz for $J_{H_3H_{11}"}$ and $J_{H_3H_4}$ respectively. Again as in the 2-chloro and 2-bromo picro derivatives no coupling for H_3H_{11} was evident. The remaining chemical shifts and coupling constants of 70 showed a similar pattern as found for the corresponding 2-chloro and 2-bromo compounds, 56 and 68 respectively (Tables 4 and 5). The mass spectrum of 70 clearly showed a molecular ion at m/e = 460.

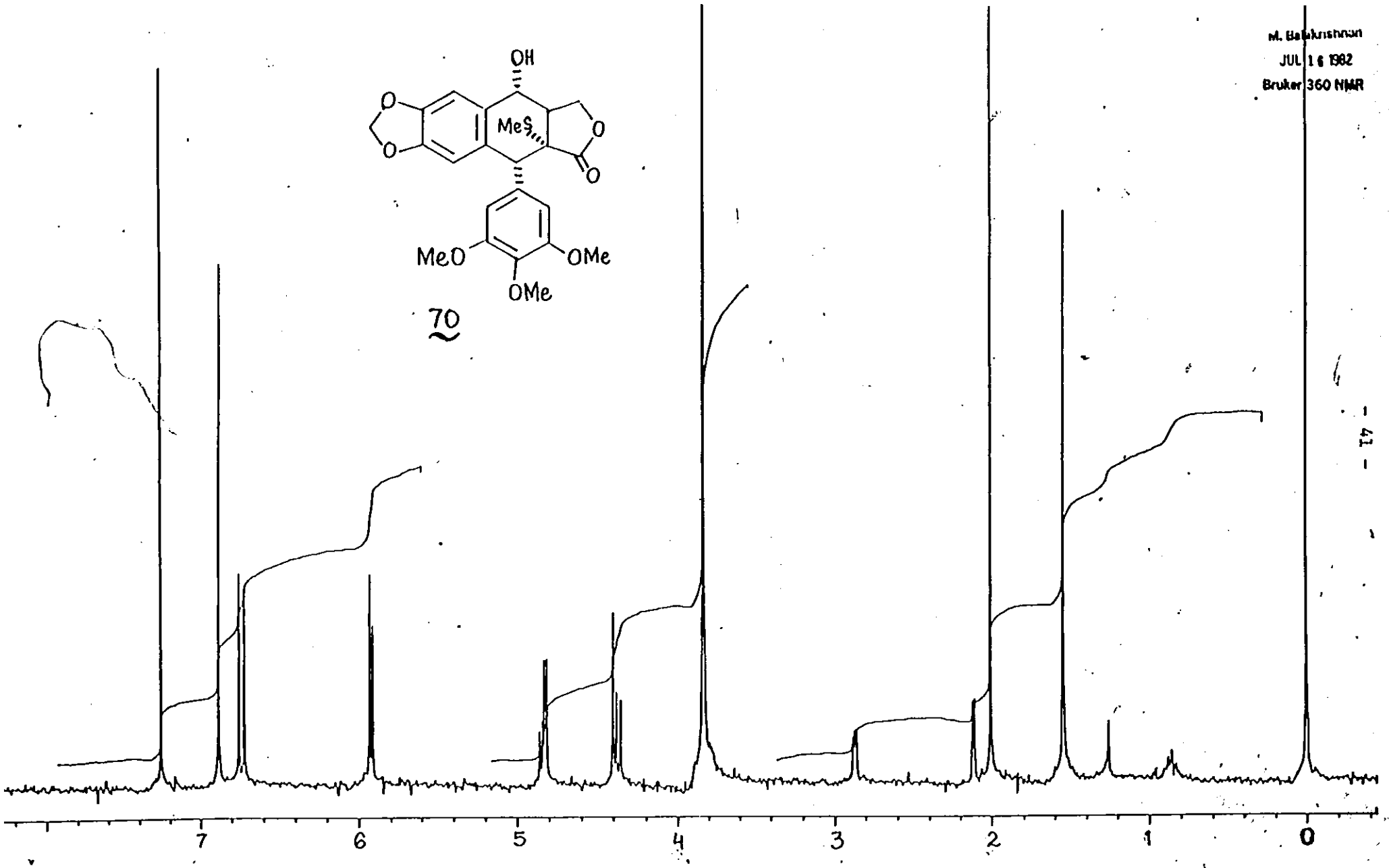
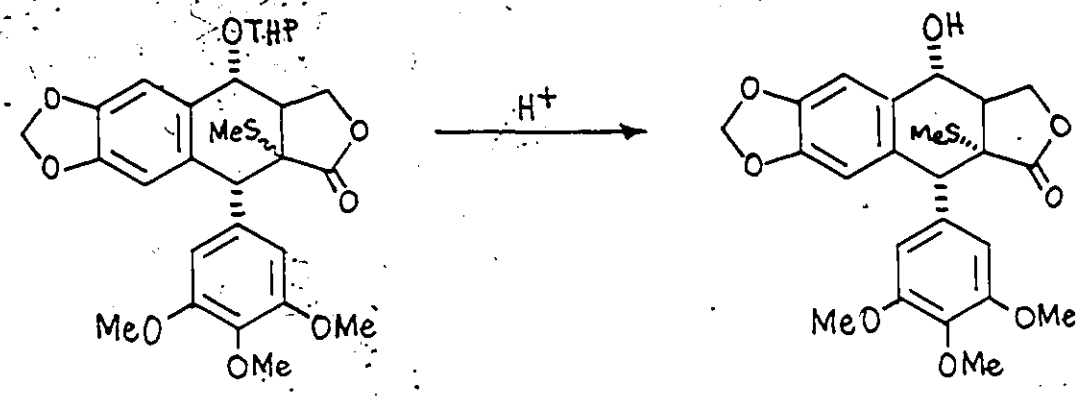


FIGURE 13: ^{1}H NMR SPECTRUM OF 2-METHYLTHIOPICROPODOPHYLLOTOXIN



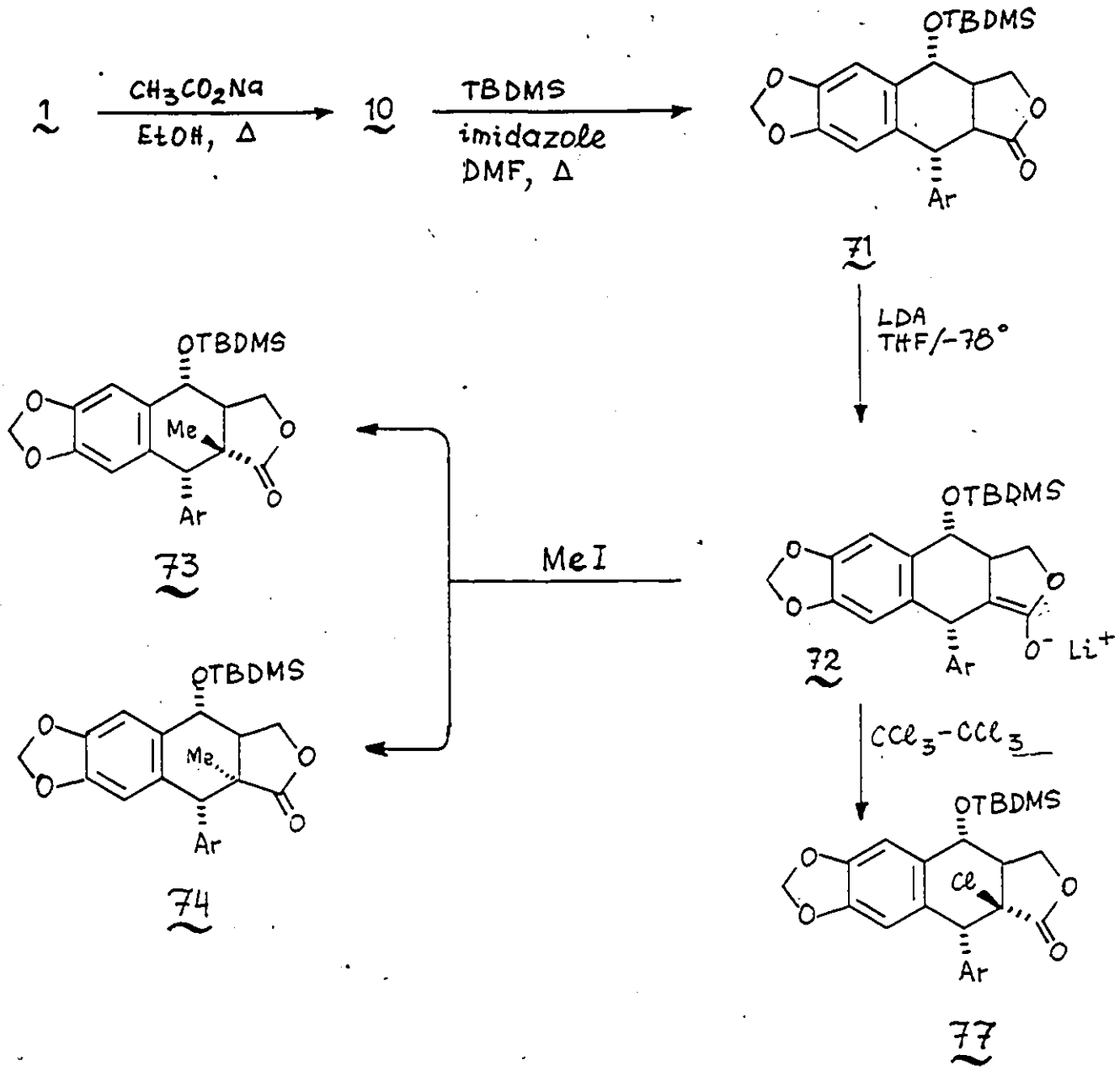
69 70

v) Reaction of the anion of 4-O-tert-butyldimethylsilylpicropodophyllotoxin with methyl iodide

The TBDMS group was also briefly investigated as a protecting group for the C-4 alcohol. Silylation of podophyllotoxin with TBDMSCl and base in DMF under various conditions failed to yield the desired 4-OTBDMS derivative.

In contrast the formation of the 4-OTBDMS derivative of picropodophyllotoxin 71 occurred in good yield under typical silylating conditions (DMF, TBDMSCl/imidazole)[24].

The enolate 72 was formed by treatment of 71 with one equivalent of LDA at -78° in dry THF and trapped with methyl iodide (-78° to rt) to give, after workup and PTLC (ethyl acetate/hexanes; 1:5; 3 runs), in order of elution, 73 and 74 in 31 and 32% yields respectively (Figures 14 and 15).



Ar = 3, 4, 5- trimethoxybenzene

SCHEME 8: PREPARATION OF 2-SUBSTITUTED DERIVATIVES OF 4-OTBDMS-PICROPODOPHYLLOTOXIN

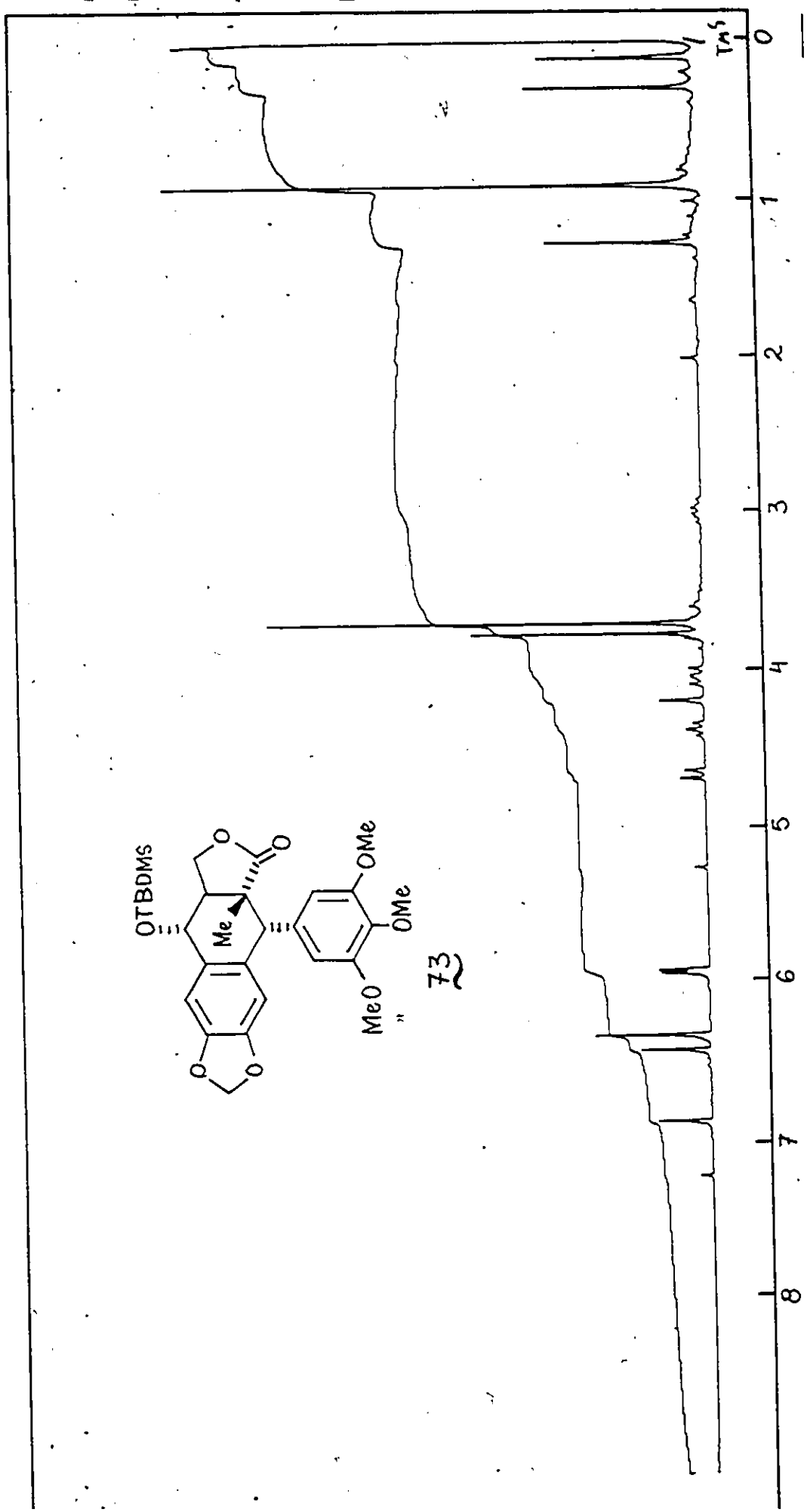


FIGURE 14: ^1H NMR SPECTRUM OF 4-OTBDMS-2-METHYLPODOPHYLLOTOXIN

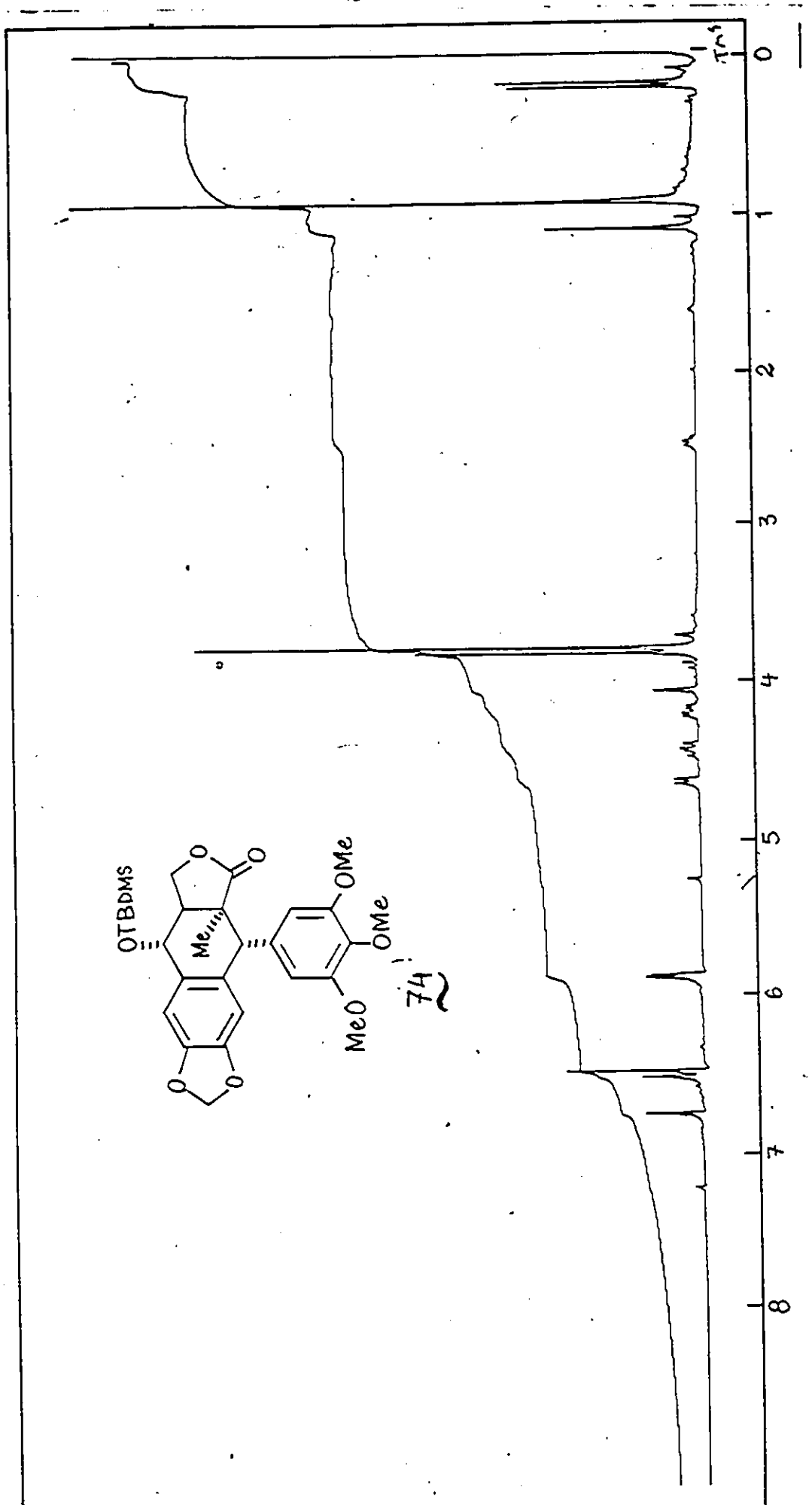


FIGURE 15: ^1H NMR SPECTRUM OF 4-OTBDMS-2-METHYLPICROPODOPHYLLOTOXIN

Substitution at C-2 was clearly evident in 73 by the presence of sharp singlets at δ = 1.31 (3H) and 4.24 (1H). These same singlets were found at δ = 1.14 (3H) and 4.10 (1H) in 74. Both compounds also showed a molecular ion of 540 consistent with the introduction of a methyl substituent.

The chemical shift pattern for the less polar compound 73, mp 44-48°, was consistent with that of a compound belonging to the podophyllotoxin series since $H_{2'}H_{6'}$ was found at higher field than both H_5 and H_8 (Table 4). The large coupling constants, J = 11.1 and 7.2 Hz for H_3H_{11} and $H_3H_{11'}$ respectively, were also in agreement with the podo stereochemical assignment. Finally, the chemical shifts and coupling constants were very similar to those of 2-methylpodophyllotoxin 75 prepared earlier by Glinski. The removal of the TBDMS group was not attempted since the protecting group did not give a higher yield of the 2-podophyllotoxin derivative than did the 4-OTHP derivative.

The aromatic pattern for the more polar compound 74, mp 55-59°, having singlets at δ=6.52 ($H_{2'}H_{6'}$), 6.56 (H_8) and 6.80 (H_5) was different from the aromatic pattern found in 2-methylpicropodophyllotoxin 76 prepared by Glinski and did not correspond to that found in any of the other compounds of the picro series (Table 4). In addition the coupling constants $J_{H_3H_{11}}$ and $J_{H_3H_{11'}}$ could not be correlated to any of the

picro compounds previously discussed (Table 5). It is believed that these differences are due to a shift in the equilibrium between the two possible conformers of this compound (see below).

vi) Reaction of the anion of 4-O-tert-butyldimethylsilylpicropodophyllotoxin with hexachloroethane

As was observed for the reaction of the 4-OTHP enolate, trapping of the enolate 72 with excess hexachloroethane (-78° to rt), followed by normal workup and plate chromatography, gave only the 2-substituted podophyllotoxin isomer 77, albeit in only 38% yield (Figure 16). The mass spectrum of 77 showed the expected molecular cluster at M^+ = 562 and $M^+ + 2$ = 564 in a 3:1 ratio. Substitution at C-2 was clearly evident by the strong singlet at δ = 4.74 for H_1. The podo stereochemistry was confirmed in the usual way (Tables 4 and 5).

vii) Structural Analysis

Trapping of the dianion 52 with electrophiles leads to a larger amount of the 2-substituted picropodophyllotoxin products in comparison to the trapping of either the 4-OTHP enolate 27 or the 4-OTBDMS enolate 72. It is suggested that in the relatively rigid structure of the dianion, the 4-alkoxy group is suitably situated to preferentially deliver the electrophile to the lower α-face resulting in the formation of the picro isomer. Protection of the 4-alkoxy group appears to remove this effect and thus allow the electrophile to enter the more open β-face to give the podo isomer.

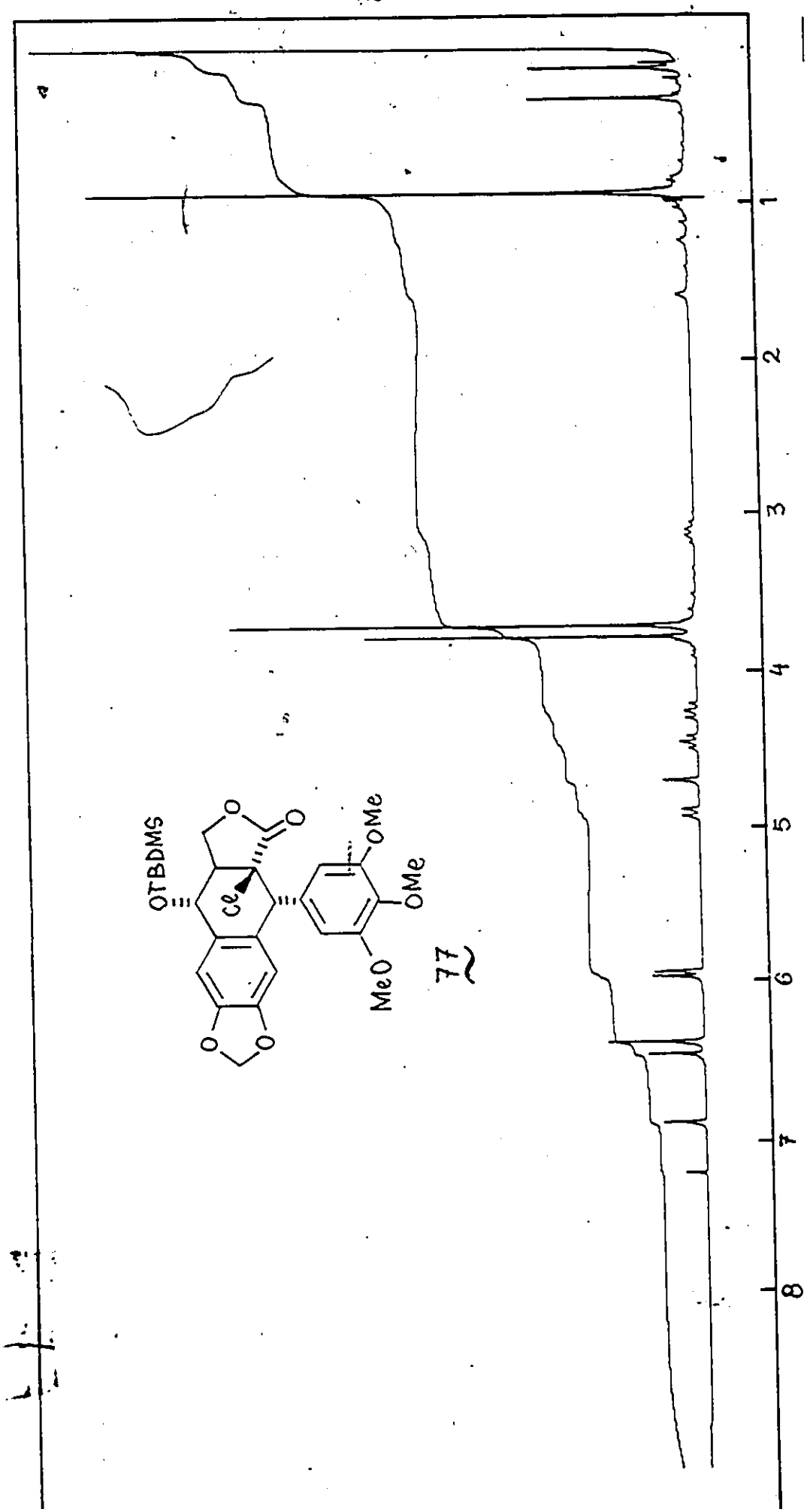


FIGURE 16: ^1H NMR SPECTRUM OF 4-OTBDMS-2-CHLOROPODOPHYLLOTOXIN

TABLE 4:

Selected Proton Chemical Shifts in Podo- and Picropodophyllotoxin Derivatives

Compound	Chemical Shift (ppm)					
	H_1	H_5	H_8	$H_{2'}H_{6'}$	H_{11}	$H_{11''}$
podophyllotoxin, 1	4.59	7.11	6.51	6.37	4.09	4.60
2-chloro-4-OTMS-, 53	4.75	6.93	6.49	6.41	4.29	4.38
2-chloro- (b), 55	4.79	7.09	6.49	6.44	4.26-4.65[a]	4.26-4.65[a]
2-chloro- (expt.), 55	4.84	7.05	6.48	6.37	4.34	4.57
2-bromo-, 67	4.91	7.10	6.52	6.42	4.25	4.56
2-methyl-4-OTBDMS-, 73	4.24	6.92	6.46	6.37	4.08	4.42
2-methyl- (b) - 75	4.26	7.10	6.48	6.36	4.17	4.51
2-chloro-4-OTBDMS-, 77	4.74	6.94	6.50	6.42	4.29	4.50
picropodophyllotoxin, 10	4.11	7.05	6.38	6.45	4.44	4.53
2-chloro-4-OTMS-, 54	4.43	6.65	6.55	6.72	4.40	4.80
2-chloro-, 56	4.51	6.75	6.61	6.77	4.49	4.84
2-bromo-, 68	4.55	6.72	6.61	6.81	4.47	4.81
2-thiomethyl-, 70	4.40	6.75	6.72	6.91	4.37	4.83-4.85[a]
4-OTBDMS-, 71	4.09	7.02	6.36	6.43	4.41	4.48-4.51[a]
2-methyl-4-OTBDMS-, 74	4.10	6.80	6.56	6.52	4.23	4.48
2-methyl- (b), 76	4.23	6.77	6.65	6.78	4.42[a]	4.42[a]

a = not measurable with accuracy
b = see reference 44

TABLE 5:

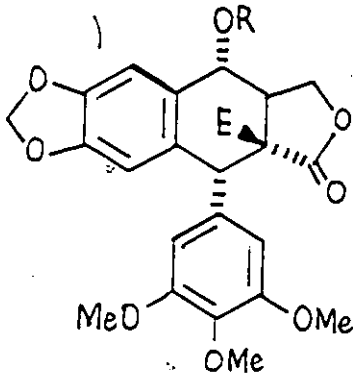
Selected Proton Coupling Constants in
Podo- and Picropodophyllotoxin Derivatives

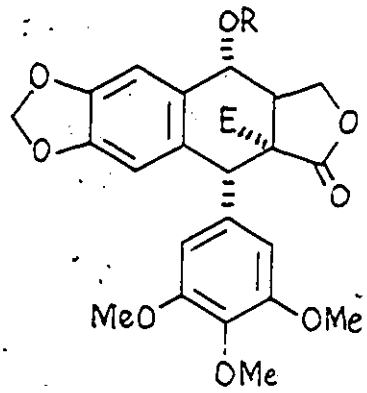
Compound	J(Hz)			
	H_3H_4	H_3H_{11}	$H_3H_{11}"$	$H_{11}H_{11}"$
podophyllotoxin, 1	9.1	9.0	8.0	8.8
2-chloro-4-OTMS-, 53	9.1	9.6	7.1	8.6
2-chloro- (c), 55	9.5	9.0	7.0	b
2-chloro- (expt.), 55	9.5	9.0	7.0	8.5
2-bromo- 67	9.0	9.6	6.8	8.8
2-methyl-4-OTBDMS-, 73	10.4	11.1	7.2	8.2
2-methyl- (c), 75	11.0	10.5	7.5	9.0
2-chloro-4-OTBDMS-, 77	8.8	10.0	6.9	8.3
picropodophyllotoxin, 10	8.3	6.0	1.5	10.2
2-chloro-4-OTMS-, 54	2.8	0	4.6	9.3
2-chloro-, 56	3.0	0	4.6	9.3
2-bromo-, 68	2.4	0	4.8	9.4
2-thiomethyl-, 70	2.6	0	4.7	10.1
4-OTBDMS-, 71	a	6.1	a	10.1
2-methyl-4-OTBDMS-, 74	5.3	6.4	4.0	9.4
2-methyl-(c), 76	7.8	6.0	1.4	b

a = not measurable with accuracy
b = not given
c = see reference 44

TABLE 6:

Results of the Enolate Trapping Experiments





E =	R =	
Cl	H	55
Cl	THP	55'(a)
Cl	TBDMS	77
Br	H	c
Br	THP	67
CH_3	H	75
CH_3	THP	75'(a,b)
CH_3	TBDMS	73

E =	R =	
Cl	H	56
Br	H	c
Br	THP	68
CH_3	H	75(a)
CH_3	THP	75(a,b)
CH_3	TBDMS	74

Electrophile/ Reagent	E	R	Products & Yields	Ratio Podo/Picro
Cl_3CCCl_3	Cl	H	55 (32%) 56 (48%)	1:1.5
Cl_3CCCl_3	Cl	THF	55' (80%)	-
Cl_3CCCl_3	Cl	TBDMS	77 (38%)	-
Br_2	Br	H	c	-
Br_2	Br	THP	67 (35%) 68 (21%)	1:1.7
CBr_4	Br	THP	67 (11%) 68 (33%)	1:3
ØS(O)Br	Br	THP	67 (16%) 68 (32%)	1:2
CH_3I	CH_3	H	75 (18%) 76 (55%)	1:2.9
CH_3I	CH_3	THP	75' (43%) 76' (10%)	2.4:1
CH_3I	CH_3	TBDMS	73 (31%) 74 (32%)	1:1

a = see reference 44
b = based on final product (R = H)
c = mainly picro (see discussion)

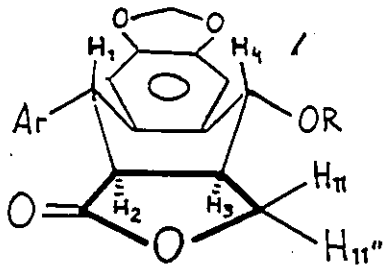
The relative position of the various aryl hydrogens and the size of the coupling constants between H_3H_{11} and $H_3H_{11"}$ have been used as indication of either the picro or podo stereochemistry. These data are gathered in Tables 4 and 5 respectively for easy comparison.

Coupling constants are considered to be reliable indicators for the stereochemical assignments utilizing the Karplus relationship. On the basis of the coupling constant for H_3H_4 one can confidently propose that picropodophyllotoxin 10 and 2-methylpicropodophyllotoxin 76 exist predominantly as conformer X (Figure 17) wherein the C-1 and C-4 substituents occupy a pseudo diequatorial relationship[46,50]. In these compounds a large coupling (8.3 and 7.8 Hz respectively) is observed between H_3 and H_4 which is consistent with a near 180° dihedral angle between these two protons.

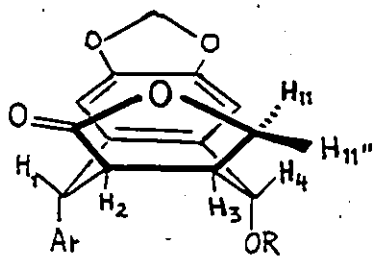
In contrast the 2-bromo, 2-chloro- and 2-thiomethyl- picropodophyllotoxin derivatives show a small coupling (2.5 - 3.0 Hz) between H_3 and H_4. Such a coupling constant suggests an equatorial-axial type relationship between these hydrogens consistent with conformer Y found in Figure 17 in which ring C is in a quasi boat conformation and the C-1 and C-4 substituents occupy the axial positions[46,50].

In these isomers one of the coupling constants between H_3 and H_{11} or $H_{11"}$ is approximately 4.6 Hz while the other is near 0 Hz. Inspection of Dreiding models, in which the C ring and the C-1 and C-4 substituents are as shown in Figure 17 (conformer Y) indicates that H_{11} and not $H_{11"}$ could

FIGURE 17: TWO POSSIBLE CONFORMERS FOR 2-SUBSTITUTED PICROPODOPHYLLOTOXINS



X



Y

form a 90° dihedral angle with H_3 and thus give rise to a negligible coupling constant. In such a conformation H_{11}" and H_3 would have an approximate 20-30° dihedral relationship which would be in agreement with the observed 4-5 Hz coupling constant[50].

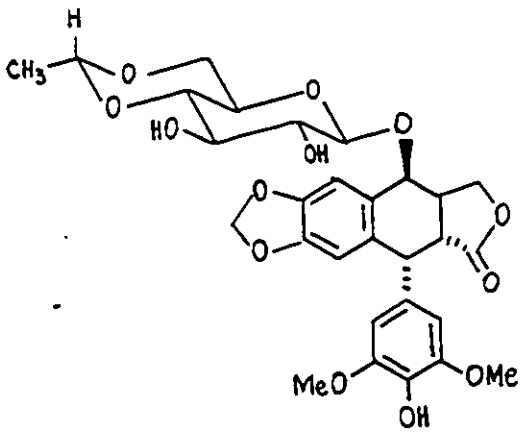
Ayres has made similar arguments regarding conformational changes and equilibria in going from picropodophyllotoxin (65± 5% conformer X) to the 4-acetoxy derivative (70± 7% conformer Y)[50]. For the 2-methyl-4-OTBDMS derivative 74 a 5.3 Hz coupling for H_3H_4 was observed indicating that this compound exists in nearly equal amounts of conformers. The coupling for H_3H_4 observed by Ayres for the acetoxypicropodophyllotoxin was 5 Hz[50].

Brewer and coworkers have suggested that the small amount of inhibition of microtubule assembly remaining in picropodophyllotoxin relative to podophyllotoxin is due to the presence of a small amount of an active conformer, one in which the trimethoxyaryl ring, the E ring, occupies a quasiaxial conformation[46]. This places this ring in the same relative orientation as that found in podophyllotoxin. The major conformation for 54, 56, 68 and 70 indicated by their NMR spectra puts the E ring in the desirable quasiaxial conformation; and, thus it may be of interest to test and compare the effects of these compounds on microtubule assembly.

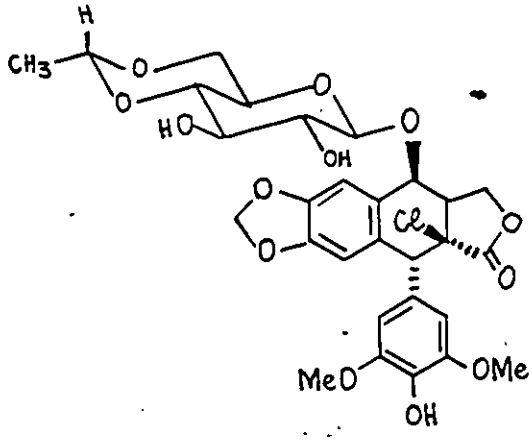
PART II

1) Introduction

In 1982 Glinski had prepared a compound which was identified as 2-chloro-4'-O-demethyl-1-O-(4,6-O-ethylidene-β-D-glucopyranosyl)epipodophyllotoxin 79[44]. Biological testing of 79 was performed by the Antitumor Division of Bristol Laboratories using Leukemia P388. The regimen had involved peritoneal implantation of ascitic fluid containing 10^6 cancer cells into female, CDF mice (six mice per test group). Treatment began 24 hours after implant and the parameter was median survival time. Results were given as percent increased survival time of test group over control group (% T/C). An initial % T/C of 125 or more is considered significant antitumor activity. The results showed that 79 compared favourably with the clinical agent, VP-16, 42 against P388 Leukemia (Table 7).



42



79

TABLE 7: EFFECT OF ETOPOSIDE DERIVATIVES ON LEUKEMIA P388

MATERIAL	TREATMENT SCHEDULE	DOSE IP mg/kg/inj	MST DAYS	EFFECT MST %T/C	AWC gm d.6	SURVIVORS DAY 5(45)
Etoposide (42)	d.1&5	40	45.0	500	-2.2	6/6 (4)
			45.0	500	-1.4	6/6 (4)
2-Chloro-Etoposide (79)	d.1&5	60	45.0	500	-1.8	6/6 (5)
		30	29.0	322	-1.2	6/6 (2)
		15	21.4	239	-1.0	6/6
		7.5	16.0	178	-0.3	6/6
CONTROL		saline	9.0	-	-0.5	10/10

Tumur inoculum : 10^6 ascites cells implanted ip.
Host : CDF_1 female mice.
Evaluation : MST = median survival time.
Effect : %T/C = (MST treated / MST control) x 100.
Criteria : %T/C > or = 125 considered significant antitumor activity.

The 2-chloro derivative 79 had been prepared by treatment of VP-16 42 with four equivalents of LDA at -78° in dry THF, followed by chlorination with hexachloroethane (-78° to rt). The chloro derivative 79 was isolated in 10% yield. This low yield was believed to be due to the insolubility of the polyanion (possibly the trianion and/or tetraanion). It was believed that the yield of 79 could be improved either by the use of the polar co-solvent HMPT or by the use of a dianion of 42 (i.e. protecting groups attached to the two glycoside hydroxyl functions).

ii) Reaction of the tetraanion of Etoposide with hexachloroethane in a THF/HMPT solution

In the event, Etoposide 42 was dissolved in a small volume of dry THF and was added slowly to a cooled solution (0°) of HMPT/THF (1:9) containing four equivalents of LDA. The approximate molarity of the polyanion of 42 was 6 mg/ml. The solution was stirred for an additional 15 minutes at 0°C, and was followed by slow warming to rt. No precipitation was observed in the solution. The solution was stirred at rt for an additional 24 h and normal workup yielded a beige solid which was initially identified as 79 since its proton NMR, mass spectra and melting point were comparable to the compound prepared by Glinski. The reaction when repeated using only a 5% solution of HMPT in THF afforded 79 in 25% yield together with a 25% recovery of the starting material, Etoposide.

Our batch of 79 was sent to the Bristol Laboratories for further biological screening under conditions where Etoposide and 79 would not give the limiting T/C > 500. This would have allowed for a better comparison of the relative activity of the chloro derivative 79 to Etoposide. Unfortunately this test and subsequent testing under the original conditions showed that our compound had only marginal activity.

Considerable efforts were made to convince ourselves that the NMR, MS and mp data of our preparation and those of Glinski's compound were virtually identical.

The reason for the difference in the biological results could not be satisfactorily explained. The possibility of an erroneous initial biological result due to inadvertent mislabelling of the Glinski sample and Etoposide was suggested to us by the Bristol scientists. However, the samples sent to Bristol on the various occasions were, in our opinion, identical substances.

Nevertheless, the eventual confirmed negative results of our compound prompted us to carefully reexamine the available structural evidence. Armed with a more complete understanding of the proton spectra of Etoposide resulting from Jardine's 470-MHz proton NMR study of VP-16 and various derivatives, we carefully reexamined our 300 MHz proton NMR spectra of 79 (Figures 18a,b)[43].

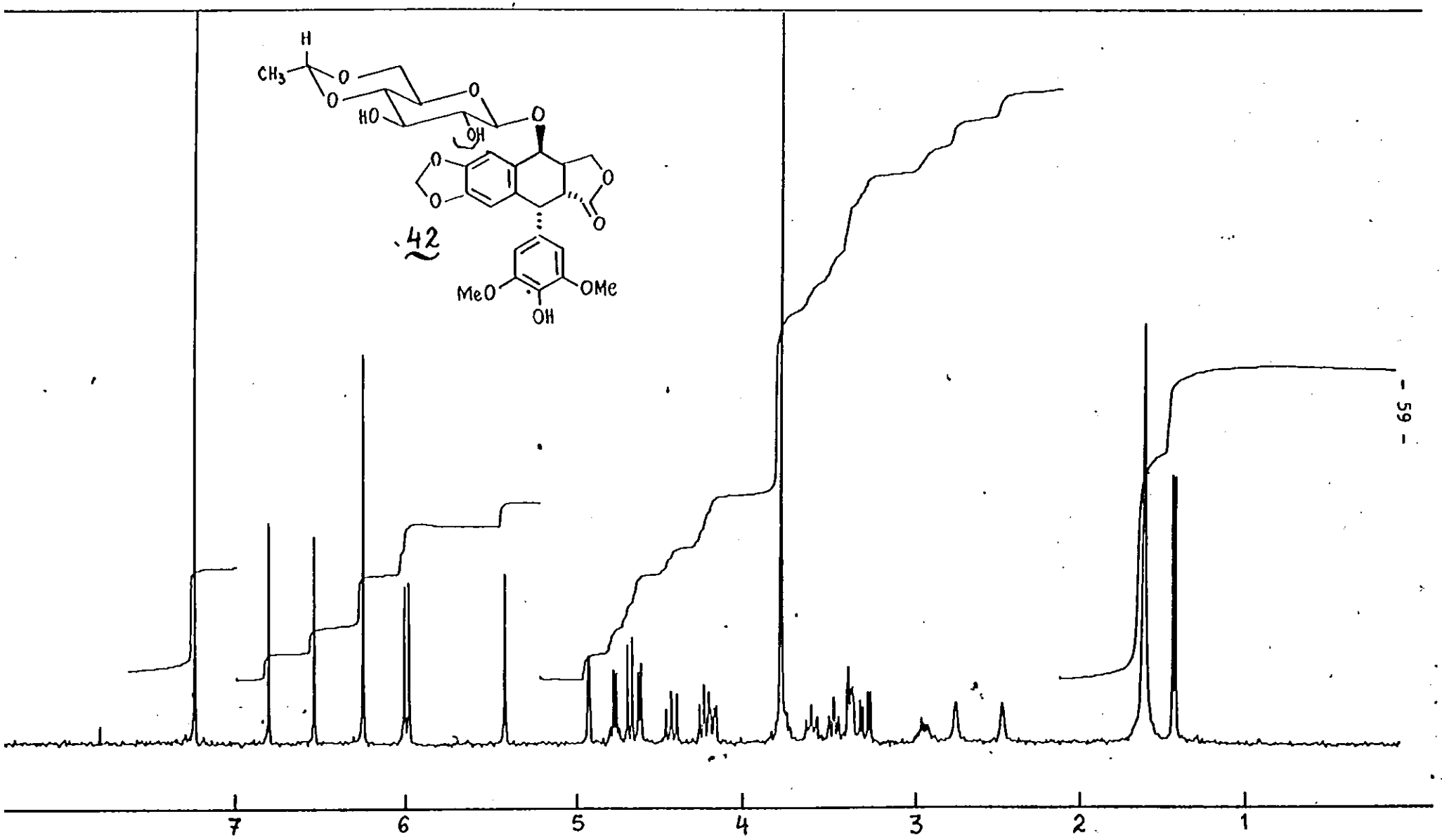


FIGURE 18A: ^{1}H NMR SPECTRUM OF ETOPOSIDE

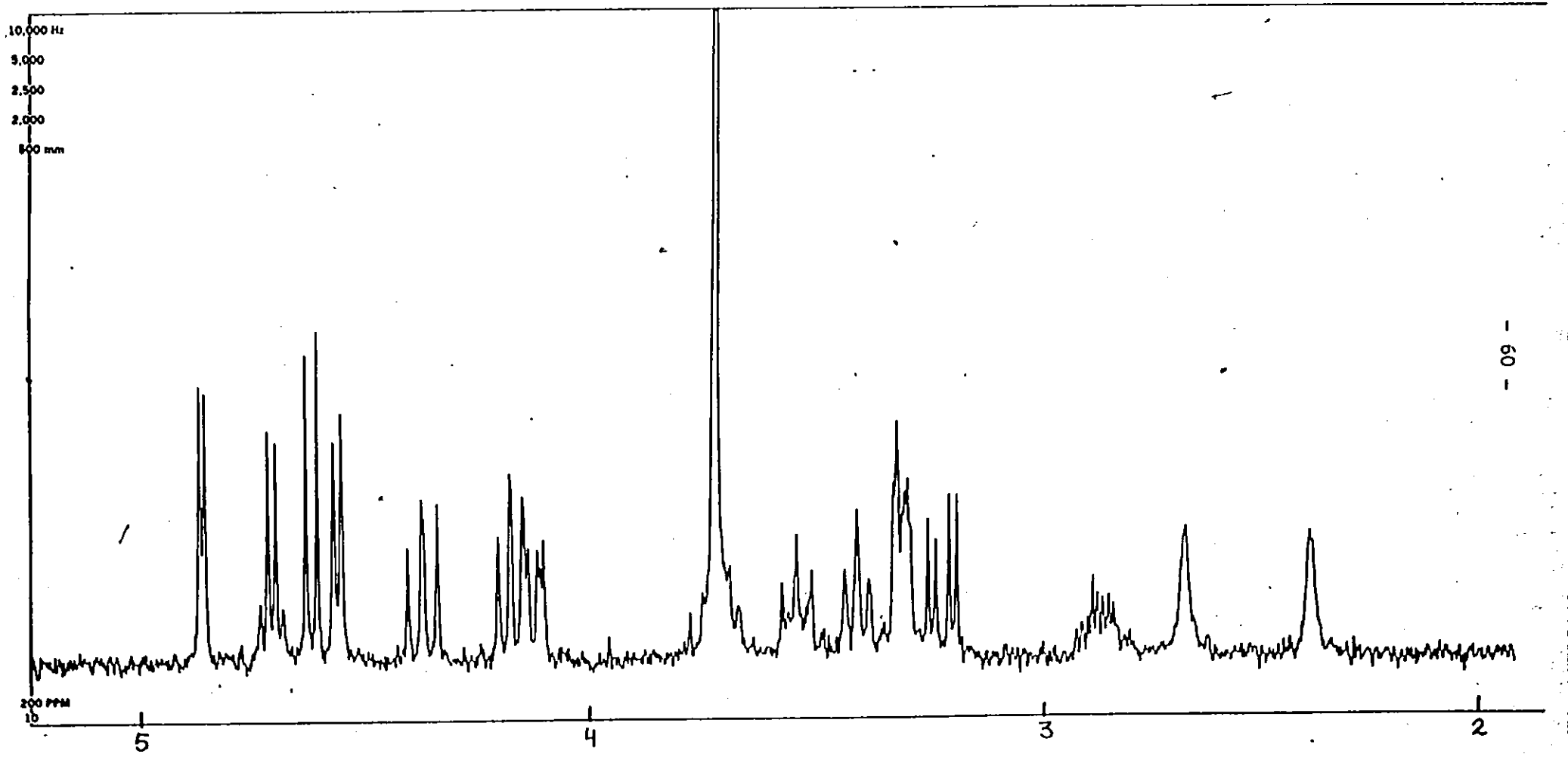


FIGURE 18B: EXPANDED ^{1}H NMR OF ETOPOSIDE

To familiarize ourselves with the complex high field NMR spectra of Etoposide and its derivatives, we first examined the spectra of Etoposide. As can be seen in Tables 8 and 9 the observed chemical shifts and coupling constants for our sample of 42 were comparable to the values obtained by Jardine. As in the podophyllotoxin series, large coupling constants were observed for $H_3H_{4"}$, H_3H_{11} and $H_3H_{11"}$.

The coupling constant that we observed for $H_3H_{11"}$ in cur 2-chloro derivative was not in agreement with the value reported by Glinski[44] (Table 9). As can be seen in the expanded spectrum (Figure 19b), the doublet of doublets at 4.63 ppm due to $H_{11"}$ cannot have coupling values of 8.7 and 8.0 Hz. We actually found the values to be 9.7 and 1.7 Hz due to $H_{11}H_{11"}$ and $H_3H_{11"}$ respectively. This small value of 1.7 Hz for $J_{H_3H_{11"}}$ was comparable to the value observed in picro-Etoposide 78 (Table 9)[43].

In retrospect it was surprising that the coupling constants for $J_{H_3H_{11}}$ and $J_{H_3H_{11"}}$ remained at the values of 7.0 and 1.7 Hz respectively. This was not in agreement to that which was observed in the 2-chloro derivatives 54 and 56 ($J_{H_3H_{11}}$=0 Hz and $J_{H_3H_{11"}}$=4.6 Hz). This suggested that conformer X was favored over conformer Y in compound 79 in contrast to 54 and 56. If the glycoside moiety sits over ring D in compound 79, then conformer X would be favoured since there is less steric interaction between ring D and the glycoside in this conformation.

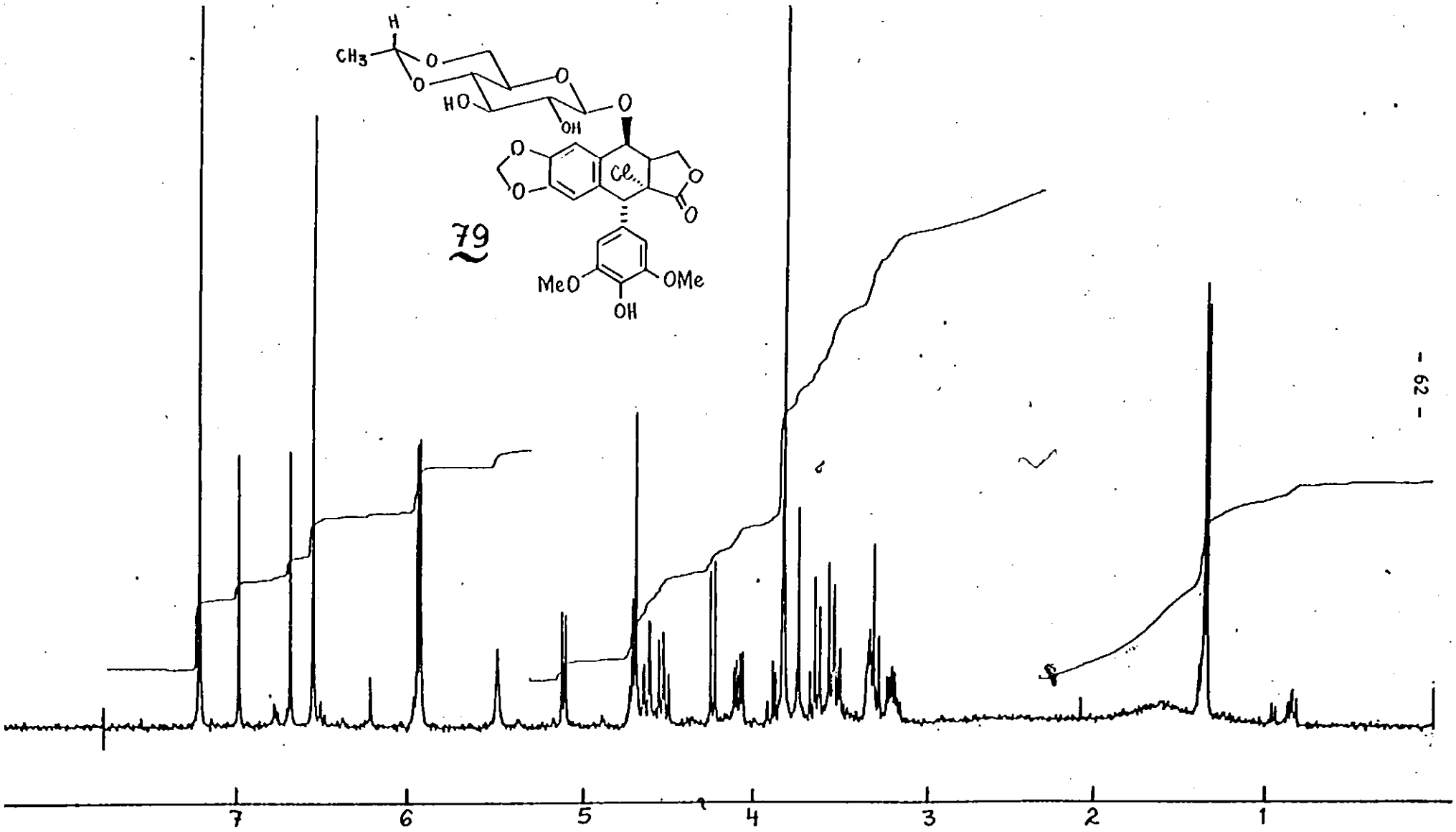


FIGURE 19A: ^{1}H NMR SPECTRUM OF 2-CHLOROPICRO-ETOPOSIDE

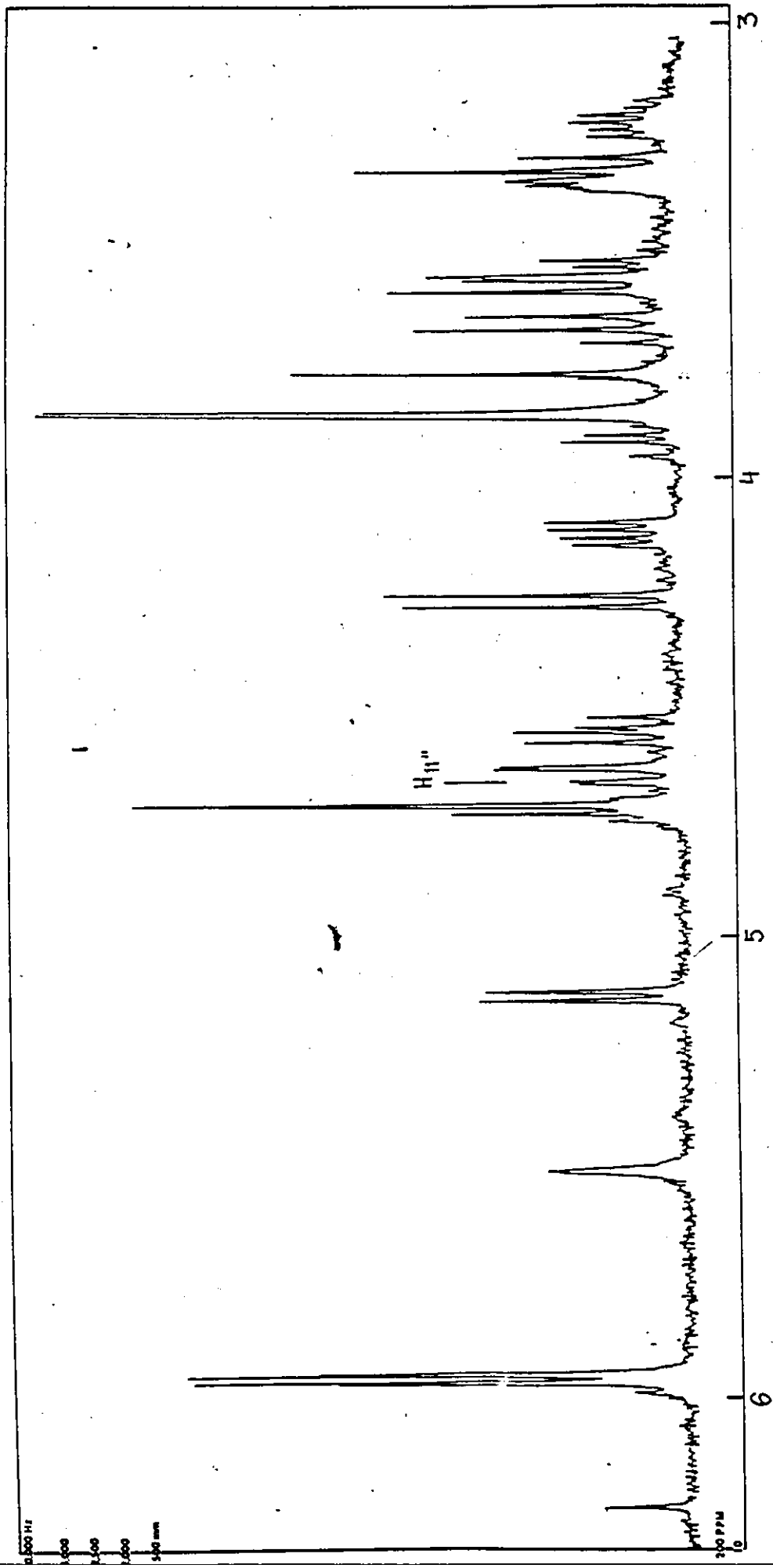
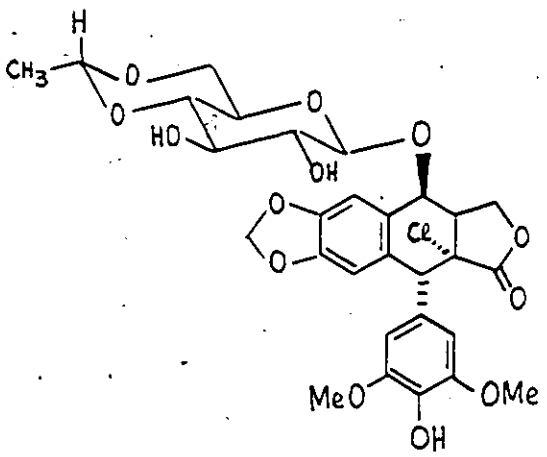


FIGURE 19B: EXPANDED ^1H NMR SPECTRUM OF 2-CHLOROPICRO-ETOPOSIDE

The remaining chemical shifts and coupling constants for the aglycon protons were similar to those observed by Glinski. This data is strongly suggestive that the original assignment of 79 was incorrect and that the "2-chloro-Etoposide" originally claimed by Glinski was in fact 2-chloro-picro-Etoposide.



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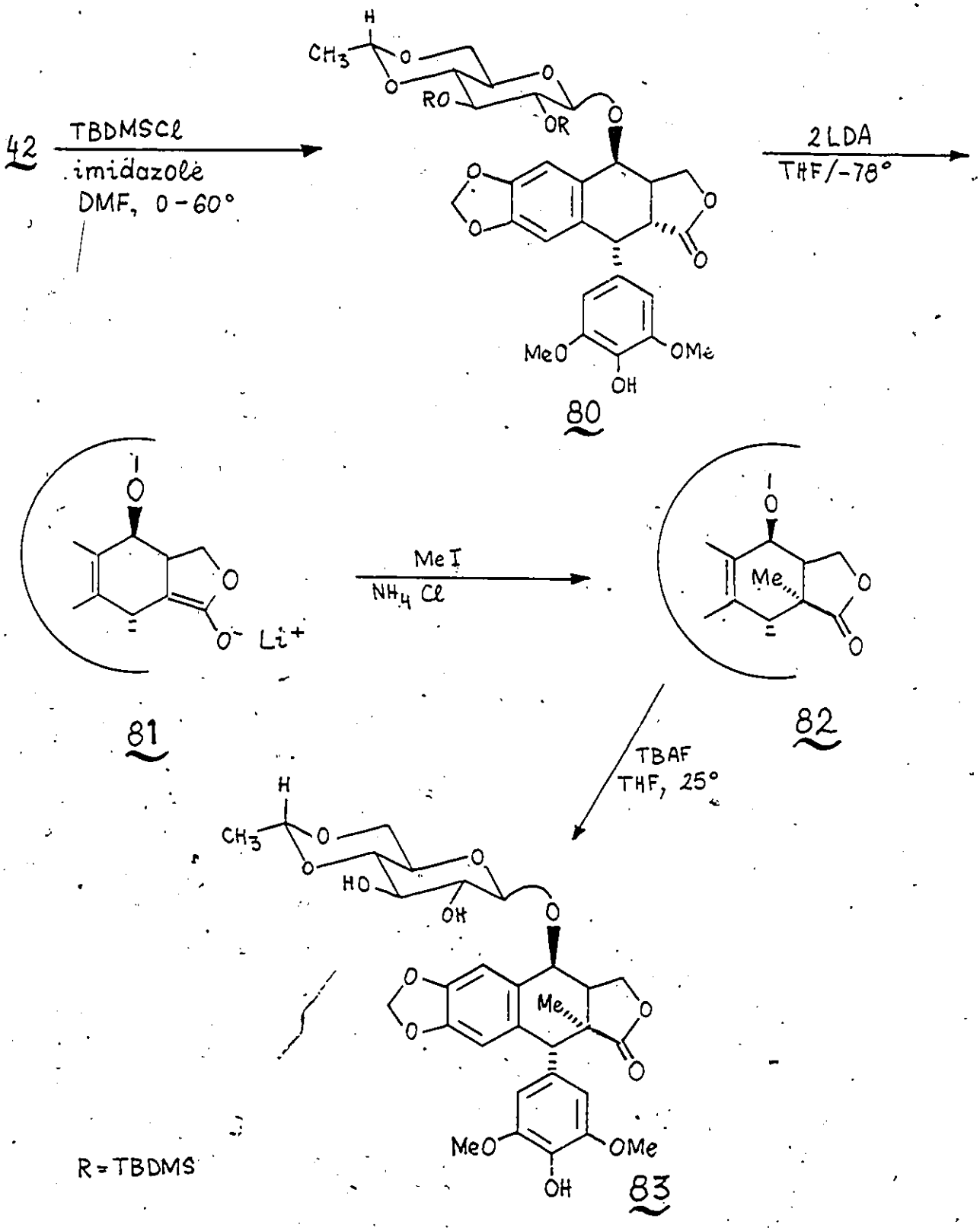
iii) Reaction of Etoposide with TBDMSCl

As part of the program for introducing a 2-substituent into Etoposide we examined the methylation of Etoposide in which the two free hydroxyl groups of the glycoside moiety had been protected as TBDMS derivatives. It was hoped that reactions in this series would be cleaner and give higher yields since the solubility of the dianion 81 in THF should be higher than that of the tetraanion.



SCHEME 9: PREPARATION OF 2-METHYLPICRO-ETOPOSIDE VIA THE DIANION ROUTE

Etoposide was dissolved in a small volume of dry DMF and this was added dropwise to a dry solution of DMF containing an excess of tert-butyldimethylsilyl chloride and imidazole. Workup followed by column chromatography gave the di-(TBDMS)-Etoposide 80 in 93% yield. The chemical shifts and proton coupling constants were similar to those observed in Etoposide (Tables 8 and 9).

Treatment of 80 with two equivalents of LDA at -78° in dry THF generated the dianion 81 with no visible precipitation (Scheme 9). This dianion was quenched with excess methyl iodide, stirred for 10 minutes at -78° and then warmed to rt and stirred overnight. Normal workup and column chromatography gave only one isomer identified as 2-methylpicro-Etoposide 82, mp 216-218°, albeit in only 33% yield. Substitution at C-2 was evident by the singlets δ = 4.34 and δ = 1.30 ppm for H_1 and CH_3(C-2).

As in 79 the aromatic pattern of 1:1:2 for H_5,H_7 and $H_{2'}$,$H_{6'}$ respectively suggested the possibility of the podophyllotoxin stereochemistry. However the proton coupling for $H_{11"}$ ($J_{H_{11"}H_{11}}$ =10.2 Hz and $J_{H_3H_{11"}}$ = 3.5 Hz) indicated the picro stereochemistry due to the relative small coupling of $H_3H_{11"}$. The increase in value of $J_{H_3H_{11"}}$ by 2 Hz appeared to show the presence of both conformers. This seemed to be corroborated by the large coupling of 6.5 Hz for $H_3H_{4"}$.

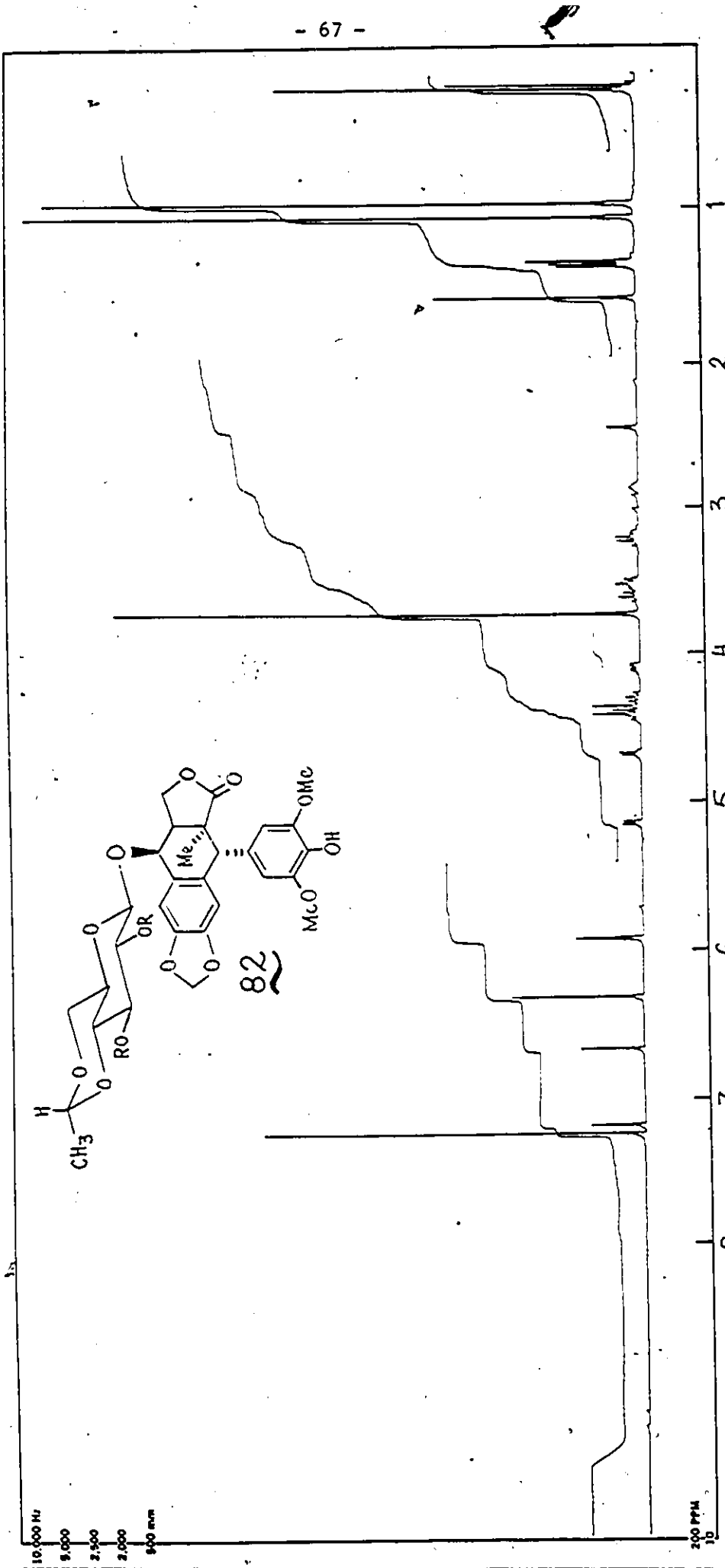


FIGURE 20A: 300 MHz-^{1}H-NMR SPECTRUM OF COMPOUND 82

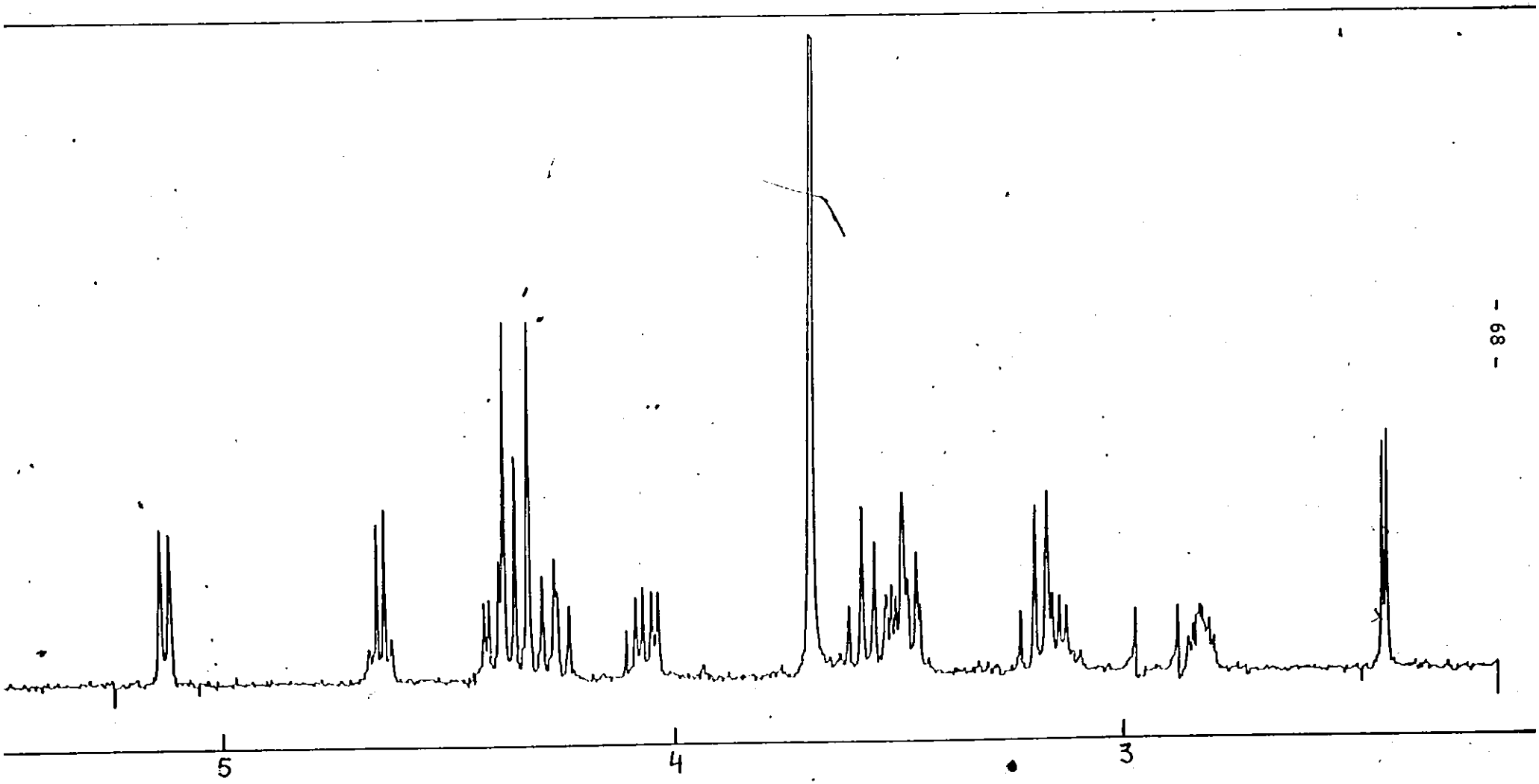


FIGURE 20B: EXPANDED ^{1}H NMR SPECTRUM OF COMPOUND 82

Desilylation of 82 was carried out in the standard method using TBAF in dry THF at rt. After 1h and normal workup followed by recrystallization (ethyl acetate/hexanes) the desilylated product 83, mp 185-190°, was isolated as a white solid in 69% yield. The MS gave a molecular ion of 602 confirming the insertion of a methyl group into the molecule. The observed coupling constants and chemical shifts for 83 were similar to those values found in 82 (Tables 8 and 9) and again to the picro.stereochemistry.

The arguments presented herein that the Glinski assignment of the chlorination product of the tetraanion of Etoposide is incorrect are probably best validated by independent synthesis from 2-chloropodophyllotoxin. Thus the application of the sequence of reactions for converting podophyllotoxin to Etoposide to 2-chloropodophyllotoxin, a compound whose structure is not in doubt, should yield unambiguously 2-chloro-Etoposide whose properties could then be compared to 79. This work is presently being attempted by others.

TABLE 8: PROTON CHEMICAL SHIFTS IN ETOPOSIDE DERIVATIVES (ppm)

Proton	COMPOUND							
	42 (c)	42 (observed)	78 (c)	79 (d)	79 (observed)	80	82	83
1	4.59(d)	4.59(d)	4.21(d)	4.72(s)	4.70(s)	4.56(d)	4.34(s)	4.37(s)
2	3.24(dd)	3.23(dd)	3.18(dd)	-	-	3.20-3.32(m)	1.30(s)	1.23(s)
3	2.86(m)	2.85(m)	2.96(m)	3.20(m)	3.20(m)	2.89(m)	2.84(m)	2.75(m)
4"	4.90(d)	4.89(d)	4.93(d)	5.12(d)	5.12(d)	4.89(d)	5.15(d)	5.08(d)
5	6.81(s)	6.80(s)	6.77(s)	7.04(s)	7.01(s)	6.83(s)	7.20(s)	7.03(s)
CH_2 — O	5.98(d)	5.99(d)	5.95(d)	5.98(d)	5.97(d)	6.00(d)	5.94(d)	5.94(d)
CH_2 — O	5.98(d)	5.96(d)	5.95(d)	6.00(d)	5.95(d)	5.97(d)	5.93(d)	5.93(d)
8	6.54(s)	6.53(s)	6.39(s)	6.75(s)	6.70(s)	6.54(s)	6.69(s)	6.68(s)
2'6'	6.25(s)	6.24(s)	6.44(s)	6.58(s)	6.57(s)	6.20(s)	6.33(s)	6.42(d)
3'5'	3.75(s)	3.74(s)	3.86(s)	3.84(s)	3.83(s)	3.66(s)	3.72(s)	3.82(s)
6'	a	5.38(s)	a	5.52(s)	5.50(s)	b	b	5.47(s)
11	4.21(t)	4.19(t)	4.48 [ABX]	4.57(dd)	4.53(dd)	4.10-4.24(m)	4.28(dd)	4.36(dd)
11"	4.40(dd)	4.40(dd)	4.53	4.65(dd)	4.63(dd)	4.42(dd)	4.42(dd)	4.45(dd)
g1	4.65(d)	4.65(d)	3.92(d)	3.28-3.77(m)	4.25(d)	4.64(d)	4.39(d)	4.24(d)
g2	3.43(t)	3.42(t)	3.44(t)	3.28-3.77(m)	3.49-3.56(m)	3.39(t)	3.48-3.55(m)	3.50-3.58(m)
g3	3.74(t)	3.74(hidden)	3.58(t)	3.28-3.77(m)	3.65(t)	3.66(hidden)	3.61(t)	3.66(t)
g4	3.34(m)	3.30-3.34(m)	3.32(t)	3.28-3.77(m)	3.27-3.34(m)	3.20-3.32(m)	3.14-3.25(m)	3.32(t)
g5	3.34(m)	3.30-3.34(m)	3.18(m)	3.28-3.77(m)	3.27-3.34(m)	3.20-3.32(m)	3.14-3.25(m)	3.18(m)
g6 ax	3.56(t)	3.56(t)	3.58(t)	3.28-3.77(m)	3.49-3.56(m)	3.55(t)	3.48-3.55(m)	3.50-3.58(m)
g6 eq	4.16(m)	4.14(dd)	4.15(dd)	4.12(dd)	4.10(dd)	4.10-4.24(m)	4.08(dd)	4.10(dd)
g7	4.74(q)	4.73(q)	4.72(q)	4.73(q)	4.71(q)	4.70(q)	4.67(q)	4.71(q)
g8	1.39(d)	1.38(d)	1.35(d)	1.37(d)	1.35(d)	1.35(d)	1.32(d)	1.35(d)
g2OH	2.44(s)	2.42(s)	2.61(s)	2.60(s)	b	-	-	2.75
g3OH	2.70(s)	2.70(s)	2.81(s)	2.60(s)	b	-	-	2.86

a = not given
b = not measurable
c = see reference 43
d = see reference 44

TABLE 9: PROTON COUPLING CONSTANTS FOR ETOPOSIDE DERIVATIVES (Hz)

J	Compound							
	42 (c)	42 (observed)	78 (c)	79 (d)	79 (observed)	80	82	83
1,2	5.1	5.2	4.8	-	-	5.3	-	-
2,3	14.0	14.1	9.4	-	-	b	-	-
3,4"	3.4	3.4	3.0	3.0	5.5	3.9	6.5	5.5
3,11	8.0	7.6	7.0	8.0	7.0	b	8.7	7.9
3,11"	10.5	10.4	1.5	8.7	1.7	10.4	3.5	3.7
11,11"	9.0	8.9	9.4	8.0	9.7	8.9	10.2	9.9
OCH_2O	1.0	1.2	1.0	0.5	1.2	1.2	1.2	1.2
g1,g2	7.6	7.6	7.7	a	7.6	8.8	7.1	7.1
g2,g3	8.0	8.7	9.0	a	8.9	9.0	9.0	9.4
g3,g4	a	b	9.0	a	8.9	b	9.0	9.4
g4,g5	a	b	a	a	b	b	9.0	9.4
g5,g6eq	4.3	4.1	4.5	5.0	4.8	b	4.3	4.7
g5,g6ax	9.5	10.0	9.0	a	b	10.0	b	b
g6eq,g6ax	10.4	10.3	10.7	8.0	10.3	10.0	10.1	10.1
g7,g8	5.0	5.1	5.0	5.0	5.1	5.1	4.7	5.0

a = not given
b = not measurable
c = see reference 43
d = see reference 44

EXPERIMENTAL

General

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^1H NMR spectra were obtained on Varian T-60, XL 200 and EM 360A spectrometers. All spectra were taken using deuterochloroform ($CDCl_3$) as solvent and trimethylsilane (TMS) as the internal standard. The chemical shifts in parts per million (ppm) are relative to the internal standard, TMS. The coupling patterns are noted as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) or broadened (br). Infrared spectra were recorded on the Unicam SP 1100 and the Beckman IR-20A spectraphotometers. Absorptions are reported in cm^{-1} and are noted as strong (s) or broadened (br). Mass spectra were obtained on a VG-7070E instrument.

Thin layer chromatography (TLC) was performed on Merck 60F 254 precoated silica plates of 0.25 mm thickness. Preparative thin layer chromatography (PTLC) was carried out on glass plates coated with a 1.0 mm layer of Kieselgel 60 GF 254. Column chromatography was performed using Baker 60-200 mesh silica gel as the adsorbant.

Tetrahydrofuran (THF) was always distilled over sodium/potassium/benzophenone under a nitrogen atmosphere immediately prior to use. All other solvents were distilled or were of reagent grade quality. All glassware was flame-dried under nitrogen before use.

Normal workup involved pouring the reaction mixture into water or a saturated ammonium chloride solution, extracting three times with methylene chloride, drying the organic extracts with magnesium sulphate and evaporating the solvents on a rotary evaporator under reduced pressure.

1. Preparation of the dianion of podophyllotoxin and subsequent reaction with hexachloroethane

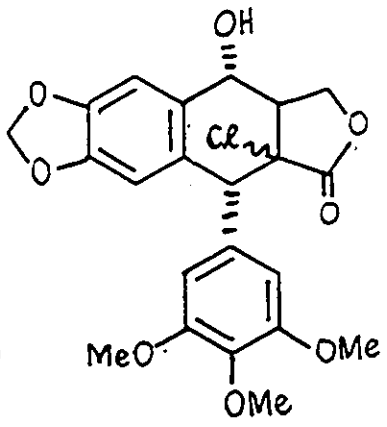
Podophyllotoxin 1 (207 mg, 0.5 mmol) was dissolved in a small volume of dry THF and added slowly to a cooled (-78°C) solution of LDA (2 equivalents), prepared from diisopropylamine (110 mg) and n-BuLi (0.42 ml, 2.4 M in hexanes) in 5 ml of dry THF. The faint yellow solution was stirred for 15 minutes at -78° and then excess hexachloroethane (500 mg) was added. The solution was stirred at -78° for 30 minutes and then it was warmed to 0° and stirred for an additional 1 h. The usual workup, followed by PTLC (ethyl acetate/hexanes; 1:1, 3 runs), afforded two inseparable components.

Precipitation from ether/hexanes furnished 180 mg (80%) of a beige powder which was shown to be a 2:3 mixture of 2-chloropodophyllotoxin 55 and 2-chloropicropodophyllotoxin 56.

55 + 56

$C_{22}H_{21}O_8Cl$

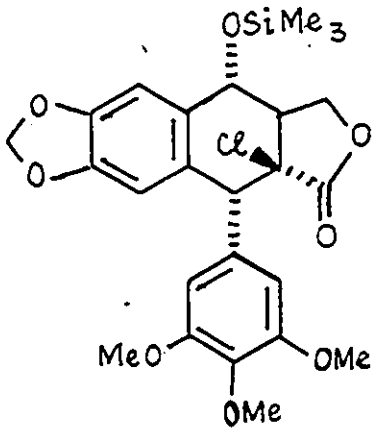
448.85



^{1}H NMR (ppm) 2.60-3.20 (m, 2H), 3.70-3.77 (br, 9H), 4.23-5.00 (m, 4H), 5.83-5.93 (br, 2H), 6.37-7.03 (br, 4H)

2. Preparation and separation of silylated 2-chloropodophyllotoxin and 2-chloropicropodophyllotoxin

The 2-chloro mixture (153 mg, 0.34 mmol), 55 and 56, pyrridine (67 mg, 0.85 mmol), and trimethylsilyl chloride (82 mg, 0.75 mmol) were combined with 10 ml CH_2Cl_2, stirred at rt for 3h and then added to a saturated brine solution. The usual workup, followed by PTLC (ethyl acetate/hexanes; 1:3, 3 runs), afforded 2 components as clear oils. Each component was identified in order of elution from the preparative plate: 4-O-trimethylsilyl-2-chloropodophyllotoxin 53 (20 mg, 24%*) and 4-O-trimethylsilyl-2-chloropicropodophyllotoxin 54 (34 mg, 32%*).



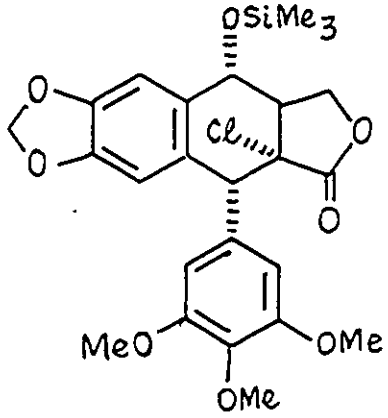
53

521.04

* yield based on only that particular isomer in mixture.

^{1}H NMR δ (ppm) 0.26 (s, 9H), 3.12 (ddd, J=9.9, 9.1, 7.1 Hz, 1H), 3.75 (s, 6H), 3.80 (s, 3H), 4.29 (dd, J=9.9, 8.6 Hz, 1H), 4.38 (dd, J=8.6, 7.1 Hz, 1H), 4.75 (s, 1H), 4.96 (d, J=9.1 Hz, 1H), 5.97 (s, 1H), 5.98 (s, 1H), 6.41 (s, 2H), 6.49 (s, 1H), 6.93 (s, 1H)

MS m/e 520 (M^+), 522 (M^++2)



54

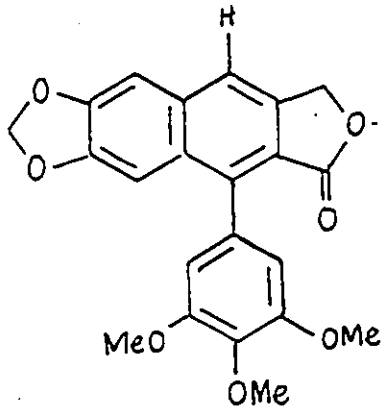
521.04

^{1}H NMR δ (ppm) 0.27 (s, 9H), 3.01 (dd, J=4.6, 2.8 Hz, 1H), 3.82 (s, 6H), 3.83 (s, 3H), 4.40 (d, J=9.3 Hz, 1H), 4.43 (s, 1H), 4.80 (dd, J=9.3, 4.6 Hz, 1H), 4.85 (d, J=2.8 Hz, 1H), 5.91 (s, 1H), 5.92 (s, 1H), 6.55 (s, 1H), 6.65 (s, 1H), 6.72 (s, 2H)

MS m/e 520 (M^+), 522 (M^++2)

3. Desilylation of 4-O-trimethylsilyl-2-chloropicropodophyllotoxin with TBAF

The silylated derivative 54 (34 mg, 0.065 mmol), was dissolved in a small volume of dry THF, treated with excess TBAF (.21 ml, 1M) and stirred at rt for 2 h. The usual workup, followed by PTLC (ethyl acetate/hexanes; 1:1, 3 runs), afforded a white solid (13 mg, 51%).



57

$C_{22}H_{18}O_7$

394.38

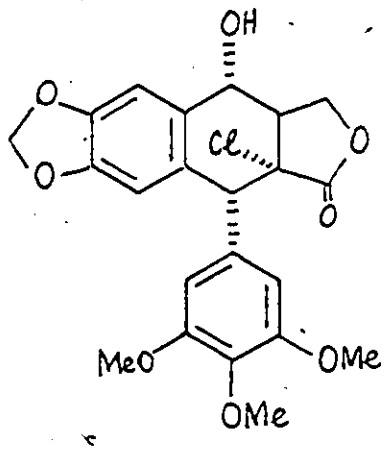
mp 283-285°C

^1H NMR δ (ppm) 3.83 (s, 6H), 3.93 (s, 3H), 5.35 (s, 2H), 6.05 (s, 2H), 6.53 (s, 2H), 7.12 (s, 1H), 7.18 (s, 1H), 7.68 (s, 1H)

MS m/e 394 (M^+)

4. Desilylation of 4-O-trimethylsilyl-2-chloropicropodophyllotoxin with AcOH

The silylated derivative 54 (35 mg, 0.067 mmol), was dissolved in a small volume of AcOH/H_2O (4:1) and stirred at rt for 1 h. The solution was diluted with H_2O and extracted three times with 15 ml of CH_2Cl_2. The combined organic fractions were washed with saturated $NaHCO_3$, dried over $MgSO_4$, and the volume reduced yielding 22 mg (73%) 56 as a beige solid.



56

$C_{22}H_{21}O_8Cl$

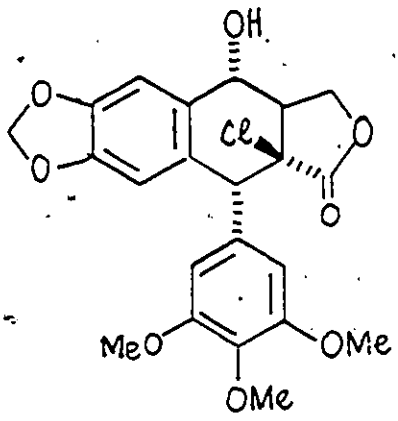
448.85

mp 99-104°C

^1H NMR δ (ppm) 2.30 (br, 1H), 3.17 (dd, J=4.6, 3.0 Hz, 1H), 3.83 (s, 9H), 4.49 (d, J=9.3 Hz, 1H), 4.51 (s, 1H), 4.84 (dd, J=9.3, 4.6 Hz, 1H), 4.90 (br s, 1H), 5.93 (s, 1H), 5.94 (s, 1H), 6.61 (s, 1H), 6.75 (s, 1H), 6.77 (s, 2H)

5. Desilylation of 4-O-trimethylsilyl-2-chloropodophyllotoxin with TBAF

The silyl derivative 53 (52 mg, 0.1 mmol) was dissolved in 10 ml dry THF and then reacted with excess TBAF (0.40 ml, 1 M) at rt for 2 hours. Normal workup, followed by PTLC (ethyl acetates:hexanes; 1:1; 3 runs), yielded 27 mg (60%) of a solid identified as 55.



55

$C_{22}H_{21}O_8Cl$

448.85

mp 105-108°C

(lit. 106-110°C)[44]

^{1}H NMR δ (ppm) 3.01 (ddd, J=9.5, 9.0, 7.0 Hz, 1H), 3.69 (s, 6H), 3.76 (s, 3H), 4.34 (dd, J=9.0, 8.5 Hz, 1H), 4.57 (dd, J=8.5, 7.0 Hz, 1H), 4.84 (s, 1H), 4.94 (d, J=9.5 Hz, 1H), 5.97 (d, J=0.5 Hz, 1H), 5.98 (d, J=0.5 Hz, 1H), 6.37 (s, 2H), 6.48 (s, 1H), 7.05 (s, 1H)

6. Desilylation of 4-O-trimethylsilyl-2-chloropodophyllotoxin with AcOH

The silylated derivative 53 (18 mg, 0.035 mmol) was dissolved in a small volume of AcOH/H_2O (4:1) and stirred at rt for 1 h. The solution was diluted with H_2O and extracted three times with 15 ml of CH_2Cl_2. The combined organic fractions were washed with saturated $NaHCO_3$, dried over $MgSO_4$, and the volume reduced yielding 13 mg (84%) of 55 as a beige solid.

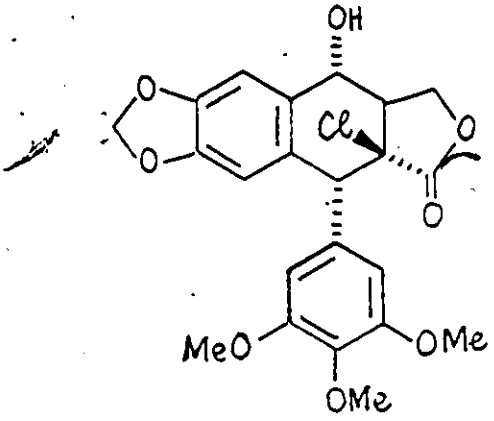
55

$C_{22}H_{21}O_8Cl$

448.85

mp 105-108°C

(lit. 106-110°C)[44]



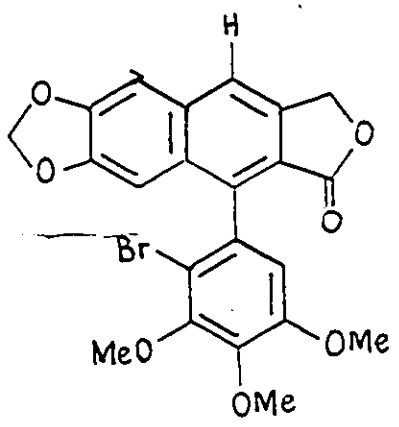
^1H NMR δ (ppm) 3.01 (ddd, J=9.5, 9.0, 7.0 Hz, 1H), 3.69 (s, 6H), 3.76 (s, 3H), 4.34 (dd, J=9.0, 8.5 Hz, 1H), 4.57 (dd, J=8.5, 7.0 Hz, 1H), 4.84 (s, 1H), 4.94 (d, J=9.5 Hz, 1H), 5.97 (d, J=0.5 Hz, 1H), 5.98 (d, J=0.5 Hz, 1H), 6.37 (s, 2H), 6.48 (s, 1H), 7.05 (s, 1H)

7. Preparation of the dianion of podophyllotoxin and subsequent reaction with bromine

Podophyllotoxin 1 (206 mg, 0.5 mmol) was dissolved in a small volume of dry THF and added slowly to a cooled (-78°C) solution of LDA (2 equivalents), prepared from diisopropylamine (110 mg) and n-BuLi (0.41 ml, 2.4M in hexanes) in 5 ml of dry THF. The faint yellow solution was stirred at -78°C for 15 minutes and then excess bromine (0.1 ml, d=3.119) was added. The solution was stirred at -78°C for 2.5 h and then warmed to 0°C for 1.5 h. The usual workup, including washing the organic layer with $Na_2S_2O_3$, afforded many inseparable components. PTLC (ethyl acetate/hexanes; 1:1, 3 runs) gave only one separable, non-polar component.

Precipitation from ether/hexanes furnished 42 mg (18%) of a white solid which was shown to be 60.

60



$C_{22}H_{17}O_7Br$

473.28

mp 228-230°C

^1H NMR δ (ppm) 3.80 (s, 3H), 3.95 (s, 3H), 4.09 (s, 3H), 5.40 (s, 1H), 5.42 (s, 1H), 6.09 (s, 2H), 6.60 (s, 1H), 6.86 (s, 1H), 7.22 (s, 1H), 7.72 (s, 1H)

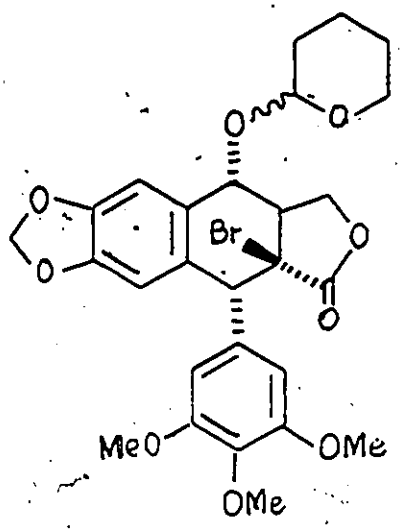
MS m/e 472 (M^+), 474 (M^++2)

8. Preparation of 4-O-tetrahydropyranylpodophyllotoxin

The THP derivative of podophyllotoxin 27 was prepared in 85% yield according to Gensler[19,20] using freshly distilled dihydropyran with p-TsOH as the acid catalyst and CH_2Cl_2 as the solvent.

9. Preparation of the enolate anion of 4-O-tetrahydropyranylpodophyllotoxin and subsequent reaction with bromine

The THP derivative 27 (501 mg, 1.00 mmol) was dissolved in a small volume of dry THF and added slowly to a cooled solution (-78°C) of LDA (one equivalent), prepared from diisopropylamine (0.154 ml) and n-BuLi (0.44 ml, 2.28 M in hexanes) in 5 ml of dry THF. The faint yellow solution was stirred for 15 minutes at -78°C and then excess Br_2 (0.06 ml 1.2 equivalents) was added. The solution was warmed to rt and stirred for an additional 15 minutes. The usual workup, including washing the organic layer with $Na_2S_2O_3$ and PTLC (ethyl acetate/hexanes; 1:3, 4 runs), afforded 2 components as beige solids. Each component was identified in order of elution from the preparative plate: 4-O-tetrahydropyranyl-2-bromopodophyllotoxin 65 (201 mg, 35%) and 4-O-tetrahydrapyranyl-2-bromopicropodophyllotoxin 66 (124 mg, 21%).



65

$C_{27}H_{29}O_9Br$

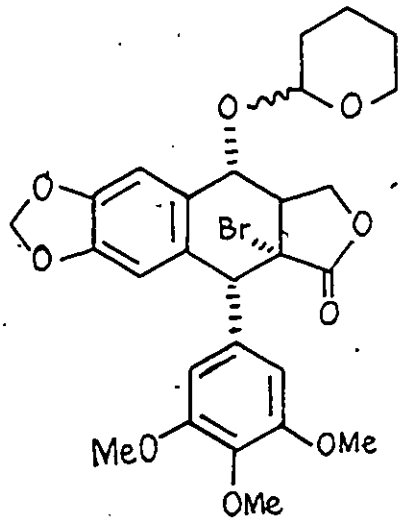
577.42

mp 86-89°C

IR ($CHCl_3$) ν max (cm^{-1}) 1785(s), 1595(s)

^1H NMR see Figure 8

MS m/e 576 (M^+), 578 (M^++2)



66

$C_{27}H_{29}O_9Br$

577.42

mp 82-85°C

IR ($CHCl_3$) ν max (cm^{-1}) 1780(s), 1592(s)

^1H NMR see Figure 9

MS m/e 576 (M^+), 578 (M^++2)

10. Preparation of the enolate anion of 4-O-tetrahydropyranylpodophyllotoxin and subsequent reaction with carbon tetrabromide

The THP derivative 27 (200 mg, 0.40 mmol) was dissolved in a small volume of dry THF and added slowly to a cooled solution (-78°C) of LDA (one equivalent), prepared from diisopropylamine (0.06 ml) and n-BuLi (0.20 ml, 2.0 M in hexanes) in 8 ml of dry THF. The faint yellow solution was stirred for 15 minutes at -78°C and then CBr_4 (133 mg, 0.40 mmol), was added. The solution was warmed to rt and stirred for an additional 2.5 days. The usual workup, including washing the organic layer with Na_2SO_3 and PTLC (ethyl acetate/hexanes; 1:3, 4 runs), afforded 2 components as beige solids. Each component was identified in order of elution from the preparative plate: 4-O-tetrahydropyranyl-2-bromopodophyllotoxin 65 (26 mg, 11%) and 4-O-tetrahydrapyranyl-2-bromopicropodophyllotoxin 66 (76 mg, 33%). Both components were identical in all respects to those products obtained via the Br_2 route.

11. Preparation of the enolate anion of-4-O-tetrahydropyranylpodophyllotoxin and subsequent reaction with benzenesulfinic bromide

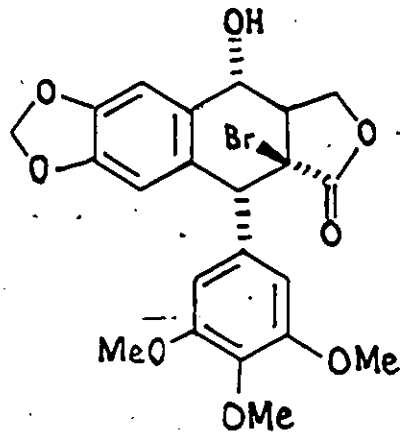
The THP derivative 27 (250 mg, 0.50 mmol) was dissolved in a small volume of dry THF and added slowly to a cooled solution (-78°C) of LDA (one equivalent), prepared from diisopropylamine (57 mg) and n-BuLi (0.22 ml, 2.27 M in hexanes) in 5 ml of dry THF. The faint yellow solution was stirred for 15 minutes at -78°C and then benzenesulfinic bromide (111 mg, 0.5 mmol) in a small volume of dry THF was added. The solution was stirred at -78°C for 1 h, warmed to rt, and stirred for an additional 2 h. The usual workup, including washing the organic layer with $Na_2S_2O_3$ and PTLC (ethyl acetate/hexanes; 1:3, 4 runs), afforded 3 components as beige solids. Each component was identified in order of elution from the preparative plate: 4-O-tetrahydropyranyl-2-bromopodophyllotoxin 65 (47 mg, 16%), 4-O-tetrahydrapyranyl-2-bromopicropodophyllotoxin 66 (93 mg, 32%) and starting material (101 mg, 40%*). All three components were identical in all respects to those components previously identified.

* based on starting material and not on desired product.

12. Hydrolysis of 4-O-tetrahydropyranyl-2-bromopodophyllotoxin

The 2-bromo derivative 65 (88 mg, 0.15 mmol) was hydrolysed at rt in 10 ml of a solution of 10% HCl and THF (1:9) for 2 h. Workup, followed by PTLC (ethyl acetate/hexanes; 1:1, 3 runs), afforded a beige powder. The beige powder was identified as being 2-bromopodophyllotoxin 67 (38 mg, 51%).

67



$C_{22}H_{21}O_8Br$

493.31

mp 89-93°C

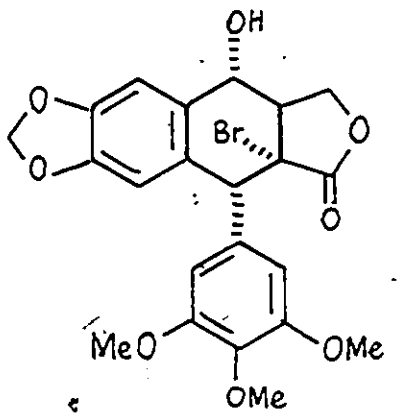
IR ($CHCl_3$) ν max (cm^{-1}) 3800-3600 (br), 1785(s), 1595(s)

^{1}H NMR δ (ppm) 2.43 (d, J=7.0 Hz, OH) 2.66 (ddd, J=9.6, 9.0, 6.8 Hz, 1H), 3.75 (s, 6H), 3.81 (s, 3H), 4.25 (dd, J=9.6, 8.8 Hz, 1H), 4.56 (dd, J=8.8, 6.8 Hz, 1H), 4.91 (s, 1H), 4.92 (dd, J=9.0, 7.0 Hz; 1H), 5.99 (d, J=0.5 Hz, 1H) 6.01 (d, J=0.5 Hz, 1H), 6.42 (s, 2H), 6.52 (s, 1H), 7.10 (s, 1H)

MS m/e 394 (M^+-HBr, H_2O)

13. Hydrolysis of 4-O-tetrahydropyranyl-2-bromopicropodophyllotoxin

The 2-bromo derivative 66 (112 mg, 0.19 mmol) was hydrolysed by refluxing in 10 ml of a solution of 10% HCl and THF (1:9) for 2 h. Workup, followed by PTLC (ethyl acetate/hexanes; 1:1, 3 runs), afforded a beige powder. The beige powder was identified as being 2-bromopicropodophyllotoxin 68 (57 mg, 60%).



68

$C_{22}H_{21}O_8Br$

493.31

mp 93-98°C

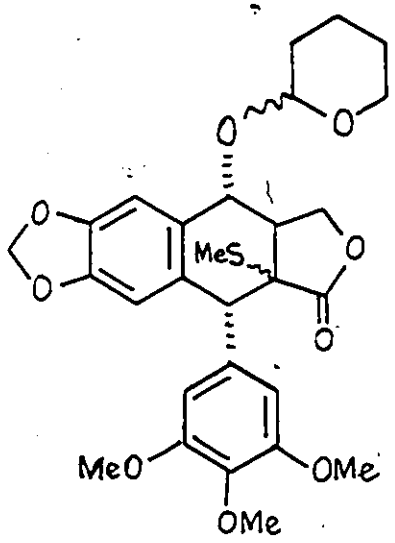
IR ($CHCl_3$) ν max (cm^{-1}) 3800-3650 (br), 1785(s), 1595(s)

^1H NMR δ (ppm) 2.57 (br s, OH), 3.22 (dd, J=4.8, 2.4 Hz, 1H), 3.83 (s, 9H), 4.47 (d, J=9.4 Hz, 1H), 4.55 (s, 1H), 4.81 (dd, J=9.4, 4.8 Hz, 1H), 4.94 (br s, 1H), 5.91 (d, J=0.5 Hz, 1H), 5.93 (d, J=0.5 Hz, 1H) 6.61 (s, 1H), 6.72 (s, 1H), 6.81 (s, 2H)

MS m/e 394 (M^+-HBr, H_2O)

14. Preparation of the enolate anion of 4-O-tetrahydropyranylpodophyllotoxin and subsequent reaction with dimethyl disulphide

The THP derivative 27 (498 mg, 1.00 mmol) was dissolved in a small volume of dry THF and added slowly to a cooled solution (-78°C) of LDA (one equivalent), prepared from diisopropylamine (0.154 ml) and n-BuLi (0.45 ml, 2.24 M in hexanes) in 5 ml dry THF. The faint yellow solution was stirred at -78°C and then excess Me_2S_2 was added next. The solution was allowed to stir at -78°C for 2 h and then was warmed to rt and allowed to stir for an additional 2 h. Normal workup and PTLC (ethyl acetate/hexanes; 1:3, 6 runs) afforded one main component as a beige solid. This component was identified as 4-O-tetrahydropyranyl-2-(methylthio)picropodophyllotoxin 69 (295 mg, 54%) after removal of the THP group.



69

$C_{28}H_{32}O_9S$

544.62

mp 77-78°C

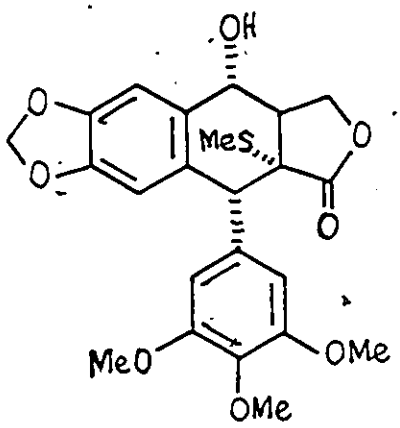
IR ($CHCl_3$) ν max (cm^{-1}) 1780(s), 1595(s)

^1H NMR see Figure 12

MS m/e 544 (M^+)

15. Hydrolysis of 4-O-tetrahydropyranyl-2-(methylthio)picropodophyllotoxin

The THP derivative 69 (99 mg, 0.18 mmol) was hydrolysed in a 10 ml solution of 5% HCl/THF (1:9). The solution was refluxed for 1 h, cooled, diluted with H_2O and extracted 3 times with 25 ml CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure. PTLC (ethyl acetate/hexanes; 3:1, 1 run), afforded 2-(methylthio)picropodophyllotoxin 70 (58 mg, 70%) as a beige solid.



70

$C_{23}H_{24}O_8S$

493.31

mp 97-101°C

IR ($CHCl_3$) ν max (cm^{-1}) 3800-3660 (br), 1780(s), 1595(s)

^1H NMR δ (ppm) 2.01 (s, 3H); 2.12 (d, J=5.0 Hz, OH), 2.88 (dd, J=4.7, 2.6 Hz; 1H), 3.84 (s, 9H), 4.37 (d, J=10.1 Hz, 1H), 4.40 (s, 1H), 4.83-4.85 (m, 2H), 5.91 (s, 1H), 5.93 (s, 1H), 6.72 (s, 1H), 6.75 (s, 1H), 6.91 (s, 1H)

MS m/e 460 (M^+)

16. Attempted Silylation of Podophyllotoxin with tert-butyldimethylsilyl chloride

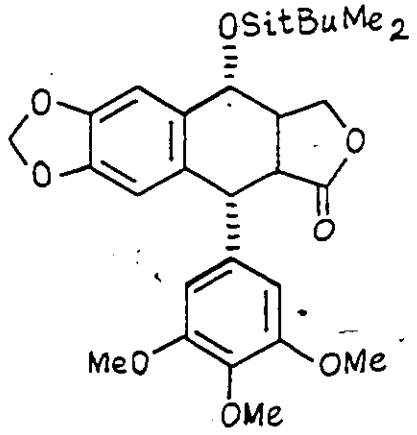
Silylation of podophyllotoxin with TBDMSCl was attempted many times using imidazole and/or triethylamine as base in DMF. The temperatures were varied from rt to 50-60° using various reaction times (1h - 1 week). After workup, starting material and/or polymeric material was recovered.

17. Epimerization of Podophyllotoxin to Picropodophyllotoxin

2 g of podophyllotoxin was dissolved in a solution of absolute ethanol (99%, 30 ml) and aqueous sodium acetate (10%, 20 ml). The solution was refluxed for 18 hrs, cooled and filtered. The precipitate was washed with H_2O, air dried and recrystallized from absolute methanol. The desired product, picropodophyllotoxin 10, was obtained in 79% yield.

18. Silylation of Picropodophyllotoxin with tert-butyldimethylsilyl chloride

Picropodophyllotoxin (1.38 g, 3.33 mmol), was dissolved in a small volume of dry DMF and added to a solution of dry DMF containing a large excess of tert-butyldimethylsilyl chloride (1.16 g, 8.1 mmol) and imidazole (1.04 g, 15.3 mmol). The solution was heated at 75° in an oil bath for 1.5 h and at rt overnight. The solution was quenched with a saturated brine solution, and normal workup followed by recrystallization (CH_2Cl_2) yielded 1.58 g (90%) of a beige powder.



71

$C_{28}H_{36}O_8Si$

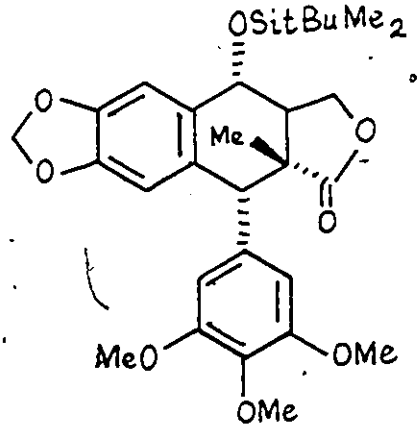
528.67

^1H NMR δ (ppm) 0.08 (s, 6H), 0.90 (s, 9H), 2.77 (m, 1H), 3.22 (dd, J=9.1, 5.1 Hz, 1H), 3.80 (s, 6H), 3.84 (s, 3H), 4.09 (d, J=5.1 Hz, 1H), 4.41 (dd, J=10.1, 6.1 Hz, 1H), 4.48 - 4.51 (m, 2H), 5.92 (d, J=1.4 Hz, 1H), 5.94 (d, J=1.4 Hz, 1H), 6.36 (s, 1H), 6.43 (s, 2H), 7.02 (s, 1H)

MS m/e 528 (M^+)

19. Preparation of the anion of 71 and subsequent reaction with methyl iodide

71 (206 mg, 0.39 mmol) was dissolved in a small volume of dry THF and added slowly to a cooled solution (-78°C) of LDA (1 equivalent), prepared from diisopropylamine (0.06 ml) and n-BuLi (0.21 ml, 1.86 M) in 3 ml of dry THF. The solution was stirred for 5 minutes at -78°C and then excess methyl iodide (1 ml) was added. The solution was stirred at -78°C for 15 minutes and then it was warmed to rt. During this time the solution turned from a faint yellow colour to a strong clear yellow with a fine, white precipitate forming. Normal workup, followed by PTLC (ethyl acetate/hexanes; 1:5, 3 runs), afforded two beige solids. Each component was identified in order of elution from the preparative plates: 4-O-t-butyldimethylsilyl-2-methylpodophyllotoxin 73 (66 mg, 31%) and 4-O-t-butyldimethylsilyl-2-methylpicropodophyllotoxin 74 (67 mg, 32%).



73

$C_{29}H_{38}O_8Si$

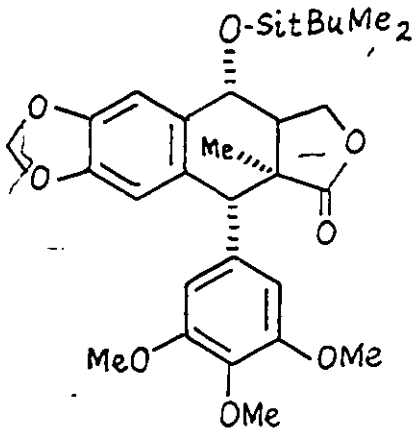
542.70

mp 44-48°C

IR ($CHCl_3$) ν max (cm^{-1}) 1785(s), 1590(s)

^{1}H NMR δ (ppm) 0.11 (s, 3H), 0.30 (s, 3H), 0.94 (s, 9H), 1.31 (s, 3H), 3.02 (ddd, J=11.1, 10.4, 7.2 Hz, 1H), 3.74 (s, 6H), 3.81 (s, 3H), 4.08 (dd, J=11.1, 8.2 Hz, 1H), 4.24 (s, 1H), 4.42 (dd, J=8.2, 7.2 Hz, 1H), 4.71 (d, J=10.4 Hz, 1H), 5.95 (d, J=1.2 Hz, 1H), 5.97 (d, J=1.2 Hz, 1H), 6.37 (s, 2H), 6.46 (s, 1H), 6.92 (s, 1H)

MS m/e 542 (M^+)



74

$C_{29}H_{38}O_8Si$

542.70

mp 55-59°C

IR ($CHCl_3$) ν max (cm^{-1}) 1780(s), 1598(s)

^{1}H NMR δ (ppm) 0.19 (s, 3H), 0.22 (s, 3H), 0.96 (s, 9H), 1.14 (s, 3H), 2.53 (ddd, J=6.4, 5.3, 4.1 Hz, 1H), 3.82 (s, 6H), 3.85 (s, 3H), 4.10 (s, 1H), 4.23 (dd, J=9.9, 4.1 Hz, 1H), 4.48 (dd, J=9.9, 6.4 Hz, 1H), 4.68 (d, J=5.3 Hz, 1H), 5.91 (d, J=1.2 Hz, 1H), 5.93 (d, J=1.2 Hz, 1H), 6.52 (s, 2H), 6.56 (s, 1H), 6.80 (s, 1H)

MS m/e 542 (M^+)

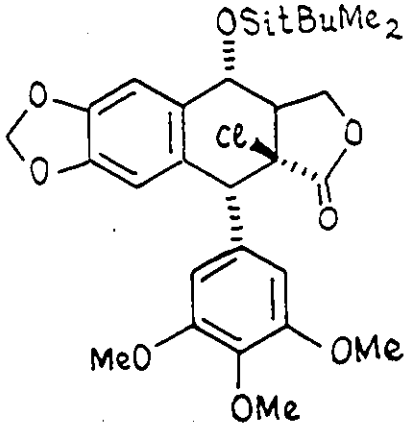
20. Preparation of the anion of 71 and subsequent reaction with hexachloroethane

71 (206 mg, 0.39 mmol) was dissolved in a small volume of dry THF and was added slowly to a cooled solution (-78°C) of LDA (1 equivalent), prepared from diisopropylamine (0.06 ml) and n-BuLi (0.34 ml, 1.16 M) in 3 ml of dry THF. The solution was stirred at -78°C for 15 minutes and then excess hexachloroethane (.789 g) was added next. The reaction was stirred at -78°C for 15 minutes, warmed to rt, and stirred for an additional 18 h. Normal workup, including washing the organic layer with Na_2SO_3, followed by PTLC (ethyl acetate/hexanes; 1:3, 1 run), afforded a beige powder (83 mg, 38%) identified as 4-O-(t-butyldimethylsilyl)-2-chloropodophyllotoxin 77.

77

$C_{28}H_{35}O_8ClSi$

563.12



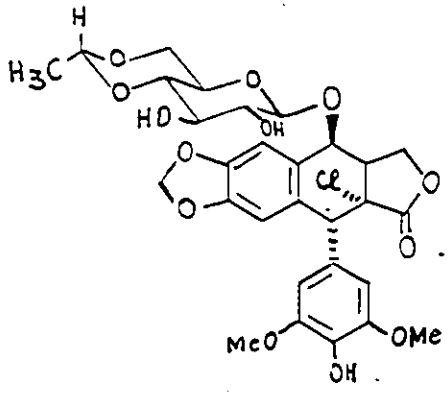
IR ($CHCl_3$) ν max (cm^{-1}) 1790(s), 1590(s)

^1H NMR δ (ppm) 0.13 (s, 3H), 0.33 (s, 3H), 0.94 (s, 9H), 3.14 (ddd, J=10.0, 8.8, 6.9 Hz, 1H), 3.74 (s, 6H), 3.81 (s, 3H), 4.29 (dd, J=10.0, 8.3 Hz, 1H), 4.50 (dd, J=8.3, 6.9 Hz, 1H), 4.74 (s, 1H), 4.95 (d, J=8.8 Hz, 1H), 5.97 (d, J=1.3 Hz, 1H), 6.00 (d, J=1.3 Hz, 1H), 6.42 (s, 2H), 6.50 (s, 1H), 6.94 (s, 1H)

MS m/e 562 (M^+) 564 (M^++2)

21. Preparation of 4'-O-demethyl-2-chloro-1-O-(4,6-ethylidene-β-D-glucopyranosyl)-epipicropodophyllotoxin 79

Etoposide 42 (295 mg, 0.50 mmol) was dissolved in a small volume of dry THF and was added slowly to a cooled (0°C) solution of LDA (4 equivalents), prepared from diisopropylamine (232 mg) and n-BuLi (1.0 ml, 2.0 M) in a dry solution of THF/HMPA (9/1). The approximate molarity of the polyanion was 6 mg/ml. The solution was stirred at 0°C for 15 minutes and then excess hexachloroethane was added. The solution was stirred at 0°C for an additional 15 minutes, warmed to rt, and stirred for an additional 24 h. Normal workup, including washing the organic layer with a saturated solution of Na_2SO_3, followed by column chromatography yielded a beige solid identified as 4'-O-demethyl-2-chloro-1-O-(4,6-ethylidene-β-D-glucopyranosyl)-epipicropodophyllotoxin 79 (220 mg, 71%).



79

$C_{29}H_{31}O_{13}Cl$

623.00

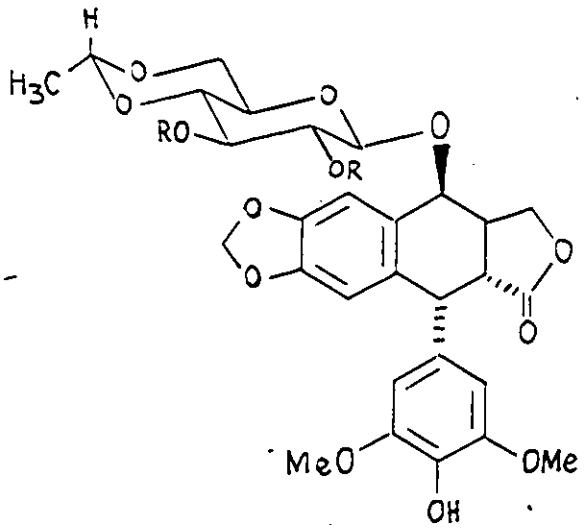
mp 160-162°C (lit. 155-160°C)

^1H NMR δ (ppm) 1.35 (d, J=5.1 Hz, 3H), 3.20 (m, 1H), 3.27-3.34 (m, 2H), 3.49-3.56 (m, 2H), 3.65 (t, J=8.9 Hz, 1H), 3.83 (s, 6H), 4.10 (dd, J=10.3, 4.8 Hz, 1H), 4.25 (d, J=7.6 Hz, 1H), 4.53 (dd, J=9.7, 7.0 Hz, 1H), 4.63 (dd, J=9.7, 1.7 Hz, 1H), 4.70 (s, 1H), 4.81 (q, J=5.1 Hz, 1H), 5.12 (d, J=5.5 Hz, 1H), 5.50 (s, OH), 5.95 (d, J=1.2 Hz, 1H), 5.97 (d, J=1.2 Hz, 1H), 6.57 (s, 2H), 6.70 (s, 1H), 7.10 (s, 1H)

MS m/e 622 (M^+), 624 (M^++2)

22. Reaction of Etoposide with tert-butyldimethylsilyl chloride

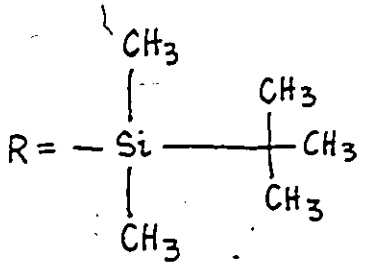
Etoposide 42 (200 mg; 0.348 mmol) was dissolved in 2 ml of dry THF and was added slowly to a solution of DMF containing TBDMSCl (210 mg, 1.40 mmol) and imidizole (119 mg, 1.74 mmol). The solution was heated for 2 hours at about 50°C to about 60°C and then was cooled to rt. 10 ml of hexanes was added followed by 15 ml CH_2Cl_2 whereupon a white powder precipitated and was discarded. The filtrate was washed four times with water, dried over sodium sulfate, and condensed to yield 355 mg of an oil. Column chromatography with ethyl acetate/hexanes (2:3) as eluant yielded 257 mg (90%) as a clear foam/oil which was identified as 80.



80

$C_{41}H_{60}O_{13}Si_2$

817.09



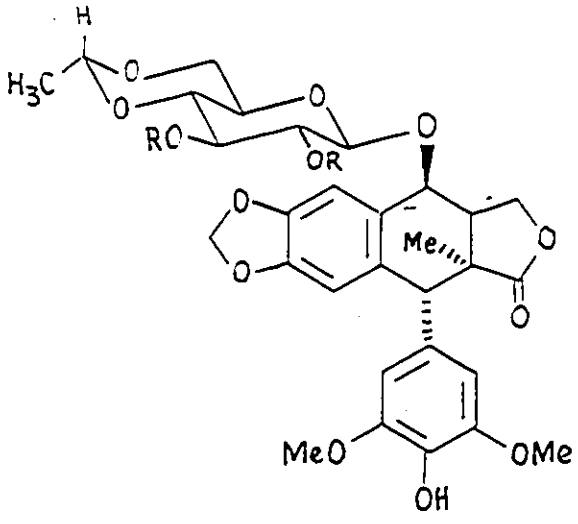
^{1}H NMR δ (ppm) 0.09 (s, 9H), 0.10 (s, 3H), 0.88 (s, 9H), 0.98 (s, 9H), 1.35 (d, J=5.1 Hz, 3H), 2.82-2.96 (m, 1H), 3.20-3.32 (m, 3H), 3.39 (t, J=8.0 Hz, 1H), 3.55 (t, J=10.0 Hz, 1H), 3.66 (hidden, 1H), 3.66 (s, 6H), 4.10-4.24 (m, 2H), 4.42 (dd, J=10.4, 8.9 Hz, 1H), 4.56 (d, J=5.3 Hz, 1H), 4.64 (d, J=8.8 Hz, 1H), 4.70 (q, J=5.1 Hz, 1H), 4.89 (d, J=3.9 Hz, 1H), 5.97 (d, J=1.2 Hz, 1H), 6.00 (d, J=1.2 Hz, 1H), 6.20 (s, 2H), 6.54 (s, 1H), 6.83 (s, 1H)

MS m/e 817 (M^+)

Analysis:	calculated	C 60.26%	H 7.40%
	found	C 60.11%	H 7.68%

23. Methylation of Disilylated Etoposide 80

Disilylated Etoposide 80 (194 mg, 0.23 mmol) was dissolved in a small volume of dry THF and added slowly to a cooled (-78°C) solution of LDA (2 equivalents), prepared from diisopropylamine (49 mg, 0.487 mmol) and n-BuLi (0.237 ml, 2.0 M, 0.475 mmol) in 10 ml of dry THF. The solution was stirred for 20 minutes at -78°C and then excess CH_3I (0.84 ml, 13.5 mmol) was added next. The solution was stirred for an additional 10 minutes and then was warmed to rt wherein the solution changed in colour from colourless to yellow. The solution was stirred overnight at rt whereupon a brown precipitate had formed. Normal workup, followed by column chromatography (ethyl acetate/hexanes; 1:4) and PTLC (ethyl acetate/hexanes; 1:3) yielded 63 mg (33%) of a white solid which was identified as 82.

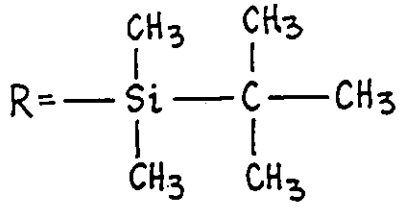


82

$C_{42}H_{62}O_{13}Si_2$

831.10

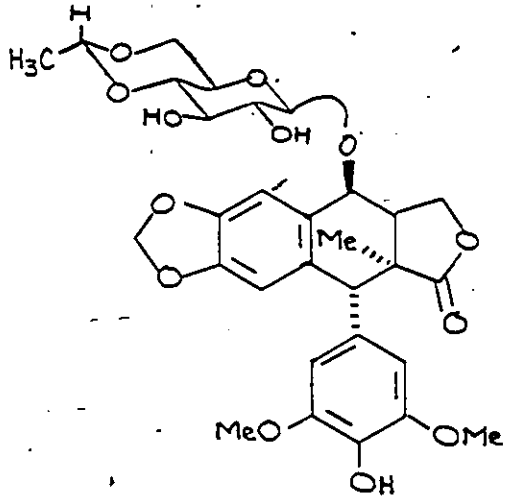
mp 216-218°C



^{1}H NMR. δ (ppm) 0.09 (s, 3H), 0.115 (s, 6H), 0.120 (s, 3H), 0.90 (s, 9H), 0.98 (s, 9H), 1.30 (s, 3H), 1.32 (d, J=4.7 Hz, 3H), 2.80-2.88 (m, 1H), 3.14-3.25 (m, 2H), 3.48-3.55 (m, 2H), 3.61 (t, J=9.0 Hz, 1H), 3.72 (s, 6H), 4.08 (dd, J=10.1, 4.3 Hz, 1H), 4.28 (dd, J=10.2, 8.7 Hz, 1H), 4.34 (s, 1H), 4.39 (d, J=7.1 Hz, 1H), 4.42 (dd, J=10.2, 3.5 Hz, 1H), 4.67 (q, J=4.7 Hz, 1H), 5.15 (d, J=6.5 Hz, 1H), 5.93 (d, J=1.2 Hz, 1H), 5.94 (d, J=1.2 Hz, 1H), 6.33 (s, 2H), 6.69 (s, 1H), 7.20 (s, 1H)

24. Desilylation of 82

82 (40 mg, 0.048 mmol) was dissolved in 5 ml of dry THF and then 0.14 ml of TBAF (0.144 mmol, 1M) was added at rt. The solution was stirred for 1 hour at rt and then poured in saturated NH_4Cl solution, extracted three times with ether, dried over $MgSO_4$, and evaporated under reduced pressure. Crystallization from ethyl acetate/hexanes yielded 20 mg (70%) of a white solid which was identified as 83.



83

$C_{30}H_{34}O_{13}$

602.57

mp 185-190°C

^1H NMR δ (ppm) 1.23 (s, 3H), 1.35 (d, J=5.0 Hz, 3H), 2.75 (m, 1H; br, OH,), 2.86 (br., OH), 3.14-3.22 (m, 1H), 3.32 (t, J=9.3 Hz, 1H), 3.50-3.58 (m, 2H), 3.66 (t, J=9.5 Hz, 1H), 3.82 (s, 6H), 4.10 (dd, J=10.1, 7.7 Hz, 1H), 4.24 (d, J=7.1 Hz, 1H), 4.36 (dd, J=9.9, 7.9 Hz, 1H), 4.37 (s, 1H), 4.45 (dd, J=9.9, 3.7 Hz, 1H), 4.71 (q, J=5.0 Hz, 1H), 5.08 (d, J=5.5 Hz, 1H), 5.47 (br s, phenolic), 5.93 (d, J=1.2 Hz, 1H), 5.94 (d, J=1.2 Hz, 1H), 6.42 (s, 2H), 6.68 (s, 1H), 7.03 (s, 1H)

MS m/e 602 (M^+)

CLAIMS TO ORIGINAL RESEARCH

1. A simple method of isolating pure 2-chloropodophyllotoxin and 2-chloropicropodophyllotoxin via their trimethylsilyl ethers was developed.

2. The synthesis of C-2 substituted podophyllotoxin and picropodophyllotoxin derivatives from the alkoxy, 4-OTMS or 4-OTBDMS enolates was reported.

3. It was found that electronegative substituents at C-2 in picropodophyllotoxin induced a conformational change.

4. The following is a list of the new compounds that were prepared and characterized:

   a) 4-OTMS-2-chloropodophyllotoxin

   b) 4-OTMS-2-chloropicropodophyllotoxin

   c) 2-chloropicropodophyllotoxin

   d) 4-OTHP-2-bromopodophyllotoxin

   e) 4-OTHP-2-bromopicropodophyllotoxin

   f) 2-bromopodophyllotoxin

   g) 2-bromopicropodophyllotoxin

   h) 4-OTHP-2-methylthiopicropodophyllotoxin

   i) 2-methylthiopicropodophyllotoxin

j) 4-OTBDMS-2-methylpodophyllotoxin

k) 4-OTBDMS-2-methylpicropodophyllotoxin

l) 4-OTBDMS-2-chloropodophyllotoxin

m) 2-chloropicro-Etoposide, 79

n) Disilylated (TBDMS)-Etoposide, 80

o) Dislylated (TBDMS)-2-methylpicro-Etoposide, 82

p) 2-methylpicro-Etoposide, 83

REFERENCES

1. Muenscher, W.C.; Poisonous Plants of the United States, New York, MacMillan Co., page 94, 1939.
2. Hartwell, J.L.; Schrecker, A.W.; The Chemistry of Podophyllum, Fortsch. Chem. Org. Natur., XV, 6-166 (1958).
3. Pharmacopoeia of the United States of America, 1st Ed. (1820).
4. King, J.; College J. Med. Sci., 2, 557 (1857).
5. Pharmacopeia of the United States of America, 15th Revision (1955).
6. Good, P.P.; The Family Flora and Materia Medica Botanica, 2 Vols., Elizabethtown, N.J. (1845).
7. Kaplan, I.W.; Condylomata Acuminata, New Orleans Med. Surg. J., 94, 388 (1942).
8. Kelly, M.G.; Hartwell, J.L.; J. Nat. Cancer Inst., 14, 967 (1954).
9. Podwyssotzki, V.; Pharmacologische Studien über Podophyllum Peltatum, Arch. Exp. Pathol. Pharmakol., 13, 29 (1880).
10. Hartwell, J.L., J. Am. Chem. Soc., 69, 2918 (1947).
11. Hartwell, J.L.; Detty, W.E., J. Am. Chem. Soc., 70, 2833 (1948).
12. Hartwell, J.L.; Detty, W.E., J. Am. Chem. Soc., 72, 246 (1950).
13. Hartwell, J.L.; Schrecker, A.W.; Greenberg, G.Y., J. Am. Chem. Soc., 74, 6285 (1952).
14. Nadkarni, M.V.; Maury, P.B.; Hartwell, J.L.; J. Am. Chem. Soc., 74, 280 (1952).
15. Nadkarni, M.V.; Hartwell, J.L.; Maury, P.B.; Leiter, J.J.; J. Am. Chem. Soc., 75, 1308 (1953).

16. Haworth, R.D., Natural Resins, Ann. Rep. Chem. Soc. (London), 33, 266 (1936).

17. Schrecker, A.W.; Hartwell, J.L.; J. Am. Chem. Soc., 75, 5916 (1953).

18. Petcher, J.L.; Weber, H.P.; Kuhn, M.; von Wartburg, A.; J. Chem. Soc. Perkin II, 288 (1973).

19. Gensler, W.J.; Wang, S.Y.; J. Am. Chem. Soc., 76, 315 (1954).

20. Gensler, W.J.; Wang, S.Y.; J. Am. Chem. Soc., 76, 5896 (1954).

21. Gensler, W.J.; Gatsonis, C.D.; J. Org. Chem., 31, 3224 (1966).

22. Gensler, W.J.; Gatsonis, C.D.; J. Org. Chem., 31, 4004 (1966).

23. Kende, A.S.; Liebeskind, L.S.; Mills, J.E.; Rutledge, P.S.; Curran, D.P.; J. Am. Chem. Soc., 99, 7082 (1977).

24. Kende, A.S.; King, M.L.; Curran, D.P.; J. Org. Chem.; 40, 2828 (1981).

25. Rodrigo, R.; J. Org. Chem., 45, 4538 (1980).

26. Plaumann, H.P.; Smith, J.G.; Rodrigo, R.; J. Chem. Soc. Chem. Comm., 354 (1980).

27. Renz, J.; Kuhn, M.; von Wartburg, A.; Liebigs Ann. Chem., 681, 207 (1965).

28. Loike, J.D.; Horwitz, S.B.; Biochemistry, 15, 5443 (1976).

29. Kelly, M.G.; Hartwell, J.L.; J. Nat. Cancer Inst., 14, 967 (1954).

30. Corman, I.; Corman, M.E.; Ann. N.Y. Acad. Sci., 51, 1443.

31. Gensler, W.J.; Judge, J.F.X.; Leeding, M.V.; J. Org. Chem., 37, 1062 (1972).

32. Gensler, W.J.; Murthy, C.D.; Trammell, M.H.; J. Med. Chem., 20, 635 (1977).

33. Loike, J.D.; Brewer, C.F.; Sternlicht, H.; Gensler, W.J.; Horwitz, S.B.; Cancer Res., 38, 2688 (1978).

34. Kuhn, M.; von Wartburg, A.; Helv. Chim. Acta , 51, 163 (1968).

35. Kuhn, M.; von Wartburg, A.; Helv. Chim. Acta , 51, 1631 (1968).

36. Keller-Juslén, C.; Kuhn, M.; von Wartburg, A.; Stähelin, H.; J. Med. Chem., 14, 936 (1971).

37. Issell, B.F.; Crooke, S.T.; Cancer Treatment Rev., 6, 107 (1979).

38. Radice, P.A.; Bunn, P.A. Jr.; Ihde, D.C.; Cancer Treatment Rep., 63, 1231 (1979).

39. Eagen, R.T.; Carr, D.T.; Frytak, S.; Rubin, J.; Lee, R.E.; Cancer Treatment Rep., 60, 949 (1976).

40. Drewinko, B.; Barlogic, B.; Cancer Treatment Rep., 60 1295 (1976).

41. Stähelin, H.; Poschmann, G.; Oncology, 35, 217 (1978).

42. Loike, J.D.; Horwitz, S.B.; Biochemistry, 15 5435 (1976).

43. Jardine, I.; Strife, R.J.; Kozlowski, J.; J. Med. Chem., 25, 1077 (1982).

44. Glinski, M.B.; Ph.D. Thesis, University of Ottawa (1982).

45. The podo/picro ratio was determined by inspection of the 60 MHz ^{1}H NMR.

46. Brewer, C.F.; Loike, J.D.; Horwitz, S.B.; Sternlicht, H.; Gensler, W.J.; J. Med. Chem., 22, 215 (1979).

47. Haworth, R.D.; Richardson, T.; J. Chem. Soc., 1936, 348.

48. Klemm, L.H.; Gopinath, D.; Hsu, L.; Kelly, F.W.; Trod, E.; McGuire, T.M.; Tetrahedron, 1966, 22, 1797.

49. Ayres, D.C.; Lim, C.K.; J. Chem. Soc., Perkin I, 1976, 832.

50. Ayres, D.C.; Harris, J.A.; Jenkins, P.N.; Phillips, L.; J. Chem. Soc., Perkin I, 1972, 1343.

51. Ayres, D.C.; Lim, C.K.; J. Chem. Soc., Perkin I, 1972, 1350.

52. Supplied by Dr. T. Doyle, Bristol Laboratories, Syracuse, New York.