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To my family here in Canada and over in Iran

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I would like to thank my research supervisor Tony Durst for all that he has taught me during my stay at this university. His patience and encouragement are greatly appreciated. I would also like to thank Raj Capoor for the ^{13}C nmr spectra, Keith Bourke for the 200 M Hz ^{1}H nmr spectra and Clem Kazakoff for the mass spectra. The biological test results from Bristol Laboratories, Syracuse, New York, are gratefully acknowledged.

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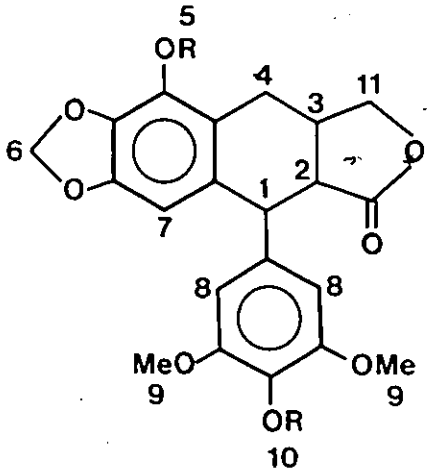
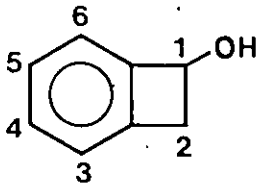
ABBREVIATION

AcOH	acetic acid
n-BuLi	n-butyllithium
DCC	dicyclohexylcarbodiimide
DMF	dimethylformamide
h	hours
HPLC	high performance liquid chromatography
LDA	lithium diisopropyl amide
MP	melting point
M.S.	mass spectra
NBS	N bromosuccinimide
nmr	nuclear magnetic resonance
PTLC	preparative thin layer chromatography
RT	room temperature
TBAF	tetrabutyl ammonium fluoride
TBDMS	tert-butyldimethyl silyl
THF	tetrahydrofuran
THP	tetrahydropyranyl
TLC	thin layer chromatography
TsOH	p-toluenesulfonic acid
°	refers to degrees Celcius (°C)
DHP	dihydropyran

NUMBERING SYSTEMS



Peltatin analogues



Benzocyclobutenol derivatives

ABSTRACT

This thesis deals with the synthesis of non-enolizable derivatives of α- and β-peltatin. A method for isolating pure α- and β-peltatin via their t-butyldimethyl.silyl ethers was developed. Substitution at C_2 was achieved by formation of the respective enolate anions with LDA followed by trapping with various electrophiles. It was found that substitution at C_2 with a methyl group produced derivatives retaining the desired trans-lactone ring stereochemistry. Desilylation of these 2-methyl (trans) derivatives by treatment with TBAF resulted in compounds which showed significant levels of antitumour activity against P388 leukemia. An attempt to prepare the tetra-acetyl glucoside of β-peltatin was also made.

INTRODUCTION

Podophyllum is the dried roots and rhizomes of species of Podophyllum (family: Berberidaceae).[1] Podophyllum peltatum Linnaeus (Figure 1), commonly called May Apple, American Mandrake, Indian Apple, Wild Lemon or Duck's Foot, is found in moist, shady woodlands and marshy meadows throughout the region east of the Mississippi from southeastern Canada to the Gulf of Mexico. The Indian species, Podophyllum emodi Wallich, was discovered by Wallich in 1824 and grows in the interior ranges of the Himalaya Mountains from Sikkim to Hazara. In 1950, Chatterjee and Mukerjee discovered a third species, growing in the Himalayan region, which was named Podophyllum sikkimensis R. Chatterjee et S. K. Mukerjee.

The early colonists learned of the medical properties of the root of Podophyllum peltatum from the North American Indians who knew of its properties as a cathartic, anthelmintic and as a mortal poison.[1] Podophyllum became so popular in the United States as a cathartic and chologogue that it was included in the first edition of the United States Pharmacopoeia in 1820 but was dropped from the twelfth revision in 1942, to be included again in the fifteenth revision in 1955.[2]

Podophyllin or podophyllum resin (also called resina podophylli or podophyllinum) refers to the alcohol-soluble water-insoluble portion of podophyllum and was first prepared by King in 1835.[3] Most of the biological activity of podophyllum resides in this resin. It became official in the fourth revision of the U. S. Pharmacopoeia

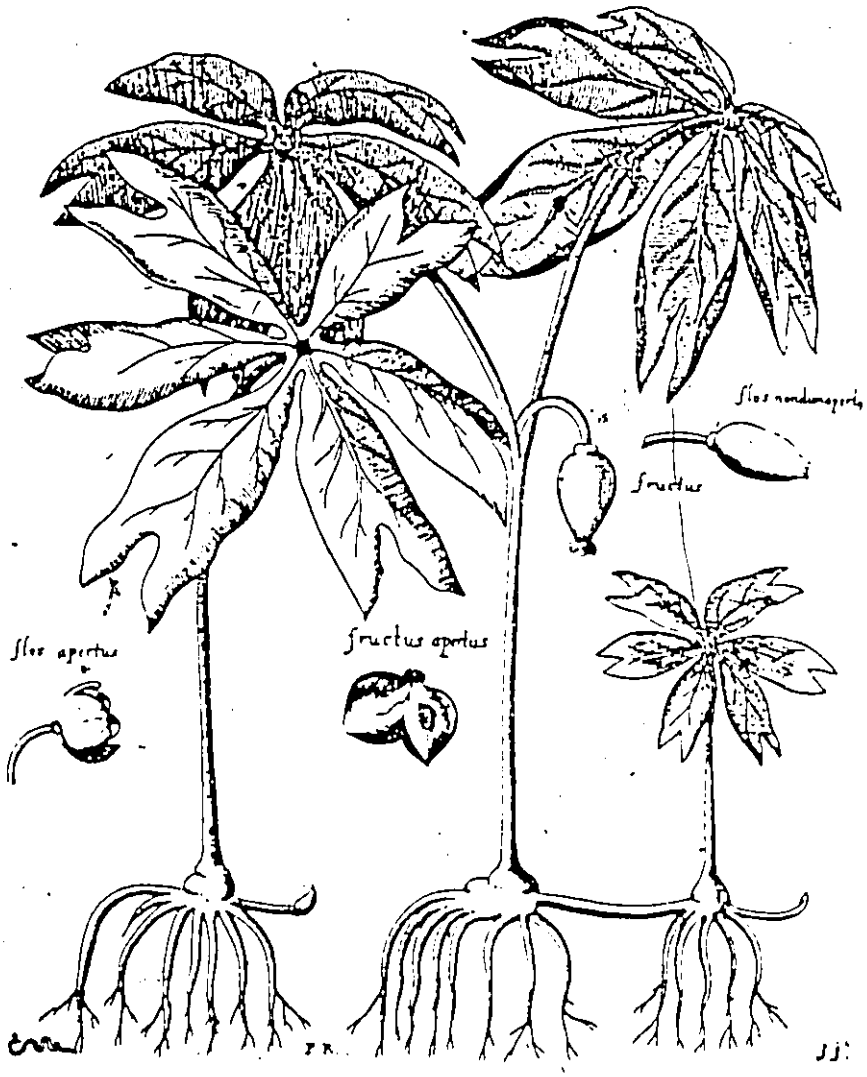


FIGURE 1 Podophyllum peltatum (May Apple)

in 1863. The official method of preparing the resin in the United States is given in the following excerpt from the U. S. Pharmacopoeia, fifteenth revision:[2]

" 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."

The same year that podophyllum and podophyllin were dropped as cathartics from the U. S. Pharmacopoeia (1942), a report by Kaplan appeared stating that the topical application of podophyllin in condyloma acuminatum, a type of venereal wart, produced excellent clinical results.[4] Earlier references to the use of podophyllum and podophyllin in folk and orthodox medicine for cancer or other growths can be found scattered through the older literature. In 1845, Good wrote:[5]

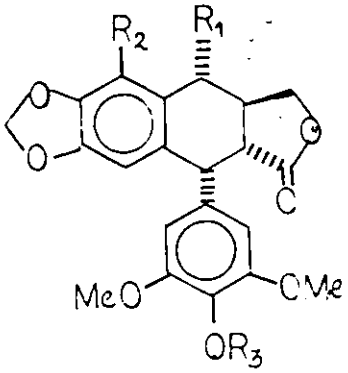
" Some Physicians and Practitioners recommend the powdered root as an escharotic to cleanse foul and ill-conditioned ulcers and dispose them to heal and to promote the exfoliation or removal of carious or rotten bones....It is also said to destroy proud flesh without any injury to the sound parts."

Kaplan's report sparked renewed medical interest in podophyllin in many fields, in particular pharmacology, biochemistry, cytology and clinical medicine. Between 1942 and 1960, podophyllin was studied in many clinical conditions including diseases of the skin due to infectious agents, non-specific dermatoses, metabolic diseases, and benign and malignant new growths.[6] However, the drug showed little therapeutic effect except in the case of condyloma acuminatum.

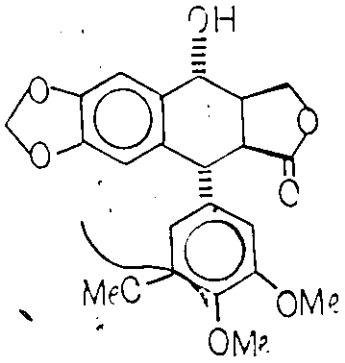
Before 1947 only two well-defined components of podophyllum were known, podophyllotoxin and quercetin. Podophyllotoxin was first isolated and named by Podwyssotzki in 1880.[7] Beginning in 1947, Hartwell and workers isolated three new tumour-damaging components of podophyllin, namely α-peltatin and β-peltatin from American podophyllin and 4'-demethylpodophyllotoxin from Indian podophyllin (Podophyllum emodi).[8,9,10] Initially it was thought that both α-peltatin and β-peltatin were isomeric to podophyllotoxin [10] but after further research it was determined that the empirical formula of α-peltatin was $C_{21}H_{20}O_8$ [11] while that of β-peltatin was $C_{22}H_{22}O_8$.[9,10] By 1958 sixteen well-characterized components of podophyllum had been isolated. Their structures are shown in Table 1. A summary of the chemistry of podophyllin shows that resins derived from different species of Podophyllum differ in composition. For instance, podophyllotoxin can be found in P. peltatum and P. emodi but not in P. sikkimenis while the peltatins are characteristic of only P. peltatum. The proportion of components also varies from species to species. For example, resin from P. emodi yields approximately 35-50% podophyllotoxin [12,13] while resin from P. peltatum yields only about 10% podophyllotoxin.[10,11]

The components of podophyllum fall into two categories, the lignans and the flavonal pigments. The term "lignan" was first used by Haworth 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.

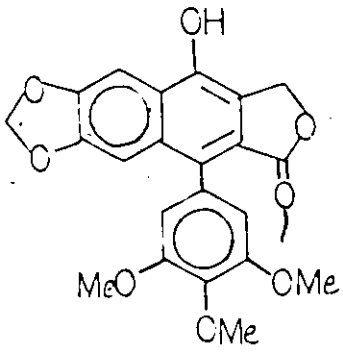
TABLE 1 : COMPOUNDS ISOLATED FROM PODOPHYLLIN



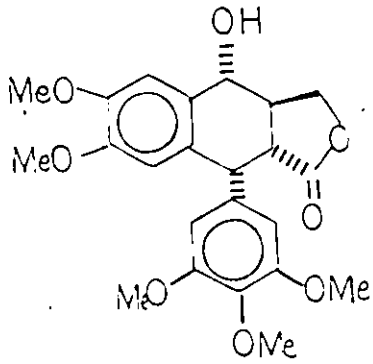
NAME	#	R_1	R_2	R_3
podophyllotoxin	1	OH	H	CH_3
α-peltatin	2	H	OH	H
β-peltatin	3	H	OH	CH_3
4'-demethylpodophyllotoxin	4	OH	H	H
deoxypodophyllotoxin	5	H	H	CH_3
podophyllotoxin glucoside	6	O-glucosyl	H	CH_3
α-peltatin glucoside	7	H	O-glucosyl	H
β-peltatin glucoside	8	H	O-glucosyl	CH_3
4'-demethylpodophyllotoxin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



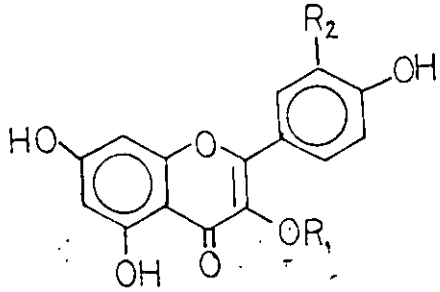
picropodophyllotoxin 10



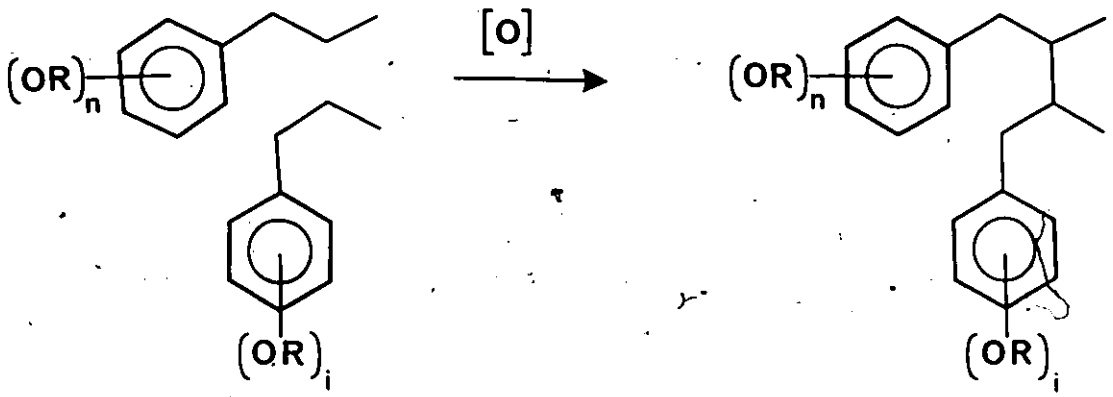
tetradehydropodophyllotoxin 11



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



Borsche and Niemann[15], in 1932, were the first researchers to determine the correct empirical formula for podophyllotoxin, $C_{22}H_{22}O_8$, but it was not until 1953 that Schrecker and Hartwell established its structure and absolute configuration by chemical means.[16] Schrecker and Hartwell postulated that the antimitotic and antitumour activity of the podophyllum lignans was closely related to the unique cis-(1:2)-trans-(2:3)-trans-(3:4) configuration and in particular, the highly strained, trans-fused γ-lactone ring.

Due to the potential of podophyllotoxin and its related compounds as anti-cancer agents, many attempts have been made to synthesize these compounds. The first synthesis of podophyllotoxin was reported by Gensler and coworkers in 1954 and 1966.[17,18] In 1977 and 1981, Kende synthesized (±)-podophyllotoxin in 12 steps from piperonal.[19,20] Both the Gensler and Kende syntheses involved the thermodynamically difficult epimerization of picropodophyllotoxin to podophyllotoxin. Rodrigo avoided this problem in his synthesis of podophyllotoxin.[21] As yet, no synthesis of the

naturally occurring peltatins has been achieved. However, Brown and coworkers have reported the syntheses of (±) iso β-peltatin and the methyl ethers of both (±) iso α- and β-peltatin.[22] Since this thesis is not primarily concerned with the synthetic aspects of these compounds, details regarding these approaches are considered outside the scope of this thesis.

In 1950, Greenspan and coworkers investigated the effect of podophyllotoxin and the peltatins on lymphomas and other transplanted tumours in mice.[23] They found that each of these compounds induced acute damage in all types of tumours studied within twenty-four hours after a single subcutaneous injection. Repeated injection of α-peltatin in mice bearing lymphatic tumours resulted in retardation of tumour growth and somewhat longer survival time of mice as compared to untreated controls. Complete regression of any of the tumours studied was never observed. However, Greenspan was encouraged by these results and decided to investigate the effect of intravenous administration of α-peltatin in patients with advanced neoplasms.[24] Although necrosis and shrinkage of tumour tissue was observed in some of the patients, there was no evidence of significant therapeutic value. According to unpublished results of Downing et al., β-peltatin was ineffective when given orally to children with acute or chronic leukemia, neuroblastoma, and other widely disseminated neoplasms.[25]

Although podophyllotoxin and the peltatins possess antitumour activity, their toxicity renders them unsuitable as anticancer agents. Workers at the Sandoz Laboratories in Basel, Switzerland

found that the glucosides of podophyllotoxin, α- and β-peltatin, and 4'-demethylpodophyllotoxin (6, 7, 8, and 9, respectively) inhibited the growth of tumours in mice.[26] Although less active than their corresponding aglycones in in vitro testing, the glucosides were found to be much less toxic at doses displaying pronounced cytostatic effect. However, clinical testing of these glucosides proved unsatisfactory. Further modifications (partial substitution of the glucose residue by condensation with various aldehydes) resulted in the preparation of a variety of cyclic acetals of 4'-demethylepipodophyllotoxin β-glycoside. Some of these cyclic acetals not only exhibited high activity in in vitro tests but also were much less toxic in in vivo testing against mouse lymphocytic leukemia (L-1210).[27] Two of these derivatives were selected for clinical trials, namely, 4'-demethyl-1-O-(4,6-O-(ethylidene-β-D-glucopyranosyl))-epipodophyllotoxin (VP 16-213 or "Etoposide"*) and 4'-demethyl-1-O-(4,6-O-(2-thenylidene)-β-D-glucopyranosyl)-epipodophyllotoxin (VM 26 or "Teniposide"*). Both drugs have proved to be very useful in the treatment of a wide variety of cancers including bladder, small cell lung, brain, non-lymphocytic leukemia, Hodgkin's disease and non-Hodgkin's lymphomas, especially reticulum-cell sarcoma.[28] These drugs are now clinically used in Western Europe and in Canada.

Under mild base catalysis or under physiological conditions[29], the highly strained, trans-fused lactone ring of podophyllotoxin is readily epimerized to give picropodophyllotoxin. This epimerization results in almost total loss of cytotoxic activity and may be the

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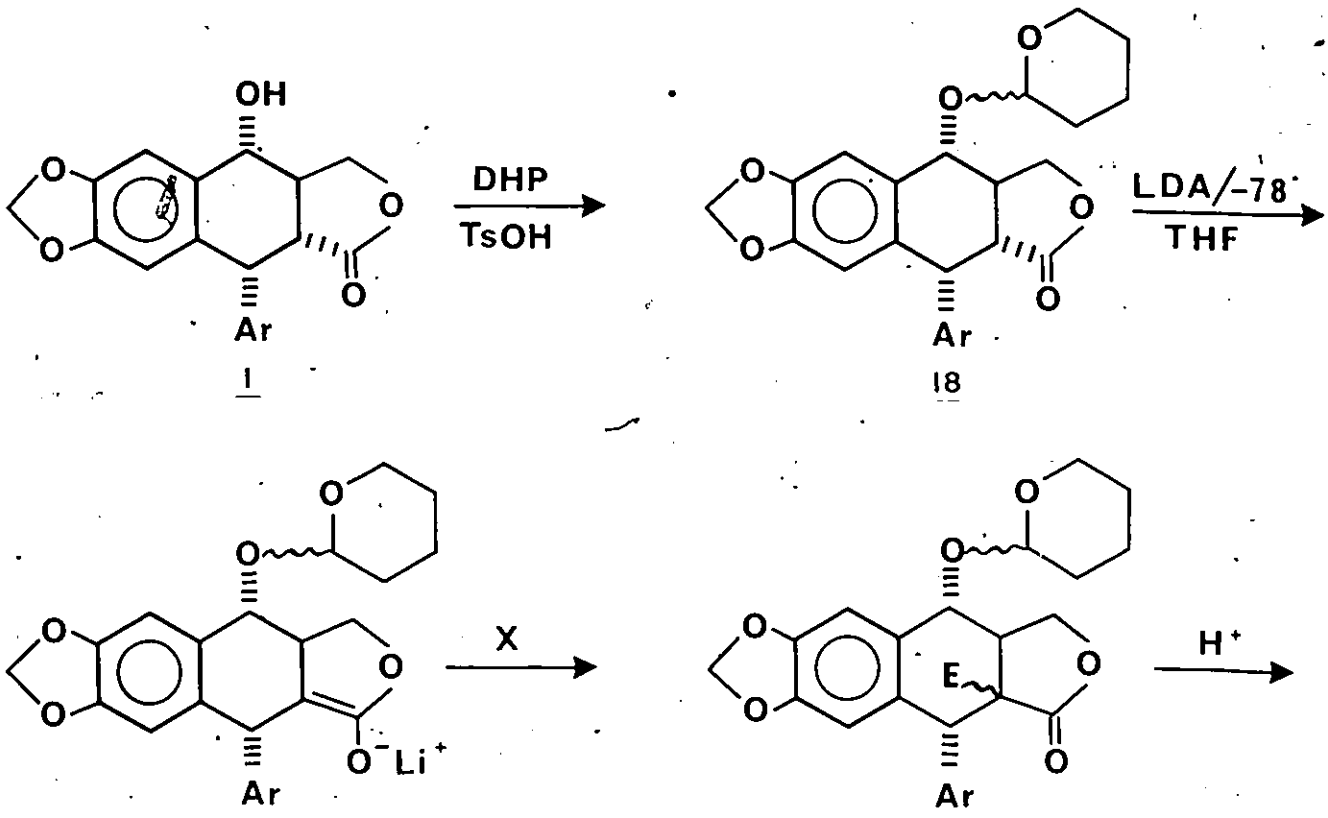
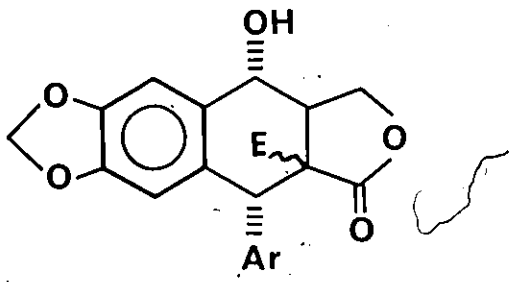
* Etoposide and Teniposide are the trade names for these compounds

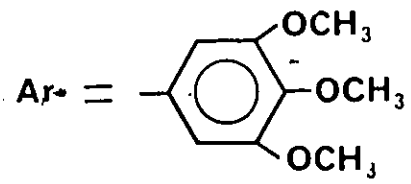
primary method of detoxification by the cell. From a chemotherapeutic standpoint this epimerization is undesirable since limiting the physiological lifetime of a drug sets an upper limit to its biological effectiveness.

Gensler attempted to synthesize non-enolizable derivatives of podophyllotoxin by replacement of the C_2 hydrogen with a variety of substituents but was unable to retain the trans-fused γ-lactone ring.[30] He then decided to eliminate the lactone group altogether by reduction of the carbonyl group to methylene and replacement of the lactone oxygen by a variety of functions: S, SO_2, CO, and CH_2.[31] However, none of these compounds proved to be more effective than podophyllotoxin or deoxpodophyllotoxin.[32]

Glinski and Durst decided to reinvestigate Gensler's problem of synthesizing C_2-substituted analogues of podophyllotoxin via the enolate anion. A very simple method of preparing non-enolizable podophyllotoxin derivatives which still contained the vitally important trans-fused γ-lactone ring was eventually developed as outlined in Scheme 1.[33] The structures of the C_2-substituted derivatives were assigned on the basis of their 360 M Hz NMR spectra. Biological testing (Bristol Laboratories, Syracuse, New York) of some of these derivatives indicated that the chlorinated analogue, 2-chloropodophyllotoxin, had a level of antitumour activity substantially higher than the accepted significant level. Knowing that the glycoside moiety was required for lower toxicity while still retaining high activity, Glinski prepared the C_2-chlorinated derivative 17 of VP 16 in a similar fashion as outlined in



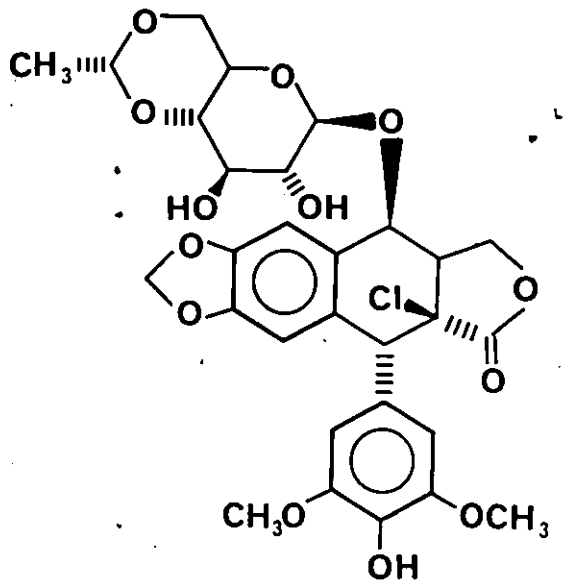




X = CH_3I, O_2, Cl_3CCCl_3

E = CH_3, OH, Cl

SCHEME 1



17

Scheme 1.[33] Biological testing of this compound showed it to be slightly less active than the current clinical agent, VP 16. The results of further studies into the effectiveness and toxicity of 17 were not available at the time this thesis was written.

RESULTS AND DISCUSSION

i. Introduction

As previously discussed in the Introduction, the podophyllum lignans are readily epimerized to their corresponding cis-fused lactone ring isomers with resulting loss of biological activity. A scale Dreiding model of podophyllotoxin or the peltatins containing the trans-fused lactone ring shows a strained, inflexible molecule. However, a model of picropodophyllotoxin or the peltatins containing the cis-fused lactone ring shows that these molecules have considerably less strain and rigidity, and may exist in several interconvertible conformations.

Gensler attempted to synthesize non-enolizable derivatives of 4'-deoxypodophyllotoxin by substituting the C_2 hydrogen with a suitable substituent via the enolate anion but was unsuccessful.

In 1980, Glinski and Durst decided to reinvestigate this problem.[33] They developed a very simple method of preparing non-enolizable derivatives of podophyllotoxin while still retaining the vitally important trans-fused γ-lactone ring (Scheme 1). Podophyllotoxin 1 was first reacted with dihydropyran in the presence of TsOH to give 4-O-tetrahydropyranylpodophyllotoxin 18 which was then treated with LDA in THF to give the corresponding enolate anion 19. The enolate anion was trapped with a variety of electrophiles (CH_3I, O_2, Cl_3CCCl_3) then treated directly with aqueous acid to remove the THP protecting group. The proportion of the cis- and trans-fused lactone ring isomers obtained depended on the electrophile used.

The best stereochemical result (highest trans/cis ratio) was obtained when hexachloroethane was used as the electrophile. Only one product, the desired trans-fused lactone ring isomer 2-chloro-podophyllotoxin, was obtained after removal of the protecting group. Biological testing of this compound against Leukemia P388 at a dosage level of 40 mg/kg indicated a significant level of antitumour activity (T/C = 156 where % T/C is the percent increased survival time of the test group over the control group). An initial T/C $\geq$ 125 is considered significant for antitumour activity. Podophyllotoxin was found to be toxic even at a lower dosage level (30 mg/kg) than that used in the testing of 2-chloropodophyllotoxin.

Encouraged by these results, Glinski synthesized the 2-chloro derivative 17 of VP 16 in a similar manner as outlined on Scheme 1, albeit in only 10% yield. Biological testing of this compound against P388 at a dosage level of 60 mg/kg gave a T/C > 500. The current clinical agent, VP 16, also had a T/C > 500 but at a lower dosage level of 40 mg/kg. Although 17 was not as active as the parent compound at the same dosage level, it was hoped that the toxicity of the derivative would be substantially lower. If this were the case then the maximum tolerated dose (MTD) could be increased. At a higher dosage level, it was hoped that 17 might be a more potent anticancer agent than VP 16. However, the results of further studies into the effectiveness and toxicity of 17 were not available at the time this thesis was written.

Although the peltatins are known to be biologically very active and highly toxic[8,9,10,34], their corresponding glucosyl derivatives

have not been synthesized, even though it is known that a glycoside moiety is required for lower toxicity while still retaining high antimitotic activity. A combination of poor response in human cancer patients, high toxicity and possibly the relative lack of availability of the peltatins resulted in these compounds being virtually ignored by researchers after Greenspan's work of the early 1950's. The peltatins are obtained only from the resin of Podophyllum peltatum and only in small proportions, 5% for α-peltatin* and 6% for β-peltatin.[10] In contrast, podophyllotoxin is present in the resins of both Podophyllum peltatum and Podophyllum emodi to the extent of 10% and 35-50%, respectively.

Encouraged by Glinski's results, we decided to synthesize non-enolizable derivatives of the peltatins via the enolate anion. It was hoped that similar substitutions at C_2 would result in compounds of lesser toxicity than the parent peltatins while still retaining their high antimitotic activity.

ii. Chlorination of α-peltatin trianion

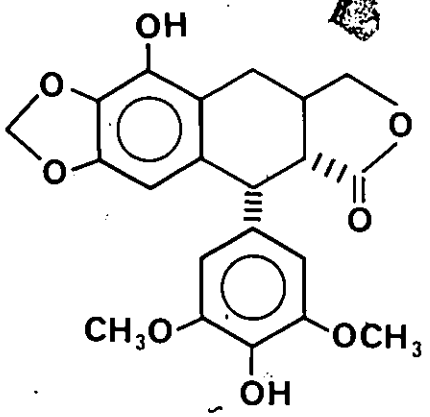
The procedure for the isolation of α- and β-peltatin from podophyllin resin was adapted from Hartwell and Detty[10] with a few modifications (see Experimental Section). The isolated yields of α- and β-peltatin were 6.4% and 6.0%, respectively. Similar yields were obtained by Hartwell and Detty. The physical properties (melting

---

*Note: The correct IUPAC name for this compound is Furo{3',4':6,7}naptho{2,3-d}-1,3-dioxol-6(5α)-one,5,8,8a,9-tetrahydro-10-hydroxy-5-(4-hydroxy,3,5-dimethoxyphenyl){5R,5aα,5aβ,8aα}, however the trivial names for α- and β-peltatin (and their derivatives) have been used in this thesis for the sake of simplicity.

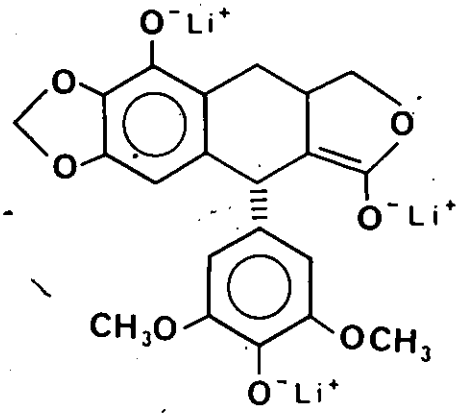
points, optical rotations, analyses) of 2 and 3 corresponded to those reported by Hartwell and Detty. All other data collected for these compounds were in agreement with the assigned structures (see Experimental Section). Similar to Hartwell and Detty, we found that the peltatins were very difficult to separate completely as they had a tendency to co-crystallize. It was necessary, therefore, to expend a great deal of energy to obtain pure samples of the two peltatins.

The first attempt to prepare a substituted peltatin was made via the trianion route (Scheme 2). We attempted to prepare the trianion 20 of α-peltatin by treatment of α-peltatin 2 with three equivalents of LDA at -78° in dry THF under a nitrogen atmosphere. A precipitate formed immediately on addition of 2 to the LDA solution. Warming of the mixture to 0° did not appear to aid in the dissolution of the precipitate. After stirring for 0.5 hours, the mixture was treated with an eightfold excess of hexachlorodthane, warmed to room temperature and stirred for an additional 18 hours. Workup and PTLC (1/1 ethyl acetate/hexanes) afforded a mixture of two components of very similar R_f values. A mass spectrum of the more mobile component indicated a chlorinated compound of the desired mass (m/e 434). The nmr spectrum of this component showed peaks at 4.70, 5.90, 6.18 and 6.38 ppm which could reasonably be assigned to H_1, H_6, H_7 and H_8, respectively in 21. Essentially the same chemical shifts for these hydrogens (taking into account that the data were obtained on EM 360A and XL 200 instruments) were observed in the spectrum of 21 obtained later (see page 39) via the disilyl ether of 2. In contrast, the corresponding chemical shifts for the less mobile component of the



2

3 eqiv. LDA/-78°
THF



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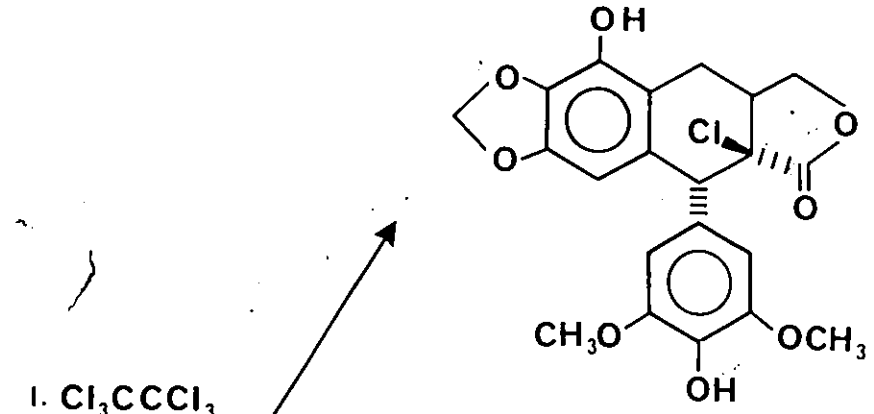

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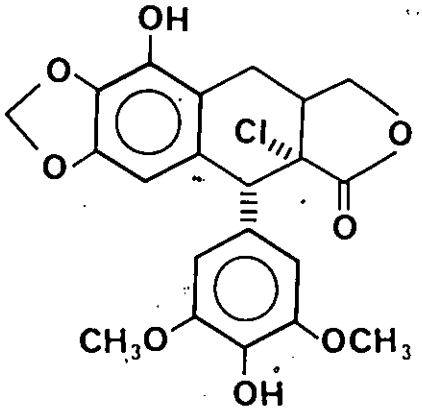


22

SCHEME 2

trianion experiment were 4.45, 5.79, 6.27 and 6.45 ppm, respectively. Based on the above data, it would seem reasonable that the more mobile component was probably the 2-chloro trans-fused lactone ring derivative 21 of α-peltatin while the less mobile component was probably the corresponding cis* isomer 22. Based on this assumption, the yields of 21 and 22 were 32% and 45%, respectively. Complete purification and identification of these two components were never made, however.

iii. Isolation of Silylated Peltatins

Due to the difficulty in obtaining pure samples of 2 and 3 uncontaminated by the other peltatin, and to the lack of solubility of the trianion of α-peltatin in THF (the usual solvent for anion chemistry in this lab), we decided to protect the hydroxyl groups of the peltatins. In this way it would only be necessary to form the THF-soluble monoanion of either peltatin.

The tert-butyldimethysilyl (TBDMS) group is stable to a wide variety of reagents including acid and base, contains no chiral centre and is rapidly cleaved by treatment with 2-3 equivalents of TBAF in THF. In addition, its derivatives have been reported to be nicely crystalline.[35] For these reasons we decided to use the TBDMS protecting group. The use of the tetrahydropyranyl (THP) group was decided against due to the formation of additional diastereomers caused by the chiral centre of the THP ring.

The general procedure for isolating the pure disilyl ether 23 of α-peltatin and the silyl ether 24 of β-peltatin is outlined in

---

*Nomenclature: Hereafter, the terms cis and trans will be used to refer to the stereochemistry of the lactone ring of the peltatins and their derivatives.

Scheme 3. The chloroform-soluble fraction of podophyllin resin was partially separated by passing it through a very short silica gel column (5g silica/ 1g mixture) using a solvent gradient (see Experimental Section). A crude mixture of α- and β-peltatin and podophyllotoxin was obtained in this way. The proportion of α- and β-peltatin present in the crude mixture was estimated from the nmr spectrum. This mixture was not purified further but treated directly with tert-butyldimethylsilyl chloride and imidazole in DMF following the procedure of Corey.[35] Normal workup followed by HPLC (1/9 ethyl acetate/hexanes) afforded pure 23 and 24 after recrystallization from hexanes and CH_2Cl_2/hexanes, respectively. The yields of 23 and 24 from podophyllin resin were 14% and 9%, respectively. This corresponds to theoretical yields of 8.9% and 7.0% for 2 and 3, respectively (7.0% and 5.6% given that the desilylation reaction is 80% efficient).

The mass spectrum of 23 showed a strong peak at m/e 571 (M^+- 57 which corresponds to loss of $\cdot C_4H_9$) while the mass spectrum of 24 indicated a strong molecular ion peak at m/e 528. In the ^1H nmr spectrum of 23, the presence of two TBDMS groups was clearly indicated by two peaks of equal intensity at 1.03 and 0.98 ppm (the t-butyl hydrogens) and three peaks at 0.29, 0.24 and 0.10 ppm (methyl groups on Si). The corresponding peaks in 24 were found at 1.03 (t-butyl group), 0.29 and 0.24 ppm (2 methyl groups).

The stereochemistry at C_2 and C_3 was assigned on the basis of the value of the coupling constant $J_{H_3-H_{11}}$. In the structurally-similar compounds podophyllotoxin 1 (trans isomer) and picro-

Complete ^1H nmr assignment of the silylated peltatins, 23 and 24

The ^1H nmr assignments for the H_7 and H_8 aromatic protons (see Table 4), the TBDMS protons (see page 18) and the methylenedioxy and methoxy protons were made on the basis of their chemical shifts as well as their integrated intensities. In the spectra of 23 and 24, the methylenedioxy protons are magnetically non-equivalent and appear as two doublets centered at 5.910 and 5.890 ppm (J=1.4 Hz) in 23 and 5.913 and 5.895 ppm (J=1.4 Hz) in 24. The presence of the methoxy groups was indicated by a single peak at 3.679 ppm for H_9 in 23 and two peaks (1:2 ratio) at 3.802 and 3.751 ppm for H_{10} and H_9, respectively, in 24. For both 23 and 24, the signal for H_1 appeared as a doublet at 4.566 ($J_{H_1-H_2}$=3.4 Hz) and 4.586 ppm ($J_{H_1-H_2}$=3.8 Hz), respectively. In silylated α-peltatin, 23, protons 2 and 3 absorbed as a multiplet at 2.58-2.72 ppm. The corresponding multiplet in silylated β-peltatin, 24, was found at 2.54-2.73 ppm.

The ^1H nmr assignments of the proton pairs 4, 4' and 11, 11' were made on the basis of their chemical shifts as well as their respective coupling constants with H_3. Each pair of protons (H_4 vs. $H_{4'}$ and H_{11} vs. $H_{11'}$) showed considerable differences in chemical shifts. Thus the two doublet of doublets occurring at 3.938 and 4.492 ppm in 24 are reasonably assigned to hydrogens on the carbon bearing an acyl oxygen while the other pair of doublet of doublets at 2.421 and 3.181 ppm are consistent with the chemical shifts of hydrogen on a benzylic carbon.

The individual assignments of H_4 at 2.421 and $H_{4'}$ at 3.181 ppm were made on the basis of the observed coupling constants. As has

already been mentioned, the peltatins and podophyllotoxin possess a highly rigid structure (see reference 16) imposed by the trans-fused lactone ring. Molecular models show clearly that H_3 has a pseudo-diaxial arrangement with H_4 and a pseudo-axial, pseudo-equatorial arrangement with $H_{4'}$. Thus these two hydrogens should show a large and relatively small vicinal coupling constant. Indeed, the observed values for $J_{H_3-H_4}$ and $J_{H_3-H_{4'}}$ were 11.6 and 4.1 Hz, respectively. The molecular models indicate dihedral angles of approximately 40° and 165° for $H_3-H_{11'}$ and H_3-H_{11}, respectively. These values are consistent with the observed coupling constants of 6.3 and 10.2 Hz, respectively. The observed geminal coupling constants were 16.0 Hz for $H_4-H_{4'}$ and 8.7 Hz for $H_{11}-H_{11'}$.

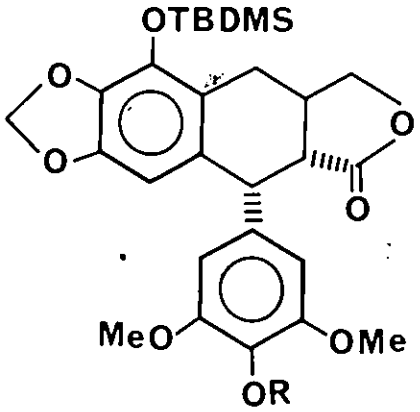
A similar argument can be used in making the individual assignments for silylated α-peltatin, 23.

The rather substantial differences in chemical shifts observed between the diastereomeric pairs of hydrogens (H_4,$H_{4'}$ and H_{11},$H_{11'}$) may be related to the fact that $H_{4'}$ and $H_{11'}$ are on the same side of the plane of the molecule as the C_1-aryl substituent and therefore are more likely to be influenced by the anisotropy of that group. In all of the trans peltatin derivatives prepared in this thesis the 4' and 11' hydrogens were consistently downfield by approximately 0.6-0.8 ppm relative to their diastereomeric partners (see Table 4).

PODOPHYLLIN RESIN ⟶ CHLOROFORM-SOLUBLE FRACTION OF RESIN

column ⟶ CRUDE PELTATIN MIXTURE ⟶ (Cl-Si(CH$_3$)$_2$-t-Bu; imidazole; DMF / 50°) ⟶ SILYLATED PELTATIN MIXTURE

HPLC ⟶

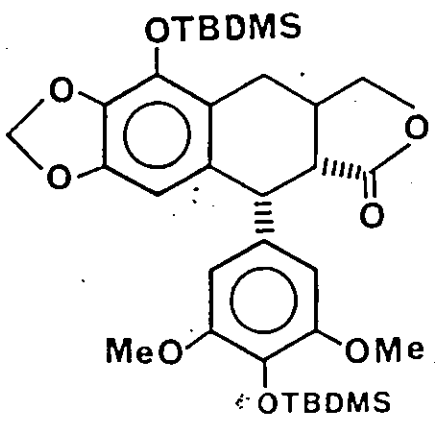


23 R = Si(CH$_3$)$_2$-t-Bu (TBDMS)

24 R = Me

SCHEME 3

FIGURE 2 ^{1}H NMR SPECTRUM OF SILYLATED α-PELTATIN (TRANS),23



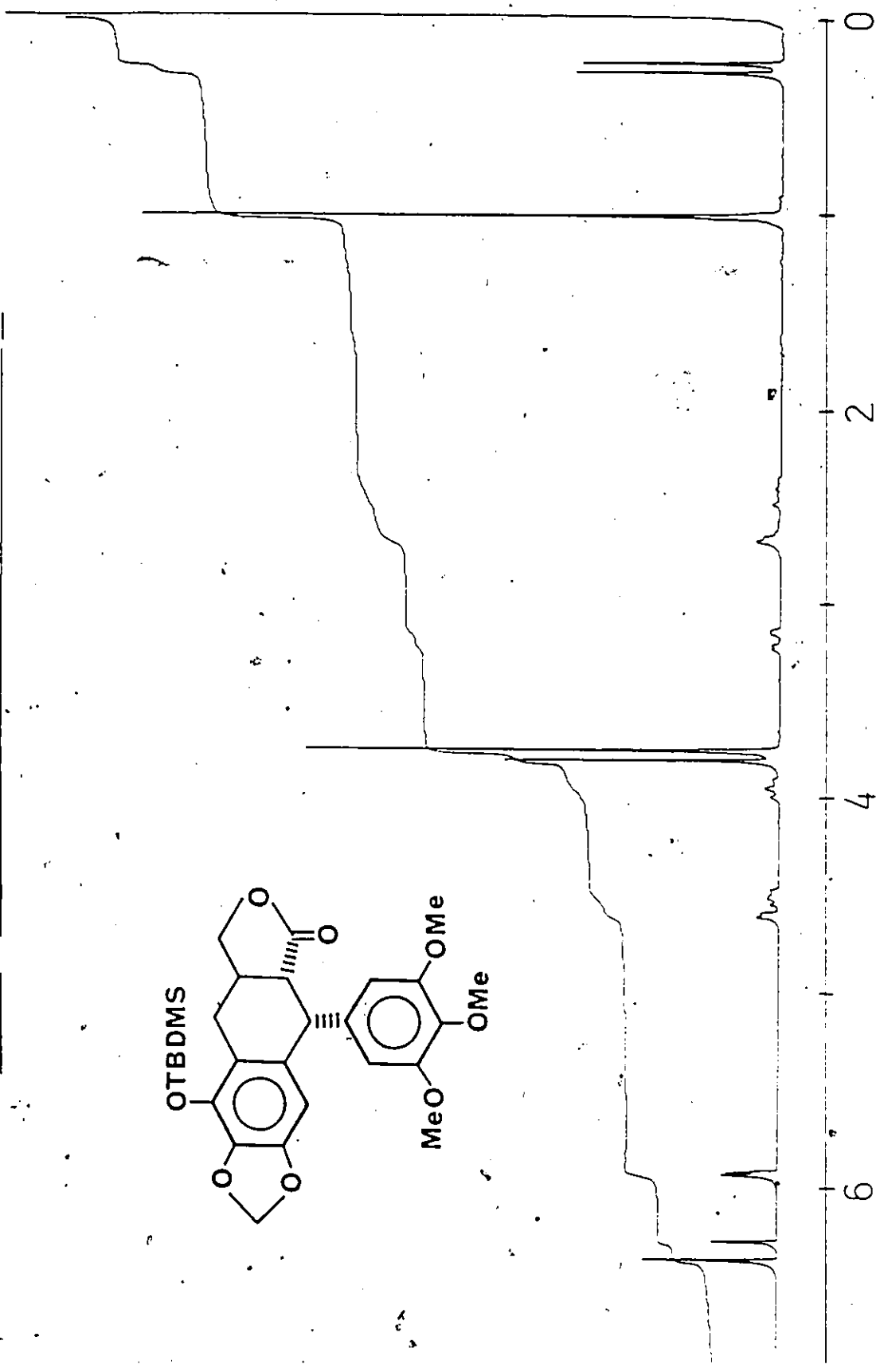


FIGURE 3 ^{1}H NMR SPECTRUM OF SILYLATED β-PELTATIN (TRANS), 24

podophyllotoxin 10 (cis isomer), the numerical value of this constant is substantially lower in the cis isomer ($J_{H_3-H_{11}} = 1.5$ Hz) than in the trans isomer ($J_{H_3-H_{11}} = 9.0$ Hz).[36] These values are consistent with the pseudo-diaxial arrangement of H_3 and H_{11} in the trans isomer and the pseudo-axial, pseudo-equatorial relationship of these hydrogens in the cis isomer. This same stereochemical relationship between H_3 and H_{11} exists in the cis and trans isomers of α- and β-peltatin and their silylated derivatives.

In order to compare the corresponding values of the coupling constant $J_{H_3-H_{11}}$ for the cis and trans isomers of the silylated peltatins, it was necessary to epimerize 23 and 24. Treatment of 23 with 1 equivalent of LDA in THF at -78° to form the enolate anion 25 was followed by warming of the solution to above 0° and addition of water. Normal workup and PTLC (1/5 ethyl acetate/hexanes) afforded the starting material 23 in 19% yield and a slightly less mobile component which was identified as the corresponding cis isomer 26 (78% yield). Similarly, treatment of 24 with LDA to form the anion 27, warming of the solution to above 0° and addition of water, followed by normal workup and PTLC (1/6 ethyl acetate/hexanes) furnished the starting material 24 and the less mobile cis isomer 28 in yields of 20% and 64%, respectively. Examination of the coupling constant $J_{H_3-H_{11}}$ for 23 (10.1 Hz), 24 (10.2 Hz), 26 (3.4 Hz) and 28 (3.4 Hz) affirmed the stereochemical assignment of 23 and 24 as the trans isomers and 26 and 28 as the corresponding cis isomers.

In addition, desilylation of 23 and 24 with TBAF in THF at 0°

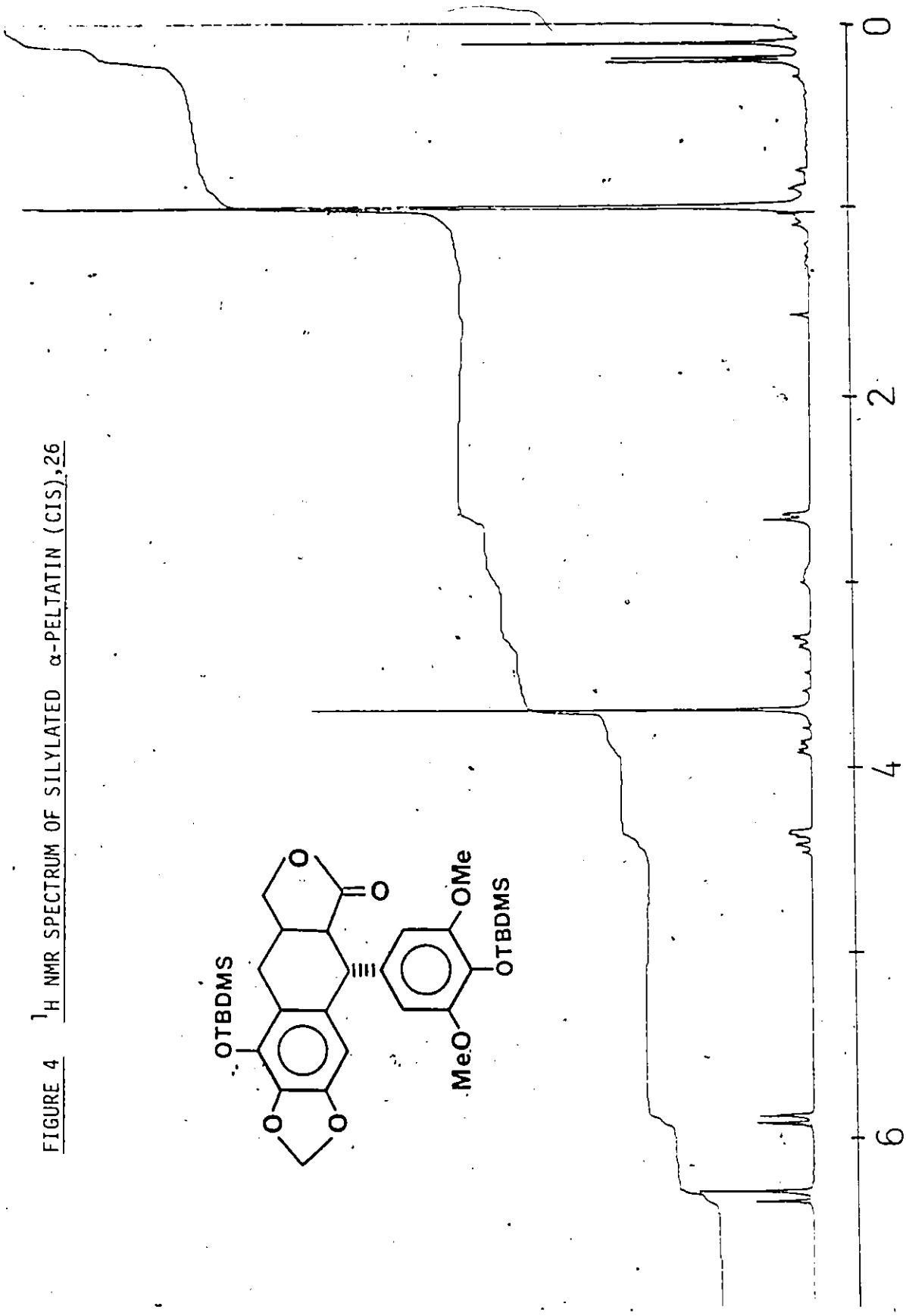
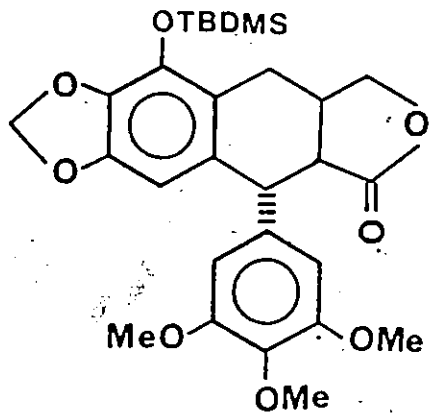


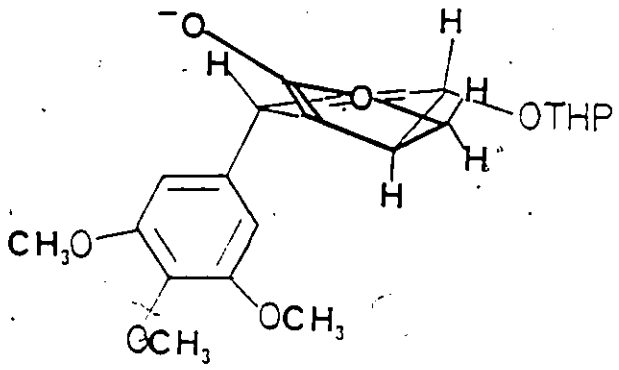
FIGURE 4 ^{1}H NMR SPECTRUM OF SILYLATED α-PELTATIN (CIS), 26

FIGURE 5 ^{1}H NMR SPECTRUM OF SILYLATED β-PELTATIN (CIS),28



followed by column chromatography on silica gel (solvent gradient used; see Experimental Section) resulted in recovery of pure 2 and 3, respectively. Therefore the silylation and desilylation processes did not affect the stereochemistry of the lactone ring.

The trans/cis isomer ratios obtained in the protonation of the anions of silylated α- and β-peltatin by H_2O were 1:4 and 1:3, respectively. In contrast, Gensler obtained a 45:55 ratio of podophyllotoxin:picropodophyllotoxin when he treated the enolate of the THP derivative of picropodophyllotoxin with acetic acid.[18] Inspection of a model of the rigid enolate of the THP derivative of picropodophyllotoxin shows that there is less steric hindrance above the plane of the enolate than below.



The same observation is made from models of the enolates of the silylated peltatins. Topside protonation affords the trans isomer. However, it appears that bottomside protonation is more favoured,

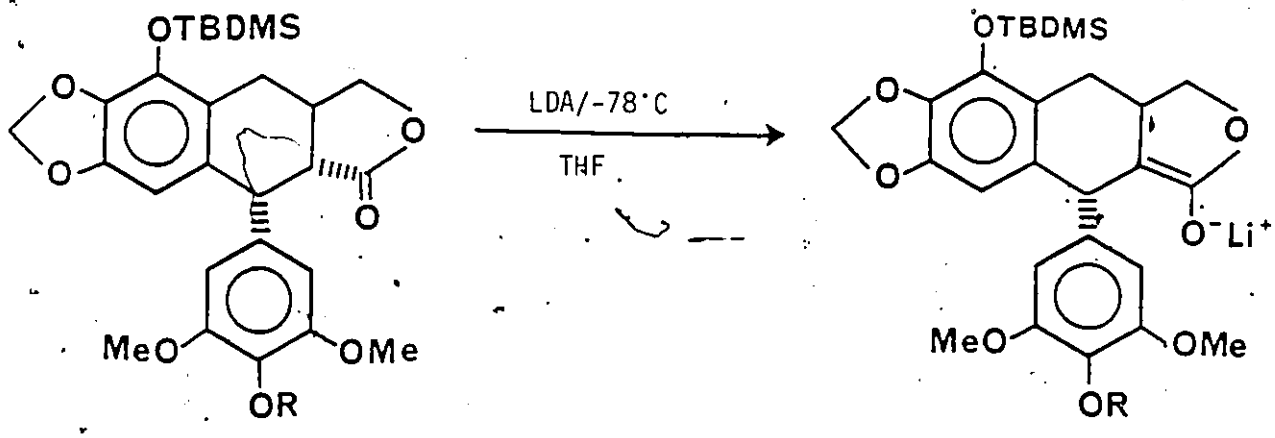
in particular for the peltatin series. Thermodynamically the cis isomer is favoured over the highly strained trans isomer. In addition, if one looks at the transition state with the enolate carbon beginning to show sp^3 geometry, one notices that this state leads to appreciably less steric hindrance below the enolate in the cis transition state. A possible explanation for the differences observed in the trans/cis ratios of the podophyllotoxin series and the peltatin series is the orientation and location of the protecting group. In the podo series, the THP group is directed below the plane of the enolate, perhaps shielding C_2 from bottomside protonation. However, in the peltatin series the silyl group is in the plane of the enolate and therfore one would not expect it to interfere with either topside or bottomside approach of the protonating agent. In addition, the silyl group of the peltatin series is farther removed from C_2 and so exerts less influence on this carbon.

iv. Synthesis of C_2-Substituted Derivatives of the Silylated Peltatins

Following the method of Glinski[33], we prepared several non-enolizable derivatives of the silylated peltatins (Scheme 4).

The enolate 25 was formed by treatment of 23 with 1 equivalent of LDA at -78° in dry THF. Trapping of the enolate with methyl iodide (-78° to RT, 18 h) followed by PTLC (1/7 ethyl acetate/hexanes) afforded the 2-methyl trans isomer 29 and the 2-methyl cis isomer 30 in yields of 38% and 56%, respectively.

Similarly, the enolate 27 was formed by treatment of 24 with LDA then trapped with methyl iodide (-78° to RT, 18 h). Normal workup followed by PTLC (1/5 ethyl acetate/hexanes) afforded the 2-methyl

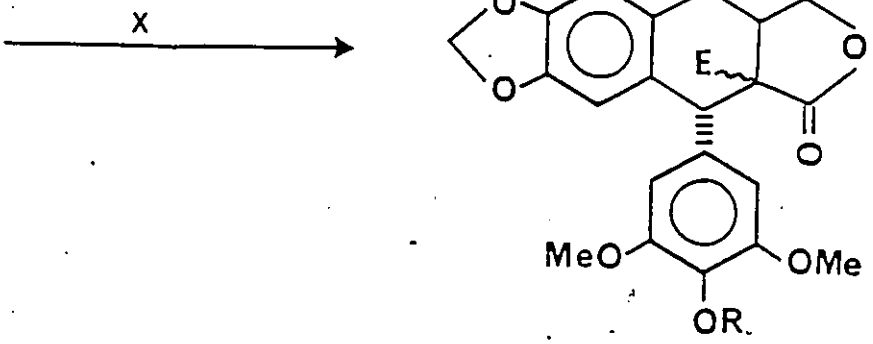


R = TBDMS 23

R = Me 24

R = TBDMS 25

R = Me 27



R = TBDMS for α-peltatin series

R = Me for β-peltatin series

SCHEME 4

trans isomer 31 (52%) and the 2-methyl cis isomer 32 (46%).

The best stereochemical (highest trans/cis ratio) results were obtained with chlorination of the enolates 25 and 27 by reaction with an eightfold excess of hexachloroethane (-78° to RT, 18 h). Essentially only one product, the desired trans isomer, was obtained in either reaction. Workup and purification by PTLC (1/6 ethyl acetate/ hexanes) of the reaction mixture from chlorination of 25 afforded the 2-chloro trans isomer 33 in 92% yield and a small amount (4%) of the corresponding cis isomer 34. Similarly, workup and purification by PTLC (1/5 ethyl acetate/hexanes) of the reaction mixture from chlorination of 27 afforded the 2-chloro trans isomer 35 (83%) and a minor amount of the corresponding cis isomer 36 (6%). The results of the enolate trapping experiments are summarized in Tables 2 and 3.

There are many possible reasons which might contribute to the differences in stereochemical outcome obtained in the enolate trapping experiments. Several of these reasons have already been discussed for the proton trapping experiments. Whether topside or bottomside approach of the electrophile predominates seems to also depend on the size of the electrophile. Hexachloroethane is a rather bulky molecule and therefore it is quite conceivable that bottomside approach is too sterically hindered for reaction to occur to any appreciable extent. Water, on the otherhand, is a much smaller molecule and therefore bottomside approach is more favourable, both sterically and thermodynamically. Methyl iodide is intermediate in size between hexachloroethane and water and thus one might expect a more even distribution of the cis and trans isomers. This was, in fact, what was observed (see Tables 2 and 3).

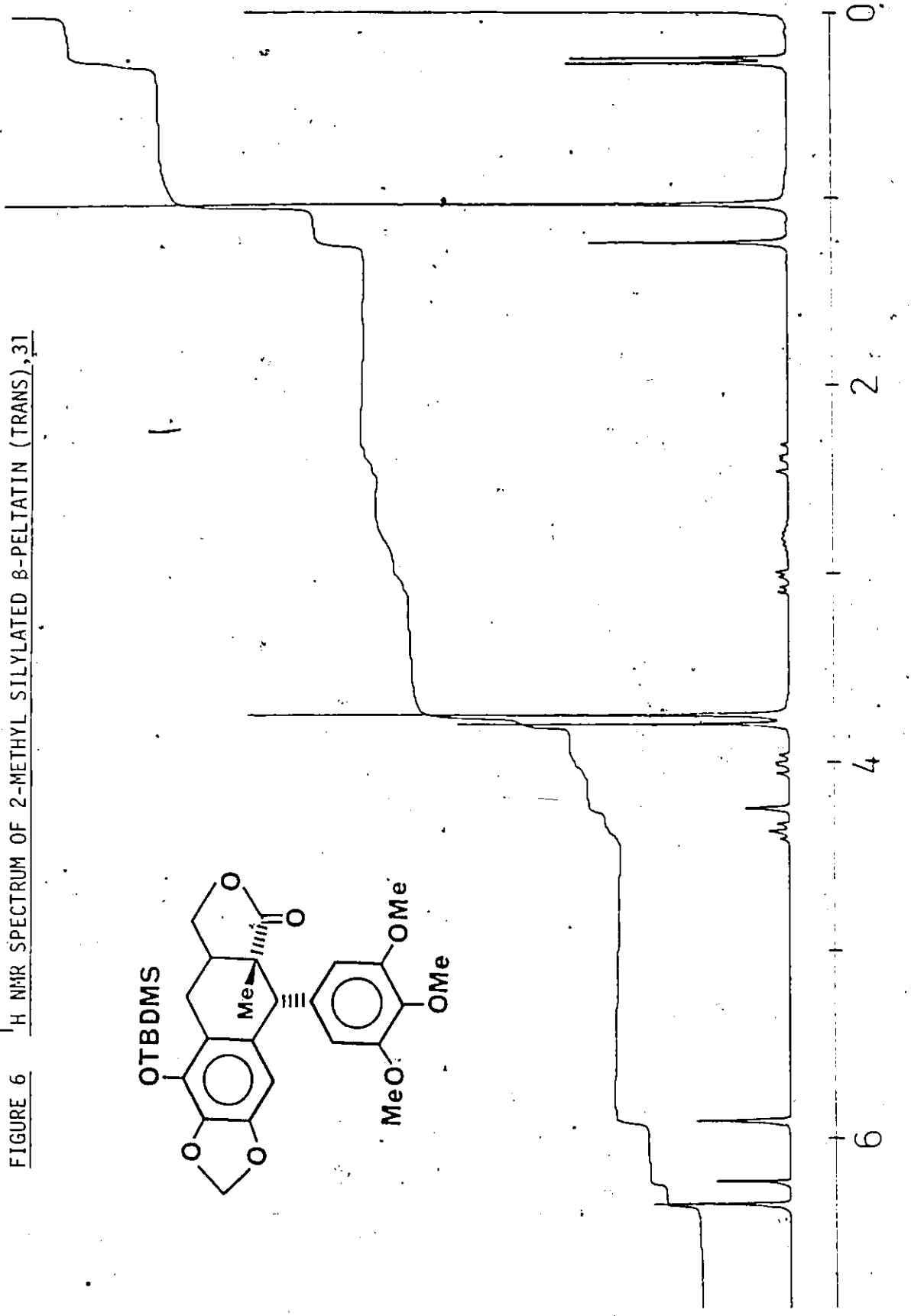


FIGURE 6 ^{1}H NMR SPECTRUM OF 2-METHYL SILYLATED β-PELTATIN (TRANS), 31

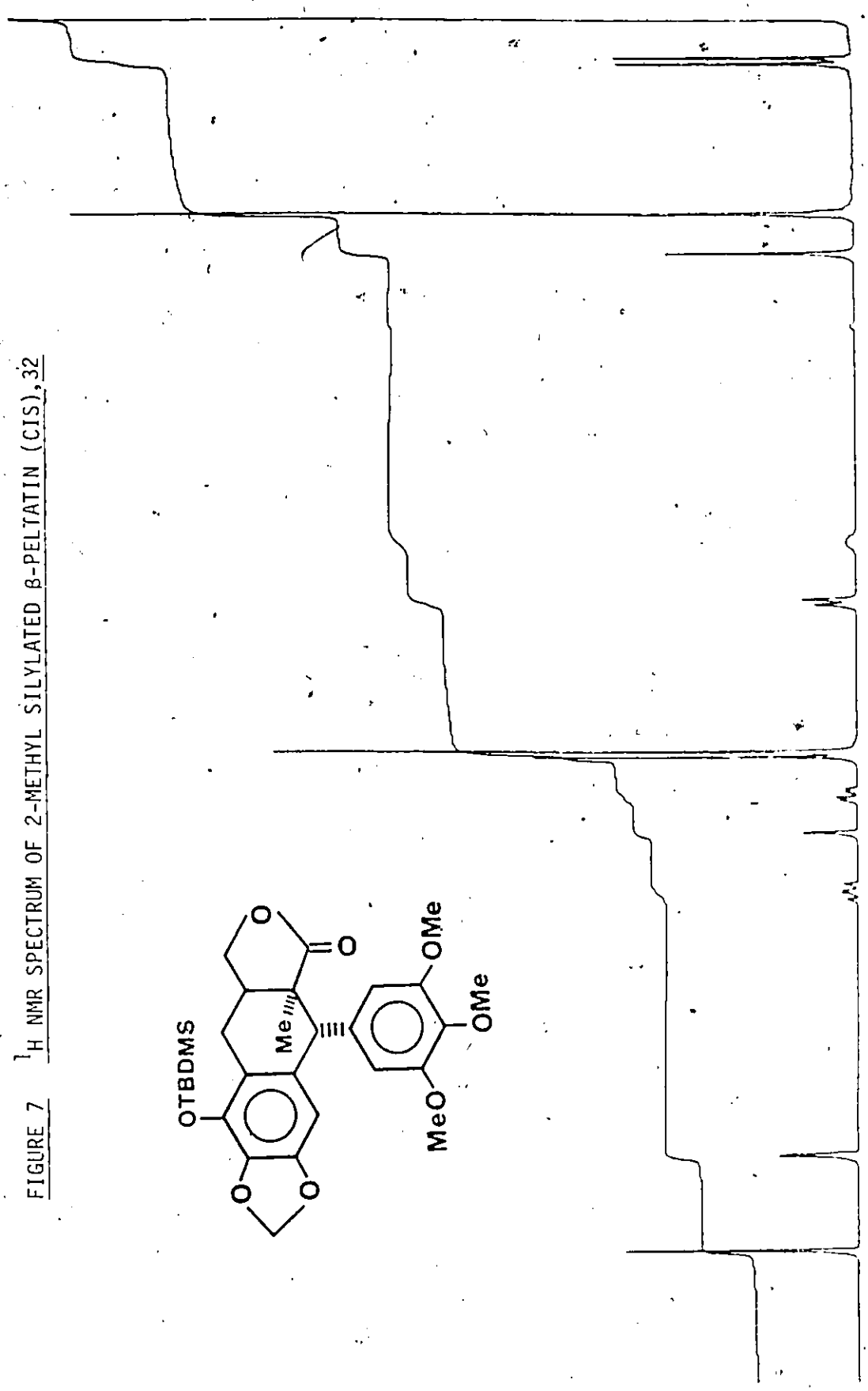
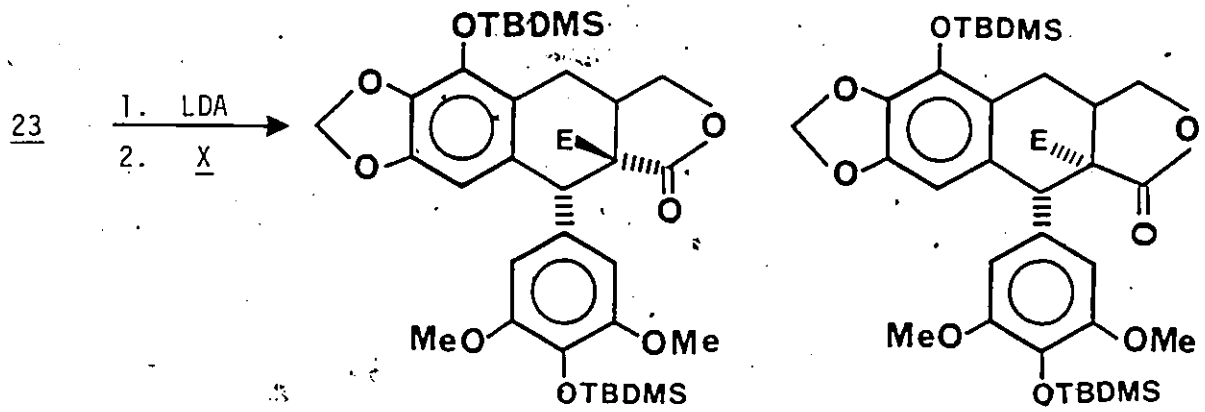


FIGURE 7 ^{1}H NMR SPECTRUM OF 2-METHYL SILYLATED β-PELTATIN (CIS), 32

TABLE 2 ENOLATE TRAPPING EXPERIMENTS α-PELTATIN SERIES

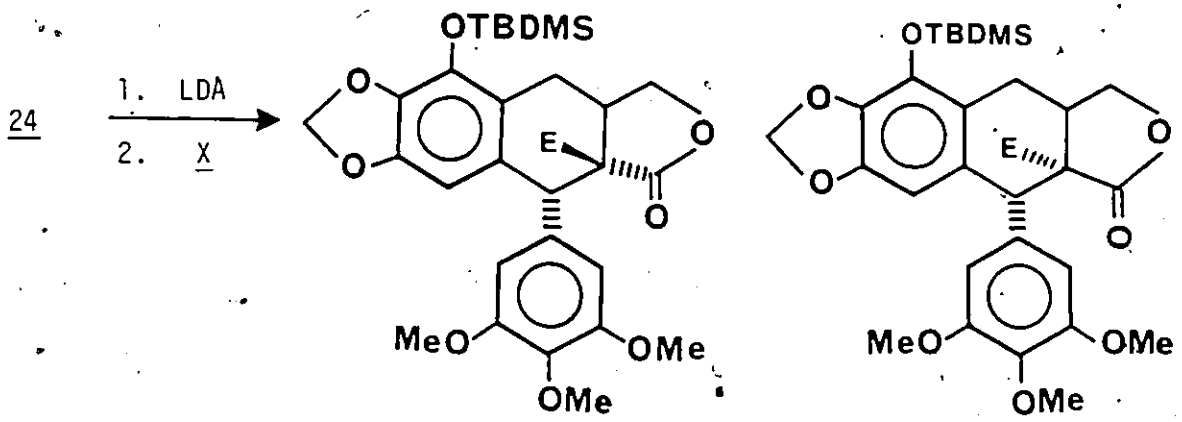


E = H	23
CH_3	29
Cl	33

E = H	26
CH_3	30
Cl	34

X	E	PRODUCTS AND YIELDS	RATIO TRANS/CIS
H_2O	H	23 (19%) 26 (78%)	1/4
CH_3I	CH_3	29 (38%) 30 (56%)	1/1.5
Cl_3CCCl_3	Cl	33 (92%) 34 (4%)	23/1

TABLE 3 ENOLATE TRAPPING EXPERIMENTS β-PELTATIN SERIES



E = H	24	E = H	28
CH_3	31	CH_3	32
Cl	35	Cl	36

X	E	PRODUCTS AND YIELDS	RATIO TRANS/CIS
H_2O	H	24 (20%) 28 (64%)	1/3
CH_3I	CH_3	31 (52%) 32 (46%)	1/1
Cl_3CCCl_3	Cl	35 (83%) 36 (6%)	14/1

v. Structure Proof of C_2-Substituted Derivatives

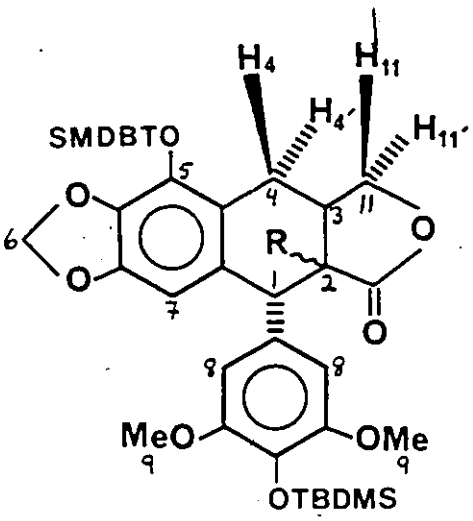
The structures of the C_2-substituted silylated peltatin derivatives were assigned on the basis of their 200 M Hz ^1H nmr spectra. The relevant nmr data for these compounds are summarized in Tables 4 and 5. Substitution at C_2 was indicated by the presence of a sharp singlet for H_1 at 4.22, 4.18, 4.24, 4.20, 4.73, 4.48, 4.75 and 4.51 ppm in 29, 30, 31, 32, 33, 34, 35 and 36, respectively.

The stereochemistry of the lactone ring was assigned on the basis of the value of the coupling constant $J_{H_3-H_{11}}$. As previously discussed, the value of the constant is lower in the cis isomers (2.9 Hz for 30, and 3.1 Hz for 32) than in the trans isomers (11.0, 11.3, 9.7 and 9.7 Hz for 29, 31, 33 and 35, respectively).

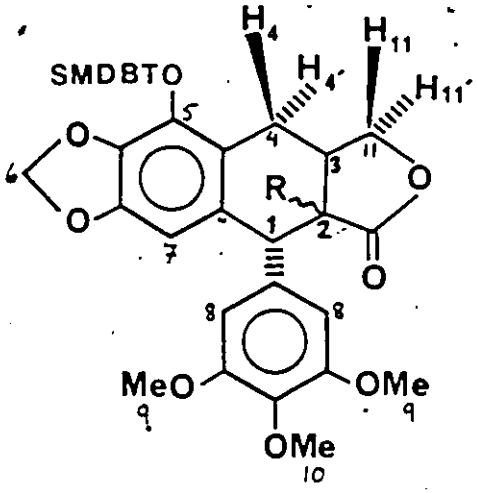
Examination of the ^{13}C nmr spectra of the silylated peltatins and their C_2-substituted derivatives indicated that the signal for the C_8 carbons was consistently shifted 2-3 ppm upfield in the cis isomers compared to the trans isomers. The observed chemical shifts for C_8 in the trans isomers 23, 24, 29, 31, 33 and 35 were 108.39, 108.23, 109.07, 108.82, 109.06 and 108.92 Hz, respectively. In the cis isomers 26, 28, 30 and 32, the values were 105.00, 104.94, 106.86 and 106.80 Hz, respectively. It was not possible to isolate sufficient quantities of the 2-chloro cis isomers 34 and 36 in order to obtain ^{13}C nmr spectra of these compounds. It would seem that the relative chemical shifts of C_8 could be used to confirm the stereochemical assignment of the lactone ring in the silylated peltatins and their derivatives.

The mass spectra of the compounds in the β-peltatin series

RELEVANT ^1H NMR DATA FOR THE SILYLATED PELTATINS AND THEIR 2-SUBSTITUTED DERIVATIVES



α-peltatin series



β-peltatin series

TABLE 4 CHEMICAL SHIFTS

COMPOUND	H_1	H_3	H_4	$H_{4'}$	H_7	H_8	H_{11}	$H_{11'}$
23	4.57	2.58-2.72(m)	2.41	3.17	6.27	6.30	3.92	4.46
24	4.59	2.54-2.73(m)	2.42	3.18	6.26	6.35	3.94	4.49
29	4.22	2.69-2.92(m)	2.36	3.01	6.23	6.30	3.98	4.33
31	4.24	2.70-2.92(m)	2.38	3.04	6.23	6.35	4.01	4.36
33	4.73	2.82-3.00(m)	2.65	3.08	6.26	6.34	4.16	4.40
35	4.75	2.80-3.04(m)	2.67	3.10	6.25	6.39	4.18	4.43
26	4.35	2.92-3.05(m)	2.63-2.71(m)		6.32	6.27	3.89	4.42
28	4.36	2.92-3.10(m)	---2.70---		6.31	6.34	3.93	4.43
30	4.18	2.63-2.75(m)	2.99-3.09(m)		6.35	6.30	3.99	4.48
32	4.20	2.64-2.77(m)	---3.02---		---6.34---		4.01	4.51
34	4.48	------2.95-3.40(m)------			6.36	6.42	4.17	4.74
36	4.51	------2.97-3.36(m)------			6.34	6.46	4.18	4.75

RELVANT ^1H NMR DATA FOR THE SILYLATED PELTATINS AND THEIR 2-SUBSTITUTED DERIVATIVES

TABLE 5 COUPLING CONSTANTS

COMPOUND	$J_{H_3-H_4}$	$J_{H_3-H_4'}$	$J_{H_4-H_4'}$	$J_{H_3-H_{11}}$	$J_{H_3-H_{11'}}$	$J_{H_{11}-H_{11'}}$
23	11.2	a	16.0	10.1	6.2	8.7
24	11.6	4.1	15.7	10.2	6.3	8.7
29	12.6	5.3	16.2	11.0	a	8.4
31	12.4	5.4	16.3	11.3	7.4	8.4
33	10.9	5.6	15.7	9.7	6.7	8.5
35	11.0	5.5	15.7	9.7	6.6	8.6
26	a	a	a	3.4	7.6	9.1
28	a	a	a	3.4	7.3	9.2
30	a	a	a	2.9	6.9	9.1
32	a	a	a	3.1	6.9	9.3
34	a	a	a	0	4.9	8.9
36	a	a	a	0	5.2	9.0

a= not measurable with accuracy

FIGURE 8 ^{13}C NMR SPECTRUM OF SILYLATED α-PELTATIN (TRANS),23

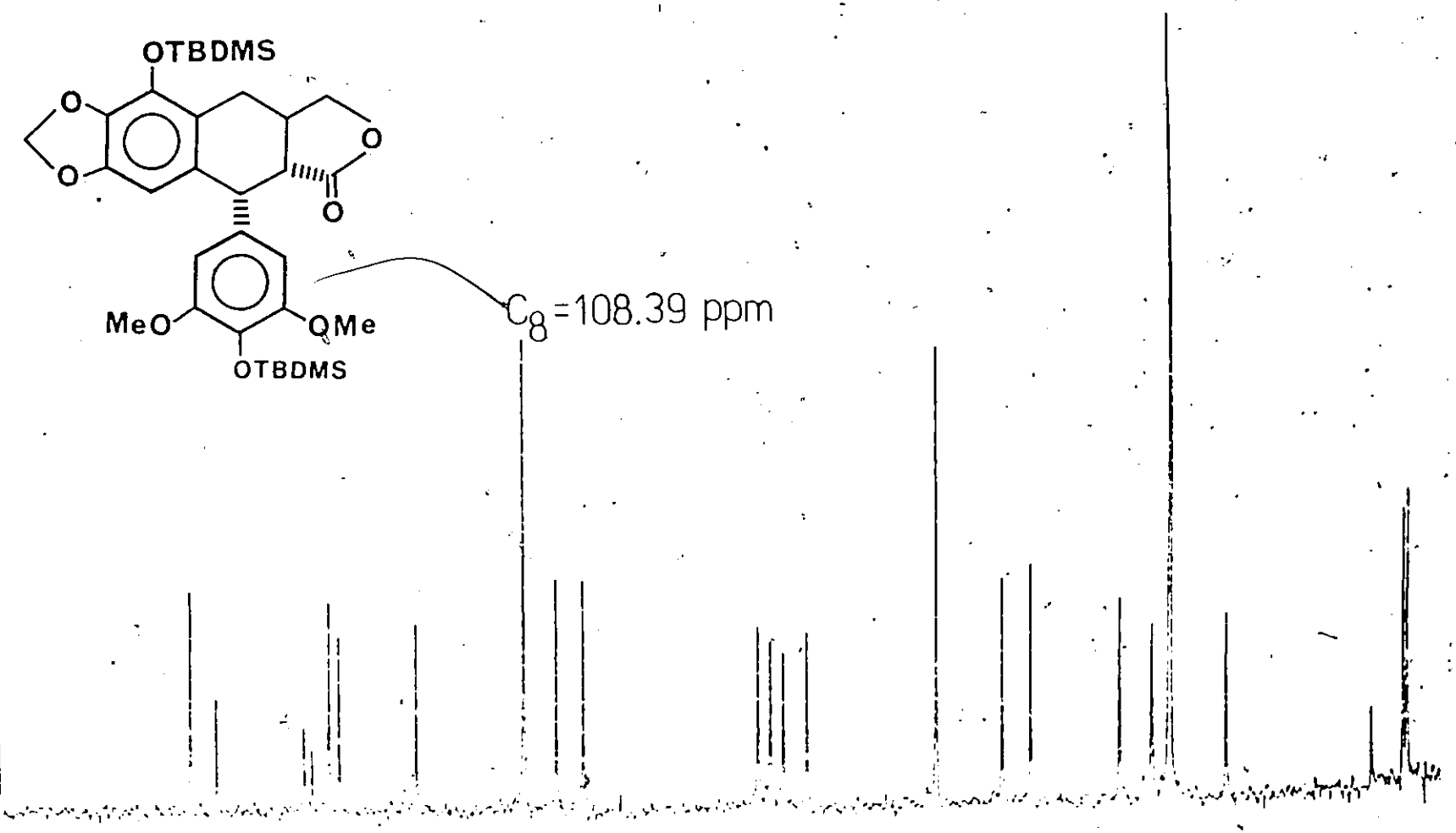
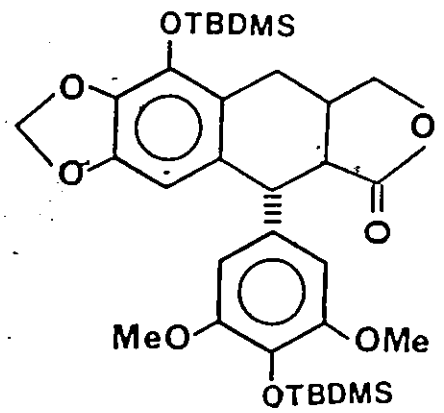


FIGURE 9. ^{13}C NMR SPECTRUM OF SILYLATED α-PELTATIN (CIS),26



showed the expected molecular ion peak. However, in the α-peltatin series the molecular ion peaks were generally very weak or not observed at all. In the latter case the highest m/e peak was observed at M^+- 57 due to loss of $^{\cdot}C_4H_9$. This type of mass spectrum behaviour for the two series has already been noted for the parent compounds 23 and 24.

Only the elemental analyses of 23, 24, 33 and 35 gave C and H values within the accepted limits. A possible reason for these erroneous results could be the presence of occluded solvent. Only 23, 24 and 35 were obtained as crystalline solids. The remaining compounds could only be obtained as foams. It is quite likely, then, that not all the solvent was stripped off during the evaporation process or after being placed on a vacuum pump for several hours at room temperature. Generally, the observed carbon content was 1-5% lower than the calculated value. Such deviations could be attributed to the presence of occluded oxygenated solvents, most likely water. For example, if for every mole of 29 there was a half mole of occluded water present, then the value obtained for the carbon content would be 62.6% instead of 63.5%. This lowered value agrees closely with the value of 62.5% obtained from the elemental analysis of 29.

vi. Desilylation Experiments

The 2-methyl derivative 37 of α-peltatin was prepared by treatment of a cooled (0°) solution of 29 in THF with 4 equivalents of TBAF (1.0 M in THF) followed by immediate quenching with a saturated ammonium chloride solution. Normal workup and PTLC (2/1

ethyl acetate/hexanes) afforded pure 37 in 71% yield.

Similarly, treatment of 30 in THF (0°) with 4 equivalents of TBAF immediately followed by normal workup, PTLC (2/1 ethyl acetate/hexanes) and recrystallization from CH_2Cl_2/hexanes afforded pure 38 in 86% yield.

The 2-methyl derivative 39 of β-peltatin was prepared by treatment of 31 in THF (0°) with 2 equivalents of TBAF followed immediately by normal workup and PTLC (3/2 ethyl acetate/hexanes) to afford pure 39 in 75% yield.

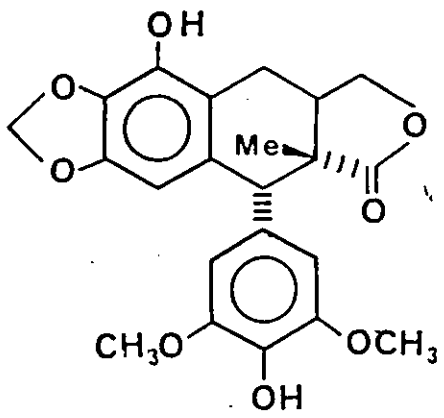
Similarly, treatment of 32 in THF (0°) with 2 equivalents of TBAF followed immediately by normal workup, PTLC (11/9 ethyl acetate/hexanes) and recrystallization from CH_2Cl_2/hexanes afforded a 67% yield of pure 40.

Initial attempts to prepare the 2-chloro derivatives 21 and 41 by treatment of 33 and 35 with 4 and 2 equivalents, respectively, of TBAF were unsuccessful. In both cases a very polar material (insoluble in acetone), possibly the lactone ring-opened hydroxy acid, was obtained. A look through the literature indicated that base-sensitive molecules could be desilylated by treatment of the silylated compound with TBAF in the presence of acetic acid.[37,38]

Successive treatment of 33 in THF (0°) with acetic acid and 4 equivalents of TBAF for 22 hours followed by washing with aqueous sodium bicarbonate, normal workup and PTLC (3/2 ethyl acetate/hexanes) afforded pure 21 in 96% yield.

Similarly, treatment of 35 with acetic acid and 2 equivalents of TBAF for 2 hours followed by washing with aqueous sodium bicarbonate,

FIGURE 10 ^{1}H NMR SPECTRUM OF 2-METHYL α-PELTATIN (TRANS),37



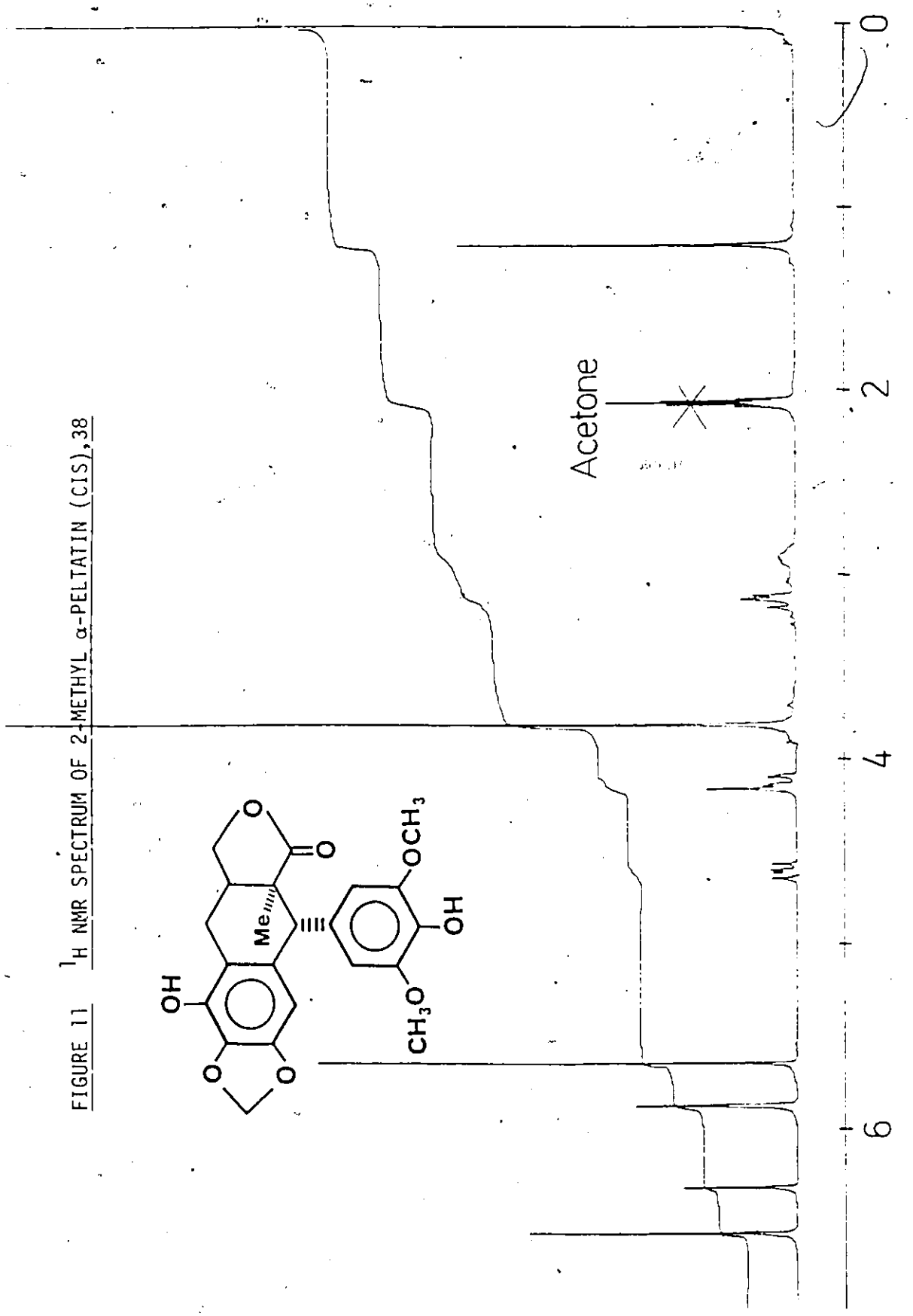


FIGURE 11 ^{1}H NMR SPECTRUM OF 2-METHYL α-PELTATIN (CIS), 38

FIGURE 12 ^{1}H NMR SPECTRUM OF 2-METHYL β-PELTATIN (TRANS),39

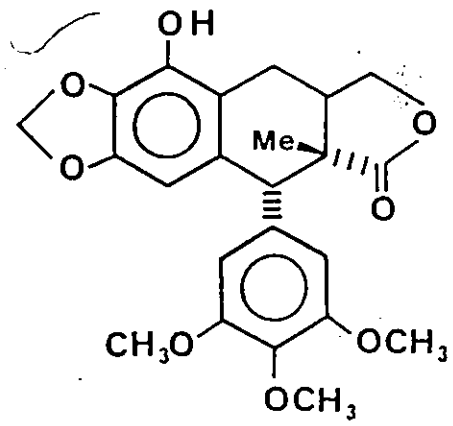
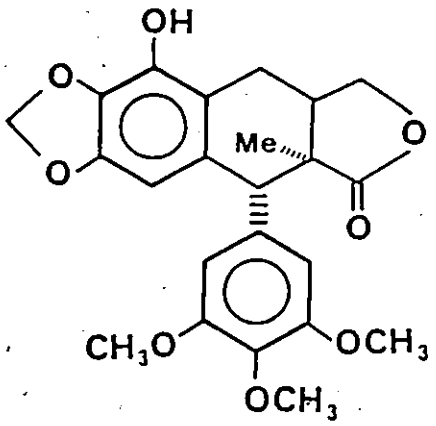


FIGURE 13 ^{1}H NMR SPECTRUM OF 2-METHYL, β-PELTATIN (CIS),40



normal workup and PTLC (3/2 ethyl acetate/hexanes) furnished a 91% yield of 41. The results of the desilylation experiments are summarized in Table 6.

The nmr data collected for the desilylated analogues confirmed the original stereochemical assignments made for the corresponding silylated derivatives. As with the silylated derivatives, the value of the coupling constant $J_{H_3-H_{11}}$ was observed to be much lower in the cis isomers than in the trans isomers. The chemical shifts of the various hydrogens were not greatly affected by the desilylation process. For example, in 33 the chemical shifts of H_1, H_4, $H_{4'}$, H_{11} and $H_{11'}$ were 4.73, 2.65, 3.08, 4.16 and 4.40 ppm, respectively, while in 21 the corresponding values were 4.75, 2.74, 3.16, 4.19 and 4.41 ppm; respectively. The value of $J_{H_3-H_{11}}$ was 9.7 Hz in 33 and 8.5 Hz in 21. The relevant nmr data for the desilylated compounds are summarized in Tables 7 and 8. The mass spectral data were also in agreement with the assigned structures. All the desilylated derivatives showed the expected molecular ion peak.

vii. Biological Screening Results

Biological testing against Leukemia P388 was performed on the peltatins, 2 and 3, and their 2-methyl derivatives, 37, 38, 39 and 40, at the antitumour division of Bristol Laboratories, Syracuse, New York. The general testing protocol was as follows.[39] Ascitic fluid containing 10^6 cancer cells was implanted intraperitonealy in female CDF_1 mice (four mice per test group). Treatment began 24 hours after implant and the parameter was median survival time.

FIGURE 14 ^{1}H NMR SPECTRUM OF 2-CHLORO α-PELTATIN (TRANS), 21

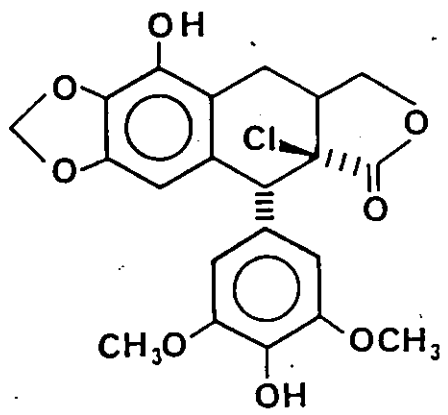


FIGURE 15 ^{1}H NMR SPECTRUM OF 2-CHLORO β-PELTATIN (TRANS), 41

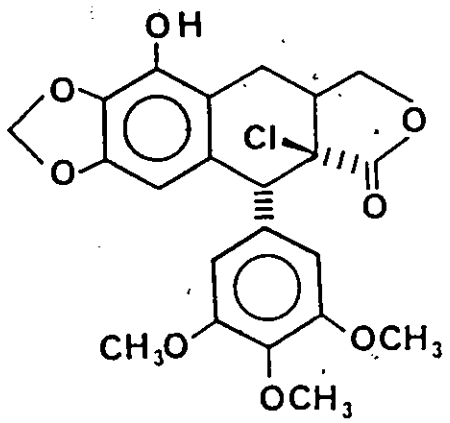
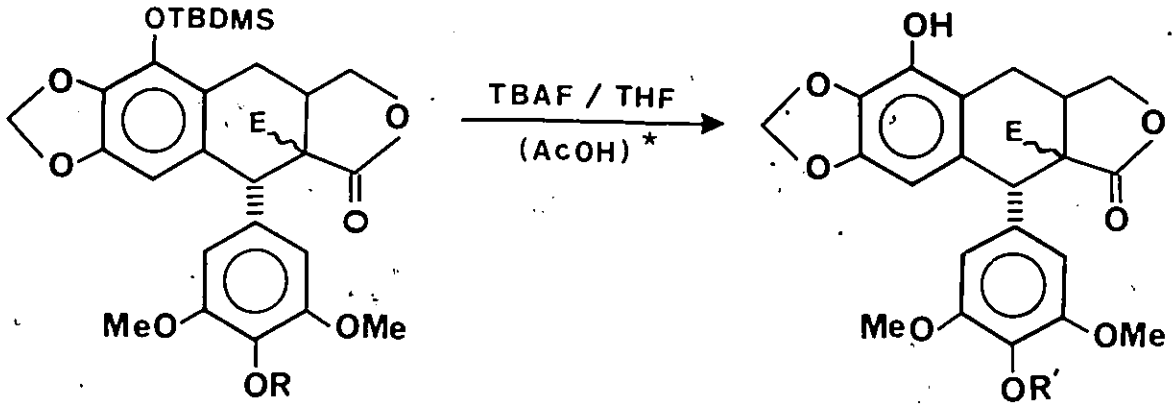


TABLE 6 RESULTS OF THE DESILYLATION EXPERIMENTS



α-peltatin series

R=TBDMS; E=H		23	R'=H, E=H		2
	CH_3	29 (trans)		CH_3	37 (trans)
	CH_3	30 (cis)		CH_3	38 (cis)
	Cl	33		Cl	21

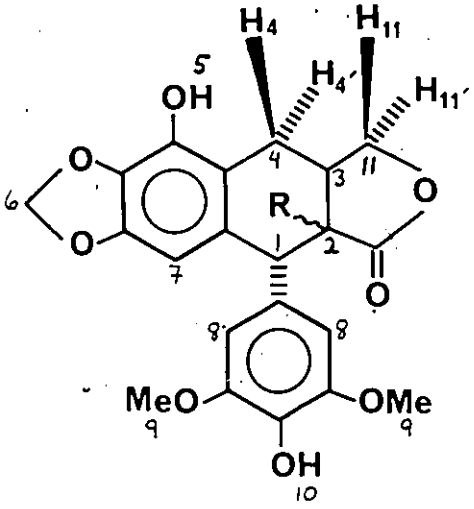
β-peltatin series

R=CH_3, E=H		24	R'=CH_3, E=H		3
	CH_3	31 (trans)		CH_3	39 (trans)
	CH_3	32 (cis)		CH_3	40 (cis)
	Cl	35		Cl	41

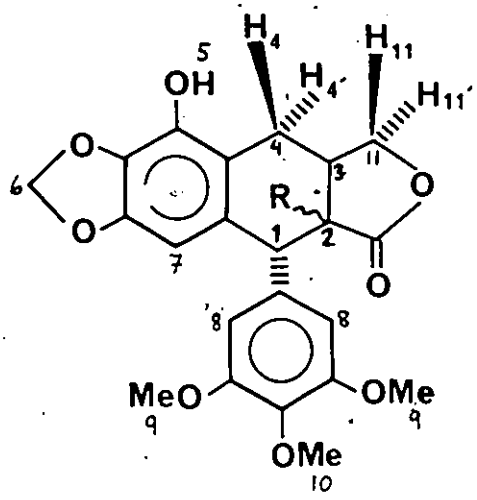
R'	E	PRODUCTS AND YIELDS	
H	H	2 (79%)	
	CH_3	37 (71%)	38 (86%)
	Cl	21 (96%)	
CH_3	H	3 (53%)	
	CH_3	39 (75%)	40 (67%)
	Cl	41 (91%)	

* Acetic acid was used only in the desilylation reactions involving the chlorinated derivatives

RELEVANT ^{1}H NMR DATA FOR THE PELTATINS AND THEIR 2-SUBSTITUTED DERIVATIVES



α-peltatin series



β-peltatin series

TABLE 7 CHEMICAL SHIFTS

COMPOUND	H_1	H_3	H_4	$H_{4'}$	H_7	H_8	H_{11}	$H_{11'}$
2	4.52	2.62-2.82(m)	2.51	3.23	6.16	6.41	3.99	4.45
3	4.54	2.63-2.89(m)	2.52	3.24	6.16	6.43	4.00	4.46
37	4.25	2.70-2.96(m)	2.45	3.08	6.19	6.36	4.02	4.30-4.40(m)
39	4.26	2.75-2.98(m)	2.46	3.08	6.19	6.36	4.02	4.31-4.45(m)
21	4.75	2.84-3.06(m)	2.74	3.15	6.22	6.40	4.19	4.41
41	4.76	2.85-3.05(m)	2.74	3.16	6.22	6.40	4.20	4.43
38	4.14	2.81-3.04(m)	3.07-3.25(m)		6.29	6.54	4.09	4.58
40	4.20	2.67-2.80(m)	3.02-3.11(m)		6.31	6.36	4.08	4.52

RELEVANT ^{1}H NMR DATA FOR THE PELTATINS AND THEIR 2-SUBSTITUTED DERIVATIVES

TABLE 8 COUPLING CONSTANTS

COMPOUND	$J_{H_3-H_4}$	$J_{H_3-H_4'}$	$J_{H_4-H_4'}$	$J_{H_3-H_{11}}$	$J_{H_3-H_{11'}}$	$J_{H_{11}-H_{11'}}$
2	a	4.0	15.2	8.4	6.4	8.3
3	10.8	4.0	15.6	9.7	6.5	8.5
37	12.4	5.4	16.2	11.2	a	8.6
39	12.4	5.3	16.2	11.3	a	8.7
21	11.0	5.4	15.5	8.5	6.8	8.4
41	11.1	5.3	15.7	9.5	6.7	8.6
38	a	a	a	2.2	6.2	9.3
40	a	a	a	2.8	6.7	9.2

a ≡ not measurable with accuracy

Results are given as % T/C (previously discussed on page 13).

The results are shown in Table 9. Both α- and β-peltatin were found to be toxic at dosage levels of 64 and 16 mg/kg. Even at a dosage level of 4 mg/kg α-peltatin was still toxic whereas β-peltatin was only marginally toxic. It was noted from Glinski's results that podophyllotoxin was not toxic (as well as inactive) at a dosage level of 15 mg/kg. Thus, of these three compounds α-peltatin is the most toxic, then β-peltatin and finally podophyllotoxin. As anticipated, the 2-methyl cis isomers, 38 and 40, of α- and β-peltatin, respectively, were inactive at all dosage levels tried. However, testing of the corresponding trans isomers, 37 and 39, at a dosage level of 64 mg/kg indicated significant levels of antitumour activity (T/C=128 and 133, respectively). It should be noted that the 2-methyl derivative of podophyllotoxin was found by Glinski to be inactive at all dosage levels tried (up to 120 mg/kg). Thus it appears that the C_2 derivatives of the peltatins are more active than the corresponding derivatives of podophyllotoxin. If this pattern is continued in the chloro-substituted derivatives then one might expect the 2-chloro trans isomers of the peltatins to have higher anticancer activity than Glinski's 2-chloropodophyllotoxin (which had a T/C=156). Unfortunately, the results of the testing of 21 and 41 against P388 were not available at the time this thesis was written.

viii. Attempted Synthesis of Glycosylated Derivatives

Knowing that the glycoside moiety is required for lower toxicity and high activity, we decided to synthesize the glucosyl

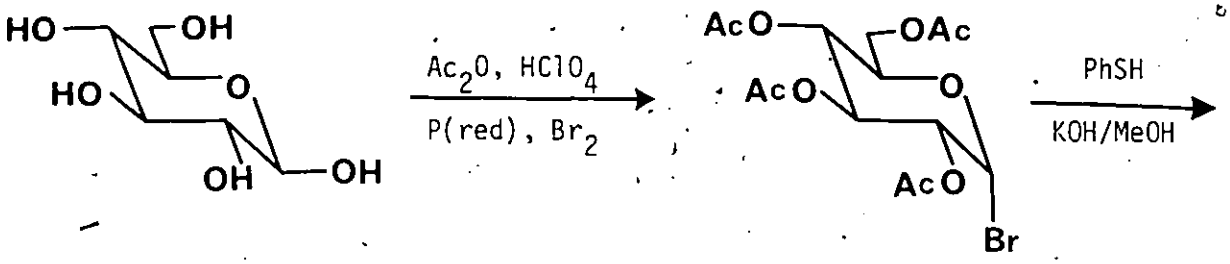
TABLE 9 EFFECT OF PELTATIN DERIVATIVES ON P388 LEUKEMIA

MATERIAL	TREATMENT SCHEDULE	DOSE IP mg/kg/inj	MST DAYS	EFFECT MST % T/C	AWC gm d.6	SURVIVORS DAY 5
NSC 38270	d.1&5	0.8	14.0	156	-1.8	6/6
		0.4	13.5	150	-1.4	6/6
2	d.1&5	64	TOX	TOX	---	0/4
		16	TOX	TOX	---	0/4
		4	TOX	TOX	---	0/4
3	d.1&5	64	TOX	TOX	---	0/4
		16	TOX	TOX	---	0/4
		4	6.0	67	---	3/4
37	d.1&5	64	11.5	128	-0.9	4/4
		16	10.0	111	0.9	4/4
		4	9.5	106	0.5	4/4
38	d.1&5	64	9.5	106	0.9	4/4
		16	9.0	100	1.0	4/4
		4	9.0	100	1.6	4/4
39	d.1&5	64	12.0	133	-2.1	4/4
		16	11.0	122	0.1	4/4
		4	9.0	100	0.5	4/4
40	d.1&5	64	9.0	100	1.0	4/4
		16	9.0	100	1.9	4/4
		4	9.0	100	1.8	4/4
CONTROL		SALINE	9.0	100	-0.5	10/10

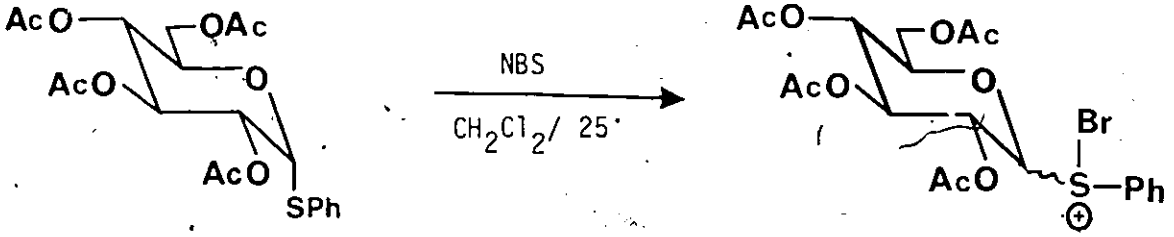
Tumour inoculum: 10^6 ascitic cells implanted intraperitonealy
Host : CDF_1 female mice
TOX : < 3/4 mice alive on Day 5
Evaluation : MST median survival time
Effect : % T/C = (MST treated / MST control) X 100
Criteria : % T/C > or = 125 considered significant antitumour activity

derivatives of these compounds. We first attempted to prepare the tetra-acetyl glucoside 42 of β-peltatin (see Scheme 5). Due to the base-sensitive nature of the trans lactone ring, it was necessary to find a method of attaching the carbohydrate unit onto the appropriate aglycone under mild, non-basic conditions. In 1982, Nicolaou reported a mild and facile procedure for the synthesis of O-glycosides which involved the activation of the appropriate phenyl thioglycoside with NBS in the presence of the hydroxy component and 4-Å molecular sieves in CH_2Cl_2 at 25°.[40] The mechanism of this glycoside bond forming reaction (see Scheme 5) is thought to involve initial electrophilic activation of the sulfur of the phenyl thioglycoside 43 to generate a reactive sulfonium species 44. Presumably oxygen-assisted departure of the activated sulfur group results in formation of an oxonium species such as 45 which is then attacked by the oxygen nucleophile (in this case β-peltatin 3) to give the desired O-glycoside.

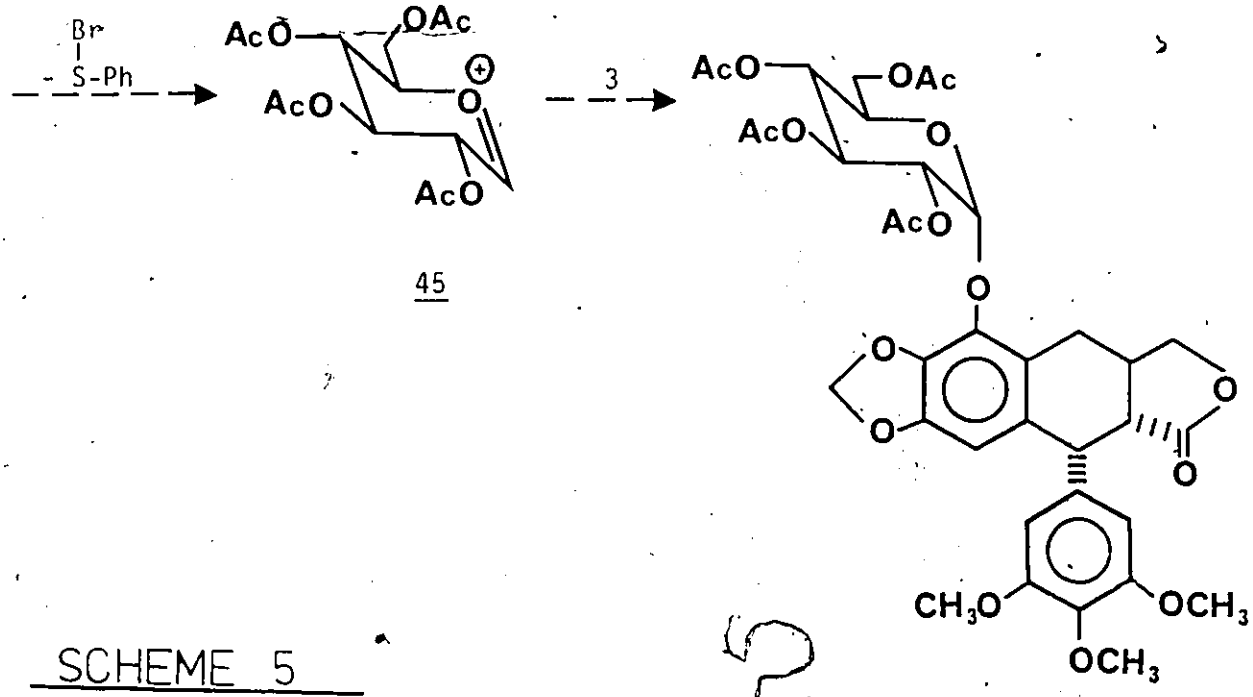
Tetra-O-acetyl-α-D-glucopyranosyl bromide 46 was prepared in 54% yield from anhydrous D-glucose in the presence of acetic anhydride, perchloric acid, red phodphorus and bromine following the procedure of Lemieux.[41] The physical properties (melting point, optical rotation) of 46 corresponded to those reported by Lemieux. All other data collected for this compound were in agreement with the assigned structure (see Experimental Section). The corresponding phenyl thioglycoside 43 was prepared by dropwise addition of 46 in THF to a solution of thiophenol and powdered KOH in methanol. Normal workup followed by PTLC (1/2 ethyl acetate/hexanes) afforded 43 in 51% yield.



46



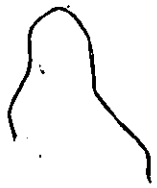
43 44



45 42

SCHEME 5

Following Nicolaou's procedure (as discussed above) we attempted to prepare the tetra-acetyl glucoside 42 of β-peltatin from β-peltatin 3 and the phenyl thioglycoside 43. The progress of the reaction was monitored by TLC. No further change in the TLC was observed after 6.5 hours so the reaction mixture was washed with a saturated sodium sulfite solution then worked up in the normal way. Purification by PTLC (2/3 ethyl acetate/hexanes) yielded three components. The most mobile component was identified as the starting phenyl thioglycoside 43. Neither of the nmr spectra of the other two components indicated the presence of both the β-peltatin moeity and the acetylated glucose moeity. Unfortunately time did not permit any further attempts to perfect this reaction.



EXPERIMENTAL

GENERAL

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations are for chloroform solutions (unless otherwise indicated), and were obtained using a Perkin-Elmer 241 polarimeter. ^{1}H nmr spectra were obtained on Varian XL 200, EM 360A and T-60 spectrometers. ^{13}C nmr spectra were obtained on a Varian FT-80 spectrometer. All spectra were taken using deuterochloroform ($CDCl_3$) as solvent (unless otherwise indicated) and tetramethylsilane (TMS) as the internal standard. The chemical shifts are relative to the internal standard, TMS. The coupling patterns are noted as singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of triplets (dt), doublet of quartets (dq), broadened (br) or multiplet (m). 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. High performance liquid chromatography (HPLC) was carried out using PrepPak^{tm}-500/silica cartridges on a Waters 500 instrument. Microanalyses were carried out by Canadian Microanalytical Service Ltd., Vancouver, B.C.

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

Normal workup involved pouring the reaction mixture into water or 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.

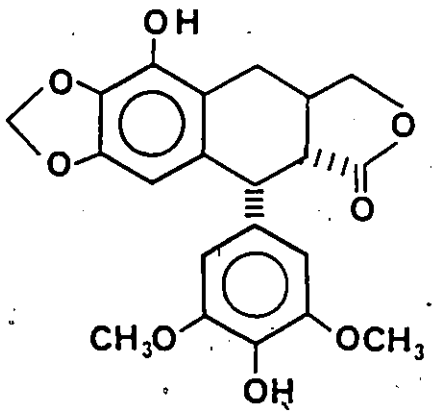
In the ^{1}H nmr spectra, the values given to three decimal places were obtained from the computer printout of the XL 200 spectrometer and are considered accurate to ± 0.001 ppm. Thus, the coupling constants obtained from these values are considered accurate to ± 0.4 Hz. The values given to two decimal places refer either to multiplets obtained from the XL 200 or to data obtained from the EM 360A spectrometer.

Preparation of Chloroform-soluble Fraction of Podophyllum Resin

Podophyllum resin (10.2g, United States Biochemical) was added to 50 ml $CHCl_3$. The resulting mixture was shaken overnight after which time the undissolved residue was filtered off. The residue was then extracted twice more with 50 ml $CHCl_3$ and the combined extracts were evaporated to yield a yellow-brown foam (5.86 g, 57%).

Isolation of 2 and 3

Pure 2 and 3 were obtained by column chromatography (100 g silica gel) on 2.0 g of the chloroform-soluble fraction of podophyllum resin using 40 ml fractions and 400 ml of each of the following solvents: CH_2Cl_2, 2% EtOAc/98% CH_2Cl_2, 5% EtOAc/95% CH_2Cl_2, 7% EtOAc/93% CH_2Cl_2, 9% EtOAc/91% CH_2Cl_2, 10% EtOAc/90% CH_2Cl_2. Fractions 30-34 yielded 0.208 g of pure β-peltatin 3 after recrystallization from benzene while fractions 47-60 yielded 0.222 g of pure α-peltatin 2 after PTLC (2/1 ethyl acetate/hexanes; 2 runs). The overall yields of α- and β-peltatin from podophyllum resin were 6.4% and 6.0%, respectively.



2

$C_{21}H_{20}O_8$

400.39

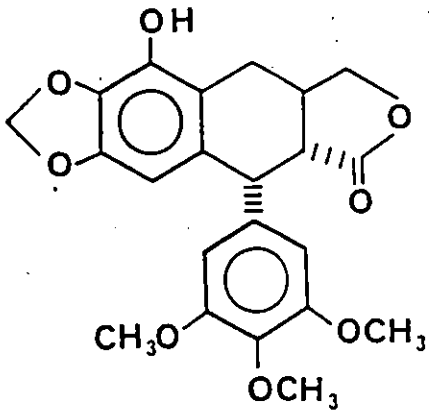
MP 241-243°C (lit. MP 230.5-232.5°C)[10]

^{1}H nmr (acetone) δ (ppm) 2.514 (dd, J=10.5, 16.1 Hz, 1H), 2.62-2.82 (m, 2H), 2.84-3.01 (br, 2H), 3.234 (dd, J=4.0, 15.2 Hz, 1H), 3.692 (s, 6H), 3.992 (dd, J=8.4, 9.6 Hz, 1H), 4.454 (dd, J=6.4, 8.2 Hz, 1H), 4.521 (d, J=4.4 Hz, 1H), 5.905 (s, 2H), 6.162 (s, 1H), 6.410 (s, 2H).

M.S. m/e 400 (M^+)

$[\alpha]_D$ (MeOH) = -115.8, c=1.02 (lit. $[\alpha]_D$ (alc.)= -115°, c=1.01)[10]

Analysis Calcd. for $C_{21}H_{20}O_8$: C, 63.00; H, 5.04.
Found : C, 63.22; H, 5.21.



3

$C_{22}H_{22}O_8$

414.41

MP 235-238°C (lit. MP 231-238°C)[10]

^{1}H nmr (acetone) δ (ppm) 2.516 (dd, J=10.8, 15.8 Hz, 1H), 2.63-2.89 (m, 2H), 2.92-3.05 (br, 1H), 3.243 (dd, J=4.0, 15.4 Hz, 1H), 3.665 (s, 3H), 3.686 (s, 6H), 3.995 (dd, J=8.5, 9.7 Hz, 1H), 4.456 (dd, J=6.5, 8.5 Hz, 1H), 4.544 (d, J=4.4 Hz, 1H), 5.907 (s, 2H); 6.156 (s, 1H), 6.426 (s, 2H).

M.S. m/e 414 (M^+)

$[\alpha]_D$ (MeOH) = -100, c=0.99 (lit. $[\alpha]_D$ (alc.)= -115°, c=1.01)[10]

Analysis Calcd. for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35.
Found : C, 63.96; H, 5.51.

Preparation of the trianion of α-peltatin and subsequent reaction with hexachloroethane

A solution of α-peltatin 2 (0.129 g, 0.32 mmol) in 5 ml dry THF was added slowly to a cooled (-78°) solution of LDA (3 equivalents) under N_2, prepared from diisopropylamine (0.16 ml) and n-BuLi (0.56 ml, 1.9 M in hexanes) in 15 ml dry THF. By the end of the addition a gelatinous mixture had formed so the flask was transferred to an ice bath and stirred for a further 0.5 h at which time a solution of hexachloroethane (629 mg, 2.65 mmol) in 1 ml dry THF was slowly added. The mixture was stirred overnight at RT then washed with saturated ammonium chloride and saturated sodium sulfite solutions, dried over $MgSO_4$, filtered and the solvent evaporated to yield 153 mg of a brown solid. Partial purification by PTLC (1/1 ethyl acetate/hexanes; 2 runs) afforded two products with very similar R_f values. Complete purification and identification of these two components was never made. However, it was postulated that the more mobile component (45 mg) was probably the trans isomer 21 of 2-chloro α-peltatin while the less mobile component was probably the corresponding cis isomer 22 (63 mg).

more mobile component

^1H nmr δ (ppm) 2.5-3.5 (m, 3H), 3.77 (s, 6H), 3.9-4.6 (m, 2H), 4.70 (s, 1H), 5.90 (s, 2H), 6.18 (s, 1H), 6.38 (s, 2H).

M.S. m/e 434, 436 (3:1 ratio)

less mobile component

^1H nmr δ (ppm) 2.5-3.5 (m, 3H), 3.80 (s, 6H), 4.0-4.4 (m, 1H), 4.45 (s, 1H), 4.5-4.9 (m, 1H), 5.79 (s, 2H), 6.27 (s, 1H), 6.45 (s, 2H).

Preparation of the Crude Peltatin Mixture

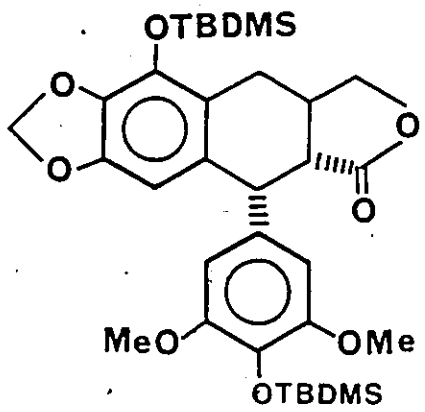
Partial separation of the chloroform-soluble fraction of podophyllum resin (6.0 g) was obtained by passing it through a short silica gel column (5 g silica / 1 g mixture) using 200 ml each of the following solvents:

Fraction 1 - hexanes
2 - 20% CH_2Cl_2/80% hexanes
3 - 40% CH_2Cl_2/60% hexanes
4 - 60% CH_2Cl_2/40% hexanes
5 - 80% CH_2Cl_2/20% hexanes
6 - CH_2Cl_2
7 - 2% EtOAc/98% CH_2Cl_2
8 - 4% EtOAc/96% CH_2Cl_2
9 - 6% EtOAc/94% CH_2Cl_2
10- 8% EtOAc/92% CH_2Cl_2

Fractions 5-10 yielded 3.5 g of the crude peltatin mixture.

Isolation of the Silylated Peltatins

The crude peltatin mixture (2.380 g), imidazole (1.609 g, 0.0236 mol), and t-butyldimethylsilyl chloride (1.709 g, 0.0113 mol) were combined with 11 ml DMF, heated overnight at 55° then poured into water. Workup yielded a yellow-brown foam (3.296 g) which was purified by HPLC (1/9 ethyl acetate/hexanes) affording two components, the disilyl ether of α-peltatin 23 and the silyl ether of β-peltatin 24. Recrystallization of 23 from hexanes yielded 0.987 g of a fine white powder. Compound 24 was obtained as a coarse off-white powder (0.630 g) after recrystallization from CH_2Cl_2/hexanes. The yields of 23 and 24 from podophyllum resin were 14% and 9%, respectively.



23

$C_{33}H_{48}O_8Si_2$

628.91

MP 136-138°C

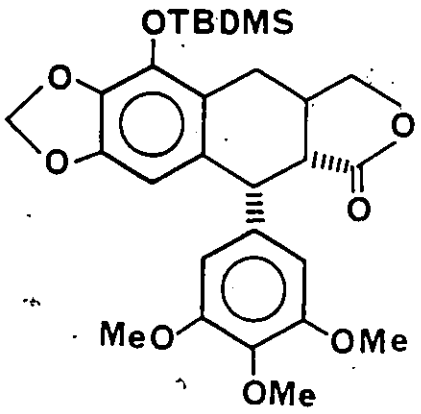
^1H nmr δ (ppm) 0.101 (s, 6H), 0.240 (s, 3H), 0.288 (s, 3H), 0.985 (s, 9H), 1.028 (s, 9H), 2.406 (dd, J=11.2, 16.0 Hz, 1H), 2.58-2.72 (m, 2H), 3.10-3.24 (m, 1H), 3.679 (s, 6H), 3.918 (dd, J=8.9, 10.1 Hz, 1H), 4.463 (dd, J=6.2, 8.4 Hz, 1H), 4.566 (d, J=3.4 Hz, 1H), 5.890 (d, J=1.4 Hz, 1H), 5.910 (d, J=1.4 Hz, 1H), 6.266 (s, 1H), 6.303 (s, 2H).

^{13}C nmr δ (ppm) -4.56 (q), -4.04 (q), 18.57 (s), 18.66 (s), 25.82 (q), 25.93 (q), 28.16 (t), 32.30 (d), 43.77 (d), 47.43 (d), 55.92 (q), 72.38 (t), 100.83 (t), 104.26 (d), 108.39 (d), 122.07 (s), 131.96 (s), 133.24 (s), 133.42 (s), 135.45 (s), 136.48 (s), 147.53 (s), 150.80 (s), 175.10 (s).

M.S. m/e 571 (M^+-t-Bu)

$[\alpha]_D$ ($CHCl_3$) = -106, c=1.00

Analysis Calcd. for $C_{33}H_{48}O_8Si_2$: C, 63.02; H, 7.69.
Found : C, 62.93; H, 8.05.



24

$C_{28}H_{36}O_8Si$

528.67

MP 172-174°C

^1H nmr δ (ppm) 0.244 (s, 3H), 0.290 (s, 3H), 1.031 (s, 9H), 2.421 (dd, J=11.6, 15.8 Hz, 1H), 2.54-2.73 (m, 2H), 3.181 (dd, J=4.1, 15.5 Hz, 1H), 3.751 (s, 6H), 3.802 (s, 3H), 3.938 (dd, J=8.8, 10.2 Hz, 1H), 4.492 (dd, J=6.3, 8.5 Hz, 1H), 4.586 (d, J=3.8 Hz, 1H), 5.895 (d, J=1.4 Hz, 1H), 5.913 (d, J=1.4 Hz, 1H), 6.256 (s, 1H), 6.350 (s, 2H).

^{13}C nmr δ (ppm) -4.04 (q), 18.56 (s), 25.93 (q), 28.13 (t), 32.42 (d), 43.91 (d), 47.29 (d), 56.20 (q), 60.71 (q), 72.41 (t), 100.89 (t), 104.18 (d), 108.23 (d), 122.07 (s), 131.59 (s), 135.58 (s), 136.38 (s), 136.51 (s), 137.00 (s), 147.64 (s), 152.48 (s), 175.04 (s).

M.S. m/e 528 (M^+)

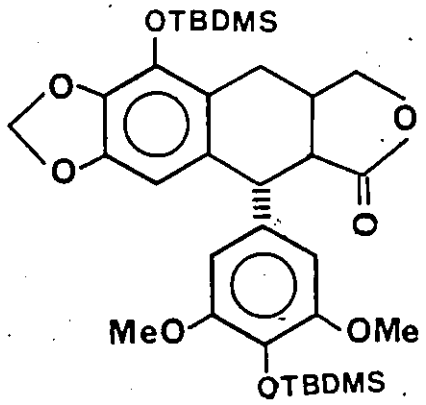
$[\alpha]_D$ ($CHCl_3$) = -106°, c=1.00

Analysis Calcd. for $C_{28}H_{36}O_8Si$: C, 63.61; H, 6.86.

Found : C, 63.47; H, 7.00.

Preparation of the enolate anion of 23 and subsequent reaction with water

Treatment of 23 (202 mg, 0.32 mmol) with 1 equivalent of LDA in 10 ml dry THF at -78° was followed by warming of the solution to above 0°, addition of water and, after 1 minute of stirring, quenching with a saturated ammonium chloride solution. Normal workup afforded a beige foam which was separated into two components by PTLC (1/5 ethyl acetate/hexanes; 2 runs). The major and less mobile product (158 mg, 78%) was identified as the cis isomer 26 of silylated α-peltatin. The minor component (39 mg, 19%) was identified as the starting material 23.



26

$C_{33}H_{48}O_8Si_2$

628.91

MP 140-142°C

^1H nmr δ (ppm) 0.105 (s, 6H), 0.184 (s, 3H), 0.206 (s, 3H), 0.988 (s, 9H), 0.995 (s, 9H), 2.63-2.71 (m, 2H), 2.92-3.05 (m, 1H), 3.329 (dd, J=2.8, 9.6 Hz, 1H), 3.692 (s, 6H), 3.892 (dd, J=3.4, 9.0 Hz, 1H), 4.350 (d, J=2.6 Hz, 1H), 4.416 (dd, J=7.6, 9.2 Hz, 1H), 5.860 (d, J=1.4 Hz, 1H), 5.904 (d, J=1.4 Hz, 1H), 6.273 (s, 2H), 6.325 (s, 1H).

^{13}C nmr —— δ (ppm) -4.58 (q), -4.24 (q), 18.48 (s), 18.69 (s), 24.80 (t), 25.83 (q), 32.61 (d), 45.44 (d), 46.28 (d), 55.85 (q), 73.07 (t), 100.68 (t), 103.89 (d), 105.00 (d), 120.98 (s), 131.57 (s), 133.09 (s), 134.94 (s), 136.01 (s), 136.65 (s), 147.25 (s), 151.59 (s), 178.59 (s).

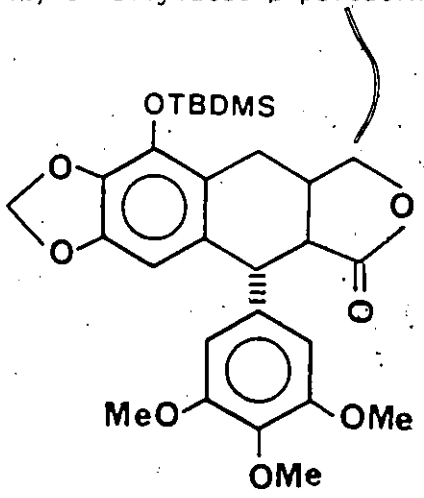
M.S. m/e 571 (M^+-t-Bu)

$[\alpha]_D$ ($CHCl_3$) = 4.7, c=0.958

Analysis Calcd. for $C_{33}H_{48}O_8Si_2$: C, 63.02; H, 7.69.
Found : C, 62.06; H, 7.49.

Trapping of the enolate anion prepared from 24 with water

Treatment of 24 (202-mg, 0.38 mmol) with 1 equivalent of LDA in 10 ml dry THF at -78° was followed by warming of the solution to above 0°, addition of water and, after 1 minute of stirring, quenching with a saturated ammonium chloride solution. Normal workup afforded two components on purification by PTLC (1/6 ethyl acetate/hexanes; 3 runs), identified as the starting material 24 (40 mg, 20%) and the slightly less mobile cis isomer 28 (130 mg, 64%) of silylated β-peltatin.



28

$C_{28}H_{36}O_8Si$

528.67

MP 55-60°C

^{1}H nmr δ (ppm) 0.212 (s, 3H), 0.226 (s, 3H), 1.013 (s, 9H), 2.705 (dd, J=1.5, 5.9 Hz, 2H), 2.92-3.10 (m, 1H), 3.318 (dd, J=2.8, 9.6 Hz, 1H), 3.777 (s, 6H), 3.825 (s, 3H), 3.926 (dd, J=3.4, 9.0 Hz, 1H), 4.364 (d, J=3.0 Hz, 1H), 4.432 (dd, J=7.3, 9.3 Hz, 1H), 5.874 (d, J=1.4 Hz, 1H), 5.915 (d, J=1.4 Hz, 1H), 6.313 (s, 1H), 6.338 (s, 2H).

^{13}C nmr δ (ppm) -4.24 (q), 18.46 (s), 24.78 (t), 25.83 (q), 32.61 (d), 45.54 (d), 46.39 (d), 56.16 (q), 60.85 (q), 73.01 (t), 100.73 (t), 103.77 (d), 104.94 (d), 120.99 (s), 131.11 (s), 136.05 (s), 136.66 (s), 136.79 (s), 138.37 (s), 147.34 (s), 153.34 (s), 178.41 (s).

M.S. m/e 528 (M^+)

$[\alpha]_D$ ($CHCl_3$) = 3.8, c=1.04

Analysis Calcd. for $C_{28}H_{36}O_8Si$: C, 63.61; H, 6.86.
Found : C, 59.89; H, 6.49.

Preparation of 2 from 23

Treatment of a cooled (0°) solution of 23 (322 mg, 0.51 mmol) in 10 ml dry THF with 4 equivalents of TBAF (2.0 ml, 1.0 M in THF) was followed, after 5 minutes of stirring, by quenching with a saturated ammonium chloride solution. Normal workup was followed by column chromatography on silica gel using the following solvents: 300 ml of CH_2Cl_2, 100 ml each of 10% EtOAc/90% CH_2Cl_2, 20% EtOAc/80% CH_2Cl_2, 30% EtOAc/70% CH_2Cl_2, 40% EtOAc/60% CH_2Cl_2. The fraction

size was 15 ml. Fractions 21-30 yielded 162 mg (79%) of pure 2. All data collected for 2 obtained by the desilylation route were in agreement with previous data obtained for α-peltatin (see pages 57 and 58).

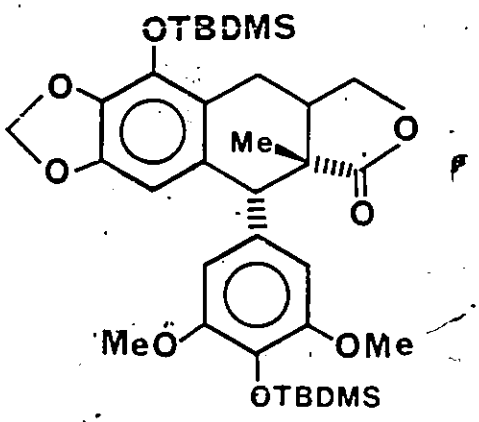
Preparation of 3 from 24

Treatment of cooled (0°) solution of 24 (222 mg, 0.42 mmol) in 10 ml dry THF with 2 equivalents of TBAF (0.85 ml, 1.0 M in THF) was followed, after 5 minutes of stirring, by quenching with a saturated ammonium chloride solution. Normal workup was followed by column chromatography on silica gel using 20 ml fractions and 200 ml of each of the following solvents: CH_2Cl_2, 10% EtOAc/90% CH_2Cl_2, 20% EtOAc/80% CH_2Cl_2, 30% EtOAc/70% CH_2Cl_2. Fractions 13-22 yielded 93 mg (53%) of pure 3. All data collected for 3 obtained by the desilylation route were in agreement with previous data obtained for β-peltatin (see page 58).

Trapping of the enolate anion prepared from 23 with methyl iodide

Compound 23 (510 mg, 0.81 mmol) was dissolved in 5 ml dry THF and added slowly to a cooled (-78°) solution of LDA (1 equivalent), prepared from diisopropylamine (0.12 ml) and n-BuLi (0.41 ml, 2.0 M in hexanes) in 10 ml dry THF. The blue solution was stirred for 15 minutes at -78° then excess CH_3I was added (0.5 ml). The resulting solution was warmed to RT and stirred overnight (22 h). Normal workup afforded two components after PTLC (1/7 ethyl acetate/ hexanes; 4 runs). The more mobile component was identified as

2-methyl silylated α-peltatin (trans) 29 (200 mg, 38%) while the less mobile component was identified as the corresponding cis isomer 30 (292 mg, 56%).



29

$C_{34}H_{50}O_8Si_2$

642.94

MP 75-83°C

^1H nmr δ (ppm) 0.097 (s, 6H), 0.243 (s, 3H), 0.273 (s, 3H), 0.982 (s, 9H), 1.032 (s, 9H), 1.220 (s, 3H), 2.355 (dd, J=12.6, 16.2 Hz, 1H), 2.69-2.92 (m, 1H), 3.014 (dd, J=5.3, 16.3 Hz, 1H), 3.675 (s, 6H), 3.984 (dd, J=8.4, 11.0 Hz, 1H), 4.219 (s, 1H), 4.28-4.40 (m, 1H), 5.897 (s, 2H), 6.230 (s, 1H), 6.296 (s, 2H).

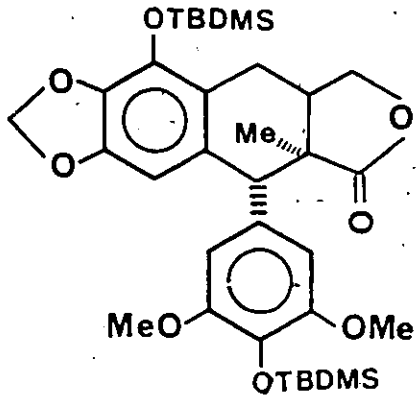
^{13}C nmr δ (ppm) -4.55 (q), -4.06 (q), 16.35 (q), 18.66 (s), 23.12 (t), 25.81 (q), 25.94 (q), 34.30 (d), 46.07 (s), 52.26 (d), 55.97 (q), 70.08 (t), 100.82 (t), 105.22 (d), 109.07 (d), 120.91 (s), 131.34 (s), 133.36 (s), 135.42 (s), 136.34 (s), 147.76 (s), 150.57 (s), 178.59 (s).

M.S. m/e 642 (M^+), 585 (M^+-t-Bu)

$[\alpha]_D$ ($CHCl_3$) = -102, c=1.00

Analysis Calcd. for $C_{34}H_{50}O_8Si_2$: C, 63.52; H, 7.84.

Found : C, 62.46; H, 7.81.



30

$C_{34}H_{50}O_8Si_2$

642.94

MP 69-75°C

^1H nmr δ (ppm) 0.111 (s, 6H), 0.212 (s, 3H), 0.241 (s, 3H), 0.994 (s, 9H), 1.026 (s, 9H), 1.190 (s, 3H), 2.63-2.75 (m, 1H), 2.99-3.09 (m, 2H), 3.712 (s, 6H), 3.988 (dd, J=2.9, 9.1 Hz, 1H), 4.175 (s, 1H), 4.484 (dd, J=6.9, 9.1 Hz, 1H), 5.850 (s, 2H), 6.301 (s, 2H), 6.353 (s, 1H).

^{13}C nmr δ (ppm) -4.58 (q), -4.17 (q), 18.49 (s), 18.67 (s), 22.87 (q), 25.84 (q), 26.98 (t), 39.66 (q), 47.04 (s), 51.27 (d), 55.79 (q), 73.50 (t), 100.64 (t), 103.57 (d), 106.86 (d), 119.56 (s), 132.26 (s), 132.56 (s), 135.74 (s), 136.29 (s), 147.31 (s), 151.24 (s), 169.31 (s), 181.39 (s).

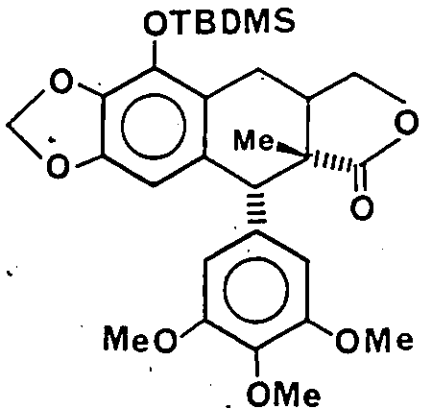
M.S. m/e 585 (M^+-t-Bu)

$[\alpha]_D$ ($CHCl_3$) = -106, c=1.00

Analysis Calcd. for $C_{34}H_{50}O_8Si_2$: C, 63.52; H, 7.84.
Found : C, 62.45; H, 7.95.

Trapping of the enolate anion prepared from 24 with methyl iodide

Treatment of 24 (544 mg, 1.03 mmol) with 1 equivalent of LDA in 10 ml dry THF at -78°, followed by trapping with excess methyl iodide (0.5 ml), warming of the solution to RT, stirring overnight (22 h) and normal workup afforded a yellow-brown foam. Purification by PTLC (1/5 ethyl acetate/hexanes; 2 runs) yielded two components which were identified as 2-methyl silylated β-peltatin (trans) 31 (292 mg, 52%) and the corresponding cis isomer 32 (258 mg, 46%).



31

$C_{29}H_{38}O_8Si$

542.70

MP 76-82.5°C

^1H nmr δ (ppm) 0.253 (s, 3H), 0.280 (s, 3H), 1.037 (s, 9H), 1.242 (s, 3H), 2.380 (dd, J=12.4, 16.2 Hz, 1H), 2.70-2.92 (m, 1H), 3.040 (dd, J=5.4, 16.4 Hz, 1H), 3.747 (s, 6H), 3.797 (s, 3H), 4.011 (dd, J=8.6, 11.3 Hz, 1H), 4.243 (s, 1H), 4.366 (dd, J=7.4, 8.2 Hz, 1H), 5.900 (s, 2H), 6.226 (s, 1H), 6.346 (s, 2H).

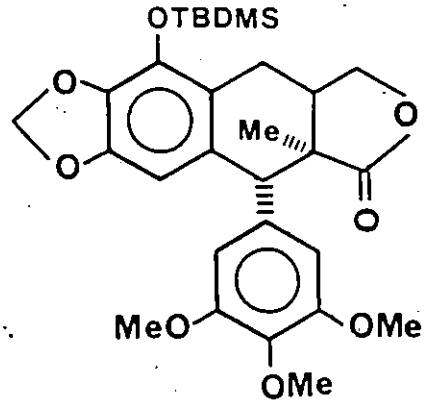
^{13}C nmr δ (ppm) -4.05 (q), 16.37 (q), 18.59 (s), 23.05 (t), 25.94 (q), 34.42 (d), 45.94 (s), 52.38 (d), 56.15 (q), 60.68 (q), 70.10 (t), 100.87 (t), 105.12 (d), 108.82 (d), 120.87 (s), 130.91 (s), 135.53 (s), 136.37 (s), 136.51 (s), 136.90 (s), 147.84 (s), 152.24 (s), 178.54 (s).

M.S. m/e 542 (M^+)

$[\alpha]_D$ ($CHCl_3$) = -107, c=1.01

Analysis Calcd. for $C_{29}H_{38}O_8Si$: C, 64.18; H, 7.06.

Found : C, 59.71; H, 6.47.



32

$C_{29}H_{38}O_8Si$

542.70

MP 71-75°C

^1H nmr δ (ppm) 0.212 (s, 3H), 0.244 (s, 3H), 1.026 (s, 9H), 1.232 (s, 3H), 2.64-2.77 (m, 1H), 3.015 (d, J=5.4 Hz, 2H), 3.785 (s, 6H), 3.814 (s, 3H), 4.007 (dd, J=3.1, 9.3 Hz, 4.205 (s, 1H), 4.506 (dd, J=6.9, 9.3 Hz, 1H), 5.852 (s, 2H), 6.345 (s, 3H).

^{13}C nmr δ (ppm) -4.17 (q), 18.49 (s), 22.83 (q), 25.87 (q), 26.91 (t), 39.64 (d), 46.97 (s), 51.38 (d), 56.10 (q), 60.79 (q), 73.43 (t), 100.67 (t), 103.44 (d), 106.80 (d), 119.56 (s), 132.19 (s), 135.50 (s), 135.80 (s), 136.34 (s), 137.02 (s), 147.39 (s), 152.94 (s), 181.21 (s).

M.S. m/e 542 (M^+)

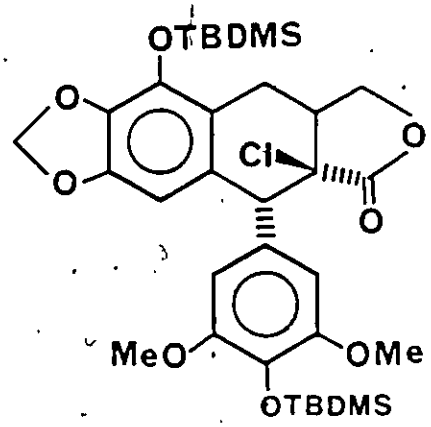
$[\alpha]_D$ ($CHCl_3$) = -107, c=1.00

Analysis Calcd. for $C_{29}H_{38}O_8Si$: C, 64.18; H, 7.06.

Found : C, 61.19; H, 6.57.

Trapping of the enolate anion prepared from 23 with hexachloroethane

Treatment of 23 (426 mg, 0.68 mmol) with 1 equivalent of LDA in 10 ml dry THF at -78° followed by trapping with excess hexachloroethane (1.288 g), warming of the solution to RT, stirring overnight (22 h) and normal workup afforded a beige foam. Purification of the crude product by PTLC (1/6 ethyl acetate/hexanes; 1 run) yielded two components, 2-chloro silylated α-peltatin (trans) 33 (414 mg, 92%) and a small proportion of the corresponding cis isomer 34 (19 mg, 4%).



33

$C_{33}H_{47}O_8Si_2Cl$

663.35

MP 79-86°C

^1H nmr δ (ppm) 0.097 (s, 6H), 0.255 (s, 3H), 0.285 (s, 3H), 0.979 (s, 9H), 1.037 (s, 9H), 2.652 (dd, J=10.9, 15.7 Hz, 1H), 2.82-3.00 (m, 1H), 3.083 (dd, J=5.6, 15.6 Hz, 1H), 3.678 (s, 6H), 4.163 (dd, J=8.5, 9.7 Hz, 1H), 4.400 (dd, J=6.7, 8.5 Hz, 1H), 4.726 (s, 1H), 5.902 (d, J=1.4 Hz, 1H), 5.928 (d, J=1.4 Hz, 1H), 6.259 (s, 1H), 6.340 (s, 2H).

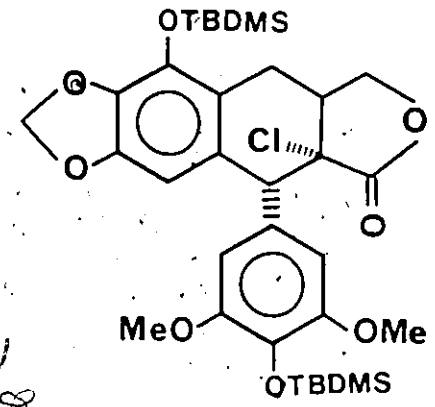
^{13}C nmr δ (ppm) -4.54 (q), -4.02 (q), 18.66 (s), 23.47 (t), 25.76 (q), 25.94 (q), 36.68 (d), 53.29 (d), 55.97 (q), 70.57 (t), 71.53 (s), 100.99 (t), 104.61 (d), 109.06 (d), 120.28 (s), 129.45 (s), 130.98 (s), 134.15 (s),

135.84 (s), 136.30 (s), 148.01 (s), 150.94 (s), 170.93 (s).

M.S. m/e 605 (M^+-t-Bu)

$[\alpha]_D$ ($CHCl_3$) = -116, c=1.01

Analysis Calcd. for $C_{33}H_{47}O_8Si_2Cl$: C, 59.75; H, 7.14.
Found : C, 59.21; H, 7.38.



34

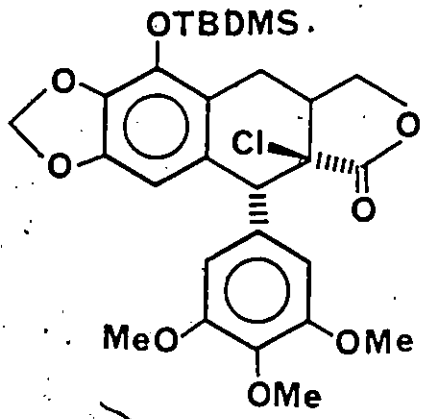
$C_{33}H_{47}O_8Si_2$

663.35

^1H nmr δ (ppm) 0.115 (s, 6H), 0.207 (s, 3H), 0.235 (s, 3H), 0.994 (s, 9H), 1.014 (s, 9H), 2.95-3.40 (m, 3H), 3.725 (s, 6H), 4.171 (d, J=9.0 Hz, 1H), 4.475 (s, 1H), 4.738 (dd, J=4.9, 8.9 Hz, 1H), 5.881 (s, 2H), 6.358 (s, 1H), 6.421 (s, 2H).

Trapping of the enolate anion prepared from 24 with hexachloroethane

Treatment of 24 (465 mg, 0.88 mmol) with 1 equivalent of LDA in 10 ml dry THF at -78° followed by trapping with excess hexachloroethane (1.627 g), warming of the solution to RT, stirring overnight (22 h) and normal workup afforded two components on purification by PTLC (1/5 ethyl acetate/hexanes), 2-chloro silylated β-peltatin (trans) 35 (409 mg, 83%) and a small proportion of the corresponding cis isomer 36 (27 mg, 6%).



35

$C_{28}H_{35}O_8SiCl$

563.12

MP 212-215°C

^{1}H-nmr δ (ppm) 0.262 (s, 3H), 0.288 (s, 3H), 1.040 (s, 9H), 2.670 (dd, J=11.0, 15.6 Hz, 1H), 2.80-3.04 (m, 1H), 3.102 (dd, J=5.5, 15.7 Hz, 1H), 3.749 (s, 6H), 3.800 (s, 3H), 4.185 (dd, J=8.7, 9.7 Hz, 1H), 4.427 (dd, J=6.6, 8.4 Hz, 1H), 4.750 (s, 1H), 5.907 (d, J=1.4 Hz, 1H), 5.930 (d, J=1.4 Hz, 1H), 6.252 (s, 1H), 6.387 (s, 2H).

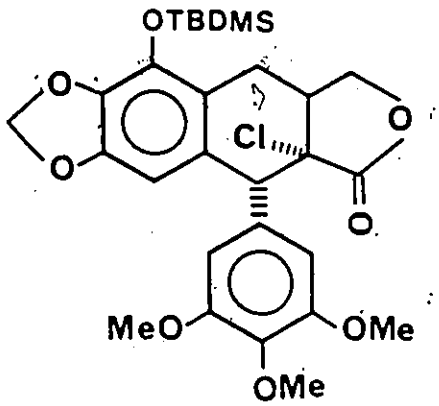
^{13}C nmr δ (ppm) -4.03 (q), 18.58 (s), 23.41 (t), 25.93 (q), 36.81 (d), 53.38 (d), 56.25 (q), 60.72 (q), 70.60 (t), 71.24 (s), 101.02 (t), 104.53 (d), 108.92 (d), 120.25 (s), 129.09 (s), 134.12 (s), 135.92 (s), 136.34 (s), 137.59 (s), 148.04 (s), 152.61 (s), 163.85 (s), 170.87 (s).

M.S. m/e 562 (M^+)

$[\alpha]_D$ ($CHCl_3$) = -133; c=0.992

Analysis Calcd. for $C_{28}H_{35}O_8SiCl$: C, 59.72; H, 6.26.

Found : C, 59.78; H, 6.36.



36

$C_{28}H_{35}O_8SiCl$

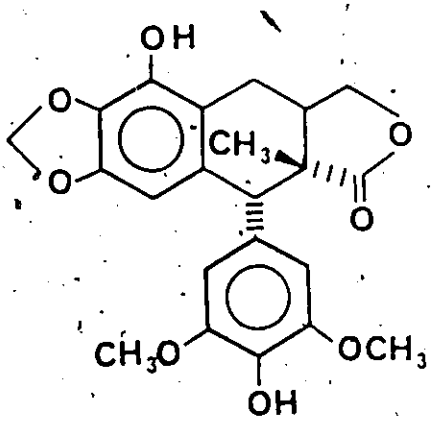
563.12

^1H nmr δ (ppm) 0.212 (s, 3H), 0.239 (s, 3H), 1.017 (s, 9H), 2.97-3.36 (m, 3H), 3.799 (s, 6H), 3.830 (s, 3H), 4.183 (d, J=9.0 Hz, 1H), 4.508 (s, 1H), 4.753 (dd, J=5.2, 9.0 Hz, 1H), 5.864 (d, J=1.4 Hz, 1H), 5.883 (d, J=1.4 Hz, 1H), 6.345 (s, 1H), 6.465 (s, 2H).

M.S. m/e 562 (M^+)

Preparation of 2-methyl α-peltatin (trans) from 29

Treatment of a cooled (0°) solution of 29 (220 mg, 0.34 mmol) in 5 ml dry THF with 4 equivalents of TBAF (1.37 ml, 1.0 M in THF) was followed by immediate quenching with a saturated ammonium chloride solution. Normal workup afforded 2-methyl α-peltatin (trans) 37 (100 mg, 71%) after PTLC (2/1 ethyl acetate/hexanes; 2 runs).



37

$C_{22}H_{22}O_8$

414.41

MP 228-233°C

^{1}H nmr δ (ppm) 1.252 (s, 3H), 2.455 (dd, J=12.4, 16.2 Hz, 1H), 2.70-2.96 (m, 1H), 3.083 (dd, J=5.4, 16.2 Hz, 1H), 3.789 (s, 6H), 4.023 (dd, J=8.6, 11.2 Hz, 1H), 4.251 (s, 1H), 4.30-4.40 (m, 1H), 5.367 (s, 1H), 5.443 (s, 1H), 5.939 (s, 2H), 6.192 (s, 1H), 6.364 (s, 2H).

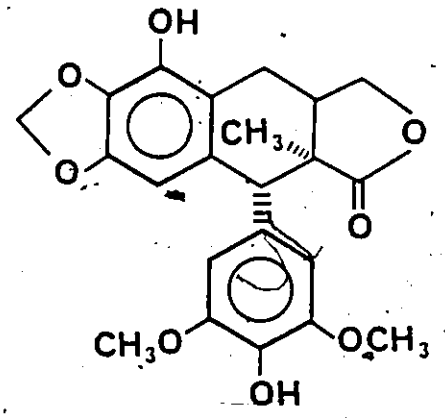
M.S. m/e 414 (M^+)

$[\alpha]_D$ (MeOH) = -123, c=0.968

Analysis Calcd. for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35.
Found : C, 63.48; H, 5.28.

Preparation of 2-methyl α-peltatin (cis) from 30

Treatment of a cooled (0°) solution of 30 (575 mg, 0.90 mmol) in 5 ml dry THF with 4 equivalents of TBAF (3.6 ml, 1.0 M in THF) was followed by immediate quenching with a saturated ammonium chloride solution. Normal workup afforded 2-methyl α-peltatin (cis) 38 (319 mg, 86%) after PTLC (2/1 ethyl acetate/hexanes; 3 runs) and recrystallization from CH_2Cl_2/hexanes as fine beige needles.



38

$C_{22}H_{22}O_8$

414.41

MP 108-111°C

^{1}H nmr (acetone) δ (ppm) 1.199 (s, 3H), 2.81-3.25 (m, 3H), 3.789 (s, 6H), 4.092 (dd, J=2.2, 9.4 Hz, 1H), 4.137 (s, 1H), 4.580 (dd, J=6.2, 9.2 Hz, 1H), 5.616 (s, 2H), 5.840 (d, J=1.0 Hz, 1H), 5.851 (d, J=1.2 Hz, 1H), 6.289 (s, 1H), 6.538 (s, 2H).

M.S. m/e 414 (M^+)

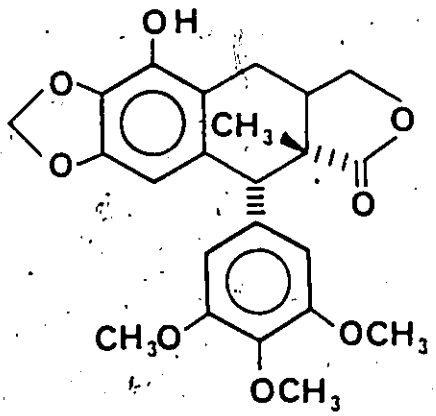
$[\alpha]_D$ (MeOH) = -104, c=1.01

Analysis Calcd. for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35.

Found : C, 54.08; H, 4.92.

Preparation of 2-methyl β-peltatin (trans) from 31

Treatment of a cooled (0°) solution of 31 (169 mg, 0.31 mmol) in 10 ml dry THF with 2 equivalents TBAF (0.63 ml, 1.0 M in THF) was followed by immediate quenching with a saturated ammonium chloride solution. Normal workup followed by PTLC (3/2 ethyl acetate/hexanes; 1 run) and recrystallization from CH_2Cl_2/hexanes afforded 2-methyl β-peltatin (trans) 39 (99 mg, 75%).



39

$C_{23}H_{24}O_8$

428.44

MP 242-244°C

^{1}H nmr δ (ppm) 1.256 (s, 3H), 2.457 (dd, J=12.4, 16.4 Hz, 1H), 2.75-2.98 (m, 1H), 3.082 (dd, J=5.3, 16.1 Hz, 1H), 3.753 (s, 6H), 3.801 (s, 3H), 4.022 (dd, J=8.7, 11.3 Hz, 1H), 4.256 (s, 1H), 4.31-4.45 (m , 1H),

5.490 (br, 1H), 5.923 (s, 2H), 6.191 (s, 1H), 6.355 (s, 2H).

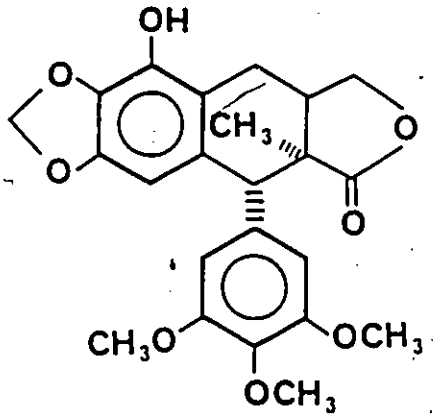
M.S. m/e 428 (M^+)

$[\alpha]_D$ ($CHCl_3$) = -119, c=0.995

Analysis Calcd. for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65.
Found : C, 64.59; H, 5.82.

Preparation of 2-methyl β-peltatin (cis) from 32

Treatment of a cooled (0°) solution of 32 (258 mg, 0.47 mmol) in 10 ml dry THF with 2 equivalents TBAF (0.95 ml, 1.0 M in THF) was followed by immediate quenching with a saturated ammonium chloride solution. Normal workup followed by PTLC (11/9 ethyl acetate/hexanes; 3 runs) and recrystallization from CH_2Cl_2/hexanes afforded 2-methyl β-peltatin (cis) 40 (137 mg, 67%) as yellow prisms.



40

$C_{23}H_{24}O_8$

428.44

MP 127-130°C

^1H nmr δ (ppm) 1.237 (s, 3H), 2.67-3.11 (m, 3H), 3.785 (s, 6H), 3.815 (s, 3H), 4.080 (dd, J=2.8, 9.4 Hz, 1H), 4.195 (s, 1H), 4.522 (dd, J=6.7, 9.1 Hz, 1H), 5.299 (s, 1H), 5.846 (d, J=1.4 Hz, 1H), 5.870 (d, J=1.4 Hz, 1H), 6 314 (s, 1H), 6.362 (s, 2H).

M.S. m/e 428 (M^+)

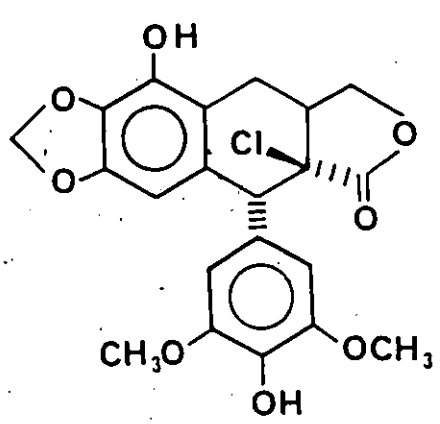
$[\alpha]_D$ ($CHCl_3$) = -113, c=0.992

Analysis Calcd. for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65.

Found : C, 61.01; H, 5.57.

Preparation of 2-chloro α-peltatin (trans) from 33

Successive treatment of a cooled solution (0°) of 33 (60 mg, 0.091 mmol) in 5 ml dry THF with 0.1 ml acetic acid and 4 equivalents of TBAF (0.35 ml, 1.0 M in THF) was followed by washing with saturated ammonium chloride and sodium bicarbonate solutions. Normal workup followed by PTLC (3/2 ethyl acetate/hexanes; 1 run) afforded 2-chloro α-peltatin (trans) 21 (38 mg, 96%) as pale yellow prisms.



21

$C_{21}H_{19}O_8Cl$

434.83

MP 208-210°C

^1H nmr δ (ppm) 2.740 (dd, J=11.0, 15.4 Hz, 1H), 2.84-3.06 (m, 1H), 3.157 (dd, J=5.4, 15.6 Hz, 1H), 3.784 (s, 6H), 4.191 (dd, J=8.5, 9.7 Hz, 1H), 4.409 (dd, J=6.8, 8.4 Hz, 1H), 4.753 (s, 1H), 5.930 (d, J=1.4 Hz, 1H), 5.957 (d, J=1.4 Hz, 1H), 6.216 (s, 1H), 6.405 (s, 2H).

M.S. m/e 434 (M^+), 398 (M^+ - HCl)

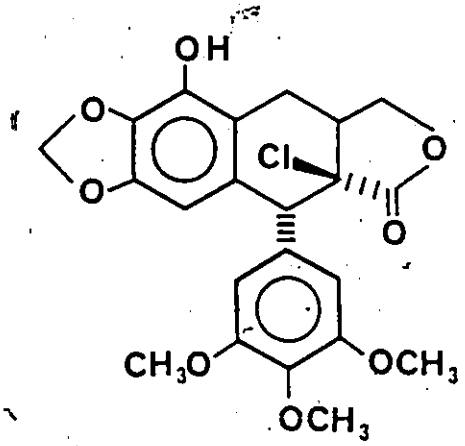
$[\alpha]_D$ (MeOH) = -136, c=0.966

Analysis Calcd. for $C_{21}H_{19}O_8Cl$: C, 58.01; H, 4.40.

Found : C, 57.70; H, 4.41.

Preparation of 2-chloro β-peltatin (trans) from 35

Successive treatment of a cooled solution (0°) of 35 (115 mg, 0.20 mmol) in 5 ml dry THF with 0.2 ml acetic acid and 2 equivalents of TBAF (0.42 ml, 1.0 M in THF) was followed by washing with saturated ammonium chloride and sodium bicarbonate solutions. Normal workup followed by PTLC (3/2 ethyl acetate/hexanes; 1 run) afforded 2-chloro β-peltatin (trans) 41 (84 mg, 91%) as pale yellow flakes.



41

$C_{22}H_{21}O_8Cl$

448.86

MP 182-184°C

^1H nmr δ (ppm) 2.745 (dd, J=11.1, 15.7 Hz, 1H); 2.85-3.05 (m, 1H), 3.162 (dd, J=5.3, 15.7 Hz, 1H), 3.756 (s, 6H), 3.807 (s, 3H), 4.201 (dd, J=8.7, 9.5 Hz, 1H), 4.429 (dd, J=6.7, 8.5 Hz, 1H), 4.765 (s, 1H), 5.926 (d, J=1.4 Hz, 1H), 5.953 (d, J=1.4 Hz, 1H), 6.220 (s, 1H), 6.398 (s, 2H).

M.S. m/e 448 (M^+)

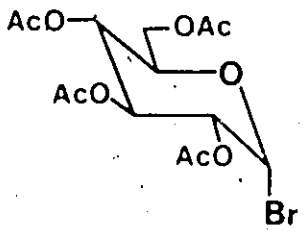
$[\alpha]_D$ (MeOH) = -129, c=1.01

Analysis Calcd. for $C_{22}H_{21}O_8Cl$: C, 58.87; H, 4.72.

Found : C, 56.85; H, 4.67.

Preparation of Tetra-O-acetyl-α-D-glucopyranosyl bromide

Perchloric acid (0.23 ml, 71-73%) was added dropwise to a cooled (0°), stirred solution of acetic anhydride (40 ml) and the resulting mixture was warmed to RT. Anhydrous D-glucose (10.0 g, 0.055 mol) was added at such a rate as to keep the reaction temperature between 30-40°. The solution was then cooled to 20° and red phosphorus (3.1 g, 0.10 mol) was added. Bromine (5.8 ml) followed by H_2O (3.6 ml) was added at such a rate as to keep the temperature below 20°. The reaction mixture was stirred for 2.5 h at RT after which time 30 ml $CHCl_3$ was added. The mixture was filtered, washed with water (0°) and extracted three times with $CHCl_3$. The organic extracts were then washed with water, saturated sodium bicarbonate and saturated sodium chloride solutions, dried over $MgSO_4$, filtered and the solvent evaporated to yield a yellow viscous oil. Crystallization was achieved by dissolving the oil in a small volume of $CHCl_3$ and adding an equal volume of petroleum ether. Recrystallization from ether afforded the glucosyl bromide 46 (5.31 g, 23%) as thin white needles.



46

$C_{14}H_{19}O_9Br$

411.20

MP 89.5-91°C (lit. MP 88-89°C)[41]

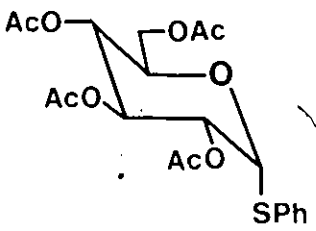
^1H nmr δ (ppm) 1.97 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 3.80-5.70 (m, 6H), 6.57 (d, J=4.0 Hz, 1H).

M.S. m/e 331 (M^+ - Br)

$[\alpha]_D$ ($CHCl_3$) = 193, c=2.00 (lit. $[\alpha]_D$ ($CHCl_3$)=198°, c=2)[41]

Preparation of the Phenyl Thioglycoside, 43

Thiophenol (0.14 ml, 1.36 mmol) was added to a stirred solution of powdered KOH (0.075 g, 1.34 mmol) and absolute methanol (10 ml) under a nitrogen atmosphere then a solution of 46 (0.502 g, 1.22 mmol) in 2 ml dry THF was added dropwise. The reaction mixture was stirred for 4 h then worked up as for 46 to afford 43 (277 mg, 51%) as a white powder after PTLC (1/2 ethyl acetate/hexanes; 4 runs).



43

$C_{20}H_{24}O_9S$

440.46

MP 97-100° (lit. MP 91-92°C)[50]

^1H nmr δ (ppm) 1.92-2.10 (m, 12H), 3.46-3.85 (m, 1H), 4.06-4.25 (m, 2H), 4.46-5.33 (m, 4H), 7.10-7.62 (m, 5H).

M.S. m/e 331 (M^+ - SPh)

Attempted Preparation of the Tetra-acetylated Glucoside of β-peltatin

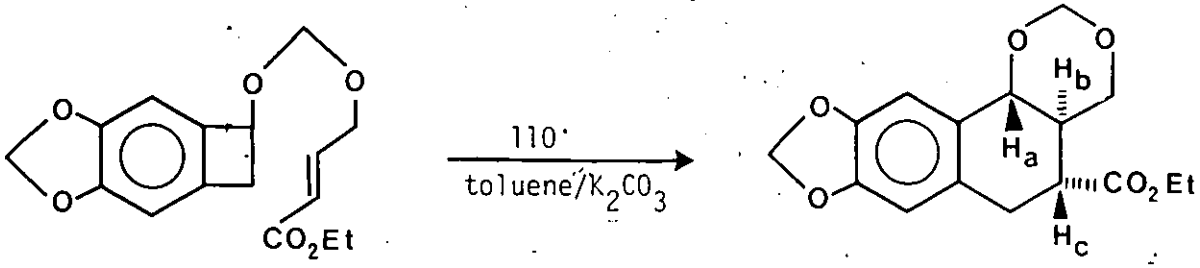
Compound 43 (257 mg, 0.58 mmol) and β-peltatin (302 mg, 0.73 mmol) were azeotropically dried three times with benzene then 5 ml CH_2Cl_2 and 156 mg pulverized 4-Å molecular sieves were added. The resulting mixture was stirred under N_2 for 0.5 h then recrystallized NBS (CH_2Cl_2/hexanes) (120 mg, 0.68 mmol) was added. The progress of the reaction was monitored by TLC. After 6.5 h when no further change was observed in the TLC, the reaction mixture was diluted with CH_2Cl_2, filtered and washed with a saturated sodium sulfite solution. Normal workup followed by PTLC (2/3 ethyl acetate/hexanes; 6 runs) afforded 3 major components. The most mobile component was identified as the starting phenyl thioglycoside 43. No attempt was made to identify the other two components as neither of the ^1H nmr spectra of these components indicated the presence of both the β-peltatin moiety and the acetylated glucose moiety.

ADDENDUM

Attempted Synthesis of Phenylsubstituted Benzocyclobutenols

Model studies of a potentially stereospecific synthesis of podophyllotoxin were reported by Glinski in 1982.[33] These studies were based on the thermolysis of the benzocyclobutenol derivative 47.

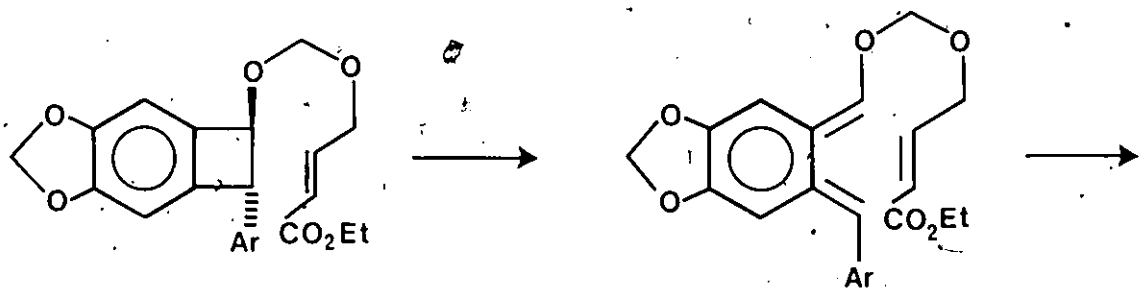
Thermolysis of 47 in refluxing toluene in the presence of K_2CO_3 gave the tetracyclic adduct 48 in 75% yield. The stereochemistry of 48 was assigned on the basis of the diaxial coupling constants $J_{H_a-H_b}$ (10.0 Hz) and $J_{H_b-H_c}$ (11.6 Hz).



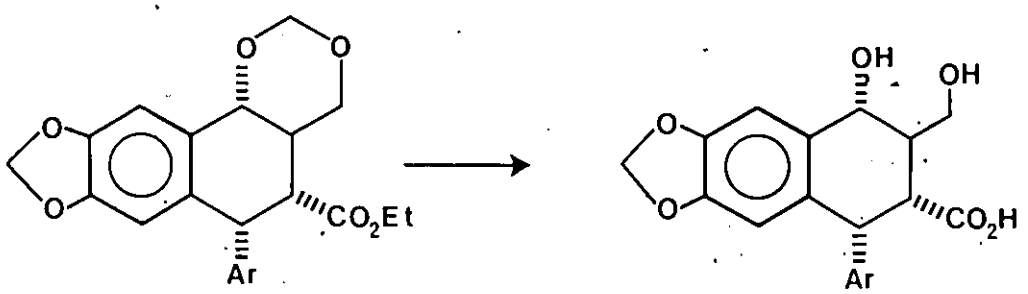
47 48

Based on the Woodward-Hoffman rules, thermal ring opening of the trans 1,2-substituted benzocyclobutene derivative 49 should occur in a conrotatory manner[42] to yield the orthoquinodimethane 50 which should then cyclize to form the pentacyclic adduct 51 (Scheme 6). Compound 51 has the correct relative stereochemistry for all of the chiral centres in podophyllotoxin 1. Conversion of 51 to 1 would

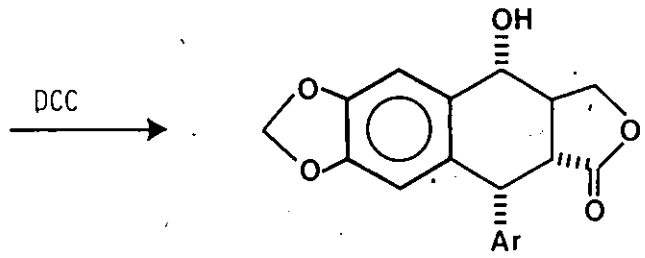
require saponification of the ester group and hydrolysis of the acetal function to form the hydroxy acid 52. In his synthesis of podophyllotoxin, Rodrigo has reported the conversion of 52 to 1 via DCC-promoted lactonization.[43]



49 50



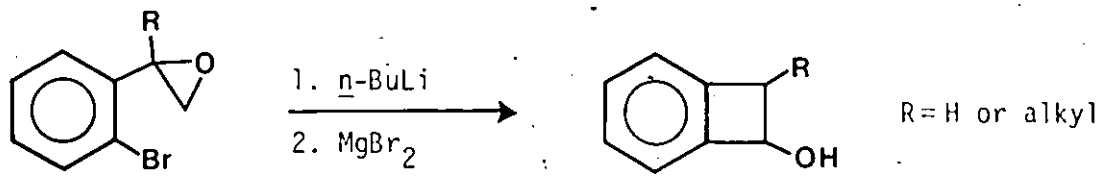
51 52

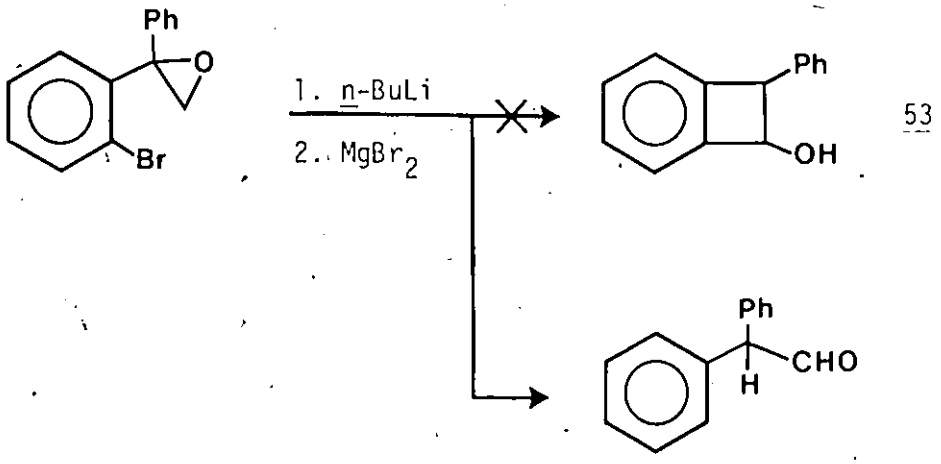


1

SCHEME 6

Unfortunately, Glinski was unable to complete the synthesis of podophyllotoxin based on this approach due to the unavailability of the precursor of 49, the 2-arylbenzocyclobutenol 53. It was found that the synthesis of benzocyclobutenols from o-halostyrene oxides developed by Durst et al [44] was not suitable for the preparation of compounds such as 53. In fact a careful search of the literature has failed to reveal any evidence that compounds such as 53 have been synthesized.

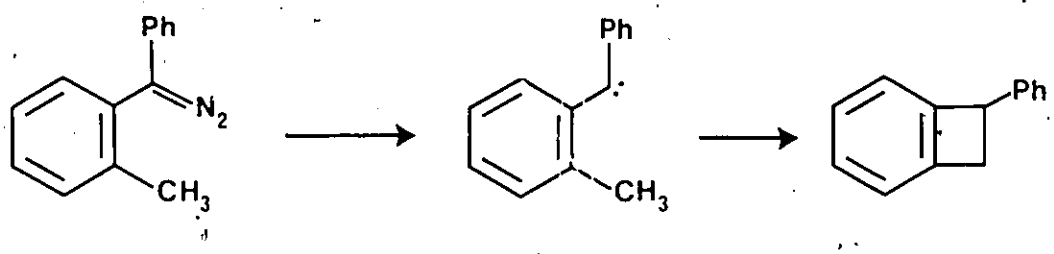




It was our intention to develop a new synthesis of compounds such as 53 and apply these to the synthesis of podophyllotoxin and its analogues.

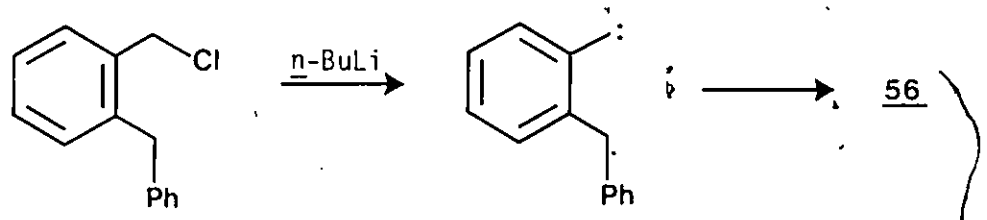
Our approach to the synthesis of 53 involved several attempts at intramolecular carbene C-H insertions.

1)



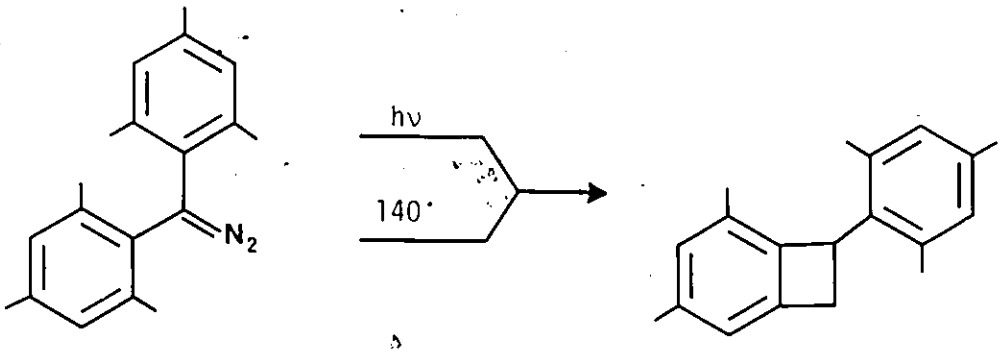
54 55 56

2)



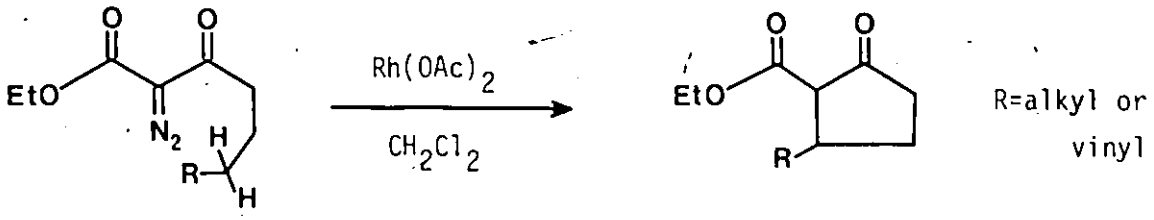
57 58

The possibility that 54 might be converted into 56 was suggested in a report by Zimmermann and Paskovich[45] in which they stated that photolysis or thermolysis of 59 afforded 60 in fair to good yield.



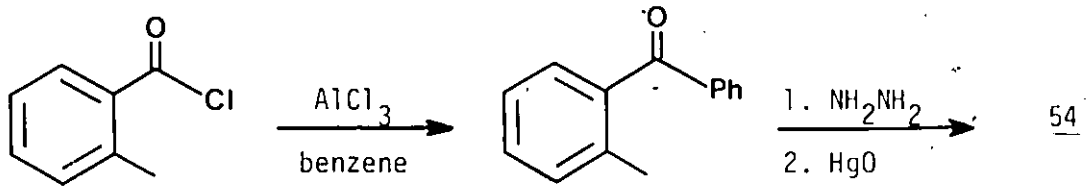
59 60 48% (hν)
78% (Δ)

In addition, Taber reported that when α-diazo β-keto esters such as 61 were treated with $Rh(OAc)_2$ in CH_2Cl_2, cyclopentanones such as 62 were obtained in 50-60% yield as a result of C-H insertion.[46]



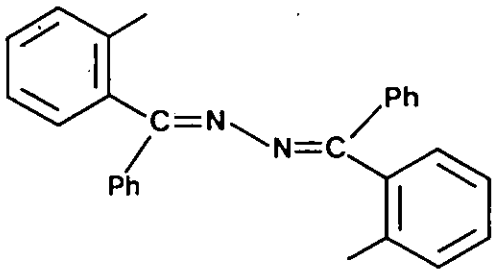
61 62 (∿ 50%)

The diazo compound 54 was prepared in routine fashion[47] as indicated below and was obtained as a dark red liquid after purification by chromatography on basic alumina using hexanes as eluant. The ^1H nmr showed a sharp singlet (3H) at 2.26 ppm in addition to a multiplet (9H) from 6.88-7.63 ppm.



Reaction of 54 with $Rh(OAc)_2$ in CH_2Cl_2 resulted in disappearance of the red colour of the starting material within 30 minutes and formation of a product with a m/e peak at 388. The ^1H nmr showed a sharp singlet (3H) at 2.25 ppm in addition to the aromatic multiplet (9H) from 6.78-7.63 ppm. These data are consistent with the formulation

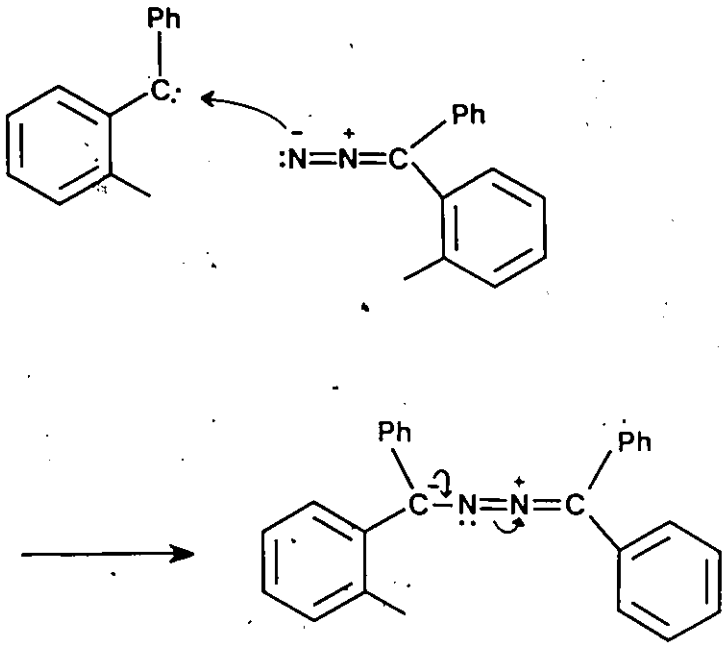
of this product as the substituted tetraphenylazine 63.



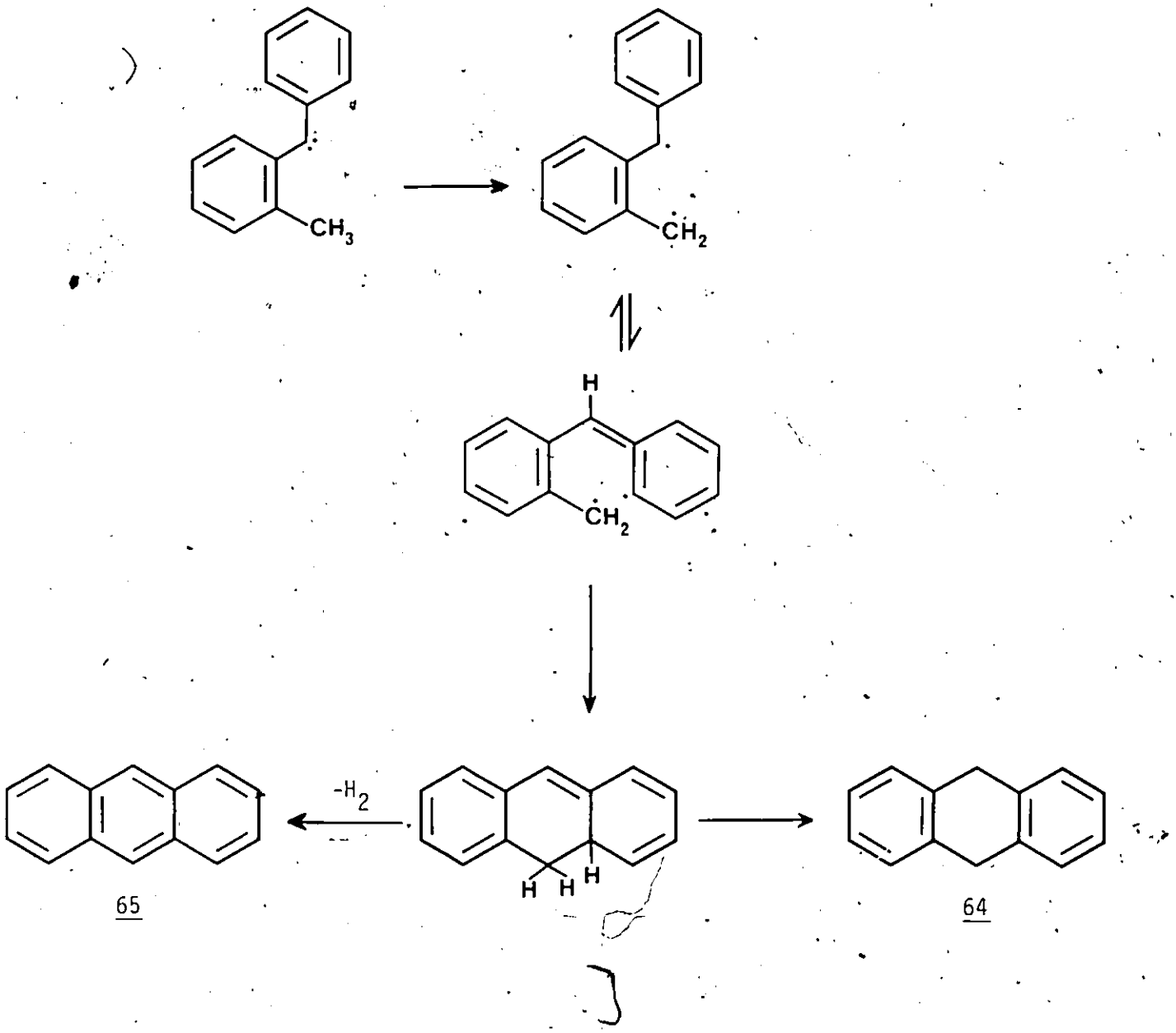
63

The exact stereochemical assignment of 63 is not known. However, the product appears to be a single stereoisomer due to the presence of only one sharp methyl singlet. The ^1H nmr spectrum of the crude reaction mixture indicated 3 sharp singlets at 1.93, 2.10 and 2.25 ppm in the ratio of 1:1:2, assignable to the methyl groups of the 3 possible stereoisomers of 63. The yield of 63 after purification by column chromatography on basic alumina (1/3 ethyl acetate/hexanes) was essentially quantitative indicating that isomerization to the most stable stereoisomer probably occurred on the alumina column.

It appears that the proposed carbene or a rhodium complex thereof, if formed, does not readily insert into a C-H bond of the o-methyl group but reacts instead with the starting diazo compound to furnish 63.

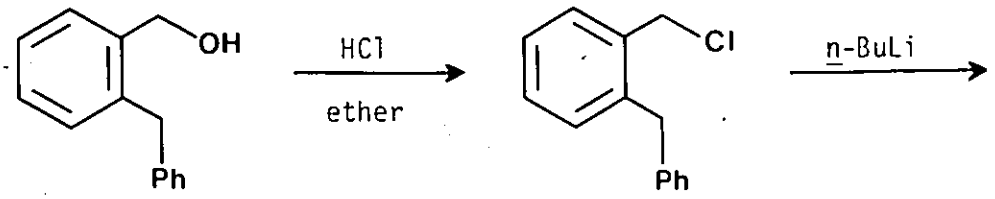


A flash vacuum thermolysis of 54 was also attempted. Compound 54 was volatilized at 90-100° then passed through a 10 cm tube at 450° under 0.3 mm Hg vacuum and trapped on a cold finger (-78°). Under these conditions some of the diazo compound decomposed prior to volatilization. Examination of this residue by ^{1}H nmr indicated the presence of essentially pure azine 63. The yellow material collected on the cold finger was shown to be a mixture of 9,10-dihydroanthracene 64 and anthracene 65 by comparison of the ^{1}H nmr with those of authentic compounds (Aldrich).[48] The ratio of 64 to 65 was approximately 1:1 based on the relative integrals of the singlet at 3.85 ppm due to the benzylic hydrogens of 64 versus the singlet at 8.37 ppm due to the C_9 and C_{10} hydrogens of 65. A plausible mechanism for the formation of 64 and 65 is shown in Scheme 7.

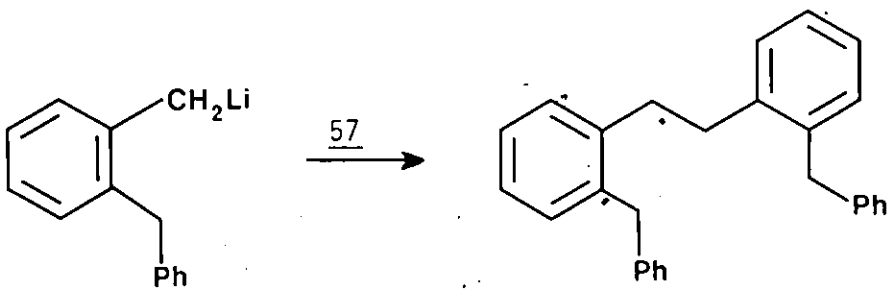


SCHEME 7

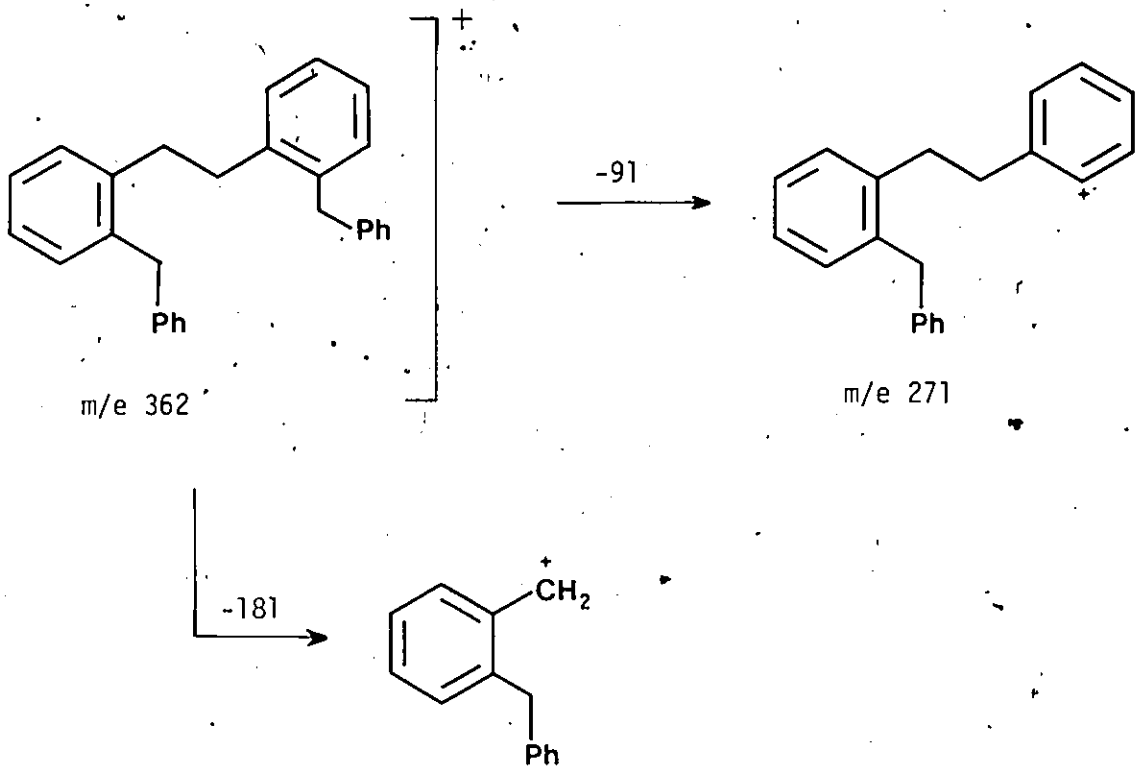
In a third attempt to produce 56, the benzyl chloride 57, prepared from the commercially available benzyl alcohol 66 upon treatment with HCl in ether, was reacted with n-BuLi in THF at 0° for 2 h. The ^{1}H nmr spectrum of the crude product showed two singlets at 3.94 and 2.76 ppm and n-butyl peaks, in addition to the aromatic protons. Part of the material crystallized from the crude reaction product. This crystalline material, obtained in about 50% yield based on the ^{1}H nmr, showed the above two singlets in addition to aromatic hydrogen absorption (relative area 2:2:9). The mass spectrum showed a strong molecular ion peak at m/e 362 consistent with the formula $C_{28}H_{16}$. The important fragmentation peaks occurred at m/e 271 and 181 (base peak). These data are consistent with the formulation of the product as 67. A reasonable mechanism for the formation of 67 is shown below.



66 57



68 67



m/e 362

m/e 271

m/e 181

Based on these results, it appears that n-BuLi was not a suitable base for the proposed α-elimination reaction. Instead halogen-metal exchange to form the benzylic carbanion 68 occurred preferentially. A better base would have been lithium tetramethyl piperidide [49] which has been shown to be an excellent reagent for carbene generation.

Preparation of 2-methyl Benzophenone Hydrazone

A solution of 2-methyl benzophenone (6.5 g, 0.033 mol) in 36 ml 99% ethanol was refluxed with 100% hydrazine (4.1, 0.13 mol) for 15.5 h then cooled on ice. Addition of distilled water (130 ml) followed by suction filtration afforded the desired hydrazone (6.2 g, 89%) as a pale yellow powder. The ^{1}H nmr showed a sharp singlet (3H) at 2.13 ppm, a broadened peak (2H) at 5.27 and a multiplet (9H) from 6.90-7.93 ppm.

Preparation of 54

The above hydrazone (4.3 g, 0.020 mol) and red HgO (4.5 g, 0.021 mol) were combined with 30 ml hexanes and stirred for 98 h. The red solution was filtered to remove any Hg, rotoevaporated to remove the solvent then placed in the refrigerator to allow any unreacted hydrazone to crystallize out. Half of the filtrate (i.e. 2 g) was purified by column chromatography on basic alumina using hexanes as the eluant to afford pure diazo compound 54 (0.96 g, 46%) as a dark red liquid. The ^{1}H nmr showed a sharp singlet (3H) at 2.26 ppm in addition to a multiplet (9H) from 6.88-7.63 ppm.

Reaction of 54 with $Rh(OAc)_2$

To a stirred solution of 54 (0.11 g, 0.53 mmol) in 10 ml CH_2Cl_2 was added 11.3 mg $Rh(OAc)_2$. After stirring for 0.5 h, the solution was filtered to remove any rhodium salts. The ^{1}H nmr of the crude product (0.10 g) showed three sharp singlets at 1.93, 2.10 and 2.25 ppm in the ratio of 1:1:2. Purification by column chromatography

on basic alumina (1/3 ethyl acetate/hexanes) afforded 63 (0.10 g, 49%) as a pale yellow solid (MP 141.5-144°C). The ^{1}H nmr of 63 showed a sharp singlet (3H) at 2.25 ppm in addition to the aromatic multiplet (9H) from 6.78-7.63 ppm. The mass spectrum indicated a strong molecular ion peak at m/e 388.

Pyrrolysis of 54

Compound 54 was volatilized at 90-100° then passed through a 10 cm tube at 450° under 0.3 mm Hg vacuum and trapped on a cold finger (-78°). The ^{1}H nmr of the residue (MP 127-136°C) remaining in the reaction flask was identical to that of 63 while the ^{1}H nmr of the yellow solid collected on the cold finger indicated a 1:1 mixture of 9,10-dihydroanthracene 64 and anthracene 65 (comparison with Aldrich spectra).

Preparation of 57

Concentrated HCl (10 ml) was added slowly to a solution of o-benzylbenzyl alcohol 66 (0.486 g, 2.46 mmol) in 10 ml ether. The resulting solution was stirred for 24 h then worked up in the usual way using ether. Purification by column chromatography on silica gel (1/7 ethyl acetate/hexanes) using 15 ml fractions afforded pure 57 (0.32 g, 60%) from fractions 2-4. The ^{1}H nmr showed sharp singlets at 4.20 (2H) and 4.58 (2H) in addition to the aromatic multiplet (9H) from 7.04-7.67 ppm.

Reaction of 57 with n-BuLi

Treatment of a cooled (0°) solution of 57 (0.362 g, 1.6 mmol) in

15 ml dry THF with 1.1 equivalents of n-BuLi (1.0 ml, 1.9 M in hexanes) was followed by stirring of the solution for 2 h. Normal workup afforded a pale yellow oil (264 mg) which crystallized on standing. The ^{1}H nmr of these white crystals showed two singlets at 2.76 and 3.94 ppm in addition to the aromatic signal from 6.86-7.50 ppm (relative area 2:2:9). The mass spectrum showed a strong molecular ion peak at m/e 362 as well as peaks at m/e 271 and 181 (base peak). The yield of the crystalline dimer 67 (MP 95-97°C) was estimated to be about 50-60% based on the ^{1}H nmr.

CLAIMS TO ORIGINAL RESEARCH

1. A simple method of isolating pure α- and β-peltatin (uncontaminated by the other peltatin) via their t-butyldimethyl silyl ethers was developed.
2. The synthesis of C_2-substituted peltatin derivatives from the TBDMS ethers of α- and β-peltatin was reported along with the biological test results of the 2-methyl derivatives against P388 leukemia.
3. The following is a list of the new compounds that were prepared and completely characterized including elemental analysis:
   1. silylated α-peltatin (trans), 23
   2. silylated β-peltatin (trans), 24
   3. silylated α-peltatin (cis), 26
   4. silylated β-peltatin (cis), 28
   5. 2-methyl silylated α-peltatin (trans), 29
   6. 2-methyl silylated α-peltatin (cis), 30
   7. 2-methyl silylated β-peltatin (trans), 31
   8. 2-methyl silylated β-peltatin (cis), 32
   9. 2-chloro silylated α-peltatin (trans), 33
   10. 2-chloro silylated α-peltatin (cis), 34
   11. 2-chloro silylated β-peltatin (trans), 35
   12. 2-chloro silylated β-peltatin (cis), 36
   13. 2-methyl α-peltatin (trans), 37
   14. 2-methyl α-peltatin (cis), 38

15. 2-methyl β-peltatin (trans), 39
16. 2-methyl β-peltatin (cis), 40
17. 2-chloro α-peltatin (trans), 21
18. 2-chloro β-peltatin (trans), 41

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