

STUDIES ON THE BIOSYNTHESIS OF PODOPHYLLOTOXIN:

Synthesis of Labelled Yatein and Matairesinol,

Two Potential Precursors of Podophyllotoxin

by

Kurt Alan Neidigh

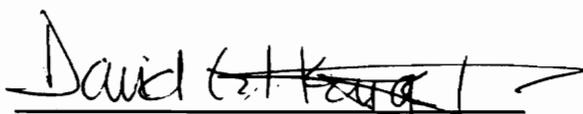
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(ABSTRACT)

Podophyllotoxin, a naturally occurring lignan isolated from several species of *Podophyllum*, is used as a precursor to the clinical chemotherapeutic agents teniposide and etoposide. The biosynthesis of podophyllotoxin is not fully understood, but its optical activity, like that of most lignans, is suggestive of enzyme-mediated processes.

It has been proposed that the formation of podophyllotoxin begins with stereo-controlled coupling of a hydroxy cinnamyl alcohol derivative and a substituted hydroxy cinnamic acid, although no "coupling" enzyme has been isolated to date. Further biosynthetic modifications of the coupled compound could lead to matairesinol and/or yatein, which have been proposed as potential biological precursors of podophyllotoxin. Although no firm evidence has been obtained to date, conversion of matairesinol to yatein has been postulated. This conversion would, however, involve biosynthetic steps which, though common for hydroxycinnamates, are unprecedented at the dimeric level. Conversion of yatein to podophyllotoxin has been demonstrated, with the conversion involving a stereo-controlled cyclization and subsequent stereospecific hydroxylation.

In order to investigate the biosynthesis of podophyllotoxin, leading from the postulated precursors matairesinol and yatein, a series of stereospecific deuterium-labelled

matairesinol and yatein derivatives was proposed and the synthetic methodology for each compound developed. The methodology used to obtain deuterium-labelled compounds can be extended to generating tritium-labelled compounds as well.

With sufficient quantities of a number of the deuterium-labelled compounds, feeding studies can now be carried out in *Podophyllum* plants. Isolation and analysis of podophyllotoxin, from plants fed with labelled yatein, will allow determination of the stereochemical nature of yatein cyclization. Isolation and analysis of yatein, from plants fed with labelled matairesinol, will indicate whether matairesinol is indeed a precursor to yatein (and, hence, podophyllotoxin). The information obtained from the synthesis and incorporation of such labelled compounds should then provide a clearer understanding of some interesting but, as yet, unestablished biotransformations.

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I. INTRODUCTION

I.1 Historical

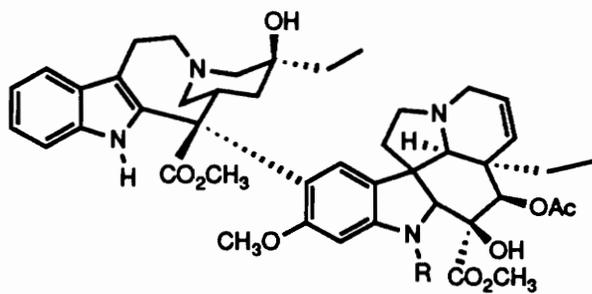
Driven by hunger, disease, or climatic changes, prehistoric people tended to be nomadic, and during the course of their wanderings, these people probably ingested nearly every form of vegetation they found. Undoubtedly, they learned that certain plants provided nourishment, while some, instead of being nourishing, produced sickness, pain, or even death. They also found that other plants gave strength and alleviated disease or illness. Gradually, people learned not only to cultivate plants for food, but also for use as poisons, as well as for the prevention and treatment of a wide variety of ailments.¹

Until fairly recently, plant materials or plant extracts -- folk remedies, as they are commonly known -- were used with little understanding of the basis for their action. It has only been within the last three centuries that serious scientific study of natural products has been carried out. With the birth of pharmacognosy (the branch of pharmacy dealing with biologically active compounds from plant sources) in the early 1800s, and the advances in organic chemistry between the mid-1700s and the turn of this century, it was discovered that the activity of traditional medicinally useful plants could be attributed most often to a single compound or series of related compounds.¹ Instead of crude plant preparations, the purified active compound(s) gradually became more popular, and presently purified compounds are nearly exclusively used in the treatment of illnesses.

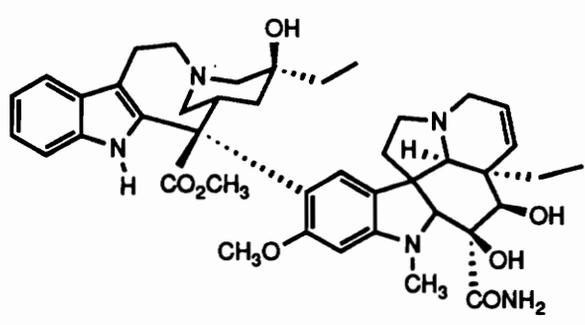
One of the most active areas of research during the past 50 years has been that of cancer chemotherapy, involving either the synthesis of potential chemotherapeutic agents, the isolation of similarly active compounds from natural sources, or a combination of both, i.e. utilizing plant-derived products as starting materials in the semi-synthesis of potential agents.² Because prospective anticancer drugs may exhibit undesirable side effects, they

must pass through a rigorous testing period. Out of thousands of test compounds only one may be found to satisfy the strict testing criteria and be allowed to be marketed as a clinically useful anticancer agent. Thus, of the large number of potential compounds isolated from plant sources and shown to have promising activities in tissue and animal trials, only a relative few have proven to be clinically useful and have become established chemotherapeutic agents. Among the most successful compounds used to date are the Vinca alkaloids obtained from *Catharanthus roseus*. Vincristine (1) has particular effectiveness in the treatment of childhood leukemia, and in allowing short-term remission of reticulum-cell sarcoma and Hodgkin's disease.^{2,3} Vinblastine (2) is useful in the treatment of generalized Hodgkin's disease, lymphocytic lymphoma, advanced testicular carcinoma, and Kaposi's sarcoma.³ Vindesine (3), a semi-synthetic derivative of vinblastine, has also shown promise as a chemotherapeutic agent, especially for use in patients who have become resistant to vincristine (1) and vinblastine (2).³

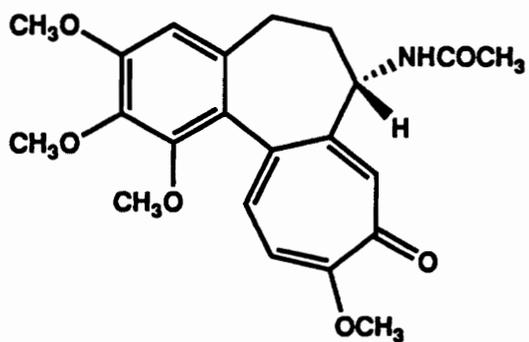
Another class of plant natural products, the lignans, has shown high potential as a source of anticancer agents as well. Such naturally occurring lignans as colchicine (4), isolated from *Colchicum autumnale*, and podophyllotoxin (5) and its derivatives, from several species of *Podophyllum*, have been found to exhibit profound cytotoxic properties. Two semi-synthetic derivatives of podophyllotoxin, teniposide (6) and etoposide (7), are currently the preferred chemotherapeutic agents in the treatment of small cell lung carcinoma, and have shown activity against Hodgkin's disease and other malignant lymphomas, pediatric leukemias, and a variety of other types of cancer.



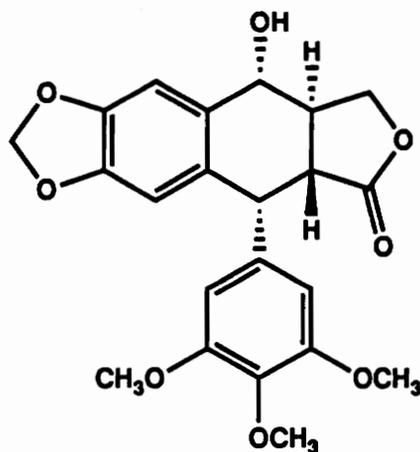
R = CHO: vincristine 1
 R = CH₃: vinblastine 2



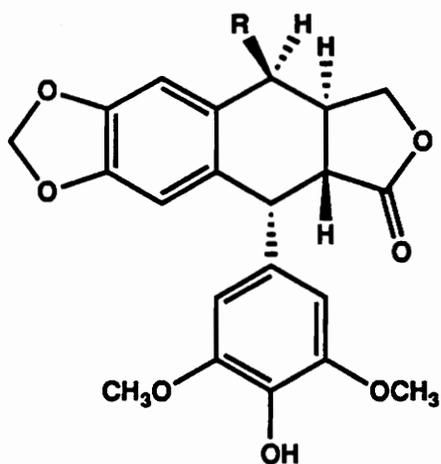
vindesine 3



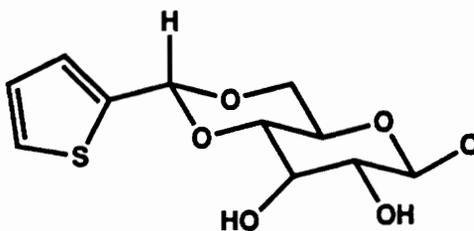
colchicine 4



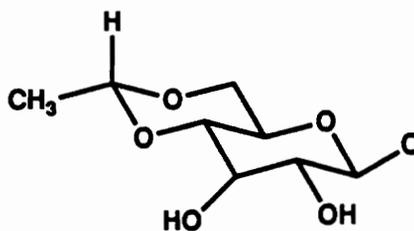
podophyllotoxin 5



teniposide 6: R =

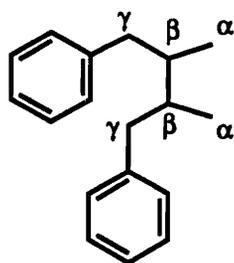


etoposide 7: R =

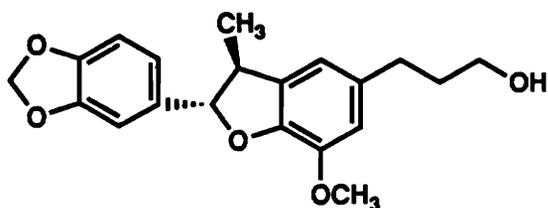


I.2 Lignans

Lignans are a class of natural products which are widely distributed among the higher species of the plant kingdom. By definition, lignans are composed of two phenylpropanoid units, stereospecifically linked at the side chain β -carbon atoms (8).⁴ Phenylpropanoid natural products with dimeric linkages other than the β - β type comprise the class of compounds known as neolignans (see 9, for example).



8



phyllnirurin 9: a neolignan

It has been shown that lignans and neolignans are produced by the coupling of *para*-hydroxy phenylpropane monomers, e.g. *para*-hydroxycinnamyl alcohol derivatives, through a phenolic oxidative coupling mechanism (Figure 1).⁵ Because lignans and neolignans are generally optically active, it has been suggested that their biosynthesis is enzyme-mediated,⁶ although to date, no coupling enzyme has been isolated.

Structurally, the lignans are quite varied, and can be classified into several main groups, all having the same carbon skeleton (8), but differing in the chemical bonding patterns of the phenylpropanoid monomers, the number and nature of aromatic substituents, and in oxidation state. These groups include diarylbutanes, e.g. isolariciresinol (10) and phyllanthin (11); tetrahydrofuran derivatives, e.g. liovil (12) and lariciresinol (13); bistetrahydrofurans, e.g. epipinoresinol (14) and phillyrin (15);

diarylbutyrolactones, e.g. matairesinol (16), arctiin (17), and yatein (18); aryltetralines, e.g. podophyllotoxin (5); and cyclooctadienes, e.g. steganone (19) (Figure 2).

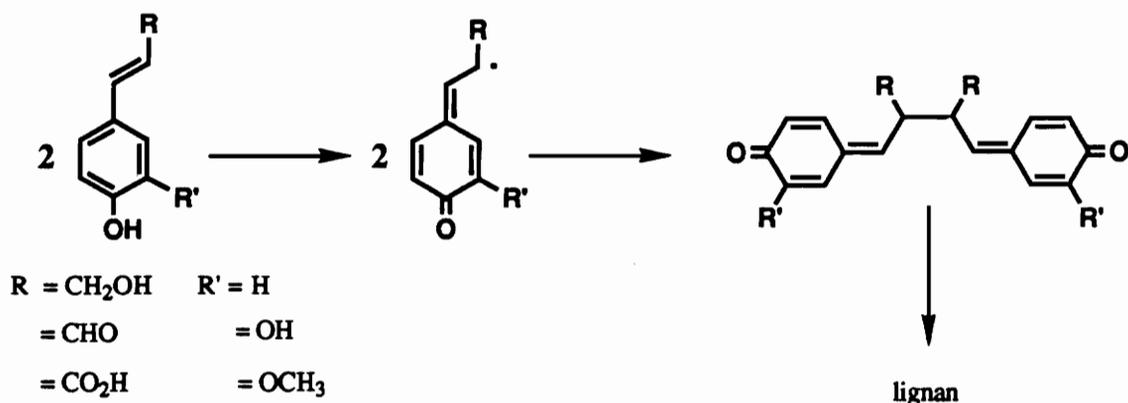
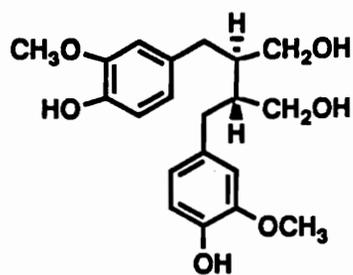
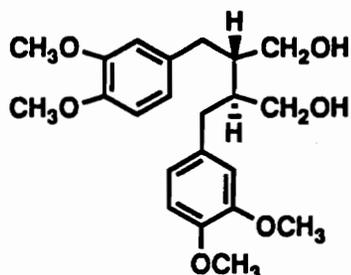


Figure 1: Generalized phenolic oxidative coupling scheme

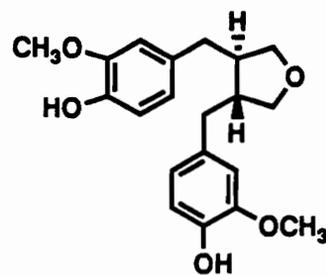
Lignans have been isolated from the bark, leaves, stems, roots, and rhizomes of many plants. By 1978, more than 200 of these natural products had been isolated, characterized, and their syntheses developed, and since that time many more have been discovered.⁷ While their exact function in plants is not fully understood, it has been proposed that plants accumulate lignans because of their ability to serve as antibacterial and antifungal agents, as well as insect antagonists. Their high capacity as chemical deterrents is due to the fact that lignans often exhibit a range of physiological effects, including antiviral, antimutagenic, and cytotoxic activities.⁸ In light of their biological activity, it is not surprising that folk medicines employing crude lignan preparations have been known and used for thousands of years by different cultures, for the treatment of a wide variety of ailments. In fact, the plant *Phyllanthus niruri* L. is well known for its liver-protecting properties, and has been used since ancient times in India. Modern analysis and testing of



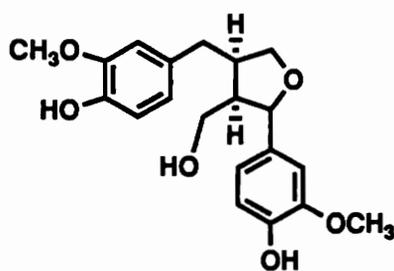
isolariciresinol 10



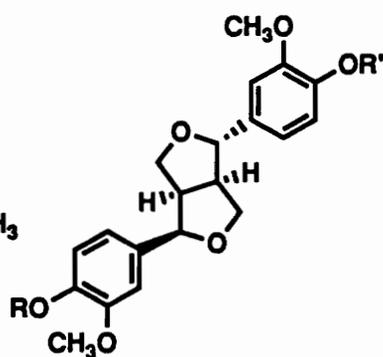
phyllanthin 11



liovil 12

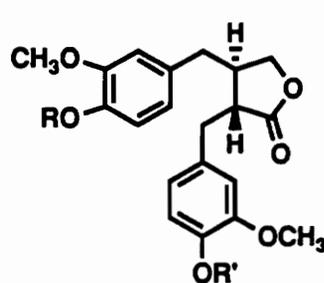


lariciresinol 13



epipinoresinol 14: R = R' = H

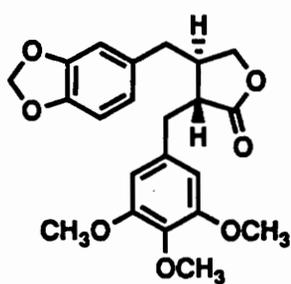
phillyrin 15: R = CH₃; R' = glucosyl



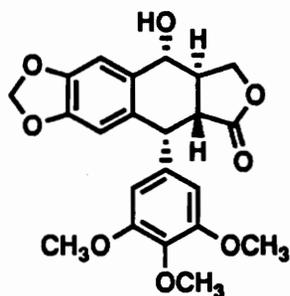
matairesinol 16: R = R' = H

arctiin 17: R = CH₃;

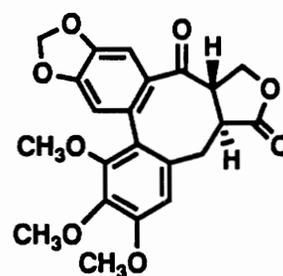
R' = glucosyl



yatein 18



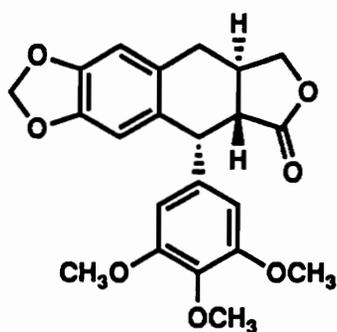
podophyllotoxin 5



steganone 19

Figure 2: General lignan types

the plant's chemical constituents shows that the antihepatotoxic activity of the plant can be attributed, in large part, to lignans.⁹ The meadow saffron, *Colchicum autumnale*, is rich in colchicine (4), which, long before knowledge of its antitumor activity, was the drug of choice in relieving acute gout, with its use dating back to the time of Alexander of Thalles, circa 550 A.D.¹⁰ An early English medical text from circa 900 to 950 A.D., the *Leech Book of BALD*, records the use of wild chervil root in the treatment of cancer (chervil contains desoxypodophyllotoxin (20), a compound now known to display antitumor activity).¹¹



desoxypodophyllotoxin 20

I.3 *Podophyllum* Lignans

When European settlers first arrived in North America, they found that the native people employed a variety of plant-derived remedies in the treatment of many types of diseases and illnesses.¹ Because supplies of medicine were often difficult to obtain from Europe, the settlers began relying on these native remedies to treat their own ailments. Several of these remedies, the conditions warranting their use, and the more recently determined active component(s) or more current uses, are given in **Table 1**.

One of the native North American remedies was derived from the roots and rhizomes of the plant *Podophyllum peltatum*, which is closely related to the species *P. hexandrum*, common to the Himalayan region of Asia, and also used by the natives of that region.^{12,13} *P. peltatum* is an herbaceous perennial known commonly as the "May apple" or "American mandrake", and is indigenous to the United States and Canada, growing in the oak-hickory forests of eastern North America from Quebec to Florida, and westward to Minnesota and Texas. *P. peltatum* plants typically appear in mid-April and grow through early October, and are characterized by a pair of multidentate leaves, a solitary white flower which appears in May, and a single fleshy, apple-like fruit containing a large number of seeds, maturing in July. The American Indians knew well the properties of the dried roots and rhizomes as a purgative (laxative), emetic (vomiting agent), anthelmintic (intestinal worm preventive), and as a deadly poison. This knowledge was passed on to the early settlers, who used the material extensively as a purgative, emetic, and cholagogue (an agent which enhances the flow of bile to the intestine). The alcohol extract of the roots and rhizomes, known as podophyllin, in addition to the properties mentioned above, was also found to be a good topical agent for treating fungal growths, and in the mid 1800s, it was

even suggested that its topical efficacy might prove useful in the treatment of cancerous growths.¹³

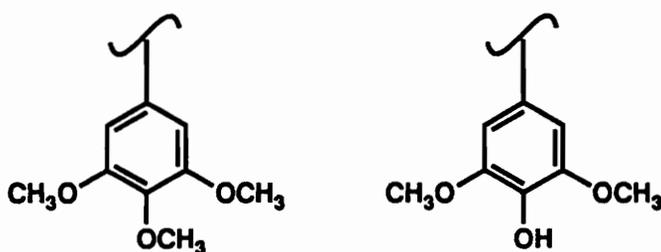
With the advent of pharmacognosy and the development of synthetic organic techniques came the knowledge that the activity of medicinally useful plants could be attributed to one compound or a series of related compounds, and that these active compounds could be synthesized and used instead of crude plant preparations. Thus, in general, "synthetic" medicines gradually grew in popularity, and by about 1900 they had virtually replaced traditional folk remedies.¹ The use of podophyllin, in particular, likewise declined, in response to other milder and more effective laxative and emetic agents on the market. However, in 1942, Kaplan¹⁴ reported that the topical application of podophyllin in the treatment of condyloma acuminatum, a type of venereal wart, produced very satisfactory clinical results. Several years later, King and Sullivan¹⁵ reported that podophyllin produced pronounced cytological changes in normal rabbit and human skin; and the following year, Belkin¹⁶ and Hartwell and Shear¹⁷ published the first reports that podophyllin exerted a strong destructive effect on experimental animal cancer cells. These reports initiated a renewed medical interest in podophyllin because of its apparent antimetabolic activity, and during the next few years, it became well established that podophyllin indeed caused mitotic arrest and cell damage.¹³ Investigations of the chemical composition of podophyllin during the late 1940s and early 1950s showed the compound podophyllotoxin (5) to be the main active constituent, with several other related aryltetralin compounds and their glycosides showing varying cytotoxic activities.¹⁸⁻²⁴ These *Podophyllum* lignans are all characterized by a central tetrahydronaphthalene moiety, with an aromatic methylenedioxy group, a highly strained trans- γ -butyrolactone system, and a pendant tri-substituted aromatic ring. They can be classified into two primary groups: compounds having a trimethoxy substitution pattern on the pendant aromatic ring,

Table 1: Native American remedies*

<u>Plant or plant material</u>	<u>Ailment or condition</u>	<u>Active compound(s) or current therapeutic usage</u>
Strips of willow bark or willow roots	Fever, headache; sore throat	Salicylic acid (the "parent" compound of acetyl salicylic acid, the active compound in aspirin)
Stoneseed (a common weed)	Birth control	Estrogens (hormonal components found in modern female contraceptives)
Jimsonweed	Asthma; cholera; epilepsy	Atropine (vasodilator, anti-spasmodic, anesthetic, and mydriatic)
Cherry tree root Blackberry root Horsetail weed	Diarrhea	Antidiarrheal
Swamp root Juniper tree twigs	Upset stomach	Diuretic

*Adapted from reference 1.

e.g. podophyllotoxin (5) and its derivatives -- this group generally predominates in *Podophyllum* species; and compounds having a dimethoxyhydroxy substitution pattern, e.g. the 4'-demethyl series.



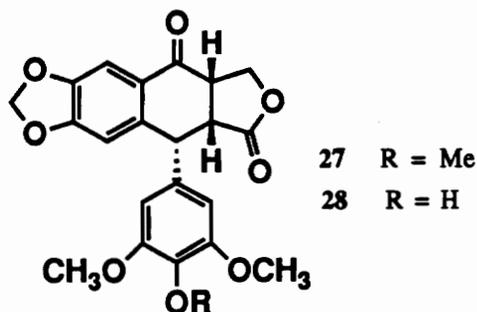
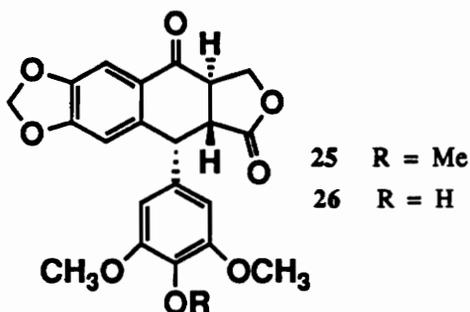
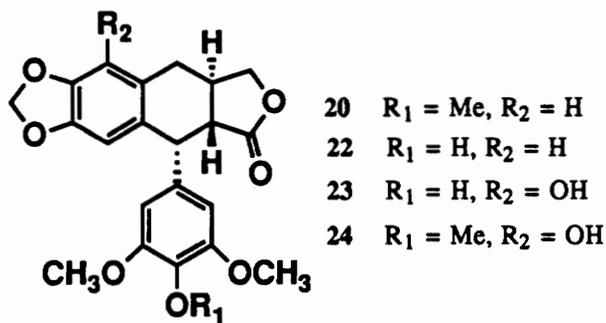
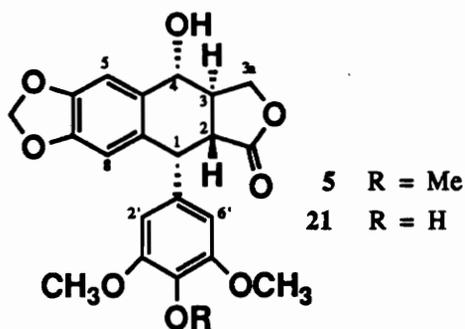
Substitution pattern of pendant aromatic rings, giving rise to classification of *Podophyllum* aryltetralin lignans

The lignan content of the roots/rhizomes of *P. peltatum*, and the related Asian species *P. hexandrum* and *P. pleianthum*, is summarized in Table 2. As the data indicate, there is a significant difference in the relative proportions of the lignans within the three species. All three *Podophyllum* species produce the same lignans, however *P. peltatum* contains roughly the same proportions of podophyllotoxin (5), α -peltatin (23), and β -peltatin (24), while the Asian species contain very low or negligible levels of 23 and 24 but relatively high levels of podophyllotoxin (5) and 4'-demethylpodophyllotoxin (21).²⁵

Table 2: Aryltetralin lignan content of *Podophyllum* species*

Lignan	mg / 10g dried root/rhizome material		
	<i>P. peltatum</i>	<i>P. hexandrum</i>	<i>P. pleianthum</i>
Podophyllotoxin 5	7.5	128	13.5
Desoxypodophyllotoxin 20	0.7	0.5	1.0
4'-Demethylpodophyllotoxin 21	0.2	13.5	4.1
4'-Demethyl-desoxy-podophyllotoxin 22	0.2	0.3	0.3
α -Peltatin 23	7.5	0.2	----
β -Peltatin 24	10.0	0.3	----
Podophyllotoxone 25	0.6	1.7	3.7
4'-Demethylpodophyllotoxone 26	0.2	0.4	1.3
Isopicropodophyllone 27	0.2	1.0	2.0
4'-Demethylisopicropodophyllone 28	0.1	0.2	0.5

*Data from reference 25.



I.4 Podophyllotoxin

I.4.i Mode of Action

It has been shown^{26,27,28} that podophyllotoxin (5) acts as a classic mitotic spindle poison, producing metaphase arrest during cell division (Figure 3). Specifically, podophyllotoxin (5) "binds competitively to the microtubule protein, tubulin, and interferes with the assembly of microtubules in the mitotic apparatus to arrest cells in metaphase."²⁶

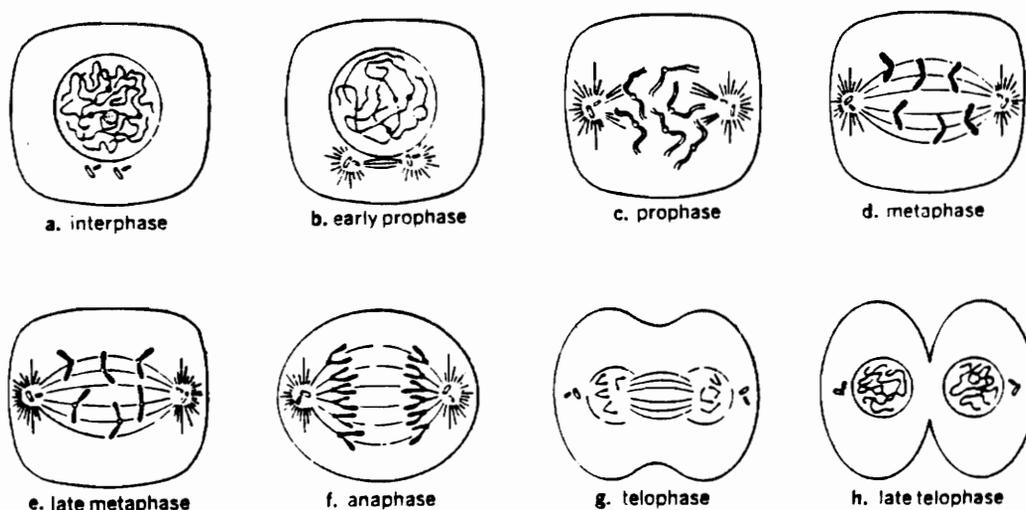


Figure 3: The stages of cell division

Tubulin has a molecular weight of approximately 110,000 daltons, consisting of two subunits, designated α - and β -tubulin, of roughly 55,000 daltons, which can assemble in a very intricate fashion, under proper conditions, to form microtubules. Microtubules are long, cylindrical "polymers" about 240 Angstroms in diameter, and have been implicated in a number of cellular processes, such as motility, mitosis, and movement of surface receptors. It has been shown²⁹ that microtubules at steady state "preferentially

assemble tubulin at one end of the microtubule and preferentially disassemble tubulin at the opposite end" ¹¹ -- a process commonly referred to as "treadmilling" (see Figure 4).

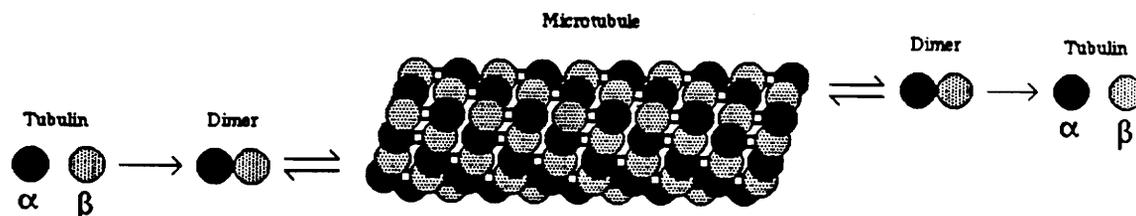


Figure 4: Schematic of microtubule assembly-disassembly equilibrium

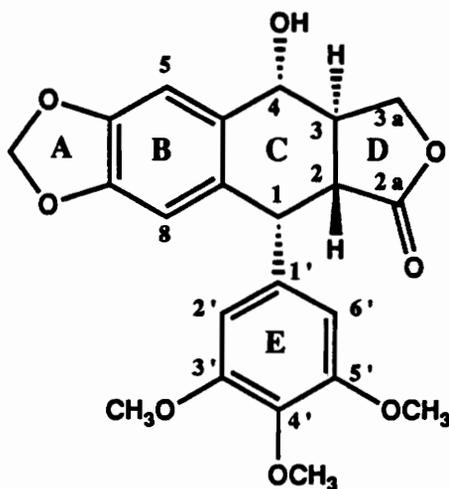
Podophyllotoxin (5) inhibits microtubule formation by adding to the tubulin dimer, preventing further assembly into microtubules, but it does not inhibit the disassembly process occurring at the opposite end.¹¹ In this regard, podophyllotoxin (5) is similar in its mode of action to the Vinca alkaloids, vincristine (1) and vinblastine (2), as well as colchicine (4). Indeed it has been found that podophyllotoxin and colchicine have nearly the same binding site on tubulin.^{30,31} The presence of a trimethoxyphenyl group in both compounds suggests a common role in tubulin binding, however podophyllotoxin (5) binds about ten times as rapidly as colchicine (4). Unlike colchicine (4), which does not bind appreciably to tubulin at 0° C, podophyllotoxin (5) binds readily at this temperature, and its binding is known to be freely reversible.¹⁰

I.4.ii Teniposide and Etoposide

Despite its initial promise as a potent chemotherapeutic agent, due to its high antimetabolic activity, podophyllotoxin (5) was found during clinical studies to have severe toxic side effects, virtually eliminating its usefulness in treating human neoplasms. In an attempt to produce less toxic but equally (or more) effective podophyllotoxin analogs, the design and synthesis of new derivatives was initiated in the 1960s. In the early 1970s, Stahelin^{32,33} reported that two semi-synthetic derivatives of podophyllotoxin, VM26 and VP16-213, known as teniposide (6) and etoposide (7), respectively, showed potential antitumor activity, with significantly less severe side effects. These compounds both differ from podophyllotoxin in having a 4'-hydroxy group rather than a 4'-methoxy group on the pendant aromatic ring; and having, in the case of teniposide (6), a β -linkage to the pendant O-thenylidene- β -D-glucopyranoside; and, in the case of etoposide (7), a β -linkage to the pendant O-ethylidene- β -D-glucopyranoside. Both compounds passed through all stages of clinical trials in the 1970s and 1980s, and have since been marketed for use as chemotherapeutic agents. Etoposide, marketed under the name Vepesid, has been reported to be the more potent agent, however both drugs appear to be effective in the treatment of several types of cancer (Table 3).³⁴

Unlike podophyllotoxin (5), teniposide (6) and etoposide (7) show no effect on the assembly of microtubules, indicating their mode of action is different from that of podophyllotoxin (5).¹¹ Experiments have indicated that the C and D rings of podophyllotoxin (5) are necessary for interaction with tubulin,¹¹ and further, that the highly strained trans-fused butyrolactone system is essential for its antitumor activity -- activity is negligible in the cis-fused lactone, picropodophyllotoxin (29).² Additionally, in experiments with 4'-demethyl podophyllotoxin derivatives, namely, 4'-

demethylpodophyllotoxin (21), 4'-demethyl-desoxypodophyllotoxin (22), and α -peltatin (23), it was found that these compounds differ from podophyllotoxin (5) in their mode of action. Instead of binding to tubulin, these compounds exhibit their cytotoxic activity by



podophyllotoxin 5

inhibiting the enzyme DNA topoisomerase II,¹¹ which is responsible for repairing DNA strand breakage and "knotting". Similarly, teniposide (6) and etoposide (7) have been shown to exert their cytotoxic activities by inhibition of DNA topoisomerase II, inducing single- and double-strand breaks in DNA.³⁶ Their mode of action can be partially explained by their structural similarity to the 4'-demethyl series, and partially by the fact that NMR analysis has shown that the glucopyranoside moiety occupies a position over the lactone ring, and could be responsible for sterically blocking any interaction of the drug with tubulin.¹¹ The question of whether cells could metabolize teniposide (6) or etoposide (7) to the corresponding aglycone, i.e. 4'-demethyl epipodophyllotoxin (30), which could then act as a microtubule poison or as a DNA topoisomerase II inhibitor, was answered in human metabolism studies^{37,38} using etoposide (7), in which it was shown that the

Table 3: Clinical effectiveness of Etoposide and Teniposide*

Etoposide (VP16-213)

is one of the most active single agents in small cell lung cancer, with a 40% single agent response rate in clinical trials, and a 6% complete response rate

is active against Hodgkin's disease and other malignant lymphomas

has some activity against some types of acute adult leukemia, with good response rates

is useful in the treatment of pediatric leukemias

has some effect on breast cancer, with a 17% partial response rate for heavily pretreated patients

has shown good activity against testicular cancer, with up to 46% response rate

is fairly active against Kaposi's sarcoma, associated with AIDS -- in fact, the most active single agent tested to date

Teniposide (VM26)

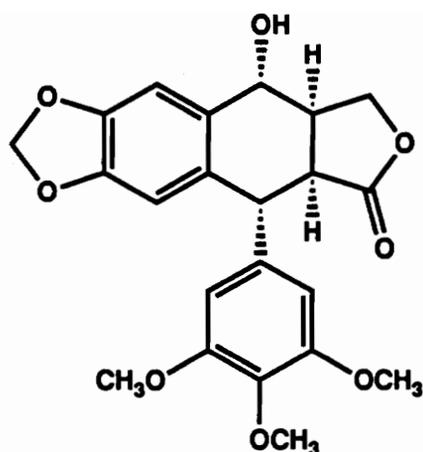
is similar to etoposide in its efficacy and has had a 28% response rate in clinical trials, and an 8% complete response rate in one trial

is also active against Hodgkin's disease and other malignant lymphomas

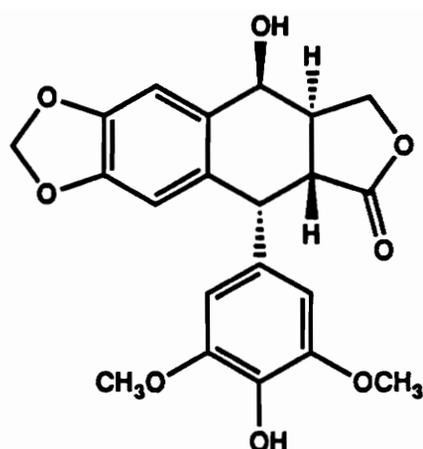
is also useful in the treatment of pediatric leukemias

has some activity in brain tumors, with responses in up to 35% of cases

*Adapted from references 34 and 35.



picropodophyllotoxin **29**



4'-demethylepipodophyllotoxin **30**

glucoside moiety is in fact retained. Apparently, it is the glucoside moiety which accounts for the less severe side effects of teniposide and etoposide, compared to their non-glucoside derivative, 4'-demethylepipodophyllotoxin (**30**). The relative antimitotic activities of podophyllotoxin derivatives and podophyllotoxin glucosides are given in **Tables 4** and **5**.^{39,40,41}

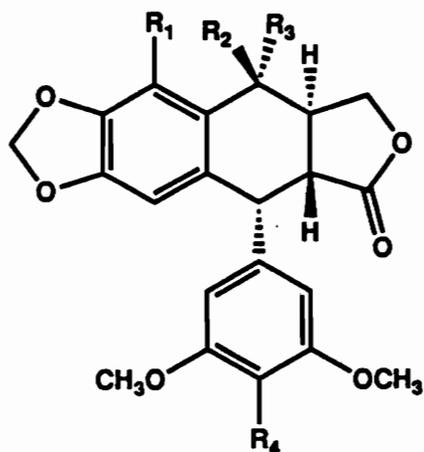
Table 4: Relative effectiveness of podophyllotoxin analogs in microtubule polymerization processes*

Compound	ED ₅₀ : inhibition of mastocytoma growth <i>in vitro</i> (μmol)	Increase in survival time (%)	Minimum dose damaging mouse sarcoma <i>in vivo</i> ($\mu\text{mol} / \text{g body wt.}$)
Podophyllotoxin 5	0.012	35	0.005
Desoxypodophyllotoxin 20	0.005		0.015
4'-Demethyl- podophyllotoxin 21	0.018	10	0.021
β -Peltatin 24	0.002		0.005
Picropodophyllotoxin 29	0.435		>1.21
Epipodophyllotoxin 30	0.082	11	0.145
Podophyllotoxin-4,6-O- Benzylidene- β -D-glucoside 46	5.720	5	-----
Podophyllotoxin- β -D- glucopyranoside 47	27.800	0	0.176
β -Peltatin- β -D- glucopyranoside 48	1.740		0.26
Colchicine 4 ⁺	0.018		0.004

*From references 39 and 40.

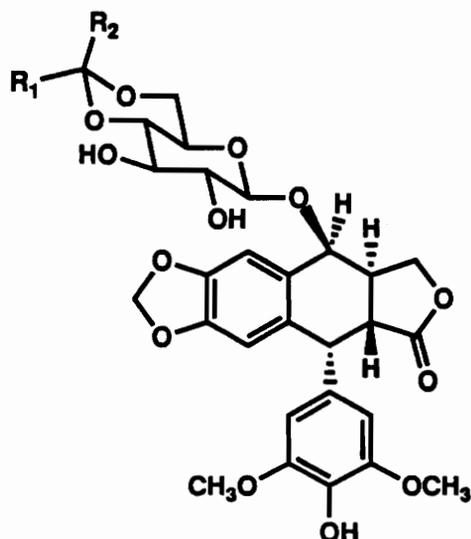
+Colchicine for comparison

Table 4 (Continued) *



Compound	R ₁	R ₂	R ₃	R ₄
Podophyllotoxin 5	H	H	OH	OCH ₃
β-Peltatin 24	OH	H	H	OCH ₃
Desoxypodophyllotoxin 20	H	H	H	OCH ₃
Epipodophyllotoxin 30	H	OH	H	OCH ₃
4'-Demethyl- podophyllotoxin 21	H	H	OH	OH
Podophyllotoxin-β-D- glucopyranoside 47	H	H	C ₆ H ₁₁ O ₆	OCH ₃
β-Peltatin-β-D- glucopyranoside 48	C ₆ H ₁₁ O ₆	H	H	OCH ₃
Podophyllotoxin-4,6-O- Benzylidene-β-D-glucoside 46	H	H	φC ₇ H ₁₀ O ₆	OCH ₃

Table 5: Relative antimitotic and antitumor activities of podophyllotoxin glucosides*



	R ₁	R ₂	ED ₅₀ : P-815 mouse mastocytoma cells, <i>in vitro</i> (mg/L)	Mouse leukemia L-1210, increase in survival time (%)
(etoposide 7)	CH ₃	H	0.031	167
	C ₂ H ₅	H	0.0085	97
	CH ₃ CH=CH	H	0.016	121
	(CH ₃) ₂ CH	H	0.0055	121
	CH ₃ CH ₂ CHCH ₃	H	0.0055	84
	(CH ₃) ₂ CHCH ₂	H	0.0048	36
	n-C ₄ H ₉	H	0.0062	85
	C ₅ H ₉	H	0.0047	39
	furanyl	H	0.018	136
(teniposide 6)	thiophenyl	H	0.0048	121
	phenyl	H	0.0068	97
	CH ₃	CH ₃	0.015	106
	C ₂ H ₅	CH ₃	0.0060	69

*From reference 41.

I.5 Biosynthesis of Podophyllotoxin

While the synthesis of teniposide (6) and etoposide (7) involve podophyllotoxin (5) as the semi-synthetic precursor, at the current time the largest source of podophyllotoxin (5) is still from the extraction of the roots and rhizomes of *Podophyllum* plants harvested from the wild.⁴² Since this is a laborious process and could eventually prove to be both economically and ecologically unsound, commercial production of podophyllotoxins will ultimately require inexpensive and readily available raw materials.⁴² To this end, many total syntheses of podophyllotoxin (5) and its derivatives have been proposed (some of which are outlined in references 43 through 61), and a substantial number of studies on the biosynthesis of this compound have been conducted in order to gain further insight into the processes responsible for its biological formation, as discussed below.

As previously mentioned, podophyllotoxin (5) is a member of the class of natural products known as lignans. Lignans are thought to be produced by the coupling of suitable phenylpropanoid monomers, *e.g.* cinnamate derivatives, through free-radical or equivalent two-electron processes in a fashion similar to that proposed for lignin biosynthesis.⁶ In contrast to the lignans, lignin is a high molecular weight biopolymer (see Figure 5) which is an important cell wall component in vascular plants, with multiple functions, including providing strength to cell walls by cementing cellulose fibers together, thus allowing plants to grow vertically while allowing the conduction of water; and providing protection from physical, biological, and chemical attack, such as insect predation and microbial infiltration.^{62,63} Unlike lignin, which is racemic due to the nature of its proposed biosynthesis via random free-radical couplings of phenylpropanoid monomers, the lignans are generally optically active.⁶ This suggests that the biosynthesis of lignans from

phenylpropanoid monomers is enzyme-mediated. However, the metabolic point at which these monomers are segregated between lignin and lignan biosynthesis is not known.

Currently, phenylpropanoids are thought to be formed along common biosynthetic pathways leading from phenylalanine (31), as shown in Figure 6.⁶⁴ Initially, ammonia is eliminated from phenylalanine (31), involving the enzyme L-phenylalanine ammonia lyase (PAL), giving cinnamic acid (32). *p*-Coumaric acid (33) is then formed from cinnamic acid (32) via an oxidation involving the so-called NIH shift. Another hydroxylation gives caffeic acid (34), which can then be O-methylated at the 3-position giving ferulic acid (35). Ferulic acid (35) can be converted to 3,4-methylenedioxy-

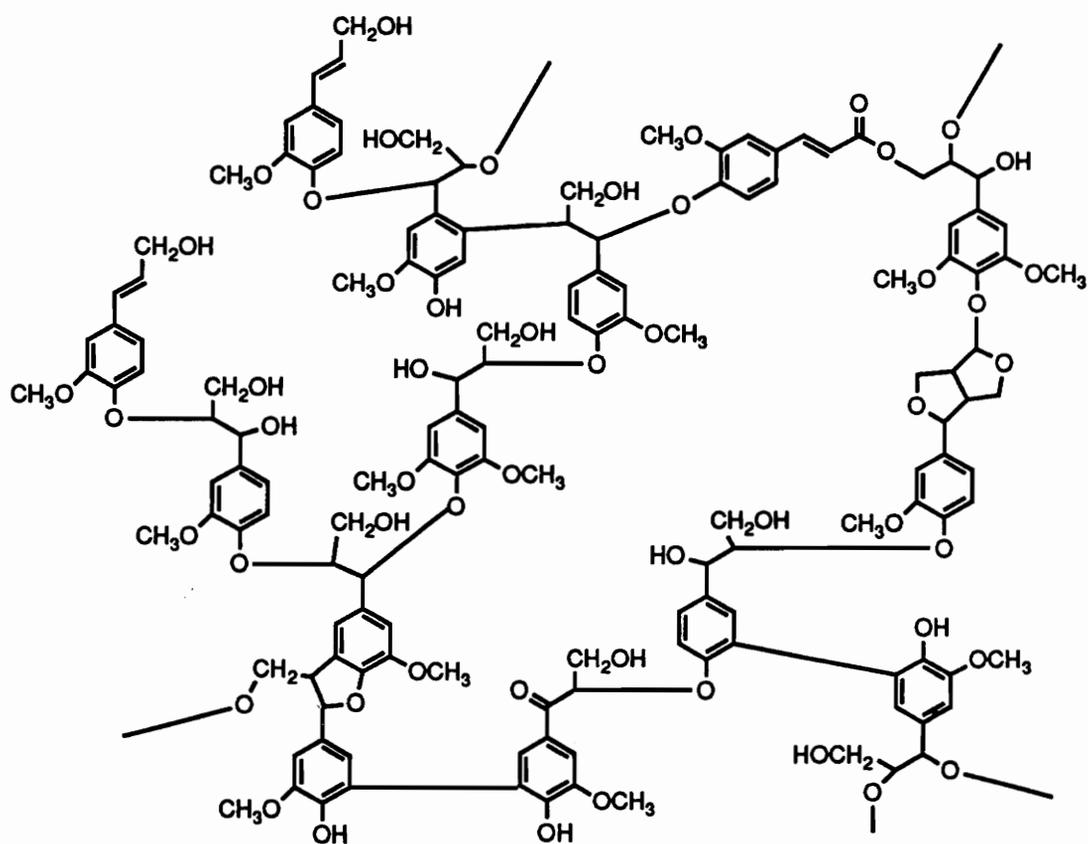


Figure 5: Hypothetical lignin structure

cinnamic acid (36) or undergo another hydroxylation giving sinapic acid (37), which can lead to the O-methylated products, syringic acid (38) and 3,4,5-trimethoxycinnamic acid (39).⁶⁴

One of the earliest experiments to investigate the biosynthesis of podophyllotoxin was published in the late 1960s.⁶⁵ It was reported that [U-¹⁴C]-L-phenylalanine (31) was wick-fed to *Podophyllum* plants to establish whether this compound can be incorporated into podophyllotoxin (5) (wick-feeding is a commonly used technique in which a wick is threaded through the plant stem and dipped into an aqueous solution containing the

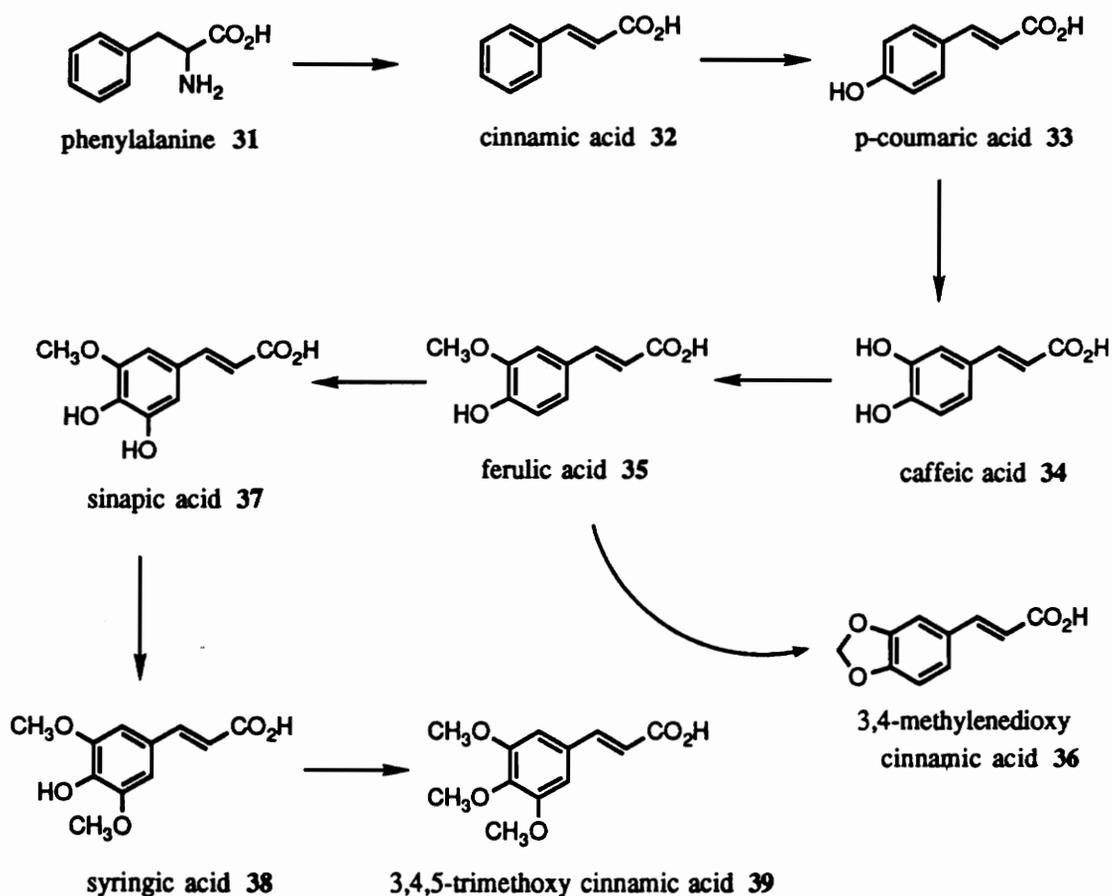


Figure 6: Proposed lignan monomer formation from phenylalanine (31)⁶⁴

precursor). Subsequent isolation of podophyllotoxin (5) from the plants showed that phenylalanine (31) is indeed incorporated into podophyllotoxin (5) at a level of 1.4% (Acceptable incorporations in plants are often below 1%, due to the fact that: (i) precursors earlier in a metabolic pathway are normally incorporated less efficiently and with a high dilution factor, i.e., they pass through more metabolic pools than precursors appearing later in the pathway; and (ii), successful incorporation often depends on the degree to which a plant is able to absorb the precursor and/or transport it to the site(s) where the biotransformation(s) can take place.⁶⁴). A closely related experiment was conducted in 1981,⁶⁶ in which DL- $[\beta\text{-}^{14}\text{C}]$ -phenylalanine (31) was administered to *Podophyllum* plants. Following isolation and oxidative degradation of podophyllotoxin (5), it was determined that two phenylpropanoid units were incorporated equally into podophyllotoxin (see Figure 7).

Stockigt and Klischies had previously demonstrated⁶⁷ that the lignan glucosides, arctiin (17) and phyllyrin (15), are formed by the coupling of two phenylpropanoid units. By feeding 3,4-[4-OCH₂³H]-dimethoxycinnamic acid (36), $[\beta\text{-}^{14}\text{C}, \text{-OCH}_2^3\text{H}]$ -glucoferulic acid (40), $[\beta\text{-}^{14}\text{C}, \text{-OCH}_2^3\text{H}]$ -coniferyl aldehyde (41), and $[\beta\text{-}^{14}\text{C}, \text{-OCH}_2^3\text{H}]$ -coniferin (42) to *Forsythia* plants, the authors were able to establish that 36 was not incorporated into the lignans 17 or 15, however, precursors 40, 41, and 42 were significantly incorporated, with no appreciable change in their ³H/ ¹⁴C ratios (see Figure 8 and Table 6), suggesting that the 4-hydroxyl group, resulting from cleavage of the glucose unit, is necessary for the proposed phenolic oxidative coupling mechanism.

In 1984 Jackson and Dewick⁶ reported the results of their experiments to establish incorporation of phenylpropanoid precursors into podophyllotoxin (5). DL-[1-¹⁴C]-phenylalanine (31) and the sodium salts of the ¹⁴C-labelled cinnamic acids 32, 35, 36, 38, and 39 were administered to the roots of *Podophyllum* plants, by suspending the

plants in the precursor solutions. Phenylalanine (31) and cinnamic acid (32) proved to be fairly good precursors of podophyllotoxin, however, ferulic acid (35), syringic acid (38), 3,4-methylenedioxcinnamic acid (36), and 3,4,5-trimethoxycinnamic acid (39) were found to be more poorly incorporated (Table 7). While the level of ferulic acid (35) incorporated into podophyllotoxin (5) was fairly low, it was reported, based on degradative studies, that both halves of the podophyllotoxin (5) isolated for this precursor were equally labelled, suggesting that two phenylpropanoid units having the 4-hydroxy-3-methoxy substitution pattern of ferulic acid (35) are involved in the coupling step leading to podophyllotoxin (5) (Figure 9).⁶ The relatively low incorporation level of ferulic acid (35) into podophyllotoxin (5) could be attributed to the likely incorporation of ferulic acid (35) into other substances within the plant, particularly lignin. The oxidation state of the two phenylpropanoid units which couple is still unknown, since coupling of the monomers could take place in either the aldehyde-aldehyde or alcohol-acid oxidation states. Combination of two aldehyde units, followed by a Cannizzaro-type reaction would lead to the alcohol and acid functions necessary for the formation of the lactone. In contrast, direct combination of an alcohol and an acid to form the lactone directly is not likely, based on the equal incorporation of the ferulic acid substitution pattern into both halves of the isolated podophyllotoxin (5).⁶ The relative non-incorporation of (38) and (39) suggests that the substitution pattern of the pendant ring is built up after the initial coupling, and in the case of (39), provides further evidence of a phenolic oxidative coupling mechanism. The level of incorporation of (36) was hypothesized to be due to *in vivo* demethylenation of (36) to caffeic acid (34) and loss of labelled formaldehyde or formic acid to the C₁-pool. Later re-emergence as a labelled methyl in S-adenosylmethionine (SAM), could allow remethylation of 34, giving the ferulic acid pattern (Figure 10).^{6,68}

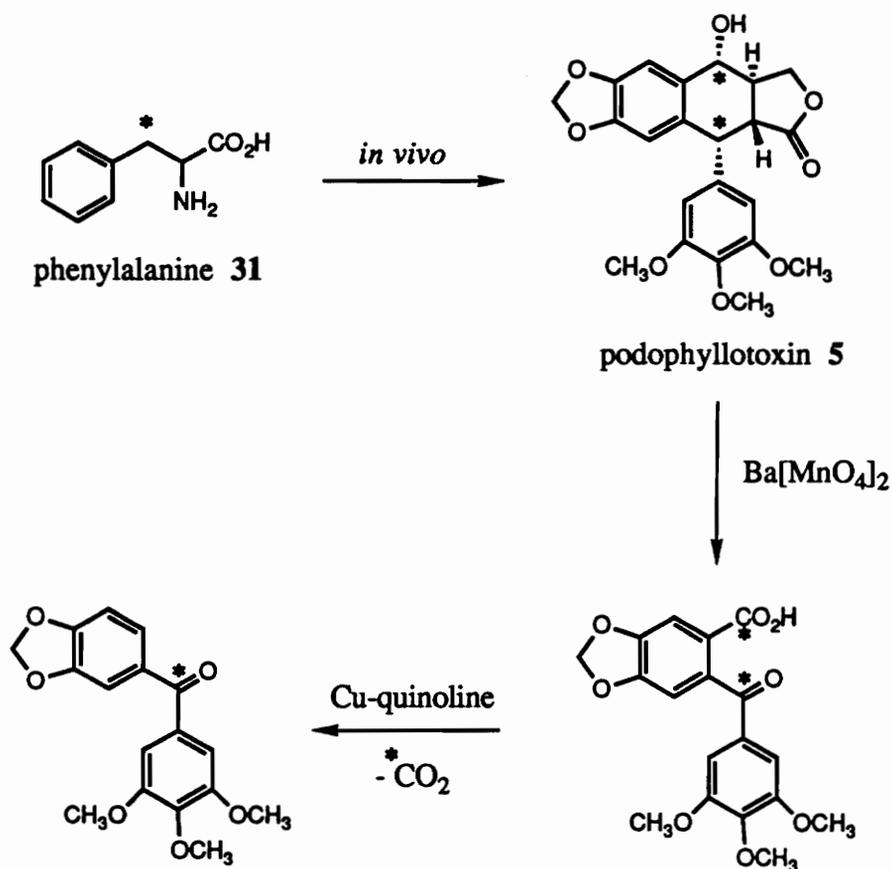


Figure 7: Oxidative degradation of podophyllotoxin isolated from DL- $[\beta\text{-}^{14}\text{C}]$ -phenylalanine-fed *Podophyllum* plants⁶⁶

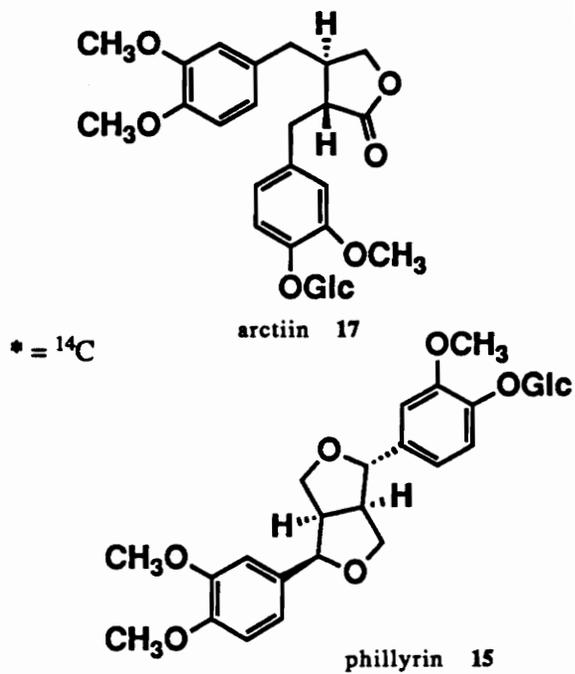
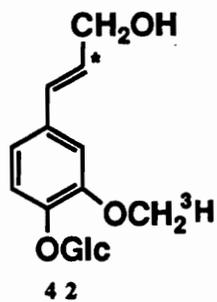
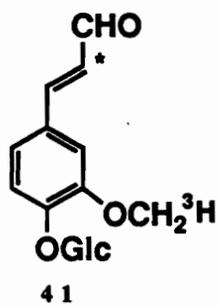
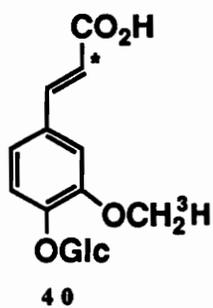
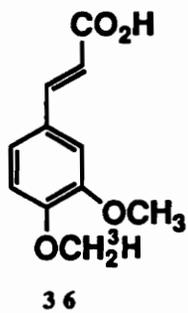


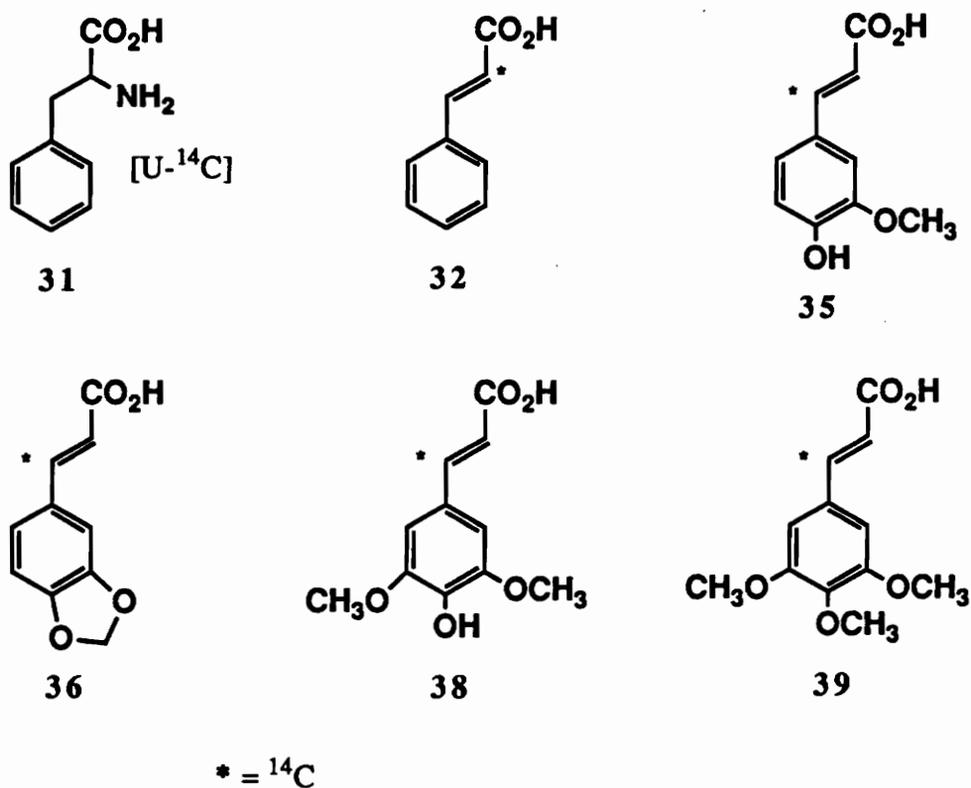
Figure 8: Precursors administered to *Forsythia* species to establish incorporation into arctiin (17) and phillyrin (15)⁶⁷

Table 6: Incorporation of [$^3\text{H}/^{14}\text{C}$]-labelled ferulic acid derivatives into arctiin (17) and phillyrin (15) in *Forsythia* species⁺

Precursor	Amount taken up (umol)	$^3\text{H}:^{14}\text{C}$	Arctiin incorp.		$^3\text{H}:^{14}\text{C}$	Phillyrin incorp.		$^3\text{H}:^{14}\text{C}$
			absol. (%)	spec. (%)		absol. (%)	spec. (%)	
[β - ^{14}C , - OCH_2^3H]-glucoferulic acid 40	2.43	10.65:1	3.66	1.9	10.37:1	0.68	1.40	10.11:1
[β - ^{14}C , - OCH_2^3H]-coniferyl aldehyde 41	0.41	12.80:1	1.37	1.2	11.63:1	0.20	0.40	11.27:1
[β - ^{14}C , - OCH_2^3H]-coniferin 42	1.41	12.90:1	0.74	0.7	11.48:1	0.16	0.40	12.25:1
3,4-[4- OCH_2^3H]-dimethoxycinnamic acid 36	0.5	—	0	0	—	0	0	—

⁺From reference 67.

Table 7: Incorporation of [¹⁴C]-labelled precursors into podophyllotoxin in *Podophyllum hexandrum* ⁺



Precursor	mg Isolated	% Incorporation
Phenylalanine 31	22.6	1.19
Cinnamic acid 32	32.2	0.17
Ferulic acid 35	34.0	0.053
Methylenedioxy-cinnamic acid 36	5.5	0.016
Syringic acid 38	25.3	0.00064
3,4,5-Trimethoxy-cinnamic acid 39	7.4	0.00039

⁺From reference 6.

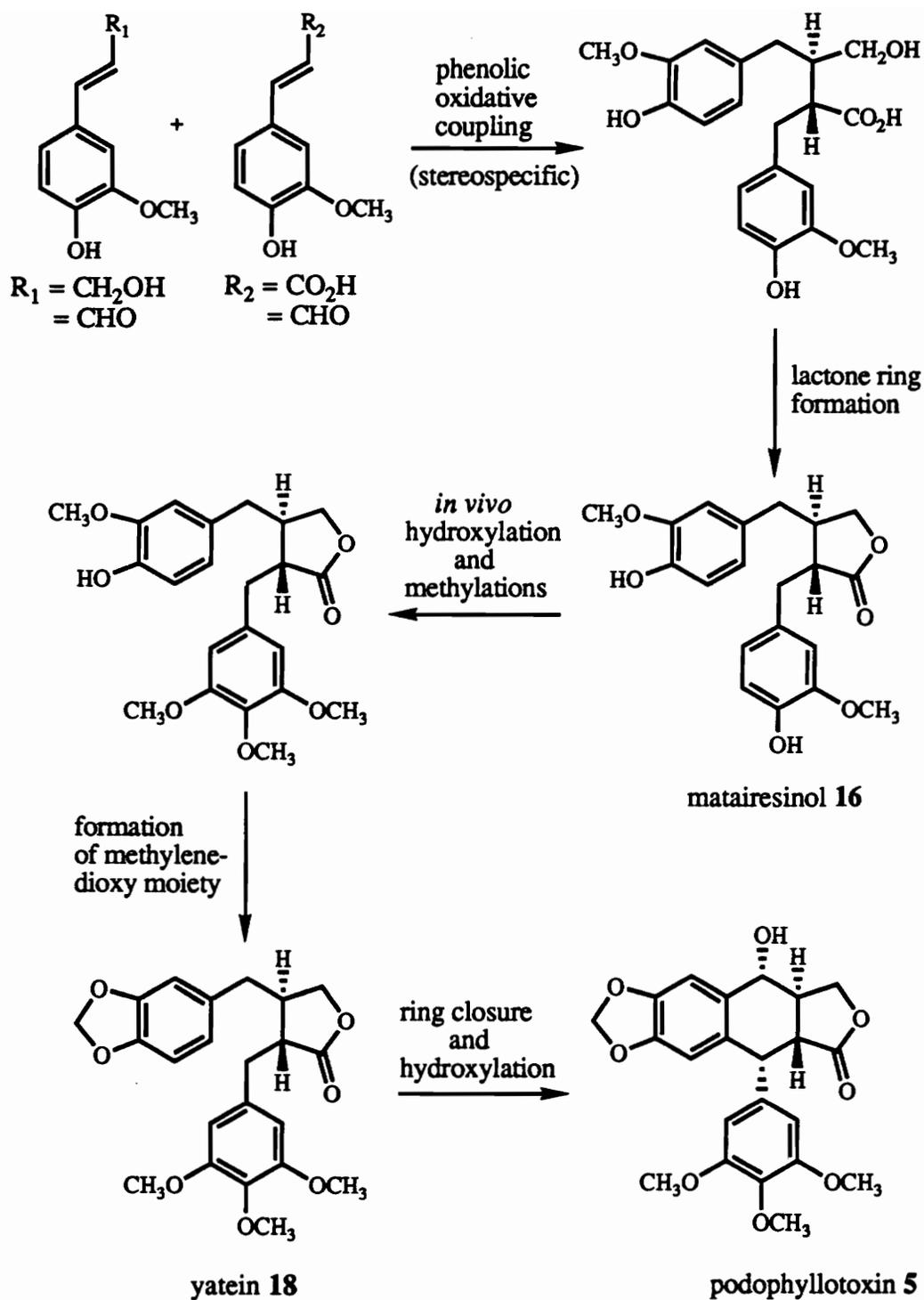


Figure 9: Postulated biosynthetic pathway to podophyllotoxin (5)⁶

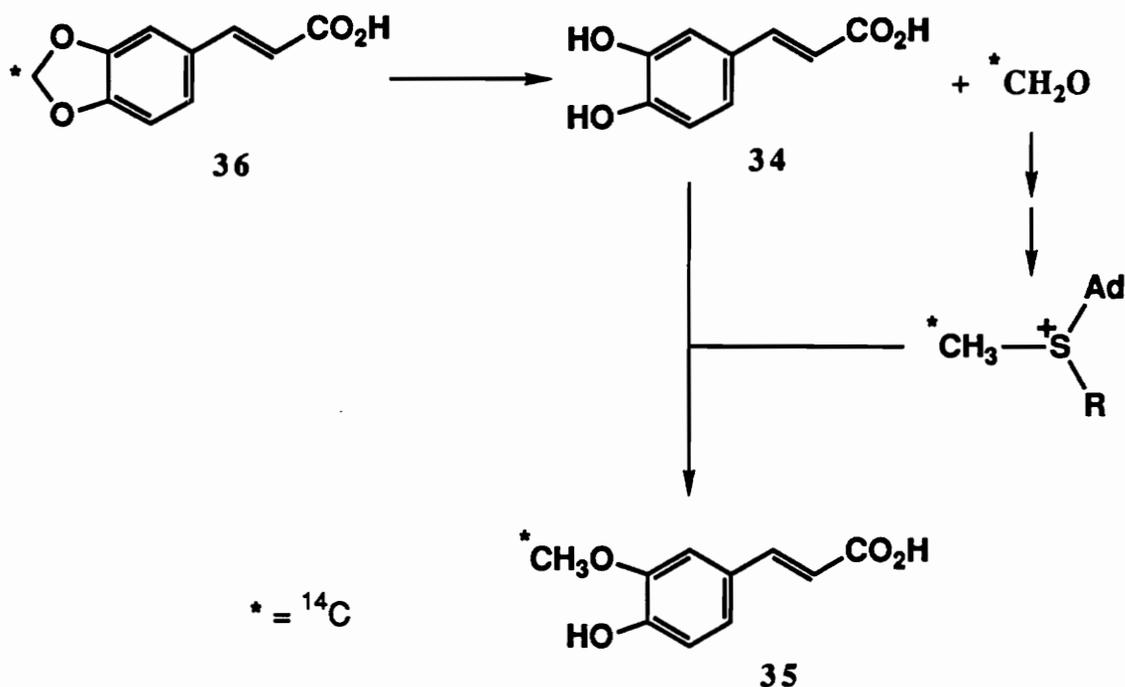
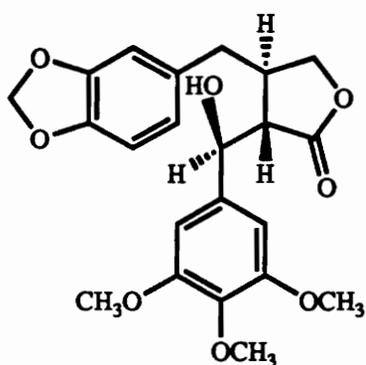


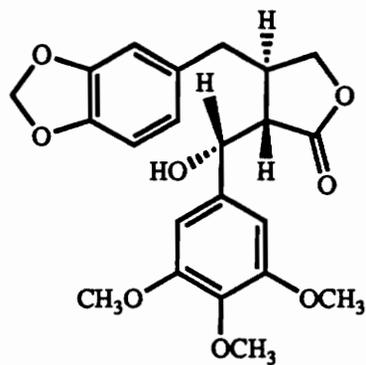
Figure 10: Metabolism of [O¹⁴CH₂O]-3,4-methylenedioxcinnamic acid, giving [¹⁴C]-methyl-labelled ferulic acid^{6,68}

Subsequently, Kamil and Dewick⁵⁹ reported the results of experiments to determine whether diarylbutyrolactone lignans could serve as precursors to podophyllotoxin (5). The authors administered the [4'-O¹⁴CH₃]-labelled diarylbutyrolactone lignans, podorhizol (43), epipodorhizol (44), anhydropodorhizol (45), and yatein (18), to *Podophyllum* roots. Yatein (18) was found to be converted to podophyllotoxin (5) to a small extent (0.19%), while podorhizol (43), epipodorhizol (44), and anhydropodorhizol (45) were not significantly incorporated (0.004%, 0.003%, and 0.001%, respectively). The biosynthetic scheme proposed by Kamil and Dewick, based on the above results, is illustrated in Figure 11. In this scheme matairesinol (16) is postulated as a precursor to

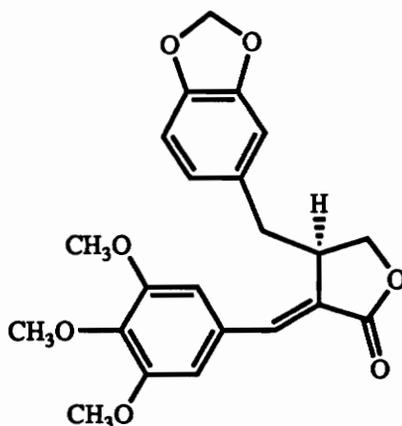
yatein (18), involving a hydroxylation, two methylations, and formation of the methylenedioxy functionality. It is speculated that yatein (18) is the common precursor of podophyllotoxin (5), podorhizol (43), and anhydropodorhizol (45),⁵⁹ although at the present time there is no data to support this hypothesis, nor is there any evidence to support the relationship between yatein (18) and matairesinol (16). While hydroxylation and methylation steps are fairly well established for the biogenesis of the monolignans ferulic acid (35) and sinapic acid (37), no such biosynthetic pathways have been shown at the dimer level and, thus, the matairesinol-to-yatein conversion would represent a very unusual circumstance.



podorhizol 43



epipodorhizol 44



anhydropodorhizol 45

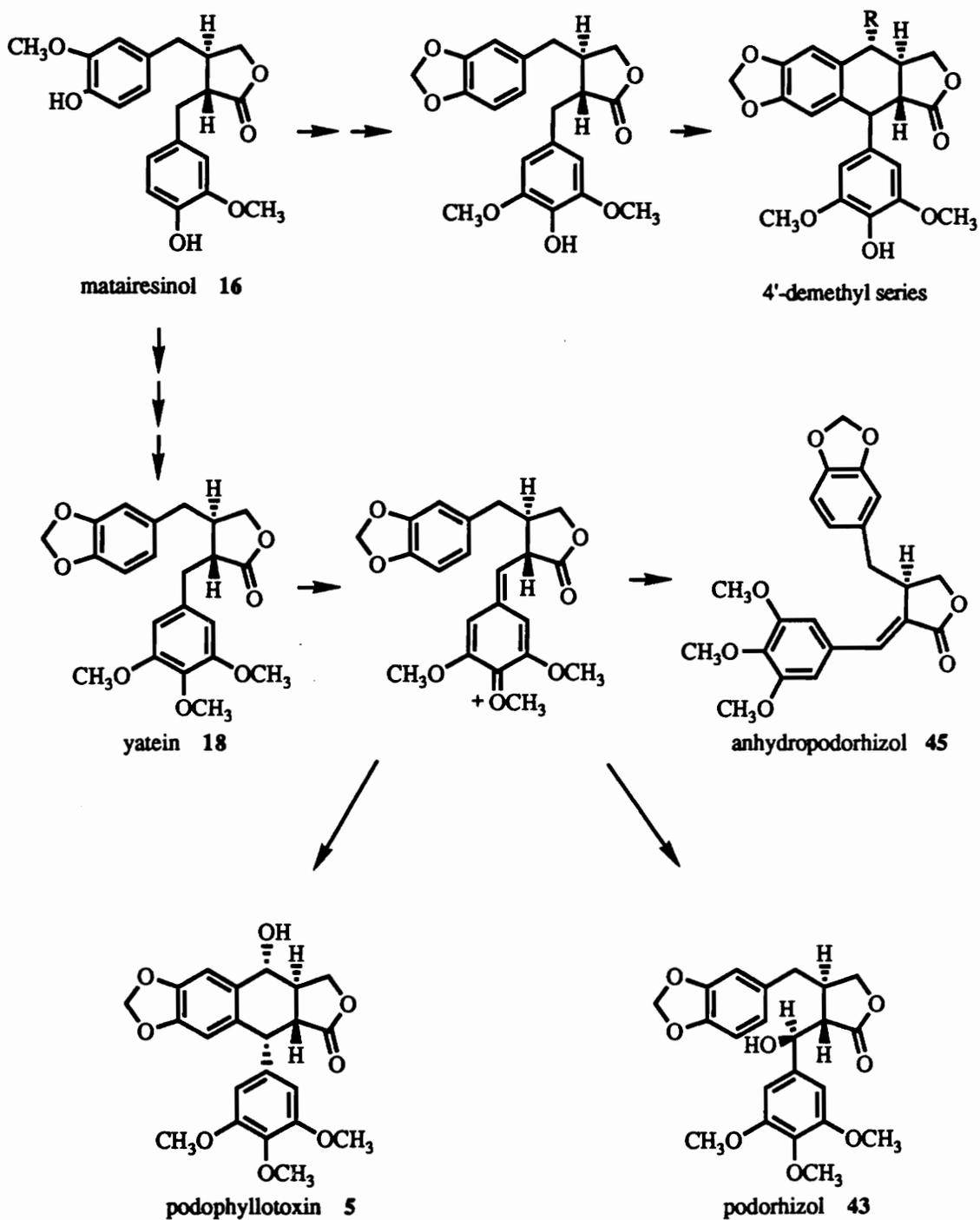


Figure 11: Postulated biosynthetic pathway to *Podophyllum* lignans⁵⁶

The relationship between two groups of aryltetralins in *Podophyllum* species, classified by the substitution pattern of the pendant aromatic ring, namely, the 4-hydroxy-3,5-dimethoxy series, e.g. 4'-demethylpodophyllotoxin (21), and the 3,4,5-trimethoxy series, e.g. podophyllotoxin (5), was investigated in 1984.^{69,70,71} From the results of this study, it was shown that the 4-hydroxy-3,5-dimethoxy series and the 3,4,5-trimethoxy series are products of pathways which diverge early in the biosynthesis of Podophyllum lignans, and that there is no interconversion of either class into the other (see Figure 12).

While much progress has been made toward establishing the biosynthesis of lignans in *Podophyllum* species, there is an inherent restriction on the investigation of plant biosyntheses, in general -- that of a limited growing season, as well as an often more restricted period in which to establish precursor incorporation levels. Jackson and Dewick⁶ reported in 1984 that administration of DL-[1-¹⁴C]-phenylalanine (31) to *Podophyllum* plants at monthly intervals during the plants' seasonal growth cycle, showed that maximum incorporation of the precursor 31 into podophyllotoxin (5) (1.19%) occurred in July, during fruit development. Incorporations earlier or later in the season resulted in substantially lower incorporation levels (Table 8).

Table 8: Incorporation of DL-[1-¹⁴C]-phenylalanine into podophyllotoxin (5) in *Podophyllum hexandrum*⁶

Date fed	Podophyllotoxin isolated (%)	% Incorporation
12 May	12.0	0.04
12 June	12.9	0.13
14 July	22.6	1.19
12 Aug	2.8	0.08
15 Sept	57.3	0.13

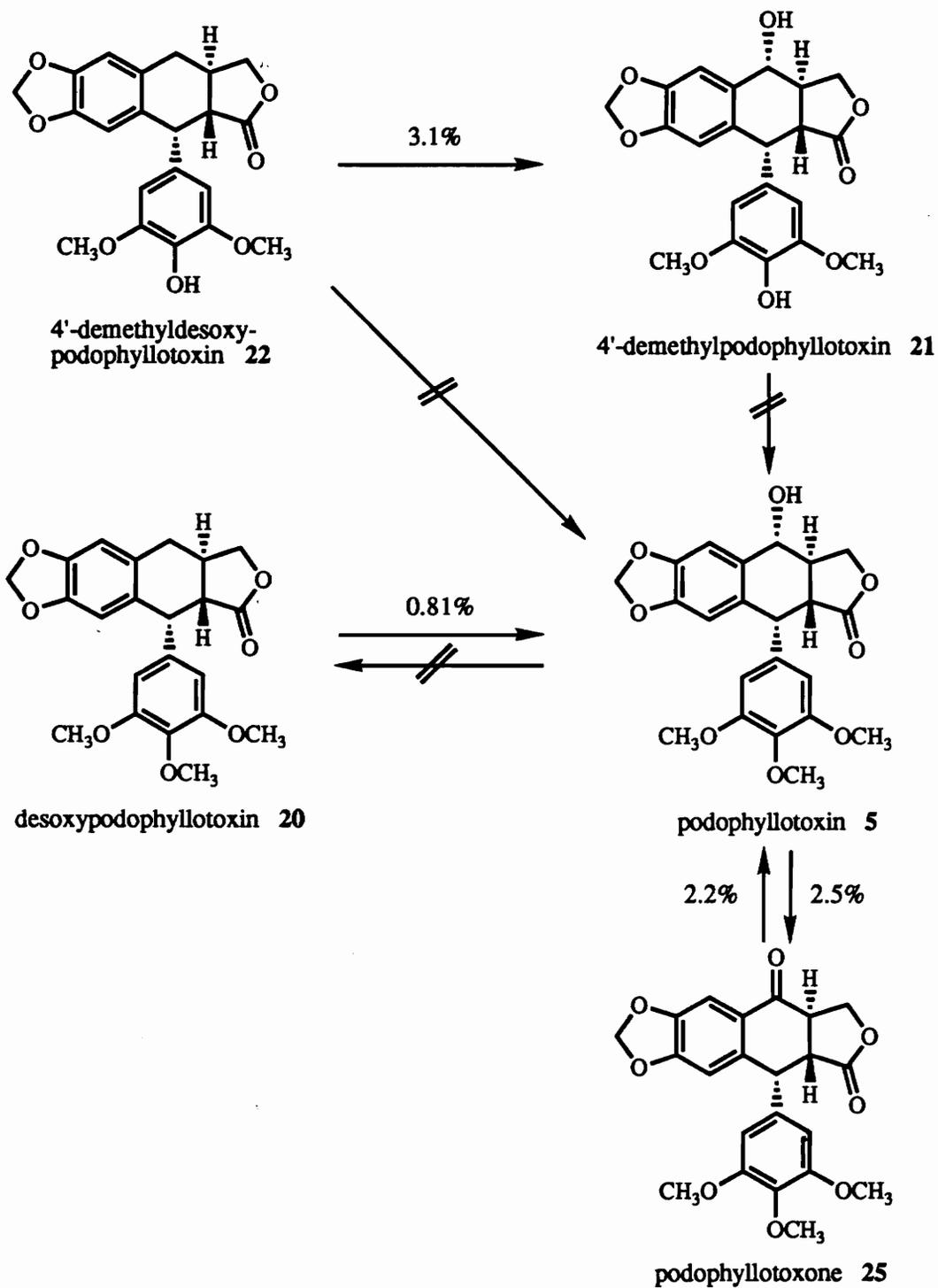


Figure 12: Lignan interconversions demonstrated in *Podophyllum hexandrum*⁶⁹

Thus, in the case of *Podophyllum* species, the collection of reasonable precursor incorporation data is dictated by the developmental stage of the plant, a point to be considered in the design and implementation of precursor incorporation studies.

It is evident from the foregoing discussion that a more detailed understanding of the biosynthesis of lignans in *Podophyllum* species has been acquired during the last two decades, with regard to the biosynthesis of lignan monomers from phenylalanine (31), the nature of the phenylpropanoid dimerization, and the biosynthetic relationships between *Podophyllum* lignans. There remain, however, a number of questions pertaining to the biosynthesis of podophyllotoxin (5), the answers to which would help to more conclusively establish the biosynthetic pathway(s) giving rise to this important compound. The goal of this thesis is to provide the molecular tools necessary to probe some of these questions.

II. RESULTS AND DISCUSSION

II.1 Research Objectives

It has been proposed that yatein (**18**) is a potential precursor of podophyllotoxin (**5**), based on a relatively small but significant level of incorporation of radiolabelled yatein (**18**) into podophyllotoxin (**5**).⁵⁹ Such a conversion would involve a cyclization step and an oxidation step, presumably in that order, based on the findings of Jackson and Dewick.⁶⁹ In 1985, Pelter, *et al.*⁷² proposed two cyclization schemes for aryltetralin lignan biogenesis such as the yatein-to-podophyllotoxin conversion. These schemes are summarized in **Figures 13** and **14**. In the first scheme, **Figure 13**, cyclization is initiated by the loss of a hydride, followed by ring formation, and finally by rearomatization by loss of a proton to give desoxypodophyllotoxin (**20**). In the second scheme, **Figure 14**, cyclization is also initiated by hydride loss, but followed by a spirocyclic ring formation, then ring expansion, and finally rearomatization, giving desoxypodophyllotoxin (**20**). In either case, the postulated oxidation of the cyclization product would then yield podophyllotoxin (**5**). One question, which to date has not been answered, is that of the stereochemistry of the ring closure of yatein (**18**) en route to podophyllotoxin (**5**), i.e. whether it is the pro-*R* or pro-*S* hydrogen which is lost during cyclization.

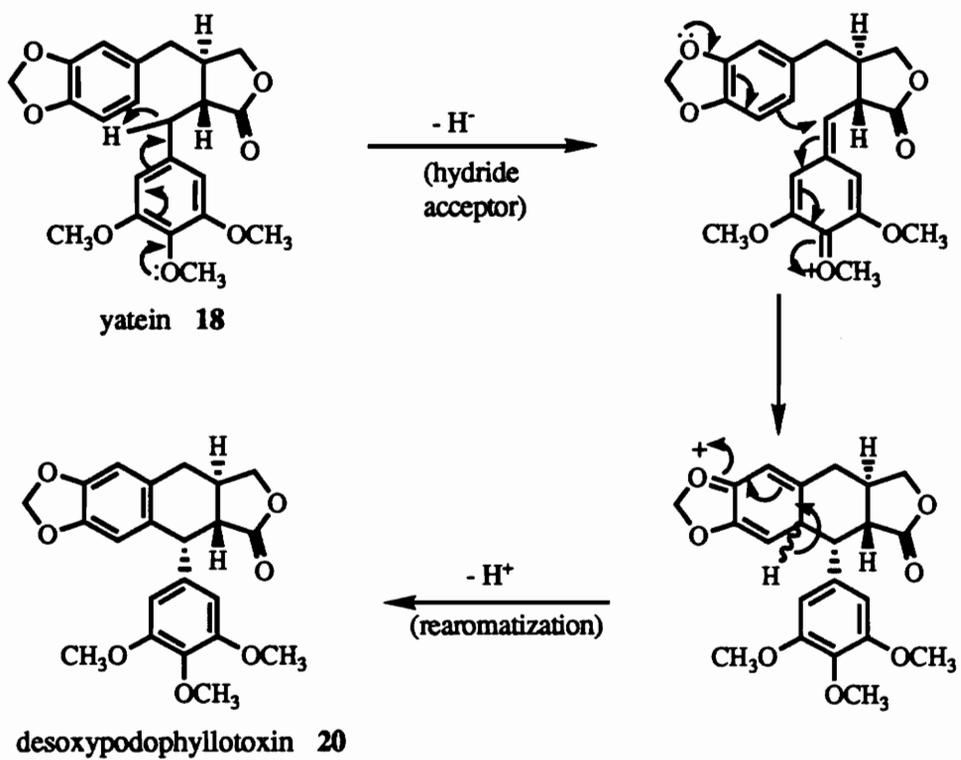


Figure 13: A possible yatein cyclization scheme

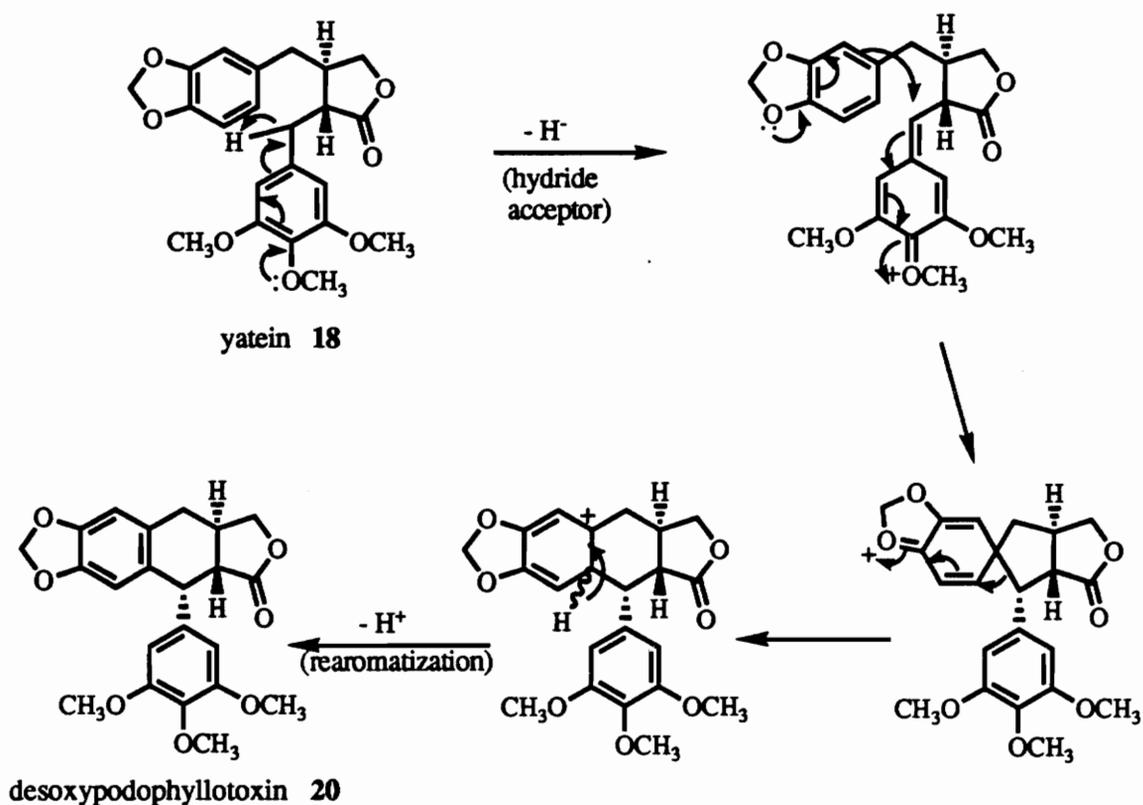
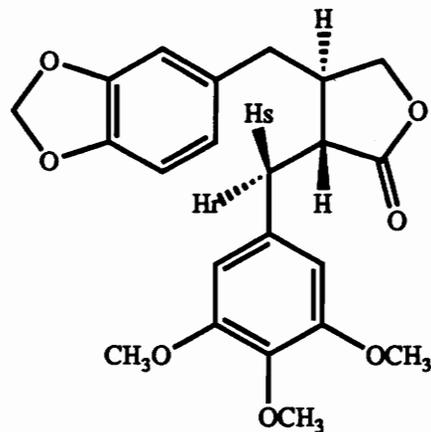


Figure 14: A possible yatein cyclization scheme

It has also been proposed that matairesinol (16) could serve as a precursor to yatein (18)⁵⁹, as shown in **Figure 15**, although no data exists to support this hypothesis. As mentioned previously, the conversion of matairesinol (16) to yatein (18) would involve



yatein 18

unusual hydroxylation and methylation steps, followed by formation of the methylenedioxy group, processes common to phenylpropanoid monomers, but having no precedent at the dimeric level.

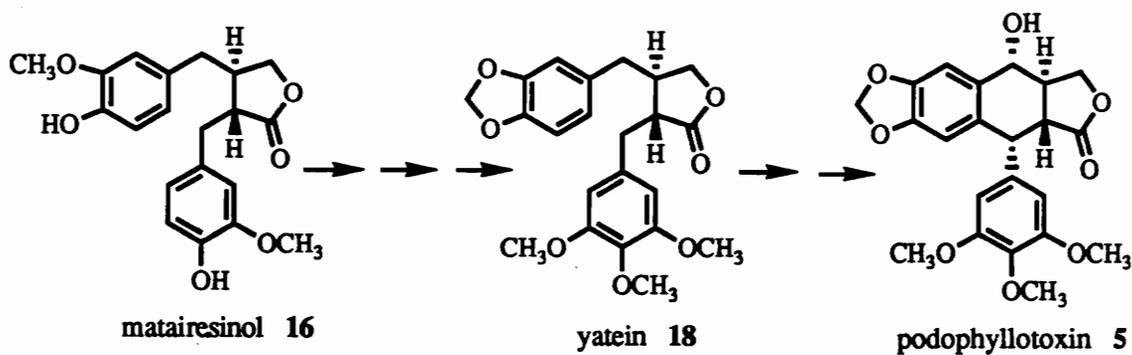


Figure 15: Matairesinol (16) and yatein (18) as possible precursors of podophyllotoxin (5)⁵⁹

Another question which has not been answered is whether matairesinol (16) is indeed converted to yatein (18) in *Podophyllum* species.

II.2 Synthesis of Yatein Derivatives

In an attempt to answer the first of the questions above, namely, the stereochemical nature of yatein cyclization, a synthetic scheme was proposed following a similar strategy reported by Kamil and Dewick.⁵⁹ The key steps in the synthesis (Figure 16) are the formation of anhydropodorhizol (45), hydrogenation of this compound with deuterium, presumably from the less hindered bottom face of 45 to give *cis*-dideuteroanhydropodorhizol (52), and epimerization of 52 to give the desired stereochemistry of yatein (18) with a single stereospecific isotopic label at the 6- β position.

As proposed, the synthesis of labelled yatein is initiated by a Stobbe condensation of piperonal (46) with dimethyl succinate, using sodium methoxide as base and methanol as solvent, under reflux conditions. The product of this reaction, α -piperonylidene succinic acid half-methyl ester (47), is then hydrogenated, giving racemic α -piperonyl succinic acid half-methyl ester (48). Treatment of this material with (-)-ephedrine (49) in 95% ethanol allows the isolation of the desired enantiomer as the (-)-ephedrine salt. Subsequent acidification of the salt affords the desired (*R*)- α -piperonyl succinic acid half-methyl ester (48a). Formation of the potassium salt and treatment with calcium borohydride permits the formation of (*R*)-(+)- β -piperonyl- γ -butyrolactone (50). The next step in the scheme is a base-catalyzed coupling of the lactone (50) with 3,4,5-trimethoxybenzaldehyde (51) to afford a mixture of podorhizol (43), epipodorhizol (44), and anhydropodorhizol (45), the latter of which, according to Kamil and Dewick, should be the predominant compound in the product mixture.⁵⁹ After separation of the components, the anhydropodorhizol is then hydrogenated with deuterium gas over Adam's catalyst affording the doubly deuterium-labelled *cis*-dihydroanhydropodorhizol (52), or "*cis*-yatein". Epimerization of compound (52) under mildly basic conditions affords the

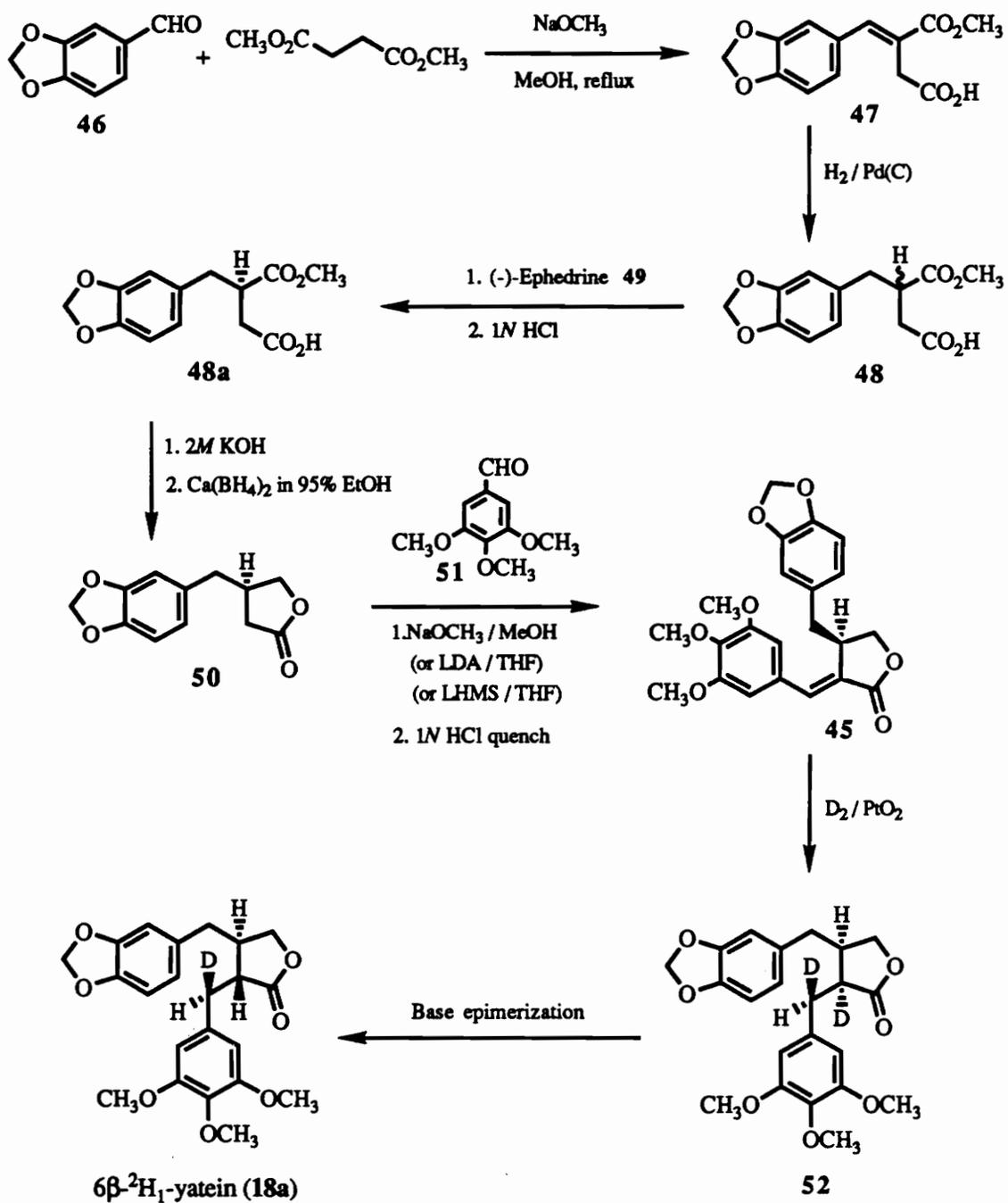
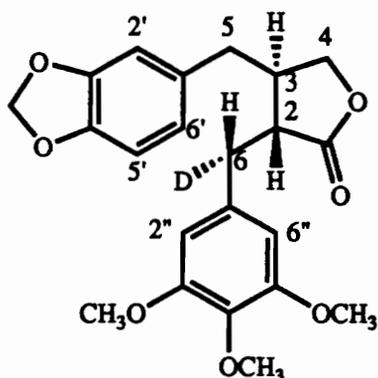


Figure 16: Proposed synthetic scheme for labelled yatein (18a)

final product 6 β -deuterium-labelled yatein (**18a**). Using the same methodology, it would be possible to prepare the 6 α -deuterium-labelled yatein (**18b**) by base-catalyzed coupling of the lactone (**50**) with 3,4,5-trimethoxybenzaldehyde (**51a**) (labelled with deuterium in place of the aldehyde hydrogen), followed by isolation of the deuterium-labelled anhydropodorhizol (**45a**), hydrogenation with hydrogen gas over Adam's catalyst, and mild base epimerization to give **18b**.



6 α -²H₁-yatein (**18b**)

Initial synthetic attempts were envisioned using deuterium as the isotopic label, with the option of extending this methodology to using tritium as the label, to aid in tracing the course of the material during incorporation studies.

The product of the lead-off reaction in the synthesis of labelled yatein, α -piperonylidene succinic half-methyl ester (**47**), was obtained via Stobbe condensation of piperonal (**46**) and dimethyl succinate (DMS), using sodium methoxide as base, in refluxing methanol. While the authors of the original synthesis of yatein⁵⁹ reported a yield of 72% for this step, the yield in our hands was typically lower, ranging from 35% to 45%. Attempts to improve the yield by modifying reaction conditions proved to be relatively unsuccessful, as summarized in Table 9.

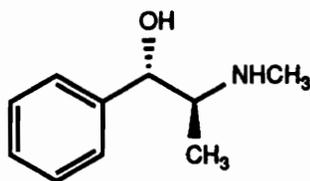
The second step in the synthesis involved hydrogenation of the α -piperonylidene succinic acid half-methyl ester (47), and was carried out in a straightforward manner using palladium-on-carbon (5 or 10%) in acetic acid. The product 48 was obtained in typical yields of 75% to 87%.

The racemic α -piperonyl succinic acid half-methyl ester (48) was treated with (-)-ephedrine (49) in 95% ethanol to achieve chiral resolution. (-)-Ephedrine (49) is widely used as a resolving agent. The principle of chiral resolution is relatively simple and is based on the fact that when a chiral resolving agent, such as (-)-ephedrine (49), is added

Table 9: Summary of the relatively unsuccessful attempts to improve the yield of the Stobbe condensation of piperonal (46) with dimethyl succinate (DMS)

Experiment #	Ratio		Solvent	Reaction time	Yield (%)
	(46 : DMS : Base)	Base			
1	1 : 2 : 1.2	NaOCH ₃	methanol	4.5 hrs	43.6
2	1 : 2 : 2.6	LiOCH ₃	methanol	2.5 hrs	34.7
3	1 : 1.5 : 1.2	KOCH ₃	methanol	6 hrs	10.5
4	1 : 1.5 : 1.2	NaOCH ₃	methanol	3.5 hrs	40.0
5	1 : 1.2 : 1.3	NaOCH ₃	methanol	18 hrs	36.1

to a mixture of racemates, such as α -piperonyl succinic acid half-methyl ester (48), two diastereomeric salts result. These salts often differ substantially in their properties, including melting point, crystal structure, and solubility.



(1*R*,2*S*)-(-)-ephedrine (49)

The last property can be exploited if conditions are such that the salt of the desired enantiomer readily crystallizes from solution, while the salt of the other enantiomer remains dissolved in solution.⁷³ This is indeed the case when (-)-ephedrine (49) is used with α -piperonyl succinic acid half-methyl ester (48): after treatment with (-)-ephedrine (49) and several recrystallizations from ethanol, the ephedrine salt of 48 was treated with acid to give the desired (*R*)- α -piperonyl succinic acid half-methyl ester (48a). Typical yields for this procedure were somewhat low, ranging from 21% to 30%, which corresponds to a range of 42% to 60% enantiomeric recovery. An enantiomeric purity of 97.7% was established on the basis of comparison with known optical rotation and melting point values. With regard to synthetic utility, the two-step, low-yield (20 %) conversion of compound 47 to compound 48a was superseded by a reported rhodium-catalyzed asymmetric hydrogenation of compound 47 to give compound 48a directly and quantitatively, in greater than 89% enantiomeric excess.⁶¹ Because the synthetic strategy outlined above had already been undertaken and had proceeded past the resolution step (affording 48a) at the time the asymmetric hydrogenation method appeared in the literature, the newer and apparently superior method was not utilized.

Preparation of (*R*)-(+)- β -piperonyl- γ -butyrolactone (50) was carried out by first forming the potassium salt of compound 48a, and then treating this salt with calcium

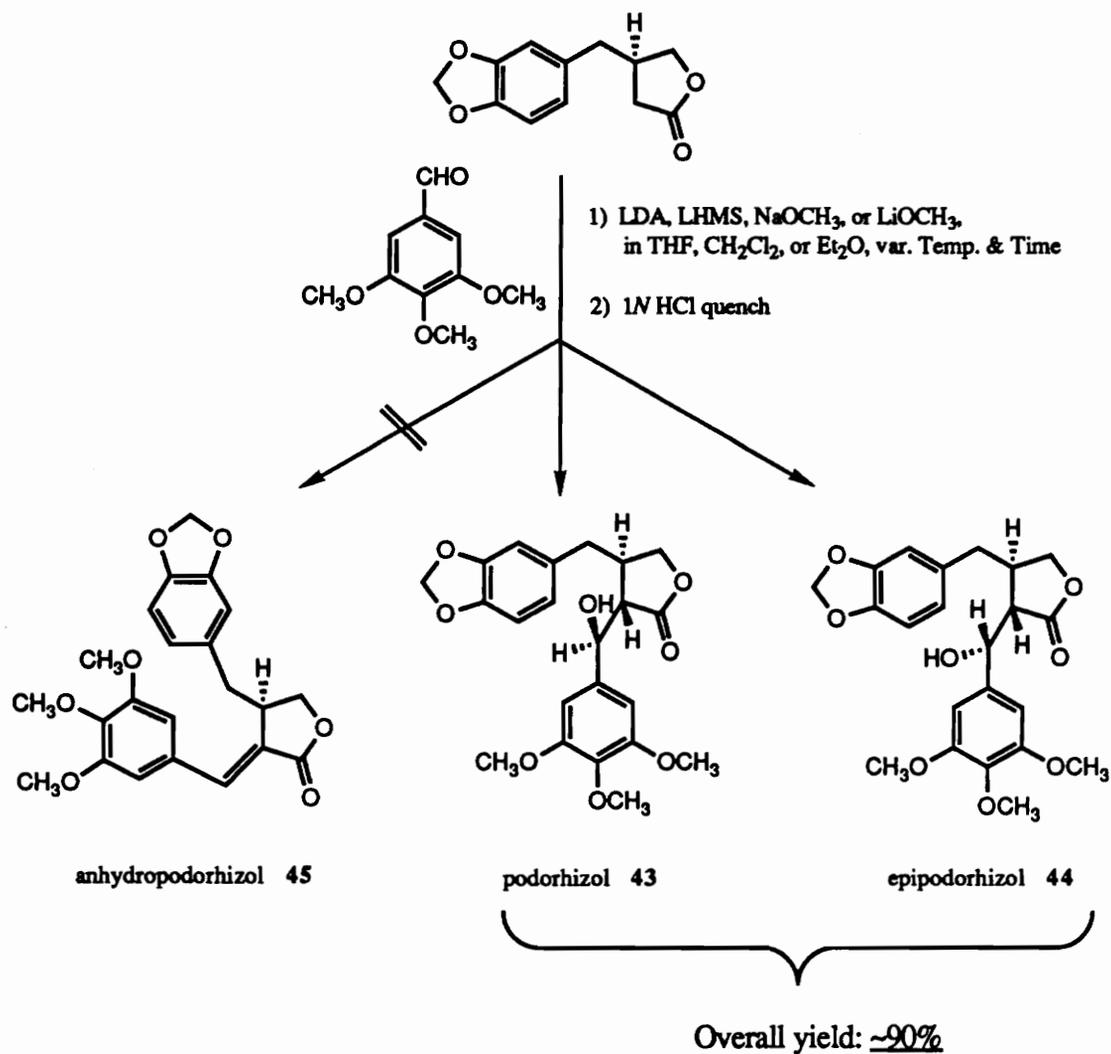
borohydride. Calcium borohydride is a very effective reagent for the reduction of esters and has a higher activity than the borohydrides of sodium, potassium, or lithium. Normally, these borohydrides show low activity toward ester reduction, unlike that found when calcium borohydride is employed. The reactivity of calcium borohydride has been ascribed to its more covalent character. Additional features of calcium borohydride are its ability to be used in hydrolytic solvents such as ethanol, methanol, and water; its higher selectivity than lithium aluminum hydride; its low potential for explosion and ignition; and its nearly neutral nature.⁷⁴ The product thus obtained from reaction with calcium borohydride was a very pale yellow oil, in 90% to 96% yield.

Using the method of Kamil and Dewick⁵⁹, several attempts were made at forming anhydropodorhizol (45). According to the authors, anhydropodorhizol (45) is the predominant product of the sodium methoxide-catalyzed condensation of (*R*)-(+)- β -piperonyl- γ -butyrolactone (50) with 3,4,5-trimethoxybenzaldehyde (51), in a ratio of 3:1.3:1 to podorhizol (43) and epipodorhizol (44), respectively, with an overall yield of about 50%. However, in all attempts made in our laboratory, no anhydropodorhizol (45) was isolated, but rather, only a mixture of podorhizol (43) and epipodorhizol (44). Changes in reaction conditions, such as base, solvent temperature, and time of reaction, failed to produce the desired compound in any isolable amount. Again, only the mixture of alcohols was produced. Table 10 summarizes the results of various unsuccessful experiments conducted toward obtaining anhydropodorhizol (45).

An attempt was made at mesylating the mixture of alcohols, in the hope that the mesylated compound would eliminate, giving a mixture of *E*- and *Z*-anhydropodorhizols (45) (although according to Kamil and Dewick,⁵⁹ the *E*-isomer is more favored, based on steric reasons as well as the tendency for the *Z*-isomer to isomerize to the *E*-form under acidic conditions, i.e. upon work-up, since acid conditions are necessary to recyclize the

lactone). However, no elimination products were noted, only an apparently unreacted starting mixture of alcohols. It is possible that elimination *did* occur but upon work-up under acidic conditions, as mentioned above, the elimination products rehydrated, giving back the mixture of alcohols. Although time did not permit a follow-up on this question, since a new strategy had been undertaken (as will be discussed below), it would have been interesting to isolate one of the alcohols, mesylate it, then check to see if elimination did occur prior to the presumed rehydration. If this indeed occurred then the single alcohol would lead to one elimination product, but rehydration would give back a mixture of both alcohols. Kamil and Dewick reported unsuccessful attempts at both mesylation and tosylation, and hypothesized that the hydroxyl group in the alcohols is hindered toward esterification, but they made no mention of the possibility of rehydration of the elimination product(s).

Table 10: Summary of unsuccessful attempts at preparing anhydropodorhizol (45)



Expt #	Base	Solvent	Temp (C)	Time	Product composition (approx. %)		
					(43)	(44)	(45)
1	LDA	THF	-78	6 hrs	50	50	--
2	LHMS	THF	-78	6 hrs	50	50	--
3	LDA	THF	-10	2 hrs	50	50	--
4	LHMS	THF	-10	2 hrs	50	50	--
5	LDA	ether	0	30 mins	55	45	--
6	LHMS	ether	0	30 mins	55	45	--
7	NaOCH ₃	CH ₂ Cl ₂	r.t.	42 hrs	55	45	--
8	LiOCH ₃	CH ₂ Cl ₂	r.t.	135 hrs	60	40	--
9	LHMS	CH ₂ Cl ₂	r.t.	5 mins	40	60	--

As noted above, the mixture of podorhizol (43) and epipodorhizol (44) was readily obtained in good yield from the base-catalyzed condensation of (*R*)-(+)- β -piperonyl- γ -butyrolactone (50) and 3,4,5-trimethoxybenzaldehyde (51). It was decided, in light of the problems associated with the formation of anhydropodorhizol (45), that the mixture of the two alcohols could be used toward the synthesis of labelled yateins. The revised strategy involved hydrogenolysis of the benzylic hydroxyl group to the corresponding methylene, as shown in the following diagram, Figure 17:

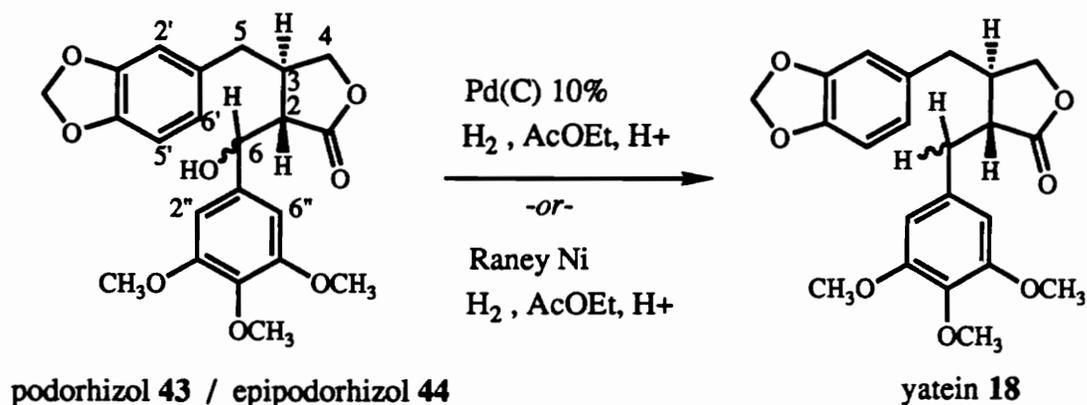
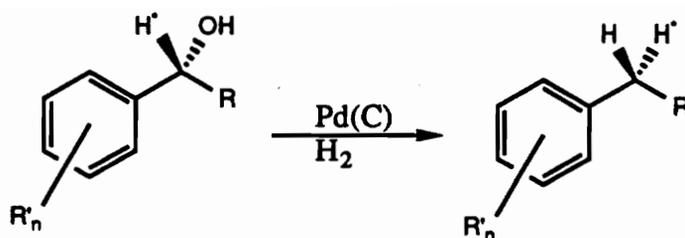
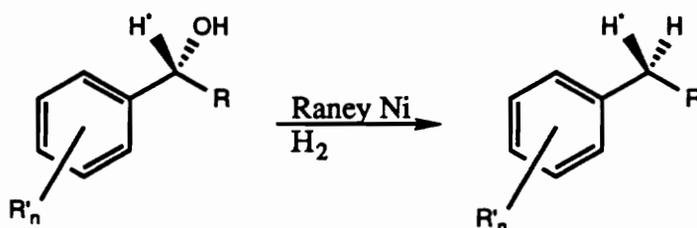


Figure 17: Hydrogenolysis of podorhizol (43) and epipodorhizol (44), leading to yatein (18)

It is known that hydrogenolysis of benzyl alcohols can be achieved by using either palladium-on-carbon (10%) or Raney nickel. In the case of palladium, it has been reported^{75,76} that hydrogenolysis proceeds with inversion of configuration, i.e.:



And, conversely, in the case of Raney nickel, it has been reported⁷⁵⁻⁸⁴ that hydrogenolysis proceeds with retention of configuration:



Hydrogenolysis over either metal is thought to proceed through π -adsorption of the phenyl group onto the metal surface, followed by formation of a π -benzylic complex with the catalyst, in a manner similar to the formation of π -allyl complexes with various transition metals. The difference in the nature of hydrogenolysis over these two metals is attributed in part to the greater affinity of nickel for oxygen, such that a given substrate adsorbs with three substituents, including the hydroxyl group, on Raney nickel; and only with two substituents, excluding the hydroxyl group, on palladium catalysts. These differences can be depicted as shown in **Figure 18**, where (*) denotes the metal surface.

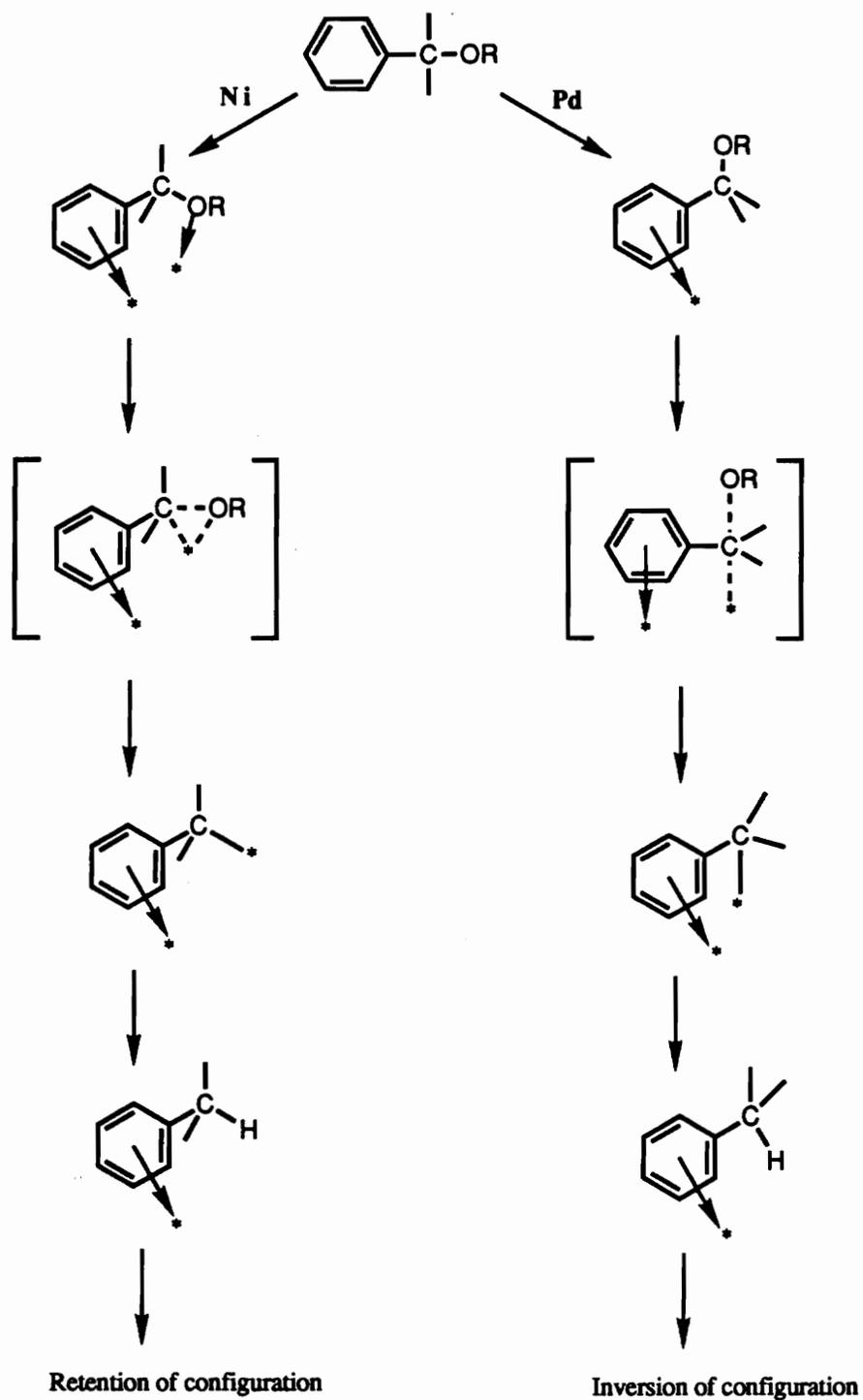


Figure 18: Mechanism of stereospecific hydrogenolysis over nickel and palladium⁷⁵

A mixture of the two alcohols podorhizol (43) and epipodorhizol (44) was subjected to hydrogenolysis over palladium-on-carbon (10%), with and without a catalytic amount of acid (HCl or HClO₄), since it has been reported that the addition of strong acid to hydrogenolysis reactions greatly enhances the reaction rate.^{83,84} Without the addition of acid the reaction times were typically quite long (i.e. > 120 hours); however the addition of acid decreased the reaction time significantly, to between 4 and 16 hours. In both cases (no acid added or acid added) the product mixture showed that one of the alcohols of the mixture had been consumed while the other remained essentially unreacted, by TLC analysis, based on the relative UV intensities of the remaining alcohol and the newly formed less polar material. By TLC comparison to podorhizol (43) and epipodorhizol (44) standards, it was determined that the unreacted alcohol was podorhizol (43). The residue was subjected to preparative TLC, and the less polar compound was isolated in 27% yield (as calculated from the starting mixture of alcohols). Characterization of this material by NMR and MS confirmed its identity as yatein (18), based upon comparison to values reported in the literature.⁸⁵

It is both an interesting and unexpected finding that only one of the alcohols, namely epipodorhizol (44), undergoes hydrogenolysis under these conditions. This may be explained by examining molecular models or suitable Newman projections, as depicted below in Figure 19. The first configuration, A, that of epipodorhizol (44), shows a situation in which formation of a hydrogen bond between the C-6 hydroxyl group and the lactone carbonyl is possible because there is little steric interaction between the bulky trimethoxyphenyl group and the lactone ring. In the second configuration, B, that of podorhizol (43), hydrogen bonding between the C-6 hydroxyl group and the lactone carbonyl is unfavorable due to the vicinal interaction of the trimethoxyphenyl and the lactone ring. This interaction results in rotation around the C-6 / C-2 bond to the conformer

C, allowing a lower energy steric arrangement, but creating a situation in which hydrogen bonding is no longer possible. These steric arguments have been reported and are supported by NMR findings.⁴⁴ Thus, the difference in hydrogen bonding ability of the two alcohols could account for the significant difference in their reactivities, since it has been well-established, by placing various substituents on the benzylic oxygen, that the relative rate of hydrogenolysis increases in proportion to the leaving group ability of the group at the benzylic position.^{76,78-82} Hydrogen bonding in epipodorhizol (**44**) would cause a slight lengthening of the benzylic carbon-oxygen bond, leading to an enhanced rate of hydrogenolysis. Furthermore, the difference in the conformations of the two alcohols would allow, in the case of epipodorhizol (**44**), the possibility of " π -stacking" of the two aromatic rings, which might serve to further stabilize the alcohol **44** in the configuration favorable for hydrogen bonding. In the case of podorhizol (**43**), π -stacking might be permitted; however this does not lead to any enhancement in hydrogen bonding ability. Thus, the difference in hydrogen bonding ability and the resulting rate enhancement may explain why there is an apparent preference for the hydrogenolysis of epipodorhizol (**44**), as depicted in **Figure 20**.

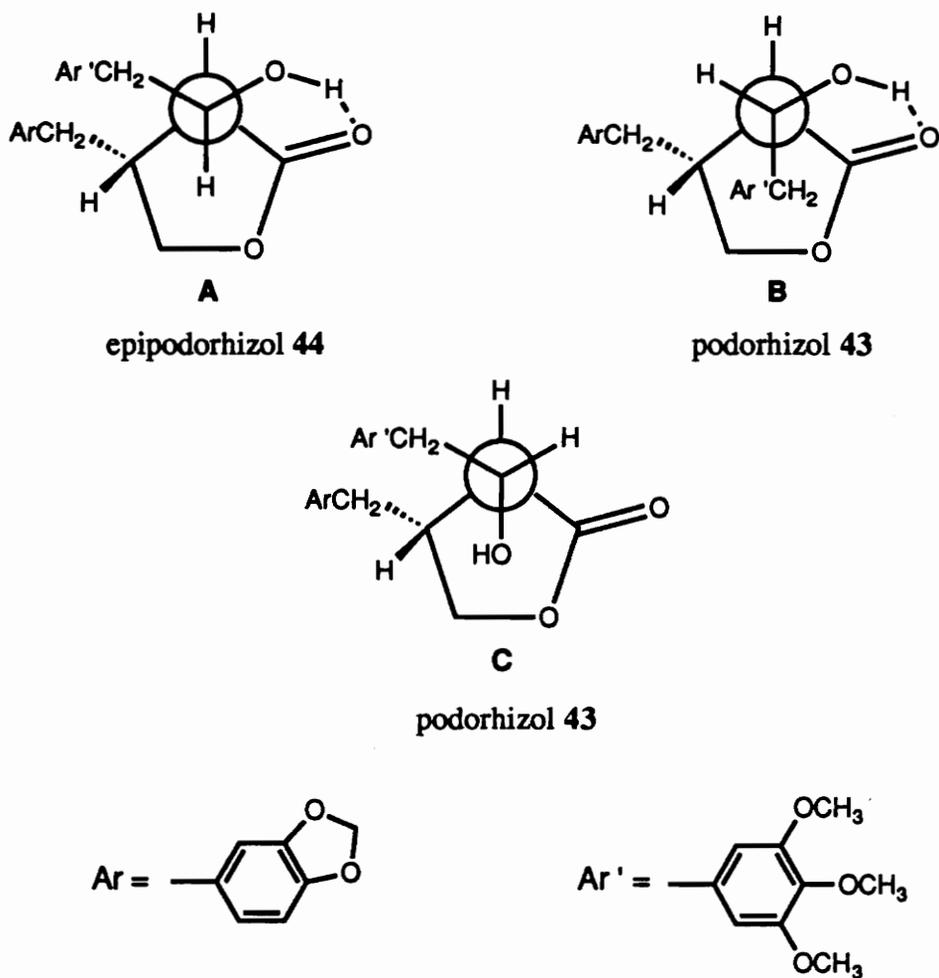


Figure 19: Newman projections showing configurations about the C-6 / C-2 bond for epipodorhizol (**44**) and podorhizol (**43**)

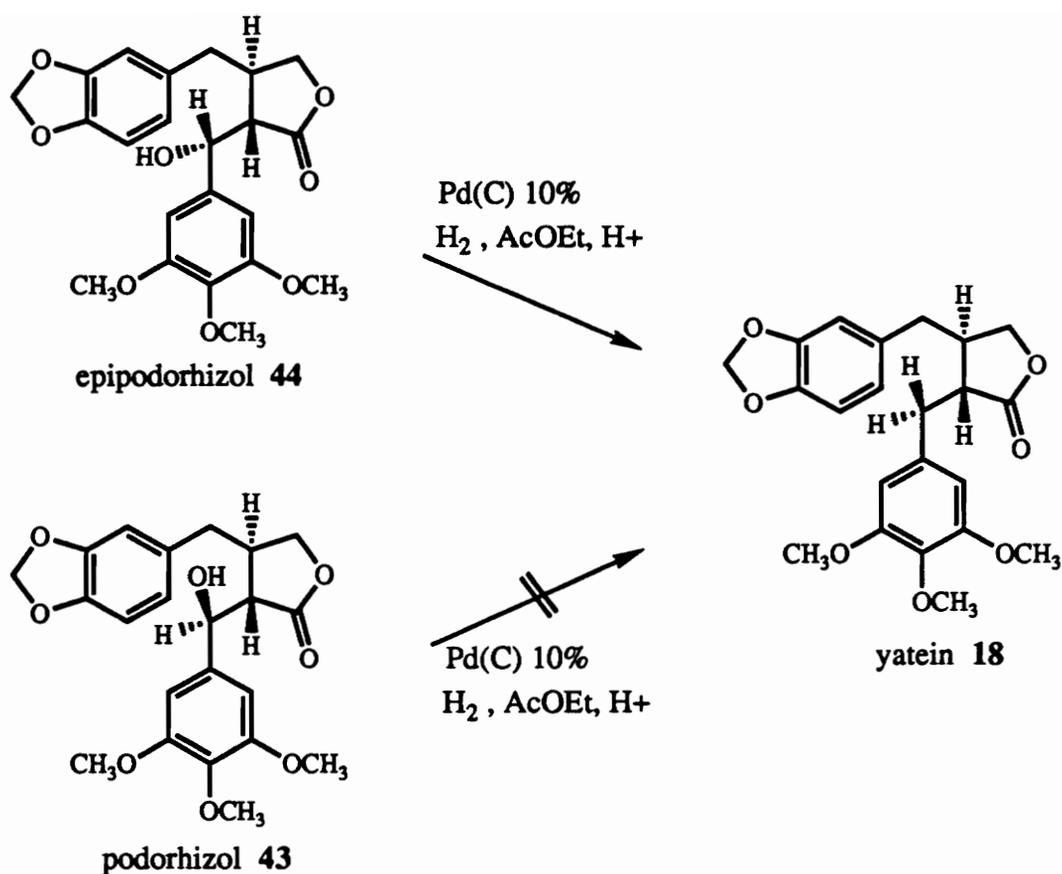


Figure 20: Preferential hydrogenolysis of epipodorhizol (44) to yatein (18)

In light of the successful hydrogenolysis of epipodorhizol (44) to yatein (18), it is interesting that Kamil and Dewick reported that attempted hydrogenolysis of either podorhizol (43) or epipodorhizol (44) proved to be unsuccessful, and that “both isomers proved extremely inert...under a variety of conditions” towards catalytic hydrogenolysis.⁵⁹

In an attempt to synthesize the desired $6\beta\text{-}^2\text{H}_1\text{-yatein}$ (18a), epipodorhizol (44) was subjected to hydrogenolysis over palladium-on-carbon (10%) using deuterium gas. The resulting product was then purified by preparative TLC and analyzed by NMR and

MS. Unexpectedly, the ^1H NMR spectrum appeared to be nearly identical to the spectrum for unlabelled yatein (18), i.e, the characteristic “doublet of doublets” (actually part of a more complex ABX pattern) at about 2.91 ppm, due to the protons on C-6 of yatein (18), remained unchanged in general appearance in the supposedly labelled material. It was expected that this signal would condense to a doublet upon placing a label at the 6-position. Additionally, the integration for the aromatic portion of the NMR spectrum for the “labelled” material appeared to be substantially less than for unlabelled yatein (18). Upon inspection of the mass spectrum for this compound, it was noted that instead of the expected sharp molecular ion peak at 401 mass units, there was instead a Gaussian distribution centered at 403 mass units. Coupled with the information from the NMR spectrum, this led to the conclusion that instead of an isotopic label being placed at the desired 6 β -position, hydrogen from the “catalytic” amount of concentrated (unlabelled) HCl used was placed at the 6-position . Upon formation of the hypothesized π -benzyl - palladium complex, deuterium was allowed to exchange for the aromatic protons. Since the aromatic protons differ in their reactivity, exchange of deuterium for the aromatic protons would presumably follow a Gaussian-type distribution, as seen in the mass spectrum. It is reasonable to assume that had the reaction been allowed to proceed for a sufficiently long time, the NMR and MS results would indicate full deuterium exchange for the five aromatic hydrogens, since once placed on the aromatic rings any re-exchange of hydrogen for the isotope would be rendered less likely, due to the primary isotope effect.

Because of the problems apparently arising from the use of non-deuterated acid, a number of new experiments was tried, using deuterated acids, i.e. 37% DCl in D₂O or 69% DCIO₄ in D₂O. However, in many of these experiments, the amount of labelled yatein formed was negligible, and precluded obtaining a reasonably conclusive NMR or mass spectrum. When enough material could be isolated, the NMR and MS spectral data

indicated no label placement at the 6 β position, but rather on the aromatic rings. In these experiments, the low amount of product formed may be due to the reduced activity of the deuterated acids relative to their unlabelled counterparts, or to the fact that the catalyst, palladium-on-carbon, supplied by Aldrich Chemical Company, typically contains 2% to 4% water (as reported in conversation with Aldrich technical representatives), and may have hydrogen already adsorbed onto its surface prior to use, depending on the method(s) of preparation. In addition, a second compound isolated from the reaction mixture was identified by ^1H NMR and mass spectral data as isodesoxy podophyllotoxin, having a structure similar to desoxy podophyllotoxin (20), but differing by its β -orientation of the pendant trimethoxyphenyl group. Formation of this compound was hypothesized to occur in a straightforward manner, since under the acidic conditions of the hydrogenolysis, protonation of the hydroxyl group of epipodophyllotoxin would readily occur. Participation by a lone pair of electrons on one of the oxygens of the methylenedioxy group could then result in nucleophilic attack by the methylenedioxy-substituted aromatic ring on the carbon carrying the protonated hydroxyl, thus displacing water and giving rise to a cyclized intermediate, which would subsequently rearomatize to give the compound identified.

At this point, it was thought that if 6- $^2\text{H}_1$ -epipodophyllotoxin (44a) could be formed, then the hydrogenolysis of this material with hydrogen and palladium-on-carbon (10%) (established as being successful previously in the synthesis of unlabelled yatein (18)) would lead to 6 α - $^2\text{H}_1$ -yatein (18b). Conversely, hydrogenolysis of this material using Raney nickel as the catalyst would lead to the retention of the isotopic label, affording 6 β - $^2\text{H}_1$ -yatein (18a), as indicated in Figure 21.

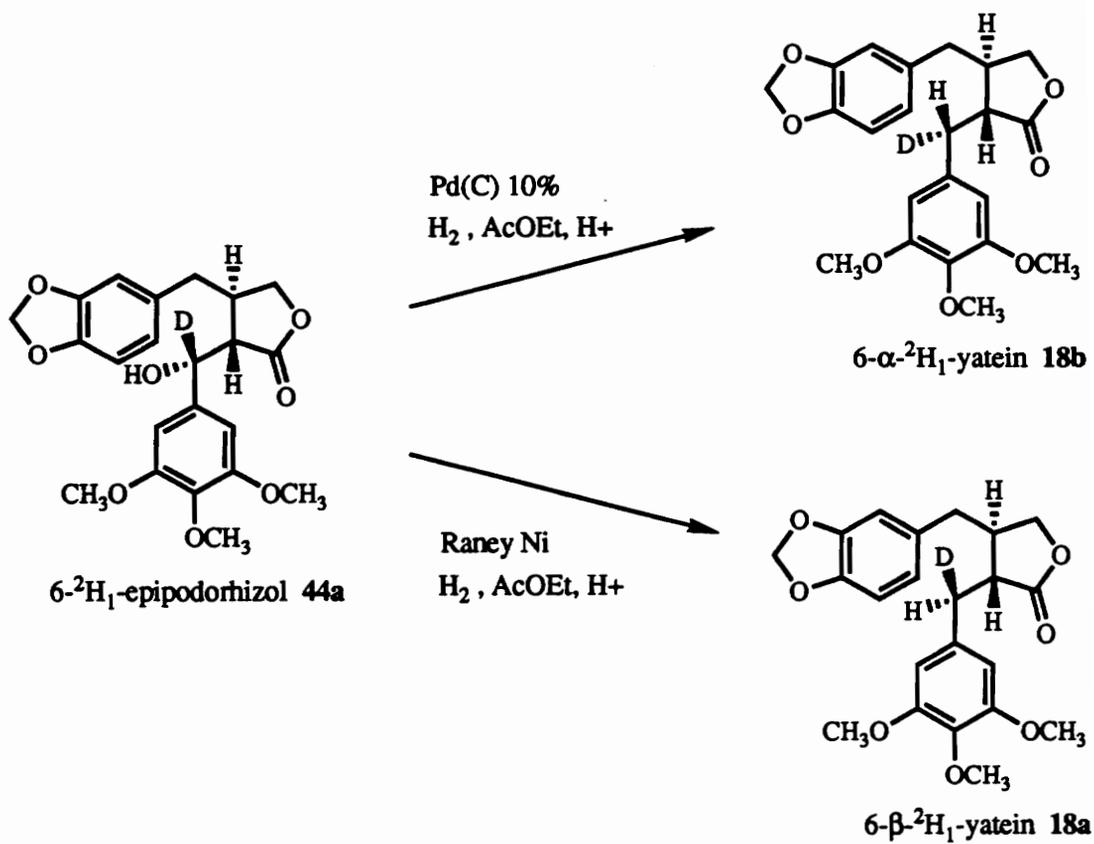


Figure 21: 6-²H₁-Epipodorhizol (44a) to 6- α -²H₁-yatein (18b),
and to 6- β -²H₁-yatein (18a)

In order to obtain 6-²H₁-epipodorhizol (**44a**), the following synthetic scheme (Figure 22) was developed:

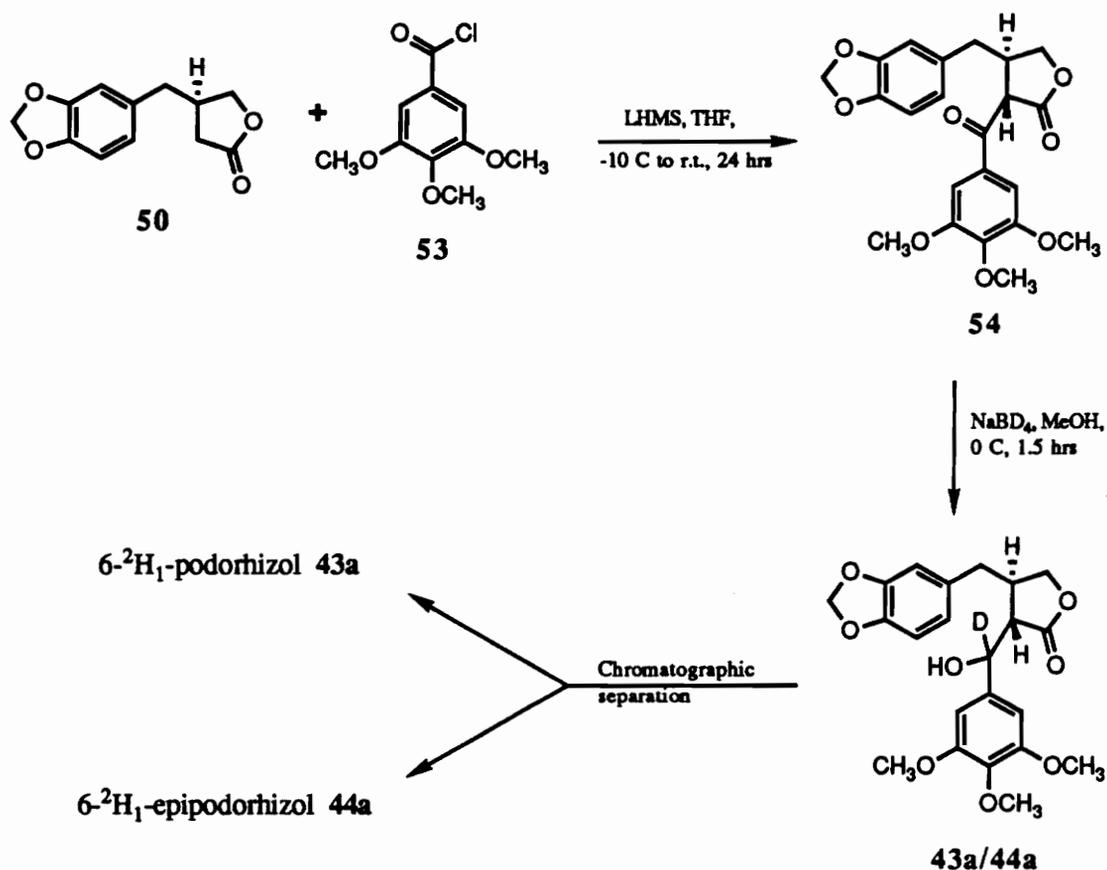


Figure 22: Proposed synthesis of 6-²H₁-epipodorhizol (**44a**) via podorhizone (**54**)

The first step involves base-catalyzed condensation of (*R*)-(+)-β-piperonyl-γ-butyrolactone (**50**) with 3,4,5-trimethoxybenzoyl chloride (**53**), to yield podorhizone (**54**). This compound can then be reduced to the corresponding mixture of deuterium-labelled alcohols (**43a** and **44a**) using sodium borodeuteride. Following chromatographic

separation, 6-²H₁-epipodorhizol (**44a**) can be obtained to be converted, via hydrogenolysis, to deuterium-labelled yatein (**18a** and **18b**).

(*R*)-(+)-β-Piperonyl-γ-butyrolactone (**50**) was treated with the base lithium hexamethyldisilazide to form the lactone enolate. The enolate was then allowed to react with 3,4,5-trimethoxybenzoyl chloride (**53**). The crude product mixture was purified by flash chromatography and upon standing, those fractions containing the product, podorhizone (**54**), yielded long, very fine, colorless needles. Yields for this reaction were typically somewhat low, usually around 30% (A later attempt, using 3,4,5-trimethoxybenzoyl imidazole instead of the benzoyl chloride, gave a substantially improved yield of 72%).

Podorhizone (**54**) was then treated with sodium borodeuteride in methanol/methylene chloride. The product mixture obtained was purified by preparative TLC. The desired alcohol, 6-²H₁-epipodorhizol (**44a**), was isolated in about 50% yield. The other alcohol, 6-²H₁-podorhizol (**43a**), was also isolated, in about 45% yield.

6-²H₁-Epipodorhizol (**44a**) was subjected to hydrogenolysis using hydrogen gas and palladium-on-carbon (10%), with a catalytic amount of HClO₄. The crude, colorless product mixture was subjected to preparative TLC, and the desired component, 6-α-²H₁-yatein (**18b**) was isolated in 30% yield. The yield was somewhat lower than expected, however enough material was obtained to allow characterization by both NMR and mass spectrometries. The spectral data appears indicates that the desired compound (**18b**) was indeed formed. Of particular note is the change in appearance of the "doublet of doublets" at about 2.91 ppm into a doublet, indicating that there is only one proton on C-6 of the yatein, and that it is being split only by the proton on the adjacent C-2 (i.e., no geminal splitting). Additionally, the mass spectrum shows a molecular ion peak at 401 mass units, apparently indicating a single deuterium label. The exact stereochemistry of the deuterium

label was not definitively established, but based on literature precedent the use of palladium in the hydrogenolysis reaction should have resulted in inversion of the pre-placed label. Thus the deuterium, which was in the β -configuration in the labelled epipodorzol, would have presumably been inverted to the α -configuration in the final product, giving 6- α - $^2\text{H}_1$ -yatein (**18b**).

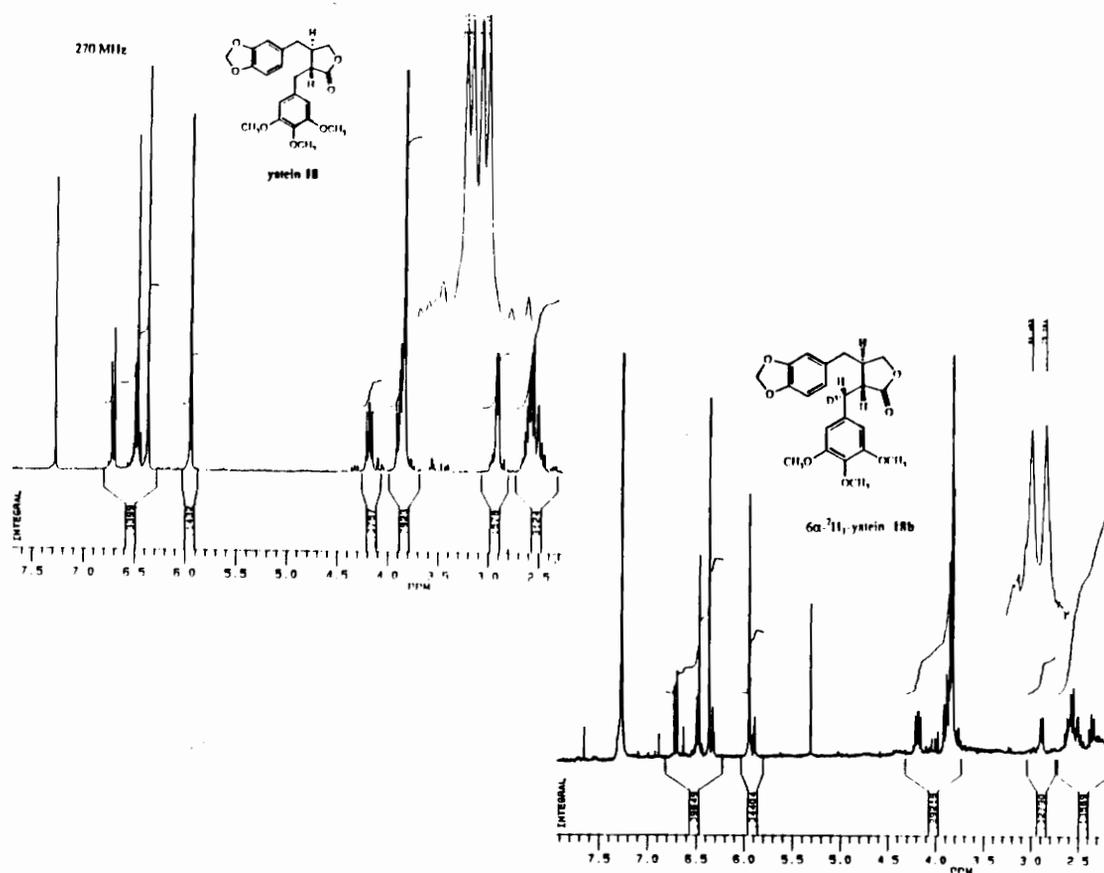


Figure 23: ^1H NMR spectra of yatein (**18**) and 6- α - $^2\text{H}_1$ -yatein (**18b**)

Due to the problems associated with obtaining $6\beta\text{-}^2\text{H}_1\text{-yatein}$ (**18a**) from the hydrogenolysis of epipodorhizol (**44**) using deuterium over palladium-on-carbon, an alternate approach was undertaken. In this approach, $6\text{-}^2\text{H}_1\text{-epipodorhizol}$ (**44a**) was envisioned to undergo hydrogenolysis over Raney nickel to give the desired yatein (**18a**). However, in several attempts at hydrogenolysis using Raney nickel, formation of the labelled yatein **18a** was negligible, even when prolonged reaction times (i.e. >135 hours) were employed. The lack of product formation was mainly attributed to the lower reactivity of the nickel catalyst relative to the palladium catalyst. This unfortunate property was reported in several papers on hydrogenolysis of benzylic alcohols over Raney nickel.^{75,76,80,81} Furthermore, unlike the case when a palladium catalyst is used, addition of catalytic amounts of acid (HCl and HClO₄) did not enhance the reaction rate of hydrogenolysis of $6\text{-}^2\text{H}_1\text{-epipodorhizol}$ (**44a**) over Raney nickel.

Another attempt at forming $6\beta\text{-}^2\text{H}_1\text{-yatein}$ (**18a**) was made, by using epipodorhizol (**44**), palladium-on-carbon (5%), and deuterium gas. In this reaction several precautions were taken to ensure that no exchangeable protons were present in the reaction mixture: the catalyst was twice stirred in 99.9+% D₂O and allowed to equilibrate with deuterium gas for 24 hours; the epipodorhizol (**44**) was twice dissolved in CDCl₃ to azeotrope with any residual exchangeable proton-containing solvent in the substrate; and the acid catalyst used was freshly obtained 70% DCIO₄ in D₂O, containing 99.9+% isotopic label. The resulting product, purified by preparative TLC, was analyzed by NMR and MS. With the exception of the aromatic proton region having a slightly lower integration value, the ¹H NMR spectrum of the isolated compound appeared identical in nearly every respect to the spectrum of unlabelled yatein (**18**), including a "doublet of doublets" appearing in the spectrum at about 2.91 ppm. The 2.91 ppm multiplet gave an integration value equivalent to two protons. The mass spectrum obtained for the compound appeared similar to that

obtained previously (page 58) by the same route, i.e., displaying a Gaussian distribution of mass peaks centered at 403 mass units. Apparently, the precautions taken were not sufficient to allow the desired yatein **18a** to be formed -- despite fairly exhaustive exchange conditions there must be some residual hydrogen remaining adsorbed on the metal surface. With the relatively large ratio of catalyst to substrate, this might explain the lack of label at carbon-6 of yatein. However, even in previous attempts when relatively small amounts of palladium-on-carbon were used, when the product could be isolated, the only difference in the ¹H NMR spectrum from that of unlabelled yatein was the reduction in the aromatic proton peak integration.

II.3 Synthesis of Matairesinol Derivatives

The question of whether matairesinol (**16**) can serve as a precursor to yatein (**18**) in *Podophyllum* species has to date eluded an answer. If such a conversion occurs, then matairesinol (**16**) could also be considered as a precursor to podophyllotoxin (**5**), since it has been established that yatein (**18**) is a precursor to podophyllotoxin (**5**).⁵⁹ In order to investigate this possibility, the synthetic scheme shown in **Figure 24** was envisioned, following the method of Brown and Daugan.⁸⁶ This scheme not only outlines the synthesis of labelled matairesinol (**16a**), but the labelled matairesinol derivatives **64a**, **64b**, and **64c** as well. The rationale for the synthesis of these derivatives is that their use in incorporation studies in *Podophyllum* plants might lend even greater insight into the postulated matairesinol-to-yatein conversion, by simulating possible biosynthetic intermediates. While the hypothesized matairesinol-to-yatein conversion was generalized in **Figure 15**, presently it is not known in which order the individual biotransformations might occur. The possible transformations are depicted in **Figure 25**. Thus the synthesis of the labelled compounds indicated by number in **Figure 25** might help to better establish, through successful incorporation into podophyllotoxin (**5**), the likelihood and order of the proposed biotransformations involving such compounds.

Another set of transformations, shown in **Figure 26**, was also envisioned, but synthesis of labelled analogs of these potential biosynthetic intermediates was regarded as being beyond the scope of this project.

Similar to the synthetic scheme followed for the synthesis of labelled yatein (**18**), the synthesis of labelled matairesinol (**16**), as shown in **Figure 24**, is initiated by a Stobbe condensation, involving vanillin (**55**), dimethyl succinate, and lithium methoxide as base, in refluxing methanol, yielding α -vanillylidene succinic acid half-methyl ester (**56**).

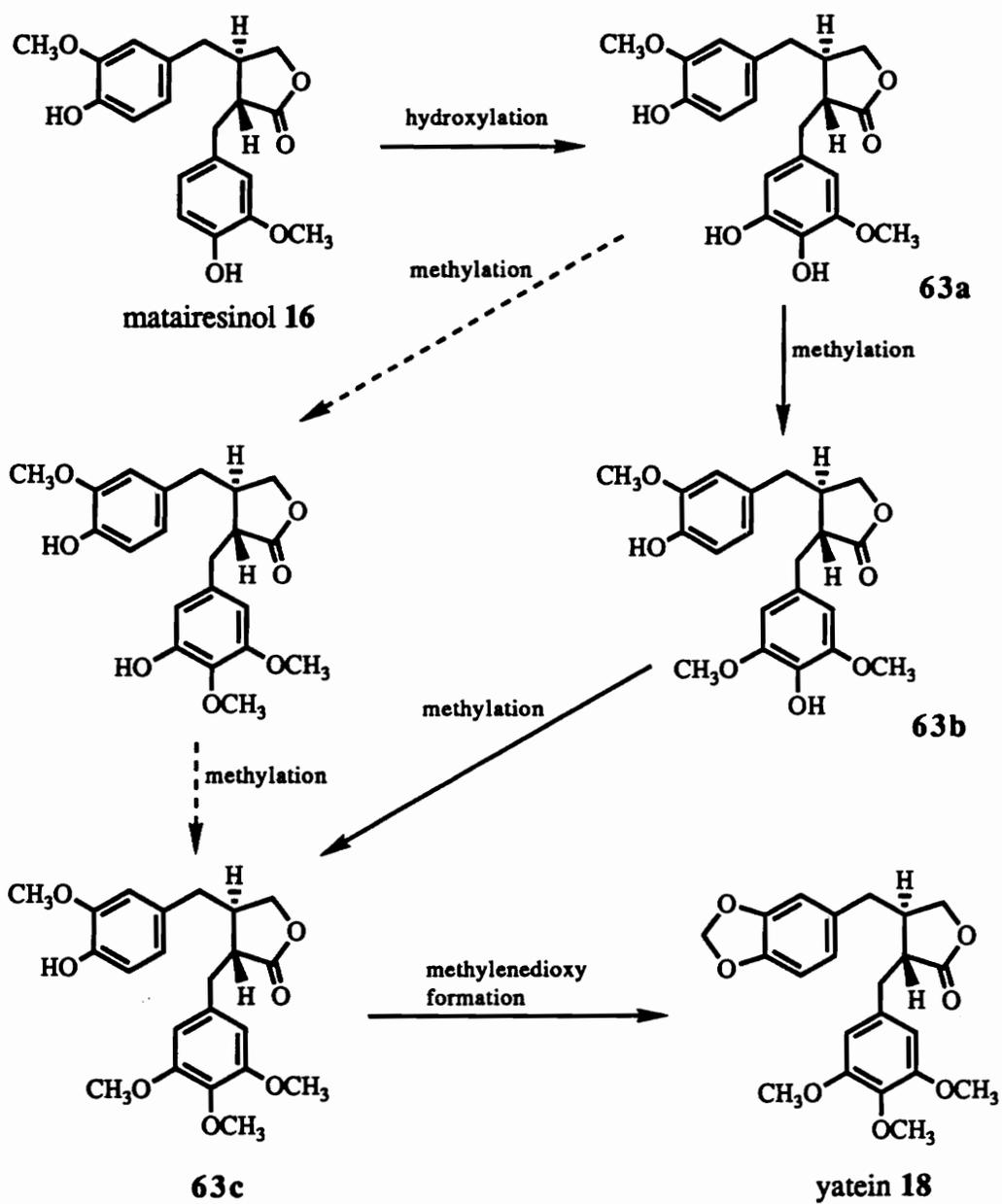


Figure 25: Possible biotransformations leading from matairesinol (16) to yatein (18)

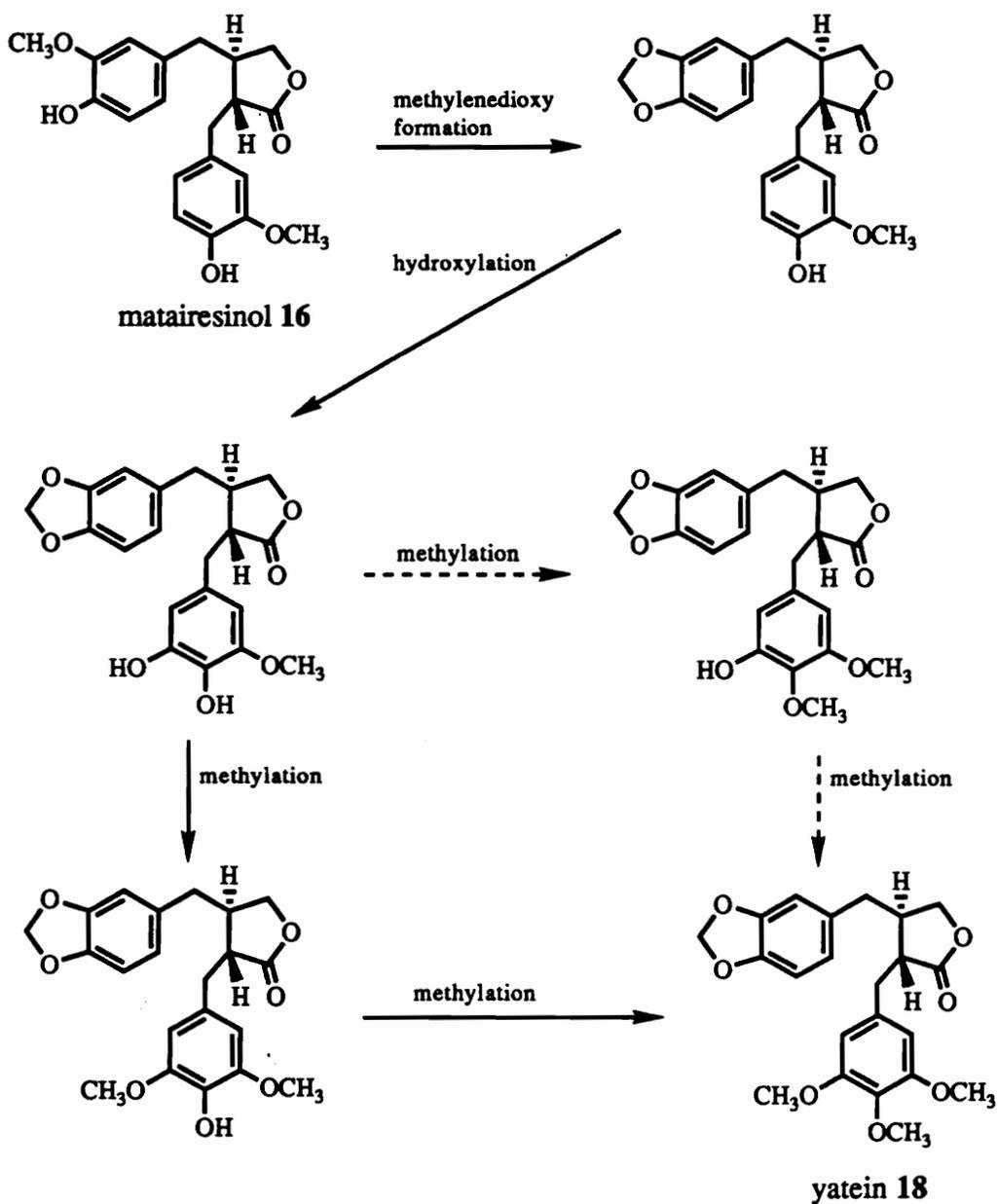
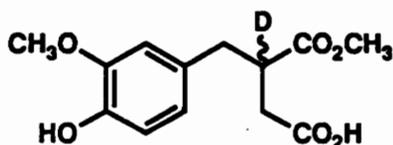


Figure 26: Possible biotransformations leading from matairesinol (16) to yatein (18)

This material is hydrogenated, giving racemic α -vanillyl succinic acid half methyl ester (**57**). It would also be possible at this point to treat compound **57** with base in order to form the ester enolate, followed by quenching of the enolate with the isotopically labelled water, deuterium oxide, in order to place a single isotopic label on the molecule:



57b

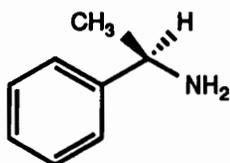
Treatment of compound **57** with (*R*)-(+)- α -methylbenzylamine (**58**) in ethyl acetate would allow the isolation of the desired enantiomer, as the (*R*)-(+)- α -methylbenzylamine salt. Subsequent acidification of the salt affords the desired (*R*)-(+)- α -vanillyl succinic acid half-methyl ester (**57a**). The potassium salt is then formed, followed by treatment with calcium borohydride to obtain the protected (*R*)-(+)- β -vanillyl- γ -butyrolactone (**59**). Protection of **59** as the benzyl ether yields compound **60**, which is subjected to basic conditions with lithium diisopropylamine to form the lactone enolate, followed by condensation with protected vanillyl bromide (**61a**), or related benzyl bromide (**61b**, **61c**, **61d**), to give protected, labelled matairesinol (or derivatives) **62a**, **62b**, **62c**, and **62d**. Alkylation of the enolate was envisioned to take place such that only the *trans* isomer resulted, based on the author's results. This stereochemistry can be attributed mainly to the steric bulk of the aromatic rings, favoring approach from the least hindered side, i.e. *trans*. Deprotection of this compound using palladium-on-carbon would afford matairesinol **16** or its derivatives **63a**, **63b**, **63c**, which can then be treated with a deuterium-labelled acid solution to exchange the aromatic protons, thus affording the series of isotopically-labelled matairesinol analogs **16a**, **64a**, **64b**, **64c**. As in the case of the labelled yatein synthesis,

this methodology could be extended to using tritium as the isotopic label. There is some question as to the degree of loss of the aromatic labels due to re-exchange with water within *Podophyllum* plants once incorporation studies are initiated; however, it is speculated that under the physiological conditions of the plant (i.e., nearly neutral pH) this occurrence should be relatively minimal. Such a situation could be avoided using compound **57b**, as discussed above, and carrying this material through the subsequent steps of the synthesis, affording matairesinol or matairesinol derivatives with a single, non-exchangeable label.

Several attempts were made at preparing α -vanillylidene succinic acid half-methyl ester (**56**) via Stobbe condensation of vanillin (**55**) and dimethyl succinate, using lithium methoxide as base, according to the procedure of Brown and Daugan.⁸⁶ The authors reported that the use of lithium methoxide allowed the condensation to be performed without the need for prior protection of the vanillin hydroxyl group, with a 90% yield compared to a 20% yield using sodium methoxide. However, in our hands, the use of lithium methoxide proved to be unsatisfactory, as yields were generally very low, in the 3% to 15% range. Use of sodium methoxide did not appreciably improve the yields (4% to 16%) without prior protection of the vanillyl hydroxyl group. However, initial protection of vanillin with benzyl bromide (methanol/potassium carbonate; 64% to 74% yield) and condensation of this material **55a** with dimethyl succinate using sodium methoxide in refluxing methanol gave more satisfactory results, with product yields in the range of 55% to 65%.

The product, **56**, from the above reaction, was hydrogenated (and deprotected, since under these conditions the benzyl group is readily cleaved) using palladium-on-carbon (5% or 10%) in acetic acid, to yield **57** in typical yields of 55% to 65% after recrystallization.

The racemic α -vanillyl succinic acid half-methyl ester (**57**) was treated with (*R*)-(+)- α -methylbenzylamine (**58**) in ethyl acetate to accomplish chiral resolution. Similar to (-)-ephedrine (**49**) in its function as a chiral resolving agent, α -methylbenzylamine (**58**) is the most widely used synthetic resolving agent.⁷³



(*R*)-(+)- α -methylbenzylamine (**58**)

After several recrystallizations of the salt from ethyl acetate, the material was treated with acid to afford the desired (*R*)-(+)- α -vanillyl succinic acid half-methyl ester (**57a**). Typical yields ranged from 19% to 33%, which corresponds to a range of 38% to 66% α -H enantiomer recovery. An enantiomeric purity of 93.9% was established on the basis of comparison with known optical rotation and melting point values. It is noteworthy that when the racemic α -vanillyl succinic acid half-methyl ester (**57**) was reprotected with benzyl bromide (methanol/potassium carbonate) prior to attempts at chiral resolution, the salts which formed failed to crystallize. The only product obtained was a brown-yellow, viscous oil, which did not produce crystals even upon prolonged (several weeks) standing at -4°C .

Formation of the (*R*)-(+)- β -vanillyl- γ -butyrolactone (**59**) proceeded as follows: the enantiomerically pure α -vanillyl succinic acid half-methyl ester (**57a**) was converted to its potassium salt by treatment with KOH and the potassium salt was then treated with

calcium borohydride, affording the product lactone **59** as colorless crystals, in about 60% yield.

Prior to subsequent steps in the proposed synthesis, it was necessary to reprotect the phenolic hydroxyl group. This was done by treating **59** with benzyl bromide (acetone/potassium carbonate, 16 hr reflux), to obtain **60** as colorless crystals in 57.9% yield.

Protected vanillin is also necessary for the preparation of the vanillyl bromide to be used in coupling to the O-benzyl-(*R*)-(+)- β -vanillyl- γ -butyrolactone (**60**), in order to obtain protected matairesinol (**62a**). The reaction sequence for obtaining the protected vanillyl bromide is shown below in Figure 27, and follows the method of Barton, *et al.*⁸⁷ As mentioned above, protection of vanillin (**55**) proves to be relatively straightforward and involves treatment with benzyl bromide. The product, O-benzyl vanillin (**55a**), is thus obtained as fine, very pale yellow crystals, in 64% to 75% overall yield. The second step in the sequence is the reduction of O-benzyl vanillin (**55a**) to the corresponding alcohol, by treatment with sodium borohydride. The product, **55b**, is obtained, as colorless crystals in about 80% yield after chromatography. The crystalline material **55b** is converted to the bromide **61a**, by reaction with N-bromosuccinimide and dimethyl sulfide. The product of this reaction is obtained in about 50% yield, after purification.

O-Benzyl-(*R*)-(+)- β -vanillyl- γ -butyrolactone (**60**) was treated with O-benzyl vanillyl bromide (**61a**), using LHMS as base, to obtain di-O-benzyl matairesinol (**62a**), as a very pale yellow oil, in 69% yield. Di-O-benzyl matairesinol (**62a**) was then deprotected by hydrogenation over palladium-on-carbon (5%), affording matairesinol (**16**), as colorless crystals (after recrystallization from ethyl acetate), in 80% yield. The deuterium-labelled matairesinol (**16a**) was obtained by treating matairesinol (**16**) with DCIO₄ in

THF/ CDCl_3 (r.t., 4hr). Confirmation of full exchange of deuterium for the aromatic hydrogens of matairesinol (**16**) was made by ^1H NMR and MS data.

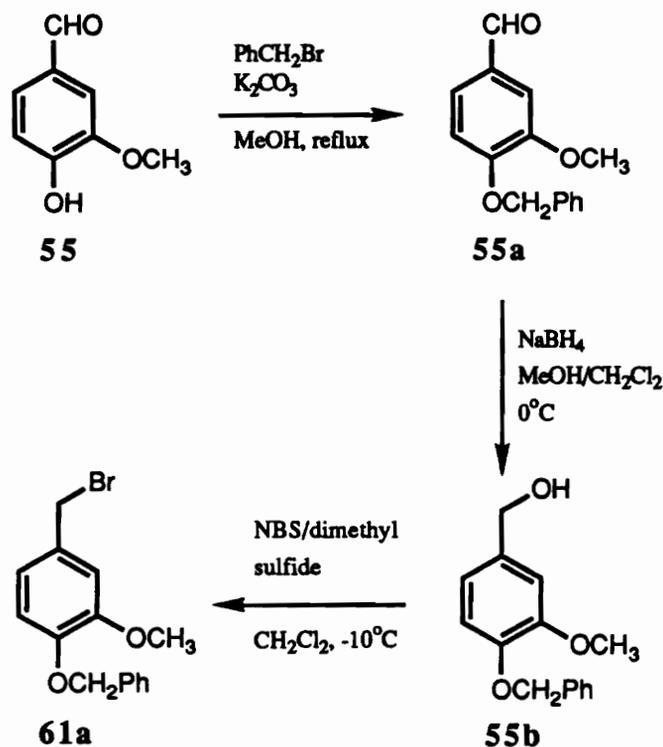
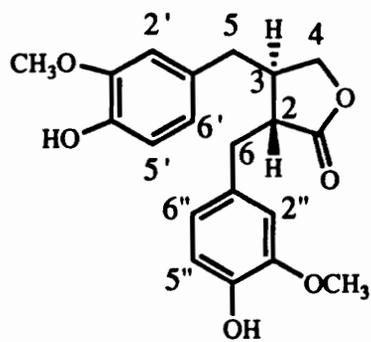


Figure 27: Synthesis of protected vanillyl bromide (**61a**).

In order to explore the incorporation potential of other matairesinol derivatives, as mentioned above, it is necessary to prepare the brominated compounds **61b**, **61c**, and **61d**, which, after coupling to O-benzyl-(*R*)-(+)- β -vanillyl- γ -butyrolactone (**60**), followed by deprotection and deuterium exchange, would afford the series of matairesinol derivatives **64a**, **64b**, and **64c**.



matairesinol **16**

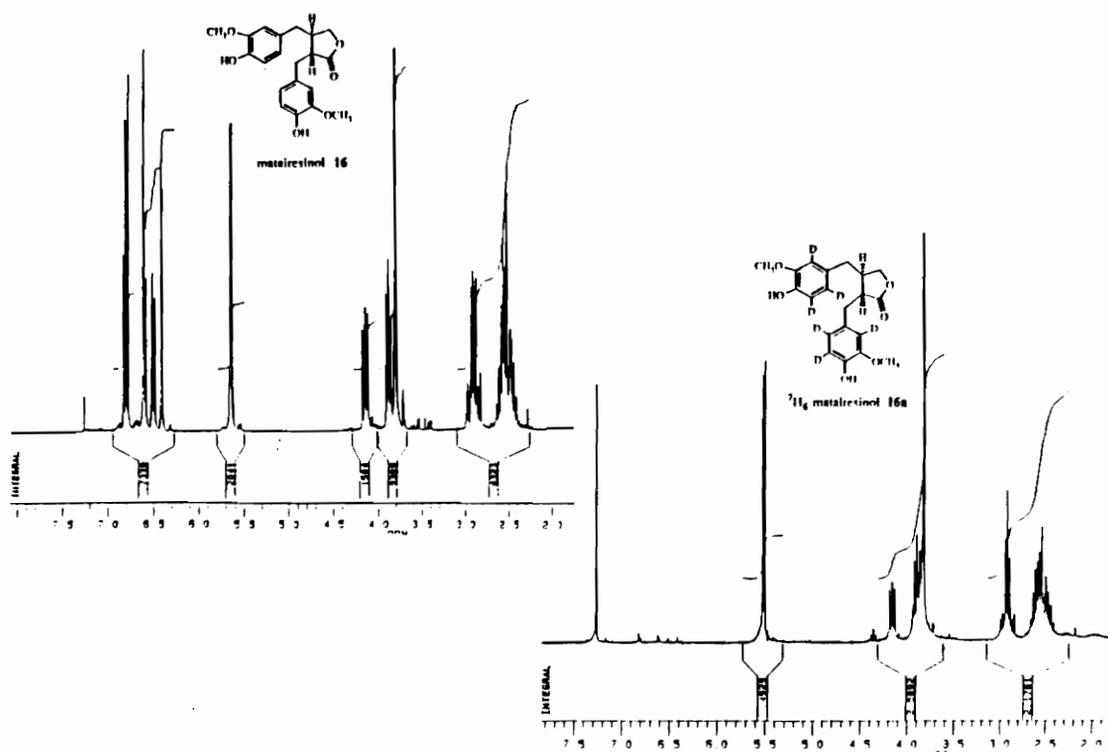


Figure 28: ^1H NMR spectra for matairesinol (**16**) and $^2\text{H}_6$ -matairesinol (**16a**)

Preparation of compounds **61b** and **61c** was envisioned to follow the reaction scheme shown in **Figure 29**, which is similar to the reaction scheme (**Figure 27**) for the preparation of O-benzyl vanillyl bromide (**61a**).

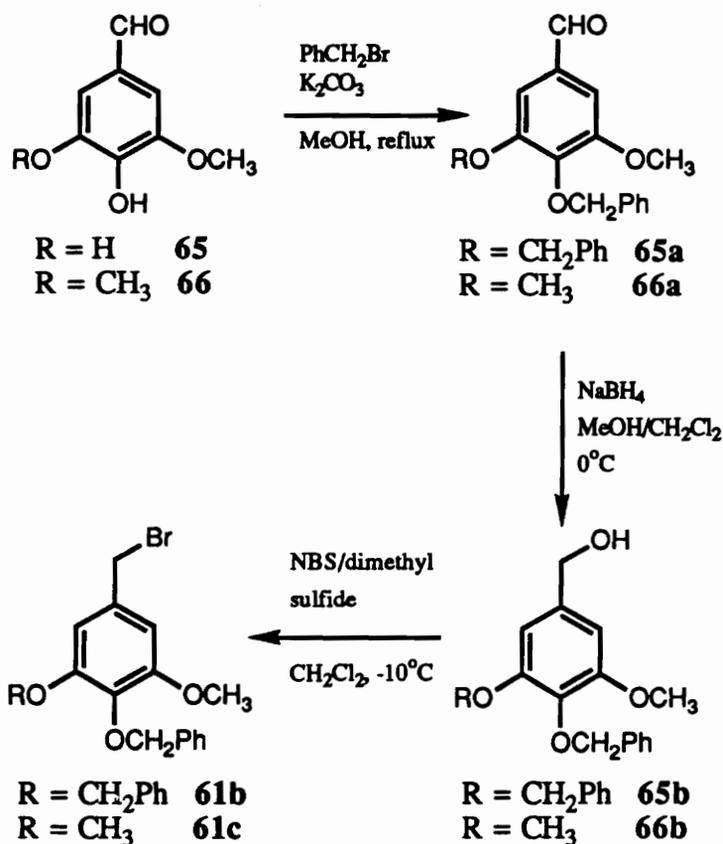


Figure 29: Synthesis of 3,4-di-O-benzyl-5-methoxybenzyl bromide (**61b**) and O-benzyl syringyl bromide (**61c**).

The first step involves protection of the phenol group of compounds **65** and **66**, using benzyl bromide (K₂CO₃, acetone, reflux) to afford compounds **65a** and **66a**, in yields of 67% and 65%, respectively. The second step is the sodium borohydride

reduction of the aldehyde of compounds **65a** and **66a** to the corresponding alcohols, **65b** and **66b**, which are obtained in 98% and 74% yield, respectively. The third step of the scheme shows the conversion of the alcohols to the corresponding benzyl bromides **61b** and **61c**. This is carried out by reaction with N-bromosuccinimide and dimethyl sulfide, giving the compounds **61b** and **61c** in 56% and 51% yield, respectively.

Preparation of compound **61d** was thought to be possible by following the same synthetic methodology as used for compounds **61a**, **61b**, and **61c**, as shown in **Figure 30**:

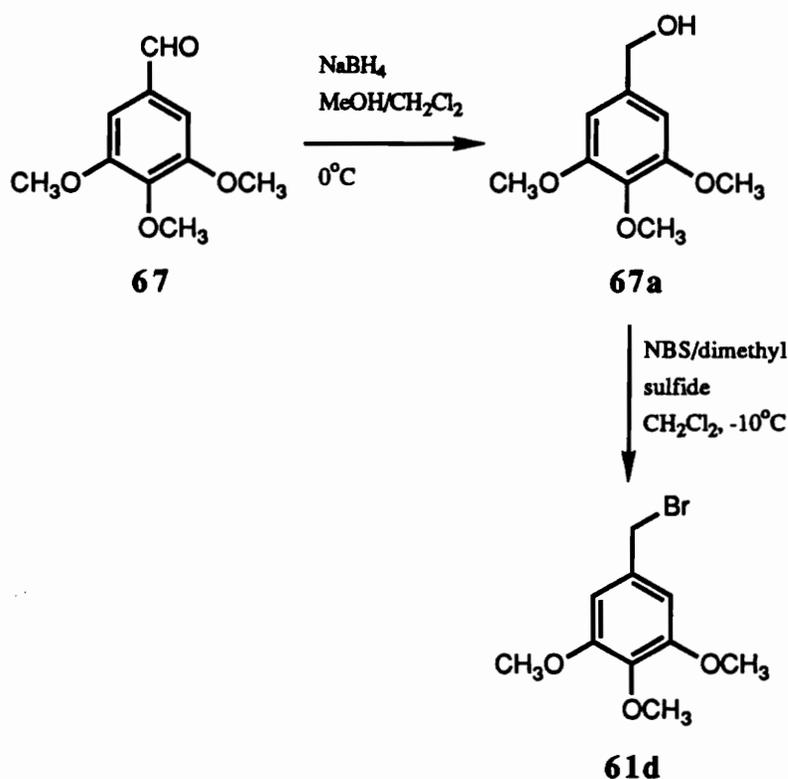
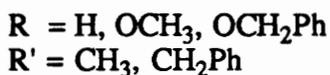
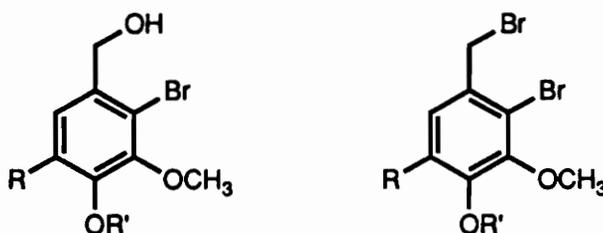


Figure 30: Synthesis of 3,4,5-trimethoxybenzyl bromide (**61d**).

The first step, reduction of 3,4,5-trimethoxybenzaldehyde (**67**) with sodium borohydride, went as planned, affording **67a** as a colorless, viscous oil, in quantitative yield. This material was converted to the corresponding benzyl bromide **61d** by reaction with N-bromosuccinimide and dimethyl sulfide, in about 55% yield.

Because of its supposedly milder conditions for converting a benzyl alcohol to the corresponding benzyl bromide, the N-bromosuccinimide / dimethyl sulfide method was initially thought to be more practical; however, as evidenced by the foregoing discussion, reaction yields were typically low (about 50%), with side products making up a large percentage of the reaction mixture. The side products of each reaction were isolated and all were determined by NMR and MS to have bromine on the aromatic ring, and either an intact benzyl alcohol group or a benzyl bromide, as depicted below:



Under the conditions of the reaction it is very likely that the bromide could undergo electrophilic attack on the aromatic ring at one of the two available sites. Once the bromine was on the ring it would destabilize the other position (*meta* to the bromine); hence, only one bromine was observed by spectral data to be on the ring. In the case of the dibromo

compounds, conversion of the benzyl alcohol to the benzyl bromide could take place either prior to or subsequent to the electrophilic attack of bromine on the ring.

Reasoning that the outcome of a reaction between the benzyl alcohols and phosphorous tribromide could be no more impractical than the above reactions involving Barton's method, an attempt was made at reacting 3,4-di-O-benzyl-5-methoxybenzyl alcohol with phosphorous tribromide in ether at room temperature. After 5 minutes the reaction appeared to be complete by TLC analysis, and after work-up, the desired benzyl bromide was isolated in nearly quantitative yield, and its identity confirmed by NMR and MS.

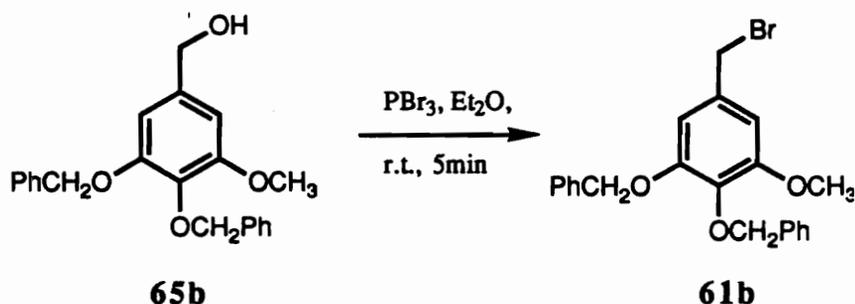


Figure 31: Conversion of 3,4-di-O-benzyl-5-methoxybenzyl alcohol (**65b**) to 3,4-di-O-benzyl-5-methoxybenzyl bromide (**61b**)

Having established the preparation of the series of substituted benzyl bromides **61b**, **61c**, and **61d**, and having successfully coupled compound **61a** with O-benzyl-(*R*)-

(+)- β -vanillyl- γ -butyrolactone (**60**), it was rationalized that coupling of **61b**, **61c**, and **61d** with the lactone **60** would prove to be equally facile. At this point it was decided that rather than performing the coupling, deprotection, and deuterium exchange reactions leading to compounds **64a**, **64b**, and **64c**, it would be more prudent to undertake preliminary incorporation studies using just the labelled matairesinol **16a**, in keeping with the project objective of establishing whether matairesinol itself can serve as a precursor to yatein (**18**) and/or podophyllotoxin (**5**).

With a deuterium-labelled yatein (**18b**) and deuterium-labelled matairesinol (**16a**) in hand, it is now possible to conduct a number of feeding studies using *Podophyllum* plants, in order to fulfill the primary goals of this project. Subsequent isolation of podophyllotoxin (**5**), in the case when the labelled yatein compound **18b** is employed, should allow the determination of whether the pro-*R* or pro-*S* label is lost or retained during the proposed cyclization. Isolation of yatein (**18**) and/or podophyllotoxin (**5**), in the case when labelled matairesinol (**16a**) is used, should allow the determination of whether yatein (**18**) and/or podophyllotoxin (**5**) can be derived from matairesinol (**16**) in *Podophyllum* species. The results of such a series of incorporation experiments will provide a much clearer understanding of the overall biosynthesis of podophyllotoxin (**5**), chiefly by identifying the precursor(s) involved in podophyllotoxin (**5**) biosynthesis, and by better establishing the nature of the metabolic processes involved, and will indicate whether further studies should be carried out using other labelled yateins and/or the labelled matairesinol derivatives **64a**, **64b**, and **64c**.

Thus, the syntheses that were performed and discussed above comprise the penultimate goal of research aimed at conducting relevant incorporation studies in *Podophyllum* plants. While 6β - $^2\text{H}_1$ -yatein (**18a**) was never successfully prepared, the

compounds 6α - $^2\text{H}_1$ -yatein (**18b**) and $^2\text{H}_6$ -matairesinol (**16a**) were prepared and can now be employed in the ultimate goal of this research, which is the use of these deuterium-labelled compounds in plant incorporation studies, and isolation of yatein (**18**) and/or podophyllotoxin (**5**), in order to determine if such compounds are indeed incorporated, the nature of the biotransformations they undergo, and the level of their incorporation. This aspect of the research will be conducted by Dr. Norman G. Lewis and his research group at the Institute of Biological Chemistry, Washington State University. Dr. Lewis, formerly a faculty member of the Department of Wood Science and Forest Products, Virginia Polytechnic Institute and State University, is a collaborator on this project. Based on the findings of Dr. Lewis' incorporation studies, it is conceivable that a broader series of incorporation studies could be initiated. In such a case, the deuterium-labelled matairesinol derivatives **64a**, **64b**, and **64c** could be employed, as well as other yatein and matairesinol derivatives carrying either deuterium or tritium labels. At this point, Dr. Lewis would be given any remaining compounds or precursors prepared in our laboratories and the synthetic methodology for the preparation of these compounds, and his group would carry out both the synthetic work and the incorporation studies.

III. EXPERIMENTAL

General

Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Optical rotation values were obtained on a Perkin-Elmer 241 polarimeter, using sodium D line illumination, at ambient temperature. Proton, deuterium, and ^{13}C NMR spectra were recorded on Bruker WP 200, WP 270, or Varian Unity 400 MHz spectrometers. Chemical shift values are given in parts per million (ppm) from the resonance of the internal reference standard, tetramethylsilane. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants are given in Hertz (Hz), and except where indicated, all spectra were recorded in deuteriochloroform (CDCl_3) at room temperature. Infrared spectra were recorded on a Perkin-Elmer 710B spectrophotometer. Low resolution electron ionization (EI) mass spectra were obtained on a VG Analytical 7070E-HF mass spectrometer. Analytical thin layer chromatography was performed on E. Merck aluminum-supported silica gel 60 F₂₅₄ (0.2mm) plates. Preparative thin layer chromatography was performed on 20cm x 20cm GF₂₅₄ (0.5mm) glass-supported plates. Silica gel for flash chromatography was E. Merck (230-400 mesh).

Formation of α -piperonylidene succinic acid half-methyl ester (47)

Sodium methoxide (25 wt.% solution in methanol; 46.77g solution; 216.5mmol) was added to 100ml methanol, and with stirring, brought to reflux. In the meantime, piperonal (46) (25.0g; 166.5mmol) and dimethyl succinate (26.8g; 183.2mmol) were dissolved in 50ml warm methanol. This solution was slowly added to the refluxing sodium methoxide solution, and the resulting solution was allowed to reflux for 42hr, at which time it was

determined by TLC analysis ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 15:1) that the piperonal (**46**) had been converted mainly to a more polar material ($R_f \sim 0.4$), although a number of side products was evident from the TLC plate. The reaction flask was allowed to cool to room temperature and the methanol was removed on a rotary evaporator. To the yellow-colored residue was added, with stirring, 150ml 1N HCl, and the solution was then extracted with diethyl ether (3 x 150ml). The combined ether portions were washed with 10% sodium bicarbonate (3 x 150ml) to separate the product **47** from starting material **46**. The combined aqueous layers were reacidified with 6N HCl and re-extracted with ether. The organic layer was washed with brine (3 x 75ml), then dried with anhydrous magnesium sulfate. After filtering off the drying agent, the solution was concentrated by rotary evaporation. Upon concentration, the solution yielded colorless crystals, which were twice recrystallized from ether. Yield: 15.6g (35.5%); melting point: 134.5-137.5°C (lit.⁵²: 136-138°C). ^1H NMR (CDCl_3) δ 7.82s, 1H, vinylic; 6.82-6.94m, 3H, aromatic; 6.01s, 2H, $-\text{OCH}_2\text{O}-$; 3.84s, 3H, CH_3 ; 3.60s, 2H, allylic. EIMS m/z (relative intensity) 264 (M^+ , 30), 232 (52), 220 (30), 160 (100), 102 (43).

Preparation of α -piperonyl succinic acid half-methyl ester (48**)**

Hydrogenation of α -piperonylidene succinic acid half-methyl ester (**47**) (15.6g; 59.0mmol) was carried out using hydrogen gas (r.t., 1 atm) and palladium-on-carbon (10%) (1.88g; 1.77mmol) as catalyst, in 150ml acetic acid. When the reaction was complete, as determined by negligible hydrogen uptake, the catalyst was filtered off and the solvent removed by rotary evaporation and high vacuum. The resultant slightly yellow-colored residue was redissolved in 50ml ether, and within minutes precipitation of colorless crystals commenced. The crystals were collected and recrystallized from ether, affording 12.1g of compound **48** (76.8%). Melting point: 90.5-93.5°C (lit.⁵²: 90-92°C). ^1H

NMR (CDCl₃) δ 6.74d, 1H, aromatic, $J = 7.8\text{Hz}$; 6.64d, 1H, aromatic, $J = 1.6\text{Hz}$; 6.59dd, 1H, aromatic, $J = 7.8, 1.6\text{Hz}$; 5.93s, 2H, -OCH₂O-; 3.68s, 3H, CH₃; $\sim 3.04\text{m}$, 1H; $\sim 2.84\text{m}$, 2H; $\sim 2.56\text{m}$, 2H, benzylic. EIMS m/z (relative intensity) 266 (M⁺, 20), 234 (5.6), 217 (4), 206 (12.3), 135 (100), 77 (13.5).

Isolation of (*R*)- α -piperonyl succinic acid half-methyl ester (48a)

(1*R*,2*S*)-(-)-Ephedrine **49** (6.66g; 40.3mmol), obtained from Aldrich Chemical Co., was dissolved with stirring in 50ml warm 95% ethanol. Compound **48** (10.5g; 39.5mmol) was likewise dissolved in 50ml warm 95% ethanol, and the two solutions were combined with stirring. After stirring under heat for 20 minutes, the solution was allowed to cool and was then stored overnight below 0°C. The resultant fluffy white crystals were filtered and recrystallized several times from 95% ethanol to a constant melting point range of 131-133.5°C (lit.⁵²: 132-134°C). The crystalline material was then dissolved in water and treated with 1*N* HCl. The product **48a** was extracted with ether (3 x 40ml), and the combined ether layers were washed with brine (3 x 30ml), then dried with anhydrous magnesium sulfate. After filtering off the drying agent, the solution was concentrated to $\sim 15\text{ml}$ and stored overnight below 0°C. The colorless crystals which formed were collected and recrystallized from ether to a constant melting point range of 101-105°C (lit.⁵²: 102-104°C). Yield: 2.87g (27.3% total, i.e. 54.6% enantiomeric recovery). Optical rotation values: $[\alpha]_{\text{D}} = +29.7^\circ$, $c = 1$, CHCl₃ (lit.⁵²: $[\alpha]_{\text{D}}^{20} = +30.4^\circ$, $c = 2$, MeOH).

Synthesis of (*R*)-(+)- β -piperonyl- γ -butyrolactone (50)

Preparation of (*R*)-(+)- β -piperonyl- γ -butyrolactone (**50**) was initiated by the formation of the potassium salt of compound **48a**, which was carried out by dissolving **48a** (1.304g;

4.9mmol) in 10ml of 95% ethanol and titrating with 2M KOH (in 95% ethanol) to neutral pH, as determined by multicolor pH indicator paper. To this solution was added an additional 50ml 95% ethanol and the stirred solution was cooled to 0°C. Calcium chloride (1.365g; 12.3mmol) was then added portionwise to the stirring solution, and the resultant slurry was allowed to stir at 0°C for 20 minutes. At this time, a solution of sodium borohydride (0.742g; 19.6mmol) in 25ml 95% ethanol was added dropwise over 30 minutes to the stirred slurry. The reaction was then allowed to proceed for 1hr, at which time TLC analysis (CH₂Cl₂/MeOH, 15:1) indicated the starting material **48a** had been completely consumed. The reaction was quenched by the addition of cold 6N HCl to pH 1. After concentration of the solution on a rotary evaporator, the product was extracted with ether (3 x 30ml). The combined ether portions were washed with brine and dried with MgSO₄. After filtering off the drying agent, the solvent was removed by rotary evaporation and high vacuum, affording a pale yellow, viscous oil (which crystallized upon storing below 0°C), in 93.4% yield. Melting point: 30.5-33.5°C (lit.⁵²: 31-33.5°C. Optical rotation values: $[\alpha]_D = +4.71^\circ$, $c = 0.76$, CHCl₃ (lit.⁵²: $[\alpha]_D^{20} = +4.87^\circ$, $c = 0.87$, CHCl₃). ¹H NMR (CDCl₃) δ 6.74d, 1H, aromatic, $J = 7.8\text{Hz}$; 6.64d, 1H, aromatic, $J = 1.7\text{Hz}$; 6.60dd, 1H, aromatic, $J = 7.8, 1.7\text{Hz}$; 5.94d, 2H, -OCH₂O-, $J = 4.9\text{Hz}$; 4.33dd, 1H, H-4 β , $J = 9.2, 6.8\text{Hz}$; 4.03dd, 1H, H-4 α , $J = 9.2, 5.9\text{Hz}$; ~2.80m, 1H, H-3; 2.69dd, 2H, H-5 α,β , $J = 7.1, 3.0\text{Hz}$; 2.59dd, 1H, H-2 β , $J = 17.4, 7.9\text{Hz}$; 2.27dd, 1H, H-2 α , $J = 17.4, 6.8\text{Hz}$. EIMS m/z (relative intensity) 220 (M⁺, 28), 135 (100), 77 (14).

Formation of podorhizol (43) and epipodorhizol (44)

(*R*)-(+)- β -Piperonyl- γ -butyrolactone (**50**) (550mg; 2.5mmol) was dissolved with stirring in 10ml THF (dry, distilled) and cooled to -78°C, under nitrogen blanket. To the stirring

solution was slowly added LDA (3mmol; 1.2 molar equiv.). The solution was allowed to warm to 0°C to ensure enolate formation, then recooled to -78°C. After 20 minutes' additional stirring, 3,4,5-trimethoxybenzaldehyde (**51**) (490.5mg; 2.5mmol), dissolved in 5ml THF, was added dropwise to the enolate solution. The reaction was allowed to proceed for 1.5hr, at which time TLC analysis (CH₂Cl₂/MeOH, 15:1) indicated complete conversion of the lactone **50** to an approximately 1:1 mixture of the alcohols **43** and **44**. The reaction was quenched by the addition of 1N HCl and the solvents were removed by rotary evaporation. The slightly yellow-colored residue was redissolved in ether and the ether layer was washed with brine, dried with anhydrous magnesium sulfate, filtered, and evaporated, leaving the mixture of alcohols **43** and **44** as a colorless oil in 89% yield (841.5mg). The alcohols **43** and **44** were separated by flash chromatography (CH₂Cl₂/MeOH, 98:2), with epipodorhizol **43** eluting first. Yield of podorhizol (**43**): 372.3mg (40.2%); yield of epipodorhizol (**44**): 397.1mg (42.9%); 93.4% total recovery after flash chromatography.

Podorhizol (**43**); melting point: 123-125.5°C (lit.⁵⁹: 124-126°C) ¹H NMR (CDCl₃) δ 6.47s, 2H, H-2", H-6"; 6.59d, 1H, H-5', J = 7.7Hz; 6.30dd, 1H, H-6', J = 7.8, 1.5Hz; 6.22d, 1H, H-2', J = 1.5Hz; 5.92dd, 2H, -OCH₂O-, J = 1.4, 1.4Hz; 5.25d, 1H, H-6, J = 2.9Hz; 4.39dd, 1H, H-4α, J = 8.7, 8.0Hz; 3.97dd, 1H, H-4β, J = 8.7, 8.9Hz; 3.83s, 3H, 4"-OMe; 3.82s, 6H, 3"-, 5"-OMe; ~2.80m, 1H, H-3; 2.61dd, 1H, H-2, J = 6.1, 2.9Hz; 2.47dd, 1H, H-5α, J = 13.7, 7.7Hz; 2.25dd, 1H, H-5β, J = 13.7, 8.1Hz. EIMS *m/z* (relative intensity) 416 (M⁺, 13.8), 399 (3.3), 264 (2.3), 220 (20.2), 198 (70.0), 197 (38.8), 182 (16), 170 (21), 155 (11), 136 (35), 135 (100), 105 (8.2), 77 (21.4).

Epipodorhizol (**44**); melting point: 132-133.5°C (lit.⁵⁹: 133-134°C) ¹H NMR (CDCl₃) δ 6.66d, 1H, H-5', J = 8.2Hz; 6.65s, 2H, H-2", H-6"; 6.34dd, 1H, H-6', J = 8.2, 1.7Hz; 6.33d, 1H, H-2', J = 1.7Hz; 5.92dd, 2H, -OCH₂O-, J = 1.4, 1.4Hz; 4.79d, 1H, H-6, J =

7.9Hz; 4.18dd, 1H, H-4 α , J = 9.3, 7.8Hz; 3.92dd, 1H, H-4 β , J = 9.3, 8.4Hz; 3.88s, 6H, 3"-, 5"-OMe; 3.83s, 3H, 4"-OMe; 2.62dd, 1H, H-2, J = 9.1, 7.8Hz; ~2.50m, 1H, H-3; 2.20dd, 1H, H-5 α , J = 13.8, 8.9Hz; 2.12dd, 1H, H-5 β , J = 13.7, 5.4Hz. EIMS *m/z* (relative intensity) 416 (M⁺, 12.9), 399 (3.2), 264 (2.2), 220 (20.2), 198 (63), 197 (38), 182 (18), 170 (21), 155 (9.0), 136 (31), 135 (100), 105 (9.1), 85 (7.1), 77 (9.3).

Synthesis of unlabelled yatein (18) from hydrogenolysis of a mixture of podorhizol (43) and epipodorhizol (44)

A 1:1 mixture of the alcohols 43 and 44 (337.1mg; 0.81mmol) was dissolved in 16ml of ethanol-CH₂Cl₂, 1:1, with stirring, in a 25-ml two-neck, septum-sealed RB flask. To this solution was added palladium-on-carbon (5%) (862.2mg; 0.41mmol). After 5 to 10 minutes' stirring, the flask was attached to a hydrogenator with a 50ml hydrogen gas capacity, and the system was purged of air by vacuum aspiration, and charged with hydrogen gas. After the addition of a few drops of 70% HClO₄ via syringe, the reaction was allowed to proceed while monitoring hydrogen uptake and periodically checking by TLC (CH₂Cl₂/MeOH, 15:1). After 8hrs the TLC showed a non-polar spot at R_f ~ 0.8, in a relative ratio of 1:1:2 with epipodorhizol (44) and podorhizol (43), respectively. At this time the catalyst was removed by filtration, and the solvent removed by rotary evaporation, leaving a pale yellow-green, amorphous solid residue. The residue was subjected to preparative TLC (CH₂Cl₂/MeOH, 98:2; developed twice), and the non-polar band was isolated, affording 87.7mg (27.1%) of a very pale yellow glassy solid, which was identified as yatein 18. Optical rotation values: [α]_D = -24.7°, c = 1.24, CHCl₃ (lit.⁸⁵: [α]_D²⁰ = -28.4°, c = 0.32, CHCl₃). IR spectrum (KBr pellet): 1764, 1243, 1590, 1506, 1489, 2777 cm⁻¹. ¹H NMR (CDCl₃) δ 6.70d, 1H, H-5', J = 6.8Hz; 6.49dd, 1H, H-6', J = 6.9, 1.6Hz; 6.46d, 1H, H-2', J = 1.6Hz; 6.36s, 2H, H-2", H-6"; 5.94dd, -OCH₂O-

$J = 2.5, 1.4\text{Hz}$; 4.18dd, 1H, H-4 α , $J = 9.3, 7.1\text{Hz}$; 3.88dd, 1H, H-4 β , $J = 9.3, 7.5\text{Hz}$; 3.85s, 3H, 4''-OMe; 3.83s, 6H, 3'', 5''-OMe; 2.94m, 1H, H-6 α ; 2.88m, 1H, H-6 β ; ~2.62m, 1H, H-5 β ; ~2.57m, 1H, H-2; ~2.51m, 1H, H-5 α ; ~2.49m, 1H, H-3. ^{13}C NMR (CDCl_3) δ C₍₁₎ 178.49, C₍₂₎ 46.49, C₍₃₎ 41.07, C₍₄₎ 71.18, C₍₅₎ 38.37, C₍₆₎ 35.28, C_(1') 131.57, C_(2') 108.79, C_(3') 147.97, C_(4') 146.45, C_(5') 108.34, C_(6') 121.57, C_(1'') 133.33, C_(2'') 106.35, C_(3'') 153.31, C_(4'') 136.80, C_(5'') 153.31, C_(6'') 106.35, -OCH₂O- 101.11, C_(3''')-OCH₃ 56.14, C_(4''')-OCH₃ 60.87, C_(5''')-OCH₃ 56.14. EIMS m/z (relative intensity) 400 (M^+ , 54), 264 (3.4), 251 (4.1), 238 (2.8), 219 (3.7), 181 (100), 167 (13), 151 (16), 135 (52), 105 (12), 84 (26), 77 (28), 65 (9).

Hydrogenolysis of epipodorhizol (44) to unlabelled yatein (18)

Epipodorhizol (44) (52mg;0.125mmol) was dissolved with stirring in 7ml ethyl acetate in a 15-ml two-neck, septum-sealed RB flask. To the solution was added palladium-on-carbon (5%) (133mg; 0.0625mmol) and the suspension was allowed to stir for an additional 5-10mins. The reaction flask was then attached to a hydrogenator with a 50ml hydrogen gas capacity, and the system was purged of air and charged with hydrogen gas. After the addition of 2 drops 70% HClO₄, the reaction was allowed to proceed. At 6hrs TLC analysis ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 15:1) indicated that the starting material had been converted to a less polar material at $R_f \sim 0.8$, presumably yatein (18). After work-up to remove residual acid, and preparative TLC purification, 14mg of yatein (18) was isolated (28.0% yield).

Attempted hydrogenolysis of podorhizol (43) to unlabelled yatein (18)

To confirm that podorhizol (43) is not converted to yatein (18) under hydrogenolysis conditions, the preceding experiment was repeated using podorhizol (43) (63mg;0.151mmol) in place of epipodorhizol (44) (0.076 mmol palladium-on-carbon,

5%). Even after 155hrs, no reaction had occurred, as noted by TLC analysis, so the starting material (43) was recovered by standard work-up and preparative TLC purification.

Synthesis of podorhizone (54)

(*R*)-(+)- β -Piperonyl- γ -butyrolactone (50) (890mg; 4.05mmol) was dissolved with stirring in 15ml THF (dry, distilled) and cooled to -78°C , under nitrogen blanket. After 10 minutes' stirring, LDA (4.65mmol; 1.15 molar equiv.) was added dropwise to the stirring lactone solution. The reaction flask was allowed to warm to 0°C to ensure enolate formation, then re-cooled to -78°C . After 20 minutes, 3,4,5-trimethoxybenzoyl chloride (53) (1.17g; 5.06mmol), dissolved in 10ml THF, was added dropwise to the stirring enolate solution. The reaction was allowed to proceed for 3hrs at -78°C , then was allowed to warm to room temperature over the next 16hrs. At this time, TLC analysis (ether/hexane, 4:1) showed the reaction to be essentially complete, i.e. no remaining lactone. The reaction was quenched with about 10ml saturated ammonium chloride solution, and the product was extracted with ether (3 x 50ml). The combined ether portions were washed with 1N HCl (50ml) and brine (3 x 50ml), then dried with anhydrous magnesium sulfate. After filtering off the drying agent, the solvent was removed by rotary evaporation, leaving an orange-yellow residue. The residue was subjected to flash chromatography (ether/hexane, 4:1). When allowed to stand for a period of time, those fractions containing the product, podorhizone (54), as determined by TLC analysis (ether/hexane, 4:1), yielded 473mg of long, thin, colorless needles (28.2% yield). Melting point: $131-134^{\circ}\text{C}$ (lit.⁶¹: $129-130^{\circ}\text{C}$). Optical rotation values: $[\alpha]_{\text{D}} = +74.8^{\circ}$, $c = 0.33$, CHCl_3 (lit.⁶¹: $[\alpha]_{\text{D}}^{23} = +79.6^{\circ}$, $c = 0.68$, CHCl_3). $^1\text{H NMR}$ (CDCl_3) δ 7.17s, 2H, H-2", H-6"; 6.60-6.70m, 3H, H-2', H-5', H-6'; 5.92dd, 2H, $-\text{OCH}_2\text{O}-$, $J = 2.5$,

1.3Hz; 4.54dd, 1H, H-4 β , J = 8.9, 7.1Hz; 4.25d, 1H, H-2, J = 6.3Hz; 4.14dd, 1H, H-4 α , J = 8.9, 5.9Hz; 3.93s, 3H, 4"-OMe; 3.89s, 6H, 3"-, 5"-OMe; 3.41m, 1H, H-3; 2.79dd, 1H, H-5 β , J = 19.0, 13.8Hz; 2.76dd, 1H, H-5 α , J = 19.0, 13.8Hz. EIMS *m/z* (relative intensity) 414 (M⁺, 25), 279 (9), 195 (64), 161 (100), 131 (48), 77 (16).

Reduction of podorhizone (54) with sodium borodeuteride

Podorhizone (54) (63.1mg; 0.152mmol) was dissolved with stirring in 5ml 95% ethanol, and cooled to 0°C. After stirring for 15 minutes, sodium borodeuteride (15.9mg; 0.38mmol) was added portionwise to the stirring solution over 30 minutes. The reaction was allowed to proceed for 2hrs, at which time TLC analysis (CH₂Cl₂/MeOH, 15:1) indicated that the starting material 54 had been completely converted to a mixture of the deuterated alcohols 43a and 44a. The reaction was quenched by the addition of 5ml 1N HCl, and the solvents were removed by rotary evaporation. The residue was redissolved in CH₂Cl₂, washed with brine (3 x 15ml), dried with magnesium sulfate, filtered, and evaporated, leaving a pale yellow, oily residue. The residue was subjected to preparative TLC (CH₂Cl₂/MeOH, 98:2) and the two major bands, corresponding to the epimeric alcohols 43a and 44a, were isolated. Yield of 6-²H₁-epipodorhizol 44a: 31.7mg (50.1%); yield of 6-²H₁-podorhizol 43a: 29.3mg (46.3%); total yield of 43a and 44a: 96%).

6-²H₁-Epipodorhizol 44a: ¹H NMR (CDCl₃) showed the same spectral data as that in the spectrum for epipodorhizol 44, with the following exceptions: missing 4.79ppm doublet, with coupling constant, J = 7.9Hz; and the 2.62 doublet of doublets, with coupling constants, J = 9.1, 7.8Hz, condensed to 2.62ppm doublet, with coupling constant, J = 9.0Hz. ²H NMR (CHCl₃) showed a broad singlet at 4.81ppm, relative to CHCl₃ peak at 7.25ppm. EIMS *m/z* (relative intensity) 417 (M⁺, 2.9), 399 (7.6), 384 (1.3), 264 (7.0),

220 (28.5), 197 (49.9), 182 (21.0), 135 (100), 126 (13.4), 111 (12.7), 96 (10.8), 77 (14.6).

6-²H₁-Podorhizol 43a: ¹H NMR (CDCl₃) showed the same spectral data as that in the spectrum for podorhizol 43, with the following exceptions: missing 5.25ppm doublet, with coupling constant, J = 2.9Hz; and the 2.61 doublet of doublets, with coupling constants, J = 6.1, 2.9Hz, condensed to 2.61ppm doublet, with coupling constant, J = 6.1Hz. ²H NMR (CHCl₃) showed a broad singlet at 5.25ppm, relative to CHCl₃ peak at 7.25ppm. EIMS *m/z* (relative intensity) 417 (M+, 2.8), 399 (0.8), 264 (1.1), 220 (20.5), 197 (38.0), 182 (20.5), 170 (7.3), 135 (100), 126 (12.1), 111 (11.9), 96 (9.5), 77 (23.7), 66 (10.9).

Hydrogenolysis of 6-²H₁-epipodorhizol (44a) to 6- α -²H₁-yatein (18b)

6-²H₁-Epipodorhizol (44a) (75mg;0.18mmol) was dissolved with stirring in 7ml ethyl acetate in a 15-ml two-neck, septum-sealed RB flask. To the solution was added palladium-on-carbon (10%) (48mg; 0.043mmol) and the suspension was allowed to stir for an additional 5-10mins. The reaction flask was then attached to a hydrogenator with a 50ml hydrogen gas capacity, and the system was purged of air and charged with hydrogen gas. After the addition of 3 drops conc. HCl, the reaction was allowed to proceed, while monitoring hydrogen uptake and checking periodically by TLC (CH₂Cl₂/MeOH, 15:1). At 48hrs TLC analysis indicated that the starting material had been converted to a less polar material at R_f ~ 0.8, presumably 6- α -²H₁-yatein (18b). After work-up to remove residual acid, the product was purified by preparative TLC, affording 21.7mg of 6- α -²H₁-yatein (18b), as a colorless, glassy solid (30.1% yield). Optical rotation values: [α]_D = -25.6°, c = 0.4, CHCl₃ (lit.⁸⁵: [α]_D²⁰ = -28.4°, c = 0.32, CHCl₃) ¹H NMR (CDCl₃) showed the same spectral data as that in the spectra for yatein 18, with the following exception:

The ABX multiplet at ~2.88-2.94ppm, integrating for the two protons, H-6 α and H-6 β , condensed to a doublet at 2.88ppm, with coupling constant, $J = 6.6\text{Hz}$, and integrating for one proton, H-6 β . EIMS m/z (relative intensity) 401 (M^+ , 52), 385 (18), 265 (3.5), 252 (4.8), 239 (3.1), 182 (100), 135 (46), 77 (18), 55 (17).

Protection of vanillin (55) with benzyl bromide

Vanillin (55) (25.56g; 0.168mol) was dissolved with stirring in 150ml MeOH. Potassium carbonate (27.92g; 0.202mol) was then added to the solution, and the suspension was heated to reflux, resulting in the color gradually changing from a pale golden color to a pale lime-green color. After 45min, benzyl bromide (28.76g; 0.168mol) was added dropwise by addition funnel, and the reaction was allowed to proceed while being monitored by TLC (ether/hexane, 1:1). After 6 hours, the reaction appeared to be complete. The reaction flask was then cooled to room temperature, 1N HCL was added to quench the reaction, and the solvent was evaporated. The residue was redissolved in a minimum of hot 95% ethanol, and upon cooling a heavy precipitation of yellow crystals resulted. The crystalline material was recrystallized twice from 95% ethanol, affording 30.2g (74% yield) of O-benzyl vanillin (55a), as fine yellow crystals. Melting point: 63.5-64.5°C (lit.⁸⁸: 64-65°C). $^1\text{H NMR}$ (CDCl_3) δ 9.83s, 1H, CHO ; 7.39m, 5H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}-$; 6.98-7.42m, 3H, aromatic; 5.24s, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}-$; 3.94s, 3H, OCH_3 . EIMS m/z (relative intensity) 242 (M^+ , 25), 151 (43), 91 (100).

Formation of α -vanillylidene succinic acid half-methyl ester (56)

Sodium methoxide (25 wt.% solution in methanol; 27.3g solution; 126.4mmol) was added to 75ml methanol, and with stirring, brought to reflux. In the meantime, O-benzyl vanillin (55a) (22.7g; 93.6mmol) and dimethyl succinate (16.4g; 112.3mmol) were dissolved in

75ml warm methanol. This solution was slowly added to the refluxing sodium methoxide solution, and the resulting solution was allowed to reflux for 12hr, at which time it was determined by TLC analysis ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 15:1) that the O-benzyl vanillin (**55a**) had been consumed. The reaction flask was allowed to cool to room temperature and the methanol was removed by rotary evaporator. To the yellow-orange, oily residue was added, with stirring, 150ml 1N HCl, and the suspension was then extracted with diethyl ether (3 x 150ml). The combined ether portions were washed with 10% sodium bicarbonate (3 x 75ml) to separate the product **56** from starting material **55a**. The combined aqueous layers were reacidified with 6N HCl and re-extracted with ether. The organic layer was washed with brine (3 x 50ml), then dried with anhydrous magnesium sulfate. After filtering off the drying agent, the solution was concentrated by rotary evaporation to about 75ml. Upon chilling overnight, the solution yielded colorless crystals, which were collected and twice recrystallized from ether. Yield of **56**: 19.0g (57%). Melting point: 112.5-115°C. ^1H NMR (CDCl_3) δ 7.85s, 1H, vinylic; 7.38m, 5H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 6.84-7.01m, 3H, aromatic; 5.20s, 2H, PhCH_2O -; 3.91s, 3H, CH_3 (ester); 3.85s, 3H, CH_3 (arom. methoxy); 3.62s, 2H, allylic. EIMS m/z (relative intensity) 356 (M^+ , 2.1), 324 (4.3), 161 (2.6), 91 (100), 77 (2.4) 65 (6.7).

Preparation of α -vanillyl succinic acid half-methyl ester (**57**)

α -Vanillylidene succinic acid half-methyl ester (**56**) (10.2g; 28.6mmol) was dissolved with stirring in 80ml of 1:1 acetic acid/ethyl acetate. To this solution was added palladium-on-carbon (10%) (0.913g; 0.86mmol) and the reaction flask was attached to a 1L hydrogenator. After purging the system of air and charging with hydrogen gas, the reduction / deprotection was allowed to proceed until hydrogen uptake was negligible and TLC analysis ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 15:1) indicated that the starting material was entirely

converted to product. The catalyst was then removed by filtration and the solvents were removed by rotary evaporation and high vacuum. The residue was redissolved in a minimum of warm ether, from which precipitated fine, colorless crystals. The crystals were collected and recrystallized from ether to afford 4.4g of the racemic compound **57** (57.6% yield). Melting point: 90-92.5°C (lit.⁸⁶: 91-93°C). ¹H NMR (CDCl₃) δ 6.83dd, 1H, aromatic, J = 8.6, 1.8Hz; 6.64d, 1H, aromatic, J = 8.6Hz; 6.62d, 1H, aromatic, J = 1.8Hz; 5.60br.s, 1H, OH (phenolic); 3.86s, 3H, CH₃ (ester); 3.68s, 3H, CH₃ (arom. methoxy); ~3.03m, 1H; ~2.86m, 2H; ~2.56m, 2H, benzylic. EIMS *m/z* (relative intensity) 268 (M⁺, 14.3), 236 (3.8), 219 (2.9), 208 (8.4), 137 (100), 122 (7.5), 77 (7.2).

Isolation of (*R*)-(+)- α -vanillyl succinic acid half-methyl ester (**57a**)

α -Vanillyl succinic acid half-methyl ester (**57**) (3.2g; 11.9mmol) was dissolved in 15ml warm ethyl acetate. (*R*)-(+)- α -Methylbenzylamine (**58**) (1.47g; 12.2mmol), obtained from Aldrich Chemical Co., was likewise dissolved in 15ml warm ethyl acetate, and the two solutions were combined. After stirring under heating for 20min, the solution was allowed to cool. Upon prolonged standing (2-3 days) at 0-4°C, precipitation of fine, colorless crystals occurred. The crystalline material was recrystallized several times from ethyl acetate, to a constant melting point range of 129-134°C (lit.⁸⁶: 126-134°C). This salt was then dissolved in 50ml distilled water, and to the solution was added 50ml 1N HCl with stirring. After 20min, the hemi-ester **57a** was extracted from the aqueous layer with ether (3 x 30ml). The combined organic layers were washed with brine (3 x 30ml), dried with magnesium sulfate, filtered, and concentrated to ~15ml. After standing at 0°C for a period of time, crystals of the enantiomerically pure compound **57a** precipitated from solution. Collection and recrystallization of this material afforded 905mg of fine, colorless crystalline **57a**, in 28.3% yield (56.6% enantiomeric recovery). Melting point: 96-99.5°C (lit.⁸⁶:

97.5-100.5°C). Optical rotation values: $[\alpha]_D = +26.3^\circ$, $c = 0.8$, CHCl_3 (lit.⁸⁶: $[\alpha]_D = +28^\circ$, $c = 1$, MeOH).

Synthesis of (*R*)-(+)- β -vanillyl- γ -butyrolactone (59)

Compound **57a** (880mg; 3.28mmol) was dissolved in 10ml of 95% ethanol and titrated with 2M KOH (in 95% ethanol) to neutral pH, as determined by multicolor pH indicator paper. To this solution was added an additional 75ml 95% ethanol and the stirring solution was cooled to 0°C. Calcium chloride (1.64g; 14.8mmol) was then added portionwise to the stirred solution, and the resultant slurry was allowed to stir at 0°C for 20 minutes. At this time, a solution of sodium borohydride (1.24g; 32.8mmol) in 40ml 95% ethanol was added dropwise, over 30 minutes, to the stirred slurry. The reaction was then allowed to proceed for 18hr, at which time TLC analysis ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 15:1) indicated the starting material **57a** had been completely consumed. The reaction was quenched by the slow addition of cold 6N HCl to pH 1. After concentration of the solution by rotary evaporation, the product was extracted with ether (3 x 30ml). The combined ether portions were washed with brine and dried with MgSO_4 . After filtering off the drying agent, the solvent was removed by rotary evaporation and high vacuum, affording 449.8mg (61.7% yield) of fine, very colorless crystals. Melting point: 119.5-122.5°C (lit.⁵³: 119-121°C). Optical rotation values: $[\alpha]_D = +9.2^\circ$, $c = 0.73$, CHCl_3 (lit.⁵³: $[\alpha]_D^{23} = +10^\circ$, $c = 1$, CHCl_3). $^1\text{H NMR}$ (CDCl_3) δ 6.86dd, 1H, aromatic, $J = 7.3, 1.3\text{Hz}$; 6.66d, 1H, aromatic, $J = 1.3\text{Hz}$; 6.65d, 1H, aromatic, $J = 7.3\text{Hz}$; 4.33dd, 1H, H-4 β , $J = 9.1, 6.8\text{Hz}$; 4.04dd, 1H, H-4 α , $J = 9.1, 5.9\text{Hz}$; 3.89s, 3H, CH_3 ; ~2.80m, 1H, H-3; 2.71dd, 2H, H-5 α,β , $J = 8.1, 3.2\text{Hz}$; 2.61dd, 1H, H-2 β , $J = 17.4, 7.9\text{Hz}$; 2.29dd, 1H, H-2 α , $J = 17.4, 6.7\text{Hz}$. EIMS m/z (relative intensity) 222 (M^+ , 18.1), 138 (11.6), 137 (100), 94 (5.8), 77 (6.5), 51 (4.2).

Protection of (*R*)-(+)- β -vanillyl- γ -butyrolactone (**59**) with benzyl bromide

(*R*)-(+)- β -Vanillyl- γ -butyrolactone (**59**) (443mg; 2.0mmol) was dissolved with stirring in 15ml dry, distilled acetone, in a flame-dried 25-ml septum-sealed, 2-necked RB flask. To the solution was added anhydrous potassium carbonate (331mg; 2.39mmol), and stirring was continued while heating to reflux. Benzyl bromide (682.2mg; 3.99mmol) was slowly added to the refluxing solution via syringe, and the reaction was allowed to proceed while being monitored by TLC (ether/hexane, 3:1). After 20hrs the reaction appeared to be essentially complete. The reaction flask was allowed to cool to room temperature, then 1*N* HCl was added to neutralize the reaction mixture. After removal of the acetone, the residue was redissolved in ether, and the organic layer was washed with brine, dried with magnesium sulfate, filtered, and evaporated, leaving a pale green-yellow, oily residue. The residue was purified by flash chromatography, yielding 360.2mg (57.9% yield) of fine, colorless crystalline **60**. Melting point: 79.5-81.5°C (lit.⁸⁶: 80-81.5°C). Optical rotation values: $[\alpha]_D = +3.7^\circ$, $c = 1.0$, CHCl₃ (lit.⁸⁶: $[\alpha]_D = +4^\circ$, $c = 2$, CHCl₃). ¹H NMR (CDCl₃) δ 7.32-7.46m, 5H, C₆H₅CH₂O-; 6.88-7.12m, 3H, aromatic; 5.13s, 2H, PhCH₂O-; 4.33dd, 1H, H-4 β , $J = 9.2$, 6.8Hz; 4.03dd, 1H, H-4 α , $J = 9.2$, 5.9Hz; 3.88s, 3H, OCH₃; 2.82m, 1H, H-3; 2.71dd, 2H, H-5 α,β , $J = 6.3$, 3.1Hz; 2.61dd, 1H, H-2 β , $J = 17.5$, 8.0Hz; 2.28dd, 1H, H-2 α , $J = 17.5$, 6.7Hz. EIMS m/z (relative intensity) 312 (M⁺, 11.7), 221 (1.2), 137 (1.7), 92 (8.1), 91 (100), 77 (2.7), 65 (8.4).

Reduction of O-benzyl vanillin (**55a**) with sodium borohydride

O-Benzyl vanillin (**55a**) (12.65g; 52.3mmol) was dissolved in 120ml MeOH/CH₂Cl₂, 2:1, and the solution was cooled to 0°C. To the stirring solution, sodium borohydride (4.15g; 109.8mmol) was added portionwise over 20min. The reaction was allowed to proceed for 1hr at 0°C, then for an additional 1hr while warming to room temperature. At this time, the

reaction appeared complete by TLC analysis ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 15:1), and the reaction was quenched by the addition of 150ml 1N HCl. The product alcohol **55b** was worked up in the usual manner, and after recrystallization from ether afforded fine, colorless needles. Yield: 10.6g (83.2%). Melting point: 73.5-78.5°C (lit.⁸⁸: 70°C). ^1H NMR (CDCl_3) δ 7.28-7.45m, 5H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 6.80-6.93m, 3H, aromatic; 5.14s, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 4.58s, 2H, CH_2OH ; 3.88s, 3H, OCH_3 ; 1.79s, 1H, OH. EIMS m/z (relative intensity) 244 (M^+ , 14), 91 (100).

Preparation of O-benzyl vanillyl bromide (61a)

N-Bromosuccinimide (11.6g; 65.2mmol) was dissolved in 120ml dry, distilled CH_2Cl_2 , in a flame-dried 500-ml septum-sealed 3-neck RB flask under nitrogen blanket. After cooling to 0°C, dimethyl sulfide (4.93g; 79.5mmol), dissolved in 40ml CH_2Cl_2 , was added dropwise over 30min to the stirring solution, which gradually turned from clear, colorless to opaque, bright orange-yellow. The reaction flask was cooled further to -23°C and allowed to stir for an additional 45min. At this time, O-benzyl vanillyl alcohol (**55b**) (10.6g; 43.4mmol), dissolved in 40ml CH_2Cl_2 , was added dropwise via addition funnel to the stirring bromide solution, over 40min. Upon completion of the addition, the reaction flask was allowed to warm gradually to room temperature and the reaction was allowed to continue for 4hr. The solution was then partitioned between hexane and brine, and after separation, the brine layer was washed with CH_2Cl_2 . The combined organic layers were washed with brine, dried, then evaporated to a yellow-orange, oily residue. The residue was purified via flash chromatography, yielding 6.93g of colorless, crystalline **61a** (52.0%). Melting point: 92-94.5°C (lit.⁸⁷: 94-95°C). ^1H NMR (CDCl_3) δ 7.30-7.44m, 5H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 6.79-6.93m, 3H, aromatic; 5.15s, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 4.48s, 2H,

CH_2Br ; 3.90s, 3H, OCH_3 . EIMS m/z (relative intensity) 308/306 (M^+ , 9), 227 (16), 91 (100), 65 (16).

Coupling of O-benzyl-(*R*)-(+)- β -vanillyl- γ -butyrolactone (60) with O-benzyl vanillyl bromide (61a)

O-Benzyl-(*R*)-(+)- β -vanillyl- γ -butyrolactone (60) (100mg; 0.321mmol) was dissolved with stirring in 5ml THF (dry, distilled) and cooled to -78°C , under nitrogen blanket. To the stirring solution was slowly added lithium hexamethyldisilazane (0.327mmol; 1.02 molar equiv.). The solution was allowed to warm to 0°C to ensure enolate formation, then recooled to -78°C . After 15 minutes, the O-benzyl vanillyl bromide (61a) (103.5mg; 0.337mmol), dissolved in 5ml THF, was added dropwise to the enolate solution. The reaction was allowed to proceed for 7.5hr, at which time TLC analysis (ether) indicated complete consumption of the lactone 60. The reaction was quenched by the addition of saturated ammonium chloride solution, and warmed to room temperature. After adding 20ml distilled water, the reaction was worked up in the usual manner, affording a yellowish, oily residue. The residue was subjected to preparative TLC (ether), and isolation of the major band, corresponding to di-O-benzyl-matairesinol (62a), yielded 119.5mg of a very pale yellow oil (69.2%). Optical rotation values: $[\alpha]_{\text{D}} = -20.6^\circ$, $c = 0.87$, CHCl_3 (lit.⁵³: $[\alpha]_{\text{D}} = -22^\circ$, $c = 1$, CHCl_3). IR (Nujol) = 3033, 2931, 1773, 1608, 1589, 1513, 1262, 1232, 1162, 1138, 1022 cm^{-1} . ^1H NMR (CDCl_3) δ 7.29-7.43m, 10H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}-$; 6.47-6.78m, 6H, H-2', H-5', H-6', H-2'', H-5'', H-6''; 5.12s, 4H, $\text{PhCH}_2\text{O}-$; 4.11dd, 1H, H-4 β , $J = 9.1$, 6.7Hz; 3.89dd, 1H, H-4 α , $J = 9.1$, 6.0Hz; 3.83s, 3H, OCH_3 ; 3.80s, 3H, OCH_3 ; 2.92m, 2H, H-6 α,β ; 2.52m, 4H, H-2, H-3, H-5 α,β (lit. ^1H NMR⁵³: δ 7.3-7.66m, 10H, aromatic; 6.43-7.0m, 6H, H-2', H-5', H-6', H-2'', H-5'', H-6''; 5.13s, 4H, $\text{PhCH}_2\text{O}-$; 4.15m, 1H, and 3.95m, 1H, H-4 α,β ; 3.85s, 3H, OCH_3 ;

3.82s, 3H, OCH₃; 2.93m, 2H, H-6 α , β ; 2.52m, 4H, H-2, H-3, H-5 α , β). EIMS *m/z* (relative intensity) 538 (M⁺, 7.7), 448 (2.3), 387 (0.7), 358 (0.9), 317 (0.8), 137 (14.1), 91 (100), 73 (9.8), 59 (10.2).

Deprotection of di-O-benzyl-matairesinol (62a)

Di-O-benzyl-matairesinol (62a) (115mg; 0.214mmol) was dissolved in 10 ml ethyl acetate in a 15-ml septum-sealed 2-neck RB flask. To the stirring solution was added palladium-on-carbon (5%) (50mg; 0.024mmol), and the reaction flask was attached to a 50-ml hydrogenator. After the system was purged of air and charged with hydrogen gas, the reaction was allowed to proceed while monitoring hydrogen uptake. After about 30min it was observed that there was negligible hydrogen being consumed, however on the addition of 2 drops 6*N* HCl, hydrogen uptake was rapid, i.e. 8ml in ~1min. Within 5min hydrogen uptake had dropped to zero, and the reaction was checked by TLC (CH₂Cl₂/MeOH, 99:1). The TLC plate showed only a single, relatively polar product spot at R_f~0.12. The catalyst was filtered and the product worked up in the usual manner, yielding a colorless, oily residue. After recrystallization from ethyl acetate, 60.7mg (79.8%yield) of colorless crystalline **16** was obtained. Melting point: 72.5-74.5°C (lit.⁵³: 73-75°C). Optical rotation values: [α]_D = -39.6°, *c* = 0.96, CHCl₃ (lit.⁵³: [α]_D²³ = -42.8°, *c* = 0.84, Me₂CO). IR (KBr pellet) = 3332, 1750, 1602, 1515, 1156, 1128, 1027 cm⁻¹. ¹H NMR (CDCl₃) δ 6.40-6.83m, 6H, aromatic; 5.65s, 1H, OH; 5.64s, 1H, OH; 4.15dd, 1H, H-4 β , *J* = 9.2, 6.9Hz; 3.88dd, 1H, H-4 α , *J* = 9.2, 6.9Hz; 3.80s, 6H, OCH₃; 2.95dd, 1H, H-6 α , *J* = 14.1, 5.3Hz; 2.86dd, 1H, H-6 β , *J* = 14.1, 6.7Hz; 2.41-2.62m, 4H, H-2, H-3, H-5 α , β . (lit. ¹H NMR⁵³: δ 6.5-7.1m, 6H, aromatic; 5.70br.s, 2H, OH; 4.15m, 1H, and 3.9m, 1H, H-4 α , β ; 3.83s, 6H, OCH₃; 2.93m, 2H, H-6 α , β ; 2.55m, 4H, H-2, H-3, H-

5 α , β). EIMS m/z (relative intensity) 358 (M^+ , 11.5), 137 (100), 122 (22.4), 105 (9.3), 94 (23.1), 77 (13.9), 65 (9.7).

Exchange of deuterium for aromatic hydrogens of matairesinol (16) giving labelled matairesinol (16a)

Matairesinol (16) (18.7mg; 0.052mmol) was dissolved in 3ml THF/ $CDCl_3$ (1:1). To the solution was then added 68% $DClO_4$ in D_2O (0.235ml; 2.62mmol; 50 molar equiv.), and the solution was allowed to stir while monitoring aliquots of the reaction by NMR (i.e., disappearance of aromatic peaks). At 4hr, the exchange appeared to be complete by NMR analysis. To the reaction flask was added 10ml CH_2Cl_2 , and the solution was washed with brine to remove residual acid. After drying and evaporation of the solvent, the residue was purified by preparative TLC. The product 16a was thus isolated in 83.5% yield (15.8mg). The 1H NMR ($CDCl_3$) appeared the same as for unlabelled matairesinol (16), with the exception that no aromatic proton peaks were evident, as expected. 1H NMR ($CDCl_3$) δ 5.52s, 1H, OH; 5.50s, 1H, OH; 4.15dd, 1H, H-4 β , $J = 9.1, 7.0$ Hz; 3.88dd, 1H, H-4 α , $J = 9.1, 7.0$ Hz; 3.82s, 6H, OCH_3 ; 2.95dd, 1H, H-6 α , $J = 14.2, 5.5$ Hz; 2.87dd, 1H, H-6 β , $J = 14.2, 6.8$ Hz; 2.41-2.63m, 4H, H-2, H-3, H-5 α , β . (lit. 1H NMR⁵³: δ 6.5-7.1m, 6H, aromatic; 5.70br.s, 2H, OH; 4.15m, 1H, and 3.9m, 1H, H-4 α , β ; 3.83s, 6H, OCH_3 ; 2.93m, 2H, H-6 α , β ; 2.55m, 4H, H-2, H-3, H-5 α , β). EIMS m/z (relative intensity) 364 (M^+ , 14.2), 224 (5.8), 197 (5.3), 167 (7.1), 140 (100), 125 (10.3), 97 (8.8), 80 (5.2), 68 (3.8).

Protection of 3,4-dihydroxy-5-methoxybenzaldehyde (65) with benzyl bromide

3,4-Dihydroxy-5-methoxybenzaldehyde (65) (0.75g; 4.46mmol) was dissolved with stirring in 40ml acetone (dry, distilled). After 15min, potassium carbonate (1.54g; 11.2mmol) was added to the solution, and the suspension was heated to reflux, resulting in the color gradually changing from colorless to a very pale yellow-green. After 35min, benzyl bromide (2.29g; 13.4mmol) was added dropwise by addition funnel, and the reaction was allowed to proceed while being monitored by TLC (ether/hexane, 1:1). After 6 hours, the reaction appeared to be complete. The reaction flask was then cooled to room temperature and 1N HCl was added to quench. The solvent was removed by rotary evaporation and the residue was redissolved in ether and worked up in the usual manner. The resulting material was purified by flash chromatography (ether/hexane, 1:1), affording 1.046g (67.4%) of 65a, as a pale yellow oil. $^1\text{H NMR}$ (CDCl_3) δ 9.82s, 1H, CHO; 7.28-7.43m, 10H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}-$; 7.14dd, 2H, aromatic, $J = 10.2, 1.7\text{Hz}$; 5.14s, 4H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}-$; 3.89s, 3H, OCH_3 . EIMS m/z (relative intensity) 348 (M^+ , 7.2), 257 (9.6), 181 (15.3), 91 (100), 65 (13.6).

Reduction of 3,4-di-O-benzyl-5-methoxybenzaldehyde (65a)

3,4-Di-O-benzyl-5-methoxybenzaldehyde (65a) (1.046g; 3.0mmol) was dissolved in 15ml MeOH/ CH_2Cl_2 , 3:2, and the solution was cooled to 0°C . Sodium borohydride (0.239g; 6.3mmol) was added portionwise over 15min, and the reaction was allowed to continue for 15min, at which time TLC analysis indicated that the starting material had been completely consumed. To the solution was added 1N HCl to quench the borohydride reagent. The organic and aqueous layers were separated and the aqueous layer was washed several times with ether. The combined organic layers were washed with brine, dried with MgSO_4 ,

filtered, and evaporated to a pale yellow oil, which was homogeneous by TLC analysis. Upon standing at 0°C, the oil crystallized into long, fine, pale yellow needles. Yield of **65b**: 1.033g (98.3%). Melting point: 52.5-54.5°C. ¹H NMR (CDCl₃) δ 7.28-7.45m, 10H, C₆H₅CH₂O-; 6.61dd, 2H, aromatic, J = 9.4, 1.7Hz; 5.08s, 2H, C₆H₅CH₂O-; 5.01s, 2H, C₆H₅CH₂O-; 4.57br.s, 2H, CH₂OH; 3.82s, 3H, OCH₃. EIMS *m/z* (relative intensity) 350 (M⁺, 8.5), 259 (8.4), 91 (100), 65 (18.2).

Preparation of 3,4-di-O-benzyl-5-methoxybenzyl bromide (61b)

N-Bromosuccimide (190.8mg; 1.07mmol) was dissolved in 4ml dry, distilled CH₂Cl₂, in a flame-dried 50-ml septum-sealed 3-neck RB flask under nitrogen blanket. After cooling to 0°C, dimethyl sulfide (66.5g; 1.07mmol), dissolved in 1.5ml CH₂Cl₂, was added dropwise over 30min to the stirring solution, which gradually turned from clear, colorless to opaque, bright orange-yellow. The reaction flask was cooled further to -23°C and allowed to stir for an additional 45min. At this time, 3,4-di-O-benzyl-5-methoxybenzyl alcohol (**65b**) (381.5mg; 1.09mmol), dissolved in 5ml CH₂Cl₂, was added dropwise via addition funnel to the stirring bromide solution, over 40min. Upon completion of the addition, the reaction flask was allowed to warm gradually to room temperature and the reaction was allowed to continue for 7.5hr. The solution was then partitioned between hexane and brine, and after separation, the brine layer was washed with CH₂Cl₂. The combined organic layers were washed with brine, dried, then evaporated to a yellow-orange, oily residue. The residue was purified via flash chromatography, yielding 251.2mg of a viscous, pale yellow oil, **61b** (55.8%). ¹H NMR (CDCl₃) δ 7.29-7.46m, 10H, C₆H₅CH₂O-; 6.61dd, 2H, aromatic, J = 9.3, 1.7Hz; 5.07s, 2H, C₆H₅CH₂O-; 5.01s, 2H, C₆H₅CH₂O-; 4.56s, 2H, CH₂Br; 3.83s, 3H, OCH₃.

Protection of 4-hydroxy-3,5-dimethoxybenzaldehyde (66) with benzyl bromide

4-Hydroxy-3,5-dimethoxybenzaldehyde (66) (0.975g; 5.35mmol) was dissolved with stirring in 40ml acetone (dry, distilled). After 15min, potassium carbonate (1.85g; 13.44mmol) was added to the solution, and the suspension was heated to reflux, resulting in the color gradually changing from colorless to a very pale lime-green color. After 30min, benzyl bromide (0.914g; 5.35mmol) was added dropwise by addition funnel, and the reaction was allowed to proceed while being monitored by TLC (ether/hexane, 1:1). After 6 hours, the reaction appeared to be complete. The reaction flask was then cooled to room temperature and 1N HCl was added to quench. The solvent was removed by rotary evaporator and the residue was redissolved in ether and worked up in the usual manner. The resulting material was purified by flash chromatography (ether/hexane, 1:1), affording 950.2mg (65.3%) of 66a, as fine, colorless crystals. Melting point: 58.5-61.5°C (lit.⁵³: 59-61°C). ¹H NMR (CDCl₃) δ 9.85s, 1H, CHO; 7.29-7.48m, 5H, C₆H₅CH₂O-; 7.11s, 2H, aromatic; 5.13s, 2H, C₆H₅CH₂O-; 3.89s, 6H, OCH₃ (lit. ¹H NMR⁵³: δ 10.0s, 1H, CHO; 7.46m, 5H, C₆H₅CH₂O-; 7.21s, 2H, aromatic; 5.2s, 2H, C₆H₅CH₂O-; 3.91s, 6H, OCH₃).

Reduction of 4-O-benzyl-3,5-dimethoxybenzaldehyde (66a)

4-O-Benzyl-3,5-dimethoxybenzaldehyde (66a) (890.2mg; 3.27mmol) was dissolved in 15ml MeOH/CH₂Cl₂, 3:2, and the solution was cooled to 0°C. Sodium borohydride (242mg; 6.54mmol) was added portionwise over 15min, and the reaction was allowed to continue for 25min, at which time TLC analysis indicated that the starting material had been completely consumed. To the solution was added 1N HCl to quench the borohydride reagent. The organic and aqueous layers were separated and the aqueous layer was washed

several times with ether. The combined organic layers were washed with brine, dried with MgSO_4 , filtered, and evaporated to a colorless residue, which was redissolved in ether. Upon standing the solution yielded 663.9mg of fine, colorless crystalline **66b** (74.1%). Melting point: 46-48.5°C (lit.⁵³: 47-49°C). $^1\text{H NMR}$ (CDCl_3) δ 7.31-7.51m, 5H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 6.58s, 2H, aromatic; 4.99s, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 4.62s, 2H, CH_2OH ; 3.83s, 6H, OCH_3 (lit. $^1\text{H NMR}$ ⁵³: δ 7.27-7.67m, 5H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 6.57s, 2H, aromatic; 5.02s, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 4.51s, 2H, CH_2OH ; 3.76s, 6H, OCH_3 ; 3.06s, 1H, OH).

Preparation of 4-O-benzyl-3,5-dimethoxybenzyl bromide (**61c**)

N-Bromosuccinimide (190.8mg; 1.07mmol) was dissolved in 4ml dry, distilled CH_2Cl_2 , in a flame-dried 15-ml septum-sealed 3-neck RB flask under nitrogen blanket. After cooling to 0°C, dimethyl sulfide (66.5mg; 1.07mmol), dissolved in 1.5ml CH_2Cl_2 , was added dropwise over 30min to the stirring solution, which gradually turned from clear, colorless to opaque, bright orange-yellow. The reaction flask was cooled further to -23°C and allowed to stir for an additional 45min. At this time, 4-O-benzyl-3,5-dimethoxybenzyl alcohol (**66b**) (300mg; 1.09mmol), dissolved in 1.5ml CH_2Cl_2 , was added dropwise via addition funnel to the stirring bromide solution, over 20min. Upon completion of the addition, the reaction flask was allowed to warm gradually to room temperature and the reaction was allowed to continue for 4hr. The solution was then partitioned between hexane and brine, and after separation, the brine layer was washed with CH_2Cl_2 . The combined organic layers were washed with brine, dried, then evaporated to a yellow-orange, oily residue. The residue was purified via flash chromatography, yielding 186.6mg of colorless crystals, **61c** (50.8%). Melting point: 42-43.5°C (lit.⁵³: 43-44°C). $^1\text{H NMR}$ (CDCl_3) δ 7.31-7.50m, 5H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 6.71s, 2H, aromatic; 5.03s, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 4.53s, 2H, CH_2Br ; 3.83s, 6H, OCH_3 (lit. $^1\text{H NMR}$ ⁵³: δ 7.33-7.70m,

5H, C₆H₅CH₂O-; 6.65s, 2H, aromatic; 5.05s, 2H, C₆H₅CH₂O-; 4.46s, 2H, CH₂Br; 3.81s, 6H, OCH₃).

Reduction of 3,4,5-trimethoxybenzaldehyde (67)

3,4,5-trimethoxybenzaldehyde (67) (2.24g; 8.58mmol) was dissolved in 25ml MeOH/CH₂Cl₂, 3:2, and the solution was cooled to 0°C. Sodium borohydride (650mg; 17.16mmol) was added portionwise over 15min, and the reaction was allowed to continue for 20min, at which time TLC analysis indicated that the starting material had been completely consumed. To the solution was added 1N HCl to quench the borohydride reagent. The organic and aqueous layers were separated and the aqueous layer was washed several times with ether. The combined organic layers were washed with brine, dried with MgSO₄, filtered, and evaporated to a colorless oil, affording 2.23g of compound 67a (98.8%). ¹H NMR (CDCl₃) δ 6.59s, 2H, aromatic; 4.62s, 2H, CH₂OH; 3.86s, 3H, 4-OCH₃; 3.83s, 6H, 3-,5-OCH₃; 1.98s, 1H, OH (lit. ¹H NMR⁸⁹: δ 6.63s, 2H, aromatic; 4.63s, 2H, CH₂OH; 3.86s, 9H, OCH₃; 2.56s, 1H, OH).

Preparation of 3,4,5-trimethoxybenzyl bromide (61d)

N-Bromosuccinimide (440mg; 2.47mmol) was dissolved in 8ml dry, distilled CH₂Cl₂, in a flame-dried 25-ml septum-sealed 3-neck RB flask under nitrogen blanket. After cooling to 0°C, dimethyl sulfide (153.5mg; 2.47mmol), dissolved in 2ml CH₂Cl₂, was added dropwise over 30min to the stirring solution, which gradually turned from clear, colorless to opaque, bright orange-yellow. The reaction flask was cooled further to -23°C and allowed to stir for an additional 45min. At this time, 3,4,5-trimethoxybenzyl alcohol (67a) (405.5mg; 2.52mmol), dissolved in 2ml CH₂Cl₂, was added dropwise via addition funnel to the stirring bromide solution, over 30min. Upon completion of the addition, the reaction

flask was allowed to warm gradually to room temperature and the reaction was allowed to continue for 6hr. The solution was then partitioned between hexane and brine, and after separation, the brine layer was washed with CH_2Cl_2 . The combined organic layers were washed with brine, dried, then evaporated to a yellow-orange, oily residue. The residue was purified via flash chromatography, yielding 359.4mg of colorless crystals, **61d** (54.6%). Melting point: 73.5-77°C (lit.⁸⁹: 75-78°C). ^1H NMR (CDCl_3) δ 6.72s, 2H, aromatic; 4.55s, 2H, CH_2Br ; 3.92s, 3H, 4- OCH_3 ; 3.87s, 6H, 3-,5- OCH_3 (lit. ^1H NMR⁸⁹: δ 6.66s, 2H, aromatic; 4.46s, 2H, CH_2Br ; 3.86s, 9H, OCH_3).

Preparation of 3,4-di-O-benzyl-5-methoxybenzyl bromide (61b) via an alternate and very efficient route

3,4-Di-O-benzyl-5-methoxybenzyl alcohol (**65b**) (233.3mg; 0.667mmol) was dissolved with stirring in 5ml anhydrous ether. To the solution was added phosphorous tribromide (60.2mg; 0.222mmol), and the solution was allowed to stir for five minutes, at which time it was determined by TLC analysis (Et_2O /hexane, 2:1) that the reaction was complete. The reaction was quenched by the addition of 10ml saturated Na_2CO_3 (aqueous). The organic layer was separated and washed with brine (3 x 15ml), dried with anhydrous magnesium sulfate, and removed by rotary evaporation, leaving 271.2mg (98.5%) of a very pale yellow, viscous oil. The oily material was confirmed to be compound **61b** by ^1H NMR analysis. ^1H NMR (CDCl_3) δ 7.28-7.47m, 10H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 6.61dd, 2H, aromatic, $J = 9.3, 1.7\text{Hz}$; 5.06s, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 5.00s, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$ -; 4.54s, 2H, CH_2Br ; 3.82s, 3H, OCH_3 .

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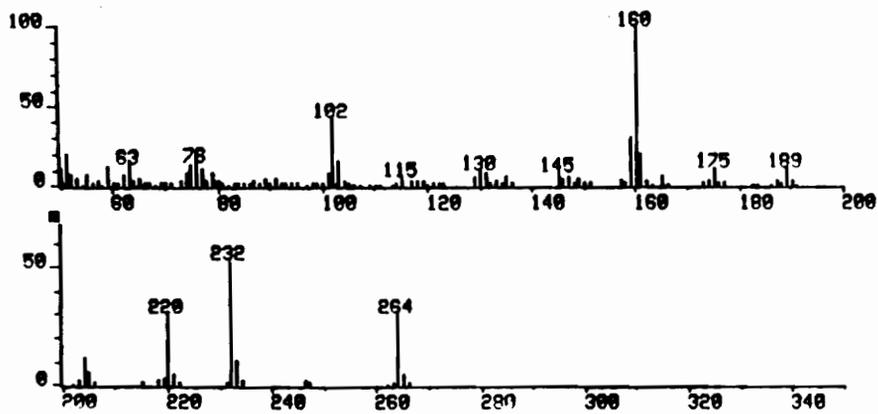
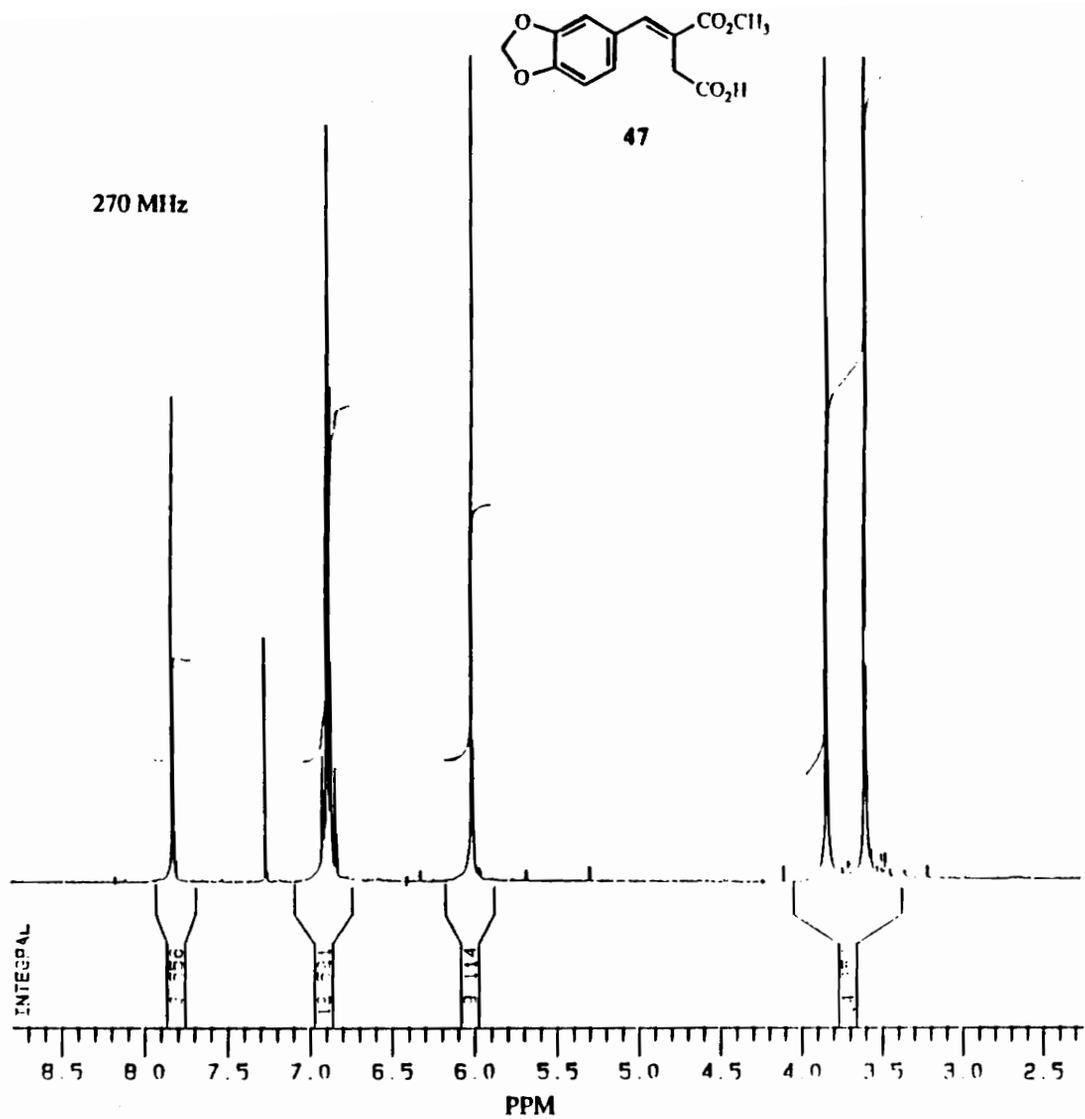
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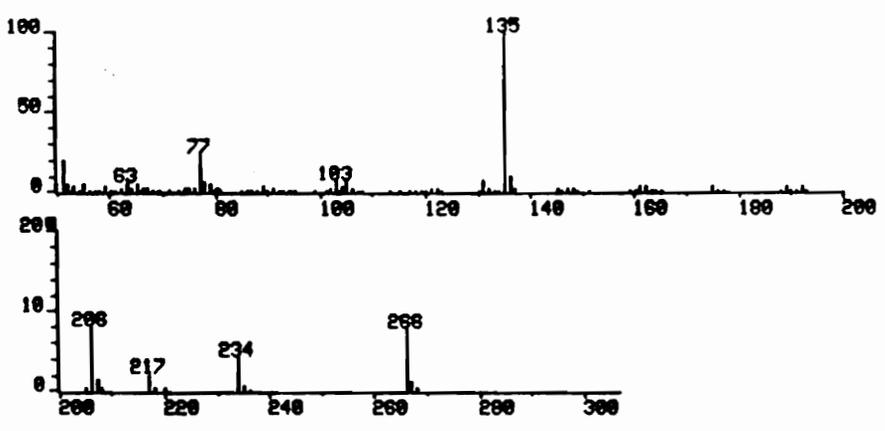
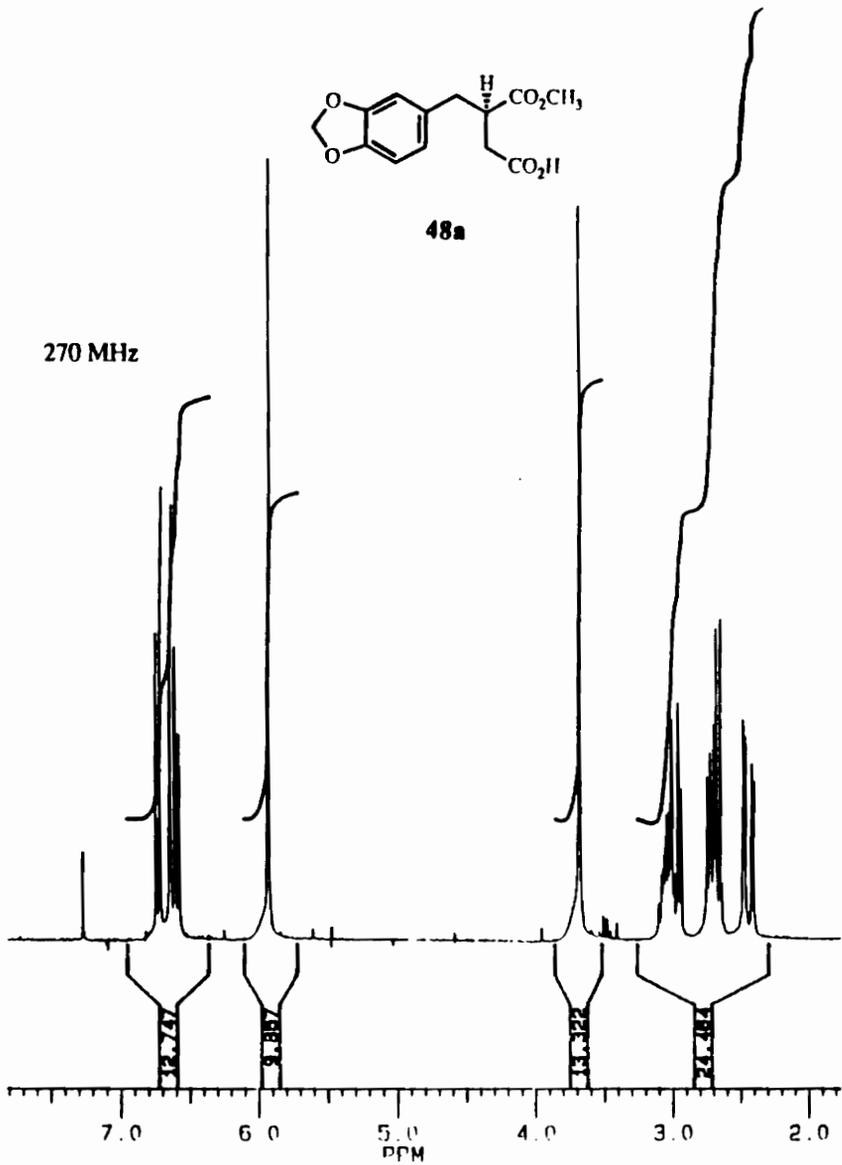
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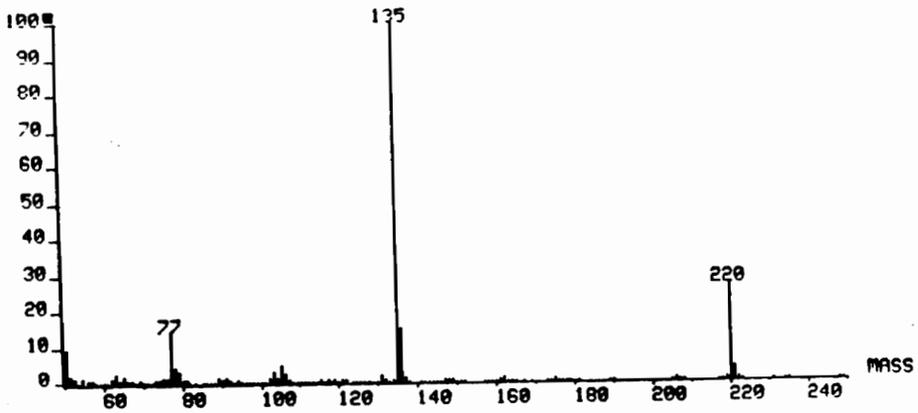
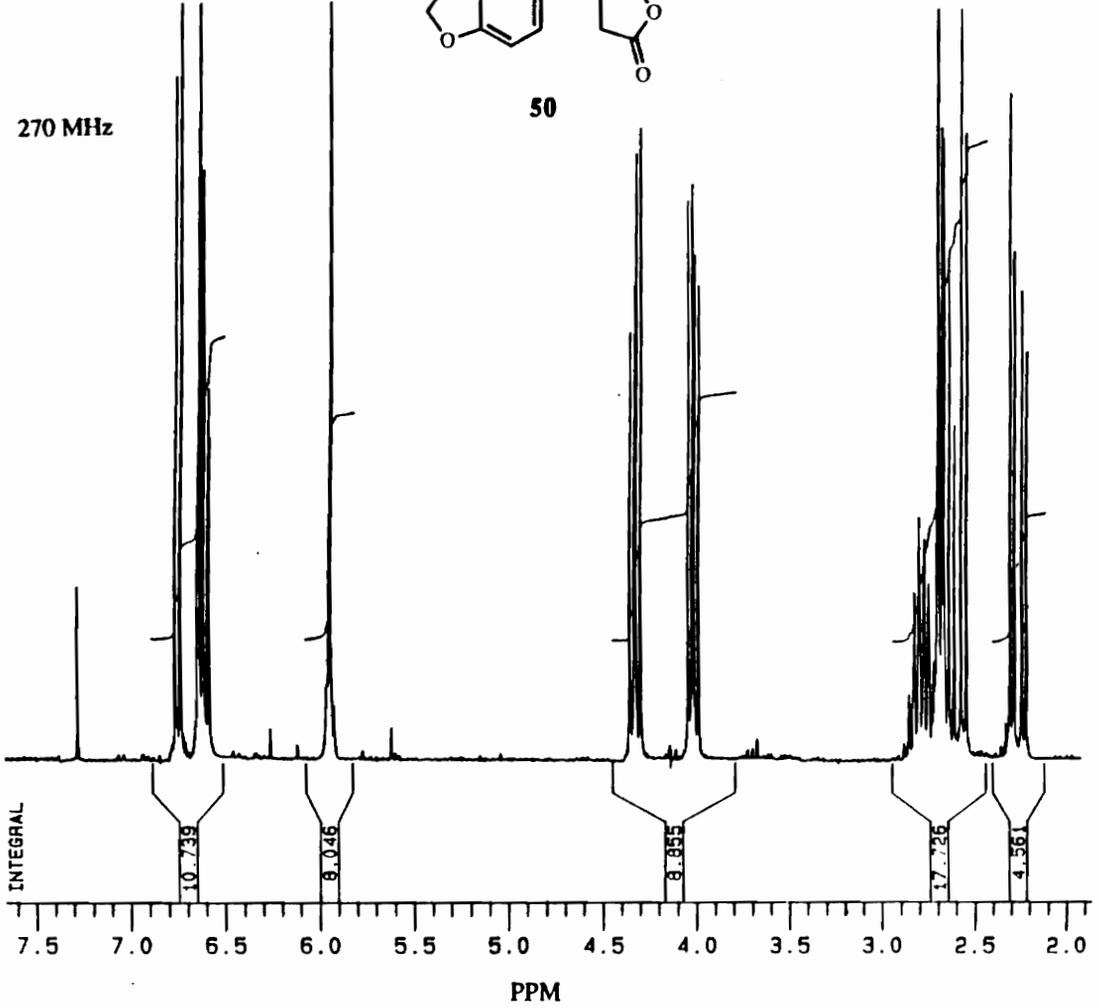
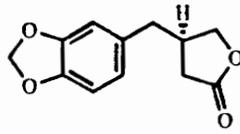
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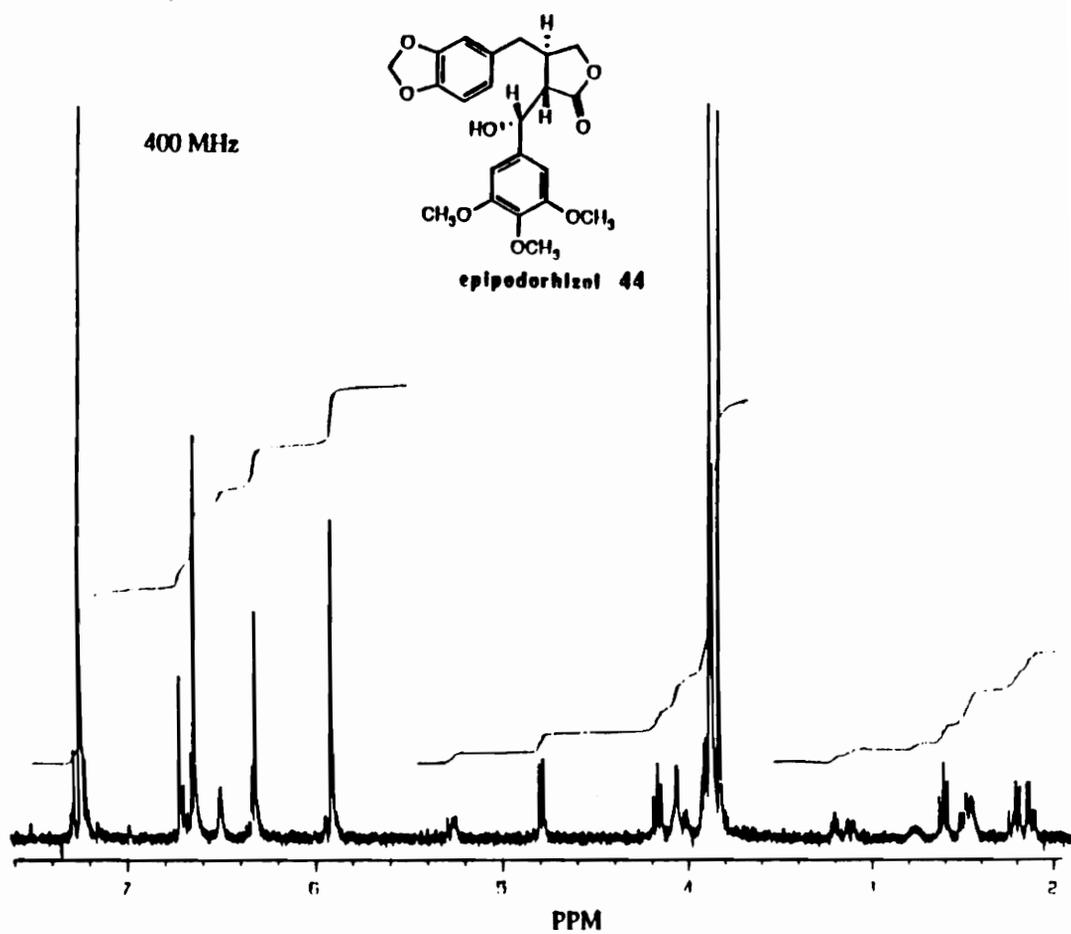
APPENDIX

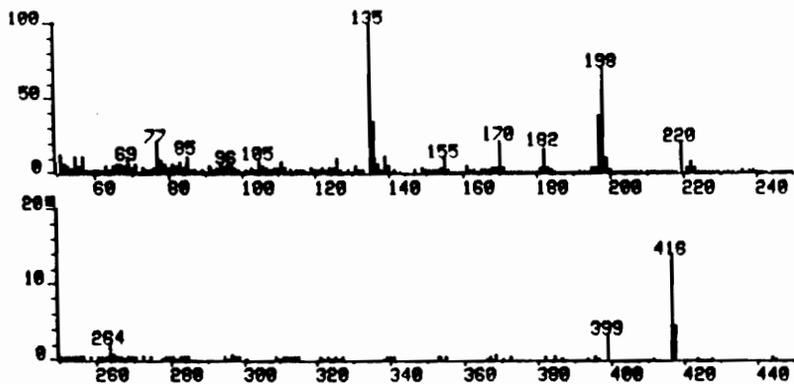
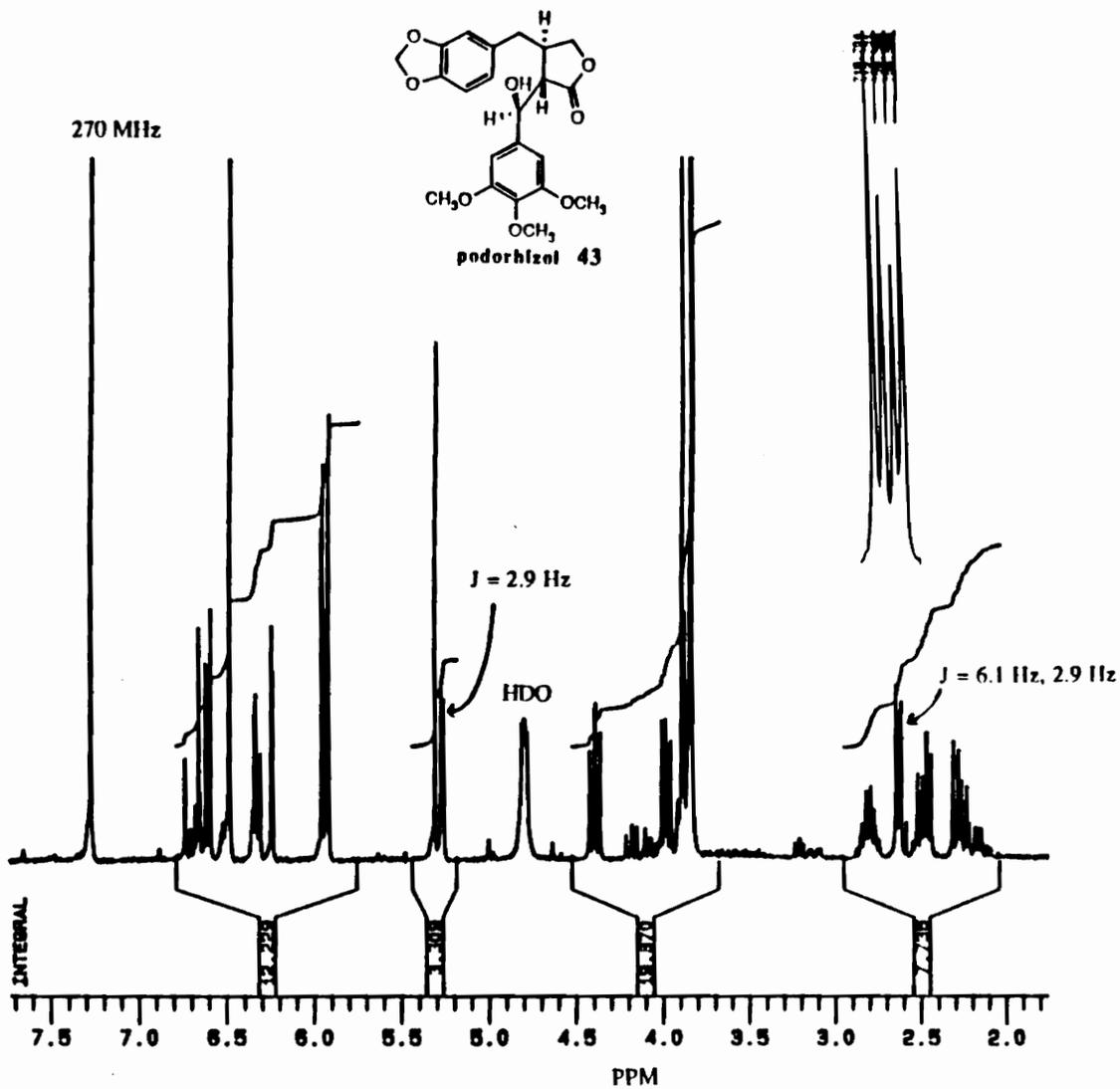


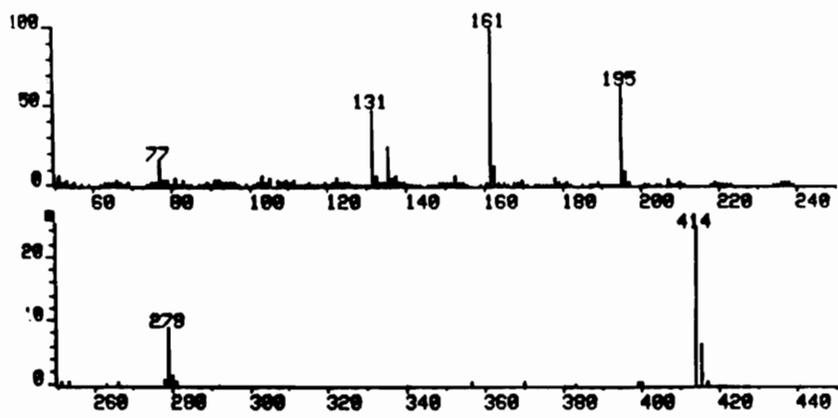
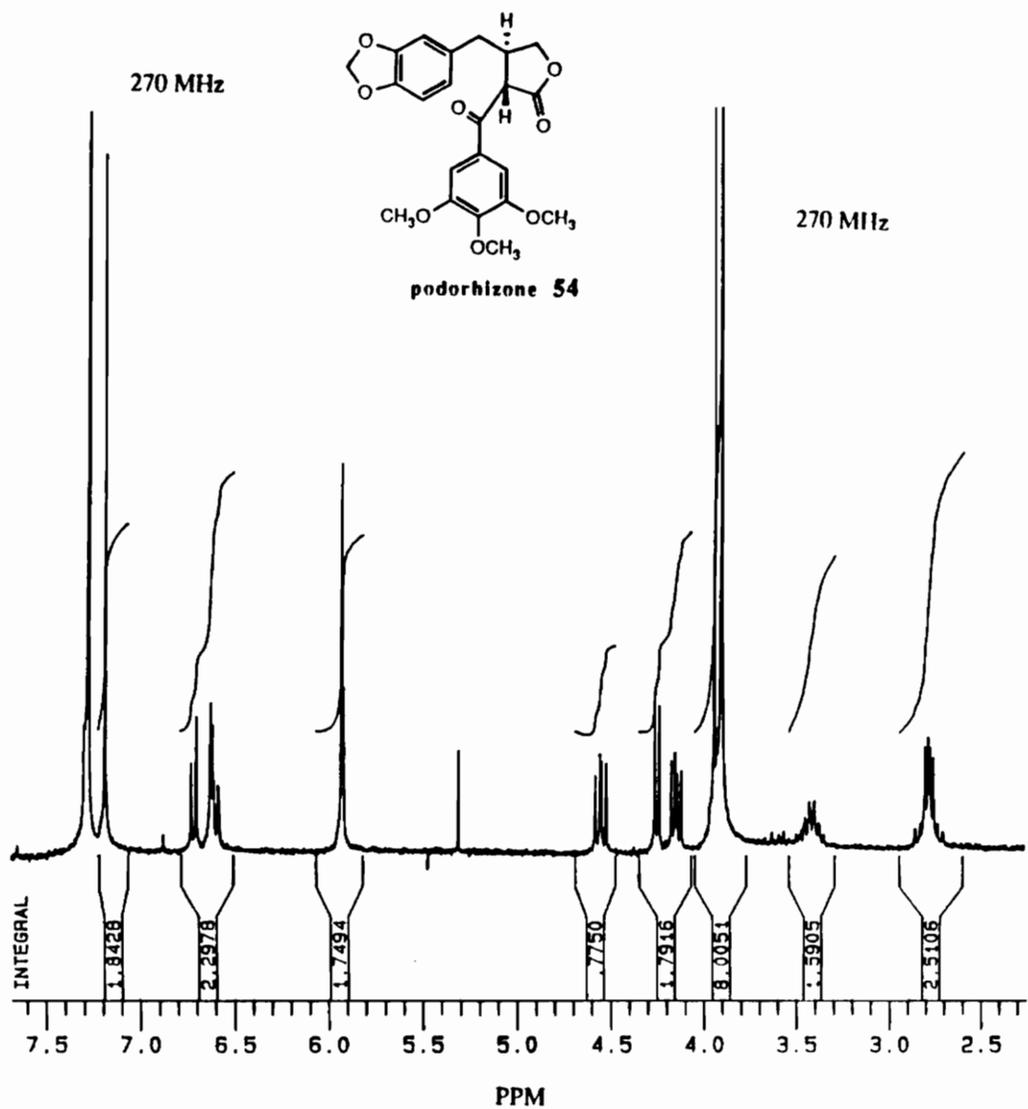


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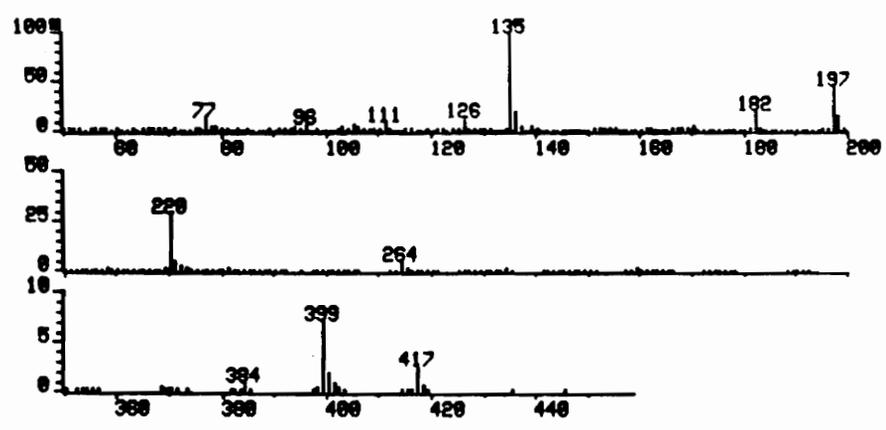
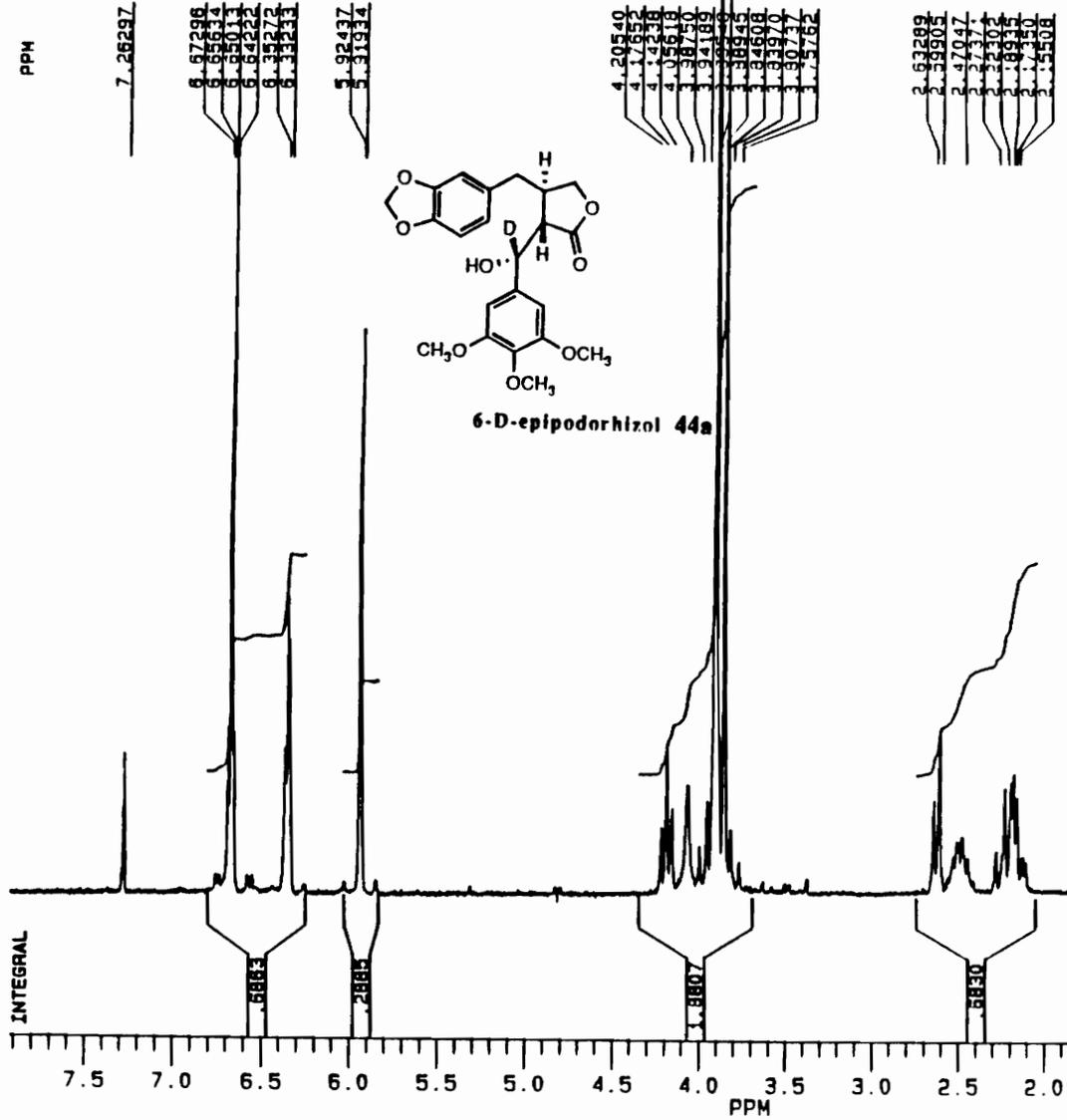


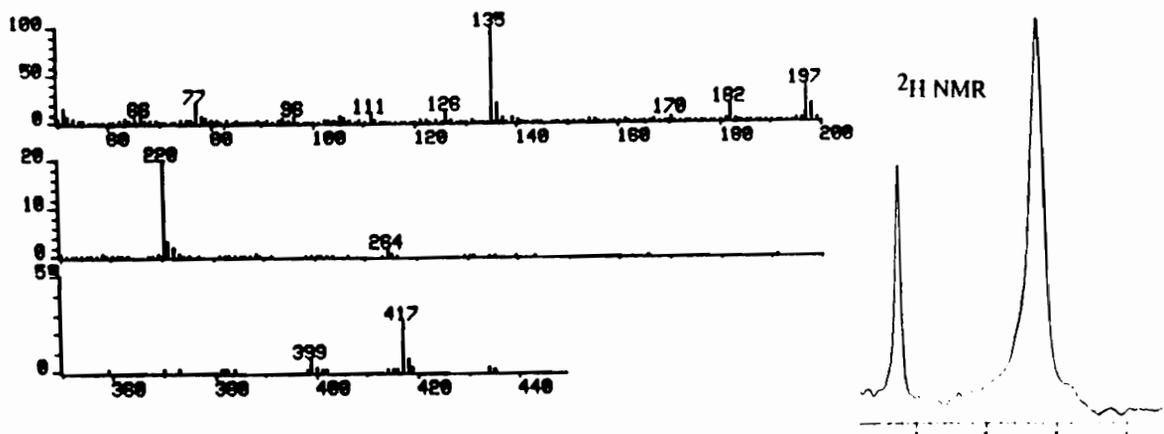
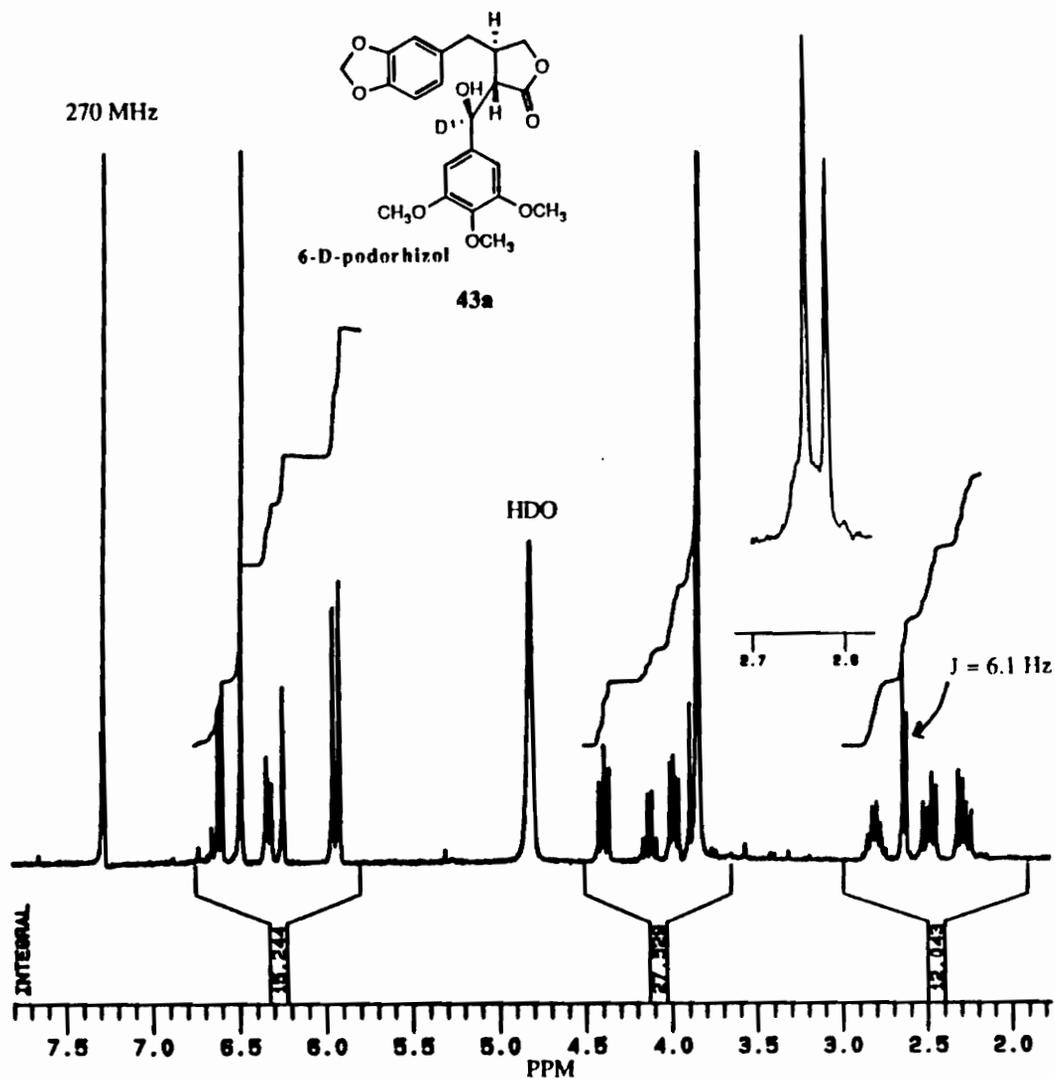






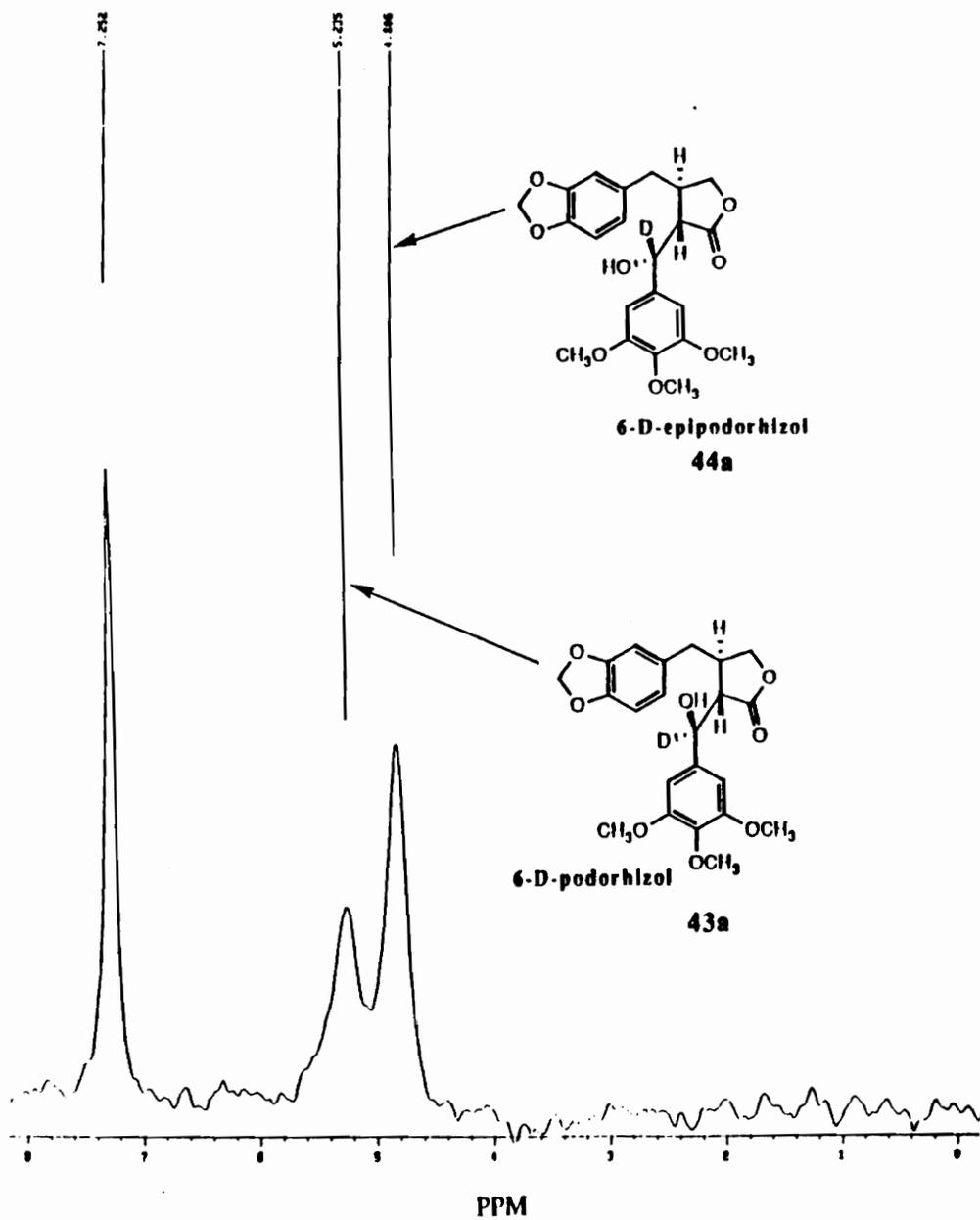
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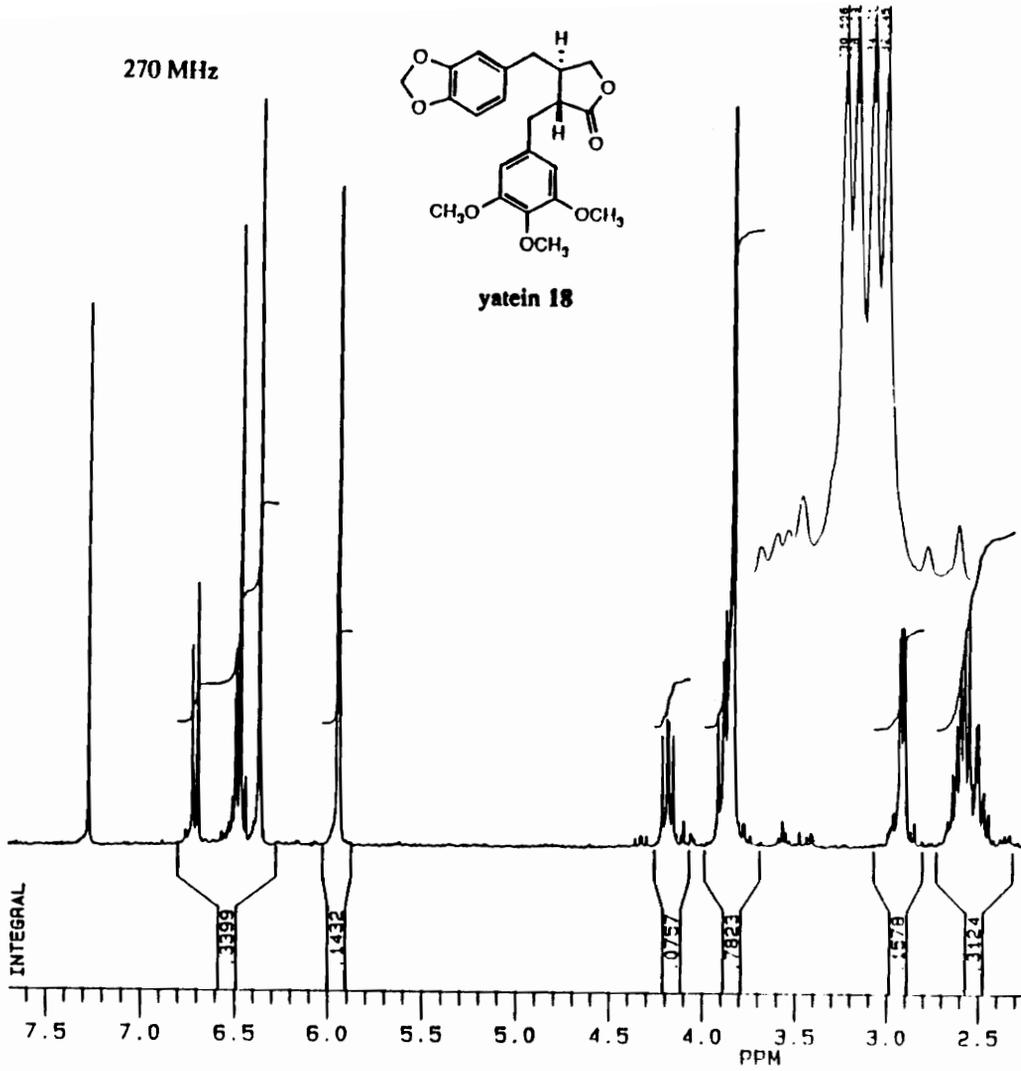




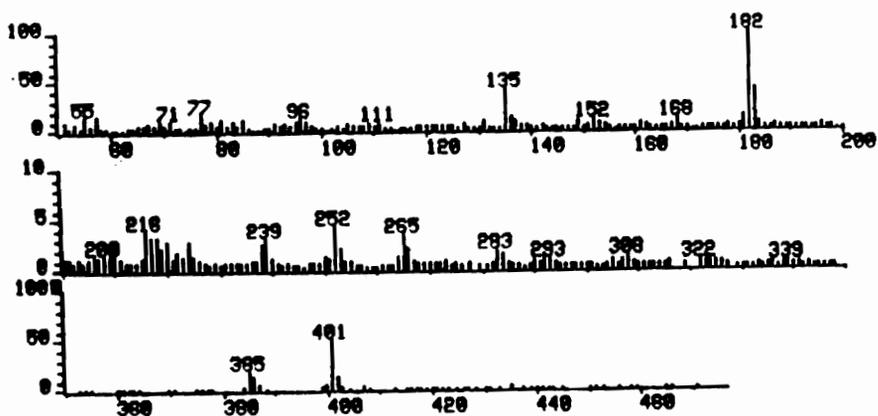
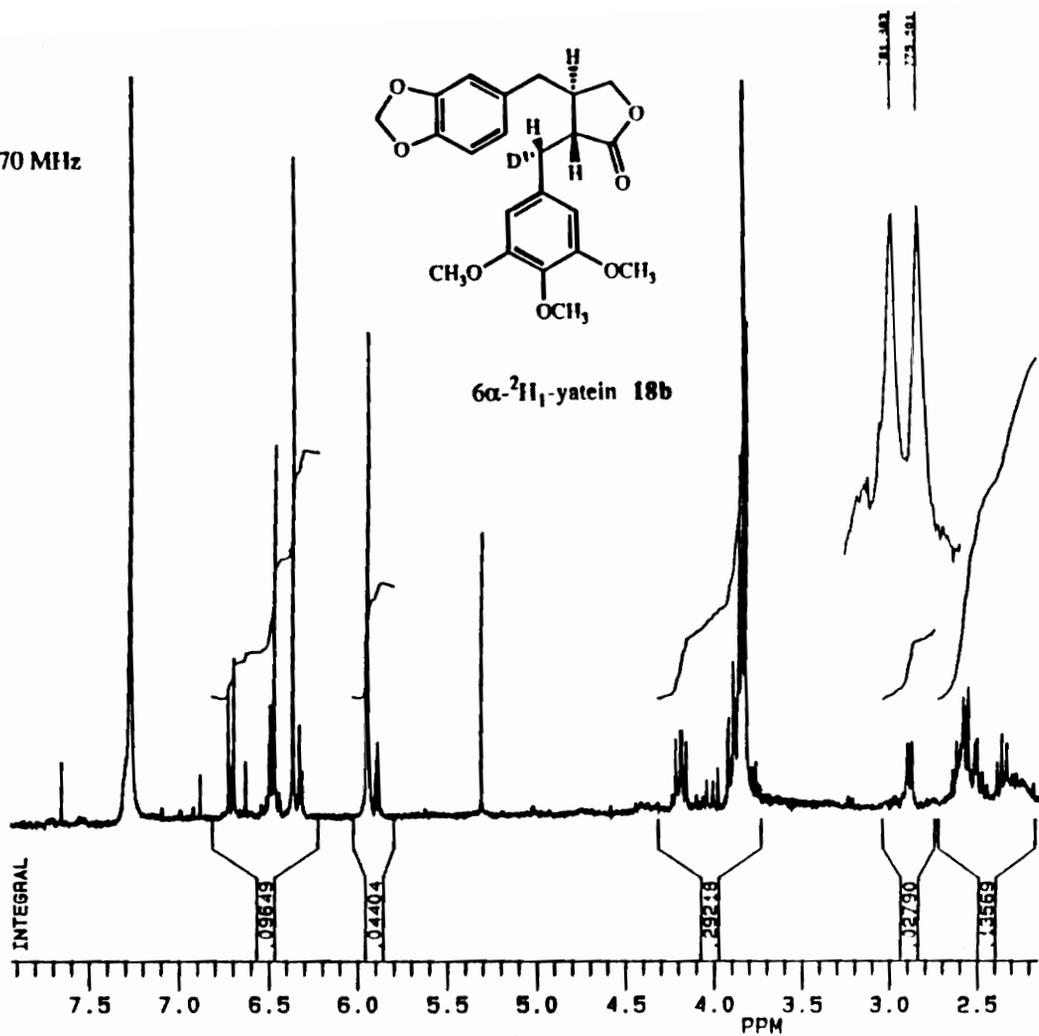
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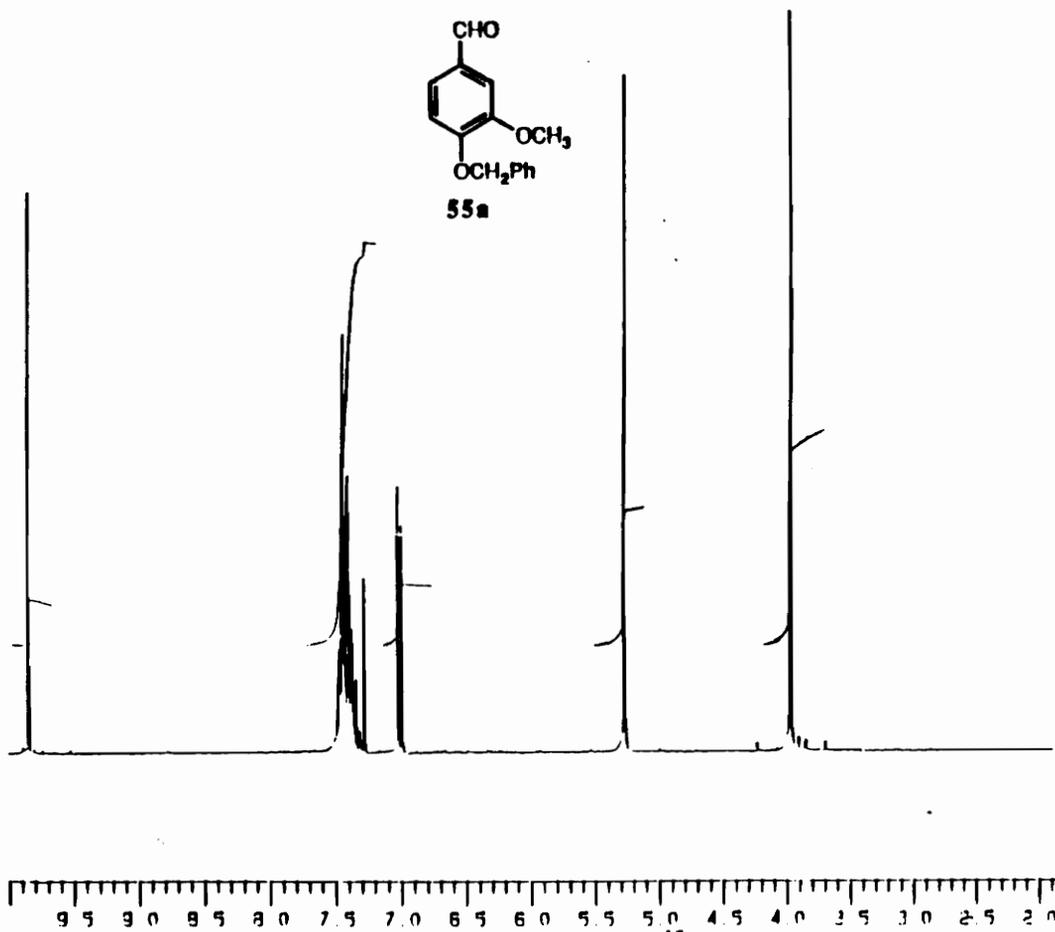
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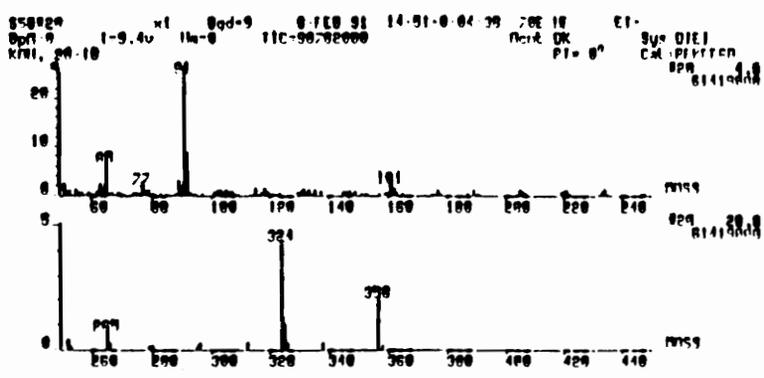
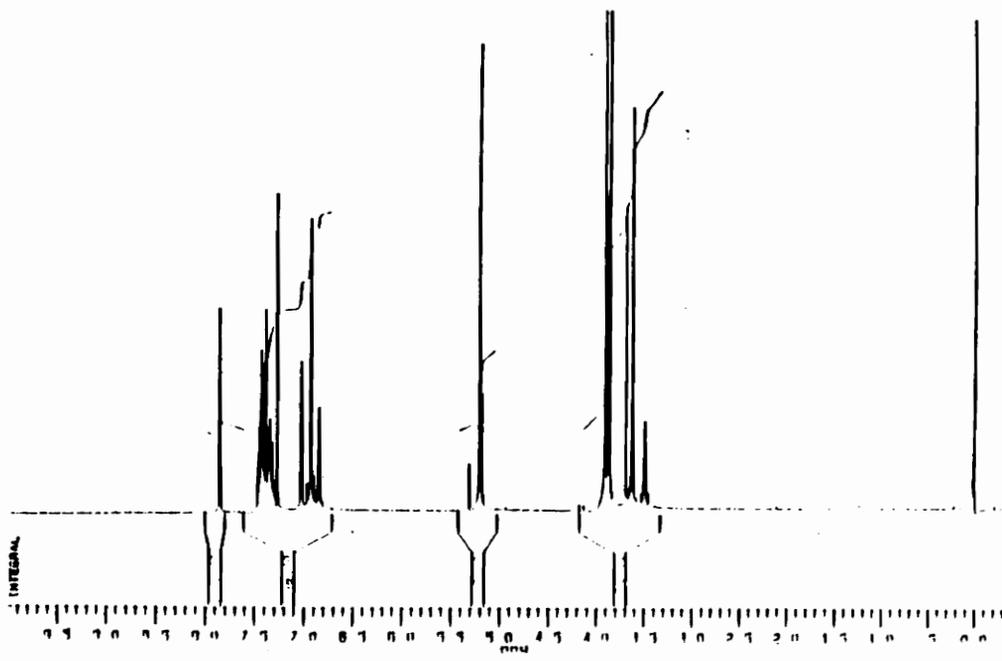
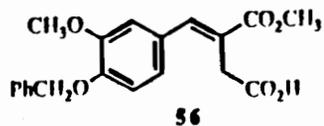


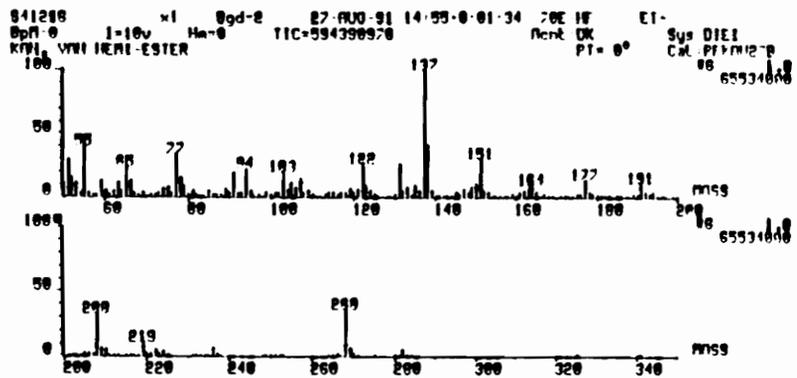
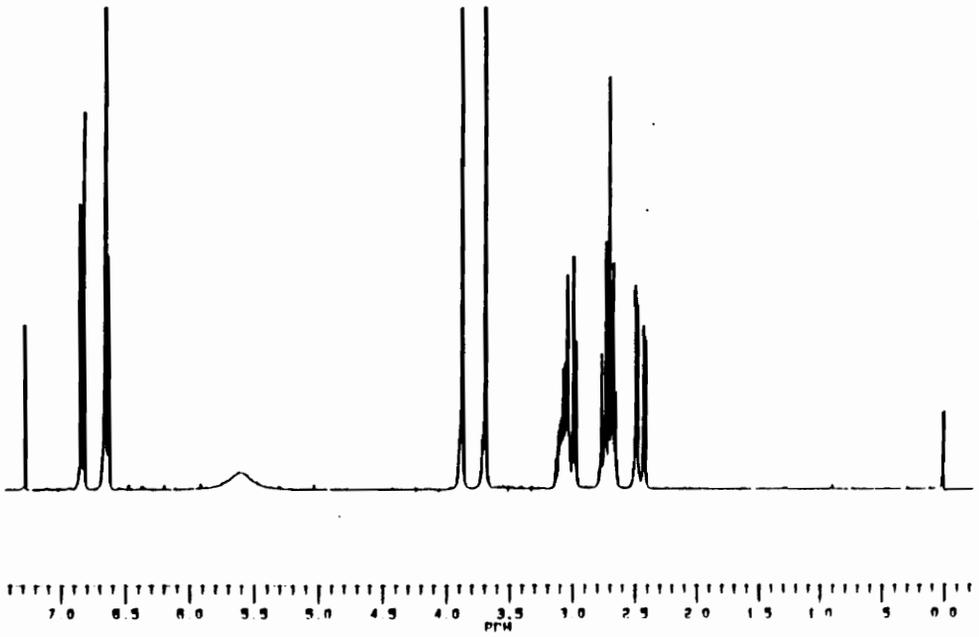
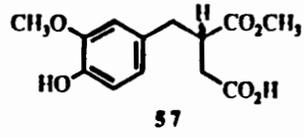


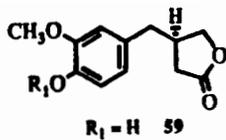
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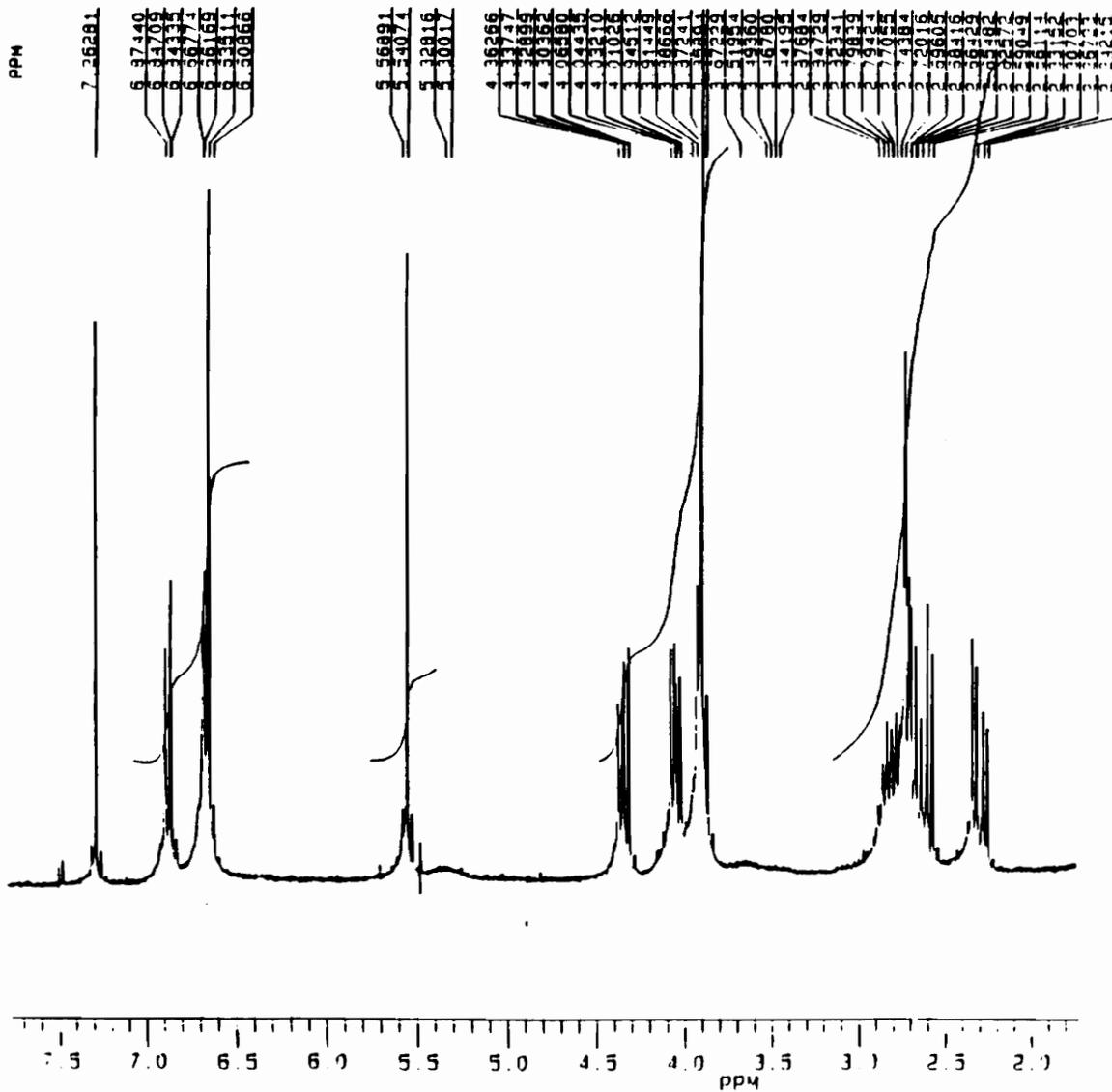




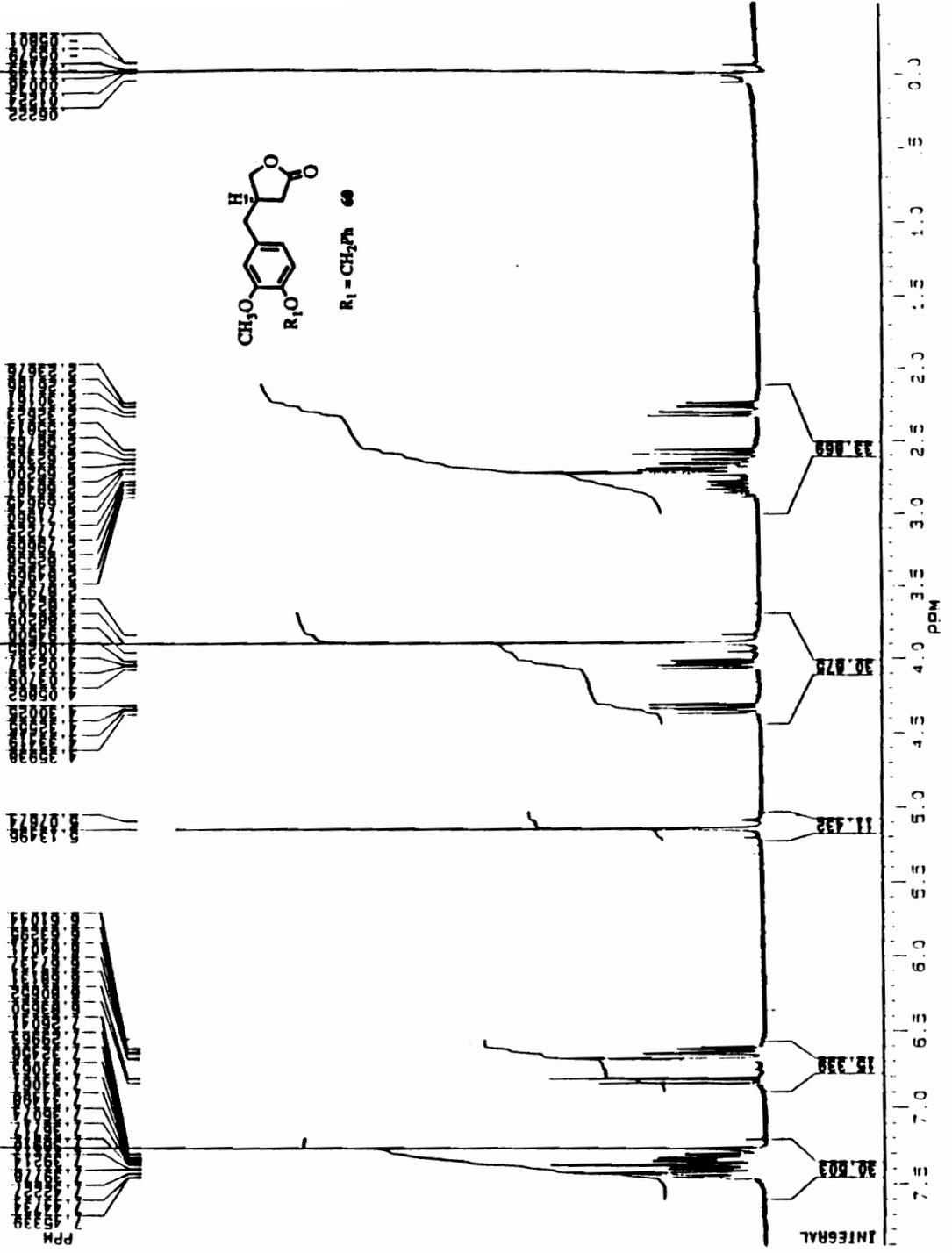


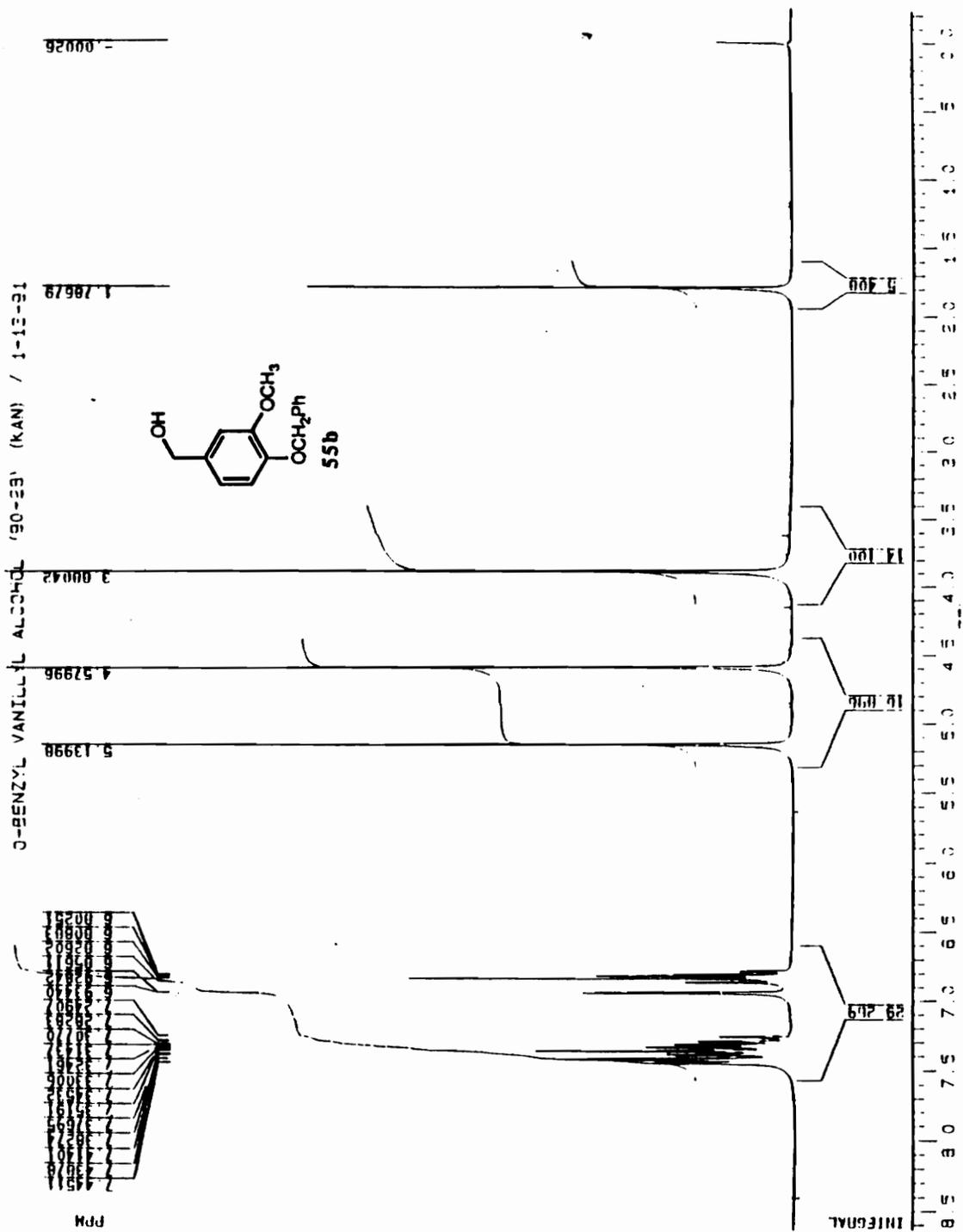


VANILLYLBUTYROLACTONE. 12-28-91

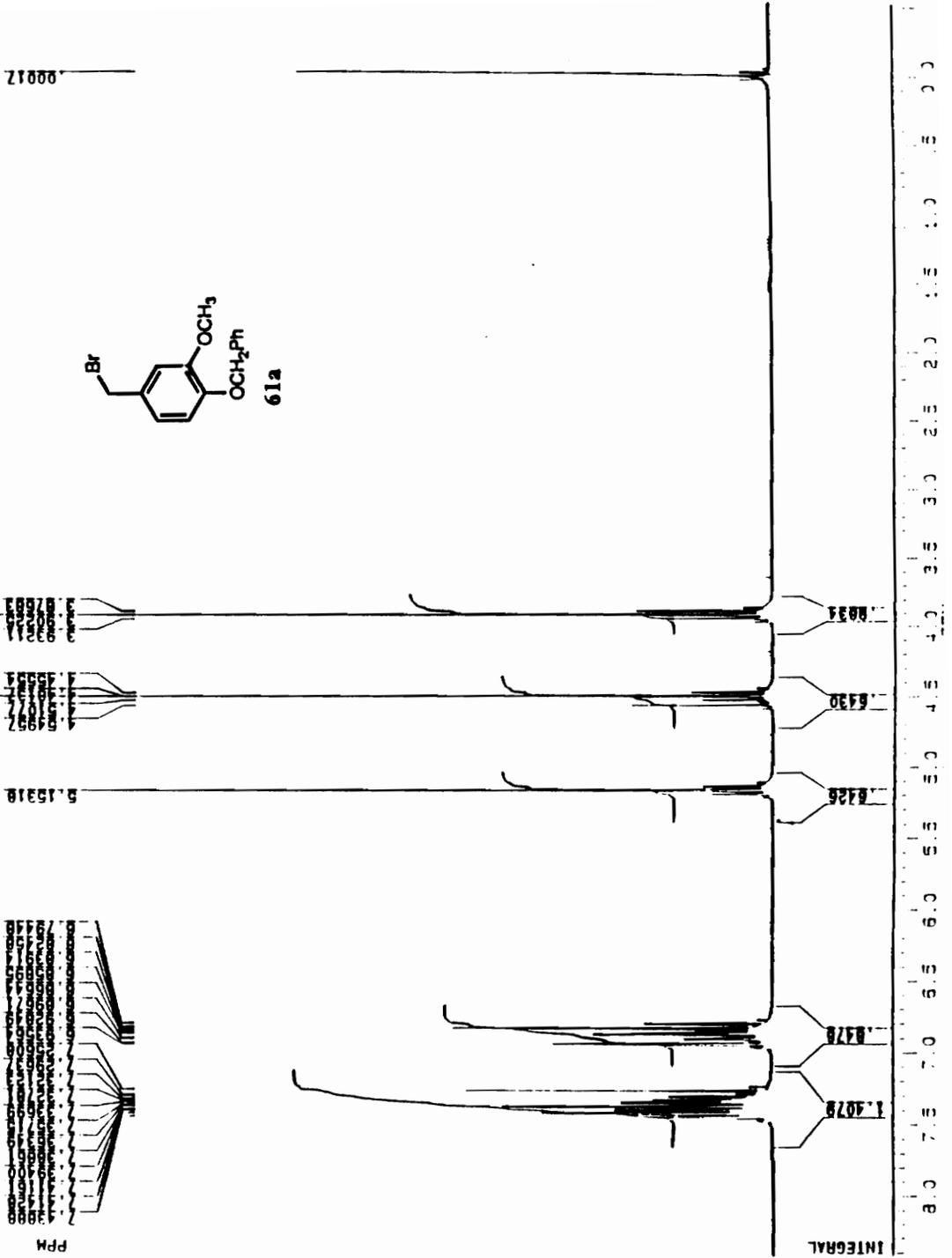
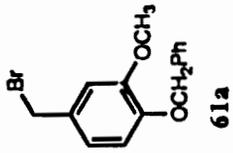


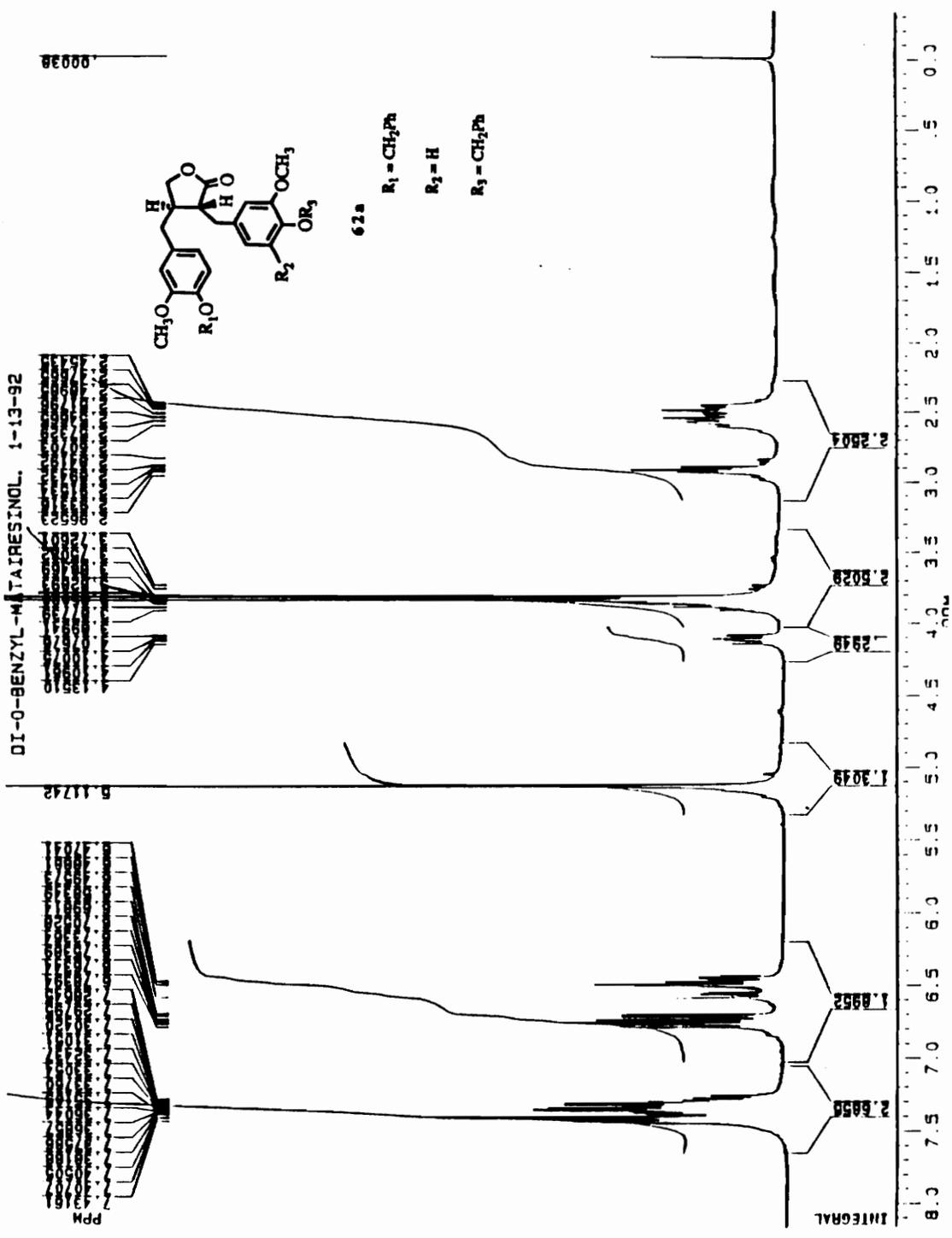
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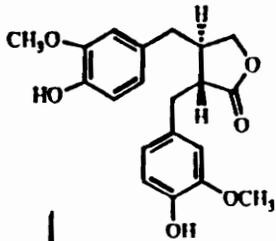




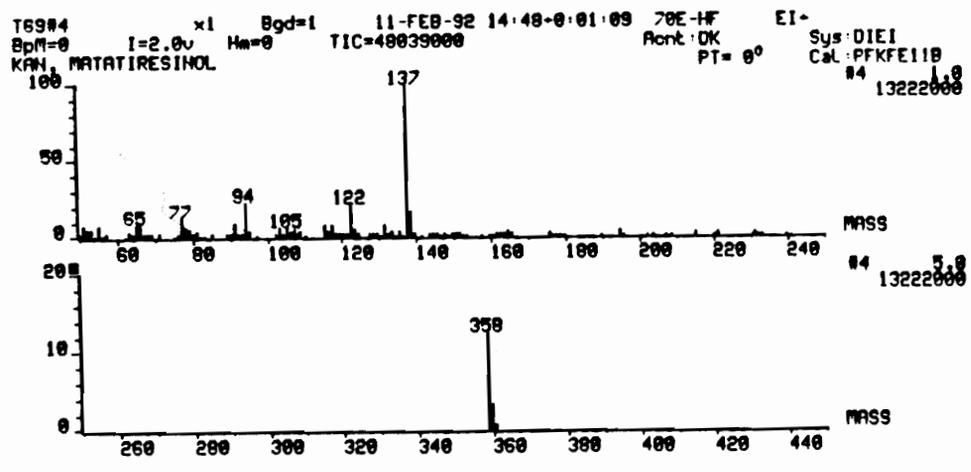
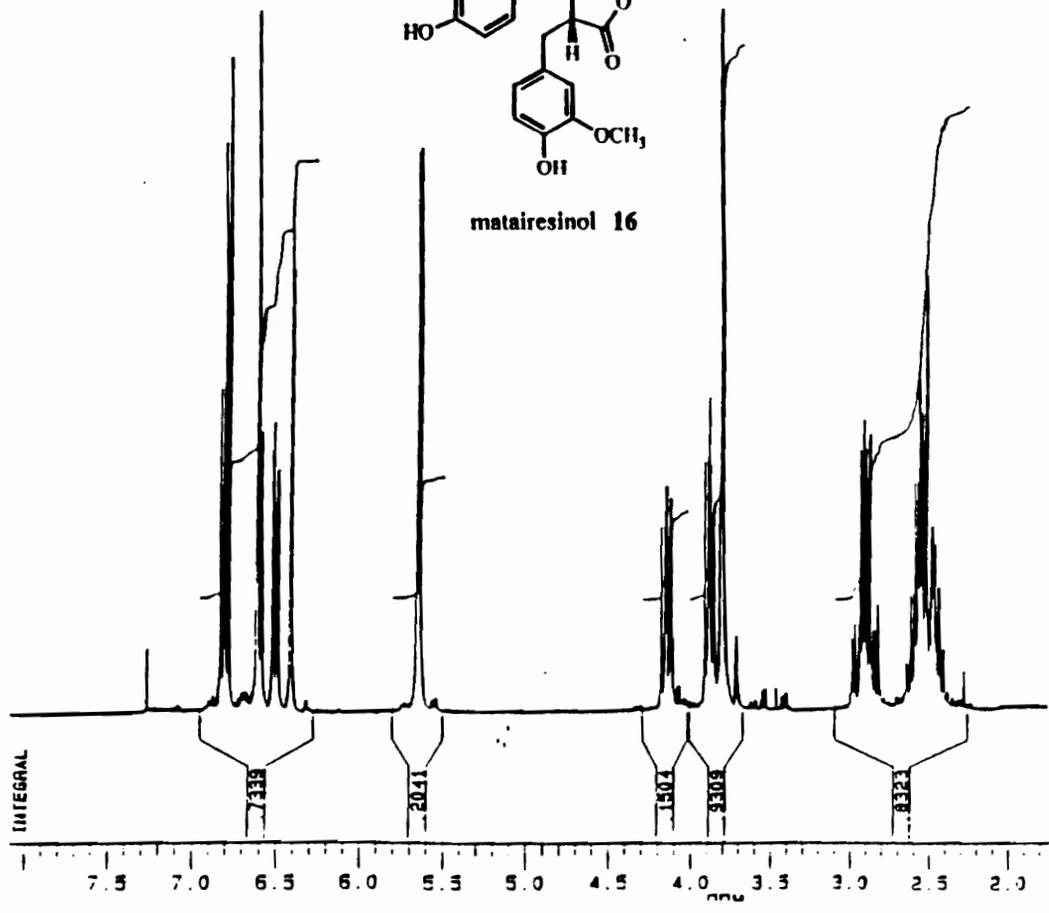
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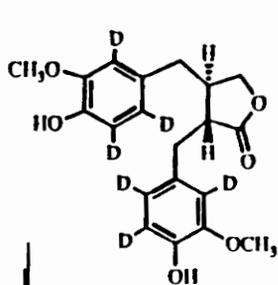




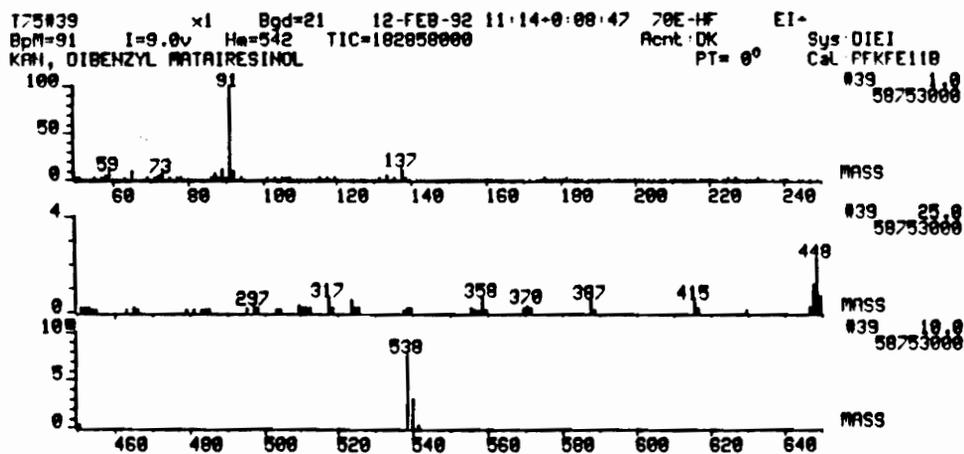
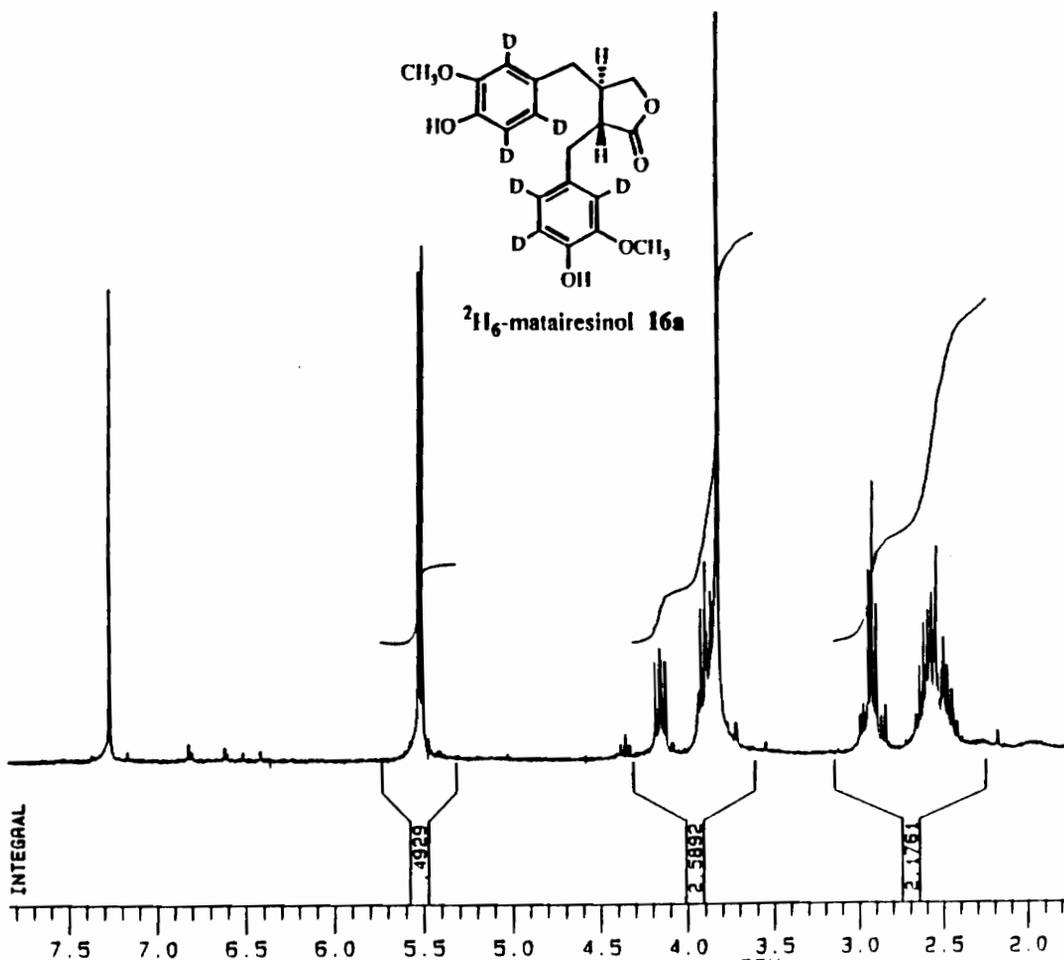


matairesinol 16





²H₆-matairesinol 16a



VITA

Kurt Alan Neidigh was born on July 18, 1962, in Washington, D.C. After graduation from Spotsylvania High School, Spotsylvania, Virginia, in June 1980, he attended Germanna Community College, Locust Grove, Virginia, where he graduated *Magna cum Laude* with Associate of Science degrees in Science, Education, and Business Administration, in June 1982. On January 12, 1984, he was married to Brenda Ann Twede, in Salt Lake City, Utah. In June 1986, he received his Bachelor of Science degree in Chemistry and Mathematics from the University of Utah, Salt Lake City, Utah. After working as a Laboratory Assistant in the Department of Bioengineering at the University of Utah, he was employed as a Laboratory Specialist in the Department of Biomedical Sciences at the Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, from November 1986 until August 1988. At this time, he enrolled as a full-time graduate student in Chemistry at VPI&SU, working under the direction of Dr. David G.I. Kingston and project collaborator, Norman G. Lewis (who, for a year, provided laboratory space and valuable advice for learning synthetic and biosynthetic techniques). In February 1992, he completed the requirements for the Master of Science degree in Organic Chemistry. Presently, he is continuing his graduate studies under Dr. Kingston, toward a Doctor of Philosophy degree, conducting research on modified taxols.

Kurt A. Neidigh