OXIDATIVE COUPLING OF SINAPIC ACID

by

Kerri-Ann S. Lee

a thesis

submitted to the Faculty of Graduate Studies

of the University of Manitoba in partial fulfillment

of the requirements for the Master's degree

University of Manitoba

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Abstract

Oxidative coupling of phenolic compounds is an important method used by synthetic organic chemists for the synthesis of various lignans. It has also been implicated in lignan biosynthesis. Recently, it was discovered that sinapic acid in a basic buffer in the presence of oxygen was quantitatively converted to the lignan, thomasidioic acid. This interesting reaction formed the basis for several studies described in this thesis.

The scope of the oxidation reaction described above was studied by applying the same reaction conditions to other 4-hydroxycinnamic acids (ferulic, caffeic and coumaric acids). The efficient oxidative coupling/cyclization appears to be limited to only sinapic acid. Oxidation of sinapic acid in more strongly basic solution (0.1 N KOH) unexpectedly produced 6-hydroxy-5,7-dimethoxy-2-naphthoic acid. The formation of this compound appeared to involve the secondary oxidation of thomasidioic acid.

As well as studying the synthetic scope of the oxidative coupling of *para*-hydroxycinnamic acids, the details of the mechanism of this interesting reaction were also studied. Various aspects of the reaction were studied, including the nature of the intermediates involved and stoichiometry of the reaction. Oxidizing agents other than oxygen were also used in order to provide more insight into the mechanism of the oxidative coupling.

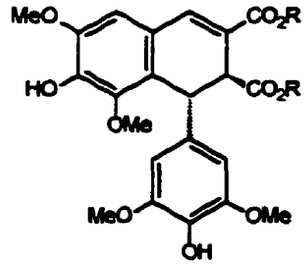
The oxidative coupling of esters of sinapic acid has also been studied. For the reaction of methyl sinapate, some of the expected dimethyl thomasidioate was formed in a mixture with a diarylbutadiene diester in a 1:2 ratio. For the reaction of (methyl (*R*)-mandelyl) sinapate in basic buffer, the products were a mixture of isomers of the diaryl

butadiene product. These results indicate that the direct asymmetric synthesis of aryltetralins by oxidative coupling of chiral cinnamate esters is probably not feasible.

Chapter 1

Introduction

Lignans are a class of naturally occurring products found in plants which have intrigued synthetic organic chemists because of their biological activities. To prepare various lignans, chemists have investigated many different synthetic methods, including oxidative coupling of phenols,[1] which is also implicated in the biosynthesis of lignans. This thesis will focus on the synthesis of thomasidioic acid (1) and its methyl ester 2 via a phenolic oxidative coupling. The introduction will give a general overview of lignans and their various biological activities followed by a discussion of thomasidioic acid in particular. Subsequently, oxidative coupling of phenols as it pertains to the synthesis of lignans will be reviewed.

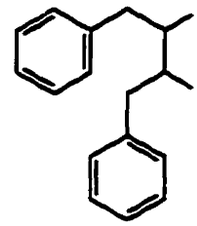


1 R = H
2 R = Me

1.1 Lignans

Lignans are a group of natural products known for their varied biological effects on humans and other species. They are formed via the shikimate pathway, a major metabolic pathway leading to formation of aromatic compounds in living systems.

Haworth first defined lignans as two propylbenzene units linked by their β-carbons, giving a 1,4 dibenzylbutane structure illustrated by structure **3**.[2] Lignans can be placed into two classes, the acyclic lignans and cyclolignans. The acyclic lignans have the general structure of **3**, while the cyclolignans have an additional C-C bond which results in



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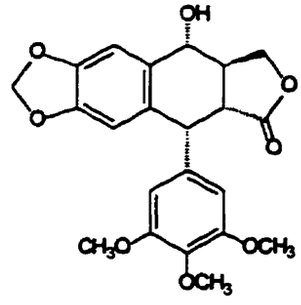
the formation of an additional ring. Also, in comparing different lignans, one can observe variable patterns of oxygen substitution on both the carbon chain and the aromatic rings, which can include heterocyclic rings. This is illustrated by the lignans podophyllotoxin



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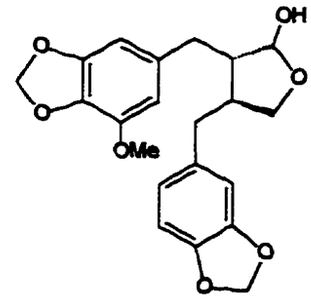

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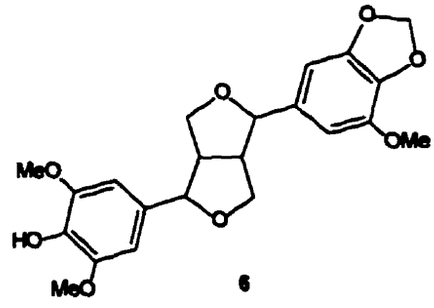

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(4), trichostin (5) and (+)-methoxypiperitol (6).[3]

1.2.1 Biological Activities of Lignans

The discovery of lignans' biological activity in the 1940's opened a new area of research into the basis and scope of this activity. Chemists have investigated many different lignans and found their biological activities to be quite diverse. The knowledge of the mechanisms behind the biological activities is steadily increasing and chemists have begun to understand the relationship between lignan structure and biological activity. The research in the area of lignans and their biological activities is constantly progressing and in time, chemists may have a complete understanding of these processes.

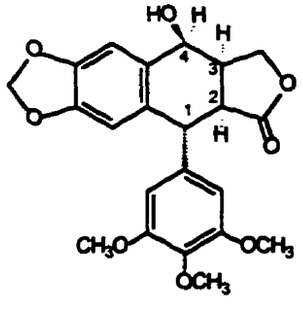
1.2.2 Lignans in Plants

Lignans isolated from plant matter have been extensively studied for their biological activities. These natural products are isolated from fifty-five families of vascular plants, most of which belong to the Gymnosperm class. Lignans have been found in almost all components of plants, such as wood, roots, flowers and seeds. The various biological activities that lignans can exhibit include enzyme inhibition and cytotoxicity. As well, they can demonstrate antitumor, antimitotic and antiviral activities.[4-10]

The reason for the initial interest in lignans was the discovery that some can act as antitumor agents. Thus far, there are 30 different naturally occurring lignans and many other lignan analogues known to have antitumor properties. The majority of the natural antitumor lignans, such as podophylltotoxin and other structurally similar lignans are isolated from the plant class, *Podophyllum*. Unfortunately, as well as having antitumor

properties, some of these naturally occurring compounds, such as podophyllotoxin itself, are extremely toxic. Chemical modifications of the various functional groups of the lignans have allowed chemists to reduce their toxicity while maintaining the antitumor activities of the lignans. Two semisynthetic derivatives of podophyllotoxin, VP-16-213 (etoposide) and VM-26 (teniposide), are currently used clinically to treat a variety of cancers.[11] It is their use in fighting cancer that has made the extraction of podophyllotoxin from *Podophyllum* a major industry.

Scientists have proposed numerous theories about the mechanism of lignans' biological activity. In many of these studies, attempts have been made to correlate activity to structure.[12-14] For example, lignans, with the podophyllotoxin general structure and able to cause chromosomal damage to a cell, usually possess a 4'-hydroxyl group, while inactive lignans do not. Also, lignans with an enhanced capability to damage DNA, such as epipodophyllotoxin (**7**) and 4'-demethyl-epipodophyllotoxin (**8**), differ as they all have an S configuration at C-4.[4]



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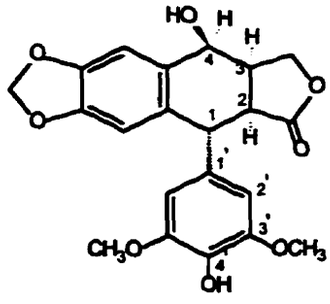

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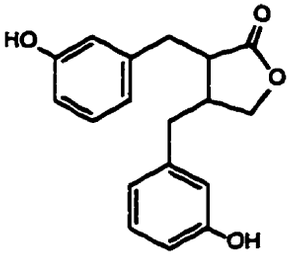
Even though scientists have been able to explain how the structure of the lignan helps it to accomplish its activity in some cases, they do not yet understand all modes of action as they pertain to the structure of the lignan and its biological activity.

1.2.3 Lignans in Animals

Recently, lignans have been found in humans and other mammals and this discovery has prompted chemists to ponder their biological and physiological roles. The primary lignans found in the urine of animals, such as rats and baboons, are enterolactone (**9**) and enterodiol (**10**).[15-18] They differ in structure from the lignans found in plants in that their aromatic rings are only meta-substituted.



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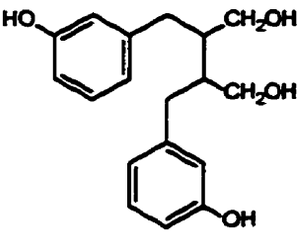

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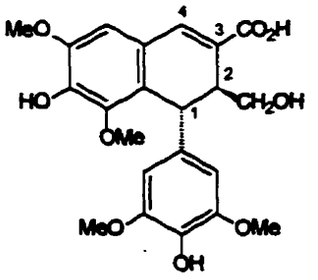
The precursors to these mammalian lignans have been traced to many common dietary foods, such as whole cereal and brans. Evidence has shown that the lignans are formed via microbial action on the two plant lignans, secoisolariciresinal diglucoside and matairesinol.[19] It was once believed that a vegetarian diet would reduce the risk of chronic diseases, such as coronary heart diseases and cancer, due to the general health benefits of dietary fiber. It has now been proposed that it is the lignans found in these foods that are indirectly involved in modulating disease. In particular, it has been proposed that these lignans can suppress estrogen-dependent cancers. The majority of estrogen in the body is bound to sex hormone-binding globulin (SHBG), because it is not readily soluble due to its lipophilicity. It has been postulated that the concentration of the unbound hormone may play a role in the occurrence of breast cancer and other hormone-related diseases. An increased concentration of mammalian lignans in the system due to

greater intake of dietary lignans has been shown to increase the concentration of SHBG and thereby decrease the amount of free estrogen in the bodily fluids. This in turn decreases the incidence of estrogen dependent breast cancer. The lignans, enterolactone and enterodiol, also demonstrate other biological effects that are currently being investigated.

1.3.1 Thomasidioic Acid

Thomasidioic acid (**1**), a unique lignan first isolated with thomasic acid (**11**) from the elm tree species, *Ulmus thomasii* Sarg. in 1969,[20,21] was recently discovered to be a biproduct formed when studying the conditions used during the processing of canola meal.[22,23] Thomasidioic acid was also found in other species of elm, such as *Ulmus alata* and *Ulmus parvifolia,* and in the brown coloration of the outer rings of trees infected with Dutch Elm disease.[24]

There are many structural characteristics of thomasidioic acid (**1**) and its congener, thomasic acid (**11**) that make them unique in their class of lignans, the aryltetralins.[25]



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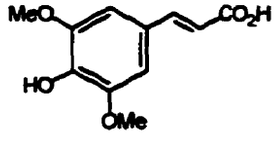
First of all, thomasidioic and thomasic acid have free carboxylic acid groups and thomasidioic acid was the first diacid lignan reported. A free acid group is unusual in lignans as the majority have a carboxyl group which has undergone lactonization.

Thomasidioic and thomasic acid were also the first lignans discovered with the 1,2-dihydro-1-phenylnaphthalene skeleton. Finally, the most unusual property of these lignans is that they are racemic despite having two asymmetric centers. Most lignans are found in an optically active form. All of these unusual attributes of thomasidioic and thomasic acid have aroused the interest of chemists.

1.3.2 Isolation of Thomasidioic Acid

The identification of thomasidioic and thomasic acids began with their isolation from *Ulmus thomasii* Sarg. heartwood.[20,21] The aqueous extracts of the heartwood were acidified with hydrochloric acid and then fractionated on a polyamide column using methanol/water as solvent. The first compound to be isolated was thomasic acid. It crystallized out of the 40% methanol eluate and was isolated in 0.2% yield.

To establish the structure of thomasic acid, the functional groups were identified and then assembled to form the molecule. Much of the determination of structure relied on the comparison of thomasic acid to sinapic acid (**12**), as they have many structural



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similarities. It was concluded that the compound was monobasic and had a chromophoric system like that of sinapic acid. The UV maxima in various solvents also provided information about conjugation in the molecule. For instance, the shift of the UV spectra in base revealed that the phenolic group and unsaturated side chain were conjugated, while the hypsochromic shift of the UV maxima in sodium acetate showed that the acid group

was also conjugated. The final structural conclusions were made using the NMR spectra of both sinapic acid and the unknown compound. The seven singlets seen in the spectrum of the newly isolated compound placed great restriction on its structure and an aryltetralin-type structure was postulated. Further proof of the structure of thomasic acid came from comparisons to other similar lignans and the preparation and study of various derivatives of thomasic acid. The original stereochemistry of the molecule was proposed to have a *cis* conformation at C-1 and C-2. Wallis later provided evidence that these centers were not *cis*, but *trans* and diaxial, as shown in structure **11**.[26]

Thomasidioic acid was eluted as a mixture with 6-hydroxy-5,7-dimethoxy-2-naphthoic acid.[21] The two compounds were refractionated on a Polyclar column. Once eluted, the 2-naphthoic acid crystallized easily out of solution, but thomasidioic acid remained amorphous. Thomasidioic acid's structure was determined using comparison of its spectral information to that of thomasic acid. The shift of their UV maxima were nearly identical, showing the phenolic and acidic nature of the molecule. The IR spectrum of thomasidioic acid differed from that of thomasic acid because the band arising from the hydroxymethyl group was missing and the carbonyl band had broadened into two bands at 1690 and 1700 cm^{-1}. These two peaks represented the conjugated and non-conjugated acid groups of the molecule. Finally, on comparison of the acids' NMR spectra, it was noted that the chemical shifts of the peaks observed for the methoxy, aromatic, and vinylic protons were nearly identical. The comparability of the various spectral data of thomasidioic and thomasic acid made it apparent that the lignans had many structural similarities. The structure proposed for thomasidioic acid was similar to that of thomasic

acid, but with replacement of the hydroxymethyl group by an unconjugated acid group, giving the diacid lignan (**1**).

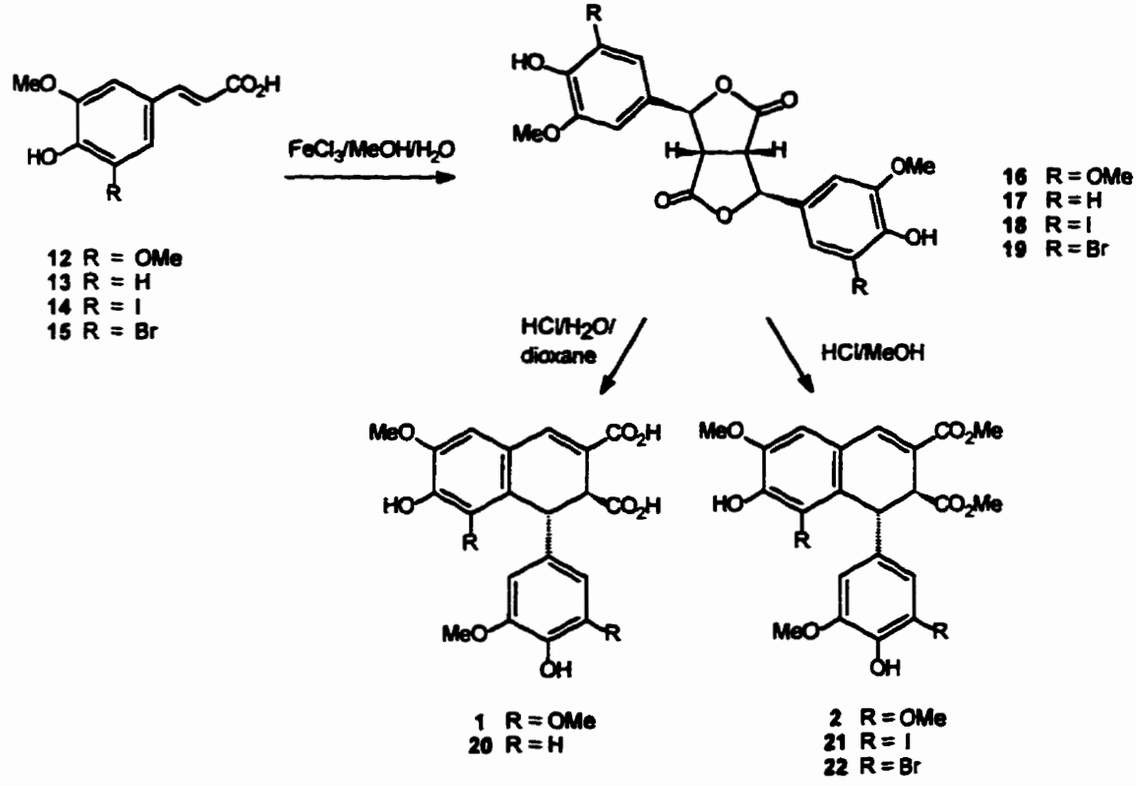
For further proof of the structure, chemical modifications of thomasic and thomasidioic acid were performed. Thomasic acid was oxidized with Jones reagent to the diacid and methylated with diazomethane giving the dimethyl ether-dimethyl ester of thomasidioic acid. This was the same compound obtained from direct methylation of thomasidioic acid. The chemists then confidently concluded that they had isolated the first aryltetralin diacid, thomasidioic acid.

1.3.3 Synthesis of Thomasidioic Acid

In the past, there have been only two articles describing the synthesis of thomasidioic acid. The first report was published by Ahmed, Lehrer and Stevenson.[25] They reacted sinapic acid with ferric chloride in aqueous methanol. Oxidative coupling of the acid yielded dehydrodisinapic acid dilactone (**16**). Its structure was determined by comparison to the spectra of dehydrodiferulic acid dilactone (**17**), which was synthesized previously by Cartwright and Haworth in the same manner.[27] The dilactone **16** was then treated with hydrochloric acid in water/dioxane. This reaction produced thomasidioic acid in 90% overall yield (Scheme 1). The structure of the product was confirmed by synthesis of the dimethyl ether-dimethyl ester and comparison to the spectra reported by Hostettler and Seikel.[21] It was also found that the reaction of dilactone **16** in methanol saturated with hydrochloric acid at 20 °C produced the dimethyl ester of thomasidioic acid (**2**). The discovery of a simple two-step procedure for the synthesis of an aryltetralin made other scientists consider the applicability of this oxidative coupling method to other *para*-

hydroxycinnamic acids. Ferulic acid (13), iodo- and bromo-ferulic acid derivatives, 14 and 15, were treated with ferric chloride as described above for sinapic acid.[28] These acids also formed dilactone-type compounds that underwent acid-catalyzed rearrangements to produce the respective aryltetralins (Scheme 1).

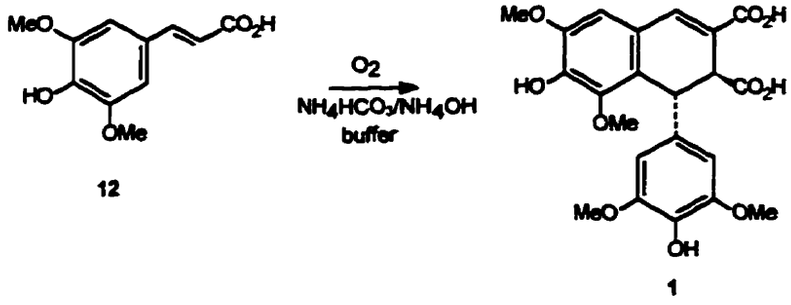
Scheme 1



Rubino, Arntfield and Charlton also discovered a simple method for the synthesis of thomasidioic acid during a study of the fate of sinapic acid during processing of canola meal.[22,23] It is known that canola meal contains phenolic compounds in their free, esterified and bound forms and that the most abundant phenolic acid is sinapic acid. On exposure of sinapic acid to alkaline conditions, a change in colour was observed and a new peak appeared in the HPLC (high-pressure liquid chromatograph) trace. The reaction was complete after 24 hours and the product was at first unidentifiable and difficult to purify.

Treatment of the compound with diazomethane produced a derivative that was tentatively identified as the dimethyl ether-dimethyl ester of thomasidioic acid, based on its ^{1}H NMR and mass spectra. A comparison of the unmethylated product's spectra to that of a pure sample of thomasidioic acid, as prepared using the method of Ahmed *et al.*, was made.[25] The product of the oxidative coupling of sinapic acid was thus identified as thomasidioic acid (Scheme 2). On further study, it was shown that oxygen played a role in the

Scheme 2

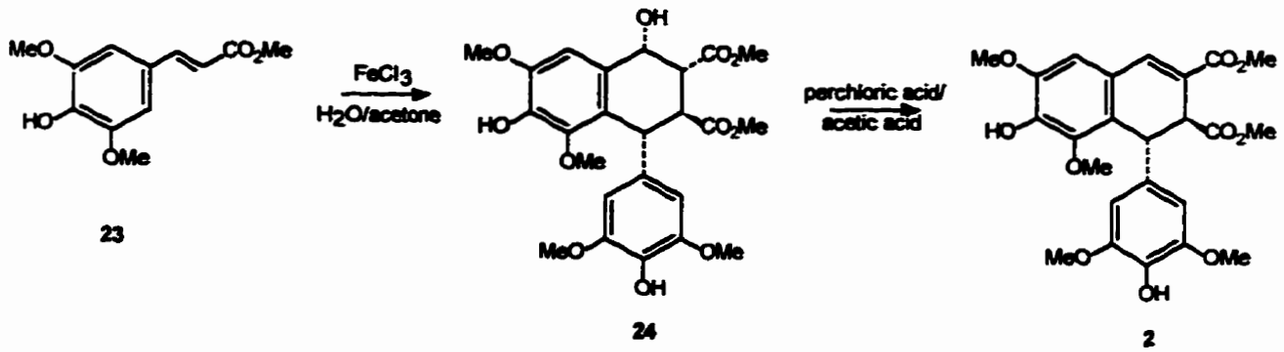


oxidation of sinapic acid because when the level of oxygen in the reaction was increased, the rate constant (k) for the reaction also rose. Under basic conditions, oxygen is known to be an effective one-electron oxidizing agent for phenolic compounds and it was concluded that that the oxidative coupling of sinapic acid to form thomasidioic acid was initiated by this reaction.

1.3.4 Synthesis of Dimethyl Thomasidioate

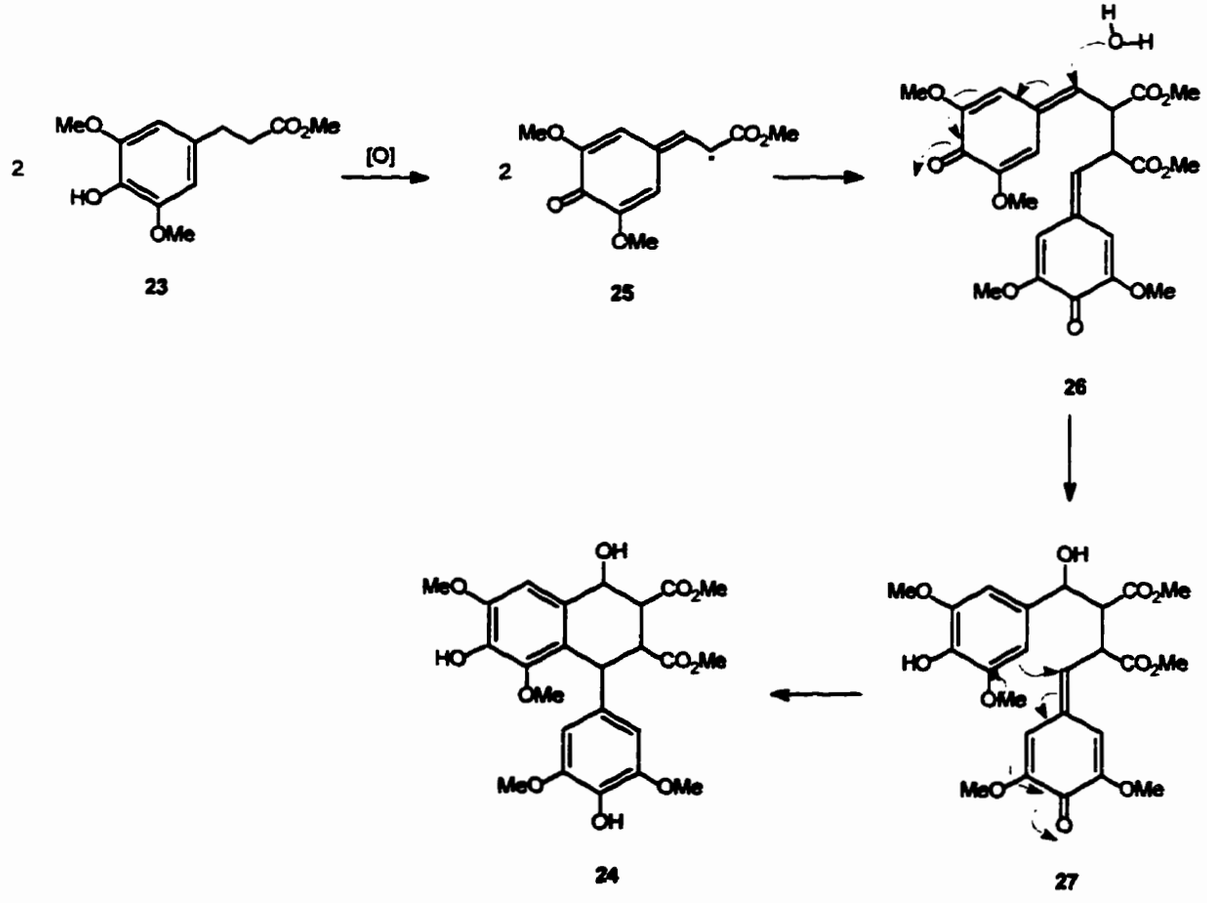
There have been two methods reported to be effective for the oxidative coupling of methyl sinapate to form the dimethyl ester of thomasidioic acid. The first method involved the use of ferric chloride as the oxidizing agent.[29] The main product of the reaction was a tetralol **24** in 61% yield as a mixture with its diastereomer and other

Scheme 3



products, such as small amounts of a lactone and the methyl ester of thomasidioic acid (Scheme 3). The mechanism for the formation of the tetralol was proposed to be: (1)

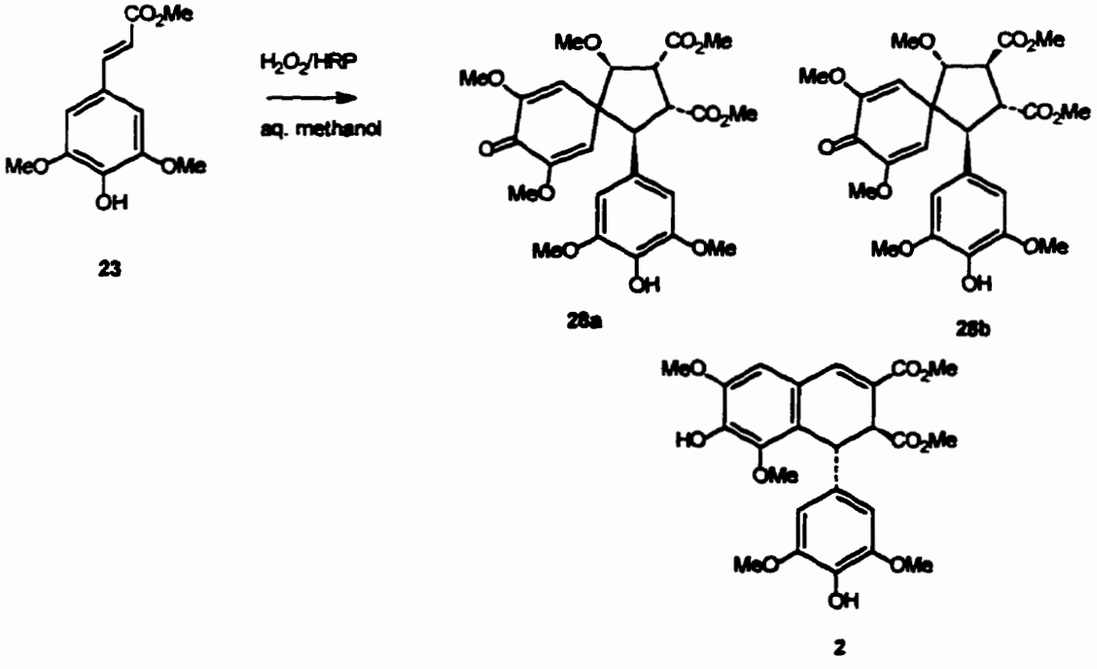
Scheme 4



1-electron oxidation of **23** to the quinone methide radical **25**, (2) dimerization of **25** to give **26**, (3) an attack of water at the benzylic carbon of the *bis*-quinone methide **26** giving **27** and (4) cyclization of the remaining quinone methide in **27** onto the aromatic ring as shown in Scheme 4. The reaction of the tetralol in perchloric acid-acetic acid at room temperature led to the loss of water from **24** to produce the dimethyl ester of thomasidioic acid (**2**) as shown in Scheme 3.

The most recent report of a synthesis of dimethyl thomasidioate (**2**) from the oxidative coupling of methyl sinapate was published by Setälä et al.[30] The reaction of methyl sinapate in hydrogen peroxide at a pH of 4, using horseradish peroxidase as a catalyst, produced dimethyl thomasidioate in 41% yield. This was the major product when the reaction solvent was aqueous acetone, but the outcome of the reaction changed when using aqueous methanol. In this case, the major product was a diastereomeric mixture of

Scheme 5



23 26a 26b 2

spiro compounds, while the thomasidioate ester was the minor product. The structures of the acetylated spiro compounds **28a** and **28b** were determined by x-ray crystallography (Scheme 5). The different result for the reaction when performed in aqueous methanol was explained by the ability of methanol to act as a nucleophile on the intermediate quinone methide. An explanation for cyclization favoring the formation of a five-membered ring over that of a six-membered ring was not given.

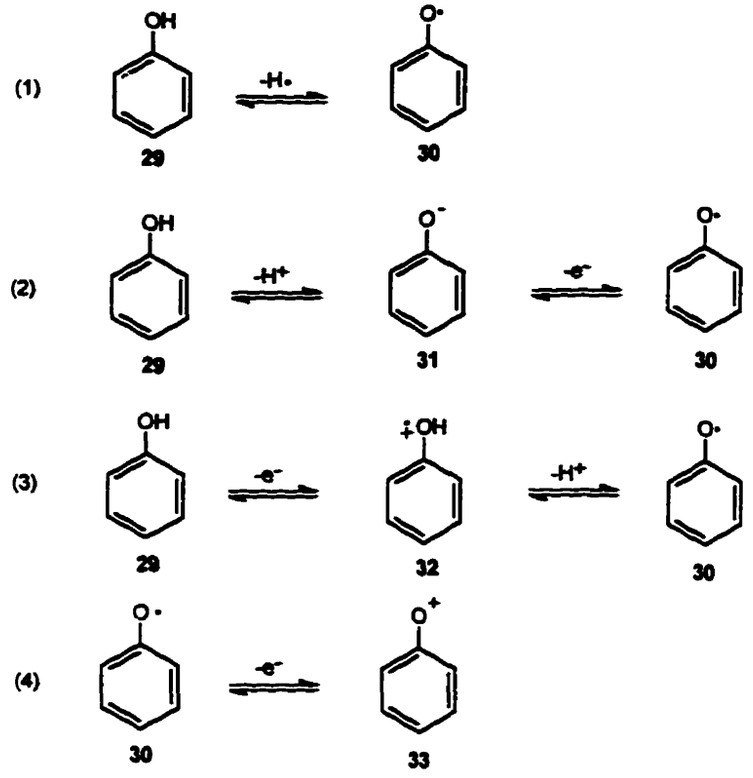
1.4.1 Oxidative Coupling of Phenols

A literature survey on oxidative coupling of phenols was made in order to obtain a better understanding of the oxidants that can be used to initiate the reaction, the mechanism of the reaction, and the intermediates involved.

1.4.2 Oxidation Step

The first step in the oxidative coupling of phenols is oxidation, or loss of electrons from the phenolic compound. Chemists have studied this aspect of the reaction for its mechanism under different reaction conditions and the ease of removal of the electrons from different phenols. Two of the mechanisms postulated for oxidation of phenols are: the abstraction of a hydrogen atom from the O-H bond, and the abstraction of an electron from a phenol or phenoxide anion.[31] With the latter mechanism, it has been postulated that the removal of an additional electron from the phenoxy radical **30** may be possible producing a phenoxonium ion **33** (Scheme 6). According to the two mechanisms, there are three different intermediates that could possibly form during the oxidation step: the

Scheme 6



phenoxy radical **30**, the protonated phenoxy radical **32**, and the phenoxonium ion **33**. Each of the mechanisms suggested involve the formation of a free radical at some stage and this was supported by electron spin resonance spectroscopy, which indicated the presence of low concentrations of reactive radicals in solution. The exact oxidation mechanism that any particular reaction follows depends on the structure of the phenol being reacted, and other conditions of the reaction, such as solvent and oxidizing agent. The reactions (1) to (4) in Scheme 6 show each of the steps of the possible mechanisms as simple reversible reactions. If the reaction is performed in water or another protic solvent, rapid protonation and deprotonation of the oxygen make it difficult to determine the exact form of the phenol during oxidation. The mechanism of oxidation is also dependent on the

character of the oxidant. For example, a peroxide, such as *t*-butyl peroxide, is known to dissociate into radicals when heated.[32] When using peroxides as oxidizing agents for the oxidative coupling of phenols at higher temperatures, it is believed that these radicals can act as initiators and abstract a hydrogen atom from the phenol. On the other hand, if the

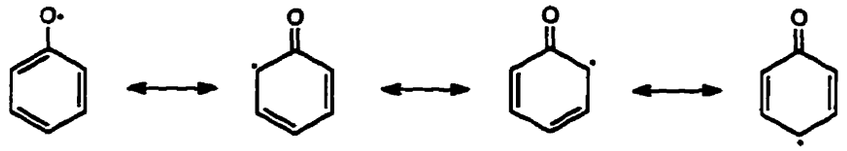
$$(tBuO)_2 \xrightarrow{\Delta} tBuO\bullet$$

$$tBuO\bullet + ArOH \rightarrow tBuOH + ArO\bullet$$

oxidizing agent is potassium ferricyanide in an alkaline medium, the ferricyanide ion is believed to behave as a single electron oxidant and the mechanism of the reaction is assumed to involve electron transfer from a phenoxide anion to the ferricyanide ion to produce the phenoxy radical.[33] Although the majority of oxidants oxidize phenols by one of the two mechanisms already mentioned, there are exceptions where some oxidants are believed to bond to the substrate and this bond is believed to influence the outcome of the reaction. One example of this is lead tetraacetate.[34,35] The attack of the phenoxy radical on the oxidant is believed to displace an acetate ion and this complex then reacts to produce dimers. Finally, when considering the effect of phenolic substituents on the oxidation step, it is found that the ease of abstraction of the electron from a phenol is dependent on the type of substituents on the aromatic ring. Ease of electron transfer can be estimated using relative oxidation potentials as determined by polarimetric methods, and these measurements have shown that the removal of an electron from the phenol occurs more easily if sterically bulky groups and/or electron donating groups are present at the *ortho* and *para* positions. Conversely, electron withdrawing substituents decrease the ease of oxidation.

Studies of the phenoxy radical have shown that it has greater stability than either alkyl or aryl radicals because its single electron is delocalized throughout the molecule. The greater stability of phenoxy radicals is evident from the length of their lifetimes and decreased rate of reaction with solvent molecules, when compared to alkyl and aryl radicals. The delocalization of the single electron can be illustrated by the various resonance contributors which can be drawn for the phenoxy radical **30**, shown in Scheme 7.[36] To determine the distribution of the single electron over the molecule, electron spin resonance spectroscopy can be used to measure the spin density at different atoms of the radical. The size of the hyperfine coupling of the electron to the ring hydrogens (a_H) is proportional to the spin density on the carbon bearing the hydrogen. From the resonance

Scheme 7



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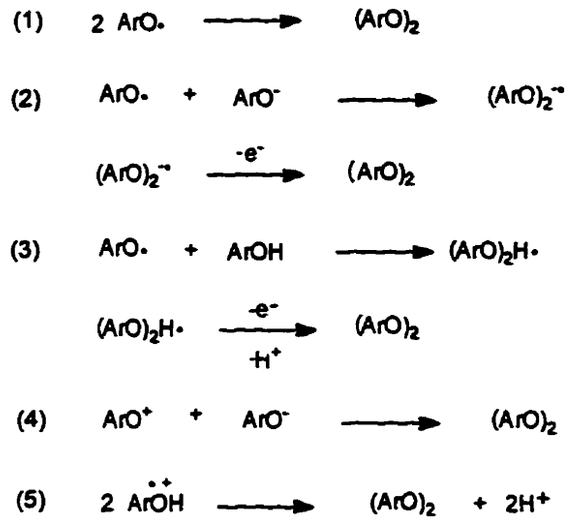
contributors to the phenoxy radical, it appears that the electron should be distributed mostly to the oxygen, and *para* and *ortho* carbon atoms. Through study of the esr spectra of various phenoxy radicals, it is generally found that the spin density is mostly concentrated at the oxygen and *para*-carbon atoms, a_H (*para*) having a value of approximately 10 gauss.[31] The *ortho*-carbon atoms have spin densities about one half the value found at the para-carbon and the remaining carbons, 1, 3 and 5, have spin densities which are quite small. Substituents on the aromatic ring will affect relative spin densities. Electron-donating groups will decrease the spin density on the ring and groups that are electron-withdrawing will increase the spin density.

1.4.3 Coupling Step

The final step in the oxidative coupling of phenols involves radical or nonradical coupling of the initially formed intermediates, or possibly reaction of the intermediates with other nonradical species present in the reaction mixture. As already discussed, the oxidation step in the reaction is complex and can produce a variety of radical intermediates, depending on the conditions of the reaction. As illustrated in Scheme 8,

Scheme 8



there are various mechanisms that should be considered for the coupling step which could involve all combinations of couplings at any of the three sites on the phenol: the oxygen atom, the aromatic carbon and the sidechain carbon. The most unlikely mechanism of these possibilities is represented by reaction 4, which is the ionic coupling of the phenoxonium ion with a phenolate anion. This reaction is unlikely for two reasons. First of all, the second electron is more difficult to abstract from the phenoxy radical than the first one. Secondly, the phenoxonium ion is more likely to couple with other nucleophiles (including the solvent) in the solution.[37,38] For example, Nishiyama *et al.* performed a

study of the electrochemical oxidation of various 4-allyl-2-methoxyphenols in methanol.[37] Through study of the phenols under these conditions, they discovered that phenoxonium ions were being formed from the respective phenols. The positive charge of these species was delocalized throughout the ion in a similar manner to the phenoxy radical such that the positive charge was concentrated at the *para* and *ortho* carbons (Scheme 9). Attack of the methanol at these sites produced various intermediates which reacted further in Diels-Alder reactions with the starting material. This provided an indication that if the

Scheme 9

33

phenoxonium ion **33** were present, nucleophilic attack on it in solution would be likely. It has been concluded in many cases that coupling of phenoxy radicals (reaction 1, Scheme 8) is most probable. Hapiot *et al.* used both cyclic voltametry and radiolysis to study the oxidative coupling of phenolic aldehydes, such as sinapaldehyde, at various pH's.[39] The voltammogram of the oxidation step indicated that only one electron was lost to produce the phenoxy radical at acidic to neutral pH's and results were consistent with the idea that the mechanism involved the coupling of phenoxy radicals. The cross-coupling of different phenols has also been attempted. Oxidative coupling of a mixture of 2,6-dimethoxyphenol and 2,6-dimethylphenol has been studied and the results showed that only symmetrical dimers were formed.[40] This result was attributed to the likelihood that the two different phenols oxidize at different rates. The rates of oxidation would differ such that only one type of phenoxy radical would be present in solution at a time and only self-coupling of

radicals would occur. Although the dimerization of phenoxy radicals may be the major reaction pathway for most reactions, it is not necessary that all reactions follow this pathway. Reactions (2) and (3) cannot be totally excluded, especially if the concentration of radicals is quite low. Under these conditions, the coupling of the phenolate and phenoxy radical or coupling of the phenol and phenoxy radical could compete with radical coupling. In a journal article published by Toda *et al.*, the use of solid ferric chloride as an oxidizing agent was reported.[41] In this case, the reacting species was believed to be the protonated phenoxy radical. Therefore, it appears that the oxidation conditions must be considered before conclusions can be drawn as to the mechanism of the coupling of phenolic species.

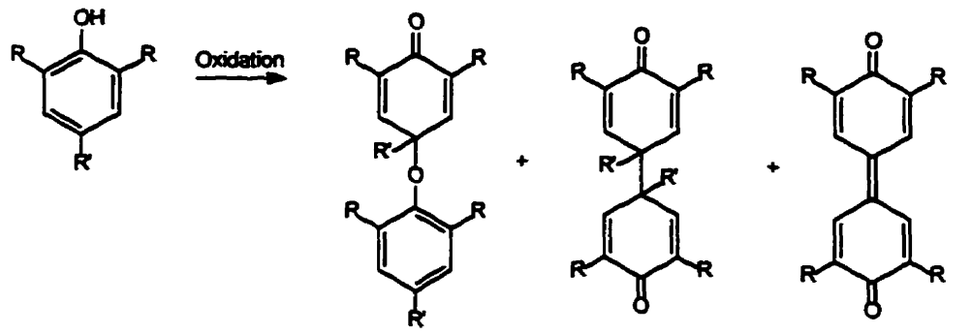
The coupling of phenols can be separated into four main categories, based on the types of bonds that are formed. These are (1) formation of a bond between the phenolic oxygens, (2) formation of a bond between an aromatic carbon and the phenolic oxygen, (3) formation of a bond between two aromatic carbons and (4) the reaction at a substituent group.

If the reacting phenol has substituents that are stable to the reaction conditions, then only the first three categories are feasible. Therefore, bonds that form will involve only the aromatic carbons and the phenoxy oxygen. The coupling between the phenoxy oxygen atoms to form a peroxide-type product has not been observed. The lack of this product is explained by the idea that it is thermally unstable with respect to the phenoxy radicals.[42] The relative yield of the various other carbon-oxygen and carbon-carbon coupling products from oxidation of phenols is often quite difficult to predict. Various theories have been postulated. Formation of products has been linked to the distribution

of the free electron on the phenol, steric hindrance of the substituents, the type of oxidizing agent used, and to polarity effects.

The effect of substituents on the outcome of oxidative coupling of phenols is most evident in 2,4,6-trisubstituted phenols.[43] If the phenol bears large, bulky groups, its exposure to oxidative conditions yields phenoxy radicals which exhibit an increased stability such that an equilibrium often exists between the radicals and dimers. For example, 2,4,6-tri-*t*-butylphenol is known to produce a stable phenoxy radical in solution. When coupling of trisubstituted phenols does occur, there are three products which

Scheme 10

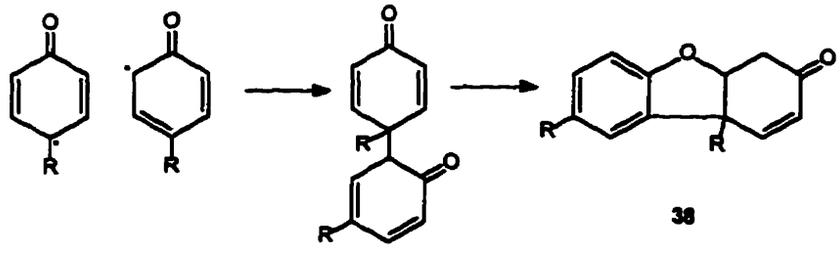


usually form, as shown in Scheme 10. These are the quinol ether **34**, the *para-para* dimer **35** and diphenoquinone **36**, which is formed by further oxidation of the *para-para* dimer. Most reactions of these phenols yield the quinol ether as the major product, although exceptions are found. For example, if 2,6-di-*t*-butyl-4-hydroxybenzoic acid is oxidized by potassium ferricyanide, the product is the diphenoquinone in 97 percent yield. On the other hand, esters of this phenol produce mainly the quinol ether.[31]

Coupling of phenols that have either the *ortho* or *para* position free of substituents shows a mix of both electron distribution effects and steric effects. In most cases, the

quinol ether is not usually produced in high yield for these compounds. For the reaction of phenols with no substituent at the *para* position, the dimerization typically occurs at this position. The oxidative coupling of 2,6-dimethylphenol using different oxidizing agents produced primarily the *para-para* diphenol and/or diphenoquinone.[44,45] These products are consistent with both decreased coupling at the *ortho* (2,6) positions due to steric hindrance, and increased coupling at the *para* position due to higher electron spin density at that position. Similar results have been attained in the majority of reactions, but exceptions have been found. When using copper-amine complexes to oxidize various 2,6-disubstituted phenols, such as 2-methyl-6-propylphenol or 2,6-dimethylphenol, the major product was a polyether.[46-48] Polyether formation has been attributed to a cationic oxidation mechanism. For phenols substituted at the *para* position, or at the *para* position and one *ortho* position, the major product from oxidative coupling tends to arise from *ortho-ortho* coupling. This is explained by the steric hindrance to coupling at the *para* position. This is observed with 4-allylphenol using ferric chloride as the oxidizing agent, where the *ortho-ortho* product is formed in about 25 percent yield.[49] Exceptions have been found to this generalization. The coupling of *p*-cresol using potassium ferricyanide in base as the oxidizing conditions produces Pummerer's ketone (**38**), which is formed

Scheme 11

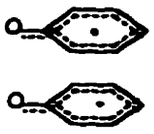


37 38

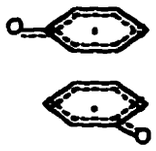
initially by the coupling at the *ortho* and *para* carbons.[40] Because the methyl group is not considered a sterically hindering group, the increased spin density at the para carbon is believed to allow *ortho-para* carbon bond formation to occur and the mechanism proceeds as shown in Scheme 11.

The last types of phenol to be discussed are those with both the *ortho* and *para* carbons free of substituents. Armstrong *et al.* carried out experiments with phenol and 3,5-dimethylphenol.[32] These phenols were oxidized with both organic peroxides and metal-ion oxidants. It was assumed that only when using the peroxides as oxidants, the phenoxy radical was likely to be formed. The results of these reactions showed that when using the peroxides as oxidants, coupling produced dimers mostly of the *ortho-ortho* and *ortho-para* variety, with relatively little of the *para-para* dimer. There was some of the C-O dimer formed, and the *para* carbon-oxygen dimer was formed in greater abundance than the *ortho* carbon-oxygen dimer in almost all cases. Because the *para-para* dimer was a minor product in these reactions, there must have been another driving force other than spin density that determines the products of the reaction. It was proposed that the outcome of a reaction was dependent on how the phenoxy radicals approached one another in the transition state. The researchers believed that there were two main modes by which the phenoxy radicals could approach each other. These two modes of approach are shown below as A and B. Approach A would give rise to the *para-para* and *ortho-ortho* products, while approach B would give the *ortho-ortho* and *ortho-para* dimers. Both of these approaches were studied theoretically to determine the variation in energy with distance between the approaching phenoxy radicals. The results of this study

Approach A

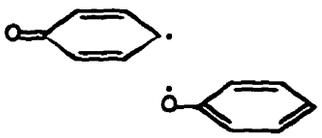


Approach B



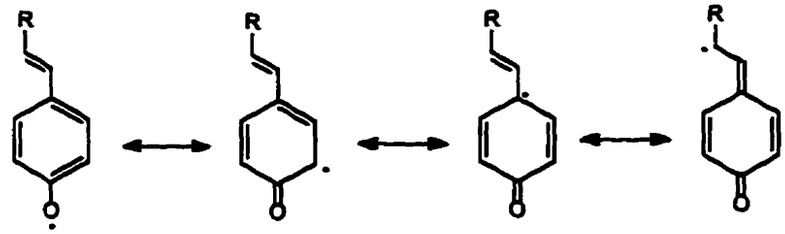
showed that the radicals preferred to approach one another by mode B. One possible explanation for this preference is related to the distribution of the single electron. There tends to be a positive charge on carbons 1 and 3, while the *ortho* carbons have negative charges. In order to reduce electrostatic repulsion, the phenoxy radicals would prefer to approach each other by pathway B. There must be another way that the phenoxy radicals can approach each other to account for the formation of quinol ethers. This is seen in approach C (see below). Even though this pathway of dimerization must play a role in this reaction, its contribution is generally quite small.

Approach C



The oxidative process that leads to lignans involves coupling in the side chain of the phenol rather than in the aromatic nucleus. Although many phenols with reactive side chains have been studied, the focus here will be on 4-hydroxyphenylpropylene derivatives because the oxidative coupling of these phenols is more directly related to the formation of lignans. Due to the conjugation of the side chain, there is another resonance contributor which can be drawn for phenoxy radical **39** (Scheme 12).[1] The additional delocalization of the single electron to the β-carbon will increase the overall stability of the radical and introduce another site at which coupling can occur.

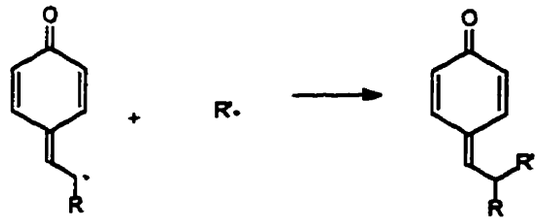
Scheme 12



39

The formation of bonds is now possible between the two β-carbons, or the β-carbon and the other sites of high spin density. If the coupling of 4-hydroxyphenylpropylenes involves initial bond formation at the β-carbon, the mechanism is more complicated than for simple phenols. The reason for this is that coupling at the β-carbon produces a quinone methide **40**, as seen in Scheme 13. This type of chemical species is known to act as a reactive intermediate in various organic processes.[50]

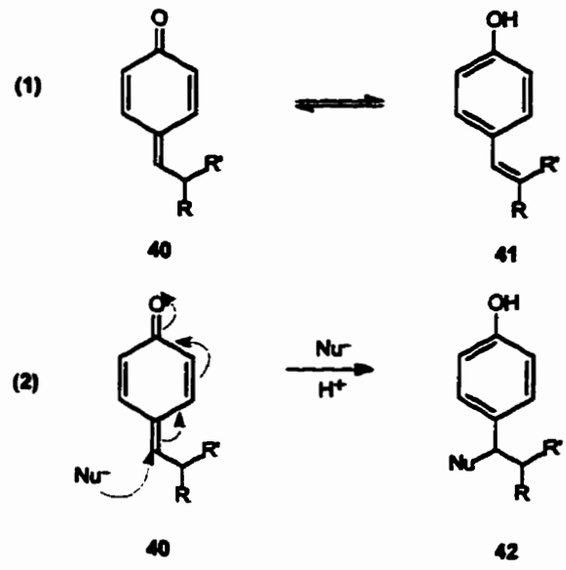
Scheme 13



39 **40**

In most cases, the quinone methide is quite reactive because of the driving force to form the more stable phenolic compound. This process can occur in two ways, as shown in Scheme 14. If the reaction occurs in a protic solvent, then tautomerization can easily occur and the conjugated phenol **41** can be formed (1). The second reaction that can occur is the attack of nucleophiles at the alpha carbon (2). The nature of the nucleophilic attack can be intermolecular or intramolecular. Intermolecular addition of solvent

Scheme 14



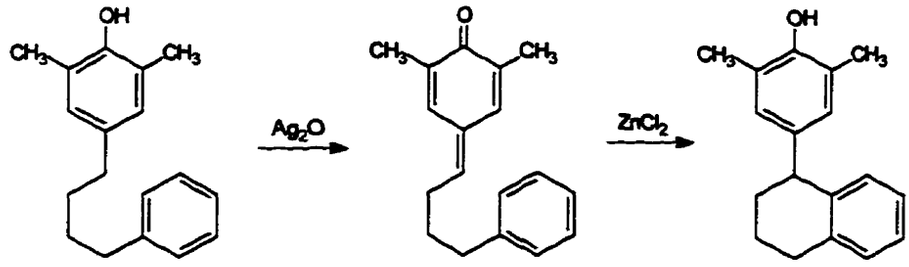
molecules can lead to monomeric products while addition of another phenol can lead to polymers. Although polymerization of 4-hydroxyphenylpropylene derivatives is an important pathway for the synthesis of lignin, it will not be discussed at this time. The intramolecular nucleophilic attack on the quinone methide that leads to cyclization of the molecule has been mentioned as a likely step in the mechanism for the formation of various lignans.[51,52]

Supporting evidence that this reaction occurs is given by Angle *et al.*, who studied reactions of *para*-quinone methides in intramolecular electrophilic substitution reactions.[53-55] In order for these cyclizations to be studied, the reacting species had to fit two criteria. First of all, the quinone methide had to be stable enough to be characterized spectroscopically. This stability was attained by synthesizing quinone methides that were substituted at both *ortho* positions. This substitution generated adequate steric hindrance to prevent intermolecular attack of the quinone methide and caused the carbonyl oxygen to twist out of the plane of the molecule. This twisting reduced the contribution of the

dipolar resonance contributor and decreased the electrophilicity of the alpha carbon. Secondly, the nucleophile that will attack the quinone methide must be stable under the conditions necessary to generate the quinone methide, but reactive enough to react before tautomerization can occur. The nucleophiles that were tested were allyl silanes, β-keto esters, benzene rings and various heterocycles.

Once the required criteria were met, the particular phenols were synthesized and then oxidized to the quinone methides using silver oxide as the oxidant. Cyclizations of the quinone methides were induced using zinc (II) chloride, which was believed to increase the electrophilicity of the quinone methide. Although not strictly a reaction of a phenylpropylene, Scheme 15 shows an example of a reaction in which an aryltetralin-type product **45** was formed. These reactions demonstrate the ability of quinone methides to react in intramolecular electrophilic substitution reactions.

Scheme 15

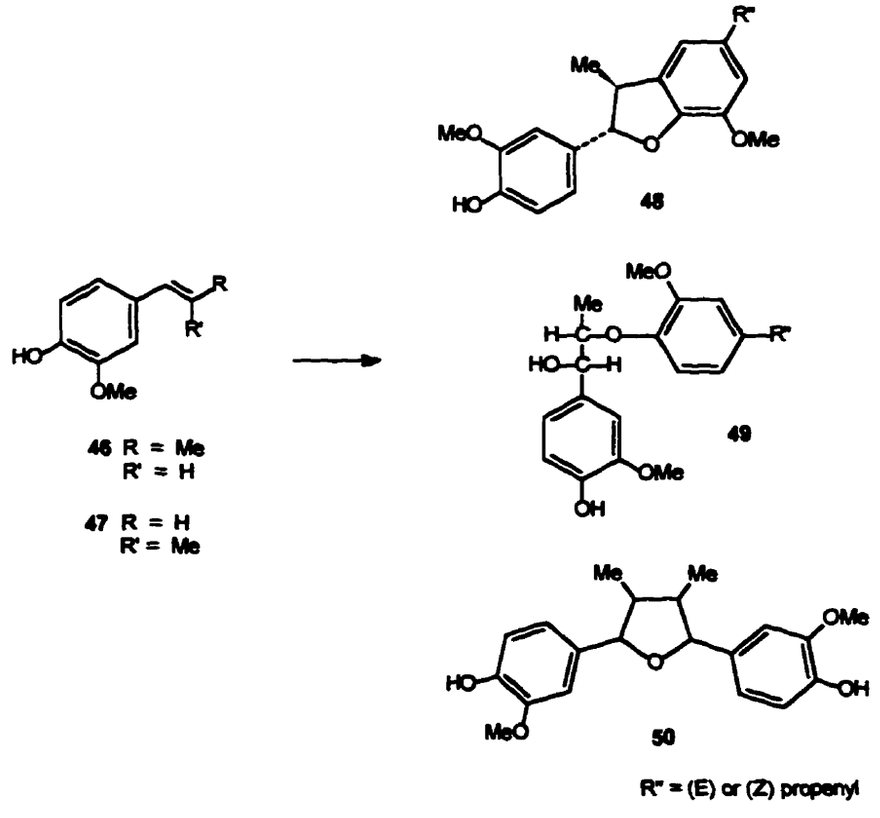


43 **44** **45**

Various studies of the oxidative coupling of 4-hydroxyphenylpropylenes, compounds more similar to those studied in this thesis work, have been reported. As previously stated, the results of these reactions vary depending on the conditions of the reaction and the phenol being reacted. This is quite evident when considering the results

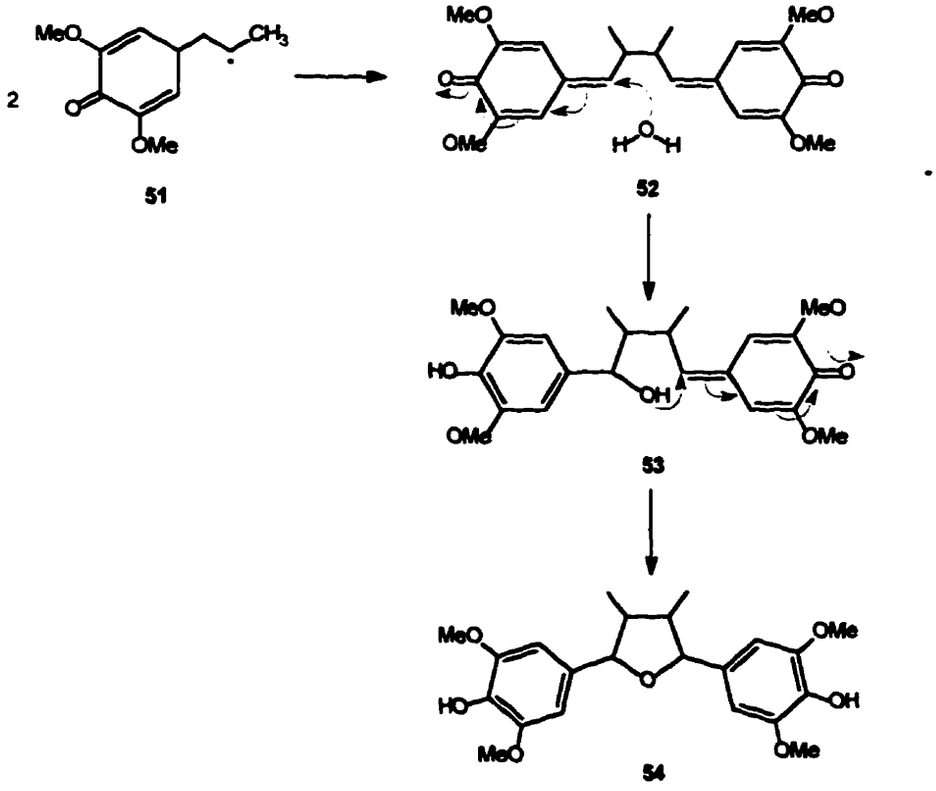
of the numerous studies performed on the oxidative coupling of phenols substituted at one of their *ortho* positions, such as (E)- and (Z)-isoeugenol, (**46**) and (**47**).[51,56-59] For example, it was determined that the reaction of these phenols using hydrogen peroxide as the oxidant, horseradish peroxidase as the catalyst, and aqueous acetone as the solvent, yielded three main products, **48**, **49**, and **50**, as shown in Scheme 16.[51] These products arise from bond formation between the β- and *ortho* carbons, the β-carbon and phenoxy oxygen, and the two β-carbons. It was found that compound **48** was the main product for (E)-isoeugenol, while the main product for (Z)-isoeugenol was compound **49**, demonstrating how different isomers of a phenol can yield distinctly different results. The

Scheme 16



reason for the difference is believed to be related to the approach of the radicals to each other during coupling. Also, it has been found that changing the solvent can alter the outcome of the reaction. Krawczyk *et al.* reacted **46** and **47** using hydrogen peroxide/horseradish peroxidase as the oxidizing system, but using aqueous methanol as the solvent in place of aqueous acetone.[56] They found only compounds **48** and **49** as products with no compound **50**. For oxidation of 2,6-disubstituted-4-propenylphenols, the products formed were very dependent on the oxidant used.[51,60,61] For example, oxidation of 2,6-dimethoxy-4-propenylphenol with hydrogen peroxide and horseradish peroxidase yielded an isomeric mixture of the tetrahydrofuran-type product **54**.[51] As shown in Scheme 17, this product was formed by the initial coupling of the phenoxy

Scheme 17

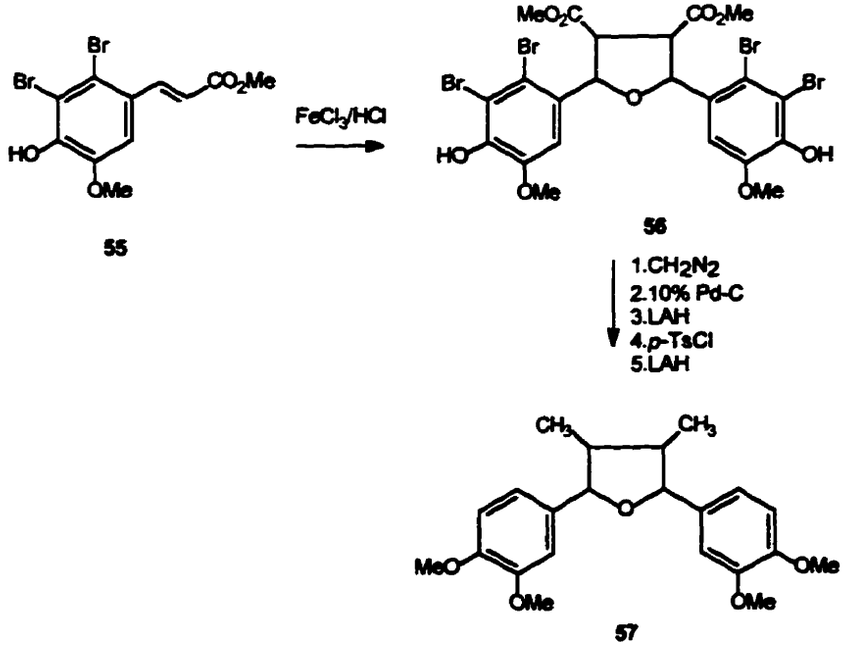


radicals **51** at the β-carbons to produce a *bis*-quinone methide **52**, followed by the attack of a water molecule on one of the quinone methides. Finally, the hydroxy group of **53** intramolecularly attacked the remaining quinone methide, to yield the tetrahydrofuran product **54**. On the other hand, the reaction of 2,6-dimethoxy-4-propenylphenol using ferric chloride as the oxidizing agent gave products arising from bond formation between the β-carbon and phenoxy oxygen, followed by attack of the quinone methide by a water molecule (similar to product **49**).[61]

1.4.4 Synthesis of Lignans using Oxidative Coupling of Phenols

Oxidative coupling of phenolic compounds is sometimes used as a method for the synthesis of various lignans. The laboratory method parallels the biosynthetic pathway, although lignans synthesized in the laboratory are usually obtained as racemic mixtures.

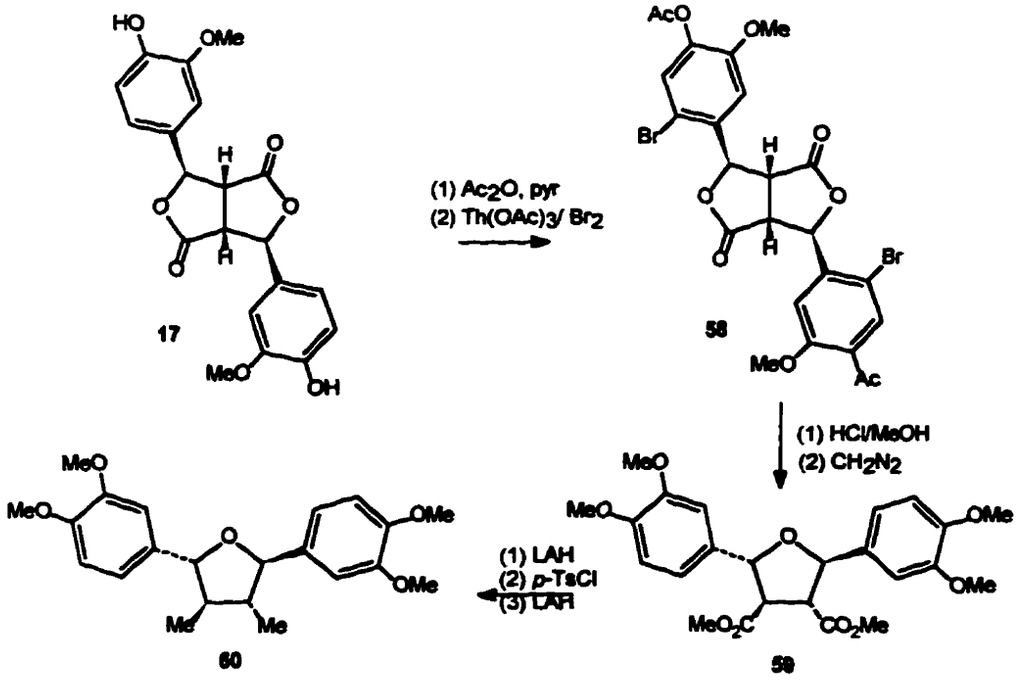
Scheme 18



55 56 57

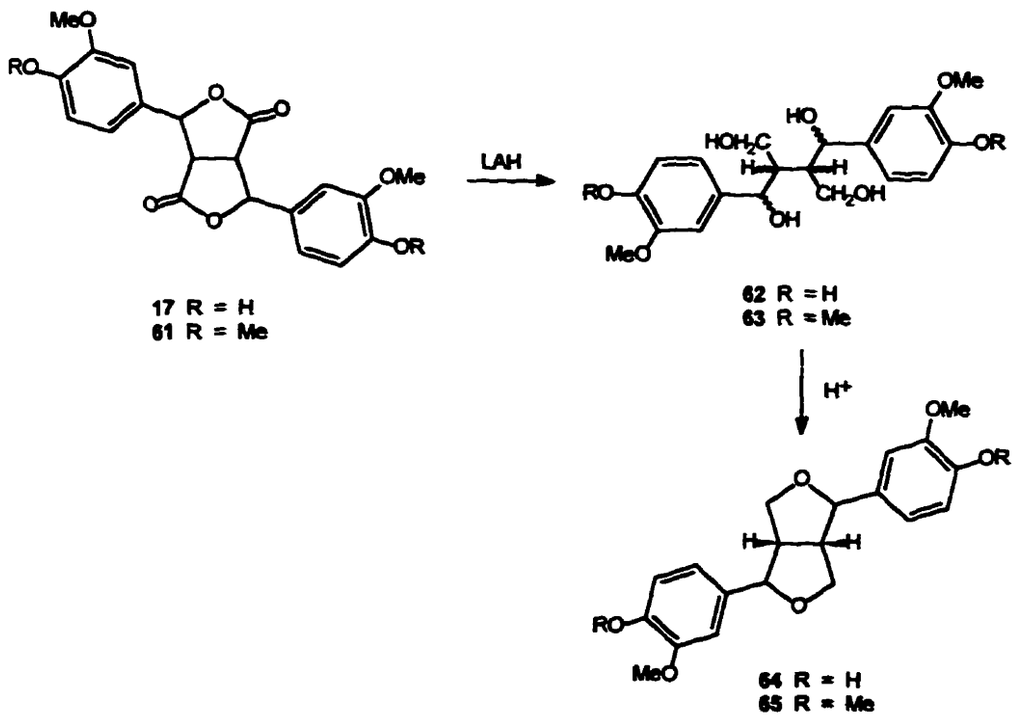
Many of the syntheses of lignans are multi-step processes with the oxidative coupling step coming early in the sequence. In one of the syntheses of thomasidioic acid, oxidative coupling of sinapic acid using ferric chloride was carried out first, with subsequent treatment of the dilactone **16** to form the cyclized product **1**.[25] The oxidative coupling of halogen-substituted cinnamate esters, such as methyl 2,3-dibromo-4-hydroxy-5-methoxycinnamate (**55**), could also be performed using ferric chloride as the oxidant.[28] The product of this reaction was the tetrahydrofuran **56**, shown in Scheme 18. Further manipulation of the functional groups yielded (±)-veraguensin (**57**), which is a lignan isolated from the wood of *Ocotea veraguensis*. The all *trans*- isomer of this lignan also occurs naturally and it is known as galbelgin. It has been synthesized from the dehydrodiferulic acid dilactone (**17**), which is easily synthesized by the oxidative coupling of ferulic acid with ferric chloride.[52] As seen in Scheme 19, dilactone **17** was acetylated,

Scheme 19



brominated, treated with methanolic hydrogen chloride and methylated to form **59**. Tetrahydrofuran **59** was reduced in three steps to finally yield (±) galbelgin **60**. Pelter *et al.* also used the dilactone **17** to synthesize lignans of the 2,6-diaryl-4,8-dihydroxy-3,7-dioxabicyclo[3.3.0]octane type.[62] For example, the treatment of nonmethylated and methylated dehydrodiferulic acid dilactone, **17** and **61**, with lithium aluminum hydride yielded the respective tetraols **62** and **63**. Acid treatment of the two tetraols gave (±)-pinoresinol (**64**) and (±)-eudesmin (**65**) respectively, as shown in Scheme 20.

Scheme 20



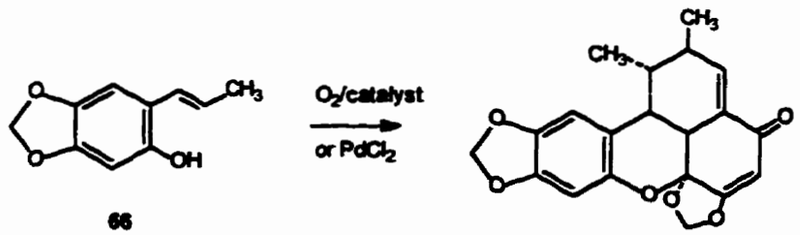
17 R = H
61 R = Me

62 R = H
63 R = Me

64 R = H
65 R = Me

It has been found that oxidative coupling of appropriate phenolic compounds can produce certain lignans in a single step. This is evident from the simple synthesis of dimethyl thomasidioate (**2**), produced by the enzyme-catalyzed oxidation of methyl sinapate (**23**). Other one-step syntheses of lignans can be found in the literature. The

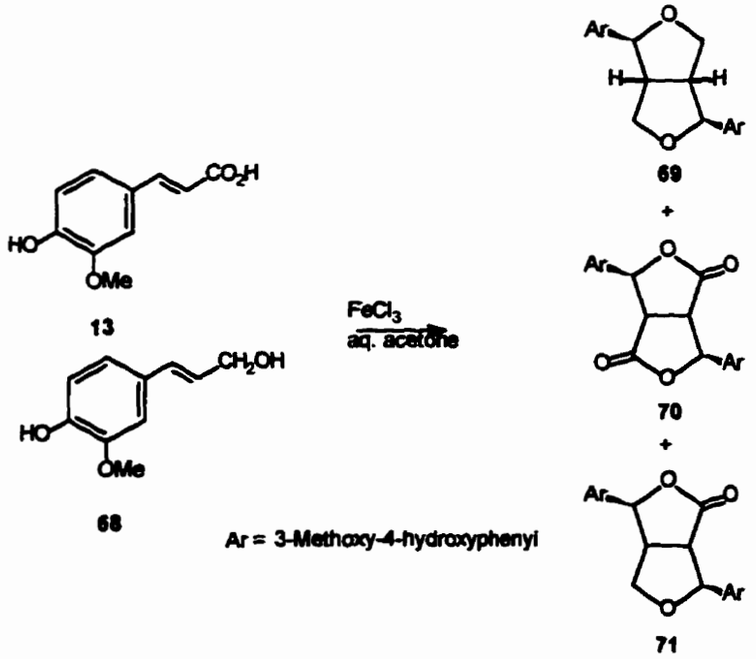
Scheme 21



66 67

lignan carpanone (**67**) was believed to be a difficult synthetic target since it has five asymmetric centers and no element of symmetry. Two different research groups carried out the synthesis of **67** by oxidative coupling of trans-2-(1-propenyl)-4,5-methylenedioxyphenol (**66**) (Scheme 21). Matsumoto and Kuroda were successful in synthesizing this lignan by using molecular oxygen as the oxidizing agent with various transition metal complexes as catalysts.[63] They attained yields ranging from 78 to 90

Scheme 22



13 68 69 70 71

percent. Chapman *et al.* used palladium (II) dichloride as the oxidant for the coupling of the phenol **66**.[64]

In many reactions, the lignan is formed as one of several oxidation products. This is true of the cross-coupling of ferulic acid (**13**) with coniferyl alcohol (**68**), using ferric chloride in aqueous acetone as the oxidant. Three different products were formed, as shown in Scheme 22. Compound **71** has spectroscopic properties identical to a novel lignan isolated from *Aegilops ovata* L.[65] Although these reactions yielded mixtures of products, they demonstrated that under the right conditions, oxidative coupling of phenolic compounds can produce naturally occurring lignans in a single step.

1.4.5 Various Oxidizing Agents

For the oxidative coupling of phenols, there are various types of oxidants that can be used. The chemical nature of each reagent determines the mechanism by which it oxidizes the phenol and how effective it is in producing the desired phenolic dimers. The oxidizing agents which will be discussed fall into the following categories: oxygen with various catalysts, organic compounds, inorganic species, enzymes, and electrochemically or photochemically initiated reactions.

The most common oxidizing agents used in the oxidative coupling of phenols are inorganic compounds. For the inorganic single-electron oxidants, there are two major mechanisms postulated to explain their ability to abstract electrons from the phenols.[66] The first mechanism is called the inner sphere mechanism. The metal is believed to form a bond to the phenol and the electron moves across the bond. The other mechanism is called the outer sphere mechanism and it requires no actual bond formation between the

two reacting species. Instead, a complex is formed between the two species and the electron moves directly from one species to the other.

The most popular inorganic oxidizing agents for the oxidative coupling of phenols are ferric chloride and potassium ferricyanide. Although ferric ion is the oxidant in both of these reagents, different mechanisms have been postulated for each oxidant which can be used to explain the formation of different products by these oxidizing agents.[67] Potassium ferricyanide is believed to oxidize by the outer sphere mechanism. In the study of the kinetics of these reactions, it has been determined that the rate of oxidation is dependent on the pH and that the substrate in the oxidation is the phenoxide anion. In the vast majority of these reactions, the products formed are the dimers of phenols. Ferric chloride, on the other hand, is believed to act via an inner sphere mechanism. Many other inorganic oxidants have also been used in the oxidative coupling of phenols. Some examples are: lead (IV) oxide,[68] lead tetraacetate,[34,35] silver oxide,[69] cupric chloride,[70,71] manganese dioxide[72,73] and vanadium-containing compounds.[74] Although the majority of these agents are believed to act as single electron oxidants yielding the phenoxy radical, evidence has shown that the oxidation process of both lead tetraacetate and the vanadium-containing compounds involves non-radical mechanisms.[34,74]

The use of organic oxidants for the coupling of phenols has been reported, although phenolic dimers are not always the major product. Various quinones have been used as oxidants, in particular 2,3-dichloro-5,6-dicyanoquinone or DDQ.[75-77] DDQ has traditionally been used for the dehydrogenation of hydroaromatic compounds, and its reaction with phenols is not as well documented. According to the few articles published, DDQ is a strong oxidizing agent often yielding dimers, although there are still unanswered

questions about its mode of action. It has been postulated that DDQ will abstract a hydride ion from the phenol and an ionic coupling reaction will occur. On the other hand, Becker postulated the formation of phenoxy radicals through removal of hydrogen atoms.[76] Both of the postulated mechanisms are possible, so further study must be performed to provide additional evidence as to the correct mechanism. Peroxides, such as benzoyl peroxide and t-butyl peroxide, can also be used as oxidizing agents for phenolic coupling.[32,78-80] It has been found that coupling of two phenoxy radicals does not always occur. Armstrong *et al.* performed a study using *t*-butyl peroxide as the oxidant at high temperatures and under these conditions the *ortho-ortho*, *ortho-para*, and *para-para* coupled dimers were the products.[32] Other studies showed that in addition to the phenolic dimers, other products arising from coupling to the oxidants were also found.[78-80]

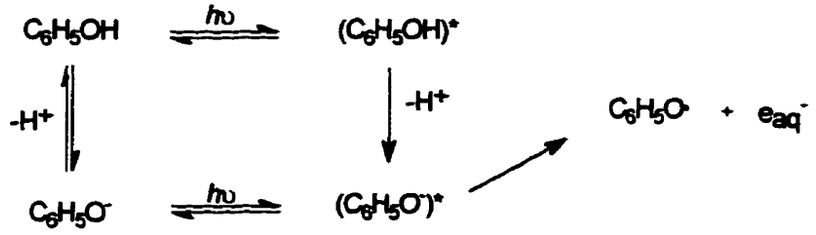
Oxidation of phenols with molecular oxygen often requires the use of a catalyst. Various inorganic catalysts, such as copper (II), cobalt (II) and iridium (III) containing compounds have been used.[81-84] The use of oxygen as an oxidizing agent in alkaline solution without an inorganic catalyst is also common for the oxidative coupling of phenols.[85-87] The pH-dependency of the reaction is related to the fact that the phenolate anion is more prone to oxidation than is the phenol. Some of the studies on the autooxidation of phenols report the formation of quinones and peroxides, along with phenolic dimers, as products.

The use of various enzymes in catalyzing the oxidative coupling of phenols parallels their participation in the biosynthesis of phenolic dimers. There are three different types of enzymes that can be used to catalyze the reaction and they are the peroxidases,[88,89] laccases[90] and tyrosinases.[91] Each type of enzyme has been isolated from

plants, bacteria, fungi and animals. All of these enzymes are believed to promote a one-electron oxidation and produce dimerized products similar to those produced by non-enzymatic one-electron oxidants. In some instances, enzymatically catalyzed reactions are more chemioselective than their non-enzymatic counterparts.

Electrochemically and photochemically initiated reactions can also lead to phenolic coupling. Photochemically, there are two pathways for the oxidation (Scheme 23).[92] Excitation of either the phenolate anion or the phenol can be followed by oxidation of the corresponding excited state to yield a radical. If the solvent is water, the photoionized electron is believed to be solvated by water molecules. The phenoxy radicals dimerize or form hydroxylated product by addition of water to the radical. For the electrochemical

Scheme 23

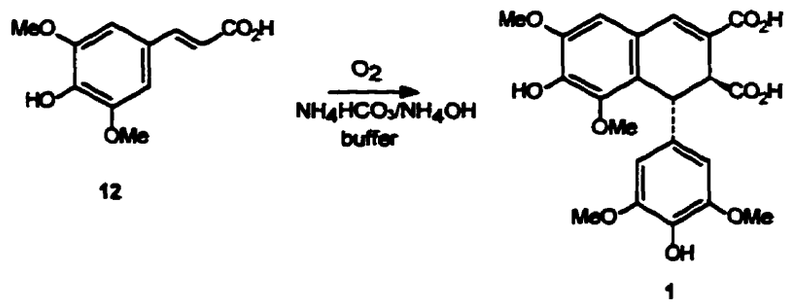


anodic oxidation of phenols, the mechanism is believed to be a fast, reversible loss of a proton, followed by the rate-determining slow removal of an electron. This method of oxidation is quite versatile as many different features of the system can be changed such as voltage, type of cell, pH and solvent.[93-95] Variable results have been attained. A study of the anodic oxidation of 2,6-di-t-butylphenol by Torii *et al.* provided evidence for the loss of two electrons in two single electron transfers.[94] On the other hand, similar studies by Hedenburg and Frieser using phenol and various tert-butyl substituted phenols as substrates showed that only one electron was removed electrochemically.[95]

Thesis Objectives

The research described in this thesis is based on the work of Rubino *et al.*[22,23] They discovered that sinapic acid, in an alkaline buffer and in the presence of oxygen, would quantitatively produce the naturally occurring lignan thomasidioic acid (Scheme 24). The purposes of the current research are outlined below.

Scheme 24



(1) To determine of the scope of the reaction

Various studies have shown that oxidative coupling of phenolic cinnamic acids and esters can be used to prepare lignans. The discovery that sinapic acid can be converted to the lignan thomasidioic acid is significant because it can be achieved under very mild conditions and in high yield. Most of the previous work on oxidation of phenolic acids, similar to sinapic acid, have given mixtures of products in low yield. The questions which can be asked are: (a) would sinapic acid react as effectively if the reaction were carried out on a preparative scale and (b) are the reaction conditions also suitable for the conversion of other phenolic cinnamic acids to aryltetralin products?

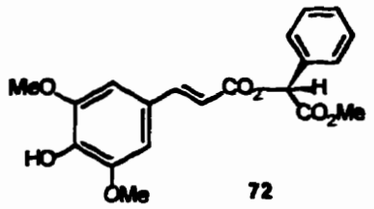
(2) To determine the mechanism of the oxidative coupling of sinapic acid

As discussed in the introduction, the mechanism of the oxidative coupling of phenols can be a complicated process for which one must consider the substrate, oxidizing agent, and other conditions of the reaction. There have been various mechanisms postulated for the oxidative coupling of hydroxycinnamic acids. Those mechanisms usually involve the coupling of cinnamyl radicals, followed by either the tautomerization of, or the nucleophilic attack on, a quinone methide. A similar mechanism can be postulated for the oxidative coupling of sinapic acid to form thomasidioic acid. The questions which must be answered are: (a) what are the intermediates involved in the coupling step, (b) what role does the solvent play, (c) what is the mechanism of the cyclization which follows coupling and (d) what is the effect of changing the oxidizing agent.

(3) To determine if esters of sinapic acid will oxidatively couple under the same mild conditions used to oxidize sinapic acid, and to determine if chiral esters will lead to asymmetric coupling/cyclization.

It has been previously established that methyl sinapate can be oxidized to form dimethyl thomasidioate in low yield by using ferric chloride in water, or by using hydrogen peroxide and a peroxidase enzyme. If the simple oxidation conditions used to oxidize sinapic acid can be used to oxidize and cyclize methyl sinapate, it could lead to a better overall method for the preparation of dimethyl thomasidioate. The past syntheses of thomasidioic acid have involved the oxidative coupling of sinapic acid producing the lignan in its natural form, as a racemic mixture. Use of a chiral ester of sinapic acid as a substrate could cause asymmetric induction, meaning that the new chiral centers in the

product would be produced with a preference for one absolute configuration. Specifically, it is proposed that the oxidative coupling of the methyl (*R*)-mandelyl ester of sinapic acid **72** be studied to determine if it will form the corresponding ester of thomasidioic acid in a diastereoselective manner.



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Chapter 2

Results and Discussion

The results of the research on the oxidative coupling of cinnamic acid derivatives and formation of lignans are presented in three sections. The first section concerns the scope of the reaction found by Rubino *et al.* for conversion of sinapic acid to thomasidioic acid.[22,23] The reaction was studied with a view to its use on a preparative scale, and its applicability to other substrates. The reaction of sinapic acid in oxygenated ammonium bicarbonate buffer was repeated with a greater concentration of substrate. The *para*-hydroxycinnamic acids ferulic, coumaric and caffeic acid, were also treated under the same conditions to see if the reaction would produce aryltetralins in good yield. In the second section, the investigation of the mechanism of the oxidative coupling of sinapic acid is discussed. The mechanism proposed for this reaction involves the formation of sinapyl radicals, their coupling and further reaction to finally yield thomasidioic acid. In order to substantiate the proposed mechanism, experiments involving the measurement of oxygen uptake, use of other known one-electron oxidants, and reaction of possible intermediates were carried out. In the last section, the oxidative coupling of esters of sinapic acid using the previously mentioned conditions, is discussed. The effects of altering these conditions (temperature, pH, and concentration of the buffer) have also been studied. The oxidative coupling of (methyl (*R*)-mandelyl) sinapate was carried out in an attempt to achieve the asymmetric synthesis of thomasidioic acid.

2.1 Scope of the reaction

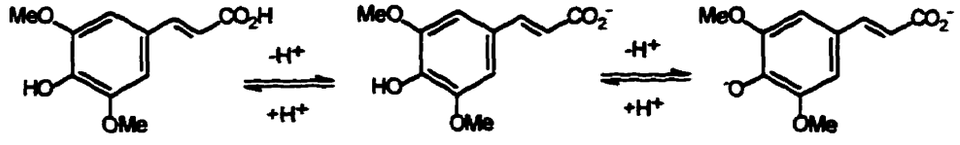
The preparative synthesis of thomasidioic acid was studied by repeating the reaction discovered by Rubino *et al.*[22,23] with a greater concentration of substrate. A 0.045 molar solution of sinapic acid was prepared in 0.287 molar ammonium carbonate buffer and stirred open to the air at room temperature. As the reaction progressed, the yellow solution darkened until it became a dark brown opaque liquid. Monitoring of the reaction by thin-layer chromatography (TLC) showed that the sinapic acid had completely disappeared after 24 hours of stirring. This was the same time of reaction as reported by Rubino *et al.*[22] This observation showed that a change in concentration of the substrate, sinapic acid, had little or no effect on the reaction time.

Isolation of the crude product from the reaction solution was achieved by extracting the acidified reaction mixture (pH 2) with ethyl acetate. The comparison of the proton NMR spectrum of the crude product to that of thomasidioic acid showed that the product was approximately 95 percent thomasidioic acid. In the previous investigation, the researchers experienced some difficulty in purifying thomasidioic acid. Purification was found possible by flash chromatography if the silica gel was pretreated with a 5:20:75 mixture of acetic acid, hexanes and ethyl acetate. The same solvent mixture was used for elution of the product. The overall yield of pure thomasidioic acid after chromatography was 41 percent. This yield was considerably lower than expected as the proton NMR spectrum of the crude product showed it to be relatively pure. Loss of the product is believed to occur during flash chromatography. There are sites on the silica gel where polar groups, such as carboxylic acids, may bind. Although treatment of the silica gel with a solvent containing 5% acetic acid before performing the chromatography was done to

block these sites with acetate groups, it is possible that not all sites were blocked. Thus, some of the molecules of thomasidioic acid still bound to the silica gel during chromatography and were not eluted.

Increasing the pH of the solution in order to form the dianion of the sinapic acid before oxidation may increase its rate of oxidation. It is believed that phenolate anion is more susceptible to oxidation than phenol. Smyk and Drabert studied the equilibrium amongst the various forms of sinapic acid in solutions of varying pH, as seen in Scheme 25.[96] They determined that the pKa of the second ionization step was 9.21. Therefore, a pH of greater than 11.5 was chosen for the reaction in order to maximize the concentration of **74** and thereby the rate of oxidation.

Scheme 25



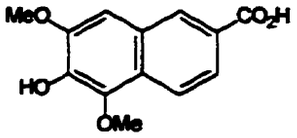
12 **73** **74**

Sinapic acid (0.02 M) was dissolved into 0.1 N potassium hydroxide solution which had a pH of approximately 13. The resulting solution was stirred at room temperature with exposure to air. Initially, an opaque brown solution was formed, but eventually the solution turned clear and deep red. When following the reaction by TLC, a fluorescent spot appeared at the top of the plate. These observations showed that this reaction progressed differently than the reaction of sinapic acid in ammonium bicarbonate buffer. Completion of the reaction was observed after approximately 20 hours. As before, the crude product was isolated by acidifying the reaction solution to a pH of 2 and then extracting the aqueous solution with ethyl acetate. This same reaction was also carried

out at 60° C. At this temperature the reaction reached completion in 6 hours. An examination of the products from the two experiments by TLC and ^{1}H NMR indicated that there was no difference in product composition.

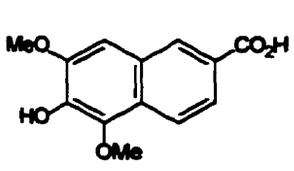
Examination of the NMR and mass spectrum of the crude product of the reaction of sinapic acid in strong base showed that it was not thomasidioic acid. The proton NMR spectrum of the unknown compound differed greatly from that of thomasidioic acid (see Appendix). For example, while the spectrum of thomasidioic acid had three peaks representing 12 protons at about 4 ppm, the unknown compound had only two peaks in this region, representing 6 protons. Considering the structure of the substrate, it was quite possible that these peaks represented two different methoxy groups on the compound. The remaining peaks for the unknown molecule appeared between 7 and 8.5 ppm implying that the other protons on the molecule were all aromatic. There were two pieces of evidence which indicated that a molecule smaller than thomasidioic had been formed. First of all, there were only thirteen different carbons seen in the ^{13}C NMR spectrum for the molecule. Also, the molecular mass of the compound seen in the mass spectra (248.07 g/mol) was less than that of thomasidioic acid. A tentative molecular formula of $C_{13}H_{12}O_5$ was calculated from the exact mass. Considering the size of this molecule and the ^{1}H NMR spectrum, a naphthalene structure was proposed. The fluorescent spot seen on TLC was also consistent with this type of structure. Considering the nature of the starting material and the ^{1}H NMR spectrum, the presence of at least two methoxyl groups and a hydroxyl group was expected. A carboxylic acid group would account for the remaining oxygen atoms of the molecule. The placement of the groups on the naphthalene ring can be deduced from the splitting of the aromatic peaks in the ^{1}H NMR spectrum. There was

a one proton singlet, a one proton doublet with *meta* coupling, a one proton double-doublet with *ortho* and *meta* coupling and finally a one proton doublet with only *ortho* coupling. Given this pattern of coupling it is likely that the protons are found at the 1, 3, 4 and 8 positions. Therefore, the structure 6-hydroxy-5,7-dimethoxy-2-naphthoic acid (**75**) was proposed, as shown below.

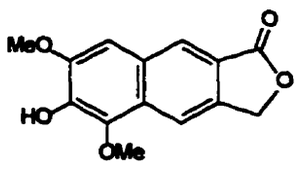


75

A literature search for 2-naphthoic acids was performed to confirm the proposed structure for the product of the reaction of sinapic acid in strong base. Coincidentally, 6-hydroxy-5,7-dimethoxy-2-naphthoic acid was found to have been isolated from *Ulmus thomasii* Sarg. by Chen and Hostettler.[97] A comparison of the spectral data of the compound obtained via reaction of sinapic acid in strong base to that of 6-hydroxy-5,7-dimethoxy-2-naphthoic acid showed that the compounds were identical. The researchers isolated two 2-naphthoic acid derivatives **75** and **76** plus various lignans from the aqueous extracts of the heartwood, as shown below. The free monoacid was first identified through analysis of its IR, UV, mass spectral and NMR data.



75



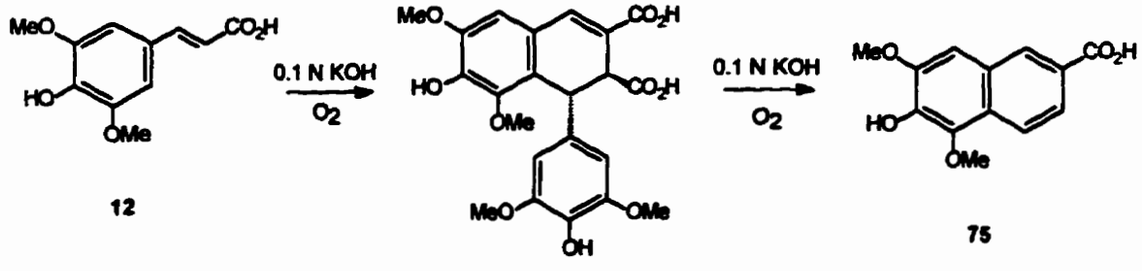
76

Having determined the structure of the product formed on air oxidation of sinapic acid in strongly alkaline solution, attention was turned to the mechanism of its formation.

At the outset, it appeared that the naphthoic acid could have been formed either from sinapic acid directly, from thomasidioic acid, or by some alternate unknown pathway. The second theory was tested by air oxidizing thomasidioic acid in 0.1 N potassium hydroxide solution. The stirred solution of thomasidioic acid in strong base turned deep red and clear. The crude product was isolated as before and identified by its proton NMR spectrum to be 6-hydroxy-5,7-dimethoxy-2-naphthoic acid. This result supported the theory that naphthoic acid **75** was formed by the further reaction of thomasidioic acid in strong base.

The necessity of oxygen for the conversion of thomasidioic acid to 6-hydroxy-5,7-dimethoxy-2-naphthoic acid had not yet been determined. Rubino *et al.* studied the conditions for the conversion of sinapic acid into thomasidioic acid and they found that this reaction required oxygen.[23] It was also possible that the conversion of thomasidioic acid to naphthoic acid **75** required oxygen. To test for this, two simultaneous reactions of thomasidioic acid were performed in 0.1 N potassium hydroxide solution. One reaction was carried out under air and the other under nitrogen. The reaction under air proceeded as previously giving a final solution that was clear and red. Only 6-hydroxy-5,7-

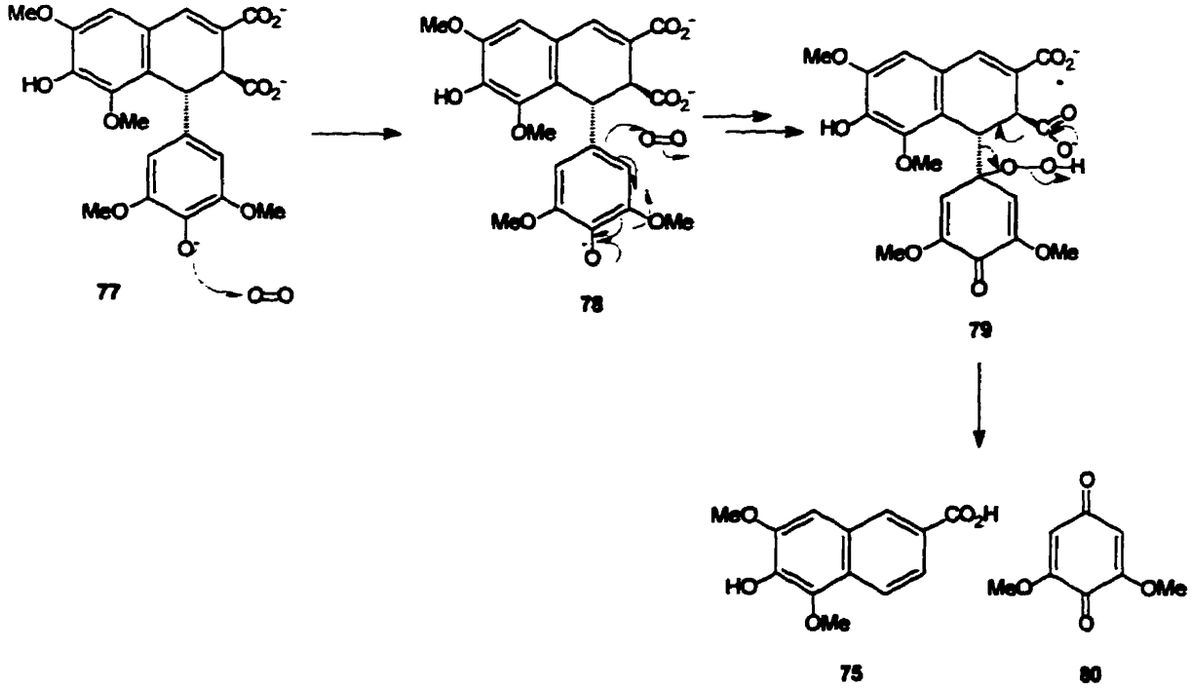
Scheme 26



dimethoxy-2-naphthoic acid was isolated. On the other hand, only the unreacted thomasidioic acid was isolated from the reaction which was performed under nitrogen.. These results confirm that the 2-naphthoic acid **75** is a secondary oxidation product of thomasidioic acid, as shown in Scheme 26.

A mechanism for the formation of 6-hydroxy-5,7-dimethoxy-2-naphthoic acid from sinapic acid can now be postulated because it is known to be formed via the oxidation of thomasidioic acid. A possible mechanism is shown in Scheme 27. The final outcome of this mechanism is the loss of the pendant aryl group as 2,6-dimethoxy-*p*-benzoquinone (**80**). If this mechanism were correct, then 2,6-dimethoxy-*p*-benzoquinone would also be a product of the reaction. The reaction of sinapic acid in 0.1 N KOH solution was repeated to determine if **80** was actually formed. Once the reaction was seen

Scheme 27

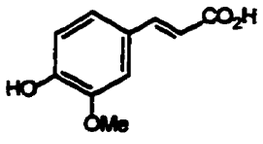


to be complete by TLC, the reaction solution was extracted with ethyl acetate before it was acidified. This initial extraction of the alkaline solution should remove any non-acidic products formed in the reaction. A reference sample of pure 2,6-dimethoxy-*p*-benzoquinone (**80**) was synthesized by the oxidation of 2,6-dimethoxyphenol with chromium trioxide. The proton NMR spectrum of pure 2,6-dimethoxy-*p*-benzoquinone (**80**) was compared to that of the material isolated from the first extraction of the reaction. The two NMR signals of **80** were found in the spectrum of the crude product, along with other peaks. Because the peaks were quite small, it was difficult to confirm the presence of the quinone by NMR spectroscopy alone. In order to confirm the formation of the quinone, an HPLC analysis of the crude and pure quinone was carried out. Peaks with very similar retention times were observed in both chromatograms. To eliminate the possibility that two different compounds might be appearing with similar elution times, the extracted solution was spiked with some of the pure 2,6-dimethoxy-*p*-benzoquinone (**80**). A definite increase in the area of the peak believed to belong to the quinone was observed in the chromatograph of the extracted material. The results of the NMR and HPLC analysis confirmed the presence of 2,6-dimethoxy-*p*-benzoquinone (**80**) and provided support for the proposed mechanism.

The formation of both thomasidioic acid and the 2-naphthoic acid on reaction of sinapic acid in alkaline solutions of varying pH's is quite coincidental with their isolation from *Ulmus thomasii* Sarg. In a series of journal articles, Hostettler and Seikel discuss the isolation of various lignans and 2-naphthoic acids from this species of elm tree.[21] Thomasidioic acid and 6-hydroxy-5,7-dimethoxy-2-naphthoic acid were two compounds that were found together in the aqueous extracts of the heartwood. One wonders if

indeed these two compounds are true natural products. Close examination of the isolation procedure used by Hostettler and Seikel revealed that the compounds were extracted into a basic medium. Sinapic acid is known to be found in various plant matter[43,98-101] and there is a distinct possibility that, if the extracts from the tree were left open to the air in an alkaline solution, thomasidioic acid and the 2-naphthoic acid **75** could have been formed. Therefore, instead of these compounds being formed naturally, they may have been formed from sinapic acid during the isolation. This theory would explain the fact that the thomasidioic acid isolated by Hostettler and Seikel was optically inactive.[21] It is one of only two lignans believed to occur naturally in a racemic form. One would have expected an optically active natural product if it had been formed biosynthetically. On the other hand, the simple air oxidation of sinapic acid to thomasidioic acid must give only racemic material. These researchers also isolated a small amount of 2,6-dimethoxy-*p*-benzoquinone from the extracts of the elm tree. The formation of this quinone could also be explained by the secondary oxidation of thomasidioic acid in alkaline solution.

Given that sinapic acid is converted cleanly to thomasidioic acid in aerated, basic buffer, the applicability of these conditions to the conversion of other *para*-hydroxycinnamic acids to aryltetralins was investigated. The *para*-hydroxycinnamic acids ferulic acid (**13**), coumaric acid (**81**) and caffeic acid (**82**) were studied. In the past, ferulic



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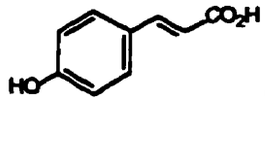

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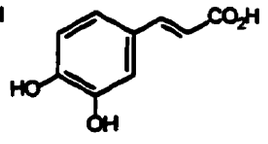
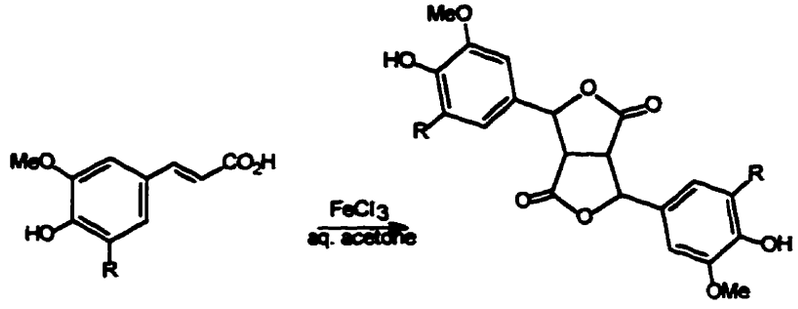


82

acid has been found to react similarly to sinapic acid under some oxidizing conditions. For example, a study by Ahmed, Lehrer, and Stevenson showed that both ferulic and sinapic acid formed their respective dehydrodilactones **16** and **17** when reacted with ferric chloride in aqueous acetone (Scheme 28).[102] In the current study, ferulic acid was stirred for two days in aerated ammonium bicarbonate buffer and no change was observed.

Scheme 28



12 R = OMe
13 R = H

16 R = OMe
17 R = H

Extraction of the acidified solution yielded only the starting material. Similar results were obtained for the attempted oxidative coupling of coumaric acid.

Considering the mechanism for the oxidation, there are two possible reasons for the lack of reaction of ferulic and coumaric acids. The first reason is that the solution may not have been basic enough to form phenolate anions, a condition that may be necessary in order for oxidation to occur. The other possible reason is that oxygen may not be a strong enough oxidant to oxidize ferulic and coumaric acids (or their anions) The first theory was tested by oxidizing the acids in a more strongly basic solution, conditions that would ensure formation of the phenolate anions. Both ferulic and coumaric acids were reacted in a 0.1 N potassium hydroxide solution with exposure to air. There was still no reaction observed under these conditions. Thus, it was concluded that

the lack of reactivity was not related to the pH of the solution. The second theory concerning the oxidation step of the reaction was then considered more seriously. It was mentioned in the introduction that the ease of oxidation of phenols is dependent on their substituents.[31,36] Electron-donating groups, such as the methoxy groups, make a phenol easier to oxidize. Comparing sinapic, ferulic and coumaric acid, the main structural difference is the number of methoxy groups. Ferulic and coumaric acids should be more difficult to oxidize as they have fewer methoxy groups.

From previous research performed on caffeic acid, it was expected that it would react differently than either ferulic or coumaric acid discussed above. The autooxidation of caffeic acid was previously studied by Cilliers and Singleton.[103,104] They reacted caffeic acid in solutions of pH ranging from 4.0 to 8.0 with exposure to 100 percent oxygen. They obtained a variety of dimers and trimers. They were only able to characterize dimeric compounds, most of which did not have lignan structures. Because the oxidation conditions used in the present research were similar to that of Cilliers and Singleton, it was expected that the reaction of caffeic acid would also yield a mixture of products. Caffeic acid was stirred in ammonium bicarbonate buffer with exposure to air, as with the other cinnamic acids. A darkening of the solution was observed. TLC of the reaction indicated that the reaction was complete after 3 days of stirring. The reaction was worked up as previously. The proton NMR spectrum of the crude material showed that a mixture of many products had formed. Caffeic acid differs from the other cinnamic acids in that it has two hydroxy groups and no methoxy groups on its aromatic ring. These hydroxy groups are not inert during an oxidative coupling reaction, providing caffeic acid with two sites for oxidation. Perhaps having two sites for oxidation increases the likelihood that

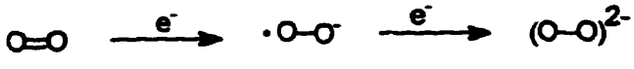
oxidation will occur. Although obtaining many products during the oxidative coupling of phenols is a common outcome, it is considered an unsatisfactory one when a useful synthetic method is being sought. Therefore the oxidative coupling of caffeic acid in basic oxygenated solution was not further investigated.

2.2 Mechanism of Oxidative Coupling of Sinapic Acid

The results of a study of the mechanism of the conversion of sinapic acid to thomasidioic acid will now be discussed. In the introduction, the mechanism for the oxidative coupling of phenols was reviewed in stages. These stages were: initial oxidation, coupling, and further reaction of coupled intermediates. The mechanism for the formation of thomasidioic acid by oxidation of sinapic acid in aerated basic buffer will be dealt with in a similar manner.

The necessity of oxygen for the conversion of sinapic acid to thomasidioic acid was previously established by Rubino *et al.* and this result indicated that the initial step in this reaction was oxidation.[22,23] Oxygen is known to act as a single-electron oxidant and is able to sequentially acquire two electrons, as shown in Scheme 29.[105] The product of the first oxidation is the oxygen radical anion, $(O_2)^{\bullet-}$, and this charged radical can acquire

Scheme 29



another electron. The possible reaction of the products of the reduction of oxygen must be considered when postulating the mechanism for the oxidation of sinapic acid by oxygen

to yield thomasidioic acid. For example, hydrogen peroxide is a known oxidizing agent, as shown in Scheme 30.[106] If it formed by the reduction of $(O_2)^{\bullet-}$, then both oxygen and

Scheme 30

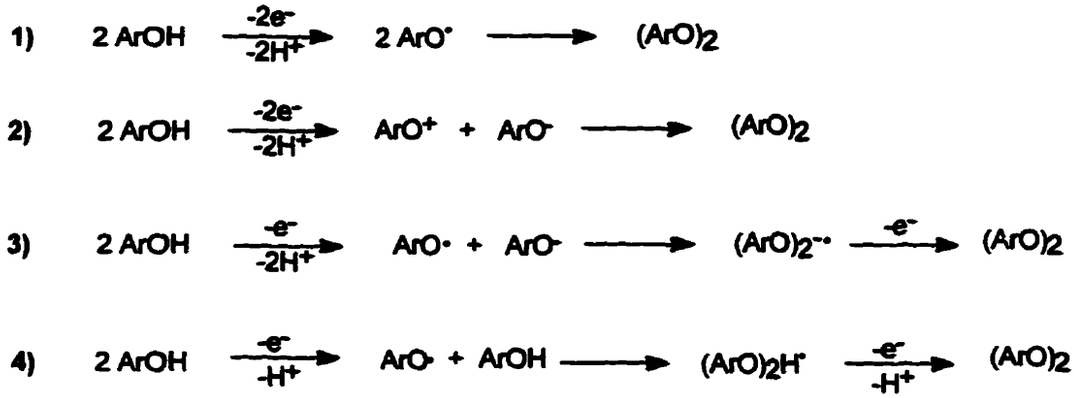
$$H_2O_2 + 2e^- \rightarrow 2HO^-$$

hydrogen peroxide could be acting as oxidants for sinapic acid. It is also possible that $(O_2)^{\bullet-}$ could couple with the sinapyl radical or other species in solution.

The study of hydrogen peroxide's ability to oxidize sinapic acid in basic buffer was carried out by performing a reaction where hydrogen peroxide was the only oxidant available to sinapic acid. Obtaining thomasidioic acid from this reaction would indicate that both oxygen and hydrogen peroxide were acting as oxidants for sinapic acid. Sinapic acid (0.02 M) was dissolved in deaerated ammonium bicarbonate buffer and this solution was stirred under nitrogen. An excess of hydrogen peroxide (1.2 equiv) was added and the reaction was allowed to stir under nitrogen for 24 hours. At this time, the TLC showed that no reaction of the sinapic acid had occurred. Workup of the reaction solution was carried out as previously, extracting the acidified solution with ethyl acetate. Only unreacted sinapic acid was isolated from the reaction solution, which indicated that hydrogen peroxide was not able to oxidize sinapic acid directly.

Determining how much oxygen is needed for the conversion of sinapic acid to thomasidioic acid would help to establish whether $(O_2)^{\bullet-}$ was forming hydrogen peroxide or coupling with other species. The consumption of oxygen will depend on the mechanism of the oxidation. The various mechanisms for the coupling step of the oxidation of phenolic compounds are shown in Scheme 31. Each mechanism requires two oxidation steps. If oxygen were reduced to hydrogen peroxide, then the overall ratio of

Scheme 31



oxygen consumed to phenol oxidized would be 1:2. There is the possibility that oxygen is being reduced to water rather than hydrogen peroxide. In this case, the ratio of oxygen consumed to phenol oxidized would be 1:4. It is clear that the measurement of the ratio of moles of oxygen to sinapic acid being consumed during the conversion of sinapic acid to thomasidioic acid would be a worthwhile experiment in the determination of the mechanism for this reaction.

To determine the ratio of moles of oxidant to substrate reacted during the oxidative coupling of sinapic acid in basic buffer, the consumption of both oxygen and sinapic acid was measured. For measurement of the uptake of oxygen, the reaction was performed at 1 atm under pure oxygen in a closed system attached to a gas burette. The disappearance of sinapic acid was followed by HPLC using an internal standard. *Para*-toluic acid was chosen as internal standard as it was unreactive under the conditions used and it had an easily measurable HPLC signal that did not interfere with the signal from sinapic acid or its oxidation products. A calibration curve of a known ratio of sinapic acid to *para*-toluic acid was plotted against the ratio of the area of their peaks in the HPLC chromatogram. The concentration of sinapic acid remaining in the solution could be

measured as a function of time from the chromatograph of the reaction solution by use of this calibration curve.

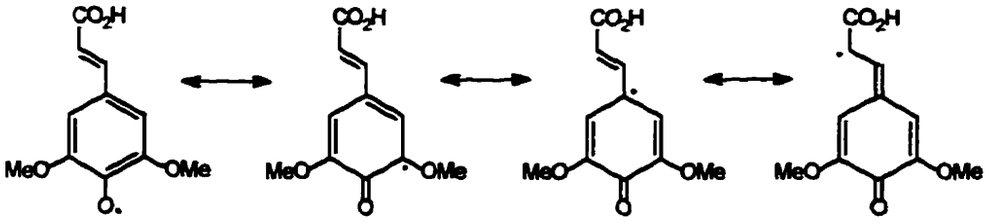
For the measurement of oxygen uptake, sinapic acid (0.02 M) and *para*-toluic acid (0.02 M) were dissolved in ammonium bicarbonate buffer which had been previously deaerated. In order to start the reaction, the reaction solution was gently bubbled with oxygen for 20 seconds and then the reaction flask was attached to the gas burette. After a reaction time of 45 minutes, the volume of oxygen consumed was determined to be 9.0 mL. At this point, the reaction was quenched by acidification of the reaction solution with concentrated hydrochloric acid. The ratio of the peaks for the sinapic and *para*-toluic acid was measured from the chromatogram of the final reaction solution. From the comparison of the amount of oxygen and sinapic acid used in this reaction, it was determined that the ratio of oxidant to substrate reacted was 0.42. This value is quite close to that of one mole of oxygen consumed for every two moles of sinapic acid oxidized. This result indicated that oxygen was most likely being reduced to hydrogen peroxide during the oxidation of sinapic acid in basic buffer.

It should be possible to titrate the hydrogen peroxide present after the air oxidation of sinapic acid. Unfortunately, the dark color of the reaction solution made such a titration impossible using the colorimetric methods available. However, the reaction solution did give a positive starch iodide test which was consistent of the presence of hydrogen peroxide. Sinapic acid (0.03 M) was dissolved in deaerated ammonium bicarbonate buffer and the solution was stirred under pure oxygen and a sample of the solution was taken every 15 minutes. The samples were acidified with 10% hydrochloric acid solution and tested with wet potassium iodide-starch indicator paper. The indicator

paper gradually turned a purple-blue colour with the fourth sample, which indicated that a small amount of hydrogen peroxide was present in solution.

There are several different intermediates that could be involved in the formation of thomasidioic acid from sinapic acid. As discussed in the introduction, air oxidation of phenols or phenolates involves the removal of a hydrogen atom or an electron and the formation of a phenoxy radical.[85-87] In the case of sinapic acid, this radical, the sinapyl radical **83**, will be quite stable due to the delocalization of the single electron. The various resonance contributors are seen in Scheme 32.

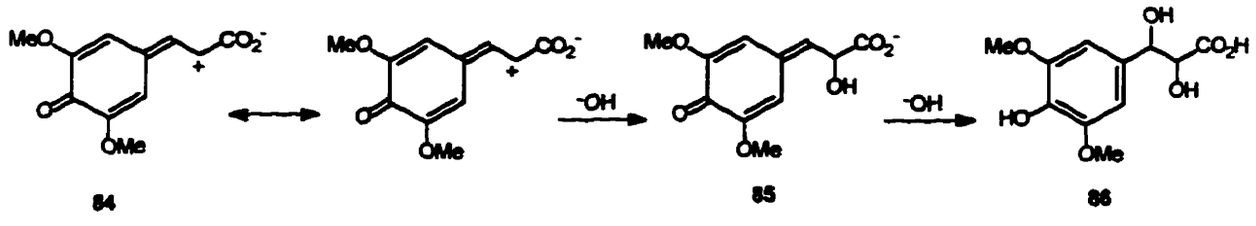
Scheme 32



83

A second oxidation of the sinapyl radical could also occur to yield the corresponding phenoxonium ion **84**. This ion is believed to be quite susceptible to nucleophilic attack. If the phenoxonium ion were to form in basic buffer, then attack by the hydroxide ion would be very likely. Distribution of the positive charge about the phenoxonium ion would be similar to that of the sinapyl radical. Therefore, the positive charge would be concentrated at the β, *para*, and *ortho* positions. The diol **86** could form

Scheme 33

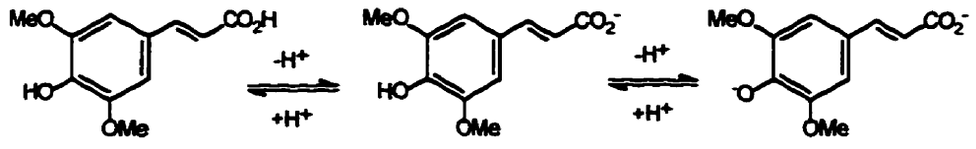


84 **85** **86**

by attack of the hydroxide ion at the β-position and then at the benzyl position of the quinone methide, as shown in Scheme 33. Since thomasidioic acid is known to be the only product of the air oxidation of sinapic acid, it seems unlikely that the phenoxonium ion is formed. Another reason for the unlikely formation of the phenoxonium ion during this reaction is that once the radical is formed, removal of a second electron becomes much more difficult.

Having tentatively excluded the phenoxonium ion from consideration, possible mechanisms involving the sinapyl radical **83** were considered. It was previously state that in basic solution, there exists an equilibrium between the various protonated forms of sinapic acid, shown below in Scheme 34.[96] Thus the mechanisms which must be

Scheme 34

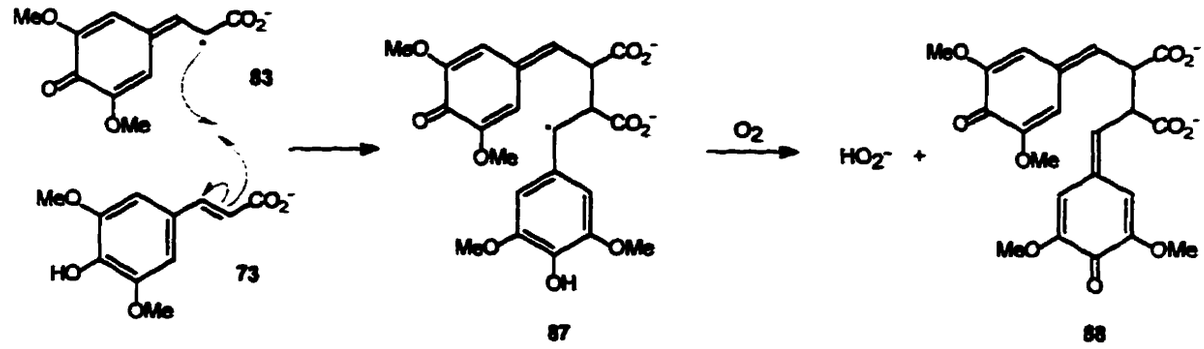


12 **73** **74**

considered for the coupling step should include reactions involving sinapate ions **73** and **74**. These mechanisms include: the coupling of two sinapyl radicals, the coupling of a sinapyl radical and sinapate anion **73**, and the coupling of a sinapyl radical and sinapate ion **74**. Certain cross-coupling reactions were carried out in order to narrow down the number of possibilities.

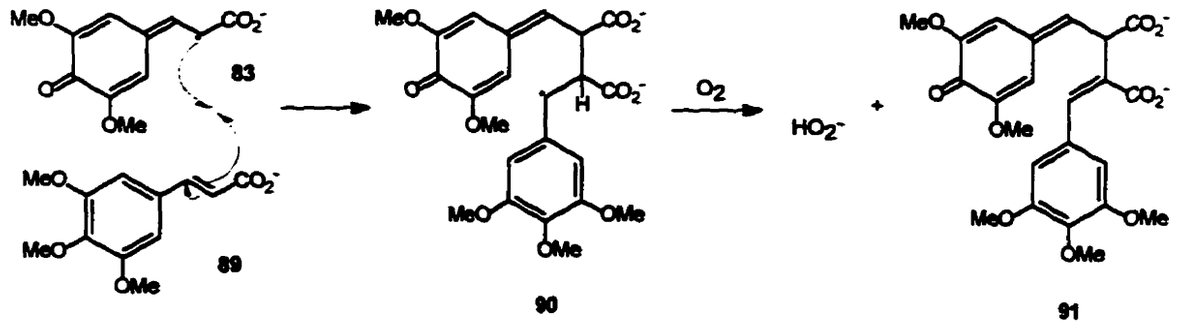
The investigation of the possibility that sinapyl radicals could add to the double bond of sinapate ion **73** was carried out. The mechanism for the addition of a sinapyl radical to the double bond of **73** is shown in Scheme 35. The radical formed from the

Scheme 35



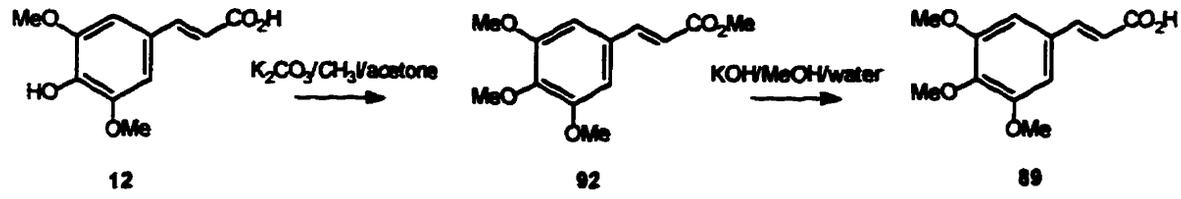
addition could be subsequently oxidized to form a *bis*-quinone methide **88**. In order to test for the possibility that sinapyl radicals could add to the un-ionized phenol **73**, coupling of sinapyl radicals with a compound incapable of ionization to a phenolate anion was attempted. The compound that was used for this study was 3,4,5-trimethoxycinnamic acid (**89**). The loss of the carboxylic acid proton is still possible for **89**, but the formation of a phenolate anion is not possible. The coupling of the sinapyl radical **83** to **89** would eventually lead to coupling product **91** as shown in Scheme 36. This result would

Scheme 36



establish that the addition of the sinapyl radical to the un-ionized phenol was a possible mechanism. 3,4,5-Trimethoxycinnamic acid (**89**) was synthesized in two steps, as seen in Scheme 37. The sinapic acid was methylated at both the phenolic and carboxylic acid positions and then the methyl ester was hydrolyzed with base.

Scheme 37



12 92 89

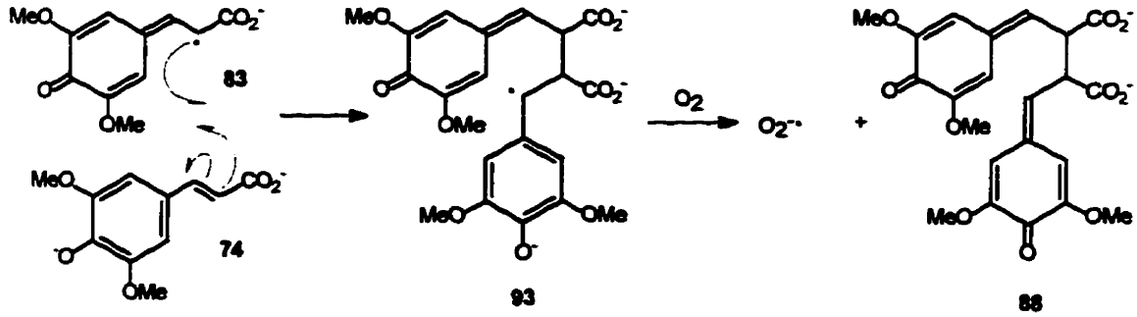
A mixture of sinapic acid (0.14 M), and an excess of both anhydrous K_2CO_3 and iodomethane, was refluxed in acetone. TLC of samples removed periodically during the reaction showed that all of the sinapic acid had disappeared after 23 hours of reflux. Water was added to the reaction mixture and the resulting solution was extracted with dichloromethane. The proton NMR spectrum of the crude product of the methylation reaction was compared to that of sinapic acid. The spectrum was similar to that of sinapic acid except for the presence of two additional signals for methoxyl groups near 4 ppm. Hydrolysis of the methyl 3,4,5-trimethoxycinnamate (**92**) was carried out in order to convert it to the desired 3,4,5-trimethoxycinnamic acid (**89**). Methyl 3,4,5-trimethoxycinnamic acid (0.13 M) was stirred at room temperature in a solution of potassium hydroxide in a solvent of methanol and water in a 1:9 ratio. By monitoring the reaction by TLC, all of the methyl ester was observed to have completely reacted after 5 hours. Isolation of the product was carried out by acidification of the reaction solution to a pH of 2 and extraction of the resulting solution with dichloromethane. The ^1H NMR spectrum of the crude product was compared to that of the starting material. The loss of one of the NMR signals at 4 ppm representing 3 protons was observed which indicated that the hydrolysis of the methyl ester had been successful. The proton NMR spectrum showed the product to be relatively pure. To remove any small impurities, the compound

was filtered through silica gel pretreated with 5% acetic acid in ethyl acetate. The yield of pure 3,4,5-trimethoxycinnamic acid was 61%.

For the cross-coupling reaction, 3,4,5-trimethoxycinnamic acid (**89**) (3.1 mM) and sinapic acid (3.4 mM) were dissolved in ammonium bicarbonate buffer and stirred under air. As the reaction proceeded, the yellow solution turned dark brown. All of the sinapic acid disappeared after 24 hours of stirring at room temperature. The reaction was worked up by acidification of the solution to pH 2 with hydrochloric acid and extraction with ethyl acetate. The proton NMR spectrum of the crude product showed that a mixture of principally two compounds was present. The two compounds were identified as thomasidioic acid (**1**) and 3,4,5-trimethoxycinnamic acid (**89**) by comparison of the crude spectrum to spectra of the pure compounds. It was concluded that no cross-coupling had occurred between the two substrates and provides evidence against the addition of the sinapyl radical to the double bond of the sinapate ion **73** as a likely mechanism for the reaction.

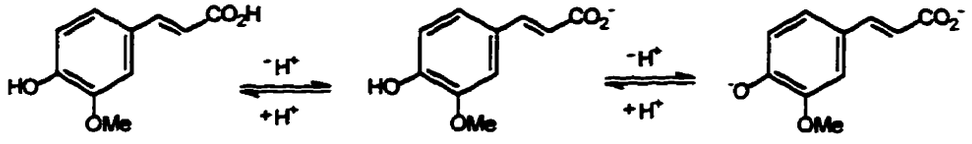
The next mechanism to be considered was bond formation between the sinapyl radical and sinapate ion **74**. The mechanism for this reaction is shown in Scheme 38. In

Scheme 38



order to determine the likelihood of this pathway, an experiment was carried out using a reactant that would mimic the action of the sinapate ion **74** coupling with the sinapyl radical **83**. The ferulate phenolate anion was the mimic chosen. The reason for choosing the ferulate phenolate anion is that its overall structure is quite similar to that of the sinapate ion **74** and it is expected to react similarly. Ferulic acid will form the phenolate anion in basic solution similar to sinapic acid and as previously shown, ferulic acid does not oxidatively couple with itself. This is interpreted to mean that it is not oxidized to a radical by oxygen. The forms of ferulic acid that will exist in ammonium carbonate buffer are shown in Scheme 39. Ferulic acid has one less methoxy substituent than sinapic acid

Scheme 39



13 **94** **95**

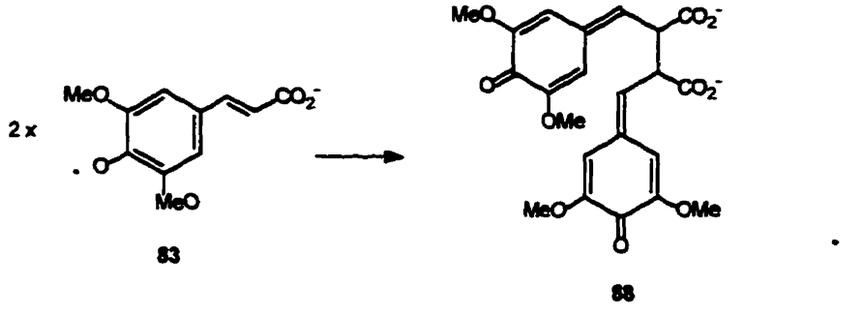
and is expected to be more acidic.[39] It will exist primarily as the ferulate dianion **95** at a pH of 8.5. Therefore, if cross-coupling occurs between ferulic and sinapic acid in aerated basic buffer, then the most likely mechanism for this coupling would be bond formation between the sinapyl radical and ferulate ion **95**. This result would also imply that the coupling of the sinapyl radical and sinapate ion **74** is a likely mechanism for the conversion of sinapic acid to thomasidioic acid.

To test this hypothesis, ferulic acid (0.03 M) and sinapic acid (0.03 M) were dissolved in ammonium bicarbonate buffer (0.28 M). The yellow solution was stirred at room temperature. After 24 hours, all of the sinapic acid was seen to have disappeared. The workup of the reaction was performed as before, with the extraction of the acidified

solution with ethyl acetate. The proton NMR spectrum of the crude material showed that the product mixture contained unreacted ferulic acid and thomasidioic acid. Therefore, no coupling between ferulic and sinapic acid had occurred during this reaction. Although this result does not provide conclusive evidence against the cross-coupling of the sinapyl radical and sinapate dianion, it does suggest that this pathway is less likely.

The final possibility for the mechanism of the oxidative coupling of sinapic acid is the dimerization of sinapyl radicals, as shown in Scheme 40. This seems to be the most likely pathway for the coupling step as the results of various experiments have provided evidence against the likelihood of the other possible mechanisms.

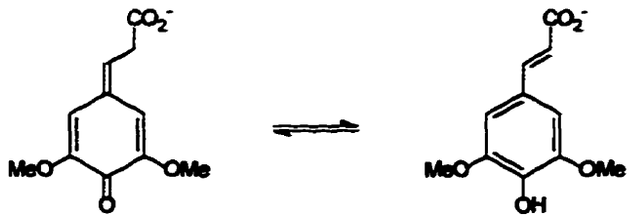
Scheme 40



There are also many other studies of oxidative coupling of phenols which show that this is the most likely mechanism for the coupling step.

The *bis*-quinone methide **88** would be the product of the coupling step and various mechanisms for the formation of thomasidioic acid could be proposed based on the formation of this intermediate. As mentioned in the introduction, quinone methides tend to be quite reactive because of the driving force for formation of more stable phenolic compounds.[50] As shown in Scheme 41, tautomerization is one manner by which the conversion of a quinone methide to a phenolic compound can occur.

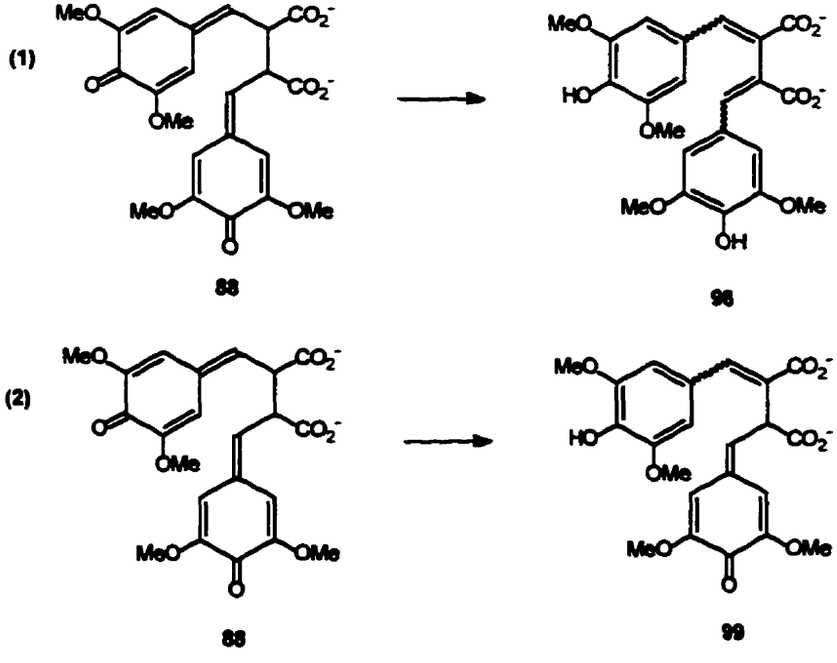
Scheme 41



96 97

There are two possible ways for the *bis*-quinone methide **88** to tautomerize. First of all, it could undergo full tautomerization (see reaction 1 of Scheme 42). The product of this reaction would be the diarylbutadiene dicarboxylic acid **98**. Secondly, partial tautomerization of **88** could occur, such that only one of the quinone methides of **88** forms the respective phenol. In this case, the *mono*-quinone methide **99** would be formed, as shown in Scheme 42.

Scheme 42

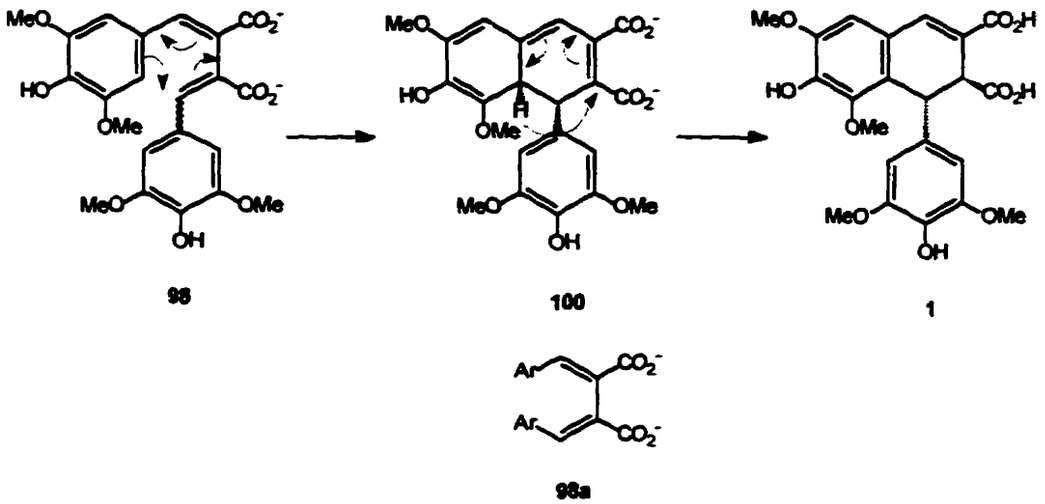


88 98

88 99

For the oxidative coupling of sinapic acid, mechanisms for formation of thomasidioic acid with butadiene **98** or *mono*-quinone methide **99** as intermediates, were considered. As shown in Scheme 43, butadiene **98** could undergo a pericyclic reaction. After cyclization, only a [1,5] sigmatropic shift of a hydride would be required to yield thomasidioic acid. The neighbouring aromatic and carboxylic acid groups of thomasidioic

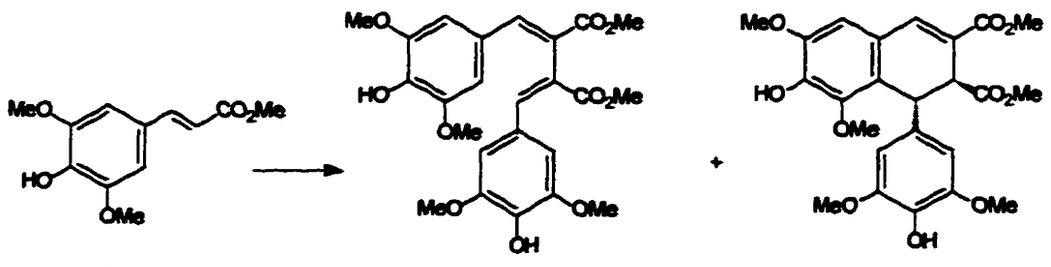
Scheme 43



acid are *trans* to one another. If the hydride shift occurs pericyclically then the migrating hydrogen and the pendant aryl group must have been *cis* to one another in intermediate **100**. Similarly the *cis* geometry of **100** implies that the butadiene intermediate **98** had the *trans, trans* geometry (**98a**).

In order to test the possibility that butadiene diacid **98** is an intermediate in the formation of thomasidioic acid, it was necessary to prepare it by an independent route. Fortunately, the corresponding dimethyl ester of this acid, **101**, was available from oxidative coupling of methyl sinapate. Although the details of this reaction will be discussed at a later time, it was determined that the products of the air oxidation of methyl

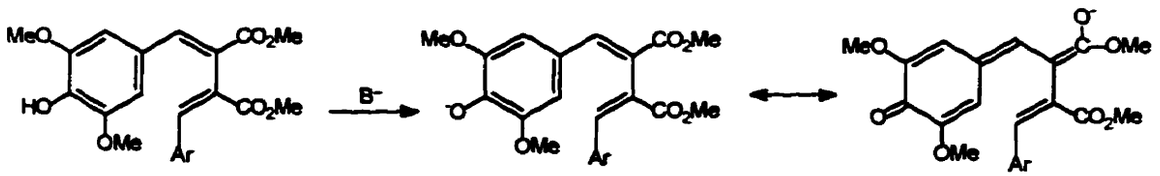
Scheme 44



23 101 2

sinapate were the diarylbutadiene dicarboxylate ester **101** and the dimethyl ester of thomasidioic acid (**2**), as shown in Scheme 44. Once **101** had been isolated from the product mixture, hydrolysis was carried out in order to prepare the diacid **98**. The hydrolysis of **101** was found to be a more difficult process than initially expected. No reaction of the ester was observed after heating the ester in strong alkaline solutions for long periods of time. A possible reason for the lack of reaction of **101** in basic solution is that the removal of the phenolic proton is likely to occur (Scheme 45). The formation of

Scheme 45



101 102 102a

the enolate **102a** will prevent the alkaline hydrolysis of **101**. After several attempts, it was finally determined that the diester **101** could only be hydrolysed under acidic conditions.

The diester **101** (0.01 M) was refluxed in sulfuric acid (0.5 M). This reaction was followed by TLC and all of **101** was observed to have disappeared after 7 days of reflux. The reaction solution was made basic with 10% sodium bicarbonate solution and

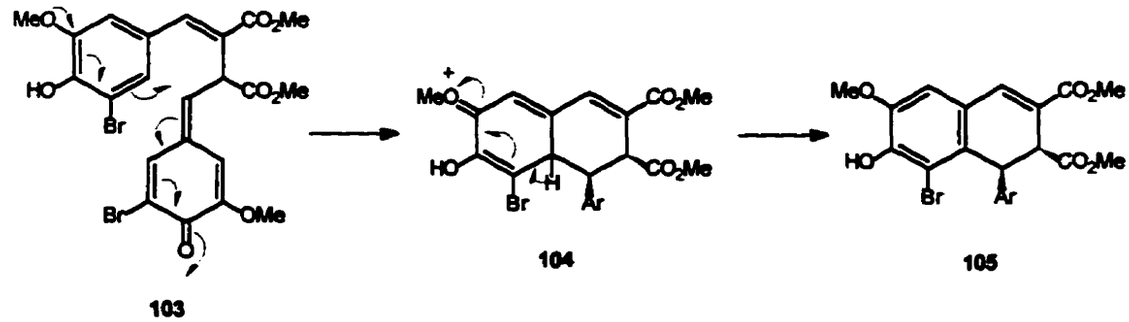
extracted with dichloromethane to isolate any non-acidic products. This was followed by acidification of the aqueous solution to pH 2 with 10% hydrochloric acid and extraction with ethyl acetate. This final extraction was expected to provide the desired diacid **98**. The proton NMR spectrum of the crude product was compared to that of **101** and it was determined that the signal belonging to the methyl ester group had disappeared. A very small amount of thomasidioic acid had also formed during the reaction.

To verify the theory that the diacid **98** was an intermediate in the formation of thomasidioic acid from sinapic acid, it was similarly reacted in aerated basic buffer. If diacid **98** acts an intermediate for the conversion of sinapic acid to thomasidioic acid, then it should cyclize under the same conditions. Initially, a small amount of the diacid **98** was reacted in aerated ammonium bicarbonate buffer for 24 hours. This reaction was worked up by extraction of the acidified solution with ethyl acetate. The proton NMR of the crude product showed that all of **98** had disappeared. Because the starting material contained a small amount of thomasidioic acid, it was difficult to confirm if the diacid also had been converted to thomasidioic acid using NMR spectrometry alone. To provide more quantitative results, HPLC was used to determine the amount of the thomasidioic acid before and after the reaction. An increase in the ratio of thomasidioic acid to an internal standard after the reaction would indicate that the diacid **98** was cyclizing in basic buffer to yield the aryltetralin. The internal standard used for this study was *para*-toluic acid. **98** (0.03 M) and *para*-toluic acid (0.08 M) were dissolved in a 1:1 solution of acetonitrile and water. This solution was divided into two portions. The first portion was analysed by HPLC. The ratio of the areas for the peaks of thomasidioic acid to *para*-toluic acid was determined to be 0.28:1. Ammonium bicarbonate buffer was added to the

second portion of the solution. This light brown solution was stirred at room temperature for 24 hours and then was acidified with 10% hydrochloric acid. On acidification of the solution, some precipitate was formed. Acetonitrile was added to dissolve any solid material and HPLC of the resulting solution showed that the peak for the diacid **98** had completely disappeared. The ratio of area of the thomasidioic acid and *para*-toluic acid peaks was 0.22:1. This result indicated that although the diacid **98** did react in aerated basic buffer, it was not being converted to thomasidioic acid. It was concluded that the diaryl butadiene dicarboxylic acid **98** was not an intermediate in the conversion of sinapic acid to thomasidioic acid.

Mono-quinone methides, similar in structure to **99**, have been shown by Angle *et al.* to undergo cyclization and yield aryltetralin-type products.[53-55] There are also various studies of aryltetralin lignans in which this mechanism is proposed. For example, Ahmed *et al.* observed the formation of an aryltetralin from oxidation of the methyl ester of 5-bromoferulic acid with ferric chloride in aqueous acetone.[28] The mechanism proposed for this conversion was the cyclization of the *mono*-quinone methide **103** (Scheme 46). It

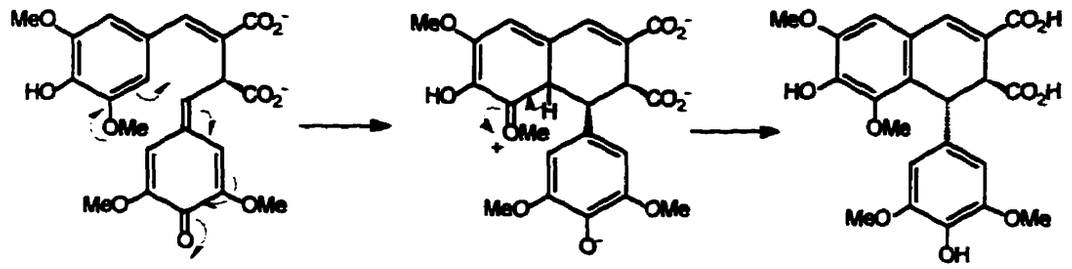
Scheme 46



103 **104** **105**

might be reasonable to postulate a similar mechanism for the formation of thomasidioic acid from intermediate **99** (see Scheme 47). Once the cyclization of **99** had occurred only the loss of a proton would be required to produce the aryltetralin.

Scheme 47



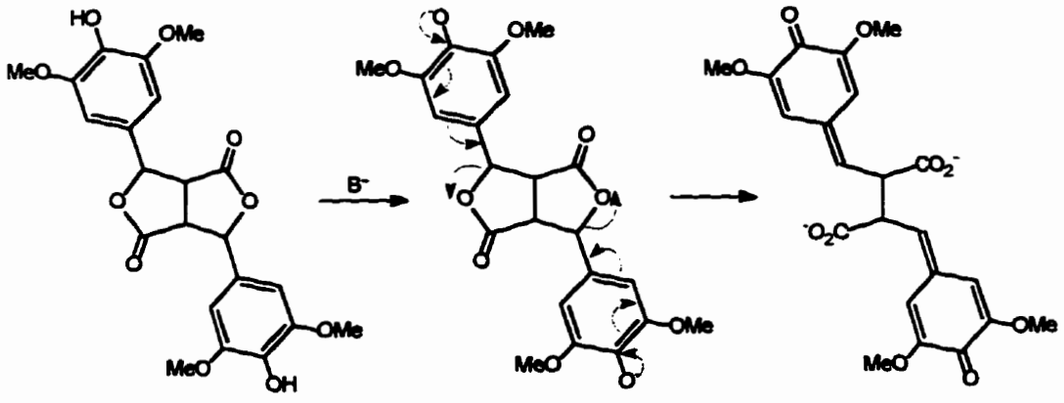
An isotope exchange study of the reaction of sinapic acid in aerated basic buffer containing D_2O was undertaken to provide more information on the mechanism of the conversion of sinapic acid to thomasidioic acid. All of the mechanisms proposed thus far do not involve proton exchange on the carbon skeleton, and the exchange study was carried out to verify this fact. The presence of deuterium atoms in the product could be determined by observing either a decrease in the peak area of a signal or the total disappearance of a signal in the ^1H NMR spectrum.

Sinapic acid (0.05 M) was dissolved in ammonium bicarbonate buffer made with D_2O. This yellow solution was stirred for 2 days with exposure to the air. On observation of the disappearance of the substrate by TLC, the solution was diluted with water and acidified with 10% hydrochloric acid. Isolation of the final product was carried out by extraction of the acidified solution with ethyl acetate. The proton NMR spectrum of the crude product was compared to that of pure thomasidioic acid. It was determined that each of the peaks from the spectrum of pure thomasidioic acid were also observed in the

spectrum of the crude product (with normal integration for one hydrogen each). These results demonstrated that deuterium exchange between the solvent and intermediate had not occurred during the conversion of sinapic acid to thomasidioic acid.

It would be beneficial to find another route to the *bis*-quinone methide in order to test the possibility that it acts as an intermediate for the oxidative coupling of sinapic acid. The dehydrodisinapic acid dilactone (**16**) (see Scheme 48) might be a possible source for this compound in basic solution. It was believed that the treatment of the dilactone **16** with base might remove both of the phenolic protons to yield the dianion **107**. If this were

Scheme 48

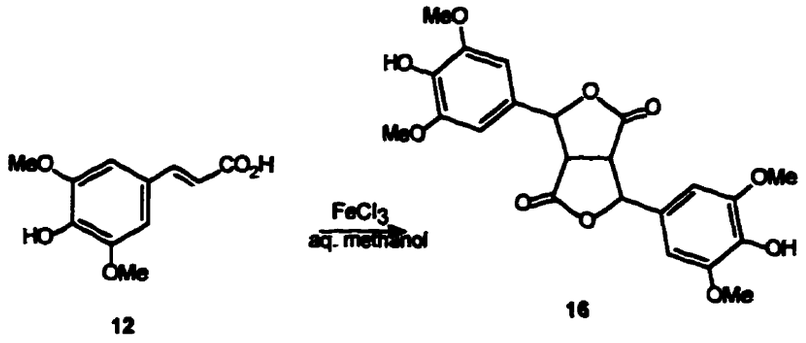


16 **107** **88**

true, then it is very likely that this dianion would undergo ring opening to yield the *bis*-quinone methide **88** (Scheme 48). If the *bis*-quinone methide is an intermediate for the conversion of sinapic acid to thomasidioic acid in aerated basic buffer, then the treatment of the dilactone with these conditions should also yield the aryltetralin lignan.

The dilactone was synthesized using the method of Ahmed *et al.* by oxidizing sinapic acid with ferric chloride in aqueous methanol (Scheme 49).[102] The dilactone **16** (1.0 mM) was then dissolved in ammonium bicarbonate buffer. This solution was stirred

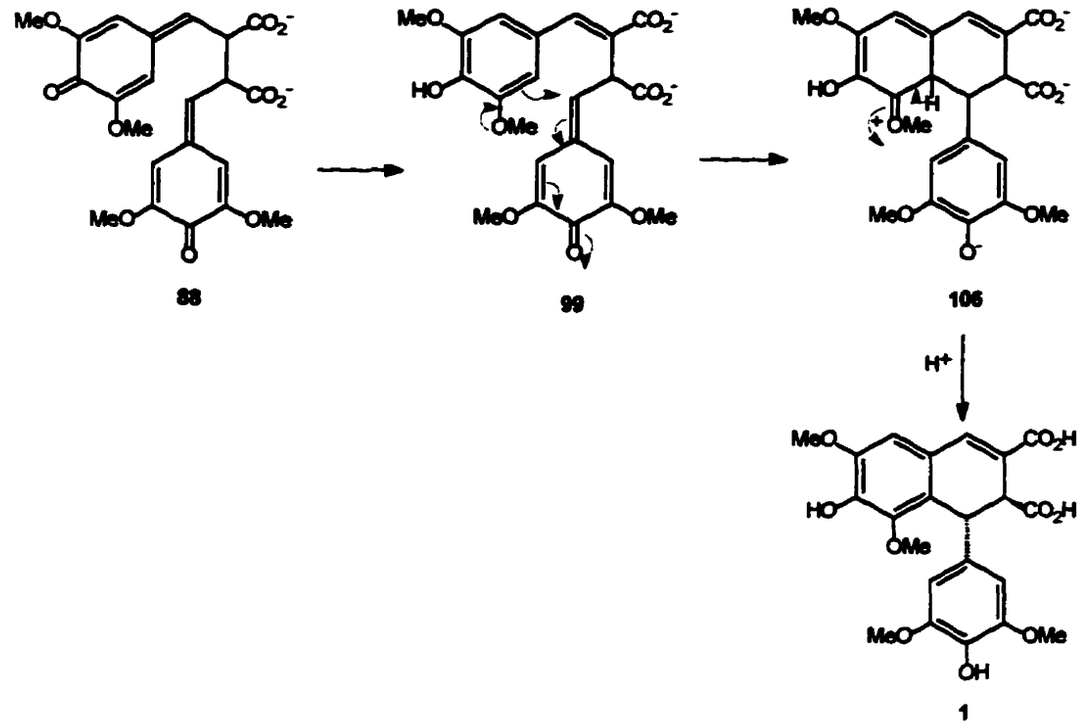
Scheme 49



at room temperature with exposure to air. The yellow solution gradually turned orange. Monitoring the reaction by TLC showed that all of the dilactone had disappeared after 20 hours of stirring. The solution was acidified to pH 2 with 10% hydrochloric acid and then extracted with ethyl acetate. The isolated product was determined to be thomasidioic acid by comparison of the proton NMR spectrum to the authentic acid. To determine the conditions necessary for the conversion of the dilactone **16** to thomasidioic acid, the reaction was repeated in basic buffer under nitrogen. The reaction was observed to progress as it did previously as the solution turned orange and all of the substrate had disappeared after 20 hours. The product of the this reaction was also determined to be thomasidioic acid.

The intermediate for the conversion of dehydrodilactone **16** to thomasidioic acid (**1**) can now be considered knowing that only alkaline conditions are necessary for this conversion. As indicated in Scheme 48, the dilactone is most likely converted to the phenolate dianion. This dianion can then open to the *bis*-quinone methide. From this point the mechanism follows that shown in Scheme 50 (and in Scheme 48) with eventual formation of thomasidioic acid. Therefore, the most likely intermediates involved in the conversion of the dilactone to thomasidioic acid are believed to be the *bis*-quinone

Scheme 50



methide **88** and *mono*-quinone methide **99**. These results are consistent with those obtained for the conversion of sinapic acid to thomasidioic acid. Other possible mechanisms for the conversion of the dehydrodilactone **16** to thomasidioic acid might be envisioned. Some of these mechanisms could involve hydrogen exchange at the carbons α to the carboxyl groups. In order to eliminate these mechanisms, the dilactone was reacted as before in a buffer made with D_2O. The occurrence of any isotopic exchange was determined by examining the ^1H NMR spectrum of the product.

Dilactone **16** (1.2 mM) was dissolved in ammonium bicarbonate buffer made with D_2O. The yellow solution was stirred at room temperature for 24 hours. Acidification and extraction of the solution was carried out as before. The proton NMR spectrum of the crude product showed that all of the protons of thomasidioic acid were present with

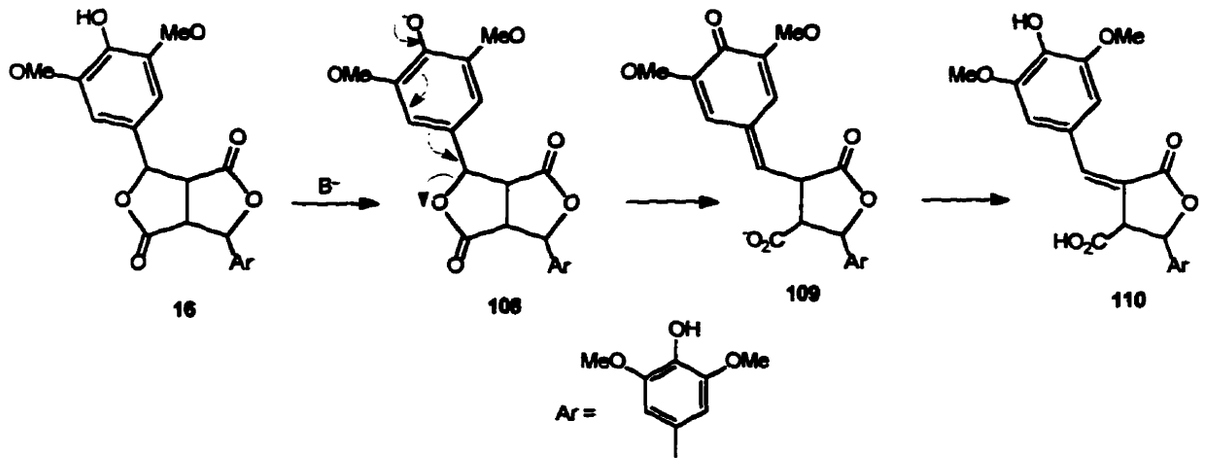
no significant change in the integration of the signals for these protons. It was concluded that no exchange of the protons α to the carboxyl groups was occurring. The most reasonable mechanism for the conversion of dehydrodilactone to thomasidioic acid is one that involves the *bis*-quinone methide as shown in Scheme 48. This result also supports the contention that sinapic acid is converted to thomasidioic acid via the *bis*-quinone methide **88**.

An attempt was also made to form the *bis*-quinone methide **88** from the dehydrodilactone **16** under non-aqueous conditions. These studies were primarily conducted in THF and a variety of bases were used (LDA, BuLi, NaHMDS). None of these reactions produced any thomasidioic acid on workup. A single product, however, was produced in high yield when the dilactone was reacted with only one equivalent of LDA in THF. Diisopropyl amine (0.12 M) was stirred in freshly distilled THF at -78 °C under nitrogen. Butyl lithium was added dropwise and the solution was stirred for 10 minutes. Dehydrosinapic acid dilactone (**16**) (0.03 M), dissolved in THF, was added dropwise to this solution. This brown solution was stirred at -78 °C for 30 minutes and then allowed to warm up to room temperature. The reaction was followed by TLC and all of the starting material was observed to have reacted after 21 hours of stirring. Water was added to the red solution and then it was acidified with 10% hydrochloric acid. The acidified solution was extracted with ethyl acetate.

The proton NMR spectrum of the crude product showed that a single product had formed which was not thomasidioic acid. Comparison of the proton NMR spectrum of the product to that of the dilactone indicated that there were some similarities. For example, both spectra contained singlets at 3.8, 4.1 and 6.7 representing 12, 1 and 2

protons respectively. Other peaks found in the unknown product's spectra were: doublets at 5.67 and 7.68 ppm and a 2-proton singlet at 6.51. In order to determine the structure of the unknown compound, possible products of the removal of one proton from the dilactone were considered. If the phenolic proton were removed, the *mono*-quinone methide **109** would be formed. This product could then tautomerize to the lactone **110** (see Scheme 51). Complete characterization of lactone **110** was not possible due to the instability of the compound, which did not allow accurate measurement of its molecular

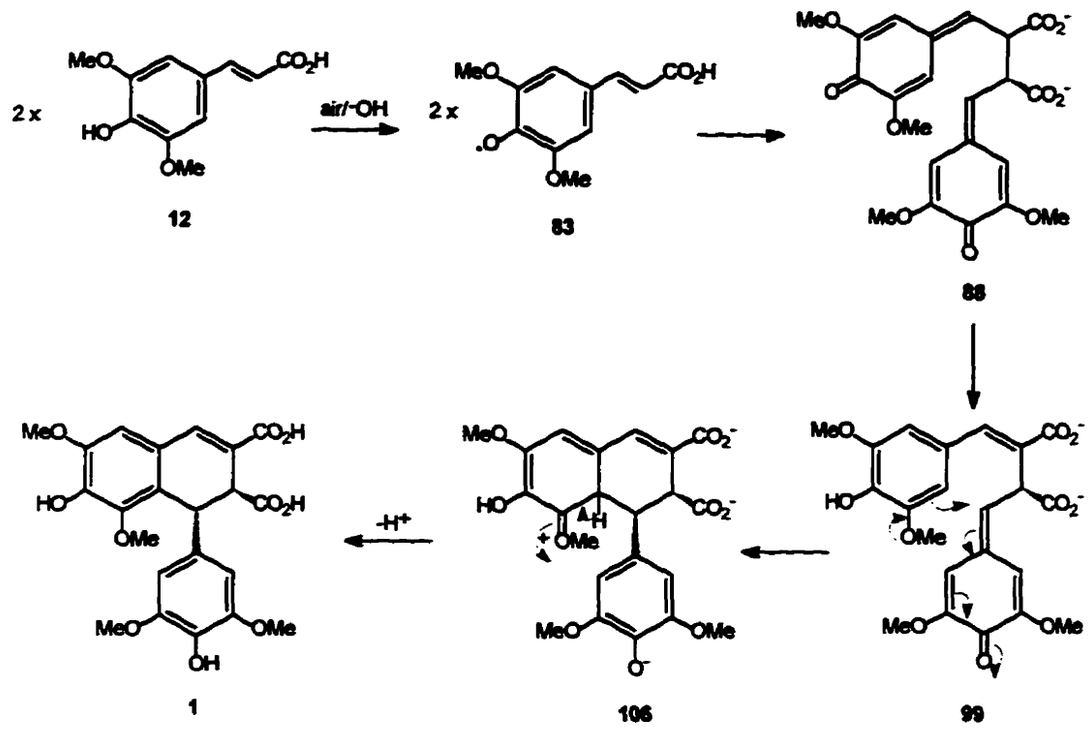
Scheme 51



mass using exact mass-mass spectrometry. The ^{1}H NMR spectrum was consistent with the proposed structure **110**, an assignment which was further substantiated by comparison to other compounds with similar structures.[29]

From all of the results of these experiments, it can be concluded that the most likely mechanism for the conversion of sinapic acid to thomasidioic acid in aerated basic buffer involves the *mono*-quinone methide **99**. The entire mechanism for this reaction is shown in Scheme 52.

Scheme 52



The reaction of sinapic acid with other single electron oxidants was carried out to determine what oxidants, other than oxygen, could convert sinapic acid to thomasidioic acid. The ultimate goal was to find an oxidant that would give high yields in a shorter time, and possibly be able to induce the same type of reaction in other phenolic cinnamic acids (coumaric, ferulic, caffeic). The oxidants that were tried were potassium triiodide (KI_3), potassium ferricyanide, ($K_3Fe(CN)_6$), and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).

The triiodide ion is formed on dissolution of iodine in an aqueous solution of potassium iodide. The reduction of triiodide to iodide requires two electrons, but the two electrons are presumably acquired in two separate oxidation steps. The equation for the reaction is as shown. Because this oxidant is able to acquire two electrons, only

$$I_3^- + 2e^- \rightarrow 3\,I^-$$

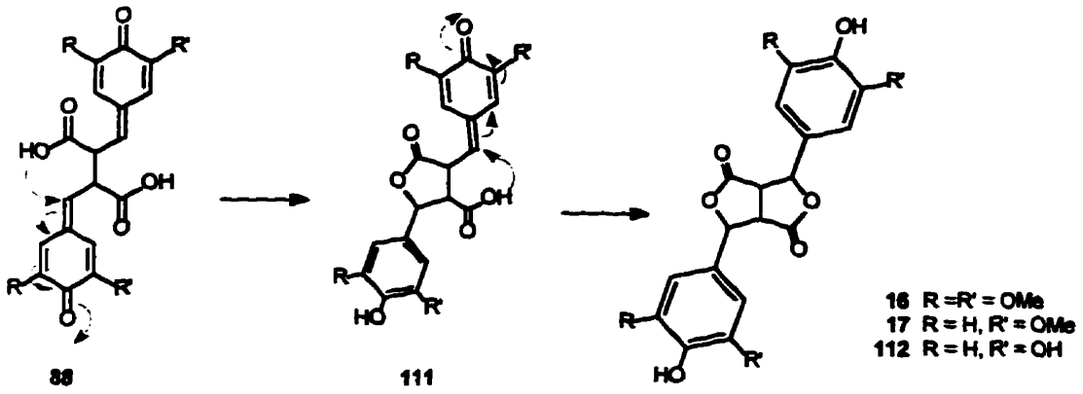
one-half of an equivalent of potassium triiodide was used to oxidize sinapic acid. Sinapic acid (0.02 M) was dissolved in the deaerated ammonium bicarbonate buffer and then deaerated potassium triiodide solution (0.06 M) was added. The dark red solution was stirred at room temperature under nitrogen for 24 hours. After this time, a TLC of the mixture showed that all of the starting material had reacted. The workup of the reaction was performed as before with the acidified solution being extracted with ethyl acetate. The proton NMR spectrum of the crude product confirmed that the product was thomasidioic acid with a purity similar to that obtained from the air oxidation. These results show that the triiodide ion is an effective oxidant for the conversion of sinapic acid to thomasidioic acid. Similar reactions were attempted with two *para*-hydroxycinnamic acids, ferulic and courmaric acid. No reaction of these acids was observed. This was true even when the reaction was carried out in strong base. Therefore it is believed that the triiodide ion is not a strong enough oxidant to form the respective phenoxy radicals and induce oxidative coupling of these acids.

Ferricyanide ion in alkaline solution was also used for the oxidative coupling of sinapic acid. This is a common oxidizing agent that has been used to oxidize a variety of phenols.[33,40] For the oxidative coupling of sinapic acid, a molar ratio of ferricyanide ion to substrate of 1:1 was used as the ferricyanide ion can only acquire one electron per molecule. Sinapic acid (0.02 M) and potassium ferricyanide (0.02 M) were dissolved in deaerated ammonium bicarbonate buffer. This solution was stirred under nitrogen at room temperature. Monitoring the reaction by TLC showed that all of the substrate had

disappeared after 20 hours of reaction. The acidified solution was extracted with ethyl acetate. The isolated product was determined to be thomasidioic acid by comparison to an authentic ^{1}H NMR spectrum. Therefore, the ferricyanide ion is also a suitable oxidizing agent for the oxidative coupling of sinapic acid.

The final oxidant used for sinapic acid was 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), which is known to be fairly strong oxidizing agent. The exact mechanism for oxidation of phenols by DDQ is unclear as DDQ has been proposed to be able to act as a one electron oxidant (accepting either an electron or a hydrogen atom) or a two electron oxidant (accepting a hydride ion).[75-77] Whatever the first step in the oxidation, the full reduction of DDQ requires a total of two electrons. One-half equivalent of DDQ was used for the oxidative coupling of sinapic acid. Sinapic acid (0.07 M) and DDQ (0.04 M) were dissolved in tetrahydrofuran and the solution was stirred at room temperature. The color of the solution changed from brown to red-green during the reaction and all of the substrate had disappeared after 6 hours of stirring. The solution was diluted with water and extracted with ethyl acetate. The organic solution was washed with sodium bisulfite solution (0.1 M) to reduce any remaining DDQ. The proton NMR spectrum of the crude product was quite simple, as there were only 4 singlets observed. A comparison of this spectrum to that of dehydrodisinapic acid dilactone showed that the dilactone **16** was the only product of the reaction. This results clearly differed from the other reactions conducted in aqueous solution. Assuming that the *bis*-quinone methide is an intermediate in this oxidation, it appears that it undergoes exclusive lactonization in THF rather than cyclization to thomasidioic acid (Scheme 53).

Scheme 53

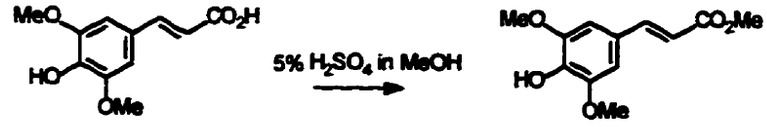


THF is non-protic and it is possible that the *bis*-quinone methide is more stable in this type of solvent. The oxidation of ferulic and caffeic acids with DDQ in THF also produced the respective dehydrodilactones **17** and **112**. The dehydrodilactones were identified by comparison of their spectra to those in the literature.[102,107] It was difficult to drive these reactions to completion regardless of the time of reaction. This result again demonstrated the increased difficulty of oxidation of *para*-hydroxycinnamic acids having fewer electron-donating groups on the aromatic ring.

2.3 Reaction of Sinapyl Esters

Several reactions were carried out to determine if the conditions for oxidative conversion of sinapic acid to an aryltetralin lignan were also applicable to the esters of sinapic acid. A preliminary study was carried out using methyl sinapate as a substrate. Methyl sinapate (**23**) was synthesized by refluxing a solution of sinapic acid in methanol containing 5% concentrated sulfuric acid. (Scheme 54)

Scheme 54

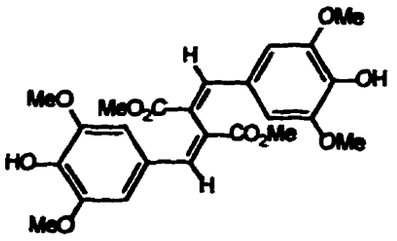


12 23

For the oxidative coupling reaction, methyl sinapate (0.014M) was dissolved in a 1:1 solution of methanol and ammonium bicarbonate buffer. The yellow solution was stirred at room temperature. Monitoring of the reaction by TLC showed that all of the methyl sinapate had disappeared after 20 hours of stirring and two spots were found on the TLC plate. Most of the methanol was removed by evaporation of the solution under reduced pressure. The solution was then acidified with 10% hydrochloric acid to a pH of 7 and extracted with ethyl acetate. A reference sample of dimethyl thomasidioate (**2**) was synthesized by methylation of thomasidioic acid in acidic methanol as described above. Comparison of the ^{1}H NMR spectrum of the crude product of the oxidation of methyl sinapate to that of pure dimethyl thomasidioate showed that both dimethyl thomasidioate and another compound were produced. In order to characterize the unknown product, separation of the two compounds was necessary. Separation by flash chromatography was difficult as the two compounds had very similar elution times. Isolation of the unknown compound was carried out by preparative HPLC.

The identity of the unknown compound was determined by analysis of its NMR and mass spectra. The proton NMR spectrum of the compound was quite simple, as it only had four singlets. Two of the singlets were observed at about 4 ppm with a ratio of 3:6. The other peaks were observed at 6.5 and 8.0 ppm and represented one and two protons respectively. The molecular mass of the compound was determined to be 474.15

g/mol by mass spectrometry. This molecular mass was the same as that of dimethyl thomasidioate, which indicated that a different dimer of methyl sinapate had probably formed. Because of the simplicity of the ^{1}H NMR spectrum and the larger molecular mass, it was likely that the molecule was a symmetrical dimer. A comparison of the proton NMR spectrum of the compound to that of methyl sinapate showed some similarities. For example, both spectra had two peaks at about 4 ppm with a ratio of 3:6 and a singlet at 6.8 ppm, representing 2 protons. There were only two differences between the two spectra. First of all, the doublet at 6.3 ppm in the spectrum of methyl sinapate (due to the proton bonded to the β-carbon of methyl sinapate (the proton α to the carboxyl group)) was not found in the other spectrum. Secondly, a singlet at about 7.6 ppm representing one proton was observed in the spectrum of unknown compound. Methyl sinapate has a doublet at about the same chemical shift (representing the vinyl proton α to the aromatic ring (β to the carboxyl group)). The loss of the proton α to the carboxyl group would cause the disappearance of the doublet at 6.3 ppm and conversion of the doublet at 7.6 ppm into a singlet, as observed in the spectrum of the unknown compound. A compound that can account for all of this spectral data is butadiene product **101**. The structure of this compound was confirmed by comparison of the spectral data to other compounds of similar structure.[60,108] In order to determine the stereochemistry about the double bonds, the coupling constant between the carbon of the carbonyl group and the vinylic proton was measured. The *J*-value for this coupling was determined to be 6.8 Hz. This value is consistent with the *cis* placement of the ester group and proton, which implies that the actual stereochemistry of both double bonds is *trans*, as shown.[109]



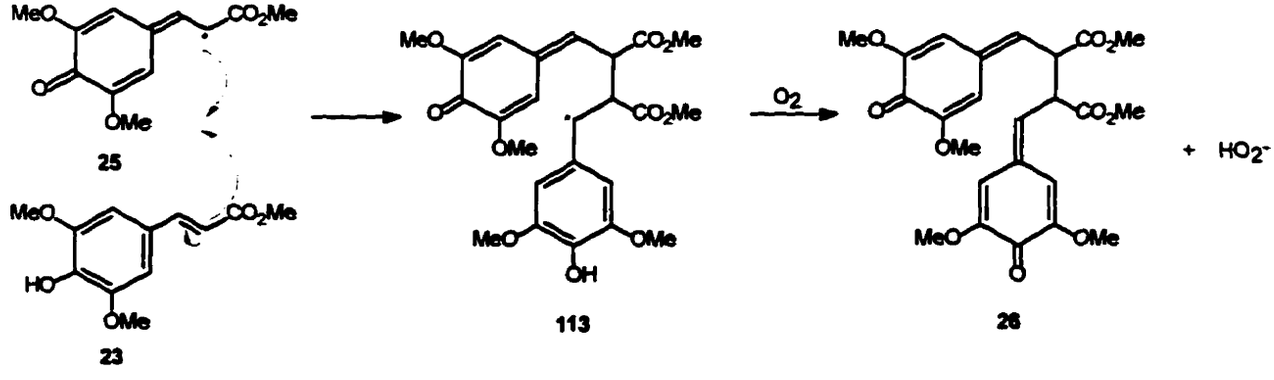
101

During the reactions of methyl sinapate in ammonium bicarbonate buffer, a slow decrease in the pH during the reaction was observed. This was probably due to a loss of ammonia from the basic solution. Due to these problems with pH control, other buffers were considered. Both the temperature and pH of the reaction solution had to be kept constant to determine accurately how any change in the conditions would affect the outcome of the reaction. The stabilities of the pH for sodium tetraborate, potassium hydrophosphate, and *tris*(hydroxymethyl)aminomethane (TRIS) buffers were studied. A 1:1 solution of methanol and the buffer (0.05 M) was stirred at room temperature while passing, through the solution, air that had been presaturated with methanol and water (by bubbling through methanol/KOH and water/KOH solutions). The pH of the solution was constantly monitored over a long period of time. Both borax and hydrophosphate buffers showed a gradual increase of pH with time. Only the pH of the TRIS buffer remained relatively stable over the time period of 3 days. A trial oxidation of methyl sinapate was carried out in a 1:1 solution of methanol and TRIS buffer (0.1 M) at a pH of 8.5. Most of the methyl sinapate was found unreacted after stirring the solution for 24 hours. The temperature of reaction was raised to 43±2 °C in an attempt to increase the rate of reaction. After 17 hours of reaction, it was observed by TLC that all of the methyl sinapate had disappeared. The acidified solution was extracted with dichloromethane. The ^{1}H NMR spectrum of the crude product showed that dimethyl thomasidioate and the

diarylbutadiene dicarboxylate ester **101** were formed in a ratio of 1:2. Most of the subsequent experiments performed to determine the mechanism of the oxidative coupling of methyl sinapate were carried out using TRIS buffered solutions.

Cross-coupling of methyl 3,4,5-trimethoxycinnamate with methyl sinapate was attempted to determine the mechanism for the oxidative coupling of methyl sinapate. As suggested for sinapic acid, the coupling step could involve bond formation between a sinapyl radical and sinapate ion **73**. A similar mechanism could be postulated for oxidative coupling of methyl sinapate (Scheme 55). To test for this mechanism, a substrate had to be chosen to mimic the methyl sinapate molecule in basic buffer. Methyl 3,4,5-trimethoxycinnamate (**92**) was chosen as it could not become ionized to the phenolate anion in basic solution. This substrate was synthesized by treatment of sinapic acid with iodomethane and potassium carbonate in acetone. Methyl sinapate (0.01 M) and methyl

Scheme 55

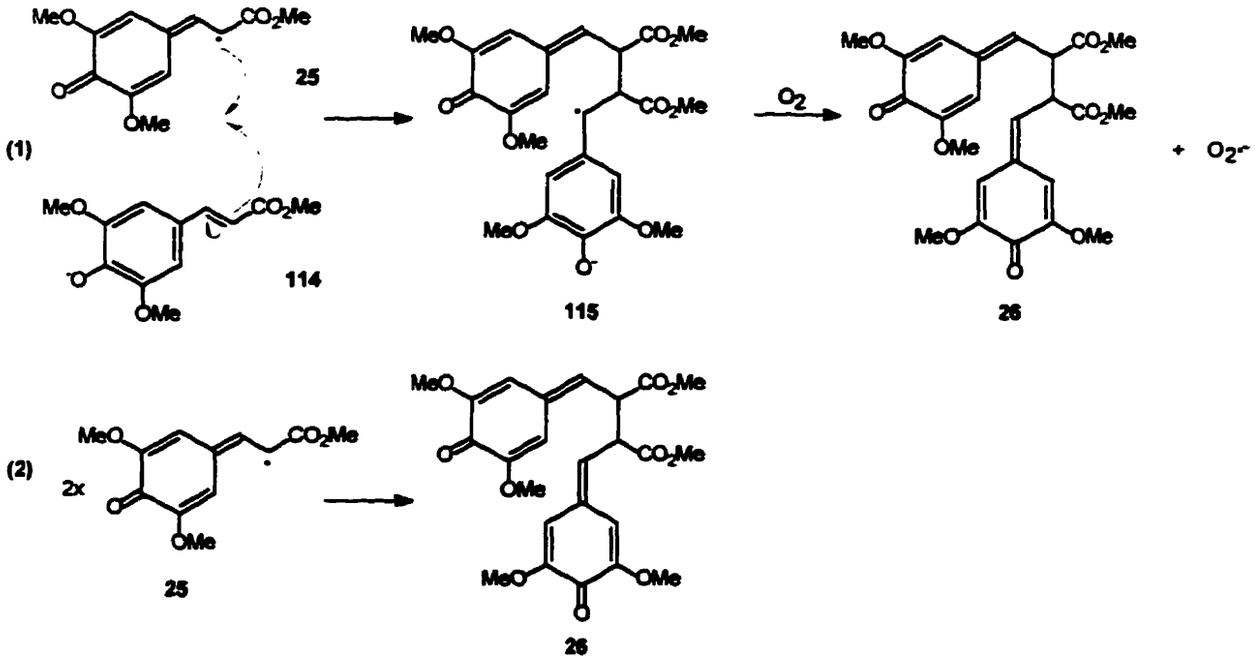


3,4,5-trimethoxycinnamate (0.01 M) were dissolved in methanol and an equal volume of TRIS buffer (0.1 M) was added. This solution was stirred at 43±2 °C and bubbled with air that was presaturated with methanol and water. After 20 hours of stirring, the acidified solution was extracted with dichloromethane. The ^{1}H NMR spectrum of the crude

product was compared to that of **101**, dimethyl thomasidioate, and methyl 3,4,5-trimethoxycinnamate and it was determined that the product mixture was comprised of these three compounds. From this result, it was seen that reaction between methyl sinapate and methyl 3,4,5-trimethoxycinnamate did not occur. Therefore, it is believed that the coupling step for methyl sinapate does not involve cross-coupling between the methyl sinapyl radical and un-ionized methyl sinapate.

Two other possible mechanisms for the coupling step involve the dimerization of two methyl sinapyl radicals or the coupling of the methyl sinapyl radical and methyl sinapate anion. The product of both of these mechanisms is the *bis*-quinone methide **26** (Scheme 56). As mentioned previously, the *bis*-quinone methide could undergo tautomerization to yield either the butadiene **101** or the respective *mono*-quinone methide. The butadiene **101** was isolated as one of the products from the oxidative coupling of

Scheme 56



methyl sinapate. It could be possible that the butadiene was an intermediate in the reaction and the conditions of the reaction were too mild for complete conversion of the butadiene to dimethyl thomasidioate. If this were true, then changes to the conditions of the reaction, such temperature and pH, should drive the cyclization of the butadiene to yield only dimethyl thomasidioate. A reaction of methyl sinapate in a solution of 1:1 methanol: ammonium bicarbonate buffer was repeated at room temperature. When all of the methyl sinapate was observed by TLC to have disappeared, the reaction solution was heated to 60 °C. The reaction was followed by TLC which showed that no further reaction of the butadiene occurred after 20 hours. The product mixture was isolated by extraction of the acidified reaction solution. ^{1}H NMR confirmed that the butadiene diester **101** was still present. Other tests were carried out by reacting the product mixture of dimethyl thomasidioate and butadiene diester **101** in solutions of various pHs and temperatures. Both products were recovered from the reaction mixtures in every case. The lack of reaction of the butadiene **101** indicated that it is not likely an intermediate in the oxidative coupling of methyl sinapate.

Various studies were carried out to determine what conditions of the reaction would favour the formation of the thomasidioate ester over that of butadiene **101**. The effect of a change in pH was studied first. The pHs chosen for the study were 8.3, 8.8 and 9.3 (TRIS buffered solutions). Methyl sinapate (**23**) (7.2 mM) was dissolved in methanol and an equal volume of the TRIS buffer was added. This solution was heated at 43±2 °C and bubbled with air presaturated with water and methanol. After 22 hours, the solution was diluted with water and acidified to a pH of 7 with 10% hydrochloric acid. The

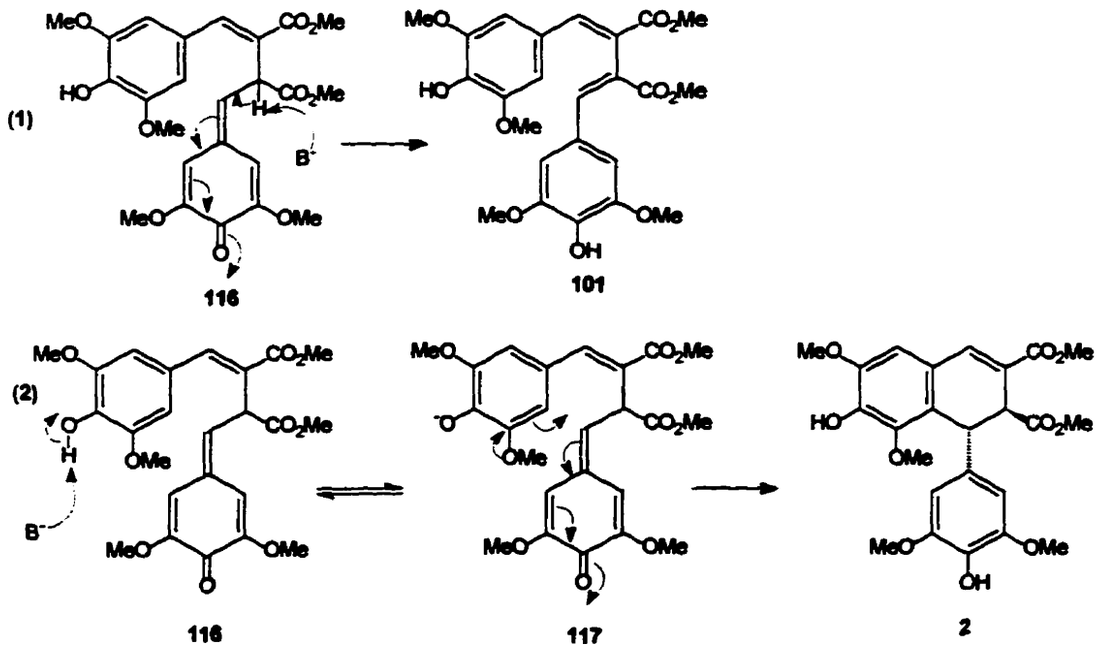
resulting solution was extracted with dichloromethane. The isolated products were analyzed by HPLC and the results are shown in Table 1. It was observed that the ratio of

pH	ratio of thomasidioiate (2) to butadiene (101)
8.3	1.29:1
8.8	2.67:1
9.3	3.34:1

Table. 1 The ratio of methyl thomasidioate to butadiene as measured by HPLC at pH values of 8.3, 8.5, and 9.3

dimethyl thomasidioate to the butadiene increased with the pH. This implied that the formation of the methyl ester of thomasidioic acid (**2**) was favored over that of the butadiene in more alkaline solutions. It is possible that there are two competing pathways for the reaction of the *mono*-quinone methide **116**. It could undergo tautomerization by removal of the α-proton to yield the butadiene **101** (reaction 1, Scheme 57). This

Scheme 57



deprotonation would be irreversible because the butadiene is more stable than the *mono*-quinone methide **116**. The *mono*-quinone methide could also undergo cyclization to form the aryltetralin **2**. The removal of the phenolic proton to yield the phenolate anion prior to this cyclization would make this cyclization more likely (reaction 2, Scheme 57). Reaction 1 of Scheme 57 is general base catalyzed and will depend on the type(s) of base(s) (and their concentration) in the solution. Reaction 2 is specific base catalyzed as the equilibrium formation of phenolate anion is dependent only on the pH of the solution. In the experiment, the buffer concentration was held roughly constant as the pH was varied. Therefore, the formation of dimethyl thomasidioate should be preferred over formation of the butadiene **101** at higher pH as observed. The increase in the ratio of dimethyl thomasidioate to butadiene was not substantial enough for this reaction to be synthetically useful.

The effect of changing the concentration of the buffer used for the oxidative coupling of methyl sinapate was also investigated. The original concentration of TRIS buffer used for the oxidation of methyl sinapate was 0.05 molar. The pH of the reaction solution used for this study was held at 9.3 while the concentration of the TRIS buffer was adjusted to 0.1, 0.2 and 0.5 molar. The general procedure was followed as for the previous study of pH. The product mixtures were analyzed by HPLC as before and the results are shown in Table 2. Although there is some scatter in the data the general trend is towards a higher yield of the butadiene **101** at higher buffer concentration. It is likely that an increase of concentration of the buffer above 0.2 molar increases the rate of removal of the α-proton thereby explaining the decrease in the ratio of dimethyl thomasidioate to butadiene diester **101** as observed.

Concentration of TRIS buffer	Ratio of thomasidioate **2** to butadiene ester **101**
0.05 M	3.34:1
0.1 M	4.17:1
0.2 M	3.22:1
0.5 M	2.00:1

Table 2 The ratio of dimethyl thomasidioate to butadiene with reaction in 0.05, 0.1, 0.2 and 0.5 molar TRIS buffer solution

The conversion of methyl sinapate to dimethyl thomasidioate was also attempted with other aerated buffers. Other buffers with a buffering range of 8.5 to 10.0 are: sodium tetraborate, $Na_2B_4O_7$/ HCl, potassium carbonate, K_2CO_3/ $KHCO_3$, glycine/ NaOH and ethanolamine/ HCl. The buffer solutions were made up at a concentration of 0.1 molar(first component) and the pH was adjusted to 9.5 (with the second component). The reaction of methyl sinapate was carried out as discussed previously, with methyl sinapate dissolved in methanol and an equal volume of the buffer added. Each of the reactions was worked up after stirring for 24 hours. It was initially anticipated that the substrate should have disappeared completely after this time. Analysis of each of the reaction solutions by HLPC (Table 3) showed that this assumption was incorrect (for comparison purposes the

Type of buffer used (0.1 M)	Ratio of thomasidioate **2**: butadiene **101**: methyl sinapate (**23**)
Borate	18: 8: 74
Carbonate	4: 32: 64
Ethanolamine	1: 14: 84
glycine	5: 45: 50
ammonium bicarbonate	1: 2: 0
TRIS	3: 1: 0

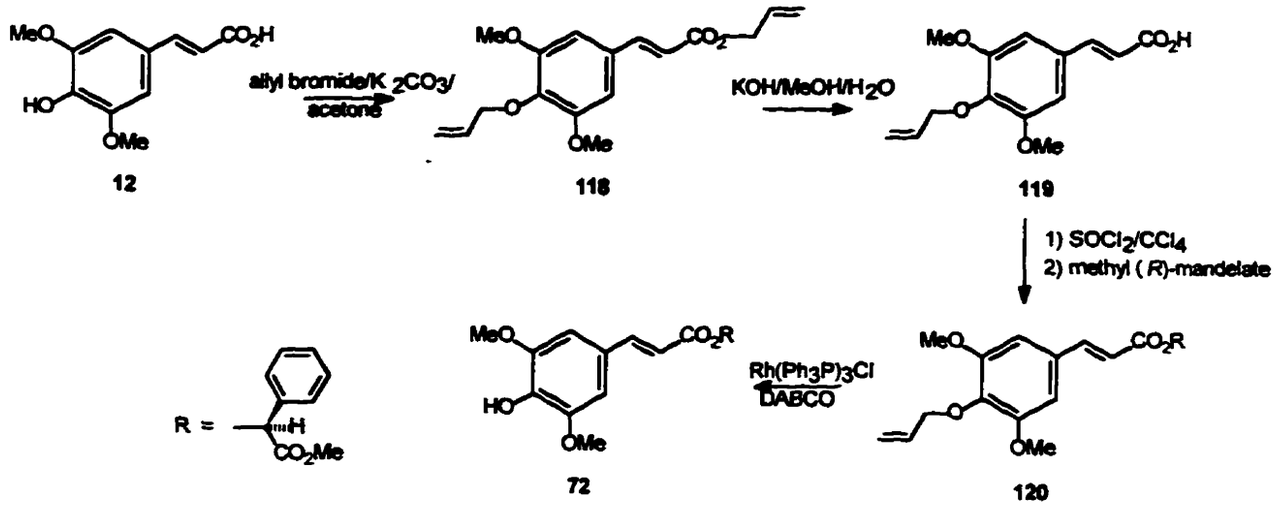
Table 3 Ratio of thomasidioate: butadiene:methyl sinapate obtained from reactions of methyl sinapate in various buffers (borate, carbonate, ethanolamine, glycine, TRIS, ammonium bicarbonate)

product ratios obtained in ammonium bicarbonate and TRIS buffer are also included in Table 3). The chromatographs for each of the reactions showed that a large portion of methyl sinapate (50 to 84 percent), remained unreacted in each solution unreacted. A comparison of the results indicates that only use of the borax and TRIS buffers favoured the formation of the dimethyl thomasidioate over the butadiene diester. The other reactions produced an excess of the butadiene in comparison to dimethyl thomasidioate (**2**). From these results, it is quite clear that oxidative coupling of methyl sinapate in basic buffer is quite dependent on the type of buffer used for the oxidation. It would be quite difficult to postulate a reason for the difference in results because there seems to be no consistent pattern. For example, there were four different amine-based buffers used. These were ethanolamine, glycine, TRIS and ammonium bicarbonate buffers. When comparing the results for these buffers, it was observed that the formation of dimethyl thomasidioate (**2**) was favoured over that of the butadiene diester **101** only when the TRIS buffer was used. The formation of the butadiene diester **101** was favoured when using the other three buffers. The reaction would also only go to completion when using TRIS and ammonium bicarbonate buffers for the oxidation. The reason for these differing results is unknown.

Even though conditions could not be found that would result in the exclusive conversion of methyl sinapate (**23**) to dimethyl thomasidioate (**2**), a similar reaction was attempted on a chiral ester of sinapic acid. The aim of this study was to determine if the chiral ester group could direct the coupling to give the thomasidioate diester diastereoselectively. (Methyl (*R*)-mandelyl) sinapate was chosen for this study. The

starting material **72** was synthesized by the esterification of the allyl-protected sinapic acid **119** with methyl (*R*)-mandelate, followed by deprotection of **120** (Scheme 58).

Scheme 58



Sinapic acid (0.03 M), with an excess of both allyl bromide and potassium carbonate, was refluxed in acetone for 24 hours. The reaction was worked up by dilution with water and extraction with dichloromethane. The allyl ester **118** was confirmed as the product by comparison of the ^{1}H NMR spectrum with that of the starting material. The hydrolysis of the allyl ester **118** was carried out in basic solution. The starting material disappeared after only 5 hours of stirring at room temperature. The acidified solution was extracted with ethyl acetate. The ^{1}H NMR spectrum of the product showed only one set of peaks representing an allyl group, indicating that **119** had been produced.

The next step in the synthesis was the esterification of acid **119**. The acid chloride was prepared by refluxing a solution of **119** (0.05 M) in carbon tetrachloride with an excess of thionyl chloride (3.0 equiv) under nitrogen for 18 hours. The product was isolated by evaporation of the solvents. The yellow solid residue was redissolved in

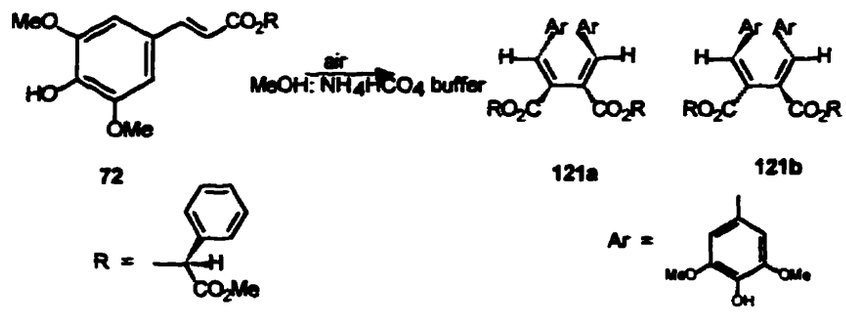
carbon tetrachloride and a slight excess of methyl (*R*)-mandelate (1.3 equiv) was added. This solution was refluxed under nitrogen. TLC indicated that all of the acid chloride had disappeared after 25 hours of refluxing. The solvent was removed by evaporation under vacuum to yield a light yellow solid. The proton NMR of the product showed that it was the ester **120**.

The final step in the synthesis was the deprotection of the phenolic group. This was carried out using chlorotris(triphenylphosphine) rhodium and DABCO. Compound **120**, the rhodium catalyst, and DABCO were refluxed in a mixture of ethyl alcohol, benzene and water in a ratio of 7: 3: 1, under nitrogen. TLC indicated that all of the substrate had disappeared after 7 hours of reaction. Water and 10% hydrochloric acid were added and the solution was extracted with dichloromethane. The ^{1}H NMR spectrum of the crude product showed the absence of any allyl signals. A large number of intense signals in the aromatic region also indicated that triphenyl phosphine was present. Flash chromatography of the mixture was carried out to obtain pure (methyl (*R*)-mandelyl) sinapate **72** in 36% yield.

(Methyl (*R*)-mandelyl) sinapate (**72**) (0.01 M) was dissolved in methanol and an equal volume of ammonium bicarbonate buffer (pH 8.5) was added. The yellow solution was stirred at room temperature for 46 hours at which time TLC indicated that all of the starting material had disappeared. The reaction solution was diluted with water, acidified with 10% hydrochloric acid and extracted with dichloromethane. A comparison of the proton NMR spectrum of the crude product was made to that of dimethyl thomasidioate (**2**). If the aryltetralin lignan had been produced, then these spectra should have been similar. None of the signals of the spectrum for the thomasidioate ester were observed in

the spectrum of the product. It was believed that this reaction did not produce any of the desired aryltetralin product. The spectrum was then compared to that of the diarylbutadiene dicarboxylate ester **101**. At the chemical shift of the peaks representing the vinylic and aromatic protons, two sets of peaks were observed in the NMR spectrum of the unknown product in the same region. Attempted separation of these compounds by flash chromatography was unsuccessful. Although the product was not fully characterized, it was quite possible that the products of the reaction were two isomers of a butadiene diester similar to **101**. If the stereochemistry about both double bonds was *trans*, then the two isomers that formed could have been the atropisomers **121a** and **121b** (Scheme 59). These isomers could be present due to hindered rotation about the central bond of the butadiene. The two isomers are diastereomers of each other and will each have a unique ^{1}H NMR spectrum. The inability to separate these two compounds by flash chromatography could be explained if they are in slow equilibrium. The oxidative coupling of the chiral ester **72** in aerated basic buffer did not produce any of the desired aryltetralin lignan. In view of these results no further research in this area was carried out.

Scheme 59



The final study carried out was the search for another oxidizing agent that would oxidize methyl sinapate and improve the yield of the thomasidioate ester. Other single-

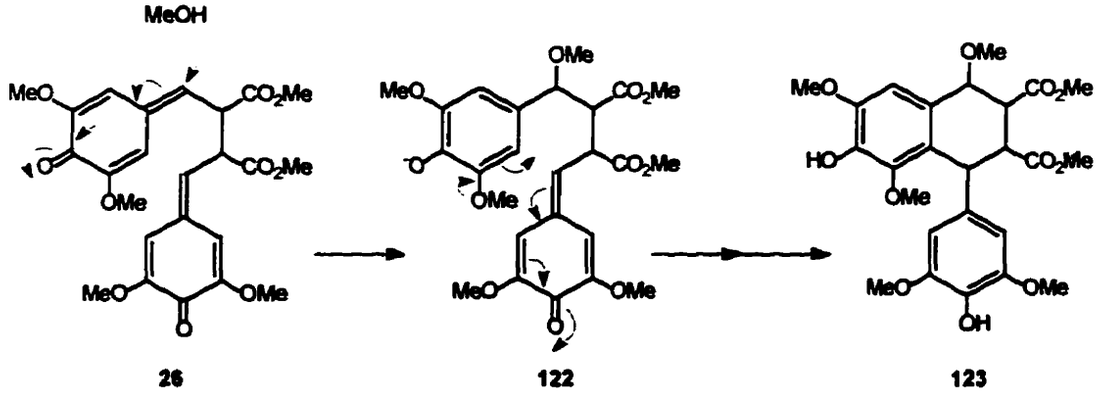
electron oxidants that were tried included triiodide ion, ferricyanide ion, DDQ, and benzoyl peroxide.

The first oxidation reaction was carried out using the triiodide ion. Methyl sinapate (0.04 M) was dissolved in methanol and deaerated ammonium bicarbonate buffer and KI_3 solution (0.02 M) was added. The red solution was stirred under nitrogen at room temperature for 48 hours. The acidified solution was extracted with dichloromethane. The proton NMR spectrum of the isolated products showed that a 1:2 mixture of the dimethyl ester of thomasidioic acid and the butadiene **101** had been produced. The formation of the mixture of the two products for this reaction showed that the triiodide ion was no more selective than oxygen in oxidizing methyl sinapate to dimethyl thomasidioate. The reaction of methyl sinapate with the ferricyanide ion was carried out using a procedure similar to that used for triiodide ion. This reaction also produced the two compounds with a similar ratio.

The oxidative coupling of methyl sinapate was also carried out using the oxidant benzoyl peroxide with DMF as the solvent. Methyl sinapate (0.06 M) was dissolved in dimethylformamide under nitrogen at room temperature. Sodium hydride (0.09 M) dissolved in DMF (1mL) was added dropwise. The solution was cooled and stirred for one hour. Benzoyl peroxide (0.06 M) was dissolved in DMF and added dropwise to this solution. The solution was then allowed to warm up to room temperature and stirred overnight. The product was isolated by extraction of the acidified solution. A comparison of the proton NMR spectrum of the crude product to that of the butadiene **101** showed that this was the product.

The final oxidative coupling reaction of methyl sinapate was carried out using DDQ as the oxidant and methanol as the solvent. Two reactions were performed simultaneously. One reaction was carried out using one molar equivalent of DDQ and the other using one-half molar equivalent of DDQ. The proton NMR spectra of the products of these reactions did not resemble that of either **101** or **2**. Previous studies of the oxidative coupling of methyl sinapate in methanol showed that methanol can act as a nucleophile and attack the benzylic carbon of the *bis*-quinone methide **26**.[30] If this happens, subsequent cyclization of **122** can lead to a 4-methoxyaryltetralin product which, in the case of methyl sinapate, would give **123** (as a mixture of diastereomers) (see Scheme 60). Heating this mixture of diastereomers with a catalytic amount of acid should

Scheme 60

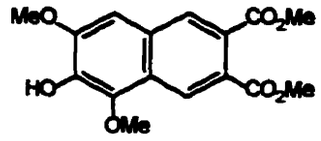


26 **122** **123**

lead to elimination of the methoxyl group and formation of dimethyl thomasidioate. To test whether the methoxylated product **123** had formed during DDQ oxidation of methyl sinapate in methanol, the products from both of the reactions described above were each dissolved in benzene with a catalytic amount of *para*-toluenesulfonic acid. Each solution was refluxed for 20 hours and then the solvent was removed to yield the final product. Only the dimethyl ester of thomasidioic acid was isolated from the reaction carried out

using one-half an equivalent of DDQ. The identity of intermediate **123** could not be confirmed because its isolation was difficult, although others have observed the formation of a similar intermediate. For example, Setälä *et al.* studied the oxidative coupling of methyl sinapate by hydrogen peroxide using horseradish peroxidase as a catalyst.[30] They mentioned that methanol can act as an nucleophile, even in aqueous solutions. As discussed in the introduction (see page 15), they isolated spiro-compounds, **28a** and **28b**, formed by the nucleophilic attack of methanol at the benzylic carbon of one of the quinone methides.

The proton NMR spectrum for the reaction carried out using one molar equivalent of DDQ, followed by acid catalyzed elimination, showed that another product was obtained in addition to dimethyl thomasidioate. The unknown product was separated from the product mixture by flash chromatography with an overall yield of 44%. The proton NMR spectrum of the compound showed four 3-proton singlets at about 4 ppm which likely represented methoxy or methyl ester groups on the molecule. There were also four one-proton singlets between 6 and 8.5 ppm. The lack of a 6-proton singlet at about 4 ppm and a 2-proton singlet at about 7 ppm showed that the molecule lacked a free aromatic ring similar to that of thomasidioic acid. Considering the structure of the starting material, it seemed likely that the product would have a similar placement of two methoxyl groups and one phenolic group on an aromatic ring. The phenolic proton could account for the singlet at about 6 ppm. The remaining protons fell between 7 and 8.5 ppm, which indicated that these protons were probably all aromatic. One possible structure for the product which would take into account all of this information is compound **124**, as shown.



124

The molecular mass of the reaction product was 320 which corresponds to the elemental composition for compound **124**. Although the exact mechanism for the formation of **124** is unknown, it is probable that the use of one full equivalent of DDQ led to oxidation of the first formed product **123**, and eventual formation of the naphthalene **124**.

Chapter 3

Conclusion

All of the objectives outlined at the beginning of this thesis have been achieved.

A detailed study of the oxidative coupling of *para*-hydroxycinnamic acids in aerated basic buffer was carried out. It was determined that the preparative synthesis of thomasidioic acid by oxidative coupling of sinapic acid under these conditions was possible. The yield of purified thomasidioic acid was 41%. Another product other than thomasidioic acid was obtained on the treatment of sinapic acid with aerated strong base. This product was determined to be 6-hydroxy-5,7-dimethoxy-2-naphthoic acid **75**. The mechanism for the conversion of sinapic acid to the 2-naphthoic acid **75** was shown to involve the secondary oxidation of the first formed thomasidioic acid. It was proposed that both thomasidioic and 2-naphthoic acid **75** may not be natural products since they could have been formed during alkaline extraction of the wood of the elm tree *Ulmus thomasii* Sarg.[21] This would explain why thomasidioic acid was obtained as a racemic mixture. Other *para*-hydroxycinnamic acids were reacted in aerated basic buffer in attempts to form the respective aryltetralins. These reactions did not cleanly produce aryltetralins, giving instead a mixture of products or no reaction at all. Other single-electron oxidants, such as the triiodide and ferricyanide ions, were also found to be good reagents for the conversion of sinapic acid to thomasidioic acid.

The mechanism for the conversion of sinapic acid to thomasidioic acid on oxidation in aerated basic buffer was also studied. It was determined that oxygen was being reduced to hydrogen peroxide during this reaction and that hydrogen peroxide was

not acting as an oxidizing agent towards sinapic acid in this process. The lack of cross-coupling between sinapic acid and other species, such as 3,4,5-trimethoxycinnamic acid and ferulic acid, in aerated basic buffer helped to establish that the dimerization of sinapyl radicals was the most likely pathway for oxidation of sinapic acid. The product of this dimerization would be the *bis*-quinone methide **88**. It was determined to be a likely intermediate on the pathway to thomasidioic acid by showing that another substrate (dilactone **16**) which can produce the *bis*-quinone methide also produces thomasidioic acid. The intermediacy of a diarylbutadiene diacid derivative was eliminated by specifically preparing the compound and showing that it was not transformed into thomasidioic acid under the reaction conditions. The most likely mechanism for the oxidative coupling of sinapic acid was determined to involve the partial tautomerization of *bis*-quinone methide **88** to form a *mono*-quinone methide **99**, and the cyclization of **99** to yield thomasidioic acid.

A study of the oxidative coupling of esters of sinapic acid was also carried out. It was determined that the reaction of methyl sinapate in aerated basic buffer produced two products. One of these products was the expected dimethyl thomasidioate and the other product was the diarylbutadiene diester **101**. The conditions for the oxidative coupling of methyl sinapate were altered in an attempt to find conditions that would favour the formation of dimethyl thomasidioate over the formation of the diarylbutadiene diester **101**. From the HPLC analysis of the products for the various reactions, it was determined that an increase in pH and a decrease in the concentration of buffer favoured the formation of dimethyl thomasidioate. However, all of the reactions still produced a significant amount of the butadiene **101**. An attempt to couple (methyl (*R*)-mandelyl) sinapate (**72**) was not

successful as the reaction did not yield any of the thomasidioate ester. It was believed that two atropisomers of the diarylbutadiene diester **121** were formed.

Experimental

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AM-300 or AMX-500 instrument using tetramethylsilane as internal standard. IR spectra were recorded on a Perkin Elmer 881 spectrometer. Aldrich silica gel (28,859-4) was used for all chromatography. HRMS/ mass spectra were obtained on an VG Analytical 7070E-HF instrument. Melting points were measured on a hot stage instrument and are uncorrected. High pressure liquid chromatography was performed on a Varian 9010 Solvent Delivery instrument on a C-18 reverse phase column with detection by a Varian 9050 Variable Wavelength UV-Vis Detector. Tetrahydrofuran (THF) was distilled under nitrogen from sodium and benzophenone. "Room temperature" during these experiments was the temperature range of 23°C to 26°C.

Scope of Reaction

Thomasidioic acid (1)

An ammonium bicarbonate buffer (0.287 M, pH 8.5) was made up by dissolving ammonium bicarbonate (2.27 g, 28.7 mmol) in distilled water (100 mL). Concentrated ammonium hydroxide was added to this solution to raise the pH to 8.5. Sinapic acid (500 mg, 2.23 mmol) was dissolved in the buffer (60 mL) and stirred at rt in presence of air for 24 h. The dark brown solution was acidified to pH 2 with HCl (10 %) and saturated with NaCl. It was extracted with ethyl acetate (3 x 25 mL). The organic layers were combined together, dried with $MgSO_4$, and evaporated under reduced pressure to give a dark brown solid. Chromatography of the solid on silica gel with 5:75:20 acetic acid/ethyl acetate/hexanes gave a colourless solid, 1 (205 mg, 0.46 mmol, 41% yield): mp 209-212 °C (dec); IR (Nujol) 1710 (CO), 1686 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.50 (s, 3H), 3.61 (s, 6H), 3.75 (d, J=1.1, 1H), 3.84 (s, 3H), 4.82 (s, 1H), 6.21 (s, 1H), 6.98 (s, 1H), 7.55 (s, 1H), 8.17 (s, 1H), 9.13 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 38.8 (CH), 46.2 (CH), 55.9 ($3CH_3$), 59.6 (CH_3), 104.9 (2CH), 108.3 (CH), 122.5 (C), 123.2 (C), 123.4 (C), 133.1 (C), 134.2 (C), 136.2 (CH), 141.3 (C), 145.3 (C), 147.5 (3C), 167.7 (CO), 172.9 (CO); ^{1}H

NMR, ^{13}C NMR and melting point data were identical to that found for thomasidioic acid in the literature.[13]

6-Hydroxy-5,7-dimethoxy-2-naphthoic acid (75)

Reaction of sinapic acid in 0.1 N KOH at room temperature

Sinapic acid (52.8 mg, 0.240 mmol) was dissolved in aqueous KOH (0.1 N, 10 mL) and stirred at rt for 20 h. The deep red solution was diluted with distilled water (40 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure, giving a colourless solid. The aqueous solution was acidified with HCl (10%) to pH 2 and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated to give a light brown solid. Chromatography of the solid on silica gel with 5:75:20 acetic acid:ethyl acetate:hexanes gave light pink crystals (23.6 mg, 0.0951 mmol, 72% yield): mp 224-227°C; IR (CH_2Cl_2) 3507 (OH), 1681 (CO) cm^{-1}; ^1H NMR (acetone-d_6) δ 4.00 (s, 3H), 4.02 (s, 3H), 7.31 (s,1H), 7.93 (dd, *J*=1.5, 8.7, 1H), 8.01 (d, *J*=8.7, 1H), 8.49 (d, *J*=1.5); ^{13}C NMR (acetone-d_6) δ 56.4 (CH_3), 60.9 (CH_3), 104.1 (CH), 121.5 (CH), 124.2 (CH), 126.6 (C), 127.8 (C), 128.2 (C), 130.5 (CH), 141.0 (C), 141.2 (C), 150.8 (C), 168.0 (CO); MS *m/z* (rel. %) 248 (M+, 100), 233 (86), 201 (47); HRMS calcd. for $C_{13}H_{12}O_5$ 248.0685, found 248.0657. ^1H NMR spectral data were consistent with those found in the literature.[97]

Reaction of sinapic acid in 0.1 N KOH at 60° C

Sinapic acid (59.0 mg, 0.263 mmol) was dissolved in aqueous KOH (0.1 N, 20 mL) and stirred at 60° C with exposure to air for 6 h. The deep red solution was acidified with 10% aqueous HCl to pH 2 and then extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated to give a light brown solid. This solid was chromatographed on silica gel with 5:20:75 acetic acid/hexanes/ethyl acetate to yield a tan solid (23.6 mg, 0.100 mmol, 72% yield). Spectral data of this product was identical to that of 2-naphthoic acid 75.

Reaction of thomasidioic acid in 0.1 N KOH at 60°C

Thomasidioic acid (53.0 mg, 0.119 mmol) was dissolved in aqueous KOH (0.1 N, 10 mL) and stirred at 60 °C, with exposure to air, for 6 h. The deep red solution was diluted with distilled water (40 mL) and extracted with ethyl acetate (3 x 10 mL). The aqueous solution was acidified with HCl (10%) and extracted with ethyl acetate (3 x 10 mL). The washings were combined, dried with $MgSO_4$ and evaporated under reduced pressure, giving a light brown solid. This solid was chromatographed on silica gel (5:20:75 acetic acid/hexanes/ethyl acetate) to yield tan crystals (23.6 mg, 0.100 mmol, 80% yield). Spectral data were consistent with 6-hydroxy-5,7-dimethoxy-2-naphthoic acid (**75**).

Reaction of thomasidioic acid in 0.1 N KOH under nitrogen

Thomasidioic acid (20.4 mg, 0.0460 mmol) was dissolved in KOH solution (0.1 N, 5 mL) that was previously deaerated by bubbling with nitrogen for 15 minutes. The solution was stirred under nitrogen at 60 °C for six hours. The clear yellow solution was diluted with distilled water (40 mL) and acidified with HCl (10%). This solution was extracted with EtOAc (3 x 10 mL). The washings were combined, dried with $MgSO_4$ and evaporated under reduced pressure, giving a colourless solid. The aqueous solution was acidified with HCl (10%) to pH 2 and then extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated to give an off-white solid (8.2 mg). The ^1H NMR spectra was identical to that of thomasidioic acid (**1**), which showed that none of starting material had reacted.

2,6-Dimethoxy-*p*-benzoquinone (80)

Chromium trioxide (317 mg, 3.17 mmol) was dissolved in water (1 mL) and acetic acid (9 mL) was added. 2,6-Dimethoxyphenol (103 mg, 0.668 mmol) was added and the solution stirred at room temperature for 90 min. Water (15 mL) was added and then the solution extracted with CH_2Cl_2 (3 x 10 mL). The organic layers were combined and washed with sodium bisulfite

solution (5%, 2 x 10 mL) and water (2 x 10 mL). The solution was dried with $MgSO_4$ and evaporated under pressure to yield **80** as a bright yellow solid. The solid was filtered through silica gel with ethyl acetate to give a yellow solid (60.7 mg, 0.361 mmol, 55% yield): mp 255-257°C, IR (NUJOL) 1698 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.82 (s, 6H), 5.85 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 56.4 ($2CH_3$), 107.3 (2CH), 157.6 (2C), 186.6 (2CO); MS *m/z* (rel.%): 168 (M+), 138 (19), 80 (40), 69 (100); HRMS calcd. for $C_8H_8O_4$ 168.0423, found 168.0425.

Reaction of sinapic acid in 0.1 N KOH, detection of 2,6-dimethoxy-*p*-benzoquinone (80)

Sinapic acid (52.5 mg, 0.234 mmol) was dissolved in aqueous KOH (0.1 N, 15 mL) and stirred under air at 60°C for 6 hours. The deep red solution was extracted with ethyl acetate (3 x 5 mL). The washings were combined, dried with $MgSO_4$ and evaporated under reduced pressure, giving a colourless solid (0.5 mg). The aqueous solution was acidified with HCl (10%) to pH 2 and then extracted with ethyl acetate (3 x 5 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated to give a light brown solid (29.1 mg). The first solid was chromatographed on HPLC (80:20 water:methanol). A peak with an elution time (7.3 min.) identical to that of pure 2,6-dimethoxy-*p*-benzoquinone (**80**) was observed.

Study of the mechanism of oxidative coupling of sinapic acid

Oxygen uptake measurement

Nitrogen was passed through NH_4HCO_3 buffer (0.287 M, pH 8.7) for 15 minutes. Sinapic acid (406 mg, 0.181 mmol) and *para*-toluic acid (251 mg, 0.184 mmol) were dissolved in the deaerated NH_4HCO_3 buffer (0.287 M, 9 mL, pH 8.7) under nitrogen and the flask sealed with a rubber septum. A barometer and syringe were inserted into the septum. Oxygen was gently passed through solution for approximately 20 seconds and then the syringe filled with oxygen. As the reaction proceeded, the syringe plunger was lowered to keep the pressure of the system at atmospheric pressure. After 45 minutes of reaction, the total amount of oxygen consumed (volume displaced from the syringe) was measured and the solution acidified with conc. HCl (0.3

The solution was analysed by HPLC (78:22 0.6% TFA in water/acetonitrile). In order to calculate the ratio of sinapic acid to toluic acid in the reaction solution, a calibration curve was constructed based on HPLC analysis of solutions of known ratios of sinapic acid and *para*-toluic acid. A calibration constant of 1.376 was calculated from the slope of this plot. The ratio of oxygen to sinapic acid reacted was determined to be 0.42: 1. The temperature during the reaction was 299°K and the pressure was 1.003 bars.

Detection of hydrogen peroxide:

Nitrogen was passed through NH_4HCO_3 buffer (0.287 M, pH 8.7) for 15 minutes in order to deaerate the solution. Sinapic acid (48.7 mg, 0.217 mmol) was dissolved in the buffer (7 mL, 0.287 M, pH 8.7). Oxygen was gently passed through the solution for 20 seconds. The yellow solution was stirred at room temperature. Samples of the reaction solution were taken every 15 minutes. The samples were acidified with HCl (10%) and tested with potassium iodide-starch paper. The fourth sample (after 1 hour of reaction) turned the indicator paper purplish-blue.

Methyl 3,4,5-trimethoxycinnamate (92)

Sinapic acid (310 mg, 1.38 mmol) and anhydrous K_2CO_3 (303 mg, 5.39 mmol) were taken up in acetone (10 mL). Iodomethane (0.22 mL, 3.5 mmol) was added to the mixture and it was refluxed for 23 hours. Water (15 mL) was added to the reaction mixture and it was extracted with CH_2Cl_2 (3 x 5 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure, giving a yellow solid, **92** (176 mg, 0.698 mmol, 51% yield): mp 112-115°C; IR (CH_2Cl_2) 1721 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.81 (s, 3H), 3.88 (s, 9H), 6.35 (d, J=16.0, 1H), 6.75 (s, 2H), 7.61 (d, J=16.0, 1H); ^{13}C NMR ($CDCl_3$) δ 51.6 (CH_3), 56.1 ($2CH_3$), 60.9 (CH_3), 105.2 (2CH), 117.0 (CH), 129.8 (C), 140.1 (C), 144.8 (CH), 153.4 (2C), 167.3 (CO); MS *m/z* (rel.%): 252 (M+, 100), 237 (59), 57 (51), 55 (37); HRMS calcd for $C_{13}H_{16}O_5$ 252.0998, found 252.1002.

3,4,5-Trimethoxycinnamic acid (89)

Methyl 3,4,5-trimethoxycinnamate (851 mg, 3.37 mmol) and KOH (2.92 g, 0.0521 mol) was dissolved in a water/MeOH (1:9, 25 mL) solution and stirred at room temperature for 5 hours. The solution was acidified with HCl (10%) and diluted with water (25 mL). It was extracted with CH_2Cl_2 (3 x 10 mL) and the organic layers were combined, dried with $MgSO_4$, and evaporated under reduced pressure to give a light brown solid. The product was filtered through silica gel that had been treated with 5:95 acetic acid/ethyl acetate to give **89** as a light yellow solid (490 mg, 2.06 mmol, 61% yield): mp 126-128°C; IR (CH_2Cl_2): 1690 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.90 (s, 9H), 6.36 (d, *J*=15.9, 1H), 6.78 (s, 2H), 7.71 (d, *J*=15.9, 1H); ^{13}C NMR ($CDCl_3$) δ 56.1 ($2CH_3$), 60.9 (CH_3), 105.5 (2CH), 116.4(CH), 129.4 (C), 140.5 (C), 146.9 (CH), 153.4 (2C), 172.3 (CO); MS *m/z* (rel. %) 238 (M+, 100), 223 (65); HRMS calcd. for $C_{12}H_{14}O_5$ 238.0841, found 238.0837.

Attempted cross coupling of 3,4,5-trimethoxycinnamic acid (89) with sinapic acid

Sinapic acid (26.5 mg, 0.118 mmol) and 3,4,5-trimethoxycinnamic acid (25.7 mg, 0.108 mmol) were dissolved in NH_4HCO_3 buffer (0.27 M, pH 8.5, 35 mL) and stirred open to the air for 20 h. The light brown solution was acidified with HCl (10%) and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to yield a brown solid (50.5 mg). The ^1H NMR spectrum was consistent with that for a mixture of thomasidioic acid and 3,4,5-trimethoxycinnamic acid.

Attempted cross coupling of ferulic acid with sinapic acid

Ferulic acid (50.2 mg, 0.260 mmol) and sinapic acid (50.2 mg, 0.224 mmol) were dissolved in NH_4HCO_3 buffer (0.287 M, pH 8.7, 7 mL). The solution was stirred open to the air at room temperature for 2 days. Acidification of the solution by HCl solution (10%) and extraction with ethyl acetate (3 x 10 mL) was carried out. The organic layers were combined and dried with

$MgSO_4$. The solvent was evaporated to yield a brown solid (84.1 mg). ^1H NMR spectrum of the product was consistent with that for a mixture of ferulic and thomasidioic acid.

1,4-(3,5-dimethoxy-4-hydroxyphenyl)-1,3-butadiene-2,3-dicarboxylic acid (98)

The methyl ester of title butadiene diacid (see below for preparation) (67.2 mg, 0.142 mmol) was refluxed in dilute sulfuric acid (0.5 M, 10 mL) for seven days. The dark solution was made basic with $NaHCO_3$ solution (10%) and washed with CH_2Cl_2 (3 x 5 mL). The aqueous solution was acidified to pH 2 with HCl (10%) and extracted with ethyl acetate (3 x 5 mL). The organic layers were combined, dried with $MgSO_4$, and evaporated to dryness under vacuum, to give a dark solid, **98** (48.3 mg): ^1H NMR (acetone-d_6) δ 3.76 (s, 12H), 7.02 (s, 4H), 7.87 (s, 2H); ^{13}C NMR (acetone-d_6) δ 56.5 ($4CH_3$), 108.6 (4CH), 126.1 (2C), 126.5 (4C), 138.6 (2C), 142.7 (2CH), 148.4 (4C), 168.3 (2CO).

Reaction of diacid 98 in NH_4HCO_3 buffer

Butadiene diacid **98** (4 mg, 0.01 mmol) and *para*-toluic acid (5 mg, 0.03 mmol) were dissolved in acetonitrile (0.2 mL) and water (0.2 mL). This solution was divided into two parts. The first part was analysed by HPLC (80:20 0.06% trifluoroacetic acid in water/acetonitrile). The ratio of thomasidioic acid/butadiene diacid **98**/*para*-toluic acid was 0.28:14.67:1. Ammonium bicarbonate buffer (8.0 mL) was added to the second part. This solution was stirred open to the air for 24 hours. It was acidified with hydrochloric acid (10%) and acetonitrile (1 mL) added. HPLC analysis of the mixture (80:20 0.06% TFA in water/acetonitrile) gave a ratio of thomasidioic acid/toluic acid of 0.22:1.

Reaction of sinapic acid in NH_4HCO_3 buffer in D_2O

In a small vial, ammonium bicarbonate (45 mg, 0.57 mmol) was dissolved in D_2O (2 mL) and 2 microdrops of concentrated ammonium hydroxide was added to provide a pH of 8.5. Sinapic acid (19.5 mg, 0.0870 mmol) was dissolved in the buffer and allowed to stir open to the air for 2 days.

The solution was diluted to 5 mL with distilled water and acidified with HCl (10%). It was extracted with ethyl acetate (3 x 10 mL) and the organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to give a reddish-brown solid (13.7 mg). ^1H NMR spectrum of the product was identical in all respects to that of thomasidioic acid.

Dehydrodisinapic acid dilactone (16)

Sinapic acid (481 mg, 2.15 mmol) was dissolved in MeOH (9 mL). Ferric chloride (1.075 g, 6.600 mmol) dissolved in distilled water (40 mL) was added to this solution over 10 minutes, while oxygen was being passed through it. Oxygen was passed through the purple solution for 5 hours and then it was left to stand overnight. The mixture was filtered and the paste was suspended in water (120 mL). This suspension was heated on the stream bath for 15 min. The mixture was acidified with water/ H_2SO_4 (1:1, 12 mL) and the resulting solution was shaken for 10 min. The mixture was cooled on ice and filtered. The solid was washed with water, methanol and ether to yield a light pink solid (151 mg, 0.338 mmol, 32% yield): mp 227-235°C; IR(CH_2Cl_2) 3520 (OH), 1781 (CO) cm^{-1}; ^1H NMR (Acetone-d_6) δ 3.84 (s, 12H), 4.11 (s, 2H), 5.76 (s, 2H), 6.74 (s, 4H), 7.45 (s, 2H); ^{13}C NMR (Acetone-d_6) δ 49.0 (2CH), 56.8 ($4CH_3$) 83.3 (2CH), 104.3 (4CH), 129.8 (2C), 137.5 (2C), 149.0 (4C), 179.2 (CO) ; MS *m/z* (rel. %): 446 (M+, 1), 149 (52), 71 (59), 57 (100); HRMS calcd. for $C_{22}H_{22}O_{10}$ 446.1213, found 446.1226.

Reaction of dilactone 16 in NH_4HCO_3 buffer

The dilactone (51.2 mg, 0.11 mmol) was dissolved in NH_4HCO_3/NH_4OH buffer (0.287 M, pH 9.0, 25 mL) and stirred at room temperature for 20 h. The reaction solution was acidified to pH 2 with HCl (10%) and extracted with ethyl acetate (3 x 10 mL). The organic extracts were combined, dried with $MgSO_4$, and evaporated to dryness to give a brown solid (29.6 mg). The ^1H NMR spectrum of this material was identical to that of thomasidioic acid.

Reaction of dilactone 16 in NH_4HCO_3 buffer under nitrogen

NH_4HCO_3 buffer (0.287 M, pH 9.0) was deaerated by stirring the solution under vacuum (*ca.* 150 mm) for one h. Dehydrodisinapic acid dilactone (12.4 mg, 0.0278 mmol) was dissolved in the buffer (50 mL). The orange solution was stirred under nitrogen at rt for 20 h. The solution was acidified with hydrochloric acid (10%) and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$, and evaporated under reduced pressure to give a light brown solid (6.2 mg). The ^1H NMR spectrum was identical to that found for thomasidioic acid (see above).

Reaction of dilactone 16 in NH_4HCO_3 buffer in D_2O

Dehydrodisinapic acid dilactone (13.5 mg, 0.0302 mmol) was dissolved in NH_4HCO_3 buffer in D_2O (0.287 M, pH 8.7, 25 mL). The yellow solution was bubbled with air while being stirred at rt. After 24 h, TLC indicated that the reaction was complete. The solution was acidified with HCl (10%) and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to give a light brown solid (9.2 mg). ^1H NMR spectral data was identical in all respects to that found for thomasidioic acid.

Reaction of dilactone 16 with 1 equivalent of LDA

LDA (0.016 mL, 0.12 mmol) was stirred in freshly distilled THF (1 mL) at -78 °C under nitrogen. BuLi (2.5 M in hexanes, 0.05 mL, 0.12 mmol) was added dropwise and the solution was stirred for 10 min. Dehydrodisinapic acid dilactone (51.5 mg, 0.120 mmol) was dissolved in dry THF (3 mL) and added dropwise to the solution followed by stirring at -78 °C for 30 min. The solution was allowed to warm up to rt and stirring was continued for 21 h. It was acidified with HCl (10%) and diluted with water (5 mL). The solution was extracted with ethyl acetate (3 x 10 mL) and the organic layers were combined, dried with $MgSO_4$, and evaporated under reduced pressure to leave a reddish brown solid: mp 133-135°C; IR (CH_2Cl_2) 3519 (OH), 1714 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.84 (s, 12H), 4.13 (m, 1H), 5.67 (d, J=2.8, 1H), 6.51 (s, 2H), 6.78 (s, 2H),

7.68 (d, J=1.9, 1H); ^{13}C NMR ($CDCl_3$) δ 53.5 (CH), 56.4 ($2CH_3$), 56.5 ($2CH_3$), 80.4 (CH), 101.9 (2CH), 107.7 (2CH), 118.2 (C), 124.5 (C), 130.3 (C), 135.2(C), 137.7 (C), 141.8(CH), 147.2 ($2CH_3$), 147.4 ($2CH_3$), 171.2 (CO), 173.5 (CO); MS m/z (rel. %): 182 (52), 149 (26), 73 (100)

Reaction of sinapic acid with potassium triiodide

Nitrogen was passed through NH_4HCO_3/NH_4OH buffer (0.287 M, pH 8.7) for 20 minutes in order to deaerate the solution. A KI_3 solution (0.058 M) was treated similarly. Sinapic acid (50.4 mg, 0.225 mmol) was dissolved in the NH_4HCO_3 buffer (5 mL), then the KI_3 solution (4 mL, 0.058 M) was added. The dark red solution was stirred at rt under nitrogen for 19 h. The solution was acidified with HCl (10%) and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$, and evaporated under reduced pressure to leave a reddish-brown solid. Chromatography of the solid on silica gel with 5: 20: 75 acetic acid: hexanes: ethyl acetate gave a light tan solid (16.6 mg, 0.0372 mmol, 33% yield). ^1H NMR spectral data of this product was identical to that found for thomasidioic acid.

Reaction of sinapic acid with $K_3Fe(CN)_6$

Sinapic acid (25.7 mg, 0.115 mmol) and $K_3Fe(CN)_6$ (35.5 mg, 0.110 mmol) were dissolved in deaerated NH_4HCO_3/NH_4OH buffer (0.287 M, 7 mL, pH 9.0). The red solution was stirred under nitrogen for 20 h. The dark brown solution was acidified with HCl (10%) and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated to dryness under reduced pressure, giving a light brown solid. This product was chromatographed on silica gel with 5:25:75 acetic acid/hexanes/ethyl acetate to give an off-white solid (8.8 mg, 0.020 mmol, 36% yield). The ^1H NMR spectrum was identical to that of thomasidioic acid.

Reaction of sinapic acid with DDQ in THF

Sinapic acid (27.1 mg, 0.121 mmol) and DDQ (16.4 mg, 0.0722 mmol) were dissolved in dry THF (7 mL) and stirred under nitrogen at rt. After 4 h of reaction, distilled water (15 mL) was added to the dark red-green solution and it was extracted with ethyl acetate (3 x 5 mL). The organic layers were combined, washed with $NaHSO_3$ solution (0.1 M), and dried with $MgSO_4$. The solvent was evaporated under reduced pressure to give a reddish-purple solid. Chromatography on silica gel treated with acetic acid (1%) in ethyl acetate, with the solvent 50:50 ethyl acetate/ hexanes gave dark-pink crystals (17.2 mg, 0.0385 mmol, 32% yield). The spectral data was identical to that found in the literature for dehydrodisinapic acid dilactone (see above)

Reaction of ferulic acid with DDQ in THF

Ferulic acid (25.3 mg, 0.131 mmol) and DDQ (30.8 mg, 0.136 mmol) was dissolved in freshly distilled THF (10 mL) and stirred at rt for 4 h. Distilled water (10 mL) was added to the dark red solution and it was extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, washed with $NaHSO_3$ solution (0.1 M), and dried with $MgSO_4$. The solvent was removed under reduced pressure to give an orange solid. Chromatography on silica gel with the solvent 5:45:50 acetic acid/ethyl acetate/hexanes gave dark-pink crystals (5.8 g, 0.015 mmol, 23% yield): mp 208-209°C; ^{1}H NMR (acetone-d_6) δ 3.84 (s, 6H), 4.07 (s, 2H), 5.75 (s, 2H), 6.87 (d, *J*=8.2, 1H), 6.92 (dd, *J*=8.2, 1.9, 1H), , 7.05 (d, *J*=1.9, 2H); ^{13}C NMR (acetone-d_6) δ 48.8 (2CH), 56.3 ($2CH_3$), 82.9 (2CH), 109.9 (2CH), 115.8 (2CH), 119.1 (2CH), 130.5 (2C), 147.8 (2C), 148.4 (2C), 175.7 (2CO); MS *m/z* (rel. %) 386 (M+, 49), 298 (70), 190 (46), 151 (100), 82 (69). ^{1}H NMR and ^{13}C NMR spectral data and melting point data of this compound were identical to that found for ferulic acid dehydrodilactone.[102]

Reaction of caffeic acid with DDQ in THF

Caffeic acid (25.1 mg, 0.141 mmol) and DDQ (30.2 mg, 0.133 mmol) were dissolved in dry THF (15 mL) and stirred at room temperature. After 18 hours, water (15 mL) was added to the dark

red solution and it was extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$, and evaporated under reduced pressure to give a dark brown solid. Chromatography on silica gel with 50:50 ethyl acetate/hexanes gave a tan solid (16.1 mg, 0.0490 mmol, 70% yield): mp 76-80°C; IR (NUJOL) 1746 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.98 (s, 2H), 5.72 (s, 2H), 6.81 (dd, *J*=8.2, 2.1), 6.87 (d, *J*=8.2, 1H), 6.91 (d, *J*=2.1); ^{13}C NMR ($CDCl_3$) δ 49.2 (CH), 83.0 (CH), 113.7 (CH), 116.3 (CH), 118.2 (CH), 146.3 (C), 146.6 (C), 175.9 (CO); MS *m/z* (rel. %) 314 (95), 270 (98), 204 (76), 123 (100), 57 (57). The spectral data for this product were identical to that found for caffeic acid dehydrodilactone.[107]

Oxidative coupling of methyl sinapate

Methyl sinapate (23)

Sinapic acid (1.030 g, 4.59 mmol) was dissolved in MeOH (50 mL). Concentrated H_2SO_4 (0.5 mL) was added and the solution was refluxed for 5 hours. Once the solution was cool, distilled water (10 mL) was added and the methanol was evaporated under reduced pressure. The solution was extracted with CH_2Cl_2 three times and the organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to give a dark brown solid. The product was crystallized from methanol to give **23** as a colourless solid (861 mg, 3.61 mmol, 79 % yield): mp 161-162°C (dec) IR (CH_2Cl_2) 1713 (CO) cm1; ^1H-NMR ($CDCl_3$) δ 3.80 (s, 3H), 3.92 (s, 6H), 6.30 (d, *J*=15.8, 1H), 6.77(s, 2H), 7.60 (d, *J*=15.8, 1H); ^{13}C NMR ($CDCl_3$) δ 51.6 (CH_3), 56.3 ($2CH_3$), 105.1 (2CH), 115.5 (CH), 125.9 (C), 137.1 (C), 145.1 (CH), 147.2 (C), 167.5 (CO); MS *m/z* (rel. %) 238 (M+, 21), 228 (31), 200 (21), 83 (43); HRMS calcd. for $C_{12}H_{14}O_5$ 238.0841, found 238.0847.

Dimethyl thomasidioate (2)

Thomasidioic acid (98.9 mg, 0.222 mmol) was dissolved in methanol containing 3% HCl and refluxed for 16 hours. The solvent was evaporated to leave a dark brown solid. Chromatography on silica gel with 40:60 hexanes/ethyl acetate gave tan coloured crystals, **2** (35.6 mg, 0.0750

mmol, 34% yield): mp 201-203°C; IR (CH_2Cl_2): 1719 (CO), 1709 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.65 (s, 6H), 3.76 (s, 9H), 3.93 (s, 3H), 4.03 (d, J=1.2, 1H), 5.00 (bs, 1H), 5.78 (s, 1H), 6.28 (s, 2H), 6.71 (s, 1H), 7.64 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 39.5 (CH), 46.5 (CH), 51.9 (CH_3), 52.5 (CH_3), 56.3 ($3CH_3$), 60.6 (CH_3), 104.4 (2CH), 107.3 (CH), 122.9 (C), 123.4 (C), 123.8 (C), 133.5 (2C), 137.5 (CH), 140.9 (C), 144.9 (C), 146.8 (3C), 167.0 (CO), 172.4 (CO); MS *m/z* (rel. %): 474 (M+, 65), 413 (56), 414 (100), 383 (71), 57 (45); HRMS calcd. for $C_{24}H_{26}O_{10}$ 474.1526, found 474.1502. ^1H NMR spectral and melting point data of the product were identical to that found in the literature.[29]

Dimethyl thomasidioate (2) / butadiene diester 101

Reaction of methyl sinapate in NH_4HCO_3 buffer

Methyl sinapate (53.0 g, 0.222 mmol) was dissolved in methanol (8 mL) and NH_4HCO_3/ NH_4OH buffer (8 mL, 0.287 M, pH 8.5) was added. The yellow solution was stirred open to air for 24 hours. Once the reaction was complete, most of the methanol was evaporated under vacuum. The resulting solution was acidified to pH 7 with HCl (10%). It was then extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to yield a light brown solid. This solid was chromatographed on silica gel with a gradient of solvents: 5:55:40 $CHCl_3$/hexanes/ethyl acetate to 5:25:70 $CHCl_3$/hexanes/ethyl acetate to give an off-white solid (19.6 g, 0.0413 mmol, 13% yield). The ^1H NMR spectrum was identical to that of dimethyl thomasidioate. The second compound to be eluted was a light brown solid (38.6 g, 0.081 mmol, 22% yield): mp 74-75°C; IR (CH_2Cl_2) 3528 (OH) , 1710 (CO) cm^{-1}; ^1H-NMR ($CDCl_3$): δ 3.69 (s, 3H), 3.80 (s, 6H), 6.81 (s, 2H), 7.82 (s,1H); ^{13}C NMR ($CDCl_3$) δ 52.4 ($2CH_3$), 56.2 ($4CH_3$), 107.1 (4CH), 124.8 (2C), 126.0 (2C), 136.7 (2C), 142.5 (2CH), 146.9 (4C), 167.6 (2CO); proton coupled ^{13}C NMR ($CDCl_3$) δ 167.6 (d, *J*=6.8); MS *m/z* (rel. %): 474 (M+, 46), 289 (92), 276 (100), 167 (94), 117 (84); HRMS calcd. for $C_{24}H_{26}O_{10}$ 474.1526, found 474.1522.

Attempted cross coupling of methyl 3,4,5-trimethoxycinnamate with methyl sinapate

Methyl 3,4,5-trimethoxycinnamate (31.5 mg, 0.125 mmol) and methyl sinapate (35.1 mg, 0.147 mmol) were dissolved in methanol (12 mL) and TRIS buffer solution (0.1 M, pH 8.7, 12 mL) was added. The solution was heated to 43±2 °C for 24 h under a reflux condenser and bubbled with air presaturated by passing through solutions of KOH/water and KOH/methanol. The solution was diluted with distilled water and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to give a tan solid (54.6 mg). The ^1H NMR spectrum was consistent with a mixture of dimethyl thomasidioate, butadiene diester **101**, and methyl 3,4,5-trimethoxycinnamate.

Oxidative coupling of methyl sinapate, pH study

pH of 8.3, 8.8, 9.3

To make up each TRIS buffer solution of pH 8.5, 9.0 and 9.5, a TRIS solution (0.1 M, 12.5 mL) was titrated with HCl solution (0.1 M) to the respective pH and then the solution was diluted to 25 mL with doubly distilled water. For each reaction, methyl sinapate (0.0158 g, 0.07 mmol) was dissolved in methanol (3 mL) and the TRIS buffer (3 mL) was added. The pH of the reaction solution was 0.2 less than the pH of original TRIS buffer solution. The solutions were heated to 43±2 °C under a reflux condenser and bubbled with air presaturated by passing through KOH/MeOH and KOH/H_2O solutions. After 22 hours, the reaction was observed to be complete by TLC. Once the solutions were cool, they were neutralized with HCl (10%), diluted with doubly distilled water (7 mL), and extracted with CH_2Cl_2 (3 x 5 mL). The organic layers were dried with $MgSO_4$ and evaporated under reduced pressure to give an orange-brown solid. The ratio of dimethyl ester of thomasidioic acid and methyl ester of butadiene for each reaction was measured using HPLC (58:42 methanol: water). The ratio of the products for reactions at pH 8.3, 8.8 and 9.3 were determined to be: 1.29:1, 2.67:1, and 3.34:1 dimethyl thomasidioate (**2**):butadiene **101**, respectively

Oxidative coupling of methyl sinapate, effect of buffer concentration

0.1, 0.2 and 0.5 molar

The TRIS buffer solution of concentrations 0.2, 0.4 and 1.0 molar were each made up by titrating a TRIS solution of chosen concentration to pH 9.5 with HCl solution of same concentration. For each reaction, methyl sinapate (17.4 g, 0.0730 mmol) was dissolved in methanol (3 mL) and the TRIS buffer solution (0.2 M, 0.4 M, or 1.0 molar, 3 mL) was added. The solution was heated to 43± 2 °C under a condenser and bubbled with air that was presaturated by passing through solutions of KOH/methanol and KOH/water. After 24 hours TLC indicated that the reaction was complete. The solution was neutralized with HCl (10%), diluted with doubly distilled water (7 mL), and extracted with CH_2Cl_2 (3 x 5 mL). The organic layers were dried with $MgSO_4$ and evaporated under reduced pressure giving a orange-brown solid. The ratio of dimethyl ester of thomasidioic acid and methyl ester of butadiene were measured for each reaction using HPLC (58:42 methanol:water). The results for 0.1, 0.2, and 0.5 molar solutions of TRIS buffer were: 4.17 :1, 3.22:1 and 2:1 (dimethyl thomasidioate (**2**): butadiene **101**), respectively.

Reaction of methyl sinapate in borax buffer

Methyl sinapate (15.4 mg, 0.0646 mmol) was dissolved in methanol (3 mL) and $Na_2B_4O_7$ solution (0.1 M, 3 mL) was added. The solution was heated to 43±2 °C under a reflux condenser and bubbled with air presaturated with solutions of KOH/water and KOH/methanol. After 21 hours of reaction, the solution was diluted with doubly distilled water (15mL) and neutralized with HCl solution (10%). It was extracted with CH_2Cl_2 three times and the organic layers were combined, dried with MgSO4 and evaporated under reduced pressure to give a light brown solid. Analysis by HPLC (58:42 methanol/water) showed compounds dimethyl thomasidioate, butadiene diester **101**, and methyl sinapate in a ratio of 18/8/74.

Reaction of methyl sinapate in K_2CO_3 buffer

A buffered solution of K_2CO_3/$KHCO_3$ was made up by titrating a K_2CO_3 solution (0.1 M, 10 mL) with $KHCO_3$ solution (0.1 M) to pH 10. Methyl sinapate (15.4 mg, 0.0646 mmol) was dissolved in methanol (3 mL) and the K_2CO_3 buffer was added (3 mL). The solution was heated to 43±2 °C under a reflux condenser and bubbled with air that had been passed through solutions of KOH/water and KOH/methanol. After 21 hours of reaction, the solution was diluted with doubly distilled water (15mL) and neutralized with HCl (10%). It was extracted with CH_2Cl_2 (3 x 5 mL) and the organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to give a light brown solid. Analysis by HPLC (58:42 methanol: water) showed the ratio of dimethyl thomasidioate/butadiene **101**/methyl sinapate to be 4/32/64.

Reaction of methyl sinapate in ethanolamine buffer

Ethanolamine/ HCl buffer (0.1 M, pH 9.5) was made up by dissolving ethanolamine (611 mg, 0.0100 mol) in distilled water (100 mL) and titrating solution with HCl (0.1 M). Methyl sinapate (15.4 mg, 0.0646 mmol) was dissolved in methanol (3 mL) and ethanolamine buffer was added (3 mL). The solution was heated to 43± 2 °C under a reflux condenser and bubbled with air that had been presaturated by passing through solutions of KOH/water and KOH/methanol. After 21 hours of reaction, the solution was diluted with doubly distilled water (15mL) and neutralized with HCl solution (10%). It was extracted with CH_2Cl_2 (3 x 5 mL) and the organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to give a light brown solid. Analysis by HPLC showed dimethyl thomasidioate/butadiene ester **101**/methyl sinapate as 1/14/85

Reaction of methyl sinapate in glycine buffer

A glycine solution (0.1 M, 10 mL) was titrated with NaOH solution (0.1 M) to pH 9.5. Methyl sinapate (15.4 mg, 0.0646 mmol) was dissolved in MeOH (3 mL) and the glycine buffer was added (3 mL). The solution was heated to 43± 2 °C under a reflux condenser and bubbled with air presaturated by passing through solutions of KOH/water and KOH/MeOH. After 21 hours of

reaction, the solution was diluted with doubly distilled water (15 mL) and neutralized with HCl solution (10%). It was extracted with CH_2Cl_2 three times and the organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to give a light brown solid. Analysis by HPLC gave a ratio of dimethyl thomasidioate/butadiene **101**/methyl sinapate of 5/45/50.

Reaction of methyl sinapate in TRIS buffer:

A TRIS solution (0.1 M, 12.5 mL) was mixed with HCl (0.1 M, 1.4 mL) and this solution was diluted to 25 mL with doubly distilled water. Methyl sinapate (15.0 g, 0.0629 mmol) was dissolved in methanol (3 mL) and buffer (3 mL) was added. The solution was heated to 43±2 °C under a reflux condenser and bubbled with air presaturated by passing through solutions of KOH in water and KOH in methanol. After 21 hours of reaction, the solution was diluted with doubly distilled water (15mL) and neutralized with HCl solution (10%). It was extracted with CH_2Cl_2 three times and the organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure to leave a light brown solid. Analysis by HPLC showed that all of starting material had reacted and that the ratio of dimethyl thomasidioate/butadiene **101** was 2.67:1.

Allyl 4-O-allyl-3,5-dimethoxycinnamate (118)

Sinapic acid (48.0 mg, 0.21 mmol) and K_2CO_3 (53.9 g, 0.960 mmol) were dissolved in allyl bromide (2.5 mL) and acetone (5mL). The mixture was refluxed for 23 hours. Distilled water (10mL) was added to the mixture and it was extracted with CH_2Cl_2 (3 x 5 mL). The organic layers were combined, dried with $MgSO_4$, and evaporated under reduced pressure to give a dark orange oil. The oil was filtered through silica gel to give an orange oil (46.6 g, 0.153 mmol, 72% yield): IR (CH_2Cl_2): 1721 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.87 (s, 6H), 4.56 (dt, *J*=6.1, 1.3, 2H), 4.71 (d, J= 5.7, 1.3, 2H), 5.14 -5.42 (m, 4H), 6.5.92-6.16 (m, 2H), 6.37 (d, *J*=15.9, 1H), 6.75 (s, 2H), 7.62 (d, *J*=15.9, 1H); ^{13}C NMR ($CDCl_3$) δ 55.1 ($2CH_3$), 64.1 (CH_2), 73.2 (CH_2), 104.2 (2CH), 116.0 (CH), 116.9 (CH_2), 117.2 (CH_2), 128.8 (C), 131.2 (CH), 133.1 (CH), 137.8

(C), 144.0 (CH), 152.6 (2C), 165.5 (CO); MS *m/z* (rel. %): 304 (M+, 14), 264 (18), 263 (100); HRMS calcd. for $C_{17}H_{20}O_5$ 304.1311, found 304.1307.

4-O-Allyl-3,5-dimethoxycinnamic acid (119)

The allyl ester of 4-O-allyl-3,5-dimethoxycinnamic acid (581 mg, 1.91 mmol) and potassium hydroxide (1.51 g, 26.7 mmol) was dissolved in a solution of water/methanol (2.5 mL:22.5 mL) and stirred for 5 h. The methanol was evaporated under vacuum and distilled water (15 mL) was added. The solution was washed with EtOAc (3 x 10 mL) and the organic layers were combined, dried with $MgSO_4$, and evaporated under pressure, to give a light yellow solid. The solid was dissolved in CH_2Cl_2 (5 mL) and passed through silica gel with ethyl acetate. The solvents were evaporated under vacuum to yield a colourless solid (457 mg, 1.73 mmol, 90% yield): mp 91-94°C; IR (CH_2Cl_2) 1689 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.88 (s, 6H), 4.57 (d, *J*=6.1, 2H), 5.17 (bd, *J*=10.4, 1H), 5.30 (dm, *J*=17.2, 1H), 6.07 (ddt, *J*=10.4, 17.2, 6.1, 1H), 6.33 (d, *J*=15.9, 1H), 6.75 (s, 2H), 7.67 (d, *J*=15.9, 1H); ^{13}C NMR ($CDCl_3$) δ 56.2 ($2CH_3$), 74.2 (CH_2), 105.4 (2CH), 116.3 (CH), 118.1 (CH_2), 129.5 (C), 133.8 (C), 134.0 (CH), 147.1 (CH), 153.7 (2C), 176.4 (CO); MS *m/z* (rel. %) 264 (M+, 5), 223 (34), 180 (100), 165 (39); HRMS calcd. for $C_{14}H_{16}O_5$ 264.0998, found 264.1014.

(Methyl-(*R*)-mandelyl) 4-O-allyl-3,5-dimethoxycinnamate (120)

4-O-Allyl protected sinapic acid (67.5 g, 0.414 mmol) was dissolved in CCl_4 (8 mL) and thionyl chloride (0.08 mL, 1.2 mmol) was added. The yellow solution was refluxed for 18 hours under nitrogen and then the solvent was evaporated under vacuum. The yellow solid was redissolved in CCl_4 (10 mL), methyl (*R*)-mandelate (50.9 mg, 0.523 mmol) added and the solution refluxed for 25 hours under nitrogen. The solvent was evaporated under vacuum to give an amorphous yellow solid. Chromatography of the solid on silica gel with toluene/ethyl acetate (10/90) gave a light yellow oil (93 mg, 0.23 mmol, 89% yield): IR (CH_2Cl_2) 1753 (CO), 1722 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.75 (s, 3H), 3.87 (s, 6H), 4.56 (dt, *J*=6.1, 1.2, 1H), 5.19 (bd, *J*=10.3, 1H), 5.31

(dq, *J*=17.2, 1.5, 1H), 6.09 (s, 1H), 6.08 (ddt, *J*=10.3, 17.2, 6.1, 1H), 6.48 (d, *J*=16.0, 1H,), 6.76 (s, 2H), 7.42 (m, 3H), 7.53 (m, 2H), 7.70 (d, *J* = 16.0, 1H); ^{13}C NMR ($CDCl_3$) δ 52.6 (CH_3), 56.1 ($2CH_3$), 74.1 (CH_2), 74.4 (CH), 105.4 (2CH), 116.0 (CH), 118.0 (CH_2), 127.5 (2CH), 128.7 (2CH), 129.2 (CH), 129.6 (C), 134.0 (C), 134.1 (CH), 139.0 (C), 146.3 (CH), 153.6 (2C), 166.0 (CO), 169.3 (CO); MS *m/z* (rel. %) 412 (M+, 7), 252 (37), 237 (41), 149 (100), 121 (51); HRMS calcd. for $C_{23}H_{24}O_7$ 412.1522, found 412.1523.

(Methyl (*R*)-mandelyl) sinapate (72)

Allyl protected (methyl (*R*)-mandelyl) sinapate (109 mg, 0.27 mmol), chlorotris-(triphenylphosphine) rhodium(0.0162 g, 0.0018 mmol) and DABCO (0.0170 g, 0.15 mmol) were dissolved in ethyl alcohol:benzene:water (7 mL:3 mL:1 mL) and refluxed for 7 hours. Distilled water (12 mL) and hydrochloric acid solution (10%, 7 mL) were added to the solution and it was then extracted with CH_2Cl_2 (3 x 10 mL). The organic layers were combined, dried with $MgSO_4$ and evaporated under vacuum to give **72** as a yellow oil. Chromatography of the oil on silica gel using 50:50 ethyl acetate/ hexanes as solvent gave a light yellow oil (35.4 mg, 0.0950 mmol, 36% yield): IR (CH_2Cl_2) 1756 (CO), 1720 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3H), 3.90 (s, 6H), 6.08 (s, 1H), 6.43 (d, *J*=15.8, 1H), 6.77 (s, 2H), 7.41 (m, 3H), 7.54 (m, 2H), 7.69 (d, *J*=15.8, 1H); ^{13}C NMR ($CDCl_3$) δ 52.6 (CH_3), 56.3 ($2CH_3$), 74.3 (CH), 105.2 (2CH), 114.6 (CH), 125.6 (C), 127.5 (CH), 128.8 (2CH), 129.4 (2CH), 134.0 (C), 137.4 (C), 146.4 (CH), 147.2 (2C), 166.2 (CO), 169.4 (CO); MS *m/z* (rel. %): 372 (M+, 2), 238 (27), 86 (68), 84 (100); HRMS calcd. for $C_{20}H_{20}O_7$ 372.1209, found 372.1192

Reaction of (methyl (*R*)-mandelyl) sinapate in NH_4HCO_3 buffer

(Methyl (*R*)-mandelyl) sinapate (40.8 mg, 0.110 mmol) was dissolved in MeOH (5 mL) and NH_4HCO_3 buffer (0.287 M, pH 8.7, 5 mL) was added. The yellow solution was stirred at rt open to the atmosphere for 46 h. The dark solution was neutralized with HCl (10%) and diluted with distilled water (15 mL). The solution was extracted with CH_2Cl_2 (3 x 10 mL). The organic layers

were dried with $MgSO_4$ and evaporated under vacuum leaving a tan-coloured solid. The solid was chromatographed on silica gel to give a mixture of compounds (12.9 mg). The proton NMR spectra of the crude and chromatographed materials were similar, and indicated the presence of at least two compounds. No further efforts were made to characterize these two compounds: ^1H NMR ($CDCl_3$) δ 3.5-4.0 (multiple singlets), 5.85 (s), 5.98 (s), 6.74 (s), 6.81 (s), 7.2-7.5 (m), 7.90 (s), 7.91 (s).

Reaction of methyl sinapate with potassium triiodide

Methyl sinapate (49.3 mg, 0.207 mmol) was dissolved in methanol (18 mL) and deaerated NH_4HCO_3/NH_4OH buffer (0.287 M, 6 mL, pH 9.0). Deaerated KI_3 solution (0.02 M , 12 mL) was added. The red solution was stirred under nitrogen at room temperature for 48 hours. It was neutralized with HCl (10%) and extracted with CH_2Cl_2 three times. The organic layers were combined, dried with $MgSO_4$ and evaporated under reduced pressure leaving a light brown solid (30.7 mg). ^1H NMR spectral data were consistent with that found for a mixture of dimethyl thomasidioate and butadiene diester **101**.

Reaction of methyl sinapate with $K_3Fe(CN)_6$

Methyl sinapate (25.9 mg, 0.109 mmol) and $K_3Fe(CN)_6$ (42.4 mg, 0.130 mmol) were dissolved in methanol (5 mL) and deaerated ammonium bicarbonate buffer (0.287 M, 5 mL, pH 9.0) was added. The solution was stirred at room temperature under nitrogen for 18 hours. The dark orange solution was acidified with HCl (10%) and diluted with water (15 mL). The solution was extracted with CH_2Cl_2 three times. The organic layers were combined, dried with $MgSO_4$ and evaporated under vacuum leaving a brown solid (18.7 mg). ^1H NMR spectral data was consistent with that found for a mixture of dimethyl thomasidioate and butadiene diester **101**.

Reaction of methyl sinapate with benzoyl peroxide

Methyl sinapate (30.8 mg, 0.129 mmol) was dissolved in DMF (2 mL) under nitrogen at room temperature and NaH (55% in oil, 4.0 mg, 0.09 mmol) dissolved in DMF (1mL) was added dropwise. The solution was cooled to 0 °C and the yellow solution was stirred for 1 hour. Benzoyl peroxide (30% water, 22 mg, 0.064 mmol) that was dissolved in DMF (1 mL) was added dropwise. The solution was warmed up to room temperature and then stirred overnight. Water (5 mL) was added and the resulting solution was extracted with CH_2Cl_2 (3 x 7 mL). The organic layers were combined, dried with $MgSO_4$, and evaporated under vacuum, to leave a dark brown oil. Chromatography of liquid on silica gel with 80/20 ethyl acetate/ hexanes gave the product as light brown crystals (25.9 mg, 0.0580 mmol, 89% yield,). The ^{1}H NMR spectrum was identical to that for the butadiene diester **101**.

Reaction of methyl sinapate with 1 equivalent DDQ in methanol

Methyl sinapate (102 mg, 0.428 mmol) and DDQ (113 mg, 0.498 mmol) were dissolved in methanol (20 mL). After being stirred at room temperature for 2 hours, the solvent was evaporated under reduced pressure to form a dark brown solid. This product and *para*-toluenesulfonic acid (0.020 g, 0.11 mmol) were dissolved in benzene (12 mL) and refluxed for 17 hours. The solvent was removed under reduced pressure to leave a brownish-red product. The ^{1}H NMR spectrum of the crude product was identical to that for a mixture of dimethyl thomasidioate and an unknown product. The mixture of products was chromatographed on silica gel with 50:50 ethyl acetate/ hexanes. The unknown compound was obtained as a colourless solid (25.5 mg, 0.10 mmol, 44% yield): mp 243-245°C (dec); IR (CH_2Cl_2) 1719 (CO), 1709 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.93 (s,3H), 3.95 (s,3H), 4.04 (s, 3H), 4.06 (s, 3H), 6.03 (bs, 1H), 7.00 (s,1H), 8.08 (s, 1H), 8.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 52.6 (CH_3), 56.3 (CH_3), 61.3 (CH_3), 102.2 (CH), 123.3 (CH), 124.8 (C), 126.8 (C), 127.0 (C), 128.4 (C), 128.8 (CH), 139.1 (C), 140.6 (C), 150.1 (C), 168.4 (CO), 168.6 (CO); MS *m/z* (rel. %): 320 (M+, 16), 230 (64), 228 (100), 200 (56), 110 (23); HRMS calcd. for $C_{16}H_{16}O_7$ 320.0896, found 320.0918.

Reaction of methyl sinapate with 1/2 equivalent of DDQ in methanol

Methyl sinapate (30.9 mg, 0.130 mmol) and DDQ (15.4 mg, 0.0678 mmol) were dissolved in methanol (7 mL). After being stirred at room temperature for 2 hours, the solvent was evaporated under reduced pressure to leave a dark brown solid. This product and *para*-toluenesulfonic acid (0.010 g, 0.05 mmol) were dissolved in benzene (10 mL) and refluxed for 20 hours. The solvent was removed under reduced pressure and leaving a brownish-red product (35.1 mg). The solid was chromatographed on silica gel with 50:50 ethyl acetate/hexanes to yield a colourless solid (5.4 mg, 0.01 mmol, 15% yield). The ^{1}H NMR spectrum was identical to that for dimethyl thomasidioate.

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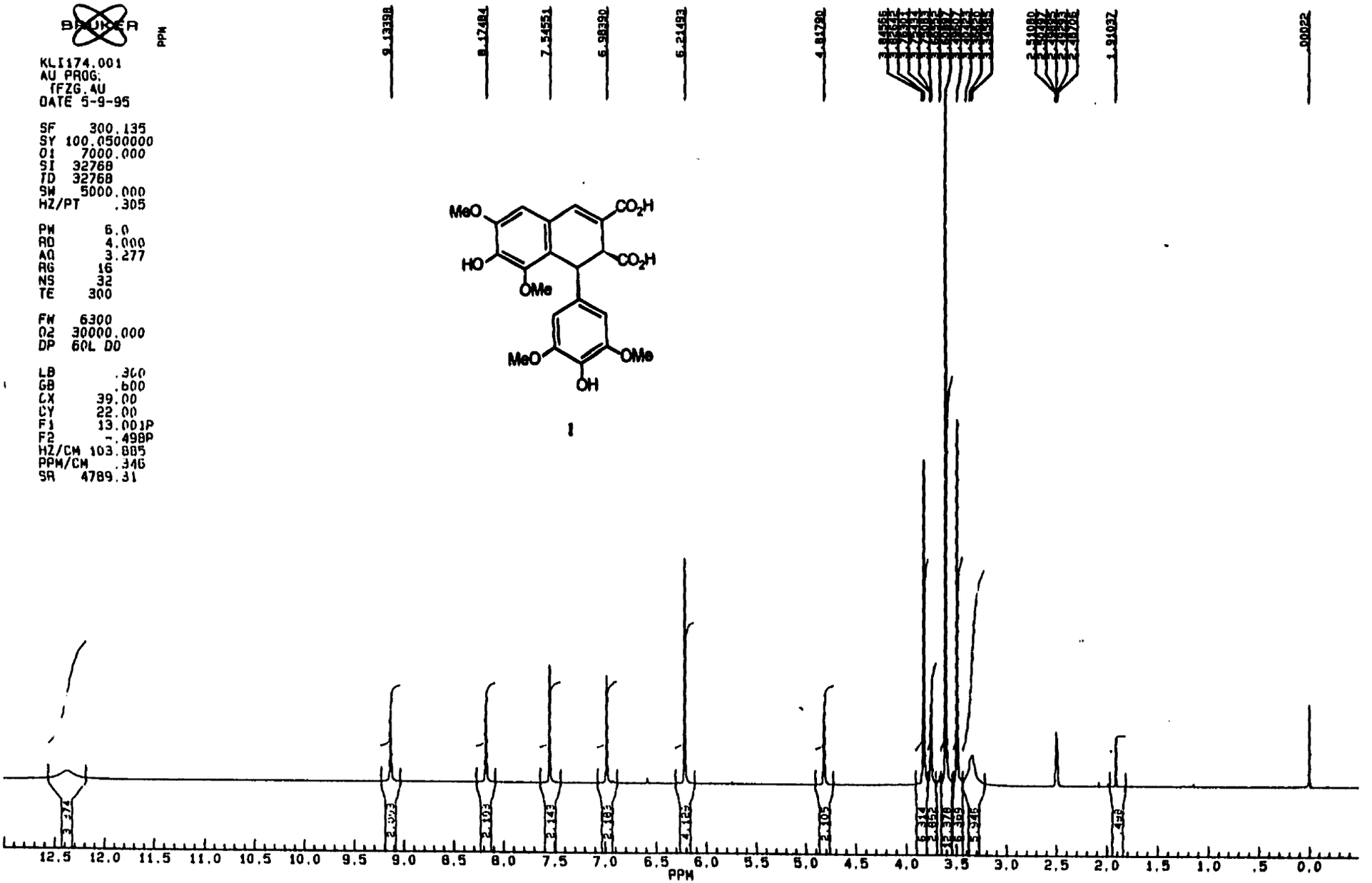
Appendix:

^{1}H and ^{13}C NMR Spectra

SAMPLE KL-I-174 1-H AT 300 MHZ IN DMSO-D6



SAMPLE KL-I-174 13-C AT 75.47 MHZ IN DMSO-D6

PPM

172.940
167.702
147.495
147.468
145.298
141.327
136.219
133.648
133.632
123.402
123.180
122.509
108.314
104.870
59.557
55.918
55.894
46.241
40.617
39.340
39.161
38.984
38.806
38.628
38.450
0.000

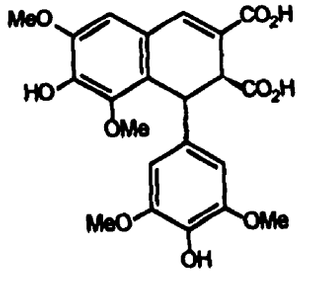
KLI174C.004
AU PROG:
AUTOC13.AU
DATE 6-9-95

SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090

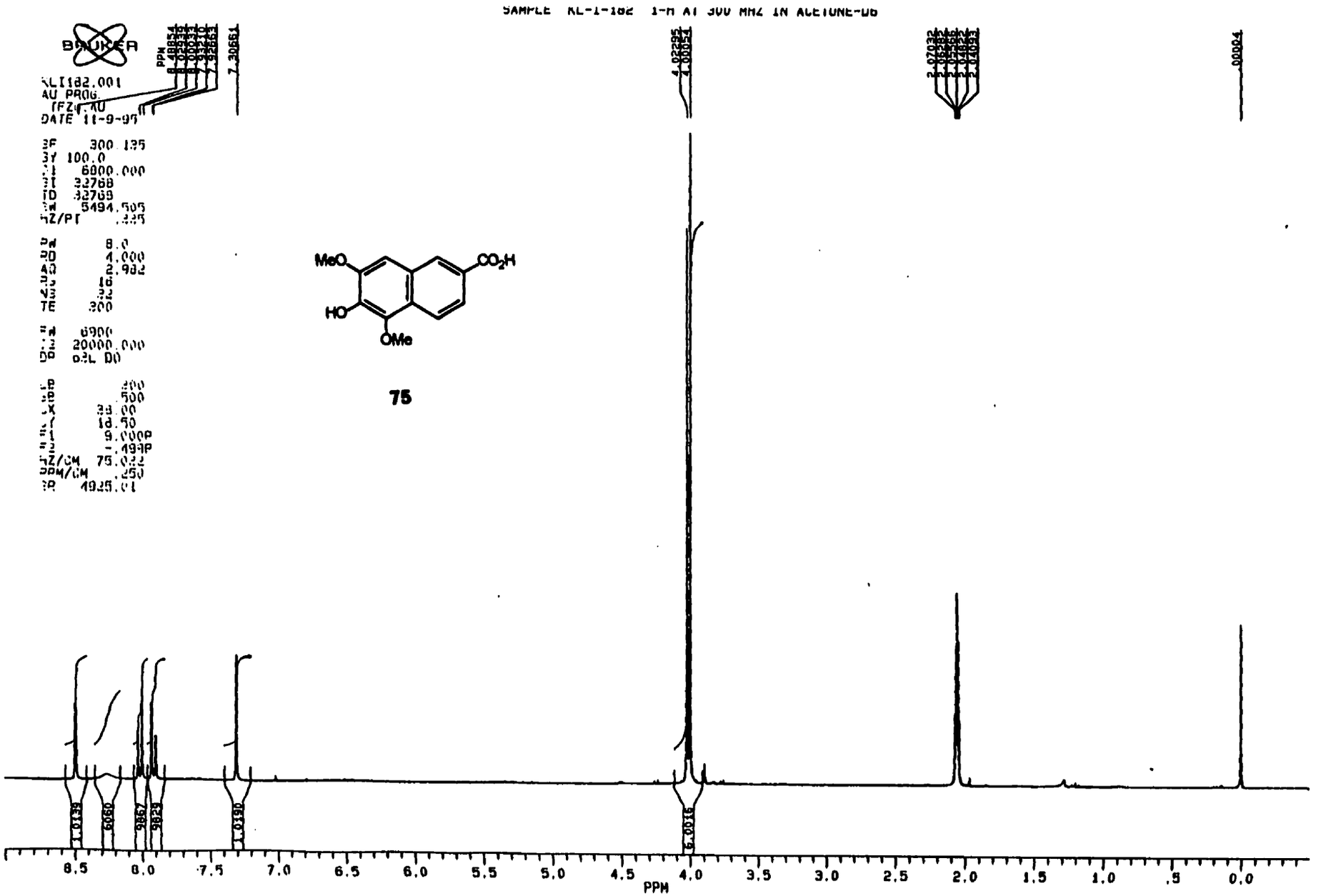
PW 5.0
RD 0.0
AQ .918
RG 200
NS 3200
TE 300

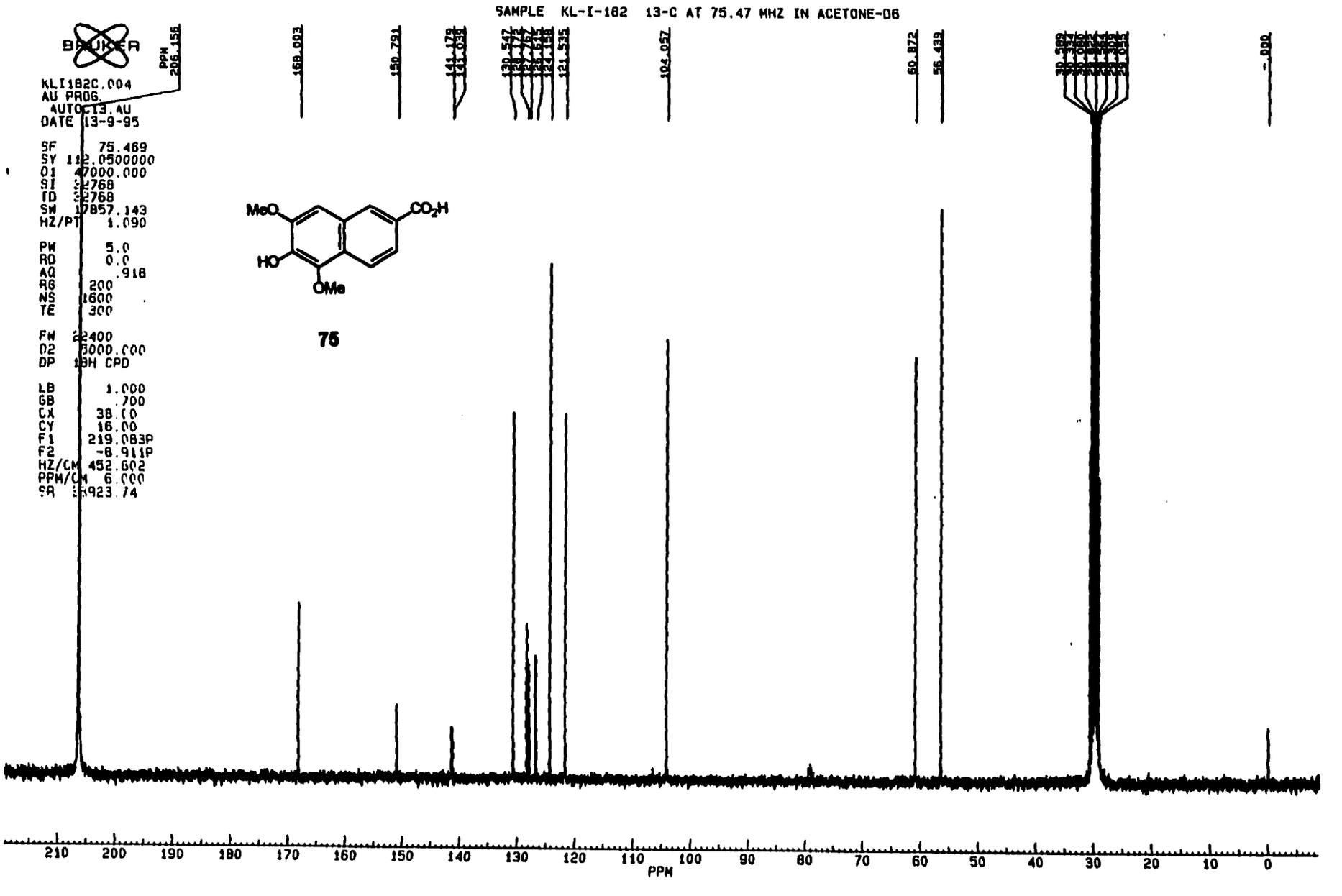
FW 22400
O2 5000.000
OP 13H CPD

LB 1.000
GB .700
CX 38.00
CY 18.00
F1 218.087P
F2 -9.907P
HZ/CM 452.602
PPM/CM 6.000
SR 33998.94



1


SAMPLE KL-1-182 1-H AT 300 MHZ IN ACETONE-D6
BRUKER
KLI182.001
AU PROG
DATE 11-9-95
MeO
HO
OMe
CO2H
75
PPM
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0


SAMPLE KL-I-182 13-C AT 75.47 MHZ IN ACETONE-D6
BRUKER
PPM
206.156
168.093
150.791
141.178
141.038
130.547
121.535
104.057
60.872
56.439
29.388
-.000
KLI182C.004
AU PROG.
AUTOC13.AU
DATE 13-9-95
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 1600
TE 300
FW 22400
O2 37000.000
DP 1BH CPD
LB 1.000
GB .700
CX 38.00
CY 16.00
F1 219.083P
F2 -8.911P
HZ/CM 452.602
PPM/CM 6.000
SR 3923.74
MeO
CO2H
HO
OMe
75
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
PPM

SAMPLE KL-II-35 1-H AT 300 MHZ IN CDCL3

PPM

KLII35.001
AU PROG.
(FZG.AU
DATE 25-10-95

SF 300.132
SY 100.0
O1 5500.000
SI 32768
TD 32768
SW 5494.505
HZ/PT .336

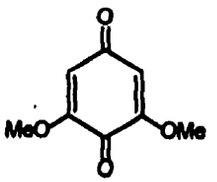
PW 8.1
RD 4.000
AQ 2.982
RG 50
NS 8
TE 300

FW 8000
DP ACQU DQ

LB .300
GB .000
WX 25.00
WY 15.50
F1 9.005P
F2 -.495P
HZ/CM 75.032
PPM/CM .250
SR 3004.44

5.84807

3.82229



80

1.0000

4.2063

8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 .5 0.0

PPM

SAMPLE KL-II-35 13-C AT 75.47 MHZ IN CDCL3

PPM

186.672
157.252
107.333
96.072
77.303
76.976
76.544
56.397
.000

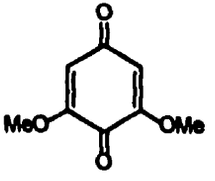
KLII35C.004
AU PROG:
AUTOC13.AU
DATE 26-10-95

SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090

PW 5.0
RD 0.0
AD .918
RG 200
NS 960
TE 300

FW 22400
O2 5000.000
DP 18H CPD

LB 1.000
GB .700
CX 38.00
CY 18.00
F1 223.402P
F2 -4.593P
HZ/CM 452.802
PPM/CM 6.000
SR 38597.86



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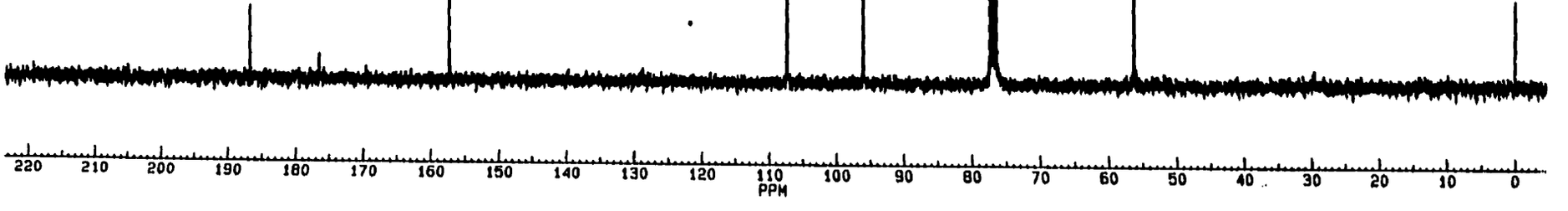


SAMPLE KL-III-84(1) 1-H AT 300 MHZ IN CDCL3

BRUKER

PPM

7.67503
7.38206
6.75001
6.37315
6.31497
3.88636
3.87933
3.80657

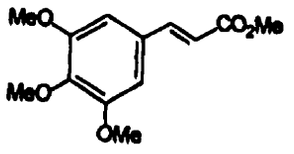
KL3841.001
DATE 21-8-96

SF 300.133
SY 100.0
O1 5500.000
SI 32768
TD 32768
SW 6494.505
HZ/PT .395

PW 8.0
RD 4.000
AQ 2.982
RG 10
NS 32
TE 300

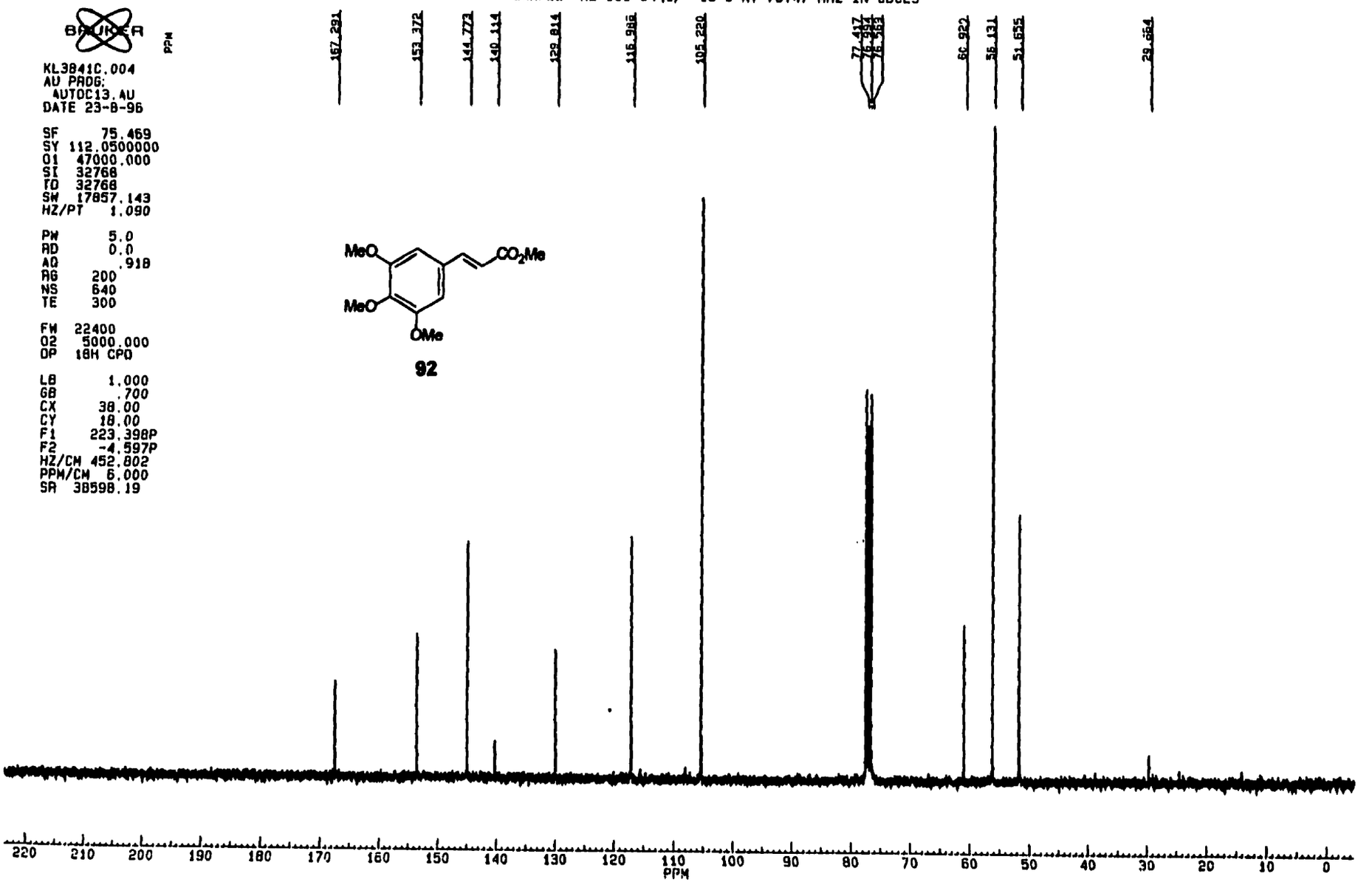
FW 6900
O2 20000.000
DP 63L DD

LB .300
GB .500
CX 38.00
CY 18.50
F1 9.005P
F2 -.495P
HZ/CM 75.032
PPM/CM .250
SR 3364.78



92

SAMPLE KL-III-84(1) 13-C AT 75.47 MHZ IN CDCL3



KL3841C.004
AU PROG:
AUTOC13.AU
DATE 23-8-96

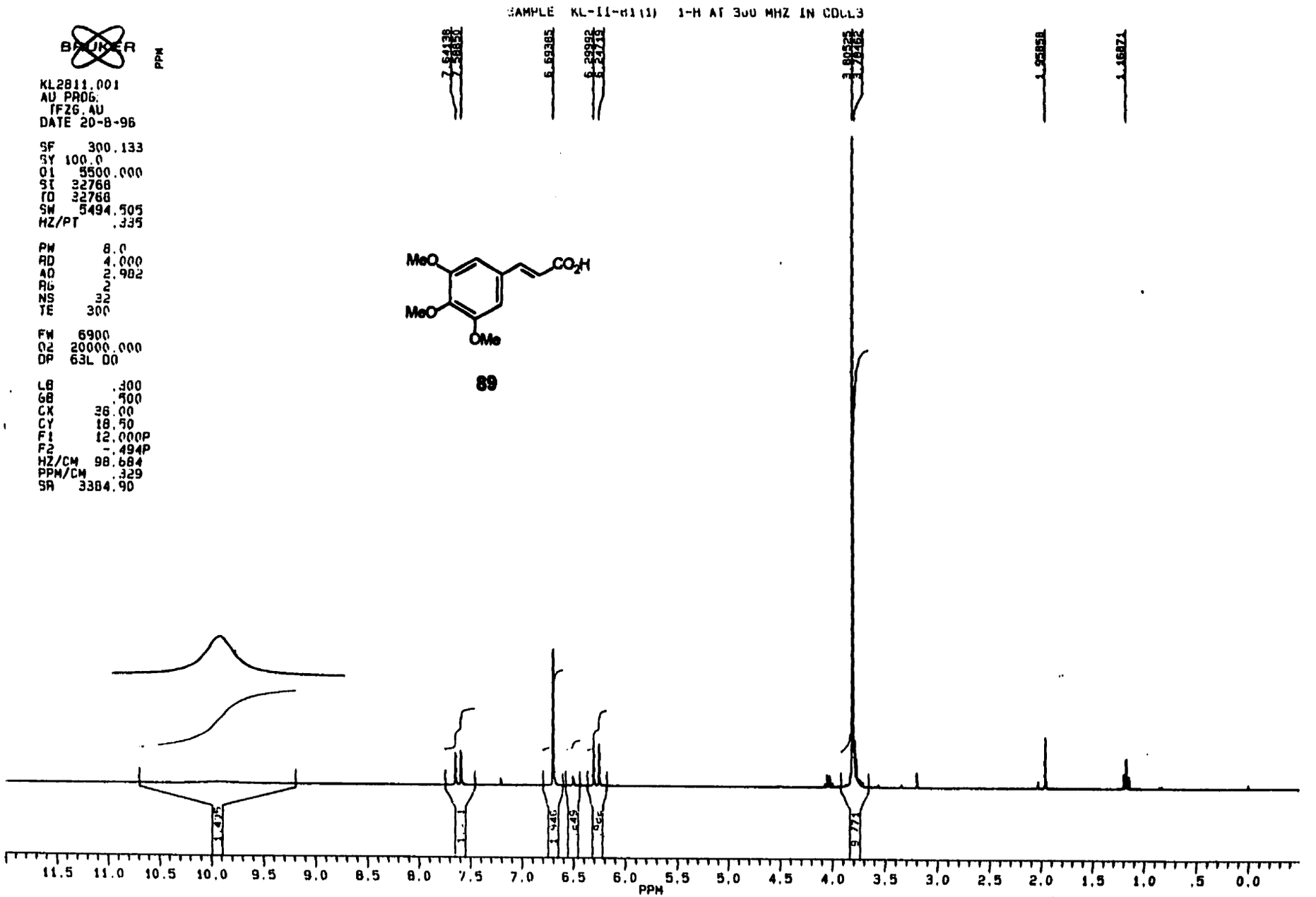
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090

PW 5.0
RD 0.0
AQ .918
RG 200
NS 640
TE 300

FW 22400
O2 5000.000
OP 18H CPD

LB 1.000
GB .700
CX 38.00
CY 18.00
F1 223.398P
F2 -4.597P
HZ/CM 452.802
PPM/CM 6.000
SR 38598.19

92


SAMPLE KL-II-81(1) 1-H AT 300 MHZ IN CDCL3
PPM
KL2811.001
AU PROG:
TFZG.AU
DATE 20-8-96
SF 300.133
SY 100.0
O1 5500.000
SI 32768
TD 32768
SW 5494.505
HZ/PT .335
PW 8.0
RD 4.000
AO 2.982
RG 2
NS 32
TE 300
FW 6900
O2 20000.000
DP 63L DO
LB .300
GB .500
CX 38.00
CY 18.50
F1 12.000P
F2 -.494P
HZ/CM 98.684
PPM/CM .329
SR 3384.90
MeO
MeO
OMe
CO2H
89
7.64138
7.58850
6.69385
6.29982
6.24418
3.80635
3.78162
1.95858
1.16871
1.405
1.000
1.946
.645
.956
9.771
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 .5 0.0
PPM

SAMPLE KL-II-81(1) 13-C AT 75.47 MHZ IN CDCL3

PPM

172.263
153.362
146.947
140.472
129.397
115.399
105.521
103.182
77.418
76.996
76.575
76.153
60.911
56.118

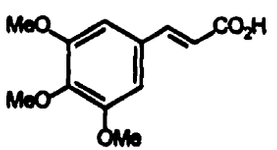
KL2B11C.004
AU PROG:
AUTOC13.AU
DATE 23-8-96

SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090

PW 5.0
RD 0.0
AD .918
RG 200
NS 640
TE 300

FW 22400
O2 5000.000
OP 18H CPD

LB 1.000
GB .700
CX 38.00
CY 18.00
F1 223.369P
F2 -4.626P
HZ/CM 452.802
PPM/CM 6.000
SR 38600.37



89

SAMPLE KL-III-102A 1-H AT 300 MHZ IN ACETONE-D6

BRUKER

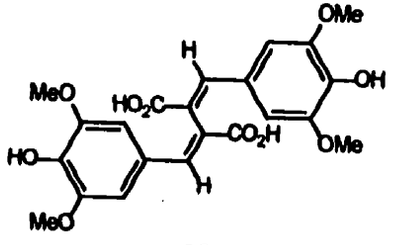
KL3102A.001
AU PROG.
IFZG.AU
DATE 22-10-96

SF 300.135
SY 100.0
D1 6800.000
SI 32768
ID 32768
SW 5494.505
HZ/PT .335

PW 8.6
RD 4.000
AQ 2.982
RG 16
NS 32
TE 300

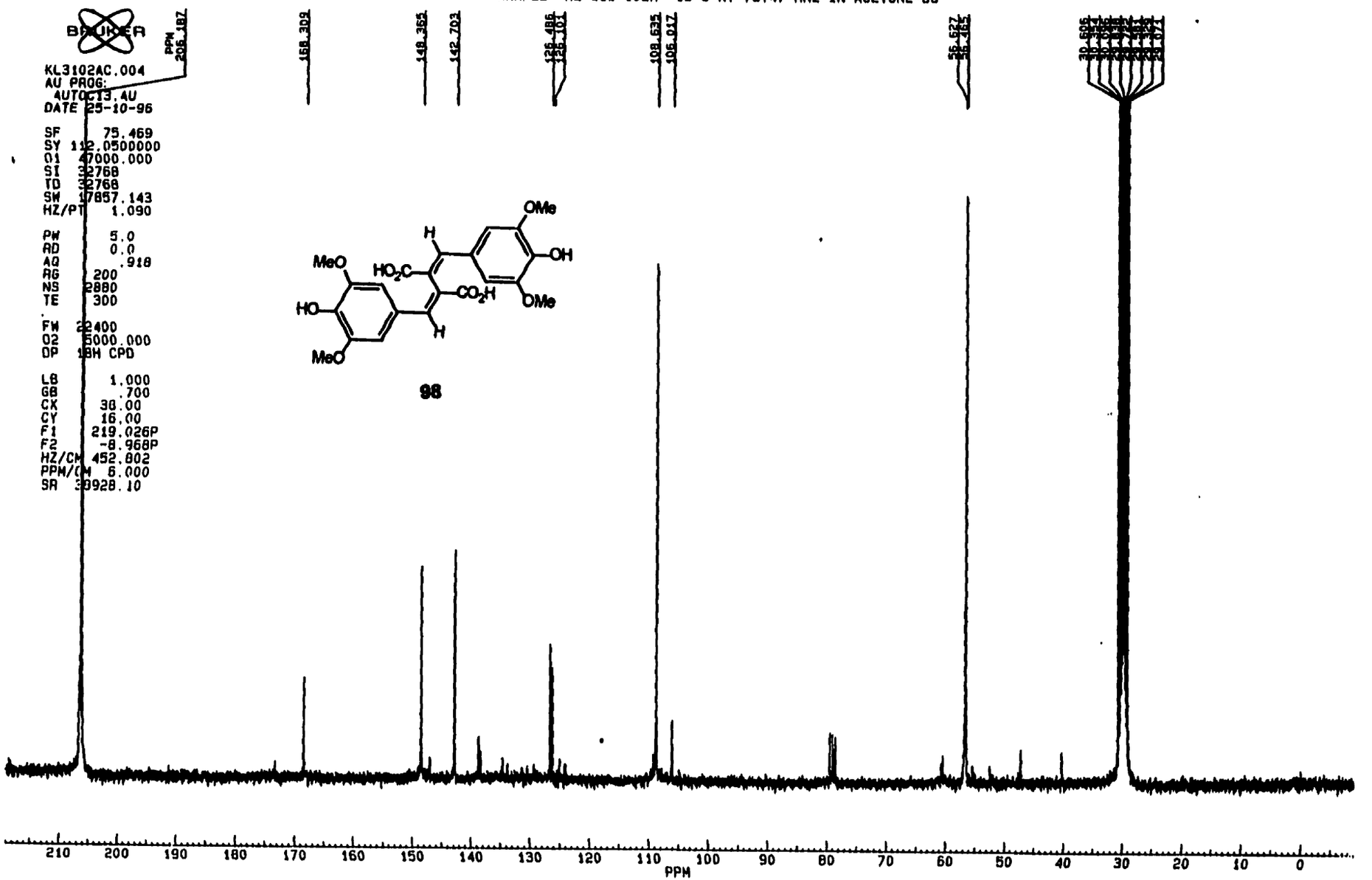
FW 6900
D2 20000.000
DP 63L DD

LB .300
GB .500
CX 38.10
CY 18.50
F1 9.000P
F2 -.499P
HZ/CM 75.032
PPM/CM .250
SR 4925.68

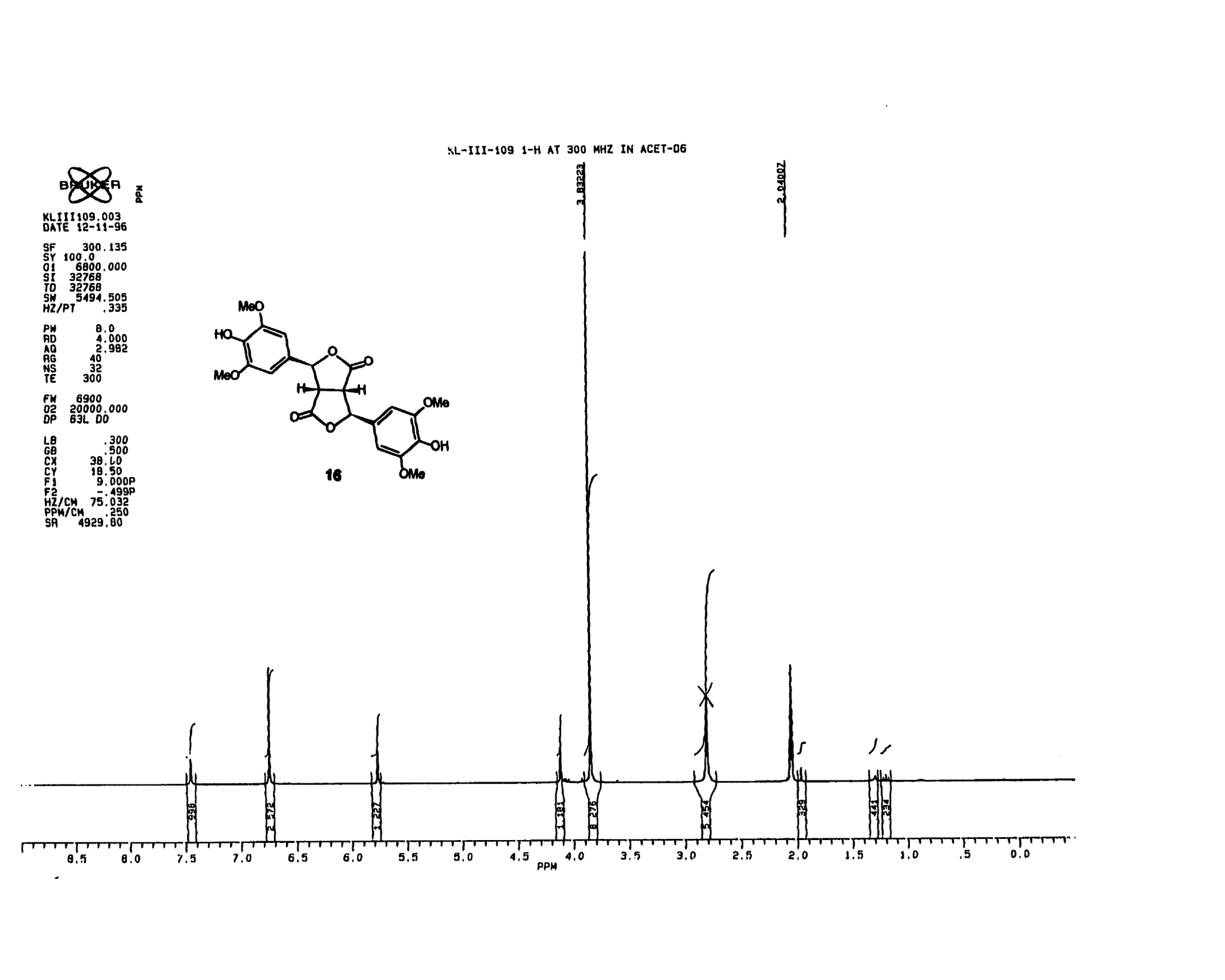


98

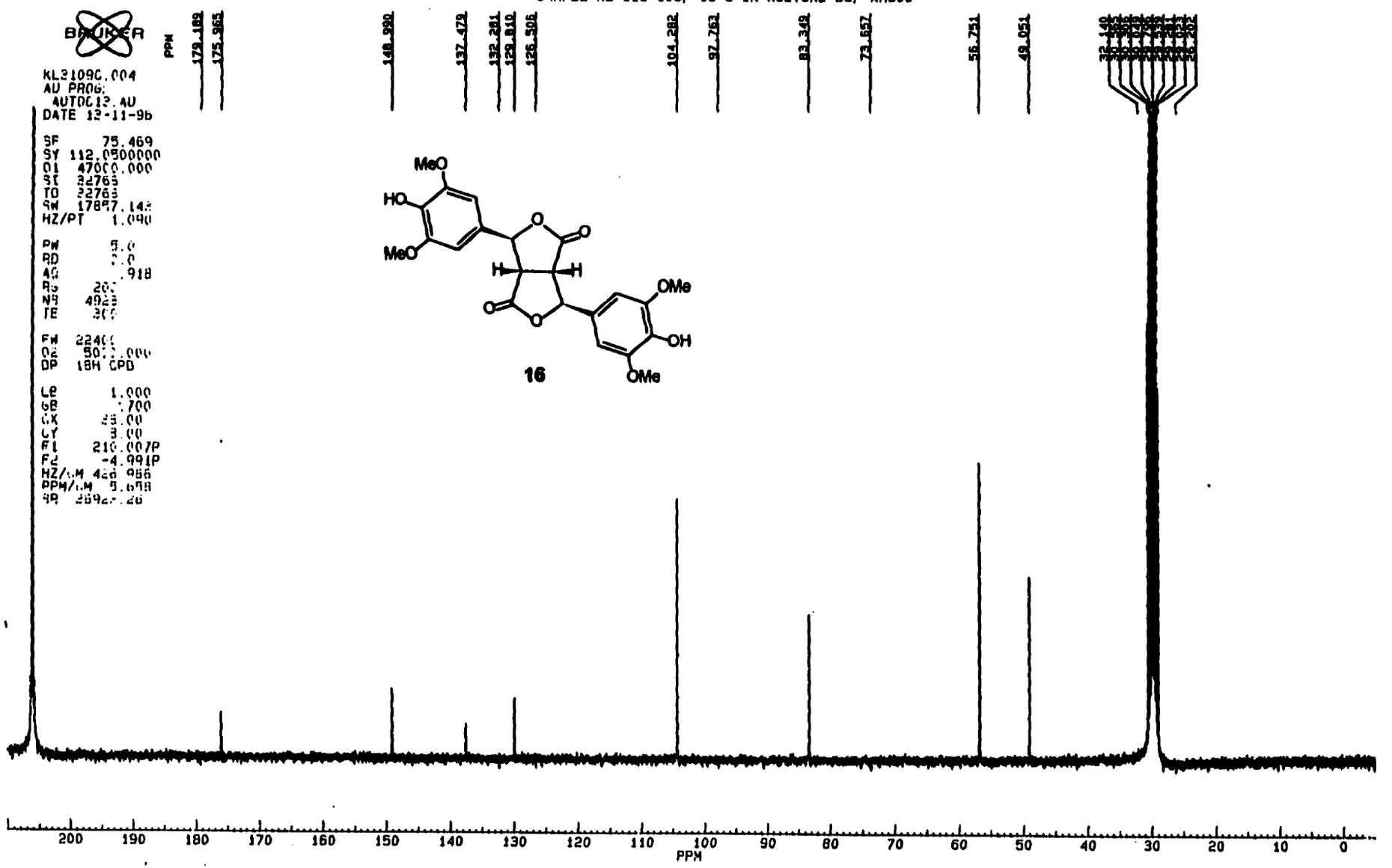
X = thomasidioic acid


SAMPLE KL-III-102A 13-C AT 75.47 MHZ IN ACETONE-D6
BRUKER
PPM
206.187
168.309
148.365
142.703
126.486
126.101
108.635
106.017
55.627
55.382
30.605
KL3102AC.004
AU PROG:
AUTOC13.AU
DATE 25-10-96
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 2880
TE 300
FW 22400
O2 5000.000
OP 18H CPD
LB 1.000
GB .700
CX 38.00
CY 16.00
F1 219.026P
F2 -8.968P
HZ/CM 452.802
PPM/CM 6.000
SR 30928.10
OMe
OH
MeO
HO2C
CO2H
HO
OMe
MeO
H
H
98
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
PPM

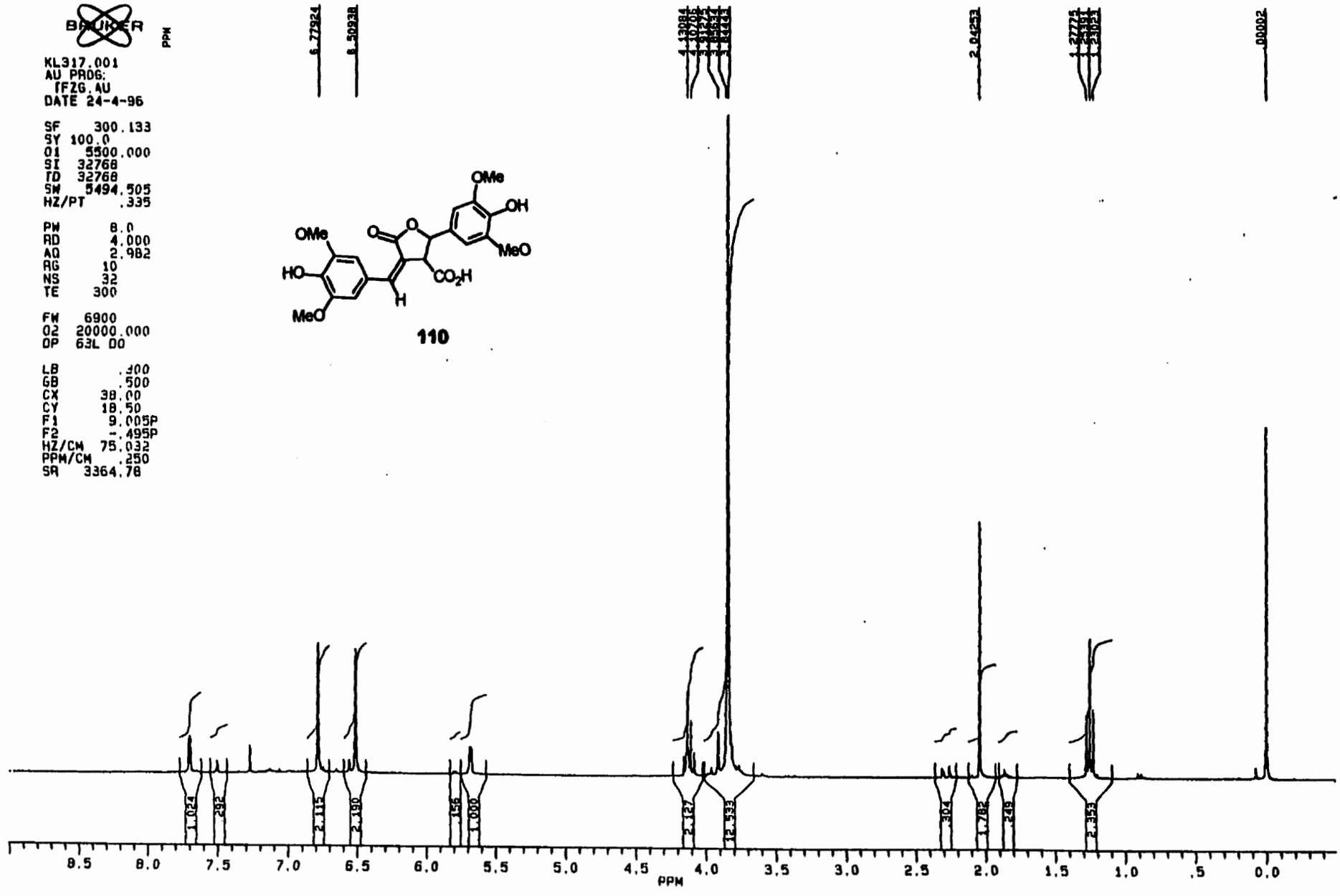
KL-III-109 1-H AT 300 MHZ IN ACET-D6

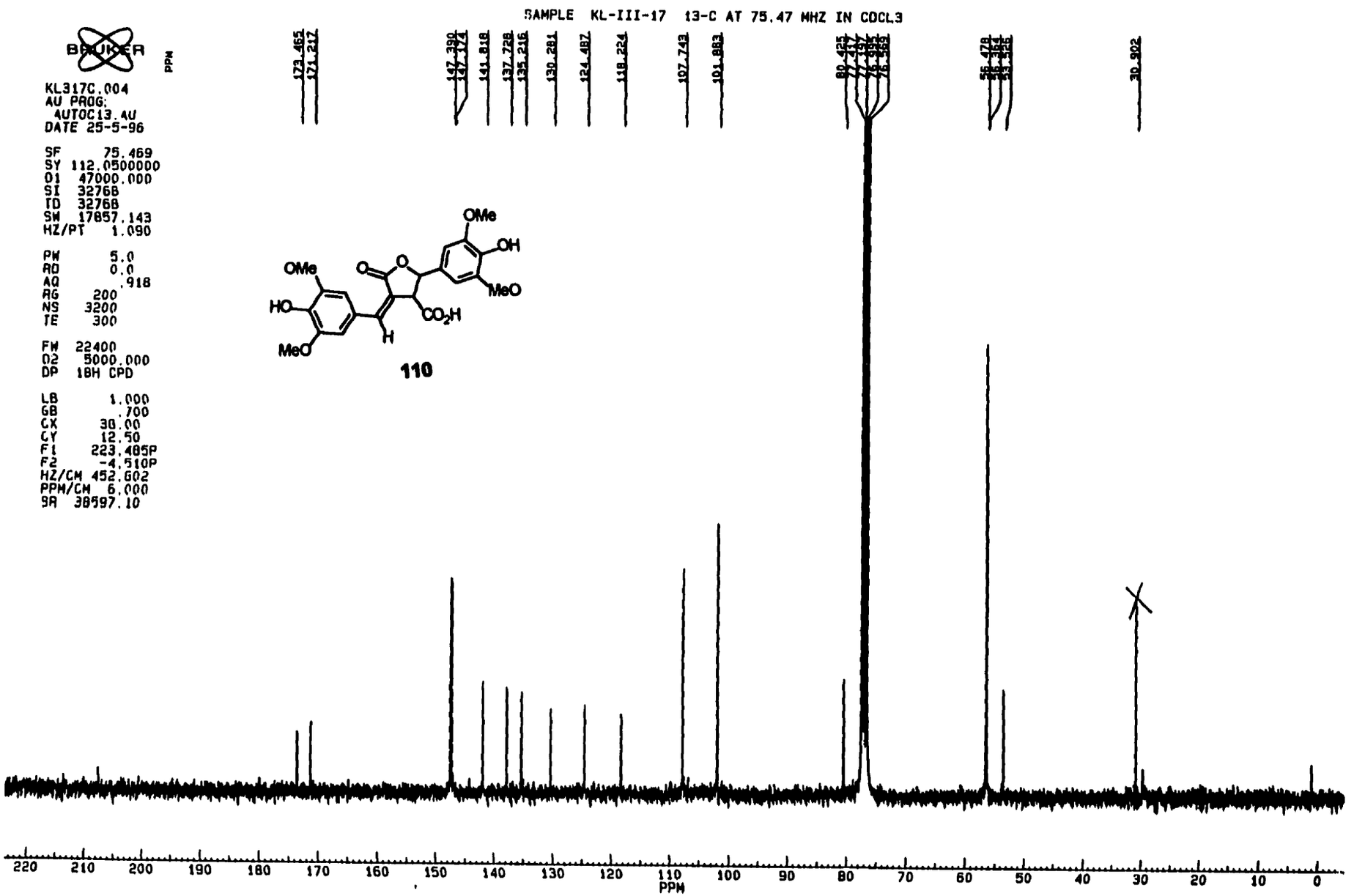


SAMPLE KL-III-109, 13-C IN ACETONE-D6, AM300

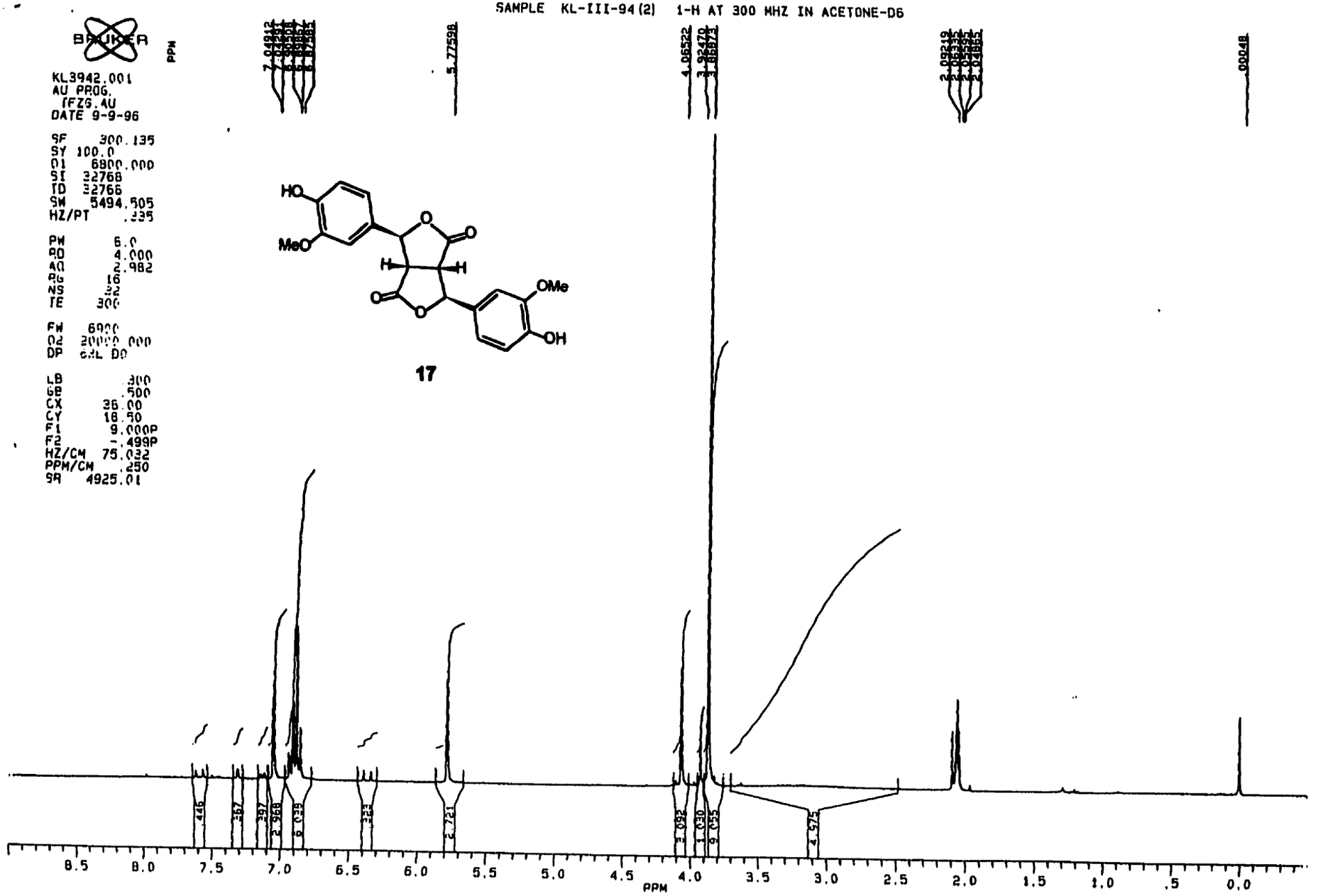


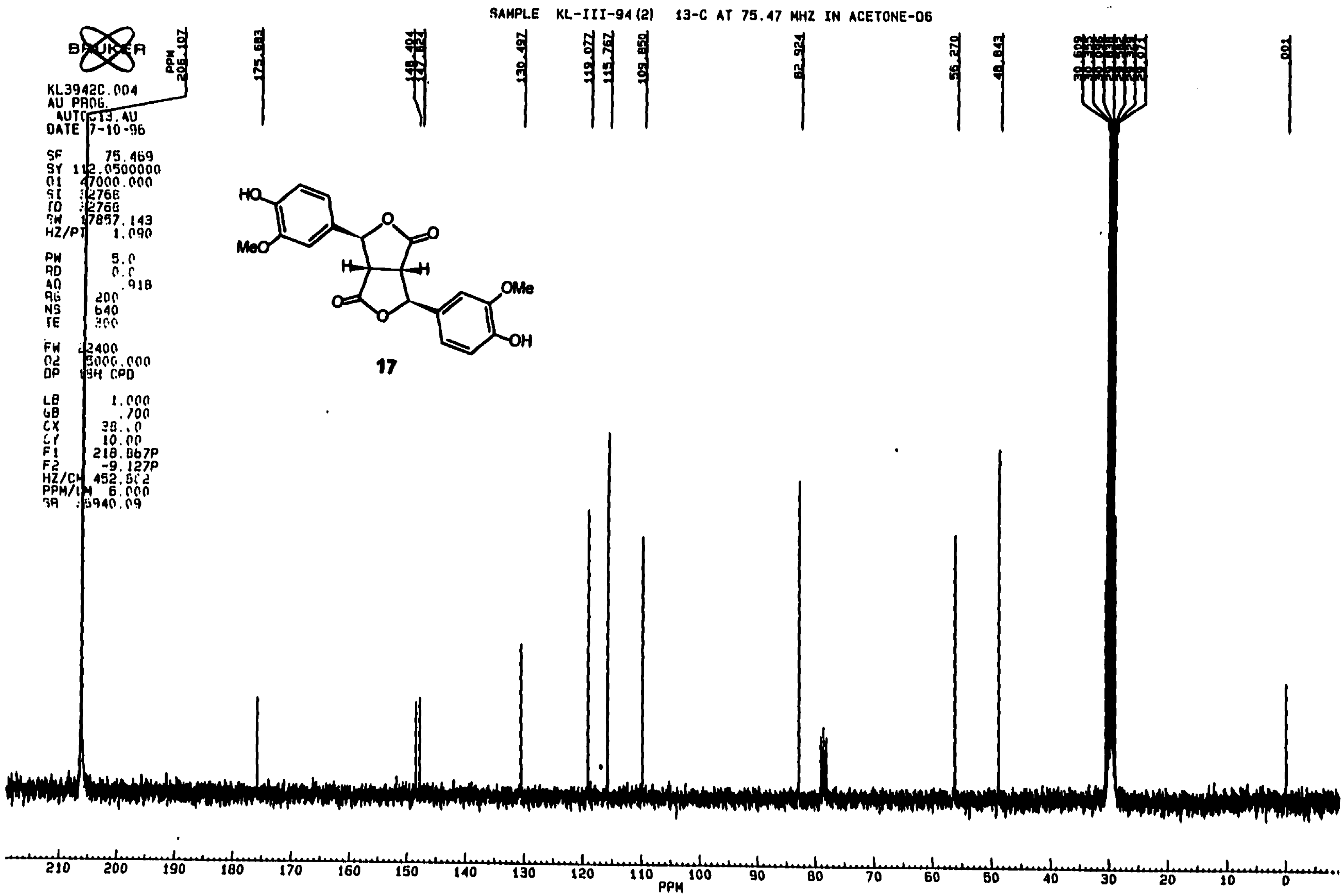
SAMPLE KL-III-17 1-H AT 300 MHZ IN CDCL3

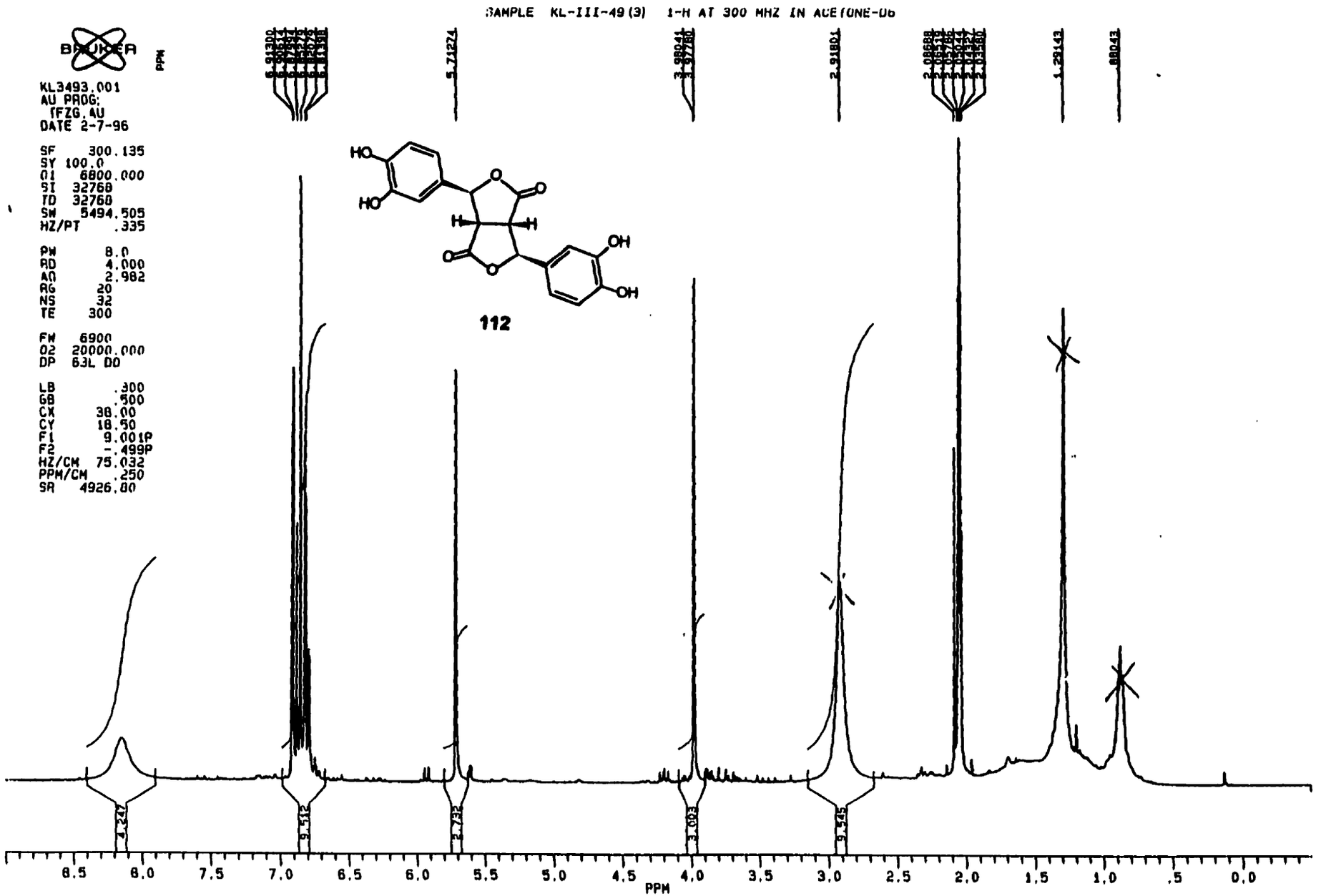


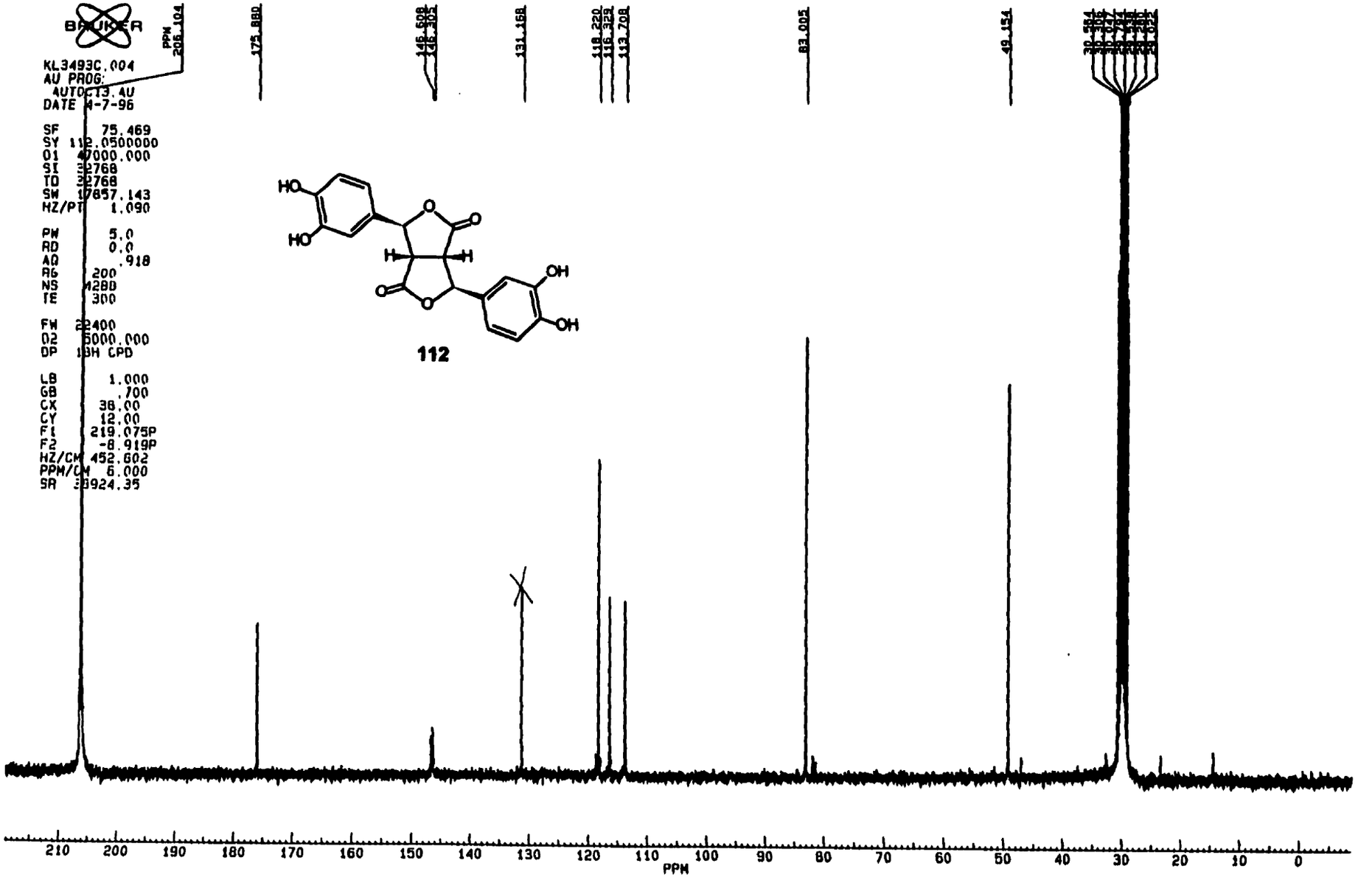

SAMPLE KL-III-17 13-C AT 75.47 MHZ IN CDCL3
BRUKER
PPM
KL317C.004
AU PROG:
AUTOC13.4U
DATE 25-5-96
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 3200
TE 300
FW 22400
O2 5000.000
DP 1BH CPD
LB 1.000
GB .700
CX 30.00
CY 12.50
F1 223.485P
F2 -4.510P
HZ/CM 452.602
PPM/CM 6.000
SR 38597.10
173.465
171.217
147.390
147.374
141.818
137.728
135.216
130.281
124.487
118.224
107.743
101.883
80.425
77.317
77.197
76.995
76.568
56.478
56.364
53.326
30.902
OMe
OH
MeO
HO
OMe
CO2H
H
MeO
110
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
PPM

SAMPLE KL-III-94(2) 1-H AT 300 MHZ IN ACETONE-D6

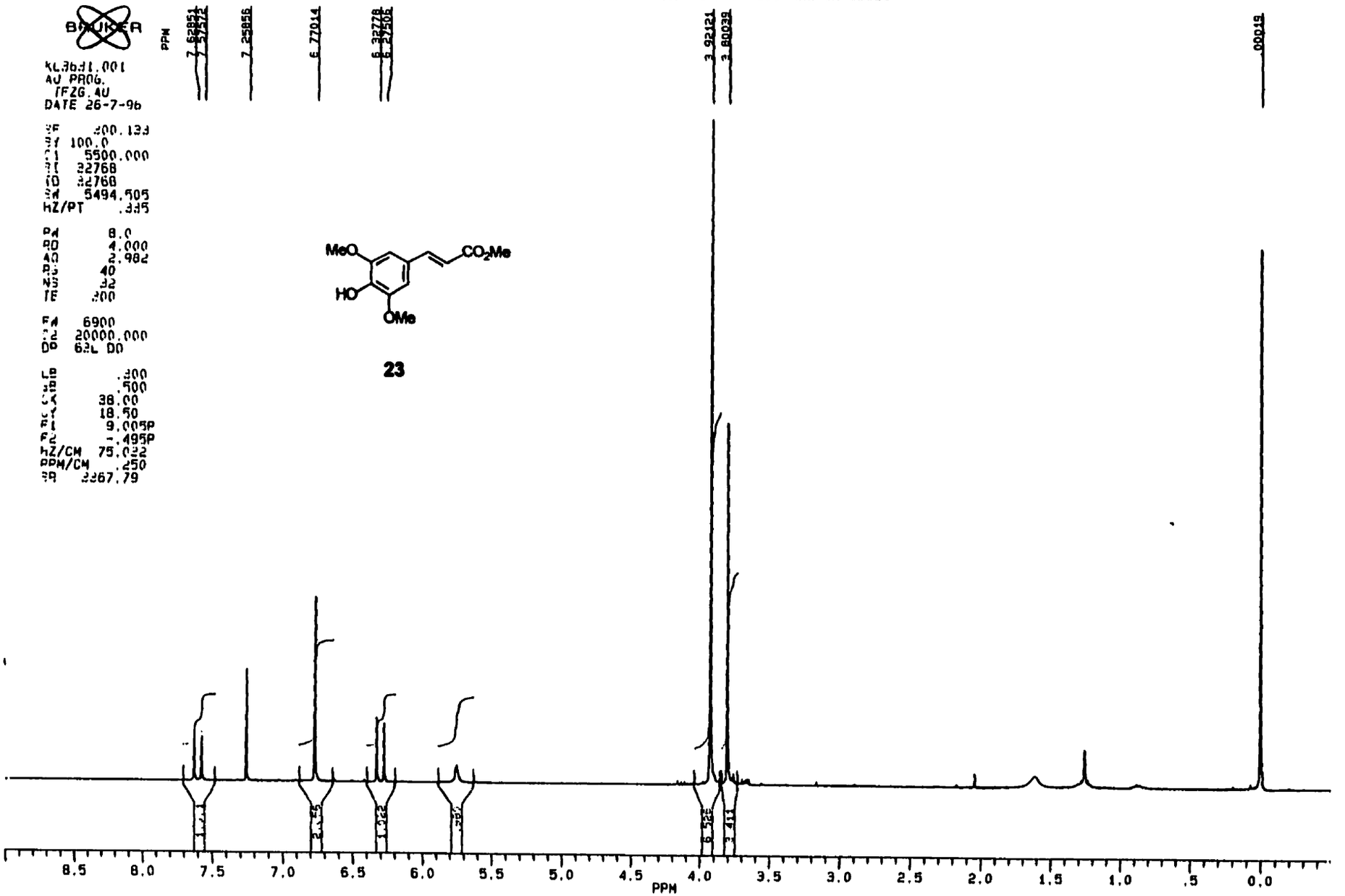


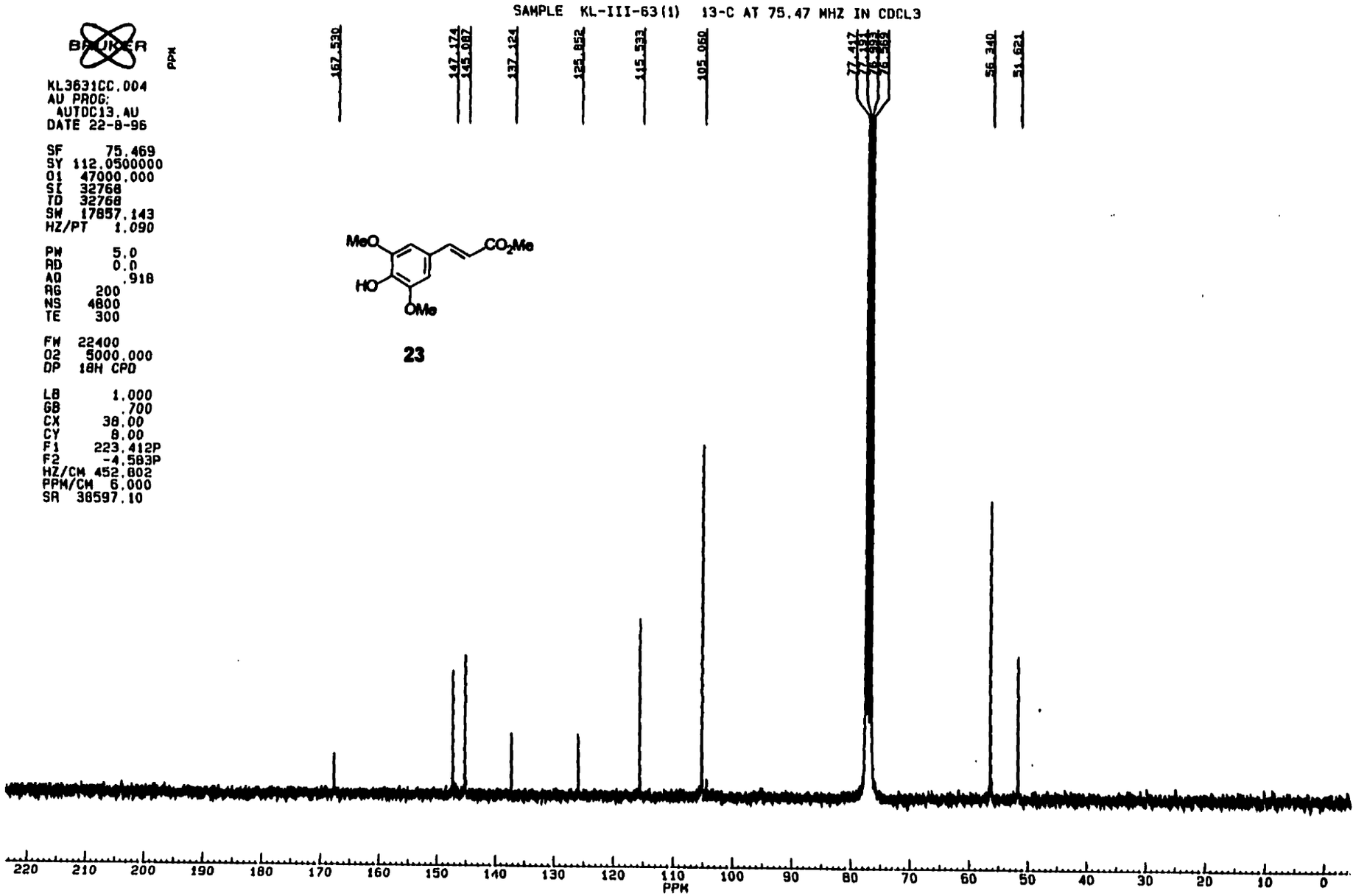

SAMPLE KL-III-94(2) 13-C AT 75.47 MHZ IN ACETONE-D6
BRUKER
KL3942C.004
AU PROG.
AUTOC13.AU
DATE 7-10-96
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 640
TE 300
FW 22400
O2 5000.000
DP 15H CPD
LB 1.000
GB .700
CX 38.0
CY 10.00
F1 218.867P
F2 -9.127P
HZ/CM 452.802
PPM/CM 6.000
SR 5940.09
PPM
206.107
175.683
148.404
147.821
130.497
119.077
115.767
109.850
82.924
56.270
48.843
30.609
29.071
.001
HO
MeO
OMe
OH
H
17
PPM

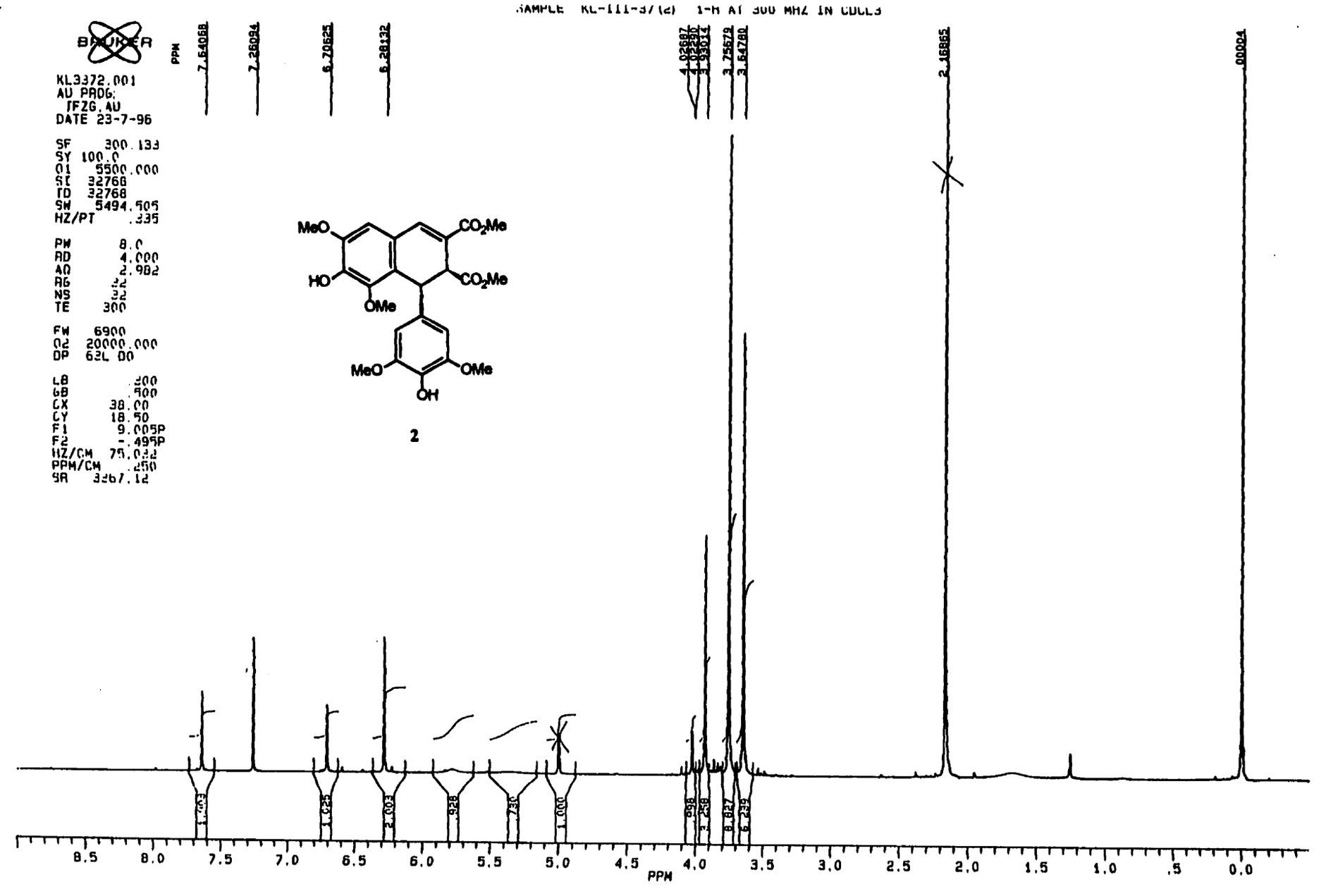

SAMPLE KL-III-49(3) 1-H AT 300 MHZ IN ACETONE-D6
BRUKER
PPM
KL3493.001
AU PROG:
1FZG.AU
DATE 2-7-96
SF 300.135
SY 100.0
O1 6800.000
SI 32768
TD 32768
SW 5494.505
HZ/PT .335
PW 8.0
RD 4.000
AQ 2.982
RG 20
NS 32
TE 300
FW 6900
O2 20000.000
DP 63L DD
LB .300
GB .500
CX 38.00
CY 18.50
F1 9.001P
F2 -.499P
HZ/CM 75.032
PPM/CM .250
SR 4926.80
HO
HO
H
H
O
O
O
OH
OH
112
5.71224
3.98041
3.97780
2.91801
1.29143
.88043
4.247
9.512
2.732
3.003
9.545
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM

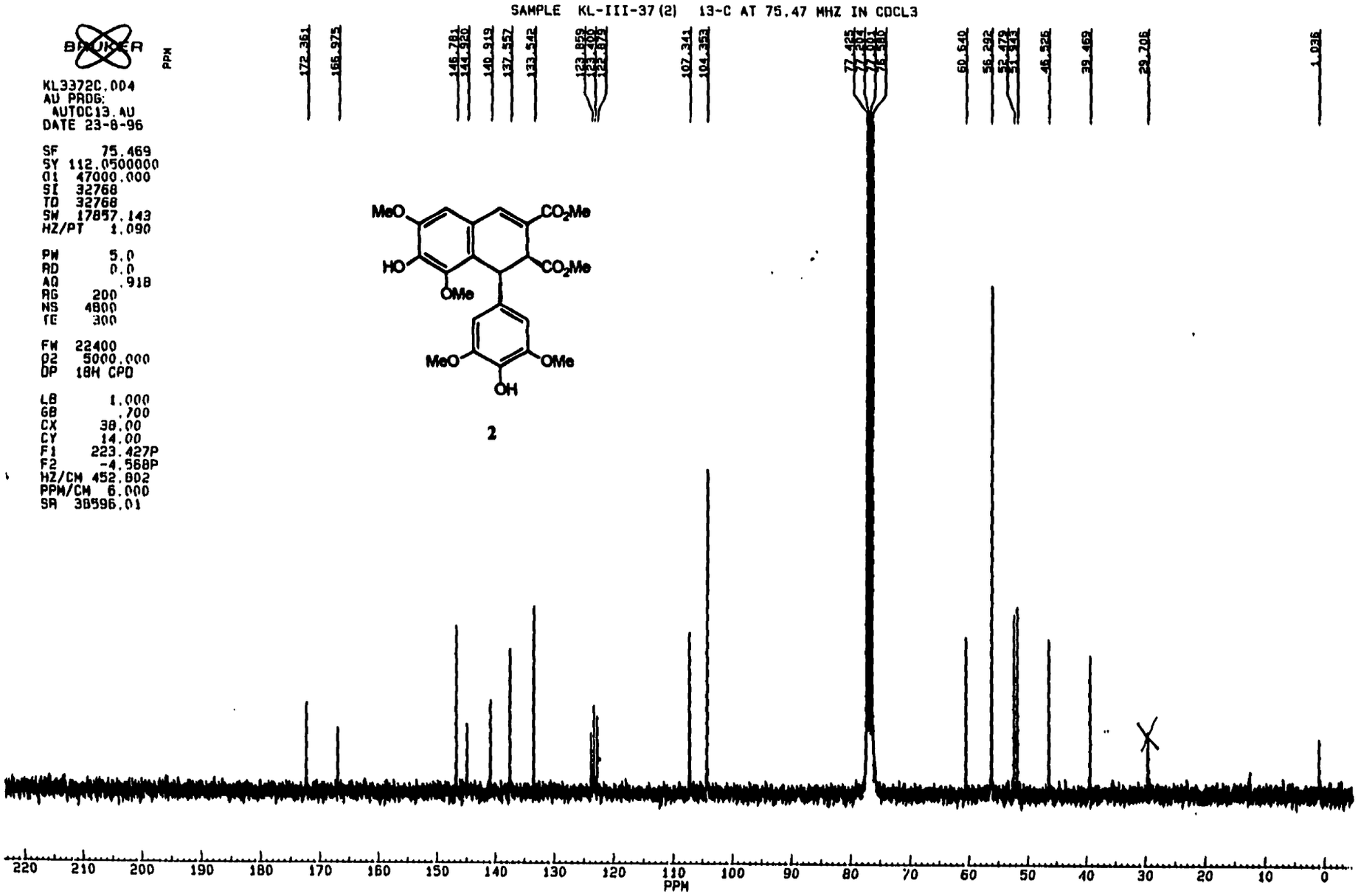

SAMPLE KL-III-49(3) 13-C AT 75.47 MHZ IN ACETONE-D6
BRUKER
PPM 206.104
175.880
146.608
146.305
131.158
118.220
116.329
113.708
83.005
49.154
KL3493C.004
AU PROG:
AUTOC13.AU
DATE 4-7-96
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 4288
TE 300
FW 22400
D2 5000.000
DP 1BH CPD
LB 1.000
GB .700
CX 38.00
CY 12.00
F1 219.075P
F2 -8.919P
HZ/CM 452.602
PPM/CM 6.000
SR 30924.35
HO
HO
O
O
O
H
H
OH
OH
112
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
PPM

SAMPLE KL-III-63(1) 1-H AT 300 MHZ IN CDCL3

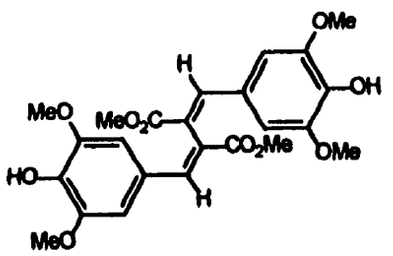



SAMPLE KL-III-63(1) 13-C AT 75.47 MHZ IN CDCL3
BRUKER
PPM
KL3631CC.004
AU PROG:
AUTOC13.AU
DATE 22-8-96
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 4800
TE 300
FW 22400
O2 5000.000
DP 18H CPD
LB 1.000
GB .700
CX 38.00
CY 8.00
F1 223.412P
F2 -4.583P
HZ/CM 452.602
PPM/CM 6.000
SR 38597.10
167.530
147.174
145.087
137.124
125.852
115.533
105.050
77.417
56.340
51.621
MeO
CO2Me
HO
OMe
23
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
PPM

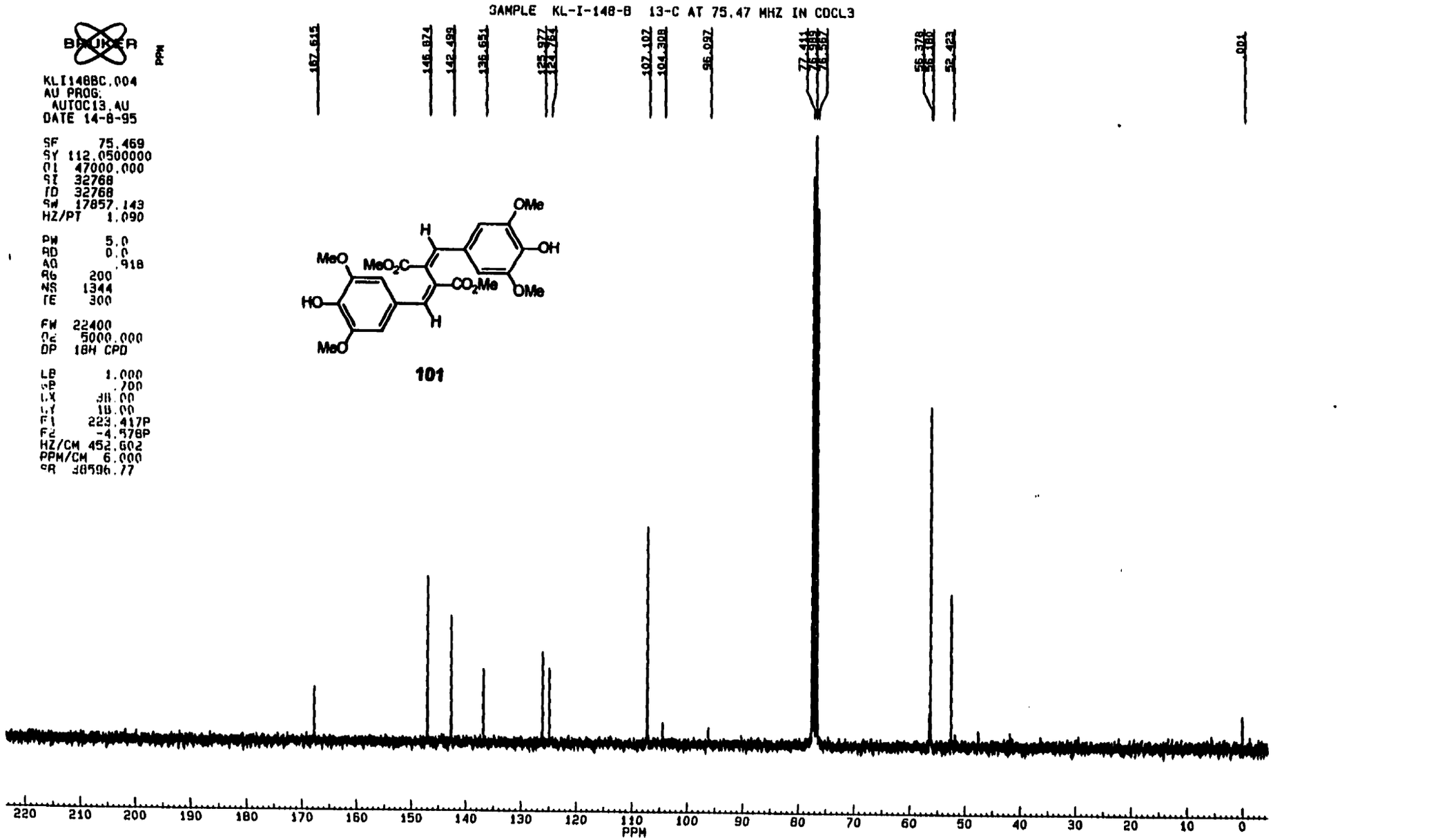

SAMPLE KL-111-37(2) 1-H AT 300 MHZ IN CDCL3
BRUKER
PPM
7.64068
7.26094
6.70623
6.28132
4.02887
4.02290
3.93614
3.75579
3.64780
2.16865
.00004
KL3372.001
AU PROG:
TFZG.AU
DATE 23-7-96
SF 300.133
SY 100.0
O1 5500.000
SI 32768
TD 32768
SW 5494.505
HZ/PT .335
PW 8.0
RD 4.000
AQ 2.982
RG 32
NS 32
TE 300
FW 6900
O2 20000.000
DP 62L 00
LB .200
GB .900
CX 38.00
CY 18.50
F1 9.005P
F2 -.495P
HZ/CM 75.032
PPM/CM .250
SR 3267.12
MeO
CO2Me
HO
CO2Me
OMe
MeO
OMe
OH
2
1.703
1.625
2.003
.928
.730
1.000
.596
1.258
5.827
6.239
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM


SAMPLE KL-III-37(2) 13-C AT 75.47 MHZ IN CDCL3
BRUKER
PPM
172.361
166.975
146.781
144.926
140.919
137.557
133.542
123.859
123.406
122.878
107.341
104.353
77.425
77.204
77.001
76.580
60.640
56.292
52.479
51.943
46.526
39.469
29.706
1.036
KL3372C.004
AU PROG:
AUTOC13.AU
DATE 23-8-96
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 4800
TE 300
FW 22400
O2 5000.000
DP 18H CPD
LB 1.000
GB .700
CX 38.00
CY 14.00
F1 223.427P
F2 -4.568P
HZ/CM 452.802
PPM/CM 6.000
SR 38596.01
MeO
CO2Me
HO
CO2Me
OMe
MeO
OMe
OH
2
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
PPM

SAMPLE KL-I-148B 1-H AT 300 MHZ IN CDCL3



101


SAMPLE KL-I-148-B 13-C AT 75.47 MHZ IN CDCL3
BRUKER
PPM
KLI148BC.004
AU PROG:
AUTOC13.AU
DATE 14-8-95
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 1344
TE 300
FW 22400
O2 5000.000
DP 18H CPD
LB 1.000
GB .700
LX 38.00
LY 18.00
F1 223.417P
F2 -4.578P
HZ/CM 452.602
PPM/CM 6.000
SR 38596.77
167.615
146.874
142.498
136.651
135.977
124.764
107.102
104.308
96.027
77.411
76.987
76.567
56.378
56.180
52.423
001
OMe
OH
H
MeO
MeO2C
CO2Me
OMe
HO
H
MeO
101
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
PPM

SAMPLE KL-I-148B 13-C 1H-COUPLED, FOCUSED ON CARBONYL C

12654.7

12647.9

BRUKER

HERTZ

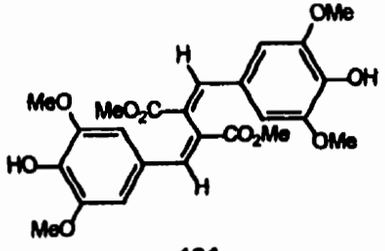
KLI148BC.200
DATE 19-8-95

SF 75.469
SY 112.0500000
O1 51249.573
SI 2048
TD 2048
SW 200.000
HZ/PT .195

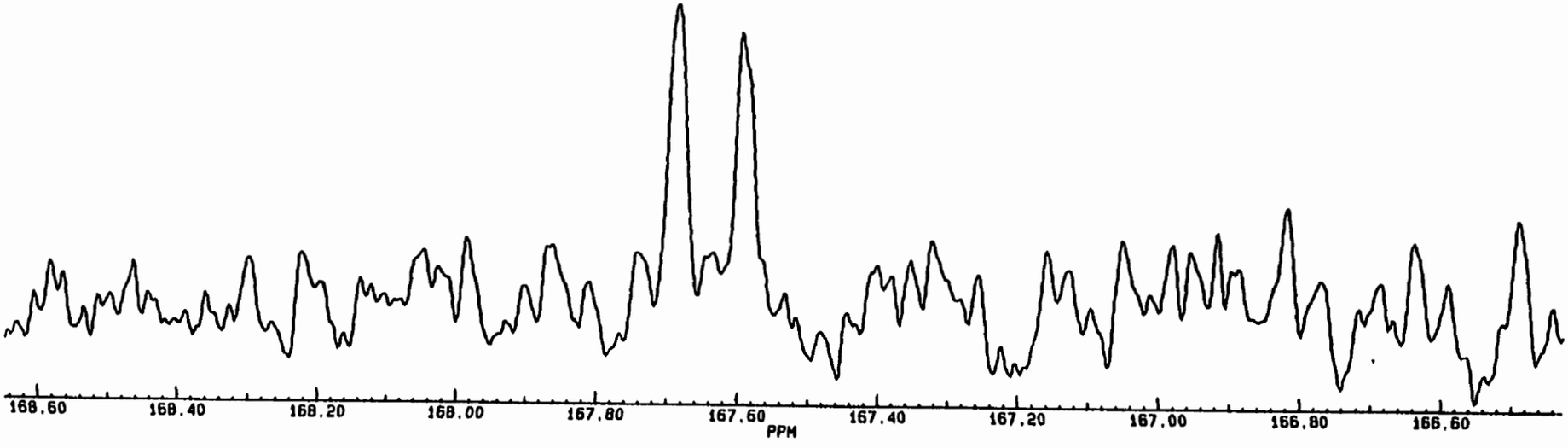
PW 6.0
RD 3.000
AQ 5.120
RG 400
NS 8000
TE 300

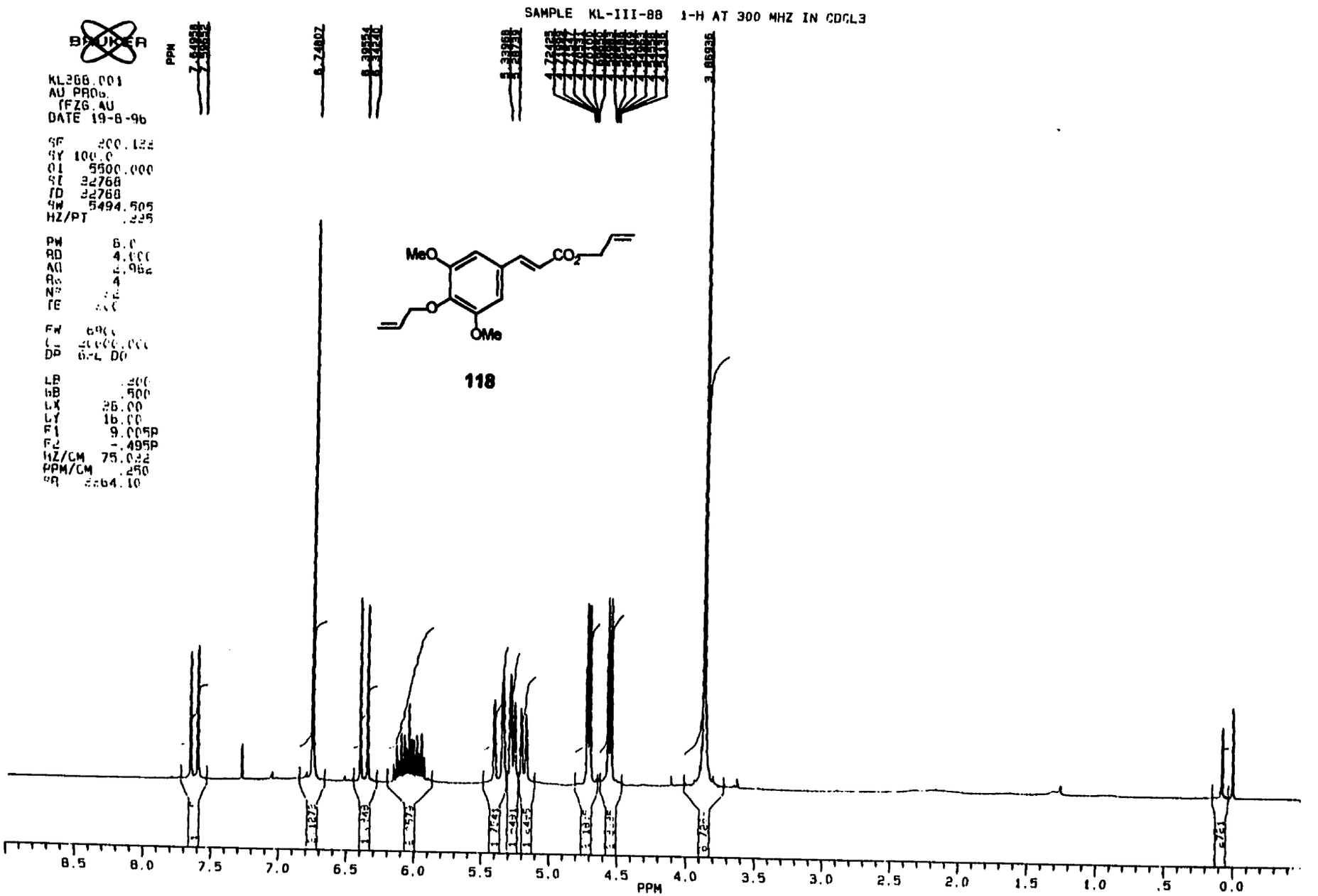
FW 300
O2 4476.150
DP 35L CW

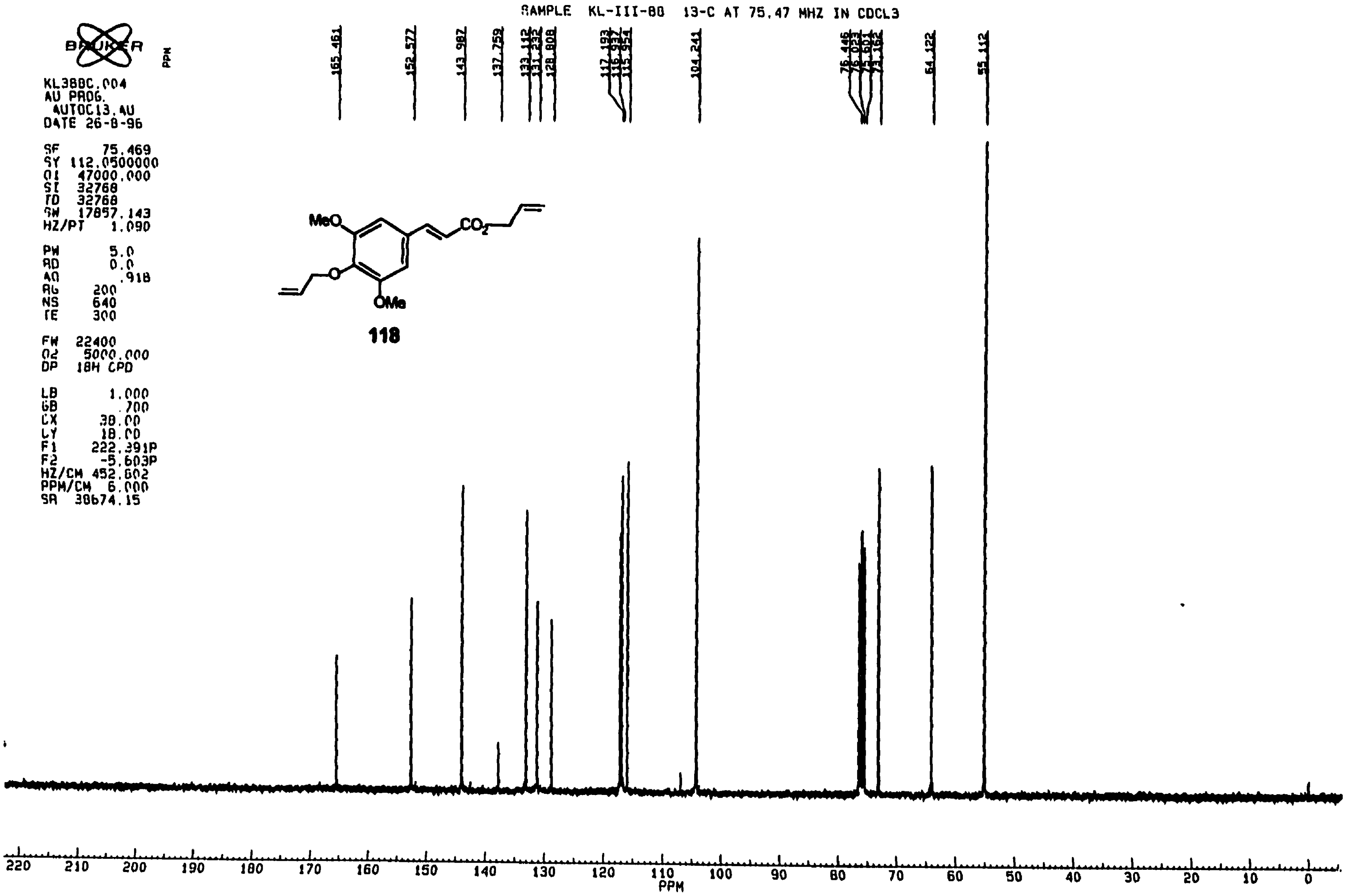
LB 1.000
GB .700
CX 30.00
CY 7.50
F1 168.646P
F2 166.426P
HZ/CM 4.410
PPM/CM .058
SR 38592.41



101

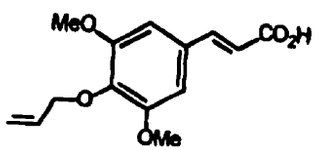



SAMPLE KL-III-88 1-H AT 300 MHZ IN CDCL3
BRUKER
PPM
KL388.001
AU PROG.
(FZG.AU
DATE 19-8-96
MeO
CO2
OMe
118
PPM

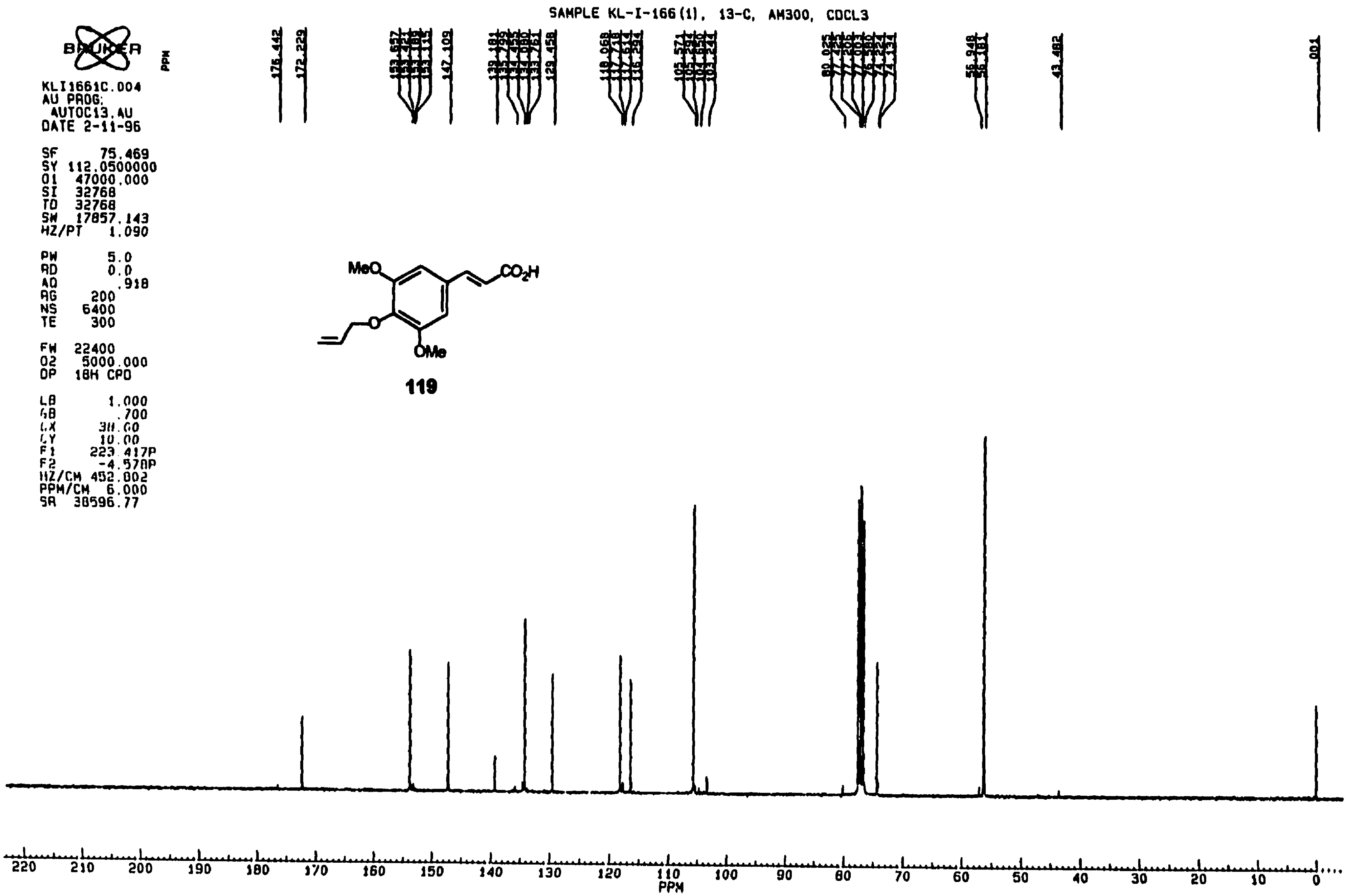


SAMPLE KL-III-80 13-C AT 75.47 MHZ IN CDCL3
BRUKER
PPM
KL380C.004
AU PROG.
AUTOC13.AU
DATE 26-8-96
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 640
TE 300
FW 22400
O2 5000.000
DP 18H CPD
LB 1.000
GB .700
CX 30.00
CY 18.00
F1 222.391P
F2 -5.603P
HZ/CM 452.602
PPM/CM 6.000
SR 30674.15
165.461
152.572
143.987
137.759
133.142
131.232
128.808
117.193
116.937
115.954
104.241
76.446
76.023
75.601
73.162
64.122
55.112
MeO
OMe
CO_2
O
118
220
210
200
190
180
170
160
150
140
130
120
110
PPM
100
90
80
70
60
50
40
30
20
10
0

SAMPLE KL-I-166(1) 1-H AT 300 MHZ IN CDCL3

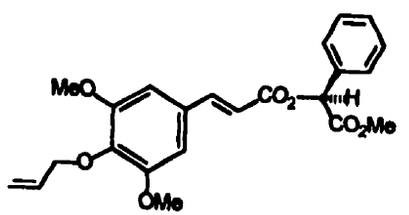


119


SAMPLE KL-I-166(1), 13-C, AM300, CDCL3
BRUKER
PPM
KLI1661C.004
AU PROG:
AUTOC13.AU
DATE 2-11-96
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 6400
TE 300
FW 22400
O2 5000.000
DP 18H CPD
LB 1.000
GB .700
CX 30.00
CY 10.00
F1 223.417P
F2 -4.570P
HZ/CM 452.802
PPM/CM 6.000
SR 30596.77
176.442
172.229
147.109
129.458
43.482
.001
MeO
OMe
CO_2H
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
PPM

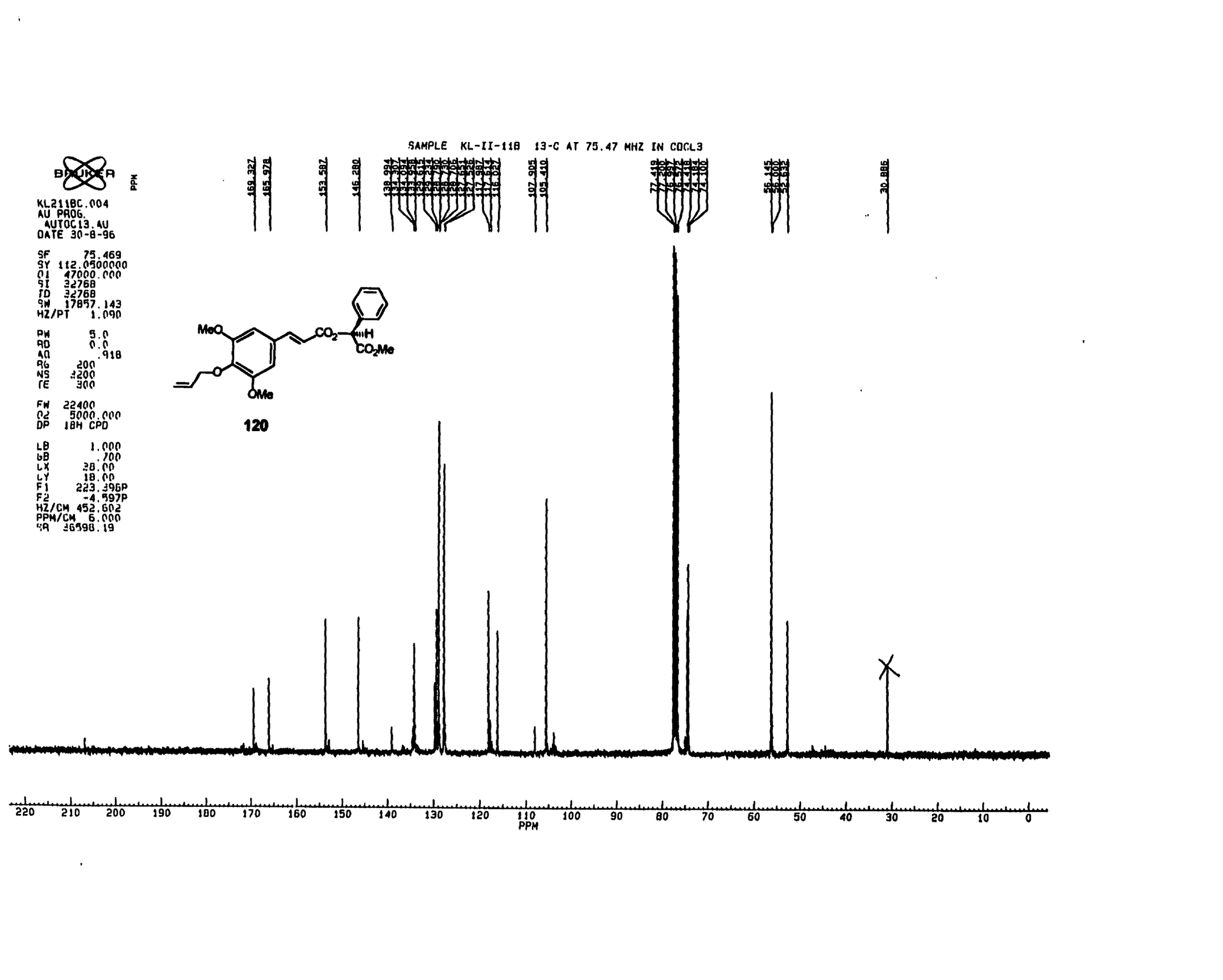
119

SAMPLE KL-II-11B 1-H AT 300 MHZ IN COCL3

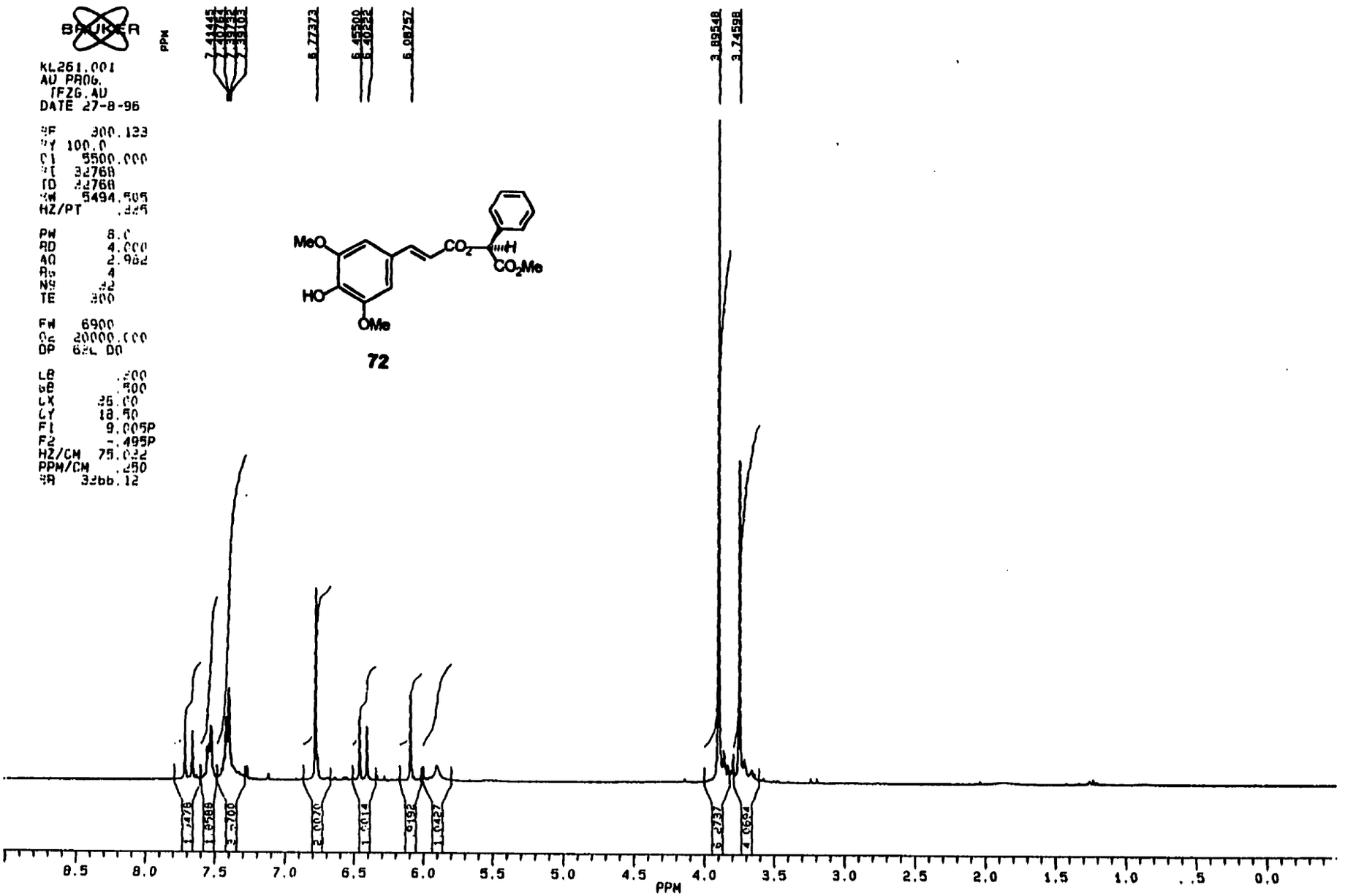


120

SAMPLE KL-II-118 13-C AT 75.47 MHZ IN CDCL3



SAMPLE KL-II-61 1-H AT 300 MHZ IN CDCL3



72

SAMPLE KL-II-61, 13-C AT 75.4 MHZ IN CDCL3

PPM

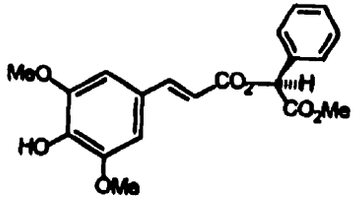
KLII61C.004
AU PROG:
AUTOC13.AU
DATE 1-11-96

SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090

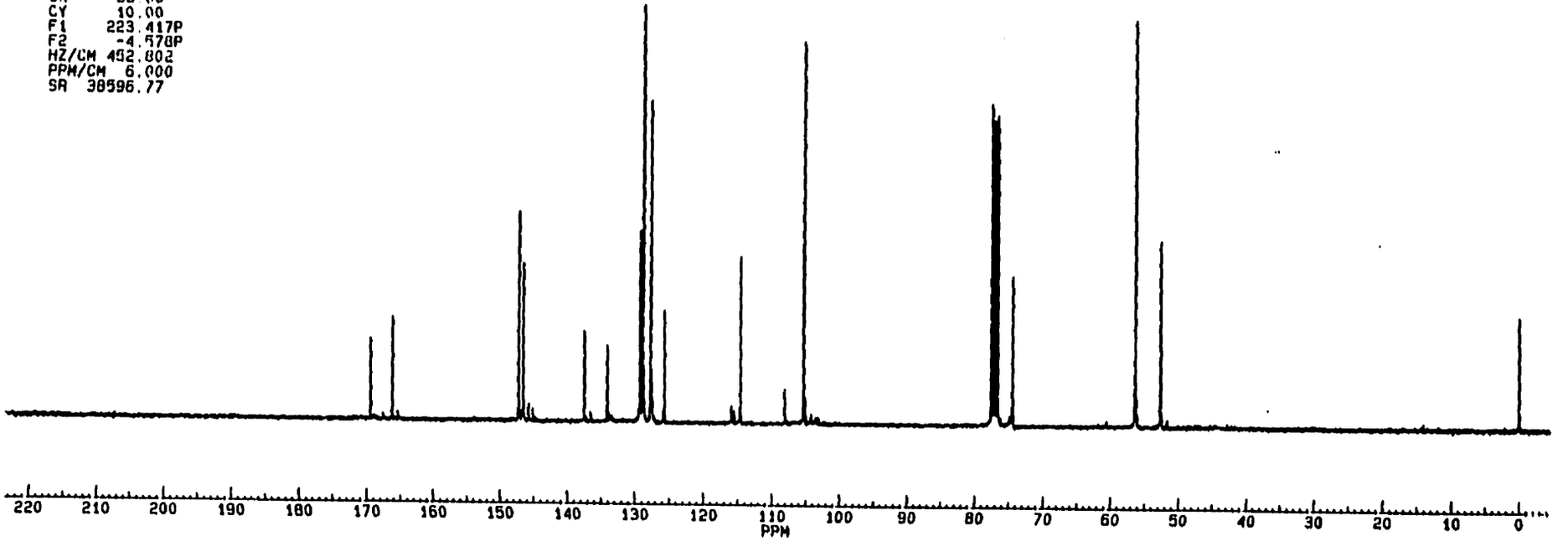
PW 5.0
RD 0.0
AQ .918
RG 200
NS 3456
TE 300

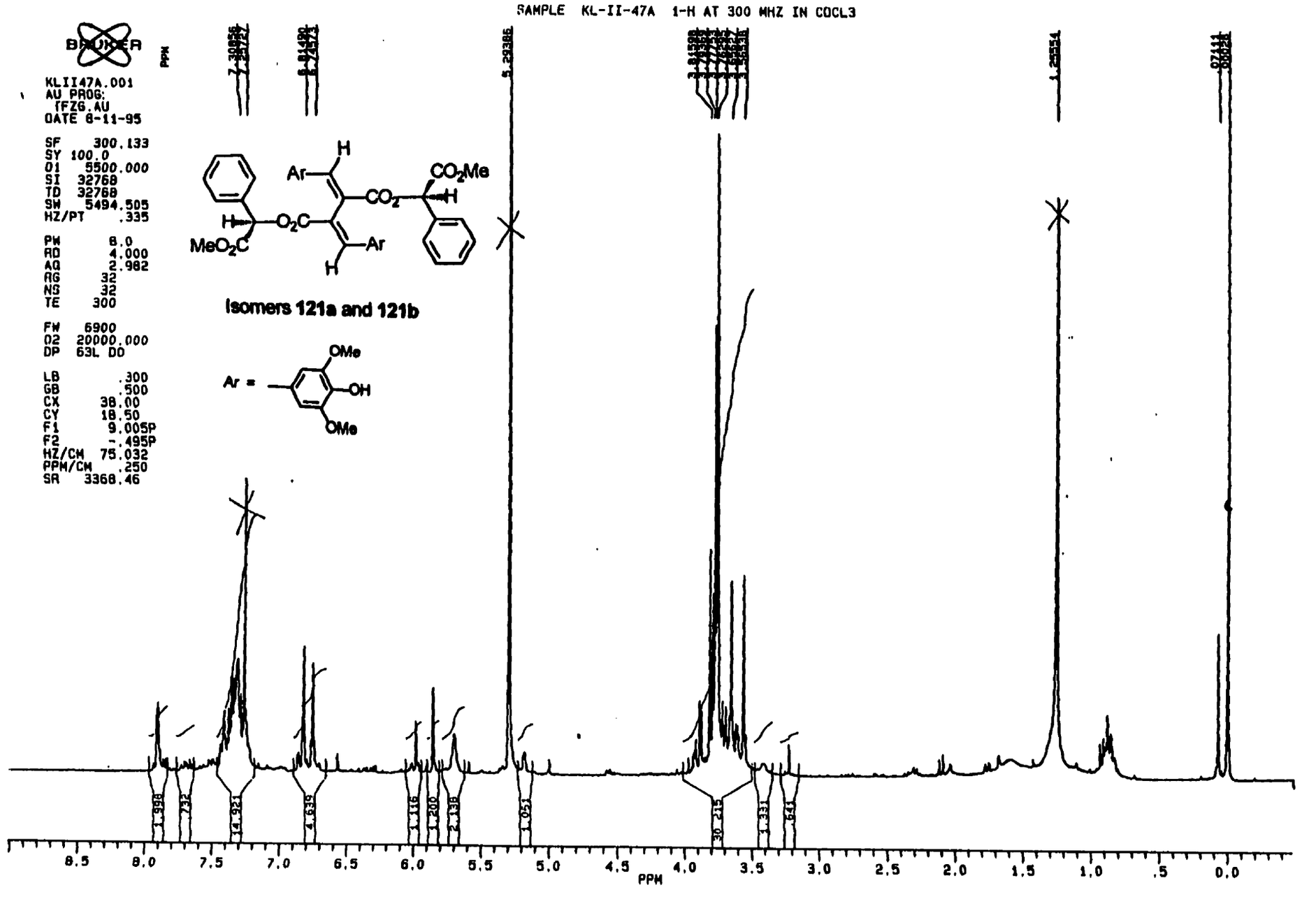
FW 22400
O2 5000.000
DP 18H CPD

LB 1.000
GB .700
CX 30.00
CY 10.00
F1 223.417P
F2 -4.578P
HZ/CM 452.802
PPM/CM 6.000
SR 38596.77

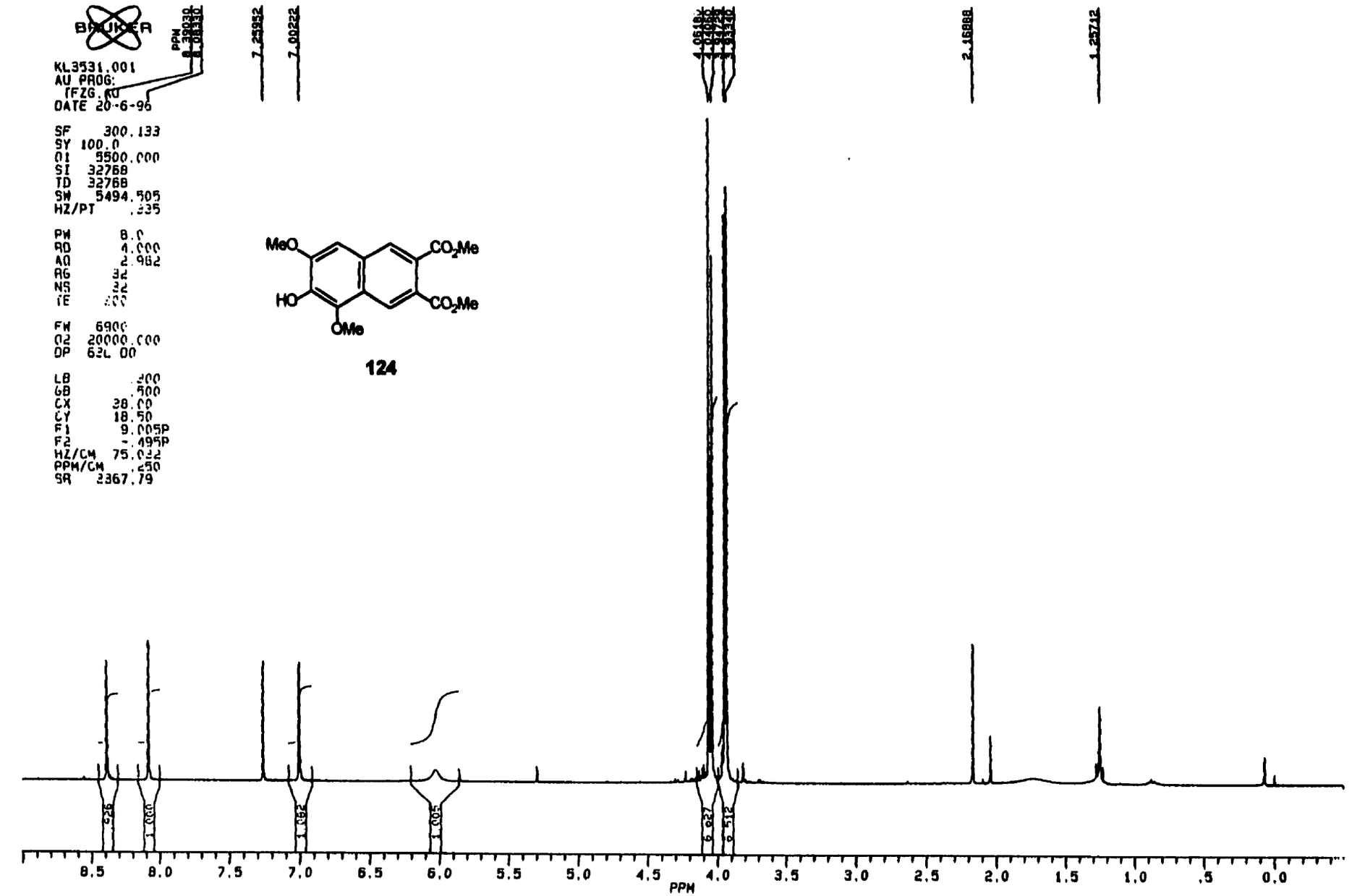


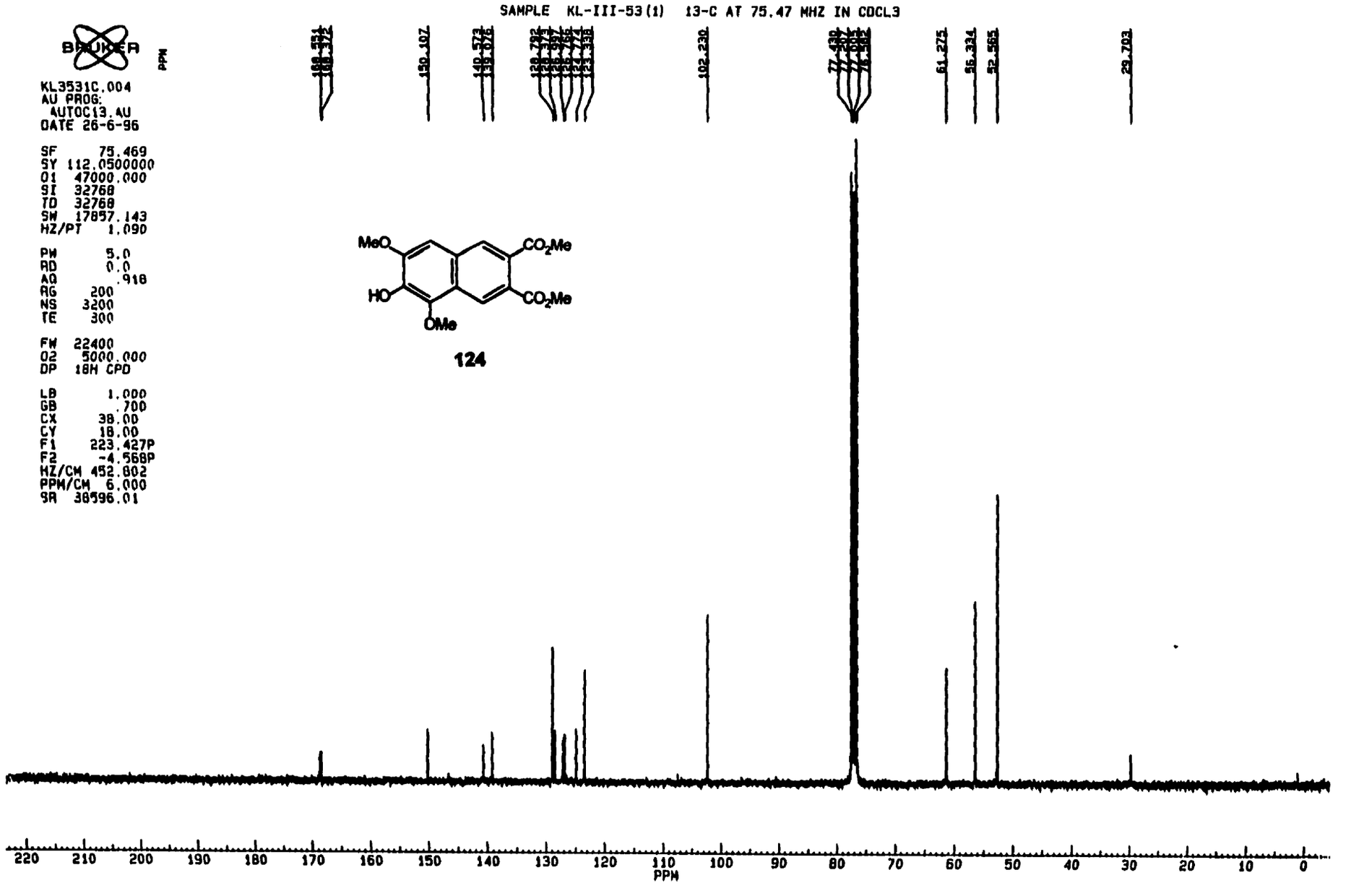
72




SAMPLE KL-II-47A 1-H AT 300 MHZ IN COCL3
BRUKER
PPM
KLII47A.001
AU PROG:
(FZG.AU
DATE 6-11-95
SF 300.133
SY 100.0
O1 5500.000
SI 32768
TD 32768
SW 5494.505
HZ/PT .335
PW 8.0
RD 4.000
AQ 2.982
RG 32
NS 32
TE 300
FW 6900
O2 20000.000
DP 63L DO
LB .300
GB .500
CX 38.00
CY 18.50
F1 9.005P
F2 -.495P
HZ/CM 75.032
PPM/CM .250
SR 3368.46
Ar
CO2Me
CO2
MeO2C
O2C
Isomers 121a and 121b
Ar =
OMe
OH
OMe
1.398
.732
14.927
4.639
1.116
1.200
2.138
1.051
30.215
1.331
.641
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM

SAMPLE KL-III-53(1) 1-H AT 300 MHZ IN CDCL3




SAMPLE KL-III-53(1) 13-C AT 75.47 MHZ IN CDCL3
BRUKER
PPM
KL3531C.004
AU PROG:
AUTOC13.AU
DATE 26-6-96
SF 75.469
SY 112.0500000
O1 47000.000
SI 32768
TD 32768
SW 17857.143
HZ/PT 1.090
PW 5.0
RD 0.0
AQ .918
RG 200
NS 3200
TE 300
FW 22400
O2 5000.000
DP 18H CPD
LB 1.000
GB .700
CX 38.00
CY 18.00
F1 223.427P
F2 -4.568P
HZ/CM 452.802
PPM/CM 6.000
SR 38596.01
150.107
102.230
61.275
56.334
52.565
29.703
MeO
CO2Me
HO
CO2Me
OMe
124
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
PPM