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THE REACTION OF CAFFEIC ACID AND COPPER(II)
IN NATURAL WATER

by

Ling Xiaozheng Xu

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Chemistry

Western Michigan University
Kalamazoo, Michigan
December 1994

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If it were only me, this dream would not have been fulfilled. This is for those who had contributed their guidance and support to the completion of this paper.

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Ling Xiaozheng Xu

THE REACTION OF CAFFEIC ACID AND COPPER(II) IN NATURAL WATER

Ling Xiaozheng Xu, M.A.

Western Michigan University, 1994

The interaction of the lignin precursor compound caffeic acid with copper(II) in aqueous solution was studied. In the presence of a reducible transition metal, such as copper, caffeic acid undergoes oxidation. Oxygen was shown to play an important role in the reaction. The UV-visible data demonstrated that the conjugated double bond in the propenoic acid side chain of caffeic acid slowly disappeared during the reaction. The molecular weight experiments indicated that the product was a dimer. FTIR spectrum agreed with two possible dimer structures involving the side chain of caffeic acid. In addition, the reaction was shown to be reversible. When heated, the dimer dissociated back into monomer. A mechanism of this reaction has been proposed. We believed the oxidation of caffeic acid in the presence of copper(II) may play an important role in the formation of humic substances in natural water.

TABLE OF CONTENTS

ACKNOWLEDGMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
CHAPTER	
I. INTRODUCTION	1
Organic Species and Transition Metals in Natural Water	1
Humic Substances	2
Lignin	3
Caffeic Acid in Natural Water	6
II. LITERATURE REVIEW	8
Caffeic Acid and Copper Complexation	8
Dimerization Mechanism	9
Ether Linkage Dimerization	9
Cyclobutane Linkage Dimerization	10
III. MATERIALS AND TECHNIQUES	13
Materials	13
Solution Preparations	13
Caffeic Acid Solution	13

Table of Contents - Continued

CHAPTER

Chloride Solution	14
Caffeic Acid and Copper(II) Chloride Mixture	14
Physical Appearance of the Mixture	14
Techniques	15
Calorimetry	15
UV-Visible Absorption Spectrometry	17
Molecular Weight Determination	17
Fourier Transform Infrared Absorption	21
Gas Chromatography/Mass Spectrometry	22
Physical Effects	23
Effect of Temperature	23
Oxygen Consumption	23
Melting Point Measurement	24
Effect of Light	24
Effect of Copper(II)	25
IV. RESULTS DISCUSSION AND PROPOSED MECHANISM ...	26
Results Discussion	26
Techniques	26

Table of Contents - Continued

CHAPTER

Physical Effects	38
Summary	43
Proposed Mechanism	46
V. CONCLUSIONS	49
BIBLIOGRAPHY	51

LIST OF TABLES

1. Molecular Weight Determination on the Product (Unit: g/mol)	34
2. FT-Infrared Data of the Dimer Product	36
3. Effect of Temperature on the Dimer Product	38
4. Oxygen Consumption for the Reaction of Caffeic Acid With Copper(II) (1:1)	39

LIST OF FIGURES

1. Three Major Structural Monomer Units of Lignin	5
2. Some Phenolic Derivatives of Lignin Degradation Products	5
3. The Biosynthetic Pathway From Caffeic Acid to Lignin	6
4. Two Dimer Products of Caffeic Acid Proposed by Cilliers	10
5. The Structure of α -Dimer and β -Dimer Proposed by Cohen	11
6. Photodimerization Reaction of o-Hydroxycinnamic Acid	12
7. Thermogram of 4-Hydroxycinnamic Acid and Copper(II)	16
8. Cooling Curve of a Pure Solvent	19
9. Warming Curve of a Solution	19
10. Viscometer	21
11. The Reaction of Caffeic Acid Derivatization	23
12. Thermogram of Caffeic Acid and Copper(II) on 1:4 Ratio	27
13. Hydrocaffeic Acid	27
14. Thermogram of Caffeic Acid and Copper(II) on 1:4, 1:8, 1:14 Ratio	28
15. UV-Visible Absorption Spectrum of Caffeic Acid and Copper(II) Mixture on Day One	30
16. UV-Visible Absorption Spectrum of Caffeic Acid and Copper(II) Mixture After Three Months	31

List of Figures - Continued

17. UV-Visible Absorption Spectrometric data of Caffeic Acid and Copper(II) at Absorbance 316 nm for Three Months	32
18. Plot of Ln(absorbance) Versus Days in the First 30 Days of Caffeic Acid and Copper(II) Reaction	33
19. The Structures of Two Dimers Illustrated From FTIR Data	35
20. Mass Spectra of the Product (Top) and Pure Caffeic Acid (Bottom)	37
21. DSC Thermogram of Pure Caffeic Acid	40
22. DSC Thermogram of Caffeic Acid and Copper(II)	41
23. Effect of Copper(II) on Caffeic Acid Dimerization	44
24. Proposed Mechanism	48
25. The Net Reaction	50

CHAPTER I

INTRODUCTION

In recent years, more and more people have become interested in studying the environmental influences of organic substances, especially humic materials, in aquatic systems. Many studies have emphasized on how these materials affect the fate of organic and inorganic pollutants in natural water. In this research copper and caffeic acid, a lignin precursor compound, have been used as a model system to study the interaction of organic materials with transition metals in natural water.

Organic Species and Transition Metals in Natural Water

The activity of transition metals in aquatic systems and their impact on aquatic life vary depending upon the species with which metals are associated (Singer, 1973). Most significant among these associations is the interaction between metals and natural organic compounds in the water. These organic compound may originate from natural processes such as vegetative decay or be the result of pollution from organic discharges of municipal and industrial sources. Many of these natural organic compounds have a remarkable affinity and

capacity to bind metals. This organic-metal complexation would naturally alter the reactivity of the metals in the aquatic system.

Humic Substances

Certain natural organic compounds, usually referred to as humic substances, often associate with transition metals. These humic substances can influence the transport of metals and the redox state of metal ions and other species. Humic substances interact with metal ions by complexation and ion-exchange mechanisms. Depending upon the circumstances, this interaction may lead to solubilization or immobilization of the metal (Suffet, 1989).

These humic substances are primarily products of natural vegetative decay and are ubiquitous throughout the aquatic environment. Because of their complex, multicomponent nature, humic substances cannot be described in specific molecular terms. They are normally defined in terms of three somewhat overlapping classifications: fulvic acid, humic acid, and humin (Suffet, 1989).

1. Humic Acid: the fraction of humic substances that is insoluble in water under acidic conditions ($\text{pH} < 2.0$) but is soluble at higher pH values.

2. Fulvic acid: The fraction of humic substances that is soluble in water under all pH conditions.

3. Humin: the fraction of humic substances that is insoluble in water at any pH.

From the quantitative point of view humic acid-type polymers are the most important constituents of natural humus deposits (Povoledo, 1973). They are believed to consist primarily of enzymatically or autoxidatively polymerized phenolic units with varying amounts of amino acids, peptides and other substances linked into the molecules. In the past investigators (Povoledo, 1973) have considered plant phenolic substances such as lignins and flavanoids as the most important source of phenolic constituent units of humic material: Lignin -----> Phenolic Constituent Unit -----> Humic acid.

Lignin

Lignin is a generic name for a group of complex aromatic polymers that are major components of vascular plant tissues. Lignin is abundant in natural water. In terms of weight it is probably second only to cellulose among renewable organic materials and in terms of energy content it might well be the single most abundant (Kirk, et al., Vol II, 1981). Lignin, however, is also a major waste product of pulp manufacture. Much of these lignins are converted to humus, thereby importing desirable acid-base and complexation properties to soil and natural waters. Studies (Suffet, 1989) have shown that humic substances have

some well established functionalities and properties. They are polyelectrolytes with various functional groups which mainly consist of carboxyl and phenolic functionalities. These carboxyl and phenolic groups are responsible for the acid-base, complexation, and salt formation capabilities of humic substances.

Lignins are generally classified into three major groups based on their structural monomer units (Kirk, et al., Vol I, 1981). The three structural monomer units are shown in Figure 1:

1. Gymnosperm lignin is a dehydrogenation polymer of coniferyl alcohol.
2. Angiosperm lignin is a mixed dehydrogenation polymer of coniferyl and sinapyl alcohols.
3. Grass lignin is a mixed dehydrogenation polymer of coniferyl, sinapyl, and p-coumaryl alcohols.

Hydrocinnamates, especially caffeic acid derivatives, are common precursors of these three structural monomer units. Caffeic acid has a structure similar to coniferyl alcohol.

Lignin is abundant in natural waters. Much of lignin is converted to humus through biodegradation. During lignin degradation different compounds are formed. Most of these degradation products are phenolic derivatives with different numbers of methoxyl groups or hydroxyl groups, see Figure 2. Among these phenolic derivatives, caffeic acid is

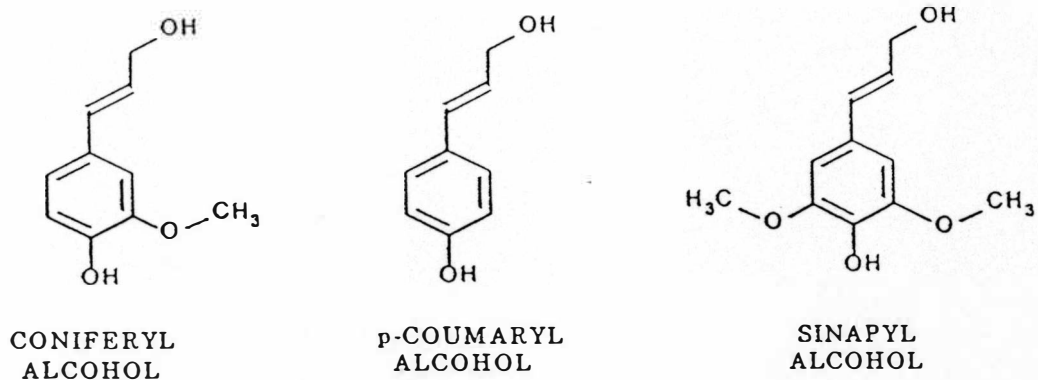


Figure 1. Three Major Structural Monomer Units of Lignin.

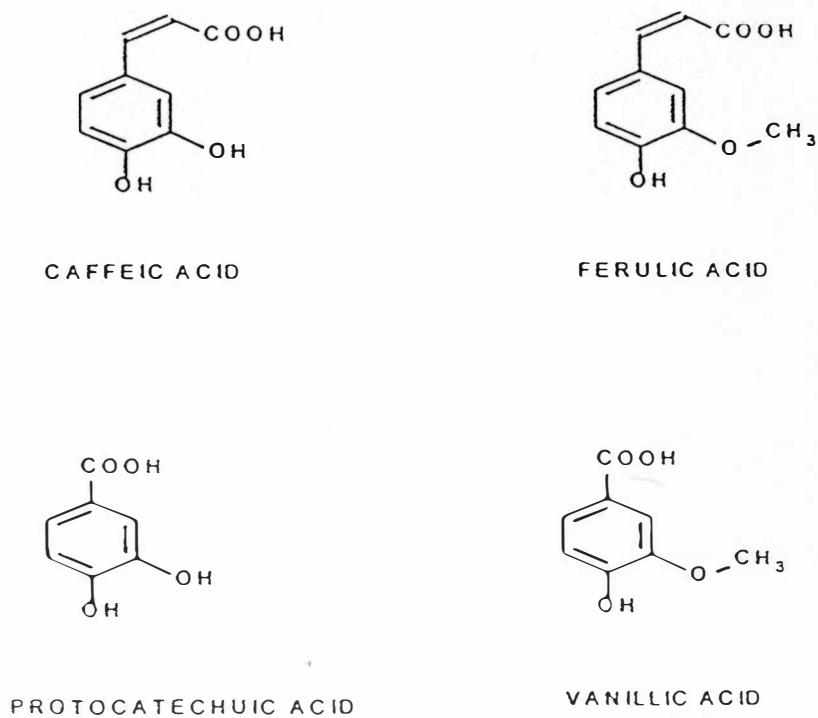


Figure 2. Some Phenolic Derivatives of Lignin Degradation Products.

one of the important oxidation products as well as a precursor of lignin.

The pathway from caffeic acid to lignin is shown in Figure 3.

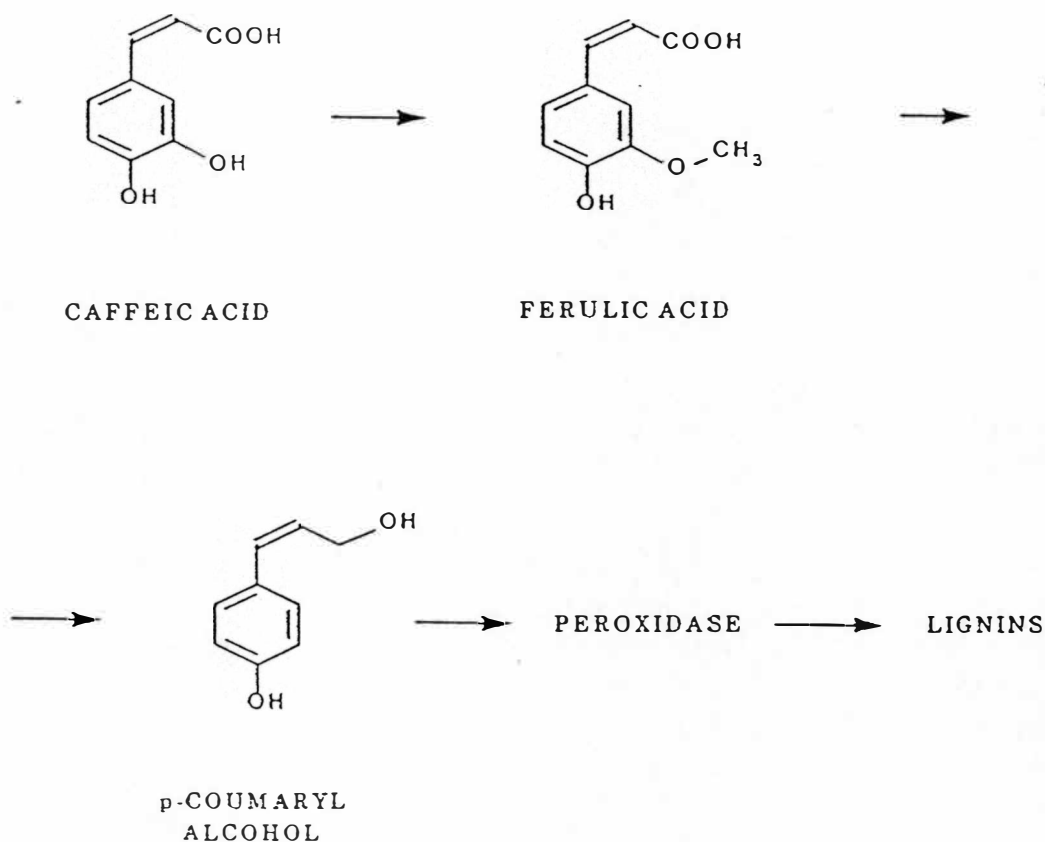


Figure 3. The Biosynthetic Pathway From Caffeic Acid to Lignin.

Caffeic Acid in Natural Water

Caffeic acid [3-(3, 4-dihydroxyphenyl)-2-propenoic acid or 3,4-dihydroxycinnamic Acid] has a molecular weight of 180.15 g/mol. It forms yellow crystals and decomposes at 223-225°C. Caffeic acid is

slightly soluble in cold water and soluble in hot water and cold alcohol. Caffeic acid is thought to be a precursor compound of lignin as well as an oxidation product of lignin as has been mentioned above.

Caffeic acid is known to be present in the root exudates (Linder and Voye, 1987). It is believed that caffeic acid is one of the most important phenolic compounds involved in the transport of metal ions from the surrounding soil to the plant roots (Linder and Voye, 1987). Studies have indicated that caffeic acid was involved not only in the transport of iron from the surrounding soil solution to roots but also in the reduction of iron(III) to iron(II). In this regard it has been established that the bioavailable form of iron in natural water is the iron(II) aquo ion (Linder and Voye, 1987). Apart from this, caffeic acid can also potentially form complexes with various other metal ions that occur in nutrient and soil solutions.

This research utilized caffeic acid and copper as a model to develop a better understanding of the interaction of organic materials and transition metals in natural waters. All experiments in this research were performed in or close to the natural conditions so that the studies could eventually be applied to natural water systems.

CHAPTER II

LITERATURE REVIEW

Caffeic Acid and Copper Complexation

In 1959, Timberlake studied the complex formation between copper and some organic species, such as phenols and phenolic acids, occurring in fruit (Timberlake, 1959). His potentiometric titration data indicated the formation of complexes between caffeic acid and copper(II) as CuA^- and CuA_2^{4-} .

Later in 1980s, several complexation studies of the binding ability of caffeic acid with copper(II) were published. One study demonstrated that caffeic acid easily forms oligomeric structures with copper(II) ions (Linder and Voye, 1987). Two other studies (Lamy et al., 1985 and Lamy et al., 1988) found only simple monomeric complexes, probably involving the catechol-type of coordination. Kiss (1989), concluded caffeic acid in complexation reaction exhibits a real ambidentate character, i.e. in addition to the chelation via the catecholate sites, the separated carboxylate site can also coordinate to the copper(II) ion. The simultaneous coordination of both types of binding sites leads to the formation of various oligomeric species.

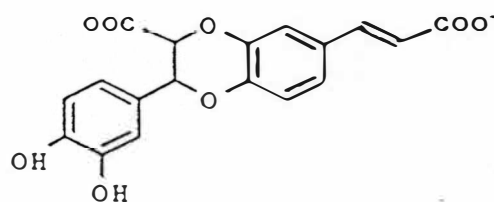
All these studies focused on metal-ligand complexation and chemical speciation. The oxidation of caffeic acid in the presence of copper(II) in natural waters has not received much attention. Soil scientists have found that caffeic acid participated not only in the metal transport but also in the reduction of iron(III) to iron(II) in the soil environment (Linder and Voye, 1987).

Dimerization Mechanism

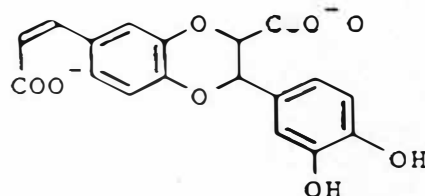
Ether Linkage Dimerization

In recent years, food scientists have also been interested in studying the oxidation of caffeic acid (Cilliers et al., 1989 and 1991). Caffeic acid and its derivatives are found in relatively high concentrations as normal metabolites in many plants and plant-derived foods. Cilliers (1989 and 1991) believed that caffeic acid and its derivatives were all susceptible to both enzymic and nonenzymic oxidation in the presence of oxygen. The oxidation products were shown to be specific dimers of caffeic acid (see Figure 4). They also noted that higher pHs could greatly accelerate the caffeic acid dimerization reaction. Under acidic conditions the nonenzymic reaction is slow, taking weeks and months, but can occur rapidly (minutes) under alkaline conditions. They concluded that the large effect of pH on the production rate indicated the involvement of

phenolate anions in the formation of these products.



(2S, 3S)



(2R, 3R)

Figure 4. Two Ether Linkage Dimer of Caffeic Acid Proposed by Cilliers.

Cyclobutane Linkage Dimerization

Photochemical studies of cinnamic acids and their derivatives also provides us with some information about dimerization of caffeic acid (Cohen, 1963 and Ghosh, 1987). In 1963, Cohen studied the photochemistry of trans-cinnamic acids, concluding that the photo-dimerization of ring-substituted cinnamic acids leads to trans- and cis-dimers by breaking the double bond of the ethylene group on the side

chain of caffeic acid. These two dimers, α -truxinic acid and β -truxinic acid, are illustrated in Figure 5. Cohen's study also indicated that there were no steric effects due to o-substituents and no intermolecular hydrogen-bonding barrier to dimerization. In 1987, Ghosh further confirmed the photodimerization of o-hydroxycinnamic acid (Figure 6).

In this thesis, we present the mechanistic and kinetic information for the oxidation reaction of caffeic acid. This reaction may involve possible ether linkage and/or cyclobutane linkage dimerization in the presence of copper(II). We believe this reaction may be one of the important steps in formation of humic acid in natural water.

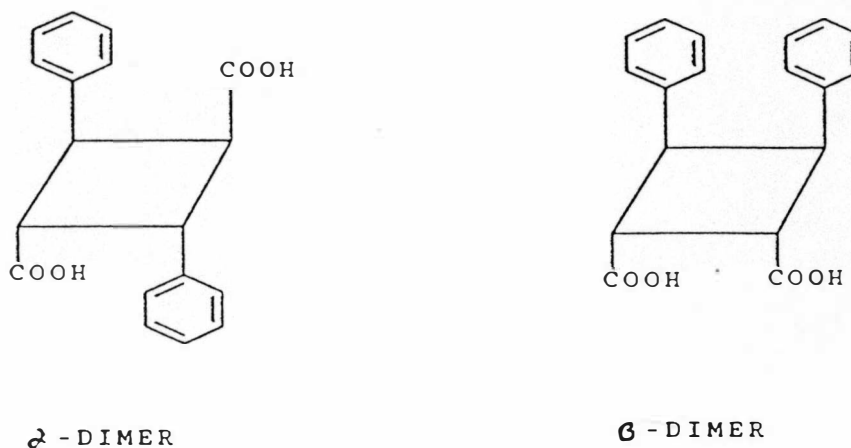


Figure 5. The Structure of α -Dimer and β -Dimer Proposed by Cohen.

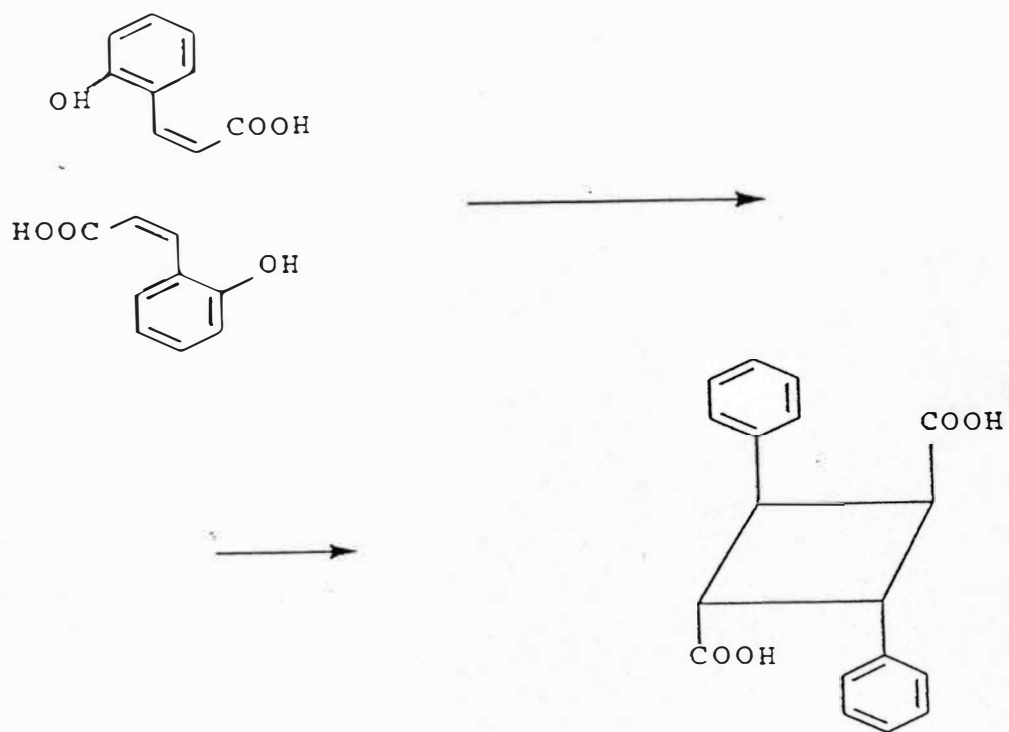


Figure 6. Photodimerization Reaction of o-Hydroxycinnamic Acid.

CHAPTER III

MATERIALS AND TECHNIQUES

Materials

This study utilized low concentration sample since the typical range of concentrations of organic compounds and metals in natural waters are approximately 0.1 to 10 mg/liter. All chemicals were reagent grade and were used without further purification. The deionized water employed in all preparations was 18 M Ω water generated from a Milli-Q[®] Water System.

Solution Preparations

Caffeic Acid Solution

A 2.0mM of caffeic acid (Aldrich, Milwaukee, WI) solution was prepared by dissolving 0.360g of caffeic acid in 1 liter water. The caffeic acid was then heated on a hot plate at about 40-50°C until it was all dissolved. The solution was stored in an amber bottle.

Copper(II) Chloride Solution

Copper(II) chloride from Fisher Scientific (Fair Lawn, NJ) was dissolved in water to make a 0.40 M solution and kept in a plastic bottle.

Caffeic Acid and Copper(II) Chloride Mixture

Caffeic acid was mixed with copper(II) chloride in a 1:1 mole ratio. Usually this solution was obtained by adding 5.0 ml of copper chloride (0.40M) to 1000 ml of caffeic acid (2.0mM). The complex solution was kept in a clear bottle at room temperature under natural room light until the solution turned dark brown (about three months). Some later experiments required the solid form of the mixture. To extract the mixture from solution, LABCONCO® 6 Liter Freeze Dryer was used. The solution was first frozen in dry ice and iso-butyl alcohol slurry and then was attached to the freeze dryer at a temperature of -50°C and vacuum pressure of 100 µmHg. A liter of mixture solution usually took about 24 hours to dry completely.

Physical Appearance of the Mixture

The aqueous solution of caffeic acid and copper(II) mixture was light green initially and become brown after a few days. After three months it turned dark brown. It has the similar solubilities as caffeic

acid. It was not soluble in organic solvents such as benzene, hexane, chloroform, or methylene chloride. It was slightly soluble in water and soluble in alcohol and DMSO.

Techniques

Calorimetry

A Tronac 450 Thermometric Titration Calorimeter with a Sargent SRG Recorder was used for determination of the heat of complexation between caffeic acid and copper(II). To measure the heat of the complexation, caffeic acid was titrated with copper(II) chloride on 1:4 ratio. An electrical calibration of the calorimeter was performed before each titration. Copper(II) solution as the titrant was added to caffeic acid using a thermostated, motor-driven syringe. The caffeic acid solution was contained in a dewar flask and it was stirred constantly to achieve and maintain thermal equilibrium in the system. The temperature was measured by a thermistor and output to the recorder.

The thermogram is recorded as a temperature versus time (T-t) diagram. The diagram starts with a preperiod, AB, before the addition of titrant. The titrant is added at point B and stopped at point C. The diagram finishes with a postperiod, CD. An example of a normal titration curve is shown in Figure 7. The slope of preperiod AB is

4-Hydroxycinnamic acid and copper

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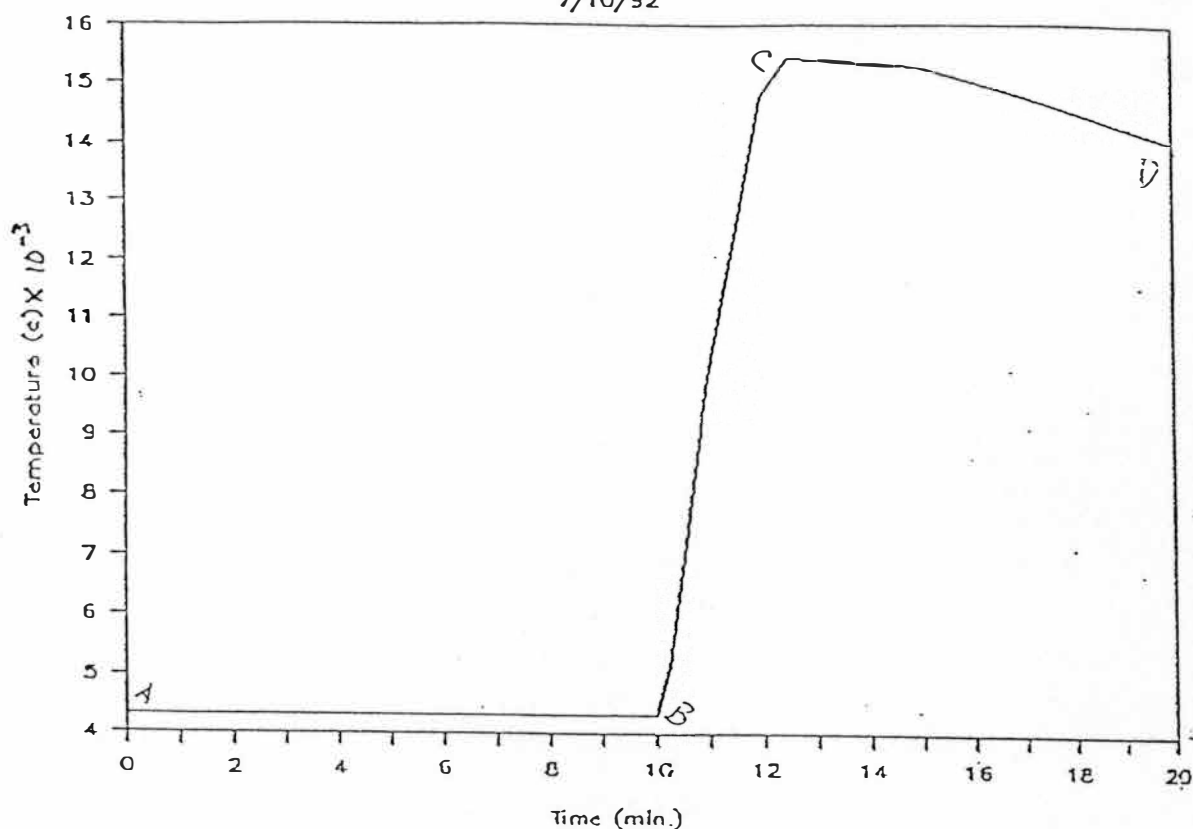


Figure 7. Thermogram of 4-Hydroxycinnamic Acid and Copper(II).

AB is the Preperiod, BC is the Titration Period, and Cd is the Postperiod.

related to heat of mixing by the stirrer and heat difference from the surrounding. The slope of BC is due to the heat of reaction. The slope of CD is again related to heat of stirring and heat difference from the environment. Since the temperature of the system is higher after the titration has been completed, the heat effect of surrounding should be less in the postperiod. The slope of CD therefore should be less than AB as Figure 6 indicates. For convenience, the T-t diagram was adjusted so

that the slope of AB was zero. This adjustment forces the CD portion to have a negative slope in a normal thermogram.

UV-Visible Absorption Spectrometry

UV-visible measurements were carried out in a HP 8451 A Diode Array Spectrophotometer with HP 7470A plotter and Think Jet Printer. Copper(II) chloride solution was mixed with caffeic acid solution at 1:1 ratio. The mixture was diluted 20 times with water and transferred to 1.0 cm fused silica cell. This solution was measured every day for three months. The sample spectra were obtained by scanning from 190-800nm wavelength and absorbance at wavelength 316nm was used to monitor the reaction.

Molecular Weight Determination

Freezing Point Depression in t-Butyl Alcohol

The presence of a solute lowers the freezing point of a solvent. The molecular weight of the solute can be calculated from the change of temperature from pure solvent to the mixture of solvent and solute. The relation between the change in freezing point to the molality of solute particles is given in equation below (Gillette and Johnson, 1988):

$$T(f) - T(f)' = K(f) \times m(c)$$

Where $T(f)$ is freezing point of the solvent and $T(f)'$ is freezing point of the solution; $K(f)$ is the molal freezing point depression constant; $m(c)$ is the molality of solute.

The freezing point of t-butyl alcohol was measured on a cooling cycle (Figure 8). T-butyl alcohol was put into the inner tube and the equipment was placed in a container of water and ice. Initially, the temperature was lowered as the alcohol cooled. Then the temperature remained constant as the liquid froze. This constant temperature was the freezing point of the pure liquid. The dip in Figure 8 is due to supercooling and can be ignored. The $K(f)$ of t-butyl alcohol was determined by measuring the freezing point change of the t-butyl alcohol when a small amount of water was added.

The freezing point of a solution was measured on the warming cycle. A known weight of caffeic acid was dissolved in a known volume of t-butyl alcohol in the inner tube. The solution was cooled and continuously stirred until the solution was semi-frozen and had a "slushy" appearance. The mixture of liquid and solid was warmed and the solvent melted. Theoretically, the plot will be a straight line of greater slope after all solvent has melted and the liquid is only being warmed, see Figure 9. The intersection of these two straight lines was the freezing point of solvent in the solution. The freezing point of the complex in t-butyl alcohol was measured as well as the freezing point of

pure caffeic acid.

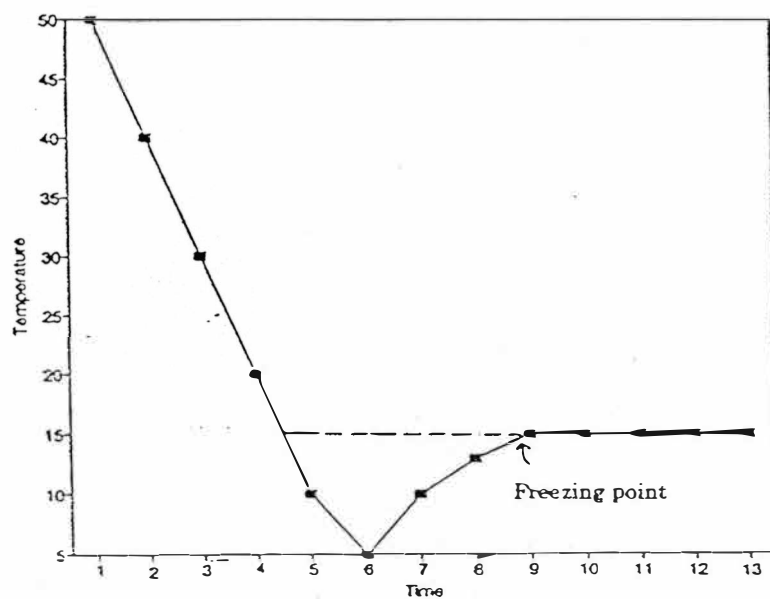


Figure 8. Cooling Curve of a Pure Solvent.

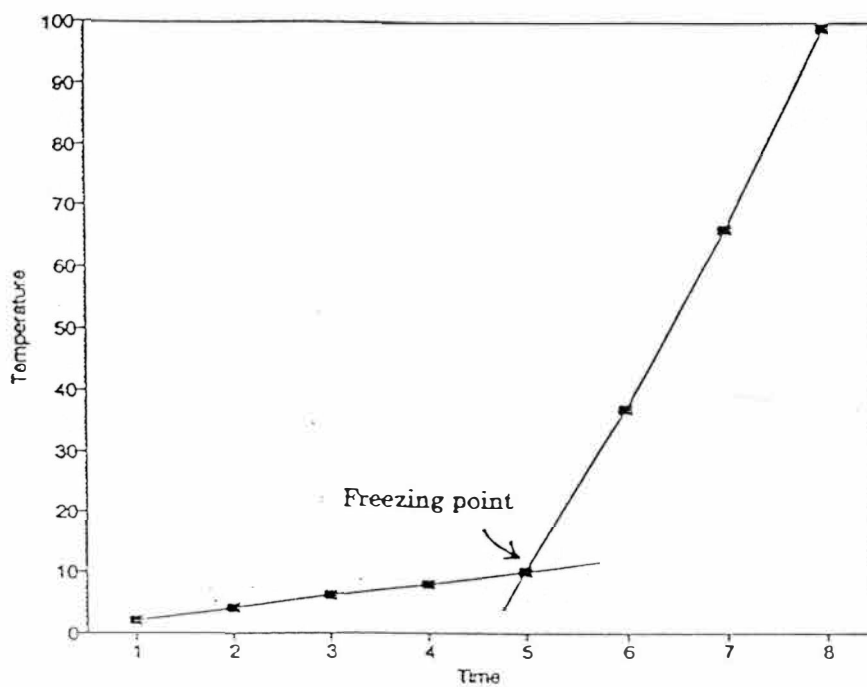


Figure 9. Warming Curve of a Solution.

Viscosity

Viscosity technique can be used to measure the molecular weight of polymers. A schematic diagram of viscometer is shown in Figure 10. In the viscosity experiment, the viscometer is filled with sample solution and is placed into a water bath at 25°C. Once the viscometer established thermal equilibrium with the water bath, high pressure air was introduced into tube A to force the solution up to a point well above upper fiducial mark. Then the pressure was released and the flow time between the upper and lower fiducial marks was measured with a timer.

The equations to determine the viscosity of the solution are given as following (Daniels, et al., 1970):

$$\eta = A\delta t \quad (1)$$

Where A is viscometer constant, η is coefficient of viscosity, t is the flow time (second) between the upper and lower fiducial marks, and δ is the density of solvent. The solvent was water at 25°C. A can be obtained from pure solvent data.

$$[\eta] = [2^{1/2} (\eta_{sp} - \ln \eta_{rel})^{1/2}] / C \quad (2)$$

$$\eta_{sp} = \eta / \eta_0 - 1 \quad (3)$$

$$\eta_{rel} = \eta / \eta_0 \quad (4)$$

Where $[\eta]$ is intrinsic viscosity and C is the concentration of solution (g/100ml). η can be obtained by equation (1). η_0 is the viscosity coefficient of water. Once the viscosity is calculated, the following Mark-Houwink equation can be used to determinate the molecular weight of the sample solution:

$$[\eta] = KM^a \quad (5)$$

Where M is the average molecular weight. K and a are coefficients and can be obtained from a viscosity-molecular weight relationship table. In this experiment K and a were 5.9×10^{-4} and 0.67, respectively.

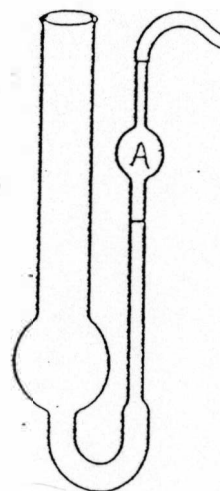


Figure 10. Viscometer.

Fourier Transform Infrared Absorption

Since the complex is very hygroscopic, the powder of caffeic acid and copper(II) chloride mixture was mixed with KBr in a dry box under nitrogen atmosphere. The sample KBr pellets were then analyzed by a Nicolet 5DXC FT-IR spectrometer. Instrument background was first

measured and then subtracted from sample spectrum. The spectrum of sample KBr pellets were then measured from 500 to 4000 cm^{-1} .

Gas Chromatography/Mass Spectrometry

A HP 5890A Gas Chromatography (GC) with HP 5970B Mass Selective Detector (MSD) was used. A HP-1 capillary column was utilized in GC. The MSD was equipped with an electron impact ion source, hyperbolic quadrupole mass filter, and electron multiplier detector. A derivatization technique (Pellizzari, 1969) was used because caffeic acid was not volatile enough to be examined by direct injection on a GC. Derivative reagents (Aldrich) TMCS (trimethylchlorosilane), TMCS (hexamethyldisilazane), and pyridine were utilized to derivatize the complex. About 5 mg of complex was weighed and placed in a sealed vial under nitrogen atmosphere. One ml of freshly prepared reagent containing HMDS, TMCS and pyridine (3:1:9) was injected into the vial. The reaction mixture was shaken vigorously for 30 seconds and then allowed to stand at room temperature for at least 5 minutes. An aliquot from the mixture was injected directly into the gas chromatograph. Using caffeic acid as an example, the derivatization reaction is as follows:

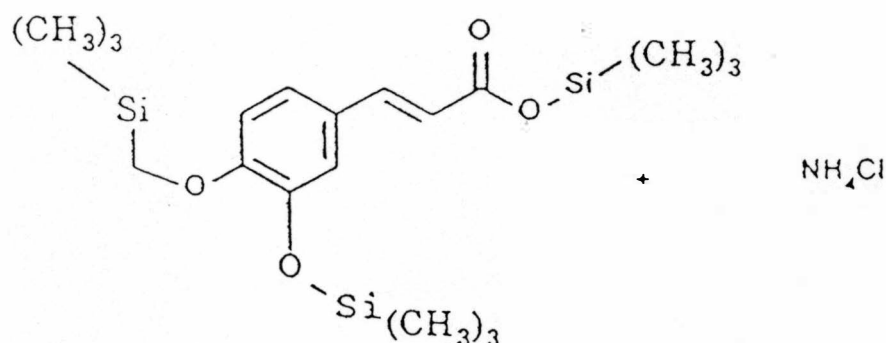


Figure 11. The Reaction of Caffeic Acid Derivatization.

Physical Effects

Effect of Temperature

Three samples of the product were prepared. One was kept at room temperature. The other two were heated, one to 60°C and the other to 100°C. Then all three samples were diluted to 20 fold with water and their absorbances were measured at wavelength 316 nm on a HP 8451 Diode Array Spectrophotometer at room temperature.

Oxygen Consumption

A YSI 54RC Oxygen Meter with Model 5420A Oxygen /

Temperature Membrane Probe was used in oxygen measurement.

Caffeic acid and copper(II) solution were mixed in a 1:1 ratio and kept in a air-tight bottle. Approximately 5.0 ml of solution was removed for the measurement every day for 7 days. Argon gas was blown over the top of the solution after each sampling.

Melting Point Measurement

A Mettler TA4000 Differential Scanning Calorimeter (DSC) was used to make melting point measurement of pure caffeic acid and the dimer product. Pure caffeic acid was measured first as a standard check. 7.042 mg of caffeic acid was placed on an aluminum plate and sealed. It then was heated in the crucible of the DSC from 25°C to 300°C at 10°C per minute rate. The melting point of pure caffeic acid, theoretically, is 223°C. Similarly, the product was measured by from 25°C to 500°C at 10°C per minute.

Effect of Light

The solution of caffeic acid and copper(II) were mixed at 1:1 ratio. This mixture was stored in the dark and its uv-visible absorption spectra were monitored at 316 nm using the HP 8451 Diode Array Spectrophotometer.

Effect of Copper(II)

Qualitative Experiment

1 liter of (2.0mM) caffeic acid was made and placed in two different bottles. In one bottle, copper(II) was added to caffeic acid at 1:1 ratio. In the other, no copper(II) was added. Two bottles were kept at room temperature for about three months. At the end of three months the absorbance of these two solutions were measured at 316 nm for comparison.

Quantitative Experiment

Caffeic acid was mixed with copper(II) at 1:0.1, 1:0.5, 1:1, 1:5, 1:10, 1:20 ratio and diluted 20 times with water. The absorbance of the mixture was measured on the HP 8451 Diode Array Spectrophotometer at 316 nm every day for 20 days.

CHAPTER IV

RESULTS DISCUSSION AND PROPOSED MECHANISM

Results Discussion

Techniques

Calorimetry

The diagram shown in Figure 12 was pre-adjusted in order to obtain a zero slope baseline AB. In this temperature verse time diagram, the slope of CD is greater than that of AB which means that when the titration stopped at point C, heat was still being generated by the system. This is an indication that the reaction continued after the titration was completed. Similar experiments were performed with non-reducible transition metals cadmium and zinc instead of copper(II). We observed a normal titration curve with no heat being generated after the titration was stopped. Normal titration curves were also observed when hydrocaffeic acid (Figure 13), which does not have a double bond on the acid side chain, was used in place of caffeic acid and also when the titration was performed under an argon atmosphere. In addition, when the ratio of caffeic acid and copper(II) was increase to 1:8 and 1:14, the

slope of CD was increased, see Figure 14.

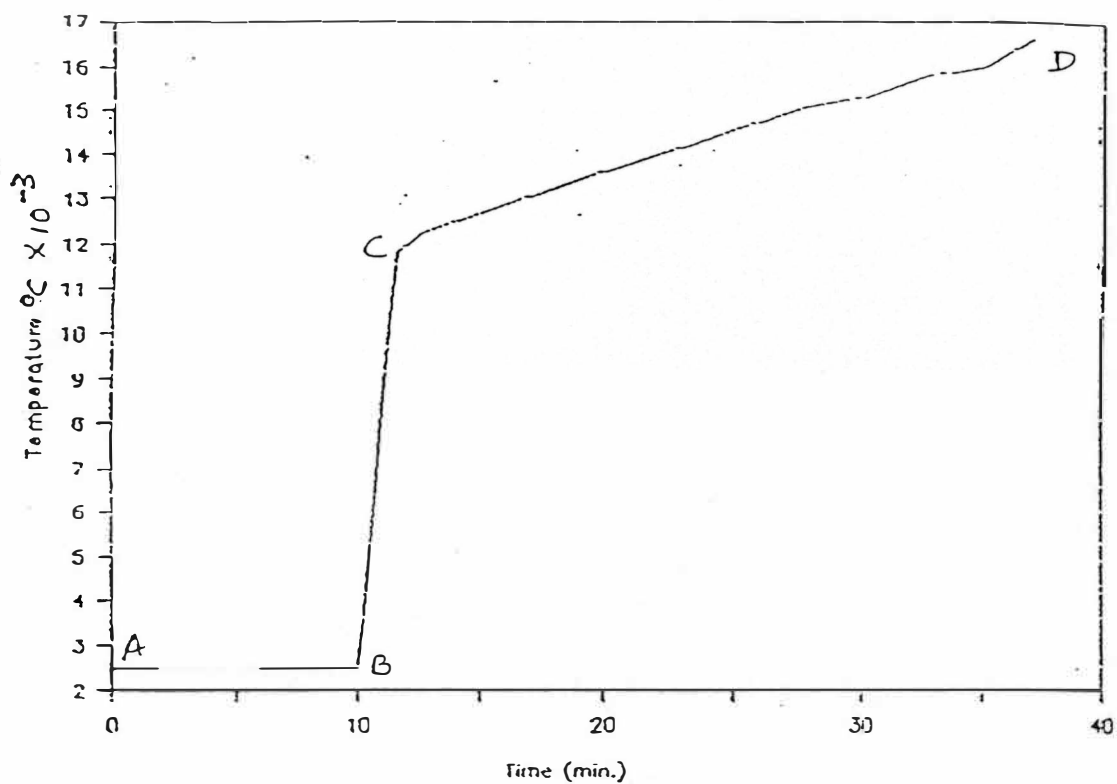


Figure 12. Thermogram of Caffeic Acid and Copper(II) on 1:4 Ratio.

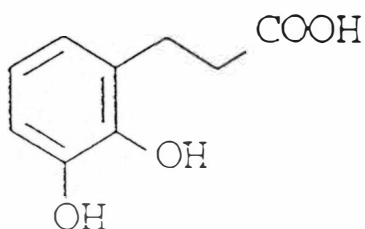


Figure 13. Hydrocaffeic Acid.

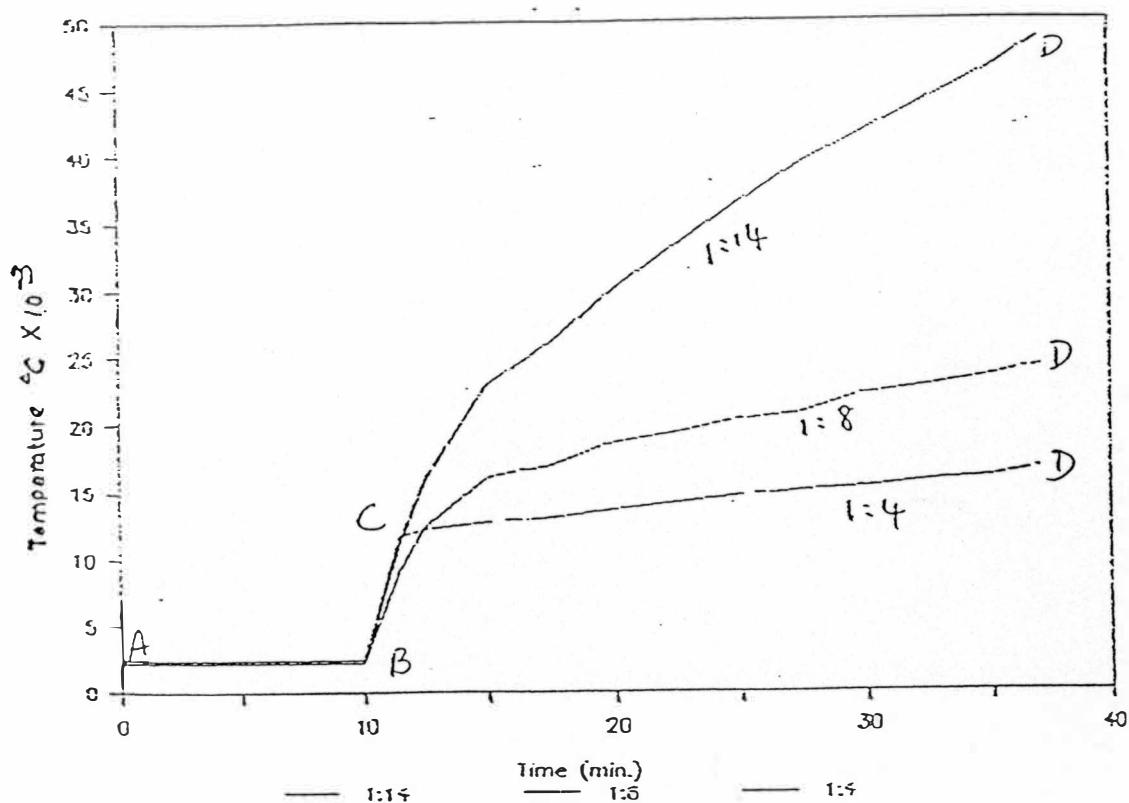


Figure 14. Thermogram of Caffeic Acid and Copper(II) on 1:4, 1:8, 1:14 Ratio.

The calorimetry data were surprising as we expected a simple complexation reaction between copper(II) and caffeic acid. Here, the first reaction appears to be complex formation with copper(II) and caffeic acid follows by the metal reduction-caffeic acid oxidation. The results indicated that the reaction took place slowly, required the reducible metal copper(II) and the conjugated double bond system on the caffeic acid side chain. In addition oxygen is also essential to the reaction.

This surprising and interesting result shifted the direction of the research from a metal-acid complexation investigation to a study of the reaction mechanism.

UV-Visible Absorption Spectrometry

Figure 15 shows that the spectrum of the copper(II) - caffeic acid mixture on day 1 was the same as pure caffeic acid. However, after three months, see Figure 16, the absorbance had decreased dramatically in the region of 310-320nm. This absorbance is due to the conjugated double bond system of caffeic acid. This indicates that the double bond on the propenoic acid side chain of caffeic was lost during the reaction. These results agree with the calorimetric data which indicated the ethylene group of caffeic acid is involved in the reaction.

A plot of the absorbance of a caffeic acid and copper solution at 315nm wavelength versus time over the three months period is shown in Figure 17. This curve demonstrates that the reaction took slowly place. It was a first order reaction with respect to caffeic acid based on the initial 30 days rate determination. The rate constant was $1.62 \times 10^{-2} \text{ day}^{-1}$ and the plot of $\ln(\text{absorbance})$ versus days is shown in Figure 18.

Molecular Weight Determination

The molecular weight of caffeic acid and the product from freezing

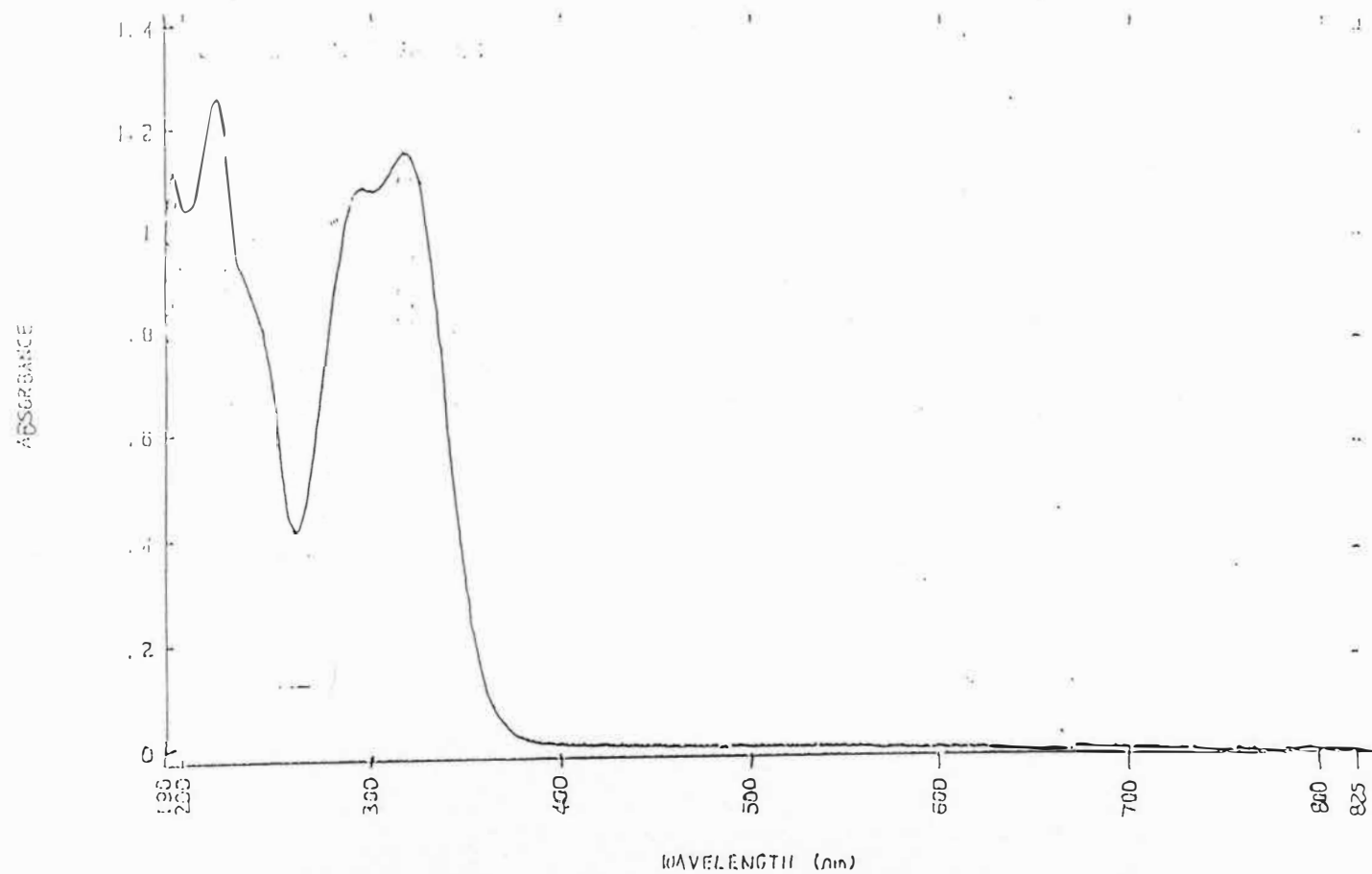


Figure 15. UV-Visible Absorption Spectrum of Caffeic Acid and Copper(II) Mixture on Day One.

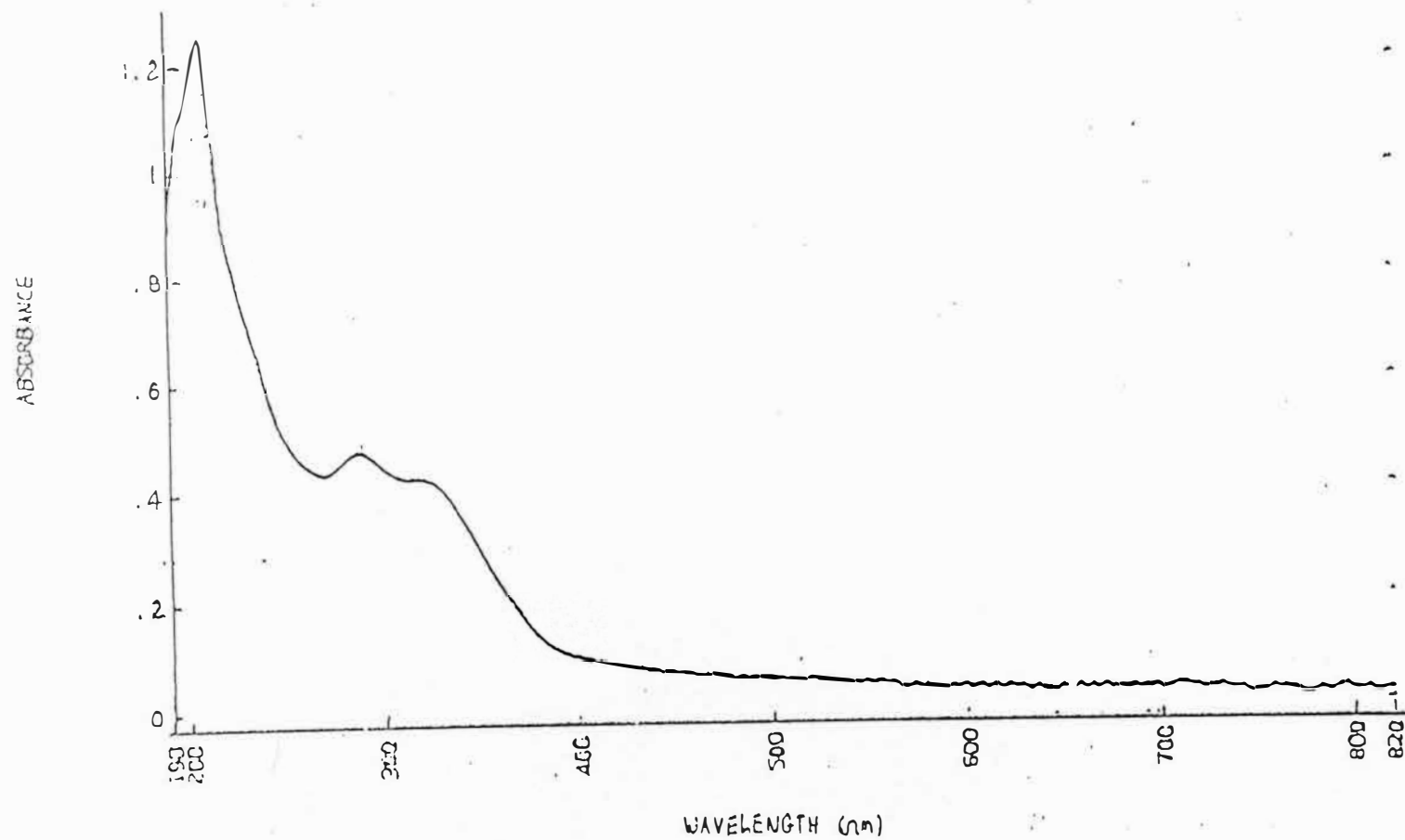


Figure 16. UV-Visible Absorption Spectrum of Caffeic Acid and Copper(II) Mixture after Three Months.

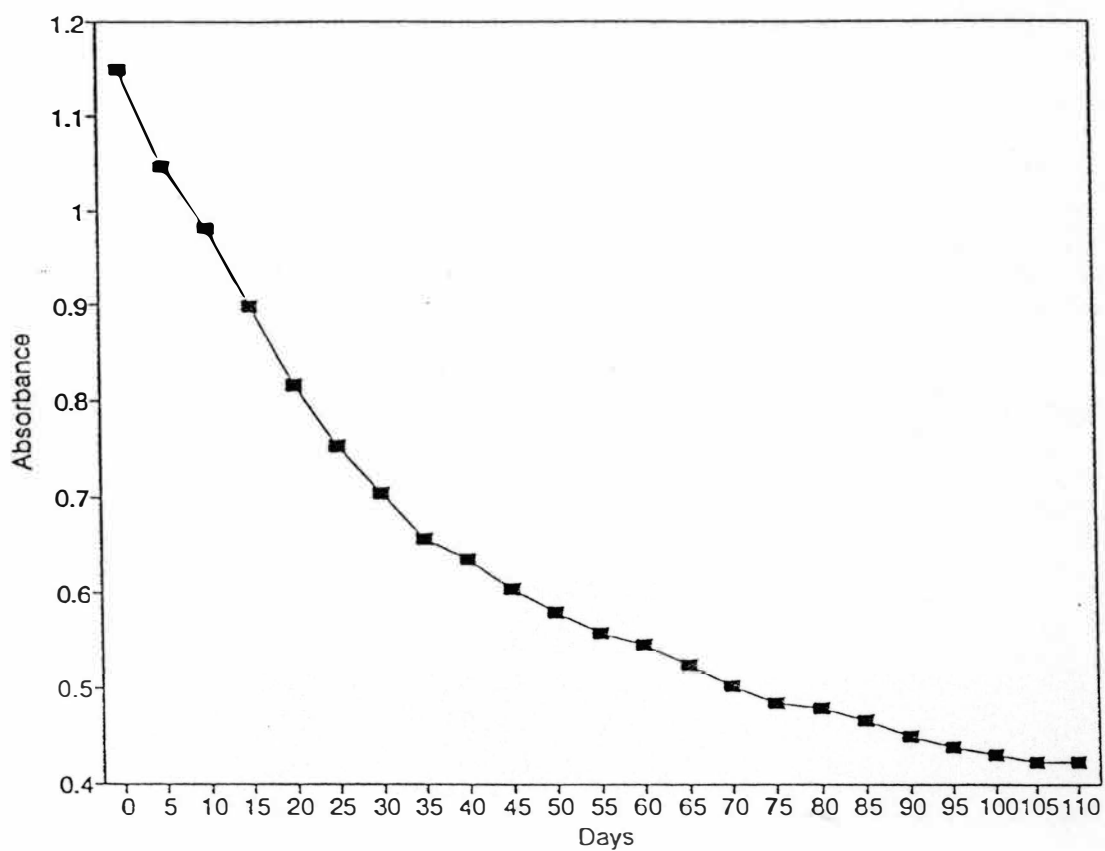


Figure 17. UV-Visible Absorption Spectrometric Data of Caffeic Acid and Copper(II) at Absorbance 316 nm for Three Months.

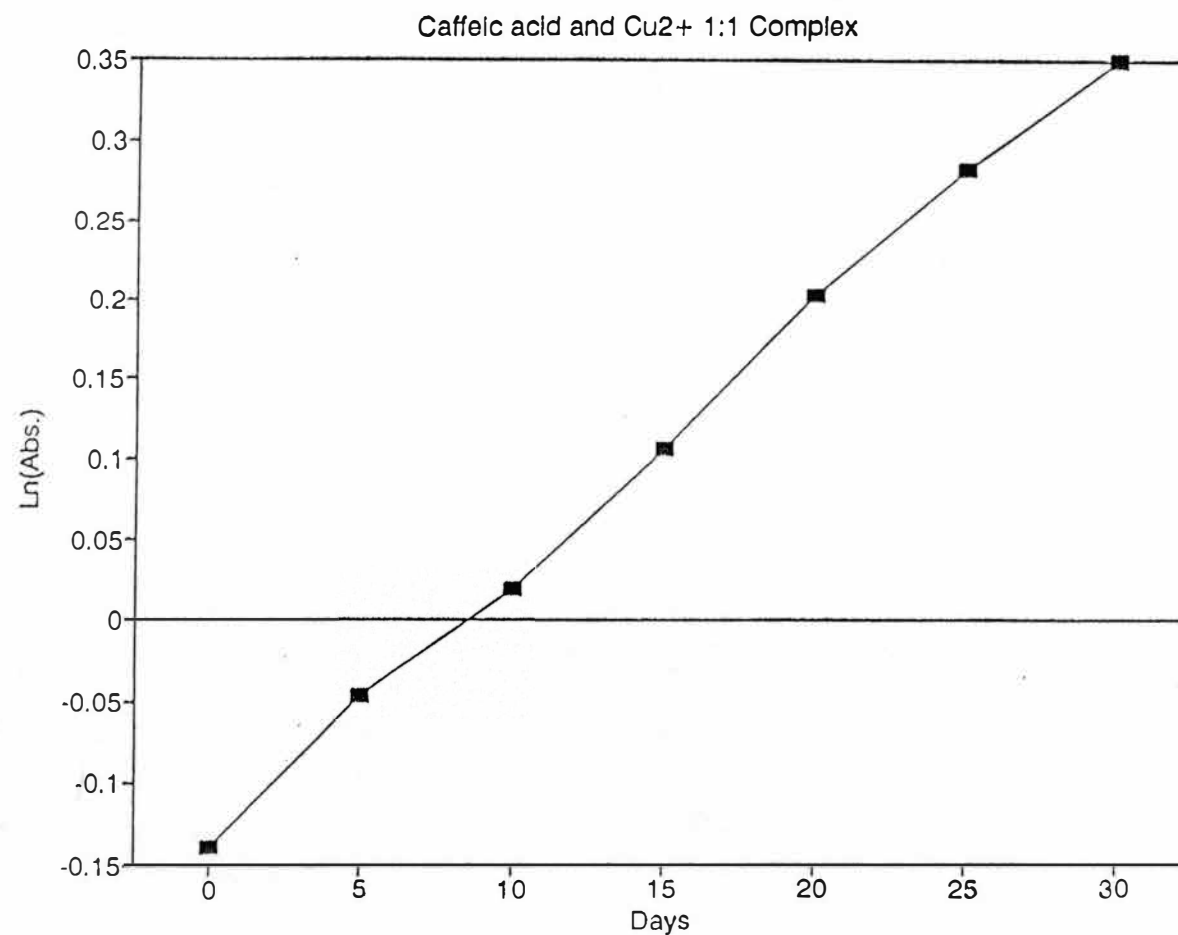


Figure 18. Plot of $\text{Ln}(\text{absorbance})$ Versus Days in the First 30 Days of Caffeic Acid and Copper(II) Reaction.

point depression experiment and viscosity experiment are given in Table

1. The ratios of product over reactant are 2.2 and 2.5, respectively.

These results demonstrate that the principle product was a dimer.

According to Cilliers' studies (1989 and 1991), caffeic acid type compounds are susceptible to both enzymic and nonenzymic oxidation in the presence of oxygen. Their work indicated that the oxidation products were ether linkage dimers. Photochemical studies of cinnamic acids and its derivatives also suggested that caffeic acid forms cyclobutane dimers (Cohen, 1964). These studies led us to believe that the oxidation product of caffeic acid and copper could be either a ether linkage dimer

Table 1
Molecular Weight Determination on the Product
(Unit: g/mol)

	Freezing Point Depression	Viscosity
Caffeic Acid	231.96	220.14
Product	510.81	555.56
Ratio	2.2	2.5

as Cilliers' studies showed or cyclobutane dimer as Cohen's studies had predicted.

Fourier Transform Infrared Absorption

As is listed in the following Table 2, cyclobutane bands and ether linkage bands for the two type of structures were present in the spectrum of product. The structures of two dimers are shown in Figure 19.

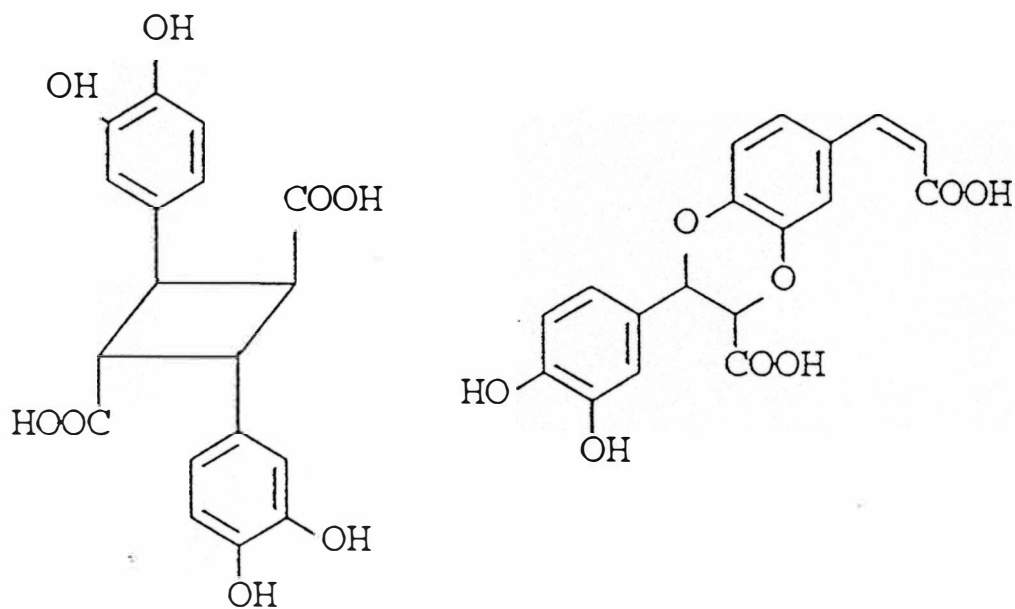


Figure 19. The Structure of Two Dimers Illustrated From FTIR Data.

Table 2
FT-Infrared Data of the Dimer Product

Wavelength	Assignments
592 cm ⁻¹	Cyclobutane ring bending
732 cm ⁻¹	Cyclobutane ring deformation
1094 cm ⁻¹	Cyclobutane ring stretching
1290 cm ⁻¹	Asymmetrical ether linkage stretch
1080 cm ⁻¹	Symmetrical ether linkage stretch

Gas Chromatography/Mass Spectrometry

The GC/MS result of the product (Figure 20) was confusing. The product's spectrum looked similar to that of caffeic acid. It implied that the reaction had been reversed. In Schonberg' studies (Schonberg et al., 1950), he found that some cyclobutane dimer would dissociated back into the monomer when heated. Our later "effect of temperature" experiment confirmed that the conjugated double bond system was regenerated upon heating. This result explains why we observed a similar product spectrum as caffeic acid. When the product was separated in the GC column, the temperature of the oven was about 200^oC. With such high temperature, we believed that the dimer was dissociated back into

monomer.

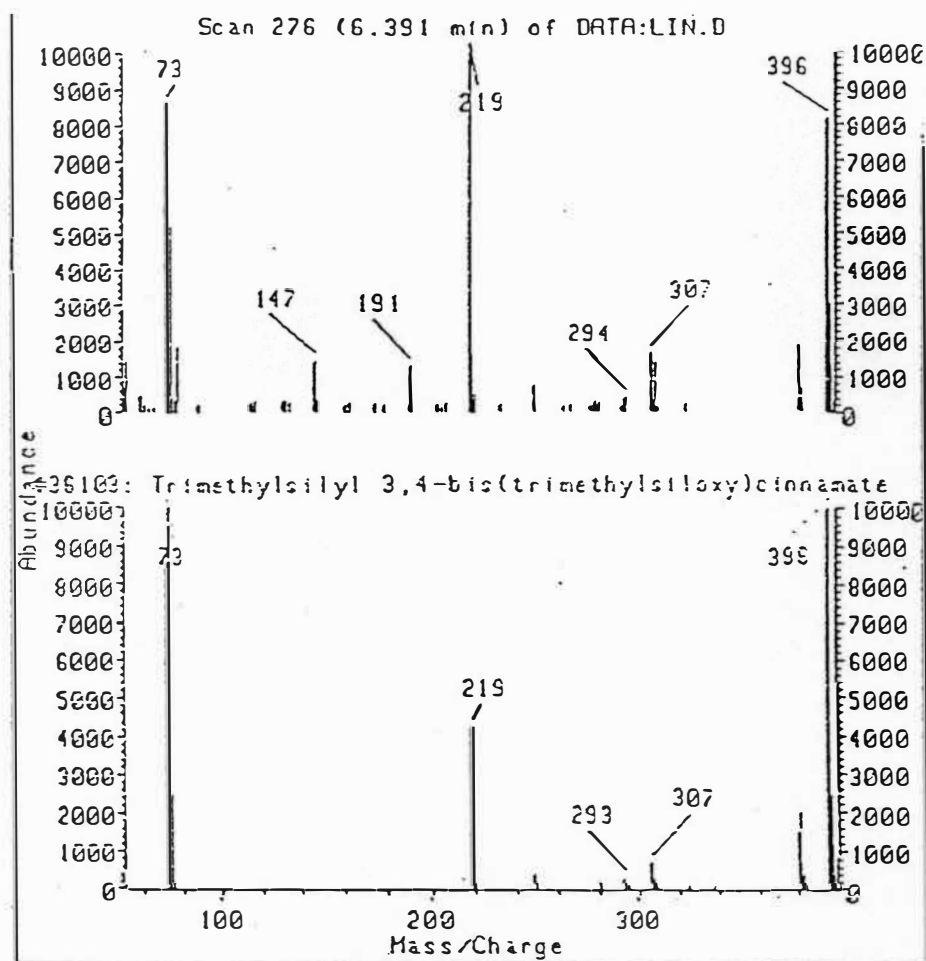


Figure 20. Mass Spectra of the Product (top) and Pure Caffeic Acid (bottom).

Nuclear Magnetic Resonance

NMR experiments were unsuccessful due to the interference of the paramagnetic of copper(II) present. Attempts to removed copper(II) by raising the pH of the solution in order to precipitate out copper(II) and

also by ion exchange techniques were not successful. Additionally an increased pH of the solution containing caffeic acid could change the rate of the reaction as shown by Cilliers' studies (1989 and 1991)

Physical Effects

Effect of Temperature

The increase in absorbance at 316 nm upon heating (Table 3) supports the GC/MS data that the conjugated double bond system was regenerated upon heating. The dimer must have dissociated back into monomer at higher temperature.

Table 3

Effect of Temperature on the Dimer Product

Temperature	Absorbance	
Room Temp	0.414	
60°C	0.441	Double Bond Regenerated
100°C	0.466	

Oxygen Consumption

The oxygen consumption data is shown in Table 4. The concen-

tration of oxygen decreased over the 7 day period of the experiment. This is in agreement with calorimetric data which showed that oxygen was required during the reaction.

Table 4
Oxygen Consumption for the Reaction of Caffeic Acid
With Copper(II) (1:1)

Days	1	2	3	4	5	6	7
Oxygen Conc. (ppm)	8.0	6.8	5.7	4.9	4.2	3.6	3.0

Melting Point Measurement

The theoretical melting point of caffeic acid is about 223°C. Figure 21 and 22 show the two thermograms. Caffeic acid began to melt at 210°C and then decomposed. The complex dehydrated at 110°C, then decomposed at about 450°C.

Effect of Light

UV-visible absorption measurements illustrated that even when the product solution was kept in the dark, the loss of conjugated system of caffeic acid was still observed. This implies that effect of light on the reaction is not significant in the caffeic acid-copper(II) reaction.

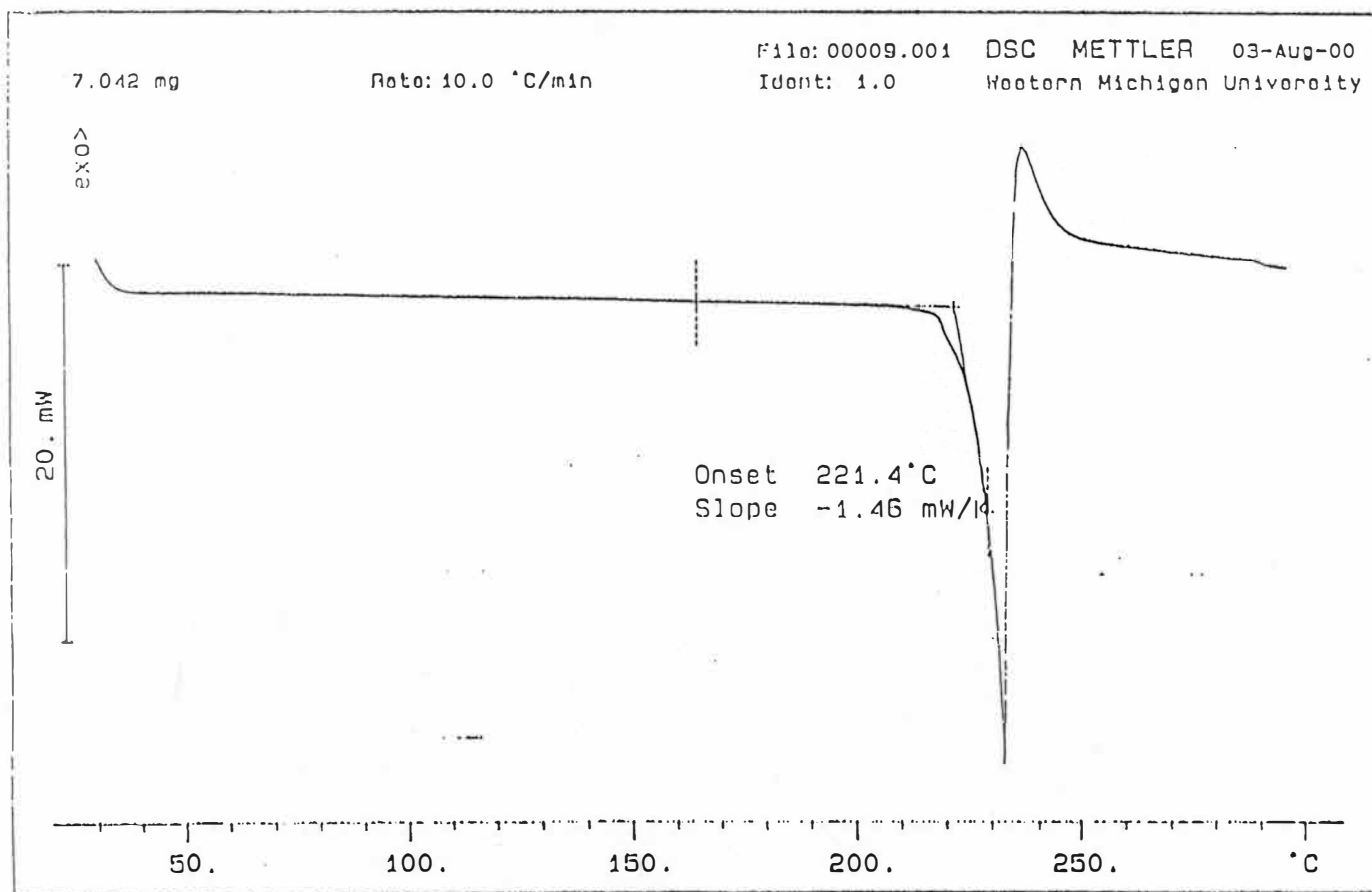


Figure 21. DSC Thermogram of Pure Caffeic Acid.

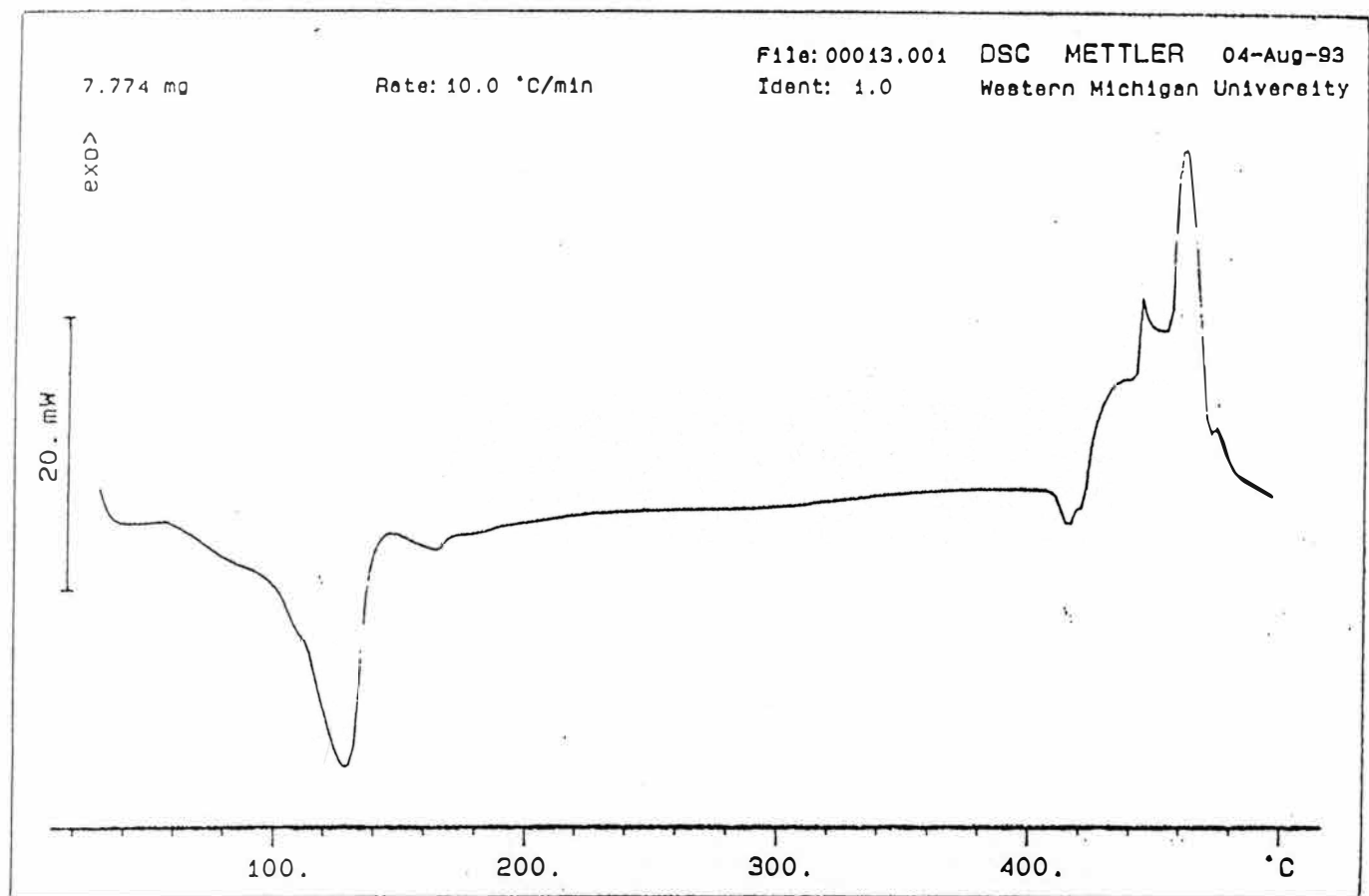


Figure 22. DSC Thermogram of Caffeic Acid and Copper(II).

Effect of Copper(II)

Qualitative Experiment. In this experiment, two bottles of caffeic acid solution were prepared. In one bottle, copper(II) was added. This bottle of caffeic acid with copper(II) turned dark brown after three months as the complex was formed while the bottle with only caffeic acid was still clear. No color change on the pure caffeic acid was observed.

Quantitative Experiment. Earlier calorimetric results had illustrated the effect of copper(II). the higher the copper(II) to caffeic acid ratio, the steeper was the slope and the faster the rate of reaction.

A plot of absorbance verse days (Figure 23) shows the effect of copper(II) on its reaction with caffeic acid. This plot illustrates that the curves may be divided into two groups. At low copper(II) concentration, the rate of the reaction was dependent on copper(II) concentration. When the ratio of caffeic acid and copper(II) reached 1:5, the rate of the reaction became independent of the copper(II) concentration. Earlier calorimetric results had illustrated the effect of copper(II) on the complexation of caffeic acid (Figure 14). The figure shows how rate of reaction changed with the concentration in the first 40 minutes. The higher the copper to caffeic acid ratio, the steeper the slope, the faster the reaction. This is an indication that copper(II) behaved as a catalyst in the reaction.

Summary

Initially, the unexpected calorimetric results indicated that the caffeic acid and copper(II) reaction was more complicated than a simple complexation. The reaction was not only influenced by thermodynamics factors but also by kinetic factors. The reaction appeared to be first complexation reaction and then a copper(II) catalyzed caffeic acid dimerization. Oxygen was shown to play an important role in the reaction. Both calorimetry and oxygen consumption measurement data support this conclusion. Such results have not been reported in previous studies. Much of the previous metal-ligand research was focused on simple complexation studies by potentiometric titration. This technique required the use of nitrogen to purge the sample before measurement in order to eliminate oxygen in the sample. Therefore, the oxidation reduction reaction would not likely have been observed.

The molecular weight determination indicated that dimers were formed in the reaction. The FTIR spectrum was consistent with two possible dimer structures: an ether linkage dimer and a cyclobutane dimer.

The catalytic effect of a reducible metal such as copper(II) in the reaction was demonstrated by the calorimetric data. In Cilliers' studies (1989 and 1991), caffeic acid was oxidized to form dimers in the presence

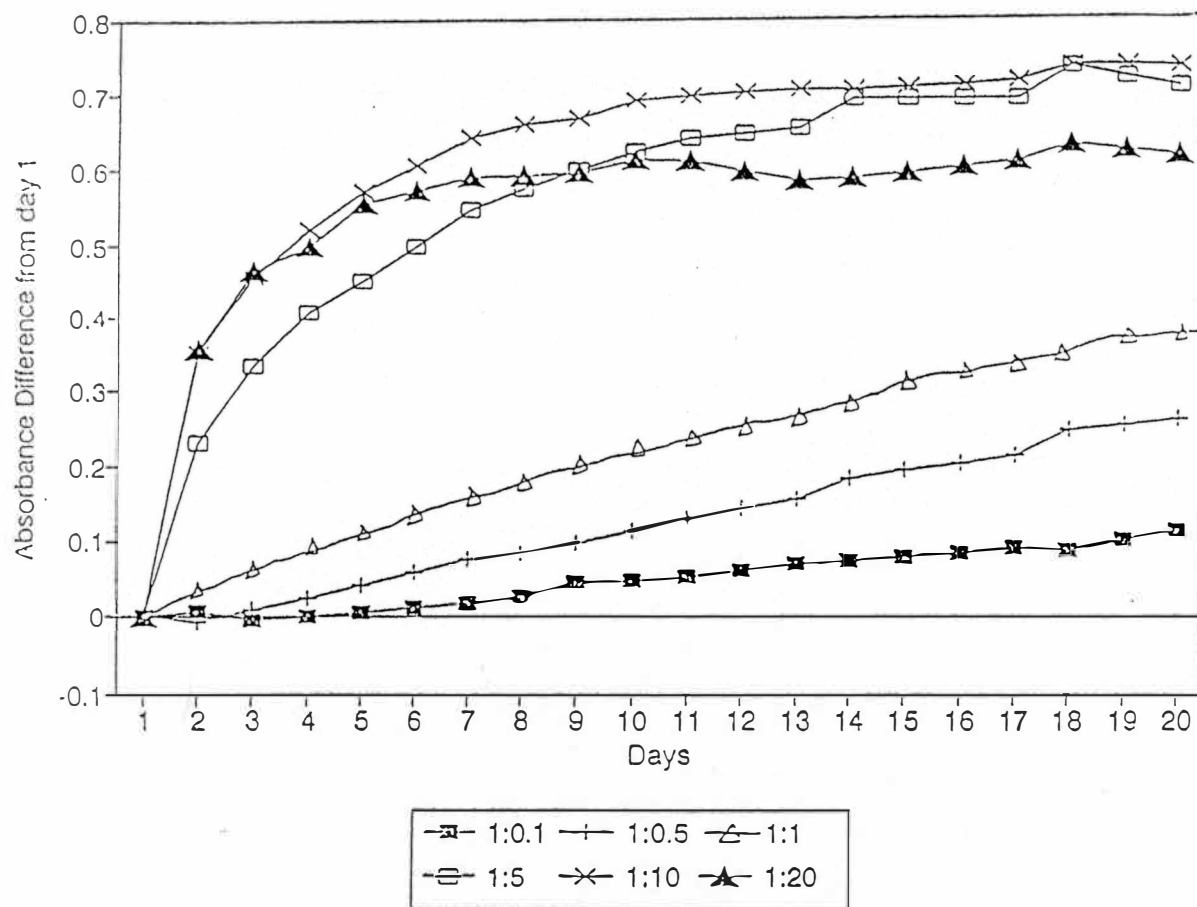


Figure 23. Effect of Copper (II) on Caffeic Acid Dimerization.

of oxygen within 6 hours at pH 11. They concluded that at high pH the reaction was accelerated. Without raising the pH, we observed that caffeic acid formed dimers in the presence of copper(II). The pH of the caffeic acid and copper(II) mixture solution was about 5-6. The molecular weight determination has confirmed this conclusion.

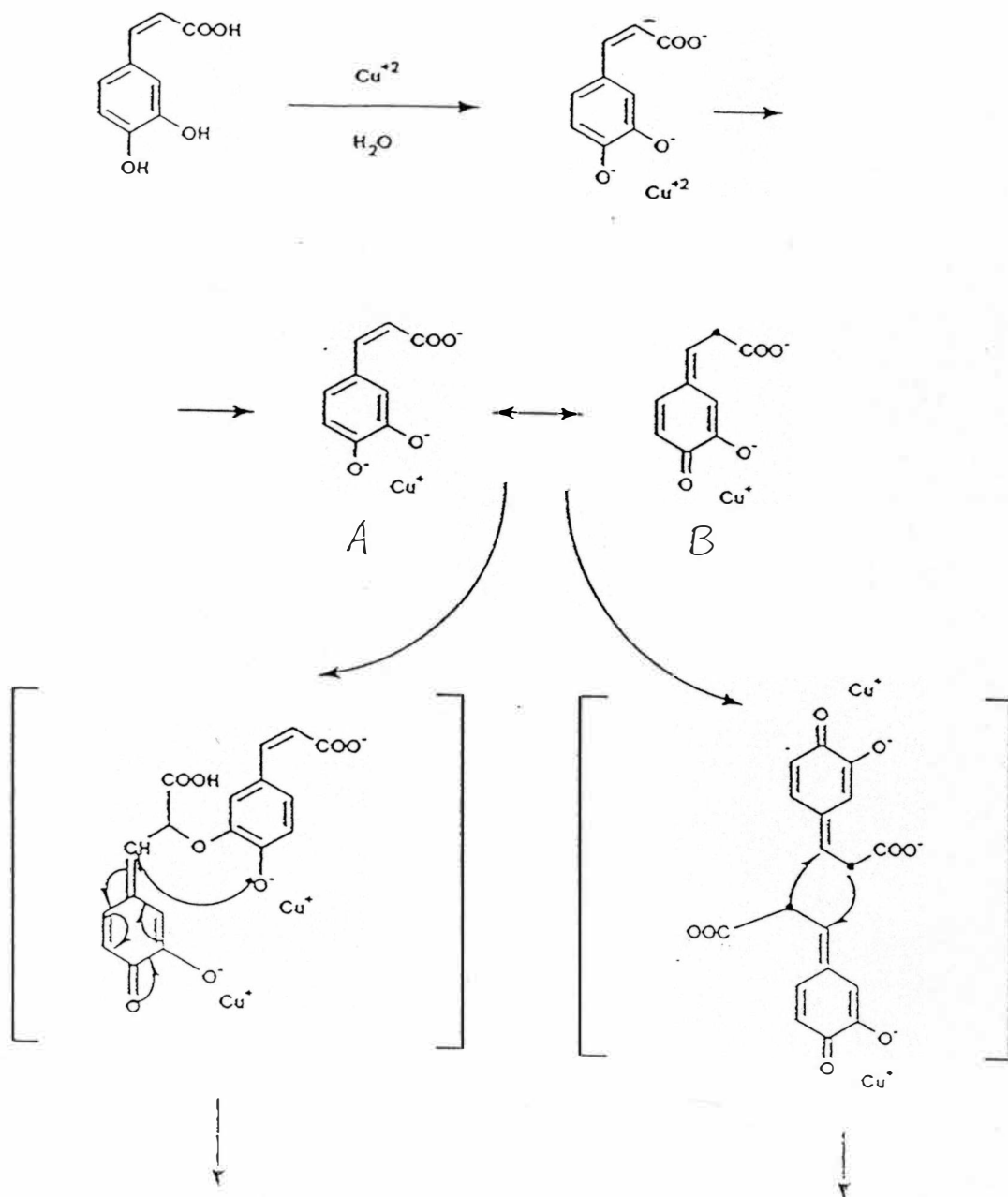
Calorimetric results indicated that the conjugated double bond system on the propenoic side chain of caffeic acid was essential for the reaction to take place. This was confirmed by the later UV-visible absorption measurements. In Cohen's photochemical studies (1964) of cinnamic acids and its derivatives, he found that when irradiated with light the cyclobutane dimers were the final products. Our studies, however, indicated that a dimer product was formed in the absence of light as well. Light did not inhibit or promote the reaction. We believe copper(II) plays an important role in formation of the caffeic acid dimer(s).

Finally GC-MS results showed that dimer dissociated to the monomer when heated. This was also suggested by results of a previous paper (Schonberg et al., 1950) and the by the effect of temperature on the UV-visible spectrum.

Based on these experimental data, the following mechanism for this reaction is proposed.

Proposed Mechanism

When copper(II) chloride solution was mixed with caffeic acid solution, copper(II) ions attach to the catechol site of protonated caffeic acid. The protonated caffeic acid structure was confirmed in Cilliers' studies. Copper(II) is then reduced to copper(I) with a corresponding loss of one electron by caffeic acid. The caffeic acid then forms a radical upon the loss of the electron. This radical has at least two resonance structures form A and B, see Figure 24. When form A and B combine together, they can form an ether linkage dimer. When form A reacts with itself, it forms the cyclobutane dimer. When the dimer was formed, the copper(I) was released from caffeic acid and oxidized back to copper(II) by oxygen.



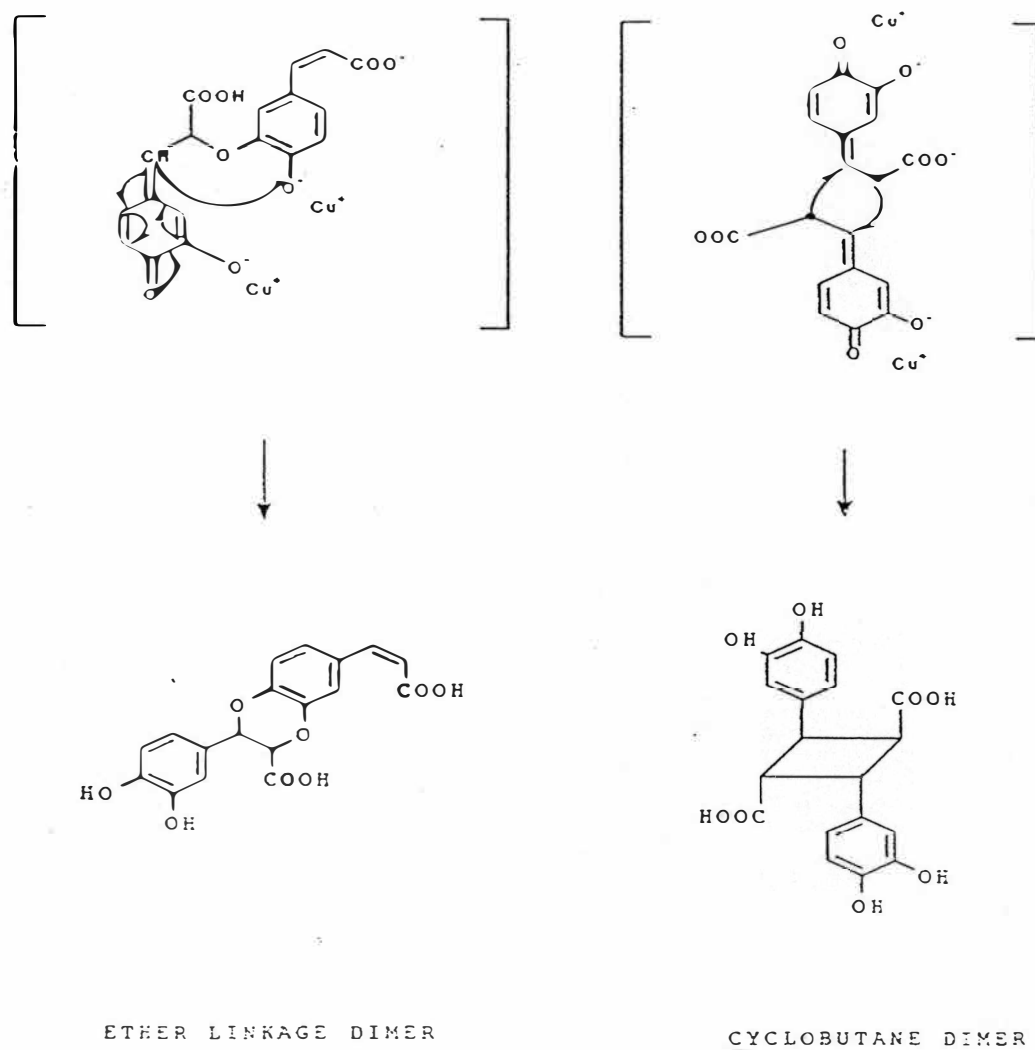


Figure 24. Proposed Mechanism.

CHAPTER V

CONCLUSIONS

In the presence of reducible transition metal such as copper, caffeic acid undergoes a dimerization reaction. The reaction was a two-step reaction, first an acid-metal complexation then a copper(II) reduction in conjunction with a caffeic acid dimerization reaction. Copper(II) is first reduced to copper(I) by caffeic acid. Then copper(I) is oxidized back to copper(II) by the presence of oxygen. The experimental data indicated that the reaction was accelerated in the presence of oxygen. This reaction takes place slowly and involves the conjugated double bond in the propenoic side chain of caffeic acid. Dimers were formed during this reaction. These dimers are pH sensitive, the ether linkage dimer, and temperature sensitive, the cyclobutane dimer.

As mentioned earlier lignin is converted to humic substance in natural waters through biodegradation. Caffeic acid is one of the oxidation products of lignin degradation. We believe the reaction of caffeic acid and copper could take place during the formation of humic substances from lignin.

The results of this research may provide some useful information

for studying organic chelator in natural waters. The fate and reactivities of organic chelator in natural waters are strongly influenced by the presence of transition metals, pH and the temperature of the system. When isolating organic species from natural water, those factors, such as pH, temperature and the presence of reducible transition metals should be taken into consideration.

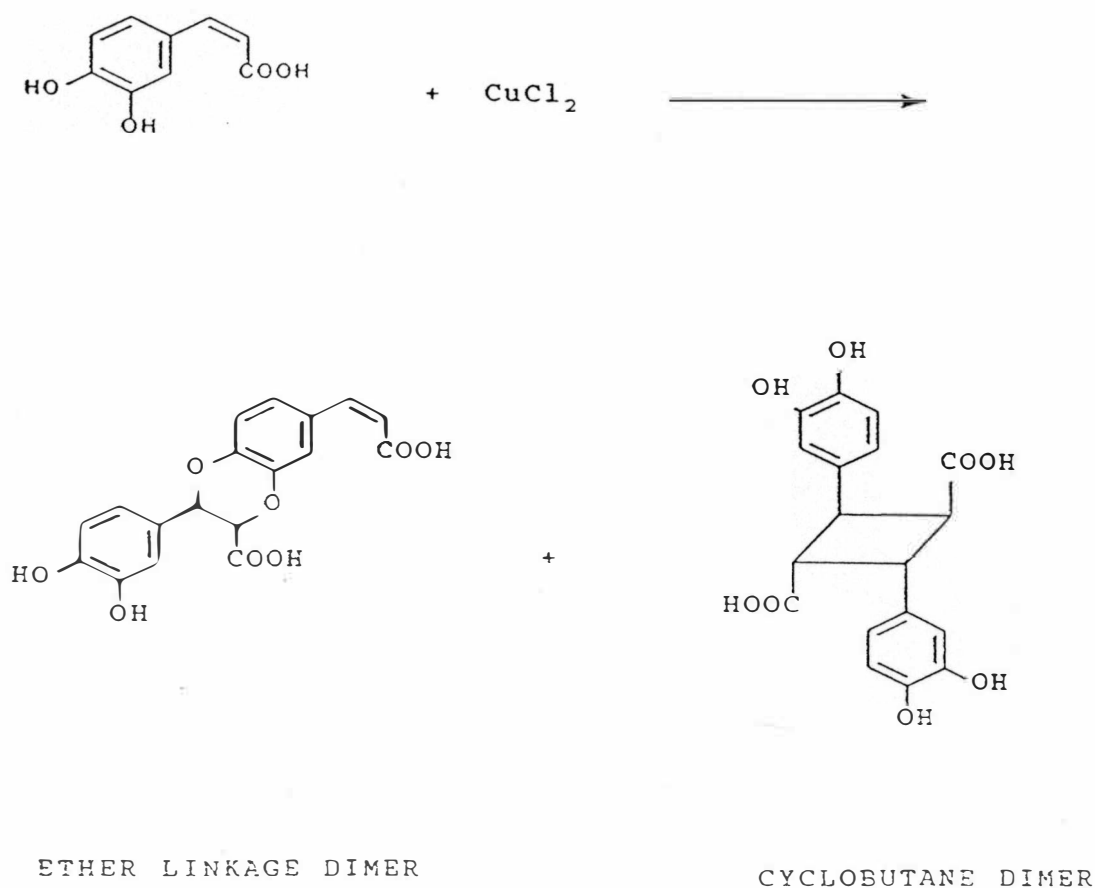


Figure 25. The Net Reaction.

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