

FIXATION MECHANISM OF AMMONIACAL COPPER  
WOOD PRESERVATIVES

by

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B.Sc. and M.Sc. Nanjing University, P. R. of China, 1982

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENT FOR THE DEGREE OF  
MASTER OF SCIENCE

in

FACULTY OF GRADUATE STUDIES

(Department of Wood Science)

We accept this thesis as conforming  
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

December, 1994

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Date Jan. 10, 1995

## ABSTRACT

This thesis describes the first comprehensive study of the chemistry of ammoniacal copper based wood preservatives, including the fixation of copper and nitrogen in treated wood; and the identification of the cause of the black color in treated Douglas-fir heartwood. The effects of enhanced nitrogen content in ammoniacal copper solution treated wood on the decay by three rotting fungi were investigated.

Taxifolin, a Douglas-fir heartwood extractive was isolated and identified using ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) and mass spectrometry (MS). It was confirmed that taxifolin reacted with ammoniacal copper solutions to form a black copper-nitrogen-taxifolin complex.

Nitrogen fixation in ammonium hydroxide treated wood was studied through the reaction of ammonium hydroxide solution with wood and its components. It was found that carbonyl and carboxylic groups in lignin and hemicelluloses can react with ammonia to fix nitrogen in wood.

The fixation mechanism of copper and nitrogen in ammoniacal copper treated wood was studied through the reaction of vanillin, a lignin model compound, with an ammoniacal copper solution. The green copper complex formed was extensively studied using infrared (IR), electron spin resonance (ESR) elemental analysis and X-ray single crystallography and identified to be a vanillin-copper ammonia complex. An X-ray structural analysis of a single crystal of the complex enabled the structure to be known. In the complex both the methoxy and phenolic oxygen atoms of guaiacyl units were coordinated to the copper, together with

nitrogen from ammonia forming a six coordinated complex. This was first determination of a crystal structure of copper-lignin model complex which may describe important bond formations occurring during the fixation of copper and nitrogen from ammoniacal copper wood preservatives.

The effect of enhanced nitrogen in ammoniacal copper treated wood on the decay was studied. Wood treated with higher concentrations of ammonium hydroxide solution showed an increased decay resistance to both *P. placenta* and a slightly enhanced resistance to *T. versicolor* fungus. However, for *G. trabeum* the weight losses of the ammonium hydroxide treated-wood was slightly increased relative to the control. Ammoniacal copper treated wood with low copper retention showed increased decay resistance to both *T. versicolor* and *G. trabeum*. The nitrogen in the complex can not be used by fungi for their growth. However, the ammoniacal copper treated wood with low copper retention was easily attacked by *P. placenta* due to its ability to form insoluble copper oxalate in copper treated wood.

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## ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. John N.R. Ruddick, my supervisor, for his patient guidance and encouragement throughout the course of the research work and in preparation of this thesis.

I would like to express my appreciation to the members of my thesis committee, Dr. L. Paszner, Dr. G. Herring and Dr. N. Richardson for their invaluable advice and support on several aspects of this thesis.

I gratefully acknowledge the NSERC/Industrial Chair in Wood preservation for financial support.

Finally, I wish to sincerely thank my wife Yi Feng and my son Lei for their patience and understanding over the past years.

## 1. INTRODUCTION

Wood is a remarkable material of great value and importance in the world economy. Canada is the world's leading exporter of forest products. In 1991 Canadian exports of forest products were valued at \$19,100 million. With a trade surplus of \$18.1 billion in 1991, the forest industry was a major contributor to Canada's positive trade balance (Canada Year Book, 1994). Clearly, the forest industry plays a major role in Canada's economic activity.

Wood is used extensively as a structural material in the world. When wood material is in use under conditions where it becomes wet, it is liable to decay. It has been estimated that the annual loss in Canada due to the biological destruction of timber is in excess of \$500 million. Decay of domestic dwellings alone has been estimated to cost Canadian homeowners over \$100 million annually (Ruddick, 1980a). With the population in Canada continuing to increase, the demand for wood supply will continue to grow. One strategy designed to achieve an adequate supply of wood for this growth is to increase the service life of wood products subject to decay. Consequently, more attention is being focused on wood preservation, since this can help conserve forests and provide economic and social benefits (Wilkinson, 1979).

The vacuum-pressure impregnation of wooden commodities with preservatives, to extend their service life, has been very successful in expanding the use of nondurable wood species such as hem-fir [a commercial mixture of western hemlock (*Tsuga heterophylla* Sarge.) and amabilis fir (*Abies amabilis* Forbes.)]. In Canada the wood preservation industry treats 1.985 million cubic meters (m<sup>3</sup>) of wood each year, with 51.5% going to consumer lumber, 21.3% to pole production and 24.1% to industrial lumber. The 1992 value of this treated

lumber was \$547 million. The value of the total volume of treated wood installed in Canada is in excess of \$10 billion (Stephens *et al.*, 1994). Among the wood preservatives used in Canada, chromated-copper-arsenate (CCA), a major waterborne chemical preservative, is widely used to protect the wood. However, it is rarely used for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) or spruce (*Picea* sp.) heartwood because of poor penetration.

Improved treatment of such refractory wood species can be achieved by ammoniacal copper based wood preservatives. Ruddick (1980b) investigated the treatability of lodgepole pine (*Pinus contorta* Dougl.) heartwood with ammoniacal copper arsenate (ACA) and CCA. Analysis of preservative penetration and retention showed that both were higher in the ACA treated lumber than in that treated with CCA. However, with some wood species with ACA treatment results in an unpleasant dark color (Ruddick, 1979). This color reduces the acceptability of certain products, e.g. decking and fencing.

In the formulations of ammoniacal copper preservative system developed, copper is the biocidely active component, but other co-biocides are also included, such as arsenic in ACA. Industrial concern with the toxicity and the cost of arsenic (V) oxide has led to the replacement of some arsenic with less toxic zinc in the development of ammoniacal copper zinc arsenate (ACZA). More recently, ammoniacal copper/quat (ACQ) and ammoniacal copper citrate (CC) have been commercialized. They exhibit relatively low mammalian toxicity and low environmental impact.

### **1.1 Black color of Douglas-fir heartwood after treatment with ACA**

It is well known that the ACA treatment of certain wood species, such as Douglas-fir, results in a darkening of the wood during treatment. The cause of this

darkening is most likely related to ammonium hydroxide in the preservatives, since treatment with either copper or arsenic containing preservatives such as CCA, does not give this reaction. It may also be a function of the high pH of the treating solution. A literature survey confirmed that no explanation has yet been found for this darkening of Douglas-fir after treatment with ammoniacal copper preservatives. It is not even known, for example, which component in Douglas-fir heartwood is responsible for the dark color.

## 1.2 Fixation of ammoniacal copper preservative in wood

The fixation mechanism of ammoniacal copper preservatives is not understood. It has been suggested that the cupriammonium ions in solution react by ion exchange with exchangeable protons in functional groups in wood, such as carboxylic acid groups (Kupchinov *et al.*, 1975). In addition, evaporation of ammonia will result in the breakdown of the tetraminocupric complexes present in solution, thus forming water-insoluble copper salts, such as arsenate in the case of ACA (Hartford, 1973). To date, this proposed fixation mechanism has not been verified experimentally.

Recently, this hypothesis was challenged when it was observed that ACA-treated spruce poles retained the enhanced nitrogen levels, more than two years after treatment. The nitrogen must therefore be strongly bound in the wood. It is possible that the ammonia may react with the wood components during impregnation, allowing the nitrogen to be fixed to the wood.

A number of investigations of the bonding of copper complexes in treated wood using electron spin resonance (ESR) have been reported (Ruddick *et al.*, 1992; Hughes *et al.*, 1992). It was found that copper-nitrogen complexes were most likely formed in ammoniacal copper treated wood. These complexes had a high

leaching resistance. Based on the above observations, a reaction between the ammonia and the copper in the preservative or the wood may be proposed which results in a complex formation, and bound nitrogen present in treated wood. However, the form of this nitrogen fixed in the wood and the nature of the copper-nitrogen complexes in treated wood remain unknown.

### **1.3 The impact of nitrogen retention in ACA-treated wood**

The main organisms responsible for deterioration of wood are bacteria, fungi and insects. In temperate regions of Canada, the most abundant and significant organisms responsible for wood decay are fungi. Fungi, like other organisms, require substantial amounts of nitrogen for synthesis of protein and other cell constituents or products such as nucleoproteins, lipoproteins, enzymes, and chitin in hyphal cell walls. Wood-inhabiting fungi are unique in their ability to obtain their nitrogen needs from the generally very small amount available in wood. The nitrogen content of wood ranges from 0.03 % and 0.1 % of the dry weight of wood (Allison *et al.*, 1963, Cowling and Merrill, 1966). Many fungi are able to use ammonia, nitrates, nitrites, and urea as sole sources of nitrogen. Several researchers have shown that increasing the nitrogen content of wood, frequently by addition of an ammonium salt, increases the rate of decay by wood-destroying fungi. Ammonium is often the best nitrogen source, but it may affect the media pH and, hence, growth responses (Zabel and Morrell, 1992).

It was found that the nitrogen content in ACA-treated spruce wood was much higher than that in untreated wood even after two years of outdoor storage. It was suggested that the nitrogen was strongly bound in the wood. Whether fungi or bacteria are capable of metabolizing the higher nitrogen content in the ammonia-treated wood to promote their growth is unknown.

#### **1.4 The objectives of this study**

Based upon the limited knowledge of the reactions of ammoniacal copper solutions with wood, and the possible enhanced effect of the ammonia treatment on fungal activity, the following studies have been planned.

**(1) Determine the cause of the darkening of Douglas-fir heartwood when treated with ACA.**

**(2) Investigate the fixation mechanism of ammoniacal copper complexes in wood and determine which wood component can take part in the fixation reaction.**

**(3) Establish whether enrichment of nitrogen in ammoniacal-copper preservative treated wood increases the decay potential.**

## **2. BACKGROUND**

### **2.1 Chemical nature of wood**

Wood is comprised primarily of cellulose (40-50%) and hemicellulose (20-35%) and lignin (15-35%). There is a minor amount of extraneous materials (2-10%) in wood, mostly in the form of organic extractives such as tannins, lignan, flavonoids, stilbenes, terpenoid, starch, lipids, pectins, alkaloids, proteins, fat and waxes and as well as trace amount of inorganic minerals (0.1-1.0%).

Cellulose is the most important component in wood and it consists of 1,4- $\beta$ -linked glucopyranose sugar units having both intermolecular and intramolecular hydrogen bonding. The average cellulose chain length (or degree of polymerization) is in the 7,000 to 10,000 glucose unit range. Cellulose consists of a crystalline area where cellulose chains are arranged in an orderly three dimensional crystal lattice as well as amorphous regions where the cellulose chains show much less orientation with respect to each other. The surfaces of the microfibrils are surrounded by hemicellulose. The hemicellulose and lignin are associated primarily with the noncrystalline zones that occur within and between the microfibrils.

Hemicelluloses are polymers of various pentose and hexose sugar units. The major sugars in the polymer backbones are glucose, xylose, mannose, galactose, arabinose, rhamnose, and uronic acids. The chemistry of hemicelluloses has been widely studied, and has been found to be much more complex than cellulose. Hemicelluloses differ from cellulose in having short chain lengths, and side chains that are sometimes branched, and sugar monomers other than glucose. Hemicellulose are either water or alkali soluble. In softwoods the galactoglucomannans and arabinoglucuronxylans predominate, while in

hardwoods the arabinoglucuronoxylans and glucomannans are the most frequently occurring structures.

The third major wood component, lignin, is a random three dimensional polymer of two basic phenylpropane monomers. The phenylpropane units are linked by biphenyl, aryl-alkyl or ether linkages, and form relatively stable and inactive polymers that are resistant to hydrolysis. Lignin can be divided into several classes according to the structural elements. Guaiacyl lignin, which occurs in almost all softwoods, is largely a polymerization product of coniferyl alcohol. The guaiacyl-syringyl lignin, typical of hardwoods, is a copolymer of coniferyl and sinapyl alcohols, the ratio varying from 4:1 to 1:2 for the two monomeric units. The concentration of lignin is higher in the middle lamella than in the secondary wall. However, because of the thickness of the secondary wall, at least 70% of the lignin in softwoods is located in this region. Lignin provides stiffness and strength. Lignin also is a very durable material, acting as a barrier against microbial attack of the more vulnerable carbohydrates in the cell wall.

Lignin has been proved to be a major fixation site for some inorganic wood preservative components, such as copper (Dahlgren, 1975). The amount, type and location of lignin within the wood structure have a significant impact on the fixation of wood preservatives and thus the microbial susceptibility of the wood.

## **2.2 Biological deterioration of wood**

Wood, as a naturally produced organic material, may be subject to decay. The principal agencies of this destruction are bacteria, fungi and insects. In general, the main agencies of biodeterioration of wood in Canada are fungi.

Not all fungi that colonize wood lead to degradation of the structural components (*i.e.* decay). Such mould and stain fungi generally do not cause loss in

strength. Decay fungi cause significant softening or weakening of wood, often to the point that wood physical characteristics are completely destroyed. Decay fungi can be further classified as brown rots and white rots (Nicholas, 1973; Zable and Morrell, 1992). The brown rots selectively attack the cellulose and hemicellulose of the cell, with little effect on the lignin (Bray and Andrews, 1924; Campbell, 1952). Wood seriously degraded by these fungi will have an abnormally brownish color. White rot fungi have the ability to degrade both the lignin and cellulose components of the cell (Kawase, 1962). They sometimes have only a limited effect upon the color of the wood but in other cases may give it a bleached or whitish color. Soft-rot fungi are Ascomycotina that attack wood that is very wet (80-100 % MC) and usually penetrate rather slowly. They gradually degrade wood from the surface. They are similar to brown rot fungi in that only degrade cellulose (Zable and Morrell, 1992).

### **2.3 Ammoniacal copper preservatives**

Ammoniacal wood preservatives have been known since the beginning of the century. In 1907 "Aczol" was one of the first to be introduced as an ammoniacal solution of copper and zinc salts with phenol (Hunt and Garratt, 1967). In 1940 the University of California patented an ammoniacal wood preservative called Chemonite® (Gordon, 1940), which contained copper salts to provide fungicidal action, and arsenic salts which functioned as a fungicide as well as an insecticide. The salts were dissolved in aqueous ammonia and the preservative was fixed in the wood by the precipitation of water-insoluble copper arsenate as the volatile ammonia evaporated. It was first ammoniacal copper arsenite preservative (ACA) which was submitted to the AWWA preservatives committee in 1949 (Baechler, 1949). The first commercial Chemonite® treating

plant was built in 1935 to preserve lumber, ties, poles, and piles, mainly of white fir. Later in 1941, a Chemonite® treating plant was built that processed almost exclusively coastal Douglas-fir (Fritz, 1947).

The composition of ACA is shown in table 2-1-1. ACA was originally prepared at the treating plant by mixing the copper chemical with arsenic trioxide in ammonium hydroxide, which was known as ammoniacal copper arsenite. In the mid-1970's, it was realized that during the mixing process, the air oxidized the arsenic to the pentavalent form. Thus the name was changed to ammoniacal copper arsenate (Ruddick, 1982). Following impregnation of the ACA in the wood, the ammonia evaporates. This causes a breakdown of the chemical complexes and formation of a water insoluble copper arsenate in the wood, which will not be washed out when the treated wood is placed in service.

With the growing pressure from environmental groups on conventional wood preservatives, ammonia-based preservatives show considerable promise for further development to increase their versatility as wood preservatives. Most of the formulations developed use copper as the active cation because of its excellent fungicidal action. Industry concern with the toxicity and leaching of arsenic led to interest in replacing some of the arsenic. Clarke and Rak (1974) found that addition of zinc oxide to ACA enhanced arsenic fixation. In this formulation, fifty percent of the arsenic oxide was replaced by zinc oxide. This led to development of ammoniacal copper zinc arsenate (ACZA) which was first introduced to Canadian Standards Association (CSA), but was never developed further. Subsequently a similar ACZA formulation was introduced in the United States in 1981 in which the Cu:Zn:As ratio was 1:1:1 (Best and Coleman, 1981). In 1983 the AWPAC approved the 1:1:1 formulation of ACZA into the preservative standard (Morgam, 1989). More recently, ammoniacal copper citrate (CC) and ammoniacal

copper/quaternary ammonium compounds formulations (ACQ) have been developed which exhibit relatively low mammalian toxicity and low environmental impact (Findlay and Richardson, 1983).

Table 2-1-1 Composition of ACA

	AWPA standard			1989 CSA standard
	1949 proposal	1953 acceptance	1969 oxide basis	1990 oxide basis
Cu as $\text{Cu}(\text{OH})_2$	60+5	55.7-59.7	49.8	-
As as $\text{As}_2\text{O}_3$	40+5	38.7-42.7	50.2	-
Cu as CuO	-	-	-	49.8-63.0
As as $\text{As}_2\text{O}_5$	-	-	-	37.0-50.2
Ammonia	2.3-3.0% in soln.	1.5-2.0 x $\text{Cu}(\text{OH})_2$	1.5-2.0 x CuO	1.5-3.5 x CuO
Acetic acid	0.05% in soln	1.6	1.7 max	-
Carbonate as $\text{CO}_2$	-	-	-	0.0-0.8 x CuO

### 2.3.1 Role of ammonia in improving preservative treatment

The reason for the increased penetration of ACA treatment is not well known, but is believed to be associated with the action of ammonia on the cellulose, which results in a separation of the hydrogen bonding (Winandy *et al.*, 1989). Therefore, ammonia is an excellent swelling agent. Ammonia is also capable of dissolving encrusting materials such as fats, waxes, resins and polygalacturonic acids (Hulme, 1979), thus enhancing preservative penetration, especially in refractory species.

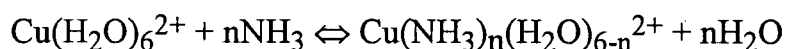
Considerable effort has been made in order to understand the effect of anhydrous ammonia on various components of the wood (Bariska and Popper, 1971 and 1975; Rak 1977). These studies indicated that ammonia penetrated the amorphous cellulose first, then the cellulose, hemicellulose and lignin, and eventually the crystalline cellulose. Water does not enter the crystalline region of cellulose and is sorbed only by the paracrystalline region of cellulose (Bariska *et al.*, 1969). Schuerch (1964) pointed out that ammonia is a superior solvent for both the major polymer systems - the phenolic lignin binder and the polysaccharide system in the cell wall. Although the lignin is a branched and crosslinked polymer, when fully penetrated by ammonia, it swells, becomes soft, but its molecules are not dissolved or completely separated. The hemicellulose may be deacetylated and acetamide may be formed during treatment with ammonia. It was reported that uronic acid groups may be converted to ammonium salts or amides (Schuerch, 1964)

Rak (1977) reported, in his studies of spruce, that the permeability of spruce roundwood in the radial direction was improved using ammoniacal solutions of inorganic salts, compared with aqueous solutions. He also noticed that the ammoniacal system penetrated spruce 1.7-1.8 times faster in the radial direction than the acidic CCA, while the permeability in the tangential direction

was 3.8 times faster. The permeability of spruce sapwood to an aqueous ammoniacal solution of inorganic salts was found to be better than a plain water solution.

### 2.3.2 Ammoniacal copper (II) complexes

Addition of ammonium hydroxide solution to an aqueous solution of the copper(II) ions results in the setting up of a complex equilibrium, involving the successive replacement of coordinated water by ammonia according to the equation:



The successive formation constants for these reactions are shown in Table 2-1-2, together with those for the  $\text{Ni}(\text{H}_2\text{O})_6^{2+}$  cation. For nickel(II) there is a smooth decrease from  $K_1$  to  $K_6$ , whereas for copper(II),  $K_6$  is zero and  $K_5$  is very small, indicating a negligible tendency to take up more than four ammonia groups.

Table 2-1-2 The successive formation constant for the copper(II) and nickel(II) ions with ammonia as a ligand (Hathaway *et al.* 1970)

	$K_1$	$K_2$	$K_{13}$	$K_{14}$	$K_{15}$	$K_{16}$
$\text{Cu}^{2+}$	12000	3000	800	120	0.3	-
$\text{Ni}^{2+}$	500	150	50	15	5	1

The most common copper-ammonia complex  $\text{Cu}(\text{NH}_3)_4(\text{H}_2\text{O})_2$  has a distorted octahedral configuration around the metal atom with the four nitrogen bonds ( $\text{Cu-N} = 2.03\text{-}2.06 \text{ \AA}$ ) closest to the copper atom in a plane and two less strongly bound water ligands ( $\text{Cu-O} = 2.59\text{-}3.37 \text{ \AA}$ ), on an axis perpendicular to

this plane (Emeleus, 1973, Mazzi, 1955). Copper (II) ions can also form complexes with amines such as ethylenediamine and pyridine.

### **2.3.3 Reaction of ammonia and ammoniacal copper with wood and its components.**

#### **2.3.3.1 Reaction of ammonia with wood components**

Treatment of wood with aqueous ammonia causes substantial changes in wood properties, (*e.g.* more hygroscopic, swelling to a high degree and doubling of internal surface area).

Mahdalik *et al.* (1971) studied the changes in the chemical properties of the wood treated with liquid ammonia. They found that the reaction of ammonia with moist wood fixed more nitrogen than when oven dried wood was reacted. They also noted that the extractives of oak fixed a noticeably greater amount of nitrogen, than the lignin-polysaccharide components. Gaseous ammonia is retained in large amounts by cellulose fiber. Cotton may occlude 115 times its volume of gaseous ammonia. When the ammonia has evaporated, the cellulose appeared to be unchanged chemically. Aqueous ammonia, even when concentrated (23-28 %  $\text{NH}_3$ ), seemed to have no effect on cellulose (Heuser, 1946). Aqueous ammonia is reported to reduce cellulose microfibrils into more elementary fragments. As a result of rupture of physical and hydrogen bonding between complexes of carbohydrate macromolecules. The cleavage of chemical bonds between the middle lamella and the cell wall in the middle lamella was observed.

Solar and Melcer (1978) have reported experiments to examine the physical and chemical changes in lignin when wood was treated with aqueous ammonia. They found that the total number of acidic groups in the lignin decreased by one third and the carboxyl group content was reduced by one half during the treatment.

The infrared spectra showed an increase in the absorption at  $1670\text{ cm}^{-1}$  which was interpreted as evidence for the formation of  $\alpha$ -keto groups in the side chain of the lignin phenylpropane units, as a result of hydrolysis of bonding between lignin and holocellulose. The band at  $1240\text{ cm}^{-1}$  decreased after treatment with ammonia solution, as a consequence of hydrolysis of C-O ester and ether bonds. The infrared spectra also contained a lowering of the band at  $1740\text{ cm}^{-1}$ , consistent with a decrease in the number of carboxyl groups in the lignin samples.

Kaplunova *et al.* (1986) studied the reaction of nitrolignin from cotton-seed husks with aqueous ammonia. At room temperature the lignin reacted with ammonia mainly through the carbonyl and carboxyl groups, forming imines and ammonium salts. Koval'chuk *et al.* (1972) reacted lignin from sunflower husks with aqueous ammonia solution. The infrared spectra showed that the carboxylic groups reacted with ammonium ions to form ammonium salts, which further converted into amides. They also pointed out that  $\beta$ -diketone structure in lignin can react with ammonia and lead to the formation of amines.

#### 2.3.3.2 Reaction of ammoniacal copper complex with cellulose

An aqueous solution of tetraamminecopper hydroxide,  $[\text{Cu}(\text{NH}_3)_4](\text{OH})_2$ , is a good solvent for dissolving cellulose, and is widely used for the viscosity determination of pulp in the paper and pulp industry. It is prepared by dissolving cupric oxide in excess ammonium hydroxide solution. The tetramminecopper hydroxide can swell, peptize, and eventually disperse cellulose. The dispersion of cellulose in cupriammonium hydroxide is accompanied by a chemical reaction. The interaction of the cupric ions in strong ammonium hydroxide solution with glycol-like hydroxyl groups to form complexes, has been reported extensively. Based on conductivity measurements. Reeves (1949) suggested that such complex

formation was critically dependent on the distances between the hydroxyl groups, which between hydroxyl groups on adjacent carbon atoms was dependent on the angles between the groups. Complex formation occurred most readily at the true *cis* position ( $0^\circ$  angles) and at  $60^\circ$  angle, but did not occur at the angles of  $120^\circ$  or  $180^\circ$ . Hinojosa and his co-workers (1974) studied the interaction of tetraamminecopper ions with cellulose using electron spin resonance (ESR). They found that the reaction between tetraamminecopper ions and cellulose was very rapid and reversible. When the concentration of ammonia was decreased in the cupric ion-ammonium hydroxide-cellulose complexes, the ESR signal due to paramagnetic resonance of the complex decreased or was lost. Similar results were obtained when potassium hydroxide was removed from the complexes formed when copper sulphate dissolved in potassium hydroxide.

It was concluded that the reaction of tetraamminecopper ions with adjacent hydroxyl groups on the cellulose to form complexes depended on an optimum distance between of the hydroxyl groups. Evidently, wetting of cellulose fibers with solutions of strong bases allowed the adjacent hydroxyl groups to favorable positions to yield this optimum arrangement. When the base was removed, rotation occurred to give less favorable positions of the hydroxyl groups for complexing with cupric ions (Hinojosa *et al.*, 1974)).

#### **2.3.4 Fixation of ammoniacal copper preservatives in wood**

Ammoniacal copper preservatives have been examined by Hulme (1979). The fixation mechanism of ammoniacal copper preservatives in wood has been reported by Eadie and Wallace (1962), Clarke and Rak (1974) and Sundman (1984). Following the impregnation of the ammoniacal copper preservative solution into the wood, it has been suggested that tetraamminecopper ions react by

ion exchange with functional groups in the wood such as carboxylic groups (Kupchinov *et al.*, 1975). In addition, the evaporation of ammonia causes the break down of the complexes in solution to form water-insoluble copper salts, such as copper arsenate in the case of ACA (Hartford, 1973). The above fixation mechanisms have not been verified experimentally.

The generally accepted fixation theory for ammoniacal copper preservatives in wood was challenged in the late 1970's, when it was observed that ACA-treated spruce pole sections stored outdoors retained enhanced nitrogen levels, more than two years after treatment (Ruddick, 1979). An analysis of four zones was made, at the surface (0 to 5 mm), at the limit of the copper penetration, the heartwood just beyond the limit of the copper penetration, and the center of the cross section. The results showed that nitrogen level was greatly enhanced in the ACA-treated wood, the nitrogen content appearing to be related to the ACA retention. In addition, there was evidence that the ammonia had penetrated beyond that achieved by the copper.

Recently electron spin resonance (ESR) was used to study the copper in ammoniacal copper treated wood (Ruddick *et al.*, 1992; Hughes *et al.*, 1994). They found that the copper-nitrogen complexes were formed in the wood after treatment with ammoniacal copper solution.

### **2.3.5 ESR spectral analysis of wood treated with ammoniacal copper preservatives.**

#### **2.3.5.1 The principal of ESR**

The electron has a spin quantum number. In the presence of an applied magnetic field, different energy states arise from the interaction of an unpaired electron spin moment with the magnetic field, resulting in the alignment of the

electron spin moment relative to the applied field. Only electromagnetic radiation with the frequency ( $\nu$ )

$$\nu = g \beta H/h$$

where  $g$  for a free electron is 2.0023  
 $\beta$  is the electron Bohn magneton  
 $H$  is the applied field strength  
 $h$  is Planck's constant

contains the right amount of energy to produce transitions between the ground and excited energy states of the electron. For that it gives an ESR signal. The paired electrons do not give an ESR signal since paired electrons have spins with opposite directions.

Unpaired electrons arise from incompletely filled electron shells, many examples are found in the d-orbitals of transition metal ions e.g.  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ , *etc.* or the excited state molecular orbitals of paramagnetic species such as radicals. The interaction of the magnetic moment of those unpaired electrons with a radio frequency electromagnetic field in the microwave region and a simultaneously applied static magnetic field leads to absorption of energy by the sample. Further, there is a frequent interaction between this unpaired electron and the magnetic moment of nuclei in the sample which gives rise to a hyperfine structure in the spectrum. As a result, a nucleus of spin  $I$  gives rise to a splitting of ESR line into  $2I + 1$  components, all of equal intensity separated by the coupling constant  $A$  (Fig. 2-1-1). Therefore, ESR results are usually reported in terms of a spin Hamiltonian and expressed as the ' $g$ ' and ' $A$ ' tensors, the principal values of which in anisotropic spectra yield information about the molecular environment of the unpaired electron responsible for the resonance and the nature of the nucleus it interacts with.

### 2.3.5.2 ESR analysis of ammoniacal copper treated wood

It is possible to observe the ESR spectrum of CCA- or ACA-treated wood, since copper (II) is paramagnetic. This arises because copper (II) contains an unpaired electron in the  $d^9$ -electronic configuration, in which the unpaired electron occupies the  $d_{x^2-y^2}$  orbital and all other d-orbitals are doubly occupied. The majority of copper (II) complexes have a distorted octahedral geometry, with the two axial bonds being longer than those in the plane.

The ESR spectral hyperfine structure results from the interaction of unpaired electron with the nucleus. For copper (II) and its nuclear spin value of  $I=3/2$ , the ESR spectrum of Cu (II) compounds will show four equally spaced features ( $2I + 1$ ) due to the hyperfine interaction. An early ESR study of CCA treated wood was carried out by Plackett *et al* (1987) who used radiata pine (*pinus radiata*) and extracted lignin impregnated with CCA and copper sulfate. The ESR results indicated that hydrated  $Cu^{2+}$  ions were residing in fixed sites within the timber. More detailed work by Ruddick *et al* (1992) and Hughes *et al* (1992) focused on other copper species as well as CCA. The copper sulphate treated wood exhibited a hyperfine structure at lower field, which suggested a  $d_{x^2-y^2}$  ground state, in which the copper was bonded to four (or six) oxygen atoms in a square planar (or distorted octahedral) configuration. Wood impregnated with copper acetate dissolved in either water or methanol gave similar spectra, which also compared well to that of copper sulphate treated wood. Clearly, in these three treatments any interaction with the substrate was independent of the solvent employed (Ruddick, 1992).

In ammoniacal-copper treated wood the copper ions had a higher  $A_{//}$  value and lower  $g_{//}$  value than those for the corresponding aquocopper ion, resulting from an increase in the electron density on the copper, since nitrogen is a more

electron-rich atom than oxygen (Peisach and Blumberg, 1974). This supports the hypothesis that in ammoniacal copper treated wood copper-nitrogen bonded complexes are formed in the wood. Hughes *et al* (1992) also reported that the copper-nitrogen bonded complexes in wood had a high leaching resistance. It has been shown that for a copper complex possessing a square planar geometry there is an inverse dependence of  $A_{//}$  on  $g_{//}$  (Peisach and Blumberg, 1974). This dependence can help to identify the copper-ligand groups in the copper complexes of known structure. Ruddick (1992) has compiled  $g_{//}$  and  $A_{//}$  for a series of copper-treated ponderosa pine samples and suggested that the ESR technique might be employed to define the geometric relationship of isostructural copper complexes formed in treated wood (Fig. 2-1-2). In the figure, complexes with four planar copper-oxygen bonding, *e.g.*  $[\text{Cu}(\text{H}_2\text{O})_4]^{2+}$ , lie to the lower right, while the corresponding molecules containing four copper-nitrogen bonds in a plane have smaller values of  $g_{//}$  and larger values of  $A_{//}$  and these complexes are located on the upper left of the plot. The parameters for copper bound to two nitrogen atoms and two oxygen atoms lie between those of the two extremes.

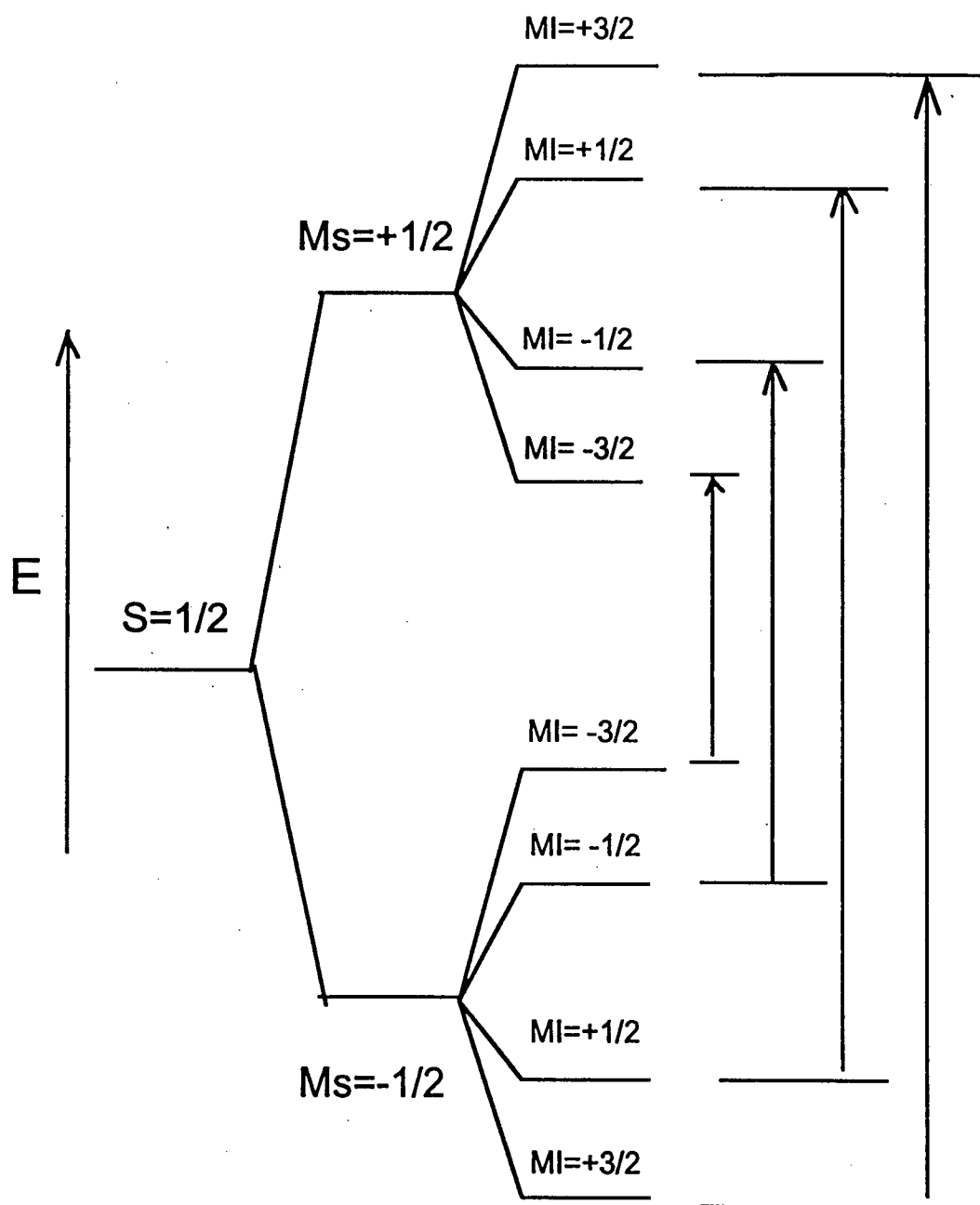


Fig. 2-1-1 Energy levels for a system with electron spin  $S=1/2$  and nuclear spin  $I=3/2$  showing the effects of magnetic field and the nuclear quadrupole moment.

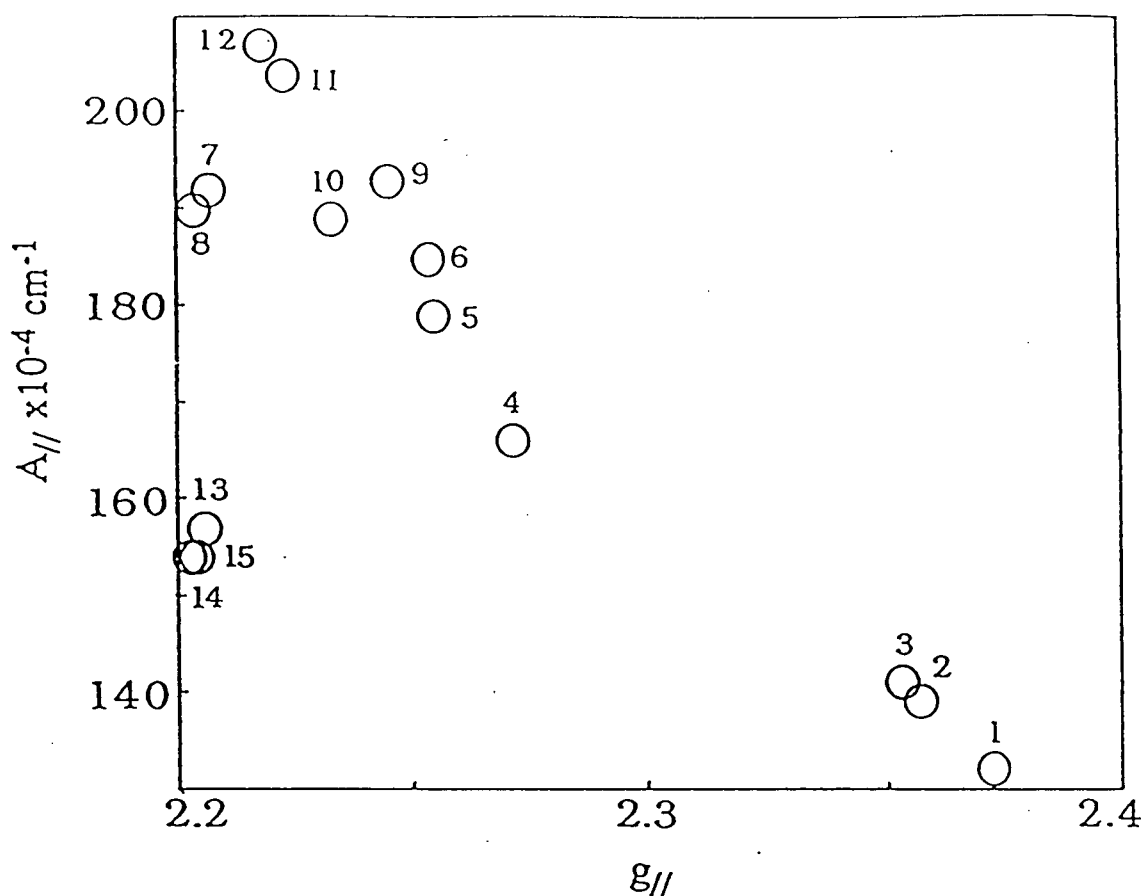


Fig. 2-1-2 Relation between the magnetic parameters  $g_{//}$  and  $A_{//}$  for copper-treated wood, Avicel and copper solutions. 1) Wood treated with  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in water at room temperature, 2) Wood treated with  $\text{Cu}(\text{CH}_3\text{COO})_2$  in water at room temperature, 3) Wood treated with  $\text{Cu}(\text{CH}_3\text{COO})_2$  in methanol at room temperature, 4) Wood treated with  $\text{CuCO}_3$  in  $\text{NH}_4\text{OH}$  at room temperature, 5) Avicel treated with  $\text{CuCO}_3$  in  $\text{NH}_4\text{OH}$  at room temperature, 6)  $\text{CuCO}_3$  in  $\text{NH}_4\text{OH}$  at 77K, 7) Wood treated with  $[\text{Cu}(\text{en})_2]^{2+}$  in water at room temperature, 8) Wood treated with  $[\text{Cu}(\text{en})_2]\text{SO}_4$  in water at room temperature, 9) Wood treated with CTST<sup>TM</sup> in water at room temperature, 10) Avicel treated with CTST<sup>TM</sup> in water at room temperature, 11) CuHDO in toluene at 77K, 12) CTM<sup>TM</sup> at 77K, 13) Wood treated with CuHDO/amine at 77K, 14) CuHDO/amine at 77K, 15) Avicel treated with CuHDO/amine in water at room temperature.\*

\*CuHDO - Bis-(N-cyclohexyldiazoniumdioxy)-copper (II); en - 1,2-diaminoethane.

## **2.4 Enhanced nitrogen content in ammoniacal copper treated wood**

### **2.4.1 Nitrogen requirement for fungal growth**

Like all living organisms, fungi have certain requirements for growth and survival. The major growth needs of wood-inhabiting fungi are water: free water on the surfaces of cell lumen; oxygen: atmospheric oxygen at a relatively low level for most fungi; a favorable temperature range from 15 to 45°C for most wood-inhabiting fungi; a digestible substrate (wood, *etc.*) provides energy and metabolites for synthesis via metabolism; a favorable pH range from 3 to 6 for most wood-inhabiting fungi; nitrogen and micronutrients such as vitamins and essential elements such as phosphorus (Zabel and Morrell, 1992).

Fungi require a substantial amount of nitrogen for the synthesis of protein and other cell constituents or products such as nucleoproteins, lipoproteins, enzymes, and the chitin in hyphal-cell walls. However, nitrogen is present in relatively small amounts in wood, comprising between 0.03 % and 0.1 % of the dry weight of wood (Allison, *et al.*, 1963). Distribution of nitrogen in wood indicates a reduction in nitrogen content from outer to inner sapwood, with the lowest amounts being found in heartwood. The pith section often contains relatively large amounts of nitrogen. Little is known concerning the nature of the nitrogenous materials in wood, principally because the small amounts present are commercially unimportant. But for wood-inhabiting microorganisms and insects that derive their nourishment primarily from wood itself, these small amounts of nitrogen are of paramount importance. The capacity of decay fungi to meet nitrogen needs wholly from the low amounts available in wood is even more surprising, because of the prodigious number of spores released (nitrogen content of about 3%).

A series of studies on the availability and roles of nitrogen in wood deterioration (Cowling and Merrill, 1966; Merrill and Cowling, 1966; Cowling 1970) showed that decay fungi probably conserve nitrogen by hyphal autolysis during which nitrogen is recycled toward the hyphal tips. The close regulation of the cellulose enzyme system in some wood-decay fungi may also serve to conserve nitrogen. Bacteria are often associated with fungi in the decay process and may play an important interactive role in nitrogen cycling and fixation in nitrogen cycling and fixation during some natural wood-decay processes (Aho *et al.*, 1974; Larsen *et al.*, 1978). Several researchers have shown that increasing the nitrogen content of wood, frequently by addition of an ammonium chemical, increased the rate of decay by wood-destroying fungi (Findlay, 1934; Amburgey and Johnson, 1978).

Fungal nitrogen sources are quite varied and may be organic or inorganic in nature. In general, fungi do not metabolize all nitrogen sources with equal ease. A fungus may have a requirement for nitrogen in a specific form. Fungi may utilize inorganic nitrogen in the form of nitrates, nitrites or ammonia, or organic nitrogen in the form of amino acids. Ammonia is often the best nitrogen source. During laboratory studies ammonia may affect media pH and hence, growth responses (Zable and Morrell, 1992). A few fungi may be able to obtain nitrogen via the direct utilization of atmospheric nitrogen (Smith, 1970).

#### **2.4.2 Nitrogen content in ammoniacal copper treated wood**

Ruddick (1979) found that the nitrogen content in ACA-treated spruce wood was much higher than that in untreated wood. In addition, the ammonia penetration was greater than that of the preservative solution, resulting in nitrogen enrichment in wood where no copper could be detected. These observations are

very important and indicate that the addition of nitrogenous materials to wood may increase its susceptibility to decay. The high nitrogen levels at surface of the treated wood are unlikely to be important, since the preservative retention will also be high and will deter fungal attack. However, at locations further from the surface, where an enhanced nitrogen level have been observed, the preservative retention is much lower than that required to prevent decay. Thus any damage extending to the inner area beyond the treated zone, either by deep checking during weathering or by mechanical damage, could expose wood with a high nitrogen content and a low preservative retention. Such a situation could lead to decay of the exposed wood.

Alkali treatment of wood (e.g., sodium hydroxide and ammonium hydroxide) increased the decay resistance in both the laboratory and the field studies, which has lead to suggestion of an alkali treatment as an alternative method of wood protection. It has been assumed that the alkali treatment may destroy thiamine (Dwivedi and Arnold, 1973), which is essential for fungal growth. But Highley (1973) found that low decay of ammonia-treated wood was not related to destruction of thiamine in wood. He found that the pH and ammoniacal nitrogen content of wood affected decay resistance, and suggested that after treatment with ammonia, toxic ammonia compounds were formed. Amburgey and Johnson (1978) reported that the increased decay resistance of ammonium hydroxide-treated wood may be due to factors that inhibit germination of basidiospores.

Recently, Ruddick (1992a) reported that soil-leached CCA-treated mini-stakes were attacked by decay fungi, causing considerable weight losses at gauge retentions above those considered to be effective. It was suggested that this

phenomenon was linked to bacterial action on the chemicals in the wood. This action caused a breakdown of the copper complexes, and made them soluble.

## **2.5 Black discoloration of Douglas-fir wood treated with ammoniacal copper preservatives**

### **2.5.1 Importance of Douglas-fir wood species in B.C.**

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), native in Canada and the Northwest of the U.S, is one of the predominant commercial timber species in British Columbia, accounting for 14 per cent of total cubic volume of softwood production in 1975. It is the strongest commercial softwood in Canada being used extensively for structural purposes, such as poles, piling and bridge timber. Much of the softwood plywood produced on the Pacific Coast contains Douglas-fir veneers. The effective preservative treatment of this heartwood will therefore be very important, particularly for sawnwood products.

### **2.5.2 Treatability of Douglas-fir**

During the last two decades, vacuum-impregnated sawnwood has become acceptable in consumer use. In western Canada, the heartwood of hemlock (*Tsuga heterophylla* (Raf.) Sarg) amabilis fir (*Abies spp*) is generally regarded as being relatively easy to treat, while that of Douglas-fir is considered to be more difficult to treat. Bramhall (1966) reported that the permeability of Douglas-fir heartwood produced in British Columbia was extremely variable, with that grown east of the coastal range being refractory, *i.e.*, untreatable, whereas that from Vancouver Island (coastal) was much easier to pressure impregnate with preservative. This observation was supported by Weaver and Levi (1979). The reasons for the refractory nature of Douglas-fir are not fully understood. Liese and Bauch (1967) have proposed that the low permeability of the ray cells is due to the relatively small proportion of ray tracheids. However, Hackbarth and Liese (1975) reported that neither the number nor the area of ray cells influenced the preservative

penetration, and rather increasing density and the proportion of latewood both reduced preservative absorption.

In general, the problem of treating refractory species has been attacked by three basic methods. There are: (1) The use of enzymes, moulds, or bacteria (biological) to enhance the permeability by biological attack of the pit structure; (2) incising, which makes use of the superior longitudinal penetration to produce a sheet of treated wood to the depth of the incision, and variation of the treating conditions (physical); and (3) preservative type and formulation (chemical).

The appropriate selection of preservative or additives for formulation can increase the penetration of wood. Hence, the problem of treating difficult-to-treat species such as Douglas-fir has been attacked by using preservatives with enhanced penetration characteristics (e.g. ACA) and modifications of these formulations. Ruddick (1989) investigated the treatability of Douglas-fir with ACA and CCA for compatible solution strength, and found that the penetration and retention of ACA were higher than those of CCA.

It is well known by wood treaters that the ACA treatment of certain wood species, such as Douglas-fir, results in a darkening of the wood during treatment. It has been postulated that the darkening is related to the presence of ammonia in ACA solution since treatment with CCA, which also contains copper and arsenic, does not give this reaction (Ruddick, 1979).

### **2.5.3 Extractive chemistry of Douglas-fir**

There are four chemical components present in wood: cellulose, lignin, hemicellulose and extractives. Cellulose, the main component in wood, is a linear macromolecule composed of (1-4)- $\beta$ -D-glucopyranose. Lignin is a complex and high molecular weight polymer built upon phenylpropane units, which are linked

by biphenyl, aryl-alkyl or ether linkages. Lignin in softwood is composed of guaiacyl units, while that in hardwood is built up with guaiacyl-syringyl units. Hemicelluloses are complex mixtures of polysaccharides, and composed of various sugar units, with shorter molecular chains and some branching. Only minor structural variations are found in the lignin and hemicelluloses of different plants. A large variety of wood components, although usually representing a minor fraction, are soluble in neutral organic solvents or water. They are called extractives, largely comprised of polyphenolic organic molecules. The extractive compositions vary widely in different wood species.

The structural constituents of Douglas-fir wood, namely cellulose, hemicellulose and lignin, occur in roughly the same proportion as in other coniferous woods. The chemical composition of Douglas-fir wood is shown in Table 2-1-3 (Isenberg, 1980). For comparison, the table also includes data on several softwoods. Foster *et al.* (1980) characterized the sapwood and heartwood extracts of Douglas-fir, and found that the diethyl ether extractive (mg/g oven-dried extract-free wood basis) recovered from the heartwood was three times that from the sapwood.

No single, universal solvent will remove all of the various extractive compounds. In order to ensure that the extractives have been quantitatively removed, a number of different solvents must be employed. Generally, to separate extractives, alcohol or acetone, ether and water are needed. Acetone leaches out the coloring matter, tannins, while ether removes oils, fats and resins. Cold water leaches the soluble short-chained carbohydrates as well as some free acids and salts. Accordingly, these three solvents were used in this study to prepare fractions containing the major extractives.

Early work on the isolation of Douglas-fir extractives was concerned with the oleoresins (Benson and McCarthy, 1925). The oleoresin collected from boring the tree was distilled with superheated steam at a temperature of 150°C for the purpose of separating the volatile oil and rosin. The volatile oil contained  $\alpha$ -pinene with small amounts of limonene and terpineol (Schorger, 1917). A systematic analysis of the entire extractive fraction was made by Graham and Kurth (1949). They investigated the ether, acetone, and cold-water extracts from three specimens of Douglas-fir heartwood. In the ether extract, oleic, linoleic, lignoceric and abietic acids, phytosterol and tannin were found. The fatty acids were present both in the free and combined states, whereas the resin acids were isolated only as the free acids. A pentahydroxyflavanone and catechol tannin were isolated from the acetone extract. Approximately 70 percent of the cold water extract was a galactan. Dihydroquercetin, a major Douglas-fir heartwood polyphenol, was first described by Pew (1948). As may be expected, the heartwood of Douglas-fir contained higher amounts of extractives (3.6%) than the corresponding sapwood (1.3%) (Harvey and Tsuneo, 1974). About a decade later Barton and Gardner (1958, 1963) and Hancock (1957) described detailed analytical methods for the determination of dihydroquercetin in Douglas-fir. The dihydroquercetin content in heartwood varied within and between trees of the same species, ranging from zero to 1.5 percent in Douglas-fir. Within a tree a general pattern of increasing concentration with increasing distance from the pith was evident in the heartwood. A leucoanthocyanidin was also found in Douglas-fir wood. Rogers and Manville (1972) isolated (-)-*cis*-4-[1'(R)-5'-dimethyl-3-oxohexyl]-cyclohexane-1-carboxylic acid from the petroleum ether extract from Douglas-fir wood.

Table 2-1-3 Chemical composition of six common coniferous woods (Isenberg, 1980)

Species	Alpha-cellulose %	Hemi-cellulose %	Lignin %	Total pentosan %	Ash %	Solubility in	
						Alcohol benzene %	Hot water %
White spruce ( <i>Picea gouca</i> )	42.6	16.4	29.4	11.8	0.3	2.0	2.6
Amabilis fir ( <i>Abies amabilis</i> )	43.8	-	28.2	9.8	0.5	2.6	3.2
Douglas-fir ( <i>Pseudotsuga menziesii</i> )	49.6	14.1	27.7	7.9	0.2	4.1	5.0
Lodgepole pine ( <i>pinus contorta</i> )	45.7	-	27.2	12.4	0.2	3.5	2.7
Western hemlock ( <i>Tsuga heteroph</i> )	49.2	15.5	29.4	9.2	0.3	2.6	2.0
Western red cedar ( <i>Thuja plicata</i> )	44.0	14.6	30.9	9.0	0.3	14.1	11.0

Note: All percentage based on moisture-free wood.

#### 2.5.4 Effect of the extractives on wood properties

The effect of the wood extractives on the properties of timber have been studied by several researchers. For example, some conifer wood species often darken during storage (Miller *et al.*, 1983, Evanta and Halvorson, 1962). The behaviour of certain classes of extractives is sufficiently well known to predict the type of color changes. The polyphenols have received more attention than other types of extractives. The tendency of polyphenols to discolor under different conditions is partly dependent on the number of vicinal hydroxyl groups (Hillis, 1962). When wood is exposed to air or sunlight, polyphenols darken, due to oxidation which produced dark-brown products. For example, the formation of the brown color stain in western hemlock is thought to result from oxidative reactions of extractives. The extractive that is believed to be the main cause of the color stain is catechin, which undergoes a two-stage enzyme-catalyzed oxidation, followed by condensation (Kreber, 1994; Avramidis *et al.*, 1993)

Another property of polyphenols containing ortho-dihydroxy and vicinal trihydroxy groupings, is their ability to bind metal ions to form chelates, which in many cases are dark colored. The degree of discoloration when metal contaminates wood depends on several factors. These include:

(1) Type and quantity of polyphenols present. In many cases, the polyphenols containing trihydroxy groupings give a darker color with metals than those containing ortho-dihydroxy groupings (Tardif, 1959).

(2) The pH of the solutions. Tardif (1959) found that the color reactions of dilute solutions of commercial tannic acid with several metals at low concentration are dependent on the pH of the solution. Ferric ion formed blue, mauve, red, and dark-red colloids between the pH ranges of 2.0-5.0, 5.0-7.0, 7.0-11.0, and greater than pH 11, respectively.

(3) The type, chemical state and quantity of metal. The most important metals were iron, aluminum, and copper. With dilute solutions of ions and tannin, the color is most intense at a pH of 4.5. With stronger ion concentration, the intense blue color is formed over a wide pH range. Copper formed dark-brown tannate complexes, while aluminum ions formed a bright-yellow color.

Troughton and Chow (1973) studied the heat-induced color-intensity change in Douglas-fir and found that its extractives contributed significantly to this change. Dihydroquercetin, when heated produced a powerful chromophore. It was also reported that dihydroquercetin possessed some fungicidal effect, completely inhibiting growth of the most sensitive fungi at concentrations of less than 0.5 per cent (Kennedy, 1956).

### **3. EXPERIMENTAL**

#### **3.1 Study of extractives in Douglas-fir heartwood responsible for black color in ammoniacal copper treated wood**

##### **3.1.1 Preliminary analysis of extractives**

In this study three kinds of solvents were used to find out whether the extractives were responsible for the discoloration, and if so, which fraction of Douglas-fir extractives was responsible for the reaction which produced the dark colour.

Douglas-fir heartwood meal was extracted with different solvents to obtain the extractive fractions (Fig. 3-1-1). In these experiments, the solvents, acetone, diethyl ether and water were used. Each fraction of extractives was reacted with ammoniacal copper solution to determine whether colored complexes were formed. When the reaction was positive, *i.e.* dark coloured materials were formed, this fraction was retained and processed to further separate the components to determine which compound in the fraction was responsible for the reaction.

Two 20 gram samples of Douglas-fir heartwood meal were extracted separately with either 100 ml of acetone or diethyl ether for 24 hours at 20°C. The two solutions were filtered and concentrated under vacuum at 20°C. The residues were dried under vacuum below 30-40°C to constant weight. The acetone-extracted wood meal was extracted again with 100 ml of distilled water at 40°C for 24 hours. The extract was filtered and concentrated at 60°C under reduced pressure to produce a third residue.

Three residues obtained were then subjected to a reaction with ammoniacal copper solutions.

The test solutions were prepared as follows:

- (i) 2% of acetone-extractive residue in methanol
- (ii) 2% of ether-extractive residue in methanol.
- (iii) 2% of water-extractive residue in water.

One ml of each solution was reacted with 1 ml of 1.5% (w/w) solution of copper sulphate in ammonium hydroxide. Upon addition of the ammoniacal copper solution, only the acetone-extracted residue in methanol formed a dark colored precipitate. The ether-extract produced a light green color solution, and water-extracted residue formed a light brown solution with a trace of precipitate. This dark color may be caused by the presence of acetone-extractive residues in the water-extracted fraction.

Based upon these observations, it was concluded that a compound was present in the acetone-extract which reacted with ammoniacal copper solution to produce a black precipitate. The subsequent experiments therefore focused on the chemicals present in the acetone extractives.

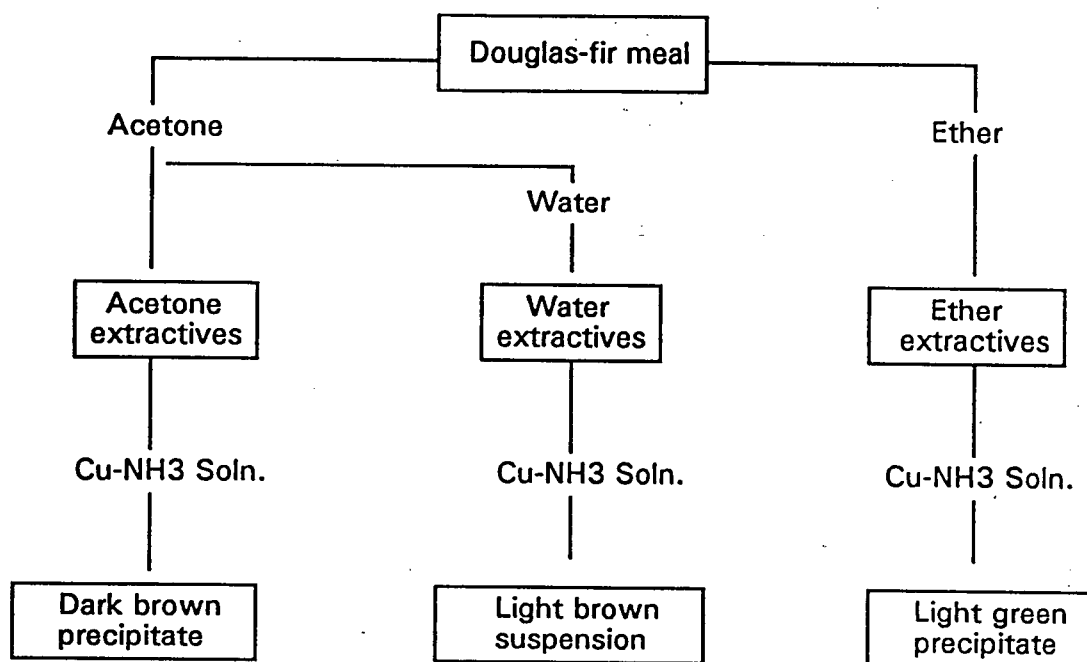


Fig. 3-1-1 Preliminary extraction procedure for isolation of extraneous compounds

### **3.1.2 Isolation and identification of the extractive reacting with ammoniacal copper solutions to produce a black color**

Because of the positive response reaction of the acetone extractive mixture with ammoniacal copper solution, further experiments were designed to isolate and identify the component in this mixture which was believed to be responsible for the black color on wood after it was treated with ammoniacal copper solution.

Column chromatography was used for preliminary purification of the components from crude acetone-extracts. The compound isolated from Douglas-fir was examined by ultraviolet (UV), fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (H-NMR) and mass spectrometry (MS).

#### **3.1.2.1 Isolation of the extractive**

Douglas-fir heartwood sawdust was prepared by grinding up thin chips cut from heartwood pieces, using a Wiley mill, until the sawdust would pass through a 20 mesh screen.

##### Extraction with acetone

A large glass flask was used to extract 800 grams of wood meal (oven-dried basis) with 1200 ml of acetone. A fresh supply of solvent was introduced into the flask every two days after filtering the extracted solutions. The extracts were combined and concentrated under reduced pressure to a volume of 300 ml. A 10 ml aliquot from the concentrated extract was dried to constant weight under vacuum at 40 °C. The total calculated yield of acetone-extract was 2.42 percent of the oven-dried weight of the wood. Silica gel thin layer chromatography (TLC) was used to examine the extracts using petroleum ether : acetone 3:1 mixture as

the developing agent. Exposing the plate under ultraviolet light revealed that the acetone extract contained a mixture of at least six compounds.

Upon addition of 150 ml of distilled water to the concentrated extract solution, a milky-white suspension was obtained. The residual acetone in the solution was removed under reduced pressure, while maintaining the temperature below 45°C. In order to separate the compound present in the mixture, it was extracted with two solvents possessing quite different solubilizing capabilities. The colloidal suspension was first extracted four times with 50 ml aliquots of chloroform. The chloroform extract was recovered. The remaining water layer was further extracted with four, 50 ml portions of diethyl ether solvent. Using these two different solvents enabled the separation of the components in the crude extracts into chloroform and ether soluble fractions. Both the ether and chloroform solutions were dried over anhydrous sodium sulfate for 30 minutes and then filtered, and concentrated under reduced pressure. The oil-like materials were obtained from the chloroform fraction.

After removing diethyl ether from the second fraction, petroleum ether was added to the residue to serve as a precipitating agent. The mixture was set aside to crystallize. After standing in the refrigerator for twenty-four hours, a light brown solid was filtered off, washed with petroleum ether and dried in the oven at 103°C for four hours.

In order to examine which part of the fraction is responsible for the darkened color in the treated heartwood, a solution was prepared from each residue by dissolving it in methanol. Ammoniacal copper solutions were then added to the solution of each extract. Only the substance from the ether fraction could react with copper-ammonia solution and form a dark colored precipitate. Further separation of the crude solid from the ether fraction was accomplished by using column chromatography.

### Column chromatography

Elution column chromatography with silica gel was used in this phase of the experiment. A plug of cotton wool was placed in the bottom of the column 50 mm in diameter and a layer of sand was added on top of the plug to provide an even base for the silica gel column. A mixture of petroleum ether and acetone (3 : 2) was chosen as the eluting solvent based upon trial experiments to determine the maximum separation of the components.

The column was packed by adding silica gel slurry in the solvent mixture. After the column had been prepared, the solvent level was lowered to the top of the silica gel column by draining solvent from the bottom of the column. The mixed residue to be separated was dissolved in acetone, and applied carefully to the top of the silica gel column. The column was then eluted with a 3:2 mixture of petroleum ether and acetone. In order to accelerate the elution speed, low nitrogen pressure was applied at the top of the column. The elution was monitored periodically by thin-layer chromatography (TLC) on silica gel using petroleum ether: acetone (3:2) as the developing solvent. The TLC results were observed under UV light. The desired fractions containing only the primary product based on the TLC evaluation were collected and consolidated. Evaporation of the eluent produced a cream white solid, which was filtered and dried.

### Product purification

The white compound was dissolved in a 50% ethanol-water solution (solid : solvent = 1:50). Activated carbon was added to the solution, and the mixture refluxed for 30 minutes before immediately being filtered. The filtered cake was washed three times with 50% warm ethanol solution. The filtrate was concentrated under vacuum to small volume and placed in the refrigerator overnight for

crystallization. A cream-white needle-crystal solid was obtained which was recovered by filtration. It was dried at 105°C in the oven for 6 hours.

### **3.1.2.2 Identification of isolated compound**

#### Melting point

The melting point was measured on a 6545-J17 microscope equipped with a Thomas model 40 hot stage melting apparatus.

#### Mass spectrometry (MS)

The mass spectrum of the white crystalline solid was obtained by Mass Spectrometry Center, Department of Chemistry, UBC. The mass spectrum was determined using chemical ionization by the addition of ammonia to the test sample.

#### Ultraviolet spectrum (UV)

The UV spectrum of the compound was recorded with a Varian Gary 3 Spectrophotometer in the range 400 to 800 nm. A solution containing 0.1 mg of the isolated compound sample in 10 ml of methanol was used for the UV measurement.

#### Fourier transform infrared spectrum (FTIR)

The FTIR spectrum of the isolated compound was obtained with a Perkin-Elmer 1600 Spectrophotometer over the range  $4000\text{ cm}^{-1}$  to  $500\text{ cm}^{-1}$ . A KBr pellet was made by mixing the compound (1 mg) with potassium bromide (200 mg) and compressing the resulting powder at 25000 psi in a 1 cm diameter pellet press.

#### Nuclear magnetic resonance (NMR) spectrum

The proton NMR spectrum of the compound was obtained using a Bruker WH-400 spectrometer, Department of Chemistry, UBC. The compound (6 mg) was dissolved in deuterio-acetone in a 5 mm thin-walled NMR glass tube.

Chemical shifts were reported in ppm downfield from the trimethylsilane (TMS) reference standard.

### 3.1.2.3 Reactivity with copper solutions

To determine under what conditions the dark coloured reaction products were produced, 0.1034 g of the purified white solid was dissolved in 100 ml of methanol and aliquots reacted with 0.0034 M copper solutions at different pH's. The copper solutions were copper sulphate in distilled water (pH=6); copper carbonate in CCA (pH=3); copper sulphate dissolved in 2% ammonium hydroxide solution (pH=10.5); and copper sulphate in excess sodium hydroxide solution (pH=10). The reaction of 2% ammonium hydroxide alone, was also examined.

Since the reaction with copper sulphate in ammonium hydroxide produced a black precipitate, it was repeated. A 14 ml aliquot of a 1.5% ammoniacal copper sulphate solution was added to 304 mg (1.0 mmol) of the white solid dissolved in anhydrous methanol (20 ml). After four hours the original white solid was no longer detected during TLC analysis. It indicated that almost all white solid compound reacted with copper ions and formed the precipitate. The black precipitate was filtered, washed three times with distilled water and oven dried at 105 °C (364 mg). The FTIR spectrum of the black precipitate was recorded.

A UV-Visible spectral analysis in the range 400 to 800 nm was performed on solutions prepared by combining 1 ml of a 2% methanol solution of the white solid, with 1 ml of each copper solution.

## 3.2 Fixation of ammoniacal copper preservative in wood

### 3.2.1 Sample preparation

#### Sample for FTIR analysis

##### a. Wood

The blocks (15 X 20 X 30 mm) of ponderosa pine (*Pinus ponderosa* Laws.) sapwood were prepared from kiln dried lumber. The blocks were soaked in distilled water under reduced pressure for 30 min, which removed the air from the wood. The vacuum was released to the atmosphere to force the water to enter the wood. Blocks which were left to soak overnight for complete impregnation.

The blocks were mounted in a microtome in order to produce 40  $\mu\text{m}$  thin earlywood sections, along the tangential surface. During microtoming water was brushed on the wood surface to avoid drying. The thin wood sections were flattened in a petri dish to allow to air dry.

##### b. Cellulose: Pure cellulose powder (Avicel™)

c. Holocellulose: Holocellulose (a mixture of cellulose and hemicellulose) was prepared according to the following procedure (Paszner, 1994).

Ponderosa pine sapwood samples were cut into thin chips and ground with a Wiley mill until the sawdust would pass through a 20 mesh screen. A 10 g portion of the wood meal was placed in an extraction thimble, and covered with a cone shaped filter paper. The thimble was placed in a Soxhlet extractor, and the sawdust was extracted with a 2:1 mixture of ethanol to benzene for 24 hours. A 2 g sample of air dried extractive free ponderosa pine wood meal was placed in a tube. Then 28 ml of buffer solution and 12 ml of 20% sodium chlorite solution were added. The tube containing the mixture was placed in a constant temperature shaking incubator (at 50 °C) overnight.

The contents of the tube were cooled and transferred to a medium porosity, tared, crucible. The solid was washed first with 1% acetic acid, and then with

acetone. The samples were conditioned in the CTH room for 1 week, before oven drying at 105 °C.

d. Klason lignin: Air-dried extractive-free ponderosa pine wood meal was used to prepare Klason lignin (Paszner, 1994). The modification involved the secondary hydrolysis step. After dilution with distilled water the concentration of sulfuric acid was reduced to 3%. The solution was autoclaved under steam pressure of 1.5 bar at 127.5 °C for one hour.

The insoluble material was allowed to settle overnight, before carefully decanting through a medium porosity filtering crucible. The solid was washed with distilled water and oven dried at 105 °C.

e. Lignin model compounds: Lignin is a complex and high molecular weight polymer built upon phenylpropane units. It is not possible to isolate lignin from wood without causing some structural changes. In this study a lignin model compound (vanillin) containing an ortho-methoxyl phenol was used to investigate the potential reaction between guaiacyl groups commonly in lignin and ammonium hydroxide/ammoniacal copper solution. Vanillin was purchased from Aldrich Chemical Company. Ammonium hydroxide and copper sulphate pentahydrate were commercial chemicals supplied by BDH.

### 3.2.2 Sample treatment

#### a. Reaction with ammonium hydroxide

Wood sections, lignin, cellulose and holocellulose were soaked in a 5% ammonium hydroxide solution or 5% on a copper oxide basis (Copper sulphate) in 10% ammonia solution for 1 hour, and filtered. The filtrate was airdried before testing.

Vanillin was dissolved in 5% ammonium hydroxide solution under stirring for eight hours, after which the ammonia was removed to produce a residue. The residue was dissolved in chloroform for gas chromatograph-mass spectrometry (GC-MS) analysis.

#### b. Reaction with ammoniacal copper solution

Vanillin (1g, 0.0066 mol) was dissolved in 20 ml 5% ammonium hydroxide solution. Twenty ml of an ammoniacal copper solution (0.0030 mol of copper sulphate in 5% ammonium hydroxide solution) was added to this, dropwise, with stirring. Evaporation of the ammonia from the vanillin-ammoniacal copper solution under reduced pressure at 40°C, produced a green crystalline precipitate. The green solid was filtered, and washed with distilled water to remove excess ammonium hydroxide until the solution attained the pH of the distilled water (pH = 6). The green polycrystalline solid was dried under vacuum at 40 °C, washed three times with ether to remove unreacted vanillin and finally vacuum dried at room temperature for eight hours. A single crystal of vanillin-copper-ammonia complex was obtained through careful recrystallization in an ammoniacal copper solution.

In a second experiment, 0.1 g of the green polycrystalline complex dissolved in 20 ml of 5 % ammonium hydroxide solution was added to 30 ml of 0.02 M sodium ethylenediaminetetraacetate (EDTA) solution to remove the copper

ions from the vanillin-copper-ammonia complex. The residue was extracted three times with diethyl ether. A white solid was obtained upon evaporating the ether.

### 3.3.3 Analysis methods

#### a. FTIR spectroscopy

The IR spectra were obtained with a Perkin-Elmer 1600 Spectrophotometer over the range  $4000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$ . A KBr pellet was made by mixing the compound (1 mg) with potassium bromide (200 mg) and compressing the resulting powder at 25000 psi in a 1 cm diameter pellet press.

#### b. Gas Chromatograph-Mass Spectrometry (GC-MS):

The GC-MS system was acquired using an HP 5890 series II gas chromatograph equipped with a VG Trio 1000 mass selective detector. The samples were analyzed on a 25 meter HP-5 capillary column (0.2 mm ID,  $0.32\text{ }\mu\text{m}$  film). The injector system was maintained at  $250\text{ }^{\circ}\text{C}$ , and the oven temperature was held at  $50\text{ }^{\circ}\text{C}$  for 2 min, and then programmed to  $300\text{ }^{\circ}\text{C}$  at a rate of  $13^{\circ}\text{C}/\text{min}$ . The components were identified by comparing the mass spectra with spectra from the available library.

c. Elemental Analysis was performed by Canadian Microanalytical Service Ltd., Delta, B.C.. The elemental analysis of the green vanillin-copper-ammonia complex was found to be: C, 47.87%; H, 4.92%; N, 6.81%; Cu, 16.0%;  $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_6\text{Cu}$  requires: C, 48.01%; H, 5.01%; N, 7.01%; Cu, 15.9%.

#### d. Mass spectroscopy (FAB):

The Fast atom bombardment mass spectrum of the green solid complex was obtained on a Kratos Concept II HQ Mass spectrometer. The Mass spectrometry

center, the Department of Chemistry, UBC. The ion source was 8 KV and scan rate was 3 to 10 sec/decade. The matrix used was thioglycerol.

e. ESR spectroscopy

The ESR spectra were recorded on a Bruker ES-160 spectrometer equipped with a variable temperature unit, operating at a frequency of 9.60 GHz (X-band) and 50 KHZ field modulation.

f. Electronic spectrum: The electron spectrum (200-2500 nm) was recorded on a Varian Cary 5 UV-Vis-Nir spectrophotometer on Nujol mull sample between quartz plates.

g. *X-ray structure determination*

The X-ray single crystal structure analysis was performed using a Rigaku AFC6S diffractometer with graphite monochromated Mo-K $\alpha$  radiation. The crystal data and details of the data collection are summarized in Table 3-1-1. The final unit-cell parameters were obtained by least-squares on the setting angles for 25 reflections with  $2\theta = 39.5-43.5^\circ$ . The intensities of three standard reflections, measured every 200 reflections throughout the data collection, showed only small random fluctuations. The data were processed using the crystal structure analysis program *teXsan* and corrected for Lorentz and polarization effects, and absorption (empirical, based on azimuthal scans for three reflections).

The structure analysis was initiated in the noncentrosymmetric space group  $P2_1$  on the basis of the  $E$ -statistics and the Patterson function. This choice was confirmed by subsequent calculations. The structure was solved by conventional heavy atom methods, the coordinates of the Cu atom being determined from the Patterson function and those of the remaining non-hydrogen atoms from subsequent difference Fourier syntheses. The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were fixed in calculated positions with  $N/C-H = 0.99 \text{ \AA}$  and  $B_H = 1.2 B_{\text{bonded atom}}$ . A parallel refinement of

the mirror-image structure gave substantially higher residuals, the  $R$  and  $R_w$  factors ratios being 1.062 and 1.070, respectively. Neutral atom scattering factors (Ibers and Hamilton, 1974) and anomalous dispersion corrections were taken from the *International Tables for X-Ray Crystallography* (Creagh and McAuley, 1992).

Table 3-1-1 Crystallographic data<sup>a</sup>

Compound	(C <sub>8</sub> H <sub>7</sub> O <sub>3</sub> ) <sub>2</sub> Cu(NH <sub>3</sub> ) <sub>2</sub>
Formula	C <sub>16</sub> H <sub>20</sub> CuN <sub>2</sub> O <sub>6</sub>
Molecular weight	399.89
Crystal color, habit	green, prism
Crystal size, mm	0.20 × 0.25 × 0.30
Crystal system	Monoclinic
Space group	<i>P</i> 2 <sub>1</sub>
<i>a</i> , Å	5.4172(9)
<i>b</i> , Å	15.452(2)
<i>c</i> , Å	10.4595(9)
β, deg	99.72(1)
<i>V</i> Å <sup>3</sup>	863.0(2)
<i>Z</i>	2
<i>D</i> <i>Calcd</i> g/cm <sup>3</sup>	1.539
<i>F</i> (000)	414
Radiation	Mo
μ, cm <sup>-1</sup>	13.00
Transmission factor (relative)	0.86-1.00
Scan type	ω-2θ
Scan range, deg in ω	1.37 + 0.35 tan θ
Scan speed, deg/min	32 (up to 8 seconds)
Data collection	+ <i>h</i> , + <i>k</i> , ± <i>l</i>
2θ <sub>max</sub> , deg	70
Crystal decay, %	Negligible
Total reflections	4225
Total unique reflections	3910
<i>R</i> <sub>merge</sub>	0.067 <sup>b</sup>
Reflection with <i>I</i> ≥ 3 σ ( <i>I</i> )	2172
Number of variables	225
<i>R</i>	0.043
<i>R</i> <sub>w</sub>	0.042
<i>gof</i>	2.32
Max Δ/σ (final cycle)	0.001
Residual density, e/Å <sup>3</sup>	-0.27, 0.73

<sup>a</sup> Temperature 294 K, Rigaku AFC6S diffractometer, Mo  $K_{\alpha}$  ( $\lambda = 0.71069 \text{ \AA}$ ) radiation, graphite monochromator, takeoff angle  $6.0^{\circ}$ , aperture  $6.0 \times 6.0 \text{ mm}$  at a distance of 285 mm from the crystal, stationary background counts at each end of the scan (scan/background time ratio 2:1),  $\sigma^2(F^2) = [S^2(C + 4B)]/Lp^2$  ( $S$  = scan rate,  $C$  = scan count,  $B$  = normalized background count), function minimized  $\sum w(|F_o| - |F_c|)^2$  where  $w = 4F_o^2/\sigma^2(F_o^2)$ ,  $R = \sum ||F_o| - |F_c|| / \sum |F_o|$ ,  $R_w = (\sum w(|F_o| - |F_c|)^2 / \sum w|F_o|^2)^{1/2}$ , and  $\text{gof} = [\sum w(|F_o| - |F_c|)^2 / (m-n)]^{1/2}$ . Values given for  $R$ ,  $R_w$ , and  $\text{gof}$  are based on those reflections with  $I \geq 3\sigma(I)$ .

<sup>b</sup> for Friedel mates  $0kl$  and  $0k-l$ ; these were not averaged

### **3.3 To determine whether the enrichment of nitrogen in ammoniacal-copper preservative treated wood increases the decay potential**

#### **3.3.1 Sample preparation:**

a. Thirty six ponderosa pine sapwood blocks (1.9 X 1.9 X 1.9 cm) were vacuum impregnated with ammonium hydroxide solutions with concentrations of 1, 5 and 10% (AWPA Standard E10-91/1992). After treatment, the blocks were removed from the solution and airdried on a wire screen for 4 weeks at ambient room temperature to evaporate before soil testing. Blocks impregnated with distilled water were used as controls.

Two blocks from each group were ground to 30 mesh sawdust. A 5 g sawdust sample of each group was soaked with 50 ml of distilled water for 24 hours. The pH values of the solutions were measured using an Orion Model 520 pH meter.

b. Southern yellow pine (*Pinus spp.*) sapwood wafers (10 cm X 3.5 cm X 0.3 cm) were vacuum treated with ammoniacal copper solutions. The treating solutions were prepared by dissolving copper sulphate containing 0.06% copper (expressed as CuO) in 1, 5 and 10% ammonium hydroxide solutions. After treatment the wafers were sealed in plastic bags for one week to allow for wet chemical fixation at ambient temperature. They were then removed from the bags and airdried at room temperature for two weeks. Wafers treated with distilled water were used as controls.

#### **Leaching test**

Eighteen wafers treated to each copper retention and ammonium hydroxide concentration were submerged in jars containing 300 ml of distilled water, and placed in a desiccator. The desiccator was evacuated for 20 min using a vacuum

pump, after which the jars were removed from the desiccator. The blocks remained submerged prior to removal. After 6, 24, 48 hours and thereafter at 48 hour intervals for a period of two weeks, the leach water was removed and replaced with fresh distilled water. At the end of this leaching period all samples were air dried. Each wafer was sawed into two pieces. One half was used for nitrogen and copper analysis, while the matching half was oven-dried, weighed and employed in a soil-block investigation.

### **3.2.2 Analysis of copper and nitrogen in treated wafers**

#### **Copper analysis:**

Analysis of copper retention in the wafers from each group, before and after leaching was carried out using an X-ray fluorescence analyzer. Each wafer was ground into 30 micro mesh sawdust. The sawdust was compressed in a sample holder using a hand press. The final analytical result was given in terms of copper oxide in test sample ( $\text{kg/m}^3$ ) using an internal commercial program developed from over 125 standards.

#### **Nitrogen analysis**

Nitrogen analysis was performed with the Kjeldahl technique using the Kjeltac Auto 1030 nitrogen analyzer.

Test procedure: 0.1 g of the milled sample was put into a Kjeldahl digestion tube. To the wood sample was added a half of a tablet of Kjeldahl catalyst ( $\text{CuSO}_4$  and  $\text{TiO}_2$ ). The tubes were set on the digestion rack in the heating mantle. Once all 12 digestion tubes were in place, 5 ml of 98% pure sulfuric acid was carefully added to each tube. After the sulfuric acid had been added, the samples were heated to about  $400^\circ\text{C}$  and the digestion allowed to proceed for two hours. The endpoint for digestion was identified by the formation of a clear lime-green liquid which showed no effervescence. The digestion tubes were then removed from the

metal mantle and allowed to cool to room temperature prior to titration. A 0.01 M HCl solution was used to titrate the distilled solutions.

The Kjeldahl apparatus was calibrated by analysis distilled water and ammonium sulfate (500 ppm) control prior to the analysis of the test samples. The nitrogen content was calculated from the equation:

$$\% \text{ nitrogen} = \frac{[\text{Vol.}_{\text{HCl}}(1) - \text{Vol.}_{\text{HCl}}(2)] \times 0.01401}{\text{mass of sample (in grams)}}$$

Where: Vol.<sub>HCl</sub>(1) was consumed for sample.

Vol.<sub>HCl</sub>(2) was consumed for blank control.

0.01401 was a factor for nitrogen.

### 3.3.3 Test Method

Two sets of samples were prepared for a soil jar experiment, which was largely based on the protocol described in the American Wood Preserver's Standard (AWPA E10-91/1992). Three fungi were used in this study: two brown rot fungi and one white rot fungus. The brown rot fungi were *Postia placenta* (120F: Fr. Cooke, *Madison* 698) and *Gloeophyllum trabeum* (47D Pers. ex Fr. Murr. *Madison* 617). *P. placenta* is an important decay fungus in timber in North America and is usually included in laboratory evaluation of preservatives in North America and Europe. It is particularly tolerant to copper and zinc compounds. *G. trabeum* is commonly found in above ground exposure and is known to be tolerant to phenolic and arsenic compounds. It is also widely used as a wood destroying test organism.

The white rot fungus employed in the investigation was *Trametes versicolor* (L.:Fr.). It too is a common standard test fungus and is frequently isolated from hardwood products.

The fungi were grown from isolates in storage using a medium consisting of 2% malt extract and 2% agar in petri plates. The medium was prepared by dissolving 5 g of malt extract and 5 g of agar in 250 ml of distilled water. The bottle containing the medium was stoppered and autoclaved for 20 min at 103.4 kPa. After sterilization, the bottle was allowed to cool to 'hand-hot' temperature and the culture medium poured into the petri plates. When the medium had solidified and cooled, the plates were inoculated with the fungi. A small plug of fungal inoculum was removed from the overgrown plate using a sterile spatula, and placed in the center of the medium in a petri plate. The culture was incubated for two weeks before being used.

#### Preparation of soil jars and soil block incubation

Soil purchased from Vantro Soil Inc., Vancouver, B.C. was sifted through a U.S. # 5 sieve. To determine the moisture content of the soil, three samples were taken from the bag of soil and weighed. They were placed in an oven at 103 °C for 24 hours, and the moisture content was calculated from the differences between the original and oven-dried weights. The soil moisture content was adjusted to 40-50% by adding the required amount of water to the soil and mixed thoroughly (AWPA Standard E10-91/1992).

The feeder strips (3 X 25 X 60 mm) were prepared from ponderosa pine sapwood for the brown rot fungi and from birch (*Betula alleghaniensis*) sapwood for the white rot fungus. The role of the feeder strip is to provide essential nutrients for the fungus to colonize the wood and soil.

The glass jars of approximately 600 ml capacity were closed with a metal lid in which a central 5 mm hole had been drilled. The hole was covered with a 25 mm diameter millipore filter, which was glued to the inside of the lid. The jars were filled to approximately half their depth with the prepared soil. Feeder strips

were placed carefully on the soil surface and were pushed lightly into the soil. So that the top edge remained slightly higher than the surface of the soil. The jars were sealed with plastic lids and steam autoclave sterilized at 103.4 kPa for one hour. They were allowed to cool and then taken to a laminar flow bench where the lids were replaced with the metal lids which had been sterilized by autoclave.

The feeder strips were inoculated at diagonally opposite corners using 6 mm diameter plugs of fungus, taken from cultures actively growing on malt agar in petri plates. The jars were incubated at 22 °C in a fungal chamber for three weeks to allow the fungus to effectively colonize the feeder strips and become established in the soil.

There are three main methods of sterilizing wood blocks, gamma radiation, steam heating and ethylene oxide treatment. Steam sterilization is easy to use and economical. However, the steam treatment may result in evaporation of extractives or chemicals from the wood. The distribution of preservatives in the treated wood samples may also be affected. The high temperature may also breakdown some organic preservatives. Gamma sterilization does not affect preservative contribution and compositions in the treated wood. In this experiment we used gamma radiation to sterilize the wood block. The wood was sterilized by exposure to 2.5 Mrad of gamma irradiation. This required approximately 48 hours of exposure.

After three weeks of incubation, the feeder strips were covered with fungal hyphae. The jars were placed on a laminar flow bench and the lids were removed to allow two sterilized blocks to be placed on the feeder strip in each soil jar. There were five replicates of each test variable. The test variables were three different ammonia concentrations, presence or absence of copper, and three species of fungi. The soil jars were incubated for twelve weeks at approximately 25 °C.

## **4.0 RESULTS AND DISCUSSION**

### **4.1 Study of extractives in Douglas-fir heartwood which is responsible for black color when treating with ammoniacal copper solution.**

#### **4.1.1 Identification of the extractive reacting with ammoniacal copper solution to produce a black colour**

The preliminary experiments showed that the chemical responsible for the dark reaction with ammoniacal copper solutions was in the acetone-extracted fraction from Douglas-fir heartwood. Further solvent extraction together with silica gel column chromatography separation of the extract lead to the isolation of a white solid which, when dissolved in methanol, produced the characteristic dark reaction with ammoniacal copper solutions.

The identification of the white solid was revealed to be taxifolin through a combination of physical and spectroscopic analyses.

The R<sub>f</sub> value for the white solid on a silica gel plate, and an eluting solution of hexane:acetone 2:3, was 0.54. The cream-white crystals prepared from the acetone extract melted with decomposition at 236-238°C, which compared favorably with reported values for taxifolin at 237 °C (Graham and Kurth, 1949) and 240-242°C (Pew, 1948).

The UV spectrum in Fig. 4-1-1 showed a maximum absorbance at 289 nm and a minimum absorbance at 249 nm, consistent with the spectrum of taxifolin published by Aft (1961) and Mabry *et al.*(1970). The FTIR spectrum of the isolated compound shown in Fig. 4-1-2(a) contained several characteristic peaks, which are identified in table 4-1-1.

The proton signal obtained in the nuclear magnetic resonance spectrum of the white solid is shown in Fig.4-1-3. The chemical shifts determined from the proton-nuclear magnetic resonance spectrum of the white solid based upon the assignment by Marby *et al.* (1970) and by Harborne *et al.*(1975) and shown in Table 4-1-2, matched those identified for taxifolin by Mabry *et al* (1970) and Mbafor and Fomum (1989). The mass spectrum of the white solid is shown in Fig. 4-1-4. The m/e fragment pattern of the mass spectrum was compared with that reported by Audier (1966). The peak at m/e = 322 resulted from ions produced by the reaction between the molecule and the ammonia used in the chemical ionization  $[M + NH_4]^+$ . The peak of m/e 305 is due to molecule ion  $[M + H]^+$ . The molecular weight of the white solid is 304. Other mass fragments observed, at m/e values of 289, 153 and 123, arise from cleavage reaction. The cleavage reactions and ionic species are illustrated in Fig. 4-1-5.

Thus, the compound was confirmed to be taxifolin (3,3',4', 5,7-pentahydroxy flavavone), the structure of which is shown in Fig 4-1-6(a). Taxifolin is a major phenolic extractive in Douglas-fir, being first isolated by Pew in 1948. The average concentration of taxifolin in Douglas-fir was found to be 1%. Gardner and Barton (1960) investigated the distribution of taxifolin in Douglas-fir and found that the content in Douglas-fir heartwood varied considerably, both within a tree and between trees. The variance ranged from zero to 1.5 percent. Within trees, a general pattern of increasing concentration with increasing distance from the pith was evident in the heartwood. Taxifolin is quite soluble in hot water (13.5% at 100 °C) and relatively insoluble in cold water (0.25 % at 25 °C).

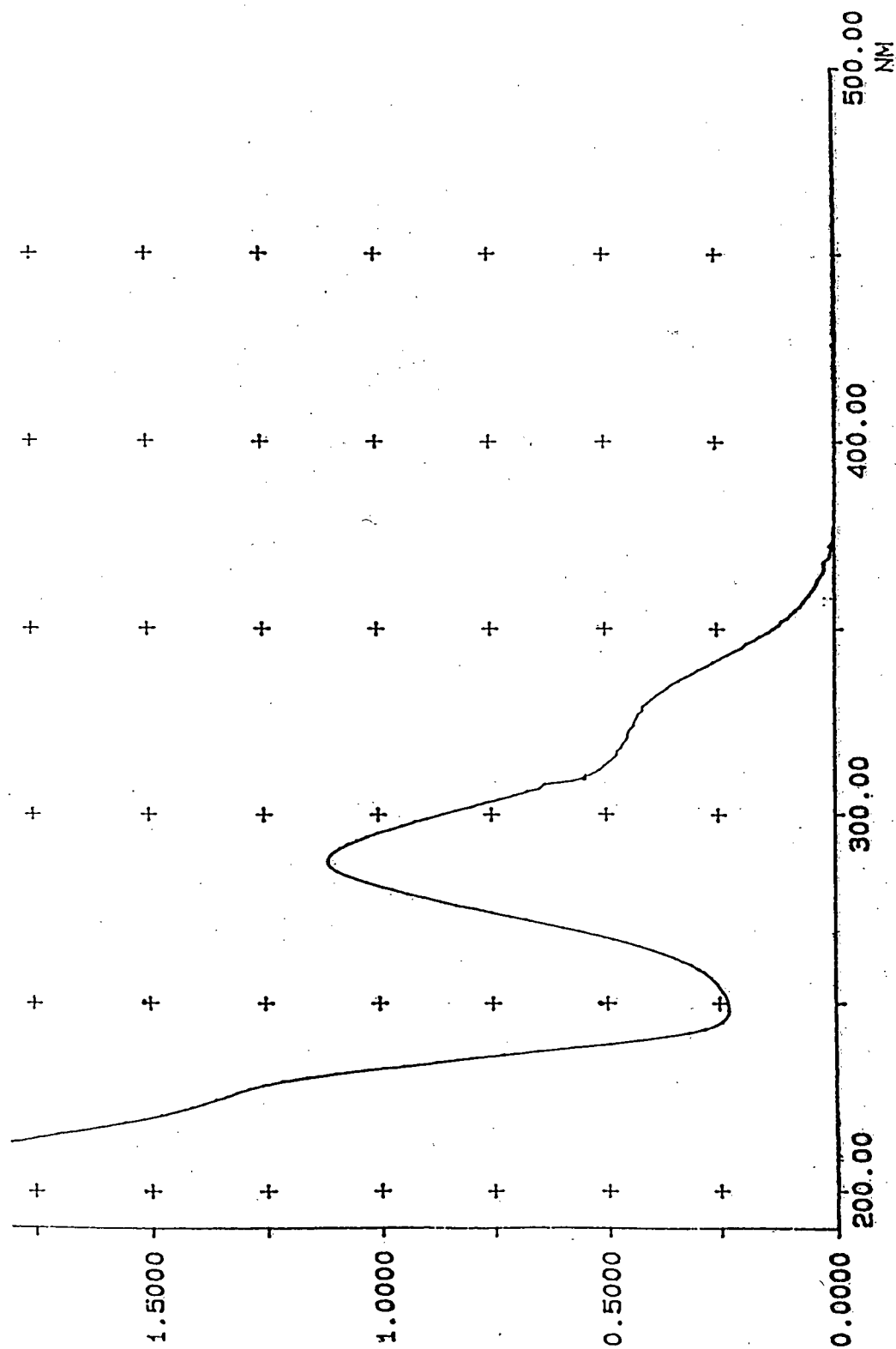


Fig 4-1-1 UV absorption spectrum of a methanol solution ( $1 \times 10^{-5} \%$ ) of the white solid isolated from the acetone extractive.

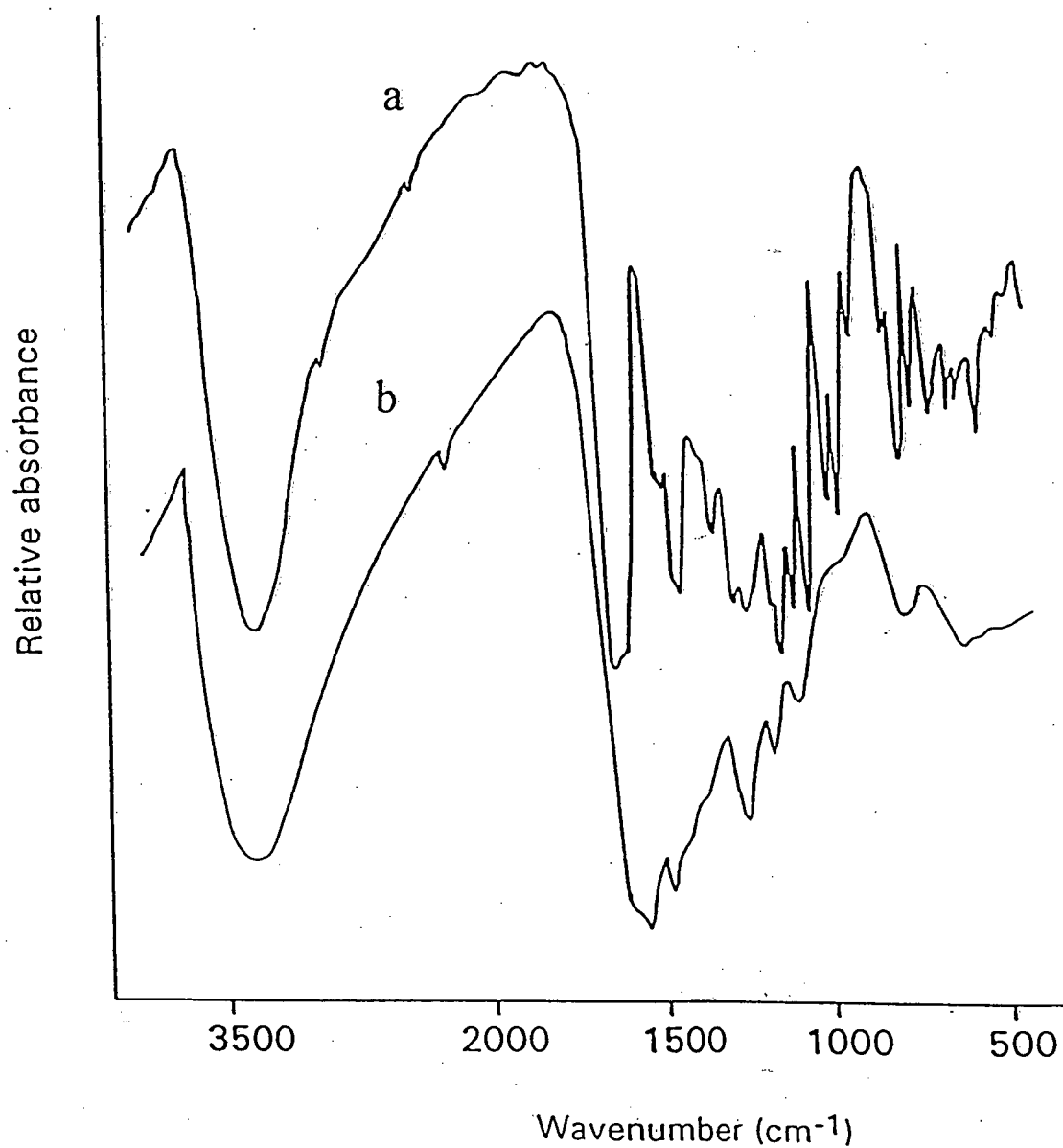


Fig. 4-1-2 FTIR spectra of a) taxifolin isolated from Douglas-fir heartwood and b) the black precipitate formed during the reaction of taxifolin and ammoniacal copper solution. Spectra were collected as KBr discs.

Table 4-1-1. Assignment of absorption bands in IR spectrum of isolated compound

Wave-number ( $\text{cm}^{-1}$ )	Assignment
3400	O-H stretching vibration
1640	C=O stretching vibration
1430-1510	C-H bending vibration/aromatic skeletal vibration
1359-1390	O-H in plane bending vibration
1000-1250	C-O-C stretching vibration
973	=CH out of plane deformation
750-875	C-H out-of-plane bending vibration
680	C-C out-of-plane bending vibration

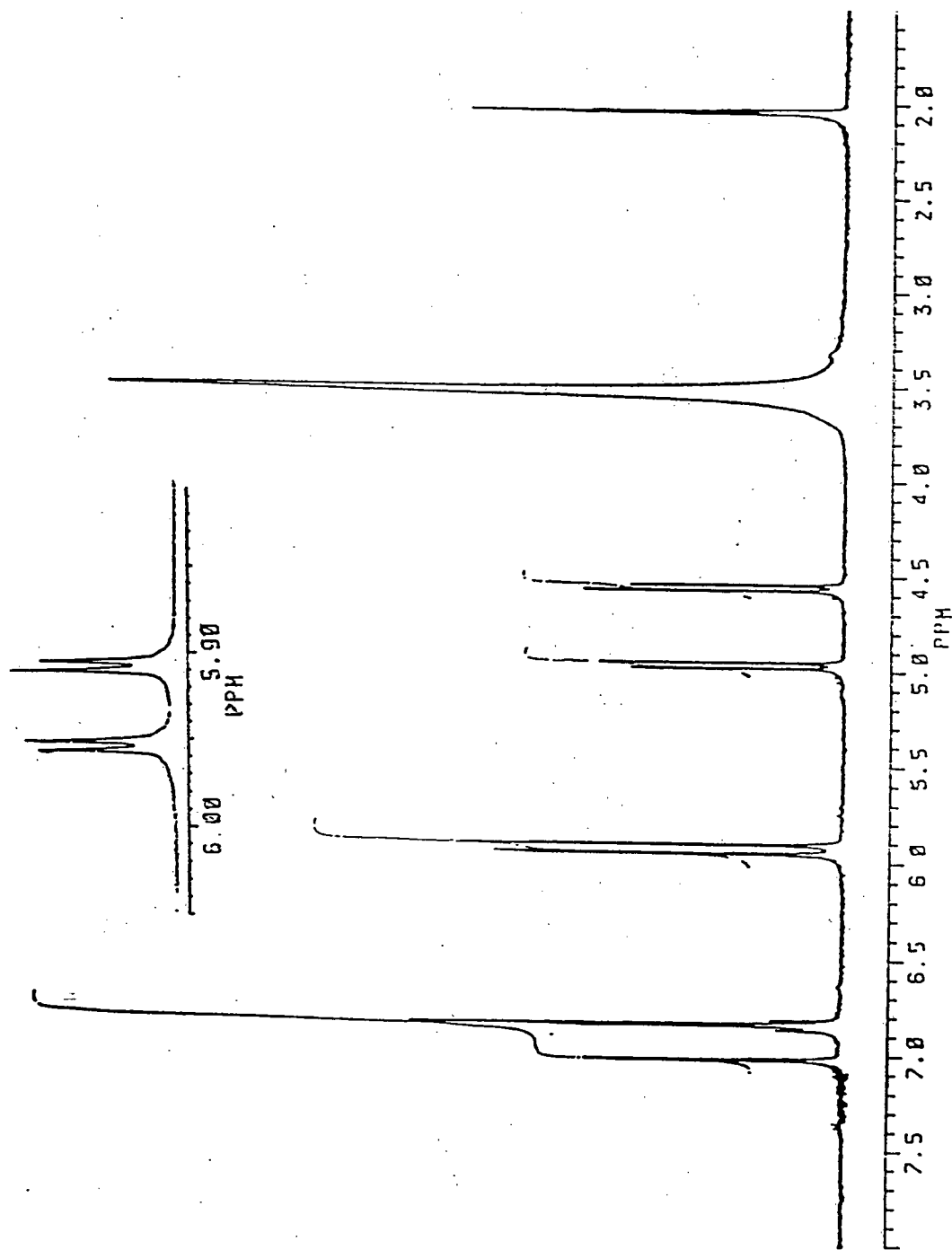


Fig 4-1-3  $^1\text{H}$ -NMR spectrum of the white solid isolated from the acetone extract of Douglas-fir heartwood dissolved in acetone- $\text{D}_6$ .

Table 4-1-2. Chemical shifts for the proton nuclear magnetic resonance spectrum of the compound extracted from Douglas-fir heartwood

Chemical shift (ppm)	Proton assignment <sup>a</sup>	Taxifolin <sup>b,c</sup>
A-ring		
5.91	H-6	5.75-5.95
5.95	H-8	5.92
B-ring		
6.80	H-2'	6.75
7.02	H-5'	6.88
6.80	H-6'	6.75
C-ring		
4.95	H-2	4.96
4.55	H-3	4.97

a. Harborne *et al.*, (1975); b. Marby *et al.*, (1970) and c. Mbafor and Fomum (1989).

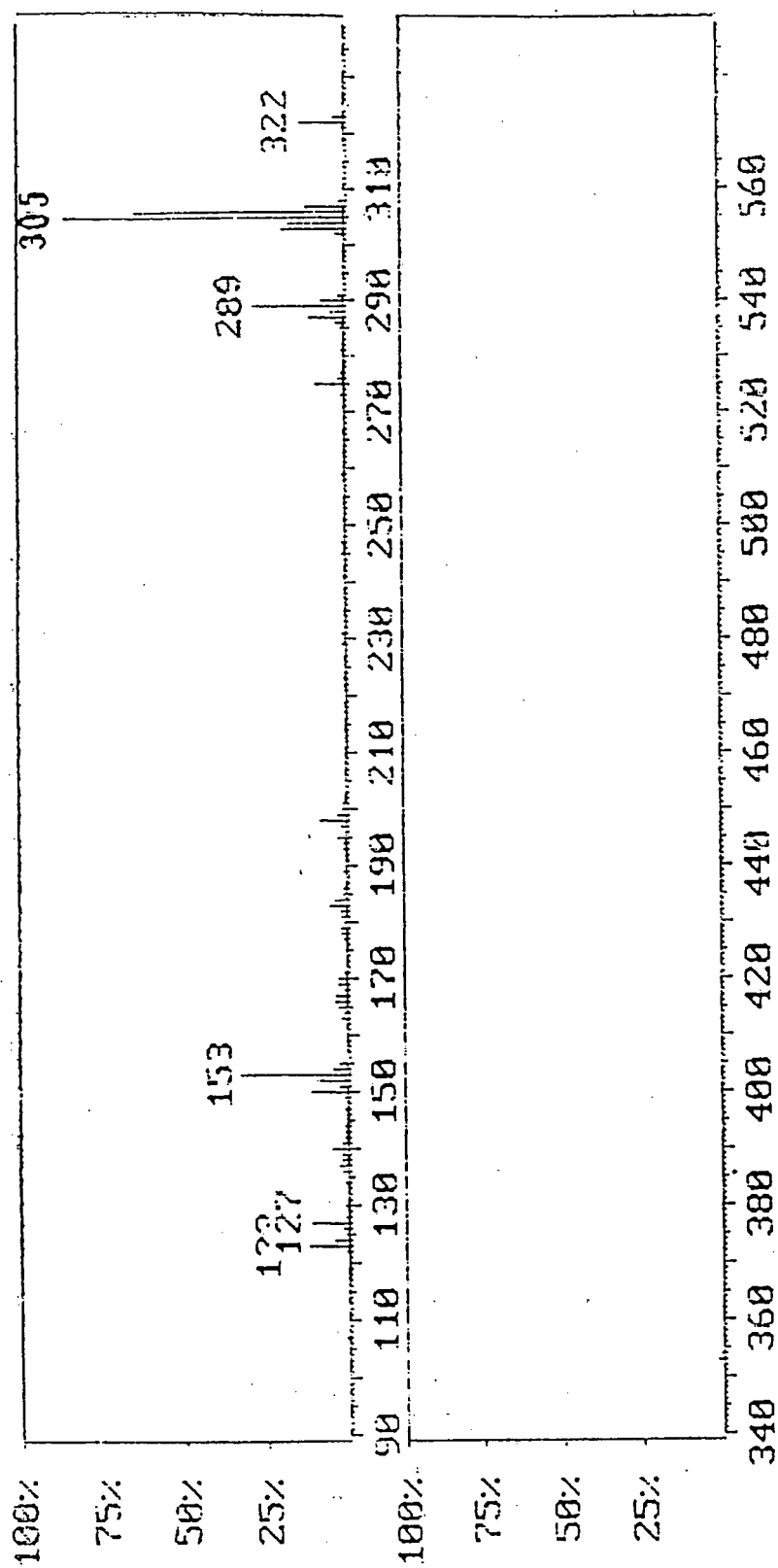


Fig 4-1-4 Mass spectrum of the white solid isolated from the acetone extract from Douglas-fir heartwood.

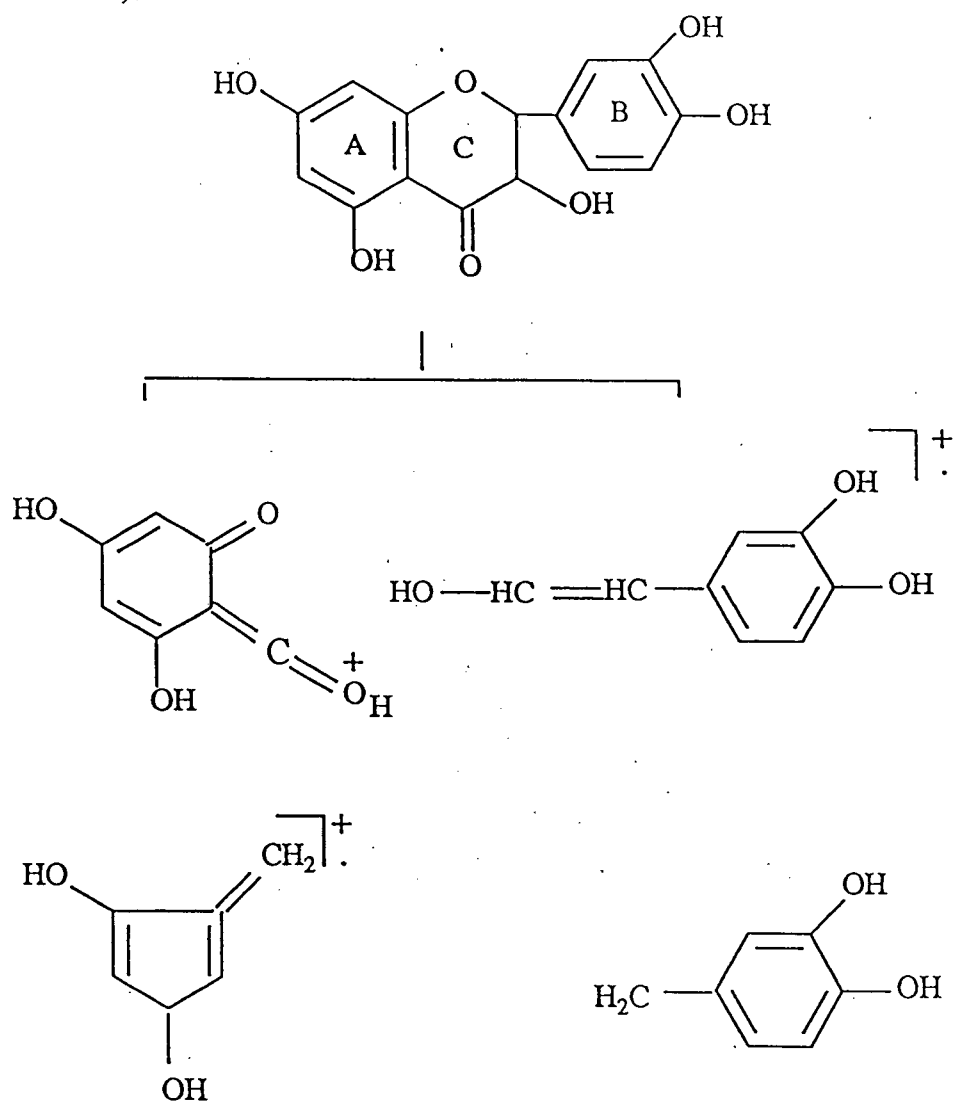


Fig. 4-1-5 Diagnostic mass spectral fragmentation from white solid extracted from Douglas-fir heartwood.

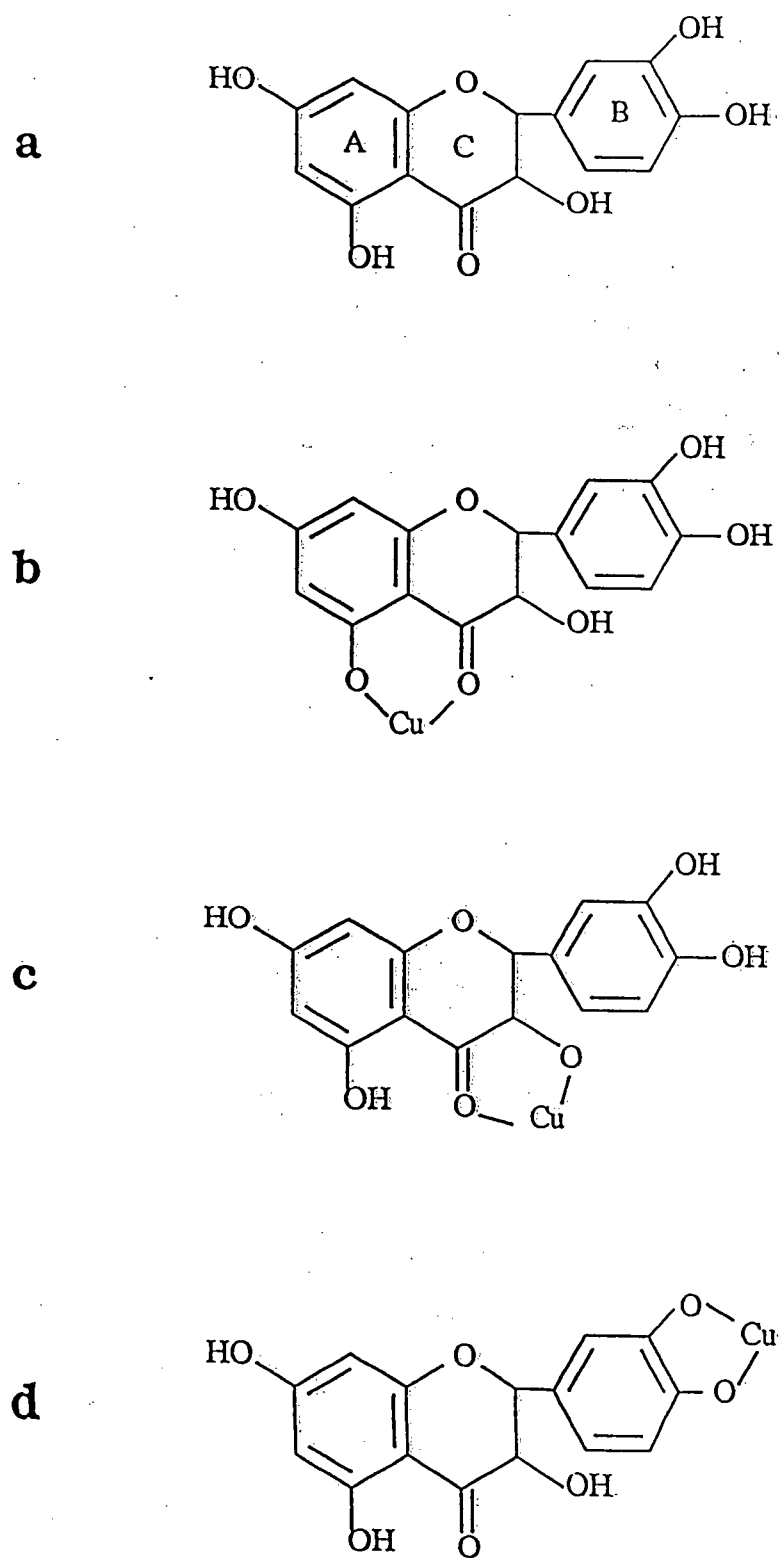


Fig 4-1-6 The molecular structures of taxifolin (a) and the three possible 1:1 copper complexes (b), (c) and (d).

#### **4.1.2 The nature of the chemical complex formed between copper solutions and taxifolin.**

Addition of a methanol solution of the white solid to ammonium hydroxide produced a pale yellow solution. Similar reactions were recorded when copper sulphate in either distilled water or in excess sodium hydroxide was added to the test solution. The CCA solution (pH=3) caused the taxifolin solution to turn dark yellow and produced a yellow precipitate after ten minutes. When ammoniacal copper sulphate was added to the test solution, the colour changed from light yellow to dark brown and a black solid slowly formed. If the reaction was made sequentially, with copper sulphate being added to the taxifolin solution first, a black product was formed only when the ammonium hydroxide was added to the mixture. The rate of formation of the black precipitate was slower than when an ammoniacal copper sulphate solution was used.

Confirmation of the visual results was made from visible spectra recorded for each of the solutions. When the methanol solution of taxifolin was mixed with ammoniacal copper sulphate, an absorption appeared at 580 nm after 20 minutes (Fig.4-1-7) which increased with time. When similar studies were made with other copper solutions, no characteristic peaks were observed (Fig. 4-1-8). These observations confirmed that only ammoniacal copper solutions of copper salts reacted with taxifolin to produce the dark colour.

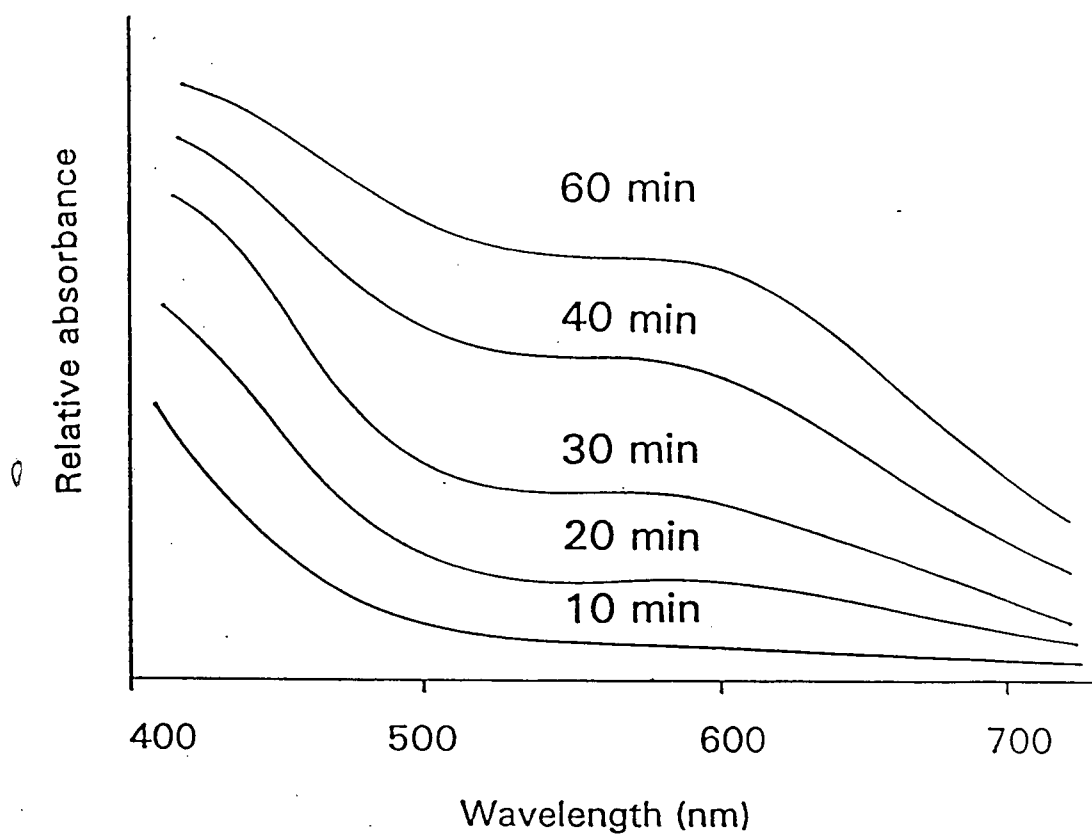


Fig. 4-1-7 Changes in the visible spectra of taxifolin in methanol to which was added ammoniacal copper solution.

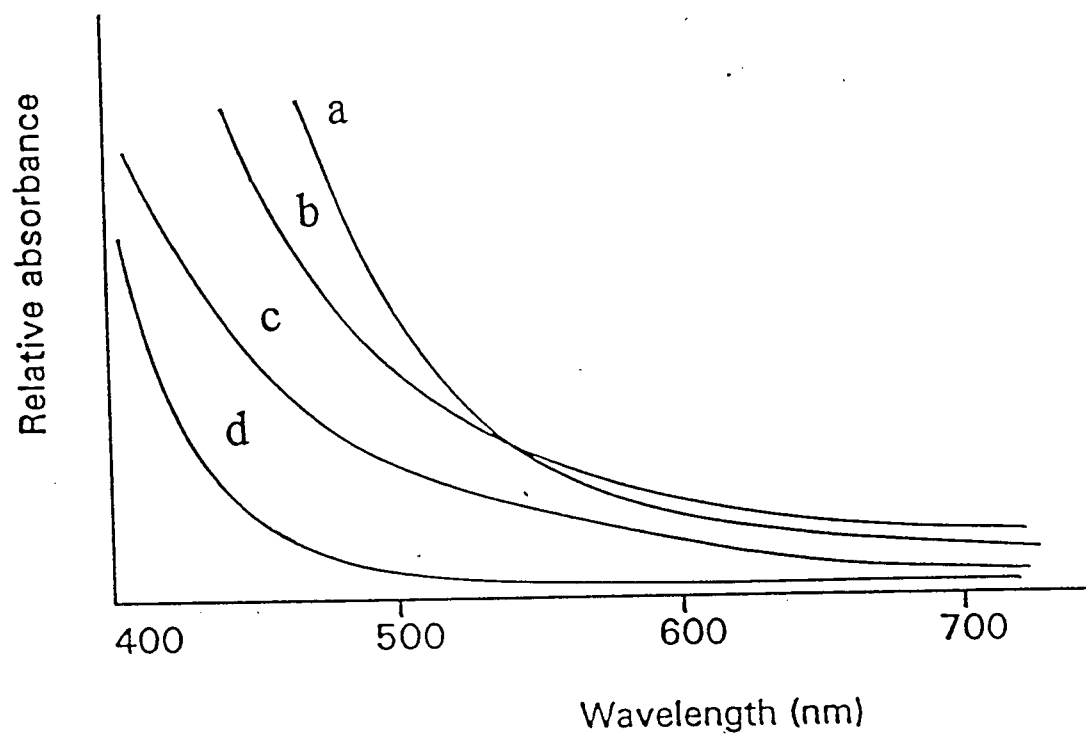


Fig. 4-1-8 The visible spectra of taxifolin plus a) CCA solution, b) copper sulphate in excess sodium hydroxide, c) copper sulphate in distilled water, and d) 2% ammonium hydroxide solution, 60 minutes after mixing.

In flavonones, copper can be chelated at either the 3-hydroxy and 4-keto or 5-hydroxy and 4-keto groups, forming a 6-membered or 5-membered ring (Fig. 4-1-6b and c) (Jurd, 1962; Sakamoto and Takamura, 1978; Takamura and Sakamoto, 1978). In taxifolin the reactivity of the proton on the 5-hydroxy group conjugated with benzene ring A, is greater than that on the 3-hydroxyl group, so that copper chelation to the A ring is anticipated. This reactivity of the 5-hydroxyl group was confirmed by Porter and Markham (1972) who demonstrated that metal ions were preferentially chelated at this position in dihydroflavonols. Following initial complex formation involving the 5-hydroxyl and the ketone groups, reaction with the 3-hydroxyl group can be eliminated. However, further coordination is possible through the 3' and 4' hydroxyl groups of taxifolin (Fig. 4-1-6d). The 3-hydroxyl and 7-hydroxyl groups are either not involved in bonding, or participate in intermolecular bonding, with the formation of a polymeric structure.

During reaction with ammoniacal copper sulphate solution, taxifolin formed a black complex, which was insoluble in both water and common organic solvents, such as diethyl ether, ethyl alcohol, chloroform and acetone. This implied that the copper was strongly bound to the taxifolin. The FTIR spectra of the taxifolin extracted from Douglas-fir heartwood and the reaction product formed with ammoniacal copper sulphate are shown in Fig 4-1-2b. An extremely broad band at  $3500\text{ cm}^{-1}$  suggested that some of the hydroxyl groups on taxifolin may be involved in chelate formation or intermolecular H-bonding, in the copper complex. The peak at  $1640\text{ cm}^{-1}$  due to the ketone bonding in taxifolin was shifted to  $1600\text{ cm}^{-1}$  in the black solid, confirming the involvement of this group during complex formation.

Two questions remained unanswered. They were a) the role of ammonia in complex formation and b) the nature of the molecular structure of the copper taxifolin complex. Based on the results of similar reactions with other copper containing solutions, the formation of the black solid appeared to require the presence of both the copper and ammonia, since other copper solutions failed to produce a black coloured product, even under alkaline conditions. Similarly, ammonium hydroxide alone did not cause a coloured reaction. Elemental analysis of the black solid confirmed the presence of nitrogen. The ammonia may be retained in the copper taxifolin product, either through the formation of a diammine complex  $[\text{Cu}(\text{NH}_3)_2 \cdot \text{taxifolin}]$  or through the formation of an imino group through a reaction with the taxifolin, which then chelated to the copper. However, since the  $\text{-C=N}$  stretching band occurs in the same region of the FTIR spectra as the carbonyl group, it was not possible to use the FTIR spectrum to confirm this reaction.

From a review of the literature of the formation of copper flavanoid complexes, it is unclear whether the ratio of copper to taxifolin would be 1:1 or 1:2. Detty *et al.* (1955) have reported that copper formed a 1:1 complex with dihydroquercetin (taxifolin) at pH 10.0 and a 1:2 complex at pH of 6.5. Conversely, Takamura and Sakamoto (1978) suggested that at a high pH, the formation of a 1:2 complex is expected, and identified the magnitude of the shifts in the maxima in absorption spectra. They also noted that the quercetin-copper(II) system could not be properly characterised because of catalytic oxidation of quercetin by copper under alkaline conditions. Delaporte and Macheix (1972) have reported that the reducing character of the flavanols is enhanced by activation of the hetrocyclic ring, when hydroxyl groups are introduced at the 5 and 4' positions of ring A and B, respectively. However, such oxidation is not anticipated

in the taxifolin-copper complexes in the current study, since the same authors have observed that no reduction occurs when the heterocyclic group is hydrogenated at the 2 and 3 positions.

The analytical results could not be resolved to confirm a copper:taxifolin ratio of either 1:1 or 1:2, but were more consistent with a 3:2 complex containing complexed nitrogen. The elemental analysis of the black solid yielded the following results. Found: C: 38.29, H: 3.48, N: 6.74, Cu: 21.04. Calculated: C: 39.96, H: 3.35, N: 6.26, Cu: 21.12, based upon the molecule  $[\text{Taxifolin}]_2\text{Cu}_3(\text{NH}_3)_4 \cdot 2\text{H}_2\text{O}$ . Further research is required to better characterize the black copper complex.

## 4.2 Fixation mechanism of ammoniacal copper wood preservatives

### 4.2.1 Reaction with ammonium hydroxide solution:

#### 4.2.1.1 Wood

The effect of ammonium hydroxide on ponderosa pine sapwood treated with ammonium hydroxide was observed by comparing the FTIR spectra of section from treated wood with those of untreated wood (Fig. 4-2-1). The peak at  $1730\text{ cm}^{-1}$ , due to carbonyl or carboxyl groups vibration, decreased in intensity, while that at  $1654\text{ cm}^{-1}$  which represents amide functionality was slightly stronger after ammonium hydroxide treatment than for the untreated wood. This may indicate that the carbonyl and carboxyl groups in wood had reacted with ammonia to form amide compounds (Wang, *et al.* 1967; Kaplunova, *et al.*, 1986).

In order to examine the effect of ammonia concentration on the possible formation of amide compounds, three ammonium hydroxide solutions (5%, 10% and 25%) were reacted with wood, and the treated wood examined by FTIR spectroscopy. The FTIR peak at  $2910\text{ cm}^{-1}$  (carbon-hydrogen stretching vibration) was used as an internal standard (Ostmeyer, *et al.*, 1989) to ensure between sample stability. To compare the spectra of different samples, the peak area ratio of the peak at  $1654\text{ cm}^{-1}$  and that at  $2910\text{ cm}^{-1}$  was used. This reduced the influence of sample heterogeneity, which may produce variations in signal energy as well as baseline errors. The peak base line was drawn from the point of transmittance on one side of the peak to the point of transmittance on the other side of the peak (Ostmeyer, *et al.*, 1989).

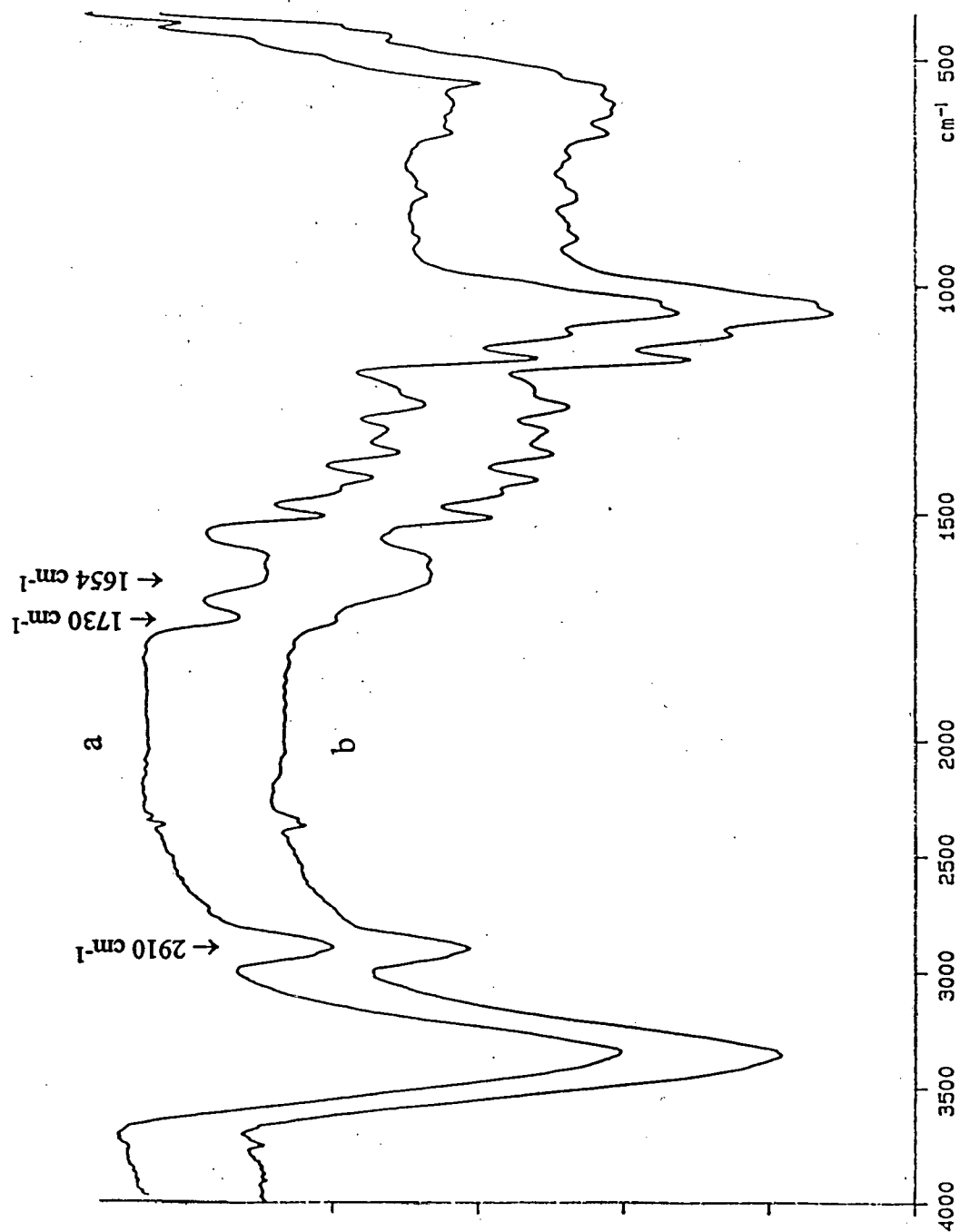


Fig 4-2-1 IR spectra of wood samples before (a) and after (b) treatment with ammonium hydroxide.

The results of the peak ratio (amide : CH) analysis are expressed in Table 4-2-1.

Table 4-2-1 Peak area ratio of amide/salt against carbon-hydrogen stretching

Ammonia concentration	Ratio (1654 cm <sup>-1</sup> /2910 cm <sup>-1</sup> )
Control	0.42
5 %	0.68
10 %	0.73
25 %	0.91

The peak ratio increased with increasing ammonia concentration, suggesting that the formation of amide compounds increased with increasing ammonia concentration.

#### 4.2.1.2 Cellulose

After soaking in ammonium hydroxide solution the FTIR spectrum of the cellulose showed no obvious change compared with untreated cellulose. This suggested that after treating with ammonium hydroxide solution, when ammonia had evaporated, the cellulose remained unchanged chemically. This observation is in good agreement with Heuser's observation (1946), who reported that the addition of aqueous ammonia, even in concentrated form (23-28 per cent) seemed to have no effect on cellulose chemically.

#### 4.2.1.3 Holocellulose

After treating with ammonium hydroxide solution, a comparison of the FTIR spectra of the untreated and treated holocellulose (Fig. 4-2-2) showed that the peaks at 1734 cm<sup>-1</sup> and 1250 cm<sup>-1</sup> disappeared. Since the peak at 1734 cm<sup>-1</sup> represented carbonyl or carboxyl groups in holocellulose, the loss of this peak

confirmed that the carboxyl ( $-\text{COOH}$ ) had reacted with ammonia to form an amide compound or an ammonium salt. The formation of an ammonium salt would result in a shift of the peak from  $1734\text{ cm}^{-1}$  to approximately  $1600\text{ cm}^{-1}$  (Zhbankov, 1966; Bellamy, 1958). In addition, a portion of the reacted hemicellulose was dissolved in ammonium hydroxide solution, since the intensities at peaks at  $1250$ ,  $1165$ , and  $1056\text{ cm}^{-1}$  which represent hemicellulose decreased. (Kuo *et al.*, 1988). The solid which was obtained from the filtrate of treated holocellulose after evaporating under vacuum was examined by FTIR. The spectrum (Fig. 4-2-3) showed a strong new peak at  $1670\text{ cm}^{-1}$ . This band corresponds to amine and amide band. The intensity of the peak at  $1402\text{ cm}^{-1}$  due to C-N stretch, increased in the spectrum. This observation confirmed that ammonia can react with carbonyl groups present in hemicellulose to form amide functional groups in wood. The loss of band of  $1250\text{ cm}^{-1}$ , which represents C-O-C vibration may be explained by the hydrolysis of the ester under the alkali condition, resulting the rupture of the C-O-C bonding.

#### 4.2.1.4 Lignin

When the FTIR spectrum of ammonium hydroxide treated lignin was compared with that of untreated lignin, the peak at  $1730\text{ cm}^{-1}$  was eliminated by the ammonium hydroxide treatment. The anticipated peak at  $1650\text{ cm}^{-1}$ , due to formation of an amide group, was not observed. One possible reason is that Klason lignin is less reactive and there is an absence of hydroxyl and carbonyl groups (Brauns, 1952). Another explanation is that the peak is not visible due to overlap by the strong benzene ring vibration at  $1600\text{ cm}^{-1}$ . However, no obvious difference in the spectra was observed in other frequencies after treating with ammonium hydroxide solution.

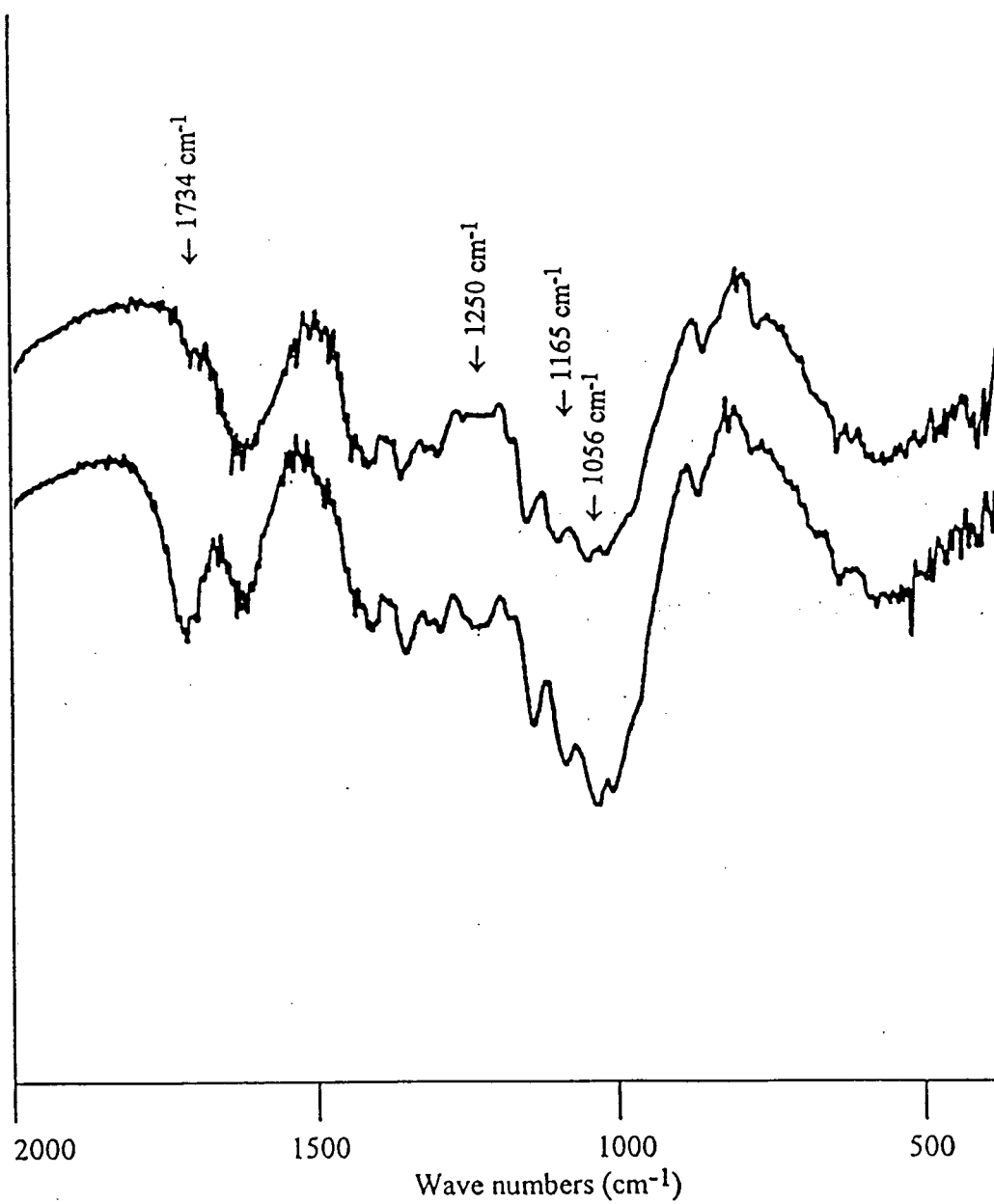


Fig 4-2-2 IR spectra of holocellulose before (lower) and after (upper) ammonium hydroxide treatment.

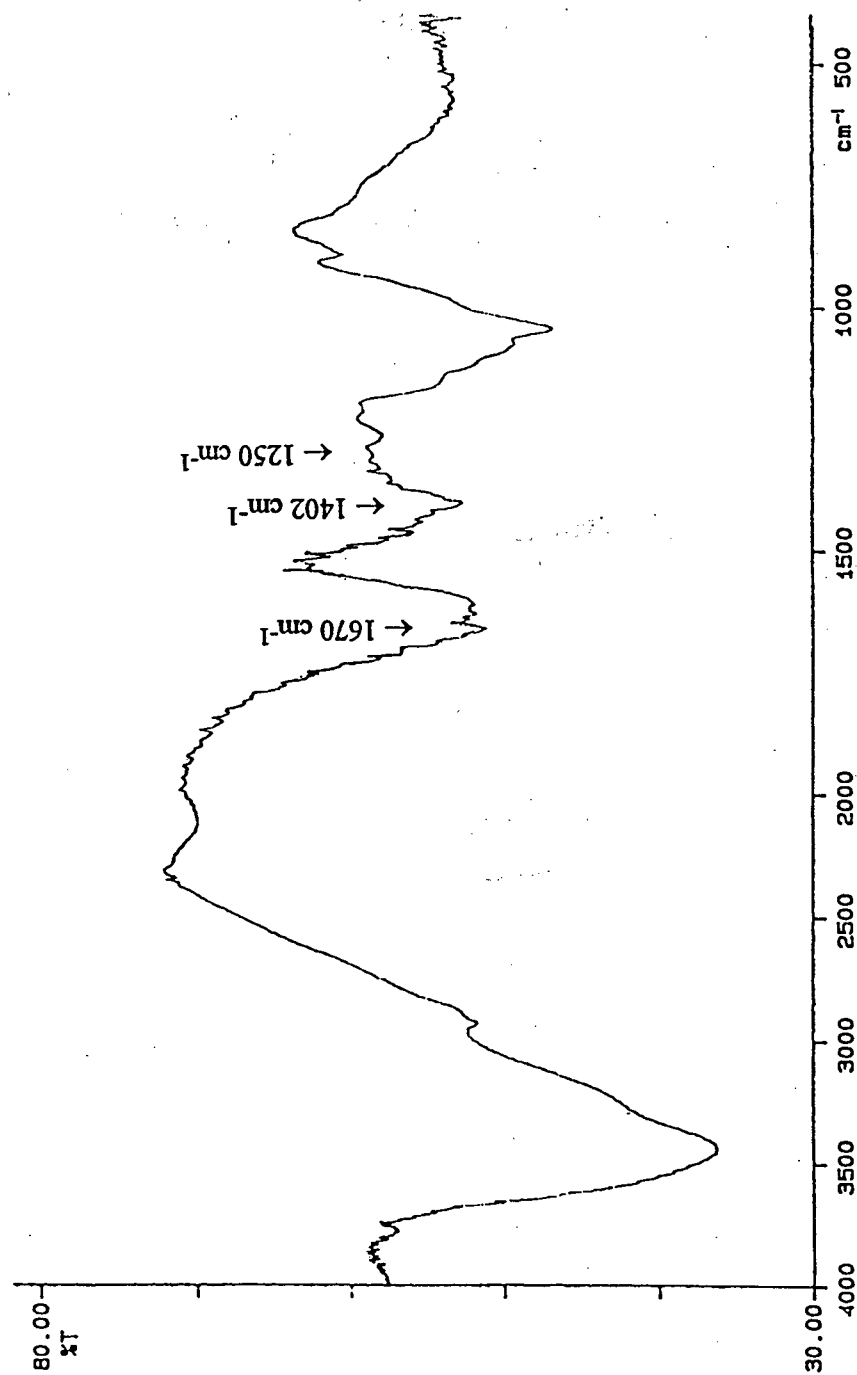


Fig 4-2-3 IR spectrum of the solid obtained from the filtrate of ammonium hydroxide treated holocellulose.

One question which may be raised concerning the interpretation of the peak at  $1654\text{ cm}^{-1}$ , in terms of amide vibration, is that this region of the FTIR spectrum is very complex, and may contain an unresolved peak due to the C-O stretching frequency.

#### 4.2.2 Reaction with ammoniacal copper solutions

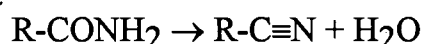
FTIR spectrum of ammoniacal copper solution treated wood sample was similar to that of ammonium hydroxide-treated wood. It is known that ESR spectral results have indicated that copper-nitrogen bonded complexes are formed in ammoniacal copper treated wood (Ruddick, 1992b). The position of the FTIR absorption peaks showed no substantial shift in frequency for the copper complex compared with the ammonium hydroxide treated wood. The IR measurements do not materially assist the identification of the complex formation.

The FTIR spectrum of ammoniacal-copper treated cellulose showed no obvious change, compared with that of untreated cellulose. It suggested that reaction between cellulose and ammoniacal copper must be very weak. This observation is in agreement with the ESR results (Ruddick *et al.*, 1992b). When holocellulose and lignin were reacted with an ammoniacal copper solution, the FTIR spectra showed a similar pattern of ammonium hydroxide-treated holocellulose or lignin.

### 4.2.3 Reaction of ammonium hydroxide/ammoniacal copper solution with lignin model compound

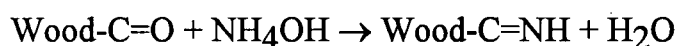
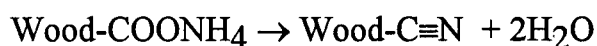
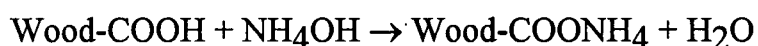
#### 4.2.3.1 Reaction of vanillin with ammonium hydroxide solution

In order to better understand the reaction between wood and ammonia, vanillin, a lignin model compound, was used to confirm the above reaction. Vanillin contains a methoxyphenol with a carbonyl group. The reaction products were analyzed using GC-MS. About 1.5% of 4-hydroxy-3-methoxy-benzonitrile was identified in the reaction products (Fig. 4-2-4). This molecule can be derived from an amide by removal of water (Merck, 1983), as described by the equation.



The observation of the nitrile compound, as one of the reaction products, is strongly supportive of the formation of some amide or amine compound due to the reaction between a carbonyl group in wood and ammonia.

The above results suggested that the some fixation of nitrogen in ammonium hydroxide-treated wood may be achieved through the reaction of ammonia with either carbonyl or carboxyl groups in hemicellulose and lignin in wood, with the formation of amide and imine compounds, as well as ammonium salts. The possible reactions may be expressed as follows:



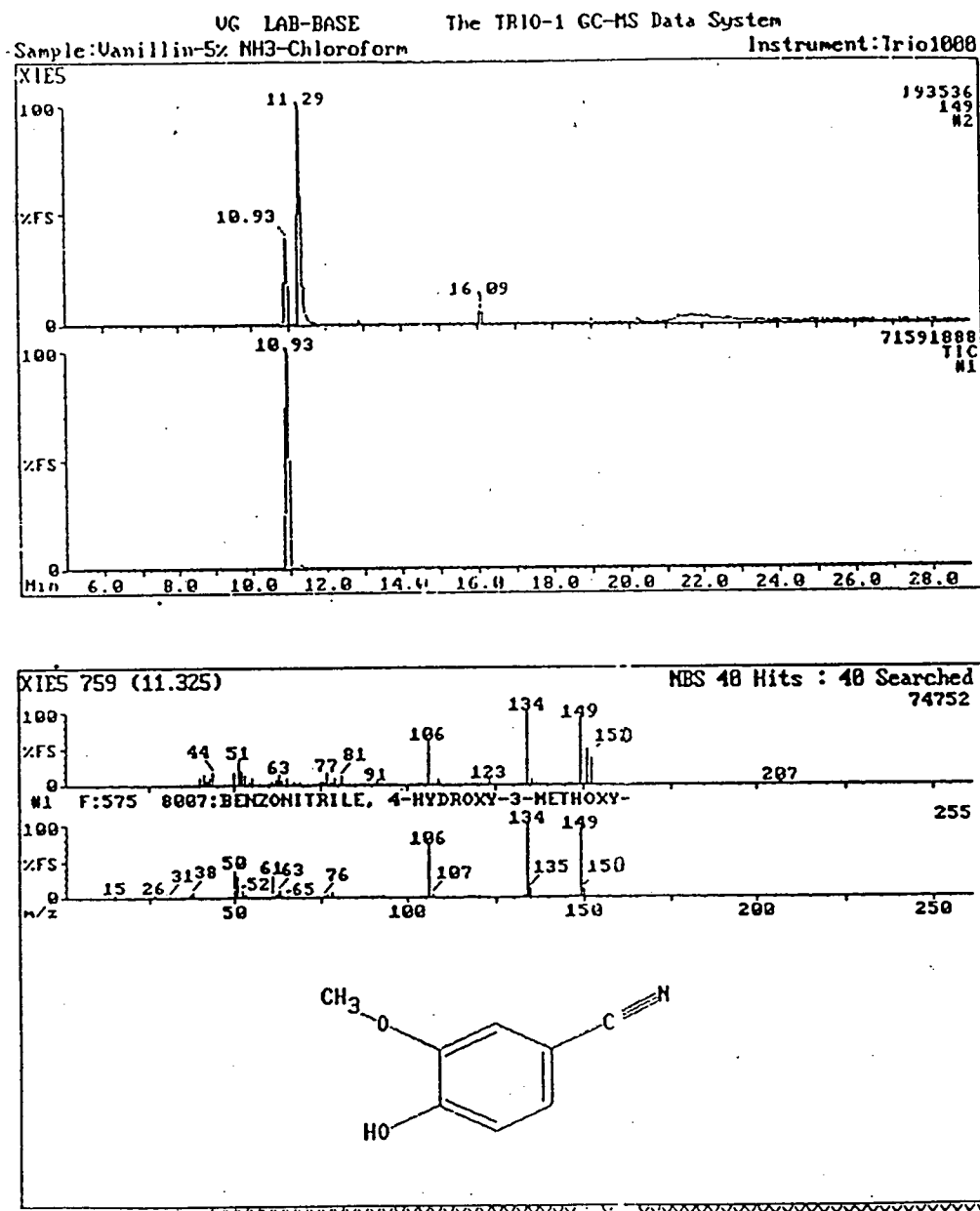


Fig 4-2-4 GC-MS spectrum of a reaction product of vanillin with ammonium hydroxide.

Although carboxylic acids containing fewer than five carbon atoms are soluble in water, many other carboxylic acids, especially the lignin type carboxylic acids of high molecular weight, are not appreciably soluble in water (Preston and Jin, 1991).

#### 4.2.3.2 Reaction of vanillin with ammoniacal copper solution

During reaction with an aqueous ammoniacal copper sulphate solution, vanillin formed a green water-insoluble complex upon evaporation to dryness. This complex was insoluble in both water and common organic solvents although it was found to be slightly soluble in DMSO. The nature of the complex was characterized using X-ray crystallography, FTIR and ESR.

##### *X-ray structural examination:*

The structure of the complex determined by X-ray crystallography is comprised of a central copper (II) ion bonded to two vanillin and two ammonia molecules. The perspective view of the complex with numbering system is presented in Fig. 4-2-5. In the complex, both the methoxy and phenolic oxygen atoms of each guaiacyl unit coordinate to the copper, together with nitrogen from two ammonia molecules to form a six coordinated molecule. The unit cell contains two symmetrically related complexes. The atomic coordinates and equivalent isotropic thermal parameters are presented in Table 4-2-2, while selected inter-atomic bond distance and angles are listed in Table 4-2-3 and 4-2-4 respectively.

The copper atom displays a distorted octahedral coordination. The Cu-O (phenolic oxygen atoms) at *ca.* 1.97 Å and the N-O (ammonia nitrogen) at *ca.* 2.02 Å form a square plane around the central copper. The very distorted octahedral configuration is completed by the Cu-O (methoxy oxygen atoms) at *ca.* 2.38 Å which are coordinated at an angle about 75° to the plane, at a greater distance than the in-plane Cu-O (phenolic) bonds. The Cu-N bond lengths of 2.014 and 2.034 Å are typical of copper(II) nitrogen bond lengths (Coughlin, *et al.*, 1984). The planar Cu-O bond lengths of 1.972 and 1.969 Å and longer axial

Cu-O lengths of 2.371 and 2.388 Å are consistent with those observed for other distorted octahedral structures (Hobson *et al.*, 1973). The Cu-O (hydroxyl) distances are shorter than those of Cu-O (methoxyl). This is due to the difference between the electron density of hydroxyl oxygen (rather ionic) and that of methoxyl oxygen (neutral). The differences between the two Cu-N bond lengths and the two Cu-O (phenolic) bond lengths were not statistically significant. In each unit hydrogen bonding occurs between the two adjacent complexes through the carbonyl and hydroxyl oxygen atoms and hydrogen on the ammonia. The hydrogen bond distances and angles are given in Table 4-2-5. The hydrogen-oxygen intermolecular bond distances are between 2.09-2.51 Å. This strong hydrogen bonding arises from the delocalization of electron to the carbonyl oxygen from the conjugated benzene ring, resulting in greater electronegativity on the oxygen atoms. A packing diagram has been depicted in Fig. 4-2-6.

The structure clearly shows that the ammonia had not reacted with the vanillin. This is consistent with the investigation of the reactions of the complex with EDTA, during which the vanillin copper complex was destroyed. The white solid produced was identified as vanillin by FTIR spectroscopy and GC-MS. This confirmed that the nitrogen was not directly bonded to vanillin, suggesting instead that two ammonia ligands of the tetrammine copper complex are replaced by copper-oxygen bonding from hydroxyl and methoxyl groups in vanillin during the complex formation.

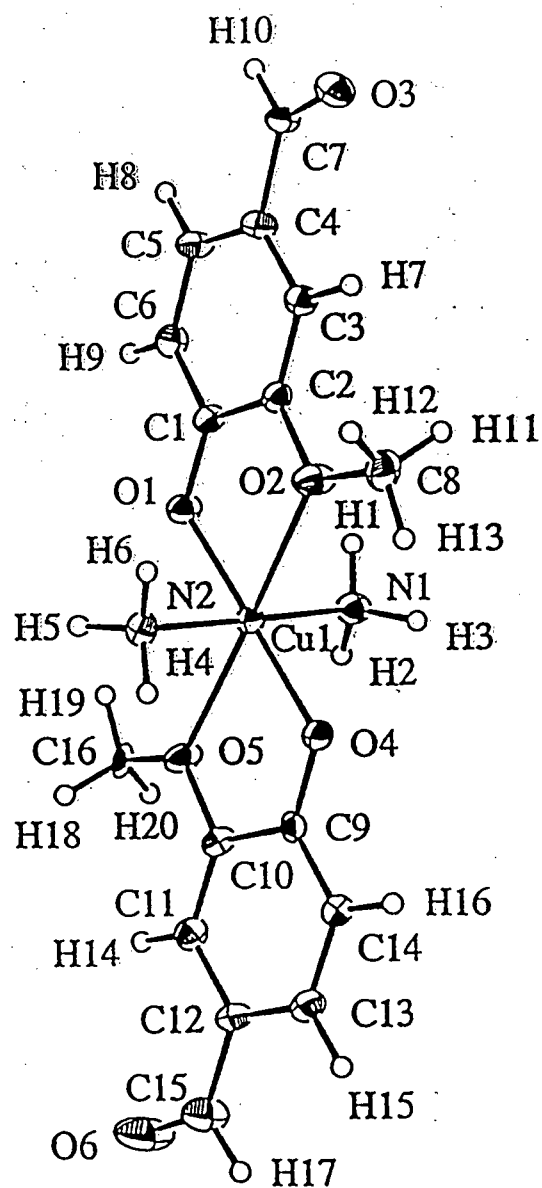


Fig. 4-2-5 Perspective view of Cu(II)-bis(vanillinato)bis(ammonia) with the atomic numbering; 33% probability thermal ellipsoid are shown for the non-hydrogen atoms.

Table 4-2-2. Atomic coordinates and Beq

Atom	x	y	z	Beq
Cu(1)	0.4061(1)	0.4928	0.18895(5)	2.379(9)
O(1)	0.6304(8)	0.5725(3)	0.1165(4)	2.80(9)
O(2)	0.2368(6)	0.5015(4)	-0.0350(3)	3.77(8)
O(3)	0.2540(7)	0.6388(3)	-0.4857(3)	3.69(9)
O(4)	0.1811(7)	0.4135(3)	0.2620(4)	2.70(8)
O(5)	0.5863(6)	0.4777(3)	0.4125(3)	3.23(9)
O(6)	0.608(1)	0.3115(4)	0.8502(5)	6.9(2)
N(1)	0.1929(10)	0.5937(3)	0.2254(5)	2.9(1)
N(2)	0.6205(10)	0.3919(3)	0.1477(5)	3.0(1)
C(1)	0.5757(9)	0.5978(3)	-0.0045(5)	2.28(10)
C(2)	0.3687(9)	0.5618(3)	-0.0916(4)	2.4(1)
C(3)	0.3155(9)	0.5861(4)	-0.2192(4)	2.6(1)
C(4)	0.462(1)	0.6472(4)	-0.2666(6)	2.8(1)
C(5)	0.633(1)	0.6852(4)	-0.1848(5)	2.8(1)
C(6)	0.7153(10)	0.6617(4)	-0.0547(5)	2.8(1)
C(7)	0.413(1)	0.6722(4)	-0.4046(5)	2.8(1)
C(8)	0.0349(10)	0.4578(4)	-0.1109(6)	3.5(1)
C(9)	0.2341(8)	0.3858(3)	0.3832(4)	2.19(10)
C(10)	0.4560(9)	0.4177(3)	0.4688(4)	2.3(1)
C(11)	0.5149(10)	0.3871(4)	0.5923(5)	2.7(1)
C(12)	0.365(1)	0.3260(4)	0.6405(6)	2.5(1)
C(13)	0.147(1)	0.2965(3)	0.5594(5)	2.8(1)
C(14)	0.0852(9)	0.3262(4)	0.4332(5)	2.6(1)
C(15)	0.427(1)	0.2928(5)	0.7706(6)	4.3(2)
C(16)	0.7862(8)	0.5172(3)	0.4816(6)	3.7(1)

Table 4-2-3. Selected bond lengths (Å) for vanillin-copper-ammonia complex

Atom	Atom	Distance	Atom	Atom	Distance
Cu(1)	O(1)	1.969(4)	Cu(1)	O(2)	2.371(3)
Cu(1)	O(4)	1.972(4)	Cu(1)	O(5)	2.388(3)
Cu(1)	N(1)	2.014(5)	Cu(1)	N(2)	2.034(5)
O(1)	C(1)	1.310(6)	O(2)	C(2)	1.368(6)
O(2)	C(8)	1.410(6)	O(3)	C(7)	1.216(7)
O(4)	C(9)	1.322(6)	O(5)	C(10)	1.359(6)
O(5)	C(16)	1.343(6)	O(6)	C(15)	1.211(9)
C(1)	C(2)	1.433(7)	C(1)	C(6)	1.398(7)
C(2)	C(3)	1.369(6)	C(3)	C(4)	1.380(7)
C(4)	C(5)	1.394(9)	C(4)	C(7)	1.474(7)
C(5)	C(6)	1.391(7)	C(9)	C(10)	1.458(7)
C(9)	C(14)	1.383(7)	C(10)	C(11)	1.363(7)
C(11)	C(12)	1.394(7)	C(12)	C(13)	1.407(8)
C(12)	C(15)	1.439(9)	C(13)	C(14)	1.384(7)

Table 4-2-4 Selected bond angles (°) for vanillin-copper-ammonia complex

Atom	Atom	Atom	Angle (°)	Atom	Atom	Atom	Angle (°)
O(1)	Cu(1)	O(2)	75.2(2)	O(1)	Cu(1)	O(4)	179.7(2)
O(1)	Cu(1)	O(5)	105.1(1)	O(1)	Cu(1)	N(1)	90.0(2)
O(1)	Cu(1)	N(2)	89.2(2)	O(2)	Cu(1)	O(4)	104.9(2)
O(2)	Cu(1)	O(5)	177.2(2)	O(2)	Cu(1)	N(1)	90.3(2)
O(2)	Cu(1)	N(2)	88.5(2)	O(4)	Cu(1)	O(5)	74.8(1)
O(4)	Cu(1)	N(1)	89.8(2)	O(4)	Cu(1)	N(2)	91.0(2)
O(5)	Cu(1)	N(1)	92.5(2)	O(5)	Cu(1)	N(2)	88.8(2)
N(1)	Cu(1)	N(2)	178.7(2)	Cu(1)	O(1)	C(1)	120.3(3)
Cu(1)	O(2)	C(2)	108.8(3)	Cu(1)	O(2)	C(8)	131.5(3)
C(2)	O(2)	C(8)	119.6(4)	Cu(1)	O(4)	C(9)	121.6(3)
Cu(1)	O(5)	C(10)	109.5(3)	Cu(1)	O(5)	C(16)	129.9(3)
C(10)	O(5)	C(16)	120.6(4)	O(1)	C(1)	C(2)	121.6(4)
O(1)	C(1)	C(16)	121.7(5)	C(2)	C(1)	C(6)	116.8(4)
O(2)	C(2)	C(1)	113.5(4)	O(2)	C(2)	C(3)	124.7(4)
C(1)	C(2)	C(3)	121.8(5)	C(2)	C(3)	C(4)	119.9(5)
C(3)	C(4)	C(5)	120.3(5)	C(3)	C(4)	C(7)	120.7(6)
C(5)	C(4)	C(7)	119.0(5)	C(4)	C(5)	C(6)	120.0(5)
C(1)	C(6)	C(5)	121.2(5)	O(3)	C(7)	C(4)	124.2(6)
O(4)	C(9)	C(10)	120.1(4)	O(4)	C(9)	C(14)	122.1(4)
C(10)	C(9)	C(14)	117.7(4)	O(5)	C(10)	C(9)	113.6(4)
O(5)	C(10)	C(11)	126.3(4)	C(9)	C(10)	C(11)	120.1(5)
C(10)	C(11)	C(12)	121.3(5)	C(11)	C(12)	C(13)	118.8(5)
C(11)	C(12)	C(15)	121.7(6)	C(13)	C(12)	C(15)	119.4(5)
C(12)	C(13)	CC(14)	120.7(5)	C(9)	C(14)	C(13)	121.2(5)
O(6)	C(15)	C(12)	126.7(7)				

Table 4-2-5 Hydrogen bonds (Å) and C-H...O interactions

A	H	B	A-H	H...B	A...B	A-H...B (°)
N(1)	H(2)	O(3)①	0.99	2.09	3.064(6)	169
N(1)	H(3)	O(1)②	0.99	2.13	3.085(8)	162
N(2)	H(5)	O(4)③	0.99	2.11	3.089(7)	171
N(2)	H(6)	O(6)④	0.99	2.40	3.341(8)	159
C(13)	H(15)	O(3)⑤	0.99	2.51	3.270(7)	133
C(16)	H(9)	O(3)⑥	0.99	2.52	3.127(6)	119

Symmetry operation: ①  $x, y, 1+z$ ; ②  $x-1, y, z$ ; ③  $1+x, y, z$ ; ④  $x, y, z-1$ ;  
 ⑤  $-x, y-1/2, -z$ ; ⑥  $1+x, y, 1+z$ .

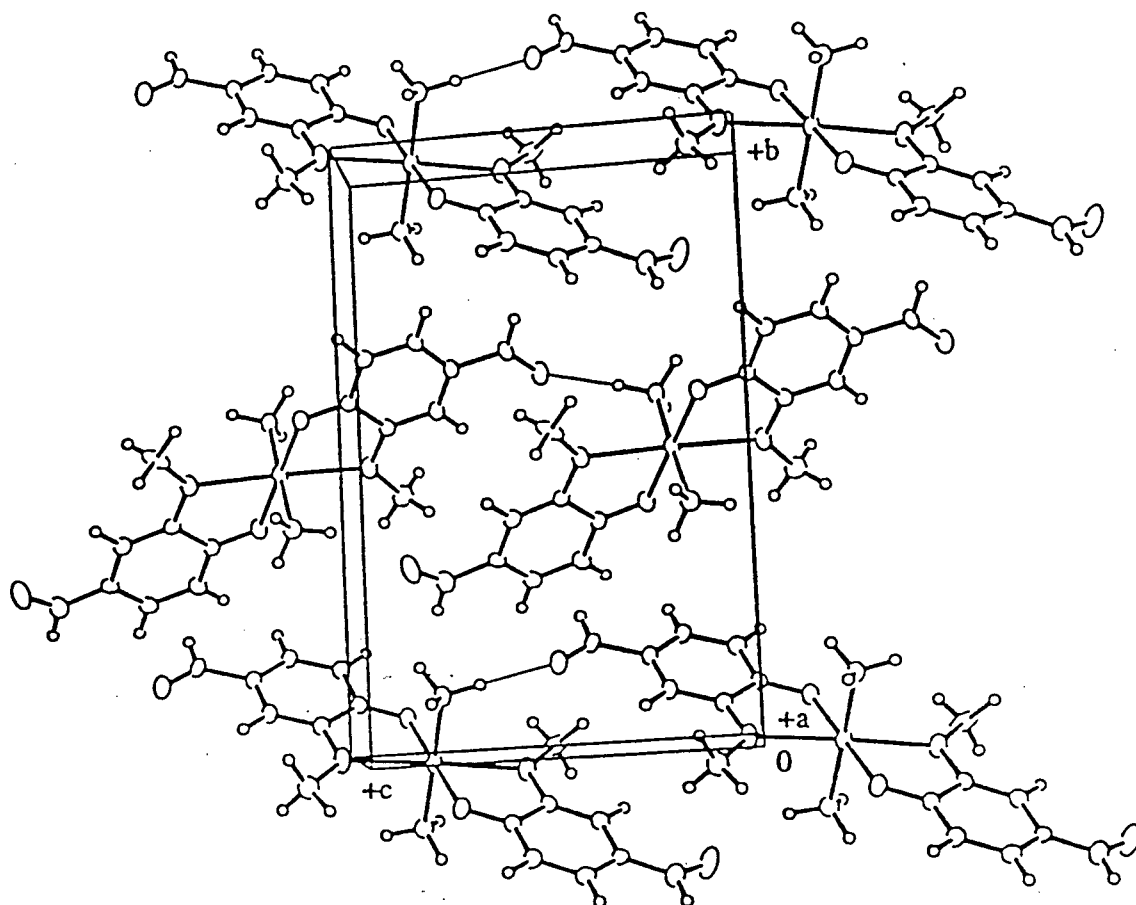


Fig 4-2-6 The packing of Cu(II)-bis(vanillinato)bis(ammonia) in a monoclinic unit cell. The hydrogen bonds are indicated by thin lines.

### *Spectroscopic analysis*

#### *FTIR spectroscopy*

The FTIR spectra of the polycrystalline green solid and pure vanillin was compared in Fig. 4-2-7 and primary FTIR vibrational assignments are present in Table 4-2-6. The peak at  $3200\text{ cm}^{-1}$  in the spectrum of pure vanillin was assigned as the OH stretching vibration. However, in the spectrum of the complex, new peaks appeared in the region of  $3200\text{-}3400\text{ cm}^{-1}$ , which were due to N-H vibration. The peak with a broad shoulder at  $730\text{ cm}^{-1}$  in the spectrum of vanillin due to the phenolic OH out of plane deformation was absent in the spectrum of the green solid. This observation supported the conclusion that the phenolic hydroxyl group was involved in the formation of the copper complex. The spectrum of the copper complex also contains a new peak at  $448\text{ cm}^{-1}$ . This was assigned to Cu-N bonding (Hathaway and Tomlinson, 1970). The peak at  $1150\text{ cm}^{-1}$  due to C-O-C bonding in pure vanillin was shifted to  $1120\text{ cm}^{-1}$  in the complex, consistent with the coordination of methoxyl oxygen.

The mass spectrum of the green crystalline solid showed a peak at  $m/e = 366$  which was assigned as an  $(M-2\text{NH}_3)^+$  fragment. From the mass spectrum and elemental analysis, the formula of the complex can be expressed as  $[\text{Cu}(\text{vanillin})_2(\text{NH}_3)_2]$ .

The electronic spectrum of a mull of the green solid (Fig. 4-2-8) shows two absorption bands at  $590\text{ nm}$  ( $16,900\text{ cm}^{-1}$ ) and  $770\text{ nm}$ . ( $13,000\text{ cm}^{-1}$ ), which were assigned as d-d electron transitions, as is typical for the presence of  $[\text{CuO}_4\text{N}_2]$  chromophores (Bullock *et al.*, 1974).

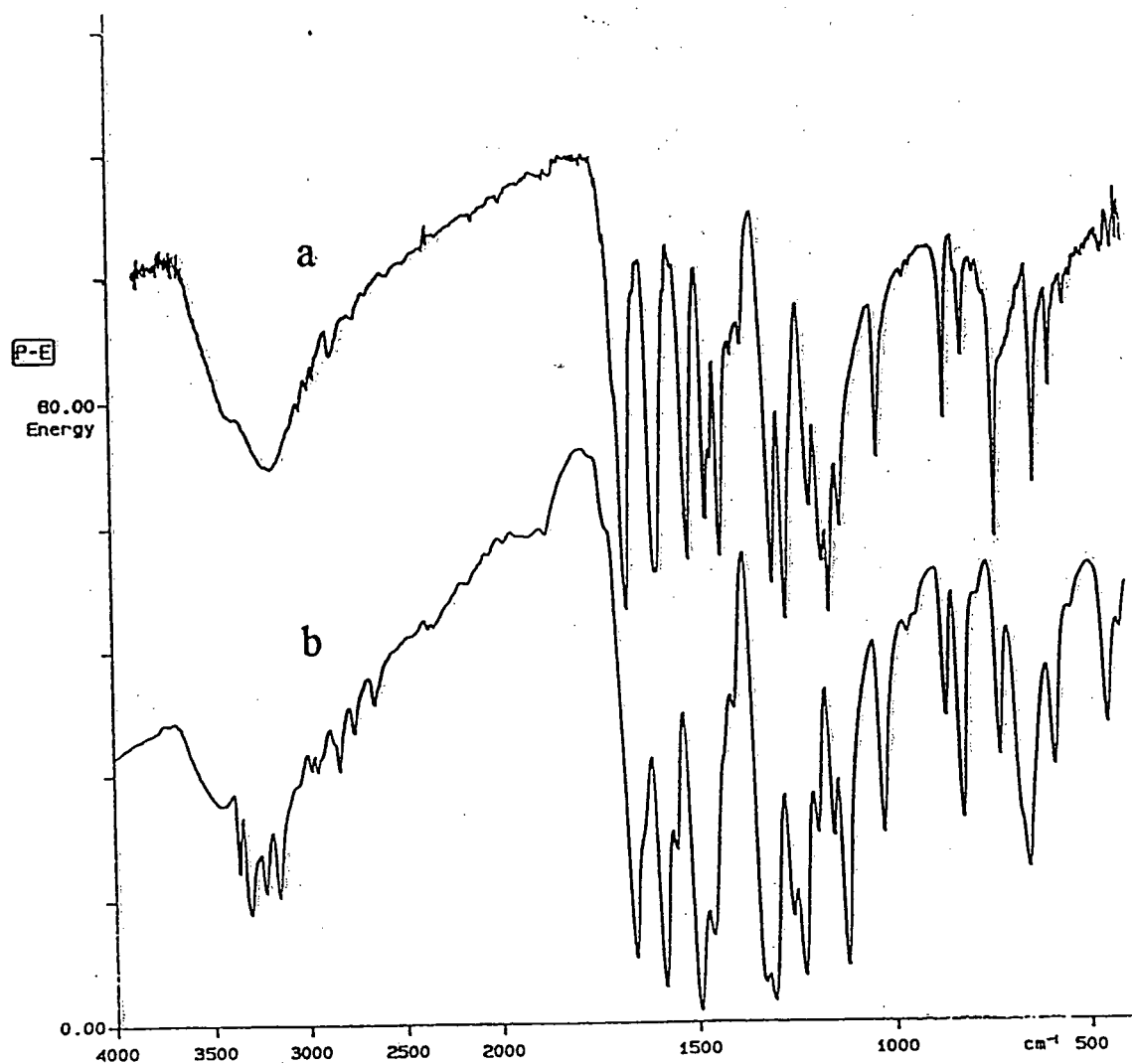


Fig 4-2-7 IR spectra of vanillin and vanillin-copper complex, a) Vanillin, b) Vanillin-copper-ammonia complex.

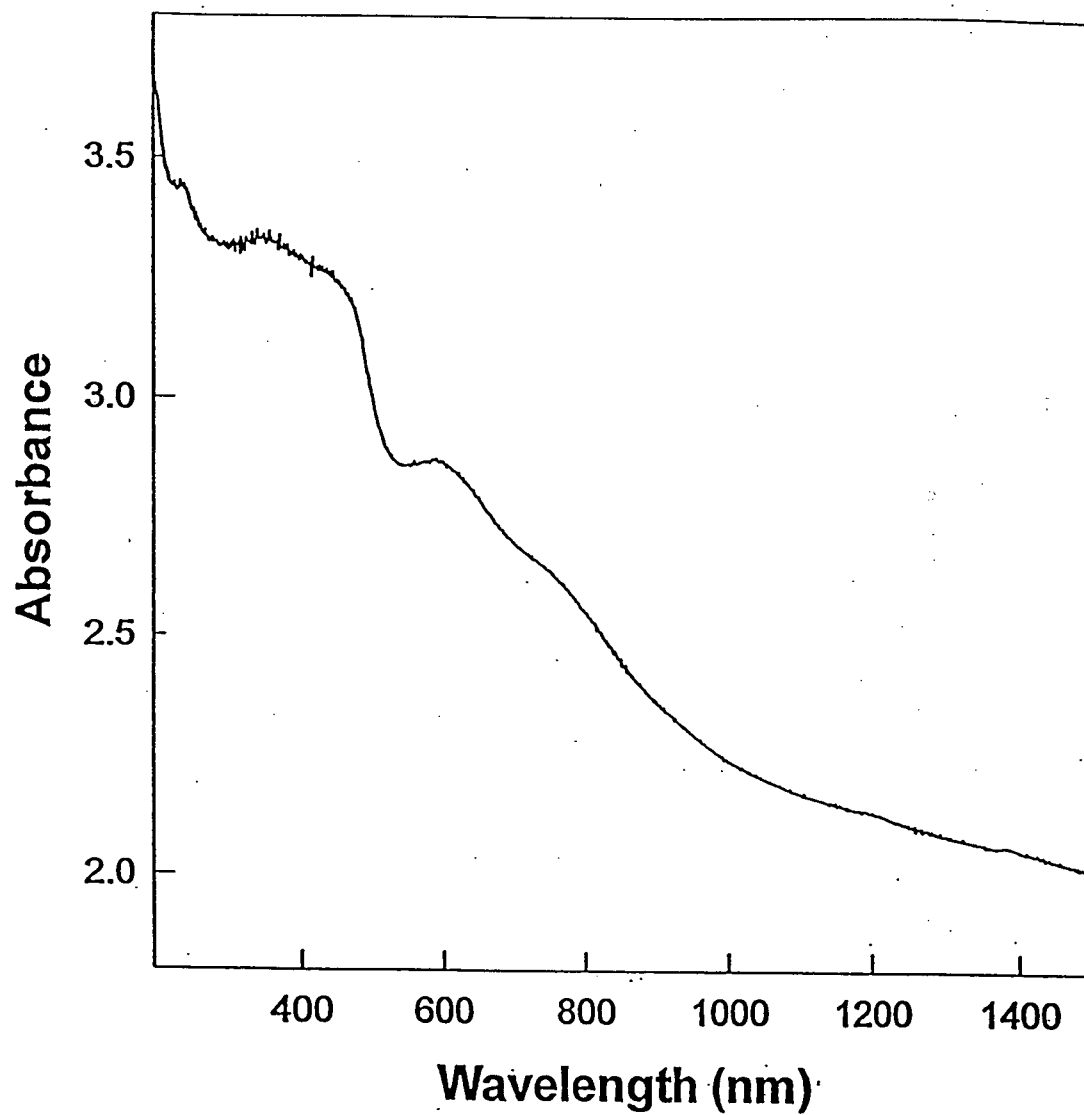


Fig 4-2-8 UV-visible spectrum of a Nujol mull of the vanillin-copper-complex.

Table 4-2-6 Assignment of main absorption bands in IR spectra of vanillin and copper-vanillin complex.

Copper-vanillin complex	Vanillin	Assignment
cm <sup>-1</sup>	cm <sup>-1</sup>	
	3174	OH stretching
3050-3350		NH stretching
1654	1666	C=O stretching
1496	1509	Aromatic skittle vibration
1310		NH symmetric deformation
1120	1160	C-O-C antisym stretching
862	859	CH out plane deformation
	730	OH out plane deformation
725, 652		NH rocking
449		Cu-N stretching

### *ESR spectroscopy*

At room temperature, the polycrystalline powdered sample of the green solid gives rise to an exchange narrowed anisotropic unresolved ESR spectrum (Fig. 4-2-9a), which does not exhibit hyperfine splitting, but provides  $g_{//}$  and  $g_{\perp}$ , values of 2.295 and 2.06 respectively. The spectra of polycrystalline  $[\text{Cu}(\text{vanillin})_2(\text{NH}_3)_2]$  show no change over the temperature range 115K to 350K, suggesting that there is no fluxional behavior. X-ray crystallographic study showed that the coordination around the Cu(II) ion is a  $\text{O}_2\text{N}_2$  square plane with two oxygen donors forming a distorted elongated octahedron; the elongation axis is at  $75^\circ$  to the  $\text{O}_2\text{N}_2$  plane. The overall geometry around Cu(II) ion is similar to that in  $\text{Cu}(\text{NH}_3)_2(\text{CH}_3\text{CO}_2)_2$  (Hathaway and Tomlinson, 1970). The elongation axes of the two crystallographic sites of elongated rhombic-octahedral coordination environment of the Cu(II) are nearly aligned in the crystal (the vectors are  $8^\circ$  apart). The alignment of this unique axis in the coordination sphere of crystallographically related sites means that the observed  $g$ -values in the solid state correspond to the molecular axes (Hathaway and Belling, 1970). The significance of this result is that the observed  $g$ -values can be used to identify this type of bonding in other systems, *e.g.* Cu(II) fixed in wood. The  $g$ -value of 2.295 corresponds to the elongated O-Cu-O axis and the magnitude of  $(g_{//} - 2)/(g_{\perp} - 2)$  suggests a ground state in which the hole resides in the  $d_{x^2-y^2}$  orbital (Hathaway and Tomlinson, 1970).

The green complex  $[\text{Cu}(\text{vanillin})_2(\text{NH}_3)_2]$  dissolved sufficiently in DMSO that an ESR spectrum was observable. The ESR spectrum of the solution at 115K is shown in Fig. 4-2-9b. Although the  $g_{//}$  and  $A_{//}$  features are well resolved, the features in the perpendicular region are less resolved. Nonetheless, the lineshape in that region is typical of an orthorhombic system. The spectrum was simulated

using the parameters given in Table 4-2-7. The  $g$ -values agree well with those from the pure green powder. The  $A_{//}$  and  $g_{//}$  values are typical of a  $\text{CuO}_2\text{N}_2\text{O}_2$  chromophore (Peisach and Blumberg, 1974; Pilbrow, 1990). All of the above suggests that the complex retains the structure  $[\text{Cu}(\text{vanillin})_2(\text{NH}_3)_2]$  in DMSO. Support for this was obtained from the electronic spectrum of the complex in DMSO which retained the features of the mull spectrum.

Based on the above assignment that the hole occupies the  $d_{x^2-y^2}$  orbital, then the electronic spectrum can be tentatively assigned as  $d_{xz}, d_{yz}, d_{z^2} \rightarrow d_{x^2-y^2}$  in the region 16.9 kK and the  $d_{xy} \rightarrow d_{x^2-y^2}$  in the region 13.0 kK by analogy with  $\text{Cu}(\text{NH}_3)_2(\text{CH}_3\text{CO}_2)_2$  (Hathaway and Tomlinson, 1970).

As shown in table 4-2-7, the vanillin-copper complex showed a much smaller  $A_{//}$  and a larger  $g_{//}$  value than those of copper sulphate in ammonium hydroxide solution. This change in the spectral parameters of the vanillin-copper complex is consistent with the replacement of two of the ammonia ligands in tetrammine copper ions by copper-oxygen bonding (Senesi, *et al.*, 1989; Ruddick, 1992), reflecting the somewhat more ionic environment. Wood treated with an aqueous  $\text{Cu}(\text{en})_2\text{SO}_4$  solution has the largest  $A_{//}$  and smallest  $g_{//}$ , indicating that copper complex in the  $\text{Cu}(\text{en})_2\text{SO}_2$  treated wood has four equatorial copper-nitrogen donor bonds (Farkas and Kurzak, 1990). ESR parameters of the vanillin-copper complex is in good agreement with that of ammoniacal copper carbonate treated wood (Ruddick, 1992), suggesting the vanillin-copper complex appears to have the same configuration as copper in the wood treated with ammoniacal copper carbonate, in which two copper-oxygen and two copper-nitrogen equatorial bonds form a plane.

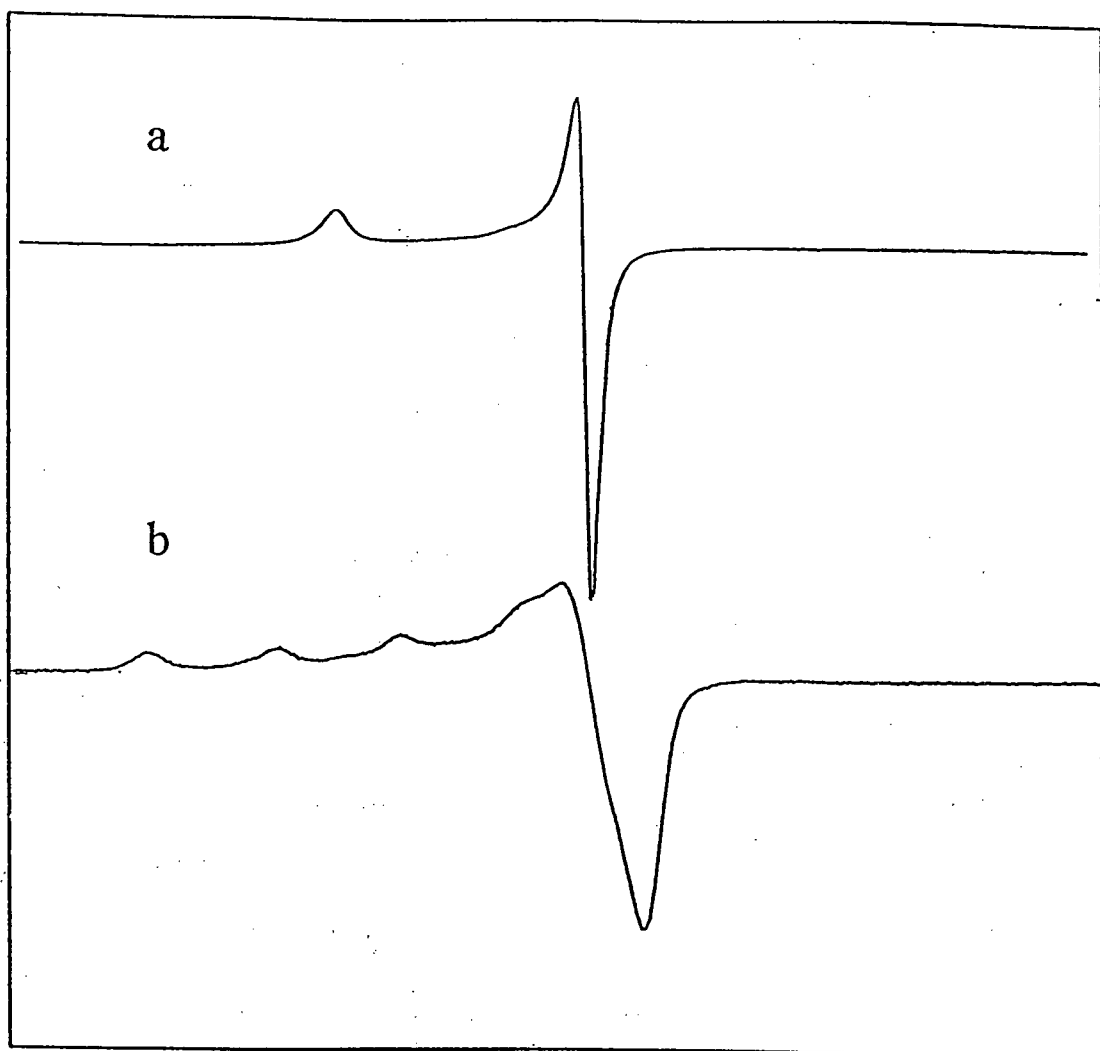


Fig 4-2-9 ESR spectra of vanillin-copper complex a) solid at room temperature. b) in DMSO solution at 115 K.

Table 4-2-7. The ESR parameters for vanillin-copper complex, copper solution and the copper treated wood at room temperature.

	$A_{//}$ $\times 10^{-4} \text{ cm}^{-1}$	$g_{//}$	$g_{\perp}$
$\text{CuSO}_4$ in $\text{NH}_4\text{OH}^1$	193	2.24	-
$\text{CuSO}_4$ in $\text{H}_2\text{O}^1$	138	2.40	2.07
Vanillin-Copper- Ammonia complex			
-in solid <sup>2</sup>	-	2.295	2.06
-in DMSO <sup>3</sup>	175	2.295	2.054
			( $g_{xx} = 2.045$ , $g_{yy} = 2.063$ )
<u>Wood treated with</u>			
$\text{CuCO}_3/\text{NH}_4\text{OH}^4$	166	2.27	2.07
$[\text{Cu}(\text{en})_2]\text{SO}_4$ in water <sup>4</sup>	190	2.20	2.06

<sup>1</sup> data from Ajiboye and Brown (1990); <sup>2</sup> at room temperature, <sup>3</sup> at 115 K, DMSO = dimethyl sulfoxide, the parameters were obtained by a simulation of the spectrum using the program CLPOW provided by the Illinois ESR Research Center. <sup>4</sup> data from Ruddick (1992).

### *Effect of complex formation on wood properties*

Ammoniacal copper solution readily formed a water insoluble complex with vanillin, providing support for the hypothesis that, during fixation, ammoniacal copper preservatives react with the guaiacyl units on lignin to form stable copper-nitrogen complexes. The formation of such complexes supports the finding of enhanced nitrogen content in ammoniacal copper treated wood observed by Ruddick (1979). It may also help explain the changes which occur in the physical properties of the ammoniacal copper treated wood. The water insoluble copper complexes formed in the treated wood will be expected to be highly leach resistant (Hughes *et al.*, 1994; Ruddick, 1992), and may also induce water repellent properties in the wood (Jin and Preston, 1992). The formation of cross-linked copper-lignin complexes in ammoniacal copper treated wood may also be responsible for the enhanced protection against photodegradation (Hon and Chang, 1985), reported to occur in ammoniacal copper treated wood during above ground weathering exposure tests (Jin, Archer and Preston; 1991).

From the review of the literature on the formation of diammine copper compounds, it is clear that after ammonia evaporating from a copper tetraammine carbonate solution, copper diammine carbonate was precipitated (Tomlinson and Hathaway, 1968). The equation is present as



The structure of the diammine showed that two nitrogen atoms from two ammonia molecules in the diammine carbonate complex were connected to the central copper ion with other oxygen atoms from the carbonate group. The geometry of the diammine was a planer-square pyramid (Tomlinson and

Hathaway, 1968), which is in agreement with the proposed structure of the copper complexes in ammoniacal copper treated wood from ESR parameters (Ruddick, 1992b).

As indicated by the above facts, the fixation of copper and nitrogen includes the reaction of cupriammonium ions with lignin, forming a diammine complex.

### 4.3 Whether the enrichment of nitrogen in ammoniacal-copper preservative treated wood increases the decay potential

#### 4.3.1 Wood treated with ammonium hydroxide solutions

The nitrogen contents in the samples for each treatment are listed in Table 4-3-1. The nitrogen content increased with increasing ammonia concentration in the treating solutions. Not surprisingly the pH of the leachate recovered from blocks showed an increase, although the magnitude of the rise in pH was small and the leachate remained slightly acid. The mean weight losses of the wood samples treated with ammonium hydroxide exposed to three different fungi were plotted against the ammonia concentration of treating solutions (Fig. 4-3-1). Analysis of variance (ANOVA) of weight losses of blocks treated with different concentration of ammonium hydroxide solutions showed that slight reduction in weight loss was significant for *T. versicolor* at the 0.05 level. For *G. trabeum* the ANOVA showed that increases in weight losses with increasing ammonia concentration were significant at the 0.10 level. The weight losses were slightly increased. No mass losses were recorded for blocks treated with 5% or 10% ammonia solutions exposed to *P. placenta*.

From the results it is clear that the ammonia treatment affected the activity of each fungus differently. *Postia placenta* was extremely sensitive to the ammonia treatment. Since the blocks were not leached, their pH was slightly increased by the ammonia treatment as demonstrated by a rise in the pH of leachate from selected blocks, from 4.99 in the control samples to 6.31 for blocks treated with 10% ammonium hydroxide. This increase in the pH was considered to be the most likely cause of inhibition of the *P. placenta* (Highley, 1973; Zabel and Morrell, 1992). The weight loss decreased immediately in the ammonia treated

blocks at the lowest nitrogen retention (0.410%) to approximately 15% and thereafter to almost zero. This sensitivity of *P. placenta* to ammonium hydroxide treatment of wood had been noted previously. Ruddick *et al.* (1982) studied the effectiveness of three preservatives for protecting Burmese hardwoods. They reported that soil blocks treated with 5% ammonium hydroxide, and leached for fourteen days with ten changes of the water during that period were not decayed by *P. placenta*. No explanation for this sensitivity of *P. placenta* was offered.

The white rot fungus, *T. versicolor* was also affected by the ammonia treatment in that, at the lowest ammonia concentration, the weight loss decreased slightly from about 35% in the control samples to 30%. As the ammonia content increased to 5%, the weight loss was reduced to 25%. This suggested that activity of *T. versicolor* was somewhat inhibited by the change in pH, but appeared to offset this inhibition as the ammonia content is increased, probably by utilizing the available nitrogen. This slight reduction in the activity of *T. versicolor* in ammonium hydroxide treated wood is consistent with observations reported previously by Ruddick *et al.* (1982) in a tropical hardwood. *Gloeophyllum trabeum* was affected by the addition of ammonia to the wood. The weight losses recorded increased slightly with increasing ammonia content. This response is consistent with a previous study utilizing ammonia treated hardwoods (Ruddick *et al.*, 1982), where weight losses were similar in ammonia and water treated controls. The magnitude of the weight losses were much greater than in the previous study.

It should be noted that since the samples were not leached before testing, some of the residual ammonium-chemicals may have been lost during the test. Amburgey and Johnson (1978) have studied the effect of ammonium hydroxide on thiamine and available micronutrients in pine sapwood during decay by *G.*

*trabeum*. They concluded that increasing decay resistance may be due to factors which inhibit the germination of basidiospores and not to thiamine depletion alone. Consistent with this study, *G. trabeum* was able to grow on wood treated with ammonium hydroxide.

Table 4-3-1 Nitrogen content in the treated wood and pH value of the leachate solutions

Treatment	Control	1% NH <sub>3</sub>	5% NH <sub>3</sub>	10% NH <sub>3</sub>
N %	0.0505 (0.004) <sup>a</sup>	0.4104 (0.002) <sup>a</sup>	0.5451 (0.08) <sup>a</sup>	0.6277 (0.05) <sup>a</sup>
pH	4.99	5.96	6.28	6.31

<sup>a</sup>Each value represents mean of four replicates. Numbers in parentheses represent standard deviation for the average nitrogen content.

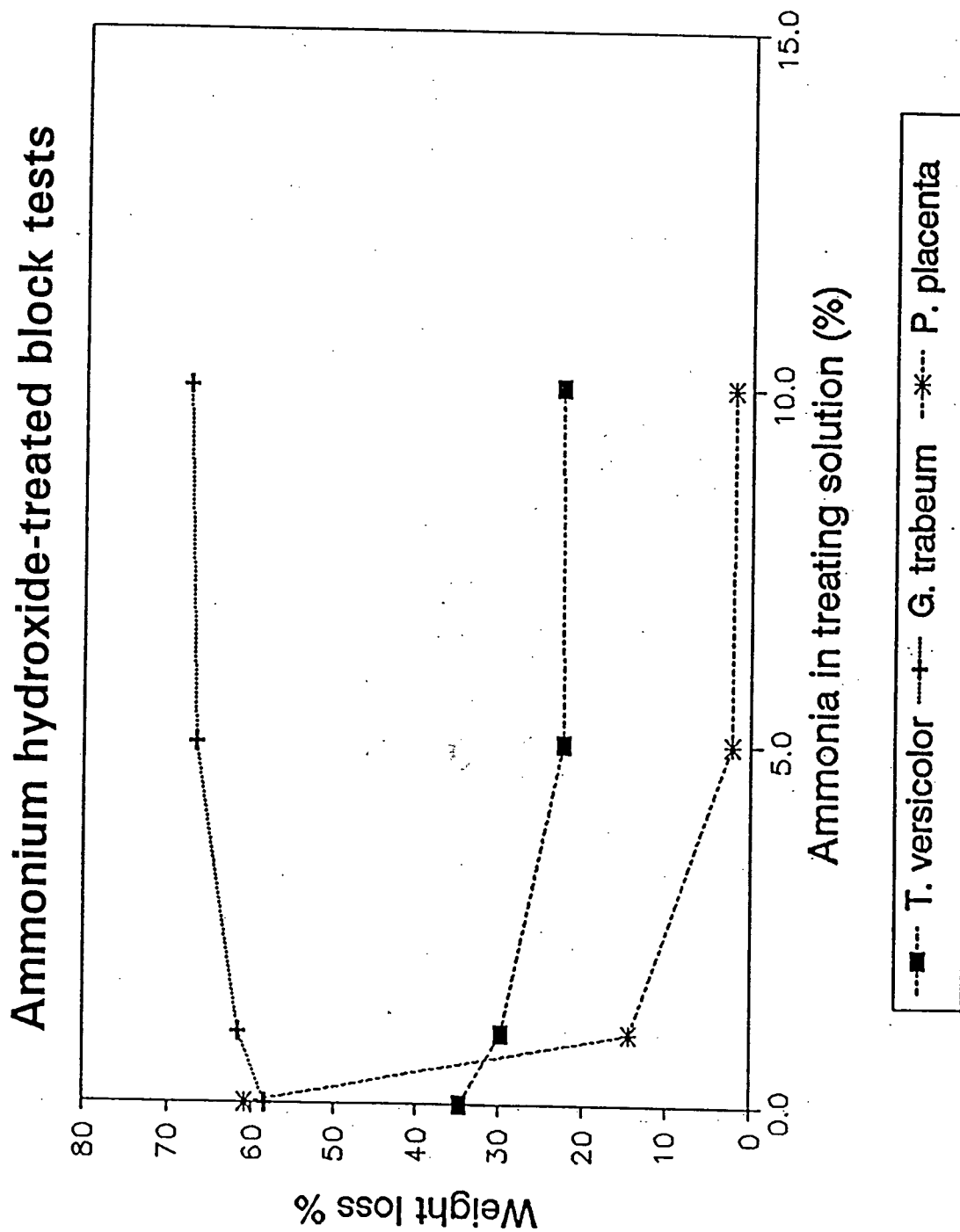


Fig. 4-3-1 Weight losses for ammonium hydroxide treated wood.

#### 4.3.2 Wood treated with ammoniacal copper solution:

All three fungi grew well on the water-treated control samples. There was some overgrowth of the ammoniacal copper-treated samples by both *T. versicolor* and *P. placenta*. The mean percent weight losses determined for the five replicates samples in each group, together with the nitrogen contents are presented in Tables 2 to 4 and Fig. 4-3-2. The weight loss data for the ammoniacal copper treated wafers were subjected to a one way ANOVA for each fungus. The statistical analysis confirmed that increase in the mean weight losses of wafers treated with ammoniacal copper solutions containing 1%, 5% and 10% ammonium hydroxide were significant at the 0.05 level for *P. placenta* and *G. trabeum*. This confirmed that the weight losses of wafers treated with copper sulphate in higher ammonia concentrations were significantly greater than the corresponding data for wafers treated with solutions containing low ammonia concentrations for these two fungi. No mass loss was observed for any of the ammoniacal copper treated wafers exposed to *T. versicolor*.

Based upon the maximum weight losses achieved, *P. placenta* was able to readily tolerate the high ammonia content in the ammoniacal copper treated blocks, while fungal activity by *T. versicolor* and *G. trabeum* was impaired. This result is somewhat surprising, given the sensitivity of *P. placenta* to ammonia treated wood. It is possible that the ammonia was mostly complexed to the copper with a relatively small amount being complexed to the wood. The leaching of the treated wood would remove any free ammonium salts. It has been well established that *P. placenta* can detoxify copper treated wood by the formation of insoluble copper oxalate which has little effect on the fungal growth (Sutter *et al.*, 1983; Murphy and Levy, 1983). This release of oxalic acid could also react with

ammonia present in the wood, including that complexed with copper. It is worth noting that the color of the wood changed from green to a natural brown color, consistent with a reaction with the copper ammonia complex formed in the wood. This distinct difference in the behavior of *P. placenta* to ammonia and ammoniacal copper treated blocks would suggest that the production of oxalic acid is an inducible reaction, which does not take place in the absence of the copper. Further research is needed to investigate this phenomenon. At the lowest ammoniacal copper treatment the weight loss of the decayed blocks decreased slightly compared to the water control, but at higher ammonia concentrations the weight losses increased. The weight losses for the blocks impregnated with copper in 10% ammonium hydroxide were comparable to the untreated wood. This increase in the fungal activity with increasing nitrogen content in the wood suggests that the form of the nitrogen is utilizable by *P. placenta*. It remains unknown whether the nitrogen being used arises from the copper-ammonia complexes or from ammonia-wood reaction products, such as amides or imines.

The weight losses for the ammoniacal copper treated wafers at the lowest ammonia concentration exposed to *G. trabeum*, were very small. The reason for this is that the copper content of ( $0.6 - 0.8 \text{ kg/m}^3$ ) is close to the threshold for this fungus ( $0.42 \text{ kg/m}^3$ ) reported by Richardson (1991). Nonetheless, as the ammonia concentration of the treating solution increases, the weight losses increase to almost 10%. This suggests that with increased ammonia concentration in the treating solution, the ability of *G. trabeum* to cause decay was enhanced. One probable explanation for this phenomenon, is that *G. trabeum* was able to metabolize the excess nitrogen in the wood. This is consistent with increased decay found in ammonia treated wood. An alternative explanation may be postulated, based upon the hypotheses that during treatment of wood with

ammoniacal copper solutions, several copper complex are formed, some of which contain ammonia. It has been observed that the depletion of copper preservative from ammoniacal copper treated wood can be reduced by increasing the ammonia content of the treating solution (Ruddick, 1992b). It is not unexpected therefore, that the efficacy of the copper preservative will be influenced by the nature of the copper complexes present in the wood. As the ammonia content is increased, the proportion of copper-ammonia complexes formed in wood, versus precipitated copper salts, will be increased. The increase in the formation of less soluble copper-ammonia complexes may allow the *G. trabeum* to tolerate the copper to a greater extent.

Although *T. versicolor* overgrew all test wood samples, no weight losses were recorded for those containing ammoniacal copper. The green color of the wafers was retained. Thus while *T. versicolor* was only partially affected by ammonia treatment of wood, its low tolerance to copper prevented it from utilizing the enhanced nitrogen. The distinctly different response of the white rot fungus *T. versicolor* to ammoniacal copper treated wood, compared to that of the brown rot fungi *G. trabeum* and *P. placenta*, both of which appeared to show enhanced decay with increasing nitrogen content, may be explained in part by their different degradative capacity for lignin. It has been proposed in the previous chapter 4.2.3.2 that the copper-ammonia complexes are formed with lignin. It would therefore not be unexpected that white rot fungi would be affected by such complex formation to a greater extent than brown rot fungi. Further investigations are required to examine this phenomenon.

Table 4-3-2 Copper and nitrogen contents and weight losses of the treated wood samples exposed to *P. placenta*.

	CuO kg/m <sup>3</sup>	Nitrogen %	Weight loss %
Control		0.0423	60.14 (1.19) <sup>a</sup>
0.06%CuO-1%NH <sub>3</sub>	0.7146	0.2373	47.13 (7.18)
0.06%CuO-5%NH <sub>3</sub>	0.7404	0.3298	50.98 (3.33)
0.06%CuO-10%NH <sub>3</sub>	0.7702	0.3401	59.90 (4.31)

<sup>a</sup> Standard deviation for the average weight loss given in parenthesis.

Table 4-3-3 Copper and nitrogen contents and weight losses of the treated wood samples exposed to *G. trabeum*.

	CuO kg/m <sup>3</sup>	Nitrogen %	Weight loss %
Control		0.0429	61.90 (4.5) <sup>a</sup>
0.06%CuO-1%NH <sub>3</sub>	0.7264	0.2460	2.52 (1.09)
0.06%CuO-5%NH <sub>3</sub>	0.7594	0.3261	7.59 (4.0)
0.06%CuO-10%NH <sub>3</sub>	0.7842	0.3386	11.3 (4.51)

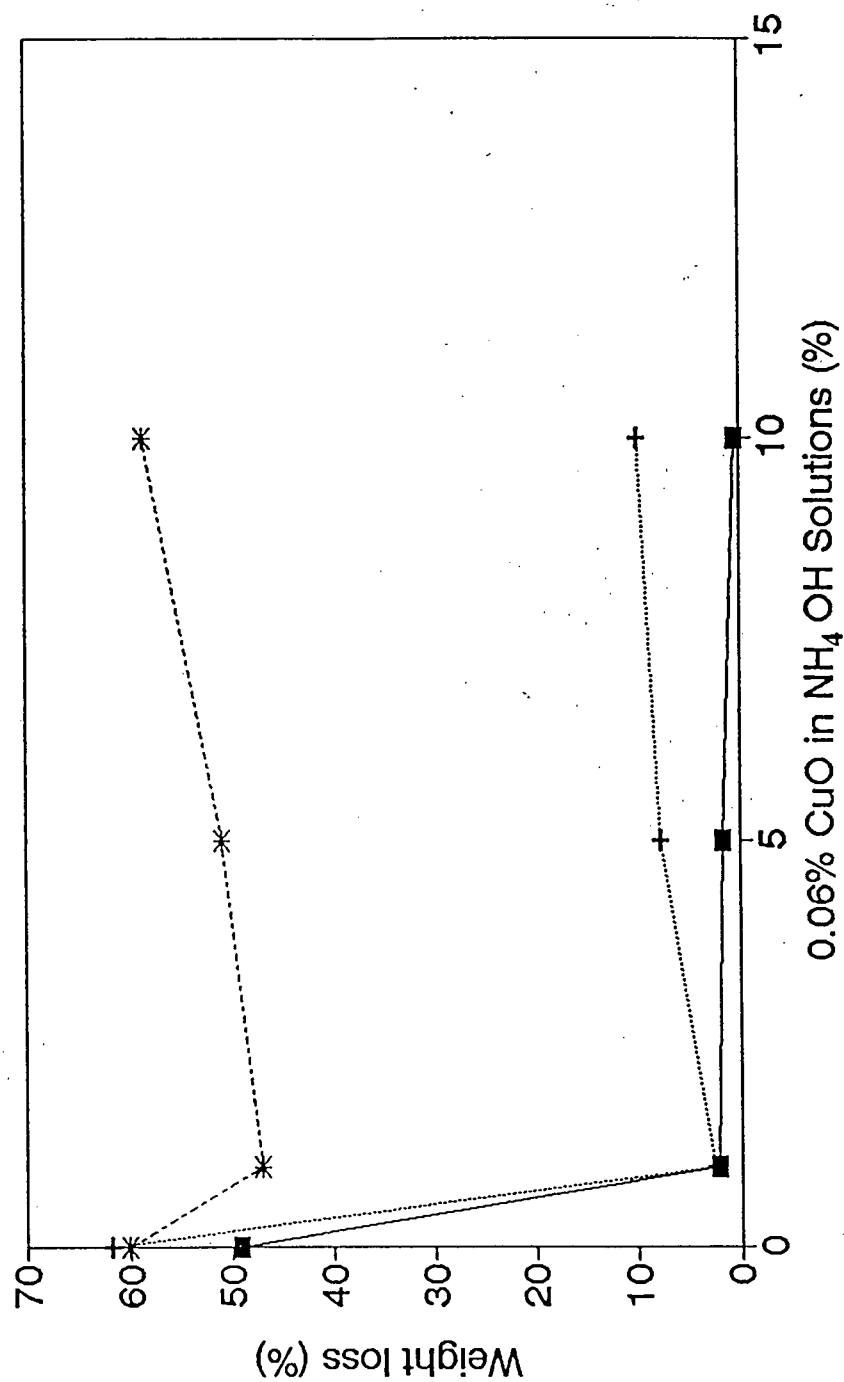
<sup>a</sup> Standard deviation for the average weight loss given in parenthesis.

Table 4-3-4 Copper and nitrogen contents and weight losses of the treated wood samples exposed to *T. versicolor*.

	CuO kg/m <sup>3</sup>	Nitrogen %	Weight loss %
Control		0.0441	49.22 (2.47) <sup>a</sup>
0.06%CuO-1%NH <sub>3</sub>	0.6944	0.2234	2.18 (3.19)
0.06%CuO-5%NH <sub>3</sub>	0.7516	0.3324	1.63 (2.81)
0.06%CuO-10%NH <sub>3</sub>	0.7838	0.3447	0.40 (1.20)

<sup>a</sup>Standard deviation for the average weight loss given in parenthesis.

# Ammoniacal Copper Treated Wood tests



—■— *T. versicolor* .....+..... *G. trabeum* ---\*--- *P. placenta*

Fig. 4-3-2 Weight losses for ammoniacal copper treated wood

## 5. CONCLUSIONS

Based upon all of the observations in this study, the following conclusions may be made.

1. The darkening of Douglas-fir wood after treatment with ammoniacal copper solution results from the formation of a taxifolin-copper-ammonia complex.
2. Lignin and hemicellulose play an important role in nitrogen fixation during the treatment of wood with ammoniacal copper based preservatives.
3. There is evidence to suggest that ammonia reacts with carbonyl and carboxyl acid groups in wood, amide and imine compounds being formed.
4. Vanillin, a lignin model compound, reacted with ammoniacal copper solution, forming a stable, water-insoluble, copper complex. It was suggested that ammoniacal copper ions can react with lignin and form diammine copper lignin complex in the wood.
5. The complex formed from the reaction of vanillin with ammoniacal copper solution was found to contain two ammonia ligands and two copper-oxygen bonds in a plane with two copper-oxygen bonds from the methoxyl group from vanillin in the axial direction.
6. Over the test period used a 5% ammonium hydroxide solution can reduce decay by *P. placenta* to almost zero. Although slightly reduced, *T. versicolor* produced a 22% weight loss in wood treated with 10% ammonium hydroxide solution. However, the ammonium hydroxide treated-wood was likely decayed by *G. trabeum* fungus.
7. Treatment of wood with ammoniacal copper solutions showed that *P. placenta* has the greatest decay capacity due to its copper tolerance, and both *G.*

*trabeum* and *T. versicolor* were unable to cause significant weight losses in wafers treated with ammoniacal copper containing 1% ammonia. As the ammonia content increased, the weight loss caused by *G. trabeum* increased.

## 7. RECOMMENDATIONS

(1) In this study the black color of Douglas-fir wood after treatment with ammoniacal copper solution was identified as resulting from the formation of a taxifolin-copper-ammonia complex. Further work should focus on remedies to this discoloration.

It was reported that chemical and physical measures were taken to remedy the brown stain problem of western hemlock, white pine and Douglas-fir (Hulme and Thomas, 1983; Miller *et al.*, 1983). The cause of the stain in western hemlock was identified to be due to the oxidation and condensation of catechin, a polyphenolic extractive in wood, which has a similar structure to taxifolin. It may be possible to carry out similar experiments with Douglas-fir.

The proposed experiments would be:

a. Examine whether chemical modification of taxifolin could be achieved, which could alter the structure and reduce the reactivity of taxifolin with ammoniacal copper solution.

b. Evaluate physical pretreatment, using steaming at high temperature to reduce the precursor content in wood.

(2) Vanillin was used as a lignin model compound, to examine the reaction of wood with ammoniacal copper solution. The structure of the complex provided useful information on the fixation mechanism of ammoniacal copper based preservatives. In practice, ammoniacal copper preservative formulations are composed of a copper salt and another active biocide such as arsenate in ACA, and quat in ACQ. Their fixation mechanisms are expected to be more complicated.

Further work should study the fixation mechanisms of combinations of ammoniacal copper ions with other active biocides.

(3) Ruddick (1992a) reported that soil-leached CCA-treated mini-stakes were attacked by fungi, causing considerable weight losses. It was explained that this depletion may be linked to bacterial action on the chemical in wood exposed to water logged soil conditions, causing a breakdown of the copper complexes and making them soluble. The higher nitrogen content in the ammoniacal copper-treated wood could provide an abundant nitrogen resource for such bacterial growth. Further work should investigate whether the enhanced nitrogen content in the treated wood could promote decay in ammoniacal copper solution treated wood, exposed to water-logged soil.

(4) It was suggested that treatment with ammoniacal copper preservatives caused the loss of soluble protein nitrogen in wood (King *et al.*, 1974) which may affect fungal colonization on the wood. The effect of ammoniacal copper solution and ammonium hydroxide on the content of soluble protein nitrogen in wood should be investigated.

## 7. LITERATURE

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