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Title: CHEMICAL BROWN STAIN OF DOUGLAS-FIR WOOD

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The cause and the mechanism of chemical brown stain on Douglas-fir wood were investigated. The discoloration of Douglas-fir wood extractives on TLC plates was observed.The extractives from both sapwood and heartwood of 12 individual trees were tested with a TLC assay. The compounds that became colored on the TLC plates were presumed to be the chromophore precursors of Douglas-fir wood discoloration. The precursors were dihydroquercetin, catechin, epicatechin, a catechin polymer and some waxy materials. Auto-discoloration of these compounds may be the cause of discoloration of wood.

The interaction of water and colored material in the wood was studied with simulation models. Fresh cut sapwood and heartwood were compared. Fresh sapwood, with a m.c. < 120%, had a much higher water soluble extractive content than did heartwood, with a m.c. < 37%. The

water insoluble chromophore from sapwood had a much higher water mobility than that from heartwood. The sapwood chromophore, and its precursor, tended to move to the surface as the sap water evaporated.

Stained wood samples were studied vs nonstained wood samples. The UV-Visible absorption curve showed that stained wood extractives had a higher absorbance than did those of nonstained wood in the range of 350-450 nm. Dihydroquercetin, which was shown to form color under mild conditions, disappeared from the stained wood. However, it was a major component in the nonstained wood. Gas chromatography-mass spectroscopy showed that the diethyl ether extract of Douglas-fir wood consisted mainly of terpene acids and fatty acids. On TLC, stained wood extractives were less hydrophilic and more polymerized.

In the process of tracing the chromophores of wood, two lignan compounds were found: 1-(3-methoxy)-phenyl-2-[4-(1-propanol)-3-methoxyphenyl] -propane-1,3-diol(A) and 2,3-dihydro-2-(3'-methoxyphenyl)-3- hydroxymethoxyl-7-methoxy-5-benzofuran propanol (B). Both were characterized with H^1 and C^{13} NMR and MS spectra. Since these lignans were closely associated with the color chromophores, they were thought to be either chromophore precursors or copigments of wood coloration and discoloration.

Chemical Brown Stain of Douglas-fir Wood

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CHEMICAL BROWN STAIN OF DOUGLAS-FIR WOOD

I. INTRODUCTION

– Douglas-fir wood discoloration has long been a problem of quality in the Pacific lumber industry (Boyce, 1930). It was accepted as natural. The investigation of the problem was not initiated until it cost Oregon lumber producers millions of dollars a year in the international market (Miller *et al*, 1983). This chemical stain is an orange to reddish-brown appearance contrasted with the surrounding light yellow colored nonstained sapwood. It is defined as a chemical stain for which non-cellular materials or extraneous materials are considered to be the cause. It does not always appear as a solid color, and is rather nonuniform in distribution. It often continues from end to end of the board but may not cover the entire width.

It is often observed in the sawmill that affected sapwood is even darker than heartwood, although normal heartwood is yellowish-brown colored by nature. This stain is more often observed during storage and kiln-drying during the warmer seasons (Niemiec, 1992). Conditions in the processing of wood that favor keeping the surface moist enhances the discoloration. For example, high relative humidity, slow air circulation, and high drying temperatures enhance discoloration.

Based on observations of the staining phenomena, a postulation of enzymatic oxidation has been suggested (Song, 1986; Musbah, 1992). At the same time, oxidation by oxygen from the air had been observed in the laboratory. It has also been known that accumulation and reaction of small molecules of wood components on the wood surface can influence the appearance of wood (Ellwood, Ecklund, and Zavarin, 1963).

The primary objectives of this study were to determine the cause of Douglas-fir wood staining and how it develops. This may result in a feasible method for the effective prevention of Douglas-fir wood discoloration. In this study, the chemistry of Douglas-fir wood extractives were studied. The light and air susceptibility and the water mobility of the wood extractives were examined. The chemical differences between stained and nonstained wood were compared.

II. BACKGROUND

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) has also been called *Pinus douglasii* (Lindl.) Carr. and *Pinus taxifolia* (Lamb.) Sudw. It is native to-the West coast of North America.

Douglas-fir has two distinct varieties. The coastal variety, *Pseudotsuga menziesii* var. *menziesii*, grows from coastal British Columbia, through Western Washington and Oregon to Northern California. This area has more fertile growing sites than those areas occupied by the Rocky Mountain variety, *Pseudotsuga menziesii* var. *glauca*, which is scattered throughout the Rocky Mountains from British Columbia to Mexico. It also grows in Eastern Washington and Oregon in the more arid areas such as the Wallowa and Blue Mountains. The Rocky Mountain variety will grow on drier sites, has a greater taper to its bole, and does not reach the great sizes of the coastal variety. A good sized Douglas-fir of the *glauca* variety would be 30 to 40 meters tall and 0.75 to 1.0 meters in diameter. Douglas-fir is by far the most important tree in the Pacific Northwest. The tree is also fine enough in both form and color for the Christmas tree trade and as an ornamental, for which purposes it is found planted all over the United States, Europe and Asia.

As a single species, Douglas-fir has been the largest lumber resource in the world. Much of the lumber from this tree is straight

grained, with growth rings delineated by pronounced bands of dark summer wood. The sapwood of a mature Douglas-fir has a white to pale yellow color, with about 20 years of growth rings from the cambium layer into the xylem. The heartwood, with an orange-red to red, sometimes yellow color, occupies the rest of the xylem next to the pith. Both sapwood and heartwood weather to a dark gray color with little or no sheen. The versatile wood of Douglas-fir is used for construction lumber, furniture material, plywood and paper making.

Although chemical discoloration of Douglas-fir wood has long been recognized, it was not systematically investigated until 1963 when Barton and Gardner (1963) reported on the color precursors in Douglas-fir wood. They outlined the isolation and attempted purification of one of the color precursor, a polymeric leucoanthocyanidin from Douglas-fir. However, after this paper no serious investigation was reported over the next twenty years.

Exports of Douglas-fir lumber into the international marketplace resulted in renewed investigations. Discolored lumber was greatly reduced in price in the export trade and interest in the discoloration increased. In 1983, Miller *et al* (1983) reported on their efforts to prevent Douglas-fir sapwood from brown staining. Dipping the wood in solutions of sodium bisulfite and azide had little or no practical effect. Steaming at 100°C was the most effective stain-preventive treatment. However, if the lumber was resawn after steaming, the cross

sections still developed objectionable brown staining during subsequent air-drying. Once the stain had formed it became necessary to use mechanical procedures such as sanding and planing to remove it from the surface.

Attempts to control the discoloration with chemicals resulted in little success. However, Kai and Kawamura (1985) found that semicarbazide was helpful in preventing Douglas-fir wood resin exudation and discoloration caused by light irradiation. It has proven difficult to find a practical chemical since both lumber processing and consumption should be safe, pleasant, and inexpensive.

Song (1988) systematically studied the phenolic precursors and the enzymatic discoloration of Douglas-fir wood. He proved that dihydroquercetin (DHQ) from the wood can develop a brownish color rapidly through enzymatic oxidation. However, dihydroquercetin has been found more in heartwood than in sapwood (Pew, 1948; Gardner and Barton, 1960).

A thorough knowledge of wood chemistry is required to fully understand the discoloration problem. There are a number of compounds in Douglas-fir wood extractives that have been isolated and identified. They are mostly phenols. The phenolic constituents in the sapwood of Douglas-fir wood include catechin, epicatechin, DHQ-3'-O-glucoside, leucocyanidins, and polymeric leucocyanidins

(Hergert, 1960: Song, 1988). The first three are known to polymerize under certain circumstances (Haslam,1989). They have ortho-phenolic groups on the aromatic rings that are known to give brownish colored polymers through ortho quinone formation by autoxidation and polymerization (Hathway, 1955; 1958). Many metal ions can form complexes with them because of their ortho-hydroxyl groups. Most of them have outstanding colors (Slabbert, 1992; Tang *et al*, 1992).

Systematic studies of Douglas-fir bark chemistry in the Forest Products Laboratory at Oregon State University have been very fruitful over the last few years. A number of novel phenolic compounds have been isolated and identified. These include procyanidin dimers, trimers, tetramers and pentamers (Foo and Karchesy, 1990; 1988) and lignans and neolignans (Gonzalez, 1993). However, to date there have been no reports describing lignans from Douglas-fir wood.

There has also been work done on the wood discoloration of numerous other species on the Pacific coast. Most of the work has focused on the extractive chemistry of the wood. Because each wood species has its own extractive composition, the cause and the mechanism of discoloration may not be the same.

Color is a sensation of the human eyes toward certain compositions of visible light. Different materials react to light differently, so we see them as different colors. The manner by which

certain materials absorb light can be described by an absorption curve. This is a record of absorption intensity while scanning different materials with radiations of different wavelengths. A peak is called an absorption band. The wavelength of the maximum absorption band is decided by the molecular structure of the pigment, which is also called the chromophore.

From what we observe, the staining of Douglas-fir wood is a continuous process. A complexity and diversity of pigments produced is expected. It should be noted that observed color changes to the human eyes do not necessarily mean chemical changes of the pigments. A color change can be observed when there is more than one absorption band for the pigments. This can cause a switch of the predominant hue. In different solvents, a different color is often observed with one chromogen. When the band is half in the visible region and half in the UV region, we see a concentration dependent color.

Regardless of physical color changes or chemical color changes, a simple way to measure color was needed, so the CIE system was developed (Commission Internationale de L'Eclairage, 1930's). There are three essential parameters describing color: Hue, Saturation, and Lightness. These give a precise description of color. The CIE system is widely used to evaluate color in many industrial practices involving color technology. However, for the study of Douglas-fir wood

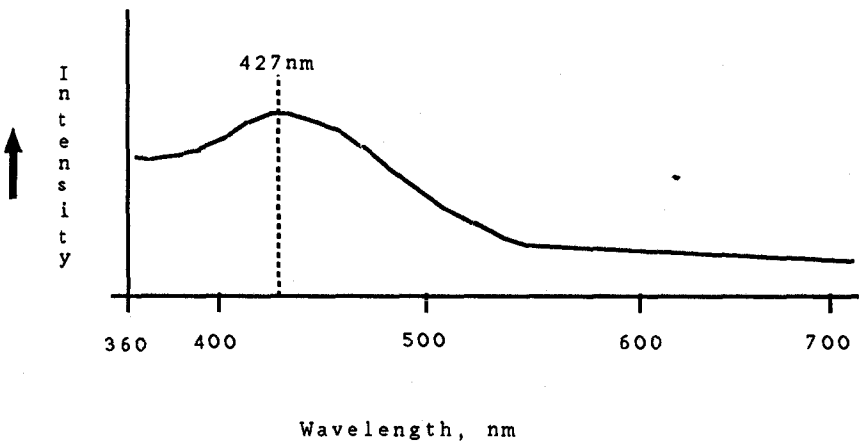
method. The UV-Visible spectral method gives direct information between color and structure. Figure 1 represents the absorption curve of a yellow dye in paper, in which the maximum absorptance is at 427 nm.

Color is believed to be caused by the energy release of an excited molecule when electrons migrate to a lower energy state. The molecular orbital theory states that electrons in the molecule occupy different orbits that have different energy levels. Electrons tend to stay at the lowest energy level available. This is called the ground state. When electrons obtain energy, ΔE, the ones in the occupied orbit will be excited, jumping up to a higher energy, but empty, orbit. The molecule is then in the excited state.

$$\Delta E = E_{\text{excited state}} - E_{\text{ground state}}$$

The excited state is not stable, and tends to release transition energy, E, in about 10^{-5} seconds and return to the ground state. If this energy is within the visible region, the human eye will sense it. We see a material as a certain color. Visible light is lower energy light and occurs only to those molecules with a large, conjugated system.

Figure 1. Absorption spectrum of yellow dye in paper. The pigment absorbs violet-blue colored light. The yellow light left out is not absorbed, so we see it.

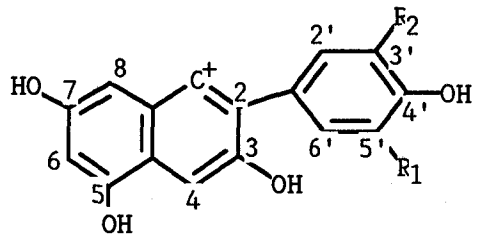


Douglas-fir sapwood is very light colored. There are a few possible chromophores in Douglas-fir wood. Quercetin, with a bright yellow color, is believed to be one of the oxidation products of dihydroquercetin. The rest of the chemicals, such as dihydroquercetin, catechin, epicatechin, etc., only show color under certain circumstances. The other chromophore precursor is a leucoanthocyanidin polymer that was reported by Barton and Gardner (1963) as a purple-colored material. Its complicated structure makes it very difficult to characterize except by its IR and UV-Visible spectra. These compounds belong to the flavonoid family. Some characteristics of flavonoids, that are interestingly related to coloration and discoloration, are reviewed.

Catechin and epicatechin previously mentioned are anthocyanidins. The anthocyanidins are phenolic substances belonging to the general group of C6-C3-C6 secondary plant metabolites known as flavonoids. In living cells they often are bonded to monosaccharrides, such as glucose, galactose,arabinose and rhamnose. They are responsible for most of the blue and red colors of leaves, flowers and fruits of plants. In high acidic media, their basic structure is a flavylium cation. The basic structure of a flavylium cation of anthocyanidin is shown in Figure 2.

One of the most remarkable reactions of anthocyanidins is their reaction with the protons of water for which they are used as classic

Figure 2. Structures of some typical anthocyanidins.



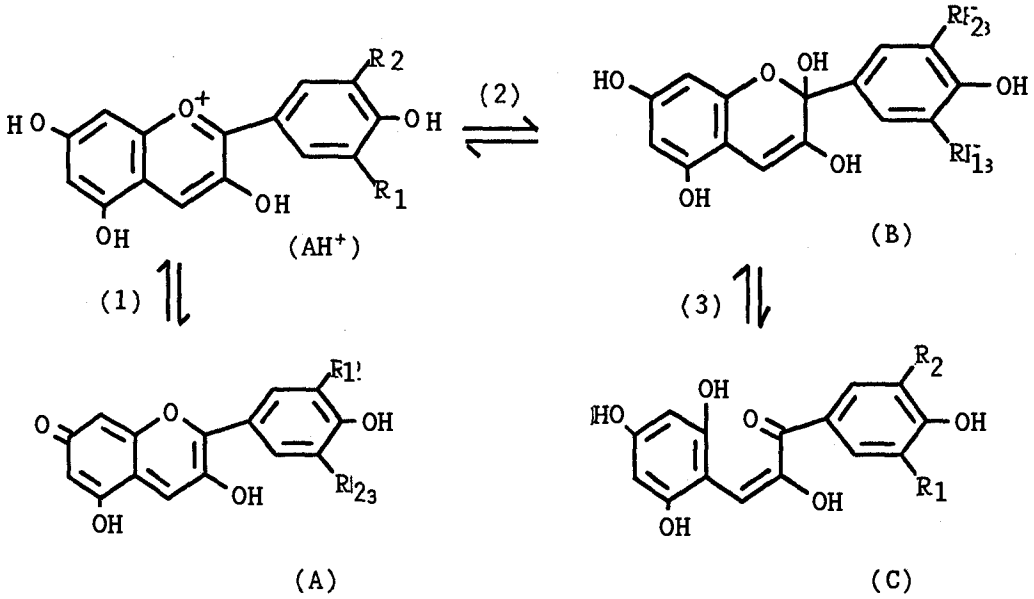
Anthocyanidin	R_1	R_2
Delphinidin	OH	OH
Cyanidin	OH	H
Petunidin	OCH_3	OH
Pelargonidin	H	H
Peonidin	OCH_3	H
Malvinidin	OCH_3	OCH_3

pH indicators. In the low pH range, water solutions of these substances are red. As the pH is raised, to 4-4.5, the color disappears. Further increasing the pH produces a purple solution which becomes blue at pH 7-8. If the pH is further raised, the solution becomes yellow. It has been shown (Brouillard, 1982) that in an acidic solution there are four anthocyanidin species (Figure 3), a quinonoidal base(A), the flavylium cation (AH+), the pseudobase or carbinol (B), and the chalcone (C).

Each of these different structures have very different spectral characteristics, and are consequently perceived as having different visual colors. At different pH levels, different structures are predominant. In highly acidic solutions, the flavylium cation (AH+) occurs and gives the colorless pseudobase (B). The further open chain reaction of (B) forms the chalcone (C), which has a yellow color. The pH not only affects the form of the anthocyanidin, but also their stability. They are fairly stable in acidic media, but destruction takes place rapidly in alkaline media (Brouillard, 1982).

Considering the vast variety of colors this group of compounds gives to the plant kingdom, the explanation of different colors due to pH change is not sufficient. Anthocyanidins give color to plant tissues only in the pH 4-6 range. Therefore, copigmentation has been suggested as a possible mechanism (Osawa, 1982), which is supported by

Figure 3. Equilibria of anthocyanidin in water solution.



the change of color to a higher wavelength and an increase in absorbance and stability. A copigment does not contribute to the visible color except by forming complexes with other compounds. The more significant ones are other phenolic compounds such as vanillin, ethyl gallate (Osawa, 1982), and the anthocyanidins themselves. Anthocyanidin molecules can form complexes with themselves which is called self-association. This is an explanation for the phenomenon that pure anthocyanidins do not follow Beers law (Timberlake and Bridle, 1983). With regard to the flavonoid system we are studying, it is an equilibrium of a series of different structures of different compounds.

In summary, wood extractives can produce or change color by accumulating to a certain concentration, by oxidizing to quinones, by changing pHs, or by the existence of other organic or inorganic substances. These aspects of the causes and the the formation of chemical brown stains in Douglas-fir wood were investigated in the present work.

III. EXPERIMENTAL

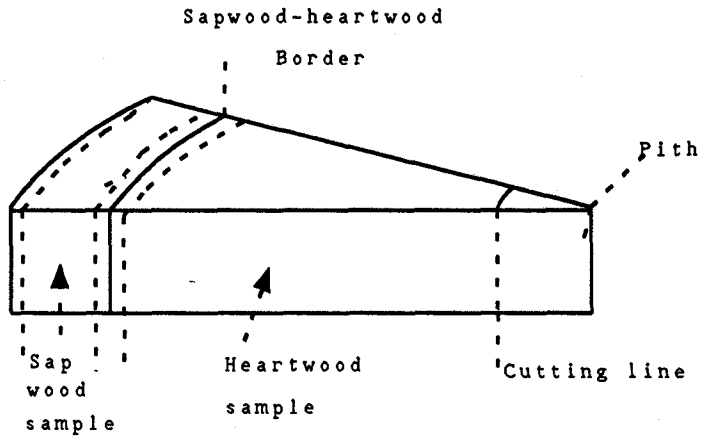
A. Collection of Wood Samples

In November 1991, twelve *ca* 120 year old Douglas-fir trees in Peavy Arboretum, Oregon State University were cut approximately 10 m above the ground. Three days later, a three inch thick cross section was cut from the top stem of each tree that had fallen to the ground. The cross sections were debarked, and placed in a plastic bag. They were stored in a cold room at -10°C.

B. Sample Preparation

A wedge-shaped piece of wood was cut from each wood block described above. Sapwood and heartwood samples were separated by cutting and discarding two years of growth rings from the outermost layer and another two years of growth rings from each side of the colored heartwood-sapwood boundary. The first four to six years of growth rings next to the pith were also removed. These sample cuts are illustrated in Figure 4. The sapwood thus obtained represented about twenty years of growth rings and the heartwood represented about thirty years of growth rings.

Figure 4. Separation of sapwood and heartwood.



Each sample of sapwood and heartwood was cut into small pieces and ground in a Thomas-Wiley lab mill to pass through the 40 mesh screen of the mill.

C. Methanol Extraction

An amount of 5 g of wood powder from each sample prepared above was treated with 10 ml of methanol in a 25 ml capped bottle at room temperature for 75 hrs. The mixture was filtered and the wood residue was rinsed with another 10 ml of methanol. The methanol extracts were combined and diluted to 25 ml in a volumetric flask by the addition of methanol.

D. Thin Layer Chromatography Systems

Two thin layer chromatography (TLC) systems were used. System 1 involved the use of precoated silica gel 60 F-254 (0.25 mm layer thickness) glass plates developed with benzene-acetone-methanol (6:3:1, v/v) (BAM) solvent. The position of the compounds were often indicated by short wave (254 nm) UV light. The developed plates were often sprayed with a solution of concentrated sulfuric acid - 37% formaldehyde (40:1, v/v) followed by heating (100-110^{0}C) to indicate the position of the compounds.

System 2 involved the use of precoated microcrystalline cellulose (0.10 mm layer thickness) glass plates. The plates were used for two-dimensional TLC. They were developed first with t-butanol-acetic acid-water (3:1:1 v/v) (BAW), air-dried, rotated 90° and developed in the second direction with 6% acetic acid. The developed plates were sprayed with vanillin-hydrochloric acid reagent (1 g vanillin dissolved in 50 ml absolute ethanol and 10 ml concentrated hydrochloric acid) followed by heating to 110°C to indicate the position of the compounds.

E. Light and Air Susceptibility Tests

Methanolic extracts from both sapwood and heartwood (section III.D) were applied to TLC plates. Four silica gel plates were loaded with several spots of sapwood and heartwood extracts plus known dihydroquercetin, catechin and epicatechin and developed with BAM (TLC system 1). Four cellulose plates were also loaded with several spots of sapwood and heartwood extracts plus known dihydroquercetin, catechin and epicatechin and developed with BAW (TLC system 2) in one direction only. These eight plates were air-dried and segregated into four pairs of plates such that each pair contained a silica gel plate loaded with spots of sapwood and heartwood extract, and a cellulose plate loaded with spots of sapwood and heartwood extract. Each pair was designated to one of the following treatments at room temperature:

Pair 1: The plates were maintained under laboratory conditions of light, air, and dust.

Pair 2: The plates were wrapped in dark paper and covered loosely with aluminum foil then placed in a lab drawer to avoid exposure to light.

Pair 3: The plates were placed in a vacuum dessicator. Air in the dessicator was removed and replaced with helium. This was repeated 9 more times to remove the residual oxygen from the atmosphere. The plates were maintained in the light.

Pair 4: The plates were wrapped with dark paper and aluminum foil. The dessicator was filled with helium as for pair 3.

All the plates with samples were subjected to a visual color check after 32 weeks.

F. Water Mobility of Douglas-fir Wood Chromophores

1. Sample Preparation and Obtaining Extractives

Additional wedge-shaped pieces of wood were cut from the same wood cross sections as described in section III.A. Sapwood and heartwood samples were separated (Figure 4) and ground to a powder as described in section III.B. The wood powders were immersed in methanol in capped bottles for three days at room temperature. The solvent was exchanged twice in the next six days. All of the methanol extracts from the same sample were combined and the methanol removed on a

rotary evaporator. A small amount of water was added to the solid residues and the resulting slurries were freeze-dried, leaving fluffy powders of sapwood methanolic extractives and of heartwood methanolic extractives.

The freeze-dried solids were weighed and partitioned (Michikazu, 1989) between the solvents ether, ethyl acetate, water and methanol as shown in Figures 5 and 6. Each solvent was evaporated and the solid residues weighed.

2. Measurement of Moisture Content of Wood Samples

Slices of solid sapwood and heartwood (4-10 g) were cut from sapwood and heartwood samples prepared as in Figure 4. They were stored at -10°C for 30 days. The moisture content (m.c.) of each sample was measured by drying in an oven at 60°C. The samples were weighed every 8 hrs until there was no further weight loss (24 hrs total). The moisture contents were calculated by use of the formula:

% m.c. = [(Weight of fresh wood - Weight of oven dried wood)/ Weight of oven dried wood] x 100

Figure 5. Partition of the sapwood extractives with solvents.

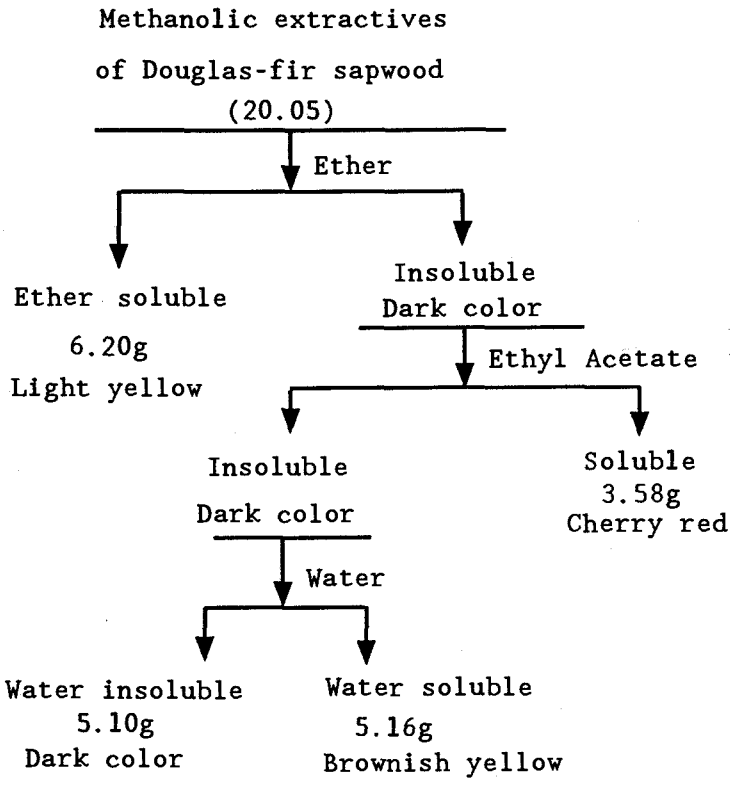
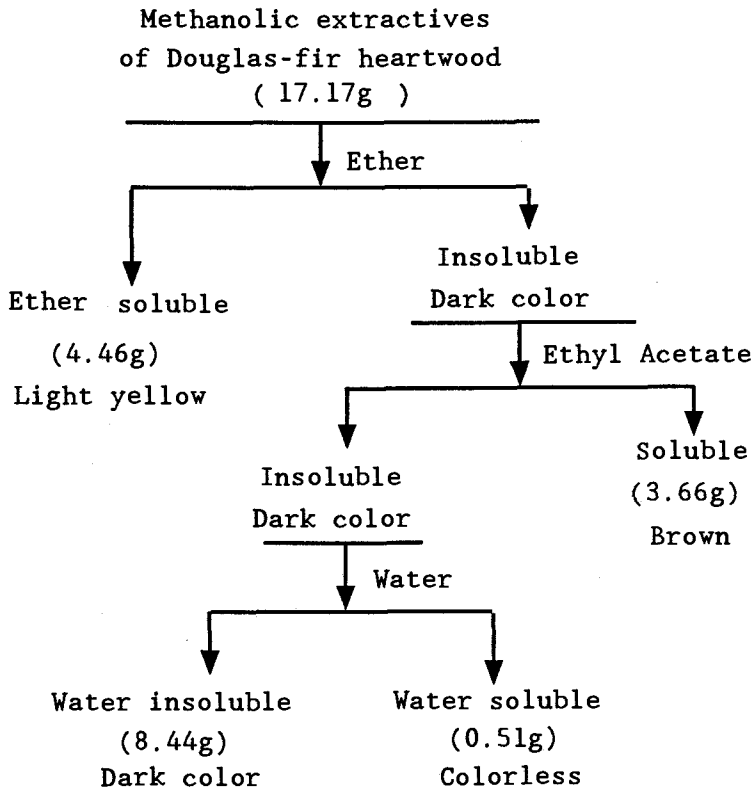


Figure 6. Partition of the heartwood extractives with solvents.



3. Monitoring Water Movement in Wood with Water-Born Blue-Colored Ink

A block of sapwood from the cross sections described in section III.A was sliced into three sections: 0.5 cm, 0.7 cm, and 6.0 cm in length. A number "5" was written on the radial and transverse faces of each piece with a blue ink pen. The block was reassembled and placed in a cardboard box in a refrigerator at 3-4^{0}C for four weeks. The pattern of the number was checked every two weeks. At the end of the four weeks, the wood was cut open to check the ink trail.

A block of heartwood from the cross section described in section III.A. was treated in a similar way at the same time.

4. Observation of the Water Mobility of the Chromogen from Douglas-fir Wood on a Sephadex LH-20 Column

An amount of 3.4 g of the ethyl acetate soluble material obtained from sapwood (Figure 5) was dissolved in a minimum amount of MeOH/H_2O (5:1 v/v). The Sephadex LH-20 column was prepared by saturating the LH-20 with H_2O for 24 hrs in a beaker. The beaker was placed in a vacuum dessicator and the dissolved air was removed by applying vacuum. The LH-20 slurry was packed into a 2.5 x 60 cm glass

column. The column was loaded with the extractive sample described above. The column was eluted with H_2O and the movement of the chromogen was observed. It was then eluted with MeOH/H_2O (1:10 v/v), followed by MeOH/H_2O (1:1 v/v) and MeOH/H_2O (9:1,v/v) and finally with 100% MeOH.

The procedure was repeated respectively with the ethyl acetate solubles of heartwood, the methanol soluble fraction of tree fresh sapwood and the tree fresh heartwood extractives obtained in Figure 6.

G. Study of Stained Wood

1. Extractives

Stained and nonstained lumber sapwood samples were supplied by the D.C. Johnson Lumber Co., Riddle, Oregon. They were kept at -10°C after arrival at the laboratory. The stained and nonstained lumber sapwood samples were visually identified and the stained and nonstained portions were separated carefully by cutting off the interspace wood. Stained and nonstained lumber samples were taken from the same piece of lumber for direct comparison.

They were put into paper bags and placed in a laboratory drawer at room temperature for one week and then ground with a Thomas-Wiley laboratory mill to smaller than 40 mesh. This provided two powder

samples from sawn lumber that were labeled as lumber nonstained sapwood and lumber stained sapwood.

Additional wedge-shaped pieces of wood were cut from the cross sections taken from Peavy Arboretum as described in section III.A. Sapwood and heartwood samples were separated (Figure 4) and ground to a powder as described in section III.B. This provided two powder samples from fresh tree wood that had never stained. They were labeled as tree nonstained sapwood and tree heartwood.

An amount of 5 g of each sample was weighed into 25 ml capped glass jars, and 15 ml of solvent was added. The samples were allowed to stand at room temperature for three days in a hood. Fresh solvents were used to replace the extractives twice in the next six days. All the extractives from the same sample in the same solvent were combined. Evaporating dishes containing the extractive solutions were left in the hood.

The solvents used were methanol, diethyl ether, and benzene. New wood samples were used with each solvent. After evaporation, the residues were weighed to constant weight (overnight evaporation followed by one day). The materials obtained were representative of nonvolatile extractives.

2. Comparison of the Extractives by UV-Visible Spectroscopy

Extractives obtained in section III.G.1 each were made up to 50 ml in volumetric flasks with their corresponding solvents. This provided a starting concentration (C_0). An amount of 10 ml of C_0 was diluted to 25 ml (C_1). An amount of 5 ml of C_1 was diluted to 25 ml (C_2).

A Shimazu-265 UV-Visible recording double beam spectrophotometer was used with a 1 cm light path quartz cuvette to obtain UV-Visible spectra. Each sample was scanned between 190-700 nm with the solvent in the reference path.

3. TLC Study of Extractives

TLC of the methanolic extractives described above (section III.G.1) were obtained. The extractives from lumber nonstained sapwood and lumber stained sapwood were applied side by side on the TLC plates along with the samples from fresh tree nonstained sapwood and tree heartwood extractives. Authentic quercetin and dihydroquercetin were also applied to the plates. The TLC systems used were as described in section III.D. The chemical components were detected with room light and short wave (254 nm) UV-light.

4. GC-MS Study of the Diethyl Ether Extracts

Diazomethane was prepared by the method described in Organic Synthesis (de Beer and Backer,1963). N-methyl-N-nitroso-p-toluene sulfonamide (Diazald from Aldrich Chemical Company, Inc.) was reacted with 40% aqueous potassium hydroxide. The product was absorbed in the upper layer (diethyl ether layer) in the round bottom three-knecked flask. The flask was slightly heated with a warm water bath to accelerate the reaction rate and distill over the diazomethane formed along with the diethyl ether to separate it from the raw material residue and the by-products. Distillation was continued until the diethyl ether solution became light yellow. Diazomethane should be prepared fresh before use.

The diethyl ether extracts of wood prepared in section III.G.1 were dissolved in 2.0 ml of diethyl ether. A 1.0 ml aliquot of this solution was transferred into a 5 ml beaker. A 1.0 ml aliquot of the diazomethane solution prepared above was added with a pipette. The reaction was allowed to proceed at room temperature in a hood. If the bright color of diazomethane disappeared immediately, some more solution was added until the bright yellow color remained.

The reaction proceeded at room temperature under a hood until most of the diethyl ether and all of the extra diazomethane had

evaporated. The reaction products were transferred to a small GC-sample bottle, sealed and stored in the refrigerator.

The GC-MS system used was a Finnigan 4000 with a 4500 ion source, equipped with a 10 m x 0.25 mm SE-54 column. The GC conditions consisted of temperature programming from 50°C and rising at 5°/min to 180°C, and then 20°/min to 300°C.

The methylated extractives of Douglas-fir wood were used directly for GC-MS analysis. The mass spectra of individual peaks were compared to mass spectra in the Galaxy 2000 database. In this way significant components were identified.

H. Separation and Identification of Lignans from Douglas-fir Sapwood Extractives

The ethyl acetate soluble fraction (3.4 g) of Douglas-fir sapwood extractives from section III.F (Figure 5) was applied onto a Sephadex LH-20 column (2.5 x 60 cm) and eluted with water. The separation was monitored with TLC and with observation under UV radiation and normal room light. A cherry red syrup was obtained (0.81 g) after 500 ml of water was used. The TLC plates used were silica gel 60 F-254, 0.25 mm. They were developed with a solvent system of benzene-acetone-methanol (6:3:1,v/v) and sprayed with H_2SO_4-HCHO (40:1,v/v), which was followed by heating at 100°C for 2-5 min. The

cherry red colored fraction (0.81 g) from the Sephadex LH-20 column was rechromatographed using 90% ethanol-water elution on a 5 x 30 cm column packed with fractogel TSK HW-40F. The colored band changed from cherry-red to orange while it was moving through the column. A fraction of a lignan mixture (0.32 g) was detected with TLC and NMR. Further rechromatographic separation of this mixture (30 mg) was carried out on a 1.0 x 30 cm Sephadex LH-20 column with ethanol-water (1:1, v/v). Two main fractions, A 12 mg and B 8 mg were obtained after freeze drying. They both presented a light yellow color in methanol solution. Fractions A and B were characterized by mass spectra, H^1-NMR and C^{13}-NMR in MeOH-d_4. The spectral data are as follows:

Fraction A: Light yellow powder, MW.378, $C_{20}H_{26}O_7$. Negative FAB-MS in DEA showed peaks $(M-H)^-$ at m/z 377 (40%).

H^1-NMR in MeOH-d_4: 6.4-7.1(m, 12H), 4.86(d, 1H, J=5.88 Hz), 4.85(s, 1H), 4.31(dt, J=5.3, 4.0 Hz), 4.24(dt,J=5.5,4.4 Hz), 3.84(s, 3H, $-OCH_3$), 3.81(s, 3H, $-OCH_3$), 3.79(s, 3H, $-OCH_3$), 3.77(s, 3H, $-OCH_3$), 3.7-4.0(m, 2H),3.42-3.47(m, 1H), 3.54(m, 4H), 2.60(m, J=6.72,9.16 Hz, 4H), 1.79(m, J=6.40,8.64 Hz, 4H).

C^{13}-NMR in MeOH-d_4: 151.9, 148.7, 147.6, 147.2, 138.2, 134.2, 122.0, 151.7, 148.8, 147.2, 147.0, 138.1, 133.8, 121.9, 121.0, 120.8, 119.6,119.6, 115.9, 115.7, 114.1, 114.0, 111.9, 111.8, 87.7, 74.2, 86.6, 74.2, 62.2, 61.9, 56.8, 56.5, 62.2, 62.2, 56.4, 56.4.

Fraction B: Light yellow powder, MW.360, $C_{20}H_{24}O_6$. MS displays the peaks $(M+1)^+$ at m/z 361 (34%), and $(M-OH)^+$ at 343 (18%).

H^1-NMR in MeOH-d_4: 6.95(d, J=1.88 Hz, 1H), 6.81(dd, J=1.96, 8.12 Hz, 1H), 6.75(d, J=8.12 Hz, 1H), 6.72(S, 2H), 5.48(d, J=6.16 Hz, 1H), 3.84(S, 3H,-OCH_3), 3.81 (S, 3H, -OCH_3), 3.59(dd, J=7.2,4.0 Hz, 1H), 3.56-3.86 (m, 1H), 3.56(t, J=6.36, 2H), 3.46(dd, J=6.40, 6.16 Hz), 2.62(m, 2H), 1.81 (t, J=7.2 Hz, 2H).

C^{13}-NMR in MeOH-d_4: 149.1, 147.5, 147.5, 145.2, 136.9, 134.8, 130.0, 119.7, 118.0, 116.2, 114.2, 110.6, 89.0, 65.0, 62,3, 56.8, 56.3, 55.4, 35.8, 32.9.

IV. Results and Analysis

A. Collection of Samples

The samples of wood collected from Peavy Arboretum represented typical Douglas-fir fresh wood specimens. It was important that they contained well defined areas of sapwood and heartwood. They were stored in a cold room to ensure that they remained fresh and did not dry out.

B. Sample Preparation

Sapwood and heartwood samples were obtained from the same cross section of wood in order to compare their respective properties. Considerable care was taken to ensure that no bark or pith contaminated the samples and that the sapwood and heartwood were well separated from each other.

C. Methanol Extraction

Methanol was used as the initial and primary extraction solvent because preliminary experiments showed that most of the colored extractives of interest were removed by methanol.

D. Thin Layer Chromatography Systems

Silica gel TLC plates developed with BAM and cellulose plates developed with BAW and 6% acetic acid were chosen as representative separation systems for wood and bark extractives. The coatings were quite different and the solvents provided a range of separation parameters.

E. Light and Air Susceptibility Tests

After exposure for 32 weeks to the different environments outlined in section III.E, the TLC plates were visually examined.

Pair 1: The developed TLC plates applied with wood extractives, catechin, epicatechin, and dihydroquercetin, had been exposed to normal laboratory conditions for 32 weeks. It was observed that the loading point of tree sapwood #3, #4 and #9 and the solvent frontier (A-direction) of each sample of wood methanolic extractives became yellow colored. All the heartwood samples of methanolic extractives developed a greenish yellow color at the position in which the R_f value is the same with dihydroquercetin. Catechin gave out a brownish yellow color and epicatechin produced an orange red color.

Pair 2: The TLC plates applied with wood extractives and reference known compounds were wrapped with dark paper and aluminum

foil after developed in solvent. Light had been avoided but the plates were exposed to laboratory air for 32 weeks before visually checked for color development. The solvent frontier of the wood extractive samples became slightly yellow. Dihydroquercetin in both heartwood extractives and reference sample gave a light yellow color. Colors developed on catechin and epicatechin were the same as with pair 1.

Pair 3: The TLC plates with developed reference and wood extractive samples were placed in a vacuum dessicator in which air was replaced by helium. They were exposed to laboratory light for 32 weeks before visual examination for color development. A yellowish red color was observed with dihydroquercetin in both reference and heartwood extractive samples on the plates. Catechin and epicatechin in the reference sample developed a purplish pink color. No other significant color was observed.

Pair 4: The TLC plates with developed reference and wood extractive samples were covered with dark paper and then aluminum foil. They were placed into a vacuum dessicator in which air was replaced by helium. Thirty-two weeks later, color on the plates was examined visually. It was found that dihydroquercetin in both the reference and wood extractive samples became reddish yellow, and brownish yellow. Catechin and epicatechin gave a pink color.

The colors formed from the discoloration of DHQ are outlined in Table 1.

F. Water Mobility of Douglas-fir Wood Chromophores

1. Sample Preparation and Obtaining Extractives

According to the solubility of the material in the solvent, the methanolic extractives of both sapwood and heartwood were separated into the following groups: ether soluble, ethyl acetate soluble, water soluble, and methanol soluble (Figures 5 and 6). The soluble composition of the methanolic extractives is listed in Table 2.

The two major differences between heartwood and sapwood extractives should be noticed. Sapwood water solubles are as much as eight times greater than heartwood water solubles and methanol solubles from heartwood are twice as great as from sapwood. This could mean that the extractives of sapwood are simpler, smaller molecules carrying more hydrophilic functional groups compared to those in the heartwood. The heartwood extractives may have been oxidized and polymerized into larger molecules or lost hydrophilic groups by any possible reaction and hence became less water soluble. The darker color of the heartwood indicates chemical change from light-colored sapwood when heartwood was formed.

Table 1. Colors formed from the discoloration of DHQ on TLC plates.

Group	Condition		Color formed
	Light	Oxygen	
1	Yes	Yes	Greenish yellow
2	No	Yes	Light yellow
3	Yes	No	Yellowish red
4	No	No	Reddish yellow

Table 2. Fractions of different solubilities of the methanolic extractives.

Samples	Total (g)	Ether (%)	Ethyl acetate (%)	Water (%)	Methanol (%)
Sapwood	20.04	30.9	17.9	25.7	25.4
Heartwood	17.17	26.0	21.3	2.97	49.2

The high water-soluble extractives content of the sapwood indicates that potential chromophoric material is easier to move in the sapwood. It is possible that in the sapwood the extractives came to the surface with the sap water and when the water evaporates the extractives accumulate in the surface with potential stain formation. Thus rinsing or extracting sapwood with water might remove the precursors before stain formation and hence reduce staining. This has proven effective in preventing Paulounia wood from discoloration in both China and Japan (Makino, 1980).

2. Measurement of Moisture Content of Wood Samples

The moisture contents obtained for the wood samples, which were stored at 10°C for 30 days, are listed in Table 3.

The average m.c. of sapwood was 88.01%; of heartwood, 26.31%. It is clear that there is a much higher amount of free water in sapwood than in heartwood. This free water in the sapwood may result in movement of the extractives and accumulation of the extractives into localized areas so that there is greater potential for color formation.

Table 3. The moisture contents of wood samples.

Sample	1	2	4	7	8	11
Sapwood	119.92	87.24	86.94	64.16	86.16	83.61
Heartwood	30.01	26.80	30.27	19.30	24.10	27.41

3. Monitoring Water Movement in Wood with Water-Born Blue-Colored Ink

As described in section III.F.3, the movement of water in wood was monitored with water-born blue ink. Sapwood has a much higher m.c.than heartwood. In sapwood it was not surprising to find that water molecules carried the ink on the cross section all through the wood blocks despite the two cross cutting surfaces, although the pattern was not maintained well. After cutting the wood longitudinally in the ink marked area, we find that ink was left in the cells all along the way. The pattern of the ink on the redial faces became blurry, but it did not penetrate much. The ink on the heartwood surface was slightly spread out.

These results indicate that free water in the wood can carry soluble chromophores into the wood from the surface. Therefore it is possible that if the pigment is formed on the surface the moisture can spread it along the wood. If the chromophore comes from the inside of the wood cells, the water should accumulate more chromophore around the water evaporation area. Sapwood has a higher water soluble chromophore content and it is possible that these are brought to the surface, darkening the wood color as the water molecules leave the surface. Conditions, such as temperature, pressure, humidity, moisture content, etc. that influence evaporation and other molecular movement

should influence the distribution of the chromophore, i.e. the discoloration.

4. Observation of the Water Mobility of the Chromogen from Douglas-fir Wood on a Sephadex LH-20 Column

As described in section III.F.4, the ethyl acetate solubles of both sapwood and heartwood were loaded on two water saturated LH-20 columns respectively. The columns were eluted with distilled de-ionized water. We found that sapwood chromophores and heartwood chromophores have different water mobilities. In sapwood, most of the colored material moved out of the column with the water. Actually, a cherry red pigment moved out of the column when 500 ml of water was used. See Figure 7 for the detailed results. The chemical composition of each group was kept for further study.

In the case of the heartwood extractives, no migration of chromophore was observed during elution with water. The coloring materials only moved when the column was eluted with methanol. The detailed results are shown in Figure 8.

The yellow chromophore shown in Figure 8 as Fraction G and H was identified by TLC. A 2" X 4" silica gel plate was developed with AcOEt/ $CHCl_3$/ 88% HCOOH (5:4:1 v/v). The yellow color was due to the presence of quercetin and an unidentified polymer. The main component

Figure 7. Mobility of sapwood chromophores
on a Sephadex LH-20 column.

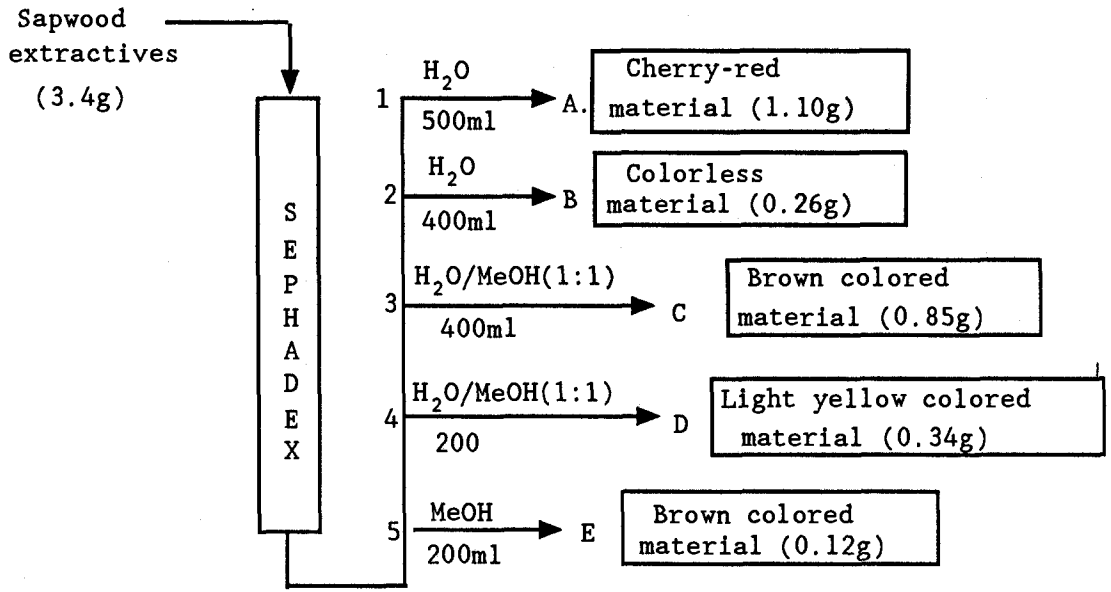
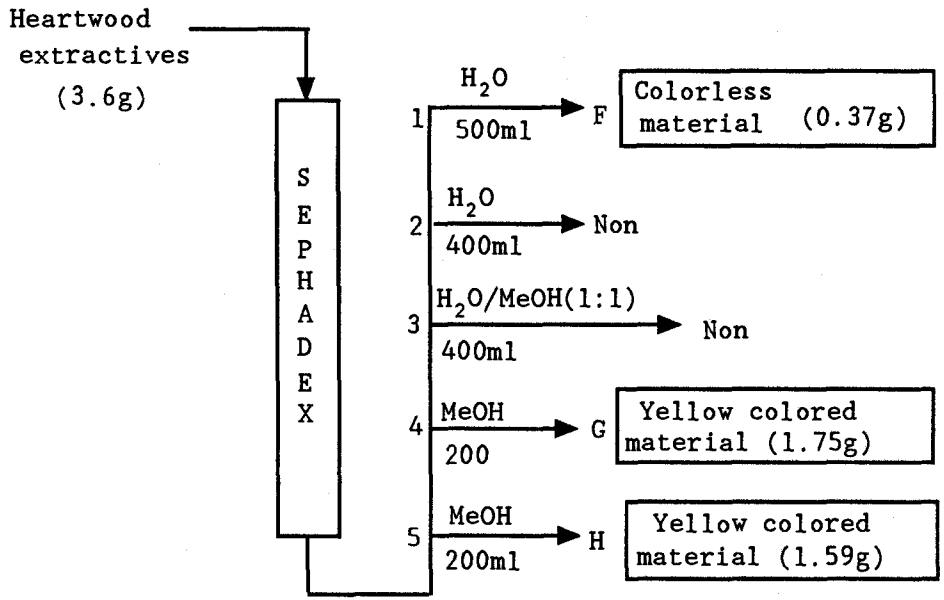


Figure 8. Mobility of heartwood chromophores on a Sephadex LH-20 column.



of both Fraction G and H (Figure 8) was dihydroquercetin. It is believed that quercetin is the result of the oxidation of dihydroquercetin. See the next section for the detailed discussion on the isolation and identification of these components.

The water mobility of the methanol soluble chromophores was similar to the ethyl acetate solubles. Sapwood chromophores moved with water and heartwood chromophores moved with methanol on the column.

G. Study of Stained Wood

1. Extractives

The extractives obtained in section III.G.1 from lumber nonstained and stained sapwood, and from fresh tree nonstained sapwood, and heartwood samples were weighed. The ratios of the amount of extractives to air-dry wood residues are listed in Table 4 in percentages (%).

It is apparent from Table 4 that methanol dissolved the most extractives out of the wood. Except for the unusual value for the benzene extractives from the lumber nonstained sapwood sample, the weight of extractives from the lumber stained sapwood sample is similar to that from tree fresh heartwood, and the extractives from

Table 4. The extractives of different wood samples.

Solvent / Sample	Methanol	Benzene	Ether
Tree fresh sapwood	2.95	0.96	1.40
Tree fresh heartwood	1.40	0.29	0.30
Lumber stained sapwood	1.10	0.71	0.54
Lumber nonstained sapwood	2.75	2.87	1.26

tree nonstained sapwood are similar to those from lumber nonstained sapwood.

Nonstained sapwood extractives, both from trees and lumber, are higher than either those from the tree heartwood or those from lumber stained sapwood samples. Thus, when sapwood becomes either heartwood or stained wood, some chemical change happens to the extractives and they became less soluble. The chemistry of the extractives will be further studied quantitatively and qualitatively.

2. Comparison of the Extractives by UV-Visible Spectroscopy

As observed, the extractives do take their color after their wood types. The extractives from the darker colored wood have darker colors. Heartwood extractives appear darker than sapwood extractives. Stained wood extractives have a darker color than nonstained wood extractives. This is especially true with the methanol extractives. These observations were supported by the UV-visible spectra of these extractives as shown in Figures 9, 10, and 11. Compared to nonstained wood extractives, stained wood methanolic extractives have a higher absorbance in the 350 nm to 450 nm range. Tree fresh heartwood extractives have a greater absorbance than tree fresh sapwood extractives in the same wavelength range.

Figure 9. UV-Visible spectra of methanolic extractives of Douglas-fir tree sapwood and tree heartwood

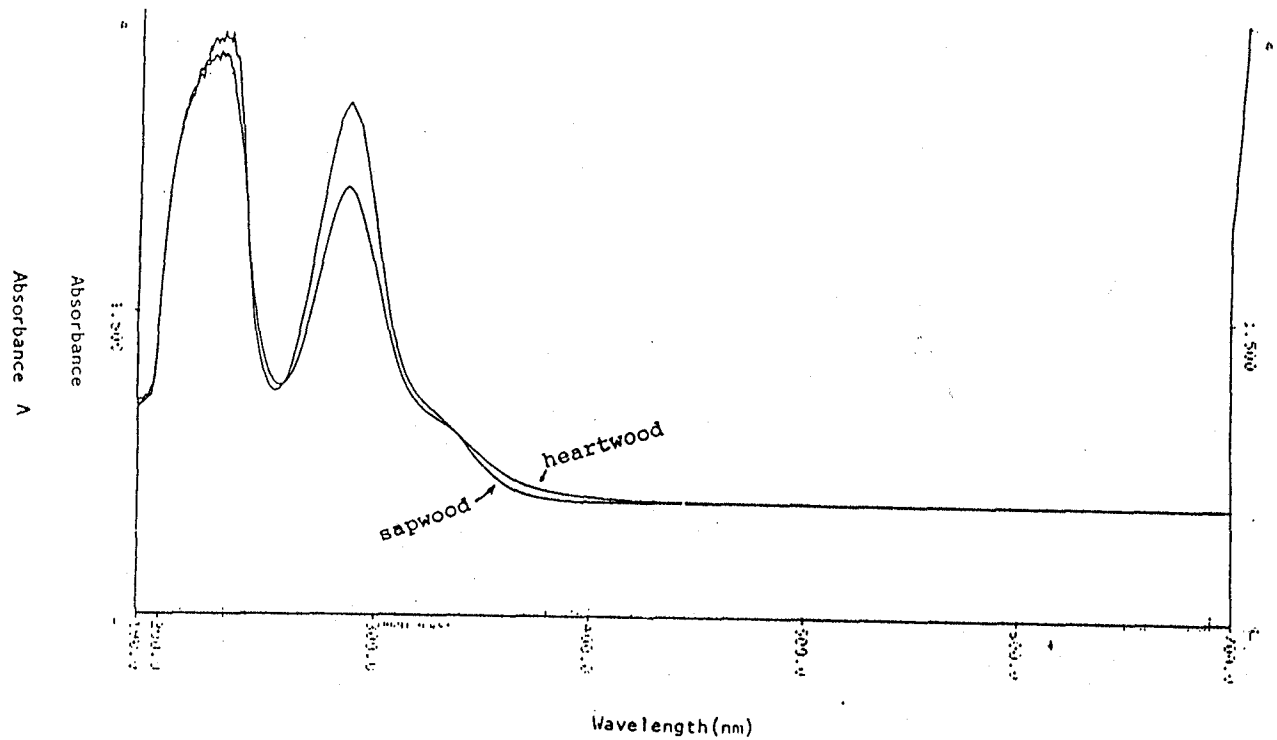


Figure 10. UV-Visible spectra of methanolic extractives of Lumber stained and nonstained sapwood

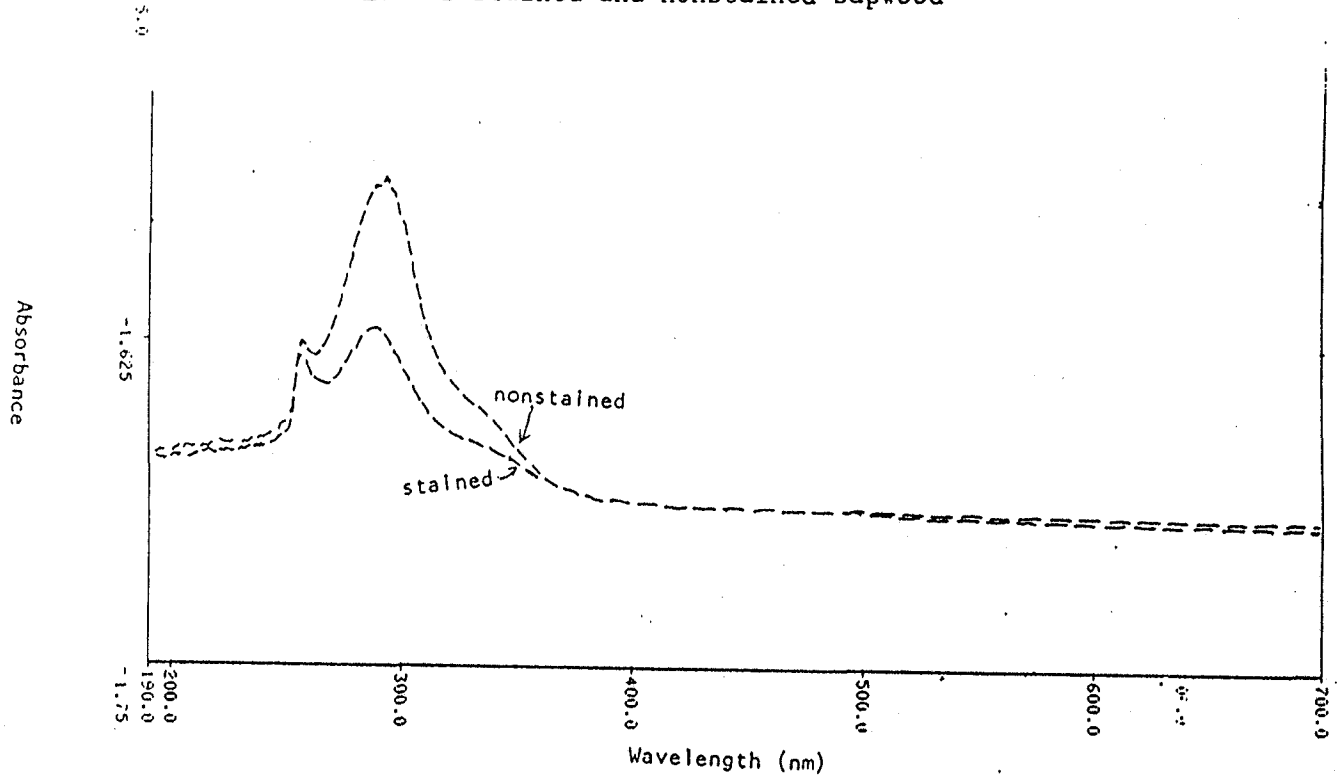
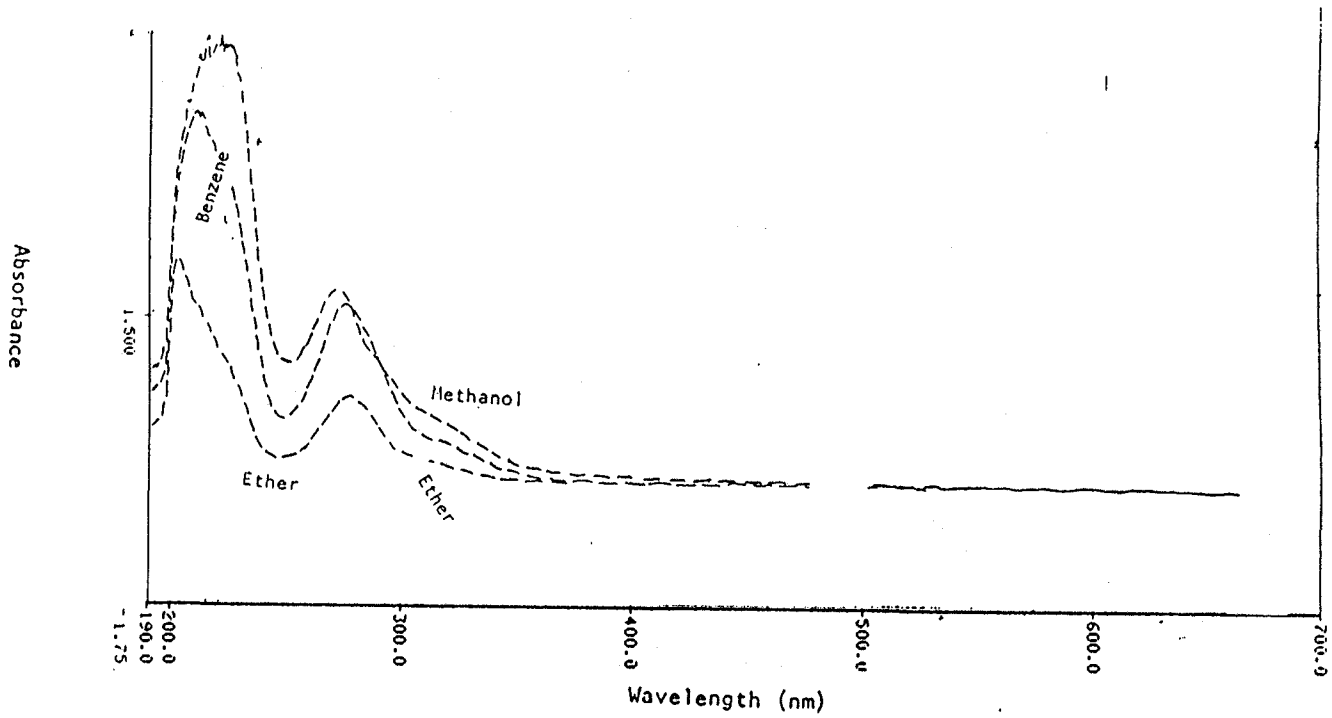


Figure 11. UV-Visible absorption of Douglas-fir wood extractives

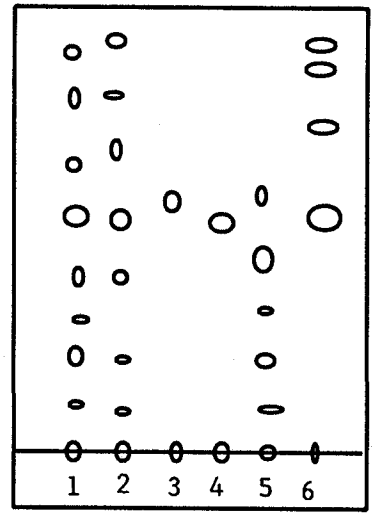


3. TLC study of Extractives

Figure 12 shows a TLC of the methanolic extractives on a silica gel thin layer plate developed with BAM solvent. The extractives were obtained as described in section III.6.1. Origin 1 is a fresh nonstained tree sapwood sample. Origin 2 is a tree heartwood sample. Origin 3 is authentic quercetin. Origin 4 is authentic dihydroquercetin. Origin 5 is a lumber stained sapwood sample. Origin 6 is a lumber nonstained sapwood sample. The spots were indicated by short wave (254 nm) UV light and by room light.

One of the main components in tree nonstained fresh sapwood 1, tree heartwood 2 and lumber nonstained sapwood 6 is dihydroquercetin, which is completely missing in the lumber stained sapwood sample 5 extractives. Song(1987) proved that dihydroquecetin forms dark brown colors rapidly when certain enzymes are present. The lack of dihydroquercetin in the stained wood supports the assumption that this compound may be involved in the discoloration as a precursor. There are other less polar compounds missing also. Compared to the extractives from tree fresh nonstained sapwood and lumber nonstained sapwood, the components in stained wood could be either more polar or possess a higher molecular weight. Because there are less extractives from the stained wood sample than from the nonstained wood samples, it is concluded that some extractive components become less soluble after

Figure 12. TLC of methanolic extractives on a silica gel plate developed with benzene-acetone-methanol (6:3:1 v/v) and detected under UV light.



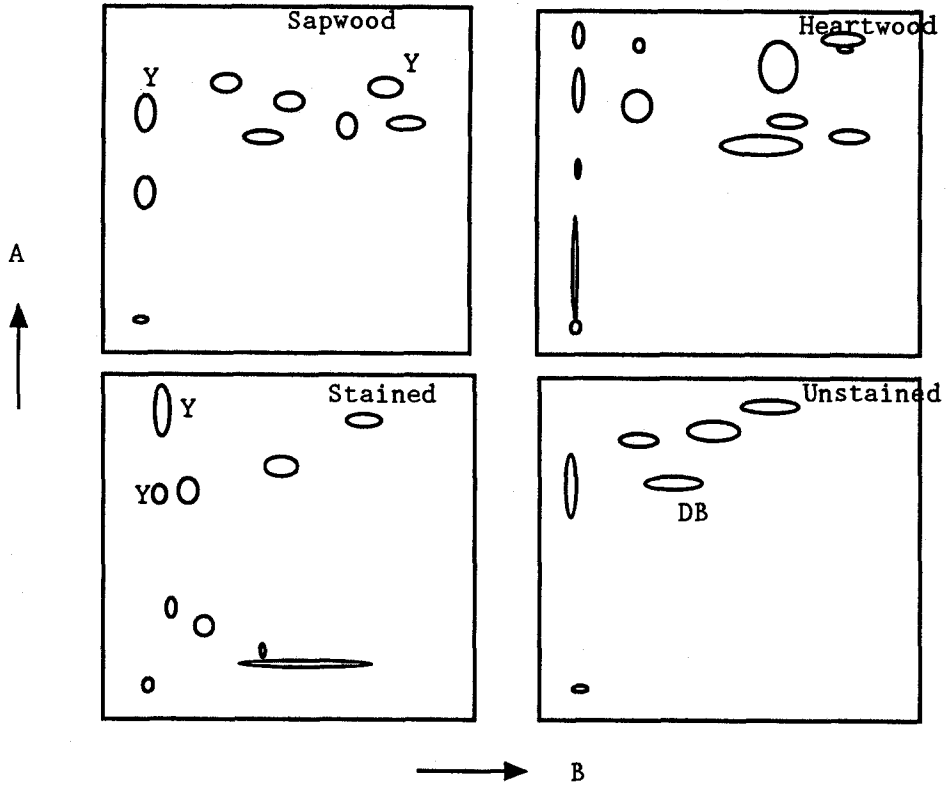
staining occurs. This supports the hypothesis of oxidization and polymerization in connection to wood stain formation.

On a cellulose plate, 2D-TLC of the extractives was obtained with the BAW-6% acetic acid solvent systems. It is shown in Figure 13. The plates were run in the A direction from the bottom up with BAW and then in the B direction from left to right with 6% AcOH/water. They seem to share little in common chemically. The chromophores are different also.

The streaking on the plates indicates that both the lumber stained wood and the tree heartwood have some higher molecular weight materials. The lumber stained sapwood has some phenolic polymers which are reasonably hydrophilic. The tree heartwood polymers are much less hydrophilic because they did not move much with AcOH/water in the B direction.

DHQ has been identified as the major contributor to color development in the samples tested, and is also the main component of the methanolic extractives of Douglas-fir heartwood (Wang and Laver, 1992). DHQ is therefore shown to be the most important component of chemical brown stain discoloration.

Figure 13. Two dimensional TLC of the methanol extractives on cellolose plates.



The observation that DHQ was one of the major components extracted from tree fresh nonstained sapwood, tree heartwood and lumber nonstained sapwood but was not found in lumber stained sapwood (Figure 12) supports the concept that DHQ is an important precursor that undergoes chemical change to give discoloration. Song (1988) also isolated and identified DHQ-3'-O-glucoside from Douglas-fir sapwood. This compound was not specifically studied in the tree nonstained sapwood sample in the present study. However, the glucoside can be easily hydrolyzed to releases free DHQ, which can then undergo reaction to form colored materials.

4. GC-MS Study of Diethyl Ether Extracts

The volatile components of Douglas-fir wood extractives and the lower oxygenated fatty acids and resin acids was investigated for their impact on discoloration or the influence on them from discoloration. This group of compounds were expected to be extracted with diethyl ether. The acids can be made volatile through their methylated derivatives. Thus, the chemical composition of this group of compounds was studied with a well developed instrumental method. The GC chromatogram of different samples are presented in Figures 14 and 15.

The major components are identified tentatively through their mass spectra by comparing the mass spectra with authentic data in the

Figure 14(a). GC of ether extracts of tree sapwood

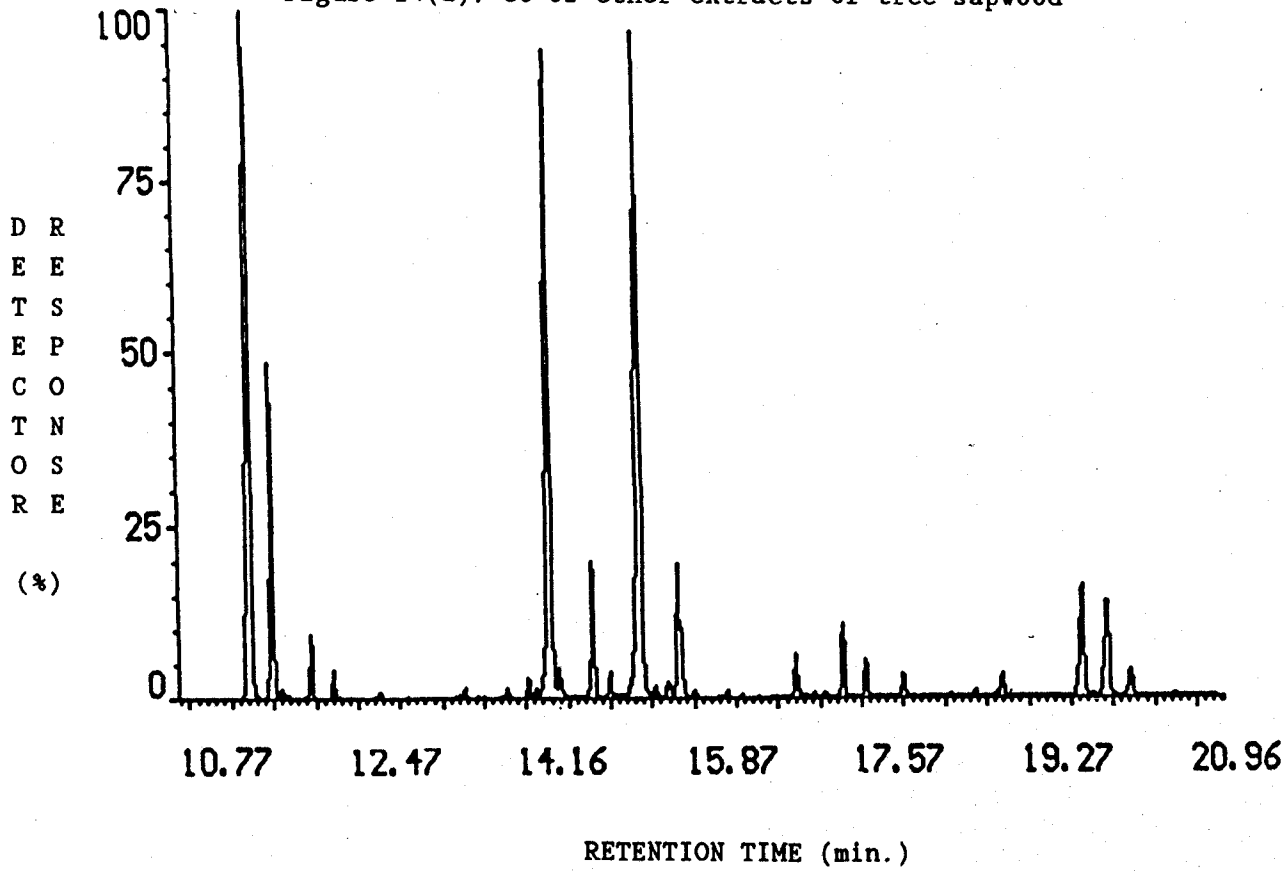


Figure 14(b). GC of ether extracts of tree heartwood

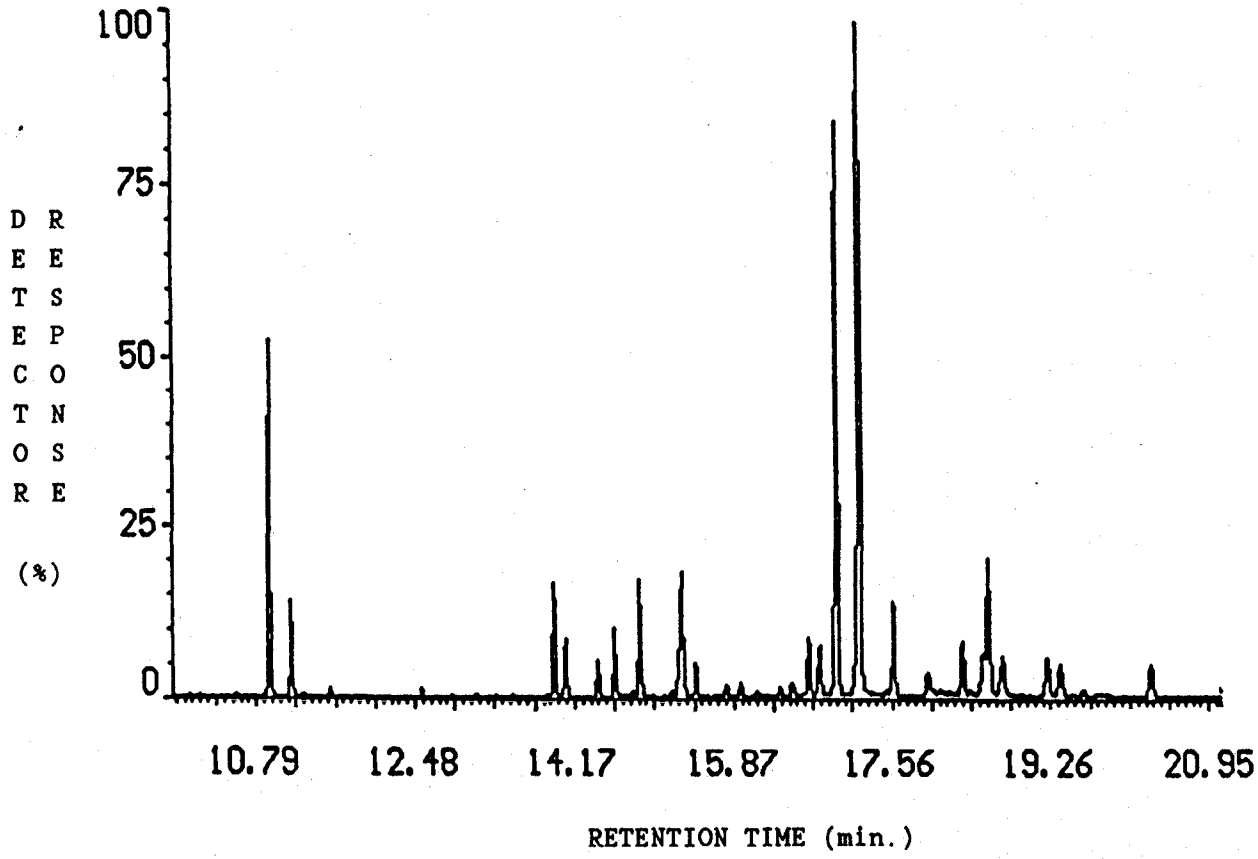


Figure 15(a). GC of ether extracts of lumber stained sapwood

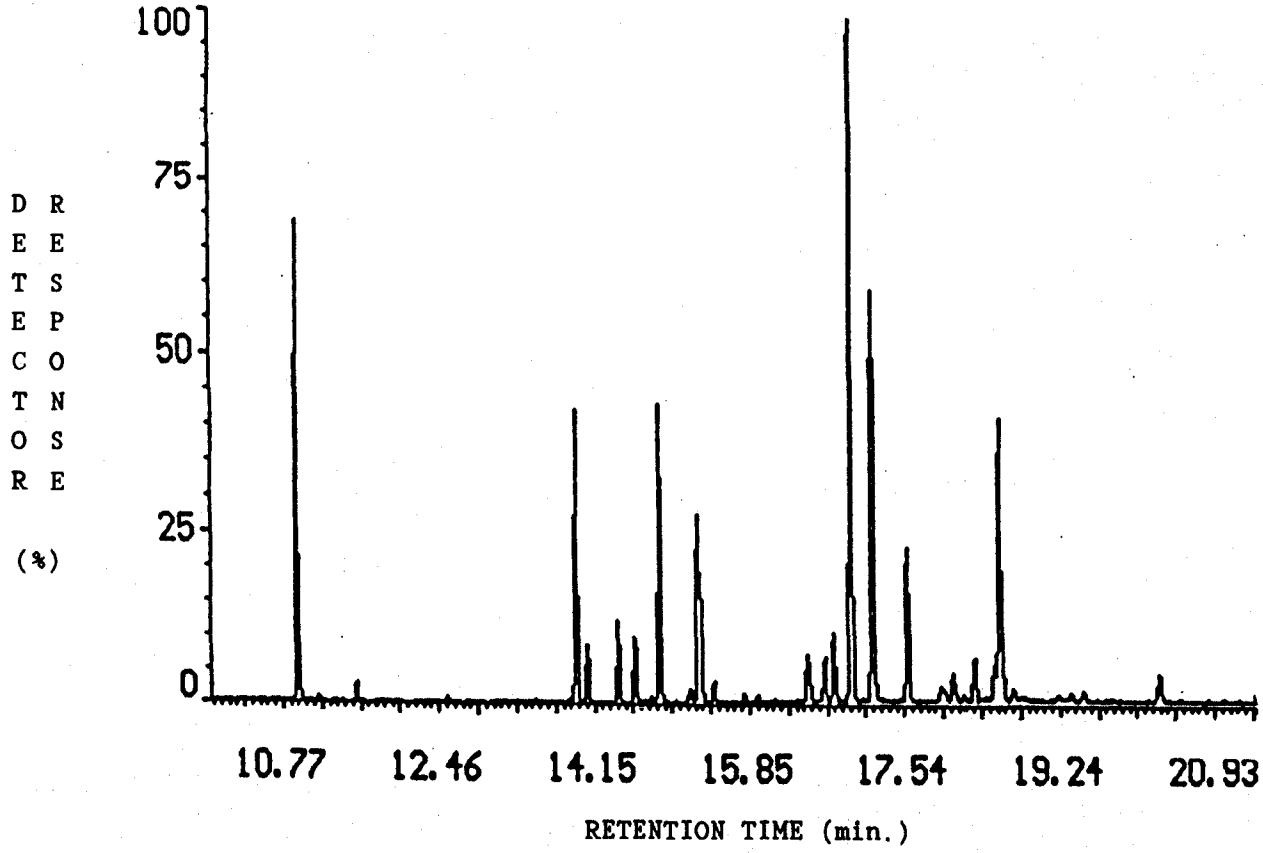
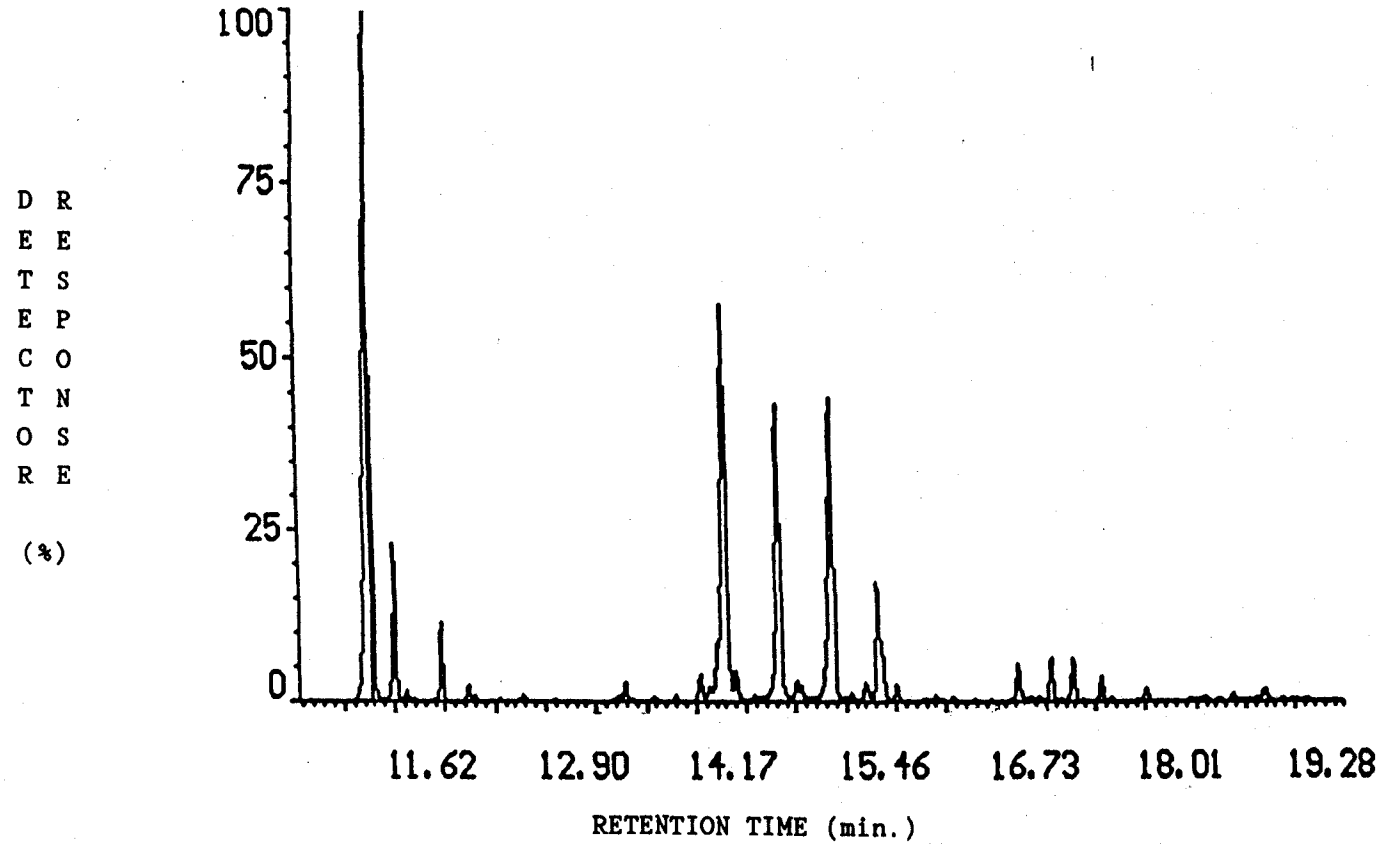


Figure 15(b). GC of ether extracts of timber nonstained sapwood



Galaxy 2000 computer system. The results of the identification are listed in Figure 16. The ratio of each peak height to the highest peak is listed in Table 5. Not all of the GC peaks were large enough or pure enough to be identified, but those that were identified are shown in Figure 16 and Table 5.

The results of the GC-MS analyses indicate that:

a. The diethyl ether extract of Douglas-fir wood was mainly composed of organic acids. They were terpene acids and fatty acids as well as 2,5-dimethylbenzenebutanoic acid (1). The terpene acids included some sesquiterpene acids, diterpene acids and a small amount of monoterpenes, which are hard to identify. The sesquiterpenes were identified as 4-(1,5-dimethyl-3-oxohexyl)-cyclohexanecarboxylic acid (3) and 4-(1,5-dimethyl-3-oxohexyl)-1-cyclohexene-1-carboxylic acid (7). The diterpene acids were pimaric acid (14), abietic acid (18), isopimaric acid (16), neoabietic acid (19), dehydroabietic acid (17), and dehydropimaric acid (22). The fatty acids were identified as docosanoic acid (21), (Z,Z)-9,12-octadecadienoic acid (8), and tetracosanoic acid (27). Quantitatively, the fatty acid (8) was among the major components in these extracts.

It is very unique for Douglas-fir, as a member of the Piceae family, to contain such a significant amount of sesquiterpene acids.

Figure 16. Chemical structures of some components identified in diethyl ether extracts of Douglas-fir wood.

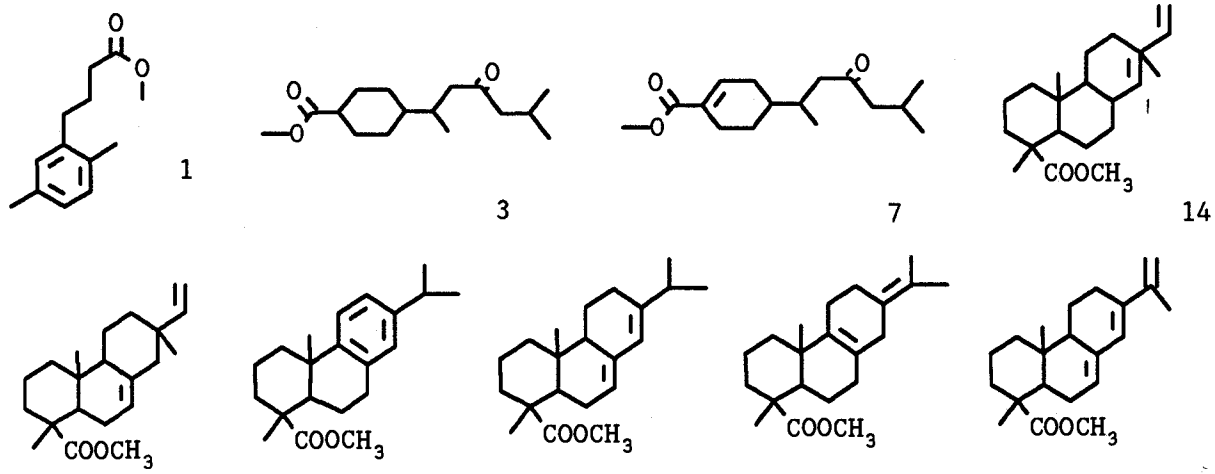
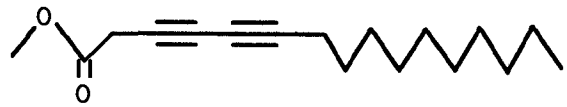


Figure 16. Chemical structures of some components identified in diethyl ether extracts of Douglas-fir wood (continued).

8

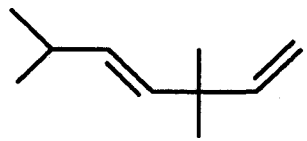


26'

27

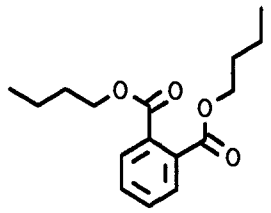
21

13

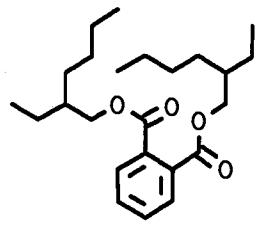


7'

Figure 16. Chemical structures of some components identified in diethyl ether extracts of Douglas-fir wood (Continued).



5



22

Table 5. GC analysis of methylated ether extracts of Douglas-fir wood.

Peak No.	US	SP	HT	ST
1	100	100	69	52
2	23	48	-	14
2'	11	10	-	t
3	67	94	42	18
4	5	4	10	8
5	40	18	13	6
6	2	3	12	12
7	40	96	43	20
7'	2	2	2	0
8	17	20	28	22

Table 5. GC analysis of methylated ether extracts of Douglas-fir wood (continues).

13	5	7	8	2
14				
15	-	-	7	8
16	7	13	100	85
17	3	7	62	100
18	2	2	24	15
19	0	0	2	3
20	-	-	5	-
21	2	1	7	8
22	2	4	43	23

Table 5. GC analysis of methylated ether extracts of Douglas-fir wood (continued).

Peak No.	US	SP	HT	ST
23	-	-	2	6
24	-	-	-	6
25	-	17	1	4
26	-	15	-	t
26'	-	4	-	t
27	-	t	4	4

b. The chemical composition of the diethyl ether extract of tree fresh sapwood is similar to that of lumber nonstained sapwood, in which sesquiterpene acids, i.e. (3) and (7) were the main components while that of lumber stained sapwood and tree heartwood, in which the diterpenes, namely, isopimaric acid (16), and dehydroabietic acid (17), were the major components. These diterpene acids are believed to be the oxidized products or the less active compounds of the diterpene acid mixture (Su, Yu, and Guo, 1986).

There were two phthalates identified. They are suspected to be impurities brought into the sample. We have no clue as to how they could be present in such a high content (Griffin, 1993).

The waxy materials including the terpene acids and fatty acids, contribute to the discoloration, but the contribution was minor compared to the phenols. They should locate at the solvent frontier of the A direction on a TLC of methanolic extractives on a cellulose plate. The discoloration of this area might have resulted from the chemical change of these materials. Although the discoloration was not as servere as the phenolic discoloration.

The natural occurrence of compounds varies in each individual tree with different seasons. Some important components might be missing in this test due to the low concentrations of the component.

Substances, which did not develop color when they were present singularly, sometimes developed color when combined. Discoloration caused by these substances in nature cannot be detected with the TLC assay we used here.

H. Separation and Identification of Lignans from Douglas-fir Sapwood Extractives

The ethyl acetate soluble portion of a methanolic extract of Douglas-fir sapwood contained material that was cherry-red in color when recovered by water elution from a Sephadex LH-20 column (section III.F.4). In an effort to trace the pigments that cause Douglas-fir wood discoloration, this red fraction was further investigated. Two separated fractions labeled Fraction A and Fraction B were isolated. These fractions were then characterized by mass spectroscopy, proton nuclear magnetic resonance, and ^{13}C nuclear magnetic resonance combined with H^1-H^1-COSY and H^1-C^{13}-HECTOR nuclear magnetic resonance.

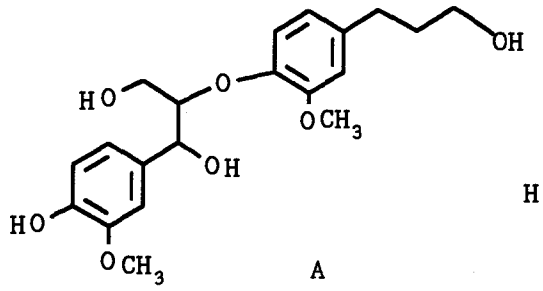
The spectral data of Fraction A indicated a lignan. On the basis of the following interpretation the structure A in Figure 17 was proposed for the material. The negative FAB-MS of A showed an $(M-H)^-$ at m/z 377, suggesting the molecular formula $C_{20}H_{26}O_7$, as estimated from signals of the NMR spectra. The H^1-H^1-COSY of A indicated a connection between the protons of the dihydroconiferyl alcohol side chain as shown in the compounds. The doublet at 4.86 (J=5.88 Hz) and the

signals at 4.31 (dt, J=5.3,4.0) are assigned to H-a and H-b of one 1-phenyl-2-phenoxypropane-1,3-diol structure, respectively. The signals at 4.85 and 4.24 are assigned to protons in a similar structure. The parallel sets of signals in C^{13}-NMR for the aromatic carbons indicates that it is a mixture of diastereoismers. The C-b at 86.6 should be the erythro and the C-b at 87.7 is for the threo isomer. On these bases the structure of A was determined as 1-(3-methoxy)-phenyl-2-[4-(1-propanol)-3-methoxyphenyl] -propane-1,3-diol. The structure of A is shown in Figure 17.

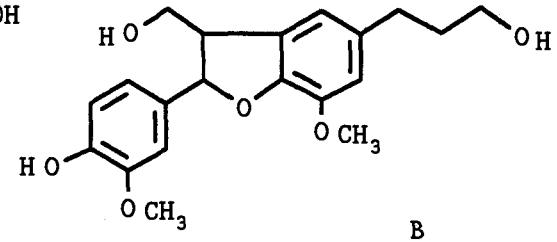
The positive MS spectra of Fraction B displayed the peak of $(M+H)^+$ at m/z 361 and $(M-OH)^+$ at 343, suggesting the formula $C_{20}H_{26}O_6$. H^1- and C^{13}-NMR spectra including 2D NMR techniques proved that B has the same structure as dihydrodehydrodiconiferyl alcohol, named as 2,3-dihydro-2-(3'-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol. The glucoside of this agycone has recently been reported by Ruben Gonzalez from the bark of Douglas-fir (Gonzalez-Laredo, 1993). Its structure is shown as structure B in Figure 17.

These lignans are very closely associated with the chromophores in the wood and are assumed to be either precursors or copigments of wood coloration and discoloration because they have the basic structure of the copigments of anthocyanins (Osawa, 1982).

Figure 17. Proposed structures of the compounds in Fraction A and Fraction B.



A



B

V. SUMMARY AND CONCLUSIONS

1. Extracts of sapwood and heartwood from fresh trees of Douglas-fir were tested for light and oxygen susceptibility for color change by application to TLC plates.

2. Phenolic compounds extracted from Douglas-fir wood did change to colored materials on TLC plates. It was observed that light and air did influence the colors formed, but that to the susceptible components some color always developed with or without either air or light.

3. Dihydroquercetin was identified as the major contributor to color development. It disappeared as color formed.

4. Catechin and epicatechin are chromophore precursors also, although they were detected only as minor components in some sapwood.

5. Waxy materials and resin acids also contributed to the discoloration, but the contribution was minor compared to the phenols.

6. The potential water mobility of chromophore in sapwood and heartwood from tree was tested. The sapwood has a higher water soluble extractive content and greater water mobility of insoluble extractive chromophores. The heartwood chromophores tend to stay in place.

7. The extractives of stained sapwood and nonstained sapwood from lumber were investigated. The stained sapwood contained much less extractives than the nonstained sapwood. This could be the result of oxidation and polymerization of the extractives. Also, stained wood extractives have a higher absorbance at 350-450 nm, which is the violet light region.

8. TLC study showed that the methanolic extractives of stained sapwood from lumber were more polymerized and oxidized than that of nonstained sapwood. Dihydroquercetin was present in the extractives of nonstained sapwood but had disappeared from the extractives of stained sapwood. Thus the dihydroquercetin possibly underwent polymerization or oxidation to form coloring materials.

9. Two lignans were isolated and their structures were elucidated to be 1-(3-methoxy)-phenyl-2-[4-(1-propanol)-3-methoxyphenyl]-propane-1,3-diol and 2,3-dihydro-2-(3'-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol. Since they were very closely associated with the color chromophore, they were assumed to be either chromophore precursors or copigments of wood coloration and discoloration.

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