

AN ABSTRACT OF THE THESIS OF

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Douglas fir bark fines which contained 74.8 percent of Klason lignin and 70.2 percent of one percent sodium hydroxide solubility and decayed Douglas fir wood which contained 53.9 percent of Klason lignin were subjected to ethanolysis. A slight modification of the Hibbert's ethanolysis procedure was used. The monomeric compounds present in the reaction products were then examined.

Two fractions of ethyl ether soluble oil were obtained from both bark and wood samples, which were mixtures of monomeric compounds produced by the ethanolysis reaction. One, which was designated "PE" fraction, was obtained by an ether extraction of a tar-like water insoluble substance of the ethanolysis products, and the other one designated "LE" fraction was obtained by the ether extraction of a water solution of the water soluble ethanolysis product.

Two dimensional paper partition chromatography of these ether soluble oils demonstrated the presence of 12 compounds in the bark "PE" fraction, four compounds in the bark "LE" fraction and nine compounds in the wood "LE" fraction. The wood "PE" fraction gave the same chromatogram as the wood "LE". In the wood "LE", the typical Hibbert's monomers, 1-ethoxy-1-guaiacyl-2-propanone, 2-ethoxy-1-guaiacyl-1-propanone, guaiacyl-acetone, vanilloyl-acetylene, vanillin, and vanillic acid were identified. Ethyl ferulate was isolated from the bark "PE", which was the only C₆-C₃, phenyl propane type compound obtained from the ethanolysis of the bark fines sample. Ethyl protococatechuate, protocatechuic acid, and vanillic acid were isolated from the bark "PE" and "LE". Ethyl vanillate was isolated from the bark "LE". Identification of the esters was confirmed by comparison with authentic samples prepared by synthesis.

From these results, it was concluded that the major monomeric compounds which were produced by the ethanolysis reaction of the bark fines were ferulic acid, protocatechuic acid, and vanillic acid. This indicates that the building units of the bark phenolic acid molecule are different from those in the lignin from wood.

ETHANOLYSIS PRODUCTS FROM DOUGLAS FIR

by

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TABLE OF CONTENTS

I.	Introduction -----	1
II.	Experimental -----	9
	Samples -----	9
	Ethanolysis procedure -----	11
	Two dimensional paper chromatography of the ethanolysis oil -----	14
	Identification of the compounds from the wood "LE" -----	14
	Isolation of the components from bark "PE" and "LE" by chromatographic method -----	16
	Spectroscopic study -----	17
	Synthesis of the authentic samples -----	18
III.	Results and discussion -----	20
	Identified compounds from the ethanolysis oil of decayed Douglas fir wood -----	20
	Isolation and identification of the compounds from the ethanolysis oil of Douglas fir bark -	21
	Discussion -----	32
IV.	Summary -----	35
	Bibliography -----	37

LIST OF FIGURES

1.	The scheme of the ethanolysis procedure	---	13
2.	Two dimensional paper partition chromatography of the ethanolysis oil from Douglas fir bark fines and decayed Douglas fir wood	---	15
3.	Ultraviolet absorption curves of ethyl ferulate isolated from Douglas fir bark ethanolysis oil	---	22
4.	Two dimensional thin layer chromatography of ethyl ferulate	---	24
5.	Ultraviolet absorption curves of ethyl protocatechuate isolated from Douglas fir bark ethanolysis oil	---	25
6.	Ultraviolet absorption curves of ethyl vanillate isolated from Douglas fir bark ethanolysis oil	---	28
7.	Ultraviolet absorption curves of vanillic acid isolated from Douglas fir bark ethanolysis oil	---	29
8.	Ultraviolet absorption curves of protocatechuic acid isolated from Douglas fir bark ethanolysis oil	---	30
9.	Ultraviolet absorption curves of compound B-1 and B-2-2-d	---	31

LIST OF TABLES

I.	Analysis of the Douglas fir bark fines by Tappi Standard Procedures -----	10
II.	Paper partition chromatogram of identified com- pounds in the ethanolysis oil of decayed Douglas fir wood -----	20
III.	Paper Partition Chromatograms of Isolated Compounds from the ether soluble oil from the ethanolysis of Douglas fir bark fines-----	34

ETHANOLYSIS PRODUCTS FROM DOUGLAS FIR

I. INTRODUCTION

The term "bark phenolic acids" has been used to designate the main portion of the Klason lignin obtained from barks. However, as Brauns has pointed out (2,p.118) it is not intended to imply that the bark phenolic acids are structurally unrelated to lignin. Therefore, the procedures such as ethanolysis, which have been used in the investigation of lignin might be very useful and the most fruitful ones in the investigation of bark phenolic acids.

As a matter of fact, many previous writers have compared the similarities and dissimilarities between bark phenolic acids and wood lignin from data that were obtained by methods which are used in lignin chemistry. Comparisons were made of the chemical and physical properties of the bark phenolic acids, analysis of the elementary composition, analysis of some functional groups, derivatives from phenolic acids and isolation of degradation products, etc.

Kiefer and Kurth (13), and Hata and Sogo (9) reported that 55 to 60 percent of carbon and 4.8 to 5.8 percent of hydrogen were obtained by the elementary analysis of the bark phenolic acids.

Ultraviolet absorption spectra were also reported by the above authors and resembled closely those of wood lignin and also of tannins and phlobaphenes (10,12). Each of these materials have a maximum absorption in the region of 280 $m\mu$, and a minimum absorption at a point about 260 $m\mu$.

These elementary analysis data and ultraviolet absorption spectra seem to suggest that bark phenolic acids are constructed with C₆-C₃, phenyl propane type building units.

The infrared spectroscopic studies show the existence of benzene nuclei, hydroxyl groups and carboxyl or ester groups in bark phenolic acids, and a lack of ketone or aldehyde carbonyl group bands which should appear in the 1660 cm^{-1} region (6,7,10,12,14).

Within the analytical data of phenolic acids a big discrepancy was shown in methoxyl group content in comparison with wood lignin. According to the literature, methoxyl group content varies from 1.7 percent to 8.2 percent depending on the source of the phenolic acids (6,7,9,10,14). Even the largest value of 8.2 percent is only approximately one half that of wood lignin.

Methylation of the phenolic acids increased their methoxyl content up to the level of methylated Braun's native spruce lignin (6,7,9, 1:p295). These methylation experiments were carried out by using dimethylsulfate and

diazomethane so that the above results show that hydroxyl groups of aliphatic, aromatic or carboxyl type exist in larger amounts in the phenolic acids than in wood lignin.

Recently Erman and Lyness produced a relatively homogeneous methylated phenolic acid and determined its molecular weight by ultracentrifugation. The methylated phenolic acid possessed an average molecular weight (ultracentrifuge) of 6000. They also treated the methylated phenolic acid with acetic anhydride-pyridine and made an acetate derivative. (6).

Hata and Sogo produced lignosulfonic acids from the outer bark of Pinus densiflora (11). A fraction equivalent to a sulfonic acid of the bark phenolic acid possessed higher amounts of carboxyl groups than the fraction which might be considered as a true lignosulfonic acid (21). They found the molecular weight of the sulfonic acid of the phenolic acid to be 500 to 4000 by ultracentrifugation (22).

Degradation studies of phenolic acids have shown interesting structural features of the materials.

By nitrobenzene oxidation of the phenolic acids of gymnosperm barks vanillin, p-hydroxybenzaldehyde, protocatechualdehyde and a trace of syringaldehyde were obtained (7,9,14). From a bark phenolic acid of an angiosperm species 5-formylvanillic acid, syringic acid and

vanillic acid were obtained in addition to the same compounds obtained from gymnosperm bark phenolic acids (10).

Cupric oxide oxidation of a phenolic acid gave vanillic acid, p-hydroxybenzoic acid, vanilloyl formic acid, 5-carboxy vanillin, vanillin, and p-hydroxybenzaldehyde (7).

Chromic acid oxidation and permanganate oxidation of methylated phenolic acid gave veratric acid and vanillic acid (6).

Alkali fusion of the phenolic acids in white fir bark was carried out by Fahey and Kurth and gave phloroglucinol, catechol, protocatechuic acid, and oxalic acid (7). Erman and Lyness found that the yield of ether soluble products from alkali fusion of pine bark could be raised after direct methylation of the alkali fusion mixture. They detected protocatechuic acid and p-hydroxy benzoic acid in the alkali fusion mixture before methylation, and after methylation they obtained methyl veratrate, phloroglucinol trimethylether and methyl anisate in large yield and identified other minor products as veratrole and veratryl alcohol (6).

In comparison of these results with wood lignin (1, p.536-570), it is obvious that the characteristic degradation products from phenolic acids are phloroglucinol, protocatechuic acid, and p-hydroxybenzoic acid besides the common guaiacyl compounds which are obtainable from wood lignin.

In the history of the investigations of lignin chemistry pyrolysis, hydrogenolysis and ethanolysis of lignin were epoch making reactions, since from the products of these reactions phenyl propane type compounds were identified and supported the hypothesis of Klason (1,p.11) who first suggested that a phenyl propane derivative of the coniferyl type might be the basic parent substance of lignin.

Phillips and Goss distilled alkali lignin from corn-cobs with zinc dust in an atmosphere of hydrogen and without any catalyst in a reduced atmosphere of carbon dioxide and obtained 1-guaiacyl-n-propane, guaiacyl ethylene and other simple phenols (18, 19).

The pyrolysis of red wood phlobaphane has been reported by Kurth and coworkers (3,15,23:p.196) and recently Zavarin and Snajberk (24) to give a mixture of mono-, and dihydroxy phenols in a good yield. Most recently Erman and Lyness reported on the pyrolysis of slash pine phenolic acid. When the pyrolysis was effected in the presence of a steady stream of gaseous formic acid, the yield of pyrolyzate was 38 percent and the principal component was pyrocatechol (10%). At the same time, they carried out the pyrolysis of quercetin and catechin as the model compounds under the same conditions as set forth above. The pyrolysis of quercetin gave 7 percent of

pyrolyzate consisting of 18 percent phloroglucinol and 60 percent pyrocatechol. On the other hand, catechin gave a 25 percent yield of pyrolyzate, which contained lesser quantities of phloroglucinol (4%) and greater quantities of pyrocatechol (76%). From these results, they suggested that the main portion of the phenolic acids might be built up of polymerized catechin (6).

Although hydrogenolysis of wood lignin gave also interesting C_6-C_3 type compounds (1,p.532), so far, the reaction has not been investigated quite well on bark phenolic acids. There is only one report in which Peterson identified p-hydroxybenzoic acid in the hydrogenolysis oil of the phenolic acid. (17).

In 1939, Hibbert and his coworkers subjected spruce wood to ethanolysis and isolated several monomeric phenol substances which have been called Hibbert's monomers, or Hibbert's building stones (5). They are 1-(4-hydroxy-3-methoxyphenyl)-1-propanone, 1-(4-hydroxy-3-methoxyphenyl)-1,2-propanedione (vanilloylacetyl), 1-ethoxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone, 1-(4-hydroxy-3-methoxyphenyl)-2-propanone (guaiacyl acetone) and vanillin. Since these compounds seem to be the reaction products from specific building units of lignin, many investigators have worked on this procedure for various wood or other plant species,

isolated lignins and biosynthesis studies of lignin
(1:p465, 2:p.459)

In regard to the ethanolysis of Douglas fir wood, Hibbert et al. have reported that the products were the same as those from other gymnosperm species (16).

Fahey and Kurth subjected the phenolic acid isolated from white fir bark to ethanolysis. However, inasmuch as only a trace of ethanolysis oil was obtained, no monomeric substance was identified (7).

Hata and Sogo also reported on the ethanolysis of phenolic acids as follows: outer bark and sapwood of Pinus densiflora were extracted successively with alcohol-benzene, ethanol, and hot water. Lignins of the extracted bark were fractionated into the phenolic acid, dioxane lignin, and residual Willstätter lignin. These isolated bark lignins, the extracted outer bark, and the extracted sapwood were subjected to ethanolysis and the petroleum ether soluble fraction of the ethanolysis oils were separated successively into the NaHSO_3 , NaHCO_3 , and NaOH soluble fractions. The fractionated ethanolysis oils were examined by paper chromatography. 3,4-dihydroxybenzaldehyde, 4-hydroxybenzaldehyde, and Hibbert's monomers which have the guaiacyl group were identified from the ethanolysis oils of the phenolic acid fraction of the outer bark and of the extractive free outer bark. Since none of the other samples

subjected to ethanolysis gave 3,4-dihydroxybenzaldehyde, it might be thought that this compound may be a particular building unit existing only in the phenolic acid. Furthermore, it should be mentioned that none of the 3,4-dihydroxy-phenylpropane derivatives could be found in their experiment (20).

The purpose of this investigation was to degrade the phenolic acid in Douglas fir bark to monomeric compounds by ethanolysis and to identify these compounds. This information is necessary in order to ascertain the chemical relationship, if any, of the bark phenolic acid to the lignin in wood. To accomplish this objective, monomeric reference compounds were similarly prepared by the ethanolysis of the lignin in Douglas fir wood.

II. EXPERIMENTAL

SAMPLES

Douglas fir bark fines (Silvacon #490 prepared by the Weyerhaeuser Co.) was used in these experiments. This sample was previously analyzed by Peterson (17) by Tappi standard procedures and his data are shown in Table 1. The most significant values in these data are 74.8 percent Klason lignin and 70.2 percent of hot one percent NaOH solubility. The high Klason lignin content and the high alkali solubility indicate that the amorphous lignin rich fraction had accumulated mechanically in the sample in much the same way that the fine particles of wood meal generally show higher lignin content than coarse particles. Most of the Klason lignin might be phenolic acid fraction since a previous report has shown that the fine powder fraction which was mechanically screened from crushed whole bark had a high yield of the phenolic acid (14).

The decayed Douglas fir wood which was used for ethanolysis was supplied by Dr. Kurth. The decayed wood was ground in a Wiley mill and the fraction which passed through a 40 mesh screen was used for further treatment. A Klason lignin content of 53.9 percent was found by the Tappi standard procedure on the extractive free sample.

Table I. Analysis of the Douglas Fir Bark Fines by Tappi
Standard Procedures

analysis	percent
(Unextracted Sample)	
Ether soluble extractives	10.54
95% ethanol soluble extractives	17.57
Water soluble extractives	4.46
Total extractives content	32.57
Ash content	3.13
(Extractive-free sample)	
Pentosans content	2.34
Klason lignin	74.80
Methoxyl content	2.71
Acetyl content	0.47
1% NaOH solubility	70.20

ETHANOLYSIS PROCEDURE

The ethanolysis procedure used in this work was a slight modification of Hibbert's procedure (4) as shown in Figure 1. The bark sample was first extracted successively with benzene-ethanol (2:1 v/v), 95% ethanol, and hot water for 72 hours respectively. Seventy-five grams of the air-dried extractive free sample were refluxed for 48 hours in 1500 ml. of 100 percent ethanol containing two percent dry hydrogen chloride. Then the alcohol solution was filtered off. Fifty-seven grams of undissolved residue were obtained. The filtrate was colored a deep purple red. When the solution was neutralized with sodium bicarbonate powder, the color turned to a greenish-black, and a greenish-black precipitate accompanied with white salts was obtained. Later the black precipitate was recognized as a red coloring matter, which is still under investigation.

The neutralized solution was again acidified with hydrochloric acid to pH 6 and evaporated at room temperature to 200 ml. The concentrated solution was poured into two liters of water, and formed a precipitate which was filtered off. Next the water-alcohol solution was evaporated in vacuo below 40°C. to 200 ml. As the evaporation proceeded a reddish brown tar separated from the water solution and

stuck to the bottom of the flask. This material may be equivalent to Hibbert et al "lignin tars" or "ethanol-water soluble lignin" (4). However, since the author found that most of the tar-like material was soluble in ethyl ether, it was extracted with this solvent. The ether solution was dried over anhydrous sodium sulfate and the ether was removed by evaporation. One hundred seventy milligrams of a yellow brown oily substance was obtained. It was designated "PE" fraction.

The residual water solution was extracted also with ethyl ether. This gave 1.2 grams of a red viscous fluid. It was designated "LE" fraction.

The total yield of the ether soluble oils was about 1.4 grams.

One hundred seventy grams of the extractive-free decayed Douglas fir wood sample were treated by the same procedure as described above except that a three percent hydrogen chloride concentration was used in the ethanolysis solution. The undissolved wood residue was 114.1 grams and the yield of the "PE" fraction was two grams and the yield of the "LE" fraction was seven grams. In this case none of the red coloring phenomena was observed in the ethanolysis solution.

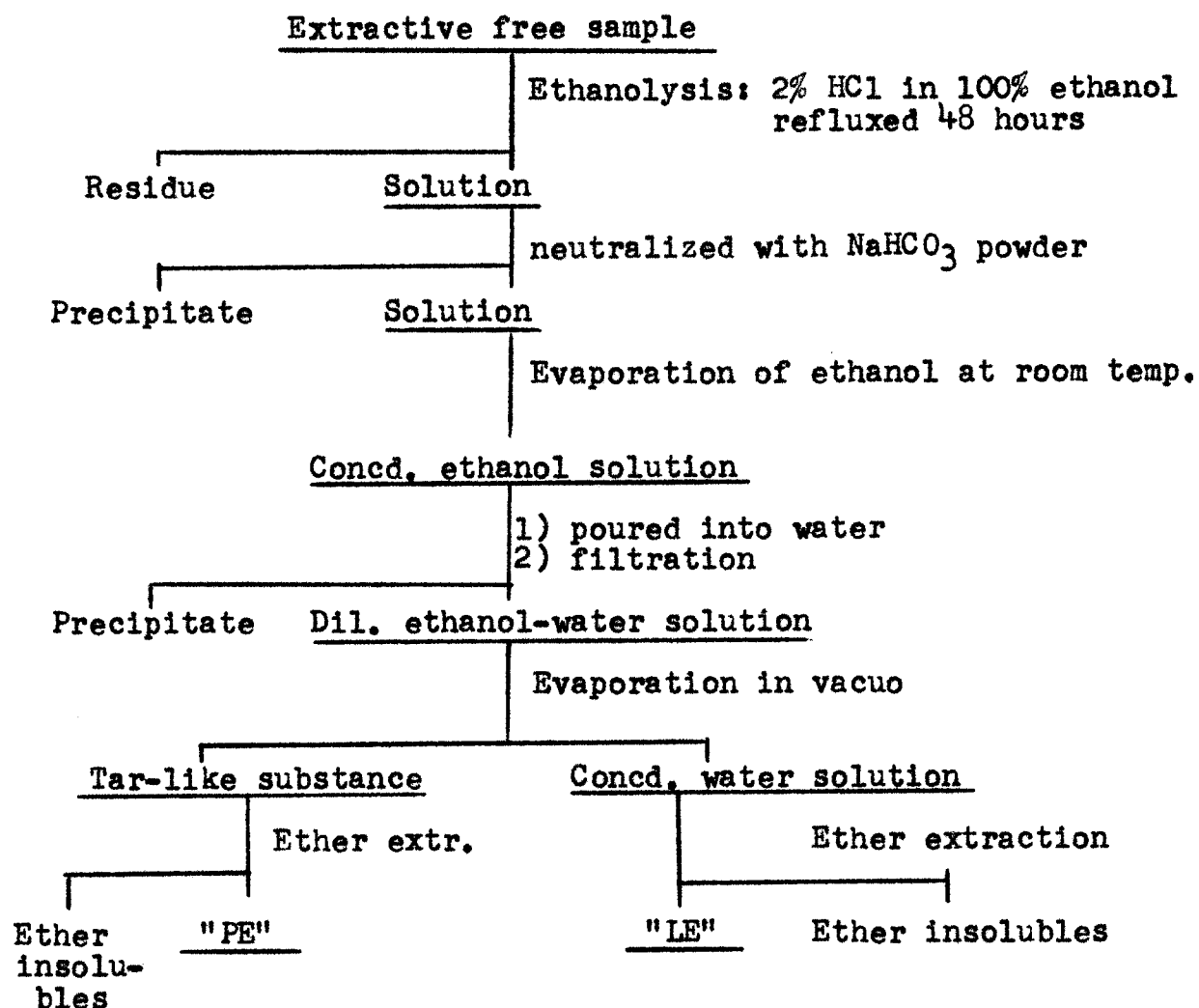


Figure 1. The scheme of the ethanolysis procedure

TWO DIMENSIONAL PAPER CHROMATOGRAPHY OF THE ETHANOLYSIS OIL

Two dimensional paper partition chromatography was carried out on each of the ethanolysis oil fractions from both bark fines and decayed wood. The chromatograms of bark "PE", bark "LE", and wood "LE" are shown in Figure 2. The wood "PE" gave the same chromatogram as the wood "LE".

For the first developing solvent, n-butanol saturated with three percent aqueous ammonia was used. The second solvent was benzene, ligroin, and methanol mixture (5:5:1 v/v). Both developments were carried out by ascendant flow on Whatman No. 1 paper.

The spots on each of the chromatograms were observed first by ultraviolet light and then sprayed with diazotized benzidine solution for coloration. Twelve spots from bark "PE", four spots from bark "LE", and nine spots from wood "LE" were obtained.

IDENTIFICATION OF THE COMPOUNDS FROM WOOD "LE"

On the base line of a wide sheet of Whatman No. 1. chromatography paper a methanol solution of wood "LE" was streaked as a solid line from a capillary glass tube. It was developed by the n-butanol-ammonia solution for 20 hours. The chromatogram was observed under the UV light, and separated in 12 streaks according to their fluorescent

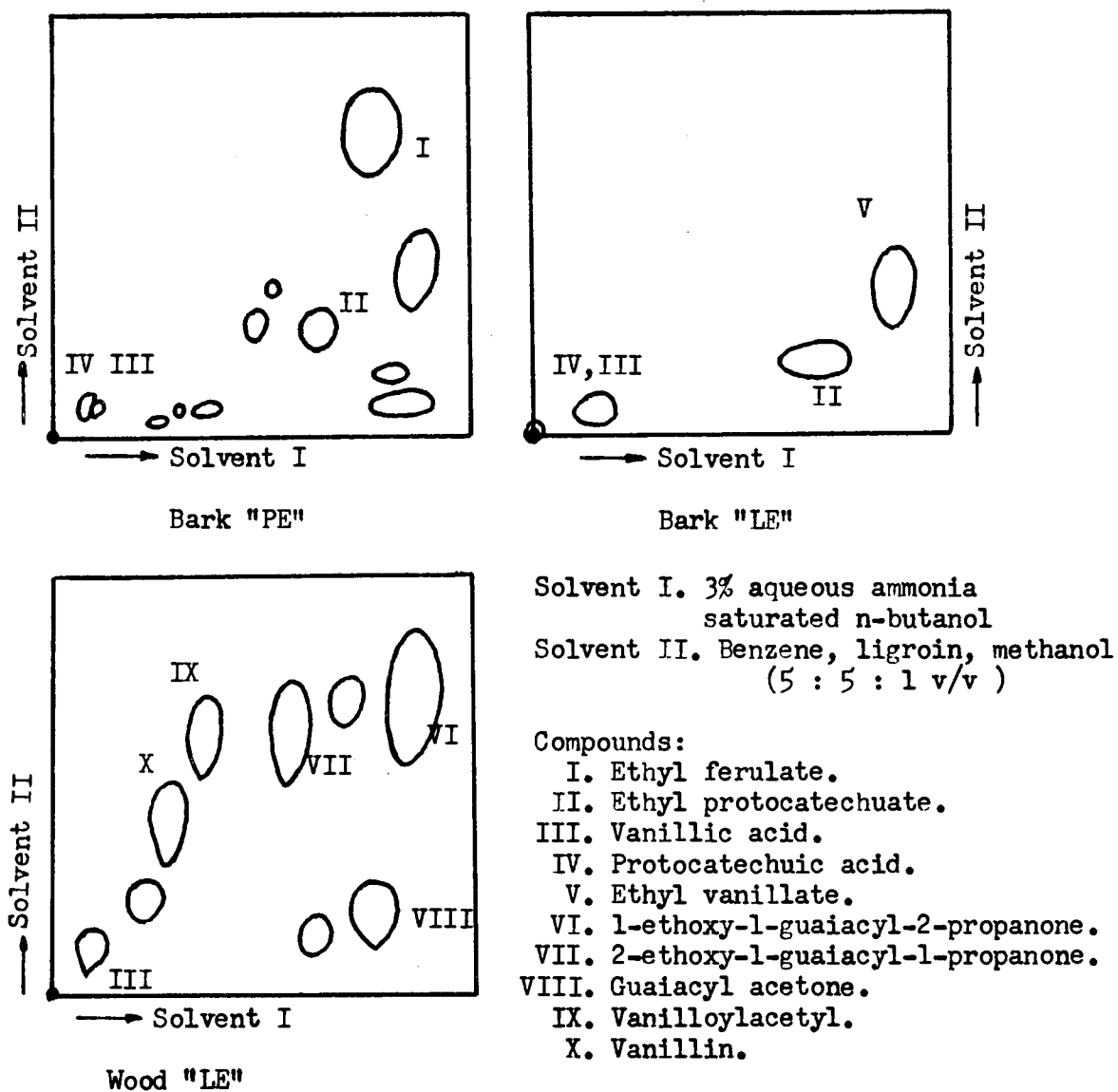


Figure 2. Two dimensional paper partition chromatography of the ethanolysis oils from Douglas fir bark fines and decayed Douglas fir wood.

color. Each of the streaks was eluted by methanol. These eluted methanol solutions were spotted on another paper and developed by benzene-ligroin-methanol solution. The spots were detected by UV light and colored by diazotized benidine solution. Their Rf values and the color of the spots were compared with authentic samples. Six of the nine spots were identified in this manner.

ISOLATION OF THE COMPONENTS FROM BARK "PE" AND BARK "LE" BY CHROMATOGRAPHIC METHOD

It was observed that there were large differences between the two dimensional chromatograms of the bark ethanolysis oil and the wood ethanolysis oil so that the isolation of each of the compounds was tried for the bark "PE" and "LE". For this purpose, the methanol solution of each oil was streaked on the base line of the Whatman No. 1. paper and developed with n-butanol-ammonia solution as described previously. Then the chromatogram was divided into strips according to the fluorescent color under UV light. Then each streak on the strips was eluted with methanol. The methanol solution was spotted on the base line of a silica gel G thin layer plate. These plates were developed by toluene, ethyl formate, and formic acid (5:4:1 v/v) solution. After the development the plates were dried at room temperature, and the fluorescent color was observed under UV light. At times dark grey streaks or yellow streaks appeared on the plates after drying without further treat--

ment. Later it was found that the most of the compounds that had a catechol group gave the dark grey color, and the yellow brown color was caused by the compounds which had a guaiacyl group.

Each of the streaks was scratched off the plates and eluted with methanol. The methanol was then evaporated at room temperature. The resulting substances were saved for the further examination. Almost all of them could be considered as pure substances because of their sharp ultraviolet absorption curves.

SPECTROSCOPIC STUDY

Ultraviolet absorption study

Ultraviolet absorption curves were taken for each of the isolated compounds by a Beckman DB recording spectrophotometer. Ninety-five percent ethanol was used for the solvent. It is known that phenolic compounds show a characteristic shift in alkaline solution (8). Therefore, after taking spectrum in neutral solution, one drop of 1.0 N NaOH solution was added to the sample cell and the reference cell to observe the alkaline shift.

Infrared absorption study

An infrared absorption curve was made on one of the unidentified crystalline compounds to obtain information

for functional groups present by the Beckman IR8 spectrophotometer using nujol mulls.

SYNTHESIS OF THE AUTHENTIC SAMPLES

Ethyl furulate

Thirty milligrams of ferulic acid were treated with five ml. of five percent hydrogen chloride in absolute ethanol solution for 72 hours at room temperature. The reaction mixture was spotted on a silica gel G thin layer plate and developed by toluene, ethyl formate, and formic acid (5:4:1 v/v) solution. The chromatogram was observed under UV light. The spot of the unreacted ferulic acid was checked by spotting pure ferulic acid on the same plate. The spot of the reaction product was scratched off the plate and eluted with methanol, and the methanol was evaporated at room temperature. A colorless oily substance remained. The ultraviolet absorption curve of this compound had the typical shape of a cinnamate type compound. It had 327 m μ of maximum absorption and 263 m μ of minimum absorption, and in 0.1 N alkaline medium the maximum absorption band shifted to 380 m μ .

Ethyl protocatechuate

Thirty milligrams of protocatechuic acid were treated by the same procedure, white crystals, m.p. 125-127°C., were obtained. Their ultraviolet absorption curve showed

maxima at 297 $m\mu$, and 263 $m\mu$, and minima at 282 $m\mu$ and 238 $m\mu$, and the alkaline shift showed maxima at 317 $m\mu$ and 240 $m\mu$, and minimum at 269 $m\mu$.

Ethyl vanillate

Five milligrams of vanillic acid were treated by the same procedure described above. A colorless solid substance was obtained. Although the quantity was too small for any sharp melting point to be observed, the ultraviolet spectrum was obtained sharply. Its maxima were at 292 $m\mu$ and 262 $m\mu$, the minima at 281 and 236 $m\mu$ in 95 percent ethanol solution, and the alkaline shift showed its maxima at 314 and 233 $m\mu$, the minima at 246 and 228 $m\mu$.

III. RESULTS AND DISCUSSION

IDENTIFIED COMPOUNDS FROM ETHANOLYSIS OIL OF DECAYED
DOUGLAS FIR WOOD.

Vanillin, vanillic acid, vanilloylacetyl, guaiacyl acetone, 1-ethoxy-1-guaiacyl-2-propanone, and 2-ethoxy-1-guaiacyl-1-propanone from the ethanolysis oil of decayed wood were identified by comparison with authentic samples by paper partition chromatography. The chromatographical data are shown in Table II.

Table II. Paper Partition Chromatogram of Identified Compounds in the Ethanolysis Oil of Decayed Douglas Fir Wood.

Identified compds.	Rf*	Rf**	Color	
			UV	Diazotized benzidine
1-ethoxy-1-guaiacyl-2-propanone	0.89	0.92	violet	yellow
2-ethoxy-1-guaiacyl-1-propanone	0.61	0.84	violet	pink
Guaiacyl acetone	0.79	0.67	white	yellow
Vanilloyl acetyl	0.42	0.77	-	pink
Vanillin	0.36	0.55	violet	pink
Vanillic acid	0.07	0.12	violet	yellow brown

*3% aqueous ammonia saturated n-butanol

** Benzene : ligroin : methanol, 5 : 5 : 1 v/v

These data are in agreement with the previous ethanolysis products from gymnosperm wood (16,20), though p-hydroxyphenyl derivatives could be detected. The author observed that vanilloylacetyl (1-(4-hydroxy-3-methoxy-phenyl)-1,2-propanedione) was easily detected by its strong yellow coloration when it was exposed to ammonia vapour. Coloration of coupling phenolic compounds with diazotized benzidine is also interesting since the compounds which have a carbonyl group conjugated to the guaiacyl group showed pink coloration, on the other hand, the compounds which have a side chain carbonyl group unconjugated to the guaiacyl nucleus showed a yellow color.

ISOLATION AND IDENTIFICATION OF THE COMPOUNDS FROM THE ETHANOLYSIS OIL OF DOUGLAS FIR BARK

Ethyl ferulate

Ethyl ferulate was isolated as a colorless oil from the bark "PE". Paper partition chromatography of this compound showed Rf 0.80 by three percent ammonia saturated butanol and Rf 0.95 by benzene, ligroin, and methanol mixture. These were the same values as given by the synthesized authentic sample. Thin layer chromatography with the authentic sample also gave the same spot. Ultraviolet absorption curves of the compound in neutral and alkaline ethanol

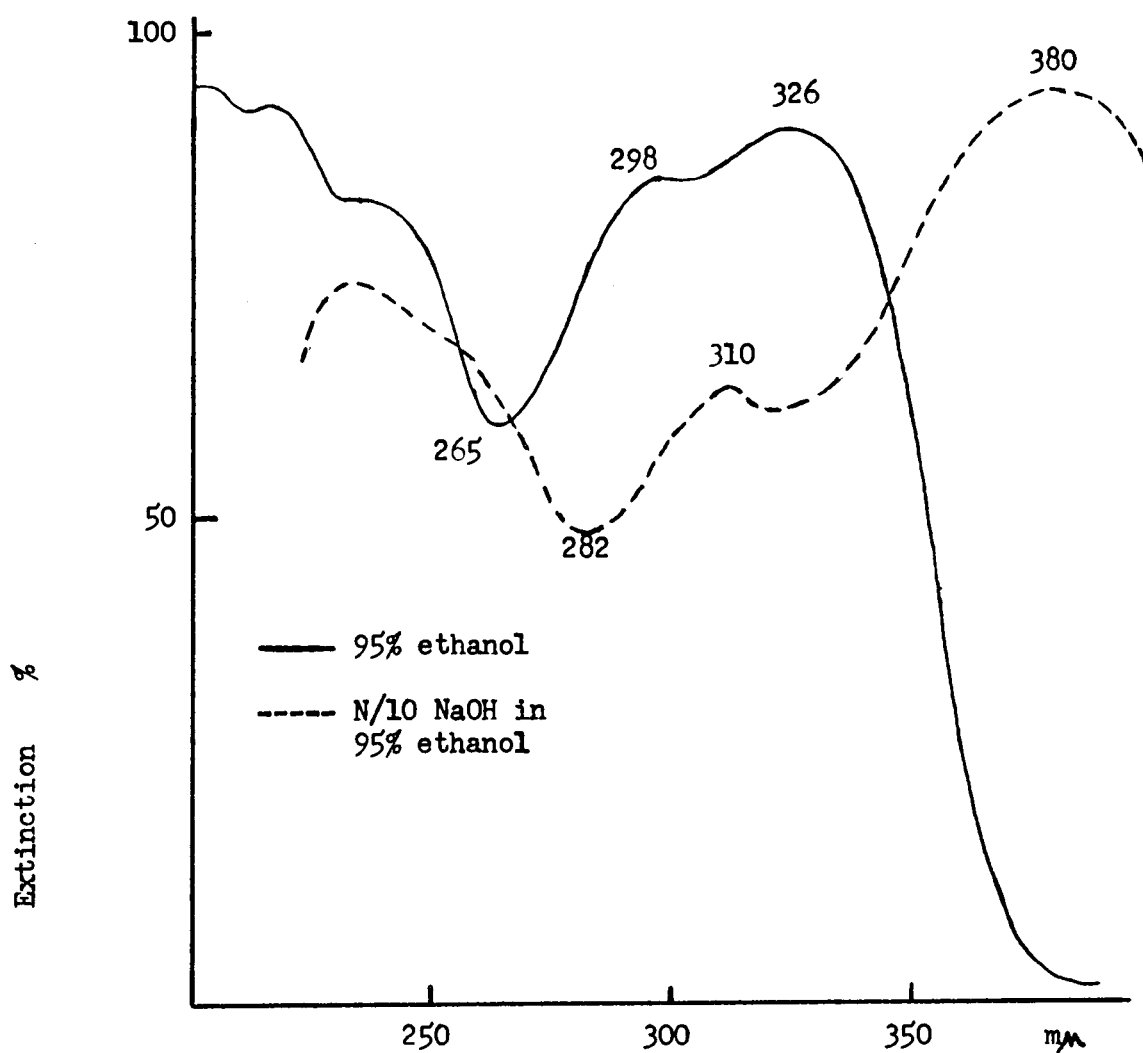


Figure 3. Ultraviolet absorption curves of ethyl ferulate isolated from Douglas fir ethanolysis oil.

solution are shown in Figure 3. These curves are also the same as for the authentic sample. The curve in neutral 95% ethanol solution has the typical shape of a cinnamate type compound with a shoulder in the 300 μ region which is caused by cis-, transisomerization (8). In this connection, the author observed very interesting phenomena in the thin layer chromatography experiment of this compound. On the thin layer chromatogram two spots were always closely together at the position of ethyl ferulate. The author wanted to isolate each of these spots and scratched each off the plate. The solution containing the spot was developed on a thin layer plate. The same two spots were obtained from each one of the spots. Next, two dimensional thin layer chromatography was carried out on the eluted solution. One direction was developed by benzene, ligroin, and methanol mixture (5:5:1 v/v) and the other direction was developed by toluene, ethyl formate, and formic acid (5:4:1 v/v). Four spots were obtained as shown in Figure 4. These facts can be explained by the cis-,transisomerization taking place during the development in both solvents.

Inasmuch as the medium used for the ethanolysis reaction was also a good condition for esterification, the source of the ethyl ferulate was ferulic acid. This was the only phenyl propane type compound obtained in the ethanolysis oil of Douglas fir bark fines.

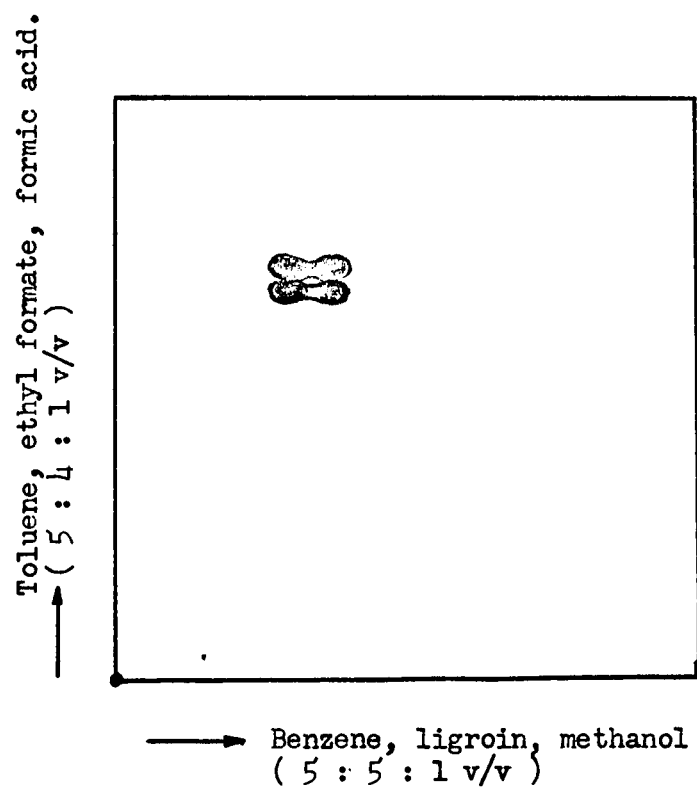


Figure 4. Two dimensional thin layer chromatography
of ethyl ferulate.

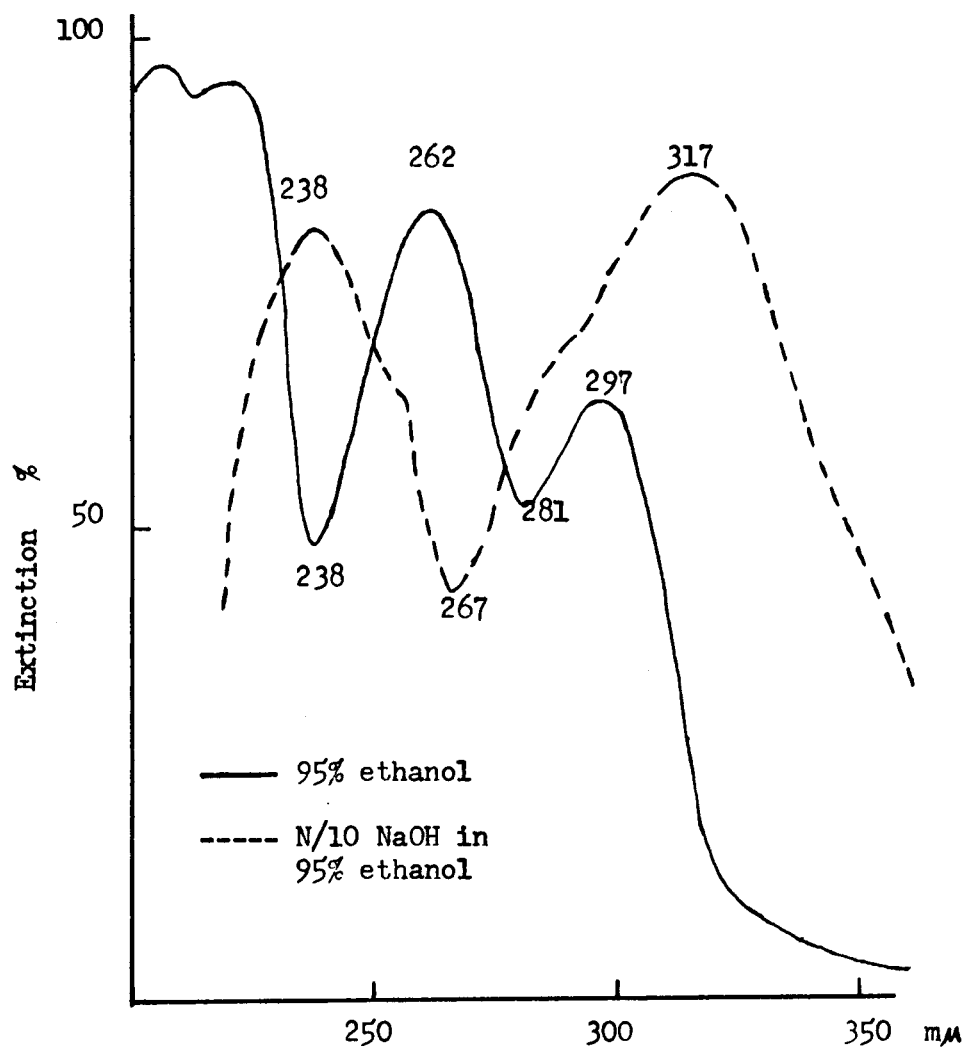


Figure 5. Ultraviolet absorption curves of ethyl protocatechuate isolated from Douglas fir bark ethanolysis oil.

Ethyl protococatechuate

Ethyl protococatechuate was obtained from both bark "PE" and "LE". The paper partition chromatography showed Rf 0.63 by three percent aqueous ammonia saturated n-butanol and Rf 0.23 by benzene, ligroin, and methanol solution. These values were the same as those obtained with the synthesized authentic sample, and a thin layer chromatogram of the sample developed with the authentic sample gave identical spots. Also, the ultraviolet absorption curves were identical with the authentic sample. These are shown in Figure 5.

Ethyl protococatechuate was also considered to have originated from protocatechuic acid. The occurrence of protocatechuic acid as an ethanolysis product is in agreement with previous degradation studies of bark phenolic acids (6,7,9,14,20).

Ethyl vanillate

Ethyl vanillate was obtained from the bark "LE" oil. Its presence was proved by chromatograms and ultraviolet absorption curves compared with synthesized ethyl vanillate. It had Rf 0.86, and Rf 0.82 on paper partition chromatograms developed by n-butanol-ammonia solution, and benzene, ligroin, and methanol mixture respectively. The ultraviolet absorption curves are shown in Figure 6. This compound was also formed by esterification of the vanillic acid which was produced by ethanolysis of the Douglas fir bark phenolic acid.

Vanillic acid

Vanillic acid was obtained from both bark "PE" and "LE". It was obtained as a colorless, needle-like crystals after evaporating the solvent which eluted the streak of the thin layer plate. The melting point was 200-203° C. and it was not depressed by mixing with an authentic sample of vanillic acid. Its ultraviolet absorption curves and chromatographical data were the same as those of the authentic sample.

Protocatechuic acid

Protocatechuic acid was obtained from bark "PE" and "LE". Its presence was proved by chromatography and ultraviolet absorption examinations.

Other isolated compounds which were unidentified

From the bark "PE" two additional compounds were isolated. One, which was designated B-1, gave a unique ultraviolet absorption curve as shown in Figure 9. The infrared absorption study showed the existence of hydroxy groups in the 3,350 cm^{-1} and phenyl nuclei in the 1,570 cm^{-1} absorption bands. The existence of a carbonyl group was uncertain since it seemed to be obscured by the phenyl absorption band. It decomposed at 198°C.

The other compound, designated B-2-2-d, showed a flavonoid type UV absorption curve which had maximum absorption at 282 m as shown in Figure 9. It decomposed at 168°C.

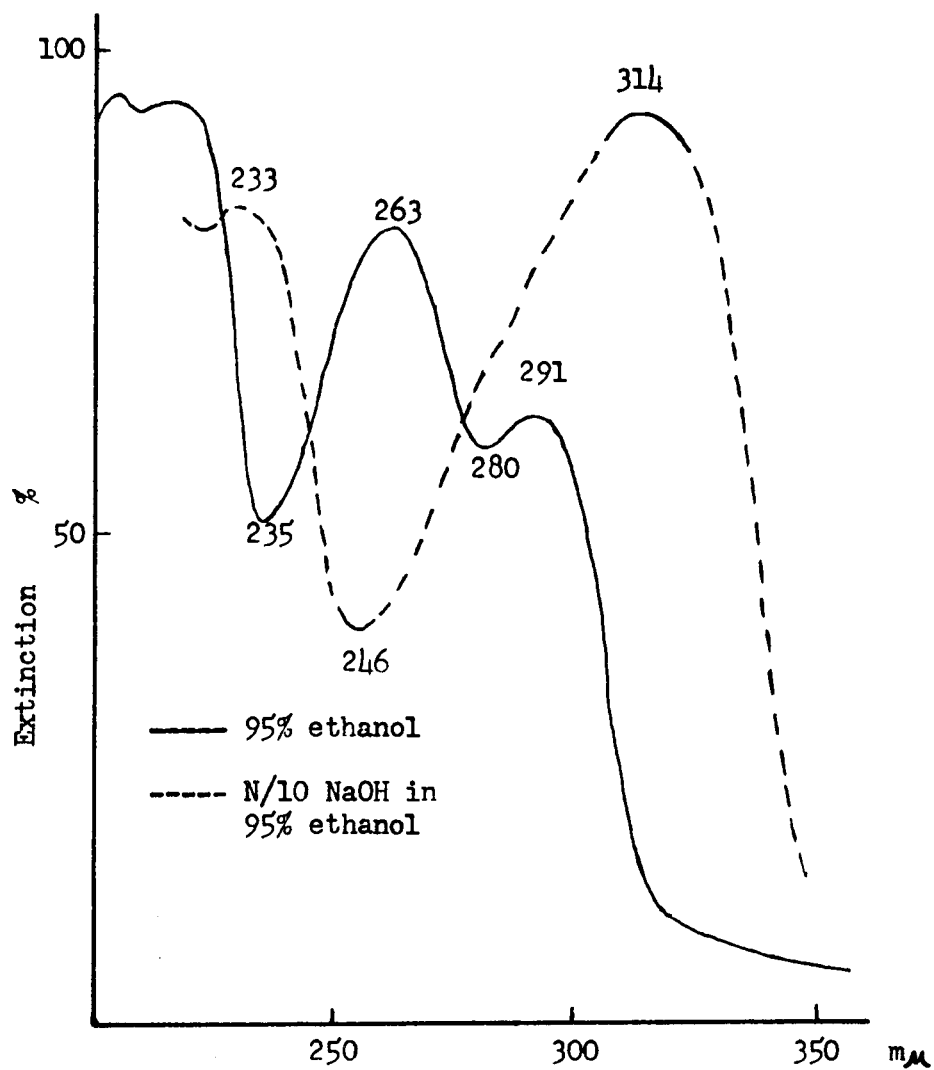


Figure 6. Ultraviolet absorption curves of ethyl vanillate isolated from Douglas fir bark ethanolysis oil.

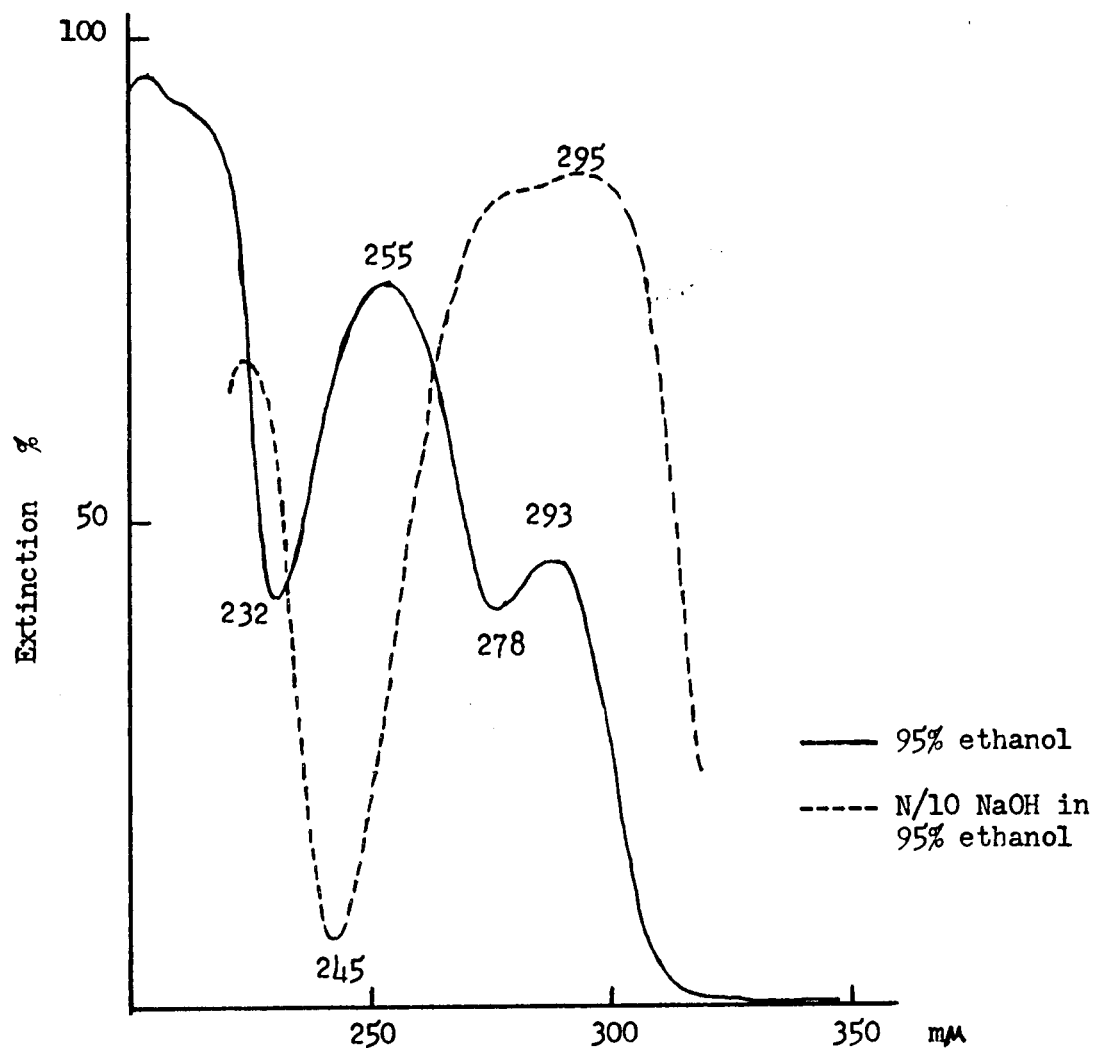


Figure 7. Ultraviolet absorption curves of vanillic acid isolated from Douglas fir bark ethanolysis oil.

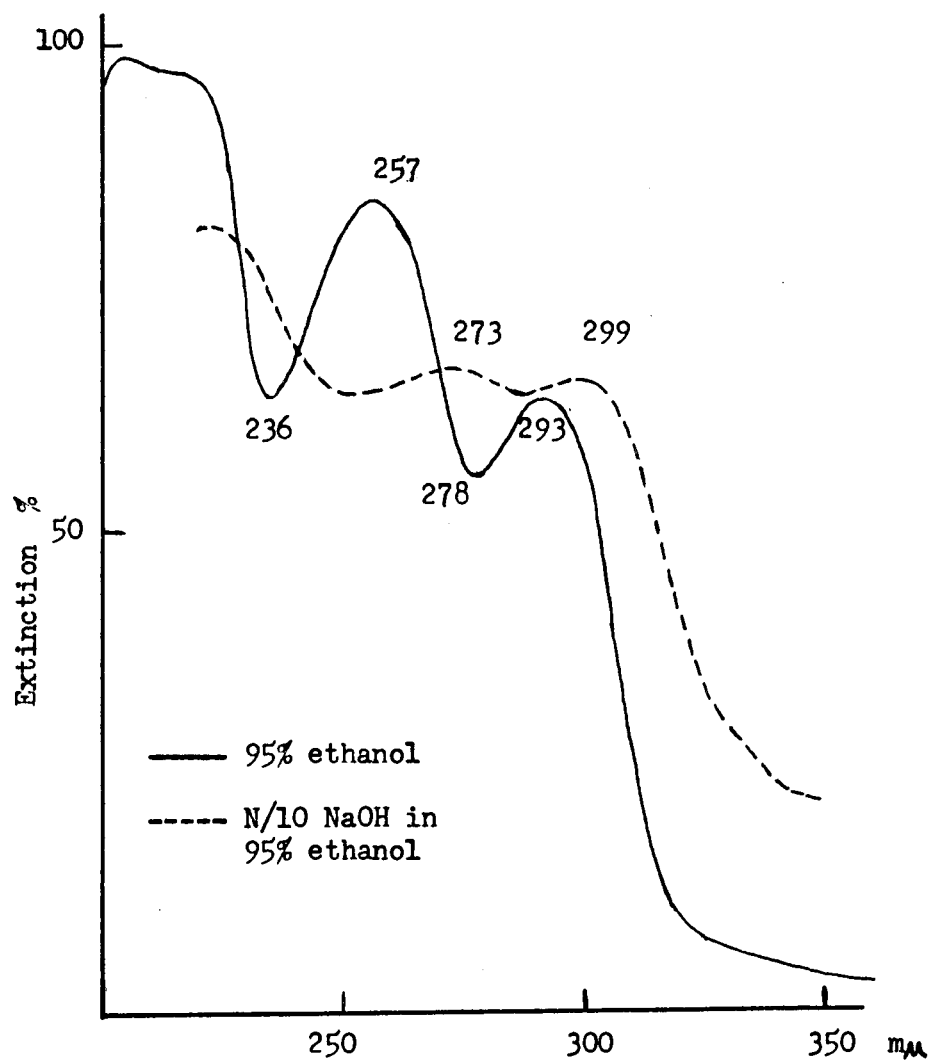


Figure 8. Ultraviolet absorption curves of protocathechuic acid isolated from Douglas fir bark ethanolysis oil.

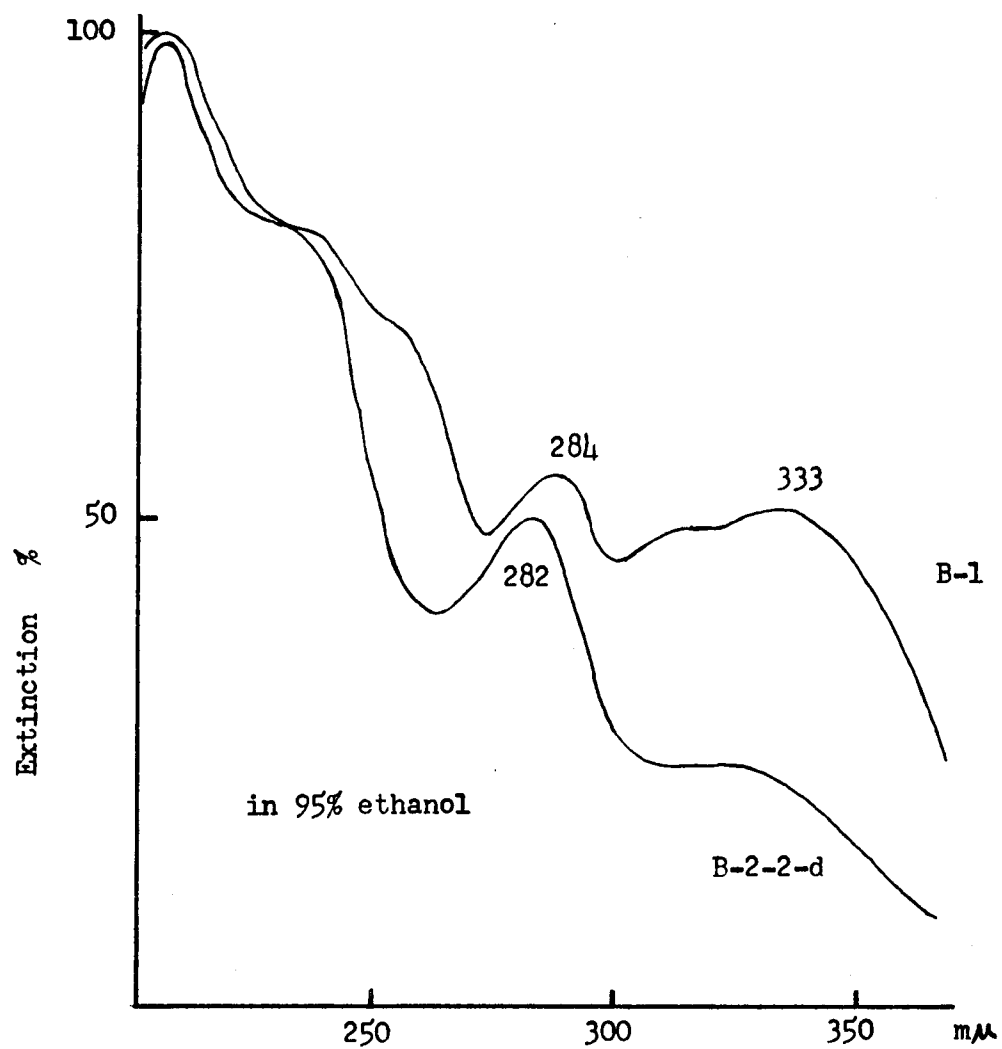


Figure 9. Ultraviolet absorption curves of compound B-1
and B-2-2-d.

DISCUSSION

The results of the experimental work on the Douglas fir bark fines are summarized in Table III.

As it was mentioned previously the origin of the ethyl esters obtained in this experimental work must be from the free acids which were split off from the phenolic acid in the bark sample. Therefore, it can be said that the major products of the ethanolysis of the Douglas fir bark fines were ferulic acid, protocatechuic acid, and vanillic acid.

Although guaiacyl group containing monomers were obtained from Douglas fir bark phenolic acid, none of the Hibbert's ketones were obtained by almost the same reaction condition as was used to obtain them from Douglas fir decayed wood. This is opposed to the previous findings that some Hibbert's ketones were obtained by ethanolysis of Japanese red pine bark phenolic acid (20). However, because the bark fines sample used in this work had a very high Klason lignin content, had almost the same value of one percent NaOH solubility, and had the very fine particle size, then one could assume that the phenolic acid fraction had accumulated through mechanical action in the preparation of the sample. Later the author detected some Hibbert's ketones in a different Douglas fir bark sample which contained some bast fibers and cork fragments. Work on this sample is still in progress.

Ferulic acid is the only C₆-C₃ type compound which was obtained in this experimental work from Douglas fir bark fines. Because no Hibbert's ketones were obtained, one could conclude the building units in the bark phenolic acid are different from those in wood lignin. The fairly large yield of protocatechuic acid, which is consistent with the low methoxyl content of the bark phenolic acids, indicates that bark phenolic acids may be a poly-flavonoid type compound and a "B" ring in the flavonoid unit may be the catechol nucleus (6).

The occurrence of vanillic acid may be caused by the oxidation of ferulic acid type building units. However, the vanillic acid may be derived from the possibility that the poly-flavonoid structure is a mixture of two types of flavonoid units; namely, a catechol type "B" ring and a guaiacyl type "B" ring.

In order to obtain a more complete picture of the chemical nature of the phenolic acids it will be necessary to isolate larger molecular fragments. In this connection, the author has obtained some interesting substances which show some anthocyanidin-like properties in the experiments still in progress. They may be the key substances to solve this problem.

Table III. Paper Partition Chromatogram of Isolated Compounds from the Ether Soluble Oil from the Ethanolysis of Douglas Fir Bark Fines.

Substance	Rf*	Rf**	Color UV	Diazotized benzidine
Identified compounds:				
Ethyl ferulate	0.80	0.95	blue	yellow
Ethyl protocatechuate	0.63	0.23	blue	orange
Ethyl vanillate	0.86	0.81	blue	orange
Protocatechuic acid	0.02	0.01	violet	yellow brown
Vanillic acid	0.07	0.12	violet	yellow brown
Unidentified:				
B-1	0.89	0.76	bright blue	pink
B-2-2-d	0.79	0.00	blue	brown

* 3% aqueous ammonia saturated n-butanol

** Benzene : ligroin : methanol (5 : 5 : 1 v/v)

IV. SUMMARY

The purpose of this research was to examine the monomeric products obtained by the ethanolysis of Douglas fir bark.

Douglas fir bark fines (Silvacon #490) were used in this work. Decayed Douglas fir wood was also subjected to the ethanolysis reaction and the products were examined in a like manner.

A yield of 2.5 percent of ether soluble oil based on the Klason lignin content was obtained from the ethanolysis of the bark. This yield includes 0.28 percent of ether soluble oil from water insoluble tar-like substance of the ethanolysis product which was designated "PE" fraction, and the rest was ether soluble oil from the water soluble fraction of the ethanolysis products which was designated the "LE" fraction. From a decayed Douglas fir wood sample with 53.9 percent lignin content, a yield of 9.8 percent of ether soluble oil was obtained based on the Klason lignin content.

Two dimensional paper partition chromatography demonstrated the presence of 12 compounds in the bark "PE" fraction, four compounds in the bark "LE" fraction, and nine compounds in the ether soluble oil from the ethanolysis of the decayed wood.

Some of Hibbert's monomers were detected in the ethanolysis oil from the wood ethanolysis products. They are

1-ethoxy-1-guaiacyl-2-propanone, 2-ethoxy-1-guaiacyl-1-propanone, guaiacyl acetone, vanilloylacetyl, vanillin, and vanillic acid.

None of the Hibbert's ketones were isolated from the ether soluble oil of the bark ethanolysis products.

Ethyl ferulate which was isolated from the bark "PE" fraction was the only phenyl propane type compound in the ether soluble oil from the bark ethanolysis products. Ethyl protococatechuate, vanillic acid, and protocatechuic acid was isolated from both the "PE" and the "LE" fractions. Ethyl vanillate was isolated from the "LE" fraction.

Two unidentified compounds were isolated from the bark "PE" fractions. They were both recognized as phenol compounds by UV or IR absorption studies.

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