

A COMPARISON OF THE EXTRACTIVES
FROM FOUR SPECIES OF TRUE FIRS

by

GARY LAWRENCE CARLBERG

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1960

APPROVED:

Redacted for privacy

Professor of Chemistry

In Charge of Major

Redacted for privacy

Chairman of Department of Chemistry

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

Date thesis is presented May 10, 1960

Typed by F. Eleanor Jones

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. E. F. Kurth for his helpful guidance during the preparation of this thesis.

Appreciation is also expressed to the Crown Zellerbach Foundation for financial aid.

TABLE OF CONTENTS

| | Page |
|--|------|
| INTRODUCTION | 1 |
| COLLECTION AND PREPARATION OF SAMPLES | 3 |
| EXPERIMENTAL PROCEDURE | 5 |
| Extractive Content of Wood Samples. | 5 |
| Volatile Oils | 9 |
| Previous Investigation | 9 |
| Extraction of Oils | 11 |
| Gas Partition Chromatographic Analysis | 15 |
| A. Preliminary Studies | 15 |
| B. Chromatography of Oils. | 24 |
| Alcohol Extract | 27 |
| Extraction and Fractionation of Extract. | 27 |
| Spectral Analysis. | 34 |
| Paper Chromatographic Analysis | 40 |
| DISCUSSION AND SUMMARY | 48 |
| BIBLIOGRAPHY | 53 |

LIST OF TABLES

| | Page |
|---|------|
| TABLE 1 - Extractive Content of Wood Samples. . . . | 7 |
| TABLE 2 - Retention Volumes With Craig Polyester | |
| Column. | 21 |
| TABLE 3 - Retention Volumes With Perkin-Elmer | |
| Column A | 23 |
| TABLE 4 - Percentage of Fractions of Alcohol | |
| Extract | 33 |

LIST OF FIGURES

| | |
|---|----|
| FIGURE 1 - Fractionation of Ether Extract | 14 |
| FIGURE 2 - Gas Chromatographic Separation of | |
| Terpene Hydrocarbons | 18 |
| FIGURE 3 - Fractionation of Alcohol Extract | 28 |
| FIGURE 4 - Ultraviolet Spectra of Alcohol Extract | |
| of Pacific Silver Fir. | 36 |
| FIGURE 5 - Paper Chromatographic Separation With | |
| Water as the Developing Solvent. | 43 |

A COMPARISON OF THE EXTRACTIVES FROM FOUR SPECIES OF TRUE FIRS

INTRODUCTION

Much interest has recently been expressed in finding a method for distinguishing among the woods of the various species of the true firs. In the forests, these species may be easily identified by examining the bark and foliage, but on the basis of wood anatomy, the true firs cannot be distinguished (6, p. 404).

The true firs, as a group, rank fourth in abundance in the Pacific Northwest, being exceeded by Douglas fir, Ponderosa pine, and Western hemlock.

In 1957 the volume of true fir sawtimber available in Washington and Oregon was estimated to be 73,500 million board feet (11). The log use for the same year totaled 624 million board feet; specifically, 380 million board feet for lumber, 220 million board feet for pulp and paper, and 24 million board feet for plywood. Although the stand of true firs represents 10 percent of the total stand of sawtimber in these two states, the log use represents only five percent of the total log usage.

The four most important species of the true firs in the Pacific Northwest have been chosen for study. These are: Pacific silver fir, (Abies amabilis Loud.); noble fir, (Abies procera Rehd.); white fir, (Abies concolor

Lindl. and Gord.); and grand fir, (Abies grandis Lindl.).

Although the wood anatomy of these species is nearly identical, the strength properties are quite different. Noble fir has the greatest strength of the true firs, and was formerly used in aircraft manufacturing. White fir, on the other hand, has poor strength properties for building materials. All of the species mentioned, except white fir, can be marketed as Douglas fir plywood, since their strength properties are quite similar to those of Douglas fir.

Therefore a definite need exists to be able to distinguish between the species of true firs. This investigation was undertaken when a literature search revealed that very little information existed concerning the extractives from the wood of the true firs. The intent of the study is to compare various fractions of the extractives of these four species in an attempt to find differences on which to base a distinguishing test.

COLLECTION AND PREPARATION OF SAMPLES

The wood samples used in this investigation were obtained and identified by various reliable sources.

White fir, (Abies concolor Lindl. and Gord.) was provided by Weyerhaeuser Timber Company, Longview, Washington. The disk provided was 7 inches in diameter and had 13 annual rings. The wood was sound throughout, although it appeared to be quite low density wood, exhibiting wide growth rings.

Noble fir, (Abies procera Rehd.), and Pacific silver fir, (Abies amabilis Loud.), were both provided by the Willamette Valley Lumber Company, and were obtained from their logging site in the Valsetz area, Oregon. The noble fir sample was about 24 inches in diameter and contained 392 annual rings. The wood was very close grained, but contained spongy pith and sapwood regions. The heartwood region was readily discernible and it contained good sound wood, thus it was this portion which was used for the investigation.

The sample of Pacific silver fir was a 17 inch diameter disk, having 140 annual rings. The wood was sound throughout, and the total wood was used.

Grand fir, (Abies grandis Lindl.), was obtained by Mr. Wade Howell, forester for the I. P. Miller Lumber

Company, Dawson, Oregon. This disk, from the butt region of the tree, was about 10 inches in diameter and had 64 annual rings. The wood was sound throughout and the total wood was used in the investigation.

All of the above mentioned samples were prepared for analysis in the same manner. After removing the bark, about 200 grams of each wood was ground in a Wiley mill to pass a 40 mesh screen. This wood was air dried and stored in airtight jars for the determination of extractive content.

To obtain enough extractives for further investigation, about 2 kilograms of sawdust from each wood was prepared by sawing the wood across the grain on a table saw.

No attempt was made to make a thorough sampling of any one of the species investigated and furthermore the samples of each species used were not comparable to each other. The results of this investigation will thus be limited by this consideration. For example, it is to be expected that the extractive content of the sample of noble fir will be higher than the other samples, because only the extractive-rich heartwood was used.

EXPERIMENTAL PROCEDURE

EXTRACTIVE CONTENT OF WOOD SAMPLES

The extractive content of grand fir, noble fir, and white fir woods have been reported (14, 16). This determination was done to substantiate the results given for these species and to compare with that of the Pacific silver fir which has not previously been reported in the literature.

Previous determinations showed the extractive content to be relatively quite low. Therefore it was felt that the Tappi Standard procedure, by using only a two gram sample, would yield inferior results due to the small amount of extractives to be weighed. Therefore, larger samples were used to increase the accuracy.

The procedures used for the ether and water solubilities were generally those of Tappi Standards T5m-45 and T1m (27), respectively, except as modified to accommodate the larger samples. Glass Soxhlet extractors having a 50 mm diameter extraction chamber and 100 mm high siphon arms were used for the extractions.

Wood samples of known moisture content, ground in a Wiley mill to pass a 40 mesh screen were used for the analysis. Samples of approximately 20 grams each were

weighed into tared fiber extraction thimbles, 43x123 mm. The samples were extracted successively with 250 ml portions of ethyl ether and 95% ethyl alcohol. The time of extraction was 9 hours for each solvent, and care was taken to completely dry out the solvent before proceeding with the next extraction.

The extract in each case, was evaporated down to about 20 ml, transferred to a previously weighed flask, and dried to constant weight by 30 minute heating periods in an oven at 105°C.

The analysis for water solubility of the wood by the Tappi Standard method is an empirical method depending on the length of time the sample is in contact with the water. The samples, previously extracted with ether and alcohol were emptied into one liter, wide mouth Erlenmeyer flasks, and 750 ml of boiling distilled water were added. The flasks, stoppered and containing "reflux" tubes of 5 mm glass tubing about 50 cm long, were then placed in a steam bath and heated for 3 hours. At the end of this time the contents were filtered into their respective extraction thimbles and washed with 200 ml of hot distilled water.

The percent hot water extractives was calculated indirectly, since the large volume of extract solution made direct weighing of the extract impractical. The extracted

samples were allowed to dry in the atmosphere, weighed and small portions of wood from each sample were taken for a determination of moisture content. The weight of total extractives was obtained by subtracting the oven dry weight of extracted wood from the oven dry weight of unextracted wood. From this, the weight of hot water extract was obtained by subtracting the weights of ether and alcohol extracts.

The results, shown in Table 1, are the average values of duplicate samples of each species. This table shows the percentage of each extract on the basis of the oven dry weight of wood. All experimental variables in the analysis were maintained constant relative to each sample, thus the results are comparable.

TABLE 1

EXTRACTIVE CONTENT OF WOOD SAMPLES
Percent of oven-dry weight of unextracted wood

| Species | Ethyl Ether | Ethyl Alcohol | Hot Water | Sum of Extractives |
|-----------------------|----------------|------------------|--------------|-----------------------|
| <u>Abies amabilis</u> | 0.22 | 1.40 | 2.22 | 3.84 |
| <u>Abies concolor</u> | 0.24 | 1.83 | 1.28 | 3.35 |
| <u>Abies grandis</u> | 0.19 | 1.43 | 1.20 | 2.82 |
| <u>Abies procera</u> | 0.21 | 2.25 | 2.28 | 4.74 |

Although minor differences can be seen among the

woods tested, no valid conclusion can be made, due to the varying ages of the trees. The values are, however, of the same order of magnitude and demonstrate that the extractive content of these fir species is low.

VOLATILE OILS

Previous Investigation

The volatile oils of the pines have been investigated thoroughly and their composition can be used to differentiate between species and sub-species of this genus. This seemed a good starting point to find differences among the species of Abies under investigation.

A search of the literature showed that a study of the volatile oils from the woods of the species under investigation had not been made. The oils from the bark, leaves and twigs, and oleoresins have been studied for each of the species. Lynn (18), in studying the leaf oils from noble fir, found that this oil consisted of 78 percent terpenes.

Schorger (24) compared the oil from the bark with that from the leaves and twigs of white fir in composition and content. Differences in content were noted for five terpenes common to both and the leaves and twigs contained 1-camphene in appreciable concentration, whereas the bark contained no camphene. This would indicate that there may well be differences in the composition of the volatile oils from the wood, compared to those from the bark and foliage.

Pacific silver fir and grand fir barks were steam distilled for their volatile oils by Trupp (29), with a yield of 0.50 percent and 0.37 percent respectively. About three fourths of this oil was found to consist of terpenes. The leaves and twigs of Pacific silver fir however, yielded only 0.08 percent volatile oils.

The leaves and twigs of grand fir were analyzed for volatile oils (23), as were the gum oleoresins of Pacific silver fir and white fir (1, 8).

All of these analyses showed the presence of 1-alpha pinene, 1-beta pinene, 1-beta phellandrene and, with the exception of Pacific silver fir, camphene. In addition grand fir and white fir contained borneol and bornyl acetate and grand fir also contained 1-cadinene. The oleoresins from Pacific silver fir and the bark of white fir both contained dipentene (racemic limonene). The remainder of the volatile oils was found to consist of aldehydes and traces of phenols and free acids.

Recently Curkal (7) investigated the oils obtained by commercial steam distillation of needles of white fir. A gas chromatographic analysis showed the presence of seven constituents, although they were not identified.

Extraction of Oils

Volatile oils can be obtained by either direct steam distillation of the wood, or by a steam distillation of an ether extract of the wood. A preliminary trial of these methods was undertaken with sawdust from a small sapling of noble fir about 3 inches in diameter. Although a slightly greater yield was obtained by direct distillation, the experimental difficulties for large batches proved to be great. Therefore the alternate method of steam distillation of the ether extract was chosen.

The extraction and further fractionation of the extract is shown diagrammatically in Figure 1. Samples of approximately 300 grams of sawdust each were placed in cloth bags and extracted with ethyl ether in a 2200 ml Borosilicate Soxhlet-type extractor. Five such batches of each species were extracted in the same solvent, only enough new solvent being added to a new batch to replace that lost by hold-up on the extracted sample just removed. Each batch was soaked in the solvent overnight for about 10 hours and then extracted for about 14 hours.

The extract was distilled while still in the Soxhlet, removing the solvent until about 500 ml of solution remained. The extract was transferred to a conventional vacuum distillation apparatus and taken to dryness at

about 135 mm pressure. The temperature was maintained at about 25-30° C, preventing heat damage and volatilization. The resulting extract was a slightly mobile yellow colored oil for all species extracted, except for white fir, which was brownish red.

Fractionation of the extract was accomplished in the following manner. Four 50 ml portions of petroleum ether, boiling range 35°-60° C, were successively shaken with the ether extract, and filtered in a suction filter apparatus. This apparatus consisted of a two-port vacuum desiccator lid fitted with a small Hirsch funnel.

The petroleum ether insoluble material was dried in a stream of air and further extracted with three 50 ml portions of hot benzene, and filtered in the suction filter apparatus.

The benzene insoluble material was found to be nearly completely soluble in alcohol and moist acetone, and should contain any flavonoid materials if present.

All of these extracts were dried under vacuum and weighed by difference to obtain a general indication of their percentage in the ether extract, and these values are shown in Figure 1.

Further fractionation of the petroleum ether soluble extract involved steam distillation to obtain the volatile

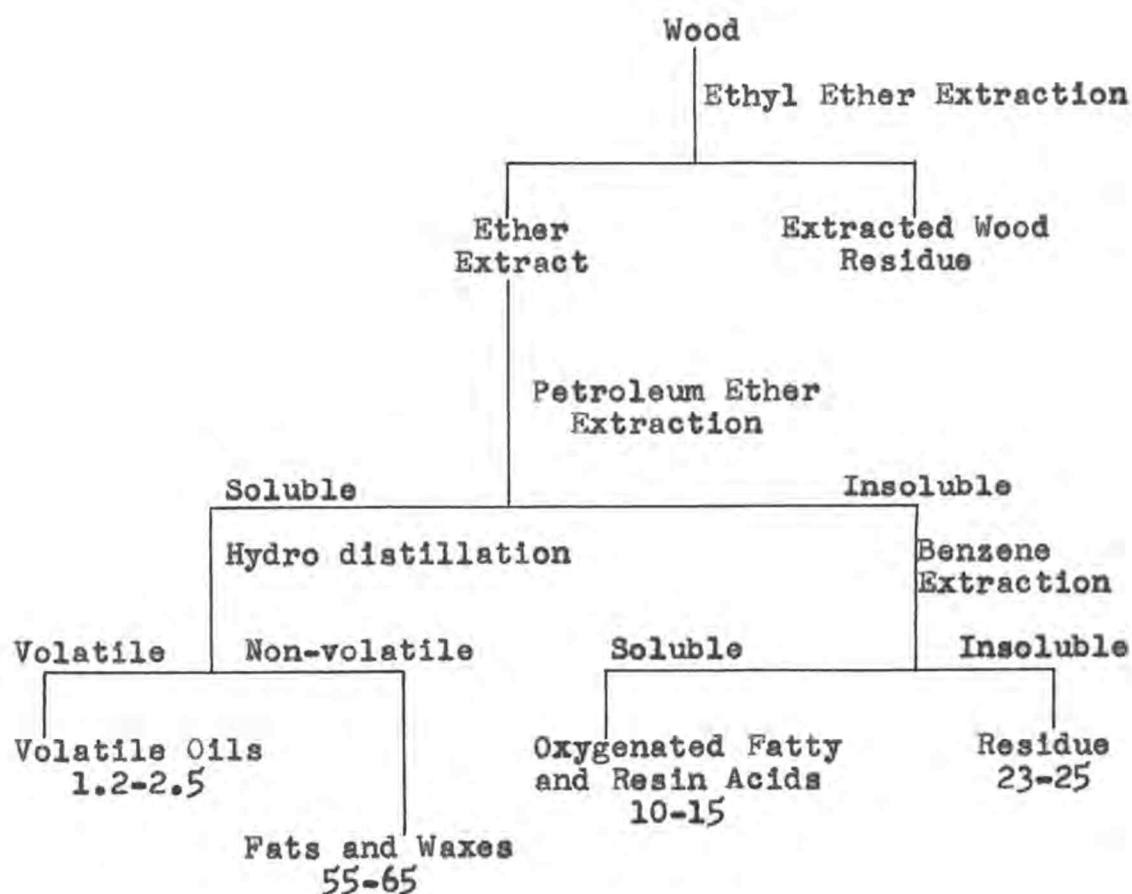
oils. The petroleum ether was evaporated by a stream of air in a one liter flask, and 200-250 ml of water added. This was then placed in a hydrodistillation apparatus composed of the one liter distillation flask which was heated by an electric flask heater, a Clevenger trap, designed for oils lighter than water, and a five-bulb Ahlin condenser. The Clevenger trap was constructed from a five ml graduated moisture test receiver, fitted with a stopcock to drain the oil sample, a sidearm to drain the water back into the flask, and standard taper joints for the flask and condenser. The distillation was continued for one hour, the solution was cooled and salted out with sodium chloride and then redistilled for another hour. Salting out was done to prevent the possible loss of the terpene alcohols which are slightly soluble in water. The yield was extremely small, 0.2 to 0.5 ml per sample distilled. To minimize losses in draining the oil from the Clevenger trap, the oil and water remaining in the trap after distillation were extracted with petroleum ether. The petroleum ether layer, containing the volatile oils, was separated and dried in a vacuum desiccator to constant weight. The samples were then subjected to analysis by gas chromatography.

The residue left after hydrodistillation consists of

the waxes and fats. This wax-like material was light tan in color with the exception of that from white fir which was a dark brown color. This fact would appear to indicate a difference in the composition of the white fir wax, and the wax from the other species, inasmuch as the treatment was similar for all extracts.

FIGURE 1

FRACTIONATION OF ETHER EXTRACT
Percentages based on weight of ethyl ether extract



Gas Partition Chromatographic Analysis

A. Preliminary Studies.

Gas partition chromatography has given the chemist a powerful new tool for analysis of small amounts of volatile materials. This method seemed ideal for detection and identification of the terpene constituents of the small amounts of volatile oils obtained in this investigation.

A Perkin-Elmer Model 154 B Vapor Fractometer with a thermistor-type detector and a 0-10 mv recorder was used in the present study. Four different column materials were tested with artificial mixtures of terpenes in an attempt to find a column which would give good resolution at a relatively low temperature. The terpenes are quite heat sensitive, thus to prevent alteration of the original components, the low temperature is quite desirable. The liquid stationary phases tested were Silicone fluid, Carbowax 600, polyester of butanediol succinate and diisodecyl phthalate.

Two one meter U-shaped columns, in series in the instrument, containing a stationary liquid phase of Silicone fluid on a support of celite gave very poor results and this material was not tested further.

A one meter column containing Carbowax 600

(Polyethylene glycol) as the stationary phase on a solid support of celite, 3:1 by weight, gave a slight separation of the terpenes, and was tested further.

W-shaped columns were constructed of six mm outside diameter pyrex glass tubing, seven feet long each. These columns were then filled with the packing material by inserting a glass wool plug and applying a vacuum to one end. The material was packed to a uniform tightness by vibrating the tube extensively. Two such columns could be installed in the heating chamber of the gas chromatograph giving 14 feet total column length. The Carbowax 600 has been successfully used by Bernhard (4) for the separation of five terpene hydrocarbons in a 20 foot column. The results in this investigation were not as good as with the longer column of Bernhard and definitely inferior to the polyester succinate column of equal length. A temperature of 145°C and flow rate of as little as 10 cc He per minute did not give sufficiently good resolution.

Craig polyester (butanediol succinate), 3:1 by weight on a solid support of Chromasorb, provided the best separation of the terpene mixture of the columns tested. As with the Carbowax column, this was packed in two W-shaped glass columns, each 7 feet long and installed in series in the chromatograph. Using artificial mixtures of terpenes, it was found that the best resolution was obtained using a

column temperature of 110°C and a He flow rate of 8.5cc per minute (He flow measured at the pressure of the column exit, 1 atmosphere, and the temperature of the column). Lowering the temperature or pressure from these optimum conditions merely broadened the peaks and stretched them out relative to one another but gave no better resolution. Increasing either temperature or pressure produced sharper peaks which were closer together, actually decreasing the resolution. Figure 2 graphically represents a chromatogram of eight terpene hydrocarbons and para-cymene separated on this column under the optimum conditions. The oxygenated terpenes required a great deal more time to elute from the column and are not shown.

The length of time required for the oxygenated terpenes, i.e. borneol, bornyl acetate, terpineol, prompted a search for a column which would give fair resolution for the lower boiling terpene hydrocarbons and at the same time resolve the oxygenated terpenes in a reasonable time. Perkin-Elmer column A, which contains diisodecyl phthalate as a liquid phase on diatomaceous earth, proved to satisfy this requirement. On this 2 meter column, these higher boiling oxygenated terpenes eluted in the reasonable time of 45-50 minutes when the column temperature was 140°C and the He flow rate was 20cc He per minute, (measured at

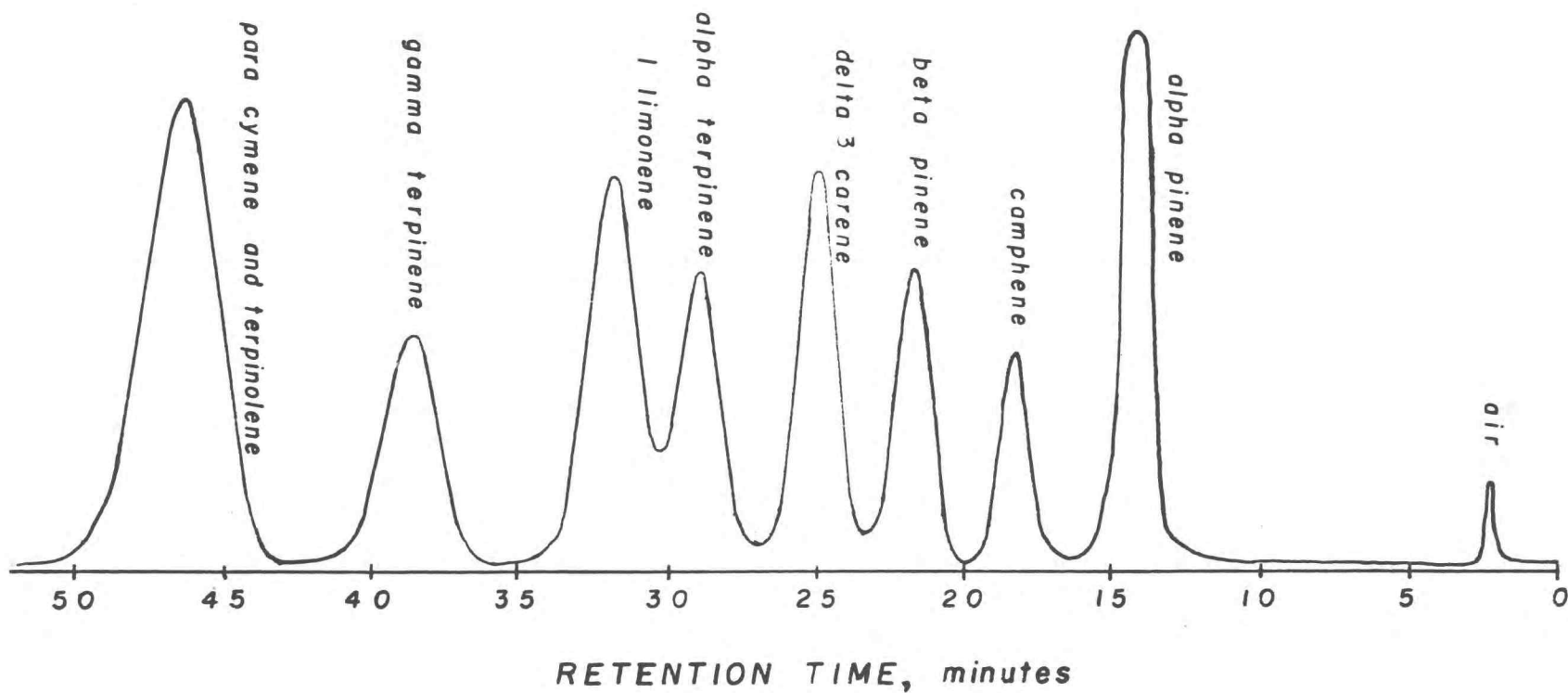


FIGURE 2 - Gas Chromatographic Separation of Terpene Hydrocarbons. Craig polyester succinate column, 14 feet long, at 110°C and flow rate of 8.5 cc per minute.

the pressure of the column exit, 1 atmosphere, and the temperature of the column).

The retention time, the time required to reach the peak maximum from the injection of the sample, is characteristic of a given component under a given set of conditions. A component can be tentatively identified by comparing its retention time to that of reference materials, under the same conditions. The retention time of a given component is dependent upon many factors. When using the same column and instrument however, the column temperature and carrier gas flow rate are the only variables. The temperature can be controlled quite accurately, but the flow rate cannot, and thus the retention time for a component may vary a few tenths of a second. A more accurate value for comparison is the ratio of the retention time of the component to that of reference compound.

The retention time however, is a relative value and applicable only when using the exact same instrument and column. The retention volume is a unit developed to make retention data transferrable to other instruments. The retention volume, V_R , is merely the product of retention time and carrier gas flow rate, when the flow rate is calculated from the pressure at the column exit and the temperature of the column (28). Relative retention volume

is a useful value, and is the ratio of the retention volume of a component to that of a reference component. The reference substance is usually chosen to have a retention volume near the middle of the range of retention volumes.

The overall performance of a given column is indicated by the theoretical plate number, n . This value may be different for each compound so is taken as the average of n for each individual component separated on the column. The theoretical plate number is calculated from the equation, (28),

$$n = 16 \left(\frac{\text{retention time}}{\text{peak width}} \right)^2$$

These values are measured on the chromatogram in consistent units so that the ratio is dimension-less. The retention time is the distance measured from the injection point to the peak maximum, and the peak width is the distance between the sides of the peak, measured at its base.

Table 2 shows the retention volumes and relative retention volumes, relative to 1-limonene, for nine terpene hydrocarbons on the Craig polyester column. This table also shows the calculated theoretical plate number for each peak, and the average value of 1370. The

TABLE 2

RETENTION VOLUMES WITH CRAIG POLYESTER COLUMN^a

| Substance | Boiling point ^b | V _r ^c | V _r relative to 1-limonene | Theoretical plates |
|--------------------------------|----------------------------|-----------------------------|---------------------------------------|--------------------|
| Alpha-pinene | 155 | 109 | .46 | 1030 |
| camphene | 160 | 138 | .58 | 1210 |
| beta-pinene | 164 | 163 | .69 | 1320 |
| delta3-carene | 171 | 186 | .79 | 1470 |
| alpha-terpinene | 174 | 216 | .91 | 1520 |
| 1-limonene | 176 | 237 | 1.00 | 1350 |
| beta phellandrene ^d | 179 | 247 | 1.04 | ---- |
| gamma-terpinene | 183 | 286 | 1.20 | 1680 |
| terpinolene | 185 | 341 | 1.44 | ---- |

Average 1370

- a. Polyester of butanediol succinate 25/75 by weight on Chromosorb. Supplied by Wilkins Instrument and Research, Walnut Creek, California. Column 14 feet long, temperature 110°, He flow rate 8.5cc per minute.
- b. Degrees C at 760 mm Hg pressure.
- c. In cc He at 760 mm at 110°C.
- d. Unavailable during study. Adapted from results of Bernhard (4).

theoretical plate number of terpinolene was not calculated because para-cymene was run in the mixture and elutes at the same time as terpinolene. Beta phellandrene was unavailable, so the values given in the table for it were calculated from the retention volume data of Bernhard (4).

Table 3 lists the retention volumes and relative retention volumes for the same terpenes as in Table 2, less beta phellandrene, and also for the three oxygenated terpenes on the Perkin-Elmer column A.

In all cases the position of each peak was identified by running individual components. Impurities in the known terpenes caused little trouble, since the main constituent could be identified from the largest peak. These values were obtained as reference materials since they represent the terpenes most probable to be found in the oils under study.

Further tests with the Craig polyester column showed that any one of the terpene hydrocarbons with a concentration of 0.1 percent of the oil could be detected on the chromatogram. This was done by diluting the known terpenes to various concentrations with Skellysolve B and noting the lowest concentration which would exhibit a peak on the chromatogram. The Skellysolve B eluted before any of the terpenes and caused no interference.

TABLE 3

RETENTION VOLUMES WITH PERKIN-ELMER COLUMN A^a

| Substance | Boiling point ^b | V _r ^c | V _r relative to 1-limonene |
|-----------------|----------------------------|-----------------------------|---------------------------------------|
| alpha-pinene | 155 | 130 | .56 |
| camphene | 160 | 150 | .64 |
| beta-pinene | 164 | 178 | .76 |
| delta 3-carene | 171 | 202 | .87 |
| alpha-terpinene | 174 | 216 | .92 |
| 1-limonene | 176 | 234 | 1.00 |
| gamma-terpinene | 183 | 266 | 1.14 |
| terpinolene | 185 | 304 | 1.30 |
| borneol | 212 | 774 | 3.30 |
| alpha-terpineol | 219 | 856 | 3.65 |
| bornyl acetate | 226 | 948 | 4.05 |

a. Diisodecyl phthalate on diatomaceous earth. Column 2 m long, temperature 150°, He flow rate 20 cc per minute.

b. Degrees C at 760 mm Hg pressure.

c. In cc He at 760 mm pressure and 150°C.

B. Chromatography of Oils.

The steam volatile oils obtained were run through both the Craig polyester succinate column and the Perkin-Elmer column A. The sample size used was 0.01 ml injected with a 0.25 ml syringe. To be able to run several trials on each column, the volume of the oil was doubled with Skellysolve B. Unfortunately the chromatograms indicated that there were no terpenes in the steam volatile material. On occasion a small peak would appear but when rerun, the peak was not duplicated. This anomaly was attributed to contaminants in the column causing a peak upon eluting at random times.

These negative results left doubt of the experimental procedures in obtaining the steam volatiles. Therefore the procedure was tested by "doping" a 250 g sample of wood with a mixture of terpenes. Various amounts of six terpenes, representing from 0.2 to 0.01 percent oil, on the basis of the oven-dry weight of wood, were placed in each of two wood samples. These were ether extracted and fractionated as shown in Figure 1, using the same procedure as that used with the large samples. The yield of steam volatiles was only 22 percent of the terpenes added, however with a total of only 1.5 g added to 250 g of wood, the loss is likely in the many extractions encountered in

the procedure, and in the possibility of adsorption of the oil on the wood. When chromatographed on both columns, even after diluting to double volume as with the other oils, all components of the mixture were resolved, although the component at 0.01 percent in the wood was at near the limit of resolution.

In another attempt to find some oils in the wood, a small sapling of noble fir about 3 inches in diameter was obtained from Mary's Peak, Oregon. This sapling was sawed on a table saw and used immediately and thus should show terpene constituents if present in the wood at appreciable concentration. 200 g of this sawdust was steam distilled in a conventional steam distillation apparatus. The distillate was extracted with petroleum ether and dried in a vacuum desiccator to constant weight. The yield of steam volatiles was 0.15 percent on the basis of oven dry weight of wood. This oil, when chromatographed, gave no indication of terpenoid compounds, as evidenced by the absence of peaks in the region of the chromatogram shown to contain terpenoid peaks.

It was concluded that the method and procedures are valid and would show any of the terpenes having a concentration in the wood of at least 0.01 percent. Although it would be good to know the composition of the

terpenes in the oil, and this could probably be done by taking a larger sample of wood and increasing the sensitivity of the recorder, a knowledge of the extremely small percentage occurrence in wood limits the use of this test as a means of differentiating the species. Therefore the volatile oils were not pursued further.

As to the actual composition of the steam volatiles, it was assumed that they contained some sesquiterpenes and aldehydes, and probably some fats and waxes carried over in the distillation.

ALCOHOL EXTRACT

The ethyl alcohol extract is largely phenolic in nature, consisting of coloring matters, phlobaphenes, tannins, and other polyphenolic substances. It was felt that this fraction might contain significantly different components among the true fir species to afford a distinguishing chemical test.

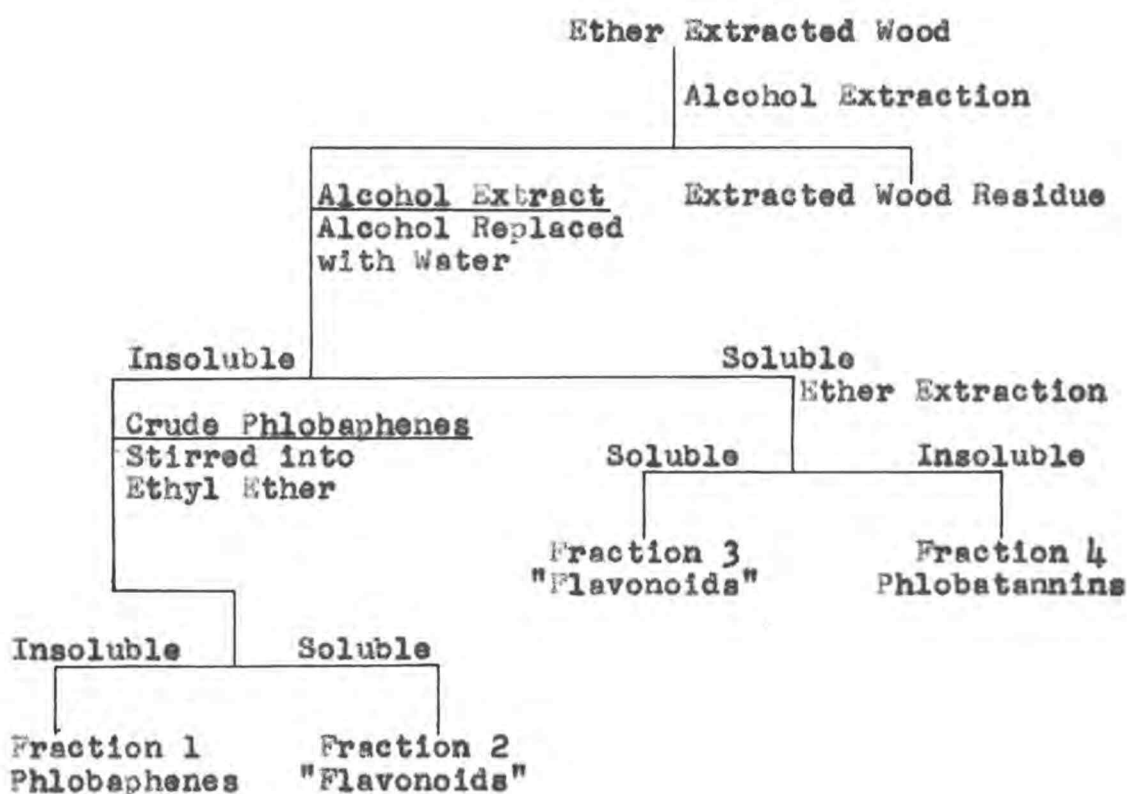
Extraction and Fractionation of Extract

The wood sawdust, previously extracted with ethyl ether, was extracted with 95 percent ethyl alcohol. As with the ether extraction, this alcohol extraction was done in five batches for each species, the sawdust being placed in a cloth bag and extracted in a 2200 ml Boro-silicate Soxhlet-type extractor. New solvent was added only to replace that lost by hold-up or evaporation. Each extraction was continued for 24 hours, at which time the solvent showed no signs of continued extraction. The dark red extract from each species was distilled in the extractor until about 800 ml of solution remained. A five ml portion of each extract was placed in a small crucible, the solvent evaporated and the residue dried to constant weight at 105°C. In this way the concentration of the extract from each species was shown to be 0.013 to 0.017

grams per ml.

The alcohol extract was fractionated as shown in Figure 3. A 500 ml portion of each extract was evaporated to one half volume in a rotary vacuum evaporator. The temperature of the extract was kept at 25°C or lower and distillation occurred at about 10 mm pressure in this apparatus.

FIGURE 3
FRACTIONATION OF ALCOHOL EXTRACT



Distilled water (325 ml) was added to the dark red brown extract and the alcohol was removed by distillation in the rotary vacuum evaporator. Once again the temperature was maintained at 25°C, and it was found that by keeping the pressure at 10 mm the alcohol would distill off leaving the precipitated phlobaphenes and a water solution of tannins and coloring matters. The use of the vacuum evaporator in this procedure proved to be much easier and more efficient than evaporation in a stream of air, as had been previously employed.

After centrifuging this mixture, the solution above the precipitated phlobaphenes remained extremely milky, indicating dispersed colloidal phlobaphenes. The solution was transferred to a 500 ml polyethylene wash bottle and frozen in a dry ice-acetone bath for six to eight hours. When thawed and centrifuged, a large amount of precipitated phlobaphene resulted and the solution remaining was clear and yellow in color. The precipitated phlobaphenes were washed with two 25 ml portions of water and these washings were added to the filtrate. Paper chromatography in several solvents demonstrated that the original precipitate and that obtained by freezing-out were identical. These two portions of crude phlobaphenes were then combined by dissolving in a small volume of acetone, and then purified

by reprecipitating into 500 ml of dry ethyl ether. The resulting precipitate was washed with ether, dried, and crushed to a fine powder. This powder was chocolate-brown in color for all species except grand fir which was light tan in color.

A large percentage of the crude phlobaphene remained soluble in the ether into which the crude was precipitated. Therefore the ether solution was evaporated down to dryness, taken up in a minimum of acetone and reprecipitated into ether, producing another small batch of phlobaphene precipitate. The ether solution still contained a large amount of material, so it was again taken to dryness to assure the removal of all acetone, in which the phlobaphenes are extremely soluble. The resulting gummy material was then extracted by shaking and stirring with three 25 ml portions of ether. This ether soluble portion, fraction 2, was retained for paper chromatography.

The water soluble portion, after removal of the phlobaphenes, was then exhaustively extracted with four 50 ml batches of ethyl ether in a separatory funnel. The resulting ether extract was evaporated down to 50 ml and the solution was dried over an excess of anhydrous sodium sulfate. This ether extract, fraction 3, was light yellow in color for all species.

If present in the wood, any flavonoid compounds would be found in fractions 2 and 3 of the alcohol extract. Therefore these two fractions were subjected to two color tests used to distinguish flavonoid compounds. The Wilson boric acid color test for flavones (30), gave negative results for both fraction 2 and 3, of all species. A much used color test for flavonoids depends upon their reduction to the red-violet colored anthocyanin with zinc and hydrochloric acid. Fraction 2 of all species when thus tested, gave a brown solution, negative for flavanones. Fraction 3 of the various species, however, exhibited distinctive colors. This fraction gave a light brown color for Pacific silver fir and grand fir, a very deep red brown color for white fir and a light cherry red color with noble fir. The test was negative for flavanones in all but noble fir, and this is doubtful, since the anthocyanin color is usually a different shade of red. The varying colors however, indicate a possible test to distinguish the various species. To be completely valid however, this test must be run on several samples of each species. The noble fir sample, it must be remembered, was composed of heartwood from an old tree, and this difference from the other samples may have caused the much different color test.

The water soluble-ether insoluble portion, fraction 4, was shown to consist of tannins. Several classification tests were performed, indicating that the tannins are of the nonhydrolyzable, phlobatannin class. This material is water, alcohol, and acetone soluble and ether insoluble. The water solution precipitated a one percent gelatin solution, and was precipitated by boiling dilute mineral acids, bromine water and formed a clear solution with lead acetate and acetic acid. Ferric chloride produced a brownish green color. All of the extracts reacted in the same manner in all of these tests. Paper chromatography in several solvents showed this fraction to be homogeneous.

If the wood being extracted with alcohol contains moisture, some carbohydrate material will be extracted along with the phenolic material and will be found in the water soluble-ether insoluble fraction. To test for sugars, fraction 4 was chromatographed on paper for 48 hours, using a solvent of n-butyl acetate: pyridine: ethanol: water, (8:2:2:1) (12), allowing the solvent to run off the serrated end of the paper. No evidence of sugars was given when sprayed with two spray reagents; anisidine-hydrochloric acid and alpha-naphthol.

A quantitative determination of the percentage of each of the four fractions of the total alcohol extract

was made for each species. One ml aliquot portions of fractions 2, 3, and 4 were dried and weighed to determine their percentage in the total extract. The percentage of fraction 1 was determined indirectly by taking an aliquot from the solution containing the crude phlobaphene before precipitation into ether. The content of fraction 1 was then taken as the difference between this value and that of fraction 2. The results, shown in Table 4, are given in terms of percentage of the total alcohol extract.

TABLE 4
PERCENTAGE OF FRACTIONS OF ALCOHOL EXTRACT

| Species | Fraction Number | | | |
|-----------------------|-----------------|------|-----|------|
| | 1 | 2 | 3 | 4 |
| <u>Abies amabilis</u> | 22.3 | 31.9 | 3.9 | 36.6 |
| <u>Abies concolor</u> | 20.5 | 28.4 | 5.6 | 37.7 |
| <u>Abies grandis</u> | 14.7 | 13.3 | 6.7 | 57.1 |
| <u>Abies procera</u> | 25.3 | 45.3 | 6.3 | 16.5 |

Although definite differences are noted, it is not possible to draw any conclusions due to the variance in the wood samples taken.

Spectral Analysis

With the hope that characteristic functional group differences might be noted, an infrared absorption curve was run for the total alcohol extract of each species. A portion (about 50 ml) of each extract was placed in an evaporation dish and carefully evaporated to near dryness on a steam bath. These were then placed in a vacuum oven and dried at 50°C and at a pressure of two inches of mercury. Infrared absorption spectra were determined for Nujol mulls of each extract, with a Perkin-Elmer Model 21 double beam infrared spectrophotometer with sodium chloride optics.

The spectra of these extracts were nearly identical, differing only in minor peaks and shoulders. These small differences however, would not indicate the presence of any different functional groups in the total alcohol extracts of the various species, and could definitely not be used to distinguish the various species.

Although the extracts are obviously quite heterogeneous, the infrared spectra demonstrated their phenolic nature. A very large hydroxyl band appeared at 3295 cm^{-1} and phenyl group absorption produced bands at 1595, 1505, and 855 cm^{-1} .

Another portion of the alcohol extract from each

species was diluted with ethyl alcohol to a concentration suitable for producing an ultraviolet spectrum, about 0.0075 g solids per 100 ml of solution. The ultraviolet absorption spectra were determined by means of a Beckman Model DU photoelectric quartz spectrophotometer. The spectrum obtained for Pacific silver fir is reproduced in Figure 4. The spectra obtained for the extracts of the other species differed only slightly in the absorbancy at the maximum and minimum and in the shape of the inflection at 310-315 m μ . The major peak appeared at 281-282 m μ and the minimum at 255 m μ for the extracts of all species.

Hergert (15) has shown the presence of small amounts of catechin and epicatechin in the wood of white fir. The ultraviolet spectra of these compounds show an absorption maximum at 280 m μ , very close to the absorption maximum at 281-282 m μ , found for the total alcohol extracts of the true firs under investigation.

The inflection in the curve indicates a concealed peak in this region. A similar peak in the spectra of compounds related to lignin has been interpreted to be the result of unsaturation, either an ethylenic double bond or a carbonyl group, in conjugation with a benzene ring (19).

Compounds such as 3-hydroxy flavones or flavanones, which contain ortho hydroxy carbonyl groups, are capable

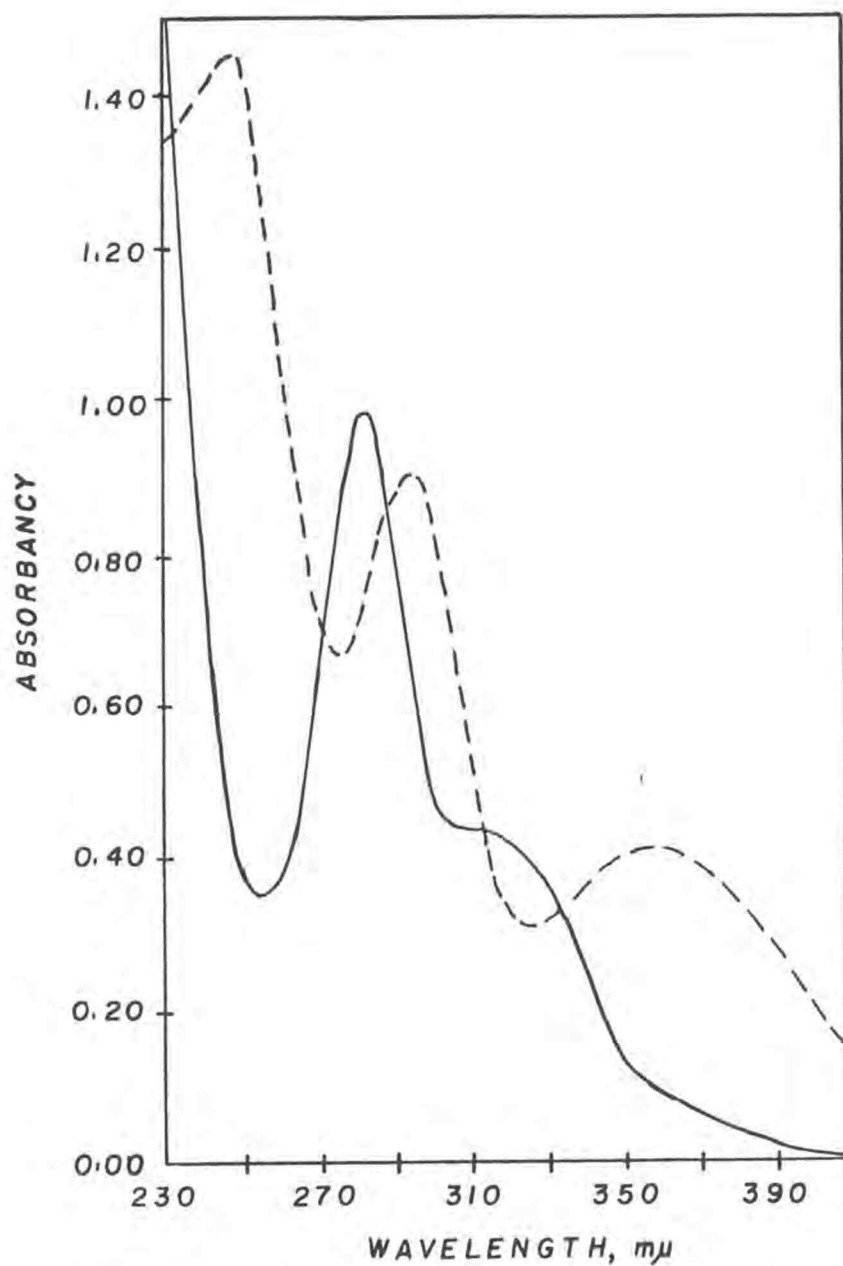


FIGURE 4 - Ultraviolet Spectra of Alcohol Extract of Pacific Silver Fir. (—) 0.0075 grams per 100 ml in 95 percent ethanol, (---) 0.0075 grams per 100 ml in 0.006 N alcoholic KOH.

of forming a complex with aluminum, causing a bathochromic shift in their ultraviolet spectra of from 10 to 70 m μ . Flavans, such as catechin, however, contain no carbonyl group to form the complex and thus show no shift (26). Therefore, 1.2 ml of one percent alcoholic aluminum chloride was added to 10 ml of each alcohol extract, which had been previously diluted to 0.0075 g solids per 100 ml solution. The ultraviolet spectra showed no shift compared to that in neutral alcohol solution.

Alkaline solutions of phenolic compounds have been shown to exhibit characteristic shifts in their ultraviolet spectra, compared to their spectra in neutral solution. These shifts depend on structural characteristics of the molecule which effect its electronic structure. The diluted alcohol extracts of each species were made approximately 0.006 N in KOH and their spectra were determined. The resulting spectra were similar for each species and showed definite bathochromic shifts of all peaks. The spectrum of the extract from Pacific silver fir in alkaline solution is reproduced in Figure 4, along with the spectrum of the same extract in neutral alcohol solution. All of the extracts in alkaline solution developed absorption peaks from the inflection at 310-315 m μ in the neutral solution, which were shifted by about 45 m μ to 360 m μ .

In addition to the total extract, fractions 1, 3, and 4 from Pacific silver fir, representing the phlobaphenes, the flavonoid fraction, and the tannins, respectively, were dissolved in alcohol and alcoholic KOH and their ultraviolet spectra were determined. It has been previously shown that similar fractions from Douglas fir give nearly identical spectra, with the major absorption appearing at 290 m μ (10). These fractions from Pacific silver fir produced similar spectra except that the major absorption occurred at 281-282 m μ , exactly the same as the total extract (Figure 4).

Although the spectra cannot be used to distinguish the woods, an indication of the basic structure can be gained from them. The flavonoids, phlobatannins, and phlobaphenes are intimately related in basic structure. The phlobatannins are thought to be condensation or hydrogen bonded products, having a catechin-like base. The phlobaphenes are further condensation products of phlobatannins. The ultraviolet spectra should thus indicate the basic structure present in all of these substances. Thus, whereas the absorption maxima in Douglas fir extracts of 290 m μ would indicate the flavone or flavanone structure as its basic nucleus, the absorption maximum for the true firs at 281-282 m μ indicates the flavan nucleus as the

basic structure. This is also corroborated by the absence of a shift in the spectra with aluminum chloride, and the definite shift in alkaline solution.

Paper Chromatographic Analysis

Phenolic compounds, typically found in alcohol or acetone extracts, have been investigated a great deal with paper chromatography (9, 13, 20, 21). Many solvents have been employed and a great number of spray reagents have been found which easily detect the phenolic substances on a paper chromatogram. It was therefore decided to investigate the alcohol extract and its fractions for each species by paper chromatography to note any differences in content. Also with the many color reactions of this group of compounds, any gross differences noted on a paper chromatogram might yield a color test to distinguish the species of true firs.

Four microliters of a one percent solution per spot was generally used throughout this work and Whatman #1 chromatography paper was used exclusively. All the chromatograms were run descendingly in a glass tank, 12 inches in diameter by 24 inches high, which was shielded from drafts and uneven temperature by enclosing the apparatus within a cardboard box.

The total alcohol extract was used to test the many developing solvents, to find the ones giving the best separation on the chromatogram. Generally speaking, the organic rich solvents were very poor for chromatographing

this material. Such developing solvents as the organic layer of n-butanol; acetic acid: water, (4:1:5); water saturated secondary butanol; methyl ethyl ketone: water: diethylamine, (921:77:2); methyl ethyl ketone: acetone: water: formic acid, (80:4:12:2); the organic layer of benzene: methyl ethyl ketone: formic acid: water, (900:100:2:98); and the organic layer of benzene: ligroin: water: methanol, (50:50:1:50), all gave very poor or no separation, and in most cases either carried the total spot along with the solvent front or did not move the spot from the origin.

In the same way, strongly acidic solvents such as 50 percent acetic acid; and acetic acid: concentrated hydrochloric acid: water, (30:3:10), carried the whole spot to very high R_f values, and gave no separation.

It was found that this mixture could be separated into fractions on the chromatogram only with solvents such as water, two percent acetic acid, and carbon dioxide in water (pH 4.2). Of these three developing solvents, water proved to give the best separation. The drawing of Figure 5-A represents a chromatogram of the alcohol extract from each species, using water as the developing solvent.

The use of water as a developing solvent has been discussed by Roberts (21). The separation of a mixture with this solvent is attributed totally to adsorption

effects of the components on the paper rather than partition effects with the developing solvent. From the results here then, it would appear that the separation of this total alcohol extract is due to differences of adsorption of the various components on the paper, and that partitioning effects with the solvent are negligible.

With reference to Figure 5-A, all of the spots were visualized with typical phenolic spray reagents, such as $\text{FeCl}_3:\text{K}_3\text{Fe}(\text{CN})_6$ (2), bis diazotized benzidine (17), and diazotized para nitroaniline (25). The latter two sprays are capable of producing distinctive colors for different phenolics, but in this case the color of corresponding spots were the same for all species. Ammoniacal silver nitrate and cinnamaldehyde - HCl developed faint colors with the spot at the origin. The cinnamaldehyde - HCl spray has been shown to be specific for catechins (flavans) or phlobatannins with a flavan nucleus (3, p. 12). None of these spots fluoresced with ultraviolet light and only the spot at the origin was visible before spraying.

As illustrated, this chromatogram shows a slight difference in the extract of noble fir compared to that for the other species. The difference in the spot, $R_f = 0.58$, is one of amount however, and therefore bears less significance than if it were a completely different spot.

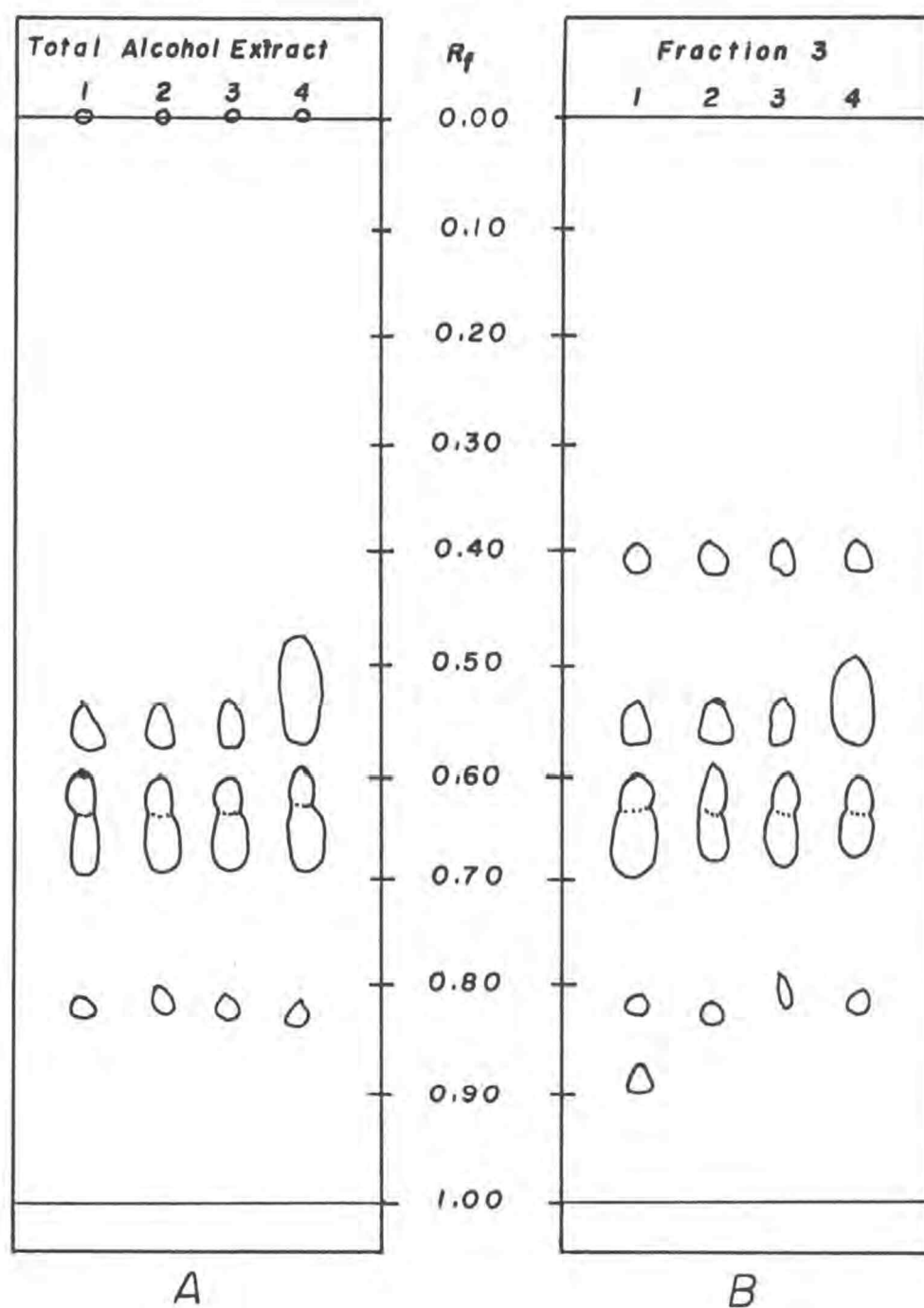


FIGURE 5 - Paper Chromatographic Separation With Water as the Developing Solvent. Spot numbers: 1) Pacific silver fir, 2) white fir, 3) grand fir, 4) noble fir.

The various fractions (Figure 3) of the alcohol extract of each species were also run in several developing solvents. As expected, the water solvent gave the best results and also gave information as to the origin of the spots in the total extracts. Fractions 1 and 4, representing phlobaphenes and phlobatannins respectively, were shown to account for the spot at the origin in the chromatograph of the total extract (Figure 5-A). In addition to the common phenolic indicators, the phlobatannins were visualized by spraying with cinnamaldehyde-HCl reagent.

Figure 5-B is a representative drawing of the chromatogram of fraction 3 of each species, using water as the developing solvent. As with the total alcohol extract, water was the best developing solvent, although carbon dioxide in water (pH=4.2) and 15 percent acetic acid in water gave fairly good results. Figure 5-B shows that this fraction, now more concentrated in these components than in the total extract, exhibits two additional spots not shown in the chromatogram of the total extracts. Each species gave a spot at $R_F=0.42$ which was visualized by its fluorescence in ultraviolet light and by its yellow color when exposed to ammonia vapor. The extract from Pacific silver fir was shown to exhibit a spot at $R_F=0.89$ which

was not shown by the other species. This spot gave an intense blue fluorescence with ultraviolet light and developed a distinctive color with diazotized para-nitroaniline. This spray turned the spot a very light yellow color which, when oversprayed with 20 percent aqueous sodium carbonate solution, changed to a golden color. The component causing this distinctive spot appeared on all chromatograms in various solvents definitely showing its occurrence in this species alone.

The remaining spots on this chromatogram were visualized with the phenolic reagents previously mentioned. In addition to these, other spray reagents were tried to gain information concerning the types of compounds present on the chromatogram. A solution of bromophenol blue indicator spray showed no indication of acids among the spots. A three percent alcoholic solution of para-toluene sulfonic acid (22) has been used to identify leuco-anthocyanins, catechins and flavan-3,4 diols on paper chromatograms. This spray developed a slight pink color with the spot at $R_f = 0.58$, especially with the heavier spot from noble fir. This color indicates a compound having the flavan-3,4 diol structure.

Fraction 2 was also chromatographed in many solvent systems, and in all of these solvents it was shown to be

similar to fraction 3, with two exceptions. The component in fraction 3 shown to be present in Pacific silver fir alone was not found in fraction 2. In addition to the spots given by fraction 3, fraction 2 also exhibited a spot at the origin. This spot was attributed to the phlobaphenes, which were not completely removed from this fraction.

Despite the many R_f values obtained from the many solvents employed, none of the components could be identified on the basis of literature R_f values. In attempting further separation of this mixture, about 100 microliters (1.0 mg solids) of fraction 3 for each species was streaked on Whatman #1 chromatography paper, and developed with water. The bands corresponding to the spots found on the original chromatogram were cut from the paper and eluted with acetone in a micro-Soxhlet extractor. These individual fractions for each species were then chromatographed in the normal manner with several solvents. No new spots appeared, and no new information was obtained concerning the other spots to allow their identification by this method.

Thus paper chromatography has shown the presence of one distinctive compound in Pacific silver fir not found in the other species. Also a much greater abundance of

another compound ($R_f=0.58$ when developed with water) exits in noble fir than in the other species. Unfortunately neither of these is distinctive enough to form the basis for a color test of the wood.

DISCUSSION AND SUMMARY

Various extracts of the wood of four species of true fir have been investigated and compared to find differences in composition. This work is preliminary to the development of a chemical test to distinguish these species.

The extractive content of these species was found to be very low, and the values were about the same for all species tested. The total ether, alcohol and water extractives amounted to about 3.5 percent on the basis of the oven dry weight of wood. This fact indicates one of the difficulties in developing a distinguishing test for these woods, since a color test would no doubt depend upon a reaction with the extractives.

Gas chromatography was used to analyze the volatile oils obtained by hydrodistillation of the petroleum ether soluble portion of an ether extract of the wood.

A method was developed to detect and tentatively identify terpene constituents of the steam volatile materials, using the gas chromatograph. This method involved the use of two different columns, a 14 foot glass column containing Craig polyester succinate as the liquid phase, and a two meter column containing a liquid phase of diisodecyl phthalate. Optimum conditions for these

columns were found and the retention parameters were determined for known terpene compounds expected to be found in the woods. The method, including extraction and hydrodistillation, was tested with a wood sample "doped" with varying amounts of six terpenes. The chromatograph detected all the terpenes with a concentration of as small as 0.01 percent of the weight of the wood.

The steam volatiles were obtained from the wood with a yield of only 0.0024 to 0.005 percent of the weight of wood. The gas chromatograph, however, was unable to detect any terpene constituents in this oil, as evidenced by the absence of peaks in the region of the chromatogram shown to contain the terpenes.

The small yield of steam volatiles and the absence of detectable amounts of terpene constituents, limited the use of this method to distinguish the various species. Therefore, further investigation was not made on the volatile oil fractions. If further work were to be done to definitely establish the terpene constituents, it would be necessary to use a much larger wood sample so that more oils could be obtained. It would also be advisable to use a more sensitive recorder with the chromatograph, allowing the detection of smaller amounts than possible with the instrument used here.

Alcohol extracts of the woods were compared by infrared and ultraviolet spectra, paper chromatography, and certain color tests.

Infrared spectra of the total extracts of the various species were very nearly identical. Although the differences were not sufficient to distinguish the species, the spectra did show the phenolic character of this extract.

Ultraviolet spectra for the total extracts and for fractions of phlobaphenes, flavonoids and phlobatannins were found to be very similar. The species can not be distinguished from their spectra, but these spectra gave more information concerning the basic structure of the components of the extracts. Spectra run in neutral and alkaline alcoholic solutions and in alcoholic solutions containing aluminum chloride indicated that the basic structural unit of this extract is the catechin (flavan) nucleus.

A water and ether soluble fraction of each extract, believed to contain flavonoids, was tested by means of two color reactions. The Wilson boric acid test gave negative results for flavones in all extracts. Reduction of this fraction, in alcoholic solution with zinc and hydrochloric acid, produced distinctive colors, even though the test was doubtful concerning the presence of

flavanones. The test produced a light brown solution with Pacific silver fir and grand fir, a dark red-brown solution and precipitate with white fir, and a light cherry-red color with noble fir. Although this fraction of the extract might be used to distinguish the species, a color test directly on the wood would not develop characteristic colors. The low concentration in the wood will not give visible evidence of this reaction.

Separation of the alcohol extract on a paper chromatogram was shown to occur because of differences in adsorption on the paper rather than by partitioning effects with the solvent. The fact that organic rich solvents moved the entire extract to very high R_f values eliminated the possibility of the occurrence, in appreciable concentration, of the more common phenolic compounds usually found in plant extracts.

Although minor differences were found in the chromatograms of the total extracts and their fractions, no definite identification could be made of any of the components of the extract. The chromatograms indicated the presence of one component in Pacific silver fir not found in the remaining species, but its concentration was so low that its use to distinguish this species is extremely limited.

With the use of selective spray reagents for the

chromatograms, it was determined that all of the spots were phenolic and none contained strongly acidic functional groups. Weak tests were obtained indicating the flavan structure for one of the spots.

The many tests performed in this investigation showed a great similarity in the extractive content and composition for these species of true firs, even though the wood samples were not from comparable trees. This fact indicates that the development of a distinguishing chemical test for these species will be indeed difficult.

BIBLIOGRAPHY

1. Aiyar, S. S. Balsam of white fir. *Journal of the American Pharmaceutical Association* 12:587-588. 1923.
2. Barton, G. M., R. S. Evans, and J. A. F. Gardner. Paper chromatography of phenolic substances. *Nature* 170:249-250. 1952.
3. Becker, Edward Samuel. The chemical nature of the extractives from the bark of Shasta red fir, Abies magnifica var Shastensis Lemm. Ph.D. thesis. Corvallis, Oregon State College, 1957. 79 numb. leaves.
4. Bernhard, Richard A. Separation and identification of some terpenes by gas partition chromatographic analysis. *Journal of the Association of Official Agricultural Chemists* 40: 915-921. 1957.
5. Block, Richard J., Emmett L. Durrum, and Gunter Zweig. A manual of paper chromatography and paper electrophoresis. 2d ed. New York, Academic Press, 1958. 710 p.
6. Brown, H. P. and A. J. Panshin. Commercial timbers of the United States. New York, McGraw Hill, 1940. 554 p.
7. Cvrkal, H. and J. Janak. Identification of some terpenes in essential oils of conifers by gas chromatography. *Collection of Czechoslovak Chemical Communications* 24:1967-1974. 1959.
8. Erdtman, H. Constitution of the resin phenols and their biogenetic connections. VIII. Conidendrin (sulfite liquor lactone) and its distribution among various conifers. *Svensk Papperstidning* 47:155-159. 1944.
9. Gage, Thomas B., Carl D. Douglass, and Simon H. Wender. Identification of flavonoid compounds by filter paper chromatography. *Analytical Chemistry* 23:1582-1585. 1951.

10. Graham, H. M. and E. F. Kurth. Constituents of extractives from Douglas fir. Industrial and Engineering Chemistry 41:409-414. 1949.
11. Grantham, John B., Chief of Forest Utilization Research. Pacific Northwest Forest and Range Experiment Station, Portland, Oregon. Private communication.
12. Hamilton, J. K., J. E. Jeffery, and E. V. Partlow. Reflectance method for estimation of sugars in wood cellulose hydrolysates. Paper presented at Northwest Regional meeting of American Chemical Society; Seattle, Washington. June 18-19, 1959.
13. Harborne, J. B. The chromatography of flavonoid pigments. Journal of Chromatography 2:581-604. 1959.
14. Hergert, H. L. and E. F. Kurth. The chemical nature of the extractives from white fir bark. Tappi 36: 137-144. 1953.
15. _____. The isolation and properties of catechol from white fir bark. Journal of Organic Chemistry 18:521-529. 1953.
16. Kurth, E. F. The chemical analysis of western woods. Part III. Tappi 33:507-508. 1950.
17. Lindstedt, Gosta. Constituents of pine heartwoods. XX Separation of phenolic heartwood constituents by paper partition chromatography. Acta Chemica Scandinavica 4:448-455. 1950.
18. Lynn, E. V. and Sukeo Nakaya. The leaf oils of Washington conifers. VI Abies nobilis. Journal of the American Pharmaceutical Association 22:305-306. 1933.
19. Patterson, R. F. and Harold J. Hibbert. Lignin and related compounds. LXXII. The ultraviolet absorption spectra of compounds related to lignin. Journal of the American Chemical Society 65:1862-1869. 1943.
20. Reio, L. A method for the paper-chromatographic

- separation and identification of phenol derivatives, mould metabolites and related compounds of biochemical interest, using a "reference system". *Journal of Chromatography* 1:338-373. 1958.
21. Roberts, E. A. H. and D. J. Wood. Separation of tea polyphenols on paper chromatograms. *Biochemical Journal* 53:332-336. 1953.
 22. Roux, D. G. Some recent advances in the identification of the leucoanthocyanins and the chemistry of the condensed tannins. *Nature* 180:973-975. 1957.
 23. Schwartz, Charles, Jr. The leaf oils of Washington conifers. *Abies grandis*. *American Journal of Pharmacy* 108:152-156. 1936.
 24. Shorger, A. W. Oils of the coniferae. II. The leaf and twig, and bark oils of white fir. *Journal of Industrial and Engineering Chemistry* 6:809-810. 1914.
 25. Swain, T. The identification of coumarins and related compounds by filter-paper chromatography. *Biochemical Journal* 53:200-207. 1953.
 26. _____. Spectral studies on phenolic compounds. *Chemistry & Industry*, 1954, p. 1480.
 27. Technical Association of the Pulp and Paper Industry. Testing methods, recommended practices, specifications. New York, n.d N.p. (loose leaf).
 28. Terminology for gas chromatography recommended by IUPAC committee. *Facts and Methods for Scientific Research* (F and M Scientific Corporation, New Castle, Delaware.) 1 (1):1-4. 1960.
 29. Trupp, Malcom S. and Louis Fischer. The chemical examination of essential oils from *Abies amabilis* and *grandis*. *Journal of the American Pharmaceutical Association* 28:433-440. 1939.
 30. Wilson, C. W. The boric acid color test for flavone derivatives. *Journal of the American Chemical Society* 61:2303-2306. 1939.

31. Wise, Louis E. and Edwin C. Jahn. (eds.). Wood Chemistry. 2d ed. vol. 1. New York, Reinhold, 1952. 688p.