Douglas-fir bark was extracted with n-hexane. A light-colored "wax-like" solid was recovered from the extract by evaporation of the solvent. Chemically intact sterol esters and ferulic acid esters were isolated from the "wax" without saponification or degradation as had been necessary in former investigations. The characterization of the intact esters provides an improved understanding of the characteristics of the extracted "wax." Previous investigations had been limited to an identification of compounds from saponified and chemically fragmented "wax."

The intact, undegraded sterol and ferulic acid esters were isolated by subjecting the n-hexane-soluble materials to column chromatography using Silica Gel G as stationary phase and chloroform-n-hexane (3:1 v/v) as developing solvent. The effluents were collected in different fractions and numbered from 1 to 75.
Fractions 1, 2, 4, 6, 9, and 11 were tested by thin-layer chromatography using diethyl ether-n-hexane (1:4 v/v) and chloroform-carbon tetrachloride (6:1 v/v) as the solvent systems. The chromatograms showed two spots ($R_f$ 0.96 and 0.76). Positive results for Liebermann-Burchard tests were found in all of the above fractions except fraction 11, with the most prominent color reaction in fraction 2. Thin-layer chromatographic and gas-liquid chromatographic results showed no free sterols in these fractions and the materials were thus considered to be sterol esters. Fraction 2 was then subjected to saponification since it showed the strongest Liebermann-Burchard test.

The unsaponifiable fractions were tested by thin-layer chromatography and gas-liquid chromatography. The results showed the existence of campesterol and $\beta$-sitosterol. Their presence was further confirmed by combined gas-liquid chromatographic-mass spectrometry analyses.

The saponifiable and diethyl ether-soluble fraction was analyzed by gas-liquid chromatography and combined gas-liquid chromatographic-mass spectrometry analyses. The results showed the existence of n-tridecanoic acid, n-hexadecanoic acid, n-heptadecanoic acid, cis-9-octadecenoic acid, n-eicosanoic acid, n-docosanoic acid, n-tetracosanoic acid and the possible existence of n-nonadecanoic acid. Any or all of these fatty acids can form esters with the two sterols.
The presence of sterol esters, especially esters of odd-numbered fatty acids, has not been previously reported in Douglas-fir bark.

Fractions 13 to 17 on the column chromatograph showed a dark blue coloration when exposed to ultraviolet light. The materials in these fractions were collected and purified by recrystallization and thin-layer chromatography. Infrared spectra of the purified material showed it to be an ester. A saponification reaction was then conducted to study the acid part and the alcohol part of the ester. The alcohol portion was found to be a mixture of behenyl alcohol and lignoceryl alcohol by studies of melting point, gas chromatography, and infrared and mass spectrometry.

The acid part was proven to be ferulic acid by melting point test, infrared spectroscopy, field desorption mass spectrometry, and nuclear magnetic resonance spectroscopy. The infrared spectrum of the ester showed the presence of hydroxyl groups. Therefore, the ester linkage was between the carboxylic acid group of ferulic acid and the hydroxyl group of the two fatty alcohols. The ferulic acid ester was thus different from the ester found in white fir bark. The present work is the first report to show that ferulic acid does not exist in the free form in n-hexane "wax" as had been suggested earlier. It exists as an ester.

The n-hexane-soluble materials were also shown to contain some terpenes, alcohols and other easily volatilized components. To
avoid heavy losses, the volatile materials were collected by steam distillation of the bark rather than to attempt to separate them from the n-hexane-soluble fraction. Combined gas-liquid chromatographic-mass spectrometry analyses showed the major volatile components to be furfural, terpinene-4-ol, α-terpineol, guaiacol, 2,5-dimethyl-3-acetylfuran, and β-cyclocitrinal. The presence of α-pinene, β-pinene, limonene, 1,5-p-menthadien-7-ol, β-citronellol, and gerniol was also tentatively identified. The presence of α-terpineol is of interest to forest entomologists because it is known to be an insect attractant for bark beetles. The present work is the first report concerning the presence of furfural, terpinene-4-ol, α-terpineol, guaiacol, 2,5-dimethyl-3-acetylfuran, and β-cyclocitrinal in Douglas-fir bark.
Douglas-fir Bark: \textit{n}-Hexane-Soluble and Volatile Materials

by

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A THESIS

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DOUGLAS-FIR BARK: *n*-HEXANE-SOLUBLE
AND VOLATILE MATERIALS

I. INTRODUCTION

Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco is the most important species of tree in the Northwest region of the United States. The wood has been extensively used in various industries. However, there are about three million tons of bark generated each year in Oregon alone that remains unused (15). The large quantity of raw material is not only wasted, but creates a solid waste disposal problem.

The research reported here shows an improved understanding of the chemical composition of Douglas-fir bark. The bark is chemically complex and difficult to analyze. However, extractions with organic solvents tend to resolve the various components. The *n*-hexane-soluble fraction investigated in this work appears to be the best separated.

Previous efforts to analyze the *n*-hexane-soluble materials have included a saponification reaction to break the larger molecules, such as esters, into smaller compounds for easier identification. The work herein reported emphasizes an analysis of the larger components as they exist in the *n*-hexane-soluble fraction when extracted from the
bark. The results more clearly demonstrate the true nature of this fraction than findings that have been previously reported.
II. HISTORICAL REVIEW

The scientific name of Douglas-fir in the early literature was *Pseudotsuga taxifolia* (Poir.) Britt. However, the presently preferred botanical name is *Pseudotsuga menziesii* (Mirb.) Franco. The names all refer to the same genus and species. Mention of this is made to avoid confusion about the exact species investigated.

From the anatomical point of view, the term "bark" means all the tissues which are outside of the vascular cambium (Figure 1). On the basis of their anatomical structure and physiological properties, bark can be divided into two parts, the "inner bark" and the "outer bark" (12). The inner bark (phloem) is the portion from the vascular cambium to the cork cambium of the innermost cork layer. The outer bark (rhytidome) comprises the tissues located outside of the innermost cork cambium (29, 30).

The inner bark of Douglas-fir consists of axial and ray parenchyma, sieve cells and sclereids. The function of the inner bark is to transport the assimilates and to serve as a storage organ for food reserves. Much of the inner bark is living in the living tree.

The outer bark consists of layers of cork and original inner bark which is interspersed among the cork layers. The cork layers form from the cork cambium, and growth increments are usually visible in the cork layers (82). When new cork cambia form in the
Figure 1. Anatomy of Douglas-fir Bark\textsuperscript{a}

\textsuperscript{a} Picture supplied by Dr. R. Krahmer, Associate Professor, School of Forestry, Oregon State University.
inner bark and "cut away" part of the inner bark, these inner bark
cells become part of the outer bark. The outer bark cells are dead
because no food passes through the layers of cork cells. Thus outer
bark is physiologically inactive and only forms a protective layer
against chemical and mechanical injuries (50).

Although the presence of "wax" in Douglas-fir bark was
recognized as early as 1923, it attracted little attention until the late
1940's. Clark and his co-workers extracted the bark with benzene
and reported "wax-like brown and black substances"; the yield was
1.5% (32). Additional investigations were made by Kurth and his co-
workers. Two important papers published in 1950 represent pioneer-
ing studies of the extractives of the bark (51, 54). The bark was
extracted with five different solvents of increasing polarity. The
results are shown in Table 1 (54).

Table 1. Extractives in Douglas-fir Bark from 80- to 95-Year-Old
Trees.

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<th>Extractive</th>
<th>Yield (%)(^a)</th>
<th>Solvent</th>
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<td>Light-colored &quot;wax&quot;</td>
<td>5.47</td>
<td>n-Hexane</td>
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<tr>
<td>Light-brown &quot;wax&quot;</td>
<td>2.52</td>
<td>Benzene</td>
</tr>
<tr>
<td>Dihydroquercetin</td>
<td>5.95</td>
<td>Ethyl ether</td>
</tr>
<tr>
<td>Tannin and phlobaphene</td>
<td>7.70</td>
<td>Acetone</td>
</tr>
<tr>
<td>Tannin and carbohydrates</td>
<td>6.68</td>
<td>Hot water</td>
</tr>
<tr>
<td>Sum of 5 extractives</td>
<td>28.32</td>
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</tr>
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\(^a\) Percentages based on oven-dry weight of bark.

Source: Kurth and Kieffer (54).
The term "wax" used by Kurth and his co-workers for n-hexane and benzene solubles (Table 1) was evidently based on the broadest practical meaning, and was not reserved for a precise chemical definition. The chemical definition of wax as described by Noller (70) is as follows:

... a practical definition of a wax might be that it is anything with a waxy feel and a melting point above body temperature and below the boiling point of water. Thus the term paraffin wax is used for a mixture of solid hydrocarbons, beeswax for a mixture of esters, and carbowax for a synthetic polyether. Chemically, however, waxes have been defined as esters of long-chain (C16 and above) monohydric (one hydroxyl group) alcohols with long-chain (C16 and above) fatty acids. Hence they have the general formula of a simple ester, RCOOR. Actually the natural waxes are mixtures of esters and frequently contain hydrocarbons as well.

Other authors similarly define waxes. Mutton (65, p. 348) writes that

... fatty acids are long, straight chain aliphatic monocarboxylic acids which usually occur in esterified form. Esters of fatty acids and glycerol are called fats or oils, whereas esters of fatty acids and high molecular weight monohydric alcohols are known as "waxes."

Hart and Schuetz (34, p. 211) contend that "waxes cannot be transformed into water-soluble products when boiled with alkali. They are saponified but, unlike glycerol from fats and oils, the long-chain alcohols are insoluble in water." They also write that "together with the esters, waxes frequently contain small quantities of saturated hydrocarbons, free fatty acids, alcohols and sterols."
Kurth (51) reported the chemical composition of the "waxes" in Douglas-fir bark. The "waxes" were first saponified and then analyzed. The results showed 60.5% lignoceric acid, 19.5% lignoceryl alcohol, 22% ferulic acid, and 0.3% "phytosterol." The yields were based on the original wax.

The \(n\)-hexane-insoluble, but benzene-soluble, fraction of the bark also has been studied and shows a more complicated composition (39, 53). After saponification, the results showed 24% dark-colored phlobaphenes, 25% fatty acids, 26% of a dark-colored, diethyl ether-soluble acid fraction, and 5% unsaponifiable matter. Recently Love-land and Laver (58, 59) studied the fatty alcohols and fatty acids in benzene wax and found \(\omega\)-hydroxy fatty acids as well.

The cork fraction was studied by Hergert and Kurth (39). The total \(n\)-hexane-soluble fraction was 5.78% which was close to the yield of \(n\)-hexane wax from the whole bark. However, the yields of the individual components were different. The analysis after saponification showed the presence of 49.3% lignoceric acid, 9.8% ferulic acid, 27.5% lignoceryl alcohol, 0.6% "phytosterol," 8.1% \(n\)-hexane-insoluble but benzene-soluble acid, and 3.6% benzene-insoluble phenolic material.

The bast fiber fraction was also studied (46). The results showed that the yield of total extractives from the bast fiber fraction (13.49%) was less than from the cork fraction, and the bark as a whole. Particularly the yield of \(n\)-hexane wax was only 1.59%
compared to 5.78% from cork, and the yield of diethyl ether solubles (mainly dihydroquercetin) was only 0.38% compared to 16% in cork. A further separation of the extractives into their individual components was not reported.

The inner bark of Douglas-fir was investigated by Holmes and Kurth (40, 41). The n-hexane solubles proved to be tan-colored, sticky waxes, which contained 3% free acid, 5% unsaponifiables, 66% saponifiables and diethyl ether solubles, and 27% saponifiable and acetone solubles. While the individual components of the n-hexane solubles were not identified, the authors (40, 41) reported an interesting result for the diethyl ether solubles. They found that the outer bark contained dihydroquercetin (I) while the inner bark contained d-catechin (II) and l-epicatechin (III) (mature inner bark contained a trace of d-dihydroquercetin). The authors suggested that the presence of catechin and epicatechin in the living inner bark and the absence in the dead outer bark implied that these compounds are the precursors of the polyphenol components of either bark or wood. However, the biosynthetic pathway of polyphenols in plants still is not understood.
The \textit{n}-hexane-soluble fraction of conifer barks other than Douglas-fir bark have also been studied (3, 11, 56). The results were, generally speaking, similar except for the yields.

The earlier studies were helpful in understanding the chemical nature of "waxes" from barks, but they had one deficiency. The authors did not investigate the compounds in the original "wax." They always saponified the "wax" and identified the products of the saponification reaction. The compounds reported might not exist in the simple, uncombined state in the "wax" itself. For example, ferulic acid has been reported in the \textit{n}-hexane solubles, but free ferulic acid is not soluble in either hot \textit{n}-hexane or hot benzene. This indicates that ferulic acid probably exists in the "wax" as a combined acid rather than as a free acid. If it exists as a combined acid, how is it combined and with what is it combined? Some publications reported the existence of fatty acids and hydroxy fatty acids. The free fatty acids and hydroxy fatty acids show different chemical properties from their relative esters. The fatty esters are soluble in \textit{n}-hexane, but if fatty acids and hydroxy fatty acids are polymerized the resulting product is insoluble in \textit{n}-hexane, as is the case with suberin (7). Sterols have also been reported in the \textit{n}-hexane solubles. Do they also exist in the free state? Saponification followed by identification of the fragments cannot answer these questions properly.
The results reported in the present work are concerned with several compounds as they exist in the original "wax." The compounds were isolated from the "wax" by column chromatography and their physical and spectral properties determined. They were then saponified and the component parts definitely identified. The positions of the linkages and the types of functional groups present in the original "wax" components were thus established.

A knowledge of the detailed composition of Douglas-fir bark "wax" has possible practical applications. The shortage of tall oils has become more and more serious, and since Douglas-fir bark "wax" is composed of about 60% fatty acids, it may be possible to produce tall oils from waste bark. Information on the composition of the fatty acid mixtures can help determine if the properties of this material can compete with the existing tall oils.

A better understanding of the chemical composition of the "wax" may also contribute to the field of chemotaxonomy and pharmacology (98). Erdtman (22) has defined chemosystematics as a study of the distribution of chemical compounds, or group of biosynthetically related compounds, in a series of related or supposedly related plants. The chemical compounds to be used in chemosystematic investigations must satisfy certain requirements. For example, the compounds must have a limited distribution in certain plant families or genera and they must be relatively insensitive to environmental
factors. Certain groups of chemically related compounds such as the flavonoids, alkaloids and terpenes are particularly suitable for chemo-systematic work (2, 97).

Erdtman and co-workers have investigated the heartwood extractives of many conifers and have observed several taxonomic correlations on the genetic level in the families of Pinaceae and Cupressaceae. Mirov studied the distribution of monoterpenes found in the gum terpentine of the genus *Pinus* (64). Zavarin extended the study to the terpenes in blister resins of the genus *Abies* (104).

Pearl and his co-workers found that the presence or absence of ferulic acid in alkaline hydrolyzates of wood extracts appear to be quite specific and can be used for differentiating species within a genus or perhaps a genus within a family (71, 72). For example, the European trembling aspen, *Populus tremula*, and its American relative, *Populus tremuloides*, appear to be substantially identical to the botanist. However, the former yields significant amounts of ferulic acid and the latter yields none whatsoever. *Salix babylonica* is different from the other two willows (*Salix nigra* and *Salix eriocephala*) in that it was the only one that did not yield ferulic acid. Red alder (*Alnus rubra*) is the only member of the Betulaceae family whose extract yielded ferulic acid.

The presence of fatty acids can also be used for chemotaxonomy. The fatty acids in the wood from European spruce (*Picea excelsa*) are
comprised of oleic, linoleic, linolenic, lauric, myristic, palmitic, stearic, arachidic, behenic, and lignoceric acids with the first two as the most abundant acids (43). The fatty acids from Norway spruce (Picea abies) consisted of about two-thirds straight chain acids with palmitic acid most abundant, followed by behenic and lignoceric. The other one-third was branched anteiso fatty acids with 14-methylhexadecanoic acid the most abundant (18). The fatty acids from the six pines commonly used in pulping showed that oleic acid was the predominant acid (8). Paper birch and European birch contained linoleic acid as the most abundant acid with a smaller amount of oleic acid (74). The fatty acids from western red cedar bark showed a narrow range of carbon chain length from $C_{16}$ to $C_{24}$ and only even-numbered acids were reported (26). In aspen wood the range is wider, from $C_{12}$ to $C_{28}$ including the odd-numbered acids with the single exception of $C_{27}$ (73).

The volatile materials in bark have not been as thoroughly studied as the non-volatiles. Kurth and Hubbard showed that the $n$-hexane extract of ponderosa pine [Pinus ponderosa (Laws.)] contained 0.2% of a volatile oil recovered by steam distillation (55). The $n$-hexane extract of the bark of incense cedar [Libocedrus decurrens (Torr.)] was shown by Smith and Kurth to contain 0.95% of a volatile material (94). These publications reported only the total content and did not identify the components. Other workers reported only a few
major components (56). All of these researchers studied pines or spruces. No one has reported research on the volatile materials in Douglas-fir bark.

Recently, entomologists have found that volatile materials in trees and barks can attract or repel insects. Heikkenen and Hrutfiord reported that $\alpha$-pinene in Douglas-fir in the Pacific coastal forest plays an essential role as host attractant for beetles while $\beta$-pinene served as a repellent (36). Laboratory and field tests showed that the attractant effect was greatly increased after the first unmated female beetle had attacked the tree (85, 86). She released a pheromone and the combination of pheromone plus $\alpha$-pinene had a synergistic effect in attracting more beetles.

The insect sex attractants, based on the source, can be divided into two types, host produced and insect produced. Based on the structural difference of the attractants they can also be classified as two groups: the bicyclic ketals such as frontalin and brevicomin (5), and terpene alcohols such as trans-verbenol and $\alpha$-terpineol. Thus far three of five volatile substances present in the hindguts of attractive female Dendroctonus pseudotsugae beetle have been identified: 1, 5-dimethyl-6, 8-dioxa-bicyclo [3. 2. 1] octane (frontalin)(IV)(48), 3-methyl-2-cyclohexen-1-one (V) (47) and trans-verbenol (VI) (86).
The compound 3-methyl-2-cyclohexen-1-one (V) has multiple functions for male Douglas-fir beetles. Field tests showed that when the concentration of the compound was below 1%, it significantly increased the number of male beetles attracted. However, when the concentration was above 1%, it significantly repressed the response of males (87, 88).

Three terpene alcohols from frass produced by male Ips confusus (Le C.) beetles were also identified (93). They were:

2-methyl-6-methylene-7-octen-4-ol (VII), cis-verbenol (VIII) and 2-methyl-6-methylene-2, 7-octadien-4-ol (IX).
Renwick showed that trans-verbenol played a role in the beetle attraction and that $\alpha$-pinene was the most effective host terpene for attracting *Dendroctonus ponderosae* and *Dendroctonus frontalis* (79). Pitman and Vite showed that frontalin plus camphene can attract *Dendroctonus pseudotsugae* (77).

Volatile materials produced from host trees or barks alone can also attract insects. Kangas, Perttunen, Oksanen, and Rinne isolated $\alpha$-terpineol from pine bark and showed attractiveness for *Blastophagus piniperda* L. (Col., Scolytidae) (44). Ethanol has been shown to be the primary attractant for the ambrosia beetle, *Trypodendron lineatum* (60) and for *Gnatbotrichus sulcatus* (10).

There are two objectives of the present work. First, the use of modern analytical methods to study the n-hexane-soluble fraction of Douglas-fir bark to provide more accurate and extensive results about the chemical composition of the "wax." Saponification reactions were carried out only after the components had been isolated as esters. Identification of the fragments thus released placed the position of the ester linkage in the original "wax" and showed the basic structures of the "wax" components. These structures are important for guidance of future research aimed at modifying or blending Douglas-fir bark "wax" for commercial use. The second objective was to identify the major volatile materials in Douglas-fir bark to assist the efforts of
entomologists who are attempting to gain an understanding and control of insect infestations in forests.
III. EXPERIMENTAL

Collection of Bark Samples

Two groups of bark samples were used in this study. The first group was collected from the George T. Gerlinger Experimental Forest, located near Falls City, Oregon, U.S.A., and operated by the School of Forestry, Oregon State University, in cooperation with the State Forestry Department of Oregon. A sample of Douglas-fir bark from this geographical region has been given the Oregon State University Herbarium Number 140826.

The tree was a dominant tree, 58 years old, with a diameter of 14.8 inches at breast height and an overall height of 102 feet. It was cut just one day before the bark was removed. The bark thickness was 1.5-2 inches. The bark was used for extraction of the n-hexane-soluble materials.

The second sample of bark was collected later from McDonald Forest, Corvallis, Oregon (Oregon State University Herbarium Number 142702). The tree was also a dominant tree, 52 years old, with a diameter of 14.2 inches at breast height and an overall height of 100 feet. The thickness of the bark was about 1.5 inches. The bark was removed immediately after the tree was cut and sealed in polyethylene bags and brought to the laboratory. This second sample of bark was used for steam distillation treatments.
Sample Preparation

**n-Hexane Extraction**

The bark was ground in a disk grinder until it passed through a screen with openings of 0.5 inches square. The ground bark (1974.76 g, moisture content 11.2%, oven-dry method) was extracted for 36 hours with n-hexane in a Soxhlet apparatus. The solvent was evaporated under aspirator vacuum in a rotary evaporator (Büchi, Rotavapor, Switzerland).

The residue was transferred to a sample jar and excess solvent was evaporated by passage of a stream of nitrogen. The yield was 5.11%, based on the oven-dry weight of bark.

**Steam Distillation**

The second sample of bark was first ground into chips and then the chips were ground to pass a screen of 10 meshes to the inch. The ground bark was put in a 2000-ml, three-necked, round-bottomed flask and steam distillation was carried out for 6 hours (99). The distillate was collected in an ice-cooled flask. The volatile organic material was recovered by extraction of the distillate with diethyl ether. The diethyl ether solution was dried over anhydrous sodium sulfate and after filtration the diethyl ether was evaporated under nitrogen. The residual material contained about 10% diethyl ether.
which could not be evaporated without a significant loss of volatile materials (100). The yield was 0.15% (exclusive of ether) based on the oven-dry weight of bark.

Chromatographic Techniques

Column Chromatographic Separations

Column chromatography was used as the first step in separating the n-hexane-soluble materials. Chloroform-n-hexane (3:1 v/v) was used as the developing solvent. A slurry was made by mixing 700.0 g of oven-dried Silica Gel G and 2000.0 ml of the developing solvent. Distilled water (8% based on the oven-dry weight of Silica Gel) was added to the slurry and mixed uniformly by using a Waring blender. The slurry was poured into a glass column 1 m in length and 75 mm in diameter. After the excess solvent had drained off, an aliquot of 17.0 g of the n-hexane-soluble materials, dissolved in 50 ml of developing solvent, was added to the top of the Silica Gel G column. The resolution of the extract was monitored by use of an ultraviolet light. As development of the column progressed, a series of bands became evident (Figure 2) under ultraviolet light.

The column effluent was collected by an automatic fraction collector. When a band which fluoresced under ultraviolet light was about to come out of the column, fraction numbers (test-tube numbers) were recorded. Fractions were collected as summarized in Table 2.
Figure 2. Column chromatogram of the n-hexane-soluble fraction from Douglas-fir bark. Solvent system: chloroform-n-hexane (3:1 v/v).
Table 2. Column Chromatographic Separation of the n-Hexane-Soluble Materials from Douglas-fir Bark.

<table>
<thead>
<tr>
<th>Fraction no.</th>
<th>Volume collected (ml)</th>
<th>Remarks a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>425</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>3 to 11</td>
<td>100 for each fraction</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>13 to 17b</td>
<td>50 for each fraction</td>
<td>Dark blue</td>
</tr>
<tr>
<td>18 to 24</td>
<td>100 for each fraction</td>
<td></td>
</tr>
<tr>
<td>25 to 27</td>
<td>100 for each fraction</td>
<td>Bright blue (lower)</td>
</tr>
<tr>
<td>28 to 64</td>
<td>100 for each fraction</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>20</td>
<td>Bright blue (upper)</td>
</tr>
<tr>
<td>66 to 72</td>
<td>100 for each fraction</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td></td>
<td>Yellow (extracted from silica gel by using the developing solvent)</td>
</tr>
<tr>
<td>74</td>
<td></td>
<td>Bluish gray (extracted from silica gel)</td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>Blue (extracted from silica gel)</td>
</tr>
</tbody>
</table>

a The colors were observed under ultraviolet light.

b These fractions were combined and in the remainder of the text are referred to as sample 15.
Thin-Layer Chromatographic Separations

Selected fractions collected by column chromatography of the n-hexane-soluble fraction were subjected to thin-layer chromatography. Silica Gel G (E. Merck A. Darmstadt, Germany) was used as the solid phase. Three solvent systems were used:

1. diethyl ether - n-hexane (1:4 v/v)
2. chloroform - carbon tetrachloride (6:1 v/v)
3. diethyl ether - n-hexane - methanol (10:40:1 v/v/v)

Three detection methods were used:

1. ultraviolet light
2. iodine vapors
3. 50% Sulfuric acid spray; plate then heated at 105° for 10 min.

Two-dimensional thin-layer chromatography was also used with the above mentioned solvent systems and detection methods. "Eastman Chromatogram Sheets" (6061 Silica Gel, Eastman Kodak Company, Rochester, N.Y.) which were already layered with Silica Gel were impregnated with silver nitrate as follows. Silver nitrate (1.58 g) was dissolved in water (4.5 ml) and absolute ethanol (40.5 ml) was added (13). The "Eastman Chromatogram Sheets" were dipped in the solution for several seconds. The sheets were air dried in the dark, activated by placing in an oven at 105° for 10 min and used within an hour with the above solvent systems.
Gas-Liquid Chromatographic Separations

Gas-liquid chromatography was used to check the purity of samples, to provide tentative identifications of unknowns, and in some cases for the collection of pure samples. The instrument was a Hewlett-Packard 5750 Research Chromatograph (Hewlett-Packard Company, Palo Alto, Calif.) equipped with a flame ionization detector. Helium was used as a carrier gas. Different columns were used in an attempt to find a column packing which would give good resolution. Details of these columns are listed in Table 3.

In some cases, samples were separated as their trimethylsilyl ether derivatives. These trimethylsilyl ethers were prepared by reacting dry samples for 5 to 10 min at room temperature with hexamethyldisilazane and trimethylchlorosilane in pyridine (2:1:10 v/v/v) (76). The pyridine solution was injected directly into the gas chromatograph.

In some cases, samples were methylated by diazomethane (61) previous to gas-liquid chromatographic resolution.

For identification purposes, authentic samples or their derivatives were injected and their peak retention times were compared with the peak retention times of materials in the unknown sample. Peak enhancement studies were conducted by adding small quantities of authentic samples to the unknown sample that was of interest. The mixture was then injected into the gas chromatograph.
Table 3. Columns Used in Gas-Liquid Chromatographic Separations.

<table>
<thead>
<tr>
<th>Liquid coating</th>
<th>Concentration (wt %)</th>
<th>Solid support</th>
<th>Mesh</th>
<th>O. D. (in)</th>
<th>Length (ft)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OV-17</td>
<td>3</td>
<td>Gas Chrom. Q</td>
<td>100/120</td>
<td>1/8</td>
<td>6</td>
</tr>
<tr>
<td>UC W-98</td>
<td>10</td>
<td>Silicone Gum</td>
<td>80/100</td>
<td>1/8</td>
<td>6</td>
</tr>
<tr>
<td>SE-30</td>
<td>5</td>
<td>Gas Chrom. Q</td>
<td>60/80</td>
<td>1/8</td>
<td>5</td>
</tr>
<tr>
<td>SE-30</td>
<td>5</td>
<td>Chromosorb G.</td>
<td>80/100</td>
<td>1/8</td>
<td>6</td>
</tr>
<tr>
<td>SE-52</td>
<td>10</td>
<td>Chromosorb G.</td>
<td>80/100</td>
<td>1/8</td>
<td>12</td>
</tr>
<tr>
<td>SAIB</td>
<td>1.5</td>
<td>Chromosorb G.</td>
<td>100/120</td>
<td>1/8</td>
<td>9</td>
</tr>
<tr>
<td>PDEAS</td>
<td>1.0</td>
<td>Chromosorb G.</td>
<td>100/120</td>
<td>1/8</td>
<td></td>
</tr>
<tr>
<td>Carbowax 20 M TPA</td>
<td>Support coated open tubular column (&quot;S. C. O. T.&quot; column)</td>
<td>0.02d</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG</td>
<td>2.0</td>
<td>Chromosorb G.</td>
<td>100/120</td>
<td>1/8</td>
<td>6</td>
</tr>
<tr>
<td>OV-17</td>
<td>1.0</td>
<td>Chromosorb G.</td>
<td>100/120</td>
<td>1/8</td>
<td></td>
</tr>
</tbody>
</table>

aSAIB = Sucrose Acetate Isobutyrate
bPDEAS = Phenyldiethanolamine Succinate
cPEG = Polyethylene Glycol
d0.02 in. I.D.
The instrument was also equipped with an effluent splitter and a collection system. After the sample was injected and had traveled through the column, the splitter at the end of the column separated the effluent so that $1/6$ of it went to the detector and the remaining $5/6$ went to the outlet and could be collected. For collection purposes, a 1/4 in. (O.D.) column was used instead of a 1/8 in. column. One column was packed with OV-17 and the other was packed with PDEAS-SAIB. The rest of the conditions were the same as in Table 3 and the operating conditions were the same as shown in Table 4.

**Liebermann-Burchard Test**

Some of the fractions collected by column chromatography (Figure 2) were tested for sterols by the Liebermann-Burchard Test (25) as follows.

A small amount of dry sample was placed in a test tube and a few milliliters of acetic anhydride were added. Concentrated sulfuric acid was added dropwise and the color change was observed. A positive test was indicated when the colorless solution first turned purple, then blue, then dark green.

**Saponification Reaction Conditions**

Since some of the unknown materials showed evidence for ester linkages, they were subjected to a saponification reaction. The procedure is shown in Chart 1.
### Table 4. Conditions for Gas-Liquid Chromatographic Separations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Figure no.</th>
<th>Detector temp. (°C)</th>
<th>Injector temp. (°C)</th>
<th>Column</th>
<th>Column temp. (°C)</th>
<th>Flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsaponifiable part of Fraction 2</td>
<td>3</td>
<td>270</td>
<td>255</td>
<td>UC W-98</td>
<td>240</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>265</td>
<td>260</td>
<td>OV-17</td>
<td>240</td>
<td>35</td>
</tr>
<tr>
<td>Saponifiable &amp; ether-soluble Fraction 2</td>
<td>7</td>
<td>270</td>
<td>255</td>
<td>UC W-98</td>
<td>150-240 at 4°/min</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>260</td>
<td>255</td>
<td>OV-17</td>
<td>140-240 at 2°/min</td>
<td>35</td>
</tr>
<tr>
<td>Unsaponifiable part of sample 15</td>
<td>15</td>
<td>250</td>
<td>245</td>
<td>SE-30</td>
<td>210</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>255</td>
<td>240</td>
<td>OV-17</td>
<td>210</td>
<td>30</td>
</tr>
<tr>
<td>Steam distillate</td>
<td>25</td>
<td>160</td>
<td>160</td>
<td>PDEAS-SAIB</td>
<td>60-140 at 2°/min</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>170</td>
<td>170</td>
<td>S. C. O. T.</td>
<td>55-145 at 3°/min</td>
<td>6</td>
</tr>
</tbody>
</table>
Sample (100 mg)

1. Dissolved in 95% EtOH with 10% KOH
2. Refluxed
3. Extracted three times with 10-ml portions of n-hexane

n-Hexane solubles
(unsaponifiable fraction)  n-Hexane insoluble
(saponifiable fraction)
or EtOH soluble

1. Neutralized to pH 3-4 with conc. H₂SO₄
2. Extracted three times with 10-ml portions of diethyl ether

Diethyl ether solubles

Aqueous portion

Chart 1. Saponification Reaction Procedure.
Spectral Analyses

Infrared, nuclear magnetic resonance, and mass spectra were obtained on purified samples. The infrared spectra were obtained by the potassium bromide disk method on a Beckman IR 20A spectrophotometer. The nuclear magnetic resonance spectrum was recorded on a Varian 100 HA spectrometer. $^6$-Acetone was the solvent used for the saponifiable part of fraction 15.

The field desorption mass spectra of standard ferulic acid and the saponifiable and diethyl ether-soluble part of sample 15 were recorded on a modified Hitachi-Perkin Elmer RMU-7 mass spectrometer. The accelerating voltages were +1.8 kV applied to the field anode and -6.8 kV applied to the cathode. Emitter heating current was 0 mA. The multiplier voltage was 2.0 kV.

All the electron bombardment mass spectra were obtained on a Finnigan 1015C Mass Spectrometer except one spectrum which was recorded on an Hitachi-Perkin Elmer RMS-4 Mass Spectrometer. For the gas chromatographic-mass spectrometry (GC-MS) work the Finnigan mass spectrometer was connected to a Varian Aerograph 1400 Gas Chromatograph and the Hitachi-Perkin Elmer Mass Spectrometer was connected to a Perkin Elmer 990 Gas Chromatograph. The exact analytical conditions are listed in Table 5.

Some samples were analyzed by direct inlet into the ion source of
Table 5. Conditions for GC-MS Study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Figure</th>
<th>Mass spectrometer</th>
<th>GLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Filament current</td>
<td>GLC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Electron voltage</td>
<td>pressure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(μA)</td>
<td>(eV)</td>
</tr>
<tr>
<td>Saponifiable part of Fraction 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of Fraction 2</td>
<td>9</td>
<td>300-500</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Unsaponifiable part of sample 15</td>
<td>17</td>
<td>250</td>
<td>70</td>
</tr>
<tr>
<td>of sample 15</td>
<td>18</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Volatile material</td>
<td>27</td>
<td>350</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

*a* All spectra were recorded on a Finnigan 1015C Mass Spectrometer except the spectrum shown in Figure 27 which was recorded on a Hitachi-Perkin Elmer RMS-4 Mass Spectrometer.

*b* The GC used was a Varian Aerograph 1400 except that the GC used for taking the spectrum shown in Figure 27 was a Perkin Elmer 990 Gas Chromatograph.

*c* Detailed column and operating conditions are given in Tables 3 and 4.
the Finnigan 1015C mass spectrometer. The filament current was 400 A, the electron voltage was 70 eV and the analyzer pressure was $10^{-7}$ atmospheres.
IV. RESULTS AND DISCUSSION

Collection of Bark Samples

Because tree to tree variations of the content of the n-hexane-soluble materials had been shown to be small (about 0.5% from 50-year-old trees to 250-year-old trees) (32), no particular effort was made to obtain a random sampling of bark specimens. Bark samples were removed from a representative tree.

Sample Preparation

n-Hexane Extraction

Soxhlet extraction is a standard method for isolating solvent-soluble materials. Detailed extraction conditions such as sample size, extraction time, and so on, closely followed the methods outlined by Kurth and Kieffer (54). The n-hexane-soluble materials were light-yellow colored, wax-like solids, the yield was 5.11%, based on the oven-dried weight of bark.

Steam Distillation

One of the objectives of this study was to determine the volatile materials in the bark. To avoid heavy loss, steam distillation of the
bark was considered to be the best method, rather than separate them from the n-hexane extractives, although the volatile materials were indeed part of the n-hexane extractives.

There are three commonly used steam distillation techniques. The Markham method and the circulatory method are suitable for small scale (1-50 g of material) distillations. The method used in the present work is suitable for large-scale distillations although the yield is lower than from the other two methods (100). However, a certain amount of volatile materials were inevitably lost during extraction of the distillate with diethyl ether and during evaporation of the diethyl ether.

**Chromatographic Techniques**

**Column Chromatographic Separations**

Previous studies (24) showed that it was difficult to separate the bright blue band and the light bluish-green band on the column chromatograph (Figure 2) (these two had been designated as the yellow band). In the present study the chromatographic conditions were modified. Distilled water (8%, based on the oven-dried weight of Silica Gel) was added. The activity of the solid phase was thus changed. The results (Figure 2) showed a great improvement in separating the above mentioned two bands.

After developing the column chromatograph for three days, there were still three bands near the top of the column which showed
little movement (Figure 2). Instead of further chromatography, each band was scratched out of the column and extracted with the developing solvent.

Thin-Layer Chromatography and Gas-Liquid Chromatography

These two methods were used for purification, collection, and identification. The results will be discussed based on the properties of the fractions.

Identification of Sterol Esters

Because no great variation could be expected between any two consecutive fractions (Figure 2), only every two or three fractions were tested by thin-layer chromatography. Diethyl ether-n-hexane (1:4, v/v) and chloroform-carbon tetrachloride (6:1, v/v) were used as solvent systems. The plates were examined under ultraviolet light and then exposed to iodine vapors to detect the spots. The results showed that fractions 1 to 6 contained two main compounds ($R_f = 0.96$ and 0.76) although each spot showed tailing. As expected, the slower moving bands (Figure 2) showed more complex compositions by thin-layer chromatography. A dark blue spot ($R_f = 0.31$) began to show up in fraction 8, although during column chromatography this dark blue band was not detected until fraction 12.
Liebermann-Burchard tests were conducted on fraction numbers 1, 2, 4, 6, 9, and 11. Positive results (dark green color) were found in all fractions 1 to 9 with the most prominent color from fraction 2 and the least prominent color from fraction 9. Since both free sterols and sterol esters will show positive Liebermann-Burchard tests, it was considered possible that one or both types of compounds might be present in these fractions. However, thin-layer chromatography and gas-liquid chromatography ruled out the possible existence of free sterols. Thin-layer chromatography showed no spots which migrated the same distance as authentic free sterols, and gas-liquid chromatography showed no peaks at all although several column systems (UC W-98, OV-17, and SE 52) were investigated.

Fraction 2 (Figure 2) was subjected to saponification (Chart 1) since it showed the strongest Liebermann-Burchard result. After saponification, the n-hexane-soluble fraction (the unsaponifiable fraction) was tested by thin-layer chromatography. A spot at $R_f = 0.15$ (solvent; diethyl ether-n-hexane 1:4 v/v) corresponded to authentic $\beta$-sitosterol. Gas-liquid chromatography using UC W-98 and OV-17 columns showed two peaks with retention times identical to authentic $\beta$-sitosterol and campesterol (Figures 3 and 4). Conditions were as shown in Tables 3 and 4. Peak enhancement studies showed no new peaks and the peaks corresponding to $\beta$-sitosterol and campesterol became larger upon the addition of authentic compounds.
Figure 3. Gas-liquid chromatographic separation on UC W-98 of the unsaponifiable material from fraction 2. Peak "a" is behenyl alcohol, "b" is lignoceryl alcohol, "c" is campesterol and "d" is β-sitosterol. Other peaks were not identified. Conditions: 10% UC W-98 on Silicone Gum 80/100; 6' x 1/8", Isotherm. 240; Detector 270, Injector 255. Flow 30 ml/min.
Figure 4. Gas-liquid chromatographic separation on OV-17 of the unsaponifiable material from fraction 2. Peak "a" is behenyl alcohol, "b" is lignoceryl alcohol, "c" is campesterol and "d" is β-sitosterol. Conditions: 3% OV-17 on Gas Chrom Q 100/120; 6' x 1/8", Isotherm, 240°; Detector 265°, Injector 260°. Flow 35 ml/min.
These two peaks were collected separately by injecting samples through a one-quarter inch O.D. OV-17 column. Mass spectra were taken by the direct ion source method. The two spectra are shown in Figures 5 and 6.

The spectra are similar to each other below m/e 200 and both show a base peak at m/e 81. The material that gave the spectrum in Figure 5 had the same retention time as authentic campesterol by gas chromatography. The mass spectrum of the material showed a parent ion peak at m/e 400. Ions at m/e 385 (P-15) corresponded to a loss of a methyl group, and at m/e 382 (P-18) showed the loss of a molecule of water. The peak at m/e 367 (P-33) indicated the loss of a methyl group and a water molecule. These are general phenomena for compounds containing hydroxy groups and for sterols.

Characteristic peaks that indicated the compound was a monounsaturated sterol were found at m/e 315, 289, 274, 273, 261, 255, 246, 231, 229 and 213. Ions at m/e 315 and 289 occur mainly in \( \Delta^5 \)-monounsaturated sterols (X) and are only intense when the sterol is in the free form (49). These ions can also serve to distinguish this class from the corresponding \( \Delta^7 \)-monounsaturated sterols.

The ion at m/e 315 may arise from the parent ion of X less a molecule of water and part of rings A and B (either C_2 to C_6 or C_3 to C_7) but would need to lose two additional hydrogen atoms. Similarly, elimination of C_1 to C_7 as a unit could furnish the ion at m/e 289.
Figure 5. Mass spectrum of campesterol. Between m/e 95 and 200, only peaks with intensities higher than 10% are shown. Peaks below m/e 95 are not shown.

*The compound under peak "c" of the gas-liquid chromatogram shown in Figure 4.*
Figure 6. Mass spectrum of $\beta$-sitosterol. a Only peaks above m/e 200 are shown.

a The compound under peak "d" of the gas-liquid chromatogram shown in Figure 4
The ion at m/e 261 consists of rings C and D together with the side chain after loss of a hydrogen atom (28). This ion is not as specific as the other two for \( \Delta^5 \)-sterols since it is also found in \( \Delta^4 \)-cholesten-3,6-dione (13). The ion at m/e 274 indicated loss of water and \( \text{C}_8\text{H}_{12} \) and is only appreciably observed for \( \Delta^5 \)- sterols (49).

The ions at m/e 273 and 255 (XI) corresponded to loss of a side chain and loss of a side chain plus water respectively (19). The ion at m/e 231 indicated loss of a side chain and ring D. The ion at m/e 213 (XII) indicated further loss of water from the previous ion (9).

The m/e 246 (XIII) ion is suggested to be due to the loss of a side chain and part of ring D. The further loss of -OH caused the ion at m/e 229 (49).
Characteristic peaks below m/e 200 were found at m/e 145, 119, 107 and 95. The m/e 145 ion (XIV) indicates the rupture of ring C. When ring C was broken at the 8-14 and 9-11 carbon to carbon bonds, the skeletal structure of rings A and B remained essentially intact. As usual, sterols which have an hydroxy group on C₃ will show an additional loss of water.

The m/e 119 ion (XV) was formed as a result of the rupture of ring B. This was good evidence that the sterol had only one double bond in ring B. The m/e 107 (XVI) and m/e 95 (XVII) fragment peaks were due to other types of rupture of ring B.
Based on the mass spectra and the gas chromatographic results, the compound was identified as campesterol.

The material that gave the spectrum shown in Figure 6 had the identical retention time by gas-liquid chromatography as authentic \( \beta \)-sitosterol. The spectrum showed a parent ion peak at m/e 414. Peaks at m/e 399, 396 and 381 were due to loss of a methyl group, a water molecule, and water plus a methyl group respectively. Characteristic peaks were found at m/e 329, 303, 288, 275, 273, 255, 246, 231, 229, 213, 145, 119, 107, and 95. These ions can be divided into two groups. One group includes all the above mentioned ions except the first four ions (m/e 329, 303, 288 and 275). This group was also shown in the spectrum of campesterol and resulted from the loss of the side chain. The remaining nucleus (rings ABCD) was bombarded into the same fragments as campesterol and formed these ions.

The other group was the ions m/e 329, 303, 288, and 275 which included the side chain. They all showed 14 mass units higher than the corresponding four ions in the spectrum of campesterol. This indicates that the side chain contained one more methylene unit.

The spectrum is very similar to the published spectrum of \( \beta \)-sitosterol (95). The intensities of some peaks were different due to different experimental conditions. The structure of \( \beta \)-sitosterol shows that it has one more methylene unit than campesterol. Based on the results of gas-liquid chromatography and mass spectroscopy, the compound was identified as \( \beta \)-sitosterol.
The percentage ratio of campesterol and \( \beta \)-sitosterol in the unsaponifiable portion (the \( n \)-hexane-soluble fraction after saponification) of fraction 2 (Figure 2) was campesterol 6.4\%, and \( \beta \)-sitosterol 29.3\%. The percentages were determined by measuring the areas under peaks "c" and "d" in Figure 4 and dividing each area by the sum of the areas under all the peaks (excluding solvent peak) and multiplying by 100.

The saponifiable and diethyl ether-soluble part of fraction 2 was methylated with diazomethane. The methyl ester was injected into the gas chromatograph using UC W-98 and OV-17 columns. The conditions are given in Table 3 and the chromatograms are shown in Figures 7 and 8. The peaks on these chromatograms are referred to in the remainder of the text as 8a, 8b, and so on. The number designates the figure and the letter designates the peak in the figure.

The chromatograms showed that the saponifiable and diethyl ether-soluble fraction (Chart 1) was a very complex mixture which contained at least eight major components. The peaks of the major components appeared in a regular pattern at about six-minute intervals which indicated that they might have similar structures.

A mixture of authentic saturated fatty acid methyl esters ranging in chain length from 14 carbons to 24 carbons (even carbon numbers only) and of methyl esters of \( C_{13} \), \( C_{17} \) and \( C_{19} \) fatty acids was injected into the gas chromatograph under the same conditions.
Figure 7. Gas-liquid chromatographic separation on UC W-98 of the saponifiable and diethyl ether-soluble materials from fraction 2. Peak "a" is methyl tridecanoate, "b" is methyl hexadecanoate, "c" is methyl heptadecanoate, "d" is methyl cis-9-octadecenoate, "e" is methyl nonadecanoate, "f" is methyl eicosanoate, "g" is methyl docosanoate, "h" is methyl tetracosanoate. Other peaks were not identified. Conditions: 10% UC W-98 on Silicone Gum 80/100, 6' x 1/8", 150°-240° at 4°/min, Injector 255°, Detector 270°, Flow 35 ml/min.
Figure 8. Gas-liquid chromatographic separation on OV-17 of the saponifiable and diethyl ether-soluble materials from fraction 2. Peak "a" is methyl tridecanoate, "b" is methyl hexadecanoate, "c" is methyl heptadecanoate, "d" is methyl cis-9-octadecenoate, "e" is methyl nonadecanoate, "f" is methyl eicosanoate, "g" is methyl docosanoate, "h" is methyl tetracosanoate. The other peaks were not identified.

Conditions: 3% OV-17 on Gas Chrom Q 100/120, 6' x 1/8", 140°-240° at 2°/min, Injector 255°, Detector 265°, Flow 35 ml/min.
and their retention times were compared with the unknowns. Standard methyl esters of oleic acid, linoleic acid, cis-5-eicosenoic acid, and erucic acid were also tested. Since methyl stearate (C\textsubscript{18}) showed a similar retention time to methyl oleate (C\textsubscript{18:1}) gas-liquid chromatographic results could not tell which one existed in the unknown. This had to be determined by mass spectrometry. The conditions were those shown in Table 5. The spectra are shown in Figures 9 through 13. These spectra showed similar patterns in the low mass ends and have base peaks at m/e 74 except the spectrum of peak 8d (Figure 13).

The mass spectrum of peak 8a from the gas chromatograph (Figure 9) showed the base peak at m/e 74. This peak was formed by a C\textsubscript{2}-C\textsubscript{3} cleavage with respect to the carbonyl group with simultaneous migration of one hydrogen atom from the fragments which were lost (13, 62). The reaction thought to occur is as shown below:

$$\text{CH}_3\text{-O-C-CH}_2\text{-CH}_2\text{-CH}_2\text{-R} \rightarrow \text{CH}_3\text{-O-C=CH}_2\text{CH}_2\text{CH} = \text{CH-R}$$

The hydrogen atom was removed from carbon atom 4 and attached to the oxygen atom (91). Meanwhile the alkyl fragment formed a stable 1-olefin.

The second strongest peak was at m/e 87, with a relative intensity of 58% compared to the base peak. The peak was due to the normal methoxycarbonyl type fragment $\text{CH}_3\text{-O-C-CH}_2\text{-CH}_2$.
Figure 9. Mass spectrum of methyl tridecanoate.\(^a\)  
\(^a\)The compound in peak "a" of Figure 8.

Figure 10. Mass spectrum of methyl hexadecanoate.\(^a\)  
\(^a\)The compound in peak "b" of Figure 8.
Significant peaks were found at m/e 171, 185, 199 and 228. No peak due to the sample was found above m/e 228. Thus m/e 228 was considered to be the parent ion peak (P^+). The small relative intensity of the P+1 peak (m/e 229) and the P+2 peak (m/e 230) supported this conclusion.

The peak at m/e 199 (P-29) was due to ions formed with loss of an ethyl group, which is also a constant feature of methyl ester spectra. Peaks at m/e 185 (P-43) and m/e 171 (P-57) corresponded to the loss of propyl and butyl fragments respectively.

Peaks at m/e 101, 115, 129, and 143 were due to the ion \([\text{CH}_3\text{-O-C-(CH}_2\text{)}_n\text{-}]^@\) where n=3, 4, 5, and 6 respectively. The ions of greater mass in this series, such as 157 and 171, are partly formed by elimination of part of the chain. One hydrogen atom is lost in addition to a number of methylene groups giving rise to an odd-numbered even-electron ion (69).

The mass spectrum of peak 8a (Figure 9) was similar to the standard published spectrum of methyl tridecanoate, although relative intensities of some peaks were different. This was due to different experimental conditions (31).

The results of the gas chromatography, mass spectrometry, and the plot of the log of retention time of isothermal gas chromatography versus chain length (Figure 11) all agreed. The compound in peak 8a (Figure 9) was identified as methyl tridecanoate.
Figure 11. Plot of the log of the retention time of isothermal gas chromatography of the fatty acid methyl esters vs. chain length. Conditions: 3% OV-17 on 100/120 Gas Chrom Q; 6' x 1/8"; Isotherm. 190°; Injection 220°, Detector 220°.
The spectrum of peak 8b (Figure 10) showed similar cracking patterns to Figure 9, with characteristic peaks at m/e 43, 57, 74 (base peak), 87 (second strongest peak, relative intensity 68%), 101, 115, 129, 143, 157, 171, 185, 199, 213 (P-57), 227 (P-43), 239 (P-31), 241 (P-29), and 270 (P+). The m/e 270 peak represented the molecular ion. The peak at m/e 241 indicated the loss of an ethyl fragment and the m/e 239 (P-31) peak was due to loss of an acylium fragment RCO.

The spectrum of peak 8c (Figure 12) had similar fragmentation patterns to those in Figure 10 in the low mass end. The characteristic peaks in the high mass end were m/e 284 (parent peak), 255 (P-29), and 241 (P-43). These characteristic peaks showed that the two compounds were fatty acid esters. The parent ion of m/e 270 for compound 8b (Figure 10) and m/e 284 for compound 8c (Figure 12) suggested the former had a chain of 16 carbons and the latter had 17 carbons.

These evidences, supported by gas chromatography results and the plotting of the log of the retention times versus chain length (Figure 11), identified the compound of peak 8b (Figure 10) as methyl hexadecanoate (methyl palmitate) and the compound of peak 8c (Figure 12) as methyl heptadecanoate.

The compound under the gas chromatographic peak of 8d showed a different spectrum (Figure 13). The base peak was at m/e 55 rather than m/e 74. Other important peaks were found at m/e 74, 87,
Figure 12. Mass spectrum of methyl heptadecanoate. a The compound in peak "c" of Figure 8

Figure 13. Mass spectrum of methyl cis-9-octadecenoate. a The compound in peak "d" of Figure 8
91, 101, 180, 222, 264, and 296. The m/e 296 was the parent peak. The peak at m/e 264 (P-32) was due to an ion formed from the loss of one molecule of methanol from the molecular ion. The peak at m/e 264 is higher than expected for an isotope peak and showed that ions are also formed with the loss of the methoxyl group only. The peak at m/e 222 (P-74) was due to loss of the ion CH$_3$-O-C=CH$_2$\(^+\). The m/e 91 peak is a typical peak for unsaturated fatty esters (89, 102). Under the same experimental conditions, the height of the peak increases with the degree of unsaturation. It was suggested that the peak was due to tropylium ions formed through extensive rearrangement and cyclization (33).

Based on the mass spectrometal data, this compound can be assumed to be a long chain fatty methyl ester with a chain length of 18 carbons which contained one double bond in the chain. The position of the double bond and whether the hydrogens are cis or trans cannot be determined directly from the mass spectrum. A pure sample was collected from the gas chromatograph and mixed with authentic methyl cis-9-octadecenoate for a melting point test. The result showed no depression. Based on the results of the mixed melting point, gas chromatography, and mass spectrometry, the compound was identified as methyl cis-9-octadecenoate (methyl oleate).

The mass spectra of compounds under the peaks 8f, 8g, and 8h (Figure 8) showed differences only in the high mass end. The spectrum
of the compound under the gas chromatographic peak 8f showed characteristic peaks at m/e 326 (P⁺), 297 (P-29), 295 (P-31), 281 (P-43) and 241 (P-85) which corresponded to the parent peak, the parent ion with loss of an ethyl fragment, the parent ion with loss of methoxyl, propyl and hexyl fragments respectively. The molecular weight corresponded to the molecular weight of methyl arachidate.

The spectrum of gas chromatographic peak 8g (Figure 8) showed characteristic peaks at m/e 354 (P⁺), 325 (P-29), 323 (P-31), 311 (P-43), and 297 (P-57). The mass spectrum of peak 8h (Figure 8) showed m/e 382 (P⁺), 339 (P-43), 325 (P-57), and 311 (P-71). The fragmentation pattern showed that these two compounds were fatty acid esters and the molecular ions at m/e 354 and m/e 382 suggested that they were saturated and had chain lengths of 22 carbons and 24 carbons respectively.

These three compounds were identified as methyl n-eicosanoate (methyl arachidate), methyl n-docosanoate (methyl behenate), and methyl n-tetracosanoate (methyl lignocerate) based on their gas chromatographic retention times, their mass spectra, and the plot of retention time versus chain length.

The retention time of gas chromatographic peak 8e (Figure 8) was the same as the retention time of standard methyl n-nonadecanoate. However, the peak was too small to obtain a good mass spectrum. Therefore, compound 8e was only tentatively identified as methyl n-nonadecanoate.
Those mass spectra that were taken before methyl arachidate (C_{20}) (peak 8f) showed relatively small parent ion peaks and more peaks in the high mass end than the published mass spectra. This was due to the high voltage of the electron beam. After gas chromatographic peak 8f (Figure 8) the experimental conditions were modified and the relative intensities of the peaks in the high mass end increased and were similar to the published spectra (23, 57, 75).

The area of each peak was measured (width at half peak height) to obtain the percentage ratio of each component in the saponifiable and ether-soluble portion of fraction 2 (Figure 2). The results were: n-tridecanoic acid, 3.5%; palmitic acid, 14.2%; n-heptadecanoic acid, 6.8%; oleic acid, 36.4%; n-nonadecanoic acid, 3.0%; arachidic acid, 8.5%; behenic acid, 17.9%; and lignoceric acid, 8.9%. The ratios were obtained by measuring the area under each peak and dividing it by the total area under all of the peaks.

No material was found in the saponifiable and water-soluble part of fraction 2 other than inorganic salts.

Prior to saponification, the materials in fraction 2 (Figure 2) had been tentatively shown to be sterol esters rather than free sterols. After saponification, the alcohol parts of the esters were shown to be β-sitosterol and campesterol. The acid parts were a greater mixture and contained at least eight kinds of fatty acids. Any or all of these fatty acids theoretically can form esters with the two sterols.
The material in fraction 2 (Figure 2) was thus proven to contain sterol esters. 

Due to the complex mixture of fatty acids, the resultant sterol esters were also a mixture. These sterol esters, although quite different in molecular weights, were very similar in chemical properties. This explains the extensive spot tailing in thin-layer chromatography, the difficulty of isolating a pure ester from the mixture, and why the percentage of each individual ester could not be measured. A typical sterol ester is as shown below (XVIII) which upon saponification would yield behenic acid and β-sitosterol.

\[
\text{XVIII. Ester of behenic acid and } \beta\text{-sitosterol.}
\]

Sterol alcohols were not the only alcohols present in fraction 2 (Figure 2). The unsaponifiable material (Chart 1) also contained behenyl alcohol (25.2%) and lignoceryl alcohol (29.7%) as shown in Figures 3 and 4. The percentages were obtained from the area under each peak x 100 divided by the sum of the area under all the peaks. These alcohols are also known to form esters with the fatty acids.
Therefore, fraction 2 (Figure 2) undoubtedly contained both sterol esters and wax esters (esters of long chain fatty acids and long chain fatty alcohols).

The presence of free β-sitosterol and campesterol has been reported in a previous study (24). The present work shows that these sterols also exist as part of esters.

β-Sitosterol was suggested to be ubiquitous in higher plants (35), and may have a function in cell wall permeability (83). Rowe (83) has reported the presence of trace amounts of various dihydrosterols, cholesterol, and 7-keto-β-sitosterols in different species of pine barks. These sterols were not found in Douglas-fir bark in either the free or esterified form.

The acid parts of the sterol esters have been shown to be complex mixtures. The most abundant acid was oleic acid (36.4%) and the second most abundant acid was behenic acid (17.9%). The results also showed the presence of fatty acids which contain an odd number of carbon atoms. The acids were n-tridecanoic (3.5%), n-heptadecanoic (6.8%) and possibly n-nonadecanoic acid (3.0%). The presence of these odd carbon-numbered acids has not been previously reported in Douglas-fir bark.

Characterization of the esters in fraction 2 (Figure 2) provided a number of indications. First, since the unsaturated oleic acid esters were the most abundant, the surface wettability of Douglas-fir
bark might be reduced because of oxidation as Hemingway (37) found in the case of yellow birchwood. This might prove significant in the use of bark in composite products and similar materials. Second, Rogers (81) found that fatty acids from spruce had a toxicity to young kokanee salmon at the 10 ppm concentration level. Since the present acids from Douglas-fir bark have a similar composition to the acids from spruce, it is possible that they too are toxic to young kokanee salmon. Third, saponification of fraction 2 (Figure 2) from Douglas-fir bark yielded mostly saturated fatty acids. Therefore, the bark cannot be used as a supply for "tall oil" compounds because the "tall oils" are composed primarily of unsaturated compounds. Fourth, the detection of odd-numbered fatty acids from Douglas-fir bark can be of value for chemotaxonomy purposes. The fatty acids from red fir bark (3), western red cedar bark (26), and grand fir bark (80) have been investigated and no odd-numbered fatty acids were found. Thus Douglas-fir bark differs in its chemical composition and these differences can be used for identification purposes.

**Identification of Ferulic Acid Esters**

Fractions 13 to 17 (Figure 2) showed a dark blue coloration on the column chromatograph when exposed to ultraviolet light. These fractions were collected from the column chromatograph into five different flasks. After the solvent evaporated, a greyish-white solid
was observed in each flask. These samples were tested by thin-layer chromatography using diethyl ether-n-hexane (1:4 v/v) as developing solvent. The resultant chromatograms were identical except that the areas of the dark-blue spots observed under ultraviolet light were different. These five fractions were thus considered to be identical and were put together and designated sample 15. The yield was 24.5% of the total n-hexane-soluble fraction of Douglas-fir bark.

Two methods were used to purify sample 15. The first method was to recrystallize the solids five times from acetone. The second method was to use thin-layer chromatography to separate the material. The dark-blue band was scraped from the thin-layer plates and extracted with n-hexane to obtain pure material. The second method proved better than the first. The melting point was 67°-70° (Fischer-Johns melting point apparatus).

Sample 15 was first thought to be lignoceryl alcohol since Kurth (51) had reported 20% of lignoceryl alcohol in the n-hexane "wax" of Douglas-fir bark and it was white in color with a melting point of 69-70°. Thin-layer chromatography (chloroform-carbon tetrachloride 6:1 v/v) of sample 15 run simultaneously with authentic lignoceryl alcohol (Applied Science Laboratory, Inc.) showed that sample 15 had an Rf value of 0.19 and showed a dark-blue coloration under ultraviolet light while authentic lignoceryl alcohol had an Rf value of 0.29 and showed no coloration under ultraviolet light. This excluded the possibility that fraction 15 was lignoceryl alcohol.
Kurth also reported 20% of ferulic acid in n-hexane wax (51). However, sample 15 was easily distinguished from ferulic acid because of the color difference and melting point difference (ferulic acid is yellowish-brown in color and has a melting point of 174°).

Both silylated and unsilylated sample 15 were tested by gas-liquid chromatography with five different columns (SE-30, UC W-98, OV-17, QF-1, and Hi-EFF 8BP) under various conditions. There was no peak which corresponded to a sample peak under any of the conditions.

The infrared spectrum (potassium bromide pellet, Figure 14) showed a strong absorption at 1730 cm⁻¹. Carbonyl groups will show a strong absorption in this region (17). It was not an aldehyde, because there was no strong absorption near 1680-1705 cm⁻¹, 1660-1680 cm⁻¹, or 1695-1715 cm⁻¹. It was not a carboxylic acid because of the lack of strong absorption peaks at 1200-1320 cm⁻¹ and the lack of a broad medium absorption between 2900-3300 cm⁻¹. The infrared spectrum could not definitely indicate whether the compound was a ketone or an ester, although the absorption patterns showed that it was more likely to be an ester. The spectrum (Figure 14) especially indicated an α,β-unsaturated aryl ester because of the strong absorption at 1730 cm⁻¹ and a medium absorption at 1269 cm⁻¹. Absorptions at 1175 cm⁻¹, 1315 cm⁻¹, and especially at 3500 cm⁻¹ indicated that a phenolic group was present. An absorption at 719 cm⁻¹ indicated the presence of a long methylene chain.
Figure 14. Infrared spectrum of the material from sample 15.
Because the compound was thought to be an ester, saponification was considered a promising way to study the acid part and the alcohol part. The unsaponifiable portion of sample 15 was recrystallized from acetone and was compared with authentic fatty alcohols ranging in chain length from \( \text{C}_{14} \) to \( \text{C}_{24} \) by thin-layer chromatography using diethyl ether-\( \text{n} \)-hexane-methanol (10:40:1 v/v/v) as solvent system. The results showed that the unsaponifiable portion of sample 15 possessed the same \( R_f \) value \( (R_f = 0.70) \) as authentic behenyl alcohol \( (\text{C}_{22}) \) and lignoceryl alcohol \( (\text{C}_{24}) \).

Trimethylsilyl ethers of the unsaponifiable part of sample 15 were subjected to gas chromatography using SE-30 and OV-17 columns. The conditions are outlined in Table 3 (Figures 15 and 16). Two peaks were evident and their retention times were identical with the trimethylsilyl ethers of authentic behenyl alcohol and lignoceryl alcohol. Peak enhancement studies supported the original observations that the peaks were due to behenyl and lignoceryl alcohols.

Since behenyl alcohol and lignoceryl alcohol are structurally very similar, gas chromatography is the most effective way to separate them and mass spectrometry is the best way to distinguish them. Therefore, gas chromatography combined with mass spectrometry was carried out to obtain spectra for these two fatty alcohols as further evidence that they were behenyl alcohol and lignoceryl alcohol.
Figure 15. Gas-liquid chromatographic separation on SE-30 of the unsaponifiable material from sample 15. Peak "a" is solvent, peak "b" is the trimethylsilyl ether of behenyl alcohol, and peak "c" is the trimethylsilyl ether of lignoceryl alcohol. Conditions: 5% SE-30 on Gas Chrom Q 60/80, 5' x 1/8", Isotherm 210°, Injector 245°, Detector 250°, Flow 30 ml/min.
Figure 16. Gas-liquid chromatographic separation on OV-17 of the unsaponifiable material from sample 15. Peak "a" is solvent, "b" is the trimethylsilyl ether of behenyl alcohol, "c" is the trimethylsilyl ether of lignoceryl alcohol. Conditions: 3% OV-17 on Gas Chrom Q 100/120; 6' x 1/8", Isotherm. 210°, Injector 240°, Detector 255°, Flow 30 ml/min.
There are two reasons for using derivatives of the two alcohols rather than the original sample for the gas chromatography-mass spectrometry work. First, because of the low volatility of long chain fatty alcohols the material was not suitable for gas chromatography. Second, because the parent mass peak of a primary alcohol is very small for chain lengths longer than eight carbons, the parent peak cannot be observed (6, 63, p. 213). It is also difficult to observe peaks at m/e = P-18 and P-20, corresponding to ions formed with a loss of water and a loss of water plus one molecule of hydrogen (27, 90). Therefore, trimethylsilyl ether derivatives of the unsaponifiable portion of sample 15 were made and were injected into the gas chromatograph-mass spectrometer. The conditions are listed in Table 5. The spectra are shown in Figures 17 and 18.

The parent ion peak of the trimethyl silyl ether derivatives might not always be present, but the P-15 peak due to cleavage of one of the Si-CH$_3$ bonds is always prominent. Because of this, they are useful for determining the molecular weight of the original alcohol (23, 27).

In Figure 17, characteristic peaks were found at m/e = 29, 41, 42, 43, 55, 56, 57 (base peak), 69, 70, 71, 83, 84, 85, 97, 98, 99, 111, 125, 247, and 383. The fragmentation pattern was typical for a long alkyl chain as indicated by the presence of specific clusters of peaks. The corresponding peaks of each cluster are 14 (CH$_2$) mass
Figure 17. Mass spectrum of the trimethylsilyl ether of behenyl alcohol.\textsuperscript{a}

\textsuperscript{a}Compound from gas-liquid chromatographic peak "15b" (Figure 15).
Figure 18. Mass spectrum of the trimethylsilyl ether of lignoceryl alcohol.\textsuperscript{a}

\textsuperscript{a} Compound from gas-liquid chromatographic peak "15c" (Figure 15).
units apart. Peaks at m/e = 29, 43, 57, 71, 85, 99, 113, and 127 corresponded to $C_nH_{2n+1}$ fragments with $n = 2$ to 9 respectively. $C_nH_{2n}$ fragments were found at m/e = 42, 56, 70 and so on and $C_nH_{2n-1}$ peaks were observed at m/e = 41, 55, 69 and so on. The fragment intensities decreased with increasing chain length. The peak at m/e = 383 represented P-15, the trimethylsilyl ether of behenyl alcohol minus a methyl group. This peak indicated the original compound had a molecular weight of 398 which corresponded to the molecular weight of silylated behenyl alcohol.

Compounds containing more than eight methylene units show fairly similar spectra in the low mass end. Identification then depends on the parent ion peak, in this case on the P-15 peak.

Figure 18 showed a similar fragmentation pattern in the low mass end to Figure 17. However, in the high mass end, a peak at m/e = 411 was observed. This was the P-15 peak. Thus, the molecular weight of the silylated original sample was 426 which was the molecular weight of the silylated, standard lignoceryl alcohol.

The spectra of silylated authentic behenyl alcohol and lignoceryl alcohol were also obtained under the same conditions and they were identical with the spectra of the two unknowns.

Pure silylated behenyl alcohol and silylated lignoceryl alcohol were collected by preparative gas-liquid chromatography using a 1/4 inch OV-17 column. Desilylation was accomplished by adding
water to the sample and the desilylated alcohol was removed by
adding n-hexane and then separating the n-hexane layer. Thus there
were several steps required in preparing a sample for analysis.

Infrared spectra of the two purified free alcohols were taken.
They are shown in Figures 19 and 20. Characteristic peaks were
observed at 3340 cm\(^{-1}\) (O-H stretching), 2900 cm\(^{-1}\), 2840 cm\(^{-1}\)
(C-H stretching for alkane), 1450 cm\(^{-1}\) (C-H bending for -CH\(_2\)-), 1040
cm\(^{-1}\) (O-H bending and C-O stretching for primary alcohol), and 700
cm\(^{-1}\) (long alkyl chain). They were different only in the finger print
region (1430 cm\(^{-1}\) to 910 cm\(^{-1}\)). The spectrum of lignoceryl alcohol
had two additional peaks at 1260 cm\(^{-1}\) and 795 cm\(^{-1}\). The spectra of
the unknowns were identical to those from the authentic compounds.

The melting point of the isolated behenyl alcohol was 71°C, and
of the isolated lignoceryl alcohol was 75°C; both were unchanged on
admixture with authentic materials. The elemental analyses were as
follows. Calcd. for C\(_{22}\)H\(_{46}\)O (behenyl alcohol): C, 80.90%; H, 14.21%.
Found: C, 81.48%; H, 14.41%. Calcd. for C\(_{24}\)H\(_{50}\)O (lignoceryl
alcohol): C, 81.26%; H, 14.22%. Found: C, 80.42%; H, 14.22%.

The above evidences proved the existence of behenyl alcohol and
lignoceryl alcohol in the unsaponifiable portion of sample 15. The
percentage ratio of these two alcohols, according to the gas-liquid
chromatographic results of Figures 15 and 16 were behenyl alcohol
42.3% and lignoceryl alcohol 56.8%. The percentages were determined
Figure 19. Infrared spectrum of behenyl alcohol.\textsuperscript{a}

\textsuperscript{a}Desilylated compound from gas-liquid chromatographic peak "15b" (Figure 15)
Figure 20. Infrared spectrum of lignoceryl alcohol.\(^a\)

\(^a\)Desilylated compound from gas-liquid chromatographic peak "15c" (Figure 15)
by measuring the areas under peaks "b" and "c" of Figures 15 and 16 and dividing each area by the sum of the areas under all the peaks (excluding solvent peaks) and multiplying by 100.

The saponifiable and ether-soluble portion of sample 15 was recrystallized twice from benzene. The melting point was 168.5°-170.0°. The infrared spectrum (Figure 21) showed a strong absorption at 3430 cm^{-1} and strong absorptions near 1200 cm^{-1} which indicated a phenolic hydroxyl group. The strong absorption at 1695 cm^{-1} indicated an α, β-unsaturated carboxylic acid. The medium broad absorptions near 2900 cm^{-1} supported this indication. Absorptions at 1665 cm^{-1}, 1620 cm^{-1} and 968 cm^{-1} indicated a conjugated trans double bond (68, p. 24). Absorptions at 3020 cm^{-1}, 1600 cm^{-1}, 1590 cm^{-1}, 1510 cm^{-1}, 1465 cm^{-1}, 1200 cm^{-1}, 1174 cm^{-1}, 1030 cm^{-1}, 848 cm^{-1}, and 800 cm^{-1} not only corresponded to an aromatic ring but also represented an unsymmetrical trisubstituted aromatic ring (17, p. 30). A potassium bromide pellet containing authentic ferulic acid (Aldrich Chemical Co.) was also prepared with the same concentration and the spectrum was identical to that from the sample of the unknown.

A nuclear magnetic resonance (NMR) spectrum (Figure 22) was taken in deuterioacetone. A pair of one proton doublets centered at 6.386 and 7.606 corresponded to olefinic protons H_b and H_c. These two doublets had a coupling constant J = 16 cps which indicated a trans
Figure 21. Infrared spectrum of the saponifiable and diethyl ether-soluble part of sample 15.
Figure 22. NMR spectrum of ferulic acid.\(^{\text{a}}\)

\(^{\text{a}}\)The compound from the saponifiable and diethyl ether-soluble part of sample 15
double bond (92, p. 127 and 145). Since $H_b$ (Figure 22) is closer to the carbonyl group, it is deshielded more than $H_c$, so the doublet at 6.388 corresponded to $H_c$ and the doublet at 7.608 corresponded to $H_b$.

The one proton doublet ($H_e$) at 6.868 was split by the one proton doublet $H_d$ at 7.148 ($J_{ed} = 8$ cps). Each doublet of $H_d$ was further split into two peaks by a sharp one proton doublet $H_h$ at 7.328 ($J_{dh} = 2$ cps). $H_d$ was further downfield than $H_e$ because $H_d$ was closer to the olefinic double bond and the effect of the carbonyl group can be more effectively transmitted to it through the conjugated system. $H_h$ was split by $H_d$ into a doublet. The coupling constant for any two aromatic protons which are ortho to each other is about 6-10 cps (92, p. 145). In this case it was $J_{ed} = 8$ cps. The coupling constant for any two protons which are meta to each other is about 1-3 cps (92, p. 145). The result in the present case was $J_{dh} = 2$ cps. The coupling constant for any two protons which are para to each other is 0-1 cps and 0 is typical (92, p. 145). This explains why there is no split observed between $H_h$ and $H_e$. The three proton singlet ($H_g$) at 3.906 corresponded to a methoxy group.

Carboxylic acids exist as stable hydrogen-bonded dimers in nonpolar solvents, even at high dilution. The carboxylic proton absorbs in a characteristically narrow range, 13.28 to 10.08. Therefore, $H_a$ is not shown in this spectrum. The phenolic proton
H$_f$ was not observed because of exchange of deuterium with the solvent (92, p. 189).

Because ferulic acid was not volatile and not thermally stable enough to obtain an electron bombardment mass spectrum, field desorption mass spectrometry was used to obtain a molecular weight. Field desorption mass spectrometry is a modification of field ionization mass spectrometry. Field ionization mass spectrometry has been established for a number of years as an alternative and complimentary technique for the study of molecules which exhibit only extremely weak or no parent ion peaks in electron bombardment mass spectra. In field ionization, the governing process is the tunneling of an electron out of a molecule under the influence of an extremely high electric field. This mode of ionization is referred to as "soft" since essentially no energy is imparted to the molecule and no fragmentation occurs (4, 45). Thus, in contrast to electron bombardment mass spectra, field ionization mass spectra generally exhibit very intense molecular ion peaks and only very weak fragment peaks. This is particularly true of highly volatile, thermally stable compounds.

A limitation to field ionization mass spectrometry is the method of getting the molecules into the high electric field. When dealing with solid or liquid organic materials the common practice is to employ the very sensitive, direct insertion heating probe. The
substances, placed in a suitable micro-crucible or capillary, are gently heated and evaporated in the ionization zone as a molecular beam. However, with thermally unstable substances a major portion of the molecules is decomposed during the evaporation prior to the ionization process. Under these circumstances, even the field ionization mass spectra exhibit a large relative intensity of fragment mass peaks, and the main field ionization feature of an intense molecular ion mass peak is lost.

Field desorption mass spectrometry overcomes most of the difficulty of getting low volatile, thermally unstable materials into the high electric field. In field desorption mass spectrometry the sample is adsorbed directly from a liquid solution onto the surface of an emitter comprised of whiskers of carbon. In the presence of an extremely high electric field, the energy barrier which must be overcome for the removal of a surface atom or molecule from such a conductor is drastically reduced. In practice, the emitter with the adsorbed material is inserted into the high electric field in the mass spectrometer, where the molecules are desorbed and immediately field ionized. Of prime importance is the fact that during the process only a negligible amount of energy is transferred to the particle, so that molecules so ionized suffer no appreciable decomposition. Thus, it is possible to obtain molecular ion spectra of thermally unstable substances of low volatility.
In the present work, authentic ferulic acid was adsorbed directly from an acetone solution onto the surface of the emitter. The spectrum of authentic ferulic acid is shown in Figure 23. A sharp peak which corresponded to the parent ion of ferulic acid was shown at 299 seconds, which was 155 seconds from the acetone dimer marker (m/e 115).

The unknown sample from the saponifiable and diethyl ether-soluble part of sample 15 was also subjected to field desorption mass spectrometry under the same conditions as the authentic ferulic acid. The resultant spectrum of the unknown sample is shown in Figure 24 and was identical to the spectrum of authentic ferulic acid. Therefore, the molecular weight of the unknown was determined as 194, the same as ferulic acid.

Based on the above pieces of evidence which included melting point tests, infrared spectroscopy, nuclear magnetic resonance spectroscopy, and field desorption mass spectrometry, the compound in the saponifiable and diethyl ether-soluble part of sample 15 was shown to be ferulic acid.

The esters of ferulic acid were spread through the column chromatograph from fraction 9 to fraction 19 (Figure 2) although they were concentrated enough in only fractions 13 to 17 to be observed by ultraviolet light. The yield of the ferulic acid esters was 24.5% of the total n-hexane-soluble materials. The ferulic acid esters are shown as XIX.
Figure 23. Field desorption mass spectrum of authentic ferulic acid. m/e 194 is the molecular weight of authentic ferulic acid; m/e 115 is the molecular weight of acetone dimer marker.
Figure 24. Field desorption mass spectrum of unknown sample from the saponifiable and diethyl ether-soluble part of fraction 15. m/e 194 is the molecular weight of the unknown; m/e 115 is the molecular weight of acetone dimer marker.
Hergert (38) extracted a wax-like substance from extractive-free white fir cork with dioxane-hydrochloric acid. The yield was 48%. After saponification, 6% ferulic acid and 72% of an aliphatic hydroxy acid were found. According to Hergert, the original complex was composed of compounds which involved esterification between the carboxyl group of the hydroxy acid and the hydroxyl group of ferulic acid (38). The infrared spectrum of the presently isolated ester (Figure 14) showed an absorption at 3500 cm\(^{-1}\), which corresponded to a free hydroxyl group, indicating that, although the compound was an ester, the ester linkage was entirely different from the ester linkage found by Hergert (38).

Hergert's ester was isolated from extractive-free white fir cork and he suggested that suberin must contain esters of aliphatic hydroxy acids and phenolic acids, and the cork cell wall consists of a lignocellulose network impregnated with this polyester. The ester found in the present work was isolated directly from the \(n\)-hexane extractives. The different sources showed different types of esters.
Characterization of the ferulic acid esters provided a number of indications. First, Kurth (51, 52) had reported the presence of ferulic acid in the n-hexane "wax" of Douglas-fir bark and many workers have thought that large quantities of free ferulic acid could be obtained by direct extraction of the bark with n-hexane. The present work shows that the ferulic acid moiety exists in the n-hexane "wax" combined as esters, and not in the free form. It is quite unlikely that free ferulic acid exists in the "wax" because the free acid is insoluble in hot n-hexane. Therefore, the acid cannot be obtained by simple extraction.

Second, Kurth (52) commented that "ferulic acid is a very reactive aromatic acid" and that "this material gives the wax many interesting and unique properties. It affords the organic chemist many opportunities to modify the properties of the wax." However, since the present work shows that the ferulic acid moiety exists in the "wax" combined as esters any reactions designed to modify free ferulic acid in order to modify the "wax" cannot be considered. If one wants to modify the properties of the "wax," such as hardness, melting point, color and so on, through the ferulic acid moiety, one must consider that the reaction will be done on an ester not a free acid.

Third, Allan (1) has reacted phenolic acids with polyalcohols and found that the resulting esters can be used for drilling muds,
cement additives, and pesticide dispersants. Since the naturally occurring ferulic acid esters are already present in quite large amounts and are fairly easy to separate, the above uses might add to the economic feasibility of extracting Douglas-fir bark "wax."

Fourth, the resistance of heartwood to decay is ascribed to the presence of compounds having phenolic properties, for example, the pinosylvins in pine heartwood (21) and the thujaplicins in the thuja species (20). Von Rudloff has suggested that tamarack owes its resistance to decay to the presence of the flavanons and ferulic acid esters (66, 67). The presence of large amounts of ferulic acid esters in Douglas-fir bark may help resistance to decay.

**Identification of Volatile Materials**

The volatile materials from Douglas-fir bark proved to be a very complex mixture which could be separated only by gas-liquid chromatography. The columns used for the gas-liquid chromatography were the best columns for separating terpenes. Four columns were tried and the best was the Support Coated Open Tubular column ("S. C. O. T. " column). The superior separating power was due to the very small column diameter (the inside diameter was 0.02 inches) and the length of the column (50 feet). However, only a very small amount of sample could be injected into it (usually less than 0.4 µl) hence the separated components could not be collected. The best
liquid phase for regular 1/8 inch columns was the mixture of 1.5% SAIB (sucrose acetate isobutyrate) and 1.0% PDEAS (phenyl diethanolamine succinate). Temperature programming was also necessary to give good results. Figures 25 and 26 show the gas-liquid chromatographic separations obtained for the volatile materials. Finnigan and Hitachi-Perkin Elmer mass spectrometers (Table 5) were attached directly to the outlets from the gas chromatographs and were used to take mass spectra of the various peaks shown in Figures 25 and 26.

The Finnigan mass spectrometer was attached to the SAIB-PDEAS column and the Hitachi-Perkin Elmer mass spectrometer was attached to the 'S. C. O. T.' column.

Gas-liquid chromatographic peaks 25a and 26d (Figures 25 and 26 respectively) had the same retention times as authentic furfural (Eastman Organic Chemicals, Rochester, N. Y.). Because the 'S. C. O. T.' column gave better resolution of the gas-liquid chromatographic peaks (Figure 26) and since it was attached to the Hitachi-Perkin Elmer mass spectrometer, the mass spectrum (Figure 27) recorded on this instrument was better and clearer than the mass spectra recorded on the Finnigan mass spectrometer.

The mass spectrum (Figure 27) showed that m/e 96 was the parent peak as well as the base peak. The second highest peak was m/e 95. The peak at m/e 39 corresponded to the fragment which contained C$_3$ to C$_5$ and m/e 29 corresponded to the aldehyde fragment.
Figure 25. Gas-liquid chromatographic separation on SAIB-PDEAS column of the volatile materials. Peak "a" is furfural, "b" is terpinene-4-ol, "c" is α-terpineol, "d" is guaiacol, "e" is citronellol, "f" is geraniol, "g" is 2,5-dimethyl-3-acetylfuran, "h" is β-cyclocitral. Other peaks were not identified. Conditions: SAIB-PDEAS on chromosorb G, H.P. 100/120, 60°-140° at 20°/min, Detector 160°, Injector 160°, Flow 30 ml/min.
Figure 26. Gas-liquid chromatographic separation on "S.C.O.T." column of the volatile materials. Peak "a" is $\alpha$-pinene, "b" is $\beta$-pinene, "c" is limonene, "d" is furfural, "e" is terpinene-4-ol, "f" is $\alpha$-terpineol, "g" is guaiacol, "h" is citronellol, "i" is geraniol, "j" is 2,5-dimethyl-3-acetylfuran, "k" is $\beta$-cyclocitral. The other peaks were not identified. Conditions: "S.C.O.T." column. 55°-145° at 3°/min, Detector 170°, Injector 170°.
Figure 27. Mass spectrum of furfural, recorded on Hitachi-Perkin Elmer mass spectrometer.

Material under GC peak 26d (Figure 26)

Figure 28. Mass spectrum of the mixture of furfural and 1,5- \( \mu \)-methadien-7-ol recorded on Finnigan mass spectrometer.

Materials under GC peak 25a (Figure 25)
The spectrum was the same as the standard published spectrum (95) for furfural. The gas chromatographic results and the mass spectrum indicated that the compound was furfural (XX). Based on the gas-liquid chromatographic spectrum shown in Figure 26, the compound amounted to 6.5% of the materials. The percentage ratio (6.5%) was determined by dividing the area of gas-liquid chromatographic peak 26d by the sum of the peak areas in Figure 26 (excluding solvent peak) and multiplying by 100.

\[
\text{CHO} \quad \overset{\text{XX}}{\text{Furfural}}
\]

The mass spectrum recorded on the Finnigan instrument from the SAIB-PDEAS gas-liquid chromatographic column (Figure 28) proved to be a spectrum of a mixture. The spectrum was recorded when gas chromatographic peak 25a (Figure 25) reached its maximum height. Based on results of a peak enhancement study with authentic furfural, and the spectrum taken on the Hitachi-Perkin Elmer instrument (Figure 28), as well as the presence of characteristic m/e peaks in this spectrum which corresponded to furfural, furfural was confirmed as one of the components in the mixture.
Other characteristic m/e peaks, not related to furfural, were found at m/e 152, 149, 137, 108, 91, 79, 77, 67, 43, and 41. The m/e 152 peak was considered to be the parent ion. The peak at m/e 137 corresponded to the loss of a methyl group. The m/e 108 peak corresponded to the loss of an isopropyl group plus one additional hydrogen. The m/e 43 peak indicated the separation of the isopropyl group as a fragment. The m/e 91 peak was due to the loss of water plus an isopropyl group. The base peak was m/e 79. The spectrum was similar to the standard spectrum of 1,5-\(\mu\)-menthadien-7-ol (XXI) (96). Since a good and clear mass spectrum of this compound was not obtained, 1,5-\(\mu\)-menthadien-7-ol can only be considered as tentatively identified.

\[
\text{XXI. 1,5-\(\mu\)-Menthadien-7-ol}
\]

Gas-liquid chromatographic peaks 25b (Figure 25) and 26e (Figure 26) showed identical retention times as authentic terpinene-4-ol. Peak enhancement studies with authentic material did not show new peaks. The mass spectrum was recorded on the Finnigan
instrument using the conditions shown in Table 5. The spectrum is shown in Figure 29. The spectrum contains the parent ion at m/e 154 and the base peak at m/e 71. The peaks at m/e 136, 111, and 93 corresponded to the loss of water, an isopropyl group, and water plus an isopropyl group respectively. The m/e 69 peak indicated that the bond between C₃ and C₄ and the bond between C₅ and C₆ were broken (101). The m/e 43 peak represented the isopropyl fragment. The spectrum was identical to the published spectrum (101) of authentic terpinene-4-ol. Figure 26 showed that 3.6% of the materials that came through the gas-chromatograph was terpinene-4-ol (XXII).

![Chemical Structures]

**XXII. Terpinene-4-ol  **

**XXIII. α-Terpineol**

Gas-liquid chromatographic peaks 25c and 26f (Figures 25 and 26 respectively) showed the same retention times as authentic α-terpineol (p-menth-1-en-8-ol, Eastman Organic Chemicals, Rochester, N. Y.) Gas chromatography-mass spectrometry work was conducted using the conditions listed in Table 5. The spectrum is shown in Figure 30. The spectrum contains a weak parent ion peak at m/e
Figure 29. Mass spectrum of terpinene-4-ol.\textsuperscript{a}

\textsuperscript{a}Material under GC peak 25b (Figure 25)

Figure 30. Mass spectrum of $\alpha$-terpineol.\textsuperscript{a}

\textsuperscript{a}Material under GC peak 25c (Figure 25).
Two stronger peaks are shown at m/e 139 and 136 which corresponded to the loss of a methyl group and a water molecule. These two peaks are good evidence that the compound was a monoterpenoid alcohol. The base peak at m/e 59 indicated that the bond between C₄ and C₈ was broken and corresponded to the fragment which included the hydroxyl group. Peaks at m/e 121 and 93 corresponded to P-18-15 and P-18-43 respectively. Other characteristic peaks are shown at m/e 67, 68 and 81. The spectrum was the same as that from authentic α-terpineol and the published spectrum (101) of authentic α-terpineol. The compound was thus identified as α-terpineol (XXIII). It was the most abundant compound (21%) that was detected after passage through the "S. C. O. T." gas-liquid chromatographic column (Figure 26).

The second most abundant component (19%) that was detected after passage through the "S. C. O. T." gas-liquid chromatographic column (Figure 26) had the same retention times as authentic guaiacol (Aldrich Chemical Co., San Leandro, Calif.) (gas-liquid chromatographic peaks 25d and 26g, Figures 25 and 26 respectively). Since the two shoulder peaks (25e and 25f, Figure 25; and 26h and 26i, Figure 26) could not be well resolved, the mass spectrum of 25d which was recorded (Figure 31) was not a spectrum of a pure compound. However, a number of characteristic peaks were observed at m/e 124, 109, 82, 81, 53, 52, 51 and 39. The parent ion was m/e 124. The base peak was m/e 109, which corresponded to the loss of
Figure 31. Mass spectrum of guaiacol.\textsuperscript{a}

\textsuperscript{a}Material under GC peak 25d (Figure 25)

Figure 32. Mass spectrum of 2,5-dimethyl-3-acetylfuran.\textsuperscript{a}

\textsuperscript{a}Material under GC peak 25g (Figure 25)
a methyl group. The exact cause of the ions at m/e 82 and 81 are not understood. They may be due to the loss of the hydroxyl group and C₁ and C₆ (57). The ions at m/e 53, 52, 51, and 39 were due to contraction of the aromatic ring. Based on the above evidence, the compound was identified as guaiacol (XXIV).

The two shoulder peaks 26h and 26i (Figure 26) could not be well resolved even by the "S. C. O. T." column. Gas-liquid chromatographic peaks 25e and 26h (Figures 25 and 26 respectively) showed identical retention times as authentic β-citronellol. Peak enhancement studies with the authentic compound did not show new peaks. Gas-liquid chromatographic peaks 25f and 26i (Figures 25 and 26 respectively) showed identical retention times as authentic geraniol. Peak enhancement studies were also carried out and did not show new peaks.

Since the two shoulder peaks 25e and 25f (Figure 25) were not well resolved, the mass spectra recorded were not spectra of each individual pure compound. The mass spectra of each gas-liquid
chromatographic peak (25e and 25f, Figure 25) showed m/e 69 as the base peak and the gas-liquid chromatographic peak 25e (Figure 25) had m/e 156 as the parent ion. The m/e 69 ion was characteristic for acyclic monoterpenes (101). The m/e 156 and 154 ions corresponded to the molecular weights of β-citronellol and geraniol respectively (101). Other ions were not clear enough to tell their origins.

Based on the comparison of gas-liquid chromatographic retention times, peak enhancement studies, and the characteristic mass spectral ions, the compound under gas-liquid chromatographic peaks 25e and 26h (Figures 25 and 26 respectively) was tentatively identified as β-citronellol (XXV) and the compound under gas-liquid chromatographic peaks 25f and 26i (Figures 25 and 26 respectively) was tentatively identified as geraniol (XXVI). Both were minor components in the volatile materials.

Gas chromatographic peaks 25g and 26j (Figures 25 and 26 respectively) had identical retention times as authentic 2, 5-dimethyl-3-acetylfuran. In order to avoid contamination from the shoulder peaks which followed gas-liquid chromatographic peak 25g (Figure 25), a mass spectrum was taken at the front side of the peak and is shown in Figure 32.

The mass spectrum showed the parent ion at m/e 138. The base peak at m/e 123 corresponded to the loss of a methyl group. The second largest peak at m/e 43 was possibly due to the loss of an
acetyl group (57). This was also the reason for the m/e 95 peak (P-43). The bond between the ring oxygen and C₂ and the bond between C₃-C₄ were broken and the further loss of a proton caused the m/e 81 ion. Other characteristic peaks were found at m/e 53, 51, and 39. The spectrum was the same as the spectrum of the authentic material (95) although not as clean. The compound was thus identified as 2, 5-dimethyl-3-acetylfuran (XXVII). The compound constituted 9.9% of the materials that were detected after passage through the "S. C. O. T." gas-liquid chromatographic column (Figure 26).

![Chemical structures]

XXVII. 2, 5-Dimethyl-3-acetylfuran

XXVIII. β-Cyclocitril

The last major component which showed in the gas-liquid chromatographic spectra was peaks 25h and 26k (Figures 25 and 26 respectively) which had identical retention times as authentic β-cyclocitril. The mass spectrum (Figure 33) was taken at the front side of gas-liquid chromatographic peak 25h in order to avoid contamination from the shoulder peaks which followed (Figure 25). The spectrum showed m/e 152 as the parent ion which was also the second
Figure 33. Mass spectrum of $\beta$-cyclocitrinal.$^a$

$^a$Material under GC peak 25h (Figure 25)
largest mass peak and m/e 137 as the base peak. The molecule also
lost an aldehyde group and formed the m/e 123 ion which was the third
largest mass peak. Other characteristic peaks were found, according
to the sequence of intensity, at m/e 81, 109, 28, 67 and 41. The
mass spectrum (Figure 33) was identical to the spectrum of the authen-
tic material. The compound was thus identified as β-cyclocitral. It
constituted 7.9% of the materials that were detected after passage
through the "S. C. O. T." gas-liquid chromatographic column (Figure 26).

The analyses of the volatile materials showed that they contained
only a small amount of monoterpenes hydrocarbons. During the sample
preparation by steam distillation and diethyl ether extraction, special
precautions had been taken to prevent the loss of monoterpenes hydro-
carbons. A small amount of the steam distillate was withdrawn from
the collection flask and was checked by gas-liquid chromatography
using an SAIB-PDEAS column even before the completion of the dis-
tillation process. The chromatogram showed only a few small peaks
in the region where monoterpenes hydrocarbons usually showed. After
completion of the steam distillation, the sample was analyzed
immediately and when it was not being used, it was kept in a tightly
capped bottle and stored in the freezer. Six months later, the sample
was checked by gas chromatography under the same conditions as the
first analysis. The resultant chromatogram showed little change.
These results indicated that the small amount of monoterpenes
hydrocarbons was due to the nature of the Douglas-fir bark rather than a loss during preparation and storage.

The two 1/8-inch gas chromatographic columns (SE-52 and the mixture of 2% PEG and 1% OV-17) either failed to give good resolution of the volatile materials or gave only a few small peaks. The support coated open tubular column ("S. C. O. T.") gave much better resolution and more sensitive responses. Authentic α-pinene, β-pinene, and limonene were tested by this column and their retention times matched with peaks 26a, 26b and 26c respectively (Figure 26). Since no mass spectra were recorded, their presence can only be tentatively indicated.

The presence of furfural, terpinene-4-ol, α-terpineol, guaiacol, 2,5-dimethyl-3-acetylfuran and β-cyclocitrinal in Douglas-fir bark has not been previously reported.

The identification of these volatile compounds will be of interest to entomologists who are investigating insect attractants. Kangas et al. (44) have shown that α-terpineol isolated from pine bark attracts Blastophagus piniperda L. (Col., Scolytidae). Since the present work shows that α-terpineol is a major component of the volatile materials in Douglas-fir bark, it may act as an attractant for insects that attack Douglas-fir. The other compounds identified will also be of interest because the entomologists will know what compounds to study for the attraction of insects.
V. SUMMARY AND CONCLUSIONS

1. The n-hexane solvent extracted a light-colored, "wax-like" fraction from Douglas-fir bark. The components of the "wax" were separated by column chromatography.

2. Fractions 1 to 9 of the column chromatographic effluent contained sterol esters and wax esters. The sterol part of the esters contained β-sitosterol and campesterol as identified by gas chromatography and combined gas-liquid chromatographic-mass spectrometry. The acid parts of the sterol esters were n-tridecanoic acid, n-hexadecanoic acid, n-heptadecanoic acid, cis-9-octadecenoic acid, n-eicosanoic acid, n-docosanoic acid and n-tetracosanoic acid. n-Nonadecanoic acid was also tentatively identified. The presence of sterol esters in Douglas-fir bark has not been previously reported.

3. Because most of the fatty acid components are saturated, they cannot be used as a source for "tall oil" compounds which are unsaturated.

4. The odd-numbered fatty acids from Douglas-fir bark can be used for chemotaxonomy purposes. For example, the barks of red fir, western red cedar and grand fir do not contain odd numbered fatty acids. Thus Douglas-fir bark differs in its chemical composition and these differences can be used for identification.
5. Fractions 13 to 17 of the column chromatographic effluent were taken from bands on the column which fluoresced a dark-blue color under ultraviolet light. The infrared spectrum of the purified material showed the presence of an ester linkage.

6. The alcohol part of the ester contained behenyl alcohol and lignoceryl alcohol. The compounds were identified by elementary analyses, melting points, gas-liquid chromatography, infrared spectroscopy, and a combined gas chromatographic-mass spectroscopy study of the trimethylsilyl ethers of the two alcohols.

7. The acid part of the ester contained ferulic acid identified by melting point, infrared and nuclear magnetic resonance spectroscopy, and field desorption mass spectrometry. The field desorption mass spectrum of ferulic acid is the first spectrum of this type presented for this compound.

8. The ferulic acid esters contained a different ester linkage from the ferulic acid ester that Hergert found in white fir bark. The presence of ferulic acid esters in Douglas-fir bark has not been previously reported.

9. The presence of ferulic acid esters showed that ferulic acid did not exist in the free form in the "n-hexane wax" as had been suggested earlier. Therefore, free ferulic acid cannot be
obtained from the "wax" or the bark without prior saponification reactions.

10. Because the ferulic acid moiety exists in the "wax" combined as esters, any reactions designed to modify free ferulic acid in order to modify the "wax" cannot be considered. To modify the properties of the "wax, " such as hardness, melting point, color and so on, through the ferulic acid moiety, consideration must be given to the fact that the reactions will be done on esters not the free acid.

11. The volatile materials in Douglas-fir bark were collected by steam distillation rather than by separation from the n-hexane-soluble fraction, although these materials were part of the n-hexane-soluble materials.

12. The major volatile materials were identified by gas chromatography and combined gas-liquid chromatography-mass spectrometry as: furfural, terpinene-4-ol, α-terpineol, guaiacol, 2,5-dimethyl-3-acetylfuran and β-cyclocitral. The presence of these materials in Douglas-fir bark has not previously been reported.

13. Other volatile materials were tentatively identified as: α-pinene, β-pinene, limonene, 1,5-p-menthadien-7-ol, β-citronellol and geraniol.
14. The identification of the volatile compounds will be helpful to entomologists investigating insect attractants. For example, α-terpineol, a major volatile compound in Douglas-fir bark, has been shown to attract insects.
BIBLIOGRAPHY


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