AN ABSTRACT OF THE THESIS OF

GARY DUANE MANNERS (Name) for the M.S. (Degree) in Forest Products (Major)

Date thesis is presented May 7, 1965

Title THE CHEMICAL COMPOSITION OF THE BARK EXTRACTIVES OF FOUR SPECIES OF THE GENUS PSEUDOTSUGA

Signature redacted for privacy.

Abstract Approved (Major professor)

This is the first detailed chromatographic examination of Pseudotsuga menzesii and three other Pseudotsuga species (P. macrocarpa, P. japonica, and P. wilsoniana). The whole bark of these four species was sequentially extracted with hexane, benzene, ethyl ether, ethyl alcohol and water. Paper and thin layer chromatographic techniques were coupled with ultraviolet spectral procedures in the isolation and identification of individual compounds.

Compounds identified and previously reported which were common to all species included: dihydroquercetin, quercetin, dihydroquercetin-3'-monoglucoside, l-epicatechin, d-catechin, vanillin, protocatechuic acid, coniferaldehyde and leucoanthocyanins. Compounds discovered in the four species which have not previously been reported include: eriodictyol, vanillic acid, vanillyl alcohol, acetovanillone, and at least two esters of ferulic acid. Some

compounds were identified which were not distributed through all four species. These included: luteolin (P. macrocarpa, P. japonica, and P. wilsoniana), and four leucoanthocyanins (variable distribution among the four species). Other unidentified compounds displayed selective distribution patterns as well.

The selective distribution patterns of these flavonoid compounds suggests their possible application in chemical taxonomic differentiation of Pseudotsuga species. However, such an application must wait for extractive analysis of the two unavailable species of Pseudotsuga (P. sinensis and P. forestii).

Comparison of the flavonoids present in the bark with those reported in other tissues of the tree supports the view that such compounds are formed in situ and certain preliminary biosynthetic observations are discussed.

The extractive distribution of the compounds found in the bark is considered in light of the possibility that such compounds may eventually make the more complete utilitation of bark a reality. Such utilization, however, is dependent upon the establishment of suitable markets for these compounds.

THE CHEMICAL COMPOSITION OF THE BARK EXTRACTIVES OF FOUR SPECIES OF THE GENUS PSEUDOTSUGA

by

GARY DUANE MANNERS

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1965

APPROVED:

Signature redacted for privacy.

Professor of Forest Products Chemistry
In Charge of Major

Signature redacted for privacy.

Head of Department of Forest Products

Signature redacted for privacy.

Dean of Graduate School

Date thesis is presented Mar. 7, 1965

Typed by Luanne Bayless

ACKNOWLEDGMENTS

Sincere appreciation is extended to Dr. Harvey Aft for his guiding efforts and understanding during this investigation.

Appreciation goes also to Dr. Kim Ching who was responsible for the aquisition of the oriental species included in this study.

Sincere thanks is extended to Mr. Terry Highley for his aid during this study and to the personnel of the Forest Research Laboratory for their co-operation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE CHEMICAL COMPOSITION OF THE BARK EXTRACTIVES OF FOUR SPECIES OF THE GENUS PSEUDOTSUGA

INTRODUCTION

Development of modern spectral and chromatographic analytical techniques has allowed more rapid and thorough investigation of the chemical compounds present in various plant tissues, thus being of great value in the areas of plant physiology, genetics, taxonomy and pathology, Comprehensive reviews of chemical studies in these particular areas have recently appeared (4, 17, 18, 73 and 86).

Chemical taxonomic studies have been applied to many vascular plants. Among the angiosperms, the genus Eucalyptus has been the object of intense chemical investigation. Recently, a chromatographic study of the occurence of cis- and trans- 3, 5, 4' trihydroxystilbene and its 3β-D glucoside in the heartwood of Eucalypts of the subsection Longiores has been reported (33, p. 80). The results of this particular investigation suggested that Eucalyptus guilfoylei be transferred to the series Transversae and that the sub-series Ochrophloiae be removed from the series Corymbosae. The genus Prunus has also been the subject of recent study with regard to taxonomy. From an analysis of the flavonoids present in the wood of six species of Prunus, Hasegawa and Shirato (30) were able to separate the six species into three distinct groups according to the nature of their chemical composition.

In the order Coniferales of the Gymnosperms, the genus Pinus has been studied extensively. The most important of these studies are the investigations of Mirov (69) and Erdtman (89, p. 463). Mirov studied the volatile "gum turpentines" of ninety-two species and varieties of pine. Based on the chemical nature of the turpentines of these pines, Mirov suggested several taxonomic changes in the genus earlier described by Shaw (81). These alterations included changing the species Pinus chinensis to P. tabulaeformis. He also considered P. jeffreyi and P. oaxacana as valid separate species and moved them from the group Australes to the group Macrocarpae. He then rearranged the groups under the subdivision Diploxylon as described by Shaw, so that all of the Pines with aliphatic hydrocarbons were together and closer to the monospecific group Pinae. Shaw's arrangement of the subgenus Haploxylon remained unchanged.

Erdtman studied compounds isolated from pine heartwood including: stilbenes, dibenzyls, flavanones, flavones, flavanonols, and cyclitols. From the study of over half of the species in the genus, he concluded that pines could be classified by their specific pattern of heartwood phenolics, into the subgenera Diploxylon and Haploxylon. However, smaller groups, with the exception of Strobi and Gerdianae (Haploxylon), were not distinguished (86, p. 107).

Aside from the comprehensive investigations of Pinus, the other genera of the family Pinaceae have received little or no

attention, although some cursory examination has been given Abies, Cedrus, Larix, Pseudolarix, Tsuga, Picea, Pseudotsuga and Pinus by Plouvier (74, p. 2377). He found pinitol and sequoyitol, two terpene alcohols, to be present in the heartwood of many of the species in the various genera in the family Pinacae studied. From the distribution of sequoyitol in gymnosperms, he concluded that there is a probable link between this compound and the basic metabolism of the gymnosperms which has allowed the retention of monophyletic character, through sequoyitol, in spite of the polyphyletic botanical classification of Gymnospermae based on morphological and other studies (86, p. 325).

In the genus Pseudotsuga, only one species, Pseudotsuga menzesii (Mirb.) Franco., the most important commercial timber species in the world (10, p. 5), has been the subject of extensive chemical investigation. Five other species are presently assigned to this genus (79, p. 497); one, Pseudotsuga macrocarpa (Mayr.), is native to the mountains of southern California, while the others, Pseudotsuga japonica (Beissn.), Pseudotsuga wilsoniana (Hay.), Pseudotsuga forestii (Craib.), Pseudotsuga sinensis (Dode), are indiginous to eastern Asia.

These species are placed in the genus Pseudotsuga according to morphological similarities (16, p. 579) although in gross anatomy they appear quite different, particularly in size and form. Some

question regarding the exact taxonomic classification of the species in this genus, based on their morphology, has persisted. Even though there are five species recognized generally, it has been stated that two of these five (P. wilsoniana and P. forestii) are actually the same species (16, p. 592). In addition, P. menzesii has been described as having a different number of varieties (81, p. 94); one of these varieties is considered a separate and distinct species by some taxonomists (16, p. 581). It would thus appear that classification based solely on a morphological basis may not be entirely satisfactory for the species of this genus.

Development of the botanical classification of this genus has involved some interesting stages. The genus is commonly called the Douglas firs, though the genus name, Pseudotsuga, implies that they are related to the hemlocks (Tsuga). The designation of firs comes from the morophological similarity to the genus Abies in which the members of Pseudotsuga were at one time placed (62, p. 305). Separation from Abies was eventually made on the basis of differences in cone characteristics (16, p. 579). However, confusion in botanical classification resulted in the placement of at least one specie (P. menzesii) of this genus in the genus Pinus at one time (62, p. 305). Finally, the common name of Pseudotsuga macrocarpa, "Big-cone spruce", implies a relationship of the Douglas firs to the genus Picea (spruce). Thus the genus

Pseudotsuga seems to represent a small group of widely variable members in terms of morphology, distribution and physical characteristics.

Certain genetic characteristics of the genus Pseudotsuga are presently under study at the Forest Research Laboratory of Oregon State University (13). In these studies, members of the genus are being crossed in the hope of producing a hybrid with characteristics more desirable from both physiological (i.e., drought resistance) and economic standpoints (i.e. accelerated growth). An initial attempt to fertilize the female strobili of Pseudotsuga menzesii with the pollen from Pseudotsuga japonica was unsuccessful (13). Seedlings of all of the species within this genus are presently being grown at the Forest Research Laboratory, but are too young to produce strobili for controlled hybridization study. Until these tree specimens are mature, the study must rely upon imported pollen for crossing with the native P. menzesii.

Biogenesis, the method of natural synthetic production of chemical compounds present in certain plant tissues, has been of intense recent interest (4). In woody plants, biosynthetic studies have been primarily concerned with the formation of simpler phenolic compounds which serve as precursors for the formation of polymers in the wood and bark of the tree. The polymers, lignins (4, p. 693-726; 2, p. 294; 40, p. 161) and tannins

(4, p. 617-639; 40, p. 191-258) have been studied extensively.

Isolation and identification of probable precursor molecules from various tissues of vascular plants has enabled postulation of synthetic routes for the formation of lignins and tannins. Confirmation of certain phenolic compounds as lignin and tannin polymer precursors has been obtained by the use of isotopically labeled compounds (31, p. 1854; 82, p. 1992).

Lignin has been shown to be formed from phenylpropane (C_6-C_3) intermediates which originate from carbohydrates. This biosynthetic route has been called the shikimic acid cycle (41, p. 264) and is shown in Figure 1.

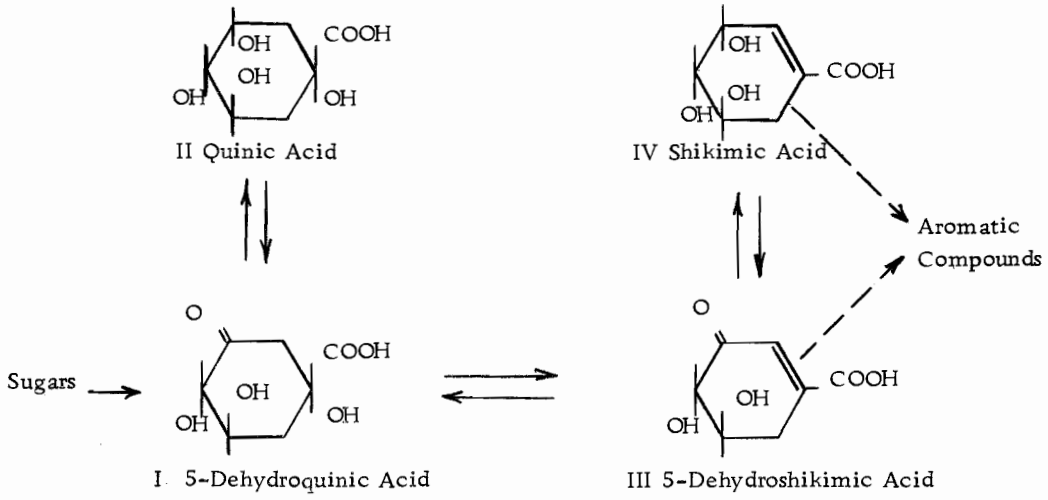

Figure 1 - The Shikimic Acid Cycle

Tannins are of two types: hydrolyzable and condensed. The compounds which form the hydrolyzable tannins have also been shown to originate through a shikimic acid pathway similar to that

of the lignins (4, p. 618). Condensed tannins, on the other hand, are polymers formed through the condensation of polyphenolic compounds, i.e. the flavonoid compounds.

The biogenesis of flavonoid compounds has been extensively reviewed (4, p. 563; 18, p. 618; 71, p. 55). Formation of these compounds has been shown to involve the formation of ring B from an oxidized cinnamic acid produced through the shikimic acid pathway (Figure 2) with subsequent linear acetate addition to form a chalcone intermediate which, through ring closure, forms the flavonoid structure (Fig. 2, IV).

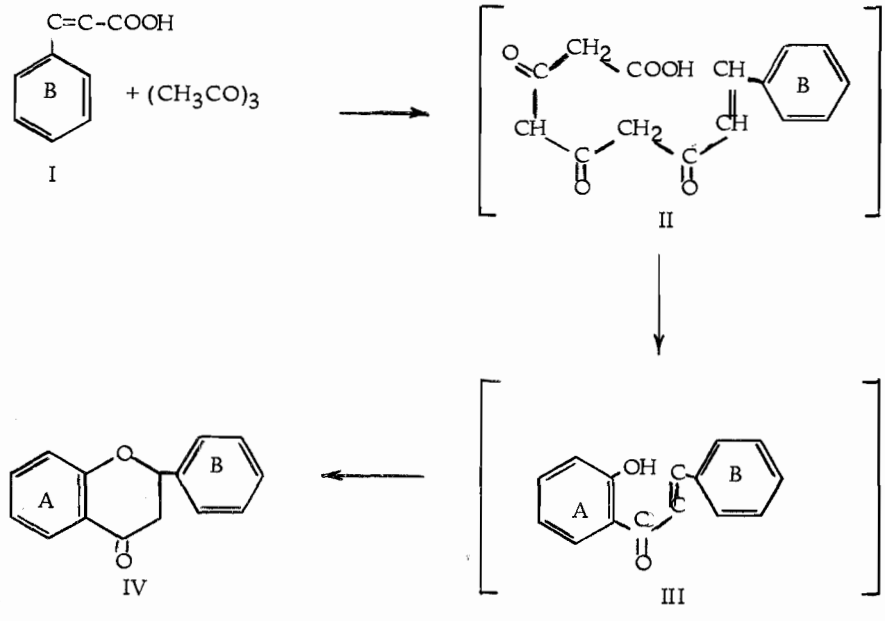

Figure 2 - The Proposed Course of Flavonoid Biosynthesis (4, p. 594)

The order in which hydroxyl groups are introduced into rings A and B is still unknown and the stages in the total synthetic course at which this hydroxylation occurs is still not clear.

Whole bark is not usually the plant tissue selected for metabolic studies. However, for the present chemical study, bark was the only tissue of the various Pseudotsuga species readily available. Because bark is considered an essentially dead tissue, the chemical compounds it contains may be considered definite end products of plant metabolism, essentially independent of seasonal variation and other external influences. Thus, the analysis of bark should offer information of value in the more accurate determination of general biosynthetic pathways of botanically related compounds. Also, analytical techniques applicable to other tissues of the Pseudotsuga species, as they eventually become available, can be developed in this bark investigation.

In vascular plants, the site of biosynthesis of the simpler phenolic compounds may not be the site of their eventual physiological use or deposition in polymer form. The movement (translocation) of the simple molecules from their site of synthesis to their site of utilization via the vascular system is well known. However, with phenolic substances, some question as to the exact nature of the translocated molecule has arisen.

Experimental evidence and some deductive reasoning has been advanced in support of the theory that polyphenolic molecules are translocated. Based on a study of the polyphenol distribution of Eucalyptus species (42, p. 597; 43, p. 263), it has been suggested

that the leucoanthocyanins from the leaves are translocated to other tissues where they are transformed to flavonoids and condensed tannins according to physiological conditions. A study of Schinopsis quebracho-colorado and Schinopsis balansae (55, p. 368), led to the conclusion that the gallocatechins in the leaves of these trees are translocated to the sapwood, however, the heartwood components were considered to have originated initially elsewhere in the trees. By analysis of various tissues of Pseudotsuga menzesii and other species, Hergert and Goldschmid (35, p. 700) determined the distribution of the flavonoids, dihydroquercetin, quercetin and their 3'-glucosides. From the distribution of these components in the various tissues, the authors suggested that the compounds were synthesized and glycosated in the leaves and were then transported, via the vascular system, down the inner bark and then moved laterally via the ray cells to the outer bark and heartwood periphery. At this point, the flavanoid glucosides are hydrolyzed to the flavonoid aglycones. Also, by comparison of the polyphenols in the leaves and bark of oak (32, p. 533) it was contended that the polyphenols were formed initially in the leaves and then translocated via the sieve tube system to the phloem where they are oxidized to the resulting condensed tannins which are stored in the bark.

In recent years, evidence contrary to the translocation theory for phenols has been presented. According to Zimmerman

(91, p. 213), who studied sieve tube sap of Fraxinus americana, the sugars and amino acids are the only major substances translocated from the photosynthetic sites to the rest of the plant. It was felt, by Hillis (44, p. 109), that the work of Zimmerman eliminated the possibility of heartwood polyphenol formation in the leaves or phloem. Zimmerman's work is further substantiated by other observations which show, for example, that the amount of tannin content of the rhizomes of Polygonum bistorta doubled during the spring season after the leaves had been removed (70, p. 940). It has also been demonstrated (28, p. 224) that the leaves and inner bark of Gingo biloba and Pinus thunbergii contained the most shikimic acid while the outer sapwood contained the lowest over a yearly period. Thus, the translocation of shikimic acid from the leaves to the heartwood periphery, before the biosynthesis of polyphenols, seems unlikely.

In a recent study of the flavonoids present in the flowers of Douglas fir (38) the following compounds were found: kampferol, quercetin-3-monoglucoside, kampferol-3-monoglucoside, d-catechin, l-epicatechin, eriodictyol-7-monoglucoside, naringenin-7-monoglucoside, kampferol-3-diglucoside, dihydroquercetin-3'-monoglucoside. These compounds present a pattern greatly different from that reported by Hergert and Goldschmid in their analysis of the flavanoid components in the needles (leaves). Since the

needles and flowers of Douglas fir originate from the same tissue and are connected by the same vascular system, it was concluded that the flavonoid compounds were formed *in situ* in the individual tissues.

The chemical composition of the extractives of different tissues of two species of *Eucalyptus* were examined chromatographically (45, p. 433). The contention of the investigators was that if translocation occurs, the polyphenolic extractives of the tissues might be biochemically inter-related. The results of the investigation showed that ellagitannins are the major monomeric components in the cambium of the two species while flavans were found to occur in the bark of one species and the heartwood of the other. The sapwood extractives of one species were found to be different than the hearwood extractives of that species, both of which differed from the extractives of the other tissues. The other species of *Eucalyptus* had a different pattern of tissue extractives. These results indicated to the workers that the polyphenols were not formed in the cambium, but rather were formed *in situ* at the heartwood-sapwood and inner bark-outer bark boundaries.

Other theories (17, p. 9) have contended that certain polyphenols are formed at the cambium and translocated to the heartwood and bark. A consideration of the leucoanthocyanin precursors of the bark and heartwood of *Schinopsis* and *Acacia mollissima*

(77, p. 1454) led to the conclusion that the precursors originated in the cambium and subsequent action of specific enzyme systems effected conversion of the leucoanthocyanins to tannins in the respective portion of the plant. More recent work has shown that lignin can be formed from glucose in the young stems of Eucalyptus nitens (26, p. 305). Additional work on lignin formation, using tissue cultures of Pinus strobus cambium cells (27, p. 173), demonstrated that lignin could be formed in situ. Swain has stated that tannins have also been shown to be synthesized by cambium cells from sugar alone (41, p. 281). A frank interpretation of this work makes it appear unlikely that translocation occurs to any great extent in plant tissues.

The majority of chemical investigations of Douglas fir bark have been done with primary emphasis on the utilization of gross extracts. These studies, for the most part, involved classical methods of chemical analysis such as, solvent separation and fractional crystalization, and essentially resulted in crude results designed to determine the potential for utilization of the extracts.

An early study of Douglas fir bark using classical analytical wood analysis techniques (57, p. 175) is summarized in Tables No. I and II.

Table I - Extractive Constituents of Douglas Fir Bark

	Solvent		
	Ethyl ether	Ethyl alcohol	Hot-water
Whole bark	7.80*	13.97	2.31

* Quantities in percent of oven dry bark

Table II - Analysis of Extractive Free Douglas Fir Bark

	Acetyl	Ash	Lignin	Methoxyl	Pentosan
Whole Bark	0.83	0.73	57.54	3.76	6.40

(Quantities in percent)

This bark analysis study was followed by an investigation of the amount of tannin available from the bark (49, p. 604). The analysis revealed that the bark contained 7.6-18.3 percent tannin, depending upon age, as determined by the hide powder method of the American Leather Chemists Association. In addition, two waxes were extracted with hexane (3.6-6.0 percent) and benzene (2-4 percent). Classical chemical analysis of the saponified waxes (58, p. 183) revealed that the hexane consisted of approximately 20 percent lignoceryl alcohol, 60 percent lignoceric acid (free and combined forms), and 24 percent ferulic acid (free and combined forms) with oleic acid and phytosterol as minor constituents (56, p. 1685). The reddish-brown benzene wax was considered to contain about 25 percent fatty acids, 24 percent

phlobaphenes, 26 percent ether solubles and five percent unsaponifiables as well as glycerol. The isolation of pure dihydroquercetin was achieved through ethyl ether extraction of benzene extracted bark.

Other investigations of the various fractions of Douglas fir bark by Kurth and co-workers followed, including chemical investigations of the cork fraction (36, p. 59) and the bast fibers (54, p. 14) which showed the cork fraction to be richest in extractive content. The investigations of neutral solvent extracts of bark led to the patenting of an extraction process by Kurth (59, 60). The chemical nature of the "lignin" of the Douglas fir bark was studied (84). The amount and character of the so called "bark phenolic acids" was found to be heterogenous, yielding vanillin and protocatechualdehyde upon oxidation.

In a more recent investigation employing modern chromatographic techniques, the chemical composition of the newly formed inner bark of Douglas fir was examined (46). In this investigation the inner bark was sequentially extracted with n-hexane, ethyl ether, methanol and water. The yields, according to the age of the tree extracted, are shown in Table III (46, p. 7).

Table III - Yield of Extractive From Douglas Fir Newly Formed Inner Bark

Sample	Hexane Sol.	Ether Sol.	Methanol Sol.	Water Sol.
40 yr. old tree	1.21*	0.92	0.75	31.2
200 yr. old tree	0.99	0.13	0.59	40.3

* Percentages based on moisture free, unextracted bark

The compounds reported in the analysis of these fractions included: ether solubles - d-catechin, l-epicatechin and dihydroquercetin; hexane solubles - no individual identification; methanol solubles - considered to be the same as the water solubles with no further investigation; water soluables - twelve amino acids, eight hydroaromatic acids (shikimic, quinic and six unidentified) and one unidentified 2, 4-dinitrophenylhydrazine, Weisner positive spot.

Douglas fir is also included in a study of the methanol extracted tannins and polyphenols of the wood and bark of several western conifers (34, p. 610) in which paper chromatography was utilized. The presence of dihydroquercetin, dihydroquercetin-3'-monoglucoside, quercetin, phloroglucinol, catechin, epicatechin, four leucocyanidins, protocatechuic acid, ferulic acid, coniferaldehyde, and vanillin in the whole bark of Douglas fir was reported.

Few other chemical studies among the species of the genus Pseudotsuga have been made. An analysis of the fatty acids

present in the pollen of three Douglas firs (P. wilsoniana, P. macrocarpa and P. menzesii) and two pines was recently reported (14, p. 890). The major fatty acid components of the fir pollens were found to be: linoleic, palmitic and stearic acids. It was also noted that the fatty acid composition of P. menzesii and P. wilsoniana were similar while that of P. macrocarpa was intermediary between that of the firs and the pines. An unknown fatty acid present to the extent of 1.7 percent, by weight, of the total isolated methyl esters, was also noted in P. macrocarpa. Wood and needles of P. japonica have recently received some cursory attention. The presence of dihydroquercetin in the heartwood has been reported (29, p. 17). The flavonoids, quercetin, kampferol and myricitin and three other unidentified compounds were found in the foliage of P. japonica (87, p. 1490). No chemical studies have been reported for P. forestii and P. sinensis.

Bark utilization continues to be one of the serious problems facing the forest products industry. It has been pointed out that 13-16 percent of the sawlog volume occurs as bark (15, p. 11) and that the major portion of the bark obtained in present milling operations is burned in "cone" burners with no effort to produce electrical or steam power (1). This uncontrolled burning of bark has produced serious air pollution problems in certain areas of Oregon. Such pressures will provide a stimulus for the

development of products with the eventual aim of total bark utilization. Some product development, using only a relatively small amount of the total bark residue available from milling operations, has been realized. These products presently produced from bark evolve from both physical and chemical components. The physical products include: composition boards and floor tiles (8, 9), fillers (67), soil amendments (5), and insulation (83). Among the chemical products are: adhesives (85, 66, 37, 38), tannin extracts (68) and crude waxes (59, 60). Excellent reviews of the present status of the chemistry and potential commercial utilization of various barks have recently appeared (22, 65, 76).

Thus, more detailed information of the chemical composition of the barks of Pseudotsuga is of value in three areas. First, it will eventually be of value in taxonomic classification for the genus Pseudotsuga as well as to closely related genera, such as Abies. Secondly, such chemical information obtained will aid in a better understanding of the metabolic pattern and physiological functions of tissue constituents. Finally, extractive constituents (or entire extracts) may eventually find application in commercial products, thus spurring further development in the chemical processing technology of bark.

EXPERIMENTAL

Collection of Bark Samples

Specimens of whole bark from the four species of Pseudotsuga used in this study were collected from indigenous sources. Details of the sources and collection data are shown in Table IV.

Table IV - Summary of Bark Collection Data

Species	Location	Age	D. B. H.	Date of Collection
P. menzesii (old growth)	McDonald Forest Benton County Corvallis, Oregon	200-300 yr.	70-80 in.	Nov. 8, 1962
P. menzesii (second growth)	same	67 yrs.	18 in.	Nov. 8, 1962
P. macrocarpa	Mt. Baldy, Angeles National Forest, L. A. County, California	300 yrs.	48 in.	March 21, 1962
P. japonica	Miyakawa-Mura, Kita-Gun, Mie Ken, Japan	216 yrs.	52 in.	- - -
P. wilsoniana	Unit 81, Ba Shen Shan, Formosa	67 yrs.	8 in.	May 16, 1962

The bark specimens were stripped from the trees, sealed in polyethylene bags and upon receipt at the Forest Research Laboratory, were placed in a cold room for temporary storage.

Sample Preparation and Extraction

In order to efficiently extract the bark samples, it was

necessary to grind them in a Wiley mill to pass a twenty mesh screen. To prevent clogging of the grinder, the bark was predried over a two-day period in a high temperature (90°), low humidity (6 percent) room.

Twenty-five to thirty grams of the milled bark was placed in alundum thimbles and oven dried to constant weight. These dried samples were then placed in soxhlet extractors and extracted for 72 hours sequentually with five neutral solvents: hexane, benzene, ethyl ether, ethyl alcohol, and water. After each extraction the samples were oven dried. The loss in weight was used to establish the amount extracted by each solvent stage.

The solvent was removed from each extract under vacuum and the solid extract was placed in individual containers and dried under vacuum. The completely extracted bark was stored in closed bottles for possible further analysis. The ultraviolet absorption spectrum was measured for each extract of all bark species in 95 percent ethyl alcohol (Figure 3).

Chromatography

Paper Chromatography

Both one and two dimensional descending paper chromatographic methods were utilized to separate the constituents of the

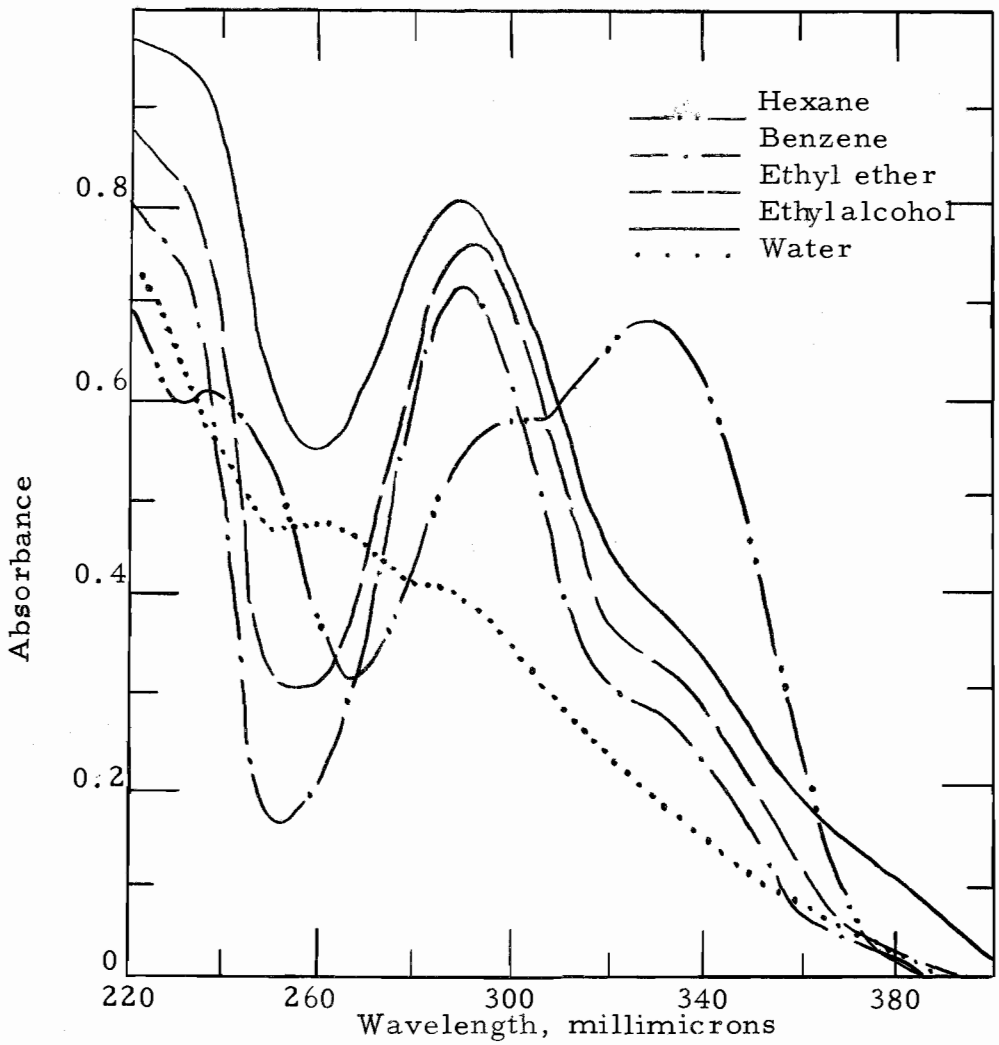

Figure 3- The ultraviolet absorption spectra of the five gross extracts of four species of Pseudotsuga.

various extracts. Whatman no. 1 filter paper was used in conjunction with the following solvents (volume-volume mixtures, unless otherwise specified):

1. Chloroform, acetic acid, water (8:12:5) (CAW) lower layer
2. Upper layer (CAW), butanol (3:1) (ULCB)
3. Butanol, acetic acid, water (4:1:5) (BAW) upper layer
4. Benzene, acetic acid, water (125:72:3) (BzW)
5. Benzene, methanol, acetic acid (45:8:4) (BMA)
6. 2% acetic acid (2% HAc)

For two dimensional chromatography, the following solvent combinations were used with the sequence of development corresponding to the order shown:

1. ULCB and 2% HAc
2. BAW and 2% HAc
3. CAW and 2% HAc
4. BzW and 2% HAc

Portions of the extracts were dissolved in volatile solvents such as chloroform, methanol or acetone and spotted on the chromatographic paper. With the exception of the acetic acid solvents, the papers were equilibrated for 8-12 hours in an atmosphere of the developing solvents. The chromatographic solvent was then added and the chromatograms developed, after which, they were removed from the developing tank and air dried. The sheets were

then examined under ultraviolet light prior to and after treatment with ammonia fumes. The following chromogenic spray reagents were also employed:

1. Two percent aluminum chloride in methanol
2. Potassium ferricyanide-ferric chloride (1% each in water) (34, p. 611)
3. Bis-diazotized benzidine (78, p. 65)
4. p-Nitroaniline (6, p. 271)
5. Diazotized sulfanilic acid (50, p. 125)
6. Sodium borohydride (47, p. 1733)
7. Cinnamaldehyde-HCl (35, p. 703)
8. 2,4-Dinitrophenylhydrazine (63, p. 95)
9. Phloroglucinol (20, p. 729)
10. Cartwright's spray (sodium periodate, piperazine, sodium nitroprusside) (12, p. 230)
11. Ninhydrin (63, p. 164)

For the identification of the simple sugars present in the water extract of the bark, the following solvents were employed in one dimensional analysis:

1. Upper layer - Chloroform, acetic acid, water (8:12:5), butanol (3:1)
2. n-Propanol, ethyl acetate, water (7:1:2)
3. Ethyl acetate, pyridine, water (2:1:2) - upper layer
4. Butanol, acetic acid, water (4:1:5)

The developed chromatograms were air dried and subjected to spraying with the following sprays:

1. Ammonical silver nitrate (63, p. 87)
2. p-Anisidine phosphate (63, p. 94)
3. Aniline hydrogen phthalate (3, p. 57)

Paper chromatographic analysis was successfully employed on all extracts except the hexane extract. No compound separation was obtained when this extract was examined using the solvent systems described. However, some separation was noted on silica gel impregnated chromatographic paper. Thin layer chromatography was also successfully employed for the separation of the compounds present in this extract.

Thin Layer Chromatography

One and two dimensional thin layer chromatography was employed in the analysis of the hexane and benzene extracts. The chromatograms consisted of eight inch square glass plates supporting a layer of Silica Gel G (Merck) applied as an aqueous solution (15 gm./35 ml. water) by spraying. The plates were dried and activated for one hour in a 105° C oven. A sample of the extract was applied to the plates and then the plates were placed in the chromatographic tank for a two-hour equilibration period in the presence of one of the following solvents:

1. Petroleum ether, ethyl ether, acetic acid (70:30:1) (PEA) (64, p. 383)

2. Chloroform ($CHCl_3$)

3. Benzene, methanol, acetic acid (45:8:4) (BMA) (75, p. 177)

The plates were then developed one dimensionally in each of the solvents or two dimensionally in the petroleum ether, ethyl ether, acetic acid and chloroform systems respectively. Following development the plates were air dried and examined in the presence of the following chromogenic spray reagents:

1. Ammonia vapors under ultraviolet light

2. 30% sulfuric acid, heated for 30 minutes at 105°C and viewed in visable and ultraviolet light

3. Iodine vapors (75 p. 126)

4. Bis-diazotized benzidine

5. Bromothymol blue (75 p. 128)

6. p-Nitroaniline

Spectral Analysis

The ultraviolet spectra of the crude bark extract and the compounds isolated from these extracts by chromatography were measured in 95 percent ethyl alcohol on a Beckman DB recording spectrophotometer. Isolated compounds were eluted from the paper or silica gel chromatograms using a minimum of ethyl alcohol. These solutions were compared with a reference solution

prepared by elution of a blank area of the chromatogram.

The techniques of Jurd (18, p. 107; 51, p. 376; 52, p. 284), Jurd and Horowitz (47, p. 2446; 53, p. 1618) and Lemon (61, p. 2998) were employed and involved the use of various chemical agents causing characteristic shifts in the spectra of phenolic compounds. These techniques have been particularly successful in the identification of flavonoid compounds (24, p. 100; 35, p. 700) and have proven to be quite effective for the identification of other phenolic compounds present in plant tissues in such small quantities as to make their isolation and identification by conventional methods almost impossible.

Positive identification of the compounds present in the various extracts was based on chromatographic behaviour, color reactions with the various chromogenic reagents, ultraviolet spectral characteristics and comparison with authentic samples, when possible.

RESULTS

The results of sequentual extraction of the four species of Pseudotsuga are summarized in Table V.

Table V - Extractive Content of Pseudotsuga Whole Barks

Specie	Percent Extract*				
	Hexane	Benzene	Ethyl Ether	Ethyl Alc.	Water
P. menzesii (O. G.)	4.75	2.84	2.77	13.89	0.72
P. menzesii (S. G.)	4.80	1.80	2.24	12.86	1.12
P. macrocarpa	5.28	4.49	8.58	10.42	5.48
P. japonica	2.08	0.29	0.24	15.60	6.71
P. wilsoniana	0.72	3.11	0.02	15.20	6.11

* Percent based on oven dry weight of unextracted bark.

These results are based on data from a single extraction and thus can only be considered generally.

There is a close agreement in the individual extractive content of the bark of old and second growth Douglas firs (P. menzesii) and these extract values in turn are in close agreement with those previously reported (57, p. 175). However, the extractive content of these native Pseudotsuga species is notably different from that of the oriental species (P. wilsoniana and P. japonica) except in the alcohol extract. The domestic Douglas firs proved to be higher in

extractives in the less polar solvents (hexane, benzene, and ether) while the orientals were notably higher in water extractives. Pseudotsuga macrocarpa seems to occupy and intermediary extractive position between P. menzesii and the oriental species. The extractive content of P. macrocarpa agrees with the amounts recorded for P. menzesii in the hexane and benzene extracts while conversely agreeing with the orientals in the amount of water extractives. In the ether extract however, P. macrocarpa exhibits a significantly higher yield than any of the other species.

Chromatographic Analysis

Paper Chromatography

Paper chromatography was employed in the separation of compounds in all but the hexane extract, whose waxy nature necessitated the use of thin layer chromatography.

For the direct comparison of extracts between species, a single two dimensional solvent system was selected for use with all extracts. This solvent system was upper layer chloroform - butyl alcohol (3:1), ULCB, in the first direction followed by two percent acetic acid in the second direction. These solvents were found to give the best results in the two dimensional separation of almost all compounds found in the benzene, ethyl ether, alcohol

and water extracts, with a minimum of streaking.

Development of ULCB (3:1) as a solvent for chromatography arose from experimentation for the utilization of the upper layer of $CHCl_3$ (8:12:5) which is ordinarily discarded, a waste of two-thirds of the solvent. The addition of n-butyl alcohol in a 1:3 ratio produces a solvent (ULCB) which exhibited much the same chromatographic properties as BAW (4:1:5), but showed less spot streaking and has a shorter development time.

The lower layer of $CHCl_3$ (8:12:5) is potentially a better solvent than both BAW (4:1:5) or ULCB (3:1) in that it showed a very good separation, with compact spots, of all compounds. However, the solvent is more volatile and very sensitive to variations in temperature and atmosphere saturation.

For the separation of several guaiacyl-type compounds, two solvents were found to be particularly effective, benzene:acetic acid:water (125:72:3), BzW (90, p. 449) and benzene:methanol: acetic acid (45:8:4), BMA (75, p. 177) both of which are one phase solvents. The latter solvent, BMA (45:8:4), proved to be particularly effective in the separation of all but alpha-carbonyl containing guaiacyl derivatives with the separated components developed as compact spots. The BzW solvent produced somewhat similar results as BMA but with less spot compactness. However, when the solvent was used in conjunction with two percent acetic acid, it

proved to be very effective in the separation of simple phenols from polyphenols. Because these two solvents were not previously reported in the separation of guaiacyl derivatives on paper, Rf values for certain standard compounds were measured and are presented in Table VI.

A summary of the extractive components of all species as determined by paper chromatography is shown in Figure 4, and is described in Table VI. Of the forty three compounds separated by paper chromatographic analysis, nineteen (spots no. 1 through 15, 18, 21, 26 and 32) were found to be common to all species (Table VII). Of the remaining twenty-four compounds, only nine (spots no. 16, 17, 20, 24, 25, 27, 30 and 31) were found to occur in greater than trace amounts. These nine compounds exhibited distinctive distribution among the four species. Compounds 16 and 17 were only in the two oriental species (P. japonica and P. wilsoniana) while compound no. 20 was present only in the old growth domestic firs (P. menzesii and P. macrocarpa). Compound no. 22 appeared only in P. menzesii and P. japonica. Compound nos. 24, 25, 30 and 31, identified as leuceanthocyanins, had the following distributions: compound no. 24 was common to all species except P. macrocarpa, compound no. 30 was common to all species except P. wilsoniana, compounds 24, 25, 30 and 31 were all present in old growth P. menzesii and P. japonica. Leucoanthocyanin

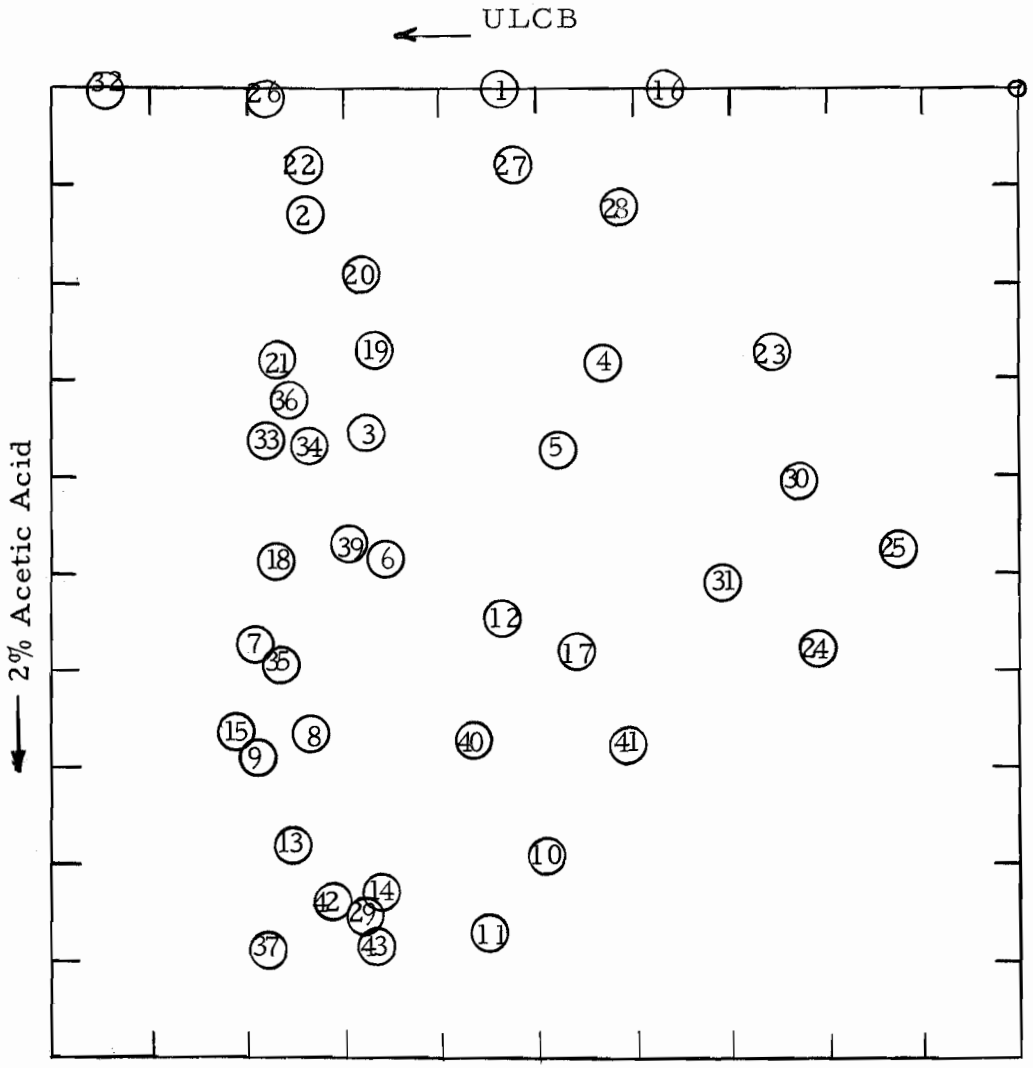

Figure 4 - A schematic paper chromatogram of the benzene, ether, alcohol and water extracts of four species of Pseudotsuga.

TABLE VI - The Results of Paper Chromatography Analysis of Four Extracts of Four Pseudotsuga Species

Sp. No.	Compound Identity	Solvent Rf Values						Chromogenic Spray Reactions											Species Distribution					Extract Distribution			
		A	B	C	D	E	F	1	2	3	4	5	6	7	8	9	10	11	OG DF	SG DF	AM	PI	PW	Bz	Et	Alc	H_2O
1	Quercetin	.55	.00	.12	.57	.12	.72	Y	brY	f1Y	Y0	B1	BrY	-	-	-	-	-	+	+	+	+	+	+	+	+	-
2	Eriodictyol	.75	.14	.25	.60	.12	.85	G	dG	pYG	0	B1	Y	-	-	Pr	-	-	+	+	+	+	+	+	++	+	-
3	DHQ	.70	.37	.11	.49	.05	.78	G	dG	-	d0	B1	pY	Y	pYB	Y	-	-	+	+	+	+	+	+	++	++	-
4	l-epicatechin	.45	.31	.05	.24	.00	.46	-	-	-	0	B1	pY	p0	Y	-	-	-	+	+	+	+	+	+	+	++	-
5	d-catechin	.50	.39	.09	.27	.06	.57	-	-	-	0	B1	pY	p0	Y	-	-	-	+	+	+	+	+	+	+	++	Tr
6	Protocat Acid	.67	.51	.23	.55	.15	.81	-	-	-	YB	Bl	BY	BlG	-	-	-	-	+	+	+	+	+	+	+	+	++
7	Vanillic Acid	.82	.59	.75	.78	.45	.86	-	-	-	pY	B1	BY	Pr	-	-	-	-	+	+	+	+	+	+	+	++	+
8	Vanillin	.75	.67	.81	.67	.33	.84	-	dBl	-	Y	B1	-	-	-	-	B	*Y	+	+	+	+	+	+	+	+	+
9	Acetovanillone	.82	.72	.84	.85	.77	.90	-	dBl	-	pY	B1	-	-	-	-	-	*Y	+	+	+	+	+	+	+	+	+
10	Unknown	.52	.82	.14	.40		.45	-	-	-	pP	B1	P	-	-	-	-	-	+	+	+	+	+	-	-	+	++
11	Unknown	.57	.88	.25	.57		.28	-	-	-	Pr	B1	-	-	-	-	-	-	+	+	+	+	+	-	-	++	+
12	DHQ 3' gluco.	.55	.55	.00	.44		.56	-	pG	pYG	p0	B1	BY	p0	-	-	-	-	+	+	+	+	+	Tr	+	++	Tr
13	Unknown	.77	.80	.57	.24		.43	-	Bl	-	-	B1	pY	-	-	-	-	-	+	+	+	+	+	-	Tr	++	+
14	Unknown	.67	.84	.43	.73		.63	-	Bl	-	-	B1	pY	-	-	-	-	-	+	+	+	+	+	-	Tr	++	+
15	Vanillyl Alc.	.83	.67	.60	.85	.47		-	-	-	pYB	B1	-	-	-	-	-	-	+	+	+	+	+	Tr	Tr	++	+
16	Luteolin	.38	.00	.08		.08	.12	-	dYG	pY	Y0	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-
17	Unknown	.47	.60	.04	.43			-	brYG	-	0	B1	-	0	-	-	-	-	-	-	-	+	+	-	-	++	-
18	Coniferaldehyde	.78	.51	.75	.83		.87	-	pG	-	-	-	-	-	-	-	-	**Pr	+	+	+	+	+	Tr	Tr	+	+
19	Unknown	.68	.28	.13	.31		.54	-	-	-	pY	B1	-	-	-	Bl	-	-	+	+	+	+	-	-	-	Tr	-
20	"	.70	.22	.37	.66		.80	-	-	-	Pr	B1	-	-	-	-	-	-	+	-	+	-	-	-	+	Tr	-
21	"	.78	.30	.67	.63			-	BlG	YG	-	-	-	-	-	-	-	-	+	+	+	+	+	Tr	Tr	+	-
22	"	.75	.10	.33	.66		.88	G	dG	-	Y	B1	-	-	-	Pr	-	-	+	+	-	+	-	-	-	+	-
23	"	.27	.39		.63		.45	-	-	-	p0	B1	pY	-	Y	-	-	-	+	+	-	+	+	-	+	Tr	-
24	Leucoanthocyanin	.23	.60		.65		.32	-	-	-	p0	B1	pY	-	Y	-	-	-	+	+	-	+	+	-	Tr	+	-
25	Leucoanthocyanin	.14	.49				.32	-	-	-	p0	B1	pY	-	Y	-	-	-	+	+	-	+	-	-	Tr	+	-

TABLE VI - (cont.)

26	Unknown	.80	.03	.78		.77	Bl	Bl	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	Tr	+	-
27	Unknown	.54	.09	.10	.30		-	-	-	p0	Bl	-	-	-	-	-	-	+	+	+	+	-	-	++	+	-
28	Unknown	.43	.14				-	-	-	0	Bl	-	-	-	-	-	-	-	-	-	+	-	-	++	-	-
29	Unknown	.70	.86				-	-	-	P	Bl	p0	-	-	-	-	-	-	-	-	+	-	-	Tr	-	-
30	Leucoanthocyanin	.24	.43				-	-	-	p0	Bl	pY	-	Y	-	-	-	+	+	+	+	-	-	Tr	-	-
31	Leucoanthocyanin	.33	.53				-	-	-	p0	Bl	pY	-	Y	-	-	-	+	-	-	+	-	-	Tr	-	-
32	Unknown	.96	.00	.78	.84		BlWh	BlWh	Y	p0	Bl	Y	-	-	-	-	-	+	+	+	+	+	+	Tr	Tr	-
33	Unknown	.80	.37				Bl	BlG	-	-	-	-	-	-	-	-	-	+	+	+	+	Tr	+	-	-	
34	Unknown	.75	.38				-	-	-	-	Bl	-	-	-	Pr	-	-	-	-	-	+	-	-	+	-	-
35	"	.77	.62				-	-	-	-	Bl	P	-	-	-	-	-	-	+	+	+	-	-	Tr	-	-
36	"	.77	.34				-	-	-	pP	-	pY	-	-	-	-	-	-	-	-	+	+	-	Tr	-	-
37	"	.80	.91				-	-	-	Y	Bl	Pr	-	-	-	-	-	-	-	-	+	-	-	Tr	-	-
38	"	.59	.41		.43	.42	Bl	Bl	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	Tr	-	-
39	"	.72	.48				Bl	Bl	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	Tr
40	"	.58	.69				-	pBlG	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	Tr
41	"	.43	.70				BlWh	BlWh	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	Tr
42	"	.83	.85			.48	-	-	-	-	Bl	-	-	-	-	-	-	+	-	+	-	-	-	-	-	Tr
43	"	.68	.91				-	-	-	-	Bl	-	-	-	-	-	-	+	-	-	-	-	-	-	-	Tr

+ = Present; - = Absent; Tr = trace

Chromatographic Solvents

A. ULCB
B. 2% HAC
C. BzW
D. CAW
E. BMA
F. BAW
G. PEA
H. $CHCl_3$

Chromogenic Spray Reagents

1. Ultraviolet light
2. Ultraviolet light and ammonia
3. 1% Aluminum chloride under U.V. light
4. Bis-diazotized benzidine
5. Ferric chloride-Potassium Ferricyanide
6. Diazotized sulfanilic acid
7. p-Nitroaniline
8. Cinnamaldehyde - HCl
9. Sodium borohydride
10. Tollen's reagent
11. *2.4 dintrophenylhydrazine
    **phloroglucinol-HCl
12. 30% Sulfuric Acid
13. 30% Sulfuric Acid and Ultraviolet light
14. Bromothymol blue
15. Iodine vapors

p - pale
br - bright
fl - fluorescent
d - dark
Y - yellow
G - green
Bl - blue
O - orange
P - pink
Pr - purple
Wh - white
B-brown
R-red
Bk-black

analysis showed only one leucoanthocyanin, compounds 24 and 30 were present in P. macrocarpa and P. wilsoniana respectively.

Twenty-three of the forty-three compounds separated by paper chromatographic examination of the benzene, ethyl ether, ethyl alcohol, and water extracts occurred in amounts sufficient for isolation and characterization. Nineteen of these compounds were positively or tenatively identified. The remaining four compounds were not identified but displayed characterisitics worthy of further investigation and identification. These twenty-three compounds will be discussed according to their extractive distribution.

Benzene Extract

The benzene extract of the four species was waxy in appearance, but unlike the hexane extract it was totally soluble in acetone and readily separated by paper chromatography. The compounds found in this extract were nos. 1 through 9, 12, 15, 18, 21, 27, 32, 33. Most of these compounds were found to occur in greater yield in subsequent extracts and will be discussed in these extracts. However, the amount of compound no. 1 appeared to be the same in this extract as in subsequent extracts.

Identification of Quercetin (3', 4', 3, 5, 7-pentahydroxyflavone). The chromatographic properties of compound no. 1 were indicative of a flavonol (73, p. 476). It failed to move in aqueous solvents,

displayed yellow coloration in both visible and ultraviolet light, and fluoresced intensely under ultraviolet light upon spraying with a 3 percent methanolic aluminum chloride solution. Spectral analysis of the compound in 95 percent ethyl alcohol showed absorption maxima at 256, 291 and 372 millimicrons. Addition of 0.006 N potassium hydroxide and 0.04M aluminum chloride resulted in spectral maxima shifts in close agreement to reported values (35, p. 704) for quercetin. The compound moved identically with authentic quercetin when compared chromatographically.

Ethyl Ether Extract

The ether extract contained thirty-one compounds including all of the compounds found in the benzene extract. The thirty-one compounds were nos. 1 through 9, 12 through 15, 18, 20 through 25, and 27 through 37. Thus, compounds no. 13, 14, 20, 22 through 25, 28 through 31 and 34 through 37 were present in this extract and were absent from the hexane or benzene extracts. Among the total thirty-one compounds in this extract, ten (21, 23, 27 through 29, 33 through 37) occurred in trace amounts and are not characterized except for Rf values and color reactions as listed in Table VI. Of the remaining 21 compounds, eight (2, 3, 20, 24, 25, 30, 31, and 32) occurred in sufficient quantity in this extract to permit isolation and identification and are discussed here.

Identification of Eriodictyol (3', 4', 5, 7-tetrahydroxyflavanone). Sodium borohydride spray produced a magenta color with compound no. 2 in the presence of hydrochloric acid fumes. This color reaction is characteristic of flavanone compounds as are the other color reaction listed in Table VI. Spectral analysis in 95 percent ethyl alcohol revealed spectral absorption maximum at 289 millimicrons with an inflection at 340 millimicrons (Figure 5). Spectral shifts in 0.006N potassium hydroxide, 0.04M aluminum chloride and anhydrous sodium acetate were in exact agreement with previously reported values for eriodictyol (48, p. 2449). The compound had chromatographic properties identical with those of an authentic eriodictyol sample. The flavanone was found to occur in greatest amount in the old growth domestic firs (P. menzesii and P. macrocarpa), with P. macrocarpa containing almost twice the amount present in P. menzesii.

Identification of Dihydroquercetin (3, 3', 4', 5, 7-pentahydroxyflavanone). Compound no. 3 produced color reactions characteristic of a flavanonol (Table VI). It was particularly sensitive to bis-diazotized benzidine spray reagent producing a yellow-orange color immediately. The ultraviolet spectrum in 95 percent ethyl alcohol exhibited an absorption maximum at 290 millimicrons with an inflection at 340 millimicrons. Addition of 0.006N potassium hydroxide, and 0.04M aluminum chloride resulted in spectral

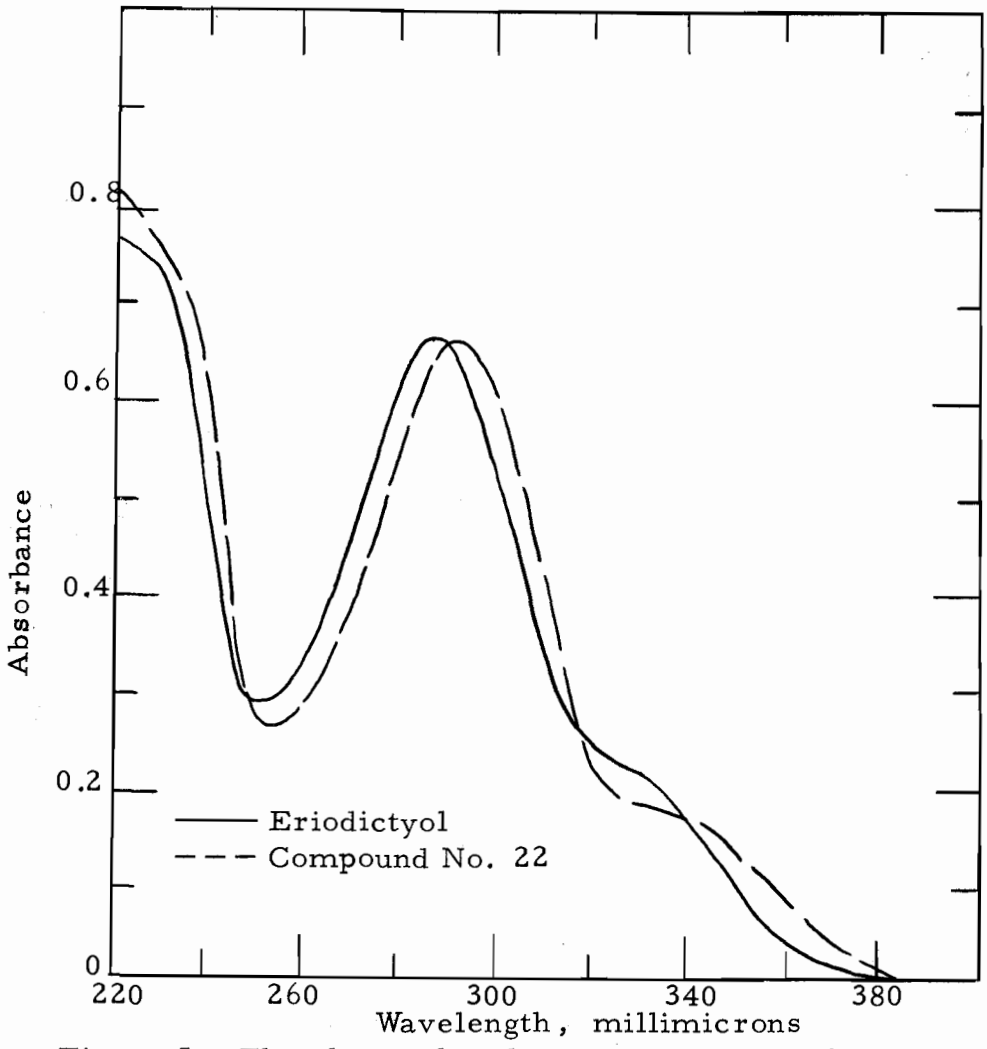

Figure 5 - The ultraviolet absorption spectra of compound no. 22 (alcohol extract of P. menzesii (O.G. and S.G.)and P. macrocarpa) and eriodictyol (benzene, ether and alcohol extract of four species of Pseudotsuga)

shifts in very close agreement with those previously reported for dihydroquercetin (35, p. 704). Co-chromatography with an authentic sample of this flavanone showed them to be identical. This flavanone was common to all species, and was present in the greatest amounts in the domestic firs (P. menzesii and P. macrocarpa).

Compound No. 20. Compound no. 20 produced an unusual purple color reaction with bis-diazotized benzidine and gave a positive reaction with the general phenolic spray ferric chloride-potassium ferricyanide. The purple color reaction with bis-diazotized benzidine is generally indicative of a compound having a phloroglucinol ring system. No color reactions were noted with the other chromogenic reagents. Attempts at elution proved unsuccessful because of the small amount of the compound present. The compound is of considerable interest since it occurred only in the domestic old growth Douglas firs (P. menzesii and P. macrocarpa).

Identification of Leucoanthocyanins. The positive reaction of compounds 24, 25, 30 and 31 with bis-diazotized benzidine and cinnamaldehyde-hyrochloric acid sprays is characteristic of leucoanthocyanins. Further confirmation of their identity was obtained from their chromatographic behaviour (Table VI) in comparison with recorded values for leucoanthocyanins present in old growth Douglas fir bark (34, p. 610). The small amounts of these compounds present made spectral analysis impossible. Standard

leucoanthocyanins, of known structure, are presently unavailable. The variation in distribution of these leucoanthocyanins, as previously described, warrants further investigation.

Compound No. 32. Compound no. 32 exhibited several color reactions and spectral properties of ferulic acid, but unlike a hydroxycinnamic acid, did not move when chromatographed in two percent acetic acid. Standard ferulic acid produced a purple color when sprayed with diazotized-sulphanilic acid, but this compound produced a dark orange-yellow color. Spectrally, the compound exhibits maxima at 235 and 323 millimicrons in 95 percent ethyl alcohol while a spectra was not obtained in base. The compound's properties seem to suggest a ferulic acid ester rather than ferulic acid itself.

Ethyl Alcohol Extract

The alcohol extract was the most carefully analyzed, with the eventual isolation of 27 compounds (no. 1 through 27). Of these 27 compounds, six (nos. 1, 2, 3, 20, 24, 25) have been described as part of the benzene and other extracts. Sixteen of the remaining twenty-one compounds occur in greatest yield in this extract. However, four of these compounds (nos. 19, 21, 26 and 27) occur in such small amounts as to prevent analysis. Seven of the remaining compounds were positively identified.

Identification of d-Catechin and l-Epicatechin. (3', 4', 5, 7-tetrahydroxyflavan-3-ols). The two compounds, numbered 4 and 5, developed characteristic and distinctive colors with the cinnamaldehyde - hydrochloric acid reagent (Table VI). Chromatographically, the compounds were in consistent agreement with authentic samples of d-catechin and l-epicatechin respectively, in all solvents. Both compounds showed a characteristic 280 millimicron absorption maximum in 95 percent ethyl alcohol and a shift of this maximum to 295 millimicrons in the presence of 0.006N potassium hydroxide. Thus, compounds numbered 4 and 5 were identified respectively as d-catechin and l-epicatechin.

The catechins were present in largest amounts in P. japonica and P. menzesii and in the least amount in P. macrocarpa and P. wilsoniana.

Identification of Vanillic Acid (4-hydroxy -3-methoxybenzoic acid). By means of identical Rf values in several solvents with reported values, and distinctive color reactions with p-nitroanaline and diazotized sulphanilic acid spray reagents, compound no. 7 was identified as vanillic acid. The compound was present in small amounts in all species and attempts to selectively elute it from paper chromatograms for spectral analysis were unsuccessful.

Compound No. 11. Spraying of two dimensional chromatograms with

bis-diazotized benzidine revealed a purple spot with a large Rf in two percent acetic acid (spot no. 11). The spot developed a positive reaction to the general phenolic spray ferric chloride-potassium ferricyanide. The compound was present in small amounts and gave no other color reaction. Elution attempts proved unsuccessful. The chromatographic behaviour was similar to that reported for quinic acid (21, p. 862) in BAW (4:1:5) and two percent acetic acid, and on that basis was considered the same compound. However, chromatographic and chromogenic (12) comparison with an authentic sample of quinic acid (Aldrich Chemical Co.) showed that this compound was not quinic acid.

Identification of Dihydroquercetin-3'-monoglucoside. Compound no. 12 displayed the same color reaction as dihydroquercetin but markedly different Rf values (Table VI). The compound had a maximum with 0.006N potassium hydroxide, 0.04 M aluminum chloride and sodium acetate were in close agreement with the recorded values (35, p. 704) for dihydroquercetin-3'-monoglucoside. The compound was co-chromatographed with an authentic sample of dihydroquercetin-3'-monoglucoside and proved identical in several solvents. Thus, compound no. 12 was identified as dihydroquercetin-3'-monoglucoside.

Identification of Vanillyl Alcohol. (4-hydroxy-3-methoxybenzylalcohol) Compound no. 15 reacted only with bis-diazotized benzidine

and ferric chloride-potassium ferricyanide, the latter spray being most sensitive. Although present in low concentration, a suitable sample was eluted for spectral analysis. This compound had its absorption maxima at 227 and 280 millimicrons in 95 percent ethyl alcohol (Figure 6). Addition of 0.006N potassium hydroxide to the alcoholic solution produced a shift of the maxima to 253 and 294 millimicrons. This spectral data was in close agreement to that of standard vanillyl alcohol and acetovanillyl alcohol. The spot was identified as vanillyl alcohol on the basis of its close agreement in Rf values to those of standard vanillyl alcohol. Two additional spray reagents were applied in an attempt to find more distinctive reactions differentiating between these alcohols. These sprays were quinone monochloroimide (19) and iodoform (80, p. 138). Quinone monochloroimide was reported to give a distinctive blue color with vanillyl alcohol. When applied to the suspected vanillyl alcohol, no reaction was noted, probably because of the low concentration of the compound on the chromatogram. The iodoform test is reported to be specific for secondary alcohols with a methyl side chain. Spray reaction with the iodoform produced no reaction, again either because of the low concentration or absence of the secondary (acetovanillyl alcohol) alcohol.

Identification of Luteolin (3', 4', 5, 7-tetrahydrodroxyflavone). In the two dimensional chromatography of P. japonica, P. wilsoniana

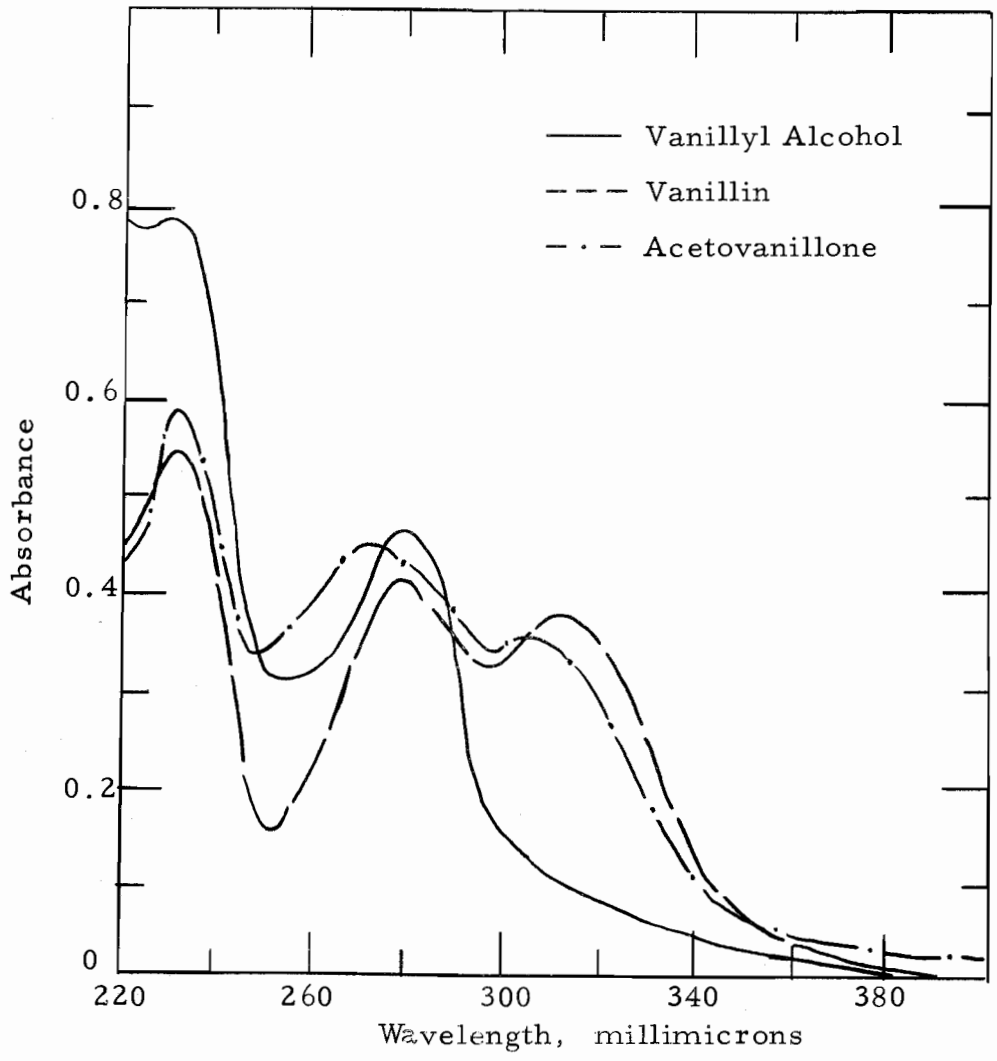

Figure 6 - The ultraviolet absorption spectra of vanillyl alcohol, vanillin and acetovanillone isolated from the ethyl alcohol and water extracts of all species and determined in 95 percent ethyl alcohol.

and P. macrocarpa, a spot appeared which had chromatographic characteristics of a flavone and fluoresced green when exposed to ammonia vapors under ultraviolet light. Elution of the compound proved difficult, but enough was finally isolated to obtain an ultraviolet absorption spectra (Figure 7). In 95 percent ethyl alcohol, the compound had absorption maxima at 227, 253, and 350 millimicrons. This absorption spectra is identical with that reported for luteolin (18, p. 111). The absorption spectra obtained in 95 percent ethyl alcohol in the presence of sodium acetate-boric acid (18, p. 111) confirmed the identity of this compound. Comparison of the chromatographic behaviour of the unknown with an authentic specimen of luteolin in several solvents showed them to be identical.

Compound No. 17. Compound no. 17 was found to occur only in the oriental species. The presence of the unknown compound was detected by a bright yellow fluorescence under ultraviolet light after treatment with ammonia. Other spray reactions (Table VI) were indicative of a flavonoid structure. Spectrally, the compound had an absorption maxima at 288 millimicrons (Figure 8) in 95 percent ethyl alcohol which shifted to 319 millimicrons in 0.006N potassium hydroxide. This maximum slowly shifted to 307 millimicrons in the presence of anhydrous aluminum chloride. Addition of sodium acetate and boric acid produced no shift. The spectra suggested a flavanone nucleus (288 maximum in ethyl alcohol) with an

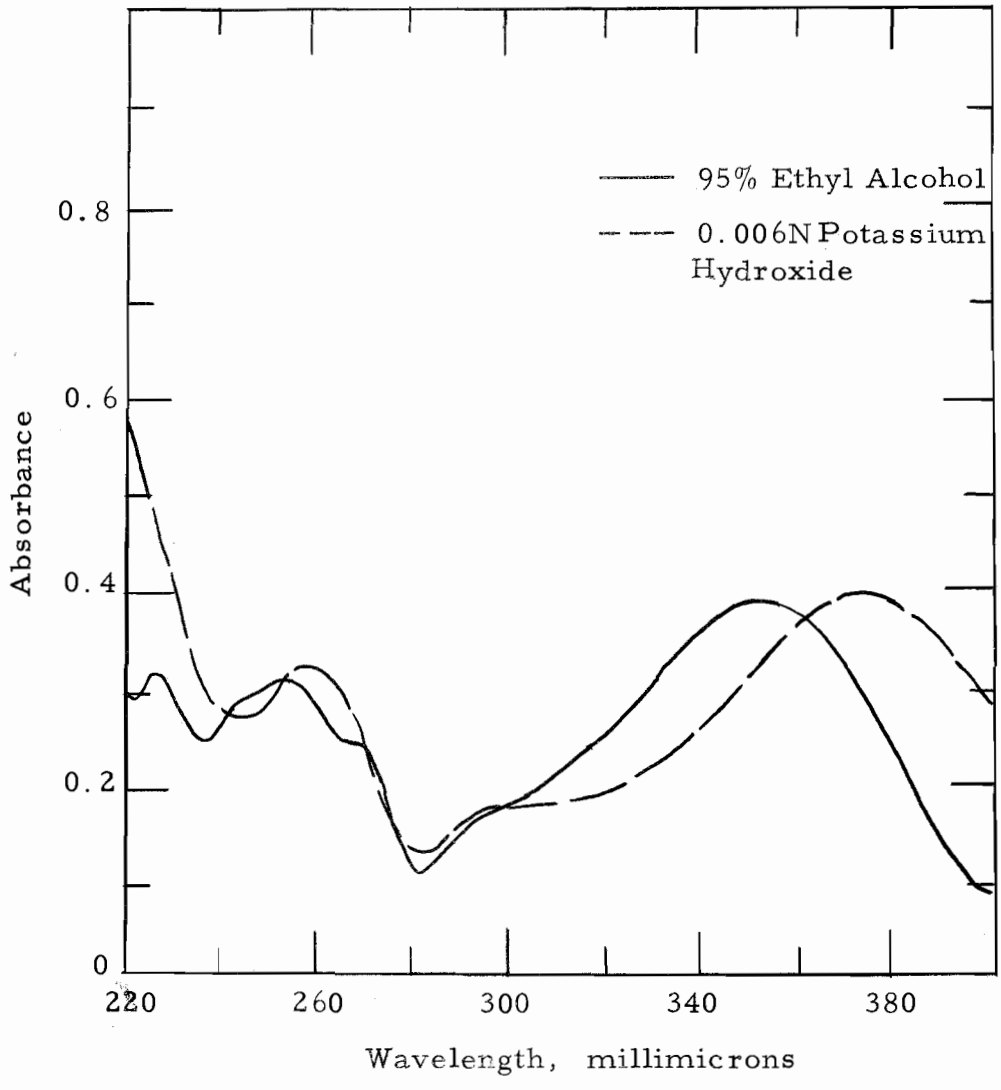

Figure 7 - The ultraviolet absorption spectra of luteolin (spot no. 16) occurring in the alcohol extract of P. macrocarpa, P. japonica and P. wilsoniana.

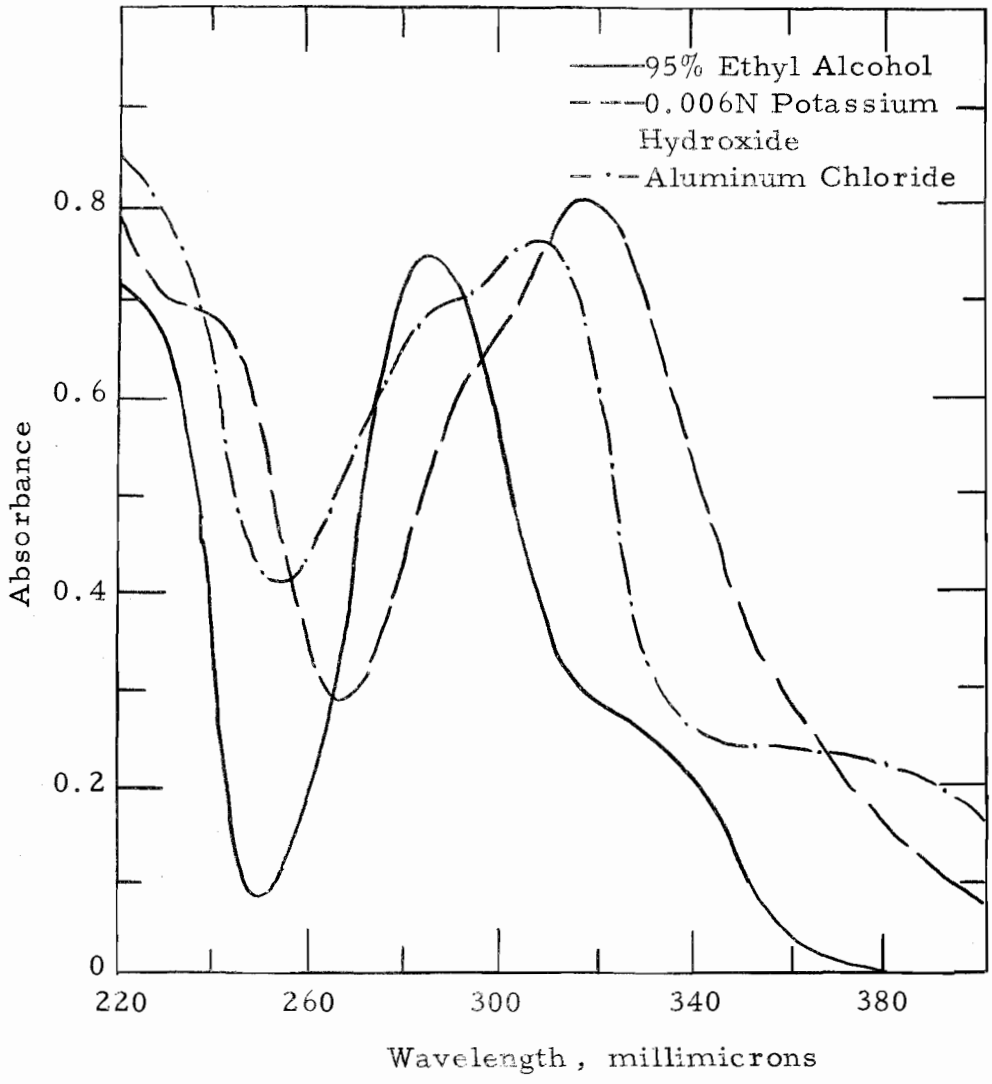

Figure 8 - The ultraviolet absorption spectra and shifts of compound no. 17 occurring in the alcohol extract of P. japonica and P. wilsoniana.

unsubstituted 5 position hydroxyl (shift with aluminum chloride), a substituted 7 position hydroxyl (no shift in sodium acetate), and a lack of an o-dihydroxyl grouping on the B ring of the structure (no shift in sodium acetate, boric acid).

To further characterize this unknown, other color reactions (18, p. 72) were investigated. Addition of aqueous sodium hydroxide to an ethyl alcohol solution of the compound produced a yellow color, while concentrated sulfuric acid produced an orange color. Addition of magnesium-hydrochloric acid gave in a brownish yellow color. Sodium amalgum followed by hydrochloric acid gave a pink color. These results suggest the compound to be either a flavanone or a isoflavanone or their glycosides, with the greatest possibility being the glycoside because of the high Rf values in aqueous solvents. Restriction of this compound to the oriental species makes its identity of some taxonomic value. Since the compound is present in fair concentration, isolation and identification should be carried out.

Identification of Coniferaldehyde (4-hydroxy - 3-methoxycinnamaldehyde). Compound no. 18 was the only compound to give a positive reaction with phloroglucinol-hydrochloric acid spray. Its purple reaction with this reagent and the compound's Rf values are characteristic of coniferaldehyde. The compound occurs in very low concentration in all species, thus making spectral analysis nearly

impossible. However, close agreement of the unknown with standard coniferaldehyde in chromatographic behaviour as well as the distinguishing color reaction satisfactorily identified this compound.

Compound No. 22. In the alcohol extract of old and second growth P. menzesii and P. japonica, a compound (No. 22) was noted which exhibited chromatographic properties similar to those of eriodictyol (Table VI). The compound moved very near to eriodictyol in all solvents though never identically. Examination spectrally (Figure 5) showed the compound to exhibit an absorption maximum at 290 millimicrons in 95 percent ethyl alcohol with a subsequent shift to 240 and 330 millimicrons with 0.006N potassium hydroxide. Addition of anhydrous aluminum chloride resulted in a shift to 223 and 310 millimicrons. No shift was noted in the presence of sodium acetate.

The chromatographic and spectral properties suggest that the compound is a 7-hydroxyl substituted derivative of eriodictyol. However, it is unlikely that it is a sugar because of its low Rf value in aqueous solvents. The possibility of the compounds no. 2 and 22 being optically active and optically inactive eriodictyol was investigated. No difference in Rf values was noted for the authentic compounds in the solvents used. No further characterization of this compound was made.

Water Extract

Paper chromatography isolated eighteen compounds (5 through 15, 18, 38 through 43) from the water extracts of the four species investigated. Among these are eight compounds (5, 7, 11 through 15) which have been discussed earlier. Of the ten remaining compounds, six occur in trace amounts with their chromatographic properties listed in Table VI. These trace amounts will not be discussed further. Thus, four compounds remain to be identified and occur in largest yield in the water extract. These compounds are nos. 6, 8, 9 and 10.

Identification of Protocatechuic Acid. (3, 4-dihydroxybenzoic acid). Distinctive spray reactions were displayed by compound number 6 with diazotized sulphanilic acid and p-nitroanaline spray reagents as shown in Table VI. The compound had an absorption maximum at 257 and 291 millimicrons in 95 percent ethyl alcohol and a characteristic shift in base to 274 and 300 millimicrons. The chromatographic and spectral properties of the compound were identical with authentic protocatechuic acid. This compound was present in fair concentration in all of the Pseudotsuga species.

Identification of Vanillin (4-hydroxy-3-methoxybenzaldehyde) and Acetovanillone (4-methoxy -3-hydroxyacetophenone). Compounds no. 8 and 9 reacted positively with 2, 4-dinitrophenylhydrazine

spray reagent, indicating a carbonyl group. Compound no. 8 gave a positive reaction with Tollen's reagent (80, p. 145) while compound no. 9 did not. Thus compound no. 8 is an aldehyde and compound no. 9 is a ketone. The ultraviolet spectra of these compounds is shown in Figure 6. Compound no. 8 has absorption maxima at 231, 280 and 312 millimicrons with a shift in absorption maxima in 0.006N potassium hydroxide to 251 and 348 millimicrons. Spot no. 9 had spectral absorption maxima at 231, 274 and 303 millimicrons which shifted to 250 and 350 millimicrons in base. This data is characteristic of vanillin (spot no. 8) and acetovanillone (spot no. 9). The chromatographic data, however, did not agree. In all solvents, authentic vanillin and acetovanillin standards were found to have fairly close Rf values. Yet, the spots no. 8 and 9 displayed unusually large differences in Rf values in some solvents, particularly BzW. In this solvent the suspect vanillin moved only to an Rf value of 0.35 while standard acetovanillone and the vanillin had values of 0.84 and 0.81 respectively. The spectra of the two compounds were identical to that reported (61, p. 2998) for authentic compounds. However, the suspect vanillin spectrum in ethyl alcohol and its alkaline shift were more like the standard acetovanillone spectrum than a standard vanillin spectra. The curve for the suspect acetovanillone, however, appeared closer to the acetovanillone standard spectrum than vanillin and thus identification of this

compound was justified. Mixing of the two compounds probably resulted in variations of both chromatographic and spectral behaviour. Vanillin was found in greater concentration in all four species investigated.

Compound No. 10. Compound no. 10 reacted with bis-diazotized benzidine spray reagent to give a purple color and yield a positive reaction to the general phenolic spray reagent. These color reactions, coupled with the chromatographic behaviour, as compared to the reported values (46, p. 40), suggested that compound no. 10 might be shikimic acid. However, chromatographic and chromogenic (12) comparison with an authentic sample of shikimic acid (Aldrich Chemical Co.) showed that the compound was not shikimic acid.

Sugar Analysis

The results of the paper chromatographic analysis of the water extracts for the detection of simple sugars is shown in Table VII.

Table VII - Sugar Distribution in Water Extracts of Four Species of Pseudotsuga

	Glucose	Galactose	Arabinose	Rhamnose	Xylose
P. menzesii (O. G.)	trace	—	+ +	trace	trace
P. menzesii (S. G.)	trace	—	+ +	trace	trace
P. macrocarpa	trace	trace	+ +	trace	trace
P. japonica	trace	trace	+ +	trace	+
P. wilsoniana	trace	trace	+ +	trace	+

+ = present - = absent

Present in this extract of all species was aribinose, glucose, rhamnose and xylose. Arabinose was present in the greatest concentration in all species, galactose was present in trace amounts in the extracts of P. macrocarpa, P. japonica and P. wilsoniana, but was not detected in the water extract of P. menzesii.

Thin Layer Chromatography

A composite two dimensional thin layer chromatogram of the hexane and benzene extracts of all of the Pseudotsuga species is shown in Figure 9 and described in Table VII. Of the 27 spots shown in the diagram, nine (spots no. 1, 2, 4, 5, 7, 17, 18, 19 and 22) occur in sufficient quantity for isolation, the remaining eighteen spots occur in trace amounts. Only two (spots no. 5 and 7) of the nine spots were tentatively identified on the basis of chromatographic and spectral characteristics. The other compounds

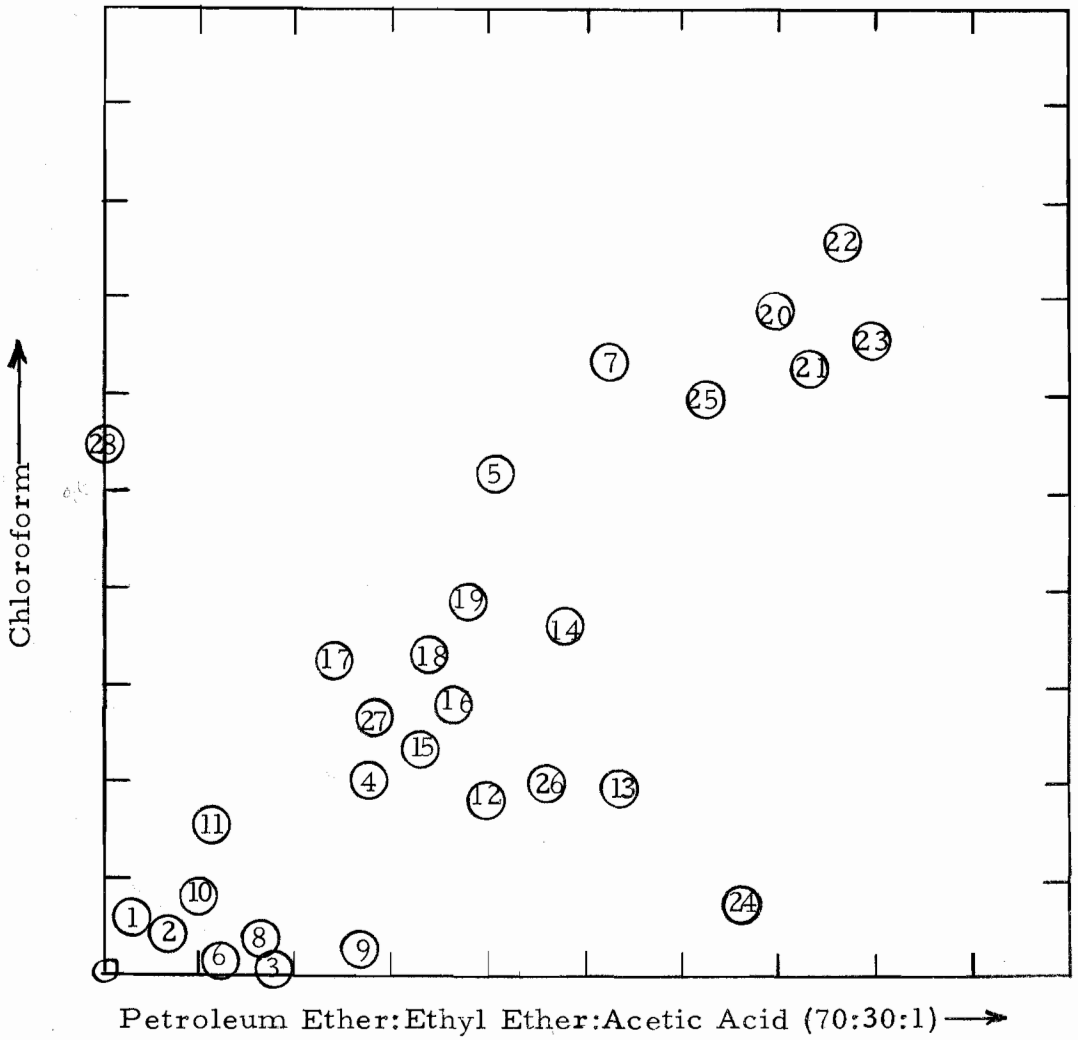

Figure 9 - A schematic thin layer chromatogram of the hexane and benzene extracts of four species of Pseudotsuga.

TABLE VIII - Thin Layer Chromatographic Results of Hexane and Benzene Extracts of Four Species of Pseudotsuga.

Sp.	Rf Values			Chromogenic Spray Reactions (T.VI)								Species Distribution					Extract	
No.	P. E. A.	$CHCl_3$	BMA	1	2	12	13	14	4	7	15	DF.O.G.	DF.S.G.	PM	PJ	PW	Hex	Benz
1	.04	.07	.46	Bl	Bl	-	pY	Bl	-	-	+	+	+	+	+	+	+	+
2	.08	.06	.52	Y	BY	RB	Bk	Bl	Y	Y	+	+	+	+	+	+	+	+
3	.10	.02		-	YG	Y	Y	YG	-	-		Tr	Tr	+	Tr	Tr	+	+
4	.30	.22	.75	-	-	Pr	Bk	Bl	-	-	+	+	+	+	+	+	+	+
5	.43	.54	.81	Bl	dG	P	flY	Y-Bl	YB	OP	+	++	++	++	++	++	+	-
6	.14	.03		pY	YG	-	-	YG	-	-		Tr	Tr	Tr	Tr	Tr	+	+
7	.54	.56	.81	Bl	dG	pP	pY	Bl	pYB	pOP	+	+	+	+	+	+	+	-
8	.18	.05	.70	-	-	-	brBl	Bl	pY	-		+	+	Tr	Tr	Tr	+	+
9	.28	.04	.68	-	-	-	flY	Bl	P	-	+	+	Tr	Tr	Tr	+	+	+
10	.12	.10		-	Bl	-	pY	Bl	Y	P	+	+	+	Tr	Tr	+	+	-
11	.13	.17		-	pY	-	pY	-	-	-		Tr	Tr	Tr	Tr	-	+	-
12	.42	.20		-	-	-	-	Bl	pYB	P	+	+	Tr	Tr	Tr	-	+	-
13	.55	.22		-	-	-	-	Bl	-	-	+	+	-	+	Tr	Tr	+	-
14	.50	.27	.74	-	pY	pY0	BMh	Bl	pB	-	+	Tr	Tr	+	Tr	Tr	+	-
15	.35	.25		pY	pY	-	-	Bl	-	-		Tr	-	-	Tr	-	+	-
16	.38	.30		-	-	-	pY	Bl	-	-	+	Tr	Tr	-	-	-	+	-
17	.26	.34		pY	-	-	pY	Bl	pB	-		+	+	Tr	+	+	+	-
18	.36	.35	.74	YG	G	-	pY	-	-	-	+	+	+	+	+	++	+	-
19	.40	.41		-	-	-	pY	Bl	-	-	+	+	+	+	+	+	+	-
20	.72	.71		-	-	pY	pY	Bl	-	-	+	+	+	Tr	Tr	+	+	-
21	.85	.75	.90	-	-	-	pY	Bl	-	-		Tr	-	-	-	-	+	-
22	.78	.77		-	-	Pr	R	Bl	-	-	+	+	+	+	++	+	+	-
23	.82	.67		-	-	-	pY	Bl	-	-		Tr	Tr	-	-	-	+	-
24	.68	.09		-	-	-	-	Bl	-	-		Tr	Tr	-	-	-	+	-
25	.64	.62		-	-	-	pY	-	-	-		Tr	Tr	+	+	Tr	+	-
26	.47	.22		-	-	P	Bl	-	P	Y		+	+	-	Tr	+	+	-
27	.31	.28	.71	-	pY	-	pY	-	-	-		Tr	Tr	Tr	-	Tr	+	-
28	.00	.56		-	-	P	-	-	-	-		Tr	Tr	Tr	-	-	+	-

displayed characteristic chromatographic behaviour but were difficult to elute from thin layer chromatograms. Extensive experimentation was needed to find solvents which gave adequate thin layer chromatographic separation. Thus, examination of these extracts was not as detailed as those by paper chromatography.

Compounds No. 5 and 7. These two compounds displayed distinctive color reaction, as shown in Table VIII. The most striking color reaction appeared in 30 percent sulfuric acid spray. After heating, the compounds appeared pink in visible light and fluoresced yellow under ultraviolet. These compounds reacted to produce colors similar to those of ferulic acid with all chromogenic reagents. Both compounds however, had much higher Rf values in the polar solvents used in the thin layer analysis.

Both compounds exhibited spectral absorption maxima at 238 and 326 millimicrons with an inflection at 295 millimicrons in 95 percent ethyl alcohol (Figure 10). These maxima are identical to those of ferulic acid. Addition of base (0.006N potassium hydroxide) to the spectral solution resulted in a maxima shift for both compounds to 250 and 383 millimicrons with a minor peak at 310 millimicrons, addition of sodium acetate produced no shift of the original maxima.

Compounds no. 5 and 7 were not positively identified from their chromatographic and spectrophotometric data, but it was

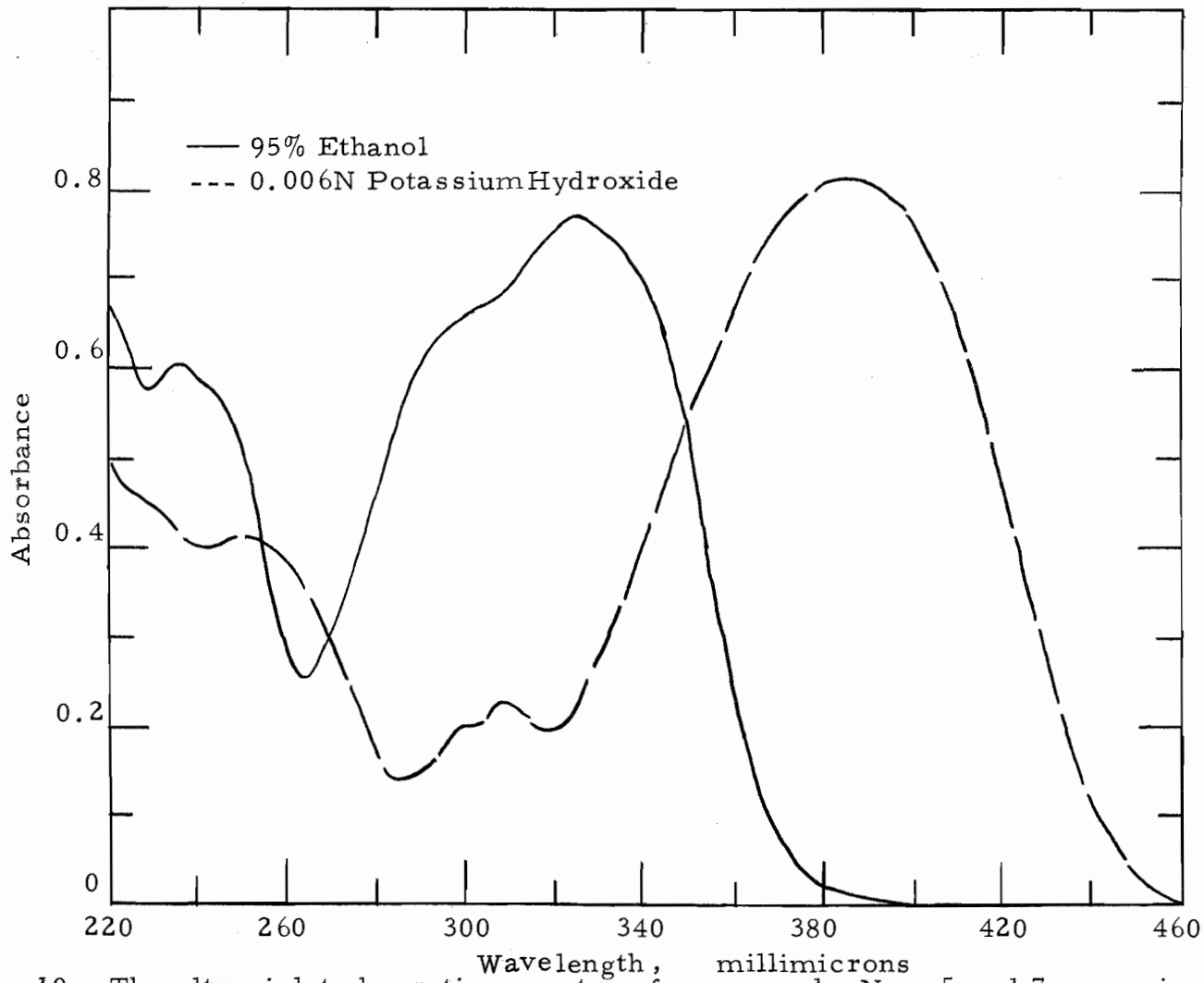

Figure 10 - The ultraviolet absorption spectra of compounds No. 5 and 7 occurring in the hexane extract of all Pseudotsuga species.

concluded that they are most likely long carbon chain alcohol esters of ferulic acid.

It is possible that each of these compounds could be a different ester of ferulic acid, i. e., ferulic acid esterified with a different alcohol. It is also possible that these could be the cis- and trans-isomers of the same ester since, it has been shown that the cis- and trans-isomers have different chromatographic behaviour and can be separated by thin layer chromatography (75, p. 219).

Both compounds are found in fairly high concentration in all species with the greatest concentration present in the old growth domestic Douglas firs (P. menzesii and P. macrocarpa).

DISCUSSION

Chromatographic analysis of the bark of Douglas fir (Pseudotsuga menzesii) has been extensive (34, 35, 36, 46, 54, 57 and 58). This investigation, however, constitutes the first detailed chromatographic examination of Pseudotsuga menzesii and three other Pseudotsuga species (P. macrocarpa, P. japonica and P. wilsoniana).

Chromatographic separation and analysis of bark extractives of the four species revealed the presence of: dihydroquercetin, quercetin, dihydroquercetin 3' monoglucoside, l-epicatechin, d-catechin, vanillin, protocatechuic acid, coniferaldehyde, and leucoanthocyanins. All of these compounds have been previously reported to occur in the bark of P. menzesii. Compounds discovered in the four species which have not previously been reported include: eriodictyol, vanillic acid, vanillyl alcohol and acetovanillone. In addition, thin layer chromatography of the hexane extract revealed the extensive occurance of ferulic acid in an esterified form in all four species rather than as the free acid as had been previously reported in Douglas fir (P. menzesii) (58, p. 105).

Several compounds were isolated which did not occur in all four species of Pseudotsuga investigated. The selective distribution

of these compounds makes them potentially important in the chemical taxonomic classification of species within the genus Pseudotsuga.

Two of the compounds (no. 16 and 17) were of particular interest. Compound no. 16 was identified as the flavone luteolin and was found to occur in low concentration in P. macrocarpa, P. japonica and P. wilsoniana. Previously, luteolin has never been reported in gymnosperms (18, p. 575) although it is known to occur in some angiosperm species (25, p. 107). It has been considered as a distinguishing compound for the Padus subgenera of Prunus (18, p. 560).

Compound no. 17 was not positively identified, but chromatographic properties and qualitative chemical tests strongly suggests that the compound was a glycoside of a flavone or isoflavone having o-dihydroxyls in the B ring. The compound occurred in good concentration in P. japonica and P. wilsoniana. The concentration and species distribution of this compound dictates the necessity of positively identifying the compound for future taxonomic purposes.

Unusual distribution patterns were noted for other compounds. Compound no. 20, suspected to contain a phloroglucinol nucleus, was found to occur only in the domestic old growth P. menzesii and P. macrocarpa while compound no. 22, displaying properties of a seven (non-sugar) substituted flavanone, was found in both specimens of the domestic Douglas fir (P. menzesii) and in the

oriental P. japonica. Four leucoanthocyanins (compound no. 24, 25, 30 and 31) were found among the species investigated, but only one occurred in P. macrocarpa (no. 30) and P. wilsoniana (no. 24). Three leucoanthocyanins were detected in P. menzesii (24, 25 and 30) and all four were present in old growth P. menzesii and P. japonica.

Other unknown compounds displaying unusual distribution were noted, but in general these compounds occurred in too small a yield to be of potential taxonomic significance. The distribution of compounds in the hexane extract has been omitted in this discussion because the late application of thin layer chromatographic techniques resulted in essentially a preliminary examination of the extract with the results subject to more comprehensive future investigation.

The selective distribution of the compounds noted is of particular interest in light of recent chemical taxonomic separations of species of Prunus based on their capacity to produce flavones (30, p. 3). The recorded distribution patterns of luteolin and the other compounds described is indicative of the possible application of chemical taxonomic separation of Pseudotsuga species based on natural capacity to form given flavonoid compounds. In order to attempt such a separation it would first be essential to know the positive identity of all of the compounds displaying unusual

distribution patterns among all six of the species in the genus. However, at this point it is possible to make a gross comparison of the species investigated on the basis of general compound distribution.

In an overall analysis of the distribution as shown in Table VI, it appears that in those compounds occurring in low concentration there is closer agreement between P. menzesii and the oriental species than between P. menzesii and P. macrocarpa. This factor was particularly noticeable in the case of the catechins and leucoanthocyanins where P. macrocarpa showed a significantly lower concentration. It should be stressed, however, that there is no overall agreement between any two species indicated in the investigation except that they all contain those compounds occurring in the greatest quantity (nos. 1-15, 18). Any future investigations should include the two unanalyzed species (P. forestii and and P. sinensis) before completely conclusive statements can be made regarding the chemical nature of the entire genus.

It is of interest to examine the compounds isolated from bark as potential biosynthetic indicators, even though the outer bark is not generally considered a tissue of biosynthetic importance. All of the compounds identified in this study were aromatic in nature. Thus, the substitution patterns of these compounds may be indicative of their placement in the overall biosynthetic

pathway leading to polyphenolic and polymeric end products.

The compounds isolated and identified fall into two general categories: the simple aromatics of the guaiacyl type (meta-methoxyl and para-hydroxyl substituted phenylpropane) and the polyphenolics (flavonoids) with o-dihydroxylation of the B ring. The only exception is protocatechuic acid (3, 4 dihydroxybenzoic acid). No mono- or trihydroxy-substituted aromatic compounds were found in the bark extracts.

Protocatechuic acid was noted to occur in good yield among all of the species of Pseudotsuga investigated. The acid was the only dihydroxy C_7 aromatic isolated and identified in the chromatographic analysis. It could be that protocatechuic acid does serve as an intermediate in the synthesis of guaiacyl compounds and/or C_6-C_3 precursors in flavonoid synthesis as suggested by several investigators (7, p. 627; 81, p. 287, 298)

The former of these alternatives seems most likely since it has been shown that protocatechuic acid can be formed from 5-dehydroshikimic acid by the elimination of water (72, p. 480; 88, p. 797) and the elimination of oxygen at C_3 (23, p. 262). The acid then can be methylated much the same as caffeic acid has been reported to be converted to ferulic acid (11, p. 633) with a methyl donor such as methionine. Thus, several C_7 guaiacyl compounds could be formed including vanillin, vanillic acid, and vanillyl

alcohol. It is also possible that the protocatechuic acid could be converted to a C_6-C_3 precursor capable of eventual flavonoid production, but is is more likely that an alternate route exists through prephenic acid (Figure 11) to a C_6-C_3 precursor.

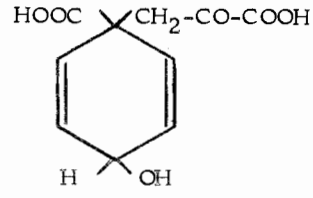

Figure 11 - Prephenic Acid

The absence of B ring methoxyl substituted flavonoid compounds suggests that a caffeoyl (3, 4 dihydroxycinnamic) C_6-C_3 precursor is involved in the biosynthesis of B ring o-dihydroxy flavonoids. Such a precursor might be a transitory intermediate in the formation of the coniferyl (4 hydroxy, 3 methoxycinnamic) C_6-C_3 intermediate, involved in the formation of lignin, and other plant constituents having this substitution pattern. The indentification of two esters of ferulic acid in the hexane extract (waxy) of the bark, would indicate that this intermediate is diversified in plant metabolism, i.e., its biosynthetic utilization.

In consideration of biosynthetic routes, it should be realized that although the origin of a large number of plant polyphenols can be described with some assurance, others are open to interpretation. Particular individual steps leading to a molecular structural methylation or hydroxylation, have not, for the most part, been

completely clarified. Such changes appear as unquestioned biosynthetic occurrences , but may not adequately be defined in terms of the point in time or order in which they occur. Thus, any biosynthetic implications of this investigation can only be considered preliminary observations for more detailed studies utilizing such precise analytical tools as radioactive tracers.

The only flavonoid sugar derivative positively identified in this investigation was dihydroquercetin 3' monoglucoside, which was also reported in the needles (35, p. 710) and flowers (39). None of the other five glucosides present in the flowers, or quercetin 3' monoglucoside reported in the needles, was found in the bark. If translocation of flavonoid compounds does occur as suggested (35, p. 710), these tissues should exhibit similar flavonoid glucoside content. Thus, it appears that flavonoid biosynthesis occurs in situ in each tissue.

The extractive distribution of compounds found in the bark is of interest. Previously, most of the flavonoid compounds were thought to occur in the ether or ethyl alcohol extracts because of solubility characteristics. This investigation revealed that almost all of the compounds present in fair concentration, occurred in the benzene and hexane extracts as well. The yield of quercetin, for instance, was not greatly different in the benzene, ether and alcohol extracts. The flavonoids were not detected in the water

extract, though the largest amount of guaiacyl compounds were found to occur in this extract. The waxy hexane extract was found to differ greatly from the other extracts, with most of its components being lipid derivatives. The benzene extract also contained some of these compounds as well as small amounts of the flavonoid compounds.

The distribution of compounds in the various extracts indicates that solvent fractionation of one particular bark component, such as the flavonoids, would be difficult. In this study it was found that the bark of old growth P. macrocarpa contained a larger amount of waxy and flavonoid compounds than the other species. The development of a substantial market for these compounds or their derivatives could possibly justify further examination of this bark as a source of these compounds. The wood of P. macrocarpa is presently of very low commercial value in terms of wood utilization. However, until such a market appears for these compounds, bark will probably continue to be a by-product of low value presenting problems of disposal.

Future chemical studies of these barks should place emphasis on complete analysis of the waxy extracts using more sophisticated methods of thin layer and gas chromatography. The exact identity and the properties of the ferulic acid ester found in the hexane extract could possibly be determined from samples eluted in large

quantity from chromatographic columns. Positive identification of those compounds which are not common to all members of this genus could be of taxonomic value.

BIBLIOGRAPHY

1. Adkison, Verner J. Air pollution control, City of Eugene, Oregon. Personal communication. 1964

2. Adler, E. Newer views of lignin formation. Tappi 40:294-301. 1957.

3. Bailey, R. W. Colour reactions between sugars and diphenylamineurea and diphenylamine-p-anisidine on paper chromatograms. Journal of Chromatography 8:57-62. 1962.

4. Bernfeld, Peter (ed.) Biogenesis of natural compounds, New York, MacMillan, 1963. 930 p.

5. Bollen, Walter B. and Douglas W. Glennie. Fortified bark for mulching and soil conditioning. Forest Products Journal 13:209-215. 1963.

6. Bray, H. G., W. V. Thorpe and K. White. The fate of certain organic acids and amides in the rabbit. 10. The application of paper chromatography to metabolic studies of hydroxybenzoic acids and amides. Biochemical Journal 46:271-275. 1950.

7. Brucker, Wolfgang. Zur Biogenese der gallus and protocatechusaure durch Phycomyces blakesleeanus. Planta 48:627-630. 1957.

8. Burrows, C. H. Particle board from Douglas-fir bark— without additives. Corvallis, 1960. 40 p. (Oregon State University. Forest Research Center. Information Circular 15).

9. Burrows, C. H. Floor tile from Douglas fir cork. Corvallis, 1959. 24 p. (Oregon State University. Forest Research Center. Information Circular 13).

10. Business Executives' Research Committee. The forest products industry in Oregon. Part I. A report sponsored by: Lewis and Clark College and Reed College, Portland, Oregon. December 1954. 36 p.

11. Byerrum, R. U. et al. Incorporation of formate and the methyl group of methionine into methoxyl groups of lignin. Journal of Biological Chemistry 210:633-643. 1954.

12. Cartwright, R. A. and E. A. H. Roberts. Theogallin as a galloyl ester of quinic acid. Chemistry and Industry 9:230-231. Feb. 26, 1955.

13. Ching, Kim K. Unpublished research on genetic investigations of the genus Pseudotsuga. Corvallis, Oregon. Oregon State University Forest Research Laboratory, 1964.

14. Ching, Te May and Kim K. Ching. Fatty acids in pollen of some coniferous species. Science 138:890-891. 1962.

15. Corder, S. E. Oregon mill residues in 1953. Corvallis, 1956. 32 p. (Oregon State University. Forest Research Center. Information Circular 7).

16. Dallimore, W. and A. Bruce Jackson. A handbook of Coniferae including Gingoaceae. London, Arnold, 1948. 682 p.

17. Erdtman, H. Conifer chemistry and taxonomy of conifers. In: Proceedings of the fourth international congress of biochemistry, Vienna, 1958. Vol. II. New York, Pergamon Press, 1959. p. 1-27.

18. Geissman, T. A. (ed.) The chemistry of flavanoid compounds. New York, MacMillan, 1962. 666 p.

19. Gierer, J. Die Reaktion von Chinonmonochlorimid mit lignin. I. Speezifitat der Reaktion auf p-Oxybenzylalkoholgruppen und deren Bestimmung in verschiedenen Ligninpraparaten. Chemica Scandinavica 8:1319-1331. 1954.

20. Goldschmid, O. Aqueous hydrolysis of lignin. I. Paper chromatographic separation of monomeric lignin degradation products. Tappi 38:728-732. 1955.

21. Goldschmid, Otto and H. L. Hergert. Examination of western hemlock for lignin precursors. Tappi 44:858-870. 1961.

22. Gregory, A. S. and D. F. Root. Status report on bark utilization. Pulp and Paper Magazine of Canada 62:T385-T391. 1960.

23. Gross, S. R. Enzymatic conversion of 5-dehydroshikimic acid to protocatechuic acid. Journal of Biological Chemistry 233:1146-1151. 1958.

24. Harborne, J. B. Plant polyphenols. The flavonol gylcosides of wild and cultivated potatoes. Biochemical Journal 84:100-106. 1962.

25. Hasegawa, Masao. Flavanoids of various Prunus species. VII. The flavanoids in the wood of Prunus ssiori and Prunus spinulosa. Journal of the Japanese Forestry Society 38:107-108. 1956.

26. Hasegawa, Masao and Takayoshi Higuchi. Formation of lignin from glucose in Eucalyptus tree. Nippon Ringaku Kaishi 42: 305-308. 1960.

27. Hasegawa, Masao, Takayoshi Higuchi and Hirotaka Ishikawa. Formation of lignin in tissue cultures of Pinus strobus. Plant and Cell Physiology 1:173-182. 1960.

28. Hasegawa, M. and T. Tateoka. Occurance of shikimic acid in plant tissue. III. Seasonal variation of shikimic acid content. Journal of the Japanese Forestry Society 42:224-225. 1960.

29. Hasegawa, Masao and Teruo Shirato. On phenolic substances of wood. IV. Distylin obtained from the wood of Pseudotusga japonica. Journal of the Japanese Forestry Society 33:17-18. 1951.

30. Hasegawa, Masao and Teruo Shirato. Genetics of flavanoids in Prunus wood. Presented to: World consultation of forest genetics and tree improvement, Stockholm, 1963. Rome, Food and Agriculture Organization of the United Nations, 1963. 4 p.

31. Haslam, E., R. D. Haworth and P. F. Knowles. Gallotannins. Part IV. The biosynthesis of gallic acid. Journal of the Chemical Society 1854-1861. 1961.

32. Hathway, D. E. Experiments on the origin of oak bark tannin. Biochemical Journal 71:533-537. 1959.

33. Hathway, D. E. The use of hydroxystilbene compounds as taxonomic tracers in the genus Eucalyptus. Biochemical Journal 83:80-84. 1963.

34. Hergert, H. L. Chemical composition of tannins and polyphenols from conifer wood and bark. Forest Products Journal 10:610-617. 1960.

35. Hergert, H. L. and Otto Goldschmid. Biogenesis of heartwood and bark constituents. I. A new taxifolin glucoside. Journal of Organic Chemistry 23:700-704. 1958.

36. Hergert, Herbert L. and E. F. Kurth. The chemical nature of the cork of Douglas-fir bark. Tappi 35:59-66. 1952.

37. Herrick, Franklin W. and Louis H. Bock. Alkali bark derivative as a partial substitute for resorcinol in resorcinol-formaldehyde adhesives. Canadian Patent 577,506. June 9, 1959.

38. Herrick, Franklin W. and Louis H. Beck. Thermosetting resin from alkali-bark derivative and polymethyl phenol compound. Canadian Patent 577,536. June 9, 1959.

39. Highley, Terry L. The flavanoid compounds of three floral phenotypes of Douglas fir. Master's thesis. Corvallis, Oregon State University, 1964. 69 numb. leaves.

40. Higuchi, Takayoshi. Studies on the biosynthesis of lignin. In: Proceedings of the fourth international congress of biochemistry, Vienna, 1958. Vol. II. New York, Pergamon Press, 1959. p. 161.

41. Hillis, W. E. Wood extractive and their significance to the pulp and paper industry. New York, Academic Press, 1962. 513 p.

42. Hillis, W. E. Formation of leuco-anthochanins in Eucalyptus tissue. Nature 175:597-598. 1955.

43. Hillis, W. E. Leucoanthocyanins as the possible precursors of extractives in woody tissue. Australian Journal of Biological Science 9:263-280. 1956.

44. Hillis, W. E. Factors influencing the formation of phloem and heartwood polyphenols. Holzforschung 14:105-110. 1962.

45. Hillis, W. E. and Ann Carle. The origin of the wood and bark polyphenols of Eucalyptus species. Biochemical Journal 82: 435-439. 1962.

46. Holmes, George W. The chemical composition of the extractives from the newly formed inner bark of Douglas fir, Pseudotsuga menzesii (Mirb.) France. Ph.D. Thesis. Corvallis, Oregon State University, 1961. 70 numb. leaves.

47. Horowitz, Robert M. Detection of flavanones by reduction with sodium borohydride. Journal of Organic Chemistry 22:1733-1735. 1957.

48. Horowitz, Robert M. and Leonard Jurd. Spectral studies on flavanoid compounds. II. Isoflavones and flavonones. Journal of Organic Chemistry 26:2446-2449. 1961.

49. Hubbard, James K. and E. F. Kurth. Douglas-fir bark tannin. Journal of the American Leather Chemists Association 44:604-614. 1949.

50. Ibrahim, R. K. and G. H. N. Towers. The identification, by chromatography, of plant phenolic acids. Archives of Biochemistry and Biophysics 87:125-128. 1960.

51. Jurd, Leonard. A spectrophotometric method for the detection of o-dihydroxyl groups in flavanoid compounds. Archives of Biochemistry and Biophysics 63:376-381. 1957.

52. Jurd, Leonard. The detection of aromatic acids in plant extracts by the ultraviolet adsorption spectra of their ions. Archives of Biochemistry and Biophysics 66:284-288. 1957.

53. Jurd, Leonard and Robert M. Horowitz. Spectral studies on flavonols. I. The structure azalein. Journal of Organic Chemistry 22:1618-1622. 1957.

54. Kiefer, Harry J. and E. F. Kurth. The chemical composition of the bast fibers of Douglas-fir bark. Tappi 36:14-19. 1953.

55. King, H. G. C. and T. White. Tannins and polyphenols of Schinopsis (Quebracho) spp., their genesis and interrelationship. Journal of the Society of the Leather Trades' Chemists 41:368-382. 1957.

56. Kurth, E. F. The composition of the wax in Douglas-fir bark. Journal of the American Chemical Society 72:1685-1686. 1950.

57. Kurth, E. F. The chemical analysis of western woods. Part II. Douglas fir bark analysis. Tappi 32:175-176. 1949.

58. Kurth, E. F. and H. J. Kiefer. Wax from Douglas fir bark. Tappi 33:183-186. 1950.

59. Kurth, Ervin F. Extraction of valuable products from bark. U. S. Patent 2,662,893. Dec. 15, 1953.

60. Kurth, Ervin F. Process of beneficiating Douglas fir wax and products thereof. U. S. Patent 2,697,717. Dec. 21, 1954.

61. Lemon, H. W. The effect of alkali on the ultraviolet adsorption spectra of hydroxyaldehydes, hydroxyketones and other phenolic compounds. Journal of the American Chemical Society 69: 2998-3000. 1947.

62. Little, Elbert, L. Jr. Check list of native and naturalized trees of the United States (including Alaska). Washington, D. C., Forest Service, 1953. (Agriculture Handbook no. 41)

63. Linskens, H. F. (ed.) Paperchromatographie in der Botanik. Berlin, Springer-Verlag, 1959. 408 p.

64. Mangold, H. K. and D. C. Malins. Fractionation of fats, oils and waxes on thin layers of silicic acid. Journal of the American Oil Chemist's Society 37:383-385. 1960.

65. Marian, J. E. and A. Wissing. The utilization of bark. I. Index to bark literature. Svensk Traforskninginstitutet Trateknik. Meddelande 90B. Stockholm 1957. 46 p.

66. Marian, J. E. and A. Wissing. The utilization of bark. III. The utilization of polyphenols in spruce bark extractives in adhesives. Svensk Papperstidning 62:187-193. 1959.

67. Marple, Elliot. Douglas fir bark utilized as a filler. Plastics 7:65-67. 1947.

68. Mell, C. D. Dyes and tans from the bark of the elm tree. Textile Colorist 61:127-129. 1939.

69. Mirov, N. T. Composition of the gum turpentines of pines. 1961. 158 p. (U. S. Department of Agriculture, Forest Service. Technical Bulletin 1239.)

70. Nick, E. Physiology of the tannins and flavone pigments in Poloygonum bistorta. Pharmazie 8:940-950. 1953.

71. Neish, A. C. Biosynthetic pathways of aromatic compounds. Annual Review of Plant Physiology 11:55-80. 1960.

72. Noguchi, Ichio and Shiro Takashima. Biological conversion of shikmic acid. Archives of Biochemistry and Biophysics 74: 480-482. 1958.

73. Paech, K. and M. V. Tracey (eds.) Modern methods of plant analysis. Vol. III. Berlin, Springer-Verlag, 1955. 761 p.

74. Plouvier, Victor. Study of sequoyitol and pinitol in several gymnosperms. Comptes Rendus 245:2377-2379. 1957.

75. Randerath, Kurt. Thin layer chromatography. Academic Press, New York. 1963. 250 p.

76. Roth, Lillian et al. Structure, extractives and utilization of bark. Appleton, Wisc., Institute of Paper Chemistry, 1960. 446 p. (Bibliographic Series no. 191)

77. Roux, David G. Biogenesis of condensed tannins from leucoanthocyanins. Nature 181:1454-1456. 1958.

78. Roux, D. G. and A. E. Maihs. Selective spray reagents for the identification and estimation of flavanoid compounds associated with condensed tannins. Journal of Chromatography 4:65-74. 1960.

79. Schenk, C. A. Fremdlandische Wald-und Parkbaume. Vol. 2. Berlin, Paul Parey, 1939. 645 p.

80. Schriner, Ralph and Reynold C. Fuson. The systematic identification of organic compounds. New York, John Wiley and Sons. 1948. 458 p.

81. Shaw, George Russell. The genus Pinus. Cambridge, Mass., Riverside Press. 1914. 96 p.

82. Shimazono, H., W. J. Schubert and F. F. Nord. Investigations of lignins and lignification. XX. The biosynthesis of methyl p-methoxycinnamate from specifically labeled d-glucose by Lentinus lepideus. Journal of the American Chemical Society 80:1992. 1958.

83. Simons, Edward. Redwood bark fiber insulation -- its properties and application. Refrigeration Engineer 59:782-787. 1951.

84. Smith, James Edward. The chemical nature of the "lignin" of the bark of Douglas fir, Pseudotsuga menzesii (Mirb.) France. Ph.D. thesis. Corvallis, Oregon State University, 1954. 109 numb. leaves.

85. Stephen, John T. Phenolic resin for adhesives. U. S. Patent 2,675,336 April 13, 1954.

86. Swain, T. (ed.) Chemical plant taxonomy. New York, Academic Press, 1963. 543 p.

87. Takahashi, Mitsuo, et al. Constituents of plants of Coniferae and allied orders. XLIII. Distribution of flavanoids and stilbenoids in Coniferae leaves. Journal of the Pharmaceutical Society of Japan 80:1489-1493. 1960.

88. Tatum, E. L. and S. R. Gross. Incorporation of carbon atoms 1 and 6 of glucose into protocatechuic acid by Neurospora. Journal of Biological Chemistry 219:797-807. 1956.

89. Todd, Sir Alexander (ed.) Perspectives in organic chemistry. New York, Interscience, 1956. 527 p.

90. Wong, E. and A. O. Taylor. The chromatography of flavanoid aglycones in the solvent system benzene-acetic acid-water. Journal of Chromatography 9:449-454. 1962.

91. Zimmerman, M. H. Translocation of organic substances in trees. III. The removal of sugars from the sieve tubes in white ash (Fraxinus americana L.) Plant Physiology 33:213-217. 1958.