A cold acetone extract of red alder bark, *Alnus rubra* Bong, Betulaceae, yielded a condensed tannin fraction which has been shown to be a polymer of epicatechin linked C-C 4, 6- (4, 8-) and a diarylheptanoid xyloside given the name oregonin. Oregonin, 1, 7-bis(3, 4-dihydroxyphenyl)heptane-3-one-5-xylopyranoside, represents a new type of glycoside compound. The condensed tannin was found not to contribute to the staining phenomenon of red alder. Oregonin, however, is implicated in the staining phenomenon because of its ability to form orange-red colors. This staining phenomenon has long been a problem in the production of lumber and to some extent in the bleaching of pulpwood.

Nine new compounds are described. In addition to the two natural products above, spectral data (n.m.r., i.r., u.v., m.s.) and
physical constants are given for seven derivatives and related synthetic products. These are: tetra-0-methylloregonin; tetra-0-methylloregonin triacetate; 1,7-bis(3,4-dimethoxyphenyl)-trans-3-heptene-5-one; 1,7-bis(3,4-dimethoxyphenyl)heptane-3,5-diol; 1,7-bis(3,4-dimethoxyphenyl)heptane-3,5-dione; 1,7-bis(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione; octa-0-methyldiepicatechin thioglycolate.

Field desorption mass spectrometry (FD-MS) of tetra-0-methylloregonin is described. In addition it was found that FD-MS could be used for controlled pyrolysis of compounds directly on the emitter surface to obtain fragment peaks which are of value for structural elucidations. It was also found that phenolic glucosides initially undergo cleavage at the acetal linkage to give peaks for the aglycone and the glucosyl moiety in the pyrolysis spectrum. Pyrolysis of monomeric flavanoids on the emitter surface produces characteristic peaks in the mass spectrum for A and B ring fragments, thus allowing assignment of hydroxylation patterns to the A and B rings of the parent compound.
Polyphenols of Red Alder: Chemistry of The Staining Phenomenon

by

Joseph John Karchesy

A THESIS

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Typed by Ilene Anderton for Joseph John Karchesy
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**SUMMARY AND CONCLUSIONS**

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POLYPHENOLS OF RED ALDER: CHEMISTRY OF THE STAINING PHENOMENON

INTRODUCTION

The Tree

*Alnus rubra* Bong., Betulaceae, previously *A. oregona* Nutt., is a hardwood species indigenous to the Pacific Coastal area of North America. Commonly known as red alder but also referred to as Oregon alder, its habitat is stream banks and shore flats from Southern Alaska to California. The tree is not one that forms permanent forests but does occur in extensive belts of pure growth as well as in mixed stands with other species. It is one of the first to invade logged and burned over land where it grows rapidly until overtaken by other species. The rapid growth of red alder is denoted by its attainment of maturity in 50 to 60 years. In the Puget Sound country of Washington State red alder reaches its largest size, commonly 80 to 130 feet in height and diameters to 3 feet (37, 26).

The Lewis and Clark expedition journal of 1805 carries the first published reference to this tree. They noted the occurrence of large alders along the Columbia River. However, long before this time the coastal Indians made use of the wood for dishes, masks, and utensils. The wood and bark were also used for fuel in the
smoking of game and fish (37). Today alder is still the preferred wood for the smoking of fish.

Presently red alder is the leading hardwood species of the Northwest with an estimated 2.5 billion board feet of sawtimber in reserve in Washington and Oregon. Oregon is the center of red alder lumber production. The wood is mainly used for furniture manufacturing, both in covered and exposed parts. It is often stained and finished to simulate many of the more expensive woods. Core stock for plywood, novelties, millwork, charcoal, and pulpwood include other uses for the wood (37, 65).

The Stain

The red alder tree derives its common name from the behavior of its pale whitish sapwood which, when cut, becomes pinkish or light reddish-brown in color. The bark similarly ages quickly from the same pale whitish color to orange or reddish-brown. This staining phenomenon has long been a problem in the production of lumber and to some extent in the bleaching of pulpwood. In freshly sawn lumber the staining often appears initially as red or orange streaks. Over a period of time these turn dark brown with a resultant depletion of product value (42).
HISTORICAL REVIEW

Kurth and Becker (52) initiated studies in the early nineteen fifties to determine the chemical nature of the coloring matters in red alder. They found the coloring matters to be in much higher concentration in the bark than in the wood and subsequently used bark as a source of their extractives. Although they did not identify any specific phenolic compounds, they did find that the major component of the ethyl acetate extract was a phenolic xyloside. They further ascertained that the xyloside had pH indicator properties, aqueous solutions being yellow below pH 4.5 and becoming red at about pH 4.8.

This was found to correlate with the respective pH's of the surface of a red alder board with yellow and red colorations. These observations strongly implicated the unidentified xyloside as the cause of the staining in red alder.

Essentially no additional work has been reported on the extractives from red alder although protocatechuic acid (I) and caffeic acid (II) have been identified by paper chromatography from the methanol extract of the leaves (44).
Other species of alder have had more thorough investigations of their phenolic compounds. European black alder, which similarly shows a staining phenomenon, yielded an unidentified phenolic glucoside (22). The lignan xyloside lyoniside (III) was isolated from grey alder (A. glutinosa) wood (28) and the flavones, quercetin and myricetin as well as several of their glycosides were isolated from the leaves of two Alnus species (44).

Recently seven new compounds of the rare C$_9$-C$_1$-C$_9$ family have been found in Alnus species. Yashabushiketol (IV), dihydro-yashabushiketol (V) from A. firm wood (2, 3), the corresponding saturated diol (VI) from A. fruticosa and A. manshurica leaves (95) and the ketone (VII) from A. pendula (91) are acyclic diarylheptanoid members of this family. Meta, meta-bridged biphenyl members
are represented by the ketone (VIII) and its derivatives (IX) and (X), which have been found in the wood of *A. japonica* (63).

\[ \begin{align*}
&\text{R} = H, \quad 4, 5\text{-epoxy} \\
&\text{R} = \text{OH}, \quad \Delta^{4, 5} \text{saturated}
\end{align*} \]

It is not immediately obvious what involvement any of the above compounds have in red or orange staining phenomena. However, the development of red colors from colorless compounds present in
plant tissues by treatment with hot mineral acid has long been known (38). For example, it was in a study of the anthocyanidin pigments of young grape vine leaves that Rosenheim (73) isolated a colorless sub-
stance which yielded the anthocyanidin (XI) (cyanidin) with such treatment. Rosenheim gave the name "leucocyanidin" to the pre-
cursor of the anthocyanidin (XI) (cyanidin) and suggested that the precursor was a pseudobase (XII) of cyanidin.

\[ \text{XI} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]

(Subsequently, extensive surveys of plant species by Robinson and Robinson (72) and Bate-Smith and Lerner (6) have pointed out the widespread natural distribution of these compounds. Cyanidin (XI) and delphinidin (XIII) were most commonly produced by the hot acid treatment.

\[ \text{XIII} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
\[ \text{OH} \]
It was also found that the leucocyanidins were confined mainly to plants with a woody habitat of growth. Indeed, *Alnus* was represented by *A. incana* and *A. cordata* which both produce cyanidin (XI) on hydrolysis of leaf tissue (8). Some of the more common phenolic compounds, such as quercetin and caffeic acid, were also found (7).

Rosenheim's structure (XII) for leucocyanidin is not correct. As a result of subsequent work over the years by many investigators, it is now accepted that two groups of substances are principally responsible for the red colors produced on mineral acid hydrolysis (92). These precursors of the red colors are flavan-3,4-diols such as (XIV) and flavan-3-ol dimers and higher oligomers such as (XV). It was proposed (97) that flavan-3,4-diols be called leucocyanidins while the latter group be named proanthocyanidins. This nomenclature is now followed in the literature. The term proanthocyanidin is not structurally explicit but refers only to the fact that these compounds yield anthocyanidins upon strong acid treatment.
Condensed tannins, which are now generally recognized as flavanoid polymers, are included in the proanthocyanidin classification (31). However, biflavonoids, such as those found in wattle bark extract, do not have significant tanning properties as do the triflavonoids and higher oligomers (80). For this reason, some lower oligomers including the monomeric flavan-3-ols such as catechin (XVI), and flavan-3,4-diols such as leucofisetinidin (XVII) are not true tannins but are invariably associated with them. Catechins and 5-deoxyflavan-3,4-diols such as (XVII) are regarded as the immediate biosynthetic precursors of the condensed tannins (80).

\[
\begin{align*}
\text{HO} & \quad \text{OH} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

(XVI)

\[
\begin{align*}
\text{HO} & \quad \text{OH} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

(XVII)

The correlation between condensed tannins and anthocyanidin production was not obvious to early workers because most of the higher oligomers condense into "phlobaphenes" or "tannin reds" without formation of anthocyanidins when treated with aqueous hydrochloric acid. It was only through the introduction by Pigman and coworkers of improved reaction conditions (high dispersion in alcoholic hydrochloric acid solution under pressure which
facilitates generation of hydrolysis products) were tannins found to yield anthocyanidins (68). The anthocyanidin producing reaction sequence is most probably an acid catalyzed elimination followed by an oxidation step as shown (96).

\[ \text{HO} \]

(XV)

\[ \rightarrow \]

(XI)

The products are cyanidin (XI) and catechin (XVI) together with "phlobaphenes" or "tannin reds" which result from a strongly competing phenol oxidative self-condensation. This latter reaction is still (with improved conditions) troublesome with increasing degrees of polymerization.

Condensed tannins represent only one half of the tannin picture. Generally, vegetable tannins are structurally divided into two groups. The above mentioned condensed tannins and a second group referred to as hydrolyzable tannins (38). Historically, the main distinction was made on the basis of the two groups actions toward hydrolysis.
As mentioned above, the condensed tannins do not readily break down with mild conditions of hydrolysis. However, the hydrolyzable tannins, which have a polyester structure, are readily hydrolyzed into a sugar or related polyhydric alcohol and a phenol carboxylic acid. Depending upon the nature of the latter, a further subdivision is made in the literature. Gallotannins are those that yield gallic acid (XVIII), and ellagitannins are those that yield ellagic acid which arises from lactonization of the liberated hexahydroxydiphenic acid (XIX). A limited number of structural modifications of the above are known.

\[
\begin{align*}
\text{(XVIII)} & \\
\text{(XIX)} & 
\end{align*}
\]

At first, a discussion of tannins is not obviously relevant to the staining of red alder. The reaction conditions necessary for the production of anthocyanidins by hot mineral acid treatment are far removed from any conditions found in red alder tissue. However, certain condensed tannins are known to cause serious reddening effects in leather manufacture when exposed to light and heat and it has been suggested that this may be of significance to the forest products industry. Roux and Drewes in 1968 (79) discussed this
reddening of flavan-3, 4-diol based tannins, particularly with regard to wattle. They showed by use of model flavan-3, 4-diol compounds that this phenomenon is associated with the 7- and 4-hydroxyl groups. Model compounds such as (XX) can lose water (induced by heat or light) to give the flav-3-ene (XXI) followed by facile oxidation to the anthocyanidin which probably exists in the 7-keto form as the anhydrobase (XXII). In short, the effect of light or pyrolysis is considered to be very similar to the well known mineral acid conversion of these compounds to anthocyanidins.

Because of the benzylic nature of the 4 position, when both 5- and 7- hydroxylation is present, there is a noticeably enhanced reactivity. It has been noted that certain tannins based on the 5, 7-dihydroxyl pattern (phloroglucinol A-ring) such as mangrove, myrtan and mallet derived tannins usually exist in vivo as pink components which redden with great rapidity when exposed to sunlight or heat (79).

Several natural anhydrobases (94), such as dracorubin (71) (XXIII), are known in nature and all possess red or orange colors
due to the same basic anhydrobenzopyranol chromophore, such as (XXII), as that generated in condensed tannins.

A condensed tannin from Rhodesian teak wood is proposed to have structure (XXIV). The property of the timber turning red when exposed to light was associated with the tannin due to its 7- and 4-hydroxylation (59). The dimeric proanthocyanidin (XV) from a *Eucalyptus* species, while possessing 5- and 7- but not 4-hydroxylation, is claimed to be one factor responsible for the red color in that wood (61, 62).

Red alder bark was shown to have a tannin content of 9.8% by the hide powder method (66). The nature of red alder tannins was unknown, however. Although hydrolyzable tannins do cause some coloration problems (43), especially in pulping reactions, there is no evidence to make them suspect for the problem in red
alders. Condensed tannins on the other hand would appear to be a possibility. Thus, the nature of red alder proanthocyanidins needed to be known in the present work.

In summary, past work on red alder gave some evidence that the staining was associated with an unidentified phenolic xyloside. In addition, the association of reddening in leather manufacture by certain condensed tannins combined with work on model leucoanthocyanidins indicated that proanthocyanidins may also be responsible for the staining in red alder. Substantiating this possibility, proanthocyanidins have been associated with reddening effects in two other species of wood, a Eucalyptus and Rhodesian teak.
RESULTS AND DISCUSSION

The purpose of this work was to isolate and identify the possible precursors to the red alder staining phenomenon (informally known in this laboratory as Oregon Orange). Condensed tannins and a phenolic xyloside were considered the likely precursors to the stain. Neither the condensed tannins nor the phenolic xyloside, which Kurth and Becker (52) had attributed to be the chief cause of the stain, have been isolated or identified previously.

Structural elucidation of the above compounds would indicate their possible involvement in the staining reaction and would provide a basis for subsequent work on elucidation of the exact mechanism of the staining reaction. Through this approach, it would become known whether or not the stain could be controlled with present science and also the exact measures necessary to do so.

Preliminary Work

Extractions. - Kurth and Becker (52) had found that the xyloside was extracted in hot ethyl acetate. Condensed tannins were usually extracted in hot water by early workers. However, in view of the known reactivity of some of the related simpler phenolics, milder extraction conditions are now practiced. Generally, acetone, methanol or ethyl acetate extracts are prepared by cold solvent
percolation of crushed plant material (38). In the present work red alder bark which had been air-dried was extracted sequentially by percolation with cold n-hexane, diethyl ether and acetone. The n-hexane and diethyl ether extractions were used to remove less polar material.

**Hydrolytic Reactions of the Acetone Extract.** - Preliminary hydrolytic reactions on the acetone extract isolated in the present work indicated that both a xyloside and a proanthocyanidin were present. Hydrolysis of the extract with 2% sulfuric acid released the sugars xylose, glucose and rhamnose. These were detected by descending paper chromatography in the usual manner (45). Xylose is the dominant sugar with lesser amounts of glucose and only trace amounts of rhamnose as judged from visual inspection of spot sizes and intensities on the paper chromatograms.

Considerable amounts of a red-orange tar were also generated. No chromatographic system was found to resolve the tar which appeared to make up most of the aglycone portion of the hydrolyzate. Thin layer chromatographic (t. l. c.) analysis did resolve three minor components. They were caffeic acid (II) and dihydroquercitin (XXV) which are nearly ubiquitous aglycones (7) and were judged to be of no consequence to this study. The third spot on the t. l. c. remains unidentified. T. l. c. analyses of the hydrolyzate also showed the absence of protocatechuic and gallic acids. This
observation is of interest, since protocatechuic acid is reported in
the hydrolyzate of red alder leaves (44). Furthermore, Bate-
Smith (8) has noted that Betulaceae is a family characterized by the
presence of gallic acid throughout; usually in combination with
glucose, e.g. tannic acid. Bate-Smith's work is apparently also
based on leaf tissue. One would suspect that phenolic prototypes
may differ with tissue. Roux and his South African school have
long noted a differentiation between bark and wood flavanoids in
Acacia spp. (23).

Treatment of the acetone extract isolated in the present work
with propane-2-ol-3N-hydrochloric acid under pressure [for
generating anthocyanidins (68)] gave a wine red solution character-
istic of proanthocyanidins. Cyanidin (XI) was identified as its
chloride by paper chromatographic and spectral analyses (36).

Chromatographic Analyses of the Acetone Extract. - After
it had been established that a xyloside and a proanthocyanidin were
present, chromatographic analyses of the acetone extract were
undertaken in order to "locate" and devise a method of isolation for
them. Traditionally, two dimensional paper chromatography has been employed in the preliminary qualitative analyses of condensed tannins and associated compounds (38). Numerous solvent systems are employed, most of which are based on solvent pairs which effect separation in one direction by partitioning effects and in the other by adsorption effects. Water saturated 2-butanol in the first direction followed by two percent acetic acid in the second direction is usually the most diagnostic for polar compounds (81). Detection of phenolic compounds on chromatograms is commonly achieved by various spray reagents or with ultraviolet light. The use of specific spray reagents which identify certain functional groups by means of color reactions often provides considerable structural information (78).

The acetone extract of red alder bark was best resolved with 2-butanol and two percent acetic acid (Figure 1). The extract, however, was unusual in that only two spots were detected by the commonly employed spray reagents. In general, similar plant extracts show many spots (27, 40, 41).

Single applications of as much as $1.5 \times 10^{-3}$ g of sample were applied to individual chromatograms with no additional spots being detected by the spray reagents. Some spray reagents used are sensitive to as little as $0.5 \times 10^{-6}$ g of material (53). Roux and Maihs utilized standard tannin applications of $10^{-3}$ g for their
Figure 1. Two-dimensional paper chromatogram of the acetone extract of red alder bark.
work (78) and much less ($10^{-6}$ g) was used for standard compounds. In common with this study, ammoniacal silver nitrate, vanillin-toluene-$p$-sulfonic acid and Roux's toluene-$p$-sulfonic acid reagents were used with adequate sensitivity.

It was considered improbable that only two substances were present in the acetone extract and that spot number 2 (Figure 1) represented a complex mixture held together by hydrogen bonding. It has been realized for some time that polyphenols are capable of a great deal of mutual solubilization, resulting in solid solutions which tend to behave as if they were single substances (29).

In support of this concept concerning spot number 2 (Figure 1) the following is noted. No neutral solvent system was found to resolve the streaking of the acetone extract which occurred on silica t.l.c. although about one hundred solvent systems, which were gleaned from sources such as Camag's thin-layer chromatography cumulative bibliographies (19), were screened. Only acid containing solvent mixtures, which helped break the hydrogen bonding, effected any separations. Only the system chloroform/methanol/acetic acid/water (85:15:10:4 v/v) and one of its modifications gave acceptable resolution (Figure 2). Ten spots were separated. $R_f$ values and selected color reactions are given in Tables 1 and 2 in the experimental section of this work.

Spot number 8 (Figure 2) is the major component of the extract
Figure 2. Thin-layer chromatogram of the acetone extract of red alder bark. Absorbant: silica gel G. Solvent: chloroform/methanol/acetic acid/water 85:15:10:4 v/v.
as judged by visual inspection of the spot intensities and sizes. It is very easily oxidized as evidenced by its response to dilute aqueous base and ammoniacal silver nitrate. This spot also gave a positive Gibbs reaction (38) (purple) indicating a free-CH-para to a phenolic hydroxyl. Spot number 8 also gave various blue to purple colorations with acids. In particular an unusual result was obtained with Roux's toluene-p-sulfonic acid reagent (76). The spot progressively developed the colors yellow → maroon → violet. The unusual aspects of the color reactions suggested structural novelty that was as yet undefined. Because of the relative concentration and the novelty of the color reactions which were suggestive of the indicator properties of the unknown xyloside (52), it was concluded that spot number 8 was probably the unknown xyloside and therefore was to be isolated.

Spot number 1 was more clearly indicated to be the condensed tannin fraction because of its pink coloration with Roux's toluene-p-sulfonic acid reagent (76). Spots number 2 and 3 are probably lower oligomers since they give similar reactions but are present in small amounts only. In agreement with preliminary findings that cyanidin is produced on acid treatment, the condensed tannin spray reagents (78) indicated a phloroglucinol A ring (red with vanillin-toluene-p-sulfonic acid reagent) and a catechol hydroxylation
pattern for the B ring of the polyflavonoid (black with ammoniacal silver nitrate reagent.)

Solvent partitioning of the acetone extract between ethyl acetate and water with monitoring by chromatography (t. l. c. and paper) revealed that enriched fractions of the materials which comprised spot number 8 could be obtained in ethyl acetate fractions whereas the materials which comprised spot number 1 remained in the aqueous phase.

Attempts to isolate spot number 8 by preparative t. l. c. failed. Once the spot was resolved it could not be removed from the silica with the usual organic solvents such as chloroform or acetone. The use of more polar solvents such as methanol plus acetic acid also removed significant amounts of colloidal silica and calcium sulfate binder.

The material of spot number 8 apparently was quite susceptible to oxidation since colorless separations rapidly yellowed. Attempts to methylate the material were unsuccessful because of detonation of the diazomethane by silica and because of solubility problems. It was concluded that acquisition of an enriched fraction by repeated solvent partitioning would be best. Methylation of that fraction would then give a methyl ether that would not only be blocked against oxidation of the phenolic groups, but could then be chromatographed and purified more easily. This practice is not uncommon with
phenolic compounds which are difficult to isolate in the free phenolic form for various reasons. A recent example is the isolation of crombenin as its tetramethyl ether (17).

Red Alder Condensed Tannin

**Isolation.** - Isolation of an acetone-soluble fraction of condensed tannin was achieved by solvent partitioning between ethyl acetate and water to obtain a tannin enriched aqueous phase. Dry pack column chromatography in nylon using cellulose and water separated the tannin from a large amount of material of higher $R_f$ believed to be glycosides. Additional purification was achieved by dissolution in minimal amounts of methanol followed by filtration to remove any carbohydrates. This was then followed by precipitation into diethyl ether and filtration to remove nonpolar material as well as monomeric polyphenols (40).

For purposes of this investigation, material obtained in this way is defined as the acetone-soluble condensed tannin fraction of red alder. The final product amounted to 0.2 percent of the dry weight of the original bark.

**Hydroxylation Pattern.** - Alkali fusion of the tannin under dry conditions (23, 27) afforded protocatechuic acid (I) and phloroglucinol (XXVI) as the two major reaction products. This traditional degradation method, which cleaves flavanoid nuclei to give A rings as the
phenol and B rings as the corresponding acid as shown below, indicated the A ring of the flavanoid monomeric units to be 5, 7-hydroxylated while the B ring possesses 3', 4'-hydroxylation.

\[
\text{OH} \quad \text{OH} \quad \text{OH} \\
\text{HO} \quad \text{1} \quad \text{I} \\
\text{KOH} \quad \Delta \quad \text{H}^+ \\
\text{CO}_2\text{H} \\
\text{OH} \\
\text{OH} \\
\text{HO} \\
\text{HO} \\
\text{HO} \\
\text{HO} \\
\text{HO} \\
\text{HO} \\
\text{HO} \\
\text{HO}
\]

(XVI) (I) (XXVI)

In agreement with A and B ring hydroxylation and further establishment of hydroxylation at the C ring 3 position, the tannin afforded cyanidin (XI) as its chloride on treatment with propane-2-ol-3N-hydrochloric acid under pressure (68). These findings indicated that the basic monomeric flavanoid unit of the condensed tannin possessed 3, 5, 7, 3', 4'-hydroxylation, in other words, that of a catechin-type nucleus (XVI). Consistent with the catechin-type chromophore, the tannin exhibited $\lambda_{\text{max}}$ at 280 nm in an ultraviolet spectrum which is very similar to the spectrum of the polymeric catechin (XXXI) from western hemlock (40, 41).

**Interflavanoid Linkage.** - Thioglycolysis of the methylated red alder condensed tannin by Sears and Casebier's procedure (86) afforded (after permethylation with diazomethane) the thioglycolate of tetra-$Q$-methylepicatechin (XXVII) which is in every way identical to an authentic sample synthesized from (-) epicatechin (XXVIII) by the
method of Betts et al. (15) (Figure 3). T.l.c. analysis of the permethylated reaction mixture revealed the absence of the corresponding thioglycolate of tetra-$\text{-}O$-methylcatechin (XXIX). An authentic sample of (XXIX) for t.l.c. analyses was kindly supplied by Mrs. Patricia Loveland of this laboratory.

The condensed tannin is thus a polymer of epicatechin linked through the 4 position as evident from the single thioglycolysis reaction product (XXVII).

Thioglycolysis was first employed by Betts and coworkers (14) to degrade the condensed tannin from common heather. The procedure represented a milestone in condensed tannin chemistry since for the first time not only could a position of interflavanoid linkage
Figure 3. Upper, n.m.r. spectrum (CDCl₃) of synthetic (+)-methyl 2, 3-cis-3, 4-trans-(3-hydroxy-3',4',5, 7-tetramethoxy-flavan-4-ylthio)acetate; lower n.m.r. spectrum (CDCl₃) of the thioglycolysis product from red alder condensed tannin.
be established, but the stereochemistry at C-2 and C-3 of the C ring was also preserved in a simple derivative. The derivative obtained from common heather (14) was (XXVII), the same as that from red alder. The structure (XXX) was proposed for the tannin from common heather. Linkage at the 4 position was certain from the derivative (XXVII), but ether linkage through the 5 and 7 oxygens was concluded since thioglycolic acid is known to cleave benzylic ethers (55).

Numerous, natural, dimeric proanthocyanidins such as (XV) have been isolated (80). A common structural feature is carbon-carbon interflavanoid linkage. This fact strongly supported the suggestions (80) that most condensed tannins are composed of flavan-3-ol nuclei in which the acid-labile carbon-carbon bond of the 4 position is linked to the 6 or 8 position of the other. Sears and Casebier
showed that thioglycolic acid was capable of cleaving the carbon-carbon bonds in model proanthocyanidins (85) and subsequently degraded the condensed tannin from western hemlock (86) to obtain the thioglycolates (XXVII) and (XXIX) in equal amounts. From these results the structure (XXXI) was proposed for the condensed tannin from western hemlock (Tsuga heterophylla).

(XXXI)

In support of a carbon-carbon linkage for the condensed tannin from red alder, hydrolysis of the methylated tannin in propane-2-ol-3N-hydrochloric acid under pressure (68) afforded tetra-O-methylcyanidin chloride (XXXII) (47, 39). No other anthocyanidin was detected.

(XXXII)
Indications that the C-3 hydroxyl is not involved in the linkage is shown by the i.r. spectrum of the methylated condensed tannin which shows a strong absorption at 3590 cm\(^{-1}\) \((\nu, \text{OH})\). Acetylation of the methylated tannin gives a product which exhibits no absorptions for hydroxyls. Figure 4 shows the i.r. spectra of tetra-0-methylepicatechin, of methylated condensed tannin from red alder and of methylated, acetylated condensed tannin from red alder. The i.r. spectrum of the methylated condensed tannin from red alder is also very similar to that published for western hemlock methylated tannin (84).

**Structure.** - On the basis of the above evidence, (Scheme 1) the condensed tannin from red alder is proposed to be a C-C 4,6-(or 4,8-) linked polymer of epicatechin (XXXIII).

![Diagram](XXXIII)

Some authors, most notably Jurd and co-workers (46), have maintained that condensed tannins must involve other structural variations in addition to the formation of repeating units simply
Figure 4. **Upper**, i. r. spectrum (CHCl₃) of tetra-0-methylepicatechin; **middle**, i. r. spectrum (CHCl₃) of methylated condensed tannin; **lower**, i. r. spectrum (CHCl₃) of methylated, acetylated condensed tannin.
Scheme 1. Main reactions of red alder condensed tannin.
linked from C-4 of one flavan unit to C-8 or C-6 of a second nucleus. This belief is based on low anthocyanidin yields compared to lower oligomers such as dimers, and perhaps the feeling that there must be more to something as complex as condensed tannin.

Other workers have noted that while the polymers are usually thought to be homogeneous in the type of flavanoid unit, they are heterogeneous in molecular weight (oligomer distribution) (14) and possess different molecular shapes (18, 69).

Red alder bark which had been exhaustively extracted as described in the experimental section yielded cyanidin (XI) as its chloride on treatment with propane-2-ol-3N-hydrochloric acid. This indicates that, in common with western hemlock bark, there is a fraction of similar structure to the bark tannin that is not removed by solvent extraction, possibly because of size or shape (86).

The structure (XXXIII) for red alder condensed tannin may only represent a backbone framework from which there is additional crosslinking. Its oligomeric distribution is not understood as yet, and certainly not its shape. The n.m.r. spectrum of its methyl ether, for example, sheds no light; as predicted by Roux (80) oligomers higher than dimers exhibit very complex spectra due to multiplicity of all signals. This effect is due to rotational isomerism about the interflavanoid linkage. The larger the oligomer, the
more rotational isomerism, and thus the more complicated and broadened the signals become.

Involvement in Stain. - Roux and Drews have discussed the reddening of flavan-3,4-diol based tannins, particularly with regard to those derived from wattle, and have shown that this property is associated with 7- and 4-hydroxylation in model flavan-3,4-diols (79). Their work indicated that the basic chromophore of the stain would be an anhydrobenzopyranol of type (XXXIV).

\[(XXXIV)\]

If red alder condensed tannin were forming the stain via the mechanism ascribed by Roux and Drewes, the chromophore of the stain in red alder would be the quinonoidal anhydrobase of cyanidin (XXXV). Presumably stabilization of (XXXV) could be afforded by substitution in the benzlylic position (90) and perhaps by reincorporation in a polymeric network. In this aspect (XXXV) closely resembles the proposed structures of the polyflavanoid derived "phlobaphenes", such as the "polymer of cyanidin" from western hemlock bark and red fir (41, 9).
Addition of acid would cause formation of the flavylium salt (anthocyanidin) (XXXVI) which is a polymer of cyanidin. The flavylium salt quinonodiol anhydrobase equilibrium is a long known pH dependent interaction (48). The extent to which flavylium salts (anthocyanidins) exist in their anhydrobase forms in plant material has yet to be defined because they are usually extracted as their salts (84) in acid media. The cyanidin-based phlobaphenes are described as reddish-purple pigments in their native form (41, 9). The cyanidin-like polymer isolated from red fir gave \( \lambda_{\text{max}} \) 575 nm in ethanol, shifting to 550 nm on addition of hydrochloric acid. This spectral observation is consistent with the relationship between chromophores (XXXV) and (XXXVI). Monomeric cyanidin chloride (XI) exhibits \( \lambda_{\text{max}} \) 545 nm in ethanol.

Examination of the stain in red alder quickly ruled out participation of chromophores such as (XXXV) and (XXXVI). Visually the orange-red color of the stain does not match the reddish-purple color expected for the anhydrobase of cyanidin (XXXV). Addition of
methanolic hydrochloric acid to the orange-colored stain in the cambial area caused an immediate decolorization of the orange color to a lighter but bright yellow color. Similarly, the darker red streaks on lumber surfaces changed to the same yellow color, indicating different shades of the stain are due to concentration effects and not different chromophores. Examination of the stain revealed $\lambda_{\text{max}}$ 433 nm in methanol, shifting to $\lambda_{\text{max}}$ 399 nm in methanolic hydrochloric acid ($\lambda_{\text{max}}$ 425 nm in ethanolic hydrochloric acid). Generation of the orange color in vitro from fresh bark by homogenizing with a phosphate buffer (pH 6) showed it to have $\lambda_{\text{max}}$ 478 nm. Clearly this behavior is inconsistent with the anhydrobenzopyranol chromophore (XXXV) (48) and in turn rules out the red alder condensed tannins as precursors to the stain.

The Thioglycolate of Octa-0-methyldiepicatechin

In the course of synthesis of an authentic sample of tetra-0-methylepicatechin thioglycolate (XXVII) by the method of Betts, Brown and Shaw (15), the previously unreported crystalline thioglycolate of octa-0-methyldiepicatechin (XXXVII) was isolated in addition to the monomer (XXVII). Presumably the dimer (XXXVII) arises via condensation of the intermediate tetra-0-methylflavan-3, 4-diol (XXXVIII) and the tetra-0-methylepicatechin thioglycolate (XXVII). Geissman and Yoshimura (32, 46) have demonstrated that
tetra-0-methyleucocyanidin condenses with phloroglucinol and with catechin under mild acidic conditions (0.1 N hydrochloric acid) to give proanthocyanidin products.

The structure (XXXVII) is proposed on the basis of u.v., mass spectral and combustion analyses. The 4,8-link has been drawn for the sake of convenience only and a 4,6-linkage must be considered an alternative possibility at this time. Combustion analysis and mass spectrometry indicate a molecular formula of $C_{41}H_{46}O_{14}S$, consistent with the proposed structure. The compound (XXXVII) exhibited $\lambda_{\text{max}}$ (ethanol) 276 nm demonstrating the same basic chromophore as the monomer (XXVII). The interflavanoid linkage is 4, 6 or 4, 8 based on the expected mechanism of the condensation reaction (31, 33, 46).

In support of structure (XXXVII) the mass spectrum shows an intense fragment peak at $m/e$ 688 for the fragment (XXXIX) remaining
after loss of the thioglycolate group. This fragmentation also occurs with the monomeric thioglycolates (85, 92). Consistent with known fragmentations of biflavanoids linked 4, 6 or 4, 8 (67, 23) intense peaks at m/e 509, 180, and 151 are due to a retro-Diels-Alder reaction. In particular the large peak at m/e 509 represents rings A and D linked via a single carbon atom (C-4 of the original biflavanoid). The total fragmentation of (XXXVII) can be seen as a composite of the fragmentation known for monomeric thioglycolates (92) and dimeric biflavanoids (67, 23).
Dimeric thiol derivatives similar to (XXXVII) have been reportedly detected by paper chromatography of the intermediate stage of thiolysis of trimeric proanthocyanidins (92). Perhaps compounds such as (XXXVII) may become useful as model compounds for such degradation reactions.

Oregonin

Continued investigation of the acetone extract of red alder bark revealed that the major component is a novel diarylheptanoid xyloside (XL). The name oregonin is given to this first known diarylheptanoid glycoside. The compound is assigned a 1,7-bis(3,4-dihydroxyphenyl) heptane-3-one-5-β-xylopyranoside structure (XL) on the basis of n.m.r., i.r., u.v. and mass spectrometry of its derivatives (XLI), (XLII), (XLIII), and (XLIV); and the total synthesis of (XLIII) and (XLIV).

\[
\begin{align*}
R_1 & \quad O \\
\text{CH}_2 & \quad \text{CH}_2 \quad \text{C} \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2 \\
R_1 & \quad \text{OR}_1 \\
\end{align*}
\]

(XL), \( R_1 = H, \ R_2 = \beta \text{ xylose} \)

(XLI), \( R_1 = \text{Me}, \ R_2 = \beta \text{ xylose} \)

(XLII), \( R_1 = \text{Me}, \ R_2 = \beta \text{xylose triacetate} \)

(XLIII), \( R_1 = \text{Me}, \ R_2 = H \)
Isolation. - Repeated distribution of the acetone extract between ethyl acetate and water resulted in fractions rich in oregonin in the ethyl acetate layer. Methylation of an enriched fraction with ethereal diazomethane gave a crystalline tetra-\(\text{O}\)-methyl ether (XLI) following separation by preparative t. l. c.

Structure. - Tetra-\(\text{O}\)-methylloregonin is a saturated ketone, as indicated by the i. r. absorption at 1713 cm\(^{-1}\) (Figure 5). Field desorption mass spectrometry (FD-MS) shows the parent ion to be \(\text{M}^+ 534\). Electron impact mass spectrometry failed to produce a peak above an intense \(\text{m/e } 384\) which corresponds to the dehydrated aglycone (XLIV). Presumably dehydration of the aglycone (XLIII) to form the \(\alpha, \beta\)-unsaturated ketone (XLIV) occurs after cleavage of the acetal linkage in the parent compound (XLI). In support of this concept, FD-MS pyrolysis (50) produces peaks at \(\text{m/e } 402\) and \(\text{m/e } 384\) corresponding to the molecular weights of (XLIII) and XLIV).

Comparative mass spectra are seen in Figure 6. Consistent with a 3,4-dimethoxyphenylpropane chromophore (30) for tetra-\(\text{O}\)-methylloregonin, a u. v. spectrum exhibited \(\lambda_{\text{max}} 229, 280\) and \(\lambda_{\text{min}} 251\) nm.
Figure 5. Upper, i.r. spectrum (CHCl₃) of tetra-α-methyloregonin; middle, i.r. spectrum (CHCl₃) of the aglycone of tetra-α-methyloregonin; lower i.r. spectrum (CHCl₃) of the dehydrated aglycone of tetra-α-methyloregonin.
Figure 6. Comparative mass spectrometry of tetra-\(\text{O-}\)methyloregonin.
Tetra-O-methyloregonin (XLI) readily affords the triacetate (XLII) from acetic anhydride and pyridine at room temperature. The n.m.r. spectrum (Figure 7) of the triacetate (XLII) clearly shows resonances for the xylopyranose ring protons which are shifted downfield on acetylation. Protons on C-2 and C-4 of the xylopyranose ring of (XLII) resonate in a multiplet (T 4.92-5.22) shifted from a multiplet centered at 6.7 in the n.m.r. spectrum of tetra-O-methyloregonin (XLI) (Figure 8). The proton on C-3 of the xylopyranose ring of (XLII) resonates as a triplet at T 4.82, shifted from ca 6.5 in the spectrum of (XLI). A doublet at T 5.46 (J = 7Hz) due to the anomeric proton indicates a β configuration (24). Two quartets, T 5.90 and 6.64 are due to the two C-5 protons. Two singlets [(T 7.97) (3H) and 7.98 (6H)] integrate for nine acetyl protons. This portion of the n.m.r. spectrum of the triacetate closely resembles the spectrum of β-D-xylopyranose tetraacetate (24) seen also in Figure 7. The relative position of the anomeric proton differs due to different substituents at C-1.

The proton signals due to the aglycone portion of the triacetate (XLII) are: a multiplet for six aromatic protons (T 3.12-3.36); a one proton multiplet at T 5.84 for -CH₂-CH₃OR₂-CH₂-; two singlets for twelve methoxyl protons (T 6.14, 6.16); an eight proton multiplet (T 7.02-7.56) due to the benzylic and α-keto -CH₂- protons; and a two proton multiplet (T 8.17) due to -CH₃OR₂-CH₂-CH₂-.
Figure 7. Upper, n.m.r. spectrum (CDCl$_3$) of tetra-$\text{O-}$methylloregonin triacetate; lower, n.m.r. spectrum (CDCl$_3$) of $\beta$-$\text{D-}$xylopyranose tetraacetate.
Figure 8. Upper, n.m.r. spectrum (acetone-d₆) of tetra-0-methyloregonin; middle, n.m.r. spectrum (acetone-d₆ plus a drop of D₂O) of tetra-0-methyloregonin; lower, n.m.r. spectrum (CDCl₃) of the aglycone of tetra-0-methyloregonin.
Hydrolysis of tetra-O-methylloregonin with 2% aqueous sulfuric acid released xylose as identified by paper chromatography in the prescribed manner (45) and field desorption mass spectrometry. The aglycone (XLIII) is produced in small amounts only while the dehydrated aglycone (XLIV) is a major product. Facile elimination of a hydroxyl group $\beta$ to the ketone group is not unexpected under hydrolysis conditions. The aglycone ketol exhibits i.r. absorptions (Figure 5) for a saturated ketone ($1710 \text{ cm}^{-1}$) and a hydroxyl group ($3540 \text{ cm}^{-1}$). The n.m.r. spectrum (Figure 8) shows signals which integrate for six aromatic protons ($\delta 3.14-3.36$, m), one proton -CHOH- ($\delta 5.94$, m), twelve methoxyl protons ($\delta 6.14$ (6H), 6.15 (6H)), a single hydroxyl proton ($\delta 6.94$, removed by $D_2O$ exchange), eight methylene protons next to carbonyl and aryl groups ($\delta 7.06$-$7.50$, m) and for the two methylene protons -CHOH-CH$_2$-CH$_2$- ($\delta 8.28$, m). The $\alpha,\beta$ unsaturated ketone (XLIV) shows i.r. absorptions (Figure 5) at $1672 \text{ cm}^{-1} (\nu C=O)$ and $1628 \text{ cm}^{-1} (\nu C=C)$. A doublet ($J = 15.8$ Hz) at $\delta 3.91$ in the n.m.r. spectrum (Figure 9) shows the double bond to be trans disubstituted. Integration of the aromatic signals ($\delta 3.01$-$3.38$) now shows seven protons, including the $\beta$-hydrogen of the conjugated ketone system. A lone singlet at $\delta 6.17$ integrates for twelve methoxyl protons and an eight proton multiplet at $\delta 7.10$-$7.58$ is due to the remaining -CH$_2-$ groups. The
Figure 9. Upper, n.m.r. spectrum (CDCl₃) of synthetic 1,7-bis (3,4-dimethoxyphenyl)-trans-3-heptene-5-one; lower, n.m.r. spectrum (CDCl₃) of the dehydrated aglycone of tetra-O-methylroregonin.
aliphatic proton resonances are very similar to those described for the cyclized C₉₋₁₋C₉ α,β-unsaturated ketone alnusone (VIII) (63).

Oregonin is totally in the free phenolic form as indicated by an n.m.r. spectrum of the enriched fraction which shows no methoxyl resonances. The mass spectrum similarly shows a fragmentation parallel to that for tetra-O-methyloregonin; that is, an intense peak which matches for (XLV) (M⁺ - xylose) with no higher peaks and a base peak for (XLVI).

\[
\begin{align*}
\text{OH} & \quad \text{CH} - \text{CH} - \text{(OH)} \\
\text{OH} & \quad \text{CH} - \text{CH} - \text{(OH)} \\
\text{(XLV)} & \quad \text{(XLVI)}
\end{align*}
\]

**Synthesis of Aglycones.** - The structures of the aglycone (XLIII) and the unsaturated aglycone (XLIV) were confirmed by total synthesis from veratryl aldehyde and acetyl acetone (Scheme 2).

Pabon's method for condensation of 2 moles of an aldehyde with one mole of acetyl acetone to yield curcumin type compounds (64) was utilized to produce (XLVII). Catalytic hydrogenation (Pd/C) of (XLVII) gave the saturated diketone (XLVIII). Both (XLVIII) and (XLVII) exist almost totally in the enolic form as seen in the n.m.r. spectra (Figure 10). Treatment of (XLVIII) with sodium borohydride (1-1/2 ketone group equivalents) in methanol at room temperature
Scheme 2. Synthesis of the aglycone and dehydrated aglycone of tetra-\(\text{O}\)-methylloregonin.
Figure 10. Upper, n.m.r. spectrum (CDCl₃) of 1,7-bis(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione; lower, n.m.r. spectrum (CDCl₃) of 1,7-bis(3,4-dimethoxyphenyl)heptane-3,5-dione.
gave the ketol (XLIII) and the diols (XLIX). Two diols are formed, presumably a meso form and a d,l pair. The two diols which migrated with very close Rf values were collected together from preparative t.l.c., and the spectra and combustion analyses of the composite material satisfied structure (XLIX). Dehydration of the synthetic ketol (XLIII) with 2% aqueous sulfuric acid produced the α,β-unsaturated ketone (XLIV). Both the synthetic products (XLIII) and (XLIV) gave identical mass, n.m.r., u.v. and i.r. spectra, and Rf values to those derived from the natural product oregonin.

Structure (XL), 1,7-bis(3,4-dihydroxyphenyl)heptane-3-one-5-β-xylopyranoside, is alone consistent with the above data for oregonin. Oregonin is unique among the diarylheptanoids with regard to its hydroxylation pattern and its glycosidic function. Oregonin thus represents the first member of a new class of glycosides.

Diarylheptanoids are becoming increasingly more common. They apparently form a group biogenetically related to curcumin (L) (57), the pigment of Curcuma longa rhizome (tumeric). Most have been found in several Alnus spp. (IV) (V) (VI) and (VII) (historical section), while centrolobol (L1), centrolobin (LII), and de-O-methylcentrolobin (LIII) have been isolated from Centrolobium spp (1, 21).

It has been postulated (53, 13) that oxidative coupling of
diarylheptanoids might lead to the \textit{meta, meta}-bridged biphenyls such as (VIII). Peroxidase may catalyse such a reaction (63). Although the biosyntheses of the \textit{meta, meta}-bridged biphenyls has not been undertaken, the close structural similarity and apparent ease of such a reaction strongly suggest such an interaction. A third group of compounds, represented by the 9-phenylperininaphthenone (LIV) and the aglycone of haemocorin (LV), can also be placed in what is apparently a family of compounds, i.e. \textit{C}_9-\textit{C}_1-\textit{C}_9. The three members of this family are believed to have a similar origin: a common biosynthetic pathway involving the combination of one acetate with two shikimate-derived \textit{C}_6-\textit{C}_3 units (25). However, work on the biosynthesis of curcumin was unable to substantiate this scheme (74). The discordant observations indicate further work is needed in this area.

**Possible Fates of Oregonin.** - The proposal that oregonin is involved in the stain is suggested by the production of a red-orange tar on acid hydrolysis and the production of a transient red-orange color on addition of peroxidase and hydrogen peroxide. Apparently the peroxidase causes further reactions, perhaps oxidative polymerization.

Adequate models are not at hand to predict the exact chromophore moiety of the stain. However, a number of natural quinone methides are known (94) which possess various yellow to red
(L), $R = \text{Me}$

(LI), $R = \text{H}$

(LII), $R = \text{Me}$

(LIII), $R = \text{H}$

(LIV), $R_1 = \text{Sugar}, R_2 = \text{OH}$

(LV), $R_1 = \text{H}, R_2 = \text{OMe}$
colorations. Some representative structures are shown below (the numbers in parenthesis are literature references). These compounds and their absorptions suggest that "Oregon Orange" may be formed through involvement of a quinone methide.

\[
\begin{align*}
\text{CO}_2^+ & \quad \text{HO} \\
\text{max} & \quad 333 (35) \\
\text{HO} & \quad \text{Ph} \\
\text{max} & \quad 355 (35) \\
\text{OMe} & \quad \text{OH} \\
\text{max} & \quad 399 (34) \\
\text{MeO} & \quad \text{O} \\
\text{max} & \quad 420 (20) \\
\end{align*}
\]

Assuming that the glycosyl function is not lost, the following states of oxidation could occur.
Clearly, establishment of the structure of oregonin now provides a sound basis for further studies into the mechanism of the staining reaction.

Considering biogenetic possibilities (25) one might speculate that formation of the stain involves loss of the glycosidic function with subsequent formation of a 9-phenylperinaphthenone. The sequence could be as follows: oregonin (XL) could lose its glycosidic function and dehydrate to the \( \alpha, \beta \) unsaturated ketone (XLV). Oxidation would lead to (LVI). A Diels-Alder reaction and subsequent oxidation would in turn give (LVII).

The aglycone of haemocorin (LV) has \( \lambda_{\text{max}} \) 505 nm which would
seem to indicate a more highly conjugated chromophore than is needed. However, it is herein suggested that (LVII) may represent the chromophore responsible for the unusual color reactions of oregonin with acids.

According to a long accepted mechanism of phenolic oxidations (31), the oxidation of oregonin would be initiated by removal of a hydrogen atom to give the radical species (LVIII). This species could represent a branching point in the biosynthetic pathways for oregonin-like compounds in nature. While most of what is shown below is speculation at this time, it is predicted by this author that most, if not all, will be found to be the case by future workers.

"oregonin-like" compounds

\[
\begin{align*}
\text{"lignin-like" polymers} & \quad \xleftarrow{\text{coupling}} \quad \text{"lignin-like" polymers} \\
\xrightarrow{\text{oxidation}} \\
\text{(LVIII)} & \quad \xrightarrow{\text{coupling}} \quad \text{meta, meta-bridged biphenyl compounds} \\
\xrightarrow{1. \text{ disproportionation,} \\
2. \text{ oxidation}} \\
\text{colored compounds}
\end{align*}
\]
Utilization of Field Desorption Mass Spectrometry

The relatively new technique of field desorption mass spectrometry (FD-MS) was utilized in this thesis work. Most notably, it was an invaluable aid to the structural elucidation of oregonin.

Examination of the FD-MS literature reveals some surprising facts. In spite of its unique potential, FD-MS literature is characterized by "show and tell" publications on organic compounds, i.e. simply "running" compounds through to show they give a spectrum. Rinehart et al., were the first to utilize FD-MS in a structural elucidation (70).

In June 1973 Professor Barofsky (Oregon Graduate Center), Professor Laver and this author entered on a collaborative effort to apply FD-MS to some of the problems encountered in wood chemistry.

Because FD-MS is not yet in widespread use, a short description of its operating principles and previous uses are felt necessary. Field ionization (FI) mass spectrometry has been established for a number of years as an alternative and complimentary technique for the study of molecules which exhibit only extremely weak or no parent ion peaks in electron impact mass spectra (12). In FI, the governing process is the tunneling of an electron out of a molecule under the influence of an extremely high electric field. This mode
of ionization is referred to as "soft" since essentially no energy is imparted to the molecule and no fragmentation occurs. Thus, in contrast to EI mass spectra, FI mass spectra generally exhibit very intense molecular ion peaks and only very weak fragment peaks. This limits the use of FI mass spectra in the solution of structure problems but makes them ideally suited to the analysis of hydrocarbon mixtures, particularly mixtures containing a large number of components, without prior separation.

FI mass spectra are also useful in measuring the products of different types of reactions. This is particularly true when one of the reactants or products is present in an extremely small amount and would, then, be easily obscured by peaks due to fragments of the major components. For example, FI mass spectrometry has been used to detect free radicals formed in photochemical reactions at a relative concentration of only $10^{-3}$ (12).

A technical feature common to both FI and EI ion sources is the methods of sample introduction. In particular, when dealing with solid and liquid organic samples of low to moderate volatility, both ion sources commonly employ the very sensitive, direct insertion heating probe. The substances, placed in a suitable microcrucible or capillary, are gently heated and evaporated in the ionization zone as a molecular beam. With thermally unstable substances a major portion of the molecules are decomposed during
the evaporation prior to the ionization process. Under these circumstances, even the FI mass spectra exhibit a large relative intensity of fragment mass peaks, and the main FI feature of an intense molecular ion mass peak is lost.

With FI ion sources, it is possible to absorb the sample directly onto the field emitter and then to remove and ionize it by a process termed field desorption (FD) (12). In the presence of an extremely high electric field, the energy barrier which must be overcome for the removal of a surface atom or molecule from a conductor is drastically reduced. Field desorption may be naively regarded as the thermal activation of the surface species over this field reduced energy hump followed by its immediate field ionization. Of prime importance is the fact that during the process only a negligible amount of energy is transferred to the particle, so that molecules so ionized suffer no appreciable decomposition. Thus, it is possible to obtain molecular ion spectra of thermally unstable substances of low volatility, such as carbohydrates and phenols.

For FD mass spectrometry the sample is absorbed directly from a liquid solution onto the surface of the emitter from which it is field desorbed. Approximately $10^{-8}$ g of sample is required for an analysis. Thus, FD mass analysis of many non-volatile substances which are difficult or impossible to analyze by conventional mass spectroscopic or chromatographic means is possible.
Since the sample is introduced directly on the ionizing electrode or emitter, thermal degradation products of the sample can be studied by heating the emitter. Hence, FD mass spectrometry is not limited to molecular weight determination, as in the case with FI mass spectrometry, but it can also be used for structure elucidation.

FD mass spectrometry has been employed on compounds such as nucleosides (87), amino acids (99), peptides (98), pesticide derivatives (88), sugars and their derivatives (10, 89), and natural phenols and glycosides (50).

In the present work the simple phenols catechol and phloroglucinol; and the acids p-hydroxybenzoic, protocatechuic and gallic all gave intense molecular ion peaks with no fragmentation. The heating current necessary for desorption was found to depend on the number of free hydroxyl groups. However, the range of desorption energies is sufficiently close that it is possible to obtain mass peaks for all of the phenols when applied in a mixture (Figure 11).

Microanalysis of polyphenols by an alkali fusion method involves co-chromatography with authentic compounds to sufficiently identify the products (77, 23). In this study an FD-MS analysis of the reaction product mixture was found to be facile and to supplement the chromatographic information. The degradation product mixtures of both (+) catechin (XVI) and red alder condensed tannin
Figure 11. Field desorption mass spectrum of a mixture of phenols.
(XXXIII) gave major peaks at m/e 126 and m/e 154 corresponding to phloroglucinol and protocatechuic acid.

Although FD-MS has great potential for determining singular parent ion peaks, workers have noted the appearance of peaks due to "thermal fragments" (89). At low emitter temperatures the molecular ion peaks and sometimes FD-induced fragmentation ion peaks have been observed. However, as the emitter temperature is raised pyrolysis fragments become more abundant, sometimes hindering the desired detection of other ion species. It occurred to our group that thermal fragmentation may be controllable and thus yield valuable structural information. It seemed reasonable to think that one should be able to control pyrolysis by manipulation of the heating current applied to the emitter. In addition to structural information, mechanistic information should also be exhibited, since the controlled pyrolysis would occur directly on the emitter surface in the instrument and hence the initially produced products would be detected before they could undergo condensation or further rearrangement reactions.

It has been shown that pyrolysis of phenyl glycosides is initiated with cleavage of the acetal linkage via a heterolytic reaction (82, 83). The aglycone abstracts a proton to form the free phenol and the glycosyl group undergoes condensation reactions. Glycosides with better leaving groups are decomposed at lower
temperatures. The p-nitrophenyl glucosides in fact show exothermic decompositions. In support of this concept fragments for the aglycone and glucosyl moiety were reported in a recent FD-MS study of p-nitrophenyl and 6-bromo-2-naphthyl glucosides (54).

In addition to tetra-O-methylorregonin, the natural glycosides arbutin (LVIX) and phloridzin (LX) were analyzed in this study by FD-MS. At an emitter heating current of 8 mA the mass spectrum of arbutin showed an intense parent ion peak and essentially no fragmentation. At a heating current of 10 mA, its mass spectrum showed mass peaks at m/e 110 and m/e 163 corresponding to p-hydroxyphenol (LXI) and the glucosyl moiety (LXII).

\[
\begin{align*}
&\text{HO-}\text{O-glucosyl} \quad 8 \text{ mA} \quad \text{(LVIX)} \\
&\text{HO-}\text{OH} \quad 10 \text{ mA} \quad \text{(LXI)} \\
&\quad \text{m/e 110} \quad \text{(LXI)} \\
&\quad \text{m/e 163} \quad \text{(LXII)} \\
&\text{parent ion} \quad \text{m/e 276}
\end{align*}
\]

Similarly, the FD-MS spectrum of phloridzin appeared at a heating current of 10 mA and showed simultaneously the parent ion peak and the expected peaks for products of acetal bond cleavage,
i.e., m/e 163 and m/e 274 for the glucosyl moiety (LXII) and the aglycone phloretin (LXIII). Increasing the heating current to 14 mA gave rise to additional peaks corresponding to the molecular weights of phenol (LXIV) (m/e 94) and phloroglucinol (XXVI) (m/e 126) which represent the B and A rings of the aglycone.

Similarly, the FD-MS of (+) catechin exhibited only a simple parent ion mass spectrum at a heating current of 8 mA. Thermal fragmentation of the flavan structure into phloroglucinol (A ring) and catechol (B ring) was indicated by major mass peaks at m/e 126 and m/e 110 respectively when the heating current was increased to 12 mA. Under similar conditions thermal fragmentation
of dihydroquercetin was not observed. In this case desorption apparently occurred at a much lower temperature than pyrolysis. This was found to be the case with other less hydroxylated flavonoids.

Flash pyrolysis cannot be performed in these cases due to scanning limitations of the mass analyser. The desorption energy is increased by decreasing the electric field strength (12). Thus, lowering the electric field strength should bring pyrolysis and desorption energies closer together. Providing the ion emission is not reduced below a useful level, it may be possible by this means to observe the pyrolysis products of the above substances. This possibility is currently being investigated.

The FD-MS analysis of tetra-\text{O}-methyloregonin and the condensed tannin were discussed in their respective sections.
EXPERIMENTAL

Melting points were taken on a Koffler hot stage and are uncorrected. Combustion analyses were performed by Micro-Tech Laboratories, Skokie, Illinois. Proton nuclear magnetic resonance ($^1$H n.m.r.) spectra were recorded at 60 MHz and 100 MHz with Varian A-60 and HA-100 spectrometers. Electronic and infrared (i.r.) spectra were taken on a Beckman ACTA TM III and IR-20A spectrophotometers respectively. Electron impact mass spectra were obtained on a CEC-2l-110 double focusing mass spectrometer. Field desorption mass spectra (FD-MS) were obtained on a modified Hitachi-Perkin Elmer RMU-7 mass spectrometer.

In the FD-MS study the field anode was an 8 μm wire activated by Barofsky and Barofsky's method (4). The accelerating voltages were +1.8 kV applied to the field anode and -8.2 kV applied to the cathode. Emitter heating currents were varied between 0 and 16 mA. Approximately $10^{-8}$ g of sample was deposited in each case on the FD emitter by the microliter syringe technique (11).

Chromatography

Materials. - Chromatographic analyses were carried out on Whatman No. 1 paper. Whatman No. 3 MM paper was used for preparative isolations. Whatman CF 11 cellulose powder and Baker
No. 3405 Silica Gel were used for column chromatography. Baker No. 3407 Silica Gel 7G was used for thin-layer chromatography (t. l. c.), 0.25 mm for analytical and 1.0 mm for preparative work. The t. l. c. plates were dried overnight and activated at 110° for 30 min and stored over phosphorus pentoxide. They had an activity of III when checked by Loev and Goodman's method (56).

**Solvents.** - The solvent systems employed for paper chromatographic analyses were: (i) water-saturated 2-butanol; and (ii) 2% acetic acid for polyphenols (27); (iii) forestol (water/acetic acid/conc. hydrochloric acid - 10:30:3 v/v); and (iv) Roux's formic acid reagent (90% formic acid/3N hydrochloric acid - 1:1 v/v) for anthocyanidins (75); (v) ethyl acetate/pyridine/water - 8:2:1 v/v for sugars (45). Solvents for silica t.l.c. were (vi) chloroform/methanol/acetic acid/water - 85:15:10:4 v/v, and (vii) benzene/acetone - 9:1 v/v (58). Other solvent systems are explained where used.

**Detection Methods.** - Iodine vapors and 40% formaldehyde-sulfuric acid-water (2:1:1 v/v) spray followed by heating at 110° for a few minutes when necessary were used as general purpose detection reagents for t. l. c. Phenols were detected with ferric chloride-potassium ferricyanide (5) (t. l. c. and paper chromatograms), or 2% 2,6-dibromoquinone-N-chlorimide (Gibbs reagent) in acetone followed (5 min) by saturated aqueous sodium bicarbonate (38) (t. l. c.
and paper chromatograms). Ortho-dihydroxy and ortho-trihydroxy phenolic groups gave black to gray spots with cold ammoniacal silver nitrate reagent (t.l.c. and paper chromatograms) while phloroglucinol nuclei gave red colors with vanillin-toluene-p-sulfonic acid reagent (78) (t.l.c. and paper chromatograms). Roux's toluene-p-sulfonic acid reagent was used to detect proanthocyanidins (76) (t.l.c. and paper chromatograms). Cold 10% aqueous sodium hydroxide was used to detect base sensitive compounds on both t.l.c. and paper chromatograms. Sugars were detected by o-aminodiphenyl reagent; pentoses gave red-brown spots and hexoses gave light brown spots (93) (paper chromatograms).

Red Alder Bark

Collection of Bark. - Red alder (Alnus rubra Bong.) bark was collected from a freshly cut tree taken from McDonald Forest, Benton County, Oregon in September of 1970. The tree was approximately 12 inches DBH and the bark was stripped from the lower trunk. The bark was air-dried and ground in a hammermill prior to extraction.

Extraction. - Dried bark (1.0 Kg) was placed in a large chromatographic column and percolated with 4 liters of cold n-hexane, 4 liters of cold diethyl ether and subsequently 4 liters of cold acetone. The extracts were evaporated under reduced pressure at

\[ ^{1} \text{OSU Herbarium voucher number 139987.} \]
25-30° and finally dried on a vacuum pump to remove the last traces of solvents. The \textit{n}-hexane extract yielded 21.1 g of a yellow green solid (2.1\% of the original bark), the diethyl ether extract gave 23.7 g of a deep green solid (2.4\% of the original bark), and the acetone extract gave 93.1 g of a tan colored, spongy solid (9.3\% of the original bark).

**Extractive-Free Bark.** - Red alder bark which had been previously cold extracted with \textit{n}-hexane, diethyl ether and acetone (above) was extracted in a Soxhlet extractor with ethanol for 90 hr (minimum of six solvent exchanges per hour), air-dried and subsequently extracted for 90 hr with water. The bark was then air-dried (50°). This material is referred to as extractive-free bark.

**Anthocyanidin Generation from Extractive-Free Bark.** - Extractive-free bark (100 mg) was heated on a steam bath in a sealed container with propane-2-ol-3N-hydrochloric acid 4:1 v/v (10 ml) for 1 hr (68) to yield cyanidin chloride (chromatographically and spectroscopically identical to an authentic, synthetic sample) \( R_f \) (iii) 0.49 [lit. (36) 0.49], \( R_f \) (iv) 0.22 [lit. (75) 0.22], \( \lambda_{\text{max}} \) (0.01\% HCl in MeOH) 535 nm, \( \lambda_{\text{max}} \) (0.01\% HCl in EtOH) 545 nm, \( \lambda_{\text{max}} \) (0.01\% HCl in EtOH plus a drop of 5\% AlCl\textsubscript{3} in EtOH) 562 nm [lit. (36, 75) 535, 545, and 563 nm respectively].

**Thioglycolysis of Extractive-Free Bark.** - Thioglycolysis of extractive-free bark (1.5 g) was performed as described by Sears.
and Casebier for *Tsuga heterophylla* (86). Permethylation, however, was done with ethereal diazomethane. Attempts to verify the presence of methyl 2,3-\textit{cis}-3,4-\textit{trans}-\((3\text{-hydroxy-3'}^\prime, 4', 5, 7\text{-tetramethoxyflavan-4-ylthio})\)acetate and methyl 2,3-\textit{trans}-\((3\text{-hydroxy-3'}^\prime, 4', 5, 7\text{-tetramethoxyflavan-4-ylthio})\)acetate by t.l.c. (vii) analysis of the permethylated thioglycolysis reaction mixture against authentic samples failed. A positive identification was complicated by large amounts of other material congesting the area on the t.l.c. plate where the authentic samples migrate.

**The Acetone Extract**

**Chromatographic Analyses.** - Two-dimensional paper chromatography with (a) water saturated 2-butanol and then (b) 2% acetic acid revealed two spots (Figure 1) which are recorded with selected color reactions in Table 1. Single applications of up to \(1.5 \times 10^{-3}\) g of extract solids, redissolved in acetone, were applied with no additional spots being detected by the employed spray reagents [Roux and Maihs utilized standard tannin applications of \(10^{-3}\) g for their work (78)]. T.l.c. analysis (Figure 2) with (vi) chloroform/methanol/acetic acid/water - 85:15:10:4 v/v is recorded in Table 2.

**Hydrolysis.** - The tan colored, spongy solid (100 mg) from the acetone extract was refluxed for 30 min in 2% aqueous sulfuric acid (5 ml). The resultant reaction mixture was light orange in color
### Table 1. Paper Chromatographic Analyses of the Acetone Extract.

<table>
<thead>
<tr>
<th>R_f (a)</th>
<th>R_f (b)</th>
<th>Spray Indicator</th>
<th>(i)</th>
<th>(ii)</th>
<th>(iii)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30</td>
<td>0.0</td>
<td>Blue</td>
<td>Pink</td>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>0.68</td>
<td>0.64</td>
<td>Blue</td>
<td>Blue</td>
<td>Black</td>
<td></td>
</tr>
</tbody>
</table>


(b) Solvent: 2% acetic acid.

(i) Ferric chloride-potassium ferricyanide (5).

(ii) Toluene-p-sulfonic acid (76).

(iii) Ammoniacal silver nitrate (78).

### Table 2. T. I. C. Analyses of the Acetone Extract.

<table>
<thead>
<tr>
<th>R_f (a)</th>
<th>(i)</th>
<th>(ii)</th>
<th>(iii)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>-</td>
<td>Pink</td>
<td>Red</td>
</tr>
<tr>
<td>0.03</td>
<td>-</td>
<td>Pink</td>
<td>Red</td>
</tr>
<tr>
<td>0.05</td>
<td>-</td>
<td>Pink</td>
<td>Red</td>
</tr>
<tr>
<td>0.13</td>
<td>Red</td>
<td>Pink</td>
<td>Brown</td>
</tr>
<tr>
<td>0.19</td>
<td>Red</td>
<td>Yellow</td>
<td>-</td>
</tr>
<tr>
<td>0.25</td>
<td>Red</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>0.30</td>
<td>Red</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.36</td>
<td>Tan</td>
<td>Violet</td>
<td>Dark Blue</td>
</tr>
<tr>
<td>0.40</td>
<td>-</td>
<td>-</td>
<td>Pink</td>
</tr>
<tr>
<td>0.48</td>
<td>Tan</td>
<td>Yellow</td>
<td>Blue</td>
</tr>
</tbody>
</table>

and contained considerable amounts of an insoluble orange-red tar. 
T. l. c. analyses of the reaction mixture showed three mobile spots. 
The orange-red tar remained at the origin. Co-chromatography with 
authentic samples and appropriate color reactions (78) indicated 
that one spot \( [R_f (vi) 0.76] \) was caffeic acid and that the other was 
dihydroquercetin \( [R_f (vi) 0.62, \text{ loss of fluorescence on sodium } \)
borohydride-aluminum chloride treatment (53)]). The third spot 
remains unidentified, \( R_f (vi) 0.19 \).

The hydrolysis reaction mixture was extracted with \( n \)-amyl 
alcohol (three 10-ml aliquots). The remaining aqueous phase was 
neutralized by the addition of saturated aqueous barium hydroxide, 
centrifuged and decanted. The decantate was evaporated to a few 
milliliters and analyzed by paper chromatography (ethyl acetate/
pyridine/water - 8:2:1 v/v, developed three times) in the usual 
manner (45). Xylose (dominant) and glucose were detected. 
Rhamnose was detected in small amounts.

**Anthocyanidin Generation.** - The tan colored, spongy solid 
(50 mg) from the acetone extract was heated in 10 ml of propane-
2-ol-3N-hydrochloric acid (4:1 v/v) in a sealed container for 40 min 
(68). The wine-red colored solution, \( \lambda_{\text{max}} \) (reaction medium) 
550 nm, was analyzed by paper chromatography and visual spectra. 
The results were identical to those for an authentic, synthesized 
sample of cyanidin chloride, \( R_f (iii) 0.49 \) [lit. (36) 0.49], \( R_f (iv) \)
0.22 [lit. (75) 0.22], $\lambda_{\text{max}}$ (0.01% HCl in MeOH) 535 nm, $\lambda_{\text{max}}$ (0.01% HCl in EtOH) 545 nm, $\lambda_{\text{max}}$ (0.01% HCl in EtOH plus a drop of 5% AlCl$_3$ in EtOH) 562 nm [lit. (36, 75) 535, 545, and 563 nm respectively].

Condensed Tannin

Isolation. - The tan colored, spongy solid (5.0 g) from the acetone extract was shaken into an emulsion with 500 ml of water. The emulsion was extracted with ethyl acetate (250 ml, repeated six times). Both the ethyl acetate and aqueous fractions were concentrated to a syrup. Acetone was added to each and they were reconcentrated to yield an off-white, spongy solid (3.1 g) from the ethyl acetate fraction, and a reddish-tan, spongy solid (1.9 g) from the aqueous fraction.

The reddish-tan spongy solid from the aqueous fraction was dissolved in a minimum amount of methanol and deposited on 5 g of cellulose which was then dried at room temperature under reduced pressure and placed on the top of a 300 g cellulose "dry packed" column contained in a nylon tube. The column was developed with water and core samples were taken to determine the location of the condensed tannin [red color with vanillin-toluene-$p$-sulfonic acid reagent (78)]. The tannin fraction was cut out ($R_f$ 0.00 to $R_f$ 0.51) and eluted from the cellulose with methanol. The
methanolic solution was filtered through sintered glass and then filter paper. The filtrate was concentrated to dryness, redissolved in methanol, refiltered, and finally evaporated to give 190 mg of a light brown solid. This material was dissolved in a minimum amount of methanol and precipitated by pouring into 100 ml of diethyl ether. The precipitate was collected and the process repeated once more. The final precipitate was dried under reduced pressure at room temperature to give 105 mg of a reddish-brown solid, which is defined here as the acetone-soluble condensed-tannin fraction.

Consistent with its proposed structure (XXXIII) the condensed tannin showed \( \lambda_{\text{max}} \) (EtOH) 280 nm (40), and afforded appropriate color reactions (78) [red with vanillin-toluene-\( p \)-sulfonic acid reagent, black with cold ammoniacal silver nitrate reagent, and pink with toluene-\( p \)-sulfonic acid reagent].

**Micro-Degradation with Potassium Hydroxide.** - Alkali degradation of the condensed tannin (4 mg) by Roux's micro fusion method (77, 23) with potassium hydroxide (1 pellet) afforded phloroglucinol and protocatechuic acid. The degradation products were identified by t.l.c. \( [R_f (iv), 0.42 \) and 0.66] through comparison with authentic reference compounds and by FD-MS mixture analyses (50) \( [m/e \) 110 and 126].

**Anthocyanidin Formation.** - The condensed tannin (2 mg) in propane-2-ol (4 ml) and 3N-hydrochloric acid (1 ml) was heated in
a sealed container for 1 hr on a steam bath (68) to yield cyanidin chloride (chromatographically and spectroscopically identical to an authentic, synthetic sample), $R_f$ (iii) 0.49 [lit. (36) 0.49], $R_f$ (iv) 0.22 [lit. (75) 0.22], $\lambda_{\text{max}}$ (0.01% HCl in MeOH) 535 nm, $\lambda_{\text{max}}$ (0.01% HCl in EtOH) 545 nm, $\lambda_{\text{max}}$ (0.01% HCl in EtOH plus a drop of 5% AlCl$_3$ in EtOH) 562 nm [lit. (36, 75) 535, 545, and 563 nm respectively].

**FD-MS Pyrolysis.** - FD-MS pyrolysis (50) of the condensed tannin (10$^{-8}$ g in MeOH) gave an intense peak at m/e 110 at 14 mA, but no peak at m/e 126 or higher.

**Methylation.** - The condensed tannin (703 mg) in acetone-methanol (5:1 v/v) (40 ml) was refluxed with stirring for 18 hr with dimethylsulfate (2.5 g) and potassium carbonate (3.5 g). The yellow reaction mixture was centrifuged, decanted and filtered. The filtrate was dissolved in chloroform (50 ml), washed with water (two 10-ml aliquots), dried with sodium sulfate, filtered, and evaporated under vacuum to give a tan colored, granular solid (579 mg), $\nu_{\text{max}}$ (CHCl$_3$) 3590, 1609, 1602, 1517, 1468, 1460, 1446, 1416, 1339, 1160, 1129, 1026 cm$^{-1}$.

**Anthocyanidin Formation From the Methylated Tannin.** - The methylated tannin (2 mg) in propane-2-ol (4 ml) and 3N hydrochloric acid (1 ml) was heated in a sealed container for 1 hr on a steam bath (68). The singularly detected anthocyanidin which was generated
was paper chromatographically coincident (three solvent systems) and showed the same $\lambda_{\text{max}}$ as authentic tetra-O-methylcyanidin chloride (XXXII), prepared (47) from 5,7,3',4'-tetramethoxyflavan-3,4-diol, $\lambda_{\text{max}}$ 532 nm [lit. (47) $\lambda_{\text{max}}$ 532 nm], $R_f$ (iv) 0.67, $R_f$ (water/acetic acid/conc. hydrochloric acid, 80:20:5 v/v) 0.39, $R_f$ (butane-1-ol/2N-hydrochloric acid 1:1 v/v) 0.59 [lit. (47, 39), 0.67, 0.38, and 0.51 respectively].

**Thioglycolysis of the Methylated Tannin.** - The methylated tannin (200 mg) was degraded by Sears and Casebier's method (86), refluxing for 4 hr under argon with water (3 ml) and thioglycolic acid (4 ml) at 135°C. The reaction was cooled and poured into 20 ml of water and extracted with ethyl acetate (two 20-ml aliquots). The ethyl acetate extract was added to 40 ml of 10% sodium bicarbonate solution and excess solid sodium bicarbonate was added until the aqueous layer became basic. The aqueous layer was acidified and extracted with diethyl ether (two 20-ml aliquots). The diethyl ether solution was dried and treated with ethereal diazomethane until the evolution of nitrogen gas ceased. T.l.c. analysis of the diethyl ether solution revealed two spots; $R_f$ (vii) 0.90 due to methyl thioglycolate, and $R_f$ (vii) 0.43 which migrated the same distance as authentic (+)-methyl 2,3-cis-3,4-trans-(3-hydroxy-3',4',5,7-tetramethoxyflavan-4-ylthio)acetate (15) when
co-chromatographed. Authentic methyl 2, 3-trans-(3-hydroxy-3', 4', 5, 7-tetramethoxyflavan-4-ylthio)acetate (XXVII) exhibited $R_f$ (vii) 0.51, which indicated the absence of this compound in the degradation reaction mixture.

Preparative t.l.c. of the diethyl ether solution on 8 plates developed in benzene/aceton 9:1 v/v and eluted from the silica with diethyl ether yielded 21 mg of (+)-methyl 2, 3-cis-3, 4-trans-(3-hydroxy-3', 4', 5, 7-tetramethoxyflavan-4-ylthio)acetate, m. p. 47-50° [lit. (15) 47-50°] (Found: C, 58.80; H, 5.98; S, 7.19; C$_{22}$H$_{26}$O$_8$S requires C, 58.65; H, 5.82; S, 7.10%), the n.m.r., i.r., u.v. and mass spectrum are identical to those recorded for a synthetic authentic sample (15).

**Acetylation of the Methylated Tannin.** - The methylated tannin (239 mg) was dissolved in pyridine (2 ml, dried over KOH) and acetic anhydride (3 ml) was added. The solution was let stand at room temperature for 48 hr and then water (25 ml) was added and the mixture cooled in the refrigerator for 2 hr and then centrifuged. The supernatent liquid was decanted and discarded and the precipitation process repeated. The residue was dissolved in chloroform (50 ml) and washed with water (two 20-ml aliquots) and the chloroform layer was dried (Na$_2$SO$_4$). The chloroform solution was evaporated to yield 172 mg of a light yellow solid, $v_{\text{max}}$ (CHCl$_3$) 1742, 1708, 1704, 1615, 1466, 1455, 1444, 1418, 1373, 1157, 1135,
1027 cm$^{-1}$. Absorptions for $\nu$ OH were absent.

Oregonin

**Isolation.** - The ethyl acetate soluble residue (3.1 g) remaining from the water-ethyl acetate partition of the acetone extract (condensed tannin isolation) was put into 500 ml of water and partitioned into the following fractions.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Solvent</th>
<th>Volume (ml)</th>
<th>Wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>diethyl ether</td>
<td>4 x 250</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>ethyl acetate</td>
<td>1 x 250</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>ethyl acetate</td>
<td>3 x 250</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>ethyl acetate</td>
<td>3 x 250</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>aqueous residue</td>
<td></td>
<td>0.6</td>
</tr>
</tbody>
</table>

Fractions 3 and 4 showed a single major spot on t. l. c., $R_f$ (vi) 0.36. Combination of fractions 3 and 4 gave 1.8 g of a fraction rich in oregonin (XL). Consistent with a totally free phenolic form, oregonin gives a black color with cold ammoniacal silver nitrate (78), exhibits no methoxyl resonances in the n. m. r. spectrum, and gives an EI-mass spectrum parallel to that of the tetra-$\Omega$-methyl ether, $m/e$ 328.135 ($M -$ xylose, 17%) $C_{19}H_{20}O_5$ requires 328.131, and $m/e$ 123 [$H_2C^+-phenyl-(OH)_2$, 100%].

_Tetra-$\Omega$-methylloregonin._ - Oregonin (14 g of the rich fraction
from above) was dissolved in a minimum amount of acetone, and excess ethereal diazomethane was added, and the solution was allowed to stand for 48 hr at -10°C. The solution was then warmed to room temperature and the solvents were evaporated by a stream of nitrogen gas. The process was repeated one more time to yield the crude methyl ether as a light yellow glass (15.8 g). The crude methyl ether was purified by column chromatography on silica (dry packed, 630 g). Elution with chloroform (2.75 l) followed by ethyl acetate (3 l) removed the non-tetra-O-methylloregonin compounds. Continued elution with ethyl acetate-acetone (10 → 20%) gave tetra-O-methylloregonin (2 g). A further purification by preparative t.l.c. (chloroform/methanol 9:1 v/v, four passes) gave the tetra-O-methyl ether as a faint yellow oil which crystallized on long standing into rosettes with very fine needles. Recrystallization from ethyl acetate gave very small white needles (which appear to lose crystallinity to varying degrees on recovery and drying), m. p. (dried 24 hr in a vacuum dessicator over phosphorus pentoxide at room temperature) 53-56°C, [Found (dried at 100°C for 24 hr over phosphorus pentoxide): C, 62.86; H, 7.11; C_{28}H_{38}O_{10} requires C, 62.89; H, 7.17%], \lambda_{\text{max}}^{\text{EtOH}} (229, 280); \lambda_{\text{min}}^{\text{EtOH}} (251 \text{nm}), \nu_{\text{max}}^{\text{CHCl}_3} (3440, 1713, 1597, 1516, 1456, 1444, 1419, 1157, 1142, 1030 \text{ cm}^{-1}. T (acetone-d_6) 3.08-3.36 (6H, m), 5.66 (1H, d, J=7 Hz, anomeric proton), 5.74-5.98 (3H, 2H removed by D_2O exchange leaving 5.83
(1H, m), 6.11 (1H, q), 6.22, 6.24, 6.26 (3H, 3H, 6H, each s),
6.40-6.96 (4H, m), 7.07-7.51 [10H, m (2H removed by D₂O
exchange)], 8.16 (2H, m), m/e 384.199 (M⁺ xylose, 32%) C₂₃H₂₈O₅
requires 384.194), 151.076 [H₂C⁺-phenyl-(OMe)₂, 100%, C₉H₁₁O₂
requires 151.076], FD-MS, M⁺ 534.

Tetra-O-methyloregonin Triacetate. - Tetra-O-methyloregonin
(100 mg) was acetylated with acetic anhydride (1 ml) and dry pyridine
(2 ml) at room temperature. After 48 hr the reaction mixture was
added to water (20 ml) and extracted with chloroform (three 20-ml
aliquots). The chloroform extract was washed with dilute hydro-
chloric acid, dilute sodium bicarbonate, and then water. The chloro-
form extract was evaporated under reduced pressure and the residue
purified by preparative t.l.c. (benzene/acetone 9:1 v/v) to give the
acetate as a glass (108 mg), λ max (EtOH) 229, 280 nm, ν max (CHCl₃)
1750, 1512, 1467, 1455, 1443, 1371, 1159, 1144 cm⁻¹. τ (CDCl₃) 3.12-
3.36 (6H, m), 4.82 (1H, t), 4.92-5.22 (2H, m), 5.46 (1H, d,
J= 7Hz), 5.84 (1H, m), 5.90 (1H, q), 6.14, 6.16 (12H, two s), 6.64
(1H, q), 7.02-7.56 (8H, m), 7.97, 7.98 (9H, two s), 8.17 (1H, m),
M⁺ 660.278 (C₃₄H₄₄O₁₃ requires 660.278).

Hydrolysis of Tetra-O-methyloregonin. - Tetra-O-
methyloregonin (100 mg) was refluxed for 30 min in 2% aqueous
sulfuric acid (15 ml). The reaction mixture was cooled and extracted
with chloroform (three 15-ml aliquots). The combined chloroform
solutions were washed free of acid with water, dried over sodium sulfate, reduced in volume and applied to preparative t.l.c. plates (two spots were resolved, Rf (vii) 0.66 and 0.38).

Collection of the material at Rf 0.66 gave 51 mg of 1,7-bis (3,4-dimethoxyphenyl)-3-heptene-5-one (XLIV) as white plates (MeOH plus a little H2O), m. p. 64-65° (Found: C, 71.66; H, 7.43. C23H28O5 requires C, 71.84; H, 7.36%).

\[ \lambda_{\text{max}} (\text{EtOH}) 228, 280 \text{ nm}, \nu_{\text{max}} (\text{CHCl}_3) 1697 (s h), 1672, 1628, 1595, 1516, 1468, 1456, 1445, 1420, 1154, 1140, 1028, 850 \text{ cm}^{-1}, T(\text{CDCl}_3) 3.01-3.37 (6H, m), 3.90 (1H, d, J_{3,4} = 15.8 \text{ Hz}, J_{2,4} = 1.8 \text{ Hz}), 6.16 (12H, s), 7.10-7.58 (8H, m), M^+ 384.189 (C23H28O5 requires 384.194).

Collection of the material at Rf 0.38 gave 15 mg of 1,7-bis(3,4-dimethoxyphenyl)heptane-3-one-5-ol (XLIII) as white plates (MeOH), \[ \lambda_{\text{max}} (\text{EtOH}) 229, 280 \text{ nm}, \nu_{\text{max}} (\text{CHCl}_3) 3560, 1710, 1612, 1596, 1515, 1468, 1459, 1445, 1421, 1159, 1144, 1030 \text{ cm}^{-1}, T(\text{CDCl}_3) 3.14-3.36 (6H, m), 5.94 (1H, m), 6.14, 6.15 (12H, two s), 6.94 (1H, removed by D2O exchange), 7.06-7.50 (8H, m), 8.28 (2H, m), M^+ 402.206 (C23H30O6 requires 402.204).

The aqueous solution remaining from the work up of the above hydrolysis was neutralized with barium carbonate. Centrifugation, decantation, and evaporation under reduced pressure gave a syrup. Paper chromatography in the prescribed manner (45) identified the sugar as xylose. Consistent with a pentose structure the spot gave a
red-brown color with o-aminodiphenyl reagent (93) and exhibited \( M^+ 150 \) (FD-MS).

**Syntheses**

**Cyanidin Chloride.** - Quercetin was reductively acetylated and the product converted to cyanidin chloride as described by King and White (51). Purification, however, was accomplished by placing the hydrolysis reaction product on a dry-packed cellulose column (500:1) contained in nylon tubing and developing with forestol solvent (iii). The cyanidin chloride band (bright red) was cut out and eluted from the cellulose with methanol which contained a trace of hydrochloric acid (0.01%). The eluant was evaporated under vacuum to give paper chromatographically pure cyanidin chloride, \( R_f \) (iii) 0.49 [lit. (36) 0.49], \( R_f \) (iv) 0.22 [lit. (75) 0.22], \( \lambda_{\text{max}} \) (0.01% HCl in MeOH) 535 nm, (0.01% HCl in EtOH) 545 nm, (0.01% HCl in EtOH plus a drop of 5% AlCl\(_3\) in EtOH) 562 nm [lit. (36, 75) 535, 545, 563 nm].

**(-)-Tetra-O-methylepicatechin.** - (-) Epicatechin (300 mg) was dissolved in 50 ml of acetone and treated with excess ethereal diazomethane (three times). T. l. c. revealed a major spot, \( R_f \) (vii) 0.47 which turned green with sulfuric acid / 40% formaldehyde / water (2:1:1 v/v) (58). Preparative t. l. c. (17 plates) gave 264 mg of white crystalline material. Recrystallization from benzene-diethyl ether
(9:1 v/v) gave rosettes, m.p. 139-140°. Two additional recrystallizations from diethyl ether-acetone also gave rosettes, m.p. 139-140° [lit. (16, 14, 92) 136-139°, 143°, 153-154°], [α]_D^{23} = -55° (c 1.7, CHCl₃) [lit. (14) [α]_D^{20} = -56°], [α]_D^{23} = -61° (c 1.7, CHCl₃) [lit. (92) [α]_D^{23} = -62°], T (CDCl₃) 2.90-3.20 (3H, m, B ring), 3.86 (2H, ABq, J = 2.5 Hz, A ring), 5.11 (1H, s, H-2), 5.80 (1H, m, H-3), 6.12, 6.14, 6.25, 6.27 (3H each, s, OCH₃), 7.05-7.16 (2H, m, C-4), 7.88 (1H, d, OH, J = 6 Hz), M⁺ 346.139 (C₁₉H₂₂O₆ requires 346.142).

(+)-Methyl 2, 3-cis-3, 4-trans-(3-hydroxy-3', 4', 5, 7-tetramethoxyflavan-4-ythio)acetate. - The synthesis follows essentially that described by Betts and co-workers (15) except that the intermediates were not characterized. (-)-Tetra-O-methylepicatechin (2.7 g) was dissolved in 75 ml of benzene (dried over sodium) and stirred with 4.5 g of lead tetraacetate at room temperature. After 9 days starch-iodide paper showed the absence of oxidizing agent. The crude reaction mixture showed a major spot on t.l.c., Rf (vii) 0.52, red with toluene-P-sulfonic acid reagent. Water (50 ml) was added to the reaction mixture and the benzene layer separated. The aqueous layer was re-extracted with an additional 50 ml of benzene. The combined benzene solution was evaporated to yield a solid which was then dissolved in 75 ml of 1N-methanolic potassium hydroxide solution and refluxed for 45
min. The reaction mixture was evaporated under reduced pressure and 50 ml of water was added and then extracted (two times) with 40 ml of ethyl acetate. The combined ethyl acetate solution was back extracted with 50 ml of water and filtered through a pad of anhydrous sodium sulfate and evaporated to about 20 ml. Preparative t.l.c. with benzene/acetone 4:1 (v/v) gave the major spot, presumably as the diol.

The above material was dissolved in 100 ml of dioxane, 50 ml of water, and 5 g of thioglycolic acid was added. The mixture was refluxed for 1 hr. The reaction mixture was cooled and an excess of 10% sodium bicarbonate was added. The mixture was then extracted with ethyl acetate (two times). The remaining aqueous layer was then acidified with 3N-hydrochloric acid and extracted with ethyl acetate (two times). This second ethyl acetate extract was evaporated to a gum and then ethereal diazomethane was added until the gum had dissolved and evolution of nitrogen gas had ceased. The reaction mixture was reduced in volume by a stream of nitrogen gas and the residue placed on a silica column (150 g dry packed) and eluted as follows:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Solvent</th>
<th>Elution Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>diethyl ether/petroleum ether (3:1 v/v)</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>diethyl ether</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>''</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>''</td>
<td>300</td>
</tr>
<tr>
<td>5</td>
<td>''</td>
<td>300</td>
</tr>
<tr>
<td>6</td>
<td>''</td>
<td>300</td>
</tr>
</tbody>
</table>
Fraction 1 contained non-flavonoid material and was discarded.

Fractions 2-4 contained a major spot on t.l.c., $R_f$ (vii) 0.43.

Fractions 2, 3, and 4 were applied to preparative t.l.c. The plates were developed 3 times in diethyl ether/petroleum ether (3:2 v/v) followed by 3 more developments in diethyl ether/petroleum ether (3:1 v/v) (multiple elution method). The major spot was removed and eluted from the silica with diethyl ether. The diethyl ether was evaporated and the material dried overnight at room temperature under reduced pressure over phosphorus pentoxide to yield 325 mg of white amorphous (+)-methyl 2, 3-cis-3', 4'-trans-(3-hydroxy-3', 4', 5, 7-tetramethoxyflavan-4-ylthio)acetate, m. p. 47-50° [lit. (15) 47-50°],

$$\lambda_{\text{max}}\text{(EtOH)} 276 \text{ nm}, \tau \text{(CDCl}_3\text{)} 2.80-3.10 \text{ (3H, m, B ring), 3.82 (2H, ABq, A ring), 4.49 (1H, s, H-2), 5.76 (2H, m, H-1, H-3), 6.05, 6.07, 6.11, 6.22, 6.24 (3H each, s, OCH}_3\text{), 6.51 (2H, s, S-CH}_2\text{), 8.06 (1H, OH), }$$

$$\nu_{\text{max}} \text{(CHCl}_3\text{)} 3575, 1745, 1470, 1269, 1150, \text{ and 1120 cm}^{-1}, \text{ m/e 450.132 (M}^+, 7\%) \text{ and 345 (100%)},$$

(C$_{22}$H$_{26}$O$_8$S requires 450.135).

Octa-O-methyldeipicatechin Thioglycolate. Fractions 5 and 6 from above gave 105 mg of crystalline material on evaporation. Recrystallization from benzene-acetone gave prisms, m. p. 165-167°. Recrystallization from ethyl acetate gave plates, m. p. 160-161° (Found: C, 61.64; H, 5.87; S, 3.81. C$_{41}$H$_{46}$O$_{14}$S requires C, 61.94; H, 5.84; S, 4.03), $\lambda_{\text{max}}\text{(EtOH)} 276 \text{ nm, M}^+ 794.230 \pm$
0.035 (variation due to lack of suitable standard in this mass region, 

\[
\text{C}_{41}\text{H}_{46}\text{O}_{14}\text{S requires 794.261).}
\]

1, 7-Bis(3, 4-dimethoxyphenyl)-1, 6-heptadiene-3, 5-dione. - 3, 4-Dimethoxybenzaldehyde (33.2 g) and tributylborate (92 g) were dissolved in 100 ml of freshly dried ethyl acetate. Acetyl acetone (10 g) and boric anhydride (5 g) were mixed into a paste and the paste was added to the ethyl acetate solution. The reaction mixture was stirred while 2 ml of butylamine was added drop by drop over a fifteen minute period. Stirring was continued for 4 hr and the mixture was let stand overnight.

Hydrochloric acid (0.4N, 150 ml) was added and the mixture was stirred and then warmed on a steam bath for 60 min. The layers were separated and the aqueous layer extracted (two times) with 100 ml of ethyl acetate. The ethyl acetate layers were combined and washed free of acid. The solution was evaporated to about 100 ml and about 50 ml of methanol was added. Light orange needles formed on standing (28 g). The mother liquor was not used for further recrystallizations. Recrystallization from ethyl acetate-methanol gave 1, 7-bis(3, 4-dimethoxyphenyl)-1, 6-heptadiene-3, 5-dione as light orange needles, m. p. 130-131\(^\circ\) (Found: C, 69.51; H, 5.95. \(\text{C}_{23}\text{H}_{24}\text{O}_{6}\) requires C, 69.67; H, 6.11), \(\lambda_{\text{max}}\) (EtOH) 262, 420 nm, \(\nu_{\text{max}}\) (CHCl\(_3\)) 1632, 1590, 1513, 1467, 1446, 1425, 1137, 1025 cm\(^{-1}\), \(\tau\) (CDCl\(_3\)) -5.86 (> 1H, br, enolic OH), 2.40
(2H, d, J = 16 Hz), 2.79-3.19 (6H, m), 3.51 (2H, d, J = 6 Hz), 4.19 (0.9 H, s), 6.08, 6.10 (12H, two s), 6.51 (~0.2 H, s), M+ 396.153 (C23H24O6 requires 396.157).

1,7-Bis(3,4-dimethoxyphenyl)heptane-3,5-dione. - 1,7-Bis(3,4-dimethoxyphenyl)-1,6-hetadiene-3,5-dione (1 g) was dissolved in 50 ml of ethyl acetate and 50 mg of 10% palladium on charcoal was added. The metallic green reaction mixture was stirred under 1 atmosphere of hydrogen overnight (the reaction mixture was colorless after 4 hr). The reaction mixture was filtered and evaporated to give 1.06 g of crystalline material. Recrystallization from methanol gave white plates, m. p. 68-69° (dried under reduced pressure at room temperature over phosphorus pentoxide) (Found: C, 68.87; H, 7.05. C23H28O6 requires C, 68.97; H, 7.05). \( \lambda_{\text{max}} \) (EtOH) 228, 280, \( \nu_{\text{max}} \) (CHCl3) 1612, 1598, 1515, 1468, 1457, 1444, 1157, 1141, 1028 cm\(^{-1}\), T (CDCl3) -5.48 (0.8 H, br), 3.13-3.37 (6H, m), 4.56 (0.8H, s), 6.15 (12H, s), 6.94 (0.4H, s), 6.95-7.54 (8H, m), M+ 400.189 (C23H28O6 requires 400.189).

1,7-Bis(3,4-dimethoxyphenyl)heptane-3-one-5-ol. - 1,7-Bis(3,4-dimethoxyphenyl)heptane-3,5-dione (2.5 g) was dissolved in 200 ml of methanol and stirred while 85 mg of sodium borohydride in 10 ml of methanol was added dropwise. The reaction mixture was allowed to stand for 30 min. Water (50 ml) was added and the reaction mixture was warmed on a steam bath for 5 min and then
evaporated to about 100 ml under reduced pressure. Diethyl ether (100 ml), was added and the layers separated. The aqueous layer was extracted twice more (100 ml each) with diethyl ether and the combined diethyl ether solutions were dried (Na$_2$SO$_4$) and reduced in volume. They were then applied to preparative silica t.l.c. plates. Four spots were resolved; $R_f$ (vii) 0.67, 0.38, 0.21, and 0.18. Collection of the material at $R_f$ 0.38 and elution with acetone-chloroform gave 0.6 g of a white crystalline material. Recrystallization from methanol gave white plates, m. p. 99-100° (dried at 78° under reduced pressure over phosphorus pentoxide) (Found: C, 68.52; H, 7.50. C$_{23}$H$_{30}$O$_6$ requires C, 68.62; H, 7.52). The n.m.r., i.r., u.v., and mass spectra were identical to those of the corresponding derivative of the natural product oregonin.

1,7-Bis(3,4-dimethoxyphenyl)heptane-3,5-diol. - Material corresponding to spots $R_f$ 0.21 and $R_f$ 0.18 from above were removed from preparative t.l.c. plates together (on preparative scale the spots merged) and were eluted from the silica with acetone. Evaporation of the acetone gave 1.2 g of a viscous oil which crystallized on standing. Recrystallization from 70% methanol gave 1,7-bis(3,4-dimethoxyphenyl)heptane-3,5-diol as a mixture of diastereomers, m. p. 82-88° (Found: C, 68.13, H, 7.97. C$_{23}$H$_{32}$O$_6$ requires C, 68.28; H, 7.98). $\lambda_{\text{max}}$ (EtOH) 229, 280, $\nu_{\text{max}}$ (CHCl$_3$) 3624, 3500, 1593, 1510, 1464, 1453, 1442, 1418,
1253, 1240, 1027, 851 cm\(^{-1}\), \(T\) (CDCl\(_3\)) 3.10-3.33 (6H, m), 5.98 (2H, m), 6.15, 6.16 (12H, two s), 7.08 (2H, br, removed by D\(_2\)O), 7.17-7.42 (4H, m), 8.23 (6H, m), \(\text{M}^+\) 404.226 (C\(_{23}\)H\(_{32}\)O\(_6\) requires 404.220). A second recrystallization from acetone gave m.p. 96°-97°, possibly due to selective crystallization of one of the diastereomers.

1,7-Bis(3,4-dimethoxyphenyl)-trans-3-heptene-5-one. -

1,7-Bis(3,4-dimethoxyphenyl)heptane-3-one-5-ol (100 mg) was refluxed for 30 min in 15 ml of 2% aqueous H\(_2\)SO\(_4\). The reaction mixture was cooled and extracted with ethyl acetate (three times 15 ml). The combined ethyl acetate fractions were washed free of acid and reduced in volume under reduced pressure. The product was purified by preparative t.l.c., \(R_f\) (vii) 0.66.

Elution from the silica with chloroform gave 72 mg of a viscous oil. Recrystallization from methanol plus a little water gave 1,7-bis(3,4-dimethoxyphenyl)-trans-3-heptene-5-one as white plates, m.p. 64-65° (Found: C, 71.7; H, 7.3. C\(_{23}\)H\(_{28}\)O\(_5\) requires C, 71.84; H, 7.36). The n.m.r., i.r., u.v. and mass spectra are identical to those of the corresponding derivatives from the natural product oregonin.

The Stain

Examination at Mill Site. - On march 12, 1973, the following
90 observations were made at Paul Barber Hardwoods, Philomath, Oregon. Freshly sawn lumber showed the usual red to orange color on bark and in cambial areas with red streaking on boards with some gold colored background. Application of 0.01% hydrochloric acid in methanol [in an attempt to show the presence of an anhydrobenzopyranol chromophore (79)] caused the orange-red stain to immediately change to a bright yellow color. All of the various shades of orange to red behaved similarly in changing to a common yellow color. This indicated a common chromophore and that the various degrees of redness are due to concentration effects. Orange colored stain material which was freshly formed was scraped from the cambial area of a piece of bark and dissolved in methanol to exhibit $\lambda_{\text{max}}$ 433 nm; $\lambda_{\text{max}}$ (MeOH plus a drop of HCl) 399 nm; $\lambda_{\text{max}}$ (0.01% HCl in EtOH) 425 nm, indicating pH sensitivity.

In Vitro Generation of Stain. - It has been the general observation in this laboratory that undried bark that has not been kept too long can be made to generate the stain by simply breaking and exposing a surface which appears to stain less willingly as the bark material becomes drier. Addition of water will increase the staining rate. It has also been a general observation that soaking the bark in aprotic solvents such as acetone only elicits a yellowing of the solution and bark surface, whereas protic solvents such as ethanol elicit a more orange shade to the solution and a contrasting dark
brown bark surface. Generation of the stain by homogenizing fresh bark in a phosphate buffer solution (pH 6) and filtration gave an orange-colored solution, $\lambda_{\text{max}}$ 478 nm.

**Decolorization of Stain.** - The orange-red stain of red alder can be decolorized (49).
SUMMARY AND CONCLUSIONS

The likely precursors to the stain in red alder were considered to be a phenolic xyloside and the condensed tannins. Preliminary examination of the cold acetone extract of red alder bark revealed the presence of a xyloside and a cyanidin yielding condensed tannin.

Isolation of the condensed tannin and structural elucidation via derivitization and degradation reactions revealed it to be a polymer of epicatechin whose constituent flavanol units are C-C 4, 6-(4, 8-) linked (XXXIII). Examination of the stain by visual spectra showed that the condensed tannin was not contributing. The spectral absorptions of the chromophore in the stain are inconsistent with an anhydrobenzopyranol chromophore that would be derived from the condensed tannin.

In the course of synthesis of a model degradation product of the condensed tannin, a crystalline thioglycolate of octa-O-methyldiepicatechin was isolated as a by-product. The compound has potential value as a model for degradation of lower oligomers of proanthocyanidins, most particularly trimeric species.

Oregonin (XL), 1, 7-bis(3, 4-dihydroxyphenyl)heptane-3-one-5-β-xylopyranoside, represents a new type of glycoside. This diarylheptanoid compound was found to be the major constituent of the acetone extract of red alder bark. Structural elucidation was
based primarily on spectrometry of tetra-\(\text{O}\)-methyloregonin (XLI) and its triacetate, and hydrolysis products. Conformation of the structure was obtained by synthesis of the tetra-\(\text{O}\)-methyl aglycone and its dehydration product.

Oregonin is implicated in the formation of the stain by its ability to form orange-red colors on hydrolysis and on exposure to peroxidase and hydrogen peroxide.

The exact mechanism of the stain remains outstanding and this work provides a sound foundation for examination of the biochemistry of the stain formation in red alder.

Utilization of field desorption mass spectrometry (FD-MS) was found to be of significant value in the structural elucidation of oregonin. In collaboration with Professor Barofsky of the Oregon Graduate Center the first application to phenolic compounds was made (50). FD-MS has proven to be of value in determining the molecular weights of underivatized polyphenols in amounts as small as \(10^{-8}\) g. The method can be used to analyze mixtures without prior isolation of the components. When combined with pyrolysis, FD-MS has shown that underivatized natural phenolic glycosides can be directly analyzed for molecular weight of the aglycone and the glycosyl moiety in submicrogram amounts. Under proper conditions flavonoids can also be analyzed for the nature of
the A and B rings by their respective fragment ion peaks. **FD-MS** is thus an effective new tool in the study of polyphenols.
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