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Title: Low Molecular Weight Polyphenols in Douglas-Fir Outer Bark: Extraction, Isolation and Structure Determination

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Joseph J. Karchesy

Low molecular weight polyphenolic compounds in Douglas-fir outer bark were investigated as possible precursors to higher molecular weight phlobaphenes. Pinoresinol, epipinoresinol, a lignan derived furolactone and eriodictyol were isolated from the 1,2-dichloroethane soluble portion of a methanol extract. Their structures were established by the use of one and two dimensional $^{1}H$ and $^{13}C$ NMR, fast atom bombardment mass spectrometry and other complementary spectroscopic means. Comparison of NMR data for these compounds to spectra for phlobaphene polymers shows corresponding functional groups and structural features strongly suggesting that these isolated compounds are phlobaphene precursors.

# Low Molecular Weight Polyphenols in Douglas-Fir Outer Bark: Extraction, Isolation and Structure Determination

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# LOW MOLECULAR WEIGHT POLYPHENOLS IN DOUGLAS-FIR OUTER BARK: EXTRACTION, ISOLATION AND STRUCTURE DETERMINATION

## INTRODUCTION

Douglas-fir [*Pseudotsuga menziesii (Mirb.) Franco*] is the main timber species of the Pacific Northwest being used to produce lumber, plywood and glue laminated beams. The considerable quantities of bark generated in these mill operations find use as a fuel and , to a lesser extent, in landscaping and as an extender in some exterior plywood adhesives. Yet, this bark is an abundant and renewable source of polyphenolic compounds that have a potential for industrial utilization to make adhesives, composites, and new polymers for the future.

Presently, petrochemical based adhesive polymers are used to make plywood and glue laminated beams (1). Phenol-formaldehyde (P-F) adhesives are used to make exterior construction grade plywood. In traditional use, the adhesive is formulated by mixing the P-F resin with various extenders, sodium hydroxide, and additives. The adhesive is then applied to veneers that have been dried to 0-7% moisture content. The assembled plywood panels

are hot pressed at 270-300 °F for several minutes to cure the adhesive. Some research has been carried out on gluing "high moisture content" veneers, but it is generally recognized that a faster curing adhesive that is cost effective may have to be developed before this comes into general use (2).

In the production of glue laminated beams, phenol-resorcinol-formaldehyde (P-R-F) is used as a cold setting adhesive. Clamp time may be 4 hours at 70 °F. Some new operations using radio-frequency curing report cure times of several minutes. It is the resorcinol moiety that allows such rapid cure times for this adhesive. However, there is a price to pay for the rapid cure rates. Phenol sells for $ 0.325/lb and resorcinol sells for $ 2.33/lb (3). P-R-F resins typically contain about 20% resorcinol. Both phenol and resorcinol are derived from petroleum. Formaldehyde is made from methanol which is derived from natural gas. With the uncertainties of petroleum supply and prices, the forest products industry needs to be looking for alternative, renewable resources to make these adhesives. The recent Kuwait-Iraq war should be evidence enough.

The phenolic functional groups present in Douglas-fir bark are not only capable of replacing phenol in reaction with formaldehyde, but they can in many cases replace resorcinol (4). There is thus the added economic incentive for fast curing rates. However, this situation

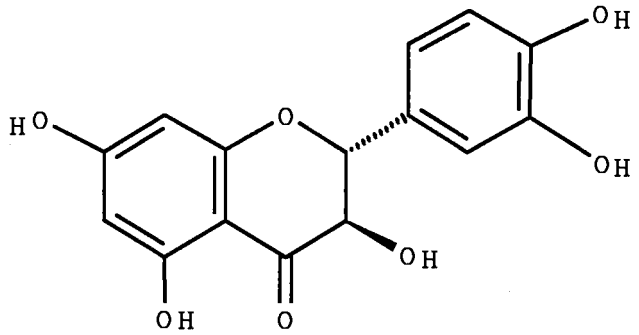
only applies to the procyanidin and associated compounds of Douglas-fir. Douglas-fir outer bark contains a significant amount of polyphenolic material that is not procyanidin in nature. Preliminary examination by Foo and Karchesy reveal that these compounds are oligomers of flavonoids, lignans, and other, as yet, unidentified compounds (5,6). The major mode of polymer linkage appears to be via phenolic oxidative coupling to produce biphenyl bonds. Often these outer bark polymers are referred to as phlobaphenes. Phlobaphenes are defined as the reddish-colored, alcohol- soluble, water-insoluble materials from bark (7). Little is known about the details of their structures and linkages. Yet, this information is important if we are to effectively utilize these materials.

The aim of this thesis research was to investigate the molecular structures of low molecular weight polyphenolic compounds in Douglas-fir outer bark that may be precursors to the phlobaphene fraction. Identification of these compounds would also help determine their potential for utilization as well. Specifically, in this work, the low molecular weight polyphenols in the 1,2-dichloroethane soluble fraction of Douglas-fir outer bark were examined.

# LITERATURE REVIEW

The investigation of polyphenols from Douglas-fir bark as a part of a program of evaluating the waste bark for commercial use has been carried out intermittently for four decades. Historically, the research can be divided into two main phases. Earlier preliminary work by Kurth (8-10), Weinges (11) and Hergert (12) provided insight into the some types of monomeric phenolic compounds present. However, few details were known about the structures and types of more complex compounds until 1970's and 1980's when major advancements were made in analytical techniques which allowed workers such as Laver(16) and Karchesy(5,6,14-18) to make major advancements in the structural determinations of the complex polyphenols of Douglas-fir bark.

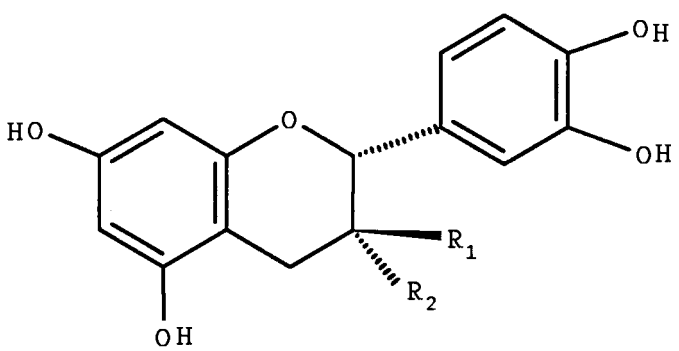
Kurth (8,9) studied the extractives of the bark and reported that they found (+)-dihydroquercetin (Structure 1) and tannins in Douglas-fir bark. Several feasible methods of extraction were reported, but the favored procedure appeared to be: first, to use an appropriate solvent, for example benzene, to remove waxes; then, to apply hot water to remove DHQ and tannins by countercurrent extraction; last, they used diethyl ether or diisopropyl ether to separate DHQ from the tannins.



Structure 1. (+)-Dihydroquercetin (DHQ)

The application of this procedure not only gave relatively higher yields of DHQ and tannins, but also had the advantage of being an easier method of purification.

In 1958, Weinges (11) isolated two monomeric flavanoid compounds from freshly dried Douglas-fir bark: d-catechin and l-epicatechin (Structure 2). In present day practice, the terms d and l have been replaced by the terms (+) and (-) respectively. Thus, d-catechin is



(A) $R_1$=OH, $R_2$=H

(B) $R_1$=H, $R_2$=OH

Structure 2 . (+)-Catechin (A) and (-)-Epicatechin (B)

(+)-catechin; l-epicatechin is (-)-epicatechin. When Holmes and Kurth (10) studied the composition of the newly formed inner bark, they isolated these same compounds. Two dimensional paper chromatography, with a solvent system of *n*-butanol-acetic acid-water (4:1:5 v/v) in one direction and 2% acetic acid in the other direction, was used as a method for qualitative analysis of these two compounds in this study. In addition, the measurement of melting points and the comparison of infrared (IR) spectra were also applied to confirm the existences of these two compounds. Therefore, they speculated that (+)-catechin and (-)-epicatechin were precursors to the higher molecular weight polyphenols in the bark.

Hergert (12) also used two dimensional paper chromatography to qualitatively analyze the methanol extracts of Douglas-fir bark. Ferric chloride-potassium ferricyanide was used as a spray reagent to detect phenolic compounds. Vanillin, coniferylaldehyde, protocatechuic acid, quercetin and leucocyanidins were determined to be present in the bark. After studying the distribution of leucocyanidins and detecting cyanidin as a degradation product, he concluded that tannins in Douglas-fir bark are predominantly polymeric leucocyanidins.

The term polymeric leucocyanidin also is no longer used in the current literature and has been replaced by

the term procyanidin (19). Procyanidins are now known to be oligomers and polymers of catechin and/or epicatechin that give cyanidin chloride on treatment with hydrochloric acid and heat in an alcohol solution (20,21).

Fang and Laver (13) worked on the *n*-hexane soluble fraction of Douglas-fir bark which was found to contain ferulic acid esters. The esters, which appeared dark blue under ultra-violet (UV) light, were saponified to give behenyl and lignoceryl alcohols. The identity of these compounds was established by melting points, gas-liquid chromatography (GLC), IR and mass spectrometry (MS). In addition to proton nuclear magnetic resonance spectroscopy ($^{1}$H-NMR), the same techniques were used to confirm the acid portion as ferulic acid. Based on the fact that the presence of free hydroxyl groups was showed by the IR spectrum, the ester linkage was believed to be between the carboxylic acid group of ferulic acid and the hydroxyl group of the fatty alcohol in each case.

More recent studies on Douglas-fir bark extracts have been carried out by Karchesy and colleagues (5,6,14-18). Alternating chromatographic separations done on columns of Sephadex LH-20 and MCI-gel CHP-20P were used to successfully isolate and purify a number of complex polyphenolic compounds. Structural elucidations of these compounds were accomplished by use of fast atom bombardment MS (FAB-MS), IR, UV and $^{1}$H- and $^{13}$C-NMR.

Two-dimensional NMR techniques played a special role in providing fine details of molecular structure.

A number of polyphenolic glycosides (14), procyanidin oligomers (15,16) and polymers (17) were isolated from the aqueous fraction of the methanol extract of the inner bark (Table 1 and Table 2). While many of these compounds are known to occur in other plant species, several of these compounds were new to the chemical literature, having been isolated for the first time from Douglas-fir. These included epicatechin-7-O-β-D-glucopyranoside, epicatechin-(4β→8)-catechin-(4α→8)-catechin, epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin, and epicatechin-(4β→8)-[epicatechin-(4β→8)-]$_3$-catechin.

Table 1. Polyphenolic Glycosides of Douglas-Fir Inner Bark (14)

| |
|---|
| 3'-O-methylepicatechin-7-O-β-D-glucopyranoside |
| epicatechin-7-O-β-D-glucopyranoside |
| catechin-7-O-β-D-glucopyranoside |
| catechin-4'-O-β-D-glucopyranoside |
| phloroglucinol-1-O-β-D-glucopyranoside |
| dihydroquercetin-3'-O-β-D-glucopyranoside |
| dihydroquercetin-7-O-β-D-glucopyranoside |
| dihydrokaempferol-7-O-β-D-glucopyranoside |

The procyanidin polymers of Douglas-fir bark were found to have predominant epicatechin monomer units as the polymer chain extending units. Smaller amounts of catechin played this role. Terminal units consisted of both catechin and epicatechin in almost even abundance.

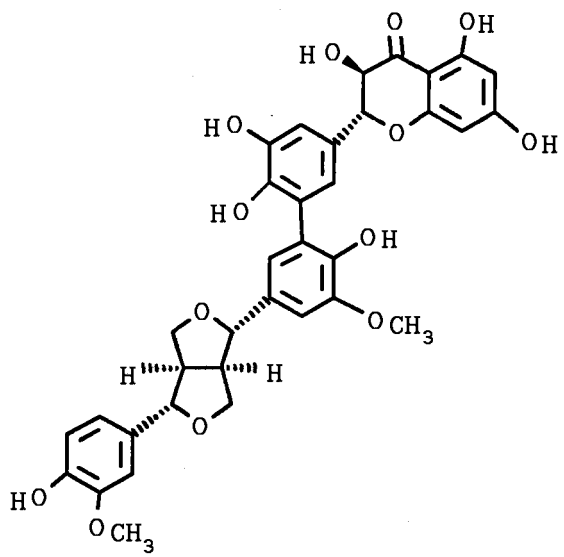
Table 2. Procyanidin Oligomers of Douglas-Fir Inner Bark (15,16)

| |
|---|
| epicatechin-(4β→8)-catechin |
| epicatechin-(4β→8)-epicatechin |
| catechin-(4α→8)-catechin |
| catechin-(4α→8)-epicatechin |
| epicatechin-(4β→8)-epicatechin-(4β→8)-catechin |
| epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin |
| epicatechin-(4β→8)-catechin-(4α→8)-catechin |
| epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin |
| epicatechin-(4β→8)-[epicatechin-(4β→8)-]$_2$-epicatechin |
| epicatechin-(4β→8)-[epicatechin-(4β→8)-]$_2$-catechin |
| epicatechin-(4β→8)-[epicatechin-(4β→8)-]$_3$-catechin |
| epicatechin-(4β→8)-[epicatechin-(4β→8)-]$_3$-epicatechin |

Polymer linkages consisted of C-4 to C-8 interflavanoid bonds predominating over C-4 to C-6 interflavanoid bonds by a ratio of 4 to 1. These results were obtained by acid catalyzed degradation of the polymers using

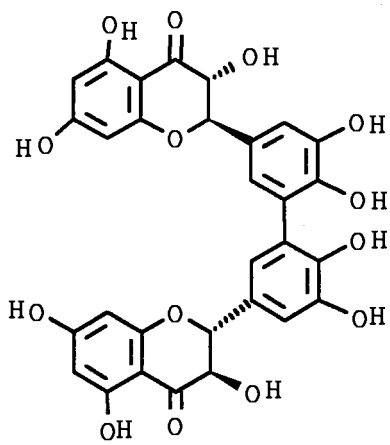
phloroglucinol as a nucleophile.

The outer bark of Douglas-fir has yielded two novel biphenyl linked dimeric polyphenols. (+)-Pseudotsuganol (Structure 3) is the first example of a new class of natural product, a true flavonolignan (6). (+)-[5',5']-Bisdihydroquercetin (Structure 4) also is novel in that it is the first example of a biflavonoid linked exclusively via B rings (18).



Structure 3. (+)-Pseudotsuganol

The phlobaphenes of Douglas-fir outer bark have been partly characterized by $^{13}C$ NMR which shows that they seem to be complex polymeric materials which are composed of condensed tannin functional groups, carbohydrates, and DHQ moieties with high content of methoxyl groups (5). The abundance of methoxyl groups suggests that lignans may also be involved in phlobaphene formation. Other



Structure 4. (+)-[5′, 5′]-Bisdihydroquercetin

structural features such as phenolic ring carbons would have been observed by similar resonances from the B-ring of DHQ and catechin or epicatechin monomer units. The isolation of pseudotsuganol also strongly suggests that lignans are likely precursors. However, fine structural details of the phlobaphene structure, such as monomer linking sequence and type of linkage, are still not completely defined. Much work remains to be done in this area including the isolation and identification of additional phlobaphene precursors.

# EXPERIMENTAL

## 1. Bark Preparation

The bark of Douglas-fir was collected from a 120 years old tree that was harvested in McDonald Forest (17). The separation of the bark to inner and outer parts was completed manually. The air-dried outer bark was then ground to smaller than 0.5 square centimeters by a disk grinder.

## 2. Extraction

The ground outer bark (9738g) was put in a conical flask and extracted with methanol five times at room temperature. In each extraction, the bark was allowed to soak in 14 liters of methanol for 24 hours before decanting. The bark residues were removed by filtration with filter paper. The methanol solution was then evaporated to one half of its volume on a rotary evaporator under reduced pressure.

The solution obtained was transferred to a separatory funnel. Hexane, which was one half of the methanol solution volume, was added. After shaking and setting, the liquid mixture was separated into hexane and

methanol solutions. The methanol solution was extracted with fresh hexane four additional times. The hexane solutions were combined and rotarily evaporated to dryness under reduced pressure to become a light yellow solid, i.e. the hexane soluble extract (35.3g). The methanol solution was also rotarily evaporated under reduced pressure. A slurry of the methanol soluble material was obtained.

The methanol soluble slurry was put into a beaker and ethyl acetate (3.8L) was added. After magnetic stirring for one hour, the insoluble material was removed by filtration. The solid was used in the same operation four more times. The resulting solid was the ethyl acetate insoluble material (109.7g).

The ethyl acetate solutions were combined and dried by evaporation under reduced pressure. The dry ethyl acetate soluble extract was dissolved into diethyl ether. Magnetic stirring was also used for one hour. The insoluble material was filtered and transferred into fresh diethyl ether again. This was repeated five times. The solid left was the diethyl ether insoluble extract (87.5g).

The diethyl ether soluble material was dried and extracted by 1,2-dichloroethane five times. 1,2-Dichloroethane soluble (59.2g) and insoluble (141.9g) extracts were obtained respectively. The 1,2-dichloroethane soluble extract was used in this

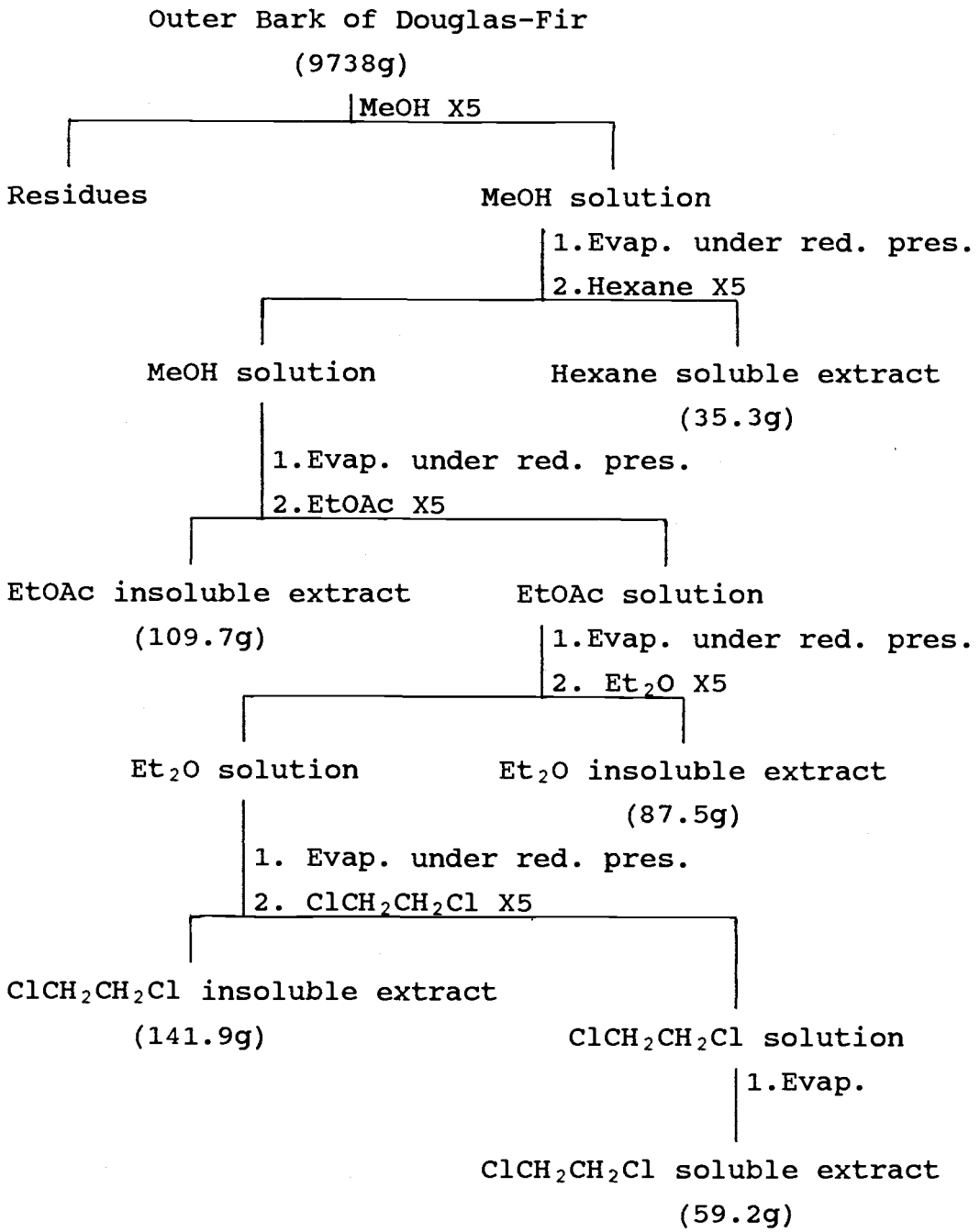



Figure 1. Extraction Procedure

study. The procedure is outlined in Figure 1.

3. Separation and Purification

Column chromatography (CC) was used for the separation of the 1,2-dichloroethane soluble extract. The procedure of packing a column is described below. A column, its diameter and height are determined by sample size, is filled partially with solvent. The stopper is slightly opened to let the solvent drain slowly. A slurry of the solvent and adsorbent is poured into the top of the column. The column is tapped gently when adding the slurry. The solvent is drained to the top of the adsorbent level and held at that level until used.

The adsorbents used in this study were Sephadex LH-20 (Pharmacia, Sweden), Kieselgel 60 (EM, Darmstadt, Germany), MCI-gel CHP20P (Mitsubishi Chemical Industries Ltd., Japan) and Toyopearl (Toso Haas, Philadelphia, PA). All columns were packed by the above method.

Thin-layer chromatography (TLC) was used for checking the results of the column separation and final purification of the compounds. Both analytical and preparative TLC plates were pre-coated with Kieselgel 60 $F_{254}$ (EM, Darmstadt, Germany). The solvent systems used for TLC were:

1. $CHCl_3$:MeOH:HOAc:$H_2O$ (85:15:10:3 v/v)
2. MeOH:$CHCl_3$ (2:98 v/v)

3. Benzene:Acetone:MeOH (90:9:1 v/v)

4. Benzene:Acetone:MeOH (6:3:1 v/v)

5. Benzene:EtOAc (4:1 v/v)

The 1,2-dichloroethane soluble extract was analysed by TLC, developed with solvent 1 and visualized under UV (254 nm) light. There were at least six spots observed.

The extract (50.1g) was dissolved in small amount of MeOH to form a slurry. The slurry was filtered to remove insoluble material (3.3g) and put on the top of Sephadex LH-20 column (7x60 cm). The eluting solvent was a mixture of MeOH-$H_2O$ used in a gradient from 80:20 (v/v) to 100% MeOH. During elution, four main fractions were obtained: fraction **1** (20.1g), fraction **2** (5.0g), fraction **3** (14.9g), and fraction **4** (3.7g).

The fraction **1** (20.1g) dissolved in 25 ml of chloroform was applied to a Kieselgel 60 column (5x90 cm) and eluted with MeOH-$CHCl_3$ (1:99 to 20:80 v/v). The column yielded five fractions: **1A** (10.9g), **1B** (8.3g), **1C** (1.8g), **1D** (0.8g), and **1E** (1.2g) after evaporation of the solvent.

**1A** was checked by TLC. When developed with solvent 2, two separated spots ($Rf_1$=0.39, $Rf_2$=0.75) were visible under UV light. A portion of the fraction (6.7g) was then rechromatographed on a Toyopearl column (5x60 cm, EtOH-$H_2O$, 90:10). Five fractions were collected: **a** (0.11g), **b** (2.28g), **c** (0.61g), **d** (1.78g), and **e** (1.77g) after removal of the solvent.

Fraction **d** (1.56g) dissolved in 10 ml of 90% ethanol was applied to a Toyopearl column (5x60 cm, EtOH-$H_2O$, 90:10 v/v) to give a more purified fraction **d** (1.19g). This fraction (1.00g) was combined with **e** (1.67g) and reapplied twice to a column (5x60 cm) of Sephadex LH-20 (EtOH-$H_2O$, 50:50 v/v) to yield compound **1** (0.54g). A mixture (1.28g) was also eluted from the column.

TLC analysis of this mixture showed that it contained two compounds ($Rf_1$=0.17, $Rf_2$=0.23 in solvent 5). However, attempts to separate them on MCI-gel column (3x60 cm) chromatography (MeOH-$H_2O$, 40:60 v/v), Spherex 10 C8 (MeOH-$H_2O$, 40:60 v/v) HPLC and PLRP-8 ($CH_3CN$-$H_2O$, 25:75 v/v) HPLC were not successful. When 0.38 gram of the mixture was applied to a Kieselgel 60 column (3X60 cm) and eluted with Benzene-EtOAc (80:20 v/v), compound **2** (0.06g) and compound **3** (0.27) were obtained. These two compounds were purified on preparative TLC plates with solvent 5.

Compound **4** (0.10g) was isolated by chromatographing fraction **b** (1.00g) on columns of Toyopearl(3x60 cm, EtOH-$H_2O$, 90:10 v/v), Sephadex LH-20 (3x60 cm, EtOH-$H_2O$, 50:50 v/v), and Kieselgel 60 (3x60 cm, Hexane-EtOAc, 90:10 v/v) successively. The final purification of the compound was carried out on preparative TLC plates with solvent 3.

The main components in fraction **2** were similar to those in fraction **d** and **e**. This result was obtained by

TLC with solvent 3. Hence, no further investigation was done on this fraction.

Fraction **3** was spotted on a TLC plate along with pure DHQ and developed in solvent 1. The result showed that the fraction contains mainly DHQ. This was confirmed by $^1H$ NMR of this fraction. Therefore, it is not necessary to study this fraction further.

Fraction **4** (0.15g) was eluted on a column (3x30 cm) of Sephadex LH-20 with EtOH-$H_2O$ (80:20 v/v), and compound 5 (0.047g) was obtained. The compound was transferred to a Kieselgel 60 column (3x30 cm) and washed with Benzene-Acetone (67:33 v/v) to yield 0.030 gram of the pure compound.

The MeOH insoluble material (3.3g) was chromatographed on a Kieselgel 60 column (5x60 cm, Benzene-EtOAc, 80:20 v/v). Three compounds (0.218g, 0.063g and 0.030g) were eluted from the column. TLC analysis with solvent 5 gave their Rf values of 0.76, 0.67 and 0.53 respectively. $^1H$ NMR spectra of these compounds indicated that they were ferulic acid esters. At present, no further investigation was done on these esters and the following fractions. The sequence of the isolation can be seen in Figure 2.

## 4. Structure Determination

The structures of the compounds were determined

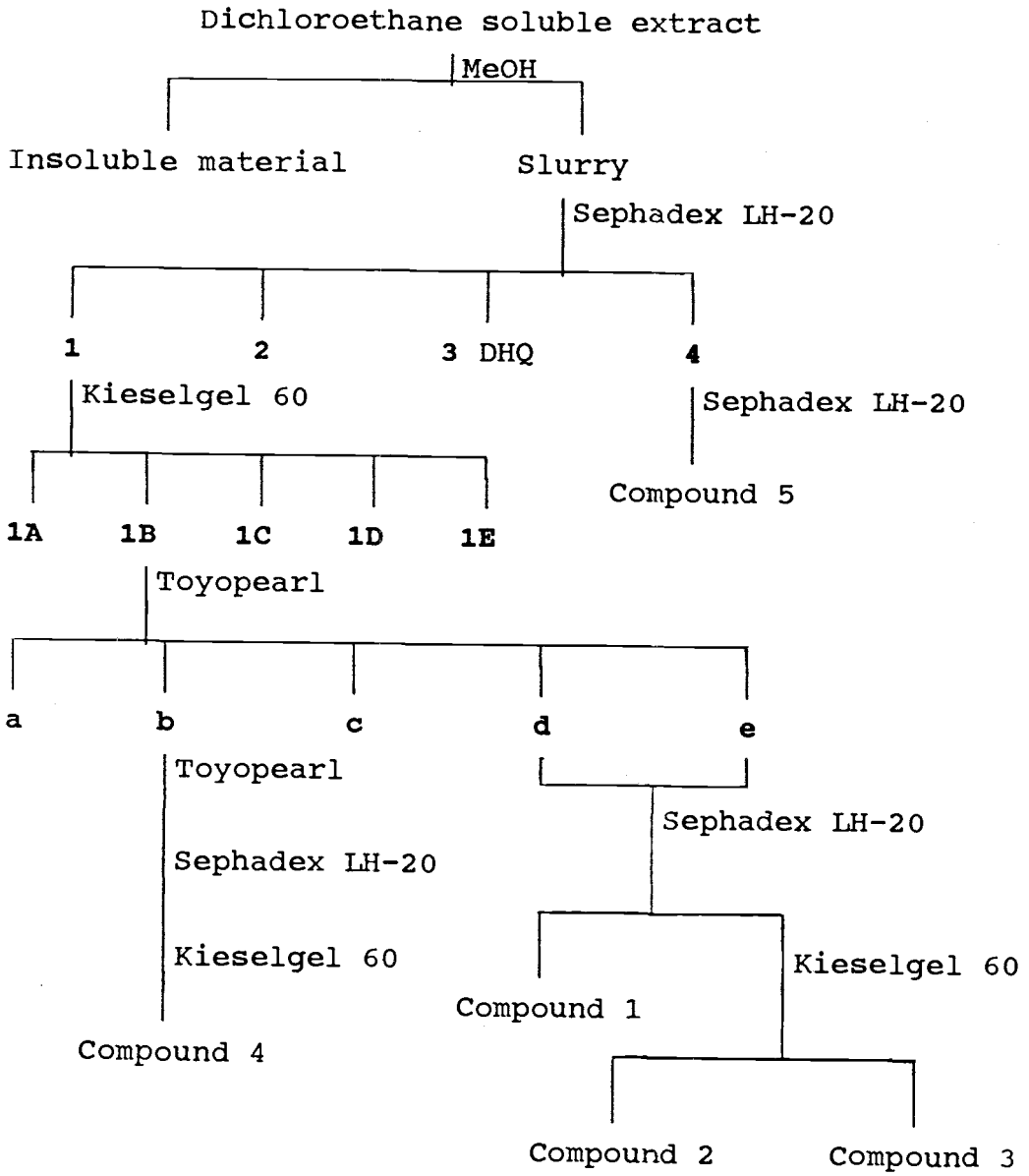



Figure 2. Sequence of Isolation

mainly by NMR spectrometry. The NMR spectra for $^{1}H$ and $^{13}C$ were obtained on a Bruker AM 400 spectrometer at 400 MHz and 100.6 MHz respectively. Two dimensional NMR spectra such as COSY, HETCOR, HETCOSY were also run on this instrument. In addition, mass spectrometry was used to substantiate molecular weights. The samples were dissolved in a mixture of dithiothreitol (DTT) and dithioerythritol (DTE) and run on a Krotos MS-50TC mass spectrometer to obtain negative ion fast atom bombardment mass spectra (22). Optical rotations were measured on a JASCO Model DIP-370 digital polarimeter. Acetone or chloroform was used as the solvent for these measurements. Infrared spectrum was measured on a Nicolet 5DXB fourier transform infrared spectrometer. The sample was mixed with potassium bromide to make a pressed pellet.

Compound 1, furolactone, (+)-2-(3'-methoxy-4'-hydroxyphenyl)-3,7-dioxa-6-oxobicyclo[3.3.0]octane. $C_{13}H_{14}O_5$. Obtained as light brown crystals (540mg), $[\alpha]_{589}$ +108° ($CHCl_3$; c=0.95), Rf 0.75 (Solvent 4). IR $\nu_{max}$ ($cm^{-1}$): 3520, 3400, 3000, 2940, 1760, 1530, 1280, 1250, 1210, 1180, 1050 and 1030. FAB-MS gave an $[M-H]^-$ ion peak at m/z 249. $^{13}C$ NMR (ppm): 45.9, 48.0, 55.9, 69.8, 69.8, 80.6, 108.5, 114.4, 119.0, 130.5, 145.8, 146.9 and 178.2. $^{1}H$ NMR ($\delta$): 3.09 (1H, m), 3.41 (1H, ddd, J=4.0, 8.5, 9.0 Hz), 3.86 (3H, s), 4.13 (1H, dd, J=4.0, 9.0 Hz), 4.28 (1H, dd, J=2.3, 10.0 Hz), 4.31 (1H, dd,

J=9.0, 9.0 Hz), 4.46 (1H, dd, J=7.0, 10.0 Hz), 4.57 (1H, d, J=6.5 Hz), 5.89 (1H, br s), 6.76 (1H, dd, J=2.0, 8.0 Hz), 6.84 (1H, d, J=2.0 Hz) and 6.85 (1H, d, J=8.0 Hz).

Compound 2, (+)-epipinoresinol. $C_{20}H_{22}O_6$. Isolated as light yellow crystals (60 mg), $[\alpha]_{589}$ +95° (Acetone; c=0.085), lit. +130° (23), Rf 0.23 (Solvent 5). FAB-MS gave an $[M-H]^-$ ion peak at m/z 357. $^{13}C$ NMR (ppm): 50.1, 54.4, 56.0, 56.0, 69.7, 71.0, 82.1, 87.7, 108.4, 108.5, 114.2, 114.2, 118.3, 119.1, 130.0, 130.3, 145.3, 146.4, 146.6 and 146.7. $^1H$ NMR (δ): 2.88 (1H, m), 3.28 (1H, dd, J=7.5, 7.5 Hz), 3.30 (1H, m), 3.80 (2H, m), 3.90 (6H, s), 4.10 (1H, dd, J=1.5, 9.0 Hz), 4.41 (1H, d, J=7.0 Hz), 4.84 (1H, d, J=5.5 Hz), 5.64 (2H, br s) and 6.74-6.93 (6H, m).

Compound 3, (+)-pinoresinol. $C_{20}H_{22}O_6$. Isolated as light yellow crystals (270 mg), $[\alpha]_{589}$ +51° (Acetone; c=0.185), lit. +82° (23), Rf 0.17 (Solvent 5). FAB-MS gave an $[M-H]^-$ ion peak at m/z 357. $^{13}C$ NMR (ppm): 54.1, 54.1, 55.9, 55.9, 71.6, 71.6, 85.8, 85.8, 108.5, 108.5, 114.2, 114.2, 118.9, 118.9, 132.9,132.9, 145.2, 145.2, 146.7 and 146.7. $^1H$ NMR (δ): 3.11 (2H, m), 3.85 (6H, s), 3.87 (2H, m), 4.25 (2H, dd, J=6.5, 9.0 Hz), 4.75 (2H, d, J=4.5 Hz), 5.79 (2H, br s) and 6.80-6.92 (6H, m).

Compound 4, unknown. Isolated as a white solid (100 mg). Rf 0.90 (Solvent 3). $^{13}C$ NMR (ppm): 24.8, 24.9, 27.1, 29.0-29.6 (congested 9 peaks), 34.0, 34.0, 129.7 and 174.2. $^1H$ NMR (δ): 0.88 (m), 1.28 (m), 1.60 (m), 2.02

(m), 2.28 (t), 3.64 (s) and 5.31 (t).

Compound 5, (-)-eriodictyol. $C_{15}H_{12}O_6$. Isolated as light brown crystals (30 mg), $[\alpha]_{589}$ -16.5° (Acetone; c=0.091), lit. -21°(24), Rf 0.64 (Solvent 4). FAB-MS gave an $[M-H]^-$ ion peak at m/z 287. $^{13}C$ NMR (ppm): 44.0, 80.4, 96.2, 97.0, 103.3, 114.7, 116.2, 119.3, 131.7, 146.5, 146.8, 164.8, 165.4, 168.3 and 197.7. $^1H$ NMR ($\delta$): 2.67 (1H, dd, J=3.0, 17.0 Hz), 3.04 (1H, dd, J=13.0, 17.0 Hz), 4.93 (4H, br s), 5.24 (1H, dd, J=3.0, 13.0 Hz), 5.87 (2H, m), 6.77 (2H, m) and 6.91 (1H, m).

# RESULTS AND DISCUSSION

## 1. Extraction and Isolation

Ground Douglas-fir outer bark was extracted exhaustively with methanol and then the methanol extract was extracted with hexane, ethyl acetate, diethyl ether and dichloroethane respectively to yield five different extracts. The percentages of these extracts based on the air-dried bark were as follows:

1. Hexane soluble extract: 0.36%
2. EtOAc insoluble extract: 1.13%
3. $Et_2O$ insoluble extract: 0.90%
4. Dichloroethane insoluble extract: 1.46%
5. Dichloroethane soluble extract: 0.61%

The 1,2-dichloroethane soluble extract of Douglas-fir outer bark, which was studied, represented less than 1% of the air-dried bark weight. At first this might seem to be low when compared to other studied fractions of Douglas-fir bark. However, two things should be remembered at this point. One: the bark was obtained from a 120-year-old tree. A younger tree might be expected to have a higher extractive content in view of transformations taking place in the outer bark to give phlobaphenes; and inner bark might also be supposed to

have a higher extractive content because of loss of extractive in the outer bark caused by weathering and fungi. Two: it was anticipated that phenolic compounds in this extract might be precursors to phlobaphenes, the phenolic compounds in this extract would then be transient products. If the chemical transformation from monomer to polymer component is very facile, then one would expect a small constant amount of monomer and larger amounts of polymers. There are demonstratably abundant amounts of phlobaphene polymers in Douglas-fir bark (5).

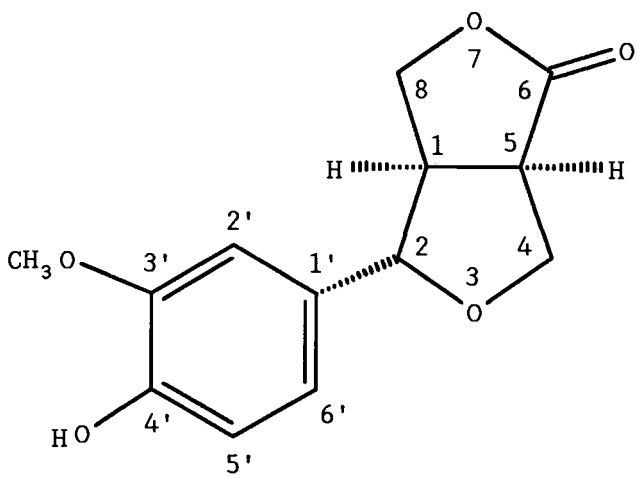
The 1,2-dichloroethane soluble extract was chromatographed repeatedly over columns of Sephadex LH-20, Kieselgel 60 and Toyopearl. During the isolations, analytical TLC techniques were used to monitor the results. Five compounds were obtained after purification by column chromatography and preparative TLC.

The availability of new chromatographic gels such as MCI-gel and Toyopearl have greatly enhanced the ability of researchers to isolate complex polyphenols. The procedure has been to alternate separations between columns of Sephadex LH-20 and one of the new gels (25). It was found in this research that Toyopearl worked well, but that column chromatography over Kieselgel 60 was also needed to complement the soft gels. In the final purification, preparative TLC or Kieselgel 60 column was still needed although it was observed that significant

losses of material sometimes still occurred on this silica material. Generally, polyphenolic natural products chemists avoid doing preparative isolation over silica gel due to excessive loss because of irreversible adsorptions. However, the compounds isolated here probably represent the limit with regard to molecular weight and number of hydroxyl groups for silica based chromatography.

## 2. Structure Determinations

Compound 1. furolactone, (+)-2-(3'-methoxy-4'-hydroxyphenyl)-3,7-dioxa-6-oxobicyclo[3.3.0]octane.




Structure 5. (+)-2-(3'-methoxy-4'-hydroxyphenyl) -3,7-dioxa-6-oxobicyclo[3.3.0]octane.

Negative ion FAB-MS of this compound gave a strong $[M-H]^-$ ion peak at m/z 249 indicating that this phenol

Table 3. $^{1}H$ NMR Assignments of (+)-Furolactone, (+)-Epipinoresinol and (+)-Pinoresinol[1,2]

| proton | furolactone δ(J)[3] | epipinoresinol δ(J)[3] | pinoresinol δ(J)[3] |
|---|---|---|---|
| 1 | 3.09m | 2.88m | 3.11m |
| 2 | 4.57d(6.5) | 4.41d(7.0) | 4.75d(4.5) |
| 4a | 4.13dd(4.0,9.0) | 3.28dd(7.5,7.5) | 3.87m |
| 4e | 4.31dd(9.0,9.0) | 3.80m | 4.2dd(6.5,9.0) |
| 5 | 3.41ddd(4.0,8.5,9.0) | 3.30m | 3.11m |
| 6 | --- | 4.84d(5.5) | 4.75d(4.5) |
| 8a | 4.28dd(2.3,10.0) | 3.80m | 3.87m |
| 8e | 4.46dd(7.0,10.0) | 4.10dd(1.5,9.0) | 4.25dd(6.5,9.0) |
| 2′/2′′ | 6.84d(2.0) | | |
| 5′/5′′ | 6.85d(8.0) | 6.74-6.93m | 6.80-6.92m |
| 6′/6′′ | 6.76dd(2.0,8.0) | | |
| OH | 5.89brs | 5.64brs | 5.79brs |
| $OCH_3$ | 3.86s | 3.90s | 3.85s |

[1]-all samples in $CDCl_3$

[2]-s=singlet, d=doublet, m=multiplet, br=broad

[3]-J in Hz

Table 4. $^{13}C$ NMR Assignments of (+)-Furolactone, (+)-Epipinoresinol and (+)-Pinoresinol[1]

| carbon | furolactone (ppm) | epipinoresinol (ppm) | pinoresinol (ppm) |
|---|---|---|---|
| 1 | 48.0 | 54.4 | 54.1 |
| 2 | 86.0 | 87.7 | 85.8 |
| 4 | 69.8 | 69.7 | 71.6 |
| 5 | 45.9 | 50.1 | 54.1 |
| 6 | 178.2 | 82.1 | 85.8 |
| 8 | 69.8 | 71.0 | 71.6 |
| 1′ | 130.5 | 133.0 | 132.9 |
| 2′ | 108.5 | 108.5 | 108.5 |
| 3′ | 146.9 | 146.7 | 146.7 |
| 4′ | 145.8 | 145.3 | 145.2 |
| 5′ | 114.4 | 114.2 | 114.2 |
| 6′ | 119.0 | 119.1 | 118.9 |
| 1′′ | --- | 130.3 | 132.9 |
| 2′′ | --- | 108.4 | 108.5 |
| 3′′ | --- | 146.4 | 146.7 |
| 4′′ | --- | 144.6 | 145.2 |
| 5′′ | --- | 114.2 | 114.2 |
| 6′′ | --- | 118.3 | 118.9 |
| $OCH_3$ | 55.9 | 56.0 | 55.9 |

[1]-all samples in $CDCl_3$

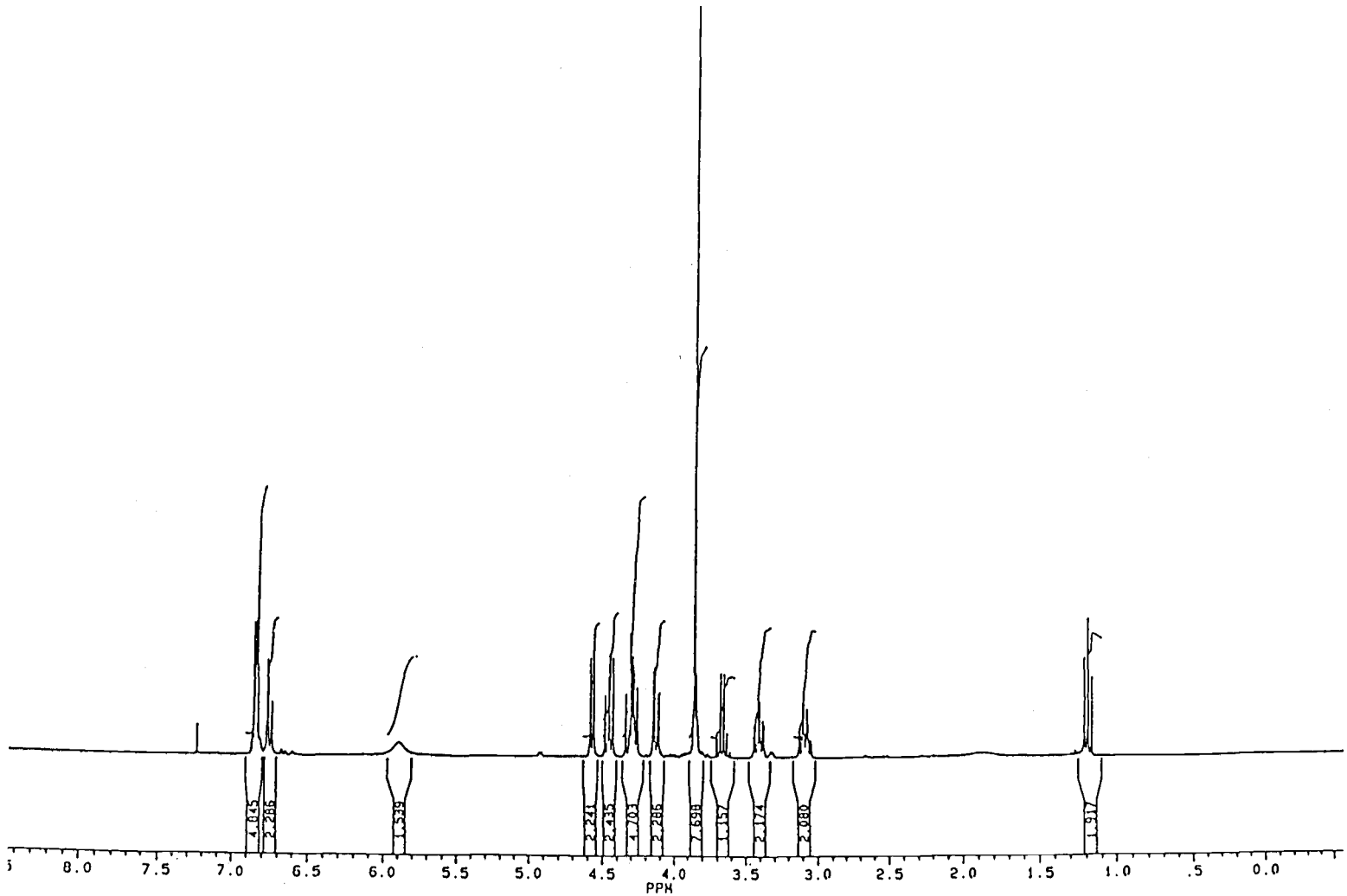



Figure 3. $^{1}H$ NMR Spectrum of Furolactone

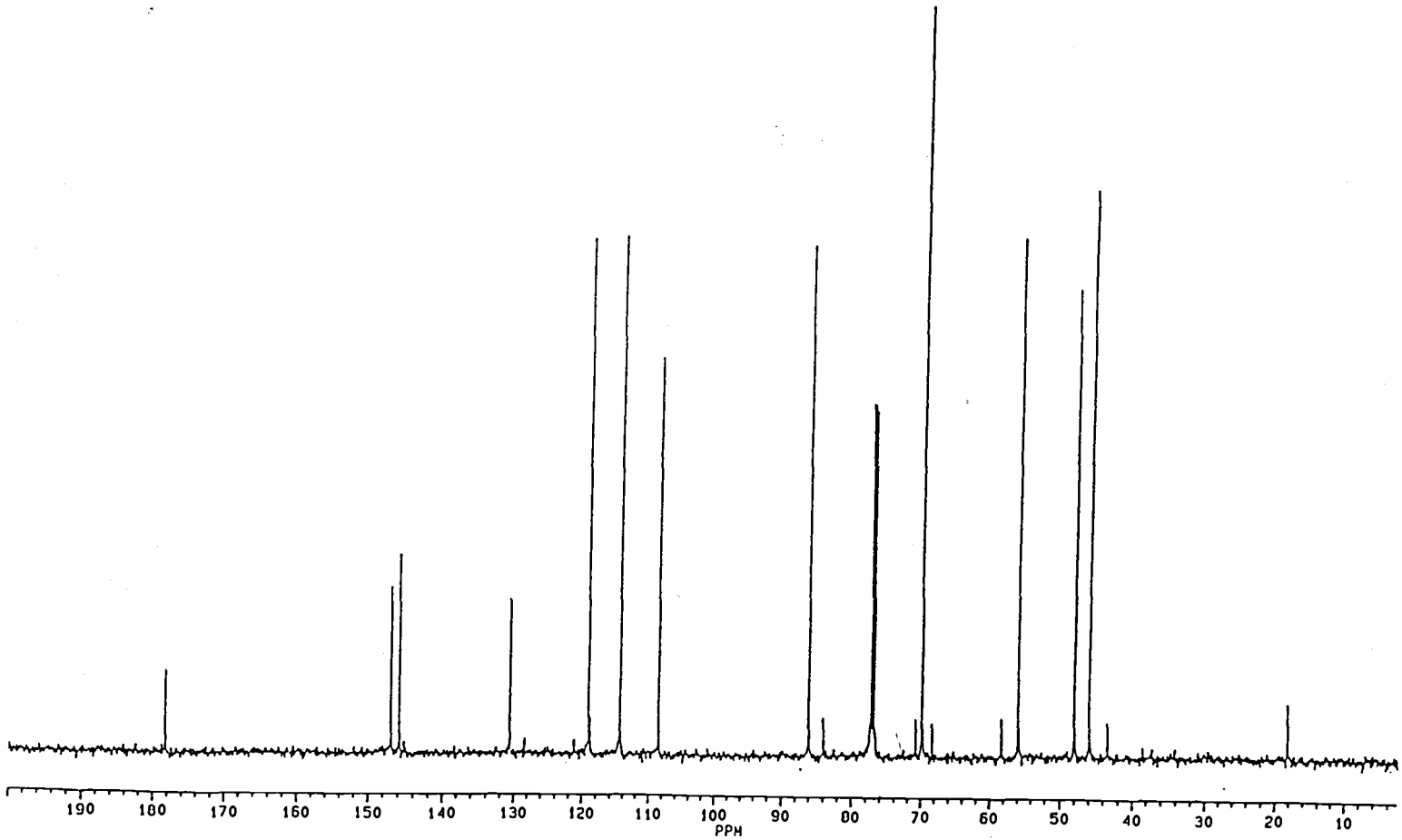



Figure 4. $^{13}C$ NMR Spectrum of Furolactone

had a molecular weight of 250. $^{1}H$ and $^{13}C$ NMR assignments are shown in Tables 3 and 4. The $^{13}C$ NMR spectrum showed signals for 13 carbons. A guaiacyl aromatic ring is evident from the series of signals at 108.5, 114.4, 119.0, 130.5, 145.8 and 146.9 ppm (26) combined with the signal at 55.9 ppm which is due to the methoxyl group. The signal at 178.2 ppm is due to the carbonyl functional group. The remaining signals at 45.9, 48.0, 69.8, 69.8 and 86.2 ppm are suggestive of a furolactone type aliphatic ring system (27). The bridge head carbons C-1 and C-5 give signals at 48.0 and 45.9 ppm. The C-2 signal at 86.0 ppm is characteristic of an equatorial substituted benzylic carbon such as in (+)-pinoresinol (28). C-4 and C-8 signals are both seen at 69.8 ppm as a double strike. These assignments are based on a two dimensional NMR HETCOR spectrum which correlated carbon and hydrogen connectivities. This spectrum showed that the protons at δ 6.84, 6.85 and 6.76 are bonded to C-2′, C-5′ and C-6′ respectively. The proton signals at δ 3.09 and 3.41 connected with the bridge head carbons signals for C-1 and C-5 separately. The proton on the benzylic carbon C-2 was at δ 4.57. Two protons at δ 4.13 and 4.31 were attached to C-4 and another two at δ 4.28 and 4.46 were attached to C-8.

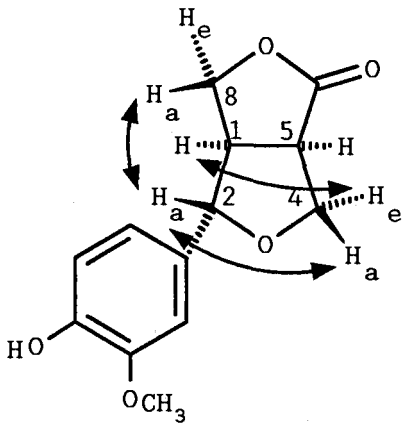
The 2-D NMR HETCOSY spectrum, which shows 2 and 3 bond long-range carbon-hydrogen coupling relationships, indicated that there was a correlation between C-3′ and

the methoxyl hydrogens, thus, establishing that the methoxyl group was at C-3′ while the hydroxyl group was at C-4′. The 2-D NMR COSY spectrum supported the assignments above.

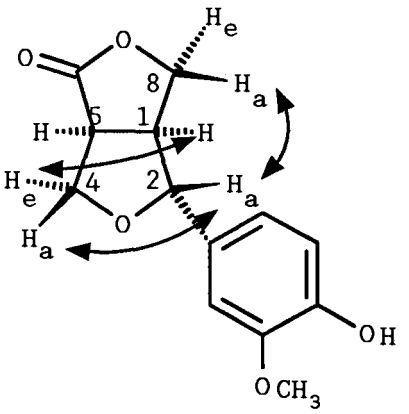
The IR spectrum showed a strong absorption at 1760 $cm^{-1}$. This further supported that the furolactone was a $\gamma$-lactone (29).

The stereochemistry of this compound was established by application of the nuclear Overhauser effect (NOE) technique. The saturation of the benzylic proton H-2 ($\delta$ 4.57) gave positive responses in the signals of H-4a ($\delta$ 4.13) and H-8a ($\delta$ 4.28), but gave no observable enhancement of the H-5 ($\delta$ 3.41) resonance. The irradiation of H-4e ($\delta$ 4.31) enhanced the response of H-1 ($\delta$ 3.09), and as expected, gave no positive response for H-2 signal. Thus, H-2, H-4a and H-8a were considered cis to one another in the molecular structure and thus established the relative stereochemistry as 5A or its enantiomer 5B.

The enantiomer 5A is preferred due to the fact that the sign of optical rotation of the furolactone is (+) which is same as those of (+)-pinoresinol and (+)-epipinoresinol. This strategy was used by Carvalho in the tentative assignment of absolute stereochemistry for the furolignan (+)-methoxypiperitol (30). In support of this contention, the furolactone 5 was shown to be produced from (+)-pinoresinol by E. typhina in culture



5A

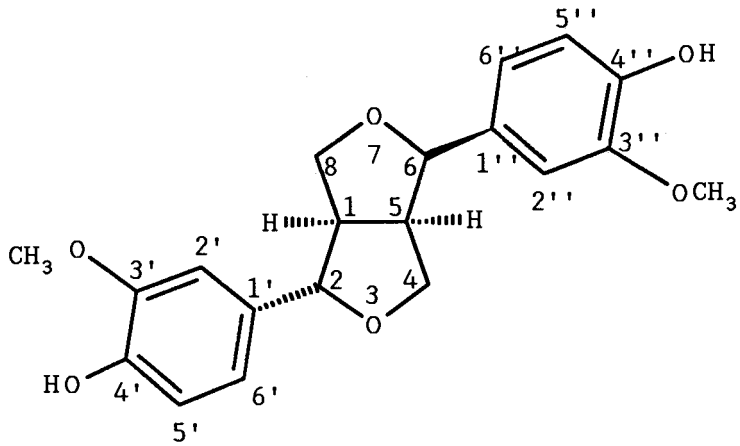


5B

(31). This has been the only other reported isolation of this rare compound in the literature. Because of the common structure of (+)-pinoresinol to 5 and the fact that it is likely derived from (+)-pinoresinol, it is reasonable to tentatively assign its absolute stereochemistry as 5A.

Compound 2. (+)-epipinoresinol

Negative ion FAB-MS of this compound gave a strong $[M-H]^-$ ion peak at m/z 357 indicating that this phenol had a molecular weight of 358. $^1H$ and $^{13}C$ NMR assignments are summerized in Tables 3 and 4. The $^{13}C$ NMR spectrum showed signals for 20 carbons. The signals at 50.1, 54.4, 69.7, 71.0, 82.1 and 87.7 ppm represent a 3,7-dioxabicyclo[3.3.0]octane lignan type ring system




Structure 6. (+)-epipinoresinol

(27). Two bridge head carbons, C-1 and C-5, show signals at 54.4 and 50.1 ppm respectively. The C-2 signal at 87.7 ppm is characteristic of an equatorial substituted benzylic carbon, while the C-6 signal at 82.1 ppm is typical of an axial substituted benzylic carbon (28). The signals of C-4 and C-8 are observed at 69.7 and 71.0 ppm respectively. The remaining signals are evidence of two guaiacyl aromatic ring systems. Due to their differences of bonding to the aliphatic bicyclo rings, the chemical shifts of the two guaiacyl ring carbons vary slightly except C-1′ and C-1′′, where the chemical shift difference is 2.7 ppm. These assignments are confirmed by 2-D NMR such as COSY, HETCOR and HETCOSY, and in agreement with the data in literature (32,33). The optical rotation of $[\alpha]_{589}$ +95° (Acetone; c=0.085) indicates that the compound is the (+) enantiomer (23) as shown in Structure 6.

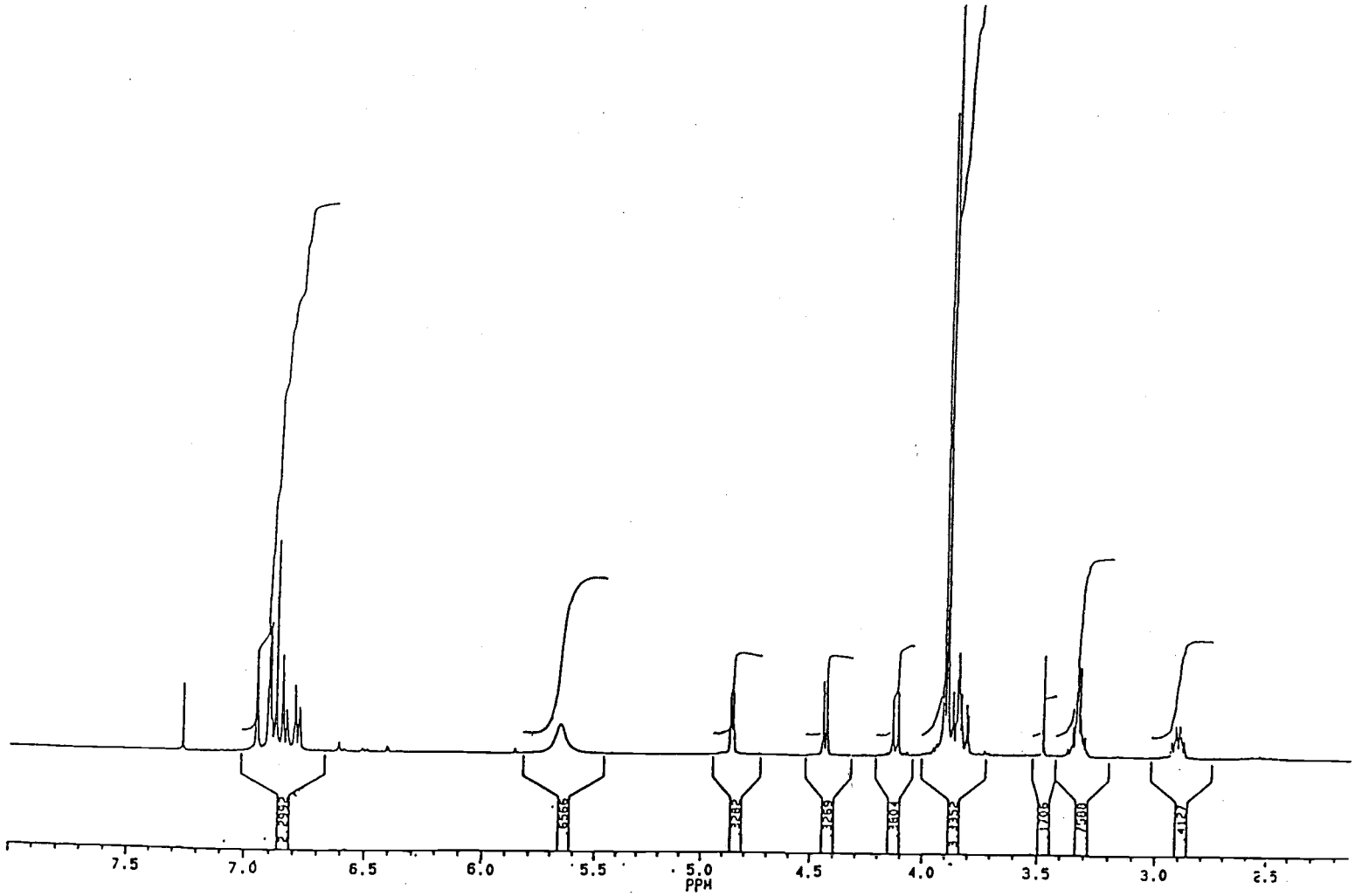



Figure 5. $^{1}H$ NMR Spectrum of Epipinoresinol

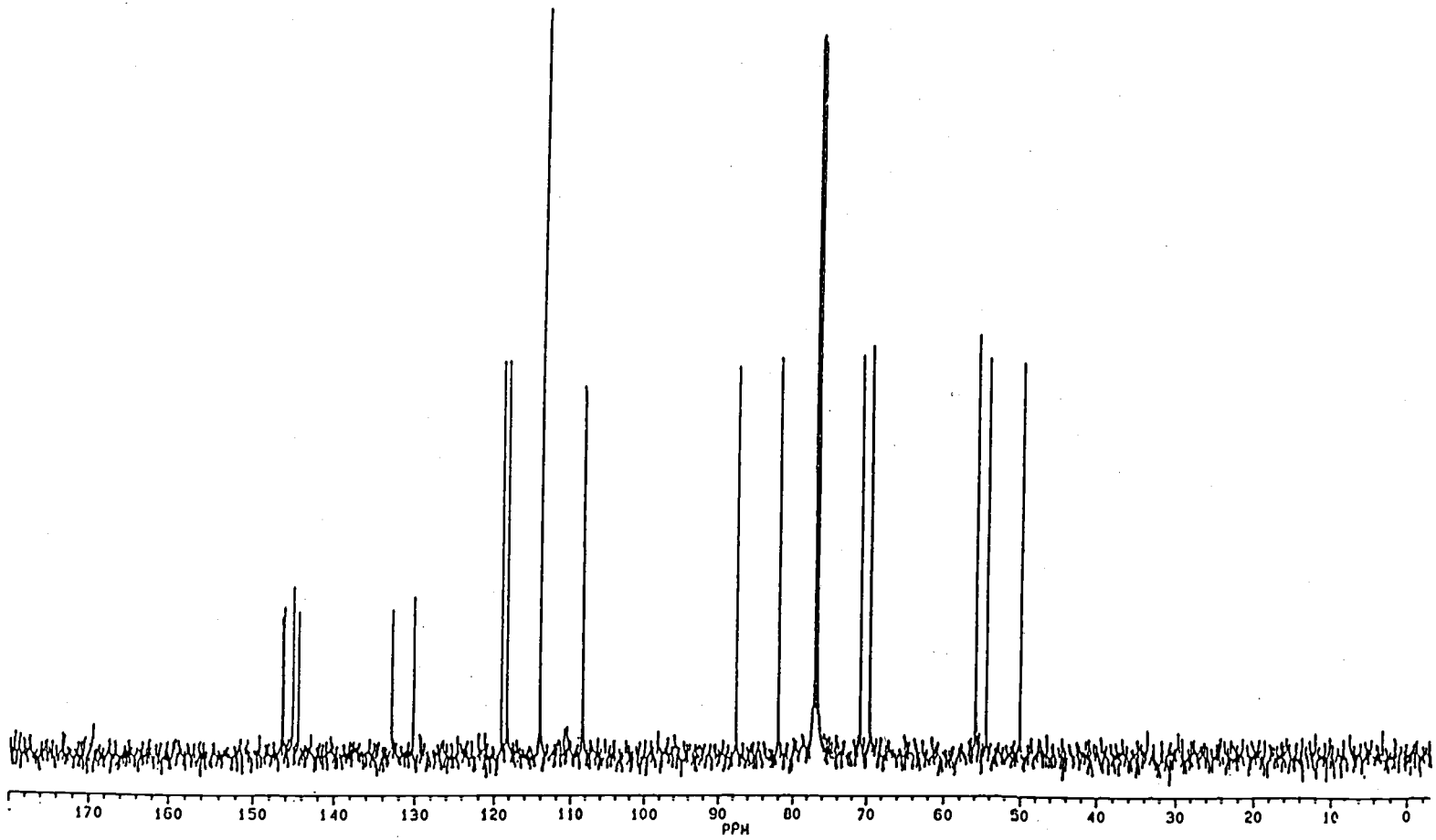



Figure 6. $^{13}C$ NMR Spectrum of Epipinoresinol

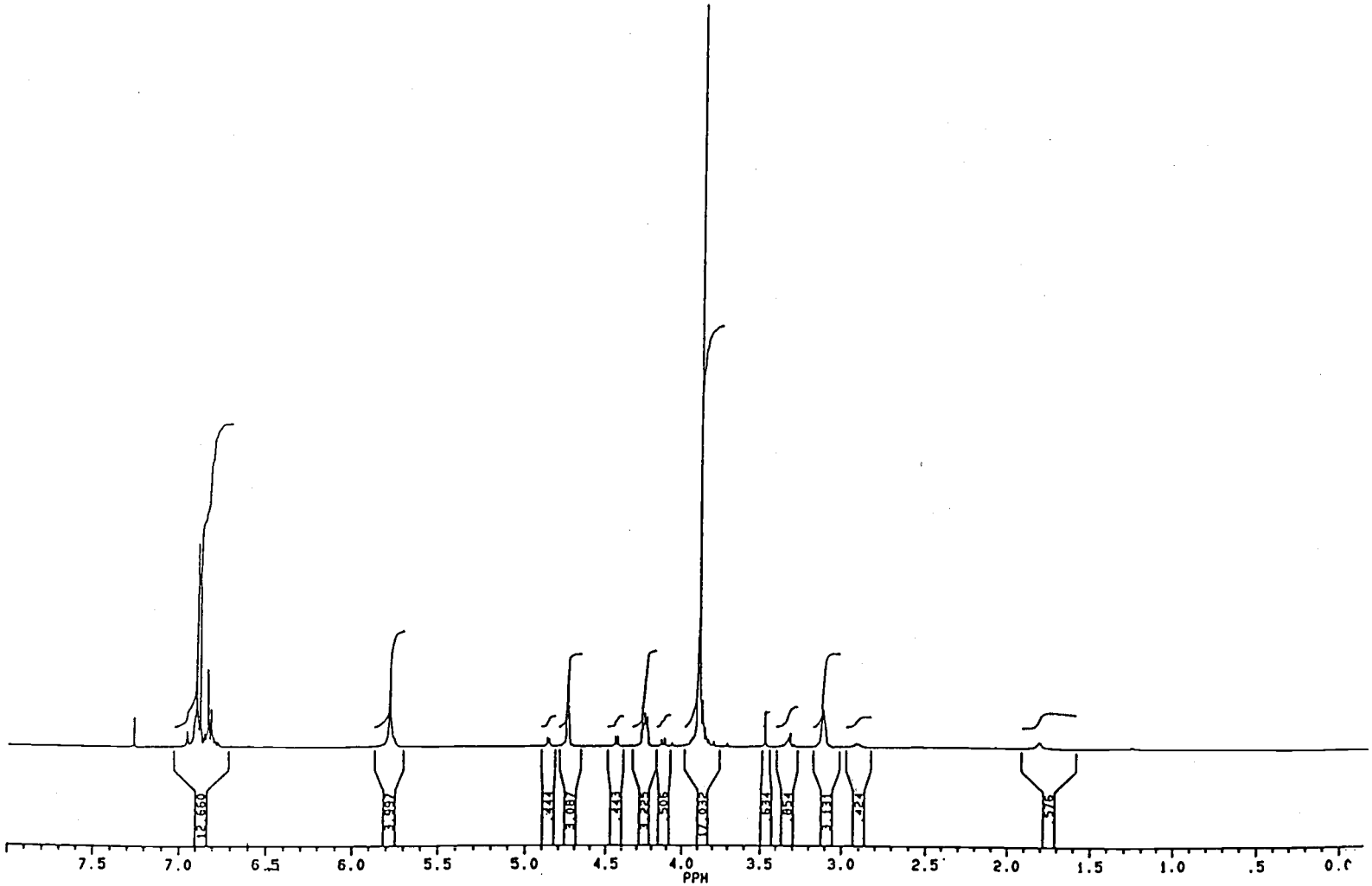



Figure 7. $^{1}H$ NMR Spectrum of Pinoresinol

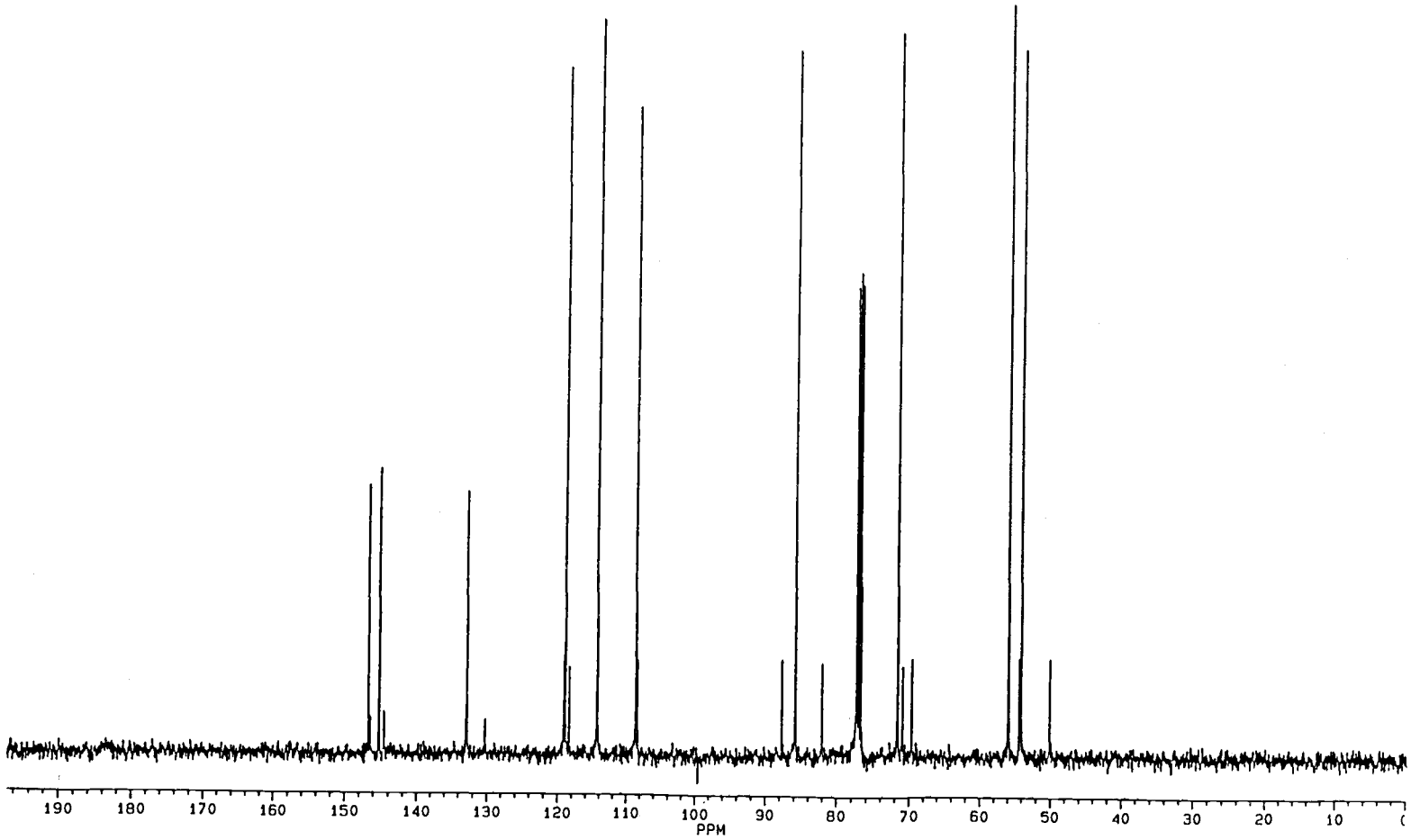
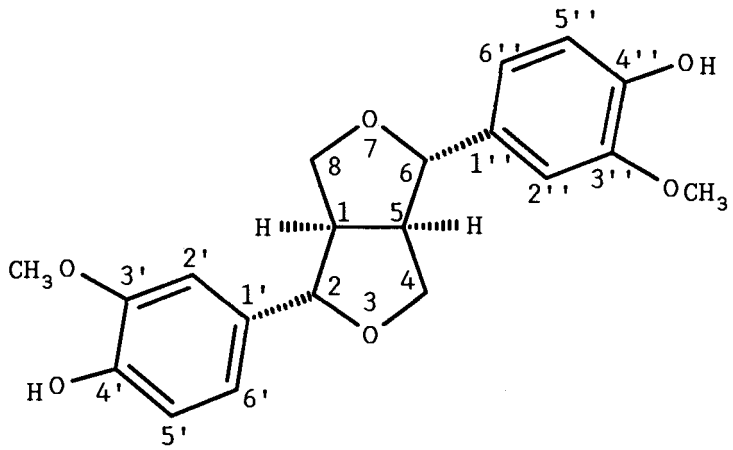



Figure 8. $^{13}C$ NMR Spectrum of Pinoresinol

Compound 3. (+)-pinoresinol



Structure 7. (+)-pinoresinol

Negative ion FAB-MS of this compound was very similar to that of epipinoresinol showing a strong $[M-H]^-$ ion peak at m/z 357. However, the $^{13}C$ NMR spectrum showed only 10 peaks due to the symmetry of this compound. The $^{1}H$ and $^{13}C$ NMR assignments are shown in Tables 3 and 4. In the $^{13}C$ NMR spectrum, signals at 108.5, 114.2, 118.9, 132.9, 145.2, 146.7 and 55.9 ppm show the characteristic signals of a guaiacyl aromatic ring system. The other signals at 54.1, 71.6 and 85.8 ppm are in agreement for pinoresinol structure (28). The signal at 85.8 ppm is assigned to both C-2 and C-6 which are equatorial substituted benzylic carbons (28). The bridge head carbons, C-1 and C-5, are observed at 54.1 ppm. The C-4 and C-8 signals are observed at 71.6 ppm. The optical rotation of $[\alpha]_{589}$ +51° (Acetone; c=0.185) indicates that

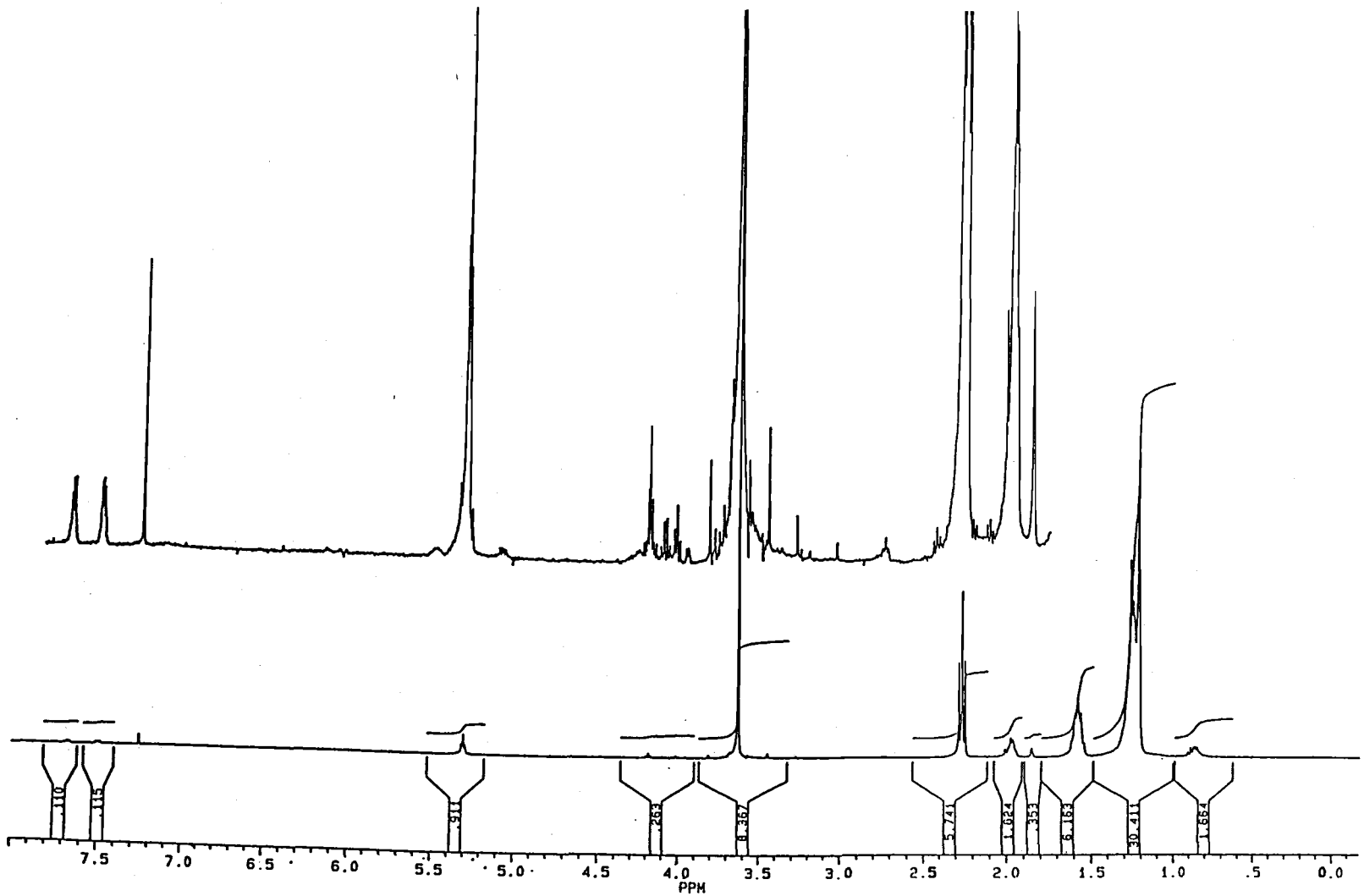



Figure 9. $^{1}H$ NMR Spectrum of Compound 4

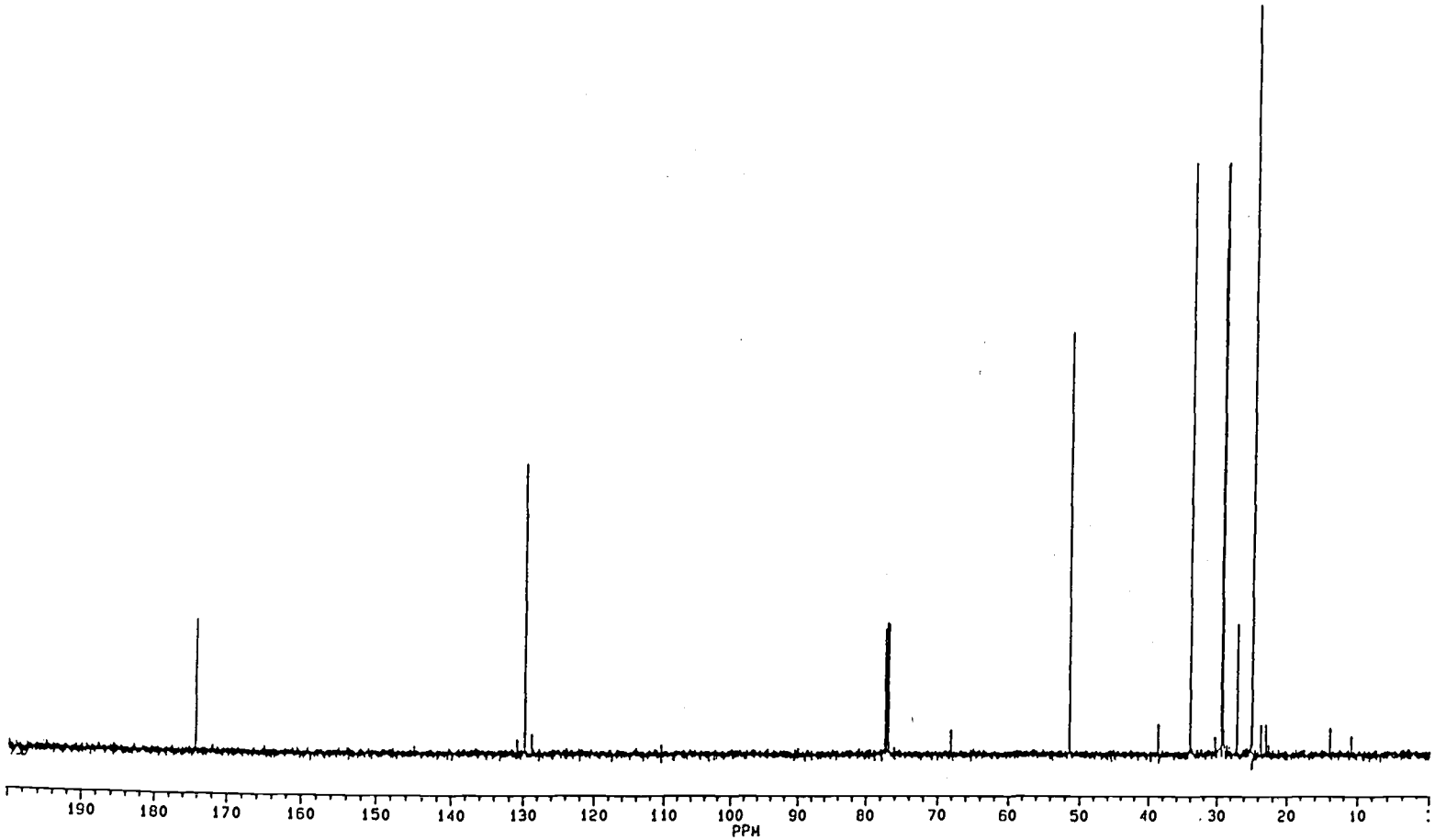
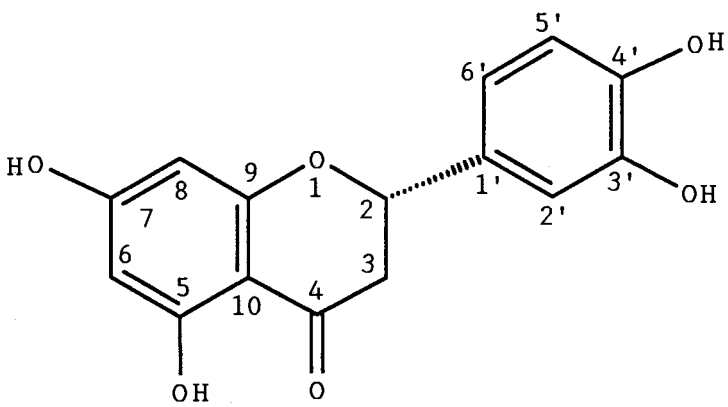



Figure 10. $^{13}C$ NMR Spectrum of Compound 4

this compound is the (+) enantiomer (23) as shown in Structure 7.

Compound 4. unidentified. According to $^{1}H$ and $C^{13}$ NMR spectra (Figures 9 and 10), this is not a phenolic compound. Hence, its structural elucidation was not further persued.

Compound 5. (-)-eriodictyol




Structure 8. (-)-eriodictyol

Negative ion FAB-MS of this compound gave a strong $[M-H]^-$ ion peak at m/z 287 indicating that this phenol had a molecular weight of 288. $^{1}H$ and $^{13}C$ NMR assignments are shown in Table 5. The $^{13}C$ NMR spectrum showed signals for 15 carbons. A flavonoid type A ring system is evident from the series of signals at 96.2, 97.0, 103.3, 164.8, 165.4 and 168.3 ppm (34). Because C-5, C-7 and C-9 are oxygen-bearing carbons, their signals are downfield to

Table 5. $^{1}H$ and $^{13}C$ NMR Assignments of (-)-Eriodictyol[1,2]

| Proton | (-)-Eriodictyol δ(J)[3] | Carbon | (-)-Eriodictyol (ppm) |
|---|---|---|---|
| 2 | 5.24dd(3.0,13.0) | 2 | 80.4 |
| 3a | 2.67dd(3.0,17.0) | 3 | 44.0 |
| 3e | 3.04dd(13.0,17.0) | 4 | 197.7 |
| 6,8 | 5.87m | 5 | 165.4 |
| 2′,5′ | 6.77m | 6 | 97.0 |
| 6′ | 6.91m | 7 | 168.3 |
| | | 8 | 96.2 |
| | | 9 | 164.8 |
| | | 10 | 103.3 |
| | | 1′ | 131.7 |
| | | 2′ | 116.2 |
| | | 3′ | 146.8 |
| | | 4′ | 146.5 |
| | | 5′ | 114.7 |
| | | 6′ | 119.3 |

[1]-spectra run in $CD_3OD$

[2]-d=doublet, m=multiplet

[3]-J in Hz

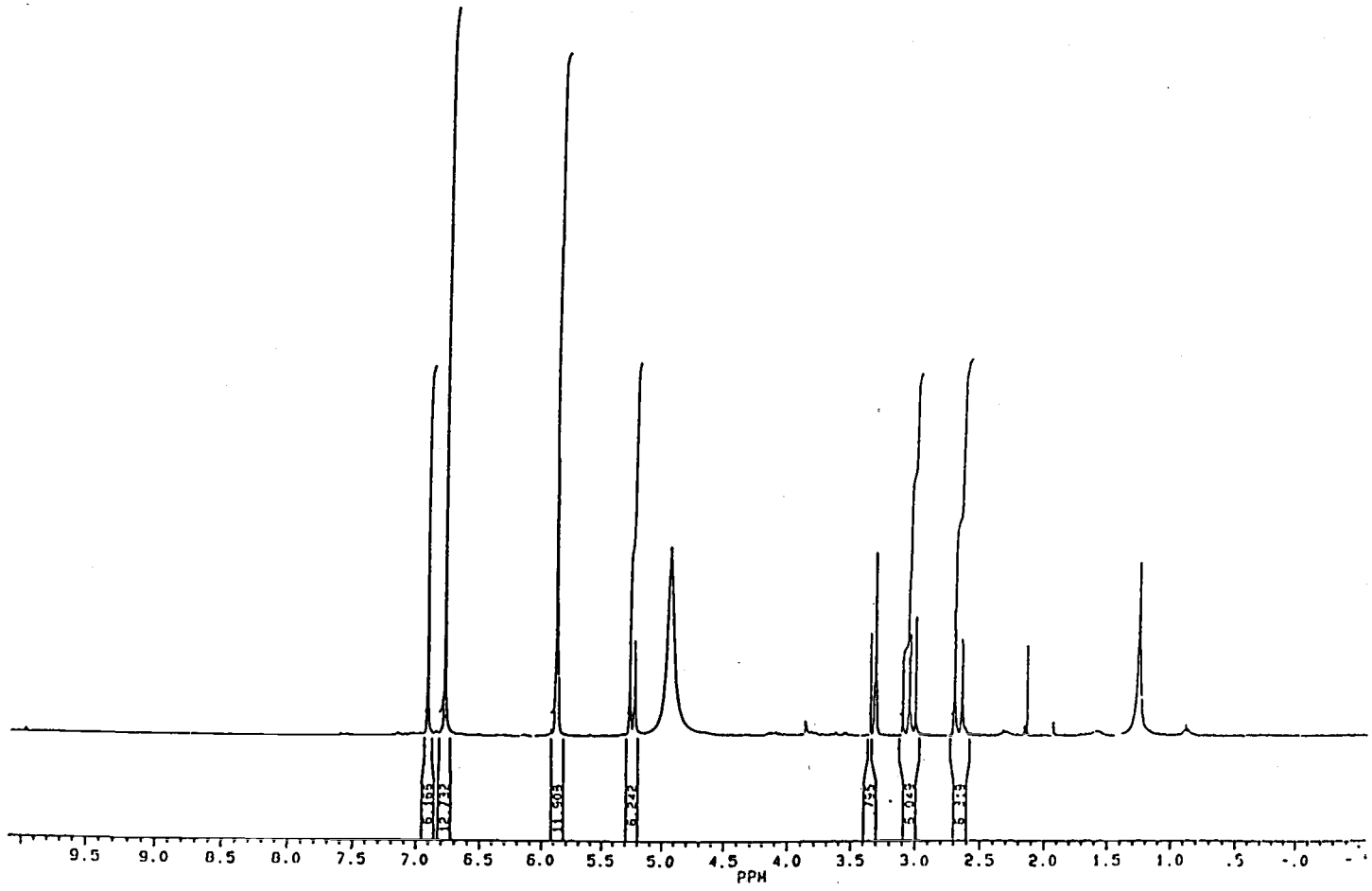



Figure 11. $^{1}H$ NMR Spectrum of Eriodictyol

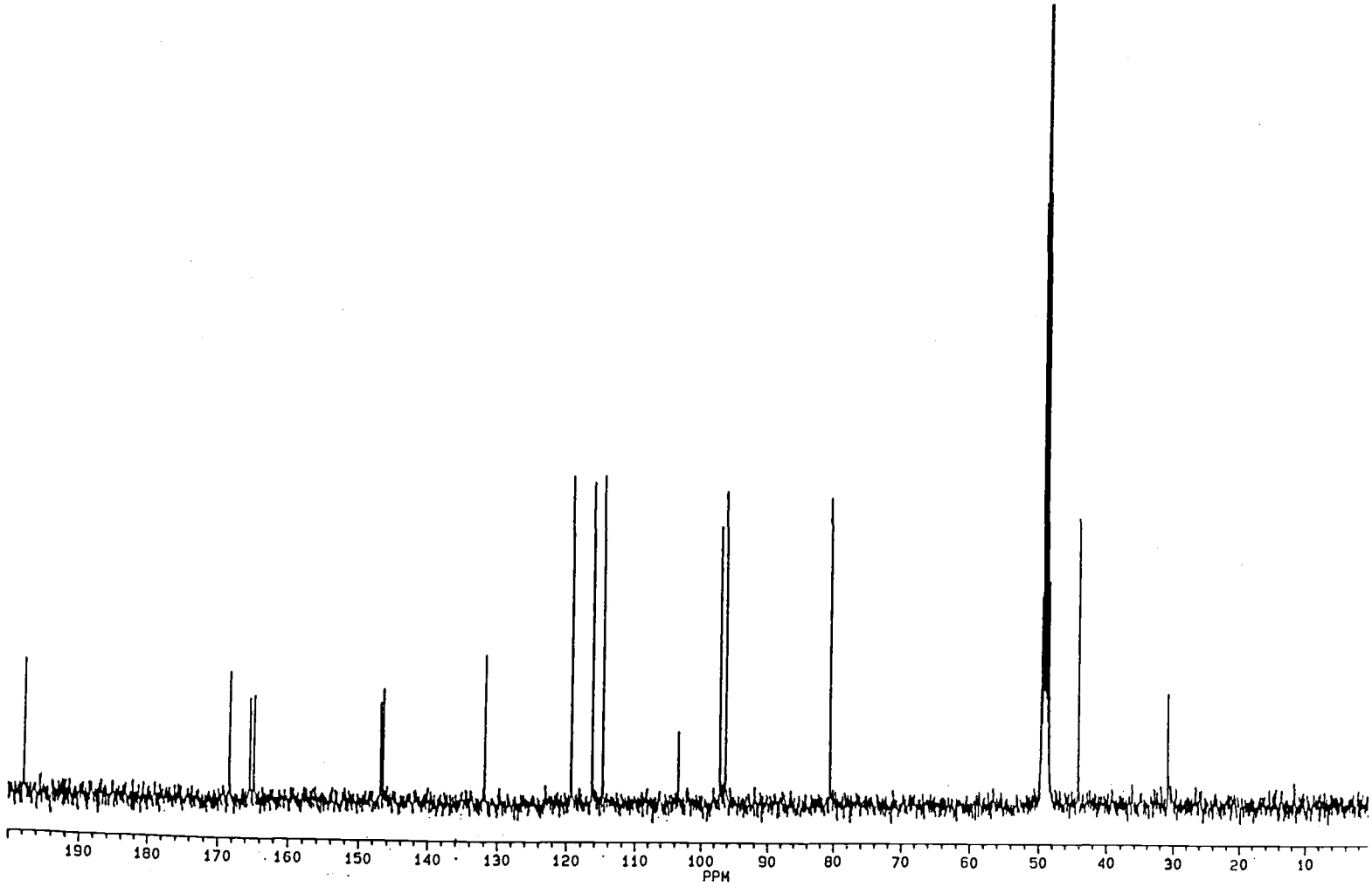


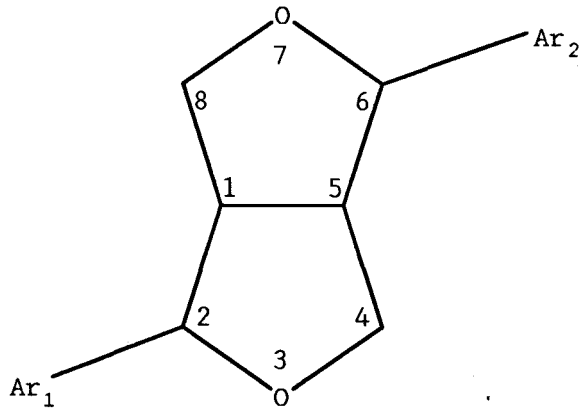

Figure 12. $^{13}C$ NMR Spectrum of Eriodictyol

the three other A ring carbons by about 70 ppm, i.e. 165.4, 168.3 and 146.8 ppm respectively. The signals at 114.7, 116.2, 119.3, 131.7, 146.5 and 146.8 ppm are characteristic of a flavonoid B ring in which C-3′ at 146.8 ppm and C-4′ at 146.5 ppm are also oxygen-bearing carbons. The signal at 197.7 ppm is due to the carbonyl group at C-4. The signals at 80.4 and 44.0 ppm are assigned as C-2 and C-3 respectively. These assignments match with those in publication (35). The optical rotation of $[\alpha]_{589}$ -16.5° (Acetone; c=0.091) indicates the stereochemistry shown in Structure 8 (24).

3. Significance of the Compounds Isolated

Lignans are widely distributed in woody plants. They are compounds whose chemical structures are made up of two $C_6C_3$ (phenylpropane) units linked by the central carbon of the side chain (36). Lignans can be divided into six subgroups depending on their exact structures (27). (+)-Pinoresinol and (+)-epipinoresinol are known as furofuran type lignans which have the general structure 9. The furofurans are one of the largest groups of lignans and are widely distributed in plants. However, this is the first report of such compounds in Douglas-fir.

Comparison of $^{13}C$ NMR data for (+)-pinoresinol and (+)-epipinoresinol to the data published for Douglas-fir




Structure 9. Skeleton of furofuran lignan

bark phlobaphenes gives evidence to the idea that these lignans are precursors (5). For example, both the lignans and the phlobaphenes have a signal around 55 ppm which is assigned as a methoxyl group; the signals of the bridge head carbons for these compounds are also similar to each other i.e. in the range of 50 to 56 ppm; furthermore, the similaries of the chemical shifts for the signals of a guaiacyl aromatic ring system can be found in both the lignans and the phlobaphenes too.

The isolation of dimeric compound pseudotsuganol (Structure 3) also supports this contention. Clearly, formation of pseudotsuganol most likely occurs via oxidative coupling of two phenolic ring systems. The formation of C-C bonds at C-5′ or C-5" position is consistent with the formation of ortho and/or para aroxyl radicals (6). Lignan molecules, such as (+)-pinoresinol, could also react with themselves. Malan has recently

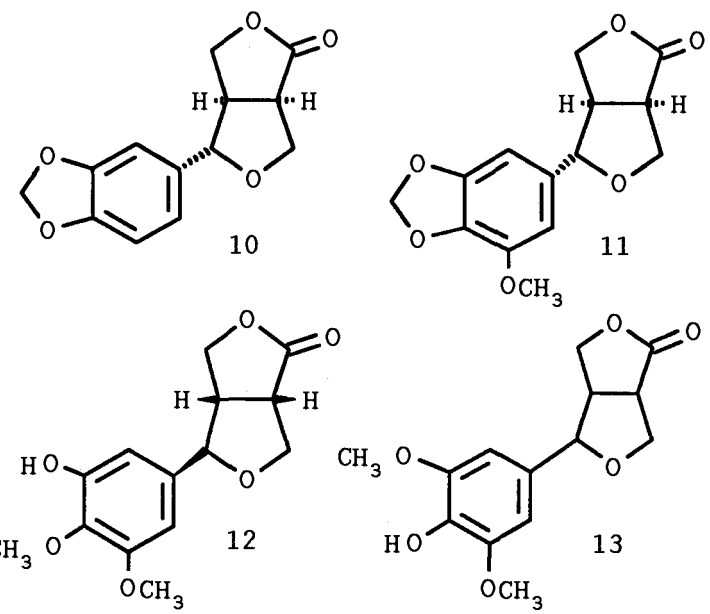
demonstrated that a pinoresinol dimer can be formed under oxidative reaction conditions. The dimer is linked through the C-5′ or C-5" position on each aromatic ring (37).

One might expect higher abundances of (+)-pinoresinol and (+)-epipinoresinol than that were found in the outer bark. Given their reactivity to oxidative coupling, they are most likely transient compounds in the bark. That is they are produced biosynthetically, as the tree grows, but they do not accumulate. Rather, they are probably constantly undergoing secondary reactions to form phlobaphenes and other compounds.

The (+)-furolactone (structure 5) is a very rare compound being reported only once before in the literature (30). It is most likely derived from (+)-pinoresinol. Koshino has showed that this reaction occurs by feeding (+)-pinoresinol to E. typhina to form the (+)-furolactone. That the (+)-furolactone is found in Douglas-fir outer bark strongly suggests that a fungi or other organisms on the bark may be doing this transformation. Other examples of these types of lignan related compounds are known in the literature (30,38-40). Their structures are shown in 10-13.

These furolactones were found to exist in various plants. 10 was isolated from the aerial parts of H. acuminatum. 11 was obtained from the trunk bark of

Nectandra turbacensis (Lauraceae). 12 was separated from Ormosia flava (Ducke) Rudd. 13 was isolated from I. pubescens glabra. Carvalho (30) suspected that 11 might be an artifact because under the influences of heat, acids and bases, the furolactone could be derived from the corresponding 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane by loss of one




10. Acuminatolide

11. (1R,2S,5R)-2-(3'-Methoxy-4',5'-methylenedioxyphenyl)-3,7-dioxa-6-oxobicyclo[3.3.0]octane

12. Ormosalin

13. glaberide

aryl group (28).

The formation of these furolactone compounds shows that we must consider the bark as an ecological unit. A

tree does not live in enviromental isolation. Outer bark has organisms growing on it naturally and they are part of the system. In the future, we are likely to see more compounds isolated that are produced by one organism and modified by a second organism living on the first.

(-)-Eriodictyol is a well known flavonoid compound. However, this is the first time it has been isolated from Douglas-fir. Like the lignans, this compound possibly undergoes oxidative coupling reactions to incorporate itself into the polymeric phlobaphenes. The coupling may occur between one of the nucleophilic sites on the A-ring (C-6 and C-8) and any of the electrophilic sites on the B-ring (C-2′, C-5′ and C-6′).

# CONCLUSIONS

Douglas-fir outer bark was extracted with methanol exhaustively and the methanol soluble material was then extracted with hexane, ethyl acetate, diethyl ether and 1,2-dichloroethane, resulting in five different extracts.

The dichloroethane soluble extract was used in this study and yielded four polyphenolic compounds that could be precursors to the phlobaphene polymers. The compounds were two furofuran type lignans, (+)-pinoresinol and (+)-epipinoresinol, a furolactone lignan derivative, (+)-2-(3'-methoxy-4'-hydroxyphenyl)-3,7-dioxa-6-oxobicyclo[3.3.0]octane and a flavonoid, (-)-eriodictyol. These compounds were isolated by column chromatography and purified on preparative TLC plates. The structural elucidations of these compounds were based on the spectral data using FAB-MS, both one and two dimensional NMR, and optical rotation. All of these compounds were separated from Douglas-fir bark for the first time. The (+)-furolactone is a rare compound whose structure was reported only once before (31). The other three compounds are well known and widely distributed in other woody plants (41,42).

The (+)-furolactone is likely formed from (+)-pinoresinol, possibly by the action of a fungi or

lichen on the outer bark. When examining the polyphenolic compounds of an outer bark, one should keep in mind that the bark does not exist in an isolated environment. It has long been suspected that polyphenols may be providing some sort of protection to the tree by absorbing ultra-violet light or defending against predators. It also seems likely that the bark compounds are beneficially used by organisms such as a lichen on the bark. In any case, modified compounds will be produced and they are part of the chemistry of that environmental unit. The isolated compounds also had NMR signals consistent with those observed in the polyphenolic phlobaphenes. Combined with the fact that all of the isolated polyphenols have functional groups able to undergo oxidative coupling reactions, it seems likely that these compounds are active precursors to the phlobaphene formation. This contention is supported by the observation that these compounds are in relatively low abundance in the outer bark compared to the phlobaphenes. They are thus likely transient products in the bark.

# BIBLIOGRAPHY

1. Knop, A. and Pilato, L. A. 1985. Phenolic resins: chemistry, application and performance future direction. Springer-Verlag. New York, 313pp.

2. Clark, R. J., Karchesy, J. J. and Krahmer, R. L. 1988. Investigation of a new resin as an exterior adhesive to bond high moisture content veneers and glulam. For. Prod. J., 38(7/8):71-75.

3. Chemical marketing reporter. Dec. 31,1990.

4. Kreibich, R. E. 1989. Tannin-based wood adhesives. In: Hemingway, R. W. and Karchesy, J. J. (eds.). Chemistry and significance of condensed tannins. Plenum Publ. Co. New York, 457-476.

5. Foo, L. Y. and Karchesy, J. J. 1989. Chemical nature of phlobaphenes. In: Hemingway, R. W. and Karchesy, J. J. (eds.). Chemistry and significance of condensed tannins. Plenum Publ. Co. New York, 109-118.

6. Foo, L. Y. and Karchesy, J. J. 1989. Pseudotsuganol, a biphenyl-linked pinoresinol-dihydroquercetin from Douglas-fir bark: isolation of the first true flavonolignan. J. Chem. Soc., Chem. Commun., 4: 217-219.

7. Stahelin, C and Hofstetter, J. 1884. Chemische untersuchung einiger Rinden. Annal. d. Chemis. u. Pharm., 51:63

8. Hubbard, J. K. and Kurth, E. F. 1949. Douglas-fir bark tannin. J. Amer. Lerther Chemists Assoc., 44: 604-614.

9. Kurth, E. F. and Chan, F. L. 1953. Extraction of tannin and dihydroquercetin from Douglas-fir bark. J. Amer. Leather Chemists Assoc., 48: 20-32.

10. Holmes, G. W. and Kurth, E. F. 1961. The chemical composition of the newly formed inner bark of Douglas-fir. Tappi, 44(12): 893-898

11. Weinges, K. 1958. Uber catechine und ihre herstellung aus leuko-anthocyanidin-hydraten. Ann. Chem., Justus Liebig's, 615: 203-209.

12. Hergert, H. L. 1960. Chemical composition of tannin and polyphenols from conifer wood and bark. Forest Prod. J., 10(11): 610-617.

13. Laver, M. L., Loveland, P. M., Chen, C.-H., Fang, H. H.-L., Zerrudo, J. V. and Liu, Y.-C. L. 1977. Chemical constituents of Douglas-fir bark: a review of more recent literature. Wood Science, 10(2):85-92.

14. Foo, L. Y. and Karchesy, J. J. 1989. Polyphenolic glycosides from Douglas-fir inner bark. Phytochemistry, 28(4): 1237-1240.

15. Foo, L. Y. and Karchesy, J. J. 1989. Procyanidin dimers and trimers from Douglas-fir inner bark. Phytochemistry, 28(6): 1743-1747.

16. Foo, L. Y. and Karchesy, J. J. 1991. Procyanidin tetramers and pentamers from Douglas-fir bark. Phytochemistry, 30(2): 667-670.

17. Foo, L. Y. and Karchesy, J. J. 1989. Procyanidin polymers of Douglas-fir bark: structure from degradation with phloroglucinol. Phytochemistry, 28(11): 3185-3190.

18. Foo, L. Y., Helm, R. and Karchesy, J. J. 1991. [5',5']-bisdihydroquercetin: A B-ring linked biflavonoid from *pseudotsuga menziesii*. Phytochemistry, in press.

19. Hemingway, R. W. 1989. Structural variations in proanthocyanidins and their derivatives. In: Hemingway, R. W. and Karchesy, J. J. (eds.). Chemistry and significance of condensed tannins. Plenum Publ. Co. New York, 83-107.

20. Pigman, W., Anderson, F., Fischer, R., Buchanan, M. and Browning, B. L. 1953. Color precursors in spruce and western hemlock woods and inner barks. Tappi, 36:4.

21. Karchesy, J. J. 1989. Analytical methods: an overview. In: Hemingway, R. W. and Karchesy, J. J. (eds.). Chemistry and significance of condensed tannins. Plenum Publ. Co. New York, 197-202.

22. Karchesy, J. J., Foo, L. Y., Barofsky, E., Arbogast, B. and Barofsky, D. F. 1989. Negative-ion fast-atom-bombardment mass spectrometry of procyanidin oligomers. J. of Wood Chem. and Tech., 9(3): 313-331.

23. Freudenberg, K. and Sidhu, G. S. 1961. Die absolute konfiguration der gruppe des sesamins und pinoresinol. Chem. Ber., 94(4): 851-862.

24. Pew, J. C. 1962. Conversion of dihydroquercetin to eriodictyol. J. Org. Chem., 27:2935-2937.

25. Karchesy, J. J., Bae, Y., Chalker-Scott, L., Helm, R. F. and Foo, L. Y. 1989. Chromatography of proanthocyanidins. In: Hemingway, R. W. and Karchesy, J. J. (eds.). Chemistry and significance of condensed tannins. Plenum Publ. Co. New York, 139-151.

26. Agrawal, P. K. and Thakur, R. S. 1985. $^{13}C$ NMR spectroscope of lignan and neolignan derivatives. Magnetic Resonance in Chemistry, 23(6): 389-418.

27.Whiting, D. A. 1985. Lignans and neolignans. Nat. Prod. Rep., 2:191-211.

28. Rao, C. B. S. (ed.). 1978. Chemistry of lignans. Andhra Univ. Press. Waltair, 227-275.

29. Silverstein, R. M., Bassler, G. C. and Morrill, T. C. 1981. Spectrometric identification of organic compound. 4th edition. John Wiley and Sons, Inc. New York, p.122.

30. Carvalho, M. G. D., Yoshida, M., Gottlieb, O. R. and Gottlieb, H. E. 1987. Lignans from Nectandra Turbacensis. Phytochemistry, 26(1): 265-267.

31. Koshino, H., Yoshihara, T., Togiya, S., Terada, S., Tsukada, S., Okuno, M., Noguchi, A., Sakamura, S., Ichihara, A. et al. 1989. Antifungal compounds from stromata of epichloe typhina on Phleum pratense. Tennen Yuki Kagobutsu Toronkai Koen Yoshishu, 31st:244-251.

32. Pelter, A., Ward, R. S., Watson, D. J. and Collins, P. 1982. Synthesis of 2,6-diaryl-4,8-dihydroxy-3,7-dioxabicyclo[3.3.0]octanes. J. Chem. Soc., Perkin I, 175-181.

33. Nishibe, S., Tsukamoto, H. and Hisada, S. 1984. Effects of O-methylation and O-glucosylation on carbon-13 nuclear magnetic resonance chemical shifts of matairesinol, (+)-pinoresinol and (+)-epipinoresinol. Chem. Pharm. Bull., 32(11): 4653-4657.

34. Markham, K. R. and Chari, V. M. 1982. Carbon-13 NMR spectroscopy of flavonoids. In: Harborne, J. B. and Mabry, T. J. (eds.). Advances in flavonoid research, 1975-80. Chapman and Hall. London, 19-133.

35. Markham, K. R. 1982. Techniques of flavonoid identification. Academic Press Inc. Ltd. London, Chapter 6.

36. Haworth, R. D. 1941. Constituents of natural phenolic resins. Nature (London), 147:255-257.

37. Malan, J. C. S. 1991. Wood Chemistry Laboratory. Oregon State University. Unpublished data.

38. Jakupovic, J., Pathak, V. P., Bohlmann, F., King, R. M. and Robinson, H. 1987. Obliquin derivatives and other constituents from Australian Helichrysum species. Phytochemistry, 26(3): 803-807.

39. Castellano, E. E., Miranda, J. M., Nagem, T. J., Böechat,A. F. B. and Gottlieb, O. R. 1986. The structure of 6-(3-hydroxy-4,5-dimethoxyphenyl)-3,7-dioxabicyclo-[3.3.0]octan-2-one. Acta Cryst., C42:168-170.

40. Peking Institute of Pharmaceutical Industry. 1980. Chemical constituents of the leaves of Ilex pubescens var. glabra. Yao Hsueh Tung Pao, 15(11):42.

41. Rowe, J. W. (ed.). 1989. Natural products of woody plants I. Springer-Verlag. Berlin Heidelberg, p.461.

42. Harborne, J. B. 1988. The flavonoids: advances in research since 1980. Chapman and Hall. London, p.367.

# APPENDICES

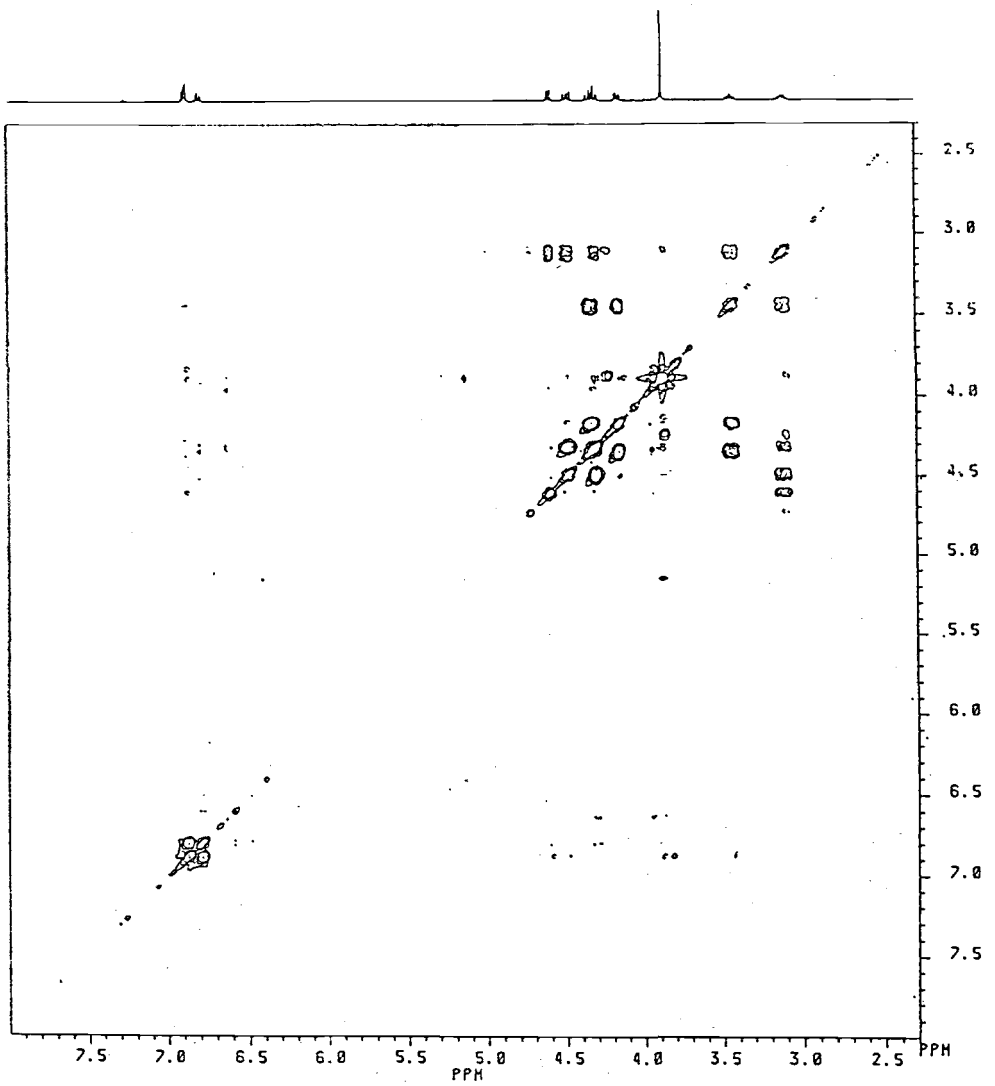



Figure 13. COSY NMR Spectrum of Furolactone

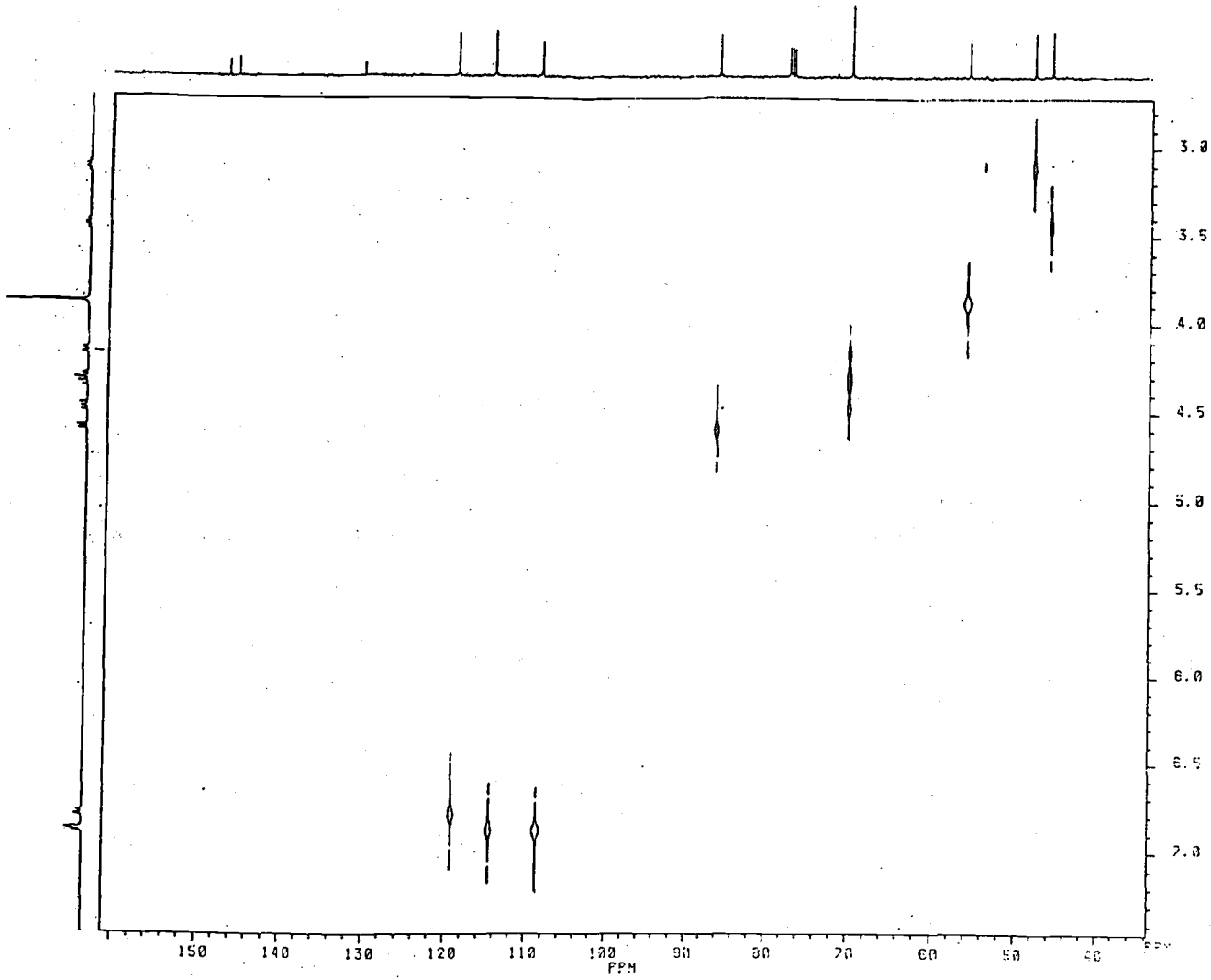



**Figure 14. HETCOR NMR Spectrum of Furolactone**

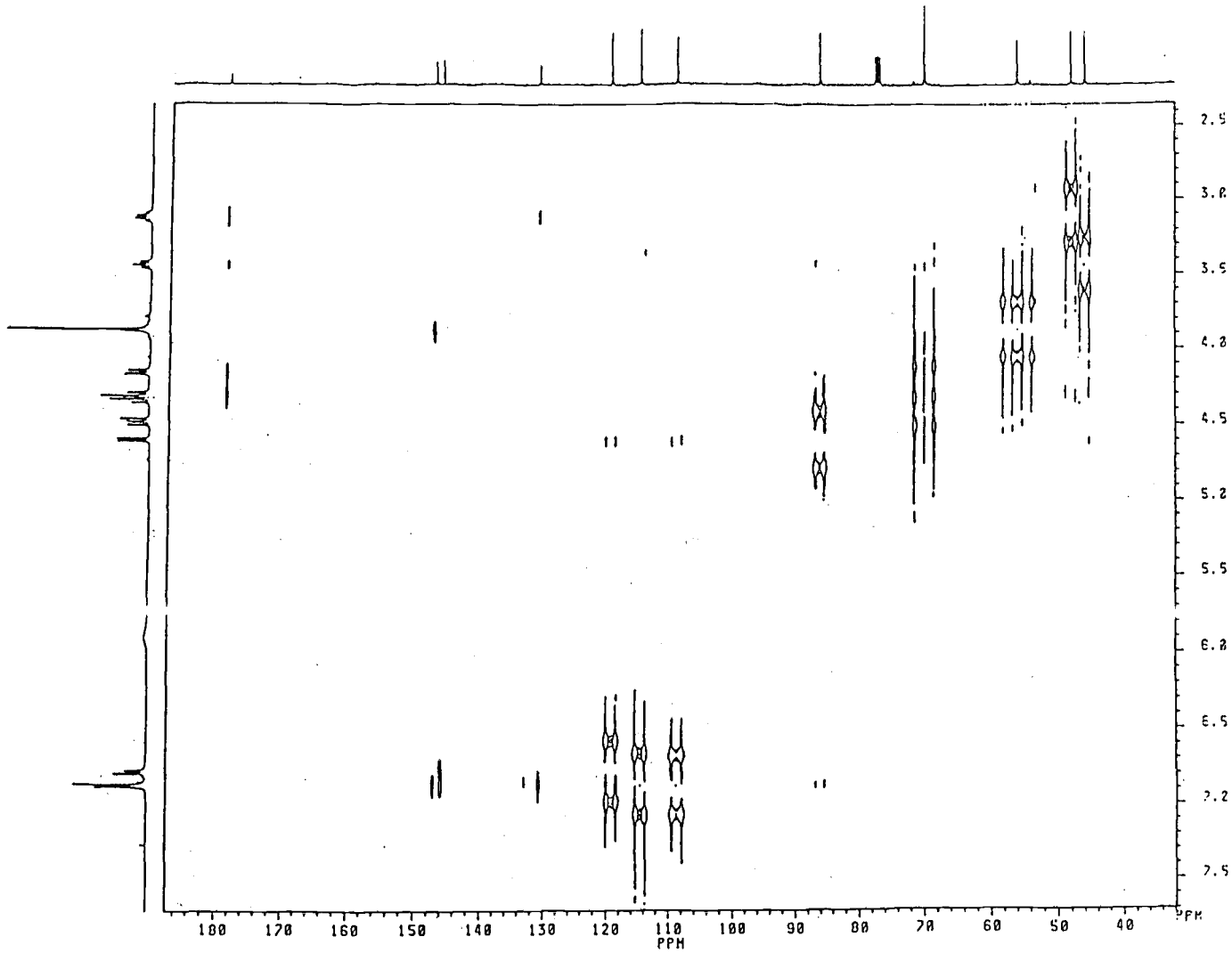



Figure 15. HETCOSY NMR Spectrum of Furolactone