AN ABSTRACT OF THE THESIS OF

Ruben Francisco Gonzalez-Laredo for the degree of Doctor of Philosophy in Forest Products presented on April 23, 1993.

Title: Polyphenols from the Bark of Douglas-fir and Red Alder

Signature redacted for privacy.

Abstract approved: Joseph J. Karchesy

The objectives of this research were to isolate and determine the molecular structures of certain polyphenols in Douglas-fir and red alder barks. The compounds of interest in Douglas-fir were the lignans which are eventually incorporated into the outer bark phlobaphenes. Phlobaphenes are the red colored, alcohol soluble, water insoluble phenolic polymers in conifer outer bark. They are commonly believed to be formed by oxidative coupling reactions of polyphenols in the bark. Four neolignan glycosides were isolated and identified from the methanol extract of Douglas-fir inner and outer bark. In addition several other non-lignan type polyphenols were isolated and identified which likely play a role in phlobaphene formation.

The compounds of interest in red alder bark were the diarylheptanoid glycosides which have potential medicinal and adhesive value. The methanol extract yielded five new

diarylheptanoids glycosides compounds.

The isolations were carried out by column chromatography and the molecular structures determined by use of ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectroscopy, Fast Atom Bombardment Mass Spectroscopy, and enzymic hydrolysis of the parent glycosides.

These results showed that a variety of polyphenol types, including neolignans which may be phlobaphene precursors, are present in Douglas-fir bark. This information suggests that most of the polyphenols in Douglas-fir bark can be used to make water-proof, formaldehyde free, wood adhesive polymers once we understand the molecular structures and reactions of these natural renewable substances.

The diarylheptanoid compounds isolated from red alder bark belong to a class of compounds which have a history of biological activity. These compounds now provide a basis for further systematic testing for pharmaceutical value in future research. The structures of the polyphenols in red alder also give insight into how these polyphenols, and red alder bark, can be used to make new types of wood adhesives copolymers.

Polyphenols from the Bark of Douglas-fir and Red Alder

by

Ruben Francisco Gonzalez-Laredo

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed April 23, 1993

Commencement June 1994

APPROVED:

Signature redacted for privacy.

Associate Professor of Wood Chemistry and Adhesives in charge of major

Signature redacted for privacy.

Head of Department of Forest Products

Signature redacted for privacy.

Dean of Graduate School

Date thesis is presented April 23, 1993

Typed by Researcher for Ruben Francisco Gonzalez-Laredo

To my parents, who besides life
gave me love and confidence
to achieve any goal.

To Maria del Pilar is the actual
credit for this Degree; in accepting
personal sacrifices she supported
and joined to this adventure.

To the four reasons of my inspiration:
Ana Paulina
Diana Ivette
Aldo Antonio
Ruben Francisco

ACKNOWLEDGMENTS

I am thankful to CONACYT (Mexico) and Instituto Tecnologico de Durango for scholarship and leave of absence to continue my education in the US.

My gratitude goes to Joe Karchesy who did not hesitate in accepting me in his research team and supported my work through these years at OSU. I deeply appreciate his friendship and generous collaboration.

I am indebted to Johannes Malan for initiating me in the secrets of the chromatographic separations at the early stages of my research. My respect to Murray Laver for his suggestions and permanent cooperation.

The assistance of Richard Helm for the NMR spectra and structure determination of *oregonoside B* is recognized. The support of Roger Kohnert for running the NMR spectra is appreciated. Also the helpful collaboration of Brian Arbogast, who ran all the mass spectra, is acknowledged. The assistance in the lab from John Lehman and Jason Sandahl is recognized.

To Vahid Ghodoussi for offering me constructive criticism and his kindly friendship. To my pears, specially Jie Chen and Shuping Wang for sharing any progress in our experimental endeavor.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SELECTED NMR SPECTRA

Spectra Page

POLYPHENOLS FROM THE BARK OF DOUGLAS-FIR AND RED ALDER

CHAPTER I

INTRODUCTION

The chemical characterization of bark extracts and other natural renewable materials can be justified from two different perspectives. Utilization of the large amounts of tannins present in many barks as a source of phenolics for the manufacture of wood adhesives and specialty polymers is one view. Another view focuses on the specific search for new pharmaceutical and biologically active compounds that are useful to mankind.

Wood adhesives.

The forest products industry is both the producer of huge tonnages of wood residues and at the same time a major consumer of phenolic adhesives. So far the oil industry supplies the raw materials demanded for manufacturing wood adhesives, but eventually these polymers will have to be produced from renewable resources, unless other synthetic or natural sources are developed.

The experience from the oil embargo in the early 70's, resulted in strongly focused efforts in many countries to replace petrochemical based materials with renewable resources. Some of the studies encouraged by industry have emphasized the extraction of resorcinol replacing compounds from a variety of tree barks, nut shells and other natural residues that have high concentrations of condensed

from a variety of tree barks, nut shells and other natural residues that have high concentrations of condensed tannins.

Much of the ongoing research, worldwide, has concentrated on the reaction of isolated and derivatized polyphenols from natural sources with formaldehyde to produce low temperature-curing thermosetting adhesives. Such resins could be formulated to be used alone or in combination with conventional resorcinol-formaldehyde or phenol-resorcinol-formaldehyde resins.

Under the present prices, adhesives from renewable sources might not be totally competitive. However their price and performance against today's oil-based products, could be improved, if the research for the development, optimization and raw material characterization is not delayed. The role that natural adhesives will play in the future will be unavoidable and more important than it is today.

Medicines from the Forest.

In the past, during the boom of the pharmaceutical industry and after the World War II, plant metabolites were studied basically from a phytochemical and chemotaxonomic point of view. However, in the last decade the interest in drugs coming directly from plants has grown steadily. In particular, the forest has become a potential source of many bioactive herbal extracts that have been used in folk medicine for centuries.

Today, *taxol*, the drug isolated from the stem bark of Pacific yew (*Taxus brevifolia* Nutt.) has become a well known example of the potential for new pharmaceuticals from

our forests. Unfortunately, the drug's success for treatment of ovarian cancer is in stark contrast to its low concentration in the raw material. The resulting limited availability of the drug has prompted considerable controversy about utilization of this forest resource. In one view, the tree has served its purpose by providing humans with the knowledge of the molecular structure that cures a form of cancer. It is now humanities turn to either synthesize the drug or learn how to manage the resource. Fortunately, it has been recently reported that laboratory synthesis is possible which relieves pressure on the tree. The search for new pharmaceuticals in the future from forest products will present similar concerns when the species in question are in limited abundance. This underlines the value of closely examining the barks of the commercially harvested timbers of the Pacific Northwest.

Red alder and Douglas-fir bark are representative examples of the challenge to achieve the goals mentioned above. Both trees have a long history of chemical research that continues to improve as better separation techniques and analytical instruments become available.

In this Thesis, chapters two to four describe the isolation and identification of polyphenols found in the bark of red alder and Douglas-fir trees. In the Appendix is shown a brief description of symbols, abbreviations and acronyms used along the document. It is included also a selected set of NMR spectra for most of the compounds reported.

CHAPTER II

DIARYLHEPTANOID GLYCOSIDES FROM RED ALDER BARK

INTRODUCTION

The tree.

Red Alder (*Alnus rubra* Bong.) is commercially the most important hardwood in the Pacific Northwest, growing on about 16% of the forest land [1]. It is known also as "Oregon alder" or "Western alder". Its range extends from Central California to Southeastern Alaska and from the Pacific Ocean cost east to Idaho.

The bark of young red alders is smooth and blue-gray with whitish blotches; on older trunks, darker and broken into flat plates. The inner bark is thin and reddish brown [1].

The anatomy of red alder shows a diffuse porous wood with little change between early and latewood. It usually presents vessel aggregates that are visible to the naked eye, but not as often and regular as in oak [2]. Its heartwood is not always easy to distinguish from sapwood and both are orange-colored due to a staining phenomenon studied by Karchesy [3]. This discoloration is observed as soon as freshly cut wood or bark are in contact with air, therefore the tree's name.

For a long time red alder has been considered a weed species for a timber industry that ignored it or destroyed

it. In recent years there has been renewed interest in utilizing this species due, in large measure, to the reduced availability of traditional timbers such as Douglas-fir. Despite that the demand for alder logs is far bellow the potential supply, which has created a low value for its wood. The tree has always found some use for pulp and paper production, because the logs typically have less than 15% bark and no twigs or foliage in the stem. However, the tree is straight and does not have branches in the first 10 to 15 meters. This clear wood is ideal for veneer production in the paneling industry and some success has been achieved in furniture manufacturing.

Red Alder is a fast growing tree outgrowing any conifer for the first 30-40 years. Although it is a short-lived tree lasting about 100 years, it plays an important role in forest health because of its nitrogen-fixing ability. Douglas-fir and other trees grow better and stronger in the surroundings of red alder stands [1].

Bark extracts.

Red Alder bark is used as hog fuel or extender in plywood adhesives. As an extender it modifies the reological properties of the adhesive enhancing the spreading on the veneer and lowers the adhesive costs. The most popular tradename of plywood adhesive extender made of red alder bark is "Modal". Traditionally the indigenous peoples of the Pacific Northwest used red alder bark to prepare red dyes and paints. Usually they mixed the bark with steelhead eggs to stabilize the color. Red alder extract is a good antioxidative and chelating agent to trap metals and free radicals, which probably occur through the formation of a conjugated quinone [3]. Indians used metal ions to accelerate color formation (e.g. ferric oxide clay)

they also drank the inner bark as tea for heart pain [4].

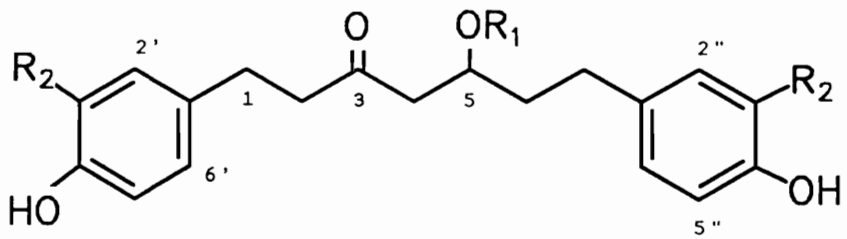
The potential use of these extracts as a renewable source of medicines should be explored. The antiviral activity of some polyphenols, especially hydrolyzable tannins and procyanidins derivatives, is well known [5,6]. A similar perspective can be taken concerning the utilization of the phenolic fractions in the formulation of wood adhesives by condensation with aldehydes. In addition, non-conventional polymers (i.e., non formaldehyde containing) can be manufactured through complexation with proteins, carbohydrates or amines.

In order to better understand how red alder might contribute to the development of new medicines, the polyphenolic extracts were further investigated for chemical structure elucidation and biological activity. Thus antitumor testing and brine shrimp bioassays were performed with some of the fractions from the chromatographic separation of red alder extract. As a consequence of chemical analyses, five novel diarylheptanoid glycosides were isolated and their molecular structure established.

The isolation of these compounds from red alder bark and their structure determination by means of Nuclear Magnetic Resonance (NMR), enzymic hydrolysis and Fast-atom Bombardment Mass Spectrometry (FAB-MS) data is reported in this chapter.

LITERATURE REVIEW

Since the early 50's, the existence of a phenolic xyloside has been known in red alder bark. Kurth [7] reported 26.9% of the ethanolic extract to be the xyloside which had properties similar to those of phlobatannins. Later in 1974, Karchesy [3,8] found that a new diarylheptanoid xyloside (*oregonin*) was the major compound, which was isolated in methylated form and given the structure **I** in figure 2.1.

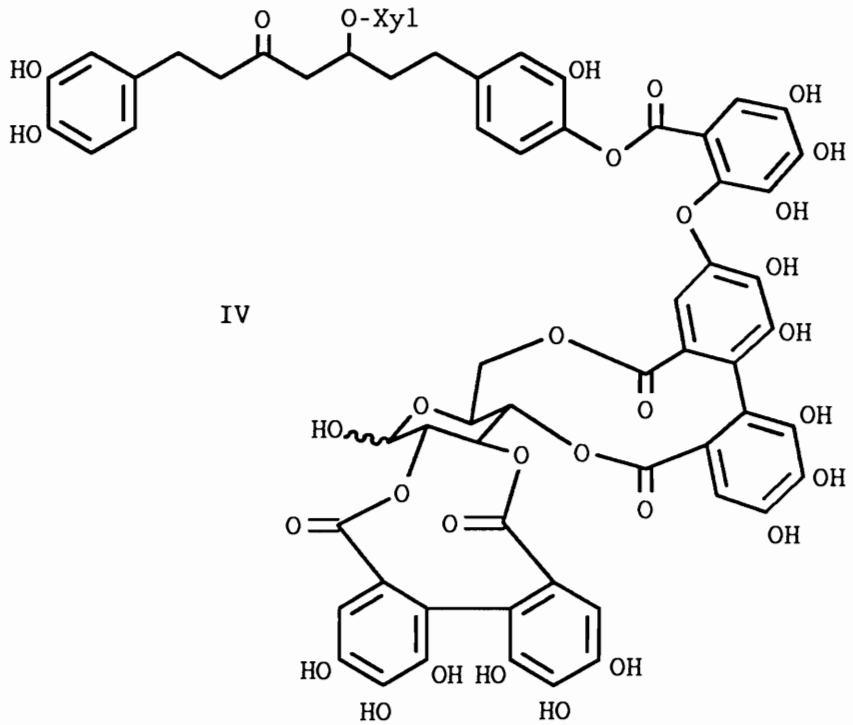


	R_1	R_2
I	β-D-Xyl	OH
II	H	OH
III	H	H

Figure 2.1 Structures of *oregonin* {**I**}, *hirsutanol* {**II**} and *platyphyllonol* {**III**}.

Almost simultaneously, Terazawa reported *hirsutanol* {**II**}, the aglycone of oregonin and *platyphyllonol* {**III**} from the green barks of *Alnus hirsuta* [9] and *Betula platyphylla* [10] respectively, see Figure 2.1. Later, Suga and Ohta [11,12] reported the absolute configuration for oregonin isolated from female flowers of *Alnus serrulatoides* as *S*. It was characterized as *(5S)-1,7-bis*

(3,4-dihydroxyphenyl)-5-(β-D-xylopyranosyloxy)-heptan-3-one. More complex structures have been found for oregonin related compounds. In a recent work [13], it was reported *hirsunin* {**IV**}, an ellagitannin-diarylheptanoid complex from *Alnus hirsuta* leaves.



IV

Figure 2.2 Structure of *hirsunin* {**IV**}.

Diarylheptanoids have two aromatic rings bridged by an heptanoid aliphatic chain (Ar-C_7-Ar). They have been found with different degrees of oxidation, saturation, methylation or acylation, and generally are isolated as glycosides. Their structures are usually linear but can cycle through an oxidative phenolic coupling process to form cyclic diarylheptanoids with biphenyl or diphenyl ether linkages as in *acerogenin A* {**VIIIa**} [14,16] and *alnusonol* {**VIIIb**} [17,18] shown in figure 2.5. Bridged biarylheptanoids usually are accompanied by their

open-chain counterparts, the supposed biogenetic precursors [19].

Nagai et. al. [14] reported in 1986 a diarylheptanoid glucoside named *aceroside VII* (**VII**) from an *Aceraceae* plant. This compound as some others, has *centrolobol* (**VI**) as its aglycone. The absolute configuration was reviewed and proposed as *R*. The structure of *centrolobol* is closely related to *rubranol* (**XII**), which is the aglycone of three of the five new glycosides reported here. Recently, several new disaccharides having *centrolobol* and *platyphyllonol* (**III**) as aglycones were isolated from the inner bark of *Betula pendula* [15].

After the first diarylheptanoids were discovered during the early 60's, in the unrelated families *Betulaceae*, *Leguminosae* and *Zingiberaceae*, it was thought that they were compounds of unique structures and little taxonomic utility [20]. Eventually interest has increased as more compounds have been reported in *Aceraceae*, *Dioscoreaceae*, and *Myricaceae* plants and more species in all the families. The major occurrence of diarylheptanoids is in three genera of the *Betulaceae* family, particularly in the *Alnus* species [14,19].

The biosynthesis of diarylheptanoids seems related to that of lignans, involving two cinnamate units and a central methylene bond supplied by malonate. This was suggested by Roughley and Whiting [21] for the biosynthesis of *curcumin* (**V**) as shown in figure 2.3.

Some diarylheptanoids have shown physiological activity as choleretic promoters in experimental animals [22], inhibitors of prostaglandin biosynthesis [23] and ileum

contraction in guinea pigs [24]. Hikino and coworkers [25] found that many diarylheptanoids have antihepatotoxic activity also.

Diarylheptanoids, as secondary metabolites, have shown chemical action in plant defense against foraging animals. *Platyphylloside* (the glucoside of *platyphyllonol*) was responsible for the *in vitro* inhibitory effect on rumiant digestibility of buds and twigs of Scandinavian birch (*Betula pendula*) [26]. The study showed that significative effects were present at concentrations bellow the naturally occurring level in birch.

Recently, McCutcheon et al. [27] found that methanolic extracts from *Alnus rubra* bark and catkins have the broadest spectra of antibiotic activity against 11 bacterial strains in a screening made with other 95 plant extracts used by Native Indians of British Columbia. The bark extract was particularly active against *Staphylococcus aureus*, while the catkin extract was the best against *Pseudomonas aeruginosa* H187. These results show a paradoxical situation because the traditional use of red alder bark is classified in the ethnobotanical literature as a non-antibiotic medicine. From eleven non-antibiotic medicinal plants, seven showed activity, including red alder.

Some diarylheptanoids have shown strong antioxidative action that prevents deterioration in foods and minimizes oxidative damage to living cells [28]. For example *curcumin* is the major compound and most active constituent of the methanolic extract of rhizome of turmeric (*Curcuma domestica*). Turmeric is a yellow-orange pigment used in food seasoning. In antioxidative tests [29], *curcumin* was

close in activity to vitamin E and one order of magnitude lower than the synthetic antioxidants BHA and BHT (butylated hydroxyanisole and butylated hydroxytoluene). Its activity mechanism may include metal ion chelation by the central β diketone group [30].

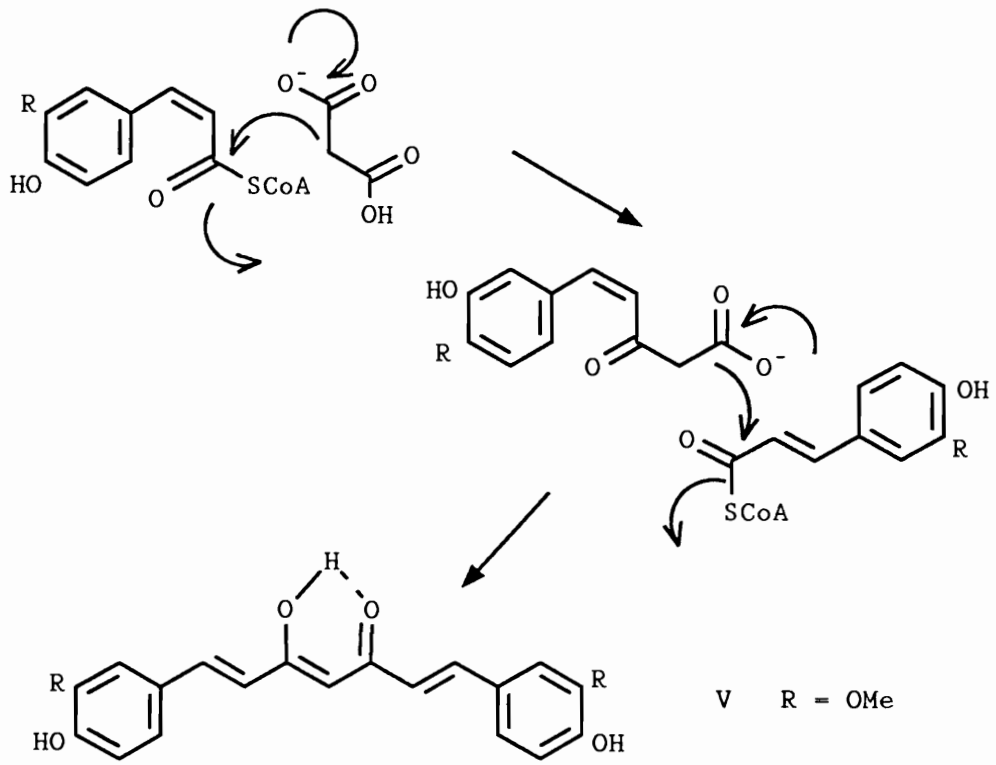


Figure 2.3 Biosynthesis of *curcumin* (**V**).

Several arylheptanoids, such as the *gingerols* [22], are present in rhizomes of ginger that are used as spices and in folk medicines. Thus diarylheptanoids from red alder have potential applications as antioxidants, if they do not show pungency (an undesired property for food applications) and exhibit strong antioxidative activity at low concentrations.

RESULTS AND DISCUSSION

The dewaxed methanolic extract of red alder bark is more than 70% *oregonin* {**I**}. However five minor constituents were isolated and identified as the novel diarylheptanoid glycosides: *Rubranol glucoside* {**IX**}. *Rubranol xyloside* {**X**}, *Rubranoside A* {**XI**}, *Oregonoside A* {**XIII**} and *Oregonoside B* {**XIV**}. Compounds **IX**, **X** and **XI** have *rubranol* {**XII**} as a common aglycone. Although *rubranol* has not been claimed as a natural product yet, it was obtained by enzymic hydrolysis from the glucoside {**IX**}.

The aqueous fraction afforded 0.86 g of carbohydrates that were not further investigated. From the methanol-water elution three major glycosidic fractions were separated. From the first and largest fraction *oregonin* (3.35g) was isolated. The other two fractions afforded, after additional chromatography, the rubranols and oregonosides reported in this work: *Rubranol glucoside*, *Rubranol xyloside*, *Rubranoside A*, *Oregonoside A* and *Oregonoside B*.

<u>The rubranols</u>.

The rubranol derivatives, *rubranol glucoside*, *rubranol xyloside and rubranoside* {**IX**,**X**,**XI**} were characterized by a common aglycone in the negative FAB-MS spectra, showing aglycone and dehydrated aglycone ions (i.e., $[aglycone-H]^-$ and $[aglycone-H_2O-H]^-$) at m/z 331 and 313 respectively. Additionally, the three compounds {**IX**,**X**,**XI**} exhibited peaks from the molecular ions (i.e., $[M-H]^-$) at the expected values of m/z 493, 463 and 625 respectively. *Rubranol* {**XII**} itself showed a major molecular peak at m/z 331 and a dehydrated ($[M-H-H_2O]^-$) molecular ion at m/z 313.

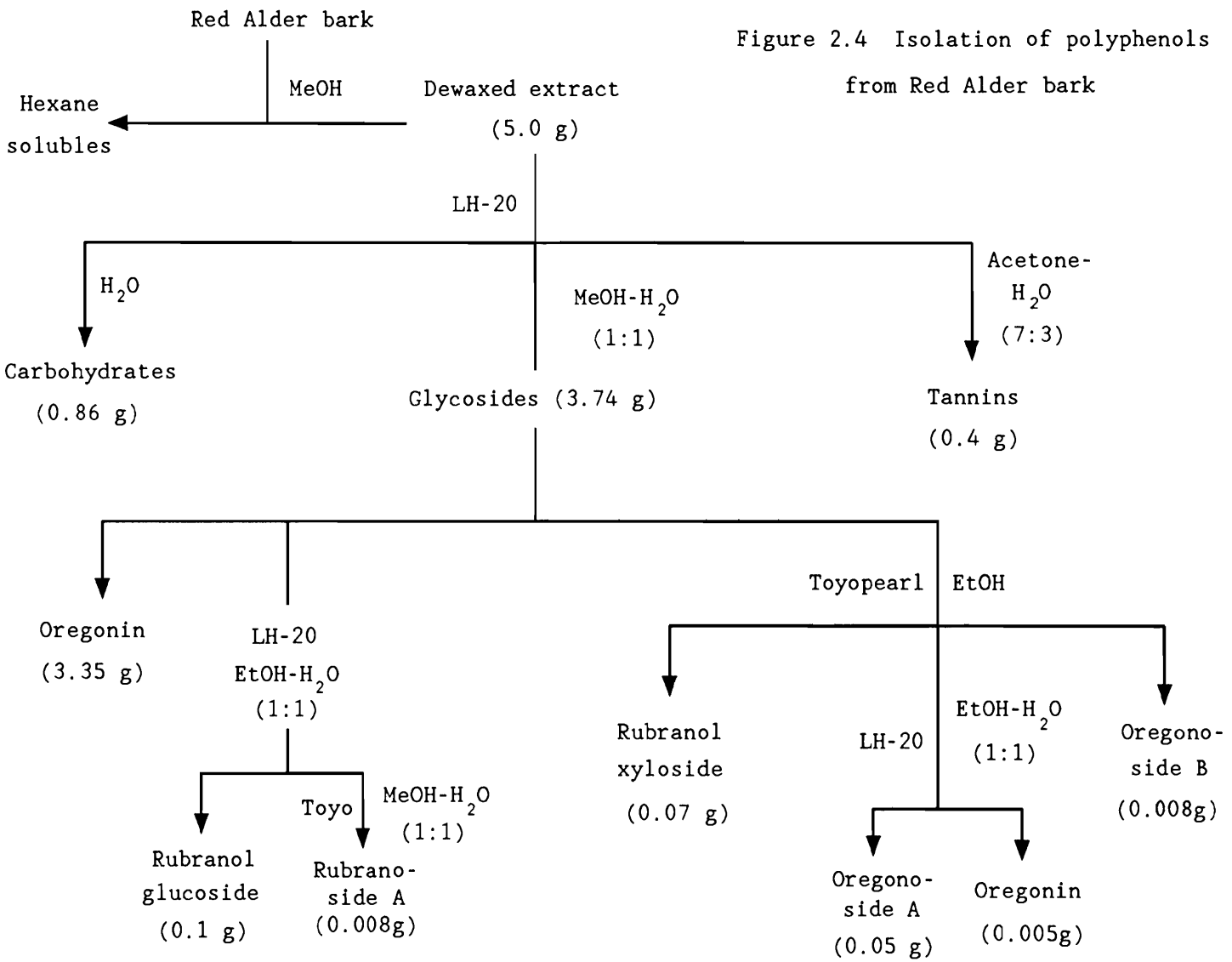
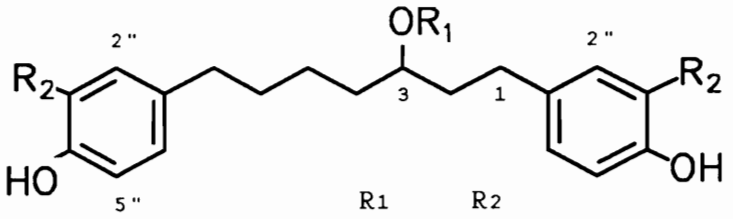


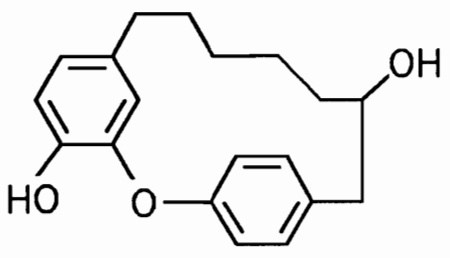
Figure 2.4 Isolation of polyphenols from Red Alder bark



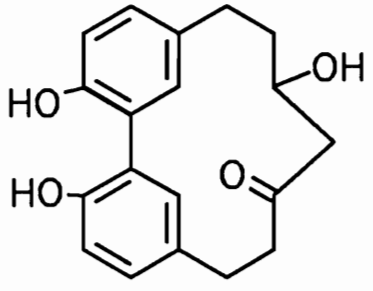
	R1	R2
VI	H	H
VII	β-D-glu	H
IX	β-D-glu	OH
X	β-D-xyl	OH

XI R1= β-D-xyl(3→1)β-D-glu; R2= OH

XII	H	OH



VIIIa

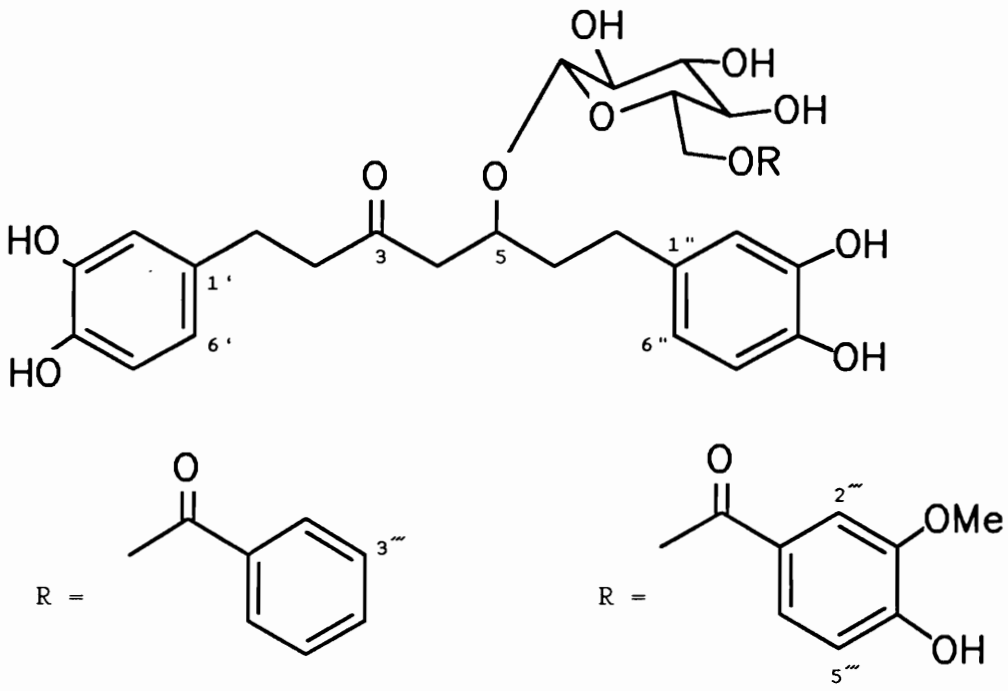


VIIIb

Figure 2.5 Structures of *centrolobol* {**VI**}, *aceroside VII* {**VII**}, *acerogenin A* {**VIIIa**}, *alnusonol* {**VIIIb**}, *rubranol glucoside* {**IX**}, *rubranol xyloside* {**X**}, *rubranoside A* {**XI**} and *rubranol* {**XII**}.

The ^{1}H NMR and ^{13}C NMR for these compounds showed the glycosidic nature of the samples. The chemical shifts of anomeric carbons (ca. 103.5 ppm for glucose and 104.5 for xylose) and the coupling constants of anomeric protons (ca. 7.6 Hz for both sugars) suggested the presence of β glycosidic linkages in the three compounds, including the disaccharide moiety in **XI**. Usually α glycosidic linkages are found about 4-5 ppm upfield, and the coupling constants (J) are smaller, around 1-2 Hz. The pyranose form in the sugars was also evident from the rest of the signals in the

carbohydrate region. It should be expected downfield shifts on the furanoses signals respect to their configurationally related pyranoses [33]. The ^{13}C NMR data (see Table 2.1) agreed with those reported in the literature for β-D-glucopyranosides and β-D-xylopyranosides [31-35].



XIII XIV

Figure 2.6 Structures of *oregonoside A* (**XIII**) and *oregonoside B* (**XIV**).

The presence of signals around 26, 32, 33, 35, 36 and 38 ppm for six aliphatic carbons in the ^{13}C NMR spectrum indicated a single and asymmetric substitution in the heptanoid backbone (see Table 2.1). The glycosidic carbon was found invariably around 80 ppm as a methide signal by the DEPT experiment, which differentiates between primary, secondary and tertiary carbons. The right position for the linkage was assigned unequivocally through 2D NMR COSY,

HETCOR and HETCOSY (i.e., homonuclear, heteronuclear and long range two and three bond ^{1}H-^{13}C correlations). The COSY spectra that shows coupling of protons on adjacent carbons, showed connectivities consistent for an heptanoid chain with a tertiary carbon at position 3. The HETCOSY spectra that supplies the long range connectivity between ^{1}H and ^{13}C's two or three bonds away, showed connectivity between the anomeric carbon and its proton with the proton and carbon at position 3.

The NMR spectrum from *rubranol* showed conclusive evidence that C_3 was the glycosidic linkage point. When the ^{13}C NMR signals for *rubranol* were compared against the corresponding to the glycosylated diarylheptanoid (i.e., *rubranol glucoside*) important differences were noticed. The signal for the C_3 carbon was shifted upfield by 7.7 ppm as a consequence of the sugar removal. So the downfield shift for the glucosylation on diarylheptanoid chains is around 8 ppm as should be expected in aliphatic chains. The C_3 proton was also shifted up for about 0.2 ppm. Additional downfield shifts were observed for C_2 and C_4 (2.6 and 3.4 ppm respectively) and smaller but significative downfield shifts in C_1 and C_5 (0.6 and 0.7 ppm).

Two catechol rings systems were characteristically observed in the NMR spectra of the rubranols. The ^{1}H NMR spectra showed a double set of signals with an ABX pattern which integrated for two sets of three protons each. In each of these systems one can readily identify a meta coupled doublet ($J \approx$ 1.8-2.0 Hz), an ortho coupled doublet ($J \approx$ 8.0 Hz) and a double of doublets ($J \approx$ 2.0 and 8.0 Hz), each integrating for one proton, as shown in Figure 2.7. The hydroxylation patterns were evident in the carbon spectra which showed four signals at about 144(2x) and

146(2x) ppm, which is characteristic of the ortho di-hydroxy pattern of a catechol ring. Again the HETCOSY spectra showed clearly the junction point of the catechol C_1 carbon to the aliphatic carbons 1 and 7 at each end of the heptanoid chain. Thus compounds **IX** and **X** were characterized as *1,7-bis(3,4-dihydroxyphenyl)-3-O-β-D-glucopyranosyl-heptane* and *1,7-bis(3,4-dihydroxyphenyl)-3-O-β-D-xylopyranosyl-heptane* respectively.

In particular, the structural analysis of rubranoside A (**XI**) was assisted by its close relation to **IX** and **X**. Its NMR spectra were almost superimposable on *rubranol xyloside* (**X**), except for additional carbohydrate signals corresponding to glucose. This fact suggested that xylose may be the intermediate unit in a monosubstituted diarylheptanoid by a glucose-xylose disaccharide. Supporting that idea, the signal for the proton at C_3 in xylose was shifted downfield slightly (~0.25 ppm).

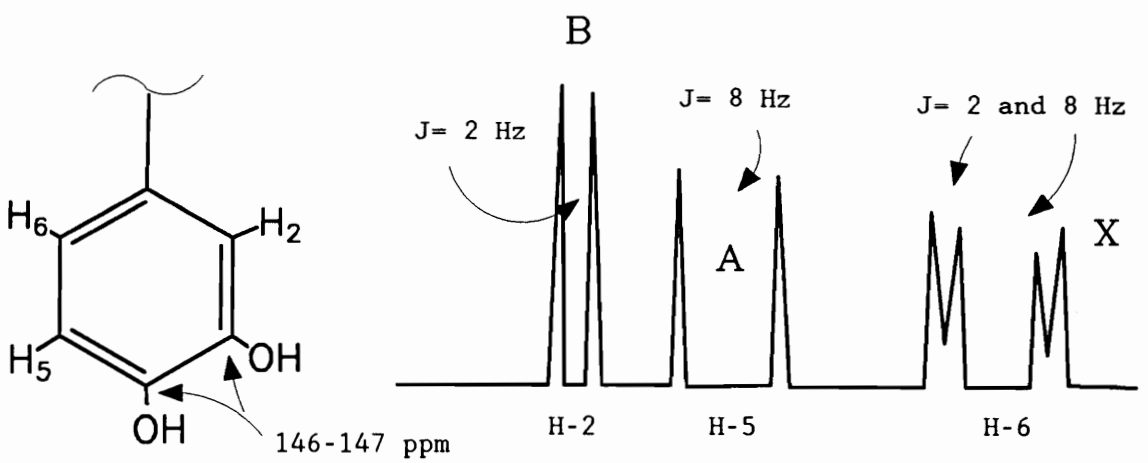


Figure 2.7 Key features in the ^1H and ^{13}C NMR of catechol rings.

The strongest evidence for defining the linkage between sugars, came from the 9.5 ppm downfield shift on the C_3 carbon of xylose. Additionally, there were small but significative upfield shifts on C_2 and C_4 (~0.9 and 1.3 ppm respectively) on the xylose moiety too. These observations plus the fact that glucose signals did not change respect to **IX** confirmed that glucosyl was the terminal unit in the disaccharide and was attached via a glucosyl (1→3) xylose linkage. Thus **XI** was characterized as *1,7-bis(3,4-dihydroxyphenyl)-3-O-[β-D-glucopyranosyl (1→3) β-D-xylopyranose]-heptane.*

The oregonosides.

Compounds **XIII** and **XIV** gave the expected molecular ($[M-H]^-$) ions in the mass spectra at m/z 611 and 657 respectively. Additionally, the now typically observed ion peaks due to dehydrated aglycone moieties ($[M-H-sugar-H_2O]^-$) were detected at m/z 325.

Oregonosides A and *B* showed typical carbonyl signals for a ketone at ca. 212 ppm and characteristic aliphatic and aromatic signals for a diaryl heptanone (see Table 2.2). Additionally their spectra presented carbonyls signals for an ester around 168 ppm, suggesting the existence of an acyl moiety in each compound. Despite of an extra set of aromatic peaks and a different carbohydrate pattern, the ^1H and ^{13}C NMR data resembled *oregonin.*

Both oregonosides appeared to be glycosylated by the same sugar moiety, that was not xylose as in *oregonin.* The chemical shifts (~4.3 ppm) and coupling constants (~7.7 Hz) of anomeric protons as well the carbon signals in the carbohydrate region agreed with a *β-D-glucopyranoside* [31-35]. However, there were noticeable downfield shifts

(~0.8 ppm) for the proton signals at the sixth position of glucose and for the carbon signal (~2.5 ppm) at the same position, suggesting a substitution at that point. Additional evidence was the small downfield shift of ~0.35 ppm in the signal from the glucosyl proton H-5 and an upfield shift of ~2.2 ppm in signal for carbon C_5.

The 2D-NMR experiments performed on **XIII** confirmed indirectly the position of the keto group at C_3. The DEPT spectra reveled five methylenic carbons, a presumably hydroxylated methine and a quaternary carbonyl carbon. Through the COSY spectrum it was deduced that there was an heptanoid backbone with successive correlations among the protons at C_4,C_5,C_6,C_7 and between C_1 and C_2. So it became evident that the quaternary carbon (i.e, ketone) was at position 3.

Through heteronuclear ^1H-^{13}C two and three bond long range correlations (HETCOSY), it was found that C_5 was the glycosylation point. As in the rubranols, this spectra showed that the two cathecol rings were connected to the opposite ends of the heptan-5-ol-3-one backbone. At this point it was clear that the identity of the aglycone in **XIII** was *hirsutanol* {**II**}.

In *oregonoside A* a set of symmetric aromatic signals was identified as the typical of a monosubstituted benzylic ring. The three signal system was integrated for five protons. The aromatic signals displayed the characteristic coupling constant of ~7.6 Hz for benzylic protons in two triplets (one for H-3 and H-5, the other for H-4) and a doublet (for H-2 and H-6). The acyl carbons were assigned through HETCOR and HETCOSY NMR experiments. In those experiments a correlation was found between the carbonyl

group and the ring. So the acylated moiety was identified as a derivative of *benzoic acid*. Thus the acyl group was attached at C_6 in the sugar and the compound characterized as the *5-O-[6-O-benzoyl]-β-D-glucopyranoside* of *1,7-bis(3,4-dihydroxyphenyl)-heptan-5-ol-3-one*.

In *oregonoside B*, an HMQC inverse-detected ^{13}C-^{1}H correlations experiment allowed for the assignment of which protons were bonded to which carbons. Additionally, it was confirmed by an HMBC inverse-detected long range ^{13}C-^{1}H experiment, that C_3 was the keto carbon. The later spectra showed correlations between protons at C_2 and C_4 with the C_3 carbon. The glycosylation at C_5 was evident from the connectivity between the anomeric proton and C_5, and between H-5 and the anomeric carbon. Finally, the same spectra showed multiple correlations of the acyl carbon with the glucose protons at the sixth position and with the aromatic protons in the aryl moiety. This implied the benzoacylated nature of the moiety and its connectivity to the sugar.

Again the ^{1}H NMR exhibited an additional set of aromatic signals with the characteristic ABX pattern that was assigned to a trisubstituted benzylic ring (i.e., cathecol type). In a long range COSY experiment, one that enhances proton to proton correlations of ca. 1.5 Hz, connectivity was found between the methoxyl protons and an ortho proton in the ring (H-2‴). This suggested that the methoxy group was attached in *para* position to the aryl ring.

The previous observations suggested an acyl moiety derived from *3-O-methyl-protocatechuic acid* or *4-hydroxy-3-methoxy-benzoic acid*. Thus, knowing that the acyl moiety

was attached to C_6 in glucose as in *oregonoside A*, the compound was characterized as the *5-O-[6-O-(4-hydroxy-3-methoxy-benzoyl)]-β-D-glucopyranoside* of *1,7-bis(3,4-dihydroxyphenyl)-heptan-5-ol-3-one*.

EXPERIMENTAL

The optical rotations were measured using a Jasco digital polarimeter Model DIP-370. The ^{1}H and C^{13} NMR experiments were run in a Bruker Model AM 400 NMR spectrometer using MeOH-d_4 as the solvent. The chemical shifts are given in ppm from TMS as an internal standard. Coupling constants are given in Hz (s, singlet; d, doublet; t, triplet; m, multiplet). FAB-MS were done with a Kratos MS-50TC mass spectrometer using thioglycerol-glycerol (2:1) or 3-NBA as matrixes.

Extraction and isolation.

The whole bark of a Red Alder tree from the McDonald Forest, Corvallis, Oregon was collected manually in the spring of 1992. A voucher specimen was deposited at the OSU Herbarium. The bark was chipped and air-dried before being soaked overnight in methanol (3x). The extracts were combined and evaporated under reduced pressure at 35°C. The crude extract was dewaxed thoroughly with hexane (3x).

A 5 g sample was taken from the methanol soluble fraction and dissolved in a minimum amount of methanol-water (1:1). The sample was applied into a Sephadex LH-20 chromatography column (5x60 cm). The column was previously washed with water, which was used as the initial eluent. The sequence of solvents, separation scheme and amounts obtained are shown on figure 2.4. The elution with water was performed to separate carbohydrates. This was continued until a colorless solution was coming out of the column.

The second solvent system was methanol-water (1:1) to elute the polyphenol glycosides reported here. This separation was monitored with a Gilson UV detector model

111B at 254 nm. Additional chromatography was accomplished with two glycosidic fractions using Toyopearl HW-40S in columns of 5x30 cm and using ethanol (95%) as the solvent. Also, Sephadex LH-20 was utilized in a 2x50 cm column with ethanol-water (1:1) as the mobile phase. The former separations were followed by TLC on precoated plastic sheets of silica gel 60 F_{254} from Merck and developed with the system (S_1) benzene-acetone-methanol (6:3:1). The spots were detected under UV and by spraying with H_2SO_4-HCHO (40:1), followed by heating.

Finally, the column was eluted with acetone-water (7:3) to remove the tannin fraction that was freeze dried for a projected antitumor promotion bioassay at Kansas State University. Some promising preliminary results were known at the time this was written.

Enzymic hydrolysis.

A sample of *rubranol glucoside* (0.022g) (R_f= 0.21, S_1) was dissolved in 3 ml of water and mixed with 0.004 g of β-glucosidase (Sigma Chemicals Co.). The suspension was stirred and warmed (35°C) for 48 Hs. Then the hydrolysate was freeze dried and extracted with ethyl acetate, which was washed with water affording 0.013g of *rubranol* (R_f= 0.60, S_1).

Oregonin {**I**}. Cream amorphous powder, highly hygroscopic, turning dark and sticky (3.35g); MW=478, $C_{24}H_{30}O_{10}$. $[\alpha]_D^{29}$= -18.6° (MeOH; c 0.6). Negative FAB-MS in thioglycerol-glycerol (2:1) gave a $[M-H]^-$ ion at m/z 477 (100%), an $[Aglycone-H]^-$ ion at 345 (6.7%) and an $[Aglycone-H_2O-H]^-$ ion at 327 (51.7%). ^1H NMR (MeOH-d_4):δ 1.74 (2H,m,H-6), 2.49(2H,,H-7), 2.55 (1H,dd,J=5.2,16.6 Hz, H-4a), 2.70 (4H,m,H-1,H-2), 2.77 (1H,dd,J=6.9,16.6 Hz, H-4b), 3.13

(2H,m,Xyl-2,5a), 3.30 (1H,m, Xyl-3), 3.46 (1H,m,Xyl-4), 3.84 (1H,dd,J=5.3,11.5 Hz, Xyl-5b), 4.09 (1H,m,H-5), 4.20 (1H,d.J=7.5 Hz, Xyl-1), 6.47 (2H,2dd,J=2.1,8.1 Hz, H-6',H-6"), 6.60 (2H,2d,J=2.1 Hz, H-2',H-2"), 6.64 (2H,2d,J=8.1 Hz, H-5',H-5").

Rubranol glucoside {**IX**}. Brown amorphous powder (0.1g); MW=494, $C_{25}H_{34}O_{10}$. $[\alpha]_D^{29}$= -28.3° (MeOH; c 0.3). Negative FAB-MS in thioglycerol-glycerol (2:1) gave a $[M-H]^-$ion at m/z 493 (100%), an $[Aglycone-H]^-$ ion at 331 (12.2%) and an $[Aglycone-H_2O-H]^-$ ion at m/z 313 (4.8%). ^1H NMR (MeOH-d_4):δ 1.34(2H,dd,J=7.3,14.7 Hz, H-5), 1.50 (2H,m,H-6), 1.55 (2H,m,H-4), 1.70 (1H,m,H-2a), 1.77 (1H,m,H-2b), 2.41 (2H,t,J=7.5 Hz, H-7), 2.53 (2H,t,J=7.5 Hz, H-1), 3.70 (1H,t,J=5.3 Hz, H-3), 3.20-3.41 (4H,m,Glc-2,3,4,5), 3.72 (1H,dd,J=5.3,11.7 Hz, Glc-6a), 3.89 (1H,dd,J=2.0,11.7 Hz, Glc-6b), 4.30 (1H,d,J=7.6 Hz, Glc-1), 6.45 (1H,dd,J=1.8,8.0 Hz, H-6"), 6.50 (1H,dd,J=1.6,7.8 Hz, H-6'), 6.60 (1H,d,J=1.9 Hz, H-2"), 6.65 (2H,d,J=8.0 Hz, H-5',H-5"), 6.70 (1H,d,J=1.8 Hz, H-2').

Rubranol xyloside {**X**}. Brown amorphous powder (0.07g); MW=464, $C_{24}H_{32}O_9$. $[\alpha]_D^{29}$= -22.5° (MeOH; c 0.4). Negative FAB-MS in thioglycerol-glycerol (2:1) gave a $[M-H]^-$ion at 463 (100%), an $[Aglycone-H]^-$ion at 331 (11.1%) and an $[Aglycone-H_2O-H]^-$ ion at m/z 313 (8.8%). ^1H NMR (MeOH-d_4):δ 1.37 (2H,m,H-5), 1.50 (2H,m,H-6), 1.52 (2H,m,H-4), 1.72 (2H,m,H-2), 2.43 (2H,t,J=7.4 Hz, H-7), 2.49 (2H,m,H-1), 3.60 (1H,t,J=5.2 Hz, H-3), 3.18 (2H,m,H-2,Xyl-5a), 3.32 (1H,t,J=8.9 Hz, Xyl-3), 3.50 (1H,m,Xyl-4), 3.86 (1H,dd, J=5.4,11.4 Hz, Xyl-5b), 4.22 (1H,d,J=7.5 Hz, Xyl-1), 6.45-6.49 (2H,m,H-6',H-6"), 6.60-6.67 (4H,m,H-2', H-2",H-5',H-5").

Rubranoside A {**XI**}. Yellowish amorphous solid (0.008g); MW=626, $C_{30}H_{42}O_{14}$. $[\alpha]_D^{25}$= -30.3° (MeOH; c 0.75). Negative FAB-MS in 3-NBA gave a $[M-H]^-$ion at 625 (81.4%), a $[M-Glc]^-$ ion at 463 (11.4%), an $[Aglycone-H]^-$ion at 331 (4.0%) and an $[Aglycone-H_2O-H]^-$ ion at m/z 313 (4.3%). ^1H NMR (MeOH-d_4):δ 1.38 (2H,m,H-5), 1.53(4H,m,H-4,H-6), 1.72 (2H,m,H-2), 2.44 (2H,mH-7), 2.54 (2H,m,H-1), 3.17-3.41 (6H,m,Glc-2,3,4,5; Xyl-2,5a), 3.48 (1H,t,J=8.8 Hz, Xyl-3), 3.56-3.65 (3H,m,H-3,Glc-6a,Xyl-4), 3.88 (1H,dd,J=2.0,11.8 Hz, Glc-6b), 3.91 (1H,dd,J=5.4,11.4 Hz, Xyl-5b), 4.26 (1H,d,J=7.6 Hz, Xyl-1), 4.55 (1H,d.J=7.8 Hz, Glc-1), 6.47 (2H,2dd,J=2.0,8.0 Hz, H-6',H-6"), 6.60 (2H,2d,J=2.0 Hz, H-2',H-2"), 6.64 (2H,2d,J=8.0 Hz, H-5',H-5").

Rubranol {**XII**}. Dark yellow oil (0.013g); MW=332, $C_{19}H_{24}O_5$. $[\alpha]_D^{27}$= -2.5° (MeOH; c 0.65). Negative FAB-MS in DEA gave a $[M-H]^-$ion at m/z 331 (100%). ^1H NMR (MeOH-d_4):δ 1.24-1.69 (8H,unresolv., H-2,H-4,H-5,H-6), 2.44 (2H,t,J=7.5 Hz, H-7), 2.56 (2H,m, H-1), 3.49 (1H,dd,J=4.5,7.6 Hz, H-3), 6.46 (1H,dd,J=1.8,8.2 Hz, H-6"), 6.48 (1H,dd,J=1.9,8.2 Hz, H-6'), 6.59 (1H,d,J=1.7 Hz, H-2"), 6.61 (1H,d,J=2.0 Hz, H-2'), 6.64 (1H,d,J=7.8 Hz, H-5'), 6.65 (1H,d,J=8.0 Hz, H-5").

Oregonoside A {**XIII**}. Brown amorphous powder (0.05g); MW= 612, $C_{32}H_{36}O_{12}$. $[\alpha]_D^{29}$= -7.45° (MeOH; c 0.07). Negative FAB-MS in 3-NBA gave a $[M-H]^-$ ion at m/z 611 (71.4%) and an $[Aglycone-H_2O-H]^-$ ion at 327 (11.4%). ^1H NMR (MeOH-d_4):δ 1.71 (2H,m,H-6), 2.39 (1H,m,H-7a), 2.46 (1H,m,H-7b), 2.50 (1H,dd,J=5.1,16.6 Hz, H-4a), 2.66 (4H,m,H-1,H-2), 2.76 (1H,dd,J=7.1,16.7 Hz, H-4b), 3.19 (1H,m,Glc-2), 3.40 (2H,m,Glc-3,4), 3.58 (1H,m,Glc-5), 4.10 (1H,m,H-5), 4.32

(1H,d,J=7.7 Hz, Glc-1), 4.45 (1H,dd,J=6.3,11.8 Hz, Glc-6a), 4.69 (1H,dd,J=2.0,11.8 Hz, Glc-6b), 6.36 (1H,dd,J=1.7,8.1 Hz, H-6"), 6.46 (1H,dd,J=1.8,8.0 Hz, H-6'), 6.54 (1H,d,J=1.8 Hz, H-2"), 6.56 (1H,d,J=7.9 Hz, H-5"), 6.60 (1H,d,J=2.0 Hz, H-2'), 6.64 (1H,d,J=8.1 Hz, H-5'), 7.37 (2H,t,J=7.6 Hz, H-3˜, H-5˜), 7.53 (1H,t,J=7.6 Hz, H-4˜), 7.96 (2H,t,J=7.6 Hz, H-2˜, H-6˜).

Oregonoside B {**XIV**}. Brown amorphous powder (0.008g); MW= 658, $C_{33}H_{38}O_{14}$. $[\alpha]_D^{29}$= -14.9° (MeOH; c 0.07). Negative FAB-MS in 3-NBA gave a $[M-H]^-$ ion at m/z 657 (86.7%), a $[M-OMe]^-$ ion at 627 (7.1%), an $[Aglycone+2H_2O-H]^-$ ion at 381 (22.9%) and an $[Aglycone-H_2O-H]^-$ ion at 327 (20.0%). ^{1}H NMR (MeOH-d_4):δ 1.70 (2H,m,H-6), 2.38 (1H,m,H-7a), 2.46 (1H,m,H-7b), 2.52 (1H,dd,J=5.3,16.6 Hz, H-4a), 2.68 (4H,m,H-1,H-2), 2.77 (1H,dd,J=7.0,16.6 Hz, H-4b), 3.17 (1H,m,Glc-2), 3.37 (2H,m,Glc-3,4), 3.59 (1H,m,Glc-5), 3.76 (3H,s,OMe), 4.11 (1H,m,H-5), 4.31 (1H,d,J=7.6 Hz, Glc-1), 4.44 (1H,dd,J=6.2,11.8 Hz, Glc-6a), 4.65 (1H,dd,J=2.0,11.8 Hz, Glc-6b), 6.38 (1H,dd,J=2.0,8.0 Hz, H-6"), 6.47 (1H,dd,J=2.0,8.0 Hz, H-6'), 6.54 (1H,d,J=2.1 Hz, H-2"), 6.56 (1H,d,J=8.1 Hz, H-5"), 6.60 (1H,d,J=1.8 Hz, H-2'), 6.64 (1H,d,J=8.0 Hz, H-5'). 6.79 (1H,d,J=8.5 Hz, H-5˜), 7.50 (1H,d,J=1.6 Hz, H-2˜), 7.55 (1H,dd,J=1.6,8.5 Hz, H-6˜).

Table 2.1 ^{13}C NMR assignments for *rubranol*, *rubranol glucoside*, *rubranol xyloside* and *rubranoside A* in MeOH-d_4.

	Rubranol	*Rubranol Glucoside*	*Rubranol Xyloside*		*Rubranoside A*
-C_7-	**XII**	**IX**	**X**		**XI**
1	32.4 ppm	31.8 ppm	31.9 ppm		32.0 ppm
2	40.5	37.9	38.1		38.2
3	71.8	79.5	80.2		80.1
4	38.2	34.8	35.0		35.0
5	26.3	25.6	25.7		25.6
6	32.9	33.0	33.0		32.9
7	36.2	36.2	36.1		36.1
Aryls					
1'	135.4[d]	135.8	135.8[a]		135.7[b]
2'	116.3[c]	117.0	116.7		116.7
3'	146.1	145.9	146.0		146.0
4'	144.1	144.0	144.0		144.1
5'	116.6	116.4	116.3		116.3
6'	120.7	121.0	120.8		120.7
1"	135.7[d]	135.8	135.7[a]		135.6[b]
2"	116.2[c]	116.7	116.6		116.6
3"	146.0	145.9	146.0		146.0
4"	144.0	144.0	144.0		144.1
5"	116.6	116.4	116.3		116.3
6"	120.7	120.8	120.8		120.7
Sugar		glucose	xylose	xylose	glucose
1		103.2	104.2	105.1	103.8
2		75.3	75.2	74.3	75.5
3		78.2	78.0	87.5	78.2
4		71.8	71.4	70.1	71.6
5		77.7	66.9	66.3	77.8
6		62.9	-	-	62.7

[a, b, c, d] These signals might be interchanged.

Table 2.2 ^{13}C NMR assignments for *oregonin*, *oregonoside A* and *oregonoside B* in MeOH-d_4.

-C_7-	*Oregonin*		*Oregonoside A*		*Oregonoside B*	
	I		**XIII**		**XIV**	
1	30.1 ppm		30.0 ppm		30.1 ppm	
2	46.4		46.3		46.4	
3	211.9		211.8		211.8	
4	49.6		49.6		49.0	
5	76.4		76.6		76.5	
6	38.6		38.4		38.6	
7	31.8		31.8		31.9	
Aryls						
1',1"	134.1	135.2	134.0	135.1	134.1	135.3
2',2"	116.6	116.6	116.4	116.5	116.6	116.6
3',3"	146.2	146.1	146.1	145.9	146.1	146.0
4',4"	144.5	144.2	144.3	144.0	144.4	144.1
5',5"	116.4	116.3	116.5	116.2	116.4	116.3
6',6"	120.6	120.7	120.7	120.6	120.6	120.8
Acylated moiety						
1‴	-		131.2		122.5	
2‴	-		130.6		113.7	
3‴	-		129.5		152.8	
4‴	-		134.2		148.7	
5‴	-		129.5		115.9	
6‴	-		130.6		125.2	
-COO-	-		168.0		168.1	
OMe	-		-		56.4	
Sugar	xylose		glucose		glucose	
1	104.3		103.6		103.6	
2	75.1		75.2		75.3	
3	77.9		77.8		77.9	
4	71.3		71.9		72.0	
5	76.0		75.2		75.5	
6	-		65.2		64.9	

REFERENCES

1. a) USDA Forest Service General Technical Report PNW-70. Utilization and management of alder, (1978). b) Little, E. L. The Audubon Society field guide to North American trees. Western region. A.A. Knopf, New York (1980). c) Brockman, F. Trees of North America. Golden Press. New York, (1986).

2. Panshin, A.J.; C. de Zeeuw. *Textbook of wood technology*. Fourth Edition. McGraw-Hill, New York, (1980).

3. Karchesy, J.J. *Polyphenols of red alder: Chemistry of the staining phenomenon*. Ph.D. Thesis, Oregon State University, (1974).

4. Forlines, D.R.; Tavenner, T.; Malan, J.C.S.; Karchesy, J.J. Plants of the Olympic coastal forests: Ancient knowledge of materials and medicines and future heritage. In Hemingway, R.W.; Laks, P.E. (eds.) *Plant polyphenols*. Plenum Press, New York, (1992).

5. Okuda, T.; Yoshida, T.; Hatano, T. Ellagitannins as active constituents of medicinal plants. *Planta Med*. 55, 117 (1989).

6. Okuda, T.; Yoshida, T.; Hatano, T. Pharmacological active tannins isolated from medicinal plants. In Hemingway, R.W.; Laks, P.E. (eds.) *Plant polyphenols*. Plenum Press, New York, (1992).

7. Kurth, E.F.; Becker, E.L. The Chemical nature of the extractives from red alder. *TAPPI*. **36**(10), 461 (1953).

8. Karchesy, J.J.; Laver, M.L.; Barofsky, D.F.; Barofsky, E.J. Structure of Oregonin, a natural diarylheptanoid xyloside. *Chem. Soc., Chem. Commun.*, 649 (1974).

9. Terazawa, M.; Okuyama, H.; Miyake, M. Isolation of Hirsutanonol and Hirsutenone, two new dyarylheptanoids from the green bark of keyamahannoki, *Alnus hirsuta* Turcz. *Mokuzai Gakkaishi*, **19**(1), 45 (1973).

10. Terazawa, M.; Koga, T.; Okuyama, H.; Miyake, M. Isolation of Platyphyllonol, a new diarylheptanoid fron the green bark of shirakanba, *Betula platyphylla* Sukatch. var japonica Hara. *Mokuzai Gakkaishi*, **19**(1), 47 (1973).

11. Suga, T.; Ohta, S.; Hirata, T.; Aoki, T. The absolute configuration of diarylheptanoid xyloside, oregonin, isolated from the female flowers of *Alnus serrulatoides*. *Chem. Lett.*, 895 (1982).

12. Ohta, S.; Aoki, T.; Hirata, T.; Suga, T. The structures of four diarylheptanoids glycosides from the female flowers of *Alnus serrulatoides*. *J. Chem. Soc. Perkin Trans.* I, 1635 (1984).

13. Lee, M; Tanaka, T.; Nonaka, G.; Nishioka, I. Hirsunin, an ellagitannin with a diarylheptanoid moiety, from *Alnus hirsuta* var. microphylla. *Phytochem.* **31**(3), 967 (1992).

14. Nagai, M; Kenmochi, N.; Fujita, M.; Furukawa, N.; Inoue, T. Studies on the constituents of Aceraceae plants. VI revised stereochemistry of (-)-centrolobol and new glycosides from *Acer nikoense*. *Chem. Pharm. Bull.* **34**(3), 1056 (1986).

15. Smite, E.; Lundgren, L.; Andersson, R. Arylbutanoid and diarylheptanoid glycosides from inner bark of *Betula pendula*. *Phytochem.* **32**(2), 365 (1993).

16. Nagai, M.; Kubo, M.; Fujita, M.; Inoue, T.; Matsuo, M. Studies on the constituents of Aceraceae plants II. Structure of Aceroside I, a glucoside of a novel cyclic diarylheptanoid from *Acer nikoense* Maxim. *Chem. Pharm. Bull.* **26**, 2805 (1978).

17. Aoki, T.; Ohta, S.; Suga, T. Triterpenoids, diarylheptanoids and their glycosides in the flowers of *Alnus* species. *Phytochem.* **29**(11), 3611 (1990).

18. Nomura, M.; Tokorayama, T.; Kubota, T. Further phenolic components from *Alnus japonica* Steud. *J.C.S. Chem Comm.* 316, (1975).

19. Nomura, M.; Tokorayama, T.; Kubota, T. Biarylheptanoids and other constituents from wood of *Alnus japonica*. *Phytochem.* **20**(5), 1097 (1981).

20. Erdtman, H. In Mabry, T.J. (Ed). *Recent Advances in Phytochemistry*. North-Holland, Amsterdam. Vol **1**, 27 (1968).

21. Roughley, P.J.; Whiting, D.A. Diarylheptanoids; The problems of the biosynthesis. *Tetrahedron Lett.* **40**, 3741 (1971).

22. Murata T.; Shinohara, M.; Miyamoto, M. Isolation of hexahydrocurcumin, dihydrogingerol and two additional pungent principles from ginger. *Chem. Pharm. Bull.* **20**(10), 2291 (1972).

23. Kiuchi, F.; Shibuya, M.; Sankawa, U. Inhibitors of prostaglandin biosynthesis from *Alpinia officinarum*. *Chem. Pharm. Bull.* **30**, 2279 (1982).

24. Itokawa, H.; Morita, M.; Mihashi, S. Two new diarylheptanoids from *Alpinia officinarum* Hance. *Chem. Pharm. Bull.* **29**(8), 2383 (1981).

25. Hikino, H.; Kiso, Y.; Kato, N.; Hamada, Y. Shioiri, T.; Aiyama, R.; Itokawa, H.; Kiuchi, F.; Sankawa, U. Series on liver-protective drugs 25. Antihepatotoxic actions of gingerols and diarylheptanoids. *Journal of Ethnopharmacology*. **14**(1), 31 (1985).

26. Sunnerheim, K.; Palo, T.; Theander, O.; Knutsson, P. Chemical defense in birch. *J. of Chem. Ecology*. **14**(2), 549 (1988).

27. McCutcheon, A.; Ellis, S.; Hancock, R.; Towers, G. Antibiotic screening of medicinal plants of the British Columbian native peoples. *Journal of Ethnopharmacology*. **37**, 213 (1992).

28. Nakatani, N. Natural antioxidants from spices. In Huang M.; Ho, C.; Lee, C. Phenolic compounds in food and their effects on health II. *ACS Symposium Series* 507, p 72 (1992).

29. Toda, S.; Miyase, T.; Arichi, H.; Tanazawa, H.; Takino, Y. Natural antioxidants, III. Antioxidative components isolated from rhizome of *Curcuma longa* L. *Chem. Pharm. Bull.* **33**, 1725 (1985).

30. Larson, R.A. The antioxidants of higher plants. *Phytochem.* **27**(4), 969 (1988).

31. Agrawal, P.K. NMR spectroscopy in the structural elucidation of oligosaccaharides and glycosides. *Phytochem.* **31**(10), 3307 (1992).

32. Gorin, P.A.; Mazarek, M. Further studies on the assignment of signals in ^{13}C magnetic resonance spectra of aldoses and derived methyl glycosides. *Can. J. Chem.* **53**, 1212 (1975).

33. Bock, K.; Pedersen C. Carbon-13 spectroscopy of monosaccharides. *Advances in Carbohydrate Chemistry and Biochemistry*. Vol **41**, 27-66. Academic Press (1983).

34. Dorman, D.E.; Roberts, J.D. Nuclear magnetic resonance spectroscopy. Carbon-13 spectra of some pentose and hexose aldopyranoses. *J. Am. Chem. Soc.* **92**(5), 1355 (1970).

35. Breitmaier, E.; Voelter, W.; Jung, G.; Tanzer, C. Konfigurations-, Konformations- und substituenteneinflusse auf die ^{13}C-chemischen verschiebungen von glykosiden. *Chem. Ber.* **104**, 1147 (1971).

CHAPTER III

POLYPHENOL GLYCOSIDES FROM DOUGLAS-FIR OUTER BARK

INTRODUCTION

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is one of the world's most important trees for the valuable timber that produces. It is widely distributed in western North America and can be found growing in different habitats, from coastal temperate rain forest to very dry mountainous sites [1]. Its natural distribution resembles an inverted V with the apex in British Columbia. The western half extends along the Pacific mountain ranges into Washington, Oregon and California for about 2200 Km. The eastern half stretches along the Rocky Mountains from Canada to central Mexico over a distance of about 4500 Km.

There are two varieties of the tree that are commonly acknowledged. The coastal variety (var. menziesii), which is the largest of the two and the smaller Rocky Mountain, variety (var. glauca). Although Douglas-fir is a native species from western North America, it has been introduced successfully to many other areas including Argentina, Australia, Chile, New Zeland and Europe [2].

Douglas-fir is the most important source of timber and wood fiber in the Pacific Northwest. Its wood is well appreciated for manufacture lumber, plywood, laminated beams, pulp and paper. Such industrial operations have historically generated almost three million tons of bark

every year creating considerable disposal problems [3]. The bark is used as fuel, in decorative landscaping, as an extender in plywood adhesives and for charcoal briquette production. These limited uses of the bark do not exploit the chemicals present in the material, especially phenolic extractives or tannins that have potential for industrial utilization. Additionally these extracts are a nuisance for some alternate applications, such as mulch, due to a natural low level of nitrogen on the organic matter. In the composting process the bark depletes the nitrogen from the soil where it is applied.

Since the early 1940's there has been an intensive search for opportunities on the better utilization of this bark [4,5]. The prospects for bark as a renewable source of condensed tannins to replace or supplement petrochemical based wood adhesives was a driving force for research during the oil crisis in the 70's.

It is known now that, for example, the concentration of tannins is higher in the living tissues of inner bark and drops sharply in the outer bark. Here the tannins have been transformed into water insoluble phlobaphenes and other complex substances.

Chemical investigations have been prolific and have resulted in much information about bark polyphenols since the initial reports by Kurth, Hillis, Barton and many others. However, there is still much to do for the natural products chemist. As part of that endeavor this chapter and the next one describe the more recent contributions to the chemical knowledge of this fascinating resource.

LITERATURE REVIEW

Among the pioneering work on natural products from Douglas-fir is the report of *(+)-dihydroquercetin* (DHQ) in the heartwood by Pew [6] and in the bark by Hubbard and Kurth [7,8]. This discovery, as many others, was driven by specific industrial problems as DHQ was interfering with pulping reactions.

In the recent years Karchesy and coworkers [9] have led an intensive chemical study of both inner and outer bark polyphenols with significant results on the identification of glycosides, procyanidin dimers, trimers, tetramers and pentamers [10-13]. Special emphasis has been put on the identification of low molecular weight polyphenols from Douglas-fir bark that might be precursors to phlobaphene formation [14,15]. It was found that some flavonoids, flavonoid glycosides, procyanidins, lignans and flavonolignans [16] that have key NMR shifts that show up in the ^{13}C NMR of phlobaphene fractions.

Phenylpropanoids, such as the one isolated {**I**} from Douglas-fir outer bark, may play an intermediate role in the biosynthetic pathway for flavonoids, lignans and neolignans. It is assumed that eventually all of them are involved in the lignification process.

Benzene derivatives as {**II**} are uncommon in nature, however it was isolated previously from the oligomeric fraction soluble in ethyl acetate of Douglas-fir outer bark [14]. Also the naringenin and eriodictyol glycosides {**III,IV**} were found in that fraction. Compound **IV** has been reported from the roots of same tree [17]. The aglycone of **III** and other related free phenols, have been found in the

diseased and living roots of Douglas-fir too [18,19].

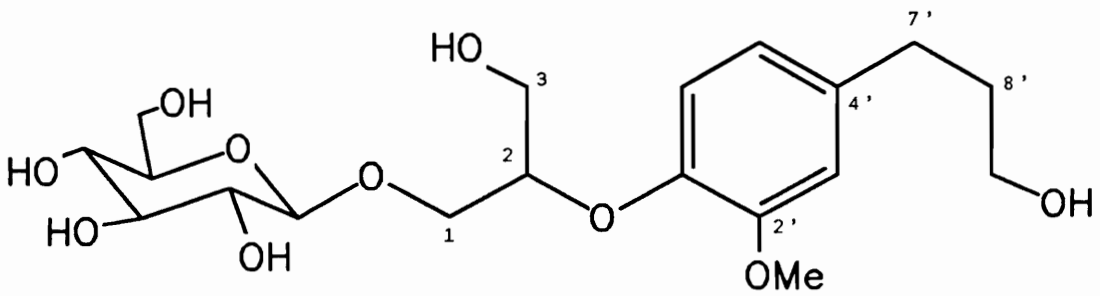


Figure 3.1 Structure of the glycerol derivative {**I**}.

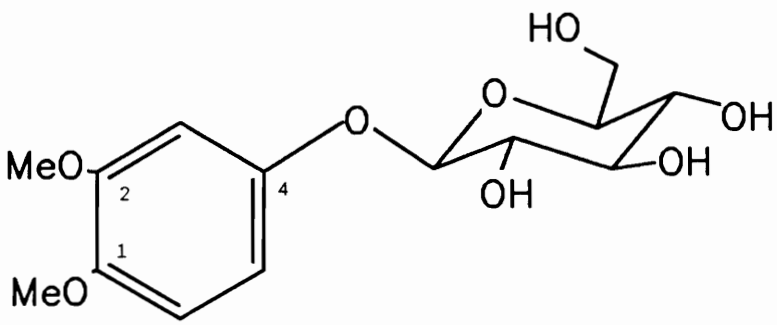
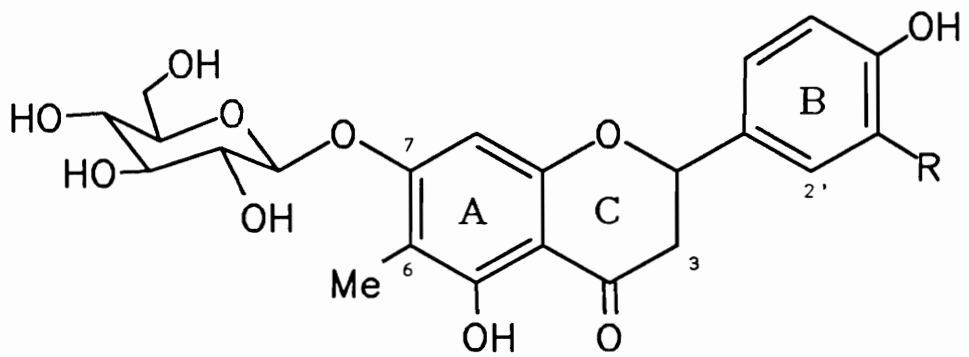


Figure 3.2 Structure of *1,2-dimethoxy-4-O-β-D-gluco-pyranoside benzene* {**II**}.

RESULTS AND DISCUSSION

Several polyphenolic compounds were isolated, including the glycerol derivative *1-O-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxyl]-propan-3-ol* {**I**}, the benzene derivative *1,2-dimethoxy-4-O-β-D-glucopyranoside benzene* {**II**}, the flavanone glycosides *6-C-methyl-naringenin-7-O-β-D-glucopyranoside* {**III**} and the *6-C-methyl-eriodictyol-7-O-β-D-glucopyranoside* {**IV**}. Other compounds isolated were the previously reported *DHQ-3'-O-β-D-glucopyranoside*, *DHQ-7-O-β-D-glucopyranoside*, *dihydrokaempferol-7-O-β-D-glucopyranoside* [10], and the well known *eriodictyol-7-O-β-D-glucopyranoside*, *dihydroquercetin* and *pinoresinol*.



III R = H

IV R = OH

Figure 3.3 Structures of the *6-C-Methyl-7-O-β-D-glucopyranosides* of *naringenin* {**III**} and *eriodictyol* {**IV**}.

The glycerol derivative **I** found in Douglas-fir outer bark has been already reported from the inner bark of *Larix leptolesis* by Miki and Sasaya [20,21]. It was isolated from the initial fraction coming out from a LH-20 column eluted

with ethanol. This separation was particularly difficult due to the presence of many low molecular phenolic and non-phenolic compounds, requiring additional steps for its purification. The ^{1}H NMR showed signals for a single aromatic ring with an ABX pattern, a short aliphatic chain, a methoxy group and a sugar moiety. From the COSY experiment an open propanolic string was clarified and the existence of another independent C_3-chain was visualized.

The ^{13}C NMR data shown in Table 3.1 confirmed the carbohydrate moiety as *β-D-glucopyranose* in agreement with the literature [22,23]. A DEPT NMR experiment confirmed a disubstituted propaneglycol string with two hydroxymethylene signals at 62.0 and 68.9 ppm, and an oxymethine signal at 81.2 ppm. The final structure was made through the analysis of ^{13}C-^{1}H heteronuclear correlations (HETCOR) and long range ^{13}C-^{1}H two and three bond couplings (HETCOSY).

From the FAB-MS the ion peak at m/z 181, which is also the base peak, was identified as corresponding to a dihydroconiferyl alcohol ion (i.e., the aglycone minus a propanetriol unit). Additional peaks were detected for the molecular ($[M-H]^-$) and aglycone ($[M-sugar-H]^-$) ions at m/z 417 and 255 respectively that supported the structure **I**, suggested from the NMR data. Thus the glycerol derivative was confirmed as *1-O-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxyl]-propan-3-ol*.

From the second outcoming fraction was isolated a light yellowish powder after evaporation of solvent. The ^{1}H NMR showed a single ABX system that was confirmed through a COSY experiment. Two methoxy signals and a carbohydrate moiety were observed. The carbon spectra showed signals

characteristic of a β-D-glucopyranose (see Table 3.2). This was confirmed later by enzymic hydrolysis and TLC along with a known sample. Thus the sugar and the methoxyls were attached to a benzylic ring. The identity of methoxyls and their position in the ring was elucidated through a Nuclear Overhauser Effect (NOE) experiment. Every methoxyl was irradiated and the enhancement on neighboring proton signals was measured. In this way the position of the substituents was precisely known. The FAB-MS of this compound exhibited a molecular ion $[M-H]^-$ at m/z 315, supporting the structure for *1,2-dimethoxy-4-O-β-D-glucopyranoside benzene* {**II**}.

The NMR spectra from two compounds isolated in the sixth and seventh fractions respectively, showed spectroscopic information for typical flavanones. The mayor differences between those compounds were in the aromatic region corresponding to the B ring. In the first case the ^1H NMR showed two symmetric doublets (J=8.5 Hz) at 6.8 and 7.3 ppm, signifying a *para* substitution on ring B. In the second compound the pattern displayed was an ABX system typical of a catechol ring. Besides the FAB-MS spectra gave molecular ions ($[M-H]^-$) at m/z at 447 and 463, confirming that the only difference was the hydroxylation pattern in the B ring.

The rest of the data presented signals for a methyl and a carbohydrate moiety (see Tables 3.3 and 3.4). Several 2-D NMR experiments showed connectivity of anomeric carbon and proton to C_7 on ring A. The same experiments confined methyl group to the position C_6. The sugar was confirmed as β-D-glucose from the ^{13}C NMR and enzymic hydrolysis as was performed before. The spectra also were compared successfully with those from their aglycones that were

isolated in free phenolic form in parallel experiments. So these compounds were characterized as the *6-C-methyl-7-O-β-D-glucopyranoside* of *naringenin* {**III**} and *eriodictyol* {**IV**}.

EXPERIMENTAL

Outer bark extract.

The outer bark of a 120-year old Douglas-fir tree was manually collected, chipped and air dried before extraction. The bark was soaked overnight at room temperature in methanol three times and the extracts combined and concentrated under reduced pressure at 35°C. The extract, 820g of a dark brown powder, was extracted exhaustively with hexane to remove waxes and high molecular weight alcohols. The alcohol soluble fraction (800g) was then treated with ether and the ether insoluble portion (650.2g) was divided into ethyl acetate soluble (153.4g) and insoluble fractions (491.4g).

Chromatographic separations.

A 90g sample from the ethyl acetate insoluble fraction was applied into two sephadex LH-20 columns (10x60 cm each) connected in series, using ethanol as eluant. The separation was monitored by TLC on precoated Merck plastic sheets, Silica gel 60 F_{254}, 0.25 mm. The plates were developed with the solvent system Benzene-Acetone-Methanol (6:3:1:) and sprayed with H_2SO_4-HCHO (40:1) followed by heating.

The proanthocyanidins were detected in 2D-TLC with cellulose plastic sheets (Schleicher and Schuell F 1440/LS 254) developing the first dimension with *ter*-BuOH-HOAc-H_2O (3:1:1) and the second with 6% HOAc [24]. Later the plates were sprayed with acidic vanillin solution (1g in 50 ml EtOH + 10 ml HCl) and heated.

The initial separation afforded 13 fractions that were successively rechromatographied using ethanol-water

mixtures and alternating with columns of Fractogel (Toyopearl) TSK HW-40F.

Enzymic hydrolysis.

An analytical enzymic hydrolysis was performed to confirm by TLC the nature of aglycone and sugar moieties in the glycosides. A small sample of glycoside was dissolved in water and stand at room temperature for 3 hs with a small amount of β-glucosidase. The solution was then freeze dried and the products extracted with methanol. Glucose was detected on cellulose plates (Schleicher & Schuell) with the system *n*-BuOH-Pyridine-H_2O (6:4:3).

1-O-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxyl]-propan-3-ol {**I**}.

Light yellow oil (0.060g); MW 418, $C_{19}H_{30}O_{10}$. $[\alpha]_D^{25}$ -14.9° [MeOH, c 1.2]. Negative FAB-MS in DEA displayed peaks for ions: $[M-H]^-$ at m/z 417 (36%), $[M-CH_3]^-$ at 403 (4.5%), $[Aglycone-H]^-$ at 255 (18%) and $[Aglycone-CH_2OHCHCH_2OH]^-$ at 181 (53.9%). ^1H NMR (MeOH-d_4) δ: 1.80 (2H,m,H-8'), 2.61 (2H,t,J=7.7 Hz, H-7'), 3.20-3.38 (4H,m,Glc-2,3,4,5), 3.55 (2H,t,J=6.5 Hz, H-9'), 3.65 (1H,dd,J=5.0,11.8 Hz, Glc-6a), 3.83 (4H,m,H-1a, H-3, Glc-6b), 3.83 (3H,s,OMe), 4.05 (1H,dd,J=4.2,10.6 Hz, H-1b), 4.32 (1H,d,J=7.5 Hz, Glc-1), 4.33 (1H,m,H-2), 6.72 (1H,dd,1.9,8.1 Hz H-6'), 6.83 (1H,d,J=1.9 Hz, H-2'), 7.00 (1H,d,J=8.1 Hz, H-5').

1,2-dimethoxy-benzene-4-O-β-D-glucopyranoside {**II**}.

Light yellow amorphous powder (0.13g), MW= 316, $C_{14}H_{20}O_8$. $[\alpha]_D^{25}$ -41.7° [MeOH, c 0.1]. Negative FAB-MS in dithiothreitol and dithioerythritol (Magic Bullet), gave a $[M-H]^-$ ion at m/z 315. ^1H NMR (MeOH-d_4) δ: 3.35-3.44 (4H,m,Glc-2,3,4,5), 3.67 (1H,dd,J=5.8,12.0 Hz, Glc-6a),

3.89(1H,dd,J=2.0,12.0 Hz, Glc-6b), 4.77 (1H,d,J=7.5 Hz, Glc-1), 6.65 (1H,dd,J=2.5,8.7 Hz, H-5), 6.81 (1H,d,J=2.5 Hz, H-6), 6.83 (1H,d,J=8.7 Hz, H-3).

6-C-methyl-naringenin-7-O-β-D-glucopyranoside (**III**). Yellow amorphous powder (0.105 g). MW 448, $C_{22}H_{24}O_{10}$. $[\alpha]_D^{25}$ -65.2° [MeOH, c 0.1]. Negative FAB-MS in dithiothreitol and dithioerythritol (Magic Bullet), displayed a peak for the molecular ion $[M-H]^-$ at m/z 447 (100%). ^1H NMR (MeOH-d_4)δ: 2.02 (3H,s,CH_3), 2.70 (1H,dd,J=3.1,17.0 Hz H-3ax), 3.15 (1H,dd,J=13.1,17.0 Hz, H-3eq), 3.38-3.50 (4H.m,Glc-2,3,4,5), 3.65 (1H,dd,J=5.0,12.0 Hz, Glc-6a), 3.85 (1H,dd,J=1.5,12.0 Hz, Glc-6b), 4.98 (1H,d,J=7.1 Hz, Glc-1), 5.32 (1H,dd,J=2.8,13.0 Hz, H-2), 6.29 (1H,s,H-8), 6.80 (2H,d,J=8.5 Hz, H-3',H-5'), 7.30 (2H,d,J=8.5 Hz, H-2',H-6').

6-C-methyl-eriodictyol-7-O-β-D-glucopyranoside {**IV**}. Brown amorphous powder (0.035g). MW 464, $C_{22}H_{24}O_{11}$. $[\alpha]_D^{25}$ -58.8° [MeOH, c 0.04]. Negative FAB-MS in DEA displayed peaks for ions: $[M-H]^-$ at m/z 463 (100%). ^1H NMR (MeOH-d_4)δ: 2.03 (3H,s,CH_3) 2.71 (1H,dd,J=3.1,17.0 Hz H-3ax), 3.10 (1H,dd,J=13.1,17.0 Hz, H-3eq), 3.35-3.51 (4H.m,Glc-2,3,4,5), 3.66 (1H,dd,J=5.0,12.0 Hz, Glc-6a), 3.86 (1H,dd,J=1.5,12.0 Hz, Glc-6b), 4.98 (1H,d,J=7.1 Hz, Glc-1), 5.27 (1H,dd,J=2.8,13.0 Hz, H-2), 6.29 (1H,s,H-8), 6.78 (2H,d,H-2',H-5'), 6.91 (1H,,H-6').

Table 3.1 ^{13}C NMR assignments of glycerol derivative **I**.

Propane chain		Phenylpropanoid	
C_1	68.9 ppm	$C_{1'}$	138.3
C_2	81.2	$C_{2'}$	114.0
C_3	62.0	$C_{3'}$	151.8
Glucose		$C_{4'}$	146.5
C_1	104.7	$C_{5'}$	119.3
C_2	75.0	$C_{6'}$	121.8
C_3	77.8	$C_{7'}$	35.5
C_4	71.5	$C_{8'}$	32.7
C_5	77.8	$C_{9'}$	62.2
C_6	62.6	OMe	56.4

Table 3.2 ^{13}C NMR assignments from benzene derivative **II**.

ring		glucose		OMe's	
C_1	151.1 ppm	$C_{1'}$	103.4	C-1	56.4
C_2	153.9	$C_{2'}$	74.9	C-2	57.1
C_3	104.1	$C_{3'}$	78.2		
C_4	146.0	$C_{4'}$	71.5		
C_5	109.3	$C_{5'}$	78.0		
C_6	113.9	$C_{6'}$	62.6		

Table 3.3 ^{13}C NMR assignments from naringenin glycoside **III.**

C_2	80.7 ppm	$C_{1'}$	131.0	glucose	
C_3	44.3	$C_{2'}$	129.0	C_1	101.2 ppm
C_4	198.8	$C_{3'}$	116.3	C_2	74.7
C_{4a}	104.5	$C_{4'}$	159.1	C_3	78.0
C_5	164.8	$C_{5'}$	116.3	C_4	71.1
C_6	107.6	$C_{6'}$	129.0	C_5	78.2
C_7	162.5			C_6	62.3
C_8	95.1				
C_{8a}	161.7	CH_3	7.3		

Table 3.4 ^{13}C NMR assignments for eriodictyol glycoside **IV.**

C_2	80.7 ppm	$C_{1'}$	131.7	glucose	
C_3	44.3	$C_{2'}$	116.3	C_1	101.3 ppm
C_4	198.7	$C_{3'}$	146.5	C_2	74.7
C_{4a}	104.7	$C_{4'}$	147.0	C_3	78.2
C_5	162.5	$C_{5'}$	114.9	C_4	71.2
C_6	107.9	$C_{6'}$	119.4	C_5	78.1
C_7	165.0			C_6	62.4
C_8	95.2				
C_{8a}	162.0	CH_3	7.3		

REFERENCES

1. Fowells, H.A. Silvics of forest trees of the United States. *Agriculture Handbook 271*, p. 546. U.S. Dept. of Agriculture. (1965).

2. Herman, R.K. The Genus *Pseudotsuga*: Historical Records and Nomenclature. Special Publication 2a, Forest Research Laboratory, Oregon State University, Corvallis, Oregon. (1982).

3. Trocino, F. *Am. Inst. Chem. Eng. Symp. Ser.* **71**(146), 46 (1975).

4. Hergert, H.L. Economic importance of flavanoid compounds: wood and bark. In Geissman, T.A. (ed.) *The Chemistry of Flavonoid compounds*. MacMillan Co. (1962).

5. Hall, J.A. Utilization of Douglas-fir bark. Pacific Northwest Forest and Range Experiment Station. Forest Service, U.S.D.A. Portland Oregon. 138p (1971).

6. Pew, J.C. A flavanone from Douglas-fir heartwood. *J. Am. Chem. Soc.* **70**, 3031 (1948).

7. Hubbard, J.K.; Kurth, E.F. *J. Am. Leather Chem. Assoc.* **44**, 604 (1949).

8. Kurth, E.F.; Kiefer, H.J. Hubbard, J.K. *The Timberman.* **49**(8), 1948.

9. Karchesy, J.J. Polyphenols of Douglas-fir bark: Structure, reactions and significance. *XVI International Symposium on Natural Products Chemistry*. Monterrey, Mexico. (1991).

10. Foo, L.Y.; Karchesy, J.J. Polyphenolic glycosides from Douglas-fir inner bark. *Phytochem.* **28**(4), 1237 (1989).

11. Foo, L.Y.; Karchesy, J.J. Procyanidin dimers and trimers from Douglas-fir inner bark. *Phytochem.* **28**(6), 1743 (1989).

12. Foo, L.Y.; Karchesy, J.J. Procyanidin polymers of Douglas fir bark: Structure from degradation with phloroglucinol. *Phytochem.* **28**(11), 3185 (1989).

13. Foo, L.Y.; Karchesy, J.J. Procyanidin tetramers and pentamers from Douglas fir bark. *Phytochem.* **30**(2), 667 (1991).

14. Malan, J.C.S.; Chen, J.; Foo, L.Y.; Karchesy, J.J. Phlobaphene precursors in Douglas-fir outer bark. In Hemingway, R.W.; Laks, P.E.(eds.) *Plant Polyphenols*. Plenum Press. (1992).

15. Foo, Y.; Karchesy, J. Chemical nature of phobaphenes. In Hemingway, R.W.; Karchesy, J.(eds.) *Chemistry and significance of condensed tannins*. Plenum Press. (1989).

16. Foo, L.Y.; Karchesy, J.J. Pseudotsuganol, a biphenyl-linked pinoresinol-dehydroquercetin from Douglas-fir bark: Isolation of the first flavonolignan. *J. Chem. Soc., Chem. Commun.* 217 (1989).

17. Barton, G.M. 7-O-β-D-Glucosyl-3',4',5-trihydroxy-6-methylflavanone - a new C-methyl flavanone glycoside from Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] roots. *Can. J. Chem.* **47**, 869 (1969).

18. Barton, G.M. A new C-methyl flavanone from diseased (*Poria weirii* Murr.) Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] roots. *Can. J. Chem.* **45**, 1020 (1967).

19. Hillis, W.E.; Ishikura, N. A new flavanone glucoside, Poriolin, from Douglas-fir roots. *Aust. J. Chem.* **22**, 483 (1969).

20. Miki, K.; Sasaya, T. A glycoside from the inner bark of *Larix leptolesis* Gord. *Mokuzai Gakkaishi*. **24**(9), 671 (1978).

21. Miki, K.; Sasaya, T. Glycerol derivative in the inner bark of *Larix leptolesis* Gord. *Mokuzai Gakkaishi*. **25**(5), 361 (1979).

22. Dorman D.E.; Roberts, J.D. Nuclear magnetic resonance spectroscopy. Carbon-13 spectra of some pentose and hexose aldopyranoses. *J. Am. Chem. Soc*. **92**, 1355 (1970).

23. Breitmaier, E.; Voelter, W.; Jung, G.; Tanzer, C. Konfigurations-, Konformations- und substituenteneinflusse auf die ^{13}C-chemischen verschiebungen von glykosiden. *Chem. Ber*. **104**, 1147 (1971).

24. Karchesy, J.; Bae, Y.S.; Chalker-Scott, L.; Helm, R.F.; Foo, L.Y. Chromatography of proanthocyanidins. In Hemingway, R.W.; Karchesy, J. (eds.) *Chemistry and significance of condensed tannins*. Plenum Press. (1989).

CHAPTER IV

NEOLIGNAN GLYCOSIDES FROM DOUGLAS-FIR BARK

INTRODUCTION

Lignans and neolignans.

Lignans and neolignans are natural products, which consist of two linked n-propylbenzene units. They are biogenetically formed through the shikimate pathway. Lignans are those compounds with the C_6-C_3 residues linked by the β carbon (i.e., central) of each side chain. This definition was given by Haworth in 1941 [1,2]. Later, McCredie [3] proposed that this definition should be expanded to cover those low molecular weights products from the oxidative coupling of p-hydroxyphenylpropane units.

The term neolignan was initially proposed by Gottlieb in 1972 to distinguish among those compounds with the same skeleton, but with a different linkage to the β-β' bond [4,5]. Later, he redefined lignans as the oxidative coupling products of cinnamyl alcohols or cinnamic acids, whereas neolignans were the oxidative dimerization products of allyl or propenyl phenols [6]. Because the later definition does not identify any fundamental chemical difference between the two types of compounds, the former definition by Gottlieb is more accepted, avoiding misinterpretations. In the past some chemical classifications based on biosynthetic origin have not worked well when new pathways became known. So it is still safer to make such assumptions based on compound structure rather than on speculations.

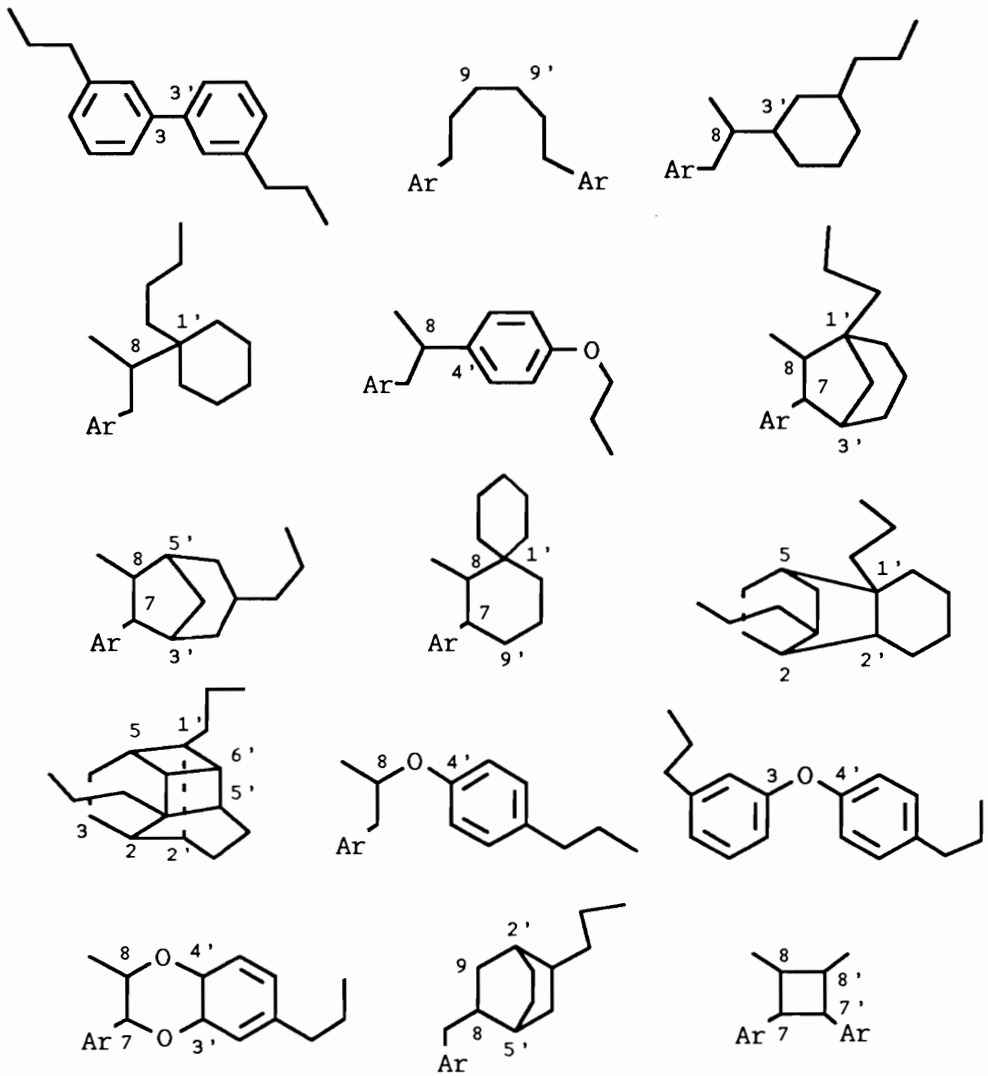


Figure 4.1 Classification of neolignans according to the union of the C_6-C_3 units.

Classification of neolignans.

Neolignans show several structural patterns that are classified in fifteen groups, each one is described by the points of union between propylbenzene units as shown in figure 4.1 [7]. Under this system, by definition, all lignans would belong to an 8-8' group. The derivatives of *dehydrodiconiferyl alcohol*, as an example, have the 8-3' linkage with an oxygen bridge between the C_6-C_3 units to form a benzofuran or dihydrobenzofuran skeleton. The absolute configuration of this group of compounds has been already studied [8] using X-ray determinations, chemical correlations, Optical Rotatory Dispersion (ORD) data and lanthanide-induced NMR shifts (LIS). The geometry was established as *2,3-trans* in most of the known cases.

As part of a systematic investigation of the chemical composition of Douglas-fir bark, as a potential source of phenolics, a series of chromatographic separations were performed on inner and outer bark methanolic extracts. These experiments lead to the isolation of four neolignan glycosides, including the novel *4-O-α-L-rhamnopyranoside* of *dihydrodehydrodiconiferyl alcohol*. Their structure were elucidated by means of ^{1}H and C^{13} NMR, 2D-NMR and FAB-MS spectroscopies. This is the first report of neolignans from Douglas-fir and the genus *Pseudotsuga*.

LITERATURE REVIEW.

Although neolignans are more diverse in structure, they are less abundant than lignans. So they have been reported only from the plants orders *Magnoliales* and *Piperales* [9]. Gottlieb reported monomeric allyl and propenylphenols from plants of those orders too [10]. Their biological function, as well as that of lignans, is not yet well identified. As secondary metabolites, it is suggested that they might have a role in plant host defense systems and serve in the regulation of plant growth.

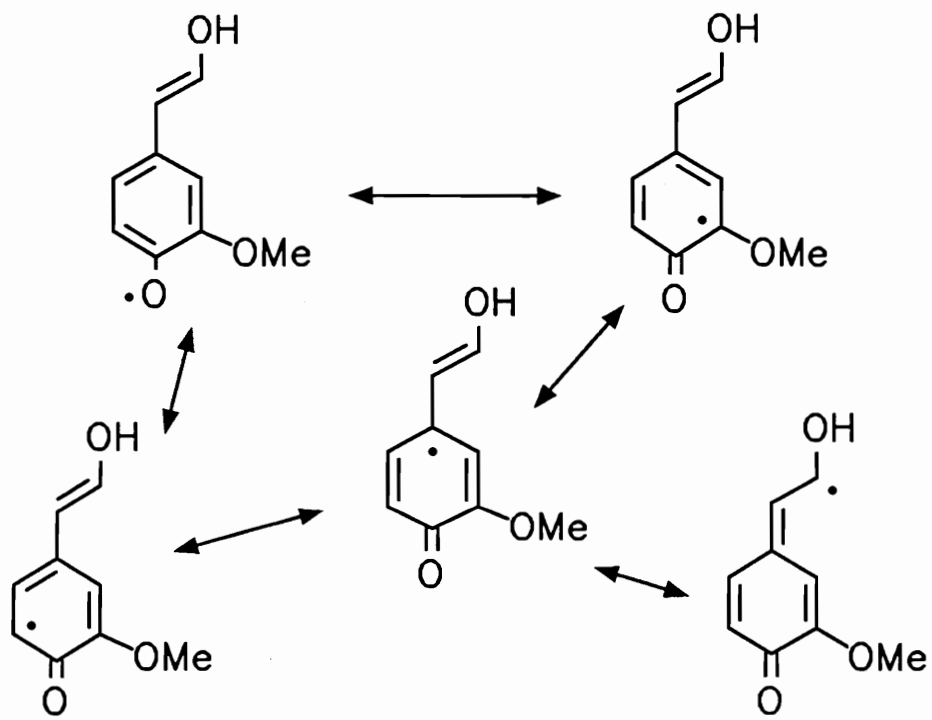


Figure 4.2 Canonical forms for the stabilized-radicals of coniferyl alcohol.

Biosynthesis of neolignans.

The formation of lignans and neolignans can be explained, as in the case of lignin, through the frequent

oxidative coupling of phenoxy radicals according to the Canonical forms proposed by Freudenberg [11]. The figure 4.2 illustrates the resonance-stabilized phenoxy radicals of the oxidation of coniferyl alcohol [12]. Thus lignan precursors will undergo additional reactions from the initial β-β' coupling, including cyclization, reduction or hydration. The neolignans will be formed through any other combination during the coupling process. In both cases it is assumed that lignan and neolignans are by-products or middle steps in the pathway of cinnamate biosynthesis to lignin.

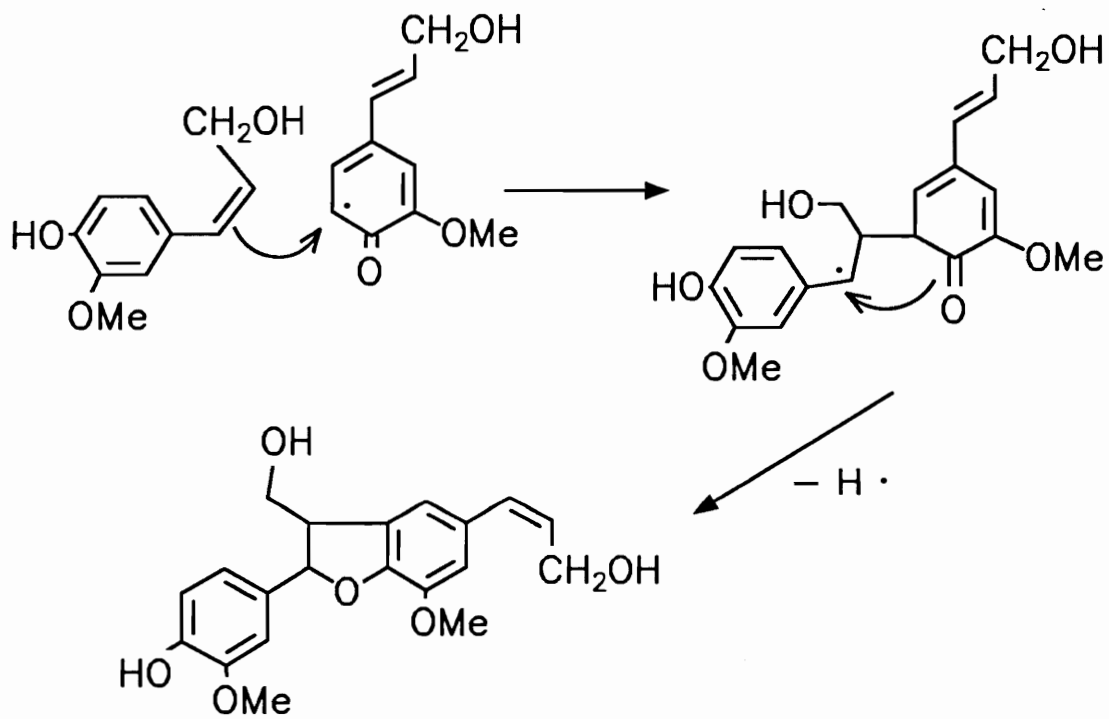


Figure 4.3 Hypothetical dimerization mechanism in the formation of dehydrodiconiferyl alcohol.

The biosynthesis of most neolignans was explained by Gottlieb [6] with the quinone methide mechanism. This mechanism explains also the formation of 8-8' linked lignan

dimers and lignol oligomers such as flavono-lignans. There is evidence that quinone methides participate in the acid-induced cleavage of lignin as partial reversal of Freudenberg's mechanisms. Figure 4.3 shows the dimerization of coniferyl alcohol through a quinone methide intermediary.

The interest in neolignans is increasing because of their widespread distribution in nature. These substances have been found in humans and other animals as well as plants. The broad range of biological activity exhibited by neolignans has also spurred recent interest [13]. Some neolignans exhibit antitumor activity while others are growth inhibitors or antifungal agents [14]. The neolignan *kadsurenone* has been studied as a potential anti-asthma drug, because it inhibits the action of Platelet Activating Factor (PAF) in aggregating human neutrophils. It also shows counteraction against induced cardiovascular changes in rabits and endotoxic shock in rats [9].

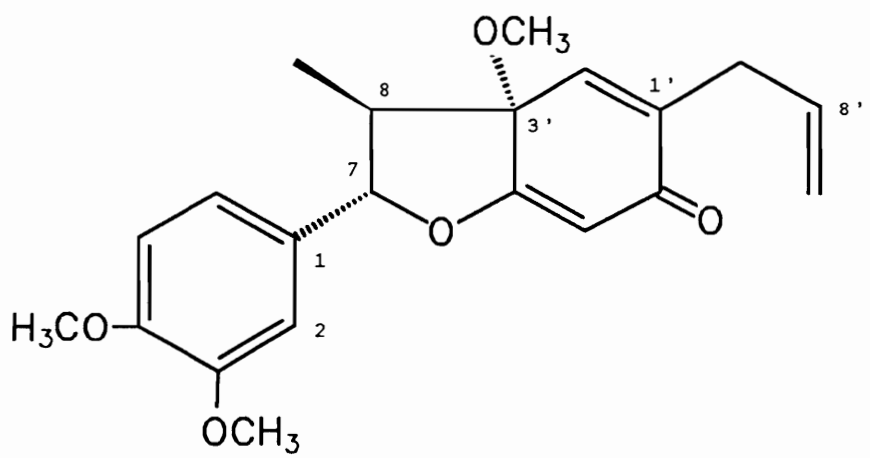


Figure 4.4 Structure of *kadsurenone*, a bioactive 8-3' neolignan.

An example of the simultaneous biosynthesis of lignans and neolignans is the oxidation of coniferyl alcohol by the enzyme *chloroperoxidase* [15,16]. This reaction produces a mixture of products including *pinoresinol* and *dehydrodiconiferyl alcohol* (DDCA) as shown in figure 4.5. This oxidative dimerization can be also accomplished by using conventional oxidating agents such as $FeCl_3$ and Ag_2O.

Lignan and neolignans have similar structures and linkages as the degradation products from protolignin. However, the former ones generally show optical activity, while the later ones remain inactive [17,18]

Extraction and isolation of related neolignans.

Experimentally, neolignans have been isolated from non polar extractions, using hexane, hexane-diethyl ether mixtures, petrol or benzene [19-21]. In many cases more polar neolignans holding free hydroxyls have been isolated with those weak solvents. However, another approach can be attempted with hot polar solvents as acetone, methanol or ethanol and removing later lipids from the extract with hexane. Some workers also have attempted steam distillation to select between volatile and non-volatile compounds [22].

Agrawal et al. reported *cedrusin* {**V**} in 1980, and *cedrusinin* {**VII**} in 1982, from *Cedrus deodara* [23,24]. Both neolignans are close relatives of *dihydrodehydrodiconiferyl alcohol* (DDDCA) {**VI**} and are present as aglycones of several glycosides reported from plants. Purification was accomplished by precipitation of those neolignans as their lead salts using lead acetate with the butanolic extract and then putting them back in solution with H_2S.

Popoff and Theander [25,26] isolated for the first time

III and **IV** from *Pinus sylvestris* needles. This was also the initial report for that type of dilignols. The neolignan *Cedrusin-4'-O-β-D-glucopyranoside* (**IV**) was isolated too from *Larix leptolepis* inner bark by Miki et. al. [18].

The literature reports on the studied neolignans use two ways for naming these compounds. One system derives their names from dihydroconiferyl alcohol and describes the numbering as two C_6-C_3 units. The other method gives the name as dihydrobenzofuran derivatives with three rings. Although the later manner might be more appropiate under IUPAC rules, the former method was chosen to display the data and the name for every compound is acknowledged according to both systems.

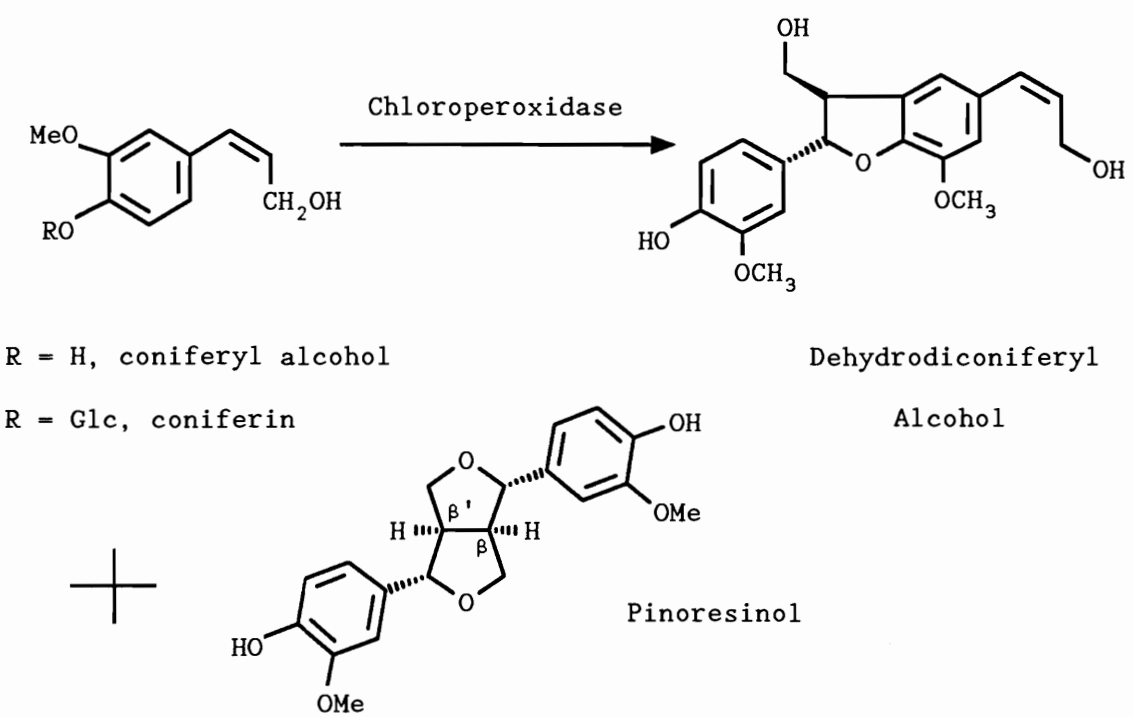


Figure 4.5 Phenolic oxidative coupling of coniferyl alcohol by enzyme chloroperoxidase.

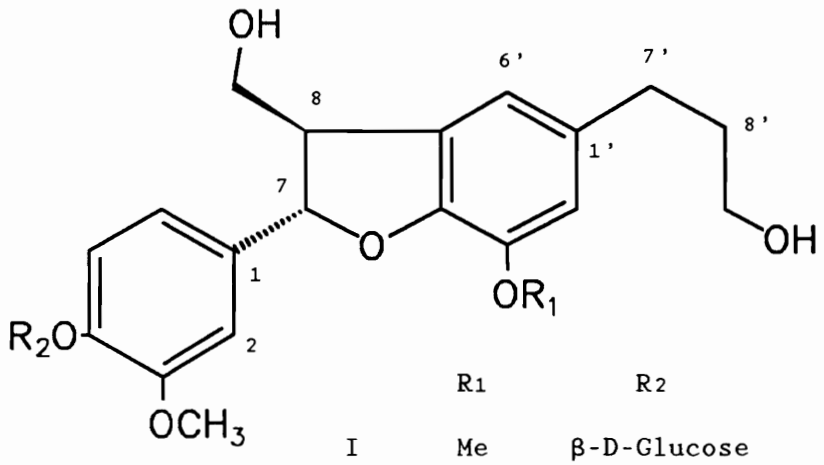
RESULTS AND DISCUSSION

Four neolignan glycosides were isolated from Douglas-fir bark methanol extracts. Two neolignan glycosides were isolated in the ethyl acetate insoluble fraction of Douglas-fir outer bark methanolic extract. They were the glucoside and rhamnoside of dihydrodehydro-diconiferyl alcohol (DDDCA-Glc and DDDCA-Rha). Both glycosides have been isolated from conifers needles and other plant barks [30,31]. The other two neolignans, the glucoside and rhamnoside of cedrusin, were found in the ethyl acetate soluble fraction from Douglas-fir inner bark methanol extract.

The ^{13}C NMR data for the known neolignan glycosides isolated from Douglas-fir outer and inner bark, were compared successfully with those reported in the literature for similar and related compounds [27,28,32-35]. In several cases, as will be noted, some former assignments should be revised. The application of high resolution 2D-NMR techniques have enhanced the precision for many assignments that previously were made intuitively.

The ^{13}C NMR spectra for *cedrusin rhamnoside* showed a close resemblance to that of DDDCA-Rha, except for the presence of only one methoxy group instead of two. The chemical shifts and variations on the benzofuran ring suggested that the differences were at position 3'. The rest of the structure was almost identical. There was a distinguishable significant shielding effect on carbon 3', which was shifted upfield for about 3.6 ppm. Complementary smaller shifts were observed in neighboring carbons 2' and 4'. The signal from C-2' was shift downfield by 2.5 ppm, while the signal from C-4' was shift upfield by 1.3 ppm.

This evidence suggested that there was no methoxy present at C-3', but a hydroxyl group. So the single methoxy was confined to be at C_3 in the B ring and the suggested aglycone structure was in agreement with the data reported by Agrawal [23,29] for *cedrusin* {**V**}, *dihydrodehydro-diconiferyl alcohol* {**VI**} and derivatives, except for some reassignments.

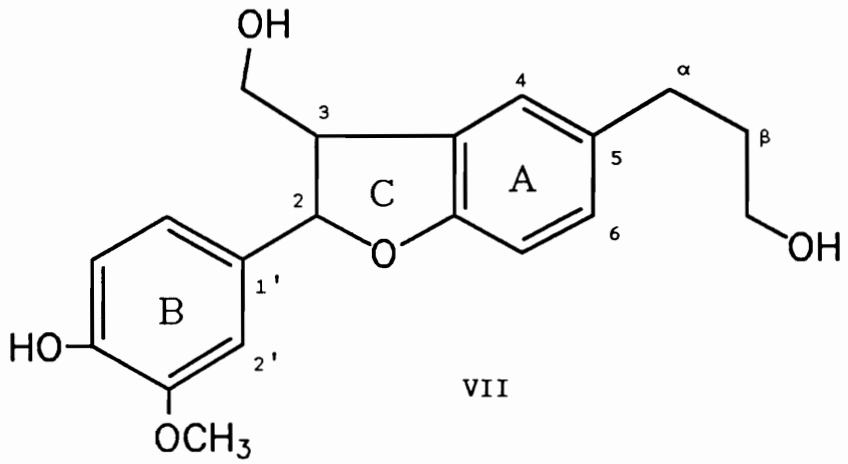


	R1	R2
I	Me	β-D-Glucose
II	Me	α-L-Rhamnose
III	H	α-L-Rhamnose
IV	H	β-D-Glucose
V	H	H
VI	Me	H

Figure 4.6 Structures of *Dihydrodehydrodiconiferyl alcohol* {**VI**}, *cedrusin* {**V**} and their glycosides.

After a careful analysis of the NMR spectra for *cedrusin rhamnoside* by heteronuclear correlations (HETCOR) and long range two and three bonds ^{1}H-^{13}C couplings (HETCOSY), some assignments have to be reviewed in *cedrusin*. The original assignments done by Agrawal [29] between C-3 and C-4, C-1' and C-5', and C-7' and C-8' may

be reversed. The same observation applies to *cedrusin* derivatives and related compounds reported in the same reference.



VII

Figure 4.7 Structure of *cedrusinin* {**VII**} and the dihydrobenzofuranpropanol skeleton.

The ^{1}H NMR for **IV** in MeOH-d_4 and for **III** either in MeOH-d_4 or Acetone-d_6, showed a couple of singlets with almost equivalent shifts around δ 6.58 ppm, corresponding to protons H-2' and H-6' (4 and 6 in ring A). In neolignans **I** and **II** the similarity was not as close, so this reinforced the proposed structure.

The FAB-MS for compounds **I,II,III** and **IV** showed the expected molecular ions $[M-H]^-$ at m/z 521,505,491 and 507. Also the aglycone ions $[Aglyc-H]^-$ at m/z 359 and 345 were detected, which correspond to the molecular ions of *DDDCA* and *cedrusin*. The dehydrated aglycone ions $[Aglyc-H_2O-H]^-$ at m/z 341 and 327 were observed too.

EXPERIMENTAL

The optical rotations were measured using a Jasco digital polarimeter Model DIP-370. The ^{1}H and C^{13} NMR experiments were run in a Bruker Model AM 400 NMR spectrometer using MeOH-d_4 as the solvent. Chemical shifts are given in ppm from TMS as an internal standard. Coupling constants are given in Hz (s, singlet; d, doublet; t, triplet; m, multiplet). FAB-MS were done with a Kratos MS-50TC mass spectrometer using DEA for the matrix.

Outer bark extract.

The outer bark and inner bark were collected from different specimens for independent experiments. The outer bark extract was prepared as mentioned previously in Chapter III. A 90 g sample from the ethyl acetate insoluble portion, was subjected to an initial separation in a sephadex column with ethanol, affording thirteen fractions. The second fraction underwent additional chromatographic separation for neolignan glycosides. Gels of Sephadex LH-20 and Toyopearl HW-40S were utilized with ethanol, ethanol-water and methanol-water mixtures as eluants

Inner bark extract.

The inner bark was extracted with methanol and dewaxed with *n*-hexane in similar procedure as stated before. Then the extract was partioned between water and ethyl acetate. A sample of 85g from the ethyl acetate fraction was chromatographied with ethanol in a sephadex LH-20 column. More than 40 one-liter fractions were collected, including the earliest six fractions (i.e., glycosides). The fifth fraction (3.05g) was chosen by TLC in searching for more neolignans and rechromatographied over LH-20 and Toyopearl gels, using mixtures of ethanol water as eluents.

All the separations were monitored by a UV detector (Gilson 111B) at 254 nm and by TLC on Silica plastic sheets (Merck). The plates were developed with the system Benzene-Acetone- MeOH (6:3:1) or (90,9,1), visualized under UV light and sprayed with H_2SO_4-HCHO (40:1), followed by heating.

2,3-dihydro-3-hydroxymethyl-7-methoxy-2-(4'-hydroxy-3'-methoxyphenyl)-5-benzofuranpropanol-4'-O-β-D-glucopyranoside) or *Dihydrodehydrodiconiferyl alcohol 4-O-β-glucopyranoside* (DDDCA-Glc) {**I**}.

Light brown oil (0.041g); MW= 522, $C_{26}H_{34}O_{11}$. $[\alpha]_D^{25}$ -15.8° [MeOH, c 0.09]. Negative FAB-MS in DEA gave the following ions: $[M-H]^-$ at m/z 521 (50.8%), $[M-CH_3]^-$ at 507 (32.3%), $[Aglycone-H]^-$ at 359 (15.4%), $[Aglycone-H_2O-H]^-$ at 341 (63.1%), and $[Aglycone-2CH_3-H]^-$ at 329 (100%). ^1H NMR (MeOH-d_4) δ: 1.80 (2H,dt,J=6.5,8.0 Hz, H-8'), 2.60 (2H,t, J=8.0 Hz, H-7'), 3.37-3.50 (4H,m, Glc-2,3,4,5), 3.45 (1H,m,H-8), 3.55 (2H,t,J=6.5 Hz, H-9'), 3.65-3.86 (4H,m, merged with MeO signals, H-9(a,b), Glc-6(a,b)), 3.81 (3H,s, 3-OMe), 3.84 (3H,s, 3'-OMe), 4.87 (1H,d,J=7.3 Hz, Glc-1), 5.54 (1H,d,J=5.8 Hz, H-7), 6.70 (1H,s,H-6'), 6.72(1H,s,H-2'), 6.92 (1H,dd,J=1.7,8.3 Hz, H-6), 7.01 (1H,d,J=1.7 Hz, H-2), 7.12 (1H,d,J=8.3 Hz, H-5)

2,3-dihydro-3-hydroxymethyl-7-methoxy-2-(4'-hydroxy-3'-methoxyphenyl)-5-benzofuranpropanol-4'-O-α-L-rhamnopyranoside or *Dihydrodehydrodiconiferyl alcohol-4-O-α-L-rhamnopyranoside* (DDDCA-Rha) {**II**}.

Light brown oil (0.026g); MW= 506, $C_{26}H_{34}O_{10}$. $[\alpha]_D^{25}$ -63.9° [MeOH, c 0.06]. Negative FAB-MS in DEA gave the following ions: $[M-H]^-$ at m/z 505 (44.6%), $[M-CH_3]^-$ at 491 (32.3%),

[Aglycone-H]$^-$ at 359 (23.1%), [Aglycone-H_2O-H]$^-$ at 341 (66.2%), and [Aglycone-2CH_3-H]$^-$ at 329 (100%). ^{1}H NMR (MeOH-d_4) δ: 1.21 (3H,d,J=6.1 Hz, Rha-6), 1.80 (2H,dt,J=6.3,7.5 Hz, H-8'), 2.61 (2H,t,J=7.5 Hz, H-7'), 3.45 (2H,m,H-8,Rha-4), 3.55 (2H,t,J=6.3 Hz, H-9'), 3.74-3.90 (4H,m, merged with MeO signals, H-9(a,b), Rha-3,5), 3.78 (3H,s,3-OMe), 3.84 (3H,s,3'-OMe), 4.05 (1H,t,J=1.5 Hz, Rha-2), 5.33 (1H,d,J=1.3 Hz, Rha-1), 5.54 (1H,d,J=6.0 Hz, H-7), 6.70 (1H,s,H-6'), 6.72 (1H,s,H-2'), 6.91 (1H,dd,J=1.9,8.2 Hz, H-6), 7.01 (1H,d,J=1.9 Hz, H-2), 7.06 (1H,d,J=8.2 Hz, H-5).

2,3-dihydro-7-hydroxy-3-hydroxymethyl-2-(4'-hydroxy-3'-methoxyphenyl)-5-benzofuranpropanol-4'-O-α-L-rhamnopyranoside or *cedrusin-4-O-α-L-rhamnopyranoside* {**III**}.

Light brown oil (0.290g); MW= 492, $C_{25}H_{32}O_{10}$. $[\alpha]_D^{29}$ -72.4° [MeOH, c 0.06] Negative FAB-MS in DEA gave the following ions: [M-H]$^-$ at m/z 491 (84.3%), [M-CH_3]$^-$ at 477 (10.0%), [M-OCH_3]$^-$ at 461 (15.7%), [Aglycone-H]$^-$ at 345 (4.3%), [Aglycone-H_2O-H]$^-$ at 327 (5.0%). ^{1}H NMR (MeOH-d_4) δ: 1.21 (3H,d,J=6.3 Hz, Rha-6), 1.77 (2H,dt,J=6.4,7.2 Hz, H-8'), 2.52 (2H,t,J=7.3 Hz, H-7'), 3.46 (2H,m,H-8,Rha-4), 3.54 (2H,t,J=6.5 Hz, H-9'), 3.71-3.85 (3H,m, merged with MeO signal, H-9(a,b), Rha-5), 3.76 (3H,s,OMe), 3.91 (1H,dd,J=3.3,9.5 Hz, Rha-3), 4.09 (1H,t,J=1.0 Hz, Rha-2), 5.35 (1H,d,J=0.9 Hz, Rha-1), 5.54 (1H,d,J=5.8 Hz, H-7), 6.58 (1H,s,H-6'), 6.59 (1H,s,H-2'), 6.90 (1H,dd,J=1.5,8.3 Hz, H-6), 7.03 (1H,d,J=1.5 hz, H-2), 7.05 (1H,d,J=8.3 Hz, H-5).

2,3-dihydro-7-hydroxy-3-hydroxymethyl-2-(4'-hydroxy-3'-methoxyphenyl)-5-benzofuranpropanol-4'-O-β-D-glucopyranoside or *cedrusin-4-O-β-D-glucopyranoside* {**IV**}.

White amorphous powder (0.065g); MW= 508, $C_{25}H_{32}O_{11}$. $[\alpha]_D^{27}$ -39.2° [MeOH, c 1.3]. Negative FAB-MS in DEA gave the following ions: $[M-H]^-$ at m/z 507 (42.3%), $[M-CH_3]^-$ at 493 (10.3%), $[M-OMe]^-$ at 477 (18.6%), [Aglycone-H]$^-$ at 345 (21.6%), [Aglycone-H_2O-H]$^-$ at 327 (32.0%), and [Aglycone-OMe]$^-$ at 315 (100%). ^1H NMR (MeOH-d_4) δ: 1.76 (2H,dt,J=6.5,7.8 Hz, H-8'), 2.53 (2H,t, J=7.8 Hz, H-7'), 3.37-3.50 (4H,m, Glc-2,3,4,5), 3.47 (1H,m,H-8), 3.54 (2H,t,J=6.5 Hz, H-9'), 3.66-3.86 (4H,m, merged with MeO signal, H-9(a,b), Glc-6(a,b)), 3.79 (3H,s, 3-OMe), 4.87 (1H,d,J=7.3 Hz, Glc-1), 5.53 (1H,d,J=5.8 Hz, H-7), 6.57 (2H,s,H-2',H-6'), 6.93 (1H,dd,J=1.7,8.3 Hz, H-6), 7.04 (1H,d,J=1.7 Hz, H-2), 7.11 (1H,d,J=8.3 Hz, H-5)

Table 4.1 ^{13}C NMR assignments for the neolignan glycosides **I,II,III** and **IV** in MeOH-d_4.

Carbon No.	DDDCA-Glc I	DDDCA-Rha II	Cedrusin-Rha III	Cedrusin-Glc IV
1	138.3	138.8	138.8	138.6
2	111.2	111.3	111.2	111.2
3	150.9	152.1	152.1	150.8
4	147.6	146.5	146.2	147.4
5	118.1	119.6	119.5	118.0
6	119.4	119.1	119.1	119.4
7	88.5	88.5	88.1	88.2
8	55.6	55.6	55.6	55.8
9	65.1	65.1	65.1	65.1
1'	137.1	137.0	136.8	136.9
2'	114.2	114.2	116.7	116.7
3'	145.2	145.2	141.6	141.8
4'	147.5	147.5	146.2	146.4
5'	129.6	129.6	129.4	129.5
6'	118.0	117.9	117.0	117.1
7'	32.9	32.9	32.5	32.6
8'	35.8	35.8	35.5	35.7
9'	62.2	62.2	62.2	62.3
Sugar	Glucose	Rhamnose	Rhamnose	Glucose
1	102.8	101.4	101.2	100.7
2	74.9	72.0	71.9	74.8
3	78.2	72.2	72.1	78.1
4	71.3	73.8	73.7	71.3
5	77.8	70.8	70.7	77.8
6	62.5	17.9	18.0	62.5
3-OMe	56.8	56.5	56.4	56.7
3'-OMe	56.7	56.8	-	-

REFERENCES

1. Haworth, R.D. *Nature* (London). **147**, 225 (1941).

2. Haworth, R.D. *J. Chem. Soc.* 448, (1942).

3. McCredie, R.S.; Ritchie, E.; Taylor, W.C. *Aust. J. Chem.* **22**, 1011 (1969).

4. Gottlieb, O.R. *Phytochem.* **11**, 1537 (1972)

5. Gottlieb, O.R. *Rev. Latinoamer. Quim.* **5**, 1 (1974).

6. Gottlieb, O.R. Neolignans. In *Progress in the Chemistry of Organic Natural Products*. Ed by Herz, W.; Grisebach, H.; Kirby, G.W. Springer Verlag. **35**, 1 (1978).

7. Whitig, D.A. Lignans and neolignans. *Natural Products Reports*. **2**, 191 (1985).

8. Gottlieb, O.R.; Mour└o, J.C.; Yoshida, M.; Mascarenhas, Y.P.; Rodrigues, M.; Rosenstein, R.D.; Tomita, K. Absolute configuration of the benzofuranoid neolignans. *Phytochem.* **16**(7), 1003 (1977).

9. Ayres, D.C.; Loike, J.D. *Lignans. Chemical, Biological and Clinical Properties*. Cambridge University Press. (1990).

10. Gottlieb, O.R. Lignans and neolignans. *Rev. Latinoamer. Quim.* **5**, 1 (1974).

11. Freudenberg, K.; Neish, A.C. Constitution and biosynthesis of lignin. Springer-Verlag. (1968).

12. Adler, E. Lignin chemistry. Past, present and future. *Wood Sci. Technol.* **11**, 169 (1977).

13. Gottlieb, O.R. In Wagner, H.; Wolf. P.M. *New Natural Products and Plant Drugs with Pharmaceutical, Biological or Therapeutical Activity*. Proceedings of the First International Congress, 1976. Springer Verlag, p 227 (1977).

14. Ward, R.S. The synthesis of lignans and neolignans. *Chemical Society Reviews*. The Royal Society of Chemistry, p 75, (1982).

15. Freudenberg, K.; Hubner, H. *Chem. Ber*. **85**, 1181 (1952).

16. Sih, C.J.; Ravikumar, P.R.; Huang, C.; Buckner, C.; Whitlock, H. *J. Am. Chem. Soc*. **98**, 5412 (1976).

17. Sakakibara, A.; Sasaya, T.; Miki, K.; Takahashi, H. Lignans and Brauns' lignins from softwoods. *Holzforschung*. **41**(1), 1 (1987).

18. Miki, K.; Sasaya, T.; Sakakibara, A. Structures of new lignan from *Larix leptolepis* Gord. *Tetrahedron Letters*. **9**, 799 (1979).

19. Barata, L.E.S.; Baker, P.M.; Gottlieb O.R.; Ruveda, E.A. Neolignans of *Virola surinamensis*. *Phytochem.* **17**, 783 (1978).

20. Fonseca, S.F.; Campello, J.P.; Barata, L.E.S.; Ruveda, E.A. CMR spectral analysis of lignans from *Araucaria angustifolia*. *Phytochem.* **17**, 499 (1978).

21. Dias A.F.; Giesbrecht, A.H.; Gottlieb, O.R. Neolignans from *Urbanodendron verrucosum*. *Phytochem.* **21**, 1137 (1982).

22. Takaoka, D.; Watanabe, K. Hirui, M. Studies on lignoids in *Lauraceae* II. *Bull. Chem. Soc. Japan.* **49**, 3564 (1976).

23. Agrawal, P.K.; Agarwal, S.K.; Rastogi, R.P. A new neolignan and other phenolic constituents from *Cedrus deodara*. *Phytochem.* **19**, 1260 (1980).

24. Agrawal, P.K.; Rastogi, R.P. Two lignans from *Cedrus deodara*. *Phytochem.* **21**(6), 1459 (1982).

25. Popoff T.; Theander, O. The constituents of conifer needles. VI. Phenolic glycosides from *Pinus sylvestris*. *Acta Chem. Scand.* B31, **4**, 329 (1977).

26. Popoff T.; Theander, O. Two glycosides of a new dilignol from *Pinus sylvestris*. *Phytochem.* **14**, 2065 (1975)

27. Agrawal, P.K.; Thakur, R.S. ^{13}C NMR spectroscopy of lignan and neolignan derivatives. *Magnetic Resonance in Chemistry*. **23**(6), 389 (1985).

28. Ludemann, H.D.; Nimz, H. ^{13}C-Kernresonanzspektren von ligninen, 1. *Die Mackromolekulare Chemie*. **175**, 2393 (1974).

29. Agrawal, P.K.; Rastogi, R.P.; Osterdahl, B.G. ^{13}C NMR spectral analysis of dehydrobenzofuran lignans. *Organic Magnetic Resonance*. **21**(2), 119 (1983).

30. Lundgreen, L.N.; Shen, Z.; Theander, O. The constituents of conifer needles, dilignol glycosides from *Pinus massoniana* Lamb. *Acta Chem. Scand.* **B39**, 241 (1985).

31. Lundgreen, L.N.; Popoff, T.; Theander, O. *Phytochem.* **20**, 1967 (1981).

32. Abe, F.; Yamauchi, T.; Lignans from *Trachelospermum asiaticum* (Tracheolospermum. II). *Chem. Pharm. Bull.* **34**(10), 4340 (1986).

33. Miyase, T.; Ueno, A.; Takizawa, N.; Kobayashi, H.; Oguchi, H. Ionone and lignan glycosides from *Epimedium diphylum*. *Phytochem.* **28**(12), 3483 (1989).

34. Achenbach, H.; Groβ, J.; Dominguez, X.A.; Cano, G.; Star, J.V.; Brussolo, C.; Munoz, G.; Salgado, F.; Lopez, L. Lignans, neolignans and norneolignans from *Krameria cystisoides*. *Phytochem.* **26**(4), 1159 (1987).

35. Kuono, I.; Yanagida, Y.; Shimono, S.; Shintomi, M.; Ito, Y.; Yang, S. Neolignans and phenylpropanoid glucoside from *Illicium difengpi*. *Phytochem.* **32**(6), 1573 (1993).

CHAPTER V

CONCLUSIONS

The industrialization of red alder wood is producing bark as by-product without a full value utilization. The high percentage of *oregonin* and presence of other polyphenols represent a technological challenge to a world less dependent on oil-derived chemicals. Besides the potential application of these extracts as antioxidants in foods, there is a need to identify and specify their bioactivity for medicinal applications.

The *rubranol* family requires determination of their absolute configuration. The apparent absence of cyclic diarylheptanoids in red alder suggests that the cathecol rings are less ease to couple each other, either in biphenyl or ether linkages, as in other diarylheptanoids with different hydroxylation pattern. However, some reports about cyclic diarylheptanoids have noted significant amounts under acidic conditions that could have inadvertently catalyzed the production of such compounds.

From the experience of the present work in trying to isolate and crystallize neolignans, it is clear that probably many of them have been overlooked in previous experiments and the compounds reported here are just a representative sample of those present in Douglas-fir bark. The glycoside neolignans were found in oligomeric and phlobaphene fractions, which suggests that they may play an active role in the lignification process and the formation of phlobaphenes. The relative absence of neolignans in free

phenolic form confirms that these compounds are very active in the biosynthetic process. Some of them may undergo an etherification process as the consequent reaction to the initial oxidative coupling of the precursors. The remaining fraction will continue polymerizing until forming propenyl or allylphenols oligomers that although have not been isolated, might be in the extract in significative concentrations.

Thus the structural variety of neolignans is increasing the importance of this kind of natural product. For the future chemists the challenge is to find out about the extension and limitations of their structural combinations, their potential utilization and their biochemical explanations.

The modern spectroscopic tools for analysis of chemical compounds are greatly enhancing our ability for structure determination, particularly the 2D-NMR experiments. More accurate assignments are becoming possible thanks to these techniques. As a consequence of applying these tools some reassignments have to be done, for example in the known neolignans glycosides reported here, which original assignments need to be revised. The high resolution of the new NMR instruments allows to make determinations on samples of just few milligrams. Such was the case in some of the diarylheptanoids that were characterized.

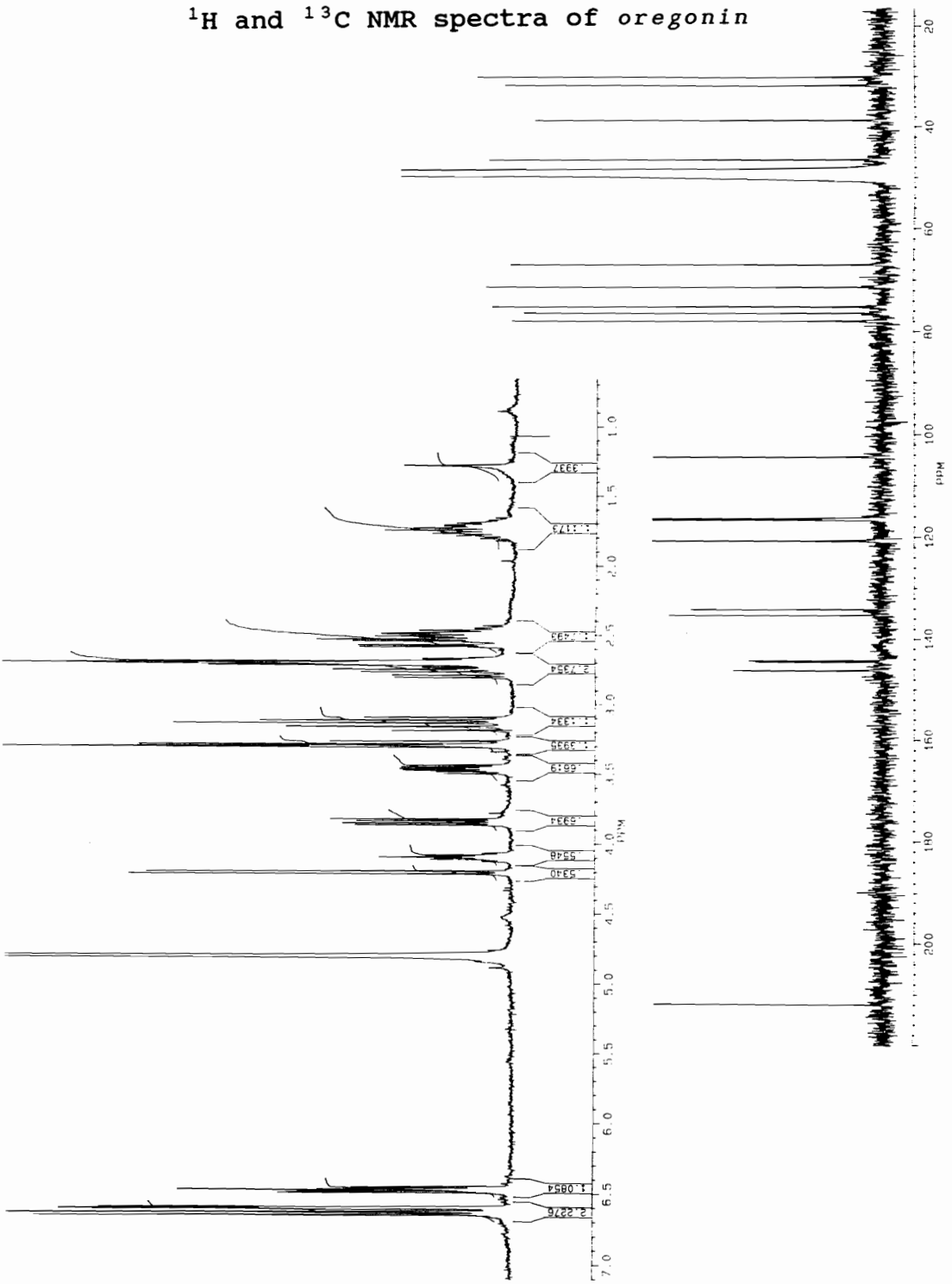
APPENDIX

SYMBOLS, ABBREVIATIONS AND ACRONYMS

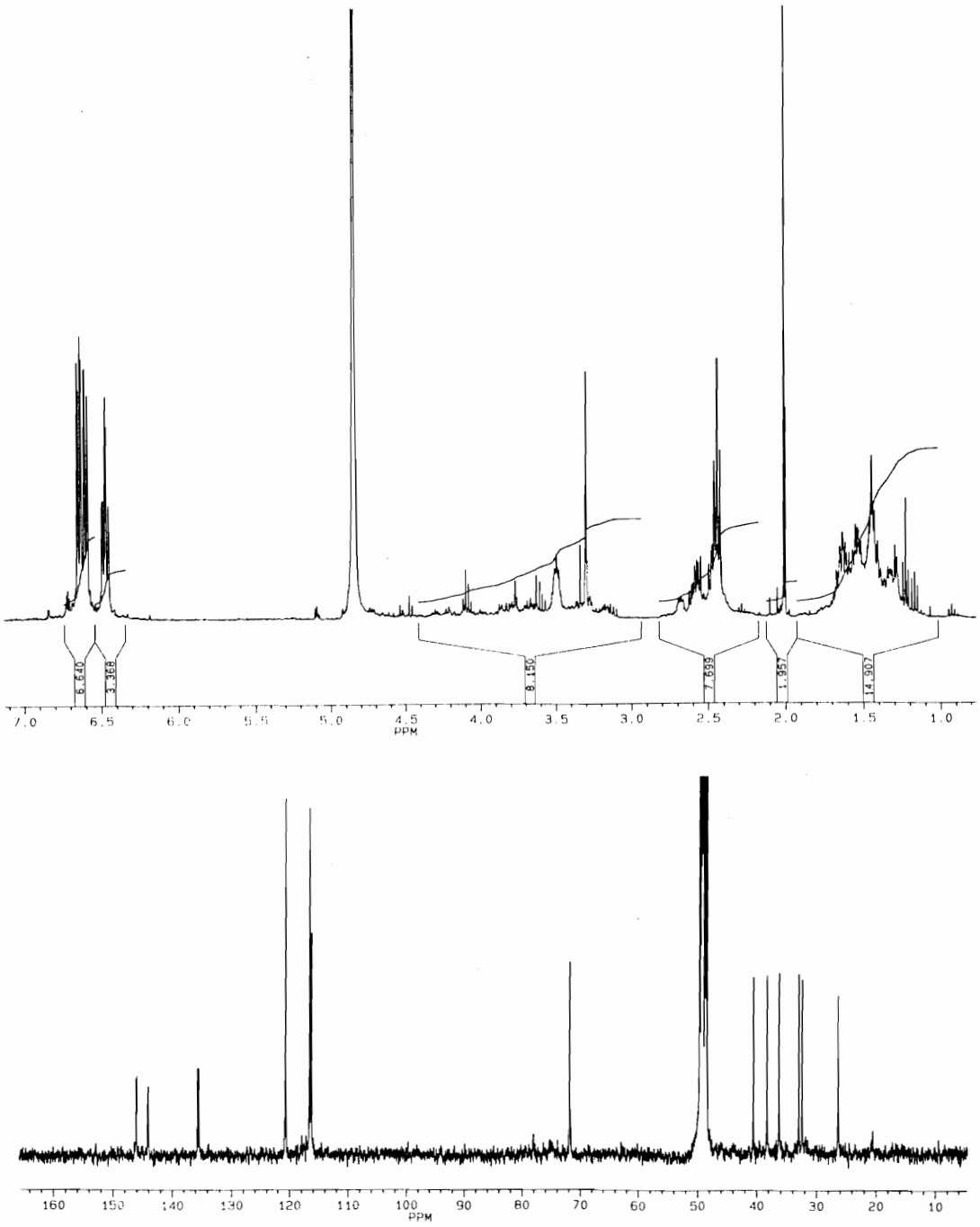
ABX	Three-spin system with two nuclei (A,B) having similar chemical shifts coupled to a third one (X) with very different resonance frequency.
$[\alpha]_D^T$	Specific rotation at temperature T°C and light with wavelenght of 589 nm (the sodium D line).
CC	Column Chromatography.
CoA	Coenzyme A.
COSY	Correlated Spectroscopy (also HOMCOR).
δ	Chemical shift for the nuclear resonance of an element (^1H or ^{13}C).
DEA	Diethylamine.
DEPT	Distortionless Enhancement by Polarization Transfer.
FAB-MS	Fast Atom Bombardment Mass Spectrometry.
HETCOR	Heteronuclear Chemical Shift Correlation (also Hetero-COSY).
HETCOSY	Long Range Heteronuclear COSY.
HMBC	Heteronuclear Multiple Bond Correlation.
HMQC	Heteronuclear Multiple Quantum Coherence.
HOMCOR	Homonuclear Correlated Spectroscopy.
Hz	Hertz.
J	Nuclear spin-spin coupling constant in Hz.
MW	Molecular Weight.
nm	Nanometers, 1 nm = 10^{-9}m.
NMR	Nuclear Magnetic Resonance.
NOE	Nuclear Overhauser Effect.
ppm	Parts per million.
TLC	Thin Layer Chromatography.
TMS	Tetramethylsilane.
2D	Two-dimensional.
UV	Ultra Violet light.

SELECTED NMR SPECTRA

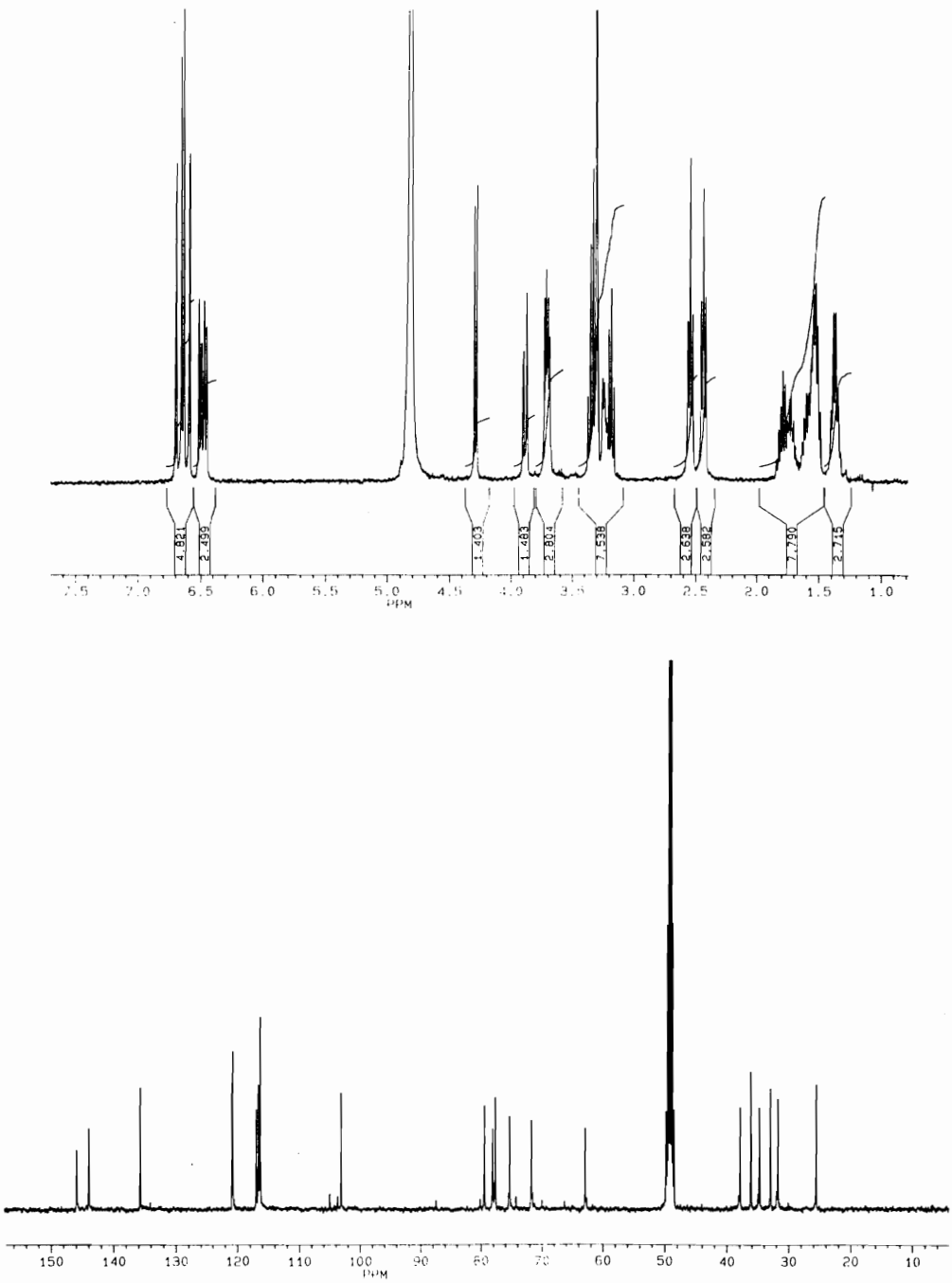
^{1}H and ^{13}C NMR spectra of *oregonin*



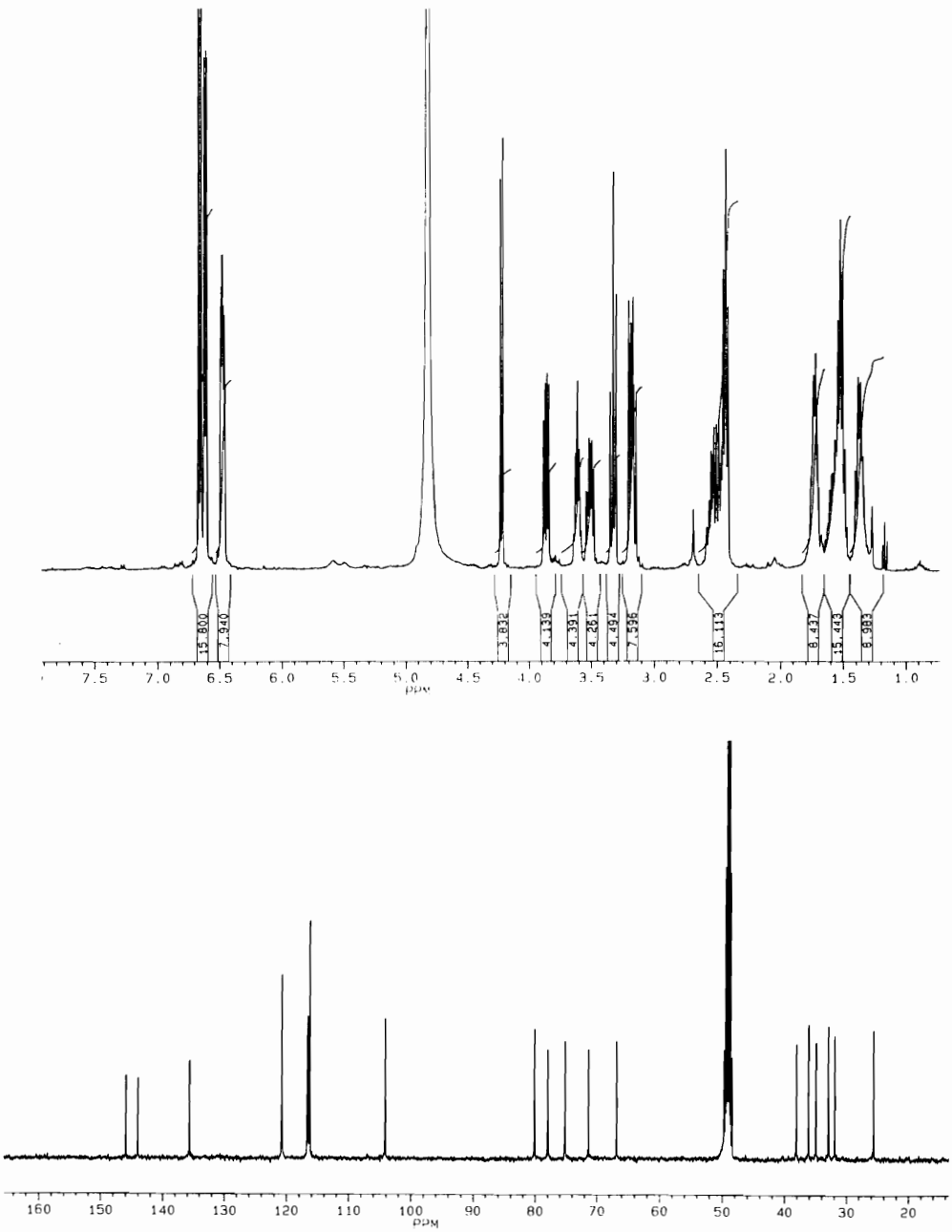
^{1}H and ^{13}C NMR spectra of *rubranol*



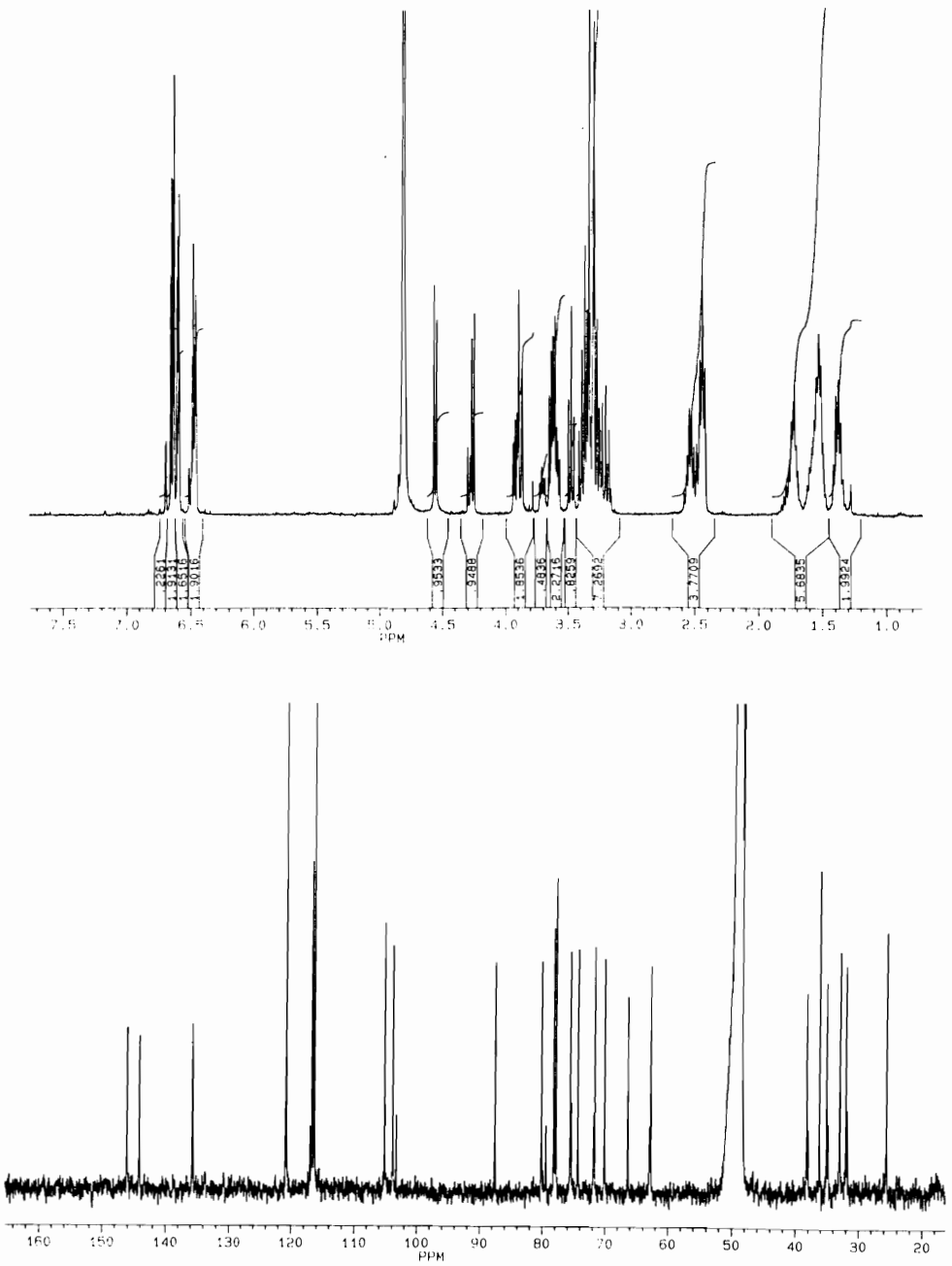
^{1}H and ^{13}C NMR spectra of *rubranol glucoside*



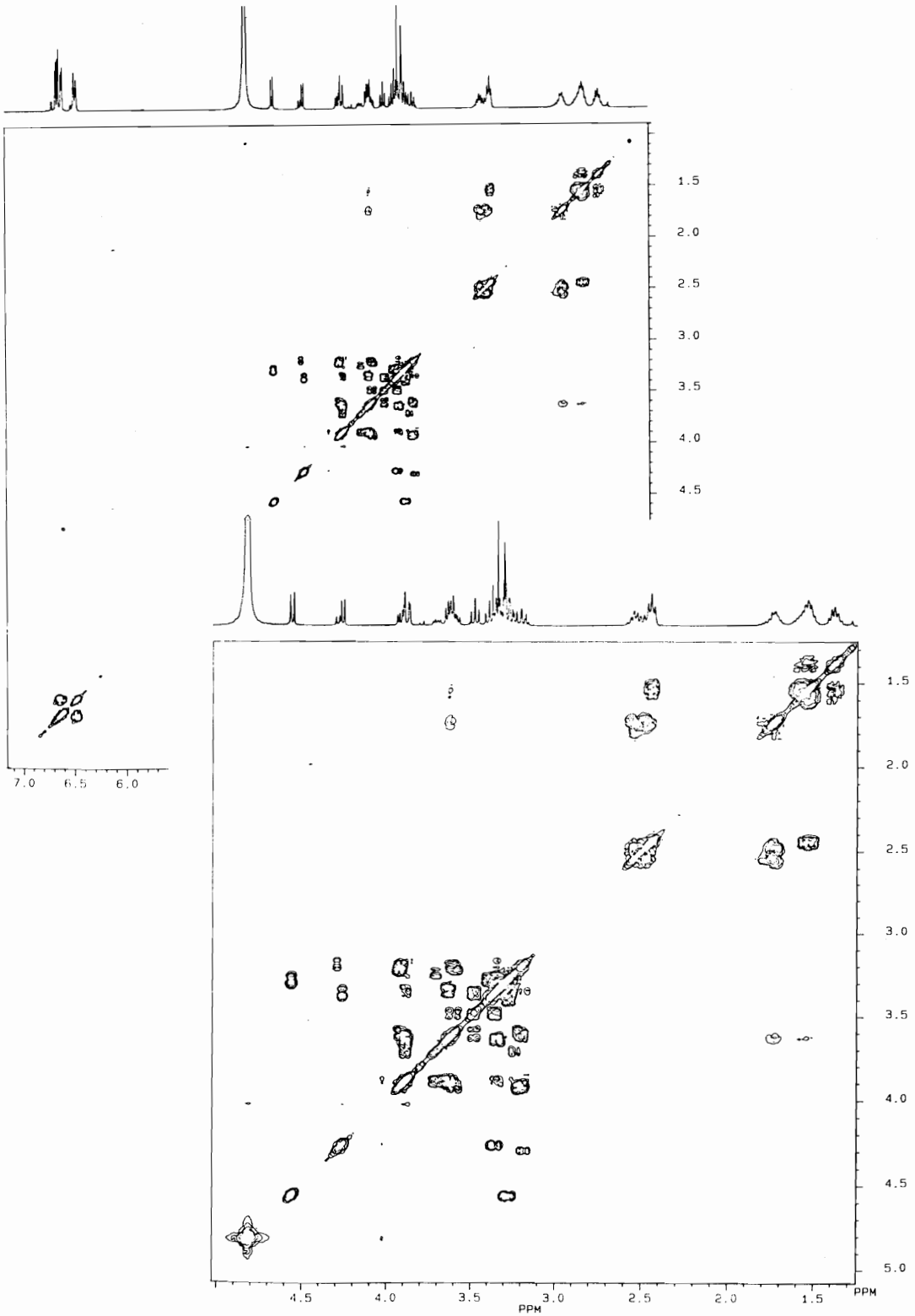
^{1}H and ^{13}C NMR spectra of *rubranol xyloside*



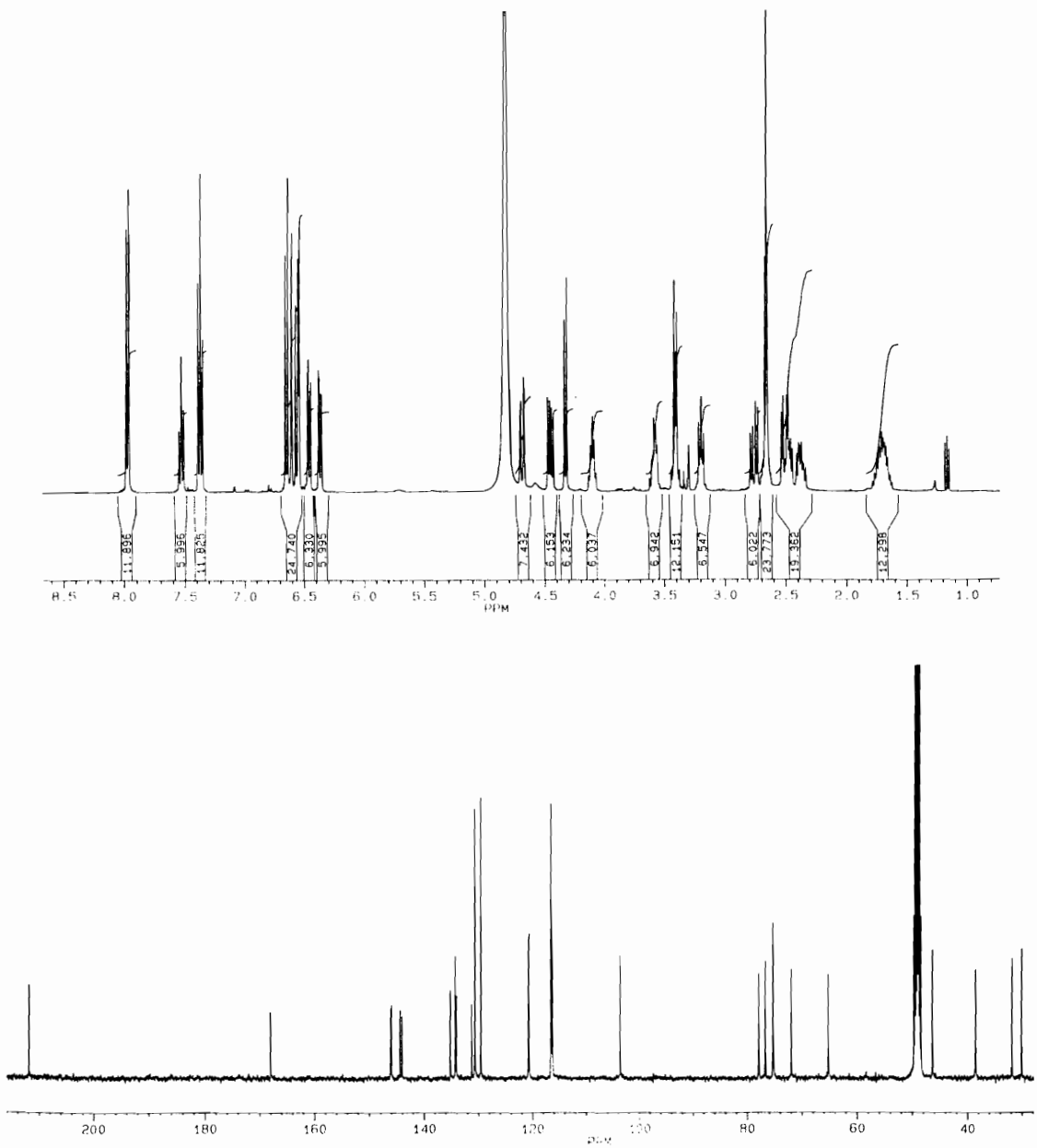
^{1}H and ^{13}C NMR spectra of *rubranoside A*



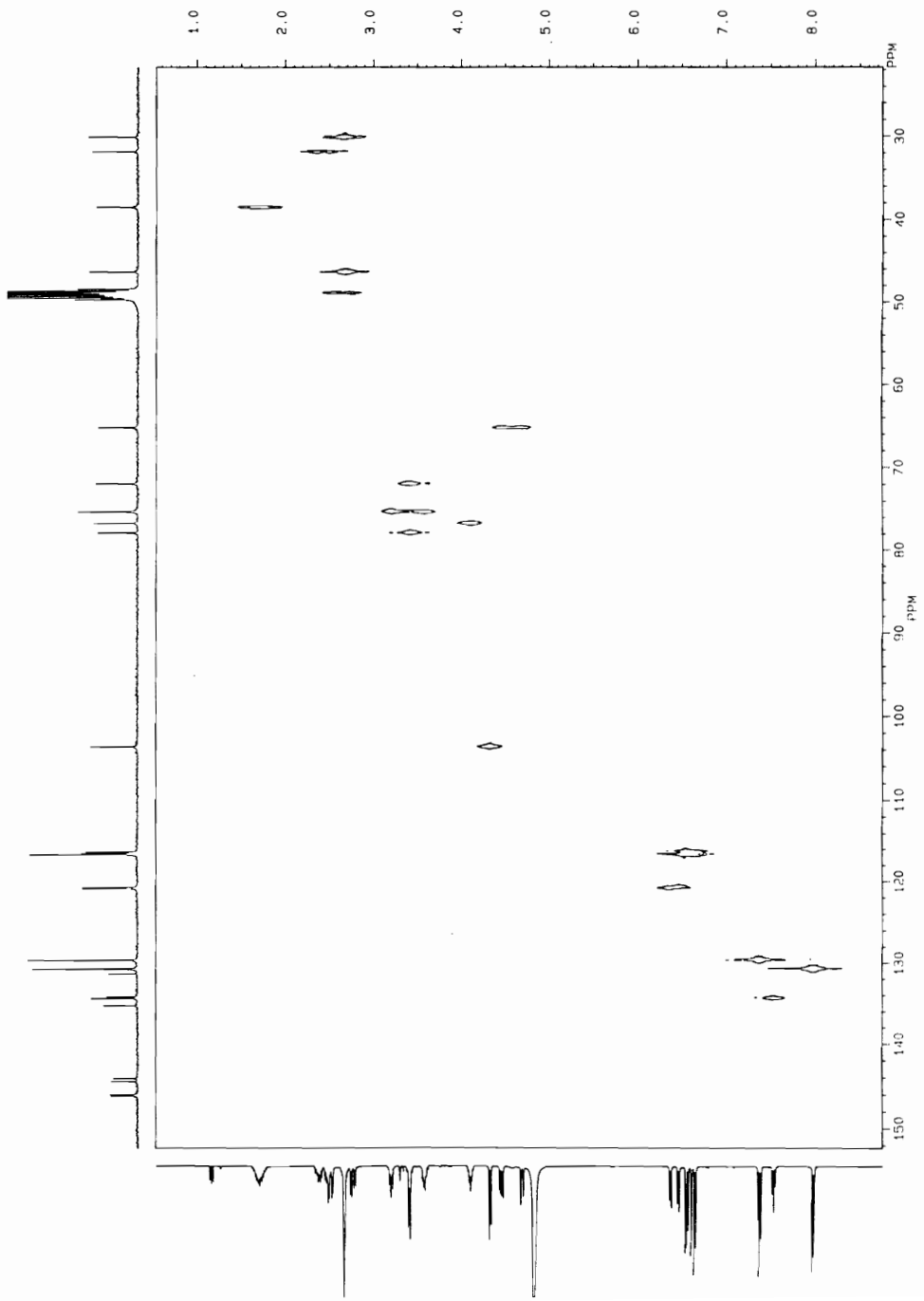
^{1}H-^{1}H COSY spectrum of *rubranoside A*



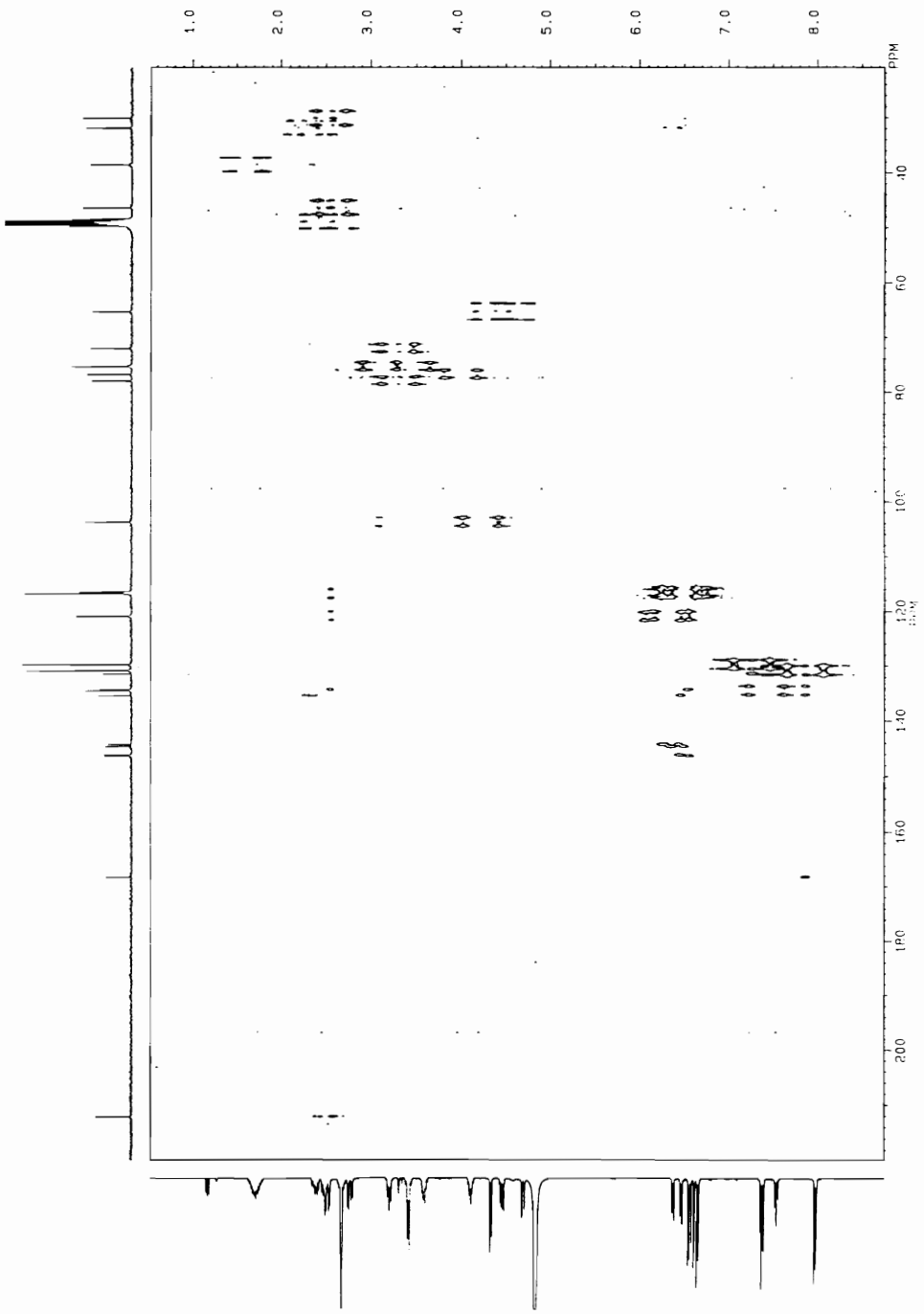
^{1}H and ^{13}C NMR spectra of *oregonoside A*



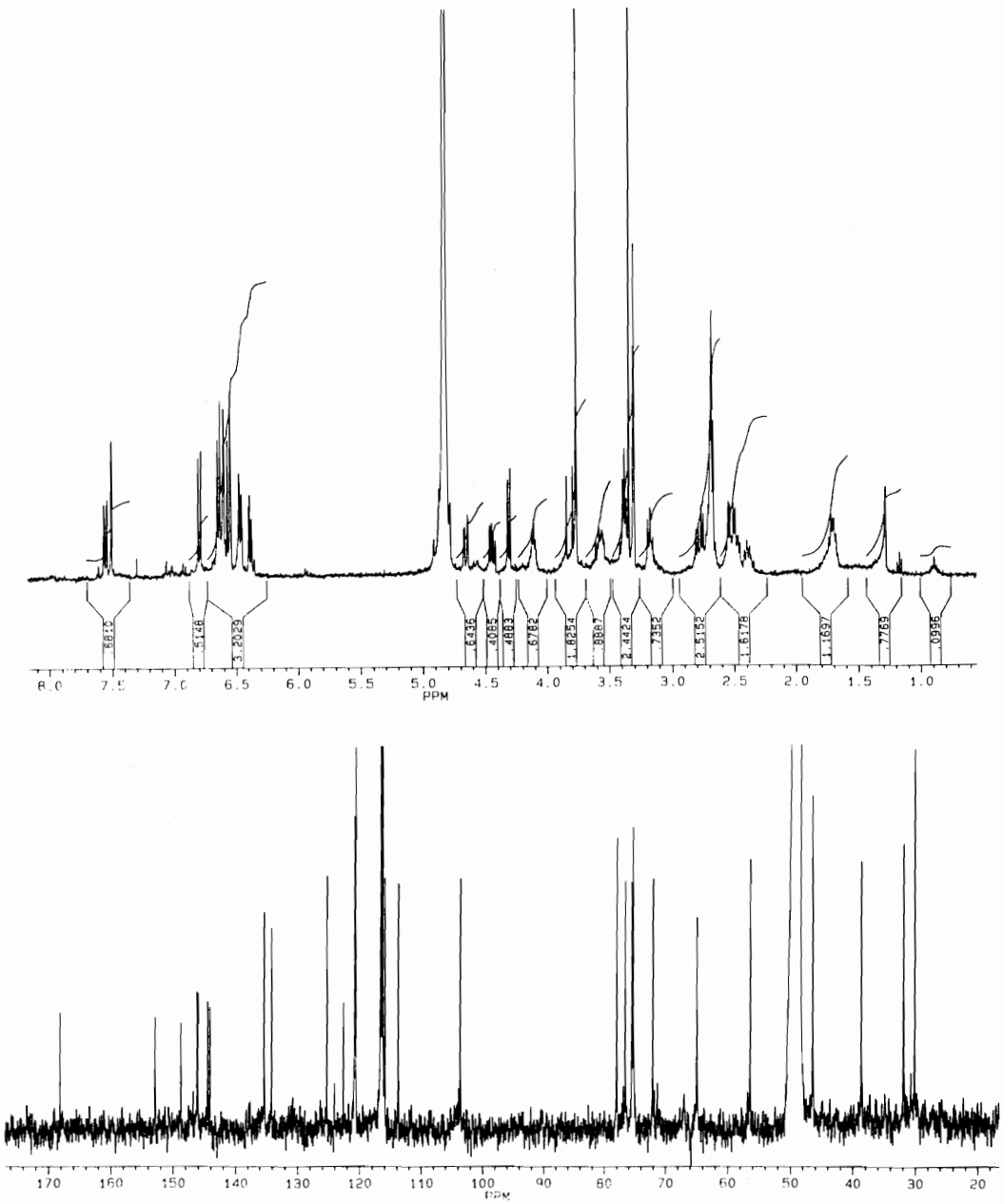
HETCOR spectrum of *oregonoside A*



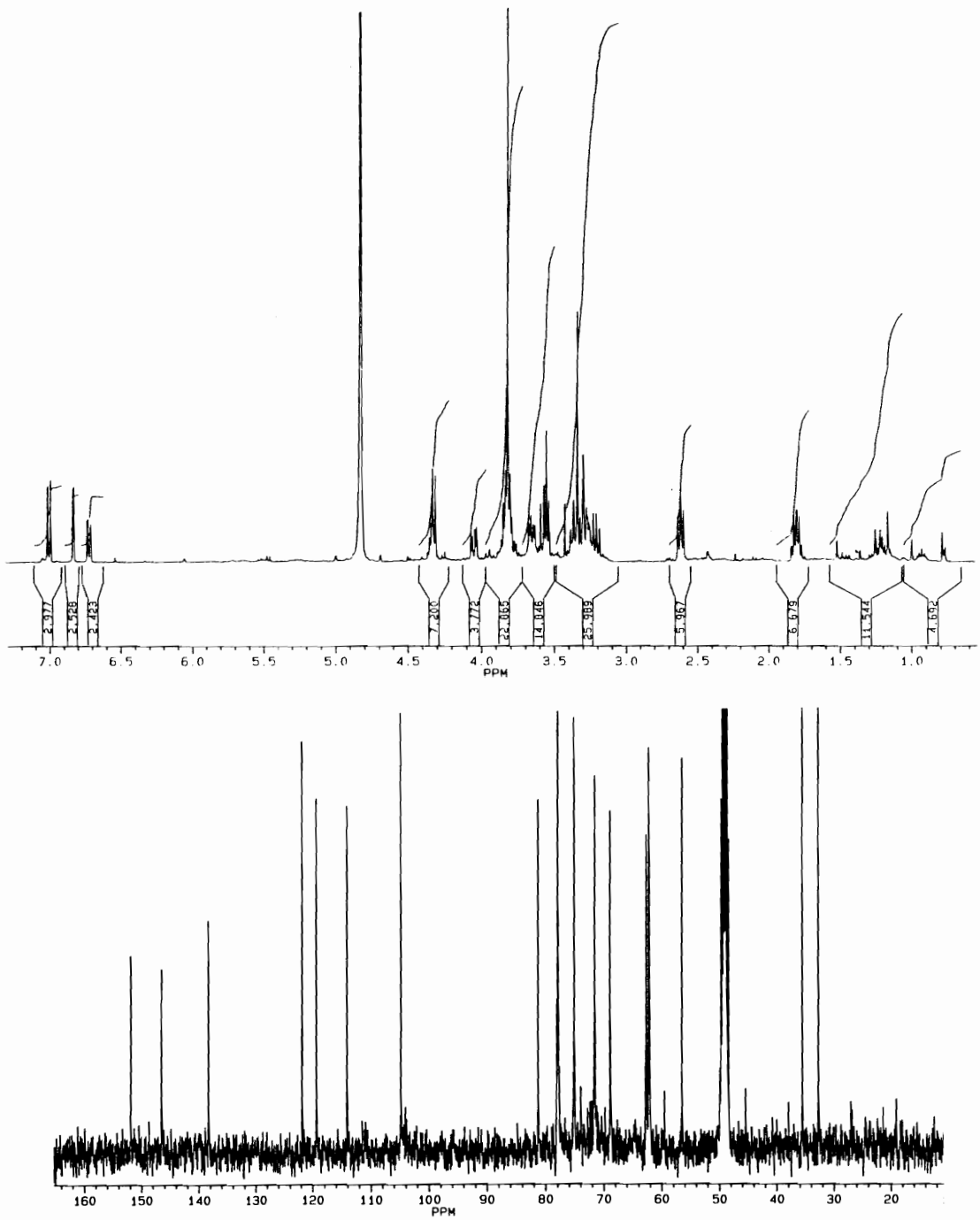
HETCOSY spectrum of *oregonoside A*



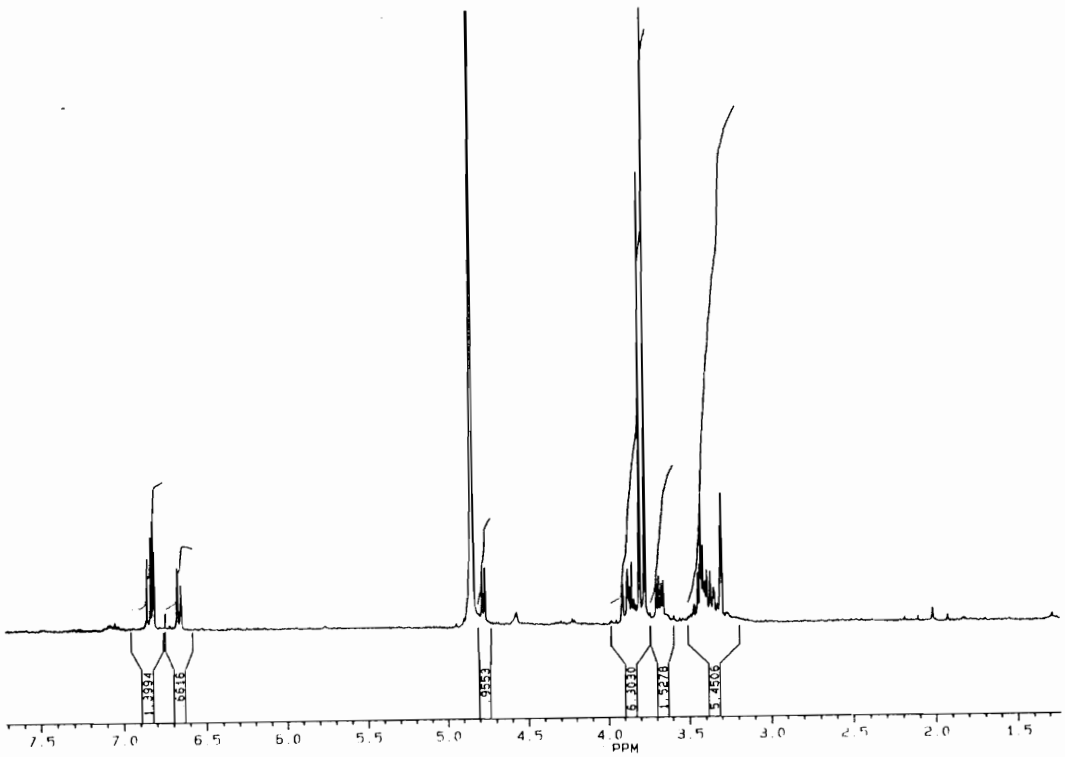
^{1}H and ^{13}C NMR spectra of *oregonoside B*
(note for missing signal at ~212 ppm)

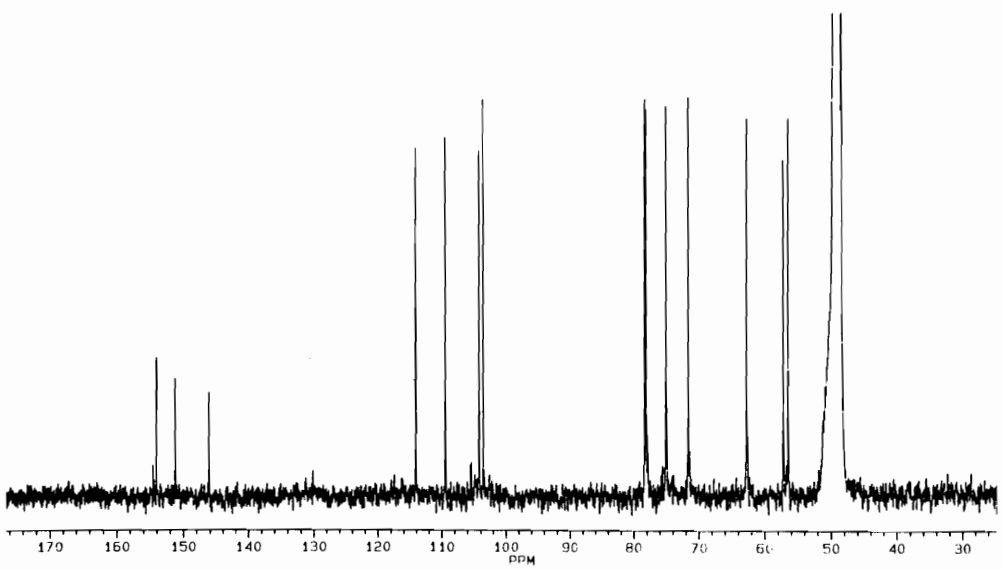


^{1}H and ^{13}C NMR spectra of the glycerol derivative

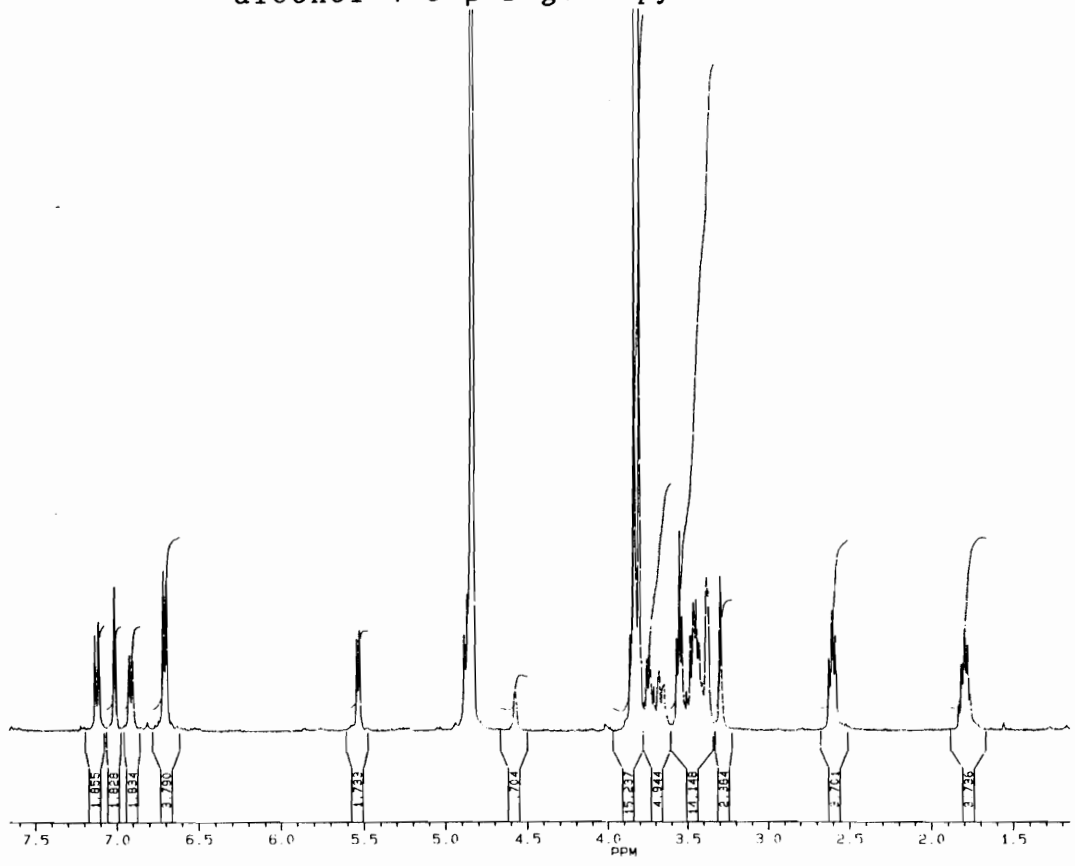


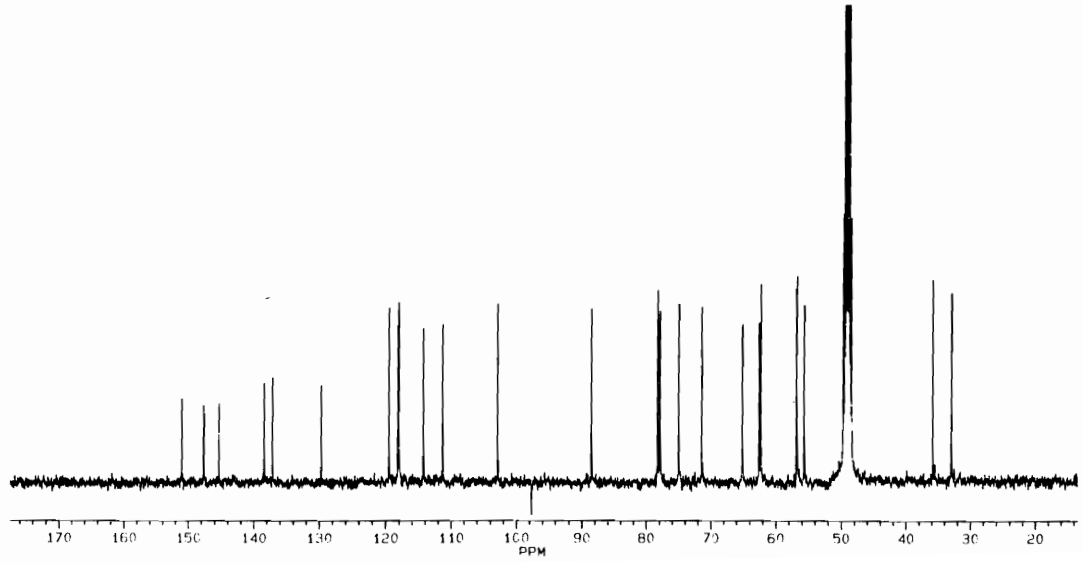
^{1}H and ^{13}C NMR spectra of *1,2-dimethoxy-4-O-β-D-glucopyranoside benzene*



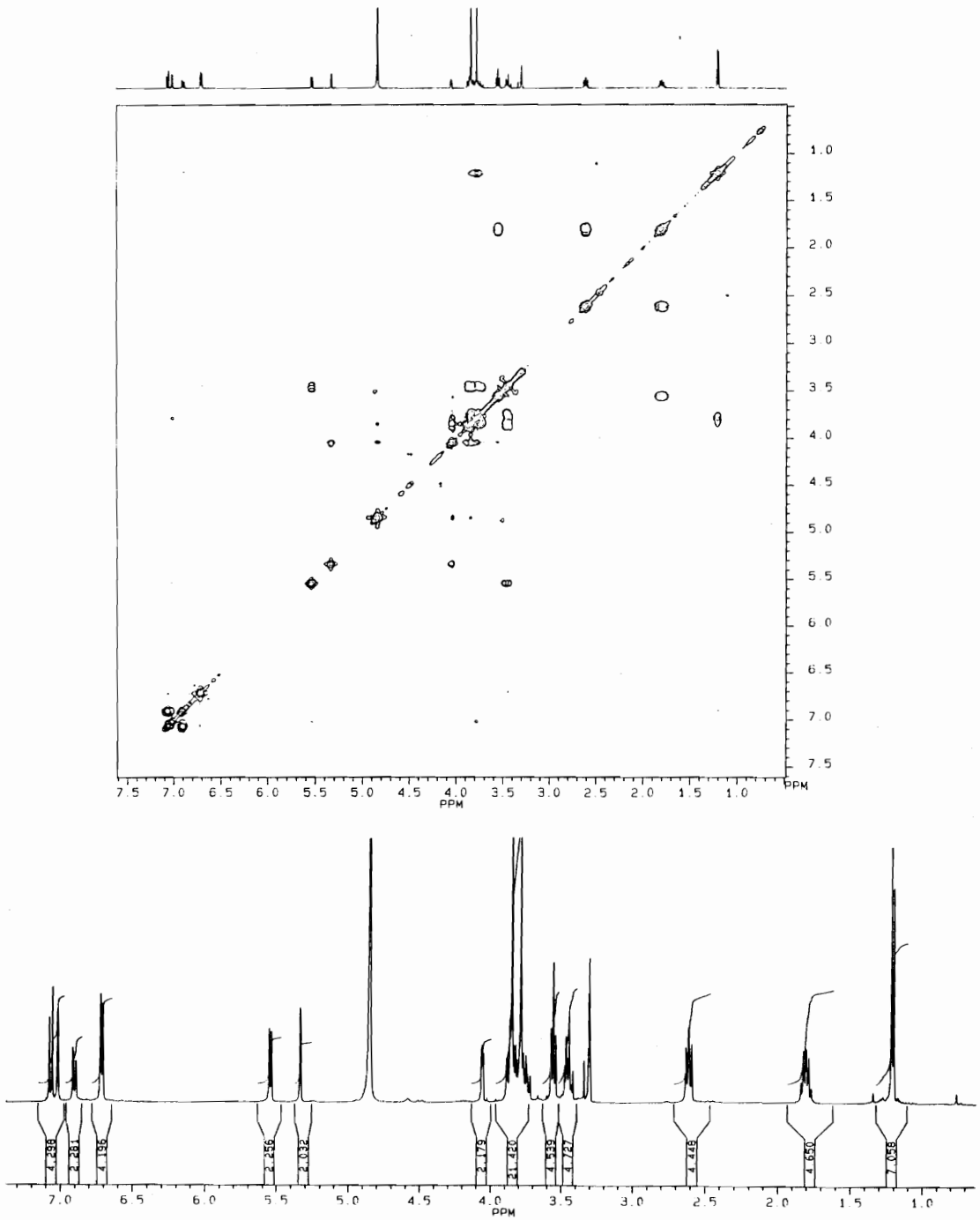


^{1}H and ^{13}C NMR spectra of *dihydrodehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside*

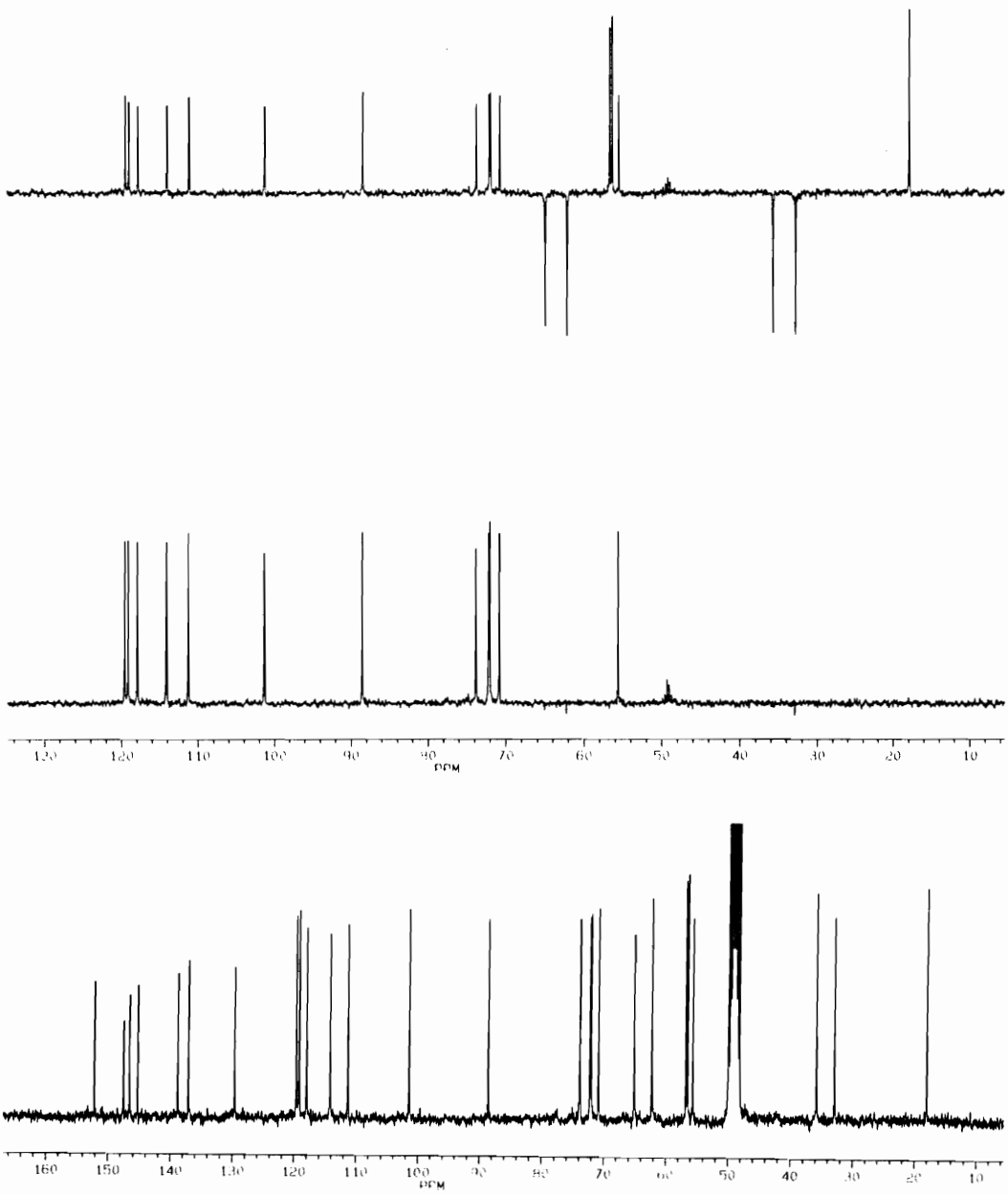




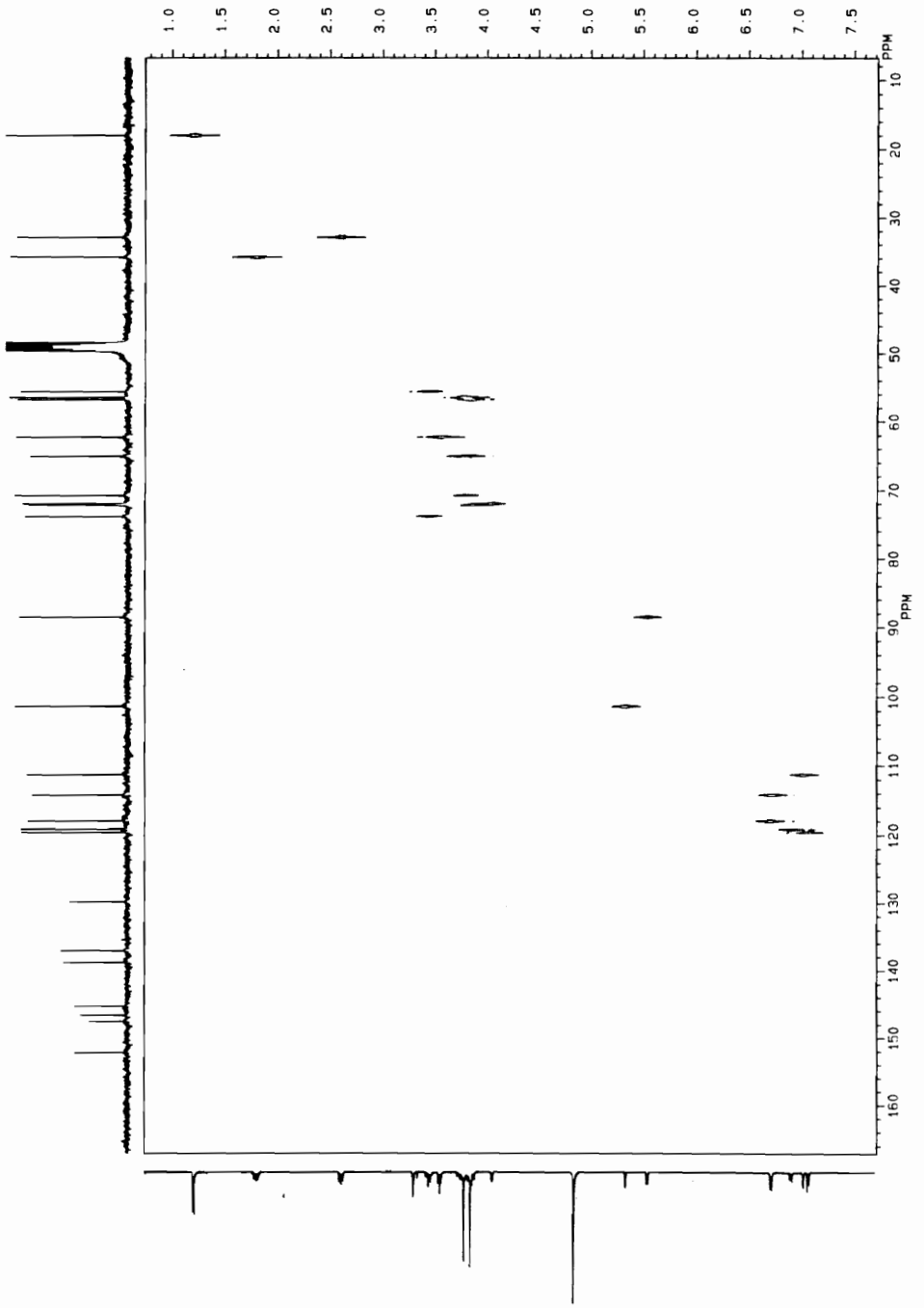
^{1}H NMR and ^{1}H-^{1}H COSY spectra of *dihydrodehydrodiconiferyl alcohol 4-O-α-L-rhamnopyranoside*



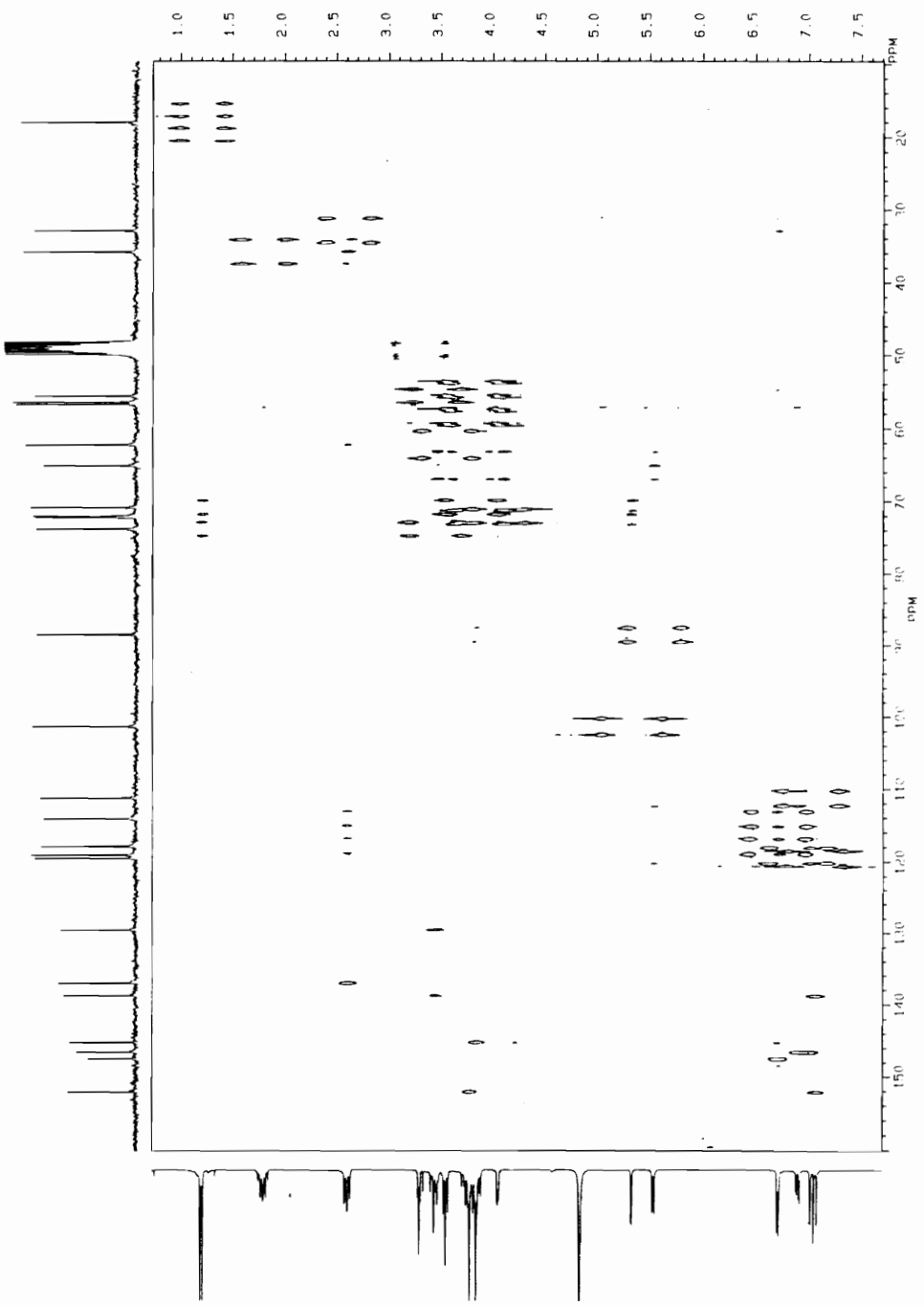
^{13}C NMR and DEPT spectra of *dihydrodehydrodiconiferyl alcohol 4-O-α-L-rhamnopyranoside*



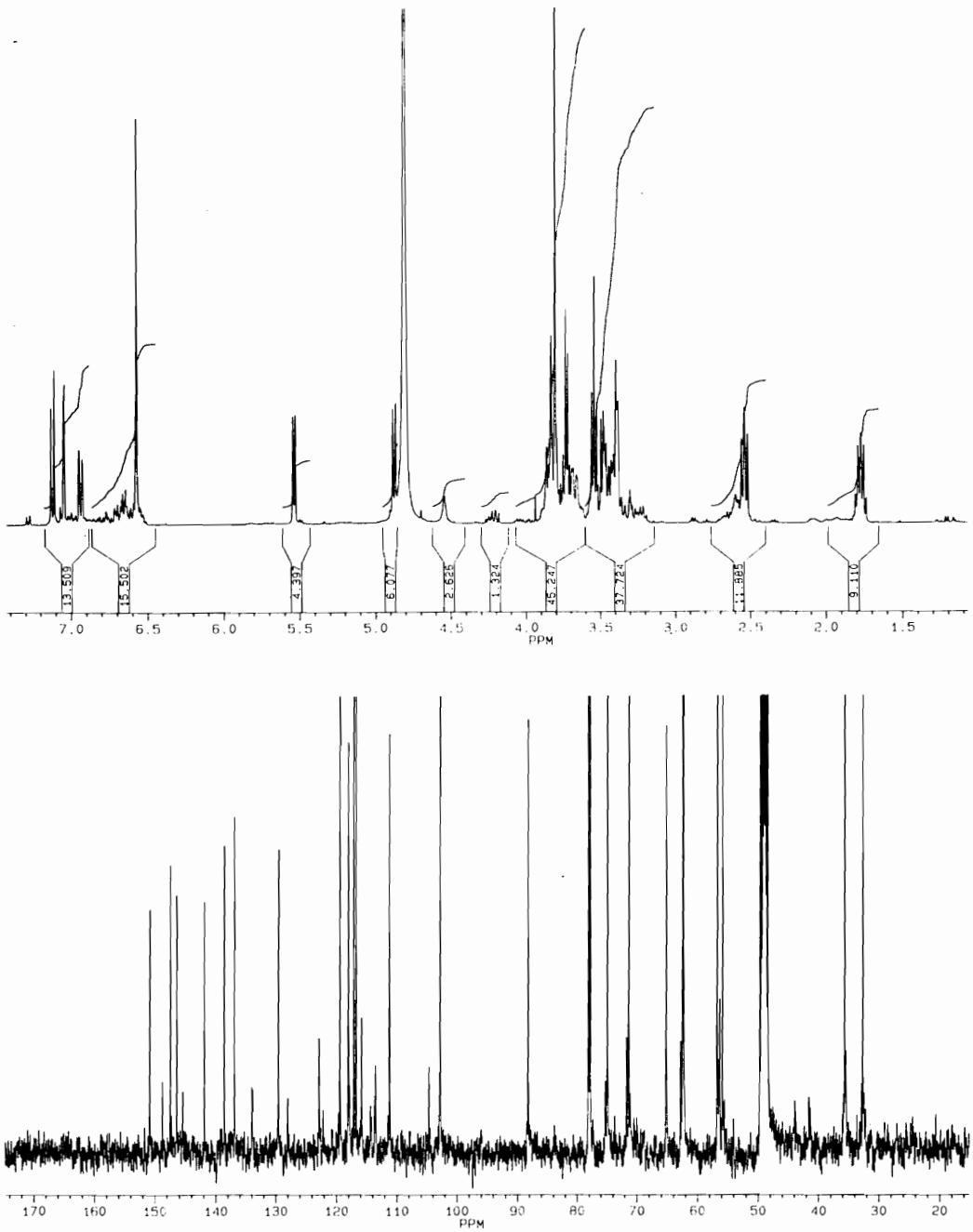
HETCOR spectrum of *dihydrodehydrodiconiferyl alcohol 4-O-α-L-rhamnopyranoside*



HETCOSY spectrum of *dihydrodehydrodiconiferyl alcohol 4-O-α-L-rhamnopyranoside*



^{1}H and ^{13}C NMR spectra of *cedrusin-4-O-β-D-glucopyranoside*



^{1}H and ^{13}C NMR spectra of *cedrusin-4-O-α-L-rhamnopyranoside*