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# EXTRACTIVES OF WESTERN LARCH (*LARIX OCCIDENTALIS* NUTT.)

by

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We accept this thesis as conforming to the
required standard.

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April, 1973

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# ABSTRACT

Western larch (*Larix occidentalis* Nutt.) heartwood meals were successively extracted with five solvents of increasing polarity and finally with absolute ethanol. The total extractable material collected was 14.94%, oven dry (OD) wood basis. Compounds in each solvent fraction were seperated, mostly by column chromatography over silica gel, Sephadex A25 and Sephadex LH20. The identities of isolated compounds, after determining relevant physical and chemical properties were confirmed by comprison with standard compounds.

Previously unreported compounds found in western larch heartwood included: Four resin acids (0.017% total yield) as sandaracopimaric, isopimaric, abietic and dehydroabietic from the petroleum ether ($65^{o}$ -$80^{o}$) extract; pinocembrin (5,7 dihdroxy-flavanone) from the benzene extract (0.003% yield); and free L-arabinose from the water extract.

Other compounds found were: β-sitosterol, β-sitosteryl palmitate, tristearin, esters of linolenic and arachidic acids, palmitic, palmitoleic, linoleic, linolenic and arachidic acids. Also found were dihydrokampferol, α-conidendrin, dihydroquercetin, quercetin and arabinogalactan (11.10% yield).

Isolates were characterized by their IR and NMR spectra, various chrmatographic behaviours and comparison with standards. Physical and chemical properties of unidentified compounds are reported as a guide for future research.

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# 1.0 INTRODUCTION

Wood consists mainly of cellulose, hemicelluloses and lignin [9]. The extractives are a minor component (on average about 5% of whole wood), but of considerable importance. Values of less than 1% and more than 20%, however, have been reported for extractives from some species [22].

Extractives affect wood usage in many ways. With present trends towards improved wood utilization, it is necessary for wood scientists and wood users to know more about wood chemical constituents. This assists utilization without expensive trial and error practices.

Wood colour, odour, taste and durability against fungal decay and insect infestation are determined often by extractive type and quantity [8]. For instance, the thujaplicins of western red cedar (*Thuja plicata* Donn.) are highly toxic to wood destroying fungi. This toxicity is of the same magnitude as sodium pentachlorophenate toxicity [3]. The natural resistance of west African iroko (*Chlorophora excelsa* Benth. and Hook.) heartwood to fungal and termite attack has been attributed to the presence of chlorophorin [43].

Oleoresin exudates from some species, e.g., western white pine (*Pinus monticola* Dougl.), affect its painting properties through discolouration and blistering. In extreme cases a complete lifting of paint may occur [28].

Where wood is used in contact with metals (especially ferrous metals) the presence of some wood polyphenols results in gradual corrosion and loosening at points of contact. Complexes formed by iron and phenolics of the catechin type are stable and impart undesirable discolouration to the lumber with which the iron, e.g., bolts, nails, is in contact [12].

In the pulp and paper industry, extractives may decrease pulp yield, increase equipment corrosion, consume chemicals and impart undesirable colour to resulting pulps [14]. Also the inhibition of pulping reactions by extractives may result in reduced penetrability of cooking liquour into the wood or reduction of lignin solubility and decomposition of cooking liquour or both. Difficulties experienced in sulphite pulping of Japanese larch (*Larix leptolepsis* (Sieb and Zucc.) Murr.) have been attributed to the presence of sulfate ion arising from liquour decomposition caused by dihydroquercetin and arabinogalactan [14].

Wood extractives have been useful in many ways. The extractive types of some woods have been used as a means of generic allocation [11, 31, 32]. Extractives (especially alkaloids) from trees have been used in medicine. This has led to syntheses of controlled potency drugs which are relatives of those obtained initially from trees and other plants.

Tannins are used extensively in the leather industry. They are obtained from the barks of woods, especially the Fagaceae, Leguminosae, Meliaceae, Anacardiaceae and Rhizophoraceae families. Condensed tannins, which are phenolic in nature are used to preserve fish nets, to control mud viscosity in oil well drilling and to increase tensile strength of ceramics clay casts. Condensed with formaldehyde, they form resins which are used in the wood industry for finishing and gluing [20, 36]. Steam distillation of pine (*Pinus* spp.) oleoresins yields turpentine, as well as pine oils used in perfumery [33]. The discovery of juvenile hormone and its mimics in some tree species may lead to a non-polluting means of insect control [39].

Various compounds of different chemical classes are known to occur in western larch (*Larix occidentalis* Nutt) wood extractives. Larch arabinogalactan has been exhaustively dealt with by many workers [10, 24, 44]. The flavonoids, dihydroquercetin (3, 3', 4', 5, 7-pentahydroxy-flavanone), dihydrokampferol (3, 4', 5, 7-tetrahydroxy-flavanone), and quercetin (3, 3', 4', 5, 7-pentahydroxy-flavanone) have been described and studied in larch wood [5, 13, 21].

The purpose of the present investigation was to isolate and characterize other western larch heartwood extractives.

# 2.0 LITERATURE REVIEW

As an aid to appreciating the extrative variety obtained from western larch wood, it is necessary to define the general classes of compounds found. Previous work on various *Larix* spp. extractives is also mentioned to serve as comparison of western larch extractives with other *Larix* spp.

## 2.1 Definitions

The general classes of compounds met with in this investigation were fatty acids, flavonoids, lignans, neutrals, resin acids and polysaccharides.

Fatty acids are long straight chain aliphatic monocarboxylic acids which are found widely in nature, chiefly as a reserve food for both plants and animals. Fatty acids may be either saturated or unsaturated and double bonds of the latter may be conjugated or not.

Flavonoids are composed of a $C_{15}$ carbon skeleton containing two distinct units as the $C_6 - C_3$ fragment (Ring B) and the $C_6$ (Ring A) fragment (Fig. 1).

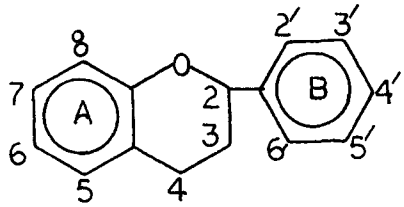
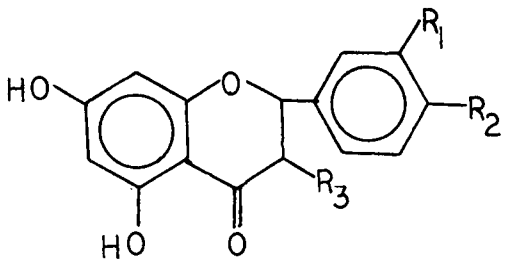
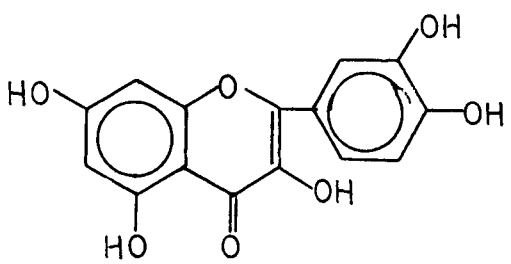



Figure 1. Basic $C_{15}$ ring structural skeleton of flavonoids.

Flavonoids classification are according to oxidation level of the oxygen containing ring or with regard to carbon linkage between the two benzene rings. Two of the flavonoid classes relating to the present investigation are flavanonols, e.g., dihydroquercetin and others (I) and flavanols, e.g., quercetin (II).




I




II

a) $R_1 = R_2 = R_3 = H$, pinocembrin

b) $R_1 = R_2 = H$, $R_3 = OH$, pinobanksin

c) $R_1 = H$, $R_2 = R_3 = OH$, dihydrokampferol

d) $R_1 = R_2 = R_3 = OH$, dihydroquercetin

Apart from degree of hydroxylation, the main difference between the two classes is the presence (II) or absence (I) of conjugation of the B ring with the carbonyl group in position 4.

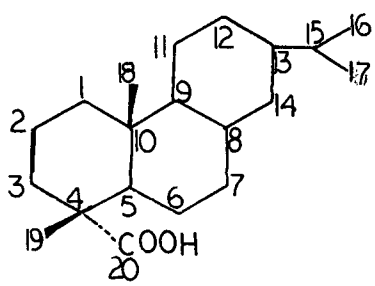
Among plants, the majority of flavonoids occur as glycosides, mostly mono-glucosides. Solubility characteristics are affected by degree of hydroxylation, as well as location and number of attached glucose units [17].

Lignans are optically active phenylpropane dimers. The carbon-carbon bond is between the middle carbon atoms (β - β') of the propyl side chains. In lignans the phenyl nuclei are substituted, and side chains exist

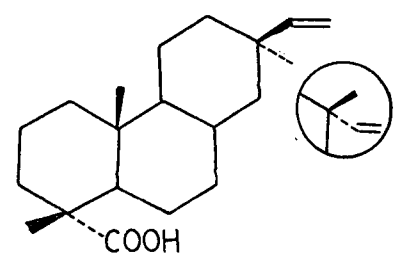
in various states of oxidation. In some cases the non-benzenoid skeleton is further modified by cyclisation to tetrahydrofuran, tetrahydrofurofuran or tetrahydronaphthalene derivatives [19].

Neutrals is a term used to represent esters of fatty acids and resin acids, fatty or terpene alcohols and hydrocarbons. The alcohols combined with fatty acids include glycerol (fats), β-sitosterol and long chain fatty alcohols (waxes) [33].

Resin acids are diterpene acids of the general formula $C_{20}H_{30}O_2$, classified as two types. The abietic type has an isopropyl side chain at position 13 (Fig. 2), while the pimaric type has methyl and vinyl substituents at this position [33].




Abietic type




Pimaric type

Figure 2. Basic $C_{20}$ structural skeleton of resin acids

## 2.2 Extractives of *Larix* spp.

The genus *Larix* includes about ten species widely scattered throughout North America, Europe and Asia [23]. Western larch occurs as the largest timber volume of the North American larches. It is a commercial species in the Columbia river region of British Columbia, and extends also into Washington, Oregon, Idaho and Montana. Its principal use is as lumber.

Several workers have investigated the extractive chemistry of various larches. In 1952, Tsvetaeva *et al* [40] extracted weeping larch (*Larix dahurica* Elwes and Henry) with a mixture of ethanol and benzene 1:1 and also with water. The yields obtained with these solvents were 5.6% and 19.9%, respectively. 'Distylin' was isolated by Hasegawa and Shirato [18] in 1952 from Japanese larch. This compound was later shown by Gripenberg [16] to be a mixture of dihydroquercetin and dihydrokampferol. He then isolated the two compounds from European larch (*Larix decidua* Mill.) in 0.25% and 0.45% yields, respectively.

The heartwood constituents of New Zealand grown Japanese and European larches were studied by Brewerton [7]. From these he isolated dihydroquercetin and dihydrokampferol. The Japanese larch yielded 2.07% dihydroquercetin and 0.43% dihydrokampferol while the European larch gave 0.52% and 0.43%, respectively. He also isolated a flavonoid of unknown structure.

Variation in composition of weeping larch heartwood constituents was studied by Tsvetaeva *et al.* [41] who found the composition to be: resin in heartwood 5.6%; ethanol-benzene 1:1 extract from 1.8 to 6.3%; and water soluble extracts 10 to 12% (up to 20% in cases) composed chiefly of arabinogalactan. Khutorshchikov (25] gave the hot water extracts of Siberian larch heartwood as 18.3% and its arabinogalactan content as 14.2%.

Work on neutrals and alcohols from *Larix* spp. was done by Nair and Von Rudloff [34] in 1959. They extracted tamarack (*L. laricinia* (Duroi) K. Koch) heartwood with acetone, and divided the extract into diethylketone, benzene and petroleum ether insolubles. They obtained dihydroquercetin

(0.3%) and dihydrokampferol (0.05%), as well as traces of quercetin and eicosanyl ferulate. The soluble fraction gave (after saponification) phthalic acid (1.9%) and long-chain fatty acids (31.7%), β-sitosterol (19.5%), eicosanol (2.2%) and 2-nonanol (3.1%). No tropolones or resin acids were detected. The same workers isolated dihydroquercetin and dihydrokampferol from the acetone extract of subalpine larch (*L. lyalli* Parl.) heartwood in 1.25% and 0.82% yields, respectively [35]. Small amounts of conidendrin were also isolated. Other compounds obtained from the extracts were β-sitosterol, 2-nonanol and phthalic, palmitic, a $C_{16}$, stearic, oleic, linoleic, linolenic and $C_{20}$ acids. Two unidentified alcohols were also obtained.

The water (12%) soluble extract of Kurile larch (*L. gmelini japonica* (Regel) Pilger) was obtained by Antonovskii *et al.* [1]. Its arabinogalactan content was found to be 11%.

Lisina *et al.* [29] analysed the petroleum ether soluble fraction of Dahurican larch (*L. gmelini* Litv.) acetone extract. The constituents were analysed by gas liquid chromatography (GLC) and from this the following acids were identified: Pelargonic, palmitic, oleic, 16 methyl octadecanoic, linoleic, octadecadienic and linolenic. The alcohols obtained from the heartwood were epimanool, larixol, β-sitosterol and cycloartenol. Larixol and larixyl acetate were also reported in European larch [37].

Using column chromatography (CC) over silica gel Leptova *et al.* [27] isolated the following lignans from weeping larch and Siberian larch (*L. sibirica* Ledeb.): Conidendrin, pinoresinol, lariciresinol, isolariciresinol secoisolariciresinol and 3,4 divanillyl-tetrahydrofuran.

# 3.0 MATERIALS AND METHODS

The following experimental procedures were adopted in the analysis.

## 3.1 Wood Source

A preliminary investigation was done with an air-dry western larch (*Larix occidentalis* Nutt.) veneer sample which had been in the laboratory for over two years. The heartwood part was cut in pieces and ground with a medium sized Wiley mill. Portions that passed through a 5 mm sieve were retained. Moisture content was determined on four samples.

Wood for the main investigation was obtained from the trunk of an 89 year old tree grown near Armstrong, B.C., cut in 1972 and shipped to the Western Forest Products Laboratory. Sections were cut perpendicular to the axis and heartwood was separated from the sapwood. The heartwood was cut into pieces, air-dried and ground as above. Portions that passed through a 5 mm sieve were retained. The wood meal was further air-dried with occasional turning to allow for uniform drying. Moisture content was determined on four samples.

## 3.2 Extraction Procedure

Wood meals were weighed (819.0 g for the preliminary investigation and 926.6 g for the main investigation) into a large cloth thimble placed in a 12 L Soxhlet. The material was successively extracted for 48 hr each with 8 L of the following solvents in the other:

i. petroleum ether (65°-80°);

ii. benzene;

iii. benzene-ethanol 1:1;

iv. ethanol;

v. distilled water; and

vi. ethanol.

Previous solvent was allowed to dry off completely before the next extraction. After extraction, solvent was removed by rotatory evaporator at reduced pressure from a tared flask. Solvent not removed by the evaporator was removed under vacuum. Flask with sample was then weighed to obtain the weight of extract. In the case of water extract, water was removed by continuous admixture with absolute ethanol to form a mixture with higher vapour pressure than water alone.

3.3 Chromatography

Thin layer chromatography (TLC) was employed to ascertain purity of compounds in column eluates, determine $R_f$ values, and compare compounds from extracts with standards. Commercially available silica-gel plates (Quantum Industries, Analytical, silica-gel GF with gypsum binder and phosphor Q4F) stored in a 70° C oven to prevent deactivation were used. Plates with a 0.25 mm thick layer were used for TLC, and thicker plates (2.5 mm) for separation and purification. This latter procedure involved depositing the mixture to be purified (in a solvent) along one side of a thick plate. Plate was developed in a suitable solvent. After viewing under UV light, the required compound plus silica gel was scraped off the plate. Another solvent dissolved the compound and the silica-gel was filtered off. Filtrate was concentrated to obtain the compound.

The developing solvents used for silica gel TLC were: methylene chloride ($CH_2Cl_2$); and benzene-ethanol, 9:1 (BE). All solvent ratios are given by volume unless otherwise stated.

For location of carbonyl compounds and esters of fatty acids, the plates were sprayed with 2,4 dinitrophenylhydrazine reagent (2,4 DNPH). This reagent was prepared by adding concentrated sulphuric acid (20 ml) to 2,4 dinitrophenylhydrazine (4 g) in a 250 ml conical flask. Water (30 ml) was added dropwise carefully with shaking. Ethanol (100 ml ) was added to the warm solution. Carbonyl compounds give yellow colour on warming the plate on a hot plate.

Diazotised sulphanilic acid spray (DSA) was used for locating phenolic compounds, such as lignans on silica gel plates. This reagent gives orange to red-orange colours with α-hydroxy guaiacyl nucleus and yellow colour with flavonoids []5]. DSA is prepared by reacting sulphanic acid solution, 5% aq sodium nitrite and 20% aq potassium bicarbonate (2:1:3). Sulphanilic acid solution was made by adding sulphanilic acid (9 g) to concentrated hydrochloric acid (90 ml) and making the solution up to 1 L with water. The procedure involved mixing the sodium nitrite solution with the sulphanilic acid first, and then adding potassium carbonate solution after 5 min. The reagent was used immediately following preparation in a cold room ($2^{\circ}$ C).

The identification of 3-OH flavanones, as distinct from other flavonoids, was made by dusting the silica gel TLC plate with powdered zinc metal. A camel hair brush was used for this. The plate was sprayed with concentrated hydrochloric acid. Bright purple colour signified the presence of 3-OH flavanone [4].

Also commercially available thin layer cellulose plates were used. The cellulose was 0.25 mm thick. Developing solvent for cellulose TLC was n-butanol, acetic acid and water, 60:15:25 ($BAW_1$). Detection of compounds on cellulose plates was done with p-anisidine hydrochloride spray. A saturated solution of p-anisidine in ethanol was sprayed on the plate followed by heating in an oven (110° C) for 15 min.

Column chromatography (CC) was the most used means for separating extract components. Chromatographic media employed were silica-gel, Sephadex A25 and Sephadex LH200. Separation on silica-gel column was always preceeded by a silica-gel TLC of the material. Petroleum ether (65°-80°) and benzene were the main solvents employed to elute the column. Stepwise increase in eluting solvent polarity was obtained by addition of varying amounts of ethyl acetate or ethanol to the main solvents. Fractions were collected in 25 ml. TLC on silica-gel and paper chromatography were employed to ascertain separation of compounds in eluates. In some cases, a 45 cm x 3.7 cm or 35 cm x 2.3 cm column was used to separate the compounds. Subsequently, a smaller column, 28 cm x 1.6 cm, was used for purification, eluting with a less polar solvent.

CC with Sephadex A25 had been very useful for separation of acids (fatty and resin) from neutrals [46]. The eluting solvents are: a mixture of diethyl ether and methanol, 9:1; diethyl ether and methanol, 9:1, saturated with carbon dioxide (using dry ice); and 4% formic acid in a mixture of diethyl ether and methanol 9:1. The first eluting solvent normally removed neutrals from the column, while the last two eluted resin and fatty acids.

Sephadex LH20 was employed to separate what was suspected to be flavonoid glycosides [38]. The eluting solvent was pure ethanol.

Silver nitrate impregnated alumina TLC was used for the fatty and resin acids methyl esters. Glass plates (20 cm x 20 cm) were coated with a slurry of alumina (aluminium oxide G with binder, Rēsearch Specialties Co.) and silver nitrate solution using a spreader set for 0.25 mm thickness. A solution of silver nitrate (12 g) in water (20 ml) was diluted with methanol (40 ml) and the resulting solution was added to alumina (30 g) and mixed by shaking. The plates were allowed to air-dry overnight, then they were dried at 110° C for 30 min before use. The developing solvent was diethyl ether and low boiling petroleum ether, 1:3. Concentrated sulphuric acid and diethyl ether, 1:4,was used as spray reagent. The plate was heated in an oven (110° C) for 15 min and then charred (200° C) for 1 hr[45].

Reverse phase TLC (RPC) was used to amplify result obtained from silica-gel TLC of β-sitosterol and neutrals. A cellulose plate was washed with 10% Nujol in petroleum ether. The petroleum ether was allowed to evaporate from the plate surface and specimens were then spotted. The developing solution was methanol saturated with Nujol; while 2,4 DNPH was used for detection.

Paper chromatography (PC) was useful for isolation and identification of flavonoids and lignans. $R_f$ values, colours under visible and ultraviolet light and ease of colour formation with reagents make such compounds readily identifiable.

Descending one-dimensional (1D) and two-dimensional (2D) PC were used in these investigations with Whatman No. 1 paper in a Shandon apparatus.

Papers of 45 cm length and 15 cm to 45 cm width were used in 1D chromatography, while papers of 45 cm square were used for 2D chromatography. The solvent systems employed for development of papers were: $BAW_1$; $BAW_2$, i.e., 10:3:7; the top layer of a mixture consisting of n-butanol, concentrated ammonia and water, 20:3:10 (BNW); and 2% acetic acid in water (AW). Detecting reagents were DSA and Bartons reagent for confirmation. Bartons reagent is a mixture of 0.5% aq ferric chloride and 0.5% aq potassium ferric cyanide (100 ml of each made up to 1 L).

Gas liquid chromatography (GLC) on a 10% EGSS-X column (5 ft x 1/8 in) gave good separations of fatty acids and resin acids (Me esters), and also of neutrals obtained in the petroleum ether extract. About 1.5 µl of material (1 g in 10 ml) was injected. Two chromatographs were used, an Aerograph 204 and a Hewlett Packard Research Chromatograph 7620A. The latter was coupled with an integrator to obtain direct reading on quantities of materials being separated by the column.

Preparative GLC was used in an attempt to quantitatively separate neutrals from the petroleum ether extract. Details of the procedure are not included since there was not enough material available to use the equipment effectively.

3.4 Derivatives Preparation

O-Methyl esters and O-methyl ethers were obtained for acids and phenols, respectively. The compound(s) to be methylated was (were) dissolved in a minimal amount of solvent and cold diazomethane in ether was added. Effervescence indicated presence of easily methylated hydroxyl groups, as in acids. The mixture in methanol was left in the cold room (2° C) overnight to

methylate phenolic hydroxyl groups. Excess diazomethane was removed by blowing nitrogen gas over the mixture. Residual solvent was removed using a vacuum pump.

Amine salts were used to separate fatty acids from resin acids. Mixture containing both was treated with 10% cyclohexamine in ethanol. The reaction mixture was put in the cold room (2° C) overnight. The resin acid amine salts precipitated, while the fatty acid amine salts remained in solution. The precipitate was collected and washed with ethanol and partitioned between chloroform and 1 N hydrochloric acid. The hydrochloric acid layer was discarded. The chloroform was evaporated and the residue was methylated with diazomethane to obtain the resin acid methyl esters. The solution containing the fatty acid amine salts was similarly partitioned between chloroform and 1 N hydrochloric acid and the chloroform layer was then methylated as above to obtain the fatty acid methyl esters.

3.5 Degradative Techniques

Saponification of esters was applied to neutrals in order to convert them to fatty acids and alcohol. The fatty acids were identified after methylation by using GLC. The saponification reagent was made by dissolving sodium metal (1.6 g) in absolute ethanol (50 ml). Water (5 ml) was added after dissolution was complete. This reagent and the material to be saponified were refluxed for 1 1/2 hr. The solution was allowed to cool and excess 1 N hydrochloric acid was added to neutralise the mixture. The fatty acids were extracted with petroleum ether (65° - 80°), and the solvent evaporated to dryness. The collected fatty acids were methylated as above.

## 3.6 Partition Between Solvents

From the results obtained with PC using $BAW_1$, initial separation before silica gel CC was performed on some mixtures. This was effected by partition between two immiscible solvents, n-butanol and water. Material extracted with n-butanol, was exhaustively extracted with water. Both solutions were evaporated to dryness on the rotary evaporator by constant admixture with absolute ethanol.

## 3.7 Spectral Techniques

Infrared spectroscopy (IR) was performed to obtain the spectra of purified samples, either from potassium bromide pellets or as smears on sodium chloride plates. A Perkin-Elmer 521 Infrared Spectrophotometer was used. The spectra of standard compounds were also taken for comparison.

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian HA-100, 100 MHz NMR spectrometer. The samples were prepared in deuterochloroform, deuterated acetone or heavy water depending on compound solubility. Tetramethylsilane was added as an internal standard and lock signal at $\tau$ = 10. Sample dilution was approximately 15 mg/300 µl of solvent.

# 4.0 RESULTS

The following are the results obtained in the experimental procedures taken in this investigation.

## 4.1 Preliminary Study

The veneer sample moisture content was 8.77% and the wood meal weight was 747.2 g (OD basis).

The petroleum ether (65° - 80°) extract (4.50 g) was 0.60% of the OD sample. Three silica-gel TLC were done on this extract. They were developed with $CH_2Cl_2$, BE and petroleum ether (65° - 80°). No movement occurred in the plate developed with petroleum ether. The other two plates revealed various compounds under UV. One plate was sprayed with 2,4 DNPH and the other with a 1:1 mixture of concentrated sulphuric acid and concentrated nitric acid. The plate sprayed with 2,4 DNPH revealed more spots than the one sprayed with mixed acids. A GLC of the methylated petroleum ether (65° - 80°) extract sample gave five major peaks.

Extraction with benzene gave 1.50 g of material (0.20% of OD sample). Two silica-gel TLC were developed in BE and viewed under UV. One plate was sprayed with 2,4 DNPH and the other with DSA. Colour of various spots signified the presence of carbonyl and phenolic compounds.

The benzene-ethanol 1:1 extract (23.12 g) was 3.09% of the OD sample. A pair of 2D, PC was done with $BAW_1$ and AW. One paper was sprayed with DSA and the other with Bartonss reagent. Spots signifying the presence of carbonyl compounds, yellow and blue respectively with the reagents, were

observed. The most conspicuous spot was shown to be dihydroquercetin.

The ethanol extract (6.85 g and 0.92% of OD sample) revealed more dihydroquercetin on 2D, PC with $BAW_1$ and AW. The water extract (117.30 g and 15.7% of OD sample) consisted mainly arabinogalactan, while the final ethanol extract (5.44 g and 0.73% of OD sample) revealed more dihydroquercetin, among other minor compounds, on 2D, PC with $BAW_1$ and AW.

The total amount of extract was 21.2% of the OD sample.

4.2 Main Study

The moisture content of the air-dry wood meal was 11.53%, and the OD weight 819.78 g. Results from each successive extraction are given below.

Petroleum ether (65° - 80°) extract was 5.27 g (0.65%) of the OD sample.

Two silica-gel TLC plates were spotted with the extract and developed in $CH_2Cl_2$ and BE. Afterwards the plates were viewed under UV. Yellow and purple spots were noted. This signified the presence of carbonyl compounds and esters. Their presence was confirmed when the plates were sprayed with 2,4 DNPH and warmed on a hot plate. Several yellow and purple spots were observed.

Separation of the neutrals from the acids (fatty and resin) was effected by CC of 1.75 g of the petroleum ether extract on Sephadex A-25. Elution of the column with diethyl ether-methanol, 9:1 gave 1.22 g of neutrals. The acids which came off the column (saturation of the solvent with carbon dioxide, and addition of 4% formic acid to the solvent) were 0.41 g.

A portion of the first neutrals fraction off the column (to avoid contamination from fatty and resin acids which might be present in the latter fraction) was dissolved in chloroform (1 g to 10 ml) and 1.3 µl of this was injected into the gas chromatograph operating under the following conditions:

i. column, 10% EGSS-X, 1/8 in x 4 ft;

ii. oven temperature, 175° C isothermal;

iii. carrier gas, nitrogen;

iv. ionisation gas, hydrogen;

v. flame ionisation detector temperature, 300° C;

vi. injection port temperature, 300° C; and

vii. chart speed 1 in/5 min.

Juvabione i.e., (+) - methyl ester of (+) - todomatuic acid ( (+) - 4 (R) - [1' (S) - 5' - dimethyl-3'-oxohexyl]-cyclohex-1-ene-1-carboxylic acid) and cis-dihydrojuvabione were injected before and after for comparison. One of the peaks gave the same retention time (38.75 min) as that obtained for juvabione, but none gave the same as its cis dihydro-derivative.

A portion of the same specimen (0.52 g) was placed on a silica-gel chromatography column and eluted with petroleum ether (65° - 80°) until very little material came off the column with this solvent. This was followed by similar elution with petroleum ether containing 1% increments of ethyl acetate. Elution continued until the solvent was a mixture of petroleum ether and ethyl acetate 9:1. Afterwards the remaining material on the column was washed down with pure ethyl acetate. TLC of fractions collected was done on silica-gel with juvabione, cis dihydrojuvabione, and β-sitosteryl palmitate as standards. The plates were developed in $CH_2Cl_2$, sprayed with 2,4 DNPH and heated on a hot plate.

Spots which gave the same $R_f$ values and colour reactions with 2,4 DNPH (yellow) as the juvabione and cis dihydrojuvabione did not give the same colour reaction (faint yellow) on heating. As a confirmation, this fraction was put in the gas chromatograph and standards were injected before and after for comparison. Retention value obtained for this fraction differed from either of the standards.

Some compounds in the fractions had the same $R_f$ (0.80 and 0.30) as β-sitosteryl palmitate and β-sitosterol, respectively. A reverse phase TLC of these compounds in the fractions and standards gave $R_f$ values of 0.35 and 0.56, respectively. Hence β-sitosteryl palmitate and β-sitosterol were both present.

A fraction of the neutrals from the silica-gel CC was found to have a trace component associated with it as seen by TLC. This fraction was put along the length of a thick silica-gel plate which was developed in $CH_2Cl_2$. The required portion was scraped off, after delineating its contours with UV light. The scrapings were extracted with chloroform. The chloroform was filtered and the filtrate was evaporated to dryness. The NMR was taken in deuterochloroform while the IR was conducted as a smear on sodium chloride plates. The spectra showed that the trace component was tristearin. This was proved by comparison of the spectra with that of the standard.

In order to identify fatty acids attached to alcohol forming fats in the neutrals, a portion (0.17 g) of the neutrals was hydrolysed with a prepared saponification reagent (20 ml) and refluxed as previously described. The resulting fatty acids were methylated, and 1.5 µl of the standard solution was injected into the gas chromatograph. Standards containing

$C_{10}$, $C_{12}$, $C_{14}$ and $C_{15}$ - $C_{19}$ fatty acid methyl esters were also injected before and after the unknown. The fatty acids identified were palmitic, stearic and two others beyond $C_{19}$, probably either linoleic, pinolic, or arachidic.

Fatty acids and resin acids were separated as amine salts after recovery from the Sephadex A25 column. Total yield of fatty acids (OD sample basis) was 0.85 g, i.e., 0.10%. Resin acids yield was 0.14g, 0.017%.

TLC of fatty and resin acids (Me esters) was performed on silver nitrate impregnated alumina plates. After developing and spraying the chromatogram, it was left in an oven (110° C) for 15 min and then charred for 1 hr [45].

The fatty acids (Me esters) $R_f$ values were 0.06, 0.31, 0.66 and 0.72. Literature values revealed the first one was linoleic acid while the last was palmitic acid (Me esters) [45]. $R_f$ values of the resin acids (Me esters) obtained were 0.13, 0.22, 0.50 and 0.63. These on comparison with standards were shown to be isopimaric, sandaracopimaric, abietic and dehydroabietic acids (Me esters), respectively.

The fatty acid methyl esters were dissolved in chloroform (1 g in 10 ml) and 1.5 µl of this solution was injected into the gas chromatograph (Aerograph 204 with oven temperature set at 155° C). All other conditions were as given previously. Standard fatty acids methyl esters which contain $C_{10}$, $C_{12}$, $C_{14}$ and $C_{15}$ - $C_{19}$ acids were injected before and after the sample. From comparative retention times, the fatty acids listed in Table 1 were identified.

| Compound | Retention time, min. | Identity |
|---|---|---|
| III | 5.31 | Palmitic |
| IV | 6.86 | Palmitoleic |
| V | 13.28 | Linoleic |
| VI | 17.50 | Possibly linolenic |
| VII | 19.53 | Possibly arachidic |

TABLE 1. Retention times of fatty acid methyl esters.

The resin acid methyl esters solution was injected into the gas chromatograph. Conditions were as described for the neutrals above. In addition, an integrator was connected so as to give a printout for calculating the ratio yield for each resin acid. Standard resin acid methyl esters mixture was injected as before. These data are given in Table 2.

| Compound | Retention time, min. | Identity | Ratio based on minimum |
|---|---|---|---|
| VIII | 13.75 | Sandaracopimaric | 1 |
| IX | 18.75 | Isopimaric | 18.29 |
| X | 25.47 | Abietic | 4.00 |
| XI | 31.72 | Dehydroabietic | 3.21 |

TABLE 2. Retention times and yield ratio of resin acid methyl esters.

Benzene extracts were recovered as 1.22 g (0.15% OD sample basis).

Two silica-gel TLC, developed in $CH_2Cl_2$ and BE were done on the sample dissolved in ethanol. One plate was sprayed with DSA and the other with 2,4 DNPH. Colour of the spots suggested the presence of carbonyl compounds and phenolics.

Methylation with diazomethane did not produce nitrogen effervescence and the mixture was left overnight in the cold. A silica-gel TLC of the unmethylated sample spotted side by side with the methylated sample was developed in BE. Spots were located by exposure to an iodine atmosphere. Various spots not present in the unmethylated sample were seen in the methylated sample. This confirmed the presence of phenols.

CC separation of the material (0.51 g) was done on silica-gel following solution in the minimum amount of benzene and ethanol mixture. The column was first eluted with pure benzene and 25 ml fractions were collected, until very little material came off by further elution with this solvent. Polarity of the eluting solvent was then increased by additions of ethyl acetate in 2% increments, and elution was continued as before. The ethyl acetate content of the eluting solvent was increased by 5% increments after elution with 10% ethyl acetate in benzene. After elution with 40% ethyl acetate in benzene, the material still remaining on the column was washed down with pure ethyl acetate followed by pure ethanol.

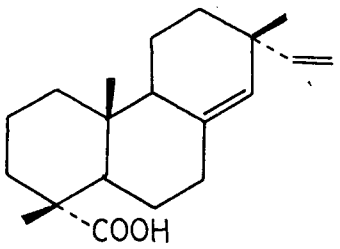
Silica-gel TLC of the fractions were made in duplicate and developed in BE. After viewing under UV light, one plate was sprayed with 2,4 DNPH and the other with DSA. Similar fractions were combined. The following compounds are reported:

Compound Ia was obtained when the column was eluted with 10% ethyl acetate in benzene. It formed about 2% of the benzene extract (0.003% of the OD sample). It crystallised out of ethanol solution after being left in the cold for some days. $R_f$ in BE was 0.62, with purple colouration under UV light. It was not sensitive to 2,4 DNPH even on warming the plate, but gave a yellow colour with DSA and no reaction with Zn/HCl. Its IR spectrum (Fig. 5) was taken in a KBr pellet and is described as follows: 3100 $cm^{-1}$, shoulder; 1650, 1570, 1490, 1290, very strong; 1250 weak; 1150 very strong; 1070, strong; 1050 and 850, weak.

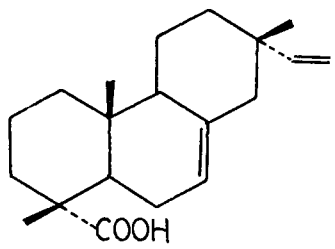
These properties were compared with those of compounds likely to have the same characteristics. Complete agreement was found with pinocembrin (Ia).

Compound Ic was collected on eluting the column with benzene and ethyl acetate mixture, 7:3. The compound with its associated contaminants was 3.6% of the benzene extract (0.005% of the OD sample). Repeated silica-gel CC did not remove all the contaminants, however this did not prevent identification. A purple fluorescence was observed when its spot (silica gel TLC) was viewed under UV. $R_f$ in BE was 0.29 and a reddish-yellow colour developed with 2,4 DNPH, while a yellow colour occurred with DSA. Reaction with Zn/HCl gave a purple colour. Comparison with standards revealed the compound to be dihydrokampferol (Ic).

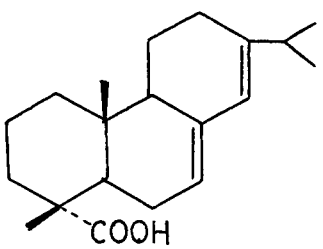
Compound XII formed about 1% of the benzene extract (0.002 of the OD sample). It came off the column with benzene-ethyl acetate (85:15). A faint pink fluorescence was observed under UV when spotted (silica-gel TLC), and orange colour with 2,4 DNPH and pink colour with DSA. It did not give a purple colour with Zn/HCl. The $R_f$ in BE was 0.36 and 0.87 in $BAW_1$.



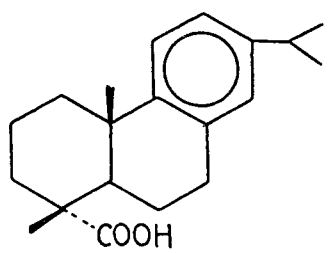

VIII



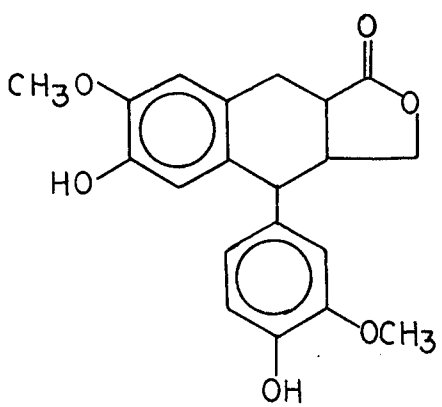

IX




X




XI




XII

Comparison with standard compounds showed that it was α-conidendrin (XII).

Some other compounds were obtained from the silica-gel CC of the benzene extract. Low yields and association with trace contaminants which were not removed by repeated chromatography prevented positive identification. The following compounds amongst them were obtained in the largest yields:

Compound XIII formed about 2% of the benzene extract (0.003% of the OD sample) and was eluted with benzene and ethyl acetate mixture, 3:1. Its $R_f$ in BE was 0.35 and a pink fluorescence occurred under UV. The compound was unsensitive to 2,4 DNPH, but on warming the plate, after spraying with this reagent, an orange colour could be noticed. A yellow colour developed with DSA and delayed purple colour with Zn/HCl. This suggested a 3-OH flavonoid structure. Its $R_f$ was slightly lower than that of pinobanksin (0.36) and did not give the dull yellow fluorescence of pinobanksin under UV.

Overnight methylation of this compound in the cold (2° C) gave two other compounds of $R_f$ 0.39 and 0.52 (silica-gel TLC, developed in BE). Both compounds gave purple colour under UV, orange and pink colours, respectively, with DSA. Methylation of pinobanksin gave only one compound of $R_f$, 0.45, yellow under UV and orange colour with DSA.

Compound XIV with associated minor compounds formed about 10% of the whole benzene extract (0.015% of OD sample). It came off with other compounds when the column was finally eluted with ethanol. This eluate on evaporation of the ethanol was dissolved in a minimum amount of ethanol and put on a silica-gel column chromatograph. Elution of the column with benzene-ethanol 95:5 yielded this unknown with associated minor compounds. Its $R_f$ (silica-gel TLC, developed in BE) was 0.50. The compound was unsensitive to

2,4 DNPH but when the plate was warmed, a green colour resulted, a pink colour was obtained with DSA. Repeated silica gel CC did not remove the carbonyl compound associated with it.

Its NMR and IR spectra were taken in deuterochloroform and potassium bromide pellet, respectively. The IR spectrum is described as follows: 3400 $cm^{-1}$, shoulder; 2900 very strong; 1600 weak; 1500 very strong; 1250 very strong; 1000 and 950, weak.

Benzene-ethanol (1:1) extracted 10.58 g (1.30% of the OD sample). No immediate reaction was noticed on methylation with diazomethane and the mixture was left overnight in the cold. A silica gel TLC of the extract and the methylated sample was developed in BE and exposed to iodine vapour. Spots not present in the unmethylated sample were observed in the methylated sample.

A 2D, PC with $BAW_1$ and AW, sprayed with DSA after viewing under UV showed three major compounds. Their PC properties are given in Table 3.

| Compound | $R_f$ | | Colour | | Identity |
|---|---|---|---|---|---|
| | $BAW_1$ | AW | UV | DSA | |
| Ic | 0.88 | 0.57 | l.y | y | Dihydrokampferol |
| Id | 0.82 | 0.37 | y | y | Dihydroquercetin |
| II | 0.71 | 0 | b.y | y | Quercetin |

TABLE 3. PC properties of compounds Ic, Id and II.
b.y = bright yellow; l.y = light yellow; y = yellow.

Silica-gel CC of a portion of the extract was first eluted with benzene and subsequently with a mixture of benzene and ethanol in 5% increments of the latter. Elution was followed by silica-gel TLC, developed in BE, viewed under UV and sprayed with DSA.

The first fraction collected from the column contained XIV, described in the benzene extract. Dihydrokampferol (Ic), dihydroquercetin (Id) and quercetin (II) were also identified from the column elutions by comparison with standards ($R_f$ in $BAW_1$ and BE). The $R_f$ values of these compounds in BE were 0.29, 0.16 and 0.06, respectively. Another silica-gel TLC plate was sprayed with Zn/HCl. Only dihydrokampferol and dihydroquercetin gave purple colours. Dihydroquercetin (eluted later and identified on PC) gave a negative result with Zn/HCl. It seems to have been masked by a compound which also gave a yellow colour with DSA.

Ethanol extract was collected in an amount of 4.59 g (0.56% of the OD sample).

A 2D, PC developed with $BAW_1$ and AW revealed the presence of dihydroquercetin and traces of quercetin on spraying with DSA. Two other spots were also observed.

Dissolving the extract in ethanol precipitated what was shown to be dihydroquercetin. It was filtered off, the filtrate was concentrated and put on a silica-gel chromatography column. Elution was first with pure benzene and then with 5% increments of ethanol added. Dihydrokampferol, dihydroquercetin and traces of quercetin were obtained. The two spots reported above were also obtained. They were not identified but their data were as given below.

Compound XIII had an $R_f$ of 0.51 in $BAW_1$ and zero in AW. It gave a yellow colour with DSA and a light purple fluorescence under UV light. Its other properties were the same as described for the unidentified compound XIII of the benzene extract. It is likely that they are identical.

Compound XV had an $R_f$ of 0.82 in $BAW_1$ and 0.69 in AW. Under UV a light purple colour was observed as was with DSA, suggesting a lignan. In BE, it had an $R_f$ of 0.45, higher than that of α- and β- conidendrin, used as comparison, but similar to them under UV and with DSA.

The ethanol extract also contained low $R_f$ resinous materials which stayed on the column until washed with pure ethanol. This material formed a brown glassy solid on evaporation of the solvent. It was about 60% of the ethanol extract.

Water extracted materials were 97.55 g, i.e., 11.90% of the OD sample. The mixture consisted mostly of crude arabinogalactan in 11.10% of the OD wood. This polysaccharide is common to *Larix* spp.

The extract was concentrated to remove as much water as possible. A portion of the concentrate was diluted 10 times with ethanol, filtered and the filtrate evaporated to dryness. The residue was redissolved in ethanol and the insoluble portion, which was arabinogalactan was discarded. The filtrate was concentrated and examined by PC. It was developed in 2D with $BAW_1$ and BNW. $R_f$ values of the spots are given in Table 4. These were possibly lignans.

| Compound | $R_f$ | | Colour with DSA |
|---|---|---|---|
| | $BAW_1$ | BNW | |
| XVI | 0.86 | 0.06 | Pink |
| XVII | 0.64 | 0.29 | Light pink |
| XVIII | 0.83 | 0.55 | Pink |
| XIX | 1.00 | 0.91 | Yellowish pink |

TABLE 4. PC properties of ethanol solubles in water extracts

Flavonoid glycosides were sought in the ethanol soluble portion of the water extract using the method of Birkofer and Kaiser [6]. A pair of 1D PC in $BAW_2$ were made. One was sprayed with concentrated ammonia and the other with DSA. The ammonia sprayed paper gave three spots of $R_f$ 0.90, 0.82 and 0.69; under UV their colours were yellowish blue, blue and light yellow, respectively. A fourth spot of $R_f$ 0.42 and light blue under UV appeared when the paper was left in the oven (100°C) for 5 min. None of these colours corresponded with those expected from glycosides of the flavonoids found. On spraying the other chromatogram with DSA, the same four spots appeared. The first three gave bright orange to orange colours, while the fourth gave a light red colour. Possibly they were lignans.

To confirm the absence of flavonoid glycosides in the ethanol soluble portion of the water extract, extract specimen was placed on a Sephadex LH20 column and eluted with ethanol. This procedure has been used successfully for the isolation of flavonoid glycosides [38]. Fractions eluted were followed by PC with $BAW_2$ and the papers were sprayed with either DSA or ammonia. Most of the compounds were recovered in the first 25 ml fraction. The second fraction collected gave a compound which crystallised out of aqueous ethanol as white needles. IR and NMR spectra showed it to be

a carbohydrate, with m.p. 159° C. Cellulose TLC with galactose and L-arabinose as standards in $BAW_1$ and spraying with p-anisidine hydrochloride reagent followed by warming in the oven (120° C) for 5 min gave a purple-brown colour for this compound (XX). This colour, $R_f$, IR (in KBr pellets), NMR (in $D_2O$) and m.p. data coincided with that of L-arabinose. Its IR spectrum is described as follows: 3250 $cm^{-1}$, shoulder; 1315, weak; 1255, 1230, 1130, strong; 1095, 1055, very strong; 1005, strong; 945, weak; 895, 845 and 785, strong.

Because of divergent $R_f$ values exhibited in $BAW_2$ by the non-arabinogalactan fraction of the water extract, these were partitioned between water and n-butanol as described. A preliminary 2D, PC with $BAW_1$ and BNW revealed several compounds when sprayed with DSA.

Each extract was put on a silica-gel CC and eluted with benzene and ethanol mixture. PC of the eluates was done in duplicate. One paper was developed in $BAW_1$ and the other in BNW. The chromatograms were sprayed with DSA after viewing under UV. Data for the n-butanol solubles obtained are given in Table 5.

| Compound | $R_f$ | | Colour with DSA |
|---|---|---|---|
| | $BAW_1$ | BNW | |
| XXI | 0.93 | 0.93 | Reddish-brown |
| XXII | 0.87 | 0.41 | Purple |
| XXIII | 0.84 | 0.04 | Orange |
| XXIV | 0.88 | 0.84 | Light red |
| XXV | 0.75 | 0.16 | Light yellow |
| XXVI | 0.73 | 0.05 | Light yellow |
| XXVII | 0.81 | 0.71 | Reddish-orange |

TABLE 5. PC properties of n-butanol solubles in water extracts.

Compound XXII was compared with ferulic acid (PC behaviour with $BAW_1$ and BNW, sprayed with DSA). Ferulic acid gave $R_f$ of 0.86 with $BAW_1$, 0.08 with BNW and purple colour with DSA.

The water solubles had the PC properties shown in Table 6.

| Compound | $R_f$ | | Colour with DSA |
|---|---|---|---|
| | $BAW_1$ | BNW | |
| XXVIII | 0.89 | 0.88 | Light orange |
| XXIX | 0.84 | 0.79 | Reddish-pink |
| XXX | 0.80 | 0.61 | Orange |
| XXXI | 0.79 | 0.68 | Reddish-pink |
| XXXII | 0.61 | 0.36 | Reddish-pink |
| XXXIII | 0.59 | 0.33 | Bright orange |

TABLE 6. PC properties of water solubles in water extracts.

Final ethanol extraction yielded 3.18 g of material (0.39% of the OD sample).

A 2D, PC was done with $BAW_1$ and AW, and sprayed with DSA. Data were obtained as given in Table 7.

| Compound | $R_f$ | | Colour with DSA |
|---|---|---|---|
| | $BAW_1$ | BNW | |
| XXXIV | 0.87 | 0 | Brownish-red |
| XXXV | 0.76 | 0.28 | Yellow |
| XXXVI | 0.82 | 0.44 | Reddish-pink |
| XXXVII | 0.82 | 0.54 | Reddish-pink |
| XXXVIII | 0.82 | 0.68 | Reddish-pink |
| XXXIX | 0.96 | 0.85 | Yellow |

TABLE 7. PC properties of final ethanol extracts.

Solubility tests were performed on portions of the specimen. No solvent completely dissolved the specimen. Acetone precipitated a white compound which was soluble in water and hot methanol. It crystallised out of aqueous ethanol and was identified as L-arabinose from IR spectra, m.p. and cellulose TLC developed in $BAW_1$ and sprayed with p-anisidine hydrochloride.

The remaining acetone solubles were chromatographed over silica gel (CC) with benzene-ethanol as the eluting solvent. A compound which crystallised out as brown flakes was obtained. IR and NMR spectra of the impure compound showed that it was a lignan. The $R_f$ in $BAW_1$ and 'AW on PC corresponded with that of compound XXXVII.

# 5.0 DISCUSSION

Various observations were made in the experimental procedures of this investigation. These results, including the physical and chemical characteristics of the compounds (especially those unreported previously) found in the wood are here discussed.

## 5.1 Experimental Procedures

Successive extraction of the wood meals with solvent of increasing polarity was most helpful for preliminary isolation of compounds. Compounds from specific chemical classes were obtained in most of the different extracts. For instance, fatty and resin acids were obtained only in the petroleum ether extract; low hydroxylated flavonoids appeared only in the benzene extract; higher hydroxylated flavonoids were found in the ethanol extract; and arabinogalactan occured only in the water extract. This assisted isolation of compounds which were present only in trace amounts.

CC procedures gave good seperations, with appropriate choice of eluting solvents. Most minor compounds of low $R_f$ values stayed at the top of the column, especially when eluting with solvents of low polarity. Washing the column finally with a strongly polar solvent removed them. Using a rubber hand pump to activate CC elution, a current procedure at the Western Forest Products Laboratory, increased the flow rate considerably. This solved a problem (low flow rate) often encountered with CC technique.

Although PC with $BAW_1$ and detection with DSA were mostly used for the identification of flavonoids and lignans, the use of silica-gel TLC with BE as the developing solvent and detection with DSA was superior. Most

compounds tried on the PC system ran close to the solvent front, resulting in similar $R_f$ values. Accurate measurement of $R_f$ values was further hampered by the large, uneven spots resulting from development. Colour developed with detecting reagents was dependent on the concentration and purity of the compound spotted. Associated trace contaminants (especially low $R_f$ value compounds) did not stay at the origin and this prevented purification on thick paper. In contrast, these minor components did not move with the compound in silica-gel TLC.

The problems mentioned above were not observed with silica-gel TLC and development with BE. In addition, all TLC data were reproducible with only slight variations in $R_f$ values for repeat TLC trials. The small differences observed might have been due to temperature variation or inaccurate mixing of the developing solvents.

The average time taken to develop a 20 cm x 20 cm TLC plate (BE) was 45 min, while PC took about 15 hr. Hence, TLC was found most convenient for following elutions from columns. The identification of sugars on cellulose TLC was also more reproducible than PC (both developed in $BAW_1$).

Bartons reagent was not used extensively in this investigation, because in earlier experiments it was found that the blue colour on the paper greatly masked the position of minor components. This was unacceptable, since in some case only trace amounts were being investigated.

Although the presence of fatty and resin acids was confirmed by comparison with standards using GLC, further confirmation using silver nitrate impregnated TLC did not proceed as anticipated [45]. Spots appeared even before the completion of the recommended 1 hr charring period.

5.2 Extracts

Petroleum ether (65° - 80°) extracted fatty and resin acids, they were easily isolated by CC with Sephadex A-25. Most probably, separation of resin acids from the rest of the petroleum ether extract (2.68% of the petroleum ether extracts), was due to the use of this absorption medium. Resin acids had not been found previously in this wood. Of all the *Larix* spp., free resin acids have been reported only in *L. sibirica* [2]. Free fatty acids, alikewise, had not been reported in most of *Larix* spp. The GLC traces of free fatty acids (Me esters) and free resin acids (Me esters) found are shown in Figs. 3 and 4, respectively. The fatty acids were palmitic (III), palmitoleic (IV), linoleic (V) and two others, possibly linolenic (VI) and arachidic (VII). The resin acids were sandaracopimaric (VIII), isopimaric (IX), abietic (X) and dehydroabietic (XI). Retention times on GLC are given in Tables 1 and 2.

Benzene extracts consisted of many carbonyl and phenolic compounds, as seen from TLC (silica-gel, BE and DSA, 2,4 DNPH). The small amount of material extracted by this solvent prevented complete separation of the compounds.

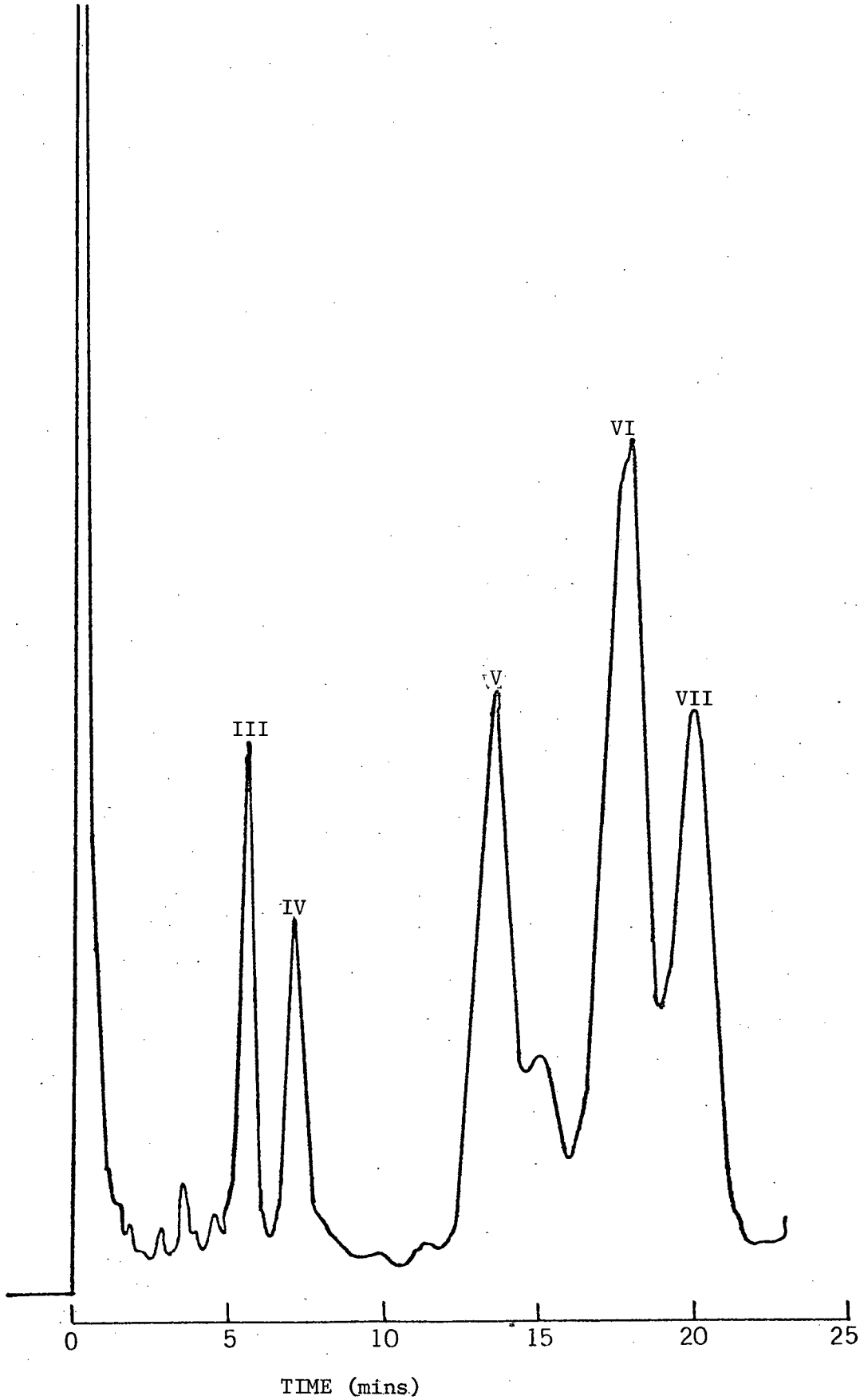



Figure 3. GLC trace of fatty acid methyl esters.

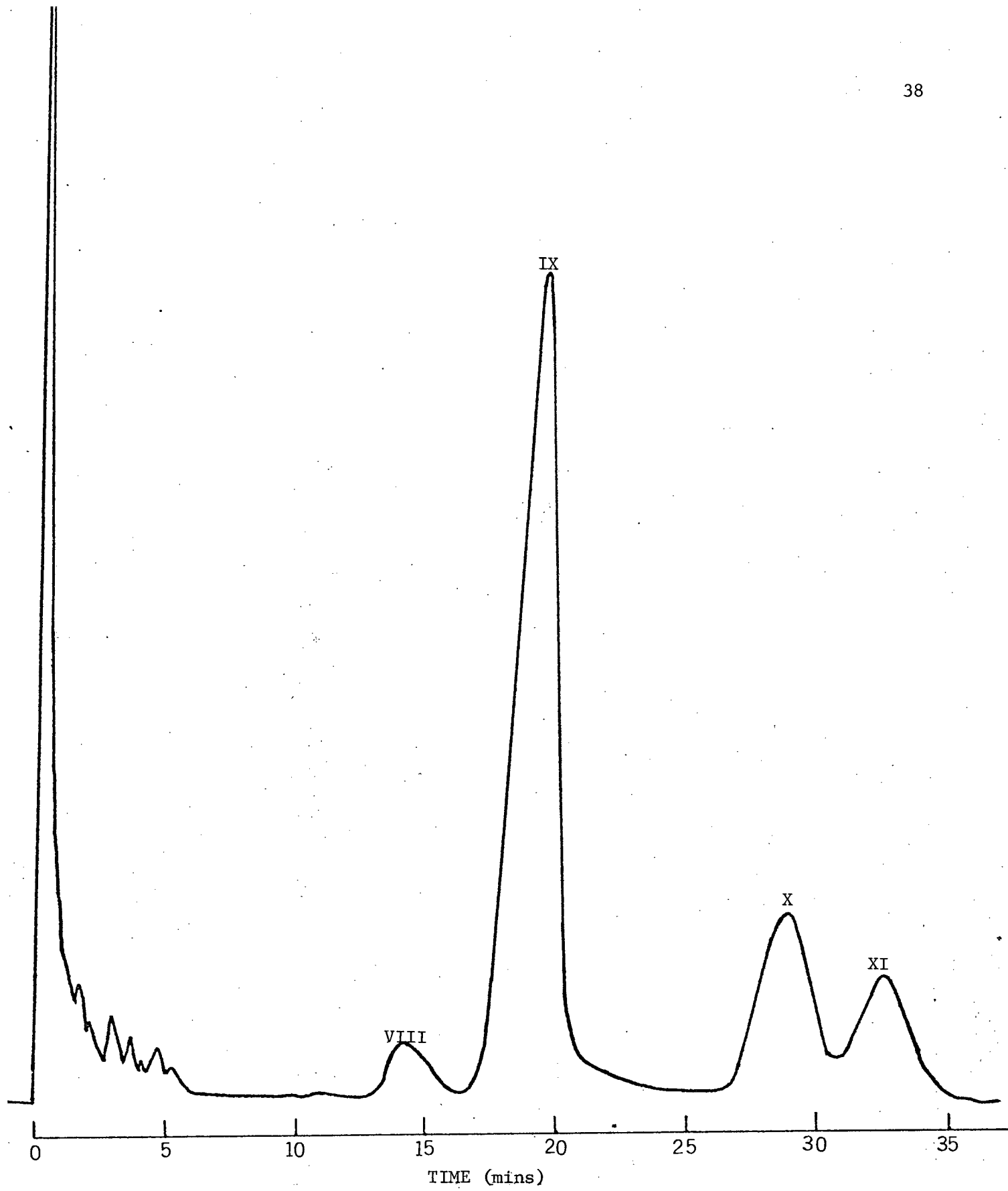



Figure 4. GLC trace of resin acid methyl esters.

Pinocembrin (Ia) previously unreported in this species, was obtained in 0.003% yield. Its IR spectrum is shown in Fig. 5. The absorption band around 3500 $cm^{-1}$ shows the presence of -OH groups while the bands at 1500 to 1650 $cm^{-1}$ are aromatic signals. Absence of an $-OCH_3$ group in the molecule is proved by lack of a band at 1400 $cm^{-1}$. The complete IR spectrum of this compound coincided with that of the standard compound, especially the bands at 1,000 to 800 $cm^{-1}$ (fingerprint region).

The $R_f$ in BE obtained for the flavanones in this wood decreased with degree of hydroxylation on the flavanone ring. Dihydroquercetin with five hydroxyl groups had an $R_f$ of 0.16, dihydrokampferol with four hydroxyl groups had an $R_f$ of 0.29 and pinocembrin with two hydroxyl groups had an $R_f$ of 0.61. The $R_f$ of pinobanksin (with three hydroxyl groups) used as standard was 0.36. This value was close to the $R_f$ (0.35) of the unidentified compound XIII, found in the benzene extract and described above. It also resembled the above flavanones by giving a purple colour with Zn/HCl, indicative of a 3-OH group. It differed from pinobanksin in the type of its fluorescence under UV (a purple colour while pinobanksin was dull yellow). Further, its methylation products differed from those of pinobanksin (both reported above). It is suggested that XIII is a trihydroxylated, 3-OH flavanone. In order to differ from pinobanksin it could have one of the two hydroxyl groups in the A ring of pinobanksin transferred to the B ring, i.e., an isomer of pinobanksin.

The NMR and IR spectra of unidentified compound XIV are shown in Fig. 6 and Fig. 7, respectively. IR signals at 3400 $cm^{-1}$ and 1400 $cm^{-1}$ are typical of -OH and aromatic $-OCH_3$ groups respectively. According to Ludwig *et al.* [30], the signal at $\tau$ 6.19 comes from the 3-OMe group in this type of compound. Integrated value for this peak indicated six protons, i.e., two

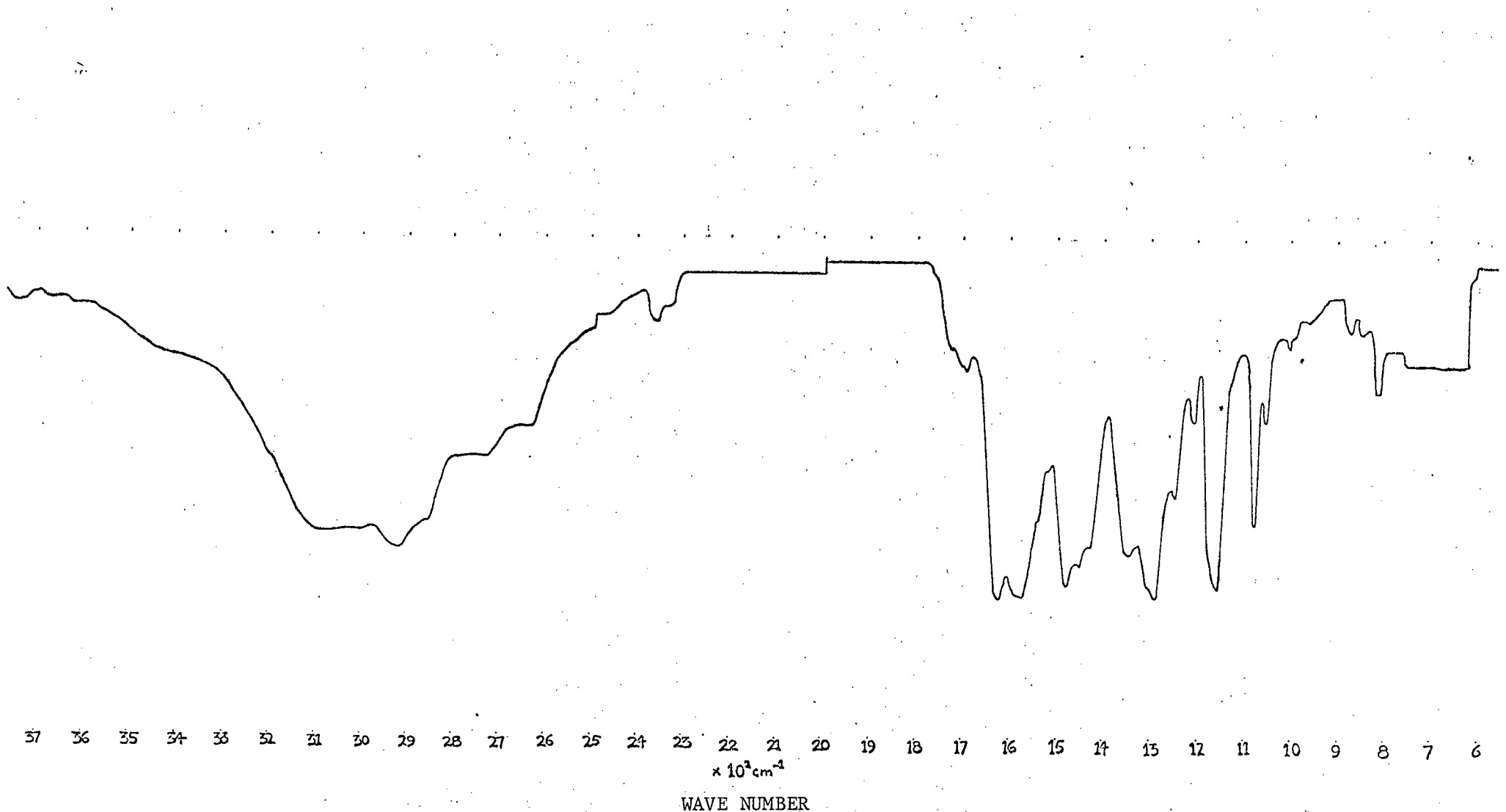
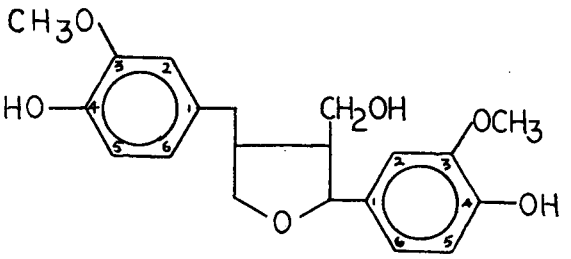
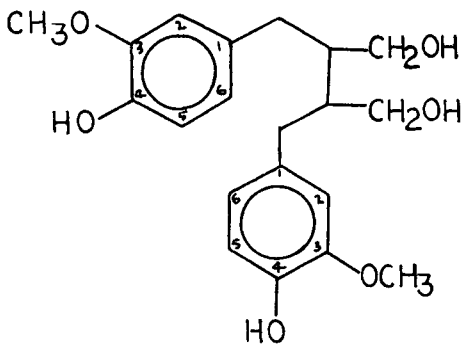



Figure 5. IR spectrum of pinocembrin (Ia).

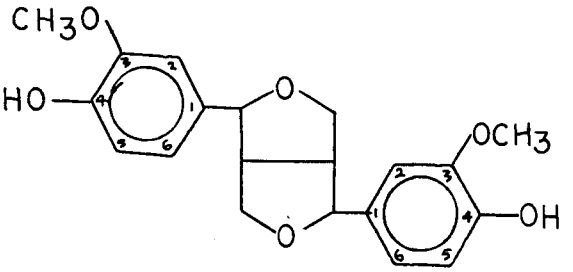
$-OCH_3$ groups. The splitting of this peak indicated that the two $-OCH_3$ were not within the same environment. Multiplets around 3.15 τ are from the 2,6 and 1,5 protons (literature values, 3.14 and 3.18 τ) respectively. Literature [30] value for the 4-OH is 4.15 τ. This signal can be indistinctly observed in Fig. 6. The absence of a singlet between 5.95 and 6.50 indicates no aliphatic -OH as in lariciresinol (XL) and secolariciresinol (XLI). It is suggested that XIV is related to pinoresinol (XLII).




XL




XLI




XLII

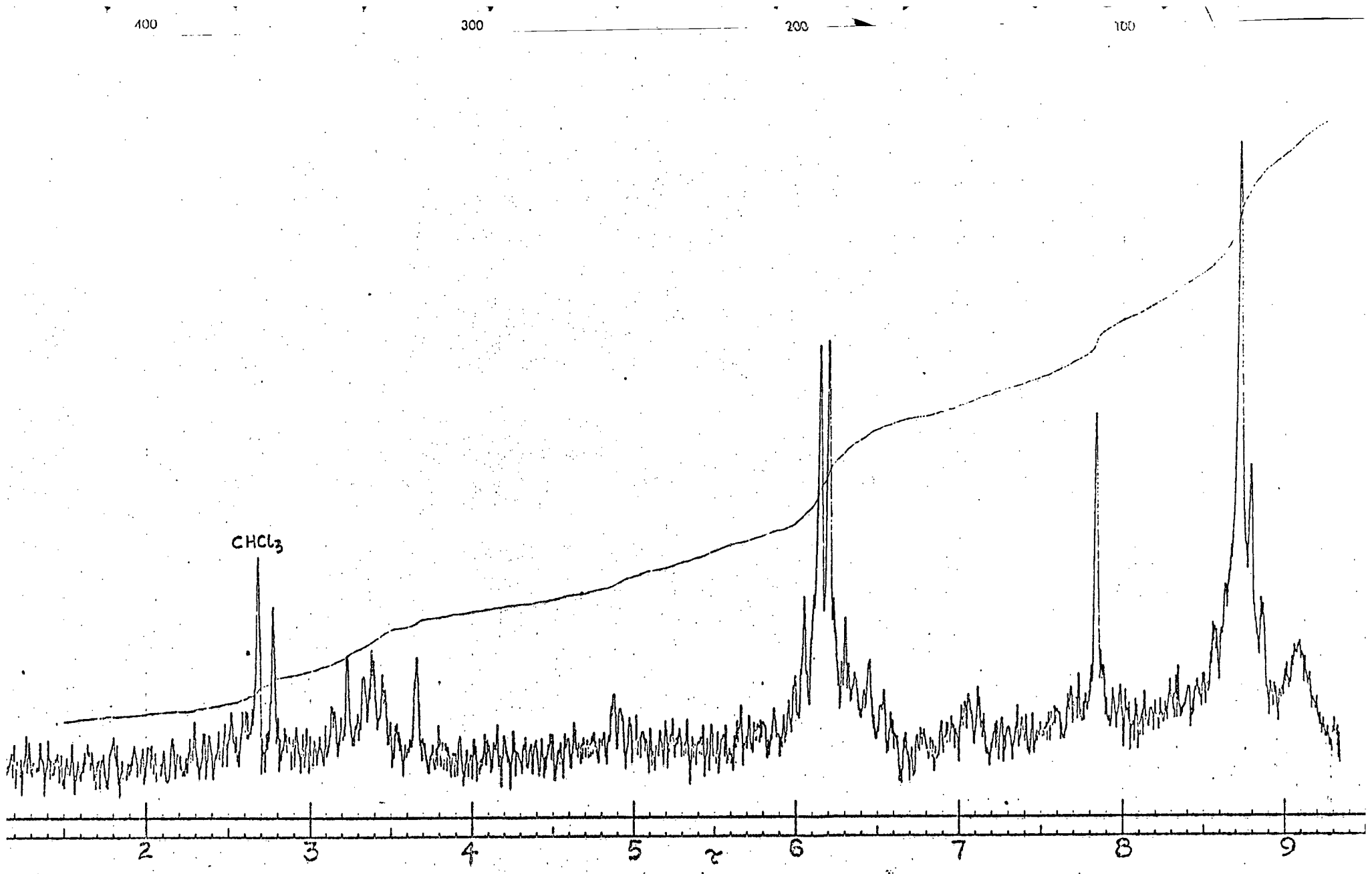



Figure 6. NMR spectrum of unidentified Compound XIV.

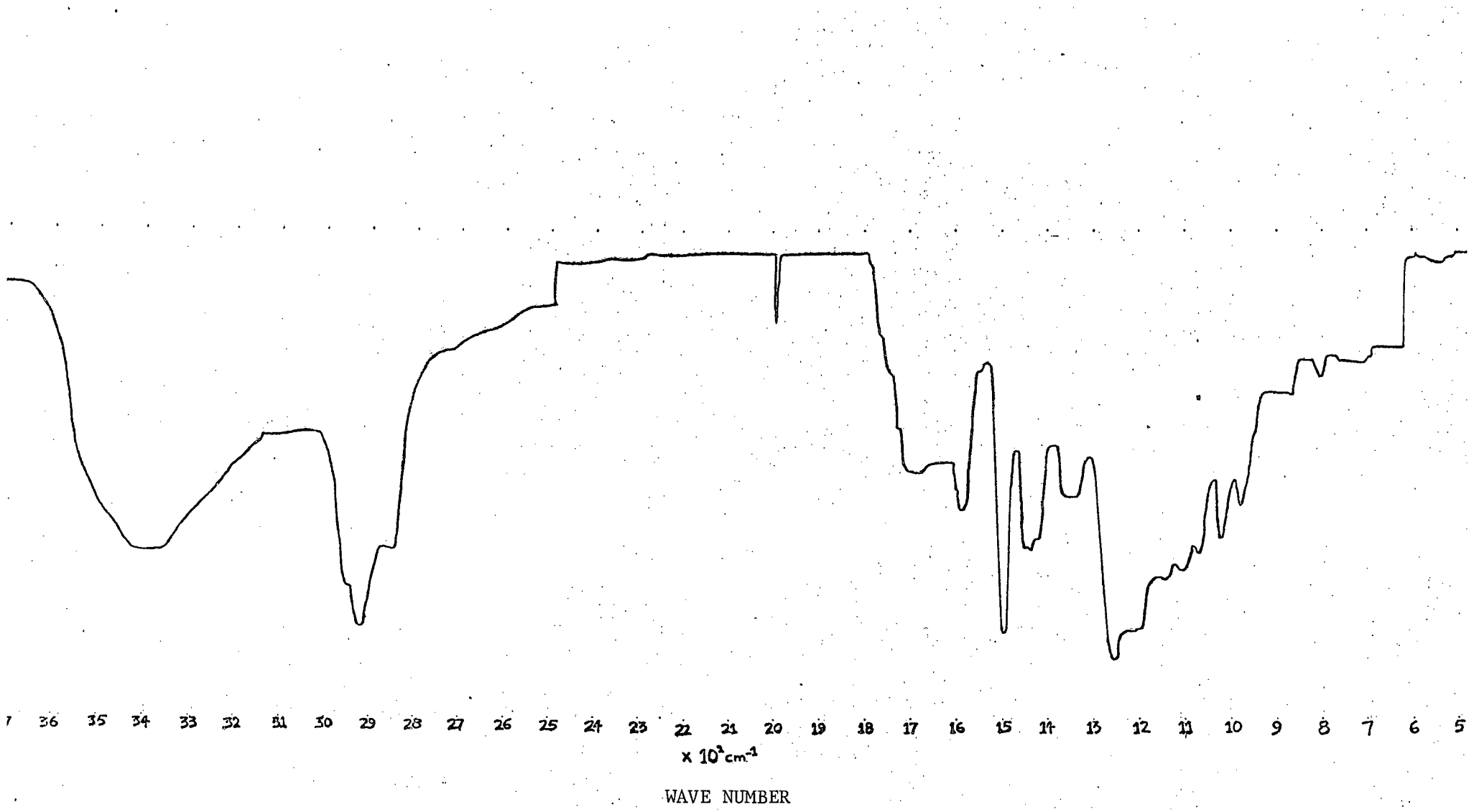



Figure 7. IR spectrum of unidentified Compound XIV.

Water extracts contained free L-arabinose, but no D-galactose. This raises the possibility of the former coming from incomplete hydrolysis of arabinogalactan. On the other hand, extraction conditions were not sufficient to cause hydrolysis. Tests, described above, also rule out the presence of flavonoid glycosides in this extract. The glycosides that have been associated with flavonoids found in this investigation, were glucosides and sophorosides [6,22]. Of course, the sugar could also exist freely in the heartwood.

Another possibility is that L-arabinose is the sugar moiety of an easily hydrolysed flavonoid glycoside in the wood. Dihydroquercetin-3'-glucoside is the only glycoside that has been reported in the sapwood of western larch [6].

The NMR and IR spectra of L-arabinose are shown in Fig. 8 and 9, respectively. The strong band at 3300 $cm^{-1}$ is typical of sugars and is due to the oxygen-hydrogen stretching frequency of hydrogen bonded hydroxy groups [26]. The IR spectrum agreed with the description of the L-arabinose IR spectrum by Urbanski *et al.* [42].

Final ethanol extract possibly contained Braun's native lignin (BNL) among the compounds reported. This is brought about by the water extraction having broken down the cell membrane to enable BNL to be extracted on subsequent ethanol extraction.

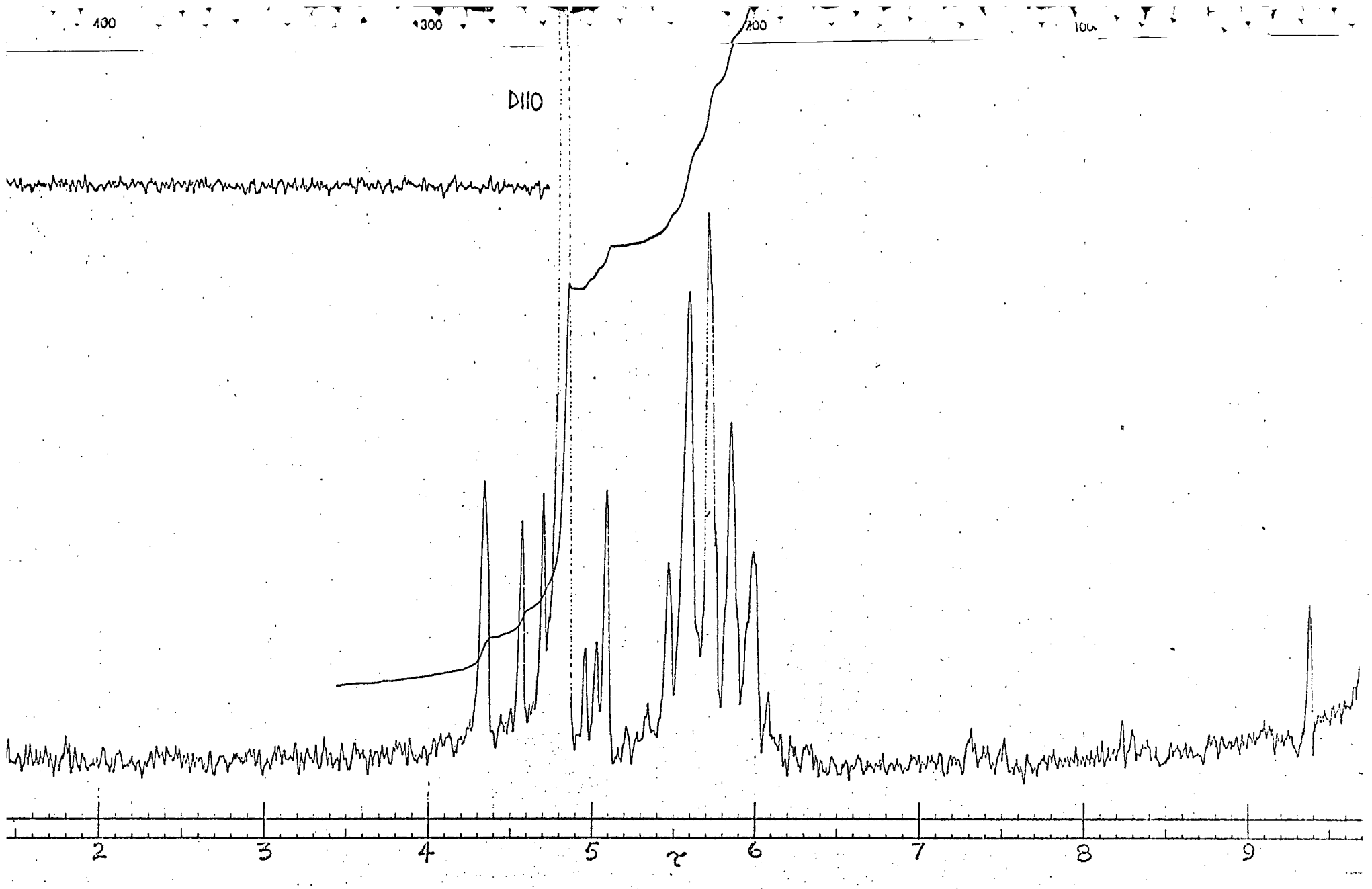



Figure 8. NMR spectrum of L-arabinose (XX).

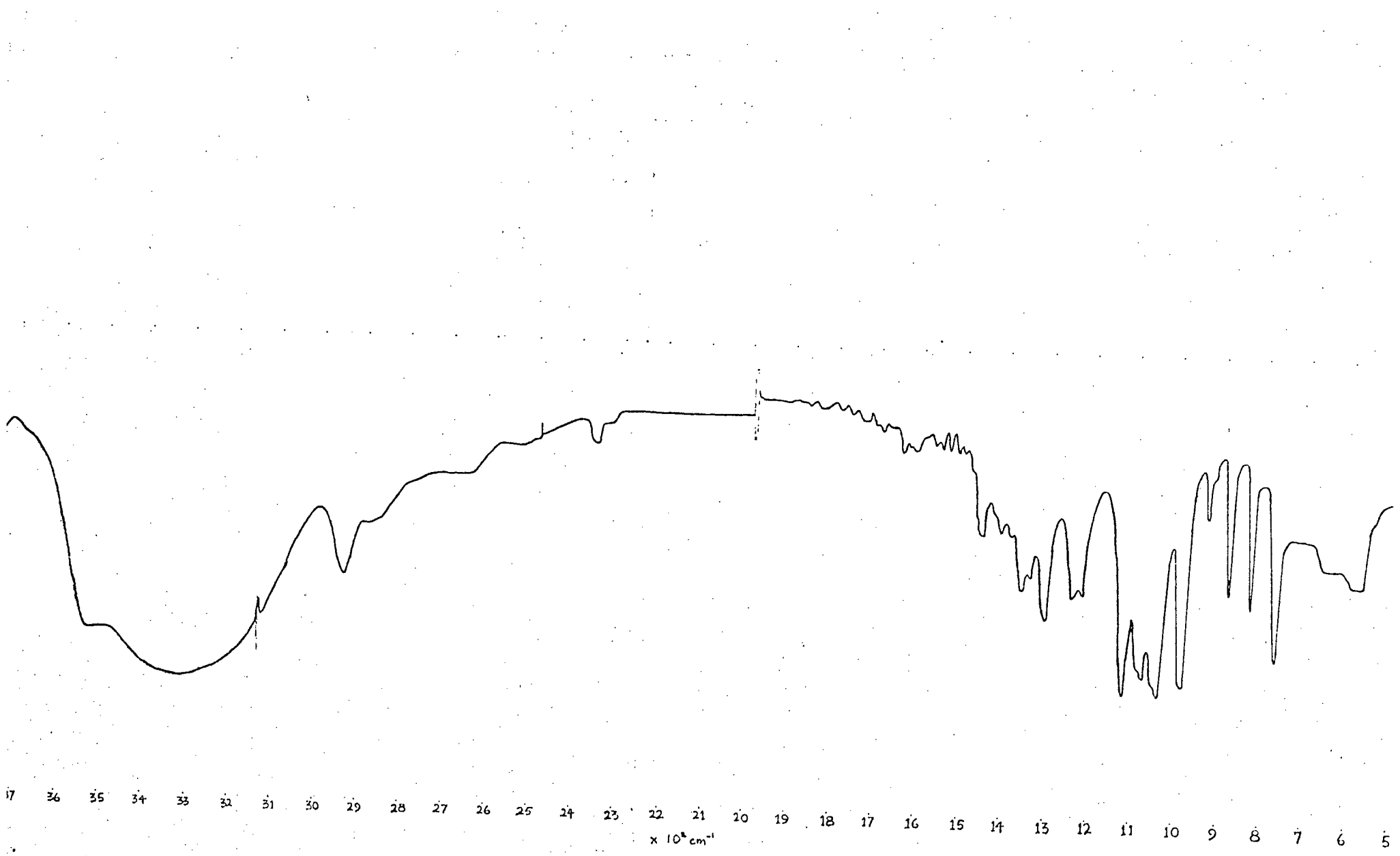



Figure 9. IR spectrum of L-arabinose (XX)

# 6.0 CONCLUSIONS

The total amount of extract obtained from the heartwood of western larch (*Larix occidentalis* Nutt) by successive extraction with five solvents of increasing polarity and finally with ethanol was 14.94% (OD wood sample basis).

Petroleum ether (65° - 80°) extracted 0.64%. The compounds found on analysis of this extract were β-sitosterol, β-sitosteryl palmitate, tristearin and esters (fats) of two acids, probably linolenic and arachidic acids. Free fatty acids found were palmitic, palmitoleic, linoleic and two acids, probably linolenic and arachidic. Free resin acids found were sandaracopimaric, isopimaric, abietic and dehydroabietic acids.

The benzene extract was 0.15% of the wood. From this extract, pinocembrin, dihydrokampferol, α conidendrin and two unidentified alcohols were obtained.

The benzene-ethanol (1:1) extract was 1.29% (OD wood sample basis) and this yielded dihydrokampferol, dihydroquercetin and quercetin.

Ethanol extracted 0.56% of the wood. This extract consisted mainly of dihydroquercetin and a glassy resinous material. Traces of dihydrokampferol and quercetin were also found. Two unidentified compounds, a flavonoid (also obtained in the benzene extract) and a lignan (from its colour reaction with DSA) also occurred in this extract.

The water extract accounted for 11.90% the wood and consisted mainly of crude arabinogalactan. It also contained L-arabinose and

unidentified lignans.

The final ethanol extract was 0.39% of the wood. It consisted mainly of guaiacyl type compounds such as lignans and possibly Braun's native lignin.

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