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The Infrared Absorption Spectrum of Native Spruce Lignin
and Related Compounds

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THE INFRARED ABSORPTION SPECTRUM OF NATIVE SPRUCE LIGNIN
AND RELATED COMPOUNDS

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INTRODUCTION

The development of a new tool that would be capable of speeding up the elucidation of the composition of lignin, the amorphous binding component of woody tissues, should be of particular value in the present state of active but inconclusive research on the subject. It was with this viewpoint that the present investigation of the infrared spectrum of lignin was undertaken.

The basic theory of infrared spectroscopy is discussed in another section. At this point, it is sufficient to say that the infrared spectrum of an organic compound may be considered its most informative physical property; the infrared spectrum offers a characteristic "fingerprint," revealing the presence of functional groups, and affording a means of easily following many chemical reactions of a compound. This property is particularly valuable with a material such as lignin, where the usual physical measurements, such as melting point, crystalline structure, etc., cannot be applied.

In spite of the extensive and, in many cases, brilliant chemical work on lignin, very little is known with certainty about its structure. The source and mechanism of its formation in wood is unknown. Although it yields aromatic decomposition products, some workers claim that the material in the wood is nonaromatic. It is not known whether the material, if a homogeneous compound, is a polymer of simple repeating units or a complex high molecular weight compound of varying build-

ing stones. There is much debate as to the nature and proportion of such functional groups as hydroxyl and carbonyl. It is believed by many that the material is chemically changed by any method of isolation.

With this background, it could not be hoped that a study of the infrared spectra of lignin preparations, as informative as it might be, would positively clarify these uncertainties. It could be hoped, however, that such a study would offer clues to the answers of a number of these questions that could be followed by intensive correlated chemical and spectrographic studies.

As the primary material for this study, Brauns' native spruce lignin (1) was selected. This preparation represents the closest approach that has been made to the isolation of lignin without chemical change, being extracted by cold neutral solvents (ethyl alcohol or dioxane) from the wood. It has been freed of foreign matter by carefully standardized methods, and has been extensively characterized chemically. Its chief weakness is the fact that it represents less than ten per cent of the total lignin in the wood. Whether the insolubility of the remainder is due to a higher molecular weight or to a chemical linkage with insoluble components of the wood is not known.

The present work covers the following points:

1. Development of techniques for obtaining the spectra of soluble and insoluble lignin preparations
2. Interpretation of the spectra obtained on the basis of established absorption band-functional group relationships
3. Extension of such interpretation through spectral changes

in lignin derivatives

4. Comparison of the spectrum of native spruce lignin with the spectra of lignin preparations isolated by other methods

5. Determination of the spectra of a large number of compounds of known structure that may be considered related to or model compounds for lignin

6. Determination of the spectra of a group of natural amorphous materials for comparison with the lignin spectrum

As a background for this investigation, it was considered desirable to evaluate the prior spectrographic work in the field. No previous published data on the infrared spectra of lignin preparations has been found. An extensive amount of work on the ultraviolet absorption spectrum exists. An analysis of this work is given in the Appendix. No organized attempt to interpret the visible spectrum of lignin can be found, although the wide variations in color of various preparations and fractions indicates significant chromophoric variations.

BACKGROUND OF INFRARED CHEMICAL SPECTROSCOPY

The fundamental principles of infrared spectrochemistry are thoroughly treated in a number of texts and reviews (2-15). Rather than attempt to digest these works, it appears desirable to present a general picture of the significance of the infrared spectrum and to follow this with a detailed study of its application to chemical structure.

The first point to be brought out is that there is nothing unique about infrared radiation. The electromagnetic spectrum, which at one end embraces the high frequency-high energy cosmic rays, moves progressively through gamma rays, x-rays, ultraviolet and visible light, and infrared radiation to the relatively low frequency-low energy radio waves. Those sections of the spectrum that have been identified by names are purely arbitrary divisions arising from distinctions in the methods of generation and detection rather than from any basic physical discontinuity of the radiation itself.

The frequency and energy content of the primary radiation emitted from an excited gaseous source is a function of the nature of the emitting material. This fact forms the basis of the field of emission spectroscopy which has found important applications in metallurgy, astronomy, inorganic analysis, etc.

In the field of organic chemistry, however, it is obvious that an organic compound cannot be heated to incandescence or made to give off a significant amount of radiant energy without being destroyed in

the process. It is necessary, therefore, to make use of a basic physical law which states that a physical structure that will emit radiant energy of a given frequency will also absorb energy of that frequency. Chemical spectroscopy might then be defined as the analysis of the relation between measured absorption of radiant energy and atomic or molecular structure. The mechanism of absorption involves the transfer of energy from the radiating source, through resonant vibration of electronic, atomic, or molecular units, to dissipation in the form of heat, re-emission at a different frequency, or chemical change. A given organic compound, if exposed to continuous electromagnetic radiation, might show strong absorption in the ultraviolet region associated with shifts in electronic energy levels in the structure; might appear strongly colored in the narrow band of visible radiation because of resonant absorption within so-called chromophoric groups; and it certainly would show a characteristic absorption pattern in the infrared region as a result of resonant vibration of atomic and molecular groups.

It is this phrase--resonant vibration of atomic and molecular groups--that holds the key to the importance of infrared spectroscopy in organic chemistry. At least in so far as chemical spectroscopy is concerned, an excellent analogy exists between the microstructure of two or more atoms held together in a chemical group and a macrostructure of metal balls elastically joined by springs and a source of friction. If the simplest case of two balls of different mass connected by a spring is considered, it is recognized that an applied vibrational force will set the balls in vibration and that, if the frequency of application of the external force is varied continuously, a frequency will be found

that corresponds to the natural frequency of the mass-spring system. At this frequency the amplitude of vibration of the system will be sharply increased and the applied energy will be utilized in maintaining the amplified motion; in other words, the system is in resonance. The masses involved and the strength of the elastic bond are the determining variables as regards the value of this resonant frequency. If, in both the macro and atomic system, these quantities are precisely known, the resonant frequencies can be mathematically predicted. Such mathematical prediction of absorption spectra is not as yet possible in any but the simplest or most symmetrical molecules, so that it is necessary in going beyond this point to rely upon empirically established fundamental absorption frequencies of specific chemical groups.

The importance of the infrared region has not been newly discovered. The fact that characteristic absorption bands of organic groups fall in this region has been recognized for at least fifty years. The reason for the current surge of activity is the fact that it is only within the past ten years that electronic amplification methods have been developed to the point that extensive use of the method is at all practical. The Perkin-Elmer instrument that is being used in the present work, for example, gives a continuous chart record of the energy received by a vacuum thermocouple with a ten-inch spread between complete absorption and 100 per cent transmission that is equivalent to only one-quarter microvolt or a difference of only a few thousandths of a degree Centigrade in thermocouple temperature.

The application of infrared analysis to problems of chemical structure has also been necessarily slow because of the empirical basis

of the relationship. This has necessitated the accumulation of spectra for a great number of compounds of each class before reliable rules of absorption band-chemical group correlations could be considered established. However, such work was begun about 1930 and has developed so rapidly that it is now possible to assemble correlation tables, such as that of Williams (11), showing the normal absorption frequency of a great number of organic groups. Unfortunately, no one has as yet published an analysis of the work on which such charts and tables are based, showing the sources of the data presented, the number of compounds of each type investigated, the relative certainty of the relationships established, etc. At this point it seems desirable to consider individually the carbon, hydrogen, oxygen groups of interest in the present study in some detail. Only those bands within the portion of the fundamental vibration region accessible to the sodium chloride prism will be considered here.

HYDROXYL

Aliphatic and aromatic hydroxyl compounds have received a great deal of attention because of the importance of the hydroxyl band in relation to hydrogen bonding. In the gaseous state or in dilute solutions, simple alcohols show a sharp fundamental O-H band at about 3650 cm.^{-1} . In concentrated solutions or in the liquid or solid state, the hydroxyl band shifts to lower frequency and is broadened. This phenomenon is perhaps the most direct demonstration of hydrogen bonding. The theory involved is that the sharing of the hydrogen nucleus (proton) between two active oxygens (or other strongly electronegative centers)

has the effect of decreasing the force constant of the hydrogen bond between the proton and the hydroxyl oxygen that exists in a "free hydroxyl." The lower force constant results in resonant vibration at lower frequency.

The work of Hilbert (16), Errera (17-19), Gordy (20), and Fox (21) has been particularly important in this field. It has been shown that, if the hydrogen bond is intermolecular, dilution of the substance in an inactive solvent breaks the bond, and the free hydroxyl band at 3650 cm.^{-1} increases in intensity with dilution (concentration $\times$ absorption path being held constant) (17). If the hydrogen bond is intramolecular, as in chelation, dilution does not break the bond (20). If association with the solvent is involved, heating will break the bond (18).

With the sodium chloride prism, it is not possible to differentiate between aliphatic, phenolic, or primary, secondary, and tertiary hydroxyl bands in this region.

CARBON-HYDROGEN

Possibly more work has been done on carbon-hydrogen stretching and bending frequencies than any other linkage as a result of its importance in hydrocarbon analysis. Aromatic and aliphatic C-H bands are found in the region $3200-2800\text{ cm.}^{-1}$. Pioneer work in this range was done by Bonino (23), who showed that separate bands could be distinguished depending upon the saturation or type of substitution. Such resolution is limited by the sodium chloride prism. Other workers have

shown a similar differentiation in the hydrogen bending region (1650-1350 cm^{-1}) (7).

CARBON-CARBON

Although a saturated aliphatic structure is identified through carbon-hydrogen bands, the aromatic ring linkage introduces two characteristic bands at 1600 cm^{-1} and 1500 cm^{-1} (7), and bands in the less specific region below 1200 cm^{-1} may be traced to the type of aromatic substitution.

The aliphatic double bond has a fundamental stretching band at 1650-1590 cm^{-1} (the frequency being toward the lower limit in the case of conjugation) (7). This is accompanied by a set of bands in the region 1000-825 cm^{-1} that varies with the type of substitution (RCH:CH_2 , RCH:CHR , etc.). These bands have been found particularly useful in following addition polymerization reactions (24).

CARBON-OXYGEN

The carbonyl group introduces a strong fundamental band in the range 1850-1550 cm^{-1} , its exact position being a close function of the type of linkage (acid, ester, aldehyde, etc.) (25). With each type, variations in aliphatic or phenyl substitution will cause some shifting (26-28). Enolization of an aldehyde or keto group causes no change in the carbonyl absorption band and no apparent hydroxyl band is observed (22). This lack of appearance of hydroxyl bands may simply be a case of strong hydrogen bonding in which the hydroxyl band is merged with

C-H bands (29).

"A C-O linkage where the carbon is unsaturated has a band between 1250 and 1200 cm^{-1} , as evidenced by esters and aromatic ethers. If the carbon atom is saturated, this band is at a lower frequency, as in aliphatic ethers, near 1100 cm^{-1} " (7).

In addition to the relatively specific bands discussed above, many bands are likely to appear in a spectrum that are either harmonics of these, or other fundamental bands, or are characteristic of some feature of molecular structure. Such "molecular bands" are particularly likely to appear below 1400 cm^{-1} . A phenomenon of particular interest and importance in the present study is the relative simplicity of the spectra of polymeric substances as compared with simple crystalline or liquid compounds. This is accounted for by Thompson (30) on the following basis:

"[In complex molecules] the changes of molecular dipole moment associated with the different vibrations will vary considerably and in consequence some vibrations are strongly excited in the infrared, others more weakly, and some so feebly as to escape detection. Also, with complex molecules built up from recurring groups, there is often a simplification of the spectrum as a whole, and while the spectra of many macromolecules remain complex and not fully interpretable in terms of vibrational assignment, others are even simpler in general appearance than the spectra of those containing only half a dozen atoms."

EXPERIMENTAL EQUIPMENT AND METHOD

The instrument employed in this work was a Perkin-Elmer Model 12-B infrared spectrometer, with a General Motors breaker type amplifier and a Brown strip chart recorder. The resolving power of the instrument may be expressed by the fact that the 4.2 micron carbon dioxide band can be separated into a doublet and that the fine structure for water vapor and ammonia was almost identical with that obtained with the Oetjen Kuo and Randall spectrometer (31). These authors' curves for the two gases mentioned and for carbon dioxide were used in calibrating the frequency scale from 3800 to 720 cm^{-1} . The frequency of sharp bands could be reproduced with an accuracy of 2 cm^{-1} from 720 to 2000 cm^{-1} and of 20 cm^{-1} from 2000 to 3800 cm^{-1} . Atmospheric frequency standards were used in every run and were checked for constancy of calibration.

The globar source was operated at 200 watts. A constant sensitivity of one microvolt full-scale was used, and actual slit-widths were varied stepwise from about 0.6 mm. at 700 cm^{-1} to 0.035 mm. at 3800 cm^{-1} . A glass shutter was used from 700 to 1600 cm^{-1} to minimize the effects of stray radiation. Recording speed was adjusted to allow adequate response time for the thermocouple.

The instrument was located in a room maintained at 80° F. and below 30 per cent relative humidity. Dishes of sodium hydroxide in the optical housing further reduced water vapor and carbon dioxide in the path. Passage of dry nitrogen through the housing was found to give little additional reduction in atmospheric bands, and its use was discontinued.



The percentage of transmission was calculated as the ratio of the recorded sample to background energy curves. Manual computation was reasonably fast and accurate except for the 2000 to 1200 cm^{-1} water vapor region. To make possible the accurate determination of weak bands in this section, a mechanical computer was devised.

This computer was simply a modification of the I.E.C. mechanical integrator, described by Van den Akker (32). The similar triangle design of the integrator is such that the positioning of followers attached to two parallel rods A and B (Figure 2, above reference) on the y values of any two 10-inch charts mounted on a traveling table produces a displacement of a third parallel rod C equal to $y_A \cdot y_B / 20$ inches. In the modification, a deadweight loaded wire was fixed to rod B, passed to the rear and around a 2X multiplying pulley, and led forward across the traveling table parallel and adjacent to rod A with a following bead positioned over the chart.

To use the computer, the spectrometer recordings of open path, I_0 , and "sample in," I , energies were superimposed. A section of such a chart was placed on the traveling table with followers B and C adjusted to corresponding I_0 and I fiduciary marks, and a plain sheet of paper, ruled with base and fiduciary lines, placed under a pen attached to follower A. As the I_0 and I curves were followed on the moving table, the pen then drew the transmittance curve, $T = I/I_0$. By this method weak bands in the water vapor region could be located with much greater certainty than by manual computation.

In the present study, it was desired to obtain qualitative

absorption spectra for liquids, crystalline solids, and soluble and insoluble amorphous materials. Several different techniques were required to handle this sample range. Volatile liquids were run in salt plate cells sealed with amalgamated lead spacers. Nonvolatile liquids were run as capillary films between salt plates, with the clamping pressure adjusted to give approximately 10 per cent transmission for the strongest absorption bands.

Somewhat special techniques were worked out for preparation of lignin samples. The most satisfactory method for soluble lignin preparations was found to be the formation of evaporated films on salt plates. Dioxane is the most useful solvent for native lignin, but dioxane solutions gave very poor results because of the high interfacial tension between the dioxane solution and the salt. It was found, however, that by filling a shallow cup comprised of a metal ring and salt plate with absolute ethyl alcohol, adding a small proportion of dioxane solution, and allowing evaporation to take place slowly, the lignin was deposited as a strongly adherent clear film. The films thus prepared were nearly transparent to visible light and showed excellent resolution of the infrared bands. This method was employed for all soluble amorphous materials and was satisfactory even in those cases where the material was not completely soluble in dioxane but in which the insoluble portion could be dispersed.

Contrary to expectations, the crystalline solids were found to give extremely satisfactory "films" by evaporation from solution onto salt plates in the manner described above for lignin. The most desirable

solvent combination varied considerably. Some compounds were found to give very satisfactory distribution from ethyl alcohol, a few were obtained in satisfactory form from dioxane, but the dioxane-ethyl alcohol combination developed for lignin was definitely the most desirable in the majority of cases. A high concentration of the starting solution appears definitely desirable. Under ideal conditions, the solution evaporates slowly to leave a uniform, transparent, amorphous film with a diameter slightly smaller than the retaining ring. In a few minutes crystallization starts at a number of separate points and proceeds at a slow rate until the entire surface is crystalline, producing a geometric pattern of interlocking discs. Such "films" are strongly adherent to the salt plates. The plates are removed from the holders, wrapped in lens paper or placed in weighing bottles, and dried at least 16 hours in a vacuum desiccator over sodium hydroxide, sulfuric acid, and paraffin. A sufficient number of runs of these materials in Nujol dispersions have been made to demonstrate that this drying procedure is adequate to remove all traces of solvents.

Blank cells were not used for these runs. It has been found that the introduction of one or two salt plates in the light path causes a general decrease in transmission throughout the range of only two or three per cent, so that the error from this source in these qualitative determinations is not appreciable.

For the insoluble lignin preparations, milled dispersions in Nujol were employed. It was found that high concentration dispersions (10 to 25 per cent) spread as capillary films between salt plates gave the most satisfactory absorption characteristics. A blank film of Nujol

was run in these cases. Although the thicknesses of the sample and blanks thus prepared were only approximately the same, the error introduced was negligible.

All the methods employed gave only qualitative percentage transmission data. Such data are entirely adequate for the exploratory work performed. For more exact comparison of relative absorption, it will be necessary to apply quantitative methods.

All the lignin, carbohydrate, and tannin preparations were prepared by carefully standardized methods and were run as received. Constancy of all infrared absorption bands through recrystallization or redistillation has been taken as the criterion of purity of the crystalline and liquid samples used. Recrystallizations were carried out on a semimicro scale. Approximately 0.1 gram samples or less were dissolved in 10-15 ml. of a suitable solvent by heating, the materials allowed to recrystallize slowly on cooling (chilling to -10 to -15° C. was required in some cases), and finally the crystals were centrifuged to the bottom of the tube and washed four or five times with cold solvent by decantation. Distillations were carried out with an all-glass multiple receiver vacuum still, using pressures of 0.5-1.0 cm. of mercury. An approximate 50 per cent center-cut was taken in each case.

Several of the samples [inactive gallic acid, α -(3,4-dimethoxyphenyl)- β -(3-propyl-5,6-dimethoxyphenyl) propane, tetrahydrodehydrodiferocourenol methyl ether, and pinocresinol dimethyl ether] were too small to permit purification treatments. Each of these, however, was from the sample especially purified by the Erdtmans for their ultraviolet spectrochemical studies.

The extensive list of materials covered in this work was made possible only through the generous co-operation of a number of people. We wish to identify the source and to express our appreciation for the samples listed below.

F. E. Brauns*: Native spruce lignin and methylated products, alkali spruce lignin, Freudenberg spruce lignin, Villstätter spruce lignin, eugenol, eugenol methyl ether, isoeugenol, isoeugenol methyl ether, flavanone, benzilidenacetophenone, quercetin, and conidendrin

L. S. Wise*: Chlorite spruce holocellulose and the spruce hemicelluloses "A" and "B" from above

Holzer Erdtman, Kungl. Tekniska Hogskolan, Stockholm: Vanillyl alcohol, tetrahydrodehydrodieugenol dimethyl ether, inactive guaiaretic acid dimethyl ether, α -(3,4-dimethylphenyl)- β -(3-propyl-5,6-dimethoxyphenyl)propane, dihydrodehydrodieugenol methyl ether, pinoresinol dimethyl ether, and barium lignosulfonates--"GIA," "GIB," and "GII"

R. A. Buchanan*: Native aspen lignin, chestnut tannin, quebracho tannin, redwood tannin, and redwood phlobaphene

W. J. Sublitz*: Chlorite spruce lignin

Conrad Schuerch, McGill University: Periodate spruce lignin

I. S. Pearl* and E. C. Lathrop, Northern Regional Research Laboratory: Russell lignin

* The Institute of Paper Chemistry

Plason spruce lignin was prepared from black sprucewood by the Institute of Paper Chemistry method 13.

Benzene, toluene, ethylbenzene, phenol, and vanillin were repurified from commercial products.

PREPARATION AND DISCUSSION OF DATA

The infrared absorption spectra to be discussed are divided into three groups. The first is the lignin preparations; the second, the liquid and crystalline compounds; and the third, tannin and carbohydrate materials.

NATIVE SPRUCE LIGNIN AND METHYLATION PRODUCTS

The absorption spectrum of native spruce lignin as determined in film form is given in Figure 1, and as a Nujol dispersion in Figure 2. In the spectra for two thicknesses shown in Figure 1, a few additional very weak bands and inflections were revealed in the lower transmission curve, but the over-all agreement seems very good. Although the Nujol dispersion curve is not as clearly defined as that from the films and does not include the aliphatic C-H regions, it serves as an excellent independent determination and permits comparison with insoluble lignin preparations.

The first observation to be made on the comparison of the film and Nujol curves is the presence of three bands in the film spectra at 872, 890, and 1123 cm.^{-1} that do not appear in the curve from the Nujol dispersion. It has been found that these three bands in the film curves gradually diminish with time on heating the films at 100° C. in vacuo, and these bands have been tentatively attributed to retained dioxane in the films. The evidence for and against this interpretation may be summarized as follows:

As mentioned above, these bands do not appear in Nujol disper-

sions of the native lignin. The absorption spectrum of native spruce lignin films from dioxane-ethyl alcohol has been found to agree exactly with spectra from similar films for Methyl Cellosolve spruce lignin, but the bands in question do not appear in films of the later material prepared from acetone (curves not shown). The three strongest bands in the absorption spectrum of dioxane correspond to those frequencies listed. All these facts would appear to be strong evidence for attributing these bands to absorbed dioxane. On the other hand, thorough washing and soaking of the salt plates and films in petroleum ether had no effect on the strength of these bands. Also in apparent conflict were the observations made on native aspen lignin (Figure 19). In this case, bands were present at 872 and 890 cm^{-1} in the dioxane-ethyl alcohol film in about the same proportion as the native spruce lignin, and a very strong band was found at 1123 cm^{-1} . The 872 and 890 bands were again absent in Mujol dispersions and decreased on heating at 100° C. in vacuo but, in this case, the third band, which was absent in Mujol and diminished on heating, was at 1085 cm^{-1} . It is for this reason that the phenomenon is considered still incompletely explained.

Once the absorption spectrum of the native spruce lignin was determined, the first question to be answered was whether the material is chemically homogeneous. This, of course, cannot be answered definitely by any one experiment or technique, but the combination of a fractional precipitation method of separation previously used (unpublished) and the determination of the infrared spectra of the fractions obtained was considered most worthy of application at this point.

One gram of native spruce lignin was dissolved in 50 ml. of

anhydrous dioxane in a centrifuge jar and successive fractions were obtained by slow addition of nonsolvents while the solution was under rapid agitation. The proportions of nonsolvents required to give approximately equal size fractions are summarized in Table I. The precipitation appeared to be almost continuous with change in solvent ratio. Each fractional precipitate was centrifuged and the centrifugate decanted. The precipitate was stirred and centrifuged with 100-ml. batches of mixed solvents of the same proportions that were used in the precipitation and with high-boiling petroleum ether, and finally dried by warming to 40-50° C. The dry material was redissolved in 10 ml. of dioxane (a few drops of methyl alcohol were required to effect solution of Fraction 1) and reprecipitated into high-boiling petroleum ether in a centrifuge jar. The product was washed twice with high-boiling petroleum ether and twice with low-boiling petroleum ether in the centrifuge jar before drying under vacuum over sodium hydroxide, sulfuric acid, and paraffin.

TABLE I

FRACTIONATION OF NATIVE SPRUCE LIGNIN

Fraction	Total Nonsolvent Added to 50 ml. 2% Solution in Dioxane, ml.	Color	Methoxyl Content, %
1	25 benzene	dull tan	12.8
2	50 benzene	dull tan	14.1
3	50 benzene - 10 H.B. petroleum ether	bright orange- yellow	14.2
4	50 benzene - 25 H.B. petroleum ether	light dull yellow	14.3
5	50 benzene - 75 H.B. petroleum ether	dull orange- yellow	14.3
6	50 benzene - 325 H.B. petroleum ether	bright orange- yellow	--

Six fractions were obtained, all of approximately equal size except for 1 and 6, which were very small. Because of the small size of the fractions after infrared analysis, the Institute analytical laboratory was asked to run methoxyl determinations. Even on a semimicro scale, it was necessary to run a single determination on Fraction 1, and Fraction 6 was too small for even a single run. The values obtained are shown in Table I. The colors of the different fractions as recorded in the table are possibly significant, since they were all reprecipitated under identical conditions. The insolubility of Fraction 1 in dioxane after isolation is consistent with previous experience with other soluble lignins.

The infrared spectra of these fractions as determined by the standard film technique (Figures 3, 4, and 5) revealed a very interesting relationship. On a quantitative basis, with the exception of the bands in the carbonyl region, the infrared spectra of the fractions show only slight and nonspecific variation from the spectrum of the original native lignin. However, a definite progressive change in the relative strength of the $1655\text{--}1660\text{ cm.}^{-1}$ band, which will be shown later to be produced by an aldehyde or keto carbonyl group, is quite apparent; this band is very weak in Fraction 6 and relatively strong in Fraction 1. A similar but possibly less pronounced trend is evident for the broad band in the acid or ester carbonyl region. A definite minimum in this region was resolved for all the fractions at $1709\text{--}1718\text{ cm.}^{-1}$. Although there is a strong temptation to estimate quantitatively the variation in proportion of the aldehyde or keto band to the adjacent aromatic nucleus bands, such a calculation would be definitely premature.

The results obtained have several important implications.

The fact that native lignin can be fractionated into products of varying physical properties on the basis of solubility must mean that the material has a considerable range of molecular weights, that it contains chemically different components, or that the variations are the result of a combination of both factors. The fact that the infrared spectra of the fractions are essentially identical, except for the carbonyl bands, definitely shows that the separation has not been effected through major chemical differences. Whether the observed variation in carbonyl content is in itself sufficient to produce the fractionation must remain an open question until a reliable method for measuring molecular weight can be applied to such fractions. If the fractionation is primarily on the basis of molecular weight, which is much more probable, these results would seem to indicate that polymerization of lignin monomers cannot be through the carbonyl group.

The important conclusion with respect to the present investigation of infrared spectra is that the basic pattern of the absorption spectrum of native spruce lignin is preserved through this fractionation. When this is added to the fact that the same characteristic pattern is obtained from total "lignin" preparations isolated by a number of different methods, and the finding that this spruce lignin spectrum is readily distinguishable from the spectra of all other materials run, the evidence is rather strong that the term "lignin" does designate a unique and basically homogeneous structure.

On this basis, it would seem justifiable to advance the following tentative interpretation of the lignin spectrum.

1. The strong bands at 1598 and 1512 cm^{-1} offer prima facie evidence of the predominant aromatic nature of the lignin.

2. The strong saturated aliphatic C-H bands at 2900 and 2830 cm^{-1} and at 1454 and 1425 cm^{-1} establish the presence of a reasonably large proportion of aliphatic groupings, the simple arrangement suggesting that these are relatively uncomplicated and not strongly cross-linked.

3. The broad, strong band at 3350 cm^{-1} shows the presence of moderately strongly bonded hydroxyl groups. This band in itself does not contribute evidence for or against the presence of one or several types of hydroxyl linkages.

Beyond this point, it is desirable simply to point out the implications of a number of other bands in the spectrum, and then present additional primary evidence before stating definite conclusions.

Use of the mechanical computer has positively established the presence of the broad inflection at 1725 cm^{-1} and the moderate band at 1663 cm^{-1} .

The obscure band at 1725 cm^{-1} has attracted considerable interest. Nothing of this type has been encountered in the simple compounds. Absorption bands at this point are rather specifically attributed in the literature to ester or acid carbonyl groups. As will be discussed later, this band is unaffected by several reactions of lignin and is absent in several other lignin products.

The band at 1663 cm^{-1} is in the region attributed to the

aldehyde or ketone carbonyl or the unconjugated aliphatic double bond. Supporting evidence for an aliphatic double bond is lacking in the 1000-900 cm^{-1} region, where only a very weak band at 918 cm^{-1} is found. The band is proportionately much weaker than those of any of the known carbonyl or unsaturated compounds examined.

The strong bands at 1269, 1256, and 1224 cm^{-1} are reliable presumptive evidence of aromatic or unsaturated C-O linkages.

The weak band at 1085 cm^{-1} and the strong band at 1139 cm^{-1} (omitting the questionable 1122 cm^{-1} band) are only possible evidence for saturated C-O-C linkages.

No useful comments on the remaining bands in the lignin spectrum can be offered at present.

An extremely valuable source of substantiating and extending evidence was obtained from the spectra of six methylation products of native spruce lignin. These will be considered individually:

1. Native spruce lignin methylated with diazomethane in ether (Figure 7). When compared with native spruce lignin (Figure 1), the curve shows a slight but definite decrease in the strength of the hydroxyl band and a new weak band at 1190 cm^{-1} (aromatic C-O region). The higher frequency aromatic nucleus band appears as a doublet at 1598-1590 cm^{-1} . In other respects the spectra are essentially identical.

2. Native spruce lignin methylated with diazomethane in dioxane (Figure 8). When compared with native spruce lignin, there is

observed an appreciable decrease in strength of the hydroxyl band, slightly deeper aliphatic C-H bands at 1418 cm.^{-1} (apparently shifted from 1427) and a distinct 1460 cm.^{-1} band, and an inflection in the higher frequency aromatic nucleus band at 1600 cm.^{-1} .

3. Native spruce lignin methylated with methyl alcohol-hydrochloric acid (Figure 6). When compared with native spruce lignin, the curve shows a definite decrease in the strength of the hydroxyl band at 3350 cm.^{-1} , complete disappearance of the carbonyl or double bond band at 1663 cm.^{-1} , no significant change in the aliphatic C-H bands, and much deeper and broader absorption going into the 1257 cm.^{-1} minimum. The 1139 cm.^{-1} minimum and 1046 inflection have disappeared. No doubling of the 1595 cm.^{-1} aromatic nucleus band is shown, as was found with the diazomethane-methylated samples. No other significant changes are apparent.

4. Methanol native spruce lignin methylated with diazomethane (Figure 10). As compared with native spruce lignin, the hydroxyl band is found to be very weak; a new aliphatic C-H band has appeared at 1464 cm.^{-1} , with the 1427 cm.^{-1} band apparently shifted to 1418 cm.^{-1} . The 1663 cm.^{-1} band is completely absent. The higher frequency aromatic nucleus band appears at 1588 cm.^{-1} with an inflection at 1598 . The 1372 cm.^{-1} band is absent but a new weak minimum is found at 1095 and an inflection at 1198 cm.^{-1} .

5. Methanol lignin from diazomethane-methylated native spruce lignin (Figure 9). As compared with native spruce lignin, very nearly the same changes that were observed with the above preparation are found,

with the exception that a very weak carbonyl band is still evident at 1670 cm.^{-1} .

6. Native spruce lignin methylated with diazomethane and dimethyl sulfate (Figure 11). As compared with native spruce lignin, a trace of hydroxyl band is found at 3420 cm.^{-1} , a weak but definite carbonyl band at 1666 cm.^{-1} is present, and there is an inflection in the higher frequency aromatic nucleus band at 1600 cm.^{-1} . In the aliphatic C-H region, the 1512 cm.^{-1} band is replaced by a $1514\text{-}1506\text{ cm.}^{-1}$ doublet, and the new 1464 cm.^{-1} band is quite strong. The 1425 band has again shifted to 1418 cm.^{-1} . In the aromatic C-O region, the 1269 and 1256 cm.^{-1} bands are replaced by a band at 1261 cm.^{-1} , and the 1231 cm.^{-1} band is possibly relatively stronger. Stronger general absorption is observed in the $1120\text{-}1200$ region and around 1140 cm.^{-1} . The 1367 and 1085 cm.^{-1} bands have disappeared, with a new band appearing at 1100 cm.^{-1} .

It should be mentioned that the broad absorption plateau in the $1725\text{-}1700\text{ cm.}^{-1}$ ester or acid carbonyl region persists throughout these spectra. In the cases where the 1660 cm.^{-1} band is weakened or eliminated, a clearly defined minimum is evident at $1715\text{-}1710\text{ cm.}^{-1}$.

The spectral changes observed occur on a background sufficiently constant to demonstrate that no general change in the material being examined has taken place.

As a supplement to the evidence previously summarized concerning the significance of the lignin spectrum, the following statements

can be added:

1. The elimination of the 1663 cm.^{-1} band by methylation with methyl alcohol-hydrochloric acid definitely establishes this band as that of an aldehyde or ketone carbonyl group.

The fact that this band is not visibly changed by diazomethane methylation is in accord with the lack of differentiation in simple compounds between normal and enolic carbonyl bands if, as concluded by Brauns (1), an enolic hydroxyl has been methylated in this reaction. The appearance of inflections in the high frequency aromatic nucleus band in a number of the methylated lignin curves is undoubtedly significant in this connection but is difficult to interpret at present. The weak carbonyl band evident in the methanol lignin from diazomethane-methylated native spruce lignin probably indicates incomplete methyl alcohol methylation.

2. In the aliphatic C-H region, the consistent gain in strength of the $1464\text{-}1460\text{ cm.}^{-1}$ band on methylation definitely establishes that this band is at least partially the result of the presence of the methoxyl CH_3 group.

3. Changes in the bands in the $1260\text{-}1190\text{ cm.}^{-1}$ aromatic or unsaturated C-O region are not clear cut. There is, however, a sufficiently consistent increase in strength of absorption throughout this region to identify these bands as resulting, at least partially, from aromatic methoxyl groups.

4. There is a definite indication of stronger absorption in

the 1125-1075 cm^{-1} aliphatic C-C-C region in the methyl sulfate-methylated lignin and some possible changes in the two diazomethane-methyl alcohol methylated lignins. Since this region is confused by the doubtful 1120 cm^{-1} band, it seemed desirable to study these materials in Nujol through this region before attempting to interpret these changes. This was done for the selected group shown in Figures 12 and 13.

Native lignin, native lignin methylated with methyl alcohol-hydrochloric acid, native lignin methylated with diazomethane in dioxane, methanol native lignin methylated with diazomethane, and native lignin methylated with diazomethane and methyl sulfate were run. The observed changes in absorption were entirely consistent, but unfortunately were not sufficiently pronounced to be acceptable as positive identification. In the case of methylation with methyl alcohol-hydrochloric acid, the only significant change is the appearance of an inflection at 1025 cm^{-1} in the saturated C-C-C region. In the case of methylation with diazomethane, the only significant change is the appearance of a weak inflection at approximately 1150 cm^{-1} (aromatic C-C). The same inflections appear in methanol native lignin methylated with diazomethane or with diazomethane and methyl sulfate. With methyl sulfate methylation, there seems to be, in addition, a marked relative gain in intensity of the 1225 and 1095 cm^{-1} bands, centered respectively in the aromatic C-O and aliphatic C-C-C regions. To confirm these observations, it will probably be necessary to find a solvent that is sufficiently transparent in this region to permit quantitative measurements.

Summary

On the basis of very strong empirical evidence, the two strong bands in the lignin spectrum at 1598 and 1512 cm.^{-1} are accepted as establishing the basic aromatic nature of native spruce lignin and the strong bands at 2900 and 2830 cm.^{-1} , and at 1454 and 1425 cm.^{-1} are accepted as representing a fairly large proportion of aliphatic C-H units. The 3350 cm.^{-1} band is accepted as that of moderately strongly bound hydroxyl groups.

With the aid of the spectra of six different methylation products of native lignin, the 1663 cm.^{-1} band has been identified as that of an aldehyde or ketone carbonyl group of relatively low concentration. These spectra also have definitely proven the hydroxyl source of the 3350 cm.^{-1} band, without yielding any evidence of different types of hydroxyl groups.

Although there are definite indications of spectral changes on methylation which suggest changes in the aromatic and aliphatic carbon-oxygen linkages, the relationships are not conclusive.

SPECTRA OF OTHER TYPES OF LIGNIN

In this section an application of the infrared absorption spectrum is reported that is nearly as useful as the identification of chemical groupings--e.g., its use as a "fingerprint" in determining the identity or differences in materials of unknown composition. The multiplicity of distinct bands in the infrared spectrum renders it an unrivaled physical tool for this purpose. The bands in the spectral

region where absorption is likely to be more a function of molecular structure than of specific groups acquires great importance in this connection.

Since most of the materials discussed below were of necessity run in Nujol, the comparisons in these cases are made to native spruce lignin in Nujol, Figure 2. This, of course, excludes the aliphatic C-H region.

Klason Spruce Lignin (Figure 14)

A one-gram sample of alcohol-benzene extracted black spruce-wood meal (approximately two years old) was treated with 72 per cent sulfuric acid according to The Institute of Paper Chemistry Method 13, and the thoroughly washed product was dried in vacuo over phosphorus pentoxide. The methoxyl content of the lignin was 15.2 per cent.

The infrared spectrum of this material as obtained in Nujol agrees very closely with that of native spruce lignin through the 1600-800 cm.^{-1} range. However, the Klason lignin shows no carbonyl band and broad, relatively weak absorption in the hydroxyl region, with no clear-cut minimum.

Willstätter Spruce Lignin (Figure 15)

Other than a displacement of the hydroxyl band to 3270 cm.^{-1} and possibly weaker hydroxyl absorption, and the presence of inflections at 1595 and 924 cm.^{-1} , the spectrum of this material is essentially

identical with that of native spruce lignin.

Alkali Spruce Lignin (Figure 16)

The hydroxyl band of this material at 3350 cm.^{-1} appears to be relatively weaker than in native spruce lignin and the aldehyde or ketone carbonyl band is completely missing. Aside from a new inflection at 1127 cm.^{-1} , the remainder of the spectrum is the same as that of native spruce lignin.

Freudenberg Spruce Lignin (Figure 17)

The hydroxyl band in this material is at a lower frequency (3290 cm.^{-1}) and is relatively weaker than in native spruce lignin. A new band is found at 3100 and an inflection at 1597 cm.^{-1} . The remainder of the spectrum is the same as that of native spruce lignin.

Periodate Spruce Lignin (Figure 18)

The absorption patterns are essentially identical with the spectrum of native spruce lignin in Nujol, except for an appreciably higher proportion of carbonyl bands in the periodate lignin. This was confirmed by comparison with various film curves for native lignin. There is no evidence of oxidation such as that found in the strong acid carbonyl band in chlorite spruce lignin (Figure 24).

Native Aspen Lignin (Figure 19)

This material was studied as dioxane-ethyl alcohol films and

in Nujol. The relationship of the absorption curves obtained was discussed on page 20. In order to obtain a comparison of the aliphatic C-H bands, the curve for the film of native aspen lignin is contrasted with that for native spruce lignin film (Figure 1). There is a far greater difference between the spectrum of this native hardwood lignin and native spruce lignin than there is within the group of four spruce lignins isolated by different methods.

The hydroxyl band of the aspen lignin is at the same position and of about the same relative strength as in native spruce lignin. No significant difference in the aliphatic C-H bands is evident. The aspen lignin, however, shows a relatively strong aldehyde or ketone carbonyl band at 1701 cm.^{-1} with no evidence of the 1725 cm.^{-1} inflection. New bands are present at 1231 , 1162 , and 1100 cm.^{-1} , and the lower frequency aromatic nucleus band appears as a doublet at 1514 - 1506 cm.^{-1} . The 1372 and 1139 cm.^{-1} bands of native spruce lignin are missing. The bands at 1328 and 1123 cm.^{-1} are relatively much stronger in the hardwood lignin. The discussion with regard to the latter band will be recalled (page 20).

Russell Synthetic "Lignin" (Figure 20)

Samples of this material were obtained from Pearl (prepared at The Institute of Paper Chemistry) and from the Northern Regional Research Laboratory of the Department of Agriculture. The spectra of these two samples as run in Nujol were essentially the same. The latter sample was also run as a film from dioxane-ethyl alcohol. Although

the material was only partially soluble in dioxane, a sufficiently dispersed suspension was obtained to produce a satisfactory film. When compared with the spectrum of the native spruce lignin film, a number of marked differences are revealed. The outstanding point of difference is the very strong carbonyl band at 1680 cm.^{-1} in the Russell "lignin" spectrum (comparable with vanillin). The hydroxyl bands are about the same, as are the C-H bands at 2910 and 2830 cm.^{-1} . In the lower frequency aliphatic C-H region, however, the synthetic product shows very strong bands at 1492 and 1465 cm.^{-1} , as compared with 1454 and 1425 cm.^{-1} in native lignin. A strong new band is found at 1295 cm.^{-1} in the synthetic material, whereas the strong 1033 cm.^{-1} band in the native lignin is very weak in the spectrum being considered. The remaining bands are quite similar.

A group of three barium lignosulfonate salts of varying sulfur content was obtained from Erdman. Because of their insolubility in organic solvents, all were run in Nujol and are compared with native spruce lignin in Nujol.

Barium Lignosulfonate "GIA" (Figure 21)

Analysis: S, 4.19 per cent; Ba, 9.22 per cent; CH_3O , 13.01 per cent; ratio of CH_3O to S, 3.2. The spectrum of this material is remarkably similar to that of native spruce lignin. The carbonyl band, if present, is very weak at 1655 cm.^{-1} , with no evidence of the 1725 cm.^{-1} inflection. Weak new bands are found at 2700 , 2650 , and 2600 cm.^{-1} . The broad band at 1150 cm.^{-1} might be partially due to the

sulfonate group. All other bands are essentially identical. (The great mass of the barium atom would definitely prohibit its absorption in these regions.)

Barium Lignosulfonate "GIE" (Figure 22)

Analysis: S, 4.75 per cent; Ba, 9.91 per cent; CH_3O , 12.52 per cent; ratio of CH_3O to S, 2.7. The spectrum of this material is essentially the same as that of "GIA." The carbonyl band, if real, is even weaker at $1662\text{--}1645\text{ cm.}^{-1}$, the 1095 cm.^{-1} band is absent, and the 1036 cm.^{-1} band appears broader and stronger.

Barium Lignosulfonate "GIB" (Figure 23)

Analysis: S, 2.24 per cent; Ba, 19.8 per cent; CH_3O , 9.75 per cent; ratio of CH_3O to S, 1.2. Compared with native spruce lignin, the hydroxyl band of this material appears possibly stronger, the carbonyl band is completely absent, and all the bands in the $1300\text{--}1000\text{ cm.}^{-1}$ region appear to be shifted and stronger: 1261 for 1269, 1210 for 1224, 1162 for 1144, 1039 for 1031 cm.^{-1} . The 1085 cm.^{-1} band of the native lignin is not present.

Compared with "GID," the carbonyl band is definitely absent, the shifts noted above apply, and the 1062 and possibly 1039 cm.^{-1} bands are stronger. The inflection at 973 cm.^{-1} is not present.

Chlorite Spruce Lignin (Figure 24)

This material represents the purified "lignin" fraction obtained by acidification and concentration of chlorite liquors. It

contained 11.2 per cent CH_3O and 6.4 per cent Cl .

The material was run as a film from dioxane-ethyl alcohol. Compared with native spruce lignin, the 3350 cm.^{-1} hydroxyl band is definitely weaker, whereas the aliphatic C-H bands are relatively the same. A strong acid or ester carbonyl band is evident at 1727 cm.^{-1} but the 1660 cm.^{-1} carbonyl band is not present. The 1257 cm.^{-1} band appears stronger in the chlorite material. New bands are present at 1209 and 1175 cm.^{-1} , the 1033 cm.^{-1} band is relatively weak, and the 1139 cm.^{-1} band of native lignin is absent. A number of the bands are comparable and the over-all character of the curves suggests that the material is a lignin derivative.

Summary

The comparison of the spectrum of native spruce lignin with those of Flason, Willstätter, alkali, periodate, and Freudenberg lignins from spruce revealed a striking over-all similarity, as well as interesting individual variations. This similarity contributes indirect evidence of uniformity among the lignin preparations and homogeneity of the native lignin. The apparent general decrease in relative strength of hydroxyl absorption among the lignins isolated by strong chemical treatments and the disappearance of carbonyl bands in the Flason and alkali lignins are of particular interest.

The synthetic material known as Russell "lignin" has been shown to have absorption characteristics quite different from those of the natural spruce lignins.

The absorption spectrum of native aspen lignin was found to be considerably more complex than that of native spruce lignin.

The series of barium linoxulfonates of increasing sulfur content showed a general over-all spectral similarity to that of native spruce lignin, and strong evidence to indicate that the carbonyl group is reacted as sulfonation takes place.

A soluble "lignin" fraction isolated from chlorite liquors showed a general absorption pattern similar to that of native lignin, but revealed a strong acid or ester carbonyl band, the disappearance of the aldehyde or keto carbonyl band, and a decrease in the strength of the hydroxyl band.

SPECTRA OF COMPOUNDS OF KNOWN STRUCTURE

The study of the spectra of compounds of known structure so far obtained has been very profitable. No special attempt has been made to determine how many of the spectra of the compounds studied have already been published. It seems definitely established that, to obtain comparisons of real value, it is necessary to obtain the curves on one instrument under similar conditions.

Each curve obtained has been carefully studied and compared with those for related compounds. It would be unprofitable to list and discuss all the bands, but it seems definitely worthwhile to call attention to the major points of interest in each spectrum. An attempt will be made to give equal weight to absorption characteristics that appear to be exceptions to general rules as to those that conform. The

chart published by Williams (11) showing the absorption frequency range of different chemical groups is used as a basic reference in this discussion.

Benzene (Figure 25)

The absorption spectrum of benzene is fairly complex, with many bands falling in regions associated with groupings not present in this compound. Possibly the only specific bands are those at 3030 and 2980 cm.^{-1} , presumably hydrogen stretching bands of aromatic C-H groups.

Toluene (Figure 26)

Substitution in the symmetrical benzene molecule leads to the appearance of the strong 1600 and 1500 cm.^{-1} aromatic nucleus bands that are found with only slight shifting in all aromatic compounds thus far studied, and also introduces strong aliphatic C-H bands at 2900 and 2830 cm.^{-1} , and at 1459 and 1378 cm.^{-1} .

Ethylbenzene (Figure 27)

The introduction of a CH_2 group leads to three strong aliphatic C-H bands at 2900, at 2880 and 2830 cm.^{-1} , and at 1459 and 1378 cm.^{-1} .

Phenol (Figure 28)

A strongly bonded hydroxyl band appears at 3270 cm.^{-1} , together

with a strong band at 1228 cm.^{-1} in the aromatic C-O region.

Vanillyl Alcohol (Figure 29)

This compound displays a curious type of absorption in the hydroxyl region, not observed in any other material. There is a relatively sharp strong band at 3390 cm.^{-1} and a second broad band at 3110 cm.^{-1} . Three aliphatic C-H bands appear at 2910, 2850, and 2820 cm.^{-1} , with the last very weak, accompanied by two strong bands at 1434 and 1382 cm.^{-1} . Absorption in the aromatic C-O region is more complex than with phenol. In addition to a strong band at 1228 cm.^{-1} corresponding to the one at 1228 cm.^{-1} in phenol, a new band appears at 1260 cm.^{-1} . This possible differentiation between aromatic C-OH and C-OR has not been encountered in the literature.

Vanillin (Figure 30)

A single strong hydroxyl band appears in the vanillin spectrum at 3120 cm.^{-1} , indicating strong hydrogen bonding. A strong, sharp band at 1662 cm.^{-1} falls in the aldehyde or ketone carbonyl region. Aliphatic C-H bands at 2930 and 2820 cm.^{-1} are relatively weak; stronger bands appear at 1430 and 1372 cm.^{-1} . Two strong bands at 1198 and 1264 cm.^{-1} possibly represent the two aromatic C-O linkages.

Eugenol (Figure 31)

The hydroxyl band in this spectrum is at a relatively high frequency, 3430 cm.^{-1} , and shows an inflection at 3380 cm.^{-1} . The

aliphatic C-H bands are strong and complex, with an arrangement peculiar to the eugenol-type compounds. Bands are found at 3000 and 2940 cm^{-1} , corresponding to $=\text{CH}_2$. Saturated C-H bands are found at 2860 and 2800 cm^{-1} and at 1461, 1452, and 1430 cm^{-1} . A sharp band at 1633 cm^{-1} falls in the C=C region, and is accompanied by strong bands at 995 and 915 cm^{-1} , a combination given by Williams (11) for $\text{RCH}=\text{CH}_2$ compounds. Three bands appear in the aromatic C-O region, at 1207, 1234, and 1262 cm^{-1} .

Eugenol Methyl Ether (Figure 32)

The spectrum is very similar to that of eugenol, with the hydroxyl band absent. The aliphatic C-H pattern is about the same, with the 1463 and 1455 cm^{-1} bands stronger in the ether. The 1207 cm^{-1} aromatic C-O band is absent in the ether spectrum.

Isoeugenol (Figure 33)

An important comparison in the C=C region is obtained between eugenol and isoeugenol. In isoeugenol, the double bond band is apparently shifted to 1602 cm^{-1} , where it appears as an inflection on the 1594 cm^{-1} aromatic nucleus band. This is in accord with observations in the literature concerning the effect of conjugation on such bands. The two 995 and 915 cm^{-1} bands of eugenol are replaced by a single strong band at 963 cm^{-1} , a position given by Williams for $\text{RCH}=\text{CHR}$ compounds. The aliphatic C-H bands are somewhat simpler in isoeugenol. Bands at 2950, 2890, and 2810 cm^{-1} are accompanied by three medium strength bands at 1451, 1442, and 1365 cm^{-1} and a weak band at

1393 cm^{-1} . The structure in the aromatic C-C region is identical with that of eugenol.

Isoeugenol Methyl Ether (Figure 34)

A very weak band at 3400 cm^{-1} in this spectrum may indicate that a trace of free hydroxyl is present. The aliphatic double bond is again at 1600 cm^{-1} but, in this case, is distinct from the aromatic nucleus band at 1578 cm^{-1} . The 964 cm^{-1} band is identical. The aliphatic C-H bands have very much the same pattern, with the exception of a much stronger band at 1461 cm^{-1} in the ether. The 1206 cm^{-1} aromatic C-O band is missing in the spectrum of the ether.

Flavanone (Figure 35)

The spectrum of this compound is exceedingly complex and difficult to interpret. A very weak band at 3270 cm^{-1} possibly indicates a trace of hydroxyl. The 1680 cm^{-1} aldehyde or ketone carbonyl band stands out very strongly. The 1494 or 1514 cm^{-1} aromatic nucleus band is abnormally weak. The aliphatic C-H bands are very weak, as would be expected, with the exception of a strong band at 1459 cm^{-1} .

Benzylidenesacetophenone (Chalcone) (Figure 36)

This material also shows a very complex spectrum, having a profusion of weak bands. Again, a very weak band at 3240 cm^{-1} possibly indicates a trace of hydroxyl. The 1659 cm^{-1} carbonyl band stands out strongly. The double 1603-1590 cm^{-1} bands possibly indicate a con-

justed double bond. A strong band at 989 cm.^{-1} is just above the range given by Williams (11) for >C=C< compounds. The aliphatic C-H bands are even weaker than in flavanone. Like flavanone, a single strong band stands out at 1046 cm.^{-1} .

Quercetin (Figure 37)

This spectrum shows a very strong and broad hydroxyl band at 3230 cm.^{-1} . The carbonyl band at 1650 cm.^{-1} stands out strongly, and a strong inflection at 1606 cm.^{-1} probably results from the conjugated double bond. A strong band at 934 cm.^{-1} is possibly also associated with this group. An anomalous characteristic of the quercetin spectrum is the weak but definite aliphatic C-H pattern at 2910, 2870, and 2820 cm.^{-1} and 1471, 1449, and 1434 cm.^{-1} . It might be suggested that this results from some rearrangement of molecular structure.

Conidendrin (Figure 38)

The outstanding point of interest is the extremely strong carbonyl band at 1771 cm.^{-1} .

Tetrahydrodehydrodieugenol Dimethyl Ether (Figure 39)

A weak band at 3200 cm.^{-1} possibly indicates a small amount of hydroxyl in this material. Compared with eugenol methyl ether (Figure 32), the aliphatic C-H bands at 2900 and 1451 cm.^{-1} are relatively stronger. The aromatic C-O bands are about the same. The 1636, 995, and 915 cm.^{-1} bands are not present in this material.

Inactive Guaiarotic Acid Dimethyl Ether (Figure 40)

Compared with isoeugenol methyl ether (Figure 34), the aliphatic C-H bands of this material are weaker. The 1601 C=C bands are about the same, but absorption in the 1000-900 cm^{-1} range is much weaker in this ether.

d-(3,4-Dimethoxyphenyl)-*l*-(3-Propyl-5,6-Dimethoxyphenyl)Propane (Figure 41)

This spectrum shows an inflection at 1602 cm^{-1} on the 1586 cm^{-1} aromatic nucleus band that resembles the isoeugenol C=C band. In this case, however, this could easily arise from the mixed type of aromatic ring substitution. Absorption in the 1000-900 cm^{-1} region is very weak.

Dihydrodehydrodiisoeugenol Methyl Ether (Figure 42)

It was hoped that the comparison of this spectrum with that of *d*-(3,4-dimethoxyphenyl)-*l*-(3-propyl-5,6-dimethoxyphenyl)propane would be particularly fruitful, since the two differ only in the presence of a Freudenberg-type furan ring in the material being considered. The difference in spectra cannot at present be specifically attributed to this grouping, however. Since the spectrum of the ether is generally more complex, it is only possible to say that new bands present in the aromatic C-O and saturated C-C-C regions may be associated with the closed ring arrangement.

Dehydrodiisoeugenol Methyl Ether (Figure 43)

This material differs from the dihydro compound only in the presence of a double bond in the 1,2-position of the propane side chain. In this case, the C=C band appears at 1678 cm.^{-1} , more closely resembling the arrangement of eugenol than of isoeugenol. This might be a logical effect of the high degree of substitution in the conjugated system. The strong band at 962 cm.^{-1} in the curve for the material being considered cannot be specifically attributed to the double bond, since a nearly equally strong band is present at this point in the saturated compound. Except for a difference in the proportion of aliphatic C-H bands and the 1678 cm.^{-1} double bond band, the spectra of the two materials are very similar.

Pinoresinol Dimethyl Ether (Figure 44)

The spectrum of this material is one of the most complex that has been encountered. The major point of interest is the anomalous appearance of a pattern of bands in the aliphatic double bond regions (the sample gives a negative bromine addition test in carbon tetrachloride). A weak band at 1638 cm.^{-1} is accompanied by a strong inflection at 1600 cm.^{-1} , and medium strength bands at 970, 935, and 914 cm.^{-1} .

Summary

An analysis of the spectra of the compounds listed above has furnished strong first-hand evidence of the characteristic absorption

bands of a wide variety of carbon, hydrogen, and oxygen groupings.

A few of the absorption patterns appear to be so specific that little evidence other than the appearance of the characteristic bands seems required to establish the presence of certain chemical groupings. This seems to be true of the pair of aromatic nucleus bands at 1600 and 1500 cm^{-1} . The maximum range of deviation of these bands in the compounds studied is from 1605 to 1584 cm^{-1} and 1517 to 1491 cm^{-1} . The aliphatic C-H bands are somewhat less sharply denoted, but the combination of several definite bands in the 3000-2800 cm^{-1} region along with proportionately strong bands in the 1480-1350 cm^{-1} region appears sufficient to definitely establish the presence of aliphatic groups. Although the relative number and strength of these bands were observed to vary markedly with the variety and proportion of aliphatic C-H arrangements, the poor dispersion of the sodium chloride prism in the higher frequency region, and the possibility of extraneous bands in the lower frequency region, makes difficult the interpretation of the arrangement of these bands in terms of saturated $>\text{C-H}$, $>\text{CH}_2$, $-\text{CH}_3$, and unsaturated groups. However, there does appear to be a consistent increase in absorption of the 1460 cm^{-1} band on methylation of phenolic hydroxyl compounds that may locate the $-\text{OCH}_3$ band.

The unique combination of mass and bond strength of the O-H linkage gives the hydroxyl group a "clear-channel" from 3700 to 3100 cm^{-1} . Although vanillyl alcohol, the only compound thus far studied that is known to contain both aliphatic and phenolic hydroxyl groups, showed two distinct bands in this region, the very poor dispersion of the sodium chloride prism in this region and the variability of location of the band

or bands within this region make it likely that several types of hydroxyls may appear as a single broad band.

In going beyond this point, it is evident that the appearance of absorption bands in a region associated with a particular grouping should be substantiated by confirming evidence obtained through removal of such bands by means of chemical reactions known to be specific for the group in question.

The carbon-oxygen and carbon-hydrogen double bond bands appear to be only slightly less specific than those discussed above. The aldehyde carbonyl band of vanillin and the ketone carbonyl bands of flavanone, chalcone, and quercetin are sharply defined within the range 1680-1650 cm^{-1} . Eugenol, isoeugenol, and their methyl ethers offer striking examples of the effect of conjugation on the aliphatic double bond absorption frequency and also of the change in the 1000-900 cm^{-1} bands in going from RCH=CH_2 to RCH=CHR . The eugenol type of isolated double bond verges on the aldehyde and ketone carbonyl region with a band at 1636 cm^{-1} , whereas in isoeugenol conjugation lowers the absorption frequency of the group to the extent that it may merge with the 1600 cm^{-1} aromatic nucleus band. The 1000-900 cm^{-1} bands are quite clearly defined in these compounds but this region is within the range of general molecular vibrations and, therefore, must be used cautiously.

Although absorption at the characteristic frequencies of other groupings must be used carefully because of the frequent presence of non-specific bands, there has been observed a fairly consistent relation of

the absorption bands in the 1200-1195 cm^{-1} aromatic or unsaturated C=C region to groupings in the compounds studied. Of particular interest is the disappearance of the 1206-1227 cm^{-1} band in eugenol and isoeugenol on methylation and the accompanying increase in strength of the 1234 cm^{-1} band. Although this does not hold invariably among the compounds studied, it appears to be a relationship worth watching in other methylation studies.

It should be observed that, in this study, no mention has been made of absorption characteristics below 900 cm^{-1} . Interesting spectral absorption patterns occur in this region that have been studied carefully for the above compounds, but no consistent relationships of band structure to chemical groupings have been found.

The first impression on going to the native lignin spectrum from those of the known compounds is the relative simplicity of the lignin absorption curve. This is a known characteristic of the spectra of high polymer materials; the large number of primary and secondary linkages apparently suppresses many of the normal modes of vibration of the single building unit without seriously impeding the resonant vibration of the functional chemical groups. This is, of course, an advantage in analyzing such a spectrum.

A second conclusion is soon reached. This is the fact that it is futile to try to compare the infrared spectrum of an unknown material with those of known compounds and to say that the spectrum of the unknown is "like" that of a particular compound unless the two curves are identical, or nearly so. Since lignin is obviously quite

different from any of the simple compounds studied, it cannot be even closely matched with any of the other curves obtained.

TANNIN AND CARBOHYDRATE MATERIALS

The infrared spectra of purified chestnut, quebracho, and redwood tannins and redwood phlobaphene are shown in Figures 45 to 47. These spectra were determined as films from dioxane-ethyl alcohol. The partial insolubility of several of the group did not prevent preparation of uniform dispersions.

This group of materials gives spectra which were of the same general type as lignin, as contrasted with low molecular weight crystalline or liquid compounds. The spectra are quite distinguishable among themselves and from lignin, however. No attempt will be made to describe these absorption curves in detail. The outstanding features of interest are the very strong acid or ester carbonyl band in the chestnut tannin and weak plateau effect in this region in both the quebracho and redwood tannin and phlobaphene. No definitely significant difference is evident between the redwood tannin and phlobaphene absorption spectra.

One of the possible interesting applications of infrared spectroscopy would be the determination of the lignin spectrum directly from wood sections. To obtain an idea of the absorption background to be expected from carbohydrate materials, a set of chlorite carbohydrate preparations were run in Nujol dispersions.

Black Spruce Holocellulose (Figure 48)

The fibrous nature of this material made sample preparation difficult. The material was ground to pass through an 80-mesh screen in the small Wiley mill and dried in vacuo over phosphorus pentoxide before mulling in Nujol and smearing between salt plates. The coarseness of the particles resulted in high general absorption and indistinct bands.

Strong absorption in the hydroxyl region centered at 3230 cm.^{-1} is evident, a strong acid or ester carbonyl band stands out at 1720 cm.^{-1} , and a very broad deep band at 1605 cm.^{-1} is probably due to liquid water (Williams: $1650\text{-}1600 \text{ cm.}^{-1}$). Other simple broad bands are vaguely defined throughout the spectrum.

Spruce Hemicellulose A (5 Per Cent Potassium Hydroxide Extract of the Holocellulose) (Figure 49)

The amorphous nature of this material made possible fine dispersion and excellent definition of the absorption bands. Hydroxyl bands are very broad and strong at 3320 and 1055 cm.^{-1} (Williams: $1040\text{-}1060 \text{ cm.}^{-1}$), as is the liquid water band at 1606 cm.^{-1} . There is no band evident in the carbonyl region.

Spruce Hemicellulose B (16 Per Cent Potassium Hydroxide Extract of the Holocellulose Residue from the Hemicellulose A Extraction) (Figure 50)

The only distinctive point of interest in this case is the presence of a strong inflection at 1650 cm.^{-1} in the liquid water band.

In addition to the interesting contrast to the spectra of the aromatic materials thus far considered, the absorption curves of these carbohydrate materials make it clear that it will not be simple to obtain the lignin spectrum in wood against this background.

SUMMARY AND CONCLUSIONS

The infrared absorption spectrum of native spruce lignin has been determined in film form and in Nujol dispersions from 720 to 3700 cm^{-1} . The curves agree throughout the range available to Nujol with the exception of three bands at 872, 890, and 1123 cm^{-1} , which appear in the film but not in Nujol. Although there is evidence to indicate that they are probably due to absorbed dioxane, this has not been conclusively demonstrated.

A basic philosophy that has been adopted in the study of this spectrum is that, in this initial phase, it is highly desirable to restrict interpretation to direct spectrochemical evidence and not to lean any more than necessary on chemical data for support.

Fractional precipitation was applied to gain evidence regarding the homogeneity of the material being studied. Separation of the native spruce lignin into six fractions and determination of the infrared absorption spectra of the fractions revealed no variation other than the progressive decrease in intensity of the 1663 cm^{-1} aldehyde or keto carbonyl band in going from the least soluble to the most soluble fraction. On this tentative basis, the material is discussed from this point on as being essentially chemically homogeneous.

In order to obtain a background for valid interpretation of the lignin spectrum, it was necessary to build up a reference library of spectra of compounds of known structure. The infrared spectra of

many organic compounds have been published, and empirical charts drawn showing the principal absorption bands of many organic groups, but the lack of standardization of instruments and methods of presentation of data made it highly desirable to work directly from spectra obtained on one instrument, as supported by accumulated data in the literature. Another consideration was the fact that the spectra of many of the compounds of direct interest in the lignin field have not been published. Therefore, the spectra of a number of compounds were determined to illustrate many of the structure-absorption relations which might be of interest in the study of lignin.

In comparing the spectra of these compounds of known structure with the spectrum of native lignin, two generalizations are evident. The first is the relative simplicity of the lignin absorption curve. This is a known characteristic of the spectra of high polymer materials. The second basic conclusion is that it is futile to try to compare the infrared spectra of an unknown material with those of known compounds unless the curves are identical or nearly so. Therefore, there is little to be gained in trying to make over-all comparisons of the lignin spectrum with the spectra of so-called model lignin compounds.

The spectra determined in this study were found to comply closely with the general empirical rules of chemical group-absorption band relations given in the literature. The most important conclusion reached, however, was that these rules must be weighed in terms of relative invariability.

The following conclusions were reached with respect to the lignin spectrum:

the pair of strong bands at 1598 and 1512 cm^{-1} in the lignin spectrum are identified as characteristic of the aromatic nucleus on the basis of strong empirical evidence. In like manner, the bands at 2900 and 2830 cm^{-1} , and at 1454 and 1425 cm^{-1} are assigned to a moderately high ratio of saturated aliphatic C-H groups. The 3350 cm^{-1} band is positively identified as being derived from hydroxyl groups by its progressive disappearance on methylation. The 1663 cm^{-1} band is demonstrated to represent an aldehyde or keto carbonyl group through its removal on methyl alcohol-hydrochloric acid methylation. Evidence suggesting, but not proving, the aromatic C-O and saturated C-O origin of some of the bands in the 1260-1075 cm^{-1} range has been discussed.

This is as far as one may proceed at present in identifying the source of the absorption bands in the native lignin spectrum. Further work will bring out other chemical structure-absorption spectrum relationships, but a number of bands, particularly at the lower frequencies, will undoubtedly have to be assigned to general characteristics of the material rather than to specific groupings.

Other spruce lignin preparations were run for comparison with the native lignin. Flason, Willstätter, alkali periodate, and Freudenberg spruce lignins were run in Muzol. The spectra obtained were surprisingly similar to that of native spruce lignin in Muzol. The spectra of the Willstätter and periodate lignins were practically identical with that of native spruce lignin; that of alkali lignin was very close except for the noteworthy absence of the 1660 cm^{-1} carbonyl band; Flason lignin showed almost complete absence of hydroxyl bands and no carbonyl band but was very similar in other regions; and the spectrum

of the Freudenberg lignin showed several important variations among the unidentified bands.

The spectrum of native aspen lignin, on the other hand, although showing a generally similar pattern to that of native spruce lignin, was found to be considerably more complex. There is a far greater difference between the spectrum of this native hardwood lignin and native spruce lignin than there is within the group of the six spruce lignins isolated by different methods.

Although the synthetic material known as Russell lignin shows a number of bands at approximately the same frequencies as those found for native spruce lignin, such as the aromatic nucleus pair, aliphatic C-H and hydroxyl bands, etc., the spectra show quite marked differences. The most striking is the very strong carbonyl band of the synthetic material.

The spectra of three barium lignosulfonates of varying sulfur content as determined in Nujol dispersions, particularly for the low sulfur (4.2 per cent) sample, were very similar to the spectrum of native spruce lignin. The outstanding evidence obtained from the series was the disappearance of absorption bands in the carbonyl region with increasing degree of sulfonation.

The spectra of a group of selected tannin and carbohydrate materials furnishes interesting comparisons and differentiations from the lignin spectrum.

It is believed that the present experimental work adequately

demonstrates the potential usefulness of infrared spectroscopy in the lignin field. It is hoped that these necessary exploratory investigations can be continued and utilized as rapidly as possible, so that the technique may assume its proper role as a powerful physical tool in the chemical investigations of the structure and reactions of lignin.

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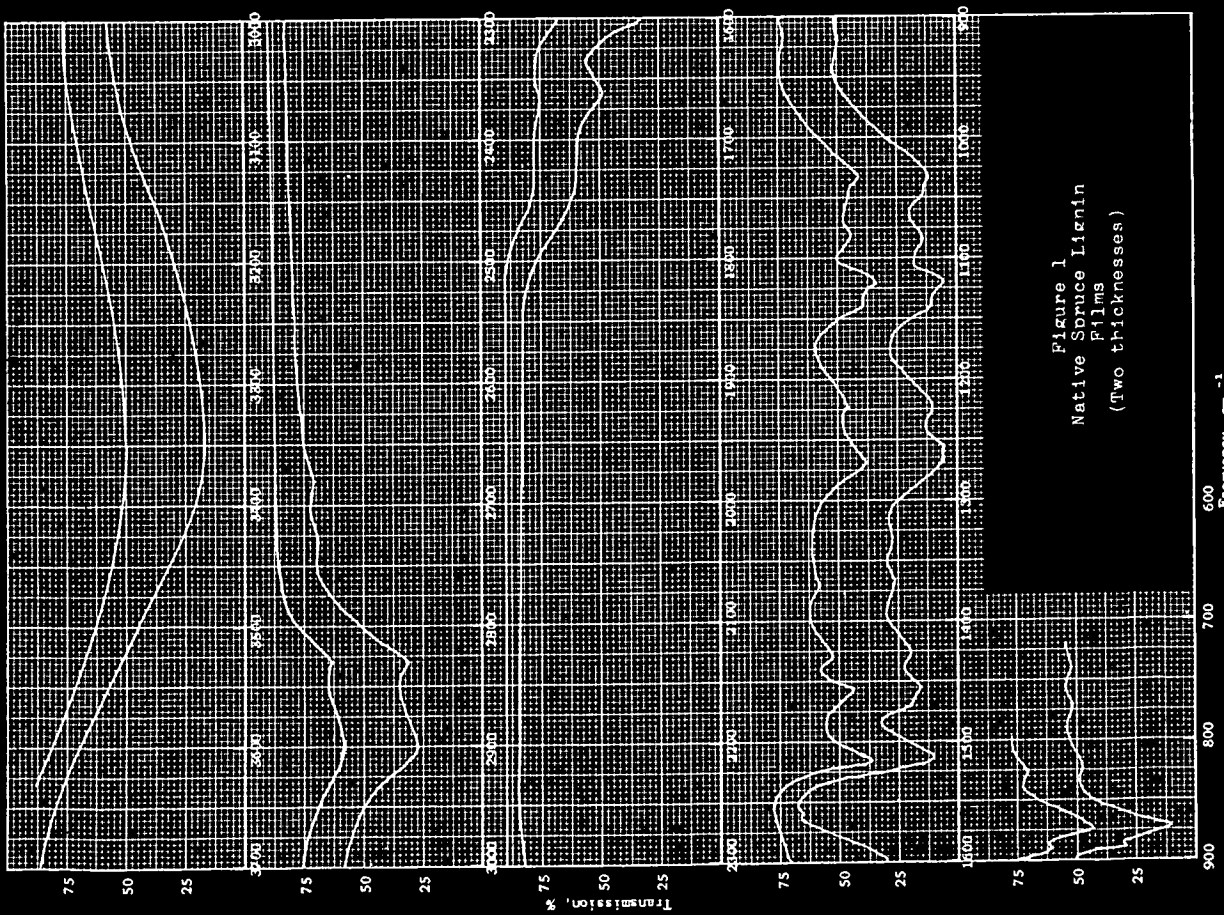


Figure 1
Native Spruce Lignin
Films
(Two thicknesses)

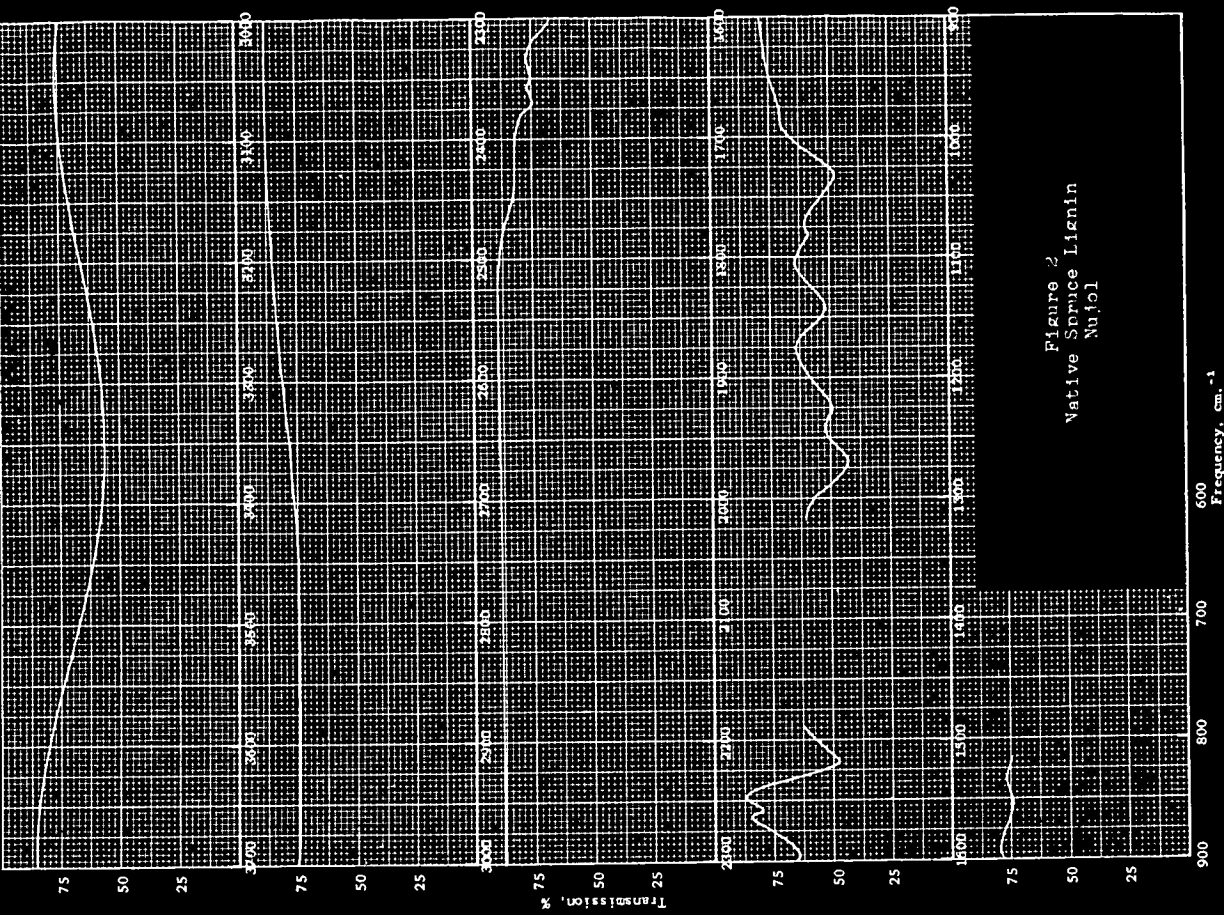


Figure 2
Native Spruce Lignin
Nujol

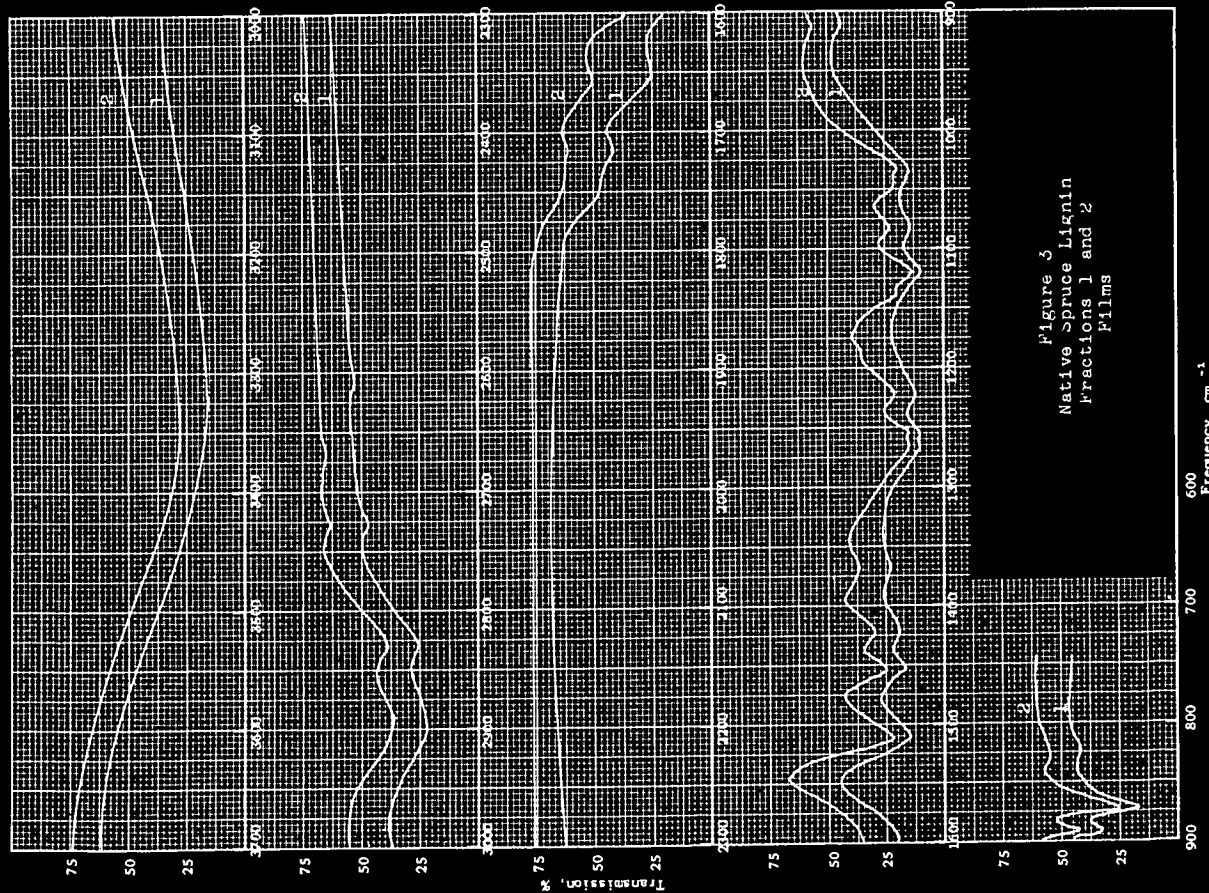


Figure 3
Native Spruce Lignin
Fractions 1 and 2
Films

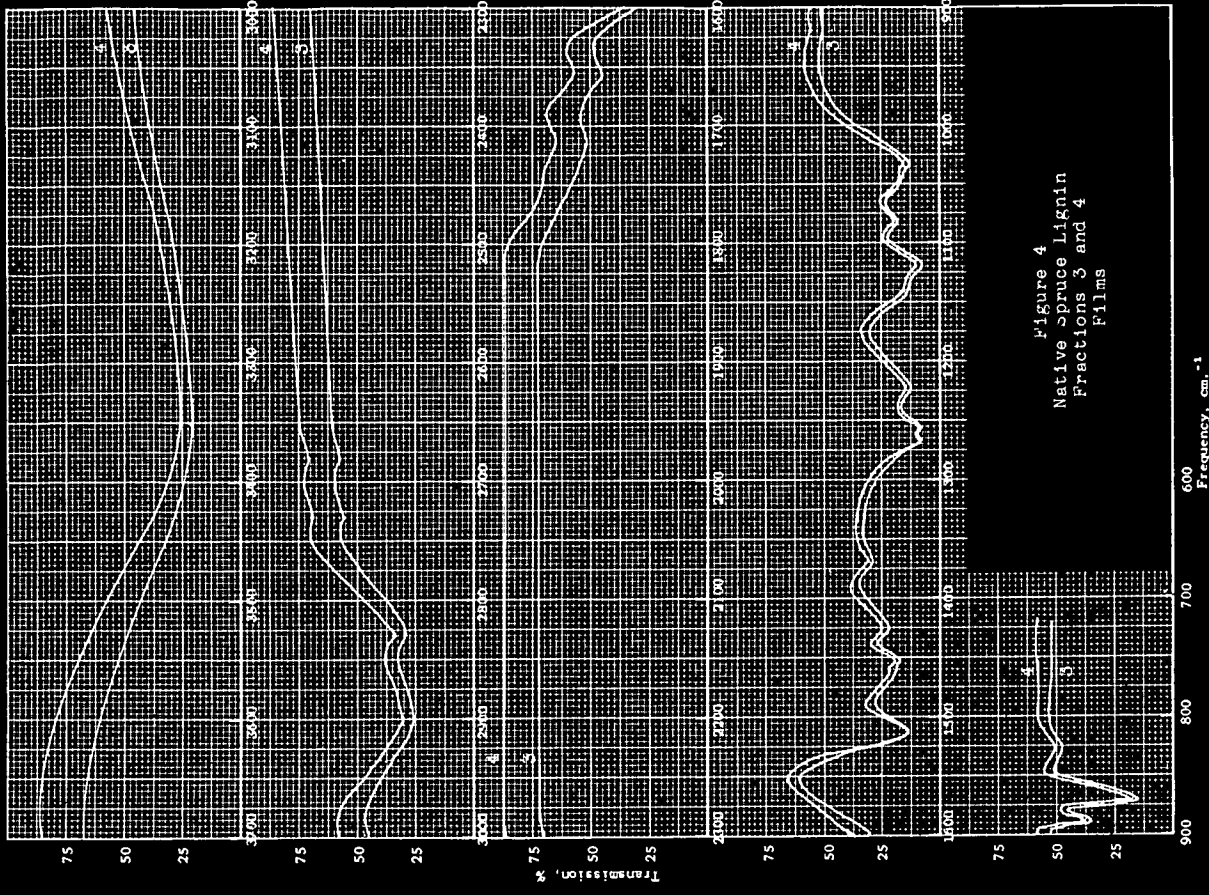


Figure 4
Native Spruce Lignin
Fractions 3 and 4
Films

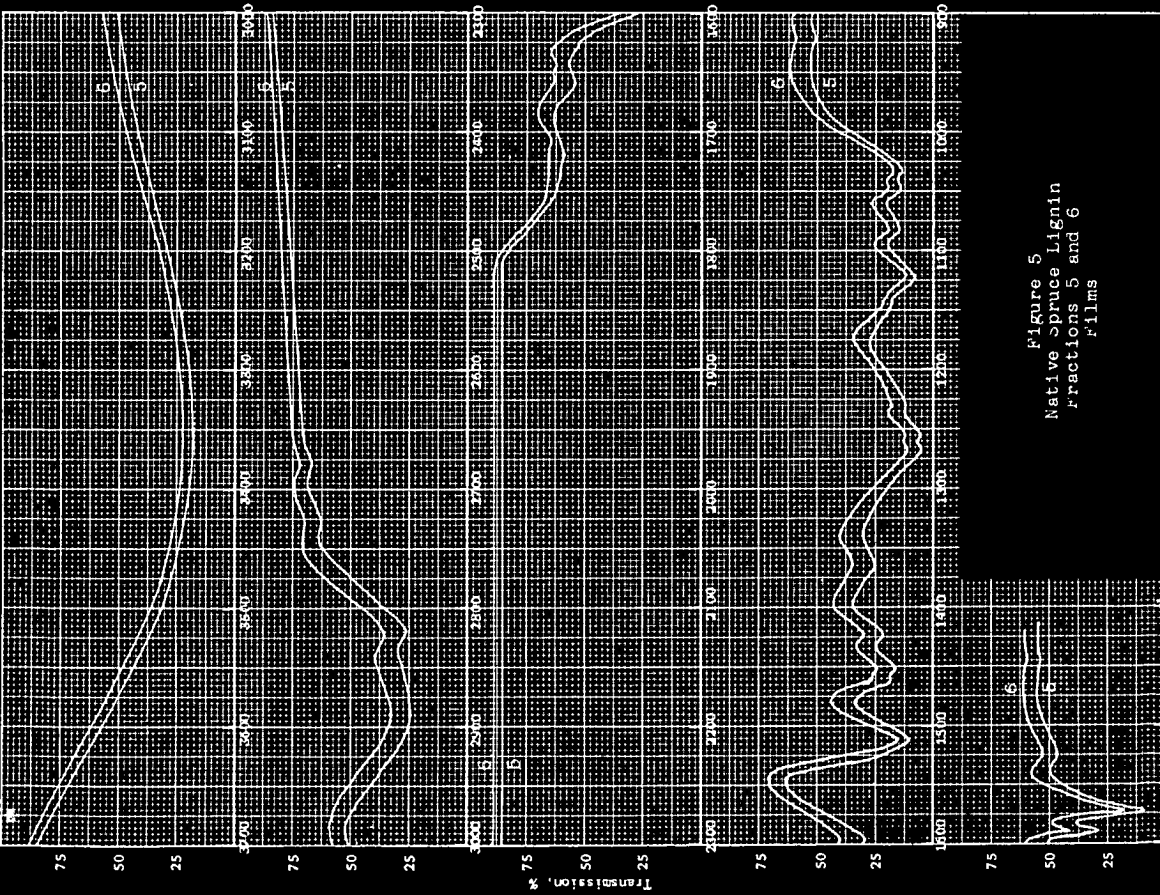


Figure 5
Native Spruce Lignin
fractions 5 and 6
films

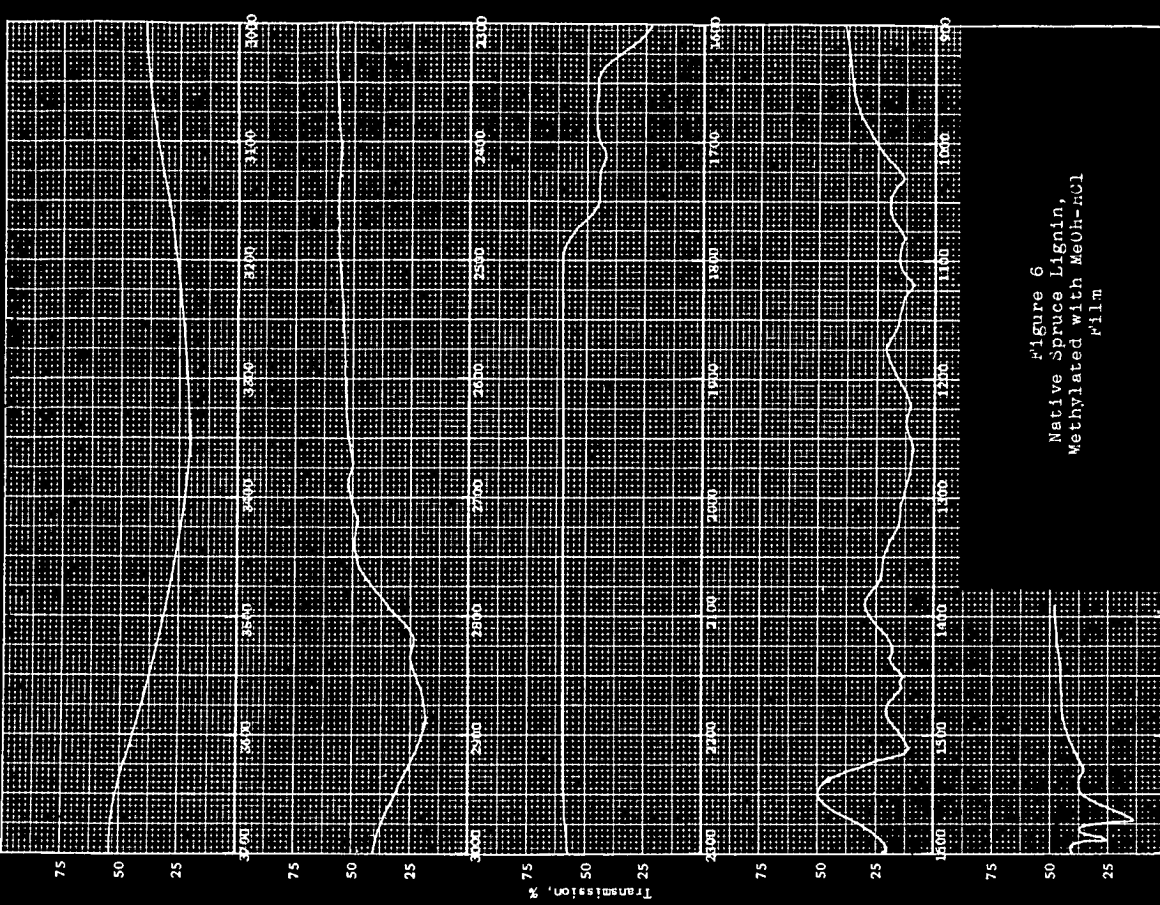
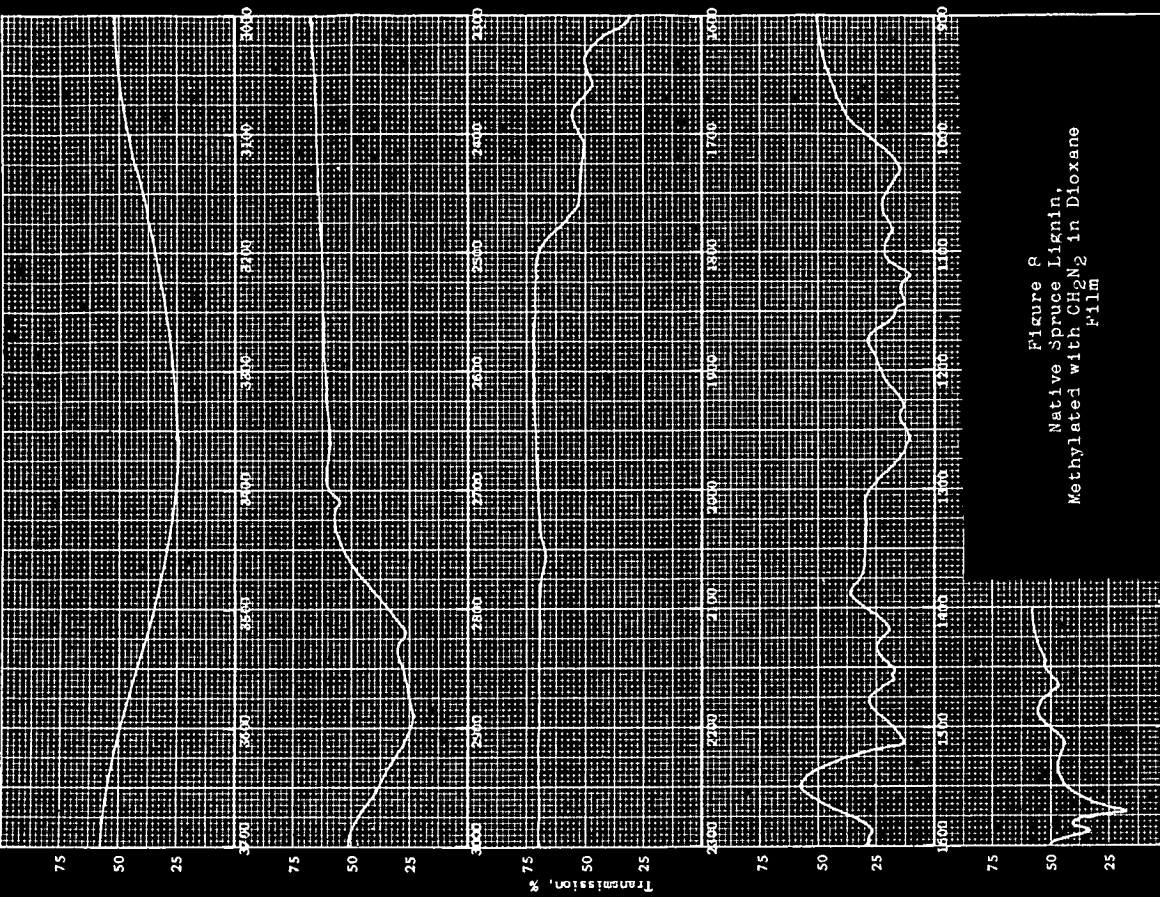
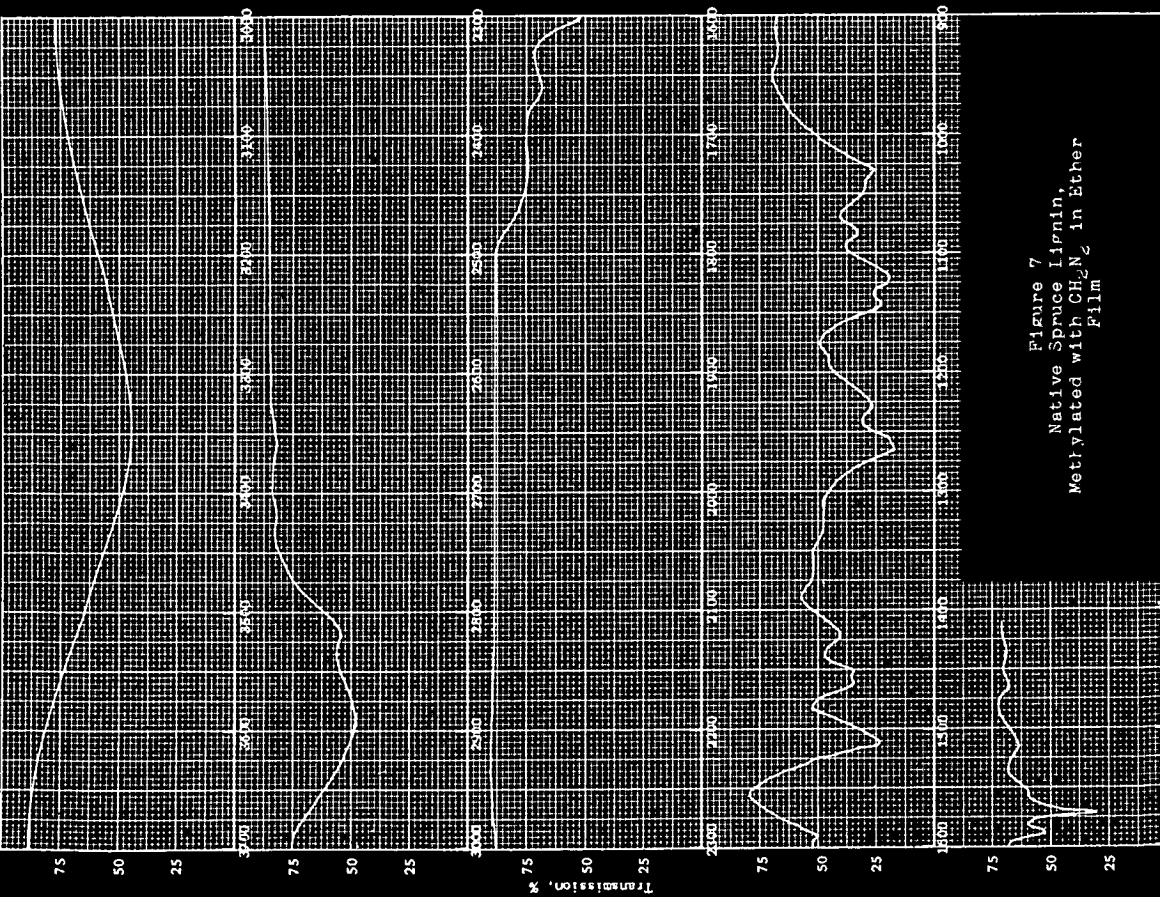


Figure 6
Native Spruce Lignin,
Methyolated with MeOH-HCl
film



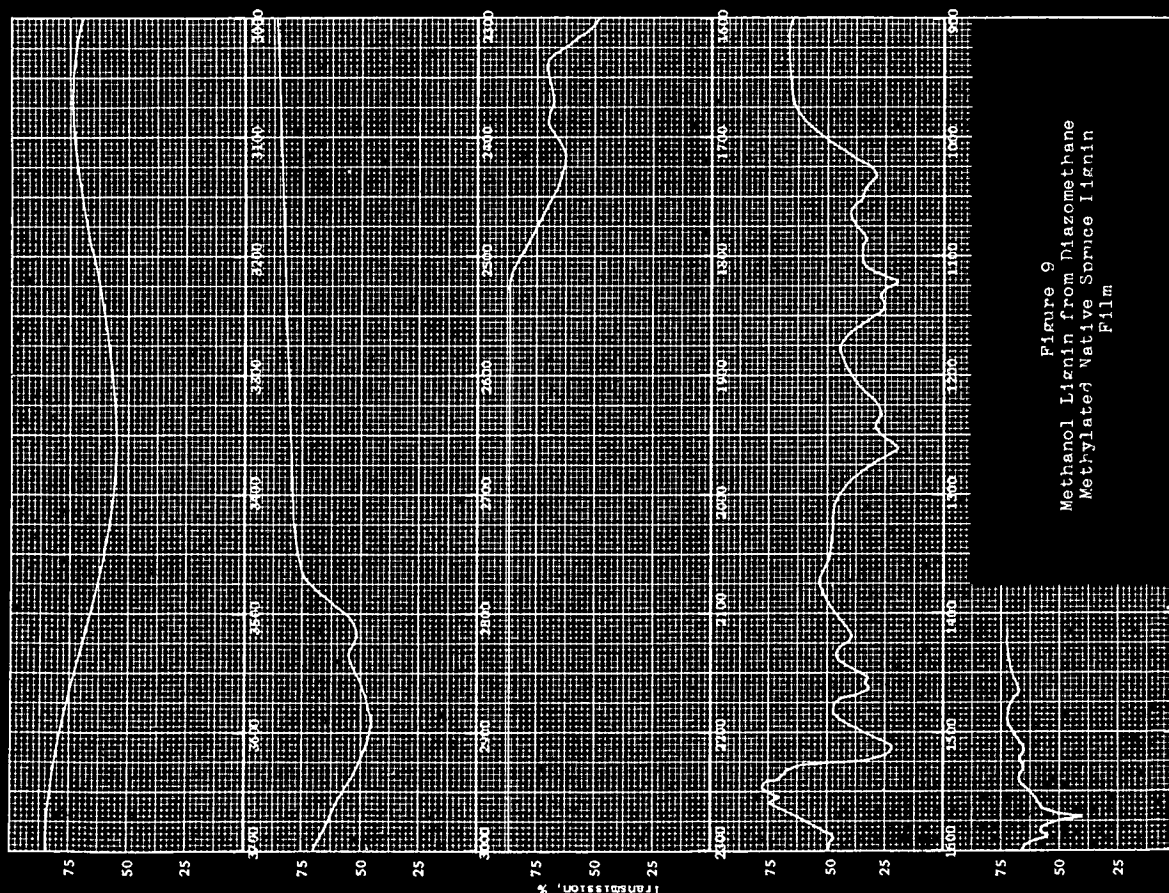


Figure 9
Methanol Lignin from Diazomethane
Methylated Native Spruce Lignin
Film

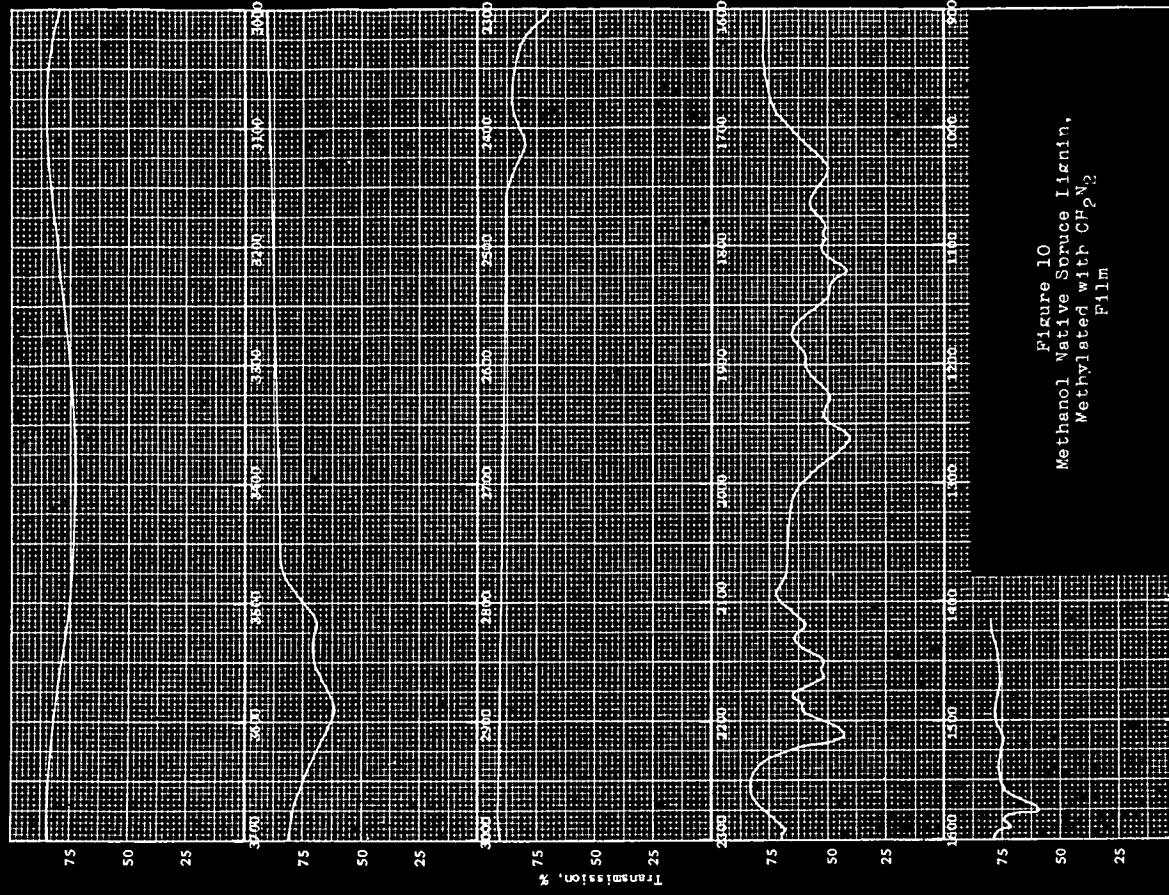


Figure 10
Methanol Native Spruce Lignin,
Methylated with CH_2N_2
Film

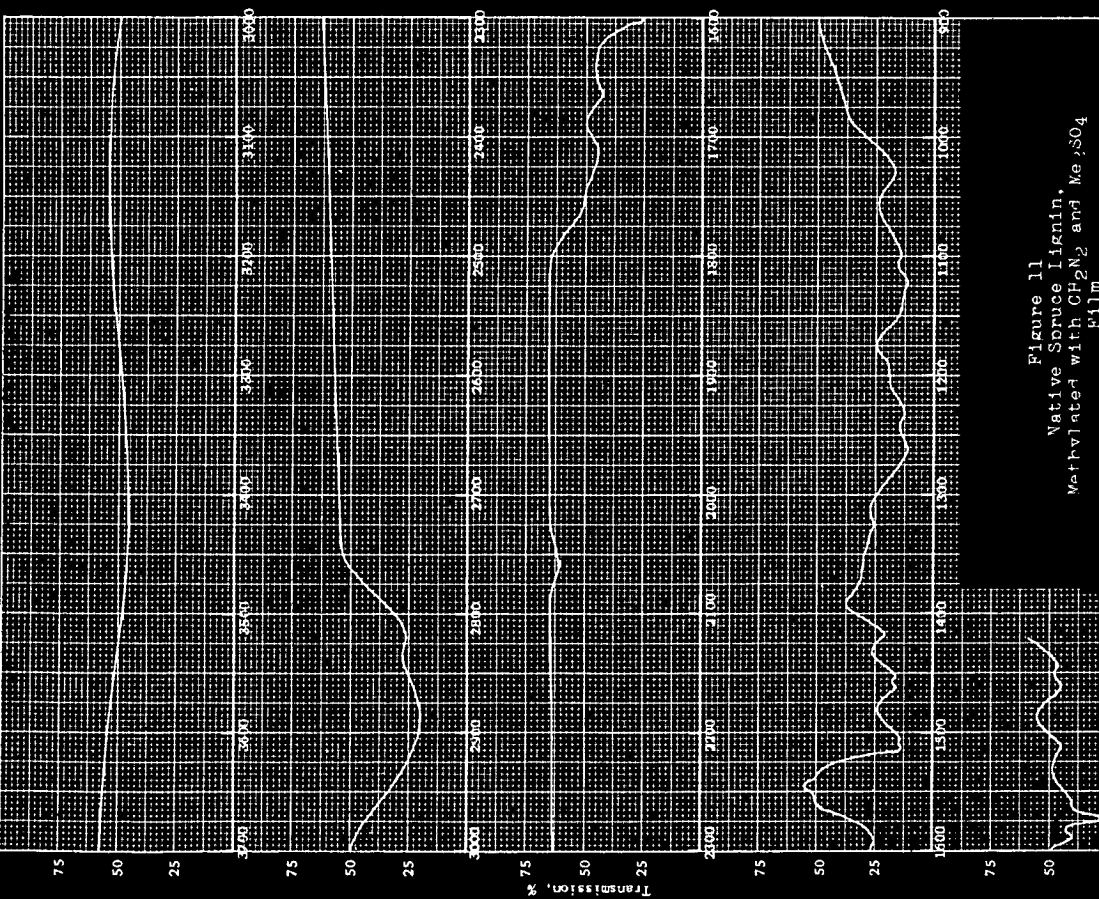


Figure 11
Native Spruce Lignin,
Methylated with CH_2N_2 and Me_2SO_4
Film

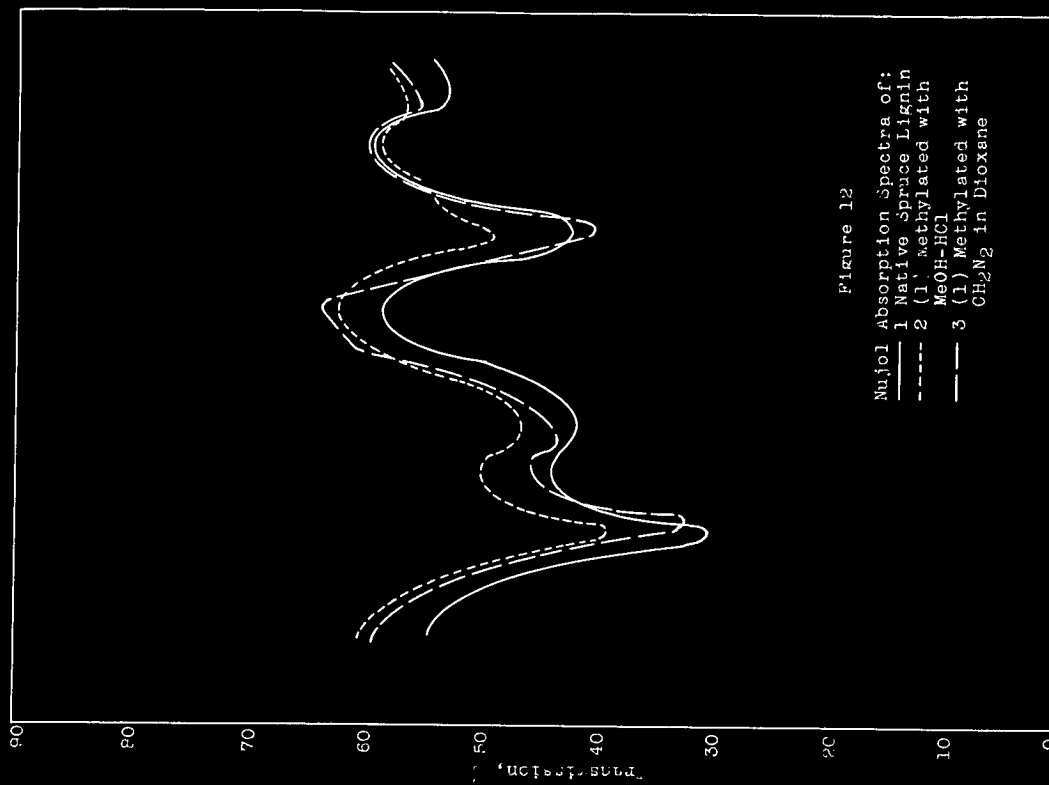
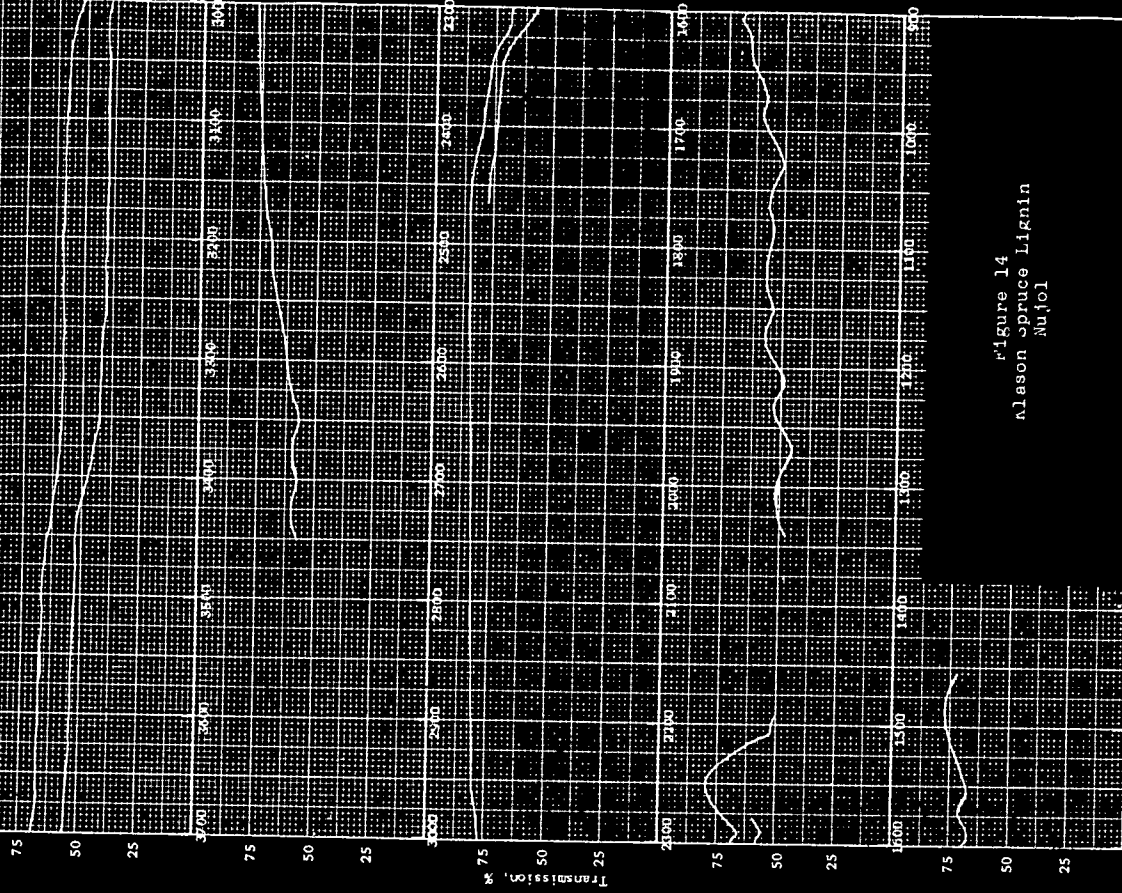
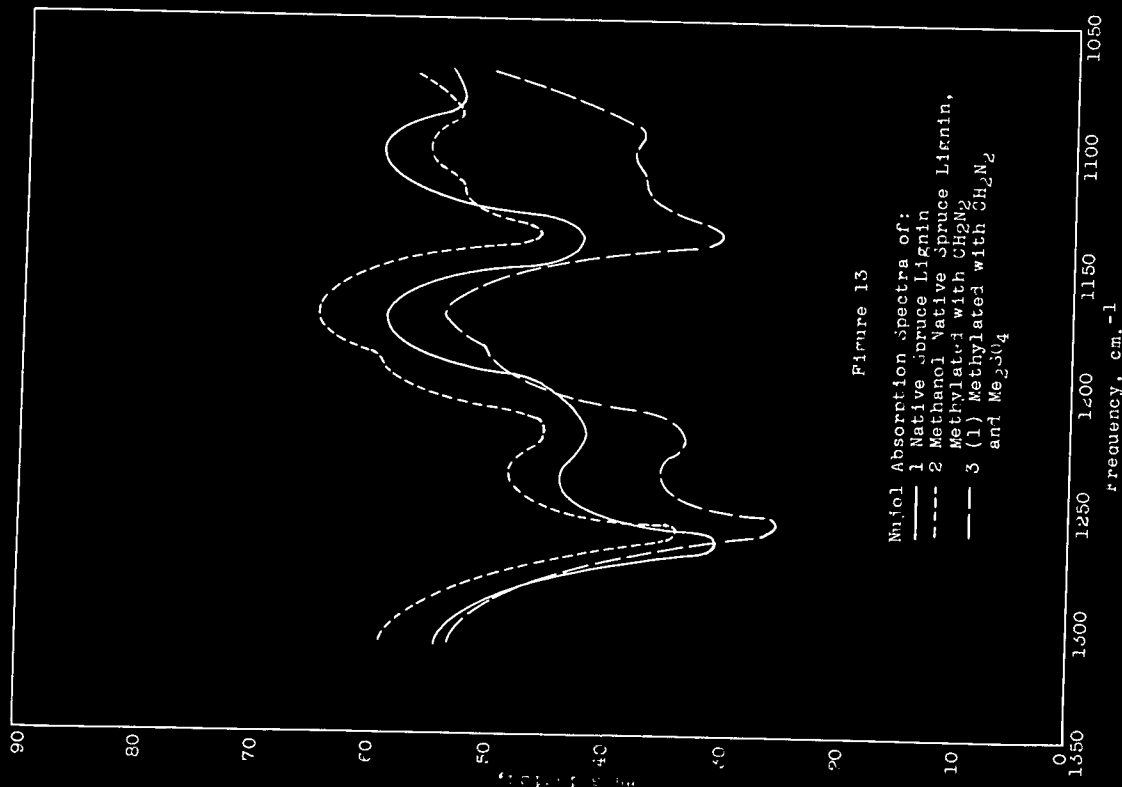


Figure 12
Nujol Absorption Spectra of:
1 Native Spruce Lignin
----- 2 (1) Methylated with
MeOH-HCl
--- 3 (1) Methylated with
 CH_2N_2 in Dioxene



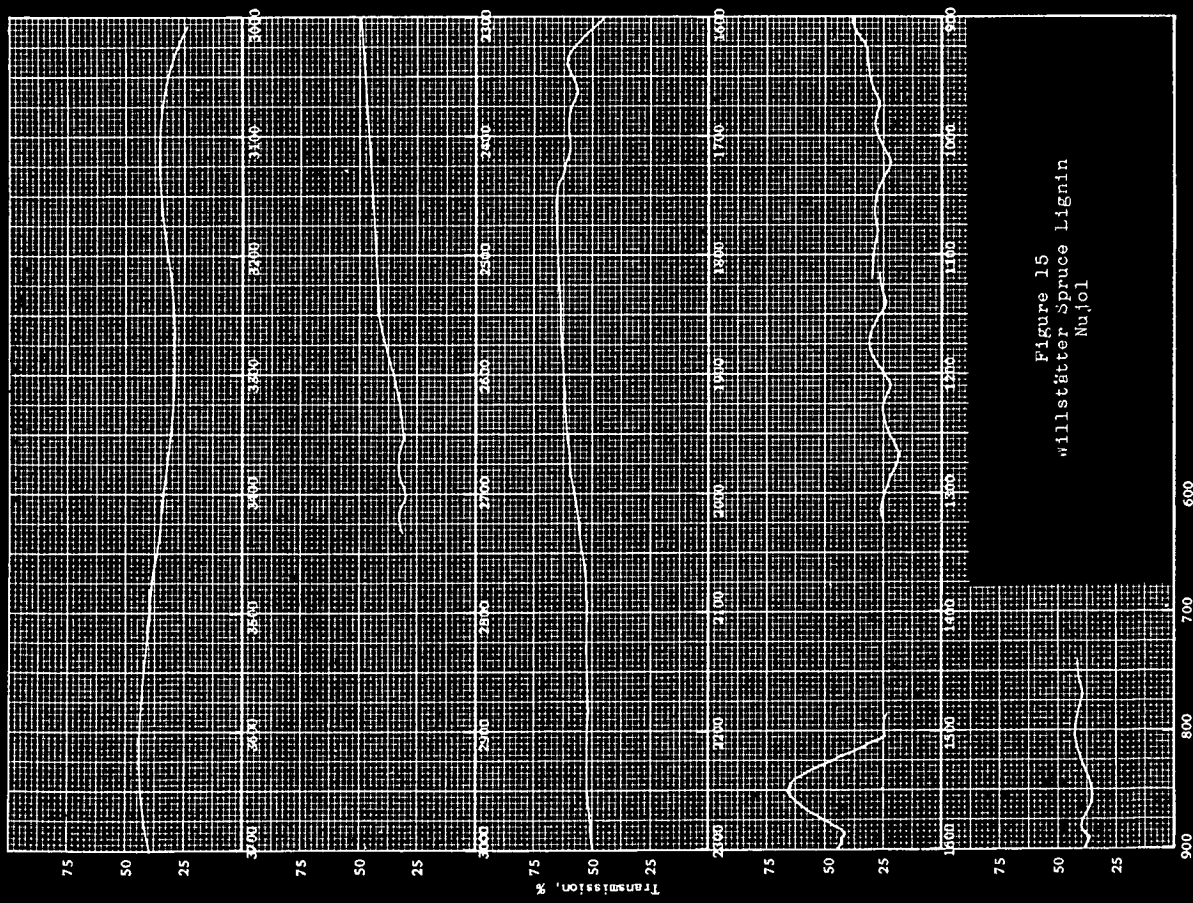


Figure 15
willstätter Spruce Lignin
Nujol

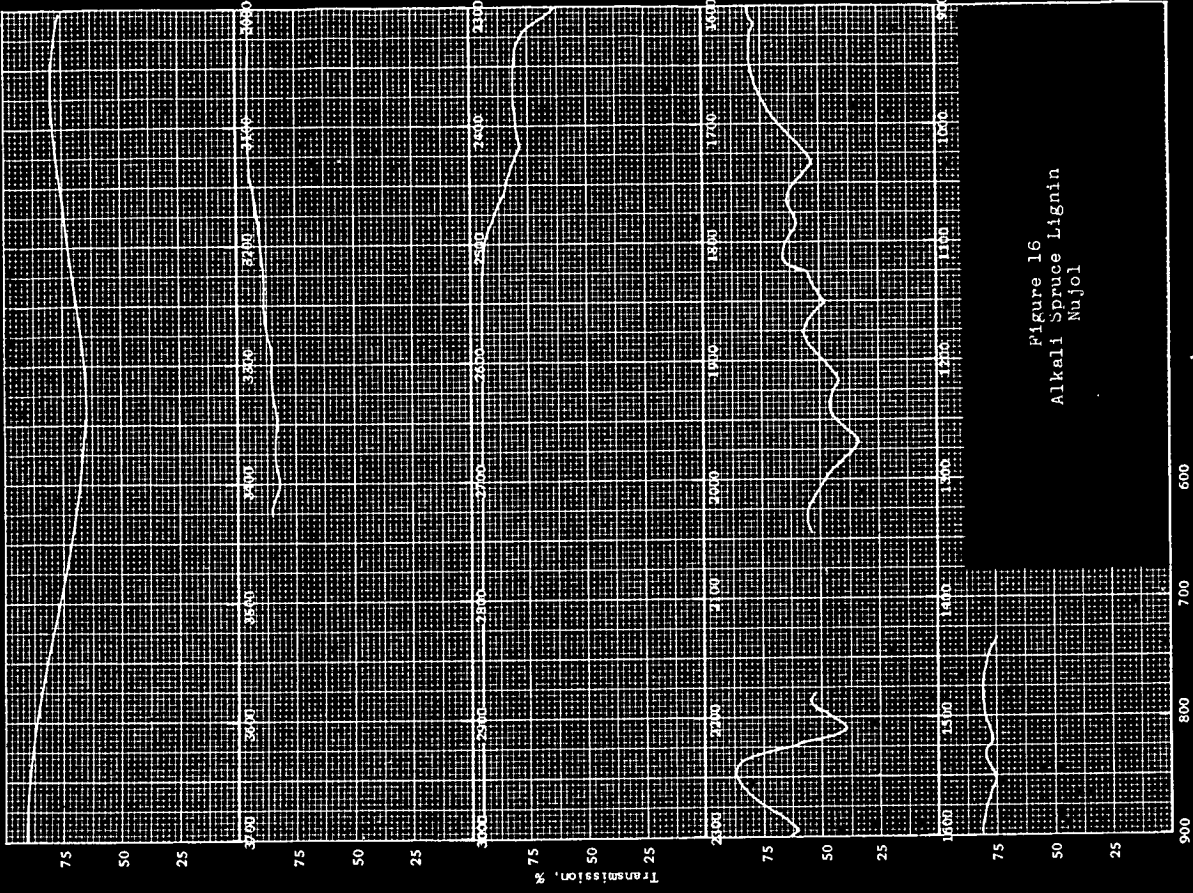


Figure 16
Alkali Spruce Lignin
Nujol

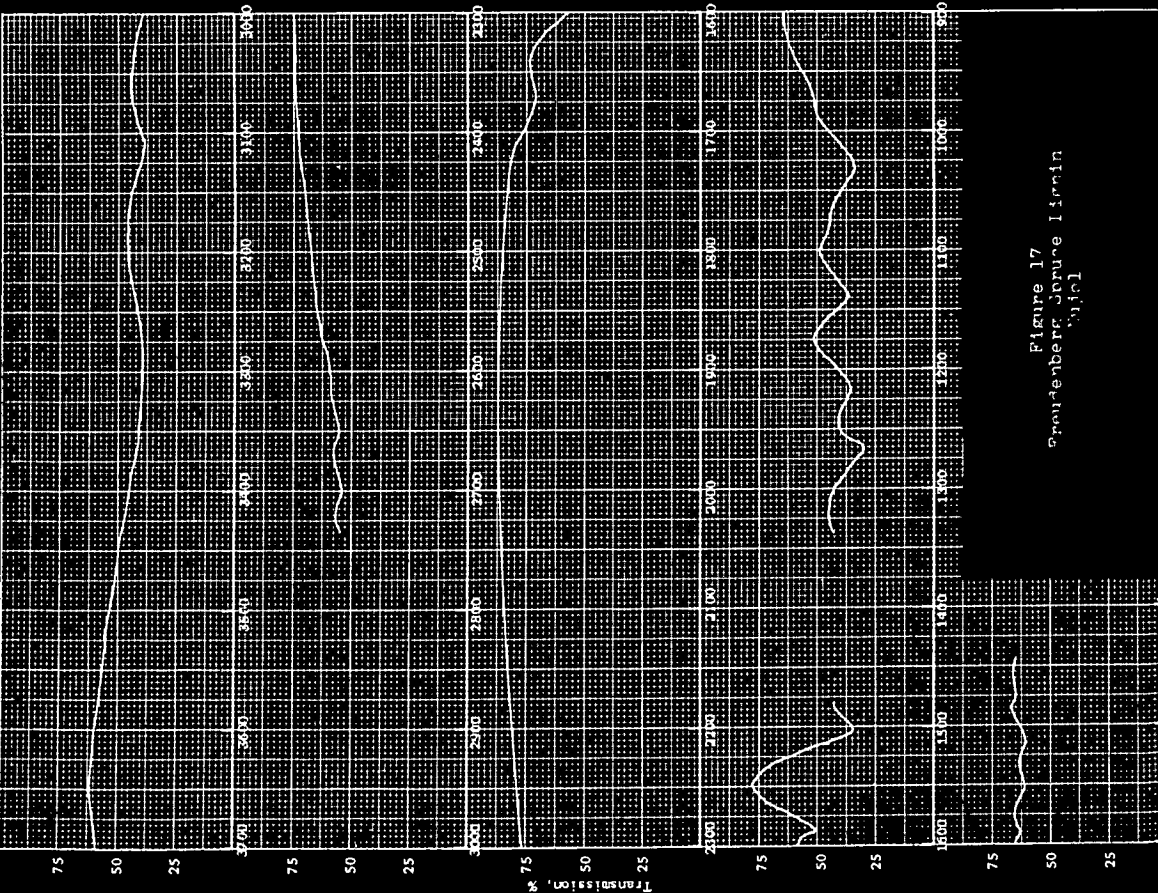


Figure 17
Periodate spruce Lirrin
Nujol

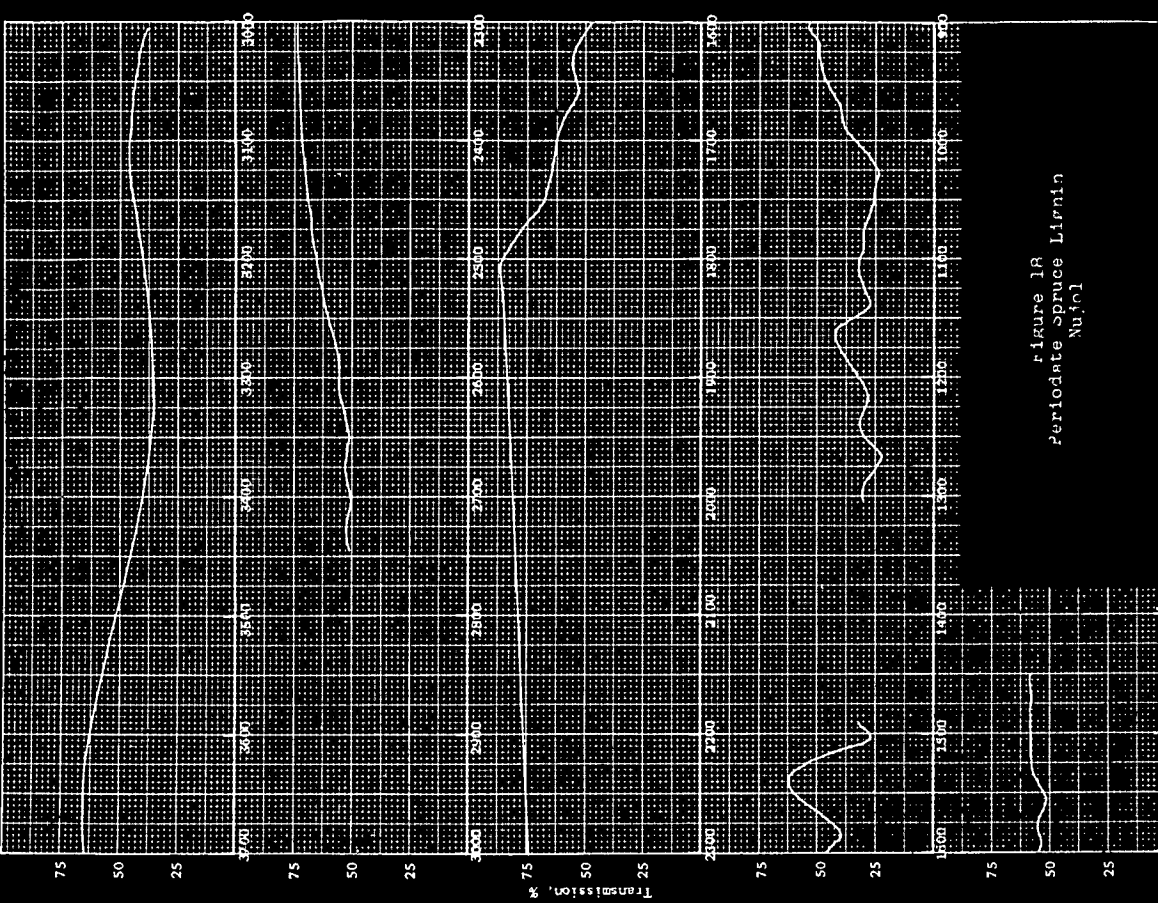
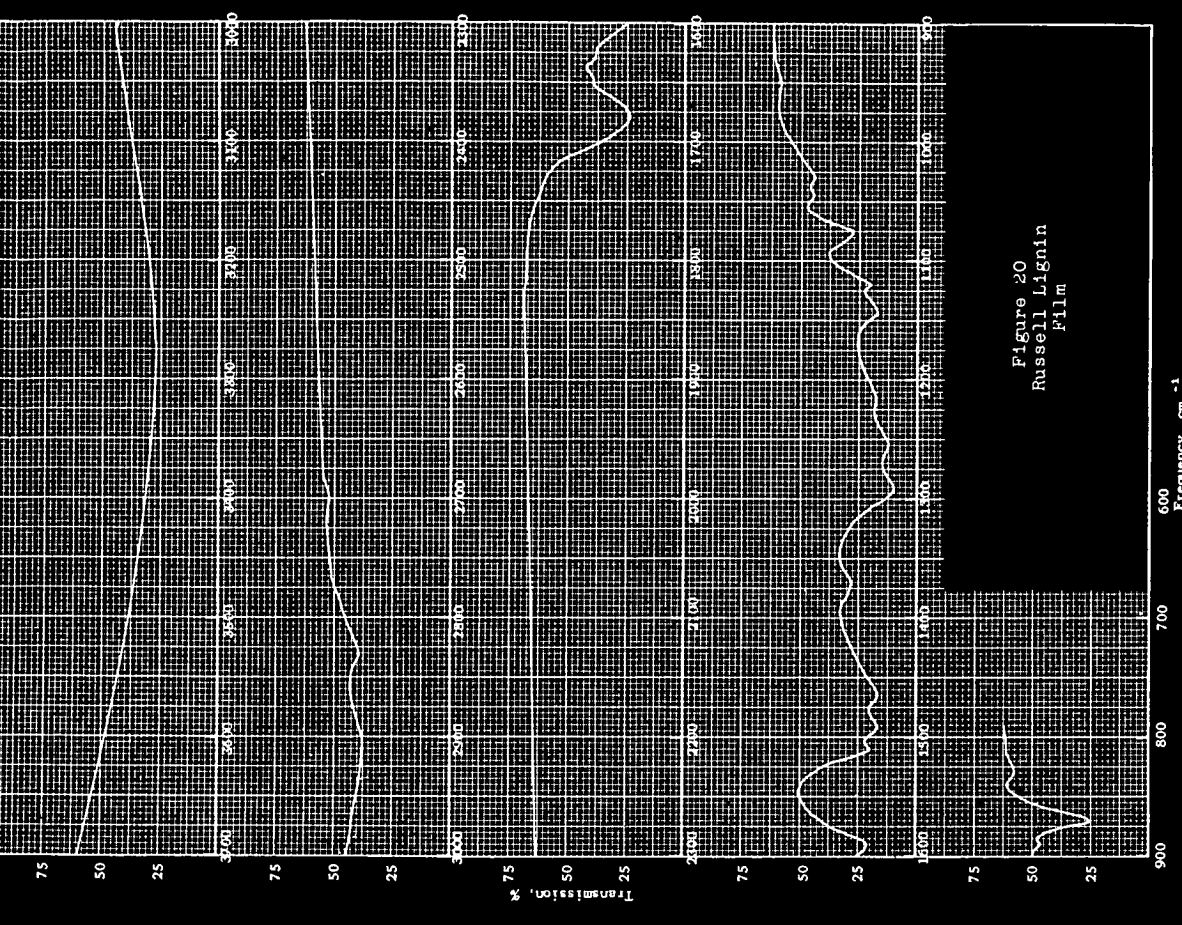
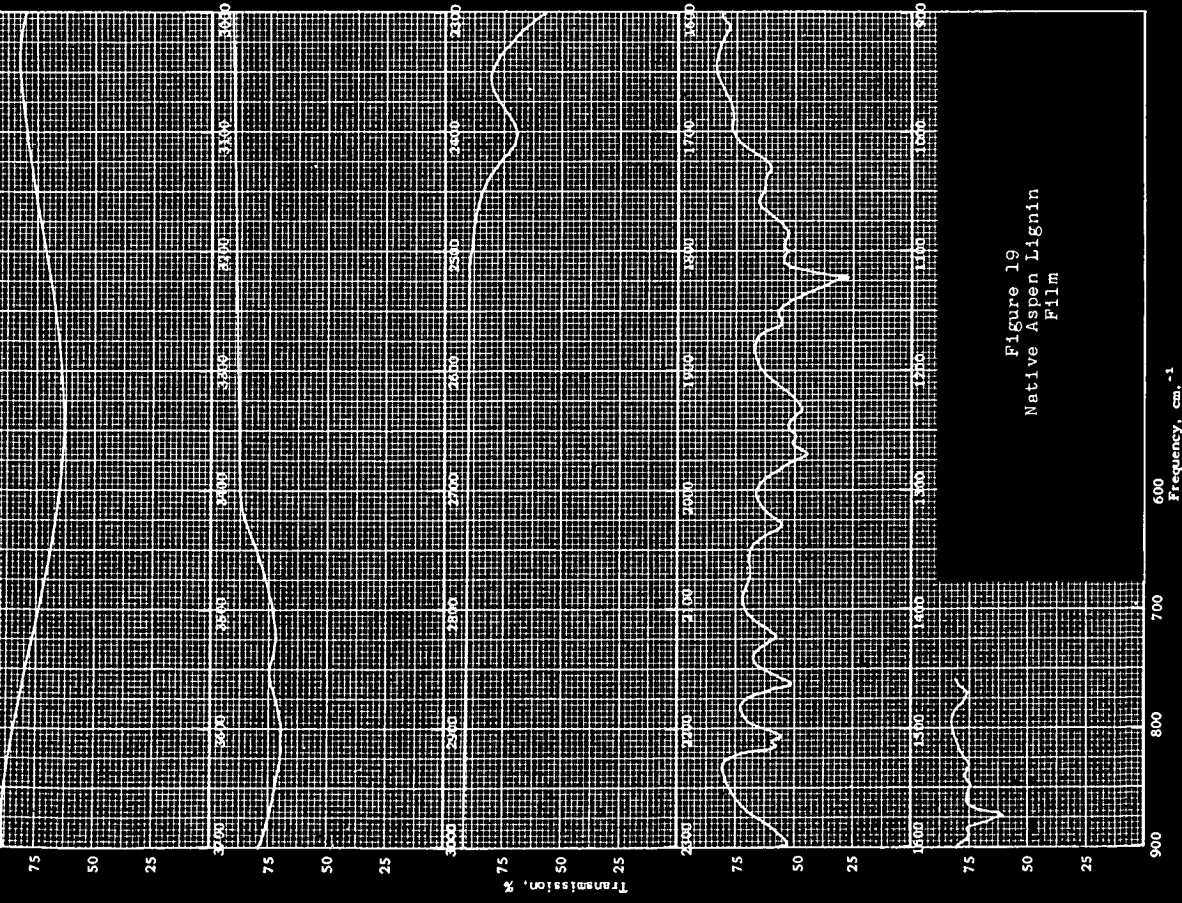


Figure 18
Periodate spruce Lirrin
Nujol



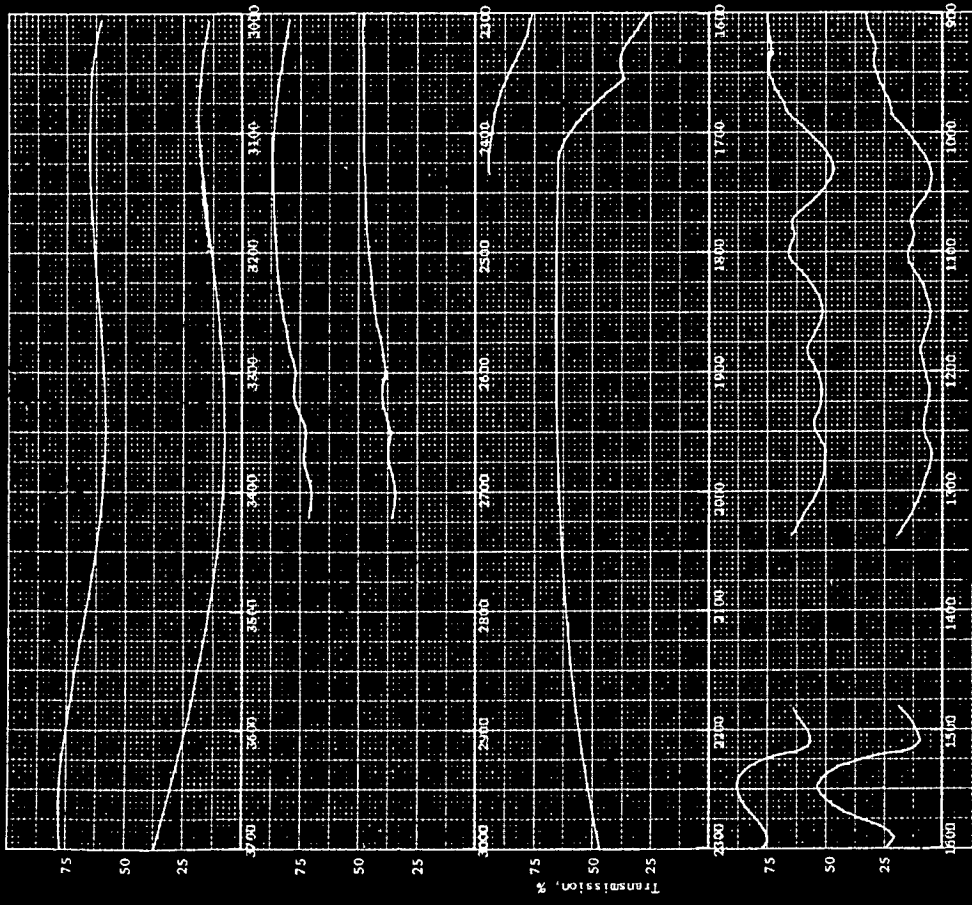


Figure 11
Lignosilicate - "J-2"
Nital
Two densities
Analysis: C = 41.0, H = 4.17,
O = 54.83 - 18.10, 0.00/5 - 4.

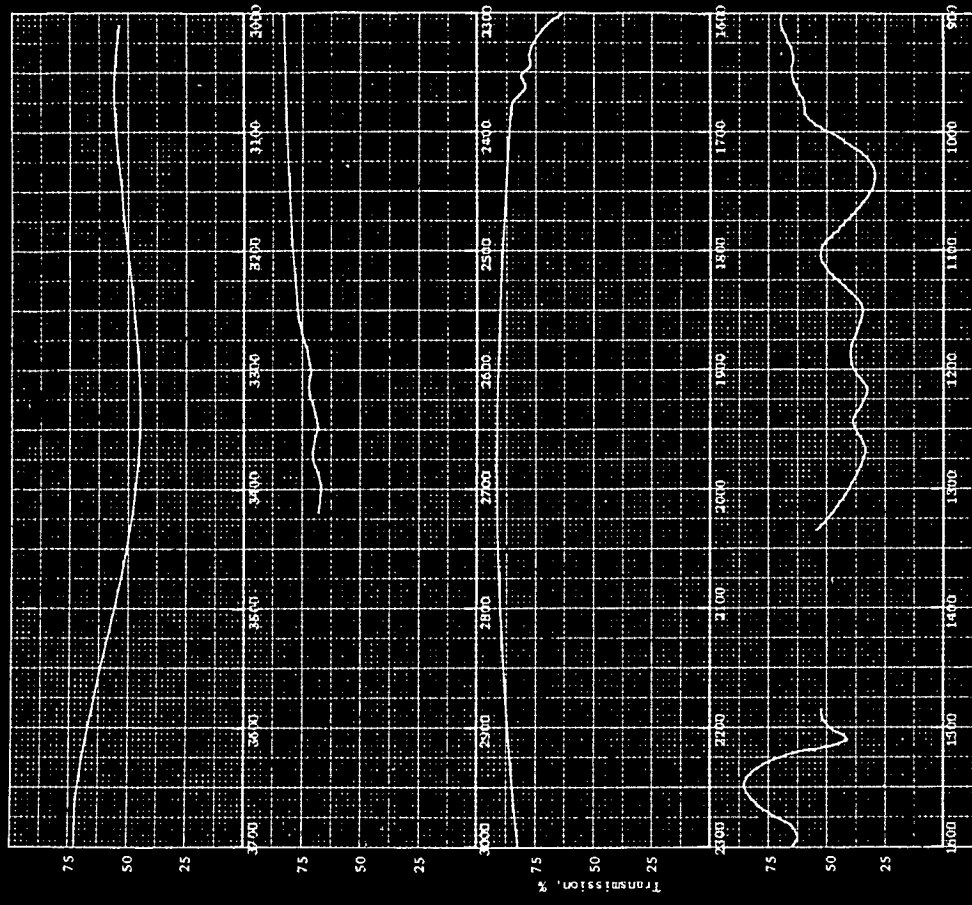


Figure 12
Lignosilicate - "J-2"
Nital
Analysis: C = 41.0, H = 4.17,
O = 54.83 - 18.10, 0.00/5 - 4.

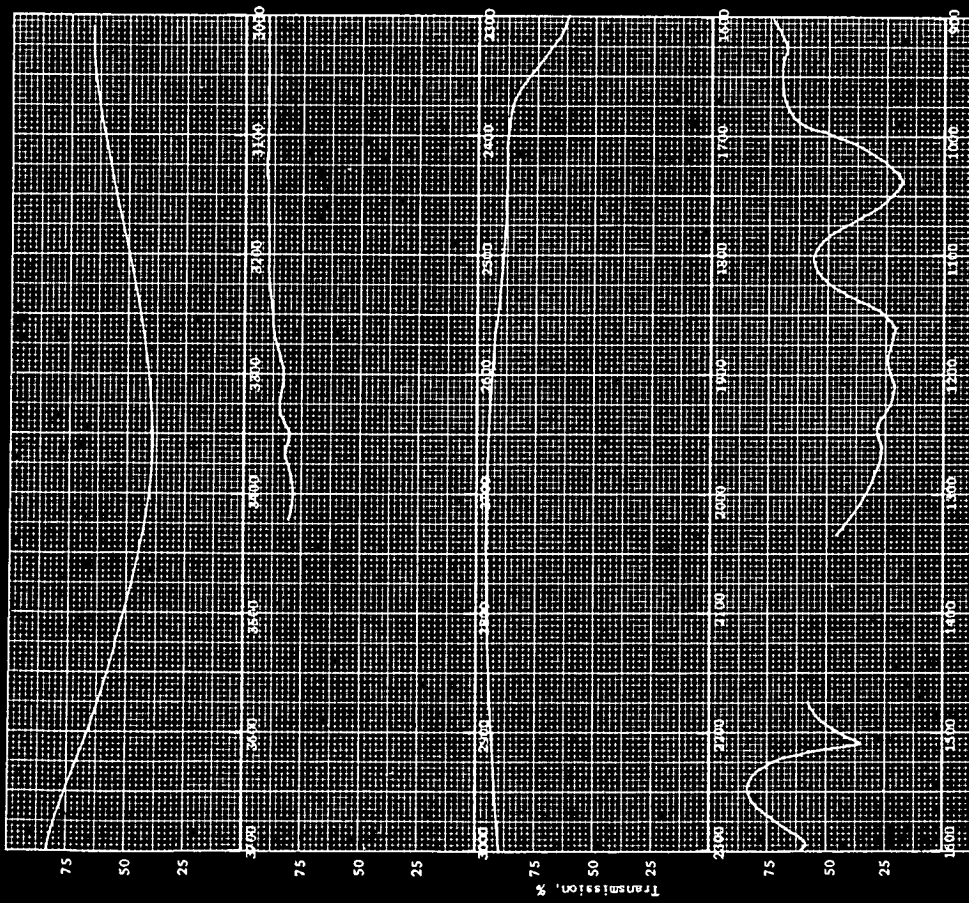


Figure 23
Barium Lignosulfonate - "cap"
Nujol
Analysis: S - 8.24, Ba - 19.8,
OCH₃ - 9.75, OCH₃/S - 1.9

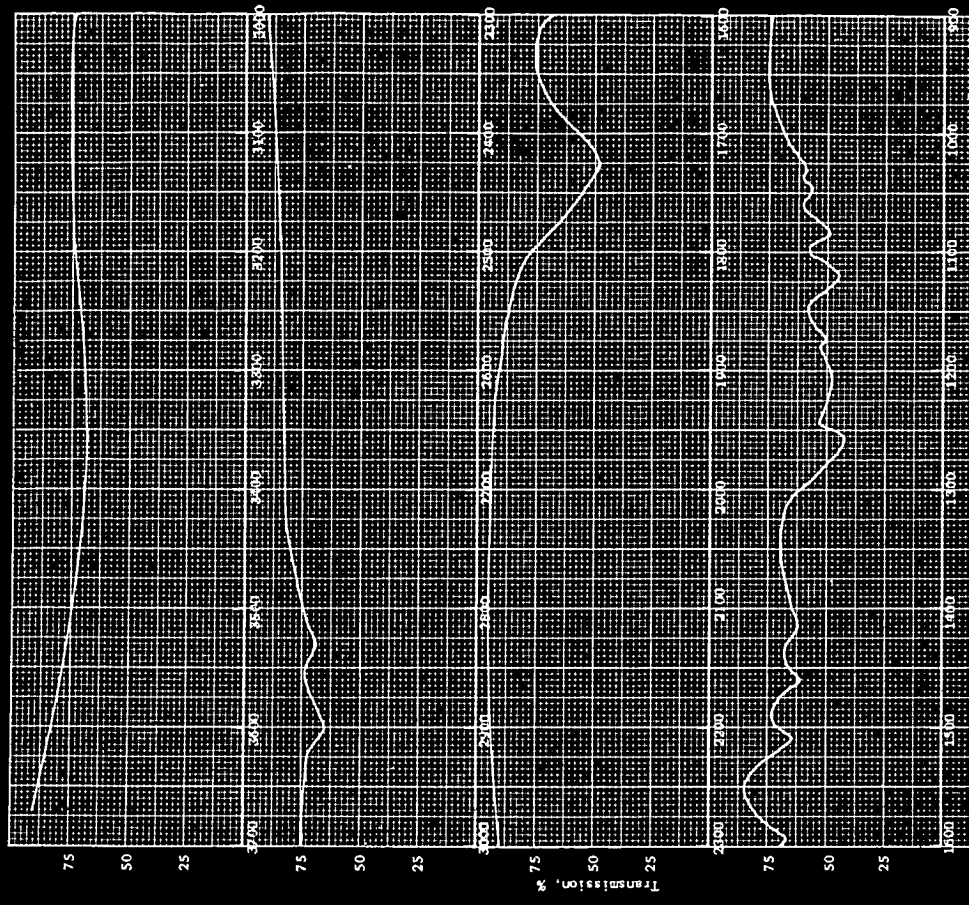


Figure 24
Chlorite spruce Lignin
Film

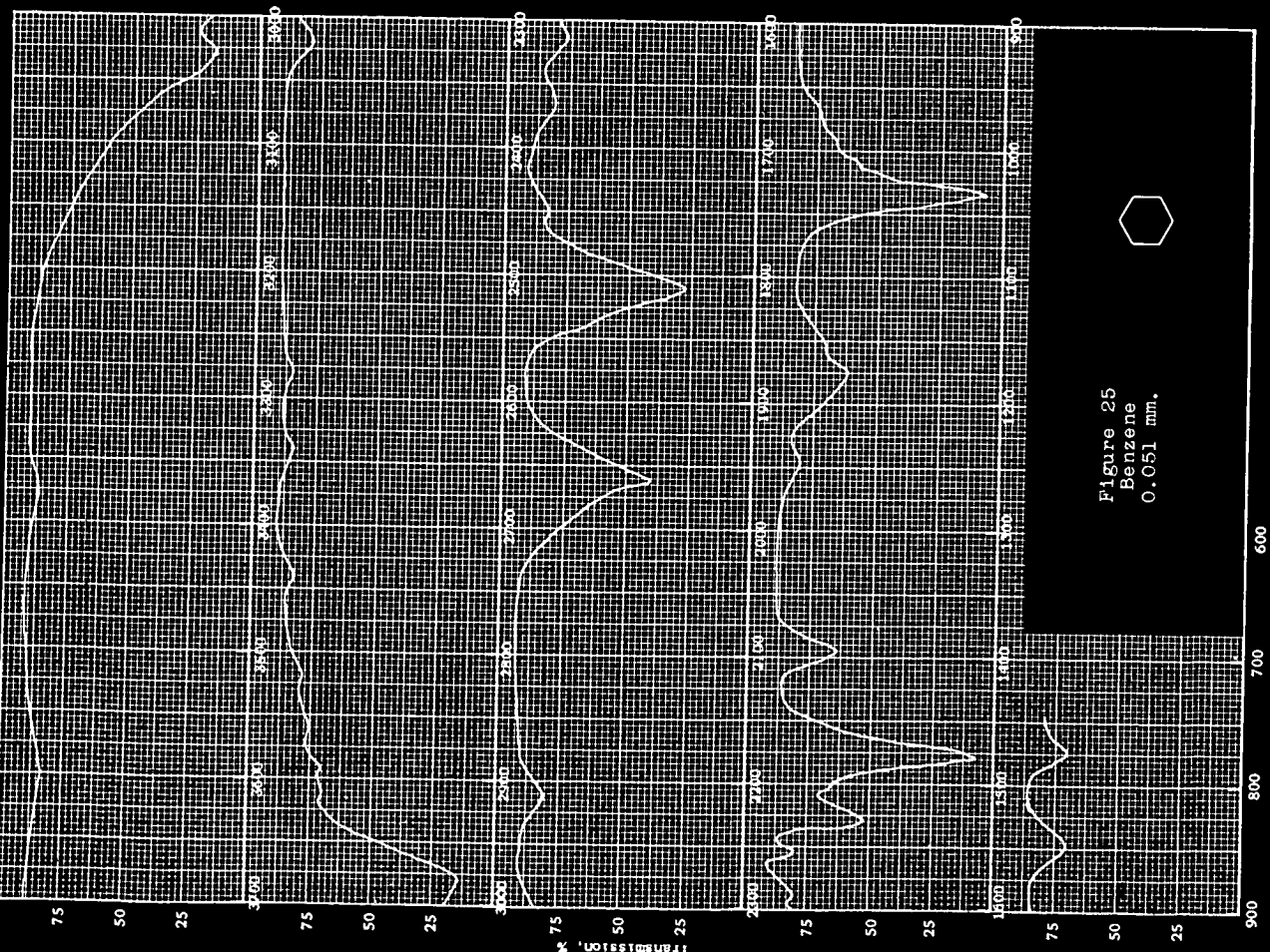


Figure 25
Benzene
0.051 mm.

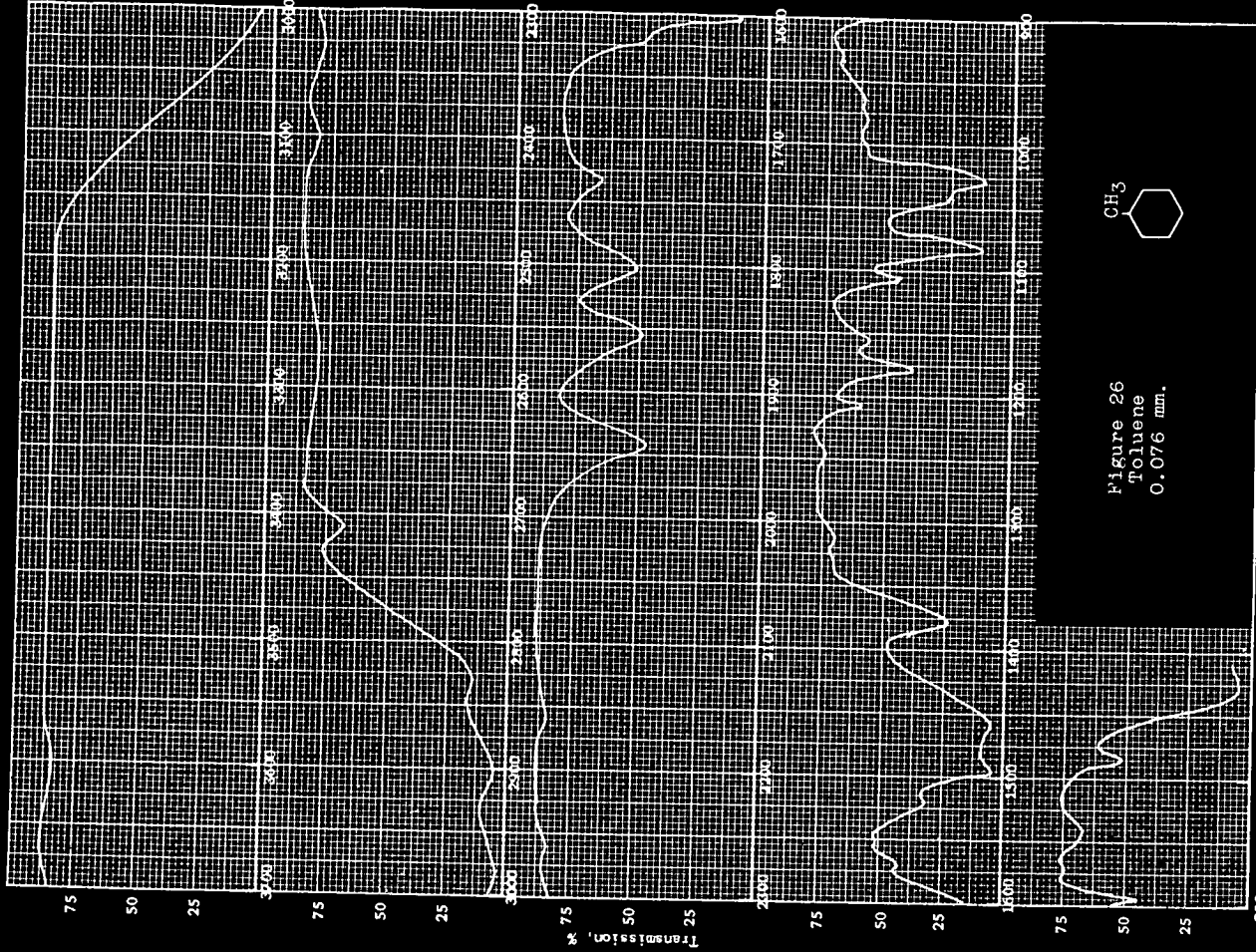
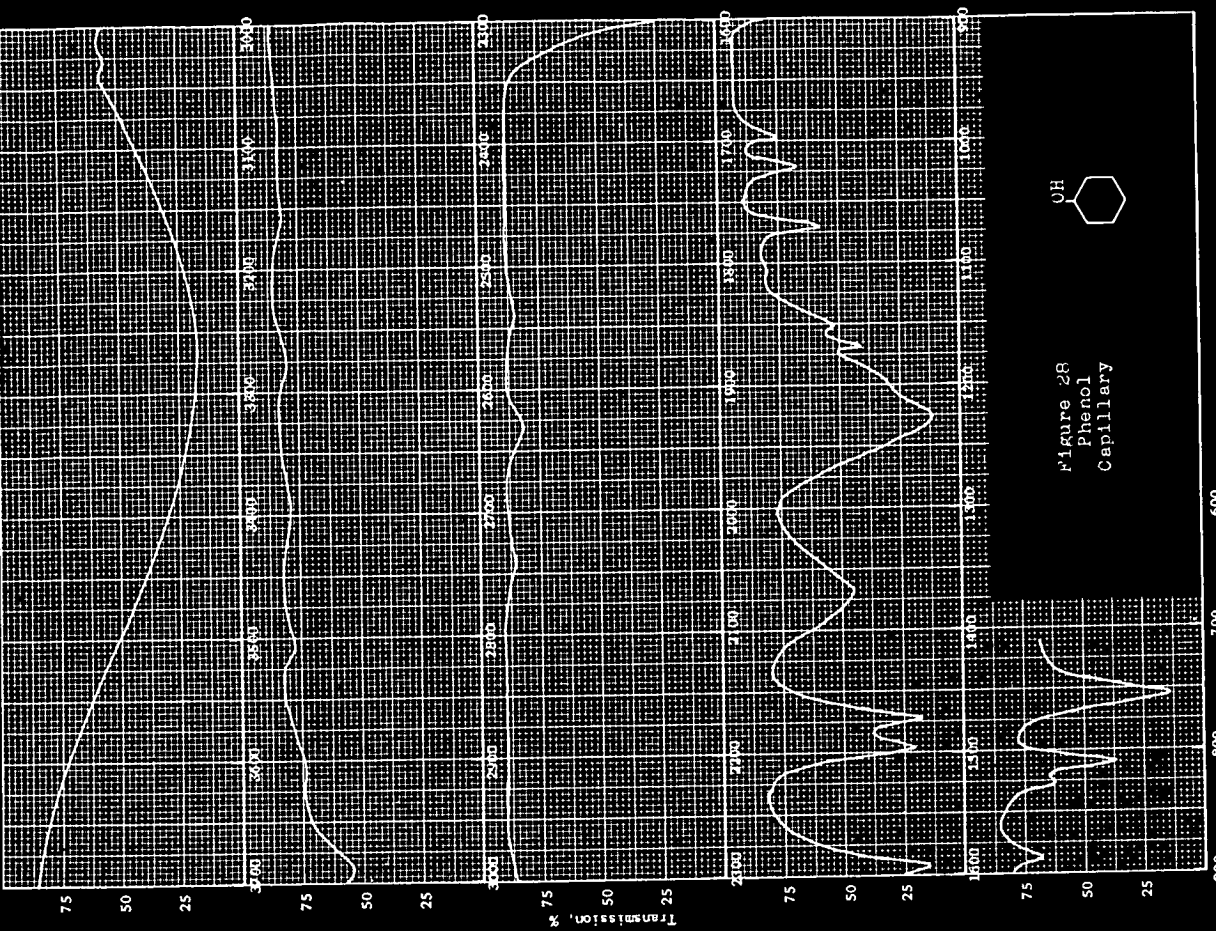
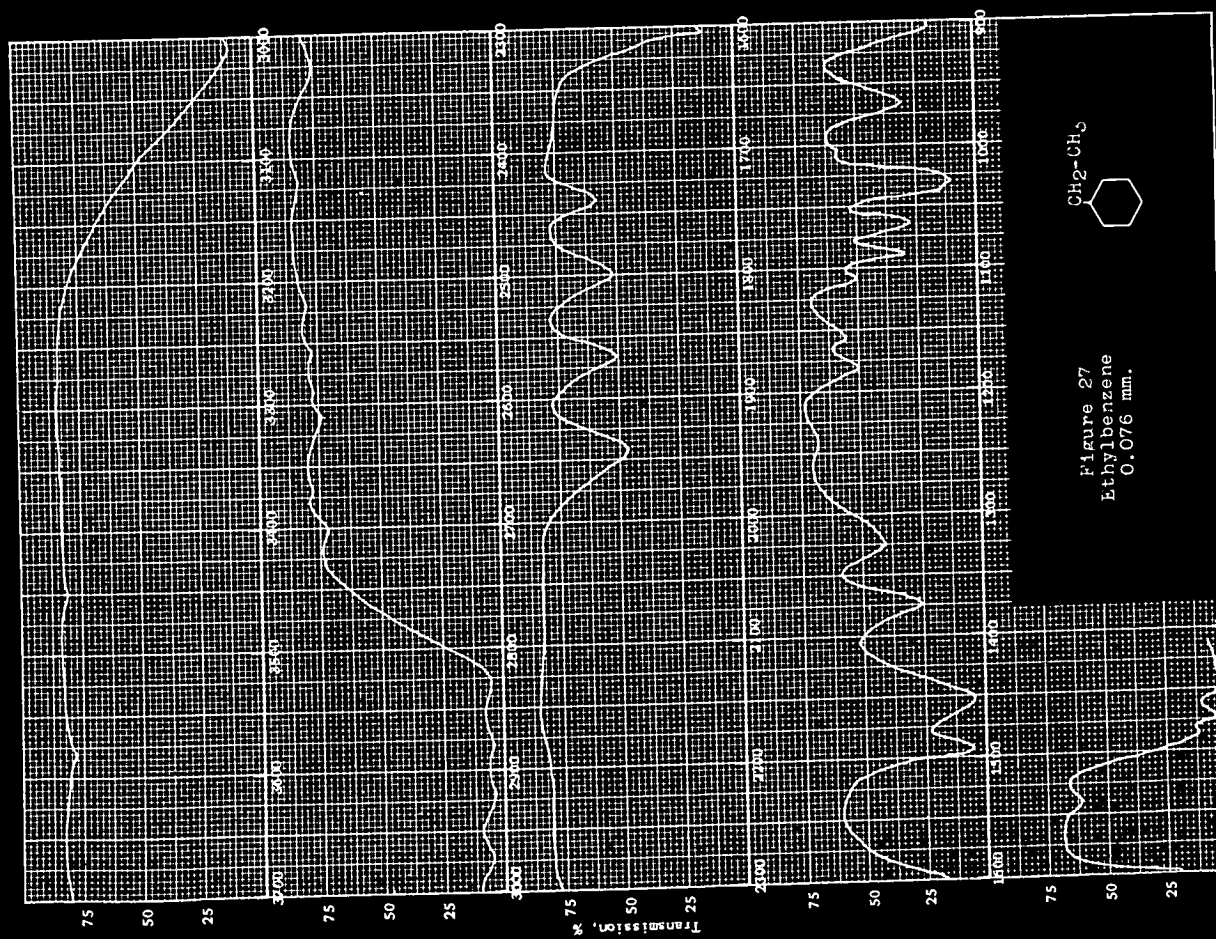


Figure 26
Toluene
0.076 mm.





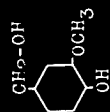
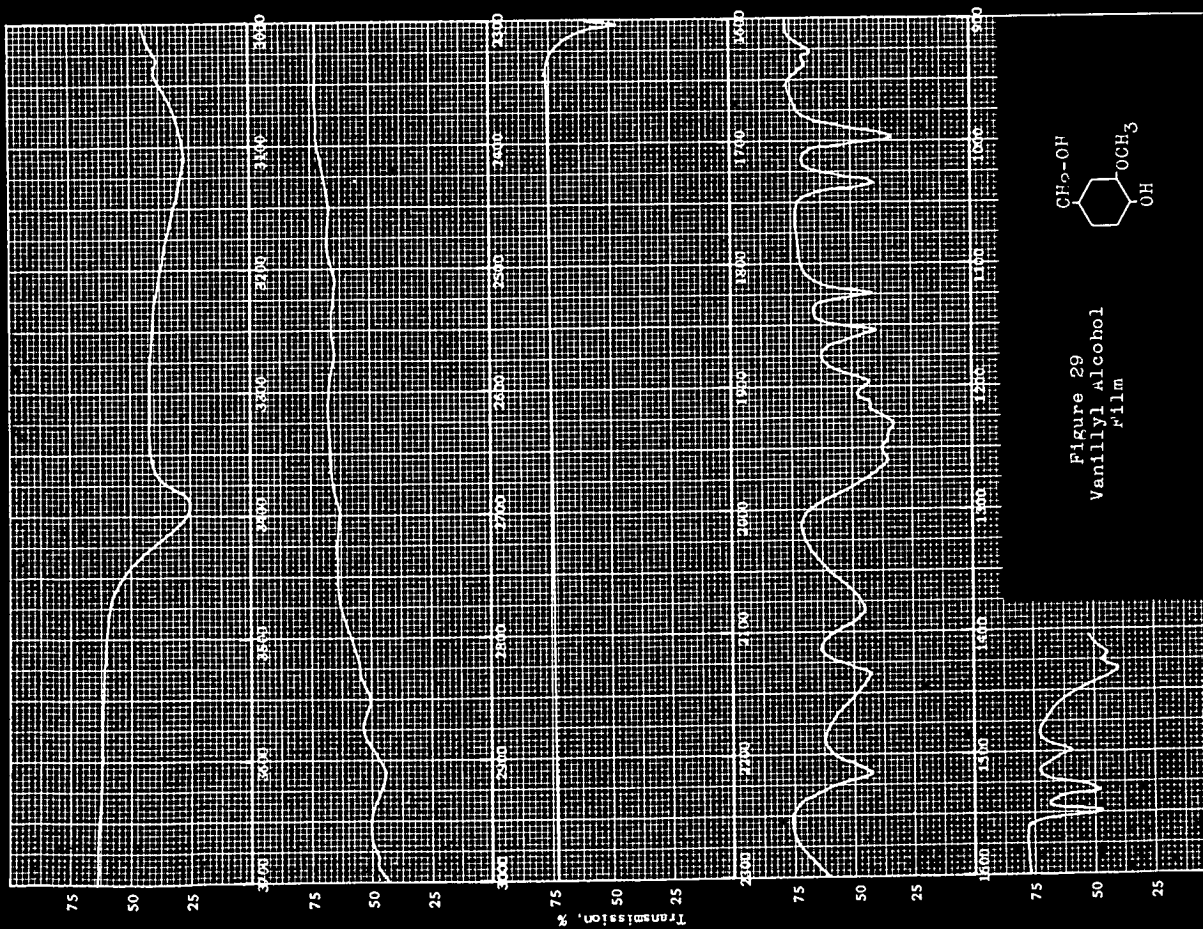


Figure 29
Vanillyl Alcohol
Film

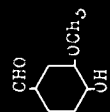
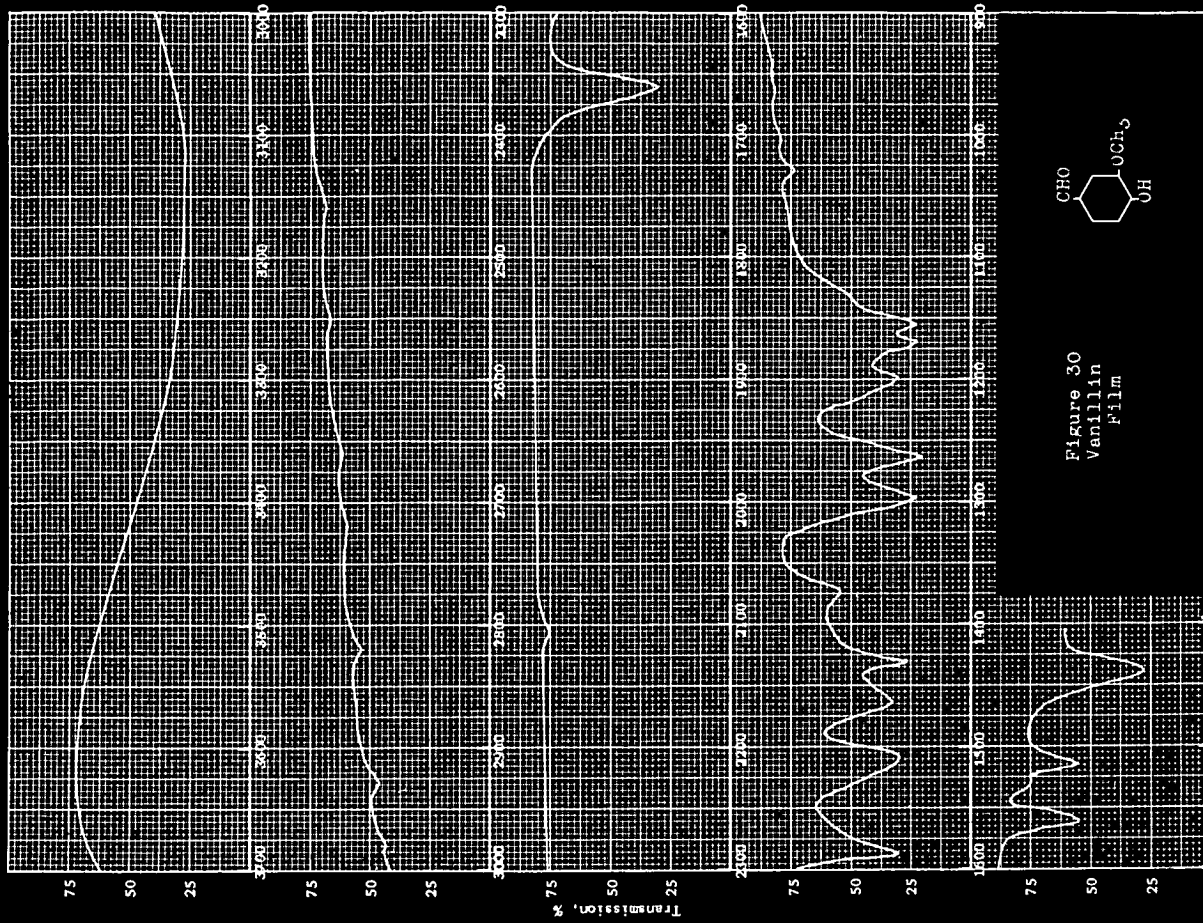
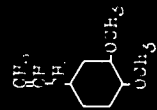
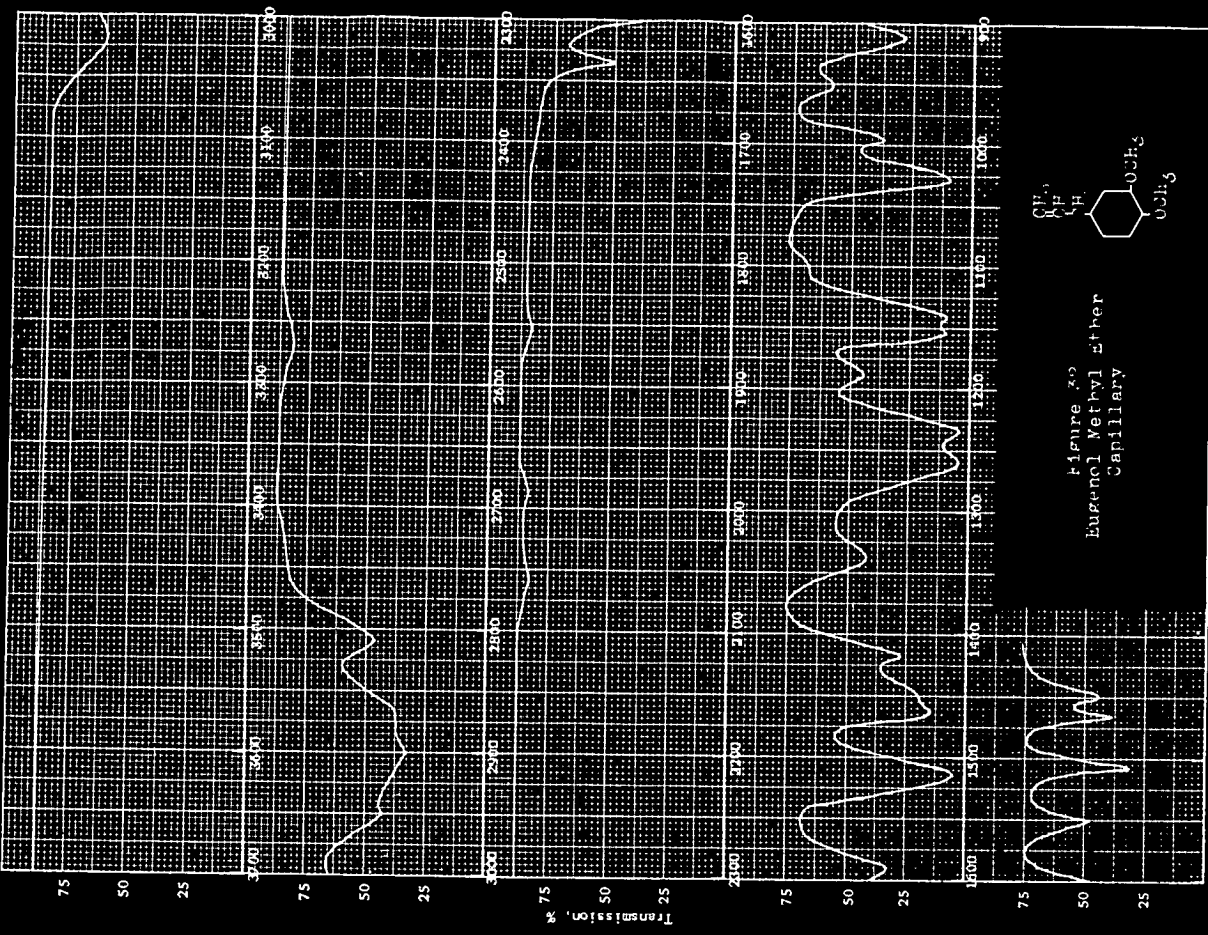
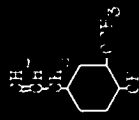
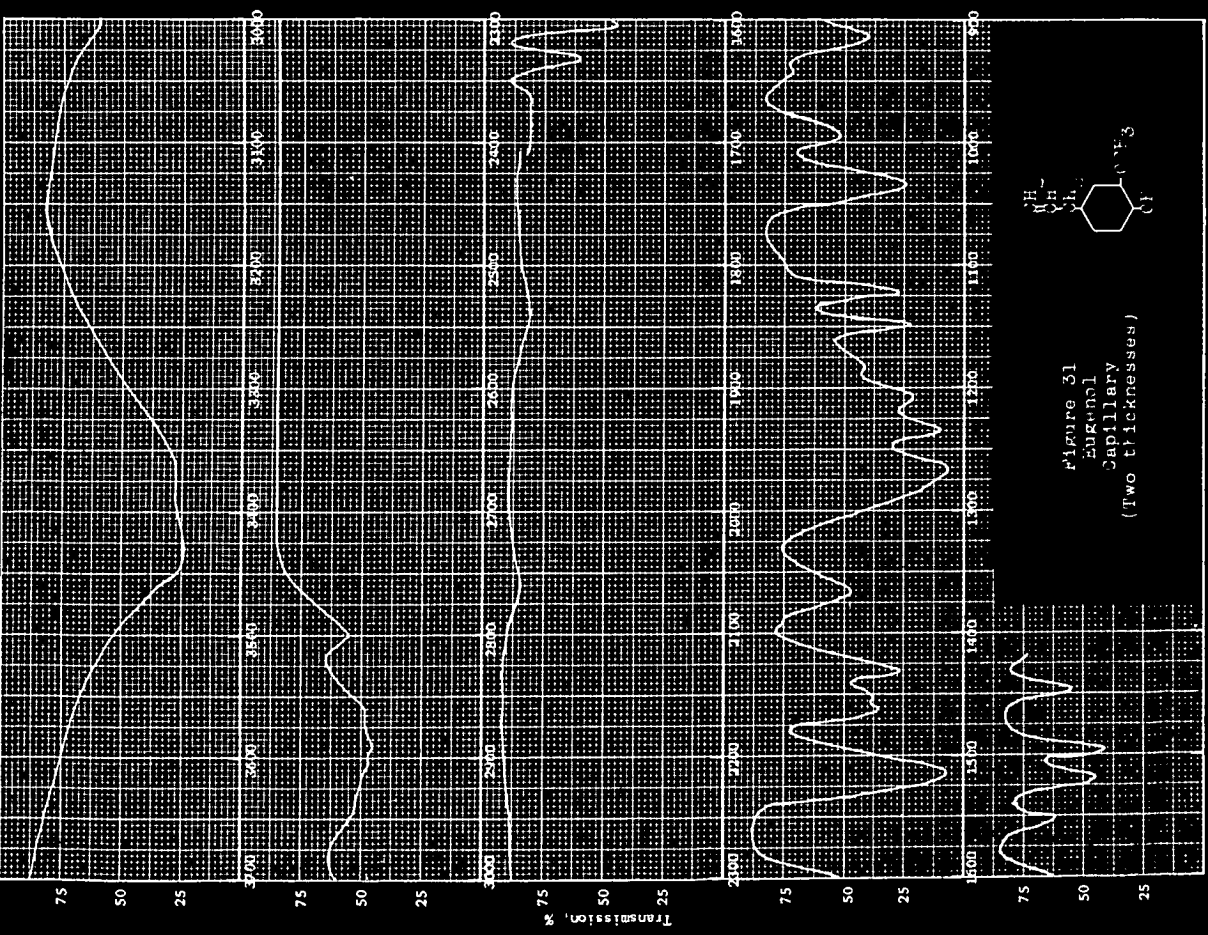
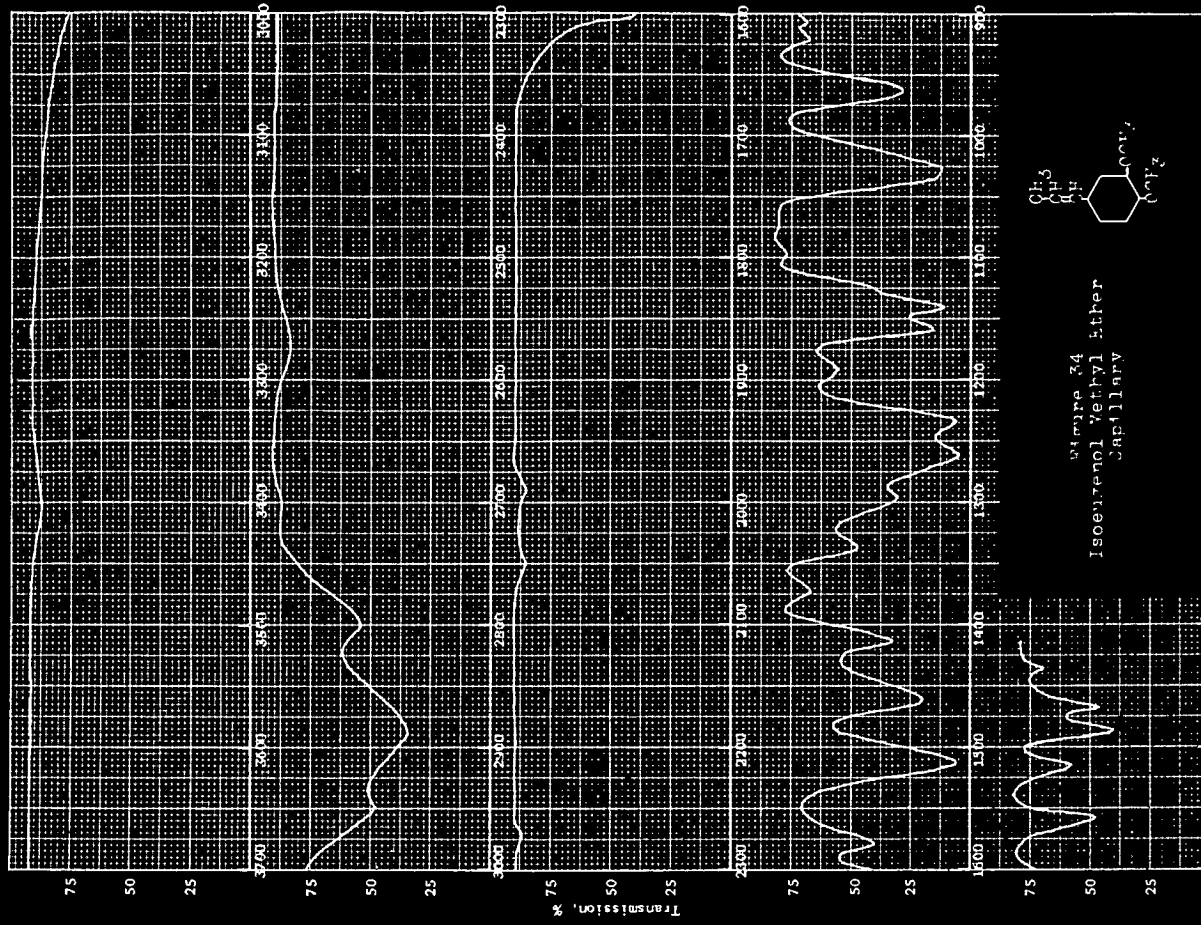
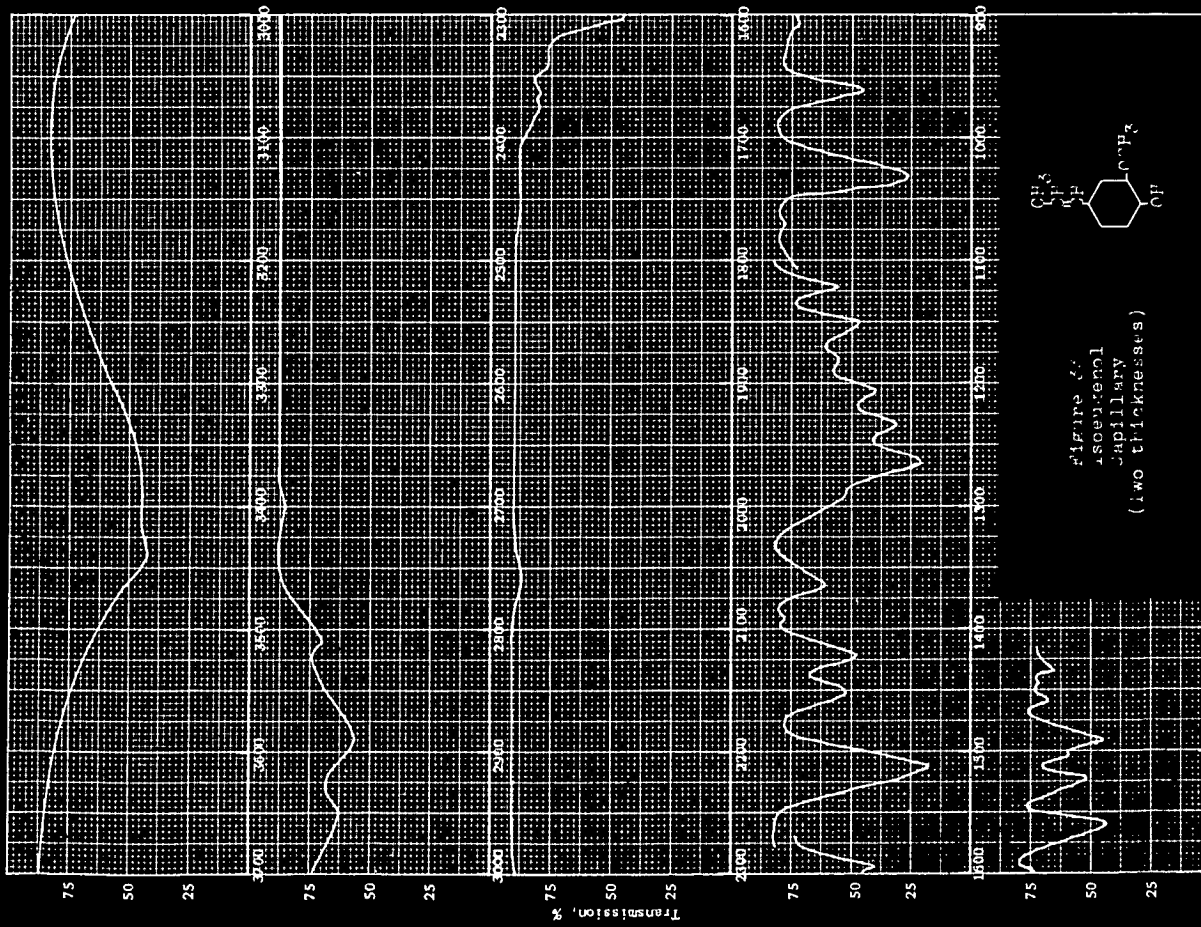


Figure 30
Vanillin
Film





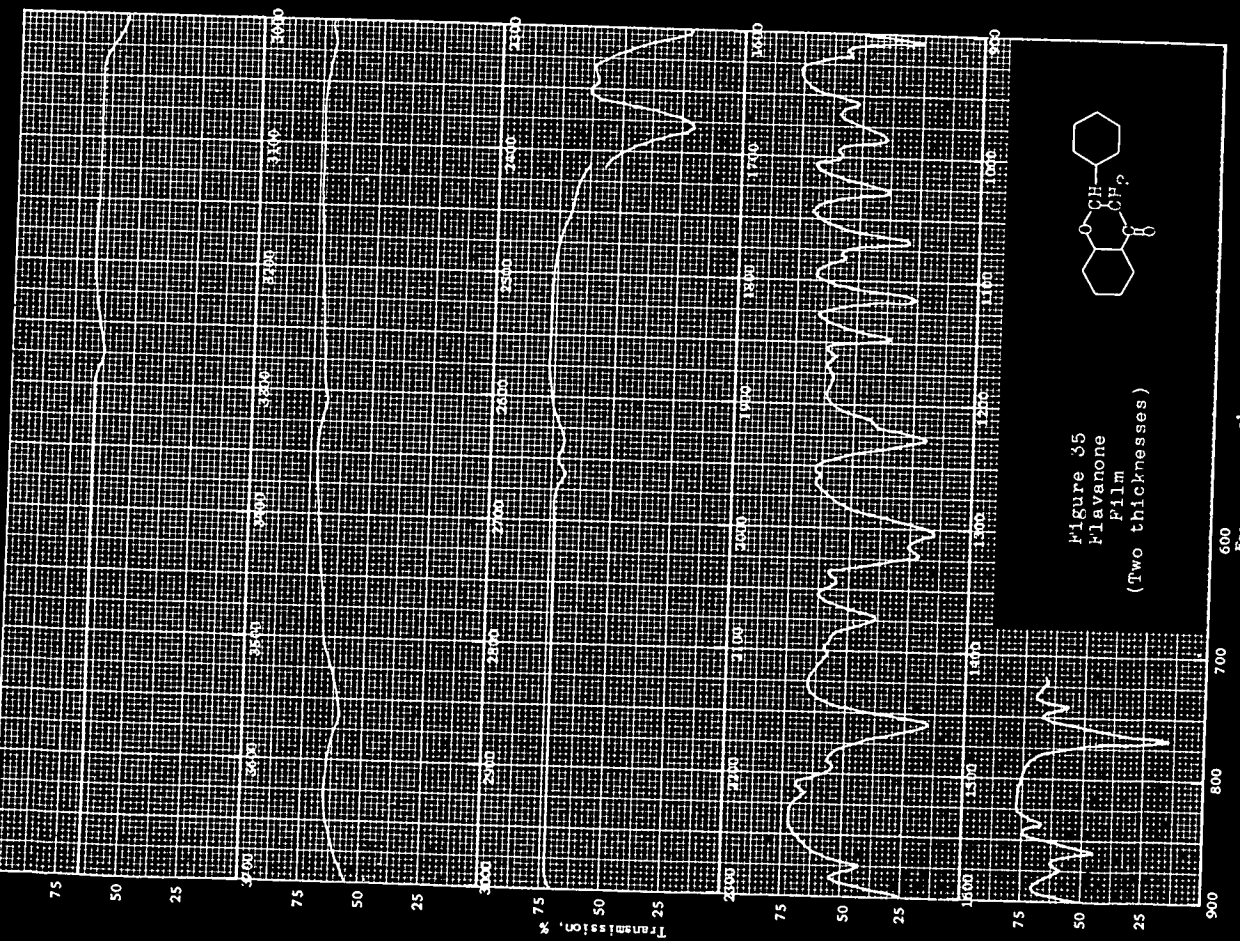


Figure 35
Flavanone
Film
(Two thicknesses)

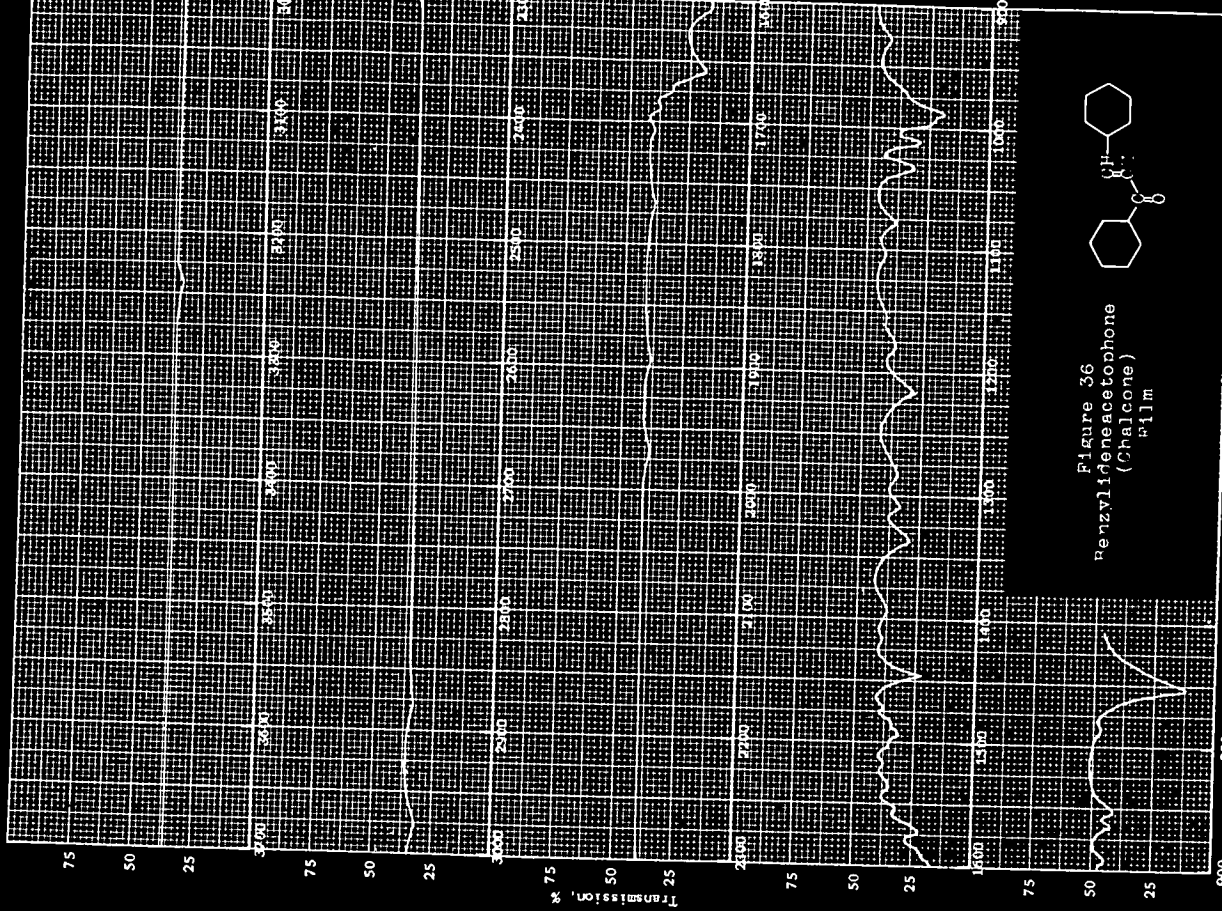


Figure 36
benzylidenacetophenone
(Chalcone)
film

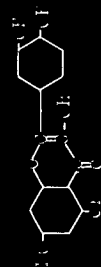
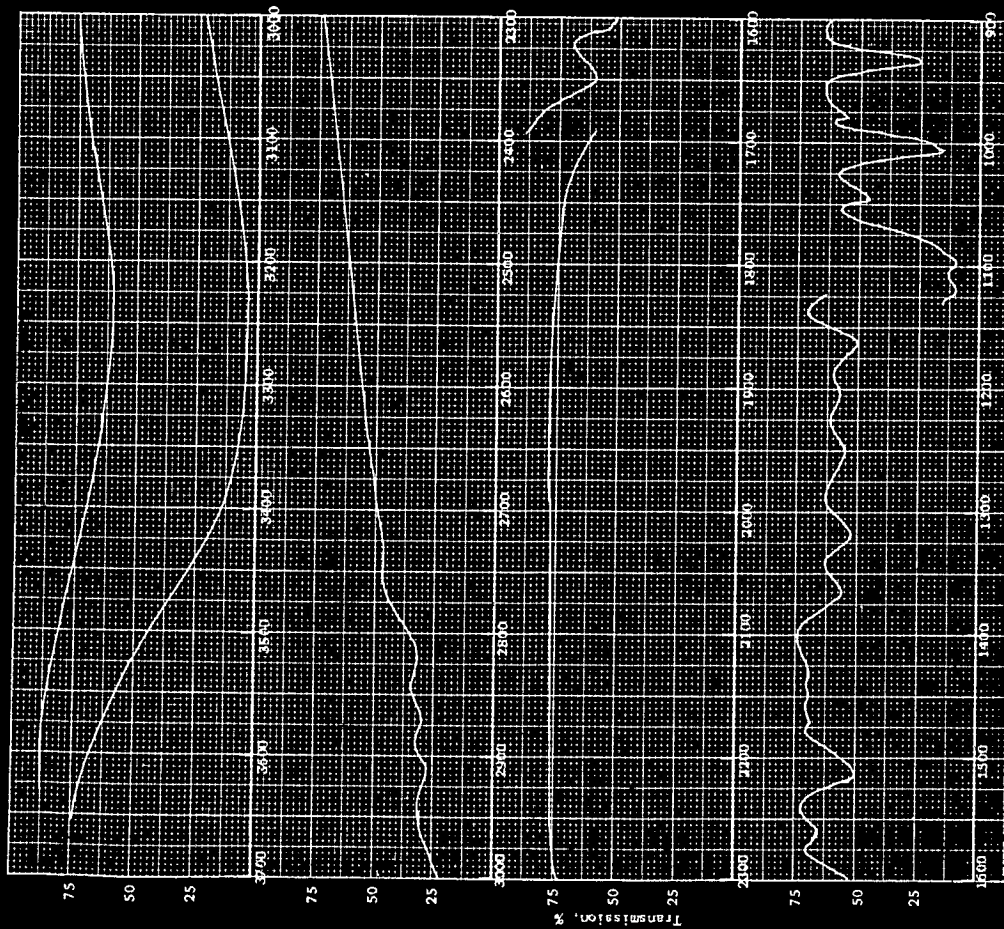


Figure 37
quercetin
film
(Two traces)

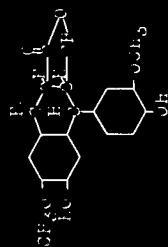
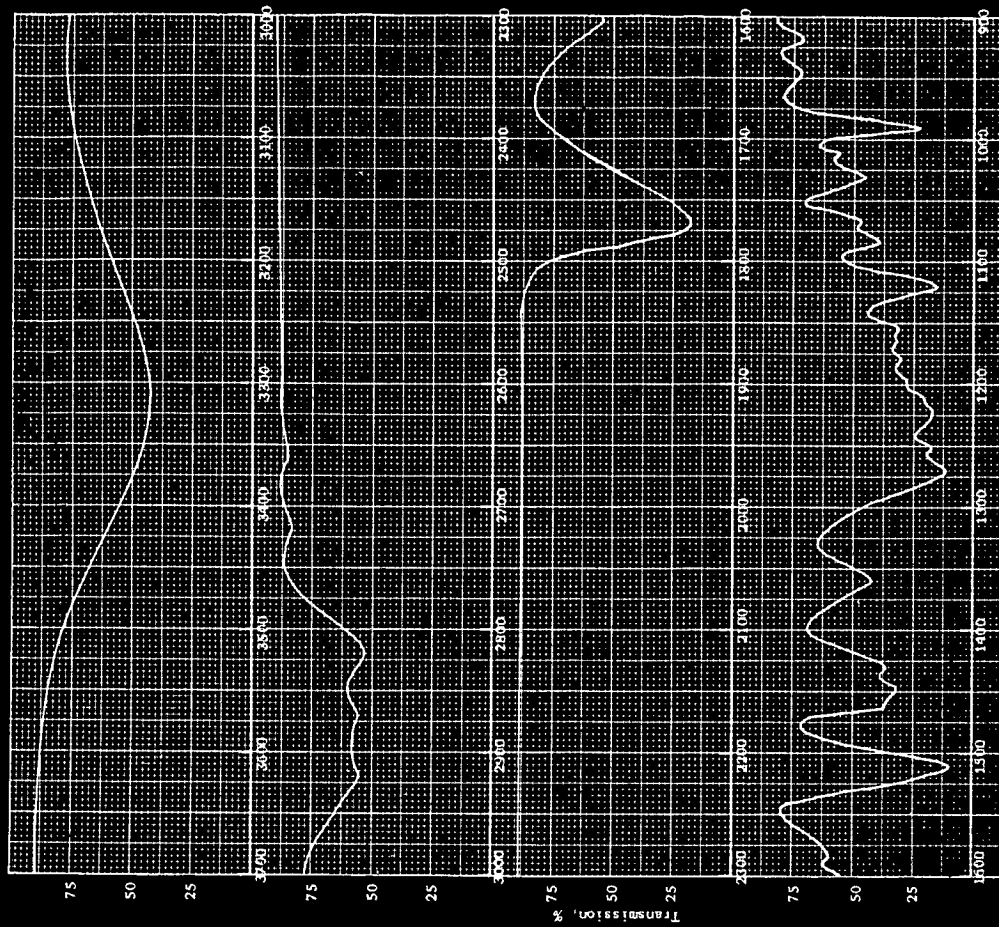
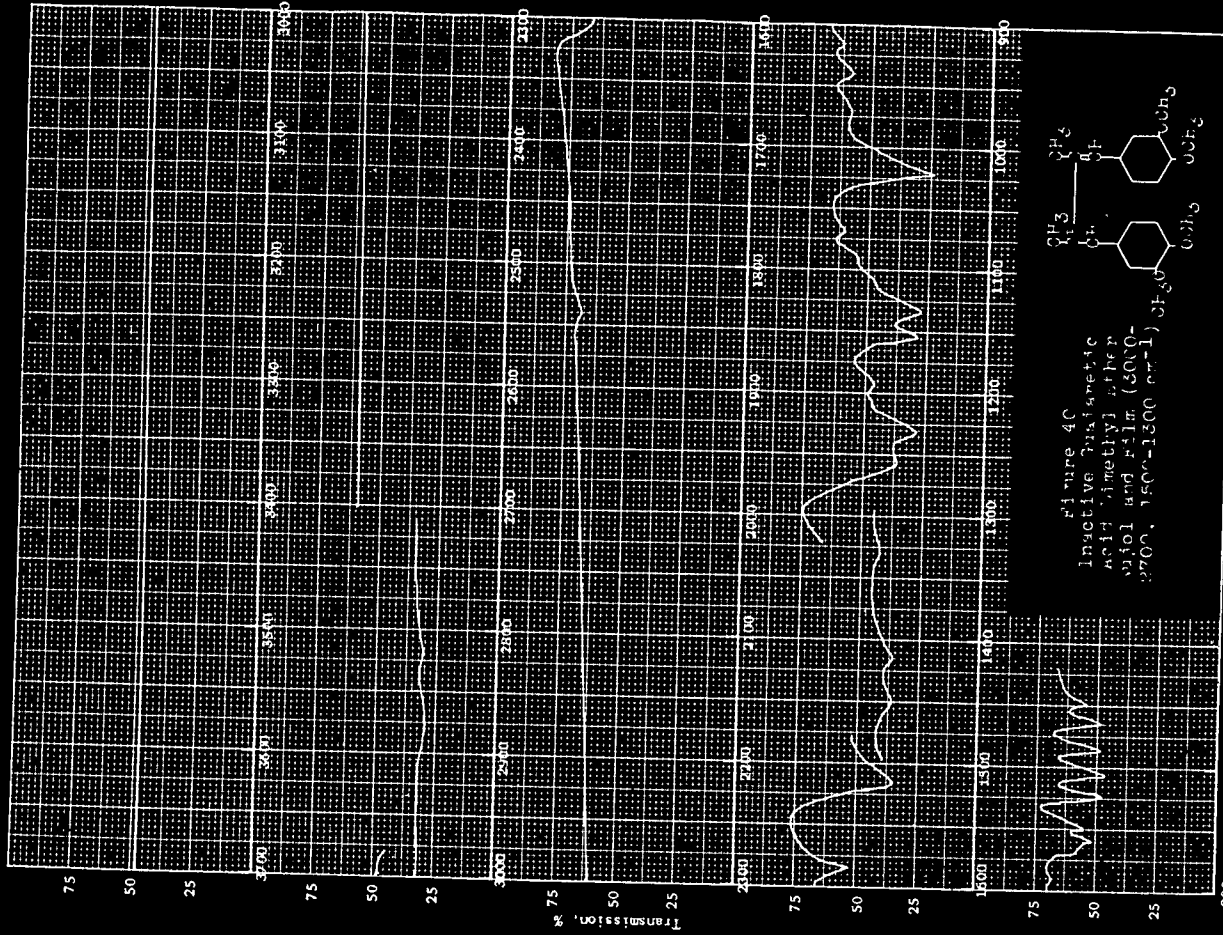
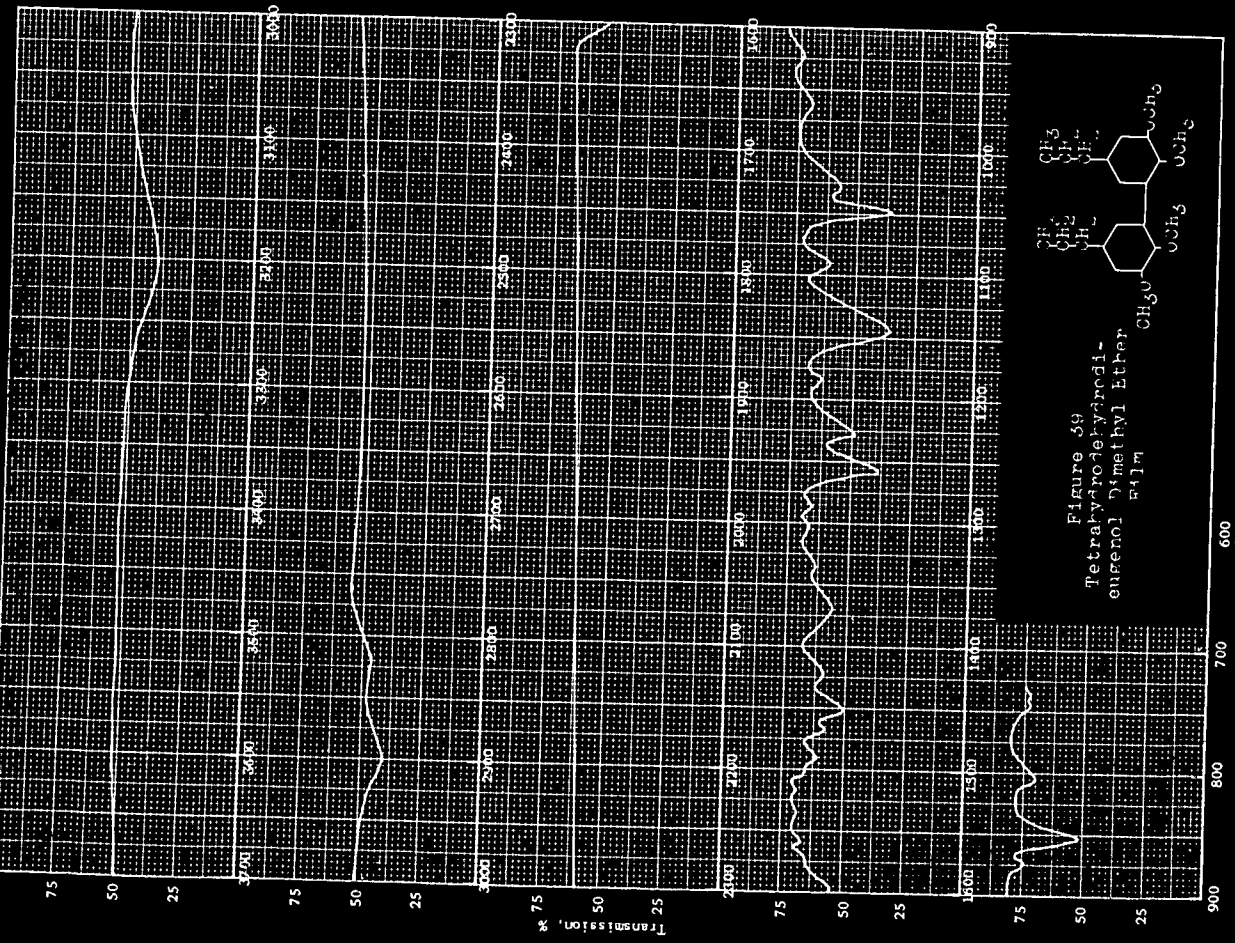


Figure 38
Compound
film



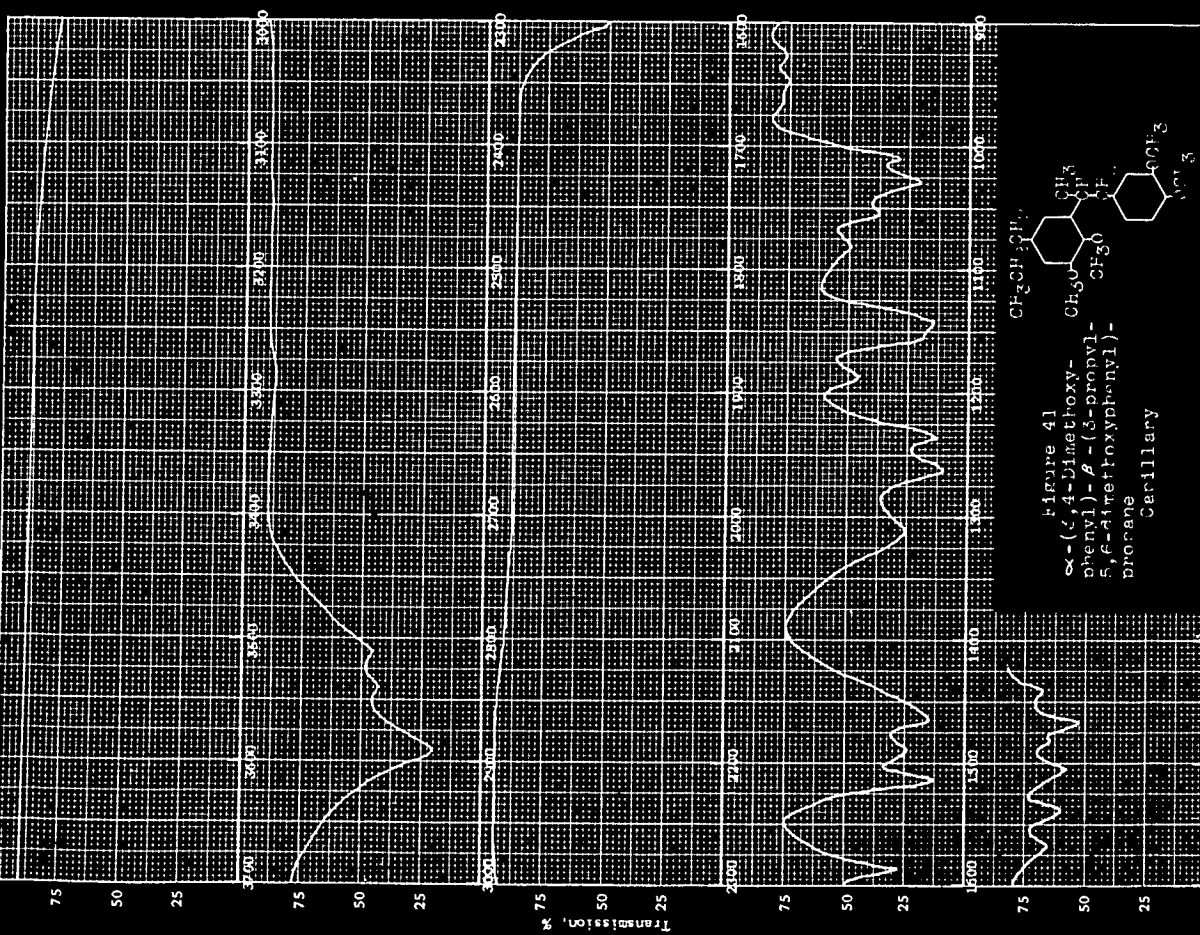
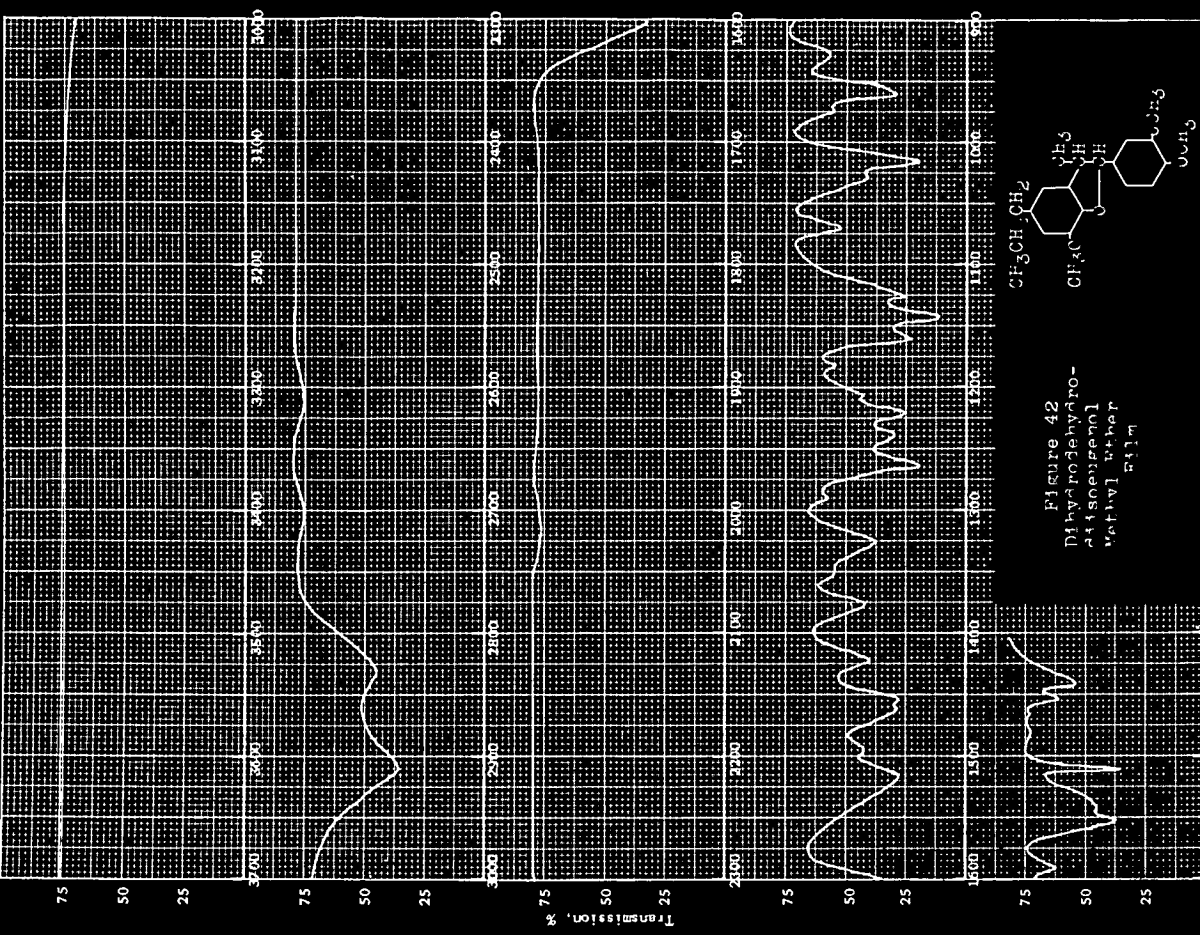
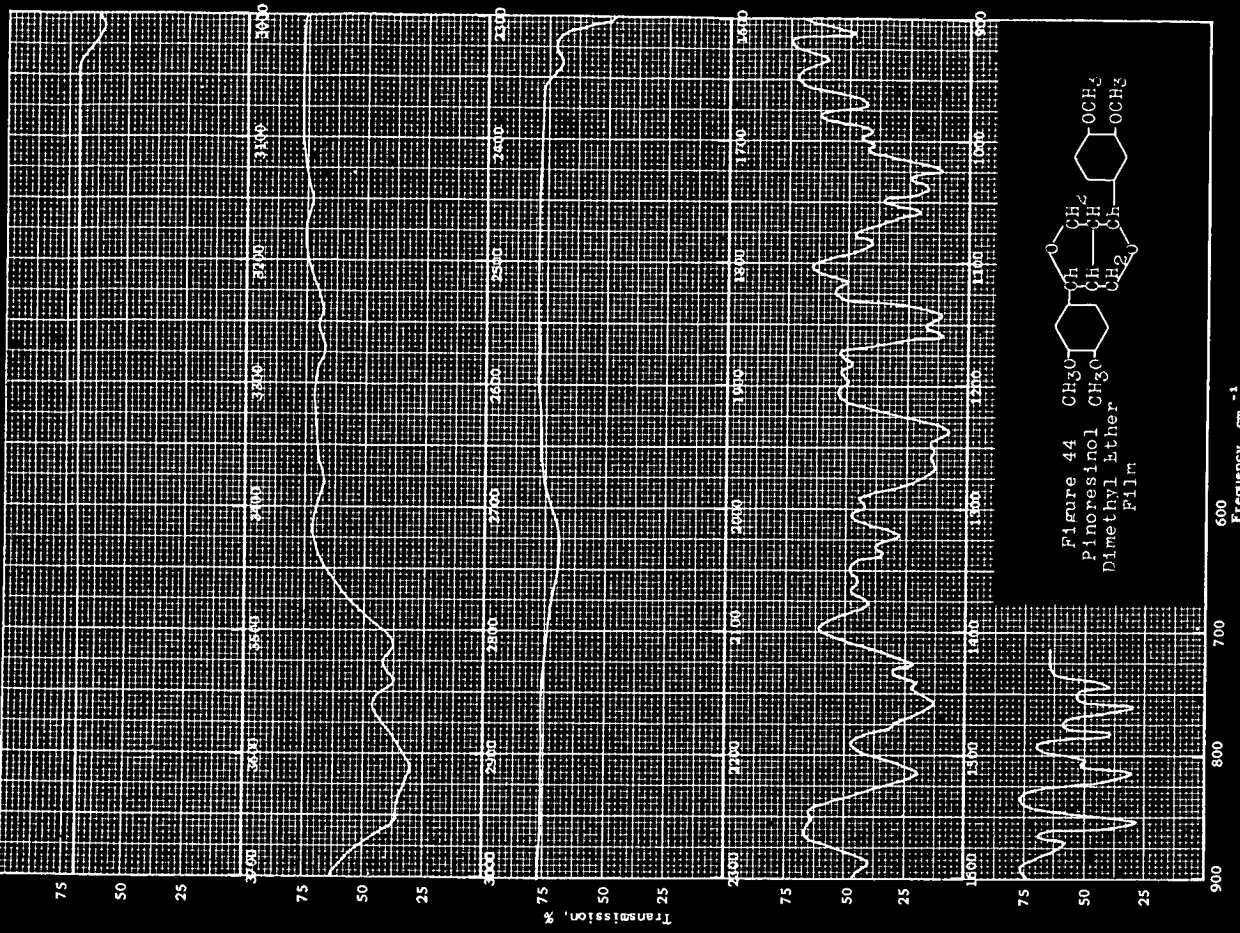
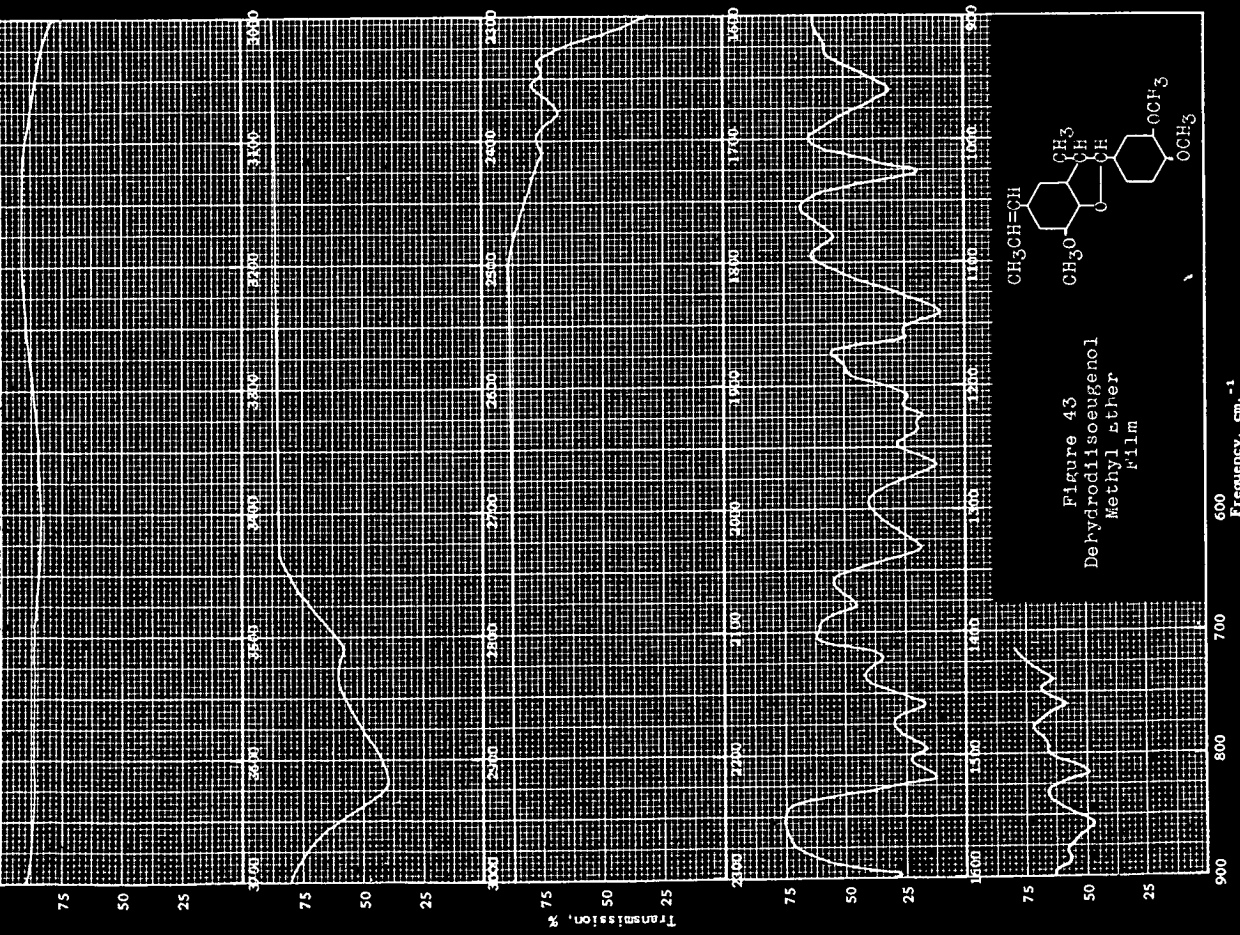


Figure 41
 α -(2,4-dimethoxy-phenyl)- β -(3-propyl-5,6-dinitroxyphenyl)-propane
 Carillary





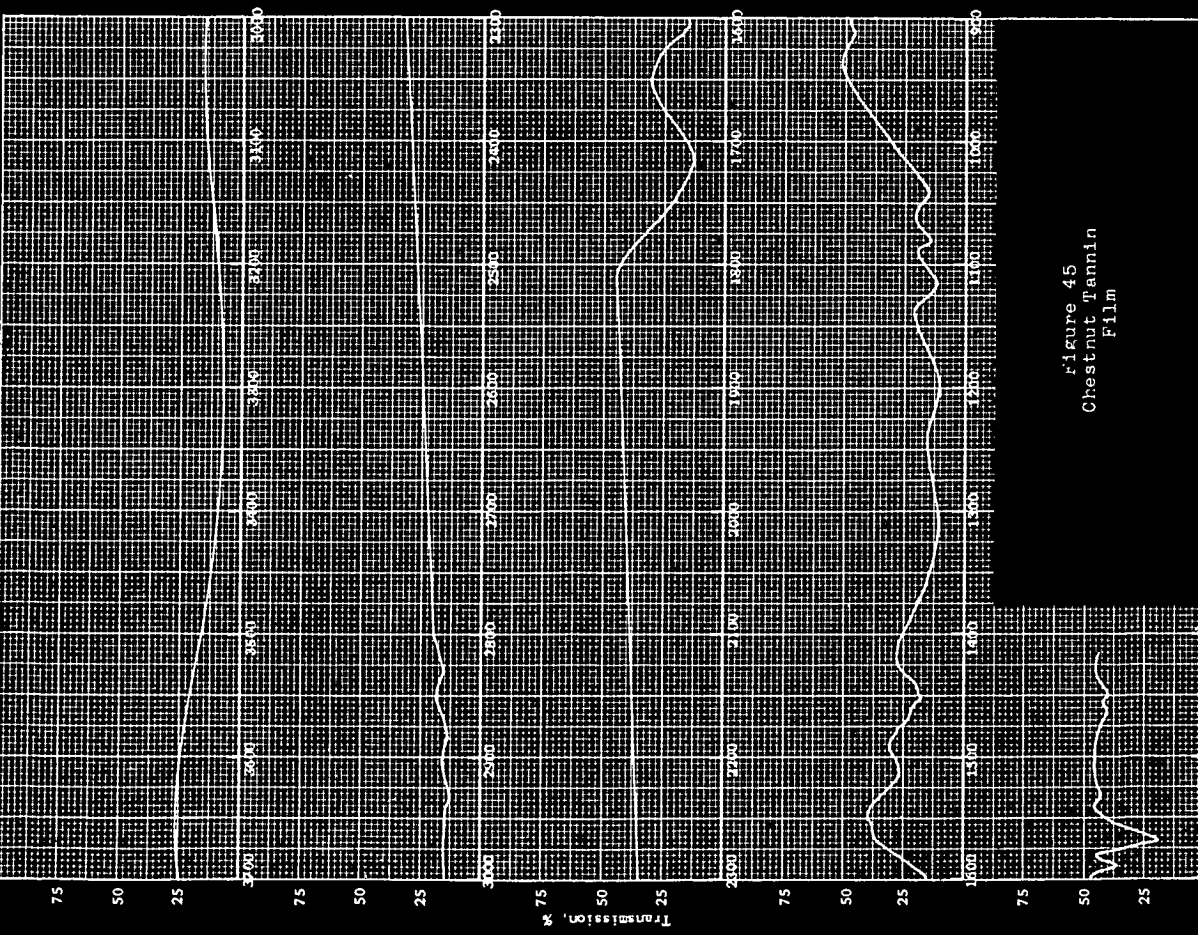


Figure 45
Chestnut Tannin
Film

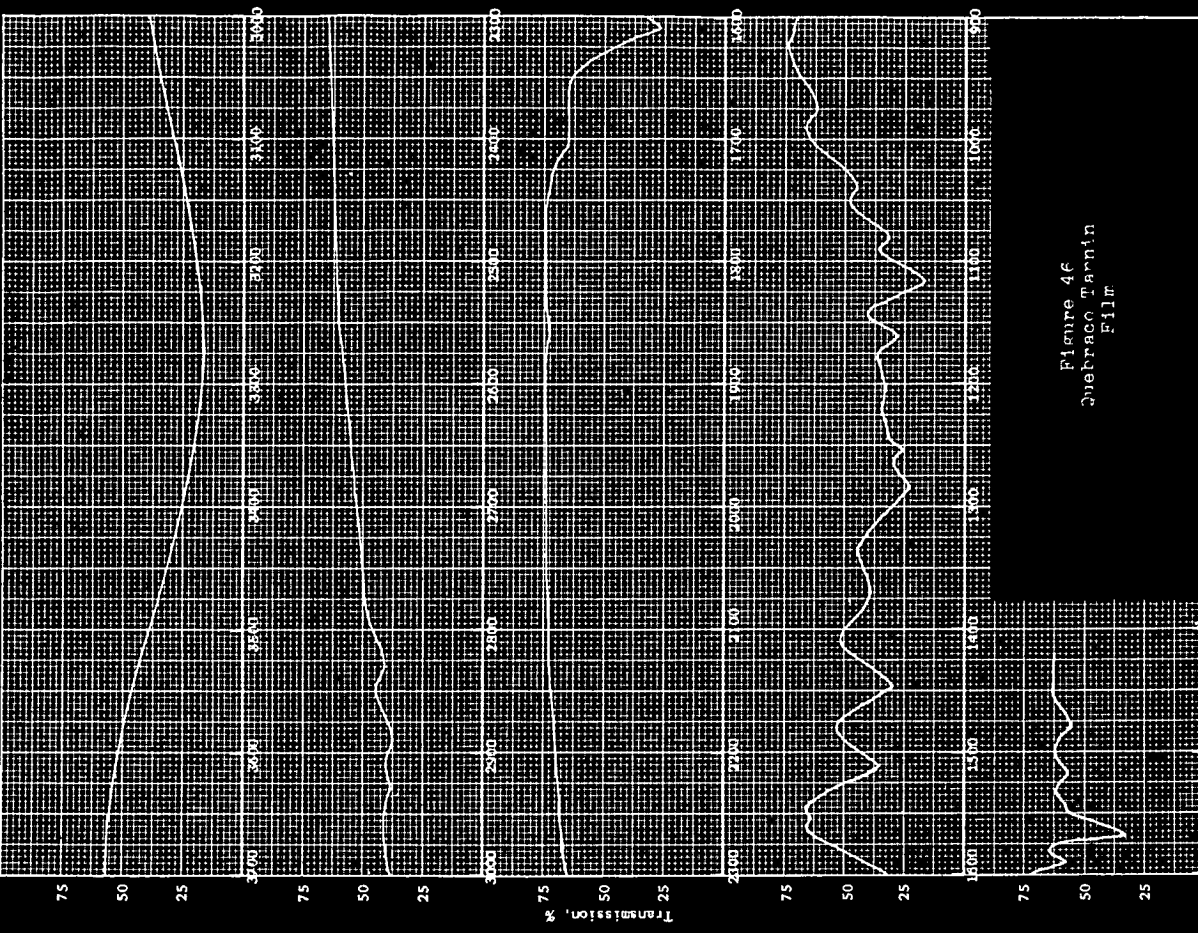


Figure 46
Quebracho Tannin
Film

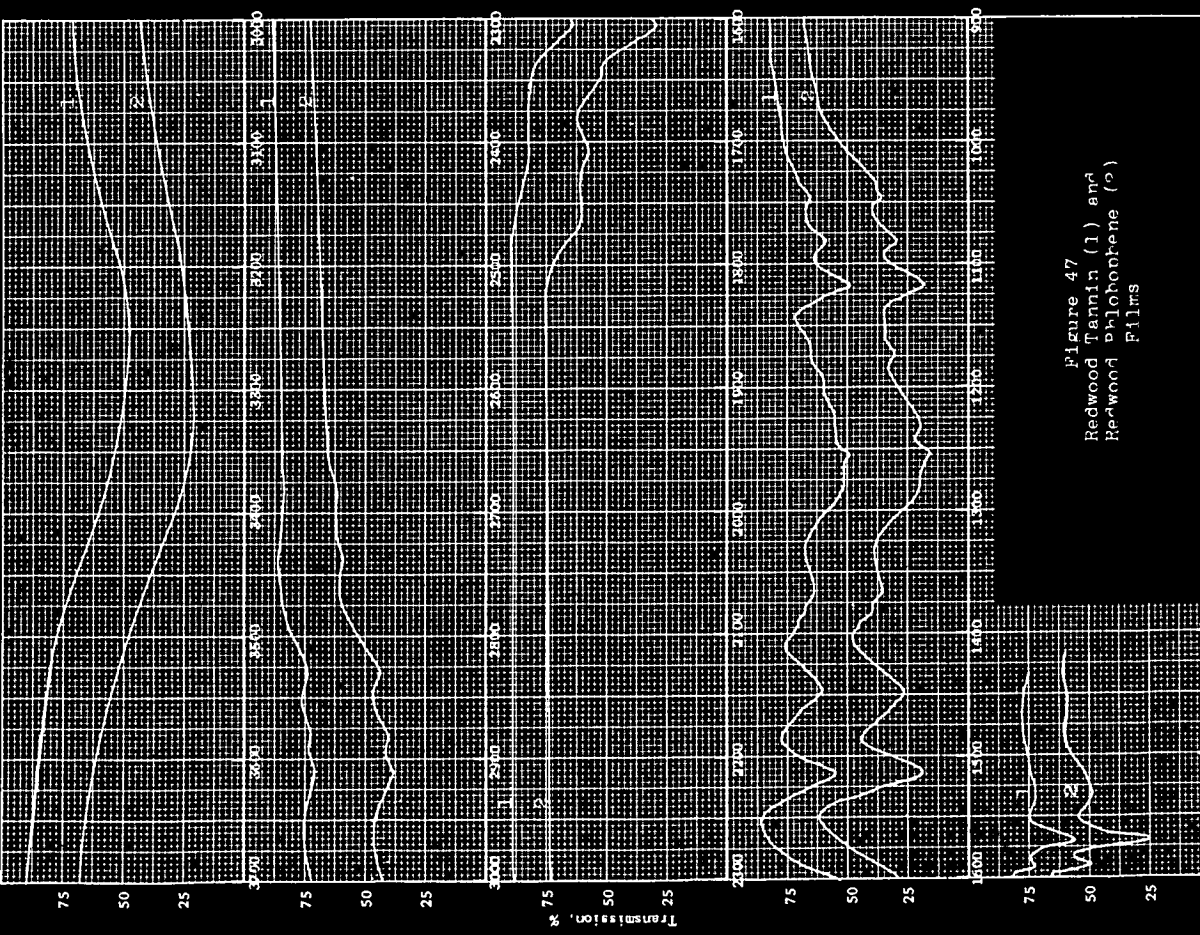


Figure 47
Redwood Tannin (1) and
Redwood Phlobaphene (2)
Films

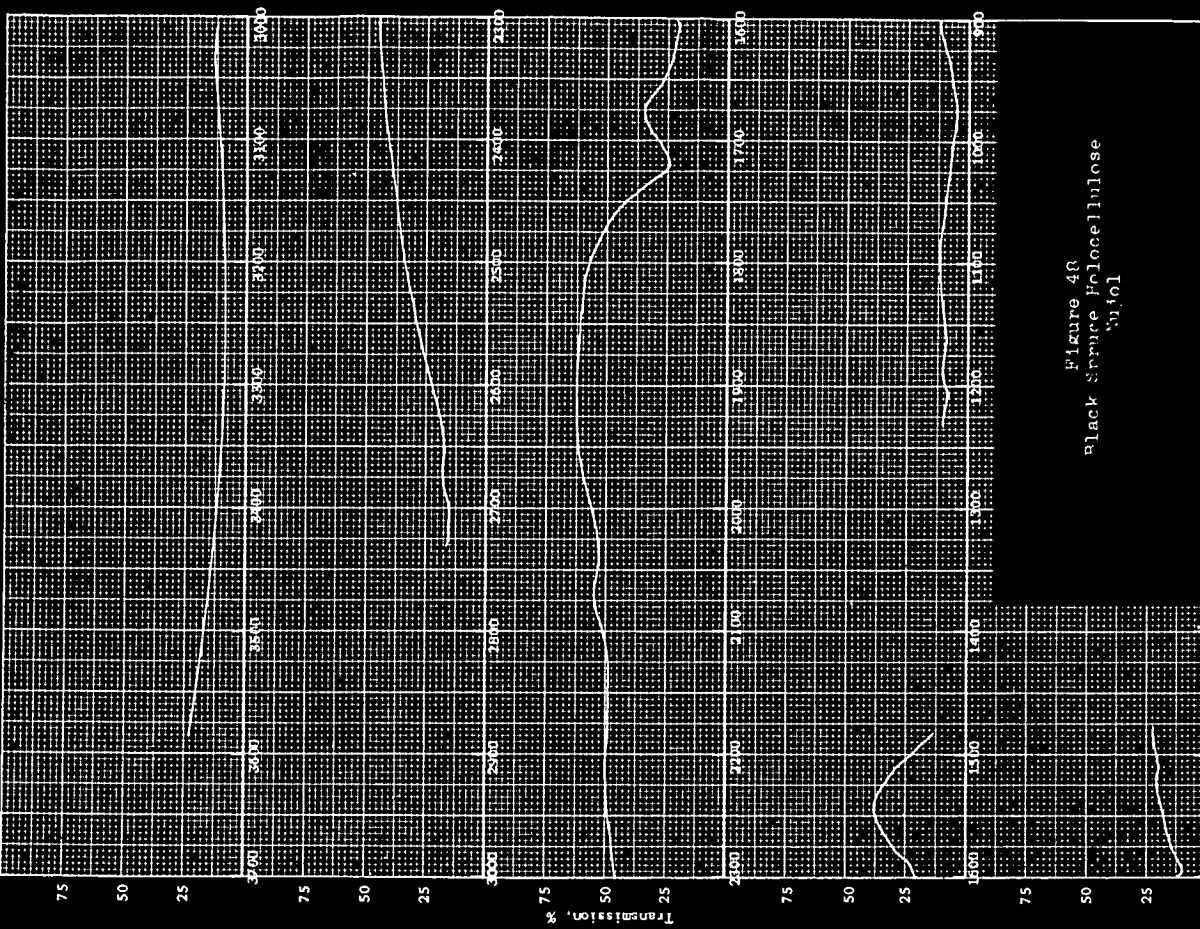
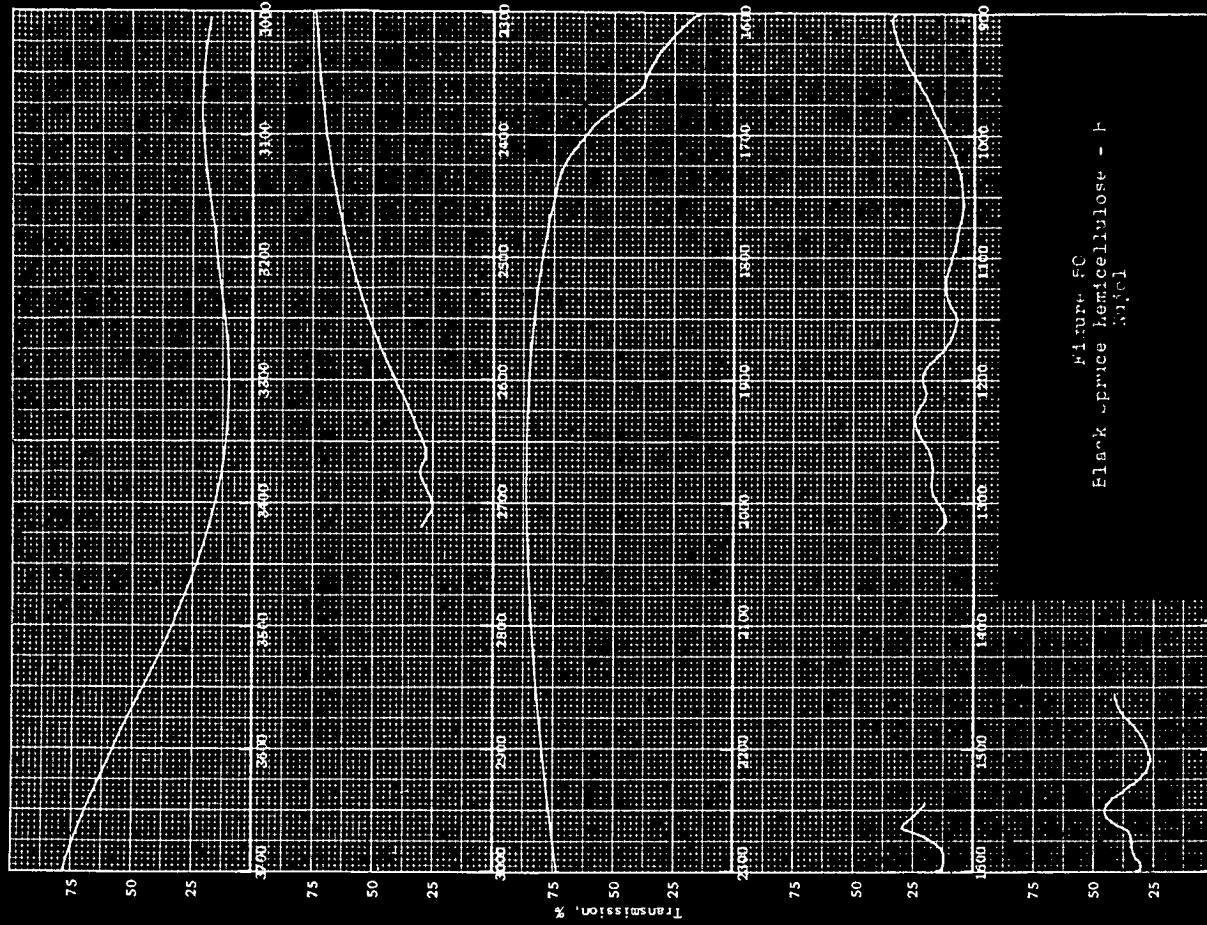
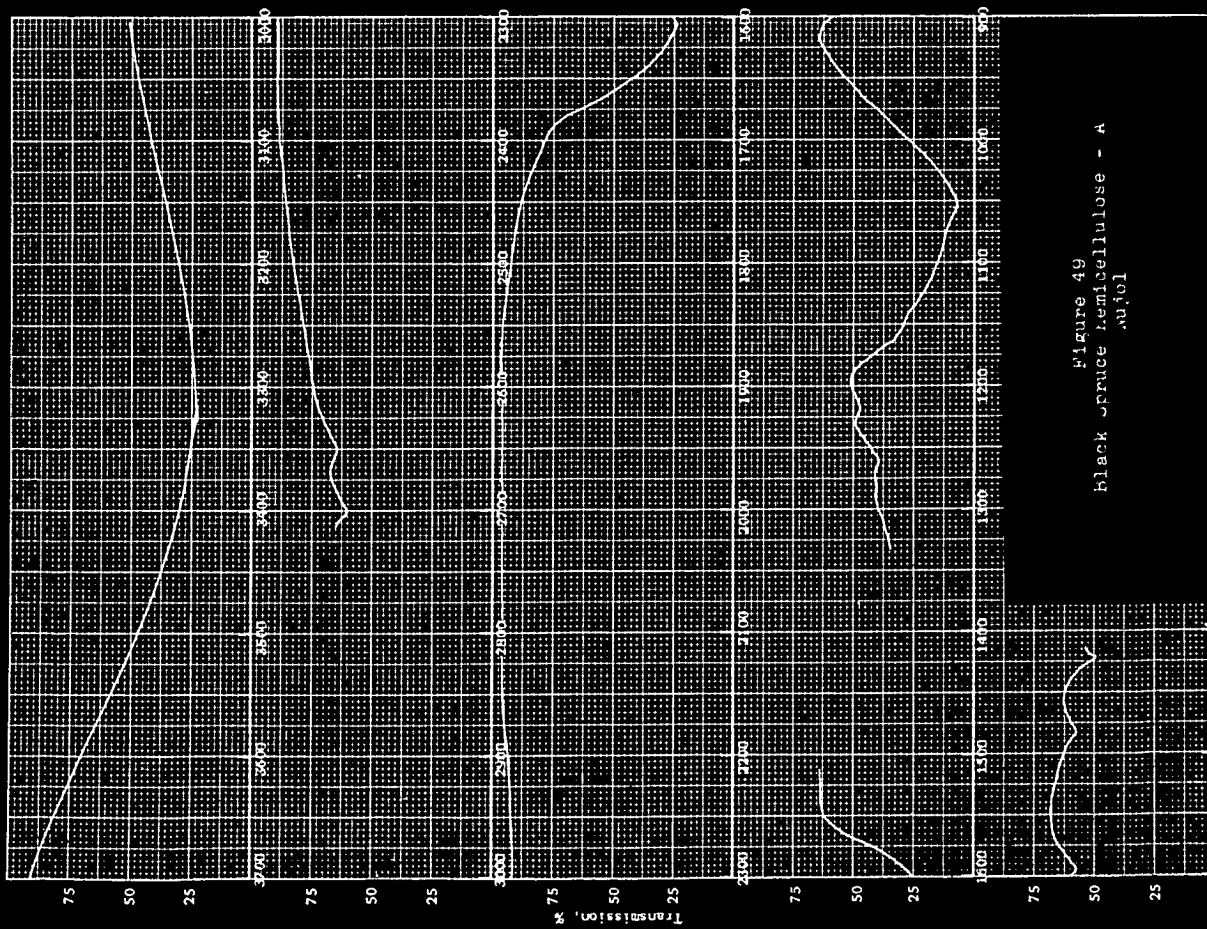


Figure 48
Black Spruce Polocellulose
Film



APPENDIX

THE ULTRAVIOLET ABSORPTION SPECTRA OF COMPLEX HYDROXY-AROMATIC COMPOUNDS
AND DERIVATIVES, WITH PARTICULAR REFERENCE TO LIGNIN

Since the development, around 1930, of relatively simple and accurate methods of quantitatively determining the ultraviolet absorption spectra of organic compounds in solution, extensive use of this tool has been made as a supplement to chemical methods in determining structural features of natural and synthetic compounds. Many important contributions have been made, particularly with respect to distinguishing between isomeric forms or possible spatial arrangements where the choice lay between a chromophoric resonating structure and a nonabsorbing form.

The ultraviolet spectrographic method has been applied extensively in the field of natural plant materials. For the purpose of this discussion, a large class of these compounds might be designated as hydroxy-aromatic compounds and derivatives in that, regardless of the complexity of their structure, they possess in common oxygen-substituted benzene rings, the oxygen being part of a hydroxyl, ether, or ester grouping. Such a broad classification would include such diverse materials as the flavylum coloring matters and flavanones, the tannins, the lignans, and lignin preparations. All these materials have been subjected to ultraviolet spectrographic studies and, in the case of the lignans, where a large group of compounds of known structure belonging to this family was available, it has been possible to relate variations in ultraviolet spectra to structural type (1).

Lignin, in particular, has received much attention, both because of its volume importance and because chemical methods have thus far failed to establish the details of its structure. During the past twenty years, four major studies of the ultraviolet spectrum of lignin have been published. Herzog and Millner (2-8), in a series of seven papers, collected the spectra of lignins from a number of sources and systematically assembled comparable data for some eighty or ninety simple aromatic compounds. Glading (9) studied the spectrum of spruce native lignin and a number of its derivatives as related to a group of model aromatic compounds. Patterson and Hibbert (10, 11) determined the spectra of a number of phenylpropane derivatives and compared these with the spectra of softwood (spruce) and hardwood (maple) lignins. Aulin-Erdtman (12) compared the spectra of ligno-sulfonic acids and spruce native lignin with a comprehensive group of synthetic model compounds.

In analyzing their experimental results, all these authors have agreed in concluding that the ultraviolet spectrum of lignin is that of a substituted benzene nucleus, yet each, in attempting to completely account for the shape of the curve, has given a different interpretation. Herzog and Millner reached the conclusion that the spectrum of lignin definitely showed the material to be made up of partly or completely methylenated catechol or pyrogallol nuclei joined together with a side chain of about three carbons, the side chain containing no ethylenic double bonds or carbonyl groups in conjugation with the benzene ring (7, 8). They also cautiously pointed out that polymerized coniferyl alcohol gives an absorption spectrum

almost identical with those of alkali lignin and lignosulphonic acids from a number of sources and suggested that this might indicate close structural similarity (5). Gladstone definitely concluded that the spruce native lignin spectrum demonstrated the presence of oxygen rings of the flavan or flavanone type in the lignin structure, and assigned the characteristic lignin band at 280 mμ to this unit. He also suggested that the shape of the lignin curve in the 350 mμ region may be the result of a "hidden" carbonyl band centered at 352 mμ. Patterson and Tibbert concluded that the spectra of amorphous lignins can be explained satisfactorily on the assumption that they are derived from lignin precursors, such as 1-(4-hydroxy-3-methoxyphenyl)-1-propanone and 1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone. They assigned the masked 310 mμ band to a carbonyl group in conjugation with the aromatic nucleus, the 280 mμ band to "meta position freedom," and the indicated 220-230 mμ band to some unexplained characteristic of the benzene nucleus. Anlin-Wittman concluded that the lignin spectrum in the 220-280 mμ region supported the Freudenberg furan or pyran ring structure for lignin, but noted that some additional chromophoric structure, such as a conjugated double bond system, would be required to account for the absorption curve at longer wavelengths.

This conflict in interpretation of the lignin spectrum, together with the growing list of materials that have practically identical spectra as lignin, has produced two very undesirable effects. On the one hand, the conclusions of these workers are being used in support or in lieu of chemical data by other workers

in the field in arriving at even more conflicting conclusions. On the other hand, the apparent confusion is creating among many a distrust of the method itself.

It would appear that, with the great amount of experimental data collected in the four basic investigations, it should now be possible to analyze the over-all picture and formulate an interpretation of the lichen spectrum that would satisfy all the experimental facts. A review of the work was made with this in mind, and an analysis drawn which seems to satisfy the requirements. To justify the conclusions reached, it is first necessary to review briefly some fundamental principles of ultraviolet absorption spectroscopy.

The source of spectral absorption bands is discussed in many texts and review papers (13, 14). It is important to emphasize the following points in the present discussion.

Strong molecular absorption in the ultraviolet is usually associated with structures capable of resonance between several configurations, such as a system of conjugated double bonds. The benzene nucleus is such a system, although its absorption becomes large only in the high frequency ultraviolet range (200-220 mμ). Substitution in the molecule may have the effect of increasing the number of possible resonant configurations, resulting in greater absorption at lower frequency. Substitution of -OH, -CH₃, or -NH₂ groups into the benzene nucleus, for example, is known to have this effect. A few chemical groups, such as C=O, -NO₂, -N=N-, are more or less self-contained resonating systems and, if isolated from strong intermolecular

influences such as conjugation, will produce characteristic absorption bands in the ultraviolet. The over-all absorption spectrum of a complex compound of unknown structure should be thought of as the integrated resultant of a number of overlapping bands. Some of these bands may be strong enough so that their maxima stand out distinctly, others may be relatively weak and hidden. The location and intensity of the maxima are the most specific data that can be obtained from the curve. The apparent broadness of consecutive bands, as reflected in the location of the minimum between them, is such a complex function that only the broadest generalizations can be drawn from their position. The over-all curve itself is a unique property of a particular compound, but the degree of differentiation within a family falls off rapidly as the complexity of the members increases, particularly when the individual variations involve inactive groups and linkages.

With these principles in mind, we can now turn to the question of the significance of the lignin spectrum. For a starting point, it is necessary to accept some basic chemical evidence regarding the structure of the material. For this purpose, it is taken as established that lignin has some type of aromatic structure and contains no large proportion of unsaturated aliphatic units. This is necessary since it is undoubtedly true that a complex or mixture of unsaturated aliphatic compounds could be found that would give an ultraviolet spectrum indistinguishable from that of lignin, and the task of classifying all such possible compounds on a purely spectrographic basis would be hopeless.

Accepting the stated chemical premise, an examination of the absorption curves of aromatic compounds in the papers being considered

and in such a collection as that given in International Critical Tables (15) at once brings out the following facts:^a

A. Benzene itself has two bands, one very strong at 200 mμ, a second of about one thirtieth the intensity at 255 mμ (Table I and Figure 1). The absorption curve drops sharply to near transparency beyond this wavelength.

B. Substitution of saturated carbon chains, as in toluene, the xylenes, and *n*-propylbenzene, causes a shift of both maxima toward the red and a slight intensification. Para disubstitution has the greatest effect (*p*-xylene, Table I).

C. Unsaturation in positions other than alpha to the ring (nonconjugated) has little effect on the ultraviolet spectrum (*o*-phenylpropylene, Table I).

D. The substitution of oxygen on the benzene ring has a pronounced effect on the position and intensity of the two maxima (phenol, Table I and Figure 1). These values are relatively unchanged for catechol, resorcinol, methylated phenols, methylene-ether linkages, or ether-linked side rings. Again, para disubstitution has the most pronounced effect (hydroquinone, Table I). The only effect of catechol disubstitution appears to be a broadening of the bands, resulting in a more shallow minimum that is shifted toward the red (7). In the case of the trisubstitution of pyrocatechol and its derivatives, however, there is, in addition to a further shift and rise of the minimum, a suppression

a. The ultraviolet curves of some of the materials considered were determined in nonpolar solvents such as hexane, others in polar solvents such as alcohol. By ignoring the fine structure sometimes shown in the nonpolar solvents, the values are comparable within the desired limits.

TABLE I

Substance	Maximum A mmu $\log_{10} \epsilon^A$		Maximum B mmu $\log_{10} \epsilon^B$		Maximum C mmu $\log_{10} \epsilon^C$		Reference
Benzene	200	3.8	255	2.3			(7)
p-Tyrene	212	3.9	263	2.8			(15)
γ -Phenylpropylene	Not reached		261	2.3			(5)
Phenol	215	3.9	275	3.4			(7)
Hydroquinone	225	3.6	295	3.4			(15)
Pyrocatechol	Not reached		255	2.9			(7)
α, α' -Phenylpropylene	Not reached		260	3.3	286	2.7	(5)
Protophenone	242	4.0	280	2.9	316 ^b	1.6	(16)
α -Indanone	242	4.1	291	3.6			(16)
Hydrocinnamaldehyde	252	2.6	269	2.6	302	1.7	(5)
β -Indanone	Not reached		268	2.8	278	3.0	(16)
p-Isobutylphenol	Not reached		260	4.3	292	3.4	(5)
Isocugenol	Not reached		262	4.2	292	3.7	(5)
2-Methoxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone	230	4.2	283	4.0	306	3.9	(10)
2-Hydroxy-1-(3,4-dimethoxyphenyl)-1-propanone	229	4.2	274	4.1	311 ^b	3.7	(10)

ϵ = molecular absorption coefficient in: $\frac{1}{\text{cm}} = \frac{1}{\text{cm}} \log_{10} \frac{I_0}{I}$

- $\frac{I}{I_0}$ - transmittance of solution (as compared with solvent)
 e - base of natural logarithms
 c - concentration of solute in moles per liter
 l - thickness of solution through which light passed, in cm.

For comparison with Figure 1, $\epsilon = k \times \text{molecular weight}$, where k is the absorption coefficient when the concentration is expressed in grams per liter.

^b Inflection point

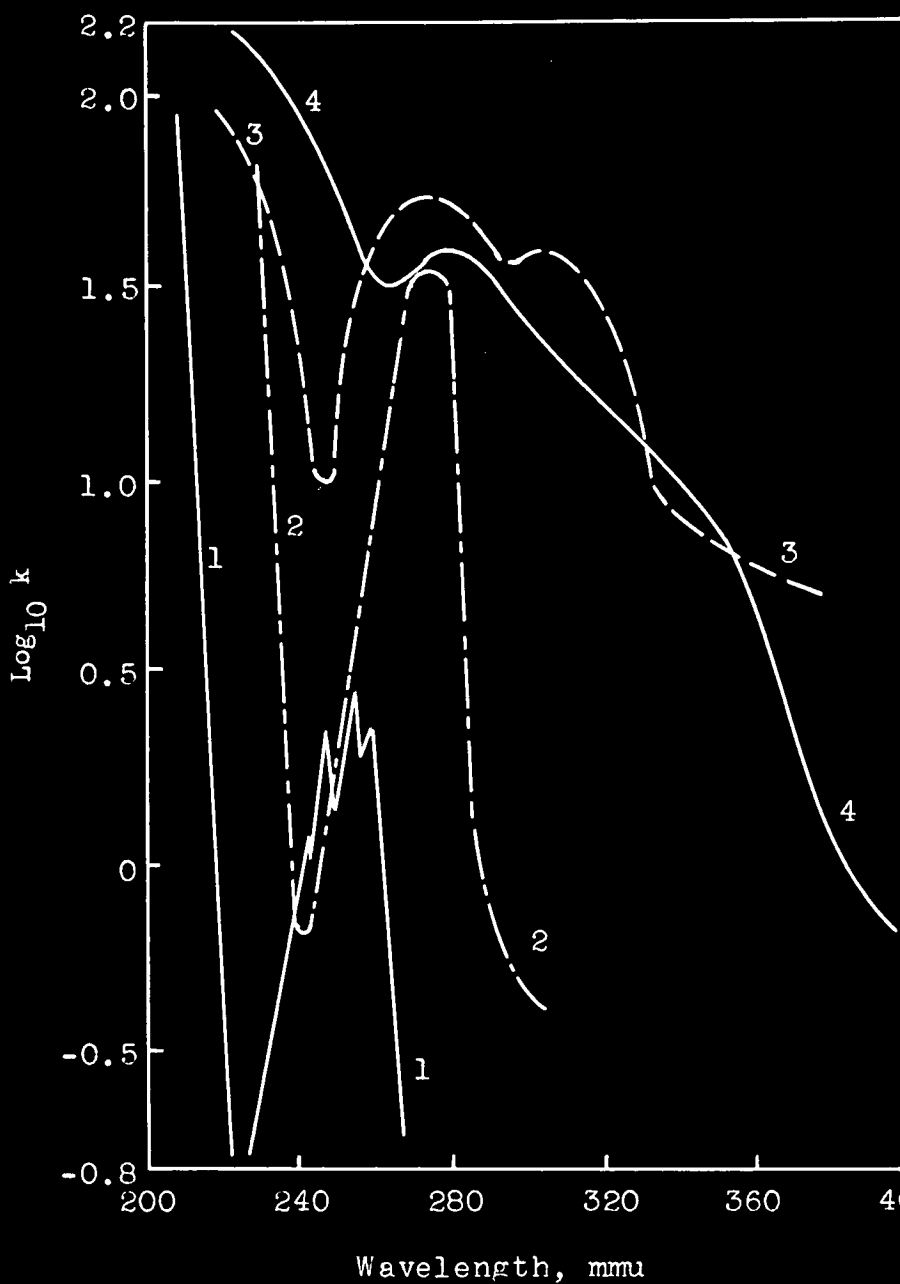


Figure 1. Curve 1, Benzene in hexane (7); Curve 2, Phenol in alcohol (9); Curve 3, 2-Hydroxy-1-(3,4-dimethoxyphenyl)-1-propanone in alcohol (10); Curve 4, Spruce native lignin in 90 per cent dioxane (9).

of the oxygen auxochrome effect in the long wavelength band (pyrocatechol, Table I).

It should be borne in mind that the increase in absorption through oxygen substitution is restricted to the two bands described. In all the compounds considered, the sharp drop in the absorption curve following the second longer wavelength maximum is characteristic.

5. Finally, there is another class of chromophoric groups that must be taken into consideration. A double bond in the alpha-beta position of a side chain or a carbonyl group in the alpha position has an effect comparable with oxygen substitution on the benzene bands. However, a unique effect of the conjugated unsaturated groupings is that a third band at longer wavelength is always introduced (α,α' -phenylpropylene and propiophenone, Table I). Conversion of this side chain to a cyclohexene appears to merge and intensify the long wavelength bands (α -indanone, Table I).

Although, as has been pointed out, nonconjugated double bonds in the side chain do not appreciably effect the benzenoid ultraviolet spectra, a carbonyl group in removed positions still produces an appreciable, though reduced, effect (hydrocinnamaldehyde and β -indanone, Table I).

When the alpha-beta double bond or carbonyl group is combined with a phenoxy nucleus, the two bands common to both groups are of such intensity that the third band associated with the unsaturated linkage may appear or may be "hidden" as an inflection in the curve or high general absorption in that region (p -isobutylphenol, isoeugenol, 2-ethoxy-

1-(4-hydroxy-3-methoxyphenyl)-1-propanone, and 2-hydroxy-1-(3,4-dimethoxyphenyl)-1-propanone, Table I and Figure 1).

In order to illustrate further the relative course of the absorption curve of the compound discussed to that of lignin, a selected group has been plotted in Figure 1. Since the spectrographic absorbing unit of lignin is not known, the plot is on the basis of weight concentration rather than mole concentration. It is interesting in this connection, and possibly significant in relation to the interpretation to follow, that Mullin-Birdman was able to demonstrate that, by reducing absorption data for a number of lignosulfonic acids of widely varying methoxyl content to absorption per unit methoxyl content, the resulting absorption curves fell very close together.

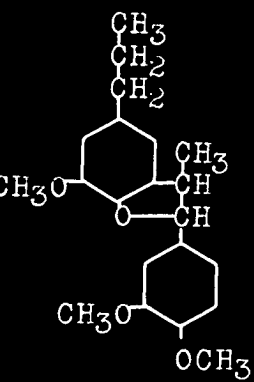
With these facts assembled, we can now re-examine the experimental data in the four works on the lignin spectrum cited.

Herzog and Hillmer quite clearly demonstrated the relationship of the spectrum of lignin to simple phenoxy compounds. There is no apparent justification from spectrographic evidence with such a high molecular weight material of unknown composition for their specific conclusion that the spectrum is definitely of the catechol or pyrocatechol type. Herzog and Hillmer's conclusion that the lignin spectrum definitely proved the absence of double bonds or carbonyl groups in conjugation with the benzene ring was apparently based on an assumption that lignin must be a polymer of chemically identical building stones. If such were true, their conclusion would probably be justified. Since later chemical studies make it at least equally possible that there may

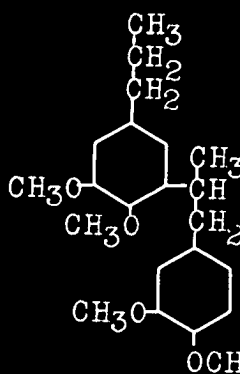
be considerable variation among the building stones of the lignin polymer, there is no basis for ruling out a small proportion of unsaturated configuration. Their observation that polymerized coniferyl alcohol gave a very similar absorption spectrum is simply another example in the long list that now exists of chemically different compounds that belong to this spectrographic class.

Glading's conclusion that the 280 mμ band in the lignin spectrum is specifically ascribable to the furan or pyran side ring of the Freudenberg formula is definitely incompatible with the well established lack of ultraviolet activity of saturated groupings. The lack of influence of such a side chain has been most clearly demonstrated by Aulin-Frdtman, although the conclusion was not stated by the author, when she showed the spectra of dihydrodehydrodiisoeugenol (I) and α -(3,4-dimethoxyphenyl)- β -(3-propyl-5,6-dimethoxyphenyl)propane (II) to be nearly identical. Glading was the first to point out that more than the phenoxy nucleus and saturated substituents is required to account for the high absorption and implied maximum in the 300-350 mμ region of the lignin spectrum. His suggestion that this may be the result of a carbonyl group is entirely consistent with the facts reviewed, but must not be taken as anything more than a hypothesis until a great deal more chemical and spectrographic work has been done.

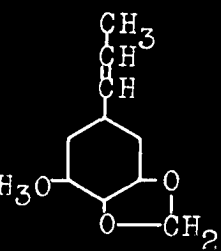
The phenyl ropanone model compounds studied by Patterson and Hibbert all belong to the spectrographic class discussed under Section E--that is, they contain the strong mixed chromophoric phenoxy and carbonyl groups which may produce either three strong ultraviolet bands or, through merger, show only two maxima. The



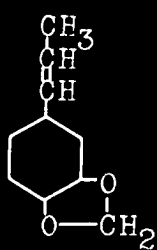
I



II



III



IV

assignment of the 295-320 mμ band in these compounds to the carbonyl group and the 220-230 mμ band to the benzene nucleus is in accord with the present general consideration. However, the assignment of the 280 mμ band to "meta position freedom," although possibly acceptable in this narrow group of compounds in a negative sense, is definitely misleading when applied generally and used for interpreting the spectrum of lignin. For example, isomyristicin (III) and isosafrole (IV) show equally strong maxima in this range, but the 310 mμ maximum of the conjugated double bond is absent in the former compound (Table I). It would appear that consideration of a restricted group of compounds of this class might lead to the erroneous conclusion that the 310 mμ band should be assigned to "meta position freedom." It seems much more correct to say that under certain structural conditions, tri-oxi substitution being one (Section D), the 260-280 mμ and 295-320 mμ bands characteristic of conjugated unsaturation substitution on the phenoxy nucleus may become fused, with a single strong maximum appearing in the range 270-300 mμ. The suppressing effect on the 275-280 mμ phenoxy band of tri-oxi substitution is evident in comparing the curves given by these authors for spruce and maple lignins. Their conclusion that the nature of the lignin curve in the 300-350 mμ region "suggests that a carbonyl group or ethylenic double bond in conjunction with the benzene ring is present to some extent," agrees with the findings of Gladstone, but still has no experimental proof.

Aulin-Brétman's conclusion that the ultraviolet absorption spectra of the model substances studied offer evidence supporting Freudenberg's furan or pyran ring structure for lignin is voided by

the same facts discussed in connection with Glavin's argument on this point.

To sum up, an evaluation of the experimental evidence regarding the ultraviolet absorption spectrum of lignin leads to the following conclusions.

1. The two characteristic bands in the lignin spectrum at 200-230 m μ and 275-285 m μ are specifically attributable to the oxygen-substituted benzene nucleus in lignin.

2. The high absorption in the 300-350 m μ range suggests the presence of a "masked maximum" and indicates the presence of other chromophoric groups in lignin--for example, carbonyl groups or double bonds conjugated with the benzene ring.

Attempts to further extend the interpretation of the lignin spectrum on the basis of slight shifts in position or intensity of the maxima or changes in position of the minimum point appear entirely unjustified at the present state of knowledge of the lignin structure. Such speculative interpretations have the harmful effect of growing to the status of proven experimental evidence and being misused in good faith by other workers in the field as unquestionable evidence in support of some currently popular hypothesis. To make this point clear, a few examples should be cited.

Early in the development of the application of ultraviolet absorption methods to lignin materials, several papers appeared in which lignin was definitely claimed to be synthesized from cellulose by simple heat treatment (17, 18, 19). This claim rested almost

entirely on the fact that the ultraviolet absorption curve of the product showed the 275-285 mμ maximum and the general shape of the lignin curve, in spite of the fact that the intensity of absorption at the maximum point of the unknown material was less than that of spruce lignosulfonic acid by a factor of six (15). Little effort has since been made to defend the thesis that such material is in any sense lignin, but the work is still cited to discredit any use of ultraviolet spectrochemistry in the lignin field. The true nature of the heated cellulose product and the source of its ultraviolet absorption curve could probably be fairly easily determined on the basis of a series of papers recently published dealing with the acid hydrolysis of simple hexose sugars (20, 21, 22). The hydroxymethylfurfural which is produced has the characteristic 280 mμ carbonyl absorption band, and it may be seen in the figures given that, under certain conditions, the absorption curve of a hydrolyzed sugar mixture is very similar to that of lignin.

Such use of the lignin absorption curve has not die out. Hitter and co-workers (23) have interpreted Patterson and Hibbert's work as identifying the 280 mμ peak with the carbonyl group and used this to support their polyfuranone formula for lignin. The demonstration by Kulin-Erdtman of the nearly identical ultraviolet curves from I and II was an experiment designed to check Freudenberg's theory that a side ring is closed in the sulfonation of lignin. The negative result of this experiment has apparently been interpreted by H. Erdtman (24) as disproving Freudenberg's theory. The fact that the experiment actually proves that the ultraviolet spectrum offers no evidence concern-

ing the presence or absence of a saturated side chain has already been shown.

Finally, it is desired to state an important conclusion arising from the two points established in regard to the lignin spectra--e.g., the assignment of the maxima to the oxygen-substituted benzene nucleus and the indicated presence of other chromophoric groups. It has been shown that the oxygen substitution on the benzene ring is responsible for at least a tenfold increase in absorption resulting in the 280 m μ maximum and that this maximum overlaps a possible band in this region associated with conjugated unsaturation. It is suggested, therefore, that, if some method were available for replacing the phenolic hydroxyl and methoxyl groups in lignin with alkyl groups or hydrogen atoms, the ultraviolet spectrum of the product would be much more illuminating in regard to other chromophoric groupings and spectroscopic comparison with analogous model compounds might be much more fruitful.

The basis for the generalization of the title of this paper to "complex hydroxy-aromatic compounds and derivatives" lies in the fact that a number of groups of such compounds, known to vary widely in chemical composition but possessing the oxygen-substituted benzene nucleus in common, have been shown to have ultraviolet absorption curves very similar to that of lignin--all showing the 220-230 m μ and 270-285 m μ maxima. These include the flavylum and flavone compounds (25), the lignans (1), and the natural tannins (26).

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