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EXAMINATION OF CELLULOSE-LIGNIN RELATIONSHIPS WITHIN CONIFEROUS GROWTH ZONES

ABSTRACT

Quantitative differences in wood growth zone chemistry were first reported forty years ago. Several studies have followed, mostly done on wide-ringed material, but always by dissection into earlywood and latewood segments usually collected as sizeable samples and analysed by standard or slightly modified procedures. An objective of the present work has been to sample and analyze multiple positions within characteristic coniferous wood growth increments, and thereby search out common patterns relating to wood biological activity and product behaviors.

Lack of selective holocellulose isolation procedures and the problem of limited material have long frustrated attempts to accurately measure and describe carbohydrate patterns within growth zones of coniferous woods. A new approach has been applied to microcellulose analysis. This arises from the observation that the corrected yield from nitration of wood pulps and whole woods closely matches that obtained as alpha-cellulose. It has been shown that 0.1g wood-meal sample treated this way in replications of three provide a determination with statistical reliability. The procedure was used to examine sixty positions within ten increments representing five Canadian coniferous woods of different genera.

Intra-incremental lignification patterns were constructed for the same materials by making ultraviolet absorptiometric measurements on products from acetyl bromide - acetic acid digestion of 20 mg wood samples in replications of three. This follows an earlier procedure of this laboratory.
The series of alpha-cellulose estimates described have shown relationship of this long-chain carbohydrate fraction to seasonal development within growth zones. New chemical evidence is provided for a physiologically significant phenomenon of some earlywoods. Therein, minimum alpha-cellulose yield occurred at considerable cellular depth following initiation of seasonal growth. It appears that the first formed earlywood arising from "over-wintered" xylary mother cells retains similarities at the chemical level of organization to last formed tissues of the preceding season, in contrast to new cambial divisions within a growing season. Both Sitka and black spruce differed from other woods examined in this regard (Douglas fir, Pacific silver fir and western red cedar) in that they did not show the phenomenon.

Among the ten growth increments studied, the alpha-cellulose estimate (45.9%; LSD = ± 2.0%) was the exact complement of lignification (27.4%; LSD = ± 1.9%) in each instance. Moreover, combining both values for the sixty positions included in the study gave less variation (73.4%; LSD = 1.2%) than considering either separately. Further evidence for this important relationship was shown by the highly significant (r = 0.785) linear regression on data combined from all increments. This suggests much closer physiological control over the combination of these chemical entities, than towards the individual components involved in such a system.

Residual nitrocelluloses were used to indicate extent of degradation introduced during preparations. Because of variability in intrinsic viscosities at each position, however, no consistent trends were found within growth zones. A limitation of the new micro alpha-cellulose method is that it can not be universally applied to all woods. Samples from four other species attempted failed to yield suitable derivatives.
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EXAMINATION OF CELLULOSE-LIGNIN

RELATIONSHIPS WITHIN CONIFEROUS GROWTH ZONES

by

Gordon Balfour Squire

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
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THE UNIVERSITY OF BRITISH COLUMBIA

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Department of Forestry

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Lack of a selective holocellulose isolation procedure and the problem of limited material have long frustrated the attempts of wood scientists to accurately measure and describe carbohydrate yields within coniferous growth zones. A new method has been devised for micro-cellulose determination, Alpha-(\(\alpha\))cellulose yield may be quantitatively estimated as the corrected yield of nitrated wood meal. Three - 0.1g wood meal samples provide a statistically reliable determination. A major limitation of the new technique, however, is that it cannot be applied to all woods.

Sixty positions within ten increments representing five Canadian coniferous woods of different genera were examined and intra-incremental patterns were constructed. Anova and Duncan's test showed latewood \(\alpha\)-cellulose yield to be greater than that of earlywood by a highly significant degree. Alpha-cellulose content throughout mature growth zones was estimated reliably by linear correlation or, more accurately, by logarithmic transformation used in a recent mathematical model. The successful application of the latter is its first reported use describing the non-linear behavior of a wood chemical property across a coniferous increment.

These patterns showed relationship of the long-chain carbohydrate fraction to seasonal development within coniferous growth zones. In addition, six of the ten patterns demonstrated new chemical evidence pertaining to a
physiologically significant phenomenon in earlywood. Therein, minimum $\alpha$-cellulose yield occurs at considerable cellular depth following cambial reactivation in the growing season. First-formed earlywood appears to retain some similarity at the chemical level of organization to last-formed tissues of the preceding season. Later-formed earlywood (i.e., from the present year) does not appear to retain such similarity.

From earlier work of this laboratory, lignification patterns were described for the same materials, using ultraviolet measurements on acetyl bromide-acetic acid digestion products of two wood meal samples.

Examination of $\alpha$-cellulose and lignification patterns provided evidence for their mutually exclusive behavior. For the ten increments studied, the $\alpha$-cellulose estimate ($x = 45.9 \pm 2.0\%$) was the exact complement of lignification ($x = 27.4 \pm 1.9\%$) at all positions but one. The linear regression for data from all increments was highly significant ($r = -0.785$). In addition, micro $\alpha$-cellulose and micro lignin values, when combined, showed a definite tendency to cluster about a central value ($x = 73.4 \pm 1.2\%$) suggesting that certain species require a common, critical measure of high molecular weight material. Dispersion about combined lignin and $\alpha$-cellulose estimates was significantly less than about either of their individual means. This suggests much closer physiological control over the combination of these chemical entities, indicating that tree physiology is oriented more towards the finished biosynthetic product than towards the individual components involved in such a system.
As a means of measuring successful nitrocellulose preparation, intrinsic viscosity $\eta$ was used to indicate presence or absence of extensive degradation. Because of the highly variable $\eta$ at each position tested, no consistent trends in chain length were found across growth zones. However, in four increments, significant differences in $\eta$ throughout the earlywood provided further evidence of two earlywood types.
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While preparation of any thesis is mainly the work of one individual, nevertheless, it represents the combined efforts of a group of people. During the writing of this thesis, I became acutely aware of the time and energy that others had willingly given to me. Therefore, it is my distinct pleasure to acknowledge the invaluable contributions made by the following persons: I wish to single out my supervisor, Dr. J. W. Wilson, Professor, Faculty of Forestry, for his patience and dedication to the highest professional standards during all phases of this work and for his truly enlightened, inspiring guidance and personal concern for my behalf. Likewise, I wish to thank Dr. R. W. Wellwood, Professor, Faculty of Forestry, Dr. G.G.S. Dutton, Professor, Department of Chemistry and Dr. R. W. Kennedy, Forest Products Laboratory (Vancouver), for their most constructive suggestions and criticisms; Dr. A. Kozak, Assistant Professor, Faculty of Forestry, for his ever-cheerful assistance in statistical analysis and computer programming; Dr. K. Sarkanen, Professor, Faculty of Forestry, University of Washington, for serving as the External Examiner of the thesis; Mrs. M. Lambden, Faculty of Forestry, for drafting the figures; Mr. S-Z. Chow, Forest Products Laboratory (Vancouver), for sample material and data; Mr. U. Rumma and Miss H. Apelt, Technicians, for help in the laboratory; Mr. E. T. Squire and Miss B. Pearcey, for preparation of the manuscript; and the Van Dusen Foundation, Pulp and Paper Research Institute of Canada, and the University of British Columbia for financial aid.
INTRODUCTION

Wood substance may be divided into three main classes of material: cell wall components, extraneous components, and tree secretions. The latter are distinguished by their insolubility in cold water and solubility in non-polar neutral solvents; oleoresins exuded from coniferous resin ducts are such an example. Extraneous components are soluble to some degree in cold water and in neutral solvents. Hot water solubility is not used to characterize extractives since this may free wood acids which can cause hydrolysis. Although the percentage of extraneous components in wood may be very low, their influence may be so great as to often characterize a wood more sharply than cell wall components; these latter are distinguished by their insolubility in cold water or neutral solvents and by their being an integral part of the cellular structure. They are chiefly high polymers, typical examples being cellulose, hemicelluloses, and lignins; pectic materials and mineral deposits (found chiefly in the middle lamella) are also included. It is obvious that these definitions are limited and far from complete and, therefore, they await considerable addition and refinement.

In wood science, many useful and sophisticated analyses have been made on carbohydrate fractions of coniferous secondary xylary tissues with the express purpose of furthering understanding on such highly complex products of plant metabolism. However, the ultimate in "purity" has never been produced in high yield, partly because of the lack of definition on what is sought in the chemical sense. This lack of absolute reference from which
to base analyses causes much confusion. Because carbohydrates separated
from wood show different chemical and physical properties dependent on
isolation method, comparison of analytical values is confounded to the point
where such carbohydrate fractions only have meaning when reported in terms
of the isolation method. Nevertheless, much useful information may be
obtained through examination of certain well-defined portions in the cell
walls.

In relation to qualitative and quantitative variations within the tree
stem, however, carbohydrate fractions have not been examined widely.
For instance, little is known regarding the pattern of holocellulose deposition
throughout growth increments of coniferous woods. This has resulted from
the problem of sampling, where the total amount of material needed exceeds
that which is available. More important, no method has been available for
reliably reporting measurement of any holocellulose, or portion thereof, at
many points within an increment. At most, only two tissues (earlywood and
latewood) have been separated and analyzed.

The immediate goals of this thesis were closely related to this
area of investigation. A reliable method had to be developed for estimating
holocellulose, or a constant portion thereof, in coniferous secondary xylary
tissues, i.e., wood. At any level of sample size, accurate measurement
of holocellulose is difficult, hence, it requires adequate replication.
Among the more important considerations affecting replication were particle
size, extraction solvents and their sequence, and limited sample size.
Since only small amounts of material were available, another requirement of the method was that it be of semi-micro nature and give statistical reliability with a reasonable replication number. The method would be applied to material sampled within several annual growth increments to determine actual patterns of cellulose deposition. Alpha-cellulose patterns determined on wood materials would be compared to lignin patterns and, along with these newly established patterns, comparisons would be made with the hope that information derived therefrom might give new insight to inter-relationships between the major secondary xylary cell wall components. This comparison would uphold or refute the hypothesis examined by this thesis which is: that lignification across coniferous growth increments is the complement to the long-chain cellulosic fraction of holocellulose, i.e., alpha-cellulose.
LITERATURE REVIEW

I. History of Chemical Organization in Wood

Due to the complex nature of wood, the history and nomenclature of wood chemistry and its nomenclature have often been confused; nevertheless, this is to be expected wherever a new branch of science emerges. In spite of shortcomings, important divisions and developments have been made in wood chemistry, mainly by its pioneers.

Purves (97) has reviewed the history of organized cellulose chemistry. This really began in 1837 with Payen, although Gay Lussac in 1811 had shown that wood contained the elements carbon, hydrogen, and oxygen. Payen brought a systematic approach to wood analysis whereby he treated oak alternately with nitric acid and alkali to get "cellulose", a solid skeletal substance, which he described as mechanically embedded in an "encrusting" substance. Chlorination was used to procure the same results with fir, a coniferous wood. Payen's work extended over a five year period and from it, he established an empirical formula for cellulose \( \left( C_6 H_{10} O_5 \right)_n \), showed this to be isomeric with starch, and because of its lack of response to colorimetric determinations, maintained that cellulose was not linked to the "encrusting" substance. Furthermore, Payen was convinced of the chemical uniformity of cellulose. Some botanists soon disputed this claim, however, on the basis that different plant tissues varied widely in staining reactions and cuprammonium solubility. In 1859, Fremy maintained the cell wall to be composed of several substances (lignin,
encrusting substance, and cellulose) which he called "vasculose", "meta", "para", and remaining celluloses. The same year, Pelouze showed that, on regeneration from cuprammonium solution, cellulose originally dissolved in conc hydrochloric acid was now soluble in a much weaker acid. Fremy's contentions were supported 25 years later, when cellulose hydrolyzates were found to contain galactose, arabinose, mannose, xylose, as well as glucose; according to Norman (94), Schulze noted in 1891 that the polysaccharides yielding these non-glucose sugars were more readily hydrolyzed or alkali-extractable; hence, he called them hemicelluloses. The more resistant polysaccharide constituent remained as cellulose. Differentiation between the two constituents, however, lacked specificity because drastic extraction treatments failed to remove all hemicelluloses (97). The non-cellulose portion of solvent-extracted plant tissue included hemicellulose as well as Payen's encrusting substance and, as these components became recognized as distinct entities, the term lignin (Fremy's "vasculose") denoted the non-carbohydrate fraction of the non-cellulosic incrustants. Such a clarification in ideas and nomenclature did much to explain the reasons whereby Fremy and his colleagues denied the chemical uniformity of cellulose.

As the isolation of cellulose required use of harsh reagents, it seemed plausible to accept cellulose as an artifact produced by chemical change during isolation. Purves (97) reports that Erdmann took this view in 1867, when he considered the concretions from fruitwood to be "gluco-drupose" ($C_{24}H_{36}O_{16}$), while spruce wood was "glycolignose" ($C_{30}H_{46}O_{21}$).
Mild acid hydrolysis left "drupose", \((C_{12}H_{20}O_8)\) and "lignose" \((C_{18}H_{26}O_{11})\), and subsequent nitric acid extraction left a nearly constant yield of wood fibre \((97)\). Erdmann's views received support from Cross and Bevan \((33)\), who claimed that bast fibres were uniform in chemical reactions, and believed that all plant components merged into one another by "insensible chemical gradations." Cellulose, they stated, was merely the aggregate resulting from a chemical breakdown of plant tissue carried to the point where reactions generally accepted for pure cellulose were obtained. Their "lignocellulose" theory was refuted in 1920 when Herzog and Janke showed that lignified non-woody fibres or cotton gave an X-ray pattern nearly identical to that of delignified wood pulp. Thus, a uniformly ordered crystalline lattice could be in uniform chemical combination with large amorphous molecules of lignin, fat, or hemicellulose.

According to Mark \((86)\), the fringe micellar theory, as first suggested by Naegele in 1858, renders the concept of "lignocellulose" (and its associated nomenclature) obsolete. Nevertheless historical controversies about the definition of cellulose have as yet not been adequately resolved. In textile or botanical circles, cellulose means the natural, carbohydrate polymer collected in fibrous form that is the structural aggregate of the cell wall. As such, it usually includes a hemicellulose fraction yielding on hydrolysis sugars other than glucose \((94)\). An alternative definition has been adopted by those studying chemical structure, which follows Payen and Schultze in accepting cotton as a standard, restricting the term cellulose to that portion of the cell wall derived exclusively from glucose.
and resembling cotton cellulose in its physical and chemical properties.

The first view of cellulose is held by Norman (94), who defined holocellulose by the chlorite method of Jayme as modified by Wise et al. (153) and differentiated this by solubility criteria which, quite expectedly, lead to overlapping of categories and contributed to a rather confused understanding of wood chemistry. This system is presented as Appendix I; dotted lines show indefinite boundaries which may shift across this area depending on intensity and number of extraction treatments. For example, separation of the hemicelluloses into cellulosans and polyuronide hemicelluloses rests primarily on the Cross and Bevan cellulose method; since this utilizes visual determination of residual lignin, the same degree of delignification will rarely be agreed upon by any two workers and, concomitantly, rarely will the same ratio be attained between hemicelluloses. Moreover, while chlorite delignification affects cellulosans and cellulose very little, the encrusting polyuronide hemicellulose fraction is rendered distinctly more soluble, with the result that variable amounts will be obtained, depending on isolation technique.

The nomenclature itself is misleading. The term hemicellulose is actually incorrect when applied to encrusting polyuronides and for this reason, its use should be discouraged; that of cellulosan is entirely misleading since it implies a long chain with glucose base and intends to mean a short chain with other-than-glucose base. Uronic acids (from polyuronides) imply terminal situation or occurrence on a side-chain of known linkage, whereas in fact only the former is present. Lastly, this nomenclature takes no account of the xylan and mannan (from cellulosans) which
are often in association with separated $\alpha$-celluloses.

In an attempt to refine these shortcomings, Stewart (117) extended Norman's original classification and nomenclature of wood substance polysaccharides (Appendix II). Analytical procedures for determining each constituent are known and, except for the fair separation of glycosan uronides from non-cellulosic glycosans, they give relatively clear-cut separations between all constituents, thus allowing approximation of the total polysaccharide fraction of wood substance. The terminology used is to be preferred to the older nomenclature (94), as it is based on systematic rather than trivial nomenclature. While use of "osan" is generally applied to macromolecular substances which hydrolyze to give sugars, Stewart has extended this definition to include oligosaccharides as well. Stewart's classification is much easier to understand and apply. Certainly it abolishes much confusion attendant between understanding actual variations in wood chemical constituents and methods used for their measurement. Much of this historical and nomenclature review is also available in a paper by Wise (152).

II. Variations in Chemical Organization in Wood

Wood is of plant origin and constitutes the secondary xylary portion of certain fibrovascular tissues. The following criteria serve to distinguish woody from non-woody plants (19, 96): woody plants have specialized conducting (vascular) tissues that produce xylem (wood) and phloem (inner bark); these plants provide a stem that persists and lives for a number of years, in fact, some woody plants (trees) are the oldest living things; and, besides exhibiting the primary growth which occurs at apical growing points
and causes elongation of tree stems, woody plants exhibit secondary thickening, a means of stem thickening achieved through the activity of a growing layer called the cambium. The term "wood" refers to part of this collected product of the cambium which, because of its origin, is also referred to as secondary xylem. Originating from apical growing points, primary xylem is formed only during the first year of growth in such plants, hence, is restricted to initial position about the stem pith.

A. Taxonomic Level

Taxonomically, there are three types of woody plants: lianas, shrubs, and trees; of these, the latter are the only ones of direct interest to Wood Science and, though sometimes difficult to separate from the others, they are usually identified from their characteristic habit of attaining a height of at least 20 feet at maturity and having a self-supporting stem. Within the plant kingdom, trees occur only in the Phylum Spermatophyta which, in turn, is subdivided into Gymnospermae and Angiospermae.

Much work has been done on the composition and chemical structure of gross wood from these two divisions. Such results are of interest because they indicate changes in the cell wall chemical structure which may have evolved during the development of terrestrial plants. Both have in common the qualitative similarity of containing the four basic wood components. Major quantitative differences between these components, however, serve to separate the two wood types. Recently, Timell (131, 132, 133) has summarized the more important differences in carbohydrate composition between Angiospermae and Gymnospermae and showed their evolutionary development
to be associated with decreasing contents of galactose, mannose, and arabinose units, and increasing amounts of glucose and xylose units. Stewart (118) notes that such trends seem to be related to increases in the extent of secondary growth, as well as to increases in the proportion of secondary to primary wall components.

The total cell-wall polysaccharides are usually obtained from wood substance in the form of holocellulose, of which Angiospermae contain from 70 to 80%, and Gymnospermae 60 to 75%, based on extractive-free wood. Glucan, as approximated by \( \alpha \)-cellulose, constitutes 45 to 50% of the former and 40 to 45% of the latter (117); this is customarily regarded as the true cellulosic fraction of wood carbohydrates. Alpha cellulose is the proportion of holocellulose or pulp insoluble in a solution of 17.5% sodium hydroxide at 20\(^\circ\)C. (124). The term, however, is strictly arbitrary, and does not imply exclusively a homogeneous glucosan for, invariably, it contains small amounts of carbon dioxide- and furfural-yielding materials, as well as significant amounts of mannans from softwoods (153).

From the literature relating to the non-cellulosic wood polysaccharides, Hamilton and Thompson (54) reached these conclusions: total non-cellulosic polysaccharides (Norman's hemicelluloses) represent about 25% of angiospermous wood, and 20% of gymnospermous wood. Each contain the following entities in common to both: galactoglucomannan, arabinogalactan and other galactose-containing polymers, pectins, and starch. In contrast, very much larger amounts of 4-O-methylglucuronoxylan (80 to 90% of total) are found in Angiospermae, whereas medium to large amounts of arabino-
4-0-methylglucuronoxylan (15 to 30% of total) and glucomannan (60 to 70% of total), respectively, occur in Gymnospermae.

In addition, many physical measurements have been performed on separated wood polysaccharides (118, 129). Further chemical investigation has revealed other differences between polysaccharides of the two major groups. Acetyl content of Angiospermae wood is about twice that of Gymnospermae wood, being in the order of 3 to 4%; most of these are known to occur as O-acetyl groups (22, 118). On the other hand, Gymnospermae contain approximately 25% lignin as compared to about 20% for Angiospermae and, of this percentage, methoxyl content of the former is about 15%, while that of the latter is closer to 20%. Extractives, although quite variable in composition, constitute an average of 3 to 5% for wood of either group.

Within the Gymnospermae four living orders are recognized by probable order of increasing evolutionary development, these are Cycadales, Ginkgoales, Coniferales and Gnetales. Only the third order, Coniferales, is of commercial importance, mainly because of the gregarious nature of its species (tending to beget tree extraction on an economic level), their excurrent mode of growth, and the diversity of their wood uses.

Among the Conifers, chemical studies of variations in polysaccharides are more limited and much more data is needed before generalizations can be made regarding differences between families or lesser taxonomic sub-divisions. While \(\alpha\)-cellulose is considered the same chemically, inter-genera variations in heteroglycans have been noted. Schwartz and Timell (109) extracted galactoglucomannan from Pacific silver fir (Abies amabilis
(Dougl.) Forb.) and found two types having difference monosaccharide ratios and degree of polymerization (DP). Timell (132) earlier studied hetero-glycans of various species of the genera **Pinus**, **Picea**, **Tsuga**, **Abies** and **Thuja**. He showed that, while galactoglucomannans were similar for all genera, each exhibited characteristic monosaccharide ratios. Furthermore, he concluded that with a few exceptions, all coniferous glucomannans contain 2 to 4% galactose, and that coniferous wood may contain 10 to 20% of a closely related series of galactoglucomannans differing mainly in their relative galactose content and perhaps also in their average DP (or molecular weight) and chain branching. Schuerch (108) reported that the latter characteristic seldom exceeds 3 branches per 100 units, and that in certain conifers, the point of O-acetyl group attachment varies. In some species of **Larix**, especially large amounts of a highly branched arabinogalactan have been found (132). An otherwise important point about arabinogalactan is that it is found only in **Pinaceae**. Schuerch (108) has listed a bibliography relating to inter-genera variations in some hemicelluloses.

Erdtman (39) has applied differences in extractive chemistry to distinguish within and between the Families **Pinaceae**, **Podocarpaceae**, **Araucariaceae**, **Taxodiaceae**, and **Cupressaceae**. Inter-species variation in all chemical components is conveniently listed by Browning (22) and Timell (135); these cover the more important commercial North American woods. Browning (22) also has shown elemental analysis of some of these species. Generally, chemical variation between species is less than that between genera; however, many exceptions have been reported.
Within a species, chemical composition varies unpredictably, due to the multiple effects of growth conditions available within and between forests and, as result of the latter, there seems to have been development of so-called races within species (96).

Some structural features and physical characteristics (hence, also chemical characteristics) are inherited (96) and, because of this, tree-breeding programs are now operated in a number of countries, with special attention being paid to these aspects.

B. Wood Zone Level

Within a single coniferous stem, the radial pattern of Cross and Bevan cellulose distribution for Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) and Monterey pine (Pinus radiata D. Don.) has been shown to rapidly increase in the first 10 to 15 years, from the pith outward, and thereafter level off (141, 160). Zobel and McElwee (160) confirmed these results for loblolly pine (Pinus taeda L.) also where cellulose content of sapwood was higher by 7% than that near the pith. Larson (76) recently determined the five principal wood sugars and lignin across radii of three mature red pine (Pinus resinosa L.) stems and showed that glucose and mannose yields increased rapidly with age, whereas yields of xylose, galactose, arabinose, and lignin decreased (the latter declined very gradually). He further showed that lignin increased by 2% from 6 ft to 18 ft in height. Klem (70) and Hata (55) reported similar results for Norway spruce (Picea abies L. (Karst.) and Japanese red pine (Pinus densiflora L.),
respectively. Wellwood et al. (147) showed that, across a stem radius of western hemlock (*Tsuga heterophylla* Raf. Sarg.), specific gravity follows the same general trend as that for cellulose content. Kennedy and Jaworsky (68) showed these two properties to be correlated in Douglas fir. Therefore, cellulose content may be expected to follow the same pattern as that of specific gravity in oblique and height series of Douglas fir and the preceding species cited for cellulose content, that is, cellulose yield could be expected to decrease from the butt upward. Extractive patterns have been established across radii of redwood (*Sequoia sempervirens* (D. Don) Endl.) (110), western larch (*Larix occidentalis* Nutt.), Douglas fir (13, 45), and western red cedar (*Thuja plicata* Donn.) (83). Both polyphenol and thujaplicin contents increased from the pith to the heartwood-sapwood periphery, after which the former decreased rapidly and the latter disappeared. In contrast, resins from a number of pines appear to be highest in the pith region and thereafter decrease gradually to the bark (5, 27, 78, 110). Luxford (81) has studied the effect of extractives on strength of whole wood.

Wood zone differences similarly reflect the above trends; juvenile wood is regarded as the central portion of the stem occupying the first 15 to 20 rings and mature wood is that formed subsequently.

The latter possesses characteristics considered regular for the species, while juvenile wood, on the other hand, has chemical properties different from those of mature wood. Trends relating cellulose, lignin,
and extractive contents between these zones have been discussed. In summary, within juvenile wood, cellulose content appears to rise abruptly, polyphenol and thujaplicin contents increase gradually, and lignin and resin contents decrease slowly; in mature heartwood, these trends continue, but at more gradual rates. Cambial zones may contain up to 20% inorganic ash, 50% ethanol solubles, 30% protein, 35% non-cellulosic polysaccharides, but little (up to 25%) cellulose or lignin (118).

In general, regular wood is that formed by normal physiological processes resulting in equal cambial division of cells throughout the tree circumference. When inspected from pith to bark along a radius, its most characteristic quality is frequently, but not always, the presence of two distinctly colored zones of growth: heartwood (duramen) and sapwood (alburnum). The latter occupies the peripheral region of secondary xylem in which a portion of the tissue (5 to 40%) is living, hence, physiologically active. Its suspected functions are stem support, sap conduction up the tree, and reserve food storage. With time and increasing girth, however, the protoplasm of all living xylary cells is thought to die and secondary changes accruing from this result in formation of heartwood, the physiologically dead, central conical core of the stem xylem. Similar death changes probably occur in the phloem.

For many species, the transition of sapwood into heartwood is characterized by a change from a creamy-white color to an appreciably darker color, the demarcation zone being either abrupt or gradual. In
other genera such as *Picea, Tsuga, Abies*, or *Populus*, this color change may be imperceptible (96). Dark heartwood visually illustrates that some chemical change has occurred in transition between the zones; various organic substances such as extractives, extraneous materials, or infil­trations are thought to pass into the lumens and cell walls. To be sure, this phenomenon occurs in species of light heartwood; however, their extrac­tives lack such a high degree of oxidation. The toxicity of some extractive substances is well-known (67, 83, 84); this, with the inhibited movement of moisture and air (caused possibly by the infiltration or morphological changes, i.e. tyloses), is thought to render heartwood more resistant to penetration than sapwood.

As indicated previously, corewood heartwood, because of its central position would be expected to contain on the average, less cellulose and more lignin than mature heartwood and sapwood, while acetyl content would be consistently higher in sapwood (22).

Irregular heartwood has been noted in Douglas fir (69) and western red cedar (84) from the interior of British Columbia. Patterns of dihydroquercetin and thujaplicin distribution appear to follow alternate light- and dark-colored concentric bands of variable width within the heartwood ("target ring"); the lighter bands appear to be more closely related by chemical extractive content to sapwood than to heartwood.

In contrast to regular wood, reaction wood is frequently correlated with a characteristic anatomy and eccentric radial growth; both result from
differential (unequal) cambial division of cells on one side of a stem, seemingly in response to gravity and/or growth hormones (142, 145). In conifers, greater cambial division occurs on the lower side, giving rise to "compression" wood; this may lead to an eccentric pith. Chemical analyses of compression wood have been summarized (89, 96).

There seems to be a certain balance which normally exists between the formation of lignin and cellulose in gross wood. This balance, within limits of numerous sources of variation, may be shifted one way or the other by a mechanism adjusting to the stress or strain to which the developing wood tissue is subjected. One expression of this shift occurs vividly with reaction wood formation. In coniferous trees, a marked change from that of regular wood is shown at the level of chemical composition. In Scots pine (Pinus sylvestris L.), Meier (89) has shown that along with higher galactan and pentosan content, the amount of lignin increases approximately 10%. This is accompanied by smaller amounts of galactoglucomannan and a reduction in cellulose content of approximately 10 to 15%. Larson (76) obtained similar results from red pine and, according to Stewart (118), Schwerin found likewise for Monterey pine. Byrd et al. (26) studied three radial positions and heights within each of four 37-year-old loblolly pine stems. Regression analysis showed chlorite alpha-cellulose and lignin to be negatively and significantly correlated (r = -0.764).
C. Growth Zone and Tissue Level

Across coniferous growth zones, chemical variations occur between longitudinal and transverse tissues. According to Timell (135), Peirila and co-workers studied carbohydrate composition of parenchyma (ray and longitudinal) and tracheid cells in Scots pine and Norway spruce. In the former, hemicellulose content of parenchyma and tracheids was 20 and 21%, respectively, while in the latter, the values were 30 and 18%, respectively. In both species, parenchyma cells contained more xylose than mannose residues, while in tracheids mannose predominated. Thompson et al. (125) showed that polysaccharides rich in galactose, arabinose, and uronic acids predominate in epithelial cells, and that these cells are lower in glucose by approximately 10%. From this evidence, it appears possible that each cell type has a characteristic carbohydrate composition. Meier (89) and Haas and Kremers (51) have compared differences in polysaccharide content for mature and immature pine tracheids.

Variations existing between and among early- and latewood tissues control the most pronounced differences found in wood quality, hence, the influence of such variations on the chemical development across a growth zone will have a most important bearing on a wood's ultimate utilization. Morphological variations within a tree reflect the influence of terminal meristems (74) and moisture availability (158); following dormancy, resumption of cambial activity is accomplished through vertical auxin translocation from the crown, causing radial enlargement of immature tracheids to form earlywood. Naturally, as the auxin reaching
the cambium decreases with distance from the crown, the magnitude and
duration of earlywood formation is least at the tree base. This condition
remains as long as moisture is highly available, but with decreasing
moisture (as in summer months), radial cell enlargement decreases and
differentiated cells remain alive longer, hence, walls become thicker, and
latewood forms (36). The mechanism by which transition occurs is still
in doubt. Zahner (159) claims it begins after the first severe water
deficit, but Larson (75) believes a decreasing auxin supply to be the cause.
Following transition, however, a mild moisture deficit favors latewood
formation (159).

Recent chemical evidence by Wu and Wilson (157) suggests the
occurrence of two earlywood types within an increment ($E_1$ and $E_2$), the
first corresponding to wood arising from "over-wintered" xylary mother
cells, the second relating to that formed by new cambial divisions within
a growing season. It is proposed, therefore, that $E_1$ might "retain some
similarity at the chemical level of organization to last-formed tissues of
the preceding season" (157). The origin of these tissues has been
demonstrated by Bannan (10) who has removed and studied developing
wood throughout single growing seasons. From northern white cedar
(*Thuja occidentalis* L.) grown in the Toronto, Ontario area, Bannan
(10) found that, on resumption of cambial activity, the first site of cell
division is in the oldest xylary mother cells contiguous to the already
differentiated xylem; furthermore, the great majority of these cells, and
not the specific layer(s) of cambial initials, are most active during the
first month(s) of radial growth. Therefore, Bannan (12) observes, a considerable portion of earlywood may be formed by redivision of (over-wintered) xylem mother cells; this portion may include anywhere between 2 to 24 (average of 8) such cells (11).

Some chemical background exists as to chemical differences across a growth zone. Early- and latewood carbohydrate differences were first noted by Ritter and Fleck (102) who reported cellulose content of western white pine (Pinus monticola Doug.), Douglas fir, and loblolly pine to be significantly higher (2 to 5%) in latewood; this same relationship has been indicated for holocellulose components of Douglas fir (9, 53), red pine (53), loblolly pine (160), Japanese red pine (55), and Scots pine (89). Hydrolyzates from growth zones have been studied also: Meier (89) and Meier and Wilkie (90) showed that in Scots pine, glucose (cellulose) content was a constant 56% across the ring, while glucomannan content increased by 4% and that for arabinoglucuronoxylan decreased by 4%. Larson (76) showed almost identical patterns in red pine; at constant age, glucose recovery from latewood consistently exceeded that of earlywood by 1 to 2%, and that for mannan in latewood was also 3 to 4% higher. Arabinan and xylan yields were higher by 1 to 2% in earlywood. Larson (76) explained that, while the increasing glucose and mannose values (throughout a radius) are age-dependent, they may be modified by the age-related increase in wall thickness. A summary of such data available from the literature is presented in Table 1. It is important to note that all these observations are based on sampling only two points within a growth increment, frequently
with unusually wide-ringed materials.

Only two previous studies have included sampling more than two points within an increment. Carbohydrate distribution was studied for six points within each of three Douglas fir increments. Using a procedure involving 0.5 g wood meal and known to have serious limitations in statistical reliability, Ifju (61) showed that for each ring, peak chlorite holocellulose as determined by the Zobel and McElwee (160) method (77 to 78%) occurred at the earlywood-latewood transition, while first and last-formed positions in the increment gave lower values (72 to 73%). In a later study, these "micro"-holocelluloses were corrected for a residual lignin determination applied for the first time (151). This micro-Kappa method (149) has been examined and reconfirmed. Hydrolyzates of glucan plus mannan (88 to 94% of the holocellulose) followed the holocellulose pattern, while galactan increased across each increment (2.5 to 6%); and pentosans decreased (xylan, 8 to 4.5%; arabinan, 0.6 to 0.2%).

Ifju also determined mean cellulose chain lengths for early- and latewood cellulose nitrates dissolved in acetone. Mean intrinsic viscosity for each of three increments was 34 dl/g in earlywood and 36 dl/g in latewood.

Mark (88) studied resistant carbohydrate by Cross and Bevan analysis in early- and late-earlywood and latewood of eastern red cedar (Juniperus virginiana L.). In order, each contained 45.0%, 49.3%, and 57.7% resistant carbohydrate.
Besides noting earlywood-latewood differences in carbohydrate yield, many of the preceding authors also reported similar quantitative differences in lignin contents, but with the higher values in each case being derived from earlywood. Wu and Wilson (157) have tabulated such earlywood-latewood values for lignin comparisons in coniferous woods available in the literature.

Using extracted wood meal from three adjacent increments of mature wood, intra-increment lignin contents of five western coniferous woods were investigated by Wu (156). In the great majority of increments studied, earlywood was higher in lignin percentage than latewood, the average difference between these being, for individual species: Douglas fir, 2.27%; Pacific silver fir, 2.06%; western red cedar, 1.28%; Sitka spruce, (Picea sitchensis (Bong.) Carr.) 1.08%; and western hemlock, 0.46%. Three different distribution patterns were evident: 1) lignin increased from Position 1 to 2 in earlywood and decreased thereafter to Position 6 in latewood; 2) lignin decreased progressively from Position 1 to 6; and 3) lignin increased from Position 1 to 2 and remained constant thereafter from Positions 3 through 6. Wu found a highly significant correlation for lignin percentages between positions within growth increments for representatives of the first pattern (Pacific silver fir, Douglas fir, and western red cedar) and the representative of the second pattern, Sitka spruce. Between growth increments, Douglas fir and western red cedar showed highly significant correlations. In order to obtain standard calibration, Klason lignin was prepared. Since a portion of this is acid-
soluble, Wu's values may be slightly low. Wu (155) showed that methoxyl contents of these species followed similar patterns within increments.

The first pattern of Wu's lignin distribution has also been shown to exist for Douglas fir polyphenols, as sampled at eight positions across a single increment (113, 114); maximum values for quercetin, dihydroquercetin, dihydrokaempferol, and pinobanksin all occurred within, but not at the initiation of, earlywood. These maxima occurred at a relative position of about 20% and thereafter fell off markedly to transition wood and levelled off through the latewood region. This position is analogous to Position 2 in Wu's lignin pattern.

There is little chance these two patterns are coincidental; the inference is that a flavonoid-oriented distribution pattern exists at least within Douglas fir growth zones and possibly in other coniferous woods having similar flavonoid extractives. This affirms the dual earlywood hypothesis of Wu and Wilson (157).

It has been suggested that both lignin and flavonoids originate from a common cinnamic acid precursor (14). This is entirely plausible. In buckwheat, for example, p-coumaric acid is the last precursor common to synthesis of both lignin and quercetin.

Some evidence exists as to physical differences across a coniferous growth zone. Intra-increment tensile and radial compressive strengths of six coniferous woods have been studied by Homoky (59); regressions of both strength properties and specific gravity showed highly significant
relationships. Compared with gross wood, it was found that, in both properties, specific gravity was responsible for greater stress increase of gross wood than of tissue. In woods having a gradual transition, distribution of specific gravity, maximum tensile stress, and maximum compression stress rises gradually from earlywood, then curves upward at the last-formed latewood. In those woods having abrupt transition, the distribution of these properties follows a sigmoid curve. The explanation of these patterns is given in terms of periodicity of, and variations in, cell wall thickness.

Ifju et al. (62) found the same sigmoid distribution of specific gravity in Douglas fir which, when correlated with tension parallel to grain elasticity, and ultimate tensile strength, gave highly significant results. Ifju (61) previously showed tension parallel to be a function of cellulose properties. Ifju et al. (62) and Homoky (59) developed specific gravity profiles for five western conifers and found that a broader range accompanied woods with abrupt earlywood-latewood transition (e.g. 0.2-0.7). Worrall (154) showed significant correlation between specific gravity and cell wall area and found cell wall density to vary directly with that of wood density.

The effect of fertilizer treatment on intra-increment strength of western red cedar has been examined (147). Following application of high nitrogen content fertilizer, growth rate increased, accompanied by a decreased specific gravity, tensile strength, stiffness and average tracheid length.
Morphological variations have been studied across coniferous growth increments. Within any one growth zone, the average length of latewood cells was greater than that of earlywood (99); also, there seems to be a definite pattern throughout the increment. This phenomenon is also found in Angiospermae (57). Bisset et al. (17) and Bisset and Dadswell (16) have shown that, following inception of growth in Douglas fir, cell length decreases to a minimum in the first portion of earlywood and thereafter increases to a maximum in the latewood, either rapidly or slowly, depending on the nature of the earlywood-latewood transition. Studies on variability of this and other cell dimensions have been summarized (36), as well as the effect of external environment upon such variability (99).

D. Cell Wall Organization

At the cellular level, the relative percentages of polysaccharides in the different cell wall layers has been estimated by Meier (89) by differentiating tissue technique. For Scots pine and Norway spruce, cellulose content was lowest in the middle lamella (M) and primary wall (P) layers (35%), but thereafter increased steadily by 15-20% over the outer, middle, and inner secondary wall (S₁, S₂, S₃). According to Timell (135) this reaffirms earlier relationships noted by Asunmaa and Lange. In the M and P, arabinan, galactan, and pectic acids were present in the order of 50%; in 1936, Bailey (9) showed M of Douglas fir to contain 14% pentosan. Meier (89) further showed that glucomannan was concentrated in the S₂ and S₃ (24 to 27%), while more than half the xylan was found in the S₃. Schuerch (108) cites Thompson et al. as having shown that carboxyl content is high near the P and decreases linearly across the cell wall to the lumen.
According to Stewart (118), Thornber and Northcote subdivided non-cellulosic polysaccharides of the P wall of Scots pine into pectic and non-pectic fractions. Of the pectic fractions (10 to 15%), one-half was composed of monosaccharide units, with the remainder being uronic acid units; the non-pectic fraction varied from 35 to 45% and contained a 4:1 ratio of monosaccharides to uronic acid units. The S wall was accompanied by polysaccharides containing glucose, xylose, glucuronic acid or 4-O-methylglucuronic acid, galactose, and mannose units.

The distribution of lignin through the cell wall of Monterey pine has been studied most thoroughly by Wardrop (143) and Wardrop and Bland (144); a lignin or its precursor is deposited initially in the P wall near the cell corners; during the formation of (cellulose) layer S_2, lignin appears along the intercellular M layer and, successively, in the tangential and then radial P walls. The majority of lignin is deposited or appears during or after formation of (cellulose) layer S_3; at the same time, layers S_2 and S_3 become lignified. During these phases of lignification, lignin continues to be deposited in the intercellular and outer layers of the cell wall.

The first attempt at a quantitative estimate of lignin distribution in the cell wall was made in 1925 by Ritter (100), who stated that 75% of the lignin in wood was located in the M, with the remainder being in the P + S layers. Three years later, he modified this finding to imply equal distribution between M and P + S, but in 1934, reverted to his first stand. In 1936, Bailey (9) showed that the middle lamella truly did consist of 71%
lignin and, in 1954, according to Frey-Wyssling (44) Lange used ultraviolet spectroscopy to establish that in spruce, 60 to 90% of the lignin was present in the M, with only 10-12% being in the S layer. Distribution throughout the latter appeared to taper off. Frey-Wyssling (44) cites Ruch and Hengartner as having shown lignification in jute fibres to be uniform throughout the S layer which contains slightly less than half as much lignin as the P layer. The conclusions of Ritter and Lange were recently disputed by Berlyn and Mark (15) who, by lucid but simple reasoning showed that most of M and P is lignin, but most of lignin is not in the M and P layers! From other published data, they calculated lignin content in M to be somewhat less than 40% of the total in wood.

The state of cellulose aggregation differs considerably from that of the encrusting constituents: Cellulose molecules are aggregated into biological units of structure termed microfibrils. From the many electron microscope studies on these units, Frey-Wyssling (43) has integrated the important conclusions into a unifying concept of microfibril structure. Dimensions of each microfibril are approximately 100 x 200 Å, within which four micelles or crystallites are located; the latter are about 30 x 70 Å in cross section, at least 600 Å in length, and are surrounded by paracrystalline material which, when undergoing lignin removal, apparently crystallizes to give a slight increase in micelle size.

According to Frey-Wyssling (44), the physical association of lignin with carbohydrates was first reported by Frey who showed that lignin was
encrusted in an amorphous state; this was confirmed by Freudenberg et al. who found that, after quantitative extraction of cellulose, lignified cell walls yielded a framework of amorphous lignin. Astbury et al. (7) have studied the physical relationship between microfibrils and associated cell wall constituents and, according to Wardrop and Bland (144), Preston and Allsop showed that the X-ray diffraction diagram of coir changed little upon lignin removal, leading to the conclusion that lignin did not penetrate the microfibrils. Hodge and Wardrop (58) obtained direct evidence of lignin penetration between crystalline regions of microfibrils; they reported that, after delignification, microfibrils (perhaps micelles) 50-100 Å in diameter could be seen with much greater clarity. Coppick and Fowler (31) studied a cold soda pulp of Australian mountain ash (Eucalyptus regnans F. Muell.) during the early stage of delignification and found lignin still adhering to the microfibril surfaces. The amorphous quality of lignin residues has been noted by Mühlethaler (92) who investigated the lignin structure of sisal after cell wall saccharification with sulfuric acid; he observed the presence of minute pores (250-400 Å in diameter) which were thought to be formed when the cellulose microfibrils were dissolved. Mühlethaler therefore concluded that cellulose and non-cellulose each form independent, but interpenetrating systems. From a cross-section of Tasmanian myrtle (Nothofagus cunninghamii, Oerst.), Wardrop and Bland (144) illustrated similar findings to this, noting the presence of pores 200 to 300 Å wide in residual lignin. As with Mühlethaler's observations, these pores could be expected to be larger than the microfibrils because of the swelling of cellulose and removal of para-crystalline areas during solution. Approximately 40 years after Frey's
work, Frey-Wyssling (44) repeated the experiments of Freudenberg et al.
and produced 'lignin ghosts' of radial cell walls of spruce tracheids
by dissolving the carbohydrates with cuoxam, leaving the proous skeleton
of lignin as a 'rodlet composite' body.

This suggests that if a (lignin) cement is added to a well-oriented
structure (cellulose) and the latter removed, the cement will then retain
its original structure and thereby the birefringence of its reflecting surface
leaves a ghost image of the original structure, cellulose.

E. Biosynthesis

While knowledge of wood biosynthesis is rather meagre in some
respects, much useful information is, however, available. Although much
is known about origin of sugars in plants, relatively little is available on
carbohydrate metabolism in trees, hence, most suggested metabolic path­
ways result from analogy with known sources in plants and/or fungi.
Regardless of their apparent physical and chemical differences, glucose
and lignin have a common origin, namely carbon dioxide (73); the mechan­
ism of conversion to sugars and polymers has been studied by many workers.
Jones (66) has shown that most hexose sugars arise in the following way:
3-$D$- phosphoglyceronic acid (the first product of photosynthesis) is split
off from a $D$ - erythropentulose diphosphate derivative and, with the enzyme
aldolase, condenses with a 3-$D$-phosphoglyceraldehyde or other triose
fragment to yield $D$-fructose - 1, 6 - diphosphate. This may then be con­
verted to pentose sugars through at least 3 pathways (66). Jones (66) cites
Krotkov et al. as showing that, besides containing mainly non-reducing hexoses, photosynthates of eastern white pine (Pinus strobus L.) seedlings contain small amounts of shikimic and quinic acids; these are known to be important lignin precursors. From this and other evidence, Jones (66) has shown that formation of lignin precursors requires the same unit as that used for hexose sugar synthesis, namely, 3-\(D\)-phosphoglyceronic acid (A). When condensed with \(D\)-erythrose - 4 - phosphate, it forms 3 - deoxy - 2 - keto - \(D\) - arabinohexulosonic acid - 7 - phosphate which then undergoes coupled reactions (73) with (A) to form shikimic and prephenic acids (66, 107). Hence, in addition to being involved in cellulose synthesis, (A) is involved in several stages of lignin biosynthesis.

Colvin (30) has given the following résumé of cellulose biosynthesis starting where Jones ended: glucose is activated intracellularly through glucose - 1 - phosphate (G - 1 - P) to uridinediphosphate glucose (UDPG) and transferred to a long-chain polyhydroxy alcohol to form a lipid-glucoselipidphosphate complex. In plants, this migrates through the cytoplasmic membrane into the plant cell wall. The glucose is transferred (by trans-glucosidase) to the end of a cellulose chain within the insoluble, elongating microfibril tips; the resulting polymerization and crystalization into the lattice are regarded as simultaneous. The lipid carrier then returns to the cell membrane for re-use in another cycle. Microfibril orientation may be subject to any of three influences; mechanical strain in the plant cell wall, lateral association with Van der Waal's forces, and direction by the protoplasm.
Stewart (118) suggests two mechanisms of cellulose biosynthesis:

It has been shown that the polysaccharide is built up of the stepwise lengthening of the non-reducing ends of a primer, which acts as the acceptor molecule. It is possible that primer molecules of saccharides are translocated into a structural mold. . . where they act as acceptors for glucosyl donors such as sucrose, UDPG, or B-1, 4-linked glucans. Thus the individual chains of cellulose may build up to a degree of polymerization of 10,000 or more, adjacent, parallelly-oriented chains perhaps being organized into crystalline micelles.

The alternate mechanism is that while . . . the average lengths of most cellulose micelles are usually within the range of 200-600 Å, these values correspond to a DP range of about 40-120. It is possible, therefore, that the primer molecules are formed within this range. They may then crystallize into micelles which, after being aligned in the structural mold, are subjected to the action of enzymes which knit together individual chains of cellulose between the micelles with incorporation of new units of glucose where necessary.

In outlining the biogenesis of cellulose, it was established that shikimic acid originated from carbohydrate material. Numerous workers have shown labelled shikimic acid to be converted via prephenic acid into aromatic amino acids, of which two pools exist: one for phenylalanine and the other for tyrosine. By this method, Neish and co-workers (93) also have established that deamination of these amino acids leads to cinnamic acids, and that derivatives of these acids (cinnamic alcohols and/or their glucosides: D - coniferin, p - coumarin, syringin) serve as intermediates in the biosynthesis of a number of phenolic metabolites peculiar to plants, i.e., lignin, flavonoids and coumarins. While these are all phenylpropanoid in structure, Neish cautions that this shows only that lignin can have a phenylpropanoid origin, not that it actually is such in structure. Nevertheless, similarity in origin and structure is, however, strong evidence for
the concept of phenylpropanoid structure in lignin.

The mechanism for conversion of cinnamic acid derivatives to lignin has been discussed by Freudenberg (42) who used Norway spruce as an example: The three cinnamic alcohols arrive at the cambium, presumably by translocation, where they may pass out as aglycones or, most frequently, combine with UDPG to form glucosides which are then stored. The greater percentage of coniferyl alcohol, the major constituent, is present as the glucoside coniferin (41). When the glucosides do pass out from the cambium to the immature cells undergoing lignification (2 to 3 cells from the cambium) they are hydrolyzed to aglycones by \( \beta- \) glucosidase, a localized enzyme. The aglycones (cinnamic alcohols now) are in turn attacked by abundant dehydrogenase present (of which laccase and peroxidase are two major sources) and converted into lignin. When the cell walls become plugged with lignin, the cell is mature.

Freudenberg (42) has also shown that conifer lignin has a methodical structure: Like other polymolecules, it is derived from single units, i.e., cinnamic alcohols. These condense or polymerize via mesomeric forms with different bond types at different points of attachment and without definite sequence. Through three major reactions, the polymer molecule grows simultaneously in at least three different ways, consequently lignin has a unique position among high polymers, because other high polymers are formed by one pathway.

Commenting on the above approach to lignification, Kremers (73) said:

In conformity with the principle that lignin must originate with photosynthesis products, the biochemical origin of
coniferyl alcohol has been related to the simple sugars by analogy to the microbial metabolism of glucose via sedoheptulose, shikimic and prephenic acids to phenylalanine and tyrosine. Biochemical reactions which would convert tyrosine into coniferyl alcohol via ferulic acid appear analogous to many transformations occurring in respiration and carbohydrate synthesis. As a whole, Freudenberg's scheme of lignification has much appeal because many steps are consistent with the generalities of plant metabolism.
III. Methods for Wood Micro-analysis

A. Carbohydrate Fraction

Wood carbohydrate analysis has evolved to the state where total polysaccharide materials or fractions thereof (whether empirically defined or not) have been prepared. Examples are firstly, the macro holocellulose preparations of Van Beckum and Ritter (138) and Wise et al (153) and secondly, the cellulose of Cross and Bevan (22, 33). The chief value of a holocellulose preparation is that it offers a beginning material for further research; it gives useful information about the starting material and allows approximate quantitative and qualitative study of hemicellulosic fractions and isolation of pectic materials.

During the past two centuries, various classical procedures have been developed for the isolation of carbohydrates from cell walls of extractive-free wood meals. Up to the 1950's, all were based on a two-stage lignin substitution and solublization using free halogens, their oxides or oxysalts. The first method to isolate cellulose to any degree was that of Cross and Bevan (33, 122). This required treatment of moist meal by alternate chlorination at 25-30° C and extraction with distilled water, 3% sulfur dioxide solution, water, then boiling 2% sodium sulfite. This cycle was continued until the fibrous material showed a faint pink tinge on further addition of sodium sulfite. The method, however, was not quantitative and contained approximately 80% of the total holocellulose (23), as at least 10% of the pentosans and 5% \( \alpha \) - cellulose were removed with the lignin (123, 138).
According to Wise et al. (153), the first method to isolate quantitatively both the cellulose and hemicellulose was that advanced by Schmidt who, in 1932, used chlorine dioxide, pyridine, and water. Although these reagents had practically no effect on the carbohydrates, his method unfortunately required one month for the isolation. In 1933, Ritter and Kurth (103) employed alternate treatment of chlorine, alcoholic pyridine, and cold calcium hypochlorite on extractive-free wood meal. When compared to Schmidt's procedure, they obtained excellent confirmation and substituted the term "holocellulose" for what Schmidt previously had called "skelettsubstanzen", the reason being that the latter did not describe the material correctly. However, this method needed refinement as the time required for analysis was two days and the use of pyridine was unpleasant (138). In 1937, Van Beckum and Ritter (138) modified this procedure to shorten the isolation time to three hr, thereby providing a method that is still in popular use today (123) as it removes nearly all the lignin. The solvent used to dissolve the chlorolignin and neutralize hydrochloric acid formed during chlorination is hot ethanolamine, with alternate chlorination and extraction made until the residue remains uncoloured on further extraction. The solvent, itself, has little if any action on the carbohydrate fraction, but in the aqueous condition, as when followed with water extraction, the solvent tends to remove small amounts of carbohydrate and become absorbed by the holocellulose itself (153). In addition, chlorination causes overheating of the meal, consequently polysaccharide degradation and hydrolysis can be expected (23).
Wise et al. (153) cite Jayme as having introduced the use of sodium chlorite with acetic acid as applied to extracted, microtomed wood sections. This required 12 hr for delignification and the residue, unlike chlorinated holocellulose, contained 3 to 5% lignin which could not be removed without loss of attendant carbohydrate; however, because of its simplicity and mild action on cellulose and hemicellulose, Jayme's method was studied intensively by Wise et al. (153) and marked improvements have been made since.

Wise et al. (153) have been able to reduce reaction time by two-thirds and apply this to analysis of wood meal. Conifers are more amenable to this treatment than to chlorination. Browning and Bublitz (23) found that the foregoing method permitted excess loss of carbohydrates as delignification approached completion. They therefore utilized the best features of both the chlorite and chlorination procedures to obtain better pentosan, hence, holocellulose yield than either. In the same article written in 1951, they (23) made an appropriate resumé of progress in cellulose isolation:

The isolation of cellulose preparations from wood by delignification with suitable reagents has been carried out for nearly a century; in early work, the differentiation between the substance now designated "cellulose" and "hemicellulose" was not clearly recognized and part of such a nature that extensive hydrolysis and oxidation of the cellulose occurred. As a result, the "cellulose" preparations obtained were of a heterogeneous character and little significance could be attached to analytical figures.

Recently, there has been a need for micro-procedures based on a wood meal sample size less than 1 g; four such procedures have been advanced, all derive from using scaled-down versions of the more popular,
though highly variable macro-methods. Zobel and McElwee (160) determined holocellulose and \( \alpha \)-cellulose yields on 0.75 g portions of wood meal and found values to be high by 1 to 2\%, due to the mild method of isolation. Though this method gave reproducible results with a replication number of two, it did not appear to be under statistical control. Erickson (38) used a multisample system employing 0.5 g samples in which extraction and chlorite holocellulose treatments were performed without transfer of samples. However, while great care was taken to study such effects as temperature, particle size, extraction procedure, and pH, no statistical verification was evident as choice of sample size appeared to be arbitrary and no replication was stipulated. Watson (146) has applied the method of Cross and Bevan to 0.5 g samples of Monterey pine. Of 50 samples having two replications, 43 were within 1\% of each other. This does not constitute statistical validity. Using weights below 0.4 g, however, error increased significantly. Leopold (77) has reported the preparation of holocellulose fibres from loblolly pine, but failed to indicate sample size or replicates. Though statistical control is lacking to a variable degree in all these methods, their great disadvantage is their large sample size and the high replication number that is obviously needed with the macro procedures upon which they rely. Also, correction for residual lignin was absent.

A different, fifth semi-micro method has been used by Meier (89) and Meier and Wilkie (90) in which secondary xylary fibres are hydrolyzed to their monosaccharides, from which the polysaccharide composition is calculated from some basic assumptions and chromatographic evidence.
Larson (76) has also used a similar method for calculating polysaccharide composition. While both methods have appeal, it is probable that they are only proximate; since reconstruction involves assumption of polysaccharide composition from their hydrolysis products and not direct experimental evidence alone, a degree of uncertainty is introduced.

About 1940, researchers were turning their attention from imperfect carbohydrate isolation to the action of nitric acid on wood. As well as substituting and solublizing the lignin, nitric acid forms a cellulose nitrate ester derivative which can be used for yield and molecular weight determination. Jahn and Coppick (64) reported that nitration of woody tissues with fuming nitric acid was first done in 1937 by Friese and Furst who nitrated a "spruce" and "red beech" in Europe. They used nitric acid mixed with sulfuric or phosphoric acids and reported a 50% solublization of spruce (hence, a solid residue of under 50%). Jahn and Coppick (64) then did a feasibility study to determine whether or not woody tissues in general could be nitrated to yield products of possible practical or scientific value. Besides three pulps, eight woods were studied successfully using a rather crude procedure; a sulfuric-nitric acid mixture at 10°C was poured onto air-dry, 60-80 mesh, unextracted wood meal and maintained at 10°C for five hours after which the product was drowned in ice water, bleached, and dried. Properties of the nitrate included a 90% acetone solubility, 11% nitrogen content, and a rather loosely defined spectrum of molecular weights. Characteristically, softwoods gave a higher yield of nitrate.
The nitration procedure has since been refined and simplified by Timell (134) and Alexander and Mitchell (2); the latter developed a nitrating mixture which, by virtue of its causing minimum degradation and high degree of nitration, is still used today.

In the late 1940's and early 1950's, work by several Norwegians showed that the \( \alpha \) - cellulose fraction of wood pulps could be obtained reliably by nitration. Timell (130) reported that Bryde pioneered this approach and later, Bryde and Smith (25) recovered 45% of a semi-chemical pulp having an intrinsic viscosity \( [\eta] \) of 36 dl/g. Abadie and Ellefsen (1) then compared \( \alpha \) - and cellulose nitrate (hereafter "nitrocellulose") yields of hydrolyzed cotton and industrial pulps between the range of 50 to 98% from 26 pairs of values. They showed a correlation coefficient of 0.99 and regression coefficient of 0.98, demonstrating nearly identical quantitative correspondence. Furthermore, they found qualitative correspondence by examining the degree of polymerization; they concluded from both approaches that the nitro\( \alpha \)-cellulose from pulp was nitrated \( \alpha \) - cellulose. Bryde et al. (24) showed a similar correlation in other commercial pulps and also found that samples as small as 0.2 g could be used.

Timell (129) has investigated the chemical purity of white spruce (\textit{Picea glauca} (Moench.) Voss) nitrocellulose as well as its chain length and polydispersity. He found chlorite \( \alpha \) - cellulose and maximum nitrocellulose yields to agree closely (i.e., 50 and 49%, respectively) and that on chromatographic analysis, both contained glucose, mannose, xylose, and uronic acid residues. Timell concluded that both methods account for
very similar portions of wood, although neither yield "true" cellulose. Snyder and Timell (111) similarly investigated Pacific silver fir (Abies balsamea L., Mill.) and showed chlorite cellulose and maximum nitrocellulose yields of 48% and 49%, respectively. The nitrocellulose product contained glucose (88%), mannose (9%) and xylose (1%) residues while, surprisingly, both the water-acetone and methanol-soluble portions contained glucose and mannose residues. Timell (130) finally compared products from both methods of cellulose recovery for four additional conifers; each gave cellulose values that agreed, ranging in yield from 40 to 45%. Timell also found that the smaller portion of total glucomannans was occluded in the nitrocellulose. Alpha-cellulose contents for most species agreed with those reported previously (129) and differences between methods showed no more variation than those observed by Abadie and Ellefsen (1). Strangely, only the latter authors indicated some semblance of statistical reliability with regard to testing hypotheses and sampling technique. As is customary with wood chemists, the remainder appear to have neglected reliability criteria altogether.

Since Staudinger, as reported by Stamm (115), and Kraemer (72) first developed empirical equations relating viscosity of high molecular weight polymers to DP, nitrocellulose has been used as an analytical tool for viscometric determination of DP and molecular weight in wood and wood pulps; this has been its most popular application. However, Abadie and Ellefsen (1) have utilized nitrocellulose to demonstrate both qualitative and
quantitative similarity with \( \alpha \) -cellulose from pulp and, after conducting similar experiments on North American conifers, Timell (130) has suggested the use of nitrocellulose as an alternate method for the isolation and estimation of \( \alpha \) -cellulose in wood. In addition, this derivative has been used to study chain-length and polydispersity of various pulp species in Scandinavia and North America. Bryde et al. (24) found the method especially suited to the study of digestion processes at various stages of delignification; since the lignin was removed it did not interfere with measurements. Hence, these diversified applications, indicate that nitrocellulose may be employed as a useful and important analytical tool in wood and cellulose science.

In considering the mechanism of nitrocellulose formation, it is important to bear in mind that cellulose is a fibrous material that may be considered at several levels of orientation, depending on the investigator and the question his analysis is meant to answer. One may progress from the sub-macroscopic level of the plant cell through the fibrillar and microfibrillar submicroscopic levels to the micellar and, finally, the molecular stage of the classic monoclinic unit cell of Meyer and Misch. Briefly, this envisages the unit cell for native cellulose as containing five cellobiose units positioned in such a way that distances between atoms of the different chains determine the nature of the forces holding the cellulose lattice together (63, 87). Each chain, of course, comprises many \( \beta \) - anhydroglucopyranose units having one primary and two secondary hydroxyls per ring. It is therefore clear that, in formation of any cellulose derivative,
the problem of hydroxyl accessibility is an important one.

In order to comprehend cellulose reactions, however, one must begin at the submicroscopic level: here, microfibrils are proposed as consisting of aggregates of cellulose chains (micelles) passing through ordered (crystalline) and disordered (amorphous) regions. If a reagent penetrates between microfibrils into amorphous regions, the extent and rate of reaction increases greatly; however, the reaction product will be non-uniform, as no reaction will have occurred with hydroxyls of the crystalline regions. Only when the latter condition is fulfilled will uniformity result, hence, in formation of cellulose derivatives, it is important that all cellulose hydroxyls are available for reaction (63).

Besides this effect of supermolecular structure on substituent distribution, two other factors must be considered, the cellulose linear polymeric nature and the hydroxyl properties. The long chain nature of cellulose makes it important that reactions occur along the chain in order to produce a homogeneous end product; recent work (63) has shown most cellulose reactions (including "nitration") to be permutoid, i.e., all hydroxyls are completely accessible. This has the overall effect of making the reaction proceed homogeneously.

When all hydroxyls are assumed to be accessible and equally reactive, the distribution of substituents with mean degree of substitution (D S) can be predicted. Spurlin (112) showed that a cellulose derivative having an intermediate overall degree of substitution always contains a certain amount of unsubstituted, mono, di- and tri-substituted anhydroglucose units,
whereas one with a high DS (2.7 to 3.0) closely approaches homogeneity. He also showed that differences in hydroxyl reactivity (which are slight) did not greatly affect the overall substituent distribution! Hence, the latter is of relatively minor importance.

In order to bring cellulose hydroxyls into contact with a reagent, hydrogen bonds must be broken to allow entry of the swelling agent. In the case of nitration, this causes limited intra-crystalline swelling characteristic of a new lattice to give a new, well-defined X-ray diagram and allows formation of the well-known addition compound of Knecht. Such compounds are important in that reagents can freely diffuse to enhance reactivity and this predisposes formation of more or less homogeneous derivatives (63).

The Knecht compound serves as the intermediate in nitration of cellulose with nitric acid above 61%; concentration; in its formation, X-ray evidence shows the cellulose lattice is attacked and expanded mainly in one direction. Whereas the periodicity along the \( b \) axis of the monoclinic cell is usually unchanged, the \( a \) and \( c \) dimensions increase (98). This is explained by an increase in the (101) interplanar distance, but almost identical (101) and (002) spacings with those of cellulose II (105). From a chemical point of view, the addition compound forms as follows: when cellulose is treated with concentrated nitric acid, the hydrogen bond between adjacent hydroxyls is destroyed. This is replaced by a new bond formed between one hydroxyl, the hydronium ion of the reagent, and either the opposite hydroxyl of the adjacent chain directly
or through another molecule or ion. Hence, one or more oxygen atoms may form a connecting bridge between hydroxyls (105). A correct amount of water in the mixed acid is important (2); enough must be present to permit reagent ionization, but not enough for competition between hydroxyls and the water molecules for the hydronium ions and, thus, prevent addition-compound formation (63). When water is present in optimum quantity and hydronium ions form hydrogen bonds, the counter ions with their water shells follow via electrostatic attraction to cause lattice expansion and intermicellar swelling (105). The cellulose hydroxyls are then capable of reacting like ordinary aliphatic hydroxyls to form esters.

Nitration is carried out in a medium of conc nitric acid, a strongly acidic swelling agent (sulfuric, phosphoric, or acetic acid), and water. This reaction is very rapid and, strictly, does not occur with cellulose itself, but with the addition compound. While the swelling acids have strong affinity for hydroxyls, nitric acid is obviously superior; this may be due, in part, to the NO$_3^-$ ion having a planar structure while ions of sulfuric and phosphoric acids are not such, but are rather voluminous (91). Substitution occurs with production of a nitrate ester and nitric acid-hydrate molecule; with increase in nitration time, more hydrate is produced and less acid is available for nitration (91). In the micellar regions, the nitrogen content of cellulose is a net result of two processes: inward "diffusion" of fresh acid and outward "diffusion" of "spent" acid (i.e., nitric acid-hydrate). For lack of knowledge and the appropriate term, the expression "diffusion" is used by Miles (91) to describe this process. The
final solid product is not simply nitrocellulose, but a nitrocellulose-nitric acid complex. The above gives a general understanding of the relative rate of nitration with time: at first, nitrogen content rises rapidly to a point within 1% of the final value, but since the rate decreases with time, the final interval is passed only after a long period. Other factors also influence speed of nitration: acid viscosity parallels nitration time markedly and with dilute acids and those of low nitrogen content (see addition compound formation) nitration is slow (91).

The presence of a swelling agent is necessary as it preserves the cellulose fibrous structure and acts as a dehydrating agent on nitric acid to form the NO\textsuperscript{2+} ion; use of nitric acid alone causes fibres to gelatinize which impedes esterification (46). Nitration carried out with sulfuric acid catalyst leads mainly to nitration; increasing the sulfuric acid content and decreasing the water content (after optimum quantity) enhances sulfation (esterification with sulfuric acid), preventing maximum nitration. This is alleviated by using phosphoric acid, an acid of lower electrophilicity. Being weaker, it forms less phosphate ester as a side product (residual phosphorus content is never more than 0.4%) (48), therefore, a higher nitrogen content is attained (e.g. 13.9%) (2, 47). Also, it is thought to yield a product of essentially the same D P as that of the original cellulose (48), hence, it is particularly suited for \text{\underline{[\eta]}} determination of plant and lignified materials. Though acetic anhydride gives slightly better \text{\underline{[\eta]}} values, it cannot swell and, hence, nitrate plant cellulose.
In summary, maximum yield of nitrocellulose is correlated with that of chlorite-cellulose to a highly significant degree either when non-corrected (1) or corrected (130) for non-glucan material. This, of course, presupposes use of the optimum nitrating mixture of Alexander and Mitchell (2) used under nitrating conditions (established for each species) as given by Timell (134). The method consists of a relatively non-degradative conversion of cellulose to a nitrate derivative, which may be performed on samples as small as 0.2 g (24). Besides indicating yield, the method will allow use of sufficient material for subsequent analysis. Herein lies an important novelty that might allow reliable measurements to be made on minute amounts of wood materials with reasonable replication number. The method could contain useful features because of the small amount of wood material needed, a requirement which even the "semi-micro" methods have failed to meet.

There is no simple or satisfactory method for the analytical determination of cellulose in wood: after reducing wood to a suitable particle size, the polysaccharide fraction may be either extracted directly from this or the meal may be delignified by a mild procedure to give holocellulose. The former is possible only in a few cases: arabino-galactans may be water-extracted (hence, their location in the cell wall is questionable) and approximately half the hemicelluloses in angiosperms may be extracted directly using 16% sodium hydroxide. Other than these instances, delignification is necessary for all coniferous woods.

Ideally, such an isolation seeks complete lignin removal without loss of, or attack on, the associated polysaccharide components. Experimentally,
this is difficult to achieve and no procedure has yet been devised to meet this aim without attacking the polysaccharides. Although indispensible for furthering knowledge of wood chemistry, present methods have also caused much confusion. Such analytical procedures are at best empirically useful for isolating groups of related substances, rather than single molecular species. The product resulting from the action of fuming nitric acid on wood, however, has been shown by Timell (130) and Abadie and Ellefsen (1) to closely approximate $\alpha$ - cellulose content. Moreover, in forming this derivative, the lignin is substituted, then solublized; the net result is that three important changes have occurred with less degradation than encountered with the conventional two stage lignin isolation procedure. Further, the derivative has double usefulness for estimation of $\alpha$ - cellulose yield and molecular weight (or D P) determination.

Because of its polymolecular nature, hence, polydispersity, the distribution of cellulose molecular weights (or that of its derivatives) is of interest to physical chemists. The methods most commonly employed for determination of the "differential distribution curve" are those of fractional precipitation or solution. The former is applied to cellulose derivatives in solution through addition of a non-solvent, by removal of solvent, or by addition of solvent non-solvent mixtures; the net result is that precipitates occur in order of decreasing chain-length. The latter method relies on the progressive extraction of fractions of increasing chain-length from undissolved cellulose derivatives as the extracting solvent becomes a better solvent.
The following studies with nitrocellulose from conifers involve the use of fractionation by precipitation. Coppick and Jahn (32) nitrated spruce and white pine, and found that the viscosity frequency curves for each species consisted of two groups of molecular sizes. The first, mainly carbohydrate in nature, had wide molecular weight distribution of a size comparable to nitrated cotton linters; the second, composed of approximately 5% of the alcohol-extracted product, had a narrow distribution of low molecular weight, which probably represented a mixture of lignin degradation products and non-cellulosic materials. Timell (129) determined that for white spruce maximum \[ n \] of cellulose nitrate dissolved in acetone was 33 dl/g using a Cannon-Fenske viscometer. Frequency distribution of D P showed two peaks within a range of 1000 to 5500, one occurring at 1400 and corresponding to 20% of the cellulose, and the other occurring at 3100; among the numerous species having this two-peak chain-length distribution are some spruces, western hemlock, and Douglas fir (129). According to Timell (129), Jorgensen has found a one-peak distribution for black spruce. Mannose residues were rather uniformly distributed over the entire range and little difference showed, chemically, between each peak. In Pacific silver fir, Snyder and Timell (111) showed that the frequency distribution of D P varied between 900 and 5500 and, as with white spruce, two maxima were present: that at 1000 represented 25% while the second, at 2500, represented most of the remainder. In location and general nature, this is almost identical to that obtained for white spruce (129) and paper birch (Betula papyrifera Marsh) (137).
Maximum $\bar{n}$ of cellulose nitrate dissolved in acetone was 40 dl/g using a Cannon-Fenske viscometer; this is higher than the 33 dl/g quoted elsewhere for this species (129), the source of this discrepancy being unknown. Throughout the frequency distribution, little chemical difference was evident.

Much information exists as to the nature and fine structure of cellulose in situ. Mainly from X-ray studies, the size and orientation of the monoclinic unit cell are known, as is the approximate size of crystallites and their regions. Because of the solid crystalline nature of cellulose, however, its polymeric or molecular properties cannot be studied in situ with present methods and, consequently, it must be solublized to disperse its molecules.

Many qualitative methods are available for cellulose characterization: osmotic pressure and sedimentation equilibrium methods may give molecular weights or D P directly; a condition for the latter is the assumption of the presence or absence of a definite lateral association of the structural chains (115). End-group and viscosity methods give primary-chain lengths which may, in turn, be converted to molecular weights by assuming a degree of association (115). In conjunction with other measurements diffusion studies yield molecular weight and shape factors, and spreading measurements, the molecular thicknesses. The poly-dispersity of cellulose and its derivatives affects some measurements differently: while various types of average can be calculated from sedimentation equilibrium data,
viscosity methods yield weight-average molecular weights and osmotic pressure and end-group methods give those on a number-average. For polydisperse materials, the latter gives smaller average molecular weights. With the light-scattering technique, weight-average molecular weights are determined along with information about the size and shape of the polymer; cellulose solutions undergo considerable degradation, however, and absolute measures are impractical. Used with cellulose derivatives, though, the technique has been successful on solutions of cellulose nitrate (8). The ultracentrifuge (120) may be used to determine not only average molecular weight, but also molecular weight distribution and sedimentation velocity.

Experimentally, viscosity measurement of a cellulose solution is the most available and simplest of the above methods, hence, it has been applied empirically to gauge plant production control and effects of various chemical treatments on cellulose, these being largely followed and controlled by measurements in cuprammonium solution (95). Evidence from reversible and degradative transformations between cellulose and its common derivatives show both are dispersed as single chains; should they be associated, such transformations would be expected to alter the association (72). Further, cellulose and such derivatives in dilute solutions with "good" solvents are dispersed in units that rightfully may be regarded as molecules (72). Linear polymers (long-chain molecules) affect viscosity to a far greater extent than spherical particles; this effect increases rapidly with an increasing minor-major axis ratio. For rigid, rodlike polymers, no simple relation-
ship exists between specific viscosity, concentration and axial ratio; a solution is possible when Brownian motion is either so great as to negate orientation (e.g. small particles) or so small as to be negligible (e.g. large particles); no solution is possible for intermediate conditions. Most relationships, however, involve the square of minor-major ratio; and some approach considering the effective particle volume equal to a sphere described by the particle length as the diameter (115).

To give a true picture of a high polymer in solution, one would expect that, as reflecting its flexible nature, its viscosity should be less than that of rigid rods, but more than that of a random coil. That this is the case has been shown from a relation between intrinsic viscosity and molecular weight, the Mark-Houwink equation (34, 105):

\[ \eta = K_M \cdot M^\alpha \]
\[ = K_M \cdot DP \]

where: \( K_M, M^\alpha \) are constants varying for solvent and cellulose derivative

\[ \eta \text{ = intrinsic viscosity} \]
\[ DP \text{ = degree of polymerization} \]

The exponent '\( \alpha \)' is a function of the geometry of the molecule in solution; theoretically, its value ranges from 0.5 for chain molecules with unrestricted rotation (a random coil) to 1.5 to 2.0 for stiff, rodlike molecules; cellulose trinitrate in acetone has a value of 1.0, showing that such molecules in solution display fairly-low flexibility, although they are
far from rodlike (105). The constants of this and other viscosity equations are obtained by comparison of viscometric measurements with one of the other molecular weight methods, e.g. ultracentrifugation. The viscosity method, then, merely expresses an empirical proportionality; it is not an absolute method used for molecular weight/DP determination, but a simple means of following such variations of members from a polymeric series.

Compared to standard viscosity methods, viscosity of nitrocellulose offers these additional advantages: it is rapid and accurate (compare the cuprammonium method); lignin, being removed from the starting material, does not interfere with measurements; and chain-length degradation is least evident. Apart from the instability of the nitrates when formed, the only further limitation of consequence is the need for development of a highly accurate and precise method for estimation of nitrogen content, since variations in this will markedly affect viscosity.

B. Other Components

Methods available for micro-analysis of lignin have been reviewed recently by Wu and Wilson (157), while those for polyphenols and resins in Douglas fir are given in papers by Squire et al. (114) and Campbell et al. (27), respectively.
MATERIALS AND METHODS

I. Wood Sample Preparation

A. Sampling Criterion

The criterion for selection of wood materials was based on a broad botanical source of wood tissues: thereby, results from the work might be interpreted over a reasonable range and more closely approach a general statement. From Pinaceae, six genera and seven species were sampled; these were jack pine (Pinus banksiana (Lamb.), Sitka spruce (Picea sitchensis (Bong.) Carr.), black spruce (Picea mariana (Mill.) B.S.P.), tamarack (Larix laricina (Du Roi) K. Koch), Douglas fir (Pseudotsuga menziesii (Mirb.) Franco), western hemlock (Tsuga heterophylla (Raf. Sarg.), and Pacific silver fir (Abies amabilis (Dougl.) Forb.). Woods from two genera and species of Cupressaceae were included; these were western red cedar (Thuja plicata Donn) and yellow cedar (Chamaecyparis nootkatensis (D. Don.) Spach.). The entire sampling plan included two families, eight genera, and nine species, with replication between trees for one species and replication of more than one increment within trees for two species. Origin and description of gross wood characteristics for some of these wood samples is given in Appendix III.

B. Preparation of Wood Meals

The general procedure for preparation of wood meals was as follows: immediately after felling, disc sections were removed from each stem at breast height, wrapped in polyethylene, and transferred to the Wood Science
Laboratory at the University of British Columbia. From these, rectangular, straight-grained specimen blocks [1/2-in (tangential) x 4-in (longitudinal) x 2-in (radial)] were split from the outer heartwood or sapwood along the major and minor axes and soaked in water for several days. Increments chosen represented characteristic widths (2 to 3 mm) for regular mature wood in the particular stem sections and for the species. As Wu and Wilson (157) point out, this is a strong point of sampling; in contrast to former work on excised earlywood and latewood, this does not require abnormally wide-ringed material.

Blocks were microtomed in the manner described by Ifju (61), whereby each specimen was placed in the jaws of a special 6-in vise, aligned, and tightened so that the top growth ring paralleled the blade cutting edge. The preceding ring was sectioned tangentially to allow for minor block adjustments in the cutting plane and the resulting extra sections were used to determine nitration time to maximum yield; between 25 to 35 sections averaging 100 μ thickness were removed from each increment and numbered individually with alcohol-benzene soluble pencil. These were air-dried and divided into six equal groups according to six sequentially spaced positions across the increment from early- to latewood. After grinding the sections through a Wiley mill, sieving and collecting a certain mesh fraction to be described, 0.5 to 1.0 g portions of the wood meals were sealed separately into small (1 1/2-in x 2-in) 100-mesh nylon organza bags with a soldering gun. All bags were grouped together and successively extracted.
As the materials had been collected and prepared over a 3-yr interval, slight differences occurred in some aspects of preparation. In 1963, Wu (156) studied Sitka spruce, Douglas fir, western hemlock, Pacific silver fir, and western red cedar. Sections were ground into meal using an intermediate Wiley mill, collected as the 40–80 mesh fraction, homogenized, conditioned to similar moisture content, and sealed under nitrogen until used for this study. The other woods were collected in 1966, and, apart from the Douglas fir sample from Increment 40 (which was microtomed two days after felling), block sections were stored in the cold in thymol solutions. Following microtoming, sections were fed gradually through a variable speed micro-Wiley mill (Wiley-F.R.I. micro model) set at approximately 3500 rpm, and ground to pass a 20-mesh screen. The latter two steps minimized fines; on screening, the 40–60 mesh fraction was retained while the oversize particles were returned to the mill and reground to pass again the 20-mesh screen. By this method, air-dry wood microsections were recovered in 75 to 80% yield as 40–60 mesh wood meal.

The 40–60 mesh fraction size was chosen because preliminary studies showed that for both early- and latewood, nitrocellulose yield reaches a maximum for this particle size and is least variable in the range of particle size between 80-mesh (0.16 mm) and 9.0 mm. Likewise, Timell (130) also recovered higher nitrocellulose yields with 40–60 mesh white spruce meal than with 60–80 mesh wood meal.
Early studies also showed that the wood extraction treatment influenced nitration. The procedure finally adopted was successive extraction of all samples as a group with four 1 hr changes of boiling distilled water and air-drying, followed with alcohol-benzene (1:2) for 24 hr in a Soxhlet apparatus. As example of extraction studies, four treatments of five replicates each were applied to the same Douglas fir latewood meal; following nitration on the moisture-free extracted meal, that giving the highest yield with least variation dictated the course of future work. In order of increasing yield, the treatments were found to be ethyl ether, ethanol, water (46.1 ± 1.0%); water, ether, alcohol (49.3 ± 1.3%); alcohol-benzene, water (49.7 ± 0.8%); and water, alcohol-benzene (51.0 ± 0.3%).

It was not the purpose of this thesis to thoroughly examine the interaction of wood extraction history with nitrocellulose preparation. Obviously, some serious effects are involved. The literature neglects these entirely.

C. Validity of Results

The application of statistics to wood chemistry procedures is primitive or often neglected, with consequence that the validity of some results is open to serious question. With this in mind, both sample size and replication number were carefully examined in the present study. Table 2 shows a typical calculation for determination of replication number. The procedure was repeated several times during development of methods for the purpose of evaluating progress in controlling experimental error. The objective was to minimize the amount of material needed for a statistically defensible determination. Thus, it was finally found that, to determine
yield reliably for a single position within a wood growth increment, only
3 x 0.1 g = 0.3 g of wood meal was needed. In addition, this sample size
supplied sufficient cellulose nitrate for nitrogen corrections and viscosity
determinations. The amount of cellulose recovered as the nitrate was
calculated from the yield corrected for the nitrogen percentage of the
derivative: Miles (91) gives a convenient equation for this:

\[ p = \frac{100 \ N}{31.13 - N} \]  \hspace{1cm} [2]

where \( p \) = increase in weight of 100 g of "cellulose"
\( N \) = nitrogen, %

The symbol "\( p \)" is really a conversion factor used to reduce weight of
nitrocellulose to weight of "cellulose" (regarded here as an estimate of
\( \alpha - \text{cellulose} \)) and is derived from the relationship (ratio) between mole-
cular weights of cellulose trinitrate and cellulose. As the former is 297 and
the latter 162 this ratio is then 1.833, which corresponds to complete
nitration of cellulose (14.15% N).

Use of 0.2 g samples gave good results using three replicates,
but required twice the amount of material as 0.1 g without large change
in absolute value or reliability. By using 0.05 g, reproducibility was lost.
The final procedure, applied to three 0.1 g replicates for samples from
four other species, gave values not unlike those for the Douglas fir early-
and latewood. Therefore, it was concluded that this number would be suffi-
cient for all wood tissues. Where the difference (range) among the three repli-
cates was much greater than the confidence interval of 1.2%, or where the absolute yield appeared questionable, duplicate sets of determinations were done and modal values were chosen from all data representing a position.

II. Nitration Procedure

A. Mixed Acid Preparation

The procedure used for preparing the nitrating mixture was that developed by Alexander and Mitchell (2) and modified by Timell (134). Additional features were discovered. Analytical grade, colorless, 90% fuming nitric acid (120 ml) was poured into a 500 ml, glass-stoppered Erlenmeyer flask. This was immersed in a Dewar flask (140 mm diameter) filled with ethylene glycol and cooled to about -15°C using a "Whirlpool" thermoelectric immersion cooler (Canlab Model 84-640) regulated by transformer.

Use of a larger diameter Dewar flask did not allow the desired temperature depression, presumably because of greater coolant surface area exposed to the atmosphere. Since the acid was slightly colored as received from the supplier, it was necessary to remove the nitrogen oxides by bubbling through dry nitrogen. To do this, a two liter round-bottomed flask was filled three-fourths full of acid, placed in a heating mantle, and heated to 40° to 50°C for only 1 to 2 hr (139). During this time, and for an hour following the heating period, a stream of nitrogen, passed consecutively through conc sulfuric acid and calcium chloride was bubbled through a gas dispersion tube into the acid. On becoming colorless, the acid was allowed
to cool and was stored in the original bottle at \(0\)\(^\circ\)C.

A total amount of 72 g of oven-dry, analytical grade phosphorus pentoxide was weighed into a beaker and then added in small increments to the acid. With each addition, the flask was swirled vigorously to dissipate the heat of solution and avoid release of brown fumes. After each addition, the flask contents were cooled to \(-15\)\(^\circ\)C. and the beaker sealed from the atmosphere with aluminum foil. Following the last addition, the cloudy white mixture was kept at room temperature for 1 to 1 1/2 hr and swirled frequently. At this stage, the mixture was composed of two distinct immiscible phases, both of which were clear and colorless. This was refrigerated at \(5\)\(^\circ\)C for 4 to 6 hr, removed and swirled vigorously into one miscible phase, and refrigerated for a second period of 5 to 6 hr. Following this, the mixture was ready for use.

Overnight storage of the nitration mixture is allowed. Maximum useful storage time, however, is one or two days after the preparation is started. Beyond this period, crystals are deposited on the flask bottom and the composition of the acid changes. On preparation, the solution contains nitric acid, phosphoric acid, and phosphorus pentoxide in a weight ratio of 64:26:10. This mixed acid must be clear and colorless the following day, and any minor particles may be removed by filtration over fritted glass. Although initial immiscibility has been encountered before, no reason has been advanced for this behavior (136), nor is one given for the required "aging" period following transition into one phase. In the
present work it was found that both features are necessary or the acid remains or separates as a two-phase system during the first stage of nitration.

B. Nitration

The procedure for nitrating wood meal is that described by Timell (134). After drying over phosphorus pentoxide under reduced pressure (1 to 2 days), wood meal (0.10 g) was weighed into a 50 ml weighing bottle and stoppered. At nitration, the bottle was inverted, opened and with the meal remaining in the lid, 6 ml of nitrating mixture at -15°C were transferred to the bottle using a clean, calibrated 20 ml glass hypodermic barrel and plunger. The meal was added gradually in small portions while the flask contents were swirled instantly and vigorously on each addition. Upon the last addition, the bottle was re-stoppered, swirled to clean the sides of adhering wood meal, and set aside at 18 ± 1°C. A water bath was kept at the proper temperature by using an electrothermal voltage regulator to control a constant-head reservoir fed by a stream of cold tap water.

The times required for development of maximum nitrocellulose yield at proper degree of substitution with minimum nitrocellulose depolymerization varied between woods of different species, ranging from 38 to 40 hr for Pacific silver fir, western red cedar, and the spruces, to 43 to 45 hr for Douglas fir. These times were in agreement with those determined by Ifju (61) and Timell (130) for similar species. Western hemlock,
tamarack, jack pine, and yellow cedar failed to nitrate properly. During the reaction period, flask contents were swirled at least six times at evenly-spaced intervals. This proved essential in order to minimize variation in yield and provide products with proper nitrogen content (i.e., 13.8% or higher). In contrast, for reasons unknown, continuous mechanical agitation is known to give measurable denitration and reduction in intrinsic viscosity after the first 20 hr of nitration (129, 137).

C. Cellulose Nitrate Recovery

Products of wood nitration were recovered according to the method of Timell (134). After completing nitration for the required time period, the flask and contents were cooled to -15°C. The acids and soluble products were removed by filtration through a 30 ml coarse, fritted-glass crucible, suction being interrupted before air entered the solid residue. This was followed by immediate addition and removal of 40 ml of 50% aq acetic acid at -15°C (104) and 40 ml of iced water.

The crucible was removed, filled with saturated aq sodium bicarbonate, and left for 5 min to solublize some of the nitrated, oxidized, and degraded lignin. The bicarbonate was displaced with 30 ml of 10% aq acetic acid at 0°C and the residue was washed with distilled water until washings were neutral. This was transferred with methanol to a 50 ml Erlenmeyer and shaken mechanically for 1 hr to remove the remaining lignin degradation products. Good lignin removal is essential in obtaining a nitrate that is completely acetone-soluble (2). The nitrated
meal was returned to the crucible, rinsed with methanol, and transferred to a 250 ml wide-mouth Erlenmeyer containing 150 ml of acetone. This was stirred at 2,200 rpm for 5 min with a mechanical stirrer. After undissolved residues (probably native xylan dinitrates) settled over 12 hr, the clear yellow supernatant liquor was decanted while the swollen residue was centrifuged at 15,000 rpm for 1 to 1 1/2 hr and washed once. The supernatant solutions were combined and poured into one liter of distilled water. Precipitated nitrocellulose fibres were collected around a spatula and pressed into a flat mass. The recovered product probably contained minor amounts of galactoglucomannan or glucomannan trinitrates occluded with the cellulose trinitrate, although most contaminants are reported to remain in solution on addition of the acetone solution to water (134). The precipitate was transferred to a 30 ml fritted-glass crucible, washed with water and methanol and, following 4 to 5 hr air-drying, the material was dried under reduced pressure over phosphorus pentoxide at room temperature for 24 hr. Following analysis for yield, nitrogen content, and viscosity, it was stored in the cold.

Nitration is a reversible process; being very rapid in the ester formation stage, but extremely slow in the de-esterification or hydrolysis stage. Hence, using high DS and proper recovery techniques lessens the chance for nitrate ester removal (denitration). However, opportunities for denitration may be increased enormously by raising temperature (91). Since major heat effects at any stage of the nitration process are those accompanying acid dilution (the heat of the nitration itself being negligible),
the sucking of air through the residue and inefficient or tardy drowning cause
clocal temperature increases which promote a serious denitrating effect (91).
Of course, this will adversely affect absolute yield and viscosity values as
well as reproducability by introducing a large experimental error.

III. Nitrogen Determination

The preferred method for determining total nitrate ester nitrogen on
cellulose trinitrate is the semi-micro Kjeldahl digestion (6, 49, 61). Gas-
volumetric methods, while suitable, are either less reproducible (37) or
more tedious (56); spectrophotometric methods may yield accurate results
(29, 79) but require elaborate, sophisticated equipment and lack the sim-
plicity of procedure essential for rapid analysis.

Briefly, the ester is digested with cold conc sulfuric acid in the
presence of a readily C - nitrated, aromatic compound (salicylic acid)
and the nitroaromatic compound thus formed is reduced with sodium thio-
sulfate to an amine (56). While the Devarda modification (56) employs
digestion in alkaline solution with a metal, a more suitable method is the
use of a trace of selenium with the thiosulfate to catalyze reduction (21).
The solution may then be treated with an excess of sodium hydroxide and
the ammonia distilled into boric acid and titrated directly with standard acid.
This method of Ma and Zuazaga (82) is commonly used. Its disadvantage is
that the buffering action of the boric acid makes the end point less sharp than
with the back-titration method using nickel ammonium sulfate (148). Nessler's
reagent gives direct colorimetric determination of ammonia in the digest.
Although more rapid than semi-micro or micro-Kjeldahl, it is reported
to be not so accurate or dependable (148).

The analytical procedure used in the present study was essentially that
of Ma and Zuazaga (82) as modified by Steyermark (119). Table 3 shows a
typical determination of replication number for the nitrogen determination
(n = 2). Where the difference between the two replicates was greater than
the confidence interval of 0.16\%, a third determination was made and the
average of the two closest values was used.

The cellulose nitrate to be analysed for nitrogen was dried \textit{in vacuo}
over phosphorus pentoxide. A 15 to 20 \pm 0.05 mg portion was weighed on a
Spoerhase semi-micro analytical balance and transferred to a 100 ml
Aminco digestion - distillation flask. Three ml of stock solution containing
0.1 g of reagent grade salicylic acid and 3.0 ml of conc sulfuric acid were
dispensed to each flask by glass hypodermic barrel and plunger. The
contents were shaken mechanically for 2 to 3 hr to ensure complete solution
and aromatic nitrination. It was noted that this provides considerable saving
in time compared with other methods which, unaccountably, specify a long
dissolution period without agitation (49, 61). Further, it was found that
shaking gave more reproduceable nitrogen values than obtained by over-
night dissolution without agitation.

Approximately 0.3 g of reagent grade sodium thiosulfate penta-
hydrate was added and, following a 15 min cooling period (119), two
selenized Hengar granules were added. The flasks were digested 2 1/2
hr at setting 6 on an Aminco rotary Kjeldahl digestion apparatus (3) that
had been warmed previously. Flasks were rotated 2 to 3 times during
digestion to ensure that splashed materials on the flask sides refluxed into
the main contents.
On cooling the digest, the flask sides were rinsed with 10 to 15 ml of distilled water, and the flask was greased and attached to an Aminco steam distillation assembly (3). A 125 ml Erlenmeyer flask containing 10 ml of 4% reagent grade boric acid solution and mixed indicator was placed under the condenser delivery tube with the tip extending beneath the liquid level. The indicators were bromcresol green (0.5 g in 100 ml of ethanol) and methyl red (0.1 g in 100 ml of ethanol). To make a stock solution for twelve determinations, five drops of the former and ten of the latter were added to 120 ml of the boric acid solution. Ten ml of 40% sodium hydroxide were run carefully into the distillation flask through the entrance tube and the resulting, strongly alkaline mixture was steam distilled for 7 min and continued 2 min longer to wash out the condenser tip. An Aminco electric steam generator was used (3), with the rate of steam generation controlled by the variable transformer.

A few ml of distilled water were used to wash the sides of the Erlenmeyer flask and the boric acid solution containing the ammonia was titrated to the end point with 0.02 N hydrochloric acid. The color was matched to that for an equal volume of boric acid, both indicators, and one drop of excess hydrochloric acid. The titration acid was standardized using recrystallized borax according to the method of Vogel (139); normality was checked in triplicate before every use. Blank determinations were made in the absence of a sample, using all reagents, and the value obtained was subtracted from experimental values of a given series. The method was standardized against recrystallized reagent potassium nitrate.
(nitrogen content = 13.85%). Calculation of nitrogen content was as follows:

\[
\% \text{ Nitrogen} = \frac{\text{ml} \times 0.02 \text{ N acid (corrected)} \times 14.008 \times 100\%}{\text{sample wt, g (oven-dry)}} \quad [4]
\]

IV. **Viscosity Determination**

The procedure used for determining intrinsic viscosity was that of Ifju (61) based on work of Davison (35). A 3 to 5 ± .01 mg sample was weighed on a Cahn gramelectric microanalytical balance and transferred to a 15 ml polyethylene test tube fitted with a perfectly closing polyethylene cap. Ten ml of reagent grade acetone were added and the tube was shaken mechanically overnight to effect solution. A 5 ml aliquot was pipetted to a clean, dry Cannon-Fenske viscometer (4) aligned vertically and suspended in a visibility jar bath at 25 ± 0.1°C. After waiting 5 min for temperature equilibrium, the viscometer capillary tube and efflux bulb were filled by attaching a rubber bulb to the open arm and squeezing gently. This technique minimized evaporation, hence errors caused by drawing the solution up the capillary by direct suction (4). Efflux time was measured to the nearest 0.1 sec between the upper and lower etch marks. Between determinations, the viscometer was rinsed exhaustively with reagent grade acetone and dried by passing through air, then placing it in an oven. Periodically, the viscometer was cleaned with **aqua regia**.
Specific viscosity, \( \eta'_{sp} \), was calculated from the expression
\[ \frac{t}{t_o} - 1 \]
where \( t \) is efflux time of the solution and \( t_o \) that of pure acetone. This assumes that densities of the solvent and the solution are equal.

Identification of flow time \((t)\) with absolute viscosity \(\nu\) is, strictly speaking, incorrect and results in error if the solution density increases significantly with concentration. The density correction, which is very small in dilute solutions of high viscosity, can be safely ignored (121).

Kinetic energy losses occur due to perturbed flow at the capillary ends, particularly when an organic solvent with rapid efflux time is used. A correction may be applied (121), but a better solution is to use a viscometer with a small bulb and a long capillary of suitable diameter. Here the kinetic energy effect would then be reduced to levels which can be disregarded.

The specific viscosity values so calculated were corrected for kinetic energy losses, according to Timell (128), in the following manner:

\[
\eta'_{sp} = \eta''_{sp} \frac{F_o}{1 - \frac{F_o}{t}} \left( \frac{t + t_o}{t} + 1 \right)
\]

where:
\[ \eta'_{sp} = \text{the corrected specific viscosity.} \]
\[ \eta''_{sp} = \text{observed value.} \]

and
\[ F_o = \text{a factor calculated for the viscometer from the expression:} \]
\[
F_o = \frac{m \, d_o \, V}{8 \eta_o \, t_o \, \pi L}
\]
where: \( m \) = the kinetic energy coefficient, (1), i.e., end effects for the capillary were neglected.

\( d_o \) = density of acetone (0.785).

\( V \) = the volume in ml of the viscometer bulb (3.65).

\( \eta_o \) = the absolute viscosity of pure acetone (0.003075 poise).

\( t_o \) = the efflux time of pure acetone (162.9 sec).

\( L \) = the length of the capillary (7.75 cm).

Substituting the above values into the above expression, \( F_o \) for the viscometer used in this experiment was calculated as 0.0293548.

When the intrinsic viscosity of cellulose nitrate in acetone exceeds approximately ten (a weight average degree of polymerization of about 2,000), the orientation of the molecules in the direction of flow lowers the apparent intrinsic viscosity. The effect increases with increasing molecular weight, and the error in intrinsic viscosity exceeds 10% for cellulose of highest D P (121). This affects shear gradient which, for each bulb at a given concentration, is calculated from the formula of Kroeplin (121):

\[
\bar{G} = \frac{8V}{3\pi r^3 t} \quad [7]
\]

where: \( r \) = capillary radius (0.02125 cm).

After correcting specific viscosity values for kinetic energy loss, the intrinsic viscosities were calculated by means of the Schultz-Blanschke and Huggins equation, as given by Davison (35):

\[
\eta^{\text{sp}}_{G} = \frac{\eta_{\text{sp}} C}{1 - K \eta^{\text{sp}}} \quad [8]
\]

where: \( \eta^{\text{sp}}_{G} \) = the intrinsic viscosity corresponding to the shear, \( G \), at which the measurement was made.

\( K \) = a factor taken as 0.30 according to Davison (35).
The intrinsic viscosity \([\eta]_G\) varies in a regular manner corresponding to shear dependence of the viscosity (126, 127). On the other hand, rate of shear depends upon efflux time which, in turn, is influenced by both concentration and nitrocellulose DP. In order to obtain comparable values for the various concentrations of different DP nitrates, all results were adjusted to the 500 sec⁻¹ velocity gradient by using the following relationship reported by Davison (35):

\[
\log [\eta]_{500} = P \log \frac{G}{500} + \log [\eta]_G \tag{9}
\]

where: \(P\) = the slope of the straight line relating the log of intrinsic viscosity to the log of rate of shear.

\(P\) may be determined by the following expression:

\[
P = \frac{d \log [\eta]_G}{d \log G} \tag{10}
\]

Davison (35) determined \(P\) experimentally and related \(P\) to intrinsic viscosity by the following equation:

\[
P = 0.0039 [\eta]_{500} - 0.8 \times 10^{-8} [\eta]_{500} \tag{11}
\]

Since \(P\) is related to intrinsic viscosity at 500 sec⁻¹ in equation[11], the value obtained by substituting \([\eta]_G\) in this equation can be used to calculate the first approximation of the slope \(P\). This value, when substituted in equation [9], gives a first approximation to \([\eta]_{500}\).
When resubstituted into equation \([11]\), \(\eta^*_{500}\) gives a better estimate of \(P\). This bracketing technique can be continued until the resulting \(\eta^*_{500}\) changes less than 0.001 in intrinsic viscosity with each successive step. The laborious calculation can be replaced by computer solution (U. B. C. Wood Science Program WS 7040-2).

The extent to which cellulose nitrate is substituted greatly affects its intrinsic viscosity. An increase in DS from 2.3 to 3.0 (12.0 to 14.15% of nitrogen) doubles the intrinsic viscosity. If nitrogen analysis shows less than full substitution, the formula of Lindsley and Frank (80) is used to convert the intrinsic viscosity to a common basis, that is, to what it would be were the sample fully nitrated:

\[
\frac{\log \eta^*_{t}}{\eta^*} = \log f_x + (14.15 - x) B \tag{12}
\]

where:

- \(\eta^*_{t}\) = the intrinsic viscosity of the fully nitrated cellulose.
- \(\eta^*\) = the intrinsic viscosity of the partially nitrated sample.
- \(f_x = 1.833 - 0.589x\), a function which accounts for the difference in molecular weights between partially and fully nitrated cellulose.
- \(x\) = the per cent nitrogen in the partially nitrated sample.
- \(B = 0.114\), an empirical constant.

Since all viscosity measurements in this study were carried out at 25° C, further adjustment of the intrinsic viscosity values is necessary. All results were converted into values corresponding to those taken at
20°C temperature by multiplying the intrinsic viscosities determined by 1.04716. The factor was obtained from the Treiber and Abrahamson relationship as reported by Ifju (61).

V. Lignin Determination

The procedure used here for ultra-violet spectrophotometric determination of lignin was originated by Johnson et al. (65). Moisture-free wood meal of accurately determined weight (0.020 g) is placed in a glass tube having a notched, ground-glass stopper. Ten ml of 25% reagent grade acetyl bromide in acetic acid is added and the tube is placed in a water bath at 70 ± 1°C and swirled gently at 10 min intervals to assist dissolution. After exactly 30 min, the contents are cooled at 13 ± 1°C for 8 to 10 min, then transferred to a 200 ml volumetric flask containing 9 ml of 2 M sodium hydroxide and 50 ml of acetic acid. The transfer is completed by rinsing with 5 to 10 ml of acetic acid and following this, 1 ml of 7.5 M hydroxylamine hydrochloride is added to the flask. Exactly 5 min following transfer, the contents are made up to volume with reagent grade acetic acid and the mixture is cooled under cold water. Peak absorbance is then measured at 282 μm, and lignin is calculated according to the following equation:

\[
\text{Lignin} \% = \frac{\text{Absorbance}}{\text{Absorptivity x moisture-free sample wt}} \quad [13]
\]

where absorptivity is determined for each species.

Since this method was first published in 1961, various aspects of the technique have been re-examined and errors not immediate inherent
have been encountered. It has been demonstrated (157) that an increase of 7 mg in sample weight lowers lignin determined by 0.1% and, similarly, increasing dilution by 50 ml or absorbance by 0.4 lowers lignin determined by the same amount. A strong interaction of sample size with dilution and absorbance is also evident. Studies on elapsed time between dilution and measurement have shown that dilute solutions may be stored in the cold for 24 hr, and still provide stable absorbances. Also noted was the rapid "aging" of acetyl bromide, such that absorptivity values became erratic only hours after preparing freshly distilled reagent; this was avoided, however, by sealing fresh stock under nitrogen in individual ampules.

Wu (156) recognized and corrected several of these inconsistencies and estimated lignin values for Sitka spruce, Douglas fir, western hemlock, Pacific silver fir, and western red cedar. These original data, as well as new data for black spruce and Douglas fir are used here to explore the important lignin-carbohydrate relationships from observations of their respective patterns.
EXPERIMENTAL RESULTS

Changes in some important chemical properties across coniferous growth zones are summarized in Table 4. Estimated lignin and \( \alpha \) - cellulose contents, and nitrocellulose viscosity are tabulated for woods from three genera and four species of Pinaceae (Sitka spruce, black spruce, Douglas fir, and Pacific silver fir) and one species of Cupressaceae (western red cedar). Estimated \( \alpha \) - cellulose is shown as that recovered as nitrocellulose and corrected for nitrogen content using the equation of Miles (91).

Averages for \( \alpha \) - cellulose (Table 4) are determined on the basis of at least three replicates and, except for two positions in Douglas fir Increment No. 40, averages for lignin are based on two replicates. Sitka spruce had the highest average yield of \( \alpha \) - cellulose (48.3%), followed in decreasing order by both Douglas fir samples (which had identical average yields of 46.7%), black spruce (45.2%), Pacific silver fir (44.9%), and western red cedar (44.0%). Western red cedar had the highest average lignin content (31.2%), followed in turn by black spruce (29.5%), Douglas fir Increment No. 40 (27.8%), Pacific silver fir (27.5%), Douglas fir Increments No. 64 to 66 (26.7%), and Sitka spruce (25.7%). Large variability was encountered among viscosity replicates and very frequently the viscosity range at one position overlapped those of contiguous positions. It was decided, therefore, that these data have little meaning other than as a qualitative measure of satisfactory derivative formation.
Examination of material from a freshly-cut Douglas fir tree (Increment No. 40) and black spruce revealed the two representative \( \alpha \) - cellulose earlywood patterns as shown in Fig. 1. Replicate values are plotted above each position examined. A recent (60) mathematical model describes each pattern by utilizing relative position within the increment, per cent latewood, and estimated \( \alpha \) - cellulose yield. The model, standard error of estimate (\( SE_E \)), and regression and correlation coefficients (b and r, respectively) were calculated from a computer solution (U. B. C. Wood Science Program WS7040-3). Linear regressions and correlation coefficients of the \( \alpha \) - cellulose content on position are listed for all increments in Table 5.

Results of analysis of variance (ANOVA) for \( \alpha \) - cellulose content within growth zones of each species are tabulated in the usual form as Tables 6 through 11. For each species, least significant ranges between position means (Duncan's multiple-range test) are presented coincidentally with ANOVA results in Tables 6a through 11a.

For all ten increments, patterns of estimated \( \alpha \) - cellulose, estimated lignin, and their combined values are plotted in Fig. 2.

Table 12 lists linear regressions and correlation coefficients of estimated \( \alpha \) - cellulose on lignin contents for all increments of each species. Figure 3 describes the highly significant linear correlation between these lignin and \( \alpha \) - cellulose estimates for all positions studied within increments.
From Fig. 2 all individual and combined lignin and cellulose values were plotted for each position and increment and are presented as Fig. 4. The latter shows that the dispersion about the combined data is significantly less than that about either of the two individual means.

Correlation coefficients from linear regressions of $\log$ on cellulose yield for four species are tabulated in Table 13. As a means of showing chain-length variation in earlywood, data corresponding to first-formed earlywood tissues are included or excluded from each regression.
DISCUSSION

The present study is not concerned with description of molecular structures or, for that matter, with development of procedures for deriving statements on ultimate chemical purity. Rather, its purpose and attention are focussed on a first estimate of important quantitative relationships of major wood constituents that exist in tissues sampled within coniferous growth zones. Answers were sought to such questions as where, how much, and how are these inter-related. This differs from the aims of basic wood chemistry. The two approaches frequently come together by interchange of methods. It is understood that future developments may provide means for making fine adjustments to the first approximations derived in this study.

Direct nitration of wood seemed a likely analytical tool for measuring an intra-increment carbohydrate yield fraction. This proved to be correct; however, limitations, as well as advantages are attendant with the method. For example, coniferous wood nitrates may contain small quantities (up to 7%) of mannans, but very little (below 1.5%) xylans (130). It may be argued, then, that the derivative does not meet the classic definition as $\alpha$ - glucan. That is, it is not pure $1,4-\beta-D$ - gluco-anhydropyranose. This is recognized in the present study, where yields corrected for nitrogen are regarded as reasonable estimates of $\alpha$ - cellulose, such as those prepared from conventional holocelluloses (124). These estimates, therefore, include the usual partial glucan products
without attempt to resolve variations by extensive analysis of constituent carbohydrates. The latter procedures, as used by Timell (130), also carry a disadvantage in that ratios between monomer sugars must be assumed in order to make corrections.

A major limitation of the micro-nitrocellulose method is that it cannot be universally applied to all woods. That is, the success of the method depends on ability to manufacture a suitable derivative. Even with many trials and adjustments of procedure, this was not accomplished with four species of the present study: jack pine, tamarack, western hemlock, and yellow cedar. Other workers (18, 129, 137) have had similar experience in chemical treatment of some of these woods. A further inherent disadvantage with nitrocellulose is that the product cannot be reduced to cellulose without collapse of the cellulose chain.

Viscosity data were mostly useful for indicating presence or absence of extensive degradation as one criterion for judging successful nitrocellulose preparation. They also provided some comparisons of intra-increment differences in chain-length. Since $\alpha$ is a more sensitive measure of degradation than $\alpha$ - cellulose yield by nitration, the higher variability associated with $\alpha$ emphasizes the need for refinement of the wood nitration procedure. For instance, the range of $\alpha$ - cellulose yield varied approximately 5%, depending on extractive pre-treatment; $\alpha$, therefore, might be expected to vary even more than this. This interaction of wood extraction history with nitrocellulose preparation has been completely
neglected in the literature!

Means for estimating lignin with small amounts of material seem to be better established since the acetyl bromide-acetic acid method for UV lignin determination was first published (65). However, certain inconsistencies have been found (157). Wu (156) recognized and corrected several of these inconsistencies, brought the technique to a statistically defensible level, and followed by estimating intra-increment lignin patterns for several species.

Wu and Wilson (157) also showed that average lignin values obtained for the woods studied compared well with values found in the literature. These data are reported as part of Table 4 and plotted in Fig. 2. These lignin values plus new data for black spruce and Douglas fir were used to explore intra-increment cellulose-lignin relationships.

In summary, data developed and used in this study are regarded as statistically defensible estimates of the two major coniferous intra-increment wood chemical components for which no absolute measure or procedures have yet been advanced.

I. Review of Data

A. Alpha-cellulose Estimates

The range of average $\alpha$-cellulose estimates found for the five species reported in this study (44.0% to 48.3%) are recorded in Table 4. These agree favorably with those found by Timell (130) who reported a
range of \( \alpha \) - cellulose by nitration from 41.3% for jack pine and eastern hemlock (Tsuga canadensis (L.) Carr) up to 46% to 48% for white spruce as given in Table 1. Close agreement is also noted by species; average values for both spruces fall in the same range as white spruce reported by Timell (130). Pacific silver fir also corresponds closely to data of Timell (130). These results affirm the consistency of the nitrocellulose method, which seems to be independent of sample size (down to 0.1 gm) and operator variability.

Except for Position 1 in western red cedar, the values do not appear to extend the range of whole wood values. This peculiar, single position was examined twice in duplicate determinations which gave the same values.

Yield of alpha-cellulose by nitration compares closely to that prepared from conventional holocellulose determinations on gross wood. The former method is generally lower by 2 to 3%. This agrees with Timell (130), who ascribed part of this difference as due to the presence of more non-glucan material retained in conventional \( \alpha \) - cellulose. The difference in yields by both methods varies by species: In Douglas fir, for instance, the yield range (44.0% to 49.4%) compares closely to that found by Kennedy and Jaworsky (68) who reported a range of chlorite \( \alpha \) - cellulose content of 45% to 51% on whole wood. The former, however, appears to have more inherent variation than the 46% to 48% range in chlorite \( \alpha \) - cellulose found by Hale and Clermont (53). The difference between both methods of \( \alpha \) - cellulose measurement, however, appears greater for Pacific
silver fir. This is at variance with what Timell (130) reported; previously he showed that yields of \( \alpha \) -cellulose from chlorite holocellulose and nitration are essentially the same value. With white spruce and eastern white cedar (\textit{Thuja occidentalis} L.), Timell (130) found that differences were evident; these were 2.5\% and 4.3\%, respectively. Therefore, for the related species of this study (both spruces and western red cedar) it is probable that \( \alpha \)-cellulose as estimated by the nitrocellulose method may be low by approximately these amounts.

B. Viscosity Estimates

Since no attempt was made to determine cellulose polymolecularity, values reported are regarded as representing average estimates. It is recognized that individual nitrocelluloses probably contained variable chain-length distributions, for instance as peaks reported by Timell (129, 137) for some of the same species.

Although nitration yields products similar to those of the original cellulose (49), it is recognized that the harsh conditions undergone by wood tissues during nitration cause some chain-length degradation (134). Except for Douglas fir Increment No. 40, it appears that little chain-length degradation occurred across any of the growth increments. As shown by black spruce viscosity data, storage of wood as a block in cold thymol solution appeared to involve less degradative effects than when wood meal was stored under nitrogen at room temperature. This focuses attention again on the influence of wood pretreatment.
What is puzzling is the lower $[\eta]$ from freshly-cut Douglas fir (Increment No. 40). This was sectioned and nitrated immediately after sampling the tree. The only reason given for its lower viscosity is that perhaps this wood was over-nitrated, hence over-exposed to the hydrolytic effect of chain-scission. While $[\eta]$ is known to decrease after early stages of nitration (129), previous experience gained in this study and elsewhere (129) indicates that $[\eta]$ does not markedly decrease until about the time of maximum nitration. The range of nitrocellulose $[\eta]$ values on stored Douglas fir meals compared favorably with that of Ifju (61), who reported average $[\eta]$ of two earlywood and latewood replicates as 34.0 and 36.0 dl/g respectively.

It is unfortunate that intra-increment $[\eta]$ patterns could not be established from the present preparations. Because of excessive variability encountered among replications, the reliability of determining an average from these would have little meaning for between-position comparisons for, very frequently, the range of $[\eta]$ at one position overlapped that of contiguous positions. In view of this limitation, therefore, it is felt that these viscosity data give indication only of the range of relative chain-length degradation across the growth increments studied. Perhaps more attention needs to be given to accuracy of the nitrogen determination. By the formula of Lindsley and Frank (80), unit changes in $[\eta]$ accompany changes in nitrogen content of approximately 0.1%. Hence, it is suggested that the method of nitrogen determination be adapted to the use of 0.1N standard acid and three replications.
C. Lignin Estimates

Estimated average lignin content of black spruce (29.5%) agrees closely with the species average of 28% (22). That for Douglas fir Increment No. 40 (27.8%) is within 1% of that reported by Wu (156) for his Douglas fir; however, both are well below the species average of 31.5% (22). These data are reported as part of Table 4 and plotted as Fig. 2. As both Douglas fir trees came from the lower mainland of B. C., it may be that Douglas fir originating from this area, is characteristically lower in lignin.

II. Intra-increment Alpha-cellulose Estimates and their Significance

A. Two Representative patterns

Yield of \( \alpha \) -celluloses from Douglas fir Increment No. 64 gave a rather definite pattern (Fig. 2), as did both adjacent growth increments, suggesting that such a pattern is a regular feature within a given coniferous stem. Examination of material from a second Douglas fir stem showed the same result. Examination of four other woods showed that a second pattern might occur (Fig. 2). The two \( \alpha \) -cellulose yield patterns are described as follows:

1. Alpha-cellulose yield dropped slightly from Position 1 (or earlywood) to 2, then rose sharply to a maximum either at Position 5 or 6 (latewood) as represented by Douglas fir.

2. Yield of \( \alpha \) - cellulose increased sharply from Position 1 to 5, whereupon it either peaked or dropped off at Position 6;
this was characteristic for both spruces examined and western red cedar.

3. Pacific silver fir varied according to increment, following either of the above two patterns.

From recent work by Ifju (61), Homoky (59), and Worrall (154) on intra-increment specific gravity profiles, it seems appropriate to consider earlywood as including Positions 1 to 3; transition wood, Position 4; and latewood as Positions 5 and 6, when increments are divided into six equal parts.

The two general patterns of $\propto$ - cellulose deposition are presented in Fig. 1. Douglas fir represents the first type of pattern, showing minimum yield at considerable cellular depth within the earlywood; this pattern also holds for six of the ten increments reported (Fig. 2). Maximum yield may be associated either with continuation or conclusion of latewood growth. Black spruce represents the second type of pattern, showing minimum yield at the initiation of earlywood. As with Douglas fir, maximum yield was associated either with continuation or conclusion of latewood growth. Fig. 1 also shows that no difference in chain-length is apparent throughout either increment.

Each pattern is most accurately described by a recent mathematical model (logarithmic equation) reported by Homoky et al. (60), based on relative position, per cent latewood, and estimated $\propto$ - cellulose yield.
For black spruce, Increment No. 40 $E_X$ and $r$ are 0.40 and 0.95, respectively; in the same order, these are 0.60 and 0.96 for Douglas fir Increment No. 40. For comparison purposes, linear regressions for both species are listed in Table 5; these do not fit the black spruce and Douglas fir data as well as does the model.

Transformations have been used with success to improve the fit in cases where non-linearity has occurred with relationships between wood properties. Wilson and Ifju (150) have described tensile strength behaviors across coniferous increments, by using an arctangent function. The application here of the particular transformation:

$$\ln Y = a + b_1 x_1 + b_2 x_2^2 + b_3 x_3^3$$

is its first use to describe non-linear behavior of a wood chemical property across a coniferous growth increment.

B. Chemical Evidence for Two Earlywood Types

The existence of minimum $\alpha$-cellulose yield deep within the earlywood (Position 2) is new chemical evidence suggesting occurrence of a major physiological change with progress of seasonal growth; this is consistent with, and thereby re-affirms the dual earlywood hypothesis of Wu and Wilson (157). To reiterate, this hypothesis states that earlywood arising from "overwintered" xylary mother cells retains a chemical pre-disposition towards latewood of the preceding season. It implies, therefore, a difference in chemical composition from that of cells formed by new cambial divisions within the present growing season. Morphological data on fibre lengths in Douglas fir carry the same suggestion (16, 17). The hypothesis relates
to evidence presented by Bannan (10) on eastern white cedar grown in southern Ontario. Bannan (10) found that, on resumption of cambial activity, the first site of cell division is in the oldest xylem mother cells contiguous to the already-differentiated xylem; furthermore, the great majority of these cells, and not the specific layer(s) of cambial initials, are most active during the first month(s) of radial growth. Hence, Bannan (12) observed that a considerable portion of earlywood may be formed by re-division of (overwintered) xylem mother cells. The portion may include anywhere between 2 to 24 (average of 8) such "overwintered" cells (11). Where many such cells occur, this first-formed earlywood is sampled as Position 1 and/or 2 within a single growth increment. Such a condition may have arisen in Douglas fir and possibly not in other woods examined although Pacific silver fir followed either pattern, as stated previously. It is not known whether the difference in patterns results from inherent species differences or is a result of climatic differences. Alpha-cellulose yields at Position 1 could relate to the combination of sampling procedure and periodicity of cambial reactivation. While the cessation time of cambial division varies widely with altitude, latitude, climate, and site (12), so also can this be said for time of cambial reactivation. Even where these factors are similar within a small forest area, reactivation time varies with species and individuals within species. At Haney, B. C., Walters and Soos (140) found that western red cedar and Douglas fir commenced shoot growth activity by the middle of April, two weeks before that for western hemlock and western white pine. At Corvallis, Oregon, Grillos
and Smith (50) claim that the cambium of Douglas fir resumes activity during the middle of March; the first divisions occur in the mother cells nearest the mature xylem. While these observations only show the effect of latitude on shoot growth and cambial reactivation in Douglas fir, one cannot deny the influence on other species. For example, both spruces used in the present study came from more northerly latitudes; the Sitka spruce from Terrace, B.C. and black spruce, from northern Quebec. It could be expected that the cambial zones for both resumed activity at later dates than trees from Haney, B.C., which may relate to frequency of xylary mother cell divisions.

Besides determining lignin by the wood digestion method of Johnson et al. (65), another method devised by Chow (28) was applied to determination of lignin within Douglas fir Increment No. 40. This provides direct measurement from Infra-Red (IR) spectroscopic absorption using thin (20 μ) tangential wood sections.

Both lignin methods correlate highly; the IR method also reveals peak lignin content at Position 2, which reaffirms by other means the existence of two earlywood types. Chow (28) has also derived an expression for relating carboxyl/carbonyl ratio on thin wood tissues. Within the earlywood of Douglas fir, Pacific silver fir, and western red cedar, where increments have been examined, this ratio peaks at approximately the same relative position as shown for lignin determined by his method. The behavior further suggests presence of different earlywood types at the chemical functional group level.
C. Alpha-cellulose Patterns and Earlywood-Latewood Variations

Fig. 2 shows estimated $\alpha$ - cellulose patterns for all ten increments reported. Verification of definite patterns for each species was achieved by two statistical tests. Analysis of variance (ANOVA) showed conclusively that highly significant differences existed among most of the positions (Tables 6 to 11). To determine significant differences between positions, Duncan's multiple-range test was used (Tables 6a to 11a). In comparison to the $F$ test, Duncan's test accounts for the number of position means compared.

In western red cedar, the freshly-cut Douglas fir, and both spruces (those specimens wherein just one increment was studied), the latewood was significantly higher than earlywood in $\alpha$ - cellulose yields. Although this was not entirely true with Sitka spruce latewood (yield for Position 6 was not statistically different from that for Positions 2 and 3), Position 4 had similar yield to that of Position 5, and both of these were significantly higher than earlywood. Within western red cedar earlywood, positions differed significantly from each other, whereas none differed within the transition-wood-latewood; this also held for the freshly-cut Douglas fir with the exception that Position 5 was significantly higher than the remaining transitionwood-latewood. Within black spruce, Position 1 was significantly lower than the remainder of earlywood, whereas Position 5 was significantly higher than transitionwood-latewood. Although in a few instances yield values fell outside the desired confidence interval of 1.0%, this had no effect on significance of the earlywood-latewood differences. For these species linear regressions of $\alpha$ - cellulose estimates on relative position within growth zones
are reported in Table 5. All are highly significant.

For stored meals from Douglas fir and Pacific silver fir (wherein three adjacent increments were studied), ANOVA showed that, as with the single increments, highly significant differences existed among most positions. This was true also between growth increments (Tables 10 and 11). For both species, latewood yields were significantly higher than those of earlywood. For Pacific silver fir, the yield pattern was the same for all three growth increments (as evidenced by negative interaction); within the earlywood, Position 3 was significantly higher and in transitionwood, Position 4 was likewise lower (Table 11a). Only one of the three growth increments was significantly different in average yield. In Douglas fir, ANOVA revealed a highly significant interaction between positions and growth increments with most interaction caused by variation at Positions 1 and 2 which, in turn, affected the shape of yield curves for each ring. Hence, while the general pattern of yield holds for these three rings, its shape is not constant throughout the six positions in every ring. By Duncan's test (Table 10a), Position 3 was significantly higher in yield within earlywood while Positions 4 and 6 were significantly different from each other, but not from Position 5. Increments No. 64 and 66 were significantly different from each other, but not from Increment No. 65.

For these same materials, linear regressions of \( \alpha \) - cellulose content on relative position within growth zones were computed and found to be highly significant. For Douglas fir and Pacific silver fir, correlation
coefficients were .86 and .83 respectively (Table 5). While regression
equations showed that although initial cellulose yields of Douglas fir were
higher by 2%, it is interesting to note the marked similarity between
regression coefficients (.75 and .76) in Table 5. This suggests that, for
the increments studied, yields for both species increased constantly and
at nearly identical rates during their respective growth periods. Perhaps
cellulose deposition across annual increments is regulated by a mechanism
common to more than one and possibly several species. In view of
significant correlation coefficients, both equations would be useful for
estimating cellulose contents throughout mature growth increments for
these species.

Results from ANOVA and Duncan's multiple-range tests for all
increments re-affirm the existence of higher cellulose or glucan
content in latewood as found by previous workers (53, 55, 76, 89, 102).
Earlier results are given in Table 1. All, however, sampled just two points
within a growth increment and analyzed wood material by methods known to
have limitations, such as cellulose prepared by the Cross and Bevan pro-
cedure (33), holocellulose by sodium chlorite (153), and cellulose by chroma-
tography of wood hydrolyzates (76, 89). Excepting the anomaly in western
red cedar, the magnitude of earlywood-latewood differences for the ten
increments reported here appears to reflect the influence of transition
type. Douglas fir, a species having characteristically abrupt transition,
shows an average difference of 4 to 5%; both spruces, on the other hand,
are characteristically woods of gradual transition and show differences of
only 2 to 3%. Hale and Clermont (53) determined chlorite holocellulose on red pine and Douglas fir early- and latetwood and found an 8 to 9% differential. It is felt that this may represent rather eccentric behavior. Although they measured Cross and Bevan cellulose in Douglas fir, Ritter and Fleck (102) showed an earlywood-latewood difference of 3-4% which seems to be in better agreement with literature values and results reported here.

Since the only previous work showing intra-increment chlorite holo-cellulose and carbohydrate patterns at more than two positions within an increment was done by Ifju (61) on Douglas fir wood, discussion and comparisons in this section are restricted to this single species. Ifju's results have been summarized earlier (151) while those from the present study are given in Fig. 2. Both sets of data show higher carbohydrate content in latetwood; however, similarity ends here. Apart from the 30% difference in absolute carbohydrate yields, what contrasts most in the comparison is the shape of the curves and the location of profile peaks. Ifju's holocellulose patterns follow the parabolic curve for all three increments examined, with the lower of the two curve ends being at Position 1 and the apex occurring at Position 4, which relates exactly to initiation of latetwood. Compared to patterns of \( \alpha \)-cellulose estimated in this study, neither Ifju's holocellulose nor glucan and mannan contents indicated minimum or maximum yield at Position 2. The second obvious difference is the peaking of holocellulose and glucan plus mannan contents at the earlywood-latewood transition, while the \( \alpha \)-cellulose yield peaks deep within the latetwood or, less commonly, at Position 6. From Ifju's work, the galactan content followed most closely
the established trend for \( \alpha \)-cellulose in this study, while the xylan content did the reverse, showing an average drop of 3% from early- to latewood.

Although patterns of holocellulose and estimated \( \alpha \)-cellulose content in Douglas fir do not agree, additional data from Ifju's work (151) and information about the acetone solubility of non-glucan nitrates (130) might explain some of the discrepancy. Timell (130) found nitrocellulose of six coniferous woods to have an average purity of 94%. Of the two major non-glucan polysaccharides which may be recovered with the nitrocellulose, xylan dinitrate is acetone-insoluble (130, 134). It is probably not included as such in the \( \alpha \)-cellulose yield. As the content of xylan in earlywood is lower by 2 to 4%, this would partly explain the lower \( \alpha \)-cellulose yield found in the earlywood. There is no obvious reason for the difference in location of peaks.

Timell (130) has demonstrated that 4 to 7% glucomannan is occluded in the nitrocellulose. If, during nitration, proportionately more of this short-chain material is solubilized or lost in the region of its greatest occurrence, this would tend to give a rather uniform, low contribution of glucomannan throughout the increment. This loss, when coupled with that of the acetone-insoluble xylan dinitrate, could account for the major difference between the curves and peak positions for holocellulose and estimated \( \alpha \)-cellulose. Residues of xylose and mannose were not measured in the present study.
In summary, \( \alpha \) - cellulose patterns were established across growth zones from several coniferous woods. These patterns suggest relationship of the long-chain carbohydrate fraction to seasonal development within coniferous growth zones. Comparing results of this study to the literature shows good agreement to determinations of \( \alpha \) - cellulose estimated by nitrocellulose preparation. The method appeals as a means for examining minute amounts of material. The use of three replicates per position is statistically defensible.

III. Relationship between Cellulose-Lignin Patterns

A. Evidence for Complementary Character

A distinct novelty of the new method reported herein is that all data arise at a level allowing comparison to other chemical and physical patterns developed at the same level. Notable relationships were found when lignin and \( \alpha \) - cellulose patterns were compared for the same positions within increments (Fig. 2). Though differences for both components between growth zones and species are evident, the pattern of one is obviously the complement of the other. This shows even at the points of changing slope and for extreme positions within any increment. The interdependency is well exemplified for Pacific silver fir and Douglas fir, where ring-to-ring positional differences modify the lignin pattern. The \( \alpha \) - cellulose complement of these is almost quantitatively perfect. Where no change appears in the slope of one component, as with both spruces for instance, so also is there no corresponding change in the slope of its complement. In both spruces, lignin contents decrease steadily from Positions 1 to 4 or
and, conversely, $\alpha$-cellulose yields increase in the same magnitude through these positions. With the sole exception of Position 1 in western red cedar, patterns for both components are continuously complementary throughout the 60 positions within the 10 increments examined. This adds new dimension for understanding the complex inter-relationships of the two major coniferous wood components.

The complementary inter-dependency of lignin and $\alpha$-cellulose was compared by regression analysis of the combined data (60 positions). In studying cell differentiation, it is known that lignification can be first noted following secondary wall formation (143), hence, lignin content was regarded as being dependent on $\alpha$-cellulose content. In Fig. 3 the relationship of this complement is shown as a highly significant linear correlation ($r = -0.785$) between the two estimates. By virtue of this strong relationship with combined data from numerous botanical sources, the hypothesis receives strong support. For the ten coniferous increments combined therein, the $\alpha$-cellulose estimate was related to lignin by the equation $Y = 63.08 - 0.773X$, where $Y = \text{lignin}$ and $X = \alpha$-cellulose estimate. The correlation coefficient compares very favorably with that of -0.764 as reported by Byrd et al. (26) for whole wood of loblolly pine.

Highly significant linear correlations were found also for eight of the ten increments when these were analyzed separately. Results of individual analyses are presented in Table 12. Except for the one anomaly in western red cedar, the "complement" hypothesis seems to hold regard-
less of species or increment. Had it been possible to treat lignin and
\( L \) - cellulose replicates as matched observations, all regressions listed
in Table 12 would have been highly significant since, as a consequence,
degrees of freedom in the analysis would have been increased two-fold.

B. Evidence for Mutually Exclusive Relationship Between Cellulose and
and Lignin

Further evidence of lignification as the complement of \( L \) - cellulose
distribution is presented in Fig. 4. All mean \( L \) - cellulose estimates
and lignin values were combined and plotted for each position with the inten-
tion of comparing inter-species variations. In Fig. 4 are shown the
mean (\( \bar{x} \)) and absolute range of one standard deviation (s) about averages
for all estimates of \( L \) - cellulose, lignin, and the sum of both.

The data treated this way provide two observations of note. First,
the sum of the component estimates clusters about the central value of
72 to 74%. Secondly, dispersion about the combined values is significantly
less than that about either of the two individual means examined separately.
These important observations suggest that for the increments studied, all
species had a common critical level that represents the sum of a unique
combination of certain cell wall components upon which the ultimate
structural nature of the cell wall depends, regardless of season of
formation. This demonstrates that some close physiological control exists
over these chemical components, as indicated by the significantly lower
dispersion about the combined estimates. Why this phenomena occurs is
not patently obvious; however, it does give strong indication of the close,
finely balanced inter-relationship existing between β-cellulose and lignin. Clearly, a pre-determined quantity of some precursor (e.g., 72 to 74% in eventual weight) is produced in metabolic pathways, and by complex biosynthetic sequences, this is directed for incorporation as either lignin or long chain glucan end-products (but never both) such that the sum for both results in a similar value, regardless of individual tree or species differences. This indicates that coniferous tree physiology is oriented more towards the finished biosynthetic product rather than towards major individual components involved in such synthesis.

The constant 26 to 28% of other material must be hemicelluloses, which seem less well related quantitatively to the basic glucan than is lignin. This amount of hemicellulose is never recovered from members of the Coniferales, which require delignification before hemicellulloses can be extracted.

As seen in Fig. 4, values for western red cedar are obviously high, being displaced 2 to 3% above the x. This is the only member examined that showed such behaviour throughout the increment; it may have a specific pre-disposition towards type of lignin treatment. For this species, lignin determination by ultra-violet spectroscopy has tended to be high in the order of 10%. MacLean and Murakami (85) have found syringyl as well as guaiacyl groups in cedar lignans. This or other undescribed phenomena may contribute to higher absorbance readings and, hence, cause artificially high UV lignin values.
From the complementary cellulose-lignin patterns (Fig. 2), their highly significant negative linear correlation (Fig. 3) and tendency to cluster about a central value when combined (Fig. 4), it is evident that where an extra amount of \( \alpha \) - cellulose is present, this is accompanied by deficiency in lignin.

This is the first quantitative evidence for such relationship within coniferous growth zones. These results support previous evidence (31, 40, 44, 58, 92, 144) confirming rather clearly that cellulose and lignin deposition is mutually exclusive. The results also affirm Freudenberg's (40) classic analogy of cell walls as re-inforced concrete, whereby the crystalline cellulose (which is customarily regarded as \( \alpha \) - cellulose) acts as re-inforcing rods surrounded, but not displaced, by non-cellulosic cell wall components which act as cement.

C. Biological Interpretations

The mutually exclusive behavior of \( \alpha \) - cellulose and lignin reflects a basic difference in chemistry of the irregular wood known as compression wood, wherein it has been noted (142) that lignin is always high and cellulose content low. There is, however, a fundamental difference in that regular wood exhibits an interchangeable balance while compression wood changes exhibit excessive lignin. Nevertheless, this does not rule out the action of a mechanism common, in part, to the chemistry of both. How it operates is a matter of conjecture.
It is known that higher auxin contents accompany earlywood and compression wood formation, although, with the latter, factors other than auxin are known to be operative at compression wood sites (74, 142, 145). Both wood types are relatively high in lignin content (142, 156). Wu and Wilson (157) have postulated that within compression wood, earlywood lignin seems to be constant and latewood lignin increases, giving a whole wood effect of higher lignin for compression wood. There may be, then, some correlation between these facts such that auxins influence formation of less cellulose and more lignin. If this were correct, then the lower lignin content of regular latewood results from a lower auxin supply. As auxin supply and translocation decrease with reduction in apical activity which, in turn, signifies latewood initiation (74, 145), this approach seems plausible. If such is the case, one would conclude also that the mechanism controlling cellulose deposition responds inversely to auxin supply or concentration. Actually, since cellulose deposition precedes lignification, it seems likely that such a mechanism would be related more directly to cellulose rather than to lignin. It might be more correct, then, to regard lignification as showing indirect rather than direct response to auxin influence on chemical constitution across the growth zone.

Other approaches have been made as to the cause of lignin and carbohydrate variations within the coniferous growth zone; these were reviewed recently by Wu and Wilson (157). Ritter (100) presented evidence that the middle lamella contained 75% of the lignin in wood; a year later,
Ritter and Fleck (102) used this evidence when they proposed that, since the middle lamella constituted a greater proportion of earlywood than latewood, the former, therefore, had a high lignin content. In reality, 75% of the middle lamella is lignin (9), but 75% of the lignin is not in the middle lamella (15). The latter contains at most 40% of the total lignin in wood (15). For this reason, the middle lamella theory advanced by Ritter and Fleck (102) is suspect.

According to Wu and Wilson (157), Phillips suggested that solar illumination on leaf surfaces controls lignification in tropical and temperate porous woods. Consequently, he showed the former to have higher lignification; however, even if applied to coniferous woods, such a hypothesis seems to contradict the major observation in that higher latewood lignification would be anticipated.

Larson (76) reported that decreasing lignification and increasing glucose yield across a growth zone are related not only to increasing cell wall thickness, but to the aging of the cambium combined with the declining influence of an upward receding crown. This led him to propose that changes in cell wall composition related to age can be typified by the earlywood, whereby at any age, its transition to latewood is superimposed on the existing earlywood matrix to represent a change of a different qualitative nature (i.e., increasing cell wall thickness). Previously, Hale and Clermont (53) indicated that higher holocellulose and \( \alpha \) -cellulose contents in latewood are associated with thicker \( S_2 \) layers within latewood prosenchyma
cell walls. This appears analogous to Larson's earlywood matrix approach, if applied at a given age.

Although cellulose and lignin have a common origin as photosynthates, the sequence of biosynthetic pathways through which precursors move has not been completely established. Much useful information, however, is available. Jones (66) has shown that the formation of lignin precursors requires the same unit as that used for synthesis of hexose sugars, namely, 3-D-phosphoglyceronic acid. Precursors of both components apparently branch off at this point to form increasingly differing entities (30, 66, 73 107, 118) in spite of the fact that both may undergo similar transformations many times. Apparently, some of these precursors may combine in the cambial zone towards the end of their biosynthetic sequence. Working with Norway spruce, Freudenberg (42) showed that, immediately prior to lignification, the greater percentage of coniferyl alcohol is present in the cambium as the glucoside coniferin. Furthermore, coniferyl alcohol is the major lignin precursor (41, 42). Freudenberg (42) goes on to say that when the glucosides pass out from the cambium to immature cells undergoing lignification, they are hydrolyzed to aglycones by β-glucosidase; the aglycones are in turn attacked by an abundant dehydrogenase and converted into lignin. What happens to the glucose on hydrolysis is not stated by Freudenberg, but it is almost certainly converted to cellulose. From a discussion with Freudenberg, Neish (93) believes that if coniferyl alcohol is overproduced in the spruce cambium it is stabilized as a glucoside by reaction with UDP-glucose. This glucoside then undergoes the
changes indicated by Freudenberg. However, where lignification is rapid, Neish (93) implies that all the coniferyl alcohol formed is converted to lignin. He does not mention formation of a glycoside as an intermediate step. Hence, it might be deduced that where lignification is rapid, the ratio of lignin to carbohydrate deposition favors the lignin. This might well hold for earlywood, where relatively higher lignin (156) and auxin contents have been found in coniferous woods (74, 142, 145). Where lignification is not so rapid, coniferyl alcohol will first be stabilized as a glucoside, which then moves out from the cambium. Here the ratio of components does not favor lignin as much as before. This might apply to latewood, since growth processes and cell maturation (e.g. including lignification) are not nearly so rapid as in earlywood, and lignin content is significantly lower (156).

IV. Relationship of Alpha-cellulose Patterns to Physical Properties

A. Strength Parameters

It was previously noted that, for the ten increments reported, the magnitude of earlywood-latewood differences in α-cellulose yield appears to reflect the influence of type of earlywood-latewood transition. While Douglas fir α-cellulose yield followed a sigmoid curve (Figs. 1 and 2), both spruces and Pacific silver fir displayed more gradual increase in α-cellulose yield across the increment. This effect in these same species has been noted for some intra-increment physical properties by Ifju et al. (62) and Homoky et al. (60).
With regard to physical properties, tensile and radial compressive strengths of Douglas fir are correlated with specific gravity to a highly significant degree (59, 60, 61, 62). That is, with a nearly three-fold increase in specific gravity, strength increases proportionately. From the newly-established pattern of cellulose distribution for the same species (though no direct evidence is established), it is unlikely that the approximately 5% yield differential between early- and latewood of any of the above species would account for the 300% strength increase with the same tissue. Hence, it would appear that such strength properties in regular wood of Douglas fir depend to a large degree upon variations reflecting degree of cell wall packing, alignment and associative properties between cell wall components rather than on those reflecting amount of long-chain cellulosic material. The material difference here is the remaining cell wall components, these being predominantly lignin and hemicellulose; thus, in influencing basic strength properties, the importance of these components is emphasized. The role of lignin (40) has already been likened to that of concrete re-inforcing surrounding steel rods (cellulose), while that of hemicellulose is, perhaps, not quite as clearly established.

B. Physical Evidence for Two Earlywood Types

Within Douglas fir growth zones Wilson and Ifju (150) discerned anomalous behaviour of two physical properties in earlywood; while specific gravity (G) remained relatively constant, ultimate tensile strength (UTS) increased progressively across this zone with as much as a three-fold change. This pronounced non-linearity was also evident (but not
discussed) in results of Kloot (71), who showed that specimens of rather uniformly low weight (earlywood) displayed a large variation in ultimate tensile load. In view of both studies, Wilson and Ifju (150) suggested that while at high specific gravity, strength depends more on amount of wood substance than on its composition, the reverse may be true at low levels of G. That is to say, earlywood strength is more affected by qualitative rather than quantitative differences in wood substance. According to Ifju et al. (62), this has apparently been implied by Hill.

It has been demonstrated here that lignin and \( \alpha \) - cellulose estimate patterns relate directly to, and positively re-affirm, the dual earlywood hypothesis of Wu and Wilson (157). Since the anomalous earlywood physical behavior noted above reflects variation within earlywood, it too must relate to the hypothesis. Thereby, results from Kloot (71) and Wilson and Ifju (150) become the first evidence showing variation in earlywood physical properties as reflecting the presence of two earlywood types.

If, within the earlywood zone, the increase in tensile strength relates to a corresponding change in cell walls and, if Freudenberg's (40) analogy is valid, then cellulose must change in some basic characteristic across the same zone. That for some species this is a quantitative change has already been shown from patterns of \( \alpha \) - cellulose content.

Qualitative evidence may be inferred from the regression of nitrocellulose \( \eta \) on \( \alpha \) - cellulose yield. For single increments of four species, nitrocellulose \( \eta \) for Position 1 appeared higher on the average
than that for the remaining earlywood. Linear regressions were determined on data plotted from all six positions and on these same data wherein Position 1 was omitted (Table 13). When the data from Position 1 were removed, a better fit occurred; when these were included, their influence was such that the slope even changed sign in one instance. This suggests further evidence of two earlywood types on the basis of average chain-length.
CONCLUSIONS

1. A method has been devised for accurate micro-cellulose determination. Alpha-cellulose yield may be quantitatively estimated from minute samples of wood meal as the corrected cellulose nitrate yield. Important variables were studied and it was shown that three-0.1 g (oven-dry) wood samples provide a statistically defensible determination. Intrinsic viscosities \( [\eta] \) of these micro-celluloses were used to indicate presence or absence of extensive degradation as a means of qualitatively deciding successful nitrocellulose preparation. A major limitation of the new method, however, is that it cannot be applied to all woods.

2. For the first time, a series of ten distinct \( \alpha \)-cellulose patterns have been established within coniferous growth increments of widely different botanical origin (5 genera). All such patterns revealed latewood to be significantly higher (2 to 3%) in \( \alpha \)-cellulose than earlywood. Six of these provide new chemical evidence pertaining to a physiologically significant phenomenon, this being the presence of two types of earlywood. Therein, minimum estimated \( \alpha \)-cellulose yield occurred at considerable cellular depth. The remaining four increments seemed different, in that they did not contain the anomaly.

3. Estimated \( \alpha \)-cellulose content varied with species. Sitka spruce had the highest average yield, 48.3% (46.5 to 50.1%), followed by
Douglas fir, 46.7% (43.4 to 49.8%), black spruce 45.2% (43.1 to 47.0%), Pacific silver fir, 44.9% (41.8 to 47.5%), and western red cedar, 44.0% (40.2 to 46.0%). Magnitude of earlywood-latewood differences in xα-cellulose yield appeared to reflect type of earlywood-latewood transition. Species having typically abrupt transition tended to display a rather sigmoidal pattern of xα-cellulose yield, whereas those having typically gradual transition show a flatter pattern.

4. Using three variables of wood quality, alpha-cellulose content throughout mature growth increments may be predicted reliably by linear regression or, more accurately, by logarithmic transformation. In two examples, black spruce and Douglas fir, correlation coefficients determined by the latter method were 0.95 and 0.96, respectively. This successful application of a logarithmic transformation is its first reported use describing the non-linear behavior of a wood chemical property across a coniferous increment.

5. Examination of xα-cellulose and lignification patterns in the same growth zones showed the xα-cellulose estimate (x = 45.9 ± 2.0%) as the exact complement of lignification (x = 27.4 ± 1.9%), regardless of species or location within growth zone (hence, season of formation). Evidence supporting this important interrelationship was demonstrated by linear correlation; for 60 pairs of xα-cellulose-lignin values, r = -0.785 was highly significant. This correlation reinforces previous theory on the mutually
exclusive nature existing between lignin and cellulose.

6. Further evidence of the mutually exclusive nature of $\alpha$ - cellulose and lignin resulted when the micro lignin and $\alpha$ - cellulose values were combined. These values clustered about a central value $(\bar{x} = 73.4 \pm 1.2\%)$, suggesting that for the increments studied, a common, critical portion of high molecular weight material was included in cell wall formation. Furthermore, dispersion about the combined values was significantly less than that about either of their individual means. This suggests closer physiological control over $\alpha$ - cellulose and lignin when combined, indicating that tree physiology is oriented more towards the finished biosynthetic product than towards the individual components involved in such a system.

7. Because of the highly variable $\mathcal{L}$ at each position, no trends in nitrocellulose chain-length variation were evident throughout growth zones. However, in four increments, significant differences in $\mathcal{L}$ throughout the earlywood provided further evidence of two earlywood types.
1. Abadie, F. A. and Ø. Ellefsen. 1952. Correspondence between the content of alpha cellulose and the content of the \( A \)-fraction, i.e., the fraction of nitrated cellulose which is precipitable in water when dissolved in acetone. Norsk Skogind. 6: 192-195.


136. ————. Personal communication.


TABLES AND FIGURES
### TABLE 1

Analyses on coniferous earlywood (E), latewood (L), and whole wood (W) carbohydrate fractions from the literature (based on oven-dry, extractive-free wood).

<table>
<thead>
<tr>
<th>Species</th>
<th>Ref.</th>
<th>Stems</th>
<th>Pairs</th>
<th>α-Cellulose by nitration, %</th>
<th>Holocellulose, E</th>
<th>α-Cellulose, L</th>
<th>Hydrolyzates, %</th>
<th>E&lt;sup&gt;a&lt;/sup&gt;</th>
<th>L&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinus monticola Dougl.</td>
<td>101&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>73.2</td>
<td>57-60</td>
<td>44</td>
<td>47.8</td>
<td>56.7</td>
<td>20.3</td>
</tr>
<tr>
<td>Pinus resinosa Ait.</td>
<td>53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>74.8</td>
<td>45.3</td>
<td>53.0</td>
<td>49.9</td>
<td>24.8</td>
<td>13.9</td>
</tr>
<tr>
<td>Pinus sylvestris L.</td>
<td>101&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>2</td>
<td>41.3</td>
<td>56-59</td>
<td>39</td>
<td>56.7</td>
<td>20.3</td>
<td>56.2</td>
</tr>
<tr>
<td>Pinus ponderosa Laws.</td>
<td>130&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>55-61</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinus banksiana Lamb.</td>
<td>106&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4</td>
<td>1</td>
<td>55-61</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinus palustris Mill.</td>
<td>160&lt;sup&gt;b&lt;/sup&gt;</td>
<td>125</td>
<td>500</td>
<td>77-84</td>
<td>52-57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinus taeda L.</td>
<td>102&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1</td>
<td>58.0</td>
<td>61.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinus densiflora S. et Z.</td>
<td>55</td>
<td>4</td>
<td>4</td>
<td>37.0-40.3</td>
<td>42.8-43.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picea abies (L.) Karst</td>
<td>52&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>51</td>
<td>60-63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picea glauca (Moench) Voss</td>
<td>106&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>1</td>
<td>46.0</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larix occidentalis Nutt.</td>
<td>106&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>1</td>
<td>43.8</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudotsuga menziesii (Mirb.) Franco</td>
<td>130&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>70.9</td>
<td>69-68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsuga canadensis (L.) Carr.</td>
<td>130&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>47.8</td>
<td>56.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abies amabilis (Doug.) Forb.</td>
<td>130&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>46-48</td>
<td>45-51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequoia sempervirens (D. Don.) Endl.</td>
<td>106&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>2</td>
<td>57-64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Libocedrus decurrens Torr.</td>
<td>106&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>2</td>
<td>40-44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thuja occidentalis L.</td>
<td>130&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>2</td>
<td>44.6</td>
<td>51-55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chamaecyparis nootkatensis</td>
<td>101&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>2</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(D. Don.) Spach</td>
<td></td>
<td></td>
<td></td>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) G = glucose, M = mannose
b) after Cross and Bevan (33)
c) after Wise et al. (153)
d) by calculation
e) as glucomannan
f) after Yundt and Bradway (160)
g) sulfite pulp species probably European species
h) sapwood
TABLE 2. Determination of replication number on cellulose yield for 40-60 mesh Douglas fir latewood meal using three sample sizes (nitrocelluloses corrected to 13.65 ± 0.30% nitrogen).

<table>
<thead>
<tr>
<th>Sample size (g oven-dry)</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>47.2</td>
<td>51.2</td>
<td>51.5</td>
</tr>
<tr>
<td>2</td>
<td>48.5</td>
<td>51.0</td>
<td>50.6</td>
</tr>
<tr>
<td>3</td>
<td>43.9</td>
<td>51.0</td>
<td>51.1</td>
</tr>
<tr>
<td>4</td>
<td>45.4</td>
<td>50.3</td>
<td>50.6</td>
</tr>
<tr>
<td>5</td>
<td>47.6</td>
<td>50.3</td>
<td>51.6</td>
</tr>
<tr>
<td>mean ((\bar{x})), %</td>
<td>46.5</td>
<td>50.8</td>
<td>51.1</td>
</tr>
<tr>
<td>variance ((s^2))</td>
<td>3.4</td>
<td>0.20</td>
<td>0.23</td>
</tr>
<tr>
<td>replication no. ((n))</td>
<td>4.3</td>
<td>2.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Total sample required, g</td>
<td>2.2</td>
<td>.3</td>
<td>.6</td>
</tr>
</tbody>
</table>

Replication number was determined by Stein's two-way sampling technique (116):

\[
n = \frac{t_1^2 s^2}{d^2}
\]

[1] where:

- \(n\) = replication number
- \(d\) = half-width of desired confidence interval
- \(s^2\) = sample variance
- \(t_1\) = tabulated \(t\) for a 90\% confidence interval

\[
f(1, 4) = t_1^2 = 4.54
\]
TABLE 3. Determination of replication number for Douglas fir latewood nitrocellulose nitrogen content.

<table>
<thead>
<tr>
<th>Item</th>
<th>Estimated Nitrogen Content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.80</td>
</tr>
<tr>
<td>2</td>
<td>13.82</td>
</tr>
<tr>
<td>3</td>
<td>13.77</td>
</tr>
<tr>
<td>4</td>
<td>13.70</td>
</tr>
<tr>
<td>5</td>
<td>13.83</td>
</tr>
</tbody>
</table>

\[ \bar{x} = 13.78 \]
\[ S^2 = 0.0027 \]
\[ n = 1.92 \]

Replication number was determined by Stein's 2-way sampling technique (116) as in Table 2.

\[ n = \frac{t^2 \cdot S^2}{d^2} = \frac{(4.54)(0.0027)}{(0.08)^2} \]
<table>
<thead>
<tr>
<th>Species</th>
<th>Stem No.</th>
<th>Increment No.</th>
<th>Position</th>
<th>Estimated alpha-cellulose, %</th>
<th>Estimated lignin, %</th>
<th>Alpha-cellulose plus Lignin, %</th>
<th>Nitrocellulose viscosity, $\frac{mL}{d/g}$</th>
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</thead>
<tbody>
<tr>
<td><em>Picea sitchensis</em></td>
<td>70</td>
<td></td>
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<tr>
<td>(Bong.) Carr</td>
<td>1</td>
<td>1</td>
<td>47.5</td>
<td>47.1</td>
<td>46.6</td>
<td>46.3</td>
<td>73.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>48.0</td>
<td>48.5</td>
<td>47.8</td>
<td>47.4</td>
<td>75.3</td>
</tr>
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<td></td>
<td>3</td>
<td>1</td>
<td>49.8</td>
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<td>49.7</td>
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<td>50.1</td>
<td>49.9</td>
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<td>74.0</td>
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<td>47.8</td>
<td>47.5</td>
<td>47.2</td>
<td>74.0</td>
</tr>
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<td>3</td>
<td>47.6</td>
<td>47.8</td>
<td>47.5</td>
<td>47.2</td>
<td>74.0</td>
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<tr>
<td><em>Picea mariana</em></td>
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<td>34</td>
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<tr>
<td>(Mill.) B.S.P.</td>
<td></td>
<td></td>
<td>43.1</td>
<td>43.5</td>
<td>43.6</td>
<td>43.4</td>
<td>73.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>44.0</td>
<td>44.7</td>
<td>45.0</td>
<td>44.6</td>
<td>73.8</td>
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<td>45.5</td>
<td>46.1</td>
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<td>45.8</td>
<td>46.0</td>
<td>45.6</td>
<td>45.2</td>
<td>73.8</td>
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<tr>
<td><em>Pseudotsuga menziesii</em></td>
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<td>(Mirb.) Franco</td>
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<td>46.3</td>
<td>46.8</td>
<td>46.4</td>
<td>73.7</td>
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<td>47.4</td>
<td>47.1</td>
<td>73.7</td>
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<td><em>Abies amabilis</em></td>
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<th>Estimated lignin, %</th>
<th>Replications</th>
<th>Alpha-cellulose plus Lignin, %</th>
<th>Replications</th>
<th>Nitrocellulose viscosity</th>
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<th>Replications</th>
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<td>1</td>
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<td>41.2</td>
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<td>29.8</td>
<td>29.6</td>
<td>29.7</td>
<td>75.4</td>
<td>30.1</td>
</tr>
</tbody>
</table>

| N                        | 60       | 60             | 60       |                              |              |                     |              |                              |              |                          |       |              |      |
| x , %                    | 45.9     | 27.6           | 73.5     |                              |              |                     |              |                              |              |                          |       |              |      |
| s , %                    | 1.98     | 1.95           | 1.23     |                              |              |                     |              |                              |              |                          |       |              |      |

a) Position 1 = first-formed earlywood; position 6 = last-formed latewood.

* repeated value
TABLE 5. Linear regression of alpha-cellulose estimates ($Y, \%$) on position ($X$) for all species and increments.

- **Picea sitchensis** (Bong.) Carr.
  
  Increment No. 70
  
  $Y = 47.17 + .33 X \quad n = 6 \quad r = .51 \quad SE_E = 1.2$

- **Picea mariana** (Mill.) B.S.P.
  
  Increment No. 34
  
  $Y = 43.19 + .59 X \quad n = 6 \quad r = .90^* \quad SE_E = .72$

- **Pseudotsuga menziesii** (Mirb.) Franco
  
  Increment No. 40
  
  $Y = 43.64 + .87 X \quad n = 6 \quad r = .83^* \quad SE_E = 1.2$

- **Increment No. 64 to 66**
  
  $Y = 44.08 + .75 X \quad n = 18 \quad r = .86^{**} \quad SE_E = .88$

- **Abies amabilis** (Dougl.) Forb.
  
  Increments No. 78 to 80
  
  $Y = 42.22 + .76 X \quad n = 18 \quad r = .83^{**} \quad SE_E = .34$

- **Thuja plicata** Donn.
  
  Increment No. 73
  
  $Y = 40.62 + .97 X \quad n = 6 \quad r = .93^{**} \quad SE_E = .78$

** highly significant

* significant
TABLE 6. Analysis of variance for alpha-cellulose estimates (%) within *Picea mariana* (Mill.) B.S.P. Increment No. 34.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>ss</th>
<th>MS</th>
<th>F</th>
<th>F.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among positions</td>
<td>5</td>
<td>21.67</td>
<td>4.33</td>
<td>47.41**</td>
<td>5.06</td>
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<td>Within positions</td>
<td>12</td>
<td>1.12</td>
<td>0.093</td>
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<td></td>
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<td>Total</td>
<td>17</td>
<td>22.79</td>
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</tbody>
</table>

** highly significant

TABLE 6A. Duncan's test of mean alpha-cellulose estimates (%) within *Picea mariana* (Mill.) B.S.P. Increment No. 34.

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Alpha-cellulose</td>
<td>43.40</td>
<td>45.57</td>
<td>44.87</td>
<td>45.90</td>
<td>45.93</td>
<td>46.77</td>
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</tbody>
</table>

no significant difference between means.

TABLE 7. Analysis of variance for alpha-cellulose estimates (%) within *Picea sitchensis* (Bong.) Carr. Increment No. 70.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
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<th>F.01</th>
</tr>
</thead>
<tbody>
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<td>Among positions</td>
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<td>4.07</td>
<td>34.27**</td>
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<td>Within positions</td>
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<td>1.40</td>
<td>0.117</td>
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<td>Total</td>
<td>17</td>
<td>21.43</td>
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TABLE 7A. Duncan's test of mean alpha-cellulose estimates (%) within *Picea sitchensis* (Bong.) Carr. Increment No. 70.

<table>
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<tr>
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<th>4</th>
<th>5</th>
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<tr>
<td>Alpha-cellulose</td>
<td>47.07</td>
<td>47.64</td>
<td>47.70</td>
<td>48.10</td>
<td>49.67</td>
<td>49.87</td>
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</table>

TABLE 8. Analysis of variance for alpha-cellulose estimates (%) within *Thuja plicata* Donn. Increment No. 73.

<table>
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<th>F</th>
<th>F.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among positions</td>
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<td>58.29</td>
<td>11.66</td>
<td>116.6**</td>
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<td>Within positions</td>
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<td>1.21</td>
<td>0.10</td>
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<td>Total</td>
<td>17</td>
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TABLE 8A. Duncan's test of mean alpha-cellulose estimates (%) within *Thuja plicata* Donn. Increment No. 73.

<table>
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<th>6</th>
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<td>Alpha-cellulose</td>
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<td>45.73</td>
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TABLE 9. Analysis of variance for alpha-cellulose estimates (%) within *Pseudotsuga menziesii* (Mirb.) Franco Increment No. 40.

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<th>F</th>
<th>F.01</th>
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<td>58.14</td>
<td>11.63</td>
<td>39.83**</td>
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<td>0.29</td>
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### TABLE 9A
Duncan's test of mean alpha-cellulose estimates (%) within *Pseudotsuga menziesii* (Mirb.) Franco Increment No. 40.

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### TABLE 10.
Analysis of variance for alpha-cellulose estimates (%) within *Pseudotsuga menziesii* (Mirb.) Franco Increments No. 64 to 66.

<table>
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<th>F.01</th>
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<tbody>
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<td>Position</td>
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<td>131.3**</td>
<td>3.54</td>
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<td>1.46</td>
<td>6.06**</td>
<td>5.23</td>
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<td>P x I</td>
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### TABLE 10A.
Duncan's test of mean alpha-cellulose estimates (%) within *Pseudotsuga menziesii* (Mirb.) Franco Increments No. 64 to 66.

<table>
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<tr>
<th>Position</th>
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<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Alpha-cellulose</td>
<td>44.89</td>
<td>44.97</td>
<td>46.73</td>
<td>47.46</td>
<td>48.07</td>
<td>48.22</td>
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<table>
<thead>
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<th>65</th>
<th>64</th>
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<td>Alpha-cellulose</td>
<td>46.26</td>
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### TABLE 11.
Analysis of variance for alpha-cellulose estimates (%) within *Abies amabilis* (Dougl.) Forb. Increments No. 78 to 80.

<table>
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<th>F</th>
<th>F .01</th>
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<td>Position</td>
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<td>130.86</td>
<td>26.17</td>
<td>108.6**</td>
<td>5.16</td>
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<tr>
<td>Increment</td>
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<td>33.66</td>
<td>16.83</td>
<td>69.80**</td>
<td>3.48</td>
</tr>
<tr>
<td>P x I</td>
<td>10</td>
<td>-9.29</td>
<td>-0.93</td>
<td>-3.85</td>
<td>2.78</td>
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<tr>
<td>Error</td>
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<td>10.13</td>
<td>.241</td>
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</tr>
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<td>Total</td>
<td>59</td>
<td>165.36</td>
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### TABLE 11A.
Duncan's test of mean alpha-cellulose estimates (%) within *Abies amabilis* (Dougl.) Forb. Increments No. 78 to 80.

<table>
<thead>
<tr>
<th>Position</th>
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<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-cellulose</td>
<td>43.05</td>
<td>43.34</td>
<td>44.31</td>
<td>45.82</td>
<td>46.19</td>
<td>46.76</td>
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</table>

<table>
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<tr>
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<th>78</th>
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</thead>
<tbody>
<tr>
<td>Alpha-cellulose</td>
<td>44.01</td>
<td>45.28</td>
<td>45.39</td>
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</table>
TABLE 12. Linear regression of lignin (Y, %) on alpha-cellulose estimates (X, %) for all species and increments.

<table>
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<tr>
<th>Species</th>
<th>Increment No.</th>
<th>Regression Equation</th>
<th>n</th>
<th>r</th>
<th>SE_E</th>
</tr>
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<tbody>
<tr>
<td>Picea sitchensis (Bong.) Carr.</td>
<td>No. 70</td>
<td>$Y = 46.10 - .422X$</td>
<td>6</td>
<td>-.820*</td>
<td>.40</td>
</tr>
<tr>
<td>Picea mariana (Mill.) B.S.P.</td>
<td>No. 34</td>
<td>$Y = 56.42 - .595X$</td>
<td>6</td>
<td>-.921**</td>
<td>.36</td>
</tr>
<tr>
<td>Pseudotsuga menziesii (Mirb.)</td>
<td>No. 40</td>
<td>$Y = 75.73 - 1.03X$</td>
<td>6</td>
<td>-.934**</td>
<td>.86</td>
</tr>
<tr>
<td></td>
<td>No. 64 to 66</td>
<td>$Y = 57.32 - .660X$</td>
<td>18</td>
<td>-.764**</td>
<td>.81</td>
</tr>
<tr>
<td>Abies amabilis (Dougl.) Forb.</td>
<td>No. 78 to 80</td>
<td>$Y = 55.43 - .622X$</td>
<td>18</td>
<td>-.945**</td>
<td>.92</td>
</tr>
<tr>
<td>Thuja plicata Donn</td>
<td>No. 73</td>
<td>$Y = 48.61 - .397X$</td>
<td>6</td>
<td>-.733 (N.S.)</td>
<td>.80</td>
</tr>
</tbody>
</table>

N.S. = not significant
Correlation coefficients ($r$) from the linear regression of $\ln (d_{1/g})$ on alpha-cellulose yield (%) for six intra-increment positions, showing effect of inclusion (+) or exclusion (-) of data from Position No. one (which corresponds to first-formed earlywood).

<table>
<thead>
<tr>
<th>Species</th>
<th>Increment</th>
<th>$+$</th>
<th>$-$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Picea sitchensis</em> (Bong.) Carr.</td>
<td>70</td>
<td>0.07</td>
<td>0.42</td>
</tr>
<tr>
<td><em>Picea mariana</em> (Mill.) B. S. P.</td>
<td>34</td>
<td>0.49</td>
<td>0.74**</td>
</tr>
<tr>
<td><em>Pseudotsuga menziesii</em> (Mirb.) Franco</td>
<td>66</td>
<td>-0.10</td>
<td>0.71*</td>
</tr>
<tr>
<td><em>Thuja plicata</em> Donn</td>
<td>73</td>
<td>-0.56*</td>
<td>0.28</td>
</tr>
</tbody>
</table>

** highly significant

* significant

↓ excludes Position No. 2 also
Figure 1. Two representative patterns of alpha-cellulose (est.), %.
Figure 2. Ten patterns showing alpha-cellulose (est.), %, lignin, %, and their sum.
Figure 3. Linear correlation of lignin, %, in combination with alpha-cellulose (est.), %, for ten coniferous increments.

Y = 63.08 - 0.773X
N = 60
r = -0.785**
Figure 4. Means ($\bar{x}$) and standard deviations ($S$) for all alpha-cellulose and lignin estimates and their sum.
APPENDICES
APPENDIX I. Relationship between the major cell wall components after Norman (94).

<table>
<thead>
<tr>
<th>Cell Wall Components</th>
<th>TRUE CELLULOSE</th>
<th>HEMICELLULOSE</th>
<th>POLYURONIDE</th>
<th>LIGNIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(long-chain 1, 4-β-D-anhydro-glucopyranose units)</td>
<td>hydrolyzed by dilute acid (soluble in dilute alkali)</td>
<td>- phenolic and enolic hydroxyls</td>
<td>(non carbohydrate) - phenyl propane units</td>
</tr>
<tr>
<td>Chlorite Holocellulose</td>
<td>Cross and Bevan &quot;Plant Cellulose&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEMICELLULOSE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRUE CELLULOSE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CELLULOSANS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>METHOXYHEXURONIC ACID</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARABINOSE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUCAN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XYLAN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHENOLIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENOLIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HYDROXYLS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- phenolic and enolic hydroxyls

amorphous

amorphous
APPENDIX II. Suggested generic classification of wood polysaccharides after Stewart (117).

Polysaccharides

- Glycosan uronides
- Non-cellulosic glycosans
  - Non-glucosic cellulosic glycosans
  - Glucosic cellulosic glycosans
- Cellulosic glycosans

Glycosans
APPENDIX III. Description of stem sections and growth increments included in the study (157).

<table>
<thead>
<tr>
<th>Species</th>
<th>Age, yr</th>
<th>Dia, in</th>
<th>Stem Growth Rate, mm/yr</th>
<th>Decadal Growth, mm/yr</th>
<th>No.</th>
<th>Growth Rate, mm/yr</th>
<th>Latewood %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Picea sitchensis</em> (Bong.) Carr.</td>
<td>92</td>
<td>16.4</td>
<td>2.3</td>
<td>1.7</td>
<td>70</td>
<td>2.2</td>
<td>18.9</td>
</tr>
<tr>
<td><em>Picea mariana</em> (Mill.) B.S. P.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4</td>
<td>43.0</td>
</tr>
<tr>
<td><em>Pseudotsuga menziesii</em> (Mirb.) Franco</td>
<td>77</td>
<td>19.2</td>
<td>3.2</td>
<td>2.5</td>
<td>64</td>
<td>2.4</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65</td>
<td>2.8</td>
<td>44.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>66</td>
<td>2.2</td>
<td>41.0</td>
</tr>
<tr>
<td><em>Pseudotsuga menziesii</em> (Mirb.) Franco</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6</td>
<td>44.0</td>
</tr>
<tr>
<td><em>Abies amabilis</em> (Dougl.) Forb.</td>
<td>143</td>
<td>23.0</td>
<td>2.0</td>
<td>3.5</td>
<td>78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79</td>
<td>3.1</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80</td>
<td>2.7</td>
<td>19.2</td>
</tr>
<tr>
<td><em>Thuja plicata</em> Donn</td>
<td>78</td>
<td>18.9</td>
<td>3.1</td>
<td>1.9</td>
<td>73</td>
<td>2.3</td>
<td>20.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Heartwood zone, all other samples from sapwood.