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Title: DOUGLAS-FIR BARK: STRUCTURE AND ALKALINE DEGRADATION OF A GLUCOMANNAN

Abstract approved: Signature redacted for privacy.

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The inner bark of a Douglas-fir [Pseudotsuga menziesii (Mirb) Franco] tree was successively extracted with ethanol-water (4:1 v/v), benzene-ethanol (2:1 v/v), hot water, 0.5% ammonium oxalate, and acidified sodium chlorite solution.

A glucomannan was isolated from the acidified-sodium chlorite-insoluble fraction (holocellulose). The glucomannan was composed of D-mannose, D-glucose, and D-galactose in the ratio of 2:1:0.3. The intrinsic viscosity of the polysaccharide in cadoxen solution at 25° was 0.46 dl/g which corresponded to a degree of polymerization of 92. The molecular weight of the glucomannan as analyzed by end-group analysis was 1.58×10^4 which, in combination with gas-liquid chromatographic analyses showed a degree of polymerization of 93.

The glucomannan was completely methylated and hydrolyzed. Gas-liquid chromatographic and mass spectrometric analyses showed the presence of 2, 3, 4, 6-tetra-O-methyl-D-glucopyranose,

2, 3, 4, 6-tetra-O-methyl-D-galactopyranose, 2, 3, 6-tri-O-methyl-D-mannopyranose, 2, 3, 6-tri-O-methyl-D-glucopyranose, 2, 3-di-O-methyl-D-mannopyranose, and 2, 3-di-O-methyl-D-glucopyranose. These data and the negative specific rotation of -35. 9° are consistent with a polysaccharide glucomannan structure consisting of anhydro-D-mannopyranose and anhydro-D-glucopyranose units attached by β-D-(1→4) glycosidic bonds in the main chain. There are side chains of anhydro-D-galactopyranose units linked (1→6) to the mannopyranose and glucopyranose units of the main chain. A possible structure for the glucomannan has been proposed.

Alkaline degradation of the glucomannan in 0. 1 N aqueous sodium hydroxide at 64. 5°, 78. 5°, and 100. 0° showed rate constants for end-group peeling of $k_1 = k_2 = 48.15\ hr^{-1}$, $96.28\ hr^{-1}$, and $103.36\ hr^{-1}$. The termination rate constants k_3 at 64. 5°, 78. 5°, and 100. 0° were $1.90\ hr^{-1}$, $2.28\ hr^{-1}$, and $1.51\ hr^{-1}$. The large amount of degradation (60%) was demonstrated by the relatively high rate of the peeling rate constant compared to the termination rate constant. The activation energy for the peeling reaction was 5. 9 kcal/mole. The results supported a possible reaction sequence involving mono- and di-anionic end-group species as the intermediates leading to end-group elimination of reducing polysaccharides.

Douglas-fir Bark: Structure and Alkaline Degradation of a Glucomannan

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DOUGLAS-FIR BARK: STRUCTURE AND ALKALINE DEGRADATION OF A GLUCOMANNAN

I. INTRODUCTION

The forest products industry in Oregon produced more than 3. 5 million tons of bark in 1972 (130, p. 19). Only 78 percent of this material was utilized. Of the 2. 76 million tons of bark utilized, about 1 percent was consumed by the pulp and board industry, 79 percent was used for fuel, and the rest was used for miscellaneous purposes such as mulch, animal bedding, and decorative uses. Before new and more valuable means of bark utilization can be introduced, a better understanding of its chemical constituents and physical properties is needed.

The bark of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] trees represents the greatest volume of bark produced in Oregon. Carbohydrates are the major constituents (50-55%) of the Douglas-fir inner bark (33). Thus the present work is concerned with the chemical and physical properties of these constituents.

The study of the structure of bark hemicelluloses requires homogeneous polymers isolated in high yields with minimal modification. This is usually accomplished by delignifying the bark and extracting the resulting holocellulose with aqueous alkali. Solubility differences can be exploited for the separation of individual

hemicelluloses by the use of cations and solution concentrations. Thus, relatively dilute alkali solutions suffice to dissolve xylans and galactoglucomannans, but higher concentrations are required for the extraction of glucomannans (59, 149, 150).

Hemicelluloses rank second to cellulose as the most abundant naturally occurring organic material. They comprise 20 to 35 percent of the weight of wood. The fate of hemicelluloses in pulping reactions is largely unknown. They are, for the most part, dissolved in the pulping liquors and are lost. The properties of the various hemicelluloses and their effects, individually and collectively, upon the ultimate end-use properties of the pulp has not been given the consideration it deserves. As wood supplies become scarcer, the need to retain the hemicelluloses with the pulp will increase.

The behavior of the hemicelluloses under alkaline conditions has not been established. It is known that cellulose undergoes a "peeling reaction" in alkaline media whereby glucose units are "peeled" one by one from the reducing-end (161). This is thought to be the primary means by which the hemicelluloses are fragmented and removed during such reactions. Information on the reaction mechanisms involved and the exact rates at which the peeling reaction progresses would provide valuable data on how to slow down and prevent dissolution of the hemicelluloses.

The specific objectives of the present work were: 1. to

determine the positions and types of chemical linkages in the glucomannan from the inner bark of Douglas-fir, 2. to determine the rate of degradation of the glucomannan in aqueous alkaline media ranging in concentration from 0.001 N to 3.16 N sodium hydroxide at temperatures of 64.5°, 78.5°, and 100.0°.

II. HISTORICAL REVIEW

The work herein reported is concerned with Douglas-fir inner bark. Previous theses on this subject are those of Lai (90), Zerrudo (169), and Chen (33). These earlier theses include the literature references to mid-1972. The present thesis reviews the literature from that time and also includes some reference to literature earlier reported by Lai (90), Zerrudo (169), and Chen (33) in order to explain the results of the present work more clearly.

A brief description of bark anatomy is included to define the specific material investigated. For a detailed anatomical description of Douglas-fir bark, reference is made to Grillos (54), Grillos and Smith (55), Chang (30), Ross and Krahmer (124), Zerrudo (169), and Chen (33). Bark consists of inner bark and outer bark. The inner bark (phloem cells) is the portion from the vascular cambium to the cork cambium of the innermost cork layer. The outer bark (rhytidome) is everything to the outside of the innermost cork cambium.

The inner bark comes from the vascular cambium, that layer of living cells between the wood and bark which divide to form wood to the inside and bark to the outside. The inner bark is composed mainly of sieve cells, axial and ray parenchyma, and sclereids. Parenchyma and sieve cells remain alive in the living tree as long as they are components of the inner bark. Kiefer and Kurth (80) and

Ross and Krahmer (124) describe and illustrate the general appearance and position of the sclereids in Douglas-fir bark.

Ross and Krahmer (124) show that Douglas-fir outer bark consists of layers of cork in which growth increments are usually visible. The cork layers form from cork cambia which are living cells that were once living parenchyma cells of the inner bark. The cork cambia produce cork cells to the outside and a few storage cells to the inside. All cells outside the innermost cork cambium are dead because no food supply can pass through this layer of cork cells. This then results in an outerbark composed of cork cells and dead phloem cells, which were once inner bark.

It is known that the major constituents of the inner bark are the carbohydrates (50-60%), just as they are the major constituents (70-80%) in wood (86, 132). During the past decades, progress in the chemistry of wood polysaccharides has been very rapid, although many minor constituents, fine structural details, and physical properties still remain to be fully elucidated. Polysaccharides such as pectic acids, galacturonogalactans, arabinogalactans, 4-O-methylglucuronoxylans, glucomannans, and cellulose have been isolated from the wood of numerous species of both gymnosperms and angiosperms (8, 60, 149, 150, 153, 162). In contrast, little attention has been devoted to the carbohydrates present in the bark of trees. One reason for this has been the greater economic importance of wood.

Another is the occurrence in bark of non-carbohydrate constituents such as tannins, suberin and additional phenolic compounds, all of which render the isolation of homogeneous polysaccharides from bark difficult. Wood contains few or none of these components although the lignin common to both xylem and phloem often has to be removed from the wood prior to a successful isolation of its various polysaccharides.

It has long been known that bark contains carbohydrates. Segall and Purves (133) reviewed the early literature to the extent of comparing the chemical composition of bark extractives as related to species and age of the tree. Kurth (86) described the early work on bark in considerable detail. He reported that the barks studied contained hemicelluloses and cellulose and that the sugars associated with the hemicelluloses were glucose, galactose, mannose, arabinose, and xylose. Kiefer and Kurth (80) isolated a holocellulose fraction from the bast fibers, or sclereids, of Douglas-fir bark. Paper chromatographs of an acid hydrolyzate of the holocellulose showed the presence of glucose, galactose, mannose and xylose. In 1961, Holmes and Kurth (69) analyzed the sugars present in the newly formed inner bark of Douglas-fir. They found that sucrose, fructose and glucose were present in varying amounts. More recently, in 1974, Laver, Chen, Zerrudo, and Lai (95) reported a systematic isolation and measurement of the various carbohydrate fractions in Douglas-fir inner bark. A holocellulose fraction was isolated from the inner bark of Douglas-fir

and analyzed. It was composed of acid-insoluble lignin (3.1%), acid-soluble lignin (4.1%), anhydro-L-arabinose residues (2.6%), anhydro-D-xylose residues (6.3%), anhydro-D-mannose residues (9.5%), anhydro-D-galactose residues (2.3%), and anhydro-D-glucose residues (61.1%). The holocellulose was fractionated into its component polysaccharides, a xylan, a galactoglucomannan, a glucomannan, and a glucan-rich residue. This paper by Laver, Chen, Zerrudo, and Lai (95) appears to be the major work published on the carbohydrates in Douglas-fir bark. There is a dearth of information in the literature relating to the carbohydrates of Douglas-fir bark.

However, the carbohydrates in the barks of other species have been investigated. Some of the more pertinent work is included for comparison purposes and because the experimental techniques reported were of use in the present study.

Barks contain a well-defined hemicellulose fraction. In 1930, Schwalbe and Neumann (131) detected large amounts of readily hydrolyzable hexosans and pentosans in the inner barks of spruce, pine and red beech; and in 1938, Buston and Hopf (28) reported the presence in ash bark of 20% of hemicellulose material which, upon hydrolysis gave galactose, mannose and arabinose. In 1947, Cram and co-workers (39) detected glucose, mannose, galactose and xylose in hydrolyzates of barks from western red cedar and reported that one-third of the glucose had originated from the hemicellulose fraction. In 1955,

Chang and Mitchell (31) carried out analyses on the barks of many North American pulpwood trees. The hydrolyzates from 72% sulfuric acid treatment of extractive-free barks were found to contain various amounts of glucose, galactose, mannose, arabinose and xylose.

In 1957, Lindberg and Meier (99) reported on the physical properties of glucomannan hemicelluloses from the bark of Norwegian spruce [Picea abies (L.) Karst.]. Meier (110) carried out similar investigations on the glucomannans and on arabinoxylan from the bark of pine (Pinus silvestris L.).

In addition to hemicelluloses, many barks are rich in pectic substances (131, 28). Ash bark (131) contained 7% of pectic material, and balsam bark was found by Hay and Lewis (64) to contain 14% of a "water-soluble mucilage" in addition to other carbohydrates. In 1938-39, Sharkov and co-workers published a series of papers (134, 135, 136) on pectic materials in the inner barks of pine, fir and birch. Pine bast fibers were reported to contain up to 35% pectin.

Anderson and co-workers also studied the pectin components of both inner bark (3) and the adjacent cambial zone (3, 4); 10% of pectic material was isolated from the inner bark of black spruce (121). In 1956, Kotasek found that hydrolysis of an aqueous extract of spruce bark yielded D-galacturonic acid, in addition to arabinose, glucose, galactose, xylose, and rhamnose (83).

The carbohydrate gums constitute another group of

polysaccharides which can be associated with barks (77). The best known of these are the "gum exudates" which in many cases arise from mechanical damage to, or parasitic infection of, the exterior of the tree, although in some instances exudation appears to take place spontaneously. It can perhaps be argued that the gum exudates are not necessarily "normal" components of bark, but similar polysaccharides are known to be present in some barks where they undoubtedly fill a normal physiological role. A well known example is "slippery elm mucilage," which occurs in the inner bark of Ulmus fulva Michx. now known as Ulmus rubra Mühl., to the extent of 16% or more (5). It is secreted in the bark "in sac-like membranes, considerably larger than the surrounding cells, and scattered irregularly throughout the tissue" (49). This mucilage has been shown by Anderson (5), and by Hirst and co-workers (49, 50, 72) to contain residues of D-galacturonic acid, D-galactose, 3-O-methyl-D-galactose, and L-rhamnose.

Another gum was isolated from the inner bark of red fir by Becker and Kurth (11). Upon hydrolysis, it yielded glucuronic acid, glucurone, an aldobiouronic acid, galactose, arabinose, and two other sugars which were tentatively identified as 6-deoxy-glucose and 6-deoxy-idose, respectively.

Inner barks also contain starch. Larsen and Lynn (93) detected starch in the bark of western larch, and Anderson and Pigman have

reported its presence in the inner bark of black spruce (3). Histological studies show very clearly that starch granules, similar to those of cereal starches, are present in the parenchymatus cells of bark (32) where they act as a food reserve.

Jabbar Mian and Timell (74) studied the polysaccharides in white birch. They found that the bark contained a pectic acid, an acidic xylan, and cellulose in addition to several other polysaccharides. The carbohydrate composition of the original bark agreed closely with previously reported values for white birch bark (31). The cellulose portion was later fully characterized (74).

In a separate study of the carbohydrates present in the phloem (inner bark) of white birch (Betula papyrifera Marsh.), Timell and Jabbar Mian (154) succeeded in isolating several polysaccharides in high yields. However, the extraction gave evidence that only a few of the polysaccharides were obtained in a state pure enough to warrant further structural investigation. Hydrolysis of these polysaccharides yielded galacturonic acid, galactose, and arabinose in a ratio of 66:7:27 and also traces of glucose, xylose, and rhamnose units. Timell (145) analyzed the carbohydrates occurring in the bark of several gymnosperms, each representing a different genus. He developed a general method for the isolation of bark polysaccharides, including cellulose and galactoglucomannans from Ginkgo biloba, Abies amabilis, Picea engelmanii, and Pinus contorta. In later studies with Abies amabilis

bark, a xylan (146) and two galactoglucomannans (148) were isolated and examined. From the same bark, Bhattacharjee and Timell (16) were able to obtain a galacturonan and a pectic acid, which were characterized. Ramalingam and Timell (122) isolated an arabino-4-O-methyl-glucuronoxylan, a "glucan" and an alkali-soluble galactoglucomannan from the bark of Engelmann spruce (Picea engelmanii Parry) and also attempted to deduce the structure of a product which was composed of galactose, glucose, and xylose residues in a ratio of 1:4:3. These reports by Timell and associates include considerable data on barks in general and provide excellent descriptions of laboratory techniques for studies of bark polysaccharide chemistry.

In 1969, Beveridge and co-workers (14) reported some outstanding work on the structural features of slippery elm mucilage. This is the mucilage first isolated by Anderson in 1933 (5) and previously studied by Hirst, Gill, Jones, and Hough (50, 49, 72). In 1971, Beveridge, Jones, Lowe and Szarek (15) investigated the structure of the mucilage. These workers concluded that the polysaccharide consists of a main chain of alternating D-galacturonic acid and L-rhamnopyranose residues joined by α-linkages through positions 4 of the galacturonic acid and 2 of the L-rhamnopyranose. The polysaccharide has side chains, containing one or two residues of 3-O-methyl-D-galactopyranose attached to some L-rhamnose residues at C-4 and C-3, respectively.

Timell and co-workers (48, 151) have recently reported work on a new facet of bark polysaccharides. This involves the β-D-(1→3)-glucan known as callose. Callose is a polysaccharide which is widely distributed in the plant kingdom and occurs especially in the sieve elements of all tree barks, where it fills the sieve pores of non-functioning or dead sieve cells (gymnosperms) and sieve tubes (angiosperms). Wounding of bark tissue also induces formation of callose. The polysaccharide has attracted much attention among botanists, but its chemical nature remained unknown until 1957.

These workers (48) proved the presence of 1,3-glucosidic bonds in callose by various means. The polysaccharide consumed only 0.11 moles of reagent on oxidation with periodate. Partial acid hydrolysis afforded glucose and the laminaribiose series of polymer-homologous oligosaccharides. No cellobiose could be detected. When the callose was treated with an endo-β-1,3-glucanase, glucose and laminaribiose as well as small amounts of laminaritriose were produced. Because of its extreme insolubility the polysaccharide could be methylated only with difficulty and in low yield. After methanolysis, gas-liquid chromatography of the methyl-O-methylglucosides and the methyl-O-methylglucoside acetates showed the presence of 2,4,6-tri-O-methylglucose and small amounts of 2,3,4,6-tetra-O-methylglucose. The low yield of methylation product prevented any further structural analysis and it remains to be established whether or not the

polysaccharide is branched.

Recent work by Litvay (103) and Litvay and Krahmer (104) report that the simple pits of the phellem cell of the Douglas-fir cork are plugged with a material that gives a callose reaction when stained with aniline blue. Therefore, callose is undoubtedly present in Douglas-fir inner bark also.

Although there were the above-mentioned reports, it appears that prior to 1960 no systematic attempt had been made to divide the total polysaccharide fraction of a bark into its components, although it had long been recognized that barks were rich in polysaccharides, some of them hot-water soluble and easily hydrolyzable hemicelluloses.

In 1960, Painter and Purves (117) reported the first systematic attempt to classify the polysaccharides present in a bark [white spruce, Picea glauca (Moench) Voss]. They isolated at least six different carbohydrate fractions from the inner bark of white spruce and concluded that the bark contained (i) starch, mainly in the form of granules, (ii) much pectin material, consisting of pectinic acid, galactan, and arabinan, and (iii) hemicellulosic material, consisting of xylan and mannan components similar to those of wood.

The glucomannan studied in the present work was isolated from the acidified-sodium chlorite-insoluble holocellulose fraction of Douglas-fir inner bark. Numerous methods have been described in

the literature for the isolation of holocellulose from plant materials with the aim of minimum alteration of the carbohydrates present (75, 76, 88, 123, 129, 156, 157, 158, 160, 166).

The delignification reaction used in the present work was the acidified sodium chlorite method (166). The method has several advantages. It requires a relatively cheap and stable oxidizing agent, it gives a product of unusual brightness, and it can easily be used for delignifying both small and large quantities of material.

The glucomannan was extracted from the holocellulose by the method of Beélik, Conca, Hamilton and Partlow (12). The procedure involved the impregnation of the holocellulose with 1-2% aqueous barium hydroxide solution followed by extraction with 10% aqueous potassium hydroxide solution. Xylans readily dissolve in this medium while the dissolution of mannose-containing polymers is largely suppressed.

After isolating the glucomannan, it becomes necessary to characterize it and determine at least some aspects of its chemical structure. The monosaccharides which serve as building units may be obtained and subsequently identified through careful depolymerization by hydrolysis, acetolysis or other reactions. This determination of the constituent sugar units is the first and one of the most fundamental operations in establishing the nature of a polysaccharide.

Depolymerization of polysaccharides is usually effected by acid or enzyme hydrolysis or by acetolysis. Sulfuric acid is often the

medium of choice because the sulfate anions can be later removed by the addition of barium hydroxide, resulting in the formation of insoluble barium sulfate.

Qualitative identification of the monosaccharides can be realized by paper chromatography or by retention times with gas-liquid chromatography. Quantitative analysis of the monosaccharides is accomplished with gas-liquid chromatography. Gas-liquid chromatography of sugars has lagged behind the technique with other families of compounds such as hydrocarbons, sterols, terpenes and so on because of their heat sensitivity and lack of volatility.

These two problems have been overcome by the preparation of heat-stable, volatile sugar derivatives. The first real improvement in this area was the excellent work published by Sweeley, Bentley, Makita and Wells (141) and their use of the trimethylsilyl ether derivatives. This procedure was quickly adapted to the analyses of monosaccharides in wood and pulp hydrolyzates (13, 25, 94). Although the method works quite well it suffers from the fact that each anomer of the sugars as well as the ring isomers yield their own individual peaks. This means a great number of peaks must be resolved by the gas-liquid chromatograph and the results show considerable overlapping of peaks which makes quantitative measurements difficult.

This problem was overcome by the "alditol acetate" procedure first applied by Gunner, Jones and Perry (56) and extended by

Sawardeker, Sloneker, and Jeanes (128), by Sloneker (137) and by Albersheim, Nevins, English, and Karr (2). The method includes a reduction of the carbonyl group of the monosaccharides to the alcohol (sugar alditol) with sodium borohydride. This prevents the formation of the anomers and ring isomers and provides only one alditol form for each monosaccharide. The alditols are acetylated to give heat sensitive, volatile derivatives. The method was adapted to wood carbohydrates by Crowell and Burnett (41), by Borchardt and Piper (23) and more recently by Hardwood (63). Modifications of the experimental conditions described by Hardwood (63) are those used for the hydrolysis of the methylated glucomannan in the present work.

To have full knowledge regarding the structure of a polysaccharide it is necessary to know the position of attachment of the glycosidic bonds and to ascertain whether the linkages are of the α- or β- type (159, p. 63). The subsequent step in the structural proof after determining the monosaccharide building blocks is to methylate the polysaccharide. The hemicellulose isolated in this work has been methylated, hydrolyzed, and the methylated fragments identified.

Until mass spectrometry (MS) was introduced, convenient micro-scale methods for structural analysis of sugars were lacking. The first comprehensive papers which demonstrated the potential of MS in this field appeared in 1963 and the early work was reviewed by Kochetkov, and Chizhov in 1966 (81). Since both gas chromatography

(GC) and mass spectrometry (MS) are gas phase methods requiring some volatility of the compounds studied, they are suitably combined. An instrument capable of recording spectra directly from the components in the effluent from a gas chromatographic column was constructed in 1964. (125).

The GC-MS combination permits simultaneous separation and structural determination of components in microgram quantities in complicated mixtures and is, in many respects, an ideal analytical method. By now, GC-MS is a standard technique in carbohydrate analyses. The MS fragmentations of sugar derivatives and the structural information obtained by MS have been reviewed by Lönngren and Svensson (105).

The mixture of partially methylated sugars obtained on hydrolysis of fully methylated polysaccharides can now be routinely analyzed by GC-MS of their alditol acetates. The mass-spectral evidence, together with the relative retention times in GC of pertinent derivatives, will, in most cases, lead to an unequivocal identification of each component. The GC-MS procedure has also been used by Bjorndal, Lindberg, Rosell and Svensson (21, 101) to locate O-acetyl groups in wood polysaccharides. The GC-MS technique was used in the present work to identify the methylated fragments following methylation and hydrolysis of the glucomannan.

Much of the pulp fiber in this country is produced by the kraft

process which involves treatment of wood with sodium hydroxide and sodium sulfide. Although the effects of these alkaline conditions on cellulose have been extensively studied, little work has been done on the hemicelluloses.

The alkaline treatment of forest materials is becoming more important due to the increased efforts to provide pollution-free pulping reactions. Many of these methods involve oxidation reactions in alkaline media (53, 78, 118). Therefore, the reaction mechanisms and reaction kinetics of hemicelluloses under alkaline conditions will become of even greater importance.

The peeling reaction of cellulose is reasonably well established (35, 106, 108, 161). This stepwise degradation of the terminal reducing group proceeds via a β-alkoxy carbonyl elimination reaction. The major acidic components from such a system have been shown to be glucoisosaccharinic acids, although considerable quantities of other acids containing a lesser number of carbon atoms are also present. The original mechanism was postulated by Isbell (73) and with minor modifications, has been shown to be correct (36, 112, 113, 114). The early kinetic analyses for the alkaline degradation of cellulose was provided by Samuelson and co-workers (47, 126) who studied the alkaline hydrolysis at 170°. A mathematical expression was derived for the ratio between the rates of the propagation and termination reaction which occur during the alkaline degradation and a value of

65 was obtained for this ratio.

Machel and Richards (107), and Colbran and Davidson (34) had observed that at lower temperatures (100°) only a part of the degrading chains terminated to meta-saccharinic acid end units, while in others, the propagation reaction appeared to come to a standstill when the degrading chain end reached the inaccessible regions of the cellulose fiber.

These observations led to a recent and greatly improved kinetic analysis of the peeling reaction by Sarkanen and his colleagues (57, 92, 168). They first studied hydrocellulose and developed mathematical expressions which included three probabilities for the conversion of each reducing end-group: (a) probability that the reducing end-group would be eliminated from the cellulose chain; (b) probability that the reducing end-group would be converted to meta-saccharinic acid and remain attached to the cellulose chain; (c) probability that the reducing end-group would become inaccessible to the alkali. An activation energy of 24 kcal/mole was found for endwise degradation while termination to a stable meta-saccharinic acid unit was 32 kcal/mole. Consequently, the number average degree of polymerization (DP_n) of the degradable chain length was highly dependent on the reaction temperature, being 1000 at 65° and 140 at 132°. At lower temperatures, the majority of degrading chains terminate to a normal reducing end-group at the crystalline-amorphous transition region.

At higher temperatures, the major reaction is the termination to a stable meta-saccharinic acid end-group.

In 1968, Lai (91) studied the kinetics of degradation of cotton cellulose. It was found that the number of peeled glucose units for each reducing end-group was approximately 68 and was independent of temperature in the range from 65° to 120°. This finding suggests that the submicroscopic structure exerts a dominating influence on the termination process of the endwise degradation reaction.

Lai and Sarkanen (91, 92) investigated the kinetics of the alkaline degradation of α-amylose, a linear polysaccharide with α-D-glucosidic bonds. The structure of this polymer is well established and it served as a completely soluble compound for their studies. They interpreted the kinetic pattern in terms of mono- and di-anions of the reducing end-groups acting as reactive intermediates. The kinetic expression involved two pseudo first-order rate constants k_1 and k_2 where k_1 was the rate constant for the propagation reaction and k_2 was the rate constant for the termination reaction. The use of these two constants explained their experimental data quite well.

In 1972, Young, Sarkanen, Johnson and Allan (168) expanded the kinetic concept to include ionization constants for the formation of the mono-anionic species (K_1) and the di-anionic species (K_2). This allowed the use of three rate constants to fully describe the reaction: k_1 was the rate constant for endwise degradation from the mono-anionic

species; k_2 was the rate constant from the di-anionic species; k_3 was the rate constant for end-group stabilization (chain termination). Using these concepts, Young, Sarkanen, Johnson and Allan (168) presented a general kinetic expression for the rate of alkaline degradation of linear polysaccharides.

The expression provided opportunities for a more complete understanding of the alkaline degradation of polysaccharides in general and was used by Zerrudo (169) to describe the kinetics of an alkaline degradation of a xylan. He showed that the kinetic data obtained for a hemicellulose could be explained in terms of the mathematical expressions proposed by Young, Sarkanen, Johnson, and Allan (168) and found that the rate constants for the xylan were $k_1 = k_2 = 5.33\ hr^{-1}$ and $k_3 = 0.66\ hr^{-1}$. This work demonstrated that the rates of alkaline degradation of hemicelluloses can now be calculated. It should be possible to apply a knowledge of these rates to the time, temperature and alkali concentration parameters of a pulping reaction to reduce the loss of hemicelluloses and increase the yield of pulp.

A change in pulping conditions to retain hemicelluloses has already demonstrated a 5% yield in pulp through a doubling of the glucomannan retained (62). This work was not based on kinetic information but was based on end-group stabilization by reaction with sodium borohydride, a very expensive approach. Mention is made here to show how much improvement in yield can be realized through

a knowledge of the hemicelluloses.

The glucomannan investigated in the present work was from Douglas-fir bark but this will have little effect on the overall results because the experiments were designed to determine the rate data for a hemicellulose type from any source. The data can be readily extrapolated to any desired hemicellulose from wood or elsewhere. The hemicelluloses from every species of tree are different and the present work outlines a working program which can be applied to any species desired.

The recent advances in chromatographic separations coupled with the physical techniques now available to characterize materials, allow for a comprehensive study of bark carbohydrates. It is possible to isolate the carbohydrate, to quantitatively analyze the amounts present, and to at least partially determine their characteristics and structural features. The experimental work reported here demonstrates the application of these techniques to a glucomannan.

III. EXPERIMENTAL

A. Collection of Bark Samples

The inner bark used in this study was taken from a freshly cut Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] tree in McDonald Forest, Benton County, Oregon, U. S. A. A voucher specimen (No. 142702) (branch and trunk section) is deposited at the Herbarium of the Department of Botany, Oregon State University, Corvallis, Oregon, U. S. A. 97331. By count of the annual rings the tree was 80 years old. It had a diameter of 2 ft 5 in at breast height. Outer bark was chipped off the tree at breast height in May, 1974. The inner bark was then carefully stripped off and immediately brought to the laboratory where the adhering cambium layer was separated from the specimen. The cambium-free inner bark (39,000.0 g, moisture content 47.9%, hot air oven at 110°) was immersed in 95.0% ethanol. Water was added later to provide a solution of ethanol-water (4:1 v/v), with adjustments being made for the moisture content.

B. Sample Preparation

The inner bark, after soaking in the ethanol-water (4:1 v/v) solution for two days, was recovered by decantation and washed well with fresh ethanol-water (4:1 v/v).

The residue of inner bark (air-dried) was ground in a Wiley Mill

and fractionated according to particle size by screening with a series of "Tyler" screens (The W. S. Tyler Company, Cleveland, Ohio). All material (14, 634. 0 g) between -20 and +60 mesh was used.

C. Benzene-Ethanol Extraction

A part (12, 000. 0 g, dry weight) of the above bark was divided into 12 portions (1000. 0 g, dry weight). Each portion was extracted with benzene-ethanol solution (2:1 v/v; 3000. 0 ml of benzene and 1500. 0 ml of ethanol) in a Soxhlet extractor. Each extraction was continued for 27. 5 hr (a minimum of 50 solvent exchanges). The residues were combined and air-dried for 4 days; dry weight 11, 060. 1 g.

D. Hot-Water Extraction

A part (11, 060. 0 g, dry weight) of the air-dried residue remaining from the benzene-ethanol (2:1 v/v) extraction was divided into ten portions. Each portion was added to 9. 0 l of distilled water and the mixture was kept at a constant temperature of 50-60° with intermittent stirring for 24 hr.

The mixture was separated by filtration using a Büchner funnel and the residue was washed with distilled water; dry weight 9609. 4 g.

E. Ammonium Oxalate Extraction

A part (9609.0 g, dry weight) of the residue, after being dried in a hot-dry room for 4 days, was divided into four equal portions. Each portion was extracted with 24.01 of 0.5% aqueous ammonium oxalate solution for 26 hr at a temperature of 70-80°. The mixture was separated by filtration using a Büchner funnel. The residue was washed with distilled water, dried in the hot-dry room for 3 days: dry weight 8418.7 g.

F. Acidified Sodium Chlorite Delignification; Isolation of a Holocellulose Fraction

The dried residue (8418.7 g dry weight) from the ammonium oxalate extraction was divided into 16 batches. Each batch was stirred into 7.5 l of distilled water at 75-80°. A steady stream of nitrogen was bubbled through the mixture to prevent the accumulation of gases and the mixture was stirred mechanically. Glacial acetic acid (50.0 ml was added, followed by sodium chlorite (150.0 g). Fresh glacial acetic acid and sodium chlorite were added two more times at one hour intervals (145, 166). Silicone defoamer (Antifoam A spray, Dow Corning Corporation, Midland, Mich. 48640, U.S.A.) was added to the mixture to prevent foaming. At the end of 4 hr, the yellow solids were recovered by filtration using a Büchner funnel with Whatman No. 1 filter paper,

and washed well with distilled water. The yellow solids were dialyzed for one week, washed with distilled water, dried with ethanol and finally air-dried for two weeks.

The yellow color of the solids indicated incomplete delignification. The acidified sodium chlorite treatment was repeated. The residue was recovered by filtration, dialyzed for one week and freeze-dried. The white insoluble material thus obtained was termed "holocellulose"; dry weight 4100.7 g.

G. Fractionation of the Holocellulose Fraction into its Component Polysaccharides

1. Impregnation with 2.0% Barium Hydroxide Followed by Extraction with 10.0% Potassium Hydroxide Solution

A portion of the freeze-dried holocellulose (50.0 g dry weight) was slurried at 25° in 782.0 g of aqueous barium hydroxide solution containing 64.0 g of barium hydroxide octahydrate (12). The mixture was stirred intermittently for 20 min. The concentration of the holocellulose in the slurry was 6.0% and the concentration of barium hydroxide in the liquor was 4.4%. At the end of 20 min, 18.5% aqueous potassium hydroxide (925.0 g) was added to the slurry, which lowered the slurry concentration of holocellulose to 2.8% and the barium hydroxide concentration to 2.0%. The mixture was stirred intermittently for another 20 min. At the end of this period,

the mixture was separated by filtration using a sintered glass funnel and the residual holocellulose was washed with an aqueous solution of barium hydroxide and potassium hydroxide (250. 0 ml) having the same concentration as the extracting liquor. The solids were washed thoroughly with distilled water, then dispersed in 3. 0% acetic acid (700. 0 ml) and allowed to stand overnight at room temperature. The acidic slurry was filtered to recover the holocellulose, then washed to neutrality with distilled water, dialyzed for 3 days, freeze-dried and weighed; dry weight 44. 0 g. They were termed "Residue A. "

The filtrate plus washings were acidified with 50. 0% acetic acid to a pH of 5. 0, and condensed to 1. 0 l on a rotary evaporator. Three volumes of methanol were added and the precipitate which resulted was allowed to settle. The precipitate was recovered by centrifugation and washed four times with 70. 0% aqueous methanol. Methanol was removed from the precipitate by the addition of water followed by evaporation using a rotary evaporator. The solids were recovered by freeze drying. The freeze-dried material had a white, fluffy appearance and was labelled "crude xylan"; dry weight 3. 3 g.

2. Extraction with 1. 0% Aqueous Sodium Hydroxide Solution

Residue A (40. 0 g dry weight) was dispersed in 990. 0 g of 1. 0% aqueous sodium hydroxide solution to give a concentration of 4. 0%. The slurry was filtered after 20 min of intermittent stirring at 25° .

The residue was washed on the filter funnel with 250.0 ml of 1.0% aqueous sodium hydroxide solution followed by 1.0 l of distilled water. The solids were dialyzed for 3 days against running tap water. The solids which remained in the dialysis bag were recovered by freeze-drying and weighed; dry weight 39.8 g. This residue was labelled "Residue B" (12).

The filtrate and combined washings were condensed to 1.0 l and acidified with acetic acid to pH 5.0. Three volumes of methanol were added and the precipitate which resulted was recovered by centrifugation. The precipitate was washed 3 times with 70.0% aqueous methanol. The methanol was removed from the final precipitate on a rotary evaporator. The solids were slurried in water and the mixture was freeze-dried. The weight of the solids recovered was 0.54 g. These solids were labelled "galactoglucomannan" (12).

3. Extraction with 15.0% Aqueous Sodium Hydroxide Solution

Residue B (39.8 g dry weight) was dispersed in 920.0 g of 15.0% aqueous sodium hydroxide solution. After stirring for 20 min at 25°, the insolubles were recovered by filtration using a sintered glass funnel. The residue was washed with 250.0 ml of 15.0% aqueous sodium hydroxide solution followed by 1.0 l of distilled water. The residue was dialyzed for 5 days and freeze-dried. The dry weight was 35.1 g. The residue was labelled "Residue C" (12).

Saturated aqueous barium hydroxide (500. 0 ml) was added to the combined filtrates and washings to precipitate the glucomannan. The precipitate was recovered by centrifugation. The recovered precipitate was dissolved in 400. 0 ml of 2 N aqueous acetic acid and recovered from the medium by precipitation with three volumes of methanol. The precipitate was redispersed in 700. 0 ml of 70. 0% aqueous methanol and recovered by centrifugation (repeated three times). The precipitate was slurried in distilled water and freeze-dried. The dry weight was 1. 43 g. These solids were labelled "crude glucomannan" (12). The above procedure was repeated a number of times to obtain adequate sample.

4. Ash Determination of the Crude Glucomannan

The ash content of the crude glucomannan was determined according to a modified procedure of Paech and Tracey (116).

Duplicate samples (0. 20200 to 0. 21164 g dry weight) were weighed into previously ignited and weighed porcelain crucibles. The samples were saturated with concentrated sulfuric acid (0. 6 ml). The mixtures were stirred thoroughly with glass rods and set aside for 1 hr. They were placed on a hot plate and heated gently until charring occurred. The samples were then heated more strongly to drive off most of the sulfuric acid and then put in a muffle furnace at 600° for 4 hr. The ashed samples were placed in a desiccator to cool and

were weighed after 1.0 hr. The samples were reignited and reweighed until constant weight was obtained. The ash content was 9.73% as sulfate (9).

5. Dialysis of the Crude Glucomannan

A part (5.10 g dry weight) of the crude glucomannan was dissolved in distilled water and dialyzed against running tap water for 6 days. The material left in the dialysis bag was concentrated on a rotary evaporator and freeze-dried to yield a fluffy white solid; dry weight 4.63 g or 90.8% of the starting material. This purified, freeze-dried material was labelled "glucomannan."

H. Characterization of the Glucomannan

1. Ash Determination

The ash content of the glucomannan was determined according to the above procedure (116). The ash content was 3.27% as sulfate, average of triplicate determinations. The ash was considered to be barium sulfate which means that the glucomannan contained 1.92% barium.

2. Determination of Lignin

a. Acid-Insoluble Lignin (Klason Lignin). A part (0.16625 g

dry weight) of the glucomannan was placed in a 500-ml round-bottom flask submerged in a cold water bath (18-20°). An amount (5.0 ml) of cold 72.0% sulfuric acid was added slowly with stirring. The sample was allowed to stand with frequent stirring for 2 hr at 18-20°. The glucomannan was completely dispersed in the acid. The transparent syrup was diluted to 3.0% sulfuric acid concentration by slowly adding 188.0 ml of distilled water. The sample was refluxed for 4 hr. The insoluble material was recovered by filtration using a Gooch-type crucible (fine porosity) which had previously been dried and weighed. The residue in the crucible was washed free of acid with 150.0 ml of hot water and dried in an oven at 105 ± 2° until the weight became constant (142). The lignin content was 1.05% (average of three determinations).

b. Acid-Soluble Lignin. The filtrates from the above Klason lignin determinations were combined to yield a solution of 1008.0 ml. The solution was analyzed for the acid-soluble lignin content by the characteristic lignin absorptions at 280 nm and 215 nm (26, 51). The instrument used was a Beckman ACTA TM III-UV-Visible spectrophotometer. The instrument was standardized by placing 3.0 ml of 3.0% sulfuric acid solution in both the sample and reference cells (cell widths 1.0 cm) and scanning over the ultraviolet spectral range

from 320 nm to 200 nm. The sample cell was cleaned, dried and 3.0 ml of the acid filtrate from the Klason lignin determination was added. The sample was scanned over the ultraviolet spectral range from 320 nm to 200 nm. Absorption peaks were recorded at 280 (absorbance 0.375) and at 215 nm (absorbance 0.655); acid-soluble lignin content 0.94%.

3. Optical Rotation

A part (0.07393 g dry weight) of the glucomannan was dissolved in 10.0% aqueous sodium hydroxide solution (50.0 ml). Ten different readings were taken on the polarimeter (Rudolf and Sons) at 25°. The measured optical rotation was -0.102° (average of ten readings). The specific rotation was calculated according to the relationship (164, p. 415):

$$[\alpha]_D^{25} = \frac{100\alpha}{bc} = \frac{100\alpha}{2c}$$

where $[\alpha]_D^{25}$ = specific rotation

α = measured optical rotation in degrees

b = cell length in dm = 2

c = conc. in g/100 ml

The specific rotation was -35.9°, (c 0.142 g/100 ml, 10.0% aqueous sodium hydroxide).

4. Hydrolysis with 77.0% Sulfuric Acid

A portion of the glucomannan (250 mg air-dry weight) was placed in a 250-ml round-bottom flask submerged in an ice bath. Sulfuric acid (77.0%, 4.5 g) was added and the mixture was kneaded with a glass rod for 2 hr. The colorless, translucent paste was maintained at ice-bath temperature, stirred, and water (84.5 ml) was added dropwise to 3.0% acid concentration. The solution was refluxed for 9 hr, cooled to room temperature, and neutralized to pH 5.0 with saturated aqueous barium hydroxide solution. The resulting barium sulfate precipitate was removed by centrifuge and washed well with water. The decantate plus washings were concentrated under vacuum on a rotary evaporator to a syrup.

5. Qualitative Uronic Acid Analysis by Color Reaction

Concentrated sulfuric acid (6.0 ml) was added slowly to an aqueous solution (1.0 ml) of the 3.0% sulfuric acid hydrolyzate of the glucomannan and cooled under tap water to prevent localized heating in the test tube. The reaction mixture was heated for 20 min in a boiling water bath and cooled. An aliquot (0.2 ml) of a 0.1% ethanolic solution of carbazole was added and the test sample allowed to stand for 2 hr at room temperature. There was no development of a purple color indicating that the glucomannan had no uronic acid moiety. The

solution was scanned in the ultraviolet spectral range and showed no absorption at 535 nm. This was interpreted to mean that the glucomannan did not possess a uronic acid portion (43, p. 497)

6. Qualitative Carbohydrate Analysis by Paper Chromatography

The hydrolyzate from the sulfuric acid hydrolysis was separated into its component monosaccharides by paper chromatography. The hydrolyzate syrup was dissolved in distilled water (1.0 ml) and spotted on Whatman No. 1 chromatographic paper, 18 x 63.5 cm. A standard solution containing about 1.0% each of the known monosaccharides mannose, glucose, galactose, arabinose, xylose, and rhamnose was spotted along with the unknown hydrolyzate on the front of the chromatographic paper 5 cm from one end. The sugars were separated by descending development with ethyl acetate-pyridine-water (8:2:1 v/v/v) (70) as developer. The solvent was allowed to migrate to the bottom of the papers (16 hr). The chromatograms were removed from the tank and air-dried. The chromatograms were sprayed with aniline hydrogen phthalate indicator (1.66 g of phthalic acid dissolved in 100.0 ml of water saturated n-butanol containing 0.93 g of freshly distilled aniline) (119) and heated in an oven at 100 ± 2° for 5 min to develop the color. The spots were investigated under ultraviolet light. The rates of movement of the hydrolyzate sugars were compared with

those of authentic samples when run simultaneously on the same chromatograms. The chromatograms showed a very strong spot for mannose, medium spot for glucose, weak spot for galactose and trace spots of xylose and arabinose.

7. Quantitative Carbohydrate Analysis by Gas-Liquid Chromatography

An amount (0. 13648 g dry weight) of glucomannan was dissolved in 2. 2 g of 77. 0% sulfuric acid in an ice bath for 2 hr with occasional stirring. The translucent paste was diluted to 3. 0% sulfuric acid by dropwise addition of 42. 0 ml of distilled water. The dilute solution was refluxed for 9 hr. The solution was diluted to 100. 0 ml from which 25. 0 ml of hydrolyzate was removed. Myo-inositol (10. 0 mg) was added to 25. 0 ml of the hydrolyzate. The hydrolyzate was neutralized to pH 5. 0 with a saturated aqueous barium hydroxide solution. The barium sulfate precipitate was removed by centrifugation. The clear supernatant solution was concentrated to 25. 0 ml and transferred to a 100. 0 ml round-bottom flask. Sodium borohydride (0. 08 g) was added to the flask and allowed to react for 2 hr at room temperature (1).

The excess sodium borohydride was decomposed by adding acetic acid until gas evolution ceased. The solution was concentrated to a syrup on a rotary evaporator, and methanol (10. 0 ml) was added

and re-evaporated. The addition and removal of methanol was repeated eight times (2). The resulting syrup was dried in an oven at 105° for 15 min to ensure complete removal of water.

Acetic anhydride (7. 5 ml) and concentrated sulfuric acid (0. 5 ml) were added to the syrup and the solution was heated for 1 hr at 50-60° in a water bath. After cooling for 5 min the acetylation mixture was poured with stirring into about 70. 0 ml of ice water. Two immiscible layers (water layer on top and acetate layer on the bottom) were formed. These were transferred to a separatory funnel and the alditol acetates were extracted with three successive amounts of methylene chloride (25 ml, 15 ml, 10 ml). The methylene chloride extract was concentrated to dryness on a rotary evaporator at 75°. Distilled water (1. 0 ml) was added to the residue and re-evaporated. The alditol acetates were dissolved in 1. 0 ml of methylene chloride. An aliquot (2 µl) of the solution was injected into the gas chromatograph for quantitative analysis (23, 136). The peaks in the resulting spectra were identified by comparison of retention times with authentic known alditol acetates. The areas under the peaks in the resulting spectra were measured by means of a planimeter.

The gas chromatograph used was a Hewlett-Packard 5751 B Research Chromatograph (Hewlett Packard Company, Palo Alto, California) equipped with dual flame ionization detector. The conditions were: column, 6. 5% ECNSS-M on Gas chrom Q 100/120 mesh,

6 ft x 1/8 in O. D. stainless steel; injection port 190° ; detector 240° ; column temperature 170° isothermal; helium flow 30 ml/min; range setting 10^2; attenuation setting 64.

8. Reducing End-Group Analysis (Somogyi Method)

a. Standardization of Sodium Thiosulfate Solution. Sodium thiosulfate (6. 5 g) and sodium carbonate (100 mg) were dissolved in 1. 0 l of freshly boiled but cooled distilled water. The solution was standardized against potassium iodate as described below.

Potassium iodate (1. 4 g) was dissolved in distilled water and the solution was diluted to 500. 0 ml in a volumetric flask. An aliquot (50. 0 ml) of the solution was pipetted into a 250 ml Erlenmeyer flask. Potassium iodide (3. 0 g) and 6 F hydrochloric acid (2. 0 ml) were added to the aliquot. The solution was titrated immediately with the sodium thiosulfate solution to be standardized (0. 005 M). Titration was continued until the yellow triiodide color was barely visible, then 5. 0 ml of 1. 0% aqueous starch solution was added. Titration was concluded by adding titrant in drops until the starch-iodine color disappeared. The exact molarity (0. 0056 M) of the sodium thiosulfate solution was calculated from the average of triplicate samples (45, p. 602).

b. Preparation of Somogyi Copper Reagent. Rochelle salt (40. 0 g) (potassium sodium tartrate), disodium hydrogen phosphate

dodecahydrate (71. 0 g) and 1. 0 N sodium hydroxide (100. 0 ml) were dissolved in 500. 0 ml of distilled water. An amount (80. 0 ml) of an aqueous solution containing 8. 0 g of cupric sulfate pentahydrate was added with stirring followed by a solution of potassium iodate (0. 372 g in 100. 0 ml water). Finally, anhydrous sodium sulfate (180. 0 g) was dissolved. The solution was diluted to 1. 0 l and allowed to stand for 3 days. The clear supernatant solution was clarified by filtration through a fritted glass filter. The pH of the solution was about 9. 5 as reported in the directions for preparation (68, p. 383, 138).

c. Determination of Total Reducing End-Groups in the Glucomannan. A sample (8. 4 mg) of the unhydrolyzed glucomannan was dissolved in distilled water (5. 0 ml) and was placed in a 25 x 200 mm test tube. Alkaline copper reagent (5. 0 ml) was added by pipette and mixed thoroughly. The test tube was closed with a glass bulb and placed in a rack. Blanks were prepared with water (5. 0 ml). Similarly, 5. 0 ml of three standard mannose/glucose (2:1) solutions containing 0. 20 mg, 0. 15 mg, 0. 5 mg, and 0.05 mg of total sugars per 5. 0 ml of solution were added to identical test tubes. Alkaline copper reagent (5. 0 ml) was pipetted into each of these test tubes. The rack of tubes was immersed in a vigorously boiling water bath to a depth of about 5 cm above the solution inside the tubes. The solutions were heated for 30 min, removed from the bath and allowed to cool. Triplicate determinations were performed in all cases.

Potassium iodide (2. 5%, 2. 0 ml) was added to each tube without mixing. From a fast flowing buret, 2 N sulfuric acid (1. 5 ml) was run into each tube with shaking so that the liberated iodine would oxidize all reduced copper. After 5 min the tubes were reshaken. The excess of liberated iodine not reduced by cuprous ions was then titrated with standardized sodium thiosulfate (0. 0056 M) to a starch end point. The difference in the amount of titer consumed by the blank and the glucomannan was attributed to the reducing end group of the polysaccharide.

9. Viscosity Measurements

A part (100. 0 mg) of the glucomannan was put into a 25-ml volumetric flask which had been previously swept free of air by a stream of nitrogen. A few drops of water were added to swell the fibers and prevent gel particle formation. After 5 min cadoxen (12. 5 ml) (cadmium ethylene diamine hydroxide) (General Chemical Division, Allied Chemical, Columbia Road and Park Ave., Morristown, N. J.) at 25° was added, and the mixture shaken until complete solution was achieved (20 min). The solution was then diluted with water up to the mark of the volumetric flask resulting in a final solution of 1:1 cadoxen-water. The filled flask was carefully weighed to determine the density of the solution in g/25 ml. An aliquot (10. 0 ml) of the solution was transferred to a Cannon-Ubbelhode dilution

viscometer (size 75) previously placed in a water bath at 25±0.1° and flushed with nitrogen. After 5 min the solution was forced into the bulb of the viscometer by applying pressure with nitrogen. The pressure was released and the time required for the miniscus to pass between the two calibration marks was measured to 0.1 sec. Triplicate measurements were made until duplicates agreed to within ±0.3% (26, p. 541, 65).

The solution was diluted directly in the viscometer with a suitable amount of solvent and mixed by stirring with a stream of nitrogen. Measurements were taken with each dilution. A total of six concentrations were measured. The viscosity of the solvent was also measured. The intrinsic viscosity was found to be 0.46 dl/g.

10. Methylation of the Glucomannan

The glucomannan (1.4 g air-dry weight, moisture content 10.0%) was dissolved in 18.0% aqueous sodium hydroxide solution (50.0 ml) at ice bath temperature with stirring followed by the addition of 6.0 g of sodium hydroxide pellets. Sodium hydroxide (100.0 ml, 30.0%) and dimethyl sulfate (50.0 ml) were added simultaneously over a period of 3 hr while maintaining the solution at ice-bath temperature. Acetone (80.0 ml) was added to prevent foaming. The mixture was stirred for an additional 45 hr at room temperature. The solution was cooled to 0° in an ice bath, neutralized with 10.0% sulfuric acid,

and dialyzed for 5 days against running tap water. The solution was concentrated on a rotary evaporator and freeze-dried. The entire methylation sequence was repeated (167).

The product was further methylated by the method of Hakomori (58, 127), wherein the methylsulfinyl anion (38) was used to generate the polysaccharide alkoxide prior to addition of methyl iodide.

The methylsulfinyl anion was prepared as follows. Sodium hydride (1. 5 g, 55% coated with mineral oil) was weighed into a dry, 300-ml three-necked round-bottom flask fitted at one neck with a rubber serum cap and containing a magnetic stirring bar. The sodium hydride was washed three times with n-pentane (30. 0 ml). After the third wash, the flask was fitted with a thermometer and stoppered condenser and residual n-pentane was removed by successive evacuations with a vacuum pump through an 18-gauge needle inserted into the serum cap. After each evacuation, the flask was regassed with nitrogen. The stopper was then removed from the condenser and nitrogen was passed continuously through the flask via the needle. Using a hypodermic syringe, dimethyl sulfoxide (15. 0 ml) distilled from calcium hydride under reduced pressure, was transferred into the flask. The flask was placed in an oil bath and stirred with a magnetic stirrer at 65-70^{o} until the solution became clear and evolution of gas ceased (about 1 hr).

The glucomannan alkoxide was generated as follows. The

glucomannan was dried in vacuo over phosphorus pentoxide at 60° overnight. The dried material (1.0 g) was dissolved in dry dimethyl sulfoxide (50.0 ml) in a 300-ml three-necked round-bottom flask containing a magnetic stirring bar and fitted with a condenser and a serum cap. Nitrogen gas was passed continuously through the flask via the needle. The suspension was heated at 60° in an oil bath and stirred with a magnetic stirrer until all of the glucomannan was dissolved (about 1 hr). After cooling to room temperature, methylsulfinyl anion (10.0 ml) was added to the mixture. Upon addition of the anion, a gel was formed immediately but gradually liquefied. After stirring at room temperature for 30 min, the reaction mixture appeared homogeneous. The solution was stirred for 7 hr. The polysaccharide solution was cooled to 20° in an ice-water bath and methyl iodide (3.0 ml) was added to the stirred solution at a rate such that the temperature did not rise above 25° (6-8 min). Within a few minutes after the addition of methyl iodide heat evolution ceased, the solution became clear and the viscosity markedly reduced. At this stage the reaction was complete. The reaction mixture was dialyzed against running tap water for 3 days. The dialyzed polysaccharide was concentrated on a rotary evaporator and freeze-dried. The product was remethylated using the procedure described above. After the fourth methylation, the dialyzed and concentrated aqueous solution was extracted with chloroform. The chloroform layer was

concentrated to a syrup which was recovered by dialysis and freeze-dried.

The final stages of the polysaccharide methylation was accomplished by the Purdie method (66, p. 294).

The partially methylated polysaccharide (0.56 g) was dissolved in methyl iodide (12.5 ml) and methanol (5.0 ml). Silver oxide (4.16 g) was added in small portions with vigorous stirring. The mixture was gently refluxed with stirring for 8 hr. The insoluble material was recovered by filtration and washed with boiling chloroform (200.0 ml). The combined filtrates were concentrated to a syrup then dialyzed for 4 days and the solids were recovered by freeze-drying. The entire methylation sequence was repeated three more times using the procedure described above. The dialyzed polysaccharide was soluble in methyl iodide and was remethylated one more time by the Purdie method (66) without the addition of methanol. The chloroform layer was concentrated to a syrup which was dissolved in acetone and filtered. The filtrate was concentrated, dissolved in chloroform (50.0 ml) and was poured into petroleum ether (b.p. 30-60°, 500 ml). The mixture was refrigerated for 3 days. The white precipitate which formed was recovered by filtration and dried in a desiccator over phosphorus pentoxide, affording a white powder. The product finally obtained (248.7 mg) had a methoxyl content of 45.3%, (calculated OCH_3=45.6 %).

11. Hydrolysis of the Methylated Glucomannan

A part of the methylated glucomannan (40. 0 mg) was hydrolyzed with 10. 0 ml of 90. 0% formic acid at 97° for 3 hr. The solution was cooled and formic acid was removed by evaporation under reduced pressure followed by the addition and removal of water. The syrup so obtained was hydrolyzed with 0. 5 N sulfuric acid (5. 0 ml) for 2. 5 hr at 97° to hydrolyze any formate esters which may have been formed (24). Upon cooling, the solution was neutralized with barium carbonate and the solids were removed by centrifugation. The centrifugate was deionized with Amberlite IR 120 (H^+), then concentrated to a syrup under reduced pressure (63).

The syrup so obtained was dissolved in a few drops of water and subjected to paper chromatography using 2-butanone saturated with water as the developer (22). The chromatograms were placed in the tank and the solvent was allowed to migrate until the solvent front had moved 32 cm from the origin where the sample was applied (5 hr). The developed chromatograms were allowed to air-dry and then sprayed with aniline hydrogen phthalate indicator (71, 119).

The papers were allowed to air-dry again for 15 min, heated in an oven at 105° for 5 min and viewed under ultraviolet light. R_f values were determined (R_f value is the distance the spot moved divided by the distance the solvent front moved, both measured from

the point of application of the material).

A second aliquot of the hydrolyzate of the methylated glucomannan was subjected to paper chromatography using n-butanol-ethanol-water (95:1:4 v/v/v) as developer (67). The solvent front was allowed to migrate 40 cm past the origin where the spot was applied (16 hr). The papers were sprayed with aniline hydrogen phthalate indicator. R_G values were determined by dividing the distance the sugars have moved from the starting line by the distance moved by 2, 3, 4, 6-tetra-O-methyl-D-glucopyranose.

A third aliquot of the hydrolyzate of the methylated glucomannan was subjected to paper chromatography using methyl ethyl ketone-water-ammonium hyrdoxide solution (30%) (100:50:3, v/v/v) (upper layer) as developer (82). The solvent front was allowed to migrate 48 cm past the origin where the spot was applied (5 hr). The papers were sprayed with aniline hydrogen phthalate indicator. R_G values were determined.

12. Gas-Liquid Chromatography and Mass Spectrometry of the Alditol Acetates of the Methylated Glucomannan

A part of the hydrolyzate containing the methylated sugars (85. 0 mg) was reduced in water (25. 0 ml) with sodium borohydride (0. 08 g) for 2 hr. After treatment with Dowex 50 (H^+) ion exchange resin the solution was concentrated to a syrup on a rotary evaporator, and

methanol (10. 0 ml) was added and re-evaporated. The addition and removal of methanol was repeated eight times. The resulting syrup was dried in an oven at 105° for 15 min to ensure complete removal of water. An aliquot (8. 0 ml) of a solution containing acetic anhydride and pyridine (1:1 v/v) was added to the dry solid and the mixture was heated for 10 min at 100°. After cooling for 5 min the acetylation mixture was poured with stirring into about 70. 0 ml of ice-water. The mixture was transferred to a separatory funnel and the alditol acetates were extracted with three successive amounts of methylene chloride (25 ml, 15 ml, 10 ml). The methylene chloride extract was concentrated to dryness on a rotary evaporator. Distilled water (1. 0 ml) was added to the residue and re-evaporated. The alditol acetates were dissolved in 1. 0 ml of methylene chloride. An aliquot (2 μl) of the solution was injected into the gas chromatograph. The peaks in the resulting spectra were identified by comparison of the relative retention times with that of authentic 2, 3, 4, 6-tetra-O-methyl-D-glucitol diacetate (19, 63).

The gas-chromatograph used was a Hewlett Packard 5751 B Research Chromatograph (Hewlett Packard Company, Palo Alto, California) equipped with dual flame ionization detectors. The conditions were: column, 6. 5% ECNSS-M on Gas Chrom Q 100/120 mesh, 6 ft x 1/8 in O. D. stainless steel; injection port 192°; detector 230°; column temperature 160° isothermal; helium flow 30 ml/min; range

setting 10^2; attenuation setting 32.

The methylated alditol acetates were also analyzed using a gas chromatograph-mass spectrometer (GC-MS) combination (20). The compounds were dissolved in methylene chloride. An aliquot (2 μl) was injected into an ECNSS-M column fitted in a Varian Aerograph Series 1200 gas chromatograph. The mass spectrometer used was a CH 7 Mass Spectrometer Varian Mat. The effluent from the chromatographic column was fed to a single stage jet separator which had a temperature of 255°. A heated glass line took the effluent from the separator to the ion source of the mass spectrometer. The connection between this line and the mass spectrometer was effected by means of a vacuum lock valve system which allowed the chromatograph to be connected or disconnected from the mass spectrometer. The point of detection for the chromatogram (strip chart recorder) and the spectrum from the mass spectrometer were the same in time, and therefore, it was possible to know exactly which position of the chromatograph peak on the recorder paper was scanned by the mass analyzer. From the spectrometric results the presence of one or more components could be identified in a certain peak. These results confirmed some of the identifications performed by other means.

The operational conditions for the gas chromatographic analysis were: injection port 270°; column temperature 160° isothermal;

detector 280°. The mass spectra were recorded at an inlet temperature of 255°, ionization potential of 70 ev, ionizing current, 300 uamp, and a temperature in the ion source of 150°.

I. Alkaline Degradation of the Glucomannan

1. Reaction in Sodium Hydroxide Solutions

A part (5 mg) of the glucomannan was placed in a volumetric flask (25 ml), which had previously been evacuated and flushed with nitrogen, and dissolved in sodium hydroxide solution of selected concentration. After the glucomannan had completely dissolved the solution was diluted to the mark with sodium hydroxide solution and shaken vigorously to attain complete mixing.

Glass ampules (5.0 ml) were evacuated and filled with nitrogen in a glove box. Aliquots (1 ml each) of the glucomannan solution were placed in the ampules. The ampules were taken out of the glove box, sealed with a flame and placed in an oil bath. The ampules were withdrawn from the constant temperature bath at definite time intervals (5, 10, 15, 20, 30, 60, 90 min and so on). Each withdrawn ampule was allowed to cool for 1 min and placed in ice water to stop the reaction. Hydrochloric acid (1.0 N, 1.0 ml) was added to the contents of the ampule to lower the alkalinity of the reaction mixture (92).

The degradations were carried out in sodium hydroxide solutions

ranging in concentration from 0.001 N to 3.16 N, at 64.5°, 78.5°, and 100°.

2. Phenol-Sulfuric Acid Method of Analysis of the Glucomannan

The analysis for the undegraded glucomannan was carried out by the phenol-sulfuric acid method of Dubois and co-workers (44). A Hitachi Perkin-Elmer Model 139 UV-Vis Spectrophotometer was used for this investigation.

Reagent grade sulfuric acid (95.5%, sp. gr. 1.84) conforming to ACS specifications was used. Phenol (80% by weight) was prepared by adding glass distilled water (20.0 g) to redistilled reagent grade phenol (80.0 g). This mixture formed a water-white liquid which was readily pipetted and has been known to stay the same color after one year of storage.

A fast delivery pipette (5.0 ml) was used to deliver the concentrated sulfuric acid. The pipette was prepared by cutting a portion off the tip of a standard 5.0 ml pipette.

Phenol (80.0% by weight, 0.05 ml) was added to an aliquot (0.8 ml) of the neutralized glucomannan solution in a test tube. Concentrated sulfuric acid (5.0 ml) was added rapidly. The stream of acid was directed against the liquid surface in order to obtain good mixing. Intense heat was generated at this time. The tubes were

allowed to stand for 10 min, then shaken and placed in a water bath at 25° for another 10 to 15 min. The test tubes were shaken again and samples were transferred to spectrophotometer cells (1.0 cm light path). Readings were taken at 490 nm. The color developed was stable for several hours. The weight of hydrolyzed hexoses was expressed as mannose equivalents by using absorbance in conjunction with a standard curve prepared from known mannose.

IV. RESULTS AND DISCUSSION

A. Collection of Bark Samples

A sample of inner bark free from outer bark and vascular cambium was desired because it provided a relatively homogeneous starting material. Outer bark contains considerable cork material which is rich in extractives (88) and these would interfere with the experimental studies of the carbohydrates. The vascular cambium contains proteins, ash, and various carbohydrates (84) and its chemical composition and physical properties are quite different from those of the inner bark. Therefore, the two are better separated and investigated separately.

The sample was collected in May because in the Spring of the year the outer bark of Douglas-fir is easily separated from the inner bark by simply chipping it off. The inner bark for the present work was taken from a freshly cut tree to reduce contamination from other sources. The vascular cambium was carefully separated from the inner bark and a relatively homogeneous sample resulted. The inner bark was immersed in ethanol-water to denature the enzymes and prevent possible enzymatic alteration of the natural materials from their native state (159, p. 27).

B. Sample Preparation

None of the polysaccharides occurs in pure form; hence, each must be carefully purified before its true characteristics may be determined. It is desirable, before proceeding with polysaccharide separation, to remove as much as possible of the low-molecular weight materials (simple sugars, organic acids, lipids, waxes, and so on) present in the bark. Some of these materials are readily oxidized and would interfere with the delignification reaction.

Chart 1 (page 53) outlines the scheme followed in the treatment of the inner bark and the isolation of the different fractions. In general this follows the scheme used by Chen (33, page 83). None of the extraction procedures shown in this chart accomplished a clear-cut separation of one type of material from another, but in general each step in the sequence performed a definite function.

The ethanol-water (4:1 v/v) extraction served two purposes. It denatured the enzymes, and it also solubilized the simple sugars. Chen (33, page 82) showed that the ethanol-water extract contained only a small amount of glucose as identified by paper chromatography.

The inner bark after the ethanol-water extraction in the present work was carefully ground, screened and reground to ensure that most of the fibers were separated and that the surface areas were exposed. All of the fine material was excluded because preliminary

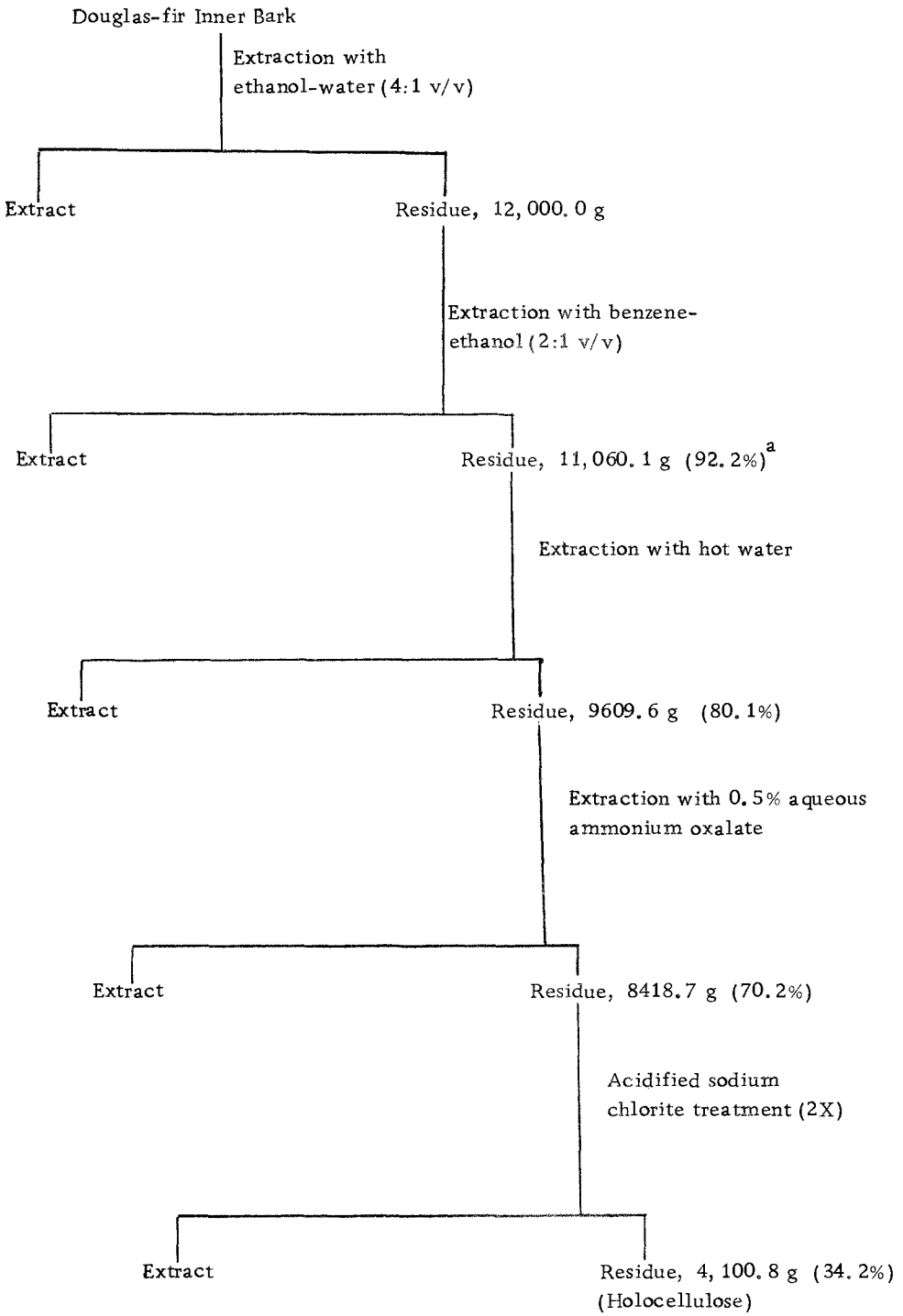


Chart 1. Isolation of the holocellulose fraction of Douglas-fir inner bark.

[a]Percentages are based on the dry weight of the residue from the ethanol-water extraction.

experiments showed that they plugged the extraction apparatus and filter systems and made experimentation difficult.

C. Benzene-Ethanol Extraction

The inner bark of Douglas-fir was known to contain appreciable quantities of lipids and waxes (86) and it was desirable to remove these prior to polysaccharide separation. Removal of the lipids and waxes not only eliminated them as sources of impurities but opened the tissue to penetration by hydrophilic solvents. Lipid extractives are not completely removed by hydrophobic solvents such as benzene or diethyl ether because these solvents are not able to penetrate all parts of the plant hydrophobic structure. Ethanol, a hydrophilic solvent, removes in addition to lipid material a portion of other constituents, such as some lignin and low molecular weight carbohydrates. A mixture of hydrophobic and hydrophilic solvents combines the advantages of each. Such a mixture provides complete penetration of the tissue but limits solvent action to non-lignin, non-carbohydrate material. The most widely used solvent mixture of this type contains ethanol and benzene in the azeotropic ratio of one to two (87; 159, p. 114). Therefore, a benzene-ethanol extraction was used in the present investigation.

D. Hot-Water Extraction

After the removal of the lipids and waxes, it was possible to carry out a hot water extraction and remove the water-soluble polysaccharides and varying amounts of non-carbohydrates.

The water-soluble components of Douglas-fir inner bark have been investigated by Zerrudo (169). Zerrudo showed that the hot-water soluble solids contained proteins, tannins, starch and hemicelluloses. He was able to remove the proteins, tannins, and the α-amylose portion of the starch by a series of enzyme hydrolyses followed by extensive dialysis. The polysaccharides which remained were composed of glucose, galactose, arabinose and traces of xylose, mannose and rhamnose. The glucose was considered to be amylopectin which did not hydrolyze with the α-amylase enzymes used to reduce the starch content. The galactose and arabinose residues were considered to exist in the inner bark as part of L-arabino-D-galactan polysaccharides. Water-soluble, highly branched L-arabino-D-galactans have been shown to be present in conifers (150, 162).

E. Ammonium Oxalate Extraction

Pectin and pectic substances are generally removed prior to hemicellulose separation. This is especially desirable in dealing with plant material containing large amounts of pectic substances. The

bark of trees is known to contain considerable amounts of pectic substances. Therefore, it was considered necessary to include an extraction procedure designed to remove the pectin from the sample of the inner bark of Douglas-fir.

Not all of the pectic material of plants is extractable with water. The insoluble part, protopectin (calcium pectate) was removed from the water-insoluble residue of Douglas-fir inner bark by extraction with a 0.5% ammonium oxalate solution (Chart 1, page 53). Presumably a cation exchange occurred in which insoluble calcium oxalate was formed along with soluble ammonium pectate. The latter was extracted with the filtrate.

F. Acidified Sodium Chlorite Delignification; Isolation of a Holocellulose Fraction

The isolation of pure polysaccharides from the wood and bark of gymnosperms is impossible without prior removal of lignin. Lignin retards or prevents the complete solution of hemicelluloses, either because of mechanical obstruction or perhaps through some type of chemical bonding mechanism. Furthermore, lignin is partially soluble in various alkaline solutions used for dissolving hemicelluloses, and, consequently, poses a purification problem in various subsequent steps designed to isolate pure polysaccharides. Therefore, it is necessary to remove this polymeric substance prior to

hemicellulose extraction. It has been shown by Meier (110) that even small amounts of residual lignin in a mixture of hemicelluloses is capable of impeding fractionation to an astonishing degree.

One of the greatest advances made in hemicellulose research has been the development of special pulping or delignification techniques in which complete or nearly complete lignin removal is attained with a minimum of removal and of physical and chemical change of the polysaccharides. The polysaccharide residue is termed "holocellulose." The various methods of holocellulose isolation have been reviewed by Chen (33).

The delignification reaction used in the present investigation of Douglas-fir inner bark was the acidified sodium chlorite method. The chlorine dioxide and chlorine gases generated during the acidified sodium chlorite reaction are toxic and the reaction should always be carried out in a well ventilated hood. The presence of these gases was indicated by yellow fumes which accumulated above the aqueous slurry as the reaction progressed. Nitrogen gas was bubbled through the mixture to carry these gases out of the reaction flask. Sodium chlorite itself is explosive in the presence of organic matter and should be kept away from material such as paper and rubber.

The holocellulose material remaining after complete delignification should, in theory, comprise the entire cellulose and hemicellulose portions of the bark. In practice, this goal is never

achieved and often not even approached. Delignification with acidified sodium chlorite reagent is known to dissolve polysaccharides (149) and so the solubilized solids were analyzed to determine the amount and nature of these dissolved polymers. Lai (90) investigated the solids dissolved in the first acidified sodium chlorite treatment of Douglas-fir inner bark. An acid hydrolyzate of the nondialyzable solids contained glucose, galactose, mannose, arabinose, xylose, and rhamnose in the ratio of 59.1:3.9:3.7:11.9:1.0:1.0 (90). These carbohydrates amounted to 56.5% of the total dissolved solids, or 12.4% of the original sample of the inner bark. These results are in agreement with Timell's (150) comment that delignification with acidified sodium chlorite reagent is known to dissolve polysaccharides. Timell (150) also commented that holocellulose isolation by any means can result in alterations of polysaccharide structures and that possible changes from the structures in the native state should be considered when investigating plant polymers.

The holocellulose isolated by the first treatment with sodium chlorite reagent was slightly yellow indicating incomplete delignification. A similar problem of delignifying barks was reported by Timell (145). A second treatment with acidified sodium chlorite reagent yielded a white holocellulose (Chart 1, page 53). The amount of holocellulose isolated in the present work was 34.2% of the residue remaining after the ethanol-water extraction. This compares

favorably with the amount of holocellulose isolated by Chen which was 36.1% (33, page 83).

Laver, Chen, Zerrudo, and Lai (95) characterized the holocellulose isolated from Douglas-fir inner bark. Table 1 shows a thorough compositional analysis of holocellulose as demonstrated by a total recovery of 89.0 percent. The loss of 11 percent was undoubtedly caused by acid-degradation during lignin and carbohydrate analyses. The presence of each monosaccharide and its correct configuration was definitely established by the preparation of crystalline derivatives (33, 95).

G. Fractionation of the Holocellulose Fraction into Its Component Polysaccharides

1. Impregnation with 2.0% Barium Hydroxide followed by Extraction with 10.0% Aqueous Potassium Hydroxide Solution

The study of the structure of bark hemicelluloses requires homogeneous polymers isolated in high yields with minimal modification. This is usually accomplished, as in the present work, by delignifying the bark and extracting the resulting holocellulose with aqueous alkali. Solubility differences can be exploited for the separation of individual hemicelluloses by the use of cations and solution concentrations. Thus, relatively dilute alkalies suffice to dissolve xylans and galactoglucomannans, but higher concentrations are required for

Table 1. Compositional analysis of holocellulose isolated from Douglas-fir inner bark. [a]

Component	Holocellulose (%)
Acid insoluble lignin	3.1
Acid-soluble lignin	4.1
L-arabinose	2.6
D-xylose	6.3
D-mannose	9.5
D-galactose	2.3
D-glucose	61.1
Total	89.0

[a] From: Laver, M. L., C.-H. Chen, Z. V. Zerrudo, and Y.-C Lai. Carbohydrates of the inner bark of Pseudotsuga menziesii. Phytochemistry 13(9):1891-1896. 1974.

the extraction of glucomannans (59, 149, 150). Potassium hydroxide is a better solvent for xylans than for glucomannans which are more effectively extracted with sodium or lithium hydroxide (59).

The procedure used in the present work to separate the polysaccharides which comprise the holocellulose from Douglas-fir inner bark is outlined in Chart 2 (page 62). The key feature was selective blocking of the dissolution of mannose-containing polysaccharides in the first extraction step. This was accomplished by impregnating the holocellulose with aqueous barium hydroxide (12). The impregnated holocellulose was then contacted with 10.0% aqueous potassium hydroxide which was known to be a good solvent for xylan-rich polysaccharides. The extract was neutralized and methanol was added to 70.0% concentration. The resulting precipitate was labelled "crude xylan." Crude xylan was recovered in 6.6% yield based on the starting holocellulose (Chart 2, page 62).

The xylan from Douglas-fir inner bark was investigated by Zerrudo (95, 169). The presence of xylose and arabinose in the xylan was demonstrated by paper chromatography and gas-liquid chromatography. Xylose was the major monosaccharide component. The presence of a uronic acid was suggested by a positive carbazole-sulfuric acid test, ash and acidity analyses, and by viscosity measurements in water and aqueous sodium hydroxide. The uronic acid moiety was identified as 4-O-methylglucuronic acid by mild acid

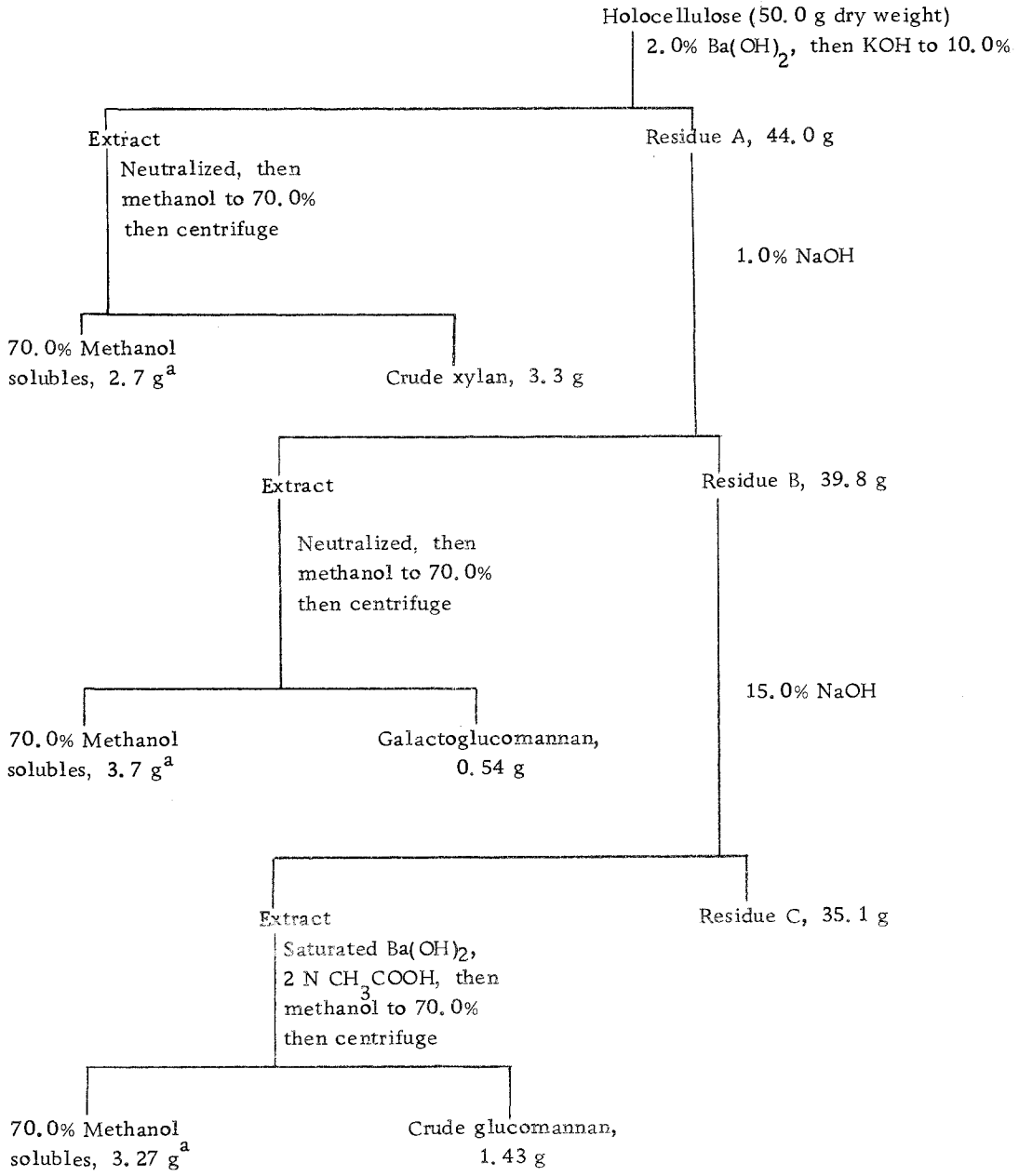


Chart 2. Fracticnation of the holocellulose from Douglas fir inner bark into its component hemicelluloses.

[a]Calculated by difference.

hydrolysis (0.3 N sulfuric acid), ion exchange resin (Dowex 50W-X4, H^+ form), and paper chromatography. A molecular weight of 1.8×10^4 and a degree of polymerization of 90 for the xylan were shown by end-group analysis and viscosity measurements. These data, in conjunction with periodate oxidation results and formic acid formation are consistent with a gross structure for the xylan consisting of a backbone of 90 xylose units attached by β-D (1→4) glycosidic linkages. The arabinose and 4-O-methylglucuronic acid moieties are considered to be branches (95, 169).

2. Extraction with 1.0% Aqueous Sodium Hydroxide Solution

Residue A (Chart 2, page 62) remaining from the 10.0% potassium hydroxide extraction was expected to contain most of the mannose-containing hemicelluloses because of the impregnation with barium hydroxide (12). After removal of the barium ions by dialysis, the residue was extracted with 1.0% aqueous sodium hydroxide which has been shown to be a good solvent for galactoglucomannan hemicelluloses (12).

The extract was neutralized and methanol was added to 70.0% concentration. The resulting precipitate was labelled "galactoglucomannan" according to the definition provided by Casebier and Hamilton (29). These workers consider galactoglucomannans to be those hemicelluloses which contain primarily glucose and mannose,

but also some galactose and which have been extracted from suitable sources with dilute alkali and are readily soluble in water. This definition is in contrast to their definition for glucomannans which can be obtained only by extraction with more concentrated sodium hydroxide solutions (9% or higher) and which are insoluble in water.

The galactoglucomannan in the present work was recovered in 1.1% yield based on the starting holocellulose (Chart 2, page 62). The monosaccharide composition of the galactoglucomannan from Douglas-fir inner bark was determined by Chen (33). Paper chromatography of an acid hydrolyzate of the galactoglucomannan showed strong spots for glucose and mannose, weak spots for galactose and xylose, and a trace spot for arabinose (33).

3. Extraction with 15.0% Aqueous Sodium Hydroxide Solution

The concentration of the sodium hydroxide extraction medium was increased to 15.0% sodium hydroxide because it has been shown (12) that polysaccharides rich in mannose residues become soluble in concentrated alkaline solutions. Saturated aqueous barium hydroxide was added to the combined filtrates to precipitate the glucomannan. The sodium hydroxide concentration was kept from dropping below 10.0% to prevent coprecipitation of any xylan which may have remained. The recovered precipitate was dissolved in 2 N aqueous acetic acid and recovered from the acidic medium after the addition

of three volumes of 70.0% aqueous methanol. The resulting precipitate was labelled "crude glucomannan." The crude glucomannan was recovered in 2.9% yield based on the original holocellulose (Chart 2, page 62). This hemicellulose fraction is the subject of a major portion of the present work.

4. Ash Determination of the Crude Glucomannan

The crude glucomannan had an ash content of 9.7%, as sulfate. The ash was probably composed mostly of barium sulfate since barium hydroxide had been used to prevent polysaccharides which contained mannose units from solubilizing in solutions of aqueous alkali (Chart 2, page 62). No further effort was made to analyze the ash.

5. Dialysis of the Crude Glucomannan

The ash content (9.7%) of the crude glucomannan was high and it was desirable that the amount of inorganic material in the glucomannan be reduced to a lower level before further investigations were performed. The barium ions were removed by dialysis. The resulting sample was labelled "glucomannan." The yield based on the moisture-free crude glucomannan was 90.8%.

H. Characterization of the Glucomannan

1. Ash Determination

The sulfated ash content of the glucomannan was 3.3% indicating that dialysis lowered the ash content of the original sample. The glucomannan obtained was pure enough for structural investigations.

2. Determination of Lignin

a. Acid-Insoluble Lignin (Klason Lignin). The separation of the carbohydrate materials from other plant substances, particularly lignin, is difficult and results in a choice between two possibilities. Most of the polymeric phenols can be removed by strong oxidation reactions but in such a reaction the degradation of the desired carbohydrates is extensive. However, when mild oxidative conditions are used, as in the present work, considerable polyphenolic substances are not separated from the carbohydrate fraction.

It was thus necessary to analyze for these polymeric phenols in order to have a complete knowledge of the glucomannan fraction. The usual analysis is the "Klason lignin" determination (142). The glucomannan isolated from Douglas-fir inner bark was found to contain 1.05% Klason lignin. The glucomannan fraction seemed to be quite free of lignin-like molecules as indicated by the low Klason lignin content.

b. Acid-Soluble Lignin. The digestion of the glucomannan with 72% sulfuric acid resulted in the solubilization of some lignin-like substances which were not measured as "Klason lignin." These materials are referred to by the term, "acid-soluble lignin" and they had to be determined to obtain a complete analysis of the glucomannan.

These materials were best analyzed by ultraviolet absorption. Lignin-like compounds strongly absorb energy at 280 nm and this band has been used for the quantitative determination of acid-soluble lignins. However, the formation of 5-hydroxymethylfurfural from hexoses and furfural from pentoses during the refluxing step in the Klason lignin determination was found to interfere with this determination. Browning and Bublitz (26) showed that interference by these compounds could be minimized by determining the absorbance of the filtrate at two wavelengths.

Using absorptivity values for lignin which were measured on Braun's spruce lignin and for the carbohydrate degradation products measured on synthetic mixtures of glucose, xylose, mannose, and glucuronolactone which had been subjected to the hydrolysis conditions of the lignin determination, Browning and Bublitz (26) were able to write the following equations:

$$A_{280} = 0.68\ C_D + 18\ C_L$$

$$A_{215} = 0.15\ C_D + 70\ C_L$$

where A_{280} and A_{215} were the absorbance values of the lignin

filtrate, 0.68 and 0.15 the absorptivities of carbohydrate degradation products, 18 and 70 the absorptivities of lignin at 280 and 215 nm, respectively, and C_D and C_L the concentrations in grams/liter of carbohydrate degradation products and of soluble lignin in the filtrate. Goldschmid (51) showed that by solving the simultaneous equations, the following expression for the soluble lignin concentration in the filtrate could be obtained:

$$C_L = \frac{4.53\ A_{215} - A_{280}}{300}$$

In the present work the acid-soluble lignin content of the glucomannan isolated from Douglas-fir inner bark was determined by scanning the filtrate from the Klason lignin determination (section III-H-2-a, page 30) over the wavelength range from 320 to 200 nm (Figure 1, page 69). Absorptivities were measured at 280 nm and 215 nm according to the procedure of Goldschmid (51).

The absorbances at 280 nm and 215 nm were 0.375 and 0.654 respectively. These values were used to calculate the acid-soluble lignin as follows:

$$C_L = \frac{4.53\ A_{215} - A_{280}}{300}\ g/l$$

$$= \frac{4.53 \times 0.655 - 0.375}{300}\ g/l$$

$$= 0.042\ g/l$$

Using this figure in conjunction with the filtrate volume (1.008 l)

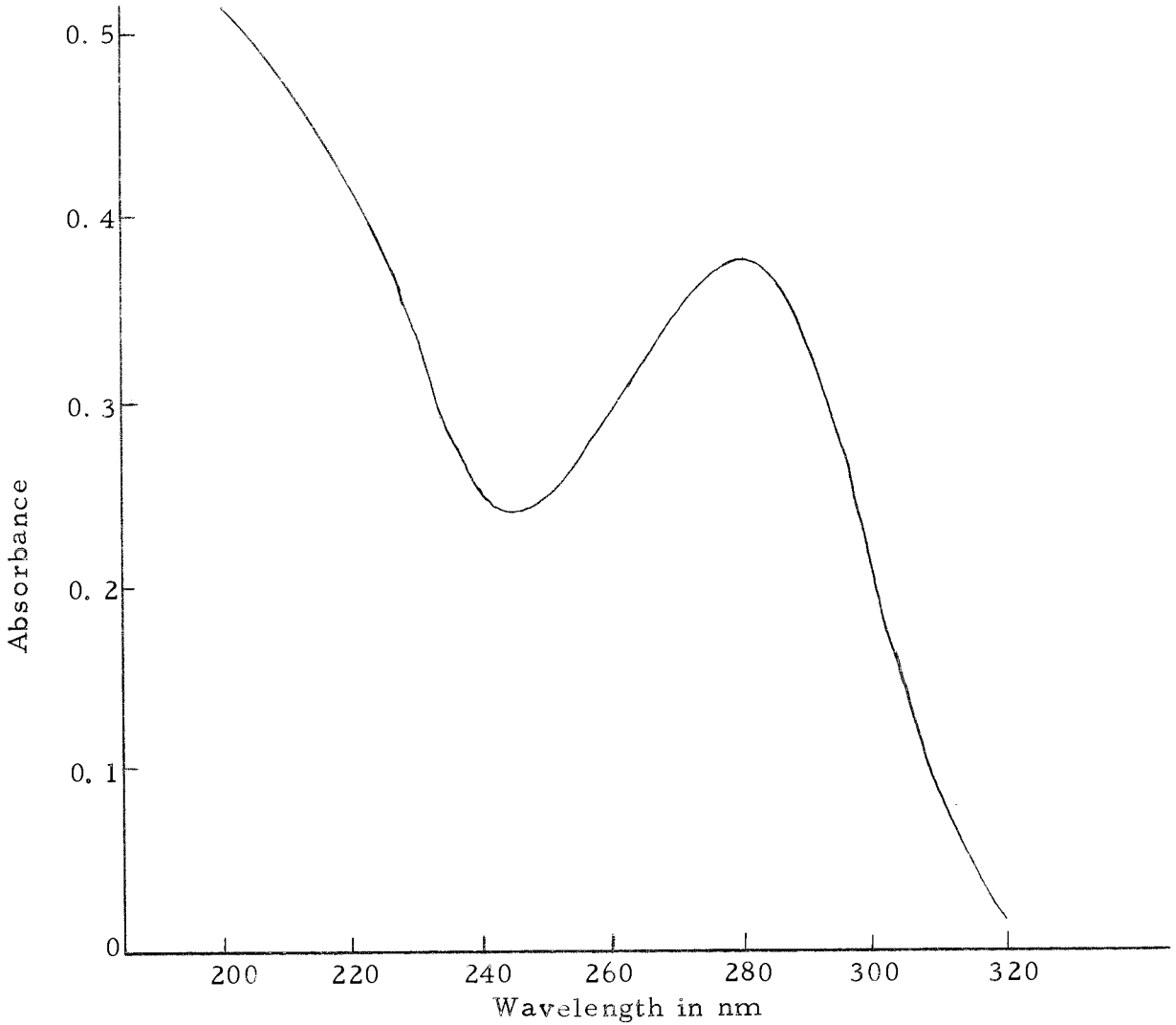


Figure 1. Ultraviolet spectrum of the acid-soluble lignin from the glucomannan.

and the dry weight of the sample (0.45050 g) (section III-H-2-a, page 30), the value of 0.94% acid-soluble lignin in the glucomannan was calculated. Thus the total lignin content was 1.05% (acid-insoluble) plus 0.94% (acid-soluble) to equal 1.99%.

3. Optical Rotation

The glucomannan sample was completely soluble in 10.0% aqueous sodium hydroxide and so optical rotation readings were taken in this medium. The glucomannan was found to have a specific rotation of -35.9°. The negative rotation of the polymer indicated that the glycosidic linkages were β-D (167). The rotation of -35.9° is close to that of -34° reported by Timell (147) for a glucomannan from the bark of Amabilis fir (Abies amabilis). Timell (150) also reported an average specific rotation in aqueous alkali of -38° ±2° for almost all of the glucomannans so far isolated from gymnosperm woods. Therefore, the present glucomannan from Douglas-fir bark may be similar in structure.

4. Hydrolysis with 77.0% Sulfuric Acid

A fundamental step in determining the structures of polysaccharides is to determine their monosaccharide components. This is achieved through hydrolysis of the polysaccharide material. Often polysaccharides contain linkages which are resistant to acid cleavage.

Thus, it is important to perform the hydrolysis with complete dissolution of the polysaccharides and with minimum decomposition of the sugars.

The glucomannan from Douglas-fir inner bark dissolved completely in 77.0% sulfuric acid. It was necessary to add water slowly to the solution with vigorous stirring to prevent local heating which might have caused degradation of the sugars. After dilution to 3.0% sulfuric acid concentration, the solution was refluxed to bring about hydrolysis.

The acid solution was neutralized with aqueous barium hydroxide resulting in a heavy precipitate of barium sulfate. This method of neutralization was preferred because the pH can be controlled easily. A final pH of about 5.0 was desired because monosaccharide solutions should not be allowed to become alkaline. The action of alkali on monosaccharides follows three general courses (94): fragmentation, isomerization, and internal oxidation and reduction. Such reactions interfere with the qualitative and quantitative results of monosaccharide analyses and considerable care was taken to avoid them.

5. Qualitative Uronic Acid Analysis by Color Reaction

Hexuronic acids react with carbazole to form 5-carboxy-2-formylfuran which, when treated with concentrated sulfuric acid, yields a colored product with an absorption maximum at 535 nm.

The carbazole-sulfuric acid color reaction is said to be reasonably specific for hexuronic acids (43). This was verified by testing monosaccharides, oxalic acid, and benzoic acid. These compounds showed no reactions indicating that the simple sugars and carboxylic acid functions other than those in uronic acids, did not interfere with the color reaction.

The 3.0% sulfuric acid hydrolyzate of the glucomannan showed no color development, indicating the absence of a uronic acid moiety. The reaction showed no absorption in the visible region at 535 ± 1 nm. This was interpreted to mean that the glucomannan did not possess a uronic acid portion.

6. Qualitative Carbohydrate Analysis by Paper Chromatography

The identification of the monosaccharides released from polysaccharides on acid hydrolysis is fundamental to an understanding of these polymers. Paper chromatography has become the standard method of tentatively identifying the monosaccharides. Several solvent systems are well established and the identification of the individual sugars can usually be achieved with relative ease.

The paper chromatogram (Figure 2, page 73) of the glucomannan hydrolyzate showed a very strong spot for mannose, a medium spot for glucose, a weak spot for galactose and trace spots of xylose and arabinose.

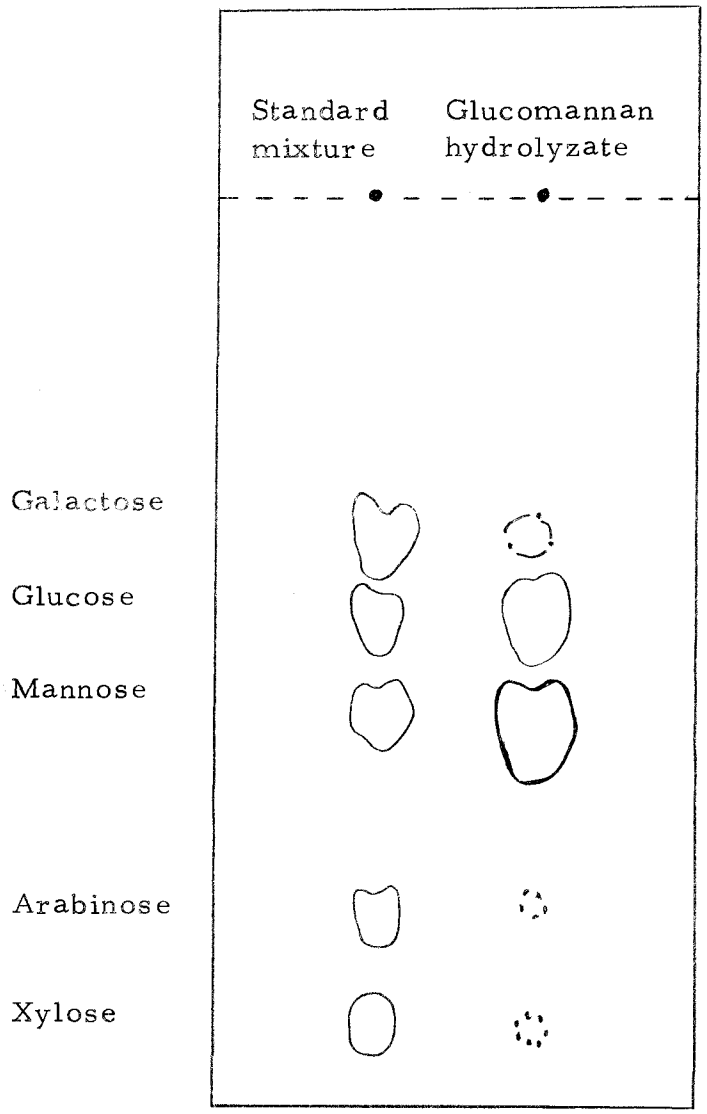


Figure 2. Paper chromatogram of the acid hydrolyzate of the glucomannan.

Solvent: ethyl acetate-pyridine-water (8:2:1 v/v/v).

represents a very strong spot

represents a medium spot

represents a weak spot

represents a trace spot

The results indicate that all of the xylan hemicellulose had been extracted by the 10.0% potassium hydroxide and the 1.0% sodium hydroxide treatments. This is in agreement with the results reported by Beélik, Conca, Hamilton and Partlow (12) for hemicelluloses from softwoods.

7. Quantitative Carbohydrate Analysis by Gas-liquid Chromatography

Carbohydrates are neither heat resistant nor volatile and so derivatives must be prepared which will volatilize without degradation in order to perform gas-liquid chromatographic analysis. There are numerous derivatives which have been tried but the ones most commonly used today are the "alditol acetates." In the preparation of the alditol acetates the monosaccharides are first treated with sodium borohydride to reduce the aldehyde function to the alcohol function (Section III-H-7, page 35). This has the result of preventing ring isomerization to the pyranose and furanose forms and so prevents the formation of alpha and beta forms of the sugars. The end result is only one form, the alditol, for each of the monosaccharide sugars. The addition and evaporation of methanol was important because Crowell and Burnett (41) had shown that the boric acid released in the reduction reaction retarded acetylation of the alditols. Albersheim, Nevins, English and Karr (2) showed that the boric acid

level could be reduced to a convenient level by a number of additions and re-evaporations of methanol. The boric acid was presumably converted to volatile methyl borate which was evaporated from the syrup. The removal of boric acid is important otherwise the acetylation reaction does not proceed. Acetylation and the gas-liquid chromatographic analysis was accomplished as outlined in section III-H-7 (page 35).

The gas-liquid chromatographic spectrum of the alditol acetates from the acid hydrolyzate of glucomannan is shown in Figure 3 (page 76). The major peak was that of mannitol hexaacetate followed by glucitol hexaacetate and galactitol hexaacetate. The myo-inositol peak shown at the end of the spectrum resulted from the addition of an accurately measured amount of myo-inositol as an internal standard. The areas under the peaks of the other sugars in the spectrum are compared to the area under the peak of myo-inositol for quantitative analysis. The areas under the peaks as measured by a planimeter were: mannitol hexaacetate, 382; glucitol hexaacetate, 170; galactitol hexaacetate, 53; xylitol pentaacetate, 11; arabinitol pentaacetate, 10; myo-inositol hexaacetate, 186. The percentages of anhydromonosaccharide residues were calculated using the following formula (23);

$$\% \text{ anhydromonosaccharide residue} = \frac{C \times I \times F \times 100}{R \times S \times H \times K}$$

where

C = chromatographic area of the component alditol acetate peak

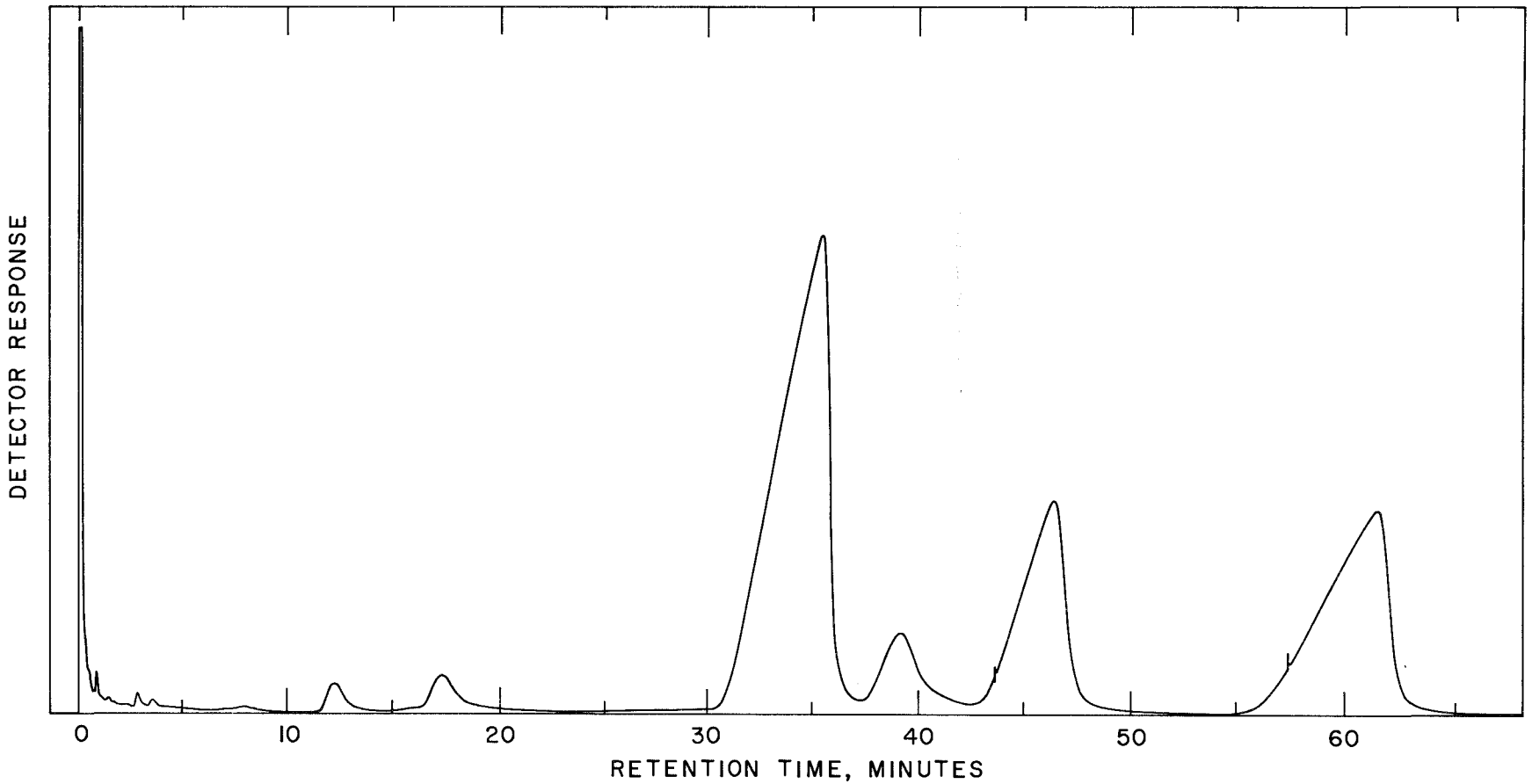


Figure 3. Gas-liquid chromatographic spectrum of the alditol acetates prepared from the acid hydrolyzate of the glucomannan. Peak "a" is from arabinose, "b" is from xylose, "c" is from mannose, "d" is from galactose, "e" is from glucose, "f" is from myo-inositol. Conditions: column, 6.5% ECNSS-M on Gas chrom Q 100/120 mesh, 6 ft x 1/8 in O.D. stainless steel; injection port 190°; detector 240°; column temperature 170° isothermal; helium flow 30 ml/min; range setting 10^2; attenuation setting 8.

R = chromatographic area of the myo-inositol hexaacetate peak

I = weight of the myo-inositol originally added, in grams

S = dry weight of the original glucomannan sample, in grams

F = factor to convert the weights of monosaccharide to anhydromonosaccharide residues (0.88 pentose) (0.90 hexose).

H = hydrolysis survival factor as used by Chen (33, page 126).

K = "Instrument k Factor" (33, page 56).

The results were: anhydromannose residues, 56.43%; anhydroglucose residues, 26.20%; anhydrogalactose residues, 7.67%; anhydroxylose residues, 1.46%; anhydroarabinose residues, 1.44%; total recovery of carbohydrates, 93.20%. The sum of the carbohydrates (93.20%), ash (3.27%), and lignin (1.99%) represents a total analysis of 98.46% of the glucomannan isolated.

The ratio of anhydrosugars in the glucomannan was therefore: anhydromannose, 2.15; anhydroglucose, 1.00; anhydrogalactose, 0.29; anhydroxylose, 0.06; and anhydroarabinose, 0.05.

Hemicelluloses extracted from gymnosperms with concentrated aqueous sodium hydroxide (15-18%) are usually glucomannans which almost always contain some galactose residues (12, 150). For example Bishop and Cooper (18) isolated a glucomannan from jack pine which contained D-mannose, D-glucose and D-galactose in the mole ratio of 49:17:2. Schwartz and Timell (132) isolated a glucomannan from amabilis fir which had a mole ratio of galactose to

glucose to mannose of 0. 1:1:3 and more recently Hardwood (63) isolated a glucomannan from Pinus radiata with a mole ratio of galactose to glucose to mannose of 0. 1:1. 0:3. 7.

Direct proof of a chemical linkage between D-galactose residues and D-mannose or D-glucose residues in alkali-soluble glucomannans is still lacking, since no oligosaccharides containing D-galactose units have thus far been obtained on partial hydrolysis. However, the fact that almost all glucomannans so far isolated have been found to contain residues of D-galactose, D-glucose, and D-mannose in a fairly constant ratio of 0. 1-0. 2:1:3, strongly suggests that the D-galactose residues are integral parts of all softwood glucomannans. The common failure to remove these residues by further fractionation also points in this direction. Reprecipitation (section III-G-3, page 28) did not remove the galactose moieties in the present work. If not attached to the glucomannan, the residues must, accordingly, originate from a galactoglucan, or more likely, a galactomannan. These polysaccharides should be water-soluble and thus easy to separate from the alkali-soluble glucomannan. However, repeated washings and precipitation did not remove the galactose moieties.

8. Reducing End-Group Analysis (Somogyi Method)

a. Standardization of Sodium Thiosulfate Solution. There are several modifications of the standard Fehling's copper reduction test

for the presence of reducing sugars. However, one of the more common methods for the quantitative analysis of reducing sugars is the Somogyi procedure (68, 138). This method is based on the ability of certain sugars (reducing sugars) to act as reducing agents. The sugars reacts with Cu^{++} in the aqueous alkaline medium to produce cuprous oxide. The cuprous oxide is oxidized by iodine back to Cu^{++} and the excess iodine is titrated with thiosulfate. The reactions are:

$$RCHO + 2\,Cu^{++} + 2\,Cu^{++} + 5OH^{-} \rightarrow RCO_2^{-} + Cu_2O + 3H_2O$$

sugar

$$IO_3^{-} + 5I^{-} + 6H^{+} \rightarrow 3\,I_2 + 3H_2O$$

$$Cu_2O + 2H^{+} + I_2 \rightarrow 2\,Cu^{++} + 2I^{-} + H_2O$$

$$I_2 + 2S_2O_3^{--} \rightarrow 2I^{-} + S_4O_6^{--}$$

This method has been widely and successfully applied on both milligram and microgram quantities, both titrimetrically and colorimetrically. Its accuracy over a wide range of sugar concentrations, the ease and rapidity of operation, and its proven reliability place it above other microoxidation methods (68).

As evidenced from the above equations, thiosulfate anion is used as the standard titer to measure the excess iodine concentration. In the present work the sodium thiosulfate solution was standardized by titration to a starch end-point of an accurately diluted solution of potassium iodate which had been prepared from recrystallized and dried potassium iodate solids.

b. Preparation of Somogyi Copper Reagent. Several different alkaline copper reagents have been recommended for use in conjunction with the iodimetric determination of reduced copper. The most common ones are Somogyi's 1945 (138) reagent and his 1952 reagent. Somogyi's 1945 phosphate-buffered reagent possesses an advantage over the 1952 carbonate-buffered reagent, in that amyloses are held in solution in the 1945 reagent but are precipitated from the 1952 reagent. Therefore, the 1945 reagent has become the standard procedure and was the method of choice in the present work. The procedure for preparing the reagent is given in section III-H-8-b (page 37). The amount of potassium iodate added to the reagent determines the amount of iodine released and hence the amount of sodium thiosulfate titer needed. Therefore some preliminary titrations are often required to determine the best amount of potassium iodate to add. When performed properly the method is very good for determining the overall reducing power of a solution and the precision is 0.01 mg for D-glucose or about ±2%, averaged throughout the range 0.3 to 3.0 mg of glucose.

c. Determination of Total Reducing End-Groups in the Glucomannan. The curve for the reducing power of mannose/glucose is shown in Figure 4 (page 81). The total weight of the mannose/glucose ratio is plotted on the abscissa. The titer of thiosulfate (0.0056 N) for the mannose/glucose solution subtracted from the titer for the

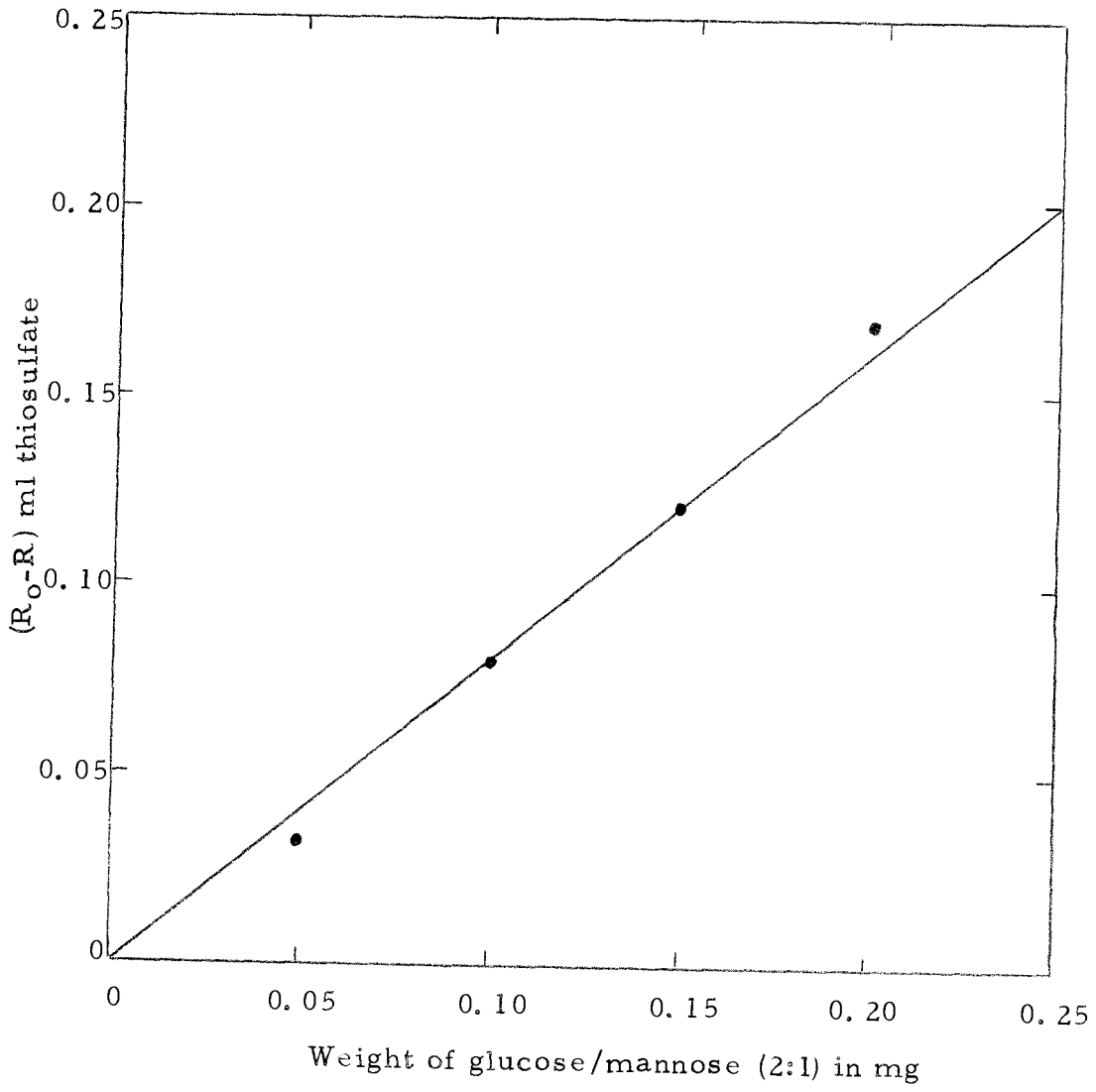


Figure 4. Standard curve for Somogyi titration of mannose/glucose (2/1).

R_o = volume thiosulfate (0. 0056 N) used to titrate blank.

R = volume thiosulfate (0. 0056 N) used to titrate reaction mixture.

blank solution is located on the ordinate. The difference in titer for the blank and the glucomannan is projected to the mannose/glucose curve and the equivalent of reducing mannose/glucose is obtained on the abscissa.

The results of the present Somogyi analysis (triplicate samples) showed an average reducing power of 0.09 mg of mannose/glucose equivalents for a sample weight of 7.95 mg of starting glucomannan on a lignin free, ash free basis.

The calculation of the molecular weight of the glucomannan from the reducing-end-group analysis is as follows:

Let A = reducing end group of glucomannan in terms of mg mannose/glucose = 0.09 mg

W = weight of glucomannan in sample = 7.95 mg

$(MW)_{GMR}$ = molecular weight of the reducing-end group of the glucomannan = 179 mg

$(MW)_{GM}$ = molecular weight of the glucomannan

M_A = moles reducing-end group

$$M_A = \frac{A}{(MW)_{GMR}} = \frac{0.09 \text{ mg}}{179 \text{ mg}}$$

$$\frac{W}{(MW)_{GM}} = M_A$$

$$(MW)_{GM} = \frac{7.95 \text{ mg}}{\frac{0.09 \text{ mg}}{179 \text{ mg}}} = \frac{7.95 \times 179}{0.09} \text{ mg}$$

$$(MW)_{GM} = 1.58 \times 10^4 \text{ mg}$$

According to this end-group analysis, the glucomannan isolated

from Douglas-fir bark has an average molecular weight of 1.58 X 10^4 mg. From this molecular weight and the analysis by gas-liquid chromatography a degree of polymerization (DP) for the glucomannan can be calculated.

The ratio of sugar residues by gas-liquid chromatography is anhydromannose:anhydroglucose (2.15:1.00). The molecular weight of anhydromannose is 162 mg. The molecular weight of anhydroglucose is 162 mg. Therefore, the molecular weight of an average repeating block of anhydromonosaccharides is (2.15 x 162 mg) + (1.00 x 162 mg) = 510.3 mg. Therefore, the number of repeating blocks in any given molecular weight of 1.58 X 10^4 mg is 1.58 X 10^4 mg divided by 510.3 mg = 31.00. These data are interpreted to mean that there are 31 repeating blocks of anhydromonosaccharides in any given glucomannan molecule.

If the glucomannan from the inner bark of Douglas-fir is similar in structure to the glucomannan so far isolated from the wood and bark of gymnosperms, then its gross framework consists of a backbone of repeating anhydromannose and anhydroglucose units (18, 63, 132, 147). Since the number of repeating blocks in each glucomannan molecule is 31 and there are two anhydromannose and one anhydroglucose moieties in each block (ratio is analyzed to be 2:1) then the DP of the anhydromannose and anhydroglucose backbone is 93.

9. Viscosity Measurements

The calculations of the viscosity data were made as follows:

$$\eta = K\rho t$$

$$\eta_r = \eta/\eta_o$$

$$\eta_{sp} = (\eta - \eta_o)/\eta_o = \eta_r - 1$$

η_{sp}/C = reduced viscosity

$$[\eta] = \lim_{C \to O} (\eta_{sp}/C)$$

where

η = viscosity in centipoise (cp)

η_o = viscosity in pure solvent

η_r = relative viscosity

η_{sp} = specific viscosity

$[\eta]$ = intrinsic viscosity in deciliters/gram (dl/g)

K = viscometer constant

ρ = density at 25.0° ± 0.01.

t = time in seconds for solution to flow through the viscometer

C = concentration in g/dl

The data for the viscosity measurements are shown in Table 2 (see page 85).

The plot of the reduced viscosities against the concentrations is shown in Figure 5 (page 86). The value for the intrinsic viscosity was obtained by extrapolating the plots to zero concentration. This was

Table 2. Viscosity measurements in cadoxen.

Conc. (g/dl)	Density (g/ml)	Time[a] (secs)	η[b] (cp)	η_r	η_{sp}	η_{sp}/C (dl/g
0.400	1.04390	249.0	2.08826	1.19793	0.19793	0.4948
0.350	1.04332	243.8	2.04328	1.17213	0.17213	0.4918
0.308	1.04318	239.2	2.00412	1.14966	0.14966	0.4859
0.250	1.04274	233.0	1.95194	1.11973	0.11973	0.4789
0.200	1.04234	228.1	1.90991	1.09562	0.09562	0.4781
0.182	1.04220	225.6	1.88872	1.08346	0.08346	0.4586
0	1.04080	208.5	1.74322			

[a] Average of triplicate determinations.

[b] η calculated from the expression $\eta = K\rho t$ where $K = 0.008033$, a viscometer constant determined from the known viscosity and density of water at $25 \pm 0.1°$.

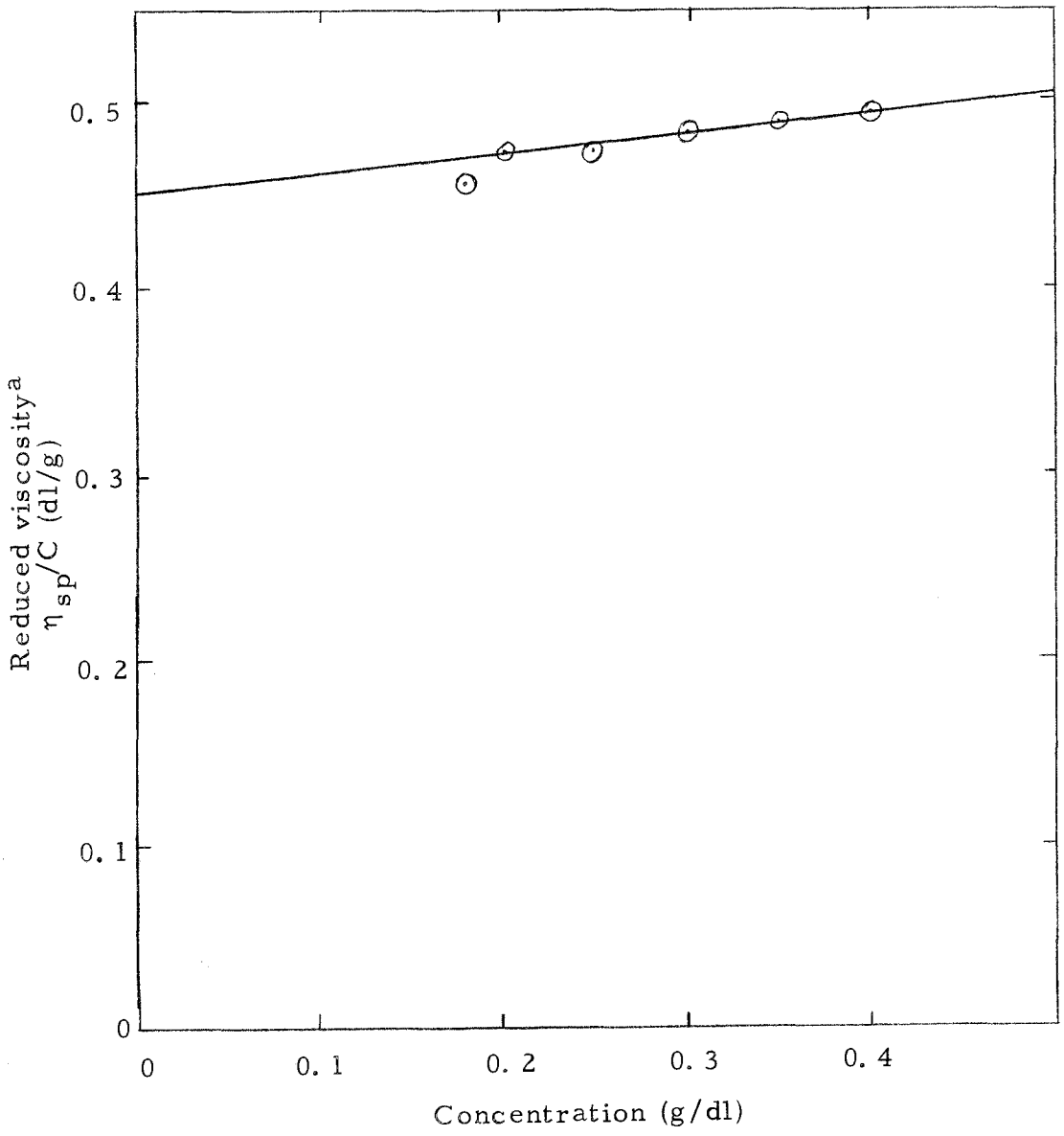


Figure 5. Relationship between the reduced viscosity and concentration in Cadoxen solution of glucomannan from Douglas-fir inner bark at 25. 0° ±0. 1°.

[a]Values are average of three replications.

determined to be 0.46 dl/g.

The degree of polymerization (DP) of glucomannan was determined using the following relationship (27, p. 529):

$$DP_n = K[\eta]$$

where DP_n = number average degree of polymerization

$[\eta]$ = intrinsic viscosity in dl/g

K = a constant

The value of the constant K has been found to be 200 for glucomannans in cadoxen (111). K was determined by measuring the viscosity of the samples of which the DP_n was known from osmometrical measurements. Using the above relationship with a value of 200 for the constant and the measured value of 0.46 dl/g for the intrinsic viscosity, a number average DP of 92 is obtained for the glucomannan isolated from the inner bark of Douglas-fir.

The DP_n of 92 by viscosity measurements is close to the DP of 93 obtained by the completely independent and quite different reducing end-group method discussed in section IV-H-8 (page 78). A degree of polymerization of 92 for the anhydromannose-anhydroglucose backbone in the glucomannan isolated from Douglas-fir inner bark is used in subsequent calculations and structural determinations.

10. Methylation of the Glucomannan

Methylation of a polysaccharide provides information about the

position of the glycosidic bonds which unite the anhydromonosaccharide residues in the polymer. The usual procedure is to form methyl ethers on all of the free hydroxyl groups. These ethers are resistant to the acid hydrolysis conditions ordinarily applied and so hydrolysis of the completely methylated polysaccharide frees only the hydroxyl groups involved in the glycosidic linkages. Location of the positions of the freed hydroxyl groups determines the point of attachment to the glycosidic bond in the original polymer.

It is important that all of the hydroxyl groups in the original polysaccharide be methylated. The methylation reaction is usually analyzed by infrared spectroscopy because the hydroxyl groups absorb readily and their disappearance as methylation progresses can be followed.

The initial methylation of polysaccharides is usually accomplished by treatment with dimethyl sulfate and a strong solution of sodium hydroxide (Haworth method), followed by methylation with methyl iodide and silver oxide. These methods remained unchanged for many years until certain aprotic solvents became commercially available. The first major advance was the use by Kuhn and associates (85) of N, N-dimethylformamide or dimethyl sulfoxide (DMSO) (Me_2SO) as solvent in conjunction with methyl iodide or dimethyl sulfate, and silver oxide or strontium oxide.

Although reaction in N, N-dimethylformamide greatly facilitates

methylation, the method suffers from the limitation that it often gives low recoveries of methylated polysaccharides and the effectiveness of the methylation appears to depend on the volume of N,N-dimethylformamide used (96). Even with monosaccharides incomplete methylation has been reported (46).

Srivastava and co-workers published a method of methylation in dimethyl sulfoxide with barium oxide and methyl iodide (139) but they found that better yields were obtained by using dimethyl sulfate and powdered sodium hydroxide (140).

The most important recent development in methylation techniques is due to Hakomori (58) who used as the base methylsulfinyl carbanion, formed by reaction of dimethyl sulfoxide with sodium hydride. The methylsulfinyl carbanion is used to generate the alkoxide prior to the addition of methyl iodide. The reactions are:

1) $CH_3\text{-}SO\text{-}CH_3 \xrightarrow[65\text{-}70^\circ]{NaH} CH_3\text{-}SO\text{-}CH_2^-Na^+$

Methylsulfinyl carbanion

2) $R\text{-}O\text{-}H + CH_3\text{-}SO\text{-}CH_2^-Na^+ \longrightarrow R\text{-}O^-Na^+ + CH_3\text{-}SO\text{-}CH_3$

sugar

3) $R\text{-}O^-Na^+ + CH_3I \longrightarrow R\text{-}O\text{-}CH_3 + NaI$

The Hakomori method has, within a short time, been applied to polysaccharides of many different types. There are a few reports where the method was found inapplicable due to insolubility of the polysaccharide in the solvent. It is probable that this insolubility

was attributable to incomplete de-ionization of the sample, a situation that may be encountered with polysaccharides containing acid groups (for example glucuronoxylans) or where inorganic complexes have been used in the isolation procedure (for example, glucomannan precipitated by barium hydroxide). In the present work, the glucomannan from Douglas-fir inner bark was found to be quite insoluble in dimethyl sulfoxide so it was methylated first by the Haworth method. When a Hakomori methylation has been preceded by Haworth methylation in order to obtain solubility in dimethyl sulfoxide, the procedure has been called the Unrau method (155).

An interesting paper by Handa and Montgomery (61) compared the results of partially methylating methyl-α-D-mannopyranoside by the methods of Haworth, Kuhn, and Hakomori. Considerable difference was found in the reactivity of the various hydroxyl groups according to the method used. Thus, the hydroxyl group at carbon-6 was found the most reactive under Haworth conditions, the least reactive with the Kuhn reagents, and intermediate in the Hakomori method. These results suggest the desirability of using various methylation techniques when difficulty is encountered in obtaining a fully methylated polysaccharide.

In the present work with the glucomannan from Douglas-fir inner bark, methylation proved difficult (section III, H-10, page 40), so the glucomannan was methylated by a number of methylation techniques.

After the eighth methylation, the methylated glucomannan was purified by fractional precipitation and the product in chloroform was poured into ten volumes of petroleum ether (boiling point 30-60°). The solution yielded 248. 7 mg (17. 7% of the starting material) of the soluble methylated glucomannan which was recovered as a white powder. However, after repeated methylations, the infrared spectrum of the methylated glucomannan still showed an absorption for hydroxyl groups in the usual regions of 3800-3300 cm^{-1} as shown in Figure 6 (page 92). Many authors consider that methylation is complete when the infrared spectrum of the product ceases to show absorption for hydroxyl group, but the importance of performing methoxyl determinations is illustrated by the work of Lee and Ballou (96). The methoxyl content of the methylated glucomannan was determined and was found to be 45. 3% on a lignin free, ash free basis (calculated methoxyl for a hexosan polysaccharide is 45. 6%). This value is comparable with the methoxyl content of methylated glucomannans reported in the literature (6, 40, 63, 82). Therefore, the methylation was considered complete.

11. Hydrolysis of the Methylated Glucomannan

Formic acid has been shown to be a good medium for the hydrolysis of mannans (167) and was used in the present work. The initial formic acid treatment was followed by hydrolysis with dilute

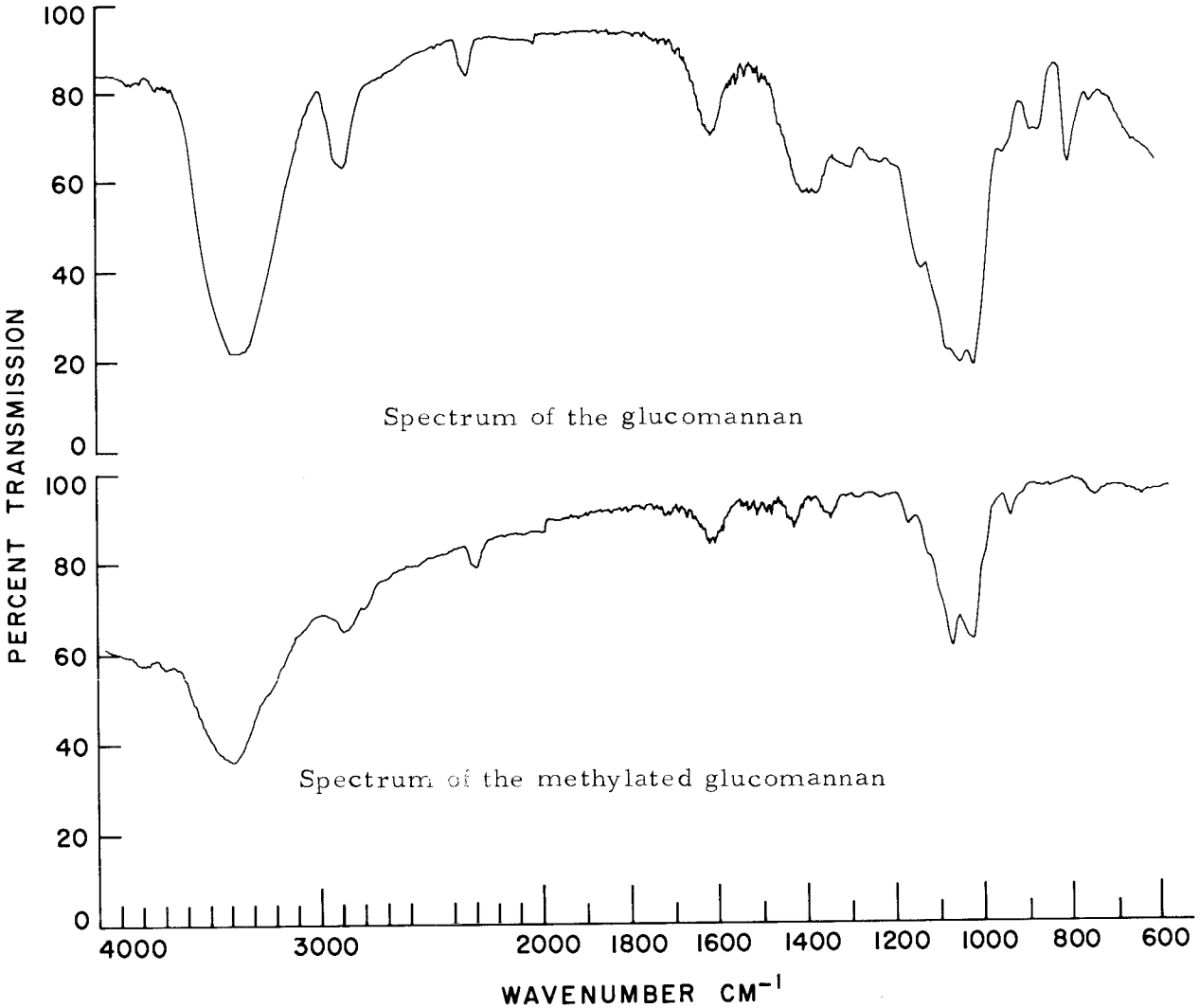


Figure 6. Infrared spectra of the glucomannan and the methylated glucomannan.

sulfuric acid to hydrolyze any formate esters which might have been formed (167).

Paper chromatography of the hydrolyzate syrup in two different solvent systems showed R_f and R_G values consistent with the presence of 2, 3, 6-tri-O-methyl-D-mannopyranose. There were no dimethyl sugars that could be detected in the paper chromatograms of the hydrolyzate syrup in two different solvent systems as shown in Figure 7 (page 94) and Figure 8 (page 95). These results suggest that the glucomannan was fully methylated. The first two solvent systems used in the present work did not fully separate the 2, 3, 6-tri-O-methyl-D-mannopyranose and 2, 3, 6-tri-O-methyl-D-glucopyranose. However, these methylated sugars were separated using the third solvent system (Figure 9, page 96). Trace amounts of slow-moving sugars were also detected. Tetra-O-methyl sugars were not detected by paper chromatography. To confirm the presence of these compounds, the hydrolyzate containing the methylated sugars was reduced with sodium borohydride and subjected to acetylation and the composition was determined by gas-liquid chromatography and mass spectrometry.

12. Gas-Liquid Chromatography and Mass Spectrometry of the Alditol Acetates of the Methylated Glucomannan

Gas-liquid chromatography (GLC) of carbohydrate derivatives is a well proven and accepted technique for both the qualitative and

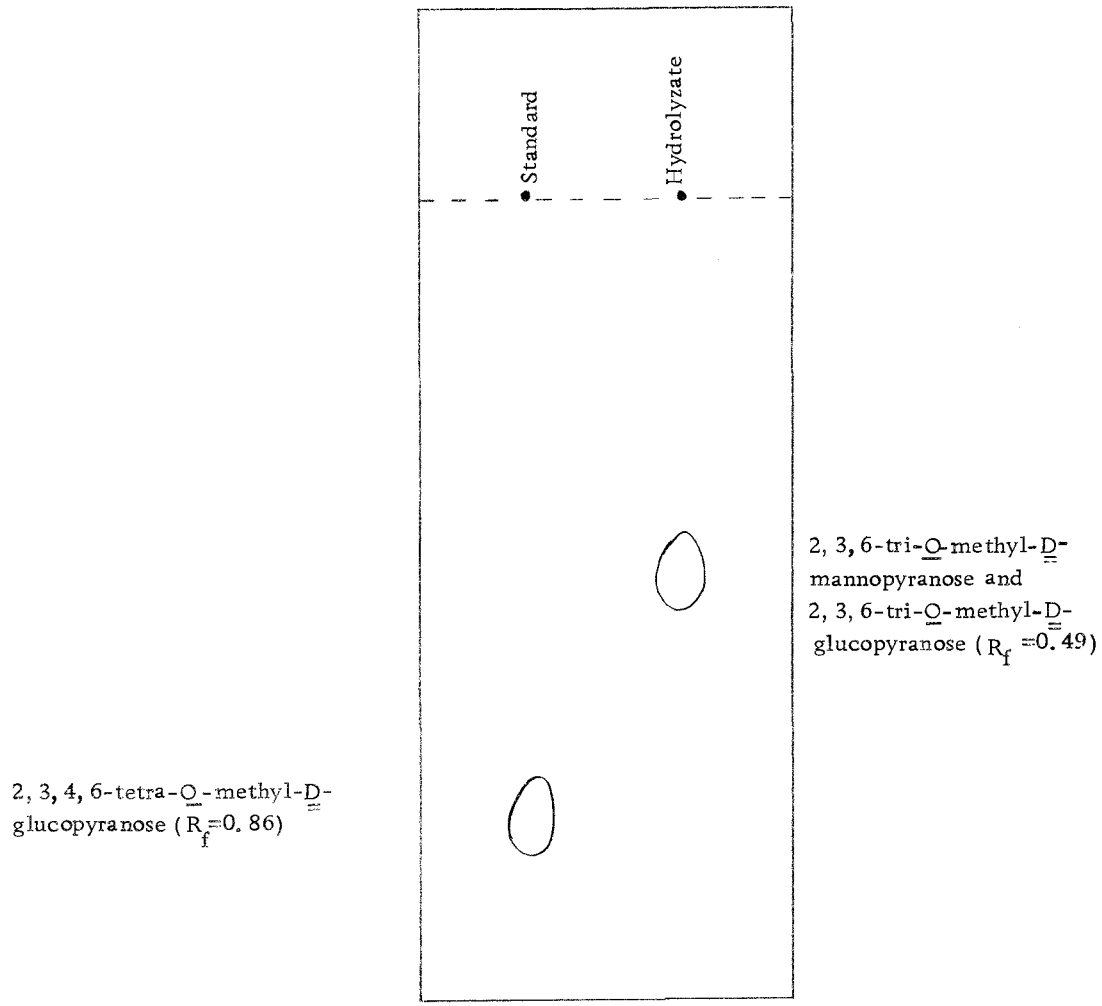


Figure 7. Paper chromatogram of the hydrolyzate of the methylated glucomannan. Solvent: 2-butanone saturated with water; for R_f values.

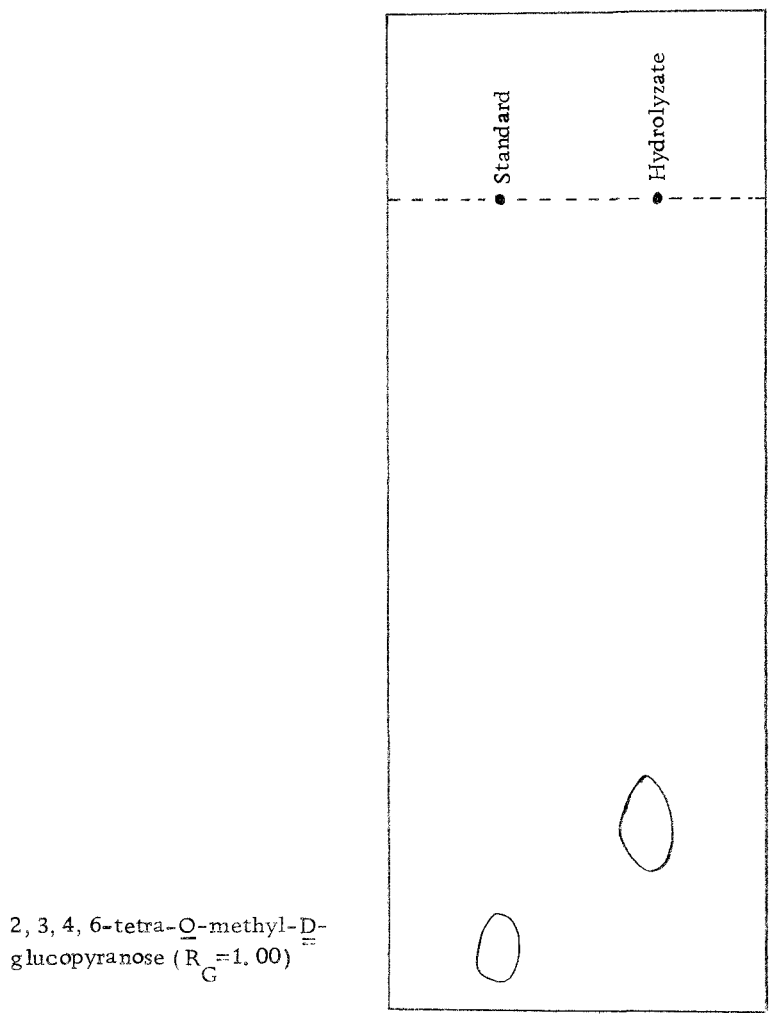


Figure 8. Paper chromatogram of the hydrolyzate of the methylated glucomannan. Solvent: n-butanol-ethanol-water (5:1:4, v/v/v) for R_G values.

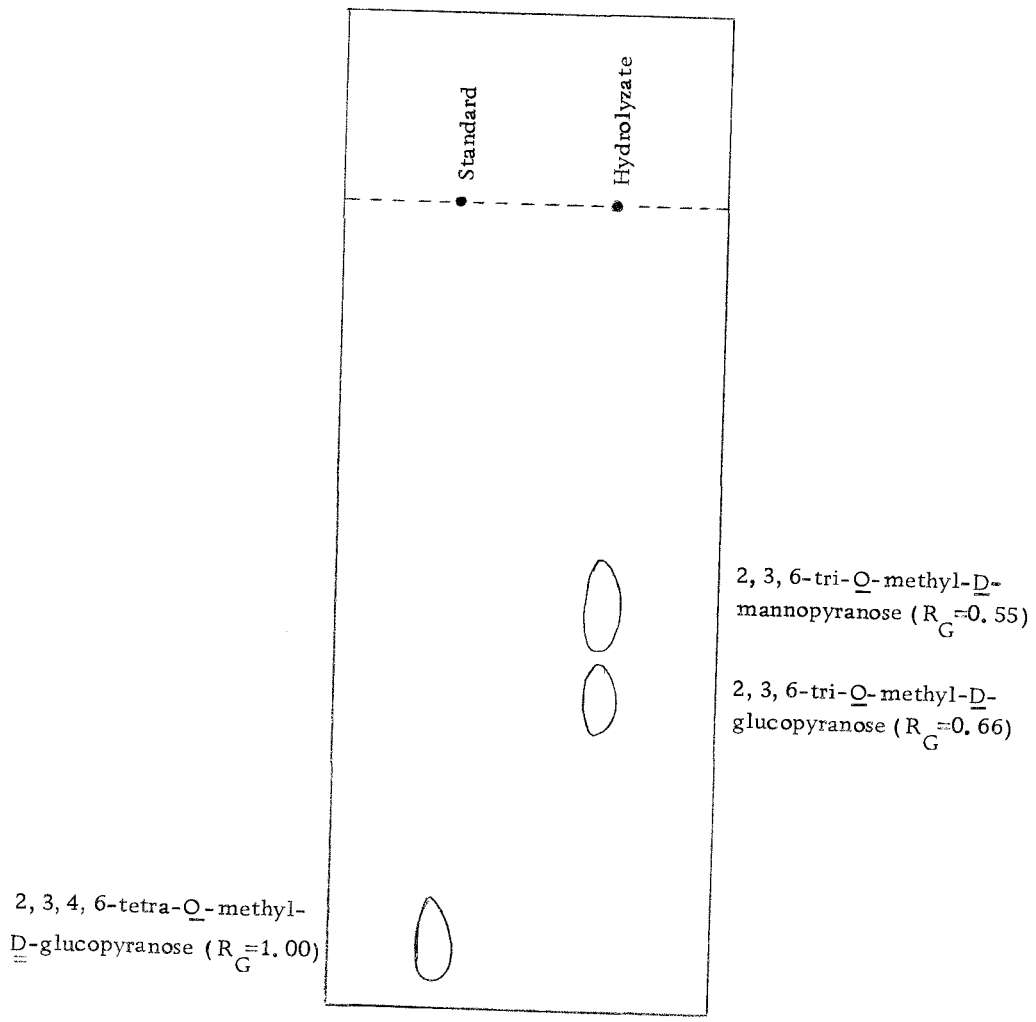


Figure 9. Paper chromatogram of the hydrolyzate of the methylated glucomannan. Solvent: methyl ethyl ketone-water-ammonium hydroxide solution (30%) (100:50:3, v/v/v) (upper layer).

quantitative analysis of sugars. In particular, the gas-liquid chromatographic separation of methylated sugars and their derivatives is useful for the investigation of oligosaccharide (42, 96, 97, 109) and polysaccharide structures (42, 52, 98, 100, 115). The free reducing methylated sugars themselves are not suitable for GLC analysis primarily due to adsorption on the stationary phase or column support (165). The derivatives which are usually prepared are the methyl glycosides which may be directly separated on the gas chromatographic column, or, where low mobility of the methyl glycoside results in exceptionally long retention times, analyzed as acetates (19, 165). Sometimes a column cannot sufficiently resolve the anomers of the different forms of certain methylated glycosides or their derivatives. In such an event, the acetates or trimethylsilyl ethers of the methylated alditols (17, 120, 165) may be suitable derivatives for a successful separation of the methylated sugars.

It is believed that transforming methylated sugars, obtained on hydrolysis of a fully methylated polysaccharide, into the acetylated alditol derivatives and analyzing the mixture by GLC offers the following advantages. First, by using a temperature gradient, alditols with all degrees of methylation, even nil, could be detected in a single experiment, and thereby undermethylation, and, or demethylation during hydrolysis would easily be detected. Secondly, improved separation of some sugars, which are not well separated as their

methyl glycosides is obtained. Thirdly, as each sugar gives a single peak, quantitative evaluation of the chromatogram is considerably facilitated (19).

There are no precise rules for the choice of a column to affect a desired separation. However, a search of the literature shows that polar liquid phases are favored by most workers as they seem to give better resolution of all types of methylated sugar derivatives. The retention time of a compound is usually expressed relative to a standard. This is to avoid the differences in absolute retention times caused by variations that may occur under standard operating conditions. Relative retention times are usually reproducible to ±2% on individual columns, but may vary up to ±5% on different columns containing the same stationary phase (7). Thus, in practice, the relative retention times should be used as a guide to indicate the order of mobility of the methylated sugars on a certain phase rather than as precise reproducible physical characteristics of these same compounds.

In the present work, the methods of Bjorndal and co-workers (19) and Hardwood (63) were used to analyze the methylated glucomannan from Douglas-fir inner bark. The alditol acetates of the methylated glucomannan was separated on an ECNSS-M column and the peaks were identified by comparison of the relative retention

times with that of authentic 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol. The results are shown in Table 3 (page 100) and Figure 10 (page 101).

Six methylated sugars were identified by comparison of their relative retention times. The early peaks (Figure 10, page 101) were due to reagents used in the preparation of the methylated alditol acetates. The major peak was that of 1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-mannitol followed by 1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-glucitol. Trace amounts of the tetramethyl and dimethyl sugars were also shown to be present in the mixture.

The methylated sugars were further characterized by mass spectrometry. The mixture was separated by the GLC and the components were directly introduced into the ionization chamber of the mass spectrometer. Table 4 gives the prominent peaks in the mass spectra of the methylated alditol acetates derived from the methylated glucomannan. Only peaks having an intensity of more than 10% of the base peak are included. The mass spectra of the different methylated alditol acetates are shown in Figures 11, 12, 13, 14, 15, and 16 (pages 103 to 108).

By using the principles of primary and secondary fragmentation of the partially methylated alditol acetates, most of the signals obtained in the mass spectra of these compounds can be rationalized. As an example, most of the signals in the mass spectrum of

Table 3. Methyl ethers obtained on methylation of the glucomannan from Douglas-fir inner bark.

Sugars	Peak	Retention[a] time (min)	T[b]	T[c]
1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol	A	5.00	1.00	1.00
1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-galactitol	B	6.25	1.25	1.26
1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-mannitol	C	11.30	2.26	2.27
1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-glucitol	D	13.25	2.65	2.62
1, 4, 5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-mannitol	E	25.25	5.05	4.87
1, 4, 5, 6, -tetra-O-acetyl-2, 3-di-O-methyl-D-glucitol	F	29.25	5.85	5.67

[a]Retention times are taken from a GLC spectrum obtained on a Hewlett Packard 5750B GC. Conditions: column, 6.5% ECNSS-M on Gas Chrom Q 100/120 mesh, 6 ft x 1/8 in O. D. stainless steel; injection port 192°; detector 230°; column temperature 160° isothermal; helium flow 30 ml/min; range setting 10^2; attenuation setting 32. The peak designations A, B, C, D, E, F are taken from Figure 10 (page 101) which is a spectrum of a computer output using a Varian GC as part of the GC-MS system.

[b]Retention time relative to 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol.

[c]From: Hardwood, V. D. Studies on the cell wall polysaccharides of Pinus radiata. II. Structure of a glucomannan. Svensk Papperstidning 76:377-379. 1973.

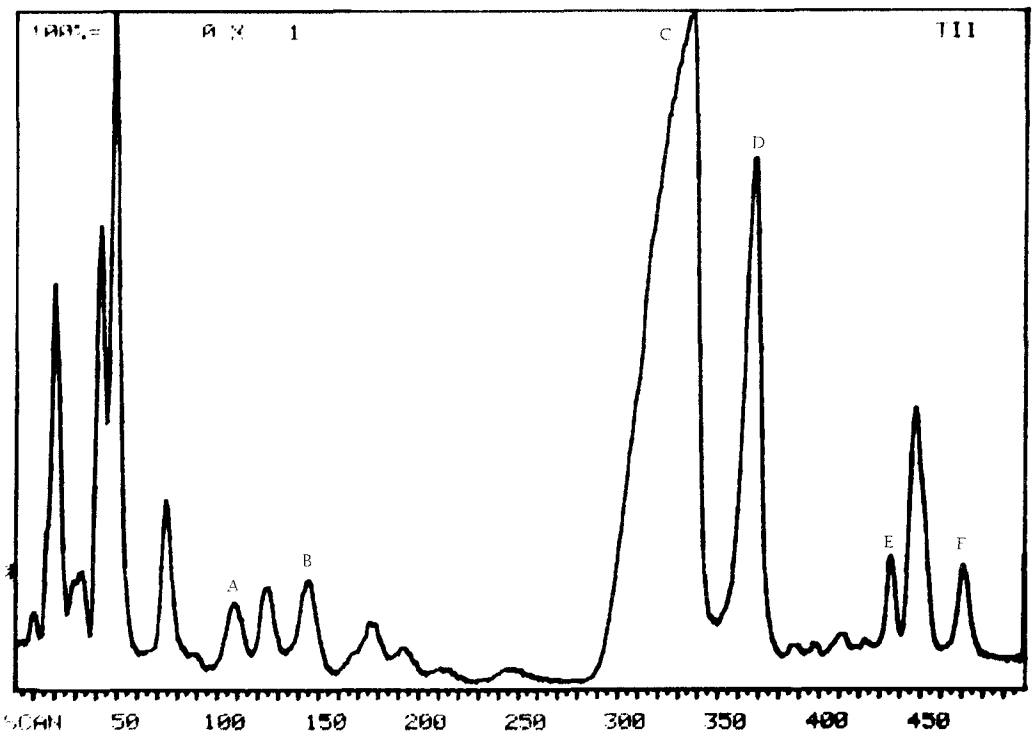


Figure 10. Computer printout of a gas-liquid chromatographic spectrum of the alditol acetates prepared from the acid hydrolyzate of the methylated glucomannan. Conditions: Varian GC; column, 6.5% ECNSS-M on Gas chrom Q 100/120 mesh, 6 ft x 1/8 in O.D. stainless steel; injection port 270°; detector 280°; column temperature 160° isothermal; helium flow 30 ml/min; range setting 10^2; attenuation setting 32.

[a]The scan numbers represent the places where mass spectrometry scans of the effluent were taken. For retention times of the compounds see Table 3 (page 100).

Table 4. Prominent peaks (m/e) in the mass spectra of the alditol acetates derived from methylated glucomannan of Douglas fir inner bark.[a]

	m/e											
	43	45	71	73	75	85	87	88	89	99	101	102
1,5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol	100.0	40.5	13.4				34.7	10.0	25.0		92.0	10.2
1,5-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-galactitol	98.3	51.2	15.8	14.3			25.5				100.0	12.3
1,4,5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-mannitol	91.0	13.2					24.8			22.9	44.0	
1,4,5- tri-O-acetyl-2, 3, 6-tri-O-methyl-D-glucitol	80.1	19.8				11.4	24.6			29.6	37.4	
1,4,5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-mannitol	60.4									14.1	32.6	
1,4,5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-glucitol	83.5					15.1	12.3			17.4	25.2	

	m/e											
	103	113	117	127	129	131	145	161	162	205	233	261
1,5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol	15.2		84.3		49.4		42.5	82.7	10.8	35.4		
1,5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-galactitol		11.8	100.0		69.6		94.1	69.8		42.5		
1,4,5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-mannitol		46.6	100.0		14.7	11.3		13.3			37.2	
1,4,5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-glucitol		46.9	100.0		19.8	13.6		11.0			47.9	
1,4,5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-mannitol			100.0	21.1								14.0
1,4,5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-glucitol			100.0	22.1								21.6

[a] The figures in the table represent percentages of the base peak.

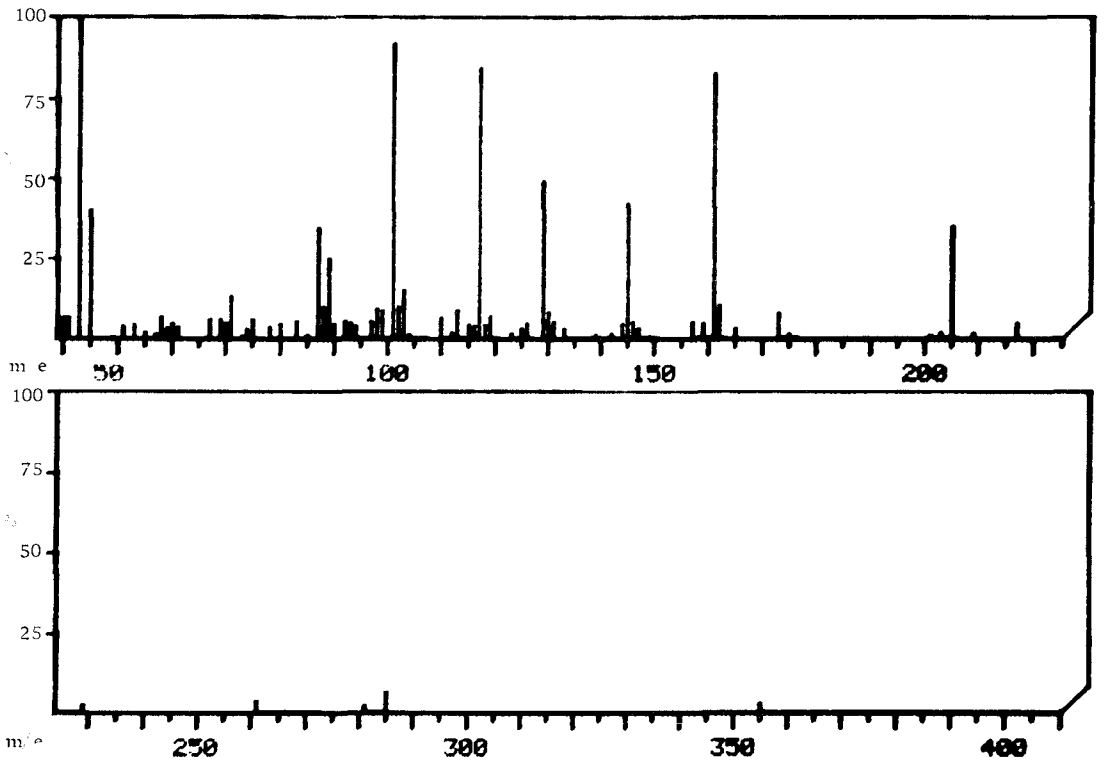


Figure 11. Mass spectrum of 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol derived from the methylated glucomannan of Douglas-fir inner bark.

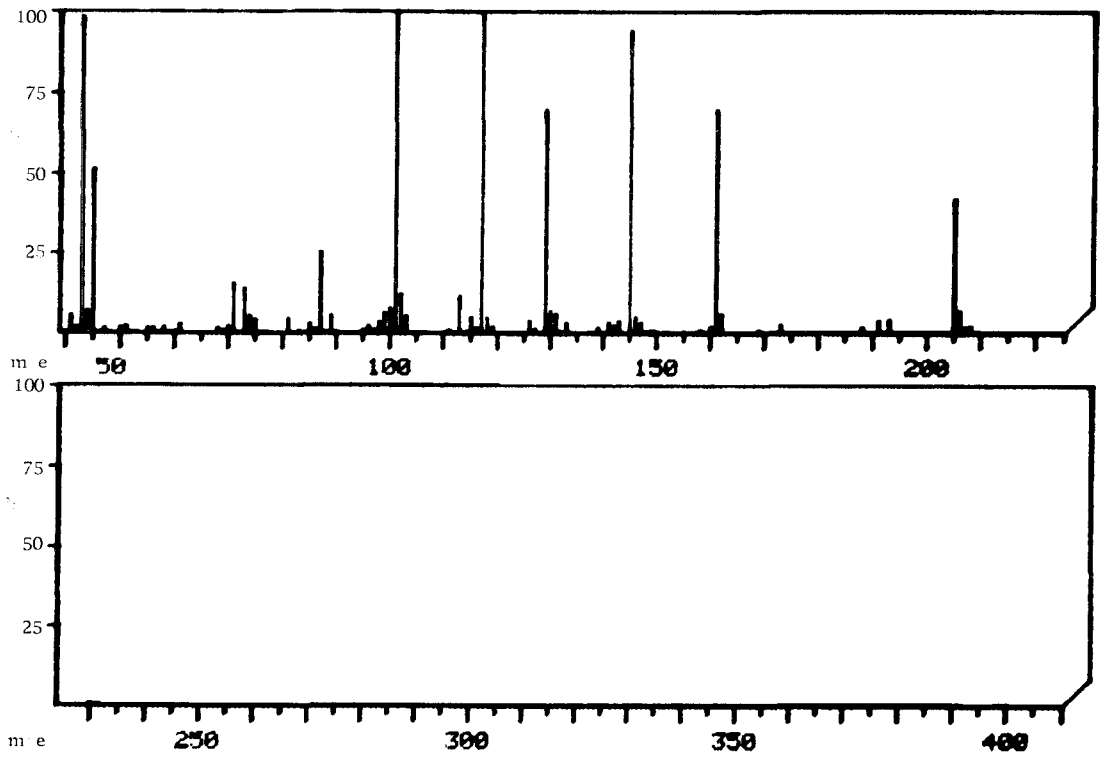


Figure 12. Mass spectrum of 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-galactitol derived from the methylated glucomannan of Douglas-fir inner bark.

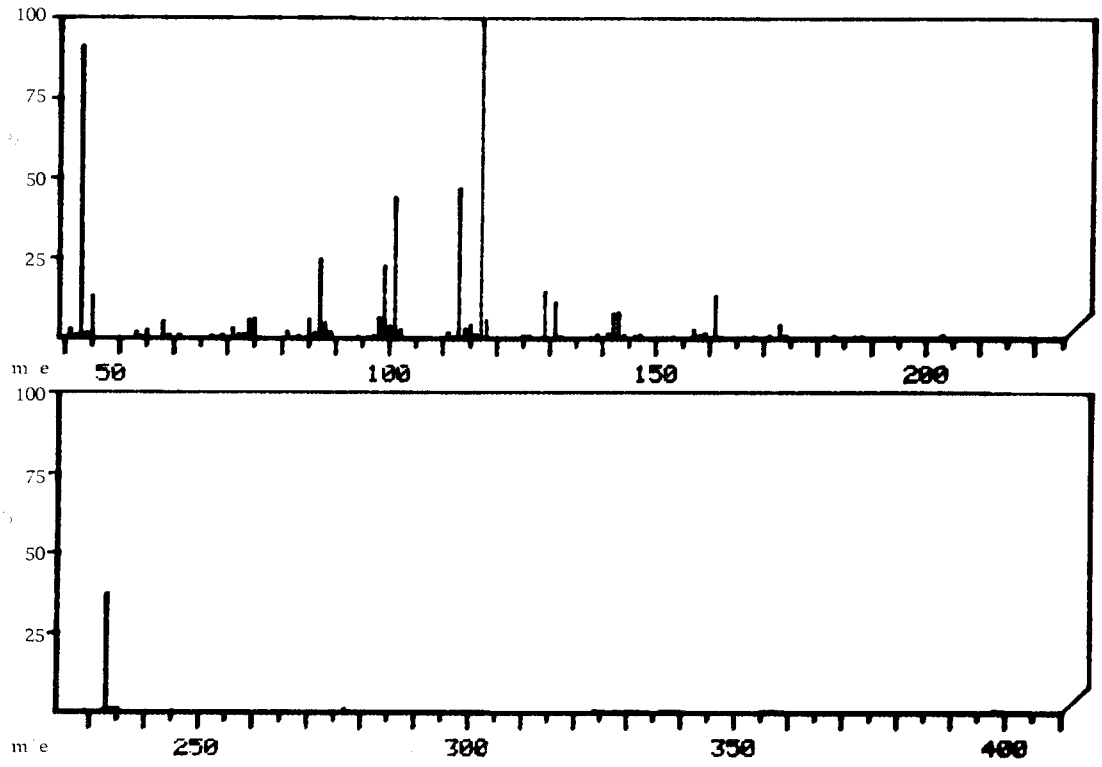


Figure 13. Mass spectrum of 1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-mannitol derived from the methylated glucomannan of Douglas-fir inner bark.

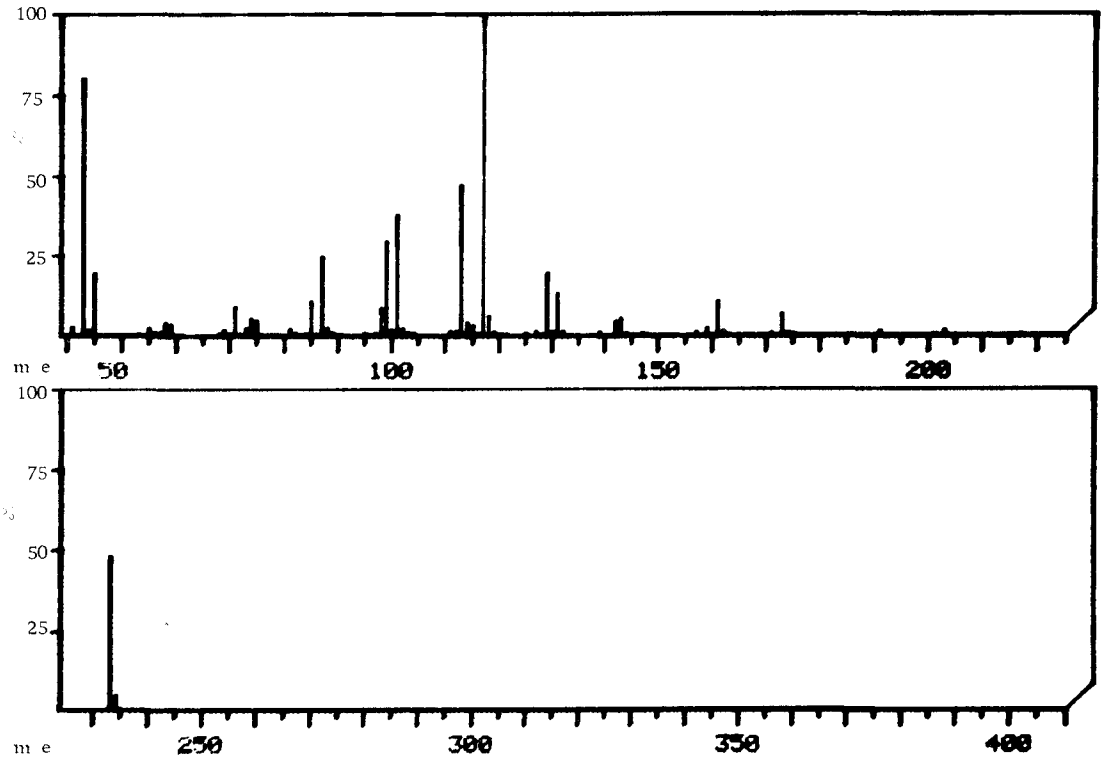


Figure 14. Mass spectrum of 1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-glucitol derived from the methylated glucomannan of Douglas-fir inner bark.

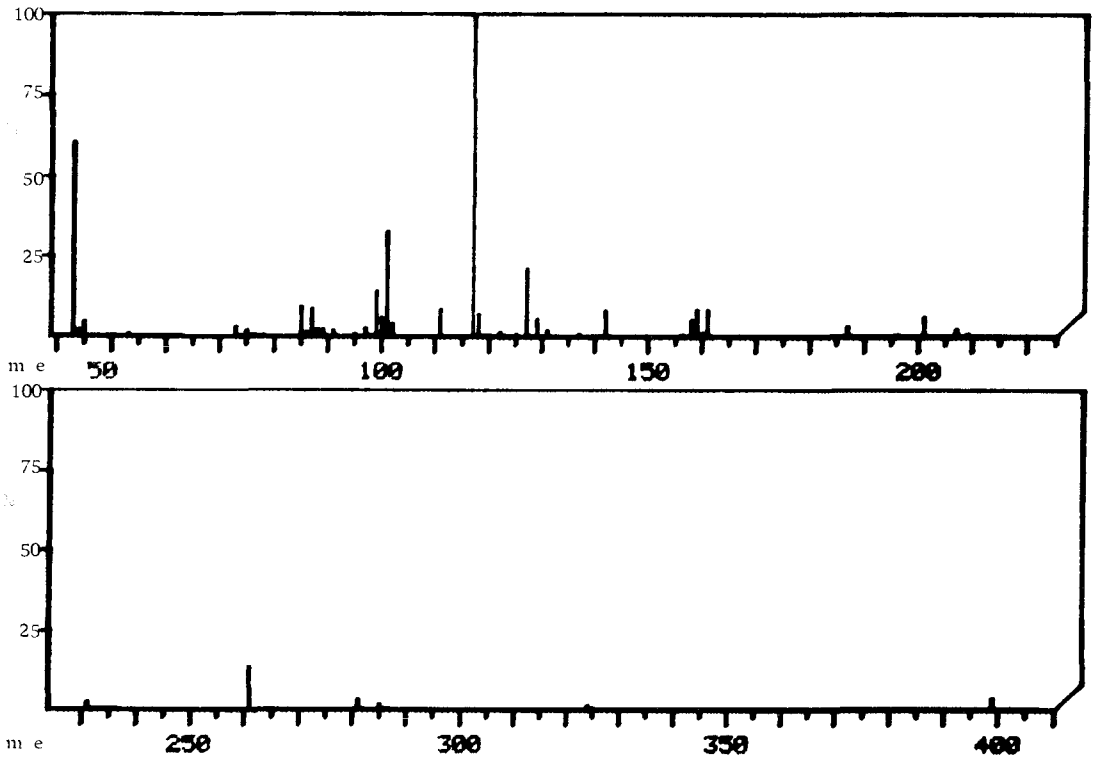


Figure 15. Mass spectrum of 1, 4, 5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-mannitol derived from the methylated glucomannan of Douglas-fir inner bark.

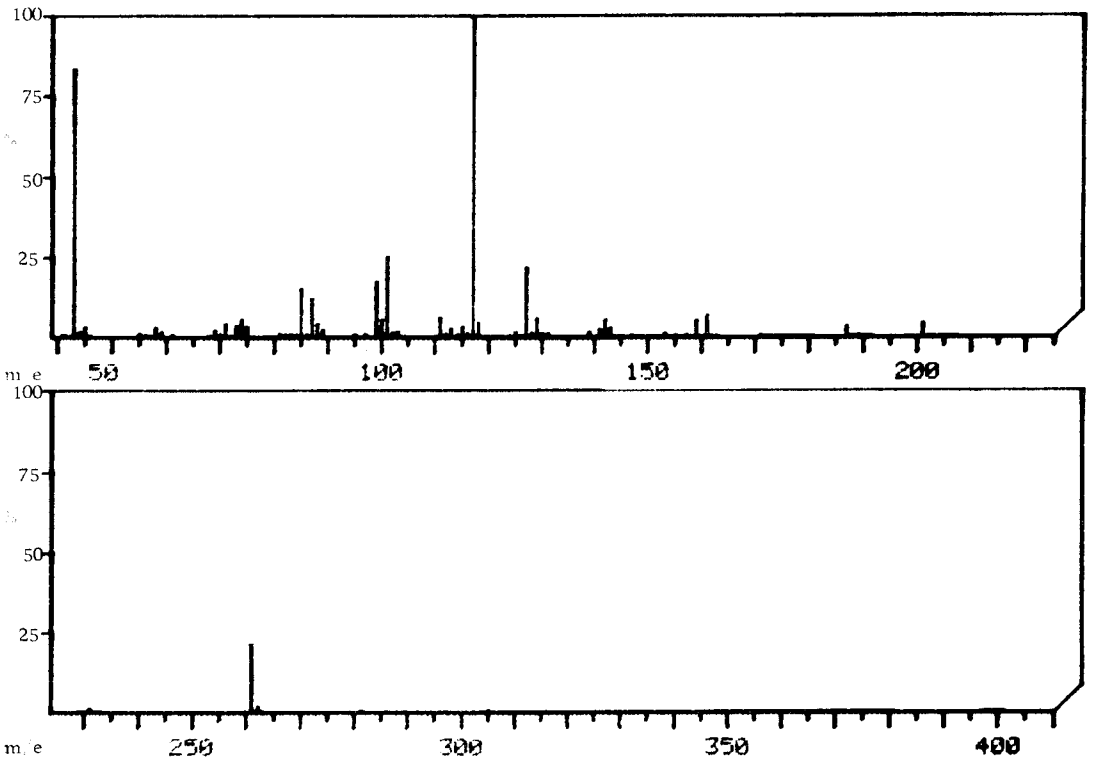
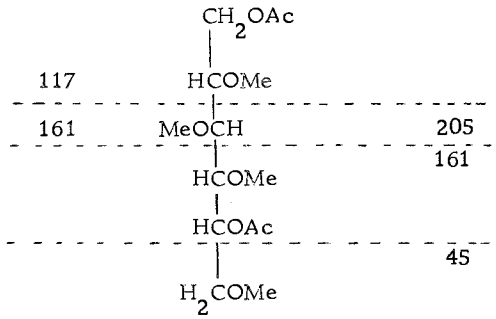


Figure 16. Mass spectrum of 1, 4, 5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-glucitol derived from the methylated glucomannan of Douglas-fir inner bark.

1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol (molecular weight 322) (Figure 11, page 103) can be accounted for as shown in Figure 17 (page 110). The mass spectra of peaks A and B (Figures 11 and 12, pages 103 and 104) are identical with the mass spectrum of an alditol methylated at positions 2, 3, 4, and 6 and acetylated at positions 1 and 5 (20). The relative retention times of peaks A and B are shown in Table 3 (page 100). From these data, peaks A and B were identified and shown to be those of 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol and 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-galactitol respectively. The peak between A and B of Figure 10 (page 101) was suspected to be 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-mannitol but the mass spectrum was not at all similar to the mass spectra from peaks A and B. In fact the mass spectrum was not that of a carbohydrate. The peak remains unidentified. The minor peaks between B and C are not carbohydrate in nature as demonstrated by their mass spectra and remain unidentified.

Different fragmentation patterns can be observed for the alditol acetates methylated at positions 2, 3, and 6 (molecular weight 350). The primary fragments are m/e 117, m/e 161, m/e 233 and m/e 189 which represent cleavages between carbons 2 and 3 and carbons 3 and 4. A prominent secondary peak, m/e 113, is most probably derived from (I) by loss of two molecules of acetic acid.

Primary fragmentation:



Secondary fragmentation:

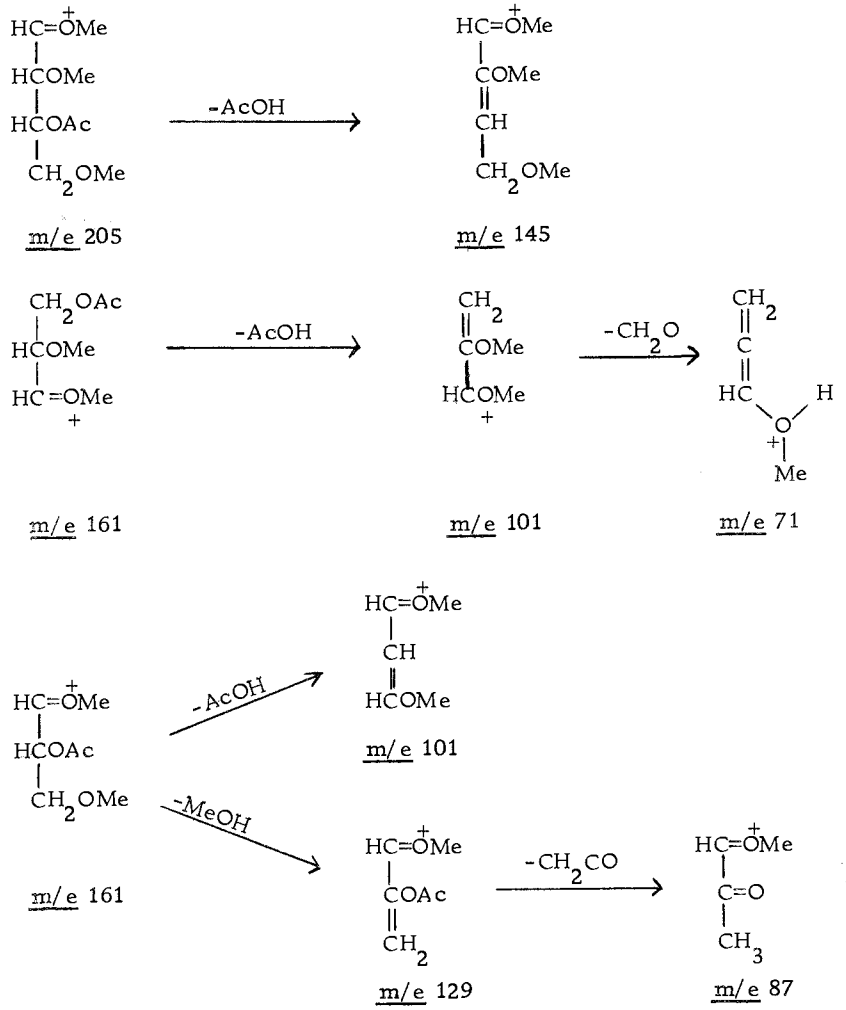
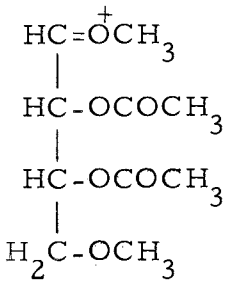


Figure 17. Fragmentation patterns of 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol.[a]

[a]From: Lönngren, J. and S. Svensson. Mass spectrometry in structural analysis of natural carbohydrates. Advances in Carbohydrate Chemistry and Biochemistry 29:41-106. 1974.

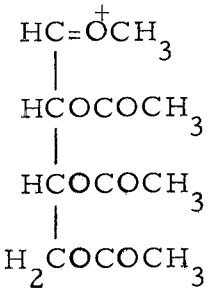


I m/e 233

The mass spectra of peaks C and D (Figures 13 and 14, pages 105, 106) are identical with that of an alditol methylated at positions 2, 3, and 6 and acetylated at positions 1, 4, and 5 (20). The relative retention times are shown in Table 3 (page 100). These data indicate that peaks C and D are those of 1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-mannitol and 1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methyl-D-glucitol respectively.

The primary peak having the highest mass number, m/e 261 (II), and a relatively high intensity, is given by alditols methylated at positions 3 and acetylated at positions 4, 5, and 6. The m/e 261 fragment results from cleavage between carbons 2 and 3. The mass number m/e 261 is present in the mass spectrum of tetraacetates of di-O-methyl-D-mannitol and di-O-methyl-D-glucitol (20). They have a base peak of m/e 117 which suggests that position 2 is also methylated (Figure 17, page 110). Thus peaks E and F in the gas-liquid chromatogram (Figure 10, page 101) correspond to 1, 4, 5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-mannitol and 1, 4, 5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-glucitol. The rather large peak between

peaks E and F (Figure 10, page 101) is not carbohydrate as demonstrated by its mass spectrum and remains unidentified.



II m/e 261

It has been shown by Bjorndal, Lindberg and Svensson (20) on their studies of the mass spectrometry of partially methylated alditol acetates that isomeric alditols having identical substitution patterns gave similar mass spectra that unambiguously determine the substitution pattern. However, the mass spectra do not show which sugar configuration is which. Each component in the mixture of the methylated polysaccharide can be properly identified when mass-spectral evidence is used in conjunction with the relative retention times from GLC because the retention times are characteristic for each sugar configuration.

From a comparison of the mass spectra and relative retention times of the different alditol acetates of methylated sugars, the peaks in the gas-liquid chromatogram (Figure 10, page 101) were identified, as shown in Table 3 (page 100).

A structure for the glucomannan isolated from the inner bark of Douglas-fir can now be proposed based on the experimental data (Figure 18, page 113). The repeating unit consists of a polymer

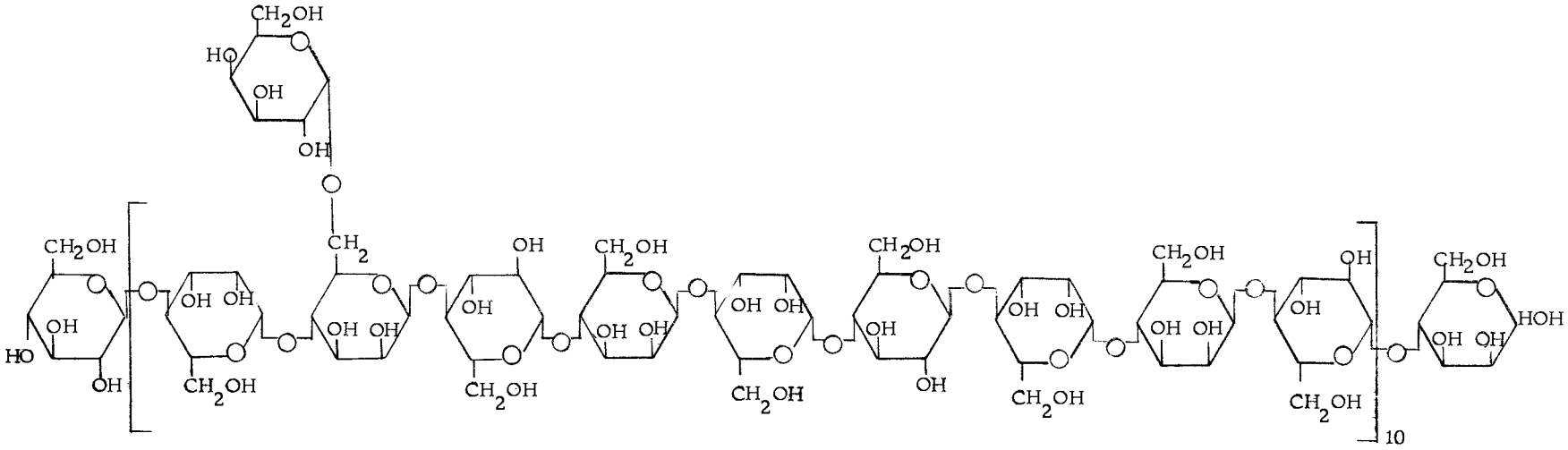


Figure 18. Proposed structure for the glucomannan isolated from the holocellulose fraction of Douglas-fir inner bark.

backbone of six anhydro-D-mannopyranose units and three anhydro-D-glucopyranose units. The sequence of the anhydro-D-mannopyranose and anhydro-D-glucopyranose units may be random. Each repeating unit contains one D-galactopyranose unit as a side chain attached to position six of an anhydro-D-mannopyranose unit. However, 1, 4, 5, 6-tetra-O-acetyl-2, 3-di-O-methyl-D-glucitol was also identified from the methylation studies (Table 3, page 100) and so the D-galactopyranose side chain is in some instances attached to position six of an anhydro-D-glucopyranose unit. The repeating unit is repeated ten times to yield a glucomannan backbone of 60 anhydro-D-mannopyranose units and 30 anhydro-D-glucopyranose units plus a reducing and non-reducing end group to yield a degree of polymerization of 92.

The presence of mannose, glucose, and galactose residues was demonstrated by paper and gas-liquid chromatography. The ratio of these monosaccharide units was determined by quantitative gas-liquid chromatography (mannose:glucose:galactose-2:1:0. 3, or in the closest whole numbers 6:3:1). The β-glycosidic linkages for the main chain were deduced from the negative optical rotation (-35. 9°) of the polymer. The linkages of the galactopyranose units to the backbone were considered to be α-glycosidic bonds because similar glucomannans containing galactopyranose side chains have been shown to possess α-linkages for these units (150). The degree of polymerization of 92

was experimentally determined by viscosity measurements and reducing end-group analysis.

Complete methylation followed by hydrolysis, sodium borohydride reduction, acetylation, gas-liquid chromatographic and mass spectrometric analyses showed that the methylated glucomannan was composed of the following partially methylated monosaccharide moieties:

2, 3, 4, 6-tetra-O-methyl-D-glucopyranose

2, 3, 4, 6-tetra-O-methyl-D-galactopyranose

2, 3, 6-tri-O-methyl-D-mannopyranose

2, 3, 6-tri-O-methyl-D-glucopyranose

2, 3-di-O-methyl-D-mannopyranose

2, 3-di-O-methyl-D-glucopyranose

The 2, 3, 4, 6-tetra-O-methyl-D-glucopyranose and 2, 3, 4, 6-tetra-O-methyl-D-galactopyranose units represent non-reducing end groups. The mannopyranose moiety terminates the main chain and the galactopyranose unit represents side chains. The 2, 3, 6-tri-O-methyl-D-mannopyranose and 2, 3, 6-tri-O-methyl-D-glucopyranose units comprise the glucomannan backbone attached by (1→4) glycosidic linkages (Figure 18, page 113). The 2, 3-di-O-methyl-D-mannopyranose and 2, 3-di-O-methyl-D-glucopyranose units indicate a degree of branching from the sixth position of the anhydromannopyranose and anhydroglucopyranose residues in the main chain. These branches can be

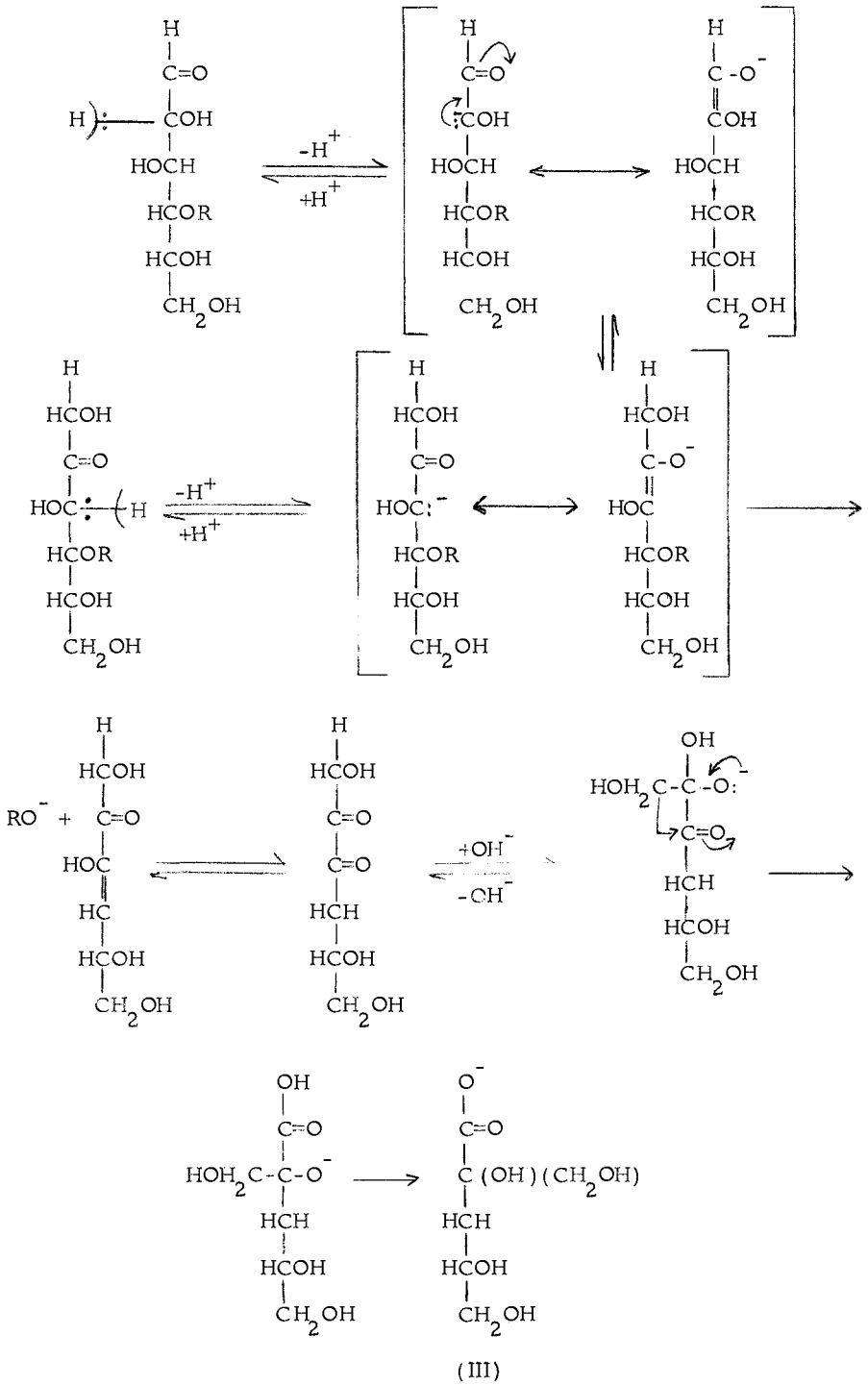
best accommodated by the galactose side chains (150).

Although variations in the fine structure of the polysaccharide are possible, the structure proposed (Figure 18, page 113) is consistent with the data. The conclusion is that the inner bark of Douglas-fir contains a glucomannan that is quite different from the glucomannans previously found in the wood and bark of gymnosperms (section II, page 4).

I. Alkaline Degradation of the Glucomannan

1. Reaction in Sodium Hydroxide Solutions

The alkaline degradation of polysaccharides linked (1→4) (Figure 19, page 117) proceeds by a peeling process in which the reducing end group is liberated from a chain by elimination of the rest of the chain as a glycoxy anion (161). Elimination takes place when the chain is in the position β to a carbonyl group of the reducing end (73). Elimination of alkoxy anions takes place under alkaline conditions when there is a carbonyl β to the alkoxide or when there is an easily removable proton on the carbon atom in the position α to the alkoxide group. An alkoxy or glycoxy ion is more easily eliminated by the ionized enediol than is a hydroxy ion, and the pyranose rings of the released glycoxy anions will open readily because of the tendency of the negatively charged oxygen to form a double bond with carbon. The released end groups form 2, 3-diketone structures which



(III)

Figure 19. The alkaline degradation of (1 → 4)-linked D-gluco- and D-mannoglycans (where R = the remaining portion of the polysaccharide molecule).[a]

[a] From: Whistler, R. L. and J. N. BeMiller. Alkaline degradation of polysaccharides. Advances in Carbohydrate Chemistry 13:289-329. 1958.

rearrange by an intramolecular type of Cannizzaro reaction which is commonly referred to as a benzilic acid rearrangement. These yield saccharinates (III) (Figure 19, page 117). However, the (1→4)-linked polysaccharides do not degrade to completion. There occurs a termination or "stopping" reaction that may be caused by (a) an alkali-resistant linkage in the polysaccharide, (b) the formation of stable, metasaccharinate end-groups in (1→4)-linked glycans (Figure 20, page 119) or, less likely, (c) formation of stable ordinary saccharinate end groups (161).

Young, Sarkanen, Johnson and Allan (168) have recently investigated the alkaline degradation of polysaccharides with specific reference to (1→3)-β-D-glucans. They propose that the rate of alkaline degradation of linear polysaccharides is best explained in terms of mono- and di-anionic species formed from the reducing end-groups. Their conclusions result in part from the investigations of Haas, Hrutfiord, and Sarkanen (57) on the alkaline degradation of cotton hydrocellulose, and the investigation of Lai and Sarkanen (92) on the alkaline degradation of amylose. Their work on (1→3)-β-D-glucans supports the mono- and di-anionic concept.

The description for the rate of alkaline degradation of polysaccharides proposed by Young, Sarkanen, Johnson and Allan (168) takes into account the termination reaction in (1→4)-linked polysaccharides. They consider that the probable sequence leading to

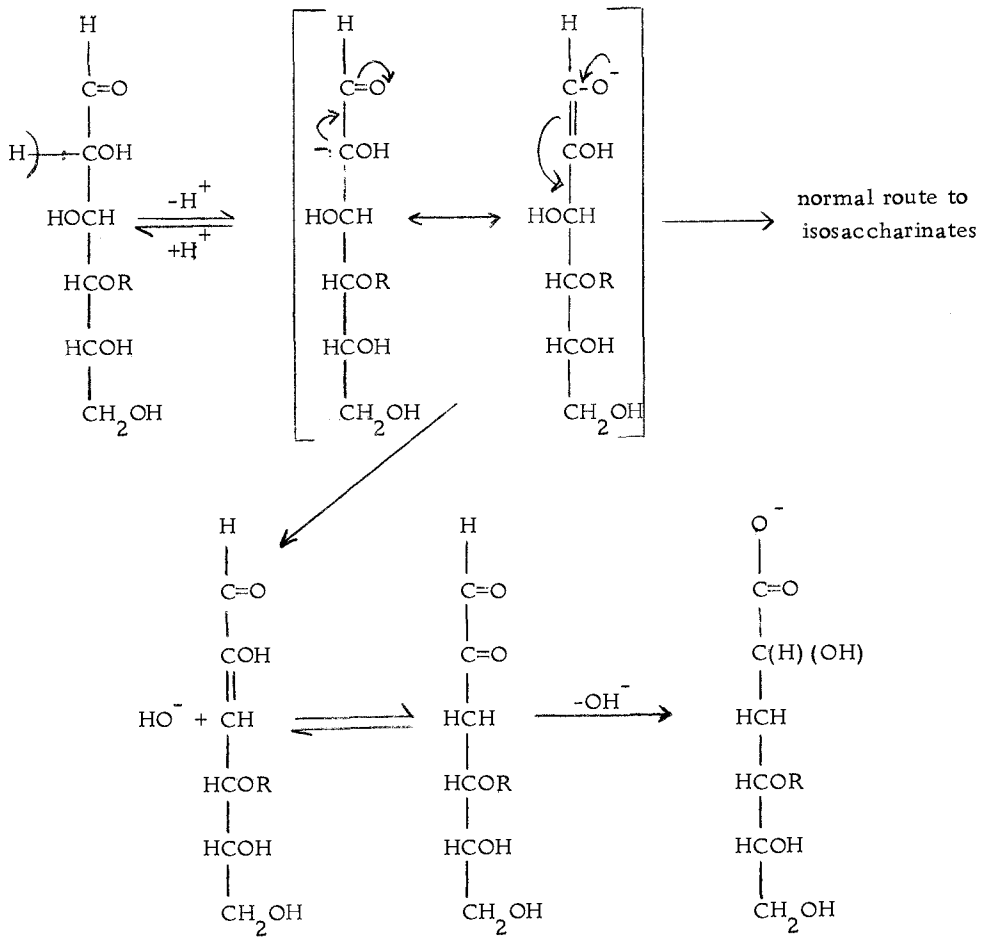


Figure 20. The formation of stable, metasaccharinate end-groups in (1 → 4) glycans (where R = the remaining portion of the polysaccharide molecule). [a]

[a] From: Whistler, R. L. and J. N. BeMiller. Alkaline degradation of polysaccharides. Advances in Carbohydrate Chemistry 13:289-329. 1958.

end-group elimination of reducing polysaccharides can be represented by the scheme illustrated in Figure 21 (page 121). It is assumed that the reaction occurs only via the anionic species, in this case the mono- and di-anionic forms of the end-group. The proposed scheme resembles the mechanism postulated by Bamford and Collins (10) for certain reactions of monomeric glucose in alkaline media, that were likewise considered to involve mono- and di-anionic intermediates. Corbett and Kenner (37) have later elaborated similar concepts for the alkaline degradation of glucose.

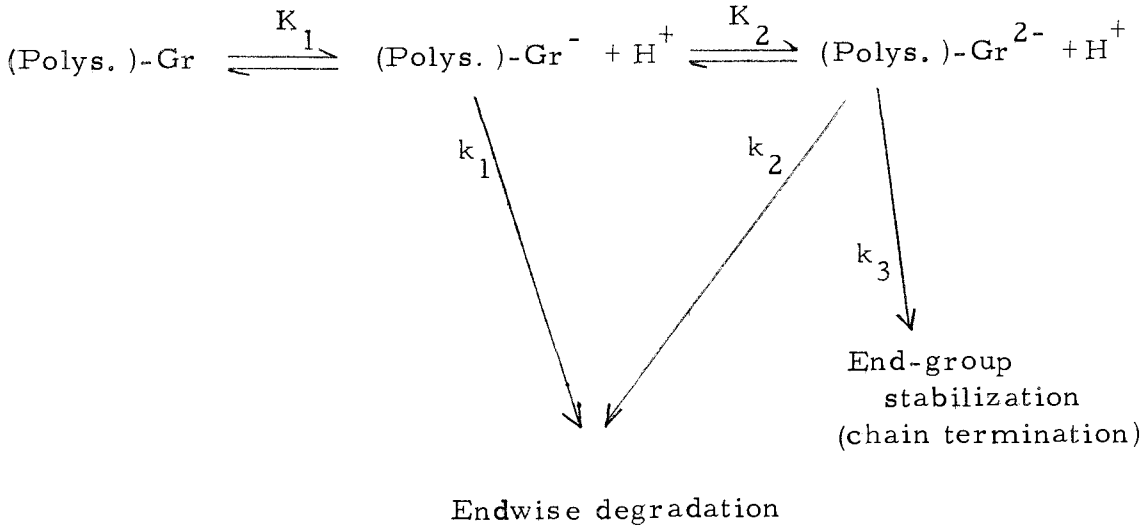
According to this scheme (Figure 21, page 121) an equilibrium is approached between neutral, mono- and di-anionic end-group species, characterized by the equilibrium constants K_1 and K_2. Both mono- and di-anions are reactive towards peeling, whereas end-group stabilization, which occurs in (1→4)-linked polymers only, is achieved via the di-anionic species by conversion into a metasaccharinic acid end-group.

Young, Sarkanen, Johnson and Allan (168) derive their kinetic expression from the reaction scheme outlined in Figure 21 (page 121). The degradative chain-propagation and chain termination are expressed by equations 1a and 1b.

$$d[Ge]/dt = k_1[Gr^-] + k_2[Gr^{2-}] = dL/dt \qquad 1a$$

$$-d[Gr]/dt = k_3[Gr^{2-}] \qquad 1b$$

where t represents the reaction time; L, the weight fraction of



Where:

(Polys.)-Gr represents a polysaccharide with a reducing end-group; K_1 and K_2 are equilibrium constants, k_1, k_2 and k_3 are rate constants for the reactions shown.

Figure 21. Reaction sequence leading to end-group elimination of reducing polysaccharides in alkaline solutions.[a]

[a]From: Young, R. A., K. V. Sarkanen, P. G. Johnson and G. G. Allan. Marine Plant Polymers Part III. A kinetic analysis of the alkaline degradation of polysaccharides with specific reference to (1→3)-β-D-glucans. Carbohydrate Research 21:111-122. 1972.

polysaccharide degraded after time t; [Ge], the mole fraction of peeled-off end-groups at time t; [Gr], the total mole fraction of remaining end-groups at time t; $[Gr^-]$ and $[Gr^{2-}]$, the mole fractions of relevant mono- and di-ionized end-groups; while k_1, k_2, and k_3 are the rate constants for the reactions depicted in the scheme in Figure 21 (page 121).

From the reaction sequence (Figure 21, page 121) it follows that the first and second ionization constants K_1 and K_2, can be represented by:

$$K_1 = [Gr^-][H^+] / \{[Gr] - ([Gr^-] + [Gr^{2-}])\} \qquad 2$$

and

$$K_2 = [Gr^{2-}][H^+]/[Gr^-], \text{ respectively} \qquad 3$$

Using equation 3 to eliminate the term $[Gr^{2-}]$ from equation 2 shows that

$$[Gr^-] = K_1[Gr][H^+]/([H^+]^2 + K_1[H^+] + K_1K_2) \qquad 4$$

Similar elimination of the term Gr^- from equation 3 by using equation 4 indicates that

$$[Gr^{2-}] = K_1K_2[Gr]/([H^+]^2 + K_1[H^+] + K_1K_2) \qquad 5$$

Combination of equations 4, 5, and 1 yields the rate expression

$$dL/dt = [Gr]\{(k_1K_1[H^+] + k_2K_1K_2)/([H^+]^2 + K_1[H^+] + K_1K_2)\} \qquad 6$$

then if $s = K_1/([H^+]^2 + K_1[H^+] + K_1K_2)$, equation 6 becomes

$$dL/dt = s[Gr]\,(k_1[H^+] + k_2K_2) \qquad 7$$

Thus, from equations 5 and 1, the rate of chain termination can be

written as $-d[Gr]/dt = sk_3K_2[Gr]$ which, on integration, becomes

$$[Gr] = [Gr]_o \exp(-sk_3K_2t) \qquad 8$$

where $[Gr]_o$ represents the mole fraction of end groups at zero time, and hence from equations 7 and 8

$$dL/dt = s(k_1[H^+] + k_2K_2)[Gr]_o \exp(-sk_3K_2t) \qquad 9$$

which is a general expression for the rate of alkaline degradation of linear polysaccharides. Evaluation of equation 9 by integration demonstrates that

$$L = \{s(k_1[H^+] + k_2K_2)[Gr]_o\}\{1-\exp(-sk_3K_2t)\}/sk_3K_2 \qquad 10$$

and so at infinite time

$$L_\infty = [Gr]_o(k_1[H^+] + k_2K_2)/k_3K_2 \qquad 11$$

where L_∞ is the weight fraction of polysaccharide degraded after an infinite time.

Zerrudo (169) used the above equations to describe the kinetics of an alkaline degradation of a xylan. He showed that the kinetic data obtained for a hemicellulose could be explained in terms of the mathematical expressions proposed by Young, Sarkanen, Johnson and Allan (168). The same equations were used in the present work to describe the kinetics for an alkaline degradation of a glucomannan.

The rate data obtained for the degradation of the glucomannan at various sodium hydroxide concentrations, at 64.5°, 78.5° and 100° are shown in Tables 5-7 (pages 124-126). The phenol-sulfuric acid reaction was used to measure the total undegraded glucomannan

Table 5. Rate data for the degradation of the glucomannan in aqueous sodium hydroxide at various pH values[a] at 64.5° C.

Time (hours)	Glucomannan remaining in µg/ml							
	pH 11.0	pH 11.5	pH 12.0	pH 12.5	pH 13.0	pH 13.5	pH 14.0	pH 14.5
0.08	91.5	90.0	88.5	85.5	91.5	91.5	91.5	90.5
0.16	90.5	87.0	87.5	85.0	92.0	92.0	89.0	90.0
0.25	91.5	86.0	86.0	80.0	80.0	91.5	88.5	92.0
0.33	88.5	85.0	87.5	81.0	92.0	82.5	89.0	93.5
0.50	86.0	84.5	86.0	79.0	85.5	87.0	87.5	89.5
1.00	85.0	83.0	83.0	80.5	82.5	81.0	76.5	90.0
1.50	84.0	82.0	82.5	76.0	75.5	83.0	89.0	93.5
2.50	83.0	79.0	78.5	81.0	78.0	81.5	85.5	92.0
4.00	81.0	81.0	77.5	76.0	69.0	78.0	75.0	92.0
6.00	79.5	78.0	78.5	75.0	77.5	75.5	71.5	78.5
9.00	79.0	79.0	77.0	74.5	78.5	76.5	78.0	76.5
9.50	79.0	79.0	76.0	71.5	78.0	69.0	67.5	77.5

[a] The pH values are calculated from the normality of the solutions assuming complete dissociation of the sodium hydroxide.

Table 6. Rate data for the degradation of the glucomannan in aqueous sodium hydroxide at various pH values[a] at 78.5° C.

Time (hours)	Glucomannan remaining in µg/ml							
	pH 11.0	pH 11.5	pH 12.0	pH 12.5	pH 13.0	pH 13.5	pH 14.0	pH 14.5
0.08	90.0	86.0	85.5	83.0	83.0	82.5	87.0	90.5
0.16	85.5	82.0	85.0	79.5	82.0	79.0	82.0	87.0
0.25	85.0	82.0	81.0	76.0	82.5	82.5	83.0	85.0
0.33	84.0	78.0	81.0	79.0	78.5	82.5	78.0	83.5
0.50	84.0	76.5	81.0	76.5	80.5	80.5	77.0	81.0
1.00	83.0	76.0	80.5	77.5	77.5	74.5	76.5	82.5
1.50	82.0	75.0	76.5	78.5	76.5	70.0	70.0	72.5
2.50	80.0	73.5	76.0	75.0	75.0	67.5	59.5	77.5
4.00	79.5	70.0	75.5	69.0	68.0	63.0	58.0	72.5
6.00	80.0	69.5	74.5	72.0	62.5	63.0	56.0	71.5
9.00	79.0	68.5	73.0	63.0	58.5	51.5	56.0	67.5
9.50	79.0	67.5	70.5	68.0	62.0	50.0	56.0	68.0

[a]The pH values are calculated from the normality of the solutions assuming complete dissociation of the sodium hydroxide.

Table 7. Rate data for the degradation of the glucomannan in aqueous sodium hydroxide at various pH values[a] at 100.0° C.

Time (hours)	Glucomannan remaining in μg/ml							
	pH 11.0	pH 11.5	pH 12.0	pH 12.5	pH 13.0	pH 13.5	pH 14.0	pH 14.5
0.08	95.0	88.5	87.5	85.5	85.5	72.5	83.5	83.5
0.16	87.0	86.0	83.0	84.0	84.5	69.5	84.0	80.5
0.25	84.0	85.5	83.0	81.0	83.0	67.5	75.0	79.0
0.33	82.5	83.0	83.0	80.5	80.5	66.0	82.5	82.5
0.50	83.0	80.5	79.5	79.5	70.0	71.0	79.5	75.0
1.00	87.0	79.5	80.5	76.5	66.5	59.5	62.5	69.0
1.50	86.0	79.0	79.0	75.0	58.0	54.5	50.0	59.5
2.50	84.5	77.5	79.0	72.5	59.0	51.0	42.5	58.0
4.00	82.0	80.0	77.5	70.0	52.5	51.0	48.5	57.5
6.00	84.5	76.5	76.5	67.5	51.0	48.5	36.0	55.5
9.00	82.0	74.5	71.5	64.0	45.0	36.0	35.0	61.5
9.50	78.0	71.5	69.0	55.5	45.0	36.0	31.0	57.5

[a]The pH values are calculated from the normality of the solutions assuming complete dissociation of the sodium hydroxide.

remaining after the times indicated. These data are plotted in Figures 22-31 (pages 128-137). The initial rates of reaction (dl/dt) were obtained from the experimental curves and are plotted in Figures 32-34 (pages 138-140) versus the pH of the sodium hydroxide solution. The shape of these curves is almost identical to that published by Young, Sarkanen, Johnson and Allan (168) for the alkaline degradation of laminaran. A similar curve was obtained by Zerrudo (169) for the alkaline degradation of a xylan from Douglas-fir inner bark. The similarity of these curves for three different polysaccharide structures supports the general reaction scheme outlined in Figure 21 (page 121) and the general rate expression for the alkaline degradation of linear polysaccharides (Equation 9, page 123).

The magnitudes of the dissociation constants K_1 and K_2 were obtained from the pK_a of these plots. At one-half the maximum rate for degradation of the glucomannan, the pK_1 values for the first ionization to mono-anion were determined as 11.40 ($K_1 = 2.51 \times 10^{-12}$) at 100°; 11.35 ($K_1 = 4.45 \times 10^{-12}$) at 78.5; and 11.40 ($K_1 = 2.51 \times 10^{-12}$) at 64.5°. The maximum rate of degradation is reached at pH 13.0 (Figures 32-34, pages 138-140). This represents the second ionization to the di-anion and so the dissociation constant pK_2 is 13.0 ($K_2 = 1 \times 10^{-13}$).

The initial rates of degradation do not increase after pH 13.0 (Figures 32-34, pages 138-140) and so subsequent calculations will be

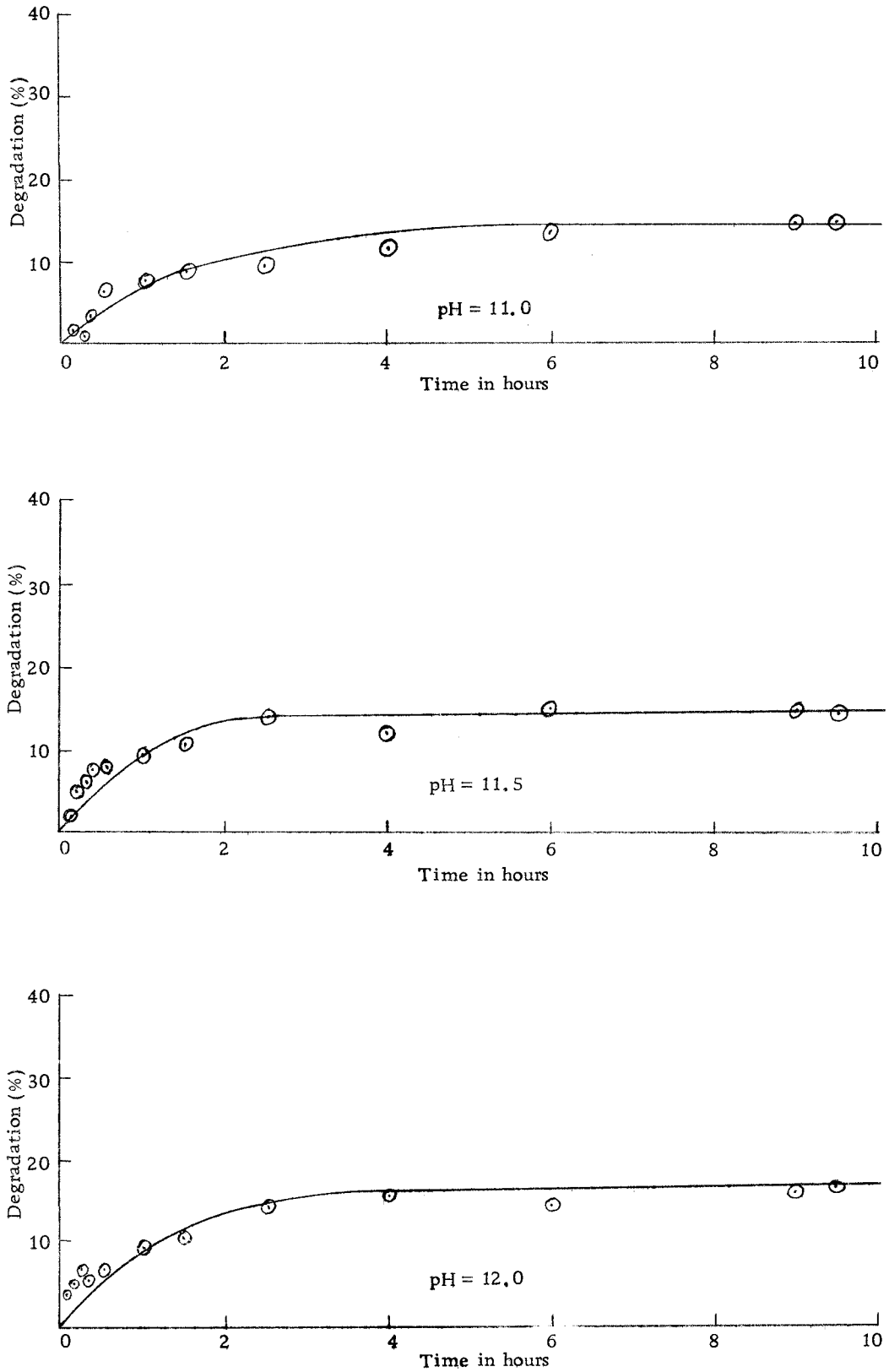
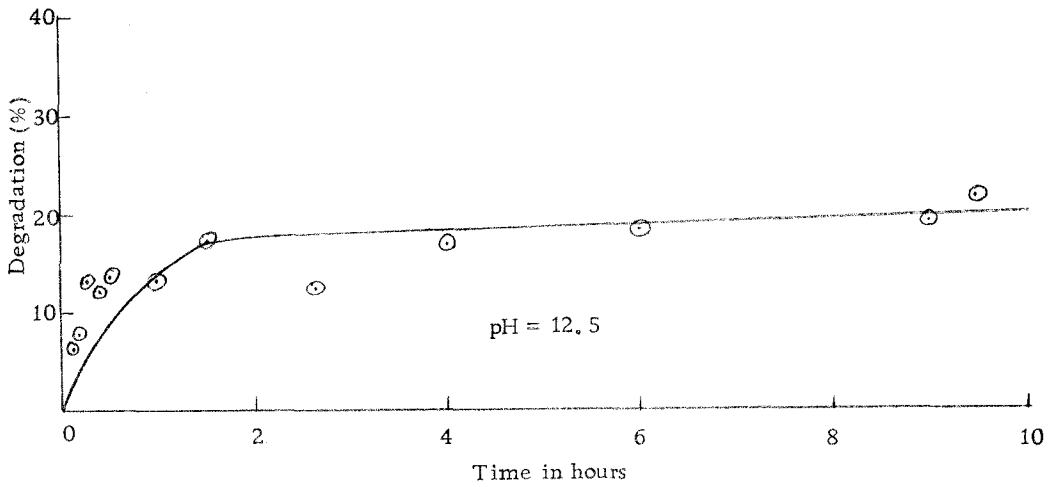
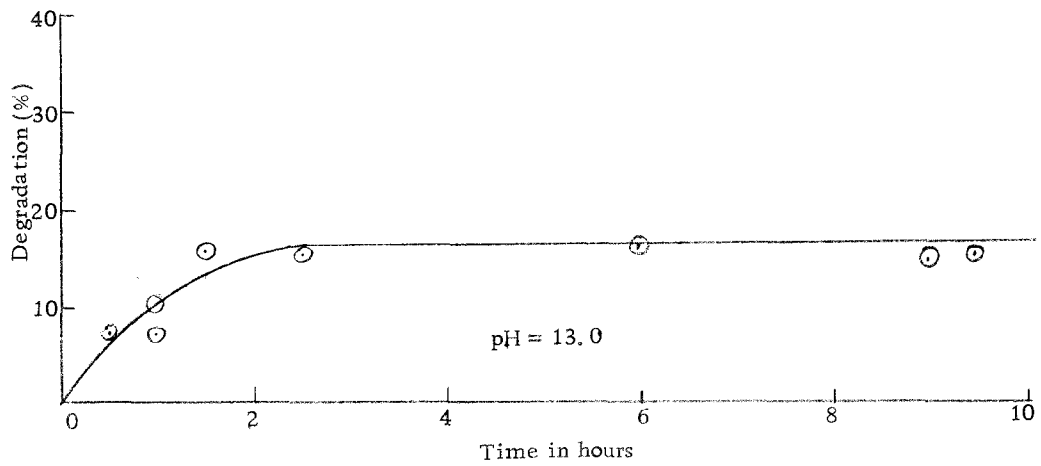


Figure 22. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 11.0-12.0 at 64.5°.





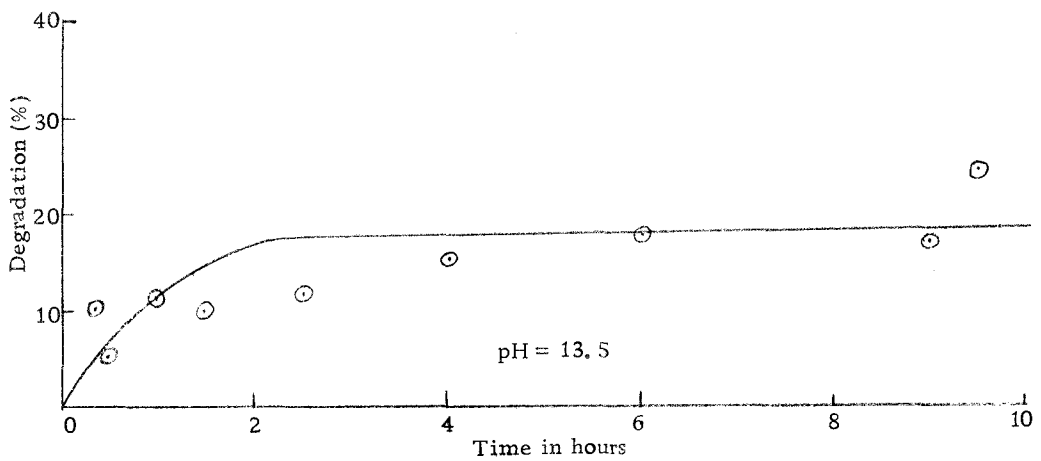


Figure 23. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 12.5-13.5 at 64.5°.

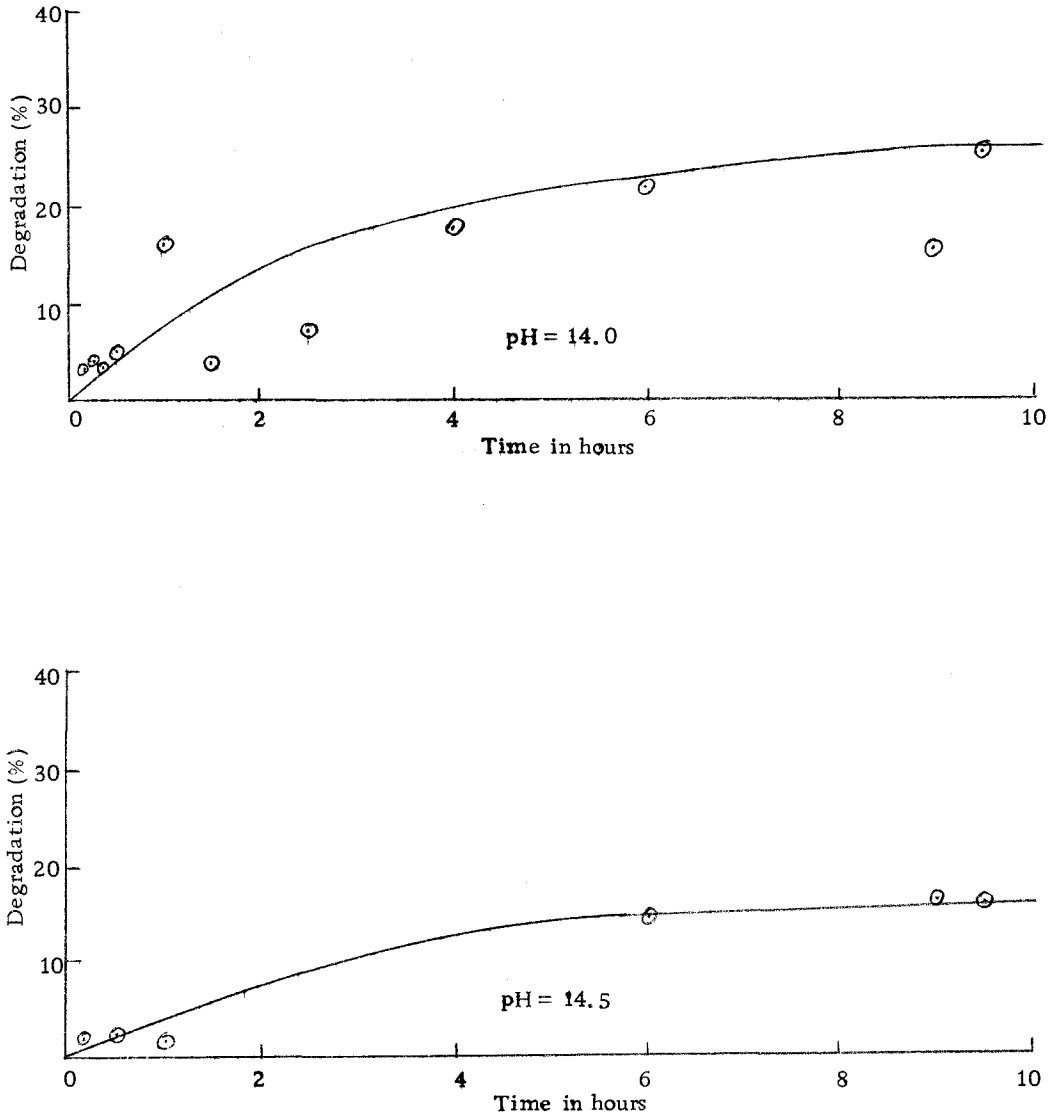


Figure 24. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 14.0-14.5 at 64.5°.

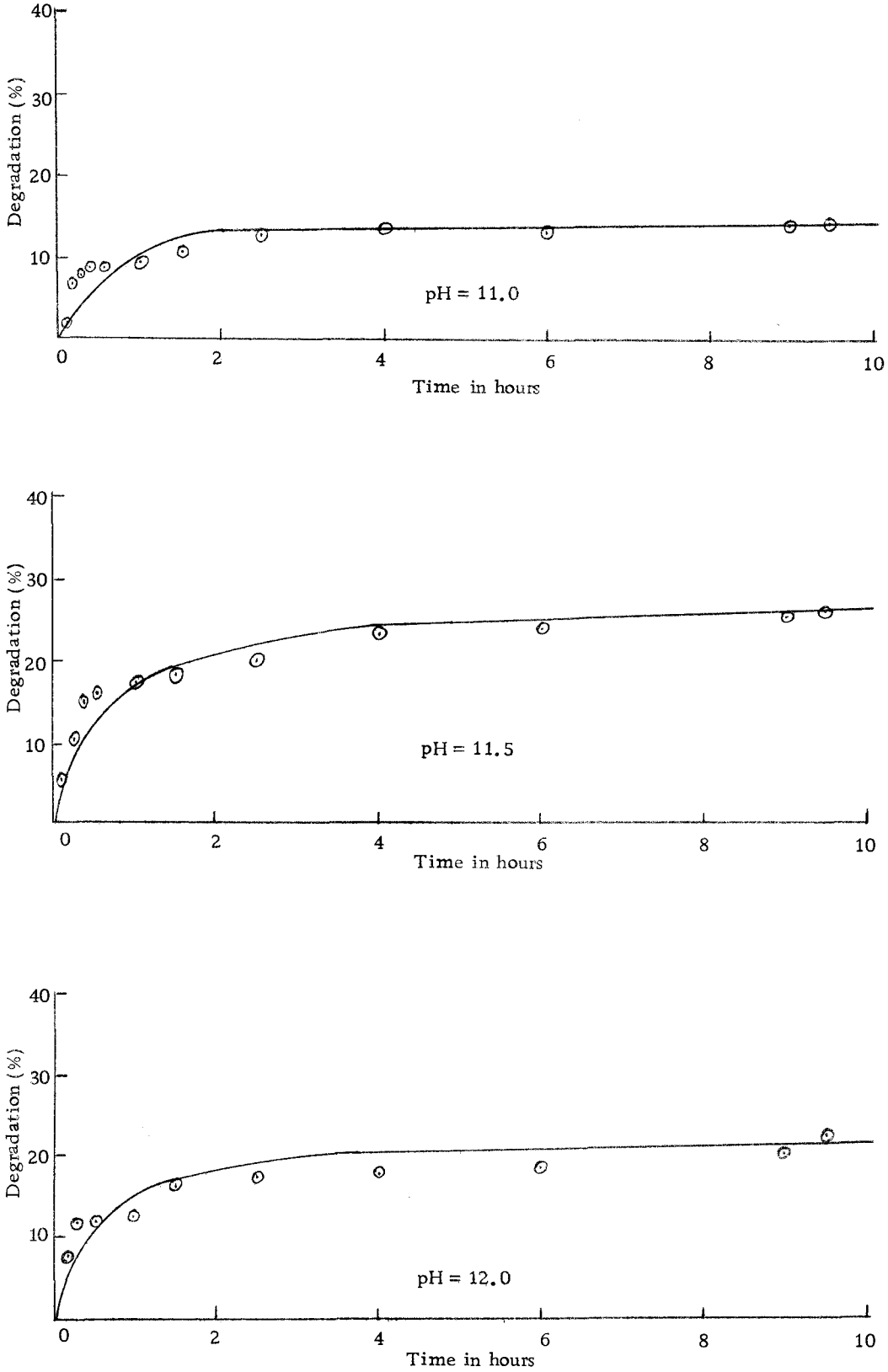
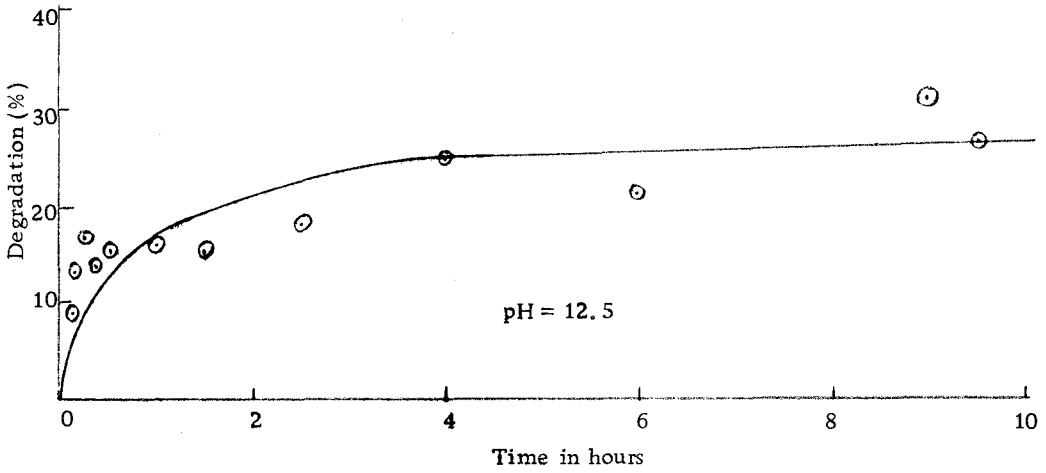
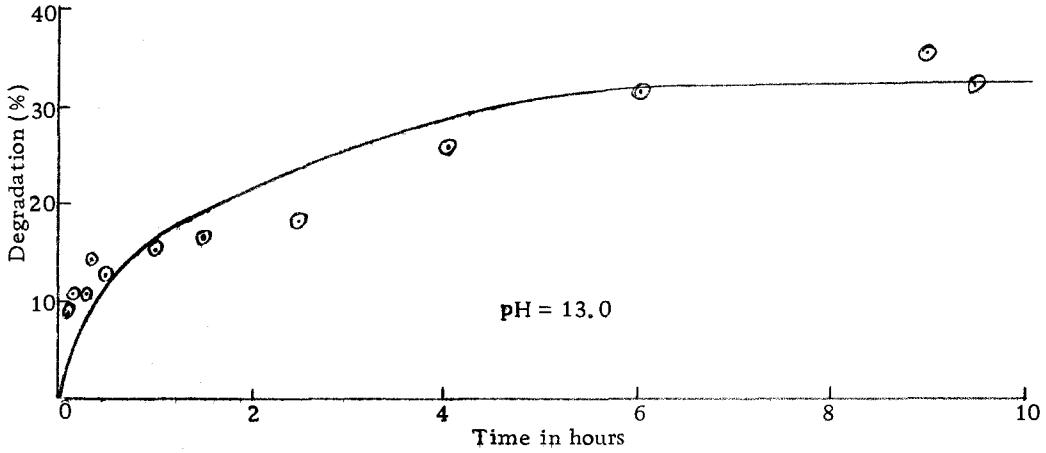


Figure 25. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 11.0-12.0 at 78.5°.





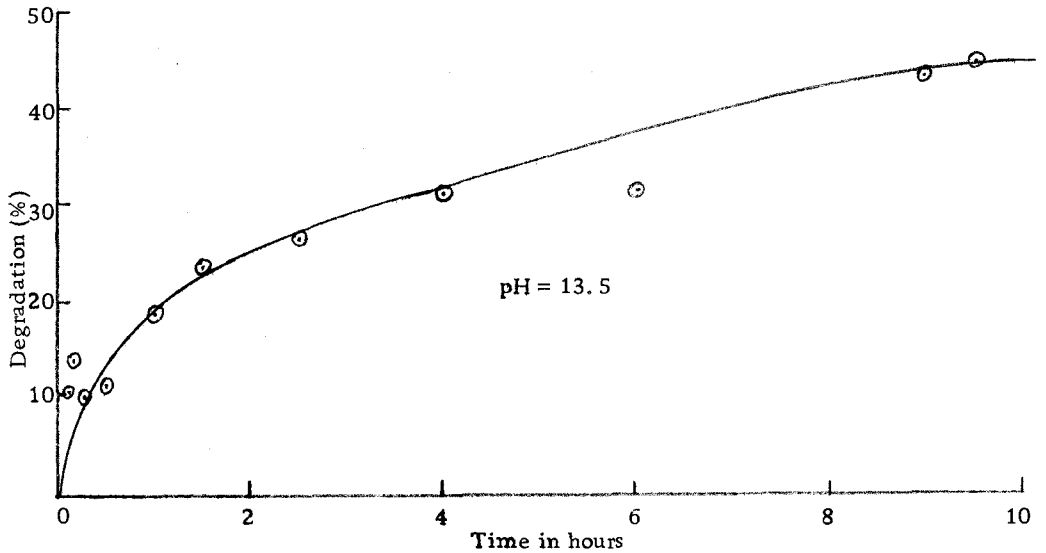


Figure 26. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 12.5-13.5 at 78.5°.

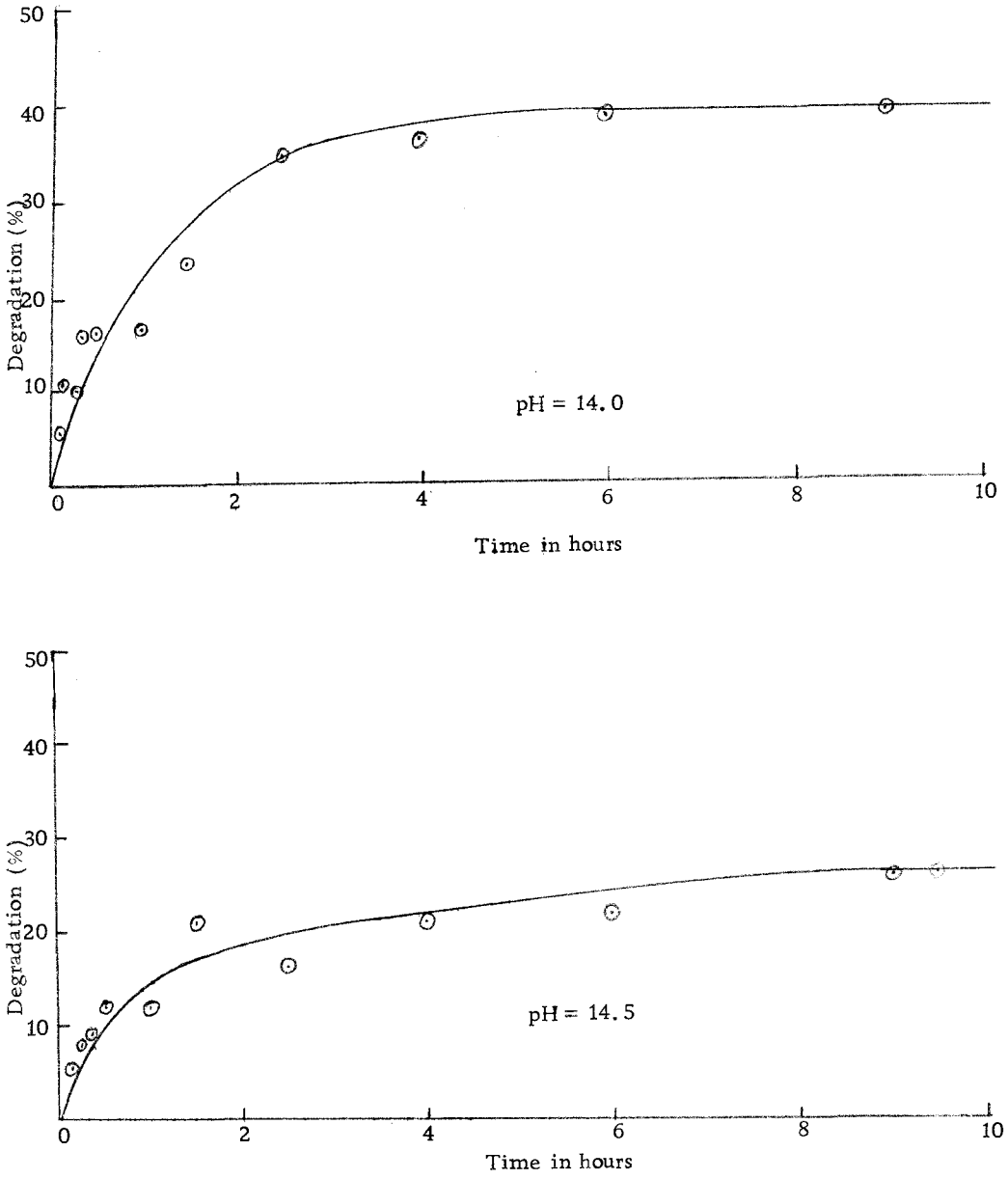


Figure 27. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 14.0-14.5 at 78.5°.

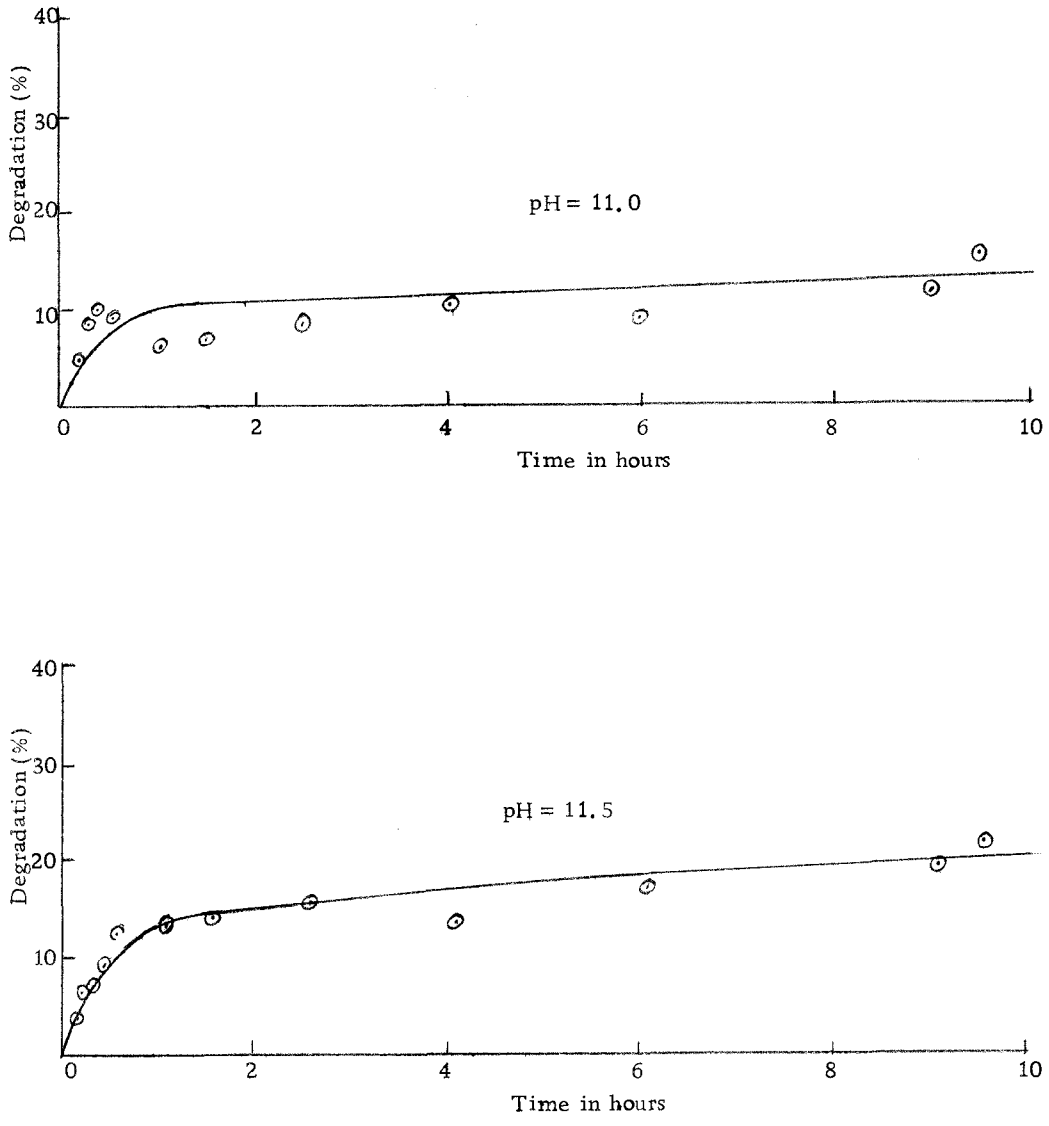


Figure 28. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 11.0-11.5 at 100°.

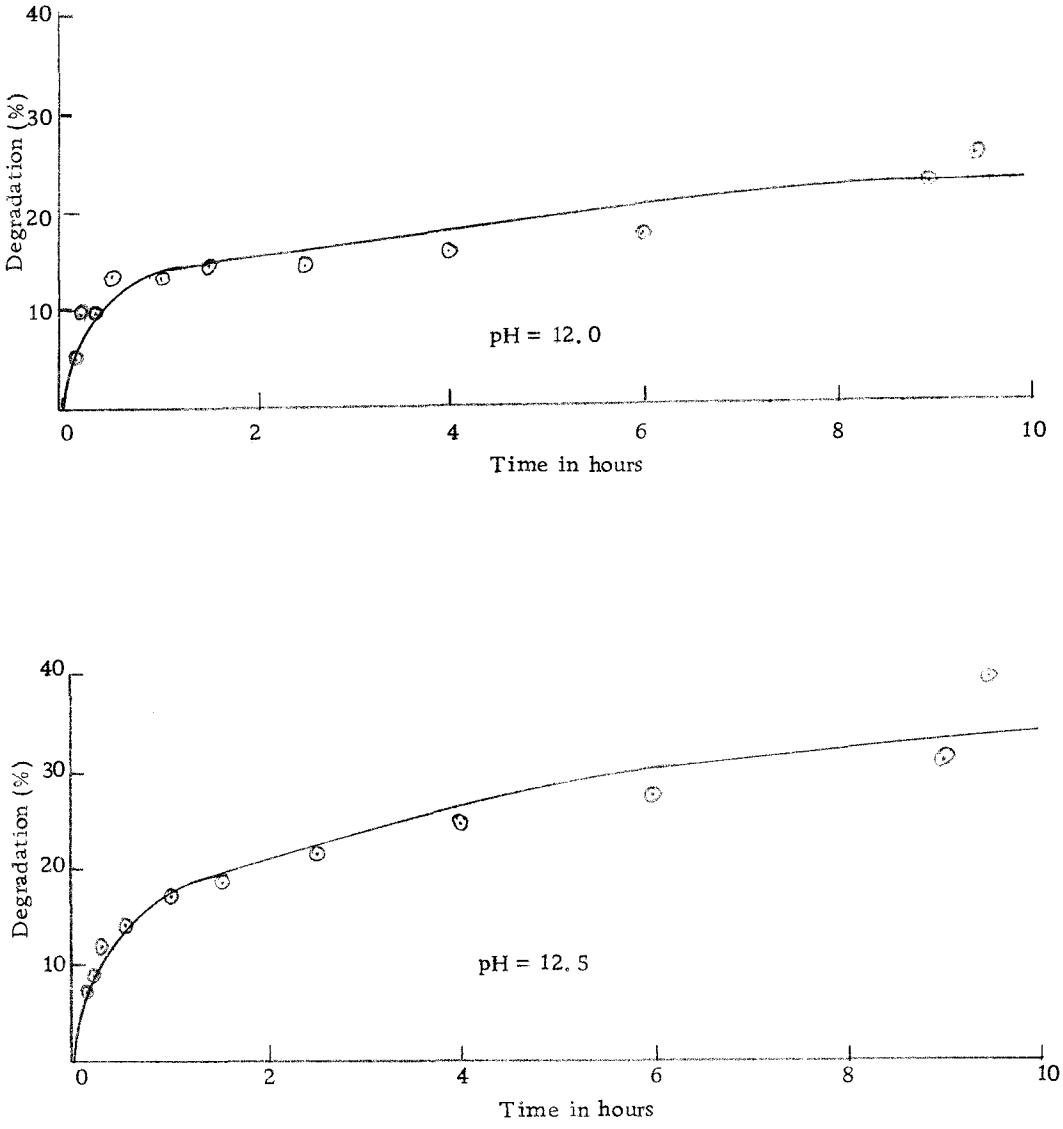


Figure 29. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 12.0-12.5 at 100°.

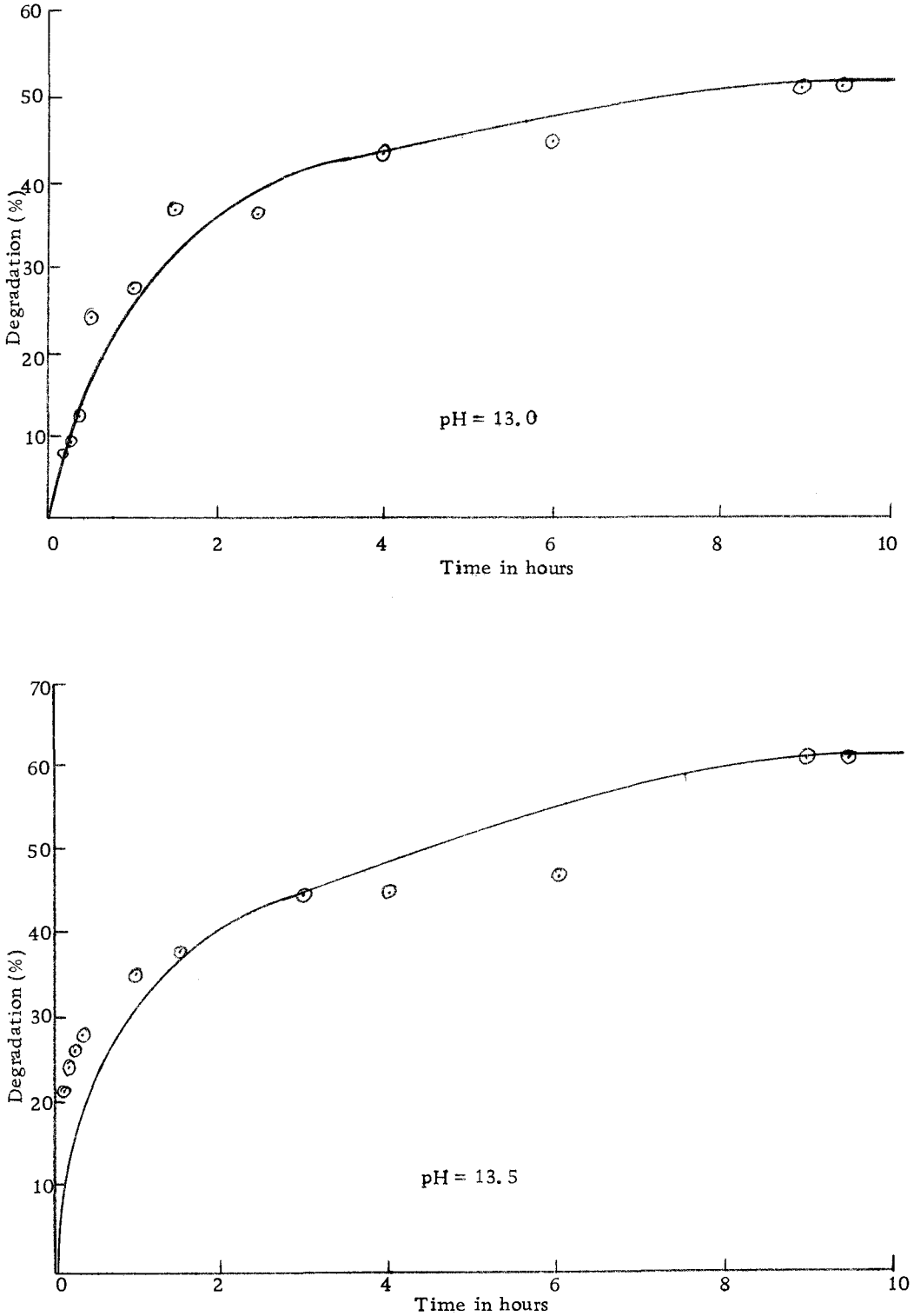
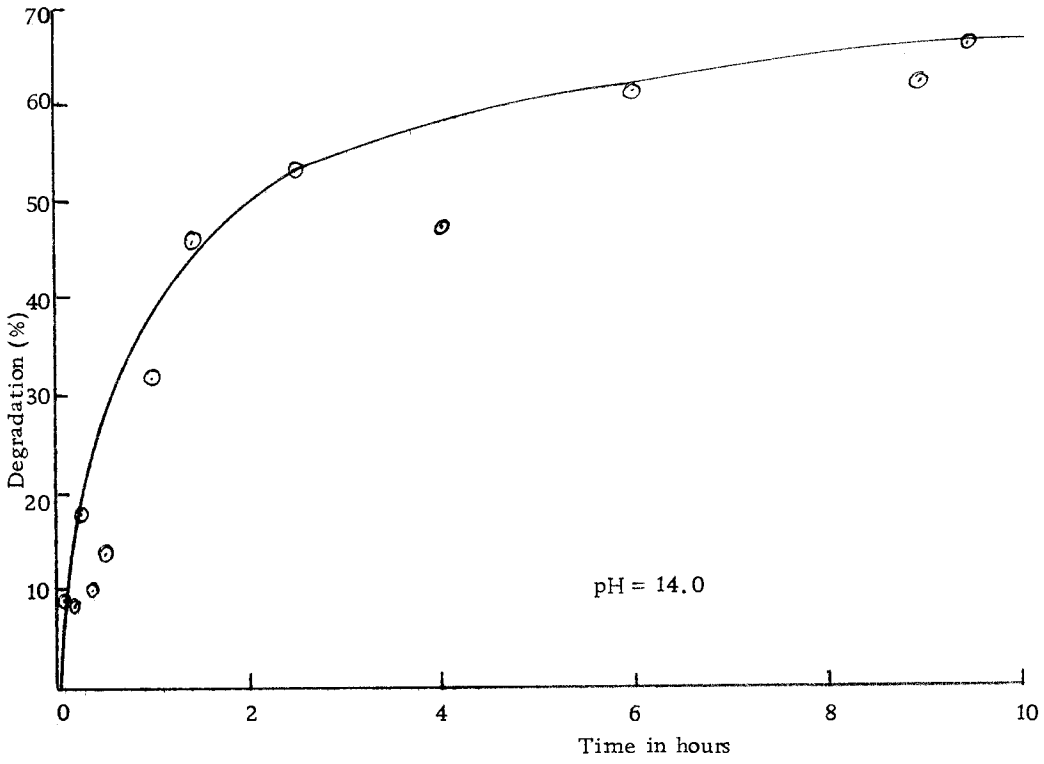


Figure 30. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 13.0-13.5 at 100°.



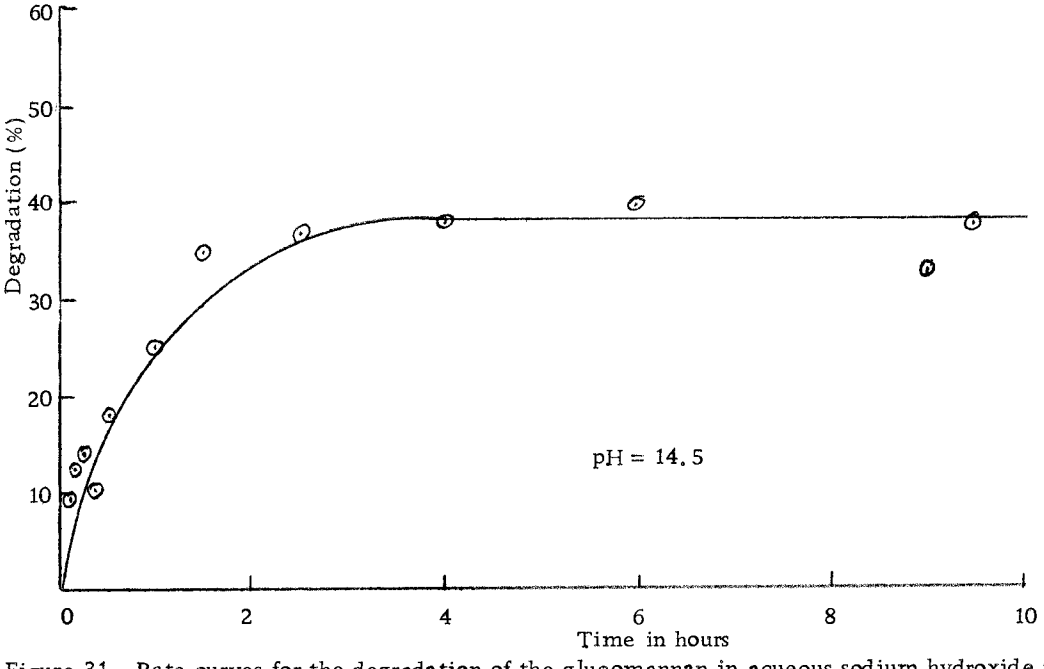


Figure 31. Rate curves for the degradation of the glucomannan in aqueous sodium hydroxide at pH values of 14.0-14.5 at 100°.

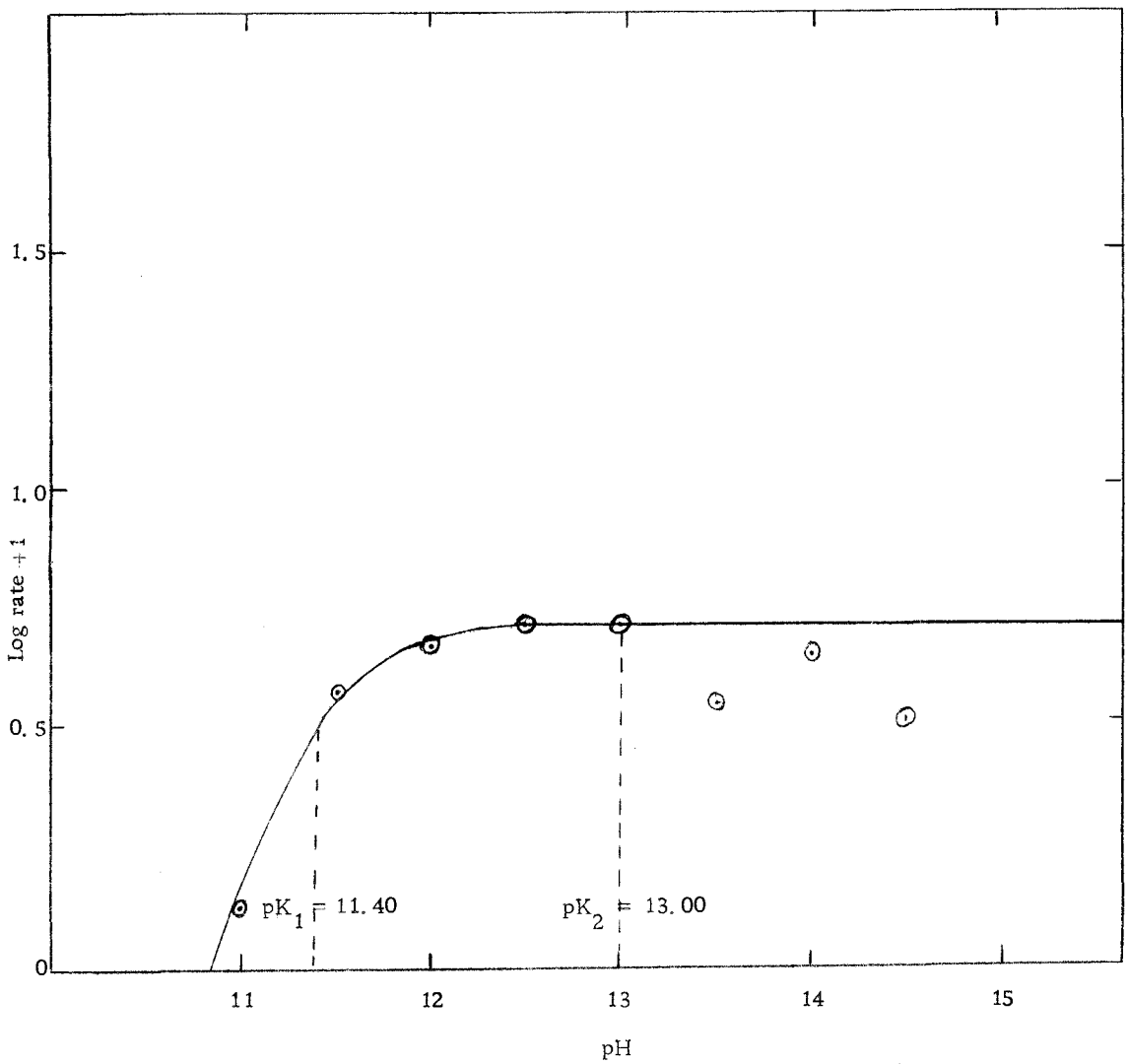


Figure 32. The effect of pH on the initial reaction rates of the alkaline degradation of the glucomannan at 64.5°.

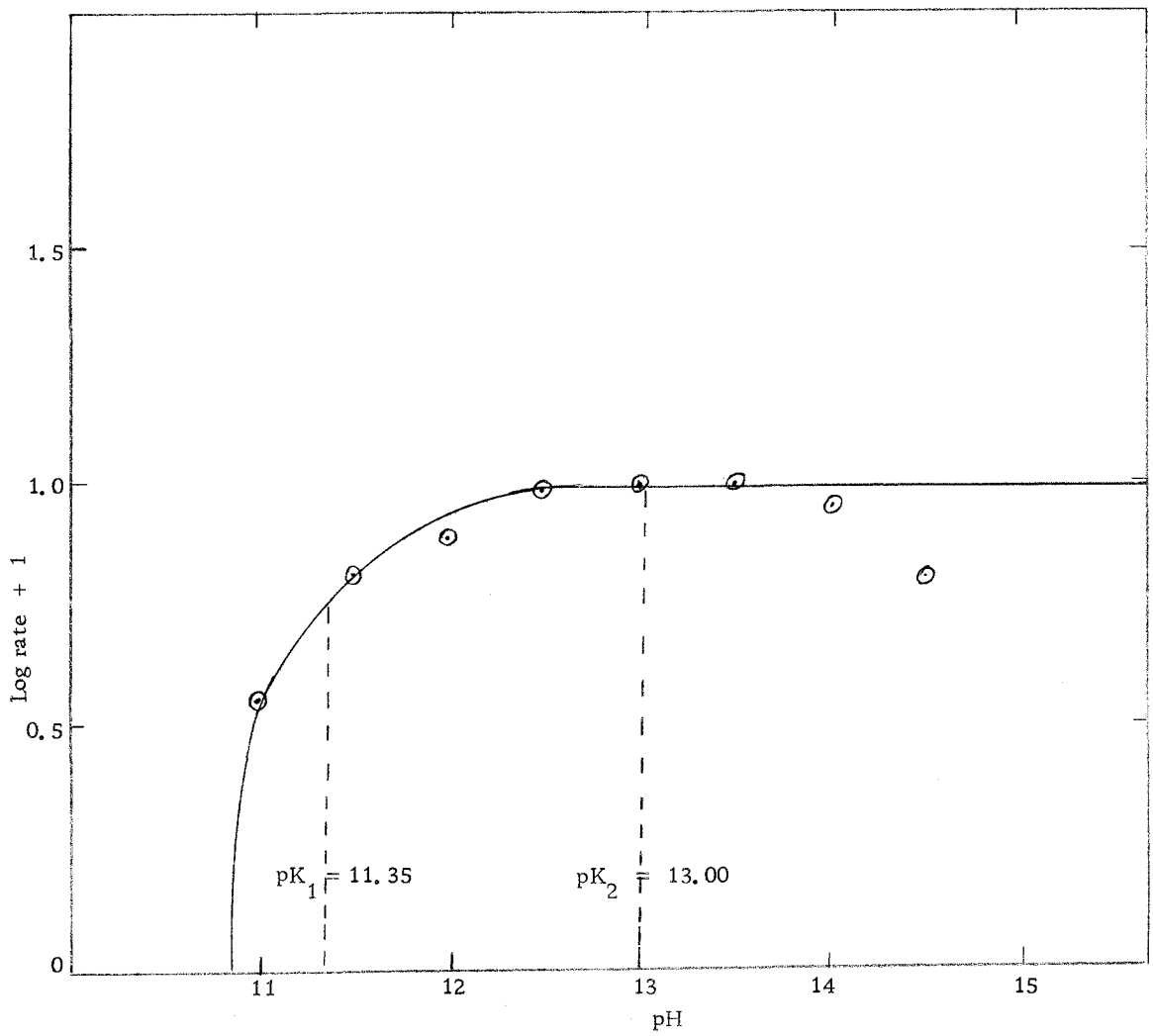


Figure 33. The effect of pH on the initial reaction rates of the alkaline degradation of the glucomannan at 78.5°.

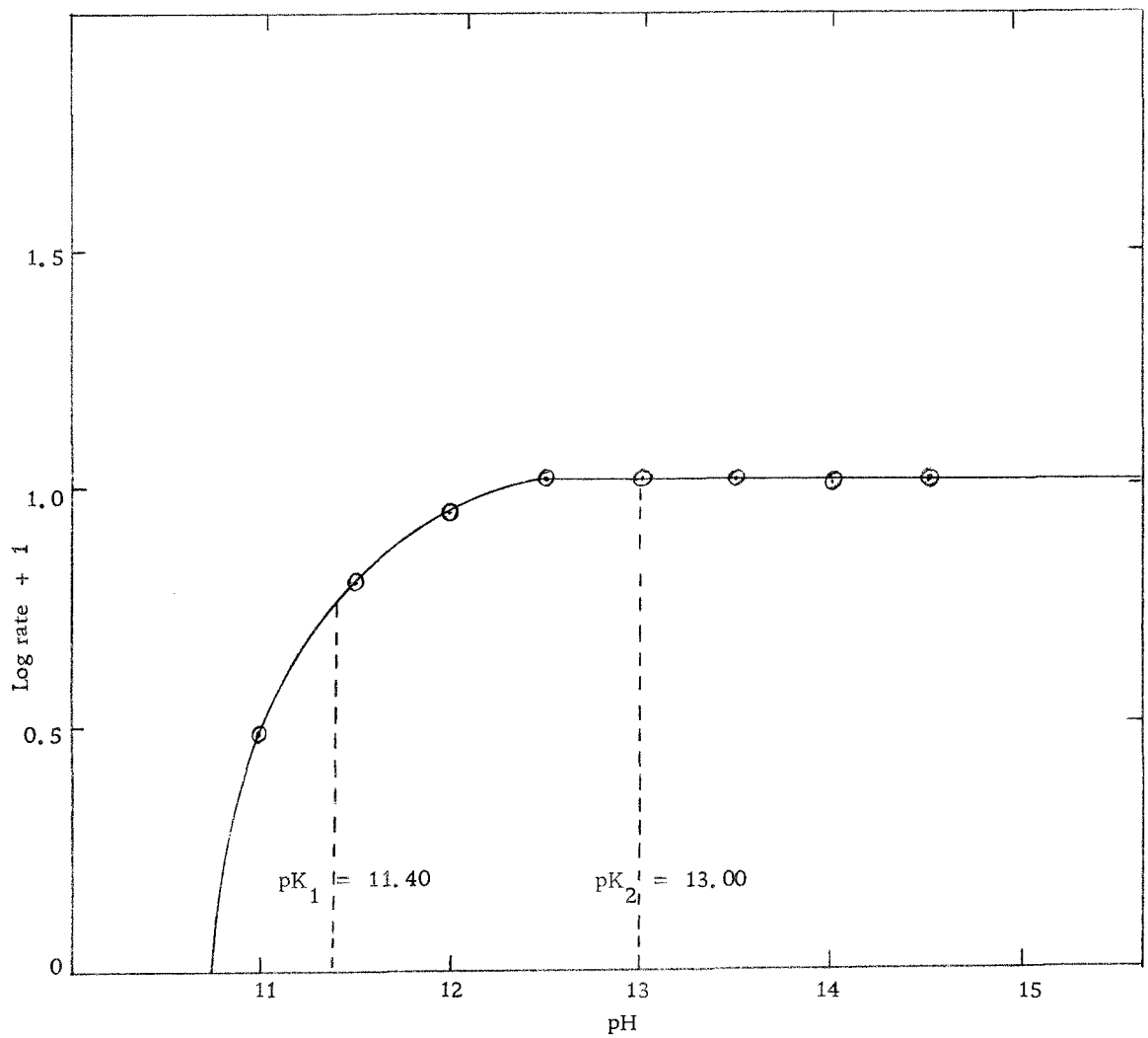


Figure 34. The effect of pH on the initial reaction rates of the alkaline degradation of the glucomannan at 100°.

considered to apply to the alkaline degradation of the glucomannan at pH 13.0 (0.1 N sodium hydroxide) and at 100°. From the data now available the constant s can be calculated from the following relationship (page 122):

$$s = K_1/([H^+]^2 + K_1[H^+] + K_1K_2) = 4.90 \times 10^{12}$$

Young, Sarkanen, Johnson and Allan (168) showed that since there was no increase in the rate of degradation of laminaran in the upper pH range where the di-anion species was the major reaction intermediate, then the rate of β-elimination from both mono- and di-anions was essentially the same. Thus the rate constants k_1 and k_2 were considered equal and were calculated for a single value. The equality of k_1 and k_2 is also valid for most (1→4)-linked polysaccharides, since the rate of degradation propagation of amylose (92) shows, like glucomannan, no inflection in the rate plot above a pH of approximately 13.0

With $k_1 = k_2$ the general expression developed for the rate of alkaline degradation (Equation 9, page 123) becomes:

$$dL/dt = s(k_1[H^+] + k_1K_2)[Gr]_o \exp(-sk_3K_2t)$$

At time t = 0 the expression $\exp -sk_3K_2t$ becomes 1.0 and the expression remaining is:

$$dL/dt = s(k_1[H^+] + k_1K_2)[Gr]_o$$

where $[Gr]_o$ is the fraction of reducing end groups in comparison to

the total number of repeating monosaccharides in the chain. For the glucomannan under the present investigation $[Gr]_o = 1/92 = 1.086 \times 10^{-2}$.

The initial rate, dL/dt, for the alkaline degradation at pH 13.0 and the temperature of 100° was 11.0×10^{-1} hr^{-1} (Figure 30, page 136).

Substituting in the values gives:

$$11.0 \times 10^{-1}\ hr^{-1} = (4.90 \times 10^{12})\ (k_1 \times 10^{-13} + k_1 \times 10^{-13})\ (1.086 \times 10^{-2})$$

$$k_1 = k_2 = 103.36\ hours^{-1}$$

The rate constants $k_1 = k_2$ were also calculated at temperatures of 78.5° and 64.5°. The values are shown in Table 8 (page 143).

The rate constant ($k_1 = k_2$) for the peeling reaction obtained for glucomannan is considerably larger than the rate constant of 5.33 hr^{-1} reported for the xylan (169) under the same conditions. The slower rate of degradation of the xylan is due to the branch points at carbon 2 (163). In order for peeling to occur on a (1→4)-linked polysaccharide, it is necessary that a ketone group be formed on carbon 2 (Figure 19, page 117). The difference in the rate of degradation between the glucomannan and the xylan is primarily due to the difference in the degree of branching.

The constant k_3 is the rate constant for the end-group stabilization (chain termination) reaction depicted in Figure 21 (page 121). It

Table 8. Calculated values of rate constants for hemicelluloses degraded in sodium hydroxide solutions.

Hemicellulose	Temp. (° C)	NaOH Conc. (N)	D. P.	$k_1 = k_2$ hr^{-1}	k_3 hr^{-1}
Glucomannan	100.0	0.1	92	103.36	1.51
	78.5	0.1	92	96.28	2.28
	64.5	0.1	92	48.15	1.90
Xylan[a]	100.0	0.1	90	5.33	0.66

[a]From: Zerrudo, J. V. Douglas-fir bark: water-soluble carbohydrates and alkaline degradation of a xylan. Doctoral thesis. Corvallis, Oregon State University. 1973. 156 numb. leaves.

is this reaction which causes degradation of the polysaccharide chain to cease. The constant can be obtained from Equation 9 (page 123) once k_1 and k_2 have been calculated.

The rate of reaction, dL/dt, at 100°, pH 13.0 and a time lapse of 2.0 hour was determined as 0.25 hr^{-1} from Figure 30 (page 136). Substituting this into Equation 9 (page 121) the expression becomes:

$$2.5 \times 10^{-1} = [4.90 \times 10^{12}] [(103.36)(10^{-13}) + (103.36)(10^{-13})]$$
$$[1.086 \times 10^{-2}] \exp^{-sk_3K_2t}$$

Solving the equation for k_3 results in a termination rate constant k_3 = 1.51 $hour^{-1}$. The termination rate constant k_3 was also calculated at temperatures of 78.5° and 64.5° and are shown in Table 8 (page 143).

It is recognized that k_3 values are calculated at some time t (Table 9, page 145) whereas the $k_1 = k_2$ values are calculated at t = 0.0 hr. However, this is required to solve the mathematical expression (Equation 9, page 123). The data in Table 9 (page 145) show that the variations in k_3 with time and temperature are quite small for rate constants of this type. A comparison between the rates of degradation (k_1=k_2) and the rates of termination (k_3) are useful in describing the final extent of polysaccharide degradation.

The rate constant k_3 for the glucomannan at 100°, pH 13.0 and a time lapse of 2.0 hr was determined as 1.51 hr^{-1} (Table 8, page 143). This rate constant is two times larger than the rate constant

Table 9. Calculated values of the termination rate constants, k_3, at pH 13.0 and various times and temperatures.

Time (hr)	k_3		
	100°	78.5°	64.5°
0.25	7.45	8.16	8.60
0.50	3.65	5.78	5.04
0.75	4.02	5.25	4.21
1.00	3.02	3.81	3.15
1.25	2.49	3.04	2.52
1.50	2.06	3.05	2.54
2.00	1.51	2.28	1.90

($k_3 = 0.66\ hr^{-1}$) reported for the xylan (169). However, the relative rate of termination compared to the rate of the peeling reaction itself is smaller for the glucomannan than for the xylan. That is, the ratio $k_3/k_1 = 1.51/103.36 = 1.46 \times 10^{-2}$ for the glucomannan is much smaller than the same ratio $k_3/k_1 = 0.66/5.33 = 1.2 \times 10^{-1}$ for the xylan. The branch points in the xylan undoubtedly are responsible for the faster relative termination rate.

Compared with the xylan the glucomannan ought to be more sensitive to the peeling reaction due to its molecular structure. There is no retarding effect of substituents except for the branches. However, branches attached to C6 of chain units would not likely disrupt the degradation of the main chain. For example, Kenner and Richards (79) have shown that substitution of the 6-hydroxyl group with an O-methyl group in both 3-O-methyl and 4-O-methyl-D-glucose enhances the rate of degradation.

In a previous investigation of the alkaline degradation of pine glucomannan, Hansson and Hartler (62) reported a loss of about 50% when the glucomannan was treated with molar sodium hydroxide at 100° for 8 hours. This value is close to the 60% loss obtained for the alkaline degradation of the glucomannan in the present work. This indicates a similarity in the structure of pine glucomannan and that of the glucomannan from Douglas-fir inner bark. The proposed structure of the glucomannan in the present work (Figure 18, page

113) is one which consists of glucopyranose and mannopyranose residues in the main chain and has branch points on carbon 6 of some units.

The effect of temperature on the rate of degradation of the glucomannan in 0.1 N sodium hydroxide is shown in Figure 35 (page 148). The activation energy can be calculated from the Arrhenius equation:

$$k = Ae^{-E/RT}$$

where

k = rate constant

A = frequency factor

E = activation energy

R = gas constant

T = absolute temperature

By plotting $\log_{10} k$ against the reciprocal of the temperature, a straight line can be obtained. Its slope is equal to $\frac{-E}{2.303\ R} = \frac{-E}{4.57}$. From a plot (Figure 36, page 149) of the corresponding rate-constants with temperature, an activation energy of 5.9 kcal/mole for the peeling reaction was calculated. This illustrates that the rate of degradation of glucomannan is highly temperature dependent.

The data from the alkaline degradation studies support the structure proposed for the glucomannan in Figure 18 (page 113). The low relative rate of termination compared to the rate of peeling

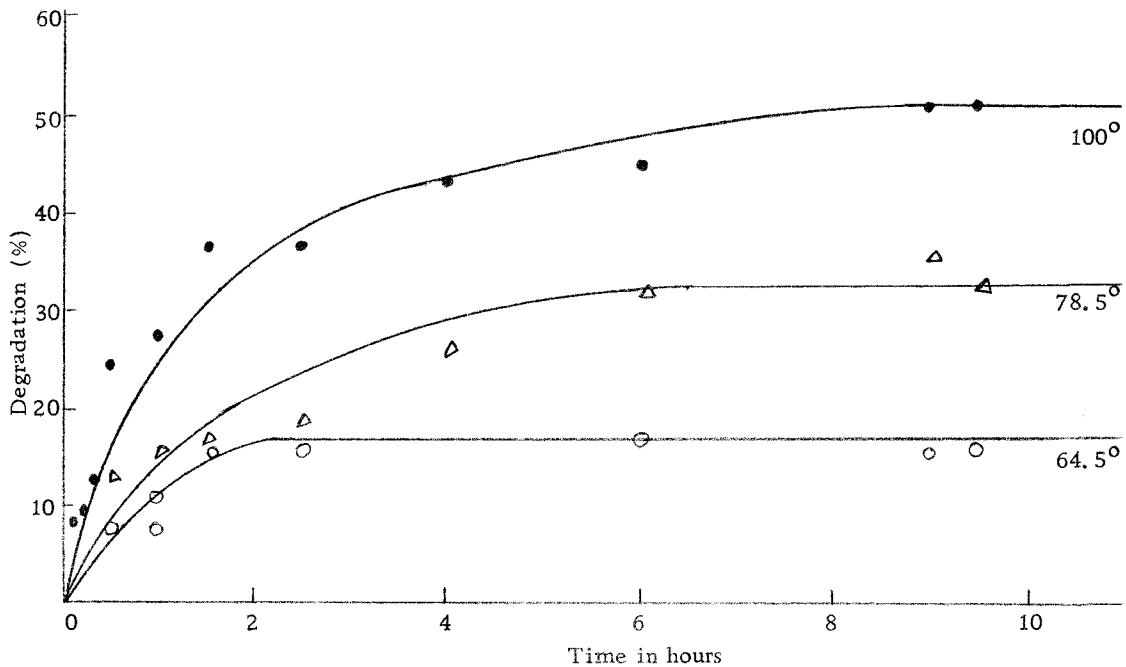


Figure 35. The effect of temperature on the degradation of the glucomannan in 0.1 N sodium hydroxide.

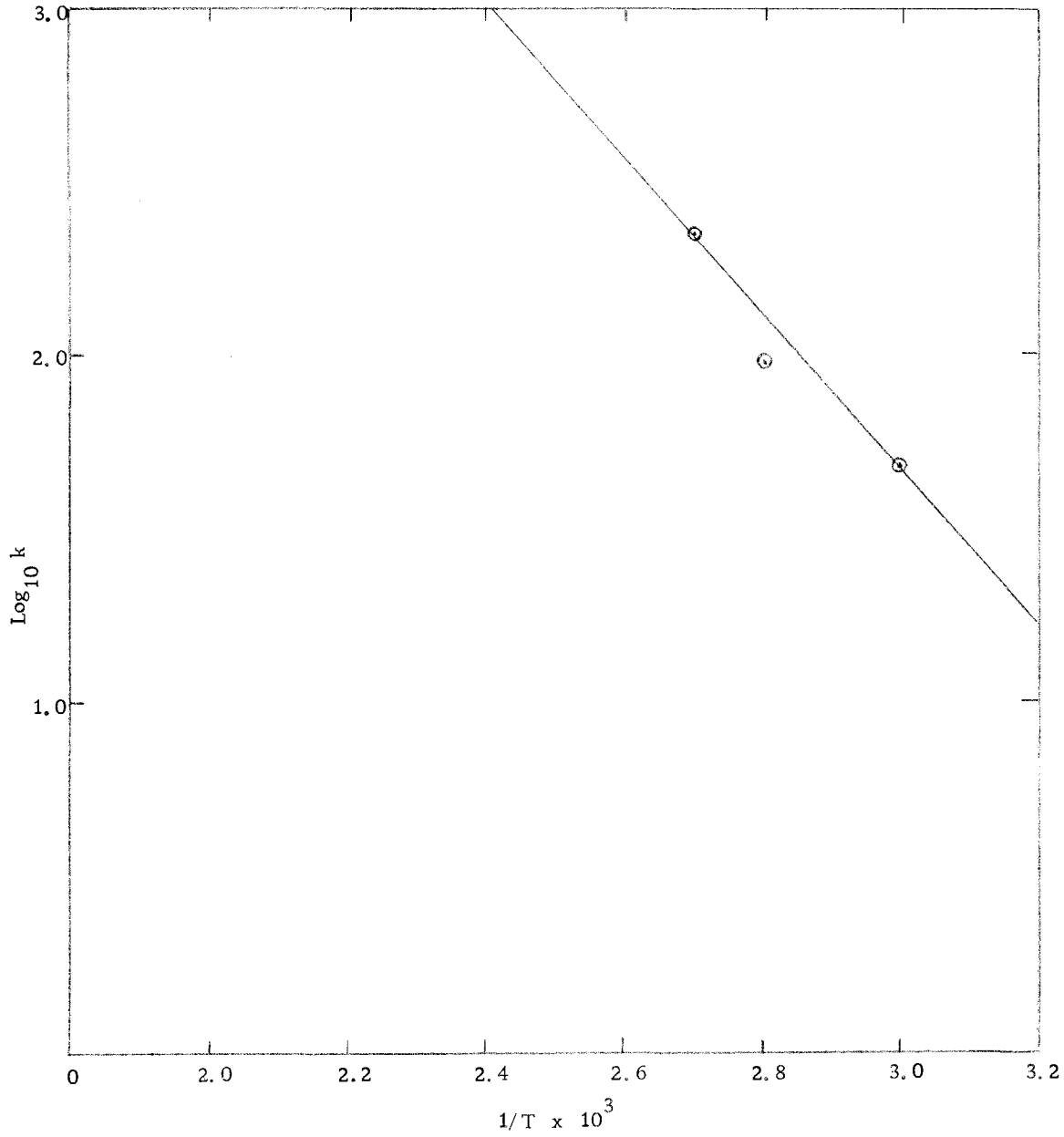


Figure 36. Arrhenius plot for the peeling reaction in 0.1 N sodium hydroxide.

supports the absence of branch points at carbons 2 and 3. Branching is presently the most probable factor causing incomplete degradation of polysaccharides. On the other hand branch points at carbon 6 enhances the rate of degradation as shown by the relatively high rate of the peeling reaction and the high amount of glucomannan degraded.

2. Phenol-Sulfuric Acid Method of Analysis of the Glucomannan

Phenol in the presence of sulfuric acid can be used for the quantitative colorimetric microdetermination of sugars and their methyl derivatives, oligosaccharides, and polysaccharides (143). The method is simple, rapid, and sensitive, and gives reproducible results. The reagent is inexpensive and stable, and a given solution requires only one standard curve for each sugar. The color produced is permanent and it is unnecessary to pay special attention to control of the conditions. Several investigators (144, 152) have investigated this method and found it satisfactory. Lindberg, Theander and Uddegard (102) have demonstrated that saccharinic acid products do not interfere with the color reactions.

The standard curve for mannose is shown in Figure 37 (page 151). Measurements were taken at 490 nm. Hexoses and their methylated derivatives have an absorption maximum at this wavelength. Pentoses, methyl pentoses, and uronic acids absorb at

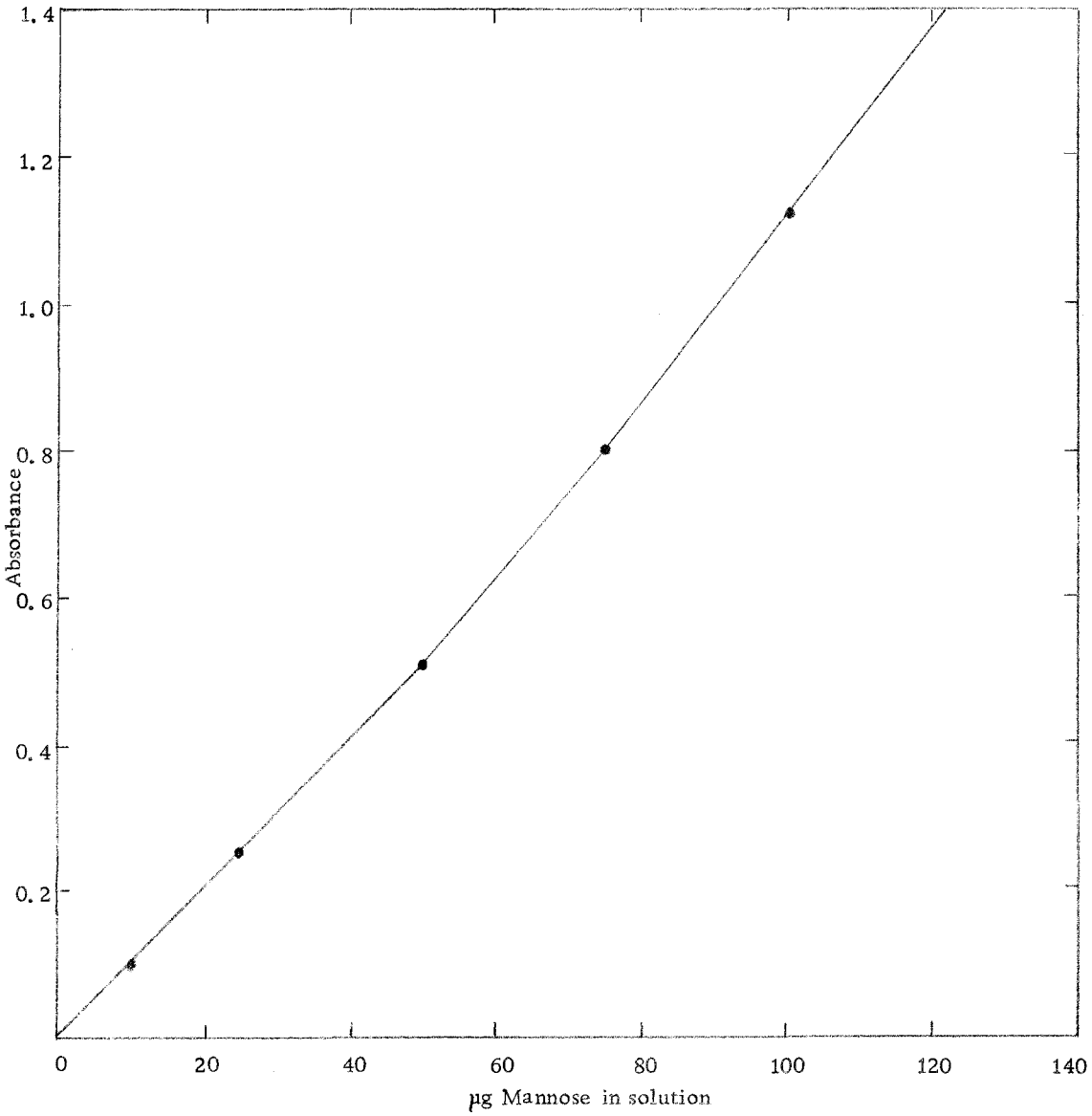


Figure 37. Standard curve for mannose in phenol and concentrated sufluric acid. Measured at 490 nm.

480 nm. Certain of the methylated pentose sugars and their methyl glycosides show selective absorption at 415 to 420 nm.

The intensity of the color is a function of the amount of phenol added. As the amount of phenol is increased, the absorbance increases to a maximum and then usually falls off. The colors produced are unusually stable, and possess a definite absorption peak. The amount of color produced at a constant phenol concentration is proportional to the amount of sugar present. The phenol-sulfuric acid method can be used to give reliable estimations of the sugar content of pure solutions. Under proper conditions the method can be expected to be accurate within ±2%.

V. SUMMARY AND CONCLUSIONS

1. A sample of Douglas-fir inner bark was successively extracted with ethanol-water (4:1 v/v), benzene-ethanol (2:1 v/v), hot water, 0.5% ammonium oxalate, and acidified sodium chlorite solution.
2. A glucomannan polysaccharide was isolated from the insoluble fraction (holocellulose) remaining after treatment of the bark residue with acidified sodium chlorite solution.
3. The ash content of the crude glucomannan was 9.70%. The high ash content was a result of the addition of barium hydroxide used in isolation of the polysaccharide. Dialysis lowered the ash content to 3.27%.
4. The glucomannan contained 1.05% acid-insoluble lignin (Klason determination) and 0.94% acid-soluble lignin.
5. The glucomannan possessed a specific rotation of -35.9° in aqueous sodium hydroxide solution indicative of a β-D-glycosidic linkage.
6. The glucomannan showed a negative carbazole-sulfuric acid color test for the presence of hexuronic acids.
7. Gas-liquid chromatographic analyses showed that the glucomannan was composed of the following sugar residues: anhydromannose (56.43%), anhydroglucose (26.20%), anhydrogalactose (7.67%), anhydroxylose (1.46%) and anhydroarabinose (1.44%).

8. End-group analysis by the Somogyi copper reduction method showed a molecular weight of 1.58×10^4. This in combination with the gas-liquid chromatographic analysis showed a degree of polymerization of 93.

9. The intrinsic viscosity of the glucomannan in cadoxen solution was 0.46 dl/g which corresponded to a degree of polymerization of 92.

10. The glucomannan was completely methylated and the methylated glucomannan was hydrolyzed. The major component in the hydrolyzate as identified by paper chromatography was 2, 3, 6-tri-O-methyl-D-mannopyranose.

11. Gas-liquid chromatography and mass spectrometry of the methylated glucomannan alditol acetates showed that the hydrolyzate from the methylated glucomannan was composed of 2, 3, 4, 6-tetra-O-methyl-D-glucopyranose, 2, 3, 4, 6-tetra-O-methyl-D-galactopyranose, 2, 3, 6-tri-O-methyl-D-mannopyranose, 2, 3, 6-tri-O-methyl-D-glucopyranose, 2, 3-di-O-methyl-D-mannopyranose, and 2, 3-di-O-methyl-D-glucopyranose.

12. The data are consistent with a polysaccharide glucomannan repeating unit composed of a backbone of anhydro-D-mannopyranose and anhydro-D-glucopyranose attached β-D-(1→4) plus a reducing and non-reducing end group. There are

side chains of anhydro-D-galactopyranose units linked (1→6) to the mannopyranose and the glucopyranose units of the main chain. The structure of this glucomannan is similar to the glucomannan previously isolated from the wood and bark of gymnosperms.

13. The glucomannan was reacted at 100°, 78.5° and 64.5°, with sodium hydroxide solutions ranging in concentrations from 0.001 N to 3.162 N. The rate of degradation did not change at alkali concentrations above 0.1 N in sodium hydroxide.

14. Alkaline degradation of the glucomannan in 0.1 N aqueous sodium hydroxide at 100°, 78.5° and 64.5° showed a rate constant for end-group peeling of $k_1 = k_2 = 103.36$, 96.28 and 48.15 hr^{-1} respectively. The termination rate constants k_3 at 100°, 78.5° and 64.5° were 1.51, 2.28, and 1.90 hr^{-1}.

15. The peeling reaction stopped when 60% of the glucomannan had been degraded. This large amount of degradation reflected the relatively large peeling rate constant compared to the termination rate constant.

16. The activation energy for the peeling reaction was 5.9 kcal/mole.

17. The results of the alkaline degradation supported a possible reaction sequence involving mono- and di-anionic end-group species as the intermediates leading to end-group elimination

of reducing polysaccharides.

18. The rate constants for degradation of the glucomannan illustrate that a lowering of the temperature and a lowering of the alkali content results in a decreased degradation of the glucomannan. An extrapolation of these results to pulping procedures might be beneficial to the recovery of a greater amount of carbohydrates.

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