THE ACTION OF LIQUID AMMONIA ON SPRUCE CHLORITE HOLOCELLULOSE

A Thesis

by

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GENERAL INTRODUCTION

In 1947 M. M. Yan found that liquid ammonia at 25° C. extracted nearly 6% by weight of sugar maple wood. The extract was separated into three fractions, acetamide 4%, "liquid ammonia lignin" 0.7%, and a polyuronide 0.8%. The liquid ammonia cleaved ester links, as shown by the nearly quantitative production of acetamide from the acetyl groups in the wood. This finding suggested that after liquid ammonia extraction, the woody residue might be chemically changed, and that previously insoluble components might then be capable of extraction.

In 1949 L. G. Neubauer investigated this possibility, and found that hot water extracted about 2% of polysaccharide material, not previously water-soluble, from the ammonia-extracted maple wood. Neubauer confirmed Yan's findings and also showed that liquid ammonia cleaved ester linkages other than those of acetyl groups. The aqueous extract was found to contain a xylan-methoxyglucuronic acid complex, contaminated by pectic materials. Further studies suggested that the "pure" polysaccharide, not isolated as such, was composed of a repeating unit consisting of a chain of 4 xylose units in 1, 4 - glycosidic union, terminated at the reducing end by a more acid-resistant aldotriuronic acid unit, consisting of 2 xylose units and a 3 - methoxy-glucuronic acid residue.

As a result of Neubauer's work, it appeared as if hemicelluloses could be extracted from woods under milder conditions than the usual procedure involving caustic soda or potash. Since delignification of wood should facilitate the extraction of the hemicelluloses, delignified black spruce wood, i.e. holocellulose, was subjected to extraction with liquid ammonia. It was found that 4.95% by weight of the wood was removed, while the subsequent cold and hot water extracts amounted to 12.7%. The greater part of the work was concerned with the purification and composition of the polysaccharides present in the water extracts.

HISTORICAL INTRODUCTION

Anhydrous liquid ammonia possesses high solvent powers and also resembles water in being neutral in reaction. These attributes, together with a marked ability to swell the cellulose fabric of wood, caused Yan to employ liquid ammonia as a solvent for lignin *in situ* (1).

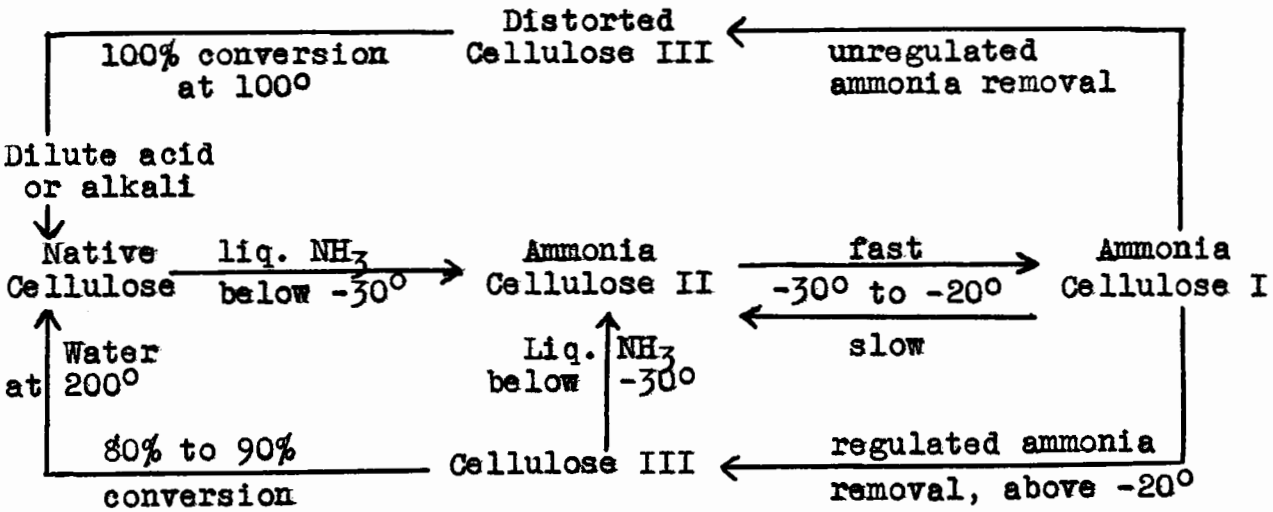
The effect of liquid ammonia on cellulose has been reviewed (1) (2), so a detailed treatment is not necessary here. Cellulose swollen by ammonia possesses different crystalline modifications, as shown by the X-ray diffraction patterns (3) (4) (5). The nature of the changes involved were clarified by Hess and Gundermann (6) who carried out the X-ray examination while the fibres were still immersed in the liquid ammonia. Ramie fibres gave two ammonia-celluloses, mutually interconvertible between -20° and -30°. When the ammonia-cellulose II, stable at lower temperatures, was warmed, it changed rapidly to ammonia-cellulose I, stable above -20°, but the reverse change was slow.

Ammonia-cellulose II was taken to be $C_6H_{10}O_5(NH_3)_6$, but inability to determine the amount of ammonia in I made it impossible to decide whether I and II were polymorphs or different chemical complexes. Loss of ammonia from

ammonia-cellulose gave a form, resembling that of cellulose mercerized by caustic soda, which Hess and Gundermann called ammonia-cellulose III. The method of production of cellulose III largely influenced its exact nature; a slow, regulated evaporation of ammonia giving a more ordered X-ray pattern and a product which reverted to natural cellulose in 80% to 90% yield when heated with water to 200°. Less ordered cellulose III was formed when the fibres were removed rapidly from the liquid ammonia and the excess ammonia was evacuated, or when the ammonia was permitted to evaporate under atmospheric pressure and the product was kept in the air for several weeks. This cellulose III was converted more easily and more completely to natural cellulose by boiling with dilute acid or alkali at ordinary pressure. The results are summarized in Fig. 1.

FIG. 1

CRYSTAL FORMS OF THE AMMONIA CELLULOSES (a)



(a) From Hess and Gundermann (ref. 6).

Barry and co-workers (7) in a later paper reported that an ammonia-cellulose, very similar to ammonia-cellulose I, was formed when cellulose was treated with liquid ammonia at room temperature. In these investigations all other modifications of cellulose obtained were considered to be more or less distorted forms of one or more of the three basic lattices:- natural cellulose, ammonia-cellulose I, and the hydrate-like cellulose III. These investigations indicated that ammonia molecules entered into the unit cell of cellulose in the regular manner of a permutoid swelling agent.

Excellent reviews on the properties and reactions of liquid ammonia have been published (8) (9), so only effects of interest to this thesis will be briefly noted. In general, liquid ammonia resembles alcohol in its solvent powers in the organic field. Most aliphatic and aromatic ethers, aldehydes and ketones are soluble in liquid ammonia, but aldehydes and ketones frequently react to give imino compounds (8). Carboxylic acids, such as acetic and benzoic acids, are dissolved as ammonium salts, but higher aliphatic acids and dicarboxylic acids are rather insoluble. Treatment of an acid, such as acetic, or an acid anhydride, with liquid ammonia under increased pressure results in the acid amide (10). Most acid amides are quite soluble, as are esters, but most of the esters

undergo ammonolysis to the corresponding amide.

All of the ordinary sugars, as well as their methylated, acetylated and isopropylidene derivatives, are quite soluble in liquid ammonia at its boiling point, -33.5°C (11) (12). Liquid ammonia has no effect on these carbohydrates at this temperature unless they have free potential carbonyl groups (12).

The reducing sugars, when dissolved in liquid ammonia at -33.5°, form the amines after first forming an aldehyde addition product. Glucose, for example, is converted into 1-amino-glucose (12). Muskat found that boiling liquid ammonia dissolved the acetylated and benzoylated derivatives of any sugar without deacetylation (13), provided the reducing group was blocked by forming the methyl glycoside or 1,2-isopropylidene compound. Liquid ammonia at room temperature and approximately 8.5 atmospheres pressure did remove all acetyl groups from the sugars.

Hess and Heumann initiated the use of cellulose-swelling liquids for the extraction of lignin from wood (14). It was hoped that the lignin could be isolated with less chemical change than was possible with other methods, since the use of heat, strong acid and alkali was avoided. Aqueous solutions of hydrazine, ethylene diamine, and amino

bases such as monoethanolamine, triethanolamine and tetramethyl ammonium hydroxide extracted lignin from rye straw. These reagents were permutoid swelling agents for cellulose (15) (16), but Hess and Heumann controverted one of their own principles, since their aqueous solutions were strongly alkaline. Hydrazine in aqueous solutions had a basicity close to that of aqueous ammonia (17), while aqueous tetramethyl ammonium hydroxide was as basic as sodium hydroxide (18).

Shortly before the work of Hess and Heumann, the action of alkali metals and alkali metal amides, dissolved in liquid ammonia, on isolated lignin and on wood was studied by Freudenberg and co-workers (19) (20). Freudenberg was attempting to isolate a lignin with its carbon skeleton relatively intact. The use of alkali metal amides caused side reactions, so in the later paper (20), Freudenberg used the alkali metals. Treatment of spruce wood meal for several hours with a solution of potassium in liquid ammonia at 20° dissolved only 1.9% of the meal. However, lignin amounting to 16% to 18% of the meal was then soluble in methanol, and the remaining 8% could be extracted by 1% caustic soda. A residue of 41.8%, supposedly in a relatively undegraded state resembling Cross and Bevan Cellulose, was recovered. Beech wood, treated in a similar manner, gave an appreciable amount of the

cellulose degraded to a methanol-soluble product. The effects of potassium in liquid ammonia on a series of model substances were also studied. Phenol ethers underwent complete or partial dealkylation, yielding free phenolic groups. Freudenberg found that this treatment increased the hydroxyl content of the lignin, and inferred that the solubilisation of lignin was connected with a similar dealkylation or with a rupture of phenolic ether links. In Franklin's ammonia system the use of an alkali amide in ammonia is the equivalent of aqueous caustic soda in a normal alkali cook.

These experiments of Hess and Freudenberg led Yan (1) to the use of liquid ammonia alone as a solvent for lignin, without the use of heat, acids or alkali. He found that hardwoods lost more lignin than softwoods, but both were less solubilised than rye straw, which lost up to half of its lignin to liquid ammonia. Since temperatures above 25°C caused no significant increase in the amount of extract, Yan adopted this temperature with an extraction period of five hours, after which time very little additional material went into solution.

Liquid ammonia extracted 5% to 7% by weight of sugar maple wood, the extract being separated into three fractions differing in solubilities. The first fraction, 0.8%,

consisting of polysaccharides almost insoluble in all the solvents tried, had a pentosan content of 6.9% and a methoxyl content of 4.4%. The second very soluble fraction was shown to be acetamide, in amount almost equivalent to the 3.4% of the acetyl groups removed from the original wood. The remaining fraction "liquid ammonia lignin", was recovered in 0.7% yield and represented 3% of the Klason lignin in the wood. This fraction yielded the sub-fractions; (a) soluble in methanol and dioxane, (b) soluble in dioxane, insoluble in methanol, and (c) insoluble in both solvents. Fraction (a) amounted to half of the extracted lignin and seemed to be quite homogeneous. Yan assigned the formula $C_{42}H_{43}O_{15}(OCH_3)_7$ to this fraction as a result of carbon, hydrogen and methoxyl analyses, together with molecular weight determinations in dioxane. Methylation with dimethyl sulphate and sodium hydroxide introduced five methoxyl groups per molecular unit. Since this fraction represented only 1.5% of the total Klason lignin, and had an elementary composition different from that of the total lignin, it was not claimed that the sample was representative of the whole. Yan found that the nitrogen content of "liquid ammonia lignin" was negligible. This observation suggested that the lignin was extracted from wood either by a purely physical process, or by a chemical process involving ammonolysis of ammonia sensitive links, probably ester links,

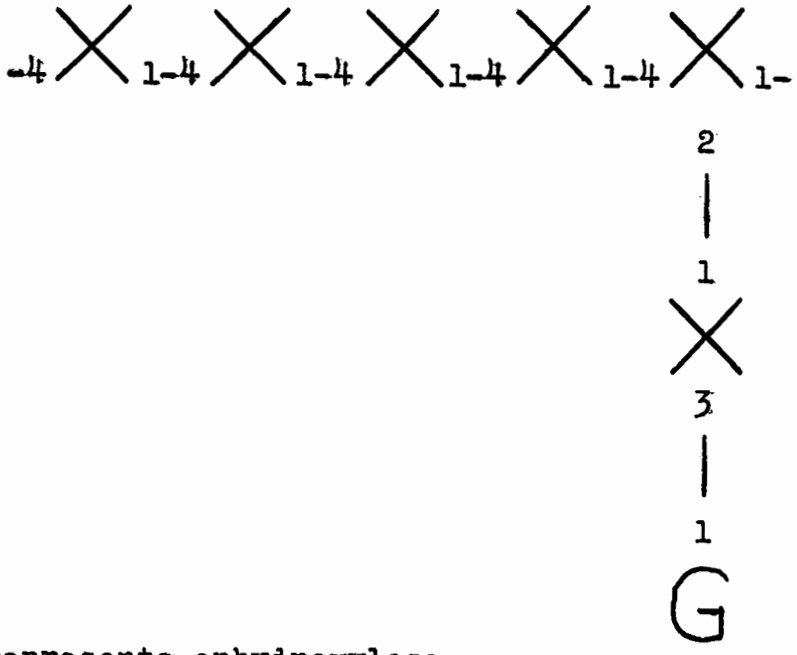
between hydroxyl groups of the lignin and carboxyl groups of the carbohydrate residue. In such a process any amide groups formed would be found with the carbohydrate portion of the wood.

The nearly quantitative production of acetamide from the acetyl groups in the wood showed that ester links were cleaved by the liquid ammonia. Cleavage of any other kind of ester groups would result in chemical change in the wood residue and might lead to solubilisation of previously insoluble components. With this consideration in mind, Neubauer (2) extracted the residue from the liquid ammonia treatment of maple wood with hot water. He found that about 2% of the residue was extracted, the extract was polysaccharide in nature and included pectic material. Fractionation of the extract by means of water and alcohol showed that very little of the pectic material could be removed. Acetylation resulted in a very clean separation, since the partially acetylated pectic substances were water soluble and the associated acetylated hemicellulose was insoluble. Acetylation, acid hydrolysis, methylation and oxidation with periodate suggested that this pure polysaccharide had as its repeating structure a main chain of xylan units linked 1-4, and on the average every fifth unit was substituted by a side chain of one xylose unit linked 1-2 and terminated by a 1-3 linkage to a

3-methoxyglucuronic acid unit. This structure (Fig. 2.)

Fig. 2

STRUCTURE OF XYLAN



represents anhydroxylose,

and G represents a 3-methylglucuronic residue.

was the simplest that satisfied the data, but others were possible and the details were not fully elucidated. No measurements of the molecular weight of the polysaccharide were made, and the resistant aldotriuronic acid resulting from hydrolysis of the hemicellulose was not isolated. Paper partition chromatography of the neutralized hydrolysate would have been very useful in detecting very small amounts of other components which might have been present.

Schulze first introduced the term "hemicellulose" to describe a class of carbohydrates found in plant materials (21). The structural role of the insoluble, difficultly hydrolysable cellulose, and the reserve role of the soluble and easily hydrolysable starch was recognized in his classification of the functions of plant carbohydrates. Between these two types was a group of polysaccharides, hydrolysable by dilute acids, and extractable only by alkaline solutions, which Schulze called hemicelluloses. Furthermore, cellulose and starch gave only glucose on complete hydrolysis, while the hemicellulose hydrolyzed to sugars such as xylose, arabinose, galactose and mannose, as well as to glucose.

The nomenclature of Schulze is not perfect as evidenced by the different names proposed for the same class of substances. Many European chemists use the term polyoses (22), while Karrer (23) designates them, according to their constituent sugars, as xylan, araban or mannan. The terms cellulosans and polyuronides are used by Norman (24) (25). Cellulosans refer to those hemicelluloses believed to be free of uronic acid groups, and closely associated with cellulose. The polyuronides, as the name implies, all have uronic acid groups, are believed to be closely associated with lignin, and are more readily extracted by alkali.

The usual definition of a hemicellulose is "a cell wall polysaccharide which may be extracted from plant tissues by treatment with dilute alkalis, either hot or cold, but not with water, and which may be hydrolysed to constituent sugar and sugar-acid units by boiling with hot dilute mineral acids" (26). After extraction, such hemicelluloses are frequently soluble or dispersable in water. This definition excludes several substances extracted from plant tissue by water which should logically be included among the hemicelluloses. Among these water-extractable materials are the arabogalactans of larch woods (27)(28)(29)(30)(31)(32)(33)(34) and Pinus Palustris (35), the "wood starches" isolated from walnut and oak by Campbell (36)(37), and the polysaccharide extracted from spruce by Brauns (38). The xylose-methoxyglucuronic acid polysaccharide described by Neubauer (2), the water soluble polysaccharides from holocellulose (e.g. 39), liquid ammonia treated wheat straw holocellulose (40) and spruce holocellulose (41), and the material described in the present thesis might also be excluded, although in these cases degradation during preparation to smaller molecules might be the cause of their observed solubility in water.

Several excellent reviews on the history, chemistry and utilization of hemicelluloses have been published recently (24)(42)(43)(44)(45)(46)(47), therefore only a

brief discussion will be given here. The pioneering work of Miss M. H. O'Dwyer formed the basis for much of the subsequent work in this field (48)(49)(50)(51)(52)(53). The alkaline extract of oak sapwood on acidification gave an insoluble polysaccharide, hemicellulose A, which she found to contain 11 xylose units for each methoxy-glucuronic acid unit. Hydrolysis of A gave a resistant nucleus, consisting of 6 xylose units to each methoxy-glucuronic acid group, closely resembling hemicellulose B, which was precipitated by adding alcohol to the filtrate from A. In addition both had a very similar optical rotation (ca-51°).

Sands and Gary obtained four fractions by the cold alkaline extract of mesquite wood (54). Xylose, hexuronic acid and ethereal methoxyl groups were found in all fractions. The most insoluble fraction had 11 xylose units to each methoxyhexuronic acid group, whereas the figure for the other three was 6 xylose units. A number of hardwoods were studied by Anderson and co-workers (55) (56) where polyuronide hemicelluloses similar in general type to those described were found. Glucose was also found attached to the xylose chain in polysaccharides from some woods. Hydrolysis of these fractions usually yielded the resistant aldobiuronic or aldotriuronic acids, but the hemicellulose from black locust sapwood gave the

methoxyhexuronic acid as the calcium salt. The methyl group in the hexuronic acid was always found in the form of an ether. Anderson (57) reported that white pine yielded hemicelluloses which contained 36% to 46% of mannan, 44% to 50% of xylan and 10.5% to 15% of methoxyuronic acid together with a qualitative test for glucose. A resistant aldotriuronic acid was obtained on hydrolysis which had xylose residues linked to the uronic acid group.

In recent years many hemicellulose preparations have been obtained from holocellulose rather than from the wood itself. Since there is much although indecisive evidence for a lignin-polyuronide union (58)(59)(60)(61)(62)(63)(64), prior delignification should facilitate the extraction of hemicelluloses.

It was early recognized that lignin contaminated hemicellulose preparations and even carefully purified materials contained from 3% to 5% of lignin, while crude preparations might have as much as 10% to 15% (e.g. 65). Other investigators found that some lignin was always removed on extraction of large amounts of carbohydrates from wood (66). In an attempt to prevent contamination of hemicellulose preparations with lignin, Norris and Preece (67) removed extractives and pectic constituents

and then refluxed the plant material with a 1% solution of sodium hydroxide in 50% ethanol. This method of pre-treatment before extraction of hemicelluloses has been widely used, but the serious degradative effect of boiling caustic soda on polyuronides (68) (69) far outweigh its advantages of partial removal of lignin and proteins. The possibility of obtaining lignin-free hemicellulose preparations is an additional argument for the use of holocellulose as a starting material.

The term "holocellulose" was first used by Ritter and Kurth (70) to describe the carbohydrate residue from woods after extraction of nearly all of the lignin by alternate treatments with chlorine and pyridine in ethanol. The carbohydrate residue corresponded to the "Skelettsubstanz" of Schmidt, isolated by a tedious treatment of wood with chlorine dioxide and pyridine (71) (72). The method of Ritter and Kurth (70) was further modified by Van Beckum and Ritter (73) in that a hot solution of 3% ethanolamine in alcohol replaced the extraction with pyridine in ethanol. The alternate extractions were continued until little or no color was formed on washing with alcoholic ethanolamine, absence of color was considered to be evidence of complete removal of lignin. Sitch (74) later showed that at this stage birch holocellulose still retained 2% to 3% of the original lignin.

These methods often gave summative analysis of holocellulose plus lignin which were remarkable in their nearness

to 100%. Insignificant loss of constituents occurred in the case of hardwoods but typical soft woods lost up to 20% of their pentoses during holocellulose preparations (75).

Thomas (76) modified the Van Beckum and Ritter method (73) by chloriting the wood meal in carbon tetrachloride at a low temperature instead of chloriting moist wood meal at room temperature. Localized over-heating was eliminated, since previous workers (77)(78)(79)(80) found that over-heating and the hydrochloric acid present degraded the carbohydrates. Sitch (74) found that water at 0°C in place of carbon tetrachloride gave smaller losses and less degradation of the carbohydrates in the case of birch holocellulose.

The beautiful summations in these preparations were first shown to be erroneous by Atchison (81) who reported that ethanolamine was retained in holocellulose. Further evidence was given by Thomas (76) who, like Atchison, reported that lignin retained in the holocellulose did not respond to analysis, and the amount retained balanced the loss of hemicellulose constituents.

A different method of preparing holocellulose was reported by Jayme (62). Thin slices of wood suspended in water were treated with sodium chlorite at pH4 and 60°C.

The residue was removed by filtration, and the process was repeated until the required lignin content was reached. The summative analysis for lignin plus holocellulose was 100% and Jayme also pointed out that from 2% to 4% of the lignin had to be left in spruce in order to avoid excessive loss of carbohydrates. This multi-stage process was altered to a very practical procedure by Wise and co-workers (63) (82). An aqueous suspension of wood meal at 70-80°C was kept at pH4 by simultaneous hourly additions of glacial acetic acid and solid sodium chlorite, the additions being repeated until the desired lignin content was reached. The residue was recovered by filtration and washing only after the last treatment. Wise also confirmed Jayme's finding that it was necessary to leave a small amount of lignin in the holocellulose in order to avoid excessive loss of carbohydrates. In a recent paper, Timell and Jahn (83) studied the effects of the different methods of preparing birch holocellulose. The methods, a modified Van Beckum and Ritter, the original Van Beckum and Ritter, and Wise's chlorite procedure, removed all but the following lignin percentages, 0.4, 2.3 and 4.9% respectively before pentosans were lost.

Because of its great convenience, Wise's method has recently been most often used for studies on cellulose (84) (85), the hemicelluloses, e.g. (40)(86)(87)(88), and

the chemical composition of various woods (89) (90). Since carbohydrates could be extracted from holocellulose with hot water, it is logical to assume that some were lost during the delignification. Evidence was soon obtained which showed that carbohydrates were indeed lost during delignification. Jayme and Hanke (91) reported the presence of glucuronic acid and tentatively identified glucose in spruce chlorite liquors. Barton (92) carefully investigated slash pine chlorite liquors and found mannose, galactose and uronic acids. Bublitz (93) reported similar results for spruce chlorite liquors and in addition found small amounts of xylose and arabinose. Evidence was also presented which showed that carbohydrates were removed well before the lignin content had been reduced to 6%.

The presence of lignin, which did not respond to the Klason procedure, in Van Beckum and Ritter holocellulose had been previously noted (76) (81). Similar results were found for chlorite holocellulose. Von Wacek and Schrott (94) (95) (96) reported the presence of soluble lignin in the acid lignin filtrates of spruce wood. Campbell and McDonald (97) (98) showed that beech and spruce holocelluloses contained modified lignin which was soluble in the Klason estimation.

These results indicated that a summative analysis of holocellulose plus lignin did not truly represent the facts,

since carbohydrate losses were nicely balanced by acid-soluble lignin. It is apparent that a true holocellulose (i.e. lignin-free) cannot be prepared without a considerable loss of carbohydrates, and that the holocelluloses available are therefore not completely representative. Nevertheless, holocellulose does give an excellent starting material for extraction of hemicelluloses since it is prepared by relatively mild methods.

Sitch (74) showed that white birch holocellulose yielded a water-soluble hemicellulose with a 5:1 pentose to uronic acid ratio, another fraction soluble in ethylenediamine had a 8:1 ratio. Both fractions had a 3:2 ratio of uronic anhydride to methoxyl groups instead of the usual 1:1 ratio. The difference between the summative analysis and 100%, especially in the water-soluble fraction, indicated the presence of hexosan.

Softwoods have not been as extensively studied as the hardwoods. Spruce wood holocellulose was hydrolyzed with 1% sulphuric acid by Kurth and Ritter (99). They found that the holocellulose contained about 7% of an easily hydrolysable hemicellulose which contained 14.6% of uronic anhydride, 3.2% methoxyl groups, 33.5% hexosan, 33.4% pentosan and 8.0% acetyl groups. Stockman and Hägglund (100) reported that spruce polyoses hydrolysable

at p H 2 gave a sugar mixture in which xylose and arabinose were found, corresponding to 5% and 0.8% respectively of the wood.

Jorgensen and Bjorkqvist (41) studied the hemicelluloses extracted from birch and spruce holocelluloses swollen in liquid ammonia. The holocelluloses were prepared by the chlorite method, and were exhaustively extracted with a solid-solvent ratio of 1:10. The different hemicellulose fractions, precipitated by acetone from solution, were obtained as follows: extractable by hot water, by 1% sodium carbonate, by 2% sodium hydroxide and by 5% sodium hydroxide. Table I gives the yields and analytical data of these hemicellulose fractions from spruce (Norway).

TABLE I

ANALYTICAL DATA FOR SPRUCE HEMICELLULOSE

Fraction	% Yield[a]	% Pentosan	% Uronic Acids
Holocellulose	---	7.8	5.1
Hot water	7.1	25.3	16.6
1% sodium carbonate	2.8	47.3	20.3
2% sodium hydroxide	7.3	40.8	8.0
5% sodium hydroxide	9.9	13.8	7.5
Residue	69.0	1.4	0.01

(a) Based on ammonia-swollen holocellulose.

The fractions were not further purified but were hydrolyzed and the constituent sugars were determined with the

results shown in Table II.

TABLE II

CONSTITUENT SUGARS IN SPRUCE HOLO-CELLULOSE AND EXTRACTED HEMICELLULOSE FRACTIONS

(RELATIVE AMOUNTS EXPRESSED IN PER CENT)

Fraction	Galactose	Glucose	Mannose	Arabinose	Xylose
Holocellulose	3.0	70.5	17.9	1.0	8.0
Hot water	5	12	45	17	21
1% sodium carbonate	5	15	24	22	34
2% sodium hydroxide	tr.	20	30	20	30
5% sodium hydroxide	tr.	27	50	5	18
Residue	0	90	10	0	tr.

Hemicellulose fractions, isolated with minimum degradation, from black spruce holocellulose prepared by a modification of the Thomas procedure (76), have been studied by Wethern (101). The fractions were obtained by extraction with alkaline solutions under an atmosphere of nitrogen. The fractions soluble in 5% and 16% potassium hydroxide were designated as the "5% extract" and "16% extract" respectively. Table III gives the yields and analytical data for these fractions.

TABLE III

YIELDS AND ANALYTICAL DATA FOR

HEMICELLULOSE FRACTIONS AND SPRUCE WOOD

	Wood	5% Extract	16% Extract
Yield, %	---	9.0	6.4
Mannan, %	8.5	7.7	22.2
Uronic anhydride,%	2.8	21.1	13.5
Pentosan, %	8.8	47.5	38.9
Sulphated ash, %	---	12.7	13.2
Insoluble ash, %	---	1.0	1.1
Klason lignin, %	29.7	negligible	negligible

Paper partition chromatography of the hydrolyzed hemicelluloses indicated that a considerable amount of glucose or galactose was present with lesser amounts of unidentified sugars.

Lignified tissues other than woods yield hemicelluloses similar to those just discussed, and only a few of the many examples are noted here. Wheat straw hemicellulose is principally of the "B" type (precipitated from aqueous solution by alcohol); hexuronic acid, arabinose and xylose in the ratios of 1:0.9:23 were reported in the hydrolysate (102). Hemicellulose fractions isolated by Bishop and Adams (40) from wheat straw holocellulose swollen in liquid ammonia had a similar composition consisting of D-xylose, L-arabinose, D-glucose, D-galactose, and a hexuronic acid in molar ratios of 40:7:2:1:4. The alkali-soluble fraction of alfalfa hay contained 12.1% of uronic

anhydride and 77.3% of pentosan, the pentosan consisting of xylose and a trace of arabinose (103). Anderson (104) found that hemicelluloses of cotton seed hulls were composed of glucuronic acid and xylose in the approximate ratio of 1:10-16. McIlroy (105) (106) investigated the hemicellulose of New Zealand flax, and suggested a main chain of 9 or 10 readily hydrolysable xylose residues, united by 1:4- β -glycosidic linkages, and terminated at the reducing end by a relatively acid-resistant aldotriuronic acid composed of 2 xylose units and 1 glucuronic acid residue. A polyuronide isolated from jute chlorite holocellulose (107), amounting to 75% of the hemicelluloses present, appeared to be composed of repeating units made up of one molecule of monomethylglucuronic acid linked with 6 molecules of anhydroxylose.

The methods of isolation and purification of hemicelluloses used by O'Dwyer have been followed in most of the subsequent investigations. Fractions obtained on extraction with increasingly strong reagents or on fractional precipitation by alcohol or acetone from aqueous solutions would naturally be rather crude. Further fractionation of hemicelluloses were sometimes carried out by means of the copper complexes discovered by Salkowski (108). Since these procedures did not give clear cut separations, difficulties in reproducing results were

not surprising.

Anderson studied a number of woods and showed that they all contained pectic material (109). Anderson and Wise (110) were accordingly led to test the uronic-rich fraction isolated by Thomas (76) from aspen for polygalacturonic acid. Oxidation of this sample by the bromine-hydrobromic acid method of Heidelberger and Goebel (111) yielded mucic acid; therefore it was highly probable that this fraction contained pectic material. This contaminant, difficult to remove, might also be present in many other hemicelluloses whose compositions have been reported.

Even today, there is no acceptable criterion of purity of any hemicellulose material.

Studies on the molecular dimensions of hemicelluloses should prove very fruitful in clarifying present uncertainties. Most of our knowledge in this field was furnished by Husemann (112) who prepared hemicelluloses from wheat straw, beech, spruce (Fichtenholz) and larch woods. The spruce hemicelluloses were obtained from Schmidt's holocellulose (71), and were fractionated from 6% caustic soda solution by addition of methanol. Viscosities and optical rotations of these fractions were very similar, so Husemann

implied that the major portion was essentially a single carbohydrate with the same molecular weight (101) (113). The water-soluble portion of this spruce "mannan" gave a degree of polymerization (D.P.) of 160 (as hexosan) on the basis of osmotic pressure measurements, which were supported by similar measurements with a mixed acetate-benzoate. He also found a D.P. of about 150 for the xylan from straw and beech.

Husemann extracted hemicelluloses by means of aqueous alkali under nitrogen, but Millett and Stamm (114) did not employ such precautions in studying hemicellulose fractions from aspen. This was probably the reason for the average D.P. of 80 reported for the fractions and their acetates. Acetylation did not cause any degradation and the solubility differences observed in these fractions were attributed to different chemical compositions, since the molecular weights were much the same. Wise and Ratliff (115) studied the hemicellulose fractions from black spruce and slash pine. All fractions contained considerable amounts of mannose, which was most plentiful in the fractions least soluble in alkali. This evidence indicated that spruce hemicelluloses were not homogeneous. Timell and Jahn (83) isolated hemicelluloses from paper birch holocellulose and found an approximate value of 220 for

the D.P., since recalculated to a value of 155 by Wise (116). The molecular weights of representative hemicellulose fractions from big tooth aspen were determined by Thompson and Wise (116). Fractions were isolated from holocellulose prepared by a modification of the Thomas procedure (76), and from the extractive-free wood itself. Viscosity measurements on 10% potassium hydroxide solutions, and osmotic pressure measurements on chloroform solutions of a butyrate, indicated an average D.P. of about 150.

A very interesting paper on the molecular properties of black spruce hemicellulose by Wethern (101) deserves considerable attention. Two hemicellulose fractions, "5% potassium hydroxide extract" and "16% potassium hydroxide extract", were fractionally precipitated from formamide solution by ethanol. The analytical data for these fractions are shown in Table IV.

TABLE IV.

ANALYTICAL DATA FOR BLACK SPRUCE HEMICELLULOSE FRACTIONS

Fraction	% of Total	Conc.[a]	$\eta_{sp.}/\%$ C.	% Pentosans[b]
		5% Extract		
Original	---	0.43	0.81	53.8
1 (c)	8.9	0.42	0.75	---
2	19.5	0.46	0.73	42.7
3	45.4	0.46	0.93	51.3
4	14.7	0.43	0.69	57.4
5	5.8	0.52	0.16	---
Total	94.3			

TABLE IV (continued)

16% Extract

Fraction	% of Total	Conc.[a]	$\eta_{sp.}$ / % C.	% Pentosans[b]
Original	---	0.47	1.03	42.7
1[c]	42.2	0.32	0.94	29.8
2	5.1	0.32	0.89	---
3	3.6	0.29	0.90	---
4	7.8	0.40	1.11	---
5	34.3	0.57	1.16	54.4
6	1.6	0.43	0.49	---
Total	94.6			

(a) Concentration in grams per 100 ml.
(b) Uncorrected for uronic acids.
(c) Insoluble in formamide.

Data presented in Table III indicated that spruce hemicelluloses did not have a uniform composition. The further evidence in Table IV leads to the conclusion that these hemicelluloses must be composed of a mixture of carbohydrates of different rather than a single type.

The corresponding butyrate esters were fractionally precipitated from acetone solutions by addition of petroleum ether. The viscosities and osmotic pressures of the fractions were determined in chloroform solution, and the molecular weights calculated from osmotic pressure data, as given in Table V.

TABLE V

MOLECULAR WEIGHTS OF SPRUCE HEMICELLULOSE

BUTYRATE FRACTIONS

Fraction	% of Total	Molecular Weights
	Butyrate of 5% Extract	
1	5.0	----
2	14.0	95,000
3	10.5	85,000
4	14.0	79,000
5	12.5	54,000
6	11.0	47,000
7	6.0	35,000
8	12.5	14,000
Total	85.5	
	Butyrate of 16% Extract	
1	42.5	----
2	1.5	----
3	29.0	65,000
4	5.0	50,000
5	5.0	52,000
6	3.0	46,000
7	7.5	31,000
Total	93.5	

The average D.P. of the butyrates, calculated as a pentosan polymer, ranged from 300 to less than 50 for the fractions studied. The butyrate esters were prepared

by the method of Carson and Maclay (117) with very little degradation, since it was possible to butyrate the hemicellulose and deacetylate the ester without causing any great change in the original viscosity. This large variation in chain length must have been present in the original hemicellulose, although fractionation from formamide solution did not show it. Fractionation from formamide was probably according to chemical composition while that from chloroform was dependent on molecular weight.

The current belief is that "pure" hemicellulose preparations may be composed of more than one simple building unit. This belief has been questioned by Isherwood as a result of studies on pear cell wall polysaccharides by electrophoretic methods (118). He obtained evidence that a complex polysaccharide was a mixture of polymers each made up of a single sugar unit.

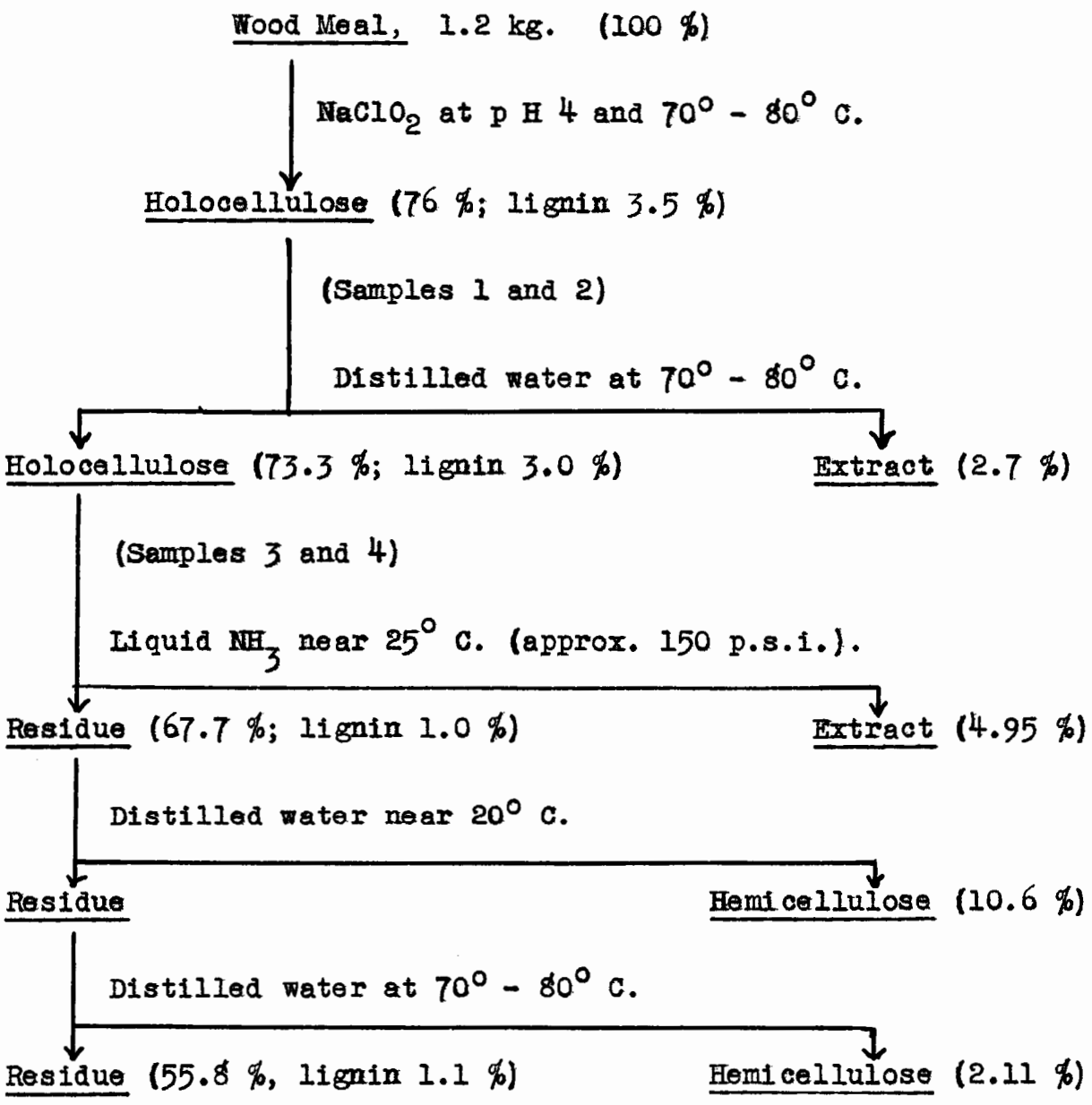
DISCUSSION OF RESULTS

In the present study, every effort was made to isolate the hemicellulose fraction from spruce wood meal under the mildest possible conditions. After being extracted exhaustively with alcohol-benzene and with alcohol, the meal was treated as shown in Fig. 3. The percentages shown in the figure are averages of two large-scale preparations made with concordant results, the holocellulose Samples 1 and 2, for example, being recovered in yields of 74.7% and 77.2%, with Klason lignin contents of 3.1% and 4% respectively.

As noted in the Introduction, Timell and Jahn (83) studied different methods of preparing birch holocellulose, and reported that the acidulated sodium chlorite procedure of Wise and co-workers (63) lowered the viscosity of the resulting holocellulose more than the procedures described by Van Beckum and Ritter (73) and by Sitch (74). Jorgensen (84) (85) found that the acid chlorite procedure (63) caused degradation of holocellulose isolated from black spruce. Wethern (101) reported that the hemicelluloses extracted from black spruce holocellulose, prepared by Thomas' modification (76) of the chlorine-ethanolamine precedure (73), had higher viscosities than those obtained from holocelluloses prepared by the procedures of Schmidt (71) and Wise (63). Although a modified chlorine-ethanolamine procedure was

FIG. 3

ISOLATION OF CRUDE HEMICELLULOSES FROM WATER-EXTRACTED SPRUCE HOLOCELLULOSE



preferred by Timell and Jahn (83) and by Wethern (101), the chlorite procedure was followed in the present research because the chance introduction of nitrogenous compounds might complicate the interpretation of the results of the subsequent extractions with liquid ammonia. Moreover, trial showed that when sucrose was substituted for the wood meal its specific rotation decreased from 66° to about 60° in the conditions of the chlorite treatment. Since complete inversion of sucrose would have changed the specific rotation to about -20°, the hydrolytic action of the chlorite was certainly minute, and presumably was negligible on the glycosidic bonds in holocellulose, which are more stable than those in sucrose. Nevertheless, no attempt was made to remove the last few percent of the lignin from the holocellulose by prolonging the treatments with chlorite.

After the delignification, the holocellulose was extracted with hot water for four hours to remove residual water-soluble materials, which amounted to 2.7 % of the wood. This extract, which yielded 5.9 % furfural, obviously contained carbohydrates and was probably similar in composition to the materials recovered from chlorite liquors from spruce (91) (93) and slash pine (92). The holocellulose, now freed of water-solubles, was then dried to less than 1% moisture content in a desiccator at room temperature. This step was

an essential preliminary to subsequent extraction with liquid ammonia, because the presence of moisture in spruce bark was found by the author's colleagues, Mr. Jablonski and Mr. Sanderson, to cause an initial rapid evolution of heat and the risk of a dangerous initial increase of pressure in the autoclave. The Experimental Portion describes in some detail the autoclaves used and the technique of conducting the extractions on a kilogram scale at room temperature and about 150 p.s.i. pressure. These methods were based on those previously used by Yan (1) and Neubauer (2) for similar extractions of maple wood.

The residual holocellulose, whose fibrous appearance was unchanged but whose color was now light brown, was then air-dried until nearly free of ammonia and extracted with ethanol in a Soxhlet apparatus. The ethanol extract was negligible and the air-dried residue was twice extracted with large volumes of distilled water at room temperature. Two similar extractions near 70° C. followed, and the removal of water-solubles was then practically complete. These extracts together removed 12.65 % of the wood and contained the hemicelluloses whose study was the principal object of the research. It will be convenient, however, first to summarize observations made on the other fractions.

Gralen and Ranby (119), Mitchell (120) and Jorgensen (84) (85) modified the early procedure of Jahn and Coppick (121) and showed that prolonged nitration with a phosphoric acid-phosphorous pentoxide-nitric acid mixture modified the lignin in several woods and made it possible to recover the cellulose trinitrate, presumably in an undegraded condition. The application of Mitchell's modification to the present samples of spruce wood and holocellulose (after extraction with water) gave such poor yields of soluble nitrates that the intention of determining their viscosities was abandoned. In conjunction with his parallel work on aspen holocellulose, the author's colleague, Mr. Milks, repeated these nitrations by a somewhat different procedure that gave good results with aspen wood. Spruce wood, however, could not be nitrated in a satisfactory manner, and any decrease in the viscosity of the nitrated holocellulose caused by the sodium chlorite treatment could not be determined. The water-extracted holocellulose had an apparent degree of polymerization of 1330 and the final residue from the extractions with liquid ammonia, cold and hot water, a D. P. of 980. These values were calculated from the intrinsic viscosities $[\eta]$ of the nitrates in acetone solution, by applying the relationship D. P. = $K[\eta]$, where a value of 100 for the constant K, corresponded to a value of 10×10^{-4} for K_m in Staudinger's equation (128). Although comparable, the degrees of

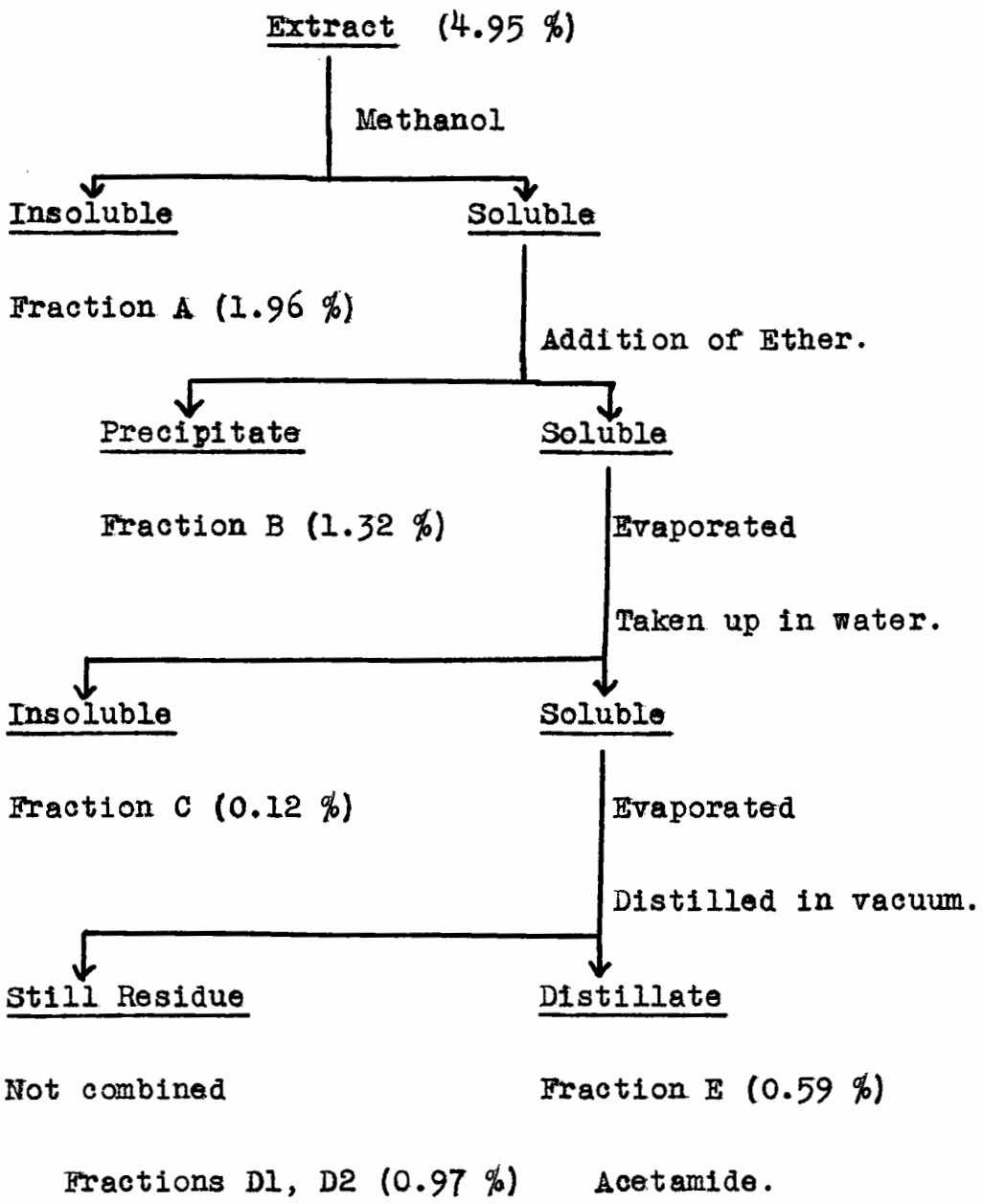
polymerization quoted were probably low by an unknown factor.

The apparent drop in D. P. after the liquid ammonia treatment suggested that a considerable degradation of the cellulose had taken place. Bjorkqvist and Jorgensen (41), however, reported no marked drop in the D. P. of the cellulose during the liquid ammonia treatment and subsequent extractions of birch and spruce holocellulose. On the other hand, Bishop (122) found that an isolated polyuronide from wheat straw holocellulose was degraded by 8.4% in anhydrous liquid ammonia. The conflicting results make it uncertain whether the reagent did exert a degradative effect on holocellulose, or whether the observed decrease in intrinsic viscosity was caused by unknown factors in the nitration. General considerations suggest that the cleavage of a normal glycosidic link by liquid ammonia is unlikely, but at 20° C. ester bonds would readily be broken.

The liquid ammonia extract from Samples 3 and 4 of the water-extracted holocellulose amounted to 4.95 % of the wood, and gave concordant results when fractionated by means of solubility differences and vacuum distillation (Fig. 4). These fractionations were originally carried out to discover evidence of an "acid-soluble" lignin or of carbohydrates. Fraction A contained 20% of Klason lignin and gave a positive Molisch test for carbohydrate. The other fractions, all

FIG. 4

FRACTIONATION OF LIQUID AMMONIA EXTRACT[a]



(a) Percentages based on original wood. Averages of two independent and concordant runs.

soluble in methanol, were not likely to contain carbohydrates, and gave negative Molisch tests. Only Fraction A showed copper reducing power, equivalent to 17% hydrolysis, when hydrolyzed in 2.5 % sulphuric acid.

The ultraviolet absorption spectra of the major fractions, A, B, D1 and D2, (Fig. 5), in which extinction coefficients for 1% aqueous solutions were plotted against wave length, all showed a slight peak at a wave length near 282 m μ, and that of B was quite similar to that found for spruce "acid-soluble" lignin by Campbell and McDonald (98). Since Fraction B was free of carbohydrate, had the relatively high methoxyl content of 8.2% and nevertheless contained only 8.6 % of Klason lignin, it presumably was lignin of the "acid-soluble" type. This supposition received support from the fact that the carbon content of Fraction B was 46.6%, similar to the low values found for "acid-soluble" lignin by Campbell and McDonald (98). Oxidation by the chlorite used in the preparation of the holocellulose was presumably extensive.

Although Fraction A contained twice as much Klason lignin as Fraction B, the extinction coefficient of A was considerably less at 282 m μ. This observation again suggested that the Klason lignin estimation gave grossly

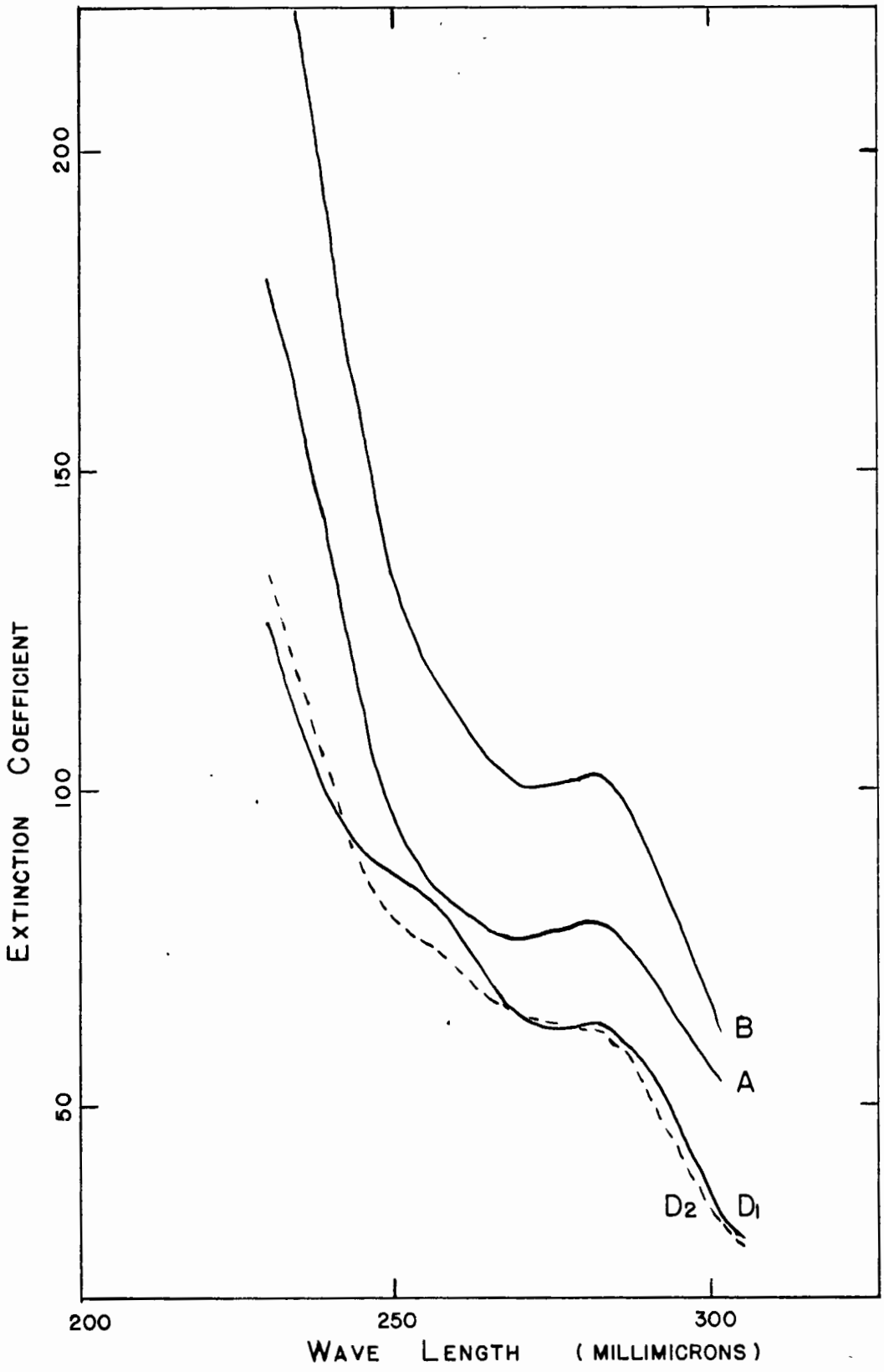


FIG. 5. ULTRAVIOLET ABSORPTION SPECTRA OF FRACTIONS A, B, D1 AND D2 FOR 1% AQUEOUS SOLUTIONS

low results with Fraction B. The minor Fraction C had 53.8% of Klason lignin, and the ultraviolet absorption plot showed that lignin-like material was also present in Fractions D1 and D2. These fractions contained about 14.5% of nitrogen, probably not derived from acetamide, which was easily recovered by distillation (Fraction E).

The material balances of the liquid ammonia extraction were interesting. Although the holocellulose lost about 2% of Klason lignin, only about one-third of this amount was recovered in Fractions A to E. The simplest explanation, previously suggested by others, e.g. (76)(97)(98)(123), was that the Klason lignin estimation was unreliable when applied to lignins that had been chemically changed. Acetyl estimations showed that the extraction removed 0.99% of the acetyl groups in the wood, but Fraction E, when recalculated to acetyl, accounted for only 0.43%. Although this recovery of about 50% would be increased by any acetamide remaining in Fractions D1 and D2, it was unlikely to equal the 85% recovery obtained by Yan (1) and Neubauer (2) in similar extractions with maple. Since the amount of acetamide present was relatively small, handling losses could be partially responsible for the low recovery.

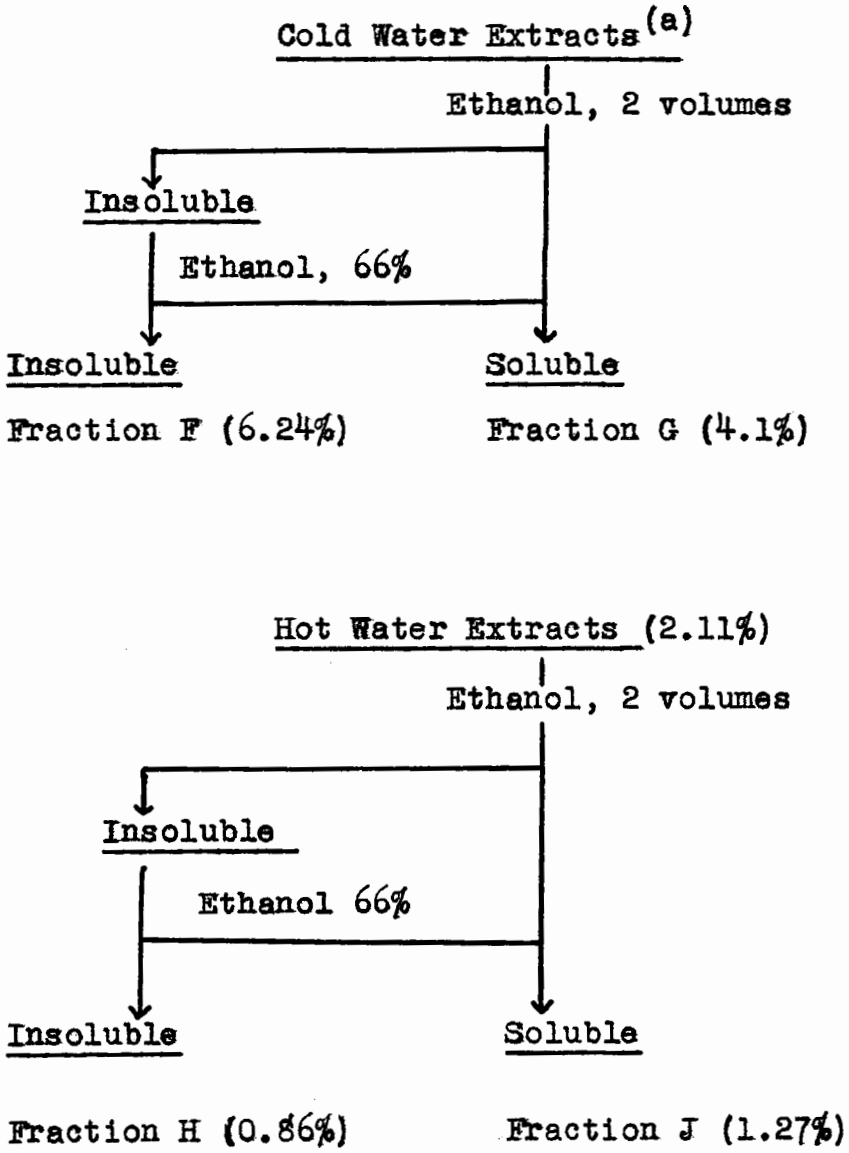
The principal observation was that liquid ammonia extracted 4.95% (based on original wood) of a dark colored

material from a white chlorite holocellulose; of this amount, only about 0.6% was Klason lignin, at least 0.59% was acetamide, and the remainder was presumably carbohydrate together with lignin in a state that did not respond to the Klason lignin estimation. The assumption that the original holocellulose consisted exclusively of carbohydrates, acetyl groups and Klason lignin was probably an over-simplification of the facts.

Two cold water extractions and 2 hot water extractions of the ammonia-treated holocellulose (Fig. 6) removed a total of 159.12 g. or 12.65% of the wood. The individual extracts were carefully concentrated under reduced pressure at a p H of about 6, and separated into insoluble and soluble fractions in 66% ethanol. The insoluble materials from the cold water extractions were combined, dissolved in water and reprecipitated by the addition of ethanol. The first fraction obtained was too gelatinous to afford a good separation, and the addition of ethanol was accordingly increased to 2 volumes to give a large amount of insoluble material, 77.26 g. (Fig. 6). The mother liquor was evaporated to dryness under reduced pressure, and the dry solid was added to the dry ethanol-soluble materials from the individual cold water extractions to give Fraction G, 51.07 g. The hot water extracts were treated in the same manner (Fig. 6) to give the alcohol-insoluble Fraction H, 10.66 g., and

FIG. 6

SEPARATION OF THE HEMICELLULOSE COLD AND HOT WATER EXTRACTS (12.65% or 159 g.)



(a) All percentages based on the original wood.

alcohol-soluble Fraction J, 15.21 g. The remainder of this thesis was concerned with the purification and study of Fractions F, G, H and J, with particular emphasis on Fraction F.

As already noted, the recovery of acetamide by extracting maple wood at room temperature with liquid ammonia cleared showed that the reagent was capable of cleaving acetic esters, and in all probability other types of esters as well. The fact that treatment with liquid ammonia rendered 12.65% of the residual holocellulose water-soluble was not inconsistent with the view that the original insolubility was caused by a transesterification of alcohol and uronic acid functions. In this event, acid amides might be present among the Fractions F to J.

The nitrogen content of these fractions was accordingly investigated by distilling separate samples with water containing magnesium oxide and with dilute sodium hydroxide according to Neubauer's procedure (2), Table VI.

TABLE VI.

DIFFERENTIATION BETWEEN AMMONIUM AND AMIDE NITROGEN IN FRACTIONS F, G, H AND J

Nitrogen, % on ash-free basis

Fraction	Total	$Mg(OH)_2$ Distillation	NaOH Distillation	Unknown
F	1.42	0.35	0.35	1.07
G	3.85	1.61	1.72	2.13
H	1.44	0.16	0.16	1.28
J	3.12	1.02	1.02	2.10

All fractions contained ammonium salts but only Fraction G contained amide nitrogen. Even this result could not be regarded as conclusive, because traces of residual acetamide would be expected to occur in this fraction. Approximately 60% of the nitrogen present in all fractions was not in the form of ammonium salts or amide nitrogen, and its nature was not investigated further.

Detection of sugars by paper partition chromatography has been so successful that it has become a standard procedure. For this reason, an extensive discussion of the method was omitted from this thesis. Although the pyridine-ethyl acetate-water system (124) did not separate mannose and arabinose, it was found to be the most efficient solvent system in other respects. The aniline phthalate

indicator (125) which colored pentoses red, hexoses brown and uronic acids pink, seemed to be superior to other reagents used to detect the separated sugars. Paper chromatography of the neutral hydrolysates from Fractions F, G, H and J showed that they all contained xylose, arabinose and uronic acids together with lesser amounts of glucose, galactose and mannose. Fractions H and J seemed to contain more mannose than F and G. Analytical data for these fractions are shown in Table VII.

TABLE VII.

ANALYTICAL DATA FOR FRACTIONS F, G, H AND J[(a)]

Fraction	F	G	H	J
% Ash	2.52	4.31	2.05	5.27
% Nitrogen	1.42	3.85	1.44	3.12
% Acetyl	trace	nil	nil	nil
% Methoxyl	4.30	9.20	3.91	7.21
% Furfural (b)	34.1	14.1	23.0	27.1
% Uronic anhydride	22.0	11.8	16.6	12.8

(a) On an ash-free basis.

(b) No correction for furfural from uronic acids.

Quantitative estimations of the individual sugars present in these crude fractions were not made, but the amount of mannose appeared to be much smaller than the 45% reported in the hot water extract of ammonia-swollen spruce holocellulose (41). Wise and Ratliff (115) studied the distribution of mannans in slash pine and black spruce

woods. They reported that the fraction most readily extracted by alkali contained the lowest percentage of mannan, whereas the least soluble fraction had the highest mannan content. On the basis of these results, it is logical to assume that the aqueous extracts of ammonia-treated spruce holocellulose would contain only small amounts of mannose. The amount of residue from the extractions, 55.8% of the wood, indicated that it retained much of the hemicelluloses including mannan. One of the author's colleagues, Mr. Watts, has just commenced to investigate the amide and mannan content of this wood residue in detail.

Fractions F and H were bleached with sodium chlorite at pH 4.7 and room temperature for 3 hours. The bleached materials, Fractions F1 and H1 respectively, were recovered in 88% yield and were cream colored. Fraction F1 had a specific rotation of -28.1° in water, while the value for H1 in water was $+11.1^{\circ}$. Fractions G and J were not bleached because of anticipated difficulties in recovery.

Neubauer (2) showed that the aqueous extract from ammonia-treated maple wood contained a considerable amount of pectic material. Acetylation of the crude extract led to a complete removal of this contaminant since it remained water-soluble, while the acetylated hemicellulose was water-insoluble. This purification seemed to be far superior to any others used up to the present time and presumably did not

degrade the hemicellulose. Wethern (101), for example, showed that hemicelluloses from black spruce could be butyrated and the ester groups could subsequently be removed without any large change in the viscosity of the hemicelluloses.

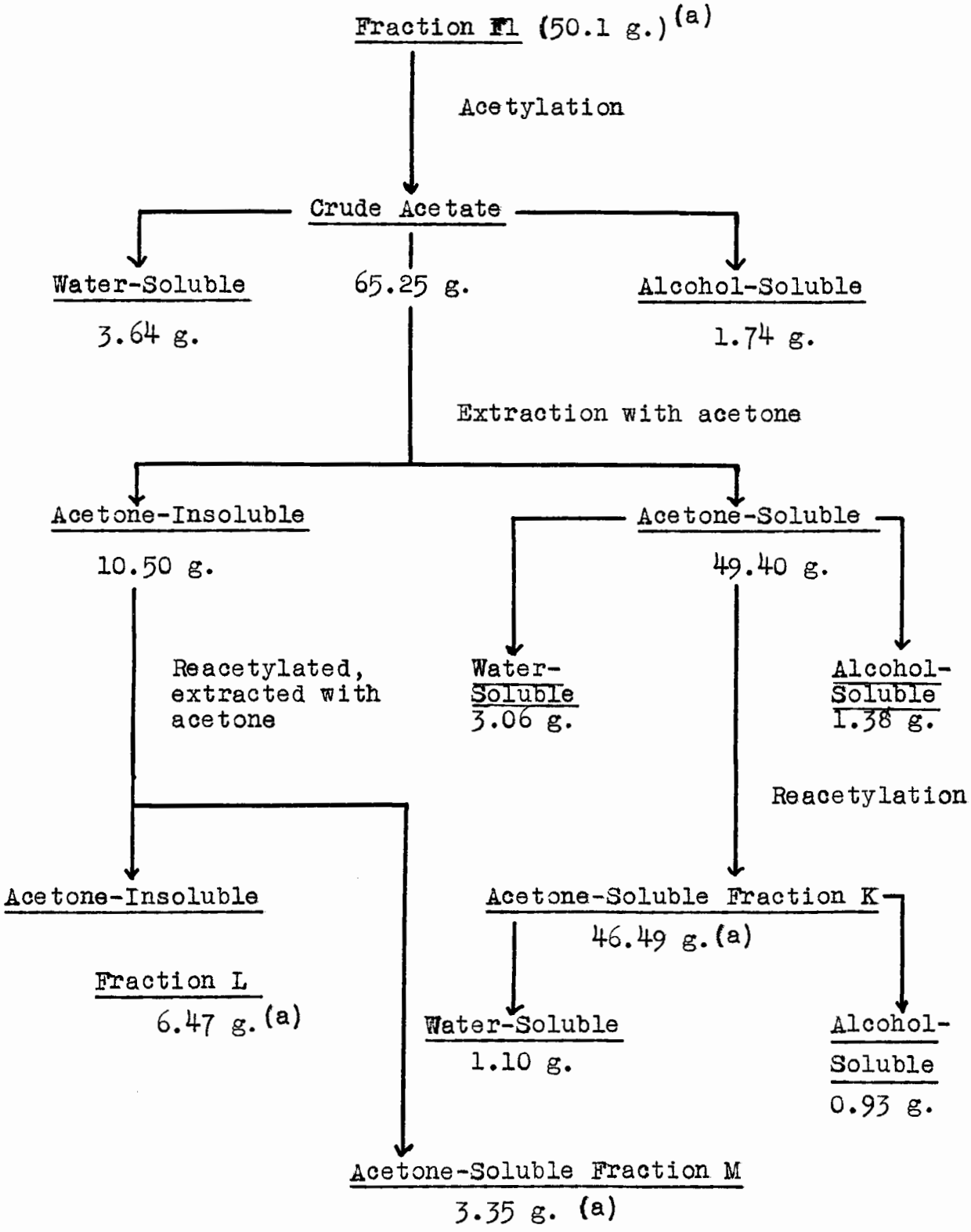
Fractions F1, G, H1 and J could not be satisfactorily acetylated by means of the pyridine and acetic anhydride procedure used by Neubauer (2). The use of formamide as a swelling and dispersing agent (117) made it possible to esterify polyuronides which were impossible or very difficult to esterify under more usual conditions. Fractions F1 and H1 were likewise acetylated very smoothly and easily when swollen in formamide. Fractions G and J gave small yields of products with low acetyl contents and were not further investigated.

Fraction F1 was completely acetylated and partially fractionated in the same series of operations which are outlined in Fig. 7. The water-soluble and alcohol-soluble materials from the acetylation of Fraction F1 had low acetyl contents, and were not further studied. The former were assumed to be partially acetylated pectic materials.

Fraction F1, when fully acetylated, was separated into Fractions K, L and M by solubility differences. Fraction K, contained 35.3% acetyl, had $[\alpha]_D^{25} = -59^\circ$ (c = 1, ℓ = 1) in chloroform, and represented 30.07 g. or 60% of

FIG. 7

ACETYLATION AND FRACTIONATION OF FRACTION F1



(a) On an ash-free basis.

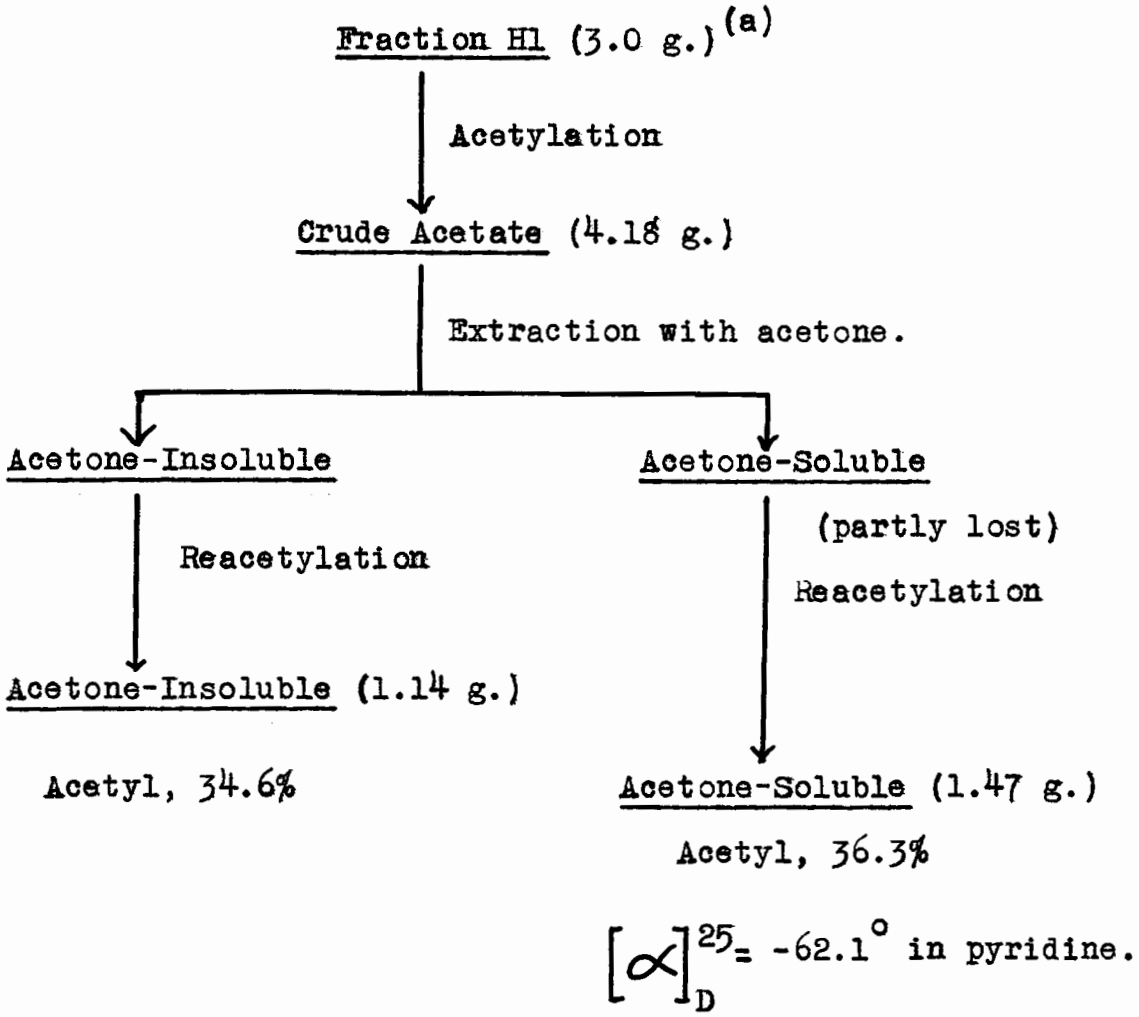
Fraction F1. Fraction M, represented 4.36% of F1, had an acetyl content of 34.8% and was soluble in acetone and pyridine but was only partially soluble in chloroform. The optical rotation in pyridine was $[\alpha]_D^{25} = -52.3^\circ$ ($c = 1$, $\ell = 1$), a value which could not be compared with that for Fraction K since its rotation had not been determined in pyridine. However, Fraction K was later fractionated and 80% (Fraction N) had a rotation in pyridine of $[\alpha]_D^{25} = -74.8^\circ$ ($c = 1$, $\ell = 2$). These results indicated that Fractions K and M were somewhat different.

Fraction L, 8.53% of F1, had 33.9% of acetyl groups, and was insoluble in pyridine, acetone and chloroform. Paper partition chromatography of the deacetylated hydrolyzed Fractions K, L and M indicated that K and M contained similar amounts of xylose, arabinose, galactose and uronic acids, but L, with the same components, had a relatively high proportion of galactose.

The fact that several different crude fractions could be obtained from Fraction F1 was additional proof that hemicellulose preparations from spruce were not homogeneous. The highest acetyl content obtained, 35.3% in Fraction K, was lower than the theoretical value of 36.4% found by Neubauer (2) for the maple hemicellulose shown in Fig. 2. Fraction H1 (Fig. 8) was treated in the same manner as F1 and the acetone-soluble acetate was isolated. The acetyl content

FIG. 8

ACETYLATION AND FRACTIONATION OF FRACTION H1



(a) All values uncorrected for ash.

and optical rotation of this acetate were different from those for the major part of Fraction K, and because of the small amount, no attempt was made to carry the fractionation further.

The bulk of the material from Fraction F1 was now concentrated in Fraction K which was chosen for further study.

A 2% solution in chloroform was fractionally precipitated by the addition of increasing amounts of petroleum ether. A total of 7 sub-fractions were obtained, the last two, amounting to 7% of the starting material, having rotations which were quite different from the other sub-fractions (Table VIII). The remaining sub-fractions had rotations

TABLE VIII.

OPTICAL ROTATIONS [a] AND INTRINSIC VISCOSITIES [b] OF SUB-FRACTIONS FROM FRACTION K [c]

Sub-Fraction	% of Fraction K	$[\alpha]_D^{25}$	Intrinsic Viscosity
1	24.5	-59.6°	0.486
2	16.5	-61.3°	0.456
3	5.5	-60.2°	0.410
4	15.5	-59.8°	0.403
5	7.5	-60.0°	0.259
6	3.5	-18.4°	0.234
7	3.5	- 9.0°	0.136
Washings	22.0	-59.5°	---

(a) Rotations in chloroform solutions.
(b) Values obtained from Fig. 9.
(c) Two grams in 100 ml. of chloroform.

which agreed within 2°, or within the experimental error for 1% solutions observed in 1 dm. tubes, and were assumed to have the same composition. A small head fraction could not be obtained. The specific viscosities of these sub-fractions in chloroform were determined, and the intrinsic viscosities calculated in the usual manner as shown in Fig. 9.

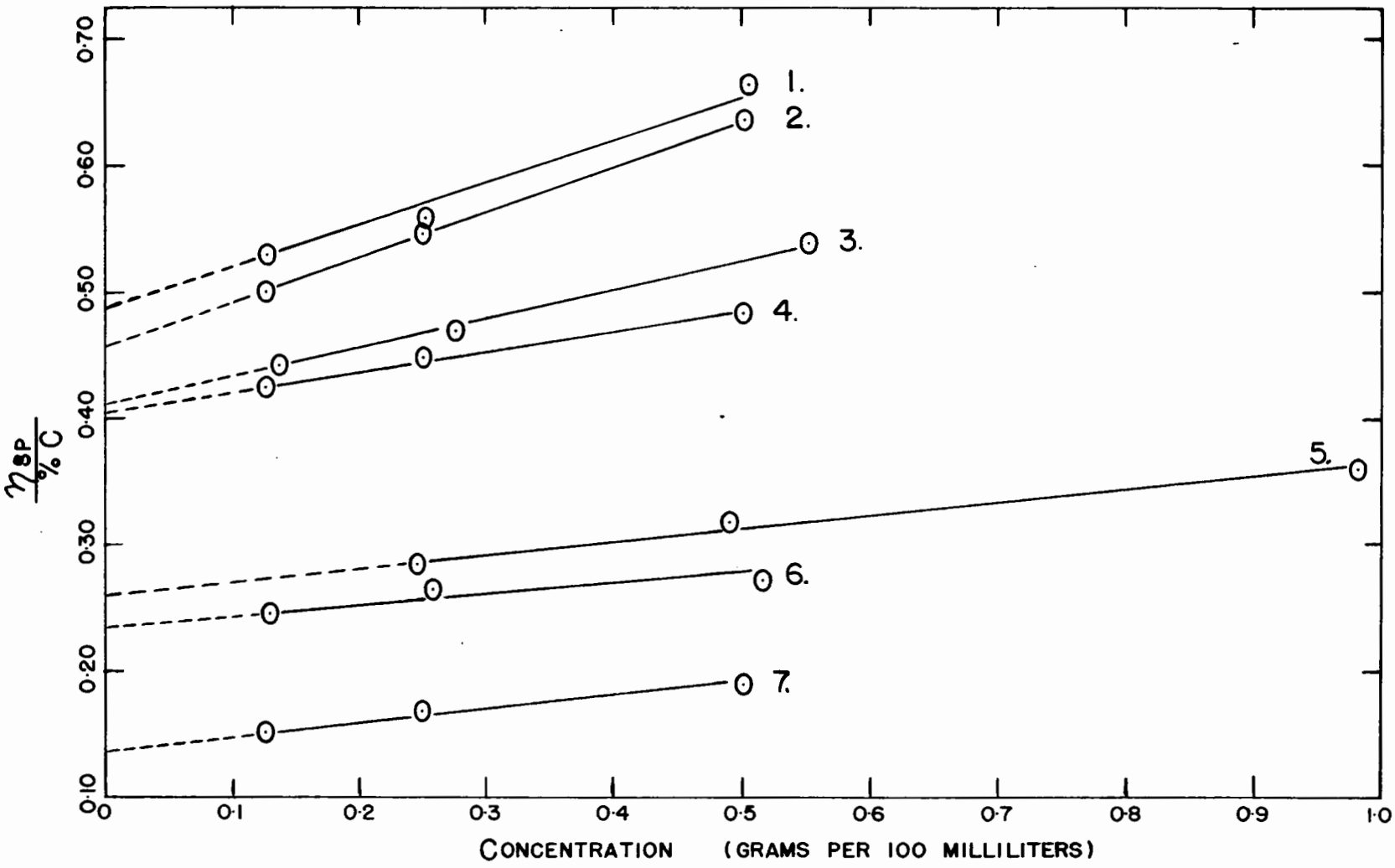


FIG.9. VISCOSITIES OF SUB-FRACTIONS FROM FRACTION K IN CHLOROFORM

The first four had similar intrinsic viscosities but sub-fraction 5, with the same rotation, had a considerably smaller value. Thus the fractionation was dependent on chain length when the composition was apparently the same, but sub-fractions 5 and 6, with different rotations, had nearly the same viscosities. In this case fractionation presumably depended on chemical composition. In order to eliminate sub-fraction 5, subsequent fractionations of Fraction K were adjusted so that about 80% of the material was obtained in a first large fraction.

These fractionations were carried out on four batches of Fraction K (Table IX), and the initial sub-fractions with the same rotations and acetyl contents (Table X) were combined to give Fraction N, 37.17 g. or 82.8% of Fraction K. The second series of sub-fractions (II) were combined and designated as Fraction O, 1.59 g. or 3.52% of Fraction K, with $[\alpha]_D^{25} = -75.8^\circ$ in pyridine ($c = 1$, $\ell = 1$). Similarily, the last sub-fractions (III), 4.26 g. or 9.48%, were combined to give Fraction P which had 36.0% of acetyl groups and $[\alpha]_D^{25} = -49.3^\circ$ in pyridine ($c = 1$, $\ell = 1$), values which were different from those of N and O. A small sample of Fraction N was again fractionally precipitated from chloroform with petroleum ether in order to test its homogeneity. The optical rotations of these sub-fractions (Table XI) were found to be similar, but slightly lower than the values in Table X.

TABLE IX

DATA FROM LARGE SCALE FRACTIONATION OF FRACTION K

BATCH [a]	1	2	3	4	Total
Fraction K	13.0	10.0	10.0	11.89	44.89
Sub-Fraction I	10.09	8.55	8.24	10.29	37.17
Sub-Fraction II	0.74	0.31	0.24	0.30	1.59
Sub-Fraction III	1.74	0.77	0.96	0.79	4.26
Washings	0.14	0.17	0.13	0.24	0.68
Recovery	12.71	9.80	9.57	11.62	43.70

(a) Weights in grams, not corrected for ash.

TABLE X

ROTATIONS [a] AND ACETYL CONTENTS OF FIRST LARGE SUB-FRACTIONS FROM FRACTION K

Sub-Fraction I [b]	$[\alpha]_D^{25}$	% Acetyl [c]
Batch 1	-75.8°	35.4
Batch 2	-74.9°	35.6
Batch 3	-75.6°	35.3
Batch 4	-75.3°	35.3

(a) In pyridine solution (c = 1, ℓ = 1).
(b) No corrections for ash.
(c) Single determinations.

TABLE XI

ROTATIONS [a] AND YIELDS OF SUB-FRACTIONS FROM FRACTION N[b]

Sub-Fractions[c]	Pet. Ether Added (ml.)	Weight (g.)	$[\alpha]_D^{25}$ in $CHCl_3$	$[\alpha]_D^{25}$
1	10	0.74	----	-74.6°
2	16	0.07	----	-74.4°
3	32	0.12	-61.6°	-74.9°
4	50	0.04	----	-74.2°

(a) In 1% pyridine solution (ℓ = 1).
(b) One g. in 100 ml. of chloroform.
(c) No corrections for ash.

The analytical data for Fraction N (Table XII) added up to 97.4% so only a small amount of hexoses could be present.

TABLE XII

ANALYTICAL DATA FOR FRACTION N

	Percent[a]	Equivalents
Ash	0.17	----
Nitrogen	0.47	0.03
Acetyl	35.45	0.815
Methoxyl	2.22	0.072
Furfural	32.65	----
Pentosans (b)	48.1	0.364
Uronic anhydride	10.96	0.062

(a) On an ash-free basis.
(b) Corrected for furfural from uronic acids.

The ratio of pentosan: uronic anhydride: methoxyl was calculated to be 5.85:1:1.15.

Fraction N was deacetylated by a 20% excess of aqueous potassium hydroxide as used by Perlin (126) and by Adams and Castagne (127). The deacetylated material contained a trace of acetyl group and was somewhat darker than Fraction N. After preliminary experiments, the product was bleached under mild conditions with sodium chlorite and ash was then removed by dialysis against water. When the dialysed solution was concentrated and 2 volumes of ethanol were added, only a part of the material could be recovered on the centrifuge. The mother liquor was concentrated to a small volume and a further fraction obtained as before. Further concentration of the mother liquor yielded a third fraction. This method of recovery made it possible to check the homogeneity of the polysaccharide.

Wethern (101) determined the viscosities of hemicellulose fractions from black spruce in 10% potassium hydroxide, and the same liquid was used for the subfractions from the deacetylated Fraction N so that a direct comparison would be possible. The data from these determinations are presented graphically in Fig. 10. Extrapolation to zero concentration gave the intrinsic

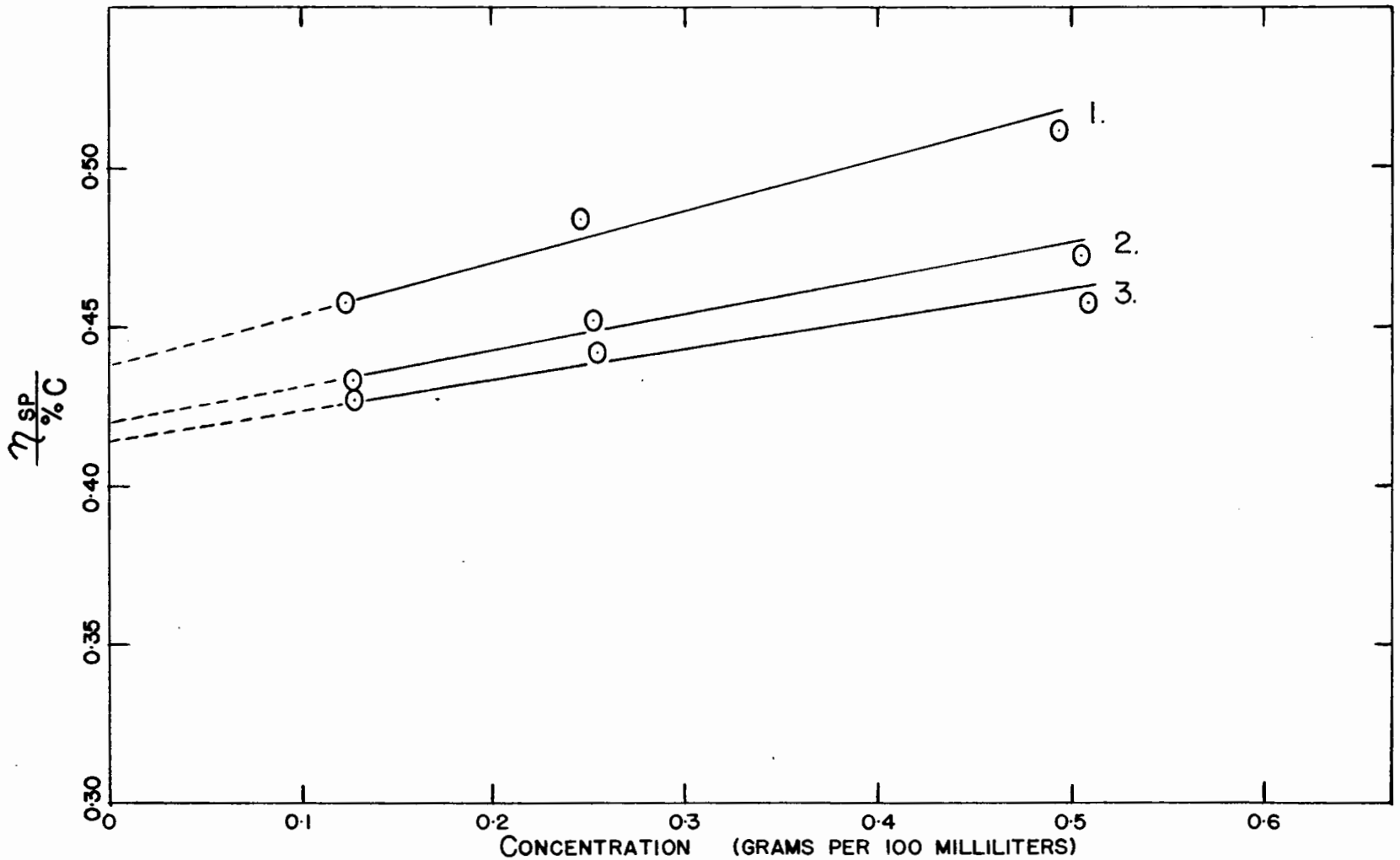


FIG. 10. VISCOSITIES OF SUB-FRACTIONS FROM DEACETYLATED FRACTION N IN 10% POTASSIUM HYDROXIDE

viscosities shown in Table XIII, which also contains the optical rotations of these sub-fractions.

TABLE XIII

INTRINSIC VISCOSITIES[a] AND OPTICAL ROTATIONS[b] OF SUB-FRACTIONS FROM DEACETYLATED FRACTION N[c]

Sub-Fraction	Intrinsic Viscosities	$[\alpha]_D^{25}$
1	0.438	-52.8°
2	0.420	-52.4°
3	0.414	-52.2°

(a) In 10% potassium hydroxide at 25° C.
(b) In 1% aqueous solutions ($\ell = 1$).
(c) All data corrected for ash.

Wethern (101) obtained approximate values of 0.65 and 0.85 for the intrinsic viscosities of the 5% and 16% potassium hydroxide extracts, respectively, in 10% potassium hydroxide at 30° C., but his preparations were obvious mixtures. As a very rough approximation from Wethern's data, it was estimated that the degree of polymerization of the deacetylated Fraction N was 80 to 90. This estimate, of course, was not relevant to the entire hemicelluloses of spruce wood, for the portion left in the cellulose residue was probably mannan of longer chain length; high viscosity material, presumably pectin, had been removed by

acetylation, and the more soluble polysaccharides by fractional precipitation. A reliable determination of the degree of polymerization of Fraction N would require an absolute method like osmotic pressure. Nevertheless, the fact that a large part of the alcohol-insoluble aqueous extract still had a relatively high viscosity even after extensive treatment indicated that excessive degradation had not taken place. It is quite possible that polyuronide hemicelluloses have shorter chain lengths than cellosans such as mannan, and that mixtures of the two types of hemicelluloses are responsible for the recently reported D. P.'s of about 150 (101)(112)(116).

The sub-fractions from deacetylated Fraction N also had very similar optical rotations (Table XIII), and preliminary analyses of the head and tail fractions (Table XIV) gave concordant results.

TABLE XIV

ANALYTICAL DATA FOR SUB-FRACTIONS FROM DEACETYLATED FRACTION N[(a)]

Sub-Fraction	Head (1)	Tail (3)
% Ash	2.44	2.47
Ash alkalinity, % Na_2O	89.4	87.9
% Acetyl	0.44	0.40
% Nitrogen	0.43	0.39
% Methoxyl	3.56	3.72
% Furfural (b)	47.3	48.4
% Uronic anhydride	15.8	15.8

(a) All data on an ash-free basis.

(b) Not corrected for furfural from uronic acid.

The three sub-fractions were recombined as Fraction R. Because of the small difference in furfural content between the head and tail sub-fractions, larger samples of Fraction R were taken for furfural and uronic anhydride determinations. The average of these values, together with the average values for other determinations on the head and tail sub-fractions (Table XIV), are reproduced in Table XV.

TABLE XV.

ANALYTICAL DATA FOR FRACTION R [a]

	Percent	Equivalents
Ash	2.45	---
Ash alkalinity, % Na_2O	88.7	0.07
Acetyl	0.42	0.01
Nitrogen	0.41	0.03
Methoxyl	3.64	0.117
Furfural	47.3	---
Pentosan (b)	69.7	0.528
Uronic anhydride	16.0	0.091

(a) All values corrected for ash.
(b) Corrected for furfural from uronic acid.

The values reported in Table XV gave a pentosan: uronic anhydride: methoxyl ratio of 5.82:1:1.29.

The analytical data added up to 92.6% of Fraction R, and addition of the 2.5% of galactose found in the quantitative estimation of the sugars increased this figure to 95.1%.

These results were rather surprising, since 97.4% of Fraction N could be accounted for without considering the amount of hexoses present. The data for Fractions N and R, however, were subject to an uncertain correction applied for furfural from uronic acid. Sarkar, Mazumdar and Pal (107) recently reported that a methoxyuronic acid believed to be methoxyglucuronic acid was incapable of yielding furfural on distillation with 12% hydrochloric acid. Moreover, the conversion of % furfural to % pentosan was by assuming that the ratio of xylan to araban units was 11:1. If the uronic anhydride correction was not applied to the % furfural, the revised pentosan content would be 75.1%, the total recovery 100.5% (including galactose), and the ratio of pentosan : uronic anhydride : methoxyl would be 6.25:1:1.29.

The presence of xylose, arabinose and galactose in the neutralized hydrolysate of Fraction R was shown by comparison with known sugars on the same chromatogram. Xylose was confirmed by the identification of the free sugar, m.p. 144° - 145° C., not depressed on admixture with an authentic sample; and $[\alpha]_D^{23} = +18.8°$ in water ($c = 0.945$, $\ell = 4$) compared to $[\alpha]_D^{23} = +18.9°$ for pure xylose under the same conditions. Arabinose was obtained in a minute amount after considerable difficulty as the benzoyl hydrazone (129), having a m.p. of 184.5° - 186° C., not depressed when admixed with an authentic sample. An attempt to prepare the methylphenylhydrazone of galactose was a

failure, and the identification of traces of galactose was based only on paper chromatography. Although the ethyl acetate-acetic acid-water developer (124) was known to separate mannose and arabinose in paper chromatography, no mannose could be detected by means of this system. Micro phenylhydrazine tests also gave negative tests for mannose.

A sample of the non-reducing Fraction R was hydrolyzed at pH_2 and 100° C. in 2 hours to a constant rotation of -24.8° from an original value of -50.6° (Fig. 11). The copper reducing power did not become constant, but after 2 hours indicated that about 11% of the material (as arabinose) had been hydrolyzed. The hydrolysate was cooled, neutralized and examined by paper chromatography. Arabinose together with a considerably smaller amount of xylose were the only free sugars detected on papers developed by the ethyl acetate-water-pyridine and the ethyl acetate - water-acetic acid systems. The neutral solution was diluted with an equal amount of 5% sulphuric acid and the hydrolysis was continued at 100° C. to constant observed rotation and reducing power (Fig. 11). Since the concentration was by then somewhat uncertain, a further sample was hydrolyzed in 2.5% sulphuric acid to a constant specific rotation of + 43.9°. This value together with the final observed rotation from the first experiment gave a calculated concentration of 0.1002 g. in 18 ml. of 2.5% sulphuric acid. This concentration was used to calculate all values in Fig. 11 for the hydrolysis of Fraction R in 2.5% sulphuric acid.

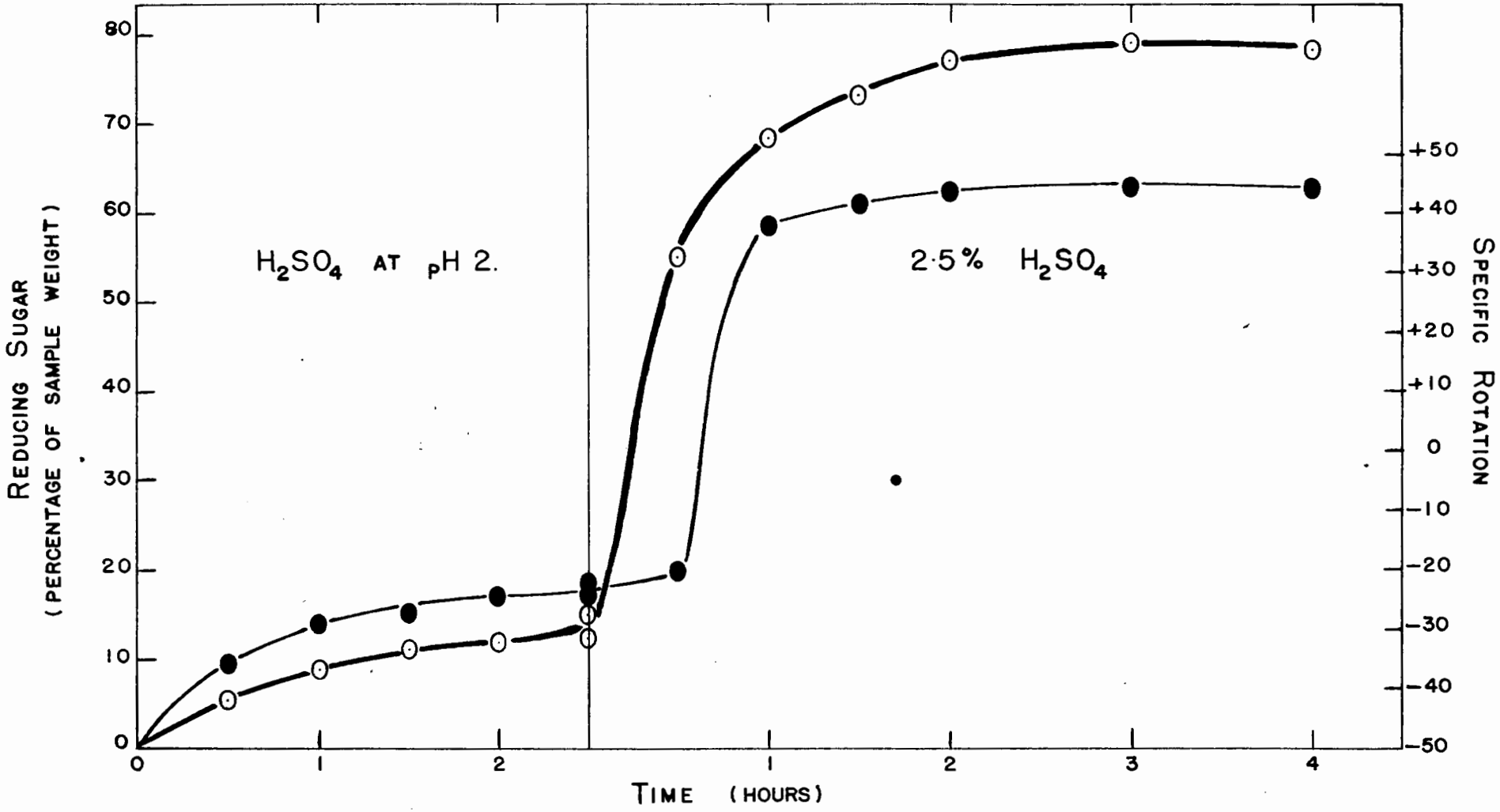


FIG. 11. HYDROLYSIS OF FRACTION R WITH 0.025 N (pH 2) AND 2.5% SULPHURIC ACID AT 100° C.

Reducing sugar **O** ; Specific rotation **●** .

The sugars in the neutralized hydrolysate of Fraction R were separated by paper chromatography and the relative amounts determined by oxidation with sodium metaperiodate as described by Hirst and Jones (130). Those found amounted to 52.7% of Fraction R, a value which was quite low. The procedure was considered to be poor when some of the free sugars were present only in relatively small amounts. A more satisfactory method was to separate the free sugars by chromatography as before and then determine their amounts with the Shaffer-Hartmann-Somogyi copper reagent (131). The results from these determinations (Table XVI) gave a ratio of arabinose to xylose of 1 to 7.2. A small amount of galactose (2.5%) was present and the sugars amounted to 64.4% of Fraction R. The ratio of arabinose to xylose indicated that 2 moles of aldotriuronic acid residues were present in addition to the free sugars, in order to account for a 6:1 pentosan : uronic anhydride ratio. Two moles of monomethyl aldotriuronic acid (M.W. 471), on the basis of the quantitative estimations of the sugars, was 42.1% of Fraction R. This value gave a total recovery of 106.5% which was considered satisfactory because of the uncertain composition of the uronic acid residue.

A larger amount of Fraction R, 7.83 g., was hydrolyzed to constant rotation, and a total of 1.18 g. of reprecipitated barium uronates were recovered in three fractions from the neutral hydrolysate. Paper chromatography showed that not all of the barium uronates had been isolated, and that the

TABLE XVI

QUANTITATIVE ESTIMATION OF SUGARS IN FRACTION R

Sugar	Titre (ml.)	Blank (ml.)	Thiosulphate (ml.) = mg. sugar	Sugar in 0.1 ml. solution (mg.)	Ratio	% of Fraction R
Xylose	9.20+9.65	0.34	10.0 = 1.328	2.46	7.03	53.4
Arabinose	2.64	0.30	3.0 = 0.451	0.35	1	7.6
Galactose	1.01	0.30	2.16 = 0.35	0.115	0.304	2.5
Total	----	----	----	2.925	----	63.5
Xylose	10.05+9.87	0.40	12.12 = 1.578	2.54	7.34	55.2
Arabinose	2.62	0.20	3.42 = 0.489	0.346	1	7.5
Galactose	0.93	0.20	2.16 = 0.35	0.118	0.34	2.6
Total	----	----	----	3.004	----	65.3

reprecipitated uronates contained no simple sugars. After preliminary analyses showed that these fractions, $[\alpha]_D^{25} = +73^\circ$ in water, had the same barium content, they were combined and re-analysed with the results given in Table XVII.

TABLE XVII

ANALYTICAL DATA FOR BARIUM URONATE

A - Theoretical values for the barium salt of monomethoxy aldobiuronic acid.

B - Theoretical values for the barium salt of monomethoxy aldotriuronic acid.

C - Values found for barium uronate.

	A	B	C	
% Barium	16.85	12.73	17.4	(a)
% Methoxyl	7.61	5.74	8.8	
% Uronic anhydride	43.15	32.6	34.6	
Equivalent Weight	408.	540.1	481.6	(b)

(a) Single large determination.
(b) Oxidation of reducing groups with hypoiodite.

The barium uronate appeared to be a mixture and the small amount remaining prevented an extensive purification. The uronic anhydride content and equivalent weight indicated that the barium salt of an aldotriuronic acid made up the bulk of the material. Calculation of the uronic anhydride: methoxyl ratio gave a value of 1:1.44, which was slightly higher than the ratios of 1:1.2 and 1:1.3 found for Fractions

N and R, respectively.

The analytical data from this preliminary study showed that the polyuronide hemicellulose isolated from spruce was in all probability homogeneous and that the ratio of pentosan to uronic anhydride was about 1 to 6. Similar types of compounds have been isolated from hardwoods and plant tissues but, until very recently, e.g. (127) (132), never in a carefully purified condition. The present preparation is believed to be the first hemicellulose fraction from softwoods to show great similarity to hemicelluloses of the "B" type found in hardwoods and plant tissues.

It was unfortunate that neither time nor the supply of the purified hemicellulose was adequate for a detailed study of its structure, for such a study would have shown among other things whether the minor constituents, araban and galactan units, were integral parts of the molecule or were very tenaciously retained impurities. These important questions must be left for future research.

EXPERIMENTAL

ANALYTICAL METHODS

All analyses were carried out in duplicate unless otherwise noted.

Moisture

The loss in weight of 1 - 2 g. samples when dried in an oven at 105° C. for 16 hours was determined.

Ash

The residue from the determination of moisture was ignited to constant weight in a tared porcelain crucible, at 600° to 650° C. in an electric muffle furnace. Samples of 0.10 to 0.15 g. of the extracted materials, dried to constant weight over phosphoric anhydride under reduced pressure, were treated in the same manner. In some cases, 0.01 to 0.03 g. samples were ashed by the procedure of Niederl and Niederl (133).

Ash Alkalinity

The ash alkalinity was determined by a standard procedure (134), and was calculated as percentage by weight of sodium oxide in the ash.

Klason Lignin

The standard TAPPI method (135), which specified a digestion of 2 hours at 18° to 20° with 72% sulphuric acid, was used. The sample weight was reduced from the 2 g. used in the official method to 0.2 g. to 1 g. The lignin was collected in a weighed, coarse sintered glass filtering crucible containing an asbestos pad.

Furfural

Furfural determinations were carried out by the TAPPI method (136). The amount of the 0.2 N bromate-bromide solution used was increased from 20 to 25 ml. Sample weights of 0.1 to 0.2 g. were used for extracted materials.

Most of the analytical results were expressed as percent furfural because of the uncertain composition of the samples as regards the pentoses and uronides present. The amount of furfural was calculated by substitution in the expression:-

$$\% \text{ Furfural} = \frac{(V_2 - V_1) \times N \times 0.048 \times 100}{W},$$

where V_2 = ml. of thiosulphate in the blank determination,

V_1 = ml. of thiosulphate in the test determination,

N = normality of thiosulphate,

And W = weight of sample in grams.

The factor 0.048 was the weight of furfural in grams corresponding to 1 ml. of N thiosulphate.

Quantitative estimations of the purified hemicellulose indicated that approximately 1 mole of arabinose was present for 11 moles of xylose. In this case the pentosan content of the polysaccharide and its acetate was calculated in the following manner.

Norris and Resch (137) reported a furfural yield of 21.5% from glucuronic acid. The furfural content was corrected for uronic anhydride furfural by substitution in the expression:-

$$\% \text{ Furfural} = A - 0.215\ U,$$

where A = % furfural found,

and U = uronic anhydride content.

When desired, the pentosan content was calculated from the expression :-

$$\% \text{ Pentosan} = \frac{\frac{11}{12} \times B}{0.727 \times 0.88} + \frac{\frac{1}{12} \times B}{0.727 \times 0.74},$$

where B = corrected furfural content, 0.727 was the correction for the difference in molecular weight between pentosan and furfural, 0.88 was the observed 88% conversion of xylose to furfural in this distillation, and 0.74 the conversion of arabinose.

Uronic Anhydride

The method of McCready, Swenson and Maclay was used (138). Samples of 0.2 g. to 1.0 g. were heated with 19% hydrochloric acid at 145° to 150° C. for 1.5 hours, a stream of carbon dioxide-free air being drawn through the apparatus as a carrier gas for the carbon dioxide generated. The carbon dioxide was absorbed in 0.25 N sodium hydroxide, the carbonate was precipitated with 10 ml. of 10% barium chloride, and the resulting solution titrated with 0.1 N hydrochloric acid. A blank run was performed without added sample. The uronic anhydride carbon dioxide was calculated from the expression:-

$$\% CO_2 = \frac{(V_2 - V_1) \times N \times 0.022 \times 100}{W},$$

where V_2 = ml. of hydrochloric acid used for blank determination,

V_1 = ml. of hydrochloric acid used for test determination,

N = normality of hydrochloric acid,

and W = weight of sample in grams.

The factor 0.022 represented the weight in grams of carbon dioxide equivalent to 1 ml. of N hydrochloric acid.

$$\% \text{ Uronic anhydride} = \% \text{ Uronic anhydride carbon dioxide} \times \frac{4.0}{\text{(mol. wt. 176)}} \quad \frac{}{\text{(mol. wt. 44)}}$$

Methoxyl

The method of Viebock and Schwappach (139) and Viebock and Brecher (140), with the modifications of Peniston and Hibbert (141) was used. The solution in the scrubber was a 1:1 mixture of 5% aqueous sodium thiosulphate and 5% aqueous cadmium sulphate, as recommended by Friedrich (142).

Acetyl

(a) Acetyl in wood was determined by hydrolysing the sample with 72% sulphuric acid. The liberated acetic acid was separated by steam distillation, and the distillate was titrated with standard sodium hydroxide. The method of Genung and Mallatt (143) modified to a semi-micro scale by Lemieux (144) was used.

(b) Acetyl in acetylated polysaccharides was determined on 0.01 g. to 0.02 g. samples by the semi-micro method of Clark (145). Saponification of the sample was effected by heating with N alcoholic potassium hydroxide, and after acidification the liberated acetic acid was removed by steam distillation and titrated with 0.02 N barium hydroxide to the phenol red end point. The distillate was increased to 100 ml. and no correction factor was applied. Complete blank determinations on cigarette paper amounted to 0.40 ml. of base.

Nitrogen

Nitrogen was determined on a semi-micro scale by an adaption of the Gunning method (146).

The sample, varying from 0.01 to 0.03 g., was weighed onto a small square of cigarette paper which was then placed in a Kjeldahl flask together with 0.1 g. of salicylic acid. After adding 2 ml. of concentrated sulphuric acid, the flask was allowed to stand at room temperature until the sample and paper had dissolved. Sodium thiosulphate, 0.3 g., was then added and after standing for 5 minutes, the flask was heated at a low temperature for 5 minutes. Finally, 0.6 g. of potassium sulphate was added, and the sample was digested by gradually raising the temperature; the flask was cooled and the clear liquid diluted with about 8 ml. of water before being poured into the distilling apparatus. The flask was washed with several small volumes of water and the solution was made strongly alkaline with about 15 ml. of 37% sodium hydroxide. Steam distillation for 5 to 10 minutes was followed by a 1-minute draining period. The distillate was collected in 25 ml. of 0.8% boric acid, and titrated with standard 0.025 N hydrochloric acid, as described by Ma and Zuazaga (147). The methyl red-bromocresol green mixed indicator was replaced by methyl purple (148) in later determinations. Blank determinations on cigarette paper amounted to 0.10 ml.

of 0.025 N hydrochloric acid.

Carbon and Hydrogen

The procedure described by Niederl and Niederl (149) was followed, the "universal" type combustion tube being used.

Chlorine

Samples, 0.01 to 0.02 g., were fused with sodium peroxide in a Paar bomb according to the directions given by the Paar Instrument Company (150). The chlorine was recovered and weighed as silver chloride.

Copper Reducing Power

The method of Shaffer and Somogyi as described by Brown and Zerban (151) was used. The alkalinity of the copper reagent was increased by using sodium carbonate in place of sodium bicarbonate as recommended. In actual determinations, 0.10 to 0.30 ml. samples of the hydrolysates were added to the reaction tubes, and then diluted to 5 ml. These solutions, which were not neutralized, gave consistent, reproducible results. The thiosulphate solutions were standardized against known sugar solutions.

Ultraviolet Absorption Spectra

Ultraviolet absorption measurements were carried out by means of a Beckmann Quartz Spectrophotometer kindly made

available by the Department of Biochemistry.

Paper Chromatography

The apparatus used was supplied by Fisher Scientific Co. The tank had a height of 24 inches, a diameter of 12 inches and contained a stainless steel stand. Strips (6 in. X 22.5 in.) of Whatman No.1 filter paper were used for the chromatograms. Five spots, 1 inch apart, were usually marked along the starting line, after which the neutralized hydrolysates and reference solutions of known sugars were added to appropriate spots. In order to keep the diameter of the spot less than 1 cm., small increments of the solution were dried by a flow of warm air from a small hair drier. The solutions were usually added to the spots by means of micro-pipettes, made by drawing out both ends of a short length of capillary tubing. The delivery end was ground so that no sharp edges were present, and the pipette was held in a short length of rubber tubing extending beyond the end of a 6-inch length of glass tubing. The pipettes, calibrated with mercury, had capacities ranging from 0.00462 ml. to 0.00873 ml. The aqueous solution was sucked into the pipette, the upper end of the pipette was then touched against the wall of the glass tubing to remove excess liquid and the contents were added to one of the spots on the paper. Larger volumes were added from a 0.20 ml. pipette graduated to 0.02 ml. Except

for the quantitative estimations, less than 1 mg. of solid was added to each spot.

The solvent system first investigated, butanol-ethanol-water, as described by Partridge (152), was much slower in its action than the pyridine-ethyl acetate-water system of Jermyn and Isherwood (124), and did not give better separations of the sugars. The ethyl acetate-acetic acid-water system (124), separated mannose and arabinose, but was quite slow and also caused "tailing". The pyridine-ethyl acetate-water solvent system was selected for most of the determinations. All papers were dried and developed with the aniline phthalate spray proposed by Partridge (125).

Preparation of Spruce Holocellulose

The black spruce (Picea mariana) used was part of a large supply stored in this laboratory. The supply came from logs approximately 70 years old which had been reduced to small chips in a Mead mill in 1948. The sample was free of knots and bark and was further reduced in size in a Wiley mill, the 40 - 60 mesh portion being retained for the present study. The meal was extracted in a large Soxhlet apparatus for 48 hours with a constant boiling 1:2 mixture of ethanol and benzene. After extracting for an additional 24 hours with ethanol, the meal was spread

out and air-dried.

The holocellulose was prepared by the general procedure of Wise, Murphy and D'Addieco (63), 100 g. of the air-dried wood meal being treated at one time. The meal, suspended in 3,200 ml. of distilled water at 70° to 80° C., was treated with 4 hourly additions of 10 ml. of glacial acetic acid and 30 g. of solid sodium chlorite. The products from several treatments were combined into one large batch. Two such large batches of holocellulose were prepared however by slightly different procedures, the first (Sample 1) at a pH maintained by hourly additions of 10 ml. of glacial acetic acid for each 100 g. of wood meal. The p H of the suspension during a typical preparation, when a total of 40 ml. of acetic acid was added, decreased from 4.0 to 3.8 after 1.5 hours and then rose slowly to 4.05 at the end. A Beckmann p H meter was used. The second large batch (Sample 2) was prepared at a p H nearer 4 by decreasing the hourly addition of glacial acetic acid to 8 ml. for each 100 g. of meal treated. During these preparations, the lowest value for the p H was 3.95 and the final value was 4.15.

Data on the holocelluloses mixed to give Sample 1 are shown in Table XVIII.

TABLE XVIII

DATA FOR HOLOCELLULOSE PREPARATIONS COMBINED AS SAMPLE 1

Lot number	1	2	3	4	5	6
Wood (g.) (a)	93.1	93.1	93.1	93.1	90.5	90.5
Holocellulose (g.) (a)	69.0	69.9	70.0	69.3	68.0	67.2
Holocellulose, %	74.2	75.1	75.2	74.5	75.1	74.2
Lignin in holocellulose, % (a)	2.8	3.2	3.0	3.0	3.2	2.9

(a) Values on moisture-free basis.

Sample 1 was prepared in an overall yield of 74.7% and had 3.1% Klason lignin, while Sample 2 amounted to 77.2% of the wood and contained 4.0% lignin.

Extraction of Holocellulose with Water

Sample 1 was extracted with hot water under the conditions used in preparing the holocellulose from the wood. A small scale extraction was first carried out on 22.8 g. (dry and ash-free) by stirring the holocellulose with 500 ml. of distilled water at a temperature of 70° - 80° C. for 4 hours. The dry, extracted material amounted to 0.69 g. or 3.03% of the holocellulose. A further treatment under the same conditions gave very little additional material.

The two large amounts of holocellulose were extracted in the same manner. In the case of Sample 1, which amounted to 336.2 g., the extract was 12.45 g. or 3.72% of the holocellulose and 2.78% of the original wood. The same treatment of Sample 2 (689 g.) gave a dry extract of 23.4 g., 3.4% of the holocellulose or 2.62% of the wood. The above values are on a moisture and ash-free basis. The analytical data for samples taken at various stages are given in Table XIX.

TABLE XIX

ANALYTICAL DATA FOR SPRUCE WOOD, WATER-EXTRACTED HOLOCELLULOSE AND THE WATER EXTRACT(a)

A - Extractive-free spruce meal.

B - Water-extracted Sample 1, designated as Sample 3.

C - Water extract from Sample 1.

D - Water-extracted Sample 2, designated as Sample 4.

	A	B	C	D
% Moisture	9.54	0.84(b)	nil(c)	0.58(b)
% Ash	0.25	0.59	8.5	0.55
% Lignin	27.5	2.5	9.25	3.60
% Furfural(d)	5.99	6.8	5.93	---
% Methoxyl	5.01	1.85	4.7	---
% Acetyl	1.28	1.3	2.7	1.4
% Uronic anhydride	3.6	4.9	11.5	---
% Nitrogen	---	0.14	----	0.15

(a) Calculated on moisture and ash-free basis.
(b) Dried in vacuum over phosphoric anhydride.
(c) Dried to constant weight over phosphoric anhydride in vacuum.
(d) Not corrected for furfural from uronic acids.

Procedure for Small Scale Liquid Ammonia Extraction

A preliminary small scale extraction was carried out on 6.81 g. of water-extracted holocellulose, a thick-walled stainless steel bomb having a capacity of about 250 ml. being used. The bomb was equipped with a thick steel lid fitted with a lead gasket, and the lid was securely fastened by a bolt threaded through a heavy steel yoke which encompassed the whole bomb. After being cooled to about -15° C. in a brine bath, the bomb containing the holocellulose was opened, weighed and 40 g. of liquid ammonia was poured in from a suction flask. The lid was replaced and fastened securely by placing the bomb in a vice and tightening the bolt with a wrench. The assembly was tested at room temperature for leaks and was placed in a shaker and shaken overnight.

The bomb was then cooled to about -60° C. by immersion to a point just below the bottom of the lid in a carbon tetrachloride-chloroform-dry ice bath. After removing the lid, the lower portion of the bomb was quickly connected to the apparatus shown in Fig. 12. This apparatus, constructed by Mr. Jablonski and Mr. Sanderson, was found to be much more convenient and efficient than the one used by Yan (1). The rubber stopper (C) was tightly forced into the mouth of the bomb by screwing down three wing-nuts (B) on the three long bolts connecting the two flanges (A). The gas dispersion tube (D) (coarse sintered glass)

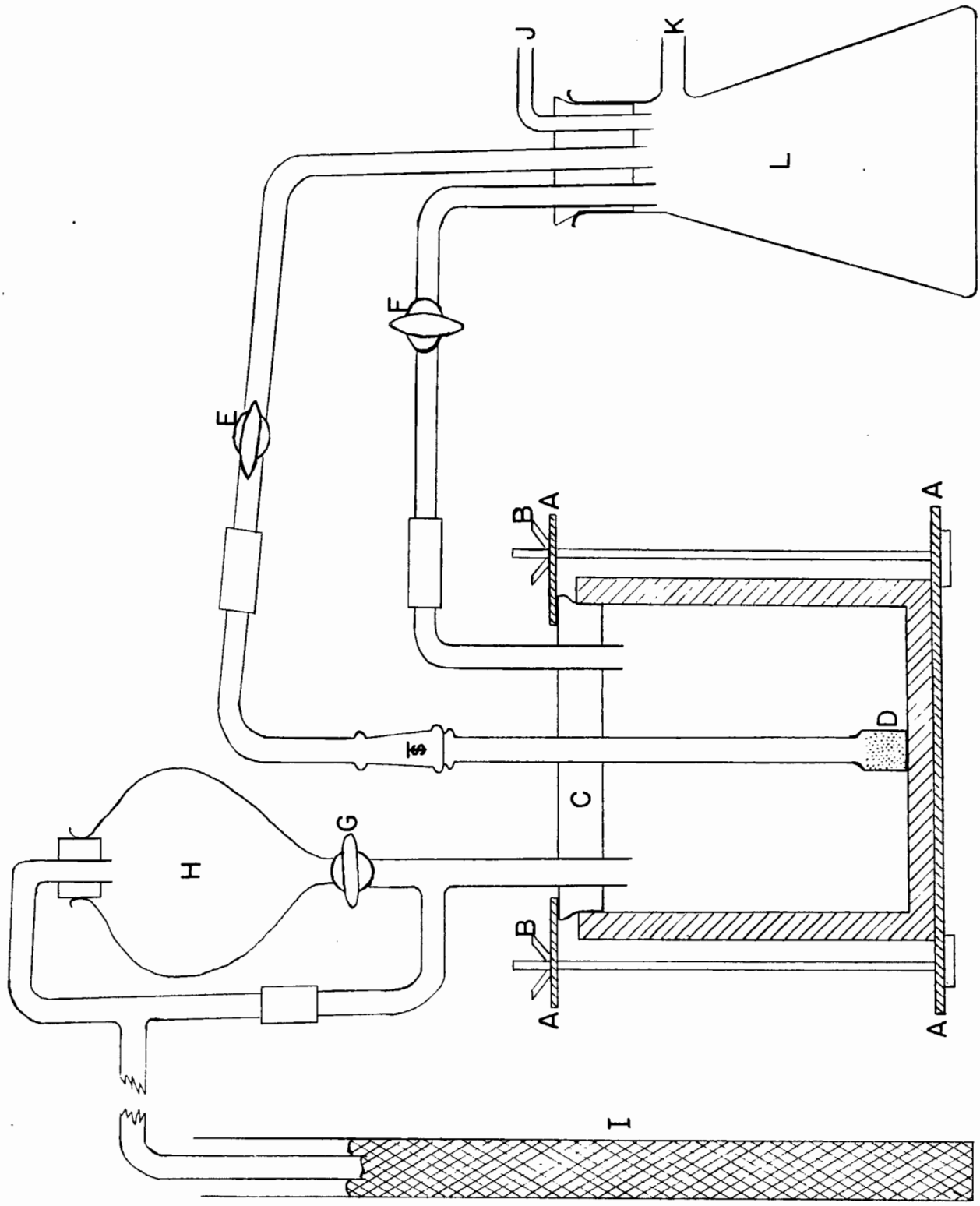


FIG. 12. APPARATUS USED FOR SMALL SCALE LIQUID AMMONIA EXTRACTION

had been adjusted previously so that it touched the bottom of the bomb and during this time, stopcocks E and F were open but stopcock G was closed. After closing stopcock F, the pressure of ammonia gas in the bomb quickly forced over the liquid ammonia in the bomb to a 1-liter suction flask (L) which contained 100 ml. of anhydrous methanol. Tubes J and K were connected to potassium hydroxide guard tubes which were vented to air at the back of the fume cupboard.

A supply of liquid ammonia had been collected in a separate suction flask and transferred to the separatory funnel (H) which had been graduated. The separatory funnel (H) was connected to a mercury safety valve (I) which contained a column of mercury approximately 50 cm. high. Stopcock E was closed, F was opened and 100 ml. of liquid ammonia was added to the bomb by opening stopcock G. This wash was removed as before and the holocellulose was washed with another portion of liquid ammonia in the same manner. Liquid ammonia amounting to approximately 40 g. was then added, the bomb was removed from the assembly, closed and shaken overnight. The second extract was removed in the same manner and added to the material from the first extraction. A third extraction was completed and this time the extract was very nearly colorless.

The bomb was vented to air through a potassium hydroxide guard tube and after most of the remaining ammonia had evaporated, the extracted holocellulose was spread out in the air to dry. Most of the liquid ammonia in the extracts was allowed to evaporate at room temperature and the remainder was removed under reduced pressure. A portion of the liquid ammonia-extracted holocellulose, 4.48 g., was re-extracted by stirring with 80 ml. of distilled water at room temperature for 2 hours. The extract was removed and the extraction was repeated with a fresh volume of water. Finally, the holocellulose was extracted in the same manner with two volumes of water at 70° - 75° C. The yields of these extracts are in Table XX.

TABLE XX

YIELDS FROM A PRELIMINARY EXTRACTION OF HOLOCELLULOSE SAMPLE 3

	Weight (g)[a]	% of Holocellulose	% of Wood
Holocellulose Sample 3	6.81	96.28	71.92
NH_3 extracted holocellulose	6.13	86.6	64.7
Ammonia extract	0.59	8.34	6.23
A	4.49	--	--
Cold water extract 1.	0.53	10.22	7.64
Cold water extract 2.	0.03	0.58	0.43
Hot water extract 1.	0.13	2.51	1.88
Hot water extract 2.	0.01	0.19	0.14
Total water extract	0.70	13.5	10.1
Residue	3.63	70.0	52.4

(a) No corrections for ash, extracts dried to constant weight in vacuum over phosphoric anhydride.

A- Amount of ammonia-extracted holocellulose used giving the water extracts shown.

Large Scale Extractions with Liquid Ammonia

The bomb used consisted of a stainless steel pipe 48 inches long and 6.5 inches in diameter, sealed at one end and threaded on the outside of the open end. A heavy top was fitted to the bomb by means of threads on the inside of the top. The top was pierced by 8 bolts which, when screwed down, pressed a thick, teflon-gasketted lid against the top of the bomb to produce a tight closure. This lid was pierced and fitted with two 1/4-inch steel tubes which passed through openings in the top, and were connected to steel needle valves serving as inlet and outlet tubes for the liquid ammonia. The ends of these tubes which opened into the bomb were covered with a disk of 60 mesh steel screen, to prevent the loss of holocellulose during the filling and emptying of the bomb.

The holocellulose, previously extracted with water, had a moisture content of less than 1% after drying over phosphoric anhydride under reduced pressure. Sample 3, 313.5 g., was added to the bomb which was closed and cooled to about -10° C. by being left outside overnight. The bomb and contents were then placed on a heavy-capacity platform scale, the inlet tube was connected to a cylinder of ammonia inverted to deliver the liquid, and a tube from the outlet led to the fume cupboard. After weighing the bomb to the nearest 1/8 kg., both valves were opened and

liquid ammonia was run in slowly. The evaporation of the first portion of liquid ammonia soon cooled the bomb and its contents to the point where the rate of addition of liquid ammonia could be increased. Two kg. of ammonia was charged in less than one-half hour, the cylinder valve, the inlet valve and outlet valve were then closed in that order. The inlet line contained an extra valve, vented in the fume cupboard, to bleed off ammonia from the line before disconnecting it from the bomb.

The bomb was then transferred to the shaking apparatus and shaken overnight at room temperature. This shaking period was longer than that used by Yan (1) and by Neubauer (2), but was more convenient. The next morning, the bomb was inverted in the shaking machine and the outlet tube was connected to a length of copper tubing which passed through a rubber stopper into a 6-liter Pyrex flask. This inlet tube extended about 3 inches further into the flask than an outlet tube which was vented to the hood by means of rubber tubing.

The outlet valve on the bomb was slowly opened and the liquid ammonia was forced into the flask, which contained 500 ml. of anhydrous methanol, by the pressure of gaseous ammonia in the bomb. When the flow of ammonia had ceased, the bomb was disconnected and charged with a

further 2 kg. of liquid ammonia and shaken as before. The Pyrex flask was transferred to the fume hood, and after attaching potassium hydroxide guard tubes to the inlet and outlet lines, the ammonia was allowed to evaporate. This process was assisted by circulating cold water around the flask. The second extract was collected in the same flask and the extraction was repeated a third time.

Much of the remaining ammonia was removed from the bomb and contents by evacuation on a water pump. The bomb was then opened and the extracted material was spread out in a fume cupboard until all ammonia had evaporated. The liquid ammonia extract was finally concentrated to a small volume under reduced pressure, at which time no ammonia could be detected in the methanol solution. The fractionation of this extract is described in a later section of this thesis. Holocellulose Sample 4, 600 g., was treated in exactly the same way except that 3 kg. of liquid ammonia was used for each extraction.

The air-dried ammonia-extracted holocellulose was then re-extracted with ethanol in a large Soxhlet apparatus, but the extract was almost negligible. The ethanol-extracted material was again spread out to dry in the air. When this operation was complete, 300 g. portions were extracted with 5 liters of distilled water at room temperature. The slurry was stirred for approximately

four hours, but the great bulk of the soluble material seemed to dissolve at once. After removing the solution by filtration, the meal was again extracted with cold water as before. Two further water extractions were carried out in a similar manner, but at a temperature of 70° - 80° C. The water extracts were concentrated under reduced pressure until the solutions appeared to be slightly viscous. All such vacuum distillations were performed under an atmosphere of nitrogen which was delivered from a nitrogen-filled balloon attached to the bubbler. Two volumes of absolute ethanol were added to each solution, thus separating the extracted material into ethanol-soluble and insoluble fractions. The ethanol-insoluble portions were recovered on the centrifuge, solvent exchanged through ethanol and ether and dried to constant weight in vacuum over phosphoric anhydride. The clear filtrates were evaporated to dryness under reduced pressure and dried to constant weight in the usual manner.

Material balances for the extractions of Samples 3 and 4 are given in Table XXI.

TABLE XXI

MATERIAL BALANCE FOR LARGE SCALE EXTRACTIONS OF HOLOCELLULOSE

	Wt. of Material (g.)		% of Holocellulose		% of Wood	
Holocellulose sample	3	4	3	4	3	4
H_2O extracted holocellulose	313.5	600.0	---	---	71.9	74.6
Liquid NH_3 extract	21.73	39.60	6.93	6.61	4.98	4.92
NH_3 extracted holocellulose	291.0	557.0	92.82	92.10	66.7	67.6
Ethanol extraction	0.26	0.39	0.09	0.07	0.06	0.05
Cold water extract 1. (a)	37.72	78.52	12.03	13.10	8.66	9.76
A	25.94	56.01	8.28	9.34	5.96	6.96
B	11.78	22.51	3.75	3.76	2.70	2.80
Cold water extract 2.	7.05	8.58	2.25	1.43	1.62	1.07
A	4.68	5.31	1.49	0.89	1.07	0.66
B	2.37	3.27	0.76	0.54	0.55	0.40
Hot water extract 1.	5.79	13.63	1.85	2.27	1.33	1.69
A	4.16	9.30	1.33	1.55	0.96	1.15
B	1.63	4.33	0.52	0.72	0.37	0.54
Hot water extract 2.	2.22	5.62	0.71	0.93	0.51	0.69
A	1.73	2.91	0.55	0.48	0.40	0.36
B	0.49	2.71	0.16	0.45	0.11	0.33
Residue	243.0	449.0	77.5	74.9	55.7	55.9
Water extracts, total	52.79	106.33	16.84	17.73	12.1	13.2
Total extracts	74.77	146.32	23.83	24.36	17.12	18.15
Recovery	317.77	595.32	101.3	99.2	----	----

(a) Extracts dried to constant weight in vacuum over phosphoric anhydride, not corrected for ash but corrected for material removed for analyses.

A - Insoluble in 66% ethanol.

B - Soluble in 66% ethanol.

Samples of the holocellulose were retained after the liquid ammonia extractions. The analytical data for these samples, together with others of completely extracted holocellulose, are shown in Table XXII.

TABLE XXII

ANALYTICAL DATA FOR AMMONIA-EXTRACTED AND COMPLETELY EXTRACTED HOLOCELLULOSE (a)

	NH_3 Extracted Holocellulose		Totally Extracted Holocellulose	
Holocellulose sample	3	4	3	4
% Moisture	6.00	5.6	4.88	5.97
% Ash	0.77	0.65	0.28	0.28
% Klason lignin	0.47	1.59	0.59	1.62
% Nitrogen	0.78	0.98	0.26	0.38
% Acetyl	0.22	0.27	0.25	0.33

(a) All data corrected for ash and moisture.

Liquid Ammonia Extract

A considerable amount of the extract was insoluble in anhydrous methanol after ammonia had been removed under reduced pressure. The product from the first extraction had a volume of 75 ml., while the second had a volume of 100 ml. The insoluble material in these volumes of methanol was removed and was washed with methanol and ether before being dried to constant weight in the usual manner. These first fractions were separately weighed and then combined to give Fraction A.

The clear, red-brown mother liquors gave a further fraction on the addition of two volumes of anhydrous ether. The light yellow precipitate was somewhat sticky and formed a very hard, compact solid in the centrifuge. It was found more convenient to allow the precipitate to settle and to pour the mother liquor off. The precipitate could then be washed with anhydrous ether and centrifuged without the formation of a dense solid. After drying and weighing, the material from the two extracts was combined (Fraction B).

The mother liquors were evaporated to dryness under reduced pressure and the residues were taken up in water. Small amounts, insoluble in water, were recovered on the centrifuge, were washed with water, dried to constant weight, weighed and combined to give Fraction C. After an unsuccessful attempt to remove acetamide from the aqueous solution by continuous extraction with ether, the liquor was concentrated to dryness under reduced pressure, and the dry solid was continuously extracted with anhydrous ether. Although the ether removed a considerable amount of acetamide, it was evident that some acetamide remained in the solid. A quicker and much more convenient method of removing acetamide was by vacuum distillation at 60° - 62° C. and 0.001 mm. pressure. The acetamide was collected in the distilling head and in a trap cooled in a carbon tetrachloride-chloroform-dry ice bath and the distillation was

continued for 3 hours after material had apparently ceased to distill. The residues were kept separate as Fractions D1 and D2, while the acetamide was weighed and combined as Fraction E.

Only Fraction A gave a positive Molisch carbohydrate test and was also the only fraction to show reducing power after hydrolysis for 5 hours in 2.5% sulphuric acid on a boiling water bath. The yields of the various fractions from the liquid ammonia extracts are given in Table XXIII, their analyses in Table XXIV and their ultraviolet absorption spectra in Table XXV.

TABLE XXIII

YIELDS OF FRACTIONS FROM LIQUID AMMONIA EXTRACTS

Fraction	Wt. of Fraction (g.)[a]		% of Holocellulose		% of Wood	
	3 (b)	4	3	4	3	4
A	8.52	15.79	2.72	2.63	1.95	1.96
B	6.25	9.66	1.99	1.61	1.43	1.20
C	0.18	1.51	0.06	0.25	0.04	0.19
D	4.42	7.58	1.41	1.26	1.01	0.94
E	2.35	5.06	0.75	0.84	0.54	0.63
Total	21.72	39.60	6.93	6.61	4.98	4.92

(a) Weights were not corrected for ash.
(b) The different samples of water-extracted holocellulose are denoted as 3 and 4.

TABLE XXIV

ANALYSES OF FRACTIONS FROM LIQUID AMMONIA EXTRACT[a]

Fractions	A	B	C	D1	D2
% Ash	3.49	1.99	0.84	1.18	1.32
% Klason lignin	20.64	8.63	53.8	2.6	3.1
% Methoxyl	7.1	8.2	9.6	3.8	4.1
% Nitrogen	4.47	6.78	3.14	14.4	15.1
% Chlorine	3.2	5.2	3.6	6.0	6.5
% Carbon	47.82	46.57	62.50	41.88	41.11
% Hydrogen	5.85	5.60	7.30	6.89	6.63

[a] All data corrected for ash.

Fraction E was acetamide since, on recrystallization from anhydrous ether, it had a m.p. of 79.5° - 80.5° C., undepressed on admixture with an authentic sample .

Analysis: Calculated for acetamide, C_2H_5ON; N, 23.7%.

Found: N, 23.3%.

Purification of Water Extracts from Ammonia-Extracted Holocellulose

The light tan-colored materials, insoluble in 66% alcohol, extracted by cold water from both large batches of ammonia-extracted holocellulose were mixed, 89.4 g., and were redissolved in 2 liters of distilled water. The solution was filtered through celite (Super-Cel) with great difficulty and had a final volume of 4 liters when the operation had been completed. After reducing the

TABLE XXV

ULTRAVIOLET ABSORPTION SPECTRA OF FRACTIONS A, B, D1 AND D2 FROM LIQUID AMMONIA EXTRACTS[a]

Wave Length (mμ)	Optical Density			
	A	B	D1	D2
230	.900	1.30	.622	.662
234	.837	1.118	.559	.598
238	.745	1.04	.509	.535
242	.638	.898	.469	.471
246	.548	.782	.442	.422
250	.481	.687	.428	.392
254	.443	.638	.417	.376
258	.418	.598	.396	.362
262	.402	.568	.368	.344
266	.390	.540	.338	.327
270	.385	.524	.317	.316
274	.388	.525	.309	.312
278	.394	.530	.309	.309
282	.396	.532	.311	.305
284	.389	.522	.307	.298
286	.383	.513	.301	.288
290	.358	.467	.275	.256
294	.328	.422	.242	.222
298	.296	.365	.197	.186
302	.267	.317	.162	.155
306	----	----	.142	.137
Concentration[b] (g./100 ml.)	0.00502	0.00519	0.0045	0.00445

[a] In aqueous solutions.
[b] Corrected for ash.

volume to 1 liter under reduced pressure, alcohol was slowly added with stirring until a definite precipitation took place. This fraction, which was precipitated by the addition of nearly one volume of ethanol, was very gelatinous, would not settle down well on centrifugation, and was very difficult to isolate by decantation.

These results indicated that any fractionation would be very crude. Since Neubauer (2) had been unable to remove pectic contaminants from hemicellulose fractions by reprecipitation from water with alcohol, no further attempts at purification were made by this method. A second volume of alcohol was added to the mixture containing the gelatinous precipitate in order to recover the polysaccharide. The resulting precipitate was recovered, solvent exchanged through alcohol and ether, and dried to constant weight in vacuum over phosphoric anhydride. The supernatant liquid was concentrated to 300 ml. under reduced pressure, and the addition of two volumes of ethanol gave a further precipitate which was treated as before. The two precipitates were combined to give Fraction F, 77.26 g. The mother liquor was evaporated to dryness under reduced pressure, and the brown solid, 11.14 g., was added to the alcohol-soluble residues from the original water extractions. The combined residues amounted to 51.07 g. and were designated as Fraction G.

The alcohol-insoluble materials from the hot water extractions were combined, 17.62 g., and dissolved in 500 ml.

of water. Filtration through celite was very slow, and the volume of the solution was increased to 1700 ml. before the operation was completed. The volume was reduced to 200 ml. under reduced pressure, and the material was reprecipitated by addition of 2 volumes of alcohol. The precipitate amounted to 10.66 g. and was designated as Fraction H. The mother liquor was concentrated to dryness under reduced pressure, and the residue, 6.05 g., was combined with the other residues from the hot water extractions as Fraction J. None of these weights were corrected for ash. Analytical data for Fractions F, G, H and J are shown in Table VII.

The large amount of nitrogen in these fractions was investigated by distilling separate 0.03 g. samples with 10 ml. of 0.01 N sodium hydroxide, and with 20 ml. of water containing 0.1 g. of magnesium oxide. In each case, 20 ml. of distillate was steam distilled into 25 ml. of 0.8% boric acid, and the whole titrated with 0.025 N hydrochloric acid, using methyl purple as indicator.

All fractions contained nitrogen as ammonium salts, but only fraction G seemed to contain a little amide nitrogen (Table VI).

Samples of Fractions F, G, H and J, 0.05 g., were hydrolyzed with 1 ml. of 1% sulphuric acid in sealed tubes for 16 hours in boiling water. After neutralizing the hydrolysates with barium carbonate to the Congo Red end

point, and removing the insoluble material by centrifuging, the neutral liquors were examined by paper chromatography. The identifications of the free sugars were made by comparison with known sugars placed on the same paper. All fractions contained a considerable amount of xylose with much smaller amounts of arabinose, glucose and galactose. The presence of mannose was uncertain, but the color of the corresponding spot indicated that only Fractions H and J contained appreciable amounts in addition to arabinose. This inference was based on the fact that the aniline phthalate spray colored pentoses red and hexoses brown, as arabinose and mannose occurred at the same position on the chromatogram. The presence of uronic acids in all fractions was indicated by heavy pink spots which had not moved very far from the starting line.

Bleaching of Fractions F and H

Although the solid Fractions F and H were light tan in color, their aqueous solutions were too dark and cloudy to permit measurement of optical rotations. Preliminary experiments indicated that a very mild bleach with sodium chlorite gave much lighter colored solutions. Since this mild treatment gave polysaccharides whose optical rotation could be determined, any further purification could be followed polarimetrically.

Fraction F, 70.0 g., was bleached in two 25 g. and one 20 g. lots. Each 25 g. lot was dissolved in 500 ml. of distilled water, and the electrodes from a Beckmann p H meter were dipped into the solution. Acetic acid was added to adjust the p H to 4.7, and 15 g. of sodium chlorite was added over a period of 1 hour. The p H of the solution was maintained between 4.7 and 4.9 by additions of acetic acid. Another 15 g. of sodium chlorite was added in the same manner. The solution was stirred during the additions, and after 3 hours at room temperature, had become light yellow in color.

Two volumes of ethanol were added and the white precipitate was recovered by centrifugation. The material was washed 7 times with 70% ethanol before the washings were free from chlorite ion on testing with potassium iodide, starch and sulphuric acid. After solvent exchange through ethanol and benzene, the precipitate was dried to constant weight in vacuum over phosphoric anhydride. The last two portions were treated in the same manner, the total recovery of Fraction F1 being 62.28 g., or 88.1% of the starting material on an ash-free basis.

The same procedure was used for Fraction H, but on a much smaller scale since only 5.88 g. (ash-free) was bleached. In this case, the recovery of Fraction H1 was 5.16 g., or 87.8% of the starting material. The ash contents

of Fractions F1 and H1 were 3.78% and 3.12% respectively. The specific rotation of Fraction F1 in water was $[\alpha]_D^{25} = -28.1^\circ$ ($c = 1.852$, $\ell = 2$). Fraction H1 gave a value of $[\alpha]_D^{25} = +11.1^\circ$ ($c = 1.805$, $\ell = 2$).

Fractions F1 and H1 were hydrolyzed in boiling 1% sulphuric acid within 10 hours to constant Shaffer-Hartmann-Somogyi reducing values which were 71.6% and 60.3% respectively, of the fractions based on glucose controls. The hydrolysates were fermented by the procedure given by Purves and Hudson (153). A sufficient amount of the acid hydrolysate was taken so that on dilution to 50 ml. and neutralization to p H 4.5, a 5 ml. aliquot gave a Shaffer-Hartmann-Somogyi reducing value equivalent to about 12 ml. of 0.005 N sodium thiosulphate. Potassium dihydrogen phosphate, 0.02 g., and ammonium acetate, 0.01 g., were added before duplicate 5 ml. samples were removed for determination of the reducing power. The containers were capped with filter paper, and the solutions were heated to approximately 80° C. on a steam cone. The flasks and contents were cooled with cold water, the covering was removed, and Fleischmann's dry yeast, 0.2 g., was added. The solutions were allowed to ferment at 38° for 3 days, the yeast was removed by centrifuging, and the reducing power was determined as before.

In order to determine whether the yeast was active, glucose was added to a portion of the solution containing yeast, and the fermentation was repeated. Fermentation of the glucose showed that the yeast was functioning. Fraction F1 lost 18% of its reducing power on fermentation, while Fraction H1 lost 51%. A control experiment indicated that the yeast used was able to ferment galactose.

Preliminary Acetylations

(a) The procedure used by Neubauer (2) in acetylating maple hemicellulose fractions was first investigated. A 1.019 g. sample of Fraction F1 was placed in a dry 125 ml. ground-glass flask. After the addition of 20 ml. of anhydrous pyridine, the mixture was allowed to stand with occasional shaking for 24 hours. Twenty ml. of freshly distilled acetic anhydride was added, and after a further 24 hours, the mixture was heated under reflux for 4 hours on a steam bath, with protection from moisture in the form of a calcium chloride guard tube. The mixture was then poured into 400 ml. of ice water and allowed to stand for 24 hours, before the insoluble acetate was recovered and washed with water. Only 0.15 g. of the dry acetate, 1.19 g., was soluble in acetone. The acetyl content of the acetone-insoluble portion was 28.1%. The dark color of the acetate, its low acetyl content and yield, together with its low

insolubility in acetone and chloroform, made a different acetylating procedure desirable.

(b) The procedure of Carson and Maclay (117), as modified by Perlin (126), was followed. A sample of Fraction F1, 1.012 g., was stirred to a smooth paste with 7 ml. of 99% formamide. Anhydrous pyridine, 14 ml., was added with thorough mixing and 7 ml. of freshly distilled acetic anhydride was dropped into the mixture over a period of 30 minutes. Twenty-four hours later the mixture was poured with stirring into 400 ml. of ice-cold distilled water. Next day the precipitated acetate was removed by centrifugation, and was washed with 2% hydrochloric acid and several changes of water before being solvent exchanged through alcohol and ether. The acetate, dried to constant weight in vacuum over phosphoric anhydride, amounted to 1.3 g. and had an acetyl content of 33.9%. No corrections for ash were applied and single acetyl determinations were made in these preliminary experiments.

The acetate, 1.22 g., was swollen in 14 ml. of pyridine for 24 hours, reacetylated by the addition of 7 ml. of acetic anhydride and recovered as before. The recovered, dried acetate, 1.16 g., was soluble in acetone with the exception of 0.12 g. of a gray-brown material (Found: acetyl, 29.0%). This acetone solution was

concentrated to a small volume, and the acetate reprecipitated by pouring into a large excess of ice water. The dried, acetone-soluble acetate weighed 0.94 g., and had an acetyl content of 35.2%. The experiment was repeated and it was found that the acetate from the first acetylation could be separated into acetone-soluble and insoluble fractions. This procedure was adopted for all subsequent acetylations. The reacetylated, acetone-soluble material from Fraction F1 was acetylated for a third time with pyridine and acetic anhydride, the conditions being 3 days at room temperature and then at 50° C. for another day. Since the product still contained 35.2% acetyl, a third acetylation was unnecessary.

(c) A sample of Fraction H1, 1.012 g., was acetylated in the same way, giving 0.40 g. of acetone-insoluble (acetyl, 30.2%) and 0.89 g. of acetone-soluble acetate (acetyl, 34.1%). Reacetylation of the acetone-soluble fraction gave 0.70 g. of acetate with an acetyl content of 35.1%. Both acetone-soluble acetates from F1 and H1 were cream-colored, and this preliminary work indicated that they were rather similar in composition.

(d) An attempt to acetylate Fraction G by the formamide procedure gave very poor results. A sample of G, 1.004 g., gave only 0.52 g. as the product on addition of the acetylation mixture to water. Much material remained in suspension

in the water, and could not be removed by filtration or centrifugation. The recovered acetate had an acetyl content of 23.4%. Reacetylation gave a gray product, 0.43 g., with an acetyl content of 24.2%. Fraction J, 1.033 g., on acetylation gave a product amounting to 0.96 g. and having an acetyl content of 18.8%. Reacetylation gave 0.65 g. of product with 21.5% acetyl. These results indicated that Fractions G and J could not be satisfactorily acetylated and as a result, they were not further studied.

Large Scale Acetylation of Fraction F1

A total of 50.1 g. (ash-free) of Fraction F1 was acetylated in two 20 g. and one 10 g. portions. The acetylations were carried out in 500 and 1000 ml. three-necked, ground-glass flasks equipped with a mercury seal stirrer, thermometer well and a dropping funnel containing a ground-glass joint which had a side arm leading to a calcium chloride guard tube. Twenty g. of Fraction F1 was added to the flask, followed by 140 ml. of 99% formamide. The mixture was stirred overnight, and 280 ml. of anhydrous pyridine was added to the smooth paste. The entire mixture was stirred for 8 hours, and 140 ml. of freshly distilled acetic anhydride was added over a period of 2 hours. This addition rapidly caused the light straw-colored mixture to become much darker, and the temperature rose to 42° C. The final mixture was stirred for 24 hours at room temperature.

The acetate was precipitated by slowly stirring the mixture into 5.5 liters of ice-cold distilled water and 24 hours later was recovered by centrifugation, washed twice with 2% hydrochloric acid, five times with distilled water, with absolute ethanol until the washings were colorless and finally with ether and petroleum ether. The combined yield of dry, light gray acetate was 65.25 g. (not ash-free) with an acetyl content of 33.7%. All aqueous mother liquors and washings were concentrated under reduced pressure to a volume small enough to suggest that only formamide remained. On addition of excess ethanol, a gray material was precipitated which was solvent exchanged through alcohol, ether and petroleum ether. The dry solid from the three portions amounted to 3.64 g. and contained 25.7% of acetyl groups. Recovery of the material in the alcohol washings gave 1.74 g. of a dark brown solid having 11.7% acetyl.

The alcohol and water-insoluble acetate was thoroughly extracted with acetone at room temperature. A residue, which was dark brown in color after drying from acetone, amounted to 10.5 g. with an acetyl content of 31.9%. Reprecipitation of the acetone solubles with distilled water recovered 49.4 g. of acetate having 34.1% acetyl. A gray solid, 3.06 g., recovered from the aqueous liquor had an acetyl content of 28.8%. Additional material, 1.38 g., with

an acetyl content of 29.1% was recovered from the alcohol washings.

The reprecipitated acetone-soluble acetate was dissolved in pyridine to give a clear, red-brown solution, and acetic anhydride was added. The ratio of 1 g. of acetate to 14 ml. of pyridine and 7 ml. of acetic anhydride was used in all reacetylations. After standing for 3 days at room temperature, the product was isolated in the usual manner as a cream-colored powder, yield 46.49 g. (ash-free) and with an acetyl content of 35.3% (Fraction K). A 1% solution in chloroform gave $[\alpha]_D^{25} = -59^\circ$ ($\ell = 1$). Additional material, 1.10 g., acetyl, 29.5%, was recovered from the aqueous solution, while the alcohol washings yielded 0.93 g. having 31.6% acetyl.

The acetone-insoluble portion was also reacetylated. This material, 10.5 g., formed a solid gel with 140 ml. of pyridine. Anhydrous benzene, 140 ml., was added to make the mixture more fluid before the addition of 70 ml. of acetic anhydride. The mixture stood for 3 days and the product was recovered as before. A portion of the product was recovered as a dry, brown, acetone-insoluble material, 6.47 g. (ash-free), with 33.9% acetyl (Fraction L). The acetone-soluble material was recovered as a cream-colored product, 3.35 g. (ash-free), with an acetyl content of

34.8%, $[\alpha]_D^{25} = -52.3^\circ$ (c = 1) in pyridine, and was called Fraction M.

Acetylation of Fraction H1

A preliminary acetylation had indicated that the acetates of Fractions F1 and H1 were very similar. A portion of H1, 2.9 g. (ash-free), was acetylated in exactly the same manner as Fraction F1, giving 4.18 g. of product. The material was separated into acetone-soluble and insoluble fractions which were reacetylated. The reacetylated, acetone-insoluble acetate, 1.14 g., had an acetyl content of 34.6%. The acetone-soluble acetate, some of which was lost, amounted to 1.47 g. having 36.3% acetyl. This value for the acetyl content was higher than that found from the preliminary experiments. The specific rotation of the acetone-soluble acetate was $[\alpha]_D^{25} = -62.1^\circ$ (c = 1) in pyridine. Because of the small amount of acetate, no attempt at fractionation was made in an effort to get material similar to Fraction K.

Preliminary Fractionation of Fraction K

Fraction K (2.0 g.) was dissolved in 80 ml. of chloroform, the solution was filtered and then diluted to 100 ml. The solution was placed in a centrifuge tube of 250 ml. capacity, and the first fraction was precipitated by the slow addition of petroleum ether, (b. p. 65° - 110° C.), cloudiness commencing with the first addition, and becoming quite

marked after 5 ml. After standing for 1 hour, the suspension was centrifuged, but as no insoluble material was deposited, an additional 2.0 ml. of petroleum ether was added with very little increase in the cloudyness. After standing for an hour, centrifugation of the solution gave the first fraction. The mother liquor was decanted into a clean centrifuge tube, and the precipitate was washed with a solution of the same composition as the mother liquor. The suspension was again centrifuged, the washing solution was poured off and the precipitate was thoroughly washed with petroleum ether.

The precipitated fraction was dried to constant weight in vacuum over sliced paraffin wax. Petroleum ether was added to the mother liquor until it became definitely cloudy, and after 1 hour, the second fraction was removed by centrifugation. This fraction was washed with a solution of the same concentration as the mother liquor, and then with petroleum ether. The fraction was dried in vacuum as before. A total of 7 fractions were obtained by the same procedure.

All chloroform-petroleum ether washing solutions were combined with the final mother liquor, and addition of excess petroleum ether gave a further fraction. The yields of these fractions together with their optical rotations in chloroform are given in Table XXVI.

TABLE XXVI

YIELDS AND OPTICAL ROTATIONS OF SUB-FRACTIONS FROM FRACTION K [a]

Sub-Fraction	Petroleum Ether Added (ml.)	Weight (g)	Conc. in $CHCl_3$ (g./ 100 ml.)	$[\alpha]_D^{25}$
1	7.0	0.49	1.001	-59.6°
2	10.05	0.33	0.979	-61.3°
3	15.25	0.11	0.980	-60.2°
4	27.30	0.31	1.003 [b]	-59.8°
5	38.20	0.15	1.000	-60.0°
6	48.80	0.07	1.034	-18.4°
7	74.50	0.07	1.004	- 9.0°
Washings	excess	0.44	1.002	-59.5°
Recovery	---	1.97	----	----
Fraction K	---	----	1.000	-59.0°

[a] Two grams in 100 ml. of chloroform.
[b] In a 2 dm. tube, all others in a 1 dm. tube.

The chloroform solutions used for the optical readings were saved for determination of the intrinsic viscosities of these fractions. An Ostwald-Cannon-Fenske viscometer was used at 25° ± 0.03° C., and the outflow time for pure chloroform was 87.44 seconds. The specific viscosities were determined at concentrations of 0.5%, 0.25% and 0.125% except for sub-fraction 5, where the concentrations were 1.0%, 0.50% and 0.25%. At least two flow times were determined at each concentration, and if they agreed within 0.02

seconds, no further readings were taken. The outflow time for the solution (in seconds) divided by 87.44 seconds gave the relative viscosity, $\eta_{rel.}$. The specific viscosity, $\eta_{sp.}$, was equal to $\eta_{rel.} - 1$. Plotting the quotient of the specific viscosity by the percent concentration (g. /100 ml.) against the concentration gave straight lines for all fractions. Extrapolation to zero concentration gave the intrinsic viscosity, $[\eta]$, shown in Table XXVII.

In the actual determinations, 5 ml. of the solution used for optical rotations was placed in a 10 ml. volumetric flask. Chloroform was added to increase the volume to 10 ml. and after a thorough mixing, 5 ml. of the solution was placed in the viscometer. The viscometer and contents were allowed to stand for 15 minutes, before the liquid was forced up the capillary arm by light pressure from a rubber bulb attached to the other arm. The residual solution in the volumetric flask was again diluted to 10 ml., and 5 ml. was taken for the determination at the second concentration.

A further dilution to 10 ml. gave the solution at the final concentration.

TABLE XXVII

ANALYTICAL DATA FOR CALCULATION OF INTRINSIC VISCOSITIES OF SUB-FRACTIONS FROM FRACTION K

Sub-Fraction	Time (sec.)	Conc. (g./100 ml.)	$\eta_{rel.}$	$\frac{\eta_{sp.}}{\%\ C (g./100\ ml.)}$	$[\eta]$
1	116.73	0.5060	1.3350	0.6619	
	99.77	0.2530	1.1410	0.5573	0.4860
	93.30	0.1265	1.0670	0.5297	
2	115.30	0.5010	1.3186	0.6349	
	99.36	0.2505	1.1363	0.5464	0.4560
	92.90	0.1253	1.0625	0.4989	
3	113.40	0.5510	1.2969	0.5388	
	98.73	0.2755	1.1291	0.4686	0.4100
	92.76	0.1378	1.0608	0.4412	
4	108.53	0.5010	1.2412	0.4815	
	97.23	0.2505	1.1120	0.4471	0.4030
	92.067	0.1253	1.0529	0.4232	
5	118.16	0.9820	1.3514	0.3578	
	101.03	0.4910	1.1554	0.3164	0.2590
	93.53	0.2455	1.0696	0.2834	
6	99.66	0.5170	1.1398	0.2705	
	93.40	0.2585	1.0682	0.2638	0.2340
	90.20	0.1293	1.0316	0.2444	
7	95.73	0.5020	1.0948	0.1889	
	91.13	0.2510	1.0422	0.1681	0.1360
	89.10	0.1255	1.0190	0.1515	

Large Scale Fractionation of Fraction K

The preliminary work, Table XXVI, showed that with the exception of sub-fractions 6 and 7 (amounting to 7%), the optical rotations of the fractions were very similar. Sub-fraction 5, 7.5%, however, had a much lower intrinsic viscosity than the first fractions (Table XXVII), so in the large scale fractionation, only about 80% of the acetate was removed as the first fraction.

The large scale fractionation was carried out in 4 batches. The acetate was slowly dissolved in chloroform, the solution was filtered to remove a small amount of insoluble impurities, and diluted to 2% concentration. The first batch, 13 g. was dissolved in 650 ml. of chloroform and another fruitless attempt was made to get a small head fraction. The solution was cloudy after the addition of 22 ml. of petroleum ether, but no precipitate was obtained on centrifugation even when the addition was increased to 40 ml. After the addition of petroleum ether was slowly increased to a total of 195 ml., the solution was allowed to stand for 1 hour before the precipitate was removed, washed with a solution of the same composition as the mother liquor, and then with several changes of petroleum ether. A further fraction was obtained on addition of 55 ml. of petroleum ether to the mother liquor. This fraction was treated in

the same way, and the addition of 250 ml. of petroleum ether to the mother liquor gave the last fraction. All fractions were dried to constant weight in vacuum over sliced paraffin wax.

The next 3 batches of Fraction K were treated in the same way, the data from the fractionations being shown in Table IX.

The optical rotations and acetyl contents of each first sub-fractions were determined before they were combined. It was very surprising to find that the chloroform solutions were too cloudy to permit the measurement of optical rotations. Filtration through Super-Cel or centrifugation at 4,000 r.p.m. for an hour did not remove any of the cloudiness. The first sub-fractions were finally dissolved in anhydrous pyridine in about 1% concentration, and the optical rotations were determined in a 1 dm. tube (Table X).

On the basis of these data the first sub-fractions were combined into the large Fraction N. All of the second fractions were combined to give Fraction O having 35.2% acetyl, and giving $[\alpha]_D^{25} = -75.8^\circ$ (c = 1) in pyridine. The third group of sub-fractions were combined to give Fraction P with 36.0% acetyl and $[\alpha]_D^{25} = -49.3^\circ$ (c = 1) in pyridine.

Investigation of Fraction N

The specific rotation of this fraction for a 1% solution in pyridine was $[\alpha]_D^{25} = -74.8^\circ$ ($\ell = 2$). In order to test the homogeneity, 1 g. was dissolved in 100 ml. of chloroform, and fractionated with the petroleum ether in the usual manner to give the fractions shown in Table XI.

Examination of Fractions L, M and P

Approximately 75 mg. of Fractions L, M and P were placed in three small tubes. Normal alcoholic potassium hydroxide, 2 ml., was added to each tube and the mixtures were heated on a water bath at 70° C. for several minutes. Deacetylation was assumed to be complete, the tubes were centriguged and the alcoholic solution was poured off. One ml. of 2.5% sulphuric acid was added to the residue in each tube, the tubes were sealed and placed in a boiling water bath for 14 hours. At the end of the hydrolysis, Fractions L and P still gave considerable amounts of dark insoluble material, while Fraction M gave a very small amount.

The residues were removed on the centrifuge and the acid hydrolysates were neutralized (Congo Red) with barium carbonate. The hydrolysates were examined by paper chromatography, comparisons being made with known sugars on the

same paper. Fraction L contained xylose, arabinose, galactose and uronic acids, but there seemed to be as much galactose and arabinose as xylose. Fraction M contained the same constituents but had much smaller amounts of arabinose and galactose. Fraction P had the same components together with a small amount of mannose and a trace of glucose.

Deacetylation of Fraction N

Since a preliminary experiment with a 15% excess of aqueous potassium hydroxide gave a product containing 2.9% acetyl, in the large scale procedure a 20% excess was used. Thirty g. (ash-free) of Fraction N was dissolved in 300 ml. of acetone to give a brown solution. The container was swept out with nitrogen and aqueous potassium hydroxide, 360 ml. of 0.837 normality, was slowly added at 5° C. with swirling. The flask was again swept with nitrogen, stoppered tightly and shaken for 24 hours at room temperature. The mixture, which had become fairly dark on the addition of the potassium hydroxide, was neutralized to p H 4 by additions of N hydrochloric acid, and the deacetylated polysaccharide was precipitated by the addition of 2 volumes of alcohol. After solvent exchange through alcohol and ether, the precipitate was dried to constant weight in vacuum over phosphoric anhydride. The dry product was light tan in color, had 4.5% ash, 0.4% acetyl

and $[\alpha]_D^{25} = -52.2^\circ$ (c = 1) in water. The yield of 19.6 g. (ash-free) compared well to the theoretical yield of 19.4 g. from 30 g. of acetate containing 35.5% of acetyl groups. No further effort was made to remove the trace of acetyl found in the polysaccharide.

The deacetylated polysaccharide was dissolved in water in order to reduce the ash content by dialysis. Since both the solid and the solution were darker than expected, a small portion was given a mild, short bleach with sodium chlorite. The solution rapidly became light yellow in color and it was decided to treat the entire solution in the same manner.

Bleaching and dialysis of the deacetylated polysaccharide were carried out on two batches. The polysaccharide 9.56 g. (ash-free), was dissolved in 300 ml. of water, the p H was adjusted to 4.5, and 5.0 g. of sodium chlorite was added to the stirred solution at room temperature. The p H was maintained at 4.5 over a period of 1.5 hours before the light yellow solution was transferred to a large cellophane sack for dialysis against tap water. After 3 days the solution was free of chlorite ion (starch-iodine), and was then concentrated to 300 ml. under reduced pressure. Addition of 2 volumes of ethanol to the concentrate produced a pure

white solution, from which only part of the polysaccharide could be recovered by centrifugation. Rather than add a small amount of a salt, such as potassium acetate, to give a much better precipitation, the first precipitate was removed, and the mother liquor concentrated to 75 ml. under reduced pressure. A middle fraction was precipitated on addition of 2 volumes of ethanol, and was recovered on the centrifuge. The mother liquor was further concentrated to 50 ml. before the addition of 2 volumes of ethanol, and 48 hours later this tail sub-fraction was recovered by centrifuging. Like the previous sub-fractions, the coagulum was solvent exchanged through alcohol and ether before being dried to constant weight in vacuum over phosphoric anhydride.

The second batch of deacetylated Fraction N amounted to 10.91 g. (ash-free), and included material from the preliminary experiment which had been deacetylated a second time. This sample was treated in the same manner to give head, middle and tail sub-fractions. The corresponding fractions from both portions were combined and the optical rotations on the combined fractions were determined with the results shown in Table XXVIII.

The solutions from the determinations of optical rotations (Table XXVIII) were used for viscosity determinations similar to those of Wethern (101). Five ml. was

TABLE XXVIII

YIELDS AND OPTICAL ROTATIONS OF SUB-FRACTIONS FROM DEACETYLATED FRACTION N

	Batch 1 (g.)	Batch 2 (g.)	Combined (g.)	$[\alpha]_D^{25}$
Deacetylated N	9.56	10.91	20.47	----
Head (a)	5.76	6.28	11.77	-52.8° (b)
Middle	2.77	1.31	3.98	-52.4°
Tail	0.90	2.44	3.26	-52.2°
Recovery	9.43	10.03	19.01	----

(a) Sub-fractions from portions 1 and 2 were not corrected for ash, all other values were ash-free.

(b) Concentration = 1% in water, ℓ = 1.

diluted to 10 ml. with 20% potassium hydroxide to give approximately 0.5% concentrations in 10% potassium hydroxide. A portion of the solution, 5 ml., was taken and the remainder diluted to 10 ml. with 10% potassium hydroxide to give a solution of one-half the original concentration. The same procedure used for the determinations shown in Table XXVII was followed. The outflow time for 10% potassium hydroxide at 25° C. was 240.75 seconds and the results are shown in Table XXIX.

TABLE XXIX

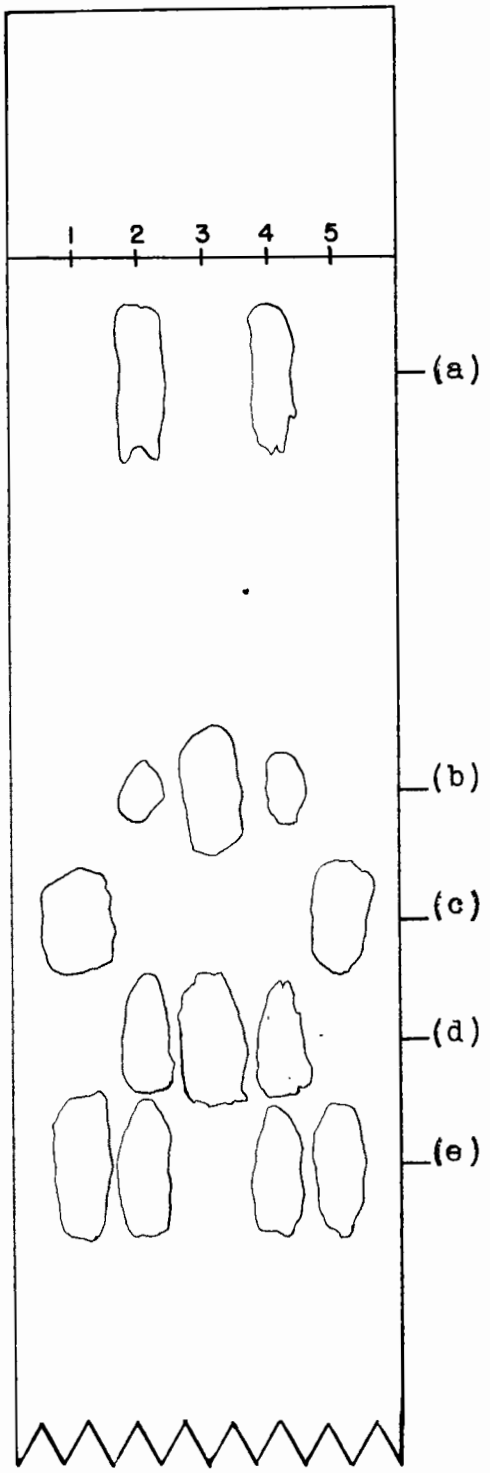
VISCOSITIES OF SUB-FRACTIONS FROM DEACETYLATED FRACTION N

Sub-Fraction	Time (secs.)	Conc. (g./100 ml.)	$\eta_{rel.}$	$\frac{\eta_{sp.}}{\%c.}$	$[\eta]$
Head	301.40	0.4925[a]	1.2519	0.5114	
	269.35	0.2462	1.1189	0.4831	0.4380
	254.30	0.1231	1.0563	0.4572	
Middle	298.10	0.5050	1.2382	0.4718	
	268.20	0.2525	1.1140	0.4514	0.4200
	253.90	0.1262	1.0546	0.4326	
Tail	269.60	0.5080	1.2320	0.4562	
	267.73	0.2542	1.1121	0.4410	0.4140
	253.80	0.1271	1.0542	0.4265	

[a] All concentrations on ash-free basis in 10% potassium hydroxide at 25° C.

To economize material, single analyses were carried out on the head and tail sub-fractions only. The polysaccharide appeared to be homogeneous (Table XIV).

Small amounts of the head and tail fractions, 50 mg., were hydrolyzed with 1% sulphuric acid in sealed tubes at 100° C. for 16 hours. The hydrolysates were neutralized with barium carbonate, centrifuged and examined by paper chromatography. The spots were compared with known sugars and both sub-fractions seemed to be exactly the same. A large amount of xylose with smaller amounts of arabinose, galactose and uronic acid were found in both sub-fractions. The chromatogram is reproduced in Fig. 13 and is a typical example of the



Spots 1, 3, 5 - reference sugars.

Spots 2, 4 - head and tail sub-fractions from Fraction R.

Developed with pyridine-ethyl acetate-water (1:2:2) for 20 hours, aniline phthalate spraying reagent.

(a) uronic acid complexes

(b) galactose

(c) glucose

(d) arabinose

(e) xylose

Scale: 1 in. = 3 in. (approx.)

FIG. 13

CHROMATOGRAM OF SUB-FRACTIONS FROM DEACETYLATED FRACTION N

many paper chromatograms obtained.

The data in Table XIV, with the possible exception of the furfural values, agreed within the limit of experimental errors. Therefore, the head, middle and tail fractions were mixed together into one large fraction called R. Samples of Fraction R were taken for duplicate determinations of pentosan and uronic anhydride contents, correction for 2.45% ash gave furfural contents of 47.2% and 47.4% or an average value of 47.3%. The uronic anhydride values were 15.92% and 16.08% and gave an average of 16.0%. These values were used in calculating a pentosan content of 69.71% corrected for furfural from uronic acids.

These results together with an average methoxyl content of 3.6% were used in calculating a pentosan: uronic anhydride: methoxyl ratio of 5.82:1:1.28.

Acid Hydrolysis of Fraction R

A sample of Fraction R, 0.2423 g. (ash-free) was dissolved in 23 ml. of 0.025 N sulphuric acid, the p H was adjusted to 2.0, and the volume increased to 25.0 ml. The solution was heated under reflux on a boiling water bath and at intervals, 2 ml. aliquots were removed for the determination of the reducing power and optical rotation. Duplicate 0.5 ml. samples, diluted to 5 ml. with distilled water, were used with

the Shaffer-Somogyi copper reagent and 7.65 ml. of 0.005 N sodium thiosulphate corresponded to 0.001223 g. of arabinose.

The remaining 1 ml. of the aliquot was used for the determination of optical rotation in a 1 dm. micro tube, specific rotations being calculated on the basis of a constant concentration and molecular weight (Table XXX).

TABLE XXX

LIBERATION OF ARABINOSE AND OPTICAL

ROTATIONS ON PARTIAL HYDROLYSIS OF FRACTION R [a]

Time (hours)	Arabinose (mg.)	% of Sample	$[\alpha]_D^{25}$
At start	trace	0	-50.6°
0.5	0.274	5.66	-36.1°
1.0	0.427	8.82	-28.9°
1.5	0.531	11.00	-26.8°
2.0	0.568	11.72	-24.8°
2.5	0.592	12.12	-24.8°

[a] A 0.969% solution in 0.025 N sulphuric acid near 100° C.

Unlike the specific rotation (Table XXX), the reducing power did not become constant after 2 hours perhaps because a slow hydrolysis was taking place after an initial rapid reaction.

The solution, 13.3 ml., remaining from the partial hydrolysis was neutralized with barium carbonate, centrifuged, and

the precipitate was washed with three volumes of warm water. The combined solution and washings were concentrated to a small volume under reduced pressure, and made up to 10 ml. in a volumetric flask. One ml. of this solution was taken and examined by paper chromatography. The only free sugars found were arabinose and a much smaller amount of xylose.

In order to complete the hydrolysis, the solution containing $(\frac{13.3}{25} \times \frac{9}{10} \times .2423)$ 0.1160 g. of original sample was diluted with an equal volume of 5% sulphuric acid by weight; barium sulphate was removed on the centrifuge and the rotation was determined in a 4 dm. tube. The calculated concentration (0.1160 g. in 18 ml.) gave $[\alpha]_D^{25} = -19.7^\circ$, compared to a previous value of -24.8°. In a separate hydrolysis of a large amount of Fraction R, Table XXXII, a final reading in 2.5% sulphuric acid of $+ 43.9^\circ$ was obtained. This value together with the final, constant observed rotation from this experiment were used to calculate a concentration of 0.5694 g. per 100 ml. or of 0.1002 g. per 18 ml. The solution (18 ml.) was hydrolyzed to constant observed rotation and reducing power, with the results shown in Table XXXI (based on a concentration of 0.1002 g. per 18 ml.) Xylose was used to calibrate the 0.005 N sodium thiosulphate in the Shaffer-Somogyi estimations, and 10.96 ml. corresponded to 0.001579 g. of the sugar.

TABLE XXXI

APPARENT XYLOSE CONTENT AND THE SPECIFIC ROTATIONS ON COMPLETE HYDROLYSIS OF FRACTION R [a]

Time (hours)	Xylose (mg.)[b]	% of Sample	$[\alpha]_D^{25}$
Start	0.167	14.95	-22.0°
0.5	0.616	55.1	-20.6°
1.0	0.765	68.5	+37.4°
1.5	0.815	73.1	+40.8°
2.0	0.861	77.2	+43.5°
3.0	0.885	79.2	+43.9°
4.0	0.881	78.2	+43.9°

(a) In 2.5% sulphuric acid near 100° C.
(b) Average from duplicate 0.2 ml. samples in Shaffer-Somogyi estimation.

The final apparent xylose content of 78.2% was slightly higher than the 76.1% found when the large scale hydrolysis of Fraction R had reached constant rotation.

Isolation of Barium Uronates from Fraction R

A large amount of Fraction R, 7.832 g. (ash-free) was hydrolyzed under reflux on a boiling water bath to constant rotation in 400 ml. of 2.5% sulphuric acid by weight. The polysaccharide required 1.5 hours at room temperature to dissolve almost completely. After one-half hour the flask and contents were cooled to 25° C. with tap water and an aliquot was taken for the polarimetric reading. Since the cooled hydrolysate was too cloudy for this purpose, it was

quickly centrifuged and the reading was taken on the clear solution. After the determination, the insoluble material was added to the aliquot, and the whole was added to the main solution. This procedure was followed until the rotation became constant (Table XXXII).

TABLE XXXII

OPTICAL ROTATIONS DURING LARGE SCALE HYDROLYSIS OF FRACTION R [a]

Time (hours)	$\alpha_{obs.}$ [b]	$[\alpha]_D^{25}$
Start	-0.94°	-48.1°
0.5	+0.08°	+ 4.1°
1	+0.58°	+29.6°
2	+0.68°	+34.7°
3	+0.83°	+42.5°
4	+0.86°	+43.9°
5	+0.86°	+43.9°

[a] In 2.5% sulphuric acid near 100° C., c = 1.958%.
[b] In a 1 dm. tube.

At the end of 5 hours the solution was cooled and centrifuged to remove insoluble material. The precipitate was washed with cold water and amounted to 0.05 g. when dried to constant weight (0.64%). The clear, light yellow liquid and washings were then stirred and neutralized near 40° C. with small additions of barium carbonate. This operation required 3 hours before the neutrality to Congo

Red (p H 6.5) was attained. The clear supernatant liquor was recovered on the centrifuge, and the insoluble residue was washed with three volumes of warm water which were added to the mother liquor. The combined solution was concentrated to 200 ml. under reduced pressure, 4 volumes of alcohol were added and after recovery, the precipitate was dried by solvent exchange through ethanol and ether, and finally in vacuum over phosphoric anhydride. The yield of crude barium uronate was 0.85 g.

The alcoholic solution was again concentrated in volume to 85 ml.; 4 volumes of ethanol were added, and an additional amount of the barium salt obtained as before. This precipitate, 0.89 g., was added to the first crop, and the combined material was extracted with water. An insoluble portion, 0.51g., was well washed with water, and the aqueous extract of the barium uronates was concentrated to 25 ml. under reduced pressure. Three volumes of ethanol were used to reprecipitate the barium uronates before they were dried through solvent exchange to a yield of 0.95 g. The mother liquor was added to the original solution which was concentrated to a final volume of 50 ml. A further precipitate, 0.35 g., obtained on addition of 4 volumes of ethanol, was reprecipitated as before to give 0.23 g. of water-soluble barium uronates and 0.01 g. of insoluble material. The mother liquor from this reprecipitation was

added to the main solution, which was evaporated to leave a white solid by repeated distillations with absolute ethanol under reduced pressure. The dry solid, 7.58 g., was reserved for identification of the constituent sugars. Examination of this solid by paper chromatography showed that it still contained a considerable amount of barium uronates.

The brown, insoluble material from the reprecipitation of the barium uronates had an ash content of 87.2%, and was not further studied. The two portions of reprecipitated barium uronates were examined separately with the similar results shown in Table XXXIII, and were then combined for further study. Paper chromatography showed that no free sugars were present in the sample.

TABLE XXXIII

ANALYTICAL DATA FOR FRACTIONS OF BARIUM URONATES

Fraction	Weight (g)	% Barium[a]	% Methoxyl[b]	$[\alpha]_D^{25}$
1	0.95	17.1	9.1	$+72.8^\circ$ (b)
2	0.23	17.3	9.2	$+73.5^\circ$

(a) On micro samples.
(b) Uncorrected for barium content, rotations in 1% aqueous solution.

Further analyses were carried out on the combined fractions after they had been exhaustively extracted with petroleum ether

to remove any traces of adsorbed alcohol. Found: barium, 17.4% (as barium sulphate on 0.1 g. sample); methoxyl, 8.7, 8.8%; uronic anhydride, 34.72, 34.48%. Calculated for a monomethoxy barium aldobiuronate, Ba $(C_{12} H_{19} O_{11})_2$; barium, 16.85%; methoxyl 7.61%; uronic anhydride 43.15%. Calculated for a monomethoxy barium aldotriuronate, Ba $(C_{17} H_{27} O_{15})_2$, barium, 12.73%; methoxyl 5.74%; uronic anhydride, 32.6%.

The inference that the sample was a mixture of these two components was strengthened by an estimation of aldehyde groups with hypoiodite as described by Hirst, Hough and Jones (154). Duplicate solutions, 0.50 ml., containing 0.00563 g. of the uronate were placed in 50 ml. flasks fitted with ground-glass stoppers. Distilled water, 4.5 ml., and exactly 1 ml. of 0.1 N iodine were added to the flasks followed by 2 ml. of carbonate buffer solution (p H 10.6). Duplicate blank runs were made without any barium uronate. The glass stoppers were moistened with 10% potassium iodide, and the solutions were allowed to stand for 1.5 hours at room temperature. After washing down the stoppers with 20 ml. of distilled water, the solutions were acidified by the addition of 2 ml. of 2 N sulphuric acid and were titrated to the starch end point with 0.01021N sodium thiosulphate. The blank solutions consumed 9.52 and 9.50 ml. of thiosulphate, while the others required 7.22 and 7.22 ml. The difference in titre, 2.29 ml. of 0.01021 N thiosulphate or iodine, gave a value of 482 for the equivalent weight of the barium

uronate. Calculated for barium monomethoxy aldobiuronate, eq. wt. 407.95; for barium monomethoxy aldotriuronate, eq. wt. 540.07. Lack of material prohibited any attempt to separate the two constituents.

Identification of the Free Sugars

A portion, 2 g., of the material from the isolation of the barium uronates, and soluble in 80% ethanol, was dissolved in water to give a thin syrup. This syrup failed to deposit crystalline xylose after being kept for several days at 0° C. A second portion, 3.5 g., was then taken up in approximately 20 ml. of hot glacial acetic acid, and the solution was filtered and allowed to stand at room temperature. After several hours, crystals started to form, and were recovered after 24 hours. The solid was washed with several small volumes of cold glacial acetic acid, a small amount of ice-cold alcohol and then with anhydrous ether. The dry, white crystals, 1.16 g. or 33% of the starting material, melted at 133° to 135° C. and when recrystallized from glacial acetic acid at 144° to 145° C., not depressed on admixture with an authentic sample of xylose. The specific rotation of a 1% aqueous solution at 23° C. in a 4 dm. tube was + 18.8° changing to + 19.0° after 24 hours. The optical rotation of pure xylose was + 18.9° under the same conditions.

The glacial acetic acid mother liquor and the washings were concentrated to a small volume under reduced pressure. A large excess of anhydrous ether was added, the light yellow precipitate was recovered, washed with ether and dried to constant weight in vacuum over potassium hydroxide pellets. Yield, 1.93 g. In order to prepare an arabinose derivative, a portion, 0.70 g., was mixed with 5 ml. of a saturated aqueous solution of benzoyl hydrazine (129). The benzoyl hydrazine had been recrystallized from water, and had the correct melting point of 112° - 113° C. The solution was kept at 30° for 48 hours with occasional shaking. A light brown, amorphous solid was deposited but the quantity was insufficient for a melting point. No more solid separated when the filtrate was kept at 0° for two hours. Repetition of the procedure with 0.07 g. of pure arabinose gave a heavy deposit after 24 hours at 30°C. This deposit was the expected benzoyl hydrazone with the correct melting point of 186° - 187° C. The same derivative was obtained from 0.07 g. of arabinose mixed with 0.4 g. of xylose.

A second portion of the dried residue from the xylose precipitation, 0.70 g., was dissolved in 3 ml. of distilled water. A small amount of insoluble brown material was removed by centrifugation, 5 volumes of ethanol were added and the insoluble material, presumably barium salts, removed by centrifugation. Paper chromatography showed that the precipitate contained very minor amounts of free sugars, while the

alcohol solution seemed to contain considerable amounts of arabinose and galactose as well as xylose. The alcoholic solution was concentrated to a small volume under reduced pressure and then dried in vacuum over phosphoric anhydride. Five ml. of the saturated benzoyl hydrazine reagent was added to the dry residue and the solution was kept at 30° C. for 48 hours. After 2 hours at 0° C. no solid had deposited. The solution was then seeded with a small amount of the benzoyl hydrazone of L - arabinose and kept at 5° C. for two days, whereupon a sufficient amount of material was deposited for melting point determinations. The dry, slightly brown solid melted at 184.5°- 186° C., with no depression on admixture with an authentic sample. These melting points were actually decomposition points as the solid darkened and turned into a very dark liquid. In a control experiment, the derivative from the synthetic mixture of pure arabinose and xylose was dissolved in hot water, the solution was filtered and kept at 5° C. Two days later there was no sign of crystals in the liquid, but after three days a considerable amount of solid had deposited. It would appear from these results that even a mixture of pure sugars containing arabinose could give an apparently negative result with benzoyl hydrazine.

The absence of mannose, already indicated by paper chromatography was confirmed by micro tests with phenylhydrazine. In a blank determination, 0.02 g. of pure mannose was dissolved in 2 ml. of water; 0.048 g. of phenylhydrazine

hydrochloride and 0.027 g. of sodium acetate were added. A considerable amount of mannose phenylhydrazone was deposited in a few minutes at room temperature. The residue from the recovery of barium uronates, 0.10 g., in 2 ml. of water gave negative results with the same procedure. Similar negative results were obtained with 0.10 g. of the residue from the recovery of xylose.

Quantitative Determination of the Free Sugars in the Hydrolysate of Fraction R.

Oxidation with Sodium Periodate -- The procedure described by Hirst and Jones (130) was used for this estimation. A sample of Fraction R, 0.05669 g. (ash-free), was hydrolyzed with 1 ml. of 2.5% sulphuric acid in a sealed tube at 100° C. for 5 hours. At the end of this period, the tube was removed, opened and the contents carefully added to 0.02029 g. of rhamnose dissolved in 1 ml. of water. Rhamnose was chosen as a standard because xylose and ribose were so close together on the chromatogram that it was difficult to decide where the paper should be cut. The combined solution was freed from acid by pouring it through a small column containing about 3 c.c. of Amberlite I R 4 B resin and was concentrated to about 2 ml. The chromatograph paper was spotted at 4 points with 0.05 ml. of the concentrated solution, the chromatographs developed by the pyridine-ethyl acetate-water solvent system, and dried at room temperature. The longitudinal panels

containing the sugars from the outside spots were cut out and were sprayed with aniline phthalate reagent, placed back in their original position in the paper, and the positions of the unsprayed sugars marked. This unsprayed portion was divided into transverse sections containing the same separated sugar from the two inside spots.

A transverse section, cut into 4 pieces, was extracted in a micro Soxhlet apparatus with water for at least an hour, which time was sufficient to remove all sugars from the strips as shown by the aniline phthalate spray. The aqueous extract and washings from the Soxhlet pot were filtered and quantitatively transferred into a special tube with a capacity of about 25 ml. This tube was made by sealing the end of the short length of tubing normally attached to an outer 24/40 standard taper joint. The liquid, about 15 ml., was concentrated to 5 ml. on a boiling water bath with a jet of nitrogen playing over the surface. A piece of the unsprayed paper (free of sugars), of the same size as that containing the sugar, was treated in the same manner to give a blank solution.

One ml. of about 0.25 M sodium metaperiodate was added to the sugar and blank solutions, the tubes were stoppered and placed in a boiling water bath for 20 minutes. The solutions were then cooled with tap water and 0.2 ml. of ethylene glycol was added to destroy the excess periodate. Two drops of

methyl red indicator (0.1 g. dissolved in 60 ml. of ethanol and diluted with 40 ml. of water) was added, and the solution was titrated to the orange end point with 0.00979 N sodium hydroxide. The difference in titre between the unknown and the blank was a measure of the amount of formic acid produced. Xylose, arabinose and rhamnose were known to yield 4 moles, and galactose 5 moles, on oxidation with periodate. These values were used in calculating the ratio of the weight of each sugar present to the weight of pure rhamnose originally added, 0.02029 g. Since this weight of rhamnose was $\frac{0.02029}{0.07698} \times 100$ or 26.36% of the total sugars present, the percentages of the other sugars in the solution could also be obtained. The results are shown in Table XXXIV.

TABLE XXXIV

QUANTITATIVE ESTIMATION OF SUGARS IN FRACTION R BY PERIODATE OXIDATION

Sugar	Titre (ml.)	Blank (ml.)	Weight (mg.)	% of Solution	% of Fraction R
Rhamnose	2.85	0.21	0.106	26.36	----
Xylose	4.02	0.22	0.1365	33.94	46.09
Arabinose	0.60	0.20	0.0147	3.656	4.96
Galactose	0.25	0.20	0.00176	0.4376	0.594

The total amount of free sugars was 51.64% and the ratio of xylose: arabinose: galactose was 9.28:1:0.084. A duplicate determination gave very similar results with the free sugars amounting to 53.82% and a ratio of xylose: arabinose: galactose of 9.44:1:0.11.

Oxidation with Somogyi's Copper Reagent:- Fraction R, 0.04605 g., was hydrolyzed in exactly 1.0 ml. of 2.5% sulphuric acid for 5 hours on a boiling water bath. The hydrolysate was warmed to 40° and neutralized with barium carbonate. The chromatograph paper was spotted with 0.050 ml. of the neutral solution on each of 4 spots and the individual sugars were marked and extracted as before. Each 5 ml. extract was oxidized with the Somogyi copper reagent (131), containing 25 ml. of N potassium iodate per liter. The procedure described by Somogyi was followed and the 0.005 N sodium thiosulphate was standardized against known solutions of the respective sugars. The individual sugar from two spots in the transverse section, obviously corresponded to that present in 0.10 ml. of the 1 ml. solution. The section containing xylose, however, was divided into two portions and the amount of xylose determined in two extractions, the values being combined to give the total amount. Duplicate determinations on the same hydrolysate are given in Table XVI. In these cases the molar ratio of xylose: arabinose: galactose was 7.03:1:0.304 and 7.34:1:0.34 respectively.

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. Black spruce chlorite holocellulose extracted with water near 70° C. yielded a soluble lignin and carbohydrate material, which amounted to 2.7% by weight of the original wood.

2. Subsequent extraction with liquid ammonia removed 4.95% of the wood and the extract was separated into fractions by solubility differences and vacuum distillation. The methanol-insoluble Fraction A, 39.6% of the extract, was the only fraction that gave a positive Molisch carbohydrate test, and showed copper reducing power on hydrolysis. Fraction B, precipitated from methanol solution by ether, 26.7%, seemed to be largely degraded lignin. The still residues, Fractions D1 and D2, 19.6%, had high nitrogen contents and contained lignin-like material. Fraction E, acetamide, 11.9%, accounted for about one-half of the acetyl groups lost during the extraction. This value was lower than the 85% recovery reported by others for acetyl groups from sugar maple wood.

3. Klason lignin estimations showed that only about one-third of the lignin lost by the holocellulose during the ammonia extraction could be accounted for in the extract. Fractions of the extract, especially Fraction B, appeared to be lignin-like material which did not respond in the Klason estimation. The observation in (1) strongly supported the view that wood lost polysaccharides during the preparation of

holocellulose with dilute chlorous acid at 70° C., since the subsequent extraction with water at 70° C. removed a residual amount. According to (2), the weight of the holocellulose was over-estimated because the residual chlorite oxy-lignin was inadequately measured by the Klason estimation. The two errors in the recovery of holocellulose therefore tended to cancel each other.

4. Material amounting to 10.6% of the wood was removed from the ammonia-extracted holocellulose by two extractions with cold water, and a further 2.1% by two subsequent extractions with water near 70° C. Since extraction with water prior to the treatment with liquid ammonia had been exhaustive, the observation supported Neubauer's conclusion that liquid ammonia had promoted chemical change other than the cleavage of acetyl groups to yield acetamide.

5. The combined cold and the combined hot water extracts were separated into soluble and insoluble fractions in 66% ethanol. Acetylation and crude fractionation of the alcohol-insoluble polysaccharides showed that several different materials were present in both extracts. The alcohol-insoluble polysaccharide from the combined cold water extracts on acetylation gave an acetone-soluble acetate, Fraction K, which accounted for 60% of the material. Fraction K was fractionated from chloroform solution by addition of petroleum ether, and approximately 80% was

found to have similar rotations, intrinsic viscosities and acetyl contents (Fraction N).

6. Intrinsic viscosities of sub-fractions from deacetylated Fraction N were similar and comparison with the uncertain data published in this field suggested a value of 80 to 90 for the degree of polymerization of the purified polysaccharide. The sub-fractions having similar compositions, specific rotations and intrinsic viscosities were combined as Fraction R, the "pure" hemicellulose. Fraction R appeared to be chemically homogeneous, and gave a pentosan: uronic anhydride: methoxyl ratio of 5.82:1:1.29, compared to 5.85:1:1.15 for Fraction N.

7. Hydrolysis of the "pure" hemicellulose at p H 2 and 100° C. yielded little but L-arabinose, and more drastic hydrolysis D-xylose. Paper chromatography of the hydrolysate revealed xylose, arabinose, galactose and uronic acids, the free sugars amounted to 64.4% of Fraction R. Xylose, arabinose and galactose were present in a ratio of 7.19:1:0.32. Isolation and examination of the acidic fraction from the hydrolysis as the barium salt showed that it was apparently a mixture of aldobiuronic and aldotriuronic acid complexes containing 1.4 methoxyl groups for each uronic anhydride residue.

8. Although the arabinose and galactose could have been impurities tenaciously retained rather than integral parts

of the molecule, the hemicellulose was believed to be the first from softwoods to be extensively purified until it satisfied the available criteria for purity. It showed great similarity to hemicelluloses of the "B" type found in hardwoods and the tissues of annual plants.

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