THE ACTION OF LIQUID AMMONIA ON MAPLE WOOD

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THE ACTION OF LIQUID AMMONIA ON MAPLE WOOD

A Thesis

by

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GENERAL INTRODUCTION

In 1947 M.M. Yan carried out an investigation in this laboratory on the solvent effects of liquid ammonia on woody materials, chiefly concerning himself with sugar maple wood. He found that liquid ammonia at 25° extracted nearly 6% by weight of maple wood, and the extract was separated into three fractions, acetamide 4%, "liquid ammonia lignin" 0.7%, and a polyuronide material 0.8%. The "liquid ammonia lignin" was investigated by Yan, and a possible formula was suggested.

The nearly quantitative production of acetamide from the acetyl groups of the wood showed that the liquid ammonia cleaved any ester links that were present. This inference suggested that the wood residue after liquid ammonia extraction might be chemically changed, and that components previously insoluble might then be capable of extraction. The present thesis is devoted to investigating this possibility.

It was found that hot water could extract about 2% of polysaccharide material, not previously water-soluble, from the ammonia-extracted wood residue. This polysaccharide was a xylan - methoxyglucuronic acid complex, contaminated with pectic material, and the greater part of the work was concerned with attempts to purify it and to determine its structure.

HISTORICAL INTRODUCTION

Anhydrous liquid ammonia is, like water, neutral in reaction, and possessed of high solvent powers. 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).

Concentrated aqueous ammonia, 23% to 28%, has no effect on cellulose under ordinary conditions (2), but by heating purified cellulose to 200° at 40 atmospheres pressure for 48 hours with 22% aqueous ammonia, Bernardy reduced it to a brown powder containing 20% of nitrogen (3). Ammonia, like most polar gases, is absorbed by cellulose in considerable amounts. Cotton will absorb 4%, and white spruce 7.4% by weight at 22° and atmospheric pressure (4). Heuser states that cotton can occlude 115 times its own volume of ammonia (2). After evaporation of the ammonia the cellulose appears to be unchanged.

Liquid ammonia, however, produces quite different effects. Bernardy (3)(5) first observed that cellulose became swollen in liquid ammonia, the fibres being mercerised in a manner similar to the action of strong aqueous alkali. Barry, Peterson and King confirmed the swelling in the anhydrous liquid, and showed that a distinct crystalline entity, called

ammonia-cellulose by them, was formed (6). They worked with bundles of ramie fibres which were immersed in liquid ammonia for 5 to 6 hours at its boiling point of -33.5°. Free evaporation at atmospheric pressure was permitted, the ramie fibres were immediately covered with paraffin oil to prevent loss of the more tenaciously held portion of the ammonia, and at once examined by the X-ray technique. Clark and Parker later showed that the use of the paraffin was ineffectual (7).

The fibres were found to be swollen, and to contain at this stage one mole of ammonia per mole of anhydro-glucose in the cellulose. X-ray analysis showed a distinct structure similar to that found by Hess and Trogus when they treated cellulose with certain diamines such as hydrazine and ethylene-diamine (8). The volume of the unit cell was increased from 671 cu. Å. for normal cellulose to 801 cu. Å. for ammonia-cellulose. Barry, Peterson and King considered this swelling to be caused by the entry of ammonia molecules into the unit cell, in the regular manner of a permutoid swelling agent. The a-axis of the cell increased from 8.3 Å. to 9.83 Å., and the c-axis from 7.9 Å. to 10.95 Å., while angle β decreased from 84° to 53.5°. The b-axis was unchanged. Ammonia-cellulose could not be prepared by soaking cellulose in saturated aqueous ammonia for several hours, or in ammonia gas at atmospheric pressure for 50 hours.

If ammonia-cellulose was heated at 105° for 15 hours instead of being coated with paraffin oil, all the ammonia was

lost, and a new cellulose, cellulose II, was formed, definitely not the original cellulose but resembling hydrate cellulose in structure and enhanced chemical reactivity. X-ray analysis showed a lattice volume of 702 cu. Å., smaller than that of ammonia-cellulose, but still larger than that of normal cellulose. The a:b:c axis ratio was found to be 7.87:10.31:10.13 compared with 8.3:10.30:7.9 for normal cellulose, while angle β was 58°. A similar cellulose II was obtained by allowing the paraffin-covered fibres to stand 48 hours at room temperature. These workers also found that ammonia-cellulose reverted to normal cellulose when immersed in dilute acetic acid, or in either dilute or concentrated aqueous ammonia. Cellulose II was unaffected by these reagents, although with liquid ammonia it gave ammonia-cellulose which could then be changed to normal cellulose. These conceptions might be expressed as in the following equation:

$$\text{n-Cellulose} \underset{H_2O}{\overset{\text{liq. } NH_3}{\rightleftharpoons}} \text{Ammonia-Cellulose} \underset{\text{liq. } NH_3}{\overset{\text{heat}}{\rightleftharpoons}} \text{Cellulose II}.$$

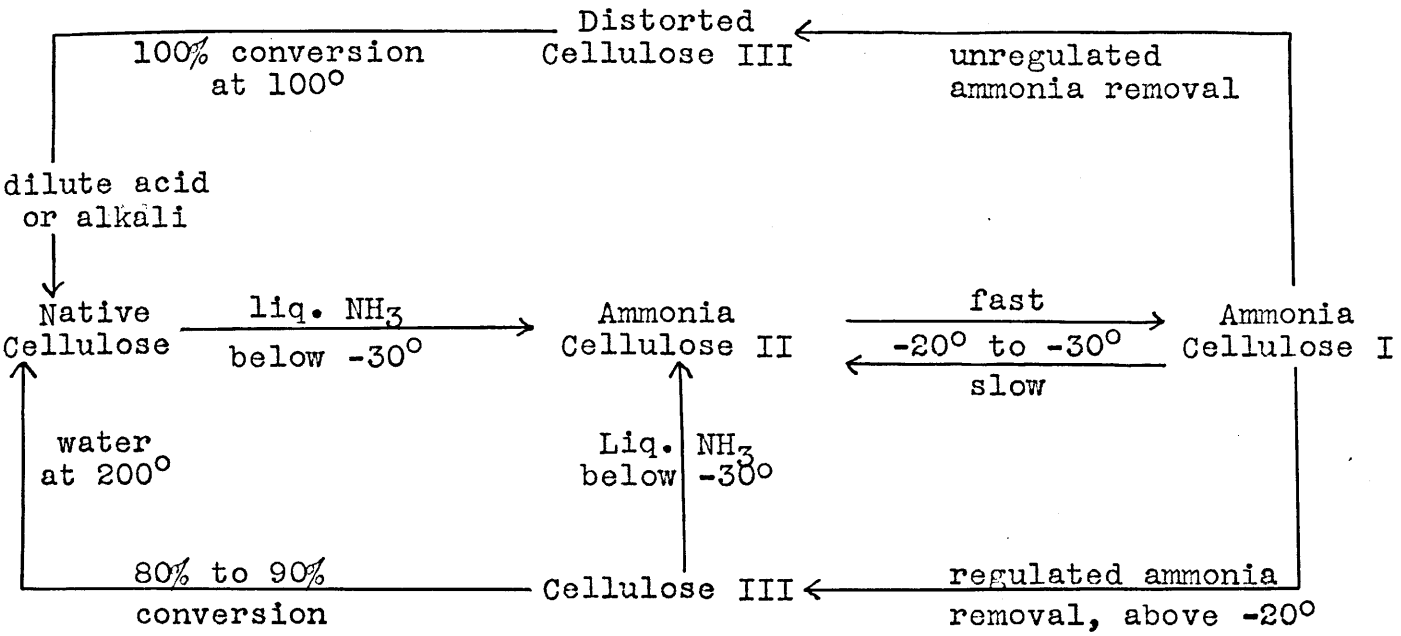
Meanwhile Hess and Trogus had been carrying out independent studies (9) simultaneously with those of Barry, Peterson and King. Hess and Trogus treated cellulose with dry liquid ammonia at -77° to -80°. The fibres did not swell, perhaps because of the very low temperature used (ammonia freezes at -77.7°). However when the ammonia was distilled, the cellulose gave a new X-ray pattern which was considered to be either ammonia - poor

ammonia - cellulose or a third modification, called provisionally cellulose III. Contrary to the behaviour of Barry, Peterson and King's preparation, neither water, methanol nor concentrated aqueous ammonia restored the normal cellulose pattern. In fact, entirely different patterns were produced, whose nature was not clear. Hess and Trogus also reported that the X-ray diffraction pattern given by their cellulose III varied with the rate of evaporation of ammonia from the fibre, and inferred that under liquid ammonia ammonia-cellulose existed in a form not identical with cellulose III. These contradictory reports led Clark and Parker to re-investigate the action of liquid ammonia on cellulose at -75° (7). They found that the cellulose fibres swelled to three times their normal size, but even when protected by paraffin oil, the fibres rapidly lost ammonia and regained their normal size when removed from the liquid ammonia. Swollen ammonia-cellulose, taken directly from liquid ammonia and immersed in concentrated aqueous ammonia, reverted to the original native cellulose.

Hess and Gundermann then considered that the X-ray examination should be carried out while the fibres were still immersed in the liquid ammonia (10), and this technique enabled them to clarify the nature of the changes involved. Ramie fibres gave two ammonia-celluloses, mutually interconvertible between -20° and -30°. When the form stable at lower temperatures, ammonia-cellulose II, 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 ammonia-cellulose I made it impossible to decide whether I and II are polymorphs or different chemical complexes. As reported by the earlier workers (6), loss of ammonia from ammonia-cellulose gave a form of cellulose, resembling hydrate-cellulose, which Hess and Gundermann called cellulose III. It was found that the exact nature of cellulose III was largely influenced by the method of its production; 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, formed when the fibres were removed rapidly from the liquid ammonia and the excess ammonia was evacuated, or by permitting the ammonia to evaporate under atmospheric pressure and the product to stand in the air for several weeks, was converted more easily and more completely to natural cellulose by boiling with dilute acid or alkali at atmospheric pressure.

The findings of Hess and Gundermann may be summarised schematically by the following figure.



Hess and Gundermann considered that the "ammonia-cellulose" and "cellulose II" of Barry, Peterson and King corresponded respectively to a partially decomposed ammonia-cellulose I, and to cellulose III mixed with natural cellulose. Later work by Barry and co-workers reported that an ammonia-cellulose, more closely equivalent to ammonia-cellulose, I, was formed when cellulose was sealed in a glass tube with liquid ammonia at room temperature (11). The dimensions of the a-axis, 11.97 Å., and the c-axis, 11.15 Å., indicated a very considerable swelling. The incorrectly named "Cellulose III" of Hess and Trogus appeared to be a partially converted ammonia-cellulose I, the result of lack of control in its production. All other modifications of cellulose obtained in these investigations were considered to be not pure types, but rather 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.

Liquid ammonia approaches water as an ionising medium. Although it does not dissolve all water-soluble salts, some salts which are quite insoluble in water, for example, the iodides of silver, lead and mercury, are easily soluble in liquid ammonia. The resemblances between liquid ammonia and water led Franklin and later workers to postulate an ammonia system of compounds where the radicle =NH is the analogue of the oxygen atom in the more familiar water system (12). Many excellent reviews of the properties and reactions of liquid ammonia have been published (13)(14)(15)(16)(17)(18)(19)(20), so only a brief summary of its behaviour with organic compounds and carbohydrates, will be given at this time.

Liquid ammonia will dissolve many organic compounds unaffected by water, and in general resembles alcohol in its powers as a solvent in the organic field. Aliphatic hydrocarbons are practically immiscible with ammonia at its boiling point, -33.5°, but become partially miscible at higher temperatures. The lower aliphatic alcohols, also benzyl and cinnamic alcohols, are completely miscible with liquid ammonia. Phenols, cresols and pyrogallol are all very soluble. Most ethers, aldehydes and ketones, aliphatic or aromatic, are soluble, although aldehydes and ketones frequently react with the ammonia by ammonolysis to give imino type compounds (16). Acetals, such as dmethyl and diethyl acetal, dissolve readily, but in contrast to their ready hydrolysis by aqueous acid, acetals

are almost unaffected by liquid ammonia even after several years solution and in the presence of ammonium salts (16), which are regarded as acids in the ammonia system. Carboxylic acids such as acetic and benzoic acids dissolve readily as ammonium salts, but the higher aliphatic acids and the dicarboxylic acids are almost insoluble. If an acid, such as acetic acid, or an acid anhydride, is treated with liquid ammonia under increased pressure the product is the acid amide (21). The amides of most acids are quite soluble. Esters are in general soluble, but most of them undergo ammonolysis to the corresponding amide.

All the ordinary sugars, arabinose, glucose, fructose, galactose, sucrose, maltose, lactose, as well as their methylated, acetylated and isopropylidene derivatives, are quite soluble in liquid ammonia at its boiling point, -33.5° (22)(23). Many polysaccharides, but not cellulose, are also soluble. With the exception of the sugars with free potential carbonyl groups, liquid ammonia at -33.5° has no effect on these carbohydrates (23).

The reducing sugars, when dissolved in liquid ammonia at its boiling point, first form an aldehyde addition product, and then the corresponding amine. Glucose, for example, forms 1-amino-glucose (22). Muskat found that liquid ammonia at -33.5° dissolved the acetylated and benzoylated derivatives of any sugar without deacylation (23), provided the reducing group was suitably blocked by forming the methyl glycoside or 1,2-isopropylidene compound. If, however, the acetyl group was attached to the aldehydic or ketonic carbon atom, it was readily removed to form an

amino sugar. At room temperature liquid ammonia removed all acetyl groups from the sugars (24). Similarly Zechmeister and Toth found that when cellobiose octa-acetate was heated with liquid ammonia at 55° for 48 hours all the acetyl groups were removed, and 1-amino-cellobiose was formed (25). Lactones of aldonic and saccharinic acids dissolve in liquid ammonia as the corresponding acid amides (26)(27)(28).

The use of cellulose-swelling liquids for the extraction of lignin from wood was initiated by Hess and Heumann in 1942 (29). With hydrazine to swell the cellulose and simultaneously to condense with the highly reactive portions of the natural lignin, it was hoped to isolate the substance with less chemical change than was possible by other methods, since the use of heat or strong acid or alkali was avoided. Three extractions of winter rye straw by a 25% aqueous solution of hydrazine hydrate almost completely delignified the straw. Ethylene diamine, and amino bases such as monoethanolamine, triethanolamine, tetramethyl ammonium hydroxide and guanidonium hydroxide, all in aqueous solution, also extracted lignin from rye straw. All of these agents are permutoid swelling agents for cellulose (8)(30), but by employing aqueous solutions Hess and Heumann controverted one of their own principles, since they were using strongly alkaline reagents. Tetramethyl ammonium hydroxide in aqueous solution is as strong a base as sodium hydroxide (31), and even hydrazine in aqueous solution has a basicity close to that of aqueous ammonia (32).

Freudenberg and co-workers studied the action of alkali metal and alkali metal amides dissolved in liquid ammonia on isolated lignin and wood (33)(34), in attempts to isolate a lignin with its carbon skeleton relatively unaltered. Spruce lignin, isolated by the Urban hydrochloric - phosphoric acid method (35), when treated for 24 hours at 20° with a solution of potassium or potassamide in ammonia, yielded a residue completely soluble in the alkaline solution produced by decomposition of the residue with water (33). Spruce wood meal, extracted with sodium dissolved in liquid ammonia, became 50% soluble in aqueous alkali, the soluble portion containing two-thirds of the lignin. In Freudenberg's later paper (34), the use of alkali amide was avoided, as its presence caused side reactions, and complicated the course of the reaction. Spruce meal was treated with a solution of potassium in ammonia for several hours at 20°. Only 1.9% of the meal dissolved, but lignin amounting to 16% to 18% of the meal was then soluble in methanol. The remaining 8% of the lignin, although methanol-insoluble, could be extracted by 1% caustic soda. A residue of 41.8% of undegraded Cross and Bevan cellulose was recovered. Beech wood, treated similarly, underwent a more complicated reaction, an appreciable amount of the cellulose being degraded to a methanol-soluble product. Isolated spruce lignin was quite stable to a solution of potassium in ammonia. Cuproxam lignin was rendered completely soluble, some 6% dissolving in the ammonia, and the rest in methanol. The effects of potassium in liquid ammonia on a series of model substances, phenol ethers, glycosides, coumarones, coumarans and

chromans were also studied. In the case of phenol ethers, complete or partial dealkylation occurred, yielding free phenolic groups. Since this treatment of wood had caused an increase in the hydroxyl content of the lignin, Freudenberg inferred that the solubilisation of wood lignin was connected with a similar dealkylation or phenolic ether linkage rupture. Freudenberg's use of alkali amide in ammonia is the equivalent in Franklin's ammonia system of aqueous caustic soda in a normal alkali cook.

The experiments of Hess and Freudenberg led Yan (1) to investigate the use of liquid ammonia alone as a solvent for lignin, without the use of heat, acids or alkali. He found that hardwoods were solubilised to a greater extent than softwoods, but both were less amenable than rye straw, which lost as much as half of its lignin to liquid ammonia. Increase in temperature beyond that of the room caused no significant increase in amount of extract, so Yan adopted an extraction temperature of 25° for a period of 5 hours, after which time little further solution was found to occur.

Liquid ammonia extracted some 5% to 7% by weight of sugar maple wood. The extract was separated into three fractions differing in solubility. The first fraction, 0.8%, almost insoluble in all the solvents tried, consisted of polysaccharides having a pentosan content of 6.9% and a methoxyl content of 4.4%. The second fraction, water- and alcohol-soluble was shown to be acetamide, and amounted to 4% of the wood weight, or equivalent to 2.9% of acetyl groups originally present. The third frac-

tion, "liquid ammonia lignin", was obtained in 0.7% yield, or 3% of the Klason lignin in the wood. The extracted lignin was further separated into three fractions, (a) soluble in methanol and dioxane, (b) insoluble in methanol, soluble in dioxane, and (c) insoluble in methanol or dioxane. Lignin fraction (a), which was approximately half of the total extracted lignin, appeared to be quite homogeneous. On the basis of carbon-hydrogen and methoxyl determinations, together with molecular weights in dioxane, Yan assigned the formula $C_{42}H_{43}O_{15}(OCH_3)_7$ to this fraction. Methylation with dimethyl sulphate and caustic soda introduced five methoxyl groups. Since this fraction of the "liquid ammonia lignin" represented only 1.5% of the total Klason lignin, and had an elementary composition different from the average for the total lignin, it was not claimed that the sample was representative of the entire lignin in sugar maple wood. The nitrogen content of "liquid ammonia lignin" was found to be negligible, a fact which Yan considered to indicate that its extraction from wood was either a purely physical process of solution, or a chemical process involving ammonolysis of ammonia-sensitive links, probably ester links between hydroxyl groups of the lignin and carboxyl groups of the holocellulose residue. In this event any amide units formed would be attached to the carbohydrate and not to the lignin constituent.

The term "hemicellulose" was introduced by Schulze to describe a class of carbohydrates found in plant materials (36). His classification of the functions of plant carbohydrates recog-

nized the structural role of the difficultly hydrolysable cellulose, and the reserve role of the soluble and easily hydrolysable starch. Intermediate to these two was a group of substances, hydrolysable by dilute acids, but extractable only by alkalis, which he classified as hemicelluloses. A further distinction was that whereas cellulose and starch gave only glucose on complete hydrolysis, the hemicelluloses hydrolysed to sugars such as xylose, arabinose and galactose, as well as to glucose.

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" (37). After extraction, however, such hemicelluloses may be soluble or at least dispersible in water. This definition is somewhat narrow, as it excludes several substances extracted from plant material by water which should apparently be included in this group of plant substances. Notable amongst these water-extractable materials are the arabogalactans of larch woods (38)(39)(40)(41)(42)(43)(44)(45) and Pinus palustris (46), the "wood starches" isolated from walnut and oak by Campbell (47)(48), the polysaccharide extracted from spruce by Brauns (49), and the xylose-methoxyglucuronic acid polysaccharide described in the present thesis.

It is of little value to summarize the present state of our knowledge of the hemicelluloses in this Introduction,

since several excellent reviews of their history, chemistry and utilisation have been published in recent years (37)(50)(51)(52)(53).

The outstanding work of Miss M.H. O'Dwyer however deserves some mention (54)(55)(56)(57)(58)(59). From oak sapwood she obtained a hemicellulose A (precipitated by acidification of an alkaline extract of the wood) which she found to consist of 11 xylose units for each methoxyglucuronic acid unit. Hydrolysis of this hemicellulose A was not smooth, and there appeared to be a more resistant nucleus consisting of 6 xylose units to each methoxyhexuronic group. The hemicellulose B fraction (precipitated by addition of alcohol to the filtrate from hemicellulose A) was found to resemble the resistant nucleus from hemicellulose A, in that it contained 6 xylose units to each methoxyhexuronic group, and had almost the same optical rotation (ca. -51°).

A hemicellulose B of the same composition was obtained by Preece by the cold alkaline extraction of boxwood (60).

By the cold alkaline extraction of mesquite wood, Sands and Gary obtained four fractions, separated by differential solubilities (61). All fractions contained only xylose, hexuronic acid and ethereal methoxyl groups, but whereas the least soluble fraction consisted of 11 xylose units to each methoxyhexuronic acid group, the figure for the other three was 6 xylose units.

Anderson and co-workers carried out studies on a number of hardwoods (62)(63), and concluded that hardwoods have polyuronide hemicelluloses similar in general type to those just described, but that some woods contain polysaccharides with glucose as well as methoxyhexuronic acid residues attached to the xylan chain. Graded acid hydrolysis of Anderson's hemicellulose fractions usually yielded aldobiuronic or aldotriuronic acids, but the hemicellulose from black locust sapwood gave the methoxyhexuronic acid by itself as the calcium salt. The methyl group in the hexuronic acid was always present in the form of an ether.

A more recent approach to the extraction of hemicelluloses is by the holocellulose route. Since there is much evidence for a lignin-polyuronide union (64)(65)(66)(67)(68)(69), it is logical to suppose that prior delignification would facilitate hemicellulose extraction.

Ritter and Mitchell have examined the hemicelluloses extracted from a holocellulose obtained in 74% yield from extractive-free sugar maple wood (70) by the method of Ritter and Van Beckum (71). They obtained five fractions by the following treatment of the holocellulose: fraction 1, by extracting with 30 parts of boiling water for 1 hours; divided into 1b, precipitated by the addition of ethanol, and 1a, precipitated by the addition of acetone to the filtrate from 1b; fraction 2, by extracting the residue from 1 with 10 parts of cold 2% aqueous sodium carbonate for 48 hours; fraction 3, by extracting the residue from 2 with 10 parts of 4% aqueous sodium hydroxide at

room temperature; fraction 4, by extracting the residue from 3 with 10 parts of boiling 10% aqueous sodium hydroxide for 1 hour. The yields and analytical data for these fractions are given in Table I.

TABLE I

Preliminary Analytical Data for Maple Hemicelluloses

Fraction	Yield %(a)	Uronic Anhydride %	Xylan %	Methoxyl %	Acetyl %	Hexosan %(b)
1a	1.6	17.1	46.1	2.7	9.3	24.7
1b	3.0	15.8	48.7	2.3	9.2	24.0
2	2.2	28.9	54.7	2.6		13.7
3	14.9	12.2	79.2	2.1		6.5
4	3.5	9.3	80.9	2.3		7.5

(a) On basis of weight of bone-dry holocellulose.
(b) By difference.

Only in the case of fraction 4 is the methoxyl content sufficient for the presence of one methoxyl group per hexuronic acid unit. The proportion of xylose to uronic acid is different from that of all previously isolated hardwood hemicelluloses. In view of the mild conditions for the extraction of fraction 1, it is unlikely that the hexosan content is non-uronide material from the cellulose, and it is probably a part of the actual polyuronide molecule. The high acetyl content of fraction 1 had also been missed previously when alkaline extraction had been employed.

In a later publication Mitchell, Rogers and Ritter reported a repetition of their previous work (72), and found that the composition of their hemicellulose fractions (Table II) was somewhat different from that noted in Table I. In this investigation they again employed Ritter and Van Beckum holocellulose from sugar maple wood. Their fractionation, using a 15:1 solvent to holocellulose ratio, gave four fractions: fraction A, extracted by water at 95° for 1 hour; fraction B, extracted from the residue from A with 2% aqueous sodium carbonate at 20° for 24 hours; fraction C, extracted from the residue from B with 4% aqueous sodium hydroxide at 20° for 24 hours; fraction D, extracted from the residue from C with boiling 10% aqueous sodium hydroxide for 1 hour.

TABLE II

Later Analytical Data for Maple Hemicelluloses

Fraction	Yield %(a)	Uronic Anhydride %	Pentosan %	Methoxyl %	Acetyl %
A	3.0	16.4	52.7	2.5	9.3
B	5.1	28.9	63.1	2.6	
C	9.6	12.2	82.7	2.1	
D	8.4	6.5	58.3	1.4	

(a) On basis of weight of extractive-free wood.

No report of hexosan by difference was made in this second publication, but it will be seen that only fractions A and D could have any appreciable hexosan content. The high hexosan content of fraction D was probably due to damage of the actual cellulose structure of the holocellulose by the severe treatment, a supposition borne out by the fact that the α-cellulose content of the residue from D was less than that from any of the earlier residues.

Summation of the yields given in Table I showed that the extracted hemicelluloses totalled 25.2% by weight of the holocellulose, yet Table II gives the total yield of hemicelluloses as 26.1% of the weight of extractive-free wood. No explanation was given, and it would appear that the close agreement of these two yields is coincidental. Mitchell, Rogers and Ritter point out that the uronic acid content of fraction A is in good agreement with the value of 17.1% reported by Thomas for a hot water-soluble aspen hemicellulose (73), and the uronic acid content of fraction C is in agreement with the value of 12.1% also reported by Thomas for an aspen hemicellulose extracted from the holocellulose by 5% aqueous sodium hydroxide. It thus appears that hemicellulose fractions from different woods may have similar compositions, an inference already suggested by Norman (37) and Anderson (63).

Rogers, Mitchell and Ritter have also examined the hemicelluloses isolated by direct extraction of sugar maple wood (74). Their procedure was to heat the meal with 20%

aqueous potassium hydroxide at 65° to 70° for 2 hours, then dilute the alkali to 3% concentration and extract at 65° to 70° for a further 2 hours. The hemicellulose-1 was precipitated by the addition of 2 volumes of methanol and bleached with aqueous sodium chlorite at 65° to 70°. Final purification was by re-solution in dilute potassium hydroxide and precipitation with 3 volumes of methanol containing sufficient acetic acid to neutralise the potassium hydroxide. This purification was repeated three times. Hemicellulose-2 was obtained by a similar treatment of the wood residue from hemicellulose-1, after it had been partially delignified by the Wise, Murphy and D'Addieco method for the preparation of holocellulose (68). The yields and composition of these two fractions are given in Table III.

TABLE III

Analytical Data for Hemicelluloses from Maple Wood

Fraction	Yield %(a)	Ash %	Uronic Anhydride %	Pentosan %	Methoxyl %	Lignin %
-1	14.6	7.10	16.67	76.60	2.15	0
-2	3.5	7.16	9.80	77.70	3.03	5.2

(a) On basis of original wood.

The hemicelluloses of aspen holocellulose have been examined by Thomas (73). Fraction A was isolated by alcohol precipitation of the washing water used in the holocellulose preparation; fraction B by extraction of the holocellulose with water at 20°; fractions C and D by extraction of the residue from B with water at 90° to 95°, fraction C being precipitated by the addition of alcohol, and fraction D by evaporation of the mother liquors from C; fraction E by extraction of the residue from C and D with 5% aqueous potassium hydroxide; and fraction F by extraction of the residue from E with 15% aqueous potassium hydroxide. Table IV gives the yields and analytical data of these hemicellulose fractions from aspen.

TABLE IV

Analytical Data
for Aspen Hemicelluloses

Fraction	Yield %(a)	Ash %	Uronic Anhydride %	Xylan %	Methoxyl %	Acetyl %	Glucosan %
A	1.7	6.9	32.6	50.0	1.9	1.7	8.0
B	3.3	4.8	17.1	57.3	2.3	8.9	5.0
C	4.2	1.2	23.4	54.2	2.5	8.1	5.5
D	2.6	1.7	12.1	8.0	2.4	8.9	7.5
E	11.3	8.2	14.6	82.7	1.7		8.6
F	5.5	16.4	11.0	85.5	1.7		7.9

(a) Based on bone-dry holocellulose.

The values for glucosan were determined by the loss in reducing power of the hydrolysate after fermentation with specific strains of yeast.

White birch holocellulose has been shown by Sitch (75) to yield a water-soluble hemicellulose with a 5:1 pentose to uronic acid ratio, and another, soluble in ethylene-diamine, with a ratio of 8:1. Both fractions showed a 3:2 ratio of uronic anhydride to methoxyl, instead of the 1:1 ratio expected for a methoxyuronic acid group. Hexosan was indicated, especially in the water-soluble hemicellulose, by the disparity between the sum of pentosan, uronic anhydride, acetyl and methoxyl percentages and 100%.

The conifers have not been studied so extensively as the hardwoods, but Anderson (76) has shown that white pine yielded hemicellulose fractions which contained 36% to 46% mannan, 50% to 44% xylan and 10.5% to 15% methoxyuronic acid, and also gave qualitative tests for glucose. Hydrolysis gave a resistant aldotriuronic acid with xylose residues linked to the uronic acid moiety. Kurth and Ritter hydrolysed spruce wood holocellulose with 1% sulphuric acid (77). Analysis of the hydrolysed solution indicated that the holocellulose contained some 7% of an easily hydrolysable hemicellulose which contained 14.6% uronic anhydride, 3.2% methoxyl groups, 33.5% hexosan, 33.4% pentosan and 8.0% acetyl groups.

Anderson's studies of a number of woods showed that they all contained pectic material (78). Anderson and Wise were accordingly led to test the uronic-rich fraction A isolated by Thomas from aspen (see Table IV) for polygalacturonic acid (79). Oxidation of this sample by the bromine-hydrobromic acid method of Heidelberger and Goebel (80) yielded mucic acid; therefore it is highly probable that this fraction contained pectic material. The same contaminant may also be present in many other hemicelluloses whose compositions have been reported.

Hemicelluloses similar to those just discussed have also been isolated from lignified tissues other than woods. Wheat straw hemicellulose is principally of the "B" type (precipitated from aqueous solution by the addition of alcohol), the hydrolysate containing a hexuronic acid, arabinose and xylose in the relative proportions of 1:0.9:23. The alkali-soluble fraction of alfalfa hay was examined by Phillips (81)(82), and was found to contain 12.1% of uronic anhydride and 77.3% of pentosan, the pentosan consisting of xylose and a trace of arabinose. Anderson examined the hemicellulose of cotton seed hulls (83), and found its composition to be glucuronic acid and xylose in the approximate ratio of 1:10-16. The hemicellulose of New Zealand flax has been partially investigated by McIlroy (84)(85), who 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.

DISCUSSION OF RESULTS

As noted in the Introduction, Yan studied the small amount of lignin extracted by liquid ammonia from maple wood in some detail, and identified other extracted material as polyuronide in nature. The latter material amounted to 0.8% of the wood, and was practically insoluble in all common liquids tested both by Yan and the present writer. Since no simple methods for the purification of the fraction were apparent, its detailed examination was not attempted in this research.

Yan also observed two noteworthy effects that liquid ammonia exerted on the residual maple wood meal, a decrease in acetyl content and a rise in nitrogen content. In the present investigation the acetamide recovered in the ammonia extract accounted for over 85% of the loss in acetyl content of the wood residue. As Yan suggested, the drop in acetyl content can thus be accounted for by a straight-forward ammonolysis of acetate ester linkages to yield soluble acetamide. The fact that the total increase in nitrogen could be liberated as ammonia by boiling the wood residue with dilute sodium hydroxide indicated that the nitrogen was present as an ammonium salt or an amide. Since aqueous magnesium hydroxide was no more effective than water in liberating ammonia, it was extremely unlikely that the residue contained an ammonium salt (86). The increase in nitrogen content was therefore credited to the formation of acid

amides which remained in the wood residue because they were insoluble in liquid ammonia. These observations appear to be the first clear evidence that wood contains esters other than acetyl esters, and it is interesting to speculate as to their nature. Ritter noted that a sample of maple wood contained 4.36% of uronic acids, or 0.25 milliequivalent per gram, and 0.24% of ash (74). Even the unlikely assumption that this ash was entirely sodium carbonate fails to raise the calculated ash alkalinity above 0.045 milliequivalent per gram. Since wood is practically neutral in reaction, at least 0.2 milliequivalent of uronic acid is neutralised in a way not dependent on salt formation. It thus seems likely that the esters yielding the amides were polyuronides.

The optimum conditions for the extraction of the wood meal with liquid ammonia, as determined by Yan (1), were adopted in the present investigation. A first extraction for 5 hours at 25° was followed by a shorter second treatment for 0.5 hour at 25°.

Although the original wood had been exhaustively extracted both with water and alcohol, re-extraction of the ammonia-treated residue with ethanol yielded 0.8% of a dark brown extract which gave a positive Mäule reaction for angiosperm lignin, but which was not examined in detail. The Mäule reaction involves treatment of the sample with dilute aqueous potassium permanganate, dilute hydrochloric acid, and immersion in ammonia solution, when angiosperm lignin will yield a red-coloured prod-

uct. The residual meal was then re-extracted three times with water at 97°. Cautious concentration of the combined aqueous extracts under vacuum, followed by addition of 2 volumes of ethanol to the concentrate caused the precipitation of a grey flocculent material, which, after isolation by filtration, and solvent-exchange through methanol and ether, was obtained as a grey powder. A total of 91.5 grams of this powder, amounting to 1.9% of the original air-dried wood, was accumulated by extracting 4.5 kilos of the wood meal in batches. Evaporation of the mother liquors yielded a further 11.4 grams, or 0.24% of the original wood, as a brown solid.

An attempt was then made to purify this water-soluble, alcohol- and liquid ammonia-insoluble material by re-solution in water, and precipitation by the addition of 2 volumes of alcohol. Each solution and precipitation increased the uronic anhydride content and caused a corresponding decrease in the pentosan content of the precipitate. The aqueous solution however, was not sufficiently clear to enable the purification to be followed by optical rotatory power. A trial filtration of a small aliquot of the solution through absorbent charcoal yielded a perfectly clear filtrate, but when this treatment was applied to the bulk of the material, half was tenaciously retained on the charcoal. Even the filtrate was not sufficiently clear for the determination of optical rotation. The filtrate was concentrated, and addition of 2 volumes of alcohol yielded 28.9 grams of a pale grey powder, fraction D (Fig. 2). Fraction D was dissolved in

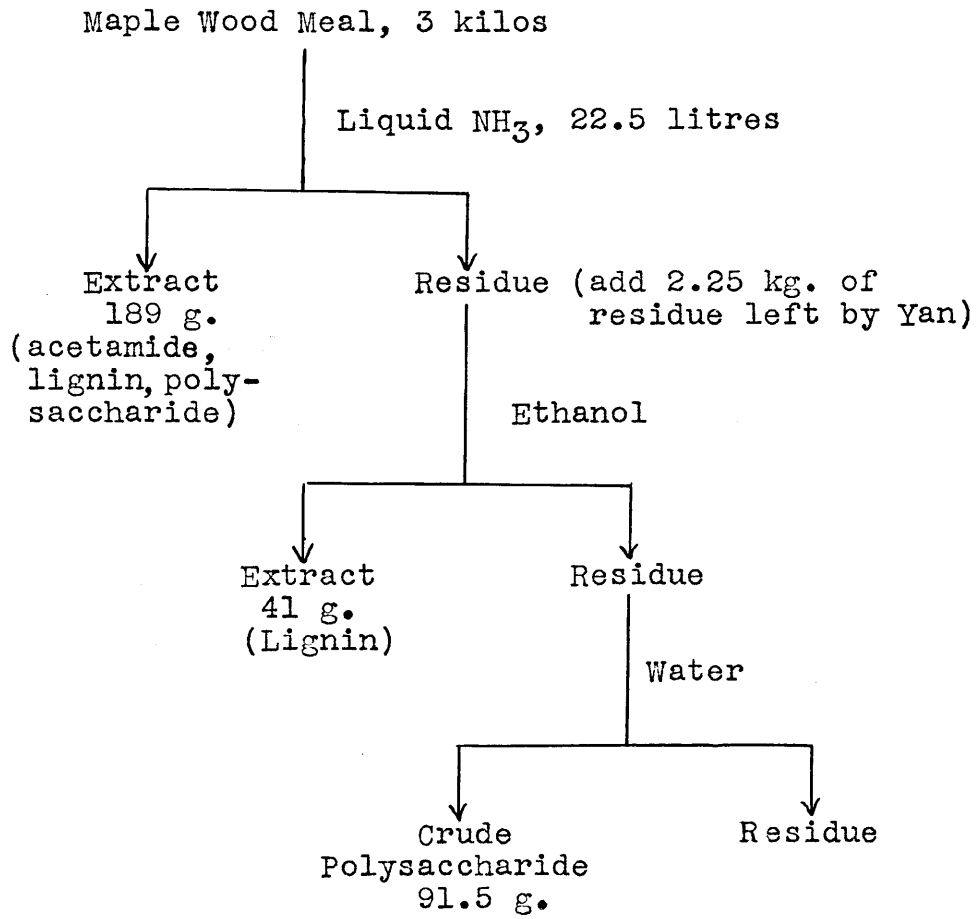


FIG. 1. Fractionation of maple wood meal, previously exhaustively extracted with water, ether and alcohol.

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water, and reprecipitated with alcohol, giving fraction H. Six further reprecipitations of fraction H yielded fraction X as the precipitate, and fraction Y as the material obtained on evaporation of the combined mother liquors.

The material which had been adsorbed on the charcoal in the attempted clarification was eventually extracted with

1% aqueous sodium hydroxide. The alkaline extract was promptly acidified, and the addition of 2 volumes of alcohol precipitated fraction F as a grey powder. This fraction was reserved for preliminary studies because of the possibility that it had been chemically altered by its brief exposure to alkali.

The complete series of fractionations undergone by the polysaccharide is represented in Fig. 2. This fractionation divided the material into several small fractions of similar properties, and fractions E, F, G and J were therefore re-united to give a large fraction M, which was then investigated, together with fractions X and Y. These three fractions, X, Y and M, represented the bulk of the original material. No fraction at any stage of the procedures reduced Fehling's solution, so it appeared that the numerous manipulations had not caused the hydrolysis of any labile sugar units.

The analytical data obtained for fractions H, X, Y and M are given in Table V. The pentosan was later shown to be xylan, and is given in the Table as such.

The uronic anhydride content of fraction H was 33.7%, when determined by the standard carbon dioxide method, but was only 1.7% by the calcium acetate method (87). As the latter estimated only free carboxyl groups, an attempt was made to de-ash the sample with 98% acetic acid. Complete de-ashing could not be effected, but the uronic anhydride content of the acetic acid - washed sample, as measured by the calcium acetate

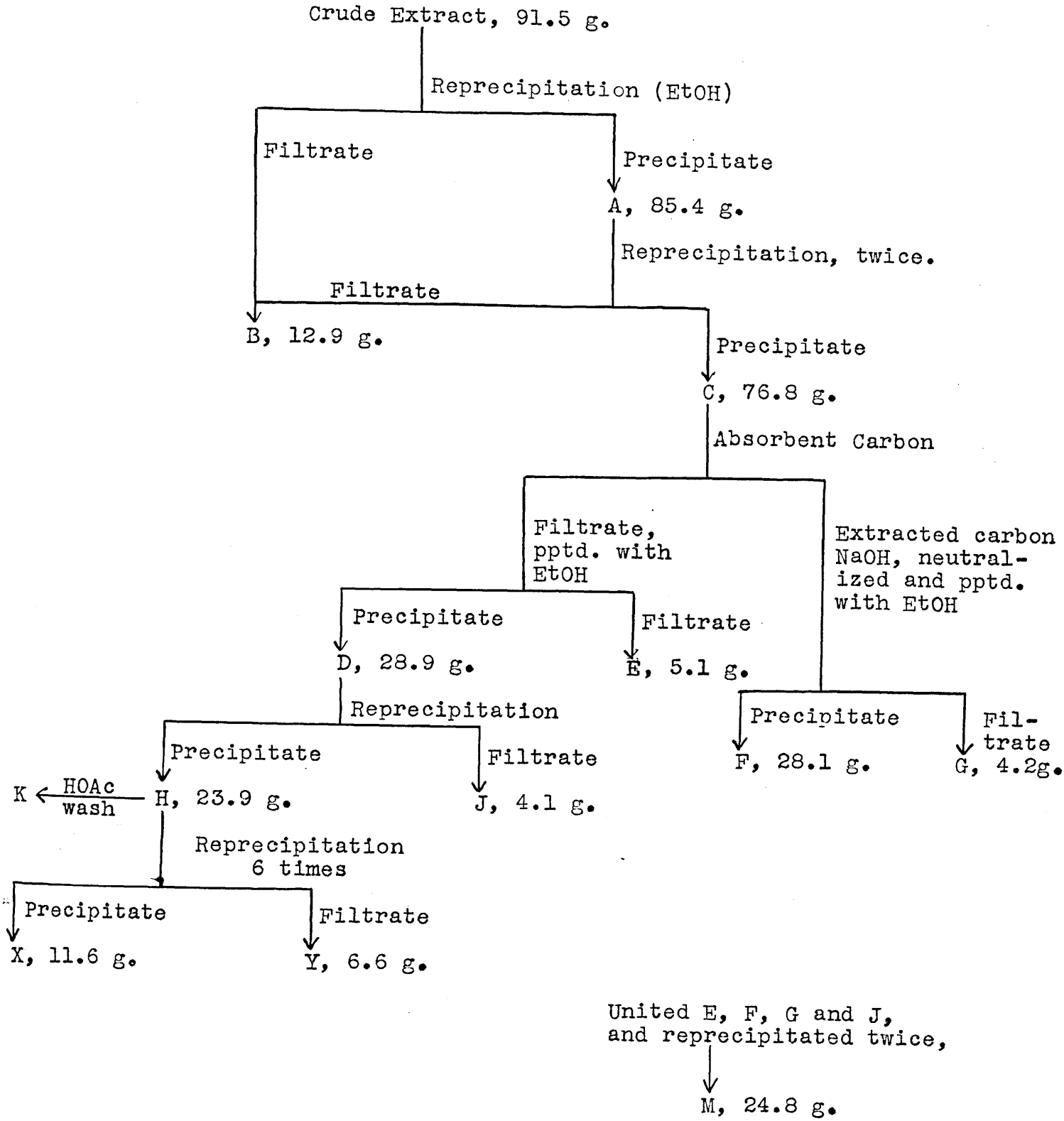


FIG. 2. Fractionation of the water-soluble polysaccharide

TABLE V

Analytical Data for Selected Fractions of Maple Polysaccharide

Fraction	Uronic Anhydride %	Xylan %	Methoxyl %	Ash %	Ash Alkalinity %
H	33.7	61.3	2.3	2.9	67.2
X	41.9	48.7	1.9	3.4	83.2
Y	22.8	72.4	2.8	2.6	73.1
M	21.8	55.5	2.1	15.8	7.9

method, was increased to 13.1%. If allowance was made for the pectic material later found in all fractions of the polysaccharide, the uronic anhydride content, calculated for a 6:1 xylan-methoxyhexuronic acid polysaccharide, was 13.2%. Whether or not this close agreement was purely fortuitous could not be decided. The ash alkalinity values for fractions H and K (the acid-washed fraction H) were of no help in this respect, since that of fraction H was equivalent to 11.0% uronic anhydride and that of fraction K to 0.93%.

Reference to Table V indicates that fractions X, Y and M had apparently quite different compositions. Acetylation of these three fractions yielded three acetates with the same composition and the same optical rotation in chloroform, and which appeared to be identical. The analyses of the acetates showed a xylan, uronic anhydride, methoxyl ratio of 6:1:1.

The yield of acetate in each case was over 90% when based on the xylan content of the fraction from which it was prepared. The uronic anhydride in the original fraction in excess of the 6:1 xylan to uronic anhydride ratio would thus appear to be polyuronic acid material, which was removed as a water-soluble, partly acetylated polyuronic acid from the products of all three acetylations.

These results indicated that the original polysaccharide fraction consisted entirely of a combination averaging 6 xylose units to each methoxyhexuronic acid residue, contaminated with a polyuronic acid. Fraction M, when hydrolysed with mineral acid, yielded a small amount of a uronic acid isolated as the alcohol-insoluble barium salt. Further oxidation of this salt with nitric acid yielded mucic acid. This observation, together with the later production of mucic acid by the oxidation of fraction X with bromine - hydrobromic acid, made it probable that the contaminant was pectic material. Fraction X had a uronic anhydride content of 41.9% (Table V). Since a uronic anhydride - xylan complex with a ratio of 1:6 requires 17.9% of the former, the polygalacturonic acid content (y%) assumed in fraction X is given by the equation $y = 41.9 - 176\ x/792$, where x is the xylan content. The result was 31.1% of the polygalacturonic acid, and three-quarters of this amount was recovered as mucic acid when fraction X was oxidised with bromine - hydrobromic acid. The agreement was considered good, because it has been reported that only

about 75% of the weight of galactose is obtained as mucic acid by oxidation with nitric acid (88). Calculation of the pectic acid content of fractions Y and M by the above equation gave values of 6.7% and 8.5% respectively.

Hydrolysis of a 3.38 gram sample of fraction M with 3% sulphuric acid at 100° was followed by determinations of the reducing power with the Schaffer-Hartmann copper reagent. The maximum value, reached after 3 hours, was equivalent to 63.7% by weight of reducing sugar, calculated as xylose (see Fig. 3). The hydrolysate was worked up in the normal manner, giving 0.12 gram of an alcohol-insoluble barium uronate, and 2.806 grams of pale yellow, alcohol-soluble, partly crystalline solid. Oxidation of the 0.12 gram of barium uronate with nitric acid yielded mucic acid as the only identifiable product. The partly crystalline material could not be induced to crystallise completely, nor could the crystals be separated from the non-crystalline portion. The mixture, however, contained at least 56.3% of D-xylose, which was isolated as the crystalline dimethyl acetal of the dibenzylidene derivative. No trace of arabinose, rhamnose nor galactose could be detected, and failure of the original hydrolysate to ferment with yeast indicated the absence of glucose or mannose. A furfural determination indicated the presence of pentosan in excess of the xylose already determined, and this value together with the uronic anhydride and methoxyl contents, was in agreement with a composition of 4 moles of xylose and 1 mole of an aldotri-

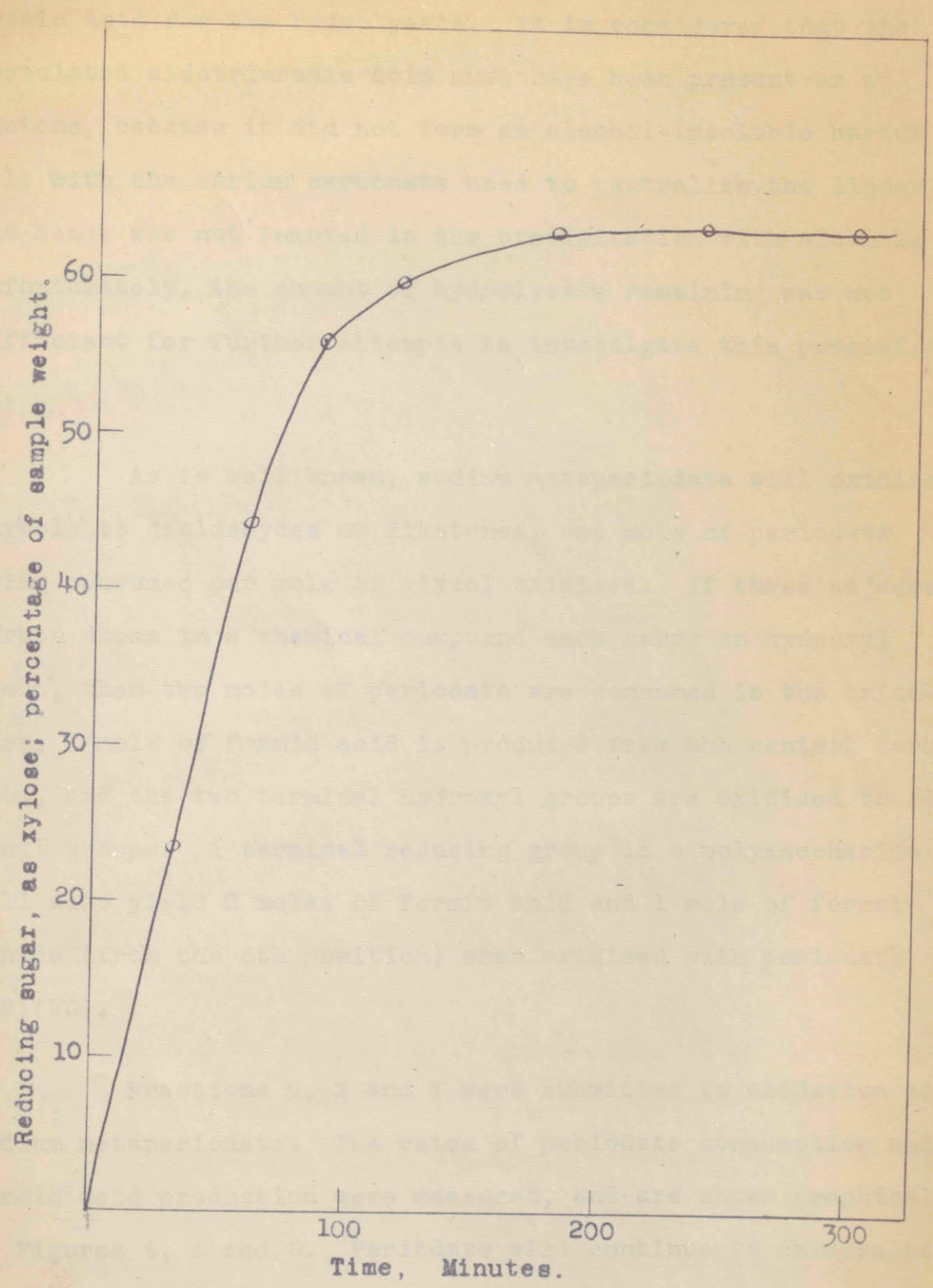


Fig.3. HYDROLYSIS OF FRACTION M WITH 3% SULPHURIC ACID AT 100°.

uronic acid for the hydrolysate. It is considered that the postulated aldotriuronic acid must have been present as a lactone, because it did not form an alcohol-insoluble barium salt with the barium carbonate used to neutralize the liquor, and hence was not removed in the precipitation with alcohol. Unfortunately, the amount of hydrolysate remaining was not sufficient for further attempts to investigate this possibility.

As is well known, sodium metaperiodate will oxidise glycols to dialdehydes or diketones, one mole of periodate being consumed per mole of glycol oxidised. If three adjacent carbon atoms in a chemical compound each carry an hydroxyl group, then two moles of periodate are consumed in the oxidation, 1 mole of formic acid is produced from the central carbon atom, and the two terminal hydroxyl groups are oxidised to carbonyl groups. A terminal reducing group in a polysaccharide will also yield 2 moles of formic acid and 1 mole of formaldehyde (from the 6th position) when oxidised with periodate (89)(90).

Fractions M, X and Y were submitted to oxidation with sodium metaperiodate. The rates of periodate consumption and formic acid production were measured, and are shown graphically in Figures 4, 5 and 6. Periodate will continue to oxidise carbohydrates very slowly after the initial rapid oxidation of glycol groups has ceased, therefore extrapolation of the flattened secondary oxidation curves back to zero time will give

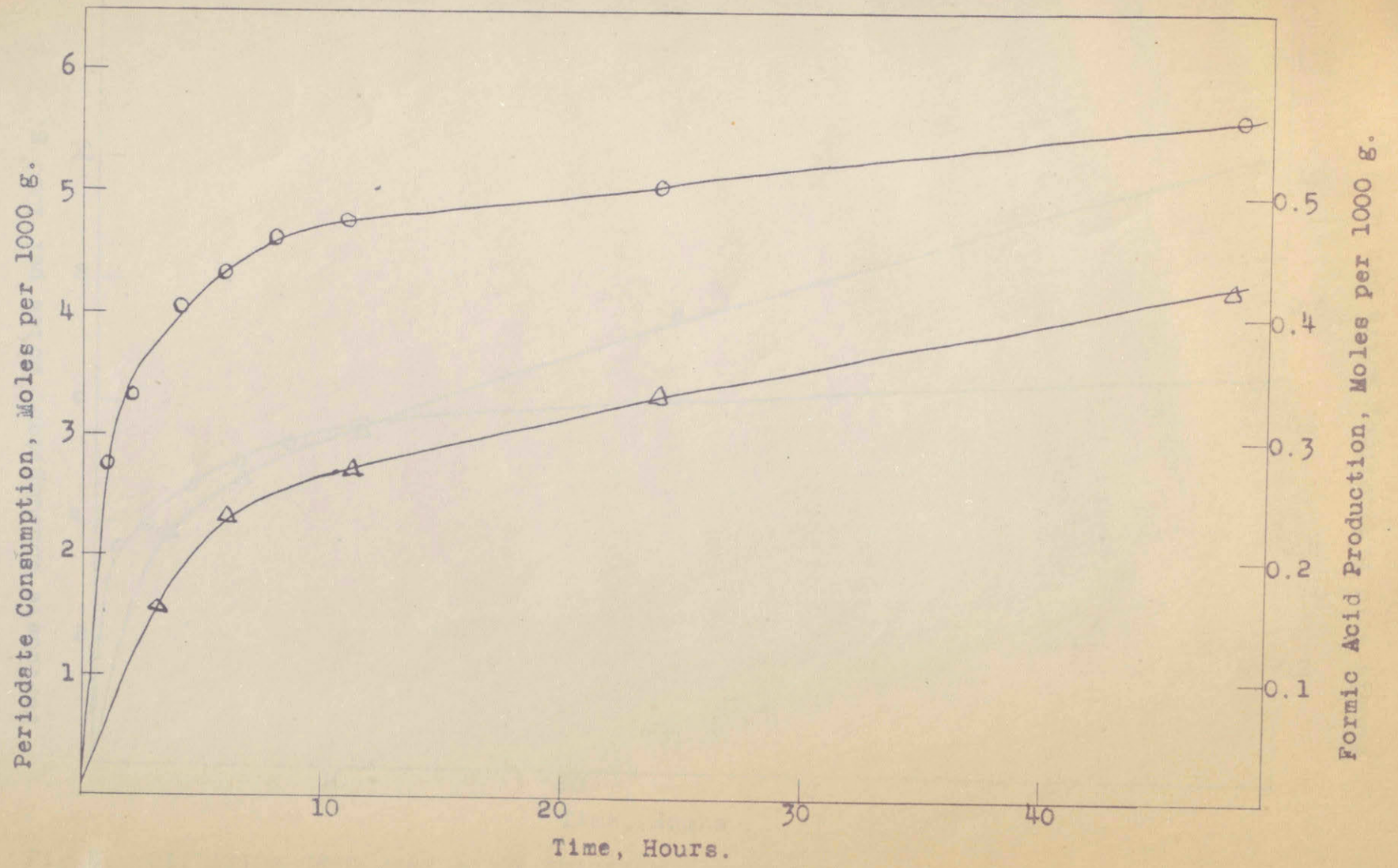


Fig.4. OXIDATION OF 0.3431 g. OF FRACTION M WITH 100 ml. OF 0.0455 M SODIUM METAPERIODATE, AT 20°.
Periodate consumption O ; Formic acid production Δ .

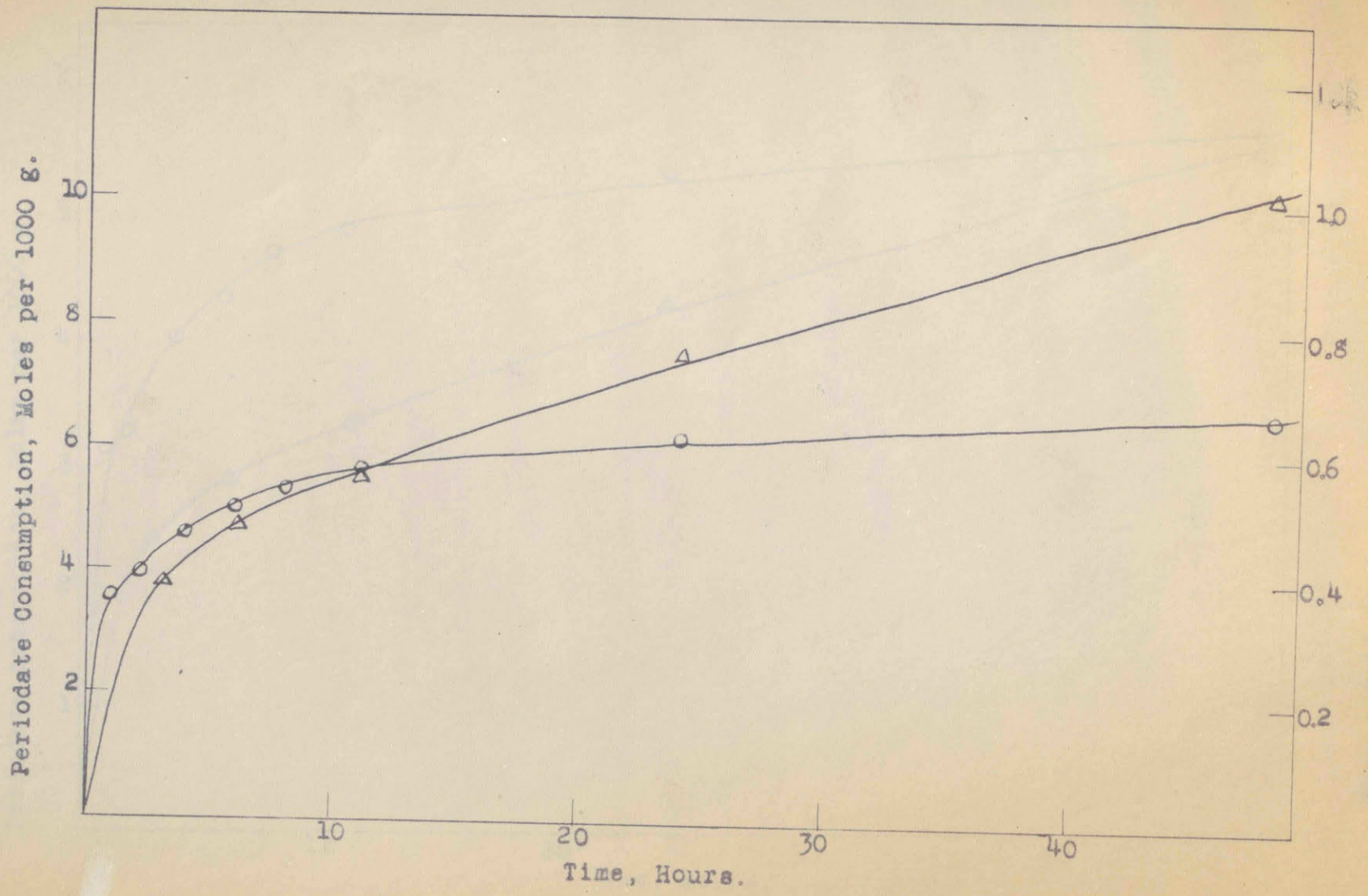


Fig.5. OXIDATION OF 0.2768 g. OF FRACTION X WITH 100 ml. OF 0.0421 M SODIUM METAPERIODATE, AT 20°.
Periodate consumption O ; Formic acid production Δ .

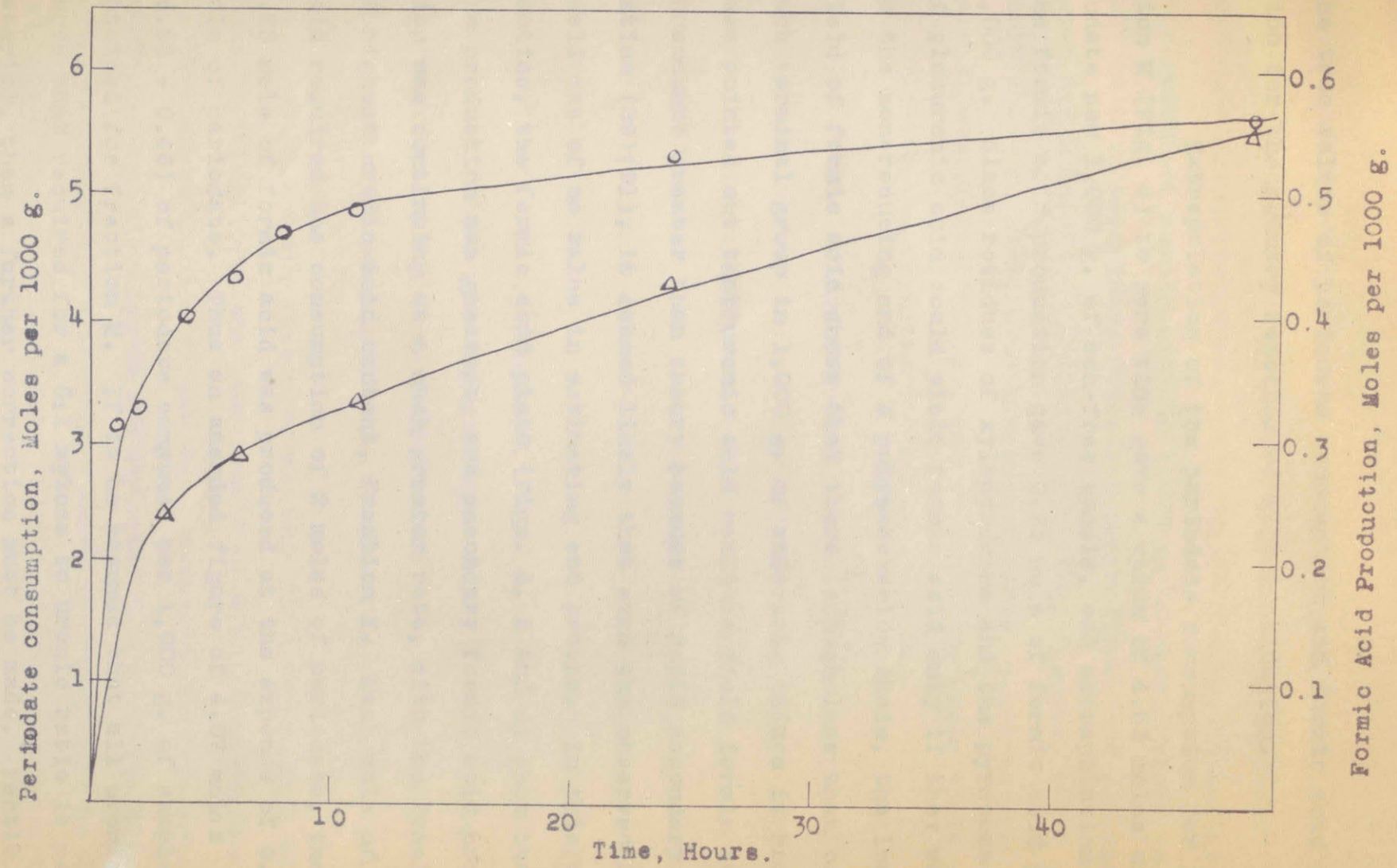


Fig.6. OXIDATION OF 0.2602 g. OF FRACTION Y WITH 100 ml. OF 0.0421 M SODIUM METAPERIODATE, AT 20°.
Periodate consumption O ; Formic acid production Δ .

the true values of periodate consumption and formic acid production for the primary reaction of glycol oxidation.

Extrapolation of the periodate consumption for fraction M (Fig. 4) to zero time gave a value of 4.53 moles of periodate per 1,000 g. of ash-free sample, and extrapolation of the formic acid production gave 0.23 mole of formic acid per 1,000 g. Since residues of xylopyranose and the pyranose form of glucuronic acid could yield formic acid only if they were at the non-reducing end of a polysaccharide chain, the low yield of formic acid shows that there is much less than one such terminal group in 1,000 g. of material. Since it has been pointed out that uronic acid residues yield formic acid in amounts greater than theory because of rapid secondary oxidation (89)(91), it seemed likely that even the observed low yield was of no value in estimating end groups. In this connection, the formic acid plots (Figs. 4, 5 and 6) show that the production was greatest, and secondary formic acid production was continuing at a much greater rate, with the fraction of highest uronic acid content, fraction X. Each mole of formic acid required the consumption of 2 moles of periodate, therefore 0.23 mole of formic acid was produced at the expense of 0.46 mole of periodate. Thus an amended figure of 4.07 moles (4.56 - 0.46) of periodate consumed per 1,000 g. of sample is obtained for fraction M. If it be assumed that all uronic acid above that required for a 6:1 xylose to uronic ratio is pectic material, then a further correction must be made. Pectic acid is composed of galacturonic acid residues in 1:4 glycosidic

union, and thus would consume 1 mole of periodate per galacturonic residue. Fraction M was previously calculated to contain 8.5% of pectic material, or 10.1% on an ash-free basis. This amount of pectic acid in 1,000 g. of sample would consume 0.57 mole of periodate, therefore the residue, 899 g., had consumed 3.5 moles (4.07 - 0.57). A unit of 6 xylose and 1 glucuronic acid residues, with 1 methoxyl group, would have a molecular weight of 982 g., and on the above basis would consume 3.9 moles of sodium metaperiodate.

The plot for fraction X (Fig. 5) gave values of 5.5 moles of periodate consumed and 0.43 mole of formic acid produced. The calculated amount of pectic material in this fraction would consume 1.82 moles of periodate. Thus after corrections for formic acid production and pectic material have been applied, a value of 2.82 moles of periodate consumed per 679 g. of material is obtained. This amount is equivalent to 4.08 moles of periodate consumed per unit of molecular weight 982. Similarly, a corrected periodate consumption of 3.98 moles per 982 g. of sample was obtained for fraction Y (Fig. 6).

In a word, after corrections for pectic acid, all three fractions gave the same result of 4 moles of periodate consumed per unit of 6 xylose and 1 methoxyglucuronic acid residues. No formaldehyde was detected in any of the oxidations, therefore the number of terminal reducing groups must be very small.

Complete methylation of fraction M by the thallous hydroxide, thallous ethylate, methyl iodide route, yielded a chloroform-soluble product which analysed for a fully methylated 6:1 xylan to hexuronic methyl ester polysaccharide, and a water-soluble residue which appeared to be a partly methylated poly-uronic acid.

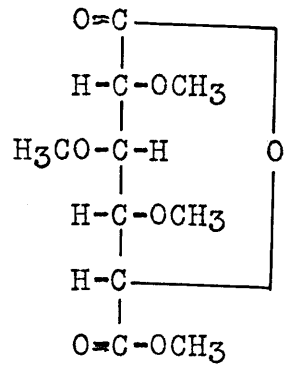
Oxidation of the fully methylated polysaccharide with nitric acid yielded several identifiable products. 2,3,4-Trimethylsaccharolactone methyl ester was definitely identified, in a yield of 11.5% of the distillable methyl esters. The presence of this compound can only be explained by the presence of a terminal glucuronic acid group in the basic 6:1 xylan to hexuronic acid unit. A crystalline diamide, isolated from all three fractions of the distillable esters in a total yield of 48.5%, had a melting point (140°), an optical rotation ($[\alpha]_D^{20}$ +28°, in water), and methoxyl and nitrogen contents which agreed closely with those of 2,3-dimethylxyloglutardiamide (84)(92). This substance could only arise from xylose units in the original polysaccharide which had linkages at the 1 and 4 or 1 and 2 carbon atoms.

A crystalline substance, which was isolated in a yield of 10% of the distillable methyl esters, had the melting point (162°), and the methoxyl and nitrogen content of 3-methylxyloglutardiamide (85). Its optical inactivity confirmed the identification because this substance is internally compensated. Such a product could arise only from a xylose unit which had

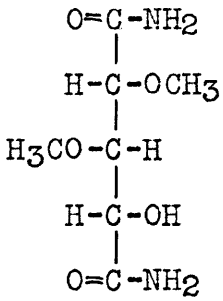
linkages at the 1, 2 and 4 carbon atoms in the original polysaccharide. Another small quantity of crystalline material had the melting point and methoxyl content of D-dimethoxysuccindiamide (93). The corresponding free acid could easily arise from the further oxidation of 2,3,4-trimethylsaccharolactone, and is of common occurrence in small yield in the preparation of this substance by the oxidation of 2,3,4-trimethyl glucose derivatives with nitric acid.

The non-crystalline material, yield 10.8% of the distillable esters, also had the nitrogen and methoxyl values for a dimethylxyloglutardiamide. There are only two such diamides, and one, the 2,3-dimethyl compound, was already isolated, melting point 140°, and would give a positive Weerman test for α-hydroxy acid amides. On the other hand, the 2,4-dimethyl compound would give a negative Weerman test, as did the syrup isolated in the present investigation. Such a 2,4-dimethylxyloglutardiamide could only arise from a xylose unit with linkages at the 1 and 3 carbon atoms.

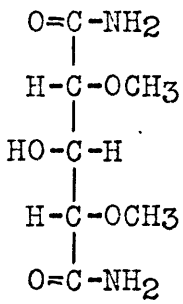
If the identification of an uncrystallized product can be accepted as correct, the yields of 2,3,4-trimethylsaccharolactone methyl ester, 3-methylxyloglutardiamide, 2,4-dimethylxyloglutardiamide and 2,3-dimethylxyloglutardiamide, which were 11.5%, 10%, 10.8% and 48.5% of the distillable methyl esters respectively, are in close agreement with the calculated 15%, 11.6%, 12.5% and 50% respectively, required for a 1:1:1:4 ratio, that would be expected by the methylation and oxidation



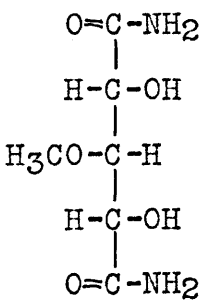
2,3,4-Trimethylsaccharo-
lactone methyl ester



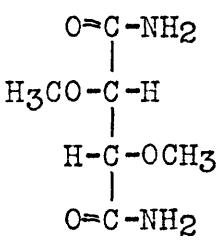
2,3-Dimethylxylo-
glutardiamide



2,4-Dimethylxylo-
glutardiamide



3-Methylxylo-
glutardiamide



D-Dimethoxy-
succindiamide

FIG. 7 Structural formulae of products isolated from the oxidation mixture of the fully methylated polysaccharide.

of a polysaccharide as depicted in Fig. 8.

Ring structures in the glucuronic and xylose residues found in nature have been of the pyran or 1,5 type in all cases hitherto examined, and the units in xylan chains have usually been in 1,4-glycosidic linkage. If these assumptions are made in the present case, then the results indicate that the original polysaccharide contained 1 terminal unit of glucuronic acid linked only at the reducing end; 4 xylose units linked 1,4; 1 xylose unit linked 1,3; and 1 xylose unit linked 1,2,4. A terminal glucuronic unit should yield at least 1 mole of formic acid per repeating unit of 6 xylose and 1 glucuronic acid residues. This amount was not nearly reached with the substance under investigation. However, it must be remembered that the material contained one methoxyl group per repeating unit. The results of the oxidation of fraction X with bromine - hydrobromic acid lent support to the view that the methoxyl group was attached to the glucuronic acid residue. Careful search recovered no crystalline potassium acid saccharate from the products, and the negative result is consistent with the presence of a methoxyglucuronic residue. Such a unit would yield a methylsaccharic acid which would not have the same physical properties as saccharic acid. If the methoxyl group were at carbon atom 3 of the glucuronic acid unit, then periodate oxidation of this unit, and hence the production of formic acid, would not occur except by side reactions.

When the postulated repeating unit (Fig. 8) is examined, it is seen that a consumption of 4 moles of periodate would be

expected. It was found that the polysaccharide under investigation consumed 4 moles of periodate. All the experimental observations, therefore, could be explained by a polysaccharide having the repeating unit shown in Fig. 8.

Several alternatives to that shown in Fig. 8, in which the xylose units substituted in the second and third positions occupy various positions in the molecule also satisfy the data. Figure 8, indeed, is merely tentative, and the structure must be confirmed by a more positive identification of the syrupy diamide found among the oxidation products of the methylated polysaccharide. The suggested aldotriuronic acid must also be isolated, and examined by oxidation with periodate, and by the methylation technique. The absence of reducing power in the polysaccharide, and the failure to isolate formaldehyde from the periodate oxidation, indicate that the molecular size of the polysaccharide is larger than Fig. 8 would suggest. These problems must be left for a future research.

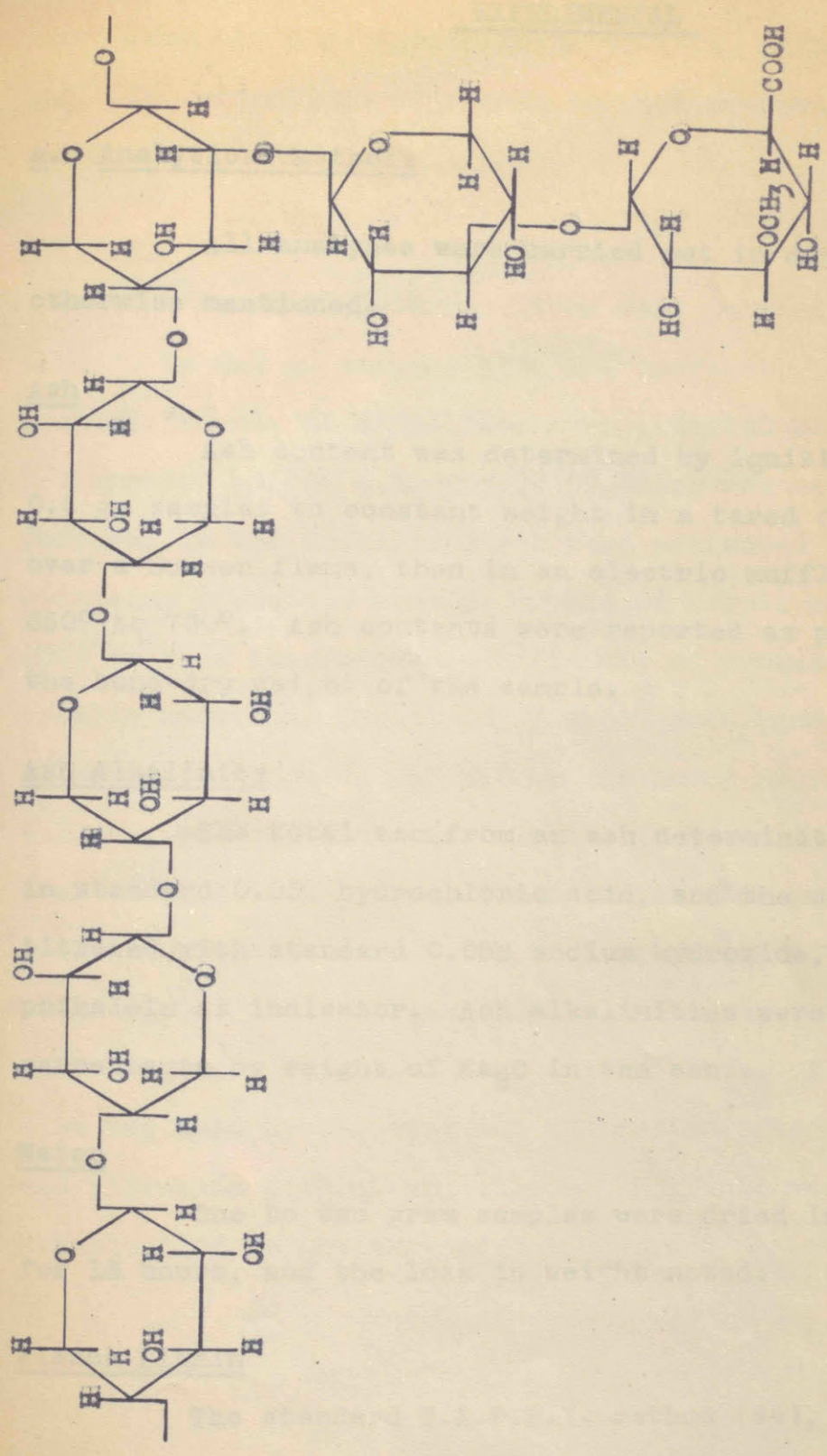


Fig. 8. TENTATIVE STRUCTURE FOR REPEATING UNIT OF MAPLE POLYSACCHARIDE.

EXPERIMENTAL

A. Analytical Methods

All analyses were carried out in duplicate unless otherwise mentioned.

Ash

Ash content was determined by ignition of 0.1 g. to 0.5 g. samples to constant weight in a tared crucible, first over a Bunsen flame, then in an electric muffle furnace at 650° to 700°. Ash contents were reported as percentages of the bone-dry weight of the sample.

Ash Alkalinity

The total ash from an ash determination was dissolved in standard 0.05N hydrochloric acid, and the solution was back-titrated with standard 0.05N sodium hydroxide, using phenolphthalein as indicator. Ash alkalinities were calculated as percentages by weight of Na_2O in the ash.

Water

One to two gram samples were dried in an oven at 105° for 16 hours, and the loss in weight noted.

Klason Lignin

The standard T.A.P.P.I. method (94), which specifies a digestion of 2 hours at 18° to 20° with 72% sulphuric acid,

was used. The sample weight for these determinations was reduced from the 2 g. specified in official method to 0.2 g. to 1 g. No corrections were made for ash content of either the sample or of the Klason lignin.

Furfural

Furfural determinations were carried out by distilling 0.06 g. to 0.1 g. samples with 12% hydrochloric acid, and collecting 360 ml. of distillate over a period of 2 hours, as recommended by the U.S. Forest Products Laboratory (95). The furfural in the distillate was then estimated by the volumetric potassium bromate - bromide method of Powell and Whittaker (96) (97) at room temperature. Fifty ml. of potassium bromate - bromide solution, containing 3 g. of potassium bromate and 50 g. of potassium bromide per litre, was added to the entire distillate, and the whole was allowed to stand in the dark for 1 hour at room temperature, during which time each mole of furfural present should consume 2 moles of bromine. After the addition of 10 ml. of 10% potassium iodide solution, the liberated iodine was titrated with 0.1N sodium thiosulphate. A blank determination was made by carrying out the entire process of distillation and titration without any sample. Furfural was calculated by substitution in the expression:

$$\% \text{ Furfural} = \frac{(v_2 - v_1) \times N \times 0.024 \times 100}{w}$$

where v_2 = ml. of thiosulphate in the blank determination,

v_1 = ml. of thiosulphate in test determination,

N = normality of thiosulphate,

and w = weight of sample in grams.

The factor 0.024 is the weight of furfural in grams equivalent to 1 ml. of N thiosulphate.

To convert furfural to xylan, corrections were applied to allow for the yield of furfural from the uronic anhydride present in the sample, and for the lower than theoretical yield of furfural from xylose. Norris and Resch (98) reported a furfural yield of 21.5% by weight from glucuronic acid, and it is generally accepted that xylose yields 88% of the theoretical quantity of furfural in the standard method used. The xylan content was therefore calculated from the weight of furfural by the following expression:

$$\% \text{ Xylan} = \frac{F - 0.215\ U}{0.73 \times 0.88} \quad ,$$

where F = % furfural,

and U = uronic anhydride content.

The factor 0.73 is the theoretical conversion factor for pentosan ($C_5H_8O_4$) to furfural ($C_5H_4O_2$).

Uronic Anhydride

The method of McCready, Swenson and Maclay was used (99). In this method 0.07 g. to 0.14 g. samples were heated with 19% hydrochloric acid at 145° 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 0.25N sodium hydroxide, which was titrated with 0.1N hydrochloric acid, after precipitation of the carbonate as barium carbonate. A blank determination was also performed on the reagents, without added sample. The uronic anhydride carbon dioxide was calculated by substitution in the expression:

$$CO_2 = \frac{(v_2 - v_1) \times N \times 0.022}{w} ,$$

where v_2 = ml. of hydrochloric acid in titration of blank determination,

v_1 = ml. of hydrochloric acid in titration of test determination,

N = normality of hydrochloric acid,

and w = weight of sample, in grams.

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

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

Methoxyl

Clark's modification (100) of the Viebock and Schwappach method (101) was used, but the customary water trap used as a scrubber was replaced by a 1:1 mixture of 5% aqueous sodium thiosulphate and 5% aqueous cadmium sulphate, as recommended by Friedrich (102).

Acetyl

(a) Acetyl in wood was determined by hydrolysing the sample with 72% sulphuric acid, and separating the acetic acid liberated in the reaction mixture by steam distillation, and by titrating the distillate with standard alkali. Lemieux's semi-micro adaptation (103) of the method of Genung and Mallatt (104) 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 (105). In this method 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 0.02N barium hydroxide to a pH of 7.0. A complete blank was carried out, and the acetyl content calculated from the difference in titre between the distillate and the blank.

Nitrogen

Nitrogen was determined on the semi-micro scale by an adaptation of the Gunning method (106). The sample, 0.2 g. to 0.3 g., was weighed into a Kjeldahl flask, together with 0.6 g.

of potassium sulphate and 0.1 g. of copper sulphate. Three ml. of concentrated sulphuric acid was added, and the sample was digested in the usual manner until the liquid was colourless or had only a faint colour of the cupric ion. The ammonia was distilled into 5 ml. of 2% boric acid, and titrated with standard 0.01N hydrochloric acid, as described by Ma and Zuazaga (107), using a methyl red - bromcresol green mixed indicator. The reagent blank, as determined in a complete run without sample, was 0.15 ml. of 0.01N hydrochloric acid.

Copper Reducing Power

Somogyi's modification (108) of the Shaffer-Hartmann method (109) was used to determine reducing power of the solution of the polysaccharide during hydrolysis. One ml. of the hydrolysate was neutralised with N sodium hydroxide, and the volume adjusted to 10 ml. with distilled water. Two ml. of this neutral, diluted solution and 3 ml. of distilled water were then heated at 100° with 5 ml. of the copper solution for 30 minutes, after which the solution was cooled to 30° and the cuprous oxide determined iodometrically in the usual manner.

B. Extractions of Maple Wood

The wood meal was obtained from the same log of sugar maple (Acer saccharum, Marsh) as used by M.M. Yan in his doctoral research (1). The log was cut about 4 feet from the ground from a tree about 90 years old. It was a solid specimen, free from rot and other visible imperfections, was 4 feet long, and had a

thickness of 9 inches, 4 inches of which was heartwood. The investigation was carried out on the whole log, no separation being made into heartwood and sapwood. The log was chipped, shredded, air-dried and then ground in a Wiley mill until all the meal passed a 40-mesh screen. The portion passing 80 mesh was discarded, and the 40-to 80-mesh portion, amounting to some 73% of the total, was retained for use. Three kg. of this meal was extracted in 500 g. portions successively with water, ether and ethanol at room temperature. These treatments, based on those of Brauns for spruce wood (110), were adopted in the hope of recovering Brauns' "native" lignin. Each 500 g. batch was treated in the following manner:-

(a) Three extractions, each for 4 days with 3 kg. of distilled water. The extract was discarded, and the meal was then air-dried.

(b) Two extractions, each for 3 days with 2.5 kg. of ether. Evaporation of the total ether extracts yielded 6 g. of a red brown, semi-solid resin, which gave a negative Maüle colour reaction (111), specific for angiosperm lignin (112)(113).

(c) Two extractions, each for 7 days with 2.5 kg. of ethanol. Evaporation of the solvent from the total ethanol extracts yielded 19.6 g. of dark resinous material, which gave a very indistinct Maüle reaction. The meal therefore contained very little or no "native" lignin.

The meal was then air-dried to remove all traces of solvent, and extracted with liquid ammonia. This extraction was done in three 900 g. batches in a stainless steel bomb. The bomb had a volume of 11 litres, and consisted of a pipe 48 inches long and 6.5 inches diameter, sealed at one end and threaded on the outside of the open end. This thread engaged a similar thread on the inside of a heavy top. The top carried 8 bolts which, when screwed down through the top, pressed a separate heavy metal lid against the body of the bomb to produce a tight, lead-gasketted closure. This lid was pierced by two openings connected to 1/4-inch steel needle valves serving as inlet and outlet tubes for the liquid ammonia. The ends of the inlet and outlet tubes opening into the bomb were both covered by a disc of 60-mesh steel screen, to prevent the loss of wood meal during filling or emptying of the bomb.

The bomb, charged with 1 kg. of meal, was sealed and cooled to about -10° by leaving outside overnight. It was then set on a heavy-capacity platform scale, the inlet connected to a cylinder of ammonia inverted to deliver the liquid, and a tube from the outlet was led to an efficient fume cupboard. After weighing the bomb to the nearest 1/8 of a kg., both valves were opened and liquid ammonia was run in slowly. The first portions of ammonia entering the bomb produced a rush of gaseous ammonia on encountering the insufficiently cooled meal and metal walls. This evaporation soon cooled the bomb and its contents so that the rate of liquid ammonia addition could be increased, and the

bomb was charged with 5 kg. of ammonia in less than 1 hour. Five kg. of ammonia was used for the first extractions, as Yan had found this quantity sufficient to cover completely 1 kg. of meal in the bomb during the extraction. An optimum extraction time of 5 hours at room temperature had been determined by Yan, so these conditions were adopted in the present investigation.

As soon as the bomb had been charged with the ammonia, the cylinder valve, inlet valve and outlet valve were closed in that order, the bomb was warmed to room temperature by immersion in warm water, and then shaken in the cradle of an hydrogenation apparatus for 5 hours, the temperature of the hydrogenation room being approximately 25°.

At the end of 5 hours the bomb was removed from the shaker, and placed in an inverted position on a stout tripod stand in the fume cupboard. The outlet tube was connected by a short length of thick-walled rubber tubing to a 12-litre Pyrex flask carrying a rubber stopper fitted with glass inlet and outlet tubes, the inlet tube projecting about 5 inches further into the flask than the outlet tube. The outlet valve on the bomb was then carefully opened, whereupon the pressure of the ammonia forced the liquid ammonia extract into the flask. The rate of flow was carefully controlled by the outlet valve on the bomb, so that the flow was never fast enough to blow any of the extract out of the receiver. Within 10 minutes the flow of ammonia had decreased to a slow drip, the outlet valve was

closed and the bomb allowed to stand for 10 minutes to permit the ammonia to drain to the bottom of the bomb, after which it was run off as before. As Yan had recommended, the bomb was then charged with a further 2.5 kg. of ammonia, shaken for a half-hour and the liquid ammonia run out as just described.

The wood meal was then removed from the bomb and allowed to stand in the air until all ammonia had evaporated. The entire liquid ammonia extract from all the extractions was collected in the same 12-litre flask, whose inlet and outlet tubes were both protected from atmospheric moisture and carbon dioxide by guard tubes containing solid potassium hydroxide. The ammonia was allowed to evaporate freely at room temperature, the bulk of the ammonia from one batch evaporating in 24 hours, leaving ample room for the addition of ammonia from the succeeding extraction.

Removal of the residual ammonia under vacuum from the extract left a crystal-studded, red brown, semi-solid resin. Fractionation by Yan's procedure yielded the same fractions as he obtained; acetamide, lignin and a water-insoluble polysaccharide. The yields of the last two fractions, 1.1% and 1.2% respectively, were some 50% higher than the 0.7% and 0.8% reported by Yan.

The ammonia-extracted wood meal, 2.25 kg., together with some 2.25 kg. of meal extracted by Yan, was then extracted in 300 g. portions with ethanol in a Soxhlet extractor. A total of 4.5 kg. of meal yielded 41 g. of dark brown resinous material,

which gave a positive "Maule reaction for angiosperm lignin. The liquid ammonia treatment had therefore rendered extractable with ethanol a lignin fraction, 0.8% of the original wood and presumably related to "native" lignin, that was not extractable from the original wood.

The meal was once again air-dried to remove alcohol, and was then extracted in 500 g. batches with water at 95° to 97°. Each batch was extracted three times with about 3 litres of water for 2 hours. The combined extracts from each batch were centrifuged to remove any fine wood meal, and were concentrated under vacuum to about 750 ml. Two volumes of ethanol were then added, causing the precipitation of a pale grey powder. The precipitate was removed by filtration, solvent-exchanged through methanol and ether, and finally dried in vacuum over phosphoric anhydride at 50°. The total yield of this grey powder from 4.5 kg. of meal was 91.5 g., or 1.9% of the original wood. Evaporation of the combined mother liquors from this precipitate yielded 11.4 g. of a pale brown solid.

C. Examination of Wood Meal at Various Stages of Extraction

Samples of the wood meal, retained at four stages of the extraction process were then analysed for Klason lignin, acetyl and nitrogen content.

TABLE VI

Analytical Data for Maple Meal

A; Original wood, 40-80 mesh.
B; A, after water, ether and ethanol.
C; B, after liquid ammonia.
D; C, after ethanol and water.

Sample	Water %	Klason Lignin %(a)	Acetyl %(a)	Nitrogen %(a)
A	7.3	23.52	4.05	0.09
B	7.7	23.49	3.92	0.09
C	4.9	22.41	0.47	0.35
D	5.7	22.07	0.46	0.36

(a) Based on bone-dry material.

The increase in nitrogen content in samples C and D was investigated by distilling separate 0.5 g. samples with 20 ml. of 0.001N sodium hydroxide, with 20 ml. of distilled water, and with 20 ml. of water containing 0.3 g. of magnesium oxide. In each case 10 ml. was distilled into 20 ml. of 0.01N hydrochloric acid, and the whole titrated with 0.01N sodium hydroxide, in the presence of methyl red as indicator.

The failure of magnesium hydroxide to displace ammonia suggested that ammonium salts were absent. Caustic soda therefore was probably displacing amide nitrogen. It was noteworthy that the alkali failed to remove 0.10% (0.35 - 0.25)

TABLE VII

Differentiation between Ammonium and Amide Nitrogen

Sample	Nitrogen, % on bone-dry weight		
	NaOH Distillation	Water Distillation	$Mg(OH)_2$ Distillation
C	0.25	0.01	0.01
D	0.25	0.01	0.01

and 0.11% (0.36 - 0.25) of nitrogen from C and D respectively. These amounts check well with the 0.09% of nitrogen in the original wood (Table VI) which probably contained traces of proteins.

D. Attempted Purification of the Water Extract from Ammonia-extracted Maple Meal.

The water-soluble, alcohol-insoluble, pale grey powder, 91.5 g., was redissolved in the minimum quantity of hot water, and reprecipitated by the addition of 2 volumes of ethanol. The precipitate was removed by filtration, solvent-exchanged through methanol and ether, and dried in vacuum over phosphoric anhydride at 50°. The yield was 85.4 g. of pale grey powder, fraction A (Fig. 2). The material was nitrogen-free, non-reducing to Fehling's solution, and gave a very faint positive naphtho-resorcin test for uronic acids.

Analysis: Klason lignin 0.79, 0.83%; Methoxyl 3.85, 3.89%; Ash 3.0, 3.0%; Ash Alkalinity 56.6, 56.9%.

Fraction A was then given two similar reprecitations, yielding 76.8 g. of an almost white powder, fraction C (Fig. 2).

Analysis: Klason lignin 0.49, 0.51%; Methoxyl 2.15, 2.17%; Ash 2.3, 2.4%; Ash Alkalinity 65.5, 65.6%.

The mother liquor from fraction A and the two mother liquors from fraction C were united and evaporated to dryness under vacuum, yielding 12.9 g. of a pale brown powder, fraction B (Fig. 2).

Analysis: Methoxyl 2.09, 2.03%; Furfural 37.2, 37.3%; Ash 5.3, 5.4%; Ash Alkalinity 16.2, 16.2%.

Although the material was being purified by solution in water, the solution was not perfect, being too cloudy to permit observation of optical activity. Addition of 0.1N alkali, although it cleared the solution somewhat, did not cause sufficient clarification to make polarimetric readings possible. A small quantity, about 0.5 g., of fraction C was dissolved in 15 ml. of hot water, and 0.5 g. of absorbent carbon was added. After filtration, the solution was perfectly clear, and an attempt was therefore made to purify the whole of fraction C in this manner. The fraction, 75 g., was dissolved in 3 litres of hot water, and 25 g. of absorbent carbon was added. Filtration was extremely difficult, even with the aid of Supercel, and was only achieved on dilution with an additional 8 litres

of water, and even then by filtering while hot. The filtrate was still cloudy, and measurement of the optical activity of this material was never realised. The filtrate was evaporated under vacuum to about 2 litres, and addition of 2 volumes of ethanol caused precipitation. Isolation of this precipitate in the usual manner yielded 28.9 g. of very pale grey powder, fraction D (Fig. 2).

Analysis: Methoxyl 2.0, 2.1%; Furfural 44.4, 44.6%; Ash 3.0, 2.9%; Ash Alkalinity 64.1, 64.3%.

Evaporation of the mother liquor from fraction D yielded 5.1 g. of a white powder, fraction E (Fig. 2).

Analysis: Methoxyl 1.8, 1.9%; Furfural 46.6, 46.7%; Ash 3.2, 3.2%.

It will be seen that only 35 g. of the original 75 g. of fraction C had been recovered. It was obvious that the residue, 40 g., had been adsorbed on the carbon. The carbon and Supercel pad was then extracted with 1 litre of cold 1% caustic soda. Filtration was again very slow, but no resort was made to dilution and heat, and eventually the operation was completed. The filtrate was neutralised with dilute hydrochloric acid, and addition of 2 volumes of ethanol caused the precipitation of 28.1 g. of pale grey powder, fraction F (Fig. 2).

Analysis: Methoxyl 1.6, 1.7%; Furfural 35.8, 35.6%; Ash 26.2, 26.1%; Ash Alkalinity 7.6, 7.7%.

Evaporation of the mother liquor from fraction F yielded 4.2 g. of white powder, fraction G (Fig. 2).

Analysis: Methoxyl 1.7, 1.8%; Furfural 37.0, 37.1%; Ash 25.9, 26.0%.

Fraction D was then dissolved in water and reprecipitated with 2 volumes of ethanol, yielding 23.9 g. of pale grey powder, fraction H (Fig. 2).

Analysis: Uronic Acid Carbon Dioxide 8.4, 8.45%, giving Uronic Anhydride 33.6, 33.8%; Furfural 46.7, 46.8%, giving Xylan 61.3%; Methoxyl 2.3, 2.3%; Ash 2.9, 2.9%; Ash Alkalinity 67.2, 67.2%.

Fraction J, (Fig. 2), 4.1 g. of white powder, was obtained by evaporation of the mother liquor from fraction H.

The free carboxyl value of fraction H was determined by the calcium acetate method of Meesook and Purves (87). A value equivalent to 1.7% of uronic anhydride was obtained, compared to 33.7% of total uronic anhydride by the carbon dioxide method. To investigate the discrepancy about 1 g. of fraction H was extracted with 98% acetic acid at room temperature. In each washing the sample was well stirred with 25 ml. of the acetic acid in a centrifuge tube. After standing for several hours the suspension was centrifuged and the supernatant liquid decanted. At the completion of 6 such washings the sample was dissolved in water, and reprecipitated with 10 volumes of alcohol, yielding a very pale grey powder, fraction K (Fig. 2).

Analysis: Ash 1.6, 1.6%; Ash Alkalinity 20.2, 20.1%.

A free carboxyl determination by the calcium acetate method now gave a value equivalent to 13.1% uronic anhydride. This experiment showed the unexpected strength of the "uronic" carboxylic ion, which competed successfully with acetic acid for the base. No attempt was made to continue the "de-ashing" with dilute mineral acid for fear of degrading the polysaccharide.

A sample of fraction H, 19 g. was given 6 successive reprecipitations from aqueous ethanol to test its homogeneity. The final precipitate, fraction X (Fig. 2) was a very pale grey powder, 11.6 g.

Analysis: Uronic Acid Carbon Dioxide 10.45, 10.5%, giving Uronic Anhydride 41.8, 42.0%; Furfural 40.3, 40.5%, giving Xylan 48.7%; Methoxyl 1.8, 1.9%; Ash 3.3, 3.4%; Ash Alkalinity 83.1, 83.2%.

Evaporation of the combined mother liquors from fraction X, yielded 6.6 g. of a white powder, fraction Y (Fig. 2).

Analysis: Uronic Acid Carbon Dioxide 5.7, 5.7%, giving Uronic Anhydride 22.7, 22.8%; Furfural 51.5, 51.7%, giving Xylan 72.4%; Methoxyl 2.8, 2.8%; Ash 2.6, 2.6%; Ash Alkalinity 73.0, 73.1%.

At this stage all of fraction X but 4 g. was accidentally lost. Since the intensive reprecipitations seemed to be accomplishing a fractionation only very slowly, it was decided to unite fractions E, F, G and J. The mixture was given 2 reprecipitations from 70% ethanol, yielding 24.8 g. of pale grey powder, fraction M (Fig. 2).

Analysis: Uronic Acid Carbon Dioxide 5.45, 5.45%, giving Uronic Anhydride 21.8, 21.7%; Furfural 40.5, 40.4%, giving Xylan 55.5%; Methoxyl 2.1, 2.1%; Ash 15.9, 15.7%; Ash Alkalinity 7.9, 7.9%.

Fraction M was then used for the main study, together with fractions X and Y.

E. Acetylation of Certain Polysaccharide Fractions

Three fractions, X (uronic rich), Y (uronic poor) and M (intermediate), were acetylated by the pyridine, acetic anhydride method. In each case 1 to 1.3 g. of material was swollen in 20 ml. of anhydrous pyridine for 24 hours at room temperature. Twenty ml. of freshly distilled acetic anhydride was then added, and the mixture again stood for 24 hours at room temperature. The acetylation mixture was then heated for 4 hours on the steam-bath, finally poured into 400 ml. of ice-water and allowed to stand overnight. The insoluble residue was removed by filtration, and extracted with acetone. Recovery of acetone-soluble material was effected by pouring a concentrated acetone solution into excess water, a procedure which yielded the acetate as a white, horny powder after drying in vacuum over phosphoric anhydride at 50°.

Any acetone-insoluble, water-insoluble material was collected as a separate fraction, and a further fraction was obtained by evaporation under vacuum of the water into which

the acetylation mixture was poured.

Fraction X

A 1.033 g. sample, on acetylation by the method described above, yielded 0.791 g. of a white acetate, soluble in acetone or chloroform, but insoluble in alcohol or water. $[\alpha]_D^{20}$ - 60.6°, in chloroform, (C = 0.91).

Analysis: Uronic Acid Carbon Dioxide 2.8, 2.85%, giving Uronic Anhydride 11.3, 11.4%; Xylan 51.0, 51.2%; Methoxyl 2.0, 2.0%; Acetyl 36.6, 36.4%; Ash 0.2, 0.2%.

Calculated for a fully acetylated 6:1 xylan to methoxyglucuronic anhydride polysaccharide: Uronic Anhydride 11.5%; Xylan 51.8%; Methoxyl 2.0%; Acetyl 36.6%.

No water-insoluble, acetone-insoluble fraction was obtained. A grey powder, 0.4 g., was obtained on evaporation of the aqueous residue from the acetylation.

Analysis: Uronic Anhydride 61.1, 61.3%; Xylan 1.7, 1.9%; Acetyl 25.9, 25.8%; Ash 10.1%.

Fraction Y

A 1.283 g. sample, when acetylated as above, yielded 1.513 g. of a white acetate, insoluble in alcohol or water, but soluble in acetone or chloroform. $[\alpha]_D^{20}$ -61.9° in chloroform, (C = 1.1).

Analysis: Uronic Acid Carbon Dioxide 2.85, 2.8%, giving Uronic Anhydride 11.5, 11.3%; Xylan 51.6, 51.7%; Methoxyl 2.0, 2.0%; Acetyl 36.4, 36.6%; Ash 0.1, 0.1%.

No water-insoluble, acetone-insoluble product was obtained. Evaporation of the aqueous solution of the acetylation mixture yielded 0.15 g. of grey powder.

Analysis: Uronic Anhydride 54.9%; Xylan 2.5%; Acetyl 24.8%; Ash 21.2%.

Fraction M

This fraction, 1.108 g., on acetylation, yielded 0.754 g. of a white acetate, soluble in acetone or chloroform, but insoluble in alcohol or water. $[\alpha]_D^{20}$ -59.8° in chloroform, (C = 1.0).

Analysis: Uronic Acid Carbon Dioxide, 2.8, 2.8%, giving Uronic Anhydride 11.3, 11.2%; Xylan 51.6, 51.7%; Methoxyl 1.9, 2.0%; Acetyl 36.5, 36.3%; Ash 0.3, 0.3%.

A pale grey product, 0.399 g., insoluble in water or acetone, was also obtained.

Analysis: Uronic Anhydride 10.3%; Xylan 45.9, 46.0%; Methoxyl 1.7, 1.7%; Acetyl 29.7, 29.6%; Ash 12.2%.

Re-acetylation failed to raise the acetyl content and reduce the ash content. It was also not found possible to reduce the

ash content appreciably, by treatment with N hydrochloric acid at 0°. The ash therefore was presumably not derived from an alkali or alkaline earth metal, but its exact nature was not determined.

Evaporation of the aqueous solution of the acetylation mixture yielded 0.329 g. of grey powder.

Analysis: Uronic Anhydride 32.9%; Xylan 1.2%; Acetyl 13.1%; Ash 47.4%.

F. Acid Hydrolysis of Fraction M

A portion of fraction M, 3.383 g. (ash-free weight), was heated on the steam-bath with 250 ml. of 3% sulphuric acid. The course of the hydrolysis was followed by withdrawing 1 ml. samples at regular intervals, and determining the copper reducing power.

The reducing power reached a maximum after 180 minutes' hydrolysis (Table VIII and Fig. 3), the maximum value being equivalent to 2.156 g. or 63.5% of xylose.

The solution was filtered from the undissolved debris, which consisted of 0.11 g. of dark material. Five ml. of the filtrate was diluted with water and neutralised with dilute sodium hydroxide. After addition of 0.2 g. of fresh brewer's yeast, the volume was adjusted to 100 ml. and the copper reducing power was measured. The value was unchanged after 48 hours' incubation at 35°. On the other hand, 0.2 g. of the same sample

TABLE VIII

Reducing Power of Hydrolysate of Fraction M

Time Minutes	Titre ml. of 0.005N thiosulphate	Apparent Xylose % of original sample
0	23.4	...
30	18.2	23.6
60	13.4	44.3
90	10.6	56.2
120	9.8	59.9
180	9.0	63.2
240	8.85	63.7
300	8.9	63.5

of yeast completely fermented 2.0 g. of glucose in less than 12 hours. Glucose, mannose and fructose were therefore absent from the hydrolysate.

The remainder of the filtered hydrolysate was then neutralised with powdered barium carbonate, and the precipitated barium sulphate removed by filtration. The filtrate was carefully concentrated under vacuum to approximately 25 ml., and addition of ethanol to a concentration of about 90% precipitated 0.12 g. of light brown material. This precipitate was oxidised by nitric acid, sp. gr. 1.15, following the Wise and Peterson modification (39) of the Van der Haar method (114) for the determination of galactose. A yield of 0.035 g. of mucic acid, m.p. 217°, mixed m.p. with authentic mucic acid, 217°, was obtained.

The filtrate from the above precipitate was evaporated to dryness under vacuum, yielding 2.806 g. of pale yellow solid material, which partly crystallised on standing. However complete crystallisation could not be induced, and the crystals could not be separated from the resinous residue.

$[\alpha]_D^{20}$ $+34.5^\circ$ in water, final value, (C = 4.1).

Analysis: Uronic Anhydride 14.9, 15.0%; Furfural 49.5, 49.6%; Ash 9.5, 9.5%; Ash Alkalinity 40.9, 41.0%.

A portion of the material was analysed for xylose by the method of Breddy and Jones (115). An 0.369 g. sample yielded 0.318 g. of white precipitate, m.p. 210°, $[\alpha]_D^{20}$ - 8.5° in chloroform, (C = 1.1), mixed m.p. with authentic dimethyl acetal of dibenzylidine-D-xylose 211°. This quantity of acetal was equivalent to 0.153 g. of xylose, or to 56.3% by weight of xylose in the sample. Tests on 0.3 g. samples for arabinose, rhamnose and galactose by the method of Hirst, Jones and Woods (116) were completely negative.

G. Oxidations with Sodium Metaperiodate

A solution of sodium metaperiodate, approximately 0.2M, was made by dissolving 4 g. of the salt in 100 ml. of carbon dioxide - free distilled water. The strength of the solution was accurately determined by the arsenite-iodine titration method of Fleury and Lange (117).

The sample, approximately 0.3 g., was dissolved in 50 ml. of carbon dioxide - free distilled water in a carefully steamed-out 100 ml. volumetric flask; 25 ml. of the periodate was added, and the mixture was made up to the mark with carbon dioxide - free water. The amount of periodate was roughly equivalent to 2 moles per sugar unit in an 0.3 g. sample. The pH of the mixture was measured immediately. At regular intervals a 5 ml. sample of the solution was withdrawn and added to 10 ml. of 0.1N sodium arsenite (containing 20 g. per litre of sodium bicarbonate), and 1.5 g. of sodium bicarbonate, and 1 ml. of 20% potassium iodide solution were added. After standing for 20 minutes the mixture was titrated with 0.1N iodine solution. A test of the periodate solution without added sample showed that there was no observable decomposition during the period of oxidation. Formic acid production was measured by withdrawing 10 ml. samples and titrating back to the original pH of the solution with 0.01N potassium hydroxide solution.

No formaldehyde was detected by the dimedon reagent (118) in the oxidised solutions of any of the fractions tested.

Fraction M

Ash-free weight, 0.343 g.
Periodate solution = 0.0455M
Iodine solution = 0.09902N
Iodine titre of blank = 5.50 ml.
Potassium Hydroxide solution = 0.01033N

TABLE IX

Oxidation of Fraction M
with Sodium Metaperiodate

Time hours	Iodine Titre ml.	pH	KOH Titre ml.
0	...	4.02	...
1	6.45	...	...
2	6.65	...	...
3	...	3.79	0.51
4	6.90	...	...
6	7.00	3.70	0.77
8	7.10	...	...
11	7.15	3.64	0.90
24	7.25	3.62	1.12
48	7.45	3.57	1.40

Fraction X

Ash-free weight, 0.2768 g.
Periodate solution = 0.0421M
Iodine solution = 0.1043N
Iodine titre of blank = 5.55ml.
Potassium hydroxide solution = 0.01033N

TABLE X

Oxidation of Fraction X
with Sodium Metaperiodate

Time hours	Iodine Titre ml.	pH	KOH Titre ml.
0	...	4.71	...
1	6.50	...	...
2	6.60	...	...
3	...	3.91	1.01
4	6.77	...	...
6	6.88	3.81	1.27
8	6.95	...	...
11	7.05	3.77	1.50
24	7.20	3.68	2.03
48	7.31	3.52	2.73

Fraction Y

Ash-free weight, 0.2602 g.
Periodate solution = 0.0421M
Iodine solution = 0.1043N
Iodine titre of blank = 5.55 ml.
Potassium Hydroxide solution = 0.01033N

TABLE XI

Oxidation of Fraction Y
with Sodium Metaperiodate

Time hours	Iodine Titre ml.	pH	KOH Titre ml.
0	...	5.22	...
1	6.32	...	...
2	6.35	...	...
3	...	4.18	0.60
4	6.54	...	...
6	6.62	4.09	0.72
8	6.71	...	...
11	6.75	4.02	0.83
24	6.88	3.92	1.08
48	6.94	3.79	1.39

H. Simultaneous Hydrolysis and Oxidation with Bromine - Hydrobromic Acid.

A sample of fraction X, 1.174 g. (ash-free weight), was oxidised by the method of Heidelberger and Goebel (80). The material was heated under reflux for 24 hours with 50 ml. of N hydrobromic acid containing 0.5 ml. of bromine. The bromine was replaced from time to time as it was removed by vaporisation.

The solution was then evaporated under vacuum to remove bromine and hydrobromic acid, and was finally evaporated to a brown liquid, about 3 ml., in a vacuum desiccator, over potassium hydroxide pellets. This liquid was placed in a room held at 8° for 24 hours, and then 0.315 g. of white crystals was removed by filtration. The crystals had m.p. 216°, mixed m.p. with authentic mucic acid, 217°.

The mother liquor was neutralised with potassium hydroxide, and concentrated under vacuum to a thick crystal-studded syrup. The syrup was acidified with a drop of concentrated hydrochloric acid, and extracted at room temperature with 95% ethanol. The extract was neutralised to pH 6 with 10% potassium hydroxide, filtered, and concentrated under vacuum to a thick syrup. The syrup was extracted with 10 ml. of absolute ethanol, filtered, and the filtrate again concentrated under vacuum to a thick syrup. This syrup was dissolved in 1 ml. of water, neutralised with powdered potassium carbonate, and 1 ml. of glacial acetic acid was added (119). No crystals

formed even after 72 hours' standing at 8°, and the only crystalline material obtained on concentration was potassium acetate, the oxidation product remaining syrupy. A trial with 0.04 g. of authentic saccharic acid showed that the above procedure would probably have caused any of this substance present in the syrup to crystallise.

J. Methylation of Fraction M

A sample of fraction M, 3.306 g. (corr. for ash), was methylated by the thallous hydroxide, thallous ethylate, methyl iodide technique (120). The ethylate, 71 g. crystalline at 0°, was prepared by the method of Assaf, Haas and Purves (121), and a 25 g. portion was dissolved in 100 ml. of pure, dry benzene, to give a stable N solution. The remainder of the thallous ethylate was redissolved in ethanol, poured into excess water, and the solution distilled in the dark under reduced pressure to a volume of 150 ml., by which time all of the ethanol had been removed. A 100 ml. portion of the thallous hydroxide solution so produced, was concentrated in the same manner to 30 ml., when it had a concentration of approximately 4N. The addition of 3.306 g. of fraction M formed a heavy grey precipitate immediately; this precipitate was removed by filtration, and dried at 35° in vacuum over phosphoric anhydride for 16 hours. The 10.3 g. of pale yellow solid which resulted was ground to pass a 120-mesh screen and heated under reflux with 25 ml. of freshly distilled methyl iodide for 18 hours. Excess methyl iodide was removed by distillation, the remaining 50 ml. of the thallous

hydroxide solution was added, and the whole was evaporated to dryness at 50° under 15 mm. pressure. The 19.3 g. of yellow residue was powdered to pass a 120-mesh screen and again methylated for 24 hours with 25 ml. of methyl iodide. After removing the excess methyl iodide by distillation, 20 ml. of anhydrous ethanol and 50 ml. of N thallous ethylate in benzene were added. Solvent was removed under vacuum, and the residue dried for 16 hours at 35° over phosphoric anhydride under vacuum. The dried material, 33.9 g., was ground to pass 120 mesh, and was once more heated under reflux for 24 hours with 30 ml. of methyl iodide. Methylation was then judged to be complete, and the residue from the methyl iodide was extracted exhaustively with acetone and then with chloroform. The solvent was evaporated from the combined acetone and chloroform extracts, yielding 2.65 g. of a pale yellow resin which had a methoxyl content of 39.0%. The residue from the solvent extractions was then extracted with hot water, the aqueous extract concentrated, and added to the pale yellow solvent-soluble resin. Once again the combined product was evaporated to dryness under vacuum and 50 ml. of N thallous ethylate in benzene was added, and the methylation was completed with 25 ml. of freshly distilled methyl iodide. Exhaustive extraction of the product with chloroform recovered 2.75 g. of very pale yellow resin.

Analysis: Uronic Anhydride 15.0, 15.0%; Methoxyl 39.3, 39.4%; Ash 0.1%.

Calculated for a fully methylated 6:1 xylan to glucuronic methyl ester: Uronic Anhydride 14.9%; Methoxyl 39.5%.

The residue from the chloroform extraction was re-extracted with hot water, and removal of the water under vacuum from this extract yielded 0.38 g. of a grey powder.

Analysis: Uronic Anhydride 51.4, 51.5%; Methoxyl 28.6, 28.7%; Ash 20.1, 20.2%.

The methylation therefore separated a chloroform-soluble fraction from another much higher in ash and uronic acid, although much lower in methoxyl content.

K. Oxidation of the Methylated Product with Nitric Acid

The remainder of the chloroform-soluble product, 2.29 g., was dissolved in 35 ml. of 32% nitric acid at 90°. The temperature was lowered to 80° when evolution of brown fumes started, and was maintained at that figure for 6 hours. The clear, pale orange solution was then diluted with 2 volumes of water, and the nitric acid removed by continuous distillation at 50° under reduced pressure, 4 litres of water being gradually added, to keep the volume in the distilling flask above 60 ml. (122). The final portion of the distillate had a pH of 6 when tested by pHydrion paper.

Concentration of the oxidised solution yielded a pale yellow syrup which was dehydrated by dissolving in anhydrous methanol and evaporating the solvent. The dehydration was repeated three times, and the residue was then heated under reflux for 8 hours with 35 ml. of anhydrous 3% methanolic hydrogen chloride. After removing the acid with solid silver carbonate,

and evaporating the solution, the residue, together with ether extracts of the precipitated silver salts, was recovered as a pale yellow syrup. This syrup was dissolved in ether, filtered from traces of undissolved inorganic salts, and after removal of the solvent yielded 1.74 g. of an almost colourless, neutral syrup.

Distillation of this syrup at 0.1 mm. pressure yielded

fraction 1, 0.22 g., bath temperature 110° to 129°;
fraction 2, 0.69 g., bath temperature 130° to 145°;
fraction 3, 0.39 g., bath temperature 146° to 160°;
and 0.31 g. of a dark brown still residue.

The fractions were all pale yellow in colour, 1 and 2 being quite mobile, and fraction 3 rather viscous. The last drop of distillate in the side-arm of the distilling flask crystallised.

Fraction 3

These crystals were used to seed this fraction, which partly crystallised on standing for 24 hours. Trituration with a 1:1:2 mixture of ethanol, ether and petroleum ether made it possible to remove 0.12 g. of the crystals by filtration. After one recrystallisation from ether, the crystals melted at 107°, and on admixture with an authentic specimen of 2,3,4-trimethyl-saccharolactone methyl ester, m.p. 109°, (prepared from 2,3,4-trimethyllevoglucosan by oxidation with nitric acid, esterification and distillation) melted at 108°. The mother liquor from the crystals, after removal of the solvent, was dissolved in 5 ml.

of anhydrous methanol, and the solution was saturated with dry gaseous ammonia at 0°. After standing for 2 days at 0°, crystals formed, and these were separated by filtration, 0.07 g. These crystals, after one recrystallisation from water and methanol, melted at 162° (dec.), were optically inactive, and gave a positive Weerman reaction (123).

Analysis: Methoxyl 16.0%; Nitrogen 14.4%.

Calculated for a methylxyloglutardiamide, $C_6H_{12}O_5N_2$: Methoxyl 16.1%; Nitrogen 14.6%.

The mother liquor was evaporated to half its volume and left a further 3 days at 0°. After this time a second crop of crystals had formed, and were separated by filtration, 0.10 g. These crystals, after one recrystallisation from water and methanol, melted at 140°, and also gave a positive Weerman test.

Analysis: Methoxyl 30.0%;

Calculated for a dimethylxyloglutardiamide, $C_7H_{14}O_5N_2$: Methoxyl 30.1%.

Fraction 2

This fraction was triturated with a 1:1:2 mixture of ethanol, ether and petroleum ether, and was left for 24 hours. Crystals separated, and were removed by filtration, 0.03 g. The crystals melted at 106°, and on admixture with authentic 2,3,4-trimethylsaccharolactone methyl ester melted at 108°. The mother liquor from the crystals, after removal of solvent, was dissolved in 8 ml. of anhydrous methanol, and the solution was saturated with dry ammonia gas at 0°. The mixture was kept

at 0° for 3 days, when crystals formed. Filtration separated 0.06 g. of crystals, which melted at 161° (dec.), both before and after admixture with the crystals of m.p. 162° from fraction 3(3-methylxyloglutardiamide). The mother liquor from these crystals was concentrated to half its volume, and left for a further 5 days at 0°, when another 0.39 g. of crystals were separated by filtration. These crystals melted at 140°, both alone and on admixture with the crystals of m.p. 140° from fraction 3(2,3-dimethylxyloglutardiamide), $[\alpha]_D^{20}$ +28° in water, (C = 0.8).

Analysis: Nitrogen 13.5%.

Calculated for a dimethylxyloglutardiamide, $C_7H_{14}O_5N_2$: Nitrogen 13.6%.

Fraction 1

A solution in 3 ml. of anhydrous methanol was saturated with dry ammonia gas at 0°. After standing at 0° for 2 days, 0.04 g. of crystals deposited, and after recovery melted at 270° (darkening at 220°). One recrystallisation from water raised the m.p. to 290°, in agreement with that for D-dimethoxysuccindiamide (93).

Analysis: Methoxyl 36.5%.

Calculated for dimethoxysuccindiamide, $C_6H_{12}O_4N_2$: Methoxyl 35.2%.

The mother liquor, kept at 0° for a further 6 days, deposited another 0.14 g. of crystals melting at 139°. On admixture with the crystals of m.p. 140° from fraction 2, the m.p.

was undepressed at 140°. They therefore constituted another fraction of 2,3-dimethylxyloglutardiamide.

No further crystallisation could be induced in the final mother liquors of any of the three main fractions of distillate. They were combined, and removal of the solvent yielded 0.14 g. of a brown syrup. This syrup, which could not be crystallised from any of the common solvents, gave a negative Weerman test.

Analysis: Methoxyl 29.0%; Nitrogen 13.8%.

Calculated for a dimethylxyloglutardiamide, $C_7H_{14}O_5N_2$:

Methoxyl 30.1%; Nitrogen 13.6%.

The total yields of crystalline products identified from 1.3 g. of distillate were 0.15 g. of 2,3,4-trimethylsaccharolactone methyl ester, 0.13 g. of 3-methylxyloglutardiamide, 0.63 g. of 2,3-dimethylxyloglutardiamide, and 0.04 g. of D-dimethoxysuccindiamide. In addition, 0.14 g. of syrup which had the methoxyl and nitrogen contents of a dimethylxyloglutardiamide, was obtained.

The brown still residue from the distillation of the neutral syrup yielded no crystalline products, either before or after treatment with methanolic ammonia, so was not further examined.

SUMMARY

Liquid ammonia extracted 6.2% by weight from 3 kilograms of sugar maple wood at 25° in 5 hours. The residual wood meal was then extracted with ethanol, yielding a further 0.8% of extract, and finally water at 97° extracted 1.9% of the original wood weight as a pale grey powder. This water-soluble material was found to be a polyuronide composed of xylan and methoxyglucuronic acid units, contaminated with pectic substances. Fractionation showed that except for the varying amount of pectic contaminant the material was homogeneous.

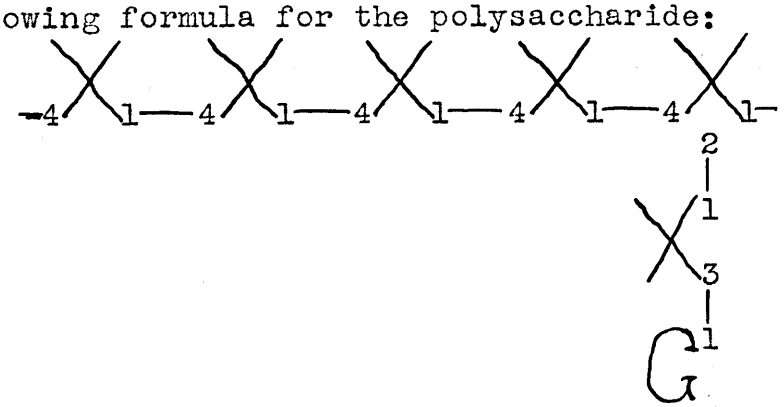
Acetylation and methylation each yielded a fully substituted polysaccharide from which the pectic contaminant could be separated by solubility differences. Analyses of the fully substituted products and of the original material indicated that the latter was based on a repeating unit of 6 xylose residues to 1 methoxyglucuronic anhydride group. Oxidation of the fractionated hemicellulose with bromine - hydrobromic acid yielded enough mucic acid to account semi-quantitatively, as pectic acid, for uronic anhydride in excess of that required for a 6:1 xylose to uronic anhydride ratio.

Hydrolysis of the polysaccharide with 3% sulphuric acid at 100° for 3 hours liberated 4 moles of xylose per repeating unit, and analysis of the hydrolysate indicated the

presence of 1 mole of an aldotriuronic acid per repeating unit.

Oxidation with sodium metaperiodate showed a consumption of 4 moles of periodate per unit of 6 xylose and 1 methoxyglucuronic anhydride residues. The consumption is in agreement with the requirements for a structure such as suggested below.

Oxidation of the fully methylated polysaccharide with nitric acid, followed by esterification of the dibasic acids formed, yielded 1 mole of the crystalline 2,3,4-trimethylsaccharolactone methyl ester. When the remaining diesters were converted to the corresponding diamides, 4 moles of 2,3-dimethylxyloglutardiamide, 1 mole of 3-methylxyloglutardiamide, and dimethoxysuccindiamide were isolated as crystalline products. There was also evidence for the presence of 1 mole of 2,4-dimethylxyloglutardiamide. These data are in agreement with the following formula for the polysaccharide:



where X represents anhydroxylose,
and G represents 3-methylglucuronic anhydride.

CLAIMS TO ORIGINAL RESEARCH

1. M.M. Yan had obtained acetamide, "liquid ammonia lignin", and a polyuronide fraction, in a total yield of 6%, by the extraction of maple wood with liquid ammonia. This result was confirmed in the present investigation.

2. Extraction of maple wood with liquid ammonia at room temperature has been shown to be accompanied by ammonolysis of ester linkages other than those of acetyl groups, since carboxyl groups in the wood substance were changed to amides rather than to ammonium salts.

3. Ethanol extracted 0.8% of a lignin fraction, not previously ethanol-soluble, from the ammonia-extracted wood residue.

4. Hot water extracted polysaccharide in 2% yield from the ammonia-extracted wood residue, together with pectic material as a contaminant.

5. A formula for this polysaccharide was suggested as a result of studies on its acetylation, acid hydrolysis, methylation and oxidation with periodate. The suggested composition was 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-methylglucuronic acid residue.

6. The thallous hydroxide technique of methylation commonly used on gums and mucilages, has been applied to a wood hemicellulose for the first time.

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