A STUDY OF THE HEMICELLULOSE OF MILKWEED FLOSS (*ASCLEPIAS SYRIACA*, L.)

A Thesis

by

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GENERAL INTRODUCTION

Seed-hairs are fibers located on the seeds of various plants, designed by nature to make it possible for the seeds to spread by the wind. One of these seed fibers, cotton, is exceptional in its high content of cellulose and has attained an importance far exceeding that of any other material of its kind. Other sources include kapok, tree cotton, cat-tail floss, milkweed floss and cotton grass. Kapok is a seed fiber, cultivated in the East Indies and is commonly used for upholstery and lifebelts. None of the others have shown much commercial importance.

The subject of the present investigation was the floss of the common milkweed (Asclepias syriaca, L.) which is a fiber of considerable interest, both in itself and because its chemical composition closely resembles that of deciduous woods, in spite of the obvious morphological differences. Milkweeds, some sixty species of which are known on this continent, are tall plants containing a milky juice in all their parts which has given them their name. The common milkweed, which is native to eastern North America, is a perennial plant, multiplying by seeds or creeping roots. It produces purple flowers, usually in July, some of which develop into fruits containing many flat, brown seeds, which are first enclosed by a pod. Each seed has a tuft of silky hairs, the so-called floss, which makes it possible for the

seed to leave the pod when the latter has opened, which usually happens in September.

In earlier studies milkweed floss was found to contain approximately 40% cellulose, 30% hemicellulose, and 15% lignin, in addition to other minor constituents. The cellulose had a very high degree of polymerization, the weight-average value being 10,500, and exhibited a rather unusual right-hand skewness in its molecular weight distribution.

The present study is concerned with the properties, and especially the structure, of the hemicellulose portion, most of which was earlier known to yield xylose and various sugar acids on hydrolysis. This investigation is paralleled by a similar one dealing with another seed-hair, namely kapok. The methods applied were analogous to those presently used for determining the structure of hemicelluloses present in mono- and dicotyledons, the composition of which approaches that of the milkweed floss.

HISTORICAL INTRODUCTION

The chemical composition of the seed-hairs mentioned in the general introduction has so far received little attention; cotton, probably because it is an almost pure cellulose, and the others because of their limited technical importance. Until recently it was generally believed that cotton contained no sugar residues other than glucose, but recent studies have shown that raw cotton is associated with other anhydrosugar units as well (1) which are very difficult to eliminate. Alpha-cellulose, purified by fractionation from a cuprammonium solution of cotton cellulose and treatment with 17.5% sodium hydroxide for removal of any co-precipitated pentosans, still contained small amounts of arabinose and xylose. The presence of the same sugars in a hydrolysate from a cotton alpha-cellulose has also been demonstrated by Das, Mitra and Wareham (2). It has been suggested (1) that since vigorous purification failed to eliminate the above sugars, they might be chemically bound to the cellulose. However, as yet, no oligosaccharides composed of glucose and arabinose or xylose have been isolated and identified from cotton cellulose, which thus leaves the above assumption open to question.

Kapok (Ceiba pentandra) is a seed fiber cultivated in the East Indies and commonly used for upholstery and lifebelts. Unlike cotton, it contains both cellulose, hemi-

cellulose and lignin, namely 40, 30 and 15% respectively (3). The pentosan (24.4%), uronic anhydride (6.6%), and acetyl (8.0%) contents of the fiber are considerable, suggesting the presence of an acidic pentosan containing acetyl ester groups. A later investigation (4), still in progress, has shown that the hemicellulose component in kapok is a methyl glucurono xylan, probably 1,4-β-linked and with every 6th to 7th anhydroxylose unit containing a single side group of 4-O-methyl-D-glucuronic acid.

The molecular properties of the alpha-cellulose component of kapok have been studied by Zapf (5). The native material was delignified with sodium chlorite and subsequently extracted with alkali to yield a "cellulose", which, however, still contained 15% hemicellulose. Viscosity measurements on the nitrate derivative indicated an average degree of polymerization of 1,500. Fractional precipitation resulted in a chain length distribution which contained three maxima and which extended from a D.P. of 70 up to 2,200. Later work suggested that the cellulose in kapok had a weight-average D.P. of approximately 10,500 (3) with a frequency distribution containing only one maximum and exhibiting a slight positive skewness. This distribution is quite different from that reported by Zapf (5). The first two peaks noticed by this worker might have been the result of the presence of both degraded cellulose and low-molecular weight hemicellulose in the nitrated product.

The seed-hairs from Eriophorum wool (Swedish cotton grass) have been investigated by Gralén, Berg and Svedberg (6). These fibers were found to contain approximately 40% cellulose, 30% pentosan, mostly xylan and 3-5% uronic anhydride. Methoxyl groups were also present but no methyl pentoses or (peculiarly enough) lignin. Sedimentation-diffusion measurements on a cuprammonium solution of the alpha-cellulose indicated a molecular weight of 710,000.

Milkweeds belong to the family of Asclepiadaceae (7,8), a distinctly marked group of dicotyledon plants, comprised chiefly of shrubs and wood vines, although many are perennial herbs with a milky juice. The fruit from each of the flowers consists of a pair of pods containing numerous seeds which carry a tuft of hair. Of the representatives native to eastern North America, the most common and conspicuous one is the common milkweed (Asclepias syriaca, L.), which reaches a height of 4-6 feet, having flowers which are violet in color (Figs. 1 and 2). The hemicellulose present in the seed-hairs of this plant, the so-called milkweed floss, forms the subject of the present investigation.

Milkweed floss is a material which attained some technical importance during the Second World War as a substitute for kapok, then unobtainable. Like kapok,

FIG. 1 The Milkweed Plant (Asclepias syriaca, L.)

FIG. 2 Milkweed Pods at Early and Late Stages of Opening

milkweed floss is not easily wetted and it is able to support a weight 35 times its own for 1-2 days. A process was developed for mechanical de-seeding of the fruit, and pods were collected and sent to a plant in the United States for further processing, the main use being in lifebelts.

The pulping characteristics of the principal fibrous components of the milkweed stem (whole stalk, bast fiber, and woody material of the stalks) have been investigated (9) from the standpoint of their potential use as a raw material in the paper industry. The bleached pulp produced was similar to flax pulp in appearance and physical properties, having a relatively high alpha-cellulose content and a low viscosity, making it suitable for the manufacture of products such as cigarette paper. It was also noted that milkweed stalks gave a higher yield of pulp than straw and had certain advantages in strength, suggesting the use of stalks in paper board manufacture. However, collection and transportation of the stalks to the mill would be a serious problem.

Various other uses, which have been either suggested or applied for the integral parts of the milkweed plant, include production of felt from the floss (10), spinnable fibers from the bast fibers (11,12), chewing gum (13) and oil from the seeds (14) for the manufacture of paints and varnishes.

The floss of the common milkweed has been reported to contain (15) 36% alpha-cellulose and 20% lignin, whereas another species (Asclepias cornuti) contains 40% alpha-cellulose, 31% pentosan and 18.2% lignin (16). The chemical composition of the stalk of the common milkweed has recently been studied (17). The data presented in Table I indicate a rather high content of cellulose. The amount of xylan present is lower than in other, similar materials, whereas the uronic anhydride content is astonishingly high, indicating the presence of a xylan polysaccharide containing a large number of uronic acid groups. The weight-average degree of polymerization of the cellulose component was 9,400 (17) which was considerably higher than that of the cellulose present in either cornstalk or wheat straw (17).

The molecular properties of milkweed floss cellulose have been studied by Timell and Snyder (18). Direct nitration of the untreated fiber gave a cellulose nitrate with glucose as the only constituent sugar in a yield corresponding to a content of 29% cellulose in the original material. In a subsequent investigation (19) this yield could be raised to 40%, corresponding to the total cellulose content of the fiber.

The weight-weight average D.P. of the cellulose nitrate was 5,800 as determined on the basis of sedimentation-

TABLE I

Chemical Composition of Milkweed Stalk

	Per Cent
Alpha-cellulose	52.6
Cellulose	49.2
Pentosan	19.2
Lignin	15.6
Ash	1.4
Acetyl	3.7
Uronic anhydride	10.2
Galactan	2.3
Glucan	53.0
Mannan	1.7
Araban	1.5
Xylan	10.6

diffusion measurements. Later work (19) has indicated a weight-average D.P. of 10,500, this time on the basis of light-scattering measurements. Fractional precipitation of the nitrate derivative was carried out with acetone-water, yielding a chain-length distribution having only one maximum and exhibiting a pronounced positive skewness. The lower D.P. limit was 2,500 and the upper 8,000 (weight-weight averages). The value of 10,500 for the D.P. was similar to that also found for native cellulose from materials such as cotton, kapok, flax, hemp, jute, ramie, pine and birch wood (17). The chain-length distribution was different from that of cotton, flax, and ramie celluloses. The first two of these materials showed a D.P. distribution with one maximum and a large negative skewness, whereas the distribution of ramie cellulose was symmetrical (20).

It is now generally accepted that in the cell wall of plants the fundamental skeletal substance consists of cellulose, the long, threadlike microfibrils of which are arranged in bundles. Few cells, however, possess a wall consisting exclusively of cellulose. In most cases the cellulose microfibrils are embedded in an amorphous mass of lignin and polysaccharides, the former of which is a three- and the latter a two-dimensional polymer. The non-cellulosic polysaccharides, usually referred to with the general term "hemicelluloses" are easily separated from the

cellulose by extraction with alkali. In many cases this treatment has to be supplemented by a prior removal of most of the lignin, which can be done with either gaseous chlorine followed by extraction with alcoholic ethanolamine or by treatment with a hot, aqueous solution of sodium chlorite.

In general, hemicelluloses contain several kinds of sugar residues, notably D-mannose, D-glucose, D-galactose, D-xylose, L-arabinose, L-rhamnose, L-fucose, D-glucuronic acid, and 4-O-methyl-D-glucuronic acid. The hemicellulose present in milkweed floss was early (17) found to be an acidic xylan, a polysaccharide encountered in all lignified plant tissues.

One of the first complete investigations dealing with a native xylan was concerned with the hemicellulose present in esparto grass (21,22). A purified fraction (23) of this material was found to consist of approximately 80 1,4-β-linked xylopyranose residues containing one branch point per molecule through position 3.

In wheat straw xylans, arabinose and D-glucuronic acid are present in addition to the xylose residues. Partial hydrolysis of the polysaccharide has yielded an aldobiouronic acid, the structure of which has been established as (24,25,26) 3-O-D-glucopyranosyluronic acid-D-xylopyranose. Side groups of L-arabinose residues are also attached to the main xylan chain through 1,3-linkages (27).

Deciduous woods such as, for example, beech (28,29) also contain an acidic xylan. This has been found to be composed of a main chain of xylose residues containing side groups of 4-O-methyl-D-glucuronic acid linked through the 2-position of the anhydroxylose units. The hemicellulose present in white birch (30) is a linear polysaccharide containing approximately 200 β-D-xylopyranose residues linked 1,4 and with every eleventh unit carrying a single side group of 4-O-methyl-D-glucuronic acid, attached by an α-glycosidic bond to the 2-position of the xylose residues. Hemicelluloses in coniferous woods, such as western hemlock (31) contain approximately twice as many 4-O-methyl-D-glucuronic acid side groups and have, in addition, L-arabofuranose residues attached to the 3-position of the main chain.

The number of xylan polysaccharides hitherto investigated is very large and the above only represents some of the more common types encountered. It should be noted that with the exception of the esparto xylan referred to above, all xylan polysaccharides so far found in nature have contained uronic acid units. Preliminary analysis of the hemicellulose present in milkweed floss indicated that this polysaccharide was no exception in this respect and that it was probably a methyl glucurono xylan.

RESULTS AND DISCUSSION

Data for the chemical composition of the floss of the common milkweed (Asclepias syriaca, L.) (18) are presented in Table II. The relative ratios of the neutral sugar residues found in the floss were determined according to the method of Timell et al. (32) and are given in Table III. These data agree closely with those reported for another species of the same family (A. cornuti) (16).

Preliminary attempts to isolate the hemicellulose indicated that direct alkaline extraction was capable of giving a hemicellulose in a yield only slightly lower than that obtained on extraction of the corresponding chlorine and chlorite holocelluloses. No use, therefore, was made of either of these delignification methods, especially since both of them, and particularly the latter, are known to cause degradation of polysaccharides.

A representative sample of milkweed floss hemicellulose was prepared by extracting the native floss, first with ethanol-benzene (1:2, v/v) to remove fats and waxes, then with 0.5% ammonium oxalate for elimination of pectin. Direct alkaline extraction of the floss was carried out with 24% potassium hydroxide, and the hemicellulose was precipitated in a mixture of ethanol-acetic acid followed by solvent exchange through ethanol and drying in vacuo from diethyl ether. The yield was 30%

TABLE II

Chemical Analysis of Milkweed Floss Based On Extractive-Free Oven-Dry Fibers

Component	Per Cent
Chlorine holocellulose	83.7
Alpha-cellulose (Chlorine method)	39.6
Alpha-cellulose (chlorite method)	38.0
Lignin	15.1
Pentosan	35.3
Uronic anhydride	5.6
Acetyl	6.1

TABLE III

Relative Composition of Neutral Sugar Residues of Milkweed Floss

	Per Cent
Galactan	2.4
Glucan	55.1
Mannan	5.2
Xylan	37.3

based on the extractive-free, dry material.

Chromatographic analysis of the hemicellulose revealed the presence of only xylose and uronic acids, indicating that the product was an acidic xylan. Purification of the hemicellulose for structural investigations by the copper complex method was, therefore, deemed unnecessary.

Analysis of the potassium salt of the milkweed xylan gave the following results:

TABLE IV

Analysis of Milkweed Xylan

Methoxyl	1.51%
Uronic anhydride	8.55%
Pentosan	86.70%
Lignin	1.59%
Equivalent weight	2087
$[\alpha]_D^{20}$	-99°

The high negative rotation of the xylan suggested that the anhydroxylose residues were linked in the β-configuration.

If it be assumed that the methoxyl originated from a monomethyl uronic acid residue, the number of anhydroxylose units present per acid group (n) can be

estimated from the methoxyl content. The following relationship will then be valid:

$$\% \, OCH_3 = \frac{100 \times 31}{132n + 190 + 38}$$

where 31 is the molecular weight of the methoxyl

132 is the molecular weight of the anhydroxylose unit

190 + 38 is the molecular weight of the potassium salt of the anhydro 4-O-methyl-D-glucuronic acid unit.

The value thus obtained was 13.8.

Similar information was also obtained from the uronic anhydride content of the hemicellulose according to the equation:

$$\% \text{ Uronic anhydride} = \frac{100 \times 176}{132n + 190 + 38}$$

where 176 is the molecular weight of the uronic anhydride unit. The number of anhydroxylose units per acid group thus obtained was 14.3, which agreed reasonably with that derived from the methoxyl content.

A minor portion of the potassium salt of the hemicellulose was converted to the free acid by reprecipitation into a mixture of ethanol and hydrochloric acid. Titration of the acid groups in duplicate determinations indicated an equivalent weight of 2087. The number of

anhydroxylose residues per acid group was, therefore:

$$\frac{2087 - 192}{132} = 14.4$$

The average value of the three determinations was 14.1 xylose residues per acid group.

The hemicellulose was oxidized with hypoiodite according to the method of Hirst, Hough and Jones (33). The result suggested the presence of one reducing end group per 62 anhydroxylose units.

Viscosity measurements were carried out with the potassium salt of the hemicellulose using M cupriethylenediamine and 10% (w/w) potassium hydroxide as solvents. A Craig-Henderson viscometer (34) was used and the measurements were thermally controlled at 25°C. The values of t, the time of flow, c, the concentration in g./dl., η_{sp}, the specific viscosity and $\eta_{sp/c}$, the reduced viscosity were determined for both solvents as shown in Table V.

The reduced viscosity was plotted against the concentration and $[\eta]$, the intrinsic viscosity, was obtained on extrapolation to zero concentration (Fig. 3). Huggins' constant, k' was 0.468 in the cupriethylenediamine solvent and 0.428 in potassium hydroxide. The intrinsic viscosities were 0.812 and 0.607, respectively. Using a relationship between the intrinsic viscosity of acidic xylans in these

TABLE V

Viscosity Data for Xylan Obtained with 10% (w/w) Potassium Hydroxide and M Cupriethylenediamine As Solvents

In M cupriethylenediamine t_o = 59.1 sec.

Time, sec.	c (g./dl.)	η_{sp}	$\eta_{sp/c}$
121.3	0.9780	1.0524	1.0760
109.0	.8150	0.8443	1.0359
97.5	.6520	.6497	0.9964
86.6	.4890	.4653	.9515
76.5	.3260	.2944	.9030
69.3	.1956	.1692	.8650

In 10% KOH t_o = 38.0 sec.

66.7	0.9780	0.7552	0.7721
61.0	.8150	.6052	.7425
55.8	.6520	.4684	.7184
50.7	.4890	.3342	.6834
46.2	.3260	.2157	.6616
42.7	.1956	.1236	.6319
41.1	.1304	.0815	.6250

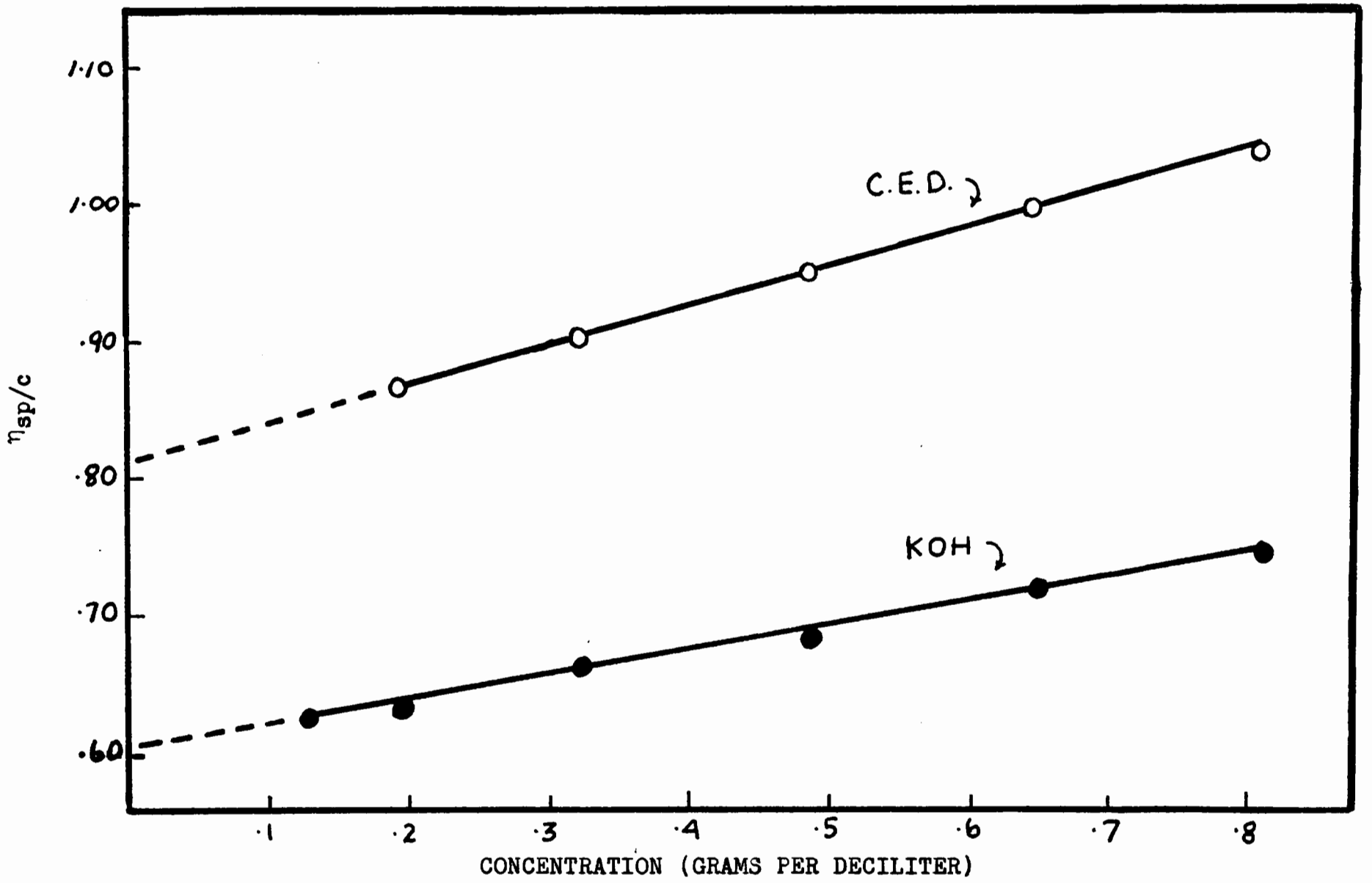


FIG. 3 Plot of Viscosity against Concentration for Milkweed Xylan in 10% (w/w) Potassium Hydroxide and M Cupriethylenediamine

solvents and their number-average degree of polymerization recently developed by Glaudemans and Timell (30), these values correspond to number-average degrees of polymerization of 172 and 196, respectively.

For a determination of the structure of the hemicellulose, the classical methylation procedure was first applied. A sample (20 g.) was subjected to five methylations according to Haworth (22), followed by seven methylations according to Adams and Falconner (35), using 90% and, later, 100% tetrahydrofuran. The polysaccharide was finally subjected to an adaptation of the methylation procedure developed by Kuhn et al. (36), involving the use of tetrahydrofuran as a solvent for the partially methylated polysaccharide. The product obtained had a methoxyl content of 37.9%, indicating complete substitution. An infra-red spectrum (Fig. 13) of the material showed only a slight trace of hydroxyl, believed to originate from moisture picked up by the potassium bromide during the pellet formation.

Osmotic pressure measurements were carried out on the fully methylated xylan with osmometers of the type designed by Zimm and Myerson (37), and later modified by Stabin and Immergut (38). The solvent was a mixture of chloroform and ethanol (9:1, v/v). Table VI gives the values obtained for C, the concentration in g./kg. chloroform, π, the osmotic pressure in centimeters of

TABLE VI

Osmometry Data for the Methylated Xylan

C[a]	π[b]	π/C
1.9037	3.168	1.6641
2.7599	4.835	1.7518
3.5111	6.061	1.7262
3.8151	6.718	1.7608
4.4151	7.897	1.7886
4.8152	9.371	1.9461

(a) Concentration in g./kg. solvent

(b) Osmotic pressure in cm. solvent

solvent mixture and π/C, the reduced osmotic pressure. The latter was plotted against the concentration; extrapolation to zero concentration giving $(\pi/C)_o$. The value of $\bar{M}n$, the number-average molecular weight, and $\bar{P}n$, the number-average degree of polymerization, were calculated assuming the presence of 14 xylose residues per methyl glucuronic acid group in the acidic xylan. The value for $(\pi/C)_o$ was 1.51 and thus:

$$\bar{M}n = \frac{25,700}{1.51} = 17,000$$

$$\bar{P}n = \frac{17,000}{2457} \times 14 = 97$$

where 2457 is the molecular weight of the fully methylated repeating unit.

The upper and lower limits of $(\pi/C)_o$ as designated by dotted lines on the graph (Fig. 4) were 1.62 and 1.44, giving values for $\bar{P}n$ of 90 and 102, respectively. The degree of polymerization obtained for the methylated xylan does not represent the average chain-length of the original polysaccharide since degradation of the polymer during methylation was unavoidable.

A portion of the methylated xylan was subjected to methanolysis under conditions which should not cleave any methylated aldobiouronic acid that might be present. The methyl glycosides were separated quantitatively into

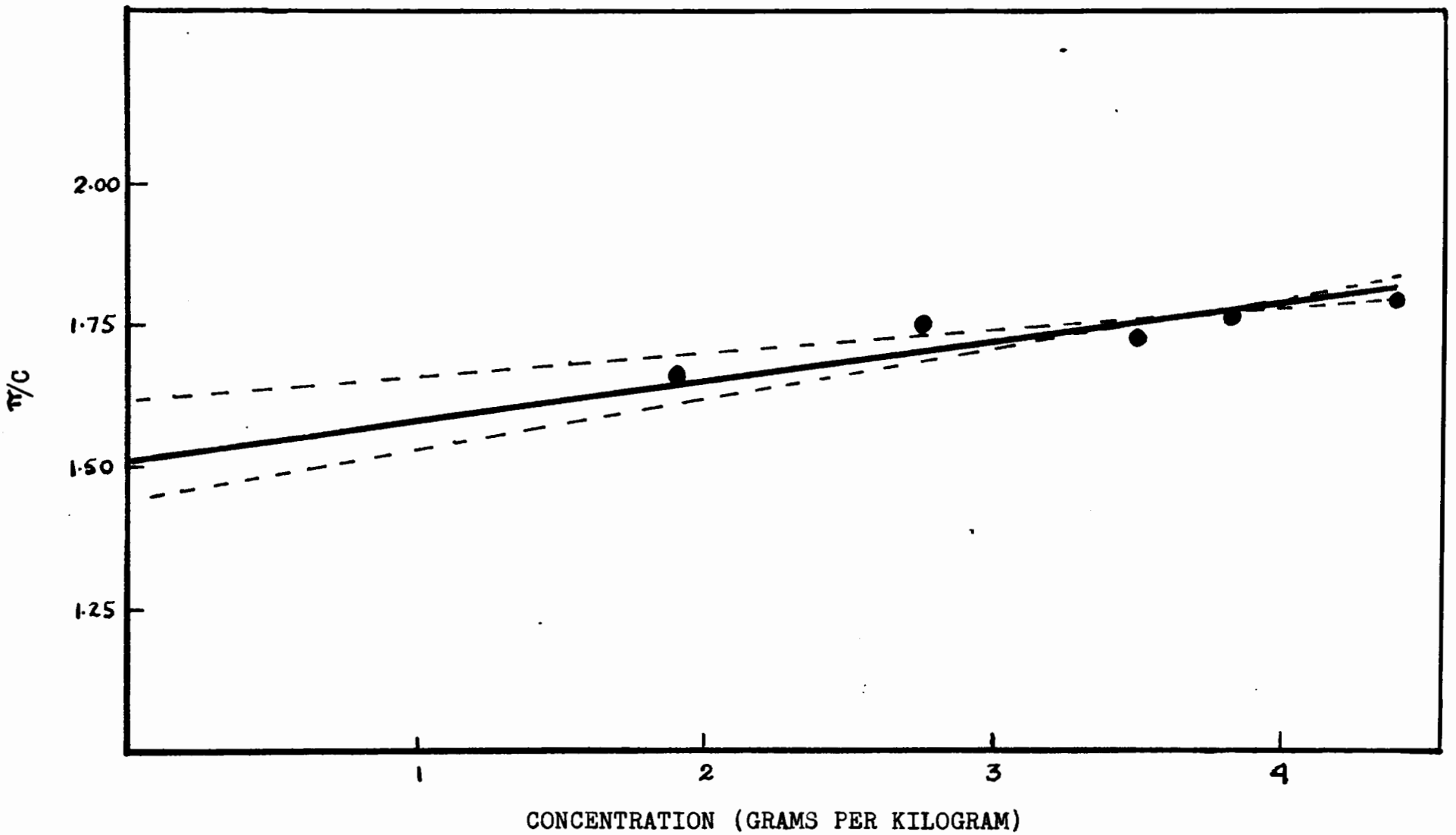


FIG. 4 Plot of Reduced Osmotic Pressure against Concentration for the Methylated Hemicellulose

acidic and neutral fractions on an anion exchange resin.

The acid fraction was characterized as methyl 2-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-3-O-methyl-D-xylopyranoside in the following way. Reduction with lithium aluminum hydride of the methyl ester-methyl glycoside yielded a mixture of two methylated sugars (Fig. 5) which was resolved by gradient elution on a charcoal column. One component was identified as 2,3,4-tri-o-methyl-D-glucose. The other was 3-O-methyl-D-xylose, identified through its crystalline aniline derivative. It was clearly distinguished from the 2-isomer in its ionopheretic behavior and exhibited an infra-red spectrum (Fig. 14) identical to that of an authentic specimen.

The neutral glycosides were converted to the corresponding free sugars and were separated by paper chromatography. Minor quantities of a mono- and a tri-O-methyl xylose, in addition to a large amount of a di-O-substituted xylose, were present.

The monomethylated component was shown by paper ionopheresis to be a mixture of 2-O- and 3-O-methyl-D-xylose in an approximate ratio of 1:1. The di-O-methylated component was identified as 2,3-di-O-methyl-D-xylose through its crystalline aniline derivative. Its infra-

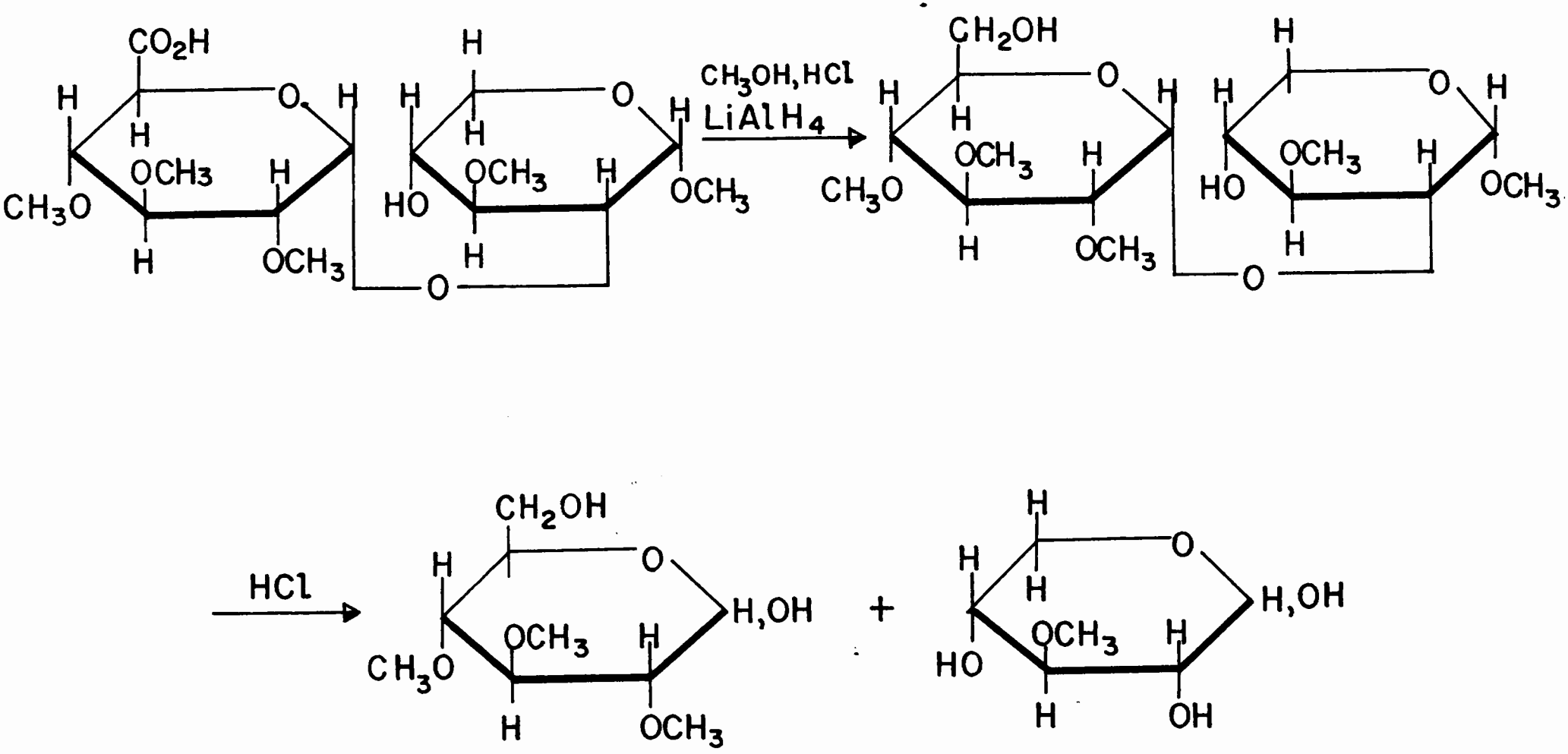


FIG. 5 Reduction and Hydrolysis of the Partially Methylated Aldobiouronic Acid Isolated from the Fully Methylated Hemicellulose

red spectrum was identical to that of an authentic specimen. The tri-O-methylated component was identified as 2,3,4-tri-O-methyl-D-xylose. The large quantity of 2,3-di-O-methyl-D-xylose indicated that the main xylan chain was composed of xylopyranose residues linked through positions 1 and 4. The 2,3,4-tri-O-methyl-D-xylose evidently originated from the non-reducing end groups of the xylan.

The relative ratio of the mono, di and tri methyl xylose derivatives was determined by the o-aminodiphenyl method of Timell, Glaudemans and Currie (32). The results are presented in Table VII; the amount of aldobiouronic acid present, as calculated from the weight of the acidic fraction, has also been included.

TABLE VII

Sugars Isolated from the Methylated Hemicellulose

Component	Molar Ratio
2-O- and 3-O-methyl-D-xylose	0.82
2,3-di-O-methyl-D-xylose	38.6
2,3,4-tri-O-methyl-D-xylose	1.0
Methyl 2-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-3-O-methyl-D-xylopyranoside	2.29

The quantity of methylated aldobiouronic acid corresponds to 18.6 anhydroxylose units per acid group, a value somewhat higher than that found by other methods (14.4). A similar preferential loss of uronic acid containing material during methylation has been observed with a similar hemicellulose by Jones and Painter (39).

Methylation of another sample of hemicellulose to a somewhat lower methoxyl content (34%), and subsequent separation of the methylated derivatives obtained on methanolysis and hydrolysis, indicated a slightly higher proportion of monomethylated xylose as could be expected. The relative amount of non-reducing end groups, as estimated from the yield of tri-O-methyl-D-xylose, as well as the number-average molecular weight, measured by osmometry, were exactly the same, thus corroborating the above results.

As a further proof of the structure of the hemicellulose, the nature of some of the uronic acids obtained on partial hydrolysis of the polysaccharide was investigated. Paper chromatography of the hemicellulose hydrolysate suggested the presence of xylose, a monouronic acid, a monomethyl monouronic acid, a monomethyl aldobiouronic acid, a monomethyl aldotriouronic acid, as well as higher uronic acids.

For isolation of a large quantity of uronic acids,

milkweed floss was subjected to partial hydrolysis to yield the above sugar acids in addition to glucose, xylose and a trace of mannose. The acid fraction was adsorbed on an anion exchange resin which did not retain any neutral sugars. A portion of the total uronic acid fraction was converted to the methyl ester-methyl glycoside, reduced with lithium aluminum hydride, and hydrolysed. Chromatographic analysis indicated the presence of galactose, xylose and 4-O-methyl-D-glucose. The latter was separated from the galactose and xylose and was identified through its crystalline osazone. The remaining mixture was resolved by paper chromatography after which the xylose crystallized. The galactose probably originated from the galacturonic acid present as pectin in the floss. On this assumption, all other uronic acids were accordingly composed of either 4-O-methyl-D-glucose and xylouronic acid, or 4-O-methyl-D-glucuronic acid and xylose.

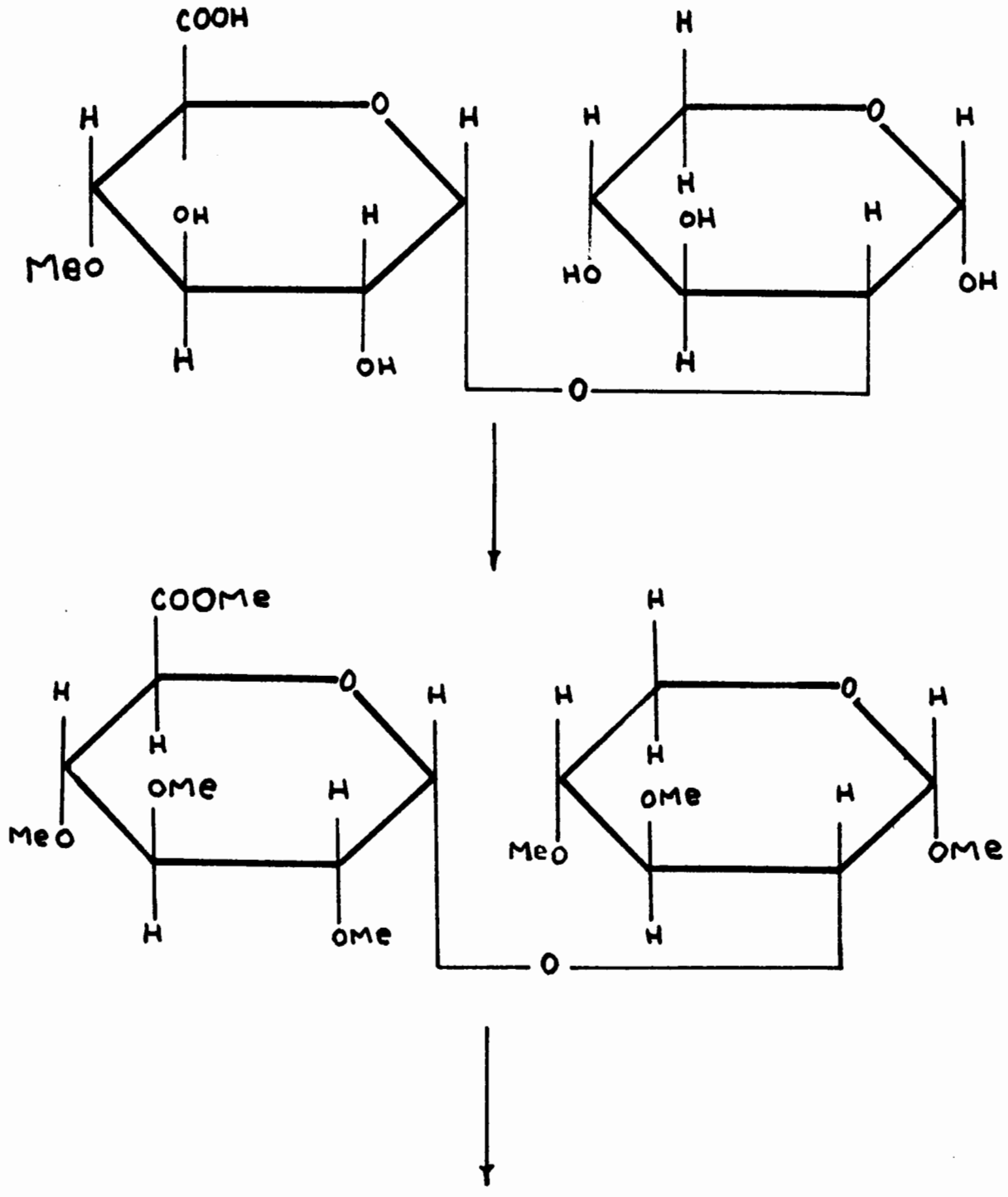
The remainder of the uronic acid mixture was resolved on the same column of anion exchange resin by elution with N and 3N acetic acid (40). Five fractions were obtained. A complete separation of the aldobiouronic and aldotriouronic acids was effected by adsorbing the second fraction on a cocoanut charcoal column and eluting with 4 and, subsequently, 7% aqueous ethanol. The aldotriouronic acid was eluted at the lower alcohol concentration and very little overlapping was observed when the

concentration of ethanol was increased to 7%.

The pure aldobiouronic acid isolated from the charcoal column had a methoxyl content (8.6%) and an equivalent weight (379) corresponding to that of a monomethylated aldobiouronic acid. The high positive rotation ($+108^{\circ}$) indicated that the glycosidic linkage was of the α-type. The methyl ester-methyl glycoside of the aldobiouronic acid was methylated three times according to Kuhn et al. (36). The infra-red spectrum (Fig. 15) of the methylated product showed the absence of any hydroxyl groups and was identical to that of an authentic sample of methyl 2-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-3,4-di-O-methyl-D-xylopyranoside methyl ester.

Reduction of the fully methylated aldobiouronic acid with lithium aluminum hydride and subsequent hydrolysis yielded 3,4-di-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-glucose (Fig. 6), which were identified through their crystalline aniline derivatives. The 3,4-di-O-methyl-D-xylose was easily distinguished from the 2,3-di-O-methyl-D-xylose by paper electrophoresis (41), only the former compound exhibiting any mobility.

The isolation of the 2,3,4-tri-O-methyl-D-glucose showed that the uronic acid group was located in the 6-position of the hexose part of the disaccharide. The isolation and identification of 3,4-di-O-methyl-D-xylose



2,3,4-TRI-O-METHYL-D-GLUCOSE 3,4-DI-O-METHYL-D-XYLOSE

FIG. 6 Reduction and Hydrolysis of a Methylated Aldobiouronic Acid

indicated that the uronic acid moiety was attached to the 2-position of the xylose component.

The fully methylated aldobiouronic acid was thus methyl 2-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-3,4-di-O-methyl-D-xylopyranoside and the aldobiouronic acid was 2-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-xylopyranose (Fig. 6). This aldobiouronic acid is identical to that isolated previously from a number of wood species, such as trembling aspen (42,43,44), European and American beech (29,45), Populus tacamahacca (46), white birch (30), white elm (40), Scots pine, black spruce (47), western hemlock (31,48), Norway spruce (49) and Loblolly pine (50). It has also been isolated from a few non-woody products, for example, flax straw (51), corn cobs (52), oat hulls (35) and kapok (53). In addition, it has been tentatively identified in hydrolysates from Finnish birch (54), Eucalyptus regnans (55,56) and Maritime pine (57).

A portion of the aldotriouronic acid separated on the charcoal column was hydrolysed in 0.5N sulphuric acid. Chromatographic analysis of the hydrolysate showed four distinct spots corresponding to unhydrolysed aldotriouronic acid (R_x = 0.31), the above aldobiouronic acid, 4-O-methyl-D-glucuronic acid and xylose. These results indicated that the aldotriouronic acid was composed of D-xylose and the aldobiouronic acid previously isolated

and characterized. The 4-O-methyl-D-glucuronic acid probably originated from the partial hydrolysis of the aldobiouronic acid. The reduction and hydrolysis of the total acid fraction (giving 4-O-methyl-D-glucose and D-xylose) corroborated these results. The methoxyl content (5.8%) corresponds to that of two xylose residues joined to one 4-O-methyl-D-glucuronic acid group.

A similar, crystalline, aldotriouronic acid has been isolated from Loblolly pine (58), trembling aspen (42,43) and western hemlock (48).

The monomethyl monouronic acid was isolated from the impure fraction 4, obtained from the exchange resin, by elution from a carbon column. The equivalent weight agreed quite well with that for a methoxy hexuronic acid, and the optical rotation corresponded to other values (47) obtained for the same acid.

Fraction 5, which contained 4-O-methyl-D-glucuronic acid and galacturonic acid was combined with fraction 4 and the methyl ester-methyl glycosides of these sugars were formed. Reduction with lithium aluminum hydride followed by hydrolysis of the reduced acid gave two spots on chromatographic analysis corresponding to 4-O-methyl-D-glucose and galactose. Separation of these sugars was effected on a cocoanut charcoal column. The

galactose was eluted with water, while the 4-O-methyl-D-glucose was removed with 2% aqueous ethanol. Its methoxyl content and specific rotation were in agreement with the published values for 4-O-methyl-D-glucose. It was further characterized through its crystalline osazone, the infrared spectrum of which was identical with that of an authentic sample.

The above evidence indicated the existence of galacturonic acid and 4-O-methyl-D-glucuronic acid in the hydrolysate from the floss. The galacturonic acid probably originated from small amounts of pectin present in the floss, while the 4-O-methyl-D-glucuronic acid was formed by partial hydrolysis of the aldobiouronic acid.

From the evidence now cited, a structure may be suggested for the hemicellulose present in milkweed floss. The large quantity of 2,3-di-O-methyl-D-xylose isolated indicates that the main portion of this methyl glucurono xylan is composed of D-xylopyranose residues linked 1,4. The high negative specific rotation of the polysaccharide (-77°) makes it probable that the anhydroxylose units are linked in the β-configuration. The 4-O-methyl-D-glucuronic acid evidently originated from terminal side groups. The partially methylated aldobiouronic acid obtained from the methylated polymer gave rise to 3-O-methyl-D-xylose on hydrolysis, whereas the substituted aldobiouronic acid

obtained by partial hydrolysis of the hemicellulose and subsequent methylation produced 3,4-di-O-methyl-D-xylose. This, together with the fact that no di-O-substituted xylose derivative could be found, strongly indicates that the 4-O-methyl-D-glucuronic acid groups were attached as single side chains through the 2-position of the units in the main xylan chain.

The 2,3,4-tri-O-methyl-D-xylose evidently originated from the non-reducing end groups of the polysaccharide, and their amount corresponded to a number-average molecular weight, $\bar{M}n$, of 42. Since almost all acid groups were accounted for by the amount of aldobiouronic acid present in the hydrolysate of the methylated product, and since no 4-O-methyl-D-glucuronic acid could be found in the latter, the 2-O- and 3-O-methyl-D-xylose found must have been due to either incomplete methylation, demethylation, or branching. That branching is probably involved is evident from the value of $\bar{M}n$ determined by osmometry, namely 97, as compared with the value of 85 calculated from the ratios of methylated xylose derivatives obtained experimentally, assuming one branch point per molecule, as shown in Table VIII.

TABLE VIII

Comparison between the Ratios of Methylated Xylose Derivatives Obtained Experimentally and Calculated From Osmotic Pressure Measurements, Assuming the Presence of one Branch Point per Molecule

Component	Found	Calculated
2-O- or 3-O-methyl-D-xylose	1.6	1
2,3-di-O-methyl-D-xylose	82	94
2,3,4-tri-O-methyl-D-xylose	2	2
Number of xylose residues per molecule (D.P.)	85	97

The data in Table VIII show a reasonable agreement between values found experimentally and calculated on the basis of this assumption. The presence of 0.6 moles of mono-O-methylated xylose in excess of that required by theory could easily be accounted for by incomplete methylation or demethylation. Such phenomena have frequently been encountered in other studies of xylan polysaccharides (29,49,31).

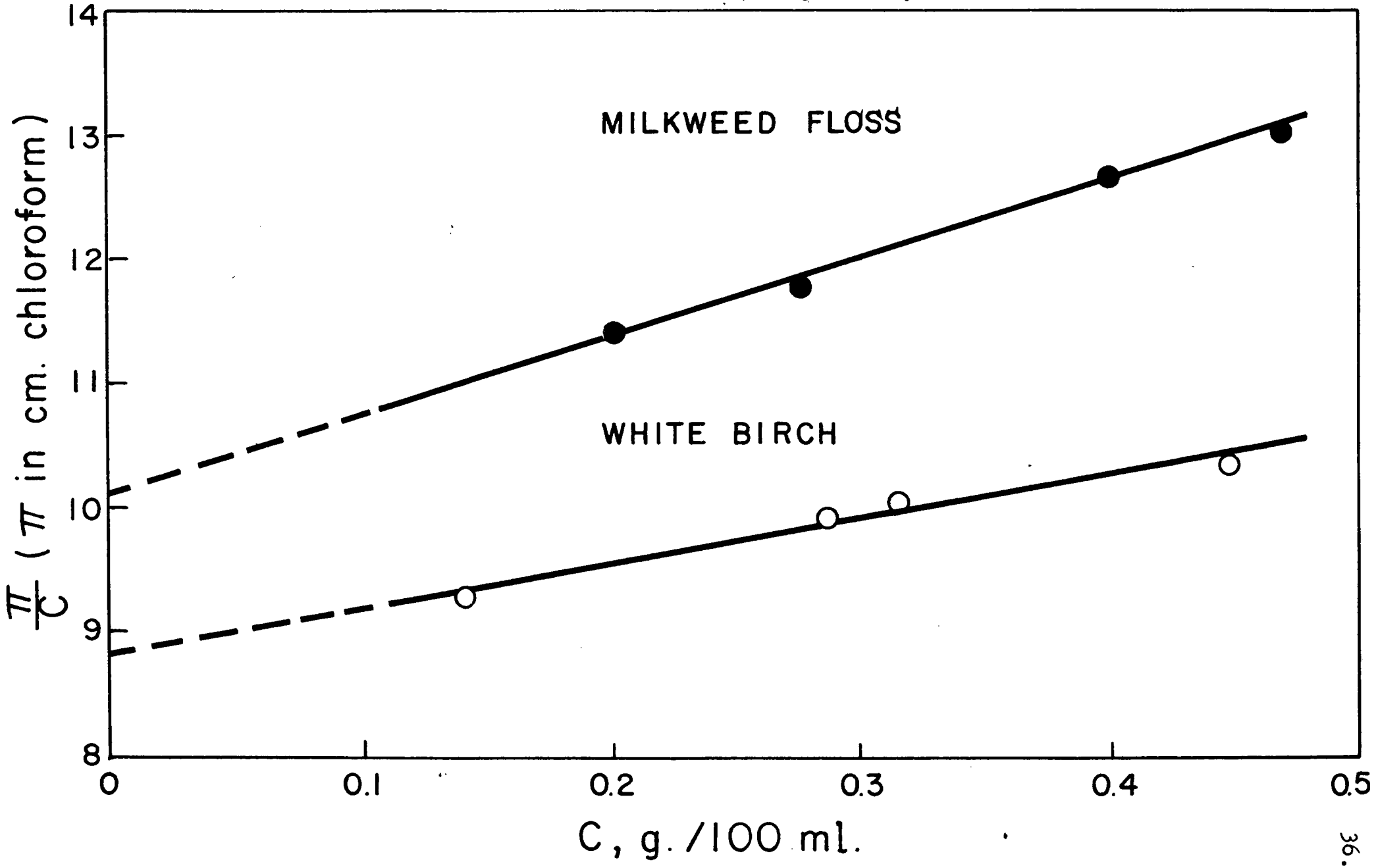
Oxidation with hypoiodite indicated the presence of 62 anhydroxylose units per reducing end group, a value considerably lower than that expected, namely, approximately 90. Overoxidation easily occurs, however, when this rather unreliable method is applied to polysaccharides. A similar

result was, for example, obtained for a birch glucurono xylan by Glaudemans and Timell (30).

In the equation relating the reduced osmotic pressure to the molecular weight of polymers forming non-ideal solutions, $\pi/C = RT/M\ (1 + Bc + Cc^2 + \ldots)$, the second vicial coefficient, B, is a measure of the interaction of the chain segments; the higher the value of B, the smaller the intermolecular attraction, that is, the greater the solubility. When the osmotic behavior of a linear methyl glucurono xylan from birch was compared with that of the milkweed floss hemicellulose (Fig. 7), it became evident that B, the slope constant, was larger in the latter case. Since a branched polymer is normally more soluble than a linear one, this is another, albeit rather indirect indication for the branched nature of the milkweed hemicellulose.

Due to the insolubility of the hemicellulose acetate, no osmotic molecular weight determinations could be carried out on the native polysaccharide. The viscosity data indicate, however, a number-average D.P. of 170-190, values only slightly lower than that obtained for a methyl glucurono xylan from white birch, or 197 (30). It should, however, be pointed out in this connection that the relationship used here for converting intrinsic viscosities to degrees of polymerization was derived for the linear birch

FIG. 7 Comparison of the Osmotic Behavior of a Methylated Hemicellulose from White Birch and Milkweed Floss, Respectively



hemicellulose, and that it also depended on the polymolecularity of the latter. The molecular weight distribution of the milkweed hemicellulose extended from a lower D.P. limit of 65 to a higher one of 174; that is, the material is polymolecular.

Summarizing, it is evident that the hemicellulose in milkweed floss contains a minimum of approximately 100 and a maximum of 180 β-D-xylopyranose residues linked together by 1,4-glycosidic bonds. To every fourteenth anhydroxylose unit there is linked, through the 2-position and by an α-glycosidic bond, a single side chain of 4-O-methyl-D-glucuronic acid (Fig. 8). Every molecule contains on the average one branch point through position 2 or 3 (Fig. 9). In its native state the methyl glucurono xylan probably contains acetyl groups, whereas the nature of the carboxyl group is yet unknown.

The similarity in chemical composition of the milkweed floss and a deciduous wood such as, for example, white birch (Betula papyrifera) is rather striking. Both contain the same amount of lignin, 16-18%. The amount of cellulose present is approximately 40% in both cases, with an identical degree of polymerization of 10,000 (17,18). The pentosan content of birch is slightly lower than that of the floss but the chemical constitution of the methyl glucurono xylan constituting this portion is on the whole

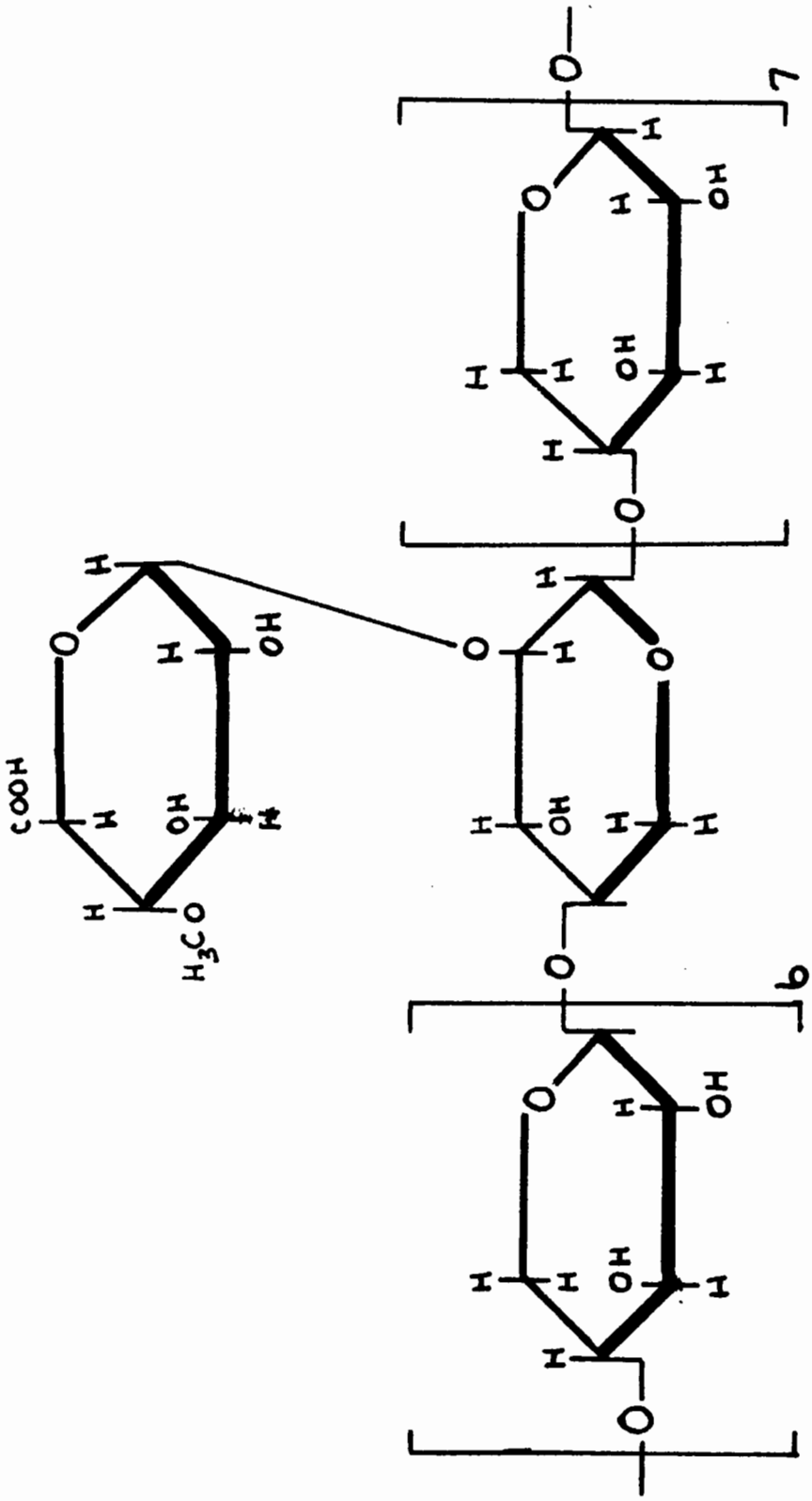
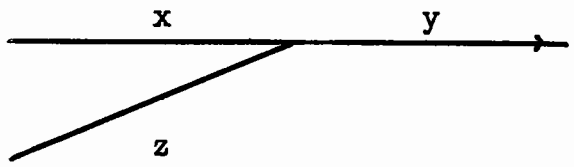


FIG. 8 Simplified Structure of Milkweed Floss Hemicellulose



$$x + y + z = 100 \text{ to } 180$$

Structure of section "x":

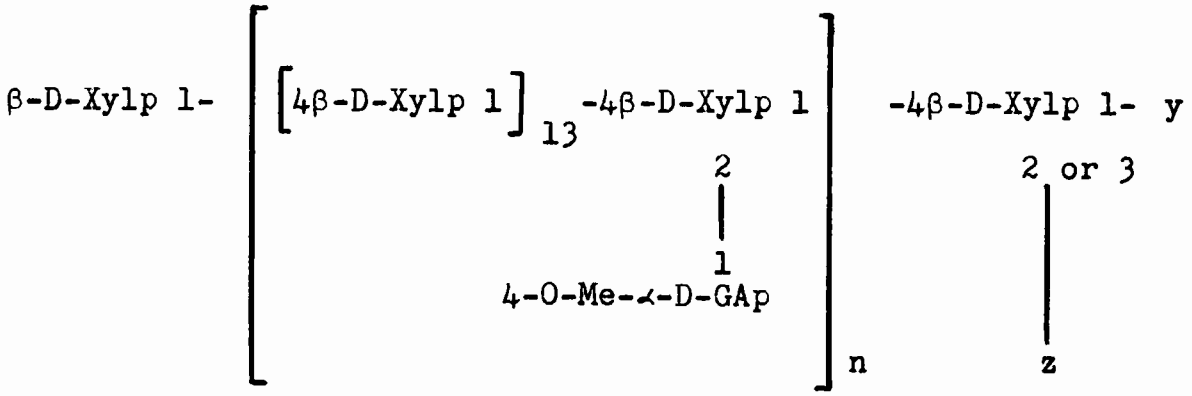


FIG. 9 Simplified Structure of Milkweed Floss Hemicellulose, Including the Single Branch Point

the same. The main differences here appear to be that the milkweed floss hemicellulose is slightly branched and contains less acid side groups than the linear birch hemicellulose. Morphologically, there is, of course, little similarity between the two materials. The lignin in the floss is probably distributed throughout the cell wall, while in the birch it is almost exclusively located in the middle lamella, a component entirely lacking in the milkweed floss.

EXPERIMENTAL

Analytical Methods and Reagents

Paper Chromatography

Solvents (v/v) used for separation of sugars were (A) ethyl acetate : acetic acid : water (9:2:2), (B) butane-1-ol : pyridine : water (10:3:3), (C) ethyl methyl ketone : ethanol : water (20:5:2), (D) butane-1-ol : ethanol : water (40:11:19), (E) butane-1-ol : ethanol : water : ammonia (100:25:127.5:2.5), (F) methyl ethyl ketone : benzene : ethanol : water (10:10:5:2) and (G) benzene : ethanol : water (169:47:15). The spots were developed with o-aminodiphenyl spray reagent which consisted of a solution of o-aminodiphenyl (3.0 g.) and 85% phosphoric acid (1.3 ml.) in glacial acetic acid (100 ml.). This reagent gave brown spots with hexoses, red spots with pentoses, and an orange color with uronic acids, which, on further heating was converted to purple for the monouronic acids, and to brick red for the aldobiouronic and aldotriouronic acids. Higher uronides gave colors intermediate between red and brown.

Amberlite IR-120 cation exchange resin

Product of Rohm and Haas Co., Philadelphia, Pa. Washed with 50% ammonium hydroxide, water, hydrogen chloride and finally with water to remove hydrogen chloride.

Amberlite IR-45 anion exchange resin

Product of Rohm and Haas Co., Philadelphia, Pa. Washed with 2N sodium hydroxide and then with water. The acetate form was prepared by washing with 8N acetic acid.

Dowex 1-4X anion exchange resin

Product of Dow Chemical Company, Midland, Michigan. Washed with 2N sodium hydroxide until the chloride ion had disappeared and later with water to remove sodium hydroxide. The acetate form was prepared by washing with 8N acetic acid and the bicarbonate form by washing with sodium bicarbonate.

Dry Methanol

Prepared according to the procedure outlined in Vogel, A.I. "Practical Organic Chemistry", sec. edition, page 168.

Celite

Product of Johns-Manville Co., New York.

Alkaline Pyrogallol

Prepared according to the procedure outlined in Vogel, A.L. "Quantitative Inorganic Analysis", page 764.

Tetrahydrofuran

Reagent grade. Product of Merck and Co., Rahway, New York. Refluxed over sodium hydroxide for 4 hours and then distilled.

Cocoanut Charcoal

Eighty mesh, activated. Product of Fisher Chemical Co., Fair Lawn, New York. Washed with 6N hydrogen chloride, water, 100% ethanol and then water until charcoal was free of ethanol.

Silver Oxide

Prepared according to the method outlined by B. Helferich and W. Klein, Annalen 450, 219 (1926).

Borate Buffer Solution

Prepared according to the method outlined by H. Bouveng and B. Lindberg, Acta. Chem. Scand. 10, 1283 (1956) and A.B. Foster, J. Chem. Soc. 982 (1953).

Analytical Procedures

The content of pentosan, lignin and ash was determined according to Tappi standard procedures (59) as described elsewhere (60). Analysis for uronic anhydride was carried out by modification of the method of Browning (61). Reducing sugars were quantitatively determined by the o-aminodiphenyl method (60,32).

Preliminary Isolation of Milkweed Floss Hemicellulose

Eighty grams of milkweed floss which had been extracted with ethanol-benzene (1:2) for 48 hours was treated with 24% potassium hydroxide (1000 ml.) under an atmosphere of nitrogen. The mixture was shaken for 2 hours and then

filtered through a sintered-glass filter funnel. The filtrate was poured with vigorous stirring into a mixture of anhydrous ethanol (5000 ml.) and acetic acid (750 ml.) previously cooled to 5°C. The precipitated hemicellulose was collected on a centrifuge at -20°C. and washed three times each with 80% ethanol, anhydrous ethanol and diethyl ether, respectively. It was then dried in vacuo over calcium chloride. Yield: 20 g.

Preliminary Chromatographic Analysis of the Hemicellulose

Milkweed floss hemicellulose (20 g.) was hydrolysed with 72% sulphuric acid (40 ml.) for 1 hour at 30°C., diluted to a concentration of 3% and heated in a pressure cooker (15 lbs. / sq. inch) for 1 hour. The hydrolysate was neutralized to pH 6 (pH-meter) with barium hydroxide and filtered through Celite to remove the insoluble barium sulphate. Barium ions were eliminated by passing the hydrolysate through a column of Amberlite IR-120 cation exchange resin.

Chromatographic analysis of the hydrolysate in system (A) irrigated for 30 hours showed spots corresponding to xylose, an aldobiouronic acid, an aldotriouronic acid and galacturonic acid.

Separation of the Uronic Acids from Xylose

The milkweed floss hemicellulose hydrolysate was slowly passed through a column of Amberlite IR-45 anion

exchange resin (acetate form) after which the resin was washed with water (20 liters) until free of sugars (Molisch test). The aqueous eluate was concentrated to a thick sirup and chromatographed in system (A) revealing only one spot, corresponding to xylose.

The uronic acids were displaced from the resin with 2N sulphuric acid (1 liter). The column was washed with water and the eluate and washings were neutralized to pH 6.5 (pH-meter) with barium hydroxide. The barium ions were removed with Amberlite IR-120 exchange resin.

Chromatographic analysis of the uronic acids in system (A) suggested the presence of a triouronic acid, an aldobiouronic acid, galacturonic acid and a monomethyl monouronic acid running at approximately the same rate as xylose. The acid fraction, irrigated in system (B), showed absolutely no trace of any free sugars.

Identification of Xylose from the Aqueous Eluate

The sirup was allowed to stand at 5°C. when it crystallized after a few days. The crystals were washed with methanol; m.p. and mixed m.p. 145°C., $[\alpha]_D^{20}$ +23.9° (initial) to +18.6° (final) (c, 3.0 in water).

The dimethyl acetal of dibenzylidene-D-xylose (62,63) was prepared by adding one gram of xylose to 10 ml. of a reagent consisting of redistilled benzaldehyde

(40 ml.), 2.5N methanolic hydrogen chloride (20 ml.) and methanol (120 ml.). After three days at room temperature the mixture crystallized in long needles. The derivative was recrystallized from methanol; m.p. and mixed m.p. 211-212°C.

Preparation of Milkweed Floss Hemicellulose for Methylation

Milkweed floss (340 g.) which had been extracted continuously for 48 hours in ethanol-benzene (1:2) and then air dried, was extracted with 0.5% ammonium oxalate (8 liters) at 70°C. for 4 hours. After cooling, the solution was decanted and the floss was washed free of traces of ammonium oxalate with large volumes of water.

The floss was extracted with 24% potassium hydroxide (5 liters) in an eight liter bottle kept under an atmosphere of nitrogen. The bottle was shaken continuously for 2 hours. The α-cellulose containing most of the lignin was removed from the potassium hydroxide by filtration using a large sintered-glass Büchner funnel. Three liters of alkali (24%) were used to wash the unextracted material and this was added to the filtrate. The hemicellulose was precipitated by pouring the alkaline extract into a mixture of methanol (25 liters), ice cubes (1000 g.), acetone (1 liter) and glacial acetic acid (3 liters) previously cooled to -16°C.

The precipitated hemicellulose was washed free of

salts and acetic acid by solvent exchange using 80% methanol, 100% methanol and finally with ethyl ether, after which it was dried in vacuo. Yield: 83 g.

Analysis of the Hemicellulose

The methoxyl content of the hemicellulose (1.51%) was determined according to the method of Hibbert (64). The equivalent weight of the hemicellulose was obtained by converting the potassium salt of the xylan to the free acid form. Ten grams of the potassium salt of the xylan was redissolved in potassium hydroxide (24%) and then precipitated in ethanol containing an excess of hydrogen chloride. The hemicellulose was washed free of salts and hydrogen chloride by solvent exchange through 80% ethanol, 100% ethanol and finally diethyl ether. A portion was dried in an Abderholden apparatus in vacuo using phosphorous pentoxide as desiccant.

Duplicate samples (200 mg.) were used for the determination and these were placed in Erlenmyer flasks (50 ml.) to which 0.01N sodium hydroxide (20 ml.) was added. The flasks were stoppered tightly and agitated on a mechanical shaker overnight. The remaining alkali was estimated by titration with 0.01N hydrogen chloride using phenolphthalein as indicator. The equivalent weight was found to be 2087.

The specific rotation of the potassium salt of

the xylan was obtained by dissolving a sample (50 mg.) in 5% potassium hydroxide (5 ml.). $[\alpha]_D^{20}$ -99° (c, 1.0 in 5% potassium hydroxide).

Viscosity Measurements

Viscosity measurements were made in M cupriethylenediamine and 10% (w/w) potassium hydroxide.

A sample of the potassium salt of the hemicellulose (150 mg.) was placed in an Erlenmyer flask (25 ml.) and exactly 15 ml. of solvent was added. The flask was stoppered tightly and put on a mechanical shaker for 1 hour to dissolve the xylan. The flask was then removed from the shaker and the contents allowed to assume a temperature of 25°C. (10 minutes) in a constant temperature bath. Exactly 10 ml. of solution was pipetted out and placed in a Craig-Henderson viscometer (34) for measurement. The solution was diluted from 10 to 75 ml. giving seven different concentrations in all. The time of flow, t, concentration, c, in g./dl., specific viscosity, η_{sp}, and reduced viscosity, $\eta_{sp/c}$, were determined. The intrinsic viscosity, $[\eta]$, was determined for both solvents as well as Huggins' constant, k' (65).

For 10% potassium hydroxide $[\eta] = 0.607$ $k' = 0.428$

M cupriethylenediamine $[\eta] = 0.812$ $k' = 0.463$

Kinetic energy corrections do not have to be applied with this type of viscometer.

Fractionation of the Hemicellulose with Alkali-Ethanol

Hemicellulose (approximately 4 g.) was dissolved in 5% potassium hydroxide (200 ml.) and the solution diluted to about 700 ml. with water and ethanol so that the dissolved polysaccharide just remained in solution. Fractionation was carried out in a 2-liter Erlenmyer flask in a constant temperature bath (25°C.). Ethanol was added dropwise, with stirring, until the solution became cloudy. The solution was then stirred for 10 minutes to insure equilibrium. The first fraction which precipitated was centrifuged off and acetic acid was added to neutralize the potassium hydroxide.

Fourteen fractions in all were collected which, after neutralization, were washed with 80% ethanol, then with anhydrous ethanol, and finally with petroleum ether (B.p. 30-60°C.) and then dried carefully *in vacuo* over calcium chloride at room temperature.

Viscosity measurements were carried out on each of the fractions by dissolving the hemicellulose (75-100 mg.) in exactly 15 ml. of M cupriethylenediamine in Erlenmyer flasks (25 ml.) which were placed on a shaker for 30 to 60 minutes.

The values of the specific viscosity, η_{sp}, the reduced viscosity, $\eta_{sp/c}$, the intrinsic viscosity $[\eta]$, and the number-average degree of polymerization, $\overline{P}n$, were calculated as shown in Table IX. The values of $\overline{P}n$, the

TABLE IX

Fractionation Viscosity Data

Fraction	η_{sp}	$\eta_{sp/c}$	$[\eta]$	$\bar{P}n$
1	0.5501	0.975	0.799	169
2	0.6872	0.984	0.775	164
3	0.6355	1.006	0.805	171
4	0.5163	0.982	0.819	174
5	0.4578	0.939	0.795	168
6	0.5184	0.974	0.811	172
7	0.5283	0.943	0.779	165
8	0.5769	0.946	0.769	163
9	0.5531	0.932	0.764	162
10	0.5273	0.918	0.759	161
11	0.4866	0.845	0.710	151
12	0.4091	0.734	0.633	134
13	0.3416	0.574	0.516	109
14	0.1380	0.322	0.306	65

the weight per cent of each fraction and the accumulative weight per cent of the fractions is given in Table X.

The intrinsic viscosity $[\eta]$ was calculated from the relation:

$$[\eta] = \frac{\eta_{sp/c}}{1 + k' . \eta_{sp}}$$

where k' is a constant.

The number-average degree of polymerization, $\overline{P}n$, was calculated using the relation:

$$\overline{P}n = [\eta] \times 212$$

Graph (Fig. 10) shows the relationship between the accumulative weight per cent of the fractions and the number-average degree of polymerization. The integral and differential distribution curves of the native hemicellulose were thus plotted.

Attempted Preparation of a Fully Acetylated Hemicellulose

The procedure followed for this preparation was that of Carson and Maclay (66,67).

Absolutely dry hemicellulose (2 g.) was swollen in formamide, (dried over sodium sulphate and redistilled shortly before use), in an Erlenmyer flask (250 ml.) for some hours at room temperature. Pyridine, (80 ml.) (dried by refluxing over barium oxide and redistilled shortly before use), was added followed by redistilled acetic anhydride

TABLE X

Fractionation Relation Between Degree of Polymerization and Accumulative Percentage of Weight

Fraction	Weight %	Σ %	$\bar{P}n$
1	1.5	1.5	65
2	3.7	5.2	109
3	3.7	8.9	134
4	7.0	15.9	151
5	36.8	52.7	162
6	22.1	74.8	165
7	4.6	79.4	167
8	14.9	94.3	171
9	5.5	99.8	174

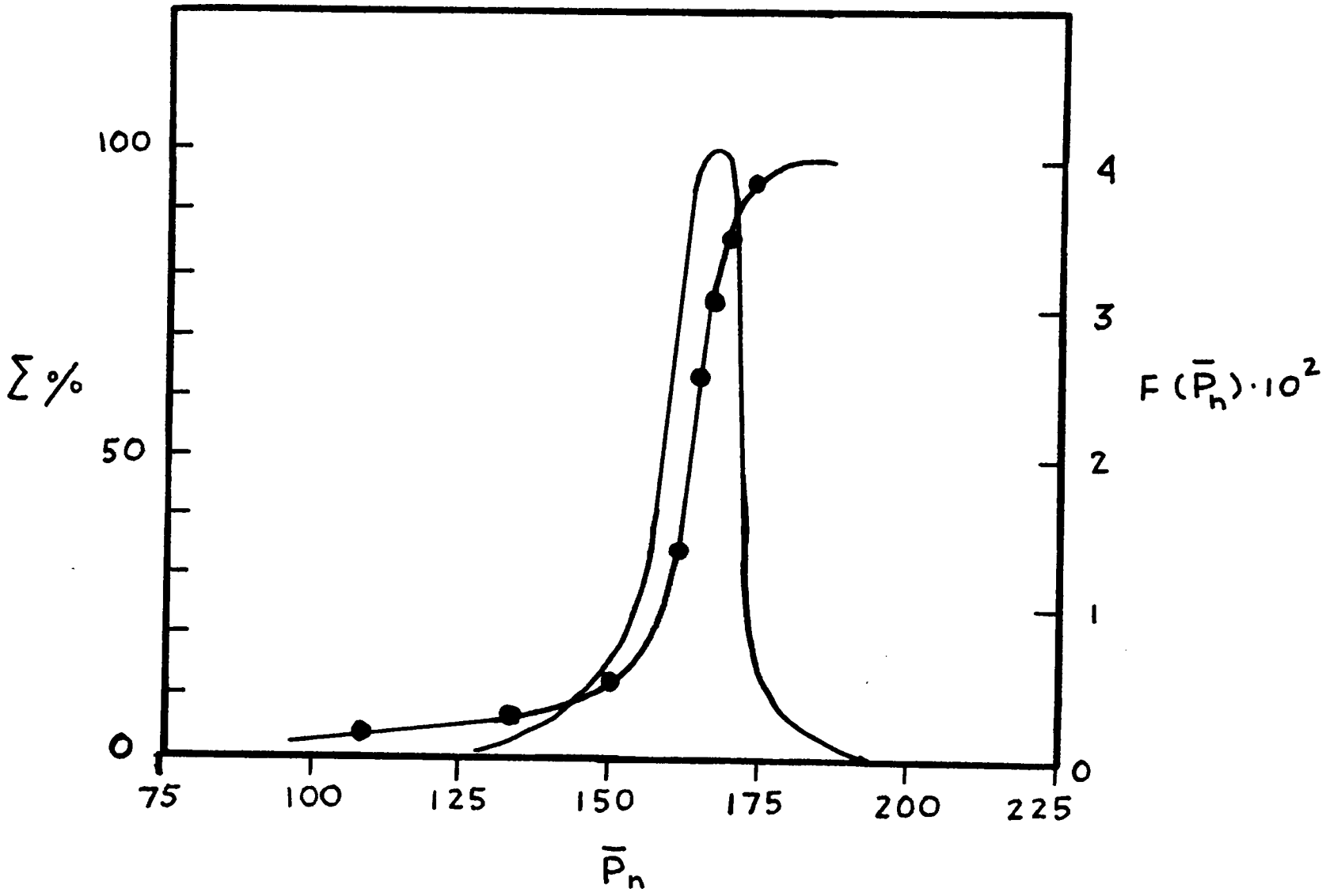


FIG. 10 Integral and Differential Distribution Curves of the Native Hemicellulose

(60 ml.). The reaction was cooled for the first 30 minutes with tap water, then stoppered and placed on a mechanical shaker for several hours at room temperature. The stopper was opened occasionally to the atmosphere. The Erlenmyer flask was then placed in the cold room and opened at least three times a day for the total reaction time (3-5 days).

The acetate was recovered by pouring the mixture with vigorous stirring into ice-water and then filtering on a large Büchner funnel supplied with filter paper. The polysaccharide was washed with 2-3 liters of 2% hydrochloric acid in ice-water, followed by ice-water until neutral. The acetate was solvent exchanged with anhydrous ethanol followed by petroleum ether (B.p. 30-60°C.).

The solubility of the acetate was tested in chloroform and chloroform containing 10% ethanol. Complete solubility was not attained and application of a second acetylation was of no avail.

Hypoiodite Oxidation of the Xylan

This determination was carried out according to the procedure of Hirst, Hough and Jones (33).

To approximately 160 mg. of hemicellulose was added water (10 ml.) and exactly 1 ml. of 0.1N iodine solution. The mixture was buffered at pH 10.6 by addition of a solution of 0.2M sodium hydrogen carbonate and 0.2M

sodium carbonate and the tubes stoppered. Blanks were run concurrently with the duplicate samples. In order to prevent loss of iodine due to evaporation, the stoppers were moistened with a little potassium iodide solution (10%). After 2-2.5 hours in the dark, the solutions were acidified carefully with 2N sulphuric acid and titrated with 0.01N thiosulphate.

Methylation of the Hemicellulose

The first stages of the methylation were carried out according to Chanda et al. (23). Twenty grams of xylan, suspended in 100 ml. water, was placed in a three liter, three-necked, round-bottomed flask fitted with a mechanical stirrer, a nitrogen inlet and an inlet for dimethyl sulphate. A constant pressure of nitrogen, deoxidized by passage through alkaline pyrogallol, was kept in the flask during the total methylation.

The mixture was stirred overnight and then 40% sodium hydroxide (200 ml.) was introduced into the flask, followed by the dropwise addition of dimethyl sulphate (180 ml.) over a 12 hour period. After stirring overnight the procedure was repeated twice. Two more methylations were carried out substituting sodium hydroxide pellets (200 g.) for the 40% sodium hydroxide solution. After stirring for 24 hours the partially methylated product was isolated by neutralization of the alkaline mixture to pH 6 with glacial acetic acid and heating to boiling,

after which the hemicellulose was recovered by filtration and air dried.

The later stages of the methylation were carried out according to the procedure of Falconmer and Adams (35). Tetrahydrofuran (500 ml.) was added to the partially methylated product followed by 40% sodium hydroxide (300 ml.) and dimethyl sulphate (180 ml.). This procedure was repeated once, followed by two methylations using pellets of sodium hydroxide (200 g.). The xylan was isolated in the manner described and then dissolved in 500 ml. 90% tetrahydrofuran. Three methylations were carried out using powdered sodium hydroxide (200 g.) and dimethyl sulphate (180 ml.) for each methylation. At the conclusion of the last methylation water was added, the mixture was neutralized with acetic acid, the tetrahydrofuran was evaporated and the precipitated xylan was collected on a Büchner funnel. It was washed with water (1 liter) and air dried, and was then dissolved in chloroform and precipitated in petroleum ether (B.p. 60-120°C.) after which the ether was decanted off and the material was dried <u>in vacuo</u>.

A modified methylation according to Kuhn et al. (36) was carried out by dissolving the methylated polysaccharide in dry tetrahydrofuran (200 ml.) and adding 500 ml. dimethyl formamide (dried over sodium sulphate and redistilled shortly before use), methyl iodide (10 ml.)

and silver oxide (10 g.). The reaction was carried out in a round-bottomed flask (1 liter) which was agitated on a mechanical shaker in the dark for 24 hours.

For isolation, the reaction mixture was poured into excess chloroform and the salts (36) which separated were removed by filtration. The chloroform layer was stirred at room temperature for 30 minutes with an aqueous solution of potassium cyanide (5 g.) in 500 ml. of water. After separation of the two layers, the chloroform solution was washed with water (800 ml.). The chloroform layer was again separated and dried over calcium sulphate for 12 hours. The fully methylated xylan was precipitated by pouring the chloroform solution (200 ml.) slowly, with vigorous stirring, into 3.5 liters of petroleum ether (B.p. 65-110°C.). The methylated xylan was washed thoroughly with petroleum ether and dried in vacuo. Yield: 14 g.

An infra-red spectrum (Fig. 13), taken on the methylated xylan, using the potassium bromide pellet technique was found to be free of hydroxyl band absorption.

Anal. Calcd. for the sodium salt of the hemicellulose: OMe, 37.9% Found: OMe, 37.9%

$[\alpha]_D^{20}$ -76.8° (c, 1.3 in chloroform)

Osmotic Pressure Measurements on the Fully Methylated Xylan

Five samples of methylated xylan, ranging from 70 mg. to 160 mg., were used for osmometry. They were dissolved in approximately 35 g. of a chloroform-ethanol mixture (9:1, v/v). The osmotic pressure, π, was determined at each of these concentrations and the values for the reduced osmotic pressure, π/C, were extrapolated to zero concentration giving $(\pi/C)_o$ from which the number-average molecular weight of the methylated polymer was calculated.

The osmometers used were of the type designed by Zimm and Myerson (37) and later improved by Stabin and Immergut (38). The cells were filled using hypodermic needles and mercury was employed for closing the instrument completely during measurements. The static method was used throughout, equilibrium being established within 3-4 hours. The membranes were prepared from cellophane which had never been dried and were received directly from the manufacturer (68). The measurements were thermostatically controlled at a temperature of 30 $\pm$ 0.01°C.

Methanolysis and Separation of the Neutral and Acidic Fractions of the Fully Methylated Xylan

Methylated hemicellulose (6.5 g.) was added to 2% methanolic hydrogen chloride (250 ml.) and the mixture refluxed for 10 hours. The solution of methyl glycosides was neutralized with silver carbonate, filtered through

Celite, treated with hydrogen sulphide and again filtered through Celite. The methanolic solution was then evaporated to a sirup.

Separation of the neutral from the acidic glycosides was accomplished by adding 25 ml. aqueous barium hydroxide (saturated at room temperature) to the above sirup, and heating at 60°C. for 2 hours, according to the procedure of Dutton and Smith (31). The solution, after neutralization with carbon dioxide, was filtered to remove insoluble barium carbonate. It was then passed successively through columns of Amberlite IR-120 and Dowex 1-4X (acetate form), the neutral fraction being collected in the aqueous eluate and concentrated to a sirup for hydrolysis.

The acidic fraction was eluted from the Dowex column with 3N acetic acid (40), concentrated to a sirup on the film evaporator and dried over potassium hydroxide in vacuo. Yield: 0.739 g.

Identification of the Acidic Fraction of the Methylated Xylan

The acidic methyl glycosides (0.643 g.) were refluxed with 2.5% methanolic hydrogen chloride (50 ml.) for 6 hours. The solution was neutralized with silver carbonate, treated with hydrogen sulphide and filtered. The filtrate was concentrated to a sirup which was dried

in vacuo over calcium chloride. Yield: 0.601 g.

The dried sirup was dissolved in 20 ml. of ether (distilled from lithium aluminum hydride) and added slowly to a mixture of ether (20 ml.) and lithium aluminum hydride (1.0 g.) according to the procedure of Abdel-Akher and Smith (69). The reaction mixture was refluxed for 2 hours, after which the lithium aluminum hydride was destroyed with ethyl acetate, followed by water. The ethyl acetate was evaporated, ethanol (200 ml.) was added and the salts were removed by filtration. The solution was deionized with Amberlite IR-120 and Dowex 1-4X (acetate form) exchange resins and then evaporated to a thick sirup. Yield: 0.571 g.

The glycosides were hydrolysed with N sulphuric acid (40 ml.) for 3.5 hours; the solution was cooled and then adsorbed on a cocoanut charcoal column. The sulphuric acid was almost completely removed from the column using 1.5-2% ethanol (1.5 liters). The sugars were eluted with 5-20% ethanol and neutralized with a dilute solution of barium hydroxide. The fractions were characterized chromatographically using solvent systems (E),(F),(G), and authentic samples of 3-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-glucose as reference compounds. The results are presented in Table XI.

TABLE XI

Chromatographic Separation of 3-O-Methyl-D-Xylose and 2,3,4-Tri-O-Methyl-D-Glucose on a Cocoanut Charcoal Column

Fraction	Ethanol,%	Chromatographic Analysis	Weight of Fraction, mg.
1	5	Pure 3-O-methyl-D-xylose	165
2	10	3-O-methyl-D-xylose plus unreduced methylated acid (trace)	34.9
3	15-20	Small amounts of 2,3,4-tri-O-methyl-D-glucose and unreduced methylated acid.	

The R_f values using system (E) for the 3-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-glucose from the hydrolysate were 0.455 and 0.709, respectively.

Fraction 1 was used for identification of the mono-O-methyl xylose. A sample of this fraction was used for two ionopheresis chromatograms irrigated with the Lindberg borate buffer (41) and run at 745 volts for 4.5 hours. Authentic samples of 3-O-methyl-D-xylose and 2-O-methyl-D-xylose were spotted on the same chromatogram. It was found that the mono-O-methyl xylose gave one large cherry-red spot corresponding to the position of 3-O-methyl-D-xylose with $R_{2\text{-O-methyl-D-xylose}}$ of 1.61 and 1.67.

Anal. Calcd. for $C_6H_{12}O_5$: OMe, 18.9% Found: OMe, 18.2%

$[\alpha]_D^{20}$ +14.7° (c, 5.5 in water)

The literature quotes (70) +14.8°.

An infra-red spectrum (Fig. 14) of 3-O-methyl-D-xylose was taken by placing the sirup between sodium chloride crystals. This spectrum was found to be identical to that of an authentic sample of 3-O-methyl-D-xylose. The 3-O-methyl-N-phenyl-D-xylosylamine was prepared by refluxing a sample of fraction 1 (109 mg.) with ethanol (3 ml.) and aniline (0.6 ml.) for 4 hours. The ethanol and part of the aniline were evaporated at 50°C. and the rest of the aniline was removed in vacuo over phosphorous pentoxide. Crystallization of the anilide took place after removal of the aniline; m.p. 132-133°C. After recrystallization from ethyl acetate the derivative had m.p. and mixed m.p. 134-135°C. Reported value (29): 136°C.

A second portion of the acidic fraction isolated from the methylated xylan was treated in the above manner with the addition of a methanolysis step before hydrolysis. Chromatographic analysis showed only two spots of approximately equal intensity corresponding to 3-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-glucose. These sugars were separated on a cocoanut charcoal column as described, giving a yield of 152 mg. of 2,3,4-tri-O-methyl-D-glucose.

Anal. Calcd. for $C_9H_{18}O_6$: OMe, 41.9% Found OMe, 40.9%

$[\alpha]_D^{20}$ +69.8° (c, 1.89 in water)

Hydrolysis of the Neutral Glycosides from the Fully Methylated Xylan

The neutral glycosides were hydrolysed with N sulphuric acid (50 ml.) for 7 hours, and after neutralization with barium hydroxide and deionization with Dowex 1-4X and Amberlite IR-120, the solution was evaporated to a sirup and dried *in vacuo* over phosphorous pentoxide. Yield: 5.87 g.

The sirup was chromatographed in systems (D),(F), (G), against authentic samples of 2,3-di-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-xylose. Three spots only were evident on the chromatogram; one very heavy spot corresponding to 2,3-di-O-methyl-D-xylose and two faint spots corresponding to 2,3,4-tri-O-methyl-D-xylose and a mono-O-methyl xylose.

Determination of the Relative Ratio of the Methyl Derivatives

The ratio of the neutral methylated sugars obtained from the fully methylated xylan was determined according to the method of Timell et al. (32). The sirup containing the neutral sugars was diluted to 150 ml. and six spots of 20 λ were applied, using an ultramicroburette, to each of three Whatman No. 1 papers, 18 cm. wide, which had been washed with solvent system (F) for 10 hours. The spotted papers were then run in solvent system (F) for 6 hours and dried. The two marginal strips (each containing a spot) were cut off and used as a guide for the relative positions of the three sugars. The mono-, di- and tri-O-methyl sugars were eluted from the papers with water and

diluted to a volume of exactly 5, 206 and 5 ml. respectively. Three 1 ml. aliquots of each sugar were placed in test tubes (20 ml.) fitted with ground-glass stoppers. Five ml. of a solution of 1.000 g. o-aminodiphenyl in 250 ml. glacial acetic acid was added to each sample. Three blanks consisting of 1 ml. of water and 5 ml. of o-aminodiphenyl solution were included with the samples. The tubes were stoppered and placed in boiling water for 2 hours. The transmission, as compared to one of the boiled blanks, was measured for each of the samples on a Beckmann D.U. spectrophotometer at a wave length of 380 mμ. The results are recorded in the following table.

TABLE XII

Spectrophotometric Determination of the Relative Ratios of Neutral Methylated Sugars

Sugar	% Transmission *)	Absorbance	μg. sugar	Molar Ratio
Mono	76.4	0.1169	154.9	0.82
Di	83.8	0.0768	7910	38.6
Tri	81.9	0.0867	221.1	1.0

*) Average of three determinations.

For determining the micrograms (μg.) of sugar in each case, the product of the absorbance and dilution was multiplied by a constant factor for the mono-, di-

and tri-O-methyl xyloses calculated from standard curves. These values are mono-O-methyl xylose 265, di-O-methyl xylose 500, and tri-O-methyl xylose 510.

Separation and Identification of the Neutral Methyl Derivatives Isolated from the Fully Methylated Xylan

For separation of a portion of the mono-O-methyl xylose, eleven sheets of Whatman No. 1 filter paper, 7¾ inches wide, were spotted with the neutral sugar hydrolysate and irrigated with solvent system (F) for 6 hours. The mono-O-methyl xylose was eluted from the sheets with water and the solution evaporated to dryness. The sirup was dissolved in chloroform and the precipitated polysaccharides from the filter paper (71,72) were removed by filtration. The chloroform was removed by evaporation and the resulting sirup redissolved in 2-3 drops of water. The sample was subjected to paper ionopheresis for 7 hours at 300-500 V. with the Lindberg borate buffer (41). Standards of 2,3,4,6-tetra-O-methyl-D-glucose and 3-O-methyl-D-xylose were spotted on the same paper. After seven hours the paper was dried and sprayed with a solution of o-aminodiphenyl. Two spots were evident; one moving at the same rate as 3-O-methyl-D-xylose and the other (2-O-methyl-D-xylose) moving slightly above the base line as defined by the position of 2,3,4,6-tetra-O-methyl-D-glucose. The 3-O-methyl-D-xylose and 2-O-methyl-D-xylose were present in approximately equal amounts by visual estimation. The

relative positions of the two mono-O-methyl xyloses, using 2,3,4,6-tetra-O-methyl-D-glucose as a base line, and measuring the distances from the center of each spot, were:

2-O-methyl-D-xylose: 1.8 cm. above 2,3,4,6-tetra-O-methyl-D-glucose

3-O-methyl-D-xylose: 8.3 cm. below 2,3,4,6-tetra-O-methyl-D-glucose

The 2,3-di-O-methyl-D-xylose was separated from the neutral hydrolysate on 24 sheets of Whatman No. 1 paper, $7\frac{3}{4}$ inches wide, using system (G). The sugar was eluted from the paper with water and the aqueous solution evaporated to a sirup and dried <u>in vacuo</u> over silica gel. Ethanol (10 ml.) was added to the sirup and the insoluble polysaccharides from the filter paper were removed by filtration. This procedure was repeated to remove all the contaminating polysaccharides. The ethanol was removed by evaporation and the sirup was dried. Yield: 0.808 g.

$$[\alpha]_D^{20}$$ +20.5° (c, 1.4 in water)

The literature quotes (73) +22° ±2°.

The 2,3-di-O-methyl-N-phenyl-D-xylosylamine was prepared by dissolving the sirup (0.452 g.) in ethanol (5 ml.) and aniline (0.8 ml.). The solution was heated

on a water bath for 4 hours. The ethanol was evaporated and ethyl acetate (5 ml.) was added. Since crystallization did not occur, the solution was evaporated to an aniline sirup and ethanol (5 ml.) was added to the latter. The solution was refluxed for 4 hours. After evaporation of the ethanol and part of the aniline, the derivative crystallized on standing, m.p. 124-125°C. Recrystallized from ethyl acetate, it had m.p. and mixed m.p. 125-126°C.

$$[\alpha]_D^{20} +180.3^\circ \text{ (c, 0.6 in ethyl acetate)}$$

The literature quotes (74) +180°.

An infra-red spectrum (Fig. 19) of the aniline derivative was identical to that of an authentic specimen.

An attempted isolation of 2,3,4-tri-O-methyl-D-xylose from the neutral hydrolysate was carried out on sheets of Whatman No. 3 m.m. paper, 7¾ inches wide, in system (G), and also by extraction of the hydrolysate with chloroform. Unfortunately, this derivative could not be isolated in a sufficient quantity from the contaminent of 2,3-di-O-methyl-D-xylose for a complete identification.

Separation of the Aldobiouronic Acid from the Acid Fraction from the Hemicellulose Hydrolysate

The aldobiouronic acid was separated on large sheets of Whatman No. 1 paper (18 inches by 22½ inches) by irrigation with solvent system (A) for 30 hours. Elution of the strips with water and concentration of the eluate to a sirup gave a yield of 111.0 mg. of aldobiouronic acid.

Reduction and Hydrolysis of the Aldobiouronic Acid

The methyl ester-methyl glycoside of the aldobiouronic acid was prepared by refluxing the dried sirup with 2% dry methanolic hydrogen chloride (50 ml.). The mixture was neutralized with silver carbonate, and, after removal of the silver chloride, the excess silver remaining in solution was precipitated as silver sulphide after passage of hydrogen sulphide through the solution for six minutes. The precipitate was filtered through Celite and washed with dry methanol, after which the filtrate and washings were concentrated to a sirup on the film evaporator. The sirup was dried over phosphorous pentoxide *in vacuo* for three days at room temperature.

The dried sirup was dissolved in 20 ml. of tetrahydrofuran (dried over sodium wire) by refluxing five to ten minutes. This solution was added dropwise to a mixture of 2 g. of lithium aluminum hydride (69)

and dry tetrahydrofuran contained in a four-necked, 500 ml. round-bottomed flask. A constant head of nitrogen (deoxidized and dried by passage through alkaline pyrogallol and sulphuric acid) was kept in the flask throughout the reaction. After addition of the sugar solution, the reaction mixture was stirred for 1 hour and the lithium aluminum hydride was decomposed by cautious addition of ethyl acetate. Water was added and the resulting aluminum hydroxide was filtered off. The filtrate was deionized by passage through Amberlite IR-45 and Amberlite IR-120 exchange resins, concentrated to a sirup and dried overnight in vacuo over phosphorous pentoxide.

The sirup was hydrolysed with N sulphuric acid by refluxing for 12 hours. The solution was neutralized to pH 5 with barium hydroxide and concentrated to a small volume.

Chromatographic analysis in systems (B) and (C) (36 and 24 hours, respectively) revealed only two spots corresponding to xylose and 4-O-methyl glucose as compared to authentic samples of the two sugars. The spot corresponding to 4-O-methyl glucose had R_x values of 1.23 and 1.27, respectively, in the above systems and was found to run directly above a spot corresponding to an authentic sample of 3-O-methyl-D-glucose.

Isolation of the Uronic Acids from Milkweed Floss

Milkweed floss (500 g.), which had been previously extracted with ethanol-benzene (1:2) and ground to 20 mesh in a Wiley mill, were hydrolysed with 72% sulphuric acid (1400 ml.) for 1.5 hours at room temperature. The solution was diluted to 3% acid concentration and refluxed for 6 hours, after which it was cooled to 0°C. and neutralized with barium hydroxide to pH 5 (pH-meter). Barium sulphate was removed by filtration through Celite. The filtrate (5 liters) was passed successively through columns of Amberlite IR-120 and Dowex 1-4X (250 g.), (bicarbonate form) exchange resins. The Dowex column was washed until free of neutral sugars as indicated by the Molisch test. A small portion of the aqueous eluate was chromatographed in system (B) showing only two spots, corresponding to glucose and xylose.

The uronic acids were eluted from the Dowex 1-4X column with N sulphuric acid (1.5 liters). The eluate was cooled to 0°C. and neutralized to pH 5 with barium hydroxide. The insoluble barium sulphate was removed as before and the filtrate was concentrated to approximately 100 ml. Chromatographic analysis of the filtrate in system (B) indicated that absolutely no neutral sugars were present in this fraction. In system (A), spots corresponding to the following sugars were evident: a triouronic acid, an

aldobiouronic acid, galacturonic acid, 4-O-methyl glucuronic acid and spots which remained near the starting line (believed to be higher uronides).

Reduction of the Uronic Acid Fraction

A portion of the uronic acid fraction (15 ml.) was evaporated to a sirup and dried over silica gel *in vacuo*. The dried sirup was refluxed for 5 hours with 2% methanolic hydrogen chloride (60 ml.) and then neutralized with silver carbonate (5 g.). The silver chloride was removed by filtration and the excess silver dissolved in the filtrate was removed with hydrogen sulphide. The insoluble silver sulphide was removed by filtration through Celite and the filtrate was concentrated to a sirup which was dried *in vacuo* to constant weight over silica gel.

The sirup (1.70 g.) was dissolved in dry tetrahydrofuran (redistilled from lithium aluminum hydride) and added slowly to a mixture of dry tetrahydrofuran (60 ml.) and lithium aluminum hydride (3 g.). The reaction mixture was refluxed for 1 hour and then placed on a mechanical shaker for 3 hours at room temperature. The lithium aluminum hydride was destroyed by cautious addition of ethyl acetate, followed by water. The tetrahydrofuran was evaporated, leaving a mixture of ethyl acetate and water from which the water layer was separated. The aqueous layer, after deionization by passage through Amberlite IR-120 and Dowex 1-4X (bicarbonate), was evapo-

rated to a sirup.

Hydrolysis and Characterization of the Reduced Uronic Acid Fraction

The above sirup was dissolved in N sulphuric acid (75 ml.) and refluxed for 6 hours. The hydrolysate was neutralized to pH 5 with barium hydroxide, and the barium sulphate removed by filtration through Celite. The filtrate was concentrated to a sirup on the film evaporator. Chromatographic analysis in system (A) revealed three spots corresponding to 4-O-methyl-D-glucose, xylose and galactose.

Separation of the 4-O-methyl-D-glucose from xylose and galactose was effected by dissolving the above sirup in water (20 ml.) and percolating the solution through a cocoanut charcoal column. The sugars were adsorbed on the column and then eluted preferentially with aqueous ethanol containing an increasing amount of ethanol. The fractions (500 ml.) from the column were collected and portions (15 ml.) of these were evaporated and chromatographed in system (A). The results of the fractionation are given in Table XIII.

TABLE XIII

Chromatographic Separation of Galactose and D-Xylose from 4-O-Methyl-D-Glucose on a Cocoanut Charcoal Column

Fractions	Ethanol, %	Components
1-4	1.5	Galactose and xylose
5-6	1.5	Xylose, galactose and 4-O-methyl glucose
7	1.5	4-O-methyl glucose and trace xylose and galactose
8-12	5 and 10	Pure 4-O-methyl glucose

Fraction seven (262 mg.) was evaporated to a sirup and dried *in vacuo* over phosphorous pentoxide in an Abderhalden drying apparatus. Fractions 8-12, used for the identification of 4-O-methyl-D-glucose, were combined and evaporated to a sirup. Yield: 252 mg.

Anal. Calcd. for $C_7H_{12}O_6$: OMe, 16.0% Found: OMe, 16.0%

$[\alpha]_D^{20}$ +47° (initial) to +51.6° (final) (c, 1.0 in water)

The literature quotes (70) +53°.

The osazone of 4-O-methyl-D-glucose (75) was prepared by heating 78.0 mg. of the sirup at 90°C. for 2 hours

in a solution of water (2 ml.), glacial acetic acid (0.3 ml.) and phenylhydrazine (0.5 ml.). The 4-O-methyl-D-glucosazone separated on cooling. The derivative was recrystallized from ethanol, m.p. and mixed m.p. 157-158°C. An infrared spectrum (Fig. 12) of the osazone was taken using the potassium bromide pellet technique. The spectrum was found to be identical to that of an authentic specimen synthesized from glucose (76).

The fractions containing the neutral sugars were combined and the xylose separated from the galactose on 60 sheets of Whatman No. 1 filter paper, 7¾ inches wide, using system (A). The xylose was eluted from the papers with water and purified from soluble polysaccharides in the filter paper by passage through a carbon column with 1.5% ethanol. The xylose was evaporated to a sirup (188 mg.) which crystallized on standing at room temperature, m.p. 143-145°C., and mixed m.p. 145°C.

$$[\alpha]_D^{20} +18.1^\circ \text{ (c, 2.0 in water)}$$

Partial Separation of Uronic Acids on a Dowex 1-4X Acetate Column

The remainder of the uronic acid fraction was diluted with water (3 liters) and adsorbed on a Dowex 1-4X acetate column 5.5 cm. in diameter by 110 cm. in length. Slow elution of the column with N acetic acid (40) followed by 3N acetic acid gave the following results using system (A) for chromatographic analysis (Table XIV).

TABLE XIV

Partial Separation of Uronic Acids

on a Dowex 1-4X (Acetate) Column

Fraction	Acetic Acid Concentration	Components
1	N	Triouronic acid, aldobiouronic acid and galacturonic acid
2	3N	Aldobiouronic acid 80%, 4-O-methyl glucuronic acid 10%, triouronic acid 10%
3	3N	Large percentage of higher uronides, triouronic acid, galacturonic acid and aldobiouronic acid
4	3N	4-O-methyl-D-glucuronic acid, galacturonic acid and higher uronides
5	3N	4-O-methyl-D-glucuronic acid and galacturonic acid

Separation and Identification of the Aldobiouronic and Aldotriouronic Acids

Fraction 2 was adsorbed on a cocoanut charcoal column 4 cm. in diameter and 12 cm. in length. The column was eluted with aqueous ethanol of increasing concentrations up to a concentration of 4% when the first traces of sugar were removed as detected by the Molisch test. The column was then placed on a fraction collector* which was regulated at the rate of one test tube (20 ml.) per twelve minutes. The results of the fractionation, as obtained by chromatography with system (A), are given in Table XV.

The fractions containing pure aldobiouronic acid were evaporated to a clear, colorless sirup.

Anal. Calcd. for $C_{12}H_{20}O_{11}$: OMe, 9.1% Found: OMe, 8.6%

$[\alpha]_D^{20}$ +103° (0 hours) to +108° (24 hours constant)
(c, 4.3 in water)

The equivalent weight of the aldobiouronic acid was obtained by adding an accurately measured volume of 0.01N NaOH (20 ml.) to the acid and titrating the excess of alkali with 0.01N HCl using phenolphthalein as indicator.

Calcd. for $C_{12}H_{20}O_{11}$: equiv. wt., 340
Found: equiv. wt., 379

*) Product of Scientific Glass Co., Bloomfield, New Jersey

TABLE XV

Separation of Uronic Acids on a Cocoanut Charcoal Column

Tube No.	Concentration of Ethanol, %	Components	Weight of Fraction, g.
1- 327	4	Triouronic acid and trace of higher uronides	0.200
328- 350	7	Aldobiouronic acid and trace of triouronic acid	0.174
351-1046	7	Pure aldobio-uronic acid	1.128
1047-1260	15	4-O-methyl glucuronic acid and aldobiouronic acid	0.450
1261-1600	15	Pure aldobiouronic acid	0.527

Extraction of the carbon column with 100% ethanol for 72 hours using a Soxhlet extractor gave 0.173 g. aldobiouronic acid.

An infra-red spectrum (Fig. 16) was taken of the aldobiouronic acid using the potassium bromide pellet technique and was found to be identical to that of an authentic sample of 2-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-xylopyranose.

Methylation of the aldobiouronic acid and identification of the methylated products were carried out according to the following procedure.

Aldobiouronic acid (1.1 g.) was refluxed with 2% methanolic hydrogen chloride (50 ml.) for 8 hours. The solution was neutralized with silver carbonate and the salts were removed by filtration through Celite. The Celite was washed with dry methanol and the filtrate and washings were treated with hydrogen sulphide. The insoluble silver sulphide was removed by filtration through Celite and the latter washed with dry methanol. The filtrate and washings were combined and evaporated to a sirup which was dried <u>in vacuo</u> over calcium chloride.

The methyl ester-methyl glycoside of the aldobiouronic acid was methylated according to Kuhn et al. (36) in the following manner.

Dimethyl formamide (30 ml.) which had been dried over sodium sulphate and redistilled was added to the above sirup in a 300 ml. round-bottomed flask.

Methyl iodide (5 ml.), which had been stored over Drierite, and silver oxide were added and the flask was stoppered tightly. The flask was agitated on a mechanical shaker for 12 hours, after which time excess chloroform (700 ml.) was added. The resulting salts (36) and silver oxide were collected on the centrifuge and the chloroform solution was evaporated to a sirup which was dried in vacuo. This procedure was repeated twice.

After the third methylation, the methylated aldobiouronic acid was isolated by pouring the mixture into chloroform (400 ml.). The salts were removed on the centrifuge, and the chloroform was extracted six times with water (50 ml.) and once with 100 ml. of water to remove dimethyl formamide. The chloroform was evaporated to a sirup and then dried in vacuo over silica gel for elimination of the last traces of chloroform and dimethyl formamide. Yield: 1.11 g.

The infra-red spectrum (Fig. 15) of the methylated aldobiouronic acid showed no absorption for hydroxyl groups. It was identical to that of an authentic sample of methyl 2-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-3,4-di-O-methyl-D-xylopyranoside methyl ester.

The dried sirup was dissolved in diethyl ether (25 ml.) which had been distilled with lithium aluminum

hydride. This solution was added dropwise to diethyl ether (100 ml.) containing lithium aluminum hydride (2 g.). The mixture was refluxed for 2 hours, after which ethyl acetate was added, followed by water. The ethyl acetate was evaporated and the volume of water increased to 200 ml. Water and salts were continuously extracted with chloroform for 48 hours. The chloroform layer was separated and evaporated to a thick sirup which was dried in vacuo over calcium chloride. Yield: 0.886 g.

Methanolysis of the fully methylated and reduced aldobiouronic acid was effected by refluxing the above sirup in 6% methanolic hydrogen chloride (60 ml.) for 12 hours. The methanol was then removed and a N solution of hydrogen chloride (60 ml.) was added. The solution was refluxed for 12 hours and then neutralized with silver carbonate. The filtrate was treated with hydrogen sulphide, and, after removal of silver sulphide, was deionized with Amberlite IR-120 and Dowex 1-4X (acetate form) resins. Evaporation of the solution yielded a thick colorless sirup. Yield: 0.564 g.

Chromatographic analysis of the sirup in system (D) (24 hours) gave spots corresponding to 3,4-di-O-methyl xylose and 2,3,4-tri-O-methyl-D-glucose, in addition to traces of lower methylated sugars.

Separation of the sugars was carried out by diluting the above sirup with water (50 ml.) and adsorbing the sugars on a cocoanut charcoal column (2 cm. in diameter and 10 cm. in length). The column was placed on a fraction collector and the sugars were eluted from the column with increasing concentrations of aqueous ethanol. The eluate was collected in test tubes (20 ml.) and every fifth tube was analysed by taking a 5 ml. aliquot, evaporating it to dryness, and chromatographing it in system (D) against the reference compounds of 2,3,4-tri-O-methyl-D-glucose and 2,3-di-O-methyl-D-xylose. The results of the fractionation are given in Table XVI.

The tubes containing pure 3,4-di-O-methyl-D-xylose were evaporated to dryness giving 0.123 g. of a clear colorless sirup.

Anal. Calcd. for $C_7H_{14}O_5$: OMe, 34.8% Found: OMe, 34.6%

$[\alpha]_D^{20}$ +21.8° (equilibrium) (c, 1.0 in water)

An infra-red spectrum (Fig. 17) was taken with the dried sirup placed on a sodium chloride prism.

The 3,4-di-O-methyl-D-xylose was distinguished from 2,3-di-O-methyl-D-xylose by paper ionopheresis, using 2,3-di-O-methyl-D-xylose as a reference compound. The two compounds were spotted on Whatman No. 1 filter paper

TABLE XVI

Separation of 3,4-Di-O-Methyl-D-Xylose and 2,3,4-Tri-O-Methyl-D-Glucose on a Cocoanut Charcoal Column

Tube No.	Ethanol Concentration, %	Components
1- 5	5	--
6- 15	5	Small amount of undermethylated products
16- 40	5	--
41- 42	10	Traces undermethylated products and 3,4-di-O-methyl-D-xylose
43- 63	10	Pure 3,4-di-O-methyl-D-xylose
64-110	15	--
111-130	25	Pure 2,3,4-tri-O-methyl-D-glucose

and separated with the Lindberg borate buffer (41) for 5 hours at 500 V. It was found that the 3,4-di-O-methyl-D-xylose sample, which was isolated from the fully methylated aldobiouronic acid, migrated toward the anode, while the 2,3-di-O-methyl-D-xylose remained stationary at the starting line, giving a clear cut separation of the two sugars. In a second ionopheresis experiment, 2,3,4,6-tetra-O-methyl-D-glucose (77) and 2,3-di-O-methyl-D-xylose were used as standards. The 3,4-di-O-methyl-D-xylose was easily separated from the 2,3-di-O-methyl-D-xylose which remained almost at the starting line as designated by the position of the 2,3,4,6-tetra-O-methyl-D-glucose. The 3,4-di-O-methyl-D-xylose moved 4.5 cm. from the starting line, while the 2,3-di-O-methyl-D-xylose moved only 0.5 cm.

The 3,4-di-O-methyl-N-phenyl-D-xylosylamine (78) was prepared by adding ethanol (2.5 ml.) and aniline (0.5 ml.) to 57 mg. of the sirup and refluxing for 3.5 hours. The ethanol was evaporated and the aniline was removed under high vacuum. Crystallization of the aniline derivative took place after removal of the aniline, m.p. 113°C. The derivative was recrystallized from 90% petroleum ether (B.p. 38.5-50°C.) and 10% ethyl acetate, m.p. 115°C.

The tubes containing pure 2,3,4-tri-O-methyl-

D-glucose were evaporated to a clear colorless sirup weighing 298 mg.

Anal. Calcd. for $C_9H_{18}O_6$: OMe, 41.9% Found: OMe, 41.6%

$[\alpha]_D^{20}$ +75.7° (initial) to +76.9° (equilibrium) (c, 2.6 in water)

An infra-red spectrum (Fig. 22) of this compound was taken with the sirup placed on a sodium chloride prism.

The 2,3,4-tri-O-methyl-N-phenyl-D-glucosylamine (79) was prepared by refluxing the sirup (75 mg.) with ethanol (2.5 ml.) and aniline (0.5 ml.) for 2¼ hours. The ethanol was evaporated and the aniline was removed under high vacuum. The aniline derivative crystallized after removal of the aniline under high vacuum (3 days) m.p. 142°C. The derivative was recrystallized from ethyl acetate m.p. 145-146°C. An infra-red spectrum (Fig. 21) of the derivative was taken using the potassium bromide technique.

The tubes containing pure aldotriouronic acid were evaporated to a sirup which partially crystallized after standing at room temperature for a few days. A portion of the acid (5 mg.) was transferred to glass tubing (4 cm. in length and 0.3 m.m. in diameter) having a small glass bulb blown in one end of the rod. After

the sirup had been placed in the bulb-end of the rod, 0.5N sulphuric acid (0.5 ml.) was added. The other end of the rod was then drawn out to a fine tip and sealed. The glass vial was suspended from a copper wire running through a straight condenser. A round-bottomed flask (50 ml.) containing enough water to immerse the glass bulb was attached to the condenser. The solution was hydrolysed under reflux for 12 hours. The vial was then removed from its copper wire holder and the fine capillary broken. The vial was inverted and the solution spotted directly on Whatman No. 1 filter paper which was irrigated with system (A) for 24 hours. Two authentic standards of xylose and 2-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-xylopyranose were spotted on the same paper as the sample.

Chromatographic analysis of the hydrolysate showed four spots corresponding to the unhydrolysed aldotriouronic acid (R_x = 0.31), the above aldobiouronic acid, D-xylose and 4-O-methyl-D-glucuronic acid.

Anal. Calcd. for $C_{17}H_{28}O_{15}$: OMe, 6.6% Found: OMe, 5.8%

$[\alpha]_D^{20}$ (aldotriouronic acid) +85.9° (0 hours) to +90.8° (equilibrium) (22 hours) (c, 3.7 in water)

Separation and Identification of Galacturonic Acid and 4-O-Methyl-D-Glucuronic Acid

Pure 4-O-methyl-D-glucuronic acid (195 mg.) was obtained by adsorbing fraction 4 from the initial separa-

tion of uronic acids (on the Dowex column), on a cocoanut charcoal column, 2 cm. in diameter and 8.5 cm. in length, and eluting with 4% aqueous ethanol. Higher concentrations of ethanol yielded mixtures of polyuronides, aldobiouronic acid, 4-O-methyl-D-glucuronic acid and galacturonic acid.

The equivalent weight of the pure sample of 4-O-methyl-D-glucuronic acid was obtained by taking an accurately weighed portion of the acid and dissolving it in 0.01N NaOH (10 ml.). The solution was kept at room temperature for 2 hours, after which the excess alkali was titrated with 0.01N HCl.

Anal. Calcd. for $C_7H_{12}O_7$: equiv. wt. 208 Found: equiv. wt. 194

$[\alpha]_D^{20}$ +42.7° (c, 1.2 in water)

The literature quotes (80,47,81) +45°.

Fraction 5 containing 4-O-methyl-D-glucuronic acid and galacturonic acid, as shown by chromatographic analysis, was combined with the remainder of fraction 4 and both were evaporated to a sirup and dried in vacuo over potassium hydroxide. Yield: 0.540 g.

The dried sirup was dissolved in 2% methanolic hydrogen chloride (60 ml.) and refluxed for 6 hours. The solution was neutralized with silver carbonate and

the salts removed. The filtrate was treated with hydrogen sulphide, concentrated to a sirup and dried *in vacuo* over calcium chloride.

Tetrahydrofuran (50 ml.), previously distilled from lithium aluminum hydride, was added to the dried sirup. The solution was introduced slowly into a mixture of tetrahydrofuran (100 ml.) and lithium aluminum hydride (2 g.). The reaction mixture was refluxed for 1 hour and left overnight at room temperature. The lithium aluminum hydride was decomposed with aqueous ethyl acetate, followed by water. The ethyl acetate and tetrahydrofuran were removed by evaporation, and the salts were centrifuged off and washed with water (3 times) and 50% ethanol (once). The aqueous solution was evaporated to a small volume and deionized with Amberlite IR-120 and Dowex 1-4X (acetate form). The eluate was then concentrated to a thick sirup.

Hydrolysis of the reduced sugars was carried out by dissolving the sirup in N hydrochloric acid (50 ml.) and refluxing for 6.5 hours. After neutralization with silver carbonate, the hydrolysate was chromatographed in system (A) using authentic samples of galactose and 4-O-methyl-D-glucose as standards. Two spots from the hydrolysate corresponding to galactose and 4-O-methyl-D-glucose were observed.

The two sugars were separated by adsorbing them on a cocoanut charcoal column, 6 cm. in length and 2 cm. in width. The aqueous eluate (400 ml.) yielded galactose, while elution of the charcoal column with 500 ml. of ethanol gave pure 4-O-methyl-D-glucose (183 mg.).

Anal. Calcd. for $C_7H_{12}O_6$: OMe, 16.0% Found: OMe, 16.0%

$[\alpha]_D^{20}$ +56.9° (20 hours) (c, 2.8 in water)

The 4-O-methyl-D-glucosazone was prepared by dissolving a portion (60 mg.) of the sample in a mixture of water (1 ml.), glacial acetic acid (0.25 ml.) and phenylhydrazine (0.35 ml.). The solution was heated for 2 hours at 90°C. in a water bath and then cooled to room temperature. On shaking, the yellow 4-O-methyl-D-glucosazone precipitated, m.p. and mixed m.p. 157-158°C.

The infra-red spectrum of 4-O-methyl-D-glucosazone (made by the potassium bromide pellet technique) was identical to the spectrum of an authentic sample of 4-O-methyl-D-glucosazone.

APPENDIX

Up to the present time only limited attention has been devoted to the infra-red spectra of carbohydrates with a view to ascertaining whether or not these spectra may be used for qualitative identification of sugars and their derivatives.

During the investigation of the hemicellulose of milkweed floss, a number of spectra were taken of various methyl sugars and certain derivatives used to characterize them. It was felt by the author that these spectra were of value for qualitative identification of these sugars and their derivatives.

It has been found that for identical sugars and their derivatives, the infra-red spectra gave exactly the same frequencies of absorption although the intensities of the peaks may vary greatly according to the amount of sample used. Compounds having similar configurations, such as isomers of methylated xylose or glucose, differ widely enough in their spectra to make a qualitative identification possible by this means.

An example is the spectra of 3,4-di-O-methyl-D-xylose and of 2,3-di-O-methyl-D-xylose (Figs. 17,18). Although the spectra for these compounds appear to be quite similar, certain definite differences exist in their absorp-

tion pattern. Distinct absorption peaks were present at 1343, 1378, 1260 cm.$^{-1}$ in the 3,4-di-O-methyl-D-xylose spectrum which were non-existent in the spectrum of 2,3-di-O-methyl-D-xylose. In the region 940 to 960 cm.$^{-1}$ two peaks were present for the 3,4-di-O-methyl-D-xylose spectrum, whereas for the 2,3-di-O-methyl-D-xylose only one at 940 cm.$^{-1}$ was noticeable.

In the case of the aniline derivatives of 2,3- and 3,4-di-O-methyl-D-xylose (Figs. 19,20) the spectra of these two compounds were so different that little doubt existed as to their identification. For example, at 3460, 1225, 1190, 1086, 1065, 1033, 1008, 910, 765 cm.$^{-1}$ definite absorption peaks were found in the spectrum of the 2,3-di-O-methyl derivative, which were non-existent in the spectrum of the 3,4 compound.

The spectra of the osazones of 4-O-methyl-D-glucose (Fig. 12) and 3-O-methyl-D-glucose were also compared, and it was found that they differed enough in their absorption so as to be distinguished from each other. For example, absorption peaks were found at 770, 1075, 1120, 1240, 1335, 1385, cm.$^{-1}$ in the spectrum of 3-O-methyl-D-glucosazone, which were absent in the spectrum of 4-O-methyl-D-glucosazone.

The compounds, 4-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose (Figs. 11,22) were also examined.

Although the spectra of these compounds were very similar, there were, nevertheless, significant differences. For example, definite absorption peaks at 893 and 957 cm.$^{-1}$ were prevalent in the spectrum of 4-O-methyl-D-glucose which were completely absent in the spectrum of 2,3,4-tri-O-methyl-D-glucose. On the other hand, absorption bands were noted at 985 and 933 cm.$^{-1}$ for 2,3,4-tri-O-methyl-D-glucose which did not exist in the spectrum of 4-O-methyl-D-glucose.

Although it would be preferable to compare a large number of sugars and their derivatives, the evidence given definitely indicates the value of infra-red spectroscopy in the identification of sugars and their derivatives.

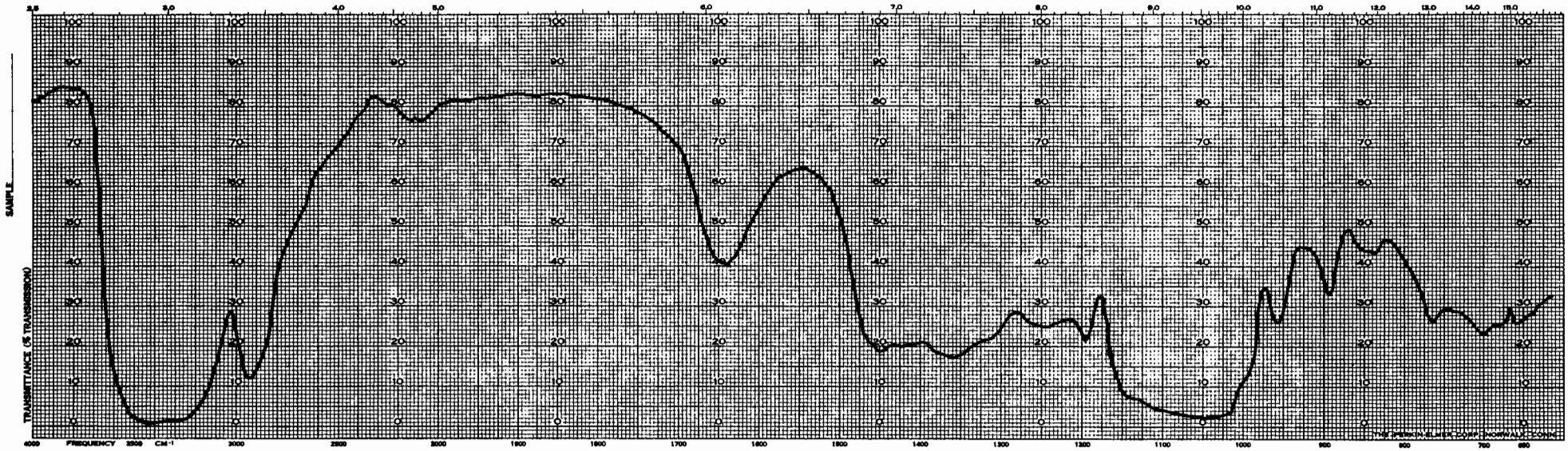


FIG. 11 Infra-Red Absorption Spectrum of 4-O-Methyl-D-Glucose

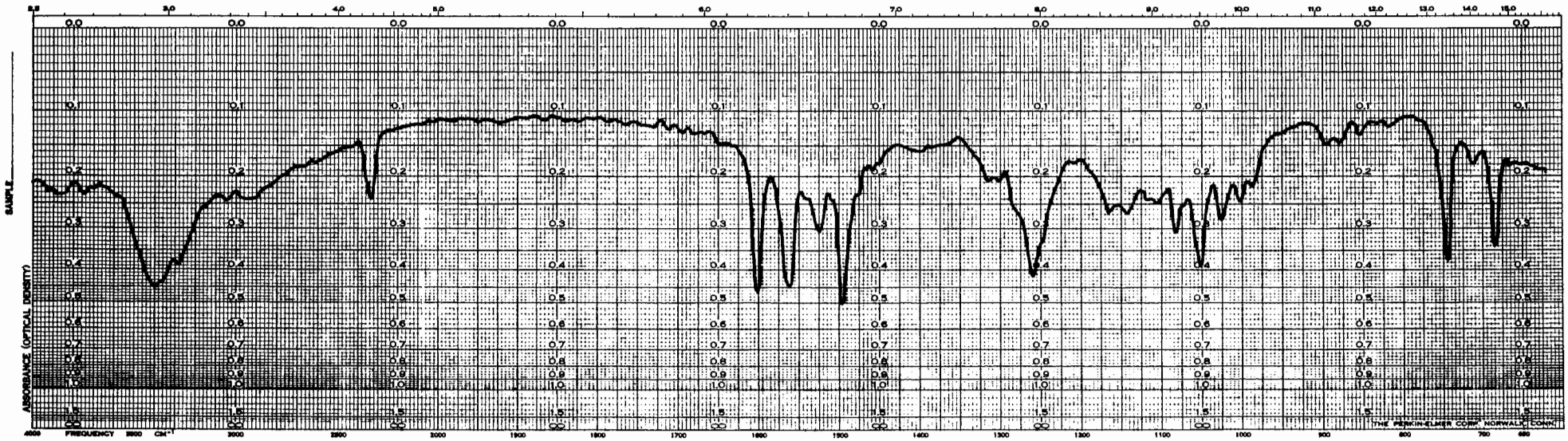


FIG. 12 Infra-Red Absorption Spectrum of 4-O-Methyl-D-Glucosazone

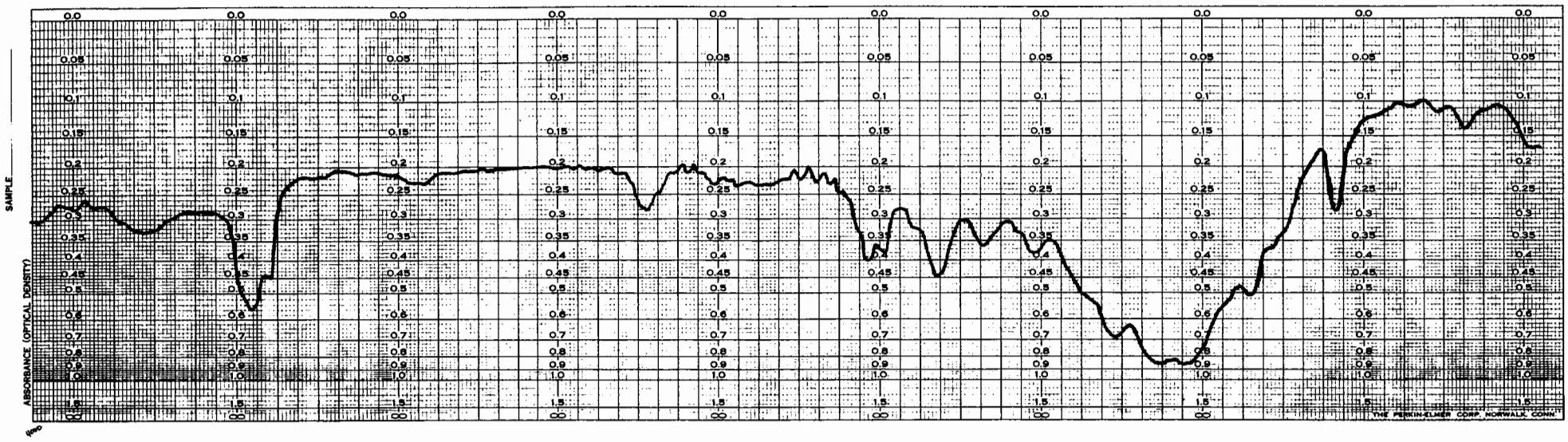


FIG. 13 Infra-Red Absorption Spectrum of the Fully Methylated Milkweed Floss Xylan

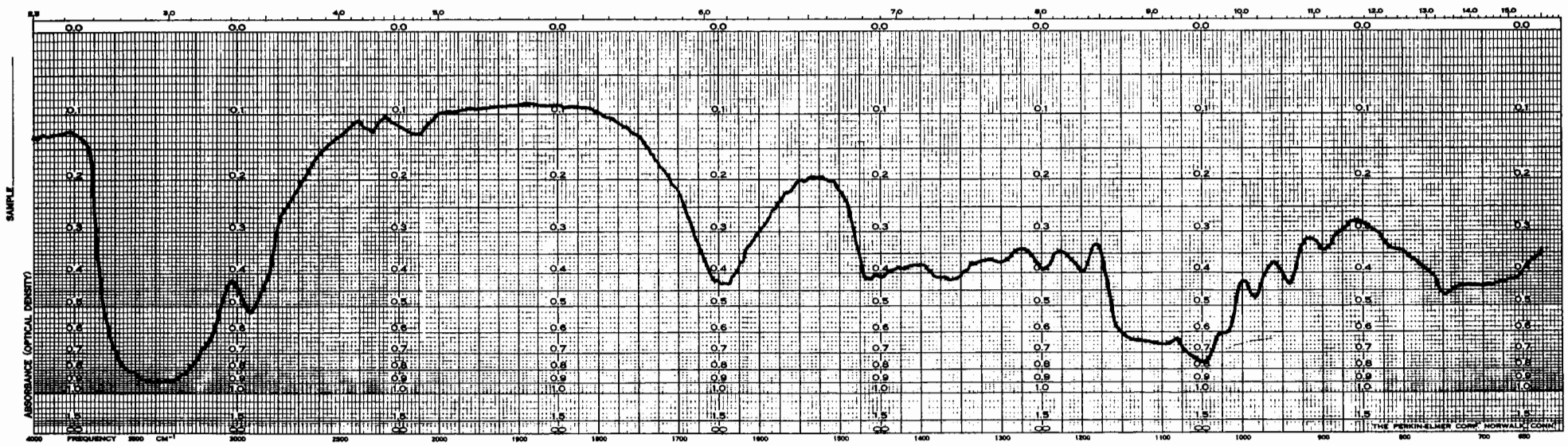


FIG. 14 Infra-Red Absorption Spectrum of 3-O-Methyl-D-Xylose

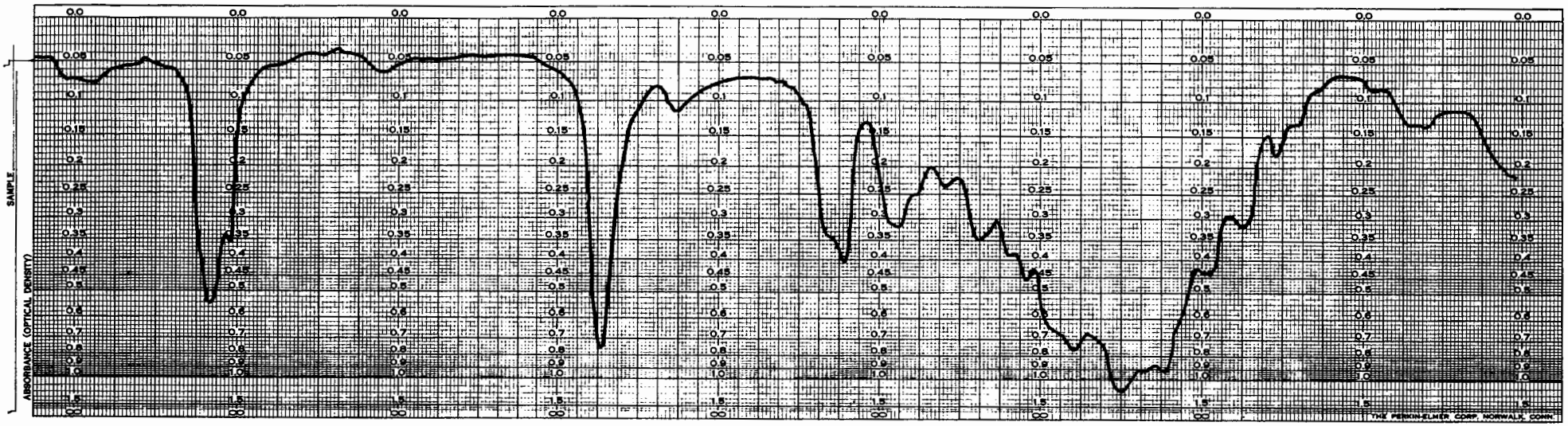


FIG. 15 Infra-Red Absorption Spectrum of Methyl 2-O-(2,3,4-Tri-O-Methyl-D-Glucopyranosyluronic Acid)-3,4-Di-O-Methyl-D-Xylopyranoside Methyl Ester

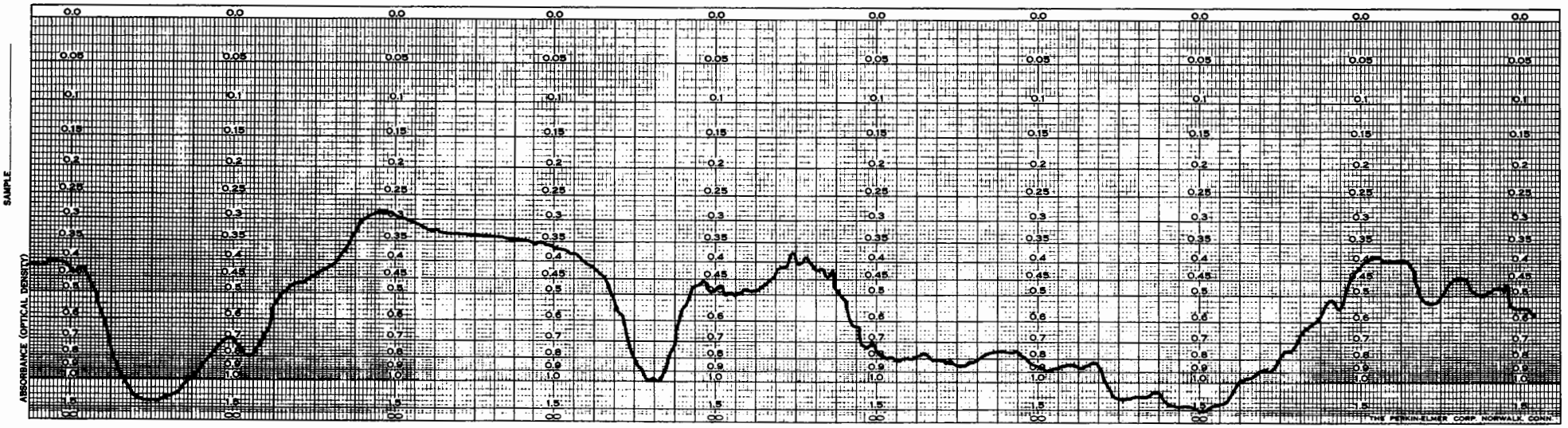


FIG. 16 Infra-Red Absorption Spectrum of 2-O-(4-O-Methyl-D-Glucopyranosyluronic Acid)-D-Xylopyranose

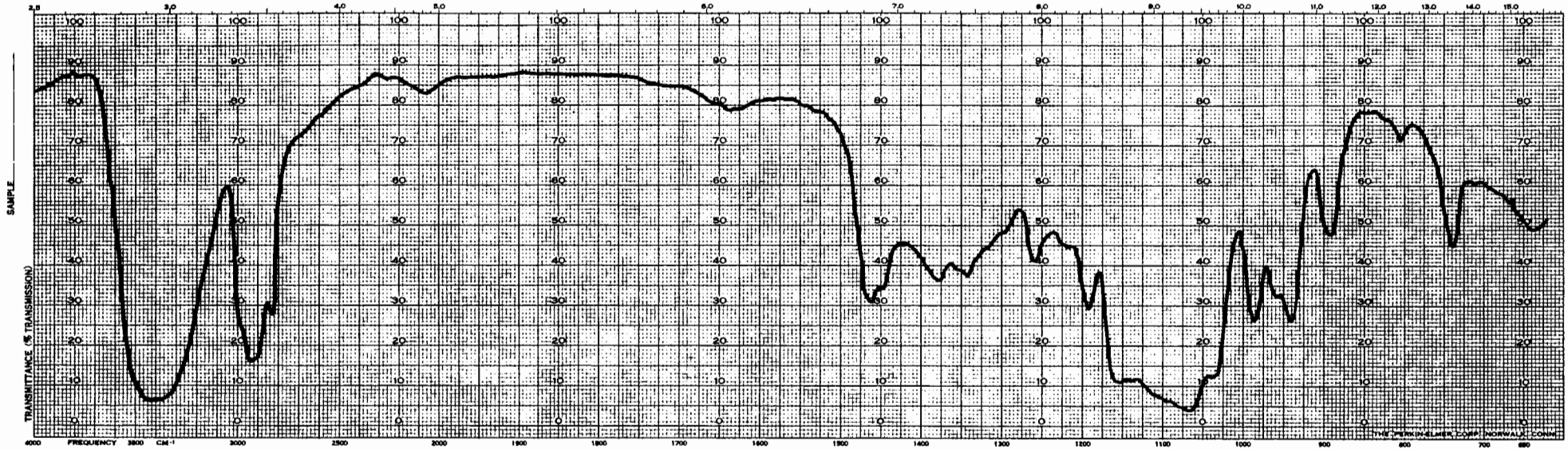


FIG. 17 Infra-Red Absorption Spectrum of 3,4-Di-O-Methyl-D-Xylose

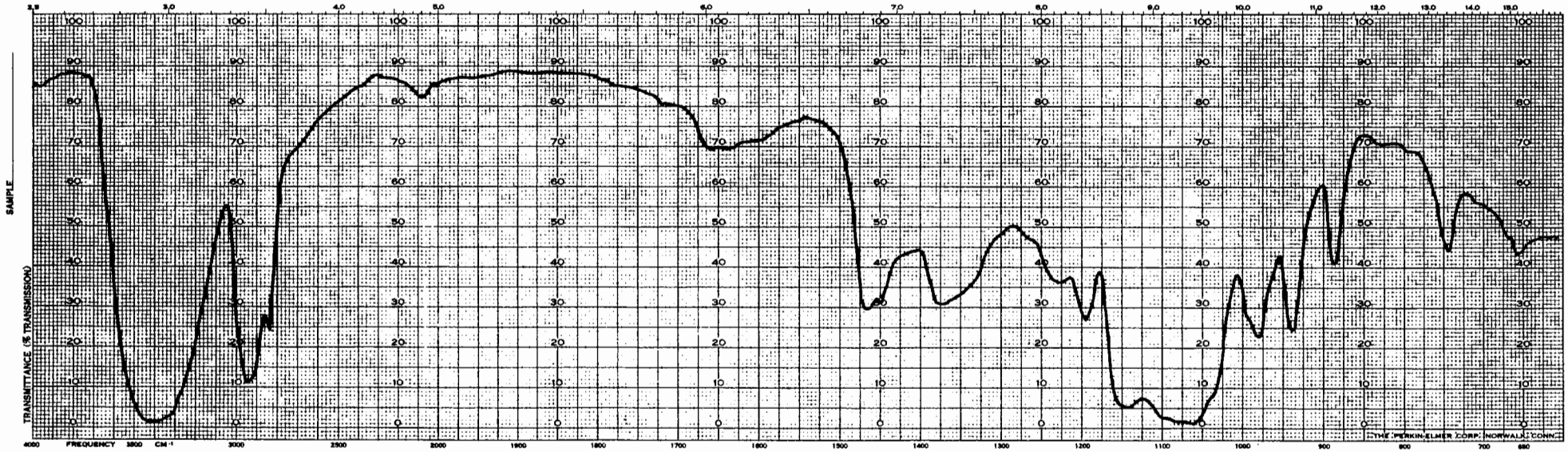


FIG. 18 Infra-Red Absorption Spectrum of 2,3-Di-O-Methyl-D-Xylose

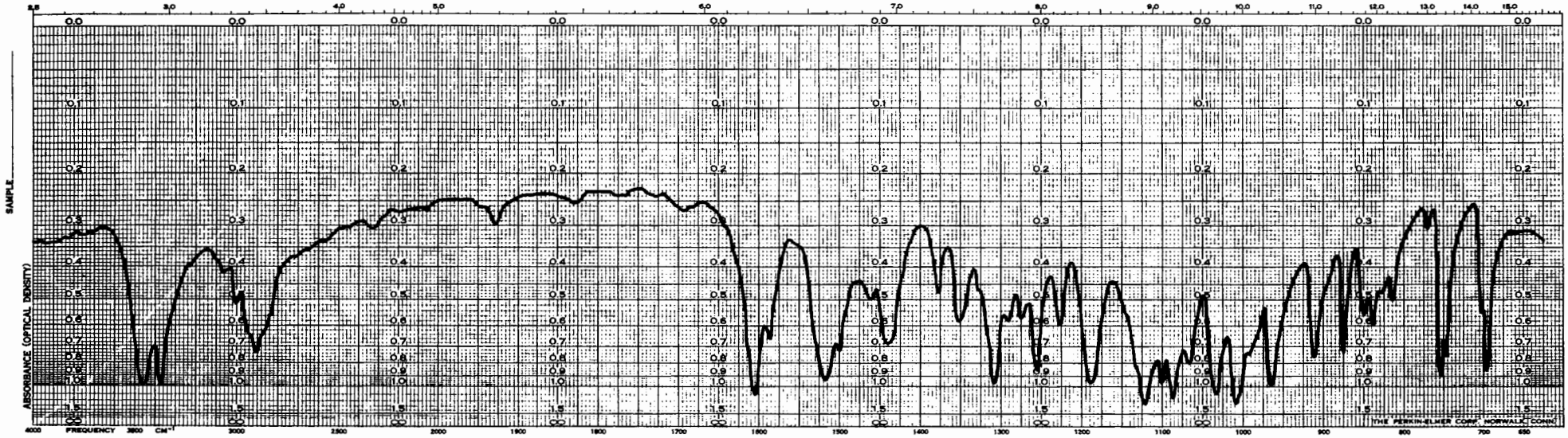


FIG. 19 Infra-Red Absorption Spectrum of 2,3-Di-O-Methyl-N-Phenyl-D-Xylosylamine

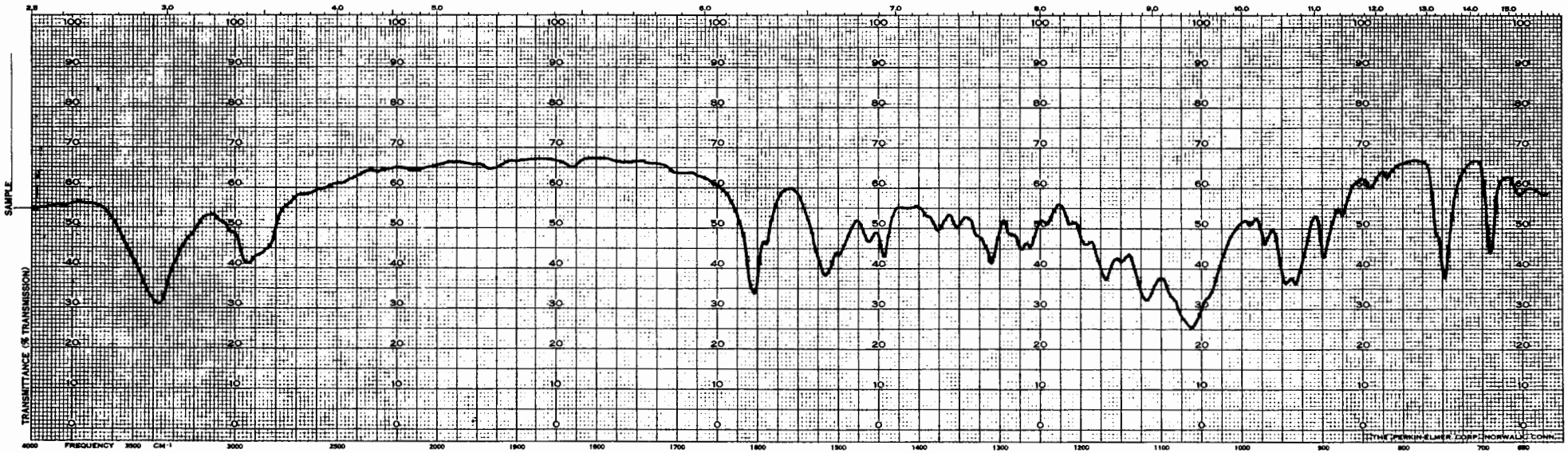


FIG. 20 Infra-Red Absorption Spectrum of 3,4-Di-O-Methyl-N-Phenyl-D-Xylosylamine

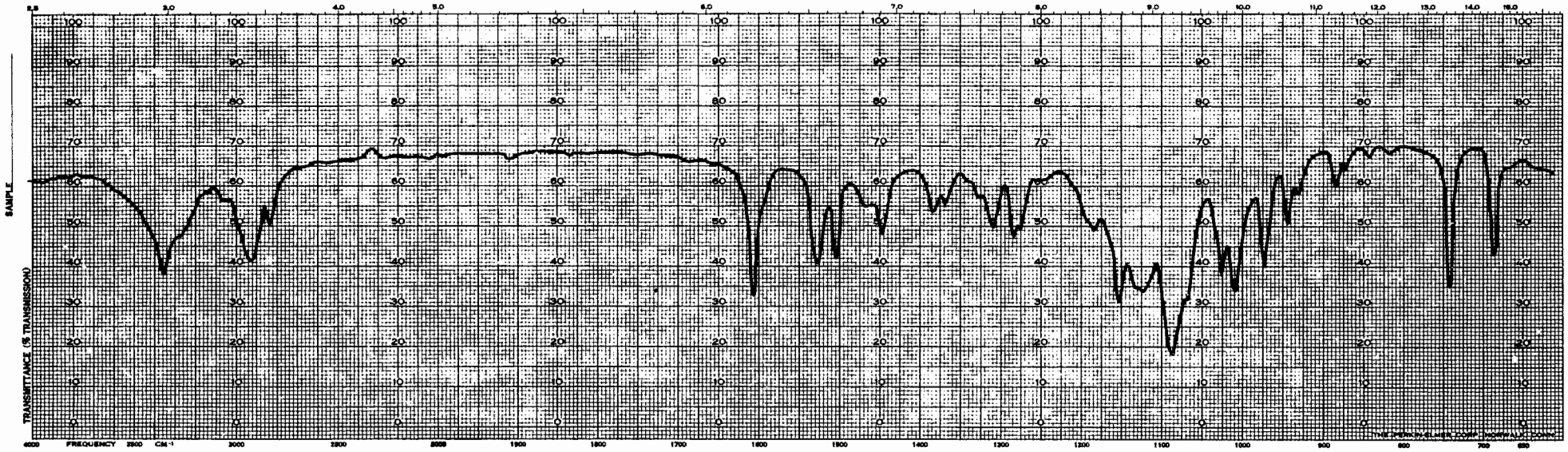


FIG. 21 Infra-Red Absorption Spectrum of 2,3,4-Tri-O-Methyl-N-Phenyl-D-Glucosylamine

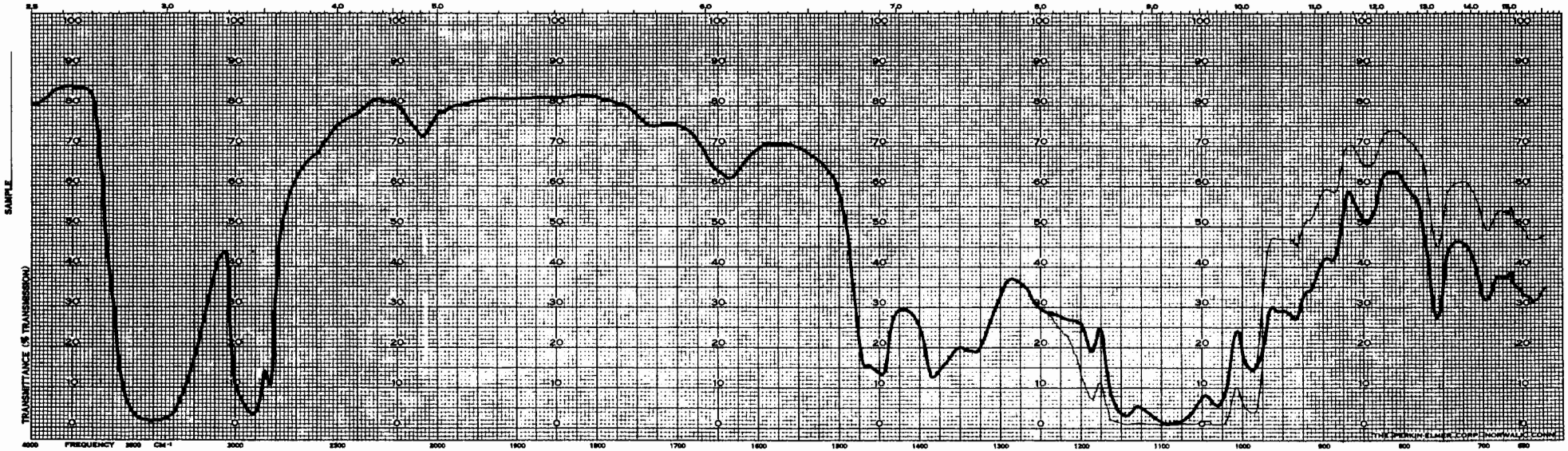


FIG. 22 Infra-Red Absorption Spectrum of 2,3,4-Tri-O-Methyl-D-Glucose

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. The floss of the common milkweed (Asclepias syriaca, L.) has been found to contain lignin (15.1), acetyl (6.1), uronic anhydride (5.6), galactan (2.4), glucan (55.1), mannan (5.2), and xylan (37.3%). The cellulose content was approximately 39%.

2. Direct alkaline extraction of the floss gave a hemicellulose in a yield of 30%, the only constituents of which were xylose and uronic acid residues.

3. The uronic anhydride content, the methoxyl content and the equivalent weight of the hemicellulose suggested the presence of approximately 14 anhydroxylose units per methoxy uronic acid unit.

4. The number of reducing end groups corresponded to a number-average degree of polymerization ($\overline{P}n$) of 62, whereas viscosity measurements indicated a value of 172 for the native polysaccharide.

5. Complete methylation of the hemicellulose was easily achieved by a modification of the method of Kuhn et al., involving the use of a mixture of tetrahydrofuran and dimethyl formamide as a solvent for the partially methylated polysaccharide.

6. Osmotic pressure measurements on the fully methylated hemicellulose gave a $\overline{P}n$ of 97. The value of the second coefficient, B, (the slope constant) suggested that the hemicellulose had a higher solubility than a similar product from birchwood.

7. Methanolysis of the methylated hemicellulose and separation of the acidic from the neutral constituents, followed by hydrolysis of the latter, gave a mixture of methylated sugars. The acid fraction contained only one component, which was reduced to the corresponding alcohol, hydrolysis of which yielded 2,3,4-tri-O-methyl-D-glucose and 3-O-methyl-D-xylose. This compound was accordingly methyl 2-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-3-O-methyl-D-xylopyranoside.

8. The neutral fraction contained a 1:1 mixture of 2-O- and 3-O-methyl-D-xylose, and 2,3-di-O-methyl-D-xylose, and 2,3,4-tri-O-methyl-D-xylose.

9. The molar ratio between the mono, di, and tri substituted sugars and the aldobiouronic acid was estimated by the o-aminodiphenyl method after separation on paper chromatograms. The ratios were 0.82: 38.6: 1: 2.29, respectively.

10. Partial hydrolysis of the milkweed floss yielded several uronic acids which on reduction and hydrolysis

gave galactose, xylose and 4-O-methyl-D-glucose.

11. Chromatographic separation on an anion exchange resin and charcoal columns gave a monouronic acid, a monomethyl aldobiouronic acid, and a monomethyl triouronic acid.

12. The monouronic acid yielded galactose on reduction and hydrolysis. The galacturonic acid was probably derived from pectic impurities in the hemicellulose.

13. The monomethyl monouronic acid on reduction and subsequent hydrolysis gave 4-O-methyl-D-glucose and was thus 4-O-methyl-D-glucuronic acid.

14. Reduction and hydrolysis of the aldobiouronic acid gave 4-O-methyl-D-glucose and D-xylose. Complete methylation followed by reduction and hydrolysis yielded 2,3,4-tri-O-methyl-D-glucose and 3,4-di-O-methyl-D-xylose. The aldobiouronic acid was accordingly 2-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-xylopyranose.

15. The aldotriouronic acid partly crystallized. Partial acid hydrolysis gave a mixture of unchanged acid, the above aldobiouronic acid, 4-O-methyl-D-glucuronic acid and xylose.

16. The above evidence indicated that the hemicellulose of milkweed floss was a methyl glucurono xylan con-

taining β-D-xylopyranose residues linked 1,4, and with, on the average, every 14th xylose residue carrying a single side group of 4-O-methyl-D-glucuronic acid attached through the 2-position with an ∠-glycosidic bond.

17. Comparison between the number of xylose residues present per non-reducing end group (42) and the number-average degree of polymerization (97) suggested one branch point per molecule.

REFERENCES

1. G.A. Adams and C.T. Bishop, Tappi, 38, 672 (1955).
2. D.B. Das, M.K. Mitra and J.F. Wareham, Nature 171, 613 (1953).
3. T.E. Timell, Textile Research J. 27, 854 (1957) Ibid. 28, (1958).
4. A.L. Currie, unpublished results.
5. F. Zapf, Makromol. Chem. 10, 71 (1953).
6. N. Gralén, S. Berg and T. Svedberg, Ber. 75, 1702 (1942).
7. Encyclopedia Britannica, vol. 2, p. 501.
8. C.A. Whitford, In H.R. Mauersberger, editor, Mathew's Textile Fibers, sixth ed., John Wiley and Sons, New York, p. 439-456 (1954).
9. L.V. Forman and D. Niesayan, Paper Trade J. 121, no. 10, 29-34 (1945).
10. B. Berkman to Milkweed Products Development. U.S. Patent 2,283,409, May 19, C.A. 366,275 (1942).
11. J.H. von Ostaer, German Patent 729,304, Nov. 19, 1942.
12. H.B. Hanson, Iowa State College J. Sci., 20, 365 (1946).
13. C.E. Hartvig, B.N. Laigovoy to American Chicle Co., U.S. Patent 2,297,651, Sept. 27, 1943.
14. L. Stauffer, Rayon Textile Monthly 23, 603-4 (1942).
15. A.E. Rheineck, Pharm. Arch. 10, 69 (1939).
16. A. Juillet and J. Delga, Mem. services chim. etat Paris 32, 378 (1948).
17. T.E. Timell, Svensk Papperstidn. 60, 836 (1957).
18. T.E. Timell and J.L. Snyder, Textile Research J. 25, 870 (1955).
19. T.E. Timell, Textile Research J., in press.

20. T.E. Timell, Ind. Eng. Chem. 47, 2166 (1955).

21. H.A. Hampton, W.N. Haworth and E.L. Hirst, J. Chem. Soc. 1739 (1929).

22. W.N. Haworth, E.L. Hirst, J.K.N. Jones and E.G.V. Percival, J. Chem. Soc. 1917 (1934).

23. S.K. Chanda, E.L. Hirst, J.K.N. Jones and E.G.V. Percival, J. Chem. Soc. 1289 (1950).

24. G.A. Adams, Can. J. Chem. 30, 698 (1952).

25. C.T. Bishop, Can. J. Chem. 31, 134 (1953).

26. G.O. Aspinall and R.S. Mahomed, J. Chem. Soc. 1731 (1954).

27. C.T. Bishop and D.R. Whitaker, Chem. and Ind. 119 (1955).

28. I.R.C. McDonald, J. Chem. Soc. 3183 (1952).

29. G.O. Aspinall, E.L. Hirst and R.S. Mahomed, J. Chem. Soc. 1734 (1954).

30. C.P.J. Glaudemans and T.E. Timell, Svensk Papperstidn. 60, 869 (1957); 61, 1 (1958); J. Chem. Soc. 80 (1958).

31. G.G.S. Dutton and F. Smith, J. Am. Chem. Soc. 78, 2505 (1956).

32. T.E. Timell, C.P.J. Glaudemans and A.L. Currie, Anal. Chem. 28, 1916 (1956).

33. E.L. Hirst, L. Hough and J.K.N. Jones, J. Chem. Soc. 928 (1949).

34. A.W. Craig and D.A. Henderson, J. Polymer Sci. 19, 215 (1956).

35. E.L. Falconner and G.A. Adams, Can. J. Chem. 34, 338 (1956).

36. R. Kuhn, H. Trischmann and I. Low, Angewandt de Chemie 67, 32 (1955).

37. B.H. Zimm and I. Myerson, J. Am. Chem. Soc. 68, 911 (1946).

38. J.V. Stabin and E.H. Immergut, J. Polymer Sci. 14, 209 (1954).

39. J.K.N. Jones and T.J. Painter, Paper presented at the 133rd Meeting of the American Chemical Society, San Francisco, Calif., April 1958.

40. J.K. Gillham and T.E. Timell, Can. J. Chem. 36, 410 (1958).

41. H. Bouveng and B. Lindberg, Acta. Chem. Scand. 8, 1283 (1956).

42. J.K.N. Jones and L.E. Wise, J. Chem. Soc. 3389 (1952).

43. J.K.N. Jones, E. Merler and L.E. Wise, Can. J. Chem. 35, 634 (1957).

44. J.E. Milks and C.B. Purves, J. Am. Chem. Soc. 78, 3738 (1956).

45. G.A. Adams, Can. J. Chem. 35, 556 (1957).

46. P.A.J. Gorin, Can. J. Chem. 35, 539 (1957).

47. A.R.N. Gorrod and J.K.N. Jones, J. Chem. Soc. 2522 (1954).

48. J.K. Hamilton and N.S. Thompson, J. Am. Chem. Soc. 79, 6464 (1957).

49. G.O. Aspinall and M.L. Carter, J. Chem. Soc. 3744 (1956).

50. D.H. Ball, J.K.N. Jones and T.J. Painter, Tappi 39, 438 (1956).

51. J.D. Geerdes and F. Smith, J. Am. Chem. Soc. 77, 3569 (1956).

52. R.L. Whistler, H.E. Conrad and L. Hough, J. Am. Chem. Soc. 76, 1668 (1954).

53. A.L. Currie, unpublished results.

54. J. Saarnie, K. Wathen and C.H. Gustafsson, Acta. Chem. Scand. 8, 825 (1954).

55. C.M. Stewart, Australian J. Chem. 6, 425-53.

56. C.M. Stewart and D.H. Foster, Nature 171, 792 (1953).

57. A. Roudier and L. Eberhard, Compt. rend. 240, 2012 (1955).

58. J.K.N. Jones and T.J. Painter, J. Chem. Soc. 669 (1957).

59. Testing Methods of the Technical Association of the Pulp and Paper Industry, New York, N.Y.

60. T.E. Timell, Tappi 40, 658 (1957).

61. B.L. Browning, Tappi 22, 119 (1949).

62. L.J. Breddy and J.K.N. Jones, J. Chem. Soc. 738 (1945).

63. L.E. Wise and E.K. Ratliff, Anal. Chem. 19, 694 (1947).

64. Q.P. Penistor and H. Hibbert, Paper Trade J. 46, 230 (1939).

65. M.L. Huggins, J. Am. Chem. Soc. 64, 2716 (1942).

66. J.F. Carson and W.D. Maclay, J. Am. Chem. Soc. 68, 1015 (1946).

67. J.F. Carson and W.D. Maclay, Ibid. 70, 293 (1948).

68. American Viscose Corporation, Marcus Hook, Pa., U.S.A.

69. M. Abdel-Akher and F. Smith, Nature 166, 1037 (1950).

70. F.J. Bates and Associates, In Polarimetry, Saccharimetry and the Sugars, U.S. Department of Commerce, National Bureau of Standards.

71. G.W. Huffman, P.A. Rebees, F. Smith and D.R. Spriesterbach, Nature 175, 990 (1955).

72. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebees and F. Smith, Anal. Chem. 28, 350 (1956).

73. C.T. Bishop, Can. J. Chem. 34, 1255 (1956).

74. F. Smith, I. Ehrenthal, M.C. Rafique, J. Am. Chem. Soc. 74, 1341 (1952).

75. F. Smith, J. Chem. Soc. 2646 (1957).

76. W.D.S. Bowering and T.E. Timell, unpublished results.

77. A.B. Foster, Chem. and Ind. 1050 (1952).

78. J.K.N. Jones and L. Hough, J. Chem. Soc. 4349 (1952).

79. S. Pert, E. Schluchterer and E.L. Hirst, J. Chem. Soc. 123, 3125 (1923).

80. J.K.N. Jones and W.H. Wadman, J. Chem. Soc. 796 (1952).

81. Advances in Carbohydrate Chemistry 9, 144 (1954).