ranosyl-2-glycerol to approximately 70% of the theoretical value in 2 hours at 30°.

The enzyme and substrate, in the appropriate buffers, were incubated at 30° and at various time intervals aliquots were withdrawn and deproteinized with barium hydroxide–zinc sulfate. Release of reducing power was determined by the Nelson[8] procedure.

No hydrolysis of the digalactosylglycerol with the β-galactosidase was observed. Incubation with α-galactosidase resulted in the release of only 50% of the potential reducing power. In order to determine the products of this hydrolysis a larger scale experiment was run. Ten mg. of the digalactosylglycerol was incubated with β-galactosidase for 3 hours at 25°. The entire reaction mixture was then deproteinized with barium hydroxide–zinc sulfate. The supernatant solution was put on a 3 × 6 cm. carbon–Celite column (Darco G-60 and Celite 545 in a 1:1 ratio). The column was first washed with 1000 ml. of water and then with approximately 200 ml. of 10% ethanol. These two fractions were concentrated *in vacuo* and finally lyophilized. Paper chromatography of the material from the water wash revealed the presence of a reducing sugar corresponding to galactose. The material from the ethanol wash was non-reducing and was identical with the monogalactosylglycerol of fraction A as judged by paper chromatography. Paper chromatography of an acid hydrolysate of this material revealed the presence of galactose and glycerol.

The monogalactosylglycerol was not hydrolyzed by α-galactosidase during 3 hours incubation at 30°. Incubation with the β-galactosidase resulted in a release of approximately 80% of the potential reducing power in 90 minutes.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY, AND THE WOOD CHEMISTRY DIVISION, PULP AND PAPER RESEARCH INSTITUTE OF CANADA]

Attempted Preparation of a Homogeneous Hemicellulose from Aspen Wood[1]

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Chlorite holocellulose from aspen wood (*Populus tremuloides*) was extracted with nearly anhydrous liquid ammonia near 20° and 150 p.s.i. The ammonia removed 10.6% of the wood in the form of acetamide, modified lignin and carbohydrate and rendered a further 9.6% soluble in subsequent extractions with water near 70°. Crude pectins were eliminated from the latter extracts by bleaching with chlorite and by acetylation followed by fractional precipitation. Deacetylation yielded about 1% of the wood as four fractions each containing one hexuronic and two 4-*O*-methylglucuronic acid residues combined with about 22, 16, 13 and 12 anhydro-D-xylose units, respectively. Partial hydrolysis yielded a crystalline aldotriuronic acid containing one 4-*O*-methylglucuronic acid and two xylose residues; also another (uncrystallized) in which one of the xylose residues was thought to be replaced by that of the unknown hexuronic acid. A chemical study by standard methods showed that the hemicellulose was based on anhydro-D-xylose units linked 1–4, probably with many branches in the second and third positions.

Although hemicelluloses extracted from aspen pulps by aqueous alkali have been examined on several occasions,[2-7] the literature records no sustained effort to separate a chemical individual from the original mixture. The present research had the isolation of such a product as one of its objectives, and another was to gain more information about the action of anhydrous, or nearly anhydrous, liquid ammonia on wood pulps. Liquid ammonia at temperatures between 25 and 100° (about 150 to 900 p.s.i.) was known to dissolve only 3.2 and 8.9% of solvent-extracted spruce and maple wood, respectively,[8] but a portion of the maple wood residue was rendered soluble in hot water.[9] The additional solubility was tentatively attributed to the cleavage of ester groups of an unknown type by the liquid ammonia, because the final wood residue contained 0.25% of additional nitrogen as amide and because at least 66% of the acetyl groups in the wood was recovered as acetamide. The acetyl groups in birch and spruce holocelluloses, however, were unaffected when the liquid ammonia was used at atmospheric pressure and −33°,[10a] or under less drastic conditions.

An aspen holocellulose, rather than the wood itself, was used on the present occasion in order to facilitate the isolation of the hemicelluloses. Chlorite holocellulose[11] was preferred to the product obtained by chlorination in water near 0°[12,13] followed by extraction with alcohol–pyridine,[14] because the former method left the holocellulose with a much lower nitrogen content (0.04 %) than did the latter (0.27%).[15] An increase in nitrogen content at this point would complicate the interpretation of any change caused by the subsequent use of liquid ammonia. The chlorite holocellulose (Fig. 1) was then thoroughly extracted with hot water to remove material that might have contaminated the hemicelluloses to be extracted later. This material (extract-1) consisted of polysaccharides and of lignin oxidized in variable degree, but no evidence of a lignin-carbohydrate compound could be found. After being dried, the residual holocellulose was extracted three times under pressure with anhydrous liquid ammonia, which removed 10.2% of the wood

(1) Abstracted from a Ph.D. Thesis submitted by J. E. Milks, October 1953. Presented before the Division of Cellulose Chemistry of the American Chemical Society, Minneapolis, September, 1955.

(2) R. E. March, *Tech. Assoc. Papers*, **31**, 240 (1948).

(3) J. O. Thompson and L. E. Wise, *Tappi*, **35**, 331 (1952).

(4) (a) J. K. N. Jones and L. E. Wise, *J. Chem. Soc.*, 2750 (1952); (b) 3389 (1952).

(5) J. Saarnio, K. Wathén and C. Gustafsson, *Paper and Timber Journal* (Finland), **36**, 209 (1954).

(6) D. C. Lea, *Tappi*, **33**, 393 (1954).

(7) M. A. Millett and A. J. Stamm, *J. Phys. Colloid Chem.*, **51**, 134 (1947).

(8) M. M. Yan and C. B. Purves, unpublished.

(9) L. G. Neubauer, M. M. Yan and C. B. Purves, unpublished.

(10) (a) K. J. Bjorkqvist and L. Jörgensen, *Acta Chem. Scand.*, **5**, 978 (1951); (b) **6**, 800 (1952).

(11) L. E. Wise, M. Murphy and A. A. D'Addieco, *Paper Trade J.*, **122**, No. 2, 35 (Jan. 10, 1946).

(12) D. A. Sitch, *Pulp and Paper Mag. Canada*, **50**, 234 (Convention Issue, 1949).

(13) T. E. Timell and E. C. Jahn, *Svensk Papperstidn.*, **54**, 831 (1951). The two methods were systematically reviewed and applied to paper birch.

(14) G. J. Ritter and E. F. Kurth, *Ind. Eng. Chem.*, **25**, 1250 (1933).

(15) (a) B. B. Thomas, *Paper Ind. and Paper World*, **26**, 1281 (1945); (b) **27**, 374 (1945), noted the increased nitrogen content of chlorine-ethanolamine holocelluloses.

as extract-2. A systematic examination of this extract showed that the methanol-soluble portion contained acetamide corresponding to 82% of the acetyl groups originally in the wood and also modified lignin; the portion insoluble in methanol consisted of modified lignin and polysaccharides, but once more no definite carbohydrate—lignin compound could be isolated.

The wood residue recovered from the liquid ammonia quickly assumed a very highly swollen, gelatinous state when brought into contact with water at room temperature (extract-3), and the removal of water-soluble substances was completed by two further extractions at 70° (extract-4). Ethanol or methanol was used to precipitate the hemicelluloses from these extracts, but the combined mother liquors retained 1.8% of the wood as soluble substances that were not examined further. The residual pulp was also neglected, although the yield of about 61% showed that it retained more than half of the hemicelluloses.

The crude hemicelluloses precipitated from extracts-3 and -4 gave brown, slightly turbid aqueous solutions, and after small-scale attempts at purification by re-precipitation, the solutions were separately bleached with sodium chlorite at *p*H 5. A portion of the hemicellulose from extract-3 was precipitated during bleaching and was dialyzed against water to remove sodium chlorite. This operation separated the hemicellulose into the insoluble and soluble fractions A and B, while the more

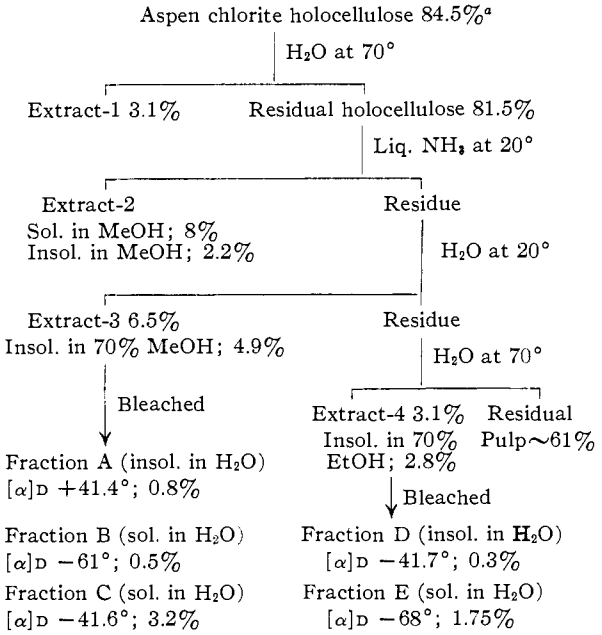

Fig. 1.—Extraction of aspen chlorite holocellulose with water, liquid ammonia and again with water. [a] (All percentages based on the original wood. Aqueous extraction and bleaching with aqueous sodium chlorite always at *p*H 5–6).

soluble fraction C was recovered from the bleach liquors. Fractions D and E were isolated in a similar way from the hot water extract-4. When small samples of the fractions were hydrolyzed with boiling *N* sulfuric acid and chromatographed, all five gave indications of much xylose with lesser amounts of glucose, arabinose, uronic acids and still less rhamnose. L-Rhamnose and its derivatives had already been isolated from hydrolysates of aspen wood.[10b,16] The dextrorotatory fraction A and the minor fraction D dissolved only incompletely during the above hydrolysis, and this evidence of pectic contaminants was supported by the recovery of much mucic acid when a sample of fraction A was oxidized with bromine in boiling *N* hydrobromic acid.[17] Both fractions were therefore rejected.

In order to eliminate pectic contaminants, the hemicellulose fraction C was acetylated with pyridine and acetic anhydride and the mixture was precipitated with water. Acetylated pectins tended to remain in the mother liquor, and the precipitated acetate amounted to only 117% by weight. Fractions B and E were combined and acetylated in the same way (yield 132%), and the product was then combined with the acetate from fraction C. Extraction of these acetates with 1000 ml. of chloroform yielded an insoluble sub-fraction 1 (Table I),

TABLE I

FRACTIONATION OF THE HEMICELLULOSE ACETATE[a]

Fraction	Pptd. from % $CHCl_3$	Yield, %	In chloroform $[\alpha]^{22}D$	$[\eta]$[b]
1	..	26.7	Insoluble	
2a	..	15.6	Insoluble	
2b	..	5.1	−74.1°	0.44
3	87	3.2	−76.2°	.47
4	85	8.7	−76.4°	.44
5	83	6.5	−79.0°	.44
6	81	5.9	−79.8°	.42
7	79	6.6	−81.8°	.40
8	77	3.3	−82.5°	.38
9	72	8.8	−84.8°	.33
10	68	3.3	−82.8°	.24
11[c]	..	2.5	−58.5°	..
	Recovery	96.6%		

[a] A 45.7-g. sample of acetates from fractions C, B and E (Fig. 1) in 1 liter of chloroform. Non-solvent: ligroin. [b] Plot of reduced specific viscosity against concentration *c*, extrapolated to $c = 0$. Plot was in all cases linear between $c = 0.25$ and $c = 1.2\%$. [c] Recovered by evaporating the final liquor.

and the addition of ligroin (150 ml.) to the extract precipitated sub-fraction 2, which was separated into portions soluble and insoluble in chloroform. As the table shows, the sub-fractions decreased steadily in intrinsic viscosity but passed through a levorotatory maximum, the final, most soluble subfraction presumably consisting of the hexosan acetates. Subfractions 2b, 3 and 4, with almost the same specific rotation and viscosity, were combined and refractionated (Table II), but proved to be mixtures of substances differing in solubility and rotation. A similar refractionation of the combined sub-fractions 9 and 10, however, failed to change the physical properties markedly, and in this case at least the method of separation was apparently approaching its limit of usefulness.

The principal sub-fractions (Tables I and II) which were either insoluble in chloroform or had similar rotations and viscosities, were collected into two groups which were separately deacetylated

(16) J. K. N. Jones and T. R. Schoettler, *Tappi*, **35**, No. 1, 102A (1952).

(17) M. Heidelberger and W. Goebel, *J. Biol. Chem.*, **74**, 613 (1927).

TABLE II

REFRACTIONATION OF CERTAIN HEMICELLULOSE ACETATE FRACTIONS[a]

Fractions[b]	Sub-fraction	Yield, g.	In chloroform $[\alpha]^{22}D$	$[\eta]$[c]
2b, 3, 4	I	3.75	Insoluble	..
	II	1.06	−70.2°	..
	III	0.73	....	..
	IV	1.36	−81.3°	0.42
9, 10	V	0.80	−83.3°	.35
	VI	1.08	−85.5°	.31
	VII	1.61	−84.0°	.33
	VIII	1.62	−81.5°	..

[a] Precipitated from chloroform solution by successive additions of ligroin. [b] See Table I, column 1. [c] See Table I, footnote *b*.

with precautions against degradation. Fractionation of the resulting two hemicelluloses (Table III) showed that each was still heterogeneous, and clearly revealed the inefficiency of the previous fractionation of the acetates. The insolubility of the first acetate in chloroform, however, was reflected in the insolubility of the deacetylated sub-fractions H-1, H-2 and H-3 in water. Since these sub-fractions were now small, they were combined for further study with the water-soluble sub-fraction H-7, whose properties were similar in other respects. The combination was designated fraction R. Fractions H-4 and H-5, with identical rotations but different viscosities, were combined as fraction S. Fractions R and S contained only xylose and uronic acid residues, but the chromatograms from hydrolyzates of fractions H-8 and H-9 showed an additional very faint spot corresponding to glucose. An examination of these four fractions (Table IV) revealed each to contain methoxyl and uronic acid residues in a molar ratio of 2:3, associated with anhydro-D-xylose units ranging in average number from 22 to about 12, and that the other properties varied in a roughly parallel way. The same ratio of methoxy groups to uronic anhydride units was observed in a hemicellulose from paper birch,[12] and it was previously known that some of the uronic anhydride units in aspen hemicellulose were not methylated.[15b]

TABLE III

FRACTIONATION OF DEACETYLATED HEMICELLULOSE ACETATES

Fraction	Yield, g.	In 10% KOH $[\alpha]^{22}D$	$[\eta]$[a]
From acetate fractions 1 and 2a (Table I)[b]			
H-1	5.02	−83.5°	0.44[c]
H-2	1.32	−81.5°	.42[c]
H-3	0.63	−79.5°	.43[c]
H-4	2.02	−71.3°	.39
H-5	1.07	−71.3°	.24
H-6	0.35	−71.3°	...
From acetate fractions 5–8 (Table I) and sub-fractions IV–VII, Table II[c,d]			
H-7	3.34	−77.0°	0.48
H-8	3.02	−63.0°	.33
H-9	1.40	−59.0°	.22
H-10	0.88	−50.8°	...
H-11	0.12	.....	...

[a] Plot of reduced specific viscosity against concentration c extrapolated to $c = 0$. Plot was in all cases linear between $c = 0.22\%$ and $c = 1\%$. [b] From 19 g. of acetate. Recovery 86% after deacetylation and fractionation from solution in 320 ml. of 2 *N* sodium hydroxide by adding ethanol. [c] Insoluble in water. [d] From 15 g. of acetate. Recovery 91% after deacetylation and fractionation.

TABLE IV

ANALYSES OF HEMICELLULOSE FRACTIONS AFTER DEACETYLATION

Fraction	$[\alpha]^{22}D$	In 10% KOH $[\eta]$	Mole ratios Xylan: OCH_3: U.A.[b]	Hydrolyzate[a] as glucose, %
R[c]	−80 ± 3°	0.45 ± 0.03	22:2:2.94	89
S[d]	−71.3°	.3 ± 0.1 (?)	16:2:2.88	90
H-8[e]	−63°	.33	13:2:3.2	79
H-9[f]	−59°	.22	12:2:3.0	68

[a] Samples, 40 mg., boiled with 3-ml. volumes of 2.5% sulfuric acid for 4 hr. and copper reducing power determined. [b] Uronic anhydride. [c] Combination of fractions H-1, H-2, H-3 and H-7 from Table III. Found: xylan, 84.7, 87.4, uronic anhydride, 15.0, 15.2; OCH_3, 1.77, 1.86; ash, 3.5, 3.7%. [d] Combination of fractions H-4, H-5 and H-6 from Table III. Found: xylan, 76.8, 76.7; uronic anhydride 17.8, 18.5; OCH_3, 2.26, 2.19; ash, 3.9, 3.8%. [e] Found: xylan, 75.4, 75.3; uronic anhydride, 24.6, 23.5; OCH_3, 2.74, 2.59; ash, 4.4, 4.2%. [f] Found: xylan, 68.3; uronic anhydride, 23.4; OCH_3, 2.66, 2.72; ash, 3.0, 2.7%.

Fractions S, H-8 and H-9 were then recombined in order to acquire enough material to study their uronic acid components. After the sample had been hydrolyzed with acid, the crude uronates were separated as barium salts from a 77% yield of crystalline D-xylose and were recovered as 1 g. of the free acids. These acids proved to have at least eight components when chromatographed quantitatively on paper, but only two were in considerable amount. Of these two, the less mobile product, 0.31 g., crystallized and was shown by accepted methods to consist of 4-*O*-methyl-D-glucuronic acid combined with the two xylose residues. Jones and Wise[4] isolated the disaccharide 2-α-(4-*O*-methyl-D-glucuronosyl)-D-xylose, apparently of specific rotation +90°, from an acid hydrolyzate of aspen wood. The new trisaccharide, with a specific rotation of only +47°, perhaps contained the same structure united through a β-xyloside bond to another xylose unit. The more mobile uronic acid fraction, 0.53 g. with a specific rotation of +81°, failed to crystallize, but the analyses approximated those of a trisaccharide based on one reducing, two acidic, one methoxyl and one anhydro-D-xylose group. As in the previous case, reduction with sodium borohydride[18] followed by acid hydrolysis, yielded 4-*O*-methylglucose and xylose on a paper chromatogram, but no spot corresponding to a sugar from the reduction of the non-methylated uronic acid could be found. The nature of this uronic acid therefore remained unknown, although the presence of glucuronic anhydride units in aspen wood has been suspected.[16]

Most of the hemicellulose fraction R (Table IV) was thoroughly methylated in several stages but in good yield. Although the fraction had been made up from various sub-fractions of closely similar viscosity and rotation, fractionation of the methylated product produced sub-fractions (Table V) varying widely in viscosity, the methoxyl content and the

(18) M. L. Wolfrom and K. Anno, THIS JOURNAL, **70**, 5583 (1952).

specific rotation remaining nearly constant. All that could be done with the available supply of material was to recombine it, submit it to methanolysis and hydrolysis, remove the uronic acids and identify the partly methylated xyloses that remained. Although the manipulations impaired the quantitative aspects of the chromatographic separations used, 93.5% of the product isolated consisted of 2,3-di-*O*-methyl-D-xylose and 6.5% of a nearly equimolecular mixture of 2- and 3-*O*-methyl-D-xylose, identified chromatographically and by selective oxidation of the 3-isomer with lead tetraacetate. No trimethylxylose could be detected, and the presence of a xylopyranoside end group was therefore improbable. The hemicellulose thus appeared to be a xylan polymer based on 1,4-linkages which were probably in the β-configuration because a xylobiose and a xylotriose of this type were recently isolated from aspen wood.[4a] The relatively high degree of branching suggested by the isolation of the monomethylxyloses was consistent with the ready consumption of only 17 moles of aqueous sodium periodate by the original hemicellulose R, which might have utilized more than 22 moles if unbranched. This oxidation produced no formaldehyde, but the presence of an unsubstituted pyranosyl end-group was indicated by the liberation of 1 mole per mole of formic acid. The unknown hexuronic acid residue probably constituted the end-group.

TABLE V

FRACTIONATION OF METHYLATED HEMICELLULOSE R[a]

Sub-fraction	Yield, g.	Methoxyl, %	In chloroform $[\alpha]^{22}D$	$[\eta]$[b]
1	0.28	37.0, 37.0	−54.2°	0.68
2	0.20	37.0, 37.0	−55.8°	.67
3	2.43	36.5, 36.5	−56.0°	.59
4	3.39	36.5, 36.5	−51.4°	.42
5	1.60	36.8, 36.8	−54.8°	.30

Recovery 7.6 g. or 90%.

[a] Sample, 8.5 g., in 200 ml. of chloroform with ligroin as the non-solvent. [b] Plots of reduced specific viscosity against c extrapolated to $c = 0$. Plots linear between $c = 0.25$ and $c = 1.4\%$.

The research also uncovered some information of general interest. The total amount of modified lignin extracted from the crude aspen holocellulose was at least as much as the 2.9% of regular Klason lignin it contained, and bleaching with chlorite failed to remove all of the lignin. Pectic materials comprised between 5 and 10% of the crude hemicellulose extracted, and their complete removal was attained only by acetylation. The fact that the intrinsic viscosities of the products remained of the same order of magnitude throughout acetylation, deacetylation and methylation argued against the occurrence of inadvertent degradation during the manipulations (Tables I, II, III, IV), and the same inference followed from the nearly constant ratio of 1:2 observed between sub-fractions of lowest and highest viscosity. Since the less soluble hemicelluloses, which presumably were of greater molecular size, were not extracted on the present occasion, the relatively low intrinsic viscosities observed were to be expected. The acidic nature of the aspen hemicelluloses, together with the probability that their structures were branched, made it prudent to defer an attempt to translate these viscosities into degrees of polymerization. Such a correlation would probably require an adequate supply of structurally homogeneous material falling in a narrow viscosity range and free of pectic contaminants.

Experimental

Materials and Methods.—A log from an aspen tree (*Populus tremuloides*) about 26 years old was de-barked, stored for a year,[19,20] chipped, ground to 40–80 mesh size, extracted with alcohol–benzene and dried in the air.

Anal. Found (on a moisture and ash-free basis): Klason lignin,[21] 19.3, 19.5; acetyl,[22] 4.25, 4.30; pentosan,[22] 20.9, 20.9; uronic anhydride,[23] 4.78, 4.60.

Ten 100-g. batches of the woodmeal were bleached[11] for a total of 3 hr. near *p*H 5 and 75° with approximately the same weight of sodium chlorite dissolved in water. The holocellulose was dried at 37°.

Anal. Found: Klason lignin, 2.8, 3.0; acetyl, 5.02, 5.02; pentosan, 23.3, 23.0; uronic anhydride, 6.08, 6.20; yield (lignin free), 81.5.

The fact that the holocellulose apparently contained 110% of the original uronic anhydride led to the discovery that a spruce periodate lignin free of carbohydrates had an apparent uronic anhydride content of 8.4% before, and of 21.3% after, oxidation with chlorine dioxide.[24] At least some of the "uronic anhydride," therefore, originated from oxidized lignin that remained in the holocellulose. Although both the acetyl content and the yield of the holocellulose were close to the values calculated from the wood, about 6% of the original pentosan was lost. It is now well known that such losses were compensated by the retention of other constituents in about the same amount.[11,15,25]

Nitration by a method supposed to cause no degradation[26] gave nitrates whose acetone-soluble portions (N, 13.2–13.4%) were recovered in yields of 83.8, 87.6 and 66.0% by weight from the aspen wood, the chlorite holocellulose and from a holocellulose prepared by the chlorination method,[12–14] respectively. Although the respective intrinsic viscosities of the nitrates in acetone, 21.1, 15.3 and 19.0, agreed with the reports[8,18,27,28] that the delignification of woods by chlorite caused more degradation than delignification by chlorine, general chemical considerations suggested that the reverse should be true, as indeed it was for the bleaching of cotton linters.[12] The anomaly might eventually prove to be related to the resistance of the pectic constituents of woods to hydrolytic degradation while the same constituents might be degraded by chlorite with unsuspected ease. In the meantime, there seems to be no compelling reason for considering that the chlorite method yields a more "degraded" cellulose or hemicellulose from wood.

Every attempt was made throughout the work to avoid degradation; aqueous solutions were evaporated near *p*H 6 under diminished pressure, operations involving caustic alkali were conducted in an atmosphere of nitrogen and products were dried *in vacuo* at room temperature over phosphorus pentoxide. All specific rotations referred to 22 or 23° and the D-line of sodium, and measurements of viscosity were made in a Cannon–Fenske viscometer, No. 50,

(19) The gift of Dr. George Tomlinson, II.

(20) Gift of the Howard Smith Paper Mills, Cornwall, Ontario.

(21) T.A.P.P.I. Official Standard, T-13m-45 (April 1945).

(22) R. U. Lemieux and C. B. Purves, *Can. J. Research*, **B25**, 485 (1947).

(23) R. M. McCready, H. A. Swenson and W. D. Maclay, *Ind. Eng. Chem. Anal. Ed.*, **18**, 290 (1946).

(24) N. Leviton, N. S. Thompson and C. B. Purves, *Pulp and Paper Mag. Canada*, **56**, No. 5, 117 (April 1955), described the β-oxylignin fraction examined. The observation confirmed the similar work on spruce chlorite lignins carried out by B. L. Browning and L. O. Bublitz, *Tappi*, **36**, 452 (1953)

(25) W. G. Campbell and I. R. C. McDonald, *J. Chem. Soc.*, 2644, 3180 (1952).

(26) W. J. Alexander and R. L. Mitchell, *Anal. Chem.*, **21**, 1497 (1949).

(27) E. Heuser and L. Jörgensen, *Tappi*, **34**, 57 (1951).

(28) J. D. Wethern, *ibid.*, **35**, 267 (1952).

A.S.T.M., the time of flow for pure acetone being 105 sec. To identify the constituent monosaccharides, 20 mg. of the polysaccharide was sealed in a glass tube with 0.6 ml. of *N* sulfuric acid and hydrolyzed for 5 hr. at 100°. After removal of the acid as the barium salt, an aliquot of the hydrolyzate was chromatographed on paper, using ethyl acetate–water–pyridine (2:2:1 vol.) as the developer[29] and aniline phthalate as the spray. Sometimes the copper-reducing power of other aliquots was determined by the Schaffer–Hartman–Somogyi method[30] and was calculated as per cent. glucose or xylose.

Preliminary Extraction with Water (Fig. 1, Extract-1).—Of the above holocellulose 740 g., on a dry basis, was extracted for 3 hr. at *p*H 5 to 6 and 65 to 70° with water, allowing 5 l. for each 200 g. After a second similar extraction, the residual holocellulose was dried; yield 81.7% of the wood.

When evaporated to dryness, the combined extracts yielded 26.8 g. of a brown, friable solid which was free of sugars. Systematic fractionations from aqueous ethanol isolated about one-fourth of this solid as brown salts of a polygalacturonic acid (OCH_3, 3.6%) which yielded 30% of crystalline mucic acid when oxidized with bromine–hydrobromic acid[17]; these salts were mixed with polysaccharides whose acid hydrolyzates yielded chromatographic evidence of galactose, xylose, arabinose and uronic acids but not of glucose and mannose. About one-third of the extract consisted of a light-brown solid (OCH_3, 6.9%) whose head sub-fractions resembled the materials already described and whose later sub-fractions were similar to the next main portion, the remaining third of the extract. This portion (OCH_3, 12.2%) was very soluble in alcohol, contained only small amounts of polysaccharides all derived from xylose and arabinose and consisted essentially of modified lignin. Since the ultraviolet spectrum of the intermediate portion retained the maximum near 2800 Å. characteristic of unoxidized lignins, at least some of the lignin in the extract had not been severely oxidized by the chlorite.[24]

Extraction of the Holocellulose with Liquid Ammonia (Fig. 1).—The bomb used[9] was constructed from a stainless steel pipe 48 inches long and 6.5 inches in diameter, whose steel inlet and outlet tubes were protected by steel screens and were closed by needle valves. After being dried to less than 1% moisture in order to reduce the considerable heat of dilution of liquid ammonia by water, the holocellulose, 668 g., was put into the bomb and the assembly was cooled to 6°. Commercial 99.9% liquid ammonia, 5 kilo, was then added, and after being closed the bomb was slowly rocked at room temperature for 16 hr. The liquid ammonia was then discharged under its own pressure into a round-bottom, 6-l. Pyrex flask containing 500 ml. of anhydrous methanol, access of atmospheric moisture being carefully prevented. A total of three extractions removed from the holocellulose almost all constituents soluble in liquid ammonia, and the three extracts were combined in the same flask (see below).

Prior to removing the residual holocellulose from the bomb, most of the ammonia was evacuated under diminished pressure as a gas. The remainder was removed by keeping the holocellulose *in vacuo* over phosphorus pentoxide for 48 hr., yield of product, 582.5 g. or 87.5%.

Anal. Found: Klason lignin, 0.77, 0.80; acetyl, 0.50, 0.51; pentosan, 26.5, 26.7; uronic anhydride, 5.1, 5.3.

Continuous extraction for 12 hr. with ethanol removed only 1.9 g. of material from the product.

Examination of the Liquid Ammonia Extract-2 (Fig. 1).—The discharge of the extract into methanol prevented the formation of an intractable lacquer when the liquid ammonia was allowed to evaporate (moisture being excluded) and separated the residue into portions soluble and insoluble in methanol. The methanol-soluble portion and the methanol washings when evaporated to dryness left 68.5 g. (9.8%) of a hard, brown residue, which was powdered and extracted three times with 200-ml. volumes of hot acetone to dissolve acetamide. After being combined and concentrated, the acetone extracts were diluted with ether until no more sirup was precipitated, and this sirup when recovered was exhaustively extracted with fresh acetone. Two or three repetitions of this cycle eliminated 10.3 g. of an insoluble lignin (OCH_3, 15.3, 15.6%) and gave a clear liquor from which 33.3 g. of nearly pure acetamide crystallized. A recrystallization from ether gave a product melting correctly at 80–81°, undepressed by admixture with an authentic sample.

Anal. Calcd. for CH_3CONH_2: N, 23.7. Found: N, 24.1.

A partly crystalline residue, 5.3 g., recovered from the mother liquors increased the yield of crude acetamide to 38.6 g., or 3.3% of the wood calculated as acetyl.

The remainder of the methanol-soluble portion consisted of modified lignin in the form of a dark-brown solid, soluble in alcohol–ether or in ether and containing no carbohydrates. Extensive fractionation of this solid yielded sub-fractions whose methoxyl contents were all near 16%, but no individual substances could be isolated. The portion insoluble in methanol was divided into three crude sub-fractions ranging in methoxyl content from 5.6 to 14.1% and in reducing power after hydrolysis from 42.5 to 6.8% as glucose. The latter sub-fraction on extensive refractionation from ethanol yielded material whose methoxyl content remained within the limits 10.5 ± 0.4% but which after acid hydrolysis gave chromatographic evidence of glucose, galactose, xylose, arabinose and uronic acids. Acid hydrolysates of the two other sub-fractions contained the same sugars, with the exception that mannose replaced arabinose.

Extraction and Bleaching of the Hemicelluloses (Fig. 1, Extracts-3 and -4).—After the extraction with liquid ammonia, the residual holocellulose, 616 g. air-dry, was stirred for 5 hr. with 5 l. of water at room temperature, the suspension being adjusted to *p*H 5–6 with a little acetic acid. The wet residue was again extracted under the same conditions and then twice more with 5-l. volumes of water at 70°. Each of the four extracts was concentrated to about 1 l.; the crude hemicelluloses were precipitated by adding alcohol to a concentration of 70% and were dried through alcohol and ether. Non-precipitated material was recovered by evaporating the mother liquors to dryness. When based on the original holocellulose, the yields of crude hemicellulose precipitated from the cold and hot water extracts were 39 g. (5.8%) and 17 g. (3.3%), respectively; those of the non-precipitated material were 13 g. (1.9%) and 2 g. (0.35%).

Three small samples of the same crude hemicellulose were then submitted to alternative methods of purification. The first method involved solution in water, the removal of a trace of undissolved material and reprecipitation with ethanol; the second, repeated reprecipitation by ethanol from solution in *N* sodium hydroxide as described by McDonald[31]; and the third, bleaching of the clarified aqueous solution with sodium chlorite near *p*H 5 and at room temperature. The last two methods caused about 14% of the products to become insoluble in water, and these fractions were not included in the data given in Table VI. Products from the last two methods gave ultraviolet absorption plots that were almost superposable and to judge from the decrease in the extinction coefficients at 2800 Å. both reduced, but failed to eliminate, slightly modified lignin. Since the specific viscosities gave no reason to think that bleaching with chlorite degraded the hemicellulose, this method of purification was selected.

TABLE VI

DATA ON HEMICELLULOSE PURIFIED BY DIFFERENT METHODS

Method	Yield, %	$[\alpha]_D$[a]	$[\eta]_{sp}/c$[b]	Ultrav. coeff.[c]	Glucose equiv.[d]
H_2O–EtOH	95	−63°	0.46	6.3	76.5
NaOH–EtOH[e]	83	−75°	.47	4.0	75.3
H_2O–$NaClO_2$[e]	82	−65°	.52	4.1	75.0

[a] At 23° in water; *c*, 0.48 to 0.97. [b] Reduced specific viscosity in 10% aqueous potassium hydroxide, *c*, 0.48 to 0.49% (*cf.* reference 28). [c] Extinction coefficients of 0.004% solutions in *N* sodium hydroxide measured at wave length 2800 Å. [d] From copper-reducing power after hydrolysis in 2.5% sulfuric acid at 100° for 5 hr. [e] Dialysis had removed 4% and caused the precipitation of about 14%.

A 3% aqueous solution of the crude hemicellulose from the cold water extract, 38.5 g., was centrifuged to remove 1.48 g. of insoluble material, and the clear liquor was bleached

(29) M. A. Jermyn and F. A. Isherwood, *Biochem. J.*, **44**, 402 (1949).

(30) C. A. Brown and F. W. Zerban, "Physical and Chemical Methods of Sugar Analysis," 3rd Ed., John Wiley and Sons, Inc., New York, N. Y., 1941, p. 846.

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

for 5 hr. at room temperature by the gradual addition of 38 g. of sodium chlorite, together with acetic acid to maintain the *p*H at 5.0 to 5.5. The mixture was centrifuged to remove material which had become insoluble, this portion being re-suspended in water and dialyzed to remove sodium chlorite. After recovery, the product was extracted twice with 100-ml. volumes of water. The water-insoluble portion, 6.47 g., was designated as fraction A, and the portion precipitated from the aqueous extract by alcohol, 4.09 g., as fraction B. Fraction C, 24.8 g., was separated from the original chlorite liquor by precipitation from 70% ethanol followed by dialysis. The total recovery was thus 36.9 g. or 96%. In the same way, the hemicellulose from the hot water extracts, 13.8 g., yielded fraction D, 1.96 g., which separated during the bleaching, and fraction E, 10.7 g., recovered from the liquor. A small portion, 0.05 g., of the original hemicellulose failed to dissolve in water.

Acetylation of the Hemicelluloses.—The dry hemicellulose C, 23.1 g., did not react well in pyridine–acetic anhydride, and it was first necessary to swell the sample for 1 hr. at 65° in 460 ml. of formamide.[32] After being diluted with an equal volume of pyridine, the suspension was cooled to room temperature and 380 ml. of acetic anhydride was added dropwise during 3 hr. Next day the product, 20.4 g., was precipitated by pouring the mixture into 3.5 l. of ice and water. Ice and water were preferable to cold ethanol as a precipitating agent. Although they gave practically the same yield of acetate, the product when deacetylated gave a much more levorotatory hemicellulose, which presumably contained less pectic material. A second acetylation involved solution of this acetate in 250 ml. of pyridine at 55°, the addition of 70 ml. of acetic anhydride and storage for 19 hr. at room temperature. A third similar acetylation brought the acetyl content to a constant value of 35.8% and the yield to 26.9 g. (117% by weight). The combined fractions B and E, 14.5 g., were acetylated in the same way to a constant acetyl content of 36.1%, yield 19.2 g. or 132%. Both acetates were then united and fractionated as described in Tables I and II.

The aqueous mother liquors from the precipitation of the acetates, on evaporation to dryness, left 4.04 g. (11%) of an amorphous pectic residue. A 1.4-g. portion of this residue when boiled with bromine–hydrobromic acid[17] yielded 0.10 g. of mucic acid, m.p. 118°, undepressed by admixture with an authentic sample.

Deacetylation of Hemicellulose Acetates (Table III).—The sub-fractions 1 and 2a (Table I), weighing 19.0 g. and insoluble in chloroform, were swollen in 450 ml. of peroxide-free dioxane at 45° for 3 hr. before being deacetylated by the addition of 1200 ml. of 0.4 *N* potassium hydroxide in ethanol.[33] To make sure that deacetylation was complete, the product was dissolved in 125 ml. of 3 *N* aqueous sodium hydroxide and was reprecipitated by an excess of ethanol. Cold 50% acetic acid was mixed with the precipitate to neutralize any base, and salts were then thoroughly extracted with 70% ethanol. The product, about 12 g., still wet with ethanol, was dissolved in 320 ml. of 2 *N* sodium hydroxide and was fractionated by adding increments of alcohol. Each sub-fraction was neutralized with cold 50% acetic acid and when necessary was reprecipitated with ethanol. Drying was after solvent-exchange through ethanol into benzene. In a similar manner, sub-fractions 5 to 8 (Table I) and IV to VII (Table II) were deacetylated and fractionated as a unit.

D-Xylose from Hemicellulose Fractions S, H-8, H-9 (Table IV).—The combined fractions, 4.47 g., were hydrolyzed with 446 ml. of 1% sulfuric acid by heating on a steam-bath for 4.5 hr. or until the copper reducing power was close to the maximum value of 85% as xylose. Sulfate ions were removed with barium carbonate, the clear, neutral filtrate was concentrated to 50 ml. and the barium uronates were precipitated by adding 500 ml. of ethanol. These salts were reprecipitated from aqueous ethanol and were saved. The combined aqueous-alcoholic mother liquors were concentrated to one-fourth volume and were de-ionized by passage through columns of Amberlite IR-120 and Amberlite IR-4B, each column being thoroughly washed with water. The eluate from the latter column when evaporated yielded a thick sirup which crystallized completely after being dried over phosphorus pentoxide; yield 3.46 g. or 77%. Recrystallization from hot, glacial acetic acid raised the m.p. to 144–145°, the correct value for β-D-xylopyranose, and a mixed m.p. with an authentic sample was not depressed. The crystals also had the correct equilibrium specific rotation of 19° in water.

Aldotriuronic Acids from Fractions S, H-8, H-9 (Table IV).—An aqueous solution of the above barium uronates was freed from barium by passage through a column of Amberlite IR-120, which was afterward well washed with water. After recovery, the dry sirup, 1.0 g. (22.4%) was diluted with water and applied along the starting lines of 10 sheets of Whatman No. 1 filter paper each 18 inches long and 22 inches wide. The chromatograms were developed for 17 hr. with a mixture of ethyl acetate, acetic acid, formic acid and water (18:3:1:4 vol.),[4a] and the use of aniline phthalate on small test strips located spots with R_f values of 0.03, 0.07, 0.10, 0.12, 0.16, 0.21, 0.29 and 0.37, L-rhamnose with R_f 0.30 being the reference. The spot with R_f 0.21 had the same mobility and color as one from D-xylose. Each component was then extracted with water from the corresponding unsprayed pieces of paper and recovered.

(a) The component with R_f 0.07, 0.31 g., was a thick sirup giving a red spot with aniline phthalate. The sirup soon crystallized extensively, and after isolation the crystals had a specific dextrorotation of +46.9° in water (*c* 1.5).

Anal. Calcd. for two xylose plus one methylhexuronic acid residue (mol. wt. 472): OCH_3, 6.57; CHO, 6.14; COOH, 9.53. Found: OCH_3, 5.90, 5.96; CHO, 6.09; COOH, 10.3, 10.0.

The aldehyde group was determined by oxidation with alkaline hypoiodide[34] and the carboxyl group by adding an excess of 0.007 *N* alkali and back-titrating with standard acid 4 hr. later. Both determinations gave satisfactory results with crystalline glucuronolactone.

Part of the aldotriuronic acid, 0.12 g., was boiled for 8 hr. with 6 ml. of 1.8% methanolic hydrogen chloride. After recovery, the sirupy methyl ester methyl glycoside was continuously stirred in 4 ml. of water for 25 min. with 0.1 g. of sodium borohydride in order to reduce the ester to primary alcohol groups.[18] The reaction mixture was then boiled with 20 ml. of 2% sulfuric acid for 5 hr.; the hydrolyzate was neutralized with barium carbonate, was de-ionized on a column and was evaporated to dryness. When chromatographed on paper, using methyl ethyl ketone saturated with aqueous ethanol (4:1 vol.) as developer and aniline phthalate as the spray, a spot appeared identical in position and color to that given by 4-*O*-methyl-D-glucose. Authentic samples of 2-, 3- and 4-*O*-methylglucose formed distinctly different spots with R_f values of 0.34, 0.36 and 0.32, respectively, in the system used.[35] The only other spot on the chromatograms was that of xylose.

(b) The other major uronic acid fraction, 0.53 g. with R_f, 0.16, gave an orange spot with aniline phthalate, failed to crystallize and had a specific dextrorotation of 81.2° in water (*c* 1.5).

Anal. Calcd. for one xylose, one hexuronic and one methylhexuronic acid residue (mol. wt. 516): OCH_3, 6.0; CHO, 5.62; COOH, 17.4. Found: OCH_3, 6.65, 6.65; CHO, 6.77, 6.68; COOH, 22.0, 21.5.

The substance was therefore impure. When a portion, 0.29 g., was reduced, hydrolyzed and chromatographed as already described, spots corresponding to 4-*O*-methylglucose and to xylose were definitely present, but no other spot was found. A parallel reduction of pure glucuronolactone resulted in an intense spot for glucose on the chromatogram.

Oxidation of Fraction R (Table IV) with Periodate.—Duplicate samples, 0.141 g. (ash-free), were oxidized in the dark by suspension in 25-ml. volumes of 0.1 *N* sodium metaperiodate with concordant results. The consumption of periodate, determined in 2-ml. aliquots by the arsenite method,[36] corresponded to 0.44, 0.55, 0.60, 0.66, 0.74, 0.77 and 0.94 m*M* after 1.4, 2.4, 3.7, 4.7, 7.2, 10.2 and 23 hr., respectively. When the later portion of a plot of these data was extrapolated to zero time, the consumption was 0.70

(32) J. F. Carson and W. D. Maclay, THIS JOURNAL, **70**, 293 (1948).

(33) F. C. Peterson, A. J. Barry, H. Unkauf and L. E. Wise, *ibid.*, **62**, 2361 (1940).

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

(35) Our colleague, E. Falconer, found this system suitable for the separation of partly methylated glucoses.

(36) E. L. Jackson, "Organic Reactions," Vol. 2, John Wiley and Sons, New York, N. Y., 1944, p. 341.

mM, or 17 moles for the base mol. wt. of 3460 assumed for fraction R. Other 5-ml. aliquots were back-titrated to the original pH of 4.89 by 0.01 N alkali, and the volumes added were 0.85, 0.85, 0.85, 1.18 and 1.35 ml. after 3.4, 4.2, 5.3, 8.4 and 26 hr., respectively. These figures corresponded to the production of 1.04 moles of formic acid per base mol. wt. of 3460 in 3 to 5 hr. and to 1.6 moles after 26 hr.

Methylation of Fraction R (Table IV).—The sample, 8.7 g., was stirred under nitrogen for 2 hr. with 140 ml. of N aqueous thallous hydroxide,[37] and after recovery the insoluble, yellow thallium derivative was thoroughly dried and finely powdered; yield 27.2 g. This product was methylated for 12 hr. with methyl iodide, but more than half was almost unchanged. The unchanged portion was remethylated in the same way and then combined with the remainder. The whole, OCH_3, 31%, was then methylated by thallous ethylate in benzene followed by methyl iodide and by silver oxide–methyl iodide to a constant methoxyl content of 36.8%; yield 8.5 g. or 97% by weight.

After being fractionated (Table V) the sub-fractions were recombined and 6.4 g. was degraded by being boiled for a total of 10 hr. with 330 ml. of 2% methanolic hydrogen chloride. Water, 300 ml., was added and boiling continued for a further 6 hr. to hydrolyze esters and glucosides. After removal of hydrogen chloride (as the silver salt) and of the solvent, the residual light yellow sirup, 6.1 g., was dissolved in 7 ml. of methyl ethyl ketone saturated with water and was applied to the top of a column of powdered cellulose, 3.7 cm. by 51 cm., previously washed with the solvent. More solvent was percolated through the column at the rate of 6 ml. in 5 min., and the progress of the separation was followed by paper chromatography. Eluates 16 to 56 contained dimethylxylose, and eluates 59 to 100 monomethylxylose, but both fractions also contained traces of uronic acids. These traces were removed by shaking aqueous solutions of the fractions with a mixture of Amberlite resins IR-120 and IR-4B, the filtrates being separately evaporated to sirups.

The dimethylxylose sirup, 2.87 g., had a specific dextrorotation of 23.8° in water (c 2.0), and when chromatographed with the methyl ethyl ketone, water–ethanol developer gave a single spot with R_f 0.65.

Anal. Calcd. for $C_5H_8O_3(OCH_3)_2$: OCH_3, 34.8. Found: OCH_3, 34.4, 34.6

The m.p. of the anilide, 124–125°, was undepressed by admixture with an authentic sample of 2,3-di-*O*-methylxylose anilide kindly supplied by Dr. G. A. Adams, of the National Research Council of Canada

The monomethylxylose sirup, 0.20 g., with a specific dextrorotation of 23° in water (c 1.0), gave a principal spot with R_f 0.43, and a slight spot with R_f 0.65 when chromatographed for 2.5 hr. The latter spot corresponded to dimethylxylose.

Anal. Calcd. for $C_5H_9O_4(OCH_3)$: OCH_3, 18.9. Found: OCH_3, 19.9, 19.8.

After 12.5 hr. the principal spot had nearly divided into two spots with centers 36.3 and 38.3 cm. from the starting line. These spots were identical in position to those pertaining to 2- and 3-*O*-methylxylose, respectively. The reduction of 200 ml. of 0.05 N lead tetraacetate in acetic acid containing 87 mg. of the original sirup was followed iodometrically[38] and within two minutes amounted to 0.52 mole per mole. This proportion of the sirup therefore consisted of 3-*O*-methylxylose. The reduction did not increase within 15 min.

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(37) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 496 (1938).

(38) R. C. Hockett and W. S. McClenahan, THIS JOURNAL, **61**, 1667 (1939).

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Constitution of the Hemicellulose of Western Hemlock (*Tsuga heterophylla*). II. Hydrolysis of the Methylated Hemicellulose[1,2]

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The hemicellulose isolated from delignified Western Hemlock wood, by extraction with alkali, has been shown to contain a branched chain arabo-methoxyglucurono-xylan. The side chains consist of single units of 4-*O*-methyl-D-glucuronic acid and single units of L-arabofuranose joined to positions 2 and 3, respectively, of D-xylopyranose units of the xylan molecular framework. The methylated hemicellulose gives upon hydrolysis: 2,3,4-tri-*O*-methyl-D-xylose (1 mole), 2,3,5-tri-*O*-methyl-L-arabinose (0.75 mole), 2,3-di-*O*-methyl-D-xylose (8 moles), 2-*O*-methyl-D-xylose (1 mole), 3-*O*-methyl-D-xylose (3 moles) and 2,3,4-tri-*O*-methyl-D-glucuronic acid (3 moles). The general structural features of the polysaccharide are discussed.

An aldobiouronic acid, 2-*O*-(4-*O*-methyl-D-glucuronosyl-D-xylose, has been shown[3] to be a component of the hemicellulose of Western Hemlock (*Tsuga heterophylla*). This paper is concerned with the main structural features of the hemicellulose itself as revealed by methylation studies.

In order to ascertain the mode of union of the building units of the hemicellulose the latter was methylated first with potassium hydroxide and methyl sulfate[4] and then with silver oxide and methyl iodide.[5] Fractional precipitation of the methylated polysaccharide from chloroform with petroleum ether indicated the presence of two principal components. The less soluble methylated polysaccharide which had $[\alpha]^{25}D$ −51° (chloroform) is the subject of the present paper. The more soluble methylated polysaccharide showing $[\alpha]^{25}D$ −13° (chloroform) will be discussed in a subsequent communication.

Following methanolysis of the methylated polysaccharide with 2% methanolic hydrogen chloride under conditions which left the sugar acid in the form of a disaccharide, the methyl glycosides were separated into neutral and acidic components using ion exchange resins.

The acidic component was shown by analysis to be a partially methylated aldobiouronic acid. This was identified as methyl 2-(2,3,4-tri-*O*-methyl-D-

(1) Paper No. 3488, Scientific Journal Series, Minnesota Agricultural Experiment Station.

(2) This paper was presented at the 128th A.C.S. Meeting in Minneapolis, Minn., September, 1955.

(3) G. G. S. Dutton and F. Smith, THIS JOURNAL, **78**, 2505 (1956).

(4) W. N. Haworth, *J. Chem. Soc.*, **107**, 8 (1915).

(5) T. Purdie and J. C. Irvine, *ibid.*, **83**, 1021 (1903).