

THE ACID COMPONENT OF JUTE HEMICELLULOSE

by

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ABSTRACT

Defatted powdered jute was delignified by chlorite treatment and the hemicellulose extracted with alkali. The precipitated material was purified by washing with alcoholic hydrochloric acid and dried by solvent exchange.

The hydrolysed hemicellulose yielded neutral sugar and sugar acids separated on ion exchange resins. The neutral sugar was identified as D-xylose. The sugar acid fraction contained mainly an aldobiouronic acid, proved by the reduction of the methyl ester methyl glycoside with lithium aluminum hydride followed by hydrolysis to consist of D-xylose linked to a monomethyl glucose. This was shown, via its anilide and osazone, to be 4-O-methyl-D-glucose.

Methanolysis of the aldobiouronic acid yielded the methyl glycoside of a uronic acid which on treatment with diazomethane and then with methanolic ammonia gave 4-O-methyl- α -D-glucuronoamide methyl glycoside, after fractional crystallization.

Reduction of the aldobiouronic acid methyl ester methyl glycoside with lithium aluminum hydride, followed by methylation and hydrolysis, gave 2,3,4,6-tetra-O-methyl-D-glucose and 3,4-di-O-methyl-D-xylose.

A search for a crystalline derivative of the purified aldobiouronic acid and of its related xylitol compound formed on reduction with potassium borohydride was unsuccessful.

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HISTORICAL INTRODUCTION

Land plants usually contain several types of polysaccharide material other than cellulose which is their main support structure. These may be described as "homoglycans" where only one type of monosaccharide is present in the structure as for instance in starch or insulin or as "heteroglycans" where more than one type of monosaccharide residue occurs. These latter materials have been classified as hemicelluloses, gums, and mucilages according to their chemical nature and origin and their distribution in nature is very wide since the composition of the polysaccharides may vary considerably in different parts of any one plant. Polysaccharides are also obtained from seaweed and from certain types of bacteria and determinations of structure are essential to many branches of science. The function which they fulfil is not always clear but it would appear that the hemicelluloses may act as reserve support structures or reserve food materials depending on their nature and situation in the plant. They occur in wood, bark, leaves, and seed pods whilst the gums are obtained as exudates on the bark of trees or on fruit and are used by the plant as a protective mechanism against bacterial invasion or physical damage (1). The mucilages are of widespread occurrence but are most commonly found in seeds, where they assist in water storage and may "solubilise" insoluble substances such as cellulose (2).

Many workers have attempted to define these three classes of polysaccharides. Generally hemicelluloses are regarded as those polysaccharides which are extractable from holocellulose by aqueous alkali. Plant gums are defined as giving a clear solution in water, whereas mucilages swell but do not dissolve in water. There are exceptions to

this definition since tragacanth gum, which is a tree exudate and a true gum, is only partly soluble in water and behaves as a mucilage (3,4).

These polysaccharide materials undergo acid hydrolysis to yield hexoses, pentoses, uronic acids and aldobiouronic acids. The methyl pentoses L-rhamnose and L-fucose are also found occasionally and occur in pyranose form as do all the other constituents except L-arabinose which prefers the furanose ring. The hexoses, uronic acids and xylose have the D-configuration. The plant gums all contain D-glucuronic acid except tragacanth gum which contains D-galacturonic acid. The seed mucilages on the other hand nearly all contain D-galacturonic acid whilst the hemicelluloses show more variation and may yield D-glucuronic, D-galacturonic and 4-O-methyl-D-glucuronic acids whilst 3-O-methyl-glucuronic acid has also been claimed to occur (5) (see later).

In the plant gums so far examined D-galactose and L-arabinose are always found whilst D-xylose is also of very common occurrence. Less usual constituents are D-mannose, L-rhamnose, and L-fucose whilst D-glucose has not yet been found. The mucilages are a more complex family than the gums and may be classified as neutral, acidic, or sulphate ester (seaweeds only). D-galactose is universally found and L-arabinose is usually present whilst D-mannose, D-glucose, D-fructose and L-rhamnose are less common. D-xylose is commonly found in the acidic mucilages which contain D-galacturonic acid. In the hemicellulose family D-xylose is very common and xylan-polyuronides are often present in woody tissue. The barks of trees often contain galactan-galacturonides whilst D-galactose is also predominant in leaves and pods and occurs together with L-arabinose in green non-lignified plant tissues (6).

Previous to 1952, the uronic and aldobiouronic acids were separated from the hexoses and pentoses by virtue of the insolubility of their barium salts in aqueous alcohol. In that year their absorption from solution on to weakly basic anion exchange resins was first utilised by Hough, Jones and Wadman (7) and this is now the normal method for the separation of sugar acids from the neutral sugars. The isolation of uronic acids from aldobiouronic, aldotriouronic, and acids of higher molecular weight is best accomplished by partition chromatography on cellulose columns or on sheets of thick paper whilst mixtures of uronic acids may be separated by fractional distillation of their methyl glycoside methyl esters in vacuo.

The present work deals with the structure of an aldobiouronic acid isolated from jute hemicellulose. Structural determinations on aldobiouronic acids are of great importance in studying the chemical nature of polysaccharides and no comprehensive table of their structures and sources exists in the literature. Table I is a literature survey of this nature giving the structures of the acids known at present. Table II comprises those acids whose structure has not been completely determined. In so far as it was possible earlier work which has been superseded is omitted. In a few cases aldotriouronic acids have also been isolated and identified.

TABLE I

Aldobiouronic Acids of Proved Structure

Structure	Config- uration	Source	Refer- ence
2- <u>Q</u> -(D-glucuronopyranosyl)- D-xylopyranose	α	maize hull hemicellulose	8
	α	oat hull hemicellulose	9
	α	corn cob hemicellulose	10
	α	wheat bran hemicellulose	11
	—	chagual gum	12
3- <u>Q</u> -(D-glucuronopyranosyl)- D-xylopyranose	α	sun flower heads	13
	α	wheat straw hemicellulose	14
	α	pear cell wall	15
	α	wheat leaf hemicellulose	16
4- <u>Q</u> -(D-glucuronopyranosyl)- D-xylopyranose	—	Kadsura Japonica mucilage	17
	α	corn cob hemicellulose	10
4- <u>Q</u> -(D-glucuronopyranosyl)- D-glucopyranose	β	Type II pneumococcus polysaccharide	18
	β	Type VIII pneumococcus polysaccharide	19
2- <u>Q</u> -(D-glucuronopyranosyl)- D-mannopyranose	β	cherry gum	20
	β	damson gum	21
	β	gum ghatti	22
4- <u>Q</u> -(D-glucuronopyranosyl)- D-galactopyranose	α	Acacia Karroo gum	23
	—	Neem gum	24
6- <u>Q</u> -(D-glucuronopyranosyl)- D-galactopyranose	β	Acacia Karroo gum	23
	—	egg plum gum	27
	β	wheat straw holocellulose	28
	β	gum arabic	29
	—	black wattle gum	30
	β	Acacia Pycnantha gum	31
	—	almond tree gum	32
	—	peach tree gum	33
	β	gum ghatti	22

TABLE I (cont'd.)

Structure	Config- uration	Source	Refer- ence
2- <u>0</u> -(4- <u>0</u> -Methyl-D-glucurono- pyranosyl)-D-xylopyranose	—	white birch hemicellulose	34
	—	white elm hemicellulose	35
	α	western hemlock hemicellulose	36
	—	beechwood hemicellulose	37
	—	black spruce hemicellulose	38
	—	Scots pine hemicellulose	38
	α	aspen wood hemicellulose	39
	α	pinus radiata hemicellulose	40
	—	American beechwood hemicellulose	41
	β	Loblolly pine hemicellulose	42
	α	wheat bran hemicellulose	11
	α	corn cob hemicellulose	43
	α	oat hulls hemicellulose	9
	—	flax	44
	—	birchwood holocellulose	45
	—	sitka spruce hemicellulose	46
3- <u>0</u> -(4- <u>0</u> -Methyl-D-glucurono- pyranosyl)-D-xylopyranose	α	Pinus radiata hemicellulose	40
4- <u>0</u> -(4- <u>0</u> -Methyl-D-glucurono- pyranosyl)-D-galactopyranose	α	gum myrrh	47
	—	mesquite gum	48,49
	—	lemon gum	25,26
	—	grapefruit gum	25,26
6- <u>0</u> -(4- <u>0</u> -Methyl-D-glucurono- pyranosyl)-D-galactopyranose	β	gum myrrh	47
	—	mesquite gum	48,49
	—	modal gum	50
4- <u>0</u> -(4- <u>0</u> -Methyl-D-glucurono- pyranosyl)-L-arabofuranose	α	lemon gum	51

TABLE I (cont'd.)

Structure	Config- uration	Source	Refer- ence
4- <u>0</u> -(D-galacturonopyranosyl)- D-xylopyranose	α	Plantago arenaria seed mucilage	52
2- <u>0</u> -(D-galacturonopyranosyl)- L-rhamnopyranose	—	flax seed mucilage	53
	α	Plantago arenaria seed mucilage	54
	—	Plantago ovata seed mucilage	55
	—	okra mucilage	56
	—	slippery elm bark mucilage	57
	—	Karaya gum	58
	α	grape juice	59
	—	sterculia setigera gum	60
4- <u>0</u> -(D-galacturonopyranosyl)- D-galactopyranose	—	Karaya gum	58
	—	sterculia setigera gum	61

TABLE II

Aldobiouronic Acids of Uncertain Structure

Structure	Source	Reference
(D-glucuronopyranosyl)-D-xylopyranose	Prunus serrulata	62
	cottonseed hull hemicellulose	63
(4-O-Methylglucuronopyranosyl)- D-xylopyranose	Eucalyptus regnans	64
3-(β -D-glucuronopyranosyl)-2-deoxy- 2-amino-D-galactopyranose	cartilage	65
(D-galacturonopyranosyl)-D-galacturonic acid	sterculia setigera gum	61
(D-galacturonopyranosyl)-D-galactopyra- nose	gum jeol	66
(D-galacturonopyranosyl)-D-gluco- pyranose	asparagus racemosus tuber mucilage	67
	cress seed mucilage	68
(D-galacturonopyranosyl)-L-rhamno- pyranose	white mustard seed mucilage	69
	cholla gum	70

THE IDENTIFICATION OF URONIC ACIDS

The characterization of the hexuronic acids and particularly of their monomethyl ethers is often difficult as their crystalline derivatives are rare and often their preparation is quite time-consuming. Three main methods are available for this purpose. Two involve the preparation of derivatives from the acid by direct and indirect means, whereas in the third method the acid is first reduced to the corresponding neutral hexose from which crystalline derivatives can be more readily obtained.

Oxidation of the Uronic Acids

In 1931, Challinor, Haworth and Hirst (29) isolated 2,3,4-tri-O-methyl-D-glucuronic acid from the hydrolysate of methylated gum arabic and proved its structure by oxidation with HNO_3 followed by methanolysis and distillation to yield the methyl ester of 2,3,4-tri-O-methyl-D-glucaro-1,5-lactone which was similarly derived from 2,3,4-tri-O-methyl-D-glucose. Similar derivatives of methylated uronic acids have been prepared by many authors following this route. Edington and Percival have synthesised 3,4-di-O-methyl-D-galacturonic acid (71) and proved its structure by oxidation with bromine water followed by esterification to yield the dimethyl ester of 3,4-di-O-methyl-D-galactaric acid. Haworth, Hirst, Isherwood and Jones (72) synthesised 2,3,4-tri-O-methyl-D-mannuronic acid which they identified by oxidation with HNO_3 followed by treatment with methanolic ammonia to yield the diamide of 2,3,4-tri-O-methyl-D-mannaric acid previously prepared from 2,3,4-tri-O-methyl-D-mannose. A more unusual proof of structure was used by White (49) who identified 4-O-methyl-D-glucuronic acid by periodate oxidation followed

by further oxidation with bromine to yield 2-hydroxy-3-methoxy-L-erythrosuccinic acid. This substance when reacted with a methanolic solution of methylamine gave the crystalline bismethyl amide.

Reduction of the Uronic Acids

The reduction of uronic acids with LiAlH_4 was first described by Lythgoe and Trippett (73) and by Abdel-Akher and Smith (74) in 1950. To confer solubility in ether or tetrahydrofuran the methyl ester methyl glycoside of the uronic acid was first prepared and the final product was the methyl glycoside of the corresponding hexose. Since aluminum salts form strong complexes with free hydroxyl groups it is now usual to isolate the reduced sugar as its acetate from which the methyl glycoside of the free sugar is easily obtained upon saponification. The reduction of uronic acids with LiAlH_4 works very well and gives good yields of the corresponding neutral sugars from which crystalline derivatives may be made. This method is probably the most widely useful means of identification of uronic acids known today.

Formation of the Amide of the Methyl Glycoside

The use of this direct derivative in characterising uronic acids was first reported by Smith (75). The methyl ester methyl glycoside of 4-O-methyl-D-glucuronic acid obtained from mesquite gum was distilled in vacuo and treated with a methanolic ammonia solution. Evaporation gave a white solid found to be a mixture of the α and β forms of the amide and these were easily separated by fractional crystallization. This derivative has since been prepared by many authors, the only objection to its use being a tedious separation of the α and β forms arising from the optical activity of C_1 .

The Identification of Aldobiouronic Acids

It is a well-known fact that the linkage between a uronic acid group and the neighbouring neutral sugar molecule in a polysaccharide is peculiarly resistant to acidic hydrolysis. This is seen from the large amounts of aldobiouronic acids produced upon acidic hydrolysis of hemicelluloses, gums and other polysaccharide materials. The reason for this resistance is not yet understood.

Due to their widespread occurrence as products of the hydrolysis of polysaccharides the identification of aldobiouronic acids has become of much importance in structural studies. Unfortunately these materials can very rarely be characterised directly as scarcely any crystalline derivatives are known. In a very few cases the amide or methyl ester of a methyl glycoside has been obtained crystalline (29,53,76,77). Smith and Srivastava (78) have obtained the crystalline pentaacetate of 2-O-(α -D-glucuronopyranosyl)-D-xylopyranose in both its α and β forms. This is the only occasion on which a xylose containing aldobiouronic acid has yielded a crystalline derivative. It is an object in this present work to search for a suitable derivative of the aldobiouronic acid from jute or of a compound easily obtained therefrom.

At the present time aldobiouronic acids are identified by reduction to neutral disaccharides followed by hydrolysis and identification of the products. It is also essential to determine the points of linkage of the constituents by methylation of the neutral disaccharide followed by hydrolysis and identification of the methylated fragments. Such a long procedure for the characterization of aldobiouronic acids requires a lot of time and, if suitable crystalline derivatives were available, this would be greatly reduced.

JUTE ALDOBIOURONIC ACID

The first study of jute hemicellulose was made by Sarkar, Mazumdar and Pal (79) in 1952. In it they determined the optimum conditions for its extraction from holocellulose and carried out detailed analyses of hemicelluloses extracted under varying conditions. They found the pure material to contain a monomethyluronic acid and xylose. From methoxyl content and neutralization equivalent measurements they deduced an equivalent weight of around 1000. In a further paper based on periodate oxidation studies on the hemicellulose Das Gupta and Sarkar (80) concluded that jute aldobiouronic acid probably had the structure 4-O-(3-O-methyl-D-glucuronopyranosyl)- β -D-xylopyranose. As further evidence for this structure the same authors in a letter to the editor (5) told how they had reduced the methyl ester methyl glycoside with lithium aluminum hydride and hydrolysed the resulting neutral disaccharide. The sugars in the hydrolysate were identified as xylose and 3-O-methyl glucose by paper chromatography in solvent 5.

The constituent sugars present in jute α -cellulose remaining after alkaline extraction of jute holocellulose have been investigated by Adams and Bishop (81). Formic acid hydrolysis and paper chromatography of the hydrolysate showed the presence of large quantities of glucose and xylose together with a definite amount of arabinose. This may indicate the occurrence of chemical bonding between the α -cellulose and the hemicellulose present in jute fibre.

The Indian workers (80) said that they would present final evidence for the presence of 3-O-methyl-D-glucuronic acid in the hemicellulose by the preparation and identification of suitable crystalline derivatives.

This evidence has not been produced and furthermore it is definitely shown in the present work that no distinction can be made by paper chromatography between 3- and 4-O-methyl-D-glucose using solvents 1 to 5.* If 3-O-methyl-D-glucuronic acid really is present in the hemicellulose it is the first time it has been found in nature whereas 4-O-methyl-D-glucuronic acid has been found in many sources (34-46). Further investigation of this question appeared to be desirable.

Powdered defatted jute was delignified readily by means of the chlorite process of Wise, Murphy and D'Addieco (81), to yield white holo-cellulose. This material was extracted with 10% aqueous sodium hydroxide and the solution neutralised. Upon addition of ethanol the hemicellulose was thrown out as a flocculent precipitate. After washing with aqueous alcohol to remove salts the material was dried by solvent exchange to give a dirty white powder in 16% yield.

A sample of crude hemicellulose was purified by precipitation as its copper salt/ⁱⁿalkaline solution. After careful washing the salt was decomposed with alcoholic hydrochloric acid and the pure hemicellulose isolated as before (yield 64%). The snow-white powder had ash content 2.33%, and was designated hemicellulose I.

Another sample of the crude material was suspended for a day in ethanolic hydrochloric acid and the hemicellulose on recovery was washed and dried as usual to give a snow-white powder (yield 70%) with an ash content of only 0.28%. This material was designated hemicellulose II.

A comparison of the optical rotations, neutralisation equivalents and methoxyl contents of hemicelluloses I and II showed them to be

* Key to this is given on p. 22.

identical. Purification as hemicellulose II was therefore adopted as a better product was obtained.

An attempt to extract the hemicellulose directly from jute without delignification was not encouraging. The yield was very poor and the product isolated after purification by the copper salt process had a brown colour. Hydrolysis of the material and examination of the hydrolysate showed the presence of a trace of glucose which might have derived from cellulose present as an impurity.

Trial experiments on the hydrolysis of jute hemicellulose showed that 1 Normal sulphuric acid for six to eight hours gave satisfactory results whereas incomplete hydrolysis resulted from the use of 45% formic acid even after twelve hours on the steam bath. The presence of oligosaccharides in this hydrolysate was demonstrated by further hydrolysis with 1 Normal sulphuric acid when chromatography showed only xylose, uronic and aldobiouronic acids to be present. It was not found possible to avoid the formation of uronic acid which occurred also in the formic acid hydrolysate.

A large batch of hemicellulose was hydrolysed with sulphuric acid and the hydrolysate separated into neutral sugar and sugar acid fractions using Amberlite IR 120 and Duolite A4 resin ion exchange columns. Chromatography on these fractions revealed only one neutral sugar which appeared to be xylose whereas the acid fraction contained uronic and aldobiouronic acids. Later a second neutral sugar was found present only in trace amounts and noticable only on heavily loaded chromatograms.

The neutral sugar was obtained crystalline and was identified as D-xylose. This was confirmed by the preparation of the dibenzylidene dimethyl acetal derivative (82). The trace sugar was identified only

by paper chromatography and ran identically with L-rhamnose in three solvents.

The sugar acid mixture was obtained as a syrup which did not crystallize. The methyl ester methyl glycoside formed by treatment with dilute methanolic hydrogen chloride was reduced with lithium aluminum hydride. The reduced sugar was freed from its aluminum complex by acetylation and the acetate extracted in chloroform and deacetylated with alkali gave the neutral disaccharide methyl glycoside as a yellow syrup which did not crystallize. This syrup was hydrolysed with 1 Normal sulphuric acid as before and the hydrolysate examined chromatographically was found to contain xylose and a monomethyl glucose which might have been 3- or 4-O-methyl-glucose.

Attempts to separate the sugars in the hydrolysate on a cellulose column using solvent 1 were a failure and the separation was finally carried out on sheets of paper using this solvent. The sugars were extracted from the paper in aqueous acetone and obtained as syrups, one of which crystallized on standing. This material was identified as D-xylose and confirmation was again obtained by preparation of the dibenzylidene dimethyl acetal derivatives (82). The other sugar was shown to be a monomethyl glucose by demethylation with hydrogen bromide and examination of the product by paper chromatography. Final and conclusive proof of the position of the methyl group was obtained by preparation of the osazone and anilide. Comparison of these derivatives against similar derivatives made from 4-O-methyl-D-glucose revealed that they were identical.

It is worthy of note that 4-O-methyl-D-glucose anilide has not previously been reported in the literature. This material was prepared

by the standard method for making sugar anilides and the yields were found to be rather variable since the formation of aniline tars often took place. The compound gave much difficulty in recrystallization from ethyl acetate and the crystals obtained by diluting with a few drops of petroleum ether were little better. Eventually a pure product was obtained after prolonged recrystallization. The maximum melting point found was 158-160°C.

In a search for suitable derivatives of 4-0-methyl-D-glucose the dibenzyl mercaptal was also prepared by the method used by Pacsu (83) in the preparation of D-glucose dibenzyl mercaptal. The sugar was condensed with α -toluenethiol in hydrochloric acid solution in the presence of zinc chloride. After addition of water the acid was removed on Duolite A4 ion exchange resin and the solution evaporated. The dibenzyl mercaptal crystallized as a white solid from ethanol and after three recrystallizations from ethanol the white needlelike crystals had a melting point of 158-159°C. This value did not rise on further crystallization. This derivative has been prepared by an indirect route by other workers (84,85) and melting points of 73°C. and 96-98°C. have been reported. It is apparent that their materials were not obtained pure and it is possible that the mercaptal was obtained as a hydrate.

The dibenzyl mercaptal of standard 3-0-methyl-D-glucose was also prepared as above. This compound did not crystallize from alcoholic solution but was obtained as white platelets from aqueous alcohol. After two recrystallizations a maximum melting point of 65-70°C. was reached. The material was apparently not pure and may have been a hydrate. The compound decomposed on heating in boiling toluene in the Abderhalden in vacuo in an attempt to remove water of crystallization.

This was unfortunate as 3-O-methyl-D-glucose dibenzyl mercaptal has never previously been reported.

A sample of jute aldobiouronic acid was hydrolysed in 10% methanolic hydrogen chloride for sixteen hours on the steam bath. After removal of hydrogen chloride the mixture containing methyl xyloside, uronic acid methyl ester methyl glycoside and the aldobiouronic acid methyl ester methyl glycoside was partially separated by continuous extraction with ether for twenty-four hours. The ether layer was shown to contain methyl xyloside and the uronic acid derivative, whilst the aqueous phase contained all three constituents. The ether extract was saponified with 0.15 Normal barium hydroxide and the uronic acid methyl glycoside separated by running the solution through Duolite A4 ion exchange resin when the acid remained on the resin whilst the methyl xyloside was removed. The recovered uronic acid methyl glycoside was re-esterified with ethereal diazomethane and the amide formed by treatment with methanolic ammonia solution. A mixture of the α and β forms of the amide methyl glycoside was obtained on evaporation. The white solid material was fractionally crystallized from aqueous ethanol solution to yield the pure α form after four recrystallizations. This was shown to be identical to an authentic sample of 4-O-methyl- α -D-glucuronamide methyl glycoside (75).

A sample of the neutral disaccharide methyl glycoside obtained by reduction of the aldobiouronic acid methyl ester methyl glycoside with lithium aluminum hydride was methylated three times with Haworth's reagents using dimethyl sulphate and 40% sodium hydroxide solution. The partly methylated disaccharide was again methylated using Purdie's reagents viz. silver oxide and methyl iodide. After three such methyl-

ations the disaccharide was found to be fully methylated. Hydrolysis of the distilled syrup with 1 Normal sulphuric acid for eleven hours was followed by separation of the methylated sugars on sheets of chromatographic paper in solvent 1.

The dimethylxylose obtained as a syrup was oxidized with bromine and on distillation in vacuo white crystalline 3,4-di-O-methyl-D-xylo-
-5-lactone was obtained. The tetramethyl glucose was not obtained pure and the contaminant appeared to be trimethyl rhamnose. An attempt to prepare the anilide of the tetramethyl glucose was successful but the crystalline anilide was not obtained pure due to the smallness of the scale on which it was made. From this derivative however and paper chromatography it was almost certain that the tetramethyl glucose obtained was 2,3,4,6-tetra-O-methyl-D-glucose.

From the evidence presented in this work it is concluded that the aldobiouronic acid present in jute must have the structure [A]. It is also evident however that a small amount of a second aldobiouronic acid occurs in which the neutral sugar unit is not D-xylose but L-rhamnose. The xylose unit to which the uronic acid is attached is part of a chain consisting of mainly xylose units with an occasional rhamnose. The length of this chain is not known but it is built up from blocks containing six xylose units to which one uronic acid group is attached.

Jute hemicellulose is unusual in that it contains no arabinose. This sugar is commonly found in hemicelluloses from annual plants. It would appear that rhamnose is involved somewhere in the biosynthetic pathways of the jute plant.

Whilst this thesis was being written Srivastava and Adams (86) published a short note on work which they had carried out on jute

hemicellulose. They also showed the presence of 4-O-methyl-D-glucuronic acid and identified the aldobiouronic acid as having structure [A]. Their characterization of the uronic acid was based on the preparation of the osazone of the monomethyl glucose obtained as already described. In addition they also separated an aldotriuronic acid in crystalline form and proved it to be 4-O-methyl-D-glucuronopyranosyl-1,2- α -D-xylopyranosyl-1,4- β -D-xylopyranose. This shows that the xylose units in the hemicellulose main chain are linked 1,4.

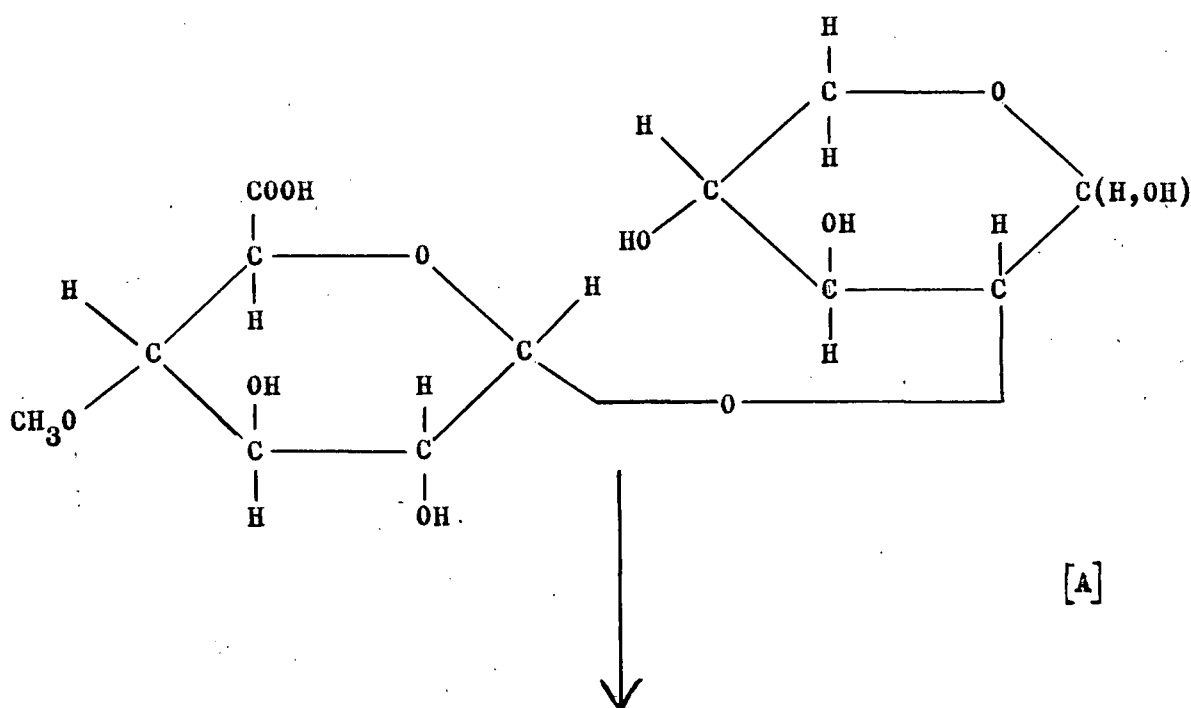
As already mentioned, the jute aldobiouronic acid used in this work contained appreciable amounts of uronic acid formed by hydrolysis and also some aldotriuronic acid which had not been completely degraded. To obtain a pure sample of aldobiouronic acid, the crude material was applied as heavy streaks on 3 mm. chromatographic paper and separated by chromatography in solvent 1. The pure acid was obtained as a pale brown glass with a high positive rotation $[\alpha]_D^{20} + 146.5^\circ$ (C0.75 in H_2O). This value is higher than any previously reported for the material and it is probably the purest sample yet prepared.

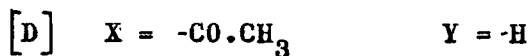
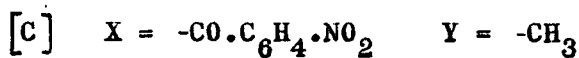
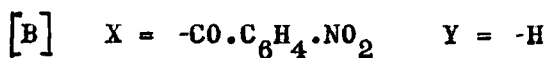
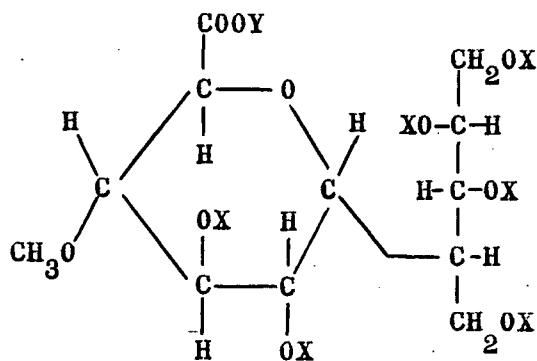
Attempts were made to prepare crystalline derivatives of this pure acid both directly and indirectly. An attempt to prepare the anilide was unsuccessful. Alcohol, which is normally used as solvent when preparing sugar anilides, could not be used here due to the danger of ester formation. The acid would have had to be dissolved in a polar solvent and none of suitable boiling point appeared to be available. An attempt to carry out the reaction without a solvent failed as the acid did not appear to dissolve in aniline.

In order to overcome the difficulty of ester formation, a sample of aldobiouronic acid was esterified with methanolic diazomethane.

The methyl ester was reacted in methanolic solution with p-nitroaniline. The latter dissolved on gentle warming but the solution turned brown in colour and no p-nitroanilide precipitated. On evaporation a brown syrup was obtained and some unchanged p-nitroaniline.

A further sample of aldobiouronic acid was allowed to react with phenylhydrazine in the same way as for osazone formation. Some amorphous brownish-yellow material precipitated. This could not have been an osazone as the 2-position on the xylose was blocked by linkage to the C1 of the 4-O-methyl-D-glucuronic acid. The material must therefore have been a phenylhydrazide salt or a phenylhydrazone or both. Attempts to recrystallize this material failed and no crystalline compound was obtained after separation on an alumina column. It appeared that the derivative did not possess a sharp melting point as it always appeared plastic in nature.





In an attempt to make a crystalline derivative indirectly the aldobiouronic acid was reduced in alkaline solution by potassium borohydride. The reaction product was dissolved in methanol and boric acid filtered off. Evaporation of the solution gave a yellow glass which was reacted with excess p-nitrobenzoyl chloride in pyridine solution. After destroying the excess reagent with sodium bicarbonate, the p-nitrobenzoate was extracted into chloroform and evaporated to yield a golden yellow glass (compound [B]). This material did not crystallise and was esterified with ethereal diazomethane to facilitate its separation on an alumina column (compound [C]). Impurities were removed on washing with dry benzene and the ester removed as a pale yellow band on washing with dry benzene containing 2% ethanol. The product obtained behaved as a glass when heated and melted over a very wide range of temperature. It appeared likely that the presence of six p-nitrobenzoyl groups in the molecule was sufficient to prevent crystallization due to their large

bulk and mutual interference.

In a further experiment the xylitol compound obtained as before by borohydride reduction was acetylated with acetic anhydride in the presence of anhydrous sodium acetate. The acetate was extracted with chloroform and evaporated to yield a viscous colourless syrup which did not crystallise on keeping (compound [D]).

No further work was carried out on the aldobionuronic acid. It might however have been possible to obtain a crystalline derivative by oxidation with nitric acid for example. Such an approach may prove successful in the future.

EXPERIMENTAL

Unless otherwise stated all paper chromatography was carried out on sheets of Whatman No. 1 chromatography paper. The developer employed was p-anisidine reagent and the irrigating solvents were as follows:-

1. ethyl acetate 18, glacial acetic acid 3, 45% formic acid 1, water 4.
2. 1-butanol 40, 95% ethanol 10, water 49, 0.880 ammonia l.
3. methyl ethyl ketone/water azeotrope.
4. 1-butanol 5, benzene 1, pyridine 3, water 3.
5. 1-butanol 4, glacial acetic acid 1, water 5, (upper layer only)

expressed as parts by volume. All evaporations of solvents were carried out under reduced pressure.

The jute used in this work was purchased from the Beamiss Bag Company of Vancouver.

Jute Hemicellulose

Jute sacking was cut into strips and reduced to powdered form in a Wiley mill. Portions of powdered jute (100 gm.) were extracted twice with 1200 ml. benzene 50/ethanol 50 in the cold, filtered and dried. The dried jute was delignified readily by the method of Wise, Murphy and D'Addieco (81) using sodium chlorite and acetic acid at 70-80°C. for three hours. The white holocellulose obtained was filtered off and washed with cold water to give yields of around 90%.

Jute holocellulose was extracted overnight in 1000 ml. 9.3% aqueous sodium hydroxide and filtered. The remaining solid material was again extracted with 500 ml. of alkali for several hours and filtered

as before. The cellulose remnant was washed well with cold water and dilute acetic acid, and dried. The orange-yellow filtrates were bulked and neutralised with glacial acetic acid to give a straw-coloured solution from which the hemicellulose did not precipitate. Upon addition of two volumes of ethanol jute hemicellulose was thrown out as a curdy precipitate and was isolated by centrifugation. The product was washed several times with 50% ethanol to remove inorganic salts, then with increasing strengths of alcohol and finally dried by solvent exchange to yield a white powder (16 gm. from 100 gm. jute).

Purification of Jute Hemicellulose

Crude hemicellulose (5 gm.) was dissolved in 30 ml. 10% aqueous sodium hydroxide and 50 ml. Fehling's solution added. The greenish-blue precipitate was left standing overnight and isolated by centrifugation. After careful washing with alcoholic sodium hydroxide followed by washing with ethanol, the complex was decomposed with dilute ethanolic hydrogen chloride and washed free of chloride ion with aqueous ethanol before isolation as a white powder by solvent exchange. This material (hemicellulose I, 3.2 gm.) had ash content 2.33%, $[\alpha]_D^{20} - 45.1^\circ$ (calculated as ash free C. 0.15 in 2N NaOH). N.E. of 953 indicated 21.7% anhydrouronic acid units.

A second batch of crude hemicellulose (5 gm.) was suspended in 150 ml. ethanol containing 4.5 ml. hydrogen chloride. After standing overnight the suspension was centrifuged and the precipitate washed free of chloride ion as before. After solvent exchange the hemicellulose was obtained as a snow-white powder (hemicellulose II, 3.5 gm.) with ash content 0.28% and $[\alpha]_D^{20} - 47.1^\circ$ (C. 0.5 in 2N NaOH). N.E. of 1005 indicated 20.6% anhydrouronic acid units. The methoxyl content of

3.18% indicated a mean E. Wt. of 975 or 21.3% anhydrouronic acid.

An attempt to prepare absolutely pure hemicellulose by electrodi-
alysis of an aqueous solution of the crude material failed as the pure
material was precipitated in gel form from which it was found impossible
to remove the last traces of water by solvent exchange.

In view of the higher purity obtained, the main batch of jute hemi-
cellulose was purified by the ethanolic hydrogen chloride method and
was obtained as hemicellulose II.

Attempted Direct Extraction of Hemicellulose from Jute

A 100 gm. sample of jute was solvent extracted and then alkali
extracted in the usual way. The hemicellulose was purified by the
copper salt method to yield a brownish-white powder (4.9 gm.). Ash con-
tent 6.38%, $[\alpha]_D^{20} - 47.0^\circ$ (calculated as ash free C. 1.25 in 2N NaOH).

A sample (304 mg.) of this material was hydrolysed on the steam
bath for sixteen hours with 1 Normal sulphuric acid and the hydrolysate
neutralised with barium carbonate, the solution obtained being deionised
in an Amberlite IR 120 resin column. The concentrated eluate showed
chromatographic spots corresponding to xylose, glucose, uronic acid,
aldobiouronic acid, and oligosaccharides when irrigated in solvents 1
and 2 and developed with p-anisidine spray reagent. The glucose was
present only in trace amounts.

Jute Aldobiouronic Acid

In a preliminary experiment hemicellulose II (2 gm.) was hydrolysed
with 30 ml. 1 Normal sulphuric acid for six hours on the steam bath
and the acids neutralised with barium carbonate. The hydrolysate was
deionised in an Amberlite IR 120 resin column and the concentrated

eluate showed spots with Rx values 0.30, 0.69, 1.00 and 1.44 corresponding to oligosaccharide, aldobiouronic acid, xylose, and uronic acid when chromatographed in solvent 1.

Another trial hydrolysis of hemicellulose II (3 gm.) was carried out in 100 ml. 45% formic acid. After twelve hours on the steam bath the formic acid was removed by evaporation under reduced pressure. Chromatography showed the same spots as before with a larger amount of aldotriuronic acid (Rx 0.35) apparently due to incomplete hydrolysis. Further hydrolysis of the material in 1 Normal sulphuric acid removed this spot with a corresponding increase in uronic and aldobiouronic acid.

A large batch of hemicellulose II (15.0 gm. expressed as dry weight) was hydrolysed on the steam bath in 400 ml. 1 Normal sulphuric acid and the reaction followed on the polarimeter. After seven hours the rotation had reached a steady value of $+57^{\circ}$ when the acids were neutralised with barium carbonate as before and deionised in an Amberlite IR 120 resin column. Upon running the eluate through a Duolite A4 resin column the sugar acids were retained and the neutral sugar fraction obtained in solution. The column was carefully washed with water and the sugar acids eluted with 1 Normal sodium hydroxide. Upon returning this eluate to the Amberlite IR 120 column a solution of the sugar acids was obtained.

The neutral sugar solution when concentrated under reduced pressure yielded a pale yellow syrup (9.976 gm.) which crystallised overnight. It appeared at first to contain only xylose when spots were irrigated in solvent 1 but later a true sugar with an Rx value greater than that of xylose was found.

The sugar acid fraction when concentrated under reduced pressure

gave a yellow glass (3.604 gm.). Spots of this material when irrigated in solvent 1 showed the presence of anappreciable amount of uronic acid and small amounts of triuronic acid besides the main fraction of aldobiouronic acid. The fraction had OMe 8.05% (calculated for $C_{12}H_{20}O_{11}$ it is 9.1%) $\left[\alpha \right]_D^{20} + 124.4^\circ$ (C. 0.5 in H_2O). N.E. 314 (calculated for $C_{12}H_{20}O_{11}$ it is 340). The low values of the methoxyl vaue and neutralisation equivalent were attributable to the uronic acid present in the fraction.

The recovery of sugars from the hydrolysate was approximately 90% and the anhydrouronic acid content of 18% was in fair agreement with the value estimated by other methods.

Identification of Neutral Sugars

The crystallised neutral sugar fraction was twice recrystallised from methanol to give white prismatic crystals melting point and mixed melting point $143-145^\circ C$. with an authentic sample of pure xylose, $\left[\alpha \right]_D^{20} + 17.0^\circ$ (equivalent C. 1.0 in H_2O).

The dibenzylidene dimethyl acetal derivative was prepared by dissolving a sample of the sugar (686 mg.) in 7 c.c. of benzaldehyde - methanolic hydrogen chloride reagent (82). This reagent was prepared by dissolving freshly distilled benzaldehyde (40 ml.) in a mixture of methanolic hydrogen chloride (20 ml., 2.5N) and methanol (120 ml.). After standing for four days a white crystalline product was obtained. Two recrystallizations from methanol/benzene gave fine white needle-like crystals melting point and mixed melting point $211^\circ C$. with a standard derivative made from crystalline D-xylose.

A sample of the true sugar was obtained when a sheet of Whatman

3 mm. chromatographic paper was heavily streaked with the neutral sugar mixture and irrigated in solvent 1. The band of sugar was extracted into 80% aqueous methanol and concentrated to give a yellow syrup. Chromatography in solvents 1, 3 and 4 against standard rhamnose and xylose showed that the unknown sugar was L-rhamnose.

Reduction of Jute Aldobiouronic Acid

The aldobiouronic acid (2.210 gm.) was treated with 3% methanolic hydrogen chloride on a steam bath under reflux and the reaction followed on the polarimeter. After five hours a constant rotation of $+112^{\circ}$ was attained. The hydrogen chloride was neutralised with lead carbonate and the precipitate removed after centrifugation. After careful washing of the precipitate with methanol the filtrate and washings were bulked and evaporated to yield the methyl glycoside methyl ester (2.697 gm.) as a thin brown syrup.

Finely crushed lithium aluminum hydride (2.8 gm.) was suspended in 120 ml. dry tetrahydrofuran and refluxed to promote dispersion. A solution of the methyl ester methyl glycoside of the aldobiouronic acid in 50 ml. dry tetrahydrofuran was added gradually to the cooled suspension and the reaction mixture refluxed for thirty minutes. Excess reagent was destroyed by the addition of ethereal ethyl acetate solution and the solvents removed under reduced pressure. Acetic anhydride (50 ml.) and anhydrous sodium acetate (3.5 gm.) was added to the reaction vessel which was heated under reflux for three hours on the steam bath. After evaporating off excess acetic anhydride the inorganic salts were dissolved in dilute hydrogen chloride and the solution extracted with 250 ml. chloroform in four separate washings. The chloroform extract was

freed of chloride ion by washing with water and the solvent removed to give a mobile yellow syrup of the disaccharide methyl glycoside pentaacetate (3.68 gm.).

Removal of the acetyl groups was accomplished by saponification in 20 ml. ethanol with 20 ml. 1 Normal sodium hydroxide for one hour on the steam bath. After deionising in Amberlite IR 120 followed by Duolite A4 resin columns the solution of the disaccharide methyl glycoside was evaporated to give a viscous yellow syrup (2.093 gm.). OMe 19.7% (calc. for $C_{13}H_{24}O_{10}$ is 18.2%), $[\alpha]_D^{20} + 98.5^\circ$ (C. 0.75 in H_2O).

Hydrolysis of the Neutral Disaccharide.

The disaccharide methyl glycoside (850 mg.) was hydrolysed in 50 ml. 1 Normal sulphuric acid on the steam bath and the reaction followed on the polarimeter. After fourteen hours a steady rotation of $+44.5^\circ$ was attained. The hydrolysate was neutralised with barium carbonate and the precipitate removed by centrifugation and washed free of carbohydrate material. The filtrate and washings were bulked and evaporated to yield a viscous yellow syrup (820 mg.).

Paper chromatograms were spotted with this material and irrigated in solvents 1, 2, 3, 4 and 5 using D-xylose, 3-O-methyl-D-glucose, and 4-O-methyl-D-glucose as standards. An excellent separation was achieved in solvent 1 giving two spots Rx 0.98 and 1.24. There was no doubt that the slower yellow spot was xylose but the faster pale brown spot might have been either 3- or 4-O-methyl-D-glucose. These sugars were found to be quite impossible to distinguish chromatographically.

Separation of the Sugars in the Hydrolysate

Attempts to separate the monomethyl glucose from the xylose using

a cellulose column irrigated with solvent 1 were unsuccessful and only a partial separation was achieved. The sugar mixture (500 mg.) was streaked on sheets of Whatman 3 mm. prewashed chromatographic paper and irrigated for sixteen hours in solvent 1. After drying the sheets the sugar zones were located by spraying strips with p-anisidine reagent. A good separation was achieved and the sugars were extracted from the paper into 80% aqueous methanol. Evaporation of the extracts yielded two chromatographically pure sugars. One fraction (120 mg.) had Rx 0.98 in solvent 1 and was apparently D-xylose whilst the other (200 mg.) had Rx 1.23 and was the monomethyl glucose.

Identification of D-xylose

The extracted sugar crystallised on standing for two days and after treatment with charcoal in aqueous solution and evaporation it was obtained as white prismatic crystals $[\alpha]_D^{20} + 22.9^\circ$ (equil. Literature value $+ 18.0^\circ$). The dibenzylidene dimethyl acetal was made as already described (82) and after recrystallization from benzene/petroleum ether was obtained in the form of white needles melting point and mixed melting point 211°C . with a standard derivative made from crystalline D-xylose.

Identification of L-rhamnose

When attempting the separation of xylose and monomethyl glucose on a cellulose column three tubes were obtained containing a sugar running faster than monomethyl glucose in solvent 1. This appeared to be present in only trace amounts and its identification was only carried out by paper chromatography. When examined in solvents 1, 2 and 4 the unknown had Rx values of 1.58, 1.88 and 1.23 respectively and ran side by side

with a standard spot of L-rhamnose. Both sugars gave a pale yellow spot with p-anisidine reagent.

Identification of 4 Methyl Glucose

(a) Demethylation

A sample of the chromatographically pure sugar (20 mg.) was heated with 1.5 ml. 40% hydrobromic acid in a sealed tube in the steam bath for five minutes when the solution in the tube was diluted with 20 ml. water. The hydrobromic acid was removed on Duolite A4 exchange resin and the deionised solution evaporated to give a pale yellow syrup. When examined by paper chromatography in solvent 4 this syrup showed spots coinciding with D-glucose and unreacted monomethyl glucose.

(b) Preparation of the osazone

A portion of monomethyl glucose (55 mg.) was dissolved in 1.8 ml. water and 0.18 ml. freshly distilled phenylhydrazine, 12. ml. 20% acetic acid and 60 mg. sodium bisulphite added. The reaction was carried out in a water bath at 70-80°C. for two hours when the osazone separated as small yellow crystals which were filtered off and recrystallized twice from aqueous alcohol to yield small yellow needlelike crystals of the osazone melting point 159-160°C. without decomposition.

The osazones of standard 3- and 4-O-methyl-D-glucose were prepared in similar fashion. 4-O-methyl-D-glucosazone was obtained as small golden needles from aqueous alcohol melting point 158.159°C. (84) but the 3-methyl analogue (yellow sheaves) did not give a sharp melting point even after many recrystallizations from aqueous alcohol and aqueous acetone. The best value obtained was 168-172°C. (values of 164-166°C. (87) and 178-179°C. (88) are reported in the literature).

The osazone from jute gave mixed melting point 158-159°C. with 4-0-methyl-D-glucosazone and 146-164°C. with 3-0-methyl-D-glucosazone.

(c) Preparation of the anilide

To a sample of monomethyl glucose (99 mg.) was added 2 ml. absolute alcohol and 0.1 ml. freshly distilled dry aniline. After refluxing on the steam bath for one and a half hours the reaction was stopped and the alcohol and excess aniline removed by evaporation in vacuo. The pale brown reaction product was treated with 20 ml. ethyl acetate under reflux and the extract upon concentration yielded pale yellow crystals of the anilide. After three recrystallizations from ethyl acetate small white needlelike crystals were obtained (15 mg.) melting point 158-160°C. Further recrystallization did not raise this value.

A sample of 4-0-methyl-D-glucose anilide was similarly prepared from the standard sugar. It yielded white mushroom like crystals melting point 156-158°C. from straight ethyl acetate solution. This material was difficult to obtain pure and the melting point quoted was the highest that could be obtained.

The mixed melting point for these derivatives was 156-159°C.

(d) Preparation of other standard derivatives

In a search for suitable derivatives of standard 3- and 4-0-methyl-D-glucose the dibenzyl mercaptals were prepared.

Standard 4-0-methyl-D-glucose (270 mg.) was dissolved in 0.35 ml. hydrogen chloride and 0.33 ml. α -toluenethiol and 150 mg. granular zinc chloride added to the solution. After placing in the mechanical shaker for six hours a one phase liquid was obtained, and this was diluted with water throwing out a white oil from solution. The hydrogen chloride was removed on Duolite A4 resin which was filtered off and

washed with alcohol. The filtrate and washings were bulked and evaporated to yield a thick yellow oil which solidified overnight. This was dissolved in absolute alcohol and treated with charcoal. After filtration and concentration of the filtrate white crystals of the mercaptal were obtained (50 mg.). These after three recrystallizations from absolute alcohol had melting point 158-159°C. Further recrystallization did not raise this value.

An attempt was made to prepare 3-O-methyl-D-glucose dibenzyl mercaptal in similar fashion. The material could not be induced to crystallize from absolute alcohol but a crop of white platelike crystals was obtained after diluting the concentrated alcoholic solution with a few drops of water. These had melting point 86-69°C. before and after recrystallization from aqueous alcohol. Upon drying this material in the Abderhalden over boiling toluene decomposition took place and a small amount of yellow oil remained.

Methanolysis of Jute Aldobiouronic Acid

A sample of aldobiouronic acid (3.06 gm.) was treated with 10% methanolic hydrogen chloride on the steam bath overnight. After neutralization with lead carbonate and evaporation of the filtrate a dark brown syrup (3.07 gm.) was obtained. This showed three spots when chromatographed in solvent 2 the R_f values being 0.23, 1.00 and 2.11. The mixture was continuously extracted into ether for one day and the ethereal and aqueous phases evaporated to yield brown syrups (0.979 gm. and 1.544 gm. respectively).

The syrup from the aqueous phase was dissolved in 50 ml. 0.15 Normal barium hydroxide and kept at 60°C. for two hours when the excess alkali

was destroyed by the addition of solid carbon dioxide. The precipitated barium carbonate was centrifuged off and the filtrate deionised with Amberlite IR 120 resin. When the eluate was run through Duolite A4 resin a solution of methyl xyloside was obtained. The acids were eluted from the column in 30 ml. 1 Normal sodium hydroxide and this on running through Amberlite IR 120 resin yielded a solution of the acid component which on evaporation gave a brown syrup (0.643 gm.). Chromatography in solvent 1 and spraying with a 1% bromophenol blue indicator solution showed two yellow spots with Rx 0.18 and 0.49 due to aldobiouronic and uronic acid methyl glycosides.

A similar treatment of the ether extracted syrup gave an acid component (0.431 gm.) Rx 0.53 corresponding to the methyl glycoside of the uronic acid.

The methyl xyloside solutions were bulked and evaporated to yield a pale yellow syrup (0.687 gm.) which gave fernlike crystals on keeping. This material was not further investigated.

Characterisation of Jute Uronic Acid

The uronic methyl glycoside (431 mg.) obtained above was esterified with ethereal diazomethane and evaporated. The methyl ester methyl glycoside was dissolved in 25 ml. cold saturated methanolic ammonia and allowed to stand for two days. Evaporation gave a white solid which was decolourised with charcoal in methanol solution. Concentration yielded white mushroomlike crystals melting point 204-213°C. After four recrystallizations from aqueous ethanol small white prismatic crystals of 4-O-methyl- α -D-glucuronamide methyl glycoside were obtained melting point and mixed melting point 234.5-236°C. with a standard sample. (Literature value 236°C. (75)).

Methylation of Neutral Disaccharide

To the neutral disaccharide methyl glycoside (864 mg.) was added 6 ml. 40% sodium hydroxide solution followed by 0.5 ml. dimethyl sulphate. The mixture was stirred at high speed at a temperature of 45-55°C. and 0.5 ml. portions of dimethyl sulphate added every fifteen minutes for one and a half hours. A further addition of 6 ml. 40% sodium hydroxide was then made and the whole cycle of operations repeated twice so that the total reaction time was four and a half hours.

The whole procedure was repeated twice more so that in all nine 6 ml. portions of 40% sodium hydroxide and 27 ml. dimethyl sulphate were used. The excess dimethyl sulphate was destroyed at the end on heating the mixture to 80°C. for half an hour. After neutralisation to pH 8.9 with sulphuric acid the reaction mixture was continuously extracted with chloroform overnight. Evaporation of the extract gave a yellow mobile syrup (675 mg.) whose infra-red spectrum showed only a weak hydroxyl function.

The partly methylated syrup was dissolved in 20 ml. dry methyl iodide and a few grains of Drierite added. Silver oxide (5 gm.) was added in small amounts to the reaction mixture kept at 50°C. under reflux in a flask fitted with mercury sealed stirrer. After nine hours acetone was added and the excess methyl iodide distilled off. The methylated sugar was filtered in acetone solution and the filtrate returned to the flask for evaporation of the solvent. After three such methylations the product gave an infra-red spectrum showing zero hydroxyl function. The methylated sugar was purified by distillation in vacuo when a pale yellow syrup (517 mg.) was obtained (bath temperature 170-180°C. at 0.01 mm. Hg). OMe content 55.7% (calculated for

$C_{18}H_{34}O_{10}$ is 52.9%) $[\alpha]_D^{20} + 108.6^\circ$ (C. 1.75 in $CHCl_3$). The high methoxyl content found was due to the presence of some rhamnose containing disaccharide present as impurity.

Hydrolysis of Methylated Disaccharide and Separation of the Methylated Sugars

The methylated disaccharide (437 mg.) was dissolved in 25 ml. 1 Normal sulphuric acid and hydrolysed on the steam bath for eleven hours when a steady rotation of $+81.0^\circ$ was attained. The acid was neutralised with barium carbonate and the precipitate centrifuged off and washed free of carbohydrate. The filtrate and washings were bulked and evaporated to yield a yellow syrup (396 mg.) showing two spots R_F 0.68 and 0.83 when chromatographed in solvent 1. The spots gave buff and pale yellow colours with p-anisidine reagent, the former resembling 3,4-dimethyl xylose whilst the latter ran identically to 2,3,4,6-tetramethyl glucose in solvents 1 and 2.

The mixture of methylated sugars (396 mg.) was streaked on pre-washed Whatman 3 mm. chromatographic paper and irrigated in solvent 1. The zones were located by spraying strips and the sugars were extracted from the dried paper using ether for the tetramethyl glucose and absolute alcohol for the dimethyl xylose. Evaporation of the extracts gave syrups of tetramethyl glucose (129 mg.) and dimethyl xylose (56 mg.).

Identification of 2,3,4,6-tetra-O-methyl-D-glucose

The extracted sugar showed traces of trimethyl rhamnose as impurity when examined on chromatograms. Attempts to crystallise the tetramethyl glucose from ether solution were unsuccessful and an attempted fractional distillation of the syrup in vacuo did not yield a pure

product. Finally a second separation on paper yielded some pure tetramethyl glucose (26 mg.).

The anilide was prepared in the usual way and a few long needle-like crystals were obtained. The material was recrystallised from absolute alcohol and gave melting point 122-132°C. (literature value 138°C. (89)). Owing to the smallness of the sample it was impossible to recrystallize the material to constant melting point. The mixed melting point with standard 2,3,4,6-tetra-0-methyl-D-glucose anilide was 130-136°C.

Identification of 3,4-di-0-methyl-D-xylose

The dimethyl xylose (56 mg.) was dissolved in 2 ml. water and 20 drops bromine added. The well-stoppered flask was kept for two days in the dark when a spot on paper gave a negative test with p-anisidine spray. The bromine was removed by aeration and hydrobromic acid neutralised with silver oxide. After centrifugation and washing of the precipitate the bulked filtrate and washings were treated with hydrogen sulphide for three minutes. After evaporation the product was taken up in a little water, centrifuged and transferred to a clean flask. Evaporation yielded a pale brown glass (47 mg.) and on distillation in vacuo white needlelike crystals were obtained (bath temperature 140-160°C. at 0.1 mm. Hg). The lactone (25 mg.) had melting point 58-64°C. and was contaminated with a small amount of colourless syrup. Redistillation gave crystals of 3,4-di-0-methyl-D-xylose- δ -lactone melting point 64-66°C. (literature value 64-66°C. (8)).

Pure Jute Aldobiouronic Acid

Impure aldobiouronic acid (4.65 gm.) prepared in the usual way was heavily streaked on Whatman 3 mm. chromatographic paper at a load of approximately 8 mg. per cm. After irrigation in solvent 1 for sixteen hours the aldobiouronic acid was located by the method of spraying strips. The dried paper was extracted with 80% aqueous acetone until a faint Molisch test was given by the extract. On evaporation the chromatographically pure aldobiouronic acid was obtained as a brownish-white glass (0.947 gm.) $\left[\alpha\right]_{\text{D}}^{20} + 146.5^{\circ}$ (C. 0.75 in H_2O).

The uronic acid zone was also retained and extracted with 80% aqueous acetone to yield on evaporation a brownish-yellow glass (0.124 gm.). The material was found to be chromatographically pure $\left[\alpha\right]_{\text{D}}^{20} + 55.0^{\circ}$ (C. 1.25 in H_2O).

Attempted Preparation of a Crystalline Derivative by Direct Methods

An attempt to make the anilide from the aldobiouronic acid was unsuccessful since the choice of solvent gave difficulty. The acid did not react with aniline without a solvent being present.

A sample of aldobiouronic acid (82 mg.) was esterified with methanolic diazomethane and evaporated to give a brown syrup. Upon reaction with recrystallized p-nitroaniline (45 mg.) in methanol (2 ml.) a dark brown solution was obtained after heating at 70°C . for fifteen minutes. No crystalline p-nitroanilide was found and when the solution was diluted with water some p-nitroaniline was recovered unchanged.

A further portion of acid (71 mg.) was dissolved in 2 ml. water and reacted at 80°C . with 0.13 ml. distilled phenylhydrazine in the presence of 20% acetic acid (1.4 ml.) and sodium bisulphite (50 mg.).

After three hours the mixture was allowed to cool when a yellow-brown solid separated. This material was filtered off and applied to the top of an alumina column after dissolving in the minimum volume of acetone. Excess phenylhydrazine was removed by washing with benzene containing 2% ethanol. The derivative remained as a brown band at the top of the column and was finally removed in 5% aqueous alcohol solution. After treatment with charcoal, filtration and evaporation the material was recrystallised from aqueous alcohol and had melting point 95° to 130° . The indefinite melting point persisted after a further recrystallization and the attempt to obtain a satisfactory crystalline derivative by this method was abandoned.

Attempted Preparation of a Crystalline Derivative by Indirect Methods

A sample of acid (107 mg.) was dissolved in 2.0 ml. water and made alkaline by the addition of 1.0 ml. 5 Normal sodium hydroxide. After the addition of potassium borohydride (55 mg.) the reduction was allowed to proceed in the cold for two hours when the excess reagent was destroyed with glacial acetic acid. The solution was deionised with Amberlite IR 120 exchange resin and filtered. Evaporation gave a mixture of the xylitol compound with white crystalline boric acid. This mixture was acetylated with acetic anhydride (5 ml.) in the presence of anhydrous sodium acetate (150 mg.) under reflux on the steam bath. After three hours the excess acetic anhydride was distilled off under reduced pressure and the inorganic salts dissolved in dilute hydrochloric acid. The solution was extracted three times with chloroform and the bulked extracts washed free of chloride ion with water. Evaporation yielded a pale yellow viscous syrup (41 mg. Compound [D]). All attempts to crystallize the acetate were unsuccessful.

A large batch of acid (655 mg.) was reduced with potassium borohydride as before and the xylitol compound dissolved in acetone. The boric acid was filtered off and the solution evaporated to yield a yellow glass (623 mg. 1 mole). This was dissolved in dry pyridine (40 ml.) and reacted with 4.23 gm. (12 moles) pure p-nitrobenzoyl chloride on the steam bath under reflux for two hours. After standing overnight a heavy precipitate probably of pyridine hydrochloride was obtained. Excess saturated sodium bicarbonate solution was added to the reaction mixture until no further effervescence was observed and the solution left for three hours. The solution was extracted three times with chloroform and the chloroform extracts washed free of bicarbonate with water. Evaporation of the extract yielded a yellow glass (1.265 gm. Compound [B]).

Only a fraction (800 mg.) of this material was soluble in chloroform the remainder having been dissolved by pyridine which had carried over in the chloroform extract. This fraction was dissolved in chloroform and methylated with ethereal diazomethane. Evaporation gave the p-nitrobenzoate methyl ester as a golden yellow glass (800 mg. Compound [C]). A sample of this compound (158 mg.) was applied to the top of an alumina column in chloroform solution (3 ml.) and the column irrigated with 400 ml. dry benzene. This was followed by irrigation with dry benzene containing 2% ethanol when the p-nitrobenzoate came off as a thin yellow band. When evaporated this gave a yellow material (69 mg.) melting point 97 to 185°C. which was apparently a glass. The benzene extract yielded a mixture of p-nitrobenzoate and what appeared to be methyl p-nitrobenzoate present as an impurity melting point 75-80°C. It did not appear that the p-nitrobenzoate of the xylitol compound could be obtained crystalline.

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