EXTRACTION AND STRUCTURAL STUDY OF HEMICELLULOSE B FROM YORKSHIRE FOG (*HOLCUS LANATUS*)

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SUMMARY

(1) Hemicellulose B has been extracted from Yorkshire Fog (*Holcus lanatus*).

(2) The homogeneity of Branched Hemicellulose B was determined by Fractional Precipitation.

(3) Gas-liquid chromatographic study showed that the polysaccharide contains xylose, arabinose and hexose.

(4) The uronic acid content was quantitatively determined by specific colour reactions.

(5) Quantitative analysis of the monosaccharides from the acid hydrolysis product by partition chromatography on cellulose column.

(6) Methylation by methods of Haworth, Kuhn and Purdie followed by hydrolysis yielded the following methyl ethers

2,3-di-O-methyl-D-xylose,

2-O-methyl-D-xylose,

2,3,4-tri-O-methyl-D-xylose,

2,3,5-tri-O-methyl-L-arabinose.

(7) The methyl ether monomers were quantitatively determined by gas-liquid chromatography.

(8) From the results a simplified structure can be suggested for the branched hemicellulose B, consisting of 16 β-D-xylo-pyranose residues linked together by 1–4 glycosidic bonds, and with a terminal side chain D-glucuronic acid residue linked through C-2 to the xylose unit. L-arabinose unit probably occurs as a side chain and is linked through position 3 to every fourth D-xylose unit.

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Introduction

1.1 Hemicellulose

The structural carbohydrates are mixtures of polysaccharides which together with lignin, constitute the cell wall. Usually they are divided into three fractions: pectic substances, hemicellulose and cellulose.[1]

Pectins, widely distributed in land plants are characterised by a main chain of 1-4 linked galacturonic acid units. They may also contain rhamnose, galactose, xylose, arabinose and fucose in varying amounts. These sugars form part of the main chain in some pectins and branch chains in others.[2]

Cellulose is generally present in the plant cell as aggregates of fibrils or partly crystalline bundles. They consist of parallel chains of β 1-4 linked glucose residues, where the chains are in perfect alignment, the cellulose has a crystalline structure. The cellulose fibrils are generally embedded in other polysaccharide material, e.g. the hemicelluloses in the higher plants.[3]

The name hemicellulose was first proposed in 1891 by Schulze, who was examining products extracted from leguminous seeds, brans and green tissues. He isolated a group of carbohydrates similar to cellulose which were characterised by their behaviour with dilute mineral acid, being more easily hydrolysed than cellulose. This group of polysaccharides are chemically and structurally related to cellulose, for which reason, Schulze designated the group hemicellulose.

The use of the term hemicellulose has changed frequently. Some investigators wish to confine the term to the non-cellulosic carbohydrates and to define hemicellulose as being composed of (1) pentosans, chiefly xylan and araban, yielding on hydrolysis the pentoses, xylose and arabinose; (2) hexosans, chiefly mannan and galactan, capable of being hydrolysed to the hexoses, mannose and galactose; and (3) polyuronides, mostly in the form of polyuronic acids. The wood and cellulose chemists have been accustomed to define the term hemicellulose as that portion of a plant material which is soluble in cold alkali solution.

In general, the name is restricted to land plants and is applied to the group of carbohydrates found in the cell walls of plants in association with lignin as an amorphous phase enveloping the cellulose strands and which can be extracted from plant material with dilute alkali, either hot or cold.[4] A wide range of molecular sizes and molecular shapes exist among hemicelluloses. Because of these differences and the differences in their acidic properties, the polysaccharides which constitute the hemicelluloses vary among themselves in solubility. As a plant tissue ages, the solubilities of the polysaccharides decrease to some extent. Decreased solubility is most pronounced when the tissue material is dried. Insolubilization is not inherent in the drying process itself because it is possible to dry plant material in such a way as to prevent loss of solubility or chemical reactivity.

The isolation of the cell wall carbohydrates is based upon their differences in solubility. The extent to which a soluble

polysaccharide is extracted from a plant tissue or holocellulose depends upon the ease with which the solvent penetrates the insoluble phase and the facility with which the soluble component can diffuse from the matrix to the surrounding solution.

	Holocellulose	
	Hemicellulose	Cellulose
Lignin containing the non-carbohydrate material of the cell wall	shorter chains containing: xylose units mannose units uronic acid units methyoxyl groups acetyl groups (possibly galactose and arabinose units)	longer chain length including: α -cellulose "True" cellulose β -d-glucopyranose units

Figure 1. Relation of Hemicellulose to other cell wall components[3]

1.2 Molecular Structure of Hemicellulose

The group of polysaccharides called hemicelluloses are usually mixtures which include some or all of the xylans, the glucomannans and the arabogalactans. Sometimes other plant polysaccharides such as the β-glucans of barley and oats, some mannans and some galactomannans are regarded as hemicelluloses.

(A) D-Xylans

Xylan is the commonest hemicellulose because it is removed by hemicellulose extraction procedures and is often the principal component of hemicelluloses. Purified hemicellulose is, in many instances, identical to xylan.[5] It occurs in practically all land plants and also in some marine algae. It is most abundant in annual crops, particularly in agricultural residues such as corn cobs, corn stalks, green hulls and stems where it occurs in amounts ranging from 15 to 30%. Hard woods contain 7 to 12% (see Tables I, II and III). Low strength vegetable fibres of commerce such as jute, sisal, manila hemp and coir may contain 5 to 20% xylan, whereas high strength fibres such as ramie, flax and cotton are almost devoid of xylan.[6] The xylan of esparto grass is of particular interest because it has been shown[7] to consist mainly of xylose residues. After complete methylation and hydrolysis the principal products obtained were 2,3,di-O-methyl-D-xylose, 2,3,4,tri-O-methyl-D-xylose, 2-O-methyl-D-xylose and a trace of 2,3,5,tri-O-methyl-L-arabinose. Acetolysis of the methylated D-xylan gave the disaccharide (I), therefore

TABLE I Chemical composition[a] of six North-American species of wood[4]

Species	Cellulose	Lignin	O-Acetyl	Hexuronic acid[b]	Residues[c] of				
					Galactose	Glucose	Mannose	Arabinose	Xylose
Acer rubrum Red maple	44.1	24.0	3.8	3.5	0.6	46.6	3.5	0.5	17.3
Betula papyrifera White birch	41.0	18.9	4.4	4.6	0.6	44.7	1.5	0.5	24.6
Fagus grandifolia American beech	42.1	22.1	3.9	4.8	1.2	47.5	2.1	0.5	17.5
Abies balsamea Balsam fir	44.8	29.4	1.5	3.4	1.0	46.8	12.4	0.5	4.8
Picea glauca White spruce	44.8	27.1	1.3	3.6	1.2	46.5	11.6	1.6	6.8
Pinus banksiana Jack pine	41.6	28.6	1.2	3.9	1.4	45.6	10.6	1.4	7.1

[a] All values in per cent of extractive-free wood

[b] As $C_6H_{10}O_7$ minus H_2O

[c] As $C_6H_{12}O_6$ minus H_2O

TABLE II Carbohydrate composition* of sixteen species of European Hardwood

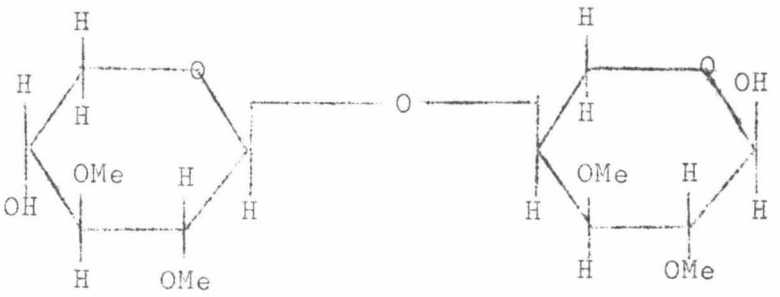
Species	Residues of:				
	Galactose	Glucose	Mannose	Arabinose	Xylose
Acer platanoides Norway maple	2.0	60.5	4.0	1.0	32.5
Alnus glutinosa Black alder	2.5	73.5	3.5	1.0	19.5
Alnus rugosa Speckled alder	3.5	67.0	1.5	1.0	27.0
Betula pubescens Birch	1.0	55.0	2.5	2.5	39.0
Betula verrucosa Silver birch	1.5	58.5	0.5	0.5	39.0
Corylus avellana European hazel	2.0	69.5	2.0	2.0	24.5
Fagus sylvatica European beech	4.0	65.0	1.5	1.5	28.0
Fraxinus excelsior European ash	3.0	60.0	2.5	2.5	32.0
Populus balsamifera Balsam poplar	3.5	68.0	2.5	2.5	23.5
Populus tremula European trembling aspen	1.5	64.5	3.0	1.0	30.0
Prunus padus European cherry	2.5	65.5	2.5	1.0	28.5
Quercus robur English oak	2.5	68.5	2.0	1.0	26.0
Salix alba White willow	3.0	74.0	2.5	1.0	19.5
Sorbus aucuparia European mountain ash	1.5	66.5	2.5	2.0	27.5
Tilia cordata Linden	1.5	58.5	3.5	2.0	34.5
Ulmus glabra Scots elm	2.5	68.5	2.0	1.0	26.0

* All values in per cent of total neutral carbohydrates.

TABLE III Percentage composition of polysaccharides[10]

Source	D Glucuronic acid	D Galactose	L-Arabinose	Other	Reference
Acacia cyanophylla	24	49	7	L-Rhamnose 20	20
Acacia Karroo (Hayne)	12	50	36	L-Rhamnose	21
Acacia mollissima (Black wattle)	9	42	42	L-Rhamnose 7	22
Acacia pycnantha	5	65	27	L-Rhamnose 1-2	43
Acacia verek (arabic)	16	52	19	L-Rhamnose 14	24
Amygdalus (Almond tree)	10	30	40	D-Xylose 20	25
Anogeissus latifolia wall (Ghatti)	12	27	41	D-Mannose 8	26
Asparagus adscendens	10			D-Glucose 40, D-Mannose 40, D-Xylose 10	
Asparagus filicinus	5			D-Mannose 45, D-Glucose 40, D-fructose 10	
Boswellia carterii (Olibanum or frankincense)		58	8	4-O-Methyl-D-glucuronic acid 33, trace of L-fucose and L-rhamnose L-Rhamnose 25	28
Brasenia schreberi Gmel (watershield)	22	47	9	D-Mannose 16	
Cetraria islandica	3	8		D-Glucose 89, D-Mannose 3	29
Citrus limonia (Lemon)	22	55	22	4-O-methyl-D-glucuronic acid	30
Citrus maxima (Grapefruit)	31	53	16	4-O-methyl-D-glucuronic acid	
Combretum verticillatum		15	38		31
Commiphora myrrha (Myrrh)		30	12	4-O-methyl-D-glucuronic acid 38	32
Curculigo orchioides	42			D-Mannose 23, D-glucose 35	
Dilsea edulis	10	80		D-xylose 7	33
Fagara xanthoyloides		61	21	4-O-methyl-D-glucuronic acid 17	34
Ferula species (Asafetida)	11	55	33	L-Rhamnose	35
Hakea acicularis	8	58		D-xylose 8, D-mannose 7	36
Hevea brasiliensis	6	4	20	D-Glucose 60, L-rhamnose 2, D-xylose 8	37
Mimosa pudica	21			D-xylose 79, trace of D-glucose	
Moringa pterygosperma (Drum stick)	10	37	53	Trace of L-rhamnose	38
Prosopis juliflora (Mesquite gum)		31	51	4-O-methyl-D-glucuronic acid 18	39
Prunus armeniaca (Apricot)	16	44	41		
Prunus cerasus (Cherry)	12	21	55	D-mannose 10, trace of L-rhamnose and D-xylose	40
Prunus domestica (Egg plum)	15	40	34	D-xylose 11	33
Prunus insits (Damson)	16	30	38	D-mannose 15, trace of D-xylose	41
Prunus persica (Peach tree)	7	36	43	D-xylose 14, trace of L-rhamnose	42
Prunus serrulata	6-8			D-xylose 92-94	
Prunus species (Purple plum)	12	38	38	D-xylose 12	25
Prunus virginiana (Chokecherry)	9	26	29	D-mannose 13, L-rhamnose 23	43
Puya chilensis (Chagual)	15	36	7	D-xylose 43	36
Ulva lactuca (Green marine algae)	19			D-xylose 9, L-rhamnose 31, D-Glucose 8	44
Zea mays (Cornhull)	7-12	7	35	D-xylose 48	45

the glycosic linkage is (1 —— 4) and the D-xylose residues are in the pyranose form.[17]



(I)

From this evidence the main features have been formulated as

β -D-xylp-(1— 4)-(β -D-xylp)$_x$—(1— 4)-(β -D-xylp)-(1— 4)
3
|
1
β -D-xylp-(1— 4)-(β -D-xylp)$_z$-(1— 4)-β -D-xylp

xylp = xylopyranose x+y+z = 75 ± 5

The presence of chains of (1— 4) β linked xylopyranose residues appears to be a general structural feature throughout most of the xylan group of polysaccharide (see Table IV).

(B) Arabino-Xylans

Perlin[8] has isolated from wheat flour a xylan fraction which contained almost as much arabinose as xylose. A study of this arabinoxylan by the methylation procedure and by other methods has shown that single L-arabofuranose residues are attached to a main chain of xylose residue, in many instances substituting the hydroxyl at both C-2 and C-3 of the same xylose residue. These arabofuranose residues

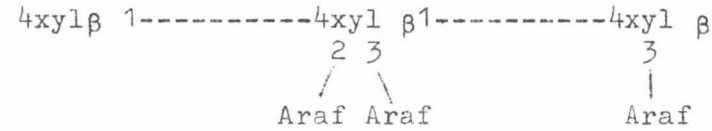
TABLE IV Xylans from the gramineae[6]

Source	Mode of linkage of L-arabinofuranose end-groups[a]	Mode of linkage of D-glucuronic acid end-groups[b]	Other structural features	References
Esparto grass	none present	none present		46
Esparto grass	(1—3)-D-xylp	none present	β-D-xylp-(1—2)-L-Ara	47
Wheat straw	(1—3)-D-xylp	(1—3)-D-xylp (Me)	branched	48
			xylan chain	48
Wheat straw	(1—3)-D-xylp	(1—2)-D-xylp	.....4-D-Gp-1.....	49
Wheat straw	(1—3)-D-xylp	none present	3	
Wheat straw	none present	(1—2)-D-xylp		
Wheat straw	(1—3)-D-xylp	(1—2)-D-xylp (Me)	some branched	50
			xylan chains	51
Oat straw	(1—3)-D-xylp	(1—2)-D-xylp (Me)		52
Cocksfoot grass	(1—3)-D-xylp	(1—2)-D-xylp (Me)		
Wheat leaf	(1—3)-D-xylp	(?)(1—3)-D-xylp		53
Barley husks	(1—3)-D-xylp	(—2)-D-xylp (Me)	β-D-xylp-(1—2)-L-Araf	
			branched	
			xylan chain	
Corn (maize) cobs	(1—3)-D-xylp	not known	.....2-L-Araf-1.....	7
Corn (maize) cobs	(1—3)-D-xylp	(1—2)D-xylp (Me)	β-D-xylp-(1—2)-L-Araf	7
		(1—4)-D-xylp		55
Corn (maize) cobs	(1—3)-D-xylp	none present	.....4-D-Gp-1.....	49
			3	
Maize fibre	(1—3)-D-xylp	not known	α-D-xylp(1—3)-L-Ara	56
			L-Galp-(1—4)-D-xylp-(1—2)-L-Ara	
Maize hulls	not known	(1—2)-D-xylp	α-D-xylp(1—3)-L-Ara	57
			β-D-Galp-(1—4)-D-xylp	58
			β-D-Galp-(1—5)-L-Araf	
Wheat bran	(1—3)-D-xylp	(1—2)-D-xylp	.....3-L-Araf-1.....+	59
	(1—3)-D-xylp-(2—1)			8
Wheat flour	(1—3)-D-xylp	none present		
	(1—3)-D-xylp-(2—1)			
Barley flour	(1—3)-D-xylp	none present		60
	(1—2)-D-xylp			
	(1—3)-D-xylp(2—1)			61
Rye flour	(1—3)-D-xylp	none present		

[a] D-xylp = D-xylopyranose, L-Araf = L-arabinofuranose, D-Gp = D-glucopyranose and Galp = galacto-pyranose residues

[b] (Me) indicates that D-glucuronic acid residues are present, wholly or in part, as the 4-methylether

were readily removed by acid hydrolysis, leaving a xylan similar to the arabinose free material from esparto. The general molecular structure of wheat flour xylan is



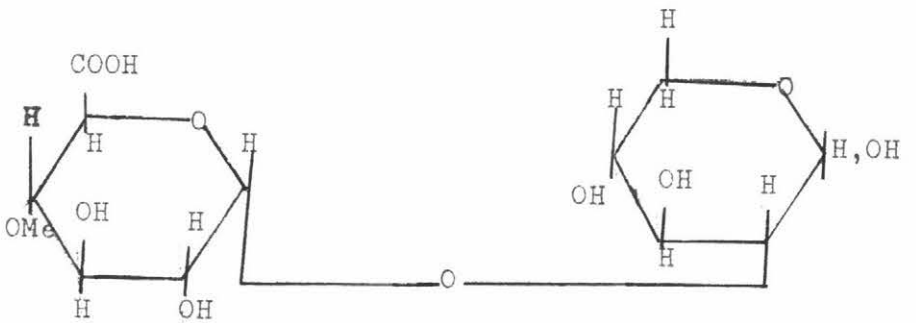
However, some xylans, such as the hemicelluloses from corn (maize) cobs, maize fibre and maize hulls and barley husks also contain non-terminal L-arabinofuranose. Such L-arabinofuranosyl linkages are easily released under mild conditions of hydrolysis, therefore oligosaccharides produced from them have L-arabinose residues at the reducing end.[18]

Bishop and Whitaker[9] have isolated an enzyme from myrothecium verrucaria which hydrolyses linear chains of β -(1— 4) linked D-xylose units. Application of the enzyme to wheat straw hemicellulose yields among other products, L-arabinofuranosyl-(1— 3) β -D-xylopyranosyl-(1— 4)-D-xylose.

(C) Glucurono-xylans

Many polysaccharides of the xylan group contain residues of D-glucouronic acid or 4-O-methyl-D-glucuronic acid.[10] As glycosiduronic acids are very resistant to mineral acid hydrolysis, aldobiouronic acids may be isolated from the graded acid hydrolysis of xylan containing hexuronic acid residues and the mode of linkage of D-glucuronic acid to D-xylose may be determined by the

identification of the hydrolysis products of the derived methylated aldobiouronic acid. Jones and Wise[11] have shown that O-(4-O-methyl-α -D-glucosyluronic acid)(1— 2)-D-xylose can be isolated from aspen wood hemicellulose.



D-glucuronic acid or its 4-methyl ether are most commonly linked to position 2 of D-xylose, although similar aldobiouronic acids containing (1— 3) (wheat straw) and (1— 4) linkages (corn cob) have also been observed.

In the structural study of hemicellulose of Lucerne, Aspinall and McGrath[12] they have found that the polysaccharide contains an essentially linear chain of 1— 4 linked β -D-xylopyranose residues with side chains of 4-O-methyl-α -D-glucopyranosyluronic acid residues attached to C-2 of approximately every ninth residue. The main chain also contains occasional rhamnose residues.

(D) Mannans

Polysaccharides based on D-mannose as the major structural unit occur in woods and in the seed of many plants. The mannans present in ivory nuts have been shown

to consist of chains of (1— 4) linked β -D-mannopyranose residues.[13] The glucomannans which comprise up to half the hemicellulose content of some coniferous woods contain units of both mannose and glucose in their molecular structure.[14] Many mannans and glucomannans contain a small proportion of D-galactose residues, which have been shown to be an integral part of the mannans or glucomannans.

(E) Arabino-galactans

L-arabino-D-galactans are the major wood glycans that can be extracted from wood with water. They are highly branched, water soluble and are found in many coniferous woods. Polysaccharides of this type have (1— 6) and (1— 3) linked D-galactopyranose residues and the L-arabinose residues appear as integral parts of arabinogalactans.

Jones and co-workers[15] have found that the addition of borate to the alkali permits the extraction of the glucomannan in reasonably pure form as the borate complexes. By controlled acid hydrolysis of Loblolly pine wood, Jones and Painter[16] have isolated 2-O-(4-O-methyl- α -D-glucuronosyl)-D-xylose and 4-O- β -D-glucopyranosyl-α -D-mannopyranose.

The greater part of cell wall material in wood, other than cellulose itself, are the xylans, (including arabinoxylans and glucuronoxylans) the galactoarabans and the glucomannans.

1.3 Isolation and Extraction of Hemicellulose from Yorkshire Fog (Holcus lanatus)

(A) Preliminary Extraction

The soluble carbohydrates are usually extracted from the dried and powdered material in two stages. Firstly, mono, di and oligo-saccharides are isolated by extraction with boiling ethanol benzene, and then the residue is extracted with warm water. Secondly, lipids and other extractives are removed. The removal of lipids and extractives before polysaccharide separation not only eliminates them as sources of impurities but opens the tissue to penetration by hydrophilic solvents.

(B) Water-soluble Polysaccharides

Fructosans and some organic acids can be extracted from the residue after benzene-ethanol extraction. The choice of 60°C for the temperature of water for extraction followed the practice of earlier work concerned with the extraction of fructosan from dried grass.[64] As the temperature increased, the amount of water soluble polysaccharide being extracted also increased. At 60°C, the extract came largely from the ionic fraction, and at the temperature higher than 60°C, an appreciable amount of extract came from non-ionic fraction. Therefore, the water at 60°C is the best condition for extracting water soluble polysaccharides.

(C) Pectin Extraction

The pectic substances consist of pectin together with

galactan and araban. They are found abundantly in the primary cell walls so that young plants may be rich in them. Mature plant material contains only small amounts and the pectic material is often associated with polymers of glucose and even of xylose. For the removal of pectic substance from the residue after extraction with ethanol-benzene and water, the method of Weihe and Phillips[65] using 0.5% ammonium oxalate at 80-85°C for twenty-four hours can be used, but has the disadvantage that, there might be some retention of ammonia by the grass fibre. Therefore, pectic substances such as polygalacturonic acids are better extracted by treating the residue with EDTA at 70°C for two hours.

(D) Extraction with Pepsin in Hydrochloric Acid

After those three previous extractions, the grass residue still contains a large amount of the original nitrogen and most of the protein. As an examination of the structural carbohydrates is a major object of this work, reagents to be employed in the removal of protein have to be mild enough not to bring into solution any appreciable quantity of the hemicellulose fraction. This precluded the use of alkali, therefore the proteolytic enzyme of pepsin was used. This has been shown to have no activity towards carbohydrates.

(E) Delignification

Lignin is a complex, three dimensional polymer of phenylpropane residues, formed by dehydrogenation polymerisation of a precursor which is probably of the coniferyl

alcohol type.[69] Lignin removal from the material to be used in structural investigation requires more than usual care because of the ease with which the water soluble hemicellulose fraction goes into solution once a substantial amount of the lignin is removed.[66] However, lignin must be removed as it often retards or prevents complete extraction of the hemicelluloses, either because of mechanical obstruction or by reason of attachement through covalent bonds. Bouveng and Lindberg[68] have observed that hemicelluloses containing lignin could not be separated into the pure components, but, after further delignification, they could be readily fractionated. Delignification may cause a loss in carbohydrate, therefore as mild treatment as possible is desirable. Delignification with chloramine in weakly acid solution was suggested by Gaillard[1] who reckoned that the treatment compares favourably with the usual acid chlorite treatment. For plant material with a moderate lignin content (6-11%) such as Yorkshire Fog, hay or fresh grass, delignification can be carried out directly after the extraction with EDTA or ammonium oxalate solution without causing any loss in carbohydrates. After the suspension was filtered and washed with ethanol, solution of ethanolamine was left in contact with the residue. The purpose of this is to remove secondary cell wall lignin without attacking that of the middle lamella.

(F) Hemicellulose Extraction

The hemicelluloses are composed of a mixture of polysaccharides of different solubility, part being readily soluble in weak alkali and the remainder being extracted only by strong alkaline solution. Alkaline extraction of holocellulose is a useful mean for the isolation of a group of polymers which can later be separated. However, alkaline extraction of the holocellulose can bring many changes in the polysaccharides, even under oxygen free conditions, alkaline degradation might result. It is also possible that any naturally occurring ester groups are saponified.[3]

Solutions of potassium hydroxide, instead of sodium hydroxide are often used, because of the high solubility of potassium acetate in ethanol. The alkaline extraction is usually carried out at or below room temperature for limited periods of time and in an atmosphere of nitrogen. Under these conditions, any changes in the xylan brought about by the alkali are kept at a minimum.

Complete subdivision of the hemicelluloses into individual molecular species has not been accomplished. Separation difficulties arise not only from the presence of different kinds of molecules, but also from the occurrence of each molecular type in different degrees of polymerisation. Therefore, separation based on solubility would not bring about a complete segregation of species. A good general method for effecting initial subdivision of the hemicellulose group is the neutralisation of an alkaline hemicell-

ulose solution which causes precipitation of the high molecular weight polymers and leaves in solution the molecules of lower degree of polymerisation.

The more soluble fraction is often called polyuronide hemicellulose or hemicellulose B and the less soluble xylan cellulosan or hemicellulose A.[67] The difference in solubility of these polysaccharides depends mostly upon the chain length and upon the presence of an uronic acid unit in the chain. A short chain length of the molecule or the presence of an uronic acid unit may raise the solubility of the polysaccharide, but the type of sugar of which a polysaccharide is constituted has no effect on its solubility.

Hemicellulose A is the fraction of the total hemicellulose which is precipitated when an alkaline extract of the holocellulose is neutralised. When the supernatant is poured into ethanol, a white precipitate (Hemicellulose B) appeared. Usually, hemicellulose B has a higher uronic acid content than hemicellulose A.

(G) Fractionation of Hemicellulose B

Hemicellulose B is a mixture of several different polymers, both linear and branched. The separation of these individual polymers in a pure state is a tedious procedure. Generally it is achieved by repeated fractional precipitation from aqueous solution with ethanol or acetone. Gaillard[98] had observed that it is possible to separate the linear from the branched polymers in hemicellulose B

by dissolving the mixture in concentrated calcium chloride solution and precipitating the linear polymers with an iodine-potassium iodide solution. The branched polymers are then recovered from the filtrate.

TABLE V Composition of the linear A and B and the branched B polymers from some gramineae and leguminosae[98]

	T.pratense (red clover) (%)	M.sativa (lucerne) (%)	G.max (soya bean) (%)	L.perenne (grass) (%)	T.vulgare (wheat) (%)	Z.mais (maize) (%)
Linear A						
uronic acid	4.7	6.6	6.6	1.9	2.1	2.4
galactose	-	-	-	-	-	-
arabinose	-	-	-	12.9	5.7	5.3
xylose	95.3	93.4	93.4	85.2	92.2	92.3
glucose	-	-	-	-	-	-
Linear B						
uronic acid	1.0	1.5	4.3	0.4	0.3	0.1
galactose	-	-	-	-	-	-
arabinose	10.8	8.3	8.3	16.5	11.1	10.9
xylose	88.2	90.2	89.2	83.1	88.6	89.0
glucose	(11.6)	(11.4)	(4.0)	(11.0)	(12.0)	(21.5)
Branched B						
uronic acid	20.6	22.3	24.6	5.4	7.9	12.8
galactose	34.5	31.1	34.3	7.7	9.8	8.7
arabinose	27.6	34.2	24.0	23.8	26.5	24.4
xylose	17.3	3.1	3.4	63.1	55.8	54.1
glucose	-	9.3	6.9	-	-	-
rhamnose	-	-	6.8	-	-	-

1.4 Homogeneity of the Polysaccharide[70,71]

Most of the naturally occurring polysaccharides are complex mixtures and for the complete structural characterisation of a polysaccharide, it is necessary to ensure that the polymer is pure, has been isolated from a single species and is chemically homogeneous. By homogeneous it is meant that further fractionation of the polysaccharide yields products having the same specific rotation and the same ratio of mono saccharide building units. There is no standard method for the isolation of a polysaccharide from a mixture, a combination of several methods usually gives the best chance of success. Each biological material presents its own problems and the task of separation and purification is often difficult and tedious, but is an essential preliminary to structural determination.

Most extraction methods yield mixtures of polysaccharides contaminated with other substances such as protein. Furthermore, the extraction procedures may modify the actual structure of the molecule and also alter its molecular weight distribution. The ease of purification depends upon the nature of the contaminating substances. As it is very difficult to obtain an absolutely pure polysaccharide.

The methods used for the separation of polysaccharides in order to obtain homogeneity include graded extraction, fractional precipitation by addition of precipitant, or by cooling, precipitation by a specific complexing agent, ultracentrifugation, electrophoresis, chromatographic adsorption, gel filtration,

the use of enzymes and immunological tests.

Fractionation of Polysaccharides

Methods of fractionation of polysaccharides are based upon many properties. Electrophoresis is one way of testing the chemical homogeneity of the material, but as with the ultracentrifuge, it can only be used to separate microquantities.

In most cases, a solution of the precipitant is added to an aqueous solution of the polymers. The separation may be poor due to the tendency towards coprecipitation and occlusion of other polysaccharides. However, the procedure is widely practised, as a useful method for purification.

Jones[72] found that cetyl trimethylammonium bromide could be used to precipitate the acidic components from a mixture of polysaccharides. Fehling solution is often employed to selectively precipitate polysaccharides, and solutions of cupriethylene diamine, copper chloride, copper sulphate and copper acetate have also been used.

1.4.1 Determination of the Homogeneity of Polysaccharide

The methods which can be used are

(a) Fractional precipitation with ethanol

(b) Precipitation using a specific complexing agent.

(a) Fractional Precipitation with Ethanol

This involves the precipitation of polysaccharide from solution by the gradual addition of ethanol to the aqueous solution of water soluble polysaccharide.

Fractionation is usually carried out at or near pH 7.0, where the polysaccharides are most stable, and

the carboxyl groups, present in the hemicelluloses containing uronic acids, are in the form of ionised salts. Under acidic conditions, significant hydrolysis of glycosidic bonds may occur and in basic solutions, alkaline degradation may take place.

(b) Precipitation using a Specific Complexing Agent

Copper salts have been widely used as precipitating agents to fractionate mixture of polysaccharides. Fehling's solution is often used possibly because it is a common reagent in the laboratory.

Usually an excess of precipitant is added, and the insoluble polysaccharide-copper complex is removed by either filtration or centrifugation. The jelly-like complex is then decomposed by an alcoholic solution of acid or a chelating agent and the precipitate is washed until free from inorganic ions.

Chanda and co-workers[46] in studying the constitution of xylan from esparto grass found that fractional precipitation as the copper complex progressively reduced the total arabinose content of xylan, suggesting that the so-called "araboxylan" or "xyloaraban" might be, not a homogeneous polysaccharide, but a mixture of a true xylan with an araban of the type present in pectic materials.

1.5 Quantitative and Qualitative Determination of Sugars by Gas-Liquid Chromatography[73,74,75,76]

Gas liquid chromatography is a valuable supplement to existing analytical methods, because of its greater selectivity, speed and simplicity. As a result it has been widely used for the separation and estimation of carbohydrates and related polyhydroxyl compounds. Since these substances are not themselves sufficiently volatile for gas chromatography, suitably volatile derivatives have to be made, and these are commonly prepared from the sugars by methylation or acetylation.

The work of Sweeley, Bentley, Makita and Wells[77,78] is one of the most significant advances in carbohydrate chemistry in the decade. Using a simple experimental procedure[79] they prepared the polytrimethysilyl ethers of sugars and chromatographed these derivatives on several different supports with spectacular qualitative results.

The sugars can be converted into their derivatives quantitatively, but problems arise when separating monosaccharides by gas liquid chromatography. The formation of as many as four glycosides per monosaccharide resulting from anomeric and ring isomerisation, and each of which produce a peak on the chromatogram. In a complex sugar mixture, containing a number of monosaccharides, the multiplicity of peaks produced would prevent complete separation of all the peaks from one another, as a result, accurate quantitative determination cannot be achieved. However, this difficulty can be overcome by converting the monosaccharides into their corresponding alditols, by reduction

with sodium borohydride and subsequent acetylation.[80] This procedure eliminates the problem of multiple peaks since the alditols cannot anomerise,[81] and the method is applicable to the quantitative analysis of the sugars in hemicellulose B.

1.6 Quantitative Determination of Uronic Acids[82,83]

The decomposition of polyuronides by hot hydrochloric acid with the formation of furfural and carbon dioxide is a common method for the determination of uronic acids. However it is not specific, as true sugars and amino sugars both give rise to the same products under the same condition, and also it usually requires a few mg of material for several determinations

Meyer, Bloch and Chaffee[84] have reported that it is possible to determine the uronic acids in certain polyuronides after isolation and methanolysis by the naphthoresorcinol reaction. However, in the presence of proteins and true sugars the results are unsatisfactory.

Since 1929, Dische's method has been widely used for the microanalysis of uronic acids. The sugars are treated with concentrated mineral acids (sulphuric acid, hydrochloric acid) to yield mixtures of products which react with various organic substances (indole, diphenylamine, carbazole and SH compounds) to give colours. Different groups of sugars such as pentoses, hexoses, desoxypentoses and even individual sugars of the same group (e.g. mannose and glucose) show marked differences in the speed of the development of the colour and in its absorption spectrum. The absorption due to the carbazole reaction of true

sugars can be deducted when the nature of the sugar and its approximate concentration are known.

Anthrone sulphuric acid and orcinol sulphuric reactions[85] are able to be used for identification of sugars, uronic acids and sugar amines, as they all give a colour reaction. The anthrone sulphuric reaction is better than orcinol sulphuric, as it has been reported that for equal quantities of glucuronic acid and galactose, the absorbance of glucuronic acid to galactose is 0.44 for the reaction with orcinol sulphuric acid, and 0.10 for the reaction with anthrone sulphuric acid. As it is impossible to obtain reproducible results with the same solution of sugars, because the absorbance varies, it is therefore necessary to introduce an internal standard into a series of determinations.

The simplicity, specificity of the carbazole and anthrone sulphuric acid reactions, and also the low degree of interferences from other constituents, encourage the application of these methods to quantitative determination of uronic acids in polysaccharides.

1.7 Paper Chromatography[19,38,86]

Since the introduction of paper partition chromatography in 1944, it has been successfully applied to the separation of sugars and also for their quantitative determination. The method gives a rapid means of separation as well as giving a strong indication of their identities. Though the final proof of their constitution depends upon their separation and ident-

ification by determination of their physical constants and the formation of characteristic derivatives.

1.8 Methylation Procedures: Which Include Methods of Methylation, Acid Hydrolysis Qualitative and Quantitative Identification of the Monomers[68,88]

Methylation analysis is a very important method for structural investigation. The procedure involves the preparation of an exhaustively methylated polysaccharide followed by hydrolysis and identification of the resulting partially-O-methylated derivatives. The purpose of methylation is to achieve an etherification of all the free hydroxyl groups in the polysaccharide. Complete methylation can convert all unsubstituted hydroxyl groups to methoxyl groups and each of free hydroxyl group present in the methylated monosaccharides that is liberated by hydrolysis corresponds to a substituted hydroxyl of the original product. Therefore, the glycosidic linkage and the ring size can be deduced.

The ideal methylating agent is one which can rapidly introduce the theoretical number of methyl groups and at the same time, cause no degradation of the sugar. However, it is very difficult to get these reagents with such properties, therefore, several methylations with different reagents are generally used in order to obtain full methylation.

The original, classical methods of Denham and Woodhouse[89] and Haworth[90] are still widely used. The polysaccharide is treated several times with 25-30% aqueous alkali and dimethyl

sulphate. It had been reported that the use of more concentrated sodium hydroxide solution[91] and an increase of the operating temperature and even the replacement of sodium hydroxide by potassium hydroxide[92] would give a more satisfactory result. In order to obtain a complete methylation, the product is usually methylated several times and different methods are applied.

In general, polysaccharides are insoluble in the organic solvents, therefore they are usually methylated first with methyl sulphate and aqueous alkali. This yields a partially methylated product which is soluble in methyl iodide required for the Purdie procedure. The method of Kuhn and his co-workers is also frequently used. The partially methylated product is treated with methyl iodide and silver oxide under reflux. The advantage of this method is the ease with which the methylated product may be recovered. Other methods such as dissolving the polysaccharides in N,N-dimethylformamide[194] and then treating the solutions with methyl iodide or treating the polysaccharide in methyl sulphoxide with sodium hydroxide and methyl iodide[93] have also been used extensively.

It is very difficult to estimate the completeness of methylation, and the usual method is the determination of the methoxyl content and a comparison of this with a theoretical determination of the suspected repeating unit which is defined as the simplest repeating structure which defines the polysaccharide molecule

The impurities, non-polysaccharide materials such as salts, condensation products and lignin and also part of the polysaccharide that is less completely methylated can be removed

by fractional precipitation from the methylated polysaccharide. Mixtures of chloroform and light petroleum or ether are usually used for this purification.

Since the methylated polysaccharides are usually insoluble in water, a non-aqueous or only partially aqueous medium is used for the initial hydrolysis. Methanolysis is usually carried out by refluxing the methylated product with methanolic hydrogen chloride. Other methods including formolysis[95] and prehydrolysis[96] in concentrated sulphuric acid can also be used.

The aim of hydrolysis is to depolymerise the methylated polysaccharide to the monomeric state under the conditions to give the least possible degradation of the resultant monomers. Because of the great resistance of glycosidic linkages to acid hydrolysis, uronic acid residues always appear as methylated aldobiouronic acids in the hydrolysate.

The mixtures of methylated sugars are separated by partition chromatography on a cellulose column, or by adsorption chromatography on carbon-celite columns. The components are tentatively identified on the basis of their mobilities on paper chromatography and electrophoresis. Since these procedures do not differentiate between D and L enantiomorphs, the components of the hydrolysate are then converted into appropriate derivatives, which can be identified by their melting points and optical rotations. Certain components of polysaccharides can be identified by specific colour reactions without previous direct hydrolysis and separation.[38]

Gas liquid chromatography is another important method in methylation analysis, both as an aid in identifying individual methylated sugars and for their quantitative measurement. It has been found that methyl ethers of the methyl glycosides of the heat labile monosaccharides were sufficiently stable and volatile to be analysed and the fully methylated methyl glycopyranosides were successfully separated by gas liquid partition chromatography.[97]

C H A P T E R II

Discussion

Hemicelluloses are present in all land plants and in some marine algae. The commonest member of this group are the xylans. Most of the xylans so far studied have the same basic structural feature, that is a main chain of β (1— 4) D-xylopyranose units to some of which may be attached by side chains sugars such as L-arabinose, D-glucuronic acid and 4-O-methyl D-glucuronic acid. Usually, the hemicellulose is composed of both linear and branched polymers with different proportions of individual sugars.

The polysaccharide under study was extracted from Yorkshire Fog (Holcus lanatus), by extracting the grass successively with boiling azeotropic ethanol-benzene (1:2) to inactive enzymes and to remove soluble sugars, with warm water which removed the water-soluble polysaccharides, with ethylene-diamine tetra-acetic acid disodium salt which removed the pectic materials and with pepsin in hydrochloric acid to remove protein. The residue was delignified with glacial acetic acid and chloro-amine T, and then it was further extracted with alkali to give a hemicellulose fraction. The hemicellulose B, dissolved in calcium chloride solution, was fractionated with iodine and potassium iodide solution into linear and branched polymer. The branched hemicellulose B was purified by forming a copper complex with Fehling's solution and further purified by

fractional precipitation. In the fractional precipitation with ethanol (see section 3.2, p43) five fractions were collected which represented 88.6% recovery of the starting material. 71.72% of the total polysaccharide precipitated in the narrow range of 30 to 35.38% ethanol by volume.

The monosaccharide compostion of the branched hemicellulose B was determined quantitatively and qualitatively by gas-liquid chromatography. The sample was first hydrolysed in sulphuric acid solution, after which the monosaccharides obtained were reduced to the corresponding alditols by sodium borohydride and subsequently converted into acetyl derivatives. Methyl-α-D-glucopyranoside was added as an internal standard. It was found that each monosaccharide resulted in only one peak, which proved that no epimersation took place during reduction with sodium borohydride. When varying amounts of alditol acetates were injected into the chromatograph, it was also found that the relationship between the peak areas and the weight of injected acetates was linear. The areas of the peaks were measured and the different components of sugar were calculated. The branched hemicellulose B contained 20.62% arabinose, 47.18% xylose and 12.26% hexose (see section 3.3, p.50).

The uronic acid content of the polysaccharide was determined quantitatively by the specific colour reactions for sugar and hexuronic acids with carbazole sulphuric acid and also with anthrone sulphuric acid. The absorptions of sugar and uronic acids were measured at a certain wavelength by the spectro-

photometer. After a series of determinations, it was found that branched hemicellulose B contained 15.6% uronic acid.

Examination of the acid hydrolysate of the polysaccharide on paper chromatographs showed the presence of xylose, arabinose, glucose, a trace of galactose and uronic acids. The neutral components were separated from the acidic component by passing the solution through ion exchange resins. The neutral sugars were further separated by partition chromatography on a cellulose column using butanol half saturated with water as the developing solvent. In this manner, xylose, arabinose, glucose and galactose were separated. The identity of xylose and arabinose was confirmed by converting them into their characteristic crystalline p-nitrophenylhydrazone derivatives. The acidic sugar was eluted from the Dowex1-X4 by alkali and then converted to free acid. The product on examination on the paper chromatogram, was found to be a single component and had the same rate as the marker, D-glucuronic acid. Therefore, the acidic component of the branched hemicellulose B was D-glucuronic acid. Its identification was further confirmed by preparing the D-glucuronic acid Brucine salt. The values obtained for optical rotation, melting point and mixed melting point were within the ranges of value obtained from the literature.

A portion of branched hemicellulose B was methylated twice with dimethyl sulphate and sodium hydroxide, after which it was dissolved in dimethyl formamide and methylated twice with methyl iodide and barium hydroxide. After another two treat-

ments with methyl iodide and silver oxide, the methylated acidic polysaccharide was now completely soluble in chloroform. The final product was a clear, yellow syrup, with an optical rotation in chloroform of $[\alpha]_D^{20}$ -105°. This large negative value indicates a high proportion of β glycosidic linkages of the main chain.

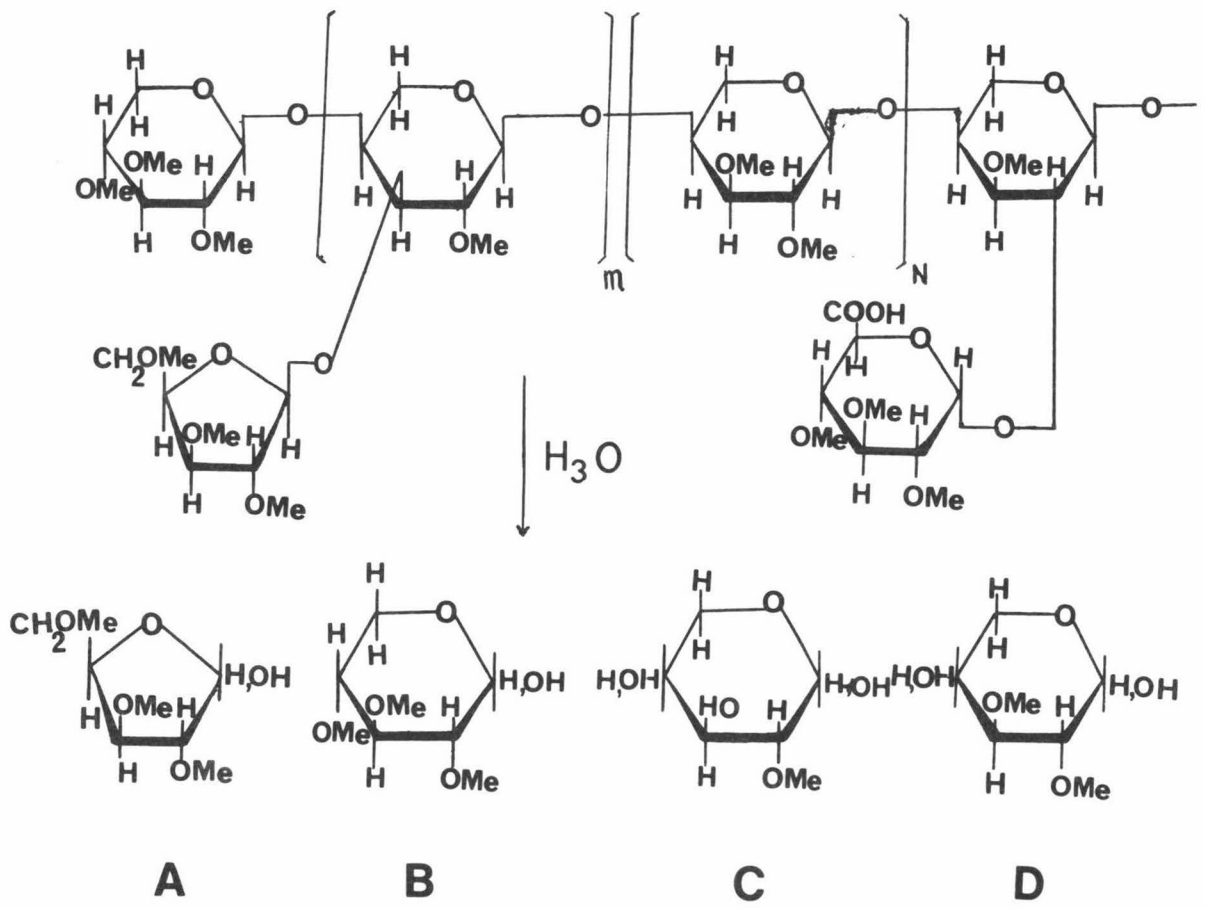
The methylated branched hemicellulose B was subjected to methanolysis, with 3% methanolic hydrogen chloride. After neutralisation, the glycosides were hydrolysed to the corresponding reducing sugars and these were resolved on a cellulose column using methyl-ethyl-ketone water azeotrope as the developing solvent. The mixture was found to contain 2,3,di-o-methyl-D-xylose, 2-O-methyl-D-xylose, 2,3,4,tri-O-methyl-D-xylose and 2,3,5 tri-O-methyl-L-arabinose. The 2,3,di-O-methyl-D-xylose was characterised by conversion into its crystalline anilide, while the rest were identified by qualitative chromatography, optical rotation and melting point of the crystalline sugars.

A portion of the hydrolysate was successfully examined by gas-liquid chromatography. A typical gas chromatogram is shown in Figure 8 (see section 3.6.3.1. 72). Five peaks were observed, four of which were identified. Peak A corresponded to the major component 2,3,di-O-methyl-D-xylose, peak C was 2-O-methyl-D-xylose. Peaks D and E were identified as 2,3,4, tri-O-methyl-D-xylose and 2,3,5-tri-O-methyl-L-arabinose respectively, by running a known sample of hydrolysed sugar under the same operating conditions as before, then comparing

FIGURE 2

Methylated Branched Hemicellulose B

And Hydrolysis Products



A. 2,3,5 – tri – O – methyl – L – arabofuranose

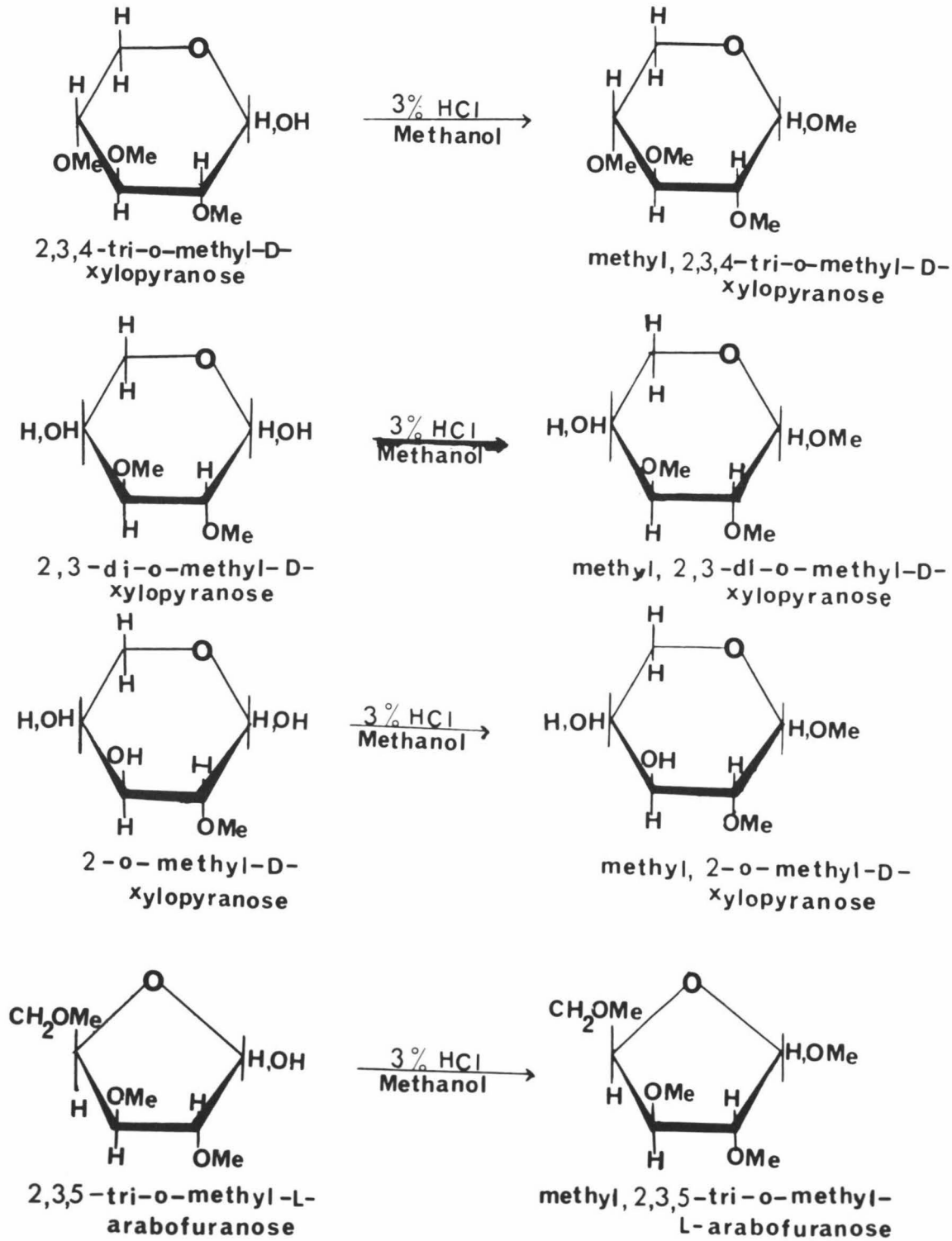
B. 2,3,4 – tri – O – methyl – D – xylopyranose

C. 2 – O – methyl – D – xylopyranose

D. 2,3 – di – O – methyl – D – **xylopyranose**

FIGURE 3

Methanolysis of Methylated Branched Hemicellulose B

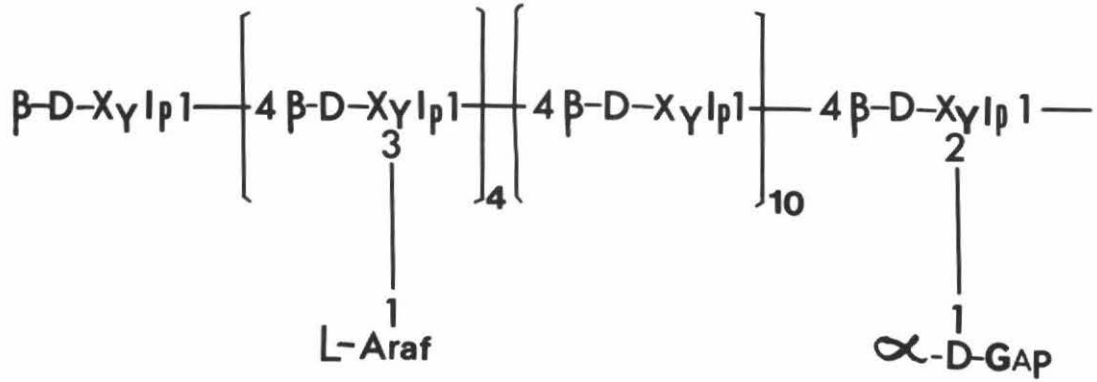


comparing the retention times. The areas of the peaks were measured and the molar ratio of the sugars was obtained (see Table 15, p.71).

The relatively large amount of 2,3-di-O-methyl-D-xylose produced from the methylated polysaccharide (see Table 15, p.71) indicated that the main portion of the hemicellulose was composed of xylopyranose residues linked together through positions 1 and 4, while the high negative rotation of the methylated polysaccharide suggested that the anhydroxylose units were present in the β configuration. The 2,3,4 tri-O-methyl-D-xylose evidently originated from the non-reducing end group of the polysaccharide. The presence of 2,3,5 tri-O-methyl-L-arabinose indicated that the hemicellulose, like many others, contained L-arabinofuranose units linked glycosidically as non-reducing end unit. The ready cleavage of the arabinose unit by mild acid hydrolysis was the evidence of the furanose form of the sugar. The 2-O-methyl-D-xylose is derived from units of xylose which form branch points in the molecule, these units are joined through position 3 in addition to positions 1 and 4. The uronic acid has been confirmed as D-glucuronic acid, linked to the main chain at position 2 by α glycosidic linkage. There is no trace of 3-O-methyl-D-xylose in the hydrolysate of the methylated polysaccharide, this could be due to the incomplete methylation of the branched hemicellulose B.

On the basis of the molar ratios of the cleavage products of the methylated polysaccharide, it is possible to propose a

structure for the branched hemicellulose B consiting of 16 D-xylopyranose units linked 1— 4 by β glycosidic bonds, with approximately four 2-mono-O-methyl-D-xylopyranose units for every ten 2,3 di-O-methyl-D-xylopyranose units. D-glucuronic acid is joined as a single terminal side chain to the xylose unit of the main structure by α 1— 2 glycosidic bond. L-arabinofuranose unit probably occurs in the side chain since L-arabinose is the first sugar obtained on hydrolysis. This unit is linked to every fourth D-xylose at position 3.



Xylp = Xylopyranose, Araf = Arabofuranose, GAp =Glucopyranosyluronic acid.

Figure 4. Proposed structure for branched Hemicellulose B of Yorkshire fog (Holcus lanatus)

CHAPTER III

Methods and Results

Melting point dterminations were carried out using a Gallenkamp electrical melting point apparatus. Optical rotations were determined using a Hilger standard polarimeter. In the uronic acid determination, the absorptions of sugar and uronic acids were measured by SP 500 series 2 ultraviolet and visible spectrophotmeter. Microanalyses were carried out at the University of Otago by Dr. A.D. Campbell.

The Plant Material

Yorkshire Fog (*Holcus lanatus*), a perennial grass was grown on the Massey Agronomy plot. After the grass was cut in spring, it was immediately frozen in "chilly bins" with dry ice and held there until freeze dried. The dried grass was ground into a powder, fine enough to pass through a sieve with holes 1 mm in diameter and then stored in brown bottles closed with waxed corks.

3.1 Extraction of Hemicellulose B from Yorkshire Fog

The grass was extracted in batches of 200 g.

Extraction Procedure

(A) Preliminary Extraction

The dried grass (200 g) was extracted with azeotropic ethanol-benzene solution (4 l) (1:2) in a macro-soxhlet for eighteen hours. The extracted material was air dried and weighed.

(B) Water-soluble Polysaccharides

The extracted grass (161.1 g) was suspended in water (12 l) at 60°C for thirty minutes with occasional shaking and then filtered. The insoluble matter was washed with water (4 l) and dried in an oven at 40°C.

(C) Pectin Extraction

The residue (147.2 g) was extracted by stirring with 2% EDTA (3 l) (sodium form) adjusted to pH 6.7 with sodium hydroxide, at 70°C for two hours. The extraction was repeated three times with fresh solvent. After filtration, the residue was washed several times with water, then dried at 40°C and weighed.

(D) Extraction with Pepsin in 0.1N HCl

The residue (139.7 g) was extracted with 0.5% pepsin in 0.1N HCl (5.6 l) for eighteen hours at 46°C. The extract was discarded and the residue was washed several times with water until the supernatant was clear and was dried and weighed.

(E) Delignification

The residue from pepsin extraction (78.9 g) was suspended in water (2 l) and held in a boiling water bath. Glacial acetic acid (5.2 ml) and chloramine T (27 g) were added slowly to the suspension. The beaker was covered with a watch glass and left with occasional stirring on the water bath for two hours. The suspension was filtered while still warm through a glass filter and washed twice with 95% ethanol. A boiling 3% solution of

ethanol amine (400 ml) was left in contact with residue on the glass filter for two minutes, then suction was applied. The residue was treated again with 95% ethanol then washed three times with water. The whole process was repeated twice, and the residue was dried and weighed.

(F) Hemicellulose Extractions

The delignified residue (69.6 g) was extracted by stirring with 10% KOH (1.4 l) under nitrogen for fifteen hours at 25°C. (The nitrogen used was deoxygenated by bubbling through a solution of pyragallol (15 g) in 50% sodium hydroxide (100 ml)). The suspension was centrifuged and the residue washed with deionised water (1 l) and centrifuged again. The extract and washings were combined.

Hemicellulose A

The extract was adjusted to pH 4.9 with glacial acetic acid and allowed to stand overnight. The precipitate (Hemicellulose A) which formed was removed by centrifugation.

Hemicellulose B

The acidified supernatant was poured into 95% ethanol (4 l) and allowed to stand until a precipitate formed. The precipitate which collected on a nylon gauze did not completely dissolve in deionised water and so was centrifuged at 10,000 g for fifteen minutes. The clear brown supernatant was poured off and the residue was re-dispersed

in deionised water and re-centrifuged at 10,000 g. The supernatants were combined and the residue was discarded.

The supernatant (400 ml) was adjusted to pH 2.5 with 1M HCl and poured into 95% ethanol (2 l). The precipitate which formed was collected and redissolved in water. The solution was adjusted to pH 2.0 with concentrated hydrochloric acid and again poured into 95% ethanol (1 l). The precipitate was washed with 80% ethanol twice and also washed with 95% ethanol until free of chloride ions. The hemicellulose B was dissolved in a minimum volume of water, frozen and freeze dried.

(G) Fractionation of Hemicellulose B[98]

The hemicellulose (12.5 g) was dissolved in calcium chloride solution (600 ml) (S.G. 1.3) and clarified by a short centrifugation at 20,000 g. 3% aqueous solution of I_2 (30 ml) and 4% aqueous solution of KI (40 ml) were added to the solution. The dark blue precipitate which formed was left to settle for two hours and then collected by centrifugation at 20,000 g. The clear brown supernatant was neutralised with sodium thiosulphate and poured with stirring into five volumes of ethanol to precipitate the branched polymer.

Branched Hemicellulose B

To remove the calcium, this precipitate was dissolved in 0.1M hydrochloric acid (70 ml) and reprecipitated with ethanol (350 ml). The polysaccharide was filtered on a nylon gauze, washed with ethanol and ether, dissolved in

a minimum volume of water and freeze dried.

Linear Hemicellulose B

The dark blue precipitate containing the linear polymer was washed with calcium chloride solution (200 ml) containing 15% iodine-potassium iodide solution. The precipitate was then dissolved in hot water (300 ml), the iodine was neutralised with sodium thiosulphate and the polymer reprecipitated by pouring into five volumes of ethanol. To remove calcium the precipitate was dissolved in 1M KOH, neutralised with hydrochloric acid and again poured into five volumes of ethanol. The precipitate was collected and dissolved in a minimum quantity of water (50 ml) then freeze-dried.

(H) Cellulose Residue

The residue from the hemicellulose extraction was washed with hydrochloric acid until neutralised and then washed with water until the effluent was above pH 5. The white material was collected, dissolved in water and freeze-dried.

3.2 Assessment of Homogeneity of Branched Hemicellulose B

(A) Fractionation using Fehling Solution[100]

The branched hemicellulose B (6.0 g) was dissolved in 0.5M NaOH (600 ml). The solution was centrifuged at 3000 r.p.m. to remove the insoluble impurity. Freshly prepared Fehling solution A (300 ml) and B (300 ml) were mixed and added to the polymer solution. No precipitate

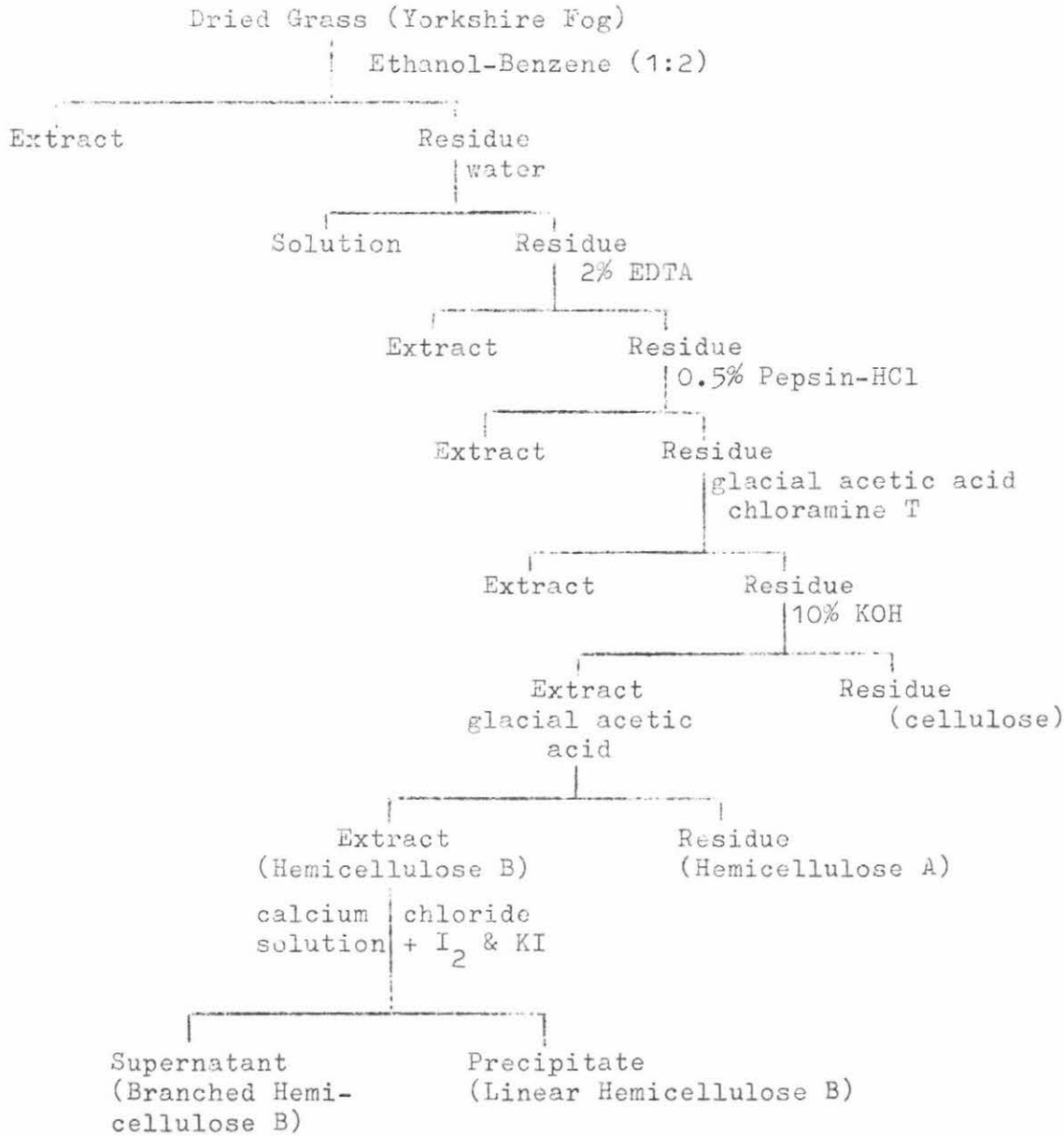


Figure 5. Scheme for Isolation of Hemicellulose[2]

TABLE VI Composition of Yorkshire Fog (*Holcus lanatus*)
(g/200 g moisture-free original grass)

Ethanol-Benzene soluble materials	38.9
Water soluble polysaccharides	13.9
Pectin	7.5
Crude proteins	60.8
Lignin	9.3
Hemicellulose A cellulose unidentified substance	58.2
Hemicellulose B	11.4

was formed. The resultant solution was neutralised with acetic acid and then dialysed against tap water for twenty-four hours. The dialysate was concentrated to a small volume in vacuo, under reduced pressure, then poured into ethanol (500 ml) at 0°. The precipitated polysaccharide was still blue green in colour, which indicated the presence of copper ions, therefore, it was collected and decomposed by maceration for one minute with cold ethanol (500 ml), which contained 5% (v/v) of concentrated hydrochloric acid. The residue was washed several times with acetone and ethanol until the washings gave a negative test for chloride ions. The fractionated polysaccharide was dissolved in a minimum amount of water and freeze-dried. Yield (5.1 g).

(B) Fractional Precipitation with Ethanol[101]

Pure branched hemicellulose B (0.6 g) was dissolved in water (30 ml). The small amount of insoluble material was removed by centrifugation at 2500 r.p.m. The solution was adjusted to pH 7 with hydrochloric acid and ethanol was added dropwise with stirring to the solution until it became milky white. After standing for five minutes, the dispersion was centrifuged. The precipitated material was washed with ethanol several times then dissolved in water and freeze-dried. The supernatant from the centrifugation was poured into another centrifuge bottle and ethanol was added dropwise as before until incipient

TABLE VII Fractional Precipitation of Branched Hemicellulose B

Fraction number	ml of ethanol added	% of ethanol by volume	Weight of precipitate in g	% of total precipitate
1	20.1	40.2	0.0467	8.78
2	30	50.0	0.0656	12.34
3	35.38	54.1	0.3158	59.38
4	52.9	63.8	0.0684	12.86
5	120	80	0.0353	6.64

FIGURE 6

Per Cent of Hemicellulose B precipitated at Various Alcohol Concentration.

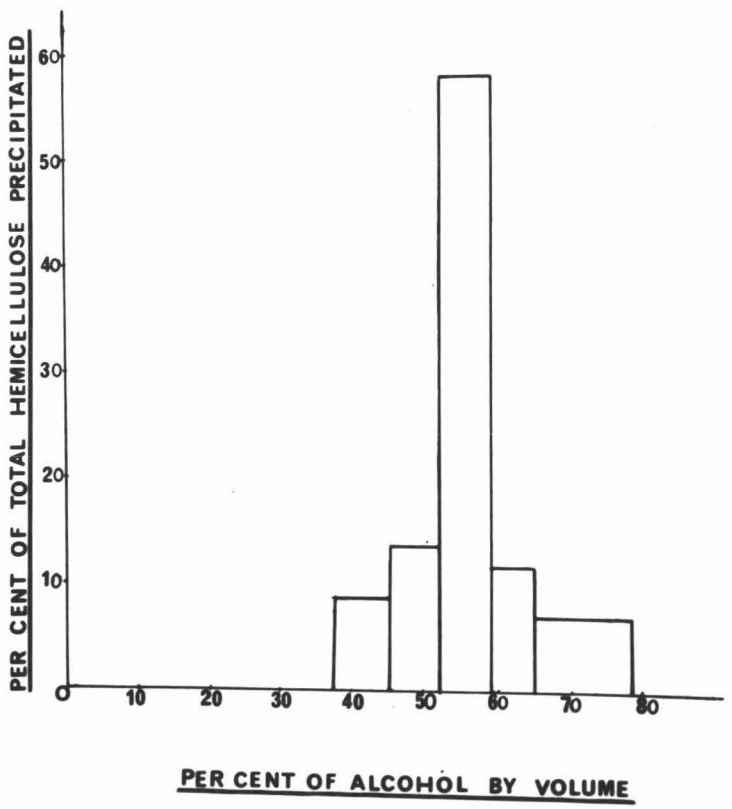
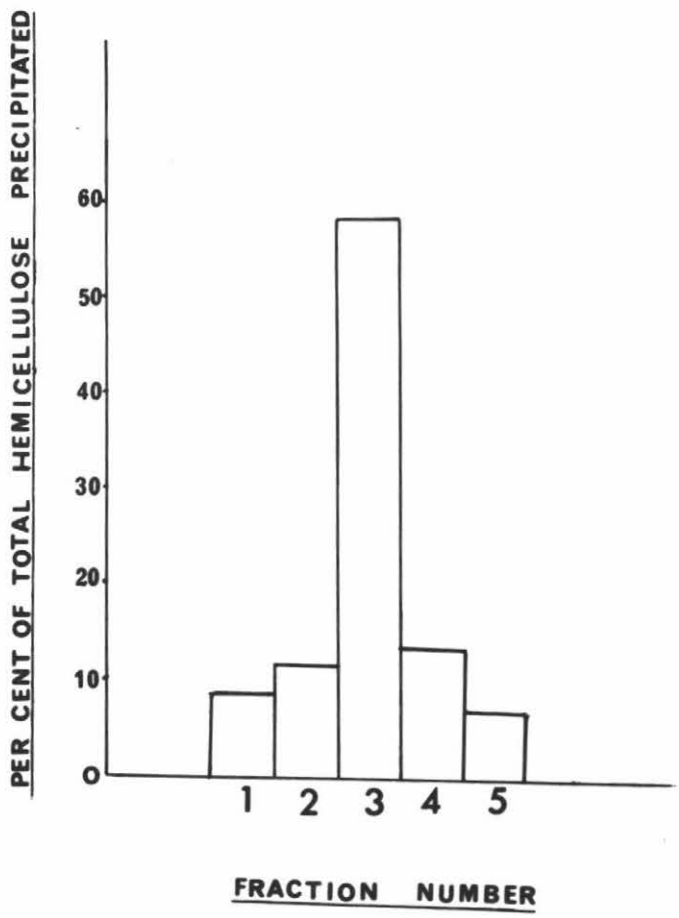


FIGURE 7

Per Cent of Hemicellulose B in Each Fraction.



turbidity appeared. This insoluble fraction was isolated as before and the precipitation procedure was repeated until a concentration of 80% ethanol was attained. From the table it can be calculated that 88.6% of polysaccharide was recovered from the starting material.

3.3 Quantitative Determination of Carbohydrates in Hemicellulose by Gas-Liquid Chromatography[77,102,103]

Apparatus

Separations were performed on a Varian Aerograph series 1740 (FID) gas chromatograph, equipped with a differential flame ionisation detector and automatic servoscribe potentiometric recorder. The coiled column was constructed from $\frac{1}{8}$" steel and packed with 3% SE-30.

Nitrogen was used as the carrier gas. The injection port was maintained at 190°C and the detector was matched to that of the recorder by means of a vibrating reed electrometer. The signal from the detector was recorded on a 100 mv full scale strip chart recorder.

Calibration

The pressure was stable and pressure gauge module was calibrated after the 3% SE-30 column was installed. The flame ionisation detector required carrier gas, hydrogen and air for proper operation. The regulator of carrier gas (nitrogen) cylinder was adjusted for 65 p.s.i., 10 p.s.i. and 20 p.s.i. for hydrogen and air cylinders respectively. The flow rate was adjusted to 20 ml/min. by using soap-bubble flowmeter, and

the gas flow was 50-60 on the flowmeter.

Procedure

Pure hemicellulose B (20 g) was hydrolysed for twenty-two hours with 0.25N sulphuric acid (10 ml) in a sealed tube in an oven at 95-100°C. The hydrolysate was neutralised to pH 6-8 with saturated barium hydroxide and methyl-α D glucopyranoside (9 mg) as a standard was added to the solution. After complete mixing and stirring, the barium sulphate was removed by centrifugation (5000 r.p.m.), and then sodium borohydride (30 mg) was added to reduce the aldoses to their corresponding alditols. The solution was left overnight and sodium ions were then removed by adding an excess of cation exchange resin (Dowex 50Wx8, hydrogen form, 50-100 mesh). After rotation for about twenty minutes in a rotatory evaporator, the supernatant was decanted and filtered. The resin was washed several times with water and these supernatants also filtered. The combined solution was evaporated under vacuum to dryness and borate was removed as the volatile methyl borate by evaporation with methanol (2 x 20 ml). The residue was acetylated by heating on a boiling water bath for twelve minutes with a 1:1 mixture (4 ml) of pyridine and acetic anhydride. The reaction mixture was concentrated on a rotatory evaporator. The thick syrup was taken up several times in ethyl acetate and re-evaporated then finally dissolved in ethyl acetate (2 ml).

Separation

A suitable quantity of the sample solution (0.4-0.5 ml) in ethyl acetate was introduced by means of a Hamilton micro-

litre syringe into a heated injection tube where they were vaporised. The initial column temperature was 144°C, and the programming was started immediately after injection. After the peak of methyl α-D-glucopyranoside was through the temperature was increased to 170°C.

Result

Since no integrator was available, the peaks areas were cut out and measured by weighing on a micro-balance (Mettler H 20). (See Figure 8, p.57).

3.4 Uronic Acid Determination[82,83]

(A) Carbazole Method

Solutions:

B is a solution containing the unknown polysaccharide 50-100 ug/ml uronic acid.

C is a standard solution containing 50 ug/ml D-glucuronic acid.

d is a solution containing 200 ug/ml of sugar (arabinose, xylose and glucose.

1 ml samples of each solution B, C and d were placed in an ice bath, and concentrated sulphuric acid (6 ml) (c.p.) was added slowly to each down the side of the tubes. The solutions were well agitated with a glass rod and heated for exactly twenty minutes in a boiling water bath. The mixtures were then cooled rapidly and a 0.1% alcoholic solution (0.2 ml) of carbazole was added with shaking. After a few minutes, a pink-purple colour appeared and the

TABLE VIII Conversion factors for the determination of sugar composition from the peak area

Sugar	Conversion Factor
Xylose	0.775
Arabinose	0.741
Hexose	0.813

TABLE IX Retention time of fully acetylated glycitols relative to methyl-α-D-glycopyranoside penta acetate

Sample	Relative Retention Time
Arabitol-penta acetate	0.706
xylitol penta acetate	0.794
methyl-α-D-glycopyranoside penta acetate	1 (standard)
sorbitol-hexa acetate	1.471 indistinguishable using column SE-30
galacitol-hexa acetate	1.471

TABLE X Arabinose composition of branched hemicellulose B

Weight of the peak area of Arabinose (in g)	Weight of the peak area of standard (in g)	Ratio = weight of area of Arabinose / weight of area of standard	Weight of standard added (in mg)	Correction factor	Product of ratio, weight of standard and correction factor	Weight of hemicellulose B (in mg)	Arabinose composition %
0.3067	0.6698	0.45789	9.67	0.741	3.28099	16.03	20.47
0.2981	0.6427	0.46382	9.67	"	3.32348	16.03	20.73
0.3035	0.6352	0.47780	9.67	"	3.42366	16.03	21.35
0.2813	0.4991	0.5636	9.26	"	3 86723	18.88	20 48
0.2779	0.5020	0.5535	9.26	"	3.79847	18.88	20.11
						Average	20.62

TABLE XI Xylose composition of branched hemicellulose B

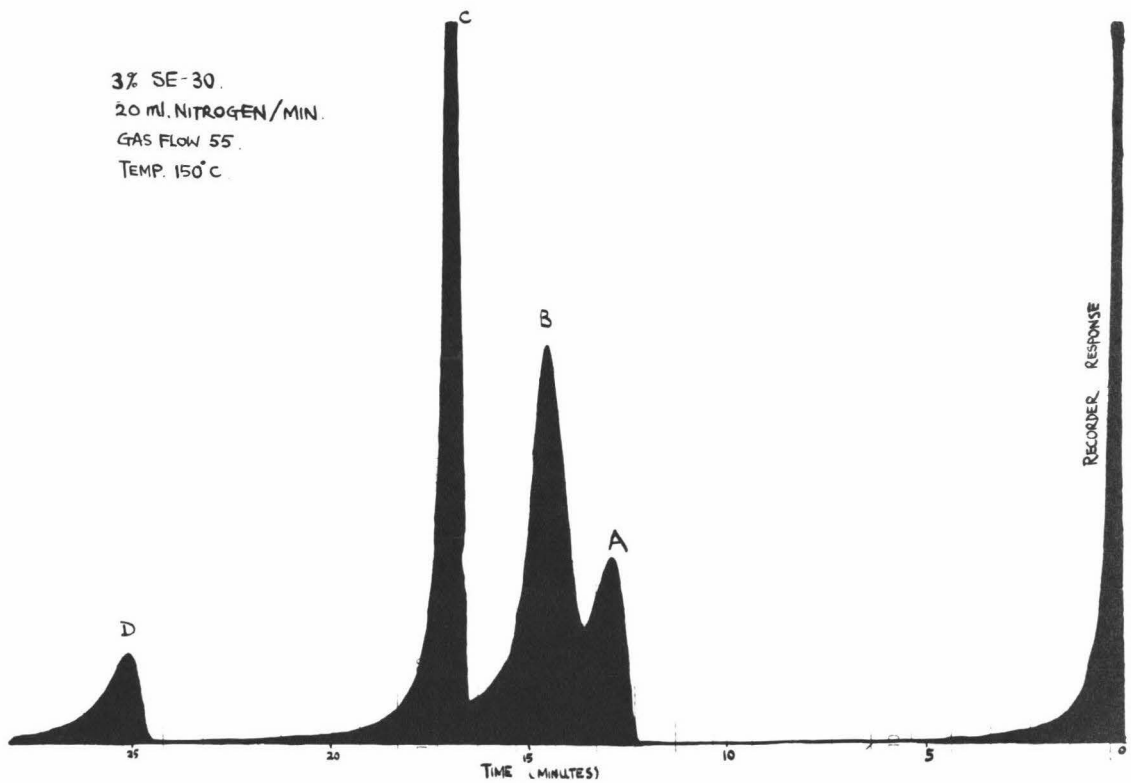
Weight of the peak area of xylose (in g)	Weight of the peak area of standard (in g)	Ratio = weight of area of xylose / weight of area of standard	Weight of standard added (in mg)	Correction factor	Product of ratio, weight of standard and correction factor	Weight of hemicellulose B (in mg)	Xylose composition %
0.6362	0.6352	1.00157	9.67	0.775	7.506016	16.03	46.83
0.6575	0.6427	1.02302	9.67	"	7.66676	16.03	47.82
0.6843	0.6698	1.02164	9.67	"	7.65642	16.03	47.45
0.8012	0.7898	1.01443	9.67	"	7.602392	16.03	47.43
0.6090	0.4991	1.22019	9.26	"	8.75669	18.88	46.38
						Average	47.18

TABLE XII Hexose composition of branched hemicellulose B

Weight of the peak area of Hexose (in g)	Weight of the peak area of standard (in g)	Ratio = weight of area of Hexose / Weight of area of standard	Weight of standard added (in mg)	Correction factor	Product of ratio, weight of standard and correction factor	Weight of hemicellulose B (in mg)	Hexose composition %
0.1466	0.5956	0.24613	9.67	0.813	1.935002	16.03	12.07
0.1509	0.6088	0.24786	9.67	"	1.948603	16.03	12.15
0.1682	0.6698	0.25119	9.67	"	1.974780	16.03	12.31
0.1525	0.5876	0.25956	9.67	"	2.040113	16.03	12.73
0.1740	0.5772	0.30145	9.26	"	2.26940	18.88	12.02
0.1343	0.4670	0.28758	9.26	"	2.16501	18.88	11.47
0.2074	0.6308	0.32878	9.26	"	2.47510	18.88	13.10
						Average	12.26

FIGURE 8

The Separation by Gas-Liquid Chromatography of Monosaccharides as Alditol acetates.



A. Arabinose.

B. Xylose.

C. Methyl-α-D-glycopyranoside.

D. Hexose.

intensity of this increased for two hours and then remained stable. The tubes were left in the dark at room temperature for 3 hours. The absorption was then measured at 530 mu by spectrophotometer.

(B) Anthrone Method

Solutions:

a is a solution containing 100 ug/ml of sugar (arabinose, xylose and glucose).

b is a solution containing 200 ug/ml D-glucuronic acid.

A is a solution containing the unknown polysaccharide, 50-100 ug/ml uronic acid.

2 ml samples of each solution a, b and A were placed in an ice bath, after fifteen minutes of cooling, a 0.2% solution (4 ml) of anthrone in concentrated sulphuric acid was added slowly to each. The mixtures were stirred with a glass rod, and the tubes were sealed with glass stoppers and heated in water bath at 92°C for eight minutes. The solutions were cooled and the tubes were left in the dark at room temperature for thirty minutes. The absorption was then measured at 585 mu by UV spectrophotometer. The quantity of uronic acid was determined in mg/ml by the following formula:

$$\frac{Ba - Ad}{\frac{Ca}{50} - \frac{bd}{200}} : 1000$$

B = 0.53 a = 1.23
c = 0.268 b = 1.78
d = 0.27 A = 1.20

Total uronic acid was 78 ug/ml

Polysaccharide was 1 ug in 2 ml (from above), i.e. 500 ug/ml

$\therefore$ % uronic acid in the polysaccharide = $\frac{78}{500}$ x 100 = 15.6%.

Paper Chromatography

Chromatographic separations of sugars were carried out on Whatman Nos. 1 and 3MM filter papers with the following solvent systems (v/v): (A) ethyl acetate - glacial acetic acid - formic acid - water (18:3:1:4), (B) n-butanol - ethanol - water (4:1:5), (C) an azeotropic mixture of 2-butanone and water, b.p. 74-75°C. Spray reagent was 3% solution of p-anisidine hydrochloride in ethanol. Rx values of neutral and acidic oligosaccharides refer to rates of movement relative to xylose R_G and R_F values of methylated sugars refer to rates of movement relative to 2,3,4,6 tetra-O-methyl-D-glucose and 2,3,4,6 tetra-O-methyl-D-galactose respectively.

3.5 Separation and Identification of Monosaccharides from the Product of Hydrolysis of Branched Hemicellulose B[104]

The branched hemicellulose B (3 g) was dissolved in 0.125M sulphuric acid (500 cm^3) and the mixture was heated at 92°C for twenty two hours. The solution was cooled and brought to pH 6 by the addition of saturated barium hydroxide and then barium carbonate. Barium sulphate was removed by filtration and the filtrate was concentrated to 250 ml and then passed through Amberlite IR-120 (Hydrogen form) exchange resin. Uronic acids were absorbed from the eluate by passing it through

a column of Dowex 1-X4 (OH^-) exchange resin, after which the resin was washed with water (2 l) until a negative anthrone test showed that all sugars had been removed. All the washings were combined together and concentrated under reduced pressure at 40°C to a syrup which contained the neutral sugars (A) (2.5 g).

The uronic acids were then eluted from Dowex 1-X4 (OH^-) exchange resin by 1M sodium hydroxide and converted to the free acid form by removal of sodium ions on Amberlite IR-120 (H^+) exchange resin, the filtrate was evaporated under reduced pressure at 40°C to a syrup (B) (0.3 g).

Separation of Sugar in (A)

The neutral fraction (A) was examined by paper chromagography using solvent A and was found to contain a mixture of xylose, arabinose and glucose. They were further separated by chromatography on a cellulose column using butanol half saturated with water as the solvent.

Separation of Acids in (B)

Paper chromatography in solvent A showed that the uronic acid had the same Rx (1.10) as the marker, D-glucuronic acid. The syrup was crystallised from methanol to give crystals m.p. 163°C, lit., D-glucuronic acid m.p. 165°, $[\alpha]_D^{20}$ + 19.5, lit., $[\alpha]_D$ + 11.7 —— + 36.3 (in water) which were further identified as D-glucuronic acid Brucine salt derivative, m.p. 154-155°, lit., m.p. 156-157°C, $[\alpha]_D^{20}$ - 13.2 (c. 1.5 in water), lit., $[\alpha]_D^{20}$ - 15.1 (in water).

3.5.1 Quantitative Analysis of Mixtures of Sugars by Partition Chromatography on Column of Powdered Cellulose[105,106,107,108,109]

The mixture of neutral sugars (A) was added as a thin syrup onto a column of cellulose (3.5x45 cm) and eluted with butanol half saturated with water. The eluate was fractionated into fractions of about 15 cm^3. After the separation had been completed, the distribution of the component sugars in the tubes was determined by paper chromatography using solvent A and p-anisidine hydrochloride spray. Four components were apparent and these corresponded to xylose, arabinose, glucose and galactose. The eluates from those tubes containing the same components were combined to give five fractions. The first group (317 mg) contained pure xylose, while the second group (140.2 mg) appeared to be a mixture of xylose and arabinose. Group III (92 mg) was the pure arabinose, group IV (94.9 mg) was glucose. Group V (27.8 mg) seemed to be a mixture of glucose and galactose.

Group II was chromatographically separated on Whatman 3MM filter paper with solvent A. Approximate zones, indicated by marker strips on both sides of the paper, were cut and the components extracted with water. The more rapid-moving component Ia (Rx 1.00) and the slow-moving component Ib (Rx 0.85) were chromatographically

identified as xylose and arabinose respectively.

Fraction I: Identification of xylose

The syrup (317 mg) (Rx 1.00), crystallised spontaneously and after recrystallisation from ethanol had m.p. 144°C, lit., m.p. 145-148° $[\alpha]_D^{20}$ + 15.2 (c. 5 in water), lit., $[\alpha]_D$ + 93.6 —— + 18.8 (c. 4 in water). p-Nitrophenylhydrazine (554 mg) dissolved in alcohol (3 ml), was added to a solution of xylose (317 mg) in water (2 ml). The mixture was warmed on a water bath and water was added until a turbidity appeared. The crystalline nitrophenylhydrazone separated on standing and was filtered off and washed with water. Recrystallisation upon aqueous alcohol gave the characteristic xylose-p-nitrophenylhydrazone, m.p. and mixed m.p. 151°C, lit., m.p. 156°C.

Fraction 3: Identification of arabinose

This component (92 mg) had Rx 0.85 after recrystallisation from ethanol, m.p. 155°C lit., m.p. 160°C, $[\alpha]_D^{20}$ + 102.8 (c. 2 in water), lit., $[\alpha]_D$ + 190.6 —— + 104.5 (c. 4.3 in water). Arabinose was also confirmed by the derivative of p-nitro-phenylhydrazone. The method was the same as that for xylose. Arabinose-p-nitro-phenylhydrazone had m.p. and mixed m.p. 166°C, lit., m.p. 187-188°C.

TABLE XIII Results of the separation of sugars by partition chromatography

Fraction number	Tube number	Yield (mg)	Rx	Components identified
1	40-63	317.0	1.00	xylose
2	64-75	140.2	1.00 0.85	xylose Arabinose
3	76-92	92.0	0.85	Arabinose
4	110-137	94.9	0.56	Glucose
5	138-180	27.8	0.56 0.53	Glucose Galactose

Rx: The distance between the centre of the sugar spot and the starting line with relative to that of standard xylose.

Solvent A was ethyl acetate-glacial acetic acid-formic acid-water (18:3:1:4).

3.6 Methylation of Branched Hemicellulose B by Methods After Haworth, Kuhn and Purdie[110,111,112]

Haworth Procedure[90,113,114,115,116]

40% sodium hydroxide (160 ml) and dimethyl sulphate (64 ml) were added to a constantly stirred solution of pure branched hemicellulose B (4 g) dissolved in water (50 ml), at room temperature over a period of six hours in such a way that the solution was always alkaline, stirring was continued for another eighteen hours. The mixture was methylated again as above but the temperature was raised to 50°C. The solution was transferred to a beaker which was heated on a sand bath. A precipitate which was the partly methylated polysaccharide was formed on the surface by stirring. The precipitate was removed and dissolved in boiling water (100 ml), then dialysed against tap water for twenty-four hours, and evaporated at 40°C in vacuo to a syrup (3.4 g).

Kuhn Procedure[94,117]

The partially methylated polysaccharide (3.4 g) was dissolved in dimethyl formamide (100 ml) and the temperature was raised to 30°C. Methyl iodide (45 ml) and barium hydroxide (45 g) were added during three hours. After another twelve hours of stirring, the partially methylated polysaccharide had precipitated. Chloroform (100 ml) was added and stirring was continued for another twelve hours. The supernatant was decanted from the barium hydroxide and the latter was extracted twice with chloroform (100 ml). The chloroform extracts were combined with the above supernatant which was then washed with

an equal valume of water (6 times). The chloroform solution was centrifuged (3000 r.p.m.) and the water layer was removed with a pipette. The extract was dried over anhydrous sodium sulphate filtered and evaporated in vacuo at 30°C to a syrup (3.2 g).

The syrup was methylated again, by dissolving it in dimethyl formamide (100 ml) and adding methyl iodide (40 ml), silver oxide (30 g) over a period of twenty four hours to the constantly stirred solution at 45°C. After another twelve hours of stirring, the reaction mixture was centrifuged (4000 r.p.m.) and the supernatant was decanted from the precipitate, the latter was washed with dimethyl formamide (50 ml) and chloroform (50 ml), and these two fractions were combined with the above supernatant. Chloroform (350 ml) was added and the solution was shaken with potassium cyanide (10 g) to remove silver ions. The filtered solution was washed with equal volumes of distilled water (8 times) and dried in anhydrous sodium sulphate, then evaporated in vacuo to a syrup (3.0 g) (Found: OCH_3, 25.44% calculated for $C_7H_{12}O_4$ (2 OCH_3 groups) OCH_3, 38.7%).

Purdie Procedure[118,119,120]

The partially methylated branched hemicellulose B from the above was dissolved in methyl iodide (50 ml) and the solution was refluxed with silver oxide (5 g) which was added in six portions during twelve hours. The mixture was refluxed for another twelve hours. The excess methyl iodide was distilled off under reduced pressure and the residue was extracted

three times with acetone (80 ml). The filtered solution was concentrated to give a light brown material which was further methylated by the Purdie procedure. The final syrup was dissolved in chloroform (300 ml) and shaken with potassium cyanide (10 g) to remove silver ions. The solution was filtered and washed with equal volumes of distilled water (6 times) before being concentrated to a syrup (2.5 g) $[\alpha]_D^{20}$ - 105° (c. 1 in chloroform). (Found: OCH_3, 32.88%, calc. OCH_3 38.7%).

3.6.1 Hydrolysis and Separation of Methylated Branched Hemicellulose B[12,121,122,123,124]

The methylated branched hemicellulose B (1.0 g) was dissolved in 3% methanolic hydrochloric acid (100 ml) and refluxed for six hours. After neutralisation with silver carbonate, centrifugation and filtration, the solution was reduced in vacuo at 30°C to a syrup (0.8 g). A portion of the syrup (0.4 g) was dissolved in 0.5M sulphuric acid (40 ml) and heated in a sealed flask at 100°C for eight hours. The hydrolysate was neutralised with barium carbonate and barium hydroxide. After filtration, the solution and washings were passed through ion exchange resins, IR-45 (OH) and IR-120 (H) respectively, and then evaporated in vacuo at 30°C to a syrup (0.26 g). Paper chromatography in solvent B and C showed that there were four major components, which corresponded to 2,3, di-methyl-D-xylose, 2 mono-methyl-D-xylose, 2,3,4, tri-methyl-D-xylose and 2,3,5 Tri-methyl L-arabinose. The

syrup (0.25 g) was separated on a cellulose column (42 x 3.5 cm), irrigated with 2-butanone-water azeotrope and the effluent collected in 15 ml aliquots. Paper chromatography with solvent B and C permitted the grouping together of aliquots with the same composition.

On the basis of R_F and R_G values, and colour reaction, Fraction I (52.2 mg) appeared to be 2,3,5,Tri-methyl-L-arabinose, Fraction II (22.8 mg) seemed to be 2,3,4,tri-methyl-D-xylose. Fraction III (72 mg) contained 2,3, di-methyl-D-xylose and Fraction IV (88.4 mg) seemed to be a mixture of 2,3,di-methyl-D-xylose and 2-mono-methyl-D-xylose. Fraction IV was chromatographically separated on sheets of Whatman 3MM filter paper with irrigant C. Marker strips at the sides of the paper indicated the zones which were cut out and the components extracted. The most rapid moving component (R_G 0.65) was 2,3,di-methyl-D-xylose and the slow-moving component (R_G 0.23) was 2-mono-methyl-D-xylose.

Fraction 3. Identification of 2,3,di-methyl-D-xylose

This component (82 mg) had $[\alpha]_D^{20}$ + 21.5 (c. 0.5 in water), lit., $[\alpha]_D^{25}$ + 25 (c. 1.0 in water)[123] + 24[124] + 23[121]. Paper chromatography showed R_F values of 0.80 (solvent C) and 0.82 (solvent B), and R_G values of 0.65 (solvent C) and 0.79 (solvent B), lit., 0.64, 0.68,[122] 0.61[121] (solvent C) and 0.79[12] (solvent B).

This fraction was refluxed for two hours in a solution of 5 ml absolute methanol containing 1 ml of

TABLE XIV Hydrolysis products from methylated branched hemicellulose B

Fraction number	Tube number	Weight (mg)	Solvent C R_F	Solvent C R_G	Solvent B R_F	Solvent B R_G	Component
1	5-18	52.2	1.30	1.02	0.96	0.94	2,3,5 tri-methyl L-arabinose
2	19-21	22.8	1.24	1.00	0.94	0.92	2,3,4 tri-methyl D-xylose
3	22-38	82	0.80	0.65	0.82	0.79	2,3,dimethyl D-xylose
4	40-80	78.4	0.28	0.23	0.51	0.47	2-mono-methyl D-xylose

Solvents: (C) 2-butanone-water azeotrope

(B) n-butanol-ethanol-water (4:1:5)

R_F is relative to 2,3,4,6 tetra-O-methyl D galactose

R_G is relative to 2,3,4,6 tetra-O-methyl D glucose

aniline. Upon evaporation of solvent, remaining aniline was removed by azeotropic distillation with water. After the last trace of aniline was removed, crystallised derivative appeared, upon recrystallisation from ethyl acetate methanol, m.p. 110°C, lit., m.p. 124°C.[124]

Fraction 4. Identification of 2 mono-methyl-D-xylose

This component (78.4 mg) had $[\alpha]_D^{20}$ + 20.2 (c. 1.0 in water), lit., $[\alpha]_D^{25}$ + 21[122] + 21.4.[121] Paper chromatography showed R_F values of 0.47 (solvent B), 0.23 (solvent C), lit., 0.47[12] (solvent B), 0.23[124] 0.22.[123]

A portion of this fraction was dissolved in absolute methanol and concentrated to a syrup which gave crystals on recrystallisation from ethyl acetate methanol, had m.p. 126°C, lit., m.p. 131°C.[123]

Fraction 2

This syrup (22.8 mg), had $[\alpha]_D^{20}$ + 17.8° (c. 1.2 in water), lit., $[\alpha]_D^{25}$ + 18.1 (c. 1.0 in water)[124] chromatography showed the presence of 2,3,4,tri-methyl D-xylose (R_G 1.00) (solvent C), (R_G 0.92) (solvent B). Attempt to prepare crystalline derivative failed.

Fraction 1

Chromatography of the syrup (52.2 mg) showed the presence of 2,3,5,Tri-methyl-L-arabinose, which had $[\alpha]_D^{20}$ - 36.5 (c. 1.0 in water), lit., $[\alpha]_D^{25}$ - 36 (c. 0.5 in water)[124] and had R_F values of 1.30 (solvent C) 0.96 (solvent B) and R_G values of 1.02 (solvent C)

and 0.94 (solvent B). Further evidence of its identification was obtained by gas chromatography.

3.6.2 Quantitative and Qualitative Determination of Methyl Ether Monomers by Gas-Liquid Chromatography

The column was the same as used before (see p50 . A portion of the methylated branched hemicellulose B was dissolved in 3% methanolic hydrochloric acid and refluxed for six hours (see p.50). After neutralisation with silver carbonate, the solution was concentrated to a syrup in vacuo at 30°C. A small portion (20 mg) of the syrup was dissolved in chloroform and the solution was injected into the gas chromatography.

The peaks were identified by injection of known samples into the chromatography under the same operating conditions as that for the product from hydrolysis of methylated hemicellulose B, and the retention times of the peaks were compared.

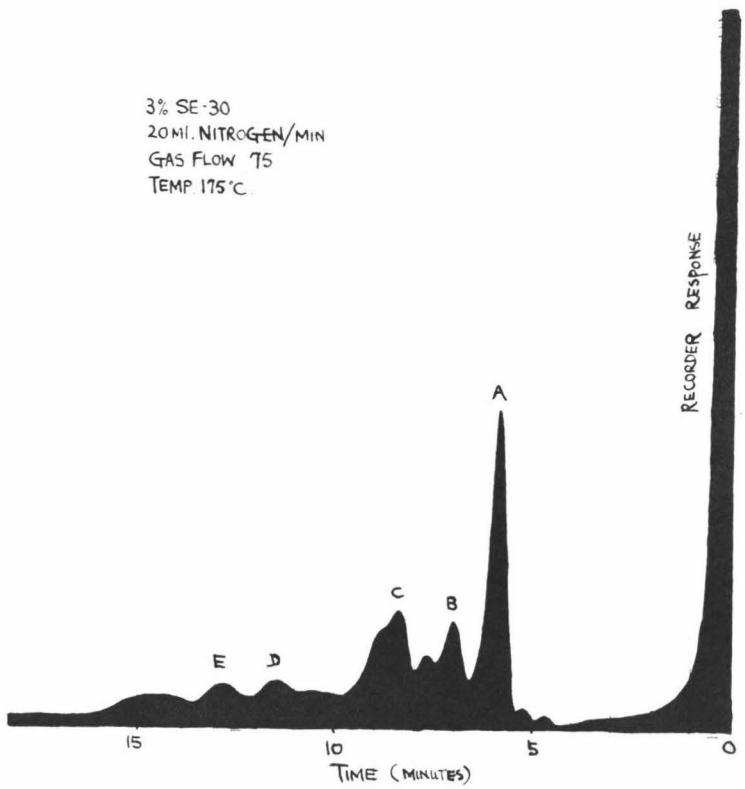
The molar ratio of the sugar were obtained by measuring the individual peak then worked out the relative ratio.

TABLE XV Products from hydrolysis of methylated branched hemicellulose B

Sugar	Molar ratio
2,3,di-o-methyl-D-xylose	9.6
2-O-methyl-D-xylose	4.3
2,3,4,Tri-O-methyl-D-xylose	1.0
2,3,5,Tri-o-methyl-L-arabinose	1.1

FIGURE 9

Gas-Liquid Chromatography of methylated Methyl Glycosides



A. 2,3, di-O-methyl-D-xylopyranose.
B. unidentified.
C. 2, mono-O-methyl-D-xylopyranose.
D. 2,3,4, tri-O-methyl-D-xylopyranose.
E. 2,3,5, tri-O-methyl-L-arabinose.

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