A SURVEY OF SOME SOUTH AFRICAN PLANT GUMS WITH SPECIAL REFERENCE TO THE CHEMISTRY OF WATSONIA CORM POLYSACCHARIDE

by

DEREK H. SHAW B.Sc.

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SUMMARY

Polysaccharide exudates from various South African plants have been investigated in order to obtain, if possible, correlation between the taxonomic origin of the plant and the chemical composition of the polysaccharide. The composition of the exudate has been shown to be genus specific though in some orders of plants the similarity does, to a lesser extent, extend to the family.

Of the polysaccharides studied, that from Watsonia pyramidata was selected for study of the fine structure. Using the techniques of partial hydrolysis, methylation, and periodate oxidation it was shown that this polysaccharide consisted of a basal chain of β1 → 4-linked xylose residues, each residue being doubly branched and carrying side chains containing galactose and arabinose. The trisaccharide O-α-D-galactopyranosyl-(1 → 3)-O-α-L-arabinofuranosyl-(1 → 2)-L-arabinose (contained in the side chains) was isolated for the first time, whilst 3-O-α-D-galactopyranosyl-L-arabinose which had previously been obtained only as a syrup was crystallised and some of the physical properties of this crystalline material were measured.

A possible structural repeating unit for Watsonia polysaccharide has been proposed in the light of experimental results obtained.

Part of the work reported here has been published as a preliminary report, viz., "A Complex Polysaccharide from Watsonia Corm- Seeds" by

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D.H. Shaw and A.M. Stephen, S.African Ind. Chemist, 1964, 65.

A further part has been accepted for publication, viz., "Isolation of Crystalline 3-O-α-D-galactopyranosyl-L-arabinose from a Polysaccharide found in Watsonia Corm-sacs." by D.H. Shaw, A.M. Stephen, and (in part), A.O. Fuller, J., 1965, in press.

INTRODUCTION

INTRODUCTION

The taxonomic relationships between various botanical classes have for many years been a source of interest to botanists. Using classical methods for deciding to which order, family, genus, and species a certain plant belonged, most plants have been satisfactorily classified. During recent years chemists have tried to classify plants taxonomically by the type of chemical components they contain. As more research work is done on the chemical constituents of plants and more results obtained, the data available for this purpose have in some cases become numerous enough to draw some conclusions.

In the field of the polysaccharide gum exudates from higher plants this is so. As early as 1854, Neubauer[1] was working on "gum arabic", which he found to be the neutral salt of an acidic polysaccharide and, following from this beginning, a great deal of knowledge concerning the chemistry of "gum arabic" has been obtained by other workers. Possibly the stimulus for studying this particular complicated substance arose from its relative abundance, commercial use, and immunological properties[2]. Over the years, with the study of other polysaccharide exudates from plants, especially from the genera Acacia and Prunus, it has become possible to obtain some general idea of the chemical constituents one would expect to find in a certain type of plant. If one considers species of Acacia as

an example/.....

an example one would expect to find that; (a) the equivalent weights are generally in the region of 1,000 to 2,000, (b) all contain D-glucuronic acid, linked normally to $C_{(6)}$, but occasionally to $C_{(4)}$ of D-galactose, (c) the backbone of the molecule consists of D-galactose units, linked 1 → 3 and sometimes 1 → 6, (d) all the exudates contain L-rhamnose and L-arabinose in addition to D-galactose and D-glucuronic acid, and (e) none of the polymers contain D- or L-xylose. If however one considers the nine species of Prunus which have been studied, a different pattern is noticed; the main differences are (a) the presence of D-xylose and D-mannose, and normally the absence of D- or L-rhamnose and (b) the occurrence of D-glucuronic acid, in this genus, linked in some cases to $C_{(2)}$ of D-mannose, and in others (as in Acacia species), to $C_{(6)}$ of D-galactose.

It appears that this type of chemical information does have taxonomical significance, and Part 1. of this thesis is an attempt to obtain more information on plant polysaccharide exudates in the genera where exudates from only a few species have been studied.

In the literature there are few references to what Stephen and Schelpe[3] have chosen to call a type C polysaccharide gum exudate; where the backbone of the molecule consists of D-xylopyranose residues linked β1 → 4 to each other and having a branch point on $C_{(2)}$ with a side chain of β-L-arabinopyranose, α-D-xylopyranose, or α-D-glucopyranuronic acid (or its 4-O-methyl ether). The type C polysaccharides

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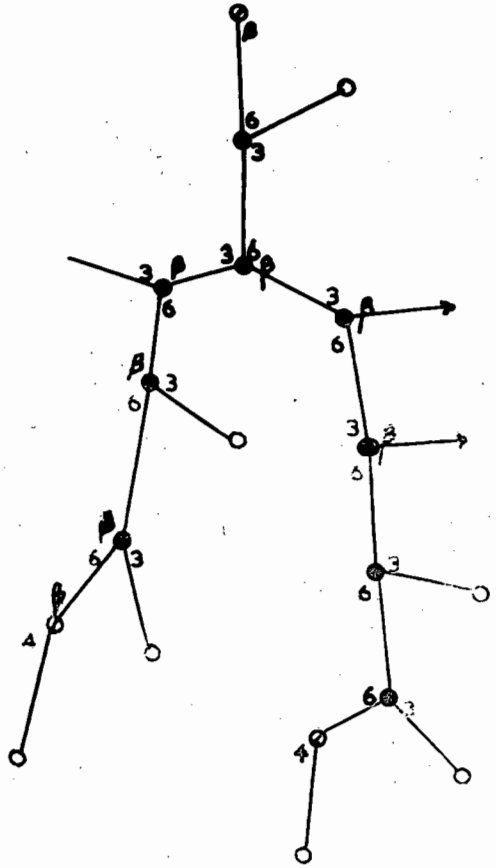
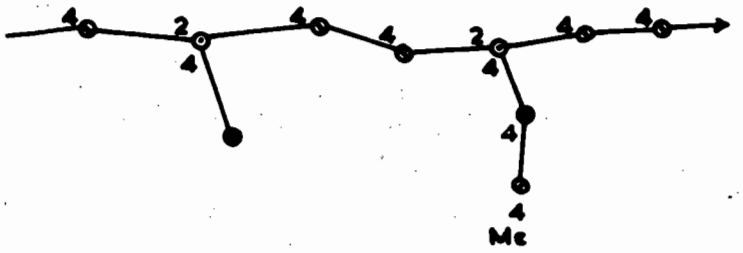


A TYPE "A" GUM



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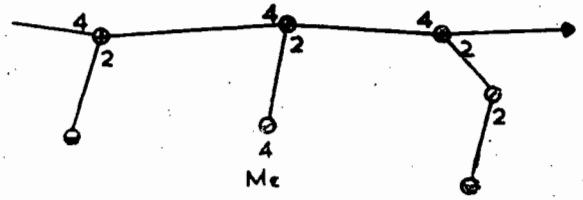

A TYPE "C" GUM

- ● D-galactopyranose
- ⊘ D-glucopyranuronic acid
- ⊘ D-galactopyranuronic acid
- ⊙ L-rhamnopyranose
- ⊕ D xylopyranose
- ⊖ α-D-xylose or β-L-arabinopyranose
- ○ L-ara$_f$, L-rh$_p$, gal$\alpha\rightarrow$3ara$_f$, or arab$_p\beta\rightarrow$3arab$_f$

are roughly similar to the hemicelluloses which have β1 → 4-linked D-xylose chains, and L-arabinose, D-glucose, D-galactose, or either of the two acids mentioned above, in the side chains, but there are many differences in detail.

In view of the small amount of information from genera other than Acacia and Prunus, the author has surveyed hitherto unexamined polysaccharide exudates from thirteen plants to find, if possible, a type C gum (as opposed to a hemicellulose) for detailed study; to see if there are major points of difference within a genus, and to see if there are points of similarity when only the order and family are the same.

Elucidation of the structure of the gum exudates from plants is an extremely complex procedure, and it is doubtful if, even with present experimental methods, one can give a better structural formula than Haworth's classical repeating unit, which is merely the statistical distribution and the position of the sugar residues over a certain number of chain units. Sir Edmund Hirst has aptly expressed the position: "The general problems involved in structural determination can be stated quite simply. It is necessary to know for each sugar residue in the macromolecule its mode of combination with its neighbours, whether it is linked glycosidically in the α- or β-form, and its exact position in the molecule. But when it is remembered that a typical polysaccharide may comprise some 10,000 or more sugar residues of various kinds, that on occasions both D- and L- varieties of the same

sugar/....

sugar may be present in the molecule and that pyranose and furanose forms may occur in the same structure, the chemist's task may be a formidable one."[4] Nevertheless, a wealth of information has been obtained for a wide range of polysaccharides by numerous workers; notably Aspinall, Hirst, and co-workers for their studies on gum Ghatti [5,6,7], Khaya grandifolia[8], Khaya senegalensis[9], Anogeissus schimperi[10], gum Tragacanth[11], and Acacia senegal[12], gum exudates from widely differing taxa; Hirst and Jones with their investigations on the polysaccharides exuded from damson[13, 14], cherry[15, 16,17], egg-plum[18,19], peach[20], and almond[21] from the genus Prunus; Smith and his collaborators from their work on Arabic acid[22,23,24,25,26]; and Stephen and colleagues investigating the structures of Acacia mollissima[27], A.cyanophylla[28], A.karroo[29] and Virgilia oroboides[30,31, 32,33,34]. These results in particular lead to the proposal that, as has been known for some time in the field of alkaloid and terpene chemistry, the chemical nature of the constituents of plants (in this case polysaccharide exudates) bears some relationship to the genus of the plant, if not to the order and family as well.

Before the introduction of chromatographic methods into the field of carbohydrate chemistry, the separation of sugars and sugar derivatives was an extremely complex and tedious procedure. Despite this, Smith and his co-workers were able to separate the very complex mixture of methyl ethers from methylated gum arabic using distillation methods[23].

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With the advent of chromatography, the separations have become greatly simplified. It often happens that very similar substances when chromatographed on paper have the same R_{gal} values when run in a variety of chromatographic solvent systems, and it is very likely that one peak on a gas-phase chromatogram does not in fact represent only one component. For this reason it is desirable to characterise all organic substances by classical methods wherever possible.

PART 1

A COMPARATIVE SURVEY OF THE CONSTITUENT SUGARS AND PROPERTIES OF THE POLYSACCHARIDE EXUDATES FROM SOME SOUTH AFRICAN PLANTS

PART 1

DISCUSSION

A comparative survey of the constituent sugars and properties of the polysaccharide exudates from some South African plants.

The polysaccharide exudates studied in this thesis, are from plants which normally grow in South Africa, though not all are necessarily indigenous to this country. This investigation is an attempt to find a chemical relationship between polysaccharide exudates in genera other than Acacia and Prunus, and to compare them with results already found for exudates, in some cases in the same genus, and in others in the same order or family. The author believes that apart from this, the main value of this study will be in the recording for the first time of equivalent weights, specific rotations, the monosaccharides which make up the polymer, and in the tentative assignment of the structure of some biouronic acids and disaccharides released on hydrolysis, of thirteen plant polysaccharide exudates which have not previously been investigated. The number of oligosaccharides obtained on acid hydrolysis gives an indication of the number of different specific linkages in the polysaccharides, presumably enzymatically formed. Thus the more components detected on paper chromatograms, the more information one can obtain about the complex structure of the exudate. Future workers may from these results, obtain a guide to exudates which are worthy of further study.

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It must be strongly emphasized that as the identities of the components released on hydrolysis were assigned solely on the basis of their having the same R_{gal} values as standards on paper chromatography in three solvent systems, the identities are tentative. The molar proportions of the constituents were only estimated visually from paper chromatograms (in the author's experience, this is within ± 20% of the true value), and as such are very approximate.

Wherever possible, the exudates which have been studied, their hydrolysis products, and the molar proportions of the sugar residues obtained on hydrolysis, have been compared with a random selection of published results for exudates from the same order, family, and genus. Whilst this by no means covers all the gum-exudates which have been studied, the ones selected are sufficient to gauge whether any relationship exists between the chemistry of the plant gums and their taxonomic origin. These results are tabulated for clarity (tables 1 → 7). The criteria for classifying an hydrolysis component in this study as acidic , are for it to be mobile when chromatographed in an acidic solvent, but immobile in a neutral or basic solvent. Components found on paper chromatograms (usually in trace amounts) and not identified against standards, are reported only in the experimental section of Part 1.

Key to tables 1 → 7 :

gal = D-galactose, gal A = D- galacturonic acid, gluc A = D-glucuronic acid, arab = L-arabinose, tag = D-tagatose, xylose = D-xylose,

fucose/....

fucose = L-fucose, rham = L-rhamnose, gluc = D-glucose, mann = D-mannose, * denotes a polysaccharide exudate whose chemical properties are reported for the first time in this study (and whose monosaccharide component configurations could be D- or L-), subscripts $_p$ and $_f$ denote pyranose and furanose forms of the sugar residues respectively, tr = trace.

(A) Order - Sapindales. (Table 1.)

In this order, comparison may be made between exudates of the same family only, as all the genera are different. Arabinose and galactose are the main components, though there is no correlation between the amounts of each; 3-O-β-D-galactopyranosyl-D-galactose is a constituent of two of the gums, whilst in Spondias cytheria the unusual hydrolysis product 3-O-α-D-xylopyranosyl-L-arabinose is found. The general pattern which emerges though, is that at the level of the same family and order, but different genera, no comparison of the polysaccharide exudates is possible.

(B) Order - Tiliales. (Table 2.)

There are very many similarities between the exudates in this order; as one might expect, Firmiana simplex being of a different genus from the other four examples, does show marked differences, especially in having arabinose as a component of the exudate. The specific rotations for all the exudates are remarkably close to one another, but the equivalent weights show a small scatter even among the Sterculia

species/....

(A) Order - Sapindales.

Family	Genus	Species	$[\alpha]_D$	equiv.	sugars identified by chromatography
Anacardiaceae	Anacardium	occidentale[35]	-	-	arab, gal, xylose, rham.
Anacardiaceae	Lannea	grandis[36]	-44°	1,150	arab(2), gal(6), gal A(2), $gal_p A \rightarrow 3gal$.
Anacardiaceae	Spondias	cytheria[37,38]	-5°	1,070	arab, gal, xylose, rham(tr), gluc A, fucose, $arab_p \beta \rightarrow 3arab$, 4Me-gluc A, $xylose_p \alpha \rightarrow 3arab$, 4Me-gluc $A\alpha \rightarrow 3arab$, 4Me-gluc $A\alpha \rightarrow 3gal$, $gal_p \beta \rightarrow 3gal$ $gal_p \beta \rightarrow 6gal$.
Anacardiaceae	Mangifera	indica L *	-	-	arab(++), gal(+).
Anacardiaceae	Rhus	viminalis*	$+11^{\circ}$	1,005	arab(+), gal(+), rham(tr), gluc A, $gal_p \beta \rightarrow 3gal$, $gluc_p A \beta \rightarrow 6gal$.

Table 1.

(B) Order - Tiliales.

Family	Genus	Species	$[\alpha]_D$	equiv.	sugars identified by chromatography
Sterculiaceae	Sterculia	setigera[39,40]	+60°	400	gal(5), tag(1), rham(5), gluc A(8), $gal_pA \rightarrow 2rham$, $gal_pA \rightarrow 4gal$.
Sterculiaceae	Sterculia	urens[41]	+64°	470	gal(6), rham(4), $gal_pA \rightarrow 2rham$, $gal_pA \rightarrow 4$ gal.
Sterculiaceae	Sterculia	caudata[42]	+69°	342	gal(1), rham(1), gluc A(2), $gluc_pA \rightarrow 2rham$.
Sterculiaceae	Sterculia	acerifolia*	+72°	271	gal(+), rham(+), gluc A(++), 4Me-gluc A, $gluc_pA\alpha \rightarrow 4gal$.
Sterculiaceae	Firmiana	simplex*	+65°	996	gal(++++), arab(++), rham(+), gal A(+), ($gal_pA\alpha \rightarrow gal$).

Table 2.

species, though when one compares these deviations with those found in Acacia species for the same property, it may be considered as well within normal limits. The unknown biouronic acid from F. simplex could well be 4-O- α -D-galactopyranuronosyl-D-galactose; this acid fragment has been found in both S. setigera and S. urens. An interesting difference is the occurrence of D-glucuronic acid in S. caudata and S. acerifolia; the former has recently been transferred to the genus Brachychiton, and one wonders whether the latter should not be reviewed as well; chemically, apart from this replacement of D-galacturonic acid with D-glucuronic acid, these two species are very similar to other Sterculia species. In this order, the exudates are chemically similar and even when only the family is the same there are many points of similarity. The high content of acid residues, coupled with the presence of galactose and rhamnose as the other major components indicate gums of type B.

(C) Order - Geraniales. (Table 3.)

Pelargonium protectum whilst interesting in have xylose as the only neutral constituent, bears little relationship to other exudates in this order. Apart from the equivalent weight there are few points of similarity; the only neutral sugar present in P. protectum is absent from the other four examples. On the evidence available the biouronic acid found in P. protectum is interesting enough for the polysaccharide exudate to be worth further investigation. This biouronic acid is certainly not 2-O-(4-O-methyl-D-glucopyranuronosyl)-D-xylose, which has

been/....

(C) Order - Geraniales.

Family	Genus	Species	$[\alpha]_D$	equiv.	sugars identified by chromatography
Rutaceae	Citrus	limonia[43]	$+21^0$	785	arab(22), gal(55), gluc A(22), $gluc_pA\ \alpha \rightarrow 4gal$.
Rutaceae	Citrus	maxima[43]	$+56^0$	590	arab(16), gal(53), gluc A(31), $gluc_pA\alpha \rightarrow 4gal$.
Meliaceae	Khaya	grandifolia[8]	$+122^0$	344	gal, rham, gal A, 4Me-gluc A, $gal_pA \rightarrow 2rham$, 4Me-$gluc_pA\ \alpha \rightarrow 4gal$, $gal_pA \rightarrow 2rham \rightarrow 4gal$.
Meliaceae	Khaya	senegalensis[9]	$+140^0$	317	gal, rham, gal A, 4Me-gluc A, $gal_pA \rightarrow 2rham$, 4Me-$gluc_pA\alpha \rightarrow 4gal$.
Geraniaceae	Pelargonium	protectum*	$+22^0$	403	xylose(++++++), gal A or gluc A(+), 4Me-gluc A, a biouronic acid R_{gal} 0.50

Table 3.

(D) Order - Ebenales.

Family	Genus	Species	$[\alpha]_D$	equiv.	sugars identified by chromatography
Sapotaceae	Sapota	achras[44]	-	679	arab, xylose, gluc A, 4Me-gluc A.
Ebenaceae	Euclea	natalensis*	-27^0	760	arab(+), gal(++), rham(+), gluc A, 4Me-gluc A, a biouronic acid R_{gal} 0.69.

Table 4.

been reported to have an R_{xylose} value of 0.79 in solvent b[42a], and it is probably different from 2- and 4-O-D-glucopyranuronosyl-D-xylose which have been reported as having the same R_{xylose} value of 0,32 in solvent b[42b]. Polysaccharide exudates of Khaya species from the order Geraniales, have been shown to be type B gums[3], but P.protectum would appear to be much more closely allied to a type C polysaccharide, possibly with D-galacturonic or D-glucuronic acid replacing α-D-xylose or β-L-arabinose as terminal unit.

(D) Order - Ebenales. (Table 4.)

Here,the order of the two plants from which the exudates were obtained is the same, but even with different family and genus one finds points of similarity between the gums, notably, the equivalent weights, and the presence of both glucuronic and 4-O-methylglucuronic acids as components. These two exudates from the order Ebenales illustrate the danger of using only the equivalent weight and some of the monosaccharide residues to draw conclusions of structure; Sapota achras has been shown to consist of a xylose backbone substituted with side chains; however the absence of xylose in Euclea natalensis precludes this type of structure. Although there are marked similarities between these two exudates, they can hardly be at all alike in structure.

(E) Order - Leguminales. (Table 5.)

The Acacia species and their chemical taxonomy have been amply discussed/....

(E) Order - Leguminales.

Family	Genus	Species	$[\alpha]_D$	equiv.	sugars identified by chromatography
Leguminosae	Acacia	cyanophylla[28]	-20°	740	arab(7), gal(50), rham(18), gluc A(25), $gal_p\alpha \rightarrow 3arab$, $gluc_pA\beta \rightarrow 6gal$.
Leguminosae	Acacia	karroo[29]	+54°	1,660	arab(36), gal(50), rham(2), gluc A(12), $gal_p\alpha \rightarrow 3arab$, $gluc_pA\beta \rightarrow 6gal$, $arab_p\beta \rightarrow 3arab$, $gluc_pA\ \alpha \rightarrow 4gal$.
Leguminosae	Acacia	mollissima[27]	-49°	1,800	arab(46), gal(38), rham(8), gluc A(8), $gluc_pA\ \beta \rightarrow 6gal$.
Leguminosae	Acacia	pycnantha[45,46]	-8°	3,700	arab(27), gal(65), rham(2), gluc A(5), $gal_p\beta \rightarrow 3gal$, $arab_f \rightarrow 3arab$, $gluc_pA\ \beta \rightarrow 6gal$.
Leguminosae	Acacia	catechu[47]	-	-	arab, gal, rham, $gluc_pA\beta \rightarrow 6gal$.
Leguminosae	Acacia	seyal[48]	+44°	1,500	arab(46), gal(38), rham(3), gluc A(12.5).
Leguminosae	Acacia	senegal[22,23,24]	-28°	1,400	arab(30), gal(37), rham(11), gluc A(14), $gal_p \rightarrow 3gal$, $gal_p\alpha \rightarrow 3arab$, $arab_p\beta \rightarrow 3arab$, $^pgluc_pA\beta \rightarrow 6gal$.
Leguminosae	Acacia	rhemaniana[49]	+51°	1,420	arab(6), gal(4), rham(0.8), gluc A, $arab_p\ \beta \rightarrow 3arab$, $gluc_pA\ \beta \rightarrow 6gal$, $gluc_pA\ \alpha \rightarrow 4gal$.
Leguminosae	Acacia	arabica[49]	+43°	1,310	arab(6), gal(4), rham(1), gluc A, $arab_p\beta \rightarrow 3arab$, $gluc_pA\beta \rightarrow 6gal$, $gluc_pA\ \alpha \rightarrow 4gal$.
Leguminosae	Acacia	sundra[50]	-29°	980	arab, gal, rham, gluc A, $gluc_pA\ \beta \rightarrow 6gal$.

Table 5.

(E) Leguminales cont.

Family	Genus	Species	$[\alpha]_D$	equiv.	sugars identified by chromatography
Leguminosae	Albizzia	zygia[51]	+21°	723	arab(6), gal(4), mann(1.5), rham(tr), gluc A(4), 4Me-gluc A(2), $gal_p \rightarrow 3gal$, $gal_p \rightarrow 6gal$, $gal_p\beta \rightarrow 3arab$, $gluc_pA\beta \rightarrow 2mann$, $4Me\text{-}gluc_pA\alpha_p \rightarrow 4\ gal.$
Leguminosae	Albizzia	procera[52]	+15°	-	arab(4), gal(6), rham(1), mann(tr), gluc A, 4Me-gluc A.
Leguminosae	Albizzia	glaberrima[53]	-	-	arab, gal, rham, mann, gluc A, 4Me-gluc A, $gluc_pA \rightarrow$ mann, $gluc_pA \rightarrow$ gal.
Leguminosae	Albizzia	flatcrown*†	-14°	865	arab(++++), gal(++++), mann(+++), xylose, 4Me-gluc A,(+), $gluc_pA\beta \rightarrow 2mann$, $gluc_pA\alpha \rightarrow 4gal$, $gal_p \rightarrow 3arab$.
Leguminosae	Prosopsis	juliflora D.C.[54]	+60°	1,350	arab, gal, 4Me-gluc A, $4Me\text{-}gluc_pA \rightarrow 4gal$, $4Me\text{-}gluc_pA \rightarrow 6gal$, $4Me\text{-}gluc_pA_p \rightarrow 3gal$.
Leguminosae	Virgilia	divaricata[55]	-29°	2,600	arab, gal, mann, gluc A.
Leguminosae	Virgilia	oroboides[31]	-38°	2,100	arab(48), gal(38), mann, gluc A(9), $gal_p \rightarrow 6gal$, $gal_p \rightarrow 6gal_p \rightarrow 3gal$, $arab_p\alpha \rightarrow 5arab\alpha_p \rightarrow 5arab_p$, $arab_f\alpha \rightarrow 3arab$, $gluc_pA\ \beta \rightarrow 2mann$, $gluc_pA\ \beta \rightarrow 6gal$, $4Me\text{-}gluc_pA \rightarrow gal$.

Table 5.(cont.)

† The author has been unable to obtain the correct botanical classification for this species; the exudate was obtained from a tree growing near Pietermaritzburg in Natal, which is known locally as Albizzia flatcrown .

discussed in the literature, but because they are probably the best examples which can be used to illustrate the chemical taxonomic correlation of the polysaccharide exudate with the species of origin, they will be dealt with in more detail here than species in other orders.

The order Leguminales, family Leguminosae, contains four genera from which polysaccharide exudates have been studied, table 5 shows the constants and components found for ten Acacia species, four Albizzia species, one exudate from the genus Prosopsis, and two Virgilia species.

It was thought at one stage that polysaccharides from the Acacias normally had negative specific rotations, but with more recent studies (shown in the table) this general observation has not been found to hold. However, in the constituent monosaccharides and disaccharides released on varying degrees of hydrolysis, all Acacia gums show a remarkable correlation. D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid are the only monosaccharides found in these polysaccharides, although there is a wide variation in the relative amounts of galactose and arabinose. Wherever a biouronic acid has been found in the hydrolysates, it is always 6-O-β-D-glucopyranuronosyl-D-galactose, though in Acacia karroo, Acacia arabica, and Acacia rhemaniana 4-O-α-D-glucopyranuronosyl-D-galactose occurs as well; the α-link in this biouronic acid in these three exudates contributes to the positive rotation of the polysaccharides as a whole The disaccharides 3-

and/.....

and 6-O-β-D-galactopyranosyl-D-galactose have been isolated, both being fragments of the backbone of the polymer, together with 3-O-α-D-galactopyranosyl-L-arabinose, 3-O-β-L-arabinopyranosyl-L-arabinose, and 3-O-L-arabinofuranosyl-L-arabinose, all fragments from the side chains.

Exudates from Albizzia species however show differences from those of Acacia species; all have 4-O-methyl-D-glucuronic acid as an acidic component in addition to the D-glucuronic acid found in Acacia gums and generally have lower equivalent weights. In the new species studied, rhamnose is absent whilst xylose appears as a constituent sugar. This is not common to other Albizzia gums, but the presence of mannose is; and all four exudates studied have mannose as a major component. Unlike Acacia gums, Albizzia gums all have 2-O-β-D-glucopyranuronosyl-D-mannose as a biouronic acid, as well as 4-O-α-D-glucopyranuronosyl-D-galactose in Albizzia "flatcrown", and 4-O-α-(4-O-methyl-D-glucuronosyl)-D-galactose in Albizzia zygia. It is probable that further investigation of Albizzia procera and Albizzia glaberrima will show 4-O-methyl-D-glucuronic acid to be the acid fragment of a biouronic acid. The backbone fragments though, appear to be the same as those in Acacia exudates but the galactose →3arabinose disaccharide whilst still found as a fragment from the side chains is now β-linked instead of α. The Prosopsis gum reported in the literature is very similar to those from Albizzia though in this case mannose is again absent from hydrolysates of the gum.

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The two reported Virgilia species again show slight changes from the Albizzia pattern; though still maintaining the basic 3- or 6-linked galactose structure common to all exudates from the family Leguminosae, Virgilia oroboides has xylose as a component sugar; and 3-O -L-arabinopyranosyl-L-arabinose appears as a new structural feature in the side chain. The biouronic acids isolated include the 6-O- β -D-glucopyranuronosyl-D-galactose common to Acacia species, and 2-O- β -D-glucopyranuronosyl-D-mannose common to the genus Albizzia .

The various structural units found in the Leguminosae have an interesting reflection in the closely related Rosaceae exudates. In this family, xylose, which appeared as a component sugar in Virgilia gums, becomes a common fragment of the hydrolysis, whilst the backbone remains the same as in gums from the Leguminosae. D-glucuronic acid reappears as the normal acid component of the gums, whilst none of the exudates contain 4-O-methyl-D-glucuronic acid, the D-glucuronic acid is now linked almost exclusively to $C_{(2)}$ of mannose (as in exudates from Albizzia species) where D-mannose is a constituent sugar; and to $C_{(6)}$ of D-galactose where no mannose is found in the gum. The appearance of L-rhamnose in a Prunus gum[20], is another link with the Acacia exudates.

Whilst all the gums exuded by plants from the families Leguminosae and Rosaceae contain similar backbone structures and are all type A gums, the nature of the side chains would appear to be genus specific;

very/....

very close correlation exists between the structures of the exudates within a genus. Chemically, no distinction can be drawn between the two families, e.g. the gum exudates from Albizzia species appear more different from those of Acacia species than from the various species of Prunus.

(F) Order - Liliales (Table 6.)

Phormium tenax produces a typical type C polysaccharide exudate with a xylose backbone, and 4-O-methylglucuronic acid as side chains. As will be shown later (part 2 of this thesis), Watsonia pyramidata gum (which occurs inside the corms and not as an exudate) also has the same type of main chain, but the rest of the molecule differs greatly from the normal type C polysaccharide. Between the two Watsonia species there are many points of similarity; both have the same constituent neutral monosaccharides, and similar specific rotations, but the exudate from the seed-boxes of W.versveldii has an uronic acid as part of its structure. It is more than likely that this difference is determined by the site in the plant where the gum forms rather than the fact that the species are different. It would be interesting to compare the corm polysaccharides from the two species, and, if they are the same or similar, to examine the hemicellulose which makes up the stem of W.pyramidata, and the seed-box exudate from the same species in an endeavour to find a connection between the three polysaccharides. The detection of xylobiose and xylotriose in the hydrolysis products of W.pyramidata corm-sac polysaccharide lead the author to classify it as

a type/....

(F) Order - Liliales.

Family	Genus	Species	$[\alpha]_D$	equiv.	sugars identified by chromatography
Liliaceae	Phormium	tenax[56]	-63°	880	xylose(45), gluc A(42).
Iridaceae	Watsonia	versveldii*	-105°	2050	arab(+++++), gal(++), xylose(++++), a biouronic acid R_{gal} 0.45.
Iridaceae	Watsonia	pyramidata*	-80°	ca 20,000	arab(++), gal(+), xylose(+), gal_p α→ 3arab, xylobiose, xylotriose.

Table 6.

(G) Order - various.

Order	Family	Genus	Species	$[\alpha]_D$	equiv.	sugars identified by chromatography
Gymnospermales	Cycadaceae	Encephalartos	latefrons*	-13°	577	arab(+), gal(++++), mann(tr), xylose(tr), gluc A, $gluc_p$Aβ → 6gal, 3Me-rham, $gluc_p$Aβ → 2mann.
Agavales	Agavaceae	Agave	(americana* (var. marginata)	-35°	1,142	arab(++), gal(++), rham(+), $gluc_p$A β → 6gal.
Palmales	Phoniceae	Phoenix	reclinata*	-33°	646	arab(++), gal(+++), mann(tr), rham(+), fucose, gluc A, $gluc_p$A α → 4gal.
Bignoniales	Pedaliaceae	Sigmatosiphon	gurichii*	-35°	1,393	gal(+++), arab(++), mann, fucose, gal_p → 3arab, $gluc_p$A β → 2mann.

Table 7.

a type C polysaccharide, and to attempt to determine its fine structure (see part 2.)

From results already published, there is evidence that the chemistry of the plant polysaccharide exudates shows a correlation with the genus of the plant from which they originated. The work reported here, for genera where fewer results were available, confirms that there appears to be a very close connection when only the genus is considered. However one must be careful in the interpretation of data where only monosaccharide components and physical data are being considered. It is important in a study of this nature to choose hydrolysis conditions such that the maximum number of di- and trisaccharides and biouronic acid fragments are obtained; only then can one begin to obtain evidence that two polysaccharides are structurally similar.

Table 7 lists the four exudates from orders which have not previously been studied; there is evidence from methylation studies in this laboratory however to show that Agave americana is structurally similar to the Acacia species[57]. The only other points of interest in these four exudates, are the presence of fucose (a sugar not often found in the polysaccharide exudates from higher plants) as a component of Phoenix reclinata and Sigmatosiphon gurichii, and the presence of 3-O-methylrhamnose in the exudate from Encephalartos latefrons. This last component has also been observed in Encephalartos longifolius[58], another species of the primative Cycads.

PART 1

EXPERIMENTAL

EXPERIMENTAL

(A) General conditions. The following solvent systems were used for paper chromatography on Whatman No. 1 paper (all v/v).

(a) butan-1-ol - ethanol - water (4:1:5, upper layer),

(b) ethyl acetate - acetic acid - formic acid - water (18:3:1:4),

(c) ethyl acetate - pyridine - water (10:4:3). The polysaccharides were purified by dissolving in water, filtering, and precipitating in four volumes of ethanol; the precipitates were washed with acetone and ether and dried for 2 hr. at 76° and 2 cm.Hg. A recent paper[59] has stressed the difficulty of completely removing solvents from polysaccharides without concomitant degradation. In view of this, drying at 76° will not afford a completely solvent free product, and this will affect the specific rotation and equivalent weight to a small extent; the specific rotations are probably slightly higher than determined experimentally, and the equivalent weights slightly lower. The equivalent weights were measured by percolating a solution of the neutral polysaccharide in water through Amberlite IR-120 ($H^{\oplus}$), freeze-drying the eluate, and titrating the freeze-dried material in water with 0.02N-sodium hydroxide using phenolphthalein as indicator. Unless otherwise indicated, specific rotations were measured in water on the neutral ethanol precipitated polysaccharide, and hydrolyses were in sulphuric acid at 96°. Where a component has been named, the identification has been made by running chromatograms in the three solvent

systems/...

systems alongside sugars, available in this laboratory, as standards. The approximate molar proportions of sugar residues in the hydrolysates were estimated visually from the chromatograms; in all cases the spray reagent used to reveal the sugars was p-anisidine hydrochloride[60]. Only the major components observed on the paper chromatograms and trace quantities of components tentatively identified are recorded in the main body of the experimental results for each gum. The number of plus signs following the name of a component is a visual estimate of the approximate molar proportion of that component. Components in trace amounts which were not tentatively identified are added at the end of each section (as $\underline{R}_{gal}$ values) under the heading of "other components".

(B) Specific rotation, equivalent weight, and major hydrolysis products of some plant gums.

(a) Mangifera indica L. (Sapindales; Anacardiaceae).

Hydrolysis of the purified gum afforded galactose (++) and arabinose (+), the small quantity of sample available was insufficient to determine the equivalent weight and specific rotation. No uronic acid was observed on the chromatogram, but this could have been due to the small amount of material available for hydrolysis.

Rhus/....

(b) Rhus viminalis (Sapindales; Anacardiaceae).

The purified gum had $[\alpha]_D$ +11° (c 1.6) and equiv., 1005. Hydrolysis in 0.1N-acid yielded galactose (+), arabinose (+), rhamnose (trace), 3-O- β -D-galactopyranosyl-D-galactose (trace), 6-O- β -D-glucopyranuronosyl-D-galactose (trace), and glucuronic acid.

Other components; 0.55 (in solvent b)

(c) Agave americana var. marginata (Agavales; Agavaceae).

The purified gum on hydrolysis for 5 hr. in N-acid afforded galactose (++), arabinose (++), rhamnose (+), and 6-O- β -D-glucopyranuronosyl-D-galactose (+), and had $[\alpha]_D$ -35° (c 1.95) and equiv., 1142.

Other components; 0.18 (in solvent a)

(d) Sterculia acerifolia (Tiliales; Sterculiaceae).

The ethanol precipitated gum, $[\alpha]_D$ +72° (c 0.59), equiv., 271, was hydrolysed for 12 hr. in N-acid, yielding galactose (++++), rhamnose (++++), D-glucuronic acid (++++++++), 4-O- α -D-glucopyranuronosyl-D-galactose (+++), 4-O-methylglucuronic acid (+), and possibly 3-O- β -D-galactopyranosyl-D-galactose, though it is unlikely that this would survive the above hydrolysis conditions.

Other components; 0.16, 0.58 (+) (in solvent b)

Firmiana/.....

(e) Firmiana simplex (Tiliales; Sterculiaceae).

The purified gum had $[\alpha]_D$ +65° (c 0.5) and equiv., 996. Hydrolysis for 3 hr. in N-acid afforded galactose (++++), arabinose (++), rhamnose (+), galacturonic acid (+), and a biouronic acid R_{gal} 0.49 (in solvent b) (++++) immobile in solvent a, and different from 6-O-β - and 4-O-α -D-glucopyranuronosyl-D-galactose in solvent b, though with a similar colour to 4-O-α -D-glucopyranuronosyl-D-galactose under ultra-violet light. A portion of the ethanol precipitate (200 mg.) was dissolved in N-sulphuric acid (14 ml.) and hydrolysed for 1.5 hr. at 96°. The hydrolysate was chromatographed on Whatman 3 mm. paper in solvent b and the component R_{gal} 0.49 was located and separated in the usual manner. On evaporation of the solution to dryness (for 2 hr. at 50° and 2 cm. Hg), a syrup (3.2 mg.) was obtained $[\alpha]_D$ +107° (c 0.64). Hydrolysis of this syrup (1.2 mg.) for 3 hr. in N-sulphuric acid at 96°, neutralization of the hydrolysate and paper chromatography, gave galactose, and (probably) galacturonic acid (in solvents a, b, and c). These results indicate that the most probable structure of the biouronic acid is galacturonic acid α → galactose (α because of the high positive rotation).

Other components: 0.35, 0.61 (in solvent b)

(f) Phoenix reclinata (Palmales; Phoniceae).

The purified gum $[\alpha]_D$ - 33° (c 0.54), equiv., 646, was hydrolysed for 3 hr. in N-acid, the hydrolysate yielding galactose (++++++), arabinose (++++), mannose (trace), rhamnose (++), fucose (+),

4-/.....

4-O- α -D-glucopyranuronosyl-D-galactose (+), and D-glucuronic acid (trace).
Other components; 0.43 (in solvent a); 0.14, 0.21, 0.27, 0.32, 0.54, 0.66, 0.79, 1.35 (in solvent b).

(g) Sigmatosiphon gurichii (Bignoniales; Pedaliaceae).

The ethanol precipitate $[\alpha]_D$ -35° (c 1.1), equiv., 1393, was hydrolysed for 24 hr. in 0.1N-acid and gave galactose (++++++), arabinose (++++), mannose (+), fucose, 3-O-D-galactopyranosyl-L-arabinose (trace), and 2-O- β -D-glucopyranuronosyl-D-mannose (++).
Other components; nil.

(h) Pelargonium protectum (Geraniales; Geraniaceae).

The purified gum had $[\alpha]_D$ +22° (c 1.02) and equiv., 403. Hydrolysis for 32 hr. in 0.1N-acid afforded xylose (+++) as the only non-acidic component, galacturonic acid or glucuronic acid (+), 4-O-methylglucuronic acid, and a component staining red on the chromatogram, having an R_{gal} value of 0.63 in solvent b, R_{xylose} 0.43 in the same solvent and being immobile in solvents a and c; this component was present to the extent of approximately two molar proportions, and was probably a biouronic acid with xylose as the reducing unit.
Other components; 0.14, 0.32 (in solvent b).

(i) Euclea natalensis (Ebenales; Ebenaceae).

The purified gum $[\alpha]_D$ -27° (c 1.04), equiv., 760 was hydrolysed for/....

for 6 hr. in N-acid giving galactose (++++), arabinose (++), rhamnose (++), 4-O-methylglucuronic acid (+), glucuronic acid, a neutral component R_{gal} 2.04 (in solvent a), and an acidic component staining red on the chromatogram and having R_{gal} 0.69 in solvent b.
Other components; 0.46 (in solvent a); 0.15, 0.25, (in solvent b)

(j) Albizzia "flatcrown" (Leguminales; Leguminosae)

The ethanol precipitated polysaccharide had $[\alpha]_D$ -14° (c 0.5) and equiv., 865. Hydrolysis of the polysaccharide for 6 hr. in N-acid yielded galactose (++++), arabinose (++++), mannose (+++), rhamnose (+), xylose, 4-O-methylglucuronic acid (+), 4-O-α-D-glucopyranuronosyl-D-galactose, 2-O-β-D-glucopyranuronosyl-D-mannose, a component R_{gal} 1.83 in solvent a (staining red), and 3-O-D-galactopyranosyl-L-arabinose (trace).
Other components; 2.72 (in solvent a); 0.25 (in solvent b).

(k) Encephalartos latefrons, (Gymnospermales; Cycadaceae).

The purified gum $[\alpha]_D$ -13° (c 1.51), equiv., 577, was hydrolysed for 20 hr. in 0.5N-acid giving galactose (++++), arabinose (+), mannose (trace), xylose (trace), 6-O-β-D-glucopyranuronosyl-D-galactose (+), 2-O-β-D-glucopyranuronosyl-D-mannose (trace), and 3-O-methylrhamnose.
Other components; nil

(l) Watsonia versveldii (Liliales; Iridaeae).

The ethanol precipitated gum from the polysaccharide found in

the/....

the seed-boxes of the above plant had $[\alpha]_D$ -105° (c 0.91) and equiv., 2050. Hydrolysis of the polysaccharide for 18 hr. in N-acid gave galactose (++), arabinose (+++++), xylose (++++), and a biouronic acid R_{gal} 0.45 (in solvent b) (++), and different from 6-O-β-D-glucopyranuronosyl-D-galactose, 4-O-α-D-glucopyranuronosyl-D-galactose, and 2-O-β-D-glucopyranuronosyl-D-mannose in solvent b. Other components; nil

(m) Watsonia pyramidata (Liliales; Iridaceae).

The ethanol precipitated polysaccharide from the gum found in the corm-sacs had $[\alpha]_D$ -80° (c 0.4) and equiv., ca. 20,000. Hydrolysis of the precipitate for 6 hr. in 0.1N-acid afforded galactose (++), arabinose (++++), xylose (++) and components chromatographically identical (in solvents a, b, and c) with 3-O-α-D-galactopyranosyl-L-arabinose, xylobiose, and xylotriose. The chromatograms showed no trace of acidic components, either as free acid, or as biouronic acids.

Apart from the above polysaccharides, exudates from the following plants were investigated but found to be non-polysaccharide in character:

(i) Pittosporales Pittosporaceae Pittasporum undulatum

(ii) Pittosporales Pittosporaceae Pittasporum tobira

(iii) Asterales Compositae Othonna merielpad

(iv) Asterales Compositae Othonna hovia

(v).....

(v) Asterales Compositae Othonna hertia

(vi) Asterales Compositae Euryops spathaceus

(vii) Liliales Liliaceae Sansevieria zevlanica

The last mentioned exudate was found to contain a mixture of glucose, sucrose, and fructose probably exuded by a natural mechanism for losing excess sugar.

PART 2

THE CHEMISTRY OF WATSONIA CORM POLYSACCHARIDE

PART 2

DISCUSSION

DISCUSSION

Many different polysaccharides have been evolved by plants; as the structural building materials, as reserve food materials, and as gum exudates from the higher plants. The first group, the building materials, consists of two closely related types of polysaccharide, the hemicelluloses and cellulose. Both are normally found closely associated with lignin, possibly covalently bonded[61]. The second group, the reserve food materials, consists mainly of starch, whilst the third group, the gum exudates, would appear to be produced in order to seal off wounds on the exterior of the plant to prevent infection, and to allow new healthy tissue to be built up under the site of the injury. Part 1 of this thesis has dealt with a cross-section of this last type of polysaccharide. It is immediately noticeable that whereas there are many possible hexose and pentose sugars only a few of these are used in building up the polysaccharides associated with land-based plants. In these polysaccharides one normally finds two or more of the sugars L-rhamnose, D-galactose, L-arabinose, D-glucose, D-xylose, D-glucuronic acid, D-galacturonic acid, 4-O-methylglucuronic acid, and D-mannose, with more rarely D-tagatose (in exudates from Sterculia species), L-fucose (in gum tragacanth[11]), and L-galactose (in maize-hull hemicellulose[62] and linseed mucilage[63]).

One of the largest groups of plant polysaccharides are the hemi-

celluloses/....

celluloses, so called because of their close association with cellulose in nature. Often the term has been applied only to polysaccharides which are insoluble in water but soluble in alkaline reagents; this however, ignores the similarity in chemical structure between water soluble and water insoluble hemicelluloses. This is possibly not the best way of classifying these polysaccharides. A classification similar to that used for gum exudates, where the types and positions of the sugar residues and their mode of linkage are taken into account would seem preferable. The hemicelluloses are part of the fibrous cell-walls of the plants and as such form one of the basic building units of the stem. Structurally they may be classified into four main types based on basal chains of mannose, galactose, glucose, and xylose.

Hemicelluloses containing more than 95% mannose are rare, and in fact the only substantiated cases are the two mannans from Phytelephas macrocarpa (vegetable ivory)[64]. These two polysaccharides named mannan A and mannan B appear to be chemically identical, but to have different molecular weights. Hence if one considers the chains to be linear, methylation end-group assay studies have shown mannan A to be of the order of 10 - 13 units long, and mannan B 38 - 40 units long[65]. A closely related group of hemicelluloses are the glucomannans, which form about half of the hemicellulose fractions from coniferous woods. The preferred structure would appear to be a linear chain of $\beta \rightarrow 4$-linked mannose residues

interrupted/....

interrupted occasionally by 4-linked β -D-glucopyranose; there is evidence to suggest that the glucomannans from Norwegian spruce are branched[66].

With the galactans and arabinogalactans however, the linear chain appears to be the exception. The only linear chain polysaccharides known in this group are both composed of β → 4-linked galactose only, which is in itself unusual, as where the backbone is composed of galactose residues they are normally linked β → 6 or β → 3. The water soluble arabinogalactan isolated from larch wood has been well investigated. As early as 1898 Trimble[67] reported its existence and numerous workers have followed up this report through the difficulties arising from the heterogeneity of the polysaccharide and methylation, to the proposal of its structure by White in 1942[68]; since then many reports using results obtained by more modern techniques have been published modifying this structure. Even now, 67 years after the initial steps were taken, it is doubtful whether the structure of the repeating unit has been finally elucidated. This then is a good example of the arabinogalactan, a highly-branched polysaccharide with a backbone of β → 6 and β → 3-linked galactose and arabinofuranose residues linked directly to the galactose. In some cases these arabinofuranose residues in turn are linked through $C_{(3)}$ to the glycosidic carbon of an arabinopyranose terminal unit. It is worth noting that, apart from the β →4-linked galactans[69,70], no

galactans/.....

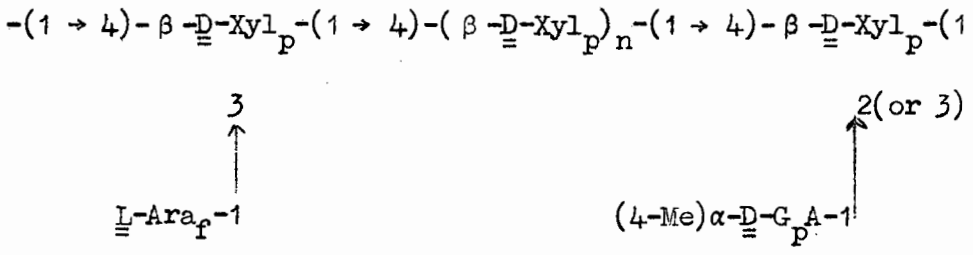
galactans have been isolated which do not contain arabinose.

The glucans which occur in the cell-walls of plants are normally chains of β → 4-linked glucose units, though in some cases β → 3-linkages do occur in the chain. On methylation and hydrolysis barley β-glucan gives equal proportions of 2,3,6- and 2,4,6,-tri-O-methyl-glucose, and it appears that in this polysaccharide β → 4 and β → 3-linkages are present in equal amounts[71]. Part of the water soluble polysaccharide found in the grain of cereals is usually a glucan, but apart from these polysaccharides, glucans are normally found in the lower forms of plant life such as lichens and seaweeds.

The last large class of hemicelluloses is that in which the basal chain of the polysaccharide consists of β → 4-linked xylose residues. In this group a polysaccharide extracted from esparto-grass[72] and another from Tamarind seeds[73] are unique in that they contain no sugar residues other than xylose. Most of the xylans, whilst having many branch-points, do not carry side chains more than three sugar units long and do not include xylose residues in these side chains, thus giving a molecule with an unbranched xylose backbone. An authentic branching of the xylose backbone has been shown to occur in wheat-straw hemicellulose, which, when autoclaved in distilled water, gave a branched xylose oligosaccharide[74]. These points illustrate one of the main difficulties encountered when dealing with hemicellulose polysaccharides;the lack of homogeneity of the polysaccharide extracted

from/.....

from the parent material. The xylan and the arabinoxylan from esparto-grass were finally separated only be repeated fractionation as the copper complex[72] under very mild conditions. The general picture which has emerged of the xylans from land-based plants is that of a molecule always having a basal chain of β → 4-linked xylose residues linked together linearly, though in one case the basal chain is branched. Attached to these basal chains are short side-chains containing up to three residues; these side chains have been shown to contain L-arabinose, L- or D-galactose, D-glucuronic acid, and 4-O-methyl-D-glucuronic acid as an integral part of the molecule. The simplest type of xylan, is that in which the arabinose residues are present as single unit, terminal arabinofuranose side chains. This type of structure is common amongst the xylans isolated from cereals and grasses. These same xylans however, do on occasion have D-glucuronic acid or 4-O-methyl-D-glucuronic acid attached as side chains. Structurally the xylans with single unit side chains may be represented by the partial structure I[75], and this is typical of the simpler type of xylan molecule.



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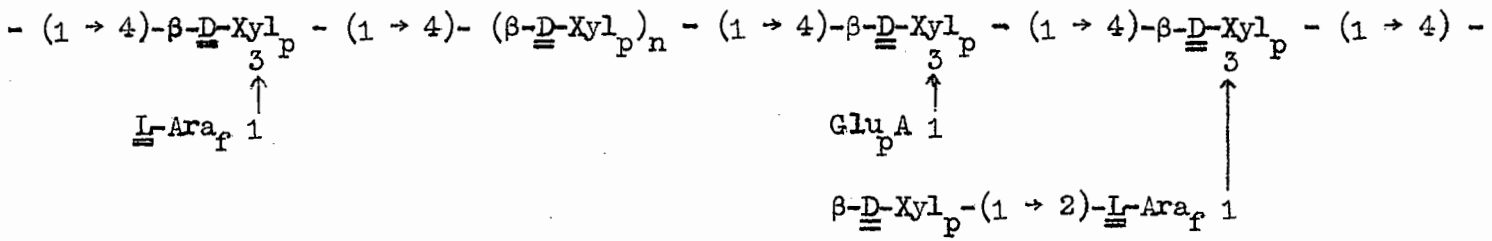

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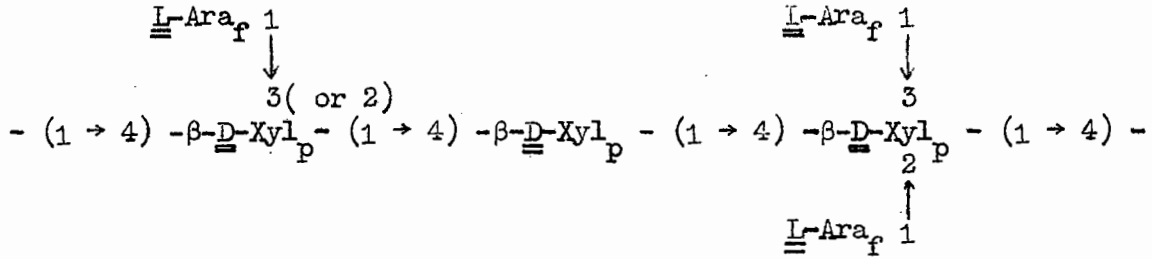
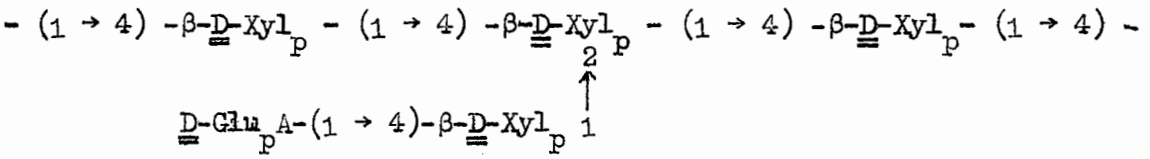


III

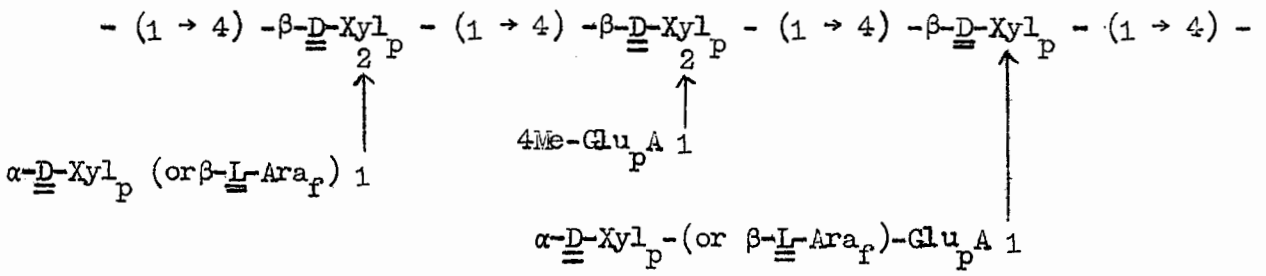
In those parts of the cereal plants associated with the grain (i.e. cobs, husks, and hulls), the xylans tend to be more complex. One of the least complex of this group is the xylan found in barley husks[76] where not all the side chains are single units of arabinofuranose or glucuronic acid but are occasionally 2-O-β-D-xylopyranosyl-L-arabinofuranose linked to $C_{(3)}$ of a xylose residue in the basal chain. The partial structure II illustrates this type of xylan. More complex is the hemicellulose from maize-hulls. Both D- and L-galactose occur as terminal non-reducing end-groups[77], the L-galactose being linked through xylose and arabinose to the main backbone of the molecule in the form of the trisaccharide unit O-L-galactopyranosyl-(1 → 4)-D-xylopyranosyl-(1 → 2)-L-arabinose. As in previous examples, the glucuronic acid residues are found as single unit side chains. This group is characterised by its multiplicity of side chain structures but there is no evidence to suppose that one xylose residue in the basal chain has more than one side chain attached to it.

In the gums extracted from the grain of cereals, L-arabinofuranose is found as single unit side chains but, in this instance, xylose residues in the basal chain are found with both $C_{(2)}$ and $C_{(3)}$ of the xylose substituted with terminal arabinofuranose, though substitution on only $C_{(3)}$ still occurs. Whilst these molecules are simpler than the preceding type, they are evidence of a new type of xylan structure III[75].

The/....


- (1 → 4) -β-D-Xyl$_p$ - (1 → 4) -β-D-Xyl$_p$ - (1 → 4) -β-D-Xyl$_p$ - (1 → 4) -
2
D-Glu$_p$A-(1 → 4)-β-D-Xyl$_p$ 1

IV


- (1 → 4) -β-D-Xyl$_p$ - (1 → 4) -β-D-Xyl$_p$ - (1 → 4) -β-D-Xyl$_p$ - (1 → 4) -
2 2
α-D-Xyl$_p$ (or β-L-Ara$_f$) 1
4Me-Glu$_p$A 1
α-D-Xyl$_p$-(or β-L-Ara$_f$)-Glu$_p$A 1

V

The xylans from hard and soft woods, whilst having the normal xylose backbone, only occasionally have arabinofuranose in the side chain but the main difference is that all have single unit side chains of 4-O-methyl-D-glucuronic acid.

There are two examples amongst the plant exudates which have been examined of polysaccharides with a basal chain of β → 4-linked xylose residues. They are the gum exudates from Phormium tenax[78] for which partial structure IV has been proposed, and from Sapota achras[44] (structure V), both of which have been classified by Stephen and Schelpe[3] as Type C gums.

On considering the different types of xylan from land-based plants discussed in the literature, marked similarities emerge which may be stated as:

(a) in the basal chain the xylose is always linked β → 4,

(b) arabinose always occurs as arabinofuranose, whether as the terminal unit in a side chain or as a non-terminal side chain unit,

(c) the basal chain of most xylans is unbranched,

(d) when galactose occurs it occurs as non-reducing end group,

(e) neutral xylans are limited to the cereal gums,

(f) because of the predominance of β -D-xylose linkages most xylans have high negative specific rotations,

(g) if arabinofuranose occurs in the side chain linked directly to the xylose backbone and as a non-terminal unit, it is often linked through $C_{(2)}$ to the glycosidic carbon of the next unit in the side chain.

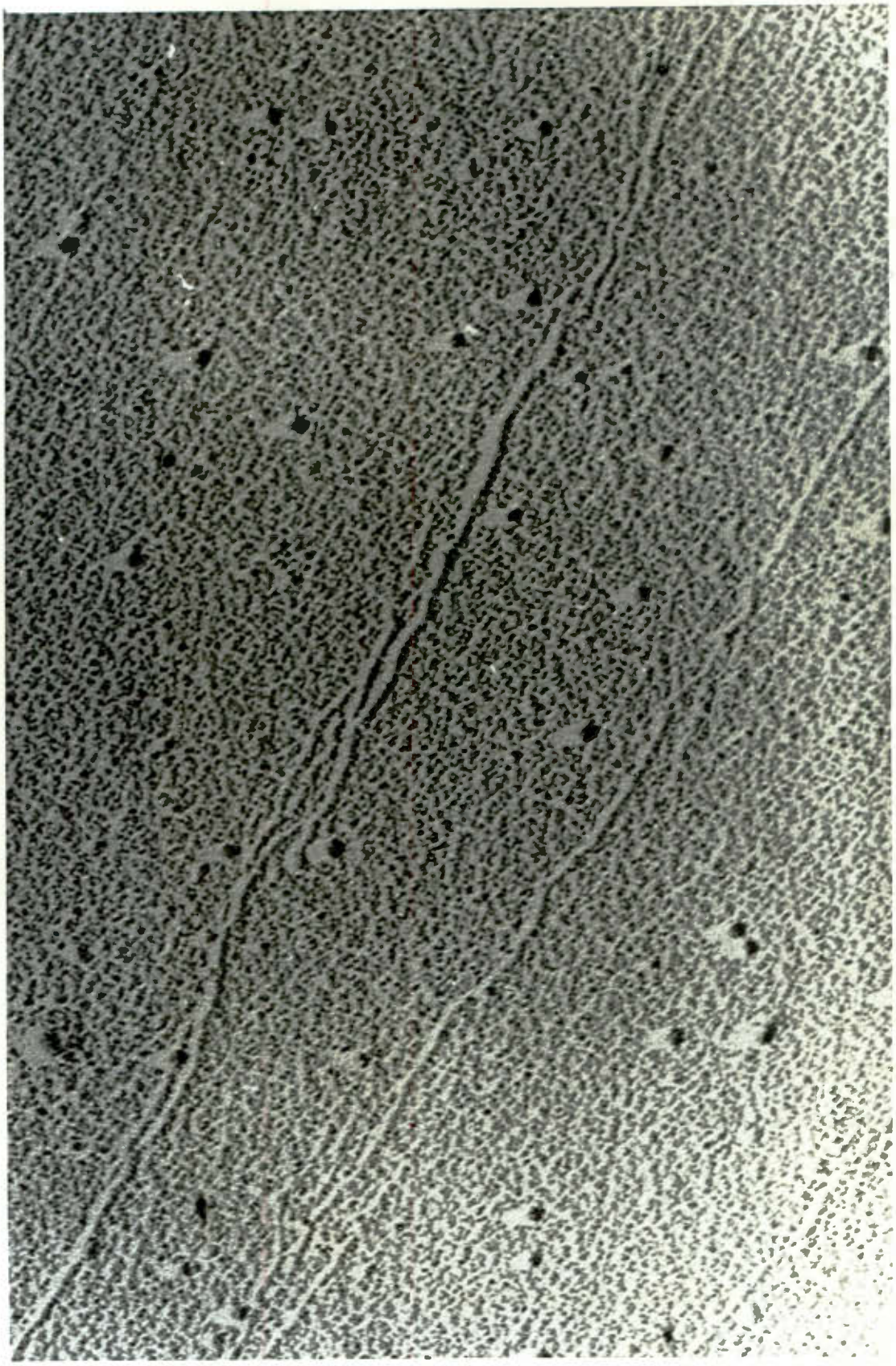
In/...

In the present research the gum from Watsonia pyramidata (Andr.) Stapf has been investigated in detail. Corms from the flowering plant Watsonia pyramidata (Andr.) Stapf were collected during March 1963, and March 1964. The plants had been growing wild for many years without annual division of the corms although, in South Africa, the Watsonia species are also grown as cultivated plants. Inside the corms of these Watsonias a polysaccharide was occluded in sacs running transversely through the corm. By studying the corm through a full year's cycle it was established that the gum was at a maximum in February or March (late summer) just prior to the beginning of new stem growth, the amount of gum falling off rapidly as the new stem was built up. By May the corm-sacs had become very small, and they contained no gum. With the formation of new corms in the ensuing months the sacs once more enlarged and filled with the polysaccharide. One of the major points of interest was the very small amount of starch in the corm, and it would appear that the polysaccharide is in fact the reserve food material of the plant performing the function normally associated with starch.

Attempts by Dr. A. Polson to measure the sedimentation constant of the polysaccharide[79], showed that a 0.05% "solution"† in water of the material was not a true solution, and therefore no sedimentation data could be measured. This was unfortunate, as it had been hoped to use this data in conjunction with the diffusion coefficient to

obtain/...

† The author will throughout this discussion refer to solutions of Watsonia corm polysaccharide, though in actual fact what is meant is a colloidal solution.



obtain the molecular weight of the gum, as has been previously achieved in the polysaccharide field with Virgilia oroboides gum[34]. Precipitation of the gum with ethanol affords a fibrous precipitate which, when redissolved in water, even in concentrations of 0.1g. per 100 ml. gives an extremely viscous solution. This could be indicative of a long basal chain with little, if any, branching of the main chain, and certainly indicates that the molecules tend to be long rather than spherical. This point is further strengthened by the electron micrograph of the corm polysaccharide[80] which shows long molecules interspersed with a number of small spherical particles. An estimate of the volumes occupied by these two types of molecule indicate that the spherical type constitues about 10% of the mixture. The background of the micrograph is too broken and the resolution is insufficient to pick out the finer structure of the molecule, but there is a suggestion of helix formation noticeable in the molecules on the left of the micrograph.

Attempts to fractionate the ethanol precipitated gum into two or more polysaccharides, either containing the same sugars in different proportions, or with different sugar residues, were singularly unsuccessful. The most meaningful result in the fractionation

experiments/...

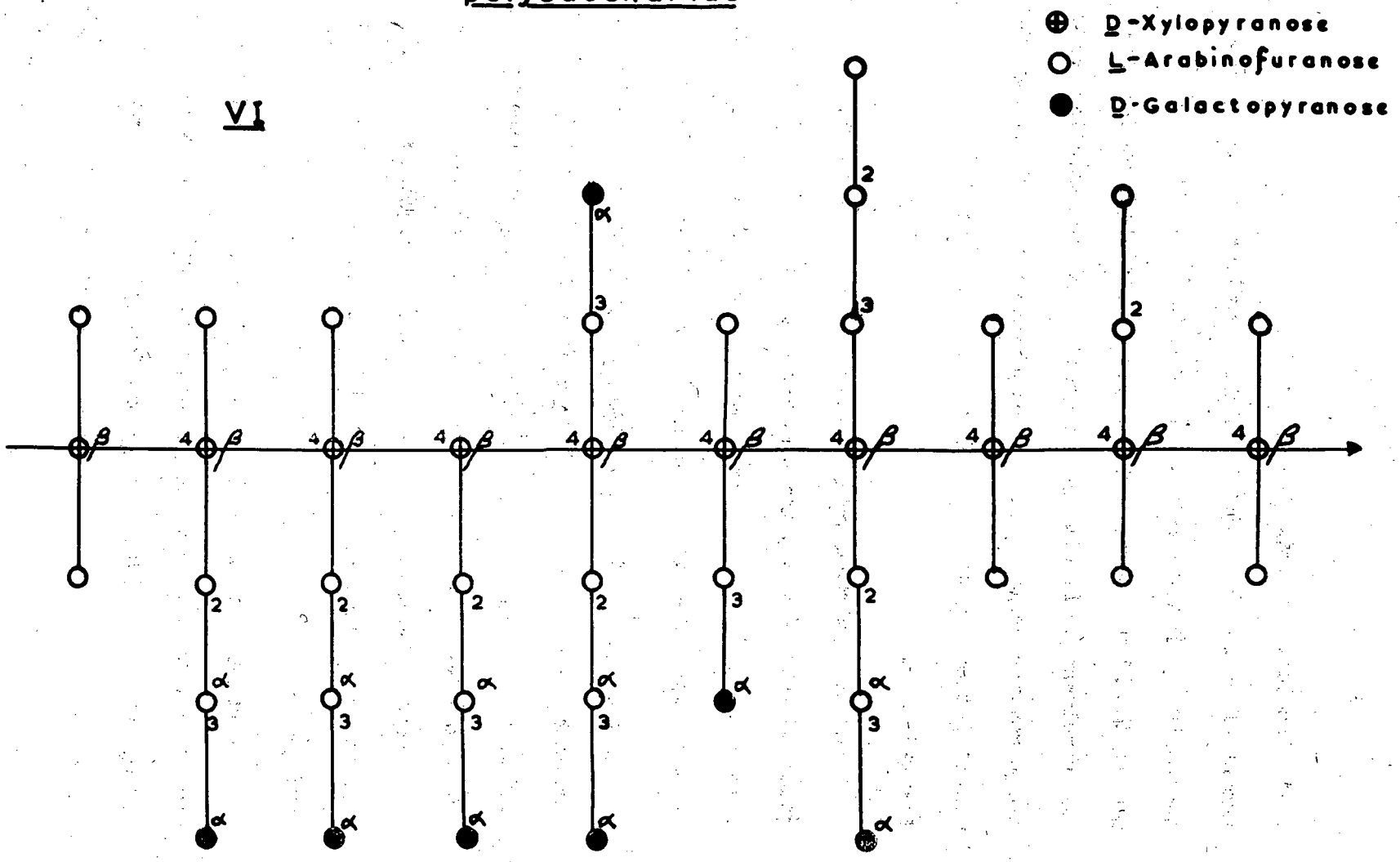
Facing page: electron micrograph of Watsonia polysaccharide.

experiments was that obtained by using a molecular sieve of cross-linked dextran gel (Sephadex). Utilising Sephadex G.50 as a fractionation medium, two polysaccharide fractions were eluted from the column, indicative of polysaccharides of differing molecular weight[81]. The same experiment using Sephadex G.25 afforded only one component. All three fractions were found to have the same sugars as components in the same ratio to each other. According to the specifications for these two grades of Sephadex this would indicate that the lower molecular weight fraction of the polysaccharide had a molecular weight between 5,000 and 10,000. The ratio of high to low molecular weight material is 9 : 1 in the Sephadex fractionation.

Other methods of fractionation proved to be confusing. Fractionation with DEAE-cellulose did give two fractions, one eluted at pH6.0 and the other at pH14.0; the second was heavily contaminated with an uninvestigated non-carbohydrate material. Duolite A4 chromatography gave two fractions similar to the above. As the corm polysaccharide contained no uronic acid one would not expect Duolite A4 or DEAE-cellulose to fractionate the material at all under the conditions used. The low equivalent weight of the second fraction from both these media indicates that the contaminant was acidic, but as no acid was used in the ethanol precipitation it must be assumed that this contaminant is due either to an acid or

salt/...

Diagrammatic representation of a possible structural repeating unit for Watsonia corm polysaccharide.



salt, insoluble in ethanol, and present in the corms. As the amount of carbohydrate in the minor fractions was low (0.74% of the total polysaccharide on the column for Duolite A4), and due to the high viscosity of the solution of the gum in water and resultant difficulty of percolating the gum solution through the chromatographic media, the polysaccharide used for structural investigation was the original ethanol precipitated material (Fraction A).

For ease of reference, the repeating unit which is tentatively proposed for Watsonia corm polysaccharide (VI) is presented on the facing page. In this discussion the experimental results will be followed by the results calculated from this model structure.

Total hydrolysis of the polysaccharide afforded galactose, arabinose, and xylose in the molar ratio 1 : 3.5 : 1.42 (model: 1 : 3.86 : 1.43), an unusual although not unknown constitution for a xylan, in that the xylose content is much lower than that of the arabinose. The same type of constitution is found in the xylan isolated from wheat-bran[82,83], where arabinose is found as the major sugar residue.

Partial hydrolysis of a polysaccharide is undertaken in order to obtain as wide a range of oligosaccharides as possible. In 1930, Kuhn[84] predicted the theoretical yield of an n-membered fragment from random degradation of a uniform linear macromolecule,

this/.....

this yield of n-mer being equal to $n\alpha^2(1-\alpha)^{n-1}$, where n = number of sugar residues in the fragment and α = the degree of scission. He further showed that the maximum possible yield of n-mer occurred when $\alpha = \frac{2}{n+1}$. The main difficulties in attaining this theoretical yield are that oligosaccharides once formed are further degraded during the hydrolysis and the lack of randomness during the degradation. As the polysaccharide from Watsonia corms contains more than one sugar residue, application of these theoretical methods is not possible but Painter, applying the expressions of Kuhn, has obtained high yields of oligomers, and prevented their further degradation during hydrolysis by using dialysis to remove the smaller molecules from the reaction mixture[85]. Although Painter used enzymatic degradation to produce the oligosaccharides, there seems to be no reason why the principle of his method (the removal of oligosaccharides after their formation) could not be adapted to the Watsonia polysaccharide hydrolysate. At various times the hydrolysis was interrupted by neutralizing the acid, and the high molecular weight material was removed by precipitation with 80% methanol. This precipitate was further hydrolysed, and the low molecular weight saccharides separated as before. Repetition of this process four times afforded four syrups containing a wide range of low molecular weight oligosaccharides in yields large enough to allow complete characterisation of each and a precipitate presumably of higher molecular weight oligosaccharides. After column chromatography of the material soluble

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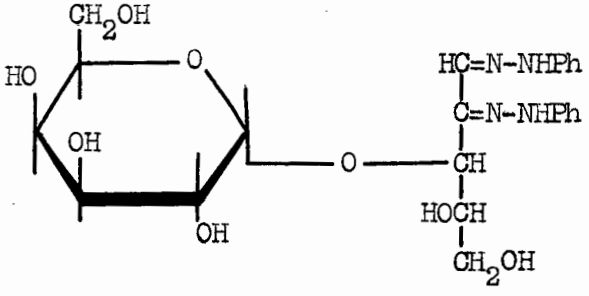
in 80% methanol after 7 hr. hydrolysis, the monosaccharides galactose and arabinose were characterised in the normal way, whilst the first disaccharide eluted from the column was chromatographically identical with a sample of 3-O-α-D-galactopyranosyl-L-arabinose prepared from carboxyl reduced Acacia cyanophylla gum. 3-O-D-galactopyranosyl-L-arabinose has been isolated before, as the β-linked disaccharide from gum Ghatti[7] and the gums of Albizzia zygia[51] and Anogeissus schimperi[10], and as the α-linked disaccharide from Arabic acid[22] (as two heptamethyl derivatives), gum Arabic Turc. variety[85a] (as a syrup), and Acacia cyanophylla gum[28] (as a syrup). Whereas the β-linked disaccharide had been crystallised (m.p. 202 - 203° [7], 204 - 205° [10], 200 - 201° [51]) and has a specific rotation variously reported as being between +60 and +67°, the α-linked disaccharide had not been isolated as a crystalline compound.

The syrupy disaccharide obtained from Watsonia polysaccharide was shown to have galactose and arabinose as the sugar residues, whilst the elegant technique developed by Hough and his co-workers[86], whereby the phenylosazone of the disaccharide is oxidised with periodate ion, was used to determine the position of linkage of the disaccharide. As hydrolysis of the phenylosazone had yielded galactose as the only sugar, arabinose must have been present as reducing end-group. The preparation of the osazone rules out $C_{(2)}$ of the arabinose moiety as the position of linkage, whilst the absence of a precipitate of mesoxalic dialdehyde 1,2-bisphenylhydrazone on periodate oxidation

proves/...

proves that the arabinose is not linked to the galactose through $C_{(5)}$. Only if the link is to $C_{(3)}$ can any formaldehyde be formed (theoretically 1 mol) from the oxidation. As experimentally 1.01 mol. was obtained, it would appear that the phenylosazone is in fact that of 3-O-α-D-galactopyranosyl-L-arabinose (3-O-α-D-galactopyranosyl-L-ribosazone VII). This was confirmed when compared with an authentic specimen of the phenylosazone, as identical X-ray diffractograms and infra-red spectra were obtained.



VII

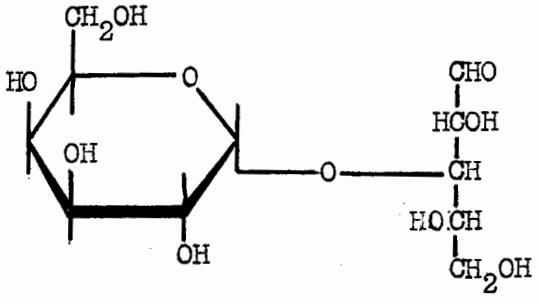
The high specific rotation of the syrupy disaccharide (+ 182°) is characteristic of an α-link between the arabinose and galactose residues, whilst the downward mutarotation shown by the crystalline disaccharide (+ 202 → +198°) is indicative of the arabinose group being more in the β- than the α- form.

Lead tetra-acetate oxidation of the disaccharide followed by borohydride reduction afforded crystalline 2-O-α-D-galactopyranosyl-L-erythritol, a compound which, accepting that the disaccharide was galactosyl-arabinose, could only have been produced from 3-O-α-D-

galactopyranosyl-/...

galactopyranosyl-L-arabinose.

The concomitant release of 2,3,4,6-tetra-O-methylgalactose and 2,4-di-O-methylarabinose on hydrolysis of the methylated disaccharide indicated that in the isolated disaccharide the arabinose and galactose residues were both present in the pyranose form, though later, methylation studies on the polysaccharide will show that, as in other xylans, the arabinose occurs in the polymer as arabinofuranose. It would appear that the disaccharide is thus 3-O-α-D-galactopyranosyl-L-arabinose (VIII).



VIII

The disaccharide was crystallised from methanol solution, apparently without any water of crystallisation; specimens dried at 100° in vacuo, and at room temperature in vacuo, had the same percentages of carbon and hydrogen. An unusual point was that, although the two specimens had identical melting point, the X-ray diffractograms and infra-red spectra of two crystalline preparations from aliquots of the same syrup were not identical. In the infra-red spectra

the/....

the only difference in the positions of the peaks was that one preparation had absorption at 796 cm.$^{-1}$; otherwise all the absorption maxima were at the same wavelength. Peak heights relative to the general contour of the spectrum did vary somewhat between the two batches of crystals, this however, is a phenomenon which has been described before for the infra-red spectra of samples of sucrose having differing degrees of crystallinity[87]. Absorption at 849 cm.$^{-1}$ in both batches indicated β-anomer[88] (of L-arabinopyranose). The differences observed in the X-ray diffractograms were probably due to two different crystalline phases being present. One can accept this as being a correct interpretation of the facts, it must be emphasised that chemically no differences could be found in the two preparations, and the melting point of a mixture of the two sets of crystals (which each had m.p. 156.5 - 157.5°) was undepressed.

Application of Hudson's rules of isorotation[89] to the disaccharide confirm both the α-link and an arabinopyranose reducing group as illustrated below.

Molecular rotations used were,

α -methyl-L-arabinopyranoside	+2,800
β -methyl-L-arabinopyranoside	+40,300
α -methyl-L-arabinofuranoside	-20,500
β -methyl-L-arabinofuranoside	+3,300
α -methyl-D-galactopyranoside	+38,000
β -methyl-D-galactopyranoside	+194

To/...

To find the A and B values for arabinose the above values are substituted in the equations $2A = [M]_\alpha - [M]_\beta$ and $2B = [M]_\alpha + [M]_\beta$ thus one calculates the values to be,

for arabinopyranoside $A = -18,750$

$B = +21,550$

and for arabinofuranoside $A = -11,900$

$B = -8,600.$

Substitution of these respective values of A and B for arabinopyranoside and arabinofuranoside in the equation

$$[M]_{disacc.} = [M]_{(\alpha \text{ or } \beta)\text{MeDgal}_p} + B_{ara} + A_{ara}$$

gives the molecular rotation of the α-anomer of the disaccharide, and substitution in the equation

$$[M]_{disacc.} = [M]_{(\alpha \text{ or } \beta)\text{MeDgal}_p} + B_{ara} - A_{ara}$$

gives the β-anomer of the disaccharide. The specific rotation is then calculated from the equation $[\alpha]_D = \frac{[M]_{disacc.}}{\text{mol. wt.}}$. Calculated values from the above equations give the following specific rotations for the four possible disaccharides (assuming that the methyl glycoside rotation is very little different from that of the disaccharide).

(i) 3-O-α-D-galactopyranosyl-L-arabinopyranose

α - anomer $[\alpha]_D = +131^\circ$

β - anomer $[\alpha]_D = +251^\circ$

(ii)/....

(ii) 3-O-β -D-galactopyranosyl-L-arabinopyranose

α -anomer $[\alpha]_D = +10^\circ$

β -anomer $[\alpha]_D = +130^\circ$

(iii) 3-O- α-D-galactopyranosyl-L-arabinofuranose

α-anomer $[\alpha]_D = +56^\circ$

β-anomer $[\alpha]_D = +132^\circ$

(iv) 3-O- β-D-galactopyranosyl-L-arabinofuranose

α-anomer $[\alpha]_D = -65^\circ$

β-anomer $[\alpha]_D = +11^\circ$

From these results it may be seen that 3-O-α-D-galactopyranosyl-L-arabinopyranose is the only form of the disaccharide in which the equilibrium specific rotation could be $+198^\circ$ (the specific rotation of the crystalline disaccharide).

The second oligosaccharide eluted from the column was shown to consist of two mols. of arabinose and one mol. of galactose. Borohydride reduction of the reducing group, and partial hydrolysis afforded arabinose, 3-O-α -D-galactopyranosyl-L-arabinose, and arabitol, whilst partial hydrolysis of the original trisaccharide yielded galactose, arabinose, and 3-O-α -D-galactopyranosyl-L-arabinose only. It is clear from these results that the trisaccharide is made up of the disaccharide previously discussed (possibly with the arabinose in the furanose form) linked to another arabinose residue which is a reducing unit. The fast rate at which arabinose is

hydrolysed/...

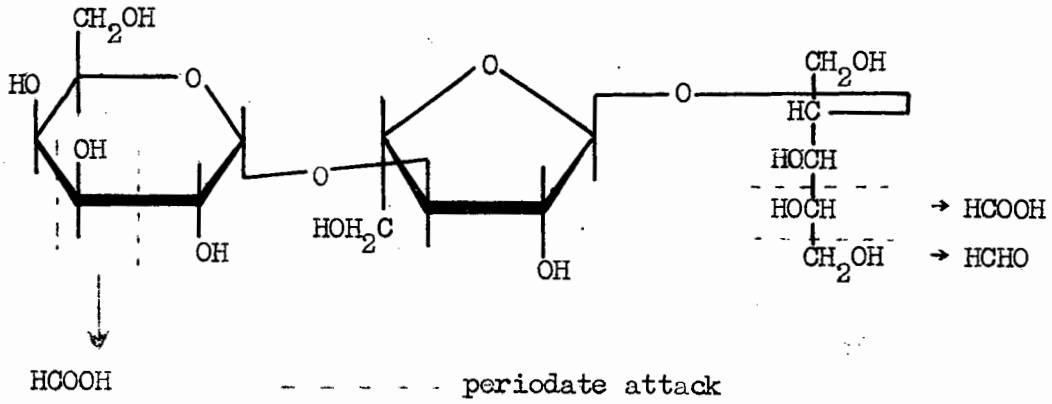
hydrolysed off this molecule in 0.01N-sulphuric acid would indicate that the non-reducing arabinose unit is in the furanose form.

Periodate oxidation studies on the borohydride reduced trisaccharide have shown that 1.06 mol. of formaldehyde was produced during the oxidation. Only if the $C_{(2)}$, $C_{(4)}$, or $C_{(5)}$ hydroxyl groups of the arabitol were involved in glycosidic linkage would one mol. of formaldehyde be obtained. If the link was through the $C_{(3)}$ hydroxyl group, two mol. of formaldehyde would be produced. The link through $C_{(4)}$ was regarded as unlikely as the fast rate at which the trisaccharide is hydrolysed from the parent polysaccharide is indicative of the reducing arabinose unit having been in the furanose form. A linkage through the hydroxyl on $C_{(5)}$ was considered more likely but one would have expected to find ethylene glycol as a product of oxidation and reduction. This was not found in an hydrolysate of the reduced periodate oxidised material; however, under certain circumstances ethylene glycol, due to its volatility, has been found difficult to detect in small quantities. A link to the non-reducing arabinose through the hydroxyl on $C_{(2)}$ of the arabitol seemed the most likely position, though at this stage experimental results did not rule out $C_{(4)}$ and $C_{(5)}$ as the positions to which the rest of the molecule could be attached. The appearance of arabinose in an hydrolysate of the reduced oxidised reduced trisaccharide is what one would expect, as the disaccharide portion of the molecule will give only glycerol, from the fragment containing $C_{(4)}$, $C_{(5)}$,

and/....

and $C_{(6)}$ of the terminal non-reducing galactose group; oxidative cleavage having taken place between the α-glycol systems on $C_{(3)}$ and $C_{(4)}$, and $C_{(2)}$ and $C_{(3)}$, with the release of one mol. of formic acid. Further formic acid (one mol.) would be produced from cleavage of the α-glycol systems on $C_{(5)}$, $C_{(4)}$, and $C_{(3)}$ of the arabitol group. Experimentally, the amount of formic acid determined was 1.9 mol. presumably made up of one mol. containing $C_{(3)}$ of the galactose unit and one mol containing $C_{(4)}$ of the arabitol. The reaction is formulated in IX.

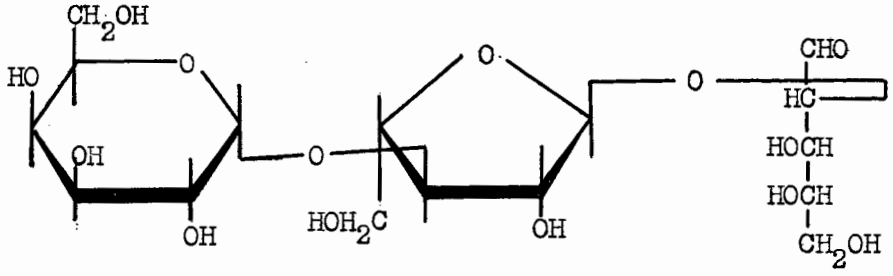
IX



Methylation of the syrup afforded a product with a specific rotation of +41°, whilst hydrolysis of this product yielded 2,3,4, 6-tetra-O-methylgalactose from the terminal non-reducing galactose group, 2,5-di-O-methylarabinose from the non-reducing arabinose residue, a large amount of 3,4-di-O-methylarabinopyranose and a small amount of 3,5-di-O-methylarabinofuranose. The presence of 2,5-di-O-methylarabinose is confirmation of the suspected furanose character

of/.....

of the non-reducing arabinose unit as $C_{(5)}$ carries a methoxyl group. The appearance of both 3,4- and 3,5-di-O-methylarabinose residues shows that in the methylated syrupy trisaccharide a mixture of the furanose and pyranose forms of the reducing group exists. This is probably the reason why attempts to crystallise the original trisaccharide from a variety of solvents were unsuccessful. The fact that these two methylated sugars do appear in the hydrolysate of the methylated trisaccharide confirms that the linkage position to the reducing arabinose is through $C_{(2)}$, and it would appear that the trisaccharide is O-α-D-galactopyranosyl-(1 → 3)-O-L-arabinofuranosyl-(1 → 2)-L-arabinose (X), with the arabinose in either the furanose or pyranose form.



X

An α-link from the non-reducing arabinose to the reducing arabinose unit is indicated by the drop in the equilibrium rotation from that of the disaccharide ($[\alpha]_D$ +198°) to that of the trisaccharide ($[\alpha]_D$ + 67°). Although Hudson's rules of isorotation take no account of the linkage positions, they provide a reasonable method of checking the configuration of glycosidic links. Application of these rules

to/...

to the trisaccharide tended to confirm that the link joining the two arabinose residues was α but certain assumptions had to be made. The main one being that furanose and pyranose forms of the arabinose reducing group were present in the original trisaccharide in much the same ratio as they were in the methylated material, and a second, that the position of the linkage made a negligable contribution to the molecular rotation. With these provisos in mind, the following specific rotations for the four possible trisaccharides were calculated using the equation

$$[M]_{trisacc} = [M]_{disacc\ (\alpha\ or\ \beta\ anomer)} + B_{ara} \begin{matrix} + A_{ara}\text{(for the } \alpha\text{-anomer)} \\ - A_{ara}\text{(for the } \beta\text{-anomer)} \end{matrix}$$

The molecular rotations used were the same as those used for the disaccharide with the addition of the two which were calculated for 3-O-α-D-galactopyranosyl-L-arabinofuranoside ($[M]_{disacc}$ in the formula). Application of these figures gave the following rotations:

(i) $Gal_p\ \alpha \rightarrow 3\ Ara_f\ \alpha \rightarrow 2\ Ara_p$ — α-anomer $[\alpha]_D = +46^\circ$; β-anomer $[\alpha]_D = +130^\circ$

(ii) $Gal_p\ \alpha \rightarrow 3\ Ara_f\ \beta \rightarrow 2\ Ara_p$ — α-anomer $[\alpha]_D = +99^\circ$; β-anomer $[\alpha]_D = +184^\circ$

(iii) $Gal_p\ \alpha \rightarrow 3\ Ara_f\ \alpha \rightarrow 2\ Ara_f$ — α-anomer $[\alpha]_D = -7^\circ$; β-anomer $[\alpha]_D = +47^\circ$

(iv) $Gal_p\ \alpha \rightarrow 3\ Ara_f\ \beta \rightarrow 2\ Ara_f$ — α-anomer $[\alpha]_D = +47^\circ$; β-anomer $[\alpha]_D = +109^\circ$

The specific rotation of the trisaccharide ($+67^\circ$) lies within the limits/....

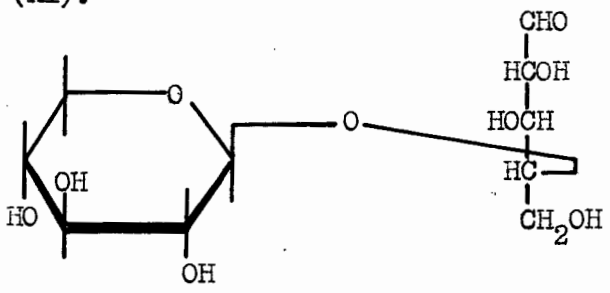
limits of either (i) or (iv) but if the pyranose form predominates, as has been assumed, the α-link as in (i) is the most likely. The presence of small quantities of α-linked arabinofuranose (as in iii) would lower the equilibrium rotation slightly from a maximum of $+130^{\circ}$ and a minimum of $+46^{\circ}$ making 67° closer to the centre of the range. Whilst this application is not as accurate as when used for the disaccharide, the rules of isorotation do still seem to give a reasonable method of calculating theoretical specific rotations for a trisaccharide.

Further elution of the column produced what appeared to be a homologous series of xylose oligomers as the relationship $\left[\log \frac{1}{\underline{R}_{gal}}\right]-1$ vs suspected degree of polymerisation proved to be a linear function.[90] The components of this fraction were not identified but added to the material chromatographed on the second charcoal / Celite column. Elution of this column afforded the same components as before, with the addition of xylose, which was characterised in the normal way, and also a disaccharide and a trisaccharide both containing xylose as the only sugar residue.

The phenylosazone of the disaccharide had a melting point 12° higher than that reported in the literature for the phenylosazone of xylobiose. However, periodate oxidation of this phenylosazone yielded 1.06 mol. of formic acid per mol. of the disaccharide phenylosazone, no formaldehyde, and no precipitate of mesoxalic

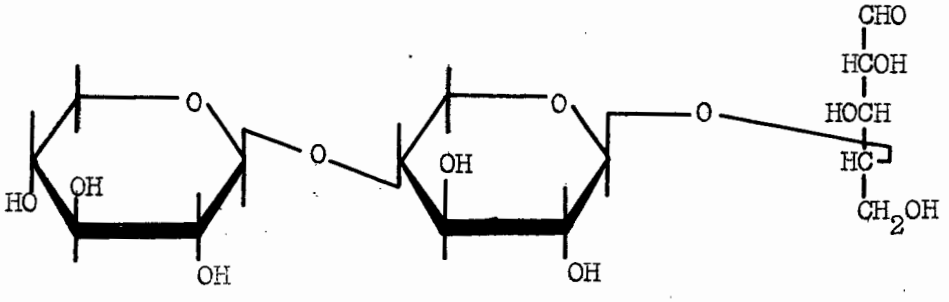
dialdehyde/....

dialdehyde 1,2-bisphenylhydrazone. These results are strong evidence that the two xylose residues were linked 1 → 4 to each other. The specific rotation is close to reported values for the β→ 4-linked xylopyranosyl-xylose, and final confirmation of the structure was obtained by preparing the acetylated disaccharide and measuring its rotation and melting point (which was undepressed on admixture with an authentic sample). The disaccharide is thus 4-O-β-D-xylopyranosyl-D-xylose (XI).



XI

The trisaccharide afforded only compound XI and xylose on mild acid hydrolysis. Crystallisation of this fraction and mixed melting point of the crystals proved it to be O-β-D-xylopyranosyl-(1 → 4)-O-β-D-xylopyranosyl-(1 → 4)-D-xylose (XII). The structure of the trisaccharide was also confirmed by comparison of its octa-acetate with an authentic sample.



XII

Finally, elution of the column with 80% methanol afforded the homologous series of xylose oligomers from xylotetraose to xylohep-taose. These were identified by paper chromatography, the spots obeying the mathematical relationship previously stated; hydrolysis yielded lower oligomers of xylose and xylose as the only products. In all probability this partial hydrolysate contains the β 1 → 4-linked series of xylose oligomers isolated by Whistler from corn-cob xylan[91] and Bishop from wheat-straw hemicellulose[74].

A second reason for degrading the original polysaccharide by a series of hydrolyses was to remove as many of the side chains as possible, leaving the basal chain intact. After hydrolysing four times, this appears to have been achieved, as a degraded polysaccharide insoluble in water but soluble in dilute alkali (a characteristic feature of xylans which are not highly substituted) was isolated. On hydrolysis this polysaccharide though showing no galactose and only traces of arabinose was found to give rise to small amounts of glucose as well as the major component, xylose. The glucose probably came from starch which must have been contaminating the polysaccharide throughout the hydrolysis. As only mild conditions of hydrolysis had been used it was unlikely that starch molecules which consist of glucopyranose units would have been seriously degraded. It must be emphasised that this glucose containing fraction was about 0.2% of the total polysaccharide which was hydrolysed and no glucose was isolated from the chromatographic separation. Methylation and then

hydrolysis/....

hydrolysis of this mixture of two polysaccharides afforded 2,3,4-tri-O-methylxylose (1 part), 2,3-di-O-methylxylose (8 parts), 2,3,4,6-tetra-O-methylglucose (trace), and 2,3,6-tri-O-methylglucose (trace). The 2,3-di-O-methylxylose confirms the 1 → 4-linked xylose, whilst its ratio to that of 2,3,4-tri-O-methylxylose indicates that the average length of the basal chain after degradation is nine xylose units, though as gas-liquid chromatography was used to calculate the molar ratios these figures must be regarded as only approximate.

The yield on column chromatography of the partial hydrolysates of Watsonia polysaccharide was only about 50%, lower than would be expected. Calculation of the amounts of arabinose, galactose, and xylose eluted from the column and comparison with that originally found in the polysaccharide showed that a large amount of xylose had still to be accounted for. In view of the small weight of xylose oligomers recovered, there is a possibility that, under the conditions used oligosaccharides of higher degree of polymerisation than seven may be irreversibly absorbed onto the charcoal and not eluted with 70% methanol.

The partial hydrolysis of the polysaccharide has shown that the basal chain of the Watsonia gum consists of β → 4-linked xylopyranose residues substituted with O-α-D-galactopyranosyl-(1 → 3)-O-α-L-arabinofuranosyl-(1 → 2)-L-arabinose and, although some of the disaccharide 3-O-α-D-galactopyranosyl-L-arabinose undoubtedly arises from side chains of the trisaccharide by hydrolysis of the furanose

link/.....

link between the two arabinose residues, some is probably linked direct to the xylose chain. The appearance of both these saccharides during the early stages of a relatively mild acid hydrolysis indicates that the reducing end group in each is probably in the furanose form, whilst the absence of saccharides longer than three sugar residues is an indication that three could be the maximum number of sugar residues in a side chain. It is interesting to note that the arabinose reducing group in the trisaccharide is joined to the next sugar residue in the molecule through $C_{(2)}$, a mode of linkage not uncommon in the xylans, c.f. $\underline{L}$-Gal_p-(1 → 4)-$\underline{D}$-Xyl_p-(1 → 2)-$\underline{L}$-Ara from maize fibre[62]. The high proportion of arabinose released on mild acid hydrolysis is indicative of a large proportion of the arabinose in the molecule occurring in the form of single unit furanose side chains, as there was no evidence of any arabinose disaccharides being released during the hydrolysis.

Normally Haworth's method of methylation requires a high aqueous concentration of polysaccharide. As Watsonia polysaccharide was only soluble in water to the extent of ca. 0.3%, this method was not very successful. However, it was found that much higher concentrations of polysaccharide could be obtained by homogenizing the material in aqueous sodium hydroxide (at 4° in an atmosphere of nitrogen); the normal Haworth procedure then worked very satisfactorily, as a product was obtained from a single methylation step with a methoxyl content of 36.8% (higher than that usually obtained when the polysaccharide

is/...

is dissolved initially in water). There are of course, inherent dangers in using this method. The main one being that alkali degradation can take place from the reducing end of the basal chain. To keep this degradation to a minimum, dimethyl sulphate was added to the reaction mixture as soon as possible in order to methylate the glycosidic hydroxyl group on the reducing residue, and stop the formation of saccharinic acids. In a later part of the discussion, it will be shown that most of the xylose residues, if not all, have the $C_{(2)}$ position substituted. Thus alkali degradation could not have taken place to any significant extent.

Further methylation by the methods of Kuhn and Purdie proved satisfactory and afforded a product with a methoxyl content of 37.5%. The use of dimethylformamide in the Kuhn methylation presented some difficulties, as higher temperatures (ca. 70°) than it is advisable to use with polysaccharides had to be employed to remove the last traces of dimethylformamide. Although no degradation appeared to take place in this case, it is probably better, if the polysaccharide is soluble in methyl iodide after methylating by the Haworth procedure, to methylate directly with Purdie's reagents. This would also obviate the step of removing the dimethylformamide-silver iodide complex, a procedure which may prove troublesome. The Kuhn procedure was not omitted in methylating the Watsonia polysaccharide, but the high methoxyl content after the initial methylation, and the fact that Kuhn methylation raised the methoxyl content by only 0.3%, and

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the similarity of the infra-red spectra in the -OH region where little absorption occurred after Kuhn or Purdie methylation, indicates that omission of the Kuhn methylation may have been justified in this case.

Hydrolysis of the fully methylated polysaccharide afforded 2,3,5-tri-O-methylarabinose, 2,3,4,6-tetra-O-methylgalactose, 3,5-di-O-methylarabinose, 2,5-di-O-methylarabinose, 2,3,6-tri-O-methylgalactose, 3-O-methylxylose, 3-O-methylarabinose, and xylose. As demethylation can take place during the hydrolysis of methylated polysaccharides, especially when the initial hydrolysis is with formic acid[92], it is advisable to modify the method normally used. The methylated polysaccharide was found to be soluble in 15% formic acid in N-sulphuric acid, and this was the hydrolysing agent employed. In the light of further investigations (see page 67) it is impossible to account for the 2,3,6-tri-O-methylgalactose which appears in an hydrolysate of the methylated gum. Whilst this could arise from the demethylation of 2,3,4,6-tetra-O-methylgalactose during hydrolysis, it is known that the axial hydroxyl group at $C_{(4)}$ of galactose is difficult to methylate. A certain amount of demethylation undoubtedly does take place as the same sample of methylated polysaccharide gave differing amounts of 2,3,6-tri-O-methylgalactose relative to the other methylated monosaccharides when hydrolysed with different reagents. The most 2,3,6-tri-O-methylgalactose was found in the hydrolysate when hydrochloric acid was used as the hydrolysing agent, a not surprising result when one considers that hydrobromic

acid/....

acid, also a halogen acid, is used as a demethylating agent[60].

The absence of any arabinose with the hydroxyl group on $C_{(4)}$ methylated coupled with the absence of 2,3-di-O-methylarabinose ($C_{(4)}$ or $C_{(5)}$ as a link position) is a final proof that all the arabinose in the gum is in the furanose form. If one accepts that the 2,3,6-tri-O-methylgalactose is produced by undermethylation or demethylation, all the galactose residues occurring in the original polysaccharide are present as non-reducing end groups. This is as would be expected from a study of other galactose containing xylans. The isolation of 2,5- and 3,5-di-O-methylarabinose from the methylated gum confirms that in the original polysaccharide the non-terminal arabinofuranose residues were linked through $C_{(3)}$ or $C_{(2)}$ respectively. The unusual point which emerges from the methylation investigation of the polysaccharide is that at least 92.5% (these figures are calculated from the molar ratios presented in Table 13, page 110) of the xylose in the original gum is resistant to methylation. The other 7.5% of the xylose is found in the hydrolysate of the methylated gum as 3-O-methylxylose, indicating that either arabinofuranose (probably as single side chains) has occasionally been removed from its point of attachment at $C_{(2)}$ of a xylose residue during the methylation, or that in fact sometimes $C_{(2)}$ on a xylose residue carries no substituent. The 3-O-methylarabinose and 2,4,6-tri-O-methylgalactose each constituting less than 1% of the products of hydrolysis are quite possibly due to under- or demethylation and may at the present stage

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be ignored in building up a structure for the polysaccharide. 2,3,5-tri-O-methylarabinose and xylose were recovered from the hydrolysate in equal molar proportions, indicating that in the original gum each xylose residue in the basal chain had an average of one arabinofuranose group attached as a single unit side chain. An interesting point is the absence of xylose with the $C_{(4)}$ hydroxyl group methylated. As this would be produced only at the non-reducing end of the basal chain, that is assuming that the chain is not terminated by arabinose or galactose, the amount found may be used as a measure of the length of the molecule, which, subject to the provision made, is consequently high. Though no upper limit can be given, 0.2 residues of 2,4,6-tri-O-methylgalactose were detected in 41.2 sugar residues (see Table 13) i.e. one residue per 206 residues. Thus one would expect to be able to isolate a similar quantity of a methylated xylose. The fact that none was detected either by cellulose column chromatography or gas-liquid chromatography presumably means that, if the terminal group on the basal chain is xylose, the polysaccharide molecule contains at most only one xylose end group per 206 sugar residues. The amounts of sugar residues isolated from the hydrolysate of the methylated polysaccharide have shown this to correspond to a basal chain length of at least 50 xylose residues. The amounts of methyl sugars and sugars found experimentally were (with the amount calculated from the proposed model in parenthesis); 2,3,5-tri-O-methylarabinose 10.0 (12), 2,3,4,6-tetra-O-methylgalactose + 2,3,6-tri-O-methylgalactose

7.35/....

7.2 (7), 2,5-di-O-methylarabinose 6.5 (8), 3,5-di-O-methylarabinose 6.2 (7), 3-O-methylxylose 0.75 (1), xylose 10.0 (9). These figures represent good agreement between theoretical and practical results, though approximately five residues of arabinose are missing for every ten xylose residues in the basal chain. This could well be due to handling losses, as 2,3,5-tri-O-methylarabinose especially is known to be volatile to a certain extent and could have been lost during the evaporation of the eluting solvent after column chromatography. This loss of arabinose is reflected in the ratio (calculated from the yields of methylated products) galactose: arabinose : xylose which was found to be 1 : 3.08 : 1.46 (1 : 3.86 : 1.43). An attempt was made to use gas-liquid chromatography to separate and identify (against standards) the methylated sugars and it was found, in agreement with other workers, that this is an excellent means of identification of the components. Quantitative gas-liquid chromatographic analysis was abandoned, (a) because the unmethylated xylose could not be eluted from the column under the conditions used and (b) because of extensive demethylation of the 2,3,5-tri-O-methylarabinose during methanolysis. Possibly acetylation of the remaining hydroxyl groups in the methanolysis products from the methanolysed polysaccharide, and gas-liquid chromatography on a mixed polar and non-polar liquid phase, would give a better idea of the quantities of the unmethylated xylose and monomethylglycosides released on methanolysis. Fully acetylated methylglycosides have been satisfactorily separated by this method[92a].

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It will be observed that the total arabinose contents found by column chromatography and gas-liquid chromatography of the methylated polysaccharide are very similar. There appears to be more dimethylarabinose and less trimethylarabinose in the products from gas-liquid chromatography. This phenomenon has been observed for other methanolysed methylated polysaccharides containing arabinose non-reducing end group[57] and it does not seem possible at the present time to put the quantitative analysis of complex methylated polysaccharides by gas-liquid chromatography onto a sound basis, when a flame-ionization detector is used.

In any methylated polysaccharide, the number of methylated end groups should equal the number of branch points in the molecule. Excellent agreement was obtained in this methylation experiment which afforded 17.2 end groups for 20.75 branch points, as against 19 to 19 respectively for the model structure. The number of groups in the chain which were neither branch points nor end groups was found to be 12.7 (15).

The investigation on the methylated polysaccharide has shown that most of the xylose residues in the β→ 4-linked basal chain are substituted on both $C_{(2)}$ and $C_{(3)}$, a type of structure which has not been observed before in a xylan; occasional double branching of a xylose residue in the backbone has been shown to occur in wheat endosperm hemicellulose[93] where positions $C_{(2)}$ and $C_{(3)}$ of the same

xylose/...

xylose residue were each substituted with a single unit side chain of L-arabinofuranose. In view of the successful methylation of the degraded polysaccharide from the partial hydrolysis investigation (see page 57) which proved to be a chain of xylose residues, it is thought highly unlikely that Watsonia polysaccharide could in fact be a mixture of an arabinogalactan and a pure xylan resistant to methylation, as much the same methylation procedures were used for each case. The unmethylated xylose recovered after methylation of the polysaccharide affords proof of the branch points in the molecule; these are essential as no other branch points had been found to provide points of attachment for the arabinose and galactose in the side chains.

Confirmation has been obtained for the earlier assumption that in both 3-O-α-D-galactopyranosyl-L-arabinose and O-α-D-galactopyranosyl-(1 → 3)-O-α-L-arabinofuranosyl-(1 → 2)-L-arabinose isolated from the partial hydrolysis mixture, all the reducing arabinose was originally in the furanose form.

Indications are that, if the basal chain is terminated by a xylose residue, the minimum length of the polysaccharide is about 50 xylose units containing a total of 206 sugar residues. The fact that most of the xylose residues carry side chains on $C_{(2)}$ and $C_{(3)}$ would make the polymer resistant to alkali degradation during the initial stages of the methylation.

Analytical periodate oxidation of the polysaccharide over 15 days/....

days indicated that the polysaccharide consumed 0.44 mol of periodate and released 0.087 mol of formic acid per 100g. of gum; the theoretical figures from the model are 0.425 mol and 0.110 mol respectively. After three months oxidation the periodate consumed was 0.515 mol.

Periodate oxidation of a large amount of polysaccharide however, produced anomolous results, as after 14 days the periodate consumed was 0.864 mol. per 100 g. of polysaccharide. As the concentration of periodate was higher in this bulk experiment the two experiments are not strictly comparable, though one would expect the consumption of periodate to be the same for each when all the α -glycol systems had been cleaved. This discrepancy in the bulk experiment was presumably due to non-Malapradian oxidation occurring; or as the reaction solution was unbuffered there was a slight chance that glyceraldehyde was being hydrolysed from the molecule by the formic acid produced, and consuming more periodate ion in cleaving the α -glycol system so formed, leading to a loss of glycerol when the oxo-polysaccharide was reduced and hydrolysed; in fact a loss of glycerol did occur (see page 67). Both of these reasons could fit the facts which have been found. A second bulk experiment (not reported in the experimental section) showed a break in the curve of periodate consumed vs time of oxidation. This has been interpreted as being the true consumption of periodate to cleave all the α -glycol systems in the polysaccharide[94]. This break occurred at a periodate consumption of 0.51 mol./100 g., a

figure/....

figure in much better agreement with that found in the analytical experiment. This point, is probably the point at which Malapradian oxidation ends.

The normal method of removing borate ion after borohydride reduction of the oxo-polysaccharide is, after removal of sodium ions, to evaporate successive portions of methanol from the product. This method has the disadvantage that temperatures above ambient temperature are used, an inadvisable procedure if it can be avoided. This difficulty was circumvented with Watsonia reduced oxo-polysaccharide by passing it in concentrated solution through a column of cross-linked dextran gel (Sephadex G.25 medium grade). This new technique was found to work admirably, affording a polysaccharide fraction completely free from inorganic ions and a fraction containing borate ion, virtually no carbohydrate, and no glycerol or other polyol. Hydrolysis of the reduced oxo-polysaccharide afforded xylose, arabinose, and glycerol in the ratio of 10 : 14 : 7 (the model requires 10 : 15 : 19). At no time during the bulk experiments was the presence of threitol in the hydrolysate detected, which confirms the assumption made earlier that the 2,3,6-tri-O-methylgalactose in the hydrolysate of the methylated gum was in fact due to under- or demethylated 2,3,4,6-tetra-O-methylgalactose; galactose linked through $C_{(1)}$ and $C_{(4)}$ must give threitol on periodate oxidation and subsequent reduction.

Smith degradation[95] of the reduced oxo-polysaccharide gave glycerol as the only polyol and a small amount of arabinose which was

shown/.....

shown to be almost entirely in the free form and not bound to a glycitol residue. Results from Smith degradation experiments must thus be carefully interpreted as it would appear that the glycosidic links of arabinofuranose residues may be cleft during hydrolysis in cold sulphuric acid. Apart from the glycerol and arabinose, which were soluble in 96% ethanol, an insoluble residue was recovered which was shown to consist of xylose and arabinose in the ratio 10 : 14.2 (model requires 10 : 15). Methylation of this material and subsequent assay of the hydrolysis products separated from paper sheets showed the following components in the approximate molar ratios given (components identified by gas-liquid and paper chromatography): 2,3,4-tri-O-methylxylose 1.0 (1), 2,3,5-tri-O-methylarabinose 9.3 (9), 2,5-di-O-methylarabinose 1.0 (1), 3,5-di-O-methylarabinose 5.6 (^{5}), 2,3-di-O-methylxylose 1.5 (2), 2- and 3-O-methylxylose 5.1 (5), xylose 3.0 (2). Use of these results gives a ratio of xylose to arabinose of 10 :15 (model requires 10 : 15).

If the basal chain is terminated by xylose at the non-reducing end, the molar proportion of terminal xylose to non- terminal xylose indicates that the approximate length of the basal chain after one Smith degradation is 10 xylose units. The number of end groups in the reduced oxo-polysaccharide (10.3) compares well with the number of branch points (11.1). A further attempt at using gas-liquid chromatography for a quantitative study of methylated polysaccharides again failed, and it was once more observed that

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whilst the amount of 2,3,5-tri-O-methylarabinose was too low, the quantity of di-O-methylarabinoses was too high.

The recovery of 3 mol. of xylose from the hydrolysate of the methylated reduced oxo-polysaccharide (the hydrolysate contained 10.6 mol. of xylose in various forms per 26.5 mol. of sugar residues) indicated that after one oxidation and reduction of the polysaccharide, 28% of the xylose residues still had side chains on $C_{(2)}$ and $C_{(3)}$ whilst the recovery of 5.1 mol. of 2- and 3-O-methylxylose showed that the same material had 48% of the xylose residues with a side chain on either $C_{(2)}$ or $C_{(3)}$ but not on both. Due to the presence of 1.5 mol. of 2,3-di-O-methylxylose, one may assume that the reduced oxo-polysaccharide has about 14% of the basal chain unsubstituted, these residues were presumably substituted on $C_{(2)}$ and $C_{(3)}$ in the original polysaccharide by L-arabinofuranose end groups.

Further periodate oxidation of the ethanol insoluble material from the Smith degraded gum showed a periodate consumption of 0.37 mol. per 100 g. of degraded polysaccharide, which is in excellent agreement with the figure of 0.395 calculated from the proposed structure. Borohydride reduction of the oxo-Smith degraded polysaccharide and hydrolysis, afforded xylose, arabinose, and glycerol in the ratio 6.8 : 5.1 : 6.0 (model requires 7 : 6 : 13). Smith degradation of the material yielded glycerol and a glycosyl-glycitol from the ethanol soluble material, and xylose and arabinose in the ratio 7.1 : 4.9 (model requires 7 : 6) from the ethanol insoluble

material/.....

material. As glycerol was the only glycitol found in the total hydrolysate, it can be assumed that a glycosyl-glycerol is present, probably with xylose as the non-reducing sugar residue. If the glycosyl-glycitol is indeed xylosyl-glycerol, this would indicate that xylose did exist in the original polysaccharide with single unit side chains on both $C_{(2)}$ and $C_{(3)}$, both these residues being destroyed during the first oxidation and Smith degradation. This would leave $C_{(2)}$ and $C_{(3)}$ on the xylose molecule open to periodate attack during the second oxidation, Smith degradation then affording xylosyl-glycerol. The recovery of glycerol from the second Smith degradation is indicative of further arabinofuranose end groups in the molecule, which could arise only from side chains which were at least two units long in the original polysaccharide.

If the proposed structural model is correct, a further oxidation and Smith degradation of the insoluble material mentioned above should break down the polymer to a maximum of four xylose units carrying no side chains, with concomitant release of glycerol (7 mol.). The small quantity of material available after two oxidations and Smith degradations in this periodate study precluded this being carried out. If, after a fourth periodate oxidation and Smith degradation of the polysaccharide (which had contained only xylose after three degradations), xylose was still present in the hydrolysate, this could only mean that, unless some of the side chains were originally more than three sugar residues long, the xylose backbone was branched with respect to xylose and was not

linear/..

linear. At no stage in this investigation has there been any evidence to show that some side chains are more than three units long, or that the basal chain is branched, though there are no experimental results which prove that this is not the case.

It is possible that at average intervals of ten xylose units in the basal chain, a residue of arabinofuranose replaces xylose. This would explain the xylan with average length ten xylose residues which appeared during partial acid hydrolysis and during periodate oxidation; this explanation in the light of published work on xylans seems, however, unlikely. A more reasonable explanation would be that on an average of every ten xylose residues in the basal chain, the stereochemistry of the molecule becomes such as to create a strain, thus weakening the glycosidic link.

The low recovery of glycerol in both Smith degradations presented a major problem in the analysis of the polysaccharide by periodate oxidation. There are two ways in which this compound could have been lost, (a) during the periodate oxidation as previously discussed or (b) during evaporation of solvents from the glycerol. As the amount of arabinose and xylose produced is the expected amount, the low glycerol recovery is attributed to loss of glycerol rather than to an incorrect model structure.

Structurally Watsonia polysaccharide appears to have many similarities with xylan hemicelluloses (see page 38). The basal chain

consists/...

consists of β 1 → 4-linked xylose residues, there is no evidence to show that arabinose is present in the pyranose form, and galactose would appear to be present only as a terminal end group in the side chains. Though the presence of some 4-linked galactose in the side chains must not be completely discounted at this stage, periodate studies do however indicate that this is not the case. The polysaccharide has, in common with other xylans, a high negative specific rotation and would appear to have an unbranched basal chain, although the latter point has not been finally proved. The arabinofuranose residue linked (presumably) direct to the xylose chain has been shown to be linked through $C_{(2)}$ to the glycosidic carbon of the next residue (arabinose) in the side chain, again a common occurrence amongst polysaccharides of the xylan type.

The absence of any uronic acid in the Watsonia polysaccharide is a point of difference from hitherto examined xylans. Apart from the cereal gums, all xylans have been found to contain D-glucuronic acid and/or 4-O-methyl-D-glucuronic acid. However, the main point of difference lies in the almost complete substitution of the hydroxyl groups on $C_{(2)}$ and $C_{(3)}$ of the xylose in the β 1 → 4-linked basal chain by side chains; a type of structure which has never been reported in the xylan series of hemicelluloses or in the type C gums.

Thus whilst Watsonia polysaccharide is closely allied to the xylan hemicelluloses, its function would not appear to be the same; though structurally it is much more similar to this group of polysaccharides/...

saccharides than to the type C gums.

The main outstanding structural feature which has not been proved is the direct attachment of the arabinofuranose groups to the basal chain. Due to the ease of hydrolysis of this furanose link, the best method to adopt would be to degrade the molecule with an enzyme preparation. This has succeeded in similar circumstances, when a hemicellulase from Myrothecium verrucaria[96] was used to obtain the fragment O-(α or β)-L-arabinofuranosyl-(1 → 3)-O-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose thus proving arabinose to be an integral part of the molecule.

Biologically, the occurrence of a polysaccharide in the corm-sacs of Watsonia pyramidata is a point of extreme interest. The virtual absence of starch in the corm and the disappearance of the polysaccharide during the growing period of the plant, is strong evidence to support an assumption that this material is in fact the reserve food supply of the plant. If this is the case, then Watsonia polysaccharide should be classed neither as a hemicellulose nor a gum exudate but as a mucilage. It has properties in common with mucilages, outstanding amongst these being the tendency to form colloidal solutions in water.

Salep mannan was one of the first neutral mucilages to be examined in detail, and has been shown to consist of a linear polymer of 1⟶4-linked mannose residues[135], structurally very similar to the mannan hemicelluloses. Salep mucilage obtained from the powdered tubers from Orchidaceae appears to bear a similar relationship to mannan hemicelluloses as does Watsonia polysaccharide to the more complex xylans. It is interesting to note that if Watsonia polysaccharide is a mucilage it is unusual in containing large quantities of D-xylose; most neutral mucilages consist of D-galactose and L-arabinose. The polysaccharide from Watsonia versveldii seed-boxes is almost certainly a mucilage, and like most seed-box mucilages contains a uronic acid (probably galacturonic acid as in other seed-box mucilages) and has the same sugar components as the mucilage from Plantago arenaria[136]. A comparison of the

chemistry/.....

chemistry of any seed-box mucilage which may be found in Watsonia pyramidata with the corm polysaccharide would be an investigation of great interest.

The insolubility of Watsonia corm polysaccharide in water raises an interesting point; for the gum to leave the corm-sacs, as presumably it must to be used as a reserve food supply, osmosis through the sac-walls will take place. Unless the polysaccharide is soluble in water this is not possible. However, possibly the polysaccharide undergoes some type of enzymatic degradation to solubilize it. The extraction of this enzyme system from the corm and its use in degrading the polysaccharide in vitro, whilst probably an extremely difficult and tedious task, would be well worth attempting to obtain a better understanding of how this reserve plant food is utilized. Conversely, the polysaccharide must be built up inside the corm-sacs from smaller units, presumably through an enzyme catalysed reaction. Starting initially with glucose, through uridine 5-(α-D-glucopyranosyl pyrophosphate), and uridine 5-(D-xylopyranosyl pyrophosphate) to the xylose backbone. It has been shown by isotopic labelling that the xylose in a xylan originates from the first five carbon atoms of D-glucose[137]. It has also been shown that L-arabinose can be incorporated into a xylan by an enzymatic reaction[138], and presumably in the same way D-galactose would also be incorporated.

PART 2

EXPERIMENTAL

EXPERIMENTAL

(A). General conditions. Paper chromatography was carried out on Whatman No.1 paper with the following solvent systems (all v/v): (a) butan-1-ol - ethanol - water (4 : 1 : 5, upper layer), (b) ethyl acetate - acetic acid - formic acid - water (18 : 3: 1 : 4), (c) ethyl acetate - pyridine - water (10 : 4: 3), (d) butan-1-ol - acetic acid - water (2: 1 : 1), (e) propan-2-ol - acetic acid- water (7 : 1 : 2), (f) butan-2-one - acetic acid - saturated aqueous boric acid (9 : 1 : 1), (g) butan-2-one - water azeotrope, (h) toluene - ethanol - water (270 : 30 : 1). Paper ionophoresis was carried out for 4 hr. at 10V/cm. in 0.2M-borate buffer[97] at pH 9.2. R_{gal}, R_G, and M_g refer to rates of movement relative to galactose, 2,3,4,6-tetra-O-methylglucopyranose, and glucose respectively. Infra-red spectra were measured with a Unicam S.P. 100 instrument, whilst d-spacings in crystalline materials were measured with a Philip's Model PW 1050 X-ray diffractometer (Co-K α and Cu-K α radiation). Gas-liquid chromatography was carried out on a Beckman GC-2A instrument, using helium as carrier gas, with a 4-foot column of ethylene glycol succinate (14% on 80 - 100 mesh Gas Chromosorb W)[98] at 166°, and a flame ionization detector. Retention times[99] were measured relative to 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside. Unless otherwise stated (a) solutions were concentrated at 40° and 20 mm. Hg in a rotary evaporator, (b) specific rotations were measured on aqueous solutions at ca.20°, (c) melting points are uncorrected and (d) sugars were revealed on paper chromatograms by spraying with the p-anisidine hydrochloride reagent[60] and assayed by

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fraction C (3.6 g.),$[\alpha]_D$ -88° (c 1.14), equiv., 17,000 (Found: sulphated ash, 4.62; Ac,0.04; OMe, 0.3; N, 0.5%).

Portions of each fraction were hydrolysed, the products being as follows: from fraction A, arabinose (3), xylose (1), galactose (1); from fraction B, arabinose (1), galactose (2), glucose (6); and from fraction C, arabinose (3), xylose (1), galactose (1). The proportions in brackets are visual estimates of mols. based upon paper chromatographic comparison with standard mixtures of sugars (solvents a,b, and c).

(ii) Fractionation of Watsonia corm polysaccharide. A solution of the gum in water was converted to the "acid" form by passing it through a column of Amberlite IR-120 (H^+) *; after concentrating to a 1.36% solution, this was used for all subsequent fractionation experiments.

(a) Ethanol fractionation. Ethanol was added to a portion of the above solution to obtain gradient precipitation of the polysaccharide. All the polysaccharide was precipitated between ethanol concentrations of 65 and 70% yielding a fibrous precipitate which, after drying (for 4 hr. at 56° and 2 cm. Hg) had$[\alpha]_D$ -78° (c 0.25) and equiv., 20,300.

(b) Cetyltrimethylammonium bromide ("Cetavlon") fractionation[101]. "Cetavlon" gave no precipitation from the aqueous solution of the gum at pH 7 but when the gum solution (25 ml. containing 340 mg. of gum) was added to an equal volume of borate buffer at pH 8.5[102] and the mixed solution poured into a 5% solution of "Cetavlon" (25 ml.) a fibrous precipitate was formed immediately. This was kept for 1 hr., centri-

-fuged/.....

* A product of Rohm and Haas Co., Philadelphia, U.S.A.

fuged, washed with water, redissolved in 2N-acetic acid, and poured into ethanol to precipitate the polysaccharide. This was washed successively with ethanol and ether and dried *in vacuo* at 18° for 3 days, when a white powder (320 mg.; 94% of original), $[\alpha]_D$ -78° (c 0.27), equiv., 21,000, was obtained. The pH of the centrifugate was then raised by the addition of N-sodium hydroxide; a further precipitate was formed between pH's 11.0 and 12.0. After being centrifuged and dried *in vacuo*, the dry precipitate (10.3 mg.; 3% of original) was recovered as described above $[\alpha]_D$ -74° (c 0.51), equiv. 20,500. The pH 12.0 supernatant liquid gave a negative response when assayed for carbohydrate. Hydrolysis of the two fractions gave arabinose, xylose, and galactose in respectively equal amounts, in the same ratio as that in the hydrolysate of the original fraction A.

(c) *Diethylaminoethyl-cellulose (DEAE) chromatography*.[103] A portion of the gum solution (containing 180 mg. of polysaccharide) was made 0.005M with respect to phosphate buffer pH 6.0 and added to the top of a column (28 x 3.3 cm.) of Whatman* DEAE-cellulose powder buffered with 0.005M-phosphate buffer pH 6.0. The carbohydrate solution eluted by the same buffer was treated with anion and cation exchange resins, precipitated with ethanol (4 volumes) and dried (for 4 hr. at 56° and 1 mm.Hg) yield 98 mg. (55%) of original), $[\alpha]_D$ -78° (c 0.28), equiv., 20,900 (Found: sulphated ash 0.12%). Stepwise elution up to 0.25M-phosphate buffer pH 6.0 gave no further carbohydrate but gradient elution with sodium

hydroxide/....

* A product of W. & R. Balston Ltd., Maidstone, England.

hydroxide gave a further small fraction eluted with 0.5N-sodium hydroxide. This latter fraction was treated with Amberlite IR-120 (H^+) to remove sodium ion, precipitated by ethanol, and dried, yielding a product (38 mg.), $[\alpha]_D$ -7° (c 1.2), equiv., 600 (Found: carbohydrate 5%). Hydrolysis gave arabinose, xylose, and galactose only from both fractions, in the same proportions as in the original fraction A. In the second fraction, there was no indication of any uronic acid which would have been expected in view of the low equivalent weight.

(d) Anion-exchange chromatography. Duolite A4 * , being granular, had superior flow characteristics to DEAE-cellulose. After small scale experiments had been performed, the optimum loading for a column of Duolite A4 (OH^-) was found to be 0.73 mg.gum / ml. of resin but in order to reduce the quantity of resin used, a loading of 1.35 mg. / ml. was used and found to be satisfactory. The polysaccharide (2.7 g.) in water (800 ml.) was passed through a column (62 x 5 cm.) of Duolite A4(OH^-) and the fraction eluted with water was collected, evaporated nearly to dryness, and added to four volumes of ethanol. The precipitate formed was washed with ethanol and ether and dried (for 4 hr. at 56° and 1mm. Hg) yielding a powder (2.58 g.) $[\alpha]_D$ -74° (c 0.15), equiv., 18,900 (Found: sulphated ash, 0.0%). Material still remaining on the column was eluted with 0.5N-sodium hydroxide, deionized on Amberlite IR-120 (H^+), and assayed for carbohydrate (Found; 19.8 mg.). The neutral solution was evaporated to a small bulk and poured into four volumes of ethanol, the precipitate formed being dried (for 4 hr. at 56° and 1 mm. Hg);

the product/.....

* A product of Chemical Process Co., Redwood City, California, U.S.A.

the product (104 mg.), $[\alpha]_D$ -7° (c 1.1), (Found: sulphated ash, 32.2%) had equiv., 610. It would appear that only 19% of the sodium hydroxide eluted fraction is carbohydrate, this constitutes only 0.74% of the total carbohydrate in fraction A. Hydrolysis of the fractions from Duolite A4, again gave only arabinose, xylose, and galactose in respectively equal amounts, the ratios being the same as in the original fraction A. As in the DEAE-cellulose fractionation, no uronic acid was found in the sodium hydroxide-eluted material. The only difference which could be detected between the two fractionation techniques (DEAE-cellulose and Duolite A4) was in the overall yield of polysaccharide, (75 and 100%, respectively).

(e) Dextran-gel chromatography. (i) Fraction A (34 mg.) in water was percolated through a column (18 x 2.5 cm.) of Sephadex G.50 * (medium grade) followed by water. Two fractions were eluted, the first containing 30 mg. and the second 4 mg. of carbohydrate (by assay). On hydrolysis each showed the same quantities of sugars as an hydrolysate of fraction A. A portion (30mg.) of the water-eluted fraction from Duolite A4 was eluted from Sephadex G.50 as a single fraction corresponding to the major peak from fraction A. (ii) Fraction A and the water-eluate from Duolite A4 each gave a single peak on percolation through Sephadex G.25 *(medium grade), indicating that the second fraction of fraction A eluted from Sephadex G.50 had molecular weight between 5,000 and 10,000.

Partial/.....

* Products of Pharmacia, Uppsala, Sweden.

(C). Partial acid hydrolysis of Watsonia corm polysaccharide.

The polysaccharide fraction A (30 g.) was homogenised in 0.01N-sulphuric acid (1 l.) and heated for 7 hr. at 96°, during which the specific rotation changed from -80° (initial) to +53° (final). The insoluble material which formed (315 mg.) was filtered off and gave a negative test for carbohydrate. The filtrate was cooled, neutralized with Duolite A4 (OH^-), filtered, and concentrated to a small volume (150 ml.) which, when added to methanol (450 ml.), afforded a precipitate. After being filtered and washed with 80% methanol, the precipitate was dried (for 5 hr. at 78° and 1 mm. Hg) yielding a white powder (5.251 g.), $[\alpha]_D$ -59° (c 2.625) (called ppt.1). The filtrate was concentrated to constant weight yielding a syrup (21.7 g.), $[\alpha]_D$ +83° (c 2.19) (syrup 1; plate 1). Ppt.1 (5.251 g.) was dissolved in 0.01N- suphuric acid (200 ml.) and hydrolysed for 6 hr. at 96°. During hydrolysis a change in specific rotation from -59° (initial) to -11° (final) was noticed. Neutralization, precipitation, and separation as previously, gave ppt.2 (2.404 g.), $[\alpha]_D$ -53° (c 1.09), and syrup 2 (2.323g.) $[\alpha]_D$ +32° (c 3.32). Hydrolysis of ppt.2 in similar manner (for 7 hr. at 96°) afforded ppt.3 (1.036 g.), $[\alpha]_D$ -39° (c 2.07) and syrup 3 (1.10 g.), $[\alpha]_D$ -8° (c 2.2). Hydrolysis of ppt.3 for 4 hr. at 96° in 0.01N-sulphuric acid gave ppt.4 (670 mg.), $[\alpha]_D$ -42° (c 0.9, 0.5N-NaOH), and syrup 4 (202 mg.), $[\alpha]_D$ -17° (c 2.2). Elution of the Duolite A4 resin used for neutralization with 0.5N-sodium hydroxide, yielded no carbohydrate-containing material. The molar ratios of mono-

and/...

PLATE 1 Chromatogram of Syrup 1 in solvent c.

A = Standard mixture of sugars

B = Syrup 1

PLATE 3 Chromatogram of Syrups 2,3,4, and Fraction VI in solvent c.

C = Syrups 2,3,4, and Fraction VI

D = Standard series of xylose oligomers

A = Standard mixture of sugars

Key to plates 1 and 3

a = origin
b = xyloheptaose
c = xylohexaose
d = xylopentaose
e = xylotetraose
f = xylotriose
g = $gal_p \alpha \rightarrow 3ara_f \alpha \rightarrow 2ara$
h = $gal_p \alpha \rightarrow 3ara$
i = galactose
j = xylobiose
k = arabinose
l = xylose
m = rhamnose

PLATE 1.

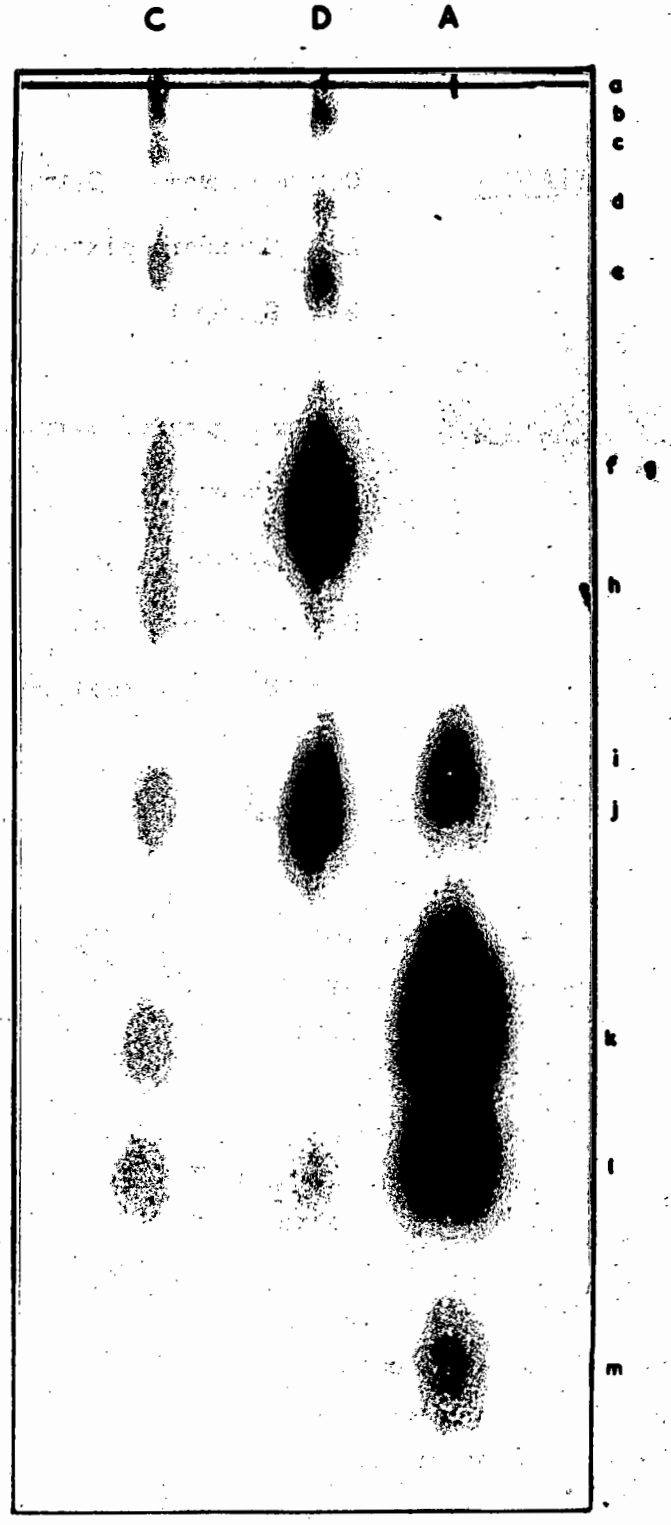


PLATE 3.

and oligosaccharides in syrups 1,2,3, and 4 are given in table 8 (syrups 1 and 2 assayed after preparative paper chromatography in solvent a; syrups 3 and 4 visually estimated from paper chromatograms in solvents a and b respectively).

Compound	R_{gal} solvent a	molar ratio			R_{gal} solvent b	molar ratio
		syrup 1	syrup 2	syrup 3		syrup 4
1	Origin*	trace		10	origin*	2
2	-	-		-	0.04 *	2
3	-	-	75	-	0.10 *	2
4	0.07*	14		3	0.24 *	3
5	0.18*			3	0.49 *	4
6	0.32	3	8	3	0.65	trace
7	0.52	8	7	6	0.78	trace
8	0.60*				0.87 *	4
9	1.00	2	3	1	1.00	trace
10	1.42	29	23	1	1.29	10
11	1.79	1	1	-	1.35	

TABLE 8.

*Estimated as xylose oligomers (all were xylose containing, as shown by spraying the chromatograms with barbituric acid in glacial acetic acid)[104].

Syrup/....

Syrup 1 (21.7 g.) was triturated with methanol, yielding crystalline arabinose (1.83 g.) and a residual syrup (19.8 g.). The sugars in this syrup were separated on a charcoal-Celite column[105] (85 x 4 cm.), using first water and then step-wise increase in ethanol concentration, as eluting solvents, yielding six main fractions over three months. Purity of the fractions was determined by paper chromatography (in solvents a and b).

Fraction I. The syrup (4.699 g.; eluted with water), $[\alpha]_D$ +104° (c 1.43), R_{gal} 1.42 and 1.29 (solvents a and b) was chromatographically homogeneous, and crystallised spontaneously. After recrystallisation from ethanol the crystals had m.p. and mixed m.p. 159.5° (with L-arabinose) and $[\alpha]_D$ +174°(6 min.) → +106° (60 min., constant; c 0.72). The derived benzoylhydrazone[106] had m.p. and mixed m.p. 199 - 200° (d) (with L-arabinose - benzoylhydrazone).

Fraction II. The syrup (396 mg.; eluted with water) gave three spots on chromatography (a) indistinguishable from fraction I, (b) chromatographically identical with xylose, and (c) identical with fraction III. Visual estimation of paper chromatograms showed a : b : c to be 7 : 4 : 2.

Fraction III. The syrup (618 mg.; eluted with water), $[\alpha]_D$ +80° (c 0.84), R_{gal} 1.00 and 1.00 (solvents a and b) and chromatographically homogeneous, was crystallised and recrystallised from aqueous methanol giving crystals m.p. 165.5° and mixed m.p. (with D-galactose) 165°, $[\alpha]_D$ +124° (15 min.) → +78° (equil.) (c 1.09). The derived 1-methyl-1-phenyl-hydrazone[106] had m.p. and mixed m.p. 186° with an authentic specimen.

Fraction/.....

Fraction IV. The syrup (3.694 g.; eluted with 4% ethanol), $[\alpha]_D$ +182° (c 5.78), M_g 0.75 had R_{gal} 0.52 (in solvent a), R_{gal} 0.78 (in solvent b), R_{gal} 0.76 (in solvent c), and was chromatographically identical with 3-O-α-D-galactopyranosyl-L-arabinose prepared from A.cyanophylla gum[28]. Hydrolysis for 5 hr. (in N-sulphuric acid at 96°), preparative scale paper chromatography, and assay of the separated arabinose and galactose (identical with standards in solvents a, b, and c), showed the molar ratio of galactose to arabinose to be 1.1 : 1.0, (the rotations of these aqueous extracts were $[\alpha]_D$ +75° and + 94° respectively).

The syrupy disaccharide (1.5 g.) was dissolved in hot methanol and after removal of a small amount of insoluble material, was kept for two months at 0°. The crystals formed were separated by filtration, washed with methanol, and dried at 100° in vacuo for 2 hr., yielding the galactose-arabinose disaccharide, m.p. 156.5 - 157.5°, $[\alpha]_D$ +202° (7 min.) → +198° (50 min., constant; c 1.08) (Found: C,42.3; H,6.1. Calc. for $C_{11}H_{20}O_{10}$: C,42.3; H,6.45%). A crystallographic report described the product as "an aggregate of fibrolamellar structure, all but extremely thin pieces of which are opaque to cloudy. Extinction is parallel to the long axis of the fibres; refractive index 1.52 for the long direction. Double refraction is moderate. The crystals are length slow". A second portion of the syrupy disaccharide (1.5 g.), dissolved in hot methanol and seeded with a crystal of the first preparation, was stored for one week at 0°. Microscopic aggregates of crystals were formed (1 g., after being dried at room termperature

in vacuo/....

in vacuo for 3 hr.) m.p. and mixed m.p. (with the first preparation) 156.5 - 157.5°, $[\alpha]_D$ +198° (equil. value, reached within 2 min.) (c 0.99) (Found: C,42.3; H,6.6%).

X-Ray powder diffractograms of the two preparations gave a large ratio of peak height to width, indicating that both samples were well crystallised. The first preparation had d-spacings (in Å) as follows: 13.3s, 10.5s, 8.36w, 6.64m, 4.96vw, 4.80vw, 4.65m, 4.40vs, 3.95vw, 3.81m, 3.39w, 3.34w, 3.15w, 3.04w, 2.82w; whilst the second preparation had all the d-spacings listed above and, in addition, d-spacings as follows: 8.19w, 6.56s, 6.19m, 4.85m, 4.53m, 4.20m, 3.65w. When the second crystalline product was heated in vacuo at 100° for 3 hr., no change in this pattern resulted.

Infra-red spectra (in Nujol mull and potassium chloride discs) of the two crystalline preparations, showed $\nu_{max.}$ in the 750 - 1200 $cm.^{-1}$ region as follows: 773w, 817s, 849w, 865vw, 898m, 923w, 949m, 972sh, 995s, 1007vw, 1020w, 1037w, 1047sh, 1058w, 1080s, 1109m, 1144vs, (peak intensities are all relative to the general contour of the spectrum); the second preparation however, absorbed very weakly at 796 $cm.^{-1}$, whilst peak heights varied from one spectrum to the next.

The syrupy disaccharide (100 mg.) was converted to the phenylosazone, by using the method adopted by Jones and Curtis (for the phenylosazone of 3-O-β-D-xylopyranosyl-D-xylose)[107]. Recrystallisation from hot water afforded crystals (12.5 mg.), $[\alpha]_D$ +82° (c 0.32 in pyridine-ethanol, 7 : 3) having an ultra-violet absorption spectrum

characteristic/...

characteristic of a disaccharide phenylosazone[108], and chromatographically homogeneous in solvent h, with m.p. 235° (lit. values for the osazone of 3-O-α-D-galactopyranosyl-L-arabinose, 240°[85a] and 235°[28]). In addition it had a mixed m.p. (with the osazone of a disaccharide (prepared from reduced A.cyanophylla gum,[109] and having identical R_{gal} values in a, b, and c) having m.p. 234°) of 235°. The infra-red spectra (potassium chloride disc) of these two osazones were identical, as were their X-ray diffractograms each of which showed well crystallised compounds, with the following d-spacings (in Å): 21.50vs, 16.30vw, 11.15w, 8.22s, 7.46m, 6.24m, 5.66w, 5.26m, 4.69 w, 4.49vw, 4.31m, 4.06vw, 3.97m, 3.66vw, 3.50vw, 3.28vw, 3.19vw, 2.98vw.

Hydrolysis of the phenylosazone (from Watsonia) (2 mg.) yielded galactose as the only sugar (in solvents a, b, and c). The phenylosazone (4.8mg.) in aqueous ethanol (10 ml.), was oxidized with periodate (for 2 hr.); no precipitate of mesoxalic dialdehyde 1,2-bisphenylhydrazone was formed but the reaction mixture yielded 1.01 mol. of formaldehyde (assayed by the chromotropic acid method[110]) and 1.00 mol. of formic acid per mol. of phenylosazone. (After 24 hr. oxidation, the formaldehyde and formic acid released were 0.97 and 1.02 mols. respectively). These results are consistent only with a 1 → 3-linkage in the disaccharide.

The method of Charlson, Gorin, and Perlin[111] was followed in converting the disaccharide (100 mg.) into the expected 2-O-α-D-galactopyranosyl-L-erythritol, by lead tetra-acetate oxidation (for 15 min. at

18° in/.....

18° in 98.4% acetic acid), followed by sodium borohydride reduction. Recrystallisation from ethanol yielded crystals (14 mg.), m.p. and mixed m.p. (with 2-O-α-D-galactopyranosyl-L-erythritol) 153°, $[\alpha]_D$ +143° (c 1.2), R_{gal} 0.71, 0.82 (in solvents a and c respectively). Hydrolysis (in 0.5N-sulphuric acid for 5 hr. at 96°) gave the expected galactose and erythritol. Infra-red spectra of this preparation and the authentic sample were identical.

Methylation of the disaccharide (300 mg.) by the Haworth[112] and Purdie[113] procedures gave a syrup (243 mg.) which crystallised spontaneously at 4°; the crystals, after recrystallisation three times from petroleum ether (b.p. 60 - 80°), appeared as needles, m.p. 88.6-88.9°, $[\alpha]_D$ +164° (c 0.86) (Found: OMe, 51.5 Calc. for $C_{18}H_{34}O_{10}$: OMe, 52.9%), and mixed m.p. (with a sample from A. cyanophylla gum) 85 - 86°, (lit. values for the hepta-O-methyl derivative of 3-O-α-D-galactopyranosyl-L-arabinopyranose are m.p. 82°, $[\alpha]_D$ +162°[22] and m.p. 87 - 88°, $[\alpha]_D$ +168°[28]). Hydrolysis of the crystalline methylated disaccharide gave 2,3,4,6-tetra-O-methylgalactose (R_G 0.89 and 0.81 in solvents a and g) and 2,4-di-O-methylarabinopyranose (R_G 0.60 and 0.33 in solvents a and g), easily distinguished from its 2,3- and 3,4- isomers by paper chromatography in solvent a (R_G 0.65 and 0.47 respectively). Methanolysis and gas-liquid chromatography of the methylated disaccharide gave the methyl-glycosides of the same two methylated sugars, with retention times of 1.88sh and 2.01 for the galactose derivatives, and 2.95sh and 3.15 for the arabinose derivatives.

Fraction V. The syrup (2.615 g.; eluted with 9% ethanol), $[\alpha]_D$ +69° (c 5.230/...

(c 5.230, equilibrium), was chromatographically homogeneous and had R_{gal} 0.32 (in solvent a), R_{gal} 0.65 (in solvent b) and R_{gal} 0.63 (in solvent c). Hydrolysis of fraction V (2 mg.) with 0.01N-sulphuric acid for 3 hr. at 96°, liberated fraction IV, galactose, and arabinose, all identical with standards (in solvents a, b, and c); after 24 hr., fraction V was completely hydrolysed to the above three components. Total hydrolysis of the syrup (N-sulphuric acid for 5 hr. at 96°), subsequent paper chromatography of the neutralized hydrolysate, and assay, showed the ratio of galactose to arabinose to be 1 : 2.1 by mols., indicating a trisaccharide.

The syrupy trisaccharide (14 mg.) was dissolved in water (1 ml.) and sodium borohydride (7 mg.) added, the resulting solution being allowed to stand for three days. After deionizing with Amberlite IR-120 (H^+) the solution was evaporated to dryness (at 25° for 30 min. at 2 cm. Hg); methanol was then repeatedly evaporated off the solid to remove boric acid, leaving a non-reducing solid (10 mg.). Hydrolysis of this material (3.3 mg.) in 0.01N-sulphuric acid for 2 and 8 hr. at 96°, gave fraction IV, galactose, arabinose, and arabitol (the latter having R_{gal} 1.51 in solvent a, and being indistinguishable from an authentic sample in solvents a and f); the arabitol was revealed by spraying the chromatogram with the periodate / benzidine spray reagent[114] to distinguish it from the reducing sugars present which were also revealed with the p-anisidine hydrochloride spray. A further portion of the solid (6.68 mg.) was oxidized with 0.021M-periodate solution (25 ml.) for 20 hr. The reaction mixture yielded 1.06 mol. of formaldehyde and 1.9 mol. of formic acid per mol. of the reduced trisaccharide; after reducing the oxidized material with sodium borohydride, deionizing

with/.....

with Amberlite IR-120 (H^+) and Duolite A4 (OH^-), removing boric acid by methanol evaporation, and hydrolysing with Amberlite IR-120 (H^+) (in a sealed tube for 16 hr. at 100°), paper chromatography revealed arabinose (when developed with p-anisidine hydrochloride) and arabinose and glycerol (when developed with periodate/benzidine).

The syrup (270 mg.) was methylated by the Haworth and Purdie procedures yielding a syrup (285 mg.; dried to constant weight at 40° and 1.6 cm. Hg), $[\alpha]_D$ +41° (c 5.207, $CHCl_3$). Hydrolysis of the methylated trisaccharide gave: 2,3,4,6-tetra-O-methylgalactose, 2,5-di-O-methylarabinose, 3,5-di-O-methylarabinofuranose, and 3,4-di-O-methylarabinopyranose (all identical with standards in solvents a and g). Gas-liquid chromatography of the methanolysed methylated trisaccharide, confirmed the above identifications giving retention times (of the methyl glycosides) as follows: 2,3,4,6-tetra-O-methylgalactose 2.02, 2,5,-di-O-methylarabinose 2.33s, 4.63w, 3,5-di-O-methylarabinofuranose 1.28, 3.21, and 3,4-di-O-methylarabinopyranose 2.82. Attempted alkali degradation of the trisaccharide by an adaptation of the method described by Fainter[114a] was unsuccessful, no degradation having taken place after heating the trisaccharide (4.7 mg.) in 0.005N-sodium hydroxide (20 ml.) for 90 min. at 74°.

The above evidence is consistent only with the trisaccharide being O-α-D-galactopyranosyl-(1 → 3)-O-L-arabinofuranosyl-(1 → 2)-L-arabinose. Hudson's rules (see discussion pg. 54) indicate that the 1 → 2 link between the two arabinose residues is α.

Fraction/....

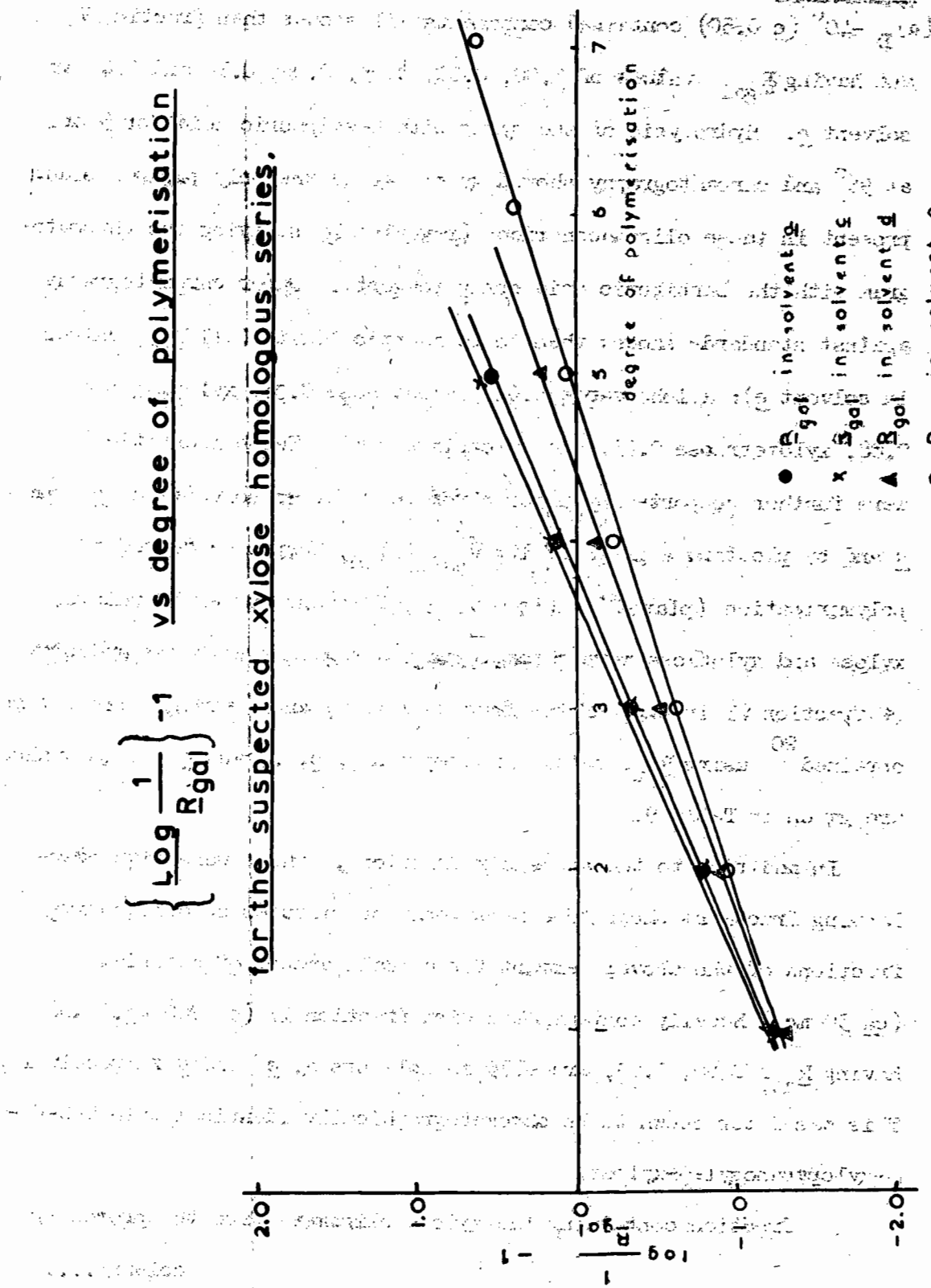

$\left\{\log \frac{1}{R_{gal}}\right\}^{-1}$ vs degree of polymerisation
for the suspected xylose homologous series.
degree of polymerisation
R_{gal} in solvent a
R_{gal} in solvent c
R_{gal} in solvent d
R_{gal} in solvent e
$\log \frac{1}{R_{gal}} - 1$
2.0
1.0
0
-1
-2.0
1
2
3
4
5
6
7

PLATE 2.

Fraction VI. The syrup (632 mg.; eluted with 70% methanol), $[\alpha]_D$ -40° (c 0.50) contained components all slower than fraction V, and having R_{gal} values of 0.00, 0.02, 0.05, 0.08, 0.13 and 0.40 in solvent e. Hydrolysis of the syrup with N-sulphuric acid for 5 hr. at 96° and chromatography showed xylose to be the only sugar residue present in these oligosaccharides (revealed by spraying the chromatogram with the barbituric acid spray reagent). Paper chromatography against standards showed them to be identical with (all R_{gal} values in solvent e): xyloheptaose 0.02, xylohexaose 0.05, xylopentaose 0.08, xylotetraose 0.13, and xylotriose 0.40. These identities were further supported by paper chromatography in solvents a, c, and d and by plotting a graph of $\left[\log \frac{1}{R_{gal}}\right]^{-1}$ vs suspected degree of polymerisation (plate 2) (to give a sufficient number of points, xylose and xylobiose were chromatographed together with the hydrolysate of fraction VI in each of the four solvents), when straight lines were obtained[90] using R_{gal} values in solvents a, c, d, and e: these values are given in Table 9.

In addition to the above six fractions, there were five overlapping fractions shown to consist only of mixtures of consecutive fractions of the above; except for a small amount of material (ca 30 mg.) heavily contaminated with fraction IV (ca 200 mg.) and having R_{gal} 0.60, 1.09, and 0.75 in solvents a, c and d respectively. This was later shown to be chromatographically identical with 4-O-β-D-xylopyranosyl-D-xylose.

The fraction containing the xylose oligomers from the preceding

column/....

Suspected degree of polymerisation	R_{gal} a	R_{gal} c	R_{gal} d	R_{gal} e
1	1.67	1.78	1.64	1.33
2	0.60	1.09	0.75	0.84
3	0.18	0.53	0.32	0.40
4	0.07	0.24	0.12	0.13
5	-	0.11	0.05	0.08
6	-	0.03	-	0.05
7	-	-	-	0.02

TABLE 9

column (fraction VI) was combined with syrups 2,3 and 4 (plate 3) (total weight 4.2 g.) and evaporated to a small bulk. This solution was charged to a charcoal / Celite column (85 x 4 cm.) and eluted first with water, and then step-wise with water containing increasing concentrations of ethanol, yielding seven major fractions and a number of overlapping fractions of these seven.

Fraction 1. The syrup (442 mg.; eluted with water) showed three components when chromatographed in solvents a, b, and c. These components were identical with authentic samples of xylose, arabinose, and galactose in the above solvents. Cellulose column chromatography (40 x 2.5 cm.) of the syrup and elution with solvent c, afforded three fractions. Fraction 1a. A syrup (67 mg.) crystallised and recrystallised

from/...

from 80% methanol was chromatographically homogeneous and had m.p. and mixed m.p. (with D-xylose) 150°, and $[\alpha]_D$ +90° (2 min.) → +21° (50 min., constant; c 1.35). The derived di-O-benzylidine dimethyl acetal had m.p. and mixed m.p. (with the di-O-benzylidine dimethyl acetal of D-xylose) 210°, and $[\alpha]_D$ -8° (c 0.7, $CHCl_3$). Fraction 1b. A syrup (330 mg.), $[\alpha]_D$ +107° (c 1.2) was chromatographically homogeneous and identical with arabinose in solvents a and c. Fraction 1c. A syrup (45 mg.) $[\alpha]_D$ +80°) (c 1.1) was chromatographically identical with galactose in solvents a, b, and c.

Fraction 2. The syrup (54 mg.; eluted with water), $[\alpha]_D$ +78° (c 0.6) was chromatographically homogeneous in solvents a,b, and c, and identical with galactose. Crystallisation and recrystallisation from glacial acetic acid yielded a compound which had m.p. and mixed m.p. (with D-galactose) 166°.

Fraction 3. The syrup (422 mg.; eluted with 5% ethanol) $[\alpha]_D$ +184° (c 2.3) was chromatographically homogeneous and identical with 3-O-α-D-galactopyranosyl-L-arabinose in solvents a, b, and c.

Fraction 4. The syrup (114 mg.; eluted with 6% ethanol) showed two components on paper chromatography in solvents a,b, and c,one identical with fraction 3 and the other identical with an authentic sample of 4-O-β-D-xylopyranosyl-D-xylose. The syrup was separated into two components on a cellulose column (40 x 2.5 cm.) when eluted with solvent c, Fraction 4a (identical with fraction 3) and fraction 4b both

chromato-/...

chromatographically homogeneous in solvents a and b.

Fraction 4b. The syrup (92 mg.), $[\alpha]_D$ -27° (c 0.67) had R_{gal} values of 0.60, 0.75, and 0.84 in solvents a, d, and e respectively. A portion of the syrup (5 mg.) was hydrolysed in N-sulphuric acid for 5 hr. at 96° yielding only xylose. The syrup (33 mg.) was mixed with phenylhydrazine hydrochloride (100 mg.), sodium acetate trihydrate (150 mg.) and distilled water (1.2 ml.). After heating in a boiling water bath for 30 min. and cooling, the derived phenylosazone crystallised and had m.p. 208.6° (d) (lit. values; 195 - 196°[c])[115]. Periodate oxidation of the osazone (1.41 mg.) for 2 hr. yielded no formaldehyde and no precipitate of the 1,2-bisphenylhydrazone of mesoxaldehyde, whilst 1.06 mol. of formic acid were liberated per mol. of phenylosazone. These results are consistent only if the disaccharide is 4-O-β-D-xylopyranosyl-D-xylose (xylobiose).

Acetylation of the syrup (33 mg.) with fused sodium acetate (60 mg.) and acetic anhydride (0.9 ml.) for 4 hr. under reflux, gave on evaporation of a chloroform extract of the reaction mixture, a syrup (29 mg.) which, on crystallisation and recrystallisation from equal volumes of ethanol and petroleum ether (b.p. 60-80°) (seeding with an authentic crystal of the hexa-acetate), gave a product m.p. and mixed m.p. 155-156° (with 4-O-β-D-xylopyranosyl-D-xylose hexa-acetate), $[\alpha]_D$ -70° (c 0.66, $CHCl_3$) (lit. value; -74°[c])[116]. Thin-layer chromatography of the acetate on Silica-gel G[117] showed it to be identical to an authentic sample.

Fraction 5. The syrup (330 mg.; eluted with 13% ethanol had $[\alpha]_D$ +70° (c 2.8)/.....

(c 2.8) and was chromatographically identical with O-α-D-galactopyranosyl-(1 → 3)-O-L-arabinofuranosyl-(1 → 2)-L-arabinose in solvents a, b and c.

Fraction 6. The syrup (246 mg.; eluted with 18% ethanol) had $[\alpha]_D$ -38° (c 2.0), was chromatographically homogeneous in three solvents, and, on hydrolysis (in 0.01N-sulphuric acid for 5 hr. at 96°) gave fraction 4b and xylose only. Total hydrolysis with N-sulphuric acid for 5 hr. at 96° gave xylose as the only component in solvents a, b and c. Crystallisation and recrystallisation of a portion of the syrup (80 mg.) from 85% ethanol afforded a product (28 mg.), $[\alpha]_D$ -45° (c 0.5) which softened at 154° and had m.p. 203° (d); an authentic sample of O-β-D-xylopyranosyl-(1 → 4)-O-β-D-xylopyranosyl-(1 → 4)-D-xylose (xylotriose) softened at 156° and had mixed m.p. 204° (d). The syrup (10 mg.) was converted to the phenylosazone by using the same conditions as suggested by Whistler and Tu[115]. The small amount of crystals which formed were drained on a porous tile and washed, yielding a crystalline product m.p. 218-219°(d) (lit. value for xylotriosazone; 214-215°[115]). A portion of the mother liquors from the crystallisation of the xylotriose (46 mg.) was acetylated by the method of Whistler and Tu[116], giving a crystalline product (crystallised from a mixture of equal volumes of ethanol and petroleum ether (b.p. 60-80°) which had m.p. and mixed m.p. (with xylotriose octa-acetate) 111° and $[\alpha]_D$ -90° (c 0.4, $CHCl_3$). Thin-layer chromatography on Silica-gel G showed the acetate to be identical with a standard.

Fraction 7. The syrup (114 mg.; eluted with 80% methanol) was chromatographically similar to fraction VI from the chromatographic separation

of/.....

of syrup 1, namely a series of xylose oligomers with degree of polymerization from four to six. The graph of $\left[\log \frac{1}{R_{gal}}\right]-1$ vs suspected degree of polymerisation (plate 2) for these three components was again linear for solvents d and e.

Precipitate 4. This degraded polysaccharide (670 mg.), $[\alpha]_D$ -42° (c 0.90, 0.5N-NaOH) (isolated after consecutive hydrolyses of the original polysaccharide; ref. page 82), was only sparingly soluble in water but soluble in dilute sodium hydroxide solution. A portion (10 mg.) was hydrolysed (in N-sulphuric acid for 5 hr. at 96°) giving products chromatographically identical with xylose, arabinose, and glucose in solvents a, b, and c. Preparative - scale paper chromatography and assay showed the ratio of xylose to arabinose to glucose to be 11 : trace : 1. Reaction of a small portion of the polysaccharide in water with a dilute solution of iodine produced the characteristic blue colour associated with starch. A further portion of the degraded polysaccharide (308 mg.) was methylated by the procedures of Haworth and Purdie yielding a product which, after drying (for 2 hr. at 60° and 0.4 mm.Hg), weighed 192 mg. and had $[\alpha]_D$ -38° (c 1.48, $CHCl_3$) and OMe, 38.0%. The methylated degraded polysaccharide (10 mg.) was hydrolysed (in N-sulphuric acid for 5 hr. at 96°), neutralized with barium carbonate, and chromatographed in solvent a against standards. The components of the hydrolysate were shown to be 2,3,4,6-tetra-O-methylglucose (R_G 1.00), 2,3,4-tri-O-methylxylose (R_G 0.94), 2,3,6-tri-O-methylglucose (R_G 0.85) and 2,3-di-O-methylxylose (R_G 0.78). Gas-liquid chromatography confirmed these identities and gave the following approximate molar proportions of

each/...

each component (table 10).

Component	T	molar proportion
2,3,4-tri-O-methylxylose	0.467, 0.607	20
2,3-di-O-methylxylose	1.78, 1.99, 2.23	160
2,3,4,6-tetra-O-methylglucose	1.00, 1.54	3
2,3,6-tri-O-methylglucose	4.22, 6.16	15

TABLE 10

These results indicate a xylan with an average chain length of approximately nine xylose units, and a "starch type" material with an average chain length of approximately five units. The xylose to glucose ratio from these figures is found to be 10 : 1.

The 2,3-di-O-methylxylose in a portion of the methylated degraded polysaccharide hydrolysate (150 mg.) was separated by preparative paper chromatography in solvent a, extracted from the paper, and evaporated to a syrup which crystallised when seeded with an authentic sample, yielding crystals m.p.76 - 78°, and mixed m.p. (with 2,3-di-O-methylxylose) 77-79°. The derived anilide, after recrystallisation from a mixture of equal volumes of ethanol and petroleum ether (b.p. 60 - 80°), had m.p. 123-123.5° (recent lit. values for 2,3-di-O-methyl-N-phenyl-D-xylopyranosylamine; 145 - 146°,[118] 122 - 123°,[119] 120 - 122°,[120] 121°[121]).

The/...

The quantities of the various mono-, di-, tri-, and oligosaccharides recovered from the columns (including the contributions from the overlapping fractions) are presented in tables 11 (fractions I → VI and I → 7) and 12 (combined totals from the first and second columns); whilst a summary of the method adopted to obtain the partial hydrolysates for separating on the charcoal / Celite columns is given (flow sheet 1.), and histograms of the elution pattern are given in Plates 4 and 5.

Sugar	weight recovered (mg.)	
	fractions I → VI	fractions 1 → 7
Xylose	39	68
arabinose	4699	330
galactose	618	100
3-O-α-D-galactopyranosyl-L-arabinose	5559	422
"xylobiose"	30	92
O-α-D-galactopyranosyl-(1 → 3)-O-α-L-arabino-furanosyl-(1 → 2)-L-arabinose	2615	332
"xylotriose"	-	246
xylose oligomers	632	114

TABLE 11

PLATE 4.

Histogram of the elution of syrup 1 from charcoal / Celite after partial hydrolysis of Watsonia polysaccharide

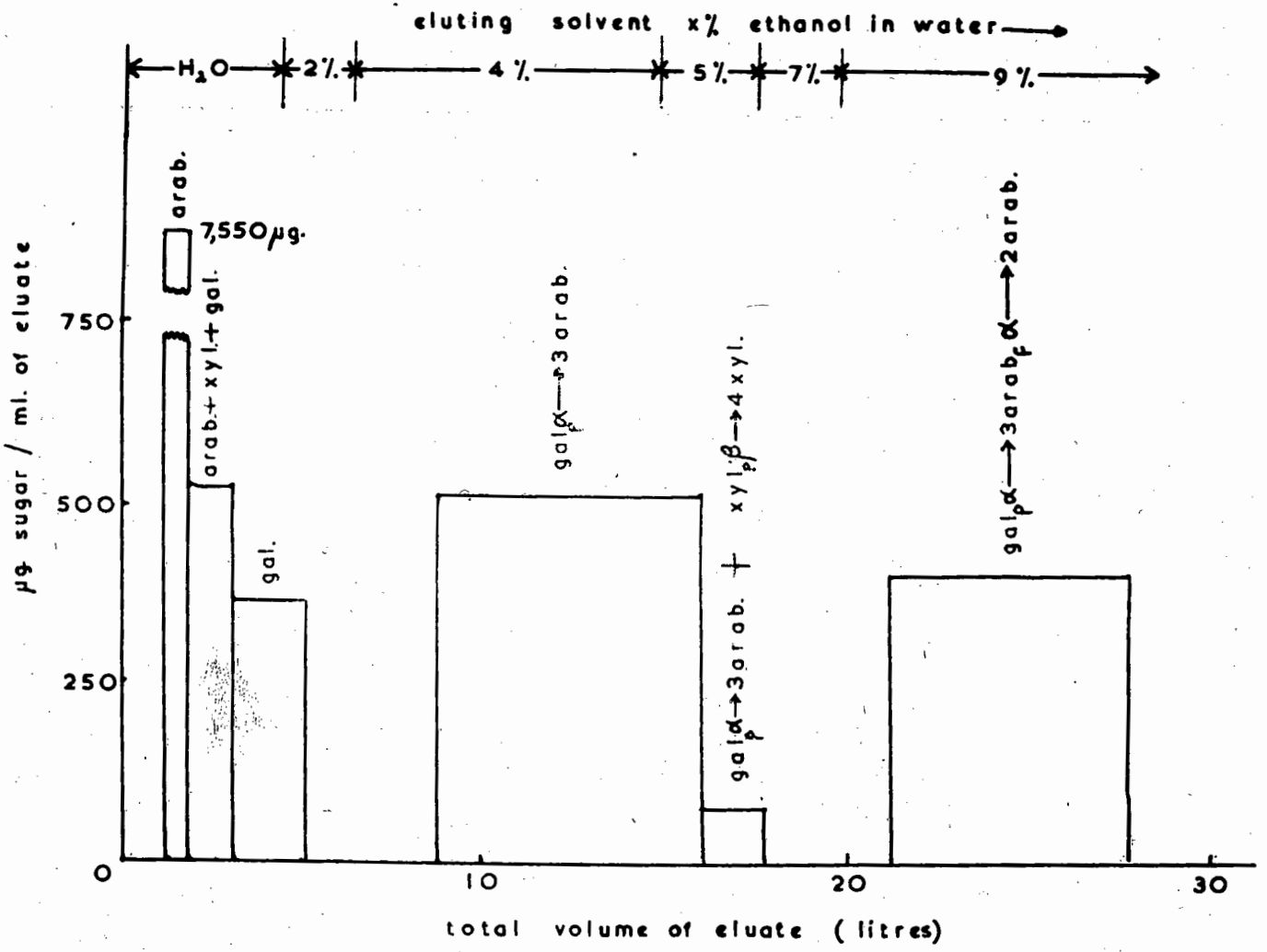
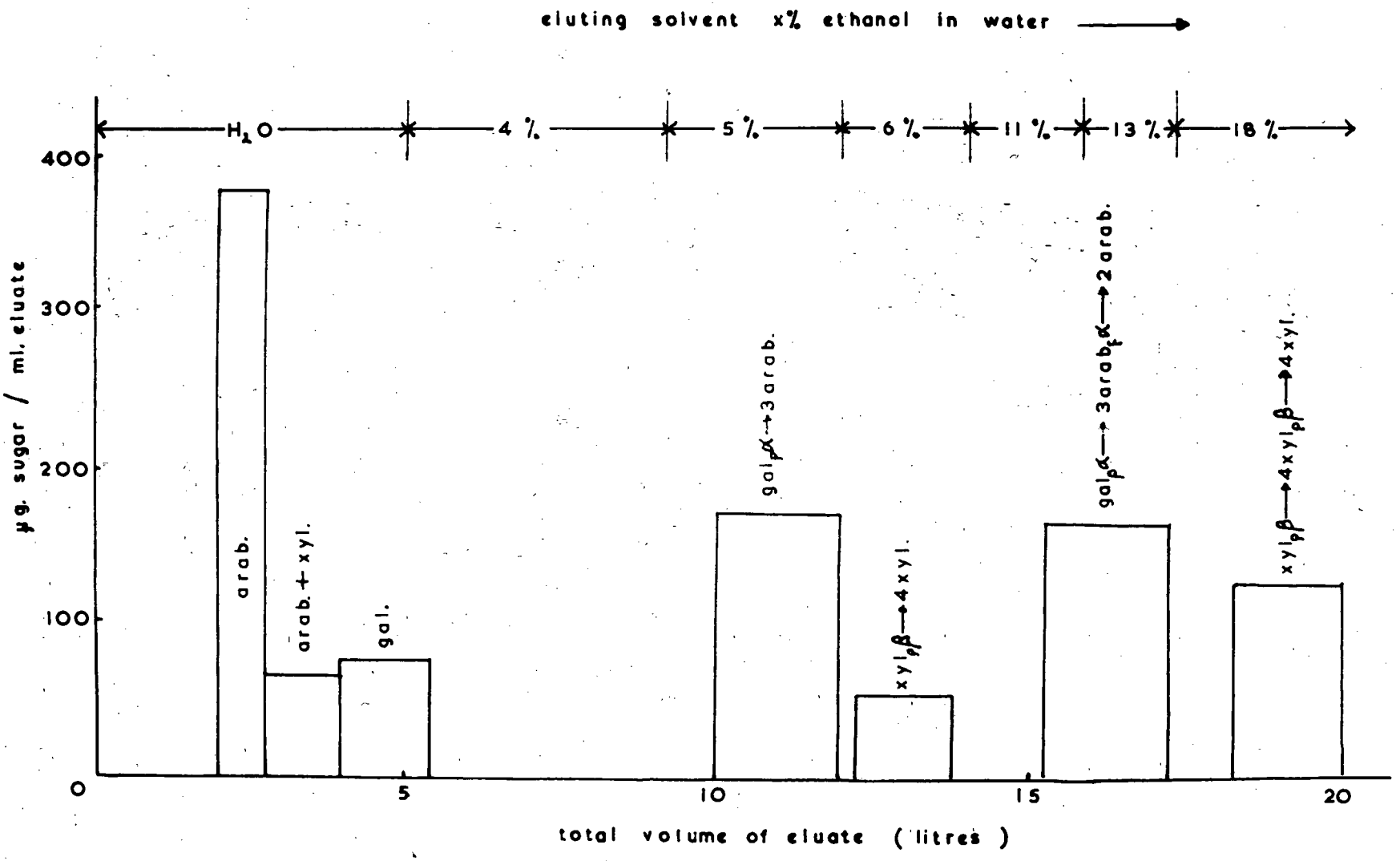


PLATE 5.

Histogram of the elution of syrups 2, 3, 4, and fraction VI from charcoal/Celite after partial acid hydrolysis of Watsonia polysaccharide



Sugar	Total weight recovered (mg.)
Xylose	107
arabinose	5029
galactose	718
3-O- α -D-galactopyranosyl-L-arabinose	5981
"xylobiose"	122
O- α -D-galactopyranosyl-(1 → 3)-O- α -L-arabinofuranosyl-(1 → 2)-L-arabinose	2947
"xylotriose"	246
xylose oligomers	114

TABLE 12

Watsonia polysaccharide

fraction A (30g.) $[\alpha]_D$ -80°

↓ 0.01N-sulphuric acid for 7 hr. at 96° then neutralization with $BaCO_3$

solution $[\alpha]_D$ +53° + non-carbohydrate residue (315 mg.)

↓ made 80% with respect to methanol

precipitate 1. (5.251 g.), $[\alpha]_D$ -59° + syrup 1. (21.7 g.), $[\alpha]_D$ +83°

↓ 0.01N-sulphuric acid for 6 hr. at 96°, followed by neutralization

syrup $[\alpha]_D$ -11°

↓ made 80% with respect to methanol

precipitate 2. (2.404 g.), $[\alpha]_D$ -53° + syrup 2. (2.323g.) $[\alpha]_D$ +32°

↓ 0.01N-sulphuric acid for 7 hr. at 96°, followed by neutralization and making 80% with respect to methanol

precipitate 3. (1.036 g.), $[\alpha]_D$ -39° + syrup 3. (1.10 g.), $[\alpha]_D$ -8°

↓ 0.01N-sulphuric acid for 4 hr. at 96°, followed by neutralization and making 80% with respect to methanol

precipitate 4. (670 mg.), $[\alpha]_D$ -42° + syrup 4. (202 mg.), $[\alpha]_D$ -17°

↓ methylation and hydrolysis

methylated sugars (Table 10)

syrup 1. (21.7 g.), $[\alpha]_D$ +83°

↓ charcoal / Celite column chromatography

fractions I → VI (Table 11)

syrup 4. (202 mg.), $[\alpha]_D$ -17°

+ syrup 3
+ syrup 2
+ fraction VI

↓ then charcoal / Celite column chromatography

fractions 1 → 7 (Table 11)

FLOW SHEET I

Methylation/....

(D). Methylation of the Watsonia corm polysaccharide and identification of the methylated monosaccharides released on acid hydrolysis.

Initially three different methods were used for the methylation of the polysaccharide which was sparingly soluble in water but all of them gave very low yields of the methylated product.

(a) The polysaccharide fraction A (10 g.) was suspended in water (100 ml.) and methylated by the procedure of Haworth, adding during 9 days, dimethylsulphate (400 ml.) and sodium hydroxide (246 g.). After heating to 60° for 1 hr. the solution was dialysed against running water until free from inorganic ion. The dialysate, after concentrating in vacuo yielded a syrup (11.2 g.) which afforded xylose, arabinose, galactose and traces of (presumably) methylated monosaccharides when hydrolysed for 3 hr. in N-sulphuric acid at 96°. The syrup was further methylated for 9 days using the same conditions as before, dialysed until salt free, and evaporated to a dry powder (12.6 g.) which was refluxed for 3 hr. with a mixture of methanol- chloroform- and dry dimethyl formamide, 5:5:2; filtration and evaporation to dryness gave a soluble fraction (4.6 g.; OMe, 24.2%) whilst the residue (7.6g.) had OMe, 32.6%. Neither of the two fractions appeared to contain any free methylated monosaccharides when subjected to paper chromatography in solvent a. Hydrolysis of the two fractions in N-sulphuric acid for 18 hr. at 100° gave the same six chromatographic components for each in solvent a, with R_G values of: 0.96, 0.89, 0.82, 0.74, 0.50 and 0.26. Further methylation of the soluble fraction (950 mg.) by the method of Kuhn[122] (with dry dimethyl-formamide, silver oxide, and methyl iodide), finally yielded a clear

yellow/...

yellow glass (120 mg.; dried at 100° for 2 hr., at 0.5 mm. Hg), $[\alpha]_D$ -90° (c 1.64, $CHCl_3$) (Found: OMe, 38.2%). Hydrolysis of this syrup (in N-sulphuric acid for 18 hr. at 100°) yielded on paper chromatography the same components as before, with the exception of that R_G 0.74 which was absent.

In view of the low overall yields (≪10%) of methylated polysaccharide in the above experiments a modification of the Kuhn procedure was used [123].

(b) Partially methylated polysaccharide(1.02 g.; from the "Haworth" methylation) was stirred for 16 hr. with dry dimethylformamide (70 ml.) before adding the silver oxide and methyl iodide, the yield from this however, was no better than in the preceeding experiment.

(c) A further method utilizing barium oxide instead of silver oxide to neutralize the hydriodic acid formed during methylation, and dimethyl sulfoxide as the solvent in place of dimethylformamide [124] was used, again giving a low yield of methylated polysaccharide. The dry powder obtained after methylation twice by the Haworth procedure (950mg.) was suspended in dimethyl sulfoxide (50 ml.), after addition of barium oxide (8 g.) and methyl iodide (25 ml.), the mixture was refluxed for 24 hr. at 45°, filtered, evaporated to a syrup, and extracted with chloroform; evaporation of this extract in vacuo yielded a syrup (80mg.) which, on hydrolysis, gave the same six components as those obtained from the polysaccharide which had been methylated twice by the method of Haworth.

By modifying the method of Haworth as follows, high yields of the methylated polysaccharide were obtained. Watsonia corm polysaccharide

fraction/....

fraction A (30 g.) was homogenised with N-sodium hydroxide (200 ml.) at 4°, methylated (for the first 8 hr. at 0° in an atmosphere of nitrogen and then over 10 days at room temperature and 4 days at 35°) with dimethyl sulphate (700 ml.) and sodium hydroxide (350 g.), dialysed against running water until free of inorganic ions, methanol, and dimethyl sulphate, and evaporated to a clear amber-coloured glass (25 g.), $[\alpha]_D$ -80° (c 1.4, $CHCl_3$) (Found: OMe, 36.8; sulphated ash, 1.4%). Hydrolysis of this material in N-sulphuric acid for 5 hr. at 96° yielded the same components as before in essentially the same amounts.

This partially methylated material (12.5 g.) was dissolved in equal volumes of dry dimethylformamide and chloroform, in which it was completely soluble (complete solution was not achieved when only dry dimethylformamide was used). Slow evaporation of the chloroform afforded a solution of the partially methylated polysaccharide in dimethylformamide. During a period of 2 weeks, silver oxide (50 g.) and methyl iodide (100 ml.) were added to this solution, the reaction taking place at ambient temperature and with constant vigorous stirring. The reaction mixture was filtered to remove unreacted silver oxide, silver iodide, and the silver iodide - dimethylformamide complex which had partially crystallised from the solution. The straw-coloured filtrate was evaporated to a small bulk; the addition of the chloroform afforded a precipitate of the silver iodide - dimethylformamide complex which was removed by filtration. Concentration of the filtrate and precipitation with chloroform was repeated until no more of the complex was removed by this method; when evaporated to dryness (at 80° and 2 cm. Hg to constant

weight/....

weight), the final filtrate afforded an amber-coloured glass (12.6 g.). The glass (12.6 g.) was dissolved in chloroform (50 ml.) and the solution extracted with a solution of aqueous potassium cyanide (5%) to remove any remaining silver iodide and silver iodide-dimethylformamide complex. This procedure was repeated three times, the chloroform layer dried (over anhydrous sodium sulphate), and evaporated in vacuo to a clear pale-yellow glass (7.85 g.), $[\alpha]_D$ -84° (c 0.74, $CHCl_3$) (Found: OMe, 37.1; sulphated ash 0.0%).

This material (7.85 g.) was dissolved in methyl iodide (100 ml.), silver oxide (20 g.) added, and refluxed at 48° (bath temperature) for ten days. The reaction mixture was then filtered to remove silver iodide and silver oxide and the filtrate was evaporated to dryness (at 60° and 2 cm. Hg to constant weight) yielding a clear glass (6.3 g.), which had $[\alpha]_D$ -86° (c 4.15, $CHCl_3$) (Found: OMe, 37.5; sulphated ash < 0.1%). Remethylation by the Purdie procedure left the methoxyl and sulphated ash contents unchanged. The methylated polysaccharide, when dissolved in chloroform, gave no precipitate on the addition of petroleum ether (b.p. 60 - 80°) to a concentration of 90%. This, coupled with the constant methoxyl content on further "Purdie" methylation, is a reasonable justification for regarding the Watsonia corm polysaccharide as being fully methylated and essentially homogeneous at this stage.

Hydrolysis of the methylated polysaccharide (20 mg.) in 98/100 formic acid for 1 hr. at 96°, and then, after evaporation of the bulk of the formic acid, in N-sulphuric acid for 5 hr. at 96° afforded the

following/....

following components (tentatively identified against standards) when chromatographed in solvents a and g (plate 6) (R_G values quoted for solvent a).

(i) 2,3,5-tri-O-methylarabinose (0.96)

(ii) 2,3,4,6-tetra-O-methylgalactose (0.87),

(iii) 2,5-di-O-methylarabinose (0.84),

(iv) 3,5-di-O-methylarabinose (0.81),

(v) 2,3,6-tri-O-methylgalactose (0.74),

(vi) and

(vii) two minor components staining pink with the p-anisidine hydrochloride spray reagent (0.48 and 0.37 respectively), and

(viii) xylose (0.26), the latter revealed by its characteristic green colour when sprayed with the barbituric acid spray reagent. In view of the possibility of demethylation of the sugar residues occurring during hydrolysis in 98/100 formic acid[92], different hydrolysing agents were used to obtain (by visual inspection of paper chromatograms) conditions which gave rise to the least amount of demethylation. (a) Initial hydrolysis with 98/100 formic acid, for 1 hr. at 96°, followed by hydrolysis with N-hydrochloric acid (for 4 hr. at 96°) yielded more of the component R_G 0.74 than was obtained with formic acid followed by N-sulphuric acid, whilst there appeared to be a decrease in the amount of component R_G 0.87. (b) Hydrolysis in N-sulphuric acid for 5 hr. at 96° yielded an hydrolysate in which the component R_G 0.74 appeared to be of the order of half that found in hydrolyses where formic acid was used initially. However, at the end of 5 hr. the original methylated poly-

saccharide/....

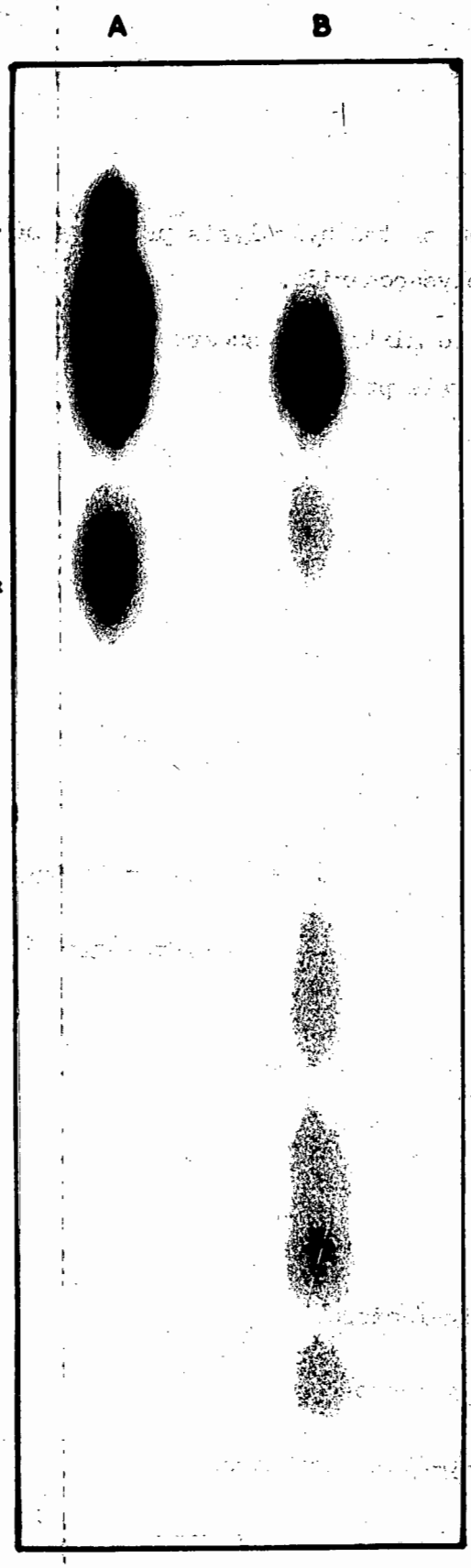


PLATE 6

PLATE 6 Chromatogram of the hydrolysis products of the methylated Watsonia polysaccharide.

A = Standard mixture of sugars

B = Hydrolysis products

Key to plate 6.

a = galactose

b = arabinose

c = xylose

d = 3-O-methylarabinose

e = rhamnose

f = 3-O-methylxylose

g = 2,3,6-tri-O-methylgalactose

h = 2,4,6-tri-O-methylgalactose

i = 2,5- and 3,5-di-O-methylarabinose

j = 2,3,4,6-tetra-O-methylgalactose

k = 2,3,5-tri-O-methylarabinose

saccharide was not completely dissolved, with consequent loss of weight of the methylated monosaccharides. (c) The method finally adopted, which gave an amount of component R_G 0.74 comparable with that in (b), was as follows: the methylated polysaccharide (5 g.) was dissolved in a mixture of N-sulphuric acid (135 ml.) and 98/100 formic acid (15 ml.), hydrolysed for 5 hr. at 96°, neutralized with barium carbonate, filtered, and the filtrate treated with Amberlite IR-120 (H^+) and Duolite A4 (OH^-) to remove barium formate (paper chromatography of the filtrate had shown no component which could have been methylated uronic acid; this, coupled with the absence of any uronic acid fragment in the complete or partial acid hydrolysis of the gum allows the use of ion-exchange resins for deionazation at this stage). Concentration of the solution of methylated monosaccharides (at 60° and 2 cm. Hg to constant weight) yielded a syrup (4 g.), which was charged to a cellulose column (98 x 4.5 cm.) and eluted with petroleum ether (b.p. 100 - 120°) and butan-1-ol, with stepwise increase in the butan-1-ol concentration, the final eluant being butan-1-ol saturated with water. The following 13 fractions were obtained:

Fraction i. The syrup (635 mg.; eluted with petrol : butanol; 7 : 2) had $[\alpha]_D$ -35° (c 1.2), R_G 0.95 (solvent a) (Found: OMe, 46.9. Calc. for $C_8H_{16}O_5$: OMe, 48.4%), and was chromatographically homogeneous (in solvents a and g). Demethylation with 48% hydrobromic acid[60] gave a series of arabinose methyl ethers and arabinose, which were identified by paper chromatography against standards. Fraction i (100 mg.), bromine (0.25 ml.), and water (2 ml.) were heated together for 15 hr. at 30°. Excess bromine was removed with a stream of nitrogen and the solution

was/....

was neutralized with silver carbonate and centrifuged. The centrifugate after treatment with a stream of hydrogen sulphide was filtered, and evaporated to the derived syrupy L-arabonolactone $[\alpha]_D$ -59° (c 0.75). Methanolic ammonia (1 ml.) was added and left for 15 hr. at 5°; crystals of the derived arabonamide formed on evaporating off the solvent. m.p. 127°. Recrystallisation from ethyl acetate afforded a product, m.p. 134.6° and mixed m.p. (with 2,3,5-tri-O-methyl-L-arabonamide) 135°.

Fraction ii. The syrup (169 mg.; eluted with petrol : butanol; 7 : 3) gave two spots on chromatography, one (a) identical with fraction i, and the other (b) with fraction iii. Gas-liquid chromatography showed the ratio a : b to be 2 : 1 (by mols.).

Fraction iii. The syrup (397 mg.; eluted with petrol : butanol; 7 : 4) had $[\alpha]_D$ + 107° (c 3.32), R_G 0.87 (solvent a) (Found: OMe, 49.4. Calc. for $C_{10}H_{20}O_6$: OMe, 52.5%) and was chromatographically homogeneous. Demethylation and paper chromatography gave a series of galactose methyl ethers and galactose. Fraction iii (100 mg.) was dissolved in dry ethanol (1 ml.) and freshly distilled aniline (0.08 ml.), after refluxing for 2 hr. at 96° (in anhydrous conditions) and cooling to room temperature, crystals of the derived anilide were obtained, m.p. 196 - 197° and mixed m.p. (with 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine) 197°.

Fraction iv. The syrup (312 mg.; eluted with petrol : butanol; 7 : 5) had $[\alpha]_D$ -57° (c 1.12), M_g 0.72, R_G 0.81 (solvent a) (Found: OMe, 34.1. Calc. for $C_7H_{14}O_5$: OMe, 34.8%) was chromatographically homogeneous and

gave/...

gave 5-O-methylarabinose, 3-O-methylarabinose, and arabinose (identified by paper chromatography against standards) on demethylation. The derived lactone after recrystallisation from ethyl acetate had m.p. 73° (lit. values for 3,5-di-O-methyl-L-arabonolactone; 75°[125], 73°[126], 78°[127]). On paper chromatography the lactone had R_f values of 0.78 and 0.88 in solvents a and g respectively. The derived amide had m.p. 145°, $[\alpha]_D$ +15° (c 0.93) (lit. values for 3,5-di-O-methyl-L-arabonamide; 144°[126], 145°[125,127], $[\alpha]_D$ +10°[125]).

Fraction v. The syrup (339 mg.; eluted with petrol : butanol; 7 : 5) gave two spots on paper chromatography and electrophoresis identical (a) with fraction iv and (b) with fraction vi. Demethylation gave only mono-methylarabinoses and arabinose, whilst gas-liquid chromatography showed a : b to be 1 : 2 (by mols.)

Fraction vi. The syrup (192 mg.; eluted with petrol : butanol; 7 : 5) had $[\alpha]_D$ -43° (c 0.6), M_g 0.05, R_G 0.84 (solvent a) (Found: OMe, 32.3. Calc. for $C_7H_{14}O_5$: OMe, 34.8%) was chromatographically homogeneous and gave 5-O-methylarabinose, 2-O-methylarabinose, and arabinose on demethylation. The derived amide had m.p. 131.6-132.3° (lit. values for 2,5-di-O-methyl-L-arabonamide, 131°[128,16], 131-132°[129]).

Fraction vii. The syrup (14 mg.; eluted with petrol : butanol; 7 : 7) showed two spots on chromatography, in equal quantities and identical with fractions vi and viii.

Fraction viii. The syrup (215 mg.; eluted with petrol : butanol; 7 : 10), $[\alpha]_D$ +87° (c 3.59), R_G 0.74 (solvent a) (Found: OMe, 38.0. Calc. for $C_9H_{18}O_6$: OMe, 41.9%) was chromatographically homogeneous and gave a

series/....

series of methyl ethers of galactose and galactose on demethylation. The derived lactone had m.p. and mixed m.p. (with 2,3,6-tri-O-methyl-D-galactonolactone) of 98°.

Fraction ix. The syrup (24 mg.; eluted with petrol : butanol; 7 : 10), $[\alpha]_D$ +97° (c 0.455), showed two components on paper chromatography, very close together. Gas-liquid chromatography showed 2,3,6-tri-O-methylgalactose (1 part) and (probably) 2,4,6-tri-O-methylgalactose (6 parts).

Fraction x. The syrup (10 mg.; eluted with petrol : butanol; 7 : 20) was shown by gas-liquid chromatography to be probably 2,4,6-tri-O-methylgalactose (1 part) and fraction xi (1 part).

Fraction xi. The syrup (40 mg.; eluted with petrol : butanol; 7 : 50), $[\alpha]_D$ +27° (c 0.78), M_g 0.84, R_G 0.52 (solvent a) was chromatographically homogeneous; demethylation gave only xylose, and paper electrophoresis showed that of the monomethyl ethers of xylose, only 3-O-methylxylose would have the correct M_g value.

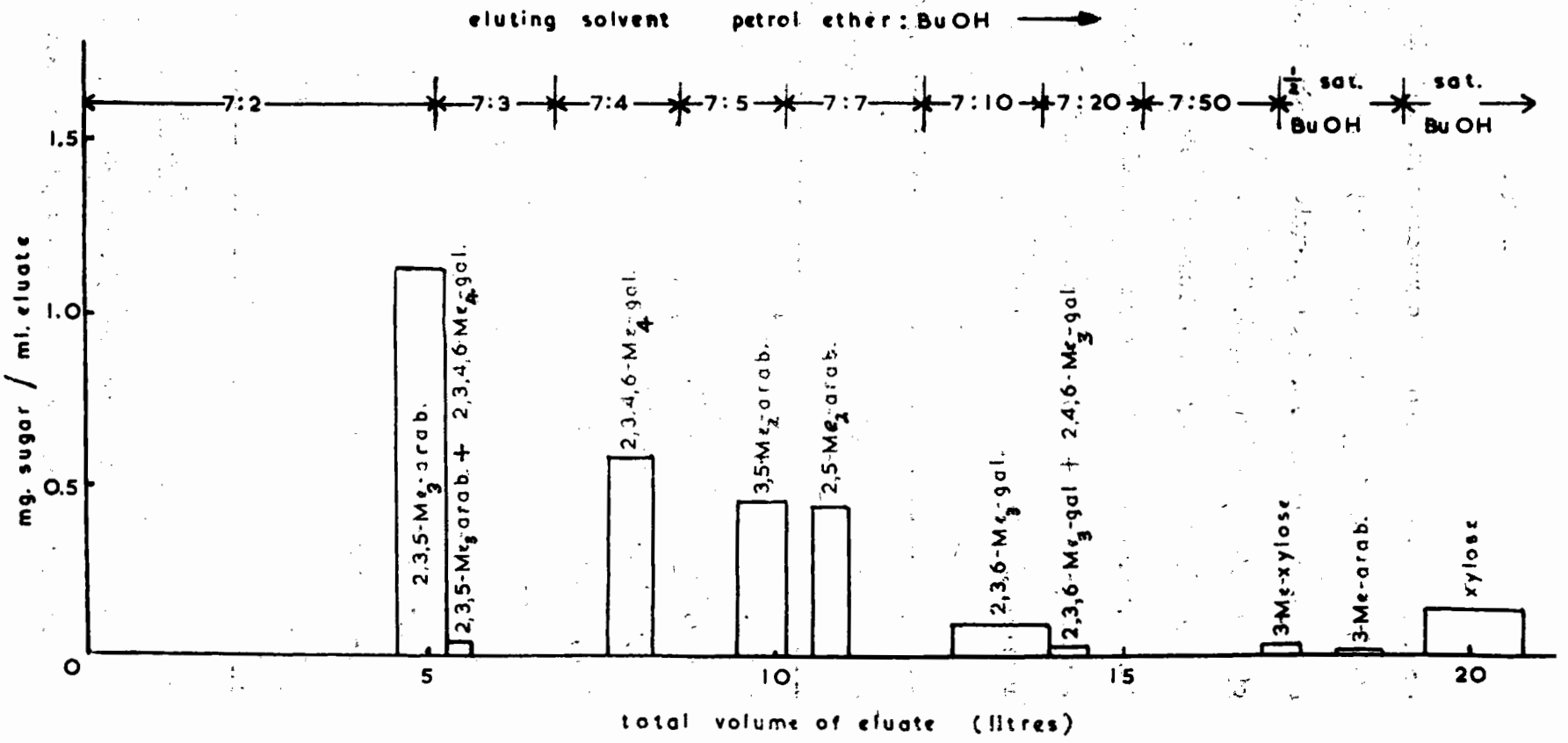
Fraction xii. The syrup (27 mg.; eluted with butanol half saturated with water), $[\alpha]_D$ +85° (c 0.42), R_G 0.37 (solvent a) gave arabinose only on demethylation and was chromatographically identical with 3-O-methylarabinose; it could not have been the 5- or 2-methyl ether of arabinose as these were ruled out by paper chromatography (R_G 0.59 and 0.46 respectively).

Fraction xiii. The syrup (594 mg.; eluted with butanol saturated with water), $[\alpha]_D$ +18° (c 5.94), R_G 0.26 (solvent a) was chromatographically identical with xylose (in solvents a, b, and c). Crystallisation

from/....

PLATE 7.

Histogram of the hydrolysis products of methylated Watsonia polysaccharide (separated on a cellulose column).



from 85% methanol afforded crystals m.p. and mixed m.p. (with D-xylose) 148°, $[\alpha]_D$ +18° (c 1.2). The derived di-O-benzylidene dimethyl acetal had m.p. and mixed m.p. (with D-xylose di-O-benzylidene dimethyl acetal of 210°; $[\alpha]_D$ -10° (c 1.40, $CHCl_3$).

Final elution of the column with ethanol : water; 1 : 1 gave no further fractions. The weights and molar proportions of the sugars eluted from the column are presented in Table 13, and diagrammatically represented in a histogram (plate 7).

Methyl ether	weight (mg.)	molar proportion
2,3,5-tri-O-methylarabinose	747	10.0
2,3,4,6-tetra-O-methylgalactose	453	4.9
3,5-di-O-methylarabinose	425	6.2
2,5-di-O-methylarabinose	418	6.5
2,3,6-tri-O-methylgalactose	218	2.3
2,4,6-tri-O-methylgalactose (probably)	26	0.2
3-O-methylxylose	45	0.75
3-O-methylarabinose	27	0.4
xylose	594	10.0

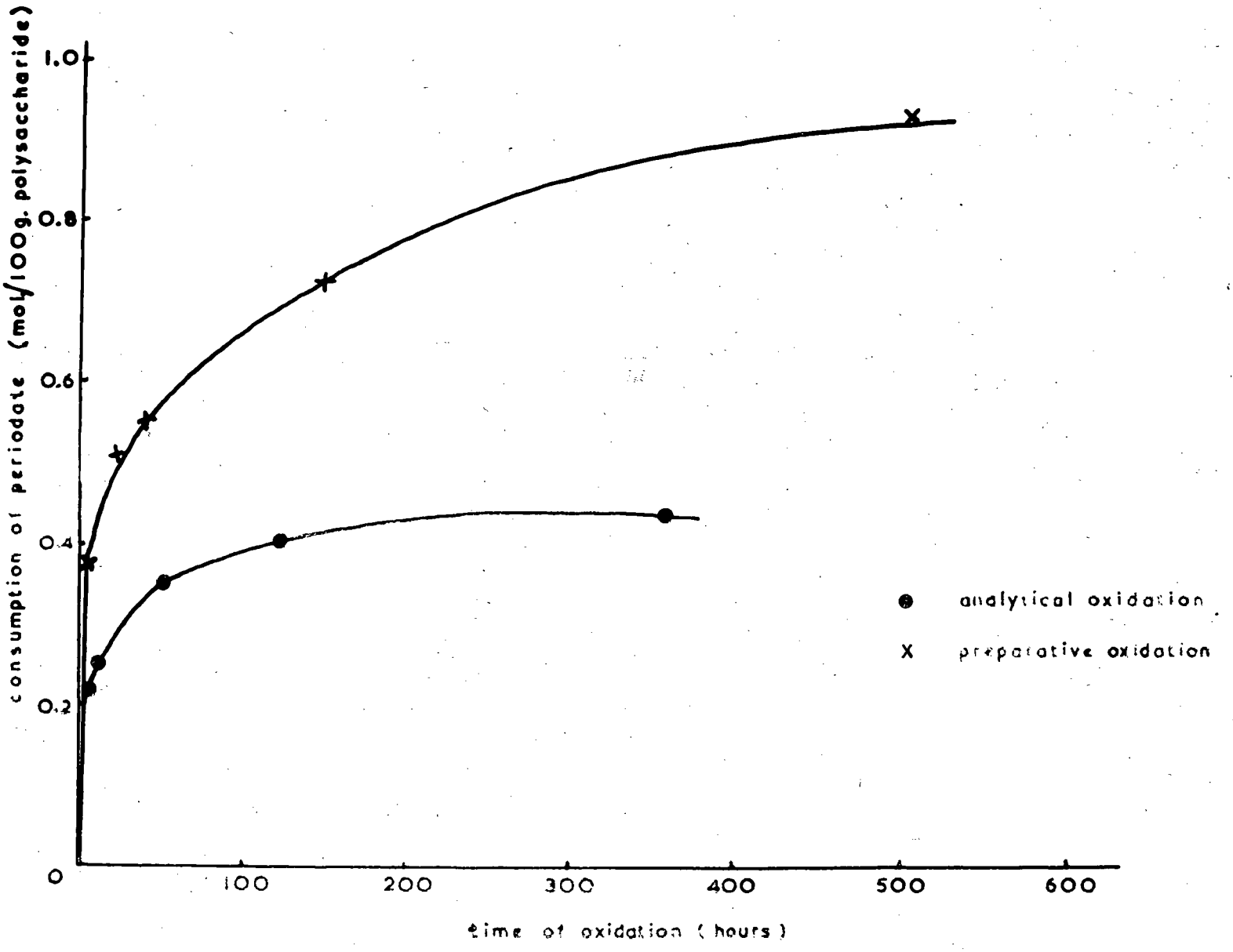
TABLE 13.

If we assume that the 2,3,6-tri-O-methylgalactose is actually from undermethylated or partially demethylated end-group galactose (see

discussion/...

PLATE 8.

Periodate oxidation of Watsonia polysaccharide.



(b) Preparative scale oxidation, reduction, and "Smith degradation".

Watsonia polysaccharide fraction A (3 g.) was dissolved in water (2 l.). To this solution sodium meta-periodate (18 g.) in water (500 ml.) was added, and the oxidation allowed to proceed for 333 hr. Aliquots of the reaction mixture were assayed for periodate uptake at various times during the oxidation (see table 16 and plate 8).

Time (hr.)	periodate consumed
2	0.312
25	0.548
50	0.564
168	0.737
192	0.756
333	0.864

TABLE 16

When the reaction had proceeded for the above time, barium carbonate (40 g.) was added and the suspension stirred for 2 days, during which time most of the iodate ion in solution was precipitated as barium iodate. After filtration through a filter-aid (Celite 535 *) the filtrate was concentrated (at 25° in vacuo) to a small bulk (40 ml.)

and/....

*A product of Johns-Manville, U.S.A.

and poly-aldehyde in the solution reduced to a poly-alcohol (over 3 days) by the addition of solid sodium borohydride (5 g.). After 3 days excess sodium borohydride was converted to sodium borate by lowering the pH of the solution to pH 6 with carbon dioxide. After filtration the solution (50 ml.) was passed through a column (3.5 x 47 cm.) of Sephadex G.25 (medium grade) to remove small molecules and ions, the eluate being checked at regular intervals for polysaccharide and borate ion content. The polysaccharide fractions free from borate ion were eluted in 175 ml., and totalled (by assay) 820 mg. of polysaccharide (i.e. 94.2% of the polysaccharide eluted from the column). Further fractions (eluted in 75 ml. and containing 51 mg. of polysaccharide) containing in addition to polysaccharide, borate ion, were de-ionized with Amberlite IR-120 (H^+) and Duolite A4 (OH^-) and concentrated (in vacuo at 25°); paper chromatography of these concentrates revealed no glycitols. The solution containing the borate free polysaccharide (175 ml.) was shell frozen and freeze-dried, yielding a white powder (1.12 g.), $[\alpha]_D$ -107° (c 1.03), a portion of which on hydrolysis (0.5N-sulphuric acid for 16 hr. at 96°) and preparative paper chromatography was found to contain xylose, arabinose, and glycerol (identified against standards in solvents a and f) in the ratic 10 : 14 : 7 by mols. (the glycerol was assayed by measuring the formaldehyde released on periodate oxidation); no spot corresponding to threitol was observed, and the above three components were the only ones noticed in the Hydrolysate.

The reduced oxidized Watsonia polysaccharide (1.0 g.) was dissolved

in/.....

in N-sulphuric acid and hydrolysed at 19° ("Smith degradation" conditions[95]); after 3 hr. arabinose started to be hydrolysed off the molecule, (determined by neutralizing a portion of the hydrolysate and spraying a spot on paper with p-anisidine hydrochloride) and the hydrolysis was terminated by adding barium carbonate to neutralize the acid. During the Smith degradation the specific rotation of the solution changed from $[\alpha]_D$ -100° (20 min.) → -93° (3 hr., c 5.01, N-sulphuric acid). The neutralized solution, after filtration, was evaporated to dryness (at 25° and 2 cm. Hg to constant weight) yielding a white solid (955 mg.) which on refluxing with absolute ethanol gave a soluble fraction (X) (230 mg. after evaporation in vacuo at 25°) and a residue (Y) (696 mg.), $[\alpha]_D$ -98° (c 0.92) (Found: ash, 1.8%).

Paper chromatography of fraction X (in solvents a and f) showed glycerol to be the only glycitol present and arabinose the only sugar; no components corresponding to sugars linked to glycitols were observed but the fraction was assayed for total sugar content (by two methods a and b) and for reducing sugar content (method (c)).

(a) By blowing steam through a solution of fraction X (14.8 mg. in water) any glycollic aldehyde in the fraction was removed, the resulting solution was assayed giving the total amount of sugar (as arabinose) present in the fraction (1.9 mg. i.e. 12.9% of fraction X).

(b) The second method used was one developed by Bahl and co-workers[131]. Fraction X (14.8 mg.) was refluxed for 2 hr. with 2.4% methanolic hydrogen chloride; when the Fehling's test was negative. Neutralization of the

solution/.....

solution with silver carbonate, centrifugation, and evaporation of the supernatant to dryness, afforded a residue from which two portions of methanol (each 20 ml.) were evaporated; after finally heating for 2 hr. at 80°, the residue was dissolved in water (25 ml.) and assayed for total sugar (as arabinose) content (Found: 1.8 mg., i.e. 12.4%).

(c) A further portion of fraction X (14.78 mg.) was assayed for reducing sugar using the micro-colorimetric method of Somogyi and Nelson[132], yielding 1.80 mg. (of reducing sugar, calculated as arabinose), i.e. 12.2%. The optical rotation of a solution of fraction X (c 1.47) was +0.10° which is equivalent to a free arabinose content of 12.9%. These results show that at most ca. 6% of the total sugars present (i.e. 0.7% of fraction X) can be bound to glycitol residues, which is in broad agreement with the absence of glycosyl-glycitols on paper chromatography.

Chromatography of fraction X (72 mg. in 0.9 ml. of water) on a column (1.2 x 30 cm.) of Dowex-1 resin * (100 - 200 mesh and 10% cross-linked) (cf. method of Austin et al[133]), elution with carbon dioxide free water, and estimation of the positions of the eluted components by measuring the periodate uptake of each fraction afforded glycerol (25.03 mg.) as the only component of fraction X eluted from the resin (identified initially by paper chromatography in solvents a and f). The derived p-nitrobenzoyl chloride[134] had m.p. 194-195°, and mixed m.p. (with an authentic specimen) 193-194°.

Hydrolysis of fraction Y (10 mg.) in N-sulphuric acid for 5 hr. at

* Product of Dow Chemical Co., Midland, Michigan, U.S.A.

96°/.....

96° gave xylose and arabinose only, in the ratio 10 : 14.2 by mols. Methylation of the fraction (279 mg.) by the procedures of Haworth and Purdie afforded a methylated degraded polysaccharide (151 mg.), $[\alpha]_D$ -120° (c 1.91, $CHCl_3$) (Found: OMe, 34.5%). Further methylation by the Purdie method did not raise the methoxyl content of this material. The methylated degraded polysaccharide (100 mg.) was hydrolysed in N-sulphuric acid for 5 hr. at 96°, neutralized with barium carbonate, filtered, and evaporated in vacuo to a syrup, a portion of which on paper chromatography (in solvent a) was shown to contain the following components (identified against standards); (i) 2,3,5-tri-O-methylarabinose, (ii) 2,5-di-O-methylarabinose, (iii) 3,5-di-O-methylarabinose, (iv) 2,3-di-O-methylxylose, (v) 3- and / or 2-O-methylxylose, (vi) xylose. Preparative paper chromatography of the syrup, and elution of the fractions from the paper with water, afforded solutions which were assayed for carbohydrate content, against an authentic standard curve for each component. Paper electrophoresis of components (ii), (iii), and (v) confirmed the identities and showed that (v) was a mixture of 3-O-methylxylose (M_g 0.66) and a trace of 2-O-methylxylose (M_g 0.39). Gas-liquid chromatography (against standards) of the methylated degraded polysaccharide, methanolysed for 18 hr. at 100° in 2.4% methanolic hydrogen chloride, confirmed all the above identities and also gave a peak corresponding to 2,3,4-tri-O-methylxylose, which has the same R_G value as 2,3,5-tri-O-methylarabinose on paper chromatography in solvent a. Molar proportions of each fraction, (a) by gas-

liquid/....

liquid chromatography (approximate), and (b) by assay after chromatography on paper sheets, are presented in table 17.

Sugar	assay from paper sheets	assay by gas-liquid chromatography
2,3,4-tri-O-methylxylose	1.0	1.0
2,3,5-tri-O-methylarabinose	9.3	6.55
2,5-di-O-methylarabinose	1.0	2.6
3,5-di-O-methylarabinose	5.6	10.2
2,3-di-O-methylxylose	1.5	1.0
2- and 3-O-methylxylose	5.1	2.6
xylose	3.0	-

TABLE 17.

The molar proportions found by the paper-strip assay method give a good agreement between the number of mols. of end group, and the number of mols. of branch points viz.;

(a) end-groups 10.3 mols. (35.0%)

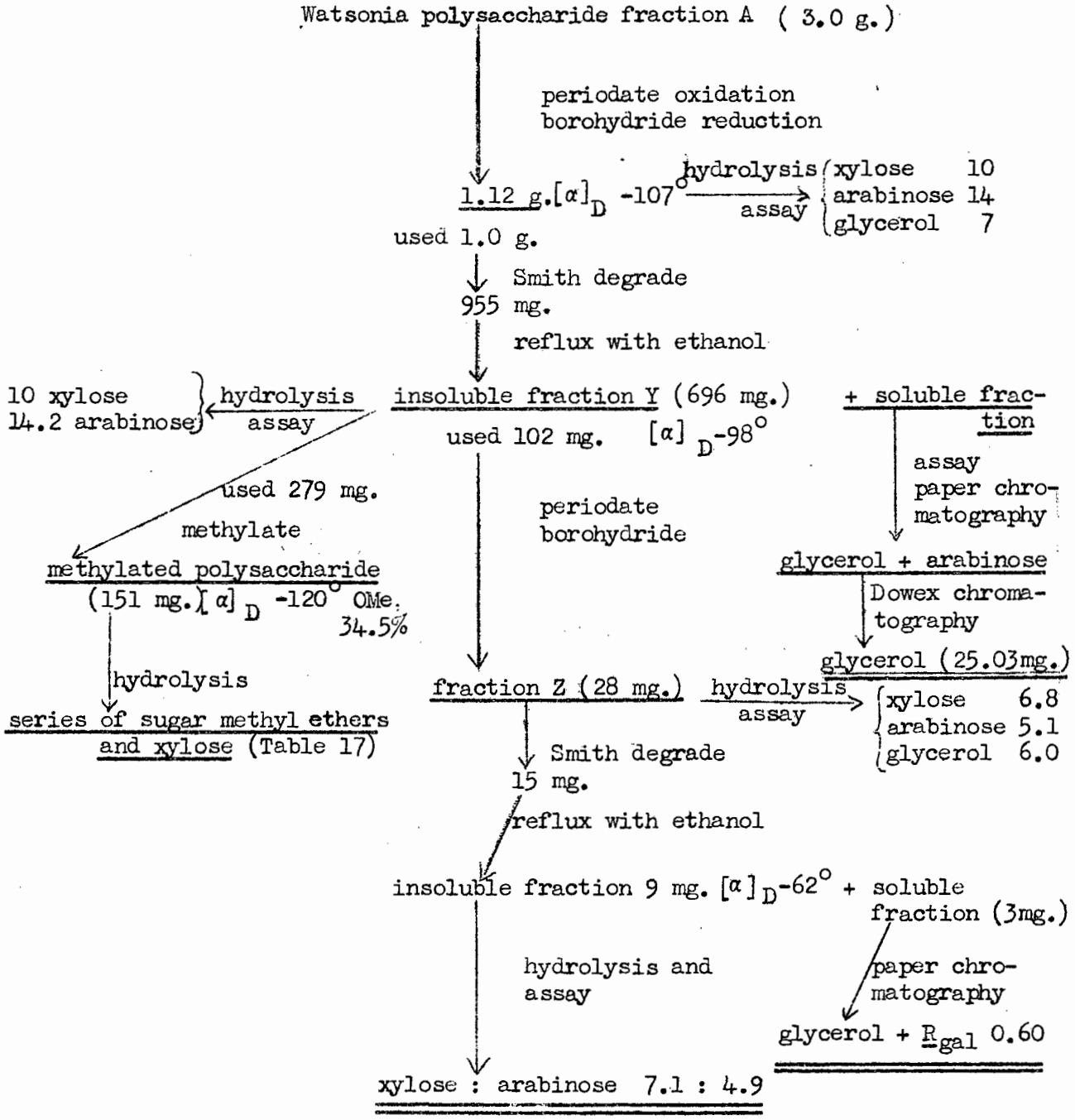
(b) chain-groups 8.1 mols. (27.4%)

(c) branch-points 11.1 mols. (37.6%)

Periodate oxidation (in the manner previously described) of the fraction Y (102 mg.) for 31 days at ambient temperature, revealed that

0.37 mols./....

0.37 mols. of periodate were consumed for 100 g. of fraction Y. Neutralization and sodium borohydride reduction of the oxidised solution, followed by conversion of the borohydride into borate with carbon dioxide, de-ionisation with mixed anion and cation exchange resins, and evaporation *in vacuo*, afforded a syrup (fraction Z) (28 mg.) which on hydrolysis (in N-sulphuric acid for 5 hr. at 96°), preparative paper chromatography, and assay of the separated components, showed xylose, arabinose, and glycerol to be the only components present, in the ratio 6.8 : 5.1 : 6 by mols. Fraction Z (19.5 mg.) was dissolved in N-sulphuric acid at room temperature ("Smith degradation" conditions) and the change in specific rotation from $[\alpha]_D$ -69° (3 min.) → -60° (3 hr., constant; *c* 0.49, N-sulphuric acid) was noted. The hydrolysate was neutralized, filtered, and concentrated (*in vacuo*) to a syrup (15 mg.). Paper chromatography in solvent *a* and revelation of the spots with *p*-anisidine hydrochloride and periodate / benzidine spray reagents, revealed glycerol (R_{gal} 4.4) as the main component of the mixture, a trace of a component R_{gal} 0.6 (not stained with *p*-anisidine hydrochloride and possibly a xylosyl-glycerol), and a component remaining at the origin; the material available was, however, insufficient to estimate the amount of glycerol, or the components of the spot R_{gal} 0.6. The remaining syrup (13 mg.) was dissolved as far as possible in ethanol, yielding two fractions (a) soluble in ethanol and containing the components R_{gal} 4.4 and 0.6 mentioned above, and (b) an insoluble fraction (9 mg.), $[\alpha]_D$ -62° (*c* 0.2) which gave xylose and arabinose in the ratio of 7.1 : 4.9 and


Watsonia polysaccharide fraction A (3.0 g.)
periodate oxidation
borohydride reduction
1.12 g. [α]D -107°
hydrolysis
assay
xylose 10
arabinose 14
glycerol 7
used 1.0 g.
Smith degrade
955 mg.
reflux with ethanol
insoluble fraction Y (696 mg.)
used 102 mg. [α]D -98°
+ soluble fraction
10 xylose
14.2 arabinose
hydrolysis
assay
used 279 mg.
methylate
methylated polysaccharide
(151 mg.) [α]D -120° OMe. 34.5%
hydrolysis
series of sugar methyl ethers and xylose (Table 17)
periodate
borohydride
assay
paper chromatography
glycerol + arabinose
Dowex chromatography
glycerol (25.03mg.)
fraction Z (28 mg.)
hydrolysis
assay
xylose 6.8
arabinose 5.1
glycerol 6.0
Smith degrade
15 mg.
reflux with ethanol
insoluble fraction 9 mg. [α]D -62°
+ soluble fraction (3mg.)
hydrolysis and assay
paper chromatography
glycerol + Rgal 0.60
xylose : arabinose 7.1 : 4.9

FLOW-SHEET 2.

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