THE CHEMISTRY OF ACACIA GUMS

A THESIS

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By

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INTRODUCTION

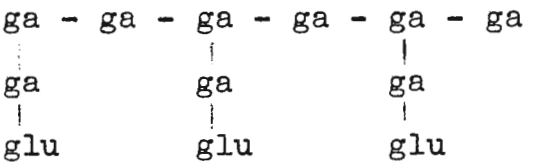
Gummy material is often exuded in response to injury by trees belonging to the Rosaceae and Leguminosae families. Acacia trees belong to the Leguminosae family, and the exudate from a variety of acacia species is known as "gum arabic" or "gum acacia". Gum arabic is collected for industrial use from acacia trees grown in hot, dry climates. The most esteemed variety is obtained from kordofan, although senegal and mogador gums are of excellent quality.

Early chemical investigations by Neubauer[1,2], Scheibler[3] and O'Sullivan[4] revealed that gum arabic is the neutral salt of an acidic polysaccharide, arabic acid. The above workers were also able to show that L-arabinose and D-galactose were produced on acid hydrolysis of gum arabic. In 1926 Heidelberger[5] reported that certain samples of gum arabic gave precipitin reactions with Type II and Type III pneumococcus antisera, and these results reawakened interest in the structure of gum arabic itself. In 1929 Butler and Cretcher[6] found that L-rhamnose, and an aldobiuronic acid, composed of D-galactose and D-glucuronic acid, were produced on acid hydrolysis of Acacia senegal gum, and in the same year Weimann[7]

isolated/...

isolated crystalline D-glucuronic acid from the degradation products of kordofan gum. Further progress was made when Challinor, Haworth and Hirst[8] proved that the aldobiuronic acid was 6-O-D-glucuronosyl-D-galactose. Hotchkiss and Goebel[9] then synthesised the aldobiuronic acid, so establishing that the configuration of the biose link was β. Smith and Jackson[10,11,12,13,14] went on to study arabic acid prepared from gum arabic.

Autohydrolysis of the gum acid gave a mixture of L-rhamnose, L-arabinose, 3-O-D-galactopyranosyl-L-arabinose and a degraded acid. The degraded acid consisted of D-galactose and D-glucuronic acid and the hydrolysis products of the methylated degraded acid were found to be 2:3:4:6-tetra-O-methyl-D-galactose, 2:3:4-tri-O-methyl-D-galactose, 2:4-di-O-methyl-D-galactose and 2:3:4-tri-O-methyl-D-glucuronic acid. It was also shown that hexamethyl-6-O-β-D-glucuronosyl-D-galactose could be split from the methylated degraded acid with cold 14N-sulphuric acid. Degraded arabic acid could then be simply represented by (I).



ga = D-galactose

glu = D-glucuronic acid

I

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The isolation of 2:4-di-O-methyl-D-galactose indicated that galactose residues could be linked together by 1-3 or 1-6 links. This was supported by the isolation of 3-O-D-galactopyranosyl-D-galactose from the mixture formed on prolonged autohydrolysis of degraded arabic acid. One possible structure for degraded arabic acid is shown by (II). The galactose residues in the main chain in (II) could be linked together by 1-3 links instead of 1-6 links. Another possibility would be alternate 1-3 and 1-6 links.

ga = D-galactose

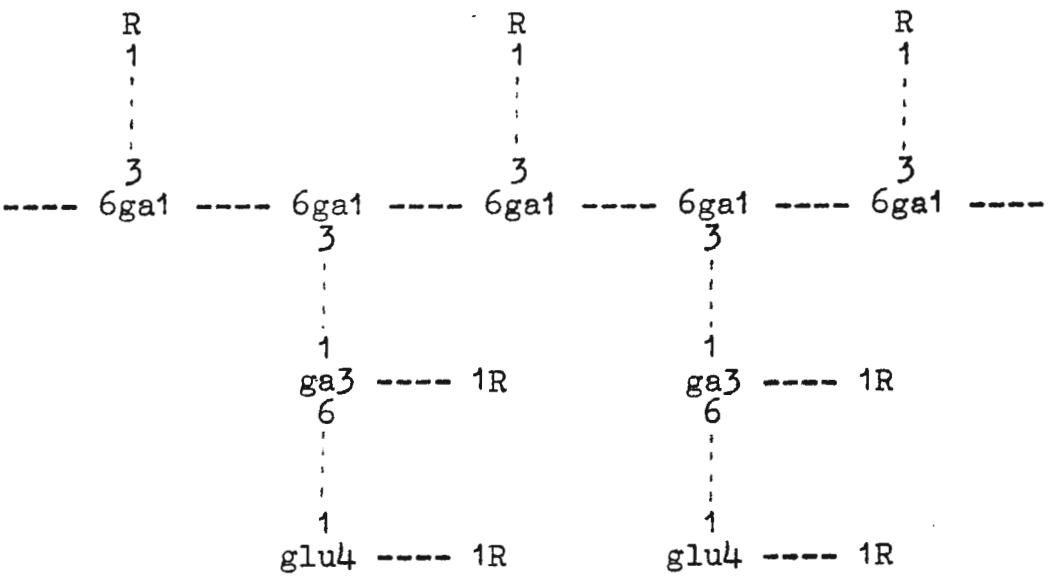
glu = D-glucuronic acid

II

Finally arabic acid itself was methylated and hydrolysed. Identification of the methylated fragments showed that arabinose, rhamnose and 3-O-D-galactopyranosyl-L-arabinose were linked in arabic acid in the form of L-arabofuranose, L-rhamnopyranose and 3-O-D-galactopyranosyl-L-arabofuranose.

The/...

The hydrolysate did not contain any tri-O-methyl-D-galactose. This indicated that the galactose units in degraded arabic acid which gave tri-O-methyl-galactose on methylation and hydrolysis, must have been linked to a labile sugar residue in the original arabic acid. Isolation of 2:3-di-O-methyl-D-glucuronic acid was evidence that labile sugar residues were also linked to glucuronic acid molecules by 1-4 bonds. A number of formulae, differing only in detail, could be assigned to arabic acid. One of these is shown by (III).



ga = D-galactose

glu = D-glucuronic acid

R = L-arabofuranose, L-rhamnopyranose or
3-O-D-galactopyranosyl-L-arabofuranose

III

The chemistry of the plant gums has attracted the attention of other carbohydrate specialists, Hirst and Jones in particular. These workers, together with their collaborators, have studied almond[15], cherry[16], cholla[17], damson[18], egg plum[19], grape fruit[20], lemon[20], peach[21] and sterculia gums[22]. From their results the following generalisations can be made:-

(i) D-Galactose, D-mannose, L-arabinose, D-xylose, L-rhamnose and D-tagatose may be constituents of gum polysaccharides.

(ii) D-Galactose, D-mannose, D-xylose and L-rhamnose are present in their pyranose forms.

(iii) L-Arabinose assumes the furanose form.

(iv) Although D-glucuronic acid is the acid constituent of most gums, D-galacturonic acid may be present instead (cholla and sterculia gums).

(v) The aldobiuronic acid present in gum arabic, 6-O-β-D-glucuronosyl-D-galactose, is also present in almond, egg plum and peach gums. 2-O-D-Glucuronosyl-D-mannose is a constituent of damson and cherry gums, and 4-O-D-glucuronosyl-D-galactose occurs in lemon and grape fruit gums.

(vi) More than one aldobiuronic acid can be present in a gum, since 4-O-D-galacturonosyl-D-galactose,

2-O-D-/...

2-O-D-galacturonosyl-L-rhamnose and 2(or 3)-O-D-galacturonosyl-D-galacturonic acid were isolated from sterculia gum.

Another industrially important polysaccharide, gum tragacanth, has been found to exhibit a number of peculiar features. O'Sullivan[23] recognised that the gum contained starch and James and Smith[24] were able to separate a neutral araban from a complex acidic polysaccharide, tragacanthic acid. Tragacanthic acid was also shown to contain D-galacturonic acid and L-fucose. It resembled the other gums, however, in that it had a highly branched structure.

Anderson and Otis'[25] experiments on mesquite gum showed that the uronic acid residue contained a methoxyl group and it has been proved[26] that the acid component is 4-O-methyl-D-glucuronic acid. Prolonged acid hydrolysis of the gum gave a mixture of aldobiuronic acids, and by methylating this mixture, Cuneen and Smith[27] obtained the methyl esters of heptamethyl 6-O-β-D-glucuronosyl-D-galactose and heptamethyl 4-O-D-glucuronosyl-D-galactose. It seems likely that the monomethyl ethers of 6-O-β-D-glucuronosyl-D-galactose and 4-O-D-glucuronosyl-D-galactose are present in mesquite gum.

Karaya gum or India gum is very similar to sterculia gum, since both gums occur as partially acetylated derivatives, possess equivalent weights of about 400, and have D-galacturonic acid, D-galactose and L-rhamnose as the major constituents[22,28]. Two other Indian gums which have not been subjected to extensive chemical investigation, are ghatti gum and jeol gum. The monosacchardie constituents of these gums are D-galactose, D-galacturonic acid and L-arabinose[29,30].

Although D-glucose has never been found to be a constituent of a gum, McIlroy[31] suggested that this monosaccharide may be present in phormium gum. In a later paper[32], however, he reported that phormium gum is composed entirely of D-glucuronic acid and D-xylose units.

In 1952 White[33] reported that 2:3:4-tri-O-methyl-L-arabinose was produced on the hydrolysis of methylated sapote gum. This proved that L-arabinose was present in the gum as L-arabopyranose. Previously it had been believed that L-arabinose is always present in its furanose form.

It can be seen that the plant gums are exceedingly complex materials and although a good deal of evidence has already been accumulated, no one formula has as yet been assigned to any gum. Useful results have been obtained

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by comparing the methanolysis products of the methylated gum acid with the methanolysis products of the methylated degraded gum acid, but it is questionable whether the information gained justifies the tedious labour involved. The modern approach to the problem of the structure of gums and other polysaccharides, therefore, is to study the oligosaccharides produced on partial degradation of the carbohydrates. From the structure of the oligosaccharides, the order and position of some of the monosaccharide units can be ascertained. At the same time, evidence for the configuration of some of the glycosidic links can be obtained. Whistler and his school[34,35] have isolated mannobiose, 4-O-β-D-mannopyranosyl-D-mannose and also 6-O-α-D-galactopyranosyl-D-mannose from the mucilage guaran. It is obvious that in the polysaccharide, therefore, D-mannose units can be mutually joined by a 1β-4 linkage, or D-galactose can be joined to D-mannose by a 1α-6 link. Whistler and Hough[36] have studied the aldobiuronic acids produced on hydrolysis of the hemicellulose of corn cobs, and Jones[37] has isolated 3-O-β-L-arabopyranosyl-L-arabopyranose from the ε-galactan of larch wood. Since the sugar portion of the last disaccharide has the pyranose form, L-arabinose can be present in its pyranose form in the galactan. This is consistent with White's result. In a most recent paper,

Jones[38] has reported the isolation of 3-O-β-L-arabopyranosyl-L-arabinose from peach and cherry gums and 4(or 5)-O-β-D-xylopyranosyl-L-arabinose from peach and cholla gums.

In 1951[39] Stephen again drew attention to the acacia gums. Although it is recognised that different acacia species produce different gums, some confusion has arisen by calling all these gums, gum arabic. Stephen's work on black wattle (Acacia mollissima) gum showed that the equivalent weight of the gum acid was much higher than that recorded for arabic acid. Black wattle gum acid resembled arabic acid in that it contained the same sugars and also 6-O-β-D-glucuronosyl-D-galactose was part of the structure. It is interesting that black wattle gum, which is derived from trees growing in wet conditions, is similar to gum arabic (Acacia senegal), which is collected from trees growing in a dry climate.

The aim of the present research was to investigate some other acacia gums found in South Africa with a view to comparing the sugar constituents and molecular proportions of the sugars with Acacia senegal gum and also to isolate and determine the structure of oligosaccharides produced on partial degradation of the gums.

SECTION I

INVESTIGATION OF THE GUMS FROM ACACIA CYANOPHYLLA AND ACACIA KARROO

Purification of Cyanophylla Gum and Physico-chemical Investigations on Cyanophylla Gum Acid

A large number of Acacia cyanophylla trees, "Port Jackson Willows", are found growing on the Cape Flats of the Western Province. These trees, indigenous to Western Australia, are characterised by their bright yellow flowers, long flat pods with constrictions between the seeds, and leaves with one medial vein. From some of these trees, cyanophylla gum exudes as a pale yellow liquid, which dries into a dark brown plastic solid. The bulk of the gum used in this work was collected from a small plantation near Retreat during the autumn months, the acacia species being identified at the Bolus Herbarium.

No attempt was made to grade the crude gum, which was converted into an amorphous gum acid of low ash content by pouring an acidified solution into ethanol. The acid had an equivalent weight of 740 and $[\alpha]_D$ -20°. Samples collected from single trees in the same plantation, or from a number of trees in different plantations, were converted into gum acids which had similar equivalent

weights/...

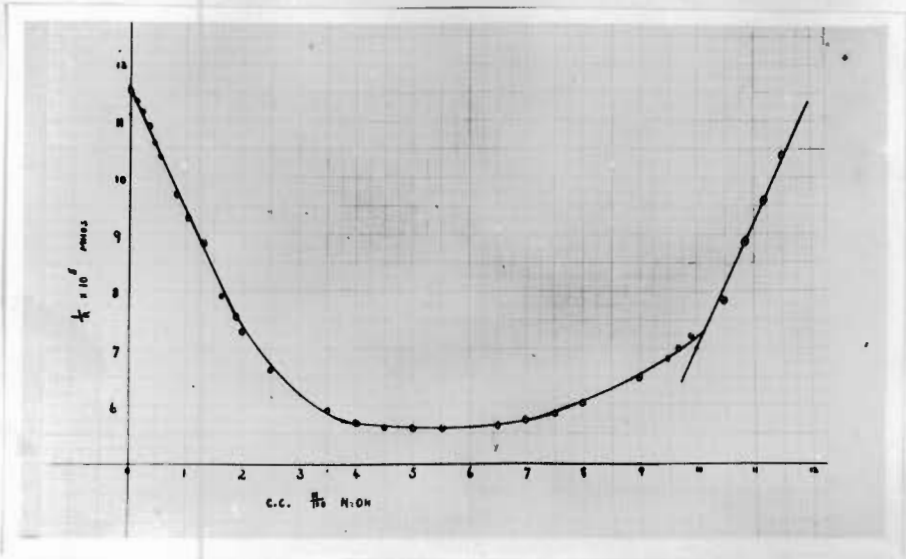

Fig. 1.
Conductimetric titration curve for cyanophylla gum acid against sodium hydroxide.

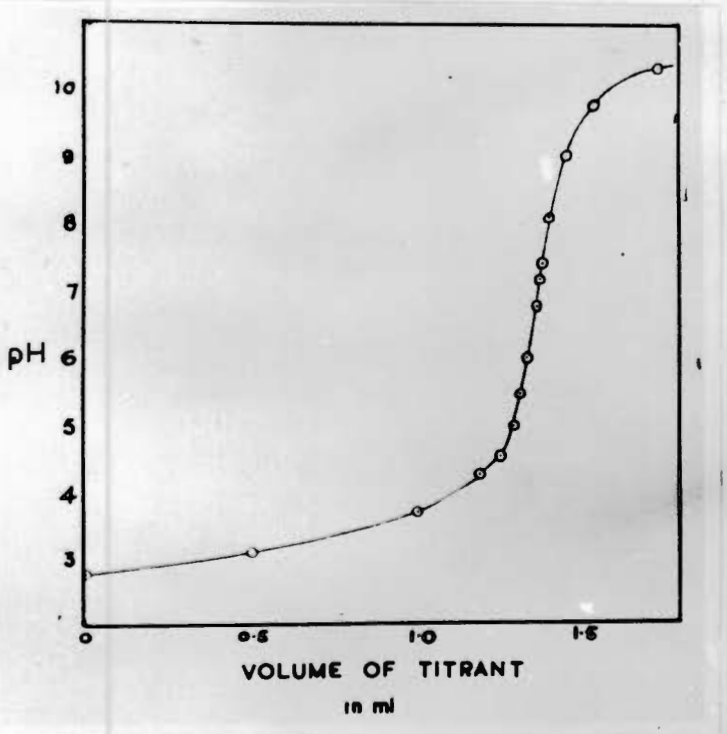

Fig. 2.
Potentiometric curve for cyanophylla gum acid against sodium hydroxide.

weights and similar specific rotations. These results indicated that the same gum is produced by all the cyanophylla trees. From similar experiments on damson, cherry, egg plum and cholla gums, Jones[40] concluded that the composition of gums is species specific. Cyanophylla gum fits in with Jones' hypothesis.

The equivalent weights quoted above were determined by direct titration using an indicator. An attempt was also made to determine the equivalent weight by conductimetric titration. Figure 1 shows the type of curve obtained. This gave an approximate equivalent weight of 700. The shape of the curve is of some interest, because it does not represent the behaviour of either a strong or a weak acid. Furthermore, Mukherjee and Ghosh[41] obtained similar curves with two discontinuities, when they titrated arabic acid against different bases. The value of their equivalent weights depended on the alkali used, barium hydroxide giving a value 10% lower than sodium hydroxide. These workers then showed that the potentiometric curves for arabic acid were typical of a strong acid. Their explanation for the shape of the conductimetric curve is not convincing and recent work by Veiss[42] has failed to confirm any difference in equivalent weight when arabic acid is titrated against different bases. Morrison[43] has

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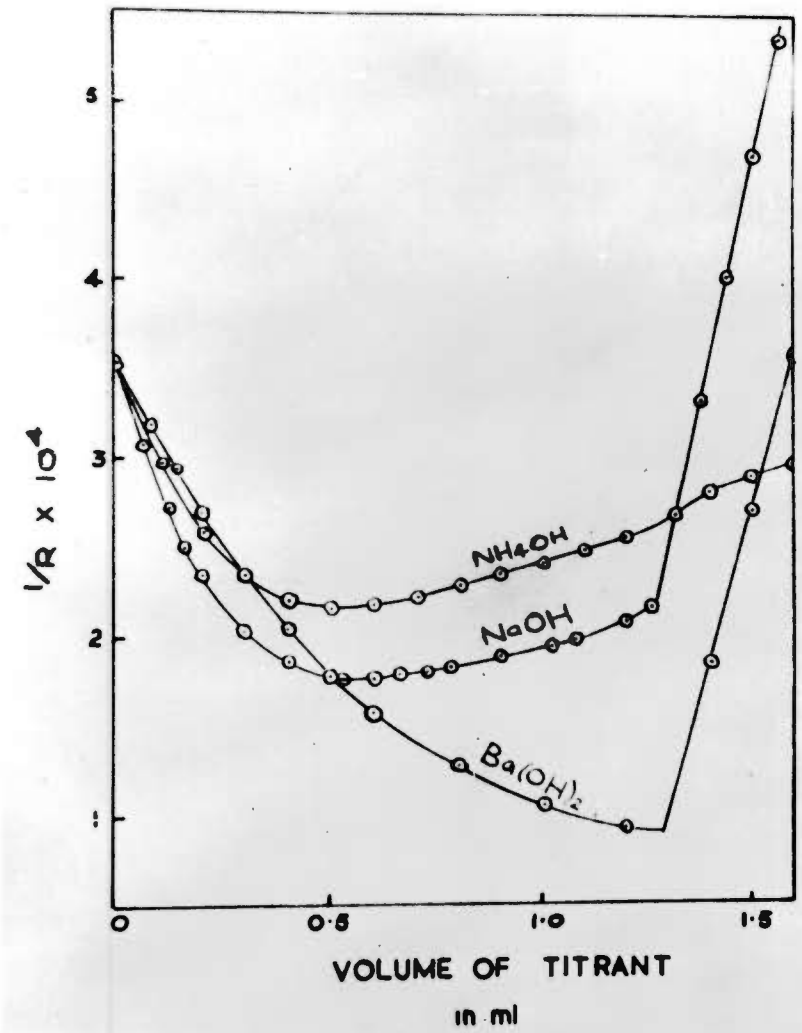


Fig. 3.

Conductimetric titration curves for cyanophylla gum acid against different bases.

therefore undertaken a more thorough investigation of the behaviour of cyanophylla gum acid with bases. His potentiometric curve, figure 2, has an end point at pH 7, indicating that the acid is fairly strong. Now Arnold and Overbeek[44] have shown how the dissociation coefficient of a polybasic acid at zero dissociation can be determined. Using similar methods, cyanophylla gum acid was found to have a dissociation coefficient of 4×10^{-2} at zero dissociation. In the case of the conductimetric curves, figure 3, only one discontinuity was obtained for sodium, barium, and ammonium hydroxides. The equivalent weight using sodium hydroxide was 708 and the value found using barium hydroxide was 715. The difference is within the experimental error. No value could be obtained for ammonium hydroxide as the line after the end point ran almost parallel to that before the end point. Morrison points out that, whereas the curves for sodium hydroxide and ammonium hydroxide show a rise in conductivity after about half neutralisation, the barium curve does not. He offers a qualitative explanation for the shape of these curves in terms of cation binding by partly neutralised polybasic acid molecules. The initial drop as hydrogen ions are replaced by cations, is characteristic of strong acids. It is known that the dissociation coefficients of polybasic

acids/...

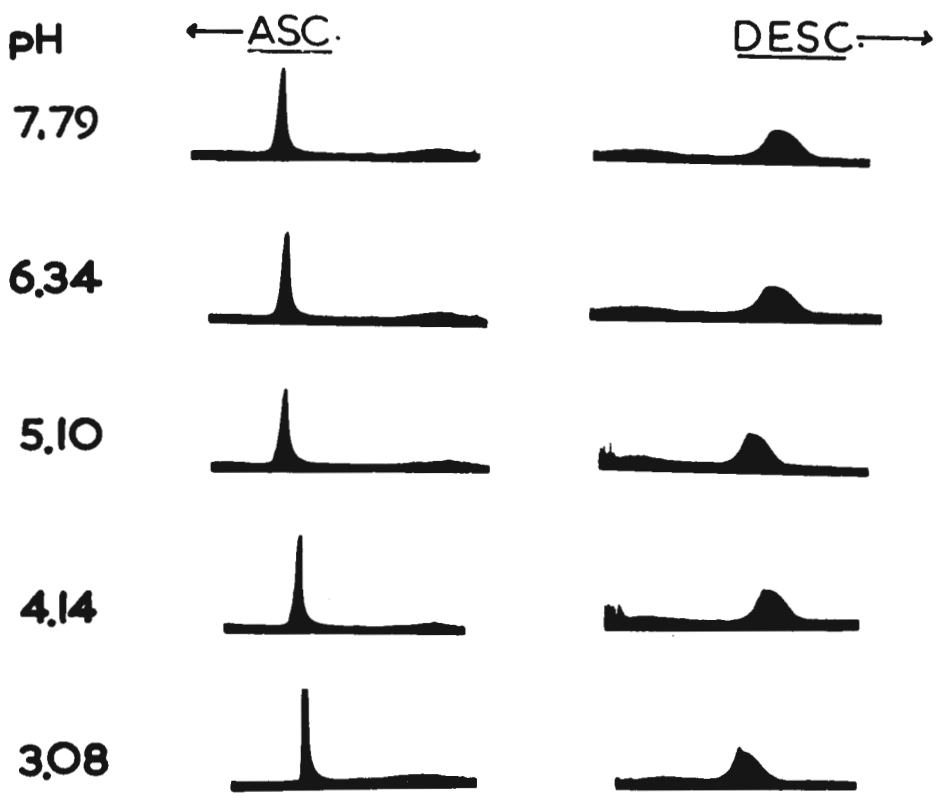


Fig. 4.

Electrophoretic diagrams of cyanophylla gum acid in buffers of ionic strength 0.20 at different pH values at 1°C. Gum acid concentration 0.66%.

acids decrease with neutralisation and a rise in conductivity would be expected after the initial drop. Owing to the cation binding, this rise will not be as marked as in the case of a weak monobasic acid. Furthermore, the barium hydroxide curve shows no rise at all before the end point, since doubly charged cations are more likely to be bound than singly charged cations.

Other physico-chemical investigations on cyanophylla gum acid have been carried out by Joubert[45] at the National Chemical Research Laboratory. Using the light scattering method, the molecular weight was found to be 172,000, whilst the osmotic pressure method gave a value of 142,000. The behaviour of the gum acid in a Tiselius electrophoresis apparatus was also examined and the electrophoretic diagrams at different pH-values are shown in figure 4. Although single boundaries are produced on both the ascending and descending sides, this is not an ideal electrophoresis pattern. In the first place, the descending boundaries show considerable spreading, and secondly the ascending and descending patterns should be mirror images. As the conditions were chosen so as to eliminate boundary anomalies as far as possible, Joubert concludes that these results must be attributed to

electrophoretic/...

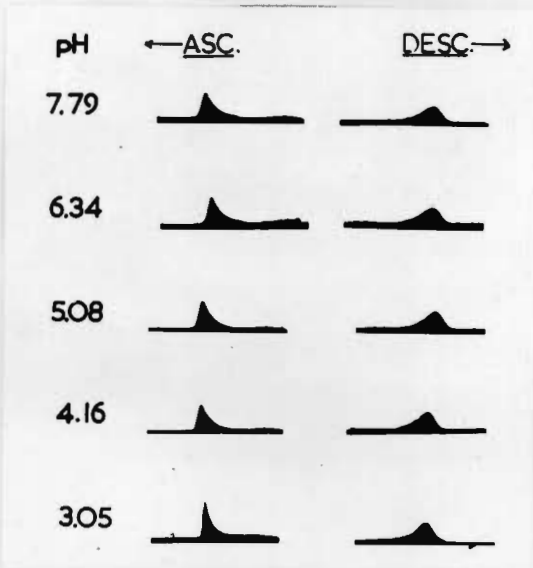


Fig. 5.
Electrophoretic diagrams of gum arabic in buffers of ionic strength 0.20 at different pH values at 1°C. Gum concentration 0.66%.

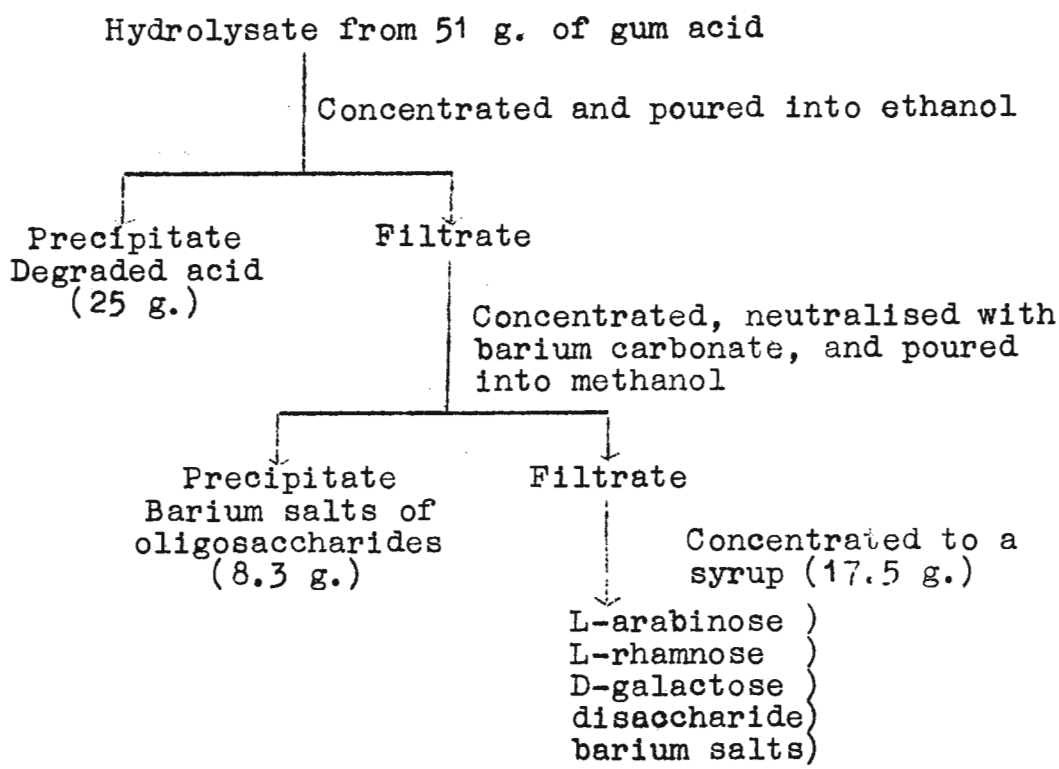
Fig. 6.
Electrophoretic diagrams of cyanophylla gum acid and gum arabic mixture in buffer of ionic strength 0.2 at pH 7.8 at 1°C. Concentration of each component 0.66%.

electrophoretic heterogeneity. A commercial sample of gum arabic (supplied by Eimer and Amend, New York) was then examined in the same way (figure 5). It can be seen that the ascending and descending patterns are rather asymmetric, but they approximate to mirror images more closely than the corresponding cyanophylla patterns do. Although the difference between the electrophoretic mobilities of cyanophylla gum acid and gum arabic was small, some separation was obtained when a mixture, containing equal weights of the two gums, was examined in the Tiselius apparatus. As shown in figure 6, the area of the slow peak, which represents gum arabic, is much smaller than the fast peak. This suggests that some of the gum arabic is incorporated into the cyanophylla gum acid boundary. Since the technique is incapable of completely separating two polysaccharides of different chemical structure, it is unlikely that it will separate polysacchardies of closely related chemical structure. If cyanophylla gum acid is a mixture of closely related polysacchardies, therefore, single boundaries could be obtained and the anomalous electrophoretic behaviour might be accounted for. It must be remembered, however, that little is known about the electrophoretic behaviour of polysaccharides, and it is therefore necessary to obtain further information before experimental results can be correctly interpreted.

Autohydrolysis/...

Autohydrolysis Products and the Aldobiuronic Acid from Cyanophylla Gum Acid

Autohydrolysis was carried out in order to find out which monosaccharide units could be easily removed and also to investigate the possibility of obtaining disaccharides. By following the hydrolysis, it was shown that rhamnose, arabinose and a disaccharide were split off during the early stages. Further hydrolysis resulted in the production of galactose and a mixture of acid oligosaccharides. The complex mixture could be separated by the methods outlined below:-



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On acid hydrolysis, both the degraded acid and the oligosaccharides gave galactose and a hexuronic acid. All the rhamnose and arabinose present in the original acid was thus concentrated in the sugar syrup. The molar ratio of rhamnose to arabinose in cyanophylla gum acid could therefore be determined by estimating the ratio of these two sugars in the above sugar syrup after the oligosaccharides had been broken down by hydrolysis. The sugars were separated on a paper chromatogram[46] and estimated by the method of Hirst and Jones[47]. The molar ratio of rhamnose to arabinose was found to be 5:1.9. From the sugar syrup, too, crystalline specimens of the monosaccharides and a syrupy disaccharide were isolated by partition chromatography on a cellulose column[48].

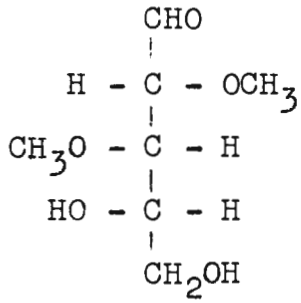
The disaccharide consisted of arabinose and galactose and by using the Kunz and Hudson bromine oxidation procedure[49], arabinose was shown to be the aglycone. Furthermore, since the disaccharide formed an osazone, the $C_{(1)}$ and $C_{(2)}$ hydroxyl groups were not involved in glycosidic linkage. Methylation and hydrolysis was then undertaken to attempt to establish the position of linkage. One of the products, 2:3:4:6-tetra-O-methyl-D-galactose, was characterised as its aniline derivative. The other product was a di-O-methyl-L-arabinose, and since the original

disaccharide/...

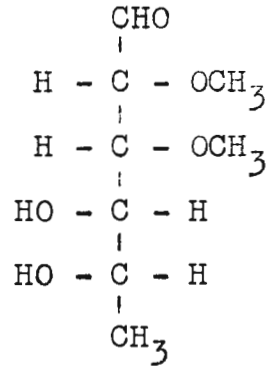
disaccharide did not have a 1-2 linkage, one of the methoxyl groups must have been on $C_{(2)}$. The three possibilities were therefore 2:3-, 2:4-, and 2:5-di-O-methyl-L-arabinose. Since the unknown di-O-methyl-L-arabinose had a specific rotation of +130°, 2:5-di-O-methyl-L-arabinose, $[\alpha]_D$ -50°, was eliminated. The di-O-methyl-L-arabinose formed a crystalline aniline derivative m.p. 136-137°. This could have been either N-phenyl-L-arabinosylamine 2:4-dimethyl ether (m.p. 142°, 145-146°) or N-phenyl-L-arabinosylamine 2:3-dimethyl ether (m.p. 139°). It is known that N-phenyl-sugar amines undergo mutarotation in solution, with the result that either α or β anomers might crystallise. This probably explains the wide range of m.ps. reported for some of these aniline derivatives; in fact Jones[38] has reported a m.p. 126° for N-phenyl-L-arabinosylamine 2:4-dimethyl ether. It was then thought that the problem of the di-O-methyl-L-arabinose might be solved by periodate oxidation. 2:3-Di-O-methyl-L-arabinose, written in its straight chain form in (IV), has both the $C_{(4)}$ and $C_{(5)}$ hydroxyl groups unprotected, and should produce formaldehyde, whereas 2:4-di-O-methyl-L-arabinose cannot evolve formaldehyde. The sugar did not evolve formaldehyde and it would appear that it was not 2:3-di-O-methyl-L-arabinose. Now Brown, Hough and Jones[50] have shown that 2:3-di-O-methyl-L-

rhamnose/...

rhamnose (V) gave anomalous results on periodate oxidation. Only 10% of the theoretical quantity of sodium periodate was consumed. Furthermore, 3:4-di-O-methyl-L-rhamnose, which consumed 70% of the theoretical amount of periodate, only liberated 10% of the calculated amount of formic acid. Hirst and Jones[47] had previously noted that 2:3-di-O-methyl-D-glucose gave two moles of formic acid instead of one, and Bell[51] could only get a 25% yield of formaldehyde from 3-O-methyl-D-glucose. Bell, however, obtained a 98% yield of formaldehyde from 2-O-methyl-D-glucose, nearly theoretical yields of formaldehyde from 2:3-di-O-methyl-D-galactose and 2:3-di-O-methyl-D-glucose, and nearly 50% yield of formaldehyde from 2:3:4-tri-O-methyl-D-glucose. The anomalous behaviour of some of the methylated sugars has lead Montgomery and Smith[52] to advise caution in the interpretation of negative periodate results.



IV



V

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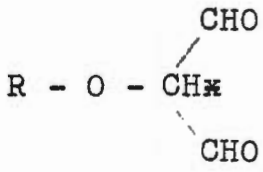
Since 2:3-di-O-methyl-L-arabinose was not available for a control experiment, the periodate evidence could therefore not be taken as conclusive. Assuming that the methylated sugar was in fact 2:4-di-O-methyl-L-arabinose, the original disaccharide would have been 3-O-D-galactopyranosyl-L-arabopyranose. This disaccharide should evolve formaldehyde on periodate oxidation since the $C_{(4)}$ and $C_{(5)}$ hydroxyl groups on the aglycone are unprotected. If the di-O-methyl-L-arabinose was the 2:3-isomer, the glycosidic link could be either 1-4 or 1-5 and the corresponding disaccharides should not evolve formaldehyde. The galactose-arabinose disaccharide was found to give 0.45 mole of formaldehyde-dimedone complex (per mole of disaccharide) when it was oxidised for three days in a phosphate buffer at pH 7[51]. Under identical conditions 1 mole of lactose yielded 0.35 mole of formaldehyde-dimedone complex and 1 mole of maltose yielded 0.5 mole of formaldehyde-dimedone complex. These experiments suggest that the link in the unknown disaccharide is 1-3, since it is unlikely that the disaccharide was hydrolysed in the neutral buffer and that the formaldehyde was thereby produced from the sugar portion. Whistler and Conrad[53] have recently shown that 4-O-D-galactopyranosyl-D-galactose yields formaldehyde on periodate oxidation, and these authors suggested that this

evidence/...

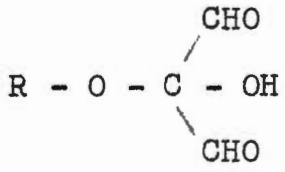
evidence eliminates a 1-5 linkage. Now a number of other workers have studied the periodate oxidation of disaccharides. Alborg[54] reported that the periodate oxidation technique can be used to distinguish between 1-4 disacchardies composed of hexopyranose units and 1-6 disacchardies composed of hexopyranose units. He found that whereas 2 moles of formaldehyde (per mole of disaccharide) were produced on periodate oxidation of the 1-4 disaccharides in acid solution, the 1-6 disaccharides did not yield formaldehyde. There is a possibility that the 1-4 disaccharides were hydrolysed in the acid solution and that the second mole of formaldehyde resulted from oxidation of the sugar portions. O'Dea[55], however, has shown that it is possible to obtain 2 moles of formaldehyde when 1 mole of maltose is oxidised with periodate in a neutral buffer. Furthermore, Head[56] has shown that 2 moles of formaldehyde are produced on prolonged oxidation of 1 mole of cellobiose with sodium metaperiodate. Although Potter and Hassid[57] had supposed that 1 mole of a 1-4 disaccharide, composed of hexopyranose units, should be quantitatively oxidised by 5 moles of periodate to yield 3 moles of formic acid, 1 mole of a substance of type (VI), and 1 mole of formic acid, Head demonstrated that the oxidation is more complicated. After 18 hours oxidation, 1 mole of cellobiose reduced

5 moles/...

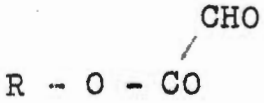
5 moles of periodate, but only 0.2 mole of formaldehyde was produced. From his analytical results, Head postulated that (VI) can be further oxidised to (VII); this in turn is oxidised to (VIII), and ultimately the molecule is completely broken down with the production of formaldehyde, carbon dioxide and formic acid.



VI

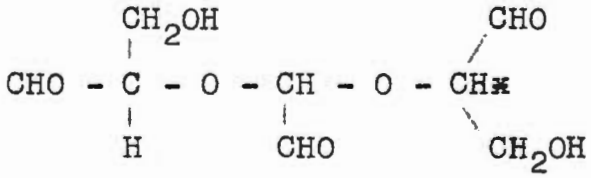


VII



VIII

The low yield of formaldehyde obtained from the galactose-arabinose disaccharide is consistent with Head's results. It must also be pointed out that the production of formaldehyde from the galactose-arabinose disaccharide does not eliminate a 1-4 linkage, since the oxidation product (IX) might undergo further oxidation.

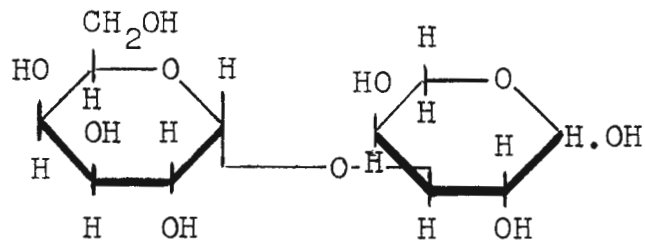


IX

Now/...

Now a crystalline heptamethyl ether of 3-O-D-galactopyranosyl-L-arabopyranose, $[\alpha]_D$ +162° and m.p. 82°, is known(10). The disaccharide from cyanophylla gum also formed a crystalline heptamethyl ether, $[\alpha]_D$ +169° and m.p. 87-88°. This also suggested that the unknown disaccharide is 3-O-D-galactopyranosyl-L-arabopyranose, and since the specific rotations of both the disaccharide and its methylated derivative are high, an α-biose link is indicated. (In Section 2, the configuration of glycosides is discussed in detail.)

The most probable structure of the disaccharide is represented by (X).



X

The Aldobiuronic Acid.

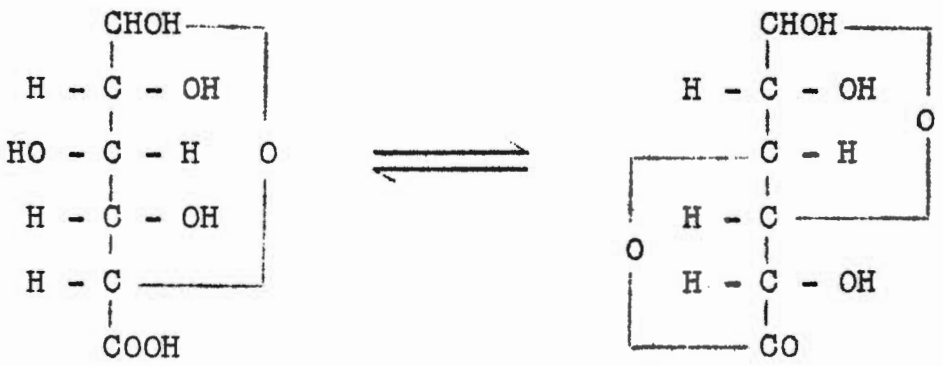
From the analytical constants and paper chromatogram patterns, it was found that the bulk of the barium oligosaccharide fraction was barium aldobiuronate. It was thought advisable to study the hydrolysis of the degraded acid before attempting a closer examination of the

oligosaccharide/...

oligosaccharide fraction, because a purer aldobiuronate might be obtained. The hydrolysis of the degraded acid was carried out in 0.5N-sulphuric acid and a relatively pure barium aldobiuronate was isolated. After removing barium from the salt, the free acid was purified on a cellulose column.

The aldobiuronic acid was known to consist of D-galactose and D-glucuronic acid, since on hydrolysis of the aldobiuronate in 2N-sulphuric acid, D-galactose, D-glucuronic acid and D-glucurone were produced.

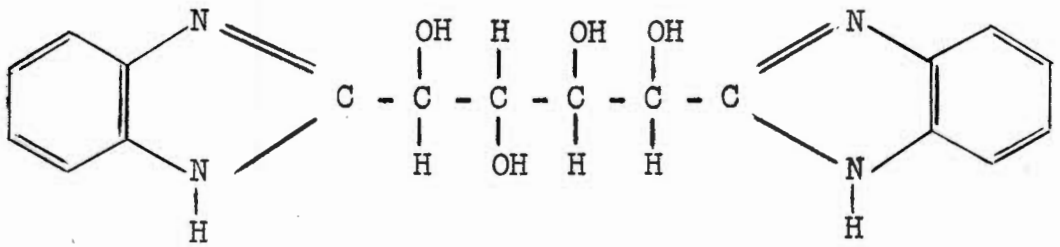
[D-Glucurone was formed by the equilibrium,



]

Both glucurone and glucuronic acid were isolated in crystalline form, the latter being characterised as the dibenzimidazole derivative of saccharic acid (XI). The oxidation of the hexuronic acid to the saccharic acid was carried out with dinitrogen tetroxide in chloroform, since bromine water proved to be unsatisfactory.

XI/...

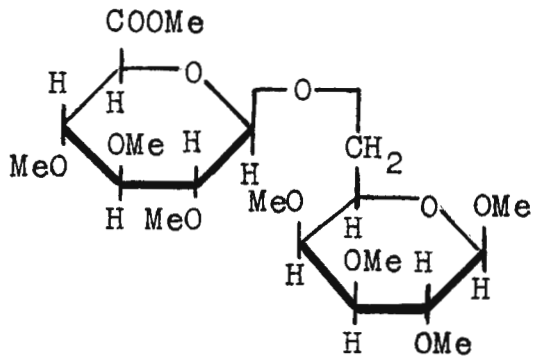


XI

Bromine oxidation experiments showed that galactose was the aglycone and this was confirmed by the production of a tri-O-methyl-D-galactose, $[\alpha]_D$ +102°, on hydrolysis of the methylated aldobiuronic acid. The high positive rotation indicated that the tri-O-methyl-D-galactose had a free hydroxyl on $C_{(5)}$ (2:3:5-tri-O-methyl-D-galactose has a specific rotation of -8°); furthermore, dimethyl sulphate and sodium hydroxide treatment generally converts the aglycone into its pyranose form. The remaining possibilities were 2:3:4-, 2:3:6-, 2:4:6-, and 3:4:6-tri-O-methyl-D-galactose, and of these the last was eliminated because of its rotation, $[\alpha]_D$ -43°. The tri-O-methyl-D-galactose gave a crystalline N-phenylamine (m.p. 162°) which suggested that it was either N-phenyl-D-galactosylamine 2:3:4-trimethyl ether (m.p. 167°) or N-phenyl-D-galactosylamine 2:4:6-trimethyl ether (m.p. 169°). A mixed melt showed that it was not N-phenyl-D-galactosylamine 2:4:6-trimethyl ether, and the methylated sugar was then proved to be 2:3:4-tri-O-methyl-D-galactose since it evolved formaldehyde

on/

on periodate oxidation. Glucuronic acid was thus joined to the galactose by a 1-6 link, and the rotation of the aldobiuronic acid, $[\alpha]_D$ -3°, indicated that the configuration of the biose link was most likely β. (See Section 2, table D.) Finally a crystalline heptamethyl methyl ester was obtained, the constants of which were in agreement with those reported for the methyl ester of hexamethyl 6-O-β-D-glucuronosyl-β-methyl-D-galactoside[8] (XII).



XII

There was a possibility that the aldobiuronic acid present in the oligosaccharide fraction might be different from the 6-O-β-D-glucuronosyl-D-galactose, which has been shown to be a constituent of the degraded acid. The aldobiuronic acid was isolated and converted into a heptamethyl methyl ester identical with the above compound (XII).

The/...

The autohydrolysis of cyanophylla gum acid resembles the autohydrolysis of arabic acid. Furthermore, both polysaccharides consist of L-arabinose, L-rhamnose, D-galactose and D-glucuronic acid, and the same aldobiuronic acid and disaccharide are produced on graded hydrolysis of both gum acids. It is reasonable to suggest, therefore, that cyanophylla gum acid also has an acid-resistant backbone consisting of D-galactose residues joined by 1-6 and 1-3 links. Furthermore, the labile arabinose and disaccharide groups are joined to the main chain as L-arabofuranose and 3-O-α-D-galactopyranosyl-L-arabofuranose, and the methyl pentose has the pyranose form in the macromolecule.

The/...

The Molecular Proportions of the Sugars in Cyanophylla Gum Acid

The proportions of the components of cyanophylla gum acid were determined by complete hydrolysis of the gum acid with 2N-sulphuric acid and estimation of the resultant sugars by paper chromatographic separation[46] and oxidation with periodate[47]. A mean of six determinations showed that the approximate molar ratio of rhamnose to arabinose to galactose was 1:0.4:2.21 or 5:2:11. The arabinose to rhamnose ratio is almost identical with the ratio found in the autohydrolysis sugar syrup.

The following calculation shows that there are approximately 2 arabinose moles, 5 rhamnose moles, 11 galactose moles and 5 glucuronic acid moles in the simplest repeating unit possible.

Sugar residue	Molecular weight in the macromolecule	Number of units	Weight in repeating unit
arabinose	132	2	264
rhamnose	146	5	730
galactose	162	11	1,782
glucuronic acid	176	5	880
			Unit wt. 3,656

As/...

As there are five hexuronic acid molecules in the repeating unit, the equivalent weight of the gum acid would be 731. This value is in good agreement with the value found (740).

It is generally assumed that polysaccharide macromolecules are built up of repeating units. Now Hassid[58] has obtained evidence that there is a repeating unit in amylopectin, and Abdel-Akher and Smith[59] have shown that thirty-seven different samples of glycogen all contained a repeating unit of approximately twelve glucose residues. The fact that cyanophylla, cherry and sterculia gums contain complicated patterns in which there are more than one uronic acid residue, seems to indicate that gum polysaccharides need not be built up of repeating units. There is always the possibility, however, that gums consist of a mixture of polysaccharides and that each component is composed of repeating units.

It is established that the components of cyanophylla gum acid are similar to those of arabic acid and the above work shows that they are combined in different proportions. Table A compares the equivalent weights, specific rotations and monosaccharide ratios of cyanophylla gum acid, arabic acid and black wattle gum acid.

Table A/...

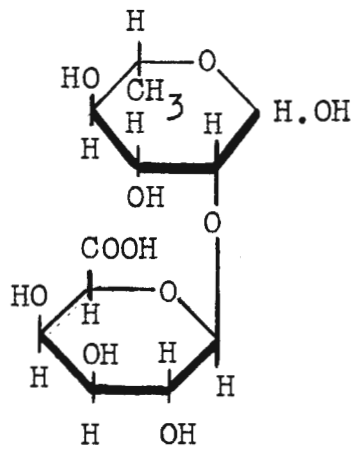
TABLE A

Gum acid	Eq. wt.	$[\alpha]_D$	Molar ratios of the monosaccharides			
			L-rhamnose	L-arabinose	D-galactose	D-glucuronic acid
arabic[6]	1030	-34°	1	3	3	1
cyanophylla	740	-20°	5	2	11	5
black wattle	1900	-49°	1	6	5	1

In all three cases the ratio of L-rhamnose to D-glucuronic acid is 1:1. This might be a coincidence or it might have a structural significance. It has been mentioned that in arabic acid the $C_{(4)}$ hydroxyl group on the glucuronic acid is linked to L-arabinose, L-rhamnose or 3-O-D-galactopyranosyl-L-arabofuranose. If L-rhamnose is the labile sugar joined to D-glucuronic acid, the ratio of 1:1 could be accounted for in arabic acid. L-Rhamnose is often closely associated with uronic acids, particularly in the plant mucilages. Thus 2-O-D-galacturonosyl-L-rhamnose (XIII) is the aldobiuronic acid constituent of flax seed mucilage[60] and the mucilage from the Ulmus fulva bark[61]. Aldobiuronic acids, consisting of D-galacturonic acid and L-rhamnose, also occur in the products of hydrolysis of the mucilages from white mustard seed[62], cress seed[63],

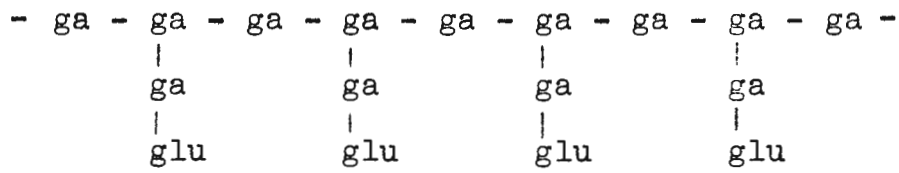
and/...

and Plantago lanceolata[64]. This lends support to the above hypothesis. It is also noteworthy that gum myrrh[65] and mesquite gum, which have a 4-O-methyl-D-glucuronic acid residue instead of D-glucuronic acid, do not contain L-rhamnose. Some gums which contain glucuronic acid, however, do not contain rhamnose and therefore the suggested position of L-rhamnose in arabic acid is only tentative. If this is correct, it is highly probable that wattle gum acid and cyanophylla gum acid have this structural feature as well. It is interesting that Hirst[66] suggested that arabinose was attached to glucuronic acid in arabic acid, but he did not give any reasons for his preference.



XIII

The polysaccharide remaining after removal of the labile sugars from arabic acid has a structure represented by (XIV).

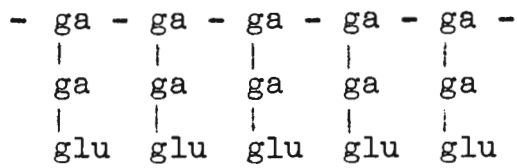


ga = D-galactose

glu = D-glucuronic acid

XIV

In his paper on black wattle gum, Stephen[39] suggested that the basic pattern of wattle gum is similar to (XIV) except that the aldobiuronic acid residues could be linked to every fifth galactose residue in the main chain instead of every third galactose residue. Since cyanophylla gum acid is also very similar to arabic acid, its basic structure could be represented by (XV).



ga = D-galactose

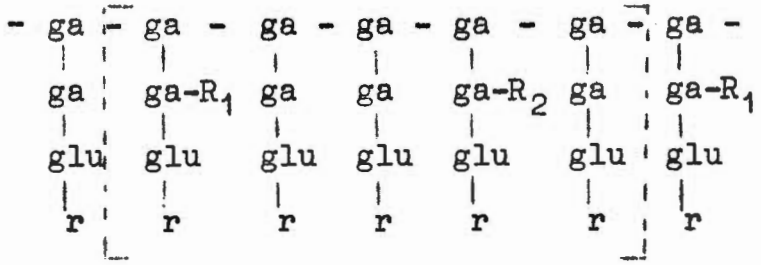
glu = D-glucuronic acid

XV

The next step is to fit in the labile sugar residues. Now in arabic acid, the maximum number of units linked to a

galactose/...

galactose molecule is three. It is unlikely, therefore, that the labile sugars are linked to the galactose residues in the main chain in (XV). This leaves the glucuronic acid and the galactose attached to it. If rhamnose is joined to the glucuronic acid, the arabinose or 3-O-α-D-galactopyranosyl-L-arabinose must be joined to the galactose. This is shown in (XVI).



ga = D-galactose

glu = D-glucuronic acid

r = L-rhamnose

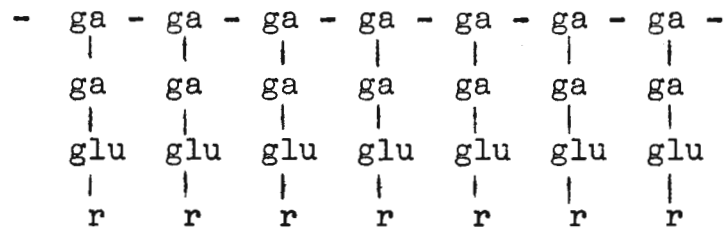
R_1 = L-arabinose

R_2 = 3-O-α-D-galactopyranosyl-L-arabinose

XVI

It can be seen that (XVI) is in agreement with the molecular proportions. Furthermore, R_1 and R_2 could be interchanged, or they could be linked to the other available galactose residues. An alternative possibility to (XVI) is that cyanophylla gum acid consists of a mixture of polysaccharides (XVII) and (XVIII).

ga/...

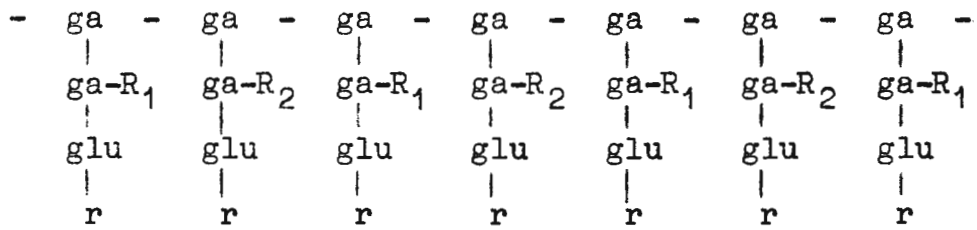


ga = D-galactose

glu = D-glucuronic acid

r = L-rhamnose

XVII



ga = D-galactose

glu = D-glucuronic acid

r = L-rhamnose

R_1 = L-arabinose

R_2 = 3-O-α-D-galactopyranosyl-L-arabinose

XVIII

The molecular proportions can be accounted for if a third of the gum consists of (XVIII). Furthermore, these polysaccharides are very similar and this may explain the electrophoretic behaviour of the gum acid.

Karroo Gum

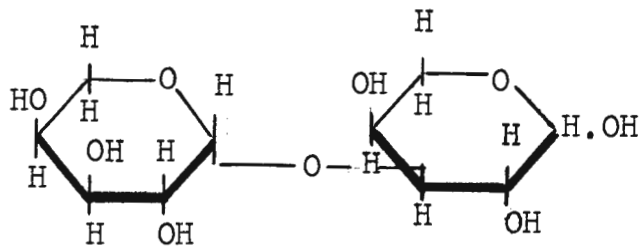
Acacia karroo is a tree indigenous to South Africa and is found in the Western and Eastern Provinces of the Cape. Some trees exude large, transparent lumps of gum, and samples of these were collected in Alice in the Eastern Province during the winter months. The gum acid was prepared by the same procedure used in the purification of cyanophylla gum. Both the equivalent weight (1660) and the specific rotation ($+54^{\circ}$) were much higher than the corresponding constants found for cyanophylla gum acid. It also differed from cyanophylla gum acid in that it showed a tendency to become insoluble on drying.

The gum acid underwent autohydrolysis with the production of a degraded acid, a mixture of acid oligosaccharides, L-arabinose, L-rhamnose, D-galactose and two neutral disaccharides. One of these disacchardies moved at the same rate on a paper chromatogram as 3-O-α-D-galactopyranosyl-L-arabinose, but as it was produced in low concentration, it was not isolated. The monosaccharides and the other neutral disaccharide were isolated by partition on a cellulose column.

The/...

The disaccharide was shown to consist of arabinose units only, and since it formed an osazone, the $C_{(1)}$ and $C_{(2)}$ hydroxyl groups of the aglycone could not be involved in glycosidic linkage. Hydrolysis of the methylated disaccharide produced both a di-O-methyl-L-arabinose and a tri-O-methyl-L-arabinose. 2:3:4-Tri-O-methyl-L-arabinose and 2:3:5-tri-O-methyl-L-arabinose were the two possibilities for the trimethyl sugar. Now the 2:3:4-tri-O-methyl ether has a specific rotation of $+158^{\circ}$ whereas the 2:3:5-isomer has $[\alpha]_D$ -30°. Since the unknown methylated sugar had $[\alpha]_D$ $+145^{\circ}$ and gave crystalline 2:3:4-tri-O-methyl-L-arabinophenylhydrazide, it must have been the 2:3:4-tri-O-methyl ether. This proved that the sugar portion of the disaccharide had the pyranose structure, and therefore L-arabinose can be present in its pyranose form in the macromolecule. The di-O-methyl-L-arabinose gave a crystalline N-phenylamine identical with that obtained from the galactose-arabinose disaccharide; this suggested a 1-3 link between arabinose units. Now since the specific rotation of the disaccharide was unusually high ($+208^{\circ}$) and the sugar portion was a member of the L series, a β-biose link was indicated. (See Section 2.) It is therefore suggested that the disaccharide is 3-O-β-L-arabopyranosyl-L-arabopyranose (XIX).

(XIX)/...



XIX

The rotation and also the m.p. of the osazone are in agreement with the constants given by Jones[37,38] for 3-O-β-L-arabopyranosyl-L-arabopyranose.

The autohydrolysis of karroo gum acid was similar to that of cyanophylla gum acid with the exception that the arabinose-arabinose disaccharide could not be detected in the hydrolysis mixture from cyanophylla gum acid. It is interesting that although Smith did not obtain an arabinose-arabinose disaccharide from arabic acid, Jones[37] has shown that a disaccharide composed of arabinose units is present in gum arabic (Turc. variety). Since cyanophylla gum acid and arabic acid are very similar, the above disaccharide would be expected to be a constituent of cyanophylla gum. The fact that it does not occur in cyanophylla gum, however, is consistent with the low arabinose content and also the presence of the galactose-arabinose disaccharide in the gum.

Very/...

Very interesting results were obtained when karroo gum acid was hydrolysed in 0.5N-sulphuric acid, since two aldobiuronic acids were produced. These were isolated by partition on cellulose and converted into the barium salts. Both aldobiuronic acids were found to consist of galactose and glucuronic acid, the one having a specific rotation of $+12^{\circ}$ and the other a specific rotation of $+110^{\circ}$. The aldobiuronic acid, $[\alpha]_D$ $+12^{\circ}$, moved at the same rate as 6-O-β-D-glucuronosyl-D-galactose on a paper chromatogram and on methylation gave the crystalline methyl ester of heptamethyl-6-O-β-D-glucuronosyl-D-galactose (XII). Bromine oxidation experiments showed that galactose was the aglycone of the other aldobiuronic acid, and an attempt was made to establish the position of linkage by the usual methylation and hydrolysis technique. The methylated derivative proved to be exceedingly resistant to acid hydrolysis and the tri-O-methyl-galactose could not be characterised. It is tentatively suggested, however, that the aldobiuronic acid is 4-O-D-glucuronosyl-D-galactose, since the specific rotation of the methylated derivative ($+96^{\circ}$) is reasonably close to the value ($+81^{\circ}$ in methanol) reported for the methyl ester of heptamethyl-4-O-D-glucuronosyl-D-galactose[20]. Furthermore, 4-O-methyl-D-glucuronic acid is most probably linked to D-galactose by 1-4 and 1-6 links in mesquite gum.

The/...

The molar ratio of arabinose to galactose in the mixture produced on complete hydrolysis of karroo gum, was found to be 5:9, and the percentage of rhamnose in karroo gum was about 2. This last value was obtained from the weight of the monosaccharide isolated from the autohydrolysis sugar mixture. The simplest repeating unit would then consist of approximately 1 mole of rhamnose, 3 moles of glucuronic acid, 10 moles of arabinose and 18 moles of galactose, giving a unit weight of 4910. As there are three hexuronic acid moles, the theoretical equivalent weight is 1637, which is in good agreement with the value found. The theoretical rhamnose percentage is 2.97.

Karroo gum thus resembles the other acacia gums in that it contains the same monosaccharides and also 6-O-β-D-glucuronosyl-D-galactose is part of the structure. It shows marked differences from the other acacia gums, however. On graded hydrolysis it gives two aldobiuronic acids. Furthermore, the proportion of rhamnose is very low. In this respect karroo gum resembles peach gum[21]. It is also similar to peach gum in that both polysaccharides yield 3-O-β-L-arabopyranosyl-L-arabopyranose on graded hydrolysis. Peach gum, however, contains xylose, which was not detected in the hydrolysis products from karroo gum. Furthermore, only one aldobiuronic acid, 6-O-β-D-glucuronosyl-D-galactose,

was/...

was obtained from peach gum. It would be of interest to re-examine the aldobiuronic acid fraction from peach gum to see whether a second aldobiuronic acid could be detected.

The above observations can be explained if both peach and karroo gums consist of a mixture of polysaccharides. One component of peach gum would be similar to a polysaccharide component of karroo gum, whilst a second karroo gum polysaccharide could be based on the "acacia pattern". Unfortunately, since karroo gum acid was very insoluble, an electrophoresis examination could not be made to test this hypothesis.

The significance of the presence of two or more aldobiuronic acids in a gum polysaccharide, merits discussion. Since galactose residues can be linked together by either 1-3 or 1-6 links in the plant gums, and glucose residues are linked by 1-4 and 1-6 links in amylopectin, it is possible that a mixture of aldobiuronic acids may be produced on degradation of a single polysaccharide. On the other hand, most of the gums which have been investigated contain only one aldobiuronic acid. It might be argued, therefore, that the presence of more than one aldobiuronic acid indicates a mixture of polysaccharides. This argument is open to question, since the detection of a mixture of

aldobiuronic acids was slight, prior to the application of the paper chromatography technique. Joubert intends carrying out an investigation of the behaviour of gum myrrh in the Tiselius apparatus. It will be of interest to see whether gum myrrh, which contains a mixture of aldobiuronic acids, gives single boundaries and if the ascending and descending patterns are mirror images.

SECTION II

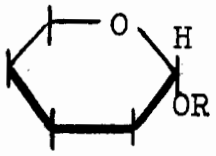
THE CONFIGURATION OF GLYCOSIDIC BONDS

The isolation and determination of the structures of disaccharides has become a most valuable method of elucidating the fine details of the structures of polysaccharides. In the case of most disaccharides, however, the question of the configuration of the biose link has not been satisfactorily settled. It was therefore considered important to make a theoretical study of this fundamental problem. The obvious approach to an investigation of this type is to start with an examination of the methyl and other simple glycosides.

Simple Glycosides

In 1909 Hudson[67] suggested that the more dextrorotatory member of a pair of glycosides in the D series of sugars be called α, and in the L series the more laevorotatory member be called α. This definition was extended by Hudson[68] in 1938 when he stated that an α-glycoside in the D series or a β-glycoside in the L series had the D configuration at the glycosidic centre, (XX) and (XXI).

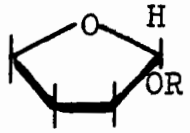
(XX)/...



α-D-aldopyranose

β-L-aldopyranose

XX



α-D-aldofuranose

β-L-aldofuranose

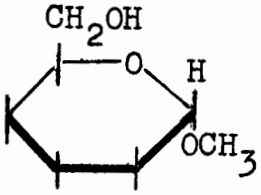
XXI

This implies that the more dextrorotatory member of a pair of glycosides in the D series has the D configuration at the glycosidic centre and that the more laevorotatory member in the D series has the L configuration at the glycosidic centre. It is of interest to review and examine the evidence for this statement.

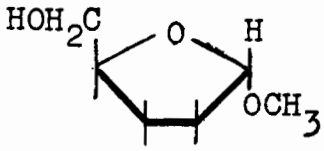
The problem resolves itself into two parts (a) to show that the more dextrorotatory (or laevorotatory) glycosides in the D series have the same configuration at the glycosidic carbon atom and (b) to prove that (XX) and (XXI) have the correct configurations at $C_{(1)}$.

Hudson and his co-workers have pointed out that the methyl glycosides of the D-aldohexopyranoses (XXII) and the methyl glycosides of the D-aldopentofuranoses (XXIII), having the same configuration at the glycosidic centre, should give the same dialdehyde (XXIV) on periodate oxidation.

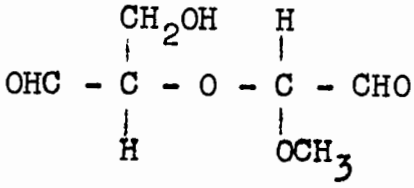
(XXII)/...



XXII



XXIII



XXIV

Table B shows the results obtained by Hudson and his co-workers[69,70]. Mc Clenahan and Hockett[71] obtained identical results when they studied the oxidation of methyl glycosides with lead tetraacetate.

TABLE B

Glycoside	$[\alpha]_D$	$[\alpha]_D$ of dialdehyde
O-methyl-α-D-glucopyranoside	+159°	+121.1°
O-methyl-α-D-galactopyranoside	+196°	+120.7°
O-methyl-α-D-gulopyranoside	+120°	+120.5°
O-methyl-α-D-mannopyranoside	+ 79°	+119.5°
O-methyl-α-D-altropyranoside	+125.8°	+120.8°
O-methyl-α-D-arabofuranoside	+123°	+117.3°
O-methyl-β-D-glucopyranoside	- 34°	-150.6°
O-methyl-β-D-galactopyranoside	+ 1°	-148.1°

Using/...

Using a similar argument, Hudson[69,72,73] showed that the more dextrorotatory O-methyl-D-aldopentopyranosides had the same configuration at $C_{(1)}$ (table C).

TABLE C

Glycoside	$[\alpha]_D$	$[\alpha]_D$ of dialdehyde
O-methyl-α-D-arabopyranoside	- 17°	+124.2°
O-methyl-α-D-xylopyranoside	+154°	+125.2°
O-methyl-α-D-lyxopyranoside	+ 59.4°	+124.1°
O-methyl-β-D-arabopyranoside	-245°	-123.7°
O-methyl-β-D-xylopyranoside	- 65°	-124.3°
O-methyl-β-D-lyxopyranoside	-128°	-125.5°
O-methyl-β-D-ribopyranoside	-105°	-123.4°

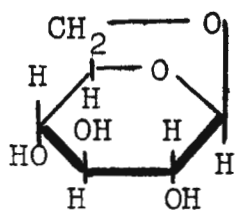
Grosheintz[74] then demonstrated that lead tetraacetate oxidation of O-methyl-α-L-arabopyranoside gave the same dialdehyde as Hudson had obtained from O-methyl-β-D-arabopyranoside. Furthermore, O-methyl-β-L-arabopyranoside, $[\alpha]_D$ +246°, gave the dialdehyde, $[\alpha]_D$ +123.2°.

Evidence for the D configuration of the glycosidic centre in α-D-glucose, has come from the work of Boëseken,

who/...

who studies the conductivity of boric acid solutions containing sugar anomers. (Boëseken reviews this work in "Advances in Carbohydrate Chemistry", 1949.) As early as 1903, Armstrong[75] had shown that O-methyl-α-D-glucoside could be enzymatically hydrolysed to give α-D-glucose, and recent experiments by Ballon, Roseman and Link[76], have proved that the reductive cleavage of benzyl glycosides of acetylated hexoses and pentoses occurs without change of configuration at $C_{(1)}$. O-Methyl-α-D-glucoside must also have the D configuration at $C_{(1)}$, therefore.

As Boëseken's work was not entirely satisfactory, Hudson[77] attacked the problem in a different way. He showed that in the presence of alkali, O-phenyl-β-D-glucoside readily lost phenol with formation of 1:6-anhydro-D-glucose (XXV).



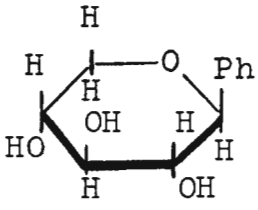
XXV

On the other hand, the anomeric α-glucoside reacted very slowly. It was therefore argued that the glycosidic group and the $C_{(6)}$ hydroxyl group had the cis relationship in

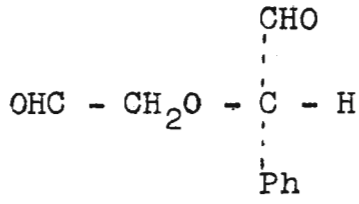
the/...

the β-anomer, i.e., $C_{(1)}$ had the L configuration. Using the same argument, O-phenyl-β-D-galactoside was shown to have the L configuration at $C_{(1)}$. This proof is not completely rigid, either, as there is a possibility of the condensation being accompanied by a Walden inversion. The results, however, are consistent with Boëseken's conductivity measurements.

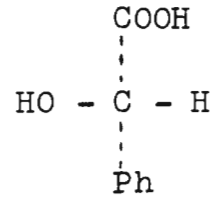
Since β-D-xylopyranosylbenzene (XXVI) has a specific rotation of -14.4°, it is considered a β-glycoside. A most elegant method of proving that $C_{(1)}$ in (XXVI) has the L configuration, has been put forward by Bonner and Hurd[78]. These workers treated the compound with periodate and showed that the oxidation product (XXVII) was related to L-mandelic acid (XXVIII).



XXVI



XXVII

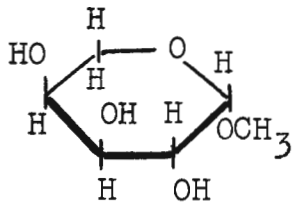


XXVIII

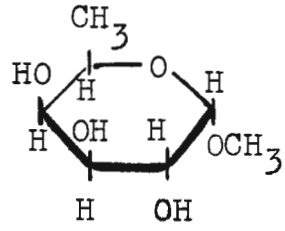
By means of X-rays, Cox[79] and his co-workers studied the crystal structures of a number of monosaccharides and their O-methyl glycosides. They pointed out the close

similarity/...

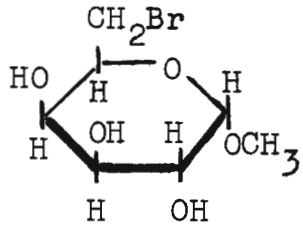
similarity between the crystal structures of O-methyl-β-L-arabopyranoside (XXIX), O-methyl-α-D-fucopyranoside (XXX) and the 6-bromohydrin of O-methyl-D-galactopyranoside (XXXI). The three glycosides must therefore have possessed the same configuration at $C_{(1)}$.



XXIX



XXX



XXXI

In a later paper[80], the above authors put forward an argument that the glycosidic hydroxyl groups in β-D-glucose and β-D-xylose had the L configuration.

It can be seen that the experimental facts obtained from a study of a wide variety of aldoses and their derivatives fit in with the Hudson formulations. It is also of interest that Gorin, Kauzmann and Walter[81] calculated the rotations of O-methyl-α-D-arabopyranoside, O-methyl-β-D-arabopyranoside, O-methyl-α-D-lyxopyranoside and O-methyl-β-D-lyxopyranoside by means of the one-electron theory. The relative signs agreed with those observed and the magnitudes were satisfactory.

In/...

In 1950 Kuhn[82] investigated the infra-red absorption spectra of seventy-five carbohydrates and he was able to show that anomers give different absorption curves. Now when only one member of an anomeric pair is available, it is sometimes difficult to assign a configuration to the glycosidic group. Whistler and House[83] therefore followed up Kuhn's work to see whether the infra-red method could be used for this purpose. They studied the spectra of aldohexoses, aldopentoses, acetyl derivatives, O-methyl glycosides and O-phenyl glycosides. The most promising results were obtained in the case of O-phenyl-D-xyloside, O-phenyl-D-glucoside and O-phenyl-D-galactoside. The α-anomers had a prominent absorption band at 11.61 to 11.73 μ, while the β-anomers did not absorb in this region. On the other hand, the β-isomers absorbed strongly in the region 12.15 to 12.25 μ, but the α-isomers did not absorb in this spectral range. Whistler and House pointed out, however, that since the absorptions of different sugars are not of the same magnitude and are not located at the same spectral position, it is necessary to have a knowledge of the sugar type and to have reference absorptions before assignments of anomeric configurations can be made.

Reducing Disaccharides

Evidence for the configuration of the biose link in the case of some of these disaccharides has been accumulated from a study of enzymatic hydrolysis and also from the methods used in the direct synthesis of these sugars.

It is known that β-glycosidases present in emulsin are capable of hydrolysing cellobiose and gentiobiose. Since these enzymes also split simple β-glycosides such as O-phenyl-β-D-glucoside, the biose links in the disaccharides were assigned the β-configuration, i.e., $C_{(1)}$ in the sugar portion had the L configuration. In the same way lactose was considered a β-disaccharide since it was hydrolysed by lactase, a β-glycoside splitting enzyme.

In 1926 Helferich and Klein[84] synthesised gentiobiose octaacetate by the reaction of acetobromoglucose on 1:2:3:4-tetra-O-acetyl-D-glucose in the presence of silver oxide. They argued that, since acetobromoglucose reacts with methanol in the presence of silver oxide to give O-methyl-β-D-glucoside[85], the configuration of the biose link in gentiobiose must also be β. This result is in agreement with enzyme studies, and is generally accepted.

Helferich/...

Helferich and his school have prepared a number of other disaccharides using the above Koenigs and Knorr reaction.

Derivatives of β-disaccharides have also been obtained[86] by the reaction of acetobromo-sugars on diacetone hexoses in the presence of silver oxide, and gentiobiose and cellobiose have been synthesised[87] by the reaction of acetobromoglucose on the sodio derivatives of 1:2:3:4-tetra-O-acetyl-D-glucose and 1:2:3:6-tetra-O-acetyl-D-glucose.

In his investigations of the structures of disaccharides, Zemplèn[88] found that he could reduce the chain length of the aglycone portion by degradation of the acetylated oxime. Cellobiose, for example, was converted into 3-O-β-D-glucopyranosyl-D-arabinose. Since cellobiose is a β-disaccharide, the biose link in the new disaccharide must also have the β-configuration.

Disaccharides have also been converted into different disaccharides by the reaction of perbenzoic acid on the lactals. Haworth[89] and his co-workers converted cellobiose into 4-O-β-D-glucopyranosyl-D-mannose by this method.

A list of β-disacchardies is given in table D.

TABLE D/...

TABLE D

Disaccharide	Structure	$[\alpha]_D$ Eqlm. val.	Evidence for configuration of biose link
gentiobiose	6-O-β-D-glucopyranosyl-D-glucose	+8.7°	Enzymes; Synthesis[84,86,87,90]
-	6-O-β-D-galactopyranosyl-D-glucose	+36.4°	Synthesis[91]
primeverose	6-O-β-D-xylopyranosyl-D-glucose	-3.4°	Synthesis[92]
-	6-O-β-D-galactopyranosyl-D-galactose	+34.0°	Synthesis[86]
-	6-O-β-D-glucopyranosyl-D-galactose	+8.2°	Synthesis[93]
-	6-O-β-D-glucuronosyl-D-galactose	-8.5°	Synthesis[9]
-	5-O-β-D-glucopyranosyl-D-arabofuranose	-3°	Zemplèn degradation[94]
lactose	4-O-β-D-galactopyranosyl-D-glucose	+55.4°	Enzymes; Synthesis[95]
cellobiose	4-O-β-D-glucopyranosyl-D-glucose	+34.6°	Enzymes; Synthesis[87]
epilactose	4-O-β-D-galactopyranosyl-D-mannose	+27°	Perbenzoic acid[96]
-	4-O-β-D-glucopyranosyl-D-mannose	+12.5°	Perbenzoic acid[89]
-	3-O-β-D-glucopyranosyl-D-arabopyranose	-100°	Zemplèn degradation[88]
-	3-O-β-D-galactopyranosyl-D-arabopyranose	-63°	Zemplèn degradation[97]

Maltose is considered an α-disaccharide because it is the anomer of cellobiose (a β-disaccharide) and also because it is hydrolysed by an α-glycosidase, maltase.

Synthetic methods have been found for the production of α-disaccharides. Melibiose[98] was formed when acetobromogalactose was reacted with 1:2:3:4-tetra-O-acetyl-D-glucose in the presence of quinoline, and α-acetochloro-isoprimeverose[99] was prepared by the reaction of acetobromoxylose on 1-chloro-2:3:4-tri-O-acetyl-D-glucose in the presence of mercuric acetate. α-Disaccharides have also been produced by the Zemplèn degradation.

6-O-α-D-Galactopyranosyl-D-mannose[35] has been shown to give the same osazone as melibiose and hence its biose link must have the α-configuration. It is obvious that this neat method is limited in application.

Table E is a list of α-disaccharides.

TABLE E/...

TABLE E

Disaccharide	Structure	$[\alpha]_D$ Eqlm. val.	Evidence for configuration of biose link
melibiose	6-O-α-D-galacto-pyranosyl-D-glucose	+129.5°	synthesis[98]
isoprimeverose	6-O-α-D-xylo-pyranosyl-D-glucose	+121.3°	synthesis[99]
-	6-O-α-D-galacto-pyranosyl-D-mannose	+124.6°	same osazone as melibiose[35]
maltose	4-O-α-D-gluco-pyranosyl-D-glucose	+130.4°	enzymes

Only disaccharides of known stereochemical structure are listed in tables D and E. An examination of the β-disaccharides shows that the maximum dextrorotation is +55.4° (lactose), whereas the minimum dextrorotation is +121° in the case of the α-disaccharides. If an unknown disaccharide has a D sugar as the glycosidic portion and a negative or low positive rotation, it is generally considered to be a β-disaccharide. On the other hand, if the specific rotation is high, it is considered to be an α-disaccharide. Although this is a useful guide, the limits are by no means

fixed/...

fixed. A disaccharide having a specific rotation of $+80^{o}$ or $+90^{o}$, for example, could be either α or β.

As early as 1916, even before the ring size of the sugar components or the position of linkage had been established, Hudson[100] noticed that the specific rotations of lactose and cellobiose were related. He first considered the general case of a galactose-glucose disaccharide (galactose < glucose <) and a glucose-glucose disaccharide (glucose < glucose <) each having the same aglycone linked to the sugar portions in the same way. If the linkages were of the β type, for example, the difference in the molecular rotations could be obtained as follows:-

β-galactose < glucose < = Ga - L + R

β-glucose < glucose < = G - L + R

difference = Ga - G

(Ga and G represent the rotations of the left hand galactose and glucose chains, L represents the rotation of their bound lactonyl carbons and R represents the rotation of the common right hand glucose residue.)

Now the molecular rotation of O-methyl-β-D-galactoside = Ga - A[67] and the molecular rotation of O-methyl-β-D-glucoside = G - A. (A represents the rotation of the

glycosidic/...

glycosidic carbon atom.) It is easy to see, therefore, that the difference in the molecular rotations of the disaccharides must be equal to the difference in the molecular rotations of the glycosides. Furthermore, since molecular rotation = specific rotation x molecular weight, the difference in the specific rotations of the disaccharides = difference in the specific rotations of the glycosides x $\frac{\text{molecular weight of the glycoside}}{\text{molecular weight of the disaccharide}}$.

This was then applied to lactose and cellobiose as shown below.

O-methyl-β-D-galactoside has $[\alpha]_D$	0°
O-methyl-β-D-glucoside has $[\alpha]_D$	-32°
difference	$+32^\circ$

The difference $+32 \times \frac{194}{342}$ i.e. $+\underline{18}^\circ$ should be equal to the difference in the specific rotations of the disaccharides. The following calculation shows that the difference in the specific rotations of β-cellobiose and β-lactose = $+19^\circ$.

β-lactose has $[\alpha]_D$	$+35^\circ$
β-cellobiose has $[\alpha]_D$	$+16^\circ$
difference	$+19^\circ$

Since it is now known that cellobiose and lactose only differ in that they have a different diastereoisomer as the glycosidic portion, it was necessary to see whether this

molecular/...

molecular difference method is applicable to any pair of disaccharides which only differ in having a different sugar portion.

Gentiobiose and 6-O-β-D-galactopyranosyl-D-glucose; epilactose and 4-O-β-D-glucopyranosyl-D-mannose are two pairs of disaccharides which are similar to the pair of disaccharides considered above. The theoretical difference in the specific rotations should again be $+18^{\circ}$.

Now, 4-O-β-D-galactopyranosyl-D-mannose has $[\alpha]_D = +27^{\circ}$

and 4-O-β-D-glucopyranosyl-D-mannose has $[\alpha]_D = \underline{+12.5^{\circ}}$

difference $= +14.5^{\circ}$

Also, 6-O-β-D-galactopyranosyl-D-glucose has $[\alpha]_D = +36.4^{\circ}$

and 6-O-β-D-glucopyranosyl-D-glucose has $[\alpha]_D = \underline{+\ 8.7^{\circ}}$

difference $= +27.7^{\circ}$

In the case of the two arabinose disaccharides listed in table D, the theoretical difference is obtained by multiplying the difference in the specific rotations of O-methyl-β-D-galactoside and O-methyl-β-D-glucoside by $\frac{194}{312}$, since the molecular weight of each disaccharide = 312. This comes out to be $+20^{\circ}$. As shown below, the actual difference is $+37^{\circ}$.

3-O-β-galactopyranosyl-D-arabinose has $[\alpha]_D = -\ 63^{\circ}$

3-O-β-glucopyranosyl-D-arabinose has $[\alpha]_D = \underline{-100^{\circ}}$

difference $= +\ 37^{\circ}$

Now/...

Now primeverose and gentiobiose differ in that a pentose is the glycosidic portion in the one case, and a hexose is the glycosidic portion in the other. A theoretical difference in the specific rotations can be calculated as follows:-

O-methyl-β-D-xylopyranoside has $[\alpha]_D = -65^\circ$. This amounts to $-65 \times \frac{164}{312}$ or $\underline{-34}^\circ$ for the disaccharide (molecular weight = 312).

O-methyl-β-D-glucopyranoside has $[\alpha]_D = -32^\circ$. This amounts to $-32 \times \frac{194}{342}$ or $\underline{-18}^\circ$ for the disaccharide (molecular weight = 342).

i.e. The theoretical difference is -16°.

Since 6-O-β-D-xylopyranosyl-D-glucose has $[\alpha]_D = -3.4^\circ$

and 6-O-β-D-glucopyranosyl-D-glucose has $[\alpha]_D = \underline{+8.7^\circ}$

the actual difference $= -12.1^\circ$

In all the above cases, the agreement between the theoretical and the practical values can be considered satisfactory, since equilibrium rotations of the disaccharides have been used, and it is questionable whether these have all been measured with a high degree of accuracy. It should be possible, therefore, roughly to calculate the specific rotation of a new disaccharide, if only the glycosidic portion is changed.

Consider/...

Consider mannobiose[34], 4-O-D-mannopyranosyl-D-mannose. Assuming this to be a β-disaccharide, its specific rotation can be predicted in the following way:-

O-methyl-β-D-glucopyranoside has $[\alpha]_D = -32^\circ$

O-methyl-β-D-mannopyranoside has $[\alpha]_D = \underline{-78^\circ}$

difference $= +46^\circ$,

which amounts to $+46 \times \frac{194}{342}$ or $\underline{+26^\circ}$ for the disaccharides (molecular weight = 342).

The difference between the specific rotations of 4-O-β-D-glucopyranosyl-D-mannose and mannobiose must therefore = + 26°.

i.e. 4-O-β-D-glucopyranosyl-D-mannose has $[\alpha]_D = +12.5^\circ$

4-O-β-D-mannopyranosyl-D-mannose has $[\alpha]_D = \underline{\alpha^\circ}$

difference $+26^\circ$

The theoretical specific rotation (α) is thus = -13.5°. A value of -2.2° has been reported. Whistler and Stein[34] suggested that the disaccharide had a β-biose link on the grounds that it was not hydrolysed by emulsin which is known to contain an α-mannosidase. The theoretical calculation confirms this rather unsatisfactory negative evidence.

Vicianose/...

Vicianose is a most interesting disaccharide because L-arabinose is the glycosidic portion. Helferich and Bredereck[98] synthesised the disaccharide by the reaction of acetobromo-L-arabinose on 1:2:3:4-tetra-O-acetyl-D-glucose using silver oxide as acid acceptor. They suggested that a β-biose link was produced, analogous to the formation of β-biose links from acetobromoglucose; and proposed the structure 6-O-β-L-arabopyranosyl-D-glucose.

Assuming this structure to be correct, its specific rotation can be calculated from the known value of primeverose (6-O-β-xylopyranosyl-D-glucose) as follows:-

O-methyl-β-D-xylopyranoside has $[\alpha]_D = -65^\circ$

O-methyl-β-L-arabopyranoside has $[\alpha]_D = \underline{+246^\circ}$

difference $= -311^\circ$, which

amounts to $-311 \times \frac{164}{312}$ or $\underline{-164^\circ}$ for the disaccharides (molecular weight = 312). The difference between the specific rotations of primeverose and vicianose must therefore $= -164^\circ$.

6-O-β-D-xylopyranosyl-D-glucose has $[\alpha]_D = -3.4^\circ$

6-O-β-L-arabopyranosyl-D-glucose has $[\alpha]_D = \underline{\alpha^\circ}$

difference $= -164^\circ$

This indicates that the specific rotation of vicianose (α) should $= +160.6^\circ$, whereas $+40.5^\circ$ was the value reported.

On/...

On the other hand, if vicianose is an α-disaccharide, its specific rotation could be predicted from the known value of isoprimeverose (6-O-α-D-xylopyranosyl-D-glucose) as shown below.

O-methyl-α-D-xylopyranoside has $[\alpha]_D = +154^\circ$

O-methyl-α-L-arabopyranoside has $[\alpha]_D = \underline{+\ 18^\circ}$

difference $= +136^\circ$, which

amounts to $+136 \times \frac{164}{312}$ or $\underline{+71.6^\circ}$ for the disaccharides (molecular weight = 312).

The difference between the specific rotations of isoprimeverose and vicianose must therefore = $+71.6^\circ$.

6-O-α-D-xylopyranosyl-D-glucose has $[\alpha]_D = +121.3^\circ$

6-O-α-L-arabopyranosyl-D-glucose has $[\alpha]_D = \underline{\quad \alpha'^\circ}$

$+\ 71.6^\circ$

The specific rotation (α') comes out to be $+49.7^\circ$. Since this value is close to the actual rotation ($+40.5^\circ$), it would appear that vicianose is an α-disaccharide. Pigman and Goepp (Carbohydrate Chemistry, 1948) also point out that vicianose should be called an α-disaccharide.

Now the acetobromo-L-arabinose used by Helferich and Bredereck had $[\alpha]_D$ $+284^\circ$, i.e. it was the β-anomer[101,102,116]. If this is reacted with an alcohol under the Koenigs and

Knorr/...

Knorr conditions, one would expect a Walden inversion with the production of an α-glycoside. Hudson[103] has shown that β-acetobromo-D-arabinose, $[\alpha]_D$ -288°, reacts with methanol in the presence of silver carbonate to give the tri-O-acetate of O-methyl-α-D-arabopyranoside. It would be of great interest to carry out the reaction of β-acetobromo-L-arabinose with methanol and silver carbonate to prove that the O-methyl-α-glycoside is produced in the L series as well.

Rutinose, 6-O-L-rhamnopyranosyl-D-glucose, is another disaccharide which has an L sugar as the glycosidic unit. The heptaacetate of rutinose was synthesised by Zemplèn and Gerecs[104] in 1934. They reacted α-acetobromo-L-rhamnose, $[\alpha]_D$ -65°, with 1-chloro-2:3:4-tri-O-acetyl-D-glucose in the presence of mercuric acetate. The 1-chloro-hexa-O-acetyl-disaccharide was then converted into rutinose heptaacetate. Zemplèn and Gerecs suggested that the biose link was β. If this is correct, the specific rotation can again be predicted from the known value of primeverose in the following way:-

O-methyl-β-D-xylopyranoside has $[\alpha]_D = -65°$. This amounts to $-65 \times \frac{164}{312}$ or $\underline{-34.2°}$ for the disaccharide (molecular weight = 312).

O-methyl-/...

6-O-α-D-xylopyranosyl-D-glucose has $[\alpha]_D$ = +121.3°

6-O-α-L-rhamnopyranosyl-D-glucose has $[\alpha]_D$ = α'°

difference = +115.4°

It can be seen that α' = +5.9° which is reasonably close to the experimental value (+0.8°). On this basis it would appear that rutinose is an α-disaccharide. Now when acetobromo sugars are reacted with alcohols using pyridine or mercuric acetate as catalyst, condensation often occurs with retention of configuration. If α-acetobromo-L-rhamnose reacts in this way, the above anomaly can be accounted for. The reaction of α-acetobromo-L-rhamnose with methanol and mercuric acetate should be carried out to see whether the α- or β-methyl glycoside is formed.

Zemplèn, Gerecs and Flesch[105] also synthesised the 1-chloro-hexaacetate of robinobiose (6-O-rhamnopyranosyl-D-galactose) by the reaction of α-acetobromo-L-rhamnose on 1-chloro-2:3:4 tri-O-acetyl-D-galactose in the presence of mercuric acetate. The authors suggested a β-biose link, but since robinobiose heptaacetate has a low rotation (-9.9° in chloroform), an α-biose link is indicated.

In 1935 Goebel and Babers[106] prepared the methyl ester of O-methyl-2:3:4-tri-O-acetyl-β-D-glucuronosyl-pyranoside. An attempt was made to deacetylate and

saponify/...

saponify the glycoside and so obtain the β-methyl glycoside of D-glucuronic acid. The latter was not obtained in crystalline form and the authors did not give the specific rotation. The specific rotation of O-methyl-D-glucuronosyl-pyranoside can be predicted in the following way:-

6-O-β-D-glucopyranosyl-D-galactose has $[\alpha]_D$ = +8.2°

6-O-β-D-glucuronosyl-D-galactose has $[\alpha]_D$ = -8.5°

difference = +16.7°

Now O-methyl-β-D-glucopyranoside has $[\alpha]_D$ = -32°, which amounts to $-32 \times \frac{194}{342}$ or -18.2° for the disaccharide (molecular weight = 342)

and O-methyl-β-D-glucuronosylpyranoside has

$[\alpha]_D = \alpha \times \frac{208^\circ}{356}$ for the aldobiuronic acid (molecular weight = 356).

Therefore, $-18.2^\circ - \alpha \times \frac{208^\circ}{356} = +16.7^\circ$

or $\alpha = -59.5^\circ$

This value could be checked by repeating Goebel and Babers work. Alternatively O-methyl-β-D-glucopyranoside could be oxidised with dinitrogen tetroxide in chloroform.

The specific rotation of the aldobiuronic acid 4-O-β-D-glucuronosyl-D-glucose can be obtained by subtracting +16.7° from the rotation of 4-O-β-D-glucopyranosyl-D-glucose (+34.6°). This comes out to be +17.9°. Now an

amorphous/...

amorphous 4-O-β-D-glucuronosyl-D-glucose, obtained by the partial hydrolysis of the specific soluble substance from the type III pneumococcus, was found to have a specific rotation of $+7.3^{o}$(107). In all probability the aldobiuronic acid was not completely pure and the agreement must be considered satisfactory.

In an analogous manner, the specific rotation of 6-O-β-D-glucuronosyl-D-glucose can be predicted by subtracting $+16.7^{\circ}$ from the value for 6-O-β-D-glucopyranosyl-D-glucose ($+8.7^{\circ}$). The theoretical rotation of the aldobiuronic acid is therefore -8°. It would be most interesting to attempt the preparation of the methyl glycoside of the aldobiuronic acid by oxidation of the methyl glycoside of gentiobiose with dinitrogen tetroxide in chloroform. The aldobiuronic acid could then be obtained and its rotation compared with the theoretical value. This investigation could be extended to the other 1-6 disaccharides as well.

This theoretical method offers a promising approach to the solution of the configuration of the biose links in reducing disaccharides. It is necessary, however, to obtain more reference disaccharides; particularly those containing aglycones of the L-series. This could be done by variation of the classical synthetic methods. A possible scheme for

the/...

the synthesis of 6-O-β-D-xylopyranosyl-L-arabinose[38], for example, would be the following:-

Acetobromo-D-xylose + 1:2:3:4-tetra-O-acetyl-L-glucose $\xrightarrow{Ag_2O}$ 6-O-β-D-xylopyranosyl-L-glucose heptaacetate $\xrightarrow[\text{degradation}]{\text{Zemplèn}}$ 5-O-β-D-xylopyranosyl-L-arabinose hexaacetate $\xrightarrow[\text{acetyl groups}]{\text{removal of}}$ disaccharide.

5-O-β-D-Glucopyranosyl-L-arabinose and 5-O-β-D-galactopyranosyl-L-arabinose could be made in an analogous way. Furthermore, Smith's method of preparing cellobiose[87] could be modified in order to obtain 3-O-β-D-glucopyranosyl-L-arabinose. This is schematically illustrated by the following:-

Acetobromo-D-glucose + 4-sodio-1:2:3:6-tetra-O-acetyl-L-glucose $\longrightarrow$ 4-O-β-D-glucopyranosyl-L-glucose octaacetate $\xrightarrow[\text{degradation}]{\text{Zemplèn}}$ 3-O-β-D-glucopyranosyl-L-arabinose heptaacetate $\xrightarrow[\text{acetyl}]{\text{remove}}$ disaccharide.

Analogous methods could be used to obtain 3-O-β-D-galactopyranosyl-L-arabinose and 3-O-α-L-arabopyranosyl-L-arabinose.

Very recent work by Stacey[108] and his school has offered an alternative approach to the problem of the configuration of biose links. These authors have

investigated/...

investigated the infra-red spectra of D-glucose derivatives and oligo- and polyglucosans. From their results they were able to show that there are three principal sets of bands.

Type I : α-anomers, 917 ±13 $cm.^{-1}$; β-anomers, 920 ±5 $cm.^{-1}$

Type II : α-anomers, 844 ±8 $cm.^{-1}$; β-anomers, 891 ±7 $cm.^{-1}$

Type III: α-anomers, 766 ±10 $cm.^{-1}$; β-anomers, 744 ±9 $cm.^{-1}$

Using the type II frequencies, it is possible to assign an anomeric configuration to an unknown polyglucose or glucoside, even although some β-glucosans have weak peaks in the frequency range 844 ±8 $cm.^{-1}$ and some of the type I absorptions of the α-compounds lie in the same range as the type II absorptions of the β-compounds.

In all probability D-galactose, D-xylose, D-mannose, L-arabinose and L-rhamnose compounds will be examined in the same way. It will then be interesting to examine disaccharides such as mannobiose, lactose, melibiose, vicianose, rutinose, and robinobiose and compare the results with those obtained by the specific rotation method.

EXPERIMENTAL

EXPERIMENTAL

Unless otherwise stated:-

M.ps. are uncorrected.

Specific rotations measured in aqueous solution.

Concentration of solutions carried out at 40°/20 mm.

Purification of Cyanophylla Gum

The crude gum (100 g.) was dissolved in water (300 c.c.) giving a dark brown, neutral solution which was filtered through a layer of cellulose powder. The filtrate was acidified with 2N-hydrochloric acid (40 c.c.) and the gum acid precipitated by pouring the acidified solution into ethanol (1.5 l.). Two further precipitations were carried out and finally an aqueous solution of the gum acid was poured into ethanol. The purified material was washed with acetone and dried in a vacuum (20 mm.) at 45° for 30 hours, yielding a white amorphous powder (65 g.). Hydrochloric acid could not be detected when a neutralised solution of gum acid was titrated against silver nitrate and potassium chromate. The gum acid had $[\alpha]_D^{16}$ -20° (C, 0.68).

Found/...

[Found: Moisture (loss at 100°/20 mm.), 10.4; sulphated ash, 0.73; N, 0.17%; equiv., 740 (by direct titration on material dried at 100°/20 mm. with 0.01N-sodium hydroxide using phenolphthalein); iodine number, 2.1 c.c. of 0.1N-iodine per g. after 20 minutes' oxidation (Baker and Hulton[109])].

Three different samples were examined (table F) I and II being collected from single trees in the same plantation near Retreat; III was a mixture from a number of trees in different plantations scattered over the Cape Flats.

TABLE F

Sample	$[\alpha]_D$	Equiv. wt.
I	-24°	750
II	-27°	740
III	-20°	742

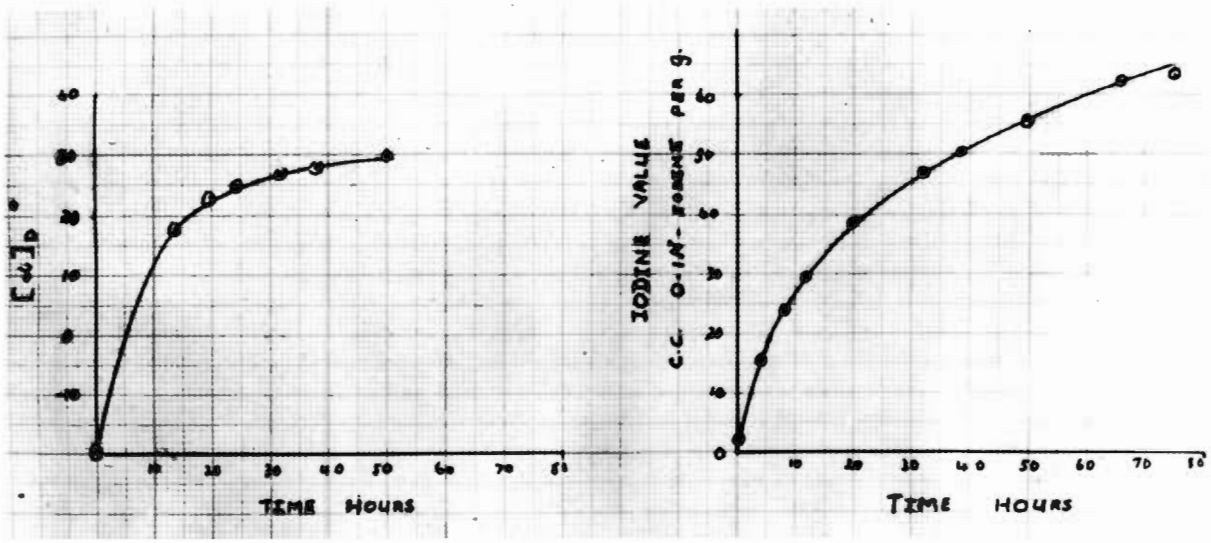


Fig. 7.

The variation of specific rotations and iodine values on autohydrolysis of cyanophylla gum acid.

Autohydrolysis

The gum acid (51 g.) in water (1 l.) was heated on a waterbath, the temperature of the solution being maintained at 90 - 95°. The hydrolysis was followed by making polarimetric and iodimetric observations: $[\alpha]_D$ -20° (initial), +18° (14 hours), +23° (20 hours), +25° (24 hours), +27° (32 hours), +28° (38 hours), ca. +30° (50 hours). Iodine numbers expressed as c.c. of 0.1N-iodine per g.: 2.3 (initial), 15.7 (4 hours), 24.0 (8 hours), 29.4 (12 hours), 38.6 (20 hours), 47.0 (32 hours), 50.1 (38 hours), 55.6 (50 hours), 62.5 (66 hours), 63.2 (75 hours). See figure 7.

The cooled solution was filtered, concentrated (to 400 c.c.) at 60° under a slightly reduced pressure, and poured into ethanol (1.5 l.). A white precipitate settled out; this turned gummy on standing. The aqueous ethanol was decanted off and the gum converted into a fibrous solid by grinding with acetone (300 c.c.). This degraded gum acid (A) was filtered at the pump, washed with ethanol and dried in vacuum (20 mm.) at 45°; yield 27.3 g. [Found: Moisture (loss at 100°/20 mm.), 6.7%]. Ethanol and acetone were removed from the combined filtrates by

distillation/...

distillation and the aqueous solution was neutralised with barium carbonate, concentrated (to 80 c.c.) and poured into methanol (1 l.). The slimy precipitate of barium salts (B) was filtered off, washed with methanol and dried immediately in vacuum over calcium chloride; yield 8.3 g. Concentration of the aqueous methanolic mother liquors gave a syrup (C); yield 17.5 g.

Examination of Syrup C.

The syrup was a mixture of reducing sugars and a small amount of barium salts B which had escaped precipitation. By comparison with authentic sugars, rhamnose, arabinose and galactose were detected on an ascending paper chromatogram[46] using butanol - ethanol - water (4 : 1 : 5, upper layer) as solvent. (Papers were sprayed with a 1% solution of P-anisidine hydrochloride in butanol.) In addition, a pink spot (R_F, 0.25) just below galactose indicated the presence of a disaccharide, and the barium salts gave a brown spot near the origin.

Quantitative Study.

Solutions (2%) of rhamnose, arabinose and galactose were standardised as follows:- A sample (60 µl.) was spotted

on/...

on a strip of filter paper (7 x 1") with a micrometer syringe. The paper was subdivided into small pieces (20 x 5 mm.) which were shaken with water (10 c.c.) in a test tube (50 c.c.) and the tube warmed in a waterbath at 40° for 10 minutes. After filtering the solution through a funnel fitted with a glasswool plug, the paper was extracted with two further aliquots of water (5 c.c.) and the sugar in the combined eluates was estimated by the method of Hirst and Jones[47] [formic acid recovery from rhamnose, 92; from arabinose, 91; from galactose, 88%]. A sample (30 µl.) of a solution of syrup C (9% in water) was spotted along a starting line (7" wide) of an ascending paper chromatogram and the solvent allowed to run for 16 hours. Sugar zones were detected by spraying longitudinal margins cut from either end of the paper, and the individual sugars were eluted and estimated as described above. The results of three such determinations are given in table G.

Syrup C (0.2 g.) was hydrolysed with 2N-sulphuric acid (5 c.c.) in a sealed tube at 100° for 14 hours. The sugars in the neutralised (barium carbonate) hydrolysate were separated on a paper chromatogram and estimated by the periodate method [Found: molar ratio of rhamnose : arabinose : galactose : : 5.0 : 1.9 : 1.2].

TABLE G/...

TABLE G

Sugar	0.0122N-NaOH c.c.	Equiv. HCOOH $x10^5$	Corrected equiv. HCOOH$x10^5$	Moles of sugar $x10^7$	Molar ratio
Rhamnose	2.68	3.00	3.27	82	1
Arabinose	0.94	1.05	1.16	29	0.35
Galactose	0.50	0.56	0.64	13	0.16
Disaccharide	0.30	-	-	-	-
Rhamnose	2.80	3.13	3.40	85	1
Arabinose	0.97	1.08	1.19	30	0.35
Galactose	0.46	0.53	0.61	12	0.14
Disaccharide	0.36	-	-	-	-
Rhamnose	2.60	2.92	3.18	80	1
Arabinose	0.90	1.02	1.12	28	0.35
Galactose	0.50	0.56	0.64	13	0.16
Disaccharide	0.25	-	-	-	-

Mean molar ratio of rhamnose : arabinose : galactose : :
1.0 : 0.35 : 0.16 or 5.0 : 1.75 : 0.8.

Isolation/...

Isolation of the Sugars.

Syrup C (16.5 g.) was triturated with methanol and kept in the refrigerator for a few days, whereupon L-rhamnose hydrate (5 g.) crystallised. The syrupy crystalline mass was washed with cold methanol, and the crystals dried on a porous tile. After recrystallisation from methanol, L-rhamnose hydrate had m.p. 93 - 94° (not depressed on admixture with an authentic sample) and $[\alpha]_D^{16}$ +9° (C, 2.1). Its osazone had m.p. and mixed m.p. 175° (decomp.).

The methanolic washings from above were concentrated to a syrup (10.7 g.). This syrup (5.1 g.), mixed with cellulose powder, was placed on a cellulose column (1¼" diam. x 10" long) packed according to directions of Hough, Jones and Wadman[49]. Butanol, half saturated with water, was used as mobile phase and 15 c.c. fractions were collected. L-Rhamnose hydrate (1.9 g.), L-arabinose (1.2 g.) and D-galactose (0.6 g.) were thereby obtained in crystalline condition. Ethanol (96%) was then substituted as mobile phase and a chromatographically pure disaccharide (0.6 g.) was eluted. The barium salts (0.5 g.) were washed off the column with water.

The L-arabinose, isolated above, had m.p. 156° (not depressed on admixture with authentic L-arabinose) and $[\alpha]_D^{20}$ +105° (C, 1.0), after recrystallisation from methanol.

Its/...

Its benzoylhydrazone[110] had m.p. and mixed m.p. 204° after recrystallisation from ethanol. D-Galactose was recrystallised from ethanol; the pure material had m.p. 164° (not depressed on admixture with authentic D-galactose), $[\alpha]_D^{18}$ +79° (C, 1.2), and oxidation with nitric acid gave mucic acid, m.p. and mixed m.p. 214°.

The disaccharide (0.1 g.) was hydrolysed with 0.5N-sulphuric acid (2 c.c.). Arabinose and galactose were produced (detected on a paper chromatogram).

Table H summarises the hydrolytic products obtained from syrup C.

TABLE H

Products	Wt. present in syrup g.	Molar ratio
L-rhamnose hydrate	9.0	5
D-galactose	1.3	0.7
L-arabinose	2.5	1.7
disaccharide	1.3	0.4
barium salts	1.1	-

Total 15.2 g. (92% recovery on 16.5 g.)

The/...

The barium salts were examined in the same way as barium salts B and were shown to be impure barium aldobiuronate. (See the following section.)

Examination of Barium Salts B.

The white amorphous powder reduced Fellings solution strongly and had $[\alpha]_D^{17^\circ} +4^\circ$ (C, 0.46). After hydrolysis of B (0.1 g.) with 2N-sulphuric acid (5 c.c.) in a sealed tube at 100° for 14 hours, the neutralised (barium carbonate) hydrolysate was examined on a paper chromatogram. Galactose and a uronic acid were detected. (The uronic acid gave a pink spot with p-anisidine hydrochloride and moved at the same rate as galacturonic acid.) The galactose was estimated by adding ribose (53.7 mg.) to a hydrolysate prepared as above from barium salts B (258 mg.) and determining the ribose to galactose molar ratio [Found: Ba, 13.8; galactose, 40%; iodine number, 38 c.c. of 0.1N-iodine per g. Calc. for $C_{12}H_{19}O_{12}Ba_{0.5}$: Ba, 16.2 : galactose, 42.5%; iodine number, 47 c.c. of 0.1N-iodine per g.]. The above results indicated that the bulk of barium salts B was barium aldobiuronate.

When examined on a descending paper chromatogram using ethyl acetate - acetic acid - formic acid - water

(18 :/...

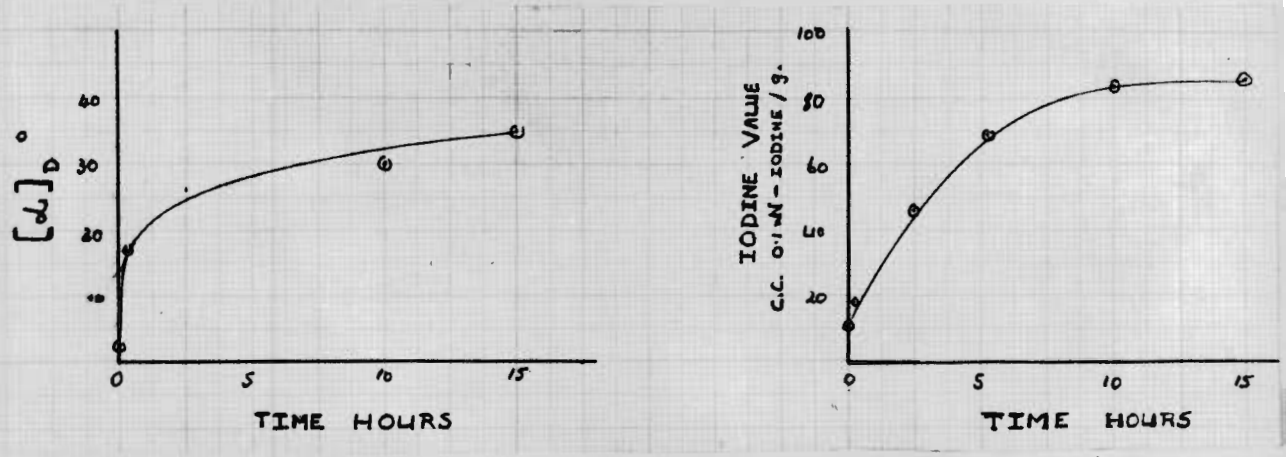
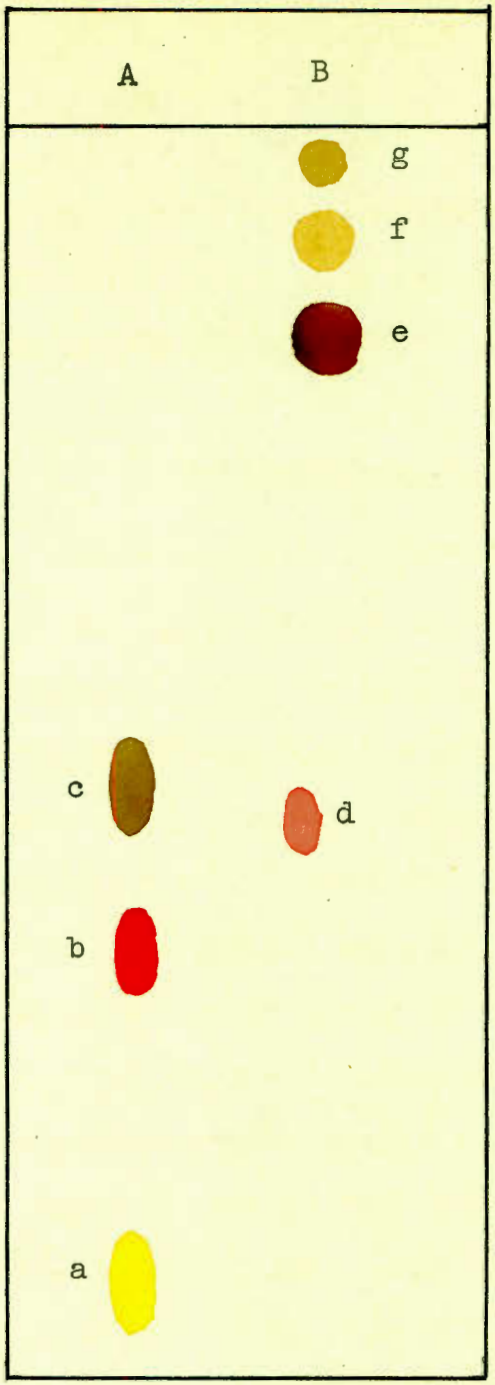


Fig. 9.
The variation of specific rotations and iodine values on acid hydrolysis of degraded polysaccharide A.



A
authentic sugar mixture

B
barium salts

Fig. 8

Paper chromatogram of barium salts B

(a, rhamnose; b, arabinose; c, galactose; d, glucuronic acid; e, aldobiuronic acid; f and g, acid oligosaccharides)

(18 : 3 : 1 : 4), a faint uronic acid spot, a dark brown spot (R gal, 0.27; where R gal indicates the rate of movement relative to galactose) and two spots (R gal $<$ 0.27) were detected. (See figure 8.) The same chromatogram pattern was produced when barium was removed with Amberlite IR-100H resin before spotting on paper.

Examination of Degraded Acid A.

The acid was a white amorphous powder which had $[\alpha]_D^{19}$ 0° (C, 0.5), and gave galactose and a uronic acid on hydrolysis with 2N-sulphuric acid (detected on a paper chromatogram) [Found: equiv., 700 (by direct titration on material dried at 100°/20 mm. with 0.01N-sodium hydroxide using phenolphthalein); iodine number, 10.7 c.c. of 0.1N-iodine per g.].

The polysaccharide A (12 g.) was heated in 0.5N-sulphuric acid (200 c.c.) on a boiling waterbath, the hydrolysis being followed iodimetrically and polarimetrically. $[\alpha]_D$: +3° (initial), + 17° (½ hour), +30° (10 hours), +35° (15 hours). Iodine numbers expressed as c.c. of 0.1N-iodine per g. : 11 (initial), 18 (½ hour), 46 (2½ hours), 69 (5½ hours), 84 (10 hours) 86 (15 hours). (See figure 9.)

A/...

A barium salt was precipitated by pouring the concentrated (50 c.c.), neutralised (barium carbonate) hydrolysate into methanol. The salt was collected by filtration, washed with methanol, and dried immediately in vacuum over calcium chloride yielding a white amorphous powder (9 g.), which had $[\alpha]_D^{17}$ +13° (C, 0.7) and gave galactose and a uronic acid on complete hydrolysis (detected on a paper chromatogram) [Found: Ba, 16.2%; iodine number, 57 c.c. of 0.1N-iodine per g.]. Galactose, a uronic acid, and a dark brown spot (R gal, 0.27) were detected when the salt was examined on a paper chromatogram using ethyl acetate - acetic acid - formic acid - water.

Concentration of the mother liquors gave crude D-galactose (5.9 g.). After recrystallisation from ethanol the pure D-galactose had m.p. 165° (not depressed on admixture with an authentic sample) and $[\alpha]_D^{22}$ +79° (C, 2.1).

Isolation of the Aldobiuronic Acid.

Barium was removed by shaking a solution of barium salt (4 g.) in water (200 c.c.) with Amberlite IR-100H resin. The filtered solution was concentrated to a syrup which was mixed with cellulose and placed on a cellulose column (1¼" diam. x 10" long). Galactose and the uronic acid were removed with half-saturated butanol, and

ethanol/...

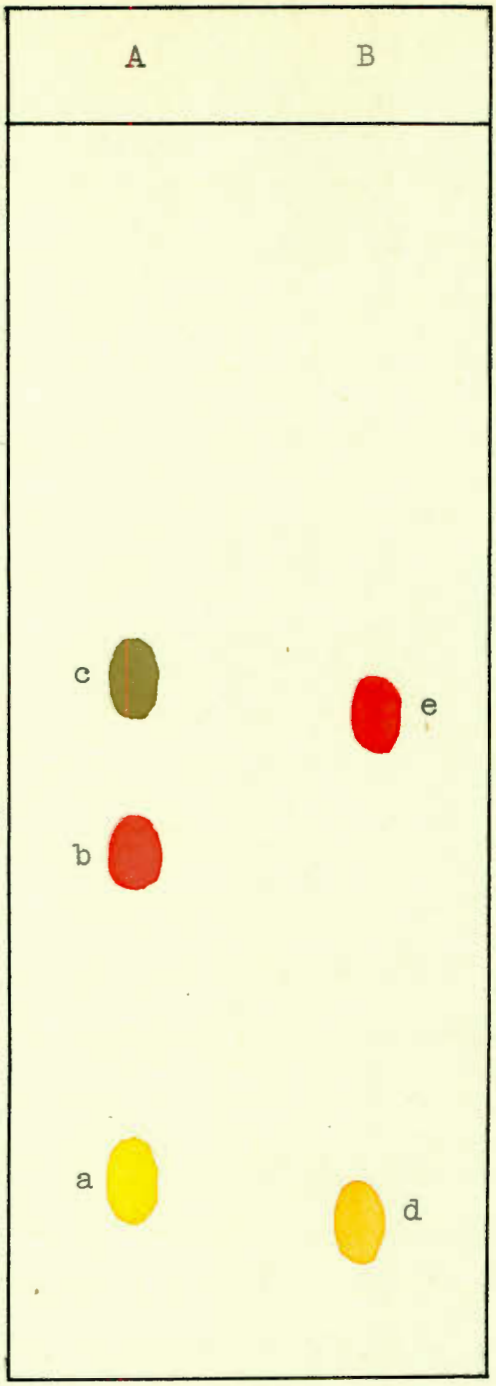
ethanol (90%) was used to elute the aldobiuronic acid (2 g.); this was a non-hygroscopic, white solid which had $[\alpha]_D^{16}$ -3° (C, 2.0); R gal, 0.27 (ethyl acetate - acetic acid - formic acid - water) [Found: equiv., 380 (by direct titration on material dried in vacuum over calcium chloride with 0.01N-sodium hydroxide using phenolphthalein). Calc. for $C_{12}H_{20}O_{12}$: equiv., 356]. As $C_{12}H_{20}O_{12} \cdot 1H_2O$ has equiv., 374, the isolated acid might be the monohydrate.

The aldobiuronic acid (0.1 g.) was oxidised with bromine water and barium benzoate according to Hudson and Isbell[111]. After removal of bromine in a stream of air, the solution was acidified with 5N-sulphuric acid (1 c.c.) and heated at 100° for 3 hours. The cooled solution was filtered and the acids were removed on Amberlite IR-4B resin. Since the neutral solution contained no sugars, galactose must have been the aglycone portion of the disaccharide. [A similar experiment on lactose produced galactose, detected on a descending chromatogram run in butanol - ethanol - water (4 :1.1 : 1.9) for 72 hours].

Identification of the Uronic Acid.

Barium aldobiuronate (11.5 g.), obtained from the hydrolysis of cyanophylla gum acid (30 g.) with 0.5N-sulphuric acid, was heated in a sealed bottle with 2N-sulphuric acid

(120 c.c.)/...



A
authentic sugar
mixture

B
fraction I

Fig. 10

Paper chromatogram showing glucuronic acid and glucurone

(a, rhamnose; b, arabinose; c, galactose; d, glucurone;
e, glucuronic acid)

(120 c.c.) at 100° for 16 hours. The filtered solution was neutralised with barium carbonate and a barium salt was precipitated by pouring the neutral solution into methanol (1 l.). Purification was effected by placing the salt on a cellulose column and washing out traces of galactose with isopropyl alcohol. The salt was eluted with water and reprecipitated from a concentrated aqueous solution with ethanol; yield 3.6 g. [Found: Ba, 26.0. Calc. for $C_6H_9O_7Ba_{0.5}$: Ba, 26.3%]. Barium was removed by shaking an aqueous solution with Amberlite IR-100H resin; the free acid was then concentrated to a syrup which was mixed with cellulose and fractionated on a cellulose column ($1\frac{1}{4}$" diam. x 10" long) using half-saturated butanol. Fraction I (0.6 g.) was a mixture of glucurone and glucuronic acid (figure 10). Crystalline glucurone was obtained when an aqueous solution was concentrated at 100°; it had m.p. 179° after recrystallisation from methanol (m.p. 175 - 176° reported by Weimann[7]). D-Glucuronic acid (1.1 g.) was obtained as a white crystalline solid from fraction II. It had m.p. 154° and $[\alpha]_D^{17}$ +33° (C, 2.0) after recrystallisation from ethanol. (Weimann[7] reported m.p. 156° and $[\alpha]_D$ +35°) [Found: equiv., 210 (by direct titration with 0.01N-sodium hydroxide). Calc. for $C_6H_{10}O_7$: equiv., 194].

D-Saccharic/...

D-Saccharic Acid Dibenzimidazole Derivative.

D-Glucuronic acid (0.2 g.), which was isolated above, was oxidised by dinitrogen tetroxide (1 c.c.) in chloroform (6 c.c.) at room temperature[112]. After 3 days, the chloroform was decanted from the syrupy saccharic acid, which was washed with chloroform, dissolved in water and concentrated to dryness in vacuum over sodium hydroxide. A dibenzimidazole derivative (0.1 g.) m.p. 234° (decomp.) was obtained from the syrup by the method of Lohmar, Dimler, Moore and Link[113]. The m.p. was not changed on admixture with a dibenzimidazole derivative prepared from potassium hydrogen-D-saccharate.

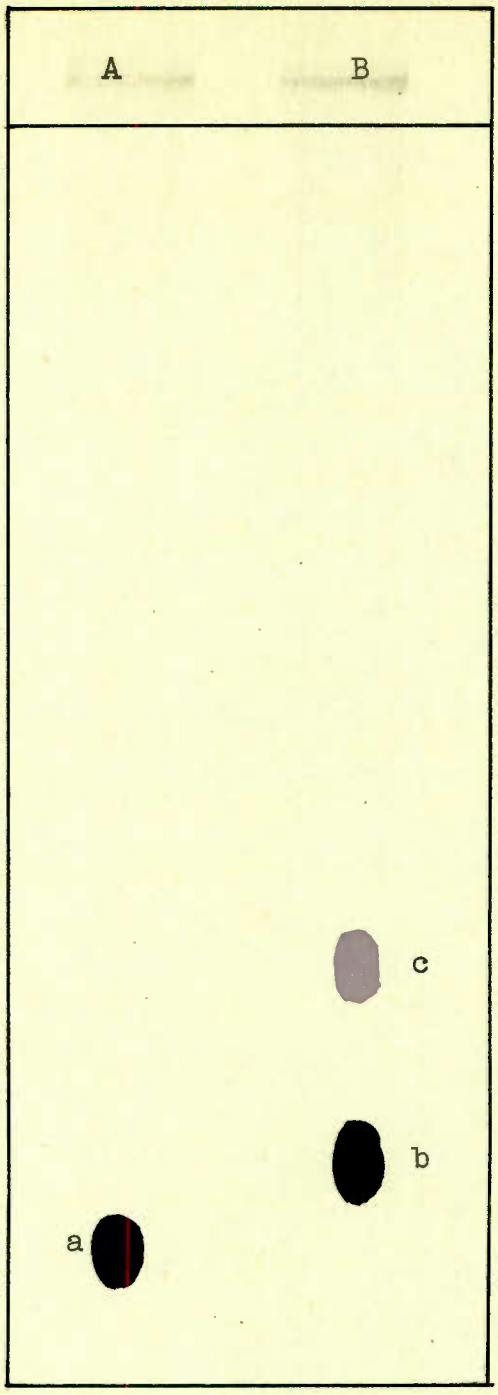
Methylation of the Aldobiuronic Acid.

The aldobiuronic acid (1.3 g.) in water (15 c.c.) was methylated at 20° by addition of dimethyl sulphate (20 c.c.) followed by dropwise addition of 30% sodium hydroxide (40 c.c.) with vigorous stirring over 7 hours. (Sodium hydroxide was added very slowly over the first 2 hours in order to prevent oxidation of the reducing end). The mixture, which was then non-reducing and alkaline, was allowed to stand overnight and the methylation completed by warming on a steambath for 1 hour. After acidifying the ice-cold solution with 50% sulphuric acid (10 c.c.) and

removing/...

removing sodium sulphate by filtration, the mixture was extracted seven times with chloroform (150 c.c. in all). The chloroform layer was dried over anhydrous sodium sulphate; removal of the solvent left a yellow syrup (0.7 g.). An increased yield of syrup (o.4 g.) was obtained from the slightly alkaline (sodium hydroxide) concentrated aqueous layer (45 c.c.) by remethylation with sodium hydroxide (20 g.) and dropwise addition of dimethyl sulphate (10 c.c.) over 4 hours. The combined syrup (1.1 g.) was converted into the methyl ester with methyl iodide (10 c.c.) and silver oxide (5 g.), the methyl iodide (2 c.c.) followed by silver oxide (1 g.) being added at 15-minute intervals[114]. After refluxing for 6 hours, the solution was filtered and the silver oxide washed with chloroform. Removal of the solvent left a viscid syrup (1.0 g.), n^{20} 1.464. When kept at room temperature, the syrupy methyl heptamethyl aldobiuronate gradually deposited crystals. The mixture of syrup and crystals (0.3 g.) was extracted with light petroleum. On cooling, the petroleum deposited needles, which after recrystallisation from light petroleum had m.p. 86 - 87° and $[\alpha]_D^{18}$ -35° (C, 1.2 in chloroform) [Found: C, 50.8; H, 7.7; OCH_3, 53.0. Calc. for $C_{20}H_{36}O_{12}$ (8 OCH_3 groups): C, 51.3; H, 7.8; OCH_3, 53.0%]. Challinor, Haworth and Hirst[8] reported m.p. 86°, $[\alpha]_D^{20}$ -43°

for/...



A
tetra-O-methyl-
galactose

B
ether extract

Fig. 11

Paper chromatogram showing tri-O-methyl-galactose

(a, tetra-O-methyl-galactose; b, tri-O-methyl-galactose;
c, di-O-methyl-galactose)

for the methyl ester of hexamethyl-6-O-D-glucuronosyl-β-methyl-D-galactoside.

Hydrolysis of the Methyl Ester of the Heptamethyl Aldobiuronate.

The syrupy crystalline material (0.6 g.) was heated in N-hydrochloric acid (8 c.c.) for 7 hours at 100°. Excess of silver carbonate was added in order to remove hydrochloric acid and the solution was filtered before and after the passage of hydrogen sulphide. Hydrogen sulphide was removed in a stream of air and the neutralised (barium carbonate) solution was filtered and concentrated to a syrup; this was exhaustively extracted with ether. Removal of the ether left a syrup (0.23 g.) which gave a dark mauve spot (R_G, 0.87; where R_G for tetra-O-methyl galactose, 1.0) and a faint mauve spot (R_G, 0.65) on a paper chromatogram run in butanol - ethanol - water (4 : 1.1 : 1.9) and sprayed with aniline phthalate in glacial acetic acid (figure 11). A tri-O-methyl-D-galactose (0.14 g.) was isolated from the above syrup, which had been placed on a paper chromatogram (8" wide) and run in butanol - ethanol - water, by eluting the appropriate strip (R_G, 0.87) with aqueous ethanol. It had $[\alpha]_D^{20}$ +100° (C, 0.9) and gave a crystalline N-phenylgalactosylamine trimethyl ether,

m.p./...

m.p. 161 - 162° after recrystallisation from ethanol. [Found: OCH_3, 31.3. Calc. for $C_{15}H_{23}O_5N$ (3 OCH_3 groups) : OCH_3, 31.3%] (lit., N-phenyl-D-galactosylamine 2:3:4-trimethyl ether, m.p. 167°, 168°, 169°, 170°; N-phenyl-D-galactosylamine 2:4:6-trimethyl ether, m.p. 169°, 179°). The aniline derivative m.p. 161 - 162° depressed the m.p. of N-phenyl-D-galactosylamine 2:4:6-trimethyl ether from 169° to 157°. The methylated sugar was proved to be 2:3:4-tri-O-methyl-D-galactose by oxidising it (15 mg.) with sodium periodate, whereupon formaldehyde (dimedone complex, m.p. and mixed m.p. 188°; yield 12 mg.) was evolved.

The original aldobiuronic acid was therefore 6-O-β-D-glucuronosyl-D-galactose.

6-O-β-D-Glucuronosyl-D-galactose from Barium Salts B.

A syrup, obtained by concentrating an aqueous solution of barium salts B (10 g.) which had been shaken with Amberlite IR-100H resin, was mixed with cellulose and fractionated on a cellulose column (1¼" diam. x 10" long). An aldobiuronic acid (2 g.; R gal 0.27) was eluted with ethanol (90%) after removal of neutral sugars with half-saturated butanol, and a crystalline heptamethyl methyl

ester/...

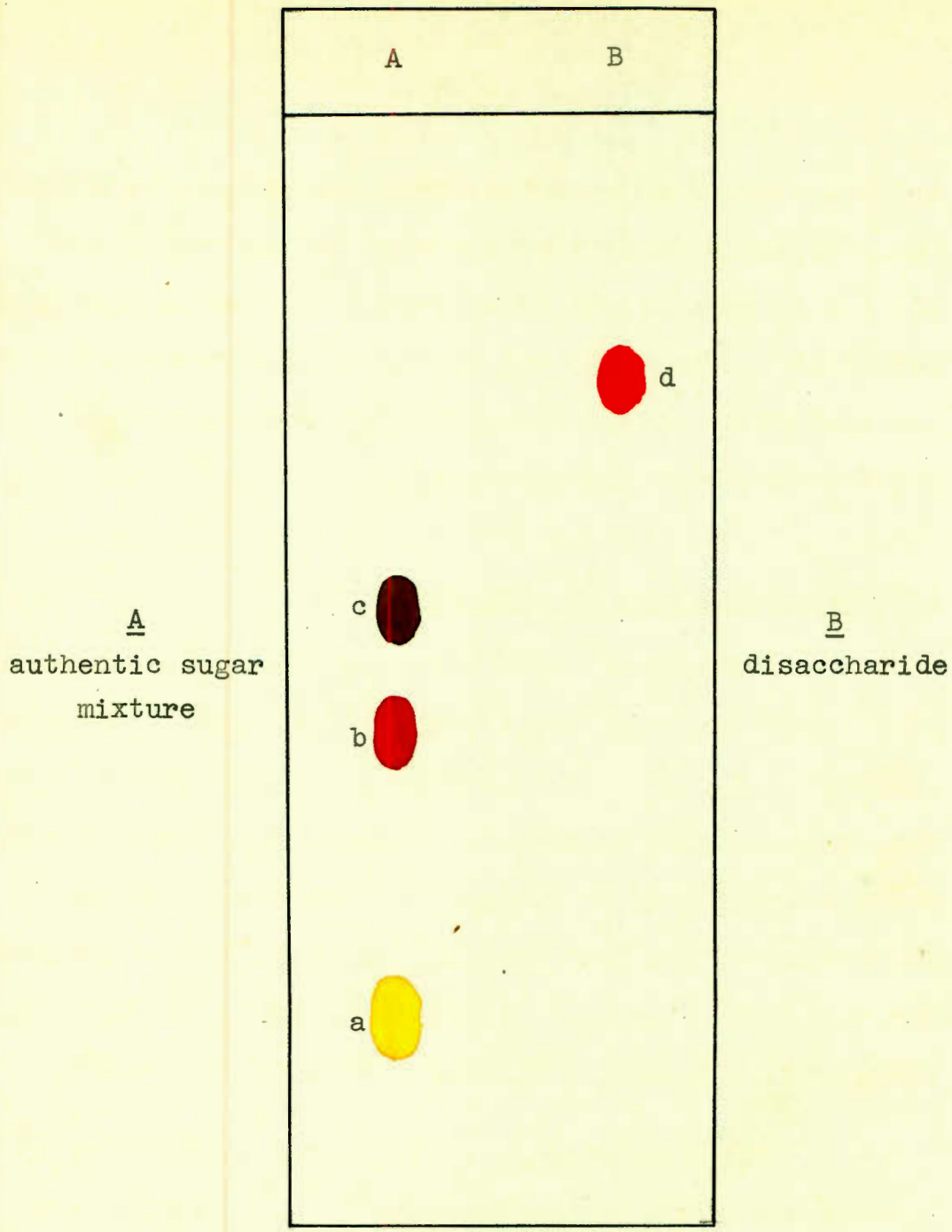


Fig. 12

Paper chromatogram showing galactose-arabinose disaccharide

(a, rhamnose; b, arabinose; c, galactose;

d, galactose-arabinose disaccharide)

ester was obtained as before from the methylated syrup. The methylated derivative had m.p. 86° and $[\alpha]_D^{17}$ -30° (C, 1.2 in chloroform) after recrystallisation from light petroleum. This m.p. was not changed on admixture with the previously obtained heptamethyl methyl ester of 6-O-β-D-glucuronosyl-D-galactose.

Arabinose-galactose Disaccharide.

The disaccharide was a reducing syrup which had $[\alpha]_D^{16}$ +152° (C, 2.3) and R gal, 0.50 in butanol - pyridine - water (9 : 2 : 2). On treating it at 100° for 30 minutes with aqueous phenylhydrazine acetate, an osazone was formed which crystallised on cooling the solution. After recrystallisation from ethanol, the pale yellow osazone had m.p. 235° (decomp.) [Found: C, 55.9; H, 6.3; N, 12.0. Calc. for $C_{23}H_{30}O_8N_4$: C, 56.4; H, 6.1; N, 11.4%].

The disaccharide (0.1 g.) was oxidised with bromine water and barium benzoate[111]. Bromine was removed in a stream of air and the solution acidified with 5N-sulphuric acid (1 c.c.). After heating at 100° for 3 hours, the solution was cooled and neutralised with Amberlite IR-4B resin. The neutral solution contained galactose (detected on a paper chromatogram). Arabinose was therefore the aglycone portion of the disaccharide.

The/...

The disaccharide (1.2 g.) in water (15 c.c.) was methylated at 20° by adding dimethyl sulphate (20 c.c.) followed by 30% sodium hydroxide (40 c.c.) during 8 hours. The solution was allowed to stand overnight and then warmed on a waterbath for 1 hour. The cooled solution was acidified with 50% sulphuric acid (10 c.c.), filtered, and extracted with chloroform (150 c.c.). Removal of the chloroform left a syrup (1.1 g.) which was remethylated with methyl iodide (10 c.c.) and silver oxide (5 g.)[114]. The mixture was filtered and the silver oxide washed with chloroform. Evaporation of the chloroform left a syrup (1.0 g.) which soon deposited crystals. The mixture of syrup and crystals (0.4 g.) was extracted with light petroleum. On cooling a syrup settled out and the clear petroleum extract was decanted off. From this solution, the heptamethyl disaccharide crystallised in needles. After recrystallisation from light petroleum (b.p. 40 - 60°), it had m.p. 87 - 88° and $[\alpha]_D^{16}$ +168° (C, 1.2) [Found: C, 52.5; H, 8.3; OCH_3, 52.8. Calc. for $C_{18}H_{34}O_{10}$ (7 OCH_3 groups) : C, 52.7; H, 8.4; OCH_3, 52.9%]. Smith[10] reported m.p. 82° and $[\alpha]_D^{18}$ +162° for heptamethyl 3-O-α-D-galactopyranosyl-L-arabopyranose.

Hydrolysis/...

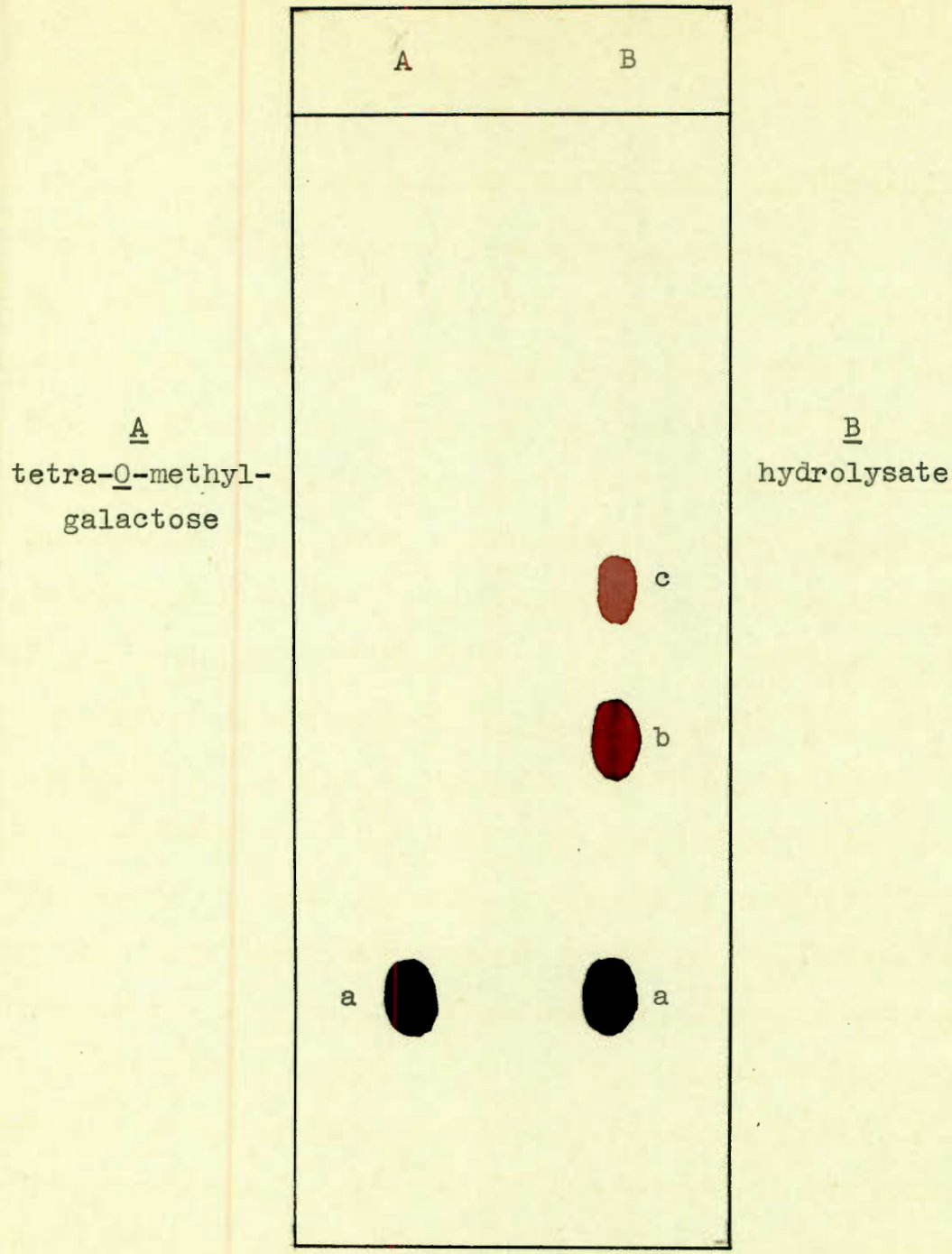


Fig. 13

Paper chromatogram of the hydrolysis of the methylated galactose-arabinose disaccharide

(a, tetra-O-methyl-galactose; b, di-O-methyl-arabinose; c, mono-O-methyl-arabinose)

Hydrolysis of the Heptamethyl Disaccharide.

The syrupy crystalline material (0.52 g.) was heated in 3% sulphuric acid (10 c.c.) on a boiling waterbath for 8 hours. After neutralisation (barium carbonate), the solution was filtered and concentrated to a syrup (0.48 g.), which gave two dark spots (R_G, 1.0 and R_G, 0.78) and a faint spot (R_G, 0.60) on a descending paper chromatogram run in butanol - pyridine - water (9 : 2 : 2) (see figure 13). The syrup was mixed with cellulose and placed on a cellulose column ($\frac{3}{4}$" diam. x 12" long) which was packed using a slurry of cellulose powder in butanol. A mixture of butanol and petroleum ether (b.p. 100 - 120°)[48] was used as mobile phase and 5 c.c. fractions were collected. The first product (0.21 g.) was a yellow syrup which gave only one spot on a paper chromatogram and formed the characteristic N-phenyl-D-galactosylamine 2:3:4:6-tetramethyl ether, m.p. 192° (unchanged on admixture with an authentic sample). A mixture of tetra-O-methyl-D-galactose and di-O-methyl-L-arabinose (0.13 g.) was then eluted and the third fraction was pure di-O-methyl-L-arabinose (0.09 g.). The di-O-methyl-L-arabinose syrup had $[\alpha]_D^{15}$ +135° (C, 1.1), did not evolve formaldehyde on periodate oxidation and formed a crystalline derivative with aniline. This N-phenyl-L-arabinosylamine dimethyl ether had m.p. 137° after

recrystallisation/...

recrystallisation from butanol [Found: OCH_3, 24.6. Calc. for $C_{13}H_{19}O_4N$ (2 OCH_3 groups) : 24.7%] (lit., N-phenyl-L-arabinosylamine 2:3-dimethyl ether m.p. 139°; N-phenyl-L-arabinosylamine 2:4-dimethyl ether m.p. 126°, 142°, 145 - 146°). The constants of the heptamethyl disaccharide suggest that the aniline derivative must be the 2:4-isomer and the rotation of the material isolated is in agreement with the value (+129°) given by Jones[37].

Periodate Oxidation of the Disaccharide.

The syrup (19 mg.) was oxidised with sodium periodate in a phosphate buffer (pH 7) for 3 days at room temperature, and treated with arsenious oxide and dimedone according to the method of Bell[51]. A formaldehyde-dimedone compound m.p. 184° was produced : yield 8 mg. (calc. yield for 1 mole of formaldehyde evolved; 18 mg.).

Using the same method, lactose (17 mg.) gave 5 mg. of formaldehyde-dimedone compound (calc. yield for 1 mole of formaldehyde evolved; 14 mg.) and maltose (18 mg.) gave a yield of 8 mg. of the dimedone compound (calc. yield for 1 mole of formaldehyde evolved; 15 mg.).

Molecular/...

Molecular Proportions of the Sugars.

Purified cyanophylla gum acid (0.23 g.) was heated in 2N-sulphuric acid at 100° for 14 hours. The solution was neutralised with barium carbonate, filtered and concentrated (to 3 c.c.). Samples (30 µl.) were separated on paper chromatograms[46] and estimated in the usual way[47] [Found: molar ratio of rhamnose : arabinose : galactose : : 5 : 2 : 11, mean of six determinations]. The simplest repeating unit would therefore consist of approximately 5 moles of rhamnose, 2 moles of arabinose, 11 moles of galactose and 5 moles of glucuronic acid; the unit weight being 3656 (unit weight, 3700 calc. on a basis of equiv., 740).

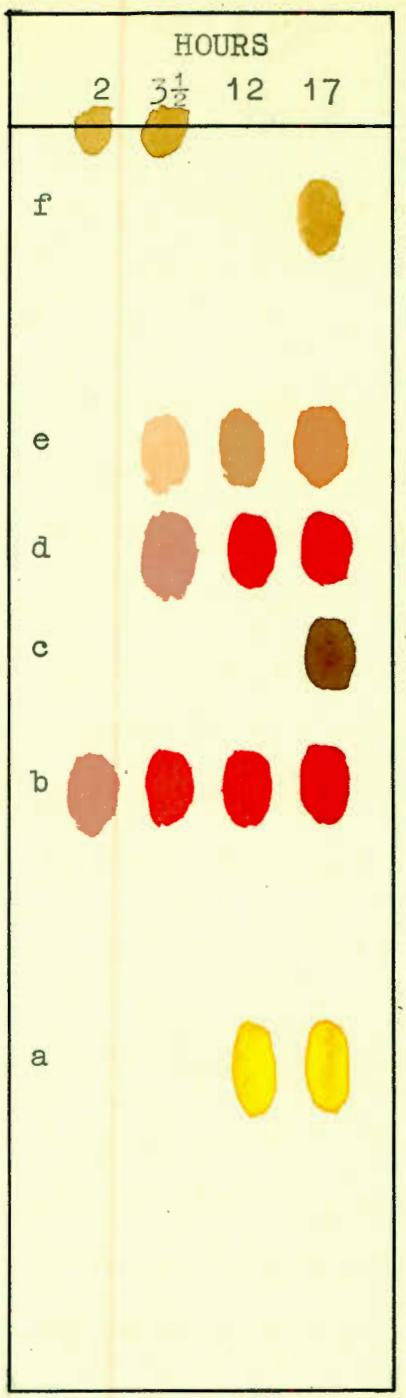


Fig. 14

Autohydrolysis of karroo gum acid

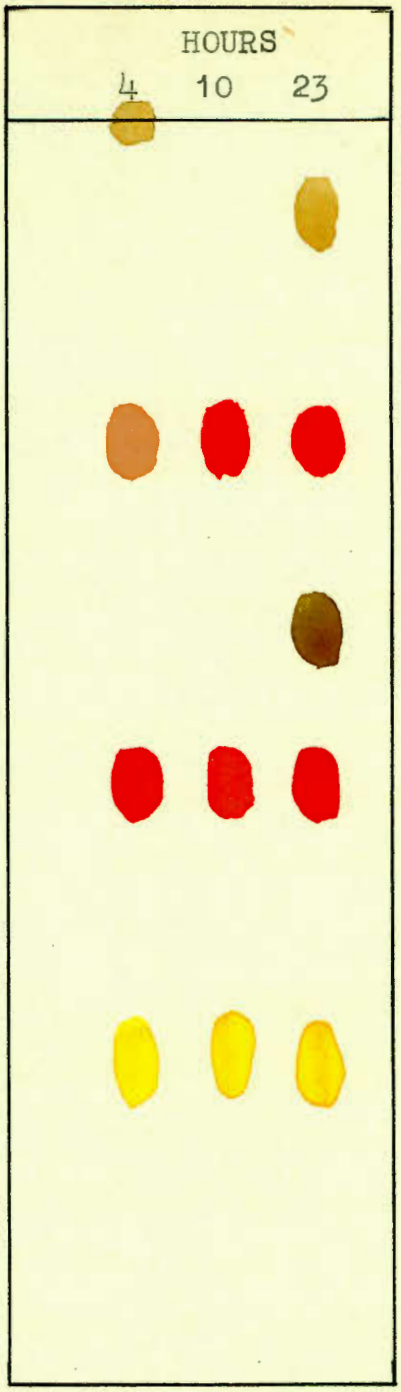


Fig. 15

Autohydrolysis of cyanophylla gum acid

(a, rhamnose; b, arabinose; c, galactose;
d, arabinose-arabinose disaccharide;
e, galactose-arabinose disaccharide;
f, aldobiuronic acid)

Karroo Gum

Purification.

The crude gum was converted into the gum acid by dissolution in water followed by acidification (hydrochloric acid) and precipitation with ethanol (cf. purification of cyanophylla gum). Traces of adsorbed hydrochloric acid were removed by pouring an aqueous solution of the gum acid into ethanol. The product was a white amorphous solid $[\alpha]_D$ +54° (C, 1.33) [Found: equiv., 1660 (on material dried at 100°/20 mm.); sulphated ash, 0.56%].

Autohydrolysis.

The gum acid (45 g.) in water (1 l.) was heated on a boiling waterbath. At intervals samples were withdrawn and examined on a paper chromatogram run in ethyl acetate - acetic acid - formic acid - water. Arabinose was detected after 2 hours and 2 disaccharides appeared at 3½ hours. The slower moving spot was very faint and moved at the same rate as the galactose - arabinose disaccharide spot. The other spot increased in intensity as the hydrolysis proceeded. Rhamnose was noticed after 12 hours and both galactose and aldobiuronic acid at 17 hours (figure 14). [In a similar hydrolysis of

cyanophylla/...

cyanophylla gum, rhamnose, arabinose and the disaccharide were detected at 4 hours and galactose and the aldobiuronic acid at 23 hours (figure 15) .

The hydrolysis was stopped after 75 hours and a degraded acid (19 g.) was precipitated by pouring the concentrated hydrolysate into ethanol [Found: equiv., 1270 on material dried at 100°/20 mm.]. Low molecular weight barium salts (4 g.) and a sugar syrup (24 g.) were obtained from the filtrate (cf. cyanophylla gum). Paper chromatograms showed that the barium salts consisted of a mixture of oligosaccharides, and that L-arabinose, L-rhamnose, D-galactose, two disaccharides and some aldobiuronic acid were the constituents of the sugar syrup. (The slow moving disaccharide gave only a very faint spot.)

The sugar syrup was fractionated on a cellulose column (1¼" diam. x 10" long) using half-saturated butanol. Crystalline L-rhamnose hydrate (0.9 g.) was obtained from the first runnings; this had m.p. 95 - 96° (not changed on admixture with an authentic sample and $[\alpha]_D^{19}$ +10° (C, 4.0) after recrystallisation from methanol. The second fraction (15 g.) was crystalline L-arabinose which had m.p. 156° (not changed on admixture with authentic L-arabinose) and $[\alpha]_D^{20}$ +107° (C, 2.1) after recrystallisation from methanol, and the third fraction (4 g.) was a syrupy mixture of

arabinose/...

arabinose, galactose and a disaccharide. The sugar syrup also contained low molecular weight barium salts (3 g.); these were eluted from the column with water and the syrup containing the disaccharide was re-fractionated on the same column. Crystalline L-arabinose (0.2 g.), crystalling D-galactose (2.0 g.) and a hygroscopic disaccharide (1.6 g.) were obtained. D-Galactose was purified by recrystallisation from ethanol. The pure material had m.p. 163 - 164° (not changed on admixture with an authentic sample) and $[\alpha]_D^{20}$ +82° (C, 2.3).

Arabinose-arabinose Disaccharide.

The disaccharide was a reducing syrup which had $[\alpha]_D^{18}$ +208° (C, 1.7) and gave a pink spot (R gal, 0.58) when examined on a paper chromatogram run in butanol - pyridine - water (9 : 2 : 2) and sprayed with p-anisidine hydrochloride. On hydrolysis with 0.5N-sulphuric acid, only arabinose was produced (detected on a paper chromatogram). The disaccharide formed an osazone which had m.p. 230° (decomp.) after recrystallisation from ethanol [Found: C, 56.6; H, 6.4; N, 12.6. Calc. for $C_{22}H_{28}O_7N_4$: C, 57.4; H, 6.1; N, 12.2%].

Dimethyl/...

Dimethyl sulphate (20 c.c.) was added to a solution of the disaccharide (1.0 g.) in water (15 c.c.). The mixture was cooled in ice and 30% sodium hydroxide (40 c.c.) added with vigorous stirring over 7 hours. The solution was then non-reducing. It was allowed to stand overnight and then stirred for a further 8 hours. The reaction was completed by heating on a steambath for 1 hour and the product isolated in the usual way; this was a viscous syrup, which was remethylated with methyl iodide (10 c.c.) and silver oxide (5 g.). The product was a syrup (1.1 g.) having n^{20} 1.469 and $[\alpha]_D^{20}$ +230° (C, 0.79). A portion (0.1 g.) of the material distilled as a colourless syrup (0.07 g.) at 130 - 150°/4 x 10^{-2} mm. [Found: OCH_3, 50.3. Calc. for $C_{16}H_{30}O_9$ (6 OCH_3 groups) : OCH_3, 50.8%].

Hydrolysis of the Methylated Disaccharide.

The methylated disaccharide (0.5 g.) was heated in N-hydrochloric acid (10 c.c.) on a boiling waterbath for 4 hours. Hydrochloric acid was removed by shaking the hydrolysate with silver carbonate and the neutralised hydrolysate was filtered before and after the passage of hydrogen sulphide and concentrated to a syrup (0.48 g.), which gave two dark spots (R_G, 0.92 and R_G, 0.78) and a faint spot (R_G, 0.60) on a paper chromatogram run in

butanol/...

butanol - pyridine - water (9 : 2 : 2). The syrup mixed with cellulose powder was placed on a cellulose column ($\frac{3}{4}$" diam. x 12" long) and a mixture of butanol and petroleum ether was used as mobile phase. Fraction I was a syrup (0.19 g.) which had $[\alpha]_D^{19}$ +145° (C, 1.1) and R_G, 0.92 [Found: OCH_3, 43.0. Calc. for $C_8H_{16}O_5$ (3 OCH_3 groups) : OCH_3, 48.4%]. The syrup (40 mg.) was oxidised with bromine water(115), and the arabonic acid produced was converted to the lactone by heating at 100°/20 mm. for 4 hours; this distilled as a colourless oil (10 mg.) at 100°/2 x 10^{-2} mm. On heating the oil with phenylhydrazine (5 mg.) in dry ether, a phenylhydrazide crystallised; this had m.p. 153 - 155° after washing with ether (lit., 2:3:4-tri-O-methyl-L-arabonophenylhydrazide m.p. 156°, 158°, 159°, 160°). The second fraction was a mixture of tri- and di-O-methyl-L-arabinose (0.12 g.) and fraction III was di-O-methyl-L-arabinose (0.11 g.), which had $[\alpha]_D^{22}$ +120° (C, 0.94) and R_G, 0.78. An N-phenyl-L-arabinosylamine dimethyl ether was prepared from the di-O-methyl-L-arabinose; this had m.p. 136 - 137° after recrystallisation from butanol and the m.p. was not changed on admixture with the N-phenyl-L-arabinosylamine dimethyl ether obtained from the methylated galactose - arabinose disaccharide (p. 87).

The/...

The above results suggest that the disaccharide is 3-O-β-L-arabopyranosyl-L-arabopyranose, and the specific rotation and the m.p. of the osazone are in agreement with the constants ($[\alpha]_D$ +204° and m.p. 233°) given by Jones[38] for 3-O-β-L-arabopyranosyl-L-arabopyranose.

The Aldobiuronic Acids.

Karroo gum acid (40 g.) was heated in 0.5N-sulphuric acid (300 c.c.) for 15 hours on a boiling waterbath. The dark solution was neutralised with barium carbonate and filtered. On pouring the neutral solution into methanol (2 l.) a barium salt (10 g.) was obtained as a white precipitate. The salt was filtered off, washed with hot methanol and dried in vacuum over calcium chloride; it had $[\alpha]_D^{20}$ +70° (C, 1.9) [Found: Ba 13.9. Calc. for $C_{12}H_{19}O_{12}Ba_{0.5}$: Ba, 16.2]. An examination of the barium salt on a paper chromatogram run in ethyl acetate - acetic acid - formic acid - water, showed a hexuronic acid spot, a large brown aldobiuronic acid spot (R gal, 0.27) and other oligosaccharide spots (p-anisidine hydrochloride spray).

Barium was removed on Amberlite IR-100H resin and the resultant syrup mixed with cellulose was placed on a cellulose column (1¼" diam. x 10" long). Glucuronic acid

and/...

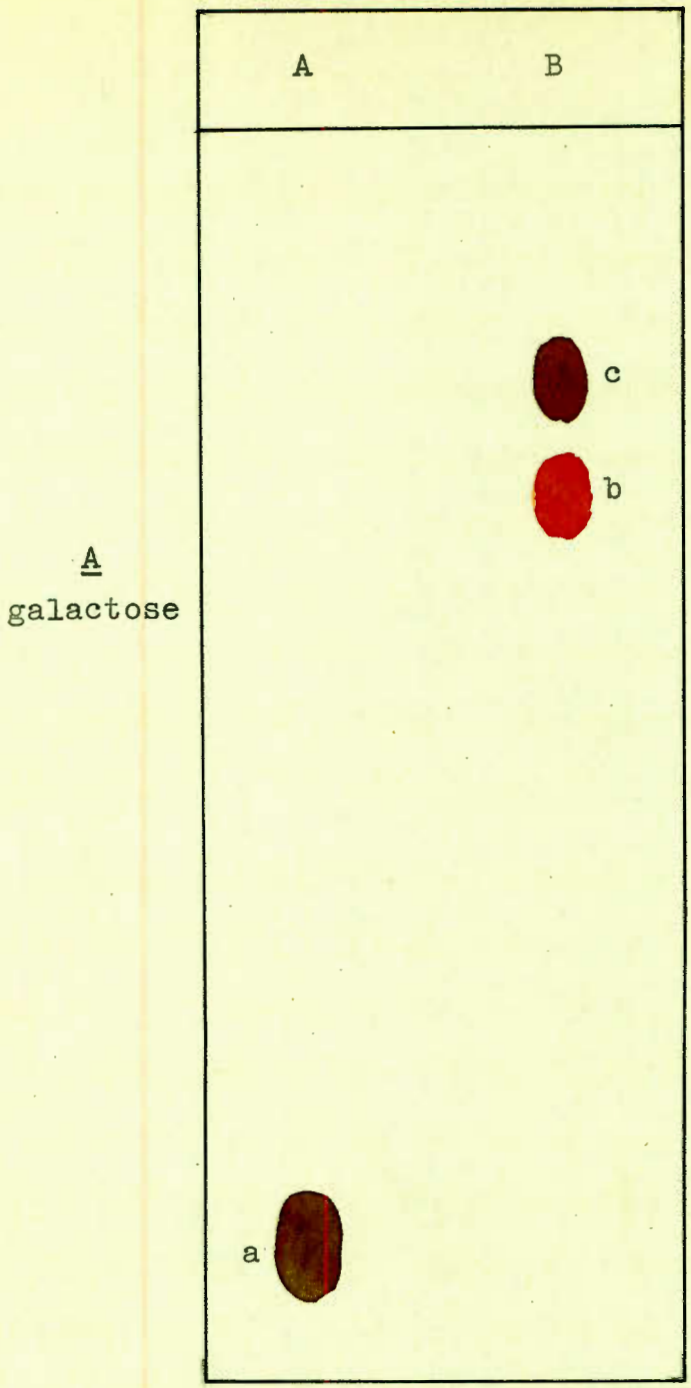


Fig. 16

Paper chromatogram showing the mixture of aldobiuronic acids

(a, galactose; b, aldobiuronic acid $[\alpha]_D$ $+110^o$; c, 6-O-β-D-glucuronosyl-D-galactose)

and glucurone were eluted with half-saturated butanol and crystalline glucurone was obtained by repeatedly concentrating an aqueous solution of the mixture at 100°. Glucurone had m.p. 176° after recrystallisation from methanol (unchanged on admixture with glucurone isolated from cyanophylla gum). A syrupy aldobiuronic acid fraction (3.5 g.) was then eluted with ethanol (90%). When examined on a paper chromatogram run in ethyl acetate - acetic acid - formic acid - water for 72 hours, two distinct spots were noticed (R gal, 0.34 and R gal, 0.27). (See figure 16).

The aldobiuronic acid mixture was refractionated on the same column using butanol (2 l.) - water (200 c.c.) - formic acid (50 c.c.) as mobile phase. The first fraction was an acidic syrup (1.6 g.) which gave only one spot (R gal, 0.34) on a paper chromatogram; this was converted into a white amorphous barium salt which had $[\alpha]_D^{21}$ +110° (C, 2.1) [Found: Ba, 15.3. Calc. for $C_{12}H_{19}O_{12}Ba_{0.5}$: Ba, 16.2%]. The second fraction (1.0 g.) was a mixture of both acids and the third fraction (0.6 g.) an acidic syrup which gave one spot (R gal, 0.27) on a paper chromatogram. The pure acid was converted into a barium salt which had $[\alpha]_D^{23}$ +12° (C, 2.0). Methylation of this salt gave a syrup from which the methyl ester of heptamethyl-6-O-β-D-glucuronosyl-D-galactose was isolated in crystalline

form/...

form. After recrystallisation from light petroleum, the methylated ester had m.p. 89° and $[\alpha]_D^{22}$ -30° (C, 0.9 in chloroform). The m.p. was unchanged on admixture with the heptamethyl methyl ester obtained from cyanophylla gum.

Examination of the Barium Salt $[\alpha]_D$ +110°.

The salt (0.1 g.) was heated with 2N-sulphuric acid (5 c.c.) for 15 hours at 100°. After neutralising the hydrolysate with barium carbonate, the solution was filtered and barium was removed with Amberlite IR-100H resin. An examination of the resultant acid solution on a paper chromatogram run in ethyl acetate - acetic acid - formic acid - water showed that galactose, glucuronic acid, glucurone and unhydrolysed aldobiuronic acid were the constituents.

A solution of the barium salt (0.1 g.) in water (5 c.c.) was oxidised with bromine water and barium benzoate[111]. After removal of bromine, 5N-sulphuric acid (1 c.c.) was added and the mixture was hydrolysed at 100° for 5 hours; this was filtered and the acids were removed on Amberlite IR-4B resin. Since the solution gave a positive Molisch test before the hydrolysis and a negative Molisch test after hydrolysis and removal of the acids,

galactose/...

galactose must have been the aglycone portion of the aldobiuronic acid.

Methylation of the Barium Salt.

Dimethyl sulphate (20 c.c.) was added to a solution of the barium salt (0.9 g.) in water (15 c.c.). The mixture was vigorously stirred and 30% sodium hydroxide (40 c.c.) was added over 8 hours. After standing overnight the methylation was completed by warming the solution on a steambath for 1 hour. The mixture was acidified and the product isolated with chloroform in the usual way. A further quantity of methylated material was obtained by remethylating the aqueous layer and the total yield was 0.6 g.; this was remethylated with methyl iodide (10 c.c.) and silver oxide (5 g.). The viscous product (0.52 g.) distilled as a colourless syrup (0.39 g.) at 130 - 140°/6 x 10^{-2} mm.; this had n^{20} 1.465 and $[\alpha]_D^{23}$ +95° (C, 1.46) [Found: OCH_3, 46.2. Calc. for $C_{20}H_{36}O_{12}$ (8 OCH_3 groups) : OCH_3, 53.0%].

Attempted Hydrolysis of the Methylated Product.

The purified syrup (0.35 g.) was heated in N-hydrochloric acid (10 c.c.) for 7 hours at 100°. The cooled solution was neutralised with silver carbonate and

filtered/...

filtered before and after the passage of hydrogen sulphide. Hydrogen sulphide was removed in a stream of air and the solution was neutralised with barium carbonate. Excess of barium carbonate was removed by filtration and the neutral solution was concentrated to dryness; this was extracted with ether. Removal of the ether left a neutral syrup (20 mg.). As the yield of the syrup was very small, the barium salt residue was re-hydrolysed with 2N-sulphuric acid (5 c.c.) for 8 hours at 100°. The hydrolysate was neutralised with barium carbonate and the neutral solution concentrated to dryness. Extraction with ether yielded a small amount of syrup (5 mg.). The syrup was combined with the previous product and a paper chromatogram showed that both a tri-O-methyl galactose and a di-O-methyl galactose were present. The tri-O-methyl galactose (10 mg.) was isolated by extracting with aqueous ethanol the appropriate strip (R_G, 0.90) from a paper chromatogram (7" wide), run in butanol - ethanol - water. An attempt was made to prepare an N-phenylamine from the tri-O-methyl galactose, but this did not crystallise.

Molecular Proportions of the Sugars.

Karroo gum acid was hydrolysed with 1.5N-sulphuric acid in a sealed tube at 100°. After neutralisation with

barium/...

barium carbonate and filtering, the sugars were separated on a descending paper chromatogram run in butanol - ethanol - water (4 : 1.1 : 1.9), and estimated using the sodium periodate procedure [Found: molar ratio of galactose : arabinose : : 6 : 5]. The gum also contained about 2% of rhamnose (0.9 g. of rhamnose hydrate isolated from 45 g. of gum acid).

SUMMARY

Cyanophylla gum, the exudate from Acacia cyanophylla, is a neutral salt of a gum acid, with equivalent weight of 740 and $[\alpha]_D$ -20°. Conductimetric and potentiometric titrations showed that the gum acid was fairly strong (dissociation coefficient at zero dissociation, 4×10^{-2}), and when examined in a Tiselius electrophoresis apparatus, the acid showed single but asymmetric boundaries on the ascending and descending sides. A degraded acidic polysaccharide, a mixture of acid oligosaccharides and a sugar syrup were produced on prolonged autohydrolysis of cyanophylla gum acid; these were separated by precipitation methods and L-arabinose, D-galactose, L-rhamnose and a galactose - arabinose disaccharide were isolated from the sugar syrup. Methylation and periodate experiments indicated that 3-O-α-D-galactopyranosyl-L-arabopyranose was the most probable structure of the disaccharide. On acid hydrolysis, the degraded polysaccharide gave D-galactose and an aldobiuronic acid. The last substance was shown to be 6-O-β-D-glucuronosyl-D-galactose and this disaccharide was also isolated from the acid oligosaccharide fraction. By estimating the ratio of the sugars produced on complete hydrolysis of the gum acid, it was established that the simplest repeating pattern consisted of rhamnose (5 moles), arabinose (2 moles), galactose (11 moles)

and/...

has been shown to hold for other disaccharides in which only the glycosidic portion has been changed. Using this method it is possible to predict the specific rotations of a number of disaccharides and also to assign anomeric configurations to the biose links. The application of infra-red absorption spectra for determining glycosidic configurations is discussed.

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