

STUDIES ON ESPARTO GRASS.

Thesis submitted for the degree of Doctor of Philosophy

by

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INTRODUCTION.

Cell-wall carbohydrate materials can be divided into three classes; "pectin", which can be removed by acid or neutral extraction, "hemicelluloses", which are removed by various strengths of alkali, and the residual cellulose. It is with the hemicellulose group of polysaccharides that the present work is concerned. As the name suggests the hemicelluloses were thought to be related to cellulose and are in fact often found in association with it.

Various theories have been put forward to account for the presence of the hemicelluloses and to explain the part they play in the metabolic functions of the plant. One of these is concerned with the association of hemicelluloses, and in particular xylan, with lignin in the woody tissues of plants. It is thought that dehydration of the pentosan groups would give rise to coniferyl alcohol units (1) and that these in turn would be built up to form the lignin molecule.

Early workers (2) failed to detect any uronic acid in the hemicelluloses and thought that the products which they obtained by precipitation of the alkaline extract with acid were pure hexosans or pentosans or more likely hexopentosans related to cellulose. Uronic acids are now known to be present in at least some of the hemicelluloses (the polyuronide hemicelluloses) and consequently the suggestion has been made that hemicelluloses are formed from the pectin and may in turn be converted/

converted into lignin (3). Until further evidence is forthcoming for the constitution of lignin no definite conclusion can be reached concerning the latter part of this theory.

Norman (4) has classified the hemicelluloses into two fractions those containing uronic acid - "polyuronide hemicelluloses" and those not containing uronic acid. A polyuronide hemicellulose which has been fairly fully studied is that extracted from beech wood (5). The aqueous alkali extract was acidified to yield a fraction, hemicellulose A, the filtrate yielding a further precipitate, hemicellulose B on the addition of excess of alcohol. Fraction A which consisted mainly of xylose with 11% glucuronic acid was more resistant to acid hydrolysis than fraction B which contained 63% galacturonic acid along with some arabinose and galactose.

The hemicelluloses which do not contain any uronic acid are either hexosans, pentosans, or hexopentosans. They are usually associated with cellulose in some way. The term "cellulosans" has been used to describe those hemicelluloses which are believed to exist in association in a cellulosic framework in the angiosperms, while the non-associated fractions are called polyoses and are thought to behave as reserve carbohydrate materials. As the carbohydrate material studied in the present work contained xylose, arabinose, and galactose it would be advantageous to examine previous work on the xylans, arabans and galactans.

XYLAN.

Xylan occurs in practically all land plants and is known to be present in some marine algae, (6). It is particularly abundant in such substances as grain hulls and straws, low strength commercial fibres such as jute, sisal, etc., woods of all sorts and also esparto grass.

In all of these materials the xylan is closely associated with cellulose in the cell wall of the plants and is often difficult to remove by solvent extraction. In fact, solubility characteristics of xylan suggest that actual chemical combination occurs between the cellulose and the xylan but rigorous proof of this is not yet available. Furthermore this chemical combination is not necessary to explain the difficulty often experienced during the extraction of xylan from the accompanying cellulose. As will be seen later the xylan chain is similar to that of cellulose and consequently one could expect the xylan to substitute for the cellulose in the cellulose matrix, and this might explain the difficulty encountered in the extraction. Additional binding forces would be present if the xylan molecules were overlaid with cellulose chains. If the xylan molecules were constituents of the crystalline micelles of the cell wall we should expect extraction of the xylan to alter the crystalline X-ray pattern. As this pattern is not appreciably altered it would seem that such a combination does not occur (7).

Before/

Before extraction of the xylan it is usually necessary to remove from the plant material waxes, pigments (chlorophyll, xanthophyll, etc.) lignin, and sometimes pectins and pectic substances. The former are generally removed by Soxhlet extraction with benzene and methanol. Lignin presents a considerable problem in the preparation of a pure xylan for two reasons. Firstly lignin prevents the complete solution of the xylan, either because of mechanical obstruction or possibly because of as yet unidentified covalent bonds. Secondly lignin is partially soluble in the aqueous alkaline reagents used for extraction and consequently gives rise to further purification problems at later stages in the isolation. Chlorine or chlorine dioxide are the reagents most commonly employed for the removal of lignin. It is necessary that this process be carefully controlled to give a final polysaccharide with a lignin content of ca 0.5 - 2.0%. This obviates the considerable loss of pentosan which may otherwise occur (8). Pectin and pectic substances may be removed by one or more extractions with 0.5% aqueous solutions of ammonium oxalate at 90 - 100°C. This reagent removes those water-insoluble pectic substances which are neither mechanically trapped nor chemically bound in the plant structure.

It is usual to employ solutions of 2 - 4% sodium or potassium hydroxide for the extraction of xylan, but it is sometimes necessary to use as high a concentration of alkali as 16% to remove the maximum amount of hemicellulose/

hemicellulose (9). Schmidt and coworkers (10), noting that some xylan could be extracted with dilute alkaline solutions while the remainder is removed only with strong solutions subdivided xylan into "easily soluble" and "difficultly soluble" fractions. There is, however, thought to be more danger of oxidation of xylan in the stronger alkali solutions, and for this reason it is probably preferable to use an alkali concentration not greater than 4%. Also because of oxidation these extractions are often carried out with the exclusion of air.

Thomsen (11) in 1879 prepared a xylan or wood-gum ("holzgummi") from beech sawdust by treatment with cold dilute sodium hydroxide, and subsequent precipitation with alcohol. He thought this to be an isomer of cellulose. It was found to have very similar properties to the wood-gum prepared in 1846 by Poumarede and Figuier (12).

Various methods have been employed for the purification of xylan and these take advantage of the facts that hemicellulose B (containing low molecular weight polysaccharides and polyuronides) is soluble in very dilute alkali solutions, and also is not so easily precipitated by neutralization of the alkali extract. Whistler and coworkers (13) removed hemicellulose B from corn cob holocellulose by extraction with 0.2 to 0.5% potassium hydroxide solutions. Other workers have employed solutions of sodium carbonate. Stronger alkali treatment then removes a relatively pure xylan. Careful control of the pH of the precipitation also enables/

enables one to prepare a fairly pure xylan.

Salkowski (14) has shown that xylan can be further purified by solution in alkali and subsequent precipitation as a gelatinous copper complex by the addition of Fehling's solution. Some araban also precipitates, more particularly if an excess of Fehling's solution is used, but it has been shown to be possible to prepare an araban-free xylan from esparto grass by two or three such treatments if the crude xylan is extracted with water between each precipitation as the copper complex (15).

Xylan on hydrolysis with dilute mineral acids has long been known to yield, in quantity, the sugar D-xylose. This sugar was shown by Wheeler and Tollens (16) to have the empirical composition $C_5H_{10}O_5$ while the parent xylan had the composition $(C_5H_8O_4)_n$. Also known to be present in the hydrolysate of some native xylans are small amounts of L-arabinose and in some cases small amounts of a uronic acid, probably D-glucuronic acid. A maximum yield of 93% of crystalline xylose has so far been reported (17). This was obtained by hydrolysis of an esparto xylan the pentosan content of which was 97 - 98%. Haworth, Hirst and Oliver (18) have reported the presence of an almost constant proportion (6%) of arabinose along with 93% xylose in the hydrolysis products of xylan from several sources. It has been reported that the hemicellulose from sugar cane bagasse contains as much as 13% of anhydroarabinose/

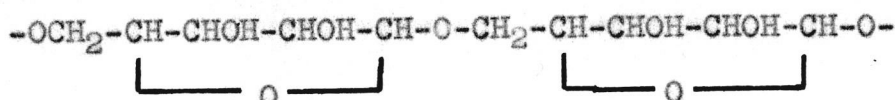
anhydroarabinose (19).

A uronic acid has been identified in the hemicellulose-A of flax, and is thought to be present as the terminal group on a chain of about ten D-xylopyranose units (20). An aldobiuronic acid consisting of D-xylose and a monomethylhexuronic acid is obtained by hydrolysis of the hemicellulose-A of English oak heartwood (21). Uronic acids have also been identified in the hydrolysis products of hemicelluloses from corn cobs (22, 23), cottonseed hulls (24), and wheat straw etc. (25).

Tollens (16) before it was proved that wood gum was a polymer, put forward the formulae:



for xylan on the evidence of the presence of two free hydroxyl groups, as shown by the formation of the diacetate and the dimethyl ether. As mentioned earlier Tollens later arrived at the empirical formula $(\text{C}_5\text{H}_8\text{O}_4)_n$. Komatsu and coworkers (26) hydrolysed methylated xylan and identified the resultant dimethyl xylose by oxidation to dimethoxy glutaric acid. This led to the formula:



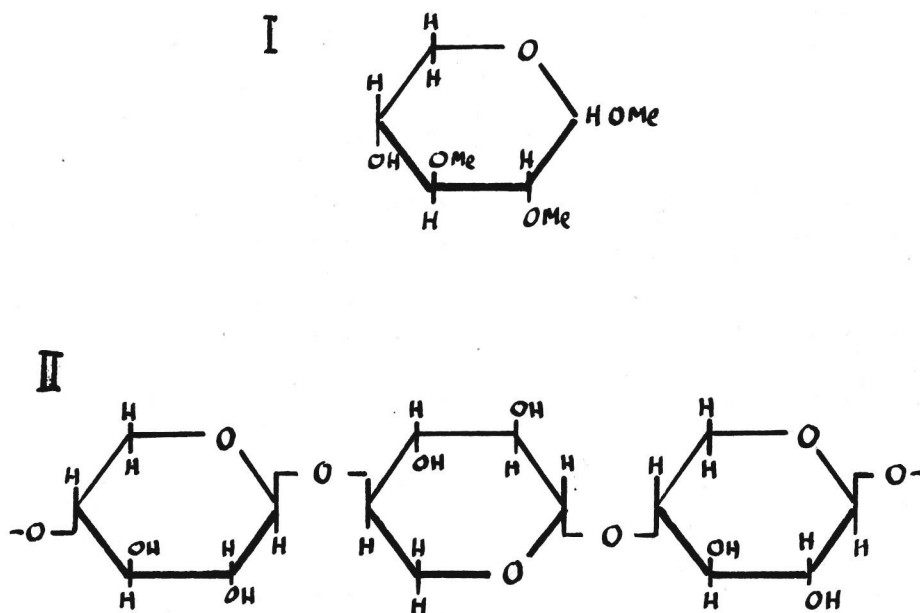
for the constitution of xylan. The experimental evidence/

evidence for this, however, is far from conclusive, owing to the several possible interpretations which can be drawn from the experimental evidence on which the identification of the oxidation product is based.

Irvine and Hirst (27) in 1924, put forward the theory that the xylan from esparto grass consists of xylose units only. A pure pentosan extracted by alkali was hydrolysed to xylose which was identified by conversion to trimethyl -methylxyloside. Acetylation of the esparto holocellulose gave a mixture of cellulose triacetate and xylan diacetate which was then hydrolysed with methanolic hydrogen chloride to yield a product which was identified as a mixture of methyl glucoside and methyl xyloside. The conclusion was reached that esparto holocellulose was a mixture of cellulose and xylan, as it was unlikely that alkali would remove the pentose units from a single polysaccharide consisting of hexose and pentose units.

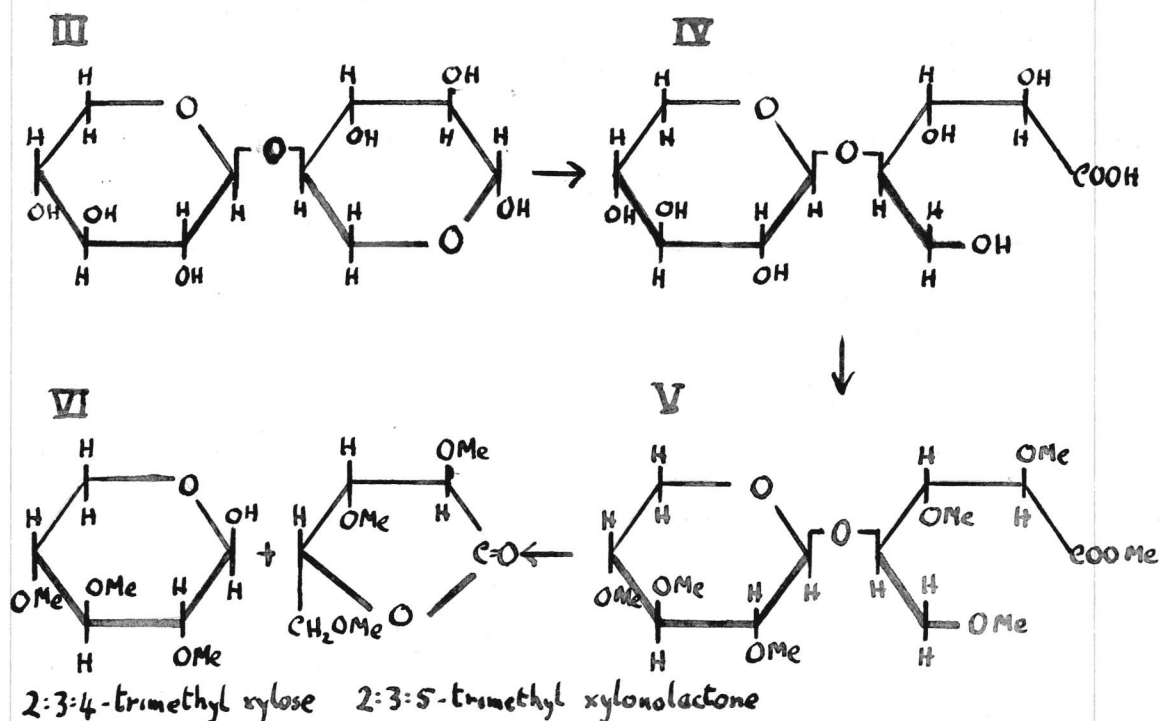
Evidence for the linkage between the xylose units was first obtained by Hampton, Haworth and Hirst (17) who methylated lignin-free esparto xylan by two treatments with caustic potash and dimethyl sulphate. The product which was insoluble in water was hydrolysed with methanolic hydrogen chloride to yield a dimethyl methyl xylopyranoside shown to be the 2:3-dimethyl sugar (1). These authors concluded therefore, that the hydroxyl groups on carbon atoms 2 and 3 were not involved/

involved in the linkage between the xylose residues. Carbon atoms 4 and 5 will then be involved in either the ring formation of the individual xylose unit or in the linkage between the xylose units. It was thought that the pyranose structure was most likely for the xylose considering the stability of the polysaccharide. Also the high laevorotatory behaviour of the xylan and of β -methylxyloside as opposed to the high dextrorotation of α -methylxyloside, suggested that the linkage in the xylan was of the β -type. From this we have a structure (II) for the polysaccharide which is seen to be similar to that of cellulose.



Further evidence concerning the linkage between the xylose units has recently been obtained by Bachrach and Whistler (28) who have succeeded in obtaining a 10% yield of xylobiose by stopping the hydrolysis of xylan at a reducing value corresponding to two-thirds of that for/

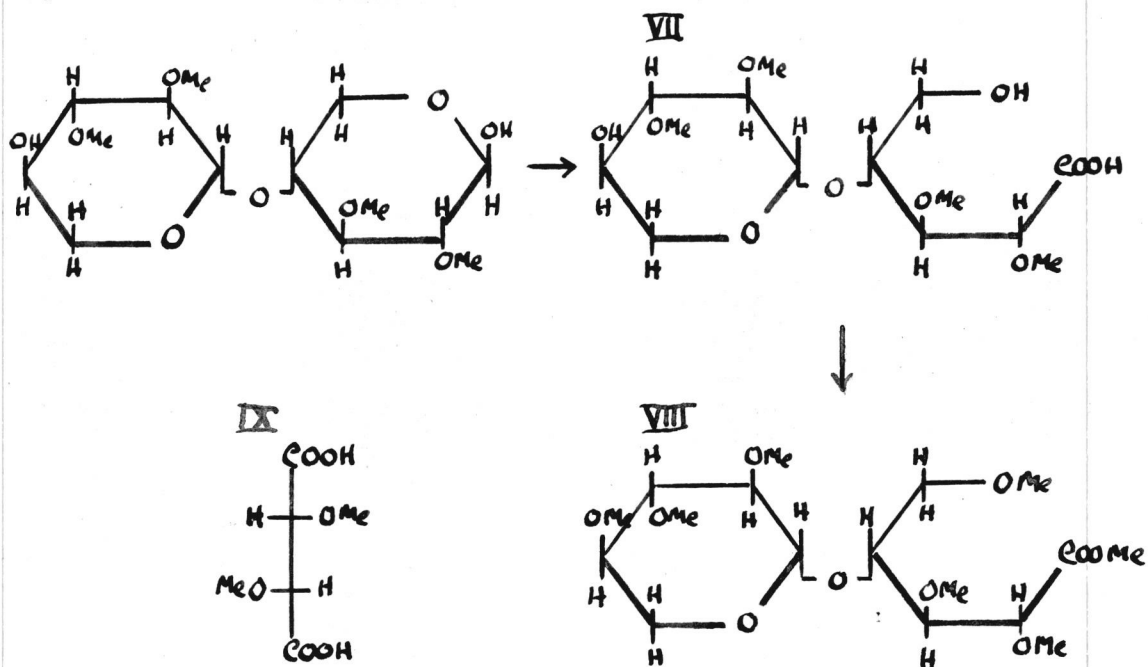
for complete hydrolysis. Crystalline derivatives have been prepared from this disaccharide. The presence of a 1:4 glycosidic linkage is indicated by the following reactions:



Xylobiose (III) was oxidised (probably with bromine water) to yield the acid (IV) which was then methylated (V) and hydrolysed to give the products (VI) which were identified as crystalline derivatives.

A similar result was obtained by Haworth and Percival (29) who subjected dimethyl xylan to partial degradation by acetolysis at 0° for 10 minutes. After deacetylation the mixture was oxidised with bromine water to yield the bionic acid (VII) which was methylated and esterified to the methyl ester of a hexamethyl di-xylobionic acid (VIII). Hydrolysis of this compound gave crystalline 2:3:4-trimethyl xylose and a liquid 2:3:5/

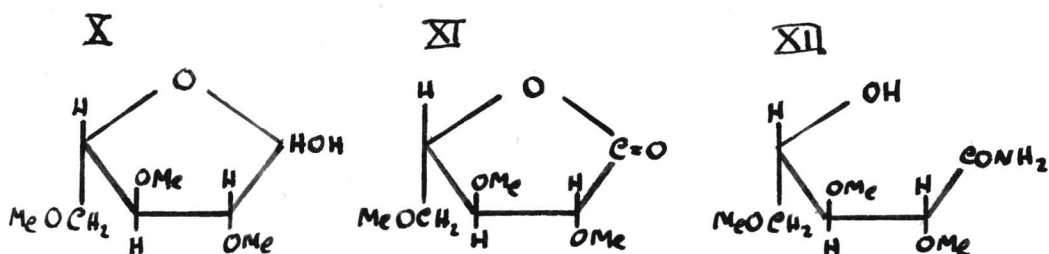
2:3:5-xylopic acid (VI) which was identified as a crystalline phenylhydrazide.



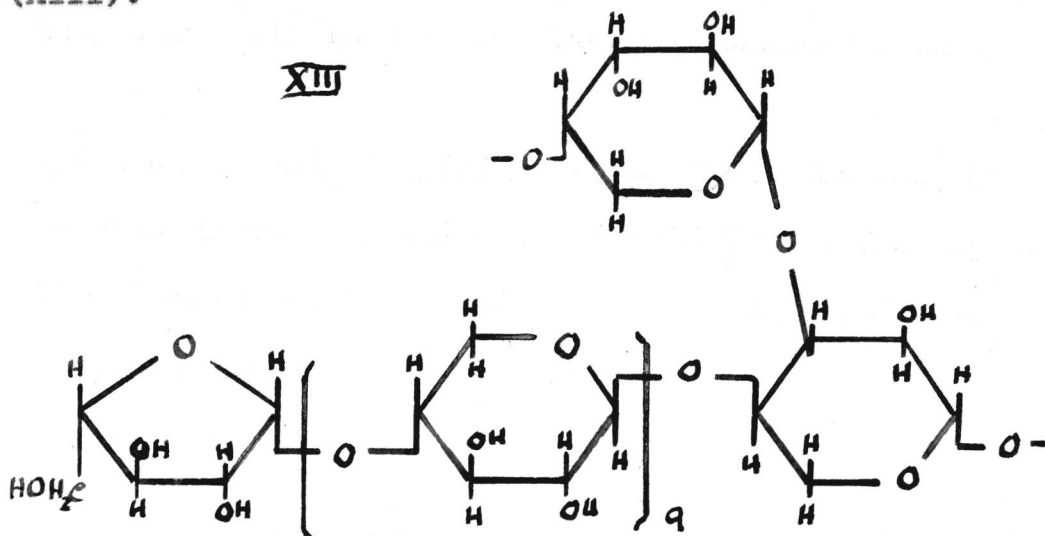
This combined with the fact that Haworth and Porter (3) had previously oxidised the lactone (VI) of 2:3:5-trimethyl xylonic acid to L(+) dimethoxy succinic acid (IX) proved beyond doubt that the linkage between the xylose units was of a 1:4 glycosidic nature.

As mentioned before Haworth, Hirst and Oliver (18) could obtain not more than 93% of xylose on hydrolysis of esparto xylan and after crystallisation of the xylose they were able to prepare from the mother liquor a crystalline arabinose benzylphenylhydrazone. Dimethyl xylan on hydrolysis yielded 6% of 2:3:5-trimethyl L-arabofuranose(X) which was identified as its crystalline lactone (XI) and amide (XII). In addition 5% of a monomethyl xylose was always found to be present in the hydrolysate/

hydrolysate of even the most exhaustively methylated xylan. This could be due to incomplete methylation or it might play a significant part in the molecule of methylated xylan. No trimethyl xylose could be identified.

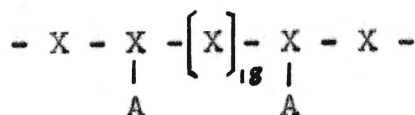


The presence of a trimethyl arabinose and no other end group suggests that the xylan molecule is composed of 18-20 β -D-xylopyranose units with an L-arabofuranose unit as end group. This is in agreement with the lack of reducing action of xylan which has been observed. On taking into account the presence of 5% of monomethyl xylose we are led to the idea of xylan having a branched chain structure with one branch for every 18-20 D-xylose units, the branches being terminated by an L-arabofuranose unit. A section of such a structure is shown in (XIII).



Bywater, Haworth, Hirst and Peat (30) later showed that the arabinose units could be removed by controlled hydrolysis at 100° with 0.2% aqueous oxalic acid. The acid-treated xylan was methylated and subsequently hydrolysed to yield 2-monomethyl-D-xylose, (5%), 2:3-dimethyl D-xylose (85 - 90%) and 2:3:4-trimethyl D-xylose (6%). It can be seen from these figures that the apparent chain length as estimated by the end group assay of the methylated derivative remains unaltered on the removal of the arabinose although the viscosity measurements suggest that a considerable shortening of the chain has occurred. As the arabinose-free xylan shows an enhanced reducing power, this constitutes additional evidence that the non-reducing terminal group of xylan is an arabinofuranose unit. It also disproves the theory put forward that xylan consists of a long chain of xylopyranose units linked through positions 1 and 4 with an arabinose residue attached as side chain after every 18 - 20 xylose residues (XIV). A similar attempt to prepare an arabinose-free xylan by hydrolysis with 0.005 N Nitric acid was only partially successful.

XIV.



Yundt/

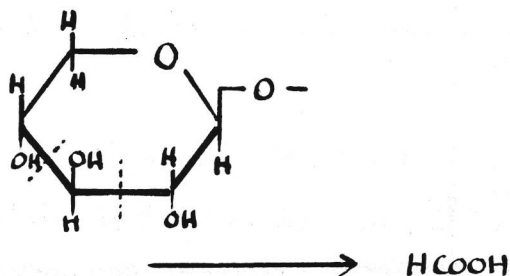
Yundt (31) has obtained a crystalline xylan from barley straw hemicellulose by hydrolysis with 0.2% aqueous oxalic acid at 100° for 5 hours. The insoluble residue was then autoclaved at 120° when 0.2% of the residue dissolved and precipitated as hexagonal platelets on cooling the filtrate to 60 - 70°. Viscosity and osmotic pressure measurements on the methylated polysaccharide show the degree of polymerisation of the crystalline material to be 35 - 39.

More recently Chanda, Hirst, Jones and Percival (15) have prepared an arabinose-free xylan from esparto grass by repeated precipitation as the copper complex. The fully methylated xylan on hydrolysis yielded 2 monomethyl D-xylose (4%), 2:3-dimethyl D-xylose (93%) and 2:3:4-trimethyl D-xylose (3%). Oxidation of the xylan with sodium periodate yielded 1 mole of formic acid for every 20 - 21 anhydro-pentose units and hydrolysis of the fully oxidised xylan showed a small amount of unattacked xylose in the hydrolysate. If branching occurs on a pentose chain unit only one free hydroxyl group would be present and consequently no oxidation would occur. On hydrolysis the pentose unit corresponding to the branching point would be recovered as the free sugar. Formic acid would be released from a terminal xylopyranose unit as in (XV). These figures led the authors to propose that xylan consists of a singly branched molecule containing 70 - 80 D-xylopyranose units. It would appear, therefore, that the/

the arabinose is present in the form of a contaminating araban. Ehrental and Smith (32) have reported the presence of small amounts of D-xylose and L-arabinose after hydrolysis of oxidised xylan from wheat straw and corn cob even after extended oxidation. This suggests that the contaminating araban if such exists, has a branched chain structure possibly similar to that of pectic araban though methylation studies do not bear this out.

The present work was initiated with the intention of elucidating a structure for this araban.

XV



At this point a brief mention might be made of some theories which have been put forward for the formation of xylan in plants. These are based on the fact that cellulose and xylan and uronic acids are often found together in nature. Examination of the xylan chain shows it to be identical to a cellulose chain from which the terminal $-\text{CH}_2\text{OH}$ groups have been removed. The linkages in both cases are 1:4 β linkages. The terminal group of the D-glucose units might then be oxidised to give the uronic acid which in turn would be decarboxylated to D-xylose.

The/

The presence of arabofuranose units however is definitely against such a theory. An additional reason why the theory is not very probable is that the materials involved are quite stable and are unlikely to undergo ready transformation. In most plants also they increase with the growth of the plant with no corresponding decrease of one component.

Another theory which is less open to criticism is that the pentoses themselves arise from an oxidation of the hexoses. This is supported by the fact that D-xylose is always found along with D-glucose and L-arabinose, accompanies the structurally similar D-galactose. In some gums and pectic substances they occur along with the corresponding acid (33). No definite proof, however, has been obtained to substantiate this theory.

ARABAN.

In 1894 Ullrich (34) isolated from beet a polysaccharide which was soluble in water but insoluble in alcohol, which did not reduce Fehling's solution, and which resembled dextrin in many of its properties. It was easily hydrolysed with dilute acids to yield arabinose while treatment with strong acids changed the optical rotation, from -83.9° to $+242.1^{\circ}$. It was suggested at the time that the polysaccharide might be an anhydride of arabinose. In the following year what was thought to be an araban was isolated from plant mucilages by concentration of the mucilage/

mucilage and subsequent precipitation of the araban with alcohol (35).

Owing to the insufficient sensitivity of the techniques available at that time these materials were not pure and in an attempt to obtain a purification Salkowski (36) noticed that araban could be precipitated from 8% aqueous sodium hydroxide solution by the addition of Fehling's solution if only one fifth of a volume of the latter was added. On the addition of more Fehling's solution (to equal parts) this precipitate redissolved to give a clear solution. The corresponding precipitate formed from xylan under the same conditions does not appear to be soluble even in excess of Fehling's solution. On decomposition of the soluble copper complex with acid and precipitation with alcohol the araban is recovered. Butler and Cretche (37), however, using more modern techniques have since shown that Salkowski's araban contains in addition to arabinose, galactose, rhamnose, and a glycuronic acid, and is therefore not a simple polysaccharide.

It was not until 1938 when Hirst and Jones (38) reported the isolation of an araban and pectic acid from the peanut that any reliable analytical results were obtained and all the arabans which have so far been examined have been isolated from pectic substances.

These authors used boiling 0.2% aqueous potassium hydroxide to extract an araban-pectic acid complex from protein- and oil-free peanut meal. Precipitation with methylated/

methylated spirits yielded a polysaccharide which gave no colour with iodine and consisted mainly of a complex of pectic acid and araban. This material was methylated by the method of Menzies (39) using thallos hydroxide and methyl iodide. The methylated material was fractionated to yield a methylated araban. This on hydrolysis gave 3-monomethyl L-arabinose, 2:3-dimethyl L-arabinose, and 2:3:5-trimethyl L-arabinose in the ratio 1:1:1. The trimethyl arabinose must have existed in the furanose form in the original polysaccharide and it is probable that all the pentose molecules were in this form as evidenced by the high rate of hydrolysis of the araban in dilute acid. Characterisation of the monomethyl derivative as 3-monomethyl L-arabinose was carried out as follows. Oxidation of the sugar to the corresponding lactone followed by treatment of the lactone with methanolic ammonia yielded an amide. Reaction of the amide with sodium hypochlorite under Weerman's conditions (40) and subsequent addition of semicarbazide gave hydrazodicarbonamide thus indicating that carbon atom number 2 had a free hydroxyl group. The free sugar possesses the high positive rotation characteristic of pyranose forms in the L-arabinose series and it gives a laevorotatory lactone characterised as a γ -lactone by its rate of hydrolysis. Positions 4 and 5 therefore contain free hydroxyl groups. The same authors (41), however, have since shown that the positive Weerman test reported above was due to the presence/

presence of a small quantity of L-arabonamide in the crude syrupy amide, a trace of L-arabinose being present in the monomethyl arabinose fraction owing to the difficulty encountered in the separation of these two high boiling fractions. The two monomethyl sugars, 2- and 3-methyl L-arabinose were therefore synthesised (42, 43), and crystalline derivatives prepared. Preparation of the crystalline phenylhydrazone, the lactone and the crystalline amide of the lactonic acid, showed that the monomethyl sugar obtained by hydrolysis of the methylated araban was 2-methyl L-arabinose and not the 3-methyl derivative.

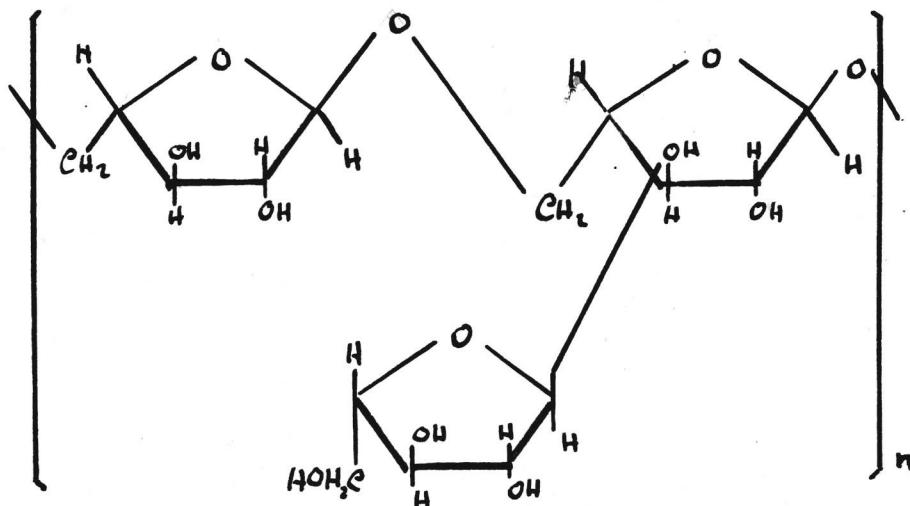
The lactone of the dimethyl sugar had a strong laevorotation and this coupled with the fact that it hydrolysed very slowly, established it as a γ -lactone. The high positive rotation of the dimethyl sugar itself showed that the sugar could exist in the pyranose form. These results indicate that positions 4 and 5 were not occupied with a methoxyl group. A Weerman test on the dimethyl arabonamide was negative showing that position 2 was occupied by a methyl group, and this constitutes additional evidence that the sugar is 2:3 dimethyl L-arabinose.

While it is not possible to formulate a unique structure for the peanut araban from the evidence available, a general type of structure consisting of a much branched chain of arabofuranose units as in (XVI) is apparent. Rotational data shows that the L-arabofuranoside/

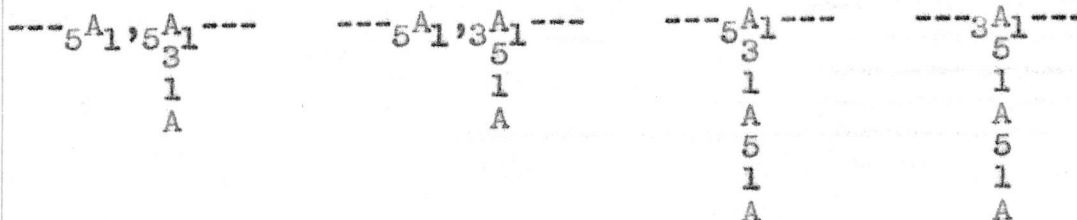
L-arabofuranoside links are of the α -configuration.

Variations of this structure as in (XVII) where A represents an L-arabofuranose unit, are also in agreement with the experimental evidence.

XVI



XVII.

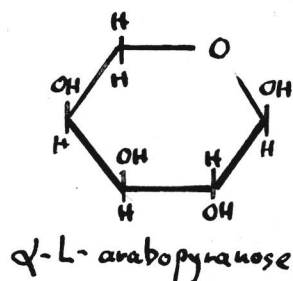
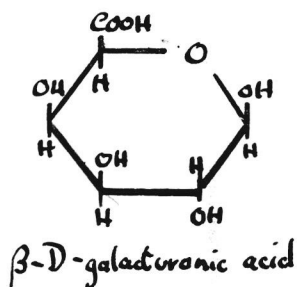
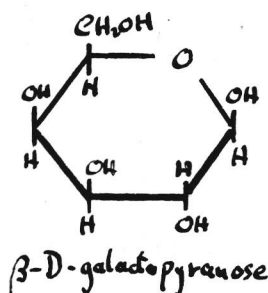


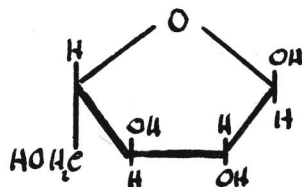
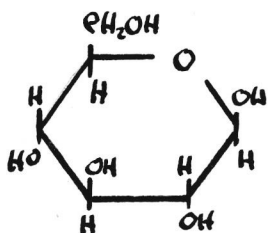
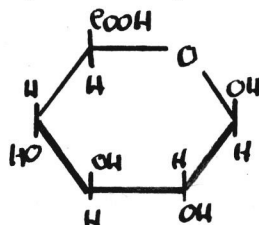
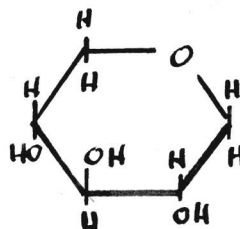
The same authors (44, 45, 46) have examined the arabans derived from apple, citrus and sugar beet pectins and have found that the methylated derivatives in all cases give on hydrolysis approximately equal proportions of 2:3:5-trimethyl L-arabofuranose, 2:3-dimethyl L-arabofuranose, and 2-monomethyl L-arabofuranose. Also in all cases the araban is associated with galactan and pectic acid. It is probable therefore that these arabans possess the same type/

type of structure.

This leads us to a further consideration of the phytochemical relationships between the carbohydrate materials in plants already discussed on p. 15. The available evidence indicates that the pectic acid portion of the peanut is identical with normal pectic acid and consists of a main chain of D-galacturonic acid residues linked through carbon atoms 1 and 4. Side chains of D-galacturonic acid units in the pyranose or furanose forms may be attached to this main chain. The galacturonic acid units must, in view of the 1:4 linkage, be of the pyranose form. If the peanut araban had been derived from the pectic acid by decarboxylation without intermediate hydrolysis of the polygalacturonide the arabinose units would also be in the pyranose form. As has been shown the arabinose units in peanut araban are of the furanose type and so any hypothesis concerned with the direct conversion of pectic acid into araban is untenable. (XVIII) below shows the structural relationships between the free sugars involved in such a hypothesis, and shows also the relationship between the sugars in the similar hypothetical formation of xylan from cellulose.

XVIII

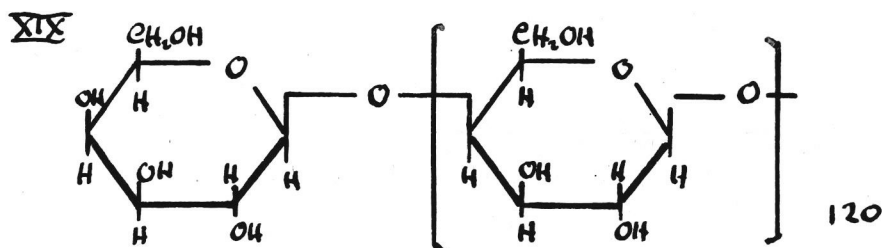


 α -L-arabofuranose β -D-glucopyranose β -D-glucuronic acid β -D-xylopyranoseGALACTAN.

Owing to the difficulty of separating galactans from accompanying arabans and pectic acid only two galactans have been isolated in any degree of purity. The first was the ϵ -galactan from the Larch (48), while the second was separated from the seeds of Lupinus albus (49). These two galactans are very different in structure and they also differ in that the ϵ -galactan contains arabinose residues as well as galactose. A galactan has also been reported to be present in sprucewood (50), but no methylation or other structural studies have so far been reported for this.

The galactan from Lupinus albus is associated with pectin from which most of the pectic acid is easily removed as the insoluble calcium salt. The araban is only partially removed by repeated precipitation from aqueous solution by the addition of alcohol. A pure methylated/

methylated galactan can, however, be prepared by separating the methylated araban from the methyl derivative of the crude galactan with ether, in which methylated galactan is insoluble. Prolonged methanolysis of the methylated galactan yielded methyl 2:3:6-trimethyl D-galactoside identified as the lactone of 2:3:6-trimethyl D-galactose along with a small amount of methyl 2:3:4:6-tetramethyl D-galactoside. The laevorotatory nature of the methylated galactan indicates that the linkages are of the β -glycosidic type. The evidence would suggest therefore, that the galactan consists of a straight chain of D-galactopyranose units linked by 1:4- β -links (XIX).



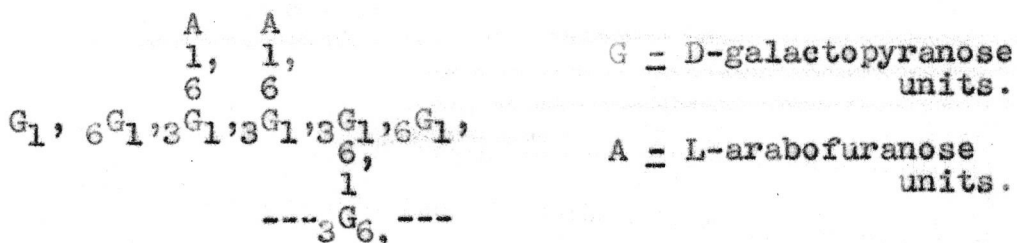
In more recent work Hirst, Jones and Walder (51) have again examined the galactan from Lupinus albus seeds. After removal of protein by extraction with 10% aqueous sodium chloride and 0.2% aqueous sodium hydroxide, boiling 0.2% aqueous sodium hydroxide was used to extract the galactan, which was precipitated from the filtrate with alcohol. Reprecipitation yielded a material giving on hydrolysis 6% galactose and 26% arabinose. No further separation was obtained.

Methylation/

Methylation studies gave similar results to those obtained previously for the galactan but the final fraction of the methylated product was found to contain some 2:3:5-trimethyl arabinose. Further experimental evidence will be necessary before any conclusion is drawn concerning the part that this arabinose plays in the polysaccharides of Lupinus albus seeds.

The ϵ -galactan mentioned above has been shown to contain D-galactopyranose and L-arabofuranose units and is a branched chain polysaccharide (52), a possible formula being shown below (XX). It can be seen that this is very different from the galactan isolated from pectin, and although pectic materials are known to be present in wood it is not known whether the ϵ -galactan is a component of wood pectin.

XX.



Finally mention might be made of attempts to isolate a galactan from the polysaccharides of the peanut. Miyama (53) has reported the isolation of a material containing L-arabinose, D-galactose and uronic acid residues. He considered that the latter was not a natural product but had arisen from alkaline oxidation/

oxidation during extraction and that the polysaccharide present was a galacto-araban. He also considered that the arabinose was present in the pyranose form. As mentioned before it has now been shown conclusively that the arabinose in peanut araban is of the furanose type (38). Hirst and Jones (54) moreover, have been unable to isolate such a galacto-araban and it would appear that Miyama was dealing with a mixture and not a pure galacto-araban.

EXPERIMENTAL.PART ONECRUDE XYLAN.Preparation of Crude Xylan from Esparto Grass.

Esparto grass (200 g.), cut into pieces 3 - 4 cms. long, was extracted with benzene (4 days) and methanol (3 days) in a Soxhlet extractor to remove respectively waxy and colouring materials and milled to obtain a fibrous product. This material was delignified in batches by the method of Wise (55). The grass (100 g.) was suspended in water (5l.) containing acetic acid (500 ml.) and sodium chlorite (500 g.). Sodium acetate (20 g.) was added and the solution heated to 60°C with continuous stirring and kept at that temperature for one minute. The flask was then transferred to a thermostat bath at 30°C and maintained at that temperature with occasional shaking for 24 hours. The mixture was filtered through muslin, the fibrous residue washed with iced water to remove the acid, and finally with acetone to remove the water. The product was dried in air at room temperature.

The impure polysaccharide was extracted from this holocellulose (100 g.) by rolling it for 24 hours at room temperature in a bottle containing glass marbles and aqueous sodium hydroxide solution (2 l.; 4%). The slurry was separated at the centrifuge and the crude xylan/

xylan precipitated from the clear solution with acetone after acidification with acetic acid. This operation was repeated on the residual solid. The small amount of lignin dissolved in the alkali was left in the acetone solution as evidenced by the brown colour of the latter. The polysaccharide was collected on the centrifuge and washed with acetone/water (1/1) to remove the acid, followed by aqueous alcohol of increasing concentration and finally with ether. The product was dried in air until most of the ether had evaporated, after which it could be powdered and dried over phosphoric oxide in a vacuum desiccator. The resultant polysaccharide was a pale cream powder (20 g.).

Ash Content of Crude Xylan.

The ash content of crude xylan was determined by the careful incineration of a weighed amount of the polysaccharide in a tared platinum crucible with lid. The black residue was then heated strongly with a blowlamp for ten minutes, the crucible and contents cooled and weighed.

Found: ash content, 2.3%.

Preliminary Hydrolysis Experiments on Crude Xylan.

In each case a small amount of the crude xylan (ca 25 mg.) was hydrolysed with acid (2 mls.) in a sealed tube in the boiling water bath. In the case of the nitric acid hydrolyses the hydrolysate was spotted directly onto a filter paper strip and subsequently examined chromatographically. When sulphuric acid was used/

used for the hydrolysis it was necessary to first neutralise the acid with barium carbonate. The insoluble barium salts were then removed by filtration and the resultant solution evaporated to a syrup under diminished pressure. The syrup was redissolved in 95% aqueous alcohol (commercial alcohol) and after filtration this solution was used for spotting onto the chromatogram paper.

Either of these processes was found to be suitable for a qualitative examination of the hydrolysate but it was necessary to use sulphuric acid for hydrolysis for quantitative estimations of the sugars present. Further removal of inorganic salts was also necessary and was carried out as follows. After removal of barium salts with alcohol as above the alcoholic solution was again evaporated to dryness under diminished pressure. The syrup was dissolved in water and the solution shaken with the ion exchange resins Amberlite IR 100H (cation exchanger) followed by Amberlite IR 4B (anion exchanger) to remove completely any ions which might interfere.

An indication as to whether the hydrolysis had gone to completion was gained from the amount of unhydrolysed material present in each case. Comparison of the hydrolysates of a complete and an incomplete hydrolysis on a ^{chromatogram} paper showed that in all cases of incomplete hydrolysis the araban present had been completely/

completely hydrolysed while the xylan was incompletely hydrolysed.

Chromatographic examination was carried out on a filter paper strip 50 cm. x 13 cm. and the paper hung from a trough containing the organic layer of a mixture of n-butanol/ethanol/water/ammonia (40/10/49/1) for two days (56). The paper was then dried and sprayed with a saturated aqueous solution of aniline oxalate (57). On heating in an oven at 110°C for five minutes this reagent reacted with hexoses to give a brown colour while pentoses gave a pink colour. It was later found that for qualitative work a mixture of benzene/butanol/pyridine/water (10/50/30/30) gave sufficient separation of galactose, glucose, arabinose and xylose in 24 hours while for quantitative estimations of these sugars this solvent gave excellent separation in 100 hours. Other solvents failed to separate the galactose and glucose sufficiently for a quantitative determination of these sugars.

Hydrolysis of crude xylan showed the presence of xylose (in quantity) arabinose and very small traces of galactose and glucose. Table I below shows the results obtained on hydrolysis of crude xylan using various strengths of nitric and sulphuric acids.

TABLE I. /

TABLE I.

<u>Acid used</u>	<u>Duration of hydrolysis</u>	<u>Result</u>
0.4N (2.5%) Nitric acid	1½ hours	complete hydrolysis of araban but incomplete hydrolysis of xylan
0.4N Nitric acid	3 hours	complete hydrolysis of araban and xylan
0.4N Nitric acid	24 hours	complete hydrolysis of araban and xylan
0.2N Nitric acid	3 hours	complete hydrolysis of araban but incomplete hydrolysis of xylan
0.2N Nitric acid	24 hours	complete hydrolysis of araban and xylan
N Sulphuric acid	24 hours	complete hydrolysis of araban and xylan
N Sulphuric acid	4 hours	complete hydrolysis of araban and xylan
0.5N Sulphuric acid	6 hours	complete hydrolysis of araban but incomplete hydrolysis of xylan

In all cases a very small amount of insoluble residue was left unhydrolysed. This was assumed not to be xylan or araban as no further hydrolysis could be effected. As a result of these experiments the procedure/

procedure adopted for subsequent hydrolyses was either that using 0.4N nitric acid for 3 hours or, if a quantitative estimation of the hydrolysis products was required, that using N sulphuric acid for 4 hours.

Estimation of the Arabinose Content of Crude Xylan.

An indication of the amount of arabinose present in the hydrolysate of crude xylan can be obtained by comparison of the intensities and areas of the sugar spots obtained on the paper chromatogram. For accurate determinations two methods were used viz. estimation of the arabinose by forming and weighing the diphenylhydrazone, and quantitative partition chromatography.

Arabinose Diphenylhydrazone.

Two methods were employed for the formation of arabinose diphenylhydrazone.

1) Diphenylhydrazone hydrochloride (5 g.) was warmed gently with sodium hydroxide (25 ml.; 2 N) the mixture cooled, extracted with ether and the ether extract washed with water and dried over anhydrous sodium sulphate. The ether was evaporated off and the residue distilled under vacuum. The fraction which distilled at 207 - 210° was used as follows (58) :-

133 mg. arabinose, ca 60 mg xylose and 200 mg. diphenylhydrazine were dissolved in 5 ml. aqueous alcohol (1 : 1) and the solution boiled under reflux at 95° for 30 minutes. Subsequent cooling to 0° for 48 hours yielded crystals of arabinose/

arabinose diphenylhydrazone which were separated and washed with 5 l ml. portions of 50% aqueous alcohol and dried for two hours at 100° m.p.

191-4° wt. 139.9 mg. This weight is equivalent to 66.4 mg. arabinose i.e. the yield of arabinose diphenylhydrazone was only 50%.

On repeating this experiment on the crude xylan hydrolysate the only solid which precipitated melted at 100° on drying.

2) Impure diphenylhydrazine hydrochloride (5 g.) (55) was heated with hydrochloric acid (37.5 ml.; 2.5%) to boiling point. After cooling to room temperature for 3 hours the solution was filtered and to the filtrate was added concentrated hydrochloric acid (750 ml.). The mixture was again cooled for 2 hours and the soft needle-like crystals of the hydrochloride filtered by suction through a sintered glass funnel. As the crystals were still slightly coloured the whole process was repeated. The crystals were finally dried and freed from hydrogen chloride by standing in a vacuum desiccator over potassium hydroxide.

The purified diphenylhydrazine hydrochloride (0.75 g.) and crystalline sodium acetate (0.75 g.) were warmed in ethyl alcohol (5 ml.; 95%). The solution was filtered from sodium chloride and washed with sufficient alcohol to bring the volume of the filtrate to 5 ml. This reagent was added to 2.5 ml. aqueous solution containing 63.6 mg. arabinose and ca 200 mg. xylose. After mixing/

mixing the solutions and warming to effect solution, the warm mixture was treated with aqueous ethyl alcohol (10 ml.; 50%) saturated with arabinose diphenylhydrazone. The mixture was again warmed carefully and then allowed to stand overnight. The crystals which had separated were washed with eight 10 ml. portions of the 50% aqueous alcoholic solution of arabinose diphenylhydrazone and dried at 100°C for 2 hours, m.p. 193-4°, weight 129.5 mg. This is equivalent to 64.7 mg. arabinose or 101% of the amount used.

When this method was repeated on the hydrolysate (0.3231 g. syrup dried in high vacuum over phosphoric oxide), a dark coloured precipitate (65 mg.) was formed m.p. 185-190°. This is equivalent to 30.9 mg. arabinose or 9.6% of the syrup.

According to Wise and Peterson it is advisable to increase the arabinose content of the syrup used to approx. 25% and so the determination was repeated adding 41.8 mg. arabinose to 225.7 mg. syrup. A crystalline precipitate was formed m.p. 192-4°, wt. 124.9 mg. This is equivalent to 59.3 mg. arabinose i.e. the arabinose in the syrup was 17.5 mg. or 7.8% of the syrup.

Quantitative Partition Chromatography (58).

1.078 g. crude xylan was hydrolysed with N sulphuric acid for 4 hours at 100° in a sealed tube, neutralized with barium carborate and 0.1887 g. ribose (recrystallized and dried) added. After the usual treatment to remove ions/

ions the solution was spotted onto a filter paper chromatogram and run with butanol/benzene/pyridine/water (50:10:30:30) as solvent at 20°. At the end of 40 hours the paper was thoroughly dried and the side strips developed with Aniline Oxalate. The centre bands corresponding to the sugars and also a paper blank of the same size were cut out, and hung at the bottom of a reflux condenser over 5 mls. boiling water in a tube fitted with a QQ ground glass socket thus allowing the water to reflux off the filter paper taking the sugar with it. Blanks were also run as follows:- 5 ml. water, 5 ml. arabinose solutions containing 17.02 mg. arabinose/500 ml. solution, and 5 ml. ribose solution containing 19.00 mg. ribose/500 ml. solution. After 30 minutes extraction 5 ml. Somogyi reagent were added to each tube with gentle mixing and the tubes were placed in a briskly boiling water bath for 25 minutes. They were then transferred to a water bath at 30° for 10 minutes at the end of which time 0.5 ml. 2.5% potassium iodide solution was run carefully down the side of each tube followed by 1.5 ml. 2 N sulphuric acid squirted into the tube to ensure that the contents were instantly mixed and acidified. The tubes were immediately closed with ground glass stoppers and chilled in cold water. Titration of the iodine formed was by means of 0.005 N sodium thiosulphate, 2 drops of 1% starch solution were used as indicator.

For/

For each sugar we have four titration figures viz:

- a - titration figure for 5 ml. water.
 b - " " " 5 ml. standard sugar solution.
 c - " " " 5 ml. extract from paper blank.
 d - " " " 5 ml. extract from paper containing sugar.

From the above (a - b) is equivalent to the copper reagent reduced by 5 ml. of standard sugar solution while (c - d) is equivalent to the copper reagent reduced by 5 ml. extract from the paper containing the sugar.

If 5 ml. of standard sugar solution contain x mg. of sugar the amount of sugar in the paper extract is

$\frac{x(c - d)}{(a - b)}$. From this equation the ribose in the paper

extract was 0.297 mg. while the arabinose was 0.147 mg.

Therefore in 1.078 g. crude xylan we have $\frac{0.147 \times 0.1887}{0.297}$ g.

arabinose, i.e. the percentage of arabinose in crude xylan

is $\frac{0.147 \times 0.1887 \times 100\%}{0.297 \times 1.078} = 8.7\%$.

Attempted Isolation of Arabinose from Crude Xylan or Esparto Holocellulose.

Extractions were carried out as shown below. The extraction mixtures were filtered and the filtrate concentrated and neutralised with acetic acid if necessary. The extracted material was precipitated by the addition of 5 volumes of acetone and the precipitate separated on the centrifuge, washed with acetone and dried in air. The product was hydrolysed with 2.5% nitric acid for 3 hours and the solution examined chromatographically. The approximate proportion of arabinose to xylose was estimated by/

by a comparison of the intensities and areas of the colour spots formed on the chromatogram by development with aniline oxalate.

- 1) Crude xylan (0.05 g.) was mixed to a paste with water (2 ml.) and calcium carbonate (0.1 g.) and the mixture boiled under reflux in a boiling water bath for 3 hours.

Found: ratio of arabinose/xylose, 1/4.

- 2) Crude xylan (0.05 g.) was mixed to a paste with water (2 ml.) and calcium carbonate (0.1 g.), and the mixture boiled under reflux in a boiling water bath for 30 minutes.

Found: ratio of arabinose/xylose, 1/4.

- 3) Esparto holocellulose (5 g.), calcium carbonate (1 g.), and water (100 ml.) were rolled gently in a dark glass bottle containing glass marbles, at room temperature for 24 hours.

Found: ratio of arabinose/xylose, 1/2.

- 4) Esparto holocellulose (20 g.) was stirred continuously in water (200 ml.) containing barium carbonate (1 g.) the flask being immersed in a boiling water bath for 24 hours.

Found: ratio of arabinose/xylose, 1/4.

- 5) Esparto holocellulose (5 g.) was extracted with water (50 ml.) containing calcium hydroxide (1 g.), the mixture being boiled under reflux in a boiling water bath for 12 hours.

Found: ratio of arabinose/xylose, 1/4.

6)/

- 6) Esparto holocellulose (5 g.), calcium hydroxide (1 g.) and water (100 ml.) were rolled gently in a dark glass bottle containing glass marbles, at room temperature for 24 hours.

Found: ratio of arabinose/xylose, 1/4.

- 7) Esparto holocellulose (5 g.) was extracted with 1% aqueous sodium hydroxide (100 ml.) the mixture being rolled with glass marbles in a dark glass bottle at room temperature for 24 hours. Air was expelled from the mixture prior to extraction by bubbling in nitrogen gas.

Found: ratio of arabinose/xylose, 1/2.

- 8) Esparto holocellulose (5 g.) was extracted as in 7) above using aqueous sodium hydroxide (100 ml.; 0.5%).

Found: ratio of arabinose/xylose, 1/2.

- 9) Esparto holocellulose (5 g.) was extracted as in 7) above using aqueous sodium hydroxide (100 ml.; 0.1%).

Found: ratio of arabinose/xylose, 1/2.

- 10) Crude xylan (5 g.) was shaken continuously with aqueous ethyl alcohol (100 ml.; 70%, v/v) at room temperature for 14 days.

Found: ratio of arabinose/xylose, 1/2.

- 11) Crude xylan (1 gm.) was extracted with aqueous ethyl alcohol (20 ml.; 70%, v/v), the mixture being stirred continuously and boiled under reflux in a hot/

hot water bath at 80°C for 24 hours.

Found: ratio of arabinose/xylose, 1/3.

- 12) Esparto holocellulose (5 g.) was extracted as in 11) above using aqueous ethyl alcohol (100 ml.; 70%, v/v) for 10 hours.

Found: ratio of arabinose/xylose, 1/2.

- 13) Esparto holocellulose (20 g.) was extracted as in 11) above using aqueous ethyl alcohol (400 ml.; 70%, v/v) for 24 hours.

Found: ratio of arabinose/xylose, 1/2.

- 14) Crude xylan (2 g.) was extracted as in 11) above using aqueous ethyl alcohol (100 ml.; 50%, v/v) for 4 hours. Peptisation occurred and subsequent precipitation with acetone gave no fractionation.

Found: ratio of arabinose/xylose, 1/10.

- 15) Esparto holocellulose (20 g.) was extracted with aqueous pyridine (200 ml.; 30%, v/v) the mixture being boiled under reflux in a boiling water bath for 3 hours.

Found: ratio of arabinose/xylose, 1/4.

- 16) Esparto holocellulose (20 g.) was extracted as in 15) above using aqueous pyridine (200 ml.; 30%, v/v) for 9 hours.

Found: ratio of arabinose/xylose, 1/10.

- 17) Esparto holocellulose (20 g.) was extracted with aqueous pyridine (200 ml.; 30%, v/v) the mixture being rolled in a dark glass bottle with glass marbles/

marbles at room temperature for 24 hours.

Found: ratio of arabinose/xylose, 1/4.

- 18) Crude xylan (2 g.) was extracted with a constant boiling mixture of alcohol, benzene and water (100 ml.) in a Soxhlet extractor for 4 days.

Found: ratio of arabinose/xylose, 1/4.

Attempted Isolation of Araban from Crude Xylan by Fractionation as the Copper Complex.

Crude xylan (20 g.) was dissolved in aqueous sodium hydroxide (1 l.; 4%), and Fehling's solution (1 l.) added with vigorous stirring. A small amount of acetone was added to improve settling of the precipitate. The clear liquid was decanted and the precipitate removed on the centrifuge. A small portion of the precipitate was decomposed with acetic acid and hydrolysed in the usual manner. Examination of the hydrolysate by paper chromatography showed this product to contain slightly less araban than the crude xylan. The soluble fraction of the copper complex was acidified with acetic acid and the resultant liquid dialysed against water with continuous stirring for seven days. At the end of this time sodium ferrocyanide gave no colour with the solution showing that the copper ions had been removed. The dialysed solution was evaporated to one and a half litres under diminished pressure, acidified with acetic acid and poured into 5 volumes of acetone. The resultant solid was filtered redissolved in aqueous sodium hydroxide (4%) and reprecipitated several times once using/

using hydrochloric acid (2 N) instead of acetic acid to facilitate the removal of any copper ions which might still be present, and finally washed with alcohol and ether and dried in the air. This material contained ca 20% arabinose, 70% xylose and 10% glucose.

A further 20 g. crude xylan were stirred with cold water and the mixture allowed to stand in the cold overnight. At the end of 24 hours the undissolved solid was filtered and fractionated as above. By this method it was hoped to remove degraded xylan in the starting material. The xylose in the final material however was only reduced to ca 60%.

The yield in both cases was 0.4 g. i.e. 2% and the yield of araban in each case amounted to ca 6% of the original.

Acetylation of Crude Xylan.

Trial Acetylations.

1. Crude xylan (0.6 g.) was swollen with pyridine (25 ml.) at 70°C for 2 hours, cooled to room temperature and acetic anhydride (7 ml.) added with shaking. The mixture was allowed to stand in the dark for 3 days at room temperature with occasional shaking. Some undissolved material was removed on the centrifuge and the centrifugate poured into excess water. The solid acetate was filtered, washed thoroughly with water and dried at room temperature over phosphoric oxide in vacuo.

Yield/

Yield 0.7 g.

- 2) Crude xylan (1 g.) was shaken with pyridine (50 ml.) containing 2% water for 2 hours at room temperature. The mixture was heated to 60°C in the water bath and acetic anhydride (25 ml.) was added with continuous stirring. The heating and stirring were continued for 18 hours. The dark solution was freed from undissolved material at the centrifuge, poured into water and the solid acetate removed, washed 5 times with water and dried with alcohol and benzene three times. Yield 1 g. Severe darkening occurred during this acetylation.
- 3) Crude xylan (0.4 g.) was ground with water in a mortar and then allowed to swell with boiling water for 2 hours. Pyridine was added continuously with distillation until the azeotrope of pyridine and water was removed. To the final volume of 100 ml., acetic anhydride (50 ml.) was added and the mixture heated in the boiling water bath for 30 mins., cooled and allowed to stand at room temperature in the dark for 20 hours. Separation of the acetate was then as described in 1) above. Yield 0.4 g. Again severe darkening occurred mainly during the azeotropic distillation.
- 4) Esparto holocellulose (20 g.) was soaked in cold pyridine (100 ml.) for 18 hours. Acetic anhydride (75 ml.) was added and the mixture shaken occasionally at room temperature during two days. Separation/

Separation of the acetate was again as in 1) above.

Yield, 2 g.

The method finally adopted for the preparation of the crude xylan was as follows:-

Crude xylan (5 g.) was heated with pure pyridine (150 ml.) in the hot water bath at 70°C for 2 hours with vigorous stirring. The mixture was cooled and acetic anhydride (60 ml.) mixed with pyridine (75 ml.) was added slowly with continued vigorous stirring. The resultant mixture was shaken occasionally at room temperature for 4 days and the acetate separated as before. Yield, 6 g.

Deacetylation of Xylan Diacetate.

Trial Deacetylations.

- a) Xylan diacetate (0.1 g.) was heated with aqueous sodium hydroxide (5 ml.; N) in a boiling water bath for 5 hours and the solution evaporated to 2.5 ml. under reduced pressure. A small amount of insoluble material was removed on the centrifuge and the centrifugate neutralised with acetic acid and poured into acetone to precipitate the deacetylated material. A large amount of inorganic material separated out making it difficult to isolate a polysaccharide without a very high ash content.
- b) Xylan diacetate (0.1 g.) was shaken with aqueous sodium hydroxide (20 ml.; N) in the cold for 2 hours and the solution evaporated to 3 ml. under reduced/

reduced pressure. The procedure was then as in a) above but the same difficulty of removing inorganic material arose.

- c) Freshly cut potassium (0.025 g.) was added to a suspension of xylan diacetate (0.1 g.) in dry methyl alcohol (10 ml.). The mixture was heated on the steam bath for 5 minutes and shaken at room temperature for 24 hours. After neutralisation with acetic acid the deacetylated material was removed by filtration, washed twice with alcohol and dried in air. This method was used in subsequent deacetylations owing to the ease with which inorganic material is removed.

Xylan Diacetate Solubility Experiments.

- 1) Pure xylan diacetate (0.5 g.) prepared by Dr. S.K. Chanda was treated with acetone (40 ml.; Analar Reagent) at the boiling point for 30 minutes. The undissolved solid was removed by filtration and the filtrate evaporated to dryness. The filtrate was found to contain no dissolved material.
- 2) A similar experiment using dry methyl alcohol (40 ml.) showed the complete insolubility of the acetylated xylan in this solvent.
- 3) Xylan diacetate (1 g.) of a sample which had been prepared some years previously by the late Dr. E.G.V. Percival, was treated with acetone (50 ml.; Analar Reagent) at the boiling point for

30 minutes. A small amount of the acetate dissolved and the undissolved material was removed by filtration. The filtrate was evaporated to dryness under reduced pressure and the resultant solid deacetylated catalytically with potassium (see method c) above) and the deacetylated material hydrolysed in the usual way. Paper chromatographic examination of the hydrolysate showed the presence of xylose but no other sugar. The partial solubility of this acetylated material may be explained by either the incomplete acetylation of the xylan or by partial deacetylation of the material due to age. Also the possibility cannot be excluded of the soluble material being the acetate of a degraded xylan.

Attempted Isolation of Araban Acetate from Acetylated Crude Xylan.

Three extractions of acetylated crude xylan were carried out as below. In each case the undissolved material was removed by filtration and after evaporation of the filtrate to dryness the resultant solid was deacetylated catalytically as before. The deacetylated material was hydrolysed with nitric acid and the hydrolysate examined chromatographically along with the hydrolysate of the crude xylan. In each case the soluble fraction of the acetate showed the same proportion of arabinose to xylose as the crude xylan. A portion of the insoluble fraction of the acetate was also deacetylated and hydrolysed in the same way. Chromatographic examination/

examination showed that arabinose was still present in the insoluble fraction.

The presence of arabinose in the insoluble fraction of the acetate suggested that the latter might not be completely acetylated as the araban acetate from pectic substances is known to be soluble in acetone and methyl alcohol. The insoluble fraction was therefore reacetylated but the major part of the araban acetate was still insoluble in the solvents below.

- a) Acetylated crude xylan (4 g.) was extracted with acetone (100 ml.; Analar Reagent) and the mixture boiled under reflux in a hot water bath for 20 hours.
- b) Acetylated crude xylan (5 g.) was extracted with acetone (100 ml.; Analar Reagent) the mixture being shaken vigorously at room temperature for 24 hours.
- c) Acetylated crude xylan (2 g.) was extracted with boiling dry methanol (25 ml.) under reflux in a hot water bath for 3 hours.

Oxidation of Crude Xylan with Sodium Periodate.

Crude xylan (1.857 g.) was shaken with aqueous sodium periodate (250 ml.; 0.1M) in a dark bottle for 7 days. At the end of this time the solution was made up to 500 ml. and 100 ml. withdrawn, neutralised with barium carbonate and the insoluble barium salts removed by filtration. The iodic acid was then removed from the filtrate/

filtrate by bubbling sulphur dioxide through the solution and the oxidised polysaccharide hydrolysed by making the solution 0.5N with respect to sulphuric acid by the addition of concentrated sulphuric acid and maintaining the mixture at 100°C in a boiling water bath for 4 hours. The solution was neutralised with barium carbonate, filtered and the filtrate, after reducing the volume by evaporation under diminished pressure, examined on a paper chromatogram. As a considerable amount of xylose was still present in the hydrolysate the oxidation was continued for a further 7 days and a second 100 ml. withdrawn and examined as before. The ratio of arabinose to xylose in the hydrolysate after 14 days oxidation appeared to be practically the same as in the hydrolysate obtained after 7 days oxidation and so it was concluded that no further oxidation would occur. The arabinose present was determined chromatographically and found to be 0.95% with respect to the original polysaccharide. This represents 11% of the original araban present in the crude xylan.

A further quantity of crude xylan (4.346 g.) was shaken in a dark bottle with aqueous sodium periodate (250 ml.; 0.3M) for 7 days. At the end of this time some insoluble material was removed by filtration. This was found to be inorganic. Treatment of the filtrate was then as for the oxidation with 0.1M sodium periodate above. A quantitative estimation of the sugars present by/

by means of paper chromatography showed the arabinose present in the hydrolysate to be 0.67% with respect to the original polysaccharide while the xylose was 1.16% of the original polysaccharide. For the arabinose this represents 7.7% of the original araban present in the crude xylan while for the xylose it corresponds to 1.3% of the pure xylan.

Methylation of Crude Xylan.

Crude xylan (15 g.) was dissolved in aqueous potassium hydroxide (300 ml.; 50% w/w) and dimethyl sulphate (150 ml.) added dropwise with vigorous stirring over a period of 6 hours. The mixture was stirred overnight and then heated to 95°C for one hour. After cooling, the mixture was treated with a further quantity of potassium hydroxide solution (200 ml.) followed by dimethyl sulphate (100 ml.) as before. After four such methylations acetone (200 ml.) was added to dissolve methylated xylan which had separated and this solvent was removed by distillation on the completion of the fifth methylation. The mixture was then cooled and treated with sulphuric acid (0.5N) until it was faintly alkaline (pH 8). On boiling with water the precipitated sodium sulphate dissolved and the methylated xylan separated. The product was filtered hot and washed with hot water. The aqueous solution and washings were extracted with chloroform, the solvent was removed and the product added to the main bulk. The partly methylated xylan was dissolved in aqueous/

aqueous acetone (80%, v/v) and subjected to a second series of five methylations to give a methylated product. Yield: 10 g. (Found: OMe, 31.1%). A further methylation yielded a methylated crude xylan. Yield: 8.5 g. (Found: OMe, 35.9%, $[\alpha]_D^{20}$, -83° , c, 1.4 in chloroform).

The product was fractionated using 200 mls. of a mixture of purified light petroleum (b.p., 60-67°C) and chloroform (Analar Reagent), the amount of the latter solvent being increased in stages. The light petroleum was purified as follows:

Light petroleum (b.p. 60-80°C; 1 l.) was shaken with concentrated sulphuric acid (500 ml.) for 16 hours at room temperature. The sulphuric acid was run off from a separating funnel and the petroleum washed with water, then with sodium bicarbonate solution and finally twice more with water. After drying the petroleum for two hours over calcium chloride it was fractionally distilled the fraction boiling between 60°C and 67°C being collected.

For each extraction the mixture was boiled gently in the water bath for 2 hours and the undissolved material removed by filtration. In each case the insoluble residue was again extracted with an equal volume of solvent of the same composition. The solvent was removed under reduced pressure and the resultant solid dried in vacuo over phosphoric oxide. The fractions obtained are tabulated below in Table II.

TABLE II.

Fraction	Solvent, Chloroform/ Light Petroleum	Yield (weight)	Yield %
1.	0/100	0.025 g.	0.30
2.	10/90	0.075 g.	0.92
3.	15/85	0.115 g.	1.40
4.	20/80	0.237 g.	2.80
5.	25/75	0.785 g.	9.55
6.	30/70	1.931 g.	23.5
7.	35/65	3.037 g.	36.9
8.	40/60	0.308 g.	3.72
9.	Residue	1.720 g.	20.9

Some of the above fractions were hydrolysed as follows:

The polysaccharide (50 mg.) was hydrolysed in a sealed tube at 100°C for 8 hours with methanolic hydrogen chloride (3 ml.; 1%). At the end of this time the solvents were removed under reduced pressure and the resultant syrup hydrolysed under reflux at 100°C for 6 hours with hydrochloric acid (5 mls.; 0.5N). The solution was neutralised with silver carbonate, insoluble silver salts removed at the centrifuge and the dissolved silver salts removed by passing hydrogen sulphide gas into the solution. After removal of the silver sulphide at the centrifuge the solution was deionised by shaking with/

with the ion exchange resins Amberlite IR 100H and IR 4B. The solution was evaporated to a small volume under reduced pressure and examined chromatographically using butanol/ethanol/water/ammonia (40/10/49/1) as solvent for 24 hours.

The results of the above hydrolyses are shown below (Table IV). The estimations are based on a comparison of the intensities and areas of the colour spots formed on a paper chromatogram using aniline oxalate as the spray reagent.

In order to distinguish between 2:4-dimethyl xylose and 2:3-dimethyl arabinose, two sugars of approximately the same RG value, a series of preliminary tests was carried out using the paper chromatography spray reagents dimethyl aniline and asym. dimethyl p-phenylene diamine (59). For the first reagent a 2% solution in glacial acetic acid containing 5% trichloroacetic acid was used. In the second case the reagent was dissolved in methylated spirit and an equal volume of water and 2% trichloroacetic acid added. After spraying with the above reagents the papers were dried in an oven at 105°C for approximately 5 minutes. The results are tabulated below (Table III):-

Table III/

TABLE III.

Sugar	Colour with dimethyl-aniline	Colour with <u>asym.</u> dimethyl p-phenylene diamine
2:3-Dimethyl arabinose	purple	grey
2:4-Dimethyl xylose	pale brown	orange
2:3-Dimethyl xylose	purple	not tested
2:3:4-Trimethyl xylose	purple	orange
2:3:5-Trimethyl arabinose	purple	grey

From the above results it can be seen that it is possible to detect by means of asym.dimethyl p-phenylene diamine the presence of trimethyl arabinose in a mixture of the trimethyl arabinose and xylose. It is also possible to distinguish 2:4-dimethyl xylose from the corresponding 2:3-dimethyl arabinose.

TABLE IV.

Fraction	Monomethyl Pentoses (Rg 0.38)	2:3-Dimethyl Xylose (Rg 0.74)	2:3-Dimethyl Arabinose (Rg 0.64)	Trimethyl Pentoses (Rg 0.95)
1	Present	Present	None	None
2	4	16	Trace	Trace
3	4	20	Trace	1
4	4	16	Trace	1
5	5	10	None	1
6	6	12	None	1

It can be seen from the above figures that no significant fractionation of the methylated polysaccharides/



polysaccharides has taken place and so a further attempt was made to fractionate the parent crude xylan.

Partial Fractionation of Crude Xylan.

a) Crude xylan (5 g.) was extracted with aqueous methylated spirit (500 ml.; 70%, v/v) in a boiling water bath for 20 hours at the end of which time the undissolved material was removed by filtration without previous cooling and the filtrate used to extract a further 5 g. of polysaccharide. This process was repeated until 7 batches of crude xylan each of 5 g. weight had been extracted with the same batch of solvent. The final filtrate was evaporated to a small volume under reduced pressure and acidified with acetic acid and poured into acetone. A small amount of a sticky dark brown material settled out. This was removed at the centrifuge, washed with acetone/alcohol and the precipitate hardened by allowing it to stand overnight in alcohol. Finally the precipitate was washed successively with absolute alcohol and ether and dried in vacuo over phosphoric oxide.

The polysaccharide was hydrolysed as before using 2.5% nitric acid for 3 hours in a sealed tube at 100°C. Paper chromatography showed that the ratio of xylose/arabinose in the hydrolysate was approximately 3/1 but that the total carbohydrate present was very small (0.1 g.).

b)/

b) Crude xylan (30 g.) was extracted with aqueous methylated spirit (1 l.; 70%, v/v) in a boiling water bath for 20 hours. The undissolved material was removed by filtration and the filtrate evaporated to a small volume under reduced pressure, acidified with acetic acid and poured into acetone. The precipitate was washed and dried as before. This process was repeated four times using each time a fresh volume of aqueous methylated spirit to extract the insoluble residue from the previous extraction. The fourth and fifth extracts showed a slight opalescence on filtering and so the filtrate was made up to 85% (v/v) with respect to the methylated spirit and allowed to stand overnight in an attempt to precipitate the suspension. No change occurred and so the colloidal solution was evaporated to dryness as before.

The first extract was small and similar in character to that in a) above. The second extract was both cleaner and larger while the third was the largest (1.24 g.). The fourth and fifth extracts were considerably smaller in size.

A few milligrams of each extract were hydrolysed and the hydrolysate examined on a paper chromatogram. The results of the hydrolyses are shown in Table V below. A hydrolysis of the final insoluble residues still showed the presence of arabinose.

TABLE V./

TABLE V.

Extract	Arabinose	Xylose
1	Spots too weak to compare sugars	
2	1	2
3	1	2
4	1	4
5	1	5

The third extract above (extract 1) was further examined. Yield: 1.24 g., $[\alpha]_D^{20}, -21^\circ$.

Quantitative paper chromatography showed the ratio of arabinose:xylose to be 35:65.

c) Crude xylan (30 g.) was extracted 3 times with aqueous methylated spirit (1 l., 70%, v/v) as in b) above and the insoluble residue was then extracted with hot aqueous methylated spirit (1 l.; 85% v/v) for 20 hours. The extract was precipitated, washed and dried as before to yield in small quantity a polysaccharide (found: $[\alpha]_D^{20}, -23^\circ$) containing a high proportion of arabinose. Quantitative chromatography showed the ratio of arabinose/xylose to be 53/47.

The first and second extracts of b) above and the 70% aqueous methylated spirit extracts of c) above were combined to give extract II (Yield: 1.60 g. found: $[\alpha]_D^{20}, -32.2^\circ$). This was dissolved in a small/

small volume of water (20 ml.). To 1.5 ml. of this solution was added absolute alcohol (4.5 ml.). The precipitate (91.8 mg.) which separated was removed at the centrifuge and the centrifugate evaporated to dryness under reduced pressure to yield a second fraction (42.6 mg.). Both of these fractions were hydrolysed as usual and the hydrolysate examined quantitatively on a paper chromatograph. The first fraction was found to have an arabinose:xylose ratio equal to 39:61 while in the second fraction the ratio was 50:50.

Other attempts at fractionation using both smaller and larger quantities of alcohol failed, smaller volumes causing no precipitation while large volumes caused almost complete precipitation.

Preliminary Methylation Studies on a "Crude Araban".

The extracts I and II together with the 85% aqueous methylated spirit extract obtained above (total weight, 3 g.) were dissolved in water (25 ml.) and saturated aqueous thallos hydroxide (approximately 3 equivalents) added (39). The mixture was evaporated to dryness at 50°C with the exclusion of carbon dioxide. The resultant solid was powdered and methyl iodide added. After heating at 45°C for 32 hours with the exclusion of light and moisture the methyl iodide was removed and the residue extracted exhaustively with dry methanol. After six such extractions 1.8 g. of methylated material had been/

been removed and the residue was extracted with water yielding a further amount of solid which has not been examined but is assumed to comprise a very incompletely methylated polysaccharide. The methanol soluble fraction was dissolved in a mixture of benzene (3 parts) and ethanol (1 part) and remethylated by adding thalious ethoxide ($2\frac{1}{2}$ equivalents), evaporating the mixture to dryness, powdering the resultant solid and heating at 45°C for a further 32 hours with methyl iodide. The methyl iodide was removed and the methylated material extracted six times with dry methanol (yield: 1.5 g.). This was methylated twice more by the method of Purdie and Irvine (60) and extracted with chloroform (yield: 1.3 g.; OMe, 31.1%). A further Purdie methylation raised the methoxyl content to 34.2% but after a fourth treatment the figure dropped to 33.4% (yield: 1.3 g.).

Attempts were made to fractionate this material from chloroform solution by means of petroleum ether but no proper precipitation occurred. The material was shaken with acetone (Analar Reagent) and a small insoluble residue removed by filtration. On addition of light petroleum to the acetone solution a sticky brown material collected at the bottom of the flask. This was removed at the centrifuge, redissolved in chloroform and the chloroform solution evaporated to dryness under reduced pressure to yield a crisp brown glass (yield: 0.9 g.; OMe, 34.6%). The petroleum solution on evaporation yielded a viscid syrup (OMe, 29%).

The/

The solid fraction was dissolved in benzene (100 ml.), thallos ethoxide ($2\frac{1}{2}$ equivalents) was added and the solution evaporated to dryness at 50°C with the exclusion of carbon dioxide. Methyl iodide was added and the mixture heated at 45°C for 32 hours. The methyl iodide was removed and the residual solid extracted with hot methanol three times and hot chloroform twice after which no further material could be extracted. Evaporation of the combined methanol and chloroform extracts yielded a crisp brown glass (yield: 0.85 g.; OMe, 34.2%).

This product was dissolved in hot methanol (100 ml.). The solution on cooling deposited a flocculent white precipitate which was removed at the centrifuge and dried in vacuo (yield: 0.188 g., OMe 34.2%). This had $[\alpha]_{\text{D}}^{16} -69.5^{\circ}$ (c 0.5 in chloroform). The methanolic mother liquor was evaporated to dryness and the resultant glass dried in vacuo (yield: 0.648 g.; OMe 34.0%). This had $[\alpha]_{\text{D}}^{16} -70^{\circ}$ (c 0.6 in chloroform).

These two products were combined and hydrolysed in a sealed tube at 100°C for 8 hours with methanolic hydrogen chloride (25 ml., 1%). The latter was removed at the pump and the resultant solid hydrolysed for 6 hours at 100°C with hydrochloric acid (30 ml., 0.5N). The hydrochloric acid was neutralised with silver carbonate and the solution filtered. The dissolved silver salts were removed by passing hydrogen sulphide gas into the solution and removing the silver sulphide formed at the centrifuge. Finally the solution was de-ionised/

deionised with Amberlite resins IR-100H and IR-4B, and evaporated to a syrup under reduced pressure.

A small part of the hydrolysate was examined on a paper chromatogram using butanol/ethanol/water/ammonia (40/10/49/1) as solvent. A trimethyl pentose (R_g 0.95), 2:3-dimethyl xylose, 2:3-dimethyl arabinose, and a monomethyl pentose, as well as several methylated hexoses were detected.

The hydrolysate was fractionated on a column of cellulose (64 cms. x 2.9 cms.) using light petroleum/butanol (70/30) saturated with water, as eluent, the proportion of butanol in the mixture being gradually increased as the sugars were removed. Finally after all the methylated sugars had passed through the column the solvent was changed to water. This removed what appeared to be a trace of a methylated uronic acid and also a small quantity of free sugars.

Eighteen fractions were obtained but most of these were too small to be examined by any means other than paper chromatography. This method indicated the results tabulated below (Table V). The colour noted is that shown on a paper chromatogram using aniline oxalate as developing agent, while the R_g value was obtained by calculation from the nearest available standard sugar of known R_g value. The solvent used in all cases was butanol/ethanol/water/ammonia (40/10/49/1).

Table V/

TABLE V.

<u>Frac- tion</u>	<u>Colour</u>	<u>Rg value</u>	<u>Possible sugar</u>
1	pink	0.95	2:3:4-trimethyl xylose 2:3:5-trimethyl arabinose
2	pink	0.95	"
	red	0.89	?
3	red	0.89	?
4	red	0.89	?
	red/brown	0.84	methylated hexose
5	red/brown	0.84	"
6	red/brown	0.84	"
	pink	0.79	?
7	pink	0.79	?
	pink	0.74	2:3-dimethyl xylose
8	pink	0.74	"
9	pink	0.74	"
	brown	0.68	?
10	brown	0.68	?
	pink	0.63	2:3-dimethyl arabinose 2:4-dimethyl xylose
11	pink	0.63	"
12	pink	0.63	"
	brown	0.47	methylated hexose
13	brown	0.47	"
14	brown	0.47	"
	pink	0.38	monomethyl pentose
15	pink	0.38	"
16	pink	0.38	"
	brown	0.36	dimethyl hexose

TABLE V (cont'd)

<u>Frac- tion</u>	<u>Colour</u>	<u>Rg value</u>	<u>Possible sugar</u>
17	brown	0.36	dimethyl hexose
18	residue containing free sugars and trace of methylated uronic acid.		

Further Examination of Sugar Fractions.

Examination of Fraction 1.

The syrup (12.5 mg.) had $[\alpha]_D^{16} -27^\circ$ (c, 0.3 in water), and showed on a paper chromatogram the presence of a sugar (Rg 0.95) corresponding to 2:3:4-trimethyl xylose on the same paper. Development of the chromatogram with asym. dimethyl p-phenylene diamine showed that 2:3:5-trimethyl arabinose was present (grey spot).

Examination of Fraction 8.

The syrup (18 mg.) had $[\alpha]_D^{16} +27^\circ$ (c 0.9 in water), and showed the presence of only one sugar (Rg 0.74) corresponding to 2:3-dimethyl xylose when examined chromatographically.

Anilide Formation. The syrup (15 mg.) was dissolved in ethanol (1 ml.) and freshly distilled aniline (15 mg.) added. The mixture was boiled under reflux in a hot water bath for two hours. The alcohol was removed by distillation and the syrup dried in a vacuum desiccator overnight. Inoculation with a crystal of the anilide of 2:3-dimethyl xylose caused complete crystallisation in three/

three days. The crystals were washed with cold dry ether and recrystallised from ethyl acetate containing some low boiling petroleum (b.p. 40-60°C). Three such recrystallisations raised the melting point to 139-142°C, alone or admixed with an authentic specimen of the anilide of 2:3-dimethyl xylose (m.p. 142°C).

Examination of Fraction 11.

The syrup (3.1 mg.) had $[\alpha]_D^{16} +71^\circ$ (c 0.15 in water) and showed the presence of only one sugar (Rg 0.63). This was distinguished as 2:3-dimethyl arabinose by spraying a chromatogram paper with asym. dimethyl p-phenylene diamine a grey colour being obtained as opposed to the orange colour shown by 2:4-dimethyl xylose on the same paper.

PART TWO.THE POLYSACCHARIDE A.Preparation of Polysaccharide A.

Esparto grass (3 kgs.) was treated as before to yield a crude xylan (300 g.). This was extracted in batches of 30 g. by means of 4 successive treatments with boiling aqueous methylated spirit (70%, v/v 1 l.) for 20 hours, the undissolved material removed by filtration from the hot solution. The filtrate was evaporated to a small volume under diminished pressure, acidified with acetic acid and poured into acetone (10 vols.). The precipitate was washed successively with absolute alcohol and ether and dried in vacuo over phosphoric oxide to yield a fawn powder (A) (yield: 35 g., ash content: 3.5%).

Hydrolysis of Polysaccharide A.

A small quantity of this material (0.915 g., dry weight) was hydrolysed with sulphuric acid (N) for 4 hours in a sealed tube at 100°C. A dark brown residue remained unhydrolysed and was removed by filtration through a sintered glass funnel (4 x G). This was washed thoroughly with water and dried in a vacuum desiccator over phosphoric oxide: dry weight, 0.1028 g. This corresponds to 11.3% of the polysaccharide.

The filtrate was neutralised with barium carbonate and dry recrystallised rhamnose hydrate (0.2652 g.) added./

added. After removal of the barium salts by filtration the solution was deionised with the Amberlite ion exchange resins IR 100H and IR 4B. Qualitative chromatography showed the presence of galactose, glucose, arabinose and xylose in addition to the added rhamnose. The sugars were separated on a paper chromatogram (100 cms. x 13 cms.) using as solvent, butanol/benzene/pyridine/water (50/10/30/30) for 100 hours. Side strips were cut as before and developed with aniline oxalate. Strips corresponding to the sugars and also a paper blank were cut out and the solvent removed in a vacuum desiccator over water overnight. Each sugar was removed from the corresponding strip by elution with cold water (5 mls.) into a QQ boiling tube. Sodium metaperiodate solution (1 ml., 0.3M) was added and the tube securely stoppered. The lower part of the tube was then heated in a boiling water bath for 20 minutes the upper part being cooled by a spiral condenser. At the end of this time the tube was cooled thoroughly and pure ethylene glycol (0.3 ml.) added to destroy excess periodate and the formic acid released titrated with sodium hydroxide solution (0.01 N) using as indicator screened methyl red/methylene blue. Under these conditions pentoses will react to yield 4 moles of formic acid while hexoses will give 5 moles of the acid. The following titration figures were obtained:-

Sugar/

<u>Sugar</u>	<u>Titre (Mls.)</u>	<u>Percentage of sugar</u>
		<u>in dry polysaccharide</u> <u>A.</u>
Galactose	0.106	2.2%
Glucose	0.123	2.5%
Arabinose	0.694	11.7%
Xylose	1.510	25.6%
Rhamnose hydrate	1.416	_____
Total ..		<u>42.0%</u>

Owing to the low total percentage of sugar found to be present in A the above estimation was repeated using polysaccharide A (dry weight, 0.5314 g.) and dry re-crystallised rhamnose hydrate (0.0969 g.). Determinations were carried out on two papers to give the following results:-

<u>Sugar</u>	<u>Titre (mls.)</u>		<u>Percentage of sugar in</u> <u>dry polysaccharide A</u>		
	I	II	I	II	Average
Galactose	0.336	0.266	2.5%	1.9%	2.2%
Glucose	0.330	0.402	2.4%	2.9%	2.6%
Arabinose	1.464	1.688	11.1%	12.6%	11.8%
Xylose	3.750	3.532	28.5%	26.5%	27.5%
Rhamnose hydrate	1.956	1.998	_____	_____	_____
Total ..			<u>44.9%</u>	<u>44.3%</u>	<u>44.1%</u>

Moisture Content of the Polysaccharide A.

The polysaccharide rapidly absorbed moisture and after/

after three weeks the moisture content was found to be 13.1%. This was determined by drying a weighed sample of the material in vacuo over phosphoric oxide for 16 hours, and weighing the sample to constant weight.

After six months the moisture content had increased to 15%.

Periodate Oxidation of the Polysaccharide A.

Periodate Uptake.

The polysaccharide (0.3690 g.) was dissolved in water and the solution made up to 50 ml. To this was added sodium metaperiodate (2.6946 g., 97% pure). The mixture was shaken in a dark bottle at room temperature and 5 ml. samples withdrawn at intervals. The samples were diluted with water, excess solid sodium bicarbonate added followed by sodium arsenite (25 ml.; 0.1 N) and excess solid potassium iodide. After 15 minutes the solution was titrated with iodine (0.0991 N).

A similar estimation was carried out on 25 ml. of the sodium arsenite solution together with the sodium bicarbonate and potassium iodide to give a blank value from which the initial titre could be calculated.

Results:-

Blank value: 25.98 ml.

Calculated initial titre/50 ml.: 16.1 ml.

Time/

<u>Time</u> (days)	<u>Titre/50 ml.</u>	<u>Periodate uptake (mols./ anhydropentose unit.)</u>
0	16.1	0
2	77.2	3.08
3	81.4	3.29
4	82.0	3.32
5	82.8	3.36

The figure for the periodate uptake is based on the figure of 40% for the total pentose present.

Formic Acid Release from Polysaccharide A.

The polysaccharide (0.423 g.) was dissolved in water (100 ml.), sodium periodate solution (25 ml.; 0.25 M) and potassium chloride (5 g.) added. The mixture was shaken in a dark bottle and 10 ml. samples withdrawn at intervals. Excess sodium periodate was destroyed by the addition of ethylene glycol and the formic acid liberated titrated with sodium hydroxide (0.0104 N), using screened methyl red/methylene blue as indicator.

The polysaccharide itself was found to be neutral to this indicator, as were the reagents.

A really satisfactory end point could not be obtained in the formic acid titrations using either the indicator specified above or ordinary methyl red. The figures given below correspond to a point midway between the beginning and end of the colour change which occurred/

occurred over a range of ca 5 ml./125 ml. of the oxidation mixture.

Results:-

<u>Time (days)</u>	<u>Titre/125 ml.</u>	<u>Formic acid release (mols./anhydro pentose unit.)</u>
1	23.5	0.216
3	28.2	0.259
5	32.1	0.295
7	38.6	0.355
9	40.4	0.372
12	41.2	0.380
14	41.5	0.382

Therefore one mole of formic acid is released for every two and a half anhydro pentose units. This is based on the figure of 40% for the total pentose present in the polysaccharide A.

Hydrolysis of the Oxidised Polysaccharide A.

The polysaccharide (1.0885 g.) was dissolved in water (75 ml.) and sodium metaperiodate (4.025 g.) added. The mixture was shaken in a dark bottle for seven days at the end of which time some insoluble inorganic material was removed by filtration. The solution was neutralised with barium carbonate to remove formic acid and excess periodate. The iodic acid was removed as in the oxidation of crude xylan and the oxidised polysaccharide/

polysaccharide hydrolysed with sulphuric acid (0.5N) at 100°C for 4 hours. Quantitative paper chromatography showed the arabinose present to be 1.88% of the original polysaccharide A, while the xylose amounted to 9.1% of A.

The arabinose unattacked therefore is 15.9% of the original arabinose, and the xylose is 33.1% of the original xylose.

Methylation of the Polysaccharide A.

The polysaccharide A (30 g.) was dissolved in water (150 ml.), thallos hydroxide (3 equivalents) added and the mixture evaporated to dryness with the exclusion of carbon dioxide. The resultant solid was powdered and methyl iodide (250 g.) added. After heating at 45°C for 32 hours with the exclusion of light and moisture the methyl iodide was removed and the resultant material extracted with dry methanol. Two such extractions gave only 4 g. of soluble polysaccharide material while a further two extractions had no effect. The thallos iodide mixture was therefore extracted twice with hot water and a further three equivalents of thallos hydroxide added and the mixture treated as before. Exhaustive extraction (9 times) with dry methanol yielded a dark brown syrup (total weight combined with previous methanol extractions above 29 g.). This syrup was dissolved in benzene/ethanol (50/50) and thallos ethoxide (2½ equivalents) added. After evaporation to dryness again with the exclusion of carbon dioxide the mixture was treated with/

with methyl iodide as before. Exhaustive extraction with methanol yielded a brown syrup (28 g.; O Me, 28.0%). This was dissolved in benzene, a fresh quantity of thallos ethoxide added, and the mixture treated as before. Exhaustive extraction yielded a brown syrup (28 g.; O Me, 30.2%).

This material was dissolved in methyl iodide and subjected to two Purdie methylations in the usual way. Extraction of the resultant material with chloroform yielded a golden brown syrup (16 g.; O Me, 34.4%). A further Purdie methylation raised the methoxyl content to 36.2% while yet another raised it to 36.4%. Two further methylations failed to raise the methoxyl content any higher.

The resultant methylated polysaccharide was purified by dissolving it in chloroform and removing insoluble silver salts at the centrifuge. The solution was then evaporated to dryness and the process repeated. The solution was finally evaporated to dryness to yield a crisp brown glass (9.33 g.; O Me, 36.6%).

This material was dissolved in hot acetone (100 ml., Analar Reagent). On cooling the solution a flocculent white precipitate formed. This was removed by filtration through glass wool and after drying in vacuo yielded a pale yellow material AMI (0.35 g.; O Me, 35%). The acetone solution on evaporation to dryness yielded a crisp brown glass, (AM II). Found: O Me, 36.6%; Ash, 0.1%).

Hydrolysis/

Hydrolysis of the Methylated Polysaccharide. (AM II).

The methylated polysaccharide (7.49 g.) was heated in a boiling water bath with methanolic hydrogen chloride (380 ml.; 1%). Portions of the solution were withdrawn at intervals, cooled and undissolved material removed at the centrifuge. The following optical rotations were observed: $[\alpha]_D^{16} +30.4^\circ$ (4 hours); $+32.5^\circ$ (8 hours); $+36.6^\circ$ (10 hours); $+38.6^\circ$ (12 hours, constant). At the end of this time the methanolic hydrogen chloride was removed under reduced pressure and the resultant syrup hydrolysed with hydrochloric acid (500 ml.; 0.5 N) and the hydrolysis followed as before, the optical rotations being $[\alpha]_D^{16} +83.0^\circ$ (3 hours); $+68.4^\circ$ (6 hours); $+64.2^\circ$ (8 hours, constant).

The unhydrolysed sticky brown residue (0.417 g., found: OMe, 18.0%) was removed on the centrifuge and the clear solution neutralised with silver carbonate. After removing undissolved silver salts by filtration soluble silver salts were removed by passing hydrogen sulphide gas into the solution and subsequent filtration of the silver sulphide formed. Finally the solution was completely deionised by means of Amberlite ion exchange resins.

Examination of the resultant solution on a paper chromatogram showed the presence of a trimethyl pentose (RG 0.95), 2:3-dimethyl xylose, 2:3-dimethyl arabinose and a monomethyl pentose (RG 0.38). In addition a small amount of free pentose could be detected.

The/

The solution was evaporated to give an almost colourless syrup. 5.5 g. of this syrup was examined chromatographically by means of a cellulose column.

Examination of the Methylated Sugar Fractions.

Separation of the Free Sugars on a Cellulose Column.

The syrupy hydrolysate (5.5 g.) from AM II was fractionated on a column of cellulose (90 cm. x 3.4 cm.) according to the method of Hough, Jones and Wadman (60). The solvents used were purified light petroleum (b.p. 100-120°C) and purified butanol. The petroleum was purified by shaking with concentrated sulphuric acid overnight. It was then washed once with water, once with saturated sodium bicarbonate solution, and finally twice with water. The petroleum was distilled without previous drying and the fraction distilling between 100° and 120° used.

The butanol was purified by boiling it (1 l.) under reflux over sodium hydroxide pellets (10 g.) for four hours. Direct distillation yielded a pure butanol (b.p. 115-118°C).

The cellulose column was washed with water (500 ml.) butanol (500 ml.) and finally with petroleum/butanol (70/30, v/v, 1 l.). The syrup was dissolved in the minimum quantity of this solvent and the solution added dropwise to the top of the column. When all the solution had soaked into the cellulose a reservoir containing petroleum/butanol (70/30) saturated with water was inverted/

inverted on the top of the column.

The eluate was collected by means of an automatic turntable in tubes containing 5-6 ml. each. The contents of every tenth tube were evaporated to dryness and the hydrolysate examined by means of paper chromatography. All the tubes containing the same sugars were combined and the solvent evaporated. The resultant syrup was purified by solution in water and filtration through a bed of filter cel. After evaporation of the water the residue was exhaustively extracted with boiling acetone (except where otherwise stated), the solution evaporated and the syrup dried in vacuo over phosphoric oxide.

Table VII shows the amount of each fraction after purification and the results obtained by examination of the fraction on a paper chromatogram using butanol/benzene/pyridine/water (50/10/30/30) as solvent. The R_g value was determined by reference to the nearest available known sugar while the colour is that obtained on development of the chromatogram with aniline oxalate.

TABLE VI.

<u>Frac- tion</u>	<u>Weight</u>	<u>Colour</u>	<u>R_g Value</u>	<u>Possible Sugar</u>
a	0.0531 g.	Grey/ Pink	0.95	2:3:5-trimethyl arabinose
b	0.2651 mg.	Grey/ Pink	0.95	"
c	0.3075 g.	Pink	0.95	2:3:5-trimethyl arabinose 2:3:4-trimethyl xylose
d/				

TABLE VI (cont'd)

<u>Frac- tion</u>	<u>Weight</u>	<u>Colour</u>	<u>Rg Value</u>	<u>Possible Sugar</u>
d	0.0228 g.	Pink	0.95	2:3:5-trimethyl arabinose 2:3:4-trimethyl xylose
		Red/ Brown	0.88	2:3:4:6 tetra- methyl galactose
e	0.0556 g.	Red/ Brown	0.88	"
f	0.1160 g.	Red/ Brown	0.88	"
		Red/ Brown	0.82	?
		Pink	0.74	2:3-dimethyl xylose
g	0.0681 g.	Red/ Brown	0.82	?
		Pink	0.74	2:3-dimethyl xylose
h	1.907 g.	Pink	0.74	"
i	0.0629 g.	Pink	0.74	"
		Red/ Brown	0.70	Methylated hexose
j	0.0657 g.	Pink	0.74	2:3-dimethyl xylose
		Red/ Brown	0.70	Methylated hexose
k	0.209 g.	Red/ Brown	0.70	2:4:6-trimethyl galactose
		Pink	0.64	2:3-dimethyl arabinose
l	0.1032 g.	Pink	0.64	"
		Pink	0.38	2-methyl xylose
m	0.5586 g.	Pink	0.38	"
n/				

TABLE VI (cont'd)

<u>Frac- tion</u>	<u>Weight</u>	<u>Colour</u>	<u>R_G Value</u>	<u>Possible Sugar</u>
n	0.0434 g.	Pink	0.38	2-methyl xylose
		Red	0.36	2:4-dimethyl galactose
o	0.1188 g.	Red	0.36	"
p	0.0498 g.	Red	0.36	"
		Pink	0.30	monomethyl pentose
q	0.0174 g.	Red	0.36	2:4-dimethyl galactose
		Pink	0.30	monomethyl pentose
		Red/ Brown	0.25	dimethyl or mon- omethyl hexose
r	0.104 g.	Pink	0.18	xylose
		Pink	0.15	arabinose
Total	4.1280 g.			

The contents of every tenth tube were used for preliminary examination of the eluate. This accounts for 0.458 g. of the hydrolysate. The total recovery is therefore 4.586 g. or 83.5%.

Examination of Fraction a.

The syrup (53.1 mg.) showed on a paper chromatogram a grey/pink spot (R_G 0.95) corresponding to 2:3:4-trimethyl xylose and a very slight trace of a hexose (R_G 1.00) which is most probably 2:3:4:6-tetramethyl glucose./

glucose.

Identification of 2:3:5-Trimethyl L-Arabinose in Fraction b.

The syrup (0.2651 g.) had $[\alpha]_D^{16} -26.8^\circ$ and showed on a paper chromatogram the presence of a sugar (R_f 0.95) corresponding to 2:3:4-trimethyl xylose on the same paper.

Lactone formation. The syrup was dissolved in water (5 ml.) and oxidised with liquid bromine for 7 days. At the end of this time a portion of the solution was withdrawn and the bromine removed by aeration. Fehling's solution showed the presence of a reducing sugar. The remaining solution was therefore heated at 60°C for 4 hours. It was then neutralised with silver carbonate, filtered and the free acid obtained by passing hydrogen sulphide gas through the solution for 15 minutes. The silver sulphide was removed by filtration and the solution evaporated to dryness. The syrup was dissolved in acetone, undissolved silver salts removed by filtration and the acetone evaporated. The resultant syrup was heated at 100°C for 2 hours and fractionally distilled to yield a syrupy distillate: 53 mg., b.p. 110-115°C (bath temperature)/0.20 mm.

Amide formation. The lactone was dissolved in methanolic ammonia at 0°C and kept at that temperature for 4 days. The solvent was removed in a vacuum desiccator to give the syrupy amide which was inoculated with a crystal/

crystal of 2:3:5-trimethyl arabinamide and left at 0°C. Partial crystallisation occurred after 3 weeks and the crystals were separated by tiling. Washing with cold ethyl acetate yielded crystalline needles m.p. 134°-136°C alone or admixed with an authentic specimen of 2:3:5-trimethyl arabinamide (m.p. 139°).

Demethylation. The syrup (10 mg.) was treated with hydrobromic acid (48%, 1 ml.) in a sealed tube at 100°C for 7 minutes. After cooling, the solution was diluted to 10 ml. with water and the acid neutralised with silver carbonate. Insoluble silver salts were removed by the addition of animal charcoal and subsequent filtration through a charcoal bed. Dissolved silver salts were precipitated by passing hydrogen sulphide gas into the solution and removed by subsequent filtration. The solution was finally deionised by means of Amberlite ion exchange resins. Excess water was evaporated under reduced pressure and the syrup examined chromatographically. The presence of arabinose (in quantity) and of traces of xylose and glucose was detected.

Identification of 2:3:5-Trimethyl L-Arabinose in Fraction c.

The syrup (307.5 mg.) had $[\alpha]_D^{16} -18.3$ and showed on a paper chromatogram the presence of a sugar ($R_G 0.95$) corresponding to 2:3:4-trimethyl xylose. Comparison of this fraction with fraction b on a paper chromatogram showed fraction c to be more closely allied to trimethyl xylose as evidenced by the colour obtained with aniline oxalate.

Demethylation. The syrup (10 mg.) was demethylated with 48% hydrobromic acid in a sealed tube at 100°C for 7 minutes and the resultant solution treated as before. Arabinose was found to be present in quantity, along with a small amount of xylose.

Lactone formation. The syrup (ca 100 mg.) was dissolved in water (5 ml.) and oxidised with liquid bromine as before. Subsequent treatment yielded a syrup which distilled to give the lactone b.p. 115-125°C (bath temperature)/0.02 mm.

Amide formation. The lactone was dissolved in methanolic ammonia at 0°C and kept at that temperature for 4 days. Removal of the solvent in a vacuum dessicator and inoculation with a crystal of 2:3:5-trimethyl arabonamide failed to yield a crystallide amide after one month at 0°C. The syrup was therefore treated with liquid ammonia for 15 minutes. Removal of the ammonia by evaporation at room temperature yielded a syrup which crystallised to give the amide m.p. 135-136°C alone or admixed with an authentic specimen of 2:3:5-trimethyl arabonamide (m.p. 139°).

Anilide formation. The syrup (ca 100 mg.) was dissolved in ethanol (5 ml.) and freshly distilled aniline (100 mg.) added. The mixture was heated under reflux on the water bath for two hours. The ethanol was removed by distillation and the syrup dried in a vacuum desiccator to yield a dark brown residue. This failed to crystallise after/

after 4 weeks at 0°C.

Examination of Fraction d.

The syrup (22.8 mg.) had $[\alpha]_D^{16} + 23^\circ$. Paper chromatography showed the presence of two sugars in approximately equal proportions. The first (R_G 0.95) corresponded to fraction c above while the second (R_G 0.88) gave a red/brown colour with aniline oxalate. This latter sugar corresponded to 2:3:4:6-tetramethyl galactose on the same paper.

Identification of Fraction e as 2:3:4:6-Tetramethyl D-Galactose.

The syrup (55.6 mg.) had $[\alpha]_D^{16} + 79.2^\circ$. Paper chromatography showed that only one sugar was present (R_G 0.88) corresponding to 2:3:4:6-tetramethyl galactose. The Anilide formation. The syrup (55 mg.) was treated with a solution of freshly distilled aniline (50 mg.) in ethanol (2 ml.). The mixture was heated under reflux on the water bath for 30 minutes. On cooling a crystalline solid separated. Recrystallisation from ethanol yielded crystals m.p. 194-195°C alone or on admixture with an authentic specimen of the anilide of 2:3:4:6-tetramethyl galactose (m.p. 195°C).

Examination of Fraction f.

The syrup (116.0 mg.) had $[\alpha]_D^{16} - 18.2^\circ$. Paper chromatography showed the presence of three sugars. Aniline oxalate gave a red/brown colour with the first (R_G 0.88)/

(R_G 0.88) which corresponded to fraction e, the second was also red/brown (R_G 0.82) and comprised the larger portion of the fraction. Also present was a pink spot corresponding to 2:3-dimethyl xylose (R_G 0.74) on the same paper.

Demethylation. The syrup (10 mg.) was demethylated with 48% hydrobromic acid in a sealed tube at 100°C for 5 minutes and the resultant solution treated as before. Arabinose was found to be present in quantity along with traces of galactose and xylose.

Lactone formation. The syrup (100 mg.) was dissolved in water (5 ml.) and oxidised with liquid bromine as before to yield a syrup which distilled to give the lactone b.p. $115-125^\circ\text{C} / 0.02$ mm. This had $[\alpha]_D^{16} -8.5^\circ$ (initial value), -8.5° (18 hours).

Amide formation. The polarimetric solution was evaporated to dryness and the syrup redistilled to give the lactone which was treated with methanolic ammonia at 0°C for 4 days. After 2 months no crystallisation had taken place.

Examination of Fraction g.

The syrup (68.1 mg.) had $[\alpha]_D^{16} +30.8^\circ$. Paper chromatography showed the presence of a large amount of a sugar (R_G 0.74). This corresponded to 2:3-dimethyl xylose on the same paper. A small amount of sugar (R_G 0.82) which gave a red/brown colour with aniline oxalate/

oxalate was also present.

Identification of Fraction h as 2:3-Dimethyl D-Xylose.

The syrup (1.907 g.) had $[\alpha]_D^{16} +30.0^\circ$. Paper chromatography showed that only one sugar (R_f 0.74) was present. This gave a pink colour with aniline oxalate and corresponded to 2:3-dimethyl xylose on the same paper.

Anilide formation. The syrup (200 mg.) was treated with a solution of freshly distilled aniline (200 mg.) in commercial alcohol (5 ml.). The mixture was boiled under reflux in a hot water bath for two hours. On removal of the alcohol by distillation a crystalline solid appeared after 4 days at 0°C. Two re-crystallisations from benzene yielded needles m.p. 120-122°C alone or admixed with an authentic specimen of the anilide of 2:3-dimethyl xylose (m.p. 121-122°C).

Lactone formation. The syrup (0.5 g.) was dissolved in water (25 ml.) and oxidised with liquid bromine as before for 8 days at the end of which time the solution was non-reducing to Fehling's solution. Subsequent treatment yielded a syrup which distilled to give the lactone b.p. 160-180°C (bath temperature)/0.2 mm. A fair amount of charring and decomposition took place. The distillate had $[\alpha]_D^{16} 86^\circ$, c 1.2 in water.

Amide formation. The polarimetric solution was evaporated to dryness and redistilled to yield the lactone, b.p. 140-160°C (bath temperature)/0.1 mm. On treatment with/

with methanolic ammonia at 0°C for 4 days, and subsequent removal of the solvent in a vacuum dessicator the syrup yielded immediately a crystalline amide. This was twice recrystallised from absolute alcohol and gave an amide m.p. 131-132°C alone or on admixture with an authentic specimen of 2:3-dimethyl xylonamide (m.p. 131-132°C).

Examination of Fraction i.

The syrup (62.9 mg.) had $[\alpha]_D^{16} +46^\circ$ (c, 0.6 in water). Paper chromatography showed the presence of a pentose (R_G 0.74) corresponding to 2:3-dimethyl xylose and a hexose (R_G 0.70) in approximately equal proportions.

Demethylation. The syrup (10 mg.) was demethylated with 48% hydrobromic acid in a sealed tube at 100°C for 5 minutes and the resultant solution treated as before. Galactose and xylose were found to be present.

Anilide formation. The syrup (50 mg.) was treated with an equal quantity of freshly distilled aniline in commercial alcohol (2 ml.) and the mixture heated under reflux in a boiling water bath for 2 hours. The alcohol was removed to leave a very dark syrup. This was redissolved in ethyl acetate and some of the dark material which had remained undissolved was removed by filtration. On allowing the ethyl acetate to evaporate slowly a syrup which failed to crystallise was obtained.

Examination/

Examination of Fraction j.

The syrup (65.7 mg.) had $[\alpha]_D^{16} + 54.7^\circ$ (c, 0.6 in water). Paper chromatography showed that 3 sugars were present. These were identified as 2:3-dimethyl xylose (R_G 0.74) (small quantity), 2:3-dimethyl arabinose (R_G 0.64) (small quantity) and a hexose (R_G 0.70).

Identification of 2:4:6-Trimethyl D-Galactose and 2:3-dimethyl L-Arabinose in Fraction k.

The syrup (0.209 g.) had $[\alpha]_D^{16} + 85.2^\circ$ (c, 2.0 in water). Paper chromatography showed the presence of a hexose (R_G 0.70) and a pentose (R_G 0.64) in approximately equal proportions. This pentose did not correspond to 2:4-dimethyl xylose on the same paper and gave a grey colour with asym.dimethyl p-phenylene diamine.

Anilide formation. The syrup (50 mg.) was treated with a solution of freshly distilled aniline (50 mg.) in commercial alcohol (2 ml.) and heated under reflux in a boiling water bath for 2 hours. The solvent was removed by evaporation and the resultant syrup allowed to crystallise at 0°C for 2 weeks. At the end of this time the crystals were removed by tiling, washed thoroughly with cold dry ether. Recrystallisation from ethyl acetate yielded crystalline needles m.p. 165-166°C alone or on admixture with an authentic specimen of 2:4:6-trimethyl galactose anilide (m.p. 170°C). The washings and the residual syrup were combined and evaporated to dryness. After a week further crystallisation took place/

place, the crystals this time being small and cubic. These were washed with ether and recrystallised from ethyl acetate. The crystals melted slowly between 90° and 100°C , and were therefore impure.

Lactone formation. The syrup (100 mg.) was oxidised with liquid bromine in the usual way. Distillation of the resultant syrup yielded the lactone b.p. $110-120^{\circ}\text{C}$ (bath temperature)/0.05 mm.

Amide formation. The lactone was dissolved in methanolic ammonia at 0°C for 4 days. The solvent was removed in a vacuum desiccator. Crystallisation occurred immediately and slow recrystallisation from dry ethanol yielded crystalline needles m.p. $159-160^{\circ}\text{C}$ alone or admixed with an authentic specimen of 2:3-dimethyl arabanamide (m.p. 160°C).

Examination of Fraction l.

The syrup (0.1032 g.) showed the presence of a trace of a pentose corresponding to that in Fraction k above and also a pentose (R_G 0.38) corresponding to 2-methyl xylose on the same paper.

Identification of Fraction m as 2-Methyl Xylose.

The syrup (0.5586 g.) had $[\alpha]_D^{16} +34^{\circ}$ (c, 2.3 in water). On dissolving the syrup in acetone and evaporating slowly to dryness complete crystallisation took place. The crystals without further purification had m.p. $134-136^{\circ}\text{C}$ alone or admixed with an authentic specimen/

specimen of 2-methyl xylose (m.p. 136°C).

Anilide formation. The syrup (50 mg.) was heated under reflux in a boiling water bath with an alcoholic solution of freshly distilled aniline (50 mg.) for 2 hours. The alcohol was removed and complete crystallisation occurred at 0°C in 4 days. The dark crystalline mass was recrystallised from ethyl acetate to yield brown crystals m.p. 124-125°C alone or on admixture with an authentic specimen of the anilide of 2-methyl xylose (m.p. 130°C).

Lactone formation. The syrup (0.4 g.) was dissolved in water and oxidised with liquid bromine for 7 days.

Treatment as before yielded a syrup which distilled to give a pale yellow lactone b.p. 150-160°C (bath temperature)/0.1 mm. Yield: 0.104 g. This had $[\alpha]_D^{16} +86.5^\circ$ (initial value), 84.6° (18 hours), 82.7° (40 hours). Crystallisation of the lactone failed to take place in a week at 0°C.

Amide formation. The polarimetric solution was evaporated to dryness and the lactone obtained by heating the syrup at 100°C for 2 hours. Distillation yielded a yellow syrup b.p. 130-140°C (bath temperature)/0.02 mm. This was dissolved in methanolic ammonia for 4 days and the solution evaporated to dryness in a vacuum desiccator. No crystallisation took place in 2 months.

Examination of Fraction n.

Chromatographic examination of the syrup (43.4 mg.) showed the presence of 2-methyl xylose (R_G 0.38) and what/

what appeared to be another pentose (R_G 0.36) in approximately equal proportions.

Identification of Fraction o as 2:4-Dimethyl D-Galactose.

The syrup was purified twice from water. Yield: 0.1188 g. This had $[\alpha]_D^{16} +71.6^\circ$ (c, 1.2 in water). Complete crystallisation occurred in a few days at room temperature. The crystals had m.p. 93-96°C. Recrystallisation from acetone containing 1% water gave crystals with the same melting point. Admixture of this material with an authentic specimen of 3-methyl xylose caused depression of the melting point to below 80°C. Admixture with an authentic specimen of 2:4-dimethyl galactose hydrate (m.p. 108°C) caused no depression.

Demethylation. The fraction (10 mg.) was demethylated with 48% hydrobromic acid in a sealed tube at 100°C for 5 minutes and the resultant solution treated as before. Galactose was the only free sugar which could be detected in the solution.

Phenylhydrazone formation. The fraction (20 mg.) was treated with an alcoholic solution of phenylhydrazine at boiling point for 2 minutes. On cooling no crystallisation occurred. The mixture was heated under reflux in a boiling water bath for 30 minutes and the solvent evaporated slowly at 0°C. No crystallisation occurred.

Anilide formation. The fraction (40 mg.) was treated with an alcoholic solution of aniline (40 mg.) in a boiling water bath for 1 hour. At the end of this time crystals/

crystals appeared in the hot solution. After cooling the solution the crystals were removed by filtration and recrystallised from ethyl acetate to give a colourless crystalline material m.p. 109-110°C alone or admixed with an authentic specimen of the anilide of 2:4-dimethyl galactose (m.p. 110°C).

Lactone formation. The fraction (50 mg.) in water (2 ml.) was oxidised with liquid bromine for 2 weeks. Treatment as before yielded a syrup which distilled to give the lactone b.p. 150-160°C (bath temperature)/0.1 mm.

Amide formation. The lactone was treated with methanolic ammonia at 0° for 4 days. The ammonia and alcohol were removed in a vacuum desiccator and the resultant syrup dissolved in ethyl acetate. This solvent was allowed to evaporate slowly at room temperature to yield the crystalline amide m.p. 163-165°C alone or on admixture with an authentic specimen of 2:4-dimethyl galactonamide (m.p. 166°C).

Examination of Fraction p.

The syrup was purified twice from water and after 4 days crystallised almost completely. Yield: 49.8 mg. Chromatographic examination showed the presence of a sugar (R_f 0.36) corresponding to 2:4-dimethyl galactose on the same paper and also what appeared to be a monomethyl pentose (R_f 0.30). The latter sugar was present in only a very small quantity amounting to approximately/

approximately one quarter of the total fraction. The crystals melted slowly between 80° and 90° C.

Examination of Fraction q.

The syrup after purification twice from water amounted to 17.4 mg. and showed on chromatographic examination the presence of small amounts of 2:4-dimethyl galactose (R_G 0.36), a monomethyl pentose (R_G 0.34), and a dimethyl or monomethyl hexose (R_G 0.30).

Examination of Fraction r.

The syrup after purification from water amounted to 0.104 g. and consisted almost entirely of free xylose in addition to a very small amount of free arabinose as evidenced by paper chromatography.

DISCUSSION.

The evidence put forward by Chanda, Hirst, Jones and Percival (15) has proved that there is present in esparto grass a pure xylan consisting entirely of xylose units, and that the average chain of this xylan is singly branched, one branch occurring for every 75 β - D - xylo-pyranose units. It was thought possible therefore that the arabinose in esparto hemicellulose was present in the form of an araban such as that which has been shown to be present in pectic materials.

The solubility characteristics of such an araban vary considerably from those of the xylan examined by Chanda et al. One would expect therefore that under favourable conditions ordinary solvent extraction would remove the araban and leave the xylan undissolved. It has been shown in the introduction (p. 3) that chemical combination between cellulose and the accompanying xylan is not necessary to explain the difficulty encountered in extracting the latter from cellulose, and that a straight chain xylan could substitute for the cellulose in the cellulose matrix. If the xylan was further overlaid with cellulose chains it would be extremely difficult to remove it without first disrupting the cellulose matrix by means of very vigorous treatment with solvents such as strong sodium hydroxide under pressure and at a high temperature.

If a multi-branched araban such as that found in pectic/

pectic substances exists in esparto holocellulose substitution of this material in the cellulose matrix would not be expected to occur and consequently solvent extraction should be effective in removing it from both the cellulose and the xylan. The experimental evidence gained in the present work in this connection has shown that such a solvent extraction is not effective and the first suggestion is made, therefore, that the arabinose is not present in the form of the usual much branched araban.

Secondly one would expect that the esparto holocellulose obtained by extraction with cold 4% sodium hydroxide solution should, by nature of its method of preparation, have no micellar structure. The difference already noted in the solubility characteristics of araban and xylan would then allow separation of these compounds by normal solvent extraction. Again the experimental evidence obtained has shown that this is not the case.

Chanda was able to effect a separation of an arabinose free xylan from esparto grass by purification of the hemicellulose as the copper complex. Decomposition of the insoluble fraction of the copper complex resulted, after several such treatments, in the preparation of a xylan which gave an almost theoretical yield of xylose on hydrolysis. An attempt was therefore made to prepare a pure araban from the soluble fraction of the copper complex. It was found that this compound still contained an appreciable quantity of xylose. This suggests that the/
the/

the xylose residues in this material are present in a form other than that which has been suggested for the xylan in esparto grass. It is not possible from this evidence to decide whether the araban is separate or whether it is part of a xylo-araban or arabo-xylan.

The acetate of pectic araban is known to be soluble in acetone and methyl alcohol while the corresponding acetate of xylan is insoluble in these solvents. An attempt to isolate an esparto araban as the acetate from acetylated esparto hemicellulose was unsuccessful, xylose being found to be present in both the soluble and insoluble fractions. This could be explained by a solubility effect on the xylan diacetate caused by the araban acetate. Alternatively, if the arabinose was present as part of a combined arabinose/xylose molecule, solution of the acetate of this combined molecule might conceivably occur. Finally the xylan, if present in some form other than that proposed by Chanda et al, might also be soluble in the solvents mentioned.

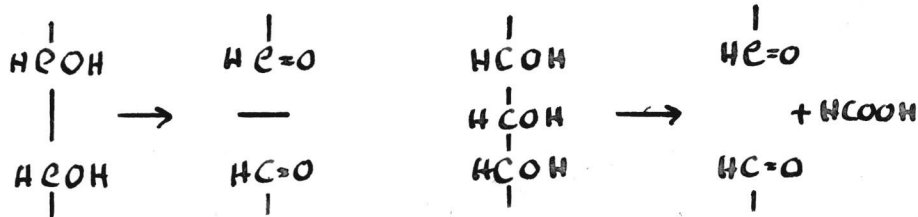
Hydrolysis of esparto hemicellulose with very weak acid (30) is known to affect the arabinose units in preference to the xylose and the suggestion has been made therefore that this points to a xylan in which the arabinose is present as end group. Perlin (62) has found that by hydrolysing the soluble pentosan material of wheat flour with very weak sulphuric acid (0.05N) at 100°C for 30 minutes and then cooling the hydrolysate, a precipitate/

precipitate was formed. This on hydrolysis showed the presence of xylose only, showing that the arabinose had been removed completely. The fact that an insoluble material is thus formed from the soluble pentosan by the simple removal of arabinose would appear to strengthen the argument in favour of a xylan with arabinose end groups. The preferential removal of arabinose does not in itself prove that the arabinose must be present as part of the pentosan molecule, as an araban such as the peanut araban is very readily hydrolysed with weak acids and could conceivably be completely hydrolysed before any measurable amount of hydrolysis of an accompanying xylan had taken place.

A precipitation similar to that observed by Perlin was not observable on cooling the hydrolysate from polysaccharide A owing to the fact that in all hydrolyses of this material precipitation occurred immediately an aqueous solution of the polysaccharide was acidified prior to hydrolysis. This precipitate gradually redissolved as the hydrolysis proceeded. Such a behaviour could be expected with either a mixture of two polysaccharides or with a combined molecule.

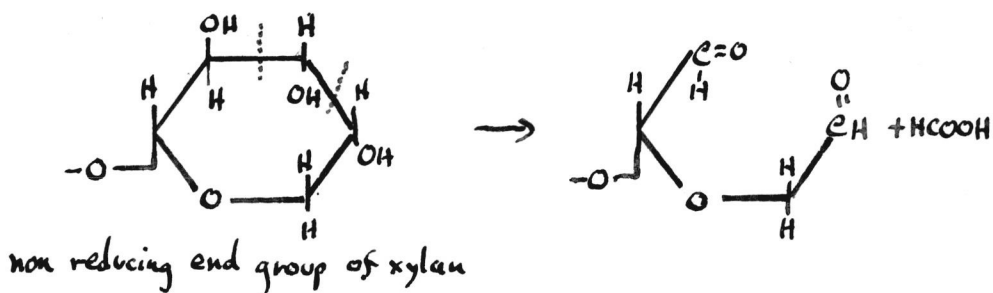
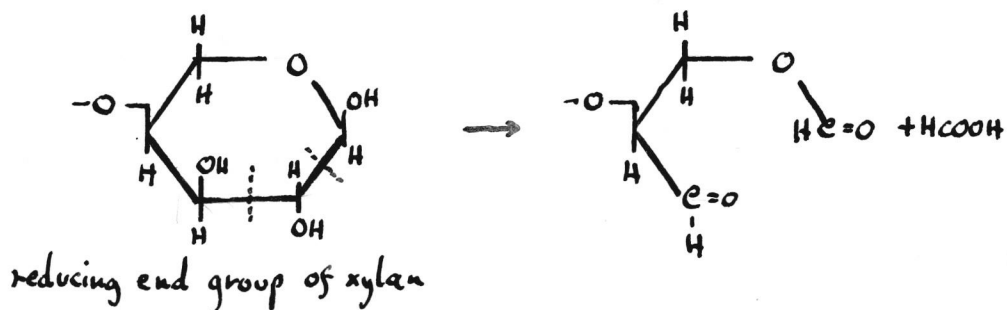
We may turn now to an examination of the use of oxidation with the periodate ion in structural carbohydrate chemistry (61). As is well known periodic acid attacks the carbon-carbon bond between carbon atoms each of which is linked to a hydroxyl group, the bond disrupting to form two aldehydes or, in the case of cyclic compounds/

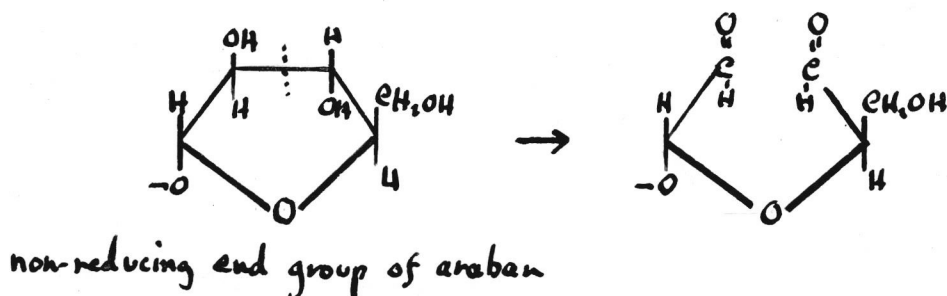
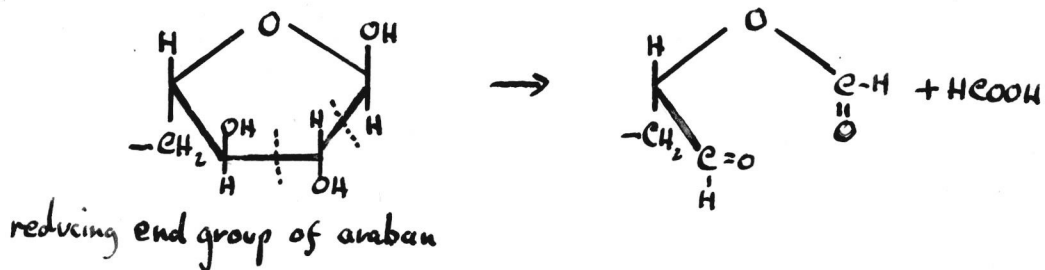
compounds such as the carbohydrates, a dialdehyde (XXI). When hydroxyl groups are located on three adjacent carbon atoms the middle carbon atom will yield formic acid.



It can be seen that in the case of a straight chain xylan in which the glycosidic linkage was 1:4 such an oxidation would result in the formation of one molecule of formic acid for every xylose residue present at the end of the chain (XXII). In the case of araban only the reducing end group would yield one molecule of formic acid while the non-reducing end group having only two adjacent hydroxyl groups would form the dialdehyde.

XXII





It is obvious then that if esparto hemicellulose existed in the form of a long chain of xylose units terminated by arabinose units the formic acid released would be very much less than in the case of a straight chain xylan with which an araban was present as an impurity. Estimation of the formic acid then would give an indication of the structure to be expected. The results obtained in the present work however, are obviously affected by the presence of substances other than polysaccharides present in the material examined, and no satisfactory conclusions can be drawn from them.

Similarly, it can be shown that in the case of a straight chain xylan linked 1:4 or 1:2 one mole of periodate will be consumed for every anhydropentose unit while if the linkage is 1:3 no periodate will be consumed except at the ends of the chain. In the case of a structure/

structure such as that put forward by Hirst and Jones (41) for peanut araban, only two out of every three arabinose units possess the necessary two adjacent hydroxyl groups to enable the periodate ion to attack the residue. Again the results obtained for the polysaccharide A can only be explained by assuming that a considerable amount of periodate has been consumed by the non-carbohydrate fraction of the material.

Finally, if a branched pentosan is subjected to periodate oxidation and the resultant oxidised polysaccharide hydrolysed in the normal way, those residues corresponding to the branching points which have not been attacked will be recovered in the hydrolysate as the free sugars. The xylose recovered in this way after oxidation of polysaccharide A amounted to 33.1% of the original xylose present suggesting that the xylan present was very much branched and consequently quite unlike that obtained from esparto grass by Chanda. The arabinose recovered was 15.9% of the original arabinose. This does not agree with the methylation results obtained for A, but it is noted that although previous workers have reported that no monomethyl arabinose can be found in the hydrolysis products of methylated esparto hemicellulose, Ehrental and Smith (32) state that even after extended oxidation of the xylan from wheat straw L-arabinose can still be detected in the hydrolysate. If the methylation results are to be considered valid it seems that the oxidation is unable to be carried to completion/

completion possibly due to some type of steric hindrance. It would, however, seem reasonable to conclude that the araban, if such exists, is not as branched as the pectic arabans, if branched at all.

Methylation studies, while not always enabling one to assign a unique structure to the polysaccharide examined, do give a very valuable information concerning the branching, if any, of the polysaccharide and the type of linkage between the residues. The methylated esparto xylan prepared by Chanda et al gave on hydrolysis 4% of 2-monomethyl D-xylose. This must have arisen from a branching point, positions 1, 3 and 4 being involved in union with neighbouring xylose units. Position 5 would be occupied by the pyranose ring. The majority of the xylose however must be in the form of a straight chain, the xylose residues being linked through positions 1 and 4 as evidenced by the presence of 93% 2:3-dimethyl xylopyranose. The remaining 2:3:4-trimethyl xylose corresponds to an end group xylose linked through position 1. This suggests that xylan consists of a singly branched molecule containing 70 - 80 D-xylopyranose units.

Similarly Hirst and Jones in the study of peanut araban concluded from the presence of equimolecular quantities of the mono-, di- and trimethyl arabinoses in the hydrolysate of the methylated polysaccharide that the molecule contained one branching point for every 3 pentose units (see p. 18).

Obviously/

Obviously an examination of the products of hydrolysis of the methylated polysaccharide A should give valuable information concerning the question of the existence of an independent araban. If a reasonable quantity of monomethyl arabinose could be found then the possibility of such an araban existing would be definitely enhanced though by no means proved. If, on the other hand, no monomethyl sugar could be detected, then it would be obvious that a multi-branched araban was not present. Also, if no dimethyl arabinose was present, then an independent araban could not exist and the arabinose would necessarily be present as part of an "araboxylan". This would be the case also if the amount of dimethyl sugar found was less than the trimethyl arabinose.

It had been hoped that the separation of methylated araban from the corresponding xylan derivative would be feasible but the experimental evidence shows that the normal method of fractionation when applied to methylated esparto hemicellulose does not give a separation. This, of course, does not necessarily mean that an esparto araban does not exist. It became obvious that in order to study the arabinose derivatives obtained by hydrolysis of the methylated hemicellulose, it would be necessary to prepare first an arabinose-rich fraction of the hemicellulose and methylate this. The attempted separation of an araban from crude xylan showed that it was not possible to increase the arabinose:xylose ratio beyond/

beyond 1:1. In a separation yielding a fraction as rich in arabinose as this the yield was found to be impractically small. It was decided therefore to extract esparto hemicellulose with 70% aqueous alcohol to give polysaccharide A. As mentioned before the pentose content of this material was only ca 40% but the ratio of arabinose:xylose was 3:7.

It was not possible to obtain a completely methylated polysaccharide A by the methods used, the final methoxyl content being 36.6% as opposed to the theoretical figure of 38.8% for a fully methylated pentosan. This fact must be borne in mind in a subsequent interpretation of the results. A further difficulty arises from the fact that complete separation of the methylated sugars could not be achieved.

The only evidence we have for the presence of trimethyl xylose is the fact that the trimethyl pentose fractions b and c yield on demethylation small quantities of xylose in addition to arabinose. A further indication that fraction c contains xylose is given by the colour obtained with aniline oxalate on a paper chromatogram. This was more similar to that obtained with an authentic specimen of 2:3:4-trimethyl xylose than was the colour obtained with fraction b. Finally the less negative optical rotation of fraction c as opposed to fraction b lends further support to the idea that a small amount of trimethyl xylose is in fact present.

It/

It has not been possible to determine the identity of the sugar (R_G 0.82) detected in fraction f. This sugar on demethylation yielded arabinose and it is possible that it may correspond to an incompletely methylated arabinose end group.

The proportions of the component sugars in those fractions containing mixtures were estimated solely by comparison of the intensities and areas of the colour spots obtained on a paper chromatogram. By this method the following figures were arrived at for the molar proportions of each sugar.

2:3:4-trimethyl xylose	5
2:3:5-trimethyl arabinose	29
2:3:4:6-tetramethyl galactose	3
2:3-dimethyl xylose	100
2:3-dimethyl arabinose	6
2:4:6-trimethyl galactose	8
2-monomethyl xylose	40
2:4-dimethyl galactose	8
xylose	6

It is obvious from these figures that even allowing for errors due to approximations etc. an araban of the type found in pectic materials is not present in polysaccharide A. The ratio of trimethyl arabinose to dimethyl arabinose is approximately 5:1, a quite impossible figure for an individual pentosan. In addition to this/

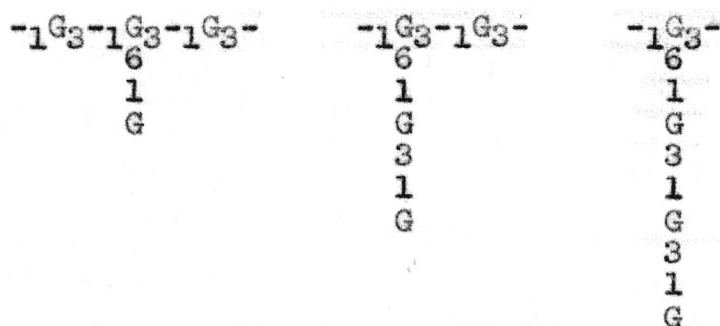
this the presence of a monomethyl arabinose could not be proved and, although a sugar which appeared to be a monomethyl pentose (R_G 0.30) was not identified, even if we assume this to be a monomethyl arabinose the amount present is far too small (1 mole) to lend any support to the suggestion of an individual araban.

On turning now to the methylated xyloses we find that the situation is reversed and we have a large yield of the monomethyl sugar and a small yield of trimethyl xylose. After taking into account the fact that incomplete methylation will give rise to the presence of an excess of monomethyl sugar, we still find that the xylan if it exists as an arabinose free compound contains approximately one branching point for every 4 xylose residues. In addition the presence of a small amount of the free sugar suggests that double branching occurs, though again under-methylation may be the reason for the presence of this free sugar. If we assume that branching does occur then we would expect to find approximately equimolecular quantities of trimethyl and dimethyl xylose. In actual fact the amount of monomethyl xylose found is 8 times greater than the trimethyl sugar and this cannot be explained by under-methylation.

It would seem reasonable to assume then, that the arabinose and xylose are combined in the same molecule in polysaccharide A. This combined polysaccharide would consist of a chain of xylose units with one branching point for every four xylose residues, the majority of these/

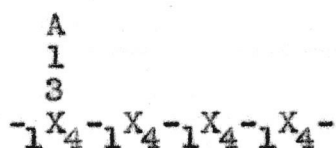
these branching points being linked to an arabinose residue. It is possible also that a double side chain of arabinose might occasionally be present.

On examining the methylated galactose fractions we find that the tetramethyl, trimethyl and dimethyl sugars are all present in reasonable quantity. Although these galactose residues could be part of a mixed polysaccharide, it is more probable that a multi-branched galactan exists as a separate entity. Branching would occur through positions 1, 3 and 6 and would probably amount to 1 branching point for every 2 units in the galactose chain. Suggested repeating units for such a galactan are shown below (XXIV).



G = D-Galactopyranose.

It would seem fairly certain then that we have in polysaccharide A an araboxylan for which a repeating unit of the type shown below is a possibility. The arabinose side chains may occasionally be replaced with xylose or with a double arabinose chain.



One/

One cannot rule out the possibility of this arabo-xylyan being a mixture of a still more branched araboxylyan and a xylyan of the type suggested by Chanda et al.

New methods of fractionation will be necessary before a further clarification of the situation can be brought about. If the galactose part of the polysaccharide mixture can be removed, a separation of the methylated sugars obtained by hydrolysis of the methylated arabo-xylyan would be very much easier and a more quantitative estimation of the sugars would be possible.

A complete removal of the non-carbohydrate material associated with polysaccharide A would be necessary before periodate oxidation results could yield any satisfactory evidence. Removal of this might assist penetration by the periodate ion and consequently remove the discrepancy noted above between the presence of an arabinose branching point as indicated by oxidation studies and the absence of the same as shown by methylation results.

Finally a better separation of the methylated sugars and in particular the trimethyl xylose and trimethyl arabinose is necessary to determine the amount of trimethyl xylose corresponding to xylose end groups and to decide whether any monomethyl arabinose corresponding to an arabinose branching point can be detected.

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